

# Evaluation of yield and water use efficiency of quinoa under irrigation regimes, gamma aminobutyric acid, and vermicompost application

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## Evaluation of yield and water use efficiency of quinoa under irrigation regimes, gamma aminobutyric acid, and vermicompost application

**Abstract:** The current study was aim to evaluate the interaction effects of gamma aminobutyric acid (GABA) and vermicompost on yield and yield components of quinoa under different levels of drought stress. For this, two experiments were similarly designed as the factorial-split with four replicates for evaluating this hypothesis. Irrigation regimes (50, 75, and 100 % of plant water requirement (PWR)) as the main plot and vermicompost V (0, 5 t ha<sup>-1</sup>) × gamma aminobutyric acid GABA levels (0, 5, 10 mg l<sup>-1</sup>) as the subplot were designed. Severe drought stress had a significant effect on plant height. Plant height reduced 31.8 % after using 50 % of PWR compared to the control conditions. Although drought stress negatively affected the 1000 seed mass and seed yield, GABA foliar application alleviated these effects. After using 50 % of PWR, 10 mg l<sup>-1</sup> of GABA increased the seed yield and harvest index up to 21.22 and 15.5 %, respectively, compared to the non-foliar application. The reduction in PWR from 100 to 50 % led to increasing in P and K concentrations, as well as sugar and proline contents. In the same conditions, the use of GABA or V had a significant effect on improving these traits. A similar trend was also recorded in relation to water use efficiency. Therefore, using 10 mg l<sup>-1</sup> of GABA and 5 t ha<sup>-1</sup> of V can be effective in alleviating water stress.

**Key words:** *Chenopodium quinoa*; proline; seed yield; sugar; drought stress

**Abbreviations:** V: vermicompost, GABA: gamma aminobutyric acid, PWR: plant water requirement, WUE: Water use efficiency

## Ovrednotenje pridelka in učinkovitosti izrabe vode kvinoje pri različnih načinih namakanja in dodatku gama aminomaslene kisline in komposta deževnikov

**Izvleček:** Namen raziskave je bil ovrednotiti vzajemne učinke gama amino maslene kisline (GABA) in komposta deževnikov (vermikomposta) na pridelek in njegove komponente kvinoje pri različnih ravneh sušnega stresa. V ta namen sta bila izvedena dva podobna poskusa kot poskusa z deljenkami s štirimi ponovitvami. Načini namakanja (50, 75 in 100 % potrebe rastlin po vodi (PWR)) so bili na glavni ploskvi in dodatki vermikomposta (V; 0, 5 t ha<sup>-1</sup>) ter gama aminomaslene kisline (GABA; (0, 5, 10 mg l<sup>-1</sup>) na podploskvah. Velik sušni stres je imel značilen učinek na višino rastlin. Višina rastlin se je zmanjšala za 31,8 % pri 50 % oskrbi rastlin z vodo v primerjavi s kontrolo. Čeprav je sušni stres negativno vplival na maso 1000 semen in pridelek semena, je foliaren nanos GABA ublažil te učinke. Pri 50 % oskrbi z vodo je dodatek 10 mg l<sup>-1</sup> of GABA povečal pridelek semena in žetevni indeks za 21,22 in 15,5 % v primerjavi z obravnavanjem brez foliarnega dodatka GABA. Zmanjšanje PWR iz 100 na 50 % je vodilo k povečanju koncentracij P in K, kot tudi k povečanju vsebnosti sladkorja in prolina. Pod enakimi pogoji je imela uporaba GABA ali V značilen učinek na izboljšanje teh lastnosti. Podoben trend je bil zabeležen glede povezave z učinkovitostjo izrabe vode. Zaradi vsega naštetega je uporba 10 mg l<sup>-1</sup> GABA in 5 t ha<sup>-1</sup> V lahko učinkovita pri blažitvi sušnega stresa.

**Ključne besede:** *Chenopodium quinoa*; prolin; pridelek semena; sladkor; sušni stres

**Okrajšave:** V: vermikompost, GABA: gama amino maslena kislina, PWR: zahteva rastline po vodi, WUE: učinkovitost izrabe vode

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## 1 INTRODUCTION

Quinoa (*Chenopodium quinoa* Willd.), an annual herbaceous plant from the *Amaranthaceae* family, is grown in arid and semi-arid regions of the world (Hinojosa et al., 2018; Bedoya-Perales et al., 2018), especially in Iran (Razzaghi et al., 2012; Ahmadi et al., 2019). The crop is well-known for its nutritional values based on the carbohydrates, lipids, and proteins nutrients (Gómez-Pando et al., 2010). Leucine, phenylalanine, and valine (822, 669, 583 mg 100 g<sup>-1</sup> of air-dried flour, respectively) were determined as the main amino acids of this crop (Agza et al., 2018). The grain flour of quinoa has been extensively used in the food industry due to its gluten free-based values (Wu et al., 2020) and highly nutritive quality based on the protein, Ca, Fe, and Zn contents (Ballester-Sánchez et al., 2019; Bazile et al., 2016; Präger et al., 2018), which can justify the quinoa as a suitable product for celiac patients (Peñas et al., 2014).

The appropriate tolerance of quinoa perceiving stressful and unfavorable environments were precisely reported (Talebnejad and Sepaskhah, 2015). Moreover, this plant can be properly considered as a pivotal candidate where water shortage is known as a crucial challenge (Talebnejad and Sepaskhah, 2015; Bedoya-Perales et al., 2018). Ahmadi et al. (2019) reported that quinoa is a well-known tolerant crop against the drought stress conditions. However, water shortage and drought stress are considered as the serious problems for crop production, especially in the east and northeast of Iran (Bannayan et al., 2011; Koocheki et al., 2014), the total annual rainfall in which is recorded about 187 and 285 mm, respectively, indicating water deficiency as the main challenge against the successful crop production (Iran meteorological organization, 2020). Drought stress may significantly affect the plant growth and production by diminishing the leaf area (Feng et al., 2013), decelerating the photosynthesis rate (Saeidi et al., 2017; Zhao et al., 2020; Nadali et al., 2021), and retarding the enzymatic activity (Xu et al., 2015; Askary et al., 2018). Deficit irrigation regimes can negatively affect the dry matter production and seed yield of quinoa compared to the normal irrigation treatment (Talebnejad and Sepaskhah, 2015). Therefore, it is necessary to use the agronomic approaches to increase the plant tolerance against the stressful conditions, which maximizes the crop yield and water use efficiency (Razzaghi et al., 2012).

Water use efficiency (WUE) is a crucial indicator related to evaluating available or applied water that can determine an actual plant function, especially in arid

and semi-arid areas. The concept of WUE indicates how much growth and performance changes for each unit of water used (Dong et al., 2011). Under dried conditions, plants generally use the available water with higher efficiency, although this may lead to a decrease in plant yield (Hosseinzadeh et al. 2018). Therefore, when the plant is faced with water limitation or suboptimal irrigation, the set of operations such as organic matter supply can directly affect the efficiency of consumed water by stimulating the growth behavior.

Soil fertility can be determined for mitigating the stress effects when the plant is affected by the adverse environmental conditions. In this regard, the use of vermicompost has been known as an appropriate media for improving the rhizosphere fertility and increasing the crop production (Joshi et al., 2015). Vermicompost can significantly promote the soil biological mechanisms (Hosseinzadeh et al., 2020) and simultaneously regulate the synchrony of minerals uptake during the growing seasons due to the balanced nutrient and high organic matter contents (Rezaei-Chiyaneh et al., 2020; Xu et al., 2016). Seyyedi et al. (2016) reported that the expanded root growth decreased soil pH, and increased microbial activity showed the positive consequences of vermicompost application.

Endogenous hormonal modifications are known as a plant self-mechanisms for repairing the cellular damage (Dobra et al., 2010), balancing the osmolytes uptake (Sarwat and Tuteja, 2017), stabilizing the cell membrane (Albacete et al., 2014), and proliferating the plant tolerance against the abiotic stresses such as drought conditions (Ni et al., 2013). However, the efficiency of internal self-mechanisms may be disturbed by increasing the severity of water shortage. Therefore, the use of exogenous phytohormone is possibly useful for mitigating the adverse consequence of water shortage. In this regard,  $\gamma$ -aminobutyric acid, a four-carbon non-protein amino acid, has been introduced as a crucial plant growth regulator for alleviating the water deficiency during the growing season (Yong et al., 2017). The use of  $\gamma$ -aminobutyric acid (GABA) is proposed for diminishing the side effects of water stress by modulating the plant signaling (Ji et al., 2018), scavenging the reactive oxygen species, and regulating the osmolytes (Vijayakumari and Puthur, 2016), which improves the plant growth under water scarcity (Rezaei-Chiyaneh et al., 2018); therefore, this study aims to evaluate GABA effects on yield and yield components of quinoa under different levels of drought stress. Furthermore, the interaction between vermicompost and GABA was assessed for finding a suitable strategy in improving water use efficiency.

## 2 MATERIALS AND METHODS

### 2.1 SITE DESCRIPTION

The experiment was conducted in two experimental stations located in Birjand [Faculty of Agriculture, University of Birjand (latitude: 32° 53'N, longitude: of 59°13'E, elevation: 1480 m above the sea level)] and Mashhad [Faculty of Agriculture, Ferdowsi University of Mashhad (latitude: 36°15'N, longitude: 59°28'E, elevation: 985 m above the sea level)] during the growing seasons 2019 (Fig. 1). Both experimental sites were located in a semi-arid region, Razavi Khorasan and South Khorasan provinces, Iran. Meteorological data during the growing season are given in Table 1.

### 2.2 EXPERIMENTAL DESIGN

Two experiments were similarly designed as the factorial-split plot with four replicates. The experimental factors were included. Irrigation regimes (50, 75, and 100 % of water requirement) and vermicompost (0, 5 t ha<sup>-1</sup>) × GABA (Sigma-Aldrich, A2129; NH<sub>2</sub>(CH<sub>2</sub>)<sub>3</sub>COOH) levels (0, 5, 10 mg l<sup>-1</sup>) were considered as the main plot and subplot, respectively (Fig. 2). The materials related to the analyses of vermicompost (produced by Arta Manufacturing Group, Iran) included N (1.45 %), P (1.16 %), K (1.2 %), and organic matter (18.65 %).

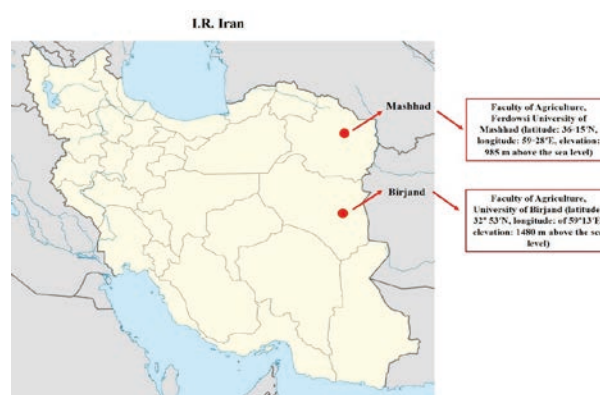
### 2.3 AGRONOMIC PRACTICES

In both Birjand and Mashhad stations, all agronomic operations were relatively similar. Before preparing the seed bed, sampling was done from 0-30 cm depth based on the soil of testing location for determining the physi-

cal and chemical properties (Table 2). In the following, vermicompost was applied according to experimental treatments.

After preparing the experimental soils (deeply plowing, twice disking, and leveling), the plots were designed (5 m width and 3 m length). Each block consisted of three main plots with six subplots. The distances among the plots and blocks were considered as 0.5 and 2 m, respectively. Seeds ('Titicaca') were sown on 29th and 26th July 2019 in Mashhad and Birjand, respectively. The final density was determined as 60 plant m<sup>-2</sup>. Foliar spraying of GABA was performed at 2-4 and 10-12 leaf stages, respectively.

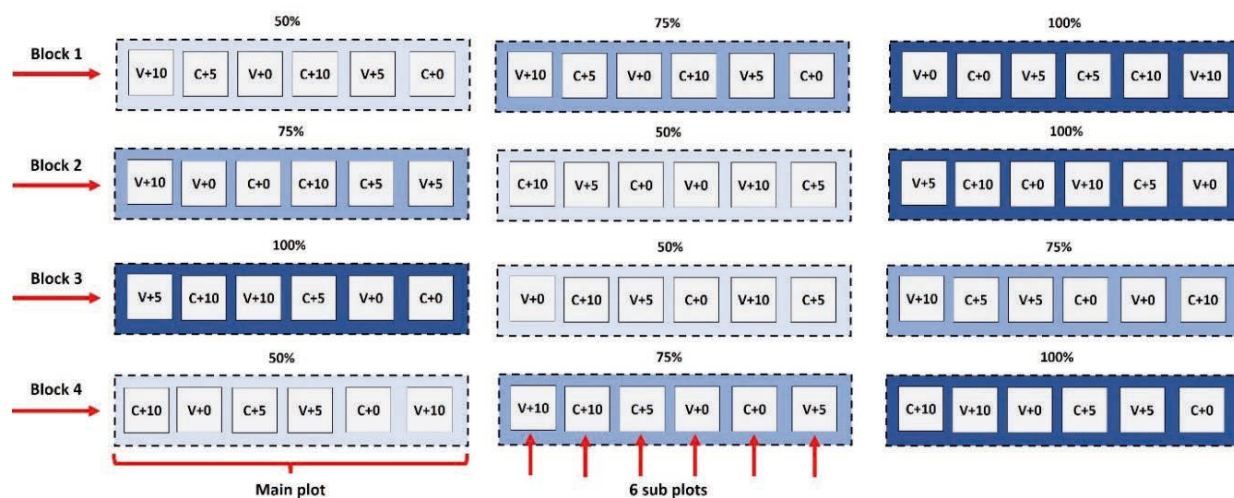
Based on the weather conditions at both sites, the plant water requirement was calculated by using CROPWAT software (FAO 1992). The first irrigation was done immediately after the seed sowing and the second irrigation was performed three days later. Weed management was conducted manually during the growing season. Af-



**Fig 1:** A map graphical presenting the position of the experimental field in Iran. The experiment was conducted in two experimental stations located in Birjand and Mashhad

**Table 1:** Weather criteria of experimental sites located at Birjand (B) and Mashhad (M), Iran in 2019

Month	Minimum temperature (°C)		Maximum temperature (°C)		Total rainfall (mm)		Relative humidity (%)	
	B	M	B	M	B	M	B	M
July	21.3	22.4	37.6	37.4	0.0	0.5	13.6	23.6
August	18.8	21.0	36.4	35.5	0.0	0.0	15.1	24.0
September	14.7	15.9	34.0	30.7	0.0	0.0	15.3	26.9
October	10.0	11.7	29.7	26.2	0.0	0.1	18.9	38.4
November	3.9	4.2	20.6	15.8	1.0	0.6	31.9	52.3
Average	13.7	15.0	31.7	29.1	-	-	19.0	33.0
Total	-	-	-	-	1.0	1.2	-	-
30 years	13.0	14.2	30.7	28.9	1.8	5.1	24.6	39.1



**Fig 2:** A schematic design of the experiment. Experiments were designed as the factorial-split plot with four replicates. Irrigation regimes (50, 75, and 100 % of water requirement) and vermicompost (0 and 5 t ha<sup>-1</sup>) × GABA levels (0, 5, 10 mg l<sup>-1</sup>) were considered as the main plot and subplot, respectively

**Table 2:** The soil quality characteristics of experimental sites located at Birjand and Mashhad, Iran

Experimental sites	%					mg/kg			pH	EC (dS/m)
	Clay	Silt	Sand	OC	N	P	K			
Birjand	18	12	70	0.16	0.031	11.75	225.3	7.80	4.58	
Mashhad	44	32	24	0.48	0.080	6.71	181.5	8.34	1.16	
Average	31	22	47	0.32	0.055	9.23	203.4	8.07	2.87	

ter establishing the plant, the use of drought stress began for this purpose.

#### 2.4 DATA COLLECTION

Six plant plot<sup>-1</sup> were randomly selected by considering the marginal effects (25 cm at the first and end of the plots and 2 sides of the rows) at the maturity stage (26<sup>th</sup> and 21<sup>st</sup> October 2019, in Birjand and Mashhad, respectively). Then, plant height, seed number/plant, seed mass/plant and 1000 seed mass were determined. Seed and biological yields and harvest index were also measured at the central part of the plots (11.25 m<sup>2</sup>). Sugar and proline contents were determined based on the studies of Dubois et al. (1956) and Bates (1973), respectively, after measuring the seed yield. Moreover, P and K concentrations were measured based on the study of Murphy and Riley (1962) and Miller (1998).

After determine yield, water use efficiency (WUE) was calculated as suggested by Dong et al. (2011). To evaluate WUE, total amount of underground water and probable run off were to be ignored (Eq. 1).

$$\text{WUE (kg. m}^{-3}\text{)} = (\text{grain yield (kg)} / \text{total amount of water used (m}^3\text{)} \times 100$$

#### 2.5 STATISTICAL ANALYSES

Finally, the measured characteristics were statistically analyzed. After Bartlett test, data analysis (ANOVA) was conducted in split plot factorial design (SPFD) based on the randomized complete block design (RCBD) in two locations (combined analysis). Means were compared by using LSD test at 5 % probability level. The SAS software (Version 9.4) and Excel were used for analyzing the data and drawing the figures.

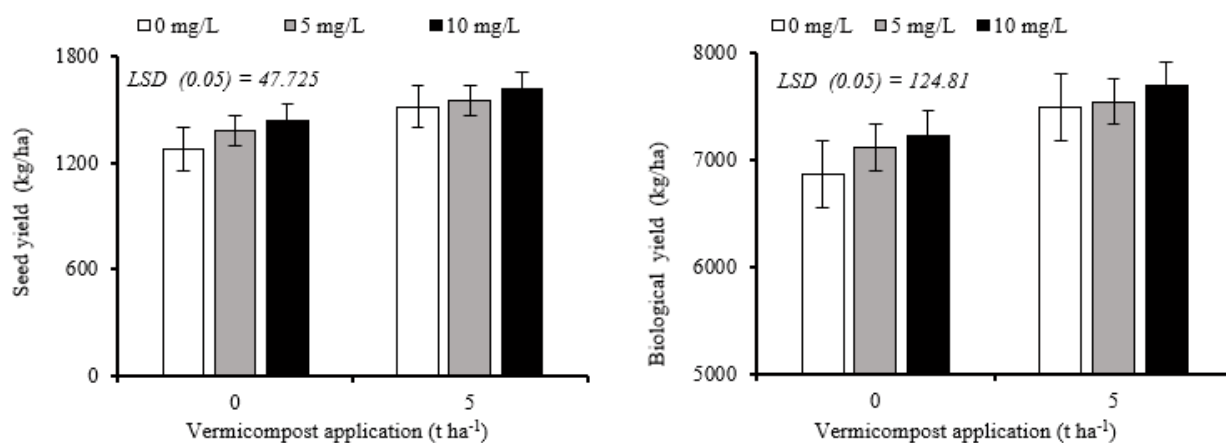


Fig 3: Seed and biological yields in quinoa as affected by interaction between vermicompost application and GABA levels

### 3 RESULTS

#### 3.1 WEATHER CRITERIA IN BIRJAND AND MASHHAD REGIONS

Table 1 indicates the average minimum and maximum temperatures, as well as total rainfall in Birjand and Mashhad regions. During the growing season, the average of maximum temperature in Birjand region (31.66 °C) was slightly higher than Mashhad region (29.12 °C). Similarly, Mashhad region received the higher relative humidity (33.03 %) compared to Birjand region (19.96 %). Therefore, it seems that quinoa plant was comparatively experienced more heat stress under Birjand climate.

#### 3.2 YIELD AND YIELD COMPONENT

As shown in Table 3, the plant height and 1000 seed mass of quinoa were affected by irrigation regimes, GABA, and vermicompost (V) under Birjand and Mashhad conditions. Irrigation treatments had the significant effects on plant height and 1000 seed mass. High level of drought stress had a significant negative effect on plant height. Thus, the plant height was reduced to 31.8 % after using 50 % of PWR compared to the control (Table 3). Reduction was observed in Birjand than Mashhad region (Table 3). Further, severe drought stress significantly decreased 1000 seed mass in Birjand and Mashhad regions. Although drought stress caused the negative effects on plant height and 1000 seed mass, GABA foliar application had the positive effect in reducing these effects. Therefore, the use of 5 and 10 mg l<sup>-1</sup> GABA increased the plant height for 6.8 and 10.5 %, respectively, compared to the non-foliar application (Table 3). V played an effective

role in decreasing the adverse effects of drought stress, similar to the GABA effect (Table 3).

Based on the results, the interaction between irrigation and V had a significant effect on seed yield, biological yield, and harvest index (Table 5). Thus, the amount of seed yield, biological yield, and harvest index decreased under the drought stress. However, V reduced the negative effects of drought on these indices (Table 5). No significant difference was observed between the interaction of 5 t ha<sup>-1</sup> of V + 75 % of PWR treatment and 0 t ha<sup>-1</sup> of V + 100 % of PWR treatment as the seed yield. Moreover, harvest index in 75 % of PWR + 5 t ha<sup>-1</sup> of V treatment (21.57 %) was significantly higher than 100 % of PWR + 0 t ha<sup>-1</sup> of V treatment (20.44 %).

GABA significantly improved the seed yield, biological yield, and harvest index, similar to the V use. Under severe drought stress, the maximum seed yield (1292.25 kg ha<sup>-1</sup>) was obtained by using 10 mg of GABA l<sup>-1</sup> (Table 6). Similar results were obtained for biological yield (6847.38 kg ha<sup>-1</sup>) and harvest index (18.82 %) (Table 6).

The maximum seed and biological yields (1724.5

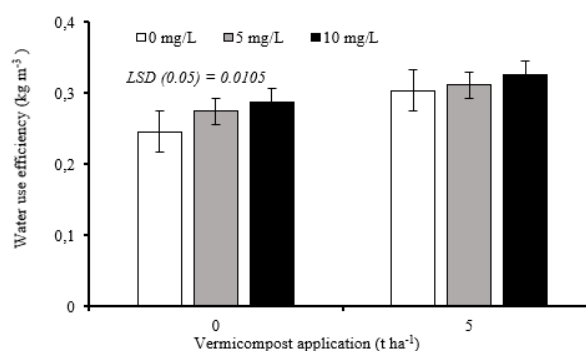


Fig 4: Water use efficiency in quinoa as affected by interaction between vermicompost application and GABA levels

**Table 3:** Plant height, 1000 seed mass, of seed yield, biological yield, and harvest index of quinoa as affected by irrigation regimes, vermicompost application, and GABA levels in Birjand and Mashhad, Iran

Treatments	Plant height (cm)	1000 seed mass (g)	Seed yield (kg ha <sup>-1</sup> )	Biological yield (kg ha <sup>-1</sup> )	Harvest index (%)
<b>Location</b>					
Birjand	89.81 (12)	1.74	1371.4	7225.2	18.85
Mashhad	104.60	1.82	1554.8	74133.1	20.83
LSD (0.05)	7.179	0.052	251.30	297.49	2.939
<b>Irrigation regimes (%)</b>					
50	76.21	1.60	1190.7	6635.6	17.81
75	103.58	1.80	1508.1	7162.8	20.69
100	111.81	1.93	1690.4	8159.1	21.01
LSD (0.05)	5.254	0.071	33.95	120.35	0.355
<b>Vermicompost application (t ha<sup>-1</sup>)</b>					
0	90.60	1.733	1364.4	7068.1	19.11
5	103.83	1.83	1561.7	7570.3	20.57
LSD (0.05)	2.629	0.031	22.03	53.51	0.356
<b>GABA levels (mg l<sup>-1</sup>)</b>					
0 (control)	91.90	1.75	1394.5	7171.4	19.22
5	98.13	1.77	1466.5	7326.8	19.90
10	101.58	1.82	1528.2	7459.3	20.40
LSD (0.05)	3.220	0.038	26.98	66.53	0.437
Average	97.20	1.78	1463.06	7319.15	19.84
<b>S.O.V</b>					
Location (L)	**	**	NS	NS	**
R (L)	-	-	-	-	-
Irrigation regimes (I)	**	**	-	-	-
L × I	NS	NS	**	**	**
R × I (L)	-	-	-	-	-
GABA levels (G)	**	**	**	**	**
Vermicompost application (V)	**	**	**	**	**
G × V	NS	*	**	**	**
I × G	NS	*	*	**	NS
I × V	*	NS	**	**	**
I × G × V	*	NS	**	**	**
L × G	NS	NS	**	**	**
L × V	NS	NS	NS	NS	NS
L × G × V	NS	NS	NS	NS	NS
L × I × G	NS	NS	*	*	*
L × I × V	NS	NS	NS	NS	NS
L × I × G × V	NS	NS	NS	NS	NS
Bartlett's test for homogeneity	0.51	0.11	0.35	0.84	0.40

\*\* : significant at  $p \leq 0.01$ ; \* : significant at  $0.01 < p \leq 0.05$ ; NS: non-significant ( $0.05 < p$ )

**Table 4:** Some quantity and quality traits of quinoa as affected by irrigation regimes, vermicompost application, and GABA levels in Birjand and Mashhad, Iran

Treatments	Sugar (mg 100 g <sup>-1</sup> )	Proline ( $\mu\text{mol g}^{-1}$ FM)	P concentration (mg g <sup>-1</sup> )	K concentration (mg g <sup>-1</sup> )	Water use ef- ficiency (kg m <sup>-3</sup> )
<b>Location</b>					
Birjand	124.75	15.47	0.98	1.58	0.2717
Mashhad	114.34	14.77	0.99	1.59	0.3121
LSD (0.05)	6.860	2.160	0.049	0.110	0.0061
<b>Irrigation regimes (%)</b>					
50	125.48	18.36	1.14	1.70	0.3421
75	118.95	15.61	1.03	1.58	0.2894
100	114.20	11.38	0.80	1.47	0.2442
LSD (0.05)	3.580	1.087	0.063	0.089	0.0074
<b>Vermicompost application (t ha<sup>-1</sup>)</b>					
0	118.57	13.59	0.93	1.51	0.2696
5	120.52	16.65	1.05	1.66	0.3142
LSD (0.05)	1.146	0.0347	0.032	0.034	0.0061
<b>GABA levels (mg l<sup>-1</sup>)</b>					
0 (control)	114.84	13.99	0.94	1.53	0.2750
5	119.29	15.10	0.99	1.57	0.2933
10	124.50	16.26	1.04	1.65	0.3037
LSD (0.05)	1.404	0.425	0.039	0.041	0.0074
Average	14.70	15.12	0.99	1.58	0.2919
<b>S.O.V</b>					
Location (L)	**	**	NS	NS	**
R (L)	-	-	-	-	-
Irrigation regimes (I)	**	**	**	**	**
L $\times$ I	NS	**	NS	NS	*
R $\times$ I (L)	-	-	-	-	-
GABA levels (G)	**	**	**	**	**
Vermicompost application (V)	**	**	**	**	**
G $\times$ V	NS	NS	*	NS	**
I $\times$ G	**	*	*	*	**
I $\times$ V	*	**	*	*	**
I $\times$ G $\times$ V	NS	NS	NS	NS	**
L $\times$ G	*	NS	*	*	NS
L $\times$ V	*	NS	NS	NS	NS
L $\times$ G $\times$ V	NS	NS	NS	NS	NS
L $\times$ I $\times$ G	NS	NS	NS	NS	NS
L $\times$ I $\times$ V	NS	NS	NS	NS	**
L $\times$ I $\times$ G $\times$ V	NS	NS	NS	NS	NS
Bartlett's test for homogeneity	0.52	0.07	0.07	0.21	0.51

\*\* : significant at  $p \leq 0.01$ ; \* : significant at  $0.01 < p \leq 0.05$ ; NS: non-significant ( $0.05 < p$ )

**Table 5:** Plant height, seed yield, biological yield, harvest index, sugar, water use efficiency (WUE), sugar content, proline, P and K concentration of quinoa as affected by interaction between irrigation regimes and vermicompost application

Irrigation regimes (%)	Vermicompost application (t ha <sup>-1</sup> )	Plant height (cm)	Seed yield (kg ha <sup>-1</sup> )	Biological yield (kg ha <sup>-1</sup> )	HI (%)	Sugar (mg 100 g <sup>-1</sup> )	Proline (μmol g <sup>-1</sup> FM)	P (mg g <sup>-1</sup> )	K (mg g <sup>-1</sup> )	WUE (kg m <sup>-3</sup> )
50	0	70.25 ± 16.61	1043.2 ± 220.6	6309.5 ± 397.7	16.43 ± 2.78	125.26 ± 9.91	16.71 ± 1.69	1.08 ± 0.09	1.63 ± 0.16	0.300 ± 0.066
	5	82.17 ± 10.22	1338.2 ± 178.9	6961.7 ± 266.6	19.19 ± 2.23	125.70 ± 10.42	20.01 ± 2.28	1.19 ± 0.08	1.78 ± 0.14	0.385 ± 0.053
75	0	96.08 ± 8.65	1445.1 ± 161.6	7052.4 ± 206.5	20.46 ± 1.89	118.34 ± 7.82	13.77 ± 2.70	0.97 ± 0.19	1.50 ± 0.16	0.277 ± 0.032
	5	111.08 ± 10.65	1571.1 ± 176.5	7273.2 ± 245.9	21.57 ± 2.01	119.56 ± 7.68	17.45 ± 2.03	1.09 ± 0.08	1.66 ± 0.08	0.302 ± 0.035
100	0	105.38 ± 9.44	1605.1 ± 176.5	7842.3 ± 268.5	20.44 ± 1.91	112.10 ± 7.58	10.29 ± 1.52	0.73 ± 0.08	1.40 ± 0.12	0.232 ± 0.027
	5	118.25 ± 14.61	1775.8 ± 192.1	8475.8 ± 279.1	20.94 ± 2.09	116.30 ± 7.19	12.47 ± 1.46	0.87 ± 0.05	1.54 ± 0.11	0.256 ± 0.029
LSD (0.05)		4.648	37.41	87.95	0.613	1.8277	0.431	0.0533	0.0565	0.0105

**Table 6:** Sugar content, proline, P and K concentration, water use efficiency (WUE), seed yield, biological yield, and harvest index of quinoa as affected by interaction between irrigation regime and GABA levels

Irrigation regimes (%)	GABA levels (mg l <sup>-1</sup> )	Sugar (mg 100 g <sup>-1</sup> )	Proline (μmol g <sup>-1</sup> FM)	P (mg g <sup>-1</sup> )	K (mg g <sup>-1</sup> )	Water use efficiency (kg m <sup>-3</sup> )	Seed yield (kg ha <sup>-1</sup> )	Biological yield (kg ha <sup>-1</sup> )	Harvest index (%)
50	0	118.63 ± 8.56	17.14 ± 2.19	1.07 ± 0.08	1.62 ± 0.2	0.305 ± 0.082	1066.1 ± 282.38	6405.4 ± 586.2	16.44 ± 3.17
	5	125.02 ± 7.89	18.41 ± 2.44	1.13 ± 0.09	1.69 ± 0.12	0.349 ± 0.057	1213.7 ± 194.05	6654.1 ± 307.4	18.17 ± 2.29
	10	132.79 ± 8.64	19.54 ± 2.69	1.22 ± 0.08	1.79 ± 0.15	0.372 ± 0.064	1292.3 ± 218.54	6847.4 ± 385.8	18.82 ± 2.66
75	0	114.62 ± 7.34	14.25 ± 2.97	0.98 ± 0.22	1.54 ± 0.2	0.282 ± 0.035	1468.5 ± 171.28	7108.5 ± 238.9	20.62 ± 1.92
	5	118.48 ± 6.51	15.64 ± 2.93	1.03 ± 0.11	1.56 ± 0.11	0.287 ± 0.033	1495.7 ± 170.03	7158.6 ± 204.4	20.86 ± 1.96
	10	123.76 ± 6.66	16.93 ± 2.67	1.07 ± 0.11	1.64 ± 0.11	0.299 ± 0.039	1560.0 ± 194.24	7221.3 ± 302.4	21.56 ± 2.16
100	0	111.27 ± 7.05	10.60 ± 1.63	0.76 ± 0.11	1.42 ± 0.13	0.238 ± 0.031	1648.9 ± 205.58	8000.2 ± 401.4	20.59 ± 2.13
	5	114.36 ± 7.48	11.24 ± 1.67	0.80 ± 0.09	1.46 ± 0.13	0.244 ± 0.030	1690.1 ± 204.12	8167.8 ± 392.1	20.67 ± 2.07
	10	116.96 ± 7.65	12.31 ± 1.88	0.84 ± 0.07	1.53 ± 0.12	0.251 ± 0.029	1732.3 ± 200.09	8309.1 ± 430.4	20.82 ± 1.9
LSD (0.05)		2.238	0.528	0.065	0.069	0.0129	45.82	107.72	0.751



and 7794.6 kg ha<sup>-1</sup>) belonged to 5 t ha<sup>-1</sup> of V + 10 mg l<sup>-1</sup> GABA treatment by considering the interaction between V and GABA (Fig. 3). Moreover, based on the three-way interactions, the highest amount of seed yield, biological yield, and harvest index at 50 % of PWR was observed in 5 t ha<sup>-1</sup> of V + 10 mg l<sup>-1</sup> of GABA treatment (Table 7).

In both Mashhad and Brjand regions, the lowest seed and biological yield, as well as harvest index, were observed in the control treatment; while the highest values were obtained when 5 t ha<sup>-1</sup> V was applied + 10 mg l<sup>-1</sup> GABA. For instance, under Mashhad region, its co-application compared to non-application caused an increase in seed yield by 28.1 % (Table 8).

### 3.3 QUALITY TRAITS IN QUINOA SEEDS

Sugar and proline contents, as well as P and K concentrations were significantly affected by irrigation regimes, V, and GABA levels (Table 4). The amounts of sugar and proline contents increased by reducing PWR from 100 to 50 %. In other words, under the high level of drought stress, these parameters increased in quinoa

seeds, which were consistent with results of P and K concentrations (Table 4).

The use of V improved the amounts of sugar and proline contents as well as P and K concentrations, regardless of drought stress levels. Under the use of 50 % of PWR, sugar and proline contents increased in the presence of 5 t ha<sup>-1</sup> of V (Table 5). Similar results were observed after increasing the amount of GABA. In fact, 10 mg l<sup>-1</sup> of GABA led to the best results when 50 % of PWR was treated (Table 6). This trend was observed in both Mashhad and Birjand regions (Table 9).

### 3.4 WATER USE EFFICIENCY

According to the results presented in Table 4, the interaction of irrigation and V had a significant effect on WUE. Irrespective of V application, reducing irrigation amount led to an increase in WUE. For example, 50 % of PWR compared to 75 % and 100 % of PWR improved WUE by 7.3 and 27.8 %, respectively (Table 5).

Similar to the effect of irrigation regime and V, the interaction of irrigation and GABA on water use ef-

**Table 7:** Seed yield, biological yield and harvest index of quinoa as affected by interaction between irrigation regimes, GABA levels, and vermicompost application

Irrigation regimes (%)	GABA levels (mg l <sup>-1</sup> )	Vermicompost application (t ha <sup>-1</sup> )	Plant height (cm)	Seed yield (kg ha <sup>-1</sup> )	Biological yield (kg ha <sup>-1</sup> )	Harvest index (%)	Water use efficiency (kg m <sup>-3</sup> )
50	0 (control)	0	61.76 ± 24.24	836.5 ± 154.55	5916.6 ± 388.5	14.11 ± 2.25	0.239 ± 0.046
		5	73.50 ± 10.72	1295.7 ± 162.74	6894.2 ± 198.3	18.77 ± 2.02	0.318 ± 0.053
	5	0	75.50 ± 8.83	1101.2 ± 172.14	6439.6 ± 211.1	17.05 ± 2.26	0.343 ± 0.050
		5	76.88 ± 8.68	1326.3 ± 148.67	6868.6 ± 229.7	19.29 ± 1.82	0.371 ± 0.049
	10	0	82.38 ± 9.68	1191.9 ± 168.55	6572.5 ± 221.9	18.11 ± 2.32	0.381 ± 0.043
		5	87.25 ± 10.61	1392.6 ± 225.7	7122.3 ± 311.2	19.53 ± 2.94	0.401 ± 0.066
75	0	0	91.25 ± 8.86	1409.3 ± 161.44	6994.2 ± 244.9	20.12 ± 1.88	0.271 ± 0.033
		5	97.00 ± 7.86	1527.8 ± 169.66	7222.8 ± 180	21.12 ± 1.94	0.275 ± 0.033
	5	0	100.00 ± 7.78	1439.7 ± 169.75	7045.7 ± 213.2	20.40 ± 2.01	0.285 ± 0.033
		5	104.63 ± 8.35	1551.7 ± 161.17	7271.4 ± 122.5	21.32 ± 1.92	0.293 ± 0.035
	10	0	112.50 ± 11.12	1486.2 ± 165.71	7117.4 ± 161.1	20.85 ± 1.98	0.299 ± 0.031
		5	116.13 ± 10.02	1633.8 ± 202.35	7325.3 ± 381.1	22.27 ± 2.22	0.314 ± 0.041
100	0	0	101.50 ± 8.64	1574.1 ± 188.58	7663.2 ± 168.8	20.52 ± 2.15	0.228 ± 0.029
		5	105.38 ± 9.24	1723.6 ± 205.52	8337.2 ± 239.0	20.66 ± 2.25	0.233 ± 0.027
	5	0	109.25 ± 9.92	1607.0 ± 179.64	7858.6 ± 196.0	20.43 ± 2.01	0.236 ± 0.027
		5	115.38 ± 9.04	1773.2 ± 203.03	8477.1 ± 269.2	20.91 ± 2.24	0.249 ± 0.031
	10	0	118.00 ± 8.4	1634.1 ± 180.28	8005.0 ± 320.7	20.39 ± 1.81	0.255 ± 0.030
		5	121.38 ± 22.98	1830.6 ± 176.67	8613.2 ± 287.7	21.26 ± 2.00	0.265 ± 0.026
LSD (0.05)			8.050	64.80	152.33	1.062	0.0182

**Table 8:** Seed yield, biological yield and harvest index of quinoa as affected by interaction between location, irrigation regimes, and GABA levels

Location	GABA levels (mg l <sup>-1</sup> )	Vermicompost application (t ha <sup>-1</sup> )	Seed yield (kg ha <sup>-1</sup> )	Biological yield (kg ha <sup>-1</sup> )	Harvest index (%)
Birjand	0 (control)	0	1200.1 ± 310.93	6800.3 ± 747.8	17.41 ± 3.07
		5	1295.8 ± 262.05	7043.0 ± 685.8	18.26 ± 2.44
	5	0	1347.2 ± 236.42	7117.9 ± 647.1	18.84 ± 2.18
		5	1417.4 ± 220.38	7379.9 ± 671.7	19.15 ± 1.95
	10	0	1454.3 ± 219.7	7431.2 ± 686.2	19.52 ± 1.9
		5	1513.4 ± 255.94	7579.2 ± 791.4	19.92 ± 2.35
Mashhad	0	0	1346.5 ± 405.1	6915.7 ± 845.9	19.10 ± 4.02
		5	1469.4 ± 263.1	7186.2 ± 581	20.33 ± 2.37
	5	0	1527.6 ± 237.2	7345.3 ± 653.3	20.73 ± 2.1
		5	1613.9 ± 243.27	7589.6 ± 662.4	21.21 ± 2.09
	10	0	1646.5 ± 247.1	7646.9 ± 782.2	21.49 ± 1.9
		5	1724.5 ± 241.94	7794.6 ± 711.4	22.12 ± 2.41
LSD (0.05)			52.91	124.38	0.867

**Table 9:** Sugar content, P and K concentration of quinoa as affected by interaction between location and GABA levels

Location	GABA levels (mg l <sup>-1</sup> )	Sugar (mg 100 g <sup>-1</sup> )	P (mg g <sup>-1</sup> )	K (mg g <sup>-1</sup> )
Birjand	0 (control)	119.98 ± 6.02	0.94 ± 0.16	1.53 ± 0.17
	5	124.72 ± 6.25	0.99 ± 0.16	1.57 ± 0.15
	10	129.54 ± 8.16	1.03 ± 0.17	1.63 ± 0.16
Birjand	0	109.70 ± 6.54	0.94 ± 0.23	1.52 ± 0.21
	5	113.85 ± 6.66	0.99 ± 0.18	1.57 ± 0.15
	10	119.47 ± 9.16	1.06 ± 0.19	1.68 ± 0.17
LSD (0.05)		1.828	0.053	0.057

**Table 10:** Water use efficiency of quinoa as affected by interaction between location and irrigation regimes

Location	Irrigation regimes (%)	Water use efficiency (kg m <sup>-3</sup> )
Birjand	50	0.316 ± 0.064
	75	0.271 ± 0.029
	100	0.228 ± 0.024
Mashhad	50	0.368 ± 0.074
	75	0.308 ± 0.032
	100	0.260 ± 0.027
LSD (0.05)		0.0105

iciency was significant (Table 6). By increasing GABA consumption in both levels of V, WUE significantly increased. As an example, 10 mg l<sup>-1</sup> of GABA under 50 % of PWR compared to no application of GABA led to an increase in WUE to 21.9 %. Under 75 and 100 % of PWR,

a positive effect was also observed in terms of GABA application.

By increasing the rate of GABA under both V levels, WUE was observed to be significantly increased, so that the lowest value was observed in the control treatment (no V + no GABA foliar spraying) and the highest value (0.33 kg m<sup>-3</sup>) was observed at 5 t ha<sup>-1</sup> of V + 10 mg l<sup>-1</sup> of GABA (Fig. 4). The results also showed that under severe stress (50 % water requirement), the quinoa plant grown in Mashhad had a higher WUE than Birjand (Table 10).

#### 4 DISCUSSION

Drought stress is an adverse factor associated with the reduced seed yield in crops, which can be imposed by unbalanced irrigation (Yuan et al., 2019). Decreased plant growth, 1000-seed mass, and harvest index are the most important consequences of water stress which were

observed in the plants such as barley and canola (Dreccer et al., 2018).

Heat stress, along with drought stress, is another disorder in agriculture leading to the reduced crop yields, especially in arid and semi-arid regions (Plazas et al., 2019). This hypothesis can justify the higher seed and biological yields in Mashhad compared to Birjand. Based on the results of Table 1, quinoa experienced the higher heat stress under Birjand climate compared to Mashhad climate. Furthermore, the low soil organic matter in Birjand compared to the Mashhad region (Table 2) may cause the soil conditions to be less favorable for quinoa growth.

Water deficiency led to a decrease in yield indices and an increase in seed quality traits. Therefore, it seems that accumulated compounds such as proline, protein, and sugar acted as a compromising mechanism for improving the adaptation of quinoa to drought conditions under severe stress. In this regard, Ebrahimian et al. (2019) reported an increase in proline concentration under the drought stress and indicated proline can create an internal resistance in the plant under water shortage, which is consistent with the results of the study of Naeem et al. (2018). These researchers considered the effective role of proline in improving the growth, regulating the water relations along with the antioxidant activity. Ghafari et al. (2019) indicated the changes in proline concentration under water-deficient conditions and described its changes as a reliable index.

The use of V under drought stress can be effective in various aspects. The plants which have a higher ability to absorb more nutrients such as V from the soil are more tolerant against the drought stress (Demir, 2020; Saba et al., 2019). Based on the results of the study of Lim et al. (2015), the positive effects of V on increasing the seed yield of crops are attributed to the improved soil criteria including physical, chemical and biological parameters. Aboelsoud and Ahmed (2020) indicated that V increased the plant height and 1000-seed mass along with seed yield in wheat. Based on the result of this study, V may proliferate the adaptive responses against the drought stress by inducing the cellular resistance and increasing the water uptake. Furthermore, V caused a significant increase in tomato yield under drought stress conditions (Chanda et al., 2011). Roberts et al. (2007) reported that V increased the number of seeds per plant, plant height, 1000-seed mass, and the seed yield in wheat. Therefore, it seems that V improved the quinoa seed yield by transition the more organic matter to seeds compared to the morphological and physiological indices. Li et al. (2019) demonstrated that the use of GABA improved the plant tolerance and adaptability to stressful conditions by increasing the content of amino acids. Based on the results of this study, the

effective role of GABA in rising the values of the mentioned parameters is mainly related to the high adaptation of morphological stages to drought stress, especially during the pollination stage. Therefore, a positive and significant relationship exists between GABA foliar and V application, which improves the mechanism of GABA in the plant by absorbing the nutrients. The use of V and GABA can reduce the consequences of drought stress when quinoa is exposed to moderate or intense drought stress which is unavoidable in semi-arid climates.

As stated before, increasing irrigation rounds led to an increase in WUE. Such behavior is probably achieved through further development of the root system which can improve the plant's ability to absorb more available water. In this regard, Feizabadi et al. (2021) reported an increase in physiological growth, root system development, and yield of rapeseed as a result of V application.

The results of the triple-interaction effects can express a precise concept for the simultaneous application of GABA and V, whether under stress or non-stress conditions (Table 16). Under severe stress, co-application of 5 t ha<sup>-1</sup> of V + 10 mg l<sup>-1</sup> of GABA recorded the highest WUE (0.401 kg m<sup>-3</sup>). As a new result, a synergistic relationship can be observed between the simultaneous application of GABA and V, which is considered useful for dealing with drought stress in quinoa plants.

Under both Birjand and Mashhad regions, a higher grain yield was observed following GABA and V. Their simultaneous application, as mentioned before, is known as the innovation of this research to improve plant resistance against drought stress. Interestingly, simultaneous application of GABA and V in Birjand region had a lower effect on increasing grain yield than Mashhad region. Therefore, it can be concluded that the co-application of these treatments may require more suitable conditions in terms of temperature to record increasing grain yield. Therefore, it can be seen as a crucial relationship between the supply of nutrients in the plant and the external environment.

Along with severe drought to quinoa, the application of V played an effective role in increasing WUE. This increase was also recorded under mild stress (75 % of PWR) and no stress (100 % of PWR). In this regard, similar results have been reported in terms of V application on increasing WUE in wheat (Azimi et al., 2018). Therefore, it can be concluded that the effective role of V in increasing WUE has been achieved through the further development of the root system and aerial parts. In fact, it can be stated that there is a positive and significant relationship between plant growth traits and the improvement of WUE, which can cause an increase in the plant's ability to deal with drought stress. Generally, any factor that can be effective in increasing the plant's ability

for water absorption can improve WUE (Hosseinzadeh et al. 2018). As mentioned earlier, GABA spraying can play an effective role in improving growth traits and plant height. According to Hosseinzadeh et al. (2018), there is a positive relationship between increasing plant growth ability and WUE. In fact, under constant water consumption, any factor that led to increasing plant performance can improve WUE. Therefore, increasing plant yield after spraying GABA will be an effective technique for increasing WUE.

According to Eq. (1), there is a positive relationship between plant performance and WUE. Considering the similar irrigation scheme applied in both regions, the higher WUE in Mashhad may be due to increased plant growth and grain yield. In other words, more suitable climatic conditions in the Mashhad region probably provide the possibility of plant performance in a better way, which has subsequently caused more water absorption.

In the present study, no significant difference was observed between 75 % of PWR + 5 t ha<sup>-1</sup> of V treatment and 100 % of PWR treatment based on the seed yield. V can decrease the negative effect of moderate drought stress by reducing the PWR to 75 %. Thus, the balance between the use of V and PWR can be effective in decreasing the intense of drought stress.

## 5 CONCLUSION

Based on the results, the foliar application of GABA can improve the plant height and seed yield of quinoa if a relative reduction occurred in PWR. 75 % of PWR can be justified as a practical treatment. The same results were observed for 1000 seed mass, harvest index, sugar, protein, P, and K contents. Moreover, the V treatment caused a similar response for improved WUE, which is consistent with the use of GABA. The co-application of V and GABA was proposed as a new approach in quinoa cultivation, which can be particularly interesting in semi-arid areas so that farmers can implement it under water-limited conditions. Therefore, the use of 10 mg l<sup>-1</sup> of GABA simultaneous with 5 t ha<sup>-1</sup> of V can be recommended when the quinoa plant is faced with moderate or severe drought stress. However, further research is yet needed regarding integrated application of GABA with other bio-stimulant inputs in quinoa production.

## 6 AUTHORS' CONTRIBUTION STATEMENT

M.A. Behdani and S. Parsa proposed the experimental idea and underwent the all stages of the study.

M. Jamshideyni conducted the experiment and did the sampling data. S. Khoramdel managed data analysis and supervised the experiment. Finally, the manuscript was proofed by all the authors.

## 7 DECLARATION OF COMPETING INTEREST

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

## 8 FUNDING AND/OR CONFLICTS OF INTERESTS/COMPETING INTERESTS

We wish to confirm that there are no known conflicts of interest associated with this publication and there has been no significant financial support for this work that could have influenced its outcome.

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