Evaluation of Grain Yield and Physiological and Biochemical Trait of Quinoa (*Chenopodium quinoa* Willd) under Irrigation and Organic Fertilizers

Hossein Tabiei[1], **Reza Baradaran***[2], **Mohammad Javad Seghatoleslami** [2]
and Seyed Gholam Reza Mousavi[2]
1- PhD Student, Department of Agriculture and Plant Breeding, Faculty of Agriculture, Birjand Branch, Islamic Azad University, Birjand, Iran
2- Associate Professor, Department of Agriculture and Plant Breeding, Faculty of Agriculture, Birjand Branch, Islamic Azad University, Birjand, Iran
*Corresponding author: r.baradaran@yahoo.com

Abstract

Quinoa received worldwide attention due to its ability to grow in different stresses. The grain protein content and its quality are better than other seeds. It is rich in the amino acids lysine, globulin, which makes it suitable for patients with celiac disease. In order to evaluate fertilizer levels under different irrigation regimes on grain yield, physiological and biochemical properties of quinoa, an experiment was conducted during the two cropping years of 2019-20 as split plots in a randomized complete block design with three replications in Birjand, IR Iran. The main factor was irrigation regimes at three levels (100%, 65%, and 30% of reference evapotranspiration) and the secondary factor was fertilizer levels at six levels (control, manure, and vermicompost, NPK, 50% manure+ 50% NPK and 50% vermicompost+ 50% NPK). The highest chlorophyll content with an average of 26.28 mg.gdw^{-1} for the first year using irrigation ET0 65% and the application of 50% M+ 50% NPK and its lowest amount with an average of 13.85 mg.gdw^{-1} belonged to the first year using ET0 30% treatment with no fertilizer application. Maximum levels of GPX, APX enzymes were observed under severe stress without fertilizer and the lowest levels were observed in 100% evapotranspiration of the reference plant (ET0) with the application of 50% M+ 50% NPK, which showed a significant difference with other treatments. Drought stress affected and reduced quinoa grain yield and the highest dry yield (2496.1 kg ha^{-1}) was obtained at the control irrigation level in 2019 and there was no significant difference between the two crop years with no stress. However, irrigation up to 65 mm evaporation from Class A pan produces acceptable dry matter, which indicates the good resistance of this plant to drought stress. We can conclude that stress mitigation along with 50% Vermicompost+ 50% NPK happened to be a corresponding hike in biochemical properties, improving physiological aspects and grain yield of quinoa.

Key words Antioxidant enzymes · Drought stress · Carotenoids · Manure · Vermicompost

Tob Regul Sci. ™ 2022;8(1): 1326-1344
DOI: doi.org/10.18001/TRS.8.1.105

Introduction

Quinoa is a plant that has received worldwide attention due to its ability to grow in different stresses, such as soil salinity, acidity, drought, cold, etc. The seed protein content of this plant is higher than seeds and its protein quality is better than seeds and legumes. It is rich in the amino acids lysine, globulin, and albumin and contains a low concentration of prolamine, which makes it suitable for patients with celiac disease (Dakhili et al., 2019). These issues have led the Food and Agriculture Organization of the United Nations to prioritize its development, especially in countries facing environmental tensions (Angeli et al., 2020).

A decrease in leaf relative water content (RWC) is one of the first effects of drought stress on plants, and then the cell membrane loses its stability and solutes leak out of the cell, and photosynthesis begins to decline. Decreasing the RWC of the leaf disrupts the physiological processes of the plant and alters the metabolism of proteins and the activity of enzymes (Goswami et al., 2020). Drought stress and nutrient deficiencies also increase the production of reactive oxygen species (Rodríguez-Rosales et al.). These compounds oxidize plant tissues and are strong inhibitors of the Calvin cycle. Under drought stress, oxidative stresses act as secondary stresses and, while reducing the rate of photosynthesis, ultimately reduce yield and have irreversible destructive effects on the plant (Umair Hassan et al., 2020). The two enzymes of peroxidase and catalase (CAT), in two different forms, inactivate a variety of ROS to continue growth under stress while improving photosynthesis and delaying aging (Forouzandeh et al., 2019). Plants with higher levels of antioxidants are more resistant to oxidative damage to biotic and non-biotic stresses (Baghizadeh et al., 2020). Antioxidant enzymes, such as superoxide dismutase (SOD), CAT, glutathione peroxidase (GPX), and peroxidase (POX) are able to oxidize stress-induced reactive oxygen species (AOS) and regenerate them into water and oxygen molecules (Gill and Tuteja, 2010). It has been reported that under drought stress, the activity of SOD and POX enzymes increases and catalase activity decreases (Guo, 2018). Changes in the activity of these enzymes under drought stress and using fertilizer have been considered by researchers. Nitrogen fertilizer has been reported to reduce the activity of ascorbate peroxidase (APX) and pyrogallol peroxidase (Giansoldati et al., 2012).

Drought stress affects nutrient uptake and imbalance in the plant. Proper nutrition during stress can help the plant to cope with various environmental stresses. In this regard, using organic fertilizers, firstly, plant yield increases, secondly, its application in agricultural products has an important role in improving the quality of food consumption (Hussain et al., 2018). On the other hand, it has been reported that under drought stress, the application of nitrogen fertilizer increases leaf chlorophyll, improves growth and plant yield stability (WU et al., 2019).

Evaluation of plant nutrition systems is one of the important needs in crop planning in order to achieve high yield and optimal quality. To produce plants, in addition to quantity, the quality of production is also of special importance. In a high-yield crop ecosystem, there is no success if the quality is poor. Although the seed yield of some crops increases due to the use of large amounts of chemical fertilizers, the yield of many other crops is stagnant due to imbalances in soil fertility and organic matter content (Moklyachuk et al., 2019). Today, due to the increasing costs of consumer organizations and increasing environmental pollution, the use of organic fertilizers to improve the supply of nutrients to achieve sustainable agriculture is essential (Park et al., 2011).

Vermicompost is an organic compound that is microbiologically active and rich in macro and micronutrients, which are able to release several organic acids, including oxalic acid, and lead to the solubility of potassium and phosphorus. Consumption of appropriate amounts of vermicompost also improves soil microbial activity and production of growth regulators by these microorganisms making more nutrients available to the plant to increase leaf chlorophyll content and yield. Increased soil nitrogen may be due to higher activity of acid phosphatase and protease in soil treated with vermicompost (Yang et al., 2015). The highest rate of photosynthesis of the Chickpea (*Cicer arietinum* L. cv. Karaj) was obtained under severe drought stress and application of vermicompost compared with the control treatment (Hosseinzadeh et al., 2016).

Due to water shortage in arid and semi-arid regions and the effect of chemical fertilizers on the environment, the use of organic fertilizers to achieve sustainable agriculture, fertility, and soil moisture is very important. Therefore, this experiment was performed to investigate the interaction of chemical and organic fertilizers and drought stress on some physiological and biochemical traits of the quinoa.

Material and experimental methods

Conditions and experiment location

An experiment was conducted during the two cropping years of 2019-20 as split plots in a randomized complete block design with three replications in the Academic Center for Education, culture, and Research (ACECR), Birjand, IR Iran in 2019 and 2020. This region is located at 69 degrees and 13 minutes east longitude and latitude 42 degrees and 52 minutes north and at an altitude of 1491 meters above sea level, which is one of the semi-arid regions in the Köppen climate classification scheme. The average annual temperature is 16°C, the average annual rainfall is 171 mm and the annual frost days are 48 days. The main factor was irrigation regimes at three levels (irrigation equivalent to 100% ET0 (control), irrigation equivalent to 65% ET0, and irrigation equivalent to 30% ET0) and Weather base the secondary factor was fertilizer levels at six levels (control, manure, vermicompost, NPK, 50% manure+ 50% NPK and 50% vermicompost+ 50% NPK).

Assessment of the tested soil properties

In order to test the soil after tillage and leveling and before planting, a composite sample at the depth of 0- 30 cm was prepared each year; the results of soil analysis are shown in (Table 1).

Table 1 Soil physical and chemical analysis in 2019 and 2020 (depth of 0 to 30 cm)

Year	pH	EC ds.m^{-1}	T.N.V	O.C	Sand	Silt	Clay	N Total	P(ave)	K(ave)	Fe	Mn	Zn	Cu
			%						P.P.M					
2019	7.9	3.73	13.5	0.08	74	14	12	0.013	4.4	168	0.68	0.94	0.24	0.36
2020	7.7	3.72	13.6	0.09	74	15	11	0.015	4.6	168	0.67	0.99	0.25	0.37

Planting and application of treatments

The cultivar used in this study was red quinoa that was planted in a row, in which each subplot consisted of 5 planting lines with a length of 5 meters with a line spacing of 50 cm and a row spacing of 10 cm and a distance between the two main plots was 2 m. Planting was done by dry planting on April 9 and manually and harvesting was done on November 10. The first irrigation was done one day after planting and in order to ensure uniform greening, the second irrigation was done 4 days after the first irrigation. Irrigation was done using a hose and a meter and the volume of water given to each plot during the whole growing period was calculated. Based on soil analysis, phosphorus fertilizer was obtained from a triple superphosphate source (250 kg ha^{-1} at planting time) and potassium fertilizer was obtained from a potash sulfate source (250 kg ha^{-1}) and added to the soil before planting and while soil preparation. Nitrogen fertilizer was used from urea (350 kg ha^{-1}) three times, including before planting, top-dressing in 6-8 leaf stage, and before flowering. For manure treatment, 35 tons per hectare of rotten cow manure and 7 tons per hectare of vermicompost in the pre-planting stage were thoroughly mixed with the soil. Weeding was performed in two stages, 4- 6 leaf and 6- 8 leaf stages, until the final density of 20 plants per square meter was reached. The chemical properties of the fertilizers used are provided in (table 2) and the results related to changes in temperature, relative humidity, and precipitation of the experiment location during the research period are presented in (Table 3).

Table 2 Fertilizer properties of the experimental

Fertilizers information	Ash	O.C	N	P	K	Fe	Mn	Zn	Cu	pH	EC	Humidity percentage
	%					ppm					1 ÷ 5	
Chemical	***	***	7.5	9.1	16.1	***	***	***	***	***	***	***
Manure	55	21.5	1.2	0.6	1.57	2482	256	71	15	8.0	16.5	***
Vermicompost	70	15.4	0.8	0.7	0.42	3795	277	105	20	8.3	3.19	44.7

Table 3 Climatic parameters of the experimental site in 2019 and 2020

Reference: I. R. of Iran Meteorological Organization (Anonymous, 2020)

Month	Mean min. Temperature ˚c		Mean max. temperature ˚c		Mean min. Humidity (%)		Mean max. Humidity (%)		Mean Precipitation (mm)	
	2019	2020	2019	2020	2019	2020	2019	2020	2019	2020
April	10.5	8.2	23.8	20.4	22.6	25.5	68.0	85.9	0	16.0
May	13.8	13.8	28.7	29.5	11.9	12.5	51.4	57.2	0	2.0
June	18.0	19.4	33.4	36.6	11.8	6.4	44.1	27.7	0	0
July	24.2	22.2	38.9	37.4	6.5	8.2	23.7	25.6	0	0
August	22.1	22.4	37.4	38.5	6.3	6.3	26.7	29.5	0	0
September	17.8	15.4	34.9	32.2	6.8	10.4	27.1	38.3	0	0
October	10.5	9.3	27.2	27.6	11.7	7.3	38.9	30.1	0	0
November	7.4	4.4	21.1	23.2	26.5	13.3	65.5	46.0	0	0

Water requirement was calculated using the FAO method using class A evaporation pan using equations 1. Then, considering the efficiency of 90% for water distribution in the field, irrigation was performed. In this method, to calculate the amount of water required by the plant, first evaporation was obtained from the daily pan evaporation (Singh et al., 2017) of the Meteorological Department and then multiplied by the pan coefficient (Kpan) (Howell et al., 2008). The obtained value was the ET0. ET0 = Kpan× Ep (1)

Chlorophyll a, b, and carotenoids

0.2 g of crushed plant tissue and 80% acetone were added to the sample, and then placed in a centrifuge at 6000 rpm for 10 minutes. The supernatant was read separately by spectrophotometer at 664 nm for chlorophyll a, 645 nm for chlorophyll b, and 470 nm for carotenoids (Arnon, 1949).

Anthocyanin content

0.1 g of fresh plant tissue was ground with methanol and kept in the dark at 25 °C for 24 hours. It was then centrifuged at 10000 rpm for 5 minutes at a wavelength of 550 nm. Concentration was calculated using Equation 2 considering the extinction coefficient (ε) of 33000 $cm.mol^{-1}$ (Wagner, 1979).

(2) A= εbc; A= Absorption, ε= Extinction coefficient, c= H_2O_2 Concentration, b= Covet length (1 cm)

Carbohydrate measurement

0.2 g of fresh tissue and 10 cc of 95% ethanol were placed in closed test tubes and then placed for 1 hour in a bain-marie at 80 °C. After cooling, half a percent phenol and 98% sulfuric acid were added. Finally, it was read at 483 nm using a spectrophotometer (Irigoyen et al., 1992).

Determination of proline

0.1 g of the leaf sample was crushed and filtered in a mortar with 3% sulfosalicylic acid. Glacial acetic acid and ninhydrin acid were added to this solution and it was placed at 100 °C for 30 minutes. Then, 6 cc of toluene was added to these samples and finally, its absorption was read at 520 nm using a spectrophotometer (Bates et al., 1973).

Antioxidant activities

Antioxidant activities were assessed using guaiacol peroxidase (GPX) (Fielding and Hall, 1978), APX (Yoshimura et al., 2000), CAT (Beers and Sizer, 1952), and polyphenol oxidase (Janovitz-Klapp et al., 1990).

Measurement of malondialdehyde (MOD) concentration

0.2 g of fresh leaf tissue was ground in a crucible containing 5 ml of 0.1% trichloroacetic acid (TCA). The resulting extract was centrifuged at 10,000 rpm for 5 min. Then, to one milliliter of the above solution, four milliliters of 20% chloroacetic acid solution containing 0.5% thiobarbituric acid (TBA) was added. The hot water bath was then heated at 95 °C for 30 minutes. It was immediately cooled in ice and centrifuged again at 10,000 rpm for 10 minutes. The absorption was read using a spectrophotometer at 532 nm (Heath and Packer, 1968).

Table 4 Combined analysis of variance for physiological characteristics of Quinoa under irrigation levels and nutrition management.

S.O.V	df	Chlorophyll a	Chlorophyll b	Total Chlorophyll	Carotenoid	Anthocyanin	Carbohydrate	Proline
Year (Y)	1	20.43ns	337.64ns	524.48*	57.52ns	0.069008ns	861.06 ns	6474.66ns
Error a	4	11.92	0.45	9.35	1.13	0.001033	91.14ns	21.95
Irrigation (I)	2	192.43*	11.54ns	214.48ns	27.17ns	0.032033ns	1.47ns	1718.59ns
Y× I	2	7.77ns	2.30ns	1842ns	39.77ns	0.040300**	59.63ns	994.71ns
Error b	8	4.88	2.32	3.74	2.12	0.002908	159.01	66.99
Fertilizers (F)	5	49.12**	26.14ns	30.14ns	26.93ns	0.001148ns	326.84**	299.06ns
Y× F	5	4.64ns	11.27ns	28.76ns	27.93ns	0.002211ns	27.46ns	166.08ns
I× F	10	7.07ns	11.98ns	18.29ns	31.99ns	0.002000ns	88.09ns	397.11ns
Y× I× F	10	9.03**	8.75**	11.06**	27.20**	0.001349ns	64.51ns	578.10**
Error c	60	3.10	2.05	4.05	2.62	0.000972	108.53	21.81
CV (%)	-	8.20	19.02	6.94	21.07	21.29	12.63	19.18

ns ,* and **: are non-significant, significant at 5 and 1% probability levels, respectively

Statistical analysis

Statistical analysis was performed using SAS software version 9.1 and the means were compared using Duncan's multiple range test at 5% probability level. Excel software was used to draw the graphs.

Result

Photosynthetic pigments: Based on the results of combined analysis of variance (Table 4), irrigation regime ($p< 0.05$), fertilizer ($p< 0.01$), and the interaction of year, irrigation regime, and fertilizer had a significant effect on chlorophyll a content ($p< 0.01$). Mean comparison of the triple interaction showed statistical difference in this trait in different groups. The highest chlorophyll a content with an average of 26.28 $mg.gdw^{-1}$was observed in the first year using 65% ET0 irrigation (mild stress) and 50% M+ 50% NPK and the lowest amount with an average of 13.85 $mg.gdw^{-1}$ belonged to the first year in 30% ET0 treatment (severe stress) in the absence of fertilizer application (control) (Fig. 1). An increasing trend was observed in the maximum amount of chlorophyll a in the absence of stress compared to severe stress with the application of fertilizer in both years.

The results of trait correlation (Table 4) showed that there is a positive and significant correlation between chlorophyll a and total chlorophyll with grain yield (r= 0.82) and (r= 0.77), respectively.

In this experiment, only the triple interaction of year× irrigation regime× fertilizer was significant in terms of chlorophyll b content ($p<0.01$) and the effect of year, irrigation regime, fertilizer and all interaction effects of year× irrigation regime, year× fertilizer, and irrigation regime× fertilizer were not significant on chlorophyll b content (Table 4). Drought stress decreased the amount of chlorophyll b in the leaves so that the application of 50% M +

50% NPK treatment increased this trait. The highest (17.12 mg $g^{-1}dw^{-1}$) and lowest (3.65 mg g^{-1} dw^{-1}) chlorophyll b content were obtained using optimal irrigation treatment with 50% M + 50% NPK and 30% ET irrigation (severe stress) without any fertilizer, which showed a 78.6% increase (Fig. 2). Leaf chlorophyll b content was not positively and significantly correlated with total chlorophyll (r= 0.70) and other traits (Table 6).

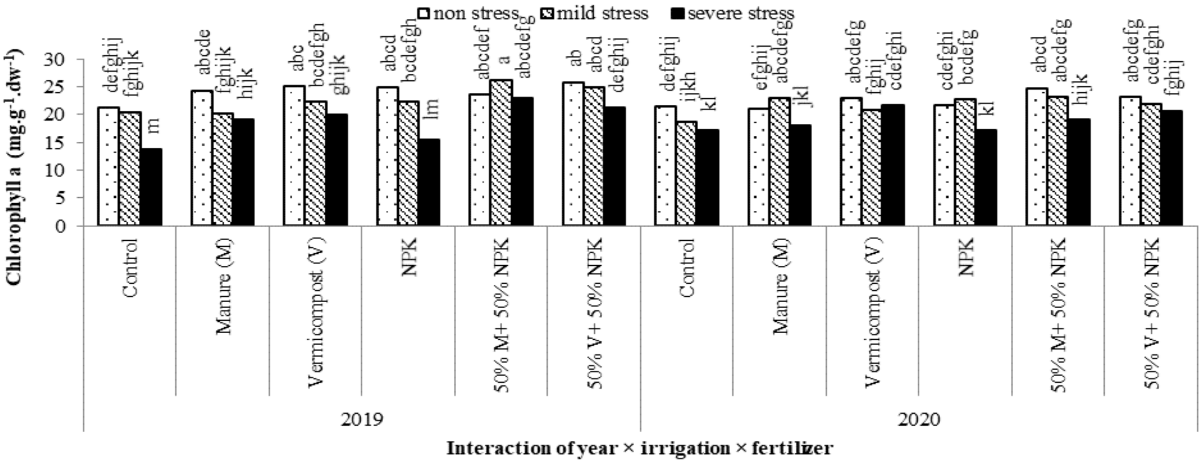

Fig. 1 Effect of different fertilizers and irrigation levels on chlorophyll a content in 2 yr. non stress, mild stress and severe stress: irrigation after 100, 65, and 30 % of reference evapotranspiration, respectively. Different letters indicate significant difference at $p \leq 0.05$.

As can be seen in the table presenting the analysis of variance, the total chlorophyll content was significantly ($p< 0.01$) affected by the interaction effects of year, irrigation regime, and fertilizer same as chlorophyll a and b (Table 4). Also, this trait was affected by the year at 5% probability level and the effect of irrigation regime, fertilizer, year× irrigation, year× fertilizer, and irrigation× fertilizer on this trait was not significant (Table 4). The mean comparison showed that the highest amount of this trait was obtained in both cropping years under 100% ET0 irrigation (no stress) and fertilizer application compared with no fertilizer application. Accordingly, the highest (40.71 mg g^{-1} dw^{-1}) and lowest (7.50 mg g^{-1} dw^{-1}) total chlorophyll content were obtained from treatment with 50% M+ 50% NPK and 100% ET0 irrigation (no stress) and no fertilizer use (control) in severe stress in the first year, respectively (Fig. 3). Total chlorophyll content was positively and significantly correlated with chlorophyll a (r= 0.89) and chlorophyll b (r= 0.70) and was negatively correlated with GPX (r= -0.54), proline (r= -0.50) and MOD (r= -0.60) (Table 6).

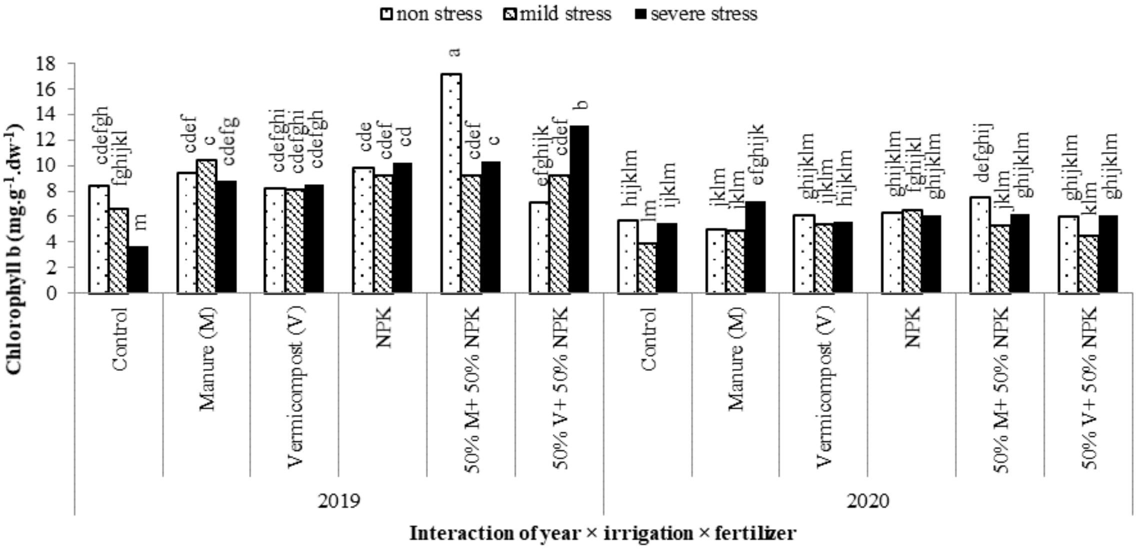

Fig. 2 Effect of different fertilizers and irrigation levels on chlorophyll b content in 2 yr. non stress, mild stress and severe stress: irrigation after 100, 65, and 30 % of reference evapotranspiration, respectively. Different letters indicate significant difference at p ≤ 0.05.

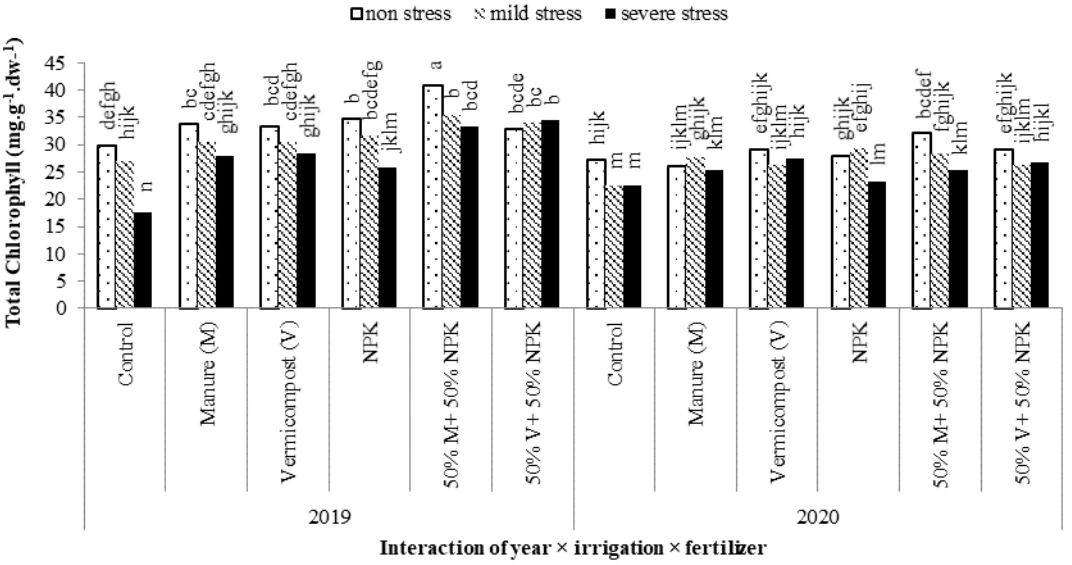

Fig. 3 Effect of different fertilizers and irrigation levels on total chlorophyll content in 2 yr. non stress, mild stress and severe stress: irrigation after 100, 65, and 30 % of reference evapotranspiration, respectively. Different letters indicate significant difference at p ≤ 0.05.

Carotenoids are considered to protect chlorophylls against optical oxidation, in order to prevent further degradation of chlorophylls by increasing synthesis under severe drought stress. Based on the results of the combined analysis of variance, the only interaction effect of year× irrigation regime× fertilizer was significant on this trait ($p < 0.01$) (Table 4). With increasing drought stress, the amount of carotenoids increases. The application of fertilizers also increases the level of leaf carotenoids and reduces oxidative damage. The results of the interaction of the treatments showed that in the 30% ET0 irrigation (severe stress) and using NPK fertilizer in the second year of the study, the highest (10.29 mg g^{-1} fw^{-1}) and under 100% ET0 irrigation (no stress) and no fertilizer (control), the lowest (4.93 mg g^{-1} fw^{-1}) value of this trait was obtained (Fig. 4).

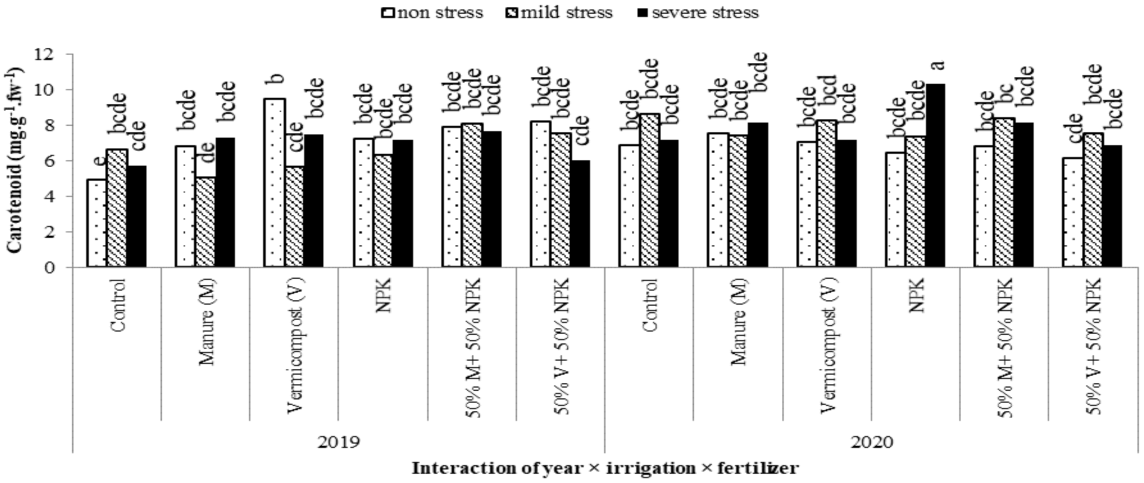

Fig. 4 Effect of different fertilizers and irrigation levels on carotenoid in 2 yr. non stress, mild stress and severe stress: irrigation after 100, 65, and 30 % of reference evapotranspiration, respectively. Different letters indicate significant difference at $p \leq 0.05$.

The results of the combined analysis of variance showed that the interaction effect of year and irrigation regime on anthocyanin content was significant ($p< 0.01$) (Table 4). Increasing the intensity of drought stress decreased the rate of this trait in both years. According to the interaction of treatments in two cropping years, the highest amount of anthocyanin with an average of 0.24 mmol gfw $^{-1}$ was obtained in the absence of stress in the first year, followed by mild stress treatment in the same year, and the lowest amount with an average of 0.11 mmol gfw $^{-1}$ was observed under severe stress in the second year that there was no significant difference between them (Fig. 5). Increasing the anthocyanin content of plant leaves increased grain yield and chlorophyll a and there was a positive and significant correlation (r= 0.62) at the level of 1% and (r= 0.46) at the level of 5% (Table 6).

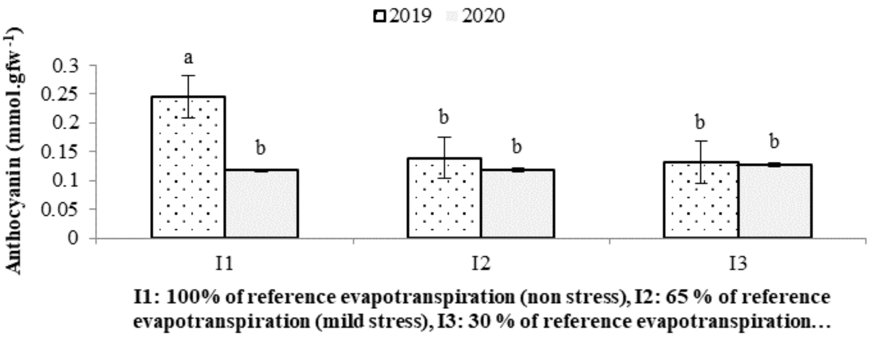

Fig. 5 Interaction of irrigation levels and year on anthocyanin. Different letters indicate significant difference at $p \leq 0.05$.

Content of osmolytes

According to the results indicated in the table of combined analysis of variance, the effect of fertilizer treatment on carbohydrate content and the interaction of year, irrigation regime, and fertilizer on proline content was significant ($p< 0.01$), and other treatments did not have a significant effect on these traits (Table 4). Under the treatments of combined application of manure and vermicompost and chemical fertilizers, organic fertilizers were effective in better absorption and availability of elements in chemical fertilizers by the plant, due to their role in the production of chlorophyll and the supply of enzymes required by the plant, leading to the increased amount of photosynthetic tissues and carbohydrates. Mean comparison of the results showed the highest amount of this trait (86 mg gfw^{-1}) using M+ 50% NPK 50% and 50% V+ 50% NPK treatments (Fig. 6). Also, the highest amount of proline with an average of 27.53 µg g^{-1} dw^{-1} in the second year was obtained under severe stress and no use of fertilizers, and the lowest amount was obtained using chemical and vermicompost fertilizers and manure and their combination (Fig. 7).

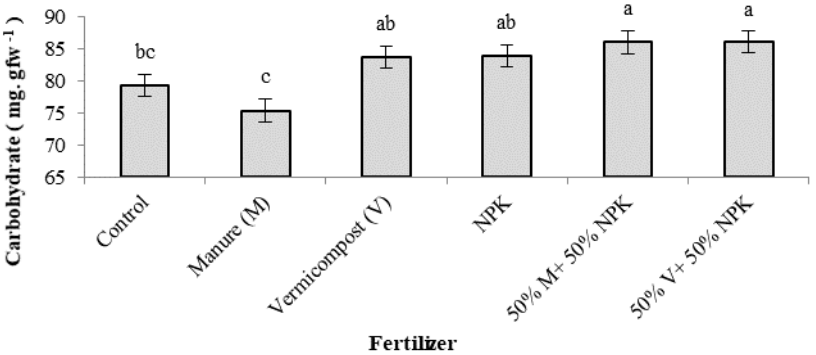

Fig. 6 Effect of fertilizer type on carbohydrate.

Different letters indicate significant difference at p ≤ 0.05.

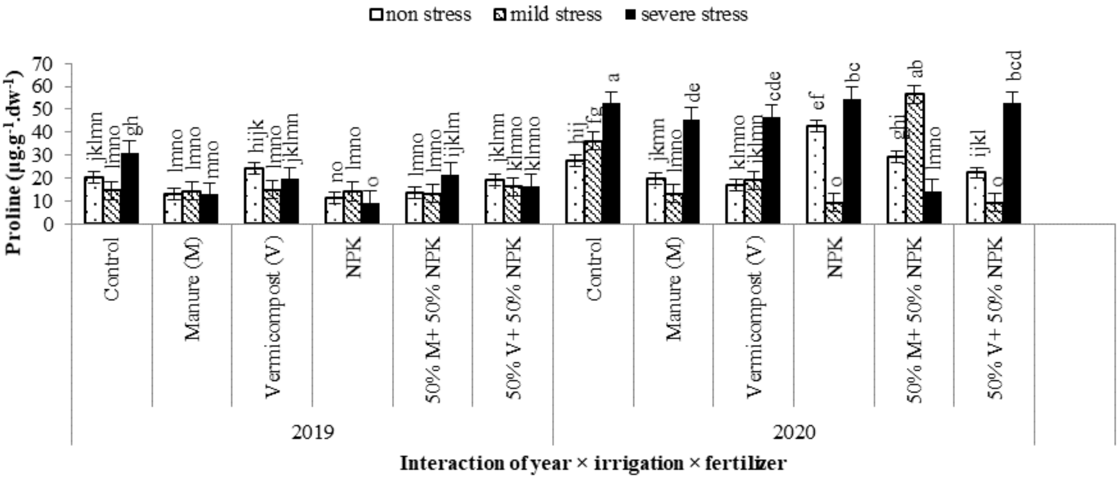

Fig. 7 Effect of different fertilizers and irrigation levels on proline in 2 yr. non stress, mild stress and severe stress: irrigation after 100, 65, and 30 % of reference evapotranspiration, respectively. Different letters indicate significant difference at p ≤ 0.05.

Antioxidant enzymes

The triple interaction of year× irrigation regime× fertilizer on the activity of antioxidant enzymes was significant ($p< 0.01$) (Table 5). Under severe stress and without fertilizer, this interaction had a positive and significant effect on the activity of GPX so that the highest level of GPX activity (0.000433 mmol min^{-1}) was obtained using a 30% ET0 irrigation regime (severe stress) without fertilizer (control) in the first year, which was able to increase the activity of this enzyme under stress compared with ideal conditions (no stress) and using 50% V+ 50% NPK 86.1% (Fig. 8).

Mean comparison showed that the application of 50% V+ 50% NPK treatment under severe drought stress increased the activity of APX. However, the activity of this enzyme decreased in the first year of the study compared with the second year (Fig. 9). The results indicated changes in the activity of antioxidant enzymes, such as CAT, glutathione peroxidase, and ascorbate peroxidase due to drought stress. There was also an inverse relationship

Table 5 Combined analysis of variance for some characteristics of Quinoa under irrigation levels and nutrition management

S.O.V	df	GPX activity	APX activity	CAT activity	PPO activity	MOD content	Grain yield
Year (Y)	1	1.44E-7ns	0.00051535ns	1.66E-7 ns	0.058800 ns	503.28ns	1025564ns
Error a	4	9.79E-10	0.00000013	1.56E-9	0.000111	9.10	986657
Irrigation (I)	2	1.75e-8ns	0.00003982ns	2.54E-8ns	0.000303ns	42.81ns	8473256*
Y× I	2	3.45E-9ns	0.00003158ns	3.18E-9ns	0.000136ns	76.38**	318829ns
Error b	8	1.06 E-9	0.00000054	1.81E-9	0.000063	4.60	943625
Fertilizers (F)	5	3.74E-8 ns	0.00016018ns	1.17E-7ns	0.000044ns	20.69ns	5265115ns
Y× F	5	8.62 E-9 ns	0.00016689ns	4.52E-8ns	0.000015ns	19.89*	1783875**
I× F	10	1.30 E-8 ns	0.00005026ns	4.23E-8ns	0.000060ns	2.67ns	264392ns
Y× I× F	10	1.00 E-8 **	0.00005815**	3.24E-8**	0.000118**	4.73**	186022ns
Error c	60	5.58 E-10	0.00000045	2.41E-9	0.000040	1.80	161652
CV (%)	-	19.15	23.22	16.60	13.51	17.48	20.34

ns ,* and **: are non-significant, significant at 5 and 1% probability levels, respectively

between catalase and APX activity. Mean comparison of the interaction effect showed that no fertilizer use (control) under severe stress and lack of drought stress increased the amount of CAT (Fig. 10). Also, polyphenol oxidase enzyme the highest activity (average: 0.0933 mmol min^{-1}) in the first year of the study using no fertilizer (control) and without drought stress, followed by using 50% M+ 50% NPK and 50% V+ 50% NPK treatments (average: 0.0766 mmol min^{-1}) (Fig. 11).

Analysis of simple correlation coefficients of the traits of the studied enzymes shows that GPX had a negative correlation ($p< 0.05$) with the grain yield ($r= -0.51$), chlorophyll a ($r= -0.50$) and total chlorophyll ($r= -0.54$) and had a positive and significant correlation with CAT ($r= 0.63$) and proline ($r= 0.64$); no significant correlation with other studied traits (Table 6).

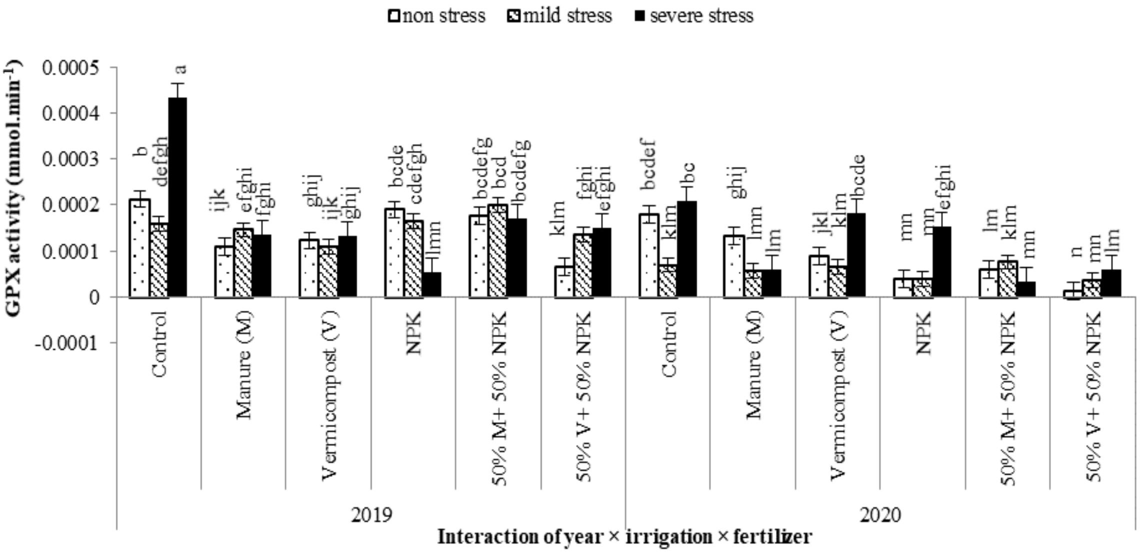

Fig. 8 Effect of different fertilizers and irrigation levels on GPX activity in 2 yr. non stress, mild stress and severe stress: irrigation after 100, 65, and 30 % of reference evapotranspiration, respectively. Different letters indicate significant difference at p ≤ 0.05.

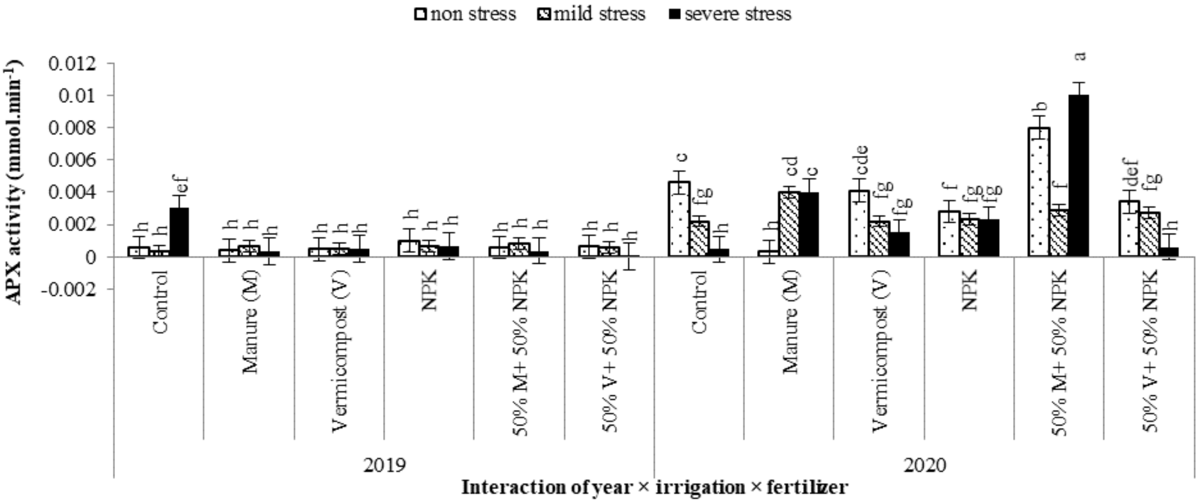

Fig. 9 Effect of different fertilizers and irrigation levels on APX activity in 2 yr. non stress, mild stress and severe stress: irrigation after 100, 65, and 30 % of reference evapotranspiration, respectively. Different letters indicate significant difference at p ≤ 0.05.

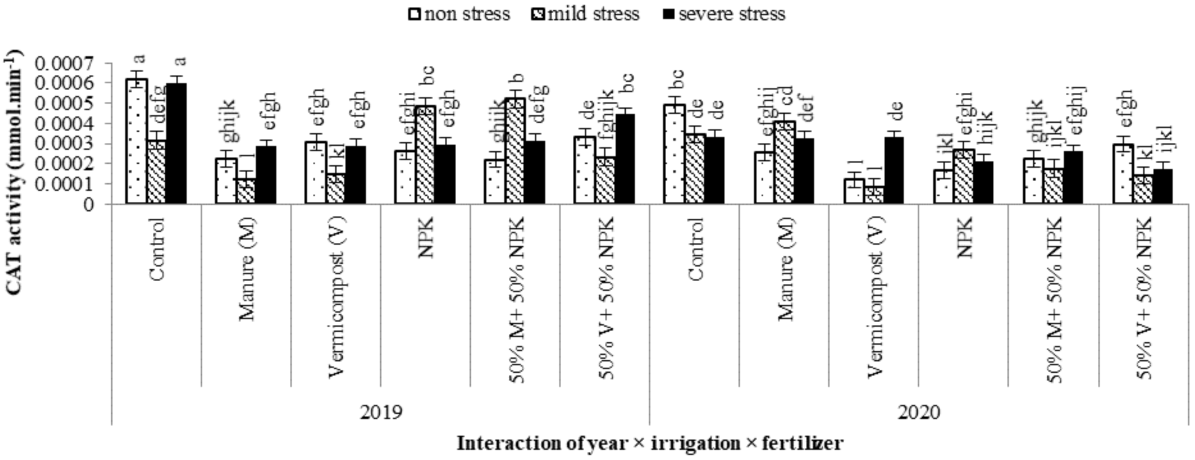

Fig. 10 Effect of different fertilizers and irrigation levels on CAT activity in 2 yr. non stress, mild stress and severe stress: irrigation after 100, 65, and 30 % of reference evapotranspiration, respectively. Different letters indicate significant difference at p ≤ 0.05.

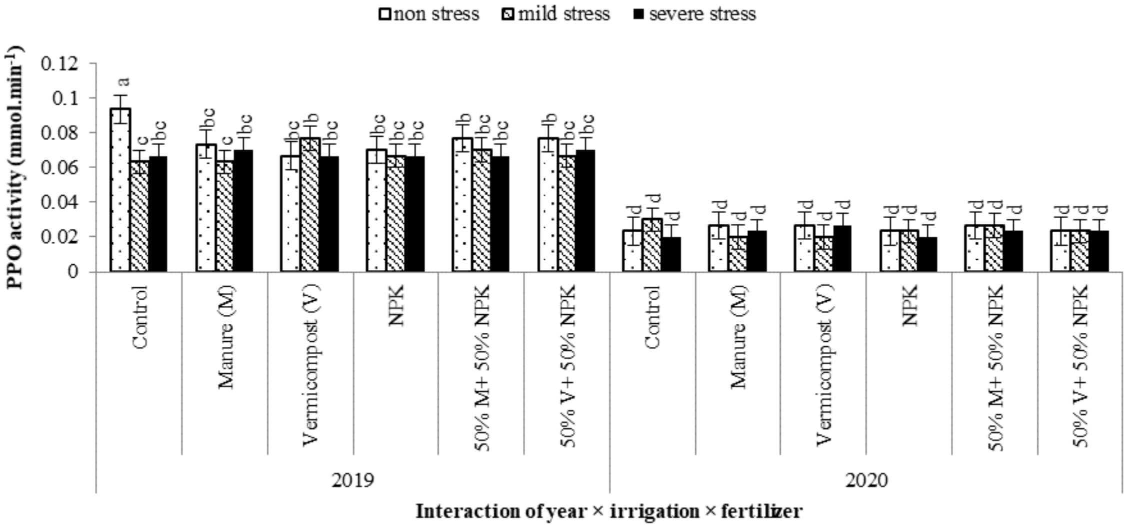

Fig. 11 Effect of different fertilizers and irrigation levels on CAT activity in 2 yr. non stress, mild stress and severe stress: irrigation after 100, 65, and 30 % of reference evapotranspiration, respectively. Different letters indicate significant difference at p ≤ 0.05.

MOD was significantly affected by the interactions of year× irrigation regime× fertilizer at a probability level of 5% (Table 5). The amount of MOD decreased significantly with increasing drought stress so that the highest amount of MOD resulted from their interaction was obtained in 2019 under severe drought stress without fertilizer (control) (average: 15.68 µg g^{-1} dw^{-1}). Also, in 2020, the highest amount (8.58 µg g^{-1}dw^{-1}) was obtained under moderate stress and no fertilizer application (control) (Fig. 12).

The correlation results of traits showed that MOD content was negatively and significantly correlated; ($p< 0.01$) with chlorophyll a (r= -0.65), total chlorophyll (r= -0.60) and grain yield (r= -0.61) and had a positive and significant correlation with proline content (r= 0.58) ($p< 0.01$) (Table 6).

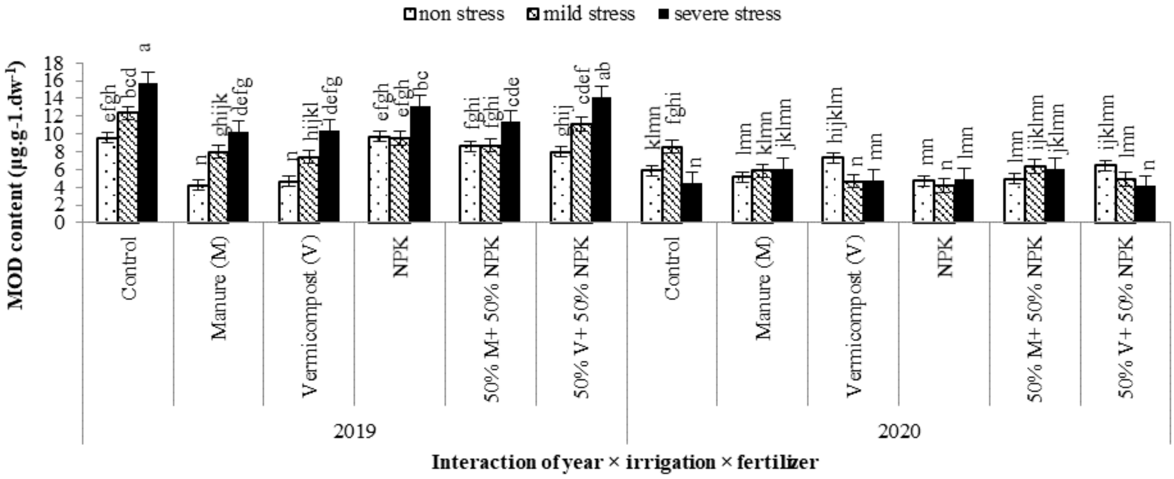

Fig. 12 Effect of different fertilizers and irrigation levels on MOD content in 2 yr. non stress, mild stress and severe stress: irrigation after 100, 65, and 30 % of reference evapotranspiration, respectively. Different letters indicate significant difference at p ≤ 0.05.

Grain yield

The results of the analysis of variance (Table 5) showed that the dual interaction effects of fertilizer× year for quinoa grain yield at 1% probability level as well as the simple effect of irrigation regime at 5% level were significant and the triple interactions of these two factors were not significant. Regarding the effect of the irrigation regime on the average yield, it was found that without stress (100% ET0), the highest yield was produced and no significant difference was observed between the two years. The level of severe stress (30% ET0) had the lowest dry yield in both cropping years (Fig. 13).

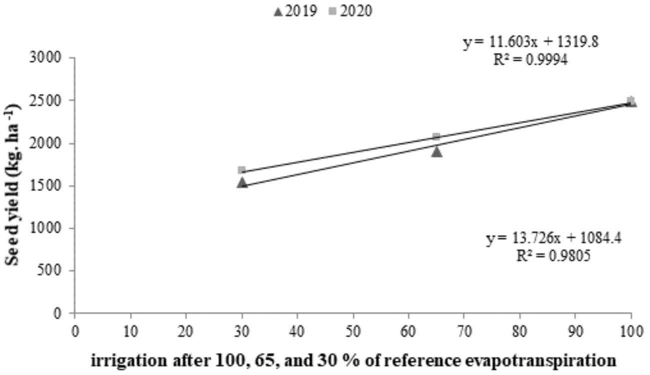

Fig. 13 Effect of irrigation levels on grain yield.

Regarding the effect of fertilizer type on grain yield (Fig. 14), the highest yield in two cropping years was obtained from the use of combined treatment with 50% V+ 50% NPK (3040.1 kg ha^{-1}), and the lowest was related to the no use of fertilizer (control).

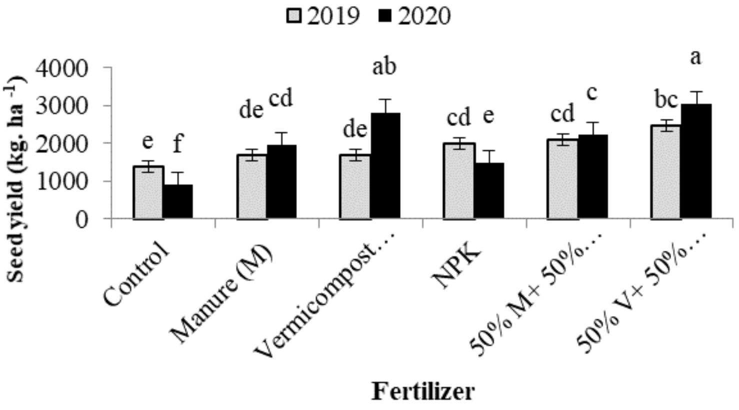

Fig. 14 Effect of fertilizer type on grain yield in 2 yr.

The results of correlation coefficients showed a positive and significant correlation between grain yield at 1 % probability level with chlorophyll a (r= 0.82), total chlorophyll (r= 0.77) and anthocyanin (r= 0.62) and a negative correlation with glutathione peroxidase (r= 0.51) and MOD (r= 0.61) (Table 6).

Table 6 Correlation between physiological characteristics of Quinoa as affected by interaction of irrigation and fertilizer

Treat	1. Grain yield	2. Chlorophyll a	3. Chlorophyll b	4. Total Chlorophyll	5. Carotenoid	6. Anthocyanin	7. Carbohydrate	8. GPX activity	9. APX activity	10. CAT activity	11. PPO activity	12. Proline	13. MOD content
1	1												

2	0.8277**	1											
3	0.3466ns	0.3276ns	1										
4	0.7785**	0.8980**	0.7097**	1									
5	-0.2677ns	-0.3883ns	0.1002ns	-0.2420ns	1								
6	0.6223**	0.4627*	0.1534ns	0.4166ns	-0.0089ns	1							
7	0.4358ns	0.3484ns	0.2175ns	0.3609ns	0.1422ns	0.1328ns	1						
8	-0.5136*	-0.5056*	-0.3654ns	-0.5476*	-0.1917ns	-0.2295ns	0.0241ns	1					
9	0.0050ns	0.0992ns	0.4565ns	0.2863ns	-0.0084ns	0.0897ns	0.0265ns	-0.0783ns	1				
10	-0.4417ns	-0.3354ns	-0.3008ns	-0.3904ns	-0.1979ns	-0.2032ns	-0.0799ns	0.6348**	-0.0664ns	1			
11	0.2661ns	0.4270ns	0.1862ns	0.4050ns	-0.3794ns	0.3909ns	0.0281ns	0.0584ns	0.0570ns	0.3118ns	1		
12	-0.3868ns	-0.5578*	-0.1883ns	-0.5037*	0.1675ns	-0.1384ns	0.1413ns	0.6409**	-0.2055ns	0.4303ns	-0.1762ns	1	
13	-0.6108**	-0.6585**	-0.2538ns	-0.6093**	0.1986ns	-0.3541ns	-0.0645ns	0.3392ns	0.0831ns	0.4395ns	-0.3558ns	0.5839**	1

ns ,* and **: are non-significant, significant at 5 and 1% probability levels, respectively

Discussion

The leaf chlorophyll content is one of the most important determinants of yield in biotic stresses in plants. Higher chlorophyll content indicates that the leaves are more efficient at absorbing light and photosynthesis, and ultimately have higher grain yield (Slattery et al., 2017).

One of the major oxidative damages caused by drought stress is the destruction of chlorophyll molecules. Decreased photosynthetic pigments, such as chlorophyll a and anthocyanins, may be due to a reduction in the synthesis of the primary complex of chlorophyll pigment, optical degradation of the protein complex of pigments a, b, and anthocyanin, which protect the photosynthetic apparatus (Zhang et al., 2020). A decrease in the level of photosynthetic pigments in rice, and an increase in chlorophyll a/b ratio at all levels of fertilizer application using the deficit irrigation compared with similar levels of fertilizer application in full flooded irrigation. One of the most important reasons for chlorophyll depletion is their degradation by ROS (Mohseni et al., 2020). Accordingly, decreased photosystem II activity decreased Rubisco enzyme activity, and ATP synthesis leads to the formation of free oxygen species in chloroplasts (Ghotbi-Ravandi et al., 2014).

With increasing drought stress, the level of chlorophyll decreases, and the amount of carotenoids increases (Mohammadkhani and Heidari, 2007). Under drought stress, the combined application of the chemical and biofertilizer increases leaf carotenoid content and reduces oxidative damage (Mahdavikia et al., 2019).

In general, it has been reported that the use of organic fertilizers as supplements due to the provision and release of essential elements in photosynthesis, in addition to increasing the efficiency of chemical fertilizers, increases the soluble carbohydrates of plants (AL Erwy, 2016). The increase in the level of carbohydrates due to the effect of different types of fertilizers has been attributed to the positive effect of these fertilizers on sugar metabolism and consequently increased plant growth. Maintaining soil fertility is achieved by providing a sufficient amount of exchangeable nutrients and creating a chemical balance between them and by the optimal use of fertilizers to prevent the negative effects of excessive use of chemical fertilizers, which in accordance with the goals of sustainable agriculture (Singh et al., 2017). The results of the present study were consistent with the results obtained for the quinoa plant (Amiryousefi and Ebrahimi, 2020).

Proline acts as a reservoir of nitrogen or as a solution to reduce the osmotic potential of the cytoplasm and helps the plant to withstand stress. It can provide the necessary conditions for the plant to continue absorbing water from the root to some extent. Compounds, such as propylene and carbohydrates have a nitrogen-based structure and are formed from glutamate as a precursor; thus, using nitrogen can greatly increase their amount in the plant. Potassium also protects cell membranes from damages caused by high ion concentrations by increasing proline concentrations (Patade et al., 2011). Reported that the interaction of drought stress and fertilizer caused the accumulation of soluble sugars and proline content in corn (*Zea mays*) leaves (Naeem et al., 2018).

According to the results of researchers, APX activities have an inverse relationship with CAT; high APX levels result in low CAT activity and vice versa, which is consistent with the results of this study (Abbasi et al., 2014). These findings suggest that plants prefer to use one of these two mechanisms to detoxify H2O2, but not both at the same time, and this may be due to the availability of their precursors.

In research reported increased activity of CAT, peroxidase, and superoxide dismutase enzymes in corn plant inoculated with a mycorrhizal fungus (Zhou et al., 2018). Mycorrhiza fungi increase antioxidant production and reduce ROS against stress and protect cells against oxidative stress. Higher levels of MOD under drought stress indicate an increase in the level of reactive oxygen species under drought stress conditions and the destruction of biological membranes by the influx of ROS (Abideen et al., 2020). In other words, under drought stress, the production of ROS increases and this toxic oxygenates cause the peroxidation of phospholipids in the cytoplasmic membrane, and finally, the amount of MOD, which is the result of the breakdown of fats, increases. In general, under drought stress, single and combined use of chemical and organic fertilizers caused a decrease in MOD of quinoa compared to no fertilizer (control) use. This indicates the positive effect of nutrition in increasing the tolerance of crops to environmental stresses, especially drought stress.

These correlations indicate the increase in the amount of ROS under drought stress and the influx of oxygen free radicals and the degradation of plant proteins, such as chlorophyll, and ultimately yield. It seems that the reason for the increase in the amount of MOD is the insufficient activity of antioxidant enzymes to prevent the peroxidation of fats and the removal of free radicals produced (Hasanuzzaman et al., 2020).

In general, nitrogen fertilizers cause a higher rate of accumulation of nitrogen in the vegetative parts and then transfer it to the seed and increase protein storage and nitrogen concentration in the seed. It has also been reported that increased nitrogen consumption activated drought tolerance mechanisms in Quinoa (Alandia et al., 2016). Water stress can indirectly affect biochemical processes related to photosynthesis and indirectly affect carbon dioxide emissions into the pores, which, by restricting photosynthetic products, plant growth, and ultimately, its yield reduction. In a report, reducing irrigation water by 50% led to a significant reduction in seed yield by 21.2%. In a study reported increased water productivity of quinoa under water stress (González-Teuber et al., 2018).

Our results of this study show that with increasing drought stress, the amount of dry matter of the studied plant decreased. In plants under water stress, the flexibility of the growing cell wall of the organ usually decreases leading to a decrease in cell development and growth. Reducing the amount of water disrupts the transport of nutrients necessary for growth and no new dry matter is produced resulting in reduced growth. Also, the reduction of water uptake through the roots is associated with a decrease in cell turbulence and reduces cell division, and inhibits cell growth. Under drought stress, photosynthesis is limited because the plant pores are closed (Pessarakli et al., 2015).

Conclusion

According to the results of this experiment, it is concluded that in quinoa, the dry matter yield decreases with increasing drought stress; however, 65 mm of Class A pan evaporation irrigation produces acceptable dry matter, which indicates the good resistance of this plant to drought stress. More dry matter was obtained by applying 50% vermicompost+ 50% NPK. An inverse relationship was observed between CAT and APX activities. Under oxidative stress, polyphenol oxidase and GPX enzymes play a parallel role in the plant defense system, so that all four enzymes are responsible for the production, detoxification, and decomposition of the plant. In the present study, the activity of the CAT enzyme under drought stress did not increase significantly. It seems that CAT is not highly active in quinoa and does not play a significant role in protecting the plant from drought stress. However, the activity of other enzymes under drought stress is increased, protecting plant cells from ROS. The highest amount of chlorophyll a, b, carotenoids, and total chlorophyll was obtained without stress and under mild stress (65 mm class A pan evaporation) and using 50% vermicompost+ 50% NPK.

Author contributions All authors contributed to the study conception.

Declarations

Conflict of interest The authors have no conficts of interest to eclare that are relevant to the content of this article.

Informed consent

Informed consent was obtained from all subjects.

All authors are agree to publish this manuscript in your valuable journal.

Reference

1. Abbasi, A., Sarvestani, R., Mohammadi, B., & Baghery, A. (2014). Drought stress-induced changes at physiological and biochemical levels in some common vetch (*vicia sativa* L.) genotypes. *Journal of Agricultural Science and Technology, 16:* 505-516. . http://jast.modares.ac.ir/article-23-812-en.html.
2. Abideen, Z., Koyro, H. W., Huchzermeyer, B., Ansari, R., Zulfiqar, F., & Gul, B. (2020). Ameliorating effects of biochar on photosynthetic efficiency and antioxidant defence of Phragmites karka under drought stress. *Plant Biology, 22*(2), 259-266. http://doi.org/10.1111/plb.13054. Epub 2020 Jan 12.
3. AL Erwy, A.S. (2016). Effect of chemical, organic and bio fertilizers on photosynthetic pigments, carbohydrates and minerals of wheat (T*riticum aestivum* L.) irrigated with sea water. *International Journal of Advanced Research in Biological Sciences, 3*: 296-310.
4. Alandia, G., Jacobsen, S. E., Kyvsgaard, N., Condori, B., & Liu, F. (2016). Nitrogen sustains seed yield of quinoa under intermediate drought. *Journal of Agronomy and Crop Science, 202*(4), 281-291. http://doi.org/doi.org/10.1111/jac.12155.
5. Amiryousefi, M., & Ebrahimi, R. (2020). Effect of biological and chemical fertilizers on some biochemical and yield traits of two quinoa cultivars. *Journal of Plant Process and Function, 9*(36), 299-314. http://jispp.iut.ac.ir/article-1-1295-fa.html.
6. Angeli, V., Miguel Silva, P., Crispim Massuela, D., Khan, M. W., Hamar, A., Khajehei, F., Piatti, C. (2020). Quinoa (*Chenopodium quinoa* Willd.): An overview of the potentials of the "Golden Grain" and socio-economic and environmental aspects of its cultivation and marketization. *Foods, 9*(2), 216. https://doi.org/10.3390/foods9020216.
7. Anonymous, (2020). Weather base: Historical Weather for Birjand, Iran. https://www.weatherbase.com/weather/weather-summary.php3?s=90804&cityname=Birjand%2C+Khorasan-e+Jonubi%2C+Iran&units=.
8. Arnon, D. I. (1949). Copper enzymes in isolated chloroplasts. Polyphenoloxidase in Beta vulgaris. *Plant physiology, 24*(1), 1. https://doi.org/10.1104/pp.24.1.1.

9. Baghizadeh, A., Hajmohammadrezaei, M., & Tohidi, Z. (2020). Evaluation of interaction effect of drought stress with ascorbate and salicylic acid on the activity of some antioxidant enzymes and flavonoids in H*ibiscus esculentus* L. *Journal of Molecular and Cellular Research*, 33: 142-152. http://cell.ijbio.ir/article_1460_en.html.
10. Bates, L. S., Waldren, R. P., & Teare, I. (1973). Rapid determination of free proline for water-stress studies. *Plant and soil, 39*(1), 205-207. https://doi.org/10.1007/BF00018060.
11. Beers, R. F., & Sizer, I. W. (1952). A spectrophotometric method for measuring the breakdown of hydrogen peroxide by catalase. *Journal of biological chemistry, 195*(1), 133-140. https://doi.org/10.1016/S0021-9258(19)50881-X.
12. Dakhili, S., Abdolalizadeh, L., Hosseini, S. M., Shojaee-Aliabadi, S., & Mirmoghtadaie, L. (2019). Quinoa protein: Composition, structure and functional properties. *Food chemistry, 299*, 125161. http://doi.org/10.1016/j.foodchem.2019.125161.
13. Fielding, J. L., & Hall, J. (1978). A biochemical and cytochemical study of peroxidase activity in roots of Pisum sativum: II. Distribution of enzymes in relation to root development. *Journal of Experimental Botany, 29*(4), 983-991. https://doi.org/10.1093/jxb/29.4.983.
14. Forouzandeh, M., Mohkami, Z., & Fazelinasab, B. (2019). Evaluation of Biotic Elicitors Foliar Application on Functional Changes, Physiological and Biochemical Parameters of Fennel (*Foeniculum vulgare*). *Journal of Plant Production Research, 25*(4), 49-65. https://doi:10.22069/jopp.2018.14077.2262
15. Ghotbi-Ravandi, A., Shahbazi, M., Shariati, M., & Mulo, P. (2014). Effects of mild and severe drought stress on photosynthetic efficiency in tolerant and susceptible barley (*Hordeum vulgare* L.) genotypes. *Journal of Agronomy and Crop Science, 200*(6), 403-415. https://doi.org/10.1111/jac.12062.
16. Giansoldati, V., Tassi, E., Morelli, E., Gabellieri, E., Pedron, F., & Barbafieri, M. (2012). Nitrogen fertilizer improves boron phytoextraction by Brassica juncea grown in contaminated sediments and alleviates plant stress. *Chemosphere, 87*(10), 1119-1125. https://doi.org/10.1016/j.chemosphere.2012.02.005.
17. Gill, S. S., & Tuteja, N. (2010). Reactive oxygen species and antioxidant machinery in abiotic stress tolerance in crop plants. *Plant physiology and biochemistry, 48*(12), 909-930. https://doi.org/10.1016/j.plaphy.2010.08.016.
18. González-Teuber, M., Urzúa, A., Plaza, P., & Bascuñán-Godoy, L. (2018). Effects of root endophytic fungi on response of Chenopodium quinoa to drought stress. *Plant ecology, 219*(3), 231-240. https://doi.org/10.1007/s11258-017-0791-1.
19. Goswami, B., Rankawat, R., & Gadi, B. R. (2020). Physiological and antioxidative responses associated with drought tolerance of Lasiurus sindicus Henr. endemic to Thar desert, India. *Brazilian Journal of Botany, 43*(4), 761-773. https://doi:10.1007/s40415-020-00666-9.
20. Guo, Y. Y. (2018). Effect of Drought Stress on Lipid Peroxidation, Osmotic Adjustment and Antioxidant Enzyme Activity of Leaves and Roots of Lycium ruthenicum Murr. Seedling. *Russian Journal of Plant Physiology, v. 65*(no. 2), pp. 244-250-2018 v.2065 no.2012. https://doi.org/10.1134/S1021443718020127.
21. Hasanuzzaman, M., Bhuyan, M., Zulfiqar, F., Raza, A., Mohsin, S. M., Mahmud, J. A., Fotopoulos, V. (2020). Reactive oxygen species and antioxidant defense in plants under abiotic stress: Revisiting the crucial role of a universal defense regulator. *Antioxidants, 9*(8), 681. https://doi.org/10.3390/antiox9080681.
22. 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.
23. Hosseinzadeh, S., Amiri, H., & Ismaili, A. (2016). Effect of vermicompost fertilizer on photosynthetic characteristics of chickpea (Cicer arietinum L.) under drought stress. *Photosynthetica, 54*(1), 87-92. https://doi.org/10.1007/s11099-015-0162-x.
24. Howell, T., Evett, S., Tolk, J., Copeland, K., Colaizzi, P., & Gowda, P. (2008). *Evapotranspiration of corn and forage sorghum for silage.* Paper presented at the World Environmental and Water Resources Congress 2008: Ahupua'A. *10,* 886-889. https://doi.org/10.1061/40976(316)88.

25. Hussain, M., Farooq, S., Hasan, W., Ul-Allah, S., Tanveer, M., Farooq, M., & Nawaz, A. (2018). Drought stress in sunflower: Physiological effects and its management through breeding and agronomic alternatives. *Agricultural water management, 201*, 152-166. https://doi.org/10.1016/j.agwat.2018.01.028.
26. Irigoyen, J., Einerich, D., & Sánchez-Díaz, M. (1992). Water stress induced changes in concentrations of proline and total soluble sugars in nodulated alfalfa (Medicago sativd) plants. *Physiologia plantarum, 84*(1), 55-60. https://doi.org/10.1111/j.1399-3054.1992.tb08764.x.
27. Janovitz-Klapp, A. H., Richard, F. C., Goupy, P. M., & Nicolas, J. J. (1990). Inhibition studies on apple polyphenol oxidase. *Journal of Agricultural and Food Chemistry, 38*(4), 926-931. https://doi.org/10.1021/jf00094a002.
28. Mahdavikia, H., Rezaei-Chiyaneh, E., Rahimi, A., & Mohammadkhani, N. (2019). Effects of fertilizer treatments on antioxidant activities and physiological traits of basil (*Ocimum basilicum* L.) under water limitation conditions. *Journal of Medicinal plants and By-product, 8*(2), 143-151. https://doi.org/10.22092/jmpb.2019.120492.
29. Mohammadkhani, N., & Heidari, R. (2007). Effects of Water Stress on Respiration, Photosynthetic Pigments and Water. *Pakistan Journal of Biological Sciences, 10*(22), 4022-4028. https://doi.org/10.3923/pjbs.2007.4022.4028.
30. Mohseni, S. H., Esmaeili, M. A., Pirdashti, H., Abbasi, R., & Nasiri, M. (2020). Effect of potassium fertilizers on chlorophyll florescence and photosynthetic pigments in rice (*Oryza sativa* L. var. Tarom Hashemi) in regulated deficit irrigation condition. *Journal of Plant Process and Function, 9*(35), 1-22. http://jispp.iut.ac.ir/article-1-982-fa.html.
31. Moklyachuk, L., Furdychko, O., Pinchuk, V., Mokliachuk, O., & Draga, M. (2019). Nitrogen balance of crop production in Ukraine. *Journal of environmental management, 246*, 860-867. https://doi.org/10.1016/j.jenvman.2019.05.108.
32. Naeem, M., Naeem, M. S., Ahmad, R., Ihsan, M. Z., Ashraf, M. Y., Hussain, Y., & Fahad, S. (2018). Foliar calcium spray confers drought stress tolerance in maize via modulation of plant growth, water relations, proline content and hydrogen peroxide activity. *Archives of Agronomy and Soil Science, 64*(1), 116-131. https://doi.org/10.1080/03650340.2017.1327713.
33. Park, J. H., Bolan, N., Megharaj, M., & Naidu, R. (2011). Isolation of phosphate solubilizing bacteria and their potential for lead immobilization in soil. *Journal of hazardous materials, 185*(2-3), 829-836. https://doi.org/10.1016/j.jhazmat.2010.09.095.
34. Patade, V. Y., Bhargava, S., & Suprasanna, P. (2011). Salt and drought tolerance of sugarcane under iso-osmotic salt and water stress: growth, osmolytes accumulation, and antioxidant defense. *Journal of Plant Interactions, 6*(4), 275-282. https://doi.org/10.1080/17429145.2011.557513.
35. Pessarakli, M., Haghighi, M., & Sheibanirad, A. (2015). Plant responses under environmental stress conditions. *Adv. Plants Agricultural Research, 2*(6), 73. https://doi.org/10.15406/apar.2015.02.00073.
36. Rodríguez-Rosales, M. P., Gálvez, F. J., Huertas, R., Aranda, M. N., Baghour, M., Cagnac, O., & Venema, K. (2009). Plant NHX cation/proton antiporters. *Plant Signaling & Behavior, 4*(4), 265-276. https://doi:10.4161/psb.4.4.7919.
37. Singh, R. P., Kumar, S., Sainger, M., Sainger, P. A., & Barnawal, D. (2017). Eco-friendly nitrogen fertilizers for sustainable agriculture. In *Adaptive Soil Management: From Theory to Practices* (pp. 227-246): Springer. https://doi.org/10.1007/978-981-10-3638-5_11.
38. Slattery, R. A., VanLoocke, A., Bernacchi, C. J., Zhu, X.-G., & Ort, D. R. (2017). Photosynthesis, light use efficiency, and yield of reduced-chlorophyll soybean mutants in field conditions. *Frontiers in Plant Science, 8*, 549. https://doi.org/10.3389/fpls.2017.00549.
39. Umair Hassan, M., Aamer, M., Umer Chattha, M., Haiying, T., Shahzad, B., Barbanti, L., Guoqin, H. (2020). The Critical Role of Zinc in Plants Facing the Drought Stress. *Agriculture, 10*(9), 396. . https://doi.org/10.3390/agriculture10090396.

40. Wagner, G. J. (1979). Content and vacuole/extravacuole distribution of neutral sugars, free amino acids, and anthocyanin in protoplasts. *Plant physiology, 64*(1), 88-93. https://doi.org/10.1104/pp.64.1.88.
41. WU, Y.W., Qiang, L., Rong, J., Wei, C., Liu, X., Kong, F., Yuan, J.C. (2019). Effect of low-nitrogen stress on photosynthesis and chlorophyll fluorescence characteristics of maize cultivars with different low-nitrogen tolerances. *Journal of Integrative Agriculture, 18*(6), 1246-1256. https://doi.org/10.1016/s2095-3119(18)62030-1.
42. Yang, L., Zhao, F., Chang, Q., Li, T., & Li, F. (2015). Effects of vermicomposts on tomato yield and quality and soil fertility in greenhouse under different soil water regimes. *Agricultural water management, 160*, 98-105. https://doi.org/10.1016/j.agwat.2015.07.002.
43. Yoshimura, K., Yabuta, Y., Ishikawa, T., & Shigeoka, S. (2000). Expression of spinach ascorbate peroxidase isoenzymes in response to oxidative stresses. *Plant physiology, 123*(1), 223-234. https://doi.org/10.1104/pp.123.1.223.
44. Zhang, X., Yang, Z., Li, Z., Zhang, F., & Hao, L. (2020). Effects of drought stress on physiology and antioxidative activity in two varieties of Cynanchum thesioides. *Brazilian Journal of Botany*, 1-10. https://doi.org/10.1007/s40415-019-00573-8.
45. Zhou, Z.-H., Wang, Y., Ye, X.-Y., & Li, Z.-G. (2018). Signaling molecule hydrogen sulfide improves seed germination and seedling growth of maize (*Zea mays* L.) under high temperature by inducing antioxidant system and osmolyte biosynthesis. *Frontiers in Plant Science, 9*, 1288. https://doi.org/10.3389/fpls.2018.01288.