Response of quinoa (*Chenopodium quinoa*) cultivars to organic acid under drought stress

Kosar Kohan, Pourang Kasraie*, Hamid Reza Larijani, Farshad Ghoshchi, and Meysam Oveisi

Department of Agriculture, Islamic Azad University, Varamin-Pishva Branch, Varamin, Iran

Abstract

The present study was an attempt to delve into the responses of two quinoa cultivars treated with humic and amino acids to drought stress. The experiment was conducted based on a split plot arrangement in a randomized complete block design with three replicates during 2019-2020. Experimental factors included drought stress at three levels (50, 80, and 110 mm evaporation from evaporation pan Class A), cultivar at two levels (Titicaca and Q29), and organic acids at four levels (control, humic acid, amino acid, and a combination of humic and amino acids). Findings suggested that extreme drought stress resulted in the decreasing of 20.33% plant height, and 30.19% of leaf area index while increasing 78.69% malondialdehyde content as well as 8.12% catalase and 31.26% peroxidase enzymes activities. Titicaca cultivar had higher plant height, leaf area index, and catalase contents (93.32%, 9.98%, and 13.38%, respectively) as compared with Q29. Applying organic compounds improved growth and biochemical properties of quinoa plants with the maximum positive effect in plants treated with a combination of humic and amino acids. These compounds also decreased the negative effects of drought stress. Application of humic and amino acids seems to improve vegetative parameters of quinoa plants under both normal and drought stress conditions through increasing antioxidant enzyme activities and regulation of abscisic acid hormone.

Keywords: drought stress, humic acid, organic acid, peroxidase, quinoa

Kohan, K., P. Kasraie, H. R. Larijani, F. Ghoshchi, and M. Oveisi. 2023. 'Response of quinoa (*Chenopodium quinoa*) cultivars to organic acid under drought stress'. *Iranian Journal of Plant Physiology 13* (4),4753- 4763.

Introduction

Quinoa (*Chenopodium quinoa* Willd) is an annual plant belonging to *Amaranthaceae* family and with applications as food and also in alcohol production industry. Grain and shoots are also used for animal feed (Peiretti and Gai, 2019). Irrigation water deficit is currently a worldwide challenge which is expected to become even worse following the global climate changes (Aziz et al., 2018). Physiological flexibility, phenotypic and genetic variation, as well as development of unique drought resistance mechanisms in quinoa have led to its high adaptability to diverse climatic conditions particularly those of dry regions, and this has attracted a global attention to this crop (Aziz et al., 2018). High adaptability to diverse climatic conditions means that quinoa has the potential of usage as an appropriate replacement for the grain crops that are low in the quality and quantity of yields, or cannot grow in dry and semidry regions (Ahmadi et al., 2019). Studies on the effects of drought stress on quinoa have shown that drought treatment reduces the growth and yields in quinoa whereas physiological

---

* *Corresponding Author*
E-mail Address: drkasraie@yahoo.com
Received: December, 2021
Accepted: May, 2023

Table 1
Results of physical/chemical analysis of the farm soil before the start of the experiment

Depth (cm)	Texture	Clay (%)	Silt (%)	Sand (%)	EC (ds/m)	PH	Water Retention Capacity (%)	Nutralizing Substance (%)	Total N (%)	Total Organic C (%)	Total Absorbable P (ppm)	Total Absorbable K (ppm)
0-30	Sandy loam	16	23	61	4.38	7.68	32	12.5	0.035	0.32	23.4	333.8

responses of the plant including increased growth in roots mitigate the negative effects of the drought (Gámez et al., 2019). Yang et al. (2019) reported that in dry conditions, plant height, shoot dry weight, leaf photosynthesis speed, stomatal conductance, and chlorophyll and nitrogen levels of leaves reduced. Generally, quinoa shows a high flexibility and tolerance to various abiotic conditions through physiological, biochemical, and morphological responses (Stoleru et al., 2019).

Plants use a sophisticated defense mechanism to prevent the adverse effects of oxidative stress. This mechanism involves making use of antioxidant enzymes, namely superoxide dismutase, catalase, peroxidase, ascorbate peroxidase, and glutathione reductase as well as non-enzyme antioxidants including molecules with low molecular mass such as ascorbic acid, glutathione, carotenoids, and tocopherol (Jain et al., 2015).

As an organic fertilizer, humic acid improves soil structure, increases rooting, enhances the soil capacity to hold more water, helps rapid growth of the useful bacteria in soils and solubility and releasing nutrients, and as a result, reduces the need to apply chemical fertilizers (Vanitha and Mohandass, 2014). Humic acid is a growth catalyst for plants through modifications in plant roots, e.g., increasing the root length and density, which increase absorption surface (Canellas and Olivares, 2014). According to Tadayyon et al. (2017) humic acid most probably improves the growth through increasing cationic exchange capacity in the plants, improving the soil potential to maintain water, and also activating perspiration, photosynthesis, and adenosine triphosphate cycle.

Amino acids, as the building blocks of life, are involved in making proteins and peptides, which are in charge of all plant procedures including structural, enzymatic, metabolic, and transportation performance (khan et al., 2016). They also increase chlorophyll content and improve plant's tolerance against abiotic stresses (Shehata et al., 2011). Biologic products including amino acids are reported to induce metabolism and metabolic procedures to improve plant performance (Nusrat et al., 2014). Also, amino acid treatment was found to significantly improve plant growth through increasing accumulation of osmolytes and increasing antioxidant potential in plants (Malekzadeh et al., 2015).

In view of the fact that Iran belongs to the dry and semidry regions of the world, it seems that efficient use of water resources and improved mechanisms of resistance against drought stress in crops can help with the general movement along the lines of sustainable development in crops. Considering the effects of humic acid and amino acids on improving resistance of various other plants to drought stress, the present study was an attempt to delve into the responses of two quinoa cultivars treated with humic and amino acids to drought stress.

Materials and Methods

The experiment was carried out based on a split plot design in a randomized complete block design with three replications in a farm located in Dastjerd, Qom, Iran, during 2019-2020. Physical/chemical analysis of the farm soil is presented in Table 1. Experimental factors were three levels of drought stress including 50, 80, and 110 mm evaporation from evaporation pan Class A, corresponding to favorable, mild, and severe stresses, respectively, two levels of cultivar (Titicaca and Q29), and four levels of humic and amino acids including control, humic acid, amino acid, and a combination of humic and amino acids. Each repetition involved 24 plots, each plot consisting of 5 rows of 4 m in length and a distance between plants of 7-9 cm. The planting was done at the depth of 1-2 cm in late March. Irrigation in all treatments was conducted normally until three-leaf stage after which drought levels were applied. Irrigation was done immediately after sowing so that first a turn of heavy irrigation was applied followed by a light turn of irrigation after 4 days. Irrigation time was determined based on daily evaporation from evaporation pan Class A. To determine the volume of water required for each irrigation turn, the plot soils were sampled out from the depth of root development before irrigation. The samples were oven dried at 80 °C for 24 h. Then, the percentage of soil moisture was calculated and the required volume of irrigation water was determined using the following equations:

$$H = \rho b\ (\Theta_{F.C} - \Theta_m)\ D$$

$$V = H \times A$$

where H is the weight of water level in plots, ρb is the apparent specific mass of soil, $\Theta_{F.C}$ is the field capacity moisture, Θ_m is the mass moisture of the plot soil during irrigation, D is the depth of root development, V is the volume of irrigation water, and A is the plot area. The volume of the consumed water was controlled using a water counter meter installed at the main water output. Spraying amino acid and application of humic acid through irrigation water (2 $g.L^{-1}$) were carried out at two stages of plantlet establishment and the beginning of generative growth. Control treatments received distilled water. Megafol Valgro fertilizer 2 $g.L^{-1}$ was used for spraying amino acid, which contains 16 amino acids. The composition of this fertilizer included 4.5% total nitrogen, 4.5% organic nitrogen, 28% amino acid, 2.9% potassium oxide, 15% organic carbon, 0.05% iron, and 0.04% phosphoric acid. It also had a volumetric weight of 1.22 grams per cubic centimeter, acidity of 6.5, and electrical conductivity of 0.3 $\mu s.cm^{-1}$.

The first harvest was done at full maturation stage in July. Ten plants were randomly sampled out from each plot at maturation stage. The two lateral rows and 0.5 meter from the beginning and end of each plot were disregarded as the margins.

Activities of catalase and peroxidase were determined using the methods of Cakmak and Horst (1991) and Nickel and Cunningham (1969), respectively. In addition, malondialdehyde content was assayed using a spectrophotometer through readings at 532 and 600 nm (De-Vos et al., 1991). Furthermore, extraction, purification, and measurement of abscisic acid were done using the method of Kelen et al. (2004) with some modifications. The extraction solution was submitted to HPLC analysis with C18 analytical column and a flow rate of 0.8 $ml.\ s^{-1}$, using 0.1 normal acetic acid and methanol 8% solvent and employing a gradient elution of 50:50 (v/v) to measure abscisic acid concentration using its various standard concentrations. Proline content was assayed following the method reported in Bates et al. (1973) and the absorptions were read at 520 nm using the spectrophotometer. The obtained data were submitted to SAS for analysis and comparison of means were performed using Duncan's Multivariate Test ($p \leq 0.05$).

Results

Plant height

Analysis of variance showed that the interaction effects of drought stress × cultivar ($p \leq 0.01$), drought stress × organic compounds ($p \leq 0.05$), and cultivar × organic compounds were significant at the probability level of $P \leq 0.01$. Treatment with organic compounds led to an increase in plant height under both normal and drought stress conditions. On the other hand, under mild drought treatment, maximum increases in plant heights, 16.56% and 20.44%, were recorded in humic acid and humic acid + amino acid treatments, respectively. Under severe drought stress condition, the highest increases in heights by 19.17% and 22.41% were recorded in plants treated with amino acid and amino acid + humic

Table 2

Comparison of mean effects of humic and amino acids on vegetative and biochemical attributes of quinoa plants under drought stress

Drought Stress (mm evaporation)	Biologic compounds	Proline (µM.g^{-1}FW)	Peroxidase (µmol min $^{-1}$ mg protein^{-1})	Catalase (µmol min $^{-1}$ mg protein^{-1})	Abscisic Acid (µM.g^{-1}FW)	Leaf Area Index	Plant Height (cm)
50	Control	13.58h	41.46e	13.29d	0.82de	4.84bcd	67.76bc
50	Humic Acid	16.14gh	43.86de	15.08cd	0.75e	5.16abc	71.96ab
50	Amino Acid	18.05fgh	41.57e	14.96cd	0.76e	5.38ab	73.18ab
50	Humic + Amino Acid	19.33efg	47.04de	16.28bcd	0.65e	5.83a	77.69a
80	Control	16.97fgh	41.55e	15.6bcd	1.14c	4.14de	60.86cd
80	Humic Acid	21.9def	44.58de	19.49ab	0.88de	4.69bcd	70.94ab
80	Amino Acid	23.61cde	46.82de	19.2abc	1.05cd	4.39cde	65.87bcd
80	Humic + Amino Acid	24.93de	49.4cde	21.91a	0.86de	5.1abc	73.3ab
110	Control	28.57cd	51.54bcd	17.69bc	1.66a	3.28f	50.82e
110	Humic Acid	36.34ab	61.76a	19.6ab	1.44ab	3.85ef	58.23de
110	Amino Acid	32.39bc	55.67abc	22.66a	1.51a	3.79ef	60.26cd
110	Humic + Amino Acid	37.8a	59.33ab	22.39a	1.24bc	3.87ef	62.21cd

Similar letters show no significant difference ($p \leq 0.05$) based on Duncan's test.

acid, respectively (Table 2). Treatment with humic and amino acid increased quinoa plant heights in both cultivars, and the maximum increase in plant height was observed in the combined treatment of humic acid + amino acid, which was not significantly different from the humic acid or amino acid treatments individually (Table 3).

Leaf area index

Results of ANOVA showed that the effects drought stress, cultivar, and organic compounds as well as the interaction effects of drought stress + cultivar on leaf area index were significant at $p \leq 0.01$. Also, the interaction effects of drought stress and organic compounds on leaf area index were significant at $p \leq 0.05$. In addition, comparison of mean leaf area index of drought-stressed cultivars showed no significant difference between the two cultivars under study in normal irrigation and severe drought condition, although drought reduced this index in both cultivars. In mild drought stress, the leaf area index in Q29 was 17.86% more than in Titicaca cultivar (Table 4). Findings showed that organic compounds increased leaf area index in quinoa cultivars under normal irrigation and drought condition, although under various drought conditions no significant difference was observed in application of humic and amino acids as compared with control (Table 2).

Abscisic acid content

Results of ANOVA showed that effects of drought stress, cultivar, organic compounds, and the interaction of drought stress + cultivar and drought stress + organic compounds on abscisic acid contents of the quinoa plants under study were significant at $p \leq 0.01$. Also, the interaction effect of cultivar + organic compounds on abscisic acid contents of the plants under study was significant at $p \leq 0.05$. Maximum level of abscisic acid under combined treatment of drought stress + cultivar (1.5 µM. g^{-1} FW) was obtained in severe drought applied to Titicaca cultivar. This means that increase in the level of drought resulted in increased concentration of abscisic acid in fresh plant material. Moreover, no differences were observed in abscisic acid contents of the plants under normal irrigation and mild drought condition (Table 4). Also, the interaction effects of

Table 3
Comparison of mean effects of humic and amino acids on vegetative and biochemical attributes of quinoa cultivars

Cultivar	Biologic Compounds	Proline (μM.g^{-1}FW)	Malondialdehyde (μM/g FW)	Catalase μmol min $^{-1}$ mg) (protein^{-1}	Abscisic Acid (μM.g^{-1}FW)	Plant Height (cm)
Titicaca	Control	20.84bc	27.41ab	14.53d	1.29a	61.92cd
Titicaca	Humic acid	25.29b	24.35abc	17.57bcd	1.09abc	66.5abc
Titicaca	Amino Acid	24.9b	24.95abc	16.68cd	1.18ab	68.3abc
Titicaca	Humic + Amino Acid	29.65a	22.13c	19.37abc	0.96cd	72.1a
Q29	Control	18.58c	28.34a	16.52cd	1.12abc	57.71d
Q29	Humic Acid	24.29b	25.38abc	18.53abc	0.95cd	67.58abc
Q29	Amino Acid	24.46b	23.78bc	21.19a	1.03bcd	64.58bc
Q29	Humic + Amino Acid	25.05b	24.6abc	21.02ab	0.87d	70.03ab

Similar letters show no significant difference ($p \leq 0.05$) based on Duncan's test.

drought stress and organic compounds on abscisic acid contents of fresh plant material were significant at $p \leq 0.05$. Under drought condition, organic compounds reduced the content of abscisic acid while in normal irrigation no differences were observed between the control and organic compound-treated plants. Treatments with humic acid alone or in combination with amino acid reduced abscisic acid contents of quinoa plants as compared with the control plants under mild and severe drought conditions (Table 2). Finally, mean abscisic acid contents of quinoa cultivars decreased in treatments with organic compounds. Combined treatment of humic acid + amino acid reduced abscisic acid contents of Titicaca and Q29 cultivars by 8.53% and 22.32%, respectively (Table 3).

Catalase activity

Based on the ANOVA table, the effects of drought, cultivar, organic compounds, and interaction effects of cultivar + organic compounds on catalase activity of the quinoa plants under study were significant at $p \leq 0.01$, and the combined effects of drought + organic compounds and also the triple effects of drought stress + cultivar + organic compounds on the catalase contents of the plants under study were significant at $p \leq 0.05$. Also, application of organic compounds increased catalase enzyme activities of the quinoa plants under drought stress. On the other hand, under normal irrigation condition, organic compound treatment did not result in a significant difference from control group while in mild drought condition, the combined treatment with humic and amino acids resulted in a decrease in catalase enzyme activity by 28.8% compared with the control. In severe drought condition, treatments with amino acid alone and in combination with humic acid increased catalase activities by 28.1% and 28.57%, respectively as compared with the control (Table 2). Furthermore, comparison of mean catalase activities as affected by cultivar showed that the maximum catalase activity in Titicaca cultivar was related to combined treatment with humic and amino acids. Q29 cultivar also experienced an increase in catalase activity under combined treatment of humic acid + amino (Table 3). Finally, the effects of organic compounds on the catalase activity of quinoa cultivars under drought stress showed an increase in the level of catalase activities under drought stress generally, and the highest level of activities of this enzyme was recorded in Q29 cultivar under severe drought stress condition (Table 5).

Peroxidase activity

Analysis of the data submitted to ANOVA showed that the effects of drought, organic compounds, and interaction effects of drought + organic

Table 4
Comparison of mean effects of humic and amino acids on vegetative and biochemical attributes of quinoa plant

Drought Stress (mm evaporation)	Cultivar	Proline ($\mu M.\ g^{-1}FW$)	Malondialdehyde ($\mu M.\ g^{-1}FW$)	Peroxidase ($\mu mol\ min^{-1}\ mg\ protein^{-1}$)	Abscisic Acid ($\mu M.\ g^{-1}FW$)	Leaf Area Index	Plant Height (cm)
50	Titicaca	15.84e	17.68c	42.15b	0.76d	5.2a	75.54a
50	Q29	17.71de	19.19c	44.82b	0.72d	5.4a	69.76ab
80	Titicaca	22.87c	24.63b	45.76b	1.05c	4.2b	67.4b
80	Q29	20.83cd	23.29b	45.41b	0.91cd	4.95a	68.09b
110	Titicaca	36.81a	31.81a	59.31a	1.58a	3.53c	58.68c
110	Q29	30.75b	34.08a	54.83a	1.34b	3.86bc	57.08c

Similar letters show no significant difference ($p \leq 0.05$) based on Duncan's test.

compounds, and drought + cultivar on peroxidase enzyme activities of the quinoa plants under study were significant at $p \leq 0.1$. Also, the triple effects of drought + cultivar + organic compounds on peroxidase enzyme activities were significant at $p \leq 0.05$. Furthermore, severe drought stress increased the activities of peroxidase in the both quinoa cultivars under study with no significant difference between them (Table 4). Also, organic compound treatments increased peroxidase activities in the plants under severe drought stress. Although application of organic compounds in normal irrigation condition did not result in a significant difference from control, treatment with humic acid in the plants under severe drought resulted in an increase by 19.86% in the activities of peroxide enzyme of the plants in comparison with the control (Table 2). Generally, the maximum activities of peroxidase in Titicaca cultivar (60.7 and 63.21 µM. min^{-1} mg $protein^{-1}$) were related to the humic acid treatment and its combination with amino acid, respectively under mild drought. On the other hand, the highest activities of peroxidase in Q29 cultivar, 62.82 µM min^{-1} mg $protein^{-1}$, belonged to the humic acid under severe drought condition (Table 5).

Malondialdehyde content

ANOVA showed that the effects of drought, organic compounds, and interaction effects of drought + cultivar on malondialdehyde content of the quinoa plants under study were significant at $p \leq 0.1$. Also, the combined effects of cultivar + organic compound treatments and the triple effects of drought + cultivar + organic compounds on malondialdehyde contents of the plants under study were significant at $p \leq 0.05$. Moreover, maximum contents of malondialdehyde, 31.81 and 34.08 µM. gFW^{-1}, were observed in Titicaca and Q29 cultivars under severe drought stress, and as the level of drought stress increased, so did the malondialdehyde content, although no significant differences were found among the cultivars under all levels of drought (Table 4). Findings also revealed that in Titicaca cultivar, the combined treatment of humic and amino acid reduced malondialdehyde content by 19.26% in comparison with the control while the other treatments were not statistically different from the control. Although, treatment of Q29 cultivar with organic compounds reduced the malondialdehyde content, no significant differences were observed in the effect of these compounds treated alone or together (Table 3). Generally, the maximum level of malondialdehyde, 38.09 µM. gFW^{-1}, was recorded in the Q29 cultivar under severe drought stress not receiving organic compounds treatment (Table 5).

Proline content

Based on the ANOVA table, the effects of drought, cultivar, organic compounds, and interaction effects of drought + cultivar and drought + organic compounds on proline contents of the quinoa plants under study were significant at $p \leq 0.01$, and the combined effect of cultivar + organic

Table 5
Comparison of mean effects of humic and amino acids on vegetative and biochemical attributes of quinoa cultivars under drought stress

Drought Stress (mm evaporation)	Cultivar	Chemical Compounds	Proline (µM. g^{-1}FW)	Malondialdehyde (µM/g FW)	Peroxidase (µmol min $^{-1}$ mg	Catalase (µmol min $^{-1}$ mg
50	Titicaca	Control	12.57l	19.44jkl	39.69i	12.28j
50	Titicaca	Humic Acid	14.87kl	17.95klm	42.32hi	14.22hij
50	Titicaca	Amino Acid	17.47ijk	17.73klm	41.41hi	13.38ij
50	Titicaca	Humic + Amino Acid	18.44ij	15.62m	45.18efghi	15.7ghi
50	Q29	Control	18.35ij	27.29def	42.91ghi	14.74hij
50	Q29	Humic Acid	23.52gh	24.88fg	46.38efgh	19.17de
50	Q29	Amino Acid	22.23gh	23.93gh	45.23efghi	17.87defg
50	Q29	Humic + Amino Acid	27.38ef	22.42ghij	48.52defg	20.11cd
80	Titicaca	Control	31.59d	35.51ab	54.61bc	16.56efgh
80	Titicaca	Humic Acid	37.49b	30.21cd	60.7a	19.32de
80	Titicaca	Amino Acid	35.01bc	33.19bc	58.73ab	18.78def
80	Titicaca	Humic + Amino Acid	43.14a	28.34de	63.21a	22.31bc
80	Q29	Control	14.59kl	21.38hij	43.24fghi	14.3hij
80	Q29	Humic Acid	17.41ijk	19.97ijkl	45.4efghi	15.93fghi
80	Q29	Amino Acid	18.62ij	18.02klm	41.73hi	16.53efgh
110	Q29	Humic + Amino Acid	20.22hi	17.37lm	48.9def	16.86efgh
110	Titicaca	Control	15.59jkl	25.54efg	40.18i	16.46efgh
110	Titicaca	Humic Acid	20.28hi	20.99hijk	42.78hi	19.8cd
110	Titicaca	Amino Acid	24.99fg	22.88ghi	48.42defg	20.52cd
110	Titicaca	Humic + Amino Acid	22.48gh	23.76gh	50.28cde	23.72b
110	Q29	Control	25.55fg	38.09a	48.46defg	18.81def
110	Q29	Humic Acid	35.19bc	35.16ab	62.82a	19.87cd
110	Q29	Amino Acid	29.78de	30.43cd	52.6cd	26.53a
110	Q29	Humic + Amino Acid	32.47cd	32.66bc	55.45bc	22.47bc

Similar letters show no significant difference ($p \leq 0.05$) based on Duncan's test

compounds and also the triple effects of drought stress + cultivar + organic compounds on the proline contents of the plants were significant at $p \leq 0.05$. Comparison of mean proline contents under effects of drought and cultivar showed that the highest level of proline (36.81 µM. g FW^{-1}) belonged to Titicaca cultivar subjected to severe drought stress. With an increase in the level of drought applied, proline contents of the quinoa plants also increased. On the other hand, in normal irrigation and mild drought condition, no significant differences were observed between the cultivars (Table 4). Also, comparison of mean proline contents in the plants under drought and organic compound treatments suggested that applying organic compounds increased proline contents in both normal irrigation and drought condition. Besides, no differences were found between various treatments of organic compounds under normal irrigation condition and mild drought stress while under severe drought, humic acid, alone and in combination with amino acid, increased proline contents by 27.15% and 32.31%, respectively as compared with the control (Table 2). Moreover, comparison of mean proline contents as affected by cultivar and organic compounds showed that applying organic compounds increased the level of proline in the cultivars under investigation. In Titicaca the maximum proline content was obtained in the combined treatment of humic and amino acids (42.27%). On the other hand, no significant differences were observed in the effects of various organic compound treatments on the proline contents of the plants under study (Table 4). Comparison of mean proline contents of quinoa cultivars under drought stress treated with organic compounds revealed that the maximum proline concentration (43.14 µM. g FW^{-1}) was obtained in Titicaca cultivar under mild drought stress and with combined treatment of humic and amino acid (Table 5).

Discussion

Findings of the present study showed that under drought stress, the plant height reduced. It seems that under drought condition, reduced photosynthesis due to the limited access of the plants to water and CO_2 results in the allocation of less photosynthetic substances to the growing organs of the plants and therefore, their growth potential significantly reduces in comparison with the condition when they have a better access to water. In other words, reduced cellular growth and turgescence, and therefore reduced leaf area, closed stomata, and limited photosynthesis in water deficit conditions are among the mean factors involved in reducing the plant height and other morphological attributes of the plants (Rady et al., 2016). With application of humic and amino acid, the plant heights improved. Amino acids are able to improve the main biochemical and metabolic processes in plants and thereby, increase plant heights (Tadayyon et al., 2017). Humic acid through increasing cationic exchange capacity, improving the soil's potential to maintain water, and also playing a role in the cell membrane permeability as the protein transporter, activator of respiration, Krebs cycle, photosynthesis, and production of amino acids and adenosine triphosphate can improve plant growth (Sidari et al., 2008). Water deficit seems to reduce cell turgescence and affect cellular growth and division, and as a result reduce plant leaf area (Rayma et al., 2016). Application of organic compounds led to increased leaf area, increased amount of nutrients available to plants, particularly nitrogen, stimulating plant growth and increasing lateral branches. Also, spraying amino acids through improved nitrogen intake and photosynthesis, increases plant leaf area (Youssef, 2014). Based on the findings, abscisic acid contents of quinoa cultivars increased under drought stress. This increase confirms the critical role of this hormone in plants' mechanism of resistance against drought (Thameur et al., 2011). Abscisic acid is an important signaling compound that stimulates plant adaptation mechanism to drought stress conditions (Wilkinson and Davies, 2010).

Drought stress led to increased catalase and peroxidase enzymatic activities. It was also observed that application of humic and amino acid increased activities of these enzymes. Drought through inducing oxidative stress in plants results in increased ROS. This in turn, increases catalase and peroxidase enzyme activities (Hayat and Ahmad, 2010). The positive effects of amino acid applied in this study on growth parameters of quinoa cultivars can be attributed to increasing the plants' antioxidant capacity or inducing other antioxidants in the plants treated with this amino acid. Also, it might be argued that with an increase in the level of humic acid applied, the plant's antioxidant system is more activated, and through increasing catalase enzyme activity, as the main defense layer against ROS, it forces the plant to resist against the damages due to drought stress (Halek et al., 2013). Humic acid treatment improved activation of the plants' antioxidant system and by increasing catalase and peroxidase enzyme activities as the first defense layer against ROS attacks, it protects the plant against the adverse effects of drought stress (Halek et al., 2013).

Malondialdehyde content of the plants under study increased in drought stress conditions. Lipid peroxidation, which damages biologic membranes, is a sign of oxidative stresses in plants under various stresses such as drought stress. Malondialdehyde content is an index of the intensity of oxidative damages to lipids (Boguszewska et al., 2010). Excessive increase in the level of free radicals results in a damage in cell membranes, with the most remarkable damage being peroxidation of fatty acids in membranes. The change in cell membrane fatty acids leads to formation of small compounds such as malondialdehyde as the end-product of lipid peroxidation, and the increase in the level of malondialdehyde is a sign of cell membrane damage (Jin et al., 2006). Drought stress results in peroxidation of chloroplast thylakoid glycolipids, which is followed by production of diacyihlycerol, triacyihlycerol, and also free fatty acids, eventually increasing the level of malondialdehyde in plant tissues (Boguszewska et al., 2010).

Osmotic adjustment is considered as one of the mechanisms of plants' adaptation to drought stress which maintains cell turgescence and the relevant processes in water deficit conditions through accumulation of soluble substances. Amino acids as osmotic regulators play a main role in plant metabolism and take part in their response to abiotic stresses. The effects of drought stress depend on the plant species and tissue (Liang et al., 2013). Accumulation of proline in drought-stressed plants is the result of synthesis and destruction of their compound. Increased proline content of the plants as a result of drought stress protects cell membranes and cytoplasmic enzymes, controlling ROS and scavenging free radicals (Jdey et al., 2014). The reason behind increased levels of proline after applying humic acid is that humic acid probably provides for favorable conditions for plants to increase nitrogen content and improve growth. Also, application of humic acid can increase production of the compounds containing nitrogen, such as proteins and amino acids (Khodadadi et al., 2020).

Conclusion

Our findings revealed that there were no significant differences between the two cultivars under different treatments of the study. Application of humic and amino acids seems to improve vegetative parameters of quinoa plants under both normal and drought stress conditions though increasing antioxidant enzyme activities and regulation of abscisic acid hormone. Therefore, humic and amino acids improve the tolerance in quinoa cultivars and increase yields in these plants. The observed effects of amino acids used in this study can be attributed to increasing the plant's antioxidant potential or inducing activities of other antioxidants in the quinoa plants treated with these amino acids.

References

Ahmadi, S. H., S. Solgi and A. R. Sepaskhah. 2019. 'Quinoa: A super or pseudo-super crop? Evidences from evapotranspiration, root growth, crop coefficients, and water productivity in a hot and semi-arid area under three planting densities'. *Agricultural Water Management*, 225: 105784.

Aziz, A., N. A. Akram and M. Ashraf. 2018. 'Influence of natural and synthetic vitamin C (ascorbic acid) on primary and secondary metabolites and associated metabolism in quinoa (*Chenopodium quinoa* Willd.) plants under water deficit regimes'. *Plant Physiology and Biochemistry*, 123: 192-203.

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

Boguszewska, D., M. Grudkowska and B. Zagdanska. 2010. 'Drought responsive antioxidant enzymes in potato (*Solanum tuberosum* L.) '. *American Journal of Potato Research*, 53: 373-382.

Cakmak, I and W. Horst. 1991. 'Effect of aluminium on lipid peroxidation, superoxide dismutase, catalase and peroxidase activities in root tip of soybean (*Glysin max*) '. *Plant Physiology*, 83: 463-468.

Canellas, L. P and F. L. Olivares. 2014. 'Physiological responses to humic substances as plant growth promoter'. *Chemical and Biological Technologies in Agriculture*, 1(3): 1-11.

De-Vos, C. H. R., H. Schat, M. A. D. De Waal, R. Vooijs and W. H. O. Ernst. 1991. 'Increased resistance to copper-induced damage of root plasma membrane in copper tolerant Cilene cucubalus'. *Physiology Plantarum*, 82: 523-528.

Halek, F., A. Delavari and A. Kavousi-rahim. 2013. 'Production of biodiesel as a renewable energy source from castor oil'. *Clean Technology Environment*, 15: 1063-1068.

Hayat, Q., S. H. Hayata, M. Irfan and A. Ahmad. 2010. 'Effect of exogenous salicylic acid under changing environment review'. *Environmental and Experimental Botany*, 68: 14–25.

Jain, V., S. Vart, E. Verma and S. P. Malhotra. 2015. 'Spermine reduces salinityinduced oxidative damage by enhancing antioxidative system and decreasing lipid peroxidation in rice seedlings'.

Journal of Plant Biochemistry and Biotechnology, 24(3): 316-323.

Jdey, A., I. Slama, A. Rouached, C. Abdelly. 2014. 'Growth, Na^+, K^+, osmolyte accumulation and lipid membrane peroxidation of two provenances of *Cakile maritima* during water deficit stress and subsequent recovery'. *Flora*, 209: 54-62.

Jin, J., S, Ningwei, B. Jinhe and G. Junping. 2006. 'Regulation of ascorbate peroxidase at the transcript level is involved in tolerance to postharvest water deficit stress in the cut Rose (*Rose Hybrida* L.) CV. Samantha'. *Postharvest Biology and Technology*, 40(3): 236-243.

Kelen, M., E. Cubukdem-Iralay, S. Sen and G. Ozkan. 2004. 'Separation of abscisic acid, indole-3-acetic acid, gibberellic acid in 99R (*Vitis berlandieri* x *Vitis rupestris*) and rose Oil (*Rosa damascena* Mill.) by reversed phase liquid chromatography'. *Turkish Journal of Chemistry*, 28: 603-610.

Khan, M. I. R., N. Iqbal, A. Masood, M. Mobin, N. A. Anjum and N. A. Khan. 2016. 'Modulation and significance of nitrogen and sulfur metabolism in cadmium challenged plants'. *Plant Growth Regul*, 78: 1–11.

Khodadadi, S., M. A. Chegini, A. Soltani, H. A. Norouza and S. Sadeghzadeh Hemayati. 2020. 'Influence of Foliar-Applied Humic Acid and Some Key Growth Regulators on Sugar Beet (*Beta vulgaris* L.) Under Drought Stress: Antioxidant Defense System, Photosynthetic Characteristics and Sugar Yield'. *Sugar Technology*, 22: 765-772.

Liang, X., L. Zhang, S. K. Natarajan and D. F. Becker. 2013. 'Proline Mechanisms of Stress Survival'. *Antioxidants and Redox Signaling*, 219 (9): 998–1011.

Malekzadeh, P. 2015. 'Inflence of exogenous application of glycine betaine on antioxidative system and growth of salt-stressed soybean seedlings (*Glycine max* L.) '. *Physiology* and *Molecular Biology* of *Plants*, 21: 225–232.

Nickel, R. S. and B. A. Cunningham. 1969. 'Improved peroxidase assay method using Ieuco 2,3,6-trichlcroindophenol and application to comparative measurements of peroxidase catalysis'. *Analytical Biochemistry*, 27: 292-299.

Nusrat, N., M. Shahbaz, S. Perveen. 2014. 'Modulation in growth, photosynthetic e_ciency, activity of antioxidants and mineral ions by foliar application of glycine betaine on pea (*Pisum sativum* L.) under salt stress'. Acta *Physiologiae Plantarum*, 36: 2985–2998.

Peiretti, P. G and F. Gai. 2019. Quinoa: Cultivation, Nutritional Properties and Effects on Health. Food Science and Technology. 328p.

Rady, M. M., T. A. Abd El-Mageed, H. A. Abdurrahman and A. H. Mahdi. 2016. 'Humic acid application improves field performance of cotton (*Gossypium barbadense* L.) under saline conditions'. *The Journal of Animal and Plant Sciences*, 26(2): 487-493.

Shehata, S. M., H. S. Abdel-Azem, A. Abou El- Yazied and A. M. El-Gizawy. 2011. 'Effect of Foliar Spraying with Amino Acids and Seaweed Extract on Growth Chemical Constitutes, Yield and its Quality of Celeriac Plant'. *European Journal* of *Scientific Research*, 58 (2): 257 - 265.

Sidari, M., G. Ronzello, G. Vecchio and A. Muscolo. 2008. 'Influence of slope aspects on soil chemical and biochemical properties in a *Pinus laricio* forest ecosystem of Aspromonte (Southern Italy) '. *European Journal of Soil Biology*, 44(4): 364-372.

Stoleru, V., C. Slabu, M. Vitanescu, C. Peres, A. Cojocaru, M. Covasa and G. Mihalache. 2019. 'Tolerance of Three Quinoa Cultivars (*Chenopodium quinoa* Willd.) to Salinity and Alkalinity Stress During Germination Stage'. *Agronomy*, 287: 1-14.

Tadayyon, A., S. Beheshti and M. Pessarakli. 2017. 'Effects of sprayed humic acid, iron and zinc on quantitative and qualitative characteristics of Niger plant (*Guizotia abyssinica* L.) '. *Journal* of *Plant Nutrition,* 40: 1644-1650.

Thameur, A., A. Ferchichi and M. López-Carbonell. 2011. 'Quantification of free and conjugated abscisic acid in five genotypes of barley (*Hordeum vulgare* L.) under water stress conditions'. *South African Journal of Botany*, 77: 222-228.

Vanitha, K and S. Mohandass. 2014. 'Effect of humic acid on plant growth characters and grain yield of drip fertigated aerobic rice (*Oryza sativa* L.)'. *The Bioscan*, 9(1): 45-50.

Wilkinson, S and W. J. Davies. 2010. 'Drought, ozone, ABA and ethylene: new insights from cell to plant to community'. *Plant, cell and environment*, 33: 510-525.

Yang, A., S. S. Akhtar, M. Amjad, S. Iqbal and S. E. Jacobsen. 2016. 'Growth and physiological responses of quinoa to drought and temperature stress'. *Journal of Agronomy and Crop Science*, 202(6), 445-453.

Youssef, A. 2014. 'Influence of some amino acids and micro-nutrients treatments on growth and chemical constituents of Echinacea purpurea plant'. *Journal of Plant Production*, 5(4): 527-543.