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INFLEUNCE OF TOLERANT STRESS COMPOUNDS ON PROTEIN PATTERNS IN CARNATION PLANT *Dianthus caryophyllus* cv. chabod

Botany

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ABSTRACT

This experiment was conducted during growing season of 2015/2016 in Lath house belong to Department of Horticulture and Landscape Design, College of Agriculture , Basra University, to study the changes in extracted Carnation protein profiles as response to spray of environmental tolerant compounds (Ascorbic acid, Proline and Paclobutrazole) based on SDS- PAGE electrophoresis . Carnation plants were sprayed with different concentrations of Ascorbic acid (0,25 and 50 mg. L^{-1}) Proline (0, 50 and 100 mg. L^{-1}) Paclobutrazole (0, 25 and 50 mg. L^{-1}) and interaction between them .

Results of the study revealed that all the treatments with these environmental tolerant compounds caused a positive changes gel electrophoresis via inducing formation of osmotic protein, which protects plants from any environmental conditions.

KEYWORDS:

Dianthus caryophyllus, Paclobutrazole, Proline, Ascorbic acid, SDS- PAGE electrophoresis

Introduction

Carnation (*Dianthus caryophyllus*) (Fig. 1) is on among the most popular commercial cut flowers of the word , native to Mediterranean region and central Asia (Roodbaraky *et al*.,2012).

Carnation is a member of the family *caryophyllaceae* which has 93 genera (Singh, 2010)

The exogenous application of tolerant stress compounds such as Paclobutrazole (pp333), Proline , and Ascorbic acid has been subjected to be an effective approach in improving biotic or environmental tolerance, this application may lead to appearance of new protein bands which considered as Osmotic protein to protect plants from abiotic stress. (Bakheta, 2004; Bakheta *et al*., 2008; Al-Taha, 2008).

(Fig. 1). Carnation plant (*Dianthus caryophyllus*) in pot

Whipkeer and Hammer (1997) reported that Paclobutrazole increasing the potentiality of many plants to tolerate to several drastic environment conditions. Al-Taha (2008) found that SDS –PAGE analysis of the extracted protein from plantlets grown under NaCl stress (10, 40 and 50 mM) showed a presence of high molecular weight proteins (82.7, 81.3, and 81.5 kDa). The addition of Proline at 80 and 75 mg/l to culture media, induced the synthesis of three new proteins (20.4- 21.6, 40.8-41.4 and 69.7-70.2 kDa), and proline treatments reduced the synthesis of salt responsive proteins. Ati (2016) reported that , the results of protein pattern showed the occurrence of differential gene expression in which a number of bands appeared on gel when date palm trees were treated with different concentrations (500 and 1000 mg) of Ascorbic acid.

The present study was aimed to find out, if the application of PP333, proline ad ascorbic acid may lead to appearance of new protein bands as osmotic protein to protect plants from abiotic or environmental stress such as temperature and drought in Basra provenance – Iraq.

Material and Methods

Pot experiments were carried out during growth seasons of 2015/2016 In Lath house-Department of Horticulture and Landscape Design College of Agriculture., Basra University – Iraq .The carnation seeds c v chabod were used in the current study. Seeds were cultured in dishes contained sterilized Patmos. Fifteen days after culturing, seeds were germinated and transplanted one plant in each pot (25 cm in diameter). Pots contained sterilized (40% formalin) soil (loam clay and Patmos 1:3). Twenty days after sowing, Carnation plants in each pot fertilized with 2 gm of NPK, after 30 days from sowing, plants were sprayed once monthly with freshly prepared solutions of PP333 (P) in concentrations .0, 25 and 50 mg. L^{-1} Proline (L) (0, 50 and 100 mg/l) or Ascorbic acid (A) (0, 25 and 50 mg/l) and interaction between them.

Electrophoresis of protein pattern.

Carnation leaves which were treated with tolerant stress compounds, were analyzed with SDS – PAGE technique by storing in deep freezer at -20 C until used for estimation of electrophonic protein using poly acryl amid Sodium dodecyl sulfate according to Weber and Osborn (1975).

Results and Discussions

SDS-polyacrylamide gel electrophoresis (SDS-PAGE) of proteins extracted from Leaves of Carnation plants subjected to different concentrations of environmental stress tolerance compounds (Ascorbic acid, Proline, Paclobutrazole) shows a different changes in gene expression via appearance and disappearance of new bands which caused changes in protein pattern (Fig 2) .

1- The results indicated that plants sprayed with different concentration of Ascorbic acid (0 , 25 and 50) mg. L^{-1} showed three types of protein bands with high molecular weights, with disappearance of fourth band when the plants were treated with 25 mg .L^{-1} as compared with control plants , whereas a four bands of protein with high molecular weights (76.54 , 131.89 , 171.98 and 225.00) KD were accumulated when the plants were treated with 50 mg. L^{-1} of ascorbic acids which was similar in their number to that obtained from control but with low molecular weights This could be, because of high concentration of Ascorbic acid (50 mg. L^{-1}) which was considered as optimal concentration in this study., moreover ,the physiological role of Ascorbic acid in the plants is inducing plants to produce nucleic acids and protein since its role

as an major enzymes. (Mahalingam and Fedoroff, 2003). These results are in agreements with those reported by Ati (2016) who found that when date palm leaves were sprayed with Ascorbic acid ,a new proteins were induced which may be responsible in the process of plant defense to environmental stress.

2- Three types of protein bands with molecular weight (218.58, 129.88, 82.99 and 236.93, 173.74 , 96.43) KD , were accumulated when plants were treated with amino acid proline in two concentrations (50 and 100) mg. L^{-1} respectively with disappearance of fourth band while four bands were appeared in control plan In addition , two protein bands showed same molecular weights in 50 mg. L^{-1} proline concentration and control (129.88 , 82.99) and (129.88 , 82.09) KD respectively .this similarity could be useful. to the plants for prolongation of plants adaptation to stress and so it's considered as osmotic proteins (Al-Taha,2008).

3- Results also revealed (Table 1) that Carnation plants treated with growth regulator Paclobutrazole in 25 mg. L^{-1} concentration induced a new 5 protein bands which had molecular weights 260.79, 226.70, 164.84 , 105.49 and 72.28 KD compared with control. Whereas two protein bands were disappeared when plants were treated with Paclobutrazole in 50 mg. L^{-1} concentration. , in addition to that , bands which appeared in the last treatment , (197.49, 100.00 and 63.70) KD had less molecular weights than those of control . which are considered as less molecular weight of protein bands that appeared in other treatments (ascorbic acid and proline) and control. This may be related to that, Paclobutrazole can protect plants from various environmentl stress , including anoxia, air pollutants , drought , extreme temperature, ultraviolet light and salinity (Davis,*et al*.,1988 ; Fleticher and Hofstra,1988 ; Bekheta *et al* .,2006 ; Kamdll and Eleiwa,2008). These results are in agreements with that reported by Bekheta,2004. ,Bekheta,*et a*l2008 , ,Bekheta and Talaat 2009 in appearance of new protein bands when wheat plants ,*Gerbera jasmonii* and *Vigna radiate* were treated with different concentrations of pp333.

4- Results also shows that, the interaction between these three compounds (Ascorbic acid, proline and Paclobutrazole), in different of concentrations . Induced appearance of new protein bands with disappearance of other bands, as compared with control. Ascorbic acid and proline in their two concentrations (50 + 50 L1A2) (100+25L2A1) mg. L^{-1} respectively encourage formation protein bands similar in their molecular weight in both interactions (166.64 KD). The reasons of increased the same protein bands may be related to that long adaptation to environmental stress such as high temperature produce these proteins which may be considered as osmotic proteins (osmotin). Al-Taha (2008) found some of the proteins band were many times accumulated In culture media when some of abiotic stress(Salt) was used with proline and was considered as osmo- protectants proteins. In addition , a six protein bands with high molecular (266.90 , 231.80, 166.64, 111.70,76.90 and 58.40) KD were accumulated when the interaction (100+25 L2A1) was performed. this was the highest interaction treatment that induced formation and appearance of these protein bands The reason of accumulation of high number of proteins in this treatment may be related to role of ascorbic acid in reducing the inhibitory effect of drought and high ambient temperature via improving the plant content through proteins anabolism (Hussein and Khursheed, 2014). Interaction of Proline with Ascorbic acid may play major role in controlling of gene expression through mRNA modification, Since all amino acids considered as protein precursors.(Kimball and Jefferson, 2004 ; Ati , 2016).

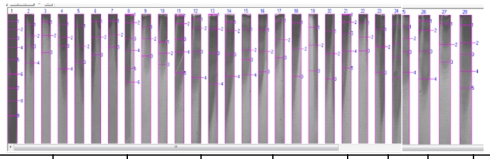
Results also show, that interaction between two treatments {(100+50)L2A2 } and {(50 +25) A2P1 } induced accumulation of a very similar protein bands in their molecular weighs and it may be the same protein {101.29,101.20) KD respectively . This protein may be considered as osmotic protein and useful to the plant to improve its efficiency in water absorption from the soil.

Results of the treatment of interaction between Paclobutrazole (50 mg. L^{-1}) and ascorbic acid (50 mg. L^{-1}) molecular weights (265.90, 233.50, 189.70, 108.50 and 72.20) KD were appeared , whereas , results of other interaction treatments I induced 3-4 protein bands as compared to control, whereas treatment of interaction between Paclobutrazole (25 mg. L^{-1}) and ascorbic acid (50 mg. L^{-1}) gave protein band with less molecular weights (55.60 KD) than other interaction treatments. the appearance of this low molecular weight could be useful to the plant as osmo protectant proteins .

5- Results of the accumulated chief proteins which were accumulated during interactions between anti stress compounds (Ascorbic acid, proline and Paclobutrazole) and presented in this study show disappearance of one of the bands and accumulation of three bands as compare to control which show accumulation of four bands in most of interaction treatments with accumulation of protein bands which similar in their molecular weight. The interaction treatments (L1A1P1 , L2A2P1, L1A1P2) had protein bands with molecular weight 73.70, 74.50. 73.70 KD respectively ,whereas , the high concentrations of the interaction treatment L2A2P2 (50+50 +100) mg. L^{-1} enhanced formation of five protein bands with low molecular weighs in comparison to other interaction treatments and control. (225.00, 150.00, 100.00, 75.00, and 50.00), The reason of accumulation of these protein band of low molecular weight as a response of Carnation plants to the environmental stress such as high ambient temperature and drought in Basra provenance. Bomer (2000) reported that , proteins of low molecular weighs are synthesized in plant tissue in response to one of the biological stress (salinity) which increase the osmotic pressure of the leaves, and then improves plant absorption efficiency .

In general , according to the protein evaluation , we found that the differences in number of proteins bands and their molecular weights may be attributed to the environmental factors that surrounding the plants (temperature and drought) or effect of the decrease the anti stress compounds such as vitamins (ascorbic acid), Bassuony *et al*.(2008) found that vitamins may cause a high variations in enzymes of protoplasm anabolism .This may refer to that vitamins could be acts as protein synthesis activators. Moreover , the amino acid proline protect these enzymes from denaturation, (Demir and Kocacaliskan, 2001).

Using of Paclobutrazole alone or interacted with other treatments , induced accumulation of low molecular weights protein bands as compared to control. These proteins are consider as osmotic protector since the Paclobutrazole as growth regulator increase the ability of most plant to tolerate the drasticen (Whipkeer and Hammer,1997). Same results were found when *Gerbera josmonii* were sprayed with different concentrations of Paclobutrazole , increased the amino acid contents which caused a positive changes in electrophoresis and these changes was combined with appearance or disappearance of some protein bands (Bekheta *et al*.,2008).



Number of bands Treatments	1	2	3	4	5	6	7	8	9
M	236.932	204.045	168.436	124.15	86.262	70.755	48.171	29.878	7.317
L0A0P0	233.379	178.964	129.88	82.09					
L0A1P0	233.523	180.686	104.047						
L0A2P0	225.00	171.985	131.843	76.546					
L1A0P0	218.584	129.888	82.997						
L1A0P0	236.932	173.744	96.435						
L1A1P0	238.636	184.105	126.04						
L2A1P0	265.909	231.818	166.646	111.754	76.908	58.407			
L2A2P0	213.761	166.646	93.324						
L2A2P0	151.901	101.296	80.467						
L0A0P1	260.795	226.705	164.844	105.497	72.285				
L0A1P1	245.455	208.917	106.995	65.59					
L0A2P1	265.909	205.673	101.296	55.642					
L1A0P1	233.523	199.137	97.571	67.398					
L1A0P1	204.045	113.422	86.262	69.677					
L1A1P1	202.413	106.995	73.703						

L2A1P1	240.341	161.207	89.803						
L2A2P1	231.818	118.644	67.398						
L2A2P1	212.149	135.804	74.582						
L0A0P2	197491	100	63.709						
L0A1P2	265.909	233.523	189.175	108.539	77.265				
L0A2P2	230.114	173.744	110.126						
L1A0P2	204.045	127.953	72.77						
L1A0P2	225	143.876	97.48	66.201					
L1A1P2	218.584	170.215	108.539	73.703					
L2A1P2	242.045	204.045	110.126	64.971					
L2A2P2	230.114	141.844	83.986						
L2A2P2	225	150	100	75	50				

(Fig.2 , Table 1). Effect of spraying of proline , paclobutrazole and ascorbic acid in pattern SDS-PAGE for leaves of carnation plant.

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