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The Effect of Foliar Application of Iron and Proline Nanoparticles on Biochemical, Physiological and Agronomic Traits of Quinoa Plant in Different Cultivation Dates

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ABSTRACT

In order to investigate the possibility of ecophysiological adaptation of quinoa plant, cultivation in different dates under the effect of foliar spraying of iron and proline nanoparticles on yield and related experimental traits in two consecutive years 2019 and 2020 in February in Ilam province, Sarableh city in the research center farm located in the north East of Ilam city was implemented in the form of a split-split plot (plots chopped twice) in the form of a basic randomized complete block design with three replications. The main factor, including the planting date, was done in three levels: February4th, February 19th and March 4th. The secondary factor includes iron nanoparticles in four levels including control or non-consumption, 0/3, 0/6 and 0/9 grams per liter of water were added to the volume. The third factor included proline in two levels: 100 mg per liter consumption and Non-use (witness) which were placed in sub-sub plots. The results showed that the main effects including year, planting date, iron and proline nanoparticles on all traits, plant height, number of flowering branches, height of the main flowering branch, weight of 1000 seeds, seed yield, biological yield and harvesting index were meaningful. Due to the difference in temperature and humidity conditions, most traits were affected. So that the maximum weight of 1000 seeds was obtained with the amount of 6.05 grams in the triple treatment of planting date in iron nanoparticles and proline consumption on the March 4th planting date and 0.9 grams of iron nanoparticles and proline consumption. Also, the lowest weight of 1,000 seeds related to the planting date of February 4th and the absence of proline and iron nanoparticles was obtained with 1.73 grams. The highest yield was obtained on the date of the third crop on the March 4th and the treatment of 0.9 grams of iron nanoparticles and the consumption of proline with the amount of 2948.17 kilograms per hectare. The lowest yield related to the planting date

of February 4th and the absence of proline and iron nanoparticles was obtained 14556.17 kg/ha. According to the obtained results, the best yield was obtained on the March 4th and the use of proline and the treatment of 0/9 grams of iron nanoparticles were obtained, which is recommended to achieve high grain production in the cultivation conditions of the region.

***Keywords**:* Planting date, Iron nanoparticles, Proline, Antioxidant enzymes and performance components

INTRODUCTION

Environmental changes have reached a critical level in recent decades and are a serious threat to the quantitative and qualitative performance of agricultural products. On the other hand, the increase in population and the need for more food puts irreparable pressure on the environment, water resources and agricultural ecosystems. Scientists believe that these changes cause climate changes, the length of seasons, the intensity and pattern of precipitation, and this factor has caused more attention to be paid to plants that grow in different latitudes and altitudes. Currently, rice, wheat and potatoes supply 50% of human food. These plants will have a lower performance in the new climatic conditions. Therefore, alternative plants should be introduced to adapt to the new conditions. Currently, crop rotation is limited to cereals (wheat and barley) in rainy lands, and crop diversification is needed in the rainy season. Quinoa is a one-year, broad-leaved plant with a height of one to two meters, and in addition to seeds, its young leaves are also used as fresh or cooked vegetables. This plant has a high tolerance against a wide range of non-living stresses such as coldness, salinity and drought. Quinoa is cultivated in many regions of the world due to its high seed quality and high tolerance in harsh conditions. Quinoa seeds contain 14-20% protein and are rich in essential amino acids such as lysine, methionine and cysteine, which are found in very small amounts in most grains. One of the important management factors is the appropriate planting date, which causes a positive effect on the physiological indicators of growth and, as a result, increases seed yield. The proper planting date makes optimal use of climatic factors such as temperature, humidity, day length, and also matching the flowering time with the desired temperature (Sepahvand *et* al., 2010).

This plant has been introduced to European countries, North America, Asia and Africa in order to increase diversity in agricultural products, and the results of experiments to introduce the plant as fodder and seed have been promising. Global attention is on this plant due to its high nutritional value is a lot.

Quinoa has a high amount of protein and the balance of its amino acids is better than wheat and rice, and it has a high amount of iron and calcium. The seeds of this plant have a high amount of antioxidants and polyphenols. Quinoa is gluten-free and is a suitable food for people with celiac disease. Its whole grain prevents type 2 diabetes due to its low glycemic index 1. Proline is considered as an anti-stress amino acid. An increase in the accumulation of proline leads to resistance to drought in the plant so that its growth can continue under water limitation. Proline, participating in the composition of hydroxyproline, is one of the important components of the Extensin protein, which plays an important role in the growth and development and defense of plants. Proline is even in honey of plants that thrive in cold regions (Liu *et al.*, 2016). The accumulation of proline in plants under stress is due to the

synthesis of proline and the deactivation of its destruction. The increase in proline content in stress conditions protects the cell membrane, proteins, cytoplasmic enzymes and inhibits active oxygen species and removes free radicals, so it is one of the responses of plants against environmental stresses and increasing the level of proline (Layang *et al.*, 2013).

One of the reasons that environmental stresses such as drought reduce the growth and photosynthetic ability of plants is the disturbance in the balance between the production of free oxygen radicals and the defense mechanisms that eliminate these radicals, which lead to the accumulation of reactive oxygen species (ROS), induction of oxidative stress, damage to proteins, membrane lipids and other cell components. In order to prevent the effects of oxidative stress, plants have several biochemical mechanisms, among them Mehler's cycle, glutathione ascorbate cycle, xanthophyll cycle and photorespiration. Also, plants have enzymatic and non-enzymatic antioxidant systems that deactivate ROS and reduce oxidative damage caused by ROS activity. Enzyme antioxidant defense system including superoxide dismutase (SOD), peroxidases (POX), ascorbate peroxidase (APX), catalase (CAT), and glutathione reductase (GR), which are key antioxidant enzymes in fighting against (ROS) and the most important non-enzymatic antioxidant compounds are glutathione, ascorbic acid, alpha-tocopherol, zeaxanthin and antheraxanthin. Ascorbate peroxidase enzyme plays an effective role in collecting hydrogen peroxide in Mehler and glutathione-ascorbate cycles. In the glutathione-ascorbate cycle, with the activity of the enzyme (APX), ascorbate is oxidized to monodehydroascorbate and is necessary for the continuation of the ascorbate production cycle. For this purpose, in this cycle, the enzymes monodehydroascorbate reductase (MDHAR), dehydroascorbate reductase (DHAR), and glutathione reductase (GR), work and reduce ascorbate using (NADPH) and glutathione. Glutathione reductase also plays an important role in adapting to oxidative stress. This enzyme is responsible for converting oxidized glutathione (GSSG) to reduced glutathione (GSH) and maintaining a high ratio of GSH to GSSHG. Glutathione plays an effective role in collecting hydrogen peroxide and maintaining GSH in the xanthophyll, Mehler and ascorbate-glutathione cycles.

GSH can act as an antioxidant (non-enzymatic) like ascorbate and scavenge singlet oxygen (O2) directly, therefore increasing GR is very important due to the regeneration of oxidized glutathione (Cruz de Carvalho 2008). Researches have shown that there is a strong correlation between tolerance to oxidative stress caused by environmental stress and an increase in the concentration of antioxidant enzymes in photosynthetic plants (Mittler 2002).

MATERIALS AND METHODS

This research was conducted in two consecutive years 2019 and 2020 in February in Sarablah city of Ilam province in the field of the research center located in the northeast of Ilam city between latitude 33 degrees 45 minutes to 33 degrees 41 minutes and longitude 46 degrees 35 minutes to 46 degrees 27 minutes. It was done at an altitude of 1000 meters above sea level.

Before conducting the test, a soil sample was randomly taken from the place where the test was conducted, and it was taken to the laboratory to determine the characteristics of the soil, the results of which are shown in Table 1-3.

Table 1. Physical and chemical characteristics of experimental soil

soil pattern	Sand (%)	clay (%)	Silt (%)	Electrical conductivity ds/m	acidity	Organic carbon %	total nitrogen %	Absorbable phosphorus (ppm)	Absorbable potassium (ppm)
clay loam	22	35	43	.85	7.49	.3	11.	9.5	245

Table 2. aerology statistics during implementation of the plan in 2019

Months of the year (AD)	Rainfall (Mm)	Average monthly temperature (degrees Celsius)	Average maximum temperature (degrees Celsius)	Average minimum temperature (degrees Celsius)	relative humidity Monthly average %	Monthly evaporation %
January	166	6.4	12.1	2.8	61	0
February	274	11.8	17.6	6	65	0
March	178.4	12.6	18.1	7	68	61
April	128.6	18.8	27.3	10.30	48	68
May	4.3	28.1	37.3	18.8	26	48
June	0	31	41	22.4	21	26
July	0	32.1	41.4	23.1	23	21

Land preparation operations and planting

First, a plot of 84 x 24 meters was selected. The mentioned land was under wheat cultivation in the 2018-2019 crop year. Land preparation was done in June. At first, the land was plowed by a reversible plow. Then two times, disks were placed perpendicular to each other, and the ground was leveled using a trowel. The weeding operation was done by hand in two stages. The most important weeds observed in the field are shirtighak, toq, soroof, ivy, oyarsalam, oat, and no pests were observed. Then according to the plan, planting was done by hand.

Experimental plan

The experiment was carried out in the form of split, split plot (double chopped plots) in the form of a basic plan of randomized complete blocks with three replications in two consecutive years. This plan has 3 main or repeated blocks, each of them had 4 meters wide and 84 meters long. In the distance between the blocks (3 meters of the corridor), the experimental plan used to investigate the quantitative and qualitative traits of quinoa was designed, and iron nanoparticles were prepared from science-based Sodour Ahrar Sharq (Khazara Kalate Nano

Fertilizers). In four levels including witness or non-use, 3/. 6/. and 9/. Gram per liter of water, it was added to the volume and then three weeks after planting, foliar spraying was done at the 4-6 leaf stage. And also, proline was obtained from Teb Shahr Company (importer of Merck chemicals from Europe and America). After 72 hours, proline foliar spraying of 100 mg per liter of water was done in one step.

Allocation of treatments in experimental plots is as follows:

The first factor (A) includes "different dates of cultivation" in 3 levels (February 4th, February 19th and March 4th)

The second factor (B iron nanoparticles were applied at four levels including (no use (control) 0.3, 0.6. and 0.9 grams per liter of water).

The third factor includes proline in two levels (100 mg/liter consumption and non-consumption (control)

Table 3.Variance analysis of the effect of planting date, iron nanoparticles and proline on yield and related traits

Sources of changes	Degrees of freedom	Bush height	grain performance	the weight of one thousand seeds
Year	1	282.80^{ns}	7421.82^{ns}	0.520^{ns}
repeat (year)	4	895.07	30595.36	5.419
planting date	2	33454.85^{**}	90808.89^{*}	78.13^{*}
Planting year × date	2	217.76^{ns}	3036.94^{ns}	1.745^{ns}
Original error	8	80.083	2635.96	1.1909
Iron nanoparticles	3	3074.68^{**}	4959.47^{*}	6.752^{**}
Year × iron nanoparticles	3	8.22^{ns}	271.84^{ns}	0.050^{ns}
Planting date × iron nanoparticles	6	24.18^{*}	507.79^{ns}	0.221^{ns}
Year×planting date×iron nanoparticles	6	4.67^{ns}	349.8^{ns}	0.278^{**}
Minor error	36	17.49	139.31	0.044
Proline	1	294.12^{ns}	2310.40^{ns}	1.368^{*}
Year x Proline	1	10.56^{ns}	27.38^{ns}	0.0046^{ns}
Planting date × proline	2	36.14^{ns}	233.84^{ns}	0.060^{ns}
Year×planting date×proline	2	21.89^{ns}	250.8^{ns}	0.0428^{ns}
Iron x proline nanoparticles	3	17.61^{ns}	310.20^{ns}	0.0143^{ns}
Year x iron nanoparticles x proline	3	17.73^{ns}	252.11^{ns}	0.0424^{ns}
Planting date × iron nanoparticles × proline	6	10.57^{ns}	433.47^{*}	0.0512^{ns}
Year×planting date×iron nanoparticles×proline	6	23.54^{**}	86.35^{ns}	0.0148^{ns}
Total error	48	6.49	96.78	0.0178
Coefficient of variation		3.04	4.870	3.562

ns.* and ** are non-significant, significant at the five and one percent probability level, respectively..

Table 4. Variance analysis of the effect of planting date, iron nanoparticles and proline on yield and related traits

Sources of changes	Degrees of freedom	Proline	Superoxide dismutase	Peroxidase	Catalase
Year	1	152.98^{ns}	0.18^{ns}	1440.8^{ns}	0.023^{ns}
repeat (year)	4	6.72	0.018	20.20	0.025
planting date	2	3785.99**	70.05**	9270.86*	0.82*
Planting year × date	2	3.73^{ns}	0.014^{ns}	483.42*	0.0097^{ns}
Original error	8	2.520	0.00098	19.07	0.0077
Iron nanoparticles	3	383.70**	5.97**	577.09*	0.057**
Year × iron nanoparticles	3	1.757^{ns}	0.0005^{ns}	31.74^{ns}	0.0013*
Planting date × iron nanoparticles	6	18.56^{ns}	0.19**	57.24^{ns}	0.00053*
Year×planting date×iron nanoparticles	6	4.48*	0.0010^{ns}	37.27^{ns}	0.00008^{ns}
Minor error	36	1.70	0.0010	1.89	0.0005
Proline	1	48.51^{ns}	0.99**	236.38^{ns}	0.008**
Year x Proline	1	0.34^{ns}	0.0000001^{ns}	48.63^{ns}	0.000002^{ns}
Planting date × proline	2	1.908^{ns}	0.035^{ns}	28.24^{ns}	0.00017**
Year×planting date×proline	2	0.250^{ns}	0.002^{ns}	23.25^{ns}	0.000048^{ns}
Iron x proline nanoparticles	3	0.16^{ns}	0.012*	20.89^{ns}	0.000021^{ns}
Year x iron nanoparticles x proline	3	0.31^{ns}	0.0011^{ns}	25.02^{ns}	0.000028^{ns}
Planting date × iron nanoparticles × proline	6	0.16^{ns}	0.013**	28.75^{ns}	0.00012^{ns}
Year×planting date×iron nanoparticles×proline	6	0.18^{ns}	0.0012^{ns}	29.74**	0.000048^{ns}
Total error	48	0.089	0.0006	0.41	0.000027
Coefficient of variation		1.25	1.13	2.45	2.31

Catalase measurement by Beers and Sizer 1952

First, 0.5g of frozen leaf sample is homogenized with liquid nitrogen (in an ice-cold mortar) and crushed, then add 5 ml of cold phosphate buffer with pH=7.5 containing 50 mM of EDTA. After transferring the homogenates to test tubes, they were centrifuged at 12,000 rpm and at a temperature of 4 degrees Celsius for 10 minutes. Use the clear supernatant liquid for catalase assay (to prevent the harmful e

ffects of consecutive freezing and thawing of the supernatant samples, absorbance of the complex was recorded immediately at the wavelength of 240 nm and after 1 minute, the absorbance was measured again. Absorption changes obtained in one minute are calculated by the quenching coefficient of this reaction which is equal to 36.6mmol-1cm-1 and is calculated from the following formula:

$$\text{Catalase activity(U)} = \frac{(\text{A1} - \text{A2}) \times Vt \times d}{\varepsilon \times Ve} \quad (1)$$

A1: Initial absorption

A2: Absorption after one minute

Vt: total volume

Ve: volume of extract

ε: extinction coefficient

d: dilution factor

The amount of APX activity was measured using the method of Nakano and Asada 1999.

The 3 mL reaction mixture included: 0.7 mL of 50 mM phosphate buffer, 1 mL of 0.1 mM EDTA, 1 mL of 5 mM ascorbic acid, 0.1 mL of 0.1 mM hydrogen peroxide, and 0.2 mL of enzyme extract. µ Enzyme reaction speed in the form of changes in absorbance over time (OD/min) at a wavelength of 290 nm with the extinction coefficient of ascorbic acid 2.8mMol-1cm-1 from the relationship A=εbc (A is the read absorption rate, ε is the extinction coefficient, c is the concentration of ascorbic acid and (b) the length of the tube is 1 cm) the amount of ascorbic acid remaining after one minute of enzymatic reaction was recorded. Decreased absorption is due to peroxidation of ascorbic acid. One enzyme unit is equivalent to breaking down one micromole of ascorbate in one minute at 25 degrees Celsius (Nakano and Asada 1999). Finally, it was expressed in micromoles per gram of fresh tissue per minute.

Calculation: ascorbate peroxidase activity

$$\text{Ascorbate peroxidase activity(U)} = \frac{(\text{A1 - A2}) \times Vt \times d}{\varepsilon \times Ve} \quad (2)$$

Table 5. Variance analysis of the effect of planting date, iron nanoparticles and proline on physiological characteristics

Sources of changes	Degrees of freedom	TC Canopy temperature	TL leaf temperature	RH relative humidity
Year	1	51.72^{ns}	8.41^{ns}	0.78^{ns}
repeat (year)	4	9.81	2.58	1.42
planting date	2	4829.6^{**}	2095.30^{**}	8117.25^{**}
Planting year × date	2	23.87^{ns}	1.65^{ns}	0.017^{ns}
Original error	8	10.76	1.36	1.26
Iron nanoparticles	3	360.69^{**}	437.038^{**}	785.66^{**}
Year × iron nanoparticles	3	0.27^{ns}	1.45^{ns}	0.21^{ns}
Planting date × iron nanoparticles	6	10.20^{ns}	18.51^{**}	87.75^{**}
Year×planting date×iron nanoparticles	6	4.14^{ns}	0.99^{ns}	0.09^{ns}
Minor error	36	1.63	1.31	0.97
Proline	1	63.33^{*}	181.80^{ns}	155.83^{*}
Year x Proline	1	0.180^{ns}	1.21^{ns}	0.09^{ns}
Planting date × proline	2	1.56^{ns}	21.77^{**}	24.76^{**}
Year×planting date×proline	2	0.89^{ns}	0.705^{ns}	0.11^{ns}
Iron x proline nanoparticles	3	0.99^{ns}	11.10^{*}	3.59^{**}
Year x iron nanoparticles x proline	3	0.15^{ns}	1.15^{ns}	0.084^{ns}
Planting date × iron nanoparticles × proline	6	0.18^{ns}	16.60^{**}	7.36^{**}
Year×planting date×iron nanoparticles×proline	6	0.65^{ns}	1.35^{ns}	0.06^{ns}
Total error	48	0.22	1.36	1.18
Coefficient of variation		1.62	4.36	2.52

Measurement of superoxide dismutase

3 ml reaction mixture (0.1 ml of 1.5 M sodium carbonate, 0.1 ml of 3 M EDTA, 1.5 ml of 50 Ml potassium phosphate buffer (pH=7.5) and 0.2 ml of 200 Ml methionine and 1 ml of distilled water and 1/ 0 ml of 2.25 mM NBT and 0.05 ml of enzyme (50 µl with sampler) were extracted) The reaction was started by adding 0.1 ml of 60 µM riboflavin and placing the test tubes under two fluorescent lamps (30 W) at a distance of 30 cm will be. The test tubes were kept under the light for 15 minutes and after the mentioned time, the enzyme reaction was stopped by turning off the lamps and placing the test tubes in absolute darkness (surrounding them with a black cloth). Another complete reaction mixture, which was placed in absolute darkness from the beginning, was used as a blank. After stopping the reaction, the absorbance of the samples was recorded at 560 nm. Enzyme activity measurement requires a light source. The control contains 3 ml of the reaction solution without extract, which is placed in the light for 15 minutes at the same time as the samples. In this way, the amount of SOD enzyme activity in the samples is measured in comparison with the light control. Tube without enzyme produces maximum color.

The absorbance of the control sample was read at a wavelength of 560 nm in 1-minute intervals until it reached the linear phase. This time was about 15 minutes. Due to the lack of enzyme in the light control, the regeneration of NBT in the presence of light is done 100% and all the nitrobuterazolium in the reaction solution is converted to formazan. The absorbance of the control at 560 nm shows 100% of NBT photo regeneration and half of it is equivalent to one enzyme unit. Therefore, one enzyme unit of superoxide dismutase is the amount of enzyme that causes 50% inhibition of NBT photo regeneration. The difference in the absorbance of the samples and the light control at 560 nm indicates the inhibition of the photo regeneration of NBT in the presence of the SOD enzyme present in the sample. Using this absorption difference, the enzyme unit of the samples was calculated and the enzyme activity was expressed in terms of enzyme unit in the amount of total protein (mg) in 100 microliters of the obtained extract according to the method (Sairam *et al*, 2002).

$$\text{X\% inhibition of NBT reduction by SOD} = \frac{(0\text{Dcontrol} - 0\text{Dsampel})}{0\text{Dcontrol}} \times 100$$

One enzyme unit of superoxide dismutase is the amount of enzyme that causes 50% inhibition of NBT photo regeneration.

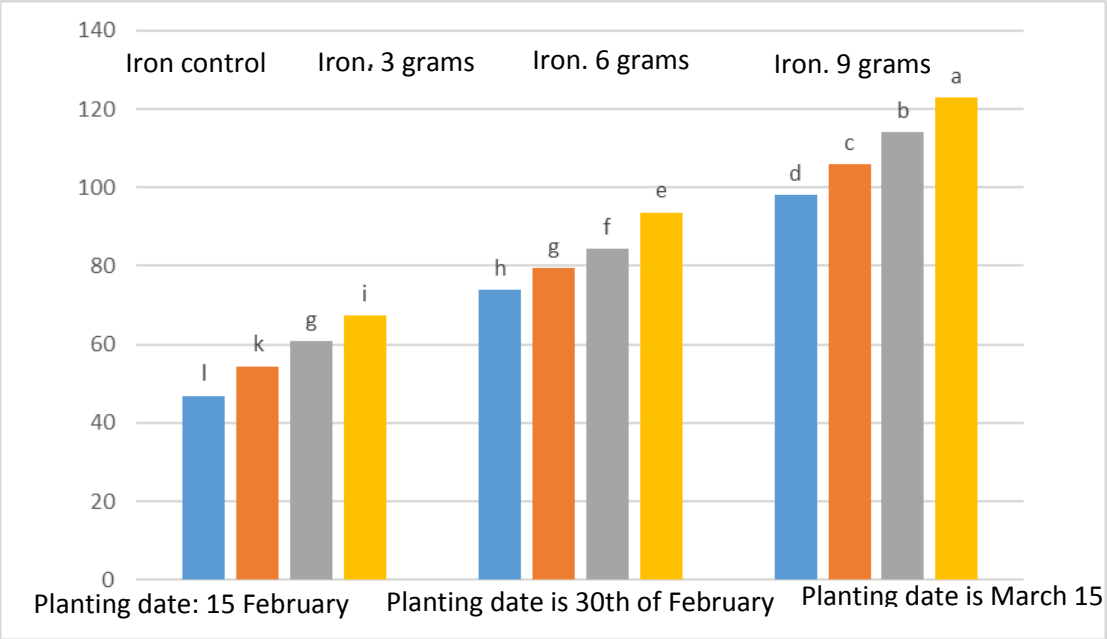

Figure 1.Comparison of the average interaction effect of iron nanoparticles and planting date on plant height

DISCUSSION AND CONCLUSION

Rasouli *et al*. (2013) reported that the use of iron nano fertilizers increases plant metabolism and more and more effective absorption of main elements and fertilizers, as well as the targeted delivery of micronutrients to specific plant tissues. Iron plays an important role in the synthesis of chlorophyll, because it is one of the main components of chlorophyll, which regulates respiration and photosynthesis and reduces nitrate and sulfate, and iron and zinc nanoparticles significantly increased plant height and yield and yield components in chamomile plants.

Karima *et al*. (2005) found in an experiment that spraying amino acids significantly increases the height of the plant, the number of secondary stems, heavier and dry chamomile plants. In an experiment on sesame, Beqaei *et al*. 2012 showed that one of the important factors in the discussion of the date of planting is the efficiency of water consumption, which was significantly affected by the increase in plant height stress, seed yield and biomass.

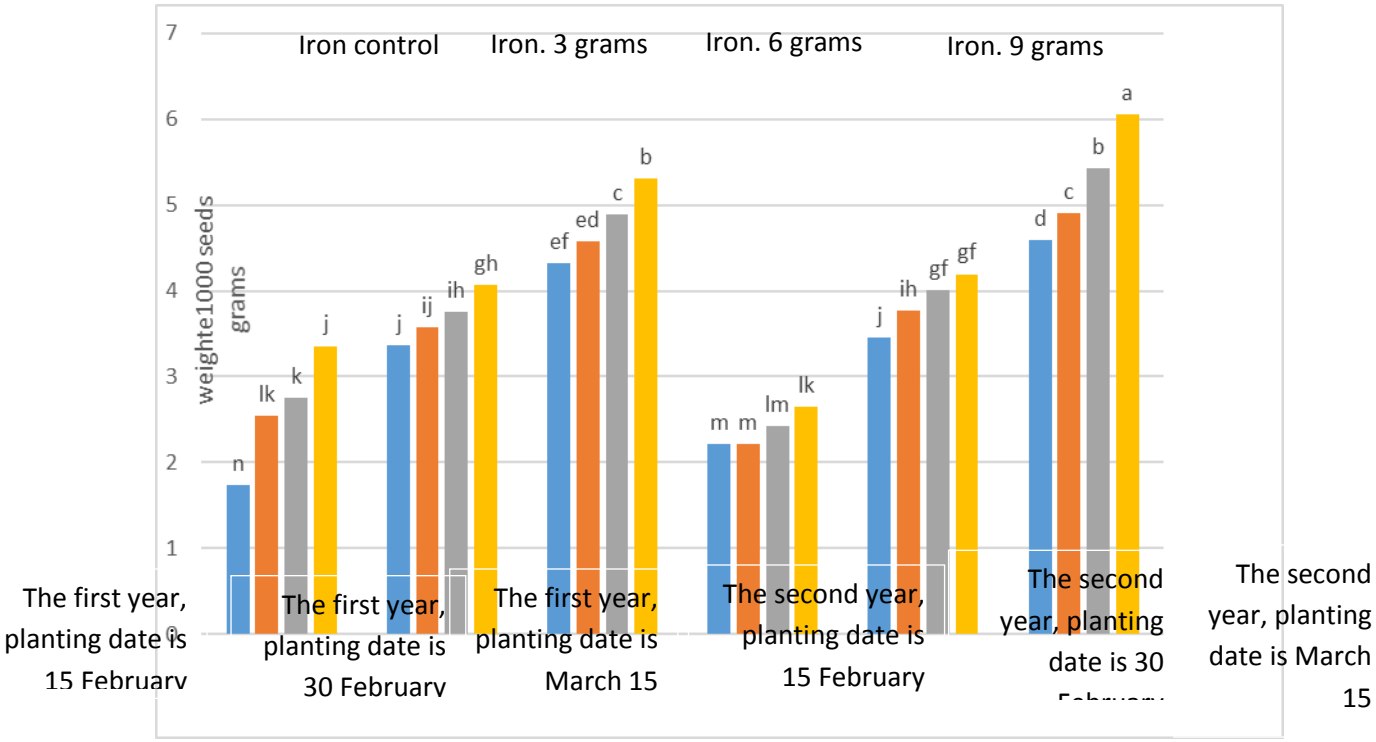

Figure 2.Comparison of the average interaction effect of year, planting date, iron nanoparticles on the weight of one thousand seeds

Also, the studies of Manjezi *et al*. (2012) showed that the effect of foliar application of iron element on the yield and yield components of wheat plant under drought stress conditions, through increasing photosynthesis and also transferring its product, i.e. sugar to the seeds, as well as increasing the rate of re-transfer of materials Compared to the control treatment, plant-to-seed photosynthesis increased the weight of 1000 seeds and seed yield. In the study of Hinojoza *et al*. (2019), high temperature improved the photosynthesis rate of quinoa plant in different planting dates, and also quinoa has high flexibility.

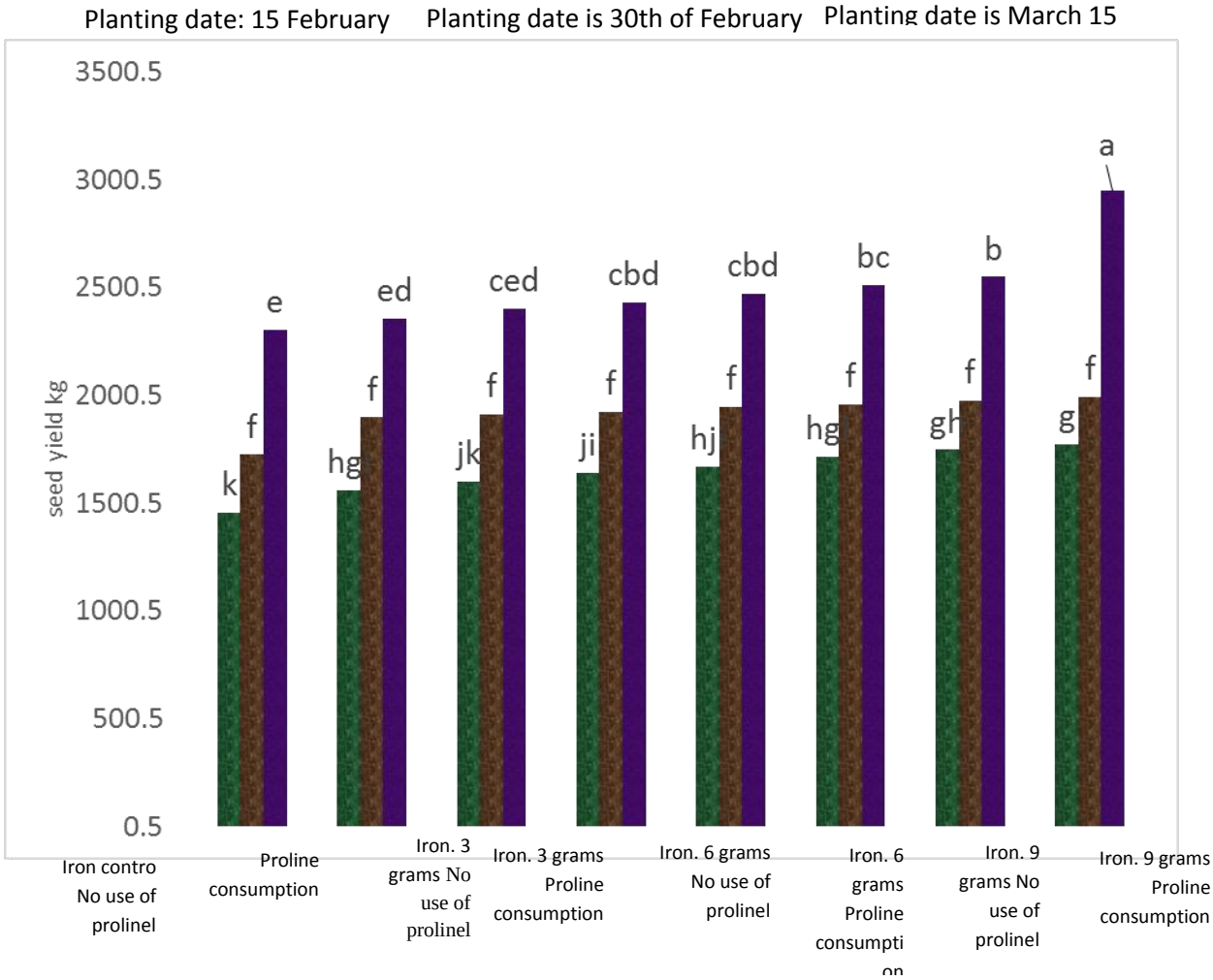

Figure 3.Comparison of the average interaction effect of planting date, iron nanoparticles and proline on seed yield

Rousta *et al*. 2015 stated that one of the most important applications of nanotechnology is the use of nano fertilizers for plant nutrition. Nano fertilizers are the best alternative to conventional fertilizers with gradual and slow release of nutrients. By using nanofertilyzes, nutrients are released slowly and at a suitable speed throughout the growing season of the plant, and due to the reduction of leaching, the plants will be able to absorb more elements, which leads to increased yield and performance.

Hassanzadeh *et al*., 2013, conducted an experiment in Minab on planting date and the results showed that the germination and vegetative growth of seedlings were optimal in the cultivations from the first of Mehr to the fifteenth of November, but with the cooling of the air, the vegetative growth rate decreased. It was reduced and the plants entered the reproductive phase at a shorter height, which led to a decrease in yield.

Rasouli *et al*., 2013, in a report stated that the benefits of using iron chelate nano fertilizers include increasing plant metabolism and more and more effective absorption of main elements and fertilizers, as well as targeted delivery of micronutrient elements to specific tissues of plants, which leads to increased yield and yield components. Abu Goj 2009 stated that proline is an amino acid that is involved in the formation of proteins and It is also

an osmolyte, which is one of the cellular mechanisms to improve plant performance during stress by regulating plant osmosis.

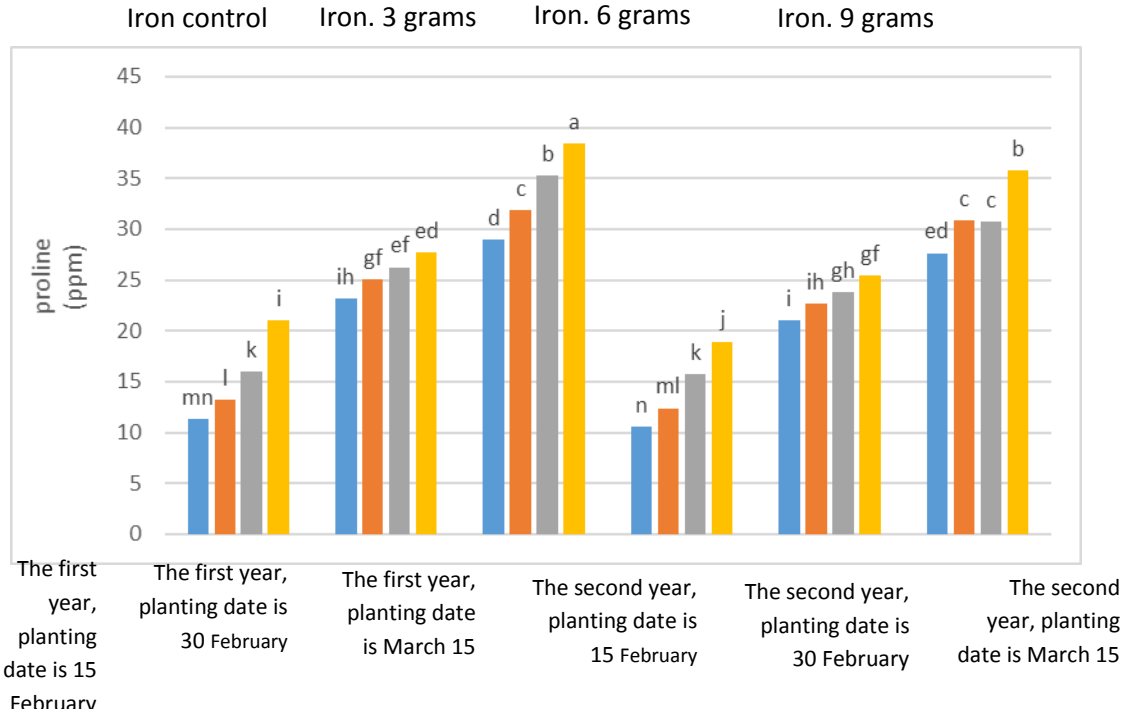

Figure 4. Comparing the average interaction effect of year, planting date, iron nanoparticles on proline

Mamdi *et al*. 2014 stated that the increase in proline accumulation under stress conditions depends on the plant species and the intensity of the stress, that environmental stresses, especially drought stress, change the nature and usually the structure of the protein. Tarahomi *et al*. 2011 stated that according to a general rule, the concentration of plant hormones also changes as the water potential decreases. In the conditions of mild or severe drought stress, the concentration of proline amino acid is increased compared to other amino acids, and this hormone does not act as a nitrogen storage tank or a soluble substance that reduces the osmotic potential of the cytoplasm, but increases the plant's tolerance to stress.

In a 2012 experiment on potatoes, Honardoost *et al* found that the use of iron nanoparticles in the form of foliar spraying and soil application resulted in the highest number of nodular stolen compared to the absence of iron nanoparticles.

In their 2011 research, Shesh Bahreh *et al*. showed that amino acids and low-use elements on peas increased protein, and as a result, significantly increased yield and performance.

Qobadi *et al*. (2010) stated that amino acid and iron nanoparticles both increase the leaf surface and increase photosynthesis, so when iron is transferred to the plant as a foliar spray, the amount of chlorophyll production (increasing photosynthesis) in the plant increases.

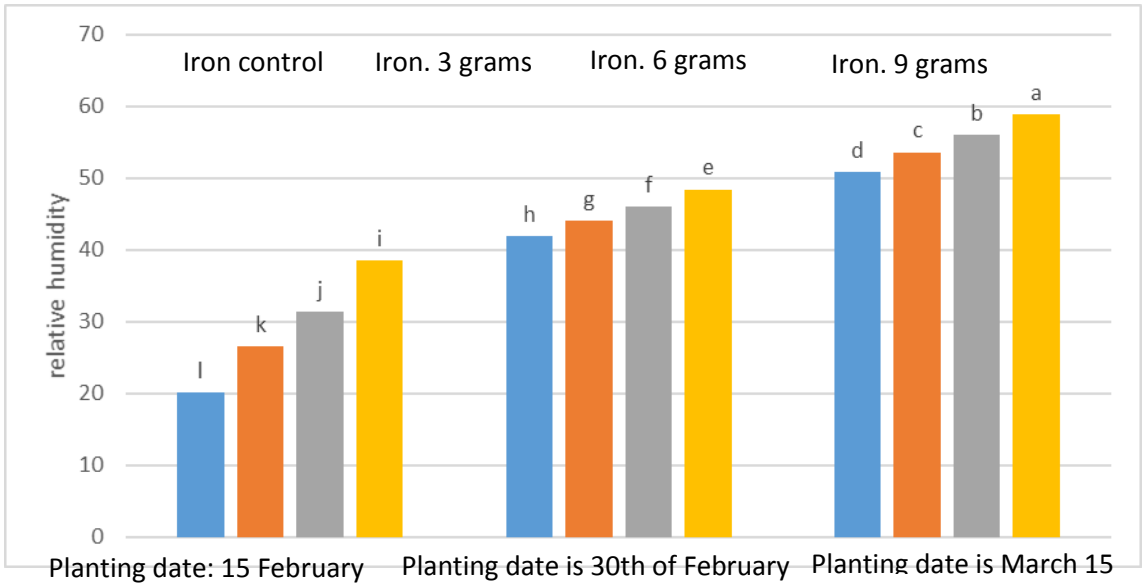

Figure 5. Comparison of the average interaction effect of planting date, iron nanoparticles on relative humidity

Karimizadeh *et al*. (2011) stated that low consumption elements such as iron reduce the effects of drought stress. Iron is part of the catalytic group of many oxidation and reduction enzymes and is required for the synthesis of chlorophyll. Iron deficiency reduces gas exchange, stomatal conductance, water consumption efficiency and increases transpiration in plants. Drought stress, while reducing the water content in plant tissues, causes limited growth and some physiological and metabolic changes in them.

Hossein *et al*. (2017) stated that plants that accumulate soluble sugars in response to stress have better osmotic regulation. Iron and zinc nanoparticles are effective and useful elements in plant growth in different dimensions. These elements are involved in chromosome synthesis, carbohydrate metabolism, chlorophyll production, photosynthesis, conversion of sugar into starch, protein metabolism, and maintenance of biological membranes. The results of Saxna *et al*. (2016) have shown that the use of nanomaterials can help in the rapid recovery of plants, improving plant tolerance and resistance to biotic and abiotic stresses, using efficient nutrients and increasing plant growth.

Low-consumption nutrients are very necessary and essential elements for the growth and development of plants. Iron is an immobile element and its deficiency is seen in young leaves (Waziri Kateshuri *et al*., 2012). By participating in the metabolism of carbohydrates such as nitrogen, iron helps to increase the soluble sugars in the plant under stress conditions (Kiani Chalmardi and Abdulzadeh, 2013).

Yadghari *et al*. 2014 have stated in a research that the reason for the effect of nanoparticles is the small diameter of nanoparticles, the speed of absorption, transfer and accumulation of nanoparticles is much higher than that of normal particles. In this way, the high absorption efficiency compared to ordinary particles justifies the greater effectiveness of these particles, since the problem of reducing atmospheric precipitation and the subsequent increase in soil

salt is a serious problem for growing plants, and also Soleimani and (2015) stated that carbohydrates play a dual role in plant cells.

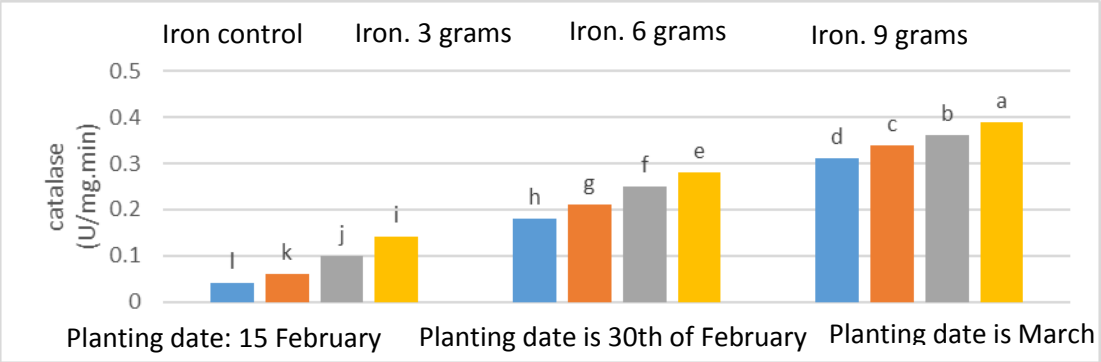

Figure 6.Comparison of mean interaction effect, planting date, iron nanoparticles, on catalase

Mohammadipour *et al*. (2013) stated in an experiment that plants have multiple defense mechanisms against environmental stress. The production of different types of secondary metabolites is a tool to deal with stress, tolerate stress and adapt to the stressful conditions of the environment, and therefore plant survival against stress, and the results showed that iron nanoparticles are quickly absorbed by the plant due to their small size and high solubility. and remove the nutrient deficiencies of plants; Therefore, with the use of these substances, optimal conditions are created for plant growth, and catalase is one of the most important antioxidant mechanisms, which increased its production due to drought stress conditions.

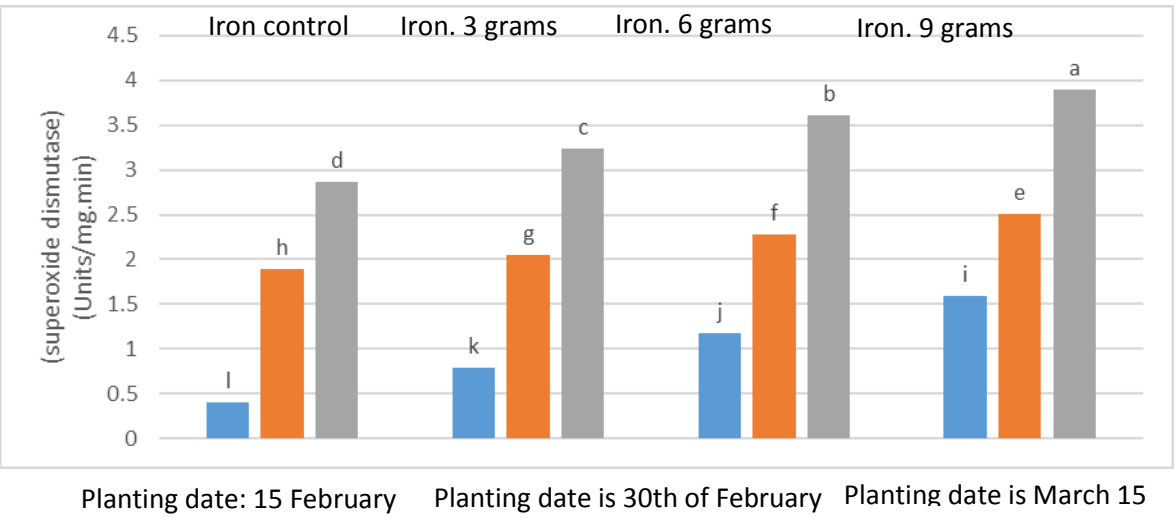

Figure 7. Comparison of the average interaction effect of planting date, iron nanoparticles on superoxide dismutase

Superoxide dismutase (SOD) is the cell's first line of defense against ROS. Usually, superoxide radical is the first free radical that is produced during stress, and SOD quickly converts superoxide radical into hydrogen peroxide and molecular oxygen, and by removing superoxide, it plays a more vital role in the antioxidant system than catalase and peroxidase enzymes. Therefore, it increases the resistance of plants to environmental stresses, and as a result, proline accumulates in the plant, which acts as an osmotic regulator and protects cytoplasmic enzymes and membrane structure, and the consumption of micronutrients increases photosynthetic activity.

Baibardi *et al*. 2012 stated that the efficiency of photosynthesis and the structure and function of the photosynthetic apparatus are strongly dependent on iron. If iron is not available to the plant in a sufficient and absorbable amount, the production of chlorophyll in the leaf will decrease. Iron is involved in the structure of cytochrome as an electron carrier in photosynthetic systems for respiration, oxidation and regeneration processes, and chlorophyll production.

Amjad *et al*., 2011, in a report on wheat, found that the consumption of micronutrients and proline on superoxide dismutase, which is an antioxidant enzyme. It is found in high concentrations in chloroplast, cytosol, vacuole as well as apoplastic spaces of leaf cells. It increased significantly with the increase of environmental tensions.

CONCLUSION

Regarding the interaction effect of iron nanoparticles and proline, the highest plant height was 95.18 cm and related to iron consumption of 0.9 grams and proline consumption, and the lowest plant height was 70.8 cm and related to no proline consumption and no iron nanoparticles consumption. . Regarding the mutual effect of planting date and proline, the highest plant height with 112.24 cm was related to the use of proline and the planting date of March 4th, and the lowest plant height with 55.59 cm was related to the planting date of February 4th without using proline. In all planting dates, the use of proline increased the height of the plant, so that there was a statistically significant difference between the use and non-use of proline in the planting date of March 4th.

The highest weight of a thousand seeds was obtained on the planting date of March 4th and the consumption of 0.9 grams of iron nanoparticles in the amount of 6.05 grams, and also the lowest weight of a thousand seeds was obtained in the planting date of the fifteenth of Bahman month and the absence of the use of iron nanoparticles in the amount of 1.73 grams. Obtained. In all planting dates, the use of proline and the use of 0.9 grams of iron nanoparticles increased the weight of 1000 seeds. The highest grain yield with the amount of 2948.17 kg/ha was obtained in the treatment of 0.9 g of iron nanoparticles and the use of proline, and the lowest amount of grain yield with the amount of 1456.17 kg/ha was obtained in the treatment of no use of proline and no use of iron nanoparticles. In all planting dates, consumption of proline and consumption of 0.9 grams of iron nanoparticles increased grain yield.

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