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Effect of calcium and ABA on growth, proline accumulation and yield parameters of wheat (*Triticum aestivum* L.) genotypes under moisture stress

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Abstract

The present investigation records response of wheat (*Triticum aestivum* L.) genotypes *viz.* C 306, PBW 154, PBW 175, PBW 396, PBW 519, PBW 520, PBW 527, PBW 528 and WH 542 to induced mild (-2 bars) and moderate (-4 bars) moistures stress. The genotypes were evaluated for seedling growth represented by dry matter accumulation in embryos axis in control and moisture stress conditions induced by polyethylene glycol (PEG- 6000). Proline content accumulated in seedlings of various genotypes was variable and recorded more in C 306, PBW 527, PBW 528, PBW 396 than PBW154, PBW175, PBW519, PBW520. These were selected for detailed experimentation in field and moisture stress was induced at tillering and anthesis stage by stopping irrigation. Foliar application of calcium chloride (20 mg l^{-1}) and ABA (5 mg l^{-1}) was given ten days after stress period. Plant height, number of spikelets ear^{-1}, grain number $plant^{-1}$ and seed weight $plant^{-1}$ showed a decreased trend except those with foliar sprayed calcium C 306 and PBW 527. ABA application was ineffective to ameliorate moisture stress affect. An increased proline accumulation in C 306 and PBW 527 than PBW 154 and PBW 519 was recorded in moisture stressed plants. Among the genotypes studied presently only C 306 and PBW 527 were found to withstand moisture deficit conditions.

Key words: *Triticum aestivum*, genotypic variation, PEG, calcium, ABA, dry matter accumulation.

Introduction

Water has several important functions to perform in the plants and is most widely operative limiting factor in crop production. Moisture stress caused a reduction in plant growth by affecting a number of plant attributes. It causes loss of water from the tissues which directly affects various biochemical parameters. In agricultural system, the availability of water can be a primary limitation to the yield. In fact, the water deficit stress was predicated to cause 80% crop reduction as compared to its potential (Boyer, 1982). A reduction in water potential affects the physiology of cells in several ways. The germination phase is critical in the growth cycle of plant species since it determines the stand establishment of final yield of the crop. The addition of non-ionic osmotic agents such as polyethylene glycol (PEG 6000) to the liquid nutrient medium has been shown to closely mimic specific levels of soil moisture stress (Lu and Neumann, 1998). PEG can not cross membranes, thus water potential gradient in the tissue is not altered (McCain, 1997). The estimation of critical external water potential, below which seed germination is declined or reduced, is of prime agricultural importance for different crops species. It is known that genotypes of a species differ in their ability to cope with water stress and in the degree of osmoregulation. The evaluation of genotype-dependent variation in critical water potential is well-documented in several cereals. It is, intended, presently to investigate the effects of variable levels of imposed moisture stress on growth and some biochemical changes in relation to their potential to withstand it.

Material and Methods

For present investigation, wheat (*Triticum aestivum* L.) cultivars PBW 154, PBW 175, PBW 396, WH 542, C 306 and lines PBW 519, PBW 520, PBW 527, PBW 528 were selected. The screening of genotypes for their potential to withstand moisture stress conditions was conducted first under laboratory conditions. The selected genotypes were grown under field conditions. The initial screening the genotypes were subjected to low (-2 bars) and moderate (-4 bars) moisture stress induced by polyethylene glycol (PEG-6000) as suggested in method of Parmar and Moore (1966). The healthy seeds of each genotype were surface sterilized with 0.1% mercuric chloride for 5 min to avoid fungal infection during seed germination. Petri dishes were sterilized in an oven at 90°C for 2 h. Fifteen seeds were sown in each Petri dish containing PEG-6000 solution providing -2 and -4 bars of moisture stress. Distilled water was added to control Petri dishes. Germination was counted after three days. Shoots and roots were separated, dried in an oven at 80°C for 48 h and weighed to determine their dry weights

(DW). The content of proline in seedling grown under control as well as stress condition was estimated by the colorimetric method are given by Singh *et al.* .(1973).The field experiment was laid out in randomized block design and moisture stress treatments were given at tillering stage and anthesis stage by withholding irrigation. Foliar application ($CaCl_2$ (20 mg l^{-1}) and ABA (5 mg l^{-1}) was given ten days after moisture stress treatment. At maturity, plants were harvested and various parameters viz. plant height, number of spikelets ear^{-1}, number of grains $plant^{-1}$ and grain yield $plant^{-1}$ were calculated.

Results and Discussion

The variability among breeding material is of primary importance in the achievement of good crop production. A near perfect crop stand can be obtained by using high quality seed that ensure high germination percentage under favourable conditions. Among seedling traits, high emergence percentage followed by a high seedling vigor is necessary for the attainment of good crop stand. Wheat breeders are continuously seeking the improvement in wheat yield through various ways to ensure a nearly perfect stand of crop, which in turn depends upon good quality seed and with good germination percentage followed by the vigorous seedling growth which can escape hazards of stress conditions.

Root and shoot dry matter accumulation showed a decreasing trend under induced moisture stress. It was significantly decreased in both the roots and shoots of PBW 154 and PBW 519 whereas PBW 520 more than in C306, PBW396.WH542, PBW527, PBW528 studied presently. Shoot growth was drastically reduced than root (Table 1) because of severe water deficit condition possibly developed in transpiring shoots. Earlier, it is reported that coleoptile growth was more adversely affected than radical under moisture stress (Sheoran *et al.*, 1979).

The root: shoot ratio was enhanced by water deficit stress, whereas absolute weight of roots generally decreased under conditions of severe water deficit stress (Dhanda *et al.*, 2003). Root growth is markedly decreased plumule and radicle development was differentially affected by water deficit stress. PEG-induced water deficit stress decreased coleoptile and radical growth and reduced weight of embryo axis in PBW 154 and PBW 519 and PBW 520. Generally coleoptile growth was more affected than radicle under water deficit stress (Singh and Bhalla 1994). This can be used as a rapid screening technique to select cultivars of this crop for relative drought tolerance. F The genotypes selected presently C 306, PBW 527, PBW 154, PBW 519 and PBW 527 were able to withstand water deficit conditions better than PBW 154, PBW 519 selected for further experimentation in field conditions. The accumulation of free proline under moisture stress is widely used as an parameter for evaluating crop varieties for relative drought tolerance. The data presented in Table 2 revealed that proline content in seedlings of various genotypes grown under moisture stress was increased as compared with control. In some genotypes *viz.* C 306, PBW, 396, PBW 527 and PBW 528 proline accumulation was further increased when moisture stress was increased to -4 bars. It can be correlated to the better performance of these genotypes under moisture stress conditions. Proline accumulation is a wide spread response to water deficit stress caused by combination of increased biosynthesis and slower oxidation in mitochondria. Most of amino acid increased, but proline increased more than any other amino acid. The results indicated that denovo synthesis is a predominant mechanism of proline accumulation in durum wheat when germinating wheat seeds were subjected to abiotic stress, proline gets accumulated. The seedlings raised under water deficit stress (PEG-6000) exhibited enhanced proline level. This proline accumulation acts as a physiological trait for drought tolerance (Mumatz *et al.*, 1995). Accumulation of free proline under moisture stress at different growth stages in sorghum and cowpea (Parameshwara and Krishnasastry 1980) indicated that proline content was high when stress was induced at initial stages. Relative ranking of the genotypes in proline accumulation varied with the stage at which stress was imposed.

Table 3 depicts the data of various physiological parameters studied under field conditions in selected genotypes. A decrease in plant height was observed in all genotype due to tillering stage stress. The decrease in plant height was more pronounced in PBW 154 and PBW 519. This decrease in plant height in response to water may be due to the decrease in relative turgidity and dehydration of protoplasm which is associated with a loss of turgor and reduced expansion of cell and cell division. This decrease in same has also been reported by Guinta *et al.* (1998) and Iqbal *et al.* (1999). Plant produce their maximum biomass under adequate water supply, whereas water stress caused a marked decrease in plant biomass production.

Table 1. Effect of moisture stress on the dry matter accumulation in wheat (*Triticum aestivum* L.) genotypes

Genotypes	Root fresh weight	Root dry weight	Shoot fresh weight	Root dry weight
C 306				
Control	0.34	0.17	0.53	0.27
-2 bar	0.20	0.10	0.38	0.19
-4 bar	0.18	0.09	0.32	0.17
CD (P=0.05)	0.02	0.02	0.02	0.02
PBW 154				
Control	0.17	0.06	0.28	0.12
-2 bar	0.10	0.05	0.18	0.09
-4 bar	0.06	0.03	0.10	0.05
CD (P=0.05)	0.020	0.06	0.01	0.06
PBW 175				
Control	0.28	0.14	0.32	0.16
-2 bar	0.22	0.12	0.28	0.14
-4 bar	0.18	0.09	0.20	0.10
CD (P=0.05)	0.03	0.02	0.02	0.06
PBW 396				
Control	0.20	0.10	0.25	0.12
-2 bar	0.16	0.08	0.22	0.11
-4 bar	0.16	0.06	0.22	0.10
CD (P=0.05)	0.07	0.04	0.09	0.02
WH 542				
Control	0.22	0.12	0.22	0.12
-2 bar	0.18	0.08	0.20	0.10
-4 bar	0.12	0.04	0.20	0.09
CD (P=0.05)	0.02	0.06	0.020	0.01
PBW 519				
Control	0.10	0.04	0.22	0.10
-2 bar	0.07	0.03	0.16	0.07
-4 bar	0.04	0.02	0.12	0.06
CD (P=0.05)	0.06	0.06	0.01	0.05
PBW 520				
Control	0.16	0.08	0.24	0.12
-2 bar	0.10	0.05	0.14	0.07
-4 bar	0.08	0.04	0.10	0.05
CD (P=0.05)	0.01	0.02	0.06	0.02
PBW 527				
Control	0.20	0.10	0.30	0.15
-2 bar	0.18	0.09	0.26	0.13
-4 bar	0.12	0.06	0.24	0.12
CD (P=0.05)	0.02	0.06	0.01	0.09
PBW 528				
Control	0.20	0.10	0.28	0.14
-2 bar	0.16	0.08	0.24	0.12
-4 bar	0.12	0.07	0.20	0.10
CD (P=0.05)	0.02	0.02	0.01	0.01

Values represent mean of three replicates

Table 2. Effect of moisture stress on proline content (mg g^{-1} FW) in shoots of seven days old wheat (*Triticum aestivum* L.)

Genotype	Control	-2 bar	-4 bar	CD (P=0.05)
C 306	0.01	0.10	0.14	0.01
PBW 154	0.02	0.04	0.06	0.01
PBW 175	0.02	0.04	0.04	0.01
PBW 396	0.02	0.06	0.06	0.01
WH 542	0.02	0.04	0.05	0.01
PBW 519	0.01	0.02	0.02	NS
PBW 520	0.01	0.01	0.01	NS
PBW 527	0.02	0.12	0.16	0.02
PBW 528	0.02	0.14	0.18	0.02

Values represents mean of three replicates

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Table 3. Effect of moisture stress and foliar spray of $CaCl_2$ and ABA on plant height in wheat (*Triticum aestivum* L.) genotypes

Genotypes	Tillering					Anthesis			
	Control	Stress	Stress+ Ca	Stress+ ABA	Mean	Stress	Stress+ Ca	Stress+ ABA	Mean
C 306	90.9	81.0	85.13	72.93	82.49	87.13	88.50	80.76	86.89
PBW 154	83.51	62.23	70.20	68.10	71.01	82.36	82.76	78.30	81.73
PBW 519	96.15	67.33	69.79	64.26	74.38	68.14	96.33	98.16	89.69
PBW 527	98.19	86.23	86.63	73.38	86.11	96.26	96.70	84.60	92.94
Mean	92.19	74.19	77.93	69.67		83.47	90.07	85.45	

CD (P=0.05)

Genotype (G) = 0.44 Genotype (G) = 0.55

Tillering stage (S) = 0.44 Anthesis stage (S) = 0.55

Interaction (G × S) = 0.89 Interaction (G × S) = 0.11

Values represent mean of five replicates.

Stress + Ca: Stress + Foliar application of $CaCl_2$ (20 ppm)

Stress + ABA: Stress + Foliar application of ABA (5 ppm)

There is unanimous agreement and no denying for fact that yield of plant in drying soil is reduced even in tolerant genotypes (Leinhos and Bergmann 1995, Far and Allam 1995). Water deficit stress when given at anthesis stage has significantly reduced number of spikelets per ear in all genotypes as compared to control. Genotypes C 306 and PBW 527 produced maximum number of spikelets per ear under stress. Foliar application of calcium had ameliorated partially the adverse effect of water deficit stress at anthesis stage (Table 4). Foliar application of ABA (5 mgl^{-1} has significantly reduced number of spikelets per ear.

Table 4. Effect of moisture stress and foliar spray of $CaCl_2$ and ABA on number of spikelets per plant in wheat (*Triticum aestivum* L.) genotypes

Genotypes	Tillering					Anthesis			
	Control	Stress	Stress + Ca	Stress + ABA	Mean	Stress	Stress + Ca	Stress + ABA	Mean
C 306	17.70	12.00	13.00	9.00	12.91	12.70	12.70	9.00	13.00
PBW 154	15.70	11.00	10.00	8.00	11.17	7.70	7.00	8.00	9.58
PBW 519	12.70	7.00	7.00	7.33	8.50	9.00	6.00	16.70	11.10
PBW 527	20.00	19.00	18.60	8.00	16.41	16.70	17.00	8.70	15.60
Mean	16.52	12.25	12.15	8.08		11.52	10.70	10.60	

CD (P=0.05)

Genotype (G) = 0.82 Genotype (G) = 0.78

Tillering stage (S) = 0.82 Anthesis stage (S) = 0.78

Interaction (G × S) = 1.64 Interaction (G × S) = 1.57

Values represent mean of five replicates.

Stress + Ca : Stress + Foliar application of $CaCl_2$ (20 ppm)

Stress + ABA : Stress + Foliar application of ABA (5 ppm)

The data in Table 5 show the grain development was reduced in all genotypes. The stress at anthesis stage had more adverse effect as compared to stress at tillering stage. Foliar application of calcium (20 mgl^{-1}) has partially ameliorated adverse effect of water stress.

The effect of ABA (5 mgl^{-1}) was inhibitory on the number of grains per year. Rewatering after tillering stage stress overcome the adverse effect of stress in all genotype but anthesis stage moisture stress had significantly reduced grain weight. Foliar application of calcium (20 mgl^{-1}) has maintained grain weight per plant during stress condition. ABA application (5 mg l^{-1}) has reduced grain weight in all genotypes (Table 6). The decrease in grain weight may be due to disturbed nutrient uptake efficiency and photosynthetic translocation within plant (Iqbal *et al.*, 1999) that produced shriveled kernels due to hastened maturity. Water deficit stress reduced number of grain per spike and grain yield. The reduction in these parameters was more pronounced in PBW 154 and PBW 519. Acevedo (1991) observed that lower yields under the pre-anthesis water stress were due to reduction in both grain number and grain weight. The anthesis stage deficit moisture stress is critical for grain growth and adversely affected grain weight. Previously, Sivakumar *et al.* (2003) observed that ABA application decreased the grain weight, grain number and dry matter partitioning between ear and seed. Grain yield generally depends upon, tillers number, fertile spikelets, number of grains per ear and grain weight. It is well established that number of productive spikelets contributes in increasing the yield under water stress conditions. Water deficit stress when given at anthesis stage has significantly reduced number of spikelets per ear in all genotypes as compared to control. Genotypes C 306 and PBW 527 produced maximum number of spikelets per ear under stress foliar application of calcium had ameliorated partially the adverse effect of water deficit stress at anthesis stage (Table 4). Foliar application of ABA has significantly reduced number of spikelets per ear. Grain yield per plant was decreased significantly ($p<0.001$) in all genotypes in response to water stress. Whereas calcium treatment had amelioration effect as compared to control conditions. Hence C 306 and PBW 527 are considered as stress tolerant genotypes and foliar applications of calcium helped in sustaining growth under moisture stress to some extent.

Table 5. Effect of moisture stress and foliar spray of $CaCl_2$ and ABA on grain number per plant in wheat (*Triticum aestivum* L.)

Genotypes	Tillering					Anthesis			
	Control	Stress	Stress + Ca	Stress + ABA	Mean	Stress	Stress + Ca	Stress + ABA	Mean
C 306	351	340	346	266	326	241	247	161	250
PBW 154	343	327	338	166	293	201	205	92.33	210
PBW 519	281	278	238	194	248	141	147	51	155
PBW 527	326	320	341	246	309	221	232	121	225
Mean	325.25	316.25	315.75	218		201	207.75	106.33	

:D (P=0.05)

Genotype (G)	=	1.57	Genotype (G)	=	0.92
Tillering stage (S)	=	1.57	Anthesis stage (S)	=	0.92
Interaction (G × S)	=	3.15	Interaction (G × S)	=	1.85

'alues represent mean of five replicates.
tress + Ca : Stress + Foliar application of $CaCl_2$ (20 ppm)
tress + ABA: Stress + Foliar application of ABA (5 ppm)

Table 6. Effect of moisture stress and foliar spray of $CaCl_2$ and ABA on grain weight per plant in wheat (*Triticum aestivum* L.)

Genotypes	Tillering					Anthesis			
	Control	Stress	Stress + Ca	Stress + ABA	Mean	Stress	Stress + Ca	Stress + ABA	Mean
C 306	13.18	13.08	14.12	10.19	12.64	10.42	10.83	8.83	10.81
PBW 154	11.96	11.53	14.98	8.81	11.82	8.65	9.54	7.87	9.50
PBW 519	12.52	13.60	14.95	9.20	12.57	7.63	8.64	8.23	9.25
PBW 527	12.98	13.04	15.12	11.8	13.08	9.60	10.91	9.11	10.65
Mean	12.66	12.81	14.79	9.84		9.08	9.98	8.51	

CD (P=0.05)

Genotype (G)	=	0.05	Genotype (G)	=	0.26
Tillering stage (S)	=	0.05	Anthesis stage (S)	=	0.26
Interaction (G × S)	=	0.11	Interaction (G × S)	=	0.53

Values represent mean of five replicates.
Stress + Ca: Stress + Foliar application of $CaCl_2$ (20 ppm)
Stress + ABA: Stress + Foliar application of ABA (5 ppm)

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