Celebrating 75 years

Souvenir

National Seminar
on
Sustainable Crop Productivity through Physiological Interventions

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Organized by

Department of Life Science
Ramnarain Ruia College, Matunga, Mumbai

&

Indian Society for Plant Physiology, New Delhi

National Seminar on Sustainable Crop Productivity through Physiological Interventions
November 24-26, 2011, Ramnarain Ruia College, Matunga, Mumbai

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HEAT STRESS SYSTEM BIOLOGY OF RICE CROP

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Introduction

Most detailed work on plant heat shock proteins has been carried out using *Arabidopsis*, a model-plant. Since 1990, our group at Delhi University is aiming to establish the characteristics of heat shock proteins of the crop plants. Employing rice as model-crop, the questions we address are: (1) genetic diversity in heat shock proteins/heat shock factors, (2) expression biology of heat shock proteins/heat shock factors, (3) biochemical roles of heat shock proteins, (4) regulation of heat shock proteins/heat shock factors expression (heat shock promoter analysis; heat shock element configurations; heat shock factor/heat shock factor interactions; heat shock factor/heat shock element binding; heat shock proteins/heat shock factors interactions) and (5) genetic transformation for higher heat tolerance

Basic tenets of heat stress system biology

Daily fluctuations in high day and/or night temperatures adversely affect crops. While some stages of plant growth may be more sensitive to high temperature than others, there is an overall reduction in plant performance when temperature is higher than the optimal temperature at specific growth stages.

Conventional breeding for high temperature stress tolerance has not been much successful due to several reasons like lack of suitable source(s) of genes in sexually-compatible gene pools, complex nature of the high temperature stress trait, lack of understanding on the genetic mechanisms of the high temperature tolerance response etc. Advent of recombinant DNA (rDNA) technology methods has opened avenues for tackling issues relating to complex genetic traits. The need for raising high temperature tolerant transgenic crops has been felt since the early days of rDNA science but however, not much has been achieved yet as the underlying physiological processes, biochemical enzymes and molecular mechanisms that impart high temperature tolerance have not been precisely defined and understood.

To possibly enable plants to grow, reproduce and set seeds at high temperatures, altering the cellular response of plants to high temperature is an important objective. To achieve this objective, it is relevant that the molecular components that underlie the high temperature stress response in plants are understood. Microarray-based transcript and two-dimensional protein gel electrophoresis coupled with microspectrometric technique-based proteome profiling data have shown that heat stress response largely involves heat shock proteins and proteins involved in metabolism of reactive oxygen species (ROS) scavengers, hormones and sugars. In recent times, it has been shown that plants for high temperature tolerance can be genetically-engineered by altering heat shock proteins (Hsps) either directly or through regulatory circuits that govern Hsp levels, levels of osmolytes, components of the cell detoxification mechanisms and components that regulate membrane fluidity.

High temperature stress is, in general, simulated in laboratory experiments by subjecting biological systems to heat shock (HS) treatment. Plants mount resistance to HS by eliciting specific metabolic adjustments. A great deal of detailed understanding has been gained on various components of the heat shock response (HSR) in living organisms including features like heat shock genes/proteins, heat shock promoters and heat shock elements (HSEs), heat shock factors (HSFs), possible receptors of the heat shock response, signaling components and chromatin re-modeling aspects. The temperature for the induction of plant HSR varies amongst different plant species but an increase of 5-10^{o}C over and above the ambient temperature is

generally sufficient to elicit the HSR. It is shown that nearly all organisms, ranging from bacteria to man, respond to HS by synthesizing Hsps. Hsps are broadly classified on the basis of their molecular weights as high molecular weight (HMW-Hsps; 70-100 kDa) and low molecular weight (sHsps; 15-20 kDa) Hsps. Under stress conditions, sHsps may comprise up to 1% of the cellular proteins. During the past nearly forty years of research, Hsps have been extensively-analyzed for their physiological, biochemical, cellular and molecular properties. It has been shown that Hsps are highly-conserved proteins across different species. Besides HS, Hsps are induced in response to several different abiotic stresses as well such as heavy metal stress, water stress, wounding stress, salt stress, cold shock stress and anoxia stress.

Plants, in general, survive lethal high temperature stress more efficiently after prior exposure to a mild high temperature stress as against direct response to lethal high temperature stress. This phenomenon is termed as acquired thermotolerance. Hsps are believed to be important for the protection of cells against heat injury both in basal thermotolerance (i.e. thermotolerance achieved without prior HS) as well as in acquired thermotolerance responses. Several groups have altered levels of sHsps in bacterial systems and shown that when over-expressed in bacterial cells, sHsps have a role in conferring thermotolerance. The involvement of Hsps in regulating thermotolerance in plants has been indicated by down-regulating their levels through antisense and RNAi approach. Mutants of *Zea mays* and *A. thaliana* plants under-expressing their respective Hsp100 proteins are observed to lack both basal as well as induced thermotolerance. Conversely, up-regulation of Hsps has been achieved in a large number of studies. There is clear evidence showing that HSEs interact with positively-acting regulatory HSF proteins to bring about increased transcription of Hsp genes. In recent years, Hsf gene induction system has emerged as a powerful target for manipulating levels of Hsps in transgenic experiments.

Hsp100 family of rice

Rice (*Oryza sativa*) is the most important world food crop. The production of rice has been severely affected with increases in mean global temperature. According to estimates made at IRRI (Manila, Philippines), yield of rice declines by 10% for every 1oC increase in growing period minimum temperature in the dry season. Processes like spikelet fertility, grain quality and yield processes in rice are especially sensitive to heat stress. Rice has emerged as a model plant species of the group monocots due to its small genome size, availability of large collection of full-length cDNAs (FL-cDNAs) and for the fact that the whole genome of this plant species is completely sequenced. This crop has attracted a great deal of efforts for the elucidation of gene functions; completion of genome sequencing in rice has paved way for comprehensive functional characterization of genes, transcription factors, signaling components and promoters. The completed rice genome sequence has been used for the characterization of a large number of gene families involved in diverse processes and pathways. However, there are ample proteins still left to be characterized in this important crop species.

Comprehensive details on HS regulated rice Hsp20, Hsp70, Hsp90 and Hsf gene families have been reported. Hsp100 is a major heat-regulated protein family in diverse organisms. Across the living systems, common features of Hsp100 chaperone action include transient interactions with non-native protein species, in the prevention of aggregation and promotion of correct folding and assembly, or in unfolding for translocation or targeting to proteases.

Nearly 20 years back, our group showed that homologues of yeast Hsp104 protein are expressed in heat shocked rice seedlings. It was subsequently established that apart from heat, rice Hsp100 expression is developmentally-controlled as seeds and developing embryos of rice show high constitutive levels of this protein. We largely work on detailed characterization of this important protein family.

Hsp100-ClpB relationship

Hsp100 proteins belong to ClpB family. Clp ATPases maintain quality of cellular proteins by performing the function of molecular chaperones and energy dependent proteases. Clp ('Caseinolytic Protease') system was first identified as a heat shock

inducible, multicomponent, ATP-dependent protease complex able to hydrolyze casein. Subsequent studies showed that the Clp system can hydrolyze numerous other proteins and peptides in both aggregated and non-aggregated forms. Clp ATPases fall within the AAA+ superfamily of ATPases associated with a substantially broader range of biological processes. Class I ATPases (ClpA, ClpB, ClpC, ClpD) have two ATP binding domains and class II Clp ATPases (ClpM, ClpN, ClpX, ClpY) have one ATP binding domain. Basically, Clp system members include three non-homologous gene families: ClpABCXY, ClpP and ClpQ. ClpACX members (but not ClpB) facilitate the activity of ClpP and some, such as ClpA and ClpX, can function as independent chaperones in roles analogous to those of DnaK and DnaJ proteins. In contrast, ClpP proteolytic subunit exhibits low levels of peptidolytic activity. Further, when ClpP is complexed with ClpA, ClpC or ClpX, active holoenzymes which are able to cleave denatured proteins are formed. ClpB is different from ClpA, ClpX and HslU as it does not associate with peptidase subunits. The function of ClpB is also distinct from that of other Clp ATPases: this protein is not involved in protein degradation, instead it disaggregates and reactivates strongly aggregated proteins. The aggregation reversing activity of ClpB requires cooperation with the Hsp70/Hsp110 chaperone machinery.

In *Arabidopsis*, ClpB proteins have been divided into 3 classes according to cytoplasmic, chloroplastic and mitochondrial isoforms. We have shown that rice genome has 9 entries (i.e. Os02g08490, Os02g32520, Os03g31300, Os04g32560, Os04g33210, Os11g16590, Os11g16770, Os12g12850 and Os05g44340) for the class I Clp ATPase protein family. From the comparative details, it appears that 9 rice loci specify 3 ClpB (ClpB-cyt, Os05g44340; ClpB-m, Os02g08490; ClpB-c, Os03g31300), 4 ClpC (ClpC1, Os04g32560; ClpC2, Os12g12580; ClpC3, Os11g16590; ClpC4, Os11g16770) and 2 ClpD (ClpD1, Os02g32520; ClpD2, Os04g33210). ClpB proteins were categorized into cytoplasmic, chloroplastic and mitochondrial isoforms based on TargetP, Predotar, LOCTREE and PSORT database.

Cross-induction of Hsp genes by diverse stresses is known. We reported that OsClpB-cyt/Hsp100 transcript in rice seedlings is induced strongly by heat and this transcript remains unaffected in response to salt stress, water stress, low temperature stress and ABA application. Microarray analysis has shown that OsClpB-cyt transcript was up-regulated more than 5-folds both during heat stress as well as oxidative stress. OsClpB-cyt transcript was induced after 48 h of low temperature stress. OsClpB-c was up-regulated during heat stress by up to 5-folds and during oxidative stress by up to 1.5 folds based on microarray analysis. Q-PCR (quantitative-PCR or real-time PCR) data showed that as with OsClpB-cyt, the relative abundance of OsClpB-c transcripts was far more responsive during heat stress as compared to oxidative stress. OsClpB-m gene was found to be heat and oxidative stress up-regulated. Q-PCR data showed that transcript abundance of OsClpB-m was highest during heat stress among the three OsClpB genes. We reported that OsClpB-m and OsClpB-c are localized to mitochondria and chloroplast, respectively. It therefore, appears that these proteins may have specific functions in cellular organelles.

Yeast expressing Hsp104 are typically 100- to 1000-fold more thermotolerant than yeast lacking Hsp104, thus demonstrating the critical requirement for this protein in cell survival during extreme heat stress. *Arabidopsis* ClpB-Cyt/Hsp101 has been shown to overcome the temperature sensitivity of yeast Ähsp104 mutant. This fact prompted us to analyze ability of diverse class I Clp genes in yeast mutant complementation assay. OsClpB-cyt showed reasonably good complementation ability. The capability of OsClpB-m appeared comparable to OsClpB-cyt in conferring tolerance.

We drew a comparable picture of *Arabidopsis* and rice class I Clp ATPases. There are some major differences in these two species for the number of ClpB, ClpCs and ClpDs nuclear genes. While *Arabidopsis* has 2 and 1 ClpC and ClpD members, respectively, rice appears to contain 4 and 2 ClpC and ClpD members, respectively. On the other hand, number of ClpB proteins appears same (3) in both rice and *Arabidopsis*. Both in rice and *Arabidopsis*, one each of ClpB type is present in cytoplasm, chloroplasts and mitochondria.

Rice Hsp100 promoter

OsClpB-cyt/Hsp100 transcript in rice seedlings is not expressed at detectable levels under unstressed control conditions but imposition of heat stress results in its rapid and predominant expression. 2kb OsClpB-cyt promoter contains an HSE, a STRE, an AP-1 binding element as well as a C/EBP binding element. Stably transformed rice seedlings with 2kbProOsClpB-cyt::Gus construct showed expression of Gus in a heat-regulated manner. From our study, the OsClpB-cyt promoter appears to be a classical HS promoter. The regulation of stress-related genes with constitutive promoters (i.e. CaMV35S promoter, actin promoter and ubiquitin promoter) in the plant biotechnology industry is considered undesirable as it may lead to unwanted and hence wasteful metabolisms, causing ultimately a reduction in the growth and yield of transgenic plants.

Heat stress and metal stress show an overlapping signal reception. 2kbProOsClpB-cyt::Gus rice seedlings in our work showed expression of Gus in response to treatment with Co, Cd and As. In reproductive tissues, a constitutive presence of transcripts/proteins for Hsp100 has been noted. A publicly available microarray database shows that the OsClpB-cyt transcript is expressed in embryo, endosperm, seed and panicle tissues (https://www.genevestigator.ethz.ch/). T1 seeds of 2kbProOsClpB-cyt::Gus rice plants showed significant Gus expression histochemically, under unstressed control conditions. In seeds, the Gus expression was seen predominantly towards the embryonal part. Earlier work showed that the expression of the OsHsp100 protein is mostly localized to the embryonal half of seeds. Rice is most susceptible to heat injury during flowering, as pollen viability is particularly sensitive to heat stress; even 1-2 h of high temperature at anthesis results in high spikelet sterility From the data on high expression of Hsp100 in anther tissues and high OsClpB-cyt promoter activity noted in this study, it may be inferred that Hsp100 is an important component in pollen physiology of rice plants at high temperature.

In transgenic rice plants raised with a deletion in HSE-273 to -280 sequence (ΔPro-HSE-273 to -280::Gus plants), HS inducibility of this promoter was not affected as Gus expression in ΔPro-HSE-273 to -280::Gus plants was increased above the control values upon HS. However, unlike in 2kbProOsClpB-cyt::Gus seedlings where Gus transcript/protein expression was at undetectable levels under unstressed control conditions, expression of Gus was noted under control conditions in ΔPro-HSE-273 to -280::Gus seedlings. It thus emerges that the HSE-273 to -280 sequence has a role in repressing expression of the downstream transcript under control, uninduced conditions. The relationship of HSEs to HS expression appears to be of a complex nature. HSE-273 to -280 deletion may be influencing the transcription apparatus such that the constitutive control has been affected while the induced control has remained unaffected. There was no change in the expression profiling of Gus in ΔUTR-HSE-like-97 to -107::Gus plants: the heat stress inducibility of Gus as well as the non-inducibility under unstressed conditions was maintained. It is thus likely that the HSE-like-97 to -107 is not an important region for regulation of the OsClpB-cyt transcription.

sHsps of rice

Hsp20 or small Hsps (sHsps) are expressed in maximal amounts under high temperature stress. The characteristic feature of the sHsps is the presence of α-crystallin domain (ACD) at the C-terminus. sHsps cooperate with Hsp100/Hsp70 and co-chaperones in ATP-dependent manner in preventing aggregation of cellular proteins and in their subsequent refolding. Database search was performed to investigate the sHsp gene family across rice genome sequence followed by comprehensive expression analysis of these genes. We identified 40 α-crystallin domain containing genes in rice. Phylogenetic analysis showed that 23 out of these 40 genes constitute sHsps. The additional 17 genes containing ACD clustered with Acd proteins of Arabidopsis. Detailed scrutiny of 23 sHsp sequences enabled us to categorize these proteins in a revised scheme of classification constituting of 16 cytoplasmic/nuclear, 2 ER, 3 mitochondrial, 1 plastid and 1 peroxisomal genes. In the new classification proposed herein nucleo-cytoplasmic class of sHsps with 9 subfamilies is more complex in rice than in Arabidopsis. Strikingly, 17 of 23 rice sHsp genes were noted to be intronless. Expression analysis based on microarray and RT-PCR showed that 19 sHsp genes were upregulated by high temperature stress. Besides heat stress,

expression of sHsp genes was up or downregulated by other abiotic and biotic stresses. In addition to stress regulation, various sHsp genes were differentially upregulated at different developmental stages of the rice plant. Majority of sHsp genes were expressed in seed. In conclusion, (1) we identified twenty three sHsp genes and seventeen Acd genes in rice, (2) three nucleocytoplasmic sHsp genes were found only in monocots and (3) analysis of expression profiling of sHsp genes revealed that these genes are differentially expressed under stress and at different stages in the life cycle of rice plant.

Heat shock factors of rice

Canonically, Hsfs have a core structure comprising of an N-terminal DNA binding domain, adjacent oligomerization domain with a heptad hydrophobic repeat (HR-A/B), a signal for nuclear localization (NLS) and export (NES) and a C-terminal AHA type activation domain. With the finished genome sequence (rice annotation release 5), we showed that 26 genes constitute OsHsf gene family. Based on the EST counts in various tissues (under unstressed condition), OsHsfs genes were noted to be expressed to variable extents in tissue specific manner. Microarray and Q-PCR results showed that OsHsfs (except OsHsfA5, OsHsfB4c, OsHsfC1a and LOC_Os06g22610) were induced by heat shock to variable extents. Q-PCR showed that OsHsfC1a was initially slightly down-regulated in the heat shock response. We suggested that OsHsfA2a, OsHsfA2c, OsHsfA2d, OsHsfA2f and OsHsfB4b may be regarded as the sensors of the heat shock response in rice. Cold stress resulted in down-regulation of all class B OsHsfs except for OsHsfB4a and OsHsfB4b genes. OsHsfB4b showed high expression levels after 5 h of cold treatment. Among the class A members, OsHsfA3, OsHsfA4d and OsHsfA9 showed up-regulation in the transcript levels after CS. In contrast, all class C OsHsfs showed inducible expression in response to low temperature with OsHsfC1b showing the strongest expression. Oxidative stress caused enhanced transcript expression to variable extents of selected OsHsfs. OsHsfA2a, OsHsfB4b and OsHsfC2a appear to be the primary players in the pathways that involve ROS accumulation and sensing. OsHsfA2a showed the strongest expression during the oxidative stress. We also proposed that OsHsfA2f and OsHsfA7 may be involved in the late response to the oxidative stress. OsHsf genes showing co-induction (such as OsHsfA2a and OsHsfA2f in HS and OS and OsHsfB4b and OsHsfC2a in all the three stress conditions) may represent pivotal points in possible cross-talk among the stress responsive pathways. The co-expression profiling of Hsfs in response to varied abiotic stresses indicates that these transcription factors might be regulating multiple mechanisms.

We did one-hybrid assays to unveil binding of rice Hsfs to OsClpB-cyt promoter. Significant increase in downstream LacZ expression was noted from OsClpB-cyt promoter in yeast cells on transformation with OsHsfA2c only in our assay, suggesting that OsHsfA2c is specifically involved in regulation of the OsClpB-cyt promoter. Apart from OsHsfA2c binding to OsClpB-cyt promoter, we noted that OsHsfA2c binds with OsClpB-cyt based on BiFC assays. OsHsfA2c thus appears to be an important player in the rice heat shock response. Earlier work has documented that OsHsfA2c is one of the most rapidly induced Hsf genes in response to heat stress, that OsHsfA2c possesses transactivation activity, that OsHsfA2c shows trimer formation activity from its monomeric forms and that OsHsfA2c binds to perfect-type HSE. Our study further noted that OsHsfB4b binds with OsClpB-cyt based on yeast two-hybrid and BiFC assays. Positive interaction was marked for all three isoforms of OsClpB isoforms namely, OsClpB-cyt, OsClpB-c and OsClpB-m with OsHsfB4b. BiFC assays confirmed binding of OsHsfB4b with OsClpB-cyt as well as OsHsfA2c. Our study suggests that heat stress transcription factors and heat shock proteins orchestrate an Hsf:Hsp circuitry which may involve several additional proteins.

Heat shock elements of rice

The ability of HS promoters to sense and respond to heat is mainly due to the presence of consensus sequences called heat shock elements (HSEs) located in the promoter region. The eukaryotic HSE consensus sequence has been defined by altering units of 5'-nGAAn-3'. HSEs are separated into three types: perfect (P), gap (G) and step (S). P-type HSE has three inverted repeats in a contiguous array

(nTTCnnGAAnnTTC). G-type HSEs have two consecutive inverted sequences, with the third sequence separated by 5bp (nTTCnnGAAnn(5bp)GAAn). S-type HSEs have 5bp gaps separating all the three modules (nTTCn(5bp)nTTCn(5bp)nTTCn). Hsfs bind with HSEs, eventually resulting in transcriptional activation of HS genes. We noted that in rice genome with estimated size of 67393 genes, 2830 genes contain at least one of the 3 HSE type configurations in their 1kb upstream promoter region. Overall, 953 genes contain P-, 695 genes G- and 1584 genes S-type HSEs. We further show that as only ~16% of HS induced genes contain the canonical HSE types in their 1kb upstream promoter regions, a major population of the HS induced genes are not associated with canonical HSE types. Our observations highlight the inadequacies in our current understanding of the relevance of HSEs in HS response, in rice.

Formation of trimeric form of Hsfs is considered important for attaining their high affinity binding to HSEs. OsHsfA2a, OsHsfA2c, OsHsfA7, OsHsfA9, OsHsfB4b and OsHsfB4c showed trimer formation activity. Notably, trimer formation activity in different members was temperature-dependent to differential extents. OsHsfB4b showed maximum trimer formation activity at lower temperature than maximum trimer formation activity noted in class A Hsfs (OsHsfA2a, OsHsfA2c, OsHsfA7 and OsHsfA9). Our study reflects first case of plant Hsfs showing this property. It remains to be seen what relevance this temperature-dependent pattern under in vitro conditions has in terms of in vivo physiological conditions. EMSA analysis showed that class A OsHsfA2a, OsHsfA2c, OsHsfA7 and OsHsfA9 and class B OsHsfB4b and OsHsfB4c proteins bind to 3P HSE. On the other hand, only class C OsHsfC1b protein tested did not show binding to this HSE probe. We noted that 3 class A proteins (OsHsfA2c, OsHsfA2d and OsHsfA9) show transactivation (TA) activity while 2 class A members (OsHsfA2a and OsHsfA7) lack TA activity. Class B OsHsfB4b and OsHsfB4c proteins lacked TA activity. Out of OsHsfC1a, OsHsfC1b and OsHsfC2a class C members tested, OsHsfC1a, and OsHsfC1b showed TA activity that appeared comparable to class A members in extent. Using yeast two-hybrid screen, we noted that OsHsfB4b forms homomers. On the other hand, homomer formation activity was lacking for OsHsfA2a, OsHsfA7, OsHsfB4c and OsHsf26 proteins. BiFC (bimolecular fluorescence complementation) analysis showed that OsHsfA2c and OsHsfB4b form homomeric state. BiFC assays further showed that homomeric OsHsfA2c and OsHsfB4b forms are clearly nucleus-localized. Notably, OsHsfB4b showed interaction with different OsHsfs tested in our study. It is possible that plasticity in functioning of HSFs is contributed by their homomeric and heteromeric associations. From the above account, we note that OsHsfB4b is predominantly involved in rice HS response. OsHsfB4b forms homomeric interactions to form a trimeric state and makes heteromeric interactions with OsHsfA2a, OsHsfA7, OsHsfB4C and OsHsf26. We have earlier noted that OsHsfB4b binds to OsClpB-cyt/ Hsp100 protein. In conclusion, we highlight that S-type HSE configuration predominates in promoter of rice genes. Temperature optima of trimer formation are differential in different OsHsfs. Transactivation activity is differential amongst OsHsfs. DNA binding activities is variable depending upon class of OsHsfs and incubation temperatures. It is likely that these differential patterns have bearing on cellular functioning of OsHsfs under a range of different physiological and environmental conditions, which influence synthesis of different target proteins governed by HSE/Hsf interactions.

Salient findings

1. Polyclonal antibodies produced against rice Hsp100 proteins. Immunological evidence presented to show that proteins related to rice Hsp100 are synthesized in response to several stress conditions and in several plants. Rice Hsp100 shown to have immunological kinship to yeast Hsp104.
2. Established that Hsp100 expression is developmentally-controlled as seeds and developing embryos of rice have constitutively high levels of this protein.
3. Isolated and sequenced rice Hsp100 gene for the first time internationally (EMBL/Gene Bank /DDBJ database accession no. AJ316025). Rice Hsp100 cDNA is shown to complement yeast hsp104 deletion and loss of thermotolerance trait in yeast. Established genetic complexity,

expression profiling and functional characterization of rice ClpB/Hsp100 proteins. Produced transgenic rice for over-expression of Arabidopsis thaliana Hsp100 gene.

4. Isolated and sequenced rice Hsp100 gene promoter (Accession number AJ302059). Showed that rice Hsp100 gene is transcriptionally and post-transcriptionally regulated.

5. Established genetic complexity and expression profiling of rice sHsp gene family members.

6. Established genetic complexity and expression profiling of rice Hsf gene family in response to multiple stresses. Established binding affinities and interactions amongst different heat shock element and heat shock factor types in rice.

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PHYSIOLOGY OF WATERLOGGING TOLERANCE IN PLANTS

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Crop plants require a free exchange of atmospheric gases for photosynthesis and respiration. Like animals, plants can be easily suffocated if this gas exchange is impeded. The most common impediment to gas diffusion is water that saturates the root environment in poorly drained soils or that accumulates above soil capacity as a result of the overflow of rivers, excessive rainfall or excessive irrigation. Flooding and submergence are major abiotic stresses and rank alongside water shortage, salinity and extreme temperatures as major determinants of species distribution worldwide. Plants invariably wilt within few hours to 2-4 d of imposing a flooding stress. This is a consequence of higher resistance to mass flow of water through the root. Wilting is caused by the inhibition of respiration and loss of ATP synthesis in the roots. This blocks the ion transport systems that normally create the gradient in water potential across the root endodermis. Plants react to an absence of oxygen by switching from an oxidative to a solely substrate-level phosphorylation of ADP to ATP; the latter reactions predominantly involve glycolysis and fermentation. An important adaptive response is formation of aerenchyma, specialized tissues in roots, which allow diffusion of gases like O_2 from aerobic shoot to hypoxic/anoxic roots. Besides, non-symbiotic-hemoglobins and nitric oxide have also been suggested as an alternative to fermentation for maintaining lower redox potential (low NADH/NAD ratio), and thereby playing an important role in anaerobic stress tolerance and signaling. A detailed review of the topic has been provided by Sairam *et al.* (2008).

Hypoxia and anoxia

Normally plant roots are in contact with oxygen at a partial pressure equivalent to the gaseous atmosphere (0.21 atmospheres). The reduction of oxygen below optimal levels, termed *hypoxia*, is the most common form of stress in wet soils and occurs during short-term flooding when the roots are submerged under water but the shoot remains in the atmosphere. Hypoxia will also occur in roots near the surface of longer-term flood water. The complete lack of oxygen, termed *anoxia*, occurs in soils that experience long-term flooding, in plants completely submerged by water, in deep roots below flood waters. Long-term flooding shifts the microbial flora in the soil in favour of anaerobic micro-organisms that use alternative electron acceptors to oxygen. As a consequence the soil tends to accumulate more reduced and phytotoxic forms of mineral ions such as nitrite (from nitrate) and ferrous (from ferric) ions and few plants are adapted to growth in these soils.

Flooding and ethylene production

Many of the adaptive growth response in hypoxic roots and shoots occur in response to ethylene. Ethylene accumulates in flooded soils and in submerged plant parts to concentrations of 10 ml L^{-1}. This is accomplished by two mechanisms. First the diffusion of ethylene from the root into the water is 10 times slower than its diffusion into air. This ethylene may be released into the internal aerenchyma channels and diffuse from the root to the shoot. Secondly, the synthesis of ethylene in the hypoxic root and in the aerobic shoot is increased.

Ethylene biosynthesis

Ethylene synthesis is stimulated in response to only a modest depression of internal oxygen concentration. The immediate precursor of ethylene is 1-amino cyclopropane 1-carboxylic acid (ACC), which is synthesized to a large extent in roots. Because the conversion of ACC to ethylene has an obligate requirement for oxygen, this reaction is blocked in an anaerobic root cell. The ACC is therefore, translocated from the anaerobic root cells towards the more aerobic portions of the root or to the shoot. ACC synthesis does not require oxygen, and in fact, ACC synthase

activity is stimulated in roots under flooding conditions. As a consequence the quantity of ACC transported to the shoot increases. The lower portions of the stems are usually the site of highest ACC levels and in the presence of oxygen ethylene is released.

Ethylene and aerenchyma formation

Ethylene synthesis was strongly enhanced in roots under hypoxia, coincident with the development of aerenchyma (Visser *et al.* 1997). Aerenchyma formation in maize roots was also stimulated by exogenous application of ethylene at rates as low as 0.1 ml L^{-1} and was inhibited in the presence of Ag^{+} ions, an inhibitor of ethylene action (Drew *et al.* 1981). The presence of a growing root tip is also essential for the formation of aerenchyma. The root tip may serve as the site of ACC synthesis (Drew *et al.* 1981).

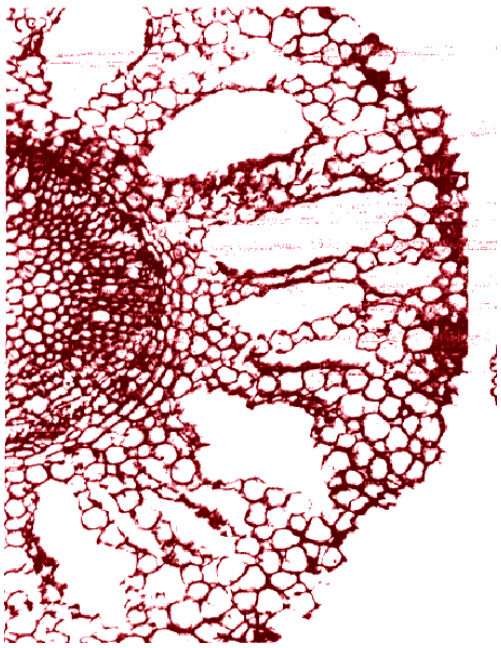

Fig. 1: Aerenchyma formations in flooded roots of Vigna luteola, a land race of marshy low lands of Kerala, India

Aerenchyma is soft tissues with large intercellular spaces to provide low resistance internal pathway for the exchange of gases between aerobic shoot to the anaerobic root (Fig. 1). Aerenchyma formation under waterlogged condition have been reported in a wide range of crop species such as, *Trifolium subterraneum* (Aschi-Smiti *et al.* 2004), wheat (Watkin *et al.* 1998), barley (Arikado and Adichi 1955), rice (Justin and Armstrong 1991), maize (Gunawardena *et al.* 2001) and *Vigna luteola* (Sairam *et al.* - unpublished).

Some of the oxygen transported through the aerenchyma to plant root tips leaks out of pores in the root and into the surrounding soil. This can result in a small zone of oxygenated soil around individual roots providing an aerobic environment for microorganisms that can prevent the influx of potentially toxic soil components such as nitrites and sulphides of ferrous, cuperous and manganous.

There is little information to date on the molecular regulation of aerenchyma formation or the identity of other enzymes involved. In maize, a flooding-induced gene (*xet1*) encodes a xyloglucan *endo*transglycosylase (XET1), a putative cell wall loosening enzyme (Saab and Sachs 1996). O_2 deprivation induces expression of XET1 in the primary root, mesocotyl, and coleoptile of maize seedlings. The induction of *xet1* appears to be specific to O_2 deprivation, since other stresses do not induce the gene. The induction of *xet1* by hypoxia was associated with aerenchyma development. Hypoxic induction of XET1 mRNA is repressed by ethylene antagonists [(aminooxy) acetic acid, 2-aminoethoxyvinyl-glycine, $AgNO_3$]. XET1 is also induced under aerobic conditions by exogenous ethylene, as is aerenchyma.

Shift in energy metabolism and anaerobiosis induced proteins (ANP)

The second major effect of oxygen depletion in flooded roots occurs because oxygen serves as the terminal electron acceptor of mitochondrial electron transport, consequently the reduced oxygen supply results in an impairment of the terminal step in mitochondrial electron transport. Lack of oxygen thus effectively blocks ATP synthesis in the mitochondria. In the absence of an electron acceptor, NADH oxidation is blocked. Once the mitochondrial respiration stops, the adenylate energy charge of the cell (ratio of ATP to ADP and AMP) declines. In the absence of an adaptive response, the flooded root cell rapidly depletes its available supply of ATP. One supplemental source of ATP for the cell is accessed through a stimulation of

glycolysis and fermentation, known as the Pasteur effect.

However, glycolysis is relatively inefficient at energy production compared to mitochondrial respiration. It also generates large quantities of pyruvate as an end-product that must be converted to alternative products to recycle NADH to NAD. These end-products of glycolysis and fermentation pathway, such as ethanol, lactic acid and carbon dioxide pose an additional hazard to the cell. Since many of the enzymes of the Kreb's cycle are regulated allosterically by the NADH/NAD ratio, the entire Kreb's cycle is blocked and glycolysis is stimulated by an increase in redox potential [NAD(P)H/ NAD(P)]. The pyruvate that accumulates from glycolysis is converted initially by LDH to lactic acid. Cytoplasmic pH consequently declines as a result of lactic acid accumulation, a process known as cytoplasmic acidosis. Low pH inactivates LDH, which has a pH optimum above 7.0 and activates PDC and ADH that are normally inactive above pH 7. Therefore, pyruvate is shunted to the production of either ethanol or lactic acid in a pH dependent manner that allows tight regulation of cytoplasmic pH around 6.8.

Alcohol dehydrogenase is a major ANP expressed under hypoxic/anoxic conditions. Its activity is critical for the recycling of NADH and thus for the continuation of glycolytic pathway (Johnson *et al.* 1994). Peng *et al.* (2001) demonstrated that *ADH* induction is linked to ethylene production. They demonstrated that hypoxic induction of *ADH* could be partially inhibited by aminooxy acetic acid, an inhibitor of ethylene biosynthesis. This partial inhibition can be reversed by the addition of ACC, a precursor of ethylene. In addition, the hypoxic induction of the *ADH* gene is also reduced in *etr*1-1 and *ein*2-1, two ethylene insensitive mutants in ethylene-signaling pathways (Peng *et al.* 2001).

Roberts *et al.* (1984a, b) reported a maize mutant deficient in one of the ADH genes, and therefore, is unable to produce a functional ADH enzyme. When this mutant was flooded, LDH synthesized lactic acid, pH declined, but ADH was not able to synthesize ethanol. As there was no counterbalance to LDH and the pH continued to decline to very low levels, consequently this mutant was more sensitive to flooding injury than the wild type plant and died after 3 days of submergence. Many of the proteins induced were subsequently identified as enzymes of the glycolytic and fermentation pathways (Dolferus *et al.* 2003). The identified ANP include sucrose synthase, phosphohexose isomerase, fructose-1,6-diphosphate aldolase, pyruvate decarboxylase (PDC), lactate dehydrogenase (LDH), and alcohol dehydrogenase (ADH) (Chung and Ferl 1999, Zeng *et al.* 1999).

Hypoxia and non-symbiotic hemoglobins

It is now known that there are several classes of hemoglobins in plants. Non-symbiotic hemoglobins, as the name implies, are not involved in symbiotic nitrogen fixation (Hill 1998). There are two classes of nonsymbiotic hemoglobins, one has oxygen-binding properties similar to symbiotic hemoglobins (class 2), the second with dramatically different oxygen-binding properties (class 1). Class 1 hemoglobins are induced by hypoxic stress and oversupply of some nutrients, and are generally referred as stress-induced hemoglobin.

Constitutive expression of barley hemoglobin in wild-type and transformed maize cells lines maintained cell adenine nucleotide levels and energy charge under hypoxic conditions, whereas wild-type cells and cells in which haemoglobin expression is suppressed had lowered adenine nucleotide levels and energy charge (Sowa *et al.* 1998). Transformed alfalfa root cultures, lines constitutively expressing barley hemoglobin maintained root growth during hypoxic treatment, whereas wild-type and lines with suppressed stress-induced hemoglobin expression had slower root growth (Dordas *et al.* 2003a, 2004). Non-symbiotic haemoglobin is a highly efficient scavenger of oxygen at low oxygen tensions, and thus, there is possibility that it may act in a metabolic reaction involving oxygen, where it could potentially interact with other enzyme proteins or molecules in an oxygen-consuming reaction in low oxygen environments (Duff *et al.* 1997).

Hemoglobin and nitric oxide interaction

While hypoxic stress-induced hemoglobins are widespread in the plant kingdom, their function has

not been elucidated. Dordas *et al.* (2003b) proposed that nitric oxide is an important metabolite in hypoxic plant cells and that at least one of the functions of hypoxic stress-induced hemoglobins is to modulate nitric oxide levels in the cell. Dordas *et al.* (2003b) demonstrated the presence of NO/haem complexes in both hypoxic maize cell cultures and alfalfa root cultures using electron paramagnetic resonance (EPR) spectroscopy. Dordas *et al.* (2003b) showed that significant amounts of NO are formed in hypoxic maize cells during the first 24 h of hypoxic treatment. Transformed lines with reduced stress-induced hemoglobin expression produced greater amounts of NO than wild-type or over expressing-hemoglobin lines, suggesting that hemoglobin may be involved in turnover of the NO. There is also the possibility that NO may be activating guanylate cyclase, as it is reputed to do in defence gene induction (Durner *et al.* 1998). The induction of stress-induced hemoglobin in *Arabidopsis* in the presence of elevated nitrate may also relate to a requirement to modulate NO levels (Wang *et al.* 2000). Stress-induced hemoglobins have also been implicated in regeneration of NAD^+ during hypoxia (Hill 1998) based on the observations that alcohol dehydrogenase activity and CO_2 production is reduced under hypoxia in maize cells constitutively expressing barley hemoglobin (Sowa *et al.* 1998).

Nitric oxide synthesis in roots may involve nitrate reductase (NR) and nitrite oxide synthase or nitrite-nitric oxide reductase ((NiR-NOR). In roots, two distinct types of nitrate reductase are present, one located in the cytosol (cNR) and the other attached to the plasma membrane and facing the apoplast (PM-NR) (Stöhr and Mäck 2001). There is a 2.5-fold activation of cNR during exposure of plant roots to hypoxia (Botrel and Kaiser 1997), with nitrite reduction being suppressed at the nitrite reductase step (Botrel *et al.* 1996). The potential maximum activity of activated nitrate reductase, although lower than alcohol dehydrogenase, exceeds the rate of hypoxic ethanol formation by more than 3-fold (Botrel and Kaiser 1997). Yamasaki *et al.* (1999) using purified cNR from maize showed that a side-reaction of cNR is the reduction of nitrite to NO with NADH as an electron donor, probably catalyzed by the same molybdenum cofactor-containing domain as in nitrate reduction.

Plasma membrane nitrate reductase (PM-NR) activity was initially demonstrated by Ward *et al.* (1989). It is present only in root tissue where it exceeds the activity of cNR, particularly during the night (Stöhr and Mack 2001). It can use both succinate and NADH, but succinate is the preferred electron donor. Plasma membrane-bound nitrite-NO reductase (Ni-NOR) is the likely enzyme that converts nitrite to NO rather than PM-NR. Ni-NOR faces the apoplast and has an activity sufficient to convert all of the nitrite formed by PM-NR to NO (Stöhr and Ulrich 2002). Ni-NOR uses reduced cytochrome c for nitrite conversion to NO (Stöhr *et al.* 2001).

Regeneration of nitrate is essential under nitrate limiting conditions of anaerobic roots for the continuation of Hb-NO cycle. It has been suggested that oxyhaemoglobin would donate negatively charged dioxygen to NO, forming nitrate and methemoglobin, a known reaction of oxyhaemoglobin (Di Iorio 1981). The reduction of methaemoglobin to haemoglobin can occur in a number of ways. A methemoglobin reductase has been demonstrated in nodules of leguminous plants (Topunov *et al.* 1980). A number of diaphorase-type enzymes, such as cytochrome b_5 reductase of the endoplasmic reticulum (Hagler *et al.* 1979) or dihydrolipoamide dehydrogenase (Moran *et al.* 2002, Igamberdiev and Hill 2004) have methaemoglobin reductase activity. Another possibility is the presence of this reaction in the haemoglobin molecule itself. Figure 2 outlines a metabolic scheme showing how

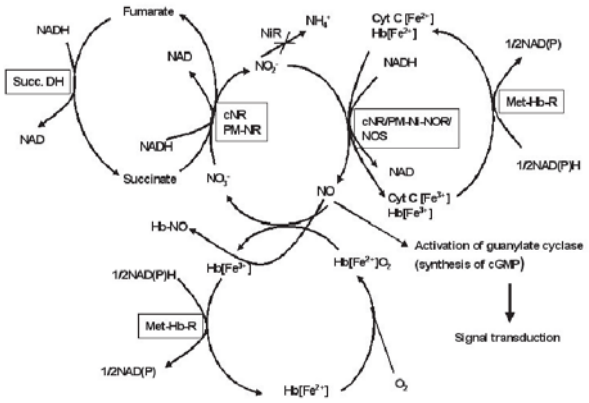

Fig. 2: Suggested pathways by which nitric oxide formation and its interaction with non-symbiotic-hemoglobin may be involved in adaptation to hypoxic stress

reactions involving both NO and haemoglobin could function in plant cells during hypoxia.

NO is also an attractive candidate for involvement in aerenchyma formation. It has been suggested that NO may interact with reactive oxygen species to produce peroxynitrite (ONOO-) directly kill plant pathogens (Durner and Klessig 1999). There is an abundance of literature on NO and programmed cell death in many mammalian tissues. Depending on NO concentration and other factors, NO may either accelerate or inhibit apoptosis (Kim *et al.* 2001). The effect may be either direct, through cell necrosis, or through regulatory pathways and it may also be selective in relation to the cells that do respond. A similar type of reaction could be responsible for selected cell death during aerenchyma formation in roots exposed to waterlogging (Drew 1997).

Conclusions and perspectives

Examination of regulatory mechanisms and signaling events responsible for triggering responses to oxygen deficient condition in plants is an interesting area of research. It is imperative to identify sensors and dissect the signaling pathways that occur at the cellular, tissue, organ and whole plant level. It will be interesting to investigate the participation of an ROS sensing mechanism involving PM-NADPH oxidase in different plants species. Again the paradoxical ROS may prove to be second messenger in the response mechanism. Further it will be interesting to determine whether observed increases in NO evolution under flooding condition from roots or soils can contribute as a positive message in root to- shoot communication. Alterations in cytosolic pH and calcium may also have a role in the signaling processes. The importance of changes in adenylate charge, redox status and carbohydrate levels must also be considered. Many questions remain to be answered about the response of individual cells. What could be the basis of differential response between stress-tolerant and intolerant organs and species? Do these differ in cellular signaling and response mechanisms? Again we need to understand what signalling transduction pathways are activated or inhibited, how do multiple and interacting pathways control adaptive responses? The involvement of growth regulators such as ethylene, auxin, gibberellins and ABA in hypoxic regulation is also an interesting possibility. The manner in which the energetic needs of meristematic cells are safeguarded and how is programmed cell death promoted or avoided, also needs examination? How do cells in roots and aerial organs communicate over a long distance when there is an oxygen crisis in the roots? Understanding the cell to cell and long-distance signalling mechanisms that determine the organ and whole plant response to oxygen deprivation, viz., regulation of leaf and internode elongation, petiole curvature, aerenchyma formation and adventitious root growth is another inviting area for research. So far we only know a part of the unfolding story, with many more questions still unanswered. Answering these questions will be of relevance to agriculture and will provide knowledge of the fundamental nature of anaerobic life.

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ENHANCEMENT OF POST-HARVEST LIFE IN HORTICULTURAL CROPS: MODULATION OF ETHYLENE-DEPENDENT AND -INDEPENDENT PATHWAYS

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Postharvest practices for the preservation of fruits, vegetables, and flowers are perhaps one of the oldest in human history. With the understanding of the molecular processes that occur during plant senescence, this discipline has developed unique features of its own. Traditionally, postharvest science is considered as an applied science focusing on the physiological aspects of enhancement of shelf life and preservation of quality of horticultural produce. However, in the past two decades biochemical and molecular biological aspects have been extensively used for analyzing postharvest issues. This work is a compilation of some of the advances in basic aspects of postharvest science. Postharvest issues are common around the world. The extent of the loss of horticultural produce after harvest can vary in different countries. In those parts of the world where the methods of agricultural production and storage employ advanced technology, postharvest losses may be minimal, and most of it occurs during the transit of produce from the production site to the destination along the consumer chain. The losses can range from 10 to 20% by volume. In tropics (say India) where the production practices are basic and based on day-to-day demand, the postharvest loses can be as high as 50% or over. It is surprising and a bit disturbing to see that fruits are considered as luxury items in some part of the world. In an era where we consider the consumption of fruits and vegetables as a means of health promotion, postharvest science gets a new meaning.

Importance of fruits, vegetables, and flowers in world economy

The production of fruits, vegetables, and flowers has been an important sector in the total world agricultural output. Although an accurate economic contribution from these segments around the world is difficult to obtain because of the reporting systems, it is estimated that the contribution from these segments could be over US$600 billion.

Importance of fruits and vegetables as food

Human evolution was potentially linked initially to the consumption of naturally available fruits and vegetables, which later might have resulted in the selection of preferred plants and varieties for agriculture. The cultivation of grapes and its processing into wine is a classic example of the use of fruits. Fruits and vegetables are also used in traditional medical systems such as Ayurveda. Fruits and vegetables are major sources of several essential nutrients that include vitamins A and C and folic acid. In addition, fruits and vegetables are rich in antioxidants such as carotenoids, polyphenols, and anthocyanins that help combat free radicals produced within the body and the excess production of which has been related to the development of cardiovascular diseases, Alzheimer's, macular degeneration, and cancers. Fruits and vegetables are integral components of food in all societies; however, in some parts of the world, this is limited due to agricultural collapse or sociopolitical conflicts. Fruits are considered as high-value items and not readily accessible to economically challenged segments of population around the world. With the results from a number of epidemiological studies spanning several countries and continents and population groups showing the relation between increased fruit and vegetable consumption and a reduced risk of developing maladies such as cardiovascular and cerebrovascular diseases, cancer, neurodegenerative diseases such as Alzheimer's, and macular degeneration, a very positive attitude toward fruit and vegetable consumption has emerged recently, especially in advanced countries.

Fruit, vegetable and flower production around the world

Every part of the world produces fruits and vegetables mostly according to the needs of domestic consumption and export. Apples, bananas, citrus fruits, grapes, tomatoes, and watermelons are the largest fruit commodities produced. Asia is the largest producer of fruits, with China being the primary producer in the whole world. Although the population of China and India are nearly the same, fruit production in India is considerably lower than that in China. This may be due to the differences in food habits between the two countries, with the Chinese population consuming a lot more fresh fruits and products. On a per capita basis, Israel produces more fruits than any other country in the world. Europe and North and Central America are also major players in fruit production. Chile is the major producer of fruits in South America. Overall, the world produces greater than 600 million metric tons of fruits (FAO Statistics, 2005).

World vegetable production also follows similar trends as in fruits, Asia being the largest producer of vegetables. Again, China is the powerhouse of vegetable production, fresh vegetables being a major component of daily diet. Europe and the Americas also contribute significantly to vegetable production. Altogether, the world vegetable production exceeds 650 million metric tons (FAO Statistics, 2005).

By contrast to fruit and vegetable production, the bulk of flower production is concentrated in specific regions of the world. The production and marketing of flowers and potted ornamentals are intricately linked across the world. Worldwide trade in floriculture products was estimated to exceed US $7.9 billion in 2001 and was made up of cut flowers (50%), plants (41%), bulbs (9%) and cut foliage (9%). Over 70% of the production of floriculture crops is concentrated in seven countries: the Netherlands, Columbia, Italy, Belgium, Denmark, the United States, and Ecuador. The Netherlands is the flower capital of the world, with over 50% of world trade (2000 estimate, www.agf.gov.bc.ca/ornamentals; British Columbia, 2003) in floriculture products that include produce grown within the Netherlands (farm gate value of US$5.4 billion), as well as floriculture products that are imported and marketed again to various destinations. Columbia was the second largest exporter with an estimated trade at 7.5%, and the rest of the flower-producing countries having export components of 2-3% each. The major flower markets (70%) include Germany, the United States, Britain, France, the Netherlands, and Japan. In the United States and Canada, the production has shifted from flowers such as carnations, chrysanthemums, and roses to specialty cut flowers such as gerbera, *Lizianthus*, snapdragons and *Alstroemeria* that are relatively more difficult to grow and transport. Also, the cultivation of potted ornamentals is on the rise. According to USDA (2002) estimates, there were over 8,000 ha in floriculture production with a wholesale value estimated at US$4.88 billion. A similar estimate by Statistics Canada (2002) provides a value of Canadian $1.4 billion for Canadian floriculture products.

Postharvest loss of fruits, vegetables and flowers

By virtue of their physiological properties, most fruits, vegetables, and flowers are highly perishable commodities. Postharvest losses can occur at any point in the production and marketing chain, and may range anywhere from >10% in advanced countries to >50% in tropical areas and where storage facilities are limited. China, the largest fruit and vegetable producing country, experiences postharvest losses in the range of 20-25%. Since a large proportion of fruits and vegetables produced are immediately consumed, the loss from long-term storage is considerably reduced. As well, processing can also reduce postharvest losses. China is the largest producer of apple juice concentrate, and this reduces the necessity for large storage facilities. Moreover, such facilities are not suitable since the sizes of farms are rather small. Cold storage facilities may be available for 10-15% of the fruit crops in China. Cooling and transportation facilities are also being developed in China. In India, the problem of storage for pulses is reduced by letting the fresh fruits mature and dry. Several fruits from the cucumber family are ripened, after which they can be stored for months without specialized storage facilities. In countries such as India and China where the weather is tropical and subtropical, fruits and vegetables characteristic to these

climates are produced. As well, the production of many of such commodities is seasonal, and this reduces the necessity of long-term storage. Thus, it is common to have peaks of availability for fruits such as apple, orange, pear, banana, mango, and guava spread throughout the year. Even then, postharvest loss of 50% or greater is common as the storage facilities at the local vendors are limited and good quality products are hard to find.

Strategies to improve quality

A recent understanding among the growers on the effect of growing conditions on the quality of produce has brought a welcome change in the attitude toward the goals of production. Simply producing a commodity in large amounts need not assure the optimum postharvest quality of the produce. In general, the quality of a produce cannot be enhanced through adopting postharvest storage technologies. The quality of a produce is determined by the growing conditions, nutritional regimes, and the genetic potential of the particular variety. Thus, increased attention is being given to these attributes. Several novel postharvest technologies developed in recent years have the potential to maintain the high quality of produce during subsequent storage at optimal conditions. These include active modified atmosphere and dynamic controlled atmosphere. In addition, there is growing concern about food safety, which is also being addressed in the postharvest area. Growers in Europe and those in other countries who export to Europe are now required to implement the standards of Good Agricultural Practice. These standards are to ensure the safety and quality of fresh produce and require accountability and traceability of produce entering the European market. This means that the farmer must keep a record of the irrigation, fertilization, and pest management treatments that he applies; the packinghouse, exporter, and the shipper must record the treatments given at the packinghouse and storage conditions of the produce along the distribution chain. It also requires safety and cleanliness conditions for the workers in the farms and packinghouse. The producer and exporter must be examined by an external evaluator to ensure that Good Agricultural Practice is being implemented.

The necessity for traceability has led to the development of barcodes and radiofrequency identification stickers that can trace a commodity from "farm to fork." Some farms and packinghouses now have barcodes on each container in the orchard that is read as it enters the packinghouse and is weighed and checked. Radiofrequency identification systems have been given to both orchard pickers and packinghouse workers. As fruit is packed, the packer prints a label that includes a barcode, grower, count, date and time, pack house and variety details, and packer. This enables fruit to be traced back to the orchard and row from which it was harvested and to know all the steps it took along the way to the consumer. In the area of produce quality, packinghouses have units of quality assessment, where samples of the produce entering the packinghouse as well as along the packing line are examined for quality. Recent innovations are instruments that can determine various aspects of quality nondestructively. On many packinghouse lines, currently fruit is automatically graded for colour and blemishes by online cameras that photograph the fruit and send it to the proper sorting line. This is combined with sorting for weight or size by automatic weighing cups that send different sizes to different lines. Newer technologies include near-infrared spectrometers that can examine internal quality, particularly soluble solids or sugars, as well as acoustic instruments that can measure firmness. These are now being supplied in new packinghouse lines, and in addition, hand-held instruments are in development for use in orchards to determine picking date.

Biotechnological approaches are also useful for enhancing the shelf life and quality of fruits, vegetables, and flowers, but the public acceptance of this technology is limited. Several information sites describing the optimal storage procedures have also increased the importance and understanding of postharvest storage (e.g., Sydney Postharvest Laboratory, www.postharvest.com.au/; www.usda.org).

Fruit ripening and flower senescence

Fruit ripening is a complex, genetically programmed process that culminates in dramatic

changes in colour, texture, flavour and aroma of the fruit flesh. Because of the economic importance of fruit crop species, the ripening processes have been, and continue to be, studied extensively at both the biochemical, molecular and genetic levels. Fruits with different ripening mechanisms can be divided into two groups: climacteric, in which ripening is accompanied by a peak in respiration and a concomitant burst of ethylene, and nonclimacteric, in which respiration shows no dramatic change and ethylene production remains at a very low level. In tomato and other climacteric fruits such as apple, avocado, banana, mango, melon, papaya, pear and peach, the ethylene burst is required for normal fruit ripening, as illustrated by the slowing or inhibition of ripening in ethylene-suppressed transgenic plants. Furthermore, it has been shown that ethylene affects the transcription and translation of many ripening-related genes. However, although ethylene is the dominant trigger for ripening in climacteric fruits, it has been suggested that both ethylene-dependent and ethylene-independent gene regulation pathways coexist to co-ordinate the process of ripening in climacteric and nonclimacteric fruits. Fruit ripening is accompanied by softening which is one of the most important determinants of fruit quality and consumer acceptability. Progressive depolymerization of the major classes of cell wall polysaccharide such as pectins, cellulose and hemicellulose during ripening can lead to the excessive softening, resulting in heavy postharvest losses, including damages during shipping and handling. These changes are brought about by cell wall modifying enzymes, like polygalacturonase (PG), pectin methyl esterase (PME), pectate lyase (PEL), cellulase, xyloglucan-endo-transglucosidase/hydrolase and protein-like expansins, which may act sequentially or synergistically.

On the other hand, plant senescence is the final event in the growth and development of a plant and ultimately leads to the death of a particular organ or whole plant. The senescence in plants is highly regulated, genetically programmed and developmentally controlled process. This phenomenon involves structural, biochemical, and molecular changes that in many cases bear the hallmarks of programmed cell death. Plant hormones and environmental factors play an important regulatory role in senescence. Flower senescence has been described as the last stage of floral development, although in the life cycle of most plant species, it is not a final event, rather an integral process that allows the removal of a metabolically costly tissue (i.e., petal), after it has attracted pollinators for sexual reproduction, and signals the initiation of ovule development and seed production. At the end of their life, petals may wilt, lose colour or abscise, or in some cases, remain on the flower stem, encasing and protecting the developing ovary. It is an actively ordered process that involves the synthesis of new RNAs and proteins and results in highly coordinated changes in metabolism and the programmed disassembly of cells.

All cut flowers are destined to die, and the challenge for postharvest researchers is to slow the processes controlling flower death to enable cut flowers to reach distant markets with a display life. Postharvest performance of cut flowers is affected by the developmental stage of a flower at harvest, pro-senescence signals that originate from specific tissues within the flower (e.g., pollination-induced petal senescence), and stress-related metabolism (in response to temperature, wounding, nutrient starvation). Cut flower stems are removed from a source of nutrients, undergo water restrictions, and may be held at undesirable temperatures in the dark for days prior to sale. Plant hormones, membrane stability, water availability, cellular proteolysis, and carbohydrate metabolism act in concert to determine the differential rate of senescence for each floral organ. Currently, flowers can be grouped into several categories based on postharvest technologies that can extend their vase life (e.g., sensitivity to ethylene, chilling sensitivity, leafy stems, multiple/single flowers per stem, and woody stems).

Flower petals are ideal tissues for cell death studies as they are short lived, the tissue is relatively homogenous, chemical manipulation can be applied without substantial wounding (i.e., feeding through the vascular tissue), and the process of flower senescence has been shown to be a genetically programmed event. A great deal of recent research in this area has led to review and reevaluation of senescence and cell death in plant tissues. To date, most genetic analyses of floral senescence have focused on changes that occur in

mature flowers just prior to wilting or colour change. However, senescence of one floral organ is part of a developmental continuum in the flower, preceded by tissue differentiation, growth and maturation of the petal, followed by growth and development of seeds, and co-coordinated by plant hormones. Cell death processes are thought to be regulated by anti- and pro-death proteins, which may be expressed throughout the life of the flower, providing for the most part a highly regulated homeostatic balance. Future genetic analyses of floral senescence are likely to identify the proteins that function to maintain a non-senescent "youthful" state, and the "pro-senescence" proteins which function to progress cell death. The past decade has seen increasingly rapid isolation and identification of senescence-associated genes from cut flower crops, with a somewhat slower movement toward understanding the function and significance of the gene products. Genome-wide searches for regulatory flower senescence genes have now been made in a number of flower species, for example, *Alstroemeria*, carnation, chrysanthemum, daffodil, daylily, rose, *Iris*, *Sandersonia*, and petunia. Characterizing generic patterns of gene expression has identified common processes that are linked with the progression of flower senescence (e.g., ethylene signaling and proteolysis). This approach will also be useful in identifying the order of molecular changes associated with flower senescence, thereby enabling researchers to accurately study cause and effect. This article briefly focuses on molecular and genetic research published within the last one decade that has increased our understanding of the processes involved in or regulating flower senescence (e.g., ethylene, water quality, cytokinin, sugar, proteolysis, membranes, and cell walls), and its significance to the postharvest industry.

Role of phytohormones in ripening and senescence

Phytohormones play an important role in ripening of fruits and flower senescence. The complete understanding of the hormonal control of ripening/ senescence is lacking because of insufficient data on endogenous phytohormones levels and the effect of different hormone ratios.

Ethylene

There are number of evidence that indicate the role of ethylene in induction of ripening in fruits and senescence in flowers. When submitted to either specific ethylene biosynthesis inhibitors or inhibitors which block the action of ethylene, tomato fruits show strong inhibition of ripening but when fruits in the green-ripe stage are exposed to exogenous ethylene, maturation can be activated. Treatment of green bananas with ethylene during the pre-climacteric phase shortens the time required for ripening initiation. Ethylene-induced ripening of banana fruit depends on the duration of ethylene treatment. The effect of ethylene and short chain saturated fatty acids on ethylene sensitivity in ripening bananas show faster ripening in fruits exposed to ethylene for a 24-h period than in fruits treated for a 6-h period. This response of banana fruit to ethylene appears to be related to the binding of ethylene to its receptor sites in the tissue. Treatment with ethylene results in the complete saturation of all the available sites. Treatment of green banana in pre-climacteric stage with octanoic acid results in a suppression of ethylene synthesis, possibly as a result of inhibition of the membrane associated ethylene forming enzyme and an increase in the sensitivity of the fruit to ethylene. Treatment of guava fruits with 1-methylcyclopropene (1-MCP) results in significant suppression of ethylene production during storage as well as ripening. When ethylene perception is avoided by using MCP, the increase in ethylene production is stopped, and softening and colour development of the papaya fruit are partially delayed, indicating that softening and colour development are dependent on ethylene. Softening of apple, melon and tomato also depends on ethylene. Continuous ethylene treatment is required for the initiation and progression of peach fruit softening; also ethylene concentration is an important factor in regulating the rate of softening.

The autocatalytic production of ethylene in climacteric fruit is perceived by ethylene receptors and a signal is transduced through a cascade, finally activating several transcription factors (TFs), which in turn activate hundreds of target genes directly or indirectly. A cumulative action of these genes bring about several changes in fruit, such as chlorophyll

degradation and pigment development, starch to sugar accumulation, cell wall degradation leading to softening, secondary metabolite accumulation and aroma production, etc. The ability to reduce ethylene biosynthesis and action could be a viable commercial method for reducing rapid softening. However, ethylene is equally important for promoting ripening processes other than softening, such as aroma and flavour volatile production, which are important attributes of fruit quality. It would be preferable to inhibit only those processes affected by ethylene that contribute to softening.

Traditionally, flowers (like fruits) are categorized as being climacteric or nonclimacteric. In climacteric or ethylene-sensitive flowers such as carnations, *Petunia*, *Gypsophila*, and orchid, senescence is accompanied by a sudden, transient increase in ethylene production and respiration, while treatment of non-senescent flowers with ethylene rapidly induces petal senescence. In nonclimacteric or ethylene-insensitive flowers such as gladiolus, tulip and iris, generally, no increases in ethylene production and respiration are apparent during flower senescence, and exogenous ethylene has little or no effect on petal senescence. In these latter species, ethylene may, however, have severe effects on other plant parts such as bulbs or corms. Knowledge about ethylene sensitivity of flower species is necessary to predict the effects of, for example, mixed storage and transport of flowers with fruit species, to predict the usefulness of anti-ethylene treatments and to direct breeding programs toward a better flower vase life.

Auxin

The characterization of a few tomato mutants unable to produce climacteric ethylene and to ripen their fruits even following treatment with exogenous ethylene, has shown that other factors also play an important role in the control of climacteric fruit ripening. In climacteric peach it has been shown that, concomitant with ethylene production, increases in the amount of auxin can also be measured (Miller *et al.,* 1987). In ripening fruits, increases in auxin content have also been reported to parallel those in climacteric ethylene production. Auxin promotes the enlargement of mesocarp disc as well as ripening processes, such as softening and anthocyanin formation in peach fruit (Ohmiya, 2000). The effect of auxin in fruit ripening is dependent on the penetration of auxin into bulk of fruit tissue. The high concentration effect of auxin is to induce early ethylene production, which in turn initiates ripening.

Auxin also plays a role of its own in fruit ripening. Auxin response factors (ARF) and Auxin/ Indole acetic acid encoding genes (Aux/IAA EG), linked to auxin signaling pathway have been reported in tomato fruits and the data suggest that auxin may be part of the mechanism that control the ripening of climacteric fruits (Jones *et al.,* 2002).

Cytokinin

Kinetin is known to prevent senescence by arresting degradation of protein and chlorophyll and also acts as a senescent retardant in fruits particularly in peel. Infiltration of kinetin into fresh banana slices enhances the ethylene production and respiration, but other ripening changes in banana fruit slices are delayed, particularly degreening of peel. Cytokinin application stimulates ethylene production in post-climacteric avocado fruit (Ferero, 2007).

Senescence is accompanied by changes in endogenous ethylene, abscisic acid (ABA), and cytokinins and these changes are believed to mediate signaling events that control the process. In ethylene-insensitive plants like daylily and gladiolus, ABA is thought to be the primary hormonal regulator of flower senescence and exogenous application of ABA accelerates visual senescence symptoms and regulates transcription of senescence-related genes. In ethylene-sensitive flowers like carnation, ABA accelerates flower senescence by increasing the endogenous production of ethylene. In contrast to the actions of ethylene and ABA, cytokinins delay senescence in vegetative and floral tissues (Van Staden *et al.*, 1988). An inverse relationship between cytokinin content and senescence occurs in some flowers. Cytokinin content in roses, carnation, and *Cosmos* is greatest in young flowers and decreases during corolla opening and development. Rose varieties with longer vase lives

have been reported to contain more cytokinins than those with shorter vase lives. Results from exogenous application of cytokinins in vase solutions have been variable. Cytokinin application delayed senescence in carnations, roses, *Gerbera* and petunia, but the response depended on the type and concentration of cytokinin and the stage of flower development.

Abscisic Acid

Abscisic acid (ABA) acts as an important regulator in natural ripening of fruits. ABA stimulates ethylene biosynthesis and shortens the time required for ripening initiation in mangoes, tomato and apple. ABA facilitates initiation and progress in the sequence of ethylene-mediated ripening events in banana. Abscisic acid is thought to have an important role in promoting climacteric ethylene production in apple and therefore may induce apple softening indirectly. ABA specific-binding proteins have been characterized in the flesh of developing apple fruit and it is hypothesized that these binding proteins may be putative ABA-receptors that mediate ABA signals during fruit development.

ABA is generally known as a strong growth inhibitor and a senescence stimulating factor, but it also controls stomata closure in certain plants. In vegetative tissues, ABA appears to be involved in the response and adaptation of plants to environmental stresses, especially in drought, salinity, and cold conditions (as may occur in storage conditions of cut flowers). It has also been proposed that under water stress, turgor pressure declines and it results in an increase in cytosolic and apoplastic ABA levels. This increase leads to (a) the closure of stomata to avoid further water stress and (b) the induction and accumulation of compatible solutes, such as proline, for water stress tolerance. Exogenous applications of ABA can serve also to increase the cold hardiness of plants. Provision of exogenous ABA in the vase solution effectively reduces vase solution usage and extends flower life. The expression of ABA genes results in the formation of, among other gene products, LEAs (late embryogenesis abundant), which are neither enzymes nor storage proteins but rather serve to protect proteins and membranes from damages during water loss in the cytoplasm due to desiccation.

Gibberellins and PGR's

Gibberellins also act as a senescence retardant. Gibberelic acid (GA) delays fruit maturation and ripening. The peach fruit pressure-infiltrated with GA had reduced ethylene emission and the respiration rate, which reflects a delay of the ripening process.

Gibberellins (GAs) are a large family of diterpenoid compounds, some of which are bioactive growth regulators, controlling such diverse processes as germination, stem elongation, and flowering. Cytokinins, and in some cases, GAs (such as GA_3, GA_4, and GA_7 in *Alstroemeria* cut flowers) delay the loss of chlorophyll, whereas ethylene and ABA enhance the rate of chlorophyll loss; the application of BA and GAs together improved the postharvest quality of cut Asiatic and Oriental lilies.

Jasmonic acid can induce senescence, while polyamines delay foliar senescence. In wax flower, the foliage of cut flowers often desiccates before flowers on the same sprig become senescent, since leaves have relatively higher turgor and lower osmotic potentials than flowers, and are less elastic. Brassinosteroids induce PCD and the formation of secondary walls. Growth retardants (e.g., uniconazole, ancymidol and paclobutrazol) reduce shoot elongation normally through inhibition of GA biosynthesis. GA effectively retains chlorophyll, while the addition of indole-3-acetic acid (IAA) in vase water has little effect on leaf yellowing, while kinetin delays it. Benzyl adenine (BA) improved the vase life of anthurium, heliconia, and ginger when applied as a spray or a dip, but inhibited that of bird-of-paradise (*Strelizia reginae*), beehive ginger (*Zinziber spectabilis*) and Uluhe fern curls (*Dicranopteris linearis*).

Membrane permeability

A consistent feature of senescence is the loss of differential permeability of cell membranes. Deterioration of cellular membranes causes increased membrane permeability, loss of ionic gradients, and decreased function of key membrane proteins (e.g., ion pumps). Changes in the properties of membranes, such as increases in microviscosity, alterations in saturation/desaturation ratios of fatty acids, and

peroxidation of lipids, are known to occur during petal senescence, with a causal link to reactive oxygen species, which are often elevated as a result of stress and have been implicated in the progression of petal senescence. Membrane deterioration is commonly associated with progressive decreases in membrane phospholipid content through phospholipase activity. Increased lipase (lipolytic acyl hydrolase) and lipoxygenase activity has been linked to the onset of membrane leakiness in carnation and rose, respectively, but a loss of membrane function in *Alstroemeria* occurs without increased activity of lipoxygenase, suggesting that loss of membrane integrity can be achieved in a number of ways. In petals, phosphatidylcholine and phosphatidylethanolamine make up 75% of the membranes' phospholipids. A senescence-induced lipase with lipolytic acyl hydrolase activity has been identified from carnation flowers. The abundance of the lipase mRNA increases just as carnation petals begin to in-roll and is enhanced by treatment with ethylene. Understanding the cause of membrane breakdown in senescing tissues also has implications for signal transduction chains, as the components of these chains are often associated with membranes.

Plant cysteine proteinases

Proteolysis in plants is a complex process involving many enzymes and multifarious proteolytic pathways in various cellular compartments, with cysteine proteinases playing an essential role. Their share in total proteolysis depends on the kind of plant and its organ. It amounts up to 30% of total proteolytic activity in mature nonsenescing organs. However, the activities of cysteine proteinases respond dramatically to different internal and external stimuli, and in some cases, they rise to 90% of the total proteolytic activity. They are involved in protein maturation, degradation, and protein rebuilt in response to different external stimuli, and they also play a housekeeping function to remove abnormal, misfolded proteins. In each case, the proteolysis by cysteine proteinases is a highly regulated process.

Role of light

Light appears to regulate pigment accumulation in tomato during fruit ripening independently of ethylene production. Ripe fruit pigments including carotenoids and flavonoids have antioxidant properties that assist in neutralizing the effects of photo-oxidation while also having nutritional significance to humans. Tomato high pigment mutations (*hp1, hp2*) result in elevated carotenoid and flavonoid accumulation with little impact on other ripening characteristics. Because mutations in the light signaling pathway positively influence pigmentation of ripe fruit, targeting the light signal pathway might be an effective means of engineering fruit nutritional quality (Adams-Phillips, 2004).

Artificial methods

Matured banana fruits are harvested from plants and stored at room temperature for natural ripening. This, however, takes a long time and fruits that are too ripened are of inferior quality. Several methods have been employed for ripening of fruits. The exposure of bananas to mast air in the presence of calcium carbide increases the rate of ripening. Carbon monoxide, coal gas and acetylene have been employed for banana ripening. Coal gas treatment is a standard Australian practice applied twice a day, depending on season and on air tightness of the room.

Physical methods

Storing tropical fruits in modified (MA) and controlled atmosphere (CA) with low oxygen levels have been shown to be effective in delaying fruit ripening. CA storage condition inhibits ethylene production and retards the rate of fruit ripening. Atmosphere reduced in O_2 and enriched in CO_2 have been used commercially in the U.S.A. Reduced O_2 and/or elevated CO_2 atmosphere reduce ethylene biosynthesis by delaying and suppressing expression of ACS at the transcriptional level and by reducing the abundance of active ACO protein in apple fruits. CA storage extends the initial slow softening phase and reduces the rate of rapid phase softening of apple fruits when applied without delay after harvest. Storing avocado fruits under CA/MA doubles the shelf life of fruits compared with storage under regular atmosphere.

The fruit ripening is significantly delayed by N_2O, as judged by both ethylene synthesis and respiration associated with changes in the colour,

acidity and softening. Combination of N_2O with low O_2 CA had a synergic effect on the ripening-delay capacity of the former. The capability of N_2O to slow down fruit ripening is thought to be due to its anti-ethylene activity. Furthermore, N_2O treatments did not cause any great change in the quality parameters assessed, except fresh weight loss which depends on the length of the pre-climacteric lag period.

Nitric oxide (NO) delays ripening and senescence in many fruits and vegetables by influencing ethylene production (Leshem *et al.,* 1998). The apple fruit softening is decreased by NO treatment indirectly by reducing ethylene production. One practical difficulty of this treatment is rapid oxidation of NO to NO_2 in the presence of oxygen.

Another method of delaying ripening is the use of sub-atmosphere pressure. MA packaging is a versatile technology which is applicable to a wide range of fruits. Addition of ethylene absorbent, potassium permanganate, to the polyethylene bags is effective in delaying ripening of pre-climacteric bananas. Banavac bags are 0.4 mm thick which lead to increase in the CO_2 to about 5% and decrease the O_2 to around 2%. A combination of 150 mmHg atmosphere pressure and one air exchange every 2 h give high quality of storage of banana fruits for 120 days. PVC packaging of ethylene-pretreated banana is effective in extending the shelf life. Storing of ethylene-pretreated banana clusters using MA and vacuum packaging is an effective treatment for delaying ripening and senescence in yellow bananas.

Heat treatment has also been employed for delayed fruit ripening. Postharvest heating is a non-contaminating physical treatment that delays the ripening process, reduces chilling injury and controls the activity of pathogens. Dipping light-red cherry tomato fruit in hot water (54°C, 5 min) and subsequently storing in polyethylene bags with various O_2 and CO_2 levels has proved effective with regard to fruit quality and delaying the maturation of tomato fruit (Akbudak *et al.,* 2007).

It was demonstrated that a judicious dose of gamma-irradiation (0.1-0.5 kGy) could enhance the shelf life of fruits by about a week to a fortnight, which could help in minimizing the spoilage during storage and transportation. Studies reveal that gamma-irradiation brings alterations/changes in metabolic pathways, which delay the production of essential precursors and energy required for ripening of fruits. Gamma irradiation in low doses satisfactorily increases the shelf life of papaya by delaying the ripening process and senescence. Irradiation of mango with 0.3 kGy shows slow ripening. Irradiation technology, however, is not widely available and is expensive.

Surface coating of fruits has been used to delay fruit ripening. The surface coating of bananas with sucrose stearate emulsion (1%) delays the ripening of the fruit by 4 days. The waxing extends the storage life of avocado. The storage life is further enhanced when waxing is carried out after 1-MCP treatment of avocado fruit. The corn-zein film delays colour change, loss of firmness and weight during storage of tomatoes with extension of the shelf life by 6 days as determined by sensory evaluation. The coating of mature green tomatoes with hydroxypropyl methylcellulose (HPMC) delays the softening of fruits during 18 days of storage at 20°C.

Role of chemicals

Chemicals have been used to control fruit ripening for a long time now. Exogenous application of ethanol vapor reversibly inhibits tomato fruit ripening *via* inhibiting ethylene biosynthesis and action. Application of acetaldehyde and ethanol have been shown to be capable of retarding senescence and inhibiting ethylene production in plants, leading to less chilling injury symptoms in various fruits. Exposure of tomato fruit to ethanol or methyl jasmonate vapour maintains firmness and less decay on storage. Treatment of banana fruits on dipping in solutions of inorganic pyrophosphate, and 2,4-D in the presence of Teepol (Primary alkyl [C6-C13] sodium sulfates) detergent shortens, while dipping in ascorbic acid, sodium metabisulfite, ferrous sulfate, IAA and GA lengthens the time for ripening initiation in the presence of detergent. A 2 min dip of fresh cut banana in a mixture of 1% (w/v) $CaCl_2$ + 1% (w/v) ascorbic acid + 0.5% (w/v) cysteine effectively prevents browning and softening of the slices. A mixture of ascorbic acid, calcium lactate and cysteine is effective in preventing

softening of fresh cut pears. Mannose also inhibits pear fruit ripening. Calcium improves the storage performance of many fruits. The mango fruit ripening is delayed on treatment with ascorbic acid, silver nitrate and sodium metabisulfite, and oxalic acid. Pre-harvest application of putrescine and storage at low temperature delays plum fruit ripening for several weeks, while putrescine treated fruit following low temperature at the ripe stage exhibits higher fruit firmness.

Various inhibitors of ethylene action have been used to delay fruit ripening. Cobalt, an ACO inhibitor, is reported to delay ripening of Saskatoon fruits. Aminoethoxyvinyl glycine (AVG) and 2,5-norbornadiene (NDB) inhibit ripening of avocado fruit discs. Ethylene actions can be blocked by some compounds, such as 2,5-norbornadiene and diazocyclopentadiene (DACP), which when linked to the ethylene binding-site, avoid its action. NDB and DACP retard the softening and ripening in apples. However, none of these products are commercially acceptable because of toxicity and manufacturing concerns. 1-MCP binds permanently to ethylene receptors and irreversibly prevents ethylene action. Generally, 1-MCP effect decreases when applied in fruits at advanced ripening stages. To achieve an increase in postharvest life, a greater exposure to 1-MCP is required for ripe tomatoes compared with green ones. The time for ripening of treated bananas varies according to the ripening stages of the bunch, with greater ripening retention in those which receive earlier application.

Growth regulator jasmonates delay ripening by interfering with ethylene biosynthesis and perception in peach fruit. Exogenously applied jasmonates leads to delay in ripening of peach fruit because of an interference with ripening- and stress\defence-related genes, as reflected in the transcriptome of treated fruit at harvest.

Molecular strategies for extending post-harvest life

With the advent of molecular biology, a number of genes have been recently identified, and a role for some enzyme families in fruit softening has been proposed, based on the correlation of mRNA accumulation and a given physiological stage or phenotype. This molecular information has been used to generate antisense or overexpressing transformants aimed to assess the physiological role of each enzyme. Several transgenic plants showing reduced expression of ripening related genes have been obtained (Stearns and Glick, 2003) using antisense RNA. The results from genetic transformation supports the role of enzymes, like PEL, one b-galactosidase isoform, protein expansins, etc., in fruit softening, as antisense fruit proved to be firmer than controls.

The use of antisense technology has shown that ethylene is the controlling factor for fruit ripening in climacteric fruits and not merely a by-product of the process. Transgenic tomato plants obtained by inhibiting ACO activity with antisense RNA had 97% reduction in ethylene production. The multigene ethylene receptor family has been shown to negatively regulate ethylene signal transduction and suppress ethylene responses. Ethylene receptor degradation controls the timing of ripening in tomato fruit. Reduction in the levels of either of two family members, *LeETR4* or *LeETR6* causes an early-ripening phenotype.

An alternative approach is the diversion of metabolic flux away from ethylene synthesis by overexpressing enzymes involved in ACC degradation. This has been achieved using constructs encoding the enzymes ACC deaminase and *S*-adenosylmethionine (SAM) hydrolase. Tomato models involved a modification of the ethylene biosynthetic pathway by the overexpression of a bacterial ACC deaminase or the expression of a bacteriophage gene encoding a SAM hydrolase which causes a reduction in ethylene production and delayed ripening.

A different approach to regulate the ethylene biosynthesis and thereby fruit ripening can be achieved by regulating the polyamine biosynthetic pathway. SAM, the substrate of ethylene biosynthetic pathway is also the precursor of polyamine biosynthesis pathway. SAM decarboxylate (SAMDC) provides aminopropyl moiety of SAM to spermidine and spermine. SAMDC gene introduction in sense orientation in tomato causes the transgenic fruits to accumulate polyamines. Some of the fruits from

transgenic line accumulate several fold higher lycopene; have longer storage life and show delay ripening and senescence than the fruit from wild type plants. The construction of transgenic plants overexpressing SAMDC with the help of sense construct can prove effective in controlling the ethylene biosynthesis and thereby fruit ripening.

In addition to transgenic regulation, virus-induced gene silencing (VIGS) also offers an attractive technology to down-regulate specific gene expression in plants. When employing this method, a recombinant viral vector carrying a partial sequence of an interested gene is used to infect the plant. As the virus spreads systemically in the plant, the endogenous gene transcripts, which are homologous to the inserted sequence in the viral vector, are degraded by post-transcriptional gene silencing. VIGS is an excellent reverse genetics tool that can be used to generate mutant phenotypes for assigning function to known and unknown genes. A significant advantage of VIGS is that it can cause a loss-of-function phenotype for a target gene within a single generation and without the need to genetically transform the plant.

To date, use of gene transfer technology to delay flower senescence has highlighted the need for tightly regulated transgene expression to avoid affecting other non target developmental processes, particularly in the modification of plant hormone levels (e.g., poor rooting and lower disease resistance in ethylene-insensitive plants). Thus, the need for tissue-specific promoters is paramount for exploiting this avenue of crop development in commercially important cultivars. Alternatively, modifying the expression of metabolic genes may produce satisfactory postharvest improvements without the need to alter hormone biosynthesis or perception, which may have pleiotrophic effects. Pollen sterility in flowers innately lowers pollen-induced senescence signals, and may make currently unsuitable flowers suitable for cut flower cropping without needing anti-ethylene treatments. The use of traditional breeding to select for genetic improvement of vase life may progress more rapidly as genetic markers for "long life" are identified and as gene transfer technologies provide a way to improve the postharvest characteristics of crops with low genetic diversity.

Future perspectives

There is little doubt that the molecular and genetic analyses of ripening/senescence made in the past one decade have raised our awareness of the complex interactions that occur to regulate ripening and senescence. Genetic technologies have enabled scientists to search for ripening/senescence-related genes in plants often described as science models (e.g., tomato, *Petunia*, *Arabidopsis*), and then translate the data into other species to determine the functional significance of the expression of specific genes in specific tissues after harvest. Interactions between ethylene, cytokinin, sugars, and various hydrolytic enzymes are now known to differentially mediate the progression of fruit ripening and flower senescence. The individual importance of each signal appears to be species specific and, in some instances, variety specific, and varies differentially between organs. The challenge for postharvest scientists is to identify a hierarchy of regulators or a specific pattern of events that progresses ripening/senescence for certain groups of fruits/flower species. Subsequent categorization of fruits and cut flowers based on their metabolism and sensitivities will enable targeted application of appropriate postharvest technologies.

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GENETIC IMPROVEMENT IN DROUGHT TOLERANCE OF CROPS: PROGRESS AND CHALLENGES

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Water is the most important agricultural input and global agricultural production mainly depends on irrigated agriculture. Irrigated agriculture produces about 40% of the global food production from 20% of the cultivated area. Globally, each year about 3.8 billion tonnes of freshwater is used by humans, of which the agriculture sector accounts for about 70 percent of the water use. To meet the food demands by 2050, about 14 percent extra water is required for agriculture (FAO Committee on Agriculture, 2007). However, the proportion of water available for irrigation is becoming increasingly scarce worldwide. In many Asian countries, per capita water availability is expected to decline by a magnitude of 15% to 54% by 2025 compared with 1990 (Guerra et al. 1998). Moreover, the Global Climate Change will further exacerbate fresh water scarcity due to the projected increase in frequencies of drought. Therefore, the agriculture must produce more from the less water. The fresh water scarcity is commonly called as drought, which is further classified in to meteorological drought (Deficit in precipitation over a long term average), hydrological drought (Reduction in water levels in rivers, lakes and groundwater), agricultural drought (soil moisture deficit), and environmental drought (a combination of all these categories of drought).

Drought Tolerance is No More a Myth

It is very common to state that drought tolerance is a "very complex trait" and it is not tractable for genetic improvement. It is often believed that "drought tolerance is a nebulous term that becomes more nebulous the more closely we look at it, much as a newspaper photograph does when viewed through a magnifying glass (Passioura 1996)". This opinion emerged rather due to a global view of yield response of plants under illdefined experimental conditions or agricultural situations. Hence the results were non-reproducible and confusion. Later, omics of plant stress response revealed massive change in epigenome, transcriptome, proteome and metabolome in response to drought that further strengthened our opinion that drought tolerance is a very complex trait (Blum 2011). In the agricultural context, drought tolerance must be defined with reference to the amount of biomass or yield produced by a genotype with the given amount of water. Yield is the culmination of complex changes in epigenome, transcriptome, proteome and metabolome that mediate various physiological, morphological and phonological changes. However, we did not leave yield improvement assuming that yield is a complex trait. Breeders have improved biomass and yield in many crops by genetic improvement. Yield improvement in green revolution is brought about by single gene mutation that resulted in gibberellin (GA) deficiency in rice (*semi-dwarf1*/*sd1*) and GA insensitivity in wheat (*Reduced height*/*Rht*). In fact, from a global view, GA regulated genes are more than 1100 in *Arabidopsis* seedlings (Cao *et al*. 2006). However, we are reaping the yield of these genes just by manipulating single genes. Therefore, all the complex traits are made up of simple component traits controlled by few major genes or limiting steps in the physiological processes. Today several genes for component traits that contribute to the yield have been identified. Significant progress has been made by plant biologist in unraveling the molecular genetic basis of drought tolerance and dissection of component traits that confer drought tolerance in plants (Chinnusamy *et al*. 2004 and 2009, Collins *et al*. 2008, Mittler and Blumwald 2010, Roy *et al*. 2011, Qin *et al*. 2011a). Since yield is amenable for genetic improvement, yield under stress can also be improved by enhancing tolerance of component traits. This review briefly summarizes the progress in understanding and genetic improvement of drought tolerance and future challenges.

Mechanisms of Drought tolerance

Drought tolerance mechanisms of plants can be broadly grouped into cellular dehydration avoidance and cellular dehydration tolerance mechanisms. Dehydration avoidance mechanisms include maximization of water uptake and minimization of water loss. Dehydration tolerance mechanisms include high water-use efficiency, phenotypic and developmental plasticity and cellular tolerance to dehydration. Xerophytes and resurrection plants are highly drought tolerant and employ cellular dehydration mechanisms for survival. Although survival under drought stress in important, we are more concerned with biomass and yield production of crops and therefore mechanisms associated with this under limited water availability.

Under drought stress, GY = WU x WUE x HI, where: WU= water transpired by the crop, WUE = water use efficiency (=biomass/unit water transpired), HI = harvest index (economic yield/total biomass) (Passioura 1977). WU depends upon the balance between the mechanisms of maximization of water uptake and minimization of water loss. WUE under drought depends upon the cellular mechanisms such as carboxylation efficiency for a given cellular CO_2 (Ci) levels, photosynthetically active leaf area (functional stay green) and cellular tolerance to low water potential. HI under drought depends upon cellular tolerance mechanisms as well as phenotypic and developmental plasticity. Here the progress in understanding and improving these traits are discussed with few examples.

Crop and the Target Environment

Agricultural drought can be defined as plant water deficit, caused by soil water availability (soil water deficit) and excess of evapotranspiration (atmospheric water deficit) that impairs the normal growth and development of plants. Therefore, agricultural drought depends upon soil moisture, atmospheric vapor pressure deficit, soil type, and the biology of plants, that is, species, genotype, and sensitivity to water deficit at various growth and development stages of the plant. Because of the varying nature of drought stress and its coincidence with a phenological phase, crop plants use diverse drought tolerance mechanisms. The rainfall pattern and water availability from other sources determine the occurrence of drought at different stages of crop growth. The intensity, duration, and occurrence of drought stress at various phenological phases differ in rainfed and limited-irrigation systems. Therefore, crop and environment specific strategies needs to be used to select component traits for improvement of drought tolerance. For example, if the water is available in deep layers of the soil, a deep root system will help, while if moisture availability is limited even in deep layers, then mechanisms that reduces water loss and cellular tolerance mechanisms are important. A genotype may be classified as drought tolerant or sensitive depending upon the stress levels. For example land races and local selections may perform when the drought stress level is very high (or rainfall is very low), while under moderate drought stress intensities, high yielding cultivars outperform the low yielding landraces.

Water Use (WU) under Drought

Total water use, one of the determinants of yield, depends upon the balance between ability of the genotype to take up moisture and mechanisms that control transpiration under moisture stress. The roots play a pivotal role in water and nutrient uptake, and act as sensors of water and nutrient status of the soil. During the interval between two rainfall event and irrigation, efficiency with which plant extracts soil moisture in different layers is critical for maintenance of water and nutrient requirements of crops. Under these conditions, the metabolism is maintained and thus the crop yields. Morphological traits such as deep root system and high root biomass at deep soil layers and physiological traits such as maintenance of hydraulic conductivity and enhancement of water uptake through aquaporins play important role in soil moisture capture from deep layers. Several QTLs for root architecture have been mapped and their effect on yield under drought stress conditions have been examined in different crops (Landi et al. 2007; Steele *et al*. 2007, for review Collins *et al*. 2008). Positional cloning of major genes these major QTLs will help in understanding the molecular basis of root growth under drought. Efforts are being made in this direction.

In addition to root morphological traits, maintenance of root hydraulic conductance is important to maintain root water uptake under stress. Symplostic water movement is regulated by aquaporins and apoplastic water movement is regulated by a change in suberization in apoplastic barriers in the exo- and endodermis. Role of aquaporins in stress response is being investigated intensely and results obtained are controversial. In some case improvement in drought tolerance was observed. Transgenic overexpression of an aquaporin gene (*RWC3* driven by the stress-inducible *SWPA2* promoter) from upland rice in lowland rice showed an increase in root osmotic hydraulic conductivity (Lp), leaf water potential, and relative cumulative transpiration at the end of 10 h of PEG treatment in the transgenic plant as compared with control plants (Lian *et al*. 2004). Aquaporins appear to be play important role in water uptake under other abiotic stresses as well (Aroca *et al*. 2011). Under intermittent rainfall conditions, seedling vigor is also an important trait to increases WU by reducing evaporation (Salekdeh *et al*. 2009).

Osmotic adjustment (OA) helps in drought avoidance as well as drought tolerance. OA is necessary for the maintenance of cell turgor, and thus maintenance of growth and metabolism, and delays leaf rolling and leaf death under drought. OA lowers root tissue water potential and thus enhances water uptake from soil. Further OA in growing root may help in root growth under drought and thus in extraction of water from deeper layers of soil. In wheat, OA trait has been successfully used to breed a drought-resistant variety Mulgara (Richards 2006). Genetic engineering approaches have demonstrated the potential use of this trait in improving multiple abiotic stress tolerance in different crops (Yang *et al*. 2010), although in most cases the osmolyte appear to protect the cells through osmoprotection rather than OA.

Water loss from the plant to the atmosphere occurs mainly through stomata. Stomatal pores allow CO_2 influx for photosynthetic carbon fixation and water loss via transpiration to the atmosphere. Thus, the rate of transpiration and photosynthesis depends upon the plant's ability to regulate its stomatal pores. The stress hormone abscisic acid (ABA), synthesized by roots under receding soil water conditions or by leaves when transpiration exceeds water uptake, acts as a signal to control stomatal responses. Transpiration depends upon leaf area (normal leaf area, drought-induced rolling and drying) and leaf reflectance characters (wax load and pubescence, leaf angle, leaf rolling). QTLs for traits that minimize water loss through plants such as controlling stomatal regulation, leaf ABA accumulation, and leaf rolling have been identified (review for Singh and Chinnusamy 2008, Salekdeh *et al*. 2009).

Water-use efficiency (WUE)

Water-use efficiency (WUE), for agronomists, WUE is the yield of harvested product per unit amount of evapotranspiration. Physiological definition or leaf level WUE is defined as the ratio of photosynthesis (A) to water loss in transpiration (E):

$$WUE = \frac{A}{E} = \frac{0.6\,(C_a - C_i)}{(W_i - W_a)}$$

Where Ca = air CO_2 concentration, Ci = leaf intracellular CO_2 concentration, Wa = air vapor density, and Wi = vapor density inside the stomatal cavity.

Among these parameters, only Ci is determined by the plant, whereas the rest of the parameters are under environmental control. Ci is determined by the balance between stomatal opening and the carboxylating capacity of the Rubisco, and photochemical reactions in the leaf. Direct measurement of WUE can be done only in a limited number of plants. The ^{13}C discrimination by Rubisco depends on Ci and thus indirectly measures WUE. High WUE has been found to be associated with low ^{13}C-isotope discrimination in rice and QTLs for ^{13}C discrimination have been mapped (Laza *et al*. 2006). The use of carbon isotope discrimination per se (rather than genetic markers linked to QTLs) in wheat grown in Australian environments, where water must be used conservatively to allow the crop to complete its life cycle, has led to the release of wheat cultivar Drystale by CSIRO, Australia (Condon *et al*. 2004). An AP2 family transcription factor from Arabidopsis AtHARDY enhanced water use efficiency in rice

transgenic plants (Karaba *et al*. 2007). An optimum combination of WUE and effective use of water (EUW) will be more effective in improving drought tolerance of crops (Blum 2009).

Cellular Tolerance

The ability of plant cells to maintain their membrane integrity and ionic-, osmotic-, and metabolic-homeostasis under drought stress determines their tolerance of cellular water deficit. Cellular water-deficit stress tolerance in plants depends on a modification of metabolism (stability of enzymes and proteins under low water potential and low water potential induced secondary stresses, change in the proportion of different metabolism), the production of organic-compatible solutes (proline, sugars, polyols, betaine, etc.), late embryogenesis abundant (LEA)-like proteins, and antioxidants. Genetic engineering efforts have sown that several transcription factors, signaling proteins, protective proteins and osmolytes can improve drought and other abiotic stress tolerance in greenhouse and field drought conditions (Yang *et al*. 2010). Plant growth and development and cellular stress tolerance is regulated by ABA. Recently, the ABA receptors, the mechanisms of action and core-components involved ABA signaling have been identified (Hubbard *et al*. 2010, Klingler *et al*. 2010, Qin *et al*. 2011a). Now the efforts are being made to engineer ABA receptor and develop cheaper chemical agonists that can be employed in improvement of crop production under drought.

Harvest Index

Maintenance of harvest index (HI) under stress is very crucial for agricultural crops. The time of flowering is very important as it determines the amount of vegetative biomass available for translocation, co-incidence of reproduce processes such as fertilization and embryo development with stress, and thus the number of effective sinks formed. Depending upon the stress intensity, plants may flower early or late. In the receding soil moisture conditions, early flowering will help the crop to complete its life cycle before severe stress comes. Spikelet fertility in rice is highly sensitive to drought and other abiotic stresses. Drought stress in rice delays flowering and low turgor under drought inhibits panicle exertion which is one of the causes for spikelet sterility. Rice genotypes that can maintain high leaf water potential under drought have shown less delay in flowering and better panicle exertion and thus high spikelet fertility. Thus, drought avoidance helps maintain better spikelet fertility. The molecular basis of spikelet fertility under low water potential needs further studies. In maize, a shorter anthesis-silking interval (ASI) is crucial for fertility and thus high sink strength. Use of QTL mapping has identified several QTLs for ASI in maize but with very less contribution to phenotypic variation (only 4% to 10% of PVE). Using both linkage–linkage disequilibrium mapping QTLs for ASI under drought, a major QTL with up to 34.7% of PVE has been mapped in maize. Further candidate genes for two significant haplotype loci were identified which included a SET domain protein (in haplotype HP71) involved in the control of flowering time and a aldo/keto reductase (in HP322) associated with detoxification pathways that contribute to cellular damage due to environmental stress (Lu *et al*. 2010). Major genes for flowering are known in rice and other crops. Expression of these genes under stress inducible promoter in transgenic plants may help early flowering. Conversely an antisense or RNAi mediated suppression of target gene can help delay flowering. Once fertilization is successful, the grain number and grain weight determine the sink strength and thus the HI. Several key genes for grain weight and grain number are known today (Miura *et al*. 2011). Regulated expression of these genes may help enhance HI under drought stress.

Grain yield

Genetic analyses revealed that grain yield under drought stress is controlled by some major genes. In rice using the Vandana/Way Rarem population, a major QTL (qtl12.1) on chromosome 12 was mapped that accounts for 51% of the genetic variance controlling grain yield under drought (Bernier *et al*. 2007). Analysis of the physiological basis of this QTL on yield improvement revealed that the Way Rarem-derived allele of qtl12.1 improve water uptake by 7% under water-limited conditions (Bernier *et al*. 2009). Another major QTL on chromosome 1 that accounted

for 32% of the variation in yield under drought stress was also mapped in rice (Kumar *et al*. 2007). Fine mapping of these QTLs to identify the underlying genes will help enhance the pace of genetic improvement of yield under drought in rice and probably in other crops.

Transgenic approaches have also been shown that overexpression of single genes with major effect can improve the yield under stress. For example, enhancement of ABA sensitivity by antisense suppression of *ENHANCED RESPONSE TO ABA1* (*ERA1*), encoding a protein farnesyl transferase â-subunit enhanced yield stability of transgenic canola under field drought stress conditions (Wang *et al*. 2005). In Arabidopsis *NF-YB2* coding for CAAT box transcription factor was found to enhance drought tolerance of Arabidopsis by maintaining high water potential and rate of photosynthesis. Overexpression of maize homolog of *NF-YB2* (*ZmNF-YB2*) in transgenic maize conferred high stability in photosynthesis and grain yield under field drought stress across growing seasons (Nelson *et al*. 2007). Several stress proteins such a LEA3 have been shown to improve abiotic stress tolerance (Yang *et al*. 2010). One of the stress proteins from *E. coli*, Cold Shock Proteins (CSPs) have been shown to protect cellular machinery under stress. Transgenic maize plant overexpressing *E. coli CspB* gene out yielded non-transgenic plants by 10% under multi-location drought field trails (Castiglioni *et al*. 2008).

The role of cytokinins in inhibition of senescence and promotion of metabolism is well known. Transgenic manipulation cytokinin levels have shown to provide very high levels of stress tolerance to tobacco (Rivero *et al. 2007*) and rice (Peleg *et al*. 2011) under pot culture conditions. Further, peanut (*Arachis hypogaea* L.) plants overexpressing an isopentenyltransferase (IPT) gene driven by promoter of senescence associated protein kinase (P_{SARK}) showed enhanced photosynthetic rate and growth under non-stress conditions. Under field stress conditions, these transgenic plants showed high stability in yield and produced higher yield than WT plants (Qin *et al*. 2011b). These results and a recent study in *Arabidopsis* suggest that maintenance of appropriate balance of plant hormones appears to be very important for stress tolerance. Phenopic and physiological analysis of CK-deficient Arabidopsis plants (oxerexpression of *CYTOKININ OXIDASE* or knockout mutants of *ISOPENTENYL-TRANSFERASE*) showed that CK-deficient plants are more stress-tolerant, and this stress tolerance was associated with enhanced cell membrane stability and ABA hypersensitivity (Nishiyama *et al*. 2011). Therefore appropriate ABA-CK balance is necessary to achieve drought tolerance in crops.

Minimum Information about a Drought Experiment (MIADE)

Most of the confusions on drought tolerance of crops and slow progress in genetic improvement can be attributed to phenotyping errors. Poor phenotyping of drought resistance traits can lead to confusing and disappointing results. Phenotyping should be preferable done under rainout shelter to avoid interruption of experiment from rainfall. The importance of a common minimal set of data for drought experiments on phenotyping of transgenics and mapping populations for proper interpretation of the results, potential comparison of the genes and QTLs, and their use in crop improvement is realized now. In analogy to the Minimum Information About a Microarray Experiment (MIAME) and Minimum Information about a Proteomics Experiment (MIAPE) established by global communities for transcriptomics and proteomics, respectively, the necessity for a "Minimum Information about a Drought Experiment (MIADE)" was suggested by Salekdeh *et al*. 2009. Here I propose the following MIADE required for phenotyping of transgenics, mapping populations and mini-core of germplasm under controlled and managed field stress environment (within rainout shelters) for proper interpretation of the results, potential comparison of the genes and QTLs, and their effective use in crop improvement:

1. Agronomic conditions of crop culture (Soil type, pH and Ec of soil, nutrient application, spacing between plants).
2. The soil moisture data (preferably soil matric potential) at different depths in the root zone for at least two time points, one each at the start and end of drought stress. Where the measurement

of soil matric potential is not possible, gravimetric or volumetric soil moisture content along with the soil type and soil moisture content at field capacity (~0.3 bars) and permanent wiling point (15 bars) needs to be given. A soil moisture release curve is more appropriate.

3. Quantity and interval of irrigation
4. The crop growth stage at which the stress was imposed
5. Duration of stress
6. Plant water status (RWC % or both leaf water potential and osmotic potential)
7. Phenology
8. Yield and yield components
9. Weather data on rainfall, temperature and VPD
10. National/regional CHECK genotype as a control

In case of pot/microplot experiments, dimensions and soil volume per pot/microplot and number of plants should be provided. In case of pot experiments, stress develops at very fast rate, and thus plants experience sudden water stress. Plants with root system as a drought tolerance mechanism may fail in pots as they quickly deplete the limited soil moisture. Consequently, plants have to survive based on their ability to minimize transpiration and cellular tolerance to low water potential. Hence, preferably large pots should be used. In field, we impose drought by withholding irrigation. In case of pots, a deficit irrigation treatment (measured percent deficit from field capacity) will be useful to evaluate the reproductive stage stress tolerance.

Summary and Challenges

- Efforts to identify stress sensors (osmosensor, ion sensor, thermosensor, etc.) and components of stress signaling pathways, and their use in improvement of abiotic stress tolerance will continue to be an active area of research.
- Drought tolerance in yield is contributed by several component traits. Molecular genetic analyses have shown that cellular stress tolerance at the vegetative stage can be easily manipulated with single genes. However, molecular basis of the component traits are poorly understood.
- The reason for this skewed knowledge in the area of abiotic stress tolerance can be attributed to the extensive use of model plants and lab/small pot phenotyping where survival is used as criteria to assess stress tolerance. Unlike in model plants, in which survival is considered as stress tolerance, in crop plants survival as well as reproduction are equally important. The reproductive phase of crop plants is often more sensitive to drought stress than the vegetative stage. Therefore more effort should be made to understand reproductive stage drought tolerance in plants
- A paradigm change in phenotyping method is necessary. Phenotyping conditions should change from less relevant lab stress methods to more relevant stress conditions that mimic field stress.
- Molecular genetic studies now should focus more on crop plants. Information generated from model plants such as Arabidopsis needs to be validated in crops. In addition, large scale forward and reverse genetic approaches must be used in combination OMICs to unravel the mechanisms of stress tolerance in crops and to identify traits and genes.
- Germplasm resources must be effectively characterized to identify sources and genes for genetic improvement programmes. For each target crop, core-germplasm must be evaluated under controlled and managed field stress to identify mini-core set of germplasm for component traits of drought tolerance. These core-set of germplasm should be effectively used in linkage disequilibrium mapping to identify haplotypes and SNPs, and finally genes for various component traits of drought tolerance.
- Contrasting germplasm for a specific component trait must be used for QTL mapping for that trait instead of using same population to map QTLs for every trait. The population size should be sufficiently large. Efforts should be made to clone

the genes that underlie the major QTLs.

- In India, only limited efforts are being made to develop functional genomics resources (T-DNA mutants/Chemical mutagenesis). These resources are important for further forward and reverse genetic approaches to identify physiological processes and genes important for drought tolerance.
- Development of a MIADE criteria and following them for drought experiments will help us in proper interpretation of the results, potential comparison of the genes and QTLs, and their effective use in crop improvement.
- Nutrient acquisition under soil moisture–deficit conditions is poorly understood. Nitrogen, phosphorous, iron and zinc uptake and use efficiency of crops under water deficit needs further stdudies.
- Combined effects of drought with other abiotic stresses such as nutrient deficiency, temperature extremes, light extremes and biotic stress needs to be studied.
- Epigenetic mechanisms of stress response and memory crops need further emphasis, specifically, it is important for countries like India, where farmers use the seeds harvested from their own fields, which are often exposed to some stresses.

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POTATO STARCH

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Potato tuber contains about 80% water and dry matter content is around 20%. A major portion of the dry matter is starch. Carbohydrates consisting of starch and sugars constitute 16% on fresh weight basis. Crude protein content is about 2.0% and the fat content is very low at 0.1%. The ash consisting of minerals constitutes 1%. In addition potato tuber contains fibre, vitamins and glycoalkaloids in small quantities. Starch content in potato ranges between 66 and 80% of the dry matter content. Therefore increase in dry matter content results in increased starch content.

Starch composition

Potato starch is present in amyloplasts and is in the form of starch grains. Potato starch is a polysaccharide consisting of two polymers, amylose and amylopectin. Normally the amylose content is around 25% (ranges between 17 and 25%) and the amylopectin content is around 75%. Both polymers consist of á-D glucose. Amylose, however, is unbranched, while amylopectin has a branched chain. Potato starch granules are generally oval in shape and the granules size shows large variation (5 to 100 µm). Native potato starch gives a B-type X-ray diffraction pattern with approximately 28% crystallinity. Potato starch granules show two distinct areas i.e. amorphous and crystalline. The crystalline areas are formed from the amylopectin molecules arranged in a cluster. Whereas the amorphous areas are largely associated with amylose molecules. When the starch molecules are heated in excess water, their crystalline structure is disrupted and water molecules become linked to the exposed hydroxyl groups of amylose and amylopectin by hydrogen bonding, which causes an increase in granule swelling and solubility. The swelling power and solubility provide evidence of magnitude of the interaction between starch chains within the amorphous and crystalline domains. The extent of this interaction is influenced by the amylose to amylopectin ratio, and by the characteristics of amylose and amylopectin in terms of molecular weight/distribution, degree and length of branching, and conformation. Potato starch has higher swelling power and solubility than other starches. These effects are due mainly to a higher content of phosphate groups on the potato starch molecule. The repulsion between phosphate groups on adjacent chains increases hydration by weakening the extent of bonding within the crystalline domains.

Starch properties

Potato starch granules are insoluble in cold water but are soluble in warm water. When starch granules are heated to a high temperature (above 60^0C) in excess water, gelatinization takes place, i.e. there is a disruption of molecular orderliness within the starch granule along with irreversible changes in properties such as granular swelling, loss of birefringence, crystallite melting, viscosity development, and solubilization. The crystalline order in starch granules is often the basic underlying factor influencing its functional properties. Collapse of crystalline order within the starch granules manifests itself as irreversible changes in properties. The gelatinization phenomenon starts at the hilum of the granule and the granule swells rapidly to the periphery. Gelatinization occurs initially in the amorphous regions as opposed to the crystalline regions of the granule, because hydrogen bonding is weakened in these areas. The order-disorder transitions that occur on heating an aqueous suspension of starch granules have been extensively investigated. Starch transition temperatures (onset, To; peak, Tp; conclusion, Tc) of gelatinization, and gelatinization enthalpy, have been related to degree of crystallinity. The onset temperature reflects the initiation of the gelatinization process, which is followed by a peak and conclusion temperature. After Tc, all amylopectin double helices have dissociated, although swollen granule structures will be retained until more extensive temperature and

shear have been applied. A high degree of crystallinity provides structural stability and makes the granule more resistant to gelatinization, ultimately resulting in higher transition temperatures; and is affected by chemical composition of starch. The gelatinization and swelling properties are controlled in part by the molecular structure of amylopectin (unit chain length, extent of branching, molecular weight, and polydispersity), starch composition (amylose to amylopectin ratio and phosphorous content), and granule architecture (crystalline to amorphous ratio). Tp gives a measure of crystallite quality (double helix length), whereas enthalpy of gelatinization, gives an overall measure of crystallinity (quality and quantity) and is an indicator of the loss of molecular order within the granule. The amount of double-helical order in native starches is strongly correlated to the amylopectin content, and granule crystallinity increases with amylopectin content.The potato amylopectin starches exhibit higher endothermic temperatures and enthalpies than the normal potato starches. The amorphous amylose in normal potato starches decreases the relative amount of crystalline material in the granule, thereby lowering the gelatinization parameters. However, the high amylose starches with longer average chain length exhibit higher transition temperatures.

Retrogradation describes physical changes following starch gelatinization. Starch retrogradation is the process that occurs when starch molecules reassociate and form an ordered structure during storage. In an initial step, two chains may associate. Ultimately, under favourable conditions, a crystalline order appears and physical phase separation occurs. Retrogradation is important in industrial uses of potato starch as it can be a desired end point in certain applications, but it also causes instability of starch pastes. During retrogradation, amylose forms double helical associations of 40-70 glucose units, whereas amylopectin recrystallizes by the association of the outermost short branches. In retrograded starch, the value of enthalpy of gelatinization provides a quantitative measure of the energy transformation that occurs during the melting of recrystallized amylopectin as well as precise measurements of the transition temperatures of the endothermic event. The endothermic peak of starches after gelatinization and storage at 4^0C appears at lower transition temperatures. Transition temperatures and retrogradation enthalpy at the end of the storage period drop considerably, compared to transition temperatures and enthalpy during gelatinization. Starch retrogradation enthalpies and transition temperatures are usually 60-80% and 10-26^0C lower, respectively, than those for gelatinization of starch granules. The crystalline forms for retrograded starch are different in nature from those present in the native starch granules and may be weaker than the latter, because recrystallization of amylopectin occurs in a less ordered manner during retrogradation than during granule formation in native raw starches.

The rheological properties of potato starch are important to both food and industrial processing. During processing, starch dispersions are subjected to combined high heating and shear rates that affect the rheological properties as well as the final characteristics of the product. Starch gelatinization, especially granular swelling, changes the rheological properties of starch. The subsequent retrogradation will further modify the rheological properties of starch. Depending on the starch concentration, the final structure of starchy products will give a thickened solution or a gelled structure. When starch is cooked, the flow behavior of the granule slurry changes markedly as the suspension becomes a dispersion of swollen granules, partially disintegrated granules, dissolved amylose, and a number of intermediate molecules. The cooked product, called a starch paste, can be described as a two-phase system composed of a dispersed phase of swollen granules and a continuous phase of leached amylose. If the amylose phase is continuous, aggregation with linear segments of amylopectin on cooling will result in the formation of a strong gel. Rheological properties of potato starch have been widely investigated. Potato starch shows the highest peak viscosity and the lowest pasting temperature with moderate final viscosity and lower setback compared with other starches.

Factors affecting properties of potato starch

Effect of cultivar

***Popular cultivars*:** Starch properties were determined in six cultivars grown at Modipuram (Uttar Pradesh),

eight cultivars grown at Patna (Bihar) and nine cultivars grown at Kufri (Himachal Pradesh). When grown at Modipuram, the dry matter and starch content were higher in Kufri Chipsona-3. The tuber dry matter content was more than 20% in Kufri Lauvkar, Kufri Lalima and Kufri Badshah. The amylose content was higher in Kufri Chipsona-3 starch while the viscosity was higher in Kufri Badshah starch. The swelling volume was higher in Kufri Lauvkar and Kufri Chipsona-3 starches. The starch clarity was higher in Kufri Chipsona-3 and lower in Kufri Sindhuri. When grown at Patna, the tuber dry matter and starch content were higher in Kufri Chipsona-2. The amylose content of starch was higher in Kufri Pushkar, while the viscosity and swelling volume were higher in Kufri Lalima starch. Least viscosity was observed in Kufri Pukhraj starch. The starch clarity was higher in Kufri Pukhraj. When grown at Kufri, maximum tuber dry matter and starch content were observed in Kufri Chandramukhi. The amylose content of starch was higher in Kufri Anand and lower in Kufri Lalima, while the starch viscosity was higher in Kufri Lalima and lower in Kufri Anand. Kufri Chandramukhi starch showed higher swelling volume, while it was lower in Kufri Anand. The starch clarity was higher in Kufri Pukhraj and lower in Kufri Chipsona-2. These results show that while significant differences were observed among cultivars, it is difficult to draw conclusions on the location effect, as comparison becomes difficult when different cultivars are grown at different locations. However, some general conclusions may be drawn. Generally, the tuber dry matter content, and amylose content and viscosity of starch were higher in tubers grown at Kufri.

***Land races of Assam and Meghalaya*:** Starch was separated from local potato cultivars grown in the North-Eastern states of Assam and Meghalaya and their properties were determined. Badami and Rongpuria are two local cultivars grown at Jorhat, and they produce small red tubers. The tuber shape is ablong in case of Badami while it is roundish in case of Rongpuria. The dry matter content of Rongpuria was higher than that of Kufri Megha, but the difference was statistically non-significant. Whereas, the dry matter content of Badami was significantly higher than that of Kufri Megha. The amylose content of starch was lower in the local cultivars but the differences were statistically non-significant. The viscosity of starch was significantly higher in Badami. While Badami starch had higher swelling volume, Kufri Megha starch had higher clarity i.e. transparency (lower optical density). Starch was separated from five local cultivars grown at Shillong and compared with two improved cultivars Kufri Megha and Kufri Giriraj. There was no significant difference in the dry matter content between Kufri Megha and Kufri Giriraj. Compared to Kufri Megha, the tuber dry matter content was significantly higher in the local cultivar Lah Sow Simit and significantly lower in Lah Sorkari. The starch content was also higher in Lah Sow Simit. The amylose content of starch was significantly lower in Lah sow Khasi and there was no significant difference among other local cultivars. The viscosity of starch was significantly higher in Lah Sow Khasi and significantly lower in the other four local cultivars, as compared to Kufri Megha. The starch viscosity was least in Lah Arpor. The swelling volume was significantly higher in Lah Sow Khasi but the starch clarity was lower. Among the five local cultivars of Meghalaya, Lah Sow Khasi had low amylose content but the starch viscosity and swelling volume were higher. Lah Sow Simit showed higher tuber dry matter content and its starch had higher amylose content and better clarity.

Effect of growing location

To study the effect of growing location on the physicochemical properties of potato starch, it is necessary to keep the cultivar common so that the observed effect can be attributed to the location. Therefore, tubers of 'Kufri Jyoti' grown at four locations were collected. The tuber dry matter and starch content were higher in 'Kufri Jyoti' tubers grown at Jorhat. This could be attributed to the high temperature which prevailed during the crop growth season at Jorhat as compared to the other three locations. The amylose content, viscosity, swelling volume and clarity were all higher in starch separated from tubers grown at Kufri and lower in starch separated from tubers grown at Modipuram.

Effect of growing season

The dry matter and starch contents were

higher in the tubers harvested from the early crop but the differences were statistically non-significant. The difference in the amylose content of starch was also statistically non-significant. Significant differences were observed for viscosity and swelling volume, with starch separated from tubers harvested from the main crop showing higher viscosity and swelling volume. However, the clarity of starch was better in the early crop. Among the cultivars, 'Kufri Chipsona-2' showed higher dry matter and starch content but the amylose content was lower. In studies dealing with different growing seasons, temperature during crop growth was found to have a strong influence on the dry matter content of the same potato variety and may vary considerably from season to season in the same locality. In the present study, during the early crop season, the temperature range was 4-20.1^{0}C min and 17-34.5^{0}C max. During the main crop season, the temperature range was 3-17^{0}C min and 17-31^{0}C max. The higher dry matter and starch content in the early crop could be due to higher temperatures which prevailed during the early crop growth period.

Effect of crop maturity

Crop maturity had a significant effect on the physicochemical properties of starch. Dry matter content increased with an increase in crop maturity. While the difference between 100 and 115 days was not significant, the dry matter content at 130 days was significantly higher than that at 100 days. Amylose content, viscosity and swelling volume showed a significant increase with crop maturity and the values were much lower in immature tubers. The differences in starch clarity were also significant with the starch from matured tubers showing lower optical density and therefore, high clarity. A significant difference in viscosity was observed only at full maturity at 130 days, with 'Kufri Jyoti' showing higher viscosity. The selling volume was significantly higher in 'Kufri Giriraj' at all three stages of maturity.

Environmental effects

From the farmer's perspective, environmental effects on crop production are measured in terms of yields. Although specific cultivars will be grown in environments to which they are broadly adapted, seasonal factors will influence the deposition (and hence yield) of starch. The farmer can do little about this. Whilst physico-chemical properties may also be affected, the primary concern in the field is to maximize yields per hectare. Hence, the priority has been to maximize output without recourse necessarily to starch quality. The synthesis of amylose and amylopectin molecules is under genetic control and is, therefore, dependent on botanical origin. However, environmental factors can influence the structure of these molecules (by regulating the activities of the key biosynthethic enzyme).Work on potato and potato microtuber starches has similarly indicated little effect of growth temperature on the branching profile of the amylopectin. The composition of different starches is susceptible to environmental variation, especially growth temperature, in common with the effects on amylose and amylopectin structure. Elevated growth temperatures decrease the amylose content. The phosphorus content of potato starch and amylopectin is not well correlated with growth temperature according to some studies although others have reported a decrease in starch phosphorus content as a function of increasing temperature. The phosphorus content of starch may also, however, depend on soil phosphorus content. Little has been reported about the influence of environment on starch protein content. Environmental effects on starch composition are mediated through appropriate control of biosynthetic enzymes. The thermoregulation of starch biosynthetic enzyme activity has also been investigated in potato microtubers, where insoluble and soluble starch synthase activity has been shown to be especially sensitive to growth temperature. The metabolic control induced by growth temperature is, however, complex. Water availability, as well as illumination also regulate starch biosynthesis. Elevation of growth temperature tends to decrease the number and/or size of starch granules.The crystalinity of starch granules is a consequence of the registration of double helices in starch granules.Constancy with respect to the polymorphic form and amount of crystallinity as a function of growth temperature, using X-ray diffraction, has been shown. In addition to the structure of amylopectin and X-ray diffraction pattern of granules remaining essentially constant as a function of growth temperature, the number of double helices remain constant. This has lead authors to conclude that the

primary effect of growth temperature of starch crystallization is mediated through improvement of the registration of double helices within starch crystallites. Elevation of growth temperature increases the gelatinization temperature and enthalpy of potato starch due primarily to the enhanced registration of amylopectin double helices and probably enhanced rigidity of amorphous regions. Planting and harvesting dates and conditions may also affect the gelatinization characteristics. Swelling factors represent the volume expansion of starch granules when heated in water. Usually the factors are determined in excess water to prevent any restriction of granule expansion due to water availability. However, as gelatinization temperatures are increased with elevated growth temperatures, the swelling factors are concurrently reduced.

Effect of storage of potatoes on properties of starch

Starches separated from eleven Indian potato cultivars stored at five temperatures (4, 8, 12, 16 and 20ºC) for 120 days were evaluated for morphological, physico-chemical, thermal, gel texture and pasting properties.

***Electron microscopy*:** Variation in granules size of starch separated from potato cultivar Kufri Chipsona-2 stored at different temperatures was observed. Starch separated from potato tubers before storage showed granule diameter between 14 to 50ìm while those separated from potato tubers stored at 4, 8, 12, 16 and 20 °C showed starch granule diameter of 9 to 36, 13 to 40, 20 to 40, 23 to 54 and 26 to 56ìm, respectively. Starch separated from tubers stored at lower temperature (4°C) showed an increase in proportion of small size granules, while those stored at higher temperature (20°C) showed a decrease in proportion of small size granules. Storage temperatures also affected the surface morphology of starch granules. The surface of the starch granules from potato tubers before storage was smooth while surface of the granules of starch from stored potatoes was rough and pitted.

***X-ray diffraction*:** The X-ray diffraction pattern of starch showed a distinctive maximum peak at around 17º, 2 è and it was not affected by the cultivar or storage temperature. The results indicated that cultivar and storage temperature had significant effects on physicochemical and pasting properties of potato starch but not on the X-ray diffraction pattern.

***Physico-chemical properties of starch*:** Among various cultivars, Kufri.Chipsona-2 starch showed the highest ash content followed by Kufri Jyoti, Kufri Sindhuri, Kufri Chipsona-1, Kufri Chandharmukhi, Kufri Bahar, Kufri Anand, Kufri Lauvkar, Kufri Lalima and Kufri Badshah, while Kufri Pukhraj showed the lowest ash content. Starches separated from potatoes stored at different temperatures showed significant difference in swelling power. Starches from potatoes stored at 16°C showed higher swelling power as compared to starches of potatoes stored at other temperatures. Swelling power of starches ranged from 16.8 to 33.0 g/g. Among cultivars, Kufri Chipsona-1 starch showed higher swelling power as compared to other cultivars. The difference in swelling power among the starches from different cultivars may be attributed to difference in phosphorous content as well as variation in the strength of associative bonding forces with granules. The higher swelling power of potato starch has been attributed to the presence of negatively charged phosphate groups, which are responsible for the swelling of starch granule. Solubility of various potato starches ranged from 6.4 to 32.7%. Among various cultivars studied, Kufri Lalima and Kufri Chandhramukhi starches showed higher solubility as compared to other cultivars. Solubility of starches reported from potatoes stored at lower temperatures was lower as compared to starches from potatoes stored at higher temperatures. Amylose content of starches ranged from 13.4 to 27.6%. Kufri Pukhraj starch showed higher amylose content as compared to other cultivars. The difference in amylose content among starches from different potato cultivars may be due to different factors such as genotype, environmental conditions and cultural practice. Starches from potatoes stored at different storage temperatures showed significant difference in amylose content, with starches from potatoes stored at 20 °C showing higher amylose content, as compared to 4°C.

***Thermal properties*:** The results of DSC analysis of starches separated from different cultivars and storage

temperatures revealed the following. The onset gelatinization temperature (T_o), peak temperature (T_p) and conclusion temperature (T_c) of starches from different storage temperatures ranged between 53.68 to 68.81 °C, 53.22 to 7.71 °C, and 55.32 to 80.03 °C, respectively. Among various cultivars studied, Kufri Sindhuri and Kufri Jyoti starches showed higher T_o, T_p and T_c as compared to other cultivars. Kufri Chipsona-2 starch showed lower transition temperatures and that may be due to the presence of lower crystallinity. Starches from potatoes stored at different temperatures showed significant difference in transition temperatures. Starches from potatoes stored at 4°C showed higher T_o, T_c and "H_{gel} as compared to starches from potatoes stored at 8, 12, 16 and 20 °C. Higher transition temperatures resulted from degree of crystallinity, which provides structural stability and makes the granules more resistant to gelatinization. The difference in "T among the starches from different potato cultivars may be due to the presence of crystalline regions of different strength in the granules.

***Transmittance (%)*:** The transmittance values during refrigerated storage of starch pastes from different potatoes stored at different temperatures were determined. The decrease in transmittance with storage duration was higher for Kufri Jyoti starch paste and lower for Kufri Lalima. This may be due to the presence of less granule remnants in starch paste that in turn depends on the morphological structure of starch granules. The low light transmittance may be due to high refraction of light by swollen granule remnants. Starches separated from potatoes stored at 4 °C showed lower transmittance values as compared to starches from potatoes stored at 8, 12, 16 and 20°C. The difference in transmittance values may be attributed to increase in proportion of small size granules at low temperature (4 °C).

***Pasting properties of starches*:** Significant differences in pasting properties of starches separated from potatoes stored at different temperatures were observed. Among various cultivars studied, Kufri Pukraj starch showed higher peak viscosity, cold paste viscosity and setback viscosity as compared to other cultivars. Peak viscosity and set back viscosity of starches separated from potatoes stored at higher temperature (20°C) showed higher values as compared to starches separated from potatoes stored at lower temperature (4°C). Peak viscosity and pasting temperatures of starches separated from potatoes stored at different temperatures ranged from 1358 to 4723 cP and 66.6 to 71.95 °C, respectively. The increase in viscosity with increase in temperature has been attributed to the removal of water from the exuded amylose by the granules on swelling of starches. Cold paste viscosity and set back viscosity of starches from potatoes stored at different temperatures varied from 163 to 2723 cP and 122 to 2006 cP, respectively. Set back viscosity was higher in starches from potatoes stored at 20°C.

***Textural properties of starch gels*:** The textural properties of gels from starches separated from potato cultivars stored at different temperatures were determined. The starch gels from potatoes showed hardness, springiness and gumminess in the range of 10.75 to 55.62 g, 0.32 to3.85 mm, and 0.26 to 14.29g, respectively. Among cultivars studied, Kufri Badshah and Kufri Lauvkar starch showed higher hardness and low springiness, as compared to other cultivars. Kufri Pukhraj starch showed higher cohesiveness, chewiness and gumminess as compared to starches separated from potatoes of other cultivars. The starch gels prepared from potatoes stored at 20°C temperature showed higher hardness, chewiness and gumminess as compared to starch gels prepared from potatoes stored at 4, 8, 12 and 16°C temperatures. The gel firmness is mainly caused by retrogradation of starch gels, which is associated with syneresis of water and crystallization of amylopectin, leading to harder gels. Starches that exhibit harder gels tends to have higher amylose content and longer amylopectin chains.

Properties of starch separated from irradiated and stored potatoes

The morphological, thermal and pasting properties of starch separated from potatoes of three cultivars (Kufri Chandramukhi, Kufri Jyoti and Kufri Chipsona-2) treated either with CIPC (Isopropyl N-(3 chlorophenyl) carbamate) or γ-irradiation (Co^{60}, 0.1 and 0.5 kGy) after storage for 90 days at 8, 12 and 16°C were studied. Mean granule size of starch separated from potatoes stored at 12°C ranged between 18-25 μm and irradiation treatment resulted in an

increase in the proportion of small size granule. The irradiation of potatoes with 0.5kGy resulted into starch with significantly lower peak-, trough- and breakdown-viscosity as compared to starch from potatoes treated with either CIPC or 0.1kGy irradiation. The irradiation of potatoes with 0.5kGy caused a significant increase in setback and pasting temperature. Pasting temperature of starch was observed to vary with the storage temperature. Starch separated from potatoes stored at higher temperature showed lower pasting temperature and vice versa. The starch from potatoes stored at 8C showed higher peak-, trough- and breakdown-viscosity and lower setback. Peak viscosity increased and swelling volume decreased with increase in storage temperature. FTIR spectra showed that the irradiation caused a significant decrease in the intensity of C-H stretch region between 2800 and 3000 cm^{-1}, which was observed to be irradiation dose dependent, higher with 0.5 than 0.1 kGy. However, a slight broadening was observed in O-H stretch (3000-3600 cm^{-1}) upon irradiation. The spectral changes caused by γ-irradiation were apparent in the O-H stretch (3000-3600 cm^{-1}), C-H stretch (2800-3000 cm^{-1}) and bending mode of water (1600-1800 cm^{-1}). Irradiation treatment, which is usually given to check sprout growth in potato tubers during storage at higher temperatures, brought about significant changes in the properties of their starches.

Changes in properties of irradiated potato starch

Changes in granule morphology, thermal, pasting, gel textural and rheological properties of starch separated from two potato cultivars (Kufri Jyoti and Kufri Chipsona-2) and exposed to gamma-irradiation (Co^{60}, 0.01, 0.05, 0.1 and 0.5 kGy) were studied. A complete disorganization of the crystalline structure and carboxyl content of 0.09-0.11% was observed in starches irradiated at 0.5 kGy. Irradiation of starch increased onset gelatinization temperature (onset-, peak- and conclusion-temperature). Peak viscosity, trough viscosity, breakdown viscosity, final viscosity and gel hardness decreased while gel cohesiveness increased with the irradiation. Irradiation effect on gumminess, chewiness, adhesiveness and retrogradation of gels varied with the cultivar. Kufri Jyoti native and irradiated starch showed greater retrogradation as compared to Kufri Chipsona native and irradiated starches. Difference in recrystallization of molecules as revealed from percent retrogradation and enthalpy of retrogradation among starch from two cultivars was also observed. Irradiation effect on the crystallinity, textural and rheological properties of starch varied with the cultivar. Kufri Jyoti starch with greater crystallinity showed greater introduction of carboxyl group and reduction in pH upon irradiation as compared to Kufri Chipsona-2 starch with lower crystallinity, particularly, at higher level of irradiation (0.5 kGy). The changes caused by irradiation in properties of potato starch were more prominent, when separated starch was exposed to irradiation as compared to exposing tubers to irradiation. Unlike chemical treatments, which are time consuming, irradiation can be a quick and efficient method for modifying the properties of starch.

Contribution of conventional breeding in modifying starch properties

The potential of conventional breeding concerning phosphorus content in potato starch was demonstrated recently. The genetically fixed contribution is important and highly significant but is additionally varied by location and year of cultivation. Phosphorus fertilization did not contribute considerably. Although amylose concentration of potato starch was said to be relatively constant at levels around 21%, much higher range of 24 to 31% with significant effects of genotype and growing conditions has been reported. In a screening programme, a similar range (25 to 32%) that depended with high significance on variety (genotype) has been observed. It may be possible, therefore, to affect the amylose/amylopectin ratio within a limited range. Particle size, as another decisive characteristic contributing to viscosity, is affected by variety with high probability and additionally by location and year of cultivation. This was the reason for researchers to initiate evaluations of potential routes in conventional breeding to increase amylose, phosphorus content as well as the fraction of large granules and thus influencing finally paste viscosity.

Contribution of genetic engineering in modifying starch properties

Complex criteria like phosphorus content, granule size distribution and viscosity behaviour are

rarely included in molecular genetic studies. Because of the different property profiles of amylose and amylopectin, a pronounced change of the well known and, within narrow limits, varying ratio became interesting within early investigations of expressing amylose-free genes in potato mutants, which led finally to potato amylopectin starches and detailed studies about their property profile deviating from the wild-types. Driving forces were outlets in commercially promising industrial applications. However, generally rising refusal to accept genetically produced plants and their products in society, and politics, impede a serious prediction of future developments.

Manipulation of starch structure to diversify its uses

The major targets for the manipulation of starch structure in plants are to modify the relative proportions of amylopectin and amylose, to change the chain length distribution in the amylopectin, or to increase the phosphate content of the starch. Due to the contrasting physico-chemical properties of amylopectin and amylose, it is generally advantageous if starches for industrial applications are composed mainly of one or the other. Many mutants have been described in various cereal and legume species that contain starches composed of only amylopectin or that have a very high proportion of amylose. Efforts have been made to introduce these traits into potato using transgenic approaches. The first transgenic potato plants with modified starch were those in which amylose was eliminated via the down regulation of GBSS. No penalties on the starch content were observed and plants seemingly suited for commercialization were produced almost 20 years ago. A mutant GBSS from a non transgenic amylose-free mutant of potato, originally identified in a diploid variety, has been bred into a commercial cultivar and commercially produced amylose-free potato starch is derived from this. It has proved more difficult to produce high-amylose potato starch. The main approach has been through downregulation of isoforms of branching enzyme. When the major isoform (BEI) was down regulated, only minor changes in starch structure and no increases in the amylose content were observed, despite the fact that more than 95% of the measurable activity was lost. Subsequently, the minor tuber isoform (BEII) was downregulated, giving an apparent amylose content of the starch of 38% compared with 30% in the starch from control plants. Only when both isoforms were simultaneously downregulated were substantial increases in the amylose content achieved (reaching 75% of the granule). Interestingly, there seems to be an interaction between the branching of starch and its phosphorylation during starch biosynthesis in potato tubers. Simultaneous downregulation of GWD and the Bes resulted in higher amylose contents (up to 90%) than downregulation of Bes alone. Downregulation of Bes led to marked increases of the phosphate content of the starch. There are numerous reports in the literature on diverse transgenic manipulations that affect amylopectin structure. Those that were done in an amylose-free background are arguably most suitable for further exploitation. Based on the observation that the simultaneous reduction of SSII and SSIII in potato led to the synthesis of an amylopectin with an elevated ratio of short to long chains, downregulation of SSII and SSIII in combination with GBSS, resulted in the production of short-chain amylopectin almost free of amylose. When heated in water this starch produces a gel that is stable through repeated cycles of freezing and thawing-a highly desirable trait. A similar approach was used to produce high-phosphate amylopectin. Based on the observation that downregulation of BEI or SSIII leads to an increased phosphate content, each has been simultaneously downregulated with GBSS.

Uses of potato starch

Food

Through effective chemical and physical modifications, potato starch has been applied in a wide variety of industrial products, including food ingredients, sizing agents for paper and textiles, and starch-based plastics. The major consumers of potato starch are the food industries, where the texture, viscosity, and colour that starch granules contribute to products are of primary interest. Much of the potato starch utilized in the food industry is used in baker's specialty items (e.g., Swedish and German style breads), in crackers, and in matzoth. It is also used as a thickener in soups and gravies. Potato starch has been pelleted

successfully to make puddings similar to those ordinarily made from tapioca starch. Pregelatinized potato starch is used in considerable quantity in instant puddings, in which its properties are preferable to those of cereal starches. The dry formulation of instant puddings is principally soluble starch, sugar, and flavouring. Upon addition of cold milk, the starch quickly dissolves and then sets to a gelled pudding. Starch is used in the confectionery industry for the following purposes: (a) as a medium of molding cast candies such as jelly beans, "orange slices", and gum drops; (b) as a bodying agent and to impart smoothness and stability to caramels and marshmallow; (c) as a thickening agent in synthetic jellies; and (d) as a dusting agent, perhaps mixed with powdered sugar, for candy gums, chewing gum, etc. Thin-boiling starch rather than thick-boiling starch (unmodified) is ordinarily used as an ingredient in candy manufacture. Starch constitutes 10-12% of the total weight of dry ingredients in candy gums. Glucose syrup, produced by the hydrolysis of starch, is widely used in candies, beverages, chewing gum, ice cream, and confections in general. Modified potato starch is used to replace the fat in flour tortillas while maintaining a soft texture and foldability. It is also used in the fat-free processed meat industry. Its clean flavor and high water binding capabilities make potato starch ideal for baking low-fat and nonfat hotdogs. Extruded potato starch can also be used to make food products for children, nonallergenic formulas, and various functional foods. Acid-modified potato starch is used in confectionery products such as starch jelly candies. Thus, modified potato starch can be labeled as "modified food starch" and provides an economical alternative to maize and wheat starches.

Paper

Modified potato starch, as a raw material, is the second largest component in papermaking by volume. Starch is used for four purposes in paper manufacture; (a) beater sizing, in which the cellulosic fibers are cemented together preparatory to sheet formation; (b) tub sizing, in which the preformed sheet is passed through a dilute size solution; (c) calender sizing, in which a smooth finish is imparted; and (d) surface coating, which is an optional step in finishing high-grade papers. Starches and dextrins are also used in combining and sealing paperboard in the fabrication of folding, corrugated, and laminated solid-fibre boxes. Cold-water-soluble potato starch is outstanding in the performance in beater sizing. This modified starch is produced by cooking a suspension of starch, drying the paste on drum driers, and grinding the flakes to a powder. This type of soluble potato starch was first manufactured in The Netherlands and has been produced for years in this country to supply a steady market. Soluble potato starch, or gum, is preferred to the corresponding products from other starches in beater sizing because its paste possesses great stringiness and cohesive strength. Futhermore, these properties are said to be affected relatively little on addition to alum. Alum is regularly used in paper manufacture, and its acidic character is detrimental to the properties of most starch pastes. Potato starch is well liked relative to cereal starches for coating smooth, white paper such as that used in magazines. The unusually strong binding power of potato starch for the white pigments and clay is advantageous here. Potato starch is said to have replaced much casein formerly used in paper coating. The process of starch modification by enzyme conversion is well established in the paper industry. Enzyme-converted starch pastes can be used in most paper surface sizing and coating applications. Oxidized starch has also been widely used for paper sizing and coating because of its excellent film-forming and binding properties. Cationic starch is an important industrial starch with superior adhesive properties that has been widely used as a wet end additive in the manufacture of paper to improve sheet strength and enhance the retention of pigments, dye, and filler. For uses in the pulp and paper industry modified starches with low degrees of substitution are generally preferred. However, for uses such as hydrophobic coatings and adhesives and as blend compatibilizers, highly substituted hydrophobic products may be of value.

Textiles

Most of the potato starch used in the textile industry is employed in the sizing of cotton, worsted, and spun rayon warps. In warp sizing, parallel threads that run lengthwise in the loom dip into a bath of hot starch paste formulation; the sized thread passes over heated drums to effect drying after leaving the bath. The function of warp sizing is to bind tightly the loose

fibers to the surface of the thread and thereby strengthen and protect the warp from abrasion during weaving. High-count warps, containing many individual fibers spun together, are difficult to size because of small interstitial space between the fibres. Potato starch is preferred to cereal starches in warp sizing because its paste penetrates farther before gelling. Deeper penetration of the starch results in formation of a film that adheres well to the warp and consequently gives it more strength and resistance to abrasion. It is well known that potato starch films have a high degree of toughness and flexibility relative to other starches. This permits potato starch-sized warps to be woven at lower humidity than those sized with cornstarch. The smooth clear pastes obtained with potato starch also have other advantages in warp sizing. Cereal starch pastes frequently contain large aggregates of gelled material, which stick to the warp and subsequently get caught in the loom to cause thread breakage. Warps sized with potato starch not only have a smoother finish but also are easier to desize after the size has served its purpose. The lesser tendency of potato starch pastes, in comparison to cereal starch pastes, to set back, or retrograde, to a gel is of advantage following shutdowns. It is also claimed that less tallow is required in potato starch sizes to minimize sticking of warp to drying drums than with other common starches. Potato starch is said to be superior for sizing warps that have been previously dyed in that it gives a brighter colour. The finishing of cotton sewing thread is similar to warp sizing. The thread is immersed in a finishing bath and then passed over brushes to provide a smooth finish. Many manufacturers of cotton thread use potato starch exclusively. Potato starch is not outstanding in its ability to bring out colour intensity of vat dyes when used as a thickener for textile printing pastes, but it possesses superior properties as a finishing agent. Cloth finished with potato starch has a better feel and smoother surface than is obtained with cereal starches.

Adhesives

In producing adhesives, it is generally advantageous to use starch that has been subjected to chemical or physical treatment to reduce its paste viscosity, thereby permitting use of higher solids concentration, and to develop so-called tackiness. Although some thin-boiling and oxidized starches are used in adhesives, generally the dextrinized form is used for this purpose. Dextrins are produced by roasting starch in the presence of an acid catalyst. Films of dextrin made from root and tuber starches, such as tapioca, sweet potato, and potato, have greater flexibility and resistance to checking than dextrins of cereal starches. Potato dextrins are used in many applications in which their specific properties make them desirable, for example, as a binder in sand paper, abrasive cloth, bookbinding, and rug sizing, each of which requires a dextrin of high paste tackiness and flexible residual film. Potato dextrin films are also outstanding for their ease in remoistening; this property is desired in mucilages used for gumming stamps, labels, envelopes, paper tape, etc.

Miscellaneous uses

Miscellaneous uses of starch include the following: (a) hygroscopic additive in baking powder; (b) fermentation raw material; (c) binder for tablets; (d) binder and extender for sausages; (e) builder for soap; (f) separator in dry cell batteries; (g) raw material for nitro-starch manufacture; (h) consistency stabilizer for oil well drilling "muds"; (i) attractant in insecticidal mixtures; (j) boiler feed water treating agent; and (k) clarifying agent for waters used in mining operations. It can also be used in the purification process for drinking water as a flocculation agent.

In addition to these applications, potato starch has been applied in the development of biodegradable plastic. Biodegradable plastic is a new generation of material that has significantly reduced environmental impact. To obtain starch-based plastic materials, granular potato starches are processed with the aid of additives and plasticizers using conventional techniques. Glycerol and water are good plasticizers for starch. Biodegradable packaging has the potential to replace synthetic, nonbiodegradable packaging for use by the food industry because of its oxygen permeability. However, the effect of moisture on the stability of such packaging limits its usefulness in other industries. To overcome this problem, starch and polyethylene, polystyrene or polyvinyl alcohol (PVOH), and polyacetic acid were blended to yield thermoplastic

materials with properties superior to those of starch alone. Some starch-PVOH blends appear to have potential to replace LDPE (low-density polyethylene) films in applications where mechanical properties are critical for the intended use and good moisture barrier properties are not necessary. Other starch-PVOH blends are being explored as replacements for polyethylene in disposable food-service items. This approach avoids the safety concerns related to longer-term food packaging application.

A comparison between potato and cereal starches

Starch granule properties

Potato starch has relatively large oval granules in contrast to the smaller round or polygonal granules of maize starch (Table 1).

Composition of starch granules

The common cereal starches (maize, wheat) contain a high percentage of fatty substances (0.7-0.8%) compared with potato (0.05%) (Table-2).The high amount of lipids in the common cereal starches reduce the water-binding capacity, the swelling and the solubilization of starches. Further, the oxidation of lipids results in the formation of undesirable flavours, and the presence of amylose-lipid inclusion compounds makes starch pastes and starch films opaque or cloudy. The cereal starch (maize, wheat, waxy maize) contain a considerable amount of proteins (0.25-0.5%) compared with potato (0.06%).The high content of protein in the cereal starches may result in formation of mealy flavours, foam building and colour formation in hydrolysates. Potato starch has a relatively high ash-content because of the presence of phosphate groups. As metals, the ash of native starches contains mainly calcium, potassium, magnesium and sodium. Potato starch is the only commercial starch which contains an appreciable amount of covalently bonded phosphate monoester groups (0.06-0.10% calculated as P). The negatively charged phosphate groups are linked exclusively to the potato amylopectin molecules (about 1 phosphate monoester group per 300 glucose units). Although the ionic charge is not high, in aqueous solutions the repulsion of like charges very likely helps to untangle the individual polymer molecules and extends their sphere of influence. This extension or uncoiling of the branches of the anionic potato starch amylopectin increases the viscosity and the thickening power of potato starch pastes. The phosphorus in the cereal starches is mainly present in lysophospholipids. Potato starch generally exhibits much less of the undesirable starchy flavours (low content of lipids and proteins). Waxy maize starch has less off-flavours than maize starch but somewhat more than potato starch. Potato starch (21%) has a much lower amylose content as compared to 28% for maize and wheat starch. The linear starch chains (amylose) have an increased tendency to line up into bundles or micelles (retrogradation). Potato amylose has a degree of polymerization (DP)-range of about 1000 to 6000 glucose-units (average about 3000). Corn and wheat amylose have a degree of polymerization ranging from 200 to 1200 glucose-units (average about 800). The long amylose molecules do not readily move into tight association with other chains (low rate of retrogradation). The small amylose molecules are more

Table 1. Starch Granule Properties (Average Values)

Starch granule properties	Potato starch	Wheat starch	Maize starch	Waxy maize starch
Type of starch shape	tuber oval, spherical	cereal round, lenticular	cereal round, polygonal	cereal round, polygonal
Diameter, range(μm)	5-100	1-45	2-30	2-30
No. of granulesper g starch x 10^6	60	2600	1300	1300
Average No. of starch molecules inone granule x 10^{12}	50	5	10	0.01

prone to quick association (high rate of retrogradation). Amylose with a high DP has a higher binding force compared to amylose with a low DP. The average DP of amylopectin is about 2 million (molecular weight about 400 million). The molecular weight of amylopectin is about 1000 times as high as the molecular retrogradation owing to its highly branched structure.It appears that the number of amylose molecules (per g of starch) in maize and wheat starch is about 5 times as high as the number of amylose molecules in potato starch. All these starches contain 100-1000 times as much amylose molecules as amylopectin molecules.Potato starch has a substantial higher average degree of polymerization as compared with maize and wheat starch. Waxy maize starch has the very high DP of amylopectin.

Gelatinization characteristics

The pasting temperature is the temperature at which the viscosity of the stirred suspension begins to rise. Maize and wheat starch have a substantial higher pasting temperature than potato and waxy maize starch (Table-3). As the temperature is increased, the starch granules swell and increase the viscosity of the starch paste until the peak viscosity is reached. Potato starch shows the highest peak viscosity, because the granules are only moderately swollen. A higher peak viscosity corresponds with a higher thickening power of a starch. Potato starch has an exceptionally high swelling power. This may be due to the presence of negatively charged phosphate groups which assist in swelling of the potato

Table 2. Composition of starch granules (Average values)

Starch components	Potato starch	Wheat starch	Maize starch	Waxy maizestarch
Moisture at 65% RH and 20^{0}C	19	13	13	13
Lipids (% on dry substance)	0.05	0.8	0.7	0.15
Proteins(% on d.s.)	0.06	0.4	0.35	0.25
Ash (% on d.s.)	0.4	0.2	0.1	0.1
Phosphorus (% on d.s.)	0.08	0.06	0.02	0.01
Amylose content (% on d.s.)	21	28	28	0
Amylopectin content	79	72	72	100
Number of amylosemolecules per g starch x10^{20}	30	130	130	0
Number of amylopectin, molecules per g starch x 10^{1-}	150	130	130	190
Ratio number of molecules amylose: amylopectin	200	1000	1000	0
Average degree of polymerization of starchmolecules	14000	3000	3000	2000000

Table 3. Gelatinization characteristics of starches

Characteristics	Potato starch	Wheat starch	Maize starch	Waxy maize starch
Pasting temperature(^{0}C)	60-65	80-85	75-80	65-70
Peak viscosity, range(Brabender units; 5% Starch concentration)	1000-5000	200-500	300-1000	600-1000
Peak viscosity, average(Brabender units; 5% Starch concentration)	3000	300	600	800
Swelling power at 95^{0}C	1153	21	24	64
Solubility (%) at 95^{0}C	82	41	25	23

starch granules. The relative low swelling power of maize and wheat starch is partly due to the presence of amylose-lipid complexes. Potato starch shows the higher solubilization. The lipids in the cereal starches reduce the solubilization. On heating in water, the granules of potato and waxy maize starch disintegrate more rapidly than the granules of maize and wheat starch and consequently they more quickly reach the homogeneous condition necessary for many uses.

Conclusions

Starch forms 66-80% of the dry matter content (20%) of a potato tuber. Potato starch is a polysaccharide consisting of two polymers, amylose (21%) and amylopectin (79%). Potato starch granules are generally oval in shape and the granules size shows large variation (5 to 100 µm). Native potato starch gives a B-type X-ray diffraction pattern with approximately 28% crystallinity. Potato starch has higher swelling power and solubility than other starches, mainly to a higher content of phosphate groups on the potato starch molecule. When starch granules are heated to a high temperature (above 60^0C) in excess water, gelatinization takes place, i.e. there is a disruption of molecular orderliness within the starch granule along with irreversible changes in properties such as granular swelling, loss of birefringence, crystallite melting, viscosity development, and solubilization. Collapse of crystalline order within the starch granules manifests itself as irreversible changes in properties. Factors affecting properties of potato starch include cultivar, growing location, season,crop maturity, environment and storage. It has been possible to change some of the properties of potato starch through conventional breeding as well as genetic engineering. Development of transgenic potatoes with amylose free starch was a significant achievement. Potato starch has several uses. Through effective chemical and physical modifications, potato starch has been applied in a wide variety of industrial products, including food ingredients, sizing agents for paper and textiles, and starch-based plastics. Potato starch is superior to cereal starches because of its unique properties therefore, there is demand for potato starch for specialized applications.

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PHYSIOLOGY OF FLOWERING OF HORTICULTURAL CROPS WITH SPECIAL REFERENCE TO MANGO (*MANGIFERA INDICA* L.)

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Flowering is the first of several events that set the stage for fruit production. Floral initiation is not only controlled by the developing meristem, but may also involve signals from other areas of the plant. The interactions between environmental stimuli and endogenous developmental signal exert some control over floral initiation. Floral initiation includes all of the developments necessary for the irreversible commitment by the meristem to produce an inflorescence (Kinet, 1993). Floral stimulus, most commonly photoperiod or temperature, leads to floral initiation. The juvenile phase, is the first which lasts for several years during which time no flowering or fruiting occurs. Thereafter, the interactions between vegetative growth, flowers, and fruit of the previous year on floral initiation in the current year, affect production through phenomena such as biennial bearing.

Two major differences exist between tropical and temperate deciduous horticultural trees with respect to floral initiation. The tropical species such as mango initiate flowers in response to an environmental stimulus, while temperate deciduous species, such as apple, initiate flowers autonomously. On the other hand temperate deciduous horticultural trees undergo a period of dormancy between floral initiation and anthesis. While in tropical species, including mango, floral development is continuous from floral induction to anthesis.

Induction of flowering in the subtropics is primarily governed by chilling temperatures from passing cold fronts during winter-spring months. The age of the previous flush modifies the cool-temperature-induced floral response, with older stems exhibiting a higher probability of a floral response and younger stems displaying a higher probability of a vegetative response. In the tropics, however, the age of the last flush is the dominant factor regulating flowering. Stems must be in rest for sufficient time, generally about four to five months to be induced to flower in the absence of chilling temperatures. This extended rest period occurs naturally as trees increase in stature, but it can also be achieved by mild plant water stress or low nitrogen fertility. Moderately cool temperatures that often reach deep into tropical dry and high elevation locations provide additional stimulus to flower in stems of a given age. Armed with the basic information provided here, growers can manage flowering to occur at any desired week of the year. Local environmental conditions may alter the expected responses, but scrutiny of all of the factors should bring consistent success.

Photoperiodic / thermal induction

Photoperiod is sensed in the leaves. The long-day (LD) and short-day (SD) plants flower in response to the change in the dark period, requiring short and long dark periods, respectively. However, in mango it was observed that the flushes occurring during low temperatures of 5-15 °C usually produce flowering and the frequency of flushes that occur depend upon cultivar and growing conditions. Photoperiods does not have any impact on flowering but the cool inductive temperatures 18 ºC / 10 °C night was found favourable for floral initiation. The role of temperature regulated florigenic promoter (FP) and an age dependent vegetative promoter (VP) for the regulation of flowering has been reported (Davenport and Nunez-Elised, 1997; Davenport, 2007). Researches have shown that mango flowering is regulated by the interaction of temperature and leaf age but not by day-length. This suggests that a florigenic signal is up-regulated under low temperature conditions and transported to buds for induction of flowering shoots. Photoperiodic induction is a common mechanism in herbaceous species but has rarely been demonstrated in trees. In avocado, both the time to flower and floral initiation were decreased by short days of 9 h compared with 15 h (Buttrose and Alexander, 1978). These

effects may be related to differences in photosynthetic period and daily carbon assimilation but not to photoperiod. The variations in light intensity can affect the amount of floral initiation, it is most likely that a secondary factor related to assimilate production and its effects on growth is responsible.

GA floral induction

The GA pathway also actively promotes flowering. Under SD conditions, GA_4, which is most likely produced in the leaves and transported to the meristem, up-regulates one or both of the genes *LEAFY* (*LFY*), a floral meristem identity gene (Blazquez *et al.*, 1998; Eriksson *et al.*, 2006), and *SUPPRESSOR OF OVEREXPRESSION OF CONSTANS 1* (*SOC1*), a 'floral integrator' (Bernier and Perilleux, 2005), leading to flowering. GA-deficient mutants have delayed flowering under short days but flower on time under long days (Wilson *et al.*, 1992). However, double mutants of the photoperiodic and GA pathways flowered later under long days than double mutants of the photoperiodic and autonomous pathways (Reeves and Coupland, 2001), suggesting some interaction between the photoperiodic and GA pathways.

Autonomous flowering

The autonomous pathway also acts upon the expression of *FLC* although independently of vernalization. In this case, several genes act additively to suppress the expression of *FLC* (Michaels and Amasino, 1999). It is unclear why they act at particular stages of plant development (Boss *et al.*, 2004). The autonomous pathway may also assist the photoperiodic and GA pathways through its action upon the floral repressors (Reeves and Coupland, 2001).

Floral induction in mango

Mango (*Mangifera indica* L.) is an evergreen tropical tree cultivated in the tropics and subtropics. With respect to floral initiation, mango has been more extensively researched than any other tropical tree species. The juvenile period for mango varies with cultivar but can be approximately three years for particular cultivars (Salomon and Reuveni, 1994). Floral initiation occurs during late autumn and early winter; flower panicles emerge from the terminal and sub-terminal buds and grow continuously until anthesis occurs in the spring.

Floral induction in mango occurs in response to cool temperatures perceived by mature leaves (L), which are necessary for floral initiation. Flower panicles (FP) originate from terminal or subterminal buds of the most recent vegetative flush. Many mango cultivars bear irregularly. This has been largely attributed to variation in flowering and fruit retention in subtropical areas (Whiley, 1993). Variations in the amount of flowering may be within trees from year to year, between trees in the same year, and between branches on the same tree besides flowering behaviour by differs markedly within the varieties(Ravishanker,1979) and even within the bearing and different units of the same tree. Thus, even in bearing trees, there are non-bearing units which can be attributed to the differential potentiality to the shoots to ferm flower buds(singh et.al.2011). Some Indian cultivars are reputedly biennially bearing, having distinct 'on' and 'off' years (Pandey, 1989). Many of the cultivars grown in Australia, including 'Kensington Pride', flower irregularly, but without predictable 'on' and 'off' years (Blaikie and Kulkarni, 2002).

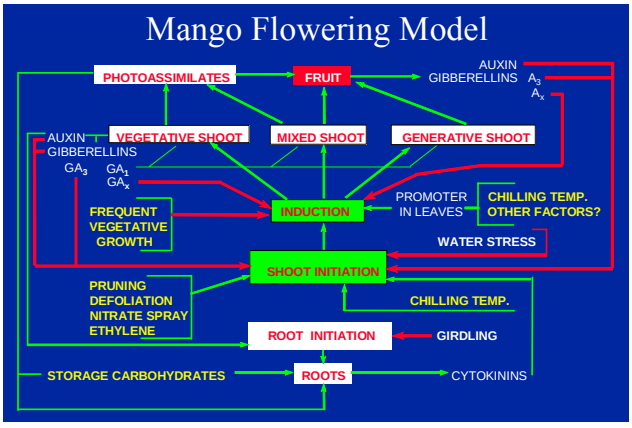
Under subtropical conditions mango flowers in response to cool temperatures (Whiley *et al.*, 1989; Batten and McConchie, 1995; Shu and Sheen, 1987; Chaikiattiyos *et al.*, 1994; Nunez-Elisea and Davenport, 1994). Whiley *et al.* (1989) reported that eight out of ten mango cultivars flowered at a day/night temperature regime of 15/10 °C, and only one of the cultivars flowered at 20/15 °C, while the other nine cultivars grew vegetatively. Shu and Sheen (1987) found that 100% of 'Haden' mangoes flowered at 19/13 °C, 60% at 25/19 °C and 0% at 31/25 °C. Interestingly, four cultivars that flowered at 30/20 °C in the work of Sukhvibal *et al.* (2000) failed to flower at 20/15 °C in the work of Whiley *et al.* (1989). It is possible that the larger diurnal temperature difference was a significant factor.

There is evidence for a phloem mobile floral stimulus (florigen) in mango. Kulkarni (1991) examined mango flowering by cross-grafting cultivars with different inductive requirements. It is likely that if the

rootstock was under inductive conditions it could promote flowering in the defoliated scion under conditions non-inductive for the scion cultivar only if the rootstock had leaves. However, when leaves remained on the scion the flowering was inhibited and subsequent growth was vegetative. The juvenile mango plants have the ability to flower after grafting to a mature plant as long as the juvenile plant is defoliated and the adult plant has leaves (Singh, 1959). This indicates that signals from the leaves of the adult plant promote flowering and can overcome juvenility, while leaves from juvenile plants inhibit flowering. When branches were girdled and decapitated, the growth from axillary buds was floral if leaves were allowed to remain on the plant for more than 4 d under inductive conditions (Reece *et al.*, 1949). Floral initiation will occur in the presence of even a fraction of a mature leaf, but the proportion of stems initiating reproductive, as opposed to vegetative, growth decreases with increasing distance from the leaves, and as the number of leaves decreases (Davenport *et al.*, 2006). There seems to be a floral stimulus in mango that is transient, graft transmissible, and generated by the leaves.

Vegetative growth in mango is through cyclic flushing. The flushing is more frequent as temperature increases (Whiley *et al.*, 1989). Episodic or recurrent flushing, common in subtropical and tropical trees, is where apical or axillary buds are released and the new shoots expand continuously through several nodes and then mature. After a period of dormancy, the cycle begins again with further bud release. The timing of flush development is important for successful flowering because bud release, for vegetative or reproductive growth, can only occur from mature flush (Nunez-Elisea and Davenport, 1995). In addition, buds appear to be receptive to the floral stimulus for only a small portion of the flush development cycle, because floral induction seems to require inductive temperatures approximately to coincide with bud release. Recently emerged buds on plants of cultivar 'Irwin', growing in warm, non-inductive conditions initiated flower panicles when they were moved to florally inductive ambient winter conditions, so long as the buds were less than approximately 10 mm in length (Batten and McConchie, 1995). Pruning treatments to manipulate the timing of flush development and synchronize canopy flushing have been successful in increasing flowering intensity (Yeshitela, 2005).

Under tropical conditions, in which cold inductive temperatures may be brief, erratic or non-existent in some seasons, it is unclear what exactly leads to floral initiation; however, some factors improve the likelihood of flowering. First, some cultivars flower at higher temperatures more reliably than others (Pandey, 1989). For example 'Florigon' was the only cultivar to flower at the higher temperature treatment of 20/15 °C of ten cultivars tested by Whiley *et al.* (1989). Secondly, the treatments which reduce vegetative vigour such as paclobutrazol (GA biosynthesis inhibitor), and manipulate the timing of flush development, such as water stress (Davenport, 2003), may help if they serve to focus bud release around the time of inductive temperatures, although this requires testing. Water stress may be used only indirectly to promote flowering in lychee. Water stress has not been shown to induce flowering or decrease the cold requirement of mango (Chaikiattiyos *et al.*, 1994). Third, potassium nitrate is thought to induce flowering (Bondad and Apostol, 1979), but seems to be ineffective in many environments (Davenport and Nunez-Elisea, 1997).



There is some correlative evidence for regulation of floral initiation in mango by plant growth regulators (PGRs), GA in particular. Evidence comes from measurements of endogenous GA, the effects of exogenous GA, and the effects of GA biosynthesis inhibitors. The concentration of GA in terminal stems of cultivar 'Khiew Sawoey' decreased during the 16 weeks before panicle emergence in trees that subsequently flowered, and increased over the same period in terminal stems of trees that remained

vegetative (Tongumpai *et al.*, 1991). It suggests the direct inhibitory role of GA in mango floral initiation. Exogenous applications of GA may indirectly regulate mango flowering by delaying bud release. This may be because applied GA delays bud release and does not inhibit flowering so long as bud release occurs under florally inductive conditions (Nunez-Elisea and Davenport, 1998). GA biosynthesis inhibitors such as paclobutrazol (Rademacher, 1995) both hasten and increase the flowering intensity of mango (Blaikie *et al.*, 2004) and also reduce vegetative vigour (Winston, 1992). So paclobutrazol may directly promote flowering, or act indirectly by increasing the likelihood of bud release during floral inductive conditions. Therefore, GA may inhibit floral initiation endogenously or may act indirectly by influencing the timing of bud release.

In summary, the only factor shown experimentally to induce flowering in mango is temperature below 15–20 °C, with florally inductive temperatures varying between cultivars. Floral initiation is affected by the cycle of flush development, and the timing and intensity of flowering can be manipulated by exogenous applications of PGRs. The evidence for the internal regulation of floral initiation by GA is not conclusive.

Mango flowering involves hormonal regulation of shoot initiation and induction events resulting in reproductive shoot formation. A balance or ratio of endogenously regulated phytohormones, thought to be auxin from leaves and cytokinins from roots, appears to govern the initiation cycle independently from inductive influences. Induction of reproductive or vegetative shoots is thought to be governed by the ratio of a temperature-regulated florigenic promoter and an age regulated vegetative promoter at the time of shoot initiation. Management of off-season flowering in mango trees is being accomplished in the tropics by successfully synchronizing shoot initiation through tip pruning and use of nitrate sprays coupled with management of the stem age to induce flowering such that it can be accomplished during any desired week of the year.

Other tree crops

Environmental control of floral initiation

A range of subtropical and tropical tree species can be induced to flower by exposure to low temperature: mango, lychee (Menzel and Simpson, 1995) and orange (*Citrus sinensis* L.) (Moss, 1976). One major difference between cool temperature induction in subtropical and tropical trees, and vernalization in herbaceous species, is the temperature required: subtropical and tropical trees often require temperatures around 15–20 °C whereas vernalization in herbaceous species requires temperatures between –1 °C and 10 °C.

Therefore cool temperatures induce flowering in several tropical and subtropical horticultural trees. In temperate deciduous horticultural trees, temperature can affect the intensity of floral initiation, but it is not clear if temperature provides an inductive stimulus.

The investigation on the site of perception of cool florally inductive temperatures has not been extensively elucidated. In mango, cool temperatures may be sensed in the leaves because mature leaves appear to be the source of an essential floral stimulus. Lychee may be similar given that mature leaves also appear necessary for floral initiation (Ying and Davenport, 2004). High root temperatures can inhibit floral initiation in lychee even when shoots are exposed to florally inductive temperatures (O'Hare, 2004), indicating either perception by the roots and long-distance signalling or heat transfer through the transpirational stream.

In citrus, leaves are not necessary for floral initiation in cool inductive temperatures (Davenport, 2000) or for floral induction through water stress (Southwick and Davenport, 1986). This indicates that both the cold temperature and water stress stimuli that induce flowering may be perceived in the stem or buds.

Water stress induces flowering in two citrus horticultural tree species. Tahitian limes, *Citrus latifolia* Tan., flowered on resumption of daily irrigation after both cyclic and constant water stress for as little as 2 weeks (Southwick and Davenport, 1986). When the same Tahitian lime plants had completed a vegetative flush 2 months later, water stress and subsequent resumption of adequate watering resulted in floral initiation again. Rewatering following water stress also caused floral initiation in lemon (*Citrus limon* (L.)

Burm. f.). This does not hold good in lychee, mango or avocado (Chaikiattiyos *et al.*, 1994). Whether the inductive stimulus is provided by the period of water stress or the subsequent watering is still not clear. Water stress can also indirectly promote floral initiation by checking vegetative flushing for e.g. in lychee (Stern *et al.*, 1998).

Vegetative growth and floral initiation

Horticultural tree species vary both in the types of shoots that produce flowers and where on these shoots the flowers are borne. The location of the flowers and the timing of floral initiation influence the interaction between flowering and vegetative growth. Several subtropical species like lychee produces inflorescences from terminal buds. Whereas, avocado produces from both terminal and axillary buds. The other species like macadamia (*Macadamia integrifolia* Maiden) and Betche (*M. tetraphylla* Johnson) produce inflorescence from axillary buds.

In the subtropical trees lychee, avocado, and macadamia, flowering is dependent on bud release during cool florally inductive temperatures (Olesen, 2005). This is largely regulated by maturity of the most recent flush, but the characteristics of the shoot affects the likelihood of bud release and flowering. Vegetative growth in lychee is through recurrent flushing, with the interval between successive flushes dependent on the prevailing weather conditions (Olesen *et al.*, 2002). There is only a small part of this cycle when the new shoots are receptive to floral induction, that being around the time of early flush development when the expanding buds are no more than a few millimetres in length (Batten and McConchie, 1995). Therefore, vegetative shoots do not flower if these are not mature by late autumn. This is because the cyclic nature of flush development and they will initiate new growth only after cool winter conditions.

The role of plant growth regulators

Endogenous GA can inhibit floral initiation and effect shoot growth. The applied GA inhibits floral initiation in citrus (Lord and Eckard, 1987). Reduced levels of endogenous GA have been correlated with floral initiation in lychee (Chen, 1990); and GA biosynthesis inhibitors have improved flowering in mango (Winston, 1992), and lychee (Menzel and Simpson, 1990).

GA applications in citrus that inhibit flowering reduce the number of buds that are released in spring but not the proportion of buds that produce floral shoots (Garcia-Luis *et al.*, 1986). Thus the effect seems to be on shoot growth rather than floral initiation. Applied GA also affects shoot growth in apple by reducing the rate of node development, lessening the chances of the buds reaching the critical appendage number (Bertelsen *et al.*, 2002).

The presence of fruit inhibits floral initiation in several species like citrus (Garcia-Luis *et al.*, 1986). Large crops can lead to poor floral initiation in the following year and induce a cycle of biennial bearing. GA exported from the seeds of citrus fruit to the buds (Garcia-Luis *et al.*, 1986) is thought to be involved in the inhibition.

Cytokinins may also be involved in floral initiation. Endogenous cytokinin levels in buds of lychee increase at the onset of florally initiation and differentiation, and exogenous applications increase floral initiation (Chen, 1991). There is no evidence that cytokinins can replace the florally inductive stimulus. Application of the growth retardant maleic hydrazide to 'Japanese pear', *Pyrus pyrifolia* Nakai, increased both endogenous cytokinin levels and floral initiation (Ito *et al.*, 2001).

Ethylene has long been used to promote flowering commercially in pineapple (*Ananas comosus* (L.) Merr.) (Turnbull *et al.*, 1999) and promotes flowering to some extent in apple (Bukovac *et al.*, 2006).

The role of carbohydrates

Carbohydrates have two roles in plant development, in the general provision of energy and carbon skeletons for growth and in the regulation of metabolism. The need for carbohydrates for floral initiation has often been investigated by measuring levels of stored carbohydrates, or imposing treatments such as girdling that modify the levels of stored

carbohydrates, and correlating these with flowering intensity. The results have been mixed. Girdling increased flowering intensity in lychee (Menzel and Simpson, 1987), and citrus (Goldschmidt *et al.*, 1985) indicating increased stored carbohydrates can increase floral initiation, because girdling has been reported to increase levels of stored carbohydrates in some horticultural trees (Goldschmidt *et al.*, 1985; Menzel *et al.*, 1995). Further, a study of stored carbohydrates in biennial bearing citrus found that high and low levels of stored carbohydrates corresponded with high and low levels of floral initiation, respectively (Goldschmidt and Golomb, 1982). However, other experiments with citrus have revealed complex interactions between cool inductive temperatures, PGRs, fruit load, and girdling treatments on the flowering intensity (Goldschmidt *et al.*, 1985). It is unclear whether increased flowering intensity in treatments that also increase the availability of carbohydrates in horticultural trees is due to the action of carbohydrates as a floral stimulus or an energy source.

Flowering genes in horticultural trees

Orthologues of flowering genes have been identified in several tree species. Studies to determine the function of these genes generally involve correlation of gene expression with floral initiation/ development or transgenic studies, where flowering genes from the perennial species are inserted into a related perennial species.

In Satsuma mandarin *FT* orthologue mRNA levels increased with the seasonal onset of cool temperatures during the time of floral induction (Nishikawa *et al.*, 2007); there is evidence that *LEAFY* orthologues isolated from sweet orange (Pillitteri *et al.*, 2004*b*) and grapevine (Boss *et al.*, 2006) act as floral promoters; and evidence that *TFL1* orthologues isolated from citrus (Pillitteri *et al.*, 2004*a*) act as floral inhibitors.

There is now some understanding of how the expression of flowering genes integrates with the environment and flowering time in horticultural trees. In sweet orange, *LFY* and *AP1* orthologue RNA levels increased during and after florally inductive cool temperatures while RNA of the *TFL* orthologue was absent (Pillitteri *et al.*, 2004*a*). In transgenic hybrid citrus, *Citrus sinensis* L. Osbeck×*Poncitrus trifoliata* L. Raf., over-expression of *LFY* and *AP1* orthologues substantially reduced the juvenile phase, but flowering still appeared to be under both environmental and endogenous control because it occurred only once a year in the spring (Pena *et al.*, 2001).

Conclusion

Flowering involves hormonal regulation of shoot initiation and induction events resulting in reproductive shoot formation especially in mango. A balance or ratio of endogenously regulated phytohormones, thought to be auxin from leaves and cytokinins from roots, appears to govern the initiation cycle independently from inductive influences. Induction of reproductive or vegetative shoots is thought to be governed by the ratio of a temperature-regulated florigenic promoter and an age regulated vegetative promoter at the time of shoot initiation. Management of off-season flowering in mango trees is being accomplished in the tropics by successfully synchronizing shoot initiation through tip pruning and use of nitrate sprays coupled with management of the stem age to induce flowering such that it can be accomplished during any desired week of the year.

Future Study

1. Investigation on the possible role of photoperiodism on the vegetative and reproductive phase of subtropical fruit crops should be critically studied.

2. Effort should be made to study the photosynthetic efficiency of the individual leaves and their contribution to flower bud formation in different type of shoots.

3. For better understanding of flowering, the exact stage of shoots at which synthesis of growth promoting and growth inhibiting substances takes place, has to be studied.

4. Translocation or synthesis of various regulatory chemicals during the growth and development of fruit should be properly determined in order to

have better understanding about fruits which set in motion a depressing effect on the tree ultimately resulting in biennial bearing.

5. The mechanism of the phenomenon of off season flowering should be studied in detail.

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APPLICATION OF REMOTE SENSING INFORMATION IN CROP WATCH AND DROUGHT

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Introduction

Accurate and timely monitoring of agricultural crop conditions and estimating potential crop yields are essential. Decreased production caused by drought or pest infestation, can be critical for countries or locales where the economy is dependent on the crop harvest. Crop area statistics generation in India date back to Kautilya's Arthasastra as well as Moghul era. The present day crop statistics form an uninterrupted series since the Government of India made a wheat assessment/forecast as early as 1884. Currently, the crop statistics cover 51 food crops and 15 non-food crops which are based on land revenue system. North eastern states rely on ad hoc surveys, while multi season full enumeration approach is adopted in the remaining part of the country. Acreage estimates from these surveys have to pass through a hierarchy of aggregation of village, taluka, district and state level, which contributes to a delay in compilation of national forecasts. Shortcomings in the present crop information system in India include delay in reporting, rigidity of definition, non-sampling errors, inadequacy for forecasting and non-responsiveness to change in growing conditions or episodic events.

Crop Acreage and Production Estimation (CAPE) activities were initiated by covering large area crop inventory and yield modeling for wheat, rice, cotton, groundnut, sorghum and mustard in India using remote sensing data. The constraint of the project has lead to FASAL (Forecasting Agricultural Output Using Space, Agrometeorology and Land-based Observations) to meet the stringent requirements of multiple, nation-wide and multi-crop forecasts. The factors affecting the accuracy of crop identification is determined by sensor parameters, scene characteristics as well as the analysis approaches. The constraints in crop inventory using space borne sensor data are a) small field sizes, b) a large diversity of crops sown in an area, c) large field-to-field variability in sowing and harvesting dates, cultural practices and crop management, d) large areas under rain fed/dry land agriculture with poor crop canopies, e) practice of inter-cropping and mixed cropping and f) extensive cloud cover during kharif crop season.

Remote sensing

Remote sensing is the science of obtaining information about an object, area or phenomenon through the analysis of data acquired by a device that is not in contact with the object under investigation. The remotely collected data can be of many forms including variations in force distribution, acoustic wave distributions or electromagnetic energy distributions. The electromagnetic energy sensors that are currently being operated from airborne and space-borne platforms to assist in mapping and monitoring of earth resources are described in this text. Data collection from multi-stage platform using multiple-view approach, from the multi-spectral sensors available onboard for the multi-temporal period would offer accurate information about the event. Scanner, radiometer and camera, positioned on the ground, truck mounted, low/ altitude air-borne and space borne platforms are used in data collection. The spectral and spatial resolution of the scanners would vary with the height from the object.

Communication, weather and earth observation satellites are available to civilian use. Some of the defense satellites are also used in emergency situation. The information from the control room to specific groups/agencies involved in the disaster mitigation is transmitted through *Communication satellites*. Commercial sectors also play an important role in global communication systems

such INTELSAT & INMARSAT programs. Additional low Earth Orbit satellites are planned by IRIDIUM (Motorola), ODYSSY (TRW), Goldstar (Loral / Qualcomm) and Project 21 (INMARSAT) in providing communication network in addition to INSAT (ISRO). Further, stationary and mobile, very small aperture terminals (VSAT) and ultra small aperture terminals (USAT) and array of antennae help in position, location and co-ordination of disaster relief operations, in addition to services. *Weather satellites* such as NOAA & INSAT provide continuous information on atmospheric and surface features. *Earth observation satellites* provide comprehensive synoptic and temporal view of large areas in real time. These orbital satellites are grouped into various categories depending on their orbital height (Geo-stationary Orbit – 22,282 km, Medium Earth Orbit – 6250-13000 km, Low Earth Orbit –500-1500 km). They are being effectively used in detecting and mapping of many types of natural disasters and planning. If the disasters are identified in the initial stages, it is easier to reduce the social and economic impacts. The availability of operational earth observational satellites, sensors and their application (remote sensing) to various disasters are discussed in detail.

Observation is typically made via different 'channels' of the Electromagnetic spectrum, in particular, the Visible and Infrared portions. Some of these channels include: Visible and Near Infrared: 0.6 ìm - 1.6 ìm - For recording cloud cover during the day; Infrared: 3.9 ìm - 7.3 ìm (Water Vapour) and 8.7 ìm, - 13.4 ìm (Thermal imaging). *Visible-light images* from weather satellites during local daylight hours are easy to interpret even by the average person; clouds, cloud systems such as fronts and tropical storms, lakes, forests, mountains, snow ice, fires, and pollution such as smoke, smog, dust and haze are readily apparent. *Infrared satellite imagery* is used effectively for tropical cyclones with a visible eye pattern, using the Dvorak technique, where the difference between the temperature of the warm eye and the surrounding cold cloud tops can be used to determine its intensity (colder cloud tops generally indicate a more intense storm).

Geostationary weather satellites orbit the Earth above the equator at altitudes of 35,880 km. They remain stationary with respect to the rotating Earth and record or transmit images of the entire hemisphere below continuously with their visible-light and infrared sensors. The United States has two in operation; GOES-11 and GOES-12. GOES-12, designated GOES-East, is located over the Amazon River and provides most of the U.S. weather information. GOES-11 is GOES-West over the eastern Pacific Ocean. Russia's new-generation weather satellite Elektro-L 1 operates at 76°E over the Indian Ocean. The Japanese MTSAT-1R over the mid Pacific at 140°E is in operation. The Europeans have Meteosat-8 (3.5°W) and Meteosat-9 (0°) over the Atlantic Ocean and have Meteosat-6 (63°E) and Meteosat-7 (57.5°E) over the Indian Ocean. India also operates geostationary satellites called INSAT which carry instruments for meteorological purposes.

Polar orbiting weather satellites circle the Earth at a typical altitude of 850 km in a north to south path, passing over the poles in their continuous flight. They are in sun-synchronous orbits and are able to observe any place on Earth and will view every location twice each day with the same general lighting conditions due to the near-constant local solar time. They offer a much better resolution than their geostationary counterparts due their closeness to the Earth. The United States has the NOAA series of polar orbiting meteorological satellites, presently NOAA 17 and NOAA 18 as primary spacecraft, NOAA 15 and NOAA 16 as secondary spacecraft, NOAA 14 in standby, and NOAA 12. Europe has the Metop-A satellite. Russia has the Meteor and RESURS series of satellites. China has FY-1D and FY-3A. India has polar orbiting satellites as well.

Harvest Index

It is the ratio of grain yield to above-ground biomass. It is a key parameter for crop yield prediction using an integrated remote sensing and ground based measurements, based on radiation use efficiency approach or crop simulation models. The process dynamic could be monitored and modeled to estimate HI could be established by combining temporal-spatial character of remote sensing information. There were three methods to estimate HI: 1) describing and

analyzing the crop growing process with high temporal resolution remote sensing data, 2) acquiring the environmental parameters with remote sensing and then to estimate the HI using these parameters and 3) crop structural parameters acquired from radar or laser radar data.

The common vegetation indices (Vis) used in HI are- normalized difference vegetation index (NDVI), ratio vegetation index (RVI), transformed vegetation index (TVI), soil adjusted vegetation index (SAVI), modified soil adjusted vegetation index (MSAVI), renormalized difference vegetation index (RDVI), difference vegetation index (DVI), enhanced vegetation index (EVI) and green chlorophyll index (CI_{green}). It is observed that HI -1) HI from canopy reflectance information based on the partitioning of crop activity before and after flowering could give good results but required long-term dataset of total growing season and 2) strong correlations with the accumulated values of VIs from anthesis to maturity period. It was found that the correlation between HI and accumulated VI after anthesis was greatly improved by introducing another variable of VI at anthesis using binary regression analysis. The coefficients of determination (R^2) of binary regression equations were higher than 0.80, and the root mean square deviations (RMSD) between the estimated HI and measured HI were as low as 0.04. It was suggested that canopy spectral reflectance from anthesis to maturity had good performances on HI estimation. With short-term data requirement and good accuracy, the method based on VI dataset of anthesis-maturity period would be more favorable for large area application since the dataset can be easily collected by means of remote sensing.

Crop classification

Crop classification is usually not available until about 4-5 months after the crops are harvested. In an operational program remote sensing data is used to predict crop yields at specific time periods. The temporal and spatial inconsistencies of the MODIS 8-day reflectance product data limit its application in crop yield models at regional scales.

Crop discrimination is based on differential spectral response of various crops in a multi-dimensional feature space produced by different spectral bands, or time domain or both and is influenced by sensor characteristics as well as pattern recognition techniques. Interaction of electromagnetic radiation with crops is influenced by chlorophyll and water content in optical region, whereas crop geometry and dielectric property influences the response in microwave region. The steps involved in the crop acreage estimation using RS data are: (a) study area extraction, (b) crop discrimination/ identification from satellite data, (c) estimation of area under a crop in the study area, and (d) assessment of accuracy of crop identification and area estimates.

The limitation in using satellite-based Leaf Area Index (LAI) methods with high and moderate (MODIS) spatial resolution data in crop yield simulation models at regional scales is that the canopy architectural parameters in the SAIL or other radiative transfer model for crop-specific LAI should be accurate. These parameters also change during the growing season. The reflectance data are used to derive LAI seasonal profile, which is used in initializing or constraining parameters in the crop

Crop Watch System

China Crop Watch System consists of seven models: crop growth monitoring, drought monitoring, grain production estimation, crop production prediction, crop planting structure inventory, cropping index monitoring, and grain supply–demand balance and early-warning. The monitoring can be carried out on different scales or levels ranging from a county through a province and the whole country to the main producing countries in the world.

Crop growth reflects the growth of seedlings, at an early stage in a crop's growth, as well as the metaphase and the anaphase of each crop as it matures and the changing trends in its yield. It is monitored by: real-time monitoring and crop-growing process monitoring. In real-time monitoring, maps of the crop condition are generated from the difference between the vegetation index of the previous year and the current year (Wu B F, 2000). Large area coverage is possible to monitor the crop continuously and dynamically. In crop growing process monitoring,

growing profile of crops can be generate by collocate the remote sensed data along time; it can reflect the change of crop parameters from planting, seedling, tassel, to maturation and harvest (Zhang F, 2004). Normalized difference vegetation index (NDVI), Leaf area index (LAI) and net primary productivity (NPP) are used to monitor the crop growth in different period during the crop-growing season. Different regions has different indicator, NDVI, LAI or NPP.

Drought Monitoring - Agricultural drought occur when the existing water supply cannot meet the needs of the growing crop. India is one of the countries in the world that suffer from serious droughts, which reduce the country's grain production considerably. Spatial distribution and progress of drought is done by computing indices from temporal satellite data, and then building drought model with these indices. Regular monitoring can not only predict droughts but also estimate their severity. The prediction and estimation of the drought development can be realized by monitoring the crop drought continuously.

Crop production prediction is achieved by monitoring the crop planting area and predicting the yield. The prediction can be made by one month before the harvest. Eight crops, including winter wheat, spring wheat, early rice, semi-late rice, late rice, spring maize, summer maize and soybean are covered in the activity.

Yield prediction - The agro-meteorological model (Meng Q Y, 2004), the remote sensing index model (Cook, Paul W, 1995), a combination of both, and the biomass model are used for this purpose. The biomass model combines inputs from three models, namely the photosynthetically active radiation (PAR) model, the light use efficiency model, and the land surface energy balance model, along with AVHRR/ TM/LISS data from remote sensing to calculate regional total crop biomass; that value is then used in conjunction with the harvest index.

Crop planting area monitoring is carried out based on two independent sampling frames for estimating the area using remote sensing together with sampling on the ground. By stratifying the main crop-growing areas into regions and applying stratified cluster sampling frames to the data from remote sensing, crop planting ratio (that is, the proportion of the total area sown to the total arable land area) are monitored, and the transect sampling frames and GVG (integration of GPS, Video and GIS) sampling system are used to estimate each crop's planting ratio (that is, the proportion of area under a given crop to the total sown area). Using these two ratios, the total sown area under each crop can be estimated.

Production prediction is based on the estimation of areas and yield per unit area. It predicts the total yield of each of the major crops. It is feasible to carry out the grain supply–demand analysis on different spatial scales. This model too can provide information for any specific area or region specified by the user.

Operation and validation of China Crop Watch System has been put into operation since 1998. Every year, 7 monthly bulletins and 20 newsletters are released, covering crop condition, crop acreage and yield, drought, agro-meteorology, cropping index and crop planting structure, as well as grain production. The results of monitoring were also initially made available on the intranet of the government and later on the Internet as well. In its 9 years' operation, CCWS is its accurate prediction that domestic grain production in 2000 would decrease by 3% and that in 2001 would remain at the same level as that of the previous year, which proved immensely useful to decision makers involved in macro control.

Validation - Each year, a few validation sites have been selected to do the field measurement for verifying specific results. The crop LAI every 10 days and crop type proportion have been measured to verify the crop condition and crop acreage. Validation shows that the accuracy is up to 97% for plant proportion and 95% for crop type proportion (He L H, 2004; Li Q Z, 2004).

Drought

Drought is the most complex phenomenon that is yet to be understood completely, irrespective of its occurrence around the world at regular interval. Most of the Government's policy in dealing with drought has been to wait till it rains and provide some

form of emergency assistance to people. Long-term impacts of this hydro-meteorological phenomenon are often ignored. An integrated approach in characterization and drought severity by linking the climate data to hydrological (e.g. stream flow, groundwater and reservoir levels, soil moisture, etc.), environmental (e.g. land use pattern, vegetation health, degradation status, etc.), and socio economic (e.g. food security situations, demographic dynamics, social behaviors, infrastructure conditions) conditions, is of critical importance in the systematic comparison of multiple indicators and short term climate variability and longer term shifts. These drought measuring parameters are not linearly related to one another. These indices often have little correlation among themselves. It is noticed that when one drought index identifies drought at a particular place, another drought index indicates a normal condition at the same place and time (Bhuiyan 2006).

Drought indices

Standardized Precipitation Index (SPI) indicates the probability of precipitation of desired time scale. It is computed by dividing the difference between the normalized seasonal precipitation and its long-term seasonal mean by standard deviation. It also provides an easy way to identify the beginning and end of drought conditions and to describe drought severity.

SPI = $X_{i,j}$ - $X_{i,m}$ / ó (where $X_{i,j}$ is the seasonal precipitation at $_i$th rain gauge station and $_j$th observation, $X_{i,m}$, the long-term seasonal mean and ó is its standard deviation).

Standardized Water level Index (SWI) represents the aquifer stress, calculated from the monitored water level (Bhuiyan 2004) It is the indirect measure of recharge and reference to drought as indicated by water- table level.

SWI = $W_{i,j}$ - $W_{i,m}$ / ó (where $W_{i,j}$ is the seasonal precipitation at $_i$th well and $_j$th observation, $W_{i,m}$, the seasonal mean and ó is its standard deviation)

Vegetation-stress is due to adverse climatic and hydrological factors. Orbital satellite observations are being used along with hydro-meteorological information in support of decision making related to drought. The use of satellite data to track phenological events complements ground observation networks. Satellites provide a unique perspective of the planet and allow for regular, even daily, monitoring of the entire global land surface.

Normalized Difference Vegetation Index (NDVI) has been in use for many years to measure and monitor plant growth (vigor), vegetation cover, and biomass production from multispectral satellite data. The NDVI image is derived from spectral data in the visible (Channel 1; 0.58-0.68ìm) and near infrared (Channel 2; 0.725-1.10ìm) regions of the electromagnetic spectrum. Channel 1 is in the red-light region of the electromagnetic spectrum where chlorophyll causes considerable absorption of incoming sunlight, whereas Channel 2 is in the near-infrared region of the spectrum where a plant's spongy mesophyll leaf structure creates considerable reflectance (Jackson *et al.,* 2004).

NDVI = (0.725-1.10µm - 0.58-0.68µm) / (0.725-1.10µm + 0.58-0.68µm)

Vigorously growing healthy vegetation has low red-light reflectance and high near-infrared reflectance, and hence, high NDVI values. This algorithm produces output values in the range of -1.0 to 1.0. Increasing positive NDVI values show the increasing shades of green on the images, indicate increasing amounts of green vegetation. NDVI values near zero and decreasing negative values indicate non-vegetated features such as barren surfaces (rock and soil) and water, snow, ice, and clouds. NDVI derived from NOAA's Advanced Very High Resolution Radiometer (AVHRR), having spatial resolution of 1- sq. km on a daily is used for regional basis. Indian Remote sensing (IRS) satellite data is also used for smaller areas. Coarser-resolution climate data is used to identify which areas of stressed vegetation in the NDVI data is experiencing dryness, which distinguishes drought impacted areas from those influenced by other environmental stressors.

Percent Average Seasonal Greenness (PASG) is calculated based on smoothed temporal NDVI curve characteristics. The *start of the season*

(SOS) and end of the season are used in the calculations for *seasonal greenness* (SG). It is selected as the dependent variable mainly because vegetation stress typically occurs after a precipitation (water) deficit affects the plant growth. For vegetation monitoring, the SG at each pixel for any given period is compared to the mean of the same period from the historical database. The measure is expressed as percentage by:

PASG = (Current SG/mean SG) * 100.

Two variables related to general vegetation conditions – the Percent Average Seasonal Greenness (PASG) and Start of Season Anomaly (SOSA) – are calculated from satellite-based observations and incorporated into the VegDRI. These metrics can be used to summarize the degree of variability in vegetation conditions from year to year. PASG compares the accumulated seasonal greenness up to a specific point in a year to the historical average seasonal greenness for that same date. A PASG value of 100% indicates that the current seasonal greenness is comparable to the average historical greenness for that location at the time. This would indicate that the vegetation conditions are near normal. PASG values less than 100% would indicate below average greenness (poorer than normal vegetation conditions) that may be linked to some form of stress (drought, flooding, hail damage, or pest infestation). PASG values greater than 100% would indicate higher than average greenness, which would reflect better than normal vegetation conditions. PASG values are not calculated for a location until the start of season has occurred.

Seasonal Greenness (SG) is a measure of growing season vegetation vigor or performance derived from the Normalized Difference Vegetation Index (NDVI). It is calculated on a 14-day basis, is based on the smoothed NDVI data distributed as part of Phenological Characterization [smoothed NDVI data]. SG is an integrated measure of NDVI from the start of the growing season to the time of observation. The integration is performed on simulated daily NDVI. The Percent of Average Seasonal Greenness (PASG) is then calculated for each pixel by: (Current Observation SG / Mean SG) * 100. Areas below the mean may result from a variety of influences including standing water, drought, deforestation, or urbanization. Climate data and drought indicators are necessary to determine where drought is causing patterns of below average SG.

The SOSA is the temporal difference in the start of season (SOS) for a given year compared to the historical average SOS for a location. SOS can be defined as the time at which vegetation initiates growth (photosynthetic activity) after winter as observed from satellite observations. There can be a delay between the SOS we observe on the ground versus the SOS that can be detected from satellite-based observations. A negative SOSA indicates that the SOS for a specific year is later than the average date and a positive SOSA appears when green up occurs earlier than normal. The SOSA is used to express the inter-annual changes that can occur from year-to-year in the VegDRI model.

Out of Season represents the over-wintering period when the vegetation is dormant and crops are unplanted or fallow land. There is often an inherent delay (by a few days to week) between what is observed on the ground and what is detected from satellite. Identification of the start of the growing season from satellite requires certain amount of photosynthetically-active plant material be present before spectral signal indicative of actively growing vegetation can be detected.

Normalized Difference Water Index (NDWI) is calculated from the near-infrared (NIR) and shortwave-infrared (SWIR) data from the Moderate Resolution Imaging Spectro-radiometer (MODIS)) is being calculated for large-area drought monitoring.

$$N_{DWI} = \frac{P_{NIR} - P_{SWIR}}{P_{NIR} + P_{SWIR}}$$

Two spectral bands used in its calculation are responsive to changes in the water content (SWIR band) and inter-cellular air spaces of the spongy mesophyll layer of leaves in the vegetation canopy. It is influenced by both the desiccation and wilting of vegetation. It is more sensitive drought indicator than traditional remote sensing-based indices such as the Normalized Difference Vegetation Index (NDVI),

which do not account for changes in the vegetation's water content.

Normalized Difference Drought Index (NDDI), combines information from both the NDWI and NDVI data derived from MODIS as shown:

$$NDDI = \frac{NDVI - NDWI}{NDVI + NDWI}$$

It is more responsive and has wider dynamic range values than a simple NDVI-NDWI differencing through drought periods. Irrigated crops have higher peak NDVI values than the non-irrigated crops.

Vegetation Drought Response Index (VegDRI) was used as an agricultural drought indicator in evaluating its relationship with crop yields during the critical periods over irrigated and rain fed areas in Nebraska, USA and found to be primarily an indicator of drought intensity and not a direct indicator of crop yield (Brown et al 2008). The crop yield is influenced by management practices, hail, flooding, and insect infestations. Periodic feedback from experts (e.g., state climatologists, USDM authors, and agricultural experts) agricultural producers and others in the general public will be used to characterize the general strengths and weaknesses of VegDRI and highlight specific locations or trends that might be in error.

It may be summarized that food security is achieved by sustained crop production, which is achieved by continuous monitoring of crops / plants on plot / village / regional / country level by way of remotely sensed and validated information. This approach would help us in the food availability / scarcity preparedness. As drought stems out of non-effective management of resources, its availability and effective utilization in the climate change scenario.

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ABIOTIC STRESS: PERCEPTION, SIGNALING AND GENE EXPRESSION

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Drought is a major abiotic stress that affects productivity of crop plants by altering the carbon and nitrogen balance in plants and causing generation of harmful reactive oxygen species. Plants show several adaptive responses to drought, which are regulated by a complex pathway of signaling and gene expression. The review attempts at highlighting recent findings with respect to drought stress perception and signaling and the gene expression related to stress-response phenotype. Since drought is a complex stress factor comprising of high temperature, high light intensity and low water availability, the plant responses to each of the stress components often result in overlapping signaling pathways.

Plant responses to stress

A primary response of plants subjected to drought stress is growth arrest. Shoot growth inhibition under drought is thought to mobilize metabolites for the synthesis of protective compounds required for osmotic adjustment, which prevent dehydration of the cytoplasm and permit cellular metabolism to continue. Root growth arrest enables the root meristem to remain functional and give rise to rapid root growth when the stress is relieved. On the other hand, fewer but deeper roots enable the plant to extract water from the lower layers of soil and enable plant survival.

Synthesis of compatible solutes like polyols, proline and trehalose under stress prevents water loss from cells and play an important role in turgor maintenance. The trehalose biosynthesis pathway, in which trehalose 6 phosphate (T6P) acts as an indicator of G6P and UDPG pool size is known to link growth and development to metabolite content (Paul *et al.,* 2008). Trehalose phosphate phosphatases are upregulated under stress conditions and in turn regulate the T6P levels.

Water deficit induced ABA synthesis brings about stomatal closure, which causes a decrease in intercellular carbon dioxide concentration and inhibits photosythesis. Carbon dioxide limitation due to prolonged stomatal closure in the face of continued photosynthetic light reactions lead to accumulation of reduced photosynthetic electron transport components, which can reduce molecular oxygen and give rise to reactive oxygen species, thus causing damage to the photosynthetic apparatus. Mitochondrial respiration in plants plays an important role in dissipating the NADPH generated during photosynthetic light reactions, through type II NADPH dehydrogenases situated on the matrix side. This prevents accumulation of reductants and reduces generation of reactive oxygen species in the chloroplasts. Plant mitochondria also prevent reactive oxygen species generation within themselves by employing the alternative oxidase pathway, in which the complex III and IV of the respiratory electron transport system are bypassed and electrons are directly transferred to oxygen, with the generation of thermal energy instead of ATP. Another stress adaptation is through the ã-aminobutyric acid (GABA) shunt, in which two steps in the TCA cycle related to generation of reducing power are bypassed (Fait *et al.,* 2007). Prohibitins are large protein complexes that localize to the inner mitochondrial membrane, where they appear to play a role in maintaining the superstructure of the inner mitochondrial membrane and the protein complexes associated with it (Van Aken *et al.,* 2010). They have been implied in stress tolerance not only because of their role in protecting mitochondrial structure, but also in triggering retrograde signaling between mitochondria and the nucleus in response to stress thus altering expression of several stress responsive transcripts, including alternative oxidase (AOX), heat shock proteins (HSP) and genes involved in hormone homeostasis.

Reactive oxygen species are generated due to metabolic perturbation of cells and these cause cell damage and death. While mechanisms to prevent

generation of reactive oxygen species have been mentioned earlier, an important adaptive mechanism consists of their effective scavenging if and when these harmful species do arise. Antioxidant enzymes like superoxide dismutase, catalase, glutathione reductase, and antioxidant substrates like ascorbate, á-tocopherol and carotenoids exist in cell organelles and the cytoplasm and play an important role in detoxifying these reactive species. Methionine sulfoxide reductases are another class of antioxidant enzymes that play a role in preventing damage to proteins due to reactive oxygen species generation in plastids (Rouhier *et al.,* 2006). These enzymes use reduced thioredoxin as cofactors and reduce the methionine sulfoxide residues generated in proteins due to oxidative stress, thus restoring protein function.

Hormonal responses to drought are characterized by a surge of abscisic acid (ABA) synthesis, particularly in the roots, which is then translocated to the leaves to bring about stomatal closure. Brassinosteroids, jasmonic acid and nitric oxide are other hormonal signals that are involved in drought stress signaling.

Stress perception and signaling

Acclimation to stress involves processes starting from perception of stress to the expression of large number of genes involved in manifestation of a morphological or physiological response that increase the chances of survival under the stress condition.

Molecular mechanisms that sense stress consist of a number of classes of cell-surface receptors like serine threonine like receptor kinases called receptor-like kinases (RLKs), ion-channel linked receptors, G-protein coupled receptors (GPCRs) and two-component histidine kinase receptors. RLKs are major contributors to the processing of a vast array of plant developmental and environmental cues. Their activity is regulated by receptor oligomerization and phosphorylation, receptor internalization and dephosphorylation, or regulation at the transcriptional level (Chae *et al.,* 2009). Brassinosteroid receptors BR1 belong to the RLK family, which in response to BR or stress, are internalized by the responding cells and the stress signal transduced. Cre 1 (cytokinin response 1) is a two-component histidine kinase receptor that transduces signal via a phosphorelay pathway. This receptor kinase, besides binding cytokinins, is also thought to act as a sensor of osmotic stress (Bartels and Sunkar, 2005). Ca^{2+} channels are responsible for influx of Ca^{2+} into the cytoplasm when activated by various stress situations (Xiong *et al.,* 2002). These channels therefore act as ion-channel linked receptors of stress. GPCRs are another group of membrane receptors, which on sensing stress, activate enzymes like phospholipase C or D which in turn release second messengers and transduce the stress signal (Tuteja and Sopory, 2008).

An intra cellular receptor for ABA, PYR/RCAR, has been shown to signal for drought stress through the activation of a serine threonine kinase SnRK2, in response to ABA binding (Sheard and Zeng, 2009). Since ABA synthesis is known to be induced in response to stress, the ABA receptor can be considered to be a stress-sensor.

Signal perception is followed by generation of secondary signaling molecules such as protein kinases and phosphatases (serine-threonine phosphatases), phospholipids like phosphoinositides (Bartels and Sunkar, 2005), reactive oxygen species, Ca^{2+}, nitric oxide, cAMP and sugars, which play an important role in signal transduction (Tuteja and Sopory, 2008).

Mitogen activated protein kinases (MAP kinases) bring about protein phosphorylation and constitute one of the major mechanisms for signal transduction. They are located in the cytoplasm and consist of three enzymes (MAPK, MAPKK and MAPKKK) that form a signaling cascade from the stress sensor located on the plasma membrane to the regulation of gene expression in the nucleus. Translocation of the MAPK into the nucleus brings about activation of transcription factors through phosphorylation (Tena *et al.,* 2001).

Calcium levels in the cytoplasm have been shown to increase transiently on stress exposure. The source of this stress-induced cytoplasmic Ca^{2+} is either from the apoplast or from the cellular reserves. Several

Ca^{2+} sensors like calmodulin (CaM) or CaM-binding proteins have been identified in the cells, which transduce the stress signal to the nucleus through other messengers like phospholipase D or Ca^{2+} dependent protein kinases (Tuteja and Sopory, 2008).

Phospholipids like phosphoinositides that are located in the plasma membranes are a source of several secondary signaling molecules like phosphotidyl inositol phosphates, which are formed by phosphorylation by kinases (eg PI3Kase) (Drobak and Watkins, 2000). Phospholipases also act on these phospholipids to generate signaling molecules like inositol 1,4,5-triphosphate (IP3), diacylglycerol (DAG) and phosphatidic acid (PA), which play a role in transmission of the signal across plasma membrane as well as in intracellular signaling.

Transcriptional regulation of gene expression

A large number of genes are seen to be involved in the expression of the stress phenotype (Xiong *et al.,* 2002; Shinozaki *et al.*, 2003). The transcriptional response initially is composed of a core set of multi-stress responsive genes and becomes increasingly stress specific as time progresses (Ma and Bohnert, 2007). DNA microarrays provide a high throughput means of analyzing gene expression at the whole genome level and have been used to study patterns of gene expression in response to drought or high-salinity stresses in several plant species (Oono *et al.,* 2003; Seki *et al.,* 2002). Some of the genes seen to be upregulated under drought stress conditions include genes involved in osmolytes synthesis, genes coding for LEA proteins, aquaporins, signalling molecules and transcription factors (TFs). Of these, the genes coding for TFs were particularly interesting since TFs act as master switches and trigger the simultaneous expression of a large number of stress-response genes that contribute to the stress phenotype (Bartels and Souer, 2004). About 104 TFs, whose expression was elevated on exposure to dehydration stress, have been identified by transcriptome analysis in Arabidopsis plants exposed to drought stress (Rhizsky *et al.,* 2004). While most of the transcription factors were up-regulated under stress, a few transcription factors that played a role in primary growth processes were down-regulated. Drought stress induced gene expression was seen to be regulated by TFs belonging to bZIP, AP2/ERF, HD-ZIP, MYB, bHLH, NAC and ZPT2 families. These TFs are activated at the transcriptional or protein level by the transduced drought signal. Since drought stress is accompanied by an increase in ABA levels, some TFs are activated specifically by ABA. The ABA-responsive TFs (ABFs) predominantly belong to the bZIP family of TFs (Jakoby *et al.,* 2002) and bind to ABA response elements (ABRE) present in the promoters of stress-response genes. TFs belonging to the AP2/ERF family play a role in ABA-independent regulation of stress-response genes and bind to the drought response element (DRE) present in their promoters(Yamaguchi-Shinozaki and Shinozaki, 2005). The HD-ZIP TFs are plant specific and show the presence of a homeodomain adjacent to leucine zipper. Among several functions attributed to this family of transcription factors, one function is the regulation of ABA-dependent genes under dehydration stress (Deng *et al.,* 2002). Most of the plant *MYBs* consist of two repeats R2R3 (Jin and Martin, 1999) and play a role in regulating expression of dehydration responsive genes (Abe *et al.,* 1997, 2003). The *ZPT2* TFs are characterized by the presence of two zinc finger motifs separated by a single long linker. These act as transcriptional repressors by down regulating the activity of other transcription factors (Sakamoto *et al.,* 2004) and are induced during dehydration stress as well as with ABA treatment. Transcription factors belonging to NAC family bind to promoters of not only dehydration response genes (Tran *et al.,* 2004) but also auxin response genes (Hegedues *et al.,* 2003).

The promoters of stress response genes are known to have several types of *cis*-elements to which TFs of the same family or different families can bind. For example, the promoter of *rd29A* gene showed the presence of both DRE and ABRE elements (Narusaka *et al.,* 2003). Similarly interactions between NAC and ZFHD transcription factors were required for the expression of the *erd1* (early response to drought 1) gene (Tran *et al.,* 2004). In some cases, transcription factors induced by different stress situations can bring about transcriptional activation of the same response gene, suggesting overlapping pathways of regulating gene expression. For example DREB1A and DREB2A

have been reported to induce the same target genes in some cases (Yamaguchi-Shinozaki and Shinozaki, 2005). Transcription factors are also known to form homo or hetero-dimers in bringing about transcriptional activation. MYC and MYB transcription factors interact with each other on binding to their respective *cis*-elements on the *rd22* gene (Abe *et al.,* 1997). Hence complex interactions between transcription factors, other proteins and their *cis*-elements play an important role in regulating gene expression in response to stress.

Epigenetic regulation of gene expression

Besides stress-induced regulation of gene expression at the transcription level, stress conditions also bring about epigenetic regulation. Stress-induced changes in histone variants, histone N-tail modifications, and DNA methylation have been shown to regulate stress-responsive gene expression and plant development under stress. Drought stress induced the expression of a variant of histone H1 called H1-S, which appeared to play a role in stomatal closure (Scippa *et al.,* 2004). ABA downregulated the expression of a histone deacetylase AtHD2C, while overexpression of this enzyme brought about enhanced expression of ABA-responsive genes and greater salt and drought tolerance than the wild type plants (Sridha and Wu, 2006). Drought-induced expression of stress-responsive genes was also seen to be associated with modifications in histones H3 and H4. Histone H3K4 trimethylation, H3K9 acetylation, H3 Ser-10 phosphorylation, H3 phosphoacetylation, and H4 acetylation was observed, which correlated to the expression of stress-induced genes (Sokol *et al.,* 2007). Histone acetyltransferases (HATs), which interact with transcription factors were also seen to be involved in activating stress responsive genes. Stresses can induce changes in gene expression through hypomethylation or hypermethylation of DNA. In tobacco stress induced-DNA demethylation was observed in the coding sequence of a glycerophosphodiesterase like protein gene, while DNA hypermethylation was induced by drought stress in pea (Chinnusamy and Zhu, 2009).

MicroRNAs (miRNAs) are ~20- to 22-nt non-coding RNAs that specifically base pair to target mRNAs and induce the cleavage of target mRNAs or repress their translation. Hence they constitute a gene-silencing mechanism that regulates expression of target genes post-transcriptionally. Regulation of stress response genes by miRNAs has been demonstrated recently (Shukla *et al.,* 2008). For example abiotic stress brought about down regulation of miR398 which targets stress-inducible Cu-Zn SOD genes that play a role in scavenging superoxide radicals generated in plants on exposure to stress. MiRNA159 was seen to be upregulated in response to ABA and this miRNA silenced several MYB transcription factors that are known to positively regulate ABA responses.

Genetic manipulation for drought stress tolerance

Genetic engineering has been used to improve abiotic stress tolerance in economically important crop plants. Engineering for osmoprotectants like proline (Δ-Pyrroline-5-Carboxylate Synthetase, *P5CS*) or glycine betaine (*coda, beta*) (Kolodyazhnaya 2009), ion transporters like cation exchanger (*CAX*), sodium antiporter (*NHX1*) (Zhang et al 2001; Yang et al., 2005), general stress proteins such as heat shock factors HSP, HSF (Lee *et al.,* 1995) and oxidative stress response protein coding genes like *SOD* (Allen 1995) has been carried out to achieve this end. However, transgenics with increased amounts of compatible solutes or antioxidant enzyme levels have not helped in improving dehydration tolerance significantly, possibly because a single gene product could not help in coping with a multifaceted stress like dehydration. Since transcription factors act as master switches in regulating expression of a large number of diverse downstream genes, they have emerged as potential candidate genes for plant transformation (Zhang *et al.,* 2004). Transgenics expressing the transcription factor coding genes, *DREB* (Dubozet *et al.,* 2003; Haake *et al.,* 2002), *MYC*, *MYB* (Abe *et al.,* 2003), *WRKY* (Wang *et al.,* 2007) were reported to show improved stress tolerance. For example Arabidopsis transgenics overexpressing *DREB2A* showed higher tolerance to water deficit stress when compared with wild type plants (Sakuma *et al.,* 2004). Wheat transgenics expressing Arabidopsis *DREB1A* showed a delay in water stress symptoms under green house conditions (Pellegrineschi *et al.,* 2004).

Concluding remarks

A number of advancements have been made in our understanding of how a plant responds to drought stress. Adaptation to drought is seen to involve metabolic and morphological alterations that prevent injury to plants. Underlying these physiological and morphological alterations are molecular mechanisms that regulate the expression of genes involved in the various adaptive processes. Though much is known now about the different type of stress sensors, the secondary signaling molecules involved and entire stress-specific signaling pathways have not been deciphered, largely due to cross-talk between different stress-signaling pathways. For examples drought stress itself is composed of three types of stress factors namely high light, high temperature and dehydration, each of which may trigger different signaling pathways.

Stress-response gene expression is regulated largely by transcription factors, which in turn are subjected to very intricate regulation at the chromatin level, RNA level and protein level. Stress-induced chromatin remodeling may mediate acclimation responses and help a plant to cope better with subsequent stress situations. Micro-RNA mediated gene silencing of stress response TFs under non-stress conditions, and their activation by down regulation of miRNA expression has emerged as another important means of regulating downstream stress response gene expression. Understanding the different layers of regulation that lead to the expression of a stress phenotype is crucial to improving stress tolerance of plants.

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ENHANCING CHICKPEA PRODUCTIVITY UNDER ABIOTIC STRESS CONDITIONS

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Chickpea (*Cicer arietinum* L.) is the third most important pulse crop of the world after dry bean and dry pea and widely cultivated in west and south Asia and north African countries. Pulses are inseparable ingredients of vegetarian diets and one of the cheapest source of dietary protein in Indian subcontinent. India, in spite, of being the largest producer in the world, imports pulses to the tune of 2 million tones every year to meet the domestic requirements. The country contributes 67% of the global chickpea production. The major constraints of low productivity are abiotic stresses like moisture, temperature, nutrients and salinity. The high temperature stress particularly at terminal stage of crop growth is the most important abiotic stress for chickpea productivity. This is because, the planting of chickpea is delayed due to late harvest of major *kharif* crops like rice, cotton, sugarcane and potato. Additionally, drought is also an important constraint for chickpea cultivation. Under resource constraints, rice-chickpea is the more remunerative than rice-wheat cropping system in north western parts of India as it requires less inputs than rice-wheat. Market price of chickpea is about four times higher than the wheat and also maintains the fertility of soil by fixing atmospheric N_2 through root nodules. Survival capacity of chickpea is also very high in comparison to wheat in rainfed condition due to long and deep root system. Under these circumstances continuous growing of wheat has shown disadvantages, viz. depletion of water table due to more requirements of irrigations and nutrients. It is, therefore, considered that chickpea is better substitute in *rabi* season in north western plains. Increase of average temperature in the crop seasons, phenological stages are advance rapidly due to availability of higher thermal units over a short period of time. (Singh *et al.,* 2005)

Chickpea is an evolutionary crop endowed with some of the important morphophysiological traits which enable it to adapt to diverse environmental conditions. These adaptive mechanisms include indeterminate habit, high response to photothermoperiods, deep root system, biological nitrogen fixing ability, phenotypic plasticity, osmotic adjustment and high degree of remobilization efficiency of stored carbon and nitrogen from subtending leaves and stems towards growing sinks. Photosynthetically, chickpea possesses a C_3 mode of carbon fixation. Hence, the yield potential is at par with any other crops such as wheat, rice, *etc.* However, harvest index (HI) is still reasonably low mainly due to partitioning of quite appreciable amount of nitrogen to growing sink which is considered to be more energy requiring physiological process than carbon fixation and its partitioning (Basu and Singh, 2003.)

Distribution, area, production and productivity

In India, chickpea crop ranks in the first position among pulses and occupying about 30% of total cultivated area and contributing about 40% of total production of pulses. More than 85% of chickpea is grown as rainfed crop mostly on the residual soil moisture after the harvesting of *kharif* crops. The crop is preferred under rainfed conditions, because of its in-built capacity of higher yields and greater economic profitability.

Chickpea is grown in over 40 countries representing all the continents over 95% of the area, production and consumption is in the developing countries. The small-seeded desi types, which account for about 85% of world production are grown in the Indian subcontinent, Ethopia, Australia, Mexico, Afghanistan and Iran. In India, 7.05 million tones of chickpea was produced from 8.25 m ha area during 2008-09 with 855 kg/ha average yield indicating all time high production and productivity the chickpea.

Notwithstanding its distribution throughout the country, six states *viz.,* Madhya Pradesh, Rajasthan, Maharashtra, Uttar Pradesh, Karnataka and Andhra Pradesh together contribute 91% of the production and 90% of the area of the country http://www.iipr.res.in 2010. Chickpea is mostly grown on residual soil moisture. The major constraints to production include abiotic stresses eg. drought, temperature and salinity. The protein content of the seed is about 20%. Chickpea leads to poor yields because the crop faces drought and heat stresses. There has been a major shift in the area of chickpea in the country. The expansion of irrigation facilities in northern India has led to replacement of chickpea with wheat and mustard in larger areas. As a result, the chickpea area reduced from 3.2 m ha to 1.0 m ha in northern states, while increased from 2.6 m ha to 4.3 m ha in central and southern states during the past three decades. Thus there has been shift in chickpea area from cooler long duration, highly productive environment to warm, short duration, rainfed and less productive environment.

Phenology

Drought and temperature stresses have direct impact on the phenology of crop plants, which affect the grain quality, yield and yield components. The in-depth knowledge of phenology is essential aspect of crop improvement and management. Most of the modern simulation models of crop production depend on the availability of appropriate data on crop phenology. Phenology indeed is an important component in the adaptation of plants to any given environment. Environmental factors such as temperature, day length and water conditions can substantially modify the phenology to a larger extent under rainfed conditions, drought and temperature prevailing during the course of chickpea ontogeny, reduces duration of phenophases, biomass and seed yield.

Drought and temperature

Leaf injury, arise from a number of factors. One, osmotic effects of salts in the soil solutions causing accelerated senescence due to leaf water deficit or hormonal effects arising from root signals. Second, there could be nutrient imbalance resulting in deficiencies or excess of other ions. Third, there could be toxic effects of salts in the leaves, due to excessive salt build up in cytoplasm or cell wall. In chickpea, biotic and abiotic stresses collectively cause up to 50% yield losses (Dua *et al.,* 2003). Moisture stress is one of the most prevalent environmental stress factor limiting plant growth, survival and productivity. Water stress causes deleterious physiological effects like membrane damage (Deshmukh *et al.,* 2000). Another important abiotic factor is temperature which affects growth of plants in many ways like reductions in root growth, nutrient and water uptake, photosynthesis, respiration and translocation of photosynthates. It also influences the morphology, development and occurrence of phenophases. In late sown chickpea crop experiences low temperature during sowing time and high temperature at the end of the cropping season. Low temperature at initial stage of crop growth results in poor and slow vegetative growth whereas high temperature at the end of cropping season leads to forced maturity and problem of poor biomass (Chaturvedi and Dua, 2003). The effect of terminal drought on pod abortion was investigated in chickpea, which is one of the key determinants of seed yield (Turner *et al.,* 2003). The priority should be given to use of wild types in chickpea breeding programme for improvement of drought and temperature tolerance and for attaining high yield potential (Yadav *et al.,* 2002). In general, *kabuli* chickpea showed greater sensitivity than *desi* chickpea to water stress. Clarke *et al.* (2002) it has been demonstrated that novel approaches can be used in combination with traditional breeding to successfully develop chilling tolerant chickpea. Clements *et al.* (2002) recorded positive correlation of yield with leaf chlorophyll concentration (r= +0.06) Higher Chlorophyll content and lower per cent decrease under stress in tolerant genotype. Biological yield and seed yield had significant positive correlation with primary branches, relative growth rate, net assimilation rate, total chlorophyll content and with relative water content (RWC) and significant negative correlation with membrane injury index (Singh *et al.* 2008).

Low temperature

Chickpea productivity records in the last four decades revealed interesting trend: productivity consistently increased in India and Mexico while it declined in Turkey, Pakistan, and Iran. This is mainly

due to more availability of seeds of high yielding varieties and mild temperature during crop season in most part of the country, especially in northern India leading optimum crop growth. Chickpea being a cool-seasons food legume faces low temperature to the tune of 0-5^{o}C for about 15-20 days in the northern states as the crop is highly sensitive to mean temperatures below 15^{o}C flowering leading to flower drop or pod abortion. The sensitivity of varieties at flowering to chilling temperature below 10^{o}C has adverse effect on chickpea yield (Ali and Kumar, 2005; Sharma *et al.,* 2005). Any advantage derived from early flowering is negated by flower drop and pod abortion/loss due to low temperature. Similarly, crop; sown after harvest of rice faces low temperature i.e. cold stress at emergence/vegetative stage leading to less biomass production. The breeding varieties possessing cold/low temperature tolerance will certainly help in enhancing chickpea production and productivity in northern India.

Salinity

Salinity (soil or water) is one of the major, limiting crop productivity under arid and semi arid regions. The world land surface area is about 13.2 x 10^9 ha, of which only 1.5 x 10^9 ha is available for cultivation and 0.34 x 10^9 ha is saline and 0.56 X 10^9 ha is sodic soils. In India about 8.6 million hectares of land is salt affected. Salinity stress retards plant growth and development. Soil salinity also affects plant morphology, anatomy and physiology in general which ultimately reduces productivity.

Heat shock proteins

High surface temperatures are common to soils during periods of drought. Seedlings frequently experience high temperature during emergence and establishment in many regions of the world, which lead to reduction in yield. When plants are exposed to excess heat, a characteristic set of cellular and metabolic response is triggered. The heat shock is characterized by a transient expression of heat shock proteins (HSPs).The expressions of HSPs positively correlate with the acquisition of thermotolerance and the over expression of HSPs often results in enhanced thermotolerence. It is well established that plants can respond defensively to heat-stress. A preliminary treatment with a moderately elevated, non-lethal temperature can temporarily render plants more resistant to a subsequent potentially lethal heat shock, this phenomenon is known as heat acclimation. Thermotolerance can be categorized as either inherent or acquired. Inherent thermotolerance relates to the ability of an organism to withstand, up to a certain degree, a rapid change in temperature away from the optimum. Acquired thermotolerance means the level of protection beyond the inherent thermotolerance that results from prior exposure to elevated, non-lethal temperatures or tolerance induced by many other putative signaling components like salicylic acid (SA), abscisic acid (ABA), $CaCl_2$, H_2O_2, ethylene, etc. In order to limit oxidative damage under stress conditions, plants have developed a series of detoxification systems which include enzymes like peroxidase, ascorbate peroxidase, catalase, superoxide dismutase, etc. (Chakraborty and Tongden, 2005)

Molecular markers

In fact, the estimated collective yield losses due to abiotic stresses (6.4 million t) have been significantly higher than for biotic stress of chickpea, causes a 40-50% reduction in yield globally. The change from spring to winter sowing of chickpea for efficient utilization of rain water in Mediterranean environments has enhanced yield, but demands tolerance to low temperature for further yield improvements. Most legumes are known to be salt sensitive. Therefore, it is becoming increasingly important to produce cultivars tolerant to high salinity in addition to other abiotic and biotic stresses for sustainable chickpea production. The cultivated chickpea has high morphological but narrow genetic variation, which makes it difficult for breeders to produce elite cultivars with durable resistance to the many major biotic and abiotic stresses. Molecular markers associated with quantitative trait loci (QTL) for resistance to biotic stresses and some morphological traits have been located on both interspecific and intraspecific linkage maps. Quantitating the effects of abiotic stresses involves measurement of various factors like survival rate, yield, dry matter production, days to maturity, flower/pod survival, root mass and transpiration ration. Their tolerances are likely to be

quantitatively controlled and this feature of abiotic stresses represents a major obstacle to developing molecular markers. (Mantri *et al.,* 2007)

Factors affecting yield components

Boimass or above ground dry matter and harvest index are the well established dominating components influencing grain yield of chickpea in addition to other growth parameters. Therefore, maximum yield variability usually observed among genotypes across the environments can be explained on the basis of biomass accumulation and partitioning. Considerable amount of work indicated four major factors of yield variation. These are the yield barriers which can be broadly grouped as (i) agronomical, (ii) phenological, (iii) morphological, and (iv) physiological.

Agronomical: Availability of conserved soil moisture, soil fertility status, time of planting, germination, seedling vigour, crop establishment, plant population, irrigation scheduling and environmental factors.

Phenological: Crop phenology such as initiation of flower and pods, duration of vegetative, reproductive phases and time of physiological maturity.

Morphological: Number of branches, pods, seeds, seed size, leaf number, leaf area, biomass, etc.

Physiological: Nodule formation, biological nitrogen fixation, rate of photosynthesis and photorespiration, root vigour, remobilization of carbon and nitrogen, probability of pod setting, pod filling rate, harvest index, sink activity, dry matter accumulation rate, water and transpiration use efficiency, leaf area development and light interception.

Primary causes for low yield potential

The main causes for low yield potential are as follows:

1. Cultivation on marginal to sub-marginal soils.
2. Poor preparatory tillage and negligence in interculturing.
3. Non-specific management of pests.
4. Chickpea and lentil are confined to drought-prone area, which have to grow on residual soil moisture, which is mostly insufficient for overall growth of the crop.
5. Inadequate sowing time.
6. Poor weed management.
7. Faulty irrigation.
8. Improper seed rate.
9. Unavailability of quality seed of modern varieties at sowing time.

Above situations need to be tackled on four fronts viz. (i) cultivar, (ii) critical input development, (iii) effective plant protection measures, and (iv) seed production.

To overcome abiotic stress following points need to be considered,

1. Evolving chickpea varieties for rainfed, irrigated and Late-sown conditions with resistance to drought and temperature.
2. Evolving chickpea varieties responsive to irrigation and application of chemical fertilizers and growth regulators.
3. Evolving high-yielding and short-duration varieties.
4. Encouraging seed village for rapid seed replacement.
5. Production of high quality nucleus, breeder, foundation, certified and truthful seed of latest varieties.

Conclusion

- Increase of average temperature in the crop seasons, phenological stages are advance rapidly due to availability of higher thermal units over a short period of time.
- The concept of ideal plant type takes into account an wholistic approach towards traits which positively or negatively influence yield for a given agro-climatic zone.
- Drought and temperature stresses have direct impact on the phenology of crop plants, which

affect the grain quality, yield and yield components.

- Early maturing genotypes can be used in crop breeding programme for improving yield potential of chickpea under resource limited conditions.
- The expressions of HSPs positively correlate with the acquisition of thermotolerance and the over expression of HSPs often results in enhanced thermotolerence.
- The breeding varieties possessing cold/low temperature tolerance will certainly help in enhancing chickpea production and productivity in northern India.
- Biological yield and seed yield had significant positive correlation with primary branches, relative growth rate, net assimilation rate, total chlorophyll content and with relative water content (RWC).
- The priority should be given to use of wild types in chickpea breeding programme for improvement of drought and temperature tolerance and for attaining high yield potential.
- Under resource constraints, rice-chickpea is the more remunerative than rice-wheat cropping system in north western parts of India as it requires less inputs than rice-wheat.

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PHYSIOLOGICAL BASIS OF PLANT PRODUCTIVITY

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Growth and productivity encompasses several of the spheres including agriculture. Productivity is related to the measure of the efficiency of production and is often expressed as the ratio between availability i.e. what is produced and what is required. In simpler term, per a given unit, total out put to input ratio is termed as productivity. Industrial and economic productivities are often treated as quantifiable entity, though qualitative parameters are integrally existed and can not be excluded in measuring the productivity. For instance, energy and time factor as every one of us know are determining factors of measurement of industrial and economic progress of nation. In a similar context, agricultural productivity of world is often measured based on three important factors, viz., area, production and yield. Food grain production enhanced from 50.8 to 218 million tones from early 1950's to 2010 a significant increase compared to that of increase in area in the same period. (97.3 to 121.3 million hectares). The daunting challenges faced by Indian Agriculture during the critical times of food deficit and reaching to surplus stage should be considered as an amazing feat as the nation stands united. Massive public investments, in irrigation, agricultural research, extension rural infrastructure farm credit are undergoing rapid changes in view of liberalized economy and new policy reforms.

India's agricultural productivity is mainly dependent on two most important cereal food crops, wheat and rice. Rice is considered as one of the unique crop with reference to its growth, development and yield potential as it can be grown under diverse ecosystems from low lying to upland, submergence, hilly regions etc., Therefore constraints for each system too varies and examination of this crop might be relevant to assess a true scenario of country's productivity. The total area of rice (44 million ha) under various ecosystems are 58% irrigated, 25% rain fed shallow, 12% rain fed upland and 5% deep and semi-deep water. The production level from the corresponding ecologies are 76%, 19%, 4% and 1% (productivity range 3.13 to 0.5 ton/ha) respectively. Apart from ecological factors, frequent floods, droughts, salinity are some of the reasons for low productivity. With this kind of low productivity levels at the backdrop for a crop like rice which relatively draws more investment for rain fed and horticultural crops the producvitywould be still lower. With increasing population levels and projected climate changesthe estimated demand for rice crop alone is 116 Mt by 2030. In recent times, several high yielding varieties, and hybrids are released for major crops whereas the yield levels appear to reach a plateau and there is no further improvement in productivity. Will it be possible to enhance crop yields further, if so, how to achieve this? Nevertheless, a brief attempt is made to identify and high light the physiological processes that are being used in plant development and identify researchable gaps where our understanding and rationale needs new ideas that might be playing significant role in increasing (!) crop productivity.

Approaches for understanding crop productivity can be broadly divided in to three areas. These are 1) **Physical Inputs**: Water, soil, seed, optimum time for sowing, matching soil, type of soil, when, where, how and what cultivar, how much fertilizer dose, plant protection measures etc., 2) **physiological process and their genetic control** and 3) **Environmental interactions**. Among these, the later two will be of significance which is "**physiological basis of productivity**" where in plant physiologists along with others will have take leads in achieving the nation's food sustainability. Delineating the principles of physiology with that of environment is rather a difficult task as productivity is an interaction between genetics and environment under which the crop is subjected to examination.

Physiological processes: Yield is complex trait and is governed by several genes. A rich gene pool for several of the crop plants has not been utilized for the

purposeful and efficient enhancement of physiological processes that limit crop productivity. Among these, increasing photosynthetic efficiency in relation to crop productivity is major target for enhancing food grain production particularly wheat, rice, pulses and oil seed crops. The high productivity of cultivated plants though with low photosynthetic rates was mainly due to leaf area increase, changes in ratio of biomass or reproductive to vegetative organs and by other morphological properties. The early work of improving crop productivity was based on mass selection by conventional breeding programs, with traits of agronomic value with increasing sink size such as spikelets, tuber size, fruit size, etc., was achieved. The photosynthesis rate of these varieties did not show appreciable increase as such. Examination of the current varieties, particularly hybrids would reveal that, an imbalance between the accumulation and partition of assimilates and negative feed back mechanism resulted in decrease in the activity of photosynthesis

Optimization of canopy structure for efficient utilization of solar energy is one strategy for increasing production. A 3-5% increase in Radiation Use Efficiency could enhance maize yield up to15-20 tons wheat grain yield up to 10-12 tons and cotton yield 8-10 tons per hectare. Crop yield is the result of complex diverse processes and reactions occurring at ontogenesis under the influence of external conditions. Optimization of canopy structure, improving photosynthetic process it self, rational partition and usage of assimilates are important areas where considerable progress was made. However, in cereals grain filling is largely dependent on current photosynthates and to a lesser extent on remobilization of pre-anthesis stored assimilates in the stems and leaf sheaths under normal conditions. RUBP oxygenase in phylo-genetic studies of blue green algae to ginkgo, revealed that large sub units comprising three polypeptides and smaller contain 1-4 polypeptides indicating they may serve as genetic markers for nuclear and cytoplasmic interactions. Use of wild relatives to develop photosynthetically efficient genotypes in the breeding research programs needs scaling up. The area of genetic control is not yet exploited despite the inference of photosynthesis in relation to crop productivity has been well established (Table 1) . On the other hand, emphasis is being laid on responses of C_3 and C_4 crops in relation to photosynthesis, adaptation and yield. It is proposed that, converting C_3 to C_4 pathways will result in radical increase inthe crop productivity based on the observations of relationships on biomass and yield in C_4 crops compared to that of C_3 species. However, few of the elegant experiments of hybridizing Atriplex, using C_3 and its intermediates proved otherwise. In fact, the photosynthetic rates were reduced. Reports on genetic transfer of PEP carboxylase are not consistent in relation to enhanced efficiency. Classical mutation experiments conducted using gamma irradiation resulted in obtaining mutants that have PEP pi dikanase activity and regeneration capacity without

Table 1. Photorespiration potential of leaf tissue in different leaf positions in *Nicotiana tobaccum sp* *(Source: Junk D 1979)*

Leaf number from top	Compensation point	RUBPC activity activity	RUBP Oxygenease (sec.cm^{-1})	Mesophyll resistance	RUBPC/RUBPO (phot. resp.pot)
1	46.2	0.422	0.231	94.1	1.8
2	47.4	0.851	0.638	20.5	1.4
3	38.7	1.394	0.369	16.6	3.8
4	24.3	1.590	0.363	19.6	4.4
5	18.7	1.133	0.255	15.3	4.4
6	19.9	1.340	0.250	18.1	5.2
7	19.2	2.165	0.405	20.5	5.3

much influence on yield. However, some of these mutants were found to have better water use efficiency. Hence, possibilities of enhancing through water productivity and help in establishing the tolerant genotypes can be expected.

Photorespiration is another important physiological process which is poorly understood. Though, it is considered to be detrimental as 50% net assimilates are lost in C_3 plants to that of C_4 crops. However, the essentiality and significance of photorespiration in C_3 plants is under estimated. In course of evolution, C_4 plants reduced photorespiration where in the autocatalytic production of free radicals is genetically regulated by activation of the antioxidant enzymes. Autocatalytic production and input especially nitrogen use efficiency are therefore, lower in C_3 plants compared to C_4 plants. Some of the wild species in spite of being C_3 were found to have lower photorespiration rates. In glycine oxidase 96 SH groups available and 12 were found to be accessible. Changing the protein structure by genetic means especially substrate acceptance at polypeptide binding site which results in formation of 2 to 3 D structure needs to be thoroughly understood.

Environmental factors: The physiological processes controlling the sequences of events starting from germination to harvest may be measured in terms of morphological attributes such as length, area, dry mass or of integrals of an environmental factor such as solar radiation intercepted and transpiration. For an organ to grow (duration or time) be changed to development (formation of a new structure) duration and quality are considered as discrete periods which are either determinate or indeterminate and depend on the base temperatures and photoperiods. In fact this is the basis of photothermic indexing of plants (Table 2) and strongly interacts with genetic background of the plants. Importance of these environmental signals in relation to the duration, responses and the extent of present understanding are mentioned here.

Principles; Responses of developmental rate (1/t) to temperature and photoperiod

Development can be expressed in terms of integral such as thermal time and photoperiod time Developmental responses of a genotype can be described in terms of smaller attributes such as base unit comparison

Responses can be defined from measurements in only a few of combinations of temperature and photoperiod.

Limitation: Between base and optimum temperature models developed might be invalid. (extremes or above optimum as happens in field).

Amongst the environmental factors, temperature responses are fairly understood well which is relatively stable in lab/controlled environments and also in field. However, it is not true in case of drought and nutrients which can be very different in stands. The control of duration is far better understood than the control of rate. Though both duration and rate are governed equally by temperature, durations are much less sensitive than rates to other factors, particularly to drought, nutrient shortage and soil physical properties (Table 3). Much is known the way water and nutrients affect cellular processes, the knowledge has to be

Table 2. Cumulative degree days (CDD) and cumulative nyctoperiods (CNP) from 2007-2010 on rice under AICRIP trials (Mean of 13 locations)

Stage	CDD				CNP			
	2007	2008	2009	2010	2007	2008	2009	2010
PI	807	864	950	889	699	743	853	782
Flowering	1150	1205	1255	1245	950	1022	1020	1065
Maturity	1437	1576	1700	1612	1261	1350	1488	1364
Yield ($g.m^2$)	475	494	462	563				

applied systematically to show how leaf area, fractional interception and conversion ratio are variably controlled in different species. Main problem is relating an effect or response to an index within the plant, when the physiological systems are reacting to conserve the waste and nutrient status of the plant tissue. Some species reduce fractional interception while others conversion ratio and is related to the general growth habit. Determinate species reduce both equally well while indeterminate sps. reduce conversion ratio. The method of analyzing dry matter conversion ratio vapour pressure deficit is more conservative.

Table 3. Pearlmillet growth and developmental stages as influenced by temperature integrals (Source: Squire 1990)

Pearlmillet developmental stage	Thermal duration (base temp 10 ^{0}C)
Between successive leaves and roots	26
Sowing to first tiller	200
Between successive tillers	80
Expansion of tiller leaves	270
Increase in mass of tillers	420
Between successive spikelets	0.23
For all spikelets	190
Floral initiation to anthesis	460
Expansion and growth of main stem	360
Increase in weight of panicle	550
Increase in weight of grain	290

Disadvantage : Technically more demanding than intercepted radiation alone i.e conversion ratio and fractional interception. When there is much water in the upper layers of soil, it is unclear how dry air restricts transpiration by reducing leaf conductance and area: and when a root system is forced to extend into lower layer of soil to obtain water, it is unclear what governs the rates of extension of the various rooting elements, and the inflow of water per unit root length, and how drought and nutrients affect the DM to transpired water ratio. These uncertainties in the physiology prevent a thorough analysis of productivity in that part of the tropics where yields are smallest and most unreliable. The control of period for which structures fill is better understood than the control of their rate of filling, as determined by partition factor. Partition factor is largely independent of plant mass and dry matter production, is inherently larger for some structures than others and is restricted for some in extreme environments. Partition factor in relation to temperature is better understood followed by photoperiod which has relatively large effects of drought are largest and least understood very poor nutrient and may be smaller. Genotypic differences in rates of sink production during drought, and mobilized metabolites from dry matter are poorly understood.

The physiological principles governing the yield are most useful in predicting the success of crop in a range of environments. Though, precision has not yet achieved, the physiological nature of genotype can be reasonable described in terms of small number of attributes that govern development, expansion, production and partition. Most of these attributes can be measured in field and are conservative, in that, they change little in response to environmental and cultural factors. Examples are the base temperature and thermal duration, the conversion ratio for solar radiation the product of WUE and VPD and the partition factor.

Some of these attributes in a few specified environments for most of the main species and in a wide range of environments for a few species is available. This invaluable information for comparing genotypes should be useful for estimating productivity on a regional scale. This is because agricultural production is governed by variations in land and soil properties, weather, water and other natural resources. All of these resources vary not only in time but also spatially leading to variability in decisions and impacts. Spatial variability of the resources therefore plays an important role in crop productivity. Geographical Information System (GIS) tools provide an effective means to represent and analyze spatially variable data. Crop productivity can be effectively estimated either with single date satellite image obtained during vegetative stage when canopies are fully developed or with GIS layers integrating with crop models. An opportunity of enhancing the crop productivity is through bridging the gap between the potential yield and the actual yields obtained. This estimated yield gap varies

from 20-30% in irrigated and greater than 50% in upland situations. Also, resource conservation technologies improved soil health management practices needs cohesion and co-ordination at macro scales. Reduction in post harvest losses is another key area which has not been dealt here.

Genetic improvement, availability of genome sequences in public domain, better management practices resulting in optimum utilization of natural resources, forging public- private, NGO partnerships, proactive roles of governmental sector, remote sensing, complex simulation models in prediction of natural disasters, studies on pest and disease dynamics, technology transfer and changing socioeconomic scenarios would greatly help in bridging the wide gap between the potential and the realized productivity. The responses of which genotypes are capable are far better understood than the responses they are most likely to take when growing in the field. Coupling experiments in controlled chambers which fluctuate as in natural environment, in which plants have unrestricted root systems, with experiments in the field, and exploiting the effects of population density and altitude is the need of the hour.

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CROP SIMULATION MODELS IN AGRICULTURAL RESEARCH AND MANAGEMENT

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Introduction

A model is a simplified representation of a complex system. Modelling of a crop has been done using approaches such as descriptive modelling, which is simple, or by explanatory modelling, which quantitatively describes the mechanisms and processes that cause the behaviour of a system. Crop growth simulation models, falling in the latter category, are based on quantitative understanding of the underlying processes, and integrate the effect of soil, weather, crop, and pest and management factor on growth and yield. The process could be crop physiological, meteorological, and soil physical, chemical or biological. Depending upon the objective, knowledge base of various agricultural disciplines can be integrated in a crop model. For instance simulating the crop-weather interaction forms the production level 1; while simulating growth rates determined by the availability of water apart from weather of a location gives production level 2. Inclusion of availability of factors such as nitrogen, other nutrients for crop growth provides production level 3…n. Addition of pests, diseases, weeds, etc. in simulating the crop growth and yield will further provide production levels more nearer to reality.

For simulating, the models need input data that mimic 'genetics' of a crop/variety. Further, the response of variety to water, nutrient, pest limited or actual productivity, knowledge base of several additional disciplines are tapped and integrated into the model. Once the integration, calibration and validation is successful, crop simulation models can help us in analyzing the effect of various climatic factors on crop growth and yield considering the interaction with edaphic, biotic and agronomic factors. Such an analysis is normally not possible with conventional experimental methods. There have been over 120 crop models or compendium of models available across the world which can simulate 151 crops which include filed crops, horticultural crops, plantations, grasses, etc.

In recent years, agricultural system models have shifted from being mainly research oriented to tools for guiding resource management and policy-making. The linkage of these models to geographic information systems (GIS) and decision support systems has added dimensions to model applications. Agricultural system model have gone through more than 40 years of development and evolution. Prior to the mid-1980's most of the modeling work focused on individual processes of agricultural systems, such as soil hydraulic properties, evapotranspiration, photosynthesis, plant growth and soil nutrients. The earlier models have served as a foundation for the development of agriculture system models in the last 20 years. Earlier examples of systems models have focused, for example, PAPRAN for pasture systems, CREAMS for soil, chemical and nutrient run off from cropping system, EPIC for soil erosion and soil productivity, CERES for crop growth, GLEAMS for ground water pollution, AquaCrop and CRPWAT for crop water requirement analysis and CENTURY for plant production, nutrient cycling and soil organic matter dynamics. Physiological growth and production models have shown to be very useful for guiding improvements in cropping systems of various annual crops. There have been several crop models and decision support systems available (Table 1). Examples include DSSAT, InfoCrop, EPIC, APSIM, CROPSYST, etc.

Calibration and validation of simulation models

Although the simulation models are flexible enough to perform under a variety of environments and farming conditions, calibration of model is necessary before running the model for study area. For this results from the detailed experiments on varietal performance can be made use of. The calibrated model can be used to

Table 1. Representative models under different categories.

Multiple crop models/decision support systems	Hydrological/irrigation/water requirement assessment models	Crop specific models	Other models
CropSyst	Soil Water Assessment Tool (SWAT)	Oryza	Agrodiversity
DSSAT	Cropwat	ORYZA2000	DNDC
ECOCROP	DRAINMOD, DRAINMOD-NII	Broom's Barn Sugar Beet Growth Model	AgPasture
STICS	Aquacrop	Potato Calculator	Animal Model
APES	SWAT	WheatGrow	
EPIC	SWB Irrigation model	GOSSIM	
APSIM	Tropical Soil Quality Model		
GLAM	WOFOST		
INFOCROP			

simulate the crop growth performance in other set of experiments consisting of various treatments for validating the model performance under a range of conditions. This validated model can used for simulating impacts in that region. Model performance can be assessed through various statistical parameters viz., model bias error (MBE), root mean square error (RMSE), index of agreement (IA) and model efficiency (ME) among others.

Application of Crop Simulation models

Crop models are increasingly being used for environmental characterization and agro-ecological zoning, defining research priorities, technology transfer, estimating potential production, strategic and anticipatory decision making. In past 20 years crop simulation models are increasingly used for projecting the effects of climate change and climate variability. Recently, they have been used for quantifying the adaptation gains in climate change scenarios for prioritizing technology dissemination and also for identification of vulnerable areas.

In a recently conducted global survey on use of crop simulation models (Rivington and Koo, 2010), it was found that the major purpose of the models are seen to be for decision support, analysis of climate change impacts and/ or adaptation, prediction or forecasting of productivity / yield and research for crop management improvement.

Crop simulation models are effective tools for the assessment of growth and yield of crops as well to suggest optimal resource management options (Kalra and Aggarwal, 1994; desired cultivar characteristics (Aggarwal *et al.,* 1997) performance evaluation of weather forecasters (Kalra and Aggarwal, 1996; Singh *et al.,* 1997). Apart from these, crop simulation models are now being seriously investigated as creditable tool for regional yield prediction (Nain *et al.,* 2002) and integration of crop simulation model with remote sensing data for farm level wheat yield prediction (Nain *et al.,* 2001).

Use of crop models in climate change studies

Analysis of impact of climate change on crop growth and yield can be carried out for individual and interaction effects of elevated temperature, rainfall, CO_2, etc. But these studies indicate the individual and interactional influence of various parameters irrespective of temporal scale. However, by using the climate scenarios, either derived from Global Climate

Table 2. Indicative use of models for several purposes and end uses

Purpose	Best used for
Decision support	Guiding current management options
Climate change impacts and / or adaptation	Climate change adaptation and mitigation guidance
Productivity / yield prediction or forecasting	Yield or productivity forecasting
Research for crop management improvement	Inter disciplinary research
Research for crop genetic improvement	Better understanding of processes,Improved crop breeding
Education / training	Improving training and education
Operations optimization	Policy development
Yield gap analysis development	Improving agricultural management/ R&D policy

Models (GCM) or from Regional Climate Models (RCMs), as inputs into the crop models, quantification of impacts on economic yields can be carried out for future climates. The adaptation analysis can be done by quantifying the response of different varieties, sowing time, nutrient management, water management, introduction of new crops, shift in cropping sequences, altered resource management and introduction of new technologies, etc. in various climate change scenarios so as to derive the best suitable technology package for reducing impacts of climate change at regional level then up-scaling to state and national level. These are called adaptation gains. The net different between impacts and adaptation gains is called net vulnerability of crop/system to climate change. Using the above approach, several studies have been conducted for quantifying the potential yields impacts, adaptation and vulnerability of coconut (Naresh Kumar *et al.,* 2008; Naresh Kumar and Aggarwal, 2009), maize (Byjesh *et al.,* 2010), sorghum (Srivastava *et al.,* 2010) and also sensitivity of fragile ecosystems (Naresh Kumar *et al.,* 2011).

Future thrust

- Model integration by linking crop models with climate, hydrological and , economic models and also integration with remote sensing and GIS
- Fine tune models based on updated thresholds of factors influencing major processes
- Fine tune/modify the models to best represent multiple stress impacts on crops in a season
- Understand the uncertainty among crop models

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PLANT MINERAL NUTRITION ESPECIALLY NITROGEN, PHOSPHORUS AND THEIR INTERACTION UNDER CHANGING CLIMATIC AND SOIL CONDITION

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Introduction

Climate change associated with increased greenhouse gas emissions is expected to cause an increase in global mean temperature. Increase in atmospheric CO_2 concentration from 280 ppm in 1750 to 380 ppm with a corresponding increase of 0.6°C in global annual surface temperature has already been recorded (Houghton *et al*. 2001). These changes in climate influences the key soil processes affecting availability of nutrients such as nitrogen (N), carbon (C), phosphorus (P), sulfur (S) and other elements, causing adverse impacts on biomass, productivity, biodiversity and environment. If air temperatures rise along with CO_2 concentration, the water requirement is also likely to increase in comparison to present-day conditions. The major concern in this context would be the below ground nutrient transformations driven by the existing flora and fauna in the ecosystem that support global primary production. Nutrient availability under changing climatic and soil condition would in turn influence the nutrient use efficiency of crops. The major concern is that the yield advantage driven by enhanced nutrient use efficiency of crops in elevated atmospheric CO_2 condition might be offset by enhanced photorespiration under elevated temperature condition.

It has been predicted that increasing atmospheric CO_2 concentration will increase the rates of carbohydrate synthesis and biomass of plants within terrestrial ecosystems primarily through stimulation of net photosynthesis and inhibition of photorespiration (Bowes, 1993). But under long-term, the increased growth rates of CO_2 enriched terrestrial plants are bound to create an enhanced demand for mineral nutrients making nutrient availability particularly important. Plant growth rates under moderate nutrient insufficiency are usually enhanced by atmospheric CO_2 enrichment, but not to the same extent as under nutrient sufficient conditions because these plants utilize nutrients more efficiently under elevated rather than ambient CO_2 (Stitt and Krapp, 1999).

Soil nutrient availability under changing climate

It has been reported that increased C inputs under elevated CO_2 stimulate growth of soil microbial biomass, thereby increasing rates of N mineralization (Zak *et al.,* 1993). On the contrary, Diaz *et al.* (1993) found that increased C inputs under elevated CO_2 stimulated competition between the soil microbial biomass and plants for soil N, leading to a decline in soil N availability. If sufficient N is available, enhanced plant growth and soil C input under elevated CO_2 are likely to be sustained, resulting in net soil C sequestration. Elevated atmospheric CO_2 also has a positive effect on soil P availability rather than leading to depletion (Khan *et al.* 2008). However, in another report, Barron-Gafford *et al.* (2005) found that elevated CO_2 accelerated depletion of soil nutrients like P, Ca and K after 3 years of plant growth, litter removal, and coppicing, especially in the upper soil profile, but total N showed no change. Under high levels of CO_2 and temperature an increase in demand for P was noted which resulted in 27% increase in fertilizer P use efficiency and greater mobilization of organic P (Kumar 2009).

Elevated CO_2 and nitrogen nutrition of plants

Nitrogen is one of the most important macronutrients required by plants for growth. Nitrate (NO_3^-), the major inorganic N form in aerobic agricultural soils is the preferred N form taken up by plants and also act as a signal molecule. The response of nitrate and ammonium uptake to elevated CO_2 may depend on the nitrogen concentration supplied. BassiriRad *et al.* (1997) found that root NO_3^-

absorption rate doubled or halved in response to doubling of atmospheric CO_2 concentration depending on the plant species. Plant N is primarily supplied by available soil NO_3^- and NH_4^+ ions, but the relative preference for each form may largely depend on ability of roots for absorption, assimilation and translocation. Elevated CO_2 causes a decrease in total N as well as nitrate concentration in plant tissues. There are reports that total nitrogen as percentage of dry weight was low under elevated CO_2 and that CO_2 enrichment decreases critical nitrate and nitrogen concentrations in wheat (Hocking and Meyer, 1991). This indicates that nitrate uptake and assimilation often fail to keep pace with photosynthesis and growth under elevated CO_2.

Root uptake and assimilation of N are energy demanding processes with carbohydrates derived from photosynthesis providing the required energy (ATP) and reductants (NADH, NADPH). Because CO_2 enrichment increases the availability of root respiratory substrates, metabolically regulated processes such as active nutrient uptake might be stimulated under high CO_2. Adjustment in uptake kinetics is only one of the potential root level mechanisms that could enable plants to meet increase in shoot demand. Kinetic parameters (V_{max} and K_m) may have a (weak or strong) regulatory role in plant nutrient budgets in response to elevated CO_2. Our work on kinetics in wheat revealed that the pattern of nitrate uptake was similar under both ambient and elevated CO_2 but rate of uptake was significantly higher in plants grown under elevated CO_2 when concentration of nitrate in the external medium was low. The V_{max} for high affinity nitrate transport system was significantly high for un-induced seedlings (116.36 ìmol g^{-1} Fw h^{-1}) grown under elevated CO_2 compared to those grown under ambient CO_2 (79.55 ìmol g^{-1} Fw h^{-1}). However, with increased nitrate concentration in the media, nitrate uptake followed linear kinetics at both CO_2 levels with V_{max} and K_m of 163.93 µmol/g FW/h and 20.16 mM under ambient CO_2, respectively and 227.27 µmol/g FW/h and 20.16 mM under elevated CO_2. Seedlings induced with nitrate had higher N uptake rate at all concentrations of nitrate irrespective of CO_2 levels (Lekshmy *et al*, 2009). The pattern of gene expression of nitrate transporters in roots was correlated with kinetics and uptake of nitrate. It is possible that the high affinity nitrate transport system may operate more efficiently under elevated CO_2 as indicated by high V_{max}, so plants will be able to take up nitrate more efficiently from soils low in nitrate and may have better N utilization efficiency.

Elevated CO_2 may affect whole network of genes that regulate nitrate uptake and assimilation. There may be changes in expression of genes required for uptake leading to changes in the activities of corresponding proteins. Elevated CO_2 also induces changes in nitrate reductase (NR) activity, the first enzyme involved in conversion of nitrate to nitrite. So, it is important to study how NR activity is affected under elevated CO_2, particularly when the plant receives varied forms of nitrogen nutrition. Lekshmy *et al* (2006) showed higher NR activity in N starved wheat seedlings exposed to high or low nitrate concentrations at elevated CO_2 in comparison to ambient CO_2. This suggests that higher rate of nitrate assimilation will presumably be required in elevated CO_2 to support higher rates of plant growth. Further, an increase in *NIA* gene transcript levels was found in wheat seedlings incubated with low concentrations (0.01 mM) of nitrate in the external media under elevated CO_2 compared to those incubated with high nitrate concentration. The CO_2 mediated increase in NR activity could be due to higher levels of sugar synthesis under such conditions.

Elevated CO_2 and phosphorus nutrition of plants

Phosphorus is a vital macronutrient for plant growth and an integral component in the formation of high-energy bonds, structure of certain biomolecules and membranes, several metabolic reactions and signal transduction pathways. The uptake and utilization efficiency of P under CO_2 enrichment in wheat showed that shoot P concentration increased by 21.2% at elevated CO_2 compared to ambient CO_2 when grown with sufficient P in the media. This resulted in 70% increase in total plant P uptake under elevated CO_2 in comparison to ambient CO_2. But the P utilization efficiency increased sharply (6-fold) in plants grown with low P as compared to sufficient P under elevated CO_2. Also, there was 57.5% increase in P utilization efficiency in plants at elevated CO_2 over ambient levels when grown with low P (Dubey 2010). This indicates

that at elevated CO_2, increase in P utilization efficiency is associated with enhanced dry matter production rather than whole plant P concentrations. On the contrary, Israel *et al.* (1990) reported that P uptake efficiency was not influenced by CO_2 enrichment in soybean. Although total P uptake in the plants were found higher with elevated CO_2 (700 µl L^{-1}), the P uptake efficiency decreased to 28% under P stress compared to control. This decrease in uptake efficiency was associated with a decreased root growth under P stress.

The kinetics of P uptake was also affected by elevated CO_2. In phosphate uninduced wheat seedlings, V_{max} increased significantly from 18.1 at ambient CO_2 to 21.5 µmol P/g root FW/h at elevated CO_2, the increase in rate of P uptake being 14.1% with a corresponding decrease in K_m value by 34.7%. This suggests that with decrease in K_m value the maximum rate of Pi influx increases which may be due to induction of high affinity P transporters. The response of high affinity phosphate transporter to elevated CO_2 in wheat under low P concentration reveals increase in high affinity phosphate transporter transcripts in the nitial 20 min of incubation under elevated CO_2 compared to ambient CO_2. But at higher P concentration, the level of expression of high affinity phosphate transporter was reduced. This result indicated that CO_2 has a regulatory role in expression of high affinity phosphate transporter gene. These finding suggests that wheat plants would be responsive to increasing atmospheric CO_2 level, by increasing the uptake of nutrients under low N and P conditions, thereby improving their nutrient acquisition efficiency.

Effect of elevated CO_2 on N & P interaction

Not much work has been conducted on the interactive effects of N and P on plant growth and nutrient uptake under elevated CO_2. The nutrient utilization efficiency of soybean plants grown with elevated CO_2 and different P levels showed that P utilization efficiency increased to 159% while N utilization efficiency decreased to 62% over control when the plants were subjected to P stress (Israel *et al*. 1990). The positive effect of P stress on P utilization efficiency suggests that the P concentration in control nutrient solution was in excess of that required for optimal P utilization efficiency. Further, at any given concentration of N and P, CO_2 enrichment resulted in increased N and P utilization efficiencies, the increase being associated with enhanced dry matter production at elevated CO_2 rather than whole plant N and P concentrations.

A study conducted in FACE (free air CO_2 enrichment) on the interactive effect of CO_2 and N supply on seasonal changes in P uptake and utilization efficiency in rice revealed that elevated CO_2 significantly increased shoot P concentration over the season but the P uptake responses to CO_2 declined gradually with crop development (Yang *et al.,* 2007). However, FACE resulted in significant decrease in P utilization efficiency with regard to biomass across the season, and grain yield and P harvest index at grain maturity. Besides the influence of elevated CO_2 on nutrient uptake and utilization by plants, the availability of nutrients in soil was also affected. In rice-wheat rotation system under FACE, the availability of soil P decreased in rice at initial growth stages but increased after heading till ripening stage whereas it was unaffected in wheat crop (Ma *et al*., 2007). This indicates that under elevated CO_2, the application of phosphatic fertilizer should be adjusted according to the requirement of specific developmental stages and also based on the crop.

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ELECTROMAGNETIC ENERGIES FOR SEED ENHANCEMENT IN AGRICULTURAL CROPS

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Crop yields can be maximized by establishment of an adequate and uniform plant population for which good quality seed is a pre requisite. The gains from agronomic inputs are drastically reduced if the seed is of poor quality resulting in a poor stand. Pre sowing seed treatment both chemical and physical are known to enhance germination by breaking dormancy, helping in uniform establishment of crop stand in the field and protecting the seed against pests and diseases. Pre sowing seed treatments like priming, pelleting, coating and artificial seeds have been successfully used for improving yields (Harris *et al*. 1999). Any kind of priming causes an effective invigoration in the dry seed which is an inception of metabolic processes that otherwise occurs during imbibition which is subsequently fixed by drying the seed. Most priming treatments involve imbibing seed with restricted amounts of water to allow sufficient hydration and advance of metabolic processes but preventing the protrusion of the radicle. All priming treatments improve germination at the cost of storage of the seed as seed is hydrated during the treatment.

Magnetic and **electromagnetic** energies are being used in agriculture for seed priming as a non invasive technique to improve the germination, vigour of seeds and finally yield. The results obtained by '**magnetopriming'** indicate that magnetic field acts as a **bio- stimulant** which can be considered as an alternative to chemical, physical and biological methods currently being used for pre-sowing treatment of the seeds with the advantage of being carried out in dry seed. Gubbels *et al.* (1982) observed that seed lots of flax (*Linum usitatissimum* L.), buckwheat (*Fagopyrum esculentum* Moench.), sunflower (*Helianthus annuus* L.) and field pea (*Pisum sativum* L.) exposed to a magnetic field produced more vigorous seedlings in some seed lots and increased the yield of sunflower. Alexander and Doijode (1995) found that onion and rice seeds exposed to a weak electromagnetic field for 12h showed significantly increased germination, shoot and root length of seedlings. Growth of the germinated *Vicia faba* seedlings was found to be enhanced by the application of power frequency magnetic fields (100 ì T) as evidenced by mitotic index and ^{3}H-thymidine uptake (Rajendra *et al.* 2005). Vashisth and Nagarajan (2007, 2008) reported significant increases in germination, seedling vigor and shoot/root growth of one month old maize plants and chickpea seeds exposed to static magnetic fields. In our experiments on magnetopriming of soybean (*Glycine max* L.) seeds, magnetic field exposure of 150 mT for 1 hour enhanced speed of germination and vigor of the seedlings. The plants emerging from the treated seeds had an enhanced performance index that contributed to higher efficiency of light harvesting and consequently increased biomass in plants from treated seeds. Total soluble protein map (SDS-Polyacrylamide gel) of leaves at this stage showed increased intensities of the bands corresponding to larger subunit [53 KDa] and smaller subunit [14 KDa] of RUBISCO in the treated plants (Shine *et al*., 2011).

Reports also show that the magnetic field exposure increases germination of **low viability seeds** and improves their quality and sprouting rate. Seed deterioration during storage results in loss of vigor and viability of the seeds that finally results in loss of expensive seed material. In the seed production chain, non-lifting of breeder seeds by indented agencies and over production of seeds in some years leads to storage and subsequent use of carry over seeds. Even with the advent of controlled storage facilities, sometimes, the seed germination falls below the minimum standard resulting in rejection of costly breeder seeds. We demonstrated the positive effect of pulsed magnetic field of 100 mT for 1h on germination and seedling vigour of carry over aged garden pea seeds variety

'Bonneville' as the adverse effect of ageing could be reversed and productivity of the crop improved. The technique can be standardized for other crop seeds and can help salvage the costly breeder seed that remains un-lifted due lower demand and improve the germination potential of long time conserved seeds in gene banks which otherwise would be discarded due to low viability (Nagarajan *et al.,* 2011).

Seed priming has been effectively used for seed enhancement under conditions of abiotic stress (Kaur *et al*., 2002, 2003). Ar study was undertaken to provide an evidence for the **ameliorative role** of magnetopriming under moisture stress conditions in maize. A significant increase in root parameters in seedlings from magnetically exposed seeds resulted in maintenance of better leaf water status in terms of increase of total water potential, turgor potential and relative content under moisture stress conditions of -0.2 and -0.4 MPa. Photosynthesis, stomatal conductance and chlorophyll content increased in plants from treated seeds compared to control under irrigated and mild stress condition (Anand *et al.*, 2011).

The hypotheses that explain biological effects of magnetic field are based on fragmentary studies and in plants cryptochromes are believed to be the possible candidate of magnetoreception (Ahmad *et al*., 2007). Two mechanisms of magnetoreception that are currently receiving attention are (1) the "radical - pair mechanism" consisting of modulation of singlet-triplet interconversion rates of a radical pair by weak magnetic fields, (2) the "ion cyclotron resonance" that revolves around the fact that ions should circulate in a plane perpendicular to an external magnetic field with their *Lamor* frequencies, which can interfere with an alternating electromagnetic field (Galland and Pazur, 2005). The biological effects may be explained as an interaction of magnetic field with ionic current in the plant embryo cell membrane that induces changes in both osmotic pressure and ionic concentrations on both sides of the membrane (Yaycili and Alikamanoglu, 2005). Changes in the ionic fluxes across cell membrane cause alterations in the mechanism of water uptake, as osmoregulation in embryo cells is controlled by the ionic transport across the membrane (Reina and Pascual, 2001).

Microwave energies for breaking hard seed coat dormancy and post harvest disinfestations

Seeds of many species in a number of families (Fabaceae, Malvaceae, Chenopodiaceae and Liliaceae) exhibit hard seededness and are impermeable to water. Hard seededness increases the number of volunteers in the subsequent crop season and also causes problem to seed analysts performing laboratory germination counts. Therefore, various mechanisms like mechanical and chemical scarification are employed to break seed dormancy and reduce hard seededness of seed lots.

Post harvest losses due to physical, physiological and pathological account for 20-35% of the agricultural produce. Conventional methods for post harvest preservation of seeds and grains include fumigation with methyl bromide, contact treatment with an appropriate pesticide, ionising radiation like gamma rays and high energy electrons, controlled atmosphere (O_2 below 1% and CO_2 above 20%) and chilled aeration. However, concerns have been raised about the health hazards of chemical pesticides and environmental pollution due to disposal of radioactive wastes. The quest has been on for safer methods of food preservation with least change in sensory qualities. Electromagnetic energies like radiofrequency and microwave have a potential to reduce/prevent post harvest losses in the commodities by controlling insect-pest infestation. Differences among various stored grain insect species in their susceptibility to RF dielectric heating exposures have been noted when they were treated in common host grains under similar conditions (Nelson, 1973). Anglade *et al*. (1979) found differences between developmental stages within species of insects exposed to RF. In general, the adult stages were more susceptible to RF treatment than immature stages. RF frequencies between 10 and 90 MHz have achieved control of insects treated in grain and grain products by exposures that raised the grain temperature to about 60 to 65°C. Reddy *et al*. (1998) observed wheat seed infection by *Fusarium graminearum* lowered seed germination and quality of the harvest. They optimized the RF treatment for which the fungus mortality is maximized while conserving 70 to 80% of the germination quality of the

seeds. We reported the efficiency of microwave treatment in breaking the hard seed coat dormancy in *Stylosanthes seabrana* compared with other conventional methods including mechanical scarification, hot water treatment (100°C for 1 min) and acid scarification (5 min). Physical scarification using sand paper, hot water and acid treatment are used on small seed lots but are not feasible for handling large seed lots and lead to seed damage (Bhatt *et al.* 2008, Mott, 1979). The microwave treatment was as efficient in breaking hard seed coat dormancy as the hot water treatment as it helped to increase germination from 6.7% in untreated control seeds to 45%. Scanning electron micrographs indicated the appearance of cracks and blisters on the seed surface of microwave - treated seeds that are most likely the sites of water entry during imbibitions (Anand *et al*., 2009, 2011).

Thus, electromagnetic energies open up a new arena for improving seed characteristics in crop plants. They are more desirable over other conventional methods as they cause less mechanical damage during handling, are safe in operation, result in uniform field emergence, decrease seed rate per hectare by increasing the germination percentage and are environment friendly.

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APPLICATIONS OF NANOTECHNOLOGY TO AGRICULTURE

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Introduction

Nanoscience/ technology, the study and manipulation of matter on an atomic and molecular scale, is considered to provide solutions to many challenges faced in the pursuit of advancement of technology. Is nano just size "below nanometers"? Microsize science and technology have been in existence for a long time – more than 30 years. These are shrinking and tending towards nanosize. Why then a sudden interest in nanoscience and technology? The reason for this is that we cannot extrapolate the knowledge at microsizes to nanosize as the science and technology are completely different in these regions. There is a clear line dividing microscience and nanoscience. There is also a line dividing microtechnology and nanotechnology. In both cases the dividing line is related to wavelength of waves. . In case of nanoscience, the De broglie wavelength of the electron (~10nm) is the dividing line. Above this line the physics is governed by conventional classical mechanics. Below this line rules of quantum mechanics are needed to explain the physics. In case of nanotechnology, the dividing line is wavelength of visible light (400 – 700 nm). Photolithography and other fabrication like photoengraving are based on use of visible light or ultra violet light. Below this size light will not focus this small, hence photofabrication processes using light will not work. Something different is needed for this technology. In summary we can say that there is a fundamental change in science below 10 nm and there is a fundamental change in technology below 100 nm. So nano is not just size below nanometers.

Why is naotechnology important?

Nanosize clusters (sizes between 10 to 100 nm) are actually intermediate stage between the world of Newtonian mechanics and quantum mechanics. It is not still clear whether the transition is sharp. But the interesting feature is that in this region surface effects play a major role in determining the physical and chemical properties. Due to this the properties of materials in the nanoscale are dependent on size and are much different from the bulk material of similar composition. The colour dependence on particle size for nanophospors or gold is a well known example. The reason for this behaviour is related to some characteristics length associated with most physical properties. For example the electrical resistivity is related to the mean free path of electrons. When particle size becomes comparable to this number, the electrical properties start behaving differently. Similar effects are seen in other properties. This is the main reason for studying nanosize materials. This change in property can be exploited to design materials with desired properties. Moreover by bottoms up approach for synthesis, it is possible to include any atom or atoms in a material at the molecular level to obtain desired properties (This is not possible in bulk materials). This has lead to the following[1]:

1. New areas of research and technology design.
2. Better understanding of matter and interactions
3. New ways to tackle important problems in various areas of technology like energy, environment, medicine, agriculture etc.

Another important issue regarding nanotechnology is that there is no single approach. There are several areas of research, which are impacted by this technology. The only thing that all these technologies share is the word "nano". Nanotechnology is thus an emerging multidisciplinary science, which integrates physics, chemistry, biology, materials science and engineering, chemical engineering, electronics and several other fields. The drawbacks are, however, as important as the benefits. The technologies are very expensive to develop as they

require top-level equipment and skills and present a high risk of failure to achieve a desired property.

Another aspect of nanotechnolgy, which is important, is the ethical, legal and social implications of nanotechnology. It is reported that in the past 10 years the US government has spent close to $12 billion to support this activity[2]. This aspect also needs to be addressed in the any research/ development programme.

Naotechnology in agriculture

Nanotechnology has the potential to revolutionize the global food system. Novel agricultural and food safety systems, disease-treatment delivery methods, tools for molecular and cellular biology, sensors for pathogen detection, pesticides, packaging materials, environmental protection, and education of the public and future workforce are examples of the important impact that nanotechnology could have on the science and engineering of agriculture and food systems [3].

Materials

Recent advances in materials science and chemistry have produced mastery in nanoparticle technology, with wide ramifications in the field of agriculture. One area that has been effected is textiles based on cotton. From harvesting the cotton to finalizing the fabric it's made into, over 25% of the cotton fiber is lost to scrap or waste [4]. Scientists at Cornell University, have developed a technique called electrospinning that makes good use of the scrap material that would otherwise be used to make low-value products like cotton balls, yarn, and cotton batting [5]. Using this technique of electrospinning, nanofibers have been spun from cellulose. The process involves dissolving cellulose in ethylene diamine, a relatively benign solvent, squeezing the liquid polymer solution through a tiny pinhole while applying a high voltage to that pinhole. This charge pulls the polymer solution through the air into a tiny fiber, which is collected on an electrical ground. The fiber that is produced is less than 100 nanometers in diameter, which is 1,000 times smaller than what is produced in conventional spinning. Possible applications of electrospun cellulose may include air filtration, protective clothing, agricultural nanotechnology, and biodegradable nanocomposites [4]. Another application that the scientists have speculated upon is using the biodegradable cellulose mats to absorb fertilizers and pesticides. These materials would then release the fertilizers or pesticides at a specific time and location for targeted application [4].

Packaging

A major problem in food science is developing an effective packaging material. What is most problematic for food packaging engineers is oxygen because it spoils the fat in food stored in containers and turns them pale. The trick is to impregnate the polymer base used for packaging with layered platelet inclusions (mainly silicates) by applying principles of nanotechnology[6]. Conventional polymerization techniques based on bulk chemical symthesis fails to incorporate the silicates homogenously. Bayer researchers instead mix the silicates in the polyamide base material. Further the silicates have to be chemically modified. The metal ions that form the bonds between the platelets are replaced by an organic acid, which increases the distance between the individual silicate stacks [6]. When the plastic is extruded into a film, the platelets orient themselves parallel to the surface, allowing minimum penetration of oxygen through the material [6]. Each platelet is only a few nanometers thick but is about 1,000 micrometers long, hence gases will have to go a long way around the platelets[6]. This shows excellent promise to the food industry, as it will allow storage and preservation of food items that expire easily, for a longer period of time.

Analysis and detection

To assess the quality of agricultural products and livestock, advanced sensors that can detect surface and airborne pathogens is needed. The key component is the nano-sensor. With this technology, large quantities of food can be readily checked for their safety of consumption. The widespread use of persistent pesticides globally over the last six decades has contaminated groundwater and soil, resulting in diseases and hardships in non-target species such as humans and animals. The first step in the removal of

disease causing microbes from food products or harmful contaminants from soil and groundwater is the effective detection of these damaging elements. Nanotechnology offers a lot of promise in the area of pollution sensing and prevention, by exploiting novel properties of nanomaterials. Nanotechnology can augment agricultural production and boost food processing industry through applications of these unique properties. Nanosensors are capable of detecting microbes, humidity and toxic pollutants at very minute levels. Organic pesticides and industrial pollutants can be degraded into harmless and often useful components, through a process called photocatalysis using metal oxide semiconductor nanostructures[7]. A newer generation of nanomaterials is represented by carbon nanotubes. Discovered in 1991 by the Japanese electron microscopist Sumio Iijima at NEC Corp., Tokyo, Japan, nanotubes are made by "winding" single sheets of graphite with honeycomb structures into very long and thin tubes that have stable, strong, and flexible structures. Nanotubes are the strongest fibers known – 10–100 times stronger than steel per unit weight – and researchers have been using them to make nanotube-reinforced composites with high fracture and thermal resistance to replace conventional ceramics, alumina, and even metals in building aircraft, gears, bearings, car parts, medical devices, sports equipment, and industrial food-processing equipment [8]. The use of CNTs in gas sensing is promising due to their high surface area, their impressive reactivity to some imflammable and toxic gases at room temperature and their porous structure [9]. Dai et al. [10] were the first to demonstrate the rapidity and high sensitivity chemical sensing of semiconducting single-walled carbon nanotubes (SWNTs) at ambient temperature. They found that when semiconducting SWCNTs were exposed to 200 ppm electron-withdrawing NO2 gas for 10 seconds, their conductivity increased up to three orders of magnitude while when they were exposed to 1% electron-donating NH3 vapour for 2 minutes, their conductivity decreased by two orders of magnitudes. Further developments have taken place in using these materials for sensors for agriculture applications by being able to detect a wide range of gases. CNTs can also be used as temperature and pressure sensors[11] and humidity sensors[12]. CNTs are considered as promising materials to be used for novel biosensors. A biosensor is an analytical device comprising a biological component and a physicochemical component able to detect a target (analyte). Wang et al. [13] made a deoxyribonucleic acid (DNA) biosensor based on self-assembled MWCNTs, produced on Au substrate by CVD. DNA probes were immobilized on the CNT surface and formed modifed Au electrodes. The sensors were able to detect the complementary DNA by a hybridization reaction.

Nanoparticles tagged to agrochemicals or other substances could reduce the damage to other plant tissues and the amount of chemicals released into the environment. The first stage is to work out the correct penetration and transport of the nanoparticles into plants. Gonza´ lezmelendi [14] have introduced core-shell magnetic nanoparticles introduced into plants and measured magnetic field gradients to assess the concentration of such magnetic nanoparticles in selected plant tissues.

A long-desired goal of farming is to maximise output (i.e. crop yields) while minimizing input (i.e. fertilisers, pesticides, herbicides, etc) through monitoring environmental variables and applying targeted action. Precision farming makes use of computers, global satellite positioning systems, and remote sensing devices to measure highly localised environmental conditions thus determining whether crops are growing at maximum efficiency or precisely identifying the nature and location of problems. By using centralised data to determine soil conditions and plant development, seeding, fertilizer, chemical and water use can be finetuned to lower production costs and potentially increase production- all benefiting the farmer. Precision farming can also help to reduce agricultural waste and thus keep environmental pollution to a minimum. Nanosensors and monitoring systems like those described in previous paragraphs will be the key requirements to meet these goals[15]. Conventional sensors are not only bulky and less sensitive, but do not have multifunctional capability of nanosensors (several sensors can be packed together).

Safety Issues

Finally a word of caution. The unique properties of nanoparticles that make them attractive

(higher reactivity) may lead to new and unforeseen risks to humans and environment. Current regulatory guidelines assume that nanoparticle toxicity is equivalent to the corresponding bulk material. This is not a valid assumption because if a nanoparticle behaves differently chemically and physically from the bulk, it may behave biologically also differently (toxicity). These issues need to be tackled with top priority, so that the mistakes made with genetically modified food is not repeated with nanotechnology enabled food products.

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POLLEN- STIGMA INTERACTION

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Pollen is the male gametophyte of angiosperm plants and is simple, semi-autonomous single or two nucleated cell that functions to deliver the male gametes to the ovule to enable fertilization. Pollen grains are neatly packed in an appropriate cover called exine and then sent off once the pollination process is completed the pollen grains are dehydrated and the process of pollen-stigma interaction begins. There are number of complex series of cellular and molecular interactions that effectively constitute a form of courtship behavior between haploid pollen and diploid pistil (Heslop-Harrison, 1975)

When molecular recognition process occurs there are active processes of discrimination and rejection of incompatible pollen at inter-specific and intra-specific levels (Hiscock and Allen, 2008). Moreover, ovules are limited in number with few exceptions. Compatible pollen tubes have to compete for ovules leading to an additional level of selection.

The pollen-pistil interaction is thus a fundamental process in the reproductive biology of flowering plants and has been the subject of intense research for many decades. Whatever species have been studied, there does not appear to be any general consensus among the types of molecules regulating a common programme of cellular-pollen-pistil interaction necessary for compatibility (Lord, 2003) There are gametophtyic self incompatibility (GSI) and sporophytic self incompatibility (SSI).

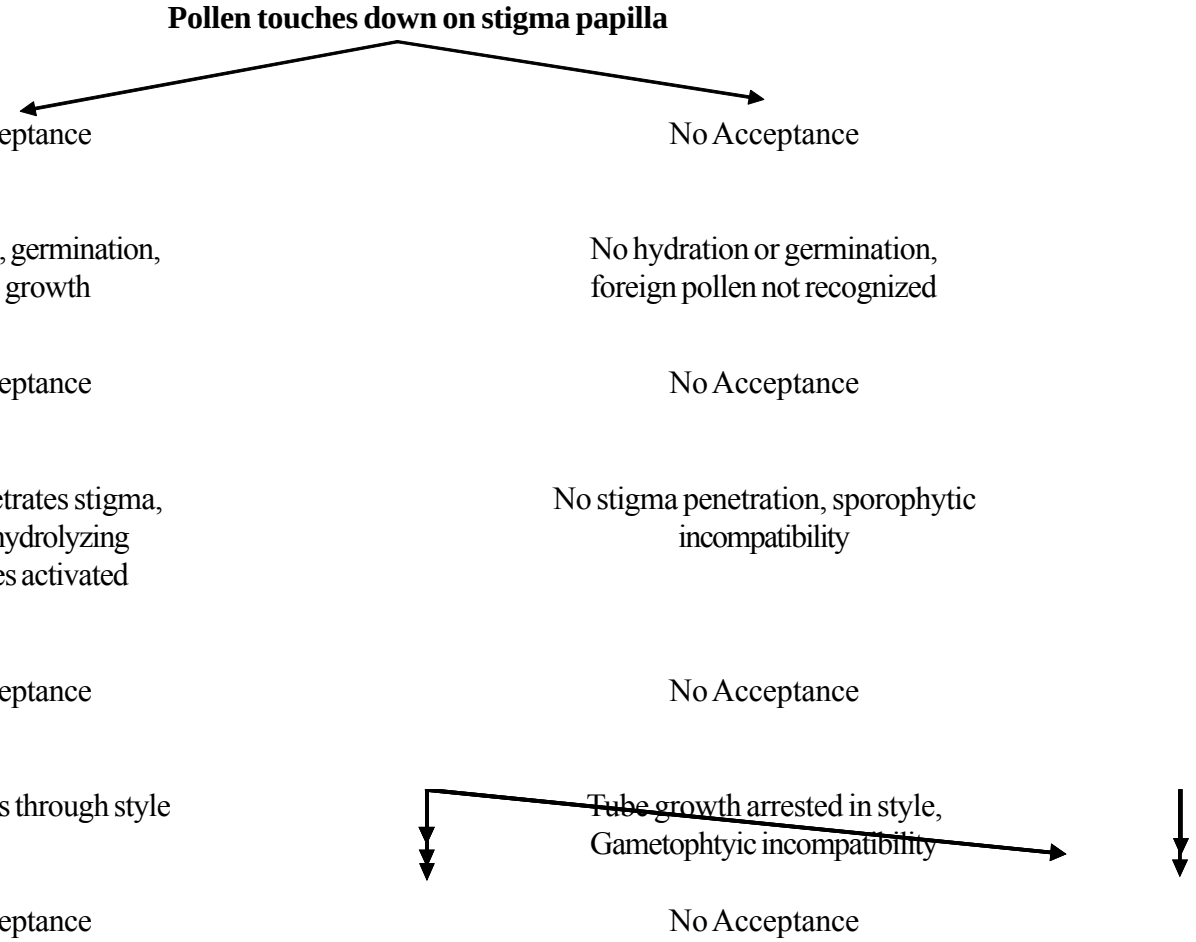
Recognition of the pollen by the stigma.

The stigmatic surface of a flower provides refuge to various pollen grains, but a physiological mechanism operates to ensure that only intra-specific pollen germinate successfully. In sporophytic self incompatibility system the recognition reaction system sets in almost immediately after the pollen comes in contact with the stigma. Recognition of compatible pollen grains by the stigmatic papillae involves a molecular interaction between substances present in the pollen wall and those present on the pellicle or in the stigmatic surface. In fact recognition mechanism is switched on with the hydration of pollen and the subsequent release of its wall proteins. The molecular events that are switched on following the acceptance or rejection of the pollen grains have been studied in *Brassica*, *Arabidopsis* from where the details are gradually emerging out.

The pollen grains following contact with the stigma, synthesize nearly forty new proteins and few of these proteins are highly phosphorylated, which are responsible for signal transduction in a compatible association (Hiscockeal, 1995). Moreover, there is a brief Ca^{2+} peak in stigma papilla cells. There is definite participation of calcium in signal perception. There is also a pollen wall based tryphine in the regulation of pollen stigma interactions (Preuss, 1993).

Self Incompatibility

Self-incompatibility or intraspecific incompatibility is a well designed genetic mechanism by which certain plants recognize and reject their own pollen thus forcing out breeding. It is defined as "inability of the plant producing functional gametes to set seed upon self-pollination", (Brewbaker, 1957). Pollen germination or pollen tube growth is blocked when the pollen grain and the stigma upon which it lands have the same allele at the same locus. This self-incompatibility is acquired nearly one or two days before anthesis as well as in open flowers (Clarke *et al.* 1990). Nearly two-thirds of the families of angiosperms exhibit self-incompatibility. The significance of self-incompatibility in the evolutionary context cannot be over slated, since its possession leads to obligate out breeding and the maintenance of heterozygosity within a species.



Self-incompatibility system can be divided into two basic-groups: i) Heteromorphic systems; ii) Homomorphic systems.

Homomorphic incompatibility

In this incompatibility, the flowers produced by different plants do not show morphological variations. The physiological barriers act in such a way that pollens do not germinate on a stigma of similar genetic constitution or their growth is so slow that by the time the pollen tube reaches the embryo sac, the latter withers out. Almost all the molecular work on self-incompatibility has come from homomorphic systems which have been reported in over 250 genera.

Homomorphic incompatibility is divided into gametophtyic and sporophytic control. In the gametophtyic self-incompatibility, phenotype is determined by the genotype of the pollen grain, whereas in the sporophytic type, pollen rejection is imposed by the genotype of the pollen parent plant and not by the pollen genotype.

In the gametophtyic type, pollen rejection mechanism operate in the style, leading to the inhibition of pollen tube growth, whereas the stigma is the site for pollen rejection in the sporophytic type due to failure of pollen germination or pollen tube penetration. Gametophytic type is common in *Solanaceae, Fabaceae, Papaveraceae, Rosaceae, Rubiaceae,*

Liliaceae, Poaceae and Commelinaceae. The sporophytic type is seen in the *Brassicaceae, Asteraceae and Convolvulaceae*. According to Lewis (1954) and Pandey (1970), there exists a difference in the time of S-gene action in the two types of incompatibility. In the sporophytic type, the S-gene is activated in meiocytes before the completion of meiosis; as a result the product of both the genes is distributed in all the four microscopes. In the gametophyte type S- gene activation is delayed until the completion of meiosis , as a result two of the four microspores receive the products of one S-alleled and the other two products of the other S-allele.

The zone of inhibition for the incompatible pollen or pollen tube is the stigmatic surface or the stylar tissue. Pollen grains that are shed at 2-celled stage show gametophtyic incompatibility and the zone of inhibition is in the style. Pollen grains shed at 3-celled stage show sporophytic incompatibility and the zone of inhibition is in the stigma. In the sporophytic system, the incompatibility substances are already present in the pollen cytoplasm, the activation of incompatibility reactions and inhibition of incompatible pollen tubes takes place in the stigma. A phenotype expression of incompatibility is seen in the form of a lenticular plug of callose between the plasma membrane and pectocellulosic layer of the stigmatic papillae, just below the point of contact with the pollen.

Molecular basis of gametophtyic self-incompatibility

The molecular biology of self-incompatibility has been worked most extensively in *Nicotiana alata*. Sequential expression of S-genes and their protein products in the female floral parts perfectly matches with the site and the time of incompatibility.

The S-gene products were found to be homologoues to the fungal RNases, thus the stylar products of the S-gene was designed as S-RNases. Several physiological factors and molecular approaches have succeeded S-RNases in a transgenic hybrid developed between two self-compatible line of *Nicotiana viz, Nicotiana alata* and *N.langsdorfii* leaves no doubt that pollen rejection is due to RNase action (Murfett et al.1994) On the other hand, antisense suppression of S-RNase activity in a transgenic hybrid between *Nicotiana plumbaginifolia* and *N.alata* allowed the acceptance of self-pollen (Murfett *et al.* 1996).

Molecular basis of sporophytic self-incompatibility

Sporophytic self-incompatibility has been best characterized in *Brassica Oleracea* and *Brassica campestris* and the stigmatic protein responsible for the incompatibility is a S-locus-specific protein called glycoproteins (SLGs). Separation of cDNA clones encoding SLGs from different homozygous lines of *Brassica oleracea* initiated the molecular analysis of sporophytic self-incompatibility. The SLG genes transcribe closely related glycoproteins that are secreted into the walls of the stigmatic papillae. It appears that another gene designated as S-locus receptor kinase (SRK) may be involved in the mechanism that activates self-pollen rejector in *Brassica oleracea*. This gene is genetically linked to the SLG gene at the S-locus and has a complex structure consisting of an SLG-like domain, presumed serine/threonine protein kinase domain, and a stretch of transmembrane domain linking the SLG-like region with the kinase moiety (Stein *et al.* 1991)

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IMPROVING HIGH TEMPERATURE TOLERANCE IN CROP PLANTS: PHYSIOLOGICAL APPROACHES

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Global warming and climatic variability is an acknowledged fact and reality as well. IPCC (2007) has provided clear evidences for changes in global climate due to human activities. These changes in climate are likely to have impacts on agricultural ecosystems and food security across the globe through their direct or indirect effects on plants. The impact of climate change would be particularly severe in the tropical areas, mainly in developing countries, including India. The concentration of greenhouse gases in the atmosphere has progressively increased over the last century. The CO_2 concentration has been increasing at the rate of 1.9 µmol mol^{-1} $year^{-1}$ and is expected to reach up to 570 µmol mol^{-1} by the middle of this century. These changes in emission of CO_2 and other green house gases have resulted in about 0.6°C increases in global temperature over the last century. IPCC (2007) has projected 1.8 to 4.0°C increase in global air temperature by the end of this century; however increase in temperature for Indian scenario will not be similar. It has been predicted that in South Asia region mean temperature could increase by 1.0-1.4°C by 2020, 2.23-2.87°C by 2050 and 3.0-4.0°C by 2100.

Plant's responses to changing environmental conditions are complex and not well understood. Various reports have shown that high CO_2 concentration in the atmosphere will have a fertilization effect on C_3 crop species and will enhance their productivity in the absence of any biotic or abiotic stresses (Kimball *et al.,* 2002). Such changes in plant growth have been attributed to enhanced photosynthesis and improved transpiration efficiency. However, prolonged exposure to high CO_2 may result in photosynthetic down regulation and inhibition of photosynthetic capacity due to various anatomical and biochemical adjustment by the plants. The rising concentration of CO_2in the atmosphere has important contribution in global warming. Therefore, CO_2 induced increase in the atmospheric temperature may lead to lower grain yield through poor seed filling and may offset the beneficial effect of CO_2.

High temperature stress is the second most important abiotic stress affecting plant productivity around the world. Temperature variation in both time and space exists. The yield losses due to high temperature are large and are often combined with losses from other environmental stresses. Compared to drought stress, heat stress is more complicated and can strike the crops at very short notice. High heat stress directly affects seed yield by reducing flowering, fertilization, and seed formation. By increasing the rate of plant development, warmer temperatures also reduce the length of the growing period, thereby reducing the yield potential. The direct effects of high temperature stress depend on the crop species and its adaptability.

Plants adopt different mechanisms for surviving under elevated temperatures, including long-term phenological and morphological adaptations and short-term avoidance or acclimation mechanisms such as changing leaf orientation, transpirational cooling, strong antioxidant system or alteration of membrane lipid compositions. Plants adaptation or their tolerance to environmental stresses can be manipulated by various approaches. Genetic improvement for development of cultivars which can tolerate environmental stresses and produce economic yield is one of the best approaches. Traditionally, most plant breeding programs have focused on development of cultivars with high yield potential in normal (i.e., non-stress) environments. Such efforts have been very successful in improving the efficiency of crop production per unit area and have resulted in significant increases in total agricultural production. However, very few attempts have been made in developing cultivars with abiotic stress tolerance. The progress in

breeding for stress tolerance depends upon an understanding of the physiological mechanisms and genetic bases of stress tolerance at the whole plant, cellular as well as molecular levels. Considerable information is presently available regarding the physiological and metabolic aspects of plant heat-stress tolerance, as discussed earlier. However, information regarding the genetic basis of heat tolerance is generally scarce, though the use of traditional plant breeding protocols and contemporary molecular biological techniques, including molecular marker technology and genetic transformation, have resulted in genetic characterization and/or development of plants with improved heat tolerance. In particular, the application of quantitative trait locus (QTL) mapping has contributed to a better understanding of the genetic relationship among tolerances to different stresses. Thus, evaluation of germplasm to identify sources of heat tolerance has regularly been accomplished by screening for fruit set under high temperature. Furthermore, although poor fruit set at high temperature cannot be attributed to a single factor, decreases in pollen germination and/or pollen tube growth are among the most commonly reported factors. Therefore, pollen viability has been suggested as an additional indirect selection criterion for heat tolerance. Screening of different genotypes (in particular wild types) for growth under high temperatures should be done on the basis of growth potential and reproductive ability/high fertility. Although genetic approaches are beneficial in the production of heat-tolerant plants, it is likely that the newly produced plants are low yielding compared to the sensitive plants. Thus, efforts should be made for inducing heat tolerance in existing high-yielding cultivars. Apart from above, following physiological traits for heat tolerance may be taken into consideration for improving high temperature tolerance in crop plants:

1. **Leaf Transpirational Cooling:** Increased water extraction in response to high temperature increases transpiration from the leaf surface and helps the plant in converting heat energy into latent energy. Thus, plant tissue remains cooler than air temperature. However, different crops have different threshold temperatures up to which leaf cooling works.

2. **Maintaining Leaf Area and Reducing Radiation Load:** Some crops maintain more leaf area even under heat stress. This helps plants to recover from heat injury by producing more flowers/pods during the recovery process. Similar to drought stress strategy, plants use strategy to reduce heat load to sustain under high temperature environment.

3. **Avoidance of Flowering Time:** Plant response to heat stress depends on its developmental stage. The reproductive stage is the most sensitive/ susceptible stage for high temperature stress in most crops (Ishimaru *et al.,* 2010). However, vegetative growth stage is least affected by high temperature stress. Therefore, some crop plants flower during early hours of the day to avoid heat stress during mid day.

4. **Size of Reproductive Structures:** Among the reproductive structures, pollens are most sensitive to high temperature. There are some genotypes which exhibit high pollen fertility despite of increased temperature. Size of the anthers is considered as important trait for high temperature tolerance. Large anther size with more number of pollen grains per anther is preferred under heat stress environment.

5. **Plant Architecture:** In some plant species, reproductive structures are surrounded by large number of leaves, which help them to have more transpirational cooling and results in less evaporation form anthers and more anther dehiscence under high temperature stress conditions.

6. **Maintaining High test weight:** Apart from the flowering/anthesis, grain filling process is also observed to be very sensitive to high temperature. But despite of sensitivity of starch synthesis to high temperature, some genotypes maintain high test weight. Therefore, during selection for tolerance under heat stress environment, genotypes with high test weight may be preferred.

In addition to genetic approaches for improving heat tolerance, attempts have been made to induce

heat tolerance in a range of plant species using various different physiological approaches. These include pre-conditioning of plants to heat stress (induction of thermotolerance) and exogenous applications of osmoprotectants or plant growth-regulating compounds on seeds or whole plants. Results from such applications are promising and further research is forthcoming. Among these approaches, foliar application with low concentrations of signalling molecules (Ca^{2+}) and growth hormones during/ or before flowering, have been tested in some crop species and found to show satisfactory performance. Low molecular weight organic compounds like glycinebetaine and polyamines have been successfully applied to induce heat tolerance in various plant species. Barley seeds pre-treated with glycinebetaine led to plants with lower membrane damage, better photosynthetic rate, improved leaf water potential and greater shoot dry mass, compared to untreated seeds (Wahid and Shabbir, 2005). Exogenous application of 4mM spermidine improved tomato heat resistance by improving chlorophyll fluorescence properties, hardening and higher resistance to thermal damage of the pigment-protein complexes structure, and the activity of PSII during linear increase in temperature (Murkowski, 2001). Thus, to improve plant heat tolerance, alternative approaches to genetic means can also be considered, however, the success of such approaches depends on plant species and genotypes studied.

It is well known that plants or other living organisms adapt to low temperature environment by increasing unsaturation in their membrane lipids. On other hand there are some reports that high temperature tolerance may be improved by reducing level of unsaturation in fatty acids (Grover *et al.,* 2000). These reports are based on some transgenic studies with silencing or overexpression of genes involved in incorporation of double or triple bonds in saturated fatty acids. Murakami *et al.* (2000) reported that tobacco transgenic plants with silenced FAD 7, contained a lower level of trienoic fatty acids compared with control, grow and performed better under high temperature. Although not much work has been attempted in this area of research but these studies raise some hope to develop plants with high temperature tolerance and to combat global warming during future climate changing.

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IRON NUTRITION OF RICE PLANT IN RELATION TO CLIMATE CHANGE

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Introduction

Rice is the most important staple food on earth today because it provides food calories for nearly half the population. According to International Rice Research Institute estimate, 750 million poor people of the world primarily consume rice to survive. The crop is cultivated on 156 million hectares world wide to yield about 680 million tonnes grain annually. USDA statistics indicated that global rice consumption exceeded production level between the years 2000- 2008. It is likely that the demand for rice will continue to increase in the long term that could lead to increase in food prices significantly. According to FAO, global rice demand increases by 8 million tonnes annually and by the year 2025 we need to reach a production level of 800 million tonnes for security of rice consumers. At present yield growth has been 5 million tonnes per annum. Average rice yield grew by 1% per annum between 1989 and 2009, but it must rise at an annual rate of 1.5%. To achieve this target rice scientist strive to follow new technological developments including higher yielding hybrid rice, super rices and rice varieties possessing resistance to a wide range of biotic and abiotic stresses. Enhancement of productivity, profitability and sustainability of the new cultivars can break yield barrier and raise the level of grain yield beyond that of that of the IR8 parented semidwarf high yielding rice. In the new technologies, rice scientists have the option for use are biotechnology, genetic engineering and modern farm management based on the principles of plant nutrition, ecology and agricultural economics. Innovative research work in genetics and genomics have opened up new opportunities. Rice breeders have successfully produced new plant type and hybrid rices that have potential to increase the yield level 15-20% more than the semidwarf rice, which revolutionised rice agriculture in late 1960s.

Effects of climate change on rice yield

As discussed, rice is not only the major source of calories for half the world's population, it is single largest source of employment and income for rural poor. No other food crop has importance for human being as much as rice. A large fraction of human population depends on growing and eating rice. But rice production world wide depend on environment conducive for growth. Current practices of promotion of high yielding rice with genetic uniformity makes the crop susceptible to the pernicious effects of both biotic and abiotic stresses. New types of stresses emerge in the rice agro-ecosystem as growing level of environmental pollution due to increased anthropogenic activities change the global climatic pattern. Climate change occurs because of several un-natural activities like mining, deforestation, refrigeration, preservation of stagnant water in dams, fossil fuel burning in automobiles and industries, and wetland rice cultivation. These activities contribute significantly to the increase of trace gases in the atmosphere, such as, carbon di-oxide, chlorofluorocarbons and nitrous oxide. The gases trap heat energy reflected from earth surface and contribute to Green house effects or Global warming. As ambient temperatures rise, day and night temperature balance changes, nights become warmer and rainfall pattern in rice agro-ecosystem alters drastically. Mild winter, erratic un-seasonal rains, unpredictable weather and excessive heat during summer are indicators of climate change. Rice yield is affected because of the pernicious effect of high night temperature. For every one degree centigrade increase of global seasonal mean temperature, rice yield goes down by 7%. Scientists forecast that the global mean temperature would increase between 1.5 and 4.5 degree centigrade and drought would grip half of the world by the middle of 21st century. Such a change would unsettle the advantages of yield benefits through introduction of new high yielding rice cultivars.

Effect of climate change on iron nutrition of rice plant

Incidence of climate change not only decelerates rice production, but changes nutritional quality of the grains as well. Iron nutrition of the plant is one of the most sensitive features that becomes destabilised due to occurrences of drought, high rain fall and high daily mean temperature. The deleterious effects of high temperature occur because it hastens evapo-transpiration and mineralization of soil and decreases fertiliser use efficiency of rice plant. Because rice is an immobile micro-nutrient, the amount translocated to the edible grains is very poor. Further, the amount of iron transported to grains remains confined to the outermost parts of the caryopsis, such as, husk, pericarp, aleurone and embryo, and milling removes them all. Therefore, people sustaining exclusively on rice for food calories become victims of iron deficiency induced anemia. Prevalence of such disorder occurs in more than two billion people world wide, and most the hapless people live in the poor countries of Asia, who do not have any dietary choice other than rice. Moreover, nutrition improvement with food fortification technology available in developed countries is not transferred to the poor nations as of today. Thus, iron deficiency anaemia is the most common micro-nutrient deficiency worldwide. Incidence of drought accentuates bioavailability of iron in rice plant further resulting in catastrophic effects of deficiency in exclusive rice consumers.

Iron stress

Iron stress in rice plant is another facet of climate change induced inclement weather conditions. Iron is an essential element for the plant. It accepts and donates electrons during photo- and oxidative-phosphorylations. It is required for biological nitrogen fixation and DNA replication. Deficiency of the element invites chlorosis and premature senescence of leaf. Excessive absorption of the element results in generation of reactive oxygen species including the harmful hydroxyl radicals in the cells through Fenton reaction. The surplus iron in living cells causes bronzing symptom of leaf, blackening of roots and damages to cell membrane in rice. Erratic rains thanks to change in global climatic patterns increases incidence of drought and flooding. Solubility of iron decreases significantly in drought-prone environment. The aerobic environment of dry soil supports existence of Fe^{3+} (ferric) form iron. Ferric iron precipitates in soil as $Fe(OH)_3$. Because synthesis of iron-phytosiderophores is low in rice, scarcity of the element accentuates further in dry soil. In contrast, anaerobic environment of flooded soil sustains Fe^{2+} (ferrous) form of iron. It is easily absorbed by roots. When duration of flooding increases, the plant acquires excess iron causing toxicity symptoms. Because erratic monsoon brings in frequent bouts of drought and flooding in tropical rice fields, management of iron nutrition becomes crucial for the success of rice cultivation. Therefore, rice scientists consider essentiality of iron nutrition, next to that of the most required elements like nitrogen and phosphorous.

Management of iron stress in rice cultivation

Global warming continues to make rice agro-ecosystem more unstable as incidence of erratic rains and high temperature stress increase with passage of time. Innovative research in modern farm management practice is necessary to counter the adverse effects of inclement weather. Under drought-prone environment external application iron may provide a temporary relief for iron deficiency, but this is not practical and feasible in open soil. The applied iron quickly oxidises from Fe^{2+} to Fe^{3+} form and becomes unusable for the plant. Iron-dense cultivars of rice may be suitable for drought-prone environment. In contrast low-iron cultivars may be good for cultivation in flood-prone ecosystem. Such flexibility is available in rice genotypes. Rice is the unique crop, which possesses genetic permissibility for phenotypic plasticity in widely contrasting agricultural ecosystems of the world. It is cultivated in a wide range of latitudes and altitudes around the globe under different environments. There are 115000 rice cultivars available world wide and nearly 40000 of them are available in India. The varietal differentiation makes it possible to grow the crop in terraced hill tops, rain fed and irrigated low lands and flood-prone deep water swamps. Such uniqueness in adaptation might have come through the modification of genetic and eco-physiological mechanisms for stress tolerance unparalleled in other major crops. Therefore, rice can become the anchor for food security of the

world confronted with the challenges of global climate change. Species diversity in contrasting environments also endows variation in iron nutrition of rice. But knowledge is scant on the physiological/biochemical nature of variation. Although some efforts have been made for exploration of iron toxicity, less work is done for iron deficiency stress and concomitant adverse effects on rice consumers. Rice researchers should take up this issue as one of the major challenges for expanding rice production in unfavourable environments.

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PHYSIOLOGICAL INTERVENTIONS TO ADDRESS CHALLENGES IN PLANT MINERAL NUTRITION

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Understanding the science of mineral nutrition is immensely important not only for crop plants but also for humans and animals and requires a concerted and coherent effort on part of plant and soil Scientist, Agronomist, Microbiologist and Geneticist. Even the tissue culture will not be a success if nutritional supplements to the growing media are not optimized. Burgeoning population further fuels pressure on our agriculturally relevant natural resources. Challenges, therefore, are huge but scientific endeavor and progress to achieve breakthrough, may be a bit slow, is consistent. Complexities in the field of plant mineral nutrition arise mainly due to variability that we have for the number of soils, for the crops and for the cropping systems. Add to these, the abiotic stress factors that in most cases influence plant nutrition and growth either directly or indirectly. Further, we have serious issues of nutrient deficiencies, toxicities and imbalance, all of which inhibit grain/crop yields and threaten human nutrition and health. We were happy to reap the benefits of Green Revolution, but in the process forgot the bottom-line message that our resources are limited and their quality utmost important. Like, we have not bothered about sulfur and micronutrient levels of our soil. We only took care of N, P, and K as the main fertilizers, which has led to widespread nutritional imbalance in the soil and has resulted in malnutrition. Fortification of commodities is being tried in many developing countries however, its economics is debatable. Scientists are working on the idea of producing nutrient rich crops i.e., biofortification approach. Strategy is similar to the production and use edible vaccines. We need to be more farsighted to assess problems that may crop up in near future or in times to come. We are aware of the soil variability and know that these are presently low on N, P and S but not K. It does not mean we should overlook K. It is just that, at this moment, more emphasis is to be laid more on N, P and S. Talk about micronutrients, it is essentially Zn and B whose deficiencies are presently predominant in our soils and require a more focused and targeted research on them. Talk about crops such as sorghum, maize, rice, wheat, they all differ in the critical limit of deficiency and sufficiency. All these parameters put together make the task of the plant scientists, in general, and plant nutritionists in particular very - very difficult. Key question arises i.e., how much fertilizer one should apply because the optimum limits are different for different crops and are dependant on the nature of the soil.

Scenario though may appear challenging but is also promising. Plant physiologists can drive strength from the variability of our soils, species and agro climate. People are worried that if BT Brinjal is introduced the genetic variability may be lost. The concerns are logical as genetic variability is our strength and we need to preserve and exploit it judiciously. Among Monocots such as durum and bread wheat, triticale, and rye, a very high degree of variability for Zn deficiency tolerance has been reported. So what should be done? The main task for us i.e., for the nutrition scientist, or the plant agronomist, breeder is to see that how we can improve the nutrient use efficiency (NUE). For e.g., for N, we are working at a very low nutrient use efficiency (30-33%). People use Neem coated urea which results in a slow release of the N and thus improves N-use efficiency by ensuring a steady equilibrium between N-availability and N-requirement of plant. Some of the plant traits that may have considerable effect on nutrient use efficiency are rooting attributes, root uptake, root – shoot nutrient translocation and also nutrient retranslocation. The latter is often not given importance. The best analogy and example of retranslocation can be drawn in context of a grand

father giving all his money and valuables at the phage end of his life to his younger, near and dear ones so as to ensure their happiness and survival. Now equate it with a leaf that on initiation of senescence starts to translocate the nutrients and other compounds that may be useful to the developing and younger green leaves. This strategy ensures survival of plants on nutrient deficient soils provided nutrient in question is mobile within the plant.

Another major challenge for plant physiologists is to enable a higher accumulation of nutrients in seed/ grain as it is not the plants capacity for uptake of nutrients but its efficiency for accumulation of nutrients in the seed that is crucial for biofortification. It is important to determine the translocation efficiency of micronutrients for example Fe content of the rice shoot is low where as Fe content of the root is almost 8-9 times higher which means that the problem is not with respect to the uptake by the plant but it is with respect to its translocation to the shoot. If it is not been translocated from the root to the shoot it will not be utilized, and it will not contribute towards the biomass production.

Root response to the variable supply of the nutrients is another gray area where the role of hormones in relation to root development needs to be deciphered (GuoHua *et al.,* 2010). We scientists love to work with the above ground tissue for sake of convenience very often neglecting, a little bit difficult analysis of roots. There is a lot more to roots than the mass. We need to improve our understanding of the rooting behavior in response to different nutrients and nutrient availability conditions and exposure to abiotic stress. All the above mentioned conditions are likely to influence root exudation behavior of crops and consequently the nutrient availability, uptake and plant growth. Two plant species growing on same soil and environment and producing same mass of roots and root surface area, may perform differently owing to their capacity to secreting different amounts and constituents of root exudates. GuoHua *et al.* (2010), have recently shown the localized production of roots in response to nitrate N availability in soil pockets. Plants are very intelligent as they have the mechanisms by which they can detect/ sense pockets where a higher amount of N is present in the soil and then, through intervention of growth hormone, produce roots in the direction/ location of the available N. Another important area of research on N-use efficiency is to understand the differential crop response to different forms of N i.e., nitrate-N or ammonia-N. It has been shown that different N forms can alter leaf morphology and overall plant growth.

A lot needs to be done in the area of nutrient-phytohormone interaction. Krouk *et al.* (2010) provide evidence for dual functioning of nutrient transporters. NRT-1.1 was shown to act as a nitrate transporter as well as a nitrate sensor. Imagine a transporter localized on the root performing a dual function of not only transporting N (in general, nutrients) but also sensing N availability and subsequently altering, favorably, its root behavior. When nitrate is in the vicinity of the root, it senses it and stops the transport of auxin out of the root and promotes growth of lateral root in the direction of sensed nutrient. This is one very important piece of evidence in favor of coordination between nutrition and hormones which will generate new interest and will boost research on nutrient sensing and signaling.

Nutrient homeostasis is another balancing act of significance particularly under condition of low or high nutrient availability. Nutrient balance is to be so maintained to ensure optimal rate of operation for the metabolic processes in the cytosol and its embedded organelles like the mitochondria and the chloroplast. Competition between the uptake of the mineral nutrients i.e., cation competition is another key area of research. Manifestation of magnesium deficiency in plants under soil condition high Mg but also high K is well known. So are interactions between Zn and P.

Recent studies harp upon both the conventional and the molecular routes to biofortification. Using Fe-52 it has been shown that under Fe deficiency, Nicotiana amine synthase is not only involved in the production of metal-chelating phytosiderophore but that these phytosiderophore-matal complexes are absorbed by roots and transported as a moiety upto the shoot. These phytosiderophores have also been postulated to play a role in determining the heavy metal tolerance of crop species (Zhao and McGrath, 2009).

In conclusion, root response, nutrient sensing, signaling, phyto-hormone regulation, nutrient use efficiency particularly of N, P, S and B are some of the most important areas of research for the Plant Physiologists. QTL mapping for related physiological and agronomic traits must be attempted. Rate and constituents of root exudates particularly that of metal mobilizing chelates is important. The above studies must take into consideration the development of multiple nutrient deficiencies and multiple abiotic stresses which are not uncommon under natural conditions of crop cultivation.

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SUSTAINABLE AGRICULTURE IN RELATION TO GLOBAL CLIMATE CHANGE WITH RESPECT TO SORGHUM

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The earth's climate is predicted to change through the buildup of greenhouse gases -primarily carbon dioxide, methane, nitrous oxide and chlorofluorocarbons. The major impact of climate change due to this buildup of green house gases will be increase in atmosphere temperatures. Increased water shortages, rising sea levels (may result in more saline lands), reduced crop yields, more floods and increase in human and animal diseases are some of the serious problems being expected under climatic change scenario. Farmers can no longer rely on the rainy seasons as before. The rise in temperatures will left many boreholes dry and streams having no water. These impacts of climate change are due to the large amount of gases such as carbon dioxide and methane that are being pumped in the air by human activities and industries. It is said that in the near future, the ice on many mountains will disappear, and that many parts of the world will experience floods, droughts and forest fires, as a result of climate change. That means many people will have little food to eat and there will be no excess produce to sell in order to afford basic needs. Growing crops, that are drought and heat resistant, is one form of adapting to the impacts of climate change. Sorghum is one of the crops which have inherited its trait to adapt and grow in harsh climate from its origin. It grows in dry conditions, tolerates heat, salt and waterlogging, making it an ideal crop for semi-arid areas where many of the world's poor live.

Sorghum [*Sorghum bicolor* (L.) Moench] rank fifth among the world's most important crops. More than 70% of the world's total production of sorghum comes from the developing countries in Asia and Africa, where crop is grown with limited input of water and nutrients. In India, sorghum is cultivated during both kharif (rainy) and rabi (post-rainy) seasons mainly as a rainfed crop (92% of the area) with about 85% of the production concentrated in Maharashtra, Karnataka and Andhra Pradesh, all falling under warm semi-arid region. It is one of the most nutritious cereals and is an important dryland crop grown in marginal lands with minimum inputs. It grows in dry conditions, tolerates heat, salt and waterlogging, making it an ideal crop for semi-arid areas where many of the world's poor live. It is now recognized worldwide as a smart crop capable of providing food, feed, fodder and fuel ("FFFF") especially under moderate inputs, especially in water deficit environments. It is also base crop on which many inter and sequence-cropping system are built upon. It is now realized that sorghum is of prime importance for the sustainable livelihood of the rural poor farmers who cannot afford purchased inputs. Further, the urban poor consumers having limited purchasing power will benefit of nutritive millets grains are also made available as rice and wheat as low cost. Increasing industrial utilization, greater use as quality fodder and as adjunct in food and feed mixes can dramatically alter the demand of sorghum.

Sorghum as food crop: Sorghum food consumption has many potential health benefits such as high anti-oxidant levels, improved cholesterol profiles of the consumer, and as a source of safe food for persons with celiac disease. Sorghum grains have high fibre content, moderate digestibility, rich mineral content compared to other cereals such as rice and wheat. Therefore, sorghum foods are recommended for diabetic and jaundice-affected persons and for fighting obesity. High tannin sorghums reduce the risk of certain types of cancer when compared to other cereals. Sorghum wax has sterols like policosanols which

regulates cholesterol absorption and endogenous cholesterol synthesis.

Despite the fact that consumption of sorghum as direct food use is declining, market for processed foods such as multigrain flour, flakes, vermicelli, pasta and biscuit is surprisingly picking up in urban areas as there is increasing acceptability of sorghum if available in ready-to-eat form or as convenient foods as health and nutritional foods. In this context of increasing demand for sorghum, owing to their nutritionally rich character its value-addition has acquired a great importance which will have a striking impact on socio-economic conditions of dry-land farmers in long-run. All sorghum processed products generally have fairly strong acceptability among the consumers as revealed from the finding of consumer acceptance studies undertaken by an independent agency commodity India. These products have more nutritional value and health benefits as compared to similar products developed from wheat and rice. Since novel processing technologies in sorghum are introduced in the markets in an integrated manner to highlight them as a choice convenient and health products which is suitable for all age groups especially for those ailing from life style diseases such as diabetes.

Sorghum as source of feed and fodder: Dairy industry has been the driving force in the region of Punjab, Haryana, Uttaranchal, Uttar Pradesh, Bundhelkhand, South Rajasthan and North West Gujarat. Sorghum as a source of feed and fodder has the potential to meet the demand set by the dairy industry. Fodder availability during the lean season has enormous value for the livestock industry and farmers' livelihoods, particularly in areas with less-productive soils in north west Gujarat (saline soils) and eastern India (acidic soils). In the Eastern India, where rice is the first crop, the problem of acidity is common and there is a potential for single cut sorghum as a second crop in these rice fallows. Options to diversify fodder production on these less productive lands are important, as well for land use and income generation. In Deccan Plateau, where sorghum grain is more important, there is a need to improve the stover quality to support dairy industry. Owing to the above, the approach to meet the feed and fodder demand should be based on locally relevant traits in the plant

Sorghum as bio-fuel crop: Sorghum has a potential to emerge as one of the two big crops in the tropics" that supply biofuel such as ethanol, the demand for which "far exceeds the supply" on the world market. Further, sweet sorghum has emerged as a supplementary crop to sugarcane in dry land pockets for the production of ethanol. The advantages of the crop are it can be grown with limited water and minimal inputs and it can be harvested in four months with 2-3 irrigations. Its water requirement is one fourth of sugarcane on comparable time scale. Use of ethanol blended fuel is increasing because they reduce vehicular emission of CO_2, methane and other gases that contribute global warming. The dual-purpose nature of sweet sorghums—they produce both grain and sugar-rich stalks—offers new market opportunities for smallholder farmers and does not threaten food, feed and fodder value of sorghum. Sorghum is being cultivated since time immemorial in several countries of Asia and Africa. Incidentally, most of the landraces that are being grown in India in post-rainy season are sweet sorghums. In China, specific programs are underway to breed sweet sorghums for silage production. The emerging bio-fuel needs, therefore offer expanded markets for sweet sorghum in India, China and several African countries. Because sweet sorghum requires less water and has a higher fermentable sugar content than sugarcane, which contains more crystallizable sugars, it is better suited for ethanol production than sugarcane or other sources, and sweet sorghum ethanol is cleaner than sugarcane ethanol, when mixed with gasoline. Pilot studies in India indicated that ethanol production from sweet sorghum is cost-effective. Also, the net returns from sweet sorghum cultivation at the prevailing cost of cultivation and ethanol prices is about 10% higher than that from grain sorghum in India. In addition to this crop has a unique inbuilt ability of biological nitrification inhibition (BNI) in its root exudates through which it suppresses

nitrification in soil. This indicates that sorghum can play a vital role in mitigating the impact of global warming by regulating the emission of greenhouse gases like nitrous oxide N_2O, CO_2 and methane.

One of the needs to cope with the changing climate scenario of rising temperature (hence increasing evaporation) is to improve the heat and drought tolerance of major food crops like wheat, rice and maize. The progress in these areas is generally low due the complex nature of traits associated with these stresses. Sorghum and/or millets is a group of crops which have already inherited higher tolerance to heat, drought, salinity etc. Therefore, these crops have a better chance to get adapted to these supra-optimal conditions. No doubt there is still need to improve genetic potential of sorghum for higher tolerance to these abiotic stresses. The other major challenge facing sorghum research and development workers is to provide technologies that will enable the agriculture sector to affect transformation of "subsistence farming" to a sustainable "market-oriented' enterprise successfully competing with the rest of world.

BRASSINOSTEROIDS-INDISPENSABLE FOR PLANT GROWTH AND SURVIVAL

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Brassinosteroids are a new group of phytohormones with significant growth promoting activity and are essential for many processes in plant growth and development. The ability of certain pollen extracts to promote growth led to the discovery of this group of substances in plants. Collective efforts initiated by the scientists at four Agricultural Research Stations (ARS) of USDA resulted in the isolation of an active factor from the pollen grains of rape plant (*Brassica napus* Linn.) which was named as brassinolide. Four years later, Japanese scientists extracted another steroidal substance with growth promoting nature from the insect galls of chestnut (*Castenea crenata*) and named it as castasterone. As the first steroidal plant growth regulator was isolated from *Brassica napus*, a generic name "Brassinosteroids" has been given to this new group of phytohormones. Brassinosteroids are polyhydroxy steroids. They have a common- cholestane skeleton and their structural varieties come from the kind and the orientation in the A/B rings and side chain. So far, 65 brassinosteroid and 5 conjugates have been isolated from plant sources.

Brassinosteroids are ubiquitously present in plant kingdom

The presence of Brassinosteroids in all taxonomic groups of plant kingdom has been established. Brassinosteroids have been reported in algae (*Hydrodyctyon reticulata*), bryophytes (*Marchantia polymorpha*), pteridophytes (*Equisetum arvense*) and in several gymnosperms and angiosperms. Thus brassinosteroids appear to be ubiquitous plant hormones that predate the evolution of land plants. In plants young and growing tissues (eg. pollen, developing seeds) contain higher levels of brassinosteroids than mature tissues.

Mutants-valuable source for establishing the essentiality of brassinosteroids

Studies conducted with brassinosteroid deficient and brassinosteroid insensitive mutants provided compelling evidences to the essentiality of brassinosteroids for normal growth and development. In extremely dwarf mutants of *Arobidopsis thaliana* cbb_1, $cbb_2$and cbb_3, the dwarfness is because of impaired cell elongation. $cbb_1$and cbb_3 were phenotypically normalized by feeding with brassinolide. cbb_2 was proved to be brassinosteroid-insensitive as growth could not be restored by brassinolide application. The dwarf tomato mutant *dpy* (*dumpy*) is intermediate dwarf, displays a curled leaf phenotype with dark ruggose leaves, suppression of axillary buds and exogenously applied brassinolide completely rescued the phenotype to wild type. Studies conducted with *d* (*dwarf*), cu_3 (*curl*) mutant of tomato, *lkb* mutant of pea, det_2(*deetiolated*) and *cpd* (*constitutive phytomorphogenesis* and *dwarphism*) mutants of *Arabidopsis* further clarified the role of brassinosteroids in normal growth and morphogenesis. The studies conducted employing brassinozole, a specific inhibitor of biosynthetic pathway of brassinosteroids lent further support to the indispensability of brassinosteroids for plant growth.

Brassinosteroids are hormones with pleiotrophic effects

Brassinosteroids influences wide array of developmental processes in plants. They exhibit strong growth promoting influence. The growth promotion as elicited by brassinosteroids is both by cell elongation and cell division. Brassinosteroids are implicated in regulation of xyloglucon endotransglucosylase/

hydrolase (XTHs) activity and cell wall proteins *expansions* there by making cell wall susceptible to stretching. Brassinosterods also induce cell division as reported in case of cultured parenchymatous cells of *Helianthus tuberosus*, leaf protoplast of *Petunia hybrida* and Chinese cabbage. Brassinosterods break seed dormancy and induce germination. They are capable of even reversing the effect of abscisic acid on seed germination. Brassinsterods induce rhizogenesis. However their effect on root growth is reported to be inhibitory as well as stimulatory. The involvement of brassinosterods in the induction of flower primordia and flowering has been noticed. Acceleration of leaf senescence, hastening fruit ripening by brassinosteroids have been observed. The involvement of brassinosteroids in pollen germination and pollen tube growth, induction of epinasty, gravitropic bending of plant roots and shoots, promotion of xylem differentiation during vascular development and stimulation of nodule formation in legumes is also observed.

Brassinosteroids confer resistance to plants against stress

Plants are subjected to multiple abiotic and biotic stresses that adversely influence plants by inducing physiological dysfunctions. Brassinosteroids have recently gaining much attention for their capabilities to impart resistance against broad spectrum of abiotic and biotic stresses. The results of the studies involving exogenous application of brassinosteroids to plants challenged with stressful environments are quite promising. Brassinosteroids have been reported to be reducing the impact of drought stress and improves the plant growth against desiccation stress. Brassinosteroids also contain the damage caused by hypoxia in plants subjected to flooding stress. The involvement of brassinosteroids in protecting the plants facing salinity stress by way of enhancing the production of osmoprotectants such as betaine, choline is reported. Brassinosteroids have been implicated in protecting the plants against temperature extremities. Enhanced thermotolerance as conferred by brassinosteroids is attributed to the induction of heat shock protein (HSP) formation. Brassinosteroids negate the harmful effects of chilling and freezing stresses. Improving the performance of plants growing in heavy metal contaminated soils by the use of brassinostresoids is attracting much attention. The results of the preliminary studies in this area are quite promising. Brassinosteroids alleviate the toxic effects of heavy metals by increasing the activity of oxy free radical scavenging enzymes, minimizing the membrane peroxidation and accumulation of non enzymatic antioxidants like reduced glutathione and ascorbate. Brassinosteroids also combat the growth of pathogenic organisms and there by promote disease resistance in plants. Extracts of *Lychnis viscaria* very rich in brassinosteroids abate the growth of several pathogenic organisms as tested in case of tomato, tobacco and cucumber. The incidence of late blight of potato caused by *Phytophthora infestans* was found minimized by brassinosteroid application. Brassinosteroids inhibited development of blue mould rot caused by *Penicillium expansum* in jujube fruits under storage. The detoxification of residual herbicides in crop plants by brassinosteroids application is also reported.

Molecular mode of action of brassinosteroids is deciphered

Much headway has been made in unravelling the mechanism of action of brassinosteroids at cellular and molecular level. Two transmembrane receptor kinases-BRI-1 (*brassinosteroids insensitive 1*) and BAK-1 (*brassinosteroid associated receptor kinase-1*) were identified and characterized. BR stimulus facilitates combining of the two receptors leading to the formation of active heterodimer and autophosphorylation of kinase region of BRI-1. Subsequent events in signal transduction involves the dephosphorylation of cytosolic transcription factor BZR-1 (*brassinozole resistant 1*) and the resultant active transcription factor moves in to the nucleus, binds to promoter region of BR-responsive genes and trigger their expression.

Brassinosteroids-A great potential to boost crop productivity

In 1982 Maugh published a paper in Science entitled "New Chemicals Promise Larger Crops" wherein he reported the positive impact of brassinosteroid application on the yield of certain vegetable crops. Subsequent field trial results in Japan and China employing brassinosteroids were quite encouraging. The beneficial effects of natural brassinosteroids and their synthetic analogues in improving the performance of vegetable crops, fruit crops, cereals, oil seed plants, plantation crops, medicinal and aromatic plants have been reported from several quarters. Prompted by successful performance in field trails several commercial brassinosteroid formulations have been introduced by agroindustries. The use of brassinosteroids in farmer's fields is just gained momentum. By clinical experiments, scientists even annulled some of the apprehensions linked to the use of 'steroids' for edible crop plants .Now brassinostreroids are considered as safe and ecofriendly natural substances with a great promise to enhance agricultural production.

Future Perspectives

Thirty years of brassinosteroid research brought into light the multifaceted influence of this 'novel' group of hormones on plant growth and development. Much of the contemporary focus is on their stress alleviation capabilities. However most of the inferences are drawn based on the results obtained from exogenous application of brassinosteroids. Information on endogenous role of brassinosteroids in amelioration of stress is scarce. Deeper insights into the internal modulation in the levels of brassinosteroids under stressful conditions and the resultant signal cascading events are needed to understand the comprehensive mechanism of stress alleviation by this group of phytohormones.

GENETIC MANIPULATION OF NITROGEN-FIXING CYANOBACTERIUM, *ANABAENA* SP. TO ENHANCE ITS BIOFERTILISER POTENTIAL

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Cyanobacteria or Blue-Green Algae originated as a group of photoautotrophs nearly 3 billion years ago (Brock, 1973). They closely resemble the higher plant chloroplasts, which may have possibly evolved through endosymbiosis of cyanobacteria by certain eukaryotic cells (Walsby, 1986). A general similarity in the composition of plasma membrane and thylakoid membrane of cyanobacteria and those of higher plants in terms of lipid composition, assembly of proteins (Los and Murata, 1999) and the various photochemical reactions carried out by them (Stewart, 1980) has also been observed. Cyanobacteria are the only group of organisms, which have a combination of abilities to photosynthesise as well as fix atmospheric nitrogen. Their application as nitrogen biofertilisers is gaining popularity as a partial substitute for chemical nitrogen fertilizers (Venkataraman, 1979). Their cultivation is fairly simple both at laboratory and large scale and requires only light and minimal salts media (Castenholz, 1988). Among the common stresses they are exposed to, salinity and desiccation have been studied well (Apte, 2001).

Cyanobacteria possess a typical higher plant-type oxygenic photosynthesis (PSI and PSII) (Walsby, 1986). The nitrogen fixation abilities of cyanobacteria depend entirely on photosynthesis for NADPH, ATP and glutamate i.e. they are photoautotrophic diazotrophs (Stewart, 1980). However, oxygen adversely affects nitrogen-fixation by degrading nitrogenase proteins and also destroying the anoxic environment required for transcription of *nif* gens and enzyme activity (Robson and Postgate, 1980). This makes nitrogen fixation and photosynthesis mutually exclusive. The cellular location, time and mode of nitrogen fixation vary in different cyanobacteria depending on whether they are unicellular or filamentous (heterocystous or non-heterocystous). In the unicellular forms the photosynthate is generated in the presence of light and is used later for the purpose of generating electrons and ATP for nitrogen fixation in dark (Schneegurt *et al.*, 1994; Mitsui *et al.*, 1996). All heterocystous forms e.g. *Anabaena*, *Nostoc*, *Fischerella* are active nitrogen fixers under aerobic conditions, the nitrogenase activity being restricted to the heterocysts only (Haselkorn, 1978), which lack phycocyanin (Thomas, 1970) and major components of PSII (Tel-Or and Stewart, 1977). The photosynthate for generating electrons in heterocysts is made available from the vegetative cells (Wolk, 1968).

In the absence of combined nitrogen, *Anabaena* differentiates some of its vegetative cells to heterocysts to fix nitrogen (Haselkorn, 1978). The heterocyst frequency of *Anabaena* 7120 is in the range of 5-8% (Wolk, 1996) and one of the important genes involved in the early step of heterocyst differentiation is *hetR* (Buikema and Haselkorn, 1991). When expressed on a multicopy plasmid or under a cupper-inducible promoter, HetR can induce the formation of multiple heterocysts (Buikema and Haselkorn, 2001), thereby resulting in an enhanced nitrogenase activity. When used in field, biofertiliser potential of *Anabaena* is severely affected due to temperature fluctuations during the day, since both nitrogen-fixation and photosynthesis are thermosensitive (Chaurasia and Apte, 2009; Rajaram and Apte, 2003; 2008). The thermosensitivity occurs due to unfolding of the proteins involved in these machineries at high temperatures. Folding of proteins during normal growth as well as during stress is the function of heat shock proteins, which function as chaperones (Morimoto *et al.,* 1994). The two major heat shock proteins of *Anabaena* are the 59 kDa GroEL and the 61 kDa Cpn60, both of which act as chaperonins (Rajaram *et al.,* 2001;

Rajaram and Apte, 2003). Thus, overexpression of these individual proteins would be beneficial to their application as biofertiliser.

Use of cupper inducible promoters for overexpression, would not be advisable for use in fields, so it necessitated generation of another expression vector for this purpose. An integrative expression vector, pFPN was generated wherein expression of the gene is under the control of a light-inducible promoter (Chaurasia *et al.,* 2008). Using this vector, several recombinant *Anabaena* strains were generated. Overexpression of HetR in An*hetR*$^{+}$ strain enhanced the biofertiliser potential of *Anabaena* and also catered to the nitrogen demand of the rice seedlings (Chaurasia and Apte, 2011). Overexpression of the chaperones enhanced the stability of the nitrogen-fixation and photosynthetic machinery under stress conditions (Chaurasia and Apte, 2009; Rajaram and Apte, 2008), with both the proteins playing a differential role. Thus, a molecular biology approach can be taken to engineer the cyanobacterium, for better uses not only as Biofertilisers, but also in the fields of bioremediation and understanding the basics of stress biology i.e. response to several abiotic stresses, such as oxidative, radiation, desiccation stresses etc.

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ENHANCEMENT OF PRODUCTIVITY UNDER WATER LIMITED CONDITIONS – A NEW PARADIGM CALLED PHYSIOLOGICAL BREEDING ARE WE READY FOR THE CHALLENGE?

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Attainment of food security in India is strongly linked with the development of superior crop cultivars that have greater ability to capture the scarce resources like water, nutrient, light etc and use these resources efficiently for growth and biomass production. This is especially important in India as most of the crop production comes from rain-fed conditions where several biotic and abiotic factors constrain growth and productivity. Among several of these stresses, drought appears to be the most predominant stress. An estimated 40 to 50 percent of yield losses can be attributed to water limitation which is greater than all stresses put together. Therefore the daunting challenge of achieving food security in the country is strongly linked with our abilities in reducing the gap between the existing yield potential and field performance. Drought is a complex stress owing to the unpredictability of its occurrence, intensity and duration. The stress levels experienced by a plant are also dependant on several factors such as soil type, climate of the region etc. Equally complex are the varied strategies evolved by plants to combat stress and survive. The species of crop in question, the stage of the crop that experiences drought, the intensity and duration of the stress etc further add to the complexity of drought response by plants. Though management approaches have significant advantages in mitigating drought stress effects, genetic enhancement of crops to sustain productivity and/or to improve yield potential under water limited conditions have greater relevance. A seed based technology of delivering superior cultivars has far greater acceptability than management practices.

Plant breeding process in the past was greatly successful in significantly increasing crop productivity through selection of high absolute yields under stress. Despite the overwhelming success in the past, in recent years, yield levels seem to have attained a ceiling and improvement in yield under stress is not forthcoming. As more than 70% of crop production in India comes from rain-fed conditions, genetic enhancement of rain-fed crop species must be achieved if India must remain self-sufficient in food grain production in the years to come. It is estimated that the cereal grain production need to be increased by 1.92% annual growth against the current 0.62% in the next 15 years. Similar increases are also required in pulses and oilseed crops which appear like the most unprecedented and formidable challenge ever. It has been pined that the selection for yield alone may not be sufficient to achieve this task. A narrow variability among the already improved cultivars, a high GxE interaction and low heritability of yield per-se are the factors associated with the reduced success while selecting for absolute yields (Sheshshayee et al., 2003; Araus et al., 2008; Reynolds and Tuberosa, 2008). This necessitates the evolution of a very focused and orchestrated approach associated with the improvement of constitutive traits associated with crop growth and productivity, especially under water limited conditions. Reshuffling alleles to enhance stress adaptation through molecular breeding and introduction of novel genes with proven relevance in providing tolerance to cellular mechanisms through transgenic technology are the most appropriate approach to be adopted.

It is apparent that the future breeding programs must concentrate on dissecting growth and yield components and enhance those physiological traits that would lead to improved growth and productivity under a given condition. Hence, the new trend in breeding is increasingly shifting towards understanding the physiological basis of growth and yield. This shift

towards breeding for physiological traits also referred to as Physiological breeding or analytical breeding has greatest applicability in the future crop improvement attempts. Global initiatives also suggest that improving the constitutive traits associated with growth and productivity under water limited conditions alone would result in the required dividends in productivity. Plants have naturally evolved several mechanisms that help in escaping the stress effects. Most of these adaptive strategies evolved though have significance to survival, are often counter productive. Thus, several criteria have now been laid out while identifying traits.

1. The trait should have a cause-effect relation with yield
2. The traits should have greater heritability than yield
3. The traits must be more stable across environments
4. The traits should not be associated with reduced productivity when stress is not there.

Though several traits can be identified that meet these criteria, any physiological trait associated with maintenance of positive turgor and positive carbon gain alone would be useful for enhancing water productivity of crop plants. From this context, the ability of water mining associated with superior root system, enhanced efficiency of water use associated with better chloroplast mechanisms, water conservation strategy of the plants associated with the accumulation of epicuticular waxes have the greatest relevance. We provide experimental evidences that prove the relevance of these traits in sustaining growth and productivity under water limited environments. Further, we also demonstrate significant genetic variability in these traits among sets of large number of germplasm accessions in important crop species like rice and groundnut. These traits showed significantly higher heritability in the broad sense than total biomass and yield indicating the possibility of exploiting these traits in crop improvement.

Recent experimental evidences clearly point to the fact that several of these relevant traits need to be introgressed onto a single elite genetic background to be able to achieve a comprehensive improvement in productivity under water limited conditions. Introgression of complex physiological traits can be best achieved through the adoption of a focused molecular breeding approach. We demonstrated that a whole genome scan for identifying polymorphism using robust co-dominant markers among a set of diverse germplasm accessions have the greatest relevance in identifying QTL for several diverse traits simultaneously. Thus the adoption of LD based association mapping has the greatest relevance in improving productivity through trait introgression. The intricate details of phenotyping of the complex physiological traits, identification of QTL by association mapping leading to crop improvement to mitigate drought will be discussed.

UNRAVELING DROUGHT TOLERANCE MECHANISMS IN CROP PLANTS USING GENOMIC APPROACHES

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Drought is a major environmental stress factor that affects the plant growth and productivity. The effects of drought are likely to increase with global climate change and growing water shortage. Thus, for sustainable agriculture, an understanding of drought adaptive mechanisms and water use in relation to plant growth is of importance. Analysis of protective mechanisms of plants will contribute to our knowledge, which is essential for crop improvement towards drought resistance. The complex responses to drought, from perception to ultimate physiological changes, need to be considered at a global systems biology level to examine the multiple interactive components (Krishnan and Pereira, 2008). Since drought tolerance is a complex trait, targeted manipulation of crops for resistance is difficult and there is a need to examine the specific mechanisms associated with the resistance. Amongst many adaptive traits, a few characters such as water mining and water conservation, water use efficiency, and cellular tolerance to desiccation are considered to be relevant for drought adaptation. Efforts have been made to manipulate some of these characters with varied degrees of success. So far, most of the studies have concentrated on manipulation of characters associated with cellular tolerance (CT) since these are governed by one or a few genes. A number of genes have been identified to be involved in CT, and their functions were confirmed by transgenic approaches (Karaba *et al*., 2007; Nelson *et al*., 2007; Kathuria *et al*., 2009). Cellular tolerance is one of the major traits in drought acclimation, and hence we made an attempt to prospect candidate genes using drought adapted crop plants like finger millet (*Elucine coracana*) and peanut (*Arachis hypogeae*) experiencing drought stress. It is believed that stress responsive genes from adapted plants are superior and might be beneficial in inducing better acclimation response in related susceptible crops. Since genome information is limited in these crops, generation and characterization of stress specific ESTs could be a viable approach to prospect candidate genes. Such genomic resources are considered as important tools for plant functional genomics, which forms the basis to combine relevant traits of interest into a desired genetic background. Expression profiling of stress specific ESTs and full-length cDNA clones has lead to the identification of key regulators in plant adaptive responses and there has been a spurt in EST databases (Ronning *et al*., 2003; Vettore *et al*., 2003; Ramý´rez *et al*., 2005; Mishra *et al*., 2007; Govind *et al*., 2008; Li *et al.,* 2009; Maki *et al*., 2010).

The drought specific cDNA library developed from finger millet exposed to drought at whole plant level contained unique set of genes (Parvathi, 2010). Annotation of the sequenced clones revealed that stress inducible genes belonged to diverse classes like cytoskeletal structures (10%), protective proteins (10%), catalytic genes (9%) etc., which are thought to maintain and enhance CT. Analysis of a few selected genes by different approaches like e-northern, semi-quantitative and quantitative RT-PCR indicated the drought responsive nature of the genes. A few stress responsive genes include metallothionein, sinapyl alcohol dehydrogenase, farnesylated protein, protein phosphatase, and farnesyl pyrophosphate synthase. Two novel upstream regulatory genes TAF6 and SR signal recognition particle receptor (SR) were also found to be responsive to other abiotic stresses like salt, osmotic and oxidative stresses. Similarly, analysis of drought specific cDNA library developed from peanut leaf tissue yielded many useful genes, including many upstream regulatory genes (Govind *et al*., 2008; Pruthvi, 2007). Some of these groundnut genes are responsive to different abiotic stress inducers such as PEG, NaCl and methyl voilogen-induced (MV) oxidative stress. The transcription factors such as Basic Transcription Factor 3 (BTF3), Nuclear factor Y A7 (NF-YA7) and C3H zinc finger were up regulated under

different stresses. BTF3 showed two to four fold increase over control under PEG and MV treatment. Similarly, under PEG, NaCl and MV induced stress NF-YA7 and C3H zinc finger expression levels were over 15 fold more than that of control. Since drought tolerance is governed by multiple genes, we validated a few upstream regulatory genes by over expression studies in model system, tobacco (*Nicotiana tobacum)*. The transgenic plants exhibited superior phenotype under different abiotic stresses indicating that these regulatory genes play a critical role in stress tolerance. Since multiple genes and pathways contribute for over all acclimation response, it would be advantageous to stack a few validated upstream genes for achieving field level tolerance and sustain productivity under water limited conditions.

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