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Influence of Selenium and Paclobutrazole on Growth, Metabolic Activities and Anatomical Characters of *Gerbera jasmonii* L.

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Abstract: Gerbera (*Gerbera jamesonii L.*) is an economically important ornamental crop produced for cut flowers, potted plants, and bedding plants. Elongated pedicle of gerbera tend to lodge and brake, so pot experiments were carried out at the screen of the National Research Centre, Dokki, Giza during two successive growth seasons 2003/2004 and 2004/ 2005 to study the influence of selenium "Se" at concentrations of 5, 10 or 20 ppm and / or paclobutrazole "PP_{333}" at the rate of 25, 50 or 100 ppm on gerbera plants. The results obtained show that all the used levels of Se and /or PP_{333} mostly caused significant increases in all the studied vegetative & reproductive growth parameters as well as photosynthetic pigments (chl.a, chl.b and carotenoides). Generally, all the used levels of Se and / or PP_{333} led to the appearance of a new amino acid methionine, the increase in the content of endogenous amino acids which in turn led to positive changes in the picture of protein electrophoresis. These changes were accompanied by appearance and disappearance of some protein bands. All the used levels of Se and /or PP_{333} mostly caused obvious changes in the histological features e.g. increased the thickness and the number of chollenchyma cells of cortex tissue, vascular bundles tissues and pith tissues.

Key words: Selenium,Paclobutrazole,Amino acids and Anatomy

INTRODUCTION

Gerbera jasmonii L.(Transvaal Daisy or Gerbera) is one of the most important economic ornamental plant in Egypt, belongs to family *Asteracea*. It is native to South Africa and contains about 70 species. Gerbera is consider as an evergreen hairy perennial herbs, the numerous leaves come from a basal tuft are pinnately lobed and woody underneath. Flower heads are daisy-like, large, solitary and large-stalked; flower colours range from cream through yellow to coral, orange, flame, violet and red. The plants grow best in full sun, in partial shade in hottest area and under airy conditions. The garden varieties have larger flowers with longer pedicle, some varieties are semi double or double and the plants do not thrive when crowded as they need space to spread their foliage without interference.

Gerbera plants are cultivated chiefly in ground beds in green houses , they do not produce enough blooms at one time as ideal ornamental pot plant. The production of gerbera flowers with a good qualities and in large quantities depends on many factors such as media of growing, nutrients available (macro and micro elements), irrigation.. etc. which influence the processes of plant growth.

In recent years, Application of antigibberellins on many plants especially potted ones do not show malformation but provide better support for leaves and flowers (Zhou, and Leul, 1998 & Bekheta and Ramadan, 2005 and Mahgoub *et al*., 2006).

In some cases, the consumers need gerbera flowers in large size and this is achieved by using antioxidants e.g. selenium "Se". (Hartikainen and Xue, 1999). In addition, Xue *et al*. (2001) found that using Se at low concentrations stimulated the growth senescent of lettuce through its antioxidants effects.

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In most cases of gerbera production the pedicles tend to lodge and / or brake, this is considered one of the most important problems facing the producers of gerbera in Egypt and all over the world. Therefore, the present investigation was suggested to make use of the growth retardant paclobutrazole and /or the antioxidant selenium as a trial to solve this problem through its effects on the physiological processes and histological characters.

MATERIALS AND METHODS

Pot experiments were carried out during two successive growth seasons (2003 / 2004 and 2004 / 2005) at the screen of the National Research Centre, Dokki, Giza, Egypt. Seedlings of gerbera were obtained from the Horticulture Research Institute, Agriculture Research Centre, Ministry of Agriculture Egypt. The seedlings (two seedlings / pot) were transplanted on the 20th of January in clay pots 30 cm in diameter, each filled with about 8 kg loam clay soil mixed with 2 gm of supper calcium phosphate as a source of phosphorous. After 30 days from transplanting, 1g ammonium nitrate was added as a source of nitrogen in three times with four weeks intervals.

Substances Used:

- Paclobutrazole: {1-(4-chlorophenyl)-4,4-dimethyl-2-(1 H-1 ,2, 4 triazol-1-yl) pentan- 3-ol} having the common name Bonzi (PP_{333}), is one of the highly specific growth retardants which exhibits a promising role in improving growth, flowering and yield as well as increasing the potentiality of many plants to tolerate several drastic environmental conditions (Whipkeer and Hammer, 1997).
- Selenium: It is an element its atomic weight is 34, having the chemical symbol Se with the chemical symbol "Se", occurs rarely in the free state in nature and is found in different forms (e.g. oxide, sulphate, selenite, selenate….etc). The results obtained by (Seppanen *et al.,*2003 and Lyi *et al,* 2007) they suggested that Se is an antioxidant, it activates protective mechanisms, which can alleviate oxidative stress in the chloroplasts. Gerbera plants were sprayed twice (after 45 and 60 days from transplanting) with freshly prepared solutions of selenium at the rate of 5, 10 or 20 ppm and /or paclobutrazole at the rate of 25, 50 or 100 ppm.

Sampling:

Gerbera plants were collected after 2 weeks from the second treatment, the root system was discarded and the shoot system was divided into 2 groups as follow**s:-**

Group A:

The plants were used for recording vegetative and reproductive growth parameters**:** peduncle length, peduncle diameter, flower length, flower diameter, plant height (all in cm), fresh & dry weights of flowers, fresh & dry weights of shoots, fresh & dry weights of peduncles (all calculated in g /plant).The young leaves of the plants of this group were used for estimation of photosynthetic pigments.

Group B:

The young leaves of the plants of this group were weighed , dried in an electric oven at 60 C^0 till constant weight, ground to fine powder and stored in tubes until needed for estimation of amino acids.

Chemical Analysis:

Photosynthetic Pigments:

An accurate weight (0.5 g) of fresh young gerbera leaves was homogenized in 85% acetone and used for determination of photosynthetic pigments (Chl.'a', Chl.'b' and carotenoids) using spectrophotometric method developed by Metzziner *et al.* (1965). The samples were read at 663, 664 and 452.5 nm respectively

Amino Acids:

Acid Hydrolysis and HPLC Determination of Amino Acids:

This was carried out according to the method described by Gehrke *et al.* (1985) as follows:- 10 g of fine dry powder of young leaves was weighed into 25x150 mm hydrolyzing tube. Aliquot (10 ml) of 6N HCL was added purged with nitrogen for 60 sec. and the tubes were caped immediately. The tubes were placed in oven at 110 $C^0$for 24 hr., removed from the oven and allowed to cool. The contents of the tube were

quantitatively transferred to 25 ml volumetric flasks and completed to volume with HPLC grade water. (About 1ml of the solution was filtered through 0.45u sample filter). Regarding the hydrolysis of cysteine & tryptophan, about 10 g of fine dry powder of young leaves were inserted in 25x150 mm hydrolyzing tube. The tubes were covered and placed in ice bath for 30 sec., 7.5 ml of performic acid (10 ml of H_2O_2 30% + 90 ml of formic acid 88%) were added to each tube and left for 1 hr. with regular check well .The tubes were placed in ice bath for 30 sec. and used directly for detection of cysteine and tryptophan. The HPLC used is waters 600E Multisolvent Delivery System, Pico-Tag analysis column ,Water 484 Detector and workstation with 815 baseline programme. The HPLC analysis was carried out by using a gradient of Pico- Tag solvent A & B at $40C^0$ with flow rate 1 ml /min. Detection of the separated PTC- amino acids at 254 nm wavelength. Before injecting the samples the instrument was calibrated by two injections of the standards.

Electrophoretic of Protein Patterns:

Electrophoretic protein profile of gerbera leaves were analyzed according to SDS—PAGE technique (Laememli,1970) which relates polypeptide maps, molecular protein markers, percentage of band intensity using gel protein analyzer version 3 (MEDIA- CYBERNE TICE,USA).

Anatomical Studies:

The specimens of gerbera plants treated with Se and /or PP_{333} in addition to the control plants were taken from the medium of the pedicle and the leaf. The specimens were killed and fixed for one week with formalin acetic acid solution (F.A.A.10 ml formalin + 5ml glacial acetic acid + 85 ml ethanol 70%). The selected materials were washed in 50% ethanol, dehydrated in series of normal butyl alcohol, embedded in paraffin wax (melting point $56C^0$), sectioned to the thickness of 20 microns double stained with safranin-light green cleared in xylem and mounted in canada balsam (Nassar and El-Sahhar, 1998). The sections were examined to detect histological manifestations of noticeable responses resulting from the treatment of the plants.

The data of the two seasons were subjected to statistical analysis of variance and the combined analysis for the two seasons were calculated after (Steel and Torrie, 1960) since the results showed the same trend. The value of LSD were calculated whenever F values were significant at 5 % level of probability (Snedecor and Cochran, 1982).

RESULTS AND DISCUSSION

Vegetative Growth Parameters:

Data presented in table (1) show that application of PP_{333} at the rate of 50 or 100 ppm on gerbera plants caused significant reduction in the plant height as compared to the control plants. On the other hand, application of Se alone on gerbera plants at all the used levels led to non-significant increases in plant height as compared with the control plants. Regarding to the interaction effect of Se and PP_{333}, the results recorded in table (1) indicate that spraying gerbera plants with Se at 20 ppm combined with PP_{333} at 100 ppm led to significant reduction in plant height compared to the control.

Table 1: Effect of spraying gerbera plants with different concentrations of paclobutrazole and /or selenium on vegetative and reproductive parameters.

Characters	Vegetative parameters				Reproductive characters							
Treatments	Plant height (cm)	No. of leaves /Plant	Leaves F.wt ./ Plant	Leaves D.wt. / Plant	Pedicle length (cm)	pedicle diameter (cm)	Pedicle F.wt. (g)	Pedicle D.wt. (g)	Flower length (cm)	Flower diameter (cm)	No. of flowers /plant	(%) of flower water content
Control	57.5	26.0	77.17	23.50	31.0	0.37	30.57	5.06	4.10	6.10	4.5	69.81
Se 5 ppm	58.0	47.5	112.42	32.66	34.83	0.40	35.0	5.64	5.10	7.00	6.0	69.34
Se 10ppm	61.5	40.00	88.45	33.39	36.0	0.43	27.25	5.14	5.00	7.80	5.0	75.48
Se 20 ppm	60.0	49.0	133.35	33.48	36.0	0.56	33.22	5.78	5.60	8.83	6.5	76.37
PP_{333} 25ppm	52.5	33.5	79.02	24.43	28.0	0.39	32.46	5.91	4.33	7.17	6.0	74.49
PP_{333} 50ppm	47.0	51.0	103.01	32.97	25.67	0.50	20.99	4.88	5.33	9.00	6.0	82.36
PP_{333} 100ppm	44.5	55.5	108.75	27.80	20.0	0.52	43.00	7.90	6.20	9.70	7.0	75.93
5 Se +25pp_{333}	54.0	40.0	80.65	29.00	31.0	0.40	32.87	4.72	5.20	7.93	6.0	82.69
10Se + 50 pp_{333}	52.0	49.0	99.29	30.64	33.0	0.51	31.90	4.32	4.70	9.00	6.0	83.79
20Se+100 PP_{333}	48.2	52.0	139.0	34.81	26.0	0.53	42.02	7.88	6.27	10.20	7.5	74.44
LSD at 5 %	6.49	4.45	12.00	3.60	2.72	0.06	3.28	0.68	0.57	0.74	0.67	

It is clear from the data recorded in table (1) that spraying gerbera plants with Se and / or PP_{333} with all the used levels caused significant increase in the average number of leaves / plant. The maximum values 55.0 was obtained from the application of 100 ppm PP_{333} followed by value obtained by application of 20 ppm Se + 100 ppm PP_{333} (52.0).

Data in table (1) also indicate that fresh and dry weights of the leaves of gerbera plants significantly increased mostly with all the used concentrations of Se and /or $PP_{333.}$ The maximum values for fresh and dry weights of leaves were obtained by the application of 20 ppm Se +100 ppm PP_{333} (139.0, 34.81 respectively) followed by the values obtained by the application of Se at 20 ppm (133.35, 33.42 for leaves fresh and dry weights respectively).

In support of these results, significant influences of paclobutrazole or selenium in changing vegetative growth parameters has been experimentally substantiated by Valkama *et al.* (2003) and Mahgoub *et al.* (2006). Recently, Navarro *et al*. (2007) found that spraying strawberry seedlings with paclobutrazole at the rate of 60 or 100 mg / L reduced plant height.

In the present investigation the reduction in plant height due to application of PP_{333} might be attributed to its interference with gibberellin biosynthesis through inhibiting the oxidation of ent-kaurene to kauronic acid which in turn, led to inhibition of the gibberellin biosynthesis (Al-Khassawneh *et al*, 2006). In addition, PP_{333} has a heterocyclic ring containing a sp2- hybridized nitrogen with a lone electron pair, it is partially responsible for growth reduction caused by an inhibition of the GA biosynthesis (Rademacher 1991 and Grossman 1992).

Reproductive Growth Parameters:

Data recorded in table (1) indicate that foliar application of PP_{333} on gerbera plants at all the used levels caused significant decreases in the pedicle length. The highest value of reduction (11 cm) was obtained from the application of PP_{333} at 100 ppm as compared to the control. On the other hand, spraying gerbera plants with Se at all the used levels led to significant increase in the pedicle length as compared to the control plants.

As regard to the interaction between Se and PP_{333} on gerbera plants, application of Se at 20 ppm combined with PP_{333} at 100 ppm caused significant reduction in the pedicle length as compared to control plants.

Spraying gerbera plants with Se at 20 ppm caused significant increase in the pedicle diameter (in cm). In addition, application of PP_{333} at 50 or 100 ppm led to significant increase in the same criteria as compared to the control plants (Table 1).

Concerning the interaction effect of Se and PP_{333} on gerbera plants, the data recorded in table (1) reveal that Se at 10 ppm together with PP_{333} at 50 ppm and Se at 20 ppm + PP_{333} at 100 ppm led to significant increase in the diameter of the pedicle. The data also indicate that spraying the plants with Se and /or PP_{333} at all the used levels some time led to significant increase in the fresh and dry weights of pedicles. The maximum values (43.0 and 7.9 g for fresh and dry weights respectively) were obtained from the application of PP_{333} at 100 ppm.

The number of flowers / plant is considered as one of the most important targets of the ornamental plants. Application of Se and /or PP_{333} at all the used levels on gerbera plants caused significant increase in the number of flowers / plant compared to the control plants (Table 1). The maximum number of flowers / plant (7.5) was obtained by the combined application of 20 ppm Se + 100 ppm PP_{333} followed by the number obtained by the application of PP_{333} at 100 ppm (7.0). In addition, all the used levels of Se and PP_{333} mostly caused positive changes in both length and diameter of flowers as compared to the control plants. The highest value of diameter (10.2 cm) was obtained from the application of Se at 20 ppm + PP_{333} at 100 ppm.

It is realized from table (1) that all the used treatments often led to increases in the percentage of flower water content, the highest value (83.79 %) was obtained from the application of Se at 10 ppm combined with PP_{333} at 50 ppm as compared with the value (69.81) obtained with the control plants.

These results are in agreement with those obtained by Bekheta & Ramadan (2005) and Mahgoub *et al.,* (2006), as they all found that application of paclobutrazole or glutathione mostly increased all reproductive growth parameters of cotton and *Calendula officinalis* L. plants respectively. In addition, Al-Khassawneh *et al.* (2006) indicated that application of paclobutrazole at the rate of 500 or 1000 mg/l of on *Iris nigricans* Dinsm plants caused reduction in rigid stalk height, weight and delayed flowering.

Chemical Analysis:

Photosynthetic Pigments:

The process of photosynthesis is affected by photosynthetic pigments which in turn are affected by exogenous application of bioregulators. The data presented in fig (1) show that foliar application of Se and / or PP_{333} on gerbera at all the used concentrations plants mostly caused significant increases in Chl. "a", Chl."b" and total carotenoides as compared to the control plants. These results are in agreement with those reported by Valkama *et al.* (2003) who reported that application of selenium on strawberry and barley plants in the field increased the efficiency of photosynthetic process. Recently, Bekheta & Ramadan (2005) and Mahgoub *et al* .(2006) indicated that foliar application of PP_{333} on cotton and *Calendula officinalis* L. plants respectively led to significant increases in photosynthetic pigments. In the present work the increase in photosynthetic pigments due to application of PP_{333} might be attributed to more stimulation of stomatal regulation (Navarro *et al*., 2007). The changes in photosynthetic pigments due to application of selenium might be attributed to the fact that selenium might induce higher respiratory potential in the leaves after treatment, as well as higher efficiency of energy conversion in PSII expressed by a higher effective quantum yield was observed in Se treated plants as foliar application of Se was efficiently absorbed by plant leaves and transported to the flowers (Germ *et al.,* 2007).

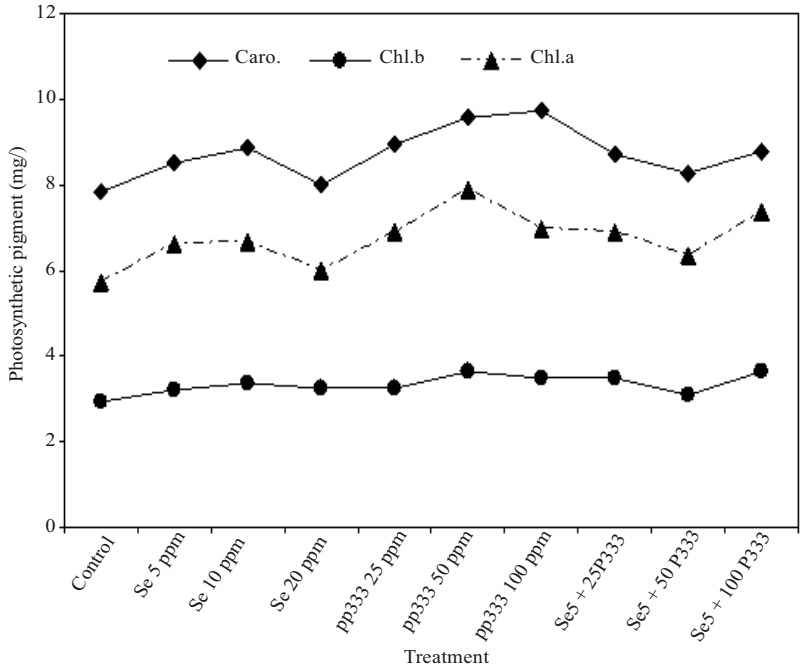

Fig. 1: Effect of selenium and / or paclobutrazole on photosynthetic pigments of gerbera plants (as mg /g fresh wt.)

Amino Acids Profile and Contents:

The effect of spraying gerbera plants with different concentrations of Se and /or PP_{333} on the profile and contents of the amino acids extracted from newly formed leaves is recorded in table (2). The data show that all the used treatments led to appearance of the new amino acid "methionine" as compared with the control plants.

Application of Se at the concentration of 10 ppm on gerbera plants led to marked increases in the profile and the amounts of all the studied amino acids as compared with the profile obtained from the control plants. Application of Se at 20 ppm caused increases in the profile and the amounts of the eleven following amino acids**:** asparatic, threonine, serine, proline, alanine, cystien, valine, leucine, isoleucine, phenylalanine and histidine. On the other hand, spraying the plants with Se at the rate of 5 ppm led to obvious decrease in the amounts of all the studied amino acids except cysteine which showed increment amounting to about 273.53 % over the control.

Table 2: Amino acids content (ug / ml) in leaves of gerbera plants treated with selenium and / paclobutrazole.

Characters / Amino acids	control	Se 5ppm	Se 10ppm	Se 20ppm	PP_{333} 25ppm	PP_{333} 50ppm	PP_{333} 100ppm	5 ppm Se + 25ppm PP_{333}	10ppm Se + 50ppm PP_{333}	20 ppm Se + 100ppm PP_{333}
Asparatic	59.09	44.03	108.18	81.68	55.51	71.47	68.12	39.02	48.13	23.79
Threonine	32.49	22.80	57.17	42.19	39.43	38.28	58.49	21.36	23.97	50.67
Serine	32.78	23.40	59.10	40.22	38.44	34.42	69.58	19.64	24.59	48.58
Glutamic	257.42	159.25	243.54	253.98	259.32	208.65	536.16	101.48	189.29	291.37
Proline	86.56	55.90	129.45	88.84	65.27	68.67	67.80	41.24	62.54	100.73
Glycine	20.11	8.72	22.72	13.90	53.21	17.23	95.45	10.99	13.08	15.92
Alanine	37.21	25.12	62.89	46.31	45.90	39.82	39.55	26.34	36.36	56.41
Cystein	1.36	5.08	14.49	1.40	0.01	7.49	0.60	3.08	7.50	2.19
Valine	34.60	22.20	57.37	44.34	43.88	42.80	86.63	21.96	30.82	52.13
Leucine	26.46	18.87	45.75	35.02	32.12	27.92	58.84	15.41	23.98	77.00
Isoleucine	55.18	34.04	91.50	64.10	62.46	52.88	108.92	28.68	24.82	26.00
Phenylalanine	15.38	10.55	26.04	22.08	18.90	21.04	30.35	10.98	13.67	54.00
Tyrosine	45.39	28.06	74.74	44.02	47.77	36.15	110.64	18.12	36.27	37.39
Histidine	28.80	18.26	48.53	30.28	31.16	28.28	66.40	16.77	26.43	62.77
Lysine	49.58	31.51	80.43	48.93	55.24	46.86	114.77	25.00	44.21	62.77
Araginine	58.53	32.27	89.92	4.00	23.57	4.55	114.87	2.50	42.45	31.76
Methionine	---	0.74	2.29	1.70	0.07	1.35	3.96	2.17	0.002	4.25

Regarding to the application of the growth retardant PP_{333} on gerbera plants, table (2) show that spraying the plants with PP_{333} at the rate of 25 or 100 ppm mostly led to increases in the profile and the content of most detected amino acids. These increments included, 12 and 13 amino acids the maximum values of the percentage were 164.59 % and 374.64 % for glycine with 25 and 100 ppm respectively. Application of PP_{333} at the rate of 50 ppm exhibited increases in nine amino acids; asparatic, threonine, serine, alanine, cystine, valine, leucine, phenylalanine and lysine as compared to the control, the maximum value of increment percentage was 450.74 % for cystin. The same treatment exhibited decreases in sex amino acids content as compared to the conterol (see table 2).

Concerning the interaction effect between Se and PP_{333} on gerbera plants, the data recorded in table (2) reveal that using Se at 20 ppm combined with PP_{333} at 100 ppm mostly led to increases in the amounts and the profile of amino acids (the increments included 11 amino acids) and the maximum value of the percentage of increments was about 191.01% over the control for phenylalanine. Application of Se at 5 ppm + PP_{333} at 25 ppm or Se at 10 ppm together with PP_{333} at 50 ppm exhibited increases only in cystin content which reached 126.47% and 451.47% respectively.

Selenium is an essential element for human and animal metabolism. Its antioxidant properties, comparable to those of vitamin E, are well known (Kabata and Pendias, 1999). However, when absorbed in higher concentrations, Se could be harmful as it catalyzes the oxidation of thiols and simultaneously generates super oxide σ_2 which means that it acts as a prooxidant (Zayed *et al.*, 1998 and Stewart *et al*., 1999). In the present investigation, the increments in the amino acids content due to application of Se is attributed to its chemical similarity to sulphur (S), therefore, Se can replace sulphur in compounds containing sulfhydryl group, such as sulphur amino acids, glutathione, cysteine, methionine and coenzyme A. When replacing sulphur in proteins, Se can modify their structure and properties (Harbone, 1997and Lyons *et al.,* 2005). Enzymes belong to the oxido-reductase class can be the most sensitive enzymes to Se owing to the antioxidant properties of the element.

Protein Electrophoresis:

Table (3) reveal the changes in the pattern of protein electrophoresis (SDS-PAGE) extracted from the newly formed leaves of gerbera plants treated with different concentrations of Se and / or PP_{333}. The molecular weights of the proteins ranged between 107. 44 -- 5.72 KDa and exhibited a maximum number of 32 bands. The results indicate that extracts of control plants contain only two bands of protein**:** band number 22 with molecular weight of 27.34 KDa and band number 23 with molecular weight 19.43 KDa. The first band (band no. 22) disappeared from the extracts of all the treatments while the second one (band no. 23) disappeared from the extract of one treatment only (Se at 10 ppm). In addition protein band number 24 having molecular weight 16.78 KDa appeared under the influences of all the used treatments compared to the control extracts. It is apparent from the table that the electrophoregram of the control plants. The scanning profile of such detected protein bands revealed that band number (23) having the molecular weight 19.43 KDa. produced the highest intensity of protein recorded (1.8 %).

Table 3: Comparative analysis of molecular weights and relative concentrations of different types of protein bands of gerbera plants treated with different concentrations of selenium and /or paclobutrazole.

Treatments											
		control	Se 5ppm	Se 10ppm	Se 20ppm	PP_{333} 25ppm	PP_{333} 50ppm	PP_{33} 100ppm	Se 5 ppm +25ppm PP_{333}	Se 10 ppm +50ppm PP_{333}	Se 20 ppm +100ppm PP_{333}
Band number	Mwt.										
1	107.44					3.1					
2	97.40										
3	79.05								1.7		
4	68.89							2.6			
5	66.00										1.3
6	65.86										
7	63.58			1.5							
8	59.18				1.7						
9	56.38						1.4				
10	55.84									0.7	
11	53.56										1.5
12	50.93										
13	47.27									1.1	
14	46.46							1.1			
15	44.95									1.7	
16	43.98		1.6			2.0					
17	41.90						2.1				
18	40.09			1.7							
19	32.02				1.4						
20	29.00										
21	27.85										1.9
22	27.34	1.6									
23	19.43	1.8	1.8		1.8	1.8	1.8	1.8	1.8	1.8	2.3
24	16.78			2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5
25	14.49			3.2	3.2						
26	12.81								1.9		
27	11.00		1.8								
28	10.84										
29	9.47				1.6						
30	7.06					2.3					
31	6.50										
32	5.72						4.2				
Total	number	2.0	3.0	4.0	6.0	5.0	5.0	4.0	4.0	4.0	6.0

Table (3) indicate that spraying gerbera plants with Se at the rate of 5 ppm induced the appearance of two new protein bands having molecular weights of 11.00 and 43.98 KDa.

The electrophoretic patterns in extracts obtained from plants treated with Se at 10 ppm showed the appearance of four new protein bands having molecular weights of 14.49, 16.78, 40.09 and 63.58 KDa respectively accompanied by disappearance of the two protein bands detected in the control extracts. The scanning profile existing protein band number 25, having molecular weight of 14.49 KDa produced the highest protein intensity (3.2 %).

Electrophoregram of the plants treated with Se at 20 ppm exhibited the presence of six protein bands having molecular weights ranging between 9.47—59.18 KDa, this treatment induced the appearance of five new protein bands having molecular weights of 59.18, 32.02, 16.78, 14.49 and 9.47 KDa.

The electrophoregram resulting from the application of PP_{333} at 25 ppm on gerbera revealed the appearance of four new protein bands having molecular weights of 7.06, 16.78, 43.98 and 107.44 KDa. The scanning profile of the existed protein band number (24) which have the molecular weight 16.78 KDa produced the highest protein intensity (2.5 %).

Application of PP_{333} at 50 ppm induced the appearance of four new protein bands having molecular weights ranging between 5.72—56.38 KDa. The electrophoregram of this treatment showed that the protein band number (32) which have the molecular weight 5.72 KDa induced a highest value (4.2 %) of protein intensity as compared with the values obtained from the all used levels of PP_{333}.

Table (3) show that spraying gerbera with PP_{333} at 100 ppm induced the appearance of three new bands of protein having molecular weights of 16.78, 46.46 and 68.89 KDa. The highest value of protein intensity (2.6 %) was obtained from the protein band number four which have the molecular weight 68.89 Kda.

The interaction effect between Se at 20 ppm and PP_{333} at 100 ppm induced the appearance of four new protein bands having molecular weights of 16.78, 27.85,44.95 and 66.00 KDa. The scanning profile of this treatment showed that the band number (24) which have the molecular weight 16.78 KDa. produced the highest intensity of protein (2.5 %).

The electrophoregram resulted from the application of PP_{333} at 50 ppm combined with Se at 10 ppm revealed the appearance of three new protein bands having the molecular weights of 16.78,48.50 and 55.84 KDa. The scanning profile of the existed protein band number (24) which have the molecular weight 16.78 KDa produced the highest protein intensity (2.5 %). The same trend was obtained from the application of Se at 5 ppm combined with PP_{333} at 25 ppm as three new protein bands were appeared having molecular weights 12.81,16.78 and 68,89 KDa. The highest value of protein intensity (2.5 %) was obtained from the band number (24) which have the molecular weight 16.78 KDa. In this respect, Bekheta (2004) showed that application of PP_{333} combined with gibberellic acid "GA_3" on wheat plants changed the electrophoretic profile of protein patterns.

The outcome of the obtained results clearly indicate that spraying gerbera plants before flowering stage with the antioxidant "Se" and /or the antigibberellin "PP_{333}" led to the appearance of new protein bands which varied according to the applied concentration. The existence of such newly formed protein bands in treated gerbera plants might be explained basing on the potentiality of Se and PP_{333} to trigger the expression of specific genes a long DNA molecule in the target cells, a process which appears to play a key role in regulating a cascade of biochemical reactions which might determine the ultimate appearance of growth patterns and yield of the produced plants. This accompanied by a persistent effect carrying over to the progeny via alteration of DNA- binding protein receptors mechanism which might amplify the signal-transduction pathway, this suggestion is reinforced by the findings of Jacobsen and Beach (1985), Hooley *et al*., (1990) and Abdel-Hamid (2002). In the present work the appearance of new protein bands due to the application of selenium could be attributed to the fact that selenium has chemical properties similar to those of sulphur and can be incorporated into proteins in place of cysteine and methionine. Insertion of selenocysteine and selenomethionine into protein could interfere with the formation of disulphide bridges, leading to slight structural changes which might lead to changes in the protein activity (Eustice *et al*., 1981; Brown and Shrif, 1982 ;Valkama *et al*.,2003 and Lyi *et al.,* 2007).

Histological Studies:

Pedicle Anatomy:

Increasing the thickness of gerbera pedicle cells is consider the main target of the present work. So, table (4) and figs (2) & (3) show that spraying the plants with Se and / or PP_{333} at all the used levels often caused increases in the thickness of cortex cells. The maximum percentage of increment in the thickness of cortex tissue (41.21%) was obtained from the application of PP_{333} at the rate of 50 and 100 ppm separately or in combination with Se at 20 ppm. On the other hand, application of Se at the rate of 5 ppm led to reduction in the thickness of cortex by -2.92% below the control.

Table (4) and figs (2) & (3) show that application of Se and /or PP_{333} at all the used concentrations led to increase in the thickness of collenchyma tissues, the maximum percentage of increments**:** 18.3%, 25.0% and 20.0 % were obtained from using PP_{333} at 50 ppm, Se at 5 ppm combined with 25 ppm PP_{333} or Se at 10 ppm combined with PP_{333} at 50 ppm respectively. The number of collenchyma cells mostly increased recording values ranging from 4.0 –6.3 according to the treatment used compared with the number obtained from the control plants (3.8 cells). The greatest number of collenchyma cells (6.3) was obtained from the application of Se at 5 ppm combined with PP_{333} at 25 ppm as compared to the control plants (3.8).

The thickness of the vascular bundles often increased under the influence of all the used treatments. The maximum percentage of increment in the thickness of vascular bundles (41.21%) over the control was obtained from the application of PP_{333} at the rate of 50 or 100 ppm. On the other hand, application of Se at 5 ppm led to reduction in the thickness of the vascular bundles by 9.1 % below the control. Outer fibrous thickness of vascular bundles of pedicle was increased by using Se at 20 ppm or PP_{333} at 25 ppm as they recorded the same value of percentage(33.3 %) over the control. In addition, mostly all the used treatments led to increases in the inner fibrous thickness of vascular tissue and the maximum value of thickness (100 %) over the control was obtained from the application of PP_{333} at the rate of 50 or 100 ppm.

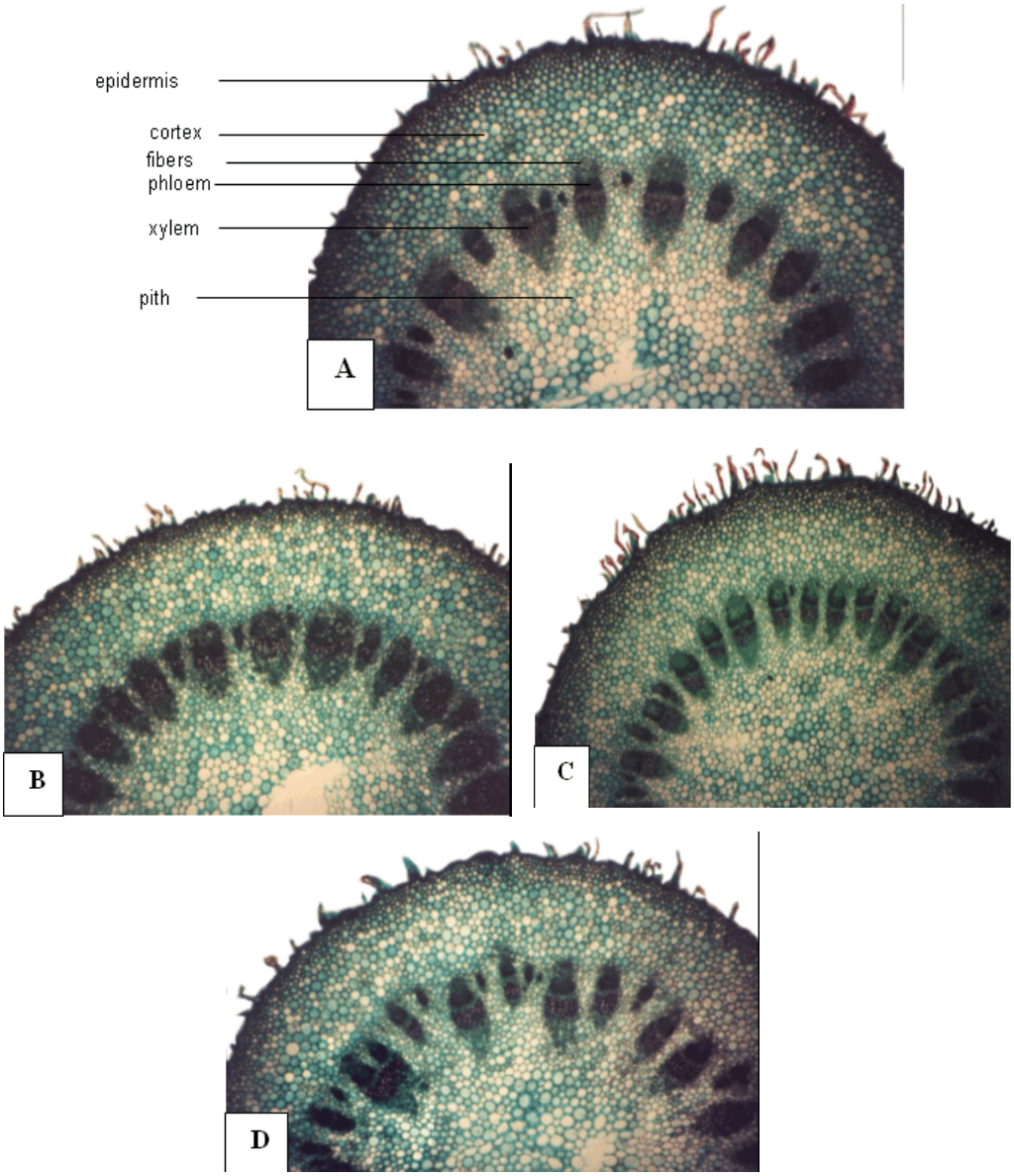

Fig. 2: Transverse sections through the middle part of pedicle of gerbera treated by selinium (Se) and / or paclobutrazol (PP_{333}). (X=100)

A- Control
B- Se (20 ppm)
C- PP_{333} (25 ppm)
D- Se (10 ppm) + PP_{333}(50 ppm)

Application of PP_{333} alone at all the used rates or at 100 ppm combined with Se at 20 ppm led to increases in the thickness of phloem tissues by (50%) over the control. Regarding to xylem and pith tissues, all the used concentrations of Se and /or PP_{333} caused increases in the thickness of these tissues. The highest percentage values (50%) for xylem and pith thickness was obtained from the application of PP_{333} at 50 &100 ppm.

Table 4: Counts and measurements (in microns) of certain histological features in transverse section through the middle part of gerbera pedicle treated with different concentrations of selenium and /or paclobutrazole.

Treatments / Characters	Control	Se 5ppm	Se 10 ppm	Se 20ppm	PP_{333} 25ppm	PP_{333} 50ppm	PP_{333} 100ppm	5 Se+25 PP_{333} ppm	10 Se+50 PP_{333} ppm	20 Se +100 PP_{333} ppm
Pedicle diameter	3581.50	4050.00	4125.00	3862.50	3675.00	4087.50	4200.00	3937.5	4125.00	3825.00
Epidermis thick.	18.75	18.75	18.75	18.75	18.75	18.75	18.75	18.75	18.75	18.75
Cortex thick.	424.90	412.5	506.25	562.50	525.00	600.00	600.00	487.50	450.00	600.00
Vasc. bundles thick.	412.50	375.00	450.00	543.80	562.50	600.00	600.00	487.50	450.00	487.50
Outer fibers thick.	112.50	75.00	75.00	150.00	150.00	112.50	112.50	112.50	75.00	75.00
Phloem tissue thick	75.00	75.00	75.00	75.00	112.50	112.50	112.50	75.00	75.00	112.50
Xylem tissue thick.	150.00	150.00	187.50	187.50	187.50	225.00	225.00	187.50	187.50	187.50
Inner fibers thick	75.00	75.00	112.50	131.30	112.50	150.00	150.00	112.50	112.50	112.50
Pith thick.	1725.00	2212.50	2062.50	1800.00	1650.00	1768.50	1950.00	2156.25	2137.50	1575.00
Chollenchyma. cortex thick	6.00	6.50	6.50	6.30	6.50	7.10	6.40	7.50	7.20	6.60
No. of choll-enchyma cells	3.80	4.75	4.25	3.30	4.00	3.70	4.10	6.30	4.50	4.25
No of Vasc. bund. thick.	29.00	36.00	32.00	32.00	33.00	36.00	46.00	34.00	36.00	31.00

Table 5a: Counts and measurements (in microns) of certain histological features in transverse section through the blade of median leaf of gerbera treated with different concentrations of selenium and /or paclobutrazole

Treatments / Characters	Control	Se 5ppm	Se 10 ppm	Se 20ppm	PP_{333} 25ppm	PP_{333} 50ppm	PP_{333} 100ppm	5 Se+25 PP_{333} ppm	10 Se+50 PP_{333} ppm	20 Se +100 PP_{333} ppm
Blade thickness	195.0	172.5	217.5	210.0	172.5	172.5	202.5	172.5	172.5	165.0
Upper Epi. thick.	15.0	15.0	15.0	15.0	15.0	15.0	15.0	15	15.0	15.0
Lower Epi. thick.	15.0	7.5	7.5	15.0	7.5	7.5	7.5	7.5	7.5	15.0
Mesophyll thick.	165.0	150.0	195.0	185.0	150.0	150.0	185.0	150.0	150.0	135.0
Palsied thick.	75.0	75.0	90.0	90.0	45.0	75.0	95.0	75.0	75.0	60.0
Spongy thick.	40.0	75.0	105.0	95.0	90.0	75.0	105.0	75.0	75.0	75.0
Midrib thick.	1290.0	1275.0	1480.0	1605.0	1170.0	1500.0	1740.0	1770.0	855.0	1380.0
Vasc.bund. thick	255.0	330.0	360.0	315.0	285.0	330.0	427.5	375.0	330.0	377.5
Outer fibers thick.	60.0	75.0	75.0	75.0	60.0	75.0	105.0	75.0	60.0	90.0
Phloem thick.	60.0	45.0	75.0	60.0	60.0	45.0	52.5	60.0	60.0	82.5
Xylem thick.	105.0	165.0	157.5	135.0	135.0	150.0	240.0	195.0	165.0	160.0
Inner fibers thick.	30.0	45.0	52.5	45.0	30.0	45.0	30.0	45.0	45.0	45.0
Vasc.bund. thick.	5.0	7.0	8.0	5.0	4.0	4.0	5.0	5.0	3.0	6.0

Table 5b: Influence of selenium or paclobutrazole on histological features of longitudinal section of gerbera pedicles (measurements in microns).

Treatments / Characters	Control	Se 20 ppm	PP_{333} 25ppm
	Cortex dimension		
Length	52.5	69.0	39.0
Width	37.5	39.5	30.0
	Pith dimension		
Length	52.5	75.0	50.5
Width	45.0	54.5	33.7

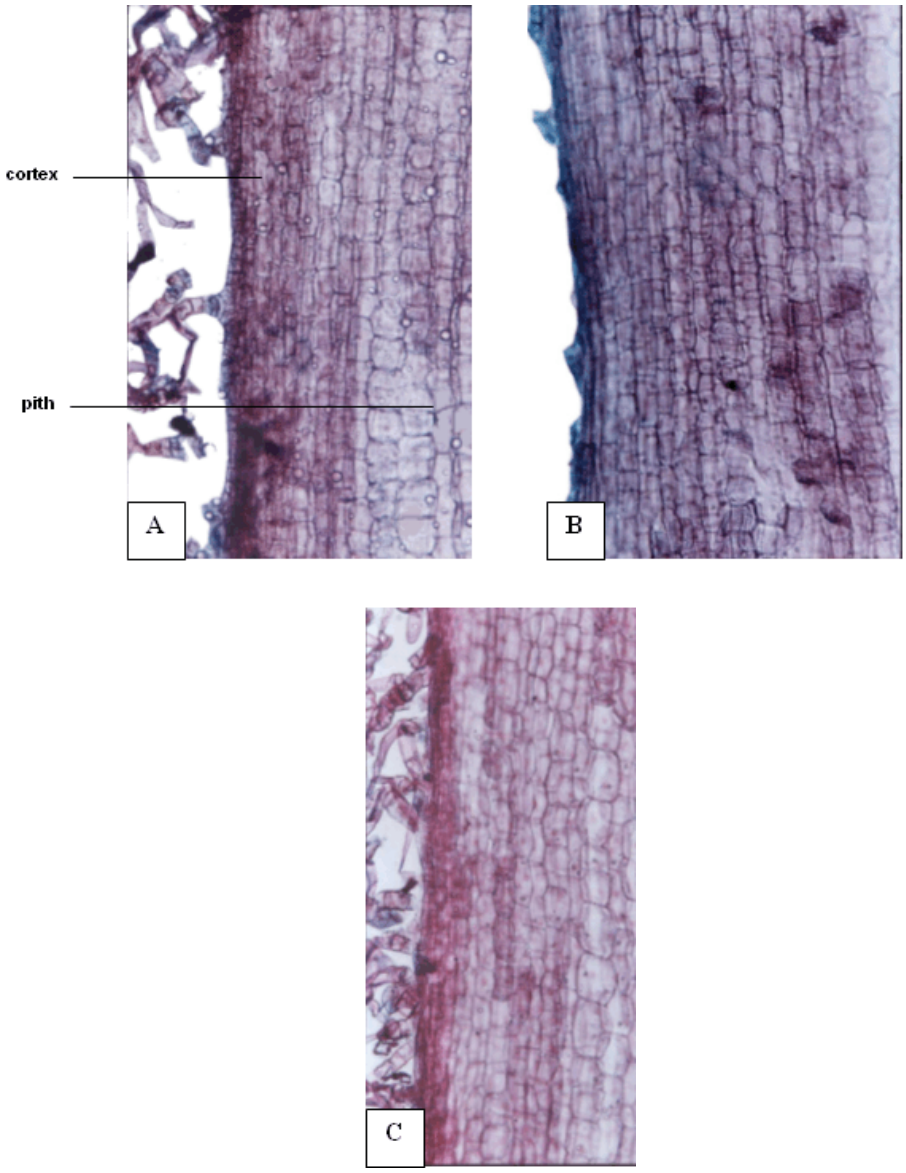

Fig. 3: Longitudinal sections of gerbera pedicle treated by Selenium or Paclobutrazole. (X= 100)
A- Control
B- Se at 20ppm
C- PP_{333} at 25 ppm

It is clear from table (4) and figs (2) & (3) that application of Se and / or PP_{333} at all the used treatments caused obvious increase in the number of vascular bundles, the increment values ranged between 32.0 – 46.0 as compared with the number of the control plants (29.0).

Concerning the longitudinal section, fig (3) illustrates that foliar application of Se at 20 ppm led to increases in the width and the length of the parenchyma cells of cortex layer by (5.3%) and (31.4%) respectively compared with the control. On the other hand, spraying gerbera plants with PP_{333} at all the used levels caused reduction in the width and length of parenchyma cells of cortex as compared to the control. From the mentioned results It can be concluded that application of PP_{333} and / or Se on gerbera plants led to increases in the thickness of cortex tissue (collenchyma cells), fibrous tissues, vascular bundles (phloem and xylem) cells. Such increments caused reduction in the pedicle

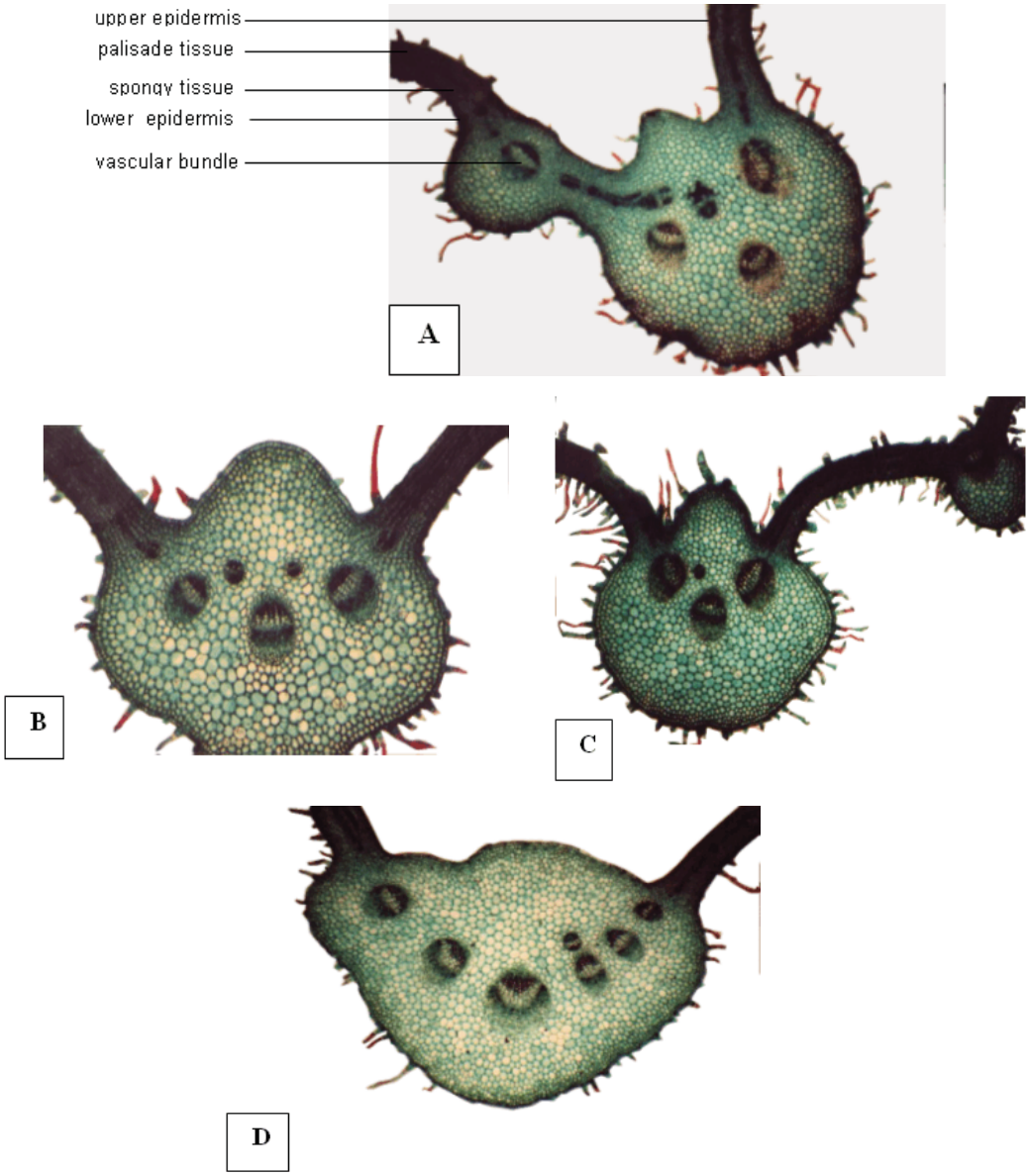

Fig. 4: Transverse sections through the blade of the median leave of gerbera as treated by Selenium and / or Paclobatrazol (PP_{333}). (X=40)

A- Control
B- Se (20 ppm)
C- PP_{333} (25 ppm)
D- Se (10 ppm)+ PP_{333} (50 ppm)

length, thickness in diameter which gave more support against lodging. The present results are in agreement with those obtained by Ali (2001) who found that application of ascorbic acid (antioxidant) at different concentrations (200, 400 or 600 ppm) on two cultivars of tomato at the age of 12 weeks induced anatomical changes in the stem of both the two cultivars 4 weeks after treatments. He also indicated that application of ascorbic acid at 200 or 400 ppm increased stem diameter and cortical layer thickness. In addition, Kurian, and Lyer (2003) also observed that higher phloem to xylem ratio in mango trees cultivar "Alphonso" dwarfed by external application of PP_{333} mainly due to fewer xylem vessels and smaller diameter of metaxylem vessels.

Leaf Anatomy:

Table (5) and fig (4) show that application of Se at all the used levels caused obvious increase in the thickness of the blade which reached a maximum percentage value (11.54 %) over the control with the rate of 10 ppm of Se, while, application of PP_{333} only at 100 ppm led to increase in the leaf blade thickness by (3.85 %) over the control. From these results, it can be concluded that the increment in the thickness of the blade might be attributed to the increase in palisade and spongy thickness.

Application of Se at 10 ppm or PP_{333} at 100 ppm led to obvious increases in mesophyll thickness, palsied thickness and spongy thickness as compared to the control plants.

Concerning midrib thickness most of the used concentrations of Se and / or PP_{333} mostly led to increase in the thickness of the midrib, exceptions resulted from the application of Se at 5 ppm, PP_{333} at 25 ppm & Se at 10 ppm combined with PP_{333} at 50 ppm as compared to the control. The thickness of leaf vascular bundles increased under the influences of all the used treatments and reached the maximum percentage value (67.5 %) with 100 ppm PP_{333} compared to the control.

Application of Se and /or PP_{333} at all the used levels led to obvious increase in the xylem tissue thickness and the highest percentage value of increment (128.57%) was obtained from the application of PP_{333} at 100 ppm. Such increment might be attributed to the increase in the outer fibrous thickness more than the phloem. Number of vascular bundles was also increased under the influence of Se at the rate of 5 and 10 ppm and Se at 20 ppm combined with PP_{333} at 100 ppm.

These results are in agreement with those obtained by Ali (2001) who found that application of ascorbic acid at the rate of 200, 400 or 600 ppm induced anatomical changes in the leaves of tomato plants. He also showed that application of ascorbic acid at 200 or 400 ppm increased the thickness of the palisade layer. In addition, Valkama *et al.,*(2003) indicated that a significant positive effect of Se on the leaf anatomy of strawberry plants was observed for the relative cross-sectional areas of vascular tissue and lower epidermis. They also found that application of Se at low concentration increased the area of low epidermis while its application at high concentration increased the thickness of vascular tissue to(6.5 %) compared to that of control plants (4.5 %).

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