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AMELIORATION OF DROUGHT STRESS BY ASCORBIC ACID, THIAMIN OR ASPIRIN IN WHEAT PLANTS

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SUMMARY

The effect of water deficit (100%, 70%, 50% and 30% field capacity) and ascorbic acid, thiamin or salicylic acid (100 ppm) and their interaction on some relevant metabolic changes of wheat plants (*Triticum aestivum* L.) were studied. Most of the cell wall components (pectin, hemicellulose, cellulose and lignin) of shoots and roots, soluble protein of shoots and other free amino acids of roots were reduced with the reduction of soil moisture content for 15 days, in the range studied. On the other side, the decreasing of soil moisture content induced progressive increase in soluble sugars, starch, proline contents of shoots and roots, other free amino acids of shoots and soluble protein of roots. Soaking of wheat grains in 100 ppm ascorbic acid, thiamin or salicylic acid before sowing did not improve pectin, hemicellulose of shoots and roots, cellulose of shoots, lignin of roots, other free amino acids of roots and soluble protein of shoots, whereas cellulose of root and lignin of shoots of soaked seedlings increased with decreasing soil moisture content. However, soluble protein, proline of roots and proline and other free amino acids of shoots was increased with the decrease of soil moisture content, but these effects were lowered to a greater extent under drought with vitamins or aspirin than drought only.

Key words : **Ascorbic acid, aspirin, drought stress, wheat**

INTRODUCTION

Drought is one of the most important environmental stresses influencing the productivity of agricultural systems around the world. The losses of production in major droughts, especially in developing countries, can be devastating. Wheat is a major crop grown in large irrigated areas as well as in rainfed areas. However, with an increase in human population, particularly in developing countries, there is an urgent need for expanding its cultivation from irrigated or relatively high rainfall areas to non-irrigated low rainfall areas. Therefore, in this investigation it was intended to test the role, if there is, of some vitamins or aspirin in counteracting the adverse effect of drought. It was intended to examine whether the soaking of seeds before sowing in vitamins (ascorbic acid or thiamin) or aspirin (sodium salt of salicylic acid) could ameliorate the inhibitory effects of drought on cell wall components, carbohydrates, proteins, proline and other free amino acid of wheat plants.

MATERIALS AND METHODS

Plastic pots (11.5 cm in diameter and 10 cm long) lined with polyethylene bags and containing soil composed of clay and sand (1:1 by volume) were used. The grains of wheat before sowing were soaked for 6 hrs in solutions containing 100 ppm of either ascorbic acid, thiamin or sodium salt of salicylic acid. After sowing (5 grains in each pot), the pots were then irrigated and the water content of the soil was adjusted regularly near to the field capacity and the plants were left to grow for 15 days. Thereafter, the pots were only watered to the desired soil moisture content (70%, 50% and 30% field capacity) that were further kept by variable daily irrigation. Some pots were left untreated (100% field capacity and 0.0 vitamins or aspirin) and regarded as absolute control. On the other side, droughted plants but non-treated with vitamins or aspirin were regarded as reference control. At the end of the experimental period (30 days) fresh shoots and roots were then dried in an oven at 70°C. Cell wall fractionation was conducted essentially according toDever *et al.* (1968) and Galbraith and Shields (1981). Aliquots of protoplast cultures were extracted twice in 80% ethanol to remove soluble metabolites. The precipitate was then extracted in 2 ml 0.5 N NaOH for starch, 0.5% ammonium oxalate-oxalic acid (90°C for 24 h) for pectin, 17.5% NaOH for hemicellulose and in 72% H_2SO_4 (with 15 min autoclaving) for cellulose extraction. After that, no precipitate was found. According to Dever *et al.* (1968) such a precipitate was ascribed to the lignin fraction. Contents of soluble sugar, starch and wall polysaccharides were determined by the anthrone sulfuric acid reagent using glucose as a standard (Fales, 1951). Free amino acids were determined according to Moore and Stein (1948), free proline according to Bates *et al.* (1973), amount of proteins according to Lowry *et al.* (1951).

RESULTS AND DISCUSSION

Cell wall metabolism is an important component in plant growth, not only because cell walls compose a large proportion of the cell biomass, but also because of the role of cell wall metabolism in determining wall extensibility for cell enlargement (Zhong and Lauchli, 1988). In this investigation, pectin, cellulose, hemicellulose and lignin as the major components of plant cell walls were quantitatively determined in stressed wheat plants. The cell wall component of shoots and roots were significantly decreased with the decrease of the soil moisture content except at 30% field capacity, the hemicellulose of shoots and at 70% feild capacity the cellulose of roots were increased (Table I and II). Osmotic stress modifies the biochemcial changes taking place in the cell walls during growth and thus preventing extension (Van Volkenburgh and Boyer, 1985). Working with tobacco cell cultures in the presence of polyethylene glycol, Iraki *et al.* (1989) described a decrease in the percentage of cellulose and an increase in the hemicellulose fractions, whereas the pectic fractions remained more or less constant. Similar results were obtained by Wakabayashi *et al.* (1997). The possible mechanisms for the inhibitory effect of salinity stress on incorporation of glucose into cell wall polysaccharides have been discussed (Hassan-Porath and Poljakoff-Mayber, 1973).

The adverse effects of drought treatments on pectin, hemicellulose and cellulose of shoots and pectin, hemicellulose and lignin of roots were partially alleviated by soaking grains in ascorbic acid, thiamin or aspirin (100 ppm). On the other hand, 100 ppm of any of the applied vitamins or aspirin, induced significant stimulatory effect on the lignin of shoots and cellulose of roots. Vitamins or aspirin may alleviated the inhibitory effect of drought on glucose incorporation into cell wall polysaccharides. Cellulose biosynthesis is particularly significant because of its interrelation with such processes as cell growth, plasma membrane function, and with the role of Ca^{2+} (Zhong and lauchli, 1988). These authors suggested that high Na^{+} concentrations reduced cellulose synthesis in cotton roots via disturbance of plasma membrane integrity. Treatment with vitamins or aspirin could, however, counteract this effect (Table I and II).

Soluble sugars and starch were increased by water stress in shoots or roots of wheat plants (Table III). Wherever affected by vitamins or aspirin, these two fractions exhibited mostly an increase. Gordon *et al.* (1986) showed that regrowth of defoliated white clover was associated with a decrease in starch and other carbohydrates in stolons and roots.

Water stress did not affect most of soluble protein content in the shoots of the stressed plants relative to their corresponding controls (Table IV). Very little changes are also observed in plant shoots when vitamins or aspirin was treated. By comparison, soluble proteins were increased in plant roots. Treatment with vitamins or aspirin increased soluble proteins in wheat roots relative to their corresponding control plants (Table IV.)

Proline accumulation is a well-known response to water deficit (Yadav *et al.*, 1997). It is clear from the test plant responses that, in the range of studied stress, the decrease in soil moisture content, generally, exerted a significant stimulatory effect on the accumulation of proline in the different organs of wheat plants (Table IV). The highest proline concentration in shoots or roots of wheat plants was consistently found in plants which grow in the lowest soil moisture content. Vitamins or aspirin

Table I. The action of ascorbic acid (AA), Thiamin (B_1) and aspirin (Asp.) treatments in ameliorating the adverse effects of water stress on cell wall of shoot of wheat plants. *Significant (P = 0.05) and **highly significant (P = 0.01) differences as compared with control.

Treatment	Shoot			
F.C. (%) + 100ppm	Pectin mg/g dry wt.	Hemicellulose mg/g dry wt.	Cellulose mg/g dry wt.	Lignin mg/g dry wt.
100+00	24.029	22.054	60.800	172.302
70+00	19.993**	16.214**	60.459	145.603**
50+00	17.210**	19.993	13.612**	132.344*
30+00	16.008**	30.127**	7.858**	125.733**
100+AA	24.836	20.353	51.092**	181.779*
70+AA	17.004**	17.391**	37.830**	189.283**
50+AA	18.894**	19.452	42.889**	185.234**
30+AA	18.859**	19.839	34.408**	178.038
100+B_1	27.962**	25.592*	53.028**	177.513
70+B_1	16.489**	20.439	23.827**	178.612
50+B_1	18.687**	23.832	27.609**	169.559
30+B_1	21.848	15.287**	23.659**	157.694**
100+Asp.	20.834*	21.693	54.975**	184.047**
70+Asp.	21.366*	24.218	20.353**	205.742**
50+Asp.	21.573	20.739	47.019**	213.751**
30+Asp.	17.416**	21.985	29.456**	235.109**
L.S.D. at 5%	2.654	3.009	2.228	7.184
L.S.D. at 1%	3.645	4.147	3.070	9.898

treatment on the other side, was significantly efficient to reduce the stimulatory effect of water stress on proline accumulation. Since proline accumulation could be regarded as an indicator of stress severity (Stewart and Larher, 1980), it can be concluded that the presence of vitamins or aspirin leads to low proline contents which means finally that vitamins or aspirin alleviated the toxic effect of water stress.

The data presented in Table IV clearly demonstrate that other free amino acids production and distribution in the different organs (shoots and roots) of wheat plants were substantially affected by the various drought levels with or without vitamins or aspirin treatments. It is interesting to note here that biphasic actions of most investigated drought levels with or without vitamins or aspirin treatments, involving stimulation of other free

Table II. The action of ascorbic acid (AA), thiamin (B_1) and aspirin (Asp.) treatment in ameliorating the adverse effects of water stress on cell wall of root of wheat plants. *Significant (P = 0.05) and ** highly significant (P = 0.01) differences compared with control.

Treatment F.C. (%) + 100ppm	Root Pectin mg/g dry wt.	Root Hemicellulose mg/g dry wt.	Root Cellulose mg/g dry wt.	Root Lignin mg/g dry wt.
100+00	65.555	47.005	51.342	189.663
70+00	42.482**	40.005**	69.133**	106.526**
50+00	41.679**	40.006**	37.071**	97.495**
30+00	40.879**	46.303	32.205**	88.786**
100+AA	39.333*	42.883**	56.172**	196.646*
70+AA	57.940**	30.487**	51.241	173.609**
50+AA	51.073**	31.773**	63.336**	168.528**
30+AA	46.601**	32.777**	72.854**	157.581**
100+B_1	24.905	77.005	53.828	192.101
70+B_1	17.680**	32.348**	101.981	163.803**
50+B_1	52.081**	37.858**	87.096**	159.971**
30+B_1	47.806**	40.649**	55.965**	147.036**
100+Asp.	47.634**	54.619**	58.023**	203.018**
70+Asp.	70.192**	30.415**	58.398**	179.392**
50+Asp.	52.844**	36.284**	90.674**	169.059**
30+Asp.	51.089**	37.787**	106.419**	163.308**
L.S.D. at 5%	1.475	1.116	3.259	5.419
L.S.D. at 1%	2.032	2.915	4.491	7.467

amino acids production in shoots and inhibition of their accumulation in roots were clearly displayed in wheat plants. This biphasic actions involving stimulation of other free amino acids accumulation in one organ and inhibition in other, clearly demonstrate the capability of drought stress with or without the three applied compound in playing a role in other free amino acids translocation from roots to shoots, a point which needs further investigation. In this context Boggess *et al.* (1976) concluded that water stress induced a promotion in proline accumulation at the expense of other free amino acids. On the other hand, Yadav *et al.* (1997) showed that free amino acids were variously accumulated under water stress. The pattern of changes in amino acids was opposite to that of soluble proteins, indicating that the increase in soluble protein is at the expense of other free amino acids

Table III. The interactive effect of drought-ascorbic acid (AA), drought-thiamin(B_1) and drought-aspirin (Asp.) on accumulation of carbohydrate in wheat shoots and roots. *Significant (P = 0.05) and **highly significant (P = 0.01) differences as compared with control.

Treatment	Shoot		Root	
F.C. (%) + 100ppm	Soluble sugars mg/g dry wt.	Starch mg/g dry wt.	Soluble sugars mg/g dry wt.	Starch mg/g dry wt.
100+00	2.727	51.756	14.471	36.653
70+00	34.009**	71.795**	39.917**	46.718**
50+00	38.417**	61.719**	44.898**	47.714**
30+00	44.199**	54.963*	48.883**	47.852**
100+AA	2.386	51.929	10.843**	38.439
70+AA	26.279**	76.776**	35.554**	47.886**
50+AA	33.093**	66.127**	40.089**	49.295**
30+AA	32.577**	58.913**	49.947**	51.356**
100+B_1	6.441**	66.528**	6.184**	30.384**
70+B_1	39.276**	83.303**	45.550**	45.894**
50+B_1	40.249**	80.956**	36.035**	71.313**
30+B_1	14.084**	67.272**	40.260**	45.585**
100+Asp.	4.437*	60.345**	5.604**	31.501**
70+Asp.	45.745**	75.975**	29.886**	38.439
50+Asp.	29.485**	78.150**	41.119**	49.398**
30+Asp.	56.394**	76.948**	66.333**	55.340**
L.S.D. at 5%	1.693	3.090	2.201	3.563
L.S.D. at !%	2.333	4.258	3.032	4.909

Table IV. Biosynthesis and accumulation of soluble protein, proline and other free amino acids in wheat shoot and root as affected by drought-ascorbic acid (AA), drought-thiamin (B_1) and drought-aspirin (Asp.) interactions. The data expressed as mg/g dry wt. *Significant (P=0.05) and **highly significant (P=0.01) difference as compared with control.

Treatment	Shoot			Root		
F.C. (%) + 100ppm	Soluble protein	Proline	Other free amino acids	Soluble protein	Proline	Other free amino acids
100+00	70.435	0.171	5.000	48.534	0.082	34.950
70+00	58.451**	0.787**	14.895**	83.225**	0.710**	11.533**
50+00	69.068	0.901**	13.376**	95.841**	0.796**	11.041**
30+00	79.266*	1.075**	16.963**	132.810**	0.911*	9.986**
100+AA	77.846	0.163	5.169	26.282**	0.106	53.516**
70+AA	52.248**	0.527**	13.249**	91.460**	0.586**	15.826**
50+AA	65.389	0.633**	17.384**	83.751**	0.458**	7.525
30+AA	76.112	0.695**	12.109**	79.196**	0.411**	13.713**
100+B_1	66.966	0.196	5.949	28.209**	0.115*	53.586**
70+B_1	66.966	0.589**	17.468**	85.328**	0.607**	33.826**
50+B_1	74.325	0.744**	15.189**	87.255**	0.562**	9.072**
30+B_1	73.589	0.839**	11.477*	81.123**	0.507**	13.221*
100+Asp.	85.205**	0.185	5.295	26.807**	0.109	37.342**
70+Asp.	43.417**	0.479**	16.540**	103.725**	0.469**	9.916**
50+Asp.	68.648	0.538**	17.089**	92.161**	0.378**	11.603**
30+Asp.	68.017	0.472**	14.219**	91.636**	0.318**	5.978**
L.S.D. at 5%	7.605	0.040	1.614	8.703	0.033	0.815
L.S.D. at 1%	10.479	0.055	2.224	11.992	0.046	1.123

through an effect of water stress and vitamins or aspirin in promoting their conversion.

In general, from the precedent discussion it could be concluded that water stress induced traits which are very complicated and involved physiological and biochemical processes deserve further consideration. Moreover, the beneficial effects of ascorbic acid or thiamin in mitigating partially or completely the adverse effects of drought stress may be one aspect of the role of these vitamins in the activation of some enzymatic reactions (Kefeli, 1981). Hamada (1998) attributed such positive effect of vitamins in counteracting the adverse effects of water stress to stabilizing and protecting the photosynthetic pigments and the photosynthetic apparatus from being oxidized. The mitigating effects of aspirin, an ubiquitous plant phenolic compound was recognized as an endogenous regulator in plants after the finding that it is involved in many plant physiological processes (Yalpini and Raskin 1993).

REFERENCES

Bates, L.S., Waldren, R.P. and Teare, I.D. (1973). Rapid determination of free proline for water stress studies. *Plant Soil,* **39** : 205-207.

Boggess, S.F., Aspinall, D. and Paleg, L.G. (1976). Stress metabolism. IX. The significance of end product inhibition of proline synthesis and of compartmentation in relation to stress-induced proline accumulation. *Aust. J. Plant Physiol.,* **3** : 513-525.

Dever, J.E. Jr., Banurski, R.S. and Kivilaan, A. (1968). Partial chemical characterization of corn root cell walls. *Plant Physiol.,* **43** : 50-56.

Fales, F.W. (1951). The assimilation and degradation of saccharides by yeast cells. *J. Biol. Chem.,* **193** : 113-124.

Galbraith, D.W. and Shields, B.A. (1981). Analysis of the initial stages of plant protoplast development using 33258 Hoechst: reactivation of cell cycle. *Physiol. Plant.,* **51** : 380-386.

Gordon, A.I., Ryle, G.J.A., Mitchell, D.F., Lowry, K.H. and Powell, C.E. (1986). The effect of defoliation on carbohydrate, protein and leghaemoglobin content of white clover nodules. *Ann. Bot.,* **58** : 141-154.

Hamada, A.M. (1998). Effect of exogenously added ascorbic acid, thiamin or aspirin on photosynthesis and some related activities of drought-stressed wheat plants. *In:* Proceeding of the XIth International Photosynthesis Congress. Budapest, Hungary, 17-22 August.

Hassan-Porath, E. and Poljakoff-Mayber, A. (1973). The effect of salinity on gluçose absorption and incorporation by pea roots. *Plant Cell Physiol.,* **14** : 361-368.

Iraki, N.M., Singh, N., Bressan, R.A. and Carpita, N.C. (1989). Alteration of the physical and chemical structure of the primary cell wall of growth-limited plant cells adapted to osmotic stress. *Plant Physiol.,* **91** : 39-47.

Kefeli, V.I. (1981). Vitamins and some other representatives of nonhormonal plant growth regulators. *PrikiBiokhim. Microbiol.,* **17** : 5-15.

Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randall, R.J. (1951). Protein measurement with the folin phenol reagent. J. Biol. Chem. **193** : 265-275

Moore, S. and Stein, W.W. (1948). Photometric ninhydrin method for use in the chromatography of amino acids. *J. Biol. Chem.,* **176** : 367-388.

Stewart, G.R. and Larher, F. (1980). Accumulation of amino acids and related compounds in relation to environmental stress. *In:* (Miflin B.J. ed): The Biochemistry of Plants. Vol. 5 pp. 609-635. Academic Press, New York.

Wakabayashi, K., Hoson, T. and Kamisaka, S. (1997). Osmotic stress suppresses cell wall stiffening and the increase in cell wall-bound ferulic and deferulic acids in wheat coleoptiles.*Plant Physiol.,* **113** : 967-973.

Van Volkenburgh, E. and Boyer J.S. (1985). Inhibitory effects of water deficit on maize leaf elongation. *Plant Physiol.,* **77** : 190-194.

Yadav, N., Gupta, V. and Yadav, V.K. (1997). Role of benzyladenine and gibberellic acid in alleviating water-stress effect in gram (*Cicer arietinum*). *Indian J. Agri. Sci.,* **67** : 381-387.

Yalpini, N. and Raskin (1993). Salicylic acid: a systematic signal in induced plant disease resistance. *Trends Microbiol.,* **1** : 88-92.

Zhong, H. and Lauchli, A. (1988). Incorporation of [^{14}C] glucose into cell wall polysaccharides of cotton roots: Effects of NaCl and $CaCl_2$. *Plant Physiol.,* **88** : 511-514.

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