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RESEARCH ARTICLE

EFFECT OF GIBBERELLIC ACID AND PROLINE ON VEGETATIVE CHARACTERISTICS OF (ZEA MAYS L.) CULTIVAR (FAJIR-1)

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ABSTRACT

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Grain crops, Growth, Phytohormons, Osmolytes. A field experiment was carried out in clay soil to investigate the effects of gibberellic acid and proline, and their interaction on the vegetative characteristics of *Zea mays* L. cultivar Fajir-1. The experiments were carried out based on the Randomised Complete Block Design. Gibberellic acid and proline were sprayed twice on the plant leaves at the concentration of 100 and 200 mg/l. The first application was during the stage of 4-6 leaves, and the other application was at the beginning of flowering stage. The results show that the addition of gibberellic acid at the concentrations of 100 and 200 mg/l, and the proline at the concentration of 200 mg/l significantly affected the studied characteristics including: plant height, number of leaves per plant, leaf area and shoot and root fresh weights. The results also show that the interaction between gibberellic acid and proline did not show any significant effects on the studied characteristics except for the leaf area.

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INTRODUCTION

Maize is one of the major human food grains that has been utilised since ancient times by people around the world. It is considered one of the most important main cereal crops after rice and wheat (Abdelmula et al., 2007). It has many industrial applications including as corn flakes, starch and oil dextrose, acetone, gluten, lactic acid and grain cakes (Nabizadeh et al., 2012). Recently, the demand for maize has increased dramatically as it has gained a lot of attention due to its unique economic importance as human food, food additive (sweetener), beverage base, oil, starch, vegetable, animal feed, petroleum fuels substitute, fibre and lipids (Ibrahim et al., 2007). Therefore, it is essential to find more advanced methods and means in order to increase corn production. Recently, great attention has been given to the use of environmentally friendly organic stimulators such as phytohormons to enhance plant growth and increase their production. Among these

Biology program, School of Distance Education, Universiti Sains Malaysia, 11800, Minden high Pulau Pinang, Malaysia. Departments of Biology, Education School for Pure Science, University of Diyala, Diyala, Iraq. phytohormons, gibberellic acid (GA₃) has been broadly applied by many researchers due to its role in increasing cell elongation, cell division or both (de Souza & Macadam, 2001; Roy et al., 2010), arousing the influence of long day lengths by increasing runner production, improving vegetative development in short day plants (Qureshi et al., 2013) and altering the source-sink metabolism via their impact on sink formation and photosynthesis (Iqbal et al., 2011). On the other hand, the application of environmentally friendly solutes such as proline has great effect on plant growth and development under salt and other environmental stress. It can be used to overcome the high salinity in soil (Wang et al., 2003; Ashraf et al., 2008). Therefore, the aim of this study is to investigate the effects of the addition of GA and proline individually and as a combination on the growth of Zea mayz L. cultivar (Fajir-1).

MATERIALS AND METHODS

A field experiment was conducted in clay soil from March to July 2014, in the northeast of Baghdad to study the response of *Zea mays* L. cultivar (Fajir-1) to the addition of gibberellic acid and proline regarding to the vegetative characteristics. The experiments were carried out based on Randomised Complete

Block Design (RCBD). The design included nine treatments (Table 1), and each treatment was replicated three times. The treatments consisted of three concentrations of GA_3 (0, 100, and 200 mg/l) and three concentrations of proline (0, 100, and 200 mg/l).

Table 1. Experimental treatments design for Fajir-1

Treatments	Gibberellic acid	Proline
T1	0 mg/l	0 mg/l
T2	0 mg/l	100 mg/l
T3	0 mg/l	200 mg/l
T4	100 mg/l	0 mg/l
T5	100 mg/l	100 mg/l
T6	100 mg/l	200 mg/l
T7	200 mg/l	0 mg/l
T8	200 mg/l	100 mg/l
Т9	200 mg/l	200 mg/l

Twenty seven experimental plots were created; each plot had an area of 6 m² including 4 planting rows of 2 m in length at a distance of 75 cm from one another. Each two plants were 20 cm apart according to the method of Elsahookie (1990). Soil analysis of the experimental field is shown in Table 2.

Table 2. Soil physical and chemical properties of the experimental field

Soil textur Particle size distribution		Soil texture	Ec	PH	CEC		vailable tri ents		anic atter	
ratter	(%)	Istiiouti		(dsm ⁻¹)		(meq/ 100g)	(ppm)	(%)
Sand	Clay	Silt					Ν	Р	K	
22.8	42.0	35.2	Clay	7.6	7.1	20.1	84.0	9.75	45.8	0.8

Nitrogen and potassium fertilisers in the form of urea (46% N) and potassium sulphate (51% K) were applied at a concentration of 240 and 120 kg/hac respectively, during planting and 45 days after planting. Triple super phosphate (P_2O_5) (21% P) was applied during soil preparation at a concentration of 160 kg/hac. Maize grains were hand sown 3 seeds/hole on the 1st of March 2014. Granular diazinon pesticide (10% active ingredient) was used at a concentration of 1.5 kg/hac to protect the plant from maize stalk borer disease (Yousif, 2012). Gibberellic acid (GA₃) and proline at two concentrations (100 and 200 mg/l) were sprayed twice on the leaves. The first application was during the stage of 4-6 leaves, and the other application was at the beginning of flowering stage. Tween 20, at a concentration of 0.05%, was added to the foliar solution to act as a cohesive agent. Plants in the control treatment were sprayed with water and Tween 20 only. Harvesting was done manually during the mature stage of the plant on 10th of July 2014. At the flowering stage, 10 plants were taken randomly from each plot in order to measure the following characteristics.

Plant height (cm)

Plant height was measured from the soil level (base of the plant) up to the base of the flag leaf by using a tailor's tape (Elsahookie, 1990).

Number of leaves per plant

Number of leaves per plant was calculated from the same 10 plants mentioned above.

Leaf area (cm²)

Leaf area was determined by the non destructive length \times width method using the formula:

Leaf area = $0.75(\text{length} \times \text{width})$ (Saxena and singh, 1965).

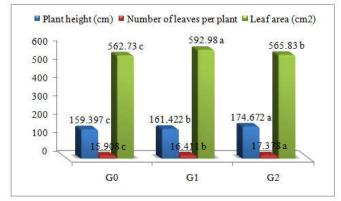
Shoot and root fresh weights (g)

Ten plants were harvested and washed with tap water. Each sample was then separated into shoots and roots and cut into small pieces to measure the fresh weights of the shoot and root using an electronic balance. The data were analysed using variance analysis (ANOVA) by SAS software (version 9.0). The means of treatments were compared using Duncan's test at 0.01 and 0.05 levels of probability (Duncan, 1955).

RESULTS AND DISCUSSION

Plant height (cm), number of leaves per plant and leaf area (cm²)

The results in Figure 1 showed significant differences in plant height, number of leaves per plant and leaf area due to the addition of GA_3 at concentrations of 100 and 200 mg/l. The highest averages of the plant height and number of leaves per plant were observed using 200 mg/l with increment rates of 8.2% and 9.6% in plant height, and 5.9% and 9.2% in number of leaves per plant as compared with 100 mg/l and the control respectively. While the highest average for the leaf area was obtained from the concentration of 100 mg/l with increment rates of 4.8% and 5.4% as compared with concentration of 200 mg/l and control respectively.



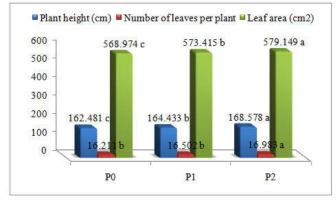
 $G0 = 0 \text{ mg/l GA}_3$; $G1 = 100 \text{ mg/l GA}_3$; $G2 = 200 \text{ mg/l GA}_3$.

Figure 1. Effect of GA₃ on plant height, number of leaves and leaf area

The significant increments which was obtained in plant height, the number of leaves per plant and leaf area due to the addition of gibberellic acid are in agreement with previous findings from Afzal *et al.* (2008), Surendra *et al.* (2010) and Rohamare *et al.*, 2013. A study by Qureshi *et al.* (2013) on *Fragaria*

ananassa also found a significant increment in the plant height, number of leaves and leaf area with an average of 18.37 cm, 4.877, and 63.63 cm² respectively compared with the control group which recorded an average of 10.93 cm, 3.970 and 34.28 cm² respectively, as a result of adding gibberellic acid at a concentration of 50 ppm.

This increment is attributed to the role of GA in increasing cell division and elongation (Khan et al., 2006). This might be occurring through the indirect influence of GA in increasing the elasticity of cell walls, as the hormone works to maintain the level of auxin in the cells by increasing auxin formation, reduces its demolition rate by turning the tryptophan to IAA, and stop the action of IAA peroxidase and oxidase enzymes in destroying the auxin. It has been speculated that auxin plays an important role in reducing the calcium ion bonds with pectic acid, and forming calcium pectate which helps in increasing the cohesion of the walls to each other (Cleland, 1960; Buckhout et al., 1981). With regard to proline application on Zea mays, the results in Figure 2 show significant differences in plant height, number of leaves per plant and leaf area due to the addition of proline at concentrations of 100, 200 mg/l. The highest averages were recorded at 200 mg/l with increment rates of 2.5% and 3.8% in plant height, 2.9% and 4.8% in the number of leaves per plant and 1% and 1.8% in leaf area, as compared to the concentration of 100 mg/l and the control treatment respectively.



P0= 0 mg/l proline; P1= 100 mg/l proline; P2= 200 mg/l proline.

Figure 2. Effect of proline on plant height, number of leaves per plant and leaf area

The findings of this study are in agreement with previous findings from HM *et al.* (2010), Faraj and Jumily (2012) and Al-Hamdany and Mohammad (2014). Mohamad (2007) found significant increments in plant height, number of leaves and leaf area of *Ziziphus* cv. Tuffahi by the addition of proline at concentrations of 75 and 150 mg/l, and that the increment effects were in a dose-dependent manner. The reason behind the increments in plant height and number of leaves per plant in this study attributed to the role of amino acids in changing the osmotic potential of the plant tissue. It has been found that amino acids reduce the osmotic potential which leads to reduced cell water potential and increases cell viability to pull water and nutrients dissolved in it from the centre of growth and thereby increases the vegetative growth of plants (Amini and Ehsanpour, 2005; Claussen, 2005).

Table 3. Effect of double interaction between gibberellic acidand proline on plant height, number of leaves per plant andleaf area

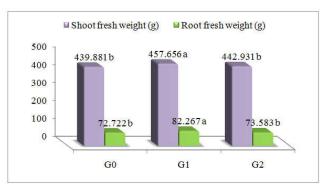
Gibberellic acid (mg/l)	Proline (mg/l)	Plant height (cm)	Number of leaves	Leaf area (cm ²)
	P0	156.917 a	15.583 a	558.327 f
G0	P1	158.450 a	15.917 a	563.572 de
	P2	162.825 a	16.225 a	566.289 cd
	P0	171.775 a	17.042 a	587.298 b
G1	P1	174.883 a	17.225 a	589.468 b
	P2	177.358 a	17.867 a	602.172 a
	P0	158.750 a	16.008 a	561.298 ef
G2	P1	159.967 a	16.367 a	567.205 cd
	P2	165.550 a	16.858 a	568.987 c

Each value is the mean of three replicates. Values with the same letters within a column are not significantly different at 0.01 and 0.05 probability levels. G0 = 0 mg/l GA3; G1= 100 mg/l GA3; G2= 200 mg/l GA3, P0= 0 mg/l proline; P1= 100 mg/l proline; P2= 200 mg/l proline.

 Table 4. Effect of double interaction between gibberellic acid and proline on shoot and root fresh weights

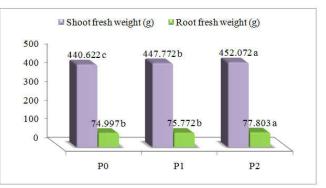
Gibberellic acid (mg/l)	Proline (mg/l)	Shoot fresh weight (g)	Root fresh weight (g)
	P0	431.317 a	71.817 a
G0	P1	440.775 a	72.442 a
	P2	447.550 a	73.908 a
	PO	453.816 a	80.442 a
G1	P1	460.242 a	81.750 a
	P2	458.908 a	84.608 a
	PO	436.733 a	72.733 a
G2	P1	442.300 a	73.125 a
	P2	449.758 a	74.892 a

Each value is the mean of three replicates. Values with the same letters within a column are not significantly different at 0.01 and 0.05 probability levels. $G0 = 0 \text{ mg/l } GA_3$; $G1=100 \text{ mg/l } GA_3$; $G2=200 \text{ mg/l } GA_3$, P0=0 mg/l proline; P1=100 mg/l proline; P2=200 mg/l proline.



G0 = 0 mg/l GA₃; G1= 100 mg/l GA₃; G2= 200 mg/l GA₃.

Figure 3. Effect of GA₃ on shoot and root fresh weights



Values with the same letters are not significantly different at 0.01 and 0.05 probability levels. P0= 0 mg/l proline; P1= 100 mg/l proline; P2= 200 mg/l proline.

Figure 4. Effect of proline on shoot and root fresh weights

While the significant increment that occurred in the leaf area as a result of proline addition, might be attributed to the effect of proline in increasing the plant's ability to photosynthesise by controlling the opening and closing of stomata which helps to balance between the taking in of CO_2 and the loss of water during transpiration, and preventing chlorophyll pigment decomposition, thus increasing leaf area per plant (Raven, 2002). The results also showed that there was no significant difference from the double interaction of these factors in the plant height and number of leaves per plant, while significant differences in leaf area were obtained from the combination of (G 100 mg/l + P 200 mg/l). The lowest average was from the combination of (G 0 mg / l + P 0 mg/l) (Table 3).

Shoot and root fresh weight (g)

Significant differences in shoot and root fresh weights were found due to the addition of GA₃ at concentrations of 100, and 200 mg/l. The highest averages of shoot and root weights were obtained using the concentration of 100 mg/l with increment rates of 3.3% and 4.0% for shoot fresh weight and 11.8% and 13.1% for root fresh weight , compared to 200 mg/l and the control treatment respectively (Figure 3). This result confirmed previous results from Srinivasa (2006), Hussein (2009) and Ghoname *et al.* (2011). Ghodrat and Rousta (2012) confirmed that soaking the seeds of *Zea mays* L. in different concentrations of gibberellic acid before planting led to a significant increment in the fresh weight of the shoots of the developing plants under salinity and natural conditions equally.

The reasons of these increments in the fresh weights of shoots and roots with the use of gibberellic acid, are attributed to the effect of the hormone in stimulating the plant growth, increase the division and elongation of plant cells which lead to increased plant height, number of leaves per plant and leaf area with the positive impact on the fresh weight increment as the final result. The stimulation of gibberellic acid also builds the DNA and RNA nucleic acids, and thus an increment in protein synthesis and biological processes within the plant cells, which lead to the fresh weight increment (Devlin et al., 1998). Furthermore, a significant increment was obtained in shoot and root fresh weights due to the addition of proline at concentrations 100, 200 mg/l. The concentration of 200 mg/l gave the highest averages of these characteristics with increment rates of 1% and 2.6% of fresh weight of shoot, and 2.7% and 3.7% of fresh weight of root compared with the concentration of 100 mg/l and control respectively (Figure 4).

This result was in tandem with results from Khedr *et al.* (2003) and Jain *et al.* (2010). Nounjan *et al.* (2012) found a significant increment in the vegetative weight of *Oryza sativa* L. seedlings after the plants resisted the negative impact of salts for 5 days by the addition of proline at dfferent concentrations. These changes in the fresh weight of plants when treated with proline are due to the role of proline as a source of nitrogen which is essential in building proteins and to generate the necessary power for various vital activities. The double interaction of (GA + proline) did not show any significant effect on fresh weight of shoot and fresh weight of root. The highest average of fresh weight of shoot was obtained from the combinations of (G 100 mg/l + P 200 mg/l), while the lowest average was from

the combination of (G0 mg / l + P 0 mg/l) as shown in Table 4. It can be concluded that the addition of GA and proline at a concentration of 100 mg/l and 200 mg/l respectively plays a major role in plant growth and development. The properties and characteristics of the plant were influenced positively by the addition of these factors. While the addition of these factors as a compound caused noticeable increments in the studied characteristics, but they were not significant.

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