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Effect of Exogenous Application of Osmolytes on Growth and Yield of Wheat under Drought Conditions

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Abstract: The experiment was performed at Agronomic Research Institute, Plant Physiology Section, Ayub Agricultural Research Institute (AARI), Faisalabad, Pakistan. The trial was conducted in split plot design with 3 replications. Plot size was used 1.8 m × 5.0 m. The aim of this study was to examine the effect of various osmolytes on physiological mechanisms conferring drought resistance in wheat. Treatments were moisture conditions (Control/irrigation at 60% available water, moisture stress/irrigation at 35% available water). Osmoprotectants (Check, Water Spray, Salicyliacid @100 mM, Ascorbic acid @1.0 mM, Calcium chloride @ 100mM, Glycine betaine @ 0.7 mM, Proline@ 30 mM, Proline@ 60 mM). The recorded parameters during experiment were number of fertile tillers/m^2, plant height (cm), spike length (cm), 1000 grain weight (g), spike length (cm) and grain yield (kg ha^{-1}). The results of the experiment showed that grain yield reduced by the increase in water stress. Grain yield depends upon number of fertile tillers, number of grains per spike and 1000 grain weight. Maximum grain yield (4510 kg ha^{-1} and 4496 kg ha^{-1}), was obtained where salicylic acid and ascorbic acid were applied at the rate of 100 mM and 1.0 mM. Thus application of osmoprotectants significantly enhanced the grain yield, which indicated their potential role in crop improvement under stress environment.

Keywords: Wheat, osmoprotectants, plant height, biological yield, grain yield, water stress

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1. Introduction

Wheat (*Triticum aestivum* L.) is major cereal crop which is cultivated on more than 200 million hectares globally (Giraldo et al., 2019; Okada and Whitford, 2019; Tsvetanov et al., 2016) with production of 730 million tons (Mondal et al., 2016). It is predicted that 85% of global population relies on wheat to fulfill protein and calories requirement (Chaves et al., 2013). The wheat demands increase to 70% by 2050 (Chenu et al., 2017). Wheat is staple food of Pakistan (Rehmani et al., 2016) which is grown on 8.734 million hectares while its production was 25.492 million tons. The production of wheat was reduced to 4.4% than last year. The shortfall in wheat production is attributed to decrease in cultivated area, prolonged crushing season of sugarcane and critical water shortage. Wheat contributes around 10.3% to value added in agriculture sector economy and 2.2% in Gross Domestic Product (GDP) (GOP, 2017).

Osmolytes are small chemicals that affect osmosis and are soluble in cell solution. They perform a pivotal role in balancing the cell fluid and retaining the cell volume. Organic osmolytes are classifieds into four categories: carbohydrates $(CH_2O)_x$ e.g. amino acids e.g. taurine, proline, glycine and their derivatives (ectoine), polyols (inositols, sorbitol and glycerol) and solutes of methylsulfonium e.g. glycine betaine and urea (Yancey, 2005). Trehalose is white powder which is 45 percent sweet in taste as compared to sucrose (Jain and Roy, 2009) and keep their stability on hydrolysis (Walmagh et al., 2015). It is source of energy for initiating signaling pathways in stress and in sprouting spore (Elbein et al., 2003) and (John et al., 2017). During the stress environment, trehalose acts as osmoprotectant (Paul et al., 2008)

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and it stabilizes the membranes, microbial cells, DNA and enzymes (Jain and Roy, 2010). Naturally it is produced in small amount but it concentration increases when plant face stress (Kempa et al., 2008). It plays supreme role as signaling molecule during drought stress (Schluepmann et al., 2003).

Osmolytes controls the biological process like protein folding, interaction between proteins, causes hindrance in protein aggregation and inverse their miss-folding (Rabbani and Choi, 2018). It stabilizes protein structure and preserve the intracellular enzyme functions (Ishrat et al., 2018). Osmolytes act as anti-oxidants and involve in many bioprocesses such as immunological adaptation (Kumar et al., 2016), host-pathogen interactions, regulation of metabolism and volume of cell (Rahaman et al., 2018). Osmolytes prevent the protein denaturation persuaded by temperature (Adamczak et al., 2018). Osmolytes are neutral molecules (zwitterions) and are inert in nature hence don't have interactions with biological molecules. Due to inert properties of osmolytes, they stabilizes the proteins and boost activities of enzymes to protect the biomolecules from stress. Methylamines augments the protein folding and stabilization of nucleic acid and protein complexes (Ueda et al., 2016).

Glycine betaine (GB) is zwitterionic quaternary amine, whichprotects the biomolecules during drought condition (Chen and Murata, 2011) and its application encourages the growth of many plants under stress (Alasvandyari et al., 2017). Glycine betaine maintains the antioxidant enzyme activities, thus decreasing the antagonistic impacts of oxidative stress in wheat (Ma et al., 2006). Exogenous application of GB surges the K^+ contents and activities of enzymes and reduces the Na^+ contents in salt-stress plants (Alasvandyari et al., 2017). The synthesis of protein decreases due to osmotic stress while GB is synthesized. In higher plants, proline has key role in osmotic process (Hasegawa et al., 2000) to enhance drought tolerance. Proline acts as free radical to relieve the drought effects (Okuma et al., 2000). External use of ascorbic acid improves plant growth, phytohormone signaling, transport of ions and cell expansion under non-stress and stress condition (Darvishan et al., 2013).

Environmental stresses are major cause of yield reduction of many crops. The land suffers 20% due to mineral stress, 15% due to freezing stress and 26% by drought stress. Environmental stresses maybe biotic or abiotic. Salinity, flooding, heat, cold and drought are main abiotic stresses (Gontia et al., 2014). Drought is the major factor which confines the crop productivity (Naveed et al., 2014). Drought changes the plant morphological and physiological traits as leaf wilting, decrease in leaf area, root elongation and chlorophyll contents (Lata et al., 2011) by disturbing the turgor and water potential of plant (Vurukonda et al., 2016). Drought stress is everlasting and widespread environmental problem across the globe, including Pakistan (Hussain et al., 2014; Nezhadahmadi et al., 2013). Uncertainity regarding availability of water is increasing, with spatiotemporal variations (Bashir et al., 2019; Nawaz et al., 2016). With increasing drought wide range plant processes like photosynthesis, protein synthesis, photosynthetic pigments synthesis and lipid metabolism compromised leading to signigfcant reduction in yield. Drought is responsible for diminishing of mineral nutrients, water balance and permeability of membrane. So plants develop alteration in biochemical process such as growth rate, morphology and osmotic potential and enhancement of defense mechanism (Duan et al., 2007).

Drought leads to oxidative stress due to reactive oxygen species (ROS) accumulation in chloroplast (Ashraf, 2009). So the plants produce antioxidants e.g. tocopherol, phenolics and ascorbic acid to reclaim the effect of ROS (Weng et al., 2015). Ascorbic acid, an osmolytes, is non-enzymatic antioxidants that defends the plants against stress by inhibiting ROS (Shafiq et al., 2014) and is effective in enhancing plant biomass (Ejaz et al., 2012). Salicyclic acid performs major role in abiotic stress such as osmotic stress (Borsani et al., 2001) and in drought stress condition (Senaratna et al., 2000). Salicylic acid along with their derivatives control stress tolerance. Accretion of proline is common plant response to stress environment e.g. water shortage. Proline acts as osmoticum and compatible solute due to its high hydrophilic and zwitterionic characteristics. It functions as sink of nitrogen and carbon for the use after respite of drought. Glycine betaine is cellular osmolytes that increases the intracellular osmolarity during hyperosmotic environment. It soothes the protein and enzymes activities thus preventing the membrane from abiotic stress. Foliar application of glycine betaine restricts the photo-inhibition in wheat during freezing stress (France et al., 1998).

Drought has key role in limiting the crop yield across the world. The abiotic stress e.g. drought stimulate the gene expression that increase the osmolytes level in cell to stand with the stress. Osmolytes are neutral molecules that safeguard the proteins and other cell membranes against various stress factors on cellular metabolism (Yancey, 1994).

By keeping the above facts in assessment, the experiment was conducted in field having particular objectives; i) to evaluate the efficiency of foliar application of water and osmolytes on wheat crop under water stress ii) to assess the role of foliar applied water and osmolytes in increasing water deficit stress tolerance.

2. Materials and Methods

A field trial was carried out at Agronomic Research Institute, Plant Physiology Section, Ayub Agricultural Research Institute (AARI), Faisalabad, Pakistan during 2014-15 in Rabi season. The soil of experimental site was clay loam. The soil analysis was done from soil and water testing laboratory, Ayub Agricultural Research Institute, Faisalaabd and its data is given below.

Table 1. Soil Chemical Analysis

Soil Depth	pH	ECe	OM	P	K
cm		dS m^{-1}	%	mg kg^{-1}	mg kg^{-1}
0-15	8.2	1.57	0.61	8.2	195
15-30	8.0	1.50	0.59	7.9	180

The trial was laid out by using split plot design with three replications. Date of sowing was 20th November 2014. The size of plot was 1.8 m × 5.0 m. The fertilizer application was done at the rate of 160-120-60 N:P_2O5: K_2O kg ha^{-1}. All of the phosphorus (P) and potash (K) fertilizers were applied at time of sowing while the nitrogen (N) was applied in two split doses. Treatments were moisture conditions (Control/irrigation at 60% avaliable water, moisture stress/irrigation at 35% available water). Osmoprotectants (check, water spray, salicylic acid @100 mM, ascorbic acid @1.0 mM, calcium chloride @ 100mM, glycine betaine @0.7 mM, proline@ 30 mM and proline@ 60 mM). The recorded parameters and their procedures are described.

At maturity, numbers of tillers were counted from an area of 1 m^2 of 3 samples from each experimental plot. Then average was calculated for statistical analysis. The plant height (cm) of ten plants was calculated by using the meter rod. Ten spikes were selected at randomly from each plot and the spike length (cm) and average spike length was measured by using measuring scale. The number of grains/spike was counted from randomly selected spikes. 1000-Grains weight (g) was calculated from randomly selected three samples of 1000 grains, counted by using seed counter from three samples and then weighed using electrical balance. At harvest maturity, 4.8 m^2 area was harvested and tied into bundles. After manual threshing grains were weighed to calculate grain yield (kg ha^{-1}).

3. Results

3.1. Plant height

It is the main essential growth parameter which adds to the total plant yield. Plant height reduced significantly by increasing the drought stress. Height of wheat plants cultivated in normal and drought conditions indicates significant difference among treatments as shown in Table1. Among all the treatments, the maximum plant height (76.27 cm) was gained where proline was applied @ 60 mM followed by Proline spray @ 30 mM, glycine betaine and calcium chloride spray, whereas, the plant height was minimum (72.20 cm) in control under moisture stress conditions.

3.2. Number of productive tillers/ m^2

Fertile tillers are a significant parameter which indicates the total plant in an area. It determines the crop (LAI) which in turn affects the grain yield. In wheat, crop density is one critical yield determining factor. Water availability is associated with stand establishment. Total number of tillers declines as enhancing drought. The data about number of tillers is shown in table 1. The statistical analysis of the data shows that maximum no of tillers (284.00 m^{-2}) were obtained where salicylic acid was applied @ 100 mM followed by check (276.335m^{-2}) under moisture stress conditions while no of tillers were minimum (248.333 m^{-2}) in case where no foliar application done. The interaction shows that there is significant difference among the means of normal moisture level and stress.

3.3. Spike Length (cm)

Generally spike length is a factor which is mostly genetically controlled. Spike length influences plant biomass as well as yield. Data regarding spike length is given in the following (Table 1). The statistical analysis of the data shows that maximum spike length (8.866cm) was obtained in case of salicylic acid spray @ 100 mM while the minimum spike length (7.66 cm) was obtained in case of control under moisture stress conditions. The results obtained by water spray, ascorbic acid spray and proline spray do not show much difference. Spike length decreased significantly under stress conditions.

Table 2. Effect of moisture levels and foliar spray of water and osmolytes on plant height (cm), no. of tillers (m^{-2}) and spike length (cm) on wheat crop

Treatments	Plant height(cm)		No. of tillers (m^{-2})		Spike Length(cm)	
	Normal	Stress	Normal	Stress	Normal	Stress
Control	74.13 c	72.20 d	276.3 a	248.3 i	8.30 c	7.67 e
Water Spray	75.30 b	75.13 b	284.7 d	254.3 h	9.30 a	8.10 d
Salicylic acid spray@100mM	75.23 b	75.13 b	302.7 a	284.0 d	9.70 a	8.87 cd
Ascorbic acid @1.0mM	74.30 c	74.13 c	291.7 c	263.3 f	9.23 a	8.33 bc
Calcium Chloride @100mM	75.50 ab	75.27 b	287.3 d	263.7 fg	9.37 a	8.43 b
Glycine betain @0.7mM	75.30 b	75.13 b	297.7 b	272.7 e	9.43 a	8.63 b
Proline @30mM	76.27 a	74.57 bc	298.3 b	266.3 f	9.53 a	8.53 b
Proline @60mM	76.67 a	76.27 a	292.0 c	261.3 g	9.20 ab	8.47 cd
Mean	74.76 A	75.18 A	291.3 A	264.2 B	9.26 A	8.37 B

3.4. Number of grains/spike

The grains number is a vital factor giving rise to plant yield. Number of grains/spike are reduced due to drought stress. Data relating to number of grains/spike in the table indicated that the means of treatment vary significantly. Maximum grains per spike (48.33) were recorded in treatment of proline spray (@ 60mM) under moisture stress conditions. Salicylic acid and ascorbic acid help to mitigate the water stress and increase the no of grains per spike. Minimum no of grains were obtained in case of check and water spray under normal moisture conditions.

3.5. 1000 grain weight (g)

It is vital yield promoting parameter and reduces in drought stress. The weight of grains decreased significantly in the treatment on which drought was applied. The result shows that there is significant difference among the means of treatments. 1000 grain weight was maximum (35.467 g) where salicylic acid spray was applied @ 100 mM under normal moisture condition. While the minimum weight (31.633 g) was obtained in case of control under moisture stress.

3.6. Grain yield (kg ha^{-1})

It is the result of yield factors such as fertile tillers/plant, grains/spike and the weight of 1000-grain. Any variation in these factors will influence the crop grain yield. Grain yield is mostly connected to the availability of water. Plants sown under the well irrigated condition showed higher yield than drought stress plants.

Table 3. Effect of moisture levels and foliar spray of water and osmolytes on no. of grains/spike, 1000 grain wt (g), and grain yield (kg ha^{-1}) of wheat crop

Treatments	No. of grains/spike		1000 grain wt (g)		Grain yield (kg ha^{-1})	
	Normal	Stress	Normal	Stress	Normal	Stress
Check	52.3 abc	42.3 i	39.5 d	31.633 g	4250 c	3323 h
Water Spray	53.0 ab	44.3 hi	40.2 c	33.300 g	4520 a	3450fg
Salicylic acid @100mM	54.0 a	45.0 fghi	42.6 a	35.467 e	4510 a	3680 d
Ascorbic acid @1.0mM	54.3 a	46.3efgh	41.5 b	34.267 f	4496 a	3523ef
Calcium Chloride @100mM	52.0 abcd	44.7 ghi	41.6 b	34.100 f	4386 b	3460fg
Glycine betain @0.7mM	48.7 cdef	46.0 efghi	41.4 b	34.500 f	4356 b	3443 g
Proline @30mM	49.0 cde	47.3 efgh	40.6 c	34.200 f	4367 b	3473efg
Proline @60mM	49.7 cdef	48.3 defg	40.5 c	34.100 f	4383 b	3543 e
Mean	51.6 A	45.5 B	1.0 A	33.946 B	4412 A	3487 B

The result shows there is significant difference among their means. Under stress conditions, the grain yield was maximum (3680 kg ha^{-1}) where salicylic acid spray@100mM was applied, followed by ascorbic acid spray@1.0mM and proline spray@30mM (3523 kg ha^{-1} and 4373 kg ha^{-1}). Grain yield was significantly reduced under water stress.

4. Discussion

Drought is one of the main limitations for higher growth and yield of field crops. To overcome significant yield losses under water stress need promoting drought tolerance mechanisms in plants to overcome drought-induced hurdles (Singh et al., 2015). El-Monayeri et al. (1984) detected that plant height of wheat is affected by drought stress. The decrease caused by protoplasm dehydration, low relative turgidity linked to turgor loss and reduced cell expansion and cell division (Arnon, 1972). The external application of osmolytes improved the plant height under water deficit condition both at vegetative and at flowering stage because it increases the tolerance mechanism of plant under adverse environmental conditions. A marked increase of plant height was recorded in plant height of wheat by applying proline whereas; the plant height was minimum under normal moisture conditions.

In this study, we acquired the osmolyte accumulation which is based on differences in growth and yield production of wheat under drought stress. Drought stress harshly stopped growth, yield and characteristics which are related to wheat; however the results showed that maximum no of tillers were obtained where salicylic acid was applied as compared to drought stress. A decreasing trend was observed regarding number of tillers in drought stress levels Water stress during the stem elongation period prevents some tillers from producing spikes, although it does not show a whole loss of income, because before senescence, all assimilated are transported to the fertile tillers. The stress during the tillering stage has a negative influence on the production of tillers (Teruel and Smiderle, 1999).

Drought stress inhibited plant growth by decrease in biomass. Reduce soil water contents at reproductive stage had caused significant decrease in spike length. Decreases in these agronomic characters are linked with decline in the activity of meristematic tissues which are reliable for elongation as well as inefficiency of photosynthetic tissues under insufficient availability of water (Siddique et al., 1999). Under terminal drought stress, soil moisture reduces towards crop maturity. Plant height, number of spikes, spike length, grains and thousand grain weights were decreased significantly in various genotypes under drought stress (Mirbahar et al., 2009). We observed similar findings under water drought stress that maximum spike length was obtained in case of salicylic acid spray while the minimum spike length was obtained in case of control under normal moisture conditions. So b using osmoleytes significantly affected the spike length of the wheat plant.

Spike length determines the production potential of wheat. Moreover the spike size and higher the number of grains/spike are responsible for high yield. The plants gave maximum spike length which were kept in well watered and a result indicates that external applied enhanced the length of spike in the plants which faced the drought. Higher the length of spike was noted in plants on which ascorbic acid spray was applied at water stress condition. Deficit water significantly restricted the spike length. A similar finding was observed by Raza et al. (2014) under drought stress, the minimum spike length was observed. Foliar application with osmolytes increased spike length and spikelets number per spike in addition to number of grains/spike and seed weight in other wheat genotypes under stress.

Decrease in 1000 grain weight and grains/spike were observed under water stress which can be linked to reduced photosynthesis. The reduction in photosynthates production and their translocation to reproductive organs e.g grains due to drought stress (Asch et al., 2005). Reduced 1000 grain weight was observed due to water deficit condition (Iqbal et al., 1999). Khannachopra et al. (1994) reported that early maturity and shriveled kernels occur due to less effective uptake of mineral nutrient and inadequate photosynthetic translation. Under drought conditions, the 1000-grain weight was reduced (Sinaki et al., 2007). We also observed similar findings that 1000-grain weight and yield affected under drought stress but its impact reduced when we applying osmolytes.

5. Conclusion

The results of the experiment showed that grain yield was reduced by the increase in water stress. Grain yield depends upon the number of fertile tillers, number of grains per spike and thousand grain weights. Grain yield depends upon number of fertile tillers, number of grains per spike and 1000 grain weight. Maximum grain yield (4510 kg ha^{-1} and 4496 kg ha^{-1}), was obtained where salicylic acid and ascorbic acid were applied at the rate of 100 mM and 1.0 mM. It was, therefore, concluded that application of osmolite under drought conditions significantly enhanced the grain yield of wheat. More study is required to precise the beneficial role of osmolytes on the water deficient stress tolerance of crops. More emphasis is needed to explore the beneficial role of osmolytes in crops under water logging and salt stress conditions.

List of Abbreviations: cm: centimeter; M: meter; mM: milli Molar; g: gram; kg ha^{-1}: kilogram per hectare; pH: power of hydrogen; ECe: electrical conductivity; OM: organic matter; P: Phosphorus; K: Potassium; dS m^{-1}: deciSiemens per metre; %: percent; mg: milligram; No.: Number; wt; weight.

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