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POLYAMINES IN RESPONSE TO ARTIFICIAL WATER STRESS IN GROUNDNUT SEEDLINGS

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SUMMARY

The groundnut seeds of different varieties in each habit group i.e. bunch, spreading and semi-spreading were germinated for 15 days. Irrespective of habit groups total polyamines level in root and shoot varied from 2.5 to 4.9 μmol/g fw and 2.8 to4.6 μmol/g fw, respectively. In each habit group, roots of GG-2 (bunch), G-13 (spreading) and G-20 (semi-spreading) had greater amount of total polyamines. In shoot similar results were obtained except that among spreading varieties instead of G-13, M-13 had higher concentration of total polyamines. Artificial stress (PEG 6000) treatment caused increase in total polyamine levels of both root and shoot tissues but the increase varied from variety to variety. Putrescine application just before PEG stress prevented the fall in tissue moisture content in water deficit seedlings.

Key words: **Drought, groundnut, moisture, putrescine, seedling**

INTRODUCTION

Polyamines are ubiquitous compounds in higher plants. They play an important regulatory role in plant growth and development. Earlier work revealed that polyamine biosynthesis increased as a result of water deficit stress in wheat (Flores and Glaston 1982), in ragi (Kandpal and Rao 1985), and in oat (Tiburcio *et al.* 1993). Higher levels of polyamines in stressed plants are adaptive in nature because of their role in the regulation of cellular ionic balance, maintenance of physical and chemical properties of membrane, prevention of chlorophyll ions and stimulation of synthesis of protein and nucleic acid (Glaston 1983, Slocum *et al.* 1984, Evans and Malmberg 1989, Kumar *et al.* 1997). The accumulation of polyamines in relation to stress is well documented in a number of crops. Very little information is available on their role in the mechanism of stress tolerance. In the present investigation an attempt has been made to study the variability in total polyamines levels and effect of foliar application of putrescine under drought conditions on different habit groups of groundnut.

MATERIALS AND METHODS

Groundnut seeds of different varieties in each habit group i.e. bunch (GG 2, GG 4, GG 5, TG 26), spreading (GG 11, GG 12, GG 13, ICGS 37, M 13 and Somnath) and semi-spreading (GG 20, Kadiri 3) were obtained from Oilseed Research Station, Gujarat Agricultural University, Junagadh. Seeds of each genotype were sown in plastic trays (45 cm × 30 cm) filled with sand. After 15 days of germination, seedlings were uprooted, washed and transferred in a 250 ml beaker filled with either 100 ml distilled water or 25% PEG-6000 solution for 24 hours. Immediately after transferring the seedlings to 25% PEG 6000 solution, one set of seedlings was sprayed with 0.1 mM Putrescine. After 24 h, seedlings were removed from the beaker and root and shoot tissues were separated.

Moisture content : Moisture content of root and shoot tissues were estimated as loss of weight gravimetrically by drying the samples in an oven (A.O.A.C. 1980).

Total polyamines : Total polyamines were extracted from root and shoot tissues with 4% perchloric acid (1 : 10 w/v) using mortar and pestle and estimated as described by Friedman *et al.* (1982). The extract was centrifuged and supernatant was collected. A suitable quantity of extract was transferred in glass vials and neutralized with saturated freshly prepared sodium carbonate solution. In the vials 0.4 ml dansylchloride was added. The content was mixed by vortexing and incubated overnight in dark. To the vials, 100 µl solution of L- proline was added and incubated for 30 minutes to remove excess fluorescence. Total polyamines were extracted with 2 × 0.5 ml toluene. Suitable quantity of extracted polyamines was taken in a cuvette and volume was made upto 4.0 ml with toluene. The fluorescence was measured in photofluorimeter (Systronics) using primary filter no. 5840 and secondary filter no. 4308.

RESULTS AND DISCUSSION

Root moisture content of groundnut varieties varied from 70.2-85.8% in control tissue (Fig. 1). Average moisture content of bunch and semi-spreading habit group has almost similar value (82%) while spreading habit group had a lower root moisture content (77%).

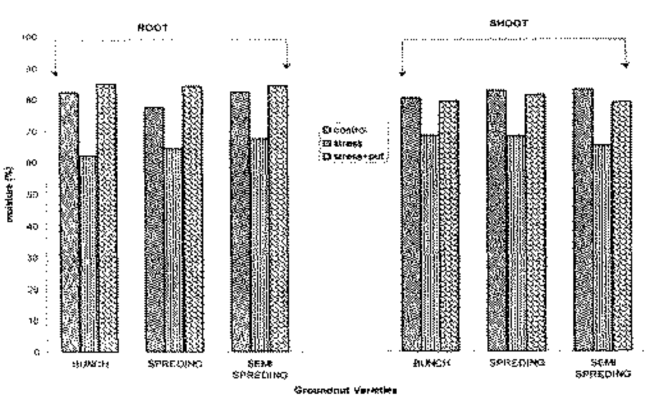

Fig. 1. Effect of PEG and putrescine application on root and shoot moisture content in different groundnut varieties

Among the bunch type variety, variety GG-4 (79%) had lowest root moisture content (Fig. 2A) while highest moisture content was recorded in GG-2 (85.8%). In case of spreading habit group, variety M-13 (70.2%) had lowest root moisture content while maximum moisture content was recorded in the variety G-11 (84%). Semi-spreading type variety Kadiri (84.4%) had higher moisture content than the variety GG-20 (80.2%).

In response to PEG 6000 stress, roots of all the varieties showed varied loss of moisture (Fig. 2A). Variety GG-4 (bunch), GG-12 (spreading) and GG-20 (semi-spreading) showed minimum loss of water in each group (Fig. 2A). Bunch type varieties showed greater loss (20.0%) of moisture (water content) when exposed to drought treatment as compared to semi-spreading (15.0%) and spreading habit group (12.9%).

Seedlings treated with 0.1mM putrescine just before imposition of PEG treatment had greater retention of moisture content in all the varieties as compared to seedlings not treated with putrescine (Fig. 2A).

Moisture content in shoot tissues of 12 groundnut varieties varied from 78.9-86.8% in control Spreading (83%) and semi-spreading (83%) group of varieties had only slightly higher moisture content in shoot tissues as compared to the bunch (81%) type habit group (Fig. 1).

Irrespective of habit group, PEG stress treatment resulted in loss of moisture content in shoot tissues and the loss of water varied in different varieties (Fig. 2B). Bunch type varieties showed an average 12% loss of water in response to PEG treatment. Variety GG-4 lost minimum water (5%) while in variety TG-26 loss of water content was maximum (18%). In spreading varieties the mean loss of water in response to PEG treatment was (14.3%). Among the spreading variety GG-11 (7%) and Somnath (7.3%) lost minimum water due to PEG treatment. In contrast to this, greater reduction in water content was noticed in varieties GG-13 (19.7%), GG-5 (18.4%) and GG-1 (18.6%). In case of semi-spreading varieties about 18% moisture was lost due to PEG stress treatment. The loss of moisture content in shoot tissues was almost similar in both the semi-spreading varieties i.e. GG-20 (17.5%) and Kadiri (18.0%).

Irrespective of habit group, seedlings treated with 0.1mM putrescine just before imposing PEG 6000 stress resulted in higher shoot moisture content, as compared to the control.

Total polyamine level in root tissues of groundnut varieties from control treatment varied from 2.52-4.90 µmoles/g/fr.wt. Various habit groups of groundnut showed

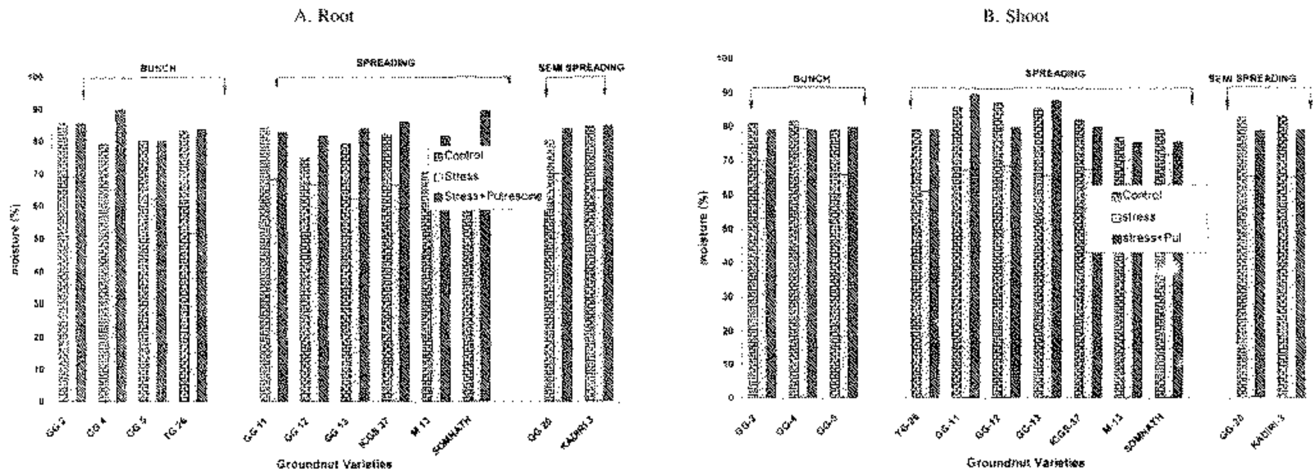

Fig. 2. Effect of PEG and Putrescine on A: root and B: shoot moisture content

narrow variation in their total polyamines level i.e. 3.4-3.9 μmoles/g/fr. wt (Fig. 3). Among bunch type GG-2 (4.95

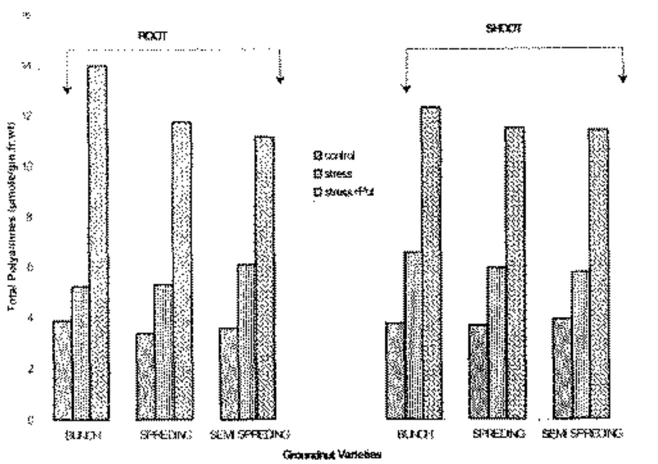

Fig. 3. Effect of PEG and Putrescine application on root and shoot total polyamines level in different types of groundnut varieties

μmol), spreading type GG-13 (4.15 μmol) and semi-spreading type varieties GG-20 (4.42 μmol) had maximum amount of polyamines (Fig. 4A).

In response to PEG stress, the total polyamines accumulated in root tissues and the rate of accumulation varied in different habit groups (Fig. 4A). Root tissues of bunch type varieties showed less accumulation of total polyamines (35%) while highest accumulation was recorded with semi-spreading group (70%).

Foliar application of 0.1 mM putrescine prior to stress treatment resulted in accumulation of total polyamines in the root tissues.

Irrespective of habit groups, total polyamines levels in shoot tissues varied from 2.8 to 4.6 μmoles/g/fr.wt., bunch varieties GG-2 (4.69), spreading variety M-13 (4.42) and semi-spreading variety GG-20 (4.56) had maximum amount of total polyamines (Fig. 3).

Artificial water deficit stress resulted in accumulation of total polyamines in shoot tissue. About 10-12% increase in total polyamines over the control value was recorded between the bunch and semi-spreading group of varieties. In contrast to this spreading varieties showed greater variations i.e. 8-16% (Fig. 4B). Overall it was observed that bunch type varieties showed maximum accumulation (74%) followed by spreading (61.9%) and semi-spreading (47.1%).

Seedlings treated with 0.1 mM putrescine prior to stress treatment resulted in accumulation of total polyamines in shoot tissues in general.

Calculating polyamine accumulation per gram water loss under PEG stress condition showed that greater accumulation of polyamines was associated with reduction in the amount of water lost (Table 1).

Thus the accumulation of polyamines in groundnut root and shoot tissues under drought condition may be of adaptive nature where it fulfils the protective and osmoregulatory role. Chan and Kao (1993) have reported that excised rice leaves accumulated putrescine under osmotic stress. Greater accumulation of diamine putrescine

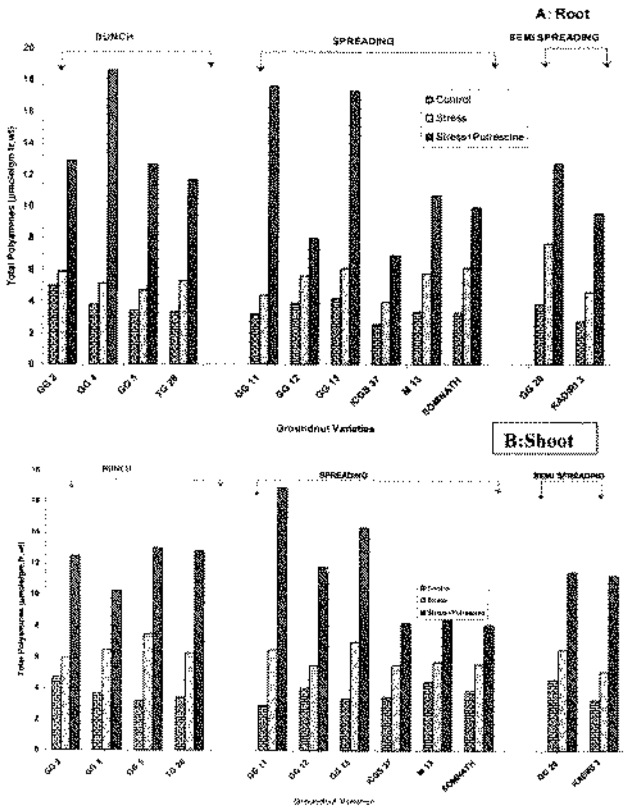

Fig. 4. Effect of PEG stress and Putrescine application on A: Root and B: Shoot polyamine level in groundnut seedlings

Table 1. Per cent loss of moisture in shoot & root tissue and polyamine accumulation

	% loss of moisture		**µmole polyamines Increased per gram loss of water**	
	Shoot	**Root**	**Shoot**	**Root**
Bunch				
GG 2	10.9	16.9	0.116	.054
GG 4	4.9	13.6	0.569	.164
GG 5	13.3	18.0	0.319	.069
TG 26	18.0	31.6	0.158	.062
Spreading				
GG 11	18.6	15.9	0.192	.073
GG 12	18.4	8.4	0.077	.205
GG 13	19.7	16.6	0.052	.113
ICGS 37	14.7	15.5	0.135	.092
M 13	6.9	7.6	0.183	.314
SOMNATH	7.3	13.4	0.233	.210
Semi-spreading				
GG 20	17.5	10.2	0.109	.310
KADIRI 3	18.0	19.8	0.099	.093

in potassium deficiency and under a variety of stresses have been reported by Flores *et al.* (1985) and Smith (1985). They suggested that polyamines at physiological pH might serve as counter ions and play important role in maintaining cellular homeostasis (Smith 1985). It is believed that polyamines acts as osmotica, stabilizing macro molecules and membrances and detoxifying tissues of excess of nitrogen (Rabe 1990, Hung *et al.* 1994).

Polyamines also play a major role in altering the level of the endogenous hormones ABA, cytokinin and ethylene and also help in maintenance of water balance. Our data showed that putrescine spray just before stress conditions prevented the loss of moisture content in root and shoot tissues. This may suggest a change in membrane permeability (Naik and Srivastava, 1978). The capacity to maintain higher moisture in root and shoot under water deficit stress is consistent with the ability to postpone dehydration in *Phaseolus* species (Castonguay and Murkhart 1991, 1992) and in pea seedlings (Upreti and Murti 1999).

Present results showed that the checking water loss and higher accumulation of polyamine might be an adaptive/tolerant nature of the varieties. Application of putrescine just before stress treatment also improved the moisture retention capacity.

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