Journal of Agricultural Sciences
(Tarim Bilimleri Dergisi)

J Agr Sci-Tarim Bili
e-ISSN: 2148-9297
jas.ankara.edu.tr

JAS

Effect of the Application of Foliar Jasmonic Acid and Drought Stress on Grain Yield and Some Physiological and Biochemical Characteristics of *Chenopodium quinoa* Cultivars

Azadeh KESHTKAR[a], Ahmad AIEN[b*], Hormozd NAGHAVI[c], Hamid NAJAFI NEZHAD[d], Mohammad Hassan SHIRZADI[a]

[a]*Department of Agronomy, Faculty of Agriculture, Jiroft Branch, Islamic Azad University, Jiroft, IRAN*

[b]*Agricultural and Horticultural Research Department, South Kerman Agricultural and Natural Resources Research and Education Center, Agricultural Research, Education and Extension Organization (AREEO), Jiroft, IRAN*

[c]*Soil and Water Research Department, Kerman Agricultural and Natural Resources Research and Education Center, Agricultural Research, Education and Extension Organization (AREEO), Kerman, IRAN*

[d]*Agricultural and Horticultural Research Department, Agricultural and Natural Resources Research and Education Center, Agricultural Research, Education and Extension Organization (AREEO), Kerman, IRAN*

ARTICLE INFO
Research Article
Corresponding Author: Ahmad AIEN, E-mail: aien_1348@yahoo.com
Received: 23 April 2020 / Revised: 25 July 2020 / Accepted: 29 September 2020 / Online: 25 March 2022

ABSTRACT

Water shortage is a critical problem touching plant growth and yield in arid and semi-arid areas such as Iran. Plant hormones such as jasmonic acid (JA) play a crucial role in altering plant morphology in response to stress. To investigate the effect of JA and drought stress on grain yield and some physiological and biochemical characteristics of quinoa cultivars, a split-plot factorial experiment based on randomized complete block design with three replications was conducted at Kerman Agricultural and Natural Resources Research and Education Center over two crop years (2018-2019). In this experiment, drought stress as the main factor in two levels including non-stress and stress based on 60% and 90% soil available moisture depletion and JA foliar application (0, 1 and 2 mg L^{-1}) and cultivars (Giza$_1$, Titicaca, Q$_{29}$) respectively, as factorial were sub factor. The maximum grain yield (3775 kg ha^{-1}) was obtained in Giza$_1$ cultivar under non-stress condition and 1 mg L^{-1} JA foliar application. The greatest grain protein and total chlorophyll content were obtained in Titicaca cultivar under non-stress and 1 mg L^{-1} JA foliar application by 18.17% and 1.83 mg g^{-1} fresh leaf weight, respectively. In the opposite trend, the maximum amount of malondialdehyde was observed under drought stress and non-use of JA. In general, given the results of this study, it can be stated that JA caused an increase in grain yield in *quinoa* cultivars by reducing the harmful effects of drought stress and improving plant growth.

Keywords: Quinoa, Abiotic stress, Growth regulator, Grain yield, Protein

1. Introduction

Chenopodium quinoa is an herbaceous dicotyledonous crop belonging to the C3 group of plants. It is a member of the Amaranthaceae family and is called a pseudo-grain plant (Adolf et al. 2012). Quinoa is plant-based caviar and is gluten-free and suitable for people with gluten intolerance. Food and Agriculture Organization has compared it with powdered milk because of its high nutritional value. If all conditions are favorable, it has been reported to be 1.5-6 t ha^{-1} depending on cultivar and crop area (FAO 2011). Drought stress is one of the most important limiting factors for plant growth and production which, reduces more than 50% the average production of most products worldwide (Lata et al. 2011). The resistance of plants to drought stress is very complex due to the complicated interactions between environmental factors and the variety of physiological, biochemical, molecular phenomena affecting plant growth, so it is quintessential to recognize the effects of drought stress on plants (Hui-Ping et al. 2012). Prolongation of the duration of the water stress causes significant damage and consequently plant cell death due to the generation of excess reactive oxygen species (ROS) which causes lipid peroxidation, degradation of proteins and nucleic acids causing an alteration in plant metabolism (Hossam et al. 2020; Al-Khateeb et al. 2019). Increasing plant resistance to drought stress would be the most economical approach to improving agricultural productivity and reducing agricultural use of freshwater resources (Sofy 2015; Sperdouli & Moustakas 2012). The initial symptoms of water stress realize at stomatal level and stomas close to prevent further moisture loss through transpiration (Farooq et al. 2009). High resistance of quinoa to drought stress and salinity is caused quinoa to be adapted to different climatic conditions (Bhargava et al. 2007).

Iran is centrally located in the arid and semi-arid regions of the Earth, with more than 60% of its land area being classed as arid and semi-arid, therefore, drought is one of the most important abiotic stresses in this region, that cultivation of drought-resistant crops such as quinoa is the best way to prevent crop failure (Vega-Galves et al. 2010). Drought-tolerant plants retain more water by absorbing water from the protoplast; accordingly, they have higher relative water content (Silva et al. 2007). Jasmonates (JA and methyl ester, methyl jasmonate) are a group of plant growth regulators that participate in many physiological processes and play a defensive role in the plant (Bari & Jones 2009). It is produced from lipid peroxide by increasing lipoxygenase activity (Maksymiec & Krupa 2002). JA can be used in limited amounts for enhancing plant growth, gene expression, osmolytes, antioxidant enzymes, and carotenoids (Sofy et al. 2020).

On the other hand, as key messengers introduce in the induction process, these compounds lead to the accumulation of secondary metabolites (Rubio et al. 2009). JA is the most important hormone for resistance to biotic and abiotic stresses. It accumulates rapidly in the wound and non-wound sites after plant injury (Bari & Jones 2009a). It has reported that JA exerts its protective role against drought stress by changes in protein, malondialdehyde content, and antioxidant activity (Yun-xia et al. 2010). In an experiment, the application of JA at a concentration of 0.5 mM on several rapeseed species *(Brassica napus)* exposed to drought stress for 10 days resulted in higher weight grain, chlorophyll content and leaf relative water content in all tested species compared to control (Mahabub Alam et al. 2014).

In this work recognizing the effects of different environmental stresses on the physiology of quinoa plant was studied for knowledge of its resistance mechanism and its survival in order to increase tolerance to stress. Due to the role of JA in increasing resistance in plants under stress, many features such as high grain quality of protein, high tolerance to drought and other stresses investigated. Therefore, the present study primary purpose was to investigate the variance effect of JA on grain yield and some physiological and biochemical parameters of quinoa cultivars under drought stress.

2. Material and Methods

This experiment has been carried out at the research farm of Joupar Station affiliated with Kerman Agricultural and Natural Resources Research and Education Center with 30° 17’ N and 57° 5’ E and 1900 m above sea level during two consecutive years (August 7^{th} 2018 and 2019). This region's climate is cold and mild, with an average annual rainfall of 145 mm and an average annual minimum and maximum temperatures of 4 and 23 °C. Meteorological observations for two years in Joopar research station has been shown in Table 1. Before experimenting, to determine the soil's physical and chemical properties, soil samples were collected from the field for laboratory analysis and the results have been shown in Table 2. Land preparation included tillage, fertilization and furrow. Based on soil test results the amount of 46 kg ha^{-1} P_2O_5 from triple super phosphate source was given to the soil before planting also, 92 kg ha^{-1} nitrogen fertilizer from urea source of one third before planting, was given a third in the stem of longitudinal growth stage (4 to 6 leaves) and one third of the soil bud stage. The experiment was conducted as a split-plot factorial based on randomized complete block design with 3 replications. In this experiment, drought stress as the main factor in two levels including non-stress (normal irrigation) and drought stress based on 60% and 90% soil available moisture depletion and JA foliar application at three levels (0, 1 and 2 mg L^{-1}) and three varieties of quinoa ($Giza_1$, Titicaca, Q_{29}) as factorial were sub-factor. Planting operations were arranged in the middle of the stack on August 7^{th} and the experimental designed based on number of lines 162, their distance 0.5 m, plant to plant distance 10 cm, variety to variety distance o.6 m, plant size 1 m, sample size from 30 cm to 80 cm with 16.6 plants per m^2. Plot length was 5 m and 4 rows per plot and distance between the replications was 3 m. Weeding was performed twice by the worker by hand and thinning of the plants was performed in a 10-leaf stage at a distance of 10 cm. In order to avoid interference with irrigation treatments, three rows were applied between drought stress and optimum irrigation. Drought stress was applied 20 days after of planting. At this stage the plant height was about 12 cm. Some genotypes such as Titicaca and $Giza_1$ cultivars are early genotypes, with the cultivation period ranging from 85 to 100 days, and medium-sized genotypes such as Q_{29} can be harvested over a period of 110 to 130 days. Calibrated Time Domain Reflectometry (TDR) was used for daily drought treatments in the way that in non-stress and drought stress, soil moisture is 12.2% and 8.9%, respectively. JA foliar application was done at 50% flowering stage in three times using a hand sprayer (Volume 20 L, pressure 3 bar, nozzle size 120 micrometer made by shark company of china). The duration of flowering, dough and milking periods of quinoa are the most sensitive stages to drought stress. Therefore, JA was used in these stages to combat drought stress (Geerts et al. 2008; Sofy et al. 2016a). In this experiment, German JA (SIGMA-ALDRICH) was used with purity of more than 97%. To 100 mg of the purchased solution, the amount of 5 cc of ethanol was added to make the milky solution be achieved. Then, the soluble volume of ethanol reached 30 ml and a completely transparent solution was produced. By adding sterile distilled water to solution up to 476cc volume, the solution of stoke 1 mM JA was obtained. the control solution contains 10 liters of sterile distilled water, the first treatment of the primary treated containing 48 ml stoke in 9.52 liters of sterile distilled water (equivalent 1 mg/L JA) and the second treatment containing 96 mL of stoke in 9.05 liters of sterile distilled water (equivalent to 2 mg/L JA).

Table 1- Detailed meteorological observations for two years based on the JOOPAR station

				Non-Stress (60%)	*Drought Stress (90%)*
Year	*Month*	*Temperature (C)*	*Humidity (%)*	*Precipitation (mm)*	*Precipitation (mm)*
2018	AUG.	17-33	16	0	0
	SEP.	15-30	18	2	0
	NOV.	13.5-27	24	5	0
	DEC.	11.5-22	31	16	0
2019	AUG.	18-31	19	1.5	0
	SEP.	17-29.5	20	6	0
	NOV.	14-23	25	11	0
	DEC.	10-21	33	17	0

Table 2- Some physico-chemical properties of soil (0-30 cm)

Soil Properties	*2018*	*Sandy Loam*
Soil texture	Sandy Loam	73
Sand (%)	69	12.6
Clay (%)	13	17.7
Silt (%)	18	7.9
pH	7.7	1.91
Electric Conductivity (ds m^{-1})	1.98	0.53
Organic matter (%)	0.48	18.1
Field Capacity (%)	18.8	7.3
Permanent wilting point (%)	7.6	1.35
Soil bulk density (g cm^{-3})	1.41	

2.1. Determination of chlorophyll and carotenoid

Absorbance of leaf extract at 663, 645 and 470 nm was measured using the S2100 Diod Array Spectrophotometer made in the United Kingdom using the following equation of photosynthetic pigments in mg g^{-1} leaf fresh weight (Lichtenthaler & Wellburn 1983).

$$Chlorophyll\ a = [12.7(A_{663}) - 2.69(A_{645})] \times V / 1000 \times W \quad [1]$$

$$Chlorophyll\ b = [22.9(A_{645}) - 4.68(A_{663})] \times V / 1000 \times W \quad [2]$$

$$Total\ Chlorophyll(a+b) = [20.2(A_{645}) + 8.02(A_{663})] \times V / 1000 \times W \quad [3]$$

$$Carotenoids = [(1000 \times A_{470}) - (2.27 \times C_a) - (81.4 \times C_b) / 226] \times V / 1000W \quad [4]$$

In the above equations, A is the absorbance read, V is the diluted extract volume and W is the fresh weight of the leaf sample in grams.

2.2. Malondialdehyde (MDA):

Malondialdehyde (MDA) was extracted with 5% trichloroacetic acid and determined according to (Heath & Packer 1968). MDA level was routinely used an index of lipid peroxidation and was expressed as µmol g^{-1} fresh weight using the following Equation:

$$MDA(\mu mol\ g^{-1} Fw) = [(A_{532} - A_{600}) / 156] \times 1000 \times dilution\ factor \quad [5]$$

2.3. Relative water content (RWC)

To determine the relative water content, 10 leaves of 5 random plants were used and finally the relative water content was calculated using the following equation in terms of percentage (Mata & Lamattina 2001):

$$RWC = (FW - DW) / (TW - DW) \times 100 \quad [6]$$

In the above equation, FW is leaf fresh weight, DW leaf dry weight, TW is turgidity weight, and DW is dry weight.

2.4. Seed protein

In order to measure grain protein, Kjeldahl apparatus in the laboratory first calculated the percentage of total nitrogen in the laboratory and then multiplied the percentage of nitrogen in the coefficient of 6.25 by the amount of protein in the grain (Magomya et al. 2014). Quinoa was harvested by plant yellowing and passing through the physiological ripening stage. To determine grain yield, two midline plants in each plot were harvested after removing the margin of plot. For calculation of biologic yield, 0.6 m^2 surface area was harvested and after drying and weighing, biomass dry weight and grain weight were determined. Harvest index was calculated using the following equation.

$$\text{Harvest index} = \text{Grain yield} / \text{biologic yield} * 100 \quad [7]$$

2.5. 1000-grain weight

Four samples (250 grains) were counted for measuring 1000-grain weight using a balance with accuracy of 0.001 g. Data imported for variance analysis running SAS 9.2 software. Before performing the statistical calculations, the normality of the experimental error variance was evaluated using SAS software for each trait. Furthermore, Duncan's multiple range test (DMRT) was used to compare the mean of main effects and the least squares means (LSMEANS) and pdiff were used for interactions.

3. Results and Discussion

3.1. Grain yield

The results of the comparison of triple interaction means showed that the greatest grain yield was obtained by $Giza_1$ (3775 kg ha^{-1}) in non-stress conditions and application of 1 mg L^{-1} JA also the Titicaca cultivar by (3438 kg ha^{-1}) was the second-ranking. The lowest seed yield by $Giza_1$ (1781 kg ha^{-1}) was obtained with 53% reduction in yield under drought stress and non-use of JA (Table 4). In total, in conditions of water scarcity, the stomas are closed, as a result, the amount of carbon input to the plant reduces and photosynthesis and grain yield reduces as well. Deficit irrigation in quinoa reduced grain yield, shoot dry weight, harvest index, number of seeds and seed weight (Razzaghi et al. 2012). Application of JA improved quinoa grain yield under drought stress and non-stress conditions compared to non-application of JA in mentioned conditions (Table 4). Increasing of grain yield due to the use of JA has also been reported in a bulk of studies (Yun-xia et al. 2010a; Sofy et al. 2016a). The response of different cultivars to the foliar application of JA was affected by the plant genotype in which $Giza_1$ and Titicaca had the highest grain yield. According to the correlation table, grain yield had a positive and significant relationship with grain protein, 1000-seed weight, biologic yield, harvest index, leaf relative water content and photosynthetic pigments traits. This indicates the importance of these traits in increasing seed yield in plant so that the highest positive correlation was obtained between grain yield and harvest index. In contrast, the correlation between malondialdehyde and grain yield was negative and significant (Table 5).

3.2. 1000-seed weight

The results of the comparison of triple interaction of year, JA in cultivar for thousand seeds weight showed that the maximum 1000-seed weight in the first year of experiment was obtained from Q_{29} cultivar with 2 mg L^{-1} of JA equivalent to 3.58 g and the lowest 1000-seed weight in the second year of experiment was obtained from Q29 cultivar and non-consumption of JA equivalent to 2.54 g (Table 3). Triple interaction results also indicated that maximum thousand seeds weight under non-stress conditions and application of 2 mg L^{-1} JA in Q_{29} cultivar was 3.74 g and the lowest was obtained in Titicaca cultivar with 36% decrease under drought stress and 1 mg L^{-1}JA by 2.4 g (Table 4).

Table 3- Mean comparison of 1000-seed weight of quinoa under interaction effect of JA and cultivar

Treatments		*1000- Seed Weight (g)*	
JA (mg L^{-1})	*Cultivar*	*First year*	*Second year*
		2018	2019
0	$Giza_1$	2.82±0.013 def	2.6± 0.14 f
	Titicaca	2.81± 0.006 ef	2.82± 0.012 cde
	Q_{29}	2.81±0.008 ef	2.54± 0.106 f
1	$Giza_1$	3.001± 0.093 c	3.005± 0.091b
	Titicaca	2.79±0.176 f	2.8± 0.174e
	Q_{29}	2.98± 0.081cd	2.79±0.085 e
2	$Giza_1$	3.15± 0.023 bc	3.2± 0.002 a
	Titicaca	2.98±0.085 cde	3.28± 0.183a
	Q_{29}	3.58±0.186 a	3.13± 0.217ab

Means in each column followed by similar letters are not significantly different at the 5% probability LSD Test.

According to the results of previous research on quinoa, intensity of drought stress at seed filling stage, by reducing photosynthesis, decreases weight of thousand seeds and ultimately decreases grain yield per unit area (Gamez et al. 2019). Increasing 1000-seed weight due to the use of JA has also been reported in many studies (Swiatek et al. 2003). According to the correlation table, there was a positive and significant relationship between 1000-seed weight and biologic yield, harvest index and photosynthetic pigments traits. It should be noted that the highest correlation coefficient (r=0.63) was between 1000-seed weight and total chlorophyll content (Table 5).

3.3. Grain protein

The Comparison of triple mean interaction showed that the greatest grain protein content was obtained from Titicaca under non-stress condition and 1 mg L^{-1} JA equivalent to 18.17%. Also, the lowest seed protein content was obtained from Q29 under drought stress and 1 mg L^{-1} JA equivalent to 13.93% (Table 4). Drought stress induces the expression of genes encoding intracellular proteases and induces the breakdown of proteins and the re-mobilization of nitrogen and subsequent synthesis of soluble substances. Therefore, a decrease in protein content under drought stress is associated with a decrease in synthesis and an increase in the activity of protein-degrading enzymes (Feller 2004), which is in line with the results of this study. Application of methyl jasmonate in stress condition increased protein content and increased activity of catalase and peroxidase enzymes (Yastreb et al. 2015). According to the correlation table, there was a positive and significant relationship between grain protein and biologic yield, harvest index, relative water content and chlorophyll content traits, whereas the highest correlation coefficient (r=0.501) was between grain protein and total chlorophyll content (Table 5).

Table 4- Three-way interaction mean comparison of drought stress, JA and cultivar related to all measured traits in quinoa plants (2018-2019)

Treatments			*Seed Yield (kg ha^{-1})*	*1000-Seed Weight (g)*	*Seed Protein (%)*	*Biologic yield (kg ha^{-1})*	*Chlorophyll a (Mg g^{-1}fw)*	*Total Chlorophyll (Mg g^{-1}fw)*	*MDA (µmol g^{-1}fw)*
Drought stress	JA (mg L^{-1})	Cultivar							
Non-stress (60% evacuation humidity)	0	Giza1	2779.58±114*de*	2.83±0.012*efg*	17.88±0.1*a*	10461.45±941.47*d−g*	1.2±0.089*c*	1.49±0.091*ac*	1.56±0.1*ij*
		Titicaca	3040.83±191.7*cd*	2.82±0.013*efg*	17.2±0.094*abc*	11078.7±482.38*cde*	1.125±0.046*cde*	1.4±0.039*cde*	2.25±0.31*g*
		Q29	2521.24±56.99*efg*	2.73±0.054*ghi*	15.18±0.058*ef*	9855.4±6.9.6 *f−i*	0.97±0.085 *fgh*	1.24±0.086 *fgh*	1.31±0.033 *jk*
	1	Giza1	3774.83±295.65*a*	3.21±0.003*c*	17.17±0.021*abc*	12556.62±389.36*ab*	1.4±0.056*ab*	1.72±0.059*a*	1.36±0.057 *jk*
		Titicaca	3286.67±181.3*bc*	3.19±0.004*c*	18.17±0.014*a*	11853.07±465.72*abc*	1.48±0.093*a*	1.83±0.097*a*	5.26±0.043*d*
		Q29	2693.83±36.09*df*	2.78±0.014*gh*	18.1±0.149*a*	9990.41±413.18*e−h*	0.97±0.054 *fgh*	1.31±0.053*efg*	0.49±0.033*l*
	2	Giza1	2939.16±203.83*cd*	3.19±0.018*c*	17.4±0.034*ab*	10816.63±308.26*c−f*	1.15±0.076*c*	1.53±0.06*a*	1.09±0.037*k*
		Titicaca	3437.88±116.09*ab*	3.38±0.141*b*	16.65±0.017*bcd*	12719.75±316.49*a*	1.37±0.048*b*	1.74±0.064*a*	1.76±0.126*hi*
		Q29	2886.64±172.11*de*	3.74±0.138*a*	16.25±0.069*b−e*	11488.05±481.94*bcd*	1.09±0.005*cde*	1.44±0.034*bcd*	1.92±0.055*h*
Drought stress (90% evacuation humidity)	0	Giza1	1780.82±92.65*i*	2.59±0.136*i*	16.52±0.066*bcd*	8447.5±494.44 *j*	0.85±0.025*i*	1.07±0.03 *j*	6.39±0.043*a*
		Titicaca	2329.02±61.7 *fgh*	2.8±0.004 *fg*	15.72±0.027*de*	8733.52±481.97*ij*	1.04±0.007*def*	1.24±0.017 *fgh*	5.88±0.24*b*
		Q29	2284.5±48.34*gh*	2.62±0.12*hi*	14.25±0.026 *fg*	8403.5±408.01*j*	0.56±0.045 *j*	0.71±0.057*k*	4.58±0.088*e*
	1	Giza1	2515.76±80.78*efg*	2.79±0.003*gh*	16.1±0.029*cde*	10423.68±298.71*d−g*	0.92±0.006*ghi*	1.16±0.028*hij*	5.45±0.1*cd*
		Titicaca	2377.67±106.79 *fgh*	2.4±0.002 *j*	15.93±0.027*de*	9436.7±365.3*g−j*	1.11±0.073*cde*	1.38±0.079*cde*	5.74±0.2*bc*
		Q29	2385.26±102.75 *fg*	2.99±0.114*de*	13.93±0.059*g*	9706.04±376.45 *f−i*	0.88±0.052*hi*	1.07±0.054*ij*	1.57±0.093*ij*
	2	Giza1	2557.16±126.97*efg*	3.16±0.021*cd*	15.95±0.033*de*	10159.3±367.99*e−h*	1.02±0.06*efg*	1.33±0.051*def*	5.15±0.058*d*
		Titicaca	2205.31±135.78*gh*	2.87±0.061*efg*	14.5±0.05 *fg*	9499.37±413.63*g−j*	1.15±0.0*cd*	1.44±0.012*bcd*	4.44±0.11*e*
		Q29	2002.18±187.09*hi*	2.96±0.051*ef*	14.26±0.045 *fg*	9161.4±627.31*hij*	0.94±0.029 *f−i*	1.19±0.034*ghi*	3.51±0.089 *f*

Means in each column followed by similar letters are not significantly different at the 5% probability LSD Test.

Table 5- Correlation coefficients between plant characteristics of quinoa (2018-2019)

Traits	*Seed Yield*	*Seed protein*	*1000-seed weight*	*Biologic Yield*	*Harvest Index*	*RWC*	*Chlorophyll a*	*Chlorophyllb*	*Total Chlorophyll*	*Carotenoied*	*MDA*
Seed Yield	1										
Seed protein	0.436^{**}	1									
1,000-seed weight	0.56^{**}	0.20^{ns}	1								
Biologic Yield	0.69^{**}	0.35^{**}	0.55^{**}	1							
Harvest Index	0.72^{**}	0.28^{*}	0.27^{*}	0.017^{ns}	1						
RWC	0.27^{*}	0.47^{**}	0.21^{ns}	0.33^{*}	0.081^{ns}	1					
Chlorophyll a	0.55^{**}	0.45^{**}	0.57^{**}	0.53^{**}	0.29^{*}	0.28^{*}	1				
Chlorophyll b	0.509^{**}	0.44^{**}	0.56^{**}	0.58^{**}	0.17^{ns}	0.49^{**}	0.47^{**}	1			
Total Chlorophyll	0.61^{**}	0.501^{**}	0.63^{**}	0.605^{**}	0.301^{*}	0.37^{**}	0.97^{**}	0.67^{**}	1		
Carotenoied	0.31^{*}	0.19^{ns}	0.309^{*}	0.21^{ns}	0.23^{ns}	-0.095^{ns}	0.40^{**}	0.32^{**}	0.42^{**}	1	
MDA	-0.37^{**}	-0.206^{ns}	-0.34^{*}	-0.37^{**}	-0.18^{ns}	-0.43^{**}	-0.13^{ns}	-0.39^{**}	-0.23^{ns}	-0.13^{ns}	1

*, **, ns means significant at 5% and 1% level of probability, and non-significant, respectively

3.4. Biologic yield

The results of mean comparison of triple interaction showed the maximum biologic yield was obtained from Titicaca cultivar with 2 mg L^{-1} JA under non-stress condition by 12719.8 kg ha^{-1} and the lowest biologic yield was related to Q_{29} cultivar with 34% decrease under drought stress condition and non-consumption of JA was equivalent to 8403.5 kg ha^{-1}, which was not statistically significant with $Giza_1$ (Table 4). According to previous research, drought stress in quinoa reduced dry matter accumulation and reduced biologic yield and biomass of whole plant (Sanchez et al. 2003), which is in agreement with the results of this study. Increased biologic yield by JA growth regulators has also been reported in other studies (Harpreet & Geertika 2015; Swiatek et al. 2003a). The results of correlation table showed that there was a positive significant relationship between biologic yield, relative water content, chlorophyll a, b and total chlorophyll. It was also found that the higher the level of malondialdehyde stress index in the plant, the lower the biologic yield would be (Table 5).

3.5. Harvest index

Non-drought stress treatment with 27.32% had higher harvest index than stress treatment with 24.52%. Harvest index is a measure of the ratio of seed weight to total plant and high yielding cultivars have higher harvest index (Roshdi et al. 2009). According to the results of the researchers on quinoa plant, harvest index was significantly lower than the control conditions. Also, under drought stress, reducing grain yield, harvest index, number of seeds and grain weight in comparison with normal irrigation conditions (Razzaghi et al. 2012). These are consistent with the results of this study. According to the correlation table, there was a positive and significant relationship between harvest index and chlorophyll a and total chlorophyll, indicating that increasing chlorophyll content in plant increased harvest index, and a negative relationship between harvest index and malondialdehyde (Table 5).

3.6. Relative water content (RWC)

The results of comparison of mean triple interaction showed that the Titicaca cultivar in non-stress condition at the first year of experiment had the maximum relative water content with 91.44% whereas the lowest value was observed in Q_{29} cultivar in the second year in drought stress condition with 67.36% which showed the 26.33% reduction (Table 6). One of the most important indices of plant water balance is relative water content and plays an important role in regulating stomata conductance and consequently plant photosynthesis rate (Mitchel et al. 2001). According to the results of the researchers, increasing the amount of irrigation water in quinoa plant resulted in an increase in chlorophyll and relative water content (Sharifan et al. 2018). Correlation coefficients showed that there was a positive significant relationship between relative water content and chlorophyll a, chlorophyll b and total chlorophyll, while a negative and significant correlation was observed with malondialdehyde. As the stress index in the plant increased, the relative water content decreased significantly. The decrease in relative water content was related to the decrease in water availability under drought stress conditions and the positive and significant relationship of this trait with total chlorophyll confirms this (Table 5).

Table 6- Interaction effect of drought stress and cultivar on Relative Water Content of quinoa a_1: Non-stress, a_2: Drought stress, c_1:$Giza_1$, c_2: Titicaca, c_3:Q_{29}

Year	*Drought Stress (a)*	*Cultivar (c)*	*RWC (%)*
2018	a_1	c_1	90.61± 1.48 ab
		c_2	91.44± 0.98 a
		c_3	89.65 ± 1.58 a-c
	a_2	c_1	85.9± 1.61 b-c
		c_2	85.14± 1.38 c
		c_3	86.14± 1.28 bc
2019	a_1	c_1	79.26± 2.21 d
		c_2	76.65± 2.88 d
		c_3	86.18± 3.18 bc
	a_2	c_1	70.51± 1.70 e
		c_2	68.41± 1.38 e
		c_3	67.36± 1.48 e

3.7. Chlorophyll a, b and total

The results of mean comparison of triple interaction of drought stress, JA and cultivar showed that the highest amount of chlorophyll a with 1.48 mg g^{-1} leaf fresh weight belonged to Titicaca cultivar and application of 1 mg L^{-1} JA under non-stress conditions and the lowest chlorophyll in was observed in Q_{29} cultivar equal to 0.56 under drought stress and non-use of JA (Table 4). The results of comparison of mean triple interaction of year with drought and JA showed that the greatest amount of chlorophyll b in the second year of experiment with 2 mg L^{-1} JA from non-stress treatment equal to 0.40 mg g^{-1} fresh weight and the lowest chlorophyll content at the first year in the stress and non-use of JA treatments were equal to 0.18 (Table 7). The results of mean comparison of triple interaction of drought stress, JA and cultivar showed that the maximum total chlorophyll content equal to 1.83 mg g^{-1} leaf fresh weight was obtained from Titicaca cultivar and 1 mg L^{-1} JA under non-stress condition which had not statistically significant difference with $Giza_1$ cultivar. The lowest total chlorophyll content was obtained with 61.2% decrease from Q_{29} cultivar with 0.71 mg g^{-1} leaf fresh weight under drought stress and non-use of JA (Table 4). The content of photosynthetic pigments, such as chlorophylls and carotenoids, which are important in converting light energy to chemical energy, varies under drought stress (Jaleel et al. 2009). These changes can somehow limit the photosynthesis that complicates the direct effect of drought on stoma closure, gas exchange, and photosynthesis. The decrease in chlorophyll and carotenoids concentrations is primarily associated with the production of reactive oxygen species (ROS) (Reddy et al. 2004), which is consistent with the results of this study. The use of JA at low concentration (1 mg L^{-1}) in non-stress conditions compared to non-application of JA and 2 mg L^{-1} resulted in the greatest chlorophyll content of whole leaf. According to the findings of researchers, the jasmonates may induce the gene expression of some key enzymes involved in chlorophyll biosynthesis through the formation of 5-aminolevulinic acid (Udea & Saniewski 2006). JA and its methyl ester (methyl jasmonate) can indirectly produce carbohydrates and other substances used in plant metabolism by increasing or preventing the plant's photosynthetic performance, in this way the plant is resistant to stress conditions (Popova et al. 2003). In the present study, due to genetic potential, Titicaca cultivar had the highest total chlorophyll content among cultivars, and under severe drought stress condition, Q_{29} cultivar had the lowest chlorophyll content compared to the other two cultivars. JA increases the chlorophyll content of the plant by increasing the activity of the enzymes involved in photosynthesis as well as decreasing the reactive oxygen species by influencing biochemical processes and antioxidant enzymes activity.

Table 7- Interaction effect of drought stress and foliar application of JA on Chlorophyll b content
a_1: Non stress, a_2: Drought stress, b_1:0 mg JA L^{-1}, b_2: 1 mg JA L^{-1}, b_3: 2 mg JA L^{-1}

Year	*Drought Stress (a)*	*JA (mg L^{-1}) (b)*	*Chlorophyll b content (mg g^{-1}fw)*
2018	a_1	b_1	0.278 ± 0.010 e
		b_2	0317 ± 0.005 cd
		b_3	0.333 ± 0.014 bc
	a_2	b_1	0.184 ± 0.012 g
		b_2	0235 ± 0.0101 f
		b_3	0294 ± 0.0106 de
2019	a_1	b_1	0.29 ± 0.0109 de
		b_2	0.356 ± 0.012 b
		b_3	0.406 ±0.031 a
	a_2	b_1	0.203 ± 0.016 fg
		b_2	0.236 ± 0.024 f
		b_3	0.275 ± 0.009 e

3.8. Carotenoids

Results of comparison of the mean interaction of drought stress and JA showed that the maximum leaf carotenoid content was observed under non-stress condition and application of 2 mg JA l^{-1} by 0.47 mg g^{-1} fresh leaf weight (Table 8). Also mean comparison of JA and cultivar showed that the highest amount of carotenoids belonged to Titicaca cultivar and application of 2 mg L^{-1} of JA equivalent to 0.51 mg g^{-1} leaf fresh weight, while the lowest amount was obtained from Q_{29} cultivar and non-use of JA (Table 9). Carotenoids are auxiliary pigments that affect the absorption and transmission of light and are chlorophyll protectors during the photo oxidation process, which reduces under drought stress, one of the most important causes of chlorophyll depletion being its degradation by reactive oxygen species. Decreasing of photosystem-2 activity lead to reduce Rubisco activity and lack of ATP synthesis; thereby reactive oxygen species in chloroplasts increases (Lawlor & Cornic 2002). Some researchers have reported that leaf chlorophyll and carotenoids content decrease under drought stress (Nayyar & Gupta 2006). In a study, the content of chlorophyll and carotenoids in cotton genotypes decreased with induction of drought stress compared to control and increased with increasing drought stress (Kumar et al. 2007).

Table 8- Interaction effect of drought stress and foliar application of JA on Carotenoieds content
a_1: Non stress, a_2: Drought stress, b_1:0 mg JA l^{-1}, b_2:1 mg JA l^{-1}, b_3:2 mg JA l^{-1}

Drought stress (a)	*JA (b)*	*Carotenoieds content (Mg g^{-1}fw)*
1	1	0.393±0.033b
1	2	0.468±0.035a
1	3	0.47±0.037a
2	1	0.323±0.025c
2	2	0.328±0.026c
2	3	0.397±0.036b

Table 9- Interaction effect of foliar application of JA and cultivar on Carotenoieds content
b_1:0 mg JA l^{-1}, b_2:1 mg JA l^{-1}, b_3:2 mg JA l^{-1}, c_1:$Giza_1$, c_2: Titicaca, c_3:Q_{29}

JA (b)	*Cultivar*	*Carotenoieds content ($Mg\ g^{-1}fw$)*
1	$Giza_1$	0.388±0.042bcd
1	Titicaca	0.363±0.031de
1	Q_{29}	0.325±0.037e
2	$Giza_1$	0.414±0.046bc
2	Titicaca	0.416±0.043b
2	Q_{29}	0.365±0.040cde
3	$Giza_1$	0.375±0.038bcd
3	Titicaca	0.51±0.047a
3	Q_{29}	0.415±0.045b

3.9. Malondialdehyde (MDA)

The results of the comparison of mean triple interaction showed that the maximum amount of malondialdehyde (MDA) in quinoa leaf under drought stress was observed in $Giza_1$ cultivar and no application of JA by 6.39 μmol g^{-1} leaf fresh weight while the lowest amount of MDA was in Q_{29} cultivar under non-stress conditions associated with 1 mg L^{-1} JA equivalent to 0.49 (Table 4). In stress conditions, excessive amounts of oxygen free radicals cause damage to the cell membrane, the most prominent of which is the peroxidation of the fatty acids present in the membrane. This change in cell membrane fatty acids result in the production of small compounds such as MDA, which is the end product of lipid peroxidation, while an increase in this compound is a sign of cell membrane damage (Jia et al. 2015). The use of JA to help the plant to cope with environmental stresses, including drought, can increase the activity of plant antioxidant systems through the expression of specific genes, thereby reducing lipid peroxidation and damage to the membrane (Shan & Liang 2010). As can be seen in the results, JA at the level of 1 mg L^{-1} resulted in a significant reduction of MDA. Increasing the amount of MDA production as a major indicator of oxidative stress and the crucial role of JA in reducing drought stress and lowering the amount of MDA in other plants have also been demonstrated (Alam et al. 2014). Correlation coefficients showed that MDA had a negative and significant relationship with grain yield, 1000-seed weight, biologic yield, relative water content and chlorophyll b, indicating that water deficit stress increased MDA in plant and decreased yield, and yield components in the plant (Table 5).

4. Conclusions

Results showed that the incidence of drought stress (90% of soil moisture discharge), decreased yield and yield components quinoa, So that cause to 53%, 36% and 34% reduction in grain yield, 1000-seed weight and biologic yield respectively, but the grain yield decrease in $Giza_1$ cultivar was due to lower chlorophyll loss and consequently higher photosynthesis than other cultivars. The application of foliar JA compared to non-spraying conditions in both favorable irrigation conditions and drought stress, had the greatest effect on performance and yield components. Application of JA at 1 mg L^{-1} increased grain yield by 16% compared to non-use. The mentioned items can be concluded that the plant quinoa has a tolerance and relatively favorable resistance to drought stress and foliar of JA on the plant under stress conditions causes the plant resistance and reduces the harmful effects of dehydration on plant growth process. Also, by applying appropriate management in farm and planting of early and yielding cultivars it is possible to guarantee the establishment of this plant in low water conditions of course, it is suggested that the experiment is also studied and investigated in greenhouse conditions.

References

Adolf V I, Jacobsen S E & Shabala S (2012). Salt tolerance mechanisms in quinoa (*Chenopodium quinoa Willd.*). Environmental and Experimental Botany 92: 43-54 https://doi.org/10.1016/j.envexpbot.2012.07.004

Alam M M, Nahar K, Hasanuzzaman M & Fujita M (2014). Exogenous JA modulates the physiology, antioxidant defense & glyoxalase systems in imparting drought stress tolerance in different Brassica species. Plant Biotechnology 8: 279-293 https://doi.org/10.1007/s11816-014-0321-8

Al-Khateeb S A, Al-Khateeb A A, El-Beltagi H S & Sattar M N (2019). Genotypic variation for drought tolerance in three date palm (*Phoenix dactylifera* L.) cultivars. Fresenius Environ. Bull 28: 2957-2967. https://doi.org/10.1186/s40659-020-00305-3

Bhargava A, Shukla S, Rajan S & Ohri D (2007). Genetic diversity for morphological and quality traits in quinoa (*Chenopodium quinoa Willd.*) germplasm. Genetic Resources and Crop Evolution 54: 167-173. https://doi.org/10.1007/s10722-005-3011-0

Bari R & Jones J D G (2009a). Role of plant hormones in plant defense responses. Plant Molecular Biology 69: 473-488 https://doi.org/10.1007/s11103-008-9435-0

FAO (2011). Quinoa, an ancient crop to contribute to world food security. Food and Agriculture Organization of the United Nations. rlc-quinoa@fao.org

Farooq M, Wahid A & Lee D J (2009). Exogenously applied polyamine increase drought tolerance of rice by improving leaf water status, photosynthesis and membrane properties. Acta Physiologiae Plantarum 31(5): 937-945. https://doi.org/10.1007/s11738-009-0307-2

Feller U (2004). Plant Cell Death Processes. Elsevier Academic Press pp. 392 London

Gamez A L, Soba D, Zamarreno A M, Garcia-Mina J M, Aranjuelo I & Morales F (2019). Effect of water stress during grain filling on yield, quality and physiological traits of Illpa and Rainbow quinoa (*Chenopodium quinoa Willd*.) cultivars. *Journal of Plants* 8: 162-173 https://doi.org/10.3390/plants8060173

Geerts S, Raes D, Garcia M, Vacher J, Mamani R, Mendoza J, Huanca R, Morales B, Miranda R & Cusicanqui J (2008). Introducing deficit irrigation to stabilize yields of quinoa (*Chenopodium quinoa willd*.). *Europian Journal Agronomy* 28: 427-436. https://doi.org/10.1016/j.eja.2007.11.008

Harpreet K & Geetika S (2015). Exogenous application of jasmonic acid offers tolerance to salinity by altering stress responses in *Brassica napus* L. Variety GSC 6 International Journal of Science and Research. 2319-7064

Heath R L & Packer L (1968). Photoperoxidation in isolated chloroplasts. I. Kinetics and stoichiometry of fatty acid peroxidation. Archives of Biochemistry and Biophysics 125 (1):189-198. https://doi.org/10.1016/0003-9861(68)90654-1

Hossam S, El-Beltagi A, Heba I M & Sofy M R (2020). Role of Ascorbic acid, Glutathione and Proline Applied as Singly or in Sequence Combination in Improving Chickpea Plant through Physiological Change and Antioxidant Defense under Different Levels of Irrigation Intervals. Molecules 25: 1702-1710. https://doi.org/10.3390/molecules25071702

Hui-Ping D, Chan-Juan Sh, An-Zhi W & Tuxi Y (2012). Leaf senescence and photosynthesis in foxtail (*Setaria italica* L) varieties exposed to drought conditions. *Australian Journal of Crop Science* 6(2): 232-237

Jaleel C A, Manivannan P, Wahid A, Farooq M, Al-Juburi J, Somasundaram R & Panneerselvam R (2009). Drought stress in plants: a review on morphological characteristics and pigments composition. International Journal of Agriculture and Biology 11: 100-105

Jia X, Sun C, Li G, Li G & Chen G (2015). Effects of progressive drought stress on the physiology, antioxidative enzymes and secondary metabolites of Radix Astragali. Acta Physiology Plant 37: 262-275. https://doi.org/10.1007/s11738-015-2015-4

Kumar Parida A, Dagaonkar V S, Phalak M S, Umalkar G V, Laxman P & Aurangabadka S (2007). Alterations in photosynthetic pigments, protein and osmotic components in cotton genotypes subjected to short-term drought stress followed by recovery. Plant Biotechnology Report 1: 37-48 https://doi.org/10.1007/s11816-006-0004-1

Lata C, Sarita, J H, Prasad M & Sreenivasulu N (2011). Differential antioxidative responses to dehydration induced oxidative stress in core set of foxtail millet cultivars. Protoplasma 248: 817-828

Lawlor D W & Cornic G (2002). Photosynthetic carbon assimilation and associated metabolism in relation to water deficits in higher plants. Plant, cell and Environment 25: 275-294 https://doi.org/10.1046/j.0016-8025.2001.00814.x

Lichtenthaler H K & Wellburn A R (1983). Determinations of total carotenoids and chlorophylls a and b of leaf extracts in different solvents. Biochemical Society Transactions 11: 591-592 https://doi.org/10.1042/bst0110591

Magomya A M, Kubmarawa D, Ndahi J A & Yebpella G G (2014). Determination of plant proteins via the kjeldahl method and amino acid analysis. A comparative study. *International Journal of Scientific and Technology Research* 3(4): 68-72

Mahabub Alam M D, Kamrun N, Hasanuzzaman M & Masayuki F (2014). Exogenous jasmonic acid modulates the physiology, antioxidant defense and glyoxalase systems in imparting drought stress tolerance in different Brassica species. Plant Biotechnology Reports, 8: 279-293. https://doi.org/10.1007/s11816-014-0321-8

Maksymiec W & Krupa Z (2002). Jasmonic acid and heavy metals in Arabidopsis plants-a similar physiological response to both stressors. Journal of Plant Physiology 159: 509-515. https://doi.org/10.1078/0176-1617-00610

Mata C G & Lamattina L (2001). Nitric oxide induces stomatal closure and enhances the adaptive plant responses against drought stress. Plant Physiology 126: 1196 -1204. https://doi.org/10.1104/pp.126.3.1196

Mitchell R A, Mitchell V J & Lawlor D W (2001). Response of wheat canopy CO_2 and water gas exchange to soil water content under ambient and elevated CO_2. Global Change Biology 7: 599-611. https://doi.org/10.1046/j.1354-1013.2001.00431.x

Nayyar H & Gupta D (2006). Differential sensitivity of C_3 and C_4 plants to water deficit stress. Association with oxidative stress and antioxidants. Environment Experiment Botany 58: 106-113. https://doi.org/10.1016/j.envexpbot.2005.06.021

Popova L, Ananieva E, Hristova V, Christov K, Georgieva K, Alexieva V & Stoinova Z H (2003). Salicylic acid and methyl jasmonate induced protection on photosynthesis to paraquat oxidative stress. Bulgarian journal of Plant Physiology 18: 133-152

Razzaghi F, Plauborg F, Jacobsen S E, Jensen C R & Andersen M N (2012). Effect of nitrogen and water availability of three soil types on Yield, radiation use efficiency and evapotranspiration in field-grown quinoa. Agricultural Water Management 109: 20-29. https://doi.org/10.1016/j.agwat.2012.02.002

Razzaghi F, Ahmadi S H, Jacobsen S E, Jensen C R & Andersen M N (2012). Effects of salinity and soil-drying on radiation use efficiency, water productivity and yield of quinoa (*Chenopodium quinoa Willd*). *Journal of Agronomy and Crop Science* 198: 173-184. https://doi.org/10.1111/j.1439-037x.2011.00496.x

Reddy A R, Ramachandra R K, Chaitanya V & Vivekanandan M (2004). Drought induced responses of photosynthesis and antioxidant metabolism in higher plants. *Journal of Plant Physiology* 161: 1189-1202

Roshdi M, Rezadost S, Khalil Mahaleh J & Haji Nasab N (2009). The impact of bio-fertilizers on yield of three varieties of sunflower. *Journal of Agricultural Sciences and Technology* 10: 24-11

Rubio V, Bustos R, Luisa M L, Irigoyen X, Cardona-Lopez, Rojas-Triana M & Paz-Ares J (2009). Plant hormones and nutrient signaling. Plant Molecular Biology 69: 361-373

Sanchez H B, Lemeur R, Damme P V & Jacobsen S E (2003). Ecophysiological analysis of drought and salinity stress of quinoa (*Chenopodium Quinoa Willd*). Food Reviews International 19: 111-119. https://doi.org/10.1081/fri-120018874

Shan C & Liang Z (2010). Jasmonic acid regulates and glutathione metabolism in Agropyron cristatum leaves under water stress. Plant Science 178: 130-139

Sharifan H, Jamali S & Sajadi F (2018). The effect of different seawater and deficit irrigation regimes on leaf properties of quinoa. Water and Irrigation Management. *Scientific Journal of Agriculture* 8(2): 62-75

Silva M A, Jifon J L, Da Silva J A G & Sharma V (2007). Use of Physiological parameters as fast tools to screen for drought tolerance in sugarcane. *Brazilian Journal of Plant Physiology* 19: 193-201. https://doi.org/10.1590/s1677-04202007000300003

Sofy M R (2015). Application of salicylic acid and zinc improves wheat yield through physiological processes under different levels of irrigation intervals. *International Journal of Plant Research* 5(5): 136-156

Sofy M R, Abd El-Monem M, Sharaf H & Fouda M (2016a). Stimulatory effect of hormones, vitamin c on growth, yield and some metabolic activities of Chenopodium quinoa Plants in Egypt. *Journal of plant Biochemistry and physiology* 10(4): 2329-9029 https://doi.org/10.4172/2329-9029.1000161

Sofy M R , Seleiman M F, Bushra A, Basmah M & Heba I (2020). Minimizing Adverse Effects of Pb on Maize Plants by Combined Treatment with Jasmonic, Salicylic Acids and Proline Agronomy 10: 699-707h. https://doi.org/10.3390/agronomy10050699

Sperdouli I & Moustakas M (2012). Interaction of proline, sugars, and anthocyanins during photosynthetic acclimation of *Arabidopsis thaliana* to drought stress. *Journal of Plant Physiology* 169: 577-585. https://doi.org/10.1016/j.jplph.2011.12.015

Swiatek A, Azmi A, Witters E & Van Onckelen H (2003). Stress messengers jasmonic acid and abscisic acid negatively regulate plant cell cycle. *Journal of plant physiology* 172-178

Ueda J & Saniewski M (2006). Methyl jasmonate-induced stimulation of chlorophyll formation in the basal part of tulip bulbs kept under natural light conditions. *Journal of fruit and Ornamental Plant Reserch* 14: 199-210

Vega-Galvez A, Miranda M, Vergara J, Uribe E, Puente L & Martinez E A (2010). Nutrition Facts and functional potential of quinoa (*Chenopodium quinoa Willd*) an ancient Andean grain: a review. *Journal of the Science of Food and Agriculture* 90(15): 2541-2547

Yastreb T O, Kolupaev Y E, Shvidenko N V, Lugovaya A A & Demitriev A P (2015). Salt stress response in Arabidopsis thaliana plants with defective jasmonate signaling. Applied Biochemistry and Microbiology 51: 451-454. https://doi.org/10.1134/s000368381504016x

Yun-Xia G, Li-Jun Z, Feng-hai L, Zhi-bin C, Che W, Yun-cong Y, Zhen-hai H, Zhang Jie & Zhen-sheng S (2010a). Relationship between jasmonic acid accumulation and senescence in drought -stress. *African Journal of Agriculture Research* Vol 5(15): 1978-1983