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Investigating the effect of gibberellic acid and kinetin hormones on proline, protein and carbohydrates of leaf soluble in maize hybrids under drought stress

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Abstract. Hormones play a critical role in regulating the plant growth and responding to drought stress. In order to study the relationship between water scarcity and gibberellic acid and kinetin hormones in three experimental maize hybrids for two years, we carried out Factorial Split Plot Design and the experiment was based the randomized complete blocks design which was carried out with 3 replications. Results showed that the drought stress is an effective factor on hormone balance changes of maize. In comparison to the control (group), kinetin and gibberellin showed the highest mean amount of proline in the leaf. Also, compared to the control group, the amounts of carbohydrates increased under the hormone spraying. However, in comparison to the usage of the grain, the grain yield trait as the major economic trait showeda positive effect وso it is recommended to apply these hormones to the maize.

Keywords: Prolin,Drought stress, Gibberellic acid, Kinetin

1. INTRODUCTION

All over the world, drought is considered as one of the important factors in reducing the yield and cultivation of crops and along with other biotic and abiotic stresses reduces the yield. Some of the plants responses to environmental stresses are accompanied by morphogenetic changes, and this requires that the tress be able to create changes in the hormonal balance of the plant (Zare et.al, 2006). Drought is the main factor which limits the crop productivity worldwide. Plants have different mechanism for coping with water scarcity (Ahmad et.al, 2014). Drought reduces the maize yield more than any other abiotic factor (Boyle & Edmeades,1993). Severe drought stress reduces the expression of genes that are important in protein synthesis and plant growth. The proteins that are made in the plant will be disintegrated into amino acids during the drought stress, these amino acid leads to an increase in the concentration of substances in the plant, so the plant resistance against the stress increases (Kafi and Damghani, 2007). In general, plant hormones such as gibberellin lead to a change, release or probably production of regulator protein and as a result, the active form of this protein is only found inAleuronecells which have received the hormonal message (Taiz and Zeiger, 2005).

Cytokinin (CK) prevents the leaf from dying, so its presence prevents the plant from dying and its leaves from falling, the gibberellin (GA) is also reduced by water stress. So drought stress affects the hormonal balance, which, in turn control the plant growth forms. All plant hormones are affected by osmotic stresses (Mighati and Ebrahim Zadeh, 2003). However, the information in this field suggests that abscisic acid and cytokinins are involved in the effect of reactions affected by the stress and control the water balance (Taiz and Zeiger, 2005). Investigation on how the drought stress affect hormonal balance of the plant has revealed that abscisic acid increases in the leaves and greatly reduces cytokinin levels resulting in the closure of stomata (Kohler, 2008). In combination with cytokinin, Auxin stimulates cell division, although auxin and cytokinin responses to drought stress are still not well understood. Some researchers have suggested that auxin and gibberellin levels decrease in plants under drought

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stress (Yang et.al, 2004). Also, it has been reported that drought stress reduces cytokinin levels in phloem sap and leaves (Kohler, 2008). Nevertheless, few researches have been conducted on the use of gibberellin and kinetin hormones in agriculture especially maize, and there are different theories about the amounts of these hormones that have to be used and their efficiency in arid and semi-arid climates (Alfonso et.al, 2008, Chunrong et.al, 2008). So the plant growth regulators can be considered as possible methods for improving the effects of abiotic environmental stresses. Hence, studying hormonal systems can be helpful in modifying the climatic effects (Kafi and Damghani, 2007).

2. MATERIALS AND METHODS

This experiment was carried out in agricultural seasons of 2012 and 2013 years in the research farm of Agriculture and Natural Resources Research Center of Ardabil (Moghan). The experiment was carried out in the form of Factorial Split-Plot Design and based on the experimental design of randomized complete blocks with 3 replications. Main plot consisted of three irrigation levels: 1. Full irrigation based on water needs of the plant and custom of the area. 2. Withholding irrigation at vegetativestage (withholding irrigation after germination till the emergence of tassel and then continuing irrigation from the emergence of tassel till the end of reproductive stage). 3. Withholding irrigation during grain filling stage (irrigation from planting till the end of pollination and grain formation and withholding irrigation till the physiological maturity of the grain). The sub plot consisted of a combination of two factors: maize hybrids (S.C704, S.C647and S.C700) and applying hormonal treatments (not using the hormone, spraying 50 ppm kinetin at eight leaf- stage(Zare et.al, 1385, Hamdia et.al, 2014), spraying 50 ppm gibberellin at eight- leaf stage, spraying 25 ppm gibberellin and 25 ppm kinetin at eight-leaf stage).

- Grain yield

The maize of three middle lines were harvested after the elimination of marginal effect and they were weighed with sensitive scale, then the grain yield per hectare (a ton per hectare) was identified by determining the percentage of corncob and calculating the humidity which was 14%.

- Method of measuring the leaf proline content

In order to investigate the changes in the leaf proline content, some leaves were randomly selected during the stages of applying germination stresses and grain filling, they were immediately placed in a container full of ice and transported to the laboratory, the leaves were stored in the freezer until the measurement time. To measure the amount of proline, we used Bates et.al (1973) method.

- Measuring the soluble carbohydrates

At first, 0.5 g of the frozen green leaf tissue was completely mashed in a mortar which contained 5 ml of 95% ethanol, then, it was transferred to test tubes with lids and was placed in an intense vortex for 30 seconds. After that, the supernatant solution was removed and transferred to a tube with the volume of 20 ml. Then, 5 ml of 70% ethanol was added to the solid residue two times and it was thoroughly washed. All above steps were performed in an ice bath and with low light. Then, the supernatant solution was transferred to a test tube and the amount of soluble carbohydrates was measured by Hendricks et.al (2001) method.

- Leaf protein

With the help of liquid nitrogen, we mashed 0.5 g of leaf in each replicate in a mortar and added 1 ml of 50 mM (pH= 7) potassium phosphate buffer to it, the potassium phosphate buffer contained 0.5 M (molar) of EDTAand 2% of PVP. The obtained solution was centrifuged for 20 minutes at the temperature of 4° C with the speed of 14000 (pH= 7) and Ependorf 5417 R model of refrigerated centrifuge was used for this step. Then the supernatant solution was collected for the measurement of soluble protein and enzymes concentration.

We used the standard protein of Bovine Serum Albumin(BSA) to measure the soluble protein concentration in the leaf tissues.

3. RESULTS AND DISCUSSION

- The grain yield

Variance of the grain yield during two years of experiment had no significant difference with Bartlett's test(table 1). Combined analysis of variance of this trait's data had a significant difference for the effects of year (Y), hormone (H), year in hormone (Y × H) at 1% level of probability, the effect of drought stress (I), year in irrigation (Y × I), year in irrigation in hormone (Y × I× H), variety (V) and irrigation in variety (I × V) at 5% level of probability (table 1).

Table 1. results of analysis of variance for studied traits

		Mean Square	(M.S)		
S.O.V	df	Carbohydrates	Protein	Prolin	Yield
Year	1	$4.2^{n.s}$	$0.011^{n.s}$	$4.62^{n.s}$	42.81^{**}
Yield(Replication)	4	158.21	0.016	5.79	1.1
Irrigation	2	681.6^{**}	0.207^{**}	192.04^{**}	520.39^{*}
Y× I	2	13.08^{*}	$0.002^{n.s}$	$0.382^{n.s}$	8.97^{*}
Error(a)	8	2.47	0.009	4.22	1.2
Hormones	4	52.22^{**}	0.01^{*}	4.12^{*}	210.26^{**}
Y × H	3	$1.77^{n.s}$	$0.001^{n.s}$	$0.396^{n.s}$	3.27^{**}
I × H	6	31.07^{**}	0.01^{*}	2.49^{*}	$1.46^{n.s}$
Y × [× H	6	$0.488^{n.s}$	$0.001^{n.s}$	$0.306^{n.s}$	1.41^{*}
Variety	2	137.69^{**}	0.058^{*}	15.03^{*}	21.67^{*}
Y × V	2	$1.09^{n.s}$	$0.001^{n.s}$	$0.487^{n.s}$	$0.36^{n.s}$
I × V	4	51.69^{**}	0.006^{*}	$1.94^{n.s}$	1.75^{*}
H × V	6	59.98^{**}	0.014^{**}	5.67^{*}	$0.8^{n.s}$
I × H × V	12	14.46^{**}	0.007^{**}	2.32^{*}	$0.82^{n.s}$
Y × I × V	4	$0.21^{n.s}$	$0.003^{n.s}$	$0.014^{n.s}$	$0.06^{n.s}$
Y × H × V	6	$0.63^{n.s}$	$0.002^{n.s}$	$0.522^{n.s}$	$0.97^{n.s}$
Y × I × H × V	12	$0.75^{n.s}$	$0.001^{n.s}$	$0.606^{n.s}$	$0.46^{n.s}$
Error(b)	132	7.45	0.0023	2.094	0.68
CV(/.) ضریب تغییرات	-	12.18	4.75	11.82	12.32
Bartlett test	-	3.19	0.881	2.82	0.63

ns, * and ** mean significant and non-significant at the probability level of 5%, 1%, respectively.

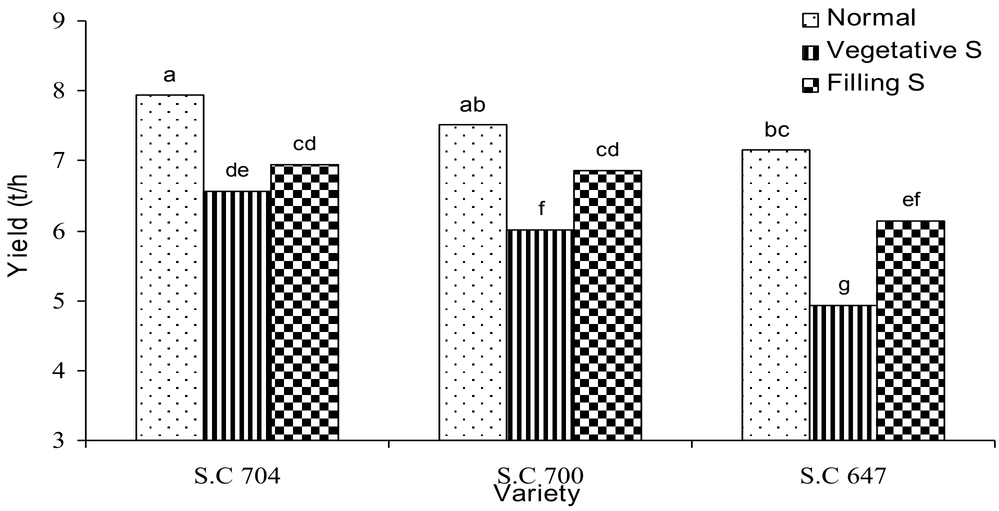

Figure1. The mutual effect of irrigation in variety on the grain yield

Comparison of the averages of drought stress mutual effects in varieties (V × I) showed that 704 and 700 hybrids had the highest grain yields under normal irrigation conditions, and the 647 hybrid had the lowest grain yield in applying stress at the vegetative stage (figure 1). Hybrid 704 had a high yield due to the high maize length, high number of kernels per row, high percentage of corncob, grain depth and high hectoliter weight. Significance of the mutual effect of irrigation in variety (I × V) shows that in different humidity conditions, hybrids has shown different responses in terms of grain yield. So it is necessary to identify top hybrids for both studied environmental conditions. A plant yield can be increased by increasing the biological yield or increasing the harvest index. In normal and free from stress conditions, the grain yield can be increased without any changes in the harvest index or it can be increased by allocating more of produced biomass to the grain (Sarmad Nia & Koochaki, 1989). The increase of maize yield in condition that the harvest index is fixed can be a result of dry matter accumulation increase during the growing season (especially during the grain filling period), this feature itself is due to the higher durability of photosynthesizer leaf area during the grain filling stage.

Khakpoor (1996), Tolenear et.al (1994) has suggested that drought stress is the main reason of harvest index reduction which limits the grain yield, leaves area, length and weight of the maize and the number of them. Since the effect of water scarcity stress on the yield is multilateral, based on the growth stages of the plant, the time of drought stress can have a large influence on the type and amount of damages. This is also proved by the results of this experiment through affecting the morphological characters and yield components. Obviously, water scarcity in all biological stages affects the plant growth. The plant response to water scarcity depends on the intensity of scarcity, duration of the stress and the plant growth stage. Germination stress resulted in a decrease in yield-related traits such as the maize length, number of kernels per row, the percentage of corncob, the hectoliter weight, stem diameter and the leaf area, and all of these reduced the grain yield. These results match the observations of Wajid et.al (2004) which suggest that grain yield decreases by the negative effect of drought stress in different stages of plant growth on the components of the yield. Comparison of the average of mutual effects of irrigation in hormone in variety (I × H × V) showed that, during two years of experiments, there is a significant difference in gibberellin hormone levels and the combination of gibberellin with kinetin. The highest grain yield with spraying gibberellin hormone was for 704 hybrid in the first year, and there was no significance difference between spraying kinetin hormone in 700, 704, 647 hybrids and not spraying the hormone (control group) in 700 hybrid. The lowest grain yield is for 647 hybrid, which was observed respectively with no hormonal treatment (control group) and the combination of gibberellin with kinetin, in the condition that no hormonal treatment was used (control group), the 647 and 700 hybrids were placed in the same group (figure 2).

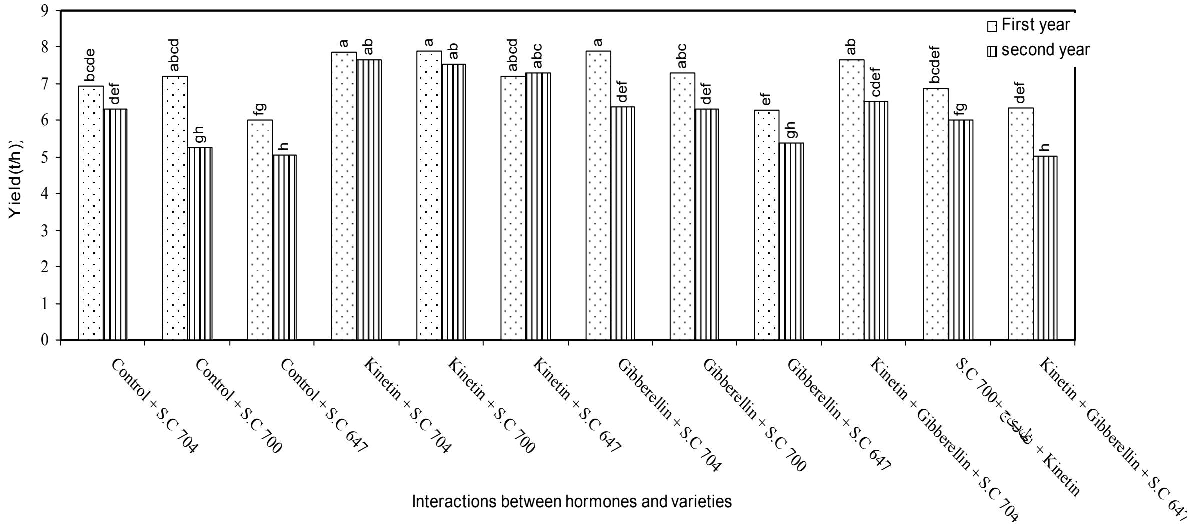

Figure2. The mutual effect of year in hormone in variety on the grain yield

In comparison to the control group, the yield through hormonal treatments showed a significant difference in kinetin (Kin) spraying. Kinetin can play an important role in substance absorption from the source through having a positive effect on cell division and increasing the reservoir potential. As Yang et.al (2004), Taiz and Zeiger (2005) have reported, cytokinins can disturb the balance between the source and reservoir. Also, gibberellic acid can play an effective role in the absorption of substances for the metabolism process due to increasing the plant growth speed, and ultimately, increases the grain yield. These results are consistent with the Yang et.al (2003) studywhich suggests that the external cytokinin (CK) applicationduring the cell division has a positive effect on the formation of thousand kernels weight and increasing the grain yield but the external application of gibberellic acid in rice played no role in the cell division and the increase of thousand kernels weight.

Iqbal and Ashraf (2010) reported that GA_3 treatment in wheat led to an increase in the grain yield because under salinity stress, the GA_3 results in the balance in absorption and sharing the ions and hormones homeostasis. The important point is that in comparison to the condition that no treatment is applied, in each of treatment groups, the kinetin, gibberellic acid regulators and the combination of these two hormones show beneficial effects on the grain yield trait since they reduce the stress levels in plants and maintain the moisture balance of them. Kinetin plays an important role in the surface expansion and number of leaf cells, it cooperate with auxin to develop the root for more effective moisture supplying, plant growth and more importantly photosynthetic support and transferring photosynthetic materials to the grains (McWilliams, 2002).

3.1. The Amount Of Leaf Protein

The comparison of the average of the mutual effects of irrigation in hormone in variety (I × H × V) showed that the highest amount of protein was for 647 hybrid by applying stress at vegetative stage and in the absence of hormone treatment (control group). There was no significant difference between this hybrid in the condition without hormonal treatment (control group) under grain filling stress using gibberellic acid and kinetin combination in the germination stress stage, and also under normal irrigation condition in treatment without hormones (control group) and 704 hybrid with the combination of gibberellic acid and kinetin combination, and with using gibberellic acid in normal irrigation conditions. The lowest amount of protein was for 704 hybrid by applying stress in the grain filling stage with using kinetin hormone which had no significant difference with 647 hybrid in the grain filling stage with hormonal treatment of gibberellic acid, and with 704 hybrid in the same stress without hormonal treatment (control group) and also with hormonal treatment by gibberellic acid + kinetin, and with 700 hybrid by hormonal treatments of gibberellic acid + kinetin without using hormone (control group) and gibberellic acid (Figure3).

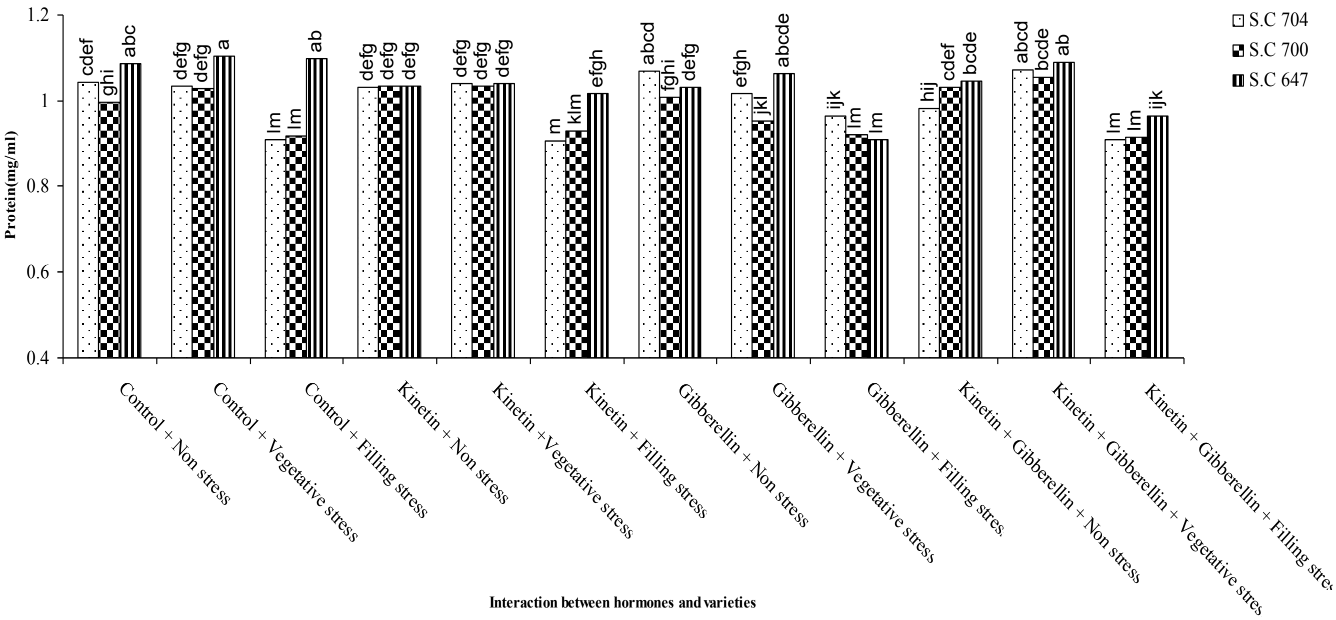

Figure2. The mutual effect of year in hormone in variety on the protein amount

In general, moisture stress changes the balance between protein and carbohydrates (glucose). Water stress increases the protein percentage in most grains. This phenomenon is due to the decrease in water potential of the cell sap in order to cope with the stress (Noor Mohammadi et.al, 1998). On the other hand, in water scarcity conditions, the formation of simpler molecules such as glucose, fructose, sucrose and amino acids like proline increase. Thus the conversion of the photosynthetic materials mainly moves toward the protein production. The obtained results indicate that the percentage of leaf protein as a qualitative trait has shown different responses to the application of spraying treatments, and in germination stress and the grain filling stages, the results of the 647 hybrid is a good proof for this. These results, somehow, match the findings of Gheisi (1988) which suggest that water scarcity conditions changes the balance between protein and carbohydrates (glucose).

It seems that hormonal spraying increases the stability of protein synthesis by increasing the internal concentration of gibberellic acid and kinetin regulators, and increase the soluble protein by affecting the related enzymes levels. Soluble protein levels and protein production are closely related to the proline activity. Stress treatments including water scarcity stress can have a great impact on it. Zand (2009) has reported that spraying the maize with auxin hormone can be effective in osmoregulation of its leaves.

3.2. The Amount Of The Leaf Proline

Comparison of the average of mutual effects (I × H × V) showed that the highest amount of proline among the irrigation levels is for applying stress at grain filling stage and the lowest was obtained in the normal irrigation condition which did not show any significant difference by applying stress in the vegetative stage. But in comparison to the control group, stress in the grain filling stage increased the amount of proline in all hormonal levels, and the increase in proline amount of the leaf was achieved respectively by spraying gibberellin hormones and combining gibberellin with kinetin. In normal irrigation condition (without stress), hormonal spraying, separately and combined, did not increase the amount of leaf proline in hybrids (figure 4). So we can say that in normal irrigation condition, spraying the intended hormones has no effect in terms of increasing the amount of proline. But in germination stress condition and with spraying the kinetin hormone, 704 hybrid had the highest amount of proline and 700 and 647 hybrids had the lowest amount of it. For the amount of leaf proline, the 700 hybrid showed the most compatibility with spraying the hormonal combination of gibberellin and kinetin (figure 3). In addition to the features such as the maize length, number of kernels per row and high weight of hectoliter, one of the reasons for the high yield of 704 hybrid is that the amount of its leaf proline under stress is higher than other hybrids.

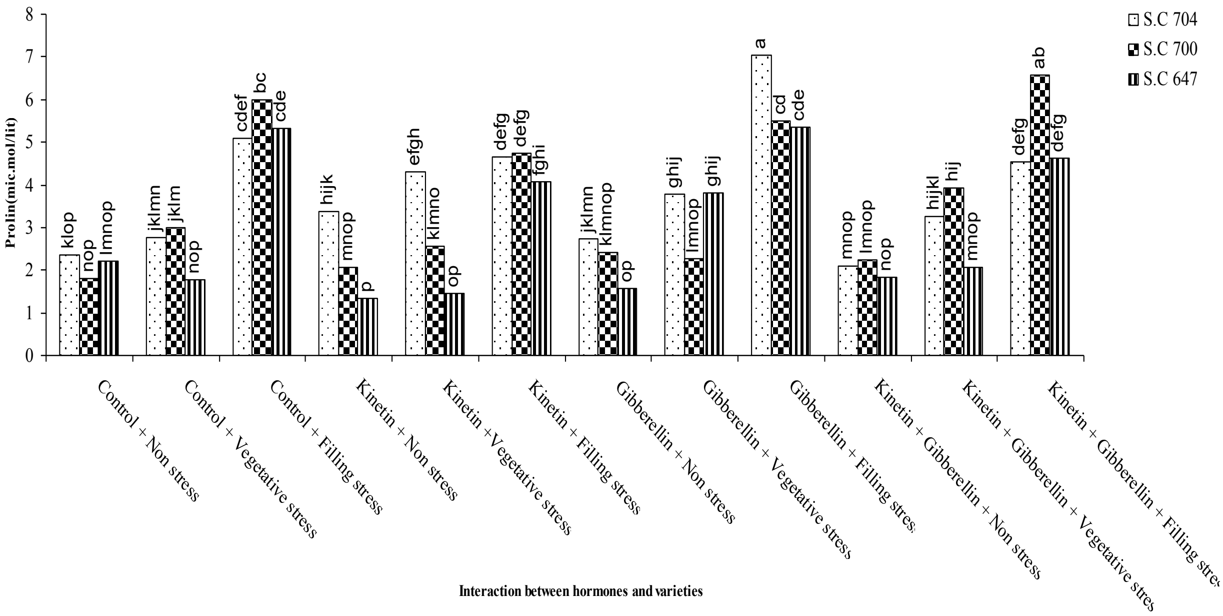

Figure4. The mutual effect of irrigation in hormone in variety on the proline amount

Among the compatible soluble compounds, proline is probably the most common and widespread osmolyte. When the plants are in drought conditions, salinity, low temperature and other environmental factors which are effective on the decrease of water potential and the cell sap, the amount of free proline in them increases. However, they are also other amino acids that are accumulated under drought and salinity stress, but their changes levels in not similar to proline which reaches very high levels within a short time after the stress.In conclusion, it can be stated that gibberellin hormone increases the amount of proline in 704 hybrid which is in line with the results of the effect of then hormone in variety and it can be concluded that 704 variety is more tolerant to drought than other hybrids (figure 4).

Since gibberellic acid increases the duration of plant's vegetative growth and also due to the grain filling stress, moisture deficiency takes place in the soil and ultimately in the bush, the intended hybrids increased the root power for absorbing water from the soil by increasing the amount of proline which is an osmotic regulator. Results show that these osmotic regulators are a common response to drought stress conditions. The increase in proline concentration of hybrids under droughts stress can be a kind of compatibility to overcome the stress condition, and 704, 700 and 647 hybrids respectively showed such compatibility in grain filling stress. Fazeli et.al (2007) also found similar results about the increase of proline amount and other osmolytes in plants.

3.3. The Amount Of Soluble Leaf Carbohydrates

Comparison of the average of mutual effects of irrigation in hormone in variety (I × H× V) showed that among irrigation levels, the highest amount of leaf carbohydrates is for applying stress in the grain filling stage and the lowest is for applying stress in the germination stress stage. In terms of hormonal levels, the highest amount of carbohydrates in the leaf is for 647 hybrid by applying stress in the grain filling stage using the hormonal combination of gibberellic acid and kinetin, and the lowest amount was respectively for 704 and 647 hybrids by applying stress in the vegetative stage using hormonal spraying of gibberellic acid and kinetin which had no significant difference with 704 hybrid by applying stress in the grain filling stage and not using hormonal spraying (control group) and gibberellic acid and combining gibberellic acid with kinetin, and it had also no significant difference with 647 hybrid in normal irrigation condition and using hormonal spraying of gibberellic acid (figure 5). According to the results of this experiment, it appearsthat, in terms of genetic characteristics, 647 hybrid is more sensitive to moisture deficiency than other hybrids. So, by applying moisture stress, it can be expected that soluble carbohydrates accumulate more in hybrids in order to prevent transpiration and to increase water absorption by increasing the osmotic potential and thus reduce the drought stress damages.

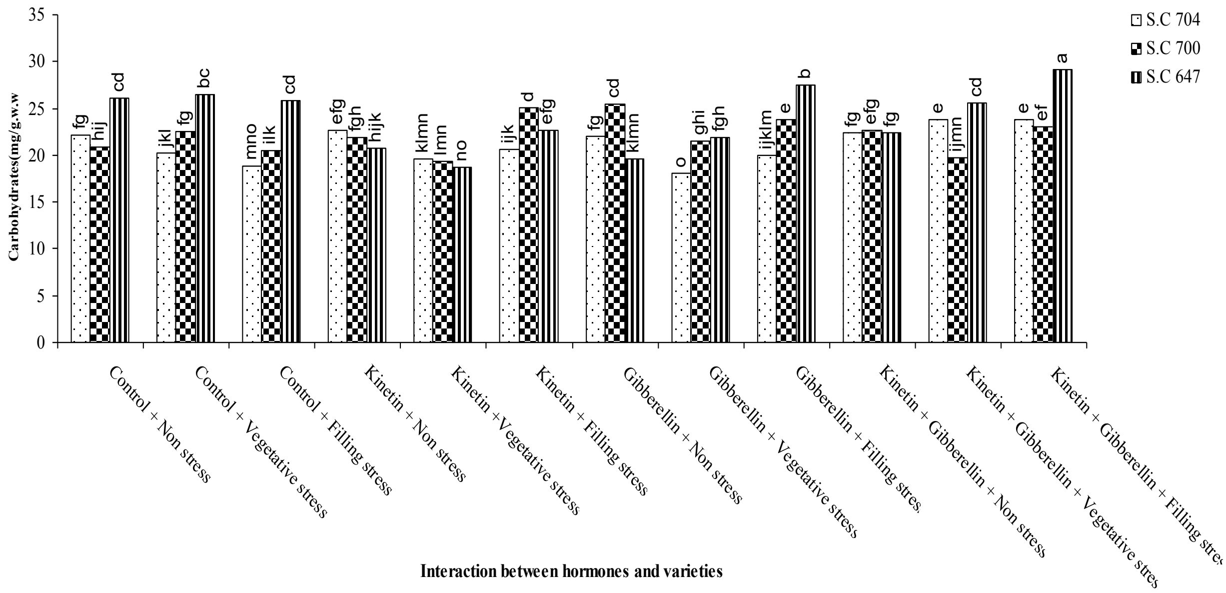

Figure5. The mutual effect of irrigation in hormone in variety on the amount of carbohydrates

Soluble sugars are a group of compatible osmolytes which accumulate in drought condition and act as an agent or osmotic protection. The increase of sugar is due to stress with osmotic regulationand maintaining the turgor, and it is related to stabilizing the membranes and proteins. Soluble sugars can act as osmotic regulators, stabilizer of cell membranes and maintainer of cells turgor. In plants such as 647 hybrid of maize, the soluble sugars accumulate in response to the drought stress thus the osmotic regulation is done better. In a study on two varieties of maize, Mohammad Khani and Heydari (2008) have reported an increase in soluble sugars concentration. According to Davies and Zhang (1991), by increasing or decreasing the amount of soil moisture, the internal concentration of hormones as signal agents increases so this can play an important role in growth processes and the accumulation of carbohydrates in the grains. The reason of increase in the leaf carbohydrates in the grain filling stress stage can be the role of hormones in increasing their internal concentration, so that the plant can perform the mechanisms of macromolecules breakdown to increase the osmotic potential of the leaf which leads to an increase the moisture absorption.

So it can be said that using gibberellic acid hormones leads to an increase in the vegetative growth of the bush and an increase in the plant height thus there is a high potential difference for moisture absorption. On the other hand, using kinetin results in development, cell division and better development of the root. In general, due to their mutual effects, the combination of gibberellic acid and kinetin leads to an increase in the leaf hydrocarbons. Yang et.al (2004) have confirmed this in their study on the effects of hormones on cytokinine concentration slope changes and on increasing the carbohydrates of maize

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