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Influence of Foliar Application with Abscisic Acid (ABA) and Vitamin C on Some Plant Hormones for Peas Plant (*Pisum sativum* L.)

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Abstract

A field experiment was conducted at botanical garden of Department of Biology, College of Education for Pure Science (Ibn Al-Haitham), University of Baghdad, during the growth winter season of 2016-2017 to study the effect of different concentrations (0, 10, 20) $mg.L^{-1}$ of abscisic acid and (0, 50, 100, 150) $mg.L^{-1}$ of vitamin C and their interaction on some plant hormones of pea plant (*Pisum sativum* L.). The results showed that ABA 20 $mg.L^{-1}$ decreased IAA about 27.44%, GA_3 about 19.73% and Kinetin 15.37% while vitamin C with 150 $mg.L^{-1}$ increased IAA 27.43%, GA_3 45.31% and Kinetin 58.53%, but ABA increased about 23.01% for ABA and 34.93% for vitamin C compared with control plants. The interaction between them is significant for four plant hormones.

Keywords:*Pisum sativum*.L ,ABA, plant hormones

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1. Introduction

Pea plant *Pisum sativum* L. belongs to the family: Papilionaceae [1] as its root grow rapidly at the soil and contains nodes which are responsible for nitrogen fixation [2]. The abscisic acid (ABA) synthesized in the leaves then translocated to the stem through phloem, ABA has the role in the abscission of leaves, flowers and fruits, it causes stomatal closure, inhibits the synthesis of IAA and GA_3 synthesis, and seeds germination of seeds, caused dormancy of bud, percentage of tuberization and senescence of leaves and inhibits fruit ripening. It becomes biologically active in Cis-ABA [3, 4]. Also it works to increase the activity of the antioxidant enzymes like: Catalase, peroxidase and superoxide dismutase under stress conditions which is exposed to the plant [5].

Vitamin C (L-Ascorbic acid), the good source of these vitamin leafy vegetables, orange, tomatoes and green peppers. It catalyzed phosphorylation and transfer the H^+ from NADPH to O_2 after coupling with oxidation and reduction of glutathione [3]. Vitamin C is derivative of monosaccharide L-glucose. Its water soluble, strong reducing activity and have cellular oxidation-reduction, it plays a role in the biosynthesis of serotonin, hydroxylation of tryptophan to 5-hydroxytryptophan [4]. Vitamin C has an important role in the increase of cell division and stimulate the building of nucleic acid and protein and consider to be scavenger free radicals harmful effects resulting from the metabolism in the cell [6].

The present study aimed to determine the effect of abscisic acid and vitamin C by foliar application at concentrations 0, 10 and 20 $mg.L^{-1}$ for first factor and 0, 50, and 100 $mg.L^{-1}$ for second factor in some plant hormones for leave of pea plant.

2. Materials and Methods

The experiment was carried out during the growing season 2016-2017 in the botanical garden of the Department of Biology in the College of Education for Pure Science (Ibn Al-Haitham), University of Baghdad. The experiment was designed according to RCBD (Randomized complete Block Design with three replicates. The experiment consisted of 12 experimental units, 1 m width and 1 m length and the land fertilized by urea (46% N) per 60 kg. Ha^{-1} and super-triphosphate (46% P_2O_5) per 200 kg. Ha^{-1} [9]. The Abscisic acid (ABA) concentrations (0, 10, 20) $mg.L^{-1}$ and vitamin C concentrations (0, 50, 100, 150) $mg.L^{-1}$, local variety pea seeds planted on 15/10/2016, the distance between the lines 33 cm and each line has 4 holes and the distance between them is 25 cm. Plant sampled on 8/1/2017, and estimated the content of some plant hormones as follows:

2.1. Extraction and estimation of the concentration of the hormones regulating the growth of the plant (auxin, gibberellin, kinetin and abscisic acid) using high performance liquid chromatography H.P.L.C:

For plant hormones extraction Ünyayar *et al.* [7] method was used by separation method Fast liquid chromatography (FLC) by taking 10 mg of the sample (plant leaves) and crushing them well until they become a paste-like block by adding 60 ml of a mixture of 12V methanol, 5V ammonia and 3V chloroform. Then filtrated the mixture and runny separated by centrifuge at speed 6000 cycle/ minute, for 15 seconds after that the runny treated by 25 ml from Non-ionic water then deposited from chloroform phase. The methanol phase was then steamed and dried by rotary evaporator and at 30 ° C. The chlorophyll phase was dissolved and the extract was modified to pH = 2.5 when injected with High performance liquid chromatography (H.P.L.C) Device type Shizmadzu LC 2010A Japanese origin.

- Liquid phase: 1% acetic acid in methanol + 1 mM tetrabutylammonium phosphate + acetonitrile, 3µm size (50x2.1 mm lid), detection by UV rays, wavelength 280 nm, specimen size 20 ml, the temperature was 30 ° C. The time of detention package areas measured for standard
specimens as shown in Table (1). The plant sample content of the hormones was estimated according to the following equation:

Concentration of unknowns material= $\frac{\text{backage area of specemen}}{\text{backage area of standared}}$ × **Concentration of standard solution × Number of dilutions**

Table (1): Retention time and package area of plant growth regulators for peas plant leaves.

No.	Standard materials	Retention time	area	Concentration of standard solution for15µg/ml
1	Kinetin	1.87	313817	
2	Indole acetic acid	3.17	487314	The standard solution obtained from Sigma Aldrich St Louis USA
3	Gibberellic acid	4.08	342280	
4	Abscisic acid	4.99	450033	

2.2. Statistical analysis

The results were statistically analyzed according to the SAS program [8] to compare the calculation means for all treatments by using the least significant difference (L.S.D.) at the probability level of 0.05.

3. Results and Discussion

3.1. Content if auxin (mg.100 gm^{-1} fresh weight) in leaves:

The results of the table (2) showed significant influence for abscisic acid in the mean of auxin content in leaves. The addition of increasing concentrations of abscisic acid led to a significant decline in the mean of this character, in particular at concentration 20 $mg.L^{-1}$ for abscisic acid, which gave less than the mean of these character reached to 2.19 mg.100 gm^{-1} fresh weight and at reduced rate 27.44% compared to the treatment of control. The concentration 10 mg. L^{-1} of abscisic acid, the mean character reached to 12.87 mg.100 gm^{-1} fresh weight and at reduced rate 23.39% this is due to the effect of abscisic acid on inhibition of plant hormone and caused cellular stress [9]. The presence of a plant hormone that can change the concentration of another hormone or inhibited their transferred, so the presence of abscisic acid causes the destruction of auxin in plant tissue as well as inhibited the biological production because of the increased activity of the IAA- oxidase enzyme and phenolic compounds which activated by presence of molecular oxygen O_2 and stress conditions [10]. The abscisic acid puts the plant under the effect of the oxidation by presence of hydrogen peroxide H_2O_2 and elements of active oxygen such as super oxide O^{-2} and hydroxyl (OH^-) radicals, which inhibited the synthesis of plant growth regulators [11]. In this sense, the treatment with abscisic acid led to a significant decline in the content of peas plant for auxin ,these results are consistent with the findings of [12] on wheat plant, as results showed the

existence of a significant decline in the content of this plant for the plant hormones including auxin when treated with abscisic acid. The results of the table also indicated the presence of a significant influence for vitamin C in the mean of auxin content on leaves, especially at concentration 150 mg.L^{-1} of vitamin C, which gave the highest mean for this character reached to 16.35 mg.100 gm^{-1} fresh weight, an increase rate of 36.07% compared to the treatment of control, while vitamin C has non-significant influence in the mean of these character at concentrations (50,100) mg.L^{-1} may by belonging to the role of vitamin C in the activation of the biological synthesis for the plant hormones because it is the first line of defense of the contents of the plant cell from damage caused by abiotic stresses [13]. Also, vitamin C have a role in the organization of the work of many plant hormones because it is an important factor in the process of construction of this plant hormones including gibberellin [14]. There has also interaction between the work of the plant hormones by the existence of gibberellin may increase the biological construction for auxin through inhibition of the effectiveness of an enzyme IAA-oxidase [15]. Therefore, the presence of non-enzymatic antioxidants, such as vitamin C have an effective role in reducing the risks arising from the conditions of oxidative stress and increase plant internal content of plant growth regulators including auxin. These results agree with the results obtained from [16] on the potato plant as the results showed the presence of high significance in the content of the potato plant from auxin when the treatment of this plant is with ascorbic acid. The results of the table also indicated the existence of a significant influence for the interaction between the experiment factors in the mean of this character as such as the concentration 0 mg.L^{-1} of abscisic acid and 150 mg.L^{-1} of vitamin C gave the highest mean of this character was 20.17 mg.100gm^{-1} fresh weight, either less than the mean of this character was 8.00 mg.100gm^{-1} fresh weight at concentration 10 mg.L^{-1} of abscisic acid and 100 mg.L^{-1} of vitamin C.

Table(2): The Effect of foliar application with abscisic acid and vitamin C and their interaction on auxin content (mg.100gm^{-1} fresh weight) in the leaves of peas plant.

Concentration of ABA (mg. L^{-1})	Concentration of Vitamin C (mg. L^{-1})				Mean
	0	50	100	150	
0	9.55	19.54	17.96	20.17	16.80
10	14.93	11.66	8.00	16.90	12.87
20	14.00	8.76	14.00	12.00	12.19
L.S.D (0.05)	Vitamin C × ABA= 1.140				ABA= 0.570
Mean	12.83	13.32	13.32	16.35	
L.S.D (0.05)	Vitamin C= 0.658				

3.2. Content of gibberellin (mg.100 gm^{-1} fresh weight) in leaves:

The results of the table (3) showed that existence of a significant influence for abscisic acid in the mean of gibberellin content. The addition of increasing concentrations for abscisic acid led to a significant decline in the mean of this character, in particular for the concentration 20 mg.L^{-1} of abscisic acid, which gave less mean of this character reached to 19.22 mg.100gm^{-1} fresh weight at reduced rate of 19.78% compared to the treatment of control. The concentration of 10 mg.L^{-1} for abscisic acid, the mean this character reached to 20.60 mg.100gm^{-1} fresh weight at reduced rate of 14.02%, which refers to the effect of abscisic acid on most of the characteristics of the vegetative growth and biological effect of plant [17]. Since the decrease of the biological effect whereby the plant from the division and elongation

of cells and the process of photosynthesis by the influence of oxidation conditions which exposure the plant for it by the presence of abscisic acid that effects on synthesis of important growth regulators inside the plant [18]. The presence of abscisic acid effects on the act of other plant hormones including gibberellin by inhibiting of their synthesis process in plant cells and stopped their activity and motivation of the plant tissue towards aging [19]. Therefore, abscisic acid treatment led to a significant decline in the gibberellin content of peas, these results agree with the results obtained from [12] for the wheat plant. Also the results of the table showed that the existence of a significant influence of vitamin C in the mean content of gibberellin and clearly influence is the greater concentration of vitamin C at concentration 150 mg.L^{-1} of vitamin C and the highest mean for this character reached to 27.32 mg.100gm^{-1} fresh weight, an increase rate of 45.31% compared to the treatment of control, while vitamin C concentrations non-significant influence in the mean of this character at concentrations (50,100) mg.L^{-1}. This is due to the effective role of vitamin C in the regulation of some of the plant hormones, because of it is the first line of defense of the contents of the plant cell from damage of oxidation conditions which increase the synthesis of plant growth regulators, including gibberellin [20].

Also, vitamin C as antioxidants plays an essential role in plant growth and development through its influence on many of the physiological events [21]. In addition, vitamin C acts as co-factor for the synthesis of many important plant hormones including gibberellin and salicylic [13, 22]. Therefore, treatment with vitamin C led to significant increase in the plant content of gibberellins. The results of the table indicated of the significant influence of the interaction between the two factor experience in the mean of this character, as the concentration 0 mg.L^{-1} of abscisic acid and 150 mg.L^{-1} of vitamin C gave the highest mean for this character reached to 35.69 mg.100G^{-1} fresh weight compared with less mean of 16.17 mg.100gm^{-1} fresh weight at the concentration 0 mg.L^{-1} of abscisic acid and vitamin C.

Table (3): The Effect of foliar application with abscisic acid and vitamin C and their interaction on gibberellin content (mg.100gm^{-1} fresh weight) in the leaves of peas plant.

Concentration of ABA (mg. L^{-1})	Concentration of Vitamin C (mg. L^{-1})				Mean
	0	50	100	150	
0	16.17	20.40	23.56	35.69	23.96
10	21.31	17.82	22.96	20.32	20.60
20	18.92	19.77	12.26	25.94	19.22
L.S.D (0.05)	Vitamin C × ABA= 3.600				ABA= 1.800
Mean	18.80	19.33	19.59	27.32	
L.S.D (0.05)	Vitamin C= 2.078				

3.3. Content of kinetin (mg.100 gm^{-1} fresh weight) in leaves:

The results of the table (4) showed that presence of a significant influence for abscisic acid in the mean of kinetin content in the leaves. The addition of increasing concentrations for abscisic acid led to a significant decline in the mean of this character, in particular for concentration 20 mg.L^{-1} of abscisic acid, which gave less mean of this character reached to 17.287 mg.100gm^{-1} fresh weight at degrease mean of 15.37% compared to the treatment of control. The concentration10 mg.L^{-1} of abscisic acid, the mean of this character reached to 18.45 mg.100gm^{-1} fresh weight and decrease rate 9.64%, this is due to the effect of abscisic acid in the content of the plant from regulation hormones, including the kinetin because of

increasing the cellular caused by oxidative stress [23]. The presence of abscisic acid effect on regulation and growth of plant by its effect in the photosynthesis an inhibit growth of side roots, which is a centre of regulator hormone, including the kinetin [24]. Thus, the addition of increasing concentrations of abscisic acid effect negatively on plant and their vegetation growth and plant internal content of plant hormones including kinetin. The results of table indicated that percent of the significant influence of vitamin C in the mean content of kinetin which increasingly influence by the increase of the concentration of vitamin, particularly concentration 150 mg.L^{-1} of vitamin C, which gave the highest mean for this character reached to 237.18 mg.100gm^{-1} fresh weight, an increase rate of 68.53% compared to the treatment of control. The two concentrations (50, 100) mg.L^{-1} of vitamin C, the mean of this character reached to (17.23, 20.33) mg.100gm^{-1} fresh weight, an increase rate of (24.04, 43.08)% respectively this is due to the role of vitamin C in the regulation of plant hormones because of it is antioxidants, roles which enhance the defense system in the plant to remove the effect of super oxide O^{-2}, or hydrogen peroxide which is formed by the stress [25, 26]
Also, vitamin C has a role in increasing the concentration of plant hormones, such as the kinetin promote the growth of the side bud [27]. Thus, vitamin C has a positive role in increasing the content of kinetin. The results of the table showed that the significant influence of the interaction between the experiment factors in the mean of this character. The concentration 10 mg.L^{-1} of abscisic acid and 150 mg.100gm^{-1} of vitamin C the highest mean for this character reached to 6.5 mg.100gm^{-1} fresh weight compared with less mean of 12.00 mg.100gm^{-1} fresh weight at the concentration 10 mg.L^{-1} of abscisic acid and 0 mg.L^{-1} of vitamin C.

Table (4): The Effect of foliar application with abscisic acid and vitamin C and their interaction on kinetin content (mg.100gm^{-1} fresh weight) in the leaves of peas plant.

Concentration of ABA (mg. L^{-1})	Concentration of Vitamin C (mg. L^{-1})				Mean
	0	50	100	150	
0	15.40	17.48	24.48	24.31	20.42
10	12.00	17.56	18.32	25.93	18.45
20	14.27	16.65	18.20	20.01	17.28
L.S.D (0.05)	Vitamin C × ABA=1.014				ABA= 0.507
Mean	13.89	17.23	20.33	23.41	
L.S.D (0.05)	Vitamin C= 0.585				

3.4. Content of abscisic acid (mg.100 gm^{-1} fresh weight) in leaves:

The results in table (5) showed the existence of significant influence in the mean of abscisic acid content. The addition of increasing concentrations of abscisic acid led to significant increase in the mean this character, in particular for the concentration 20 mg.L^{-1} of abscisic acid, which gave the highest mean in this character reached to 21.00 mg.100gm^{-1} fresh weight, an increase rate of 23.09% compared with the treatment of control. The concentration 10 mg.L^{-1} of abscisic acid, the mean of this character reached to 18.52 mg.100gm^{-1} fresh weight, an increase of 8.55%, and this shows the effect of foliar application with abscisic acid on plant peas, which work to increase leaves content of abscisic acid which increase cellular oxidation within the plant by the effect of stress condition causing an increase in the reactive oxygen species including O_2 and H_2O_2 [23]. The oxidation conditions in turn increase the effectiveness of the antioxidant, such as peroxidase, super oxide dismutase and catalase in the

plant tissue and thus increase plant stress resistance [28]. Therefore, the foliar application with abscisic acid on a peas plant led to significant increase in internal content of leaves for abscisic acid. These results are consistent with the findings of a number of researchers including [12, 29, 30] on mung bean, wheat and peas at respectively. The results of the table show significant increase in the mean of this character when the treatment plant on increasing concentrations of vitamin C and particular for the concentration 100 $mg.L^{-1}$, which gave the highest mean for this character reached to 26.37 $mg.100mg^{-1}$ fresh weight, an increase rate of 70.05% compared to the treatment of control. The two concentrations (50, 150) $mg.L^{-1}$ of vitamin C, the mean of this character reached to (15.53, 19.08) $mg.100gm^{-1}$ fresh weight, an increase rate of (7.39, 31.95)% at respectively. This shows the effective role of vitamin C as non-enzymatic antioxidant because of the external addition for vitamin C increases the content of the plant from abscisic acid which certain the role of this vitamin as the first line of defense and has an effective role in the protection of plant cells from damages resulting from oxidation condition which result from effect of the stressful factors that exposure plant for them by presence of abscisic acid [31, 32].

Also, vitamin C acts to remove the effect of hydrogen peroxide H_2O_2 and free radicals that result from the reaction of photo oxygen that occur in the first chromosome in chloroplast by the organization of metabolic for antioxidants in developing plants under stress conditions [33, 34]. Therefore, the foliar application with vitamin C increase the content of the plant for the abscisic acid. These results agree with the results obtained [16] on the potato plant. The results of the table show the existence of a significant influence of the interaction between the experiment factors in the mean of this character. The concentration 20 $mg.L^{-1}$ of abscisic acid and 100 $mg.L^{-1}$ of vitamin C the highest mean of this character was 30.17 $mg.100gm^{-1}$ fresh weight compared with less mean of 7.51 $mg.100gm^{-1}$ fresh weight at concentration 0 $mg.L^{-1}$ for each of abscisic acid and vitamin C.

The concentration 20 $mg.L^{-1}$ of abscisic acid and 100 $mg.L^{-1}$ of vitamin C the highest mean for this character reached to 30.17 $mg.100\ mg^{-1}$ fresh weight compared with less mean of 11.65 $mg.100\ mg^{-1}$ fresh weight at concentration 10 $mg.L^{-1}$ of abscisic acid and 50 $mg.L^{-1}$ of vitamin C.

Table (5): The Effect of foliar application with abscisic acid and vitamin C and their interaction on abscisic acid content ($mg.100gm^{-1}$ fresh weight) in the leaves of peas plant.

Concentration of ABA ($mg.\ L^{-1}$)	Concentration of Vitamin C ($mg.\ L^{-1}$)				Mean
	0	50	100	150	
0	7.51	22.77	22.42	15.54	17.06
10	16.31	11.65	26.53	19.57	18.52
20	19.54	12.17	30.17	22.13	21.00
L.S.D (0.05)	Vitamin C × ABA=1.431				ABA= 0.715
Mean	14.46	15.53	26.37	19.08	
L.S.D (0.05)	Vitamin C= 0.826				

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