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## Investigation of changes in corn hybrids grain protein, proline, and micronutrient content under the influence of drought and fertilizers

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**Abstract:** In order to investigate the effect of drought stress and the application of chemical and biological fertilizers of potassium and maize cultivars on micronutrient uptake, protein content, and proline changes, an experiment was conducted as a split-plot based on a randomized complete block design with three replications. The main factor was three levels of stress treatment, complete irrigation, irrigation cut at 12 leaf stage and irrigation cut at the flowering stage; first subfactor was fertilization at three levels of potassium fertilizer 100%, potassium fertilizer 70% +30% fertile-2 biofertilizer (60g/h) and fertilizer 50% potassium +50% fertile-2 fertilizer (100g/h) and the second subfactor was related to three maize hybrid cultivars AS71, NS640, and CORDONA. The results showed that drought stress significantly reduced the absorption of phosphorus and potassium elements and significantly increased zinc, copper, and iron elements in the seeds. The grain protein content of normal irrigation and irrigation at the flowering stage was the highest. Drought stress increased proline content. Also, the use of biological fertilizer increased the amount of micronutrient absorption, increased protein and proline content. Finally, the AS71 hybrid was better than other hybrids in terms of absorption of nutrients, protein, and proline content.

**Key words:** Drought stress, biofertilizer, micronutrients, proline, phosphorous, potassium

### 1. Introduction

Drought is one of the environmental constraints affecting growth and production in the world (Ashraf and Harris, 2004; Farooq et al., 2009; Niari Khamssi and Najaphy, 2018; Gholmohammadi et al., 2019; Falaknaz et al., 2019a; Estakhr et al., 2021). It is estimated that up to 45% of the world's agricultural lands are exposed to drought (Bot et al., 2000; Akbarabadi et al., 2015; Rostami Ahmadvandi et al., 2021). Irrigation water supply is one of the factors that directly affects the growth and productivity of corn plants. In agricultural conditions, water scarcity has always been one of the first factors limiting crop production (Hussain et al., 2004; Ahmadi et al., 2012; Hosseini Beryekhani and Parsa, 2021). Its effects depend on different stages of development and could have a significant effect on the crop and other physiological traits of the plant (Chaves et al., 2009; Falaknaz et al., 2019b). Aydinsakir et al. (2013) showed that different levels of drought stress had a significant effect on the yield components such as blossoming-silking intervals, plant height, ear diameter, ear length, ear number, and 1000-grain weight excluding the ears number. Another study by Jafari et al. (2012) found that different cultivars had different responses to water scarcity. Jones (1980) examined the effect of drought

stress on the absorption of some nutrients and showed that the two main factors limiting corn production in the world are water stress and nitrogen deficiency. The higher the amount of soil moisture, the higher the nitrogen is absorbed by the plant, and also the absorption of other elements such as phosphorus, iron, potassium, zinc, etc., is closely related to the available moisture content of the soil.

The induction of proline amino acid synthesis is one of the first plant responses to environmental stresses. Increasing proline results in more cellular adaptation to stress conditions and protect enzymes present in the cytosol and cellular structures. The accumulation of assimilates in cytosol provides a possibility of modulating osmotic pressure in the cell, and also makes the enzymes stable in the presence of ions. Under prolonged stress conditions, proline has several biological effects including control of gene expression, deactivation of free radicals, preserving nitrogen source, carbon source, protection of macromolecules and membranes, and osmotic regulators, which appear to be presenting in proteomics and osmotic modifications. Enzymes are also protected by proline because of their protein structure (Ashraf and Foolad, 2007). The accumulation of proline and soluble carbohydrates in osmotic regulation occurs if

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water potential is reduced by more than 1 MPa (Ashraf, 2010). In order to study the effect of drought stress on the grain filling stage in corn hybrids, Moharram Nejad et al. (2016) stated that drought stress significantly increases the amount of proline and soluble sugars in corn hybrids.

Potassium is one of the most important nutrients required by the plant, which affects the quantity and quality of the product. While involved in many physiological processes, potassium directly influences the relationships between water, photosynthesis, absorption and transport, and enzymatic activity as well as productivity. Potassium deficiency can also reduce the number and size of the leaves (Pettigrew, 2008; Rajkovic et al. 2020; Tas et al., 2022). If the reduction of this source of photosynthetic material is accompanied by a decrease in photosynthetic rates per leaf area, the result will be a reduction in the total volume of photosynthetic materials and a decrease in the transfer of absorbed material for growth.

The reduction in photosynthetic absorption material production and absorption material transfer from outside the leaf to grain development will greatly increase the negative impact of potassium reduction on product and production quality (Pettigrew, 2008).

Biofertilizers are made up of useful microorganisms, each of which is produced specifically for a particular purpose such as nitrogen fixation, phosphate, potassium, iron releasing. These microorganisms are located around the root and, by increasing soil fertility, improve the absorption of the nutrients by the plant (Singh et al., 2011; Norouzi et al., 2021; Chen and Huang, 2022). Researchers also believe that the combined application of bio-fertilizers and potassium increases the activity of acid phosphatase and alkaline phosphatase around roots and increases the phosphorus content in the soil and increases the absorption of more nitrogen, zinc, copper, and iron (Behera et al., 2014).

The purpose of this study was to investigate the effect of drought stress and the application of potassium chemical and biological fertilizers on the absorption of micronutrients, protein content, and proline changes as one of the most important factors of dehydration in plants on corn hybrids under Dehloran climate conditions.

## 2. Materials and methods

The present study was conducted on the lands of the Iranian modern company (Etko organization) located in latitude 32° and 41' N and 74° and 16' E longitude with an elevation of 350 m above sea level, with warm and dry weather, with loose soil texture (sandy loam) and located 50 km from Dehloran city. Prior to seeding, all stages of land preparation were carried out. Each plot consisted of six rows of six m, with 75 cm intervals between rows and 20 cm of plant spacing within each row. The experimental design was a split-split plot and a randomized complete

block design with three replications. The main factor was stress treatment in three levels: full irrigation, irrigation cut off at 12 leaf stage, and irrigation cut at flowering stage. Under irrigation conditions, irrigation was performed based on 37% FC discharge but under stress conditions, based on 50% FC discharge. The first subfactor including fertilization, which was 100% potassium fertilizer, 70% potassium fertilizer, 30% fertile fertilizer (60 g/h) and 50% potassium fertilizer with 50% fertile-2 fertilizer (50 g/h). It should be noted that the amount of required potassium fertilizers was determined based on field experiment so that 150 kg potassium was recommended per hectare and based on the size of the plot, 100% potassium-containing 405 g, 70% potassium treatment containing 283 g, and treatment with 50% containing 202 grams of potassium. PotaBarvar-2 bio-fertilizer procured from Zist-Fanavar-e-Sabz Co., and its proportion was determined based on the company's instruction (every 100g package is equivalent to 50% of chemical fertilizer). The second subfactor was related to three corn hybrid cultivars including AS71, NS640, and CORDONA.

### 2.1. Studied traits

#### 2.1.1. Grain protein content

In order to measure the protein content of the seeds, the nitrogen content of the grain was measured by the Kjeldahl apparatus (Venayi et al., 2012).

#### 2.1.2. Grain nutrients content

Grain nutrients such as phosphorus, potassium, iron, zinc, and copper were measured at the physiological maturity stage. To measure the amount of phosphorus and potassium, a photometer (Model 410, Sherwood Co., England) was used. The atomic absorption system (AA6300 Model) was also used to determine the iron, zinc, and copper content. For measuring the nutrients, digestion, dry burning and combining with chloride-containing acid were used (Jafardukht et al., 2015).

#### 2.1.3. Proline content

For extraction and measurement of proline method of Bates et al. (1973) were used.

### 2.2. Statistical analysis

Data were normalized before the analysis of variance by the Kolmogorov-Smirnov test. After the analysis of variance, the mean comparison of the middle-aged data was done by Duncan's test at a probability level of 5%. Statistical analyses were conducted using SAS software and graphs were also drawn up by excel software.

## 3. Results and discussion

The analysis of variance based on the measured traits for the factors applied in this study is shown in Table 1. Also, the mean values of each factor presented in Table 2 and the analysis of the variance of the interactions (breakdown analysis) related to the significant two-factor interaction

are shown in Table 3. It needs to be explained that only the two-factor interactions of the type of fertilizer and the type of stress, cultivar and stress and cultivar and fertilizer type due to their high importance have been discussed and analyzed and other interactions interpretation is avoided. In the following, the results for each attribute are examined separately.

### 3.1. Micronutrients

Analysis of variance showed that the effects of drought stress, fertilizer type, and cultivar on micronutrients were significant ( $p < 0.01$ ) (Table 1). Also, the interactions of fertilizer type and stress, cultivar and stress, and cultivar and fertilizer type were significant for all micronutrients ( $p < 0.01$ ) The interaction effects of cultivar and stress for phosphorus and copper elements were not significant. Table 2 shows that drought stress significantly reduced the absorption of phosphorus and potassium elements, and significantly increases zinc, copper, and iron in the grain so that irrigation cutting in 12 leaf stage decreases by 6.8% phosphorus and 8.8% in potassium and irrigation

cut during flowering stage decreased 19.4% in grain phosphorus and decreased 13.1% in potassium content. Similarly, irrigation cut in 12 leaf stage increased 1% in zinc, 2.8% in copper, and 7.5% in iron content, and irrigation cut during flowering stage increased by 25.7% in zinc, 47.4% in copper, and 14.98% in iron content absorbed by the grain. Among the micronutrients, zinc has an important role in the RNA metabolism in the content of ribosomes in plant cells, which triggers the metabolism of carbohydrates, proteins, and DNA, and zinc plays an important role in a large number of metabolic reactions, and the zinc acts as cofactors for several enzymes such as dehydrogenase, oxidase, and peroxidase and also protects chloroplasts. Therefore, in terms of moisture stress and potassium consumption, the amount of this element increases in grain (Ayad et al., 2010). Researchers concluded that the occurrence of drought stress leads to a decrease in potassium in different seed hybrids of maize (Jamali et al., 2012) and generally increases the absorption of nitrogen, potassium, magnesium, sodium, and chloride,

**Table 1.** Analysis of variance of the effect of year, drought stress, fertilizers, and cultivars on nutrient uptake and protein and proline content in maize.

S. O. V.	df	Mean square						
		P content	K content	Zn content	Cu content	Fe content	Grain protein	Proline
Block	2	0.05	0.303	0.007	0.0023	0.017	0.001	0.0006
Year(Y)	1	26.59	32**	0.639**	0.0064	0.59*	0.322	0.247**
E <sub>(a)</sub>	2	1.48	0.28	0.006	0.0032	0.023	0.0005	0.0009
Stress(S)	2	995.7**	277.2**	15.52**	0.49**	200.6**	19.02**	14.95**
Y*S	2	1.04	6.84**	0.049**	0.0003	0.019	0.00006	0.137**
E <sub>(b)</sub>	8	0.35	0.109	0.005	0.0018	0.038	0.0009	0.0007
Fertilizer(F)	2	285.1**	186.6**	0.19**	0.04**	11.95**	8.05**	0.304**
Y*F	2	11.575**	4.64**	0.061**	0.0002	0.063	0.0009	0.029**
S*F	4	5.57**	1.88**	0.069**	0.0025**	0.75**	1.443**	0.027**
Y*S*F	4	5247**	5.38**	0.019**	0.0004	0.052	0.088	0.018**
E <sub>(c)</sub>	24	0.12	0.115	0.008	0.0005	0.029	0.0004	0.0004
Cultivar (C)	2	1021.6**	229.7**	18.01**	1.47**	59.35**	1.81**	9.44**
Y*C	2	2.59**	2.59**	0.13**	0.005*	0.058	0.00045	0.029**
S*C	4	0.33	2.45**	0.035**	0.0016	1.95**	0.037**	1.37**
F*C	4	5.96**	6.31**	0.029**	0.0056**	0.36**	0.02**	0.0089**
Y*S*C	4	0.33	0.49**	0.012	0.0002	0.049	0.0004	0.014**
Y*F*C	4	1.27**	0.91**	0.033	0.0007	0.07	0.018**	0.0027**
S*F*C	8	0.73**	0.74**	0.014	0.0005	0.32**	0.0034**	0.0063**
Y*S*F*C	8	0.77**	0.18	0.014	0.0005	0.1**	0.0036**	0.0026**
E <sub>(d)</sub>	72	0.16	0.099	0.007	0.0015	0.03	0.0009	0.00037
CV%		1.01	1.003	0.57	0.88	0.63	0.288	0.802

\* And \*\* significant at 5% and 1% probability level, respectively.

and reduces the absorption of iron and phosphorus (Abdel-Rahman, 1971).

The fertilizer type applied in the present study caused a significant difference in the micronutrient content accumulated in the grain, which shows that the application of 100% potassium fertilizer showed the least nutrient uptake, but the application of Barvar-2 biofertilizer along with 30% and 50% potassium fertilizer has increased

nutrients uptake. Also, there is no difference between levels of Fertile-2 in terms of nutrient uptake. Therefore, the use of this fertilizer has increased the absorption of the elements. Among the elements studied, the combined application of potassium with chemical fertilizers and Fertile-2 had a significant effect on increasing the content of potassium and phosphorus in grains. These results are consistent with the finding of Leeson et al. (1998).

**Table 2.** The mean comparison results of the various levels of the year, drought stress, fertilizers, and cultivars on nutrient uptake and protein and proline content in maize.

Year	P content	K content	Zn content	Cu content	Fe content	Grain protein	Proline
Year (1)	39.55 a	31.04 b	14.51 b	4.35 a	27.63 b	10.565 b	2.369 b
Year (2)	40.36 a	31.92 a	14.63 a	4.36 a	27.75 a	10.654 a	2.448 a
Stress treatment							
Control	44.082 a	33.96 a	14.147 c	4.25 c	25.76 c	10.085 c	1.916 c
Cut irrigation in 12 leaf stage	40.271 b	30.97 b	14.386 b	4.37 b	27.69 b	10.49 b	2.347 b
Cut irrigation in flowering	35.511 c	29.52 c	15.172 a	4.44 a	29.62 a	11.25 a	2.963 a
Fertilizing treatment							
100% K	37.366 c	29.354 c	14.501 b	4.322 b	27.148 b	10.163 b	2.32 c
100% K + 30% Fertile 2	41.63 a	32.329 b	14.615 a	4.373 a	27.981 a	10.831 a	2.446 b
100% K + 50% Fertile 2	40.893 b	32.772 a	14.591 a	4.365 a	27.944 a	10.834 a	2.458 a
Cultivar treatment							
AS 71	43.24 a	33.079 a	14.948 a	4.464 a	28.319 a	10.768 a	2.701 a
Ns 640	35.018 c	29.156 c	13.904 c	4.164 c	26.481 b	10.409 c	1.929 c
Cordona	41.622 b	32.22 b	14.854 b	4.432 b	28.274 a	10.651 b	2.595 b

Means having similar letters have no significant difference at the 5% probability level.

**Table 3.** The mean comparison results of the interaction effect of fertilizer in stress, cultivar in stress, and cultivar in fertilizer on nutrient uptake and protein and proline content in maize.

Stress*Fertilizer								
S.O.V	df	P content	K content	Zn content	Cu content	Fe content	Grain protein	Proline
Control	2	135.15**	86.17**	0.303**	0.0267**	1.234**	8.757**	0.028**
Cut irrigation in 12 leaf stage	2	92.36**	47.54**	0.019	0.0057**	4.55**	0.373**	0.118**
Cut irrigation in flowering	2	68.75**	56.69**	0.011	0.0129**	7.673**	1.809**	0.212**
Stress*Cultivar								
Control	2	327.54**	106.03**	6.64**	0.462**	28.61**	0.596**	0.614**
Cut irrigation in 12 leaf stage	2	342.03**	52.01**	6.404**	0.557**	8.69**	0.458**	2.44**
Cut irrigation in flowering	2	352.71**	76.63**	5.04**	0.452**	25.97**	0.829**	9.124**
Fertilizer*Cultivar								
100% K	2	450.6**	37.55**	6.109**	0.603**	16.005**	0.41**	2.805**
100% K + 30% Fertile 2	2	296.8**	94.82**	5.32**	0.412**	22.8**	0.716**	3.267**
100% K + 50% Fertile 2	2	286.04**	110.02**	6.64**	0.463**	21.27**	0.72**	3.393**

Means having similar letters have no significant difference at the 5% probability level.

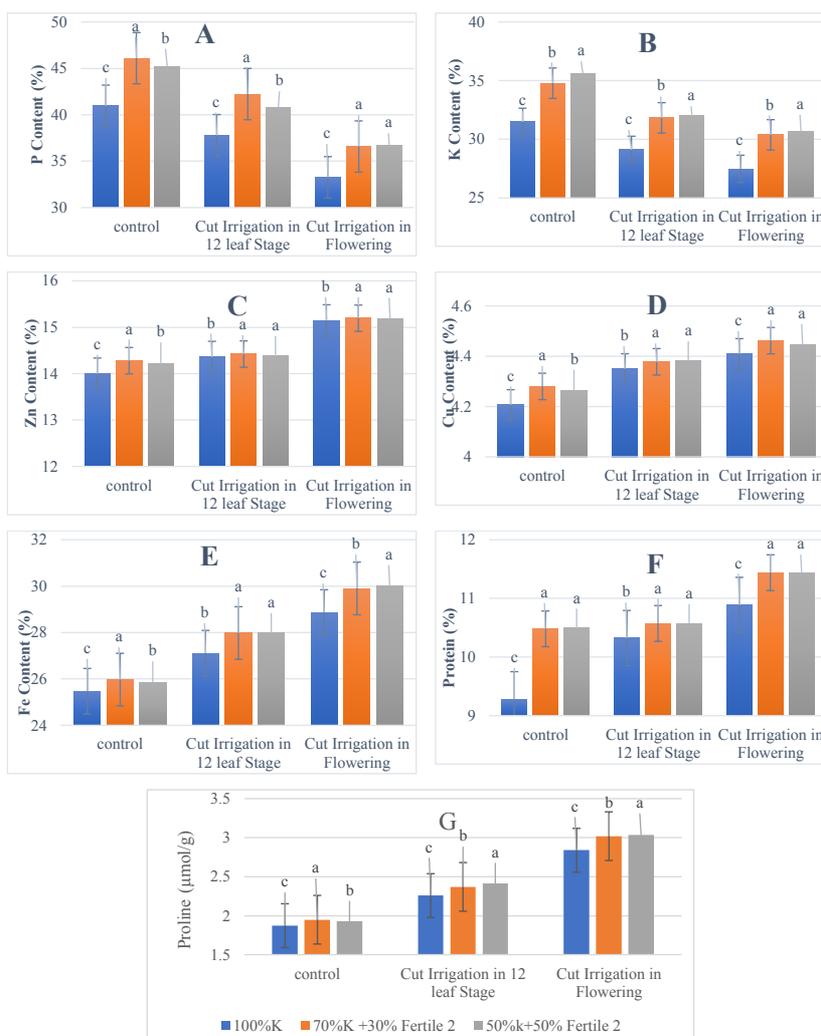
Researchers found that potassium consumption increases the amount of iron in the wheat grains (Manasek et al., 2013) and decreases the copper accumulation trend in the grain (Javanmard et al., 2015).

Among the studied cultivars, the As71 hybrid showed the highest and the Ns640 hybrid showed the least amount of elements absorbed in the seed. The Cordona hybrid is also ranked second with a smaller amount of trace elements. Jamali et al. (2012) showed that different maize hybrids show significant differences in potassium content in grain even in optimal moisture conditions.

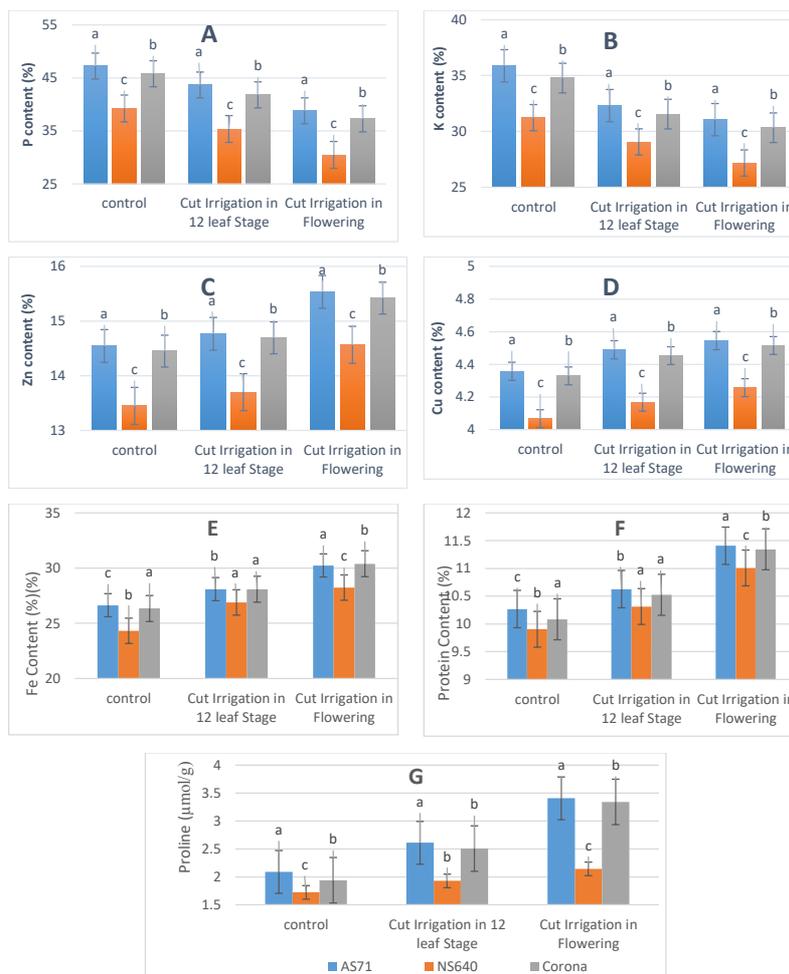
Considering the significance of interactions of fertilizer × stress level, cultivar × stress, and cultivar × fertilizer, and separate analysis of variance was observed that there was a significant difference among various fertilizer levels at any stress level and various cultivars and any stress level and among various cultivars at different fertilizer levels (Table

3). According to the graph of the interactions of fertilizer × stress for all elements, 100% potassium fertilizer and 70% potassium coupled with 30% fertile 2 show the lowest and the highest phosphorous content, respectively, at all stress levels. While for potassium, at all stress levels, 100% potassium fertilizer and 50% of potassium with 50% fertile 2 show the lowest and highest potassium content, respectively. For zinc, copper, and iron contents, there was the same trend and at all stress levels, the nutrient content increased as increasing Fertile-2 (Figure 1).

Figure 2 shows that for phosphorus and potassium, the interaction effect of cultivar and stress is a kind of variation in the amount. In these two elements, at all stress levels, NS640 hybrid and AS71 hybrids exhibit the highest uptake of these elements. Also, with increasing stress levels, the absorption of these two elements decreases. The same situation was observed for copper, zinc, and iron



**Figure 1.** Variation trends in mean values for the interaction of fertilizer and stress levels (A: Phosphorous, B: Potassium, C: Zinc, D: Copper, E: Iron, F: Protein, G: Proline). Means having similar letters have no significant difference at the 5% probability level.



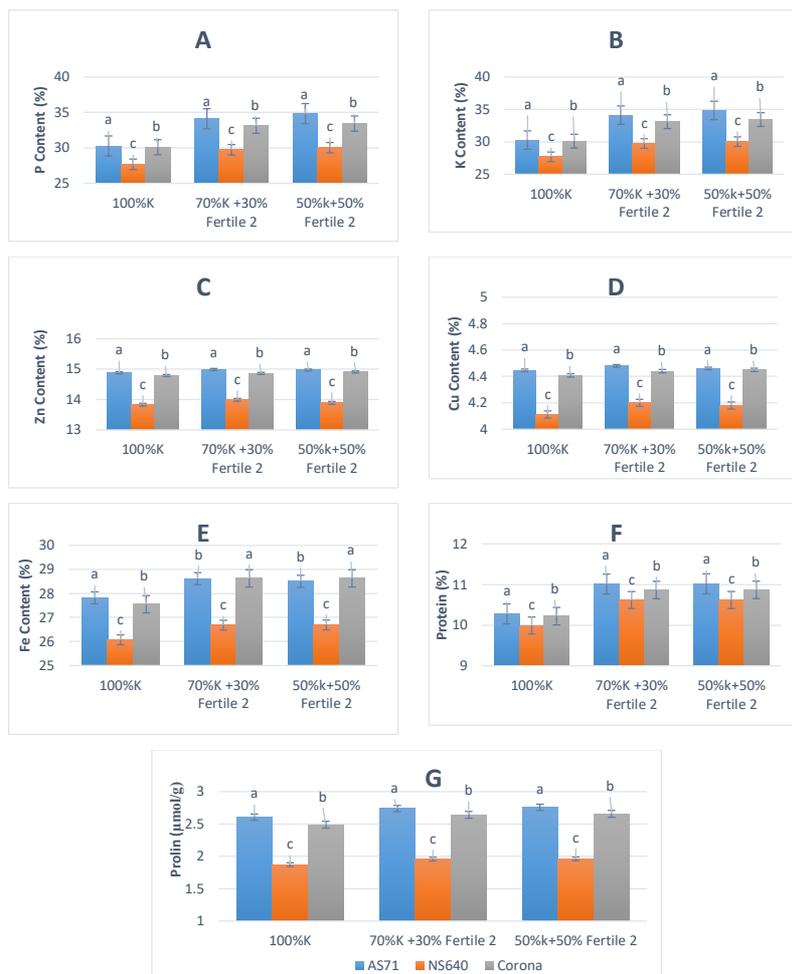
**Figure 2.** Variation trends in mean values for the interaction of cultivar and stress levels (A: Phosphorous, B: Potassium, C: Zinc, D: Copper, E: Iron, F: Protein, G: Proline). Means having similar letters have no significant difference at the 5% probability level.

elements, and the NS640 hybrid and AS71 hybrids show the highest uptake, with the increase in the stress level, the uptake of these elements increases. The interaction of cultivar in fertilizers for phosphorus, potassium, copper, and zinc (Figure 3) showed that the interactions are of a kind of variation in the amount, but for the iron element, it is a kind of change in the order. In all studied nutrients, the uptake increases with the addition of fertile-2 fertilizer. This increase is more pronounced in phosphorus, potassium, and iron. At all fertilization levels, the elements of phosphorus, potassium, copper, and zinc are the highest in the AS71 hybrid and the least amount observed in the NS640 hybrid. However, for the iron at the 100% fertilizer level, the AS71 hybrid had the highest and the NS640 hybrid had the lowest absorption rates.

### 3.2. Grain protein content

The results of the analysis of variance for grain protein (Table 1) show that there is a significant difference between

stress and fertilizer levels and among cultivars ( $p < 0.01$ ). Also, the interactions of fertilizer  $\times$  stress, cultivar  $\times$  stress, and cultivar  $\times$  fertilizer were significant ( $P < 0.01$ ). The results of mean comparisons (Table 2) show that the normal irrigation and irrigation at the flowering stage show the highest grain protein content. Under drought stress conditions, due to the shortening of the grain filling period, the transfer of photosynthetic materials to the seeds is reduced and the starch storage in them decreases, which causes the seeds to be small and increases the protein content (De-Mejia et al., 2003; Kakaie et al., 2010; Ghobadi et al., 2012; Zirgoli and Kahrizi, 2015). Regarding the reduction of seed protein content due to drought stress, Daniel and Triboi (2008), in separate experiments on corn and wheat, found that drought stress results in an increase in the seed protein content relative to the optimal irrigation conditions. They attributed this to the reduction in the transfer of photosynthetic materials, which reduces



**Figure 3.** Variation trends in mean values for the interaction of cultivar and fertilizer levels (A: Phosphorous, B: Potassium, C: Zinc, D: Copper, E: Iron, F: Protein, G: Proline). Means having similar letters have no significant difference at the 5% probability level.

the ratio of starch endosperm to the total volume of seed, and since the percentage of protein in the shell and fetus is higher than the starch endosperm, the percentage of protein in the drought stress condition increases. Under drought stress conditions, CO<sub>2</sub> absorption and fixation decrease due to the relative closure of the stomata or the reduction of their openness degree; hence, the total amount of material used for grain filling is reduced, but the drought stress reduces nitrogen transfer from leaves to seed and this increases the seed's protein content (Javadaslami et al., 2005).

In the fertilization factor, the application of 100% potassium and 50% potassium +50% Fertile-2 significantly increased the seed protein content. Research has shown that potassium activates many enzymes in the plant and these enzymes interfere with the production of materials such as starch and protein and increase the amount of these substances in the seed (Kholedebarin and Islamzade,

2005). Among the studied hybrids, the AS71 hybrid had the highest and NS640 hybrid shows the lowest protein content. A separate analysis of interactions shows that there is a significant difference between fertilizer levels at each stress level (Table 3). The trend of changes in fertilizer levels at each stress level (Figure 1) shows that at each stress level, the addition of fertile-2 fertilizer increased seed protein content. At each stress level, 100% potassium fertilizer and 70% potassium fertilizer +30% fertile-2 indicate the highest seed protein content. In the interaction of cultivar in stress, there is a significant difference between different hybrids at each stress level (Table 3). The interaction effect is the type of change in the amount and at all levels, AS71 hybrid had the highest and the NS640 hybrid showed the lowest grain protein content (Figure 2). There is a significant difference between different hybrids at each fertilizer level (Table 3). In this case, the interactions are of a kind of variation in the amount and at all fertilizer levels,

AS71 hybrid had the most and NS640 hybrid showed the lowest grain protein content (Figure 3).

### 3.3. Proline

Analysis of variance for proline (Table 1) shows that there is a significant difference between two years of experiment, stress levels, fertilizer levels, and cultivars ( $p < 0.01$ ). Also, the interactions of fertilizer type  $\times$  stress, cultivar  $\times$  stress, and cultivar  $\times$  fertilizer type were significant ( $p < 0.01$ ). The results of mean comparisons (Table 2) show that the normal irrigation and irrigation at the flowering stage show the lowest proline content. Increasing proline during stress can be the result of protein degradation, as well as declining consumption due to plant growth retardation. Proline accumulation in stressed plants may also be due to proline synthesis and inactivation of degradation. Increasing proline content under stress conditions will protect cell membranes, proteins, cytoplasmic enzymes and inhibit active oxygen species and remove free radicals (Chaghakaboodi et al., 2012a,b; Ghorbanli et al., 2013; Liang et al., 2013). Proline protects plants from stress through various mechanisms such as osmotic regulation, detoxification of reactive oxygen species, membrane integrity, and protein building (Gravandi et al., 2011; Patade et al., 2011; Jahangiri and Kahrizi, 2015). Cham et al. (2011) investigated the effect of different levels of drought stress on proline accumulation in two drought-sensitive and -tolerant hybrids and found that proline accumulation in the cell increases with increasing drought stress. However, the rate of increase in drought-tolerant hybrids is far more than the susceptible hybrid to drought stress. This suggests that these compounds play an important role in determining how to respond to stress conditions. Anjum et al. (2011) studied the effect of drought stress on proline accumulation in corn hybrids and concluded that due to drought stress, drought-tolerant genotypes rapidly increase their proline content. Moharramnejad et al. (2015) studied osmotic stress on corn lines and reported that drought stress significantly increased the proline content of corn lines, such that the drought-tolerant line had more proline content than the susceptible one. Ashraf and Foolad (2007) observed that in cases of water scarcity, proline accumulation in the root zone of corn seedlings increases rapidly, compared to the other amino acids, especially glycine. This suggests that proline may play a stimulating role in root growth under drought stress conditions.

In the fertilization factor, the application of 100% potassium and the 50% potash+ 50% fertile-2 resulted in a

significant increase in proline content. Naderi et al. (2013) reported that coapplication of chemical and biological fertilizers in corn under stressed and nondrought stresses would increase the proline content of the plant compared to the control without fertilizer, which is consistent with the results of the present study.

Among the studied hybrids, the AS71 hybrid had the highest and NS640 hybrid showed the lowest proline content. A separate analysis of interactions shows that there was a significant difference in the interaction of fertilizer in stress between fertilizer levels at each stress level (Table 3). The trend of changes in fertilizer levels at each stress level (Figure 1) shows that, at each stress level, the amount of proline increases by adding fertile-2 biofertilizer. At each level of stress, 100% potassium and 70% potassium +30% of fertile 2 show the highest amount of proline. In the interaction of variety in stress, there is a significant difference between different hybrids at each stress level (Table 3). Interactions were the variation in the amount and at all levels of AS71 hybrid had the highest and NS640 hybrid exhibited the lowest proline content (Figure 2). There is a significant difference in the interaction between different hybrids at each fertilizer level (Table 3). In this case, at all fertilizer levels, the AS71 hybrid had the most and NS640 hybrid had the lowest seed protein content (Figure 3).

### 4. Conclusion

The study showed that drought stress significantly reduced the absorption of phosphorus and potassium elements and significantly increased zinc, copper, and iron elements in the grain. Drought stress also increased grain protein content and proline content such that irrigation cut at 12 leaf stage showed the highest grain protein and proline content. The results showed that the application of Fertile-2 biofertilizer increased the amount of micronutrient absorption, increased protein, and proline content. Considering the increase in proline content, following the increase of Fertile-2, it is recommended to use this biofertilizer for increasing drought resistance. Interaction effects among the treatments were, in most cases, a change in the amount and no significant interaction was observed between the treatment levels. Finally, the AS71 hybrid was better than other hybrids in terms of nutrient uptake, protein, and proline content.

### Acknowledgment/disclaimers/conflict of interest

The authors declare that they have no conflict of interest.

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