

Drought Stress Mitigation by Foliar Feeding of Potassium and Amino Acids in Wheat

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Abstract: Wheat growth and yield are highly affected when it suffers to drought stress. The effects of drought on wheat can be alleviated by the foliar spray of potassium and amino acids. This experiment was performed in the net-house, a pot study, conditions with the objective to mitigate the drought's threat against wheat growth and yield. In this study, three treatments were applied involving two different nutrients [F₀: Control; F₁: Amino Acids @ 5ml/L; F₂: 2% Potassium] which were applied through foliar spray on wheat grown in an aptly watered (WW: 60% of the field capacity) as well as under the drought (DD: 40% of the field capacity) situations in the soil stuffed earthen's pots. Crop was harvested at maturity and several growth related (no. of productive tillers/plant, no. of spikelets/spike, no. of grains/spike, hundred grains wt. and harvest index) attributes plus nutrient assimilated (leaf's potassium, nitrogen and phosphorus contents) by the wheat were determined. Relative water as well as chlorophyll content of leaf was also determined at 60 days after sowing (DAS). The outcomes of this trial illustrated that drought severely affected the above stated attributes. The foliar sprays of potassium and amino acids significantly improved the morphological as well as biochemical characters of wheat when grown in the presence as well as absence of drought stress condition. Chlorophyll contents of leaf were enhanced up to a level where radiation use efficiency of the wheat was optimum. The recommendation deduced from this experiment showed that the foliar application of amino acids and potassium is a high value approach to alleviate the drought's effects on wheat to enhance the yield.

Keywords: Potassium, amino acid, morphology, water stress, foliar spray.

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1. Introduction

Wheat is a main food for Asian nationals and also crucial cereal crop in the world. Wheat faces numerous stresses which limit its growth, development and yield, water deficit is considered major limiting factor (Karim and Rahman, 2015; Rehmani et al., 2016; Souza et al., 2004). Moisture deficit stress prevails in those areas where amount of the rainfall is less than the crop water requirement (Minhas et al., 2018) and wheat crop is mostly grown in the low rainfall areas (Deng et al., 2004). Climate change induced anomalies in precipitation pattern aggravated water scarcity issues (Amin et al., 2018; Darand et al., 2017; Nawaz et al., 2016)

Moisture deficit stress has an injurious impacts on crop plants including wheat (Ashraf et al., 2017; Hussain et al., 2014) because it reduces growth at different stages like crown root formation, tiller development, booting, anthesis, grain formation, dry matter partitioning, harvest index and also affect normal development of plant (Manikavelu et al., 2006; Zivcak et al., 2016). Saeedipour, (2012) described that under water deficit stress leaves' senescence increase due to loss of leaf soluble proteins and pigments. Drought stress hampers photosynthesis and radiation use efficiency of plants, because of reduced synthesis and presence of photosynthetic pigments (Jaleel et al., 2008; Kunrath et al., 2018; Siddique et al., 2016; Srivastava et al., 2019).

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Globally, there are many strategies that are being followed to cope with the water scarcity (Hasanuzzaman et al., 2018; Khan et al., 2017; Upadhyaya and Panda, 2019; Ullah et al., 2017; Wimalasekera, 2016). One of them is the foliar application of nutrients which mitigate the effects of drought on the plant growth (Ahmad et al., 2018; Ashraf and Foolad, 2007; da Silva Folli-Pereira et al., 2016; Hussain et al., 2016). Foliar application of different macro as well as micro nutrients assists in alleviating drought's effects (Hu et al., 2008; Karim et al., 2016; Nawaz et al., 2015; Noreen et al., 2018). Foliar spray of various plants' nutrients is helpful because plants have ability to get nutrients also through the leaves. Stomata and epidermis is the site through which plant absorbs nutrients, in case of foliar application of nutrient but the absorption's rate is high through the stomata (Fernandez and Brown, 2013; Li et al., 2018). Various trials had proved that foliar spray of some nutrients is beneficial for plants (Ahmad et al., 2014; Siringam et al., 2011).

Application of potassium improved the ability of plant to tolerate osmotic stress, including drought by minimizing its negative effects on plant and enhancing the uptake and translocation of water to make a balance (Adhikari et al., 2019; Cakmak, 2005). Plants get nutrients once applied via plant foliage and yield of crop increased (Ashraf and Foolad, 2007). The harmful impacts of drought stress on the growth of wheat are minimized through the foliar spray of potassium. Plant transfers absorbed K to some or all parts of the plant and increases the yield/plant (El-Ashry et al., 2005). Usually, the wheat yield enhanced by the application of potassium (Pettigrew, 2008). Potassium has an essential role in the biochemical processes like osmoregulation, stomatal opening and closing, protein synthesis and transpiration etc. (Cakmak, 2005; Milford and Johnston, 2007). Plant growth is hampered due to K deficiency (Hermans et al., 2006). Entirely distinct resistances as well as tolerance system in plants have been generated to survive under stress conditions. K has an essential function in the activation of plant defense system against different stresses.

Amino acids have various important roles in plants. These act as endocrine precursors, stress reducing compounds and nitrogen source (DeLille et al., 2011; Maeda and Dudareva, 2012; Zhao, 2010). Drought tolerance enhanced by amino acids' application in plants to minimize the detrimental effects and increase the assemblage of attuned solutes. There are several forms of amino acids available in

soil but their life, in soil, is very short and plants absorb them only when roots' transporters are present (Jamtgard et al., 2010).

The main objective of this experiment is to screen a noble drought stress alleviating compound and to check its efficacy by measuring morphological and biochemical plant characteristics. In this experiment, the two excellent drought mitigator are used in both normal and moisture deficit conditions.

2. Materials and Methods

This experiment was performed in a wire house (a pot trial) to test the efficacy of foliar application of plant nutrients in the wheat (*Triticum aestivum* L.) cultivated under the properly watered as well as moisture deficit stress situations at the Ghazi University, Dera Ghazi Khan, Punjab, Pakistan (altitude: 185; latitude: 310 North) in winter season, 2015-16.

2.1. Soil Analysis

Soil sampling was done before the sowing of a crop to study the chemical and physical properties of soil. These were obtained with the help of auger from the two different depths (0-15 cm plus 15-30 cm). These soil samples were placed in the tagged polyethylene bags and transferred to Fauji Fertilizer Company (FFC), D.G. Khan, for the analysis. Detail of different Physio-chemical attributes is given below. The trial was performed in the pots to evaluate the efficacy of foliar applied potassium and amino acids in the alleviation of drought stress in wheat. There were three different nutrient's states including no foliar application of nutrients, foliar application of amino acids and foliar application of potassium in wheat. In six earthen pots, amino acids (@ 5 ml/L were applied on the foliage of wheat to investigate the function of the amino acids as a nutrient under moisture deficit condition. In further six earthen pots, potassium @ 2% was applied to test its efficacy as a drought mitigator. Remaining six earthen pots were treated as a control and there was no exogenous application of potassium and amino acids on plants of these pots. On the other hand, drought stress and well-watered were the water levels set in the experiment. So, in the nine pots, moisture deficit condition was implemented by providing water equal to forty percent of the field capacity but the remaining nine pots were properly watered by providing water equal to sixty percent of the field capacity.

Table 1. Physio-chemical attributes of analyzed soil

Parameters	0-15 cm	15-30 cm
pH (1:25)	8.8	8.7
EC (1:25) dS m ⁻¹	2.38	1.61
Exchangeable .Na(mmol/100g)	2.2	1.6
Organic matter (%)	0.92	0.41
Nitrogen (%)	0.055	0.027
Available Phosphorus (ppm)	3.0	4.0
Exchangeable potassium (ppm)	347	321
Soil texture	Sandy loam	

Water application in wheat, from sowing to maturity, was balanced by weighing the individual pot after four to five day's interval. For this purpose, digital weighing balance was utilized. Each pot had four kg of sieved soil. The recommended dose of fertilizers was applied before the sowing of crop. In each of the eighteen earthen pots, fifteen seeds of the high yielding variety of wheat, Galaxy-2013, were sown and then thinning was performed to maintain the plant population up to eight plants per pot after the germination of wheat seeds. Sowing of wheat was completed on Nov 15, 2015. The earthen pots were arranged in accordance with factorial completely randomized design (CRD) and each treatment had three replications. The treatments of the experiment: Factor A. Nutrients: F₀=Control, no nutrients' foliar application. F₁= 5ml/L amino acids application, F₂= 2% potassium application and Factor B. Water: Well-watered (WW) = 60% of the field capacity, Drought condition (DD) =40% of the field capacity.

Five plants from each pot were tagged for the measurement of height and meter rod was used for this purpose. Height was recorded from collar to shoot apex of plant. Then this raw data of plant height converted to tabulated data. Spike length (cm) of the five selected plants from each earthen pot measured by using meter rod and then the average spike length of the plants was calculated. An average number of productive tillers per plant were calculated by counting and taking average of the tagged plants from every single pot. From the tagged plants, spikelets spike⁻¹ were counted to get average. Tagged plants of each pot were harvested, removed an individual spike and threshed manually. Counted no. of the grains present in the individual spike and calculated the average grains spike⁻¹.

Hundred grains were counted very precisely after threshing and then weighed on the analytical balance to record hundred grains' weight. At physiological maturity, from every single pot, single selected plant was harvested, threshed manually to record the

economical yield by using analytical balance. Single plant was harvested near the soil level from each pot. Weight was recorded with the assistance of analytical balance. Harvest index (HI) was obtained by using the values of biological and economical yield of the single plant of all the pots. To get this value in percentage multiplied with hundred.

For nitrogen content of leaf (mmol g⁻¹dwt), 0.1 g of fully dried, ground and sieved leaf samples were taken in the tubes used for digestion. Five ml of the commercial grade sulphuric acid added in all digestion tube. These were kept overnight, about twelve hours, at normal temperature, 20-25°C. One milliliter of 35% H₂O₂, was added along the borders of the tube. Then digestion tubes containing digestion mixture were arranged in the digestion block. Digestion mixture's fumes evolved when the temperature started rising and finally reached up to 350°C. This mixture heated for about thirty minutes. Then digestion tubes were withdrawn from the digestion block cooled the partially digested material to add one ml of H₂O₂ and placed back in the digestion block. It was performed many times until material in the tubes turned colorless. Added distilled water in the colorless digested material to make final volume up to 50 ml. Nitrogen content determined from the filtered extract by using Kjeldahl's method.

For phosphorus content of leaf (mmol g⁻¹dwt), 5 ml aliquot was added in the 50 ml of the volumetric flask. 10 ml Barton reagents put in and final volume was formed equal to the mark by adding distilled H₂O. Standard solutions prepared by utilizing KH₂PO₄ and final volume prepared with the use of 10 ml of the Barton reagents plus distilled water. The color was developed in the sample while kept for the few minutes. After the development of color, the sample was run on the spectrophotometer at the wavelength of 420 nm to determine the phosphorus in the leaves by using the standard curve.

For potassium content of leaf (mmol g⁻¹dwt), 0.1 g of fully dried, ground and sieved leaf samples were taken in the digestion tubes. 5 ml of the commercial scale sulphuric acid added in all digestion tube. The samples kept for a night, about twelve hours, at normal temperature, 20-25 °C. One milliliter of the 35% H₂O₂ was added along the borders of the tubes. Then digestion tubes containing digestion mixture were kept in the digestion block. Digestion mixture's fumes emitted from the digestion tubes during heating (at 350 °C).

Table 2. Effect of foliar spray of potassium and amino acids on growth parameters of wheat

Treatment		Morphological Parameters						
		Tillers plant ⁻¹	Spikelets spike ⁻¹	Grains spike ⁻¹	TGW (g)	EY (g plant ⁻¹)	BY (g plant ⁻¹)	HI (%)
Well-watered	Control	3.96bc	20.35bc	46.03 c	2.99 c	4.44bc	13.83b	34.30 c
	Potassium	4.93ab	22.00 b	50.00 b	3.63 b	4.83 b	14.83 b	37.30 b
	Amino Acids	6.00 a	25.14a	55.10 a	4.31 a	5.93 a	16.93 a	43.03 a
Drought Stress	Control	1.36 f	10.13e	28.40e	1.19 f	1.99 e	3.86e	23.40 f
	Potassium	3.13cd	18.66c	44.13c	2.32 d	4.03c	10.21c	29.13 d
	Amino Acids	2.16de	15.33d	39.16d	1.68 e	23.05d	7.39d	26.06 e

TGW, 1000-grain weight; EY, economic yield; BY, biological yield; HI, harvest index

The samples were heated for the thirty minutes. Then the tubes withdrawn from the digestion block cooled the material in the tubes plus added 1 ml of the H₂O₂ and arranged the digestion tubes back into the digestion block. It was done many times until the material turned colorless. 50 ml was the final volume of extract that made in the flasks. Then the filtered extract used for the measurement of potassium content with the help of flame photometer.

Leaf chlorophyll content was measured with the help of chlorophyll meter. Entirely extended leaves of the wheat were cut with the blade to determine the relative water contents. Fresh weight (FW), of the leaves recorded, immediately. Leaves were placed in the plastic bags and then put into the distilled water to determine the turgid weight (TW). Under natural conditions in the laboratory, wheat leaves were permitted to imbibe water for the night, about twelve hours, by putting polythene bags in the low light. After imbibitions, the turgid leaf samples again weighed to note turgid weight. Then leaf samples were put in the oven at 70°C for about 72 hours to determine the dry weight (DW), of the leaf samples. All the recordings related to weight were made by using an analytical scale of high precision (0.0001 g). Relative water content was calculated with the use of given equation.

$Relative\ water\ content\ (\%) = [(Fresh\ wt. - Dry\ wt.) / (Turgid\ wt. - Dry\ wt.)] * 100$

Statistical analysis: The recorded data was statistically analyzed by using the Fisher's analysis of variance (ANOVA) technique. Least Significant Difference, LSD, test was applied ($p \leq 0.01$) to compare the significant treatments by means of Statistic version 8.1 [Analytical Software ©, (1985-2005) according to Steel et al. (1997)].

3. Results

Outcomes of the growth attributes and yield components {no. of tillers/plant, no. of spikelets/spike, no. of grains/spike, hundred grains wt. (g), grains yield (g plant⁻¹), biomass yield (g plant⁻¹) plus harvest index (%)} during the pot study (Table2) illustrated that above characters of crop were significantly ($P \leq 0.01$) decreased under water deficit condition than well watered condition.

However, the foliar spray of potassium as well as amino acids improved the above parameters under stress condition. Under the well-watered, condition, plants achieved maximum growth and yield parameters when supplemented with amino acids followed by the application of potassium, whereas the lowest values were achieved in the absence of exogenous nutrients' application. While under limited water condition, (DD), maximum growth was achieved in the treatment with exogenous potassium followed by the amino acids' spray and the lowest growth were noted in control.

Table 3: Effect of foliar spray of potassium and amino acids on biochemical traits of wheat leaves

		Nconc	Pconc	Kconc	Chlorophyll	RWC
		(m mol g ⁻¹ dwt ⁻¹)	(m mol g ⁻¹ dwt ⁻¹)	(m mol g ⁻¹ dwt ⁻¹)	(m mol g ⁻¹ dwt ⁻¹)	(m mol g ⁻¹ dwt ⁻¹)
Well-watered	Control	17.46b	5.31 c	4.99 c	46.06 c	34.20 c
	Potassium	18.40 b	5.61 b	5.94 a	48.43 b	36.06b
	Amino Acids	19.41 a	6.03 a	5.61 b	50.96 a	46.56 a
Drought Stress	Control	14.06e	4.31 f	4.04 f	39.30e	20.40 f
	Potassium	15.23 d	5.01 d	4.81d	44.95c	29.00 d
	Amino Acids	16.36c	4.71 e	4.66 e	41.83 d	24.20 e

RWC; relative water content

Results of the biochemical parameters [Nitrogen content of leaf ($\text{mmol g}^{-1}\text{dwt}$), phosphorous content of leaf ($\text{mmol g}^{-1}\text{dwt}$), potassium content of leaf ($\text{mmol g}^{-1}\text{dwt}$), leaf's chlorophyll content (SPAD Value), the relative water contents of leaf (in %)] (Table 3) illustrated that the above parameters were significantly, $P \leq 0.01$, decreased due to moisture deficit stress than the normal watered conditions. Potassium and amino acids' foliar spray augmented the enlisted attributes under stress conditions. Under the well-watered conditions, plants achieved the maximum biochemical traits with the applications of amino acids followed by the potassium application but the lowest values of biochemical traits were recorded where no foliar application of nutrients.

Under the drought stress (DD) conditions, it was noticed that plants attained the maximum biochemical traits under potassium application followed by the amino acids application but the lowest biochemical attributes were recorded where no exogenous nutrients application.

4. Discussion

Moisture deficit stress has harmful impacts on the production of crop. The findings of our experiment clearly stated that the foliar feeding of amino acids and potassium to the wheat under the moisture deficit stress condition at any stage of crop (juvenile, anthesis and grain filling) enhanced the stress tolerance ability, growth and yield. In this experiment, plant height was decreased in limited water condition, which was also supported by Khan et al. (2001). Drought impact either at the juvenile or reproductive stage reduced the vigor and growth of the plant; but additional harmful effects were observed at the anthesis stage. The plant height reduced because of the dearth of water in the protoplasm, reduced relative turgidity and reduced division and elongation of cell (Hussain et al., 2008).

Leaves expansion and tillers elongation are mainly completed in the vegetative stage whereas under drought stress stem elongates slowly and plant remains dwarf. Zhao et al. (2006) reported that shortage of water influenced plant height because of the hormonal imbalance (abscisic acid and cytokinin) that hampered growth because of changes in the property of cell membrane. The harmful effects of the drought may be minimized by applying water to the plant (Alfredo and Setter, 2000; Hoad et al., 2001). Yadov (2006) reported that application of mineral nutrients to plant play a crucial role in enhancing the drought tolerance ability of plant. The plant nutrients

like potassium and amino acids play crucial role in improving drought stress tolerance of plant. Many physiological and biochemical functions like photosynthesis, enzymes activation, regulation of turgor pressure, translocation of assimilates; amino acids and potassium are important nutrients for all these functions (Mengel and Kirkby, 2001). The foliar feeding of amino acids and potassium increased the plant height and it is noted more superior in increasing the plant height once foliar applied under drought condition at vegetative stage than anthesis or grain formation stage.

Spike length, no. of grains/spike, no. of spikelets/spike, hundred grains weight and grains yield were highly reduced when drought stress prevailed at reproductive stages. The two parameters, 100 grains' weight and overall grain yield were highly reduced under drought stress up to the critical level. Spike length significantly reduced under water stress conditions at sensitive stages like jointing and spike development (Fabian et al., 2019; Giunta et al., 1993). The reduced spike length is due to fewer nodes on the rachis as well as short nodal distance (Wolde et al., 2019). Lower number of spikelets spike⁻¹ can be resulted from reduced stored photosynthates before the heading stage (Taiz and Zeiger, 1991).

Drought stress at the juvenile, anthesis and post-anthesis stages rigorously affects assembly of the crop by inducing critical decline in the no. of grains/spike (Rad et al., 2005; Nasri, 2005). Number of grains/spike was severely reduced under moisture deficit stress condition (Richards et al. 2001). Reduction in the grains yield was owing to small spikes under water deficit stress (Plaut et al., 2004).

Decline in the wt. of hundred grains was also determined by the Plaut et al. (2004) under limited moisture conditions at reproductive stage due to fewer and uneconomical nutrients uptake along with less accumulation of photosynthates that hasten maturity in plant with desiccated grains. Drought stress at any particular stage or during entire growing season considerably reduced the yield and components of yield in wheat crop (Nasriet al., 2005). Under drought, biomass of the plant decreased (Zhao et al., 2006). It was also determined by the Manivannan et al. (2007) in *Helianthus annuus* and by Sankaret al. (2007) in *Abelmoschus esculentus* that total biomass of the crop plants reduced under drought stress. Typical number of irrigations required for normal growth along with yield of crop but, once, when there is less availability of H₂O. Application of nutrients through foliar spray facilitates nutrient uptake and minimize the

detrimental impacts of water deficit stress (Arif et al., 2006; Cakmak, 2005; García-Gaytán et al., 2018). The exogenous application of amino acids as well as potassium mitigates the negative effects of drought stress (Cakmak, 2005). In our study, foliar application of amino acid as well as potassium enhanced the yield, components of yield and ability of plant to withstand the drought.

5. Conclusion

Water is the major constituent of plant cells and its deficiency causes disturbance in the normal function of plant. At any growth stage, drought stress reduces the yield along with growth of wheat crop. There is plethora of ways to protect plant from the harmful effects of drought and foliar feeding is one of them. The findings of this study clearly demonstrated that exogenous application of potassium as well as amino acids enhanced the growth and yield. Under well-watered condition, amino acid application is the best option to improve growth, yield and quality of yield but under drought condition, foliar application of potassium is the best way to get an optimum yield. Potassium has an actively role in opening and closing of stomata so under limited moisture condition it proves better than amino acids' application. Under drought conditions, amino acids act as an antioxidant and protect the cellular membrane from oxidative damage instead directly regulating the opening and closing of stomata.

List of Abbreviations: DD: developed drought; WW: well-watered; DAS: days after sowing; FFC: Fauji Fertilizer Company; CRD: completely randomized design; HI: harvest index; $m\ mol\ g^{-1}\ dwt$: mili mole per gram dry weight; RWC: relative water content; ANOVA: analysis of variance; LSD: least significant difference.

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