Water relations of wheat influenced by exogenous application of growth substances under moisture stress

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Abstract

Water potential is amongst the most useful parameters for the measurement of the degree of water stress in plants. With the objective of mitigating adverse effects of moisture stress on water potential, osmotic potential and pressure potential of wheat variety Inquilab-91, a greenhouse experiment was conducted using two moisture levels viz., normal moisture supply and moisture stress at crown root stage. The moisture levels were randomized in main plots and growth substances including distilled water, Ethephon @ 400 mg l^{-1}, Paclobutrazol @300 mg l^{-1}, SADH @ 2000 mg l^{-1} and Triadimefon @ 200 mg l^{-1} were kept in sub-plots. Whereas, stages of growth substances application, i.e., pre-sowing seed treatment and foliar spray at crown root development, heading and grain filling were randomized in sub-plots. Water stress at crown root development stage was imposed simply by withholding irrigation. Moisture stress significantly lowered water potential and pressure potential and enhanced osmotic potential while growth substances application ameliorated all the traits under study; however, stages of application showed a varied response in this regard. Ethephon application @ 400 mg l^{-1} had pronounced effect with higher water potential (-1.584 MPa), osmotic potential (-1.781 MPa) and turgor potential (0.217 MPa) values. Amongst the stages of growth substances application, pre-sowing seed soaking in different growth substances was found to be the most effective in maintaining plant water balance. Interaction of moisture levels, growth substances treatments and stages of their application was highly significant. Thus, Ethephon application @ 400 mg l^{-1} as pre-sowing seed treatment under moisture stress conditions depicted the highest values of water potential (-2.080 MPa), osmotic potential (-2.268 MPa) and turgor potential (0.186 MPa).

Keywords: Triticum aestivum; exogenous application; growth substances; water potential; osmotic potential; pressure potential; Pakistan

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Introduction

Drought is the most serious abiotic stress to plants that leads to a remarkable reduction in crop yield. Pakistan is facing drought due to low rainfall and less water availability at farm gate. Drought affects plant water relations like water potential, osmotic potential, turgor potential and relative water contents. Water potential in plants is the sum of turgor potential, osmotic potential and matric potential. It is greatly lowered under water stress. A pronounced decrease in water

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potential was found in wheat due to water stress by Khan et al. (1993). Water use efficiency of wheat was observed higher in well-watered situation than in limited water supply. Water use efficiency has positive relation with stomatal closing by reducing transpiration (Abbate et al., 2004). Stomata get close in water limited condition and transpirational water loss is reduced to a greater extent. Leaf turgor and water potential are reduced in such a condition (Farooq et al., 2009). Osmotic adjustment under moisture stress conditions is said to be a drought avoidance mechanism. Under water stress conditions the plants which quickly adjust osmotically tend to survive. Osmotic adjustment is the result of accumulation of solutes within cells, which lowers the osmotic potential and helps in turgor maintenance (Ludlow, 1987; Blum 1996) which helps in stomatal conductance, cell expansion and growth of cells. Ashraf and Ahmad (1998) reported that leaf osmotic potential decreased considerably due to drought stress. Positive correlation was observed in osmotic adjustment and drought resistance and negative correlation in water retention capacity and drought resistance in four sorghum lines. Turgor potential is the first and the most sensitive component of water potential to be affected by water stress and loss of turgor may cause reduction in cell size accompanied by a reduction of leaf area resulting in reduction of the amount of photosynthates produced which in turn contributes to the reduction in overall plant growth. Moreover, many important physiological and morphological processes such as leaf enlargement, stomatal opening and associated photosynthesis are directly affected by the leaf turgor potential. Under water stress conditions plants lose their turgor to a point restricting cell expansion (Ludlow and Muchow, 1990). The plants must adjust their internal osmotic potential and sufficiently increase turgor to resume cell expansion and growth. The major mechanism of turgor maintenance is osmoregulation. Here solutes accumulate and water potential decreases allowing uptake of water for maintaining the turgor potential of the cell (Khan et al., 1992). Fisher and Cash-Clark (2000) observed that in water stressed plants there was a drop in turgor pressure in grain sieve tubes and vascular parenchyma cells. Foliar application of plant growth regulators improves growth under abiotic stresses (Taiz and Zeiger, 2006). Use of osmoprotectants helps in signaling and regulating plant responses to multiple stresses. Osmolyte accumulation is a vital mechanism for defense against drought stress. The intra-cellular osmolytes enhance water retaining capacity and help in alleviating harmful impacts of water stress on plants. Effects of drought can be minimized by introducing the drought tolerant varieties or by creating tolerance in already existing varieties. This can be done by spraying various compatible solutes to increase their endogenous level. The following study was therefore planned to decrease the damaging effects of drought on wheat crop by applying certain osmotica viz. Ethephon, Paclobutrazol, SADH and Triadimefon at various growth stages.

Materials and Methods

In this experiment five grains of wheat variety Inqulab-91 were sown in buckets employing factorial design having three replications in the greenhouse. Two moisture levels viz., no stress (M_1) and moisture stress (M_2) at crown root stage were randomized in main plots and growth substances including distilled water (T_1), Ethephon @ 400 mg l^{-1} (T_2), Paclobutrazol @ 300 mg l^{-1} (T_3), SADH @ 2000 mg l^{-1} (T_4) and Triadimefon @ 200 mg l^{-1} (T_5) in the sub-plots. Whereas, stages of growth substances application, i.e., pre-sowing seed treatment (S_1), foliar spray at crown root development (S_2), heading (S_3), and grain filling stage (S_4) were randomized in sub-plots. Water stress at crown root development stage was imposed simply by with-holding irrigation. Fertilizer NPK was applied @ 150-100-50 kg per ha^{-1} and all the other agronomic practices were kept uniform.

Data regarding Water potential, Osmotic potential and Turgor potential was recorded. For measuring water potential three fully expanded flag leaves were sampled per replicate from each treatment between 1100 and 1300 hours. Leaf water potential was measured using pressure chamber (Model OSK2710, OGAWA Seiki Japan). Pressure chamber technique is a relatively quick method for estimating the water potential of large pieces of tissues, such as whole leaves and shoots. Excised shoot with flag leaves were sealed in a pressure chamber. Before excision, the water column in the xylem was under tension. When the water column was broken by excision of shoot, the water was pulled into the xylem capillary by the then unopposed tension. The cut surface consequently appeared dull and dry. To make the measurement, the chamber was pressurized with compressed gas until the water in the xylem was brought back to the cut surface, which became wet and shiny when that pressure was attained. The pressure needed to bring the water back to the surface was detected by the change in the appearance of the cut surface. This pressure is called as balance pressure which is equal in magnitude but opposite in sign, to the negative pressure that existed in the xylem column before the plant tissue was excised.

For taking osmotic potential, plant leaves were washed in distilled water blotted dried with tissue paper and transferred to eppendorf tubes in deep freezer. The frozen samples were thawed, crushed with glass rod and the sap was centrifuged out at 600 x g. Osmotic pressure was measured with micro osmometer (Chamlab Ltd., Nuffield Raod, Cambridge UK) by calibrating the equipment in m.osmol kg^{-1} of water. The pressure was converted into potential by putting a negative sign as prefix to the figures. The concentration unit's m.osmole kg^{-1} of water was converted into pressure units, MPa using Vent Hoff relationship at 20 °C (Nobel, 1983).

Pressure potential of flag leaves was calculated putting the determined values of water potential and osmotic potential in the equation given by Kramer (1983) as follows:

$$\Psi_w = \Psi\pi + \Psi_p$$

where

Ψ_w = water potential

$\Psi\pi$ = osmotic potential

Ψ_p = turgor potential

Table 1
Effect of moisture stress and growth substances application at different ontogenesis on water relations in wheat leaves

Treatments	Water Potential - MPa	Osmotic Potential - MPa	Pressure Potential MPa
(a) Moisture Conditions.			
Normal Moisture	1.103 b	1.338 b	0.241 a
Moisture stress	2.146 a	2.313 a	0.162 b
(b) Growth Substances.			
Control	1.602 d	1.867 a	0.197 c
Ethephon	1.584 e	1.781 d	0.217 a
Paclobutrazol	1.654 b	1.800 c	0.213 b
Succinic Acid	1.621 c	1.801 c	0.181 e
Triadimefon	1.673 a	1.852 b	0.196 d
LSD at P≤5 (b)	0.00358	0.00870	0.00424
(c) Stage of G.S application			
Seed treatment	1.576 d	1.782 d	0.205 a
Spray at CRS	1.646 a	1.879 a	0.200 b
Spray at anthesis	1.629 b	1.828 b	0.200 b
Spray at G. filling	1.613 c	1.810 c	0.197 c
LSD at P≤5 (c)	0.00283	0.00201	0.00298

Results

Water potential

Water potential is the free energy available for movement of water or for the reactions involving water. Water potential is adversely affected by soil moisture deficit. A perusal of the data (Table 1a) revealed that water potential of wheat plant was significantly lowered due to water stress. It was -1.103 MPa in case of normal moisture supply and was lowered to -2.146 MPa under water stress. Growth substances application also had a significant effect on plant water potential, it was the maximum (-1.584 MPa) in Ethephon application and the minimum (-1.673 MPa) in Triadimefon application (Table 1b). Similarly, stages of growth substances application also showed a significant and differential response in this regard. As water stress was imposed at crown root development, the application of growth substances at this stage depicted more negative response with the lowest water potential of -1.646 MPa. Whereas, the highest water potential of -1.576 MPa was observed in case of pre-sowing seed soaking (Table 1c). Effect of interaction between moisture conditions and growth substances treatments varied significantly (Fig. I). Thus, the maximum water potential of -1.015 MPa was observed when distilled water was applied under normal moisture supply whereas the minimum water potential of -2.189 MPa was found in case of distilled water application under stress. This was followed by Paclobutrazol application under limited moisture supply with water potential of -2.174 MPa; whereas, the maximum water potential of -2.131 MPa under stress was noted when SADH was applied. Interaction between moisture conditions and stages of growth substances application also illustrated a significant effect on plant water potential. The maximum water potentials of -1.067 and -2.085 MPa were observed in case of pre-sowing seed soaking under normal and limited moisture conditions, respectively. Whereas, the minimum water potentials of -1.103 and -2.212 MPa were found with the application of growth substances at grain filling stage under normal moisture supply and their foliar spray at crown root development under moisture stress conditions, respectively (Fig. II). Differences among the means of interaction between growth substances treatments and stages of their application were significant. Pre-sowing seed soaking in 400 mg/l Ethephon solution exhibited the highest water potential of -1.580 MPa; whereas, the minimum water potential of -1.660 MPa was noted when Triadimefon was applied as foliar spray at crown root development (Table 2). Data in Table 3 shows the interaction between moisture conditions, growth substances treatments and stages of growth substances application where the differences among their means was significant. While pre-sowing seed soaking in distilled water under normal moisture supply led to the highest water potential (-1.041 MPa), it was the minimum (-2.215 MPa) in case of distilled water spray at crown root development under limited moisture supply.

Osmotic potential

It is obvious from the data given in Table 1a that osmotic potential of wheat plants was significantly decreased under moisture stress. It was -1.338 MPa in case of normal moisture supply and was lowered to -2.313 MPa under limited moisture conditions. Among the growth substances treatments, distilled water application showed the lowest osmotic potential of -1.867 MPa as against the highest of -1.781 MPa in case of Ethephon application (Table 1b). Stages of growth substances application expressed a differential response with the highest osmotic potential of -1.782 MPa when growth substance were applied as pre-sowing seed soaking followed by foliar spray at grain filling stage with osmotic potential of -1.810 MPa and it was the lowest (-1.879 MPa) when growth substances were sprayed at crown root development when

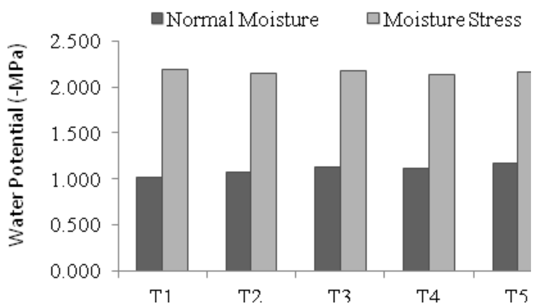

Fig. I. Effect of interaction between moisture conditions and growth substances treatments on water potential of wheat

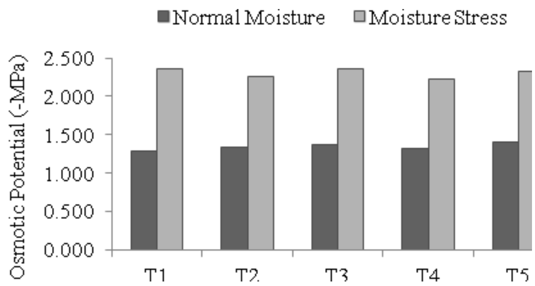

Fig. II. Effect of interaction between moisture conditions and growth substances treatments on osmotic potential of wheat

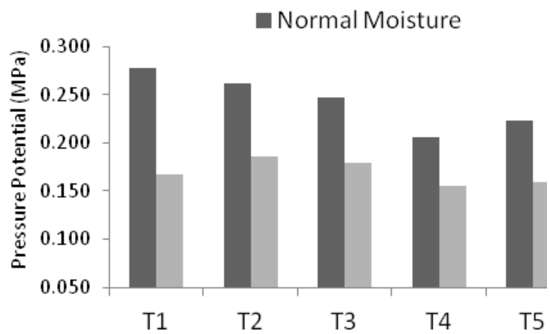

Fig. III. Effect of interaction between moisture conditions and growth substances treatments on pressure potential of wheat

Growth substance

T_1= Control

T_2= Foliar application of Ethephon @ 400 mg l^{-1}

T_3= Foliar application of Paclobutrazol @300 mg l^{-1}

T_4= Foliar application of SADH @ 2000 mg l^{-1}

T_5= Foliar application of Triadimefon @ 200 mg l^{-1}

Table 2
Effect of interaction between growth substances and stages of their application on water relations of wheat

Treatments	Stages	Water Potential (-MPa)	Osmotic Potential (-MPa)	Pressure Potential (MPa)
T_1	S_1	1.590	1.821	0.231
	S_2	1.625	1.874	0.202
	S_3	1.616	1.832	0.195
	S_4	1.608	1.806	0.172
T_2	S_1	1.580	1.775	0.208
	S_2	1.615	1.832	0.216
	S_3	1.606	1.817	0.216
	S_4	1.599	1.816	0.202
T_3	S_1	1.615	1.835	0.212
	S_2	1.650	1.821	0.216
	S_3	1.641	1.848	0.179
	S_4	1.634	1.847	0.197
T_4	S_1	1.598	1.803	0.205
	S_2	1.633	1.811	0.219
	S_3	1.624	1.812	0.180
	S_4	1.617	1.800	0.196
T_5	S_1	1.625	1.824	0.220
	S_2	1.660	1.855	0.212
	S_3	1.651	1.848	0.188
	S_4	1.643	1.837	0.194
LSD at P≤5		0.0634	0.0450	0.0666

Growth substances: T_1= Control, T_2= Foliar application of Ethephon @ 400 mg l^{-1}, T_3= Foliar application of Paclobutrazol @300 mg l^{-1}, T_4= Foliar application of SADH @ 2000 mg l^{-1}, T_5= Foliar application of Triadimefon @ 200 mg l^{-1}

Stage of growth substances application: S_1= Seed treatment, S_2= Spray at Crown root stage (CRS), S_3= Spray at anthesis, S_4= Spray at grain filling

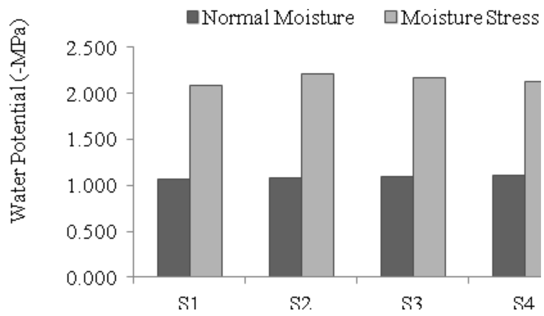

Fig. IV. Effect of interaction between moisture conditions and stages of growth substances application on water potential of wheat

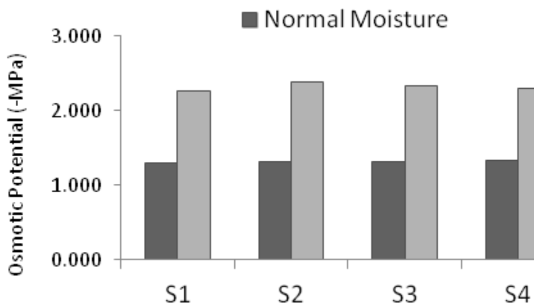

Fig. V. Effect of interaction between moisture conditions and stages of growth substances application on osmotic potential of wheat

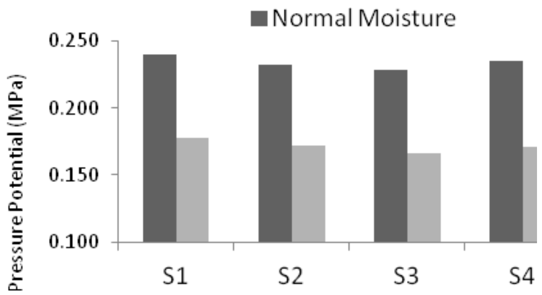

Fig. VI. Effect of interaction between moisture conditions and stages of growth substances application on pressure potential of wheat

Stage of growth substances application
S_1= Seed treatment
S_2= Spray at Crown root stage (CRS)
S_3= Spray at anthesis
S_4= Spray at grain filling

stress was employed (Table 1c). Interaction of moisture conditions and growth substances treatments had a significant effect on osmotic potential (Fig. III). It was the minimum (-2.356 MPa) when distilled water was applied under moisture stress; whereas, it was the maximum (-1.293 MPa) in case of distilled water application under normal moisture availability. Under moisture deficit stress the maximum osmotic potential of -2.226 MPa was exhibited by SADH application. Similarly, interaction of moisture conditions and stages of growth substances application predicted a significant effect on osmotic potential. Thus, it was the maximum (-1.307 MPa) in pre-sowing seed soaking under normal moisture supply. This was followed by foliar spray at crown root development with the osmotic potential of -1.311 MPa. Osmotic potential was the minimum (-2.384 MPa) in case of growth substances application at crown root stage under stress and it was the maximum (-.266 MPa) when pre-sowing seeds were soaked (Fig. IV). The differences among the means of interaction between growth substances treatments and stages of their application were significant on osmotic potential. Spray of distilled water at crown root development produced the minimum osmotic potential of -1.874 MPa, whereas, pre-sowing seed soaking in Ethephon solution depicted the maximum osmotic potential of -1.775 (Table 2). Effect on osmotic potential because of interaction between moisture condition, growth substances treatments and stages of their application varied significantly. Eventually, the maximum osmotic potential of -1.288 MPa was found in case of pre-sowing seed soaking in distilled water under normal soil moisture supply. Osmotic potential was the lowest (-2.368 MPa) when distilled water was sprayed at crown root stage when the plants were subjected to water stress. The highest osmotic potential of -2.262 MPa under stress was observed in case of pre-sowing seed soaking in SADH solution. This was followed by pre-sowing seed hardening in Ethephon solution with osmotic potential of -2.268 MPa (Table 3).

Pressure potential

It is evident from the data (Table 1a) that pressure potential (turgor potential) of wheat leaves was significantly lowered by water stress. It was 0.241 MPa in normal moisture supply and was decreased to 0.162 MPa under moisture stress. Thus, the decrease in pressure potential was amounted to 32 per cent. Pressure potential showed a varied response to various growth substances treatments. The maximum pressure potential of 0.217 MPa was observed with the application of Ethephon. This was followed by the application of Paclobutrazol with the pressure potential of 0.213 MPa. It was the lowest (0.181 MPa) in case of SADH application (Table 1b). Stages of growth substances application had a significant effect on pressure potential being the maximum (0.205 MPa) when growth substances were applied as pre-sowing seed soaking treatment. This was followed by foliar spray at crown root development and anthesis stage with pressure potentials of 0.201 and 0.200 MPa, respectively; while pressure potential was minimum 0.197 MPa when growth substances were sprayed at grain filling stage (Table 1c).

Differences among the means of

Table 3
Effect of moisture conditions, growth substances and stages of their application on water relations of wheat

Treatments	Stages	Water Potential (-MPa)		Osmotic Potential (-MPa)		Pressure Potential (MPa)	
		M_1	M_2	M_1	M_2	M_1	M_2
Control	S_1	1.041	1.804	1.288	2.297	0.287	0.167
	S_2	1.047	2.215	1.322	2.368	0.265	0.169
	S_3	1.054	2.177	1.319	2.343	0.264	0.169
	S_4	1.059	2.157	1.310	2.322	0.253	0.169
Ethephon @ 400 mg l^{-1}	S_1	1.072	2.080	1.351	2.268	0.279	0.186
	S_2	1.079	2.143	1.336	2.340	0.257	0.181
	S_3	1.085	2.119	1.341	2.309	0.255	0.180
	S_4	1.091	2.098	1.342	2.279	0.251	0.178
Paclobutrazol @300 mg l^{-1}	S_1	1.099	2.130	1.356	2.305	0.257	0.173
	S_2	1.105	2.193	1.352	2.366	0.247	0.173
	S_3	1.112	2.152	1.347	2.329	0.240	0.175
	S_4	1.117	2.131	1.363	2.303	0.250	0.172
SADH @ 2000 mg l^{-1}	S_1	1.088	2.109	1.329	2.262	0.203	0.155
	S_2	1.094	2.172	1.294	2.327	0.203	0.154
	S_3	1.101	2.148	1.311	2.308	0.212	0.160
	S_4	1.106	2.127	1.319	2.280	0.213	0.152
Triadimefon @ 200 mg l^{-1}	S_1	1.126	2.123	1.362	2.286	0.233	0.163
	S_2	1.132	2.186	1.351	2.347	0.216	0.162
	S_3	1.139	2.163	1.377	2.286	0.233	0.154
	S_4	1.144	2.142	1.372	2.303	0.222	0.158
LSD at $P \leq 5$		0.0897		0.0636		0.0943	

Moisture Condition: M_1= Normal moisture, M_2= Moisture stress
Growth substances: T_1= Control, T_2= Foliar application of Ethephon @ 400 mg l^{-1}, T_3= Foliar application of Paclobutrazol @300 mg l^{-1}, T_4= Foliar application of SADH @ 2000 mg l^{-1}, T_5= Foliar application of Triadimefon @ 200 mg l^{-1}
Stage of growth substances application: S_1= Seed treatment, S_2= Spray at crown root stage (CRS), S_3= Spray at anthesis, S_4= Spray at grain filling

interaction between moisture conditions and growth substances treatments were also significant. Under normal moisture supply, application of distilled water exhibited the highest pressure potential of 0.277 MPa. On the contrary, under moisture stress conditions the maximum pressure potential of 0.185 MPa was exhibited with Ethephon spray. Fig. (V) depicts the interaction between moisture conditions and growth substances treatments. Effect on pressure potential due to interaction between moisture conditions and stages of growth substances applications were significant. Pre-sowing seed soaking under both the moisture conditions proved to be the best with the maximum pressure potential of 0.240 MPa under normal and 0.178 MPa under restricted moisture supply (Fig. VI). Means of interaction between growth substances treatments and stages of their application varied significantly, the maximum pressure potential of 0.231 MPa was noted in case of pre-sowing seed soaking in distilled water and was followed by seed soaking in Paclobutrazol with pressure potential of 0.220 MPa (Table 2). As for interactions between moisture conditions, growth substances treatments and stages of their application, differences among their means were significant. Pre-sowing seed soaking in distilled water under normal supply showed the maximum pressure potential of 0.287 MPa followed by seed soaking in Ethephon with pressure potential of 0.279 MPa. It was the minimum 0.203 MPa each in case of seed treatment and foliar spray of SADH at crown root development under normal moisture supply. Whereas, under moisture stress conditions the maximum pressure potential of 0.186 MPa was found with the application of Ethephon as pre-sowing seed soaking. The minimum pressure potential of 0.152 MPa was noted in case of SADH spray at grain filling stage (Table 3).

Discussion

Maintenance of the physiologically active state in individual cells and the whole multi-cellular plants is dependent upon relative consistency of a number of conditions, one of which being favorable water balance. Under inadequate water supply, plants have reduced water content and on all vital functions proceeded at a reduced rate. Hence, proper plant water status is of prime importance for maintenance of turgidity required for plant growth and development. Under drought stress, osmotic adjustment resulting from accumulation of solutes lowers the osmotic potential helping to maintain turgor pressure of plants. Water potential which is one of the most useful parameters to measure the degree of water stress in plants, osmotic potential and turgor potential of plants experiencing water stress were adversely affected. There was a notable fall in water potential, osmotic potential and turgor potential in plants subjected to water stress at crown root development. This was associated with reduced water supply from the soil to the roots and ultimately to the leaves. Whereas, excessive accumulation of solutes, i.e., proline, ethylene and ABA was the cause of low osmotic potential and turgor potential created by water molecules, bombarding the surfaces of membranes and cell wall was attributed to lowering in water and osmotic potential. The results are in line with those of Pleijel et al. (2000), Zhang et al. (2001) and Tambussi et al. (2000) who also reported a remarkable decline in water potential, osmotic potential and turgor potential in plants subjected to water stress.

Growth substances treatments and stages of their application also had a significant effect on plant water relations. Ethephon application was noted to have a pronounced effect in this regard as it showed higher water potential, osmotic potential and turgor potential values. Among the stages of growth substances application, pre-sowing seed soaking in different growth substances was found to be the most effective in maintaining plant water balance. Growth substances when applied under water stress conditions possibly appeared to have acted as modulators of plant responses and helped the plants in better osmotic adjustment. The findings are in consistence with those of Chippa and Lal (1988), Pastor et al. (1999) and Blum (1996) who also reported a significant positive role of growth substances in maintaining water potential, osmotic potential and turgor potential under water stress conditions.

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