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ORIGINAL ARTICLES

Anatomical Aspects of Gerbera Leaves under the Effect of Progestrone and Irridition Treatments

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ABSTRACT

In the present work off shoots of gebera plants were exposed to laser treatments (He-Ne and Ar.) with different exposure time, gamma rays (100,150 and 250 rad) progesterone with 10-20 and 30 ppm in single treatments or incombination.The effect of such treatments were investigated on leaf area and leaf structure. The obtained results from this study proved that: Single treatments with progesterone, gamma rays and laser treatments was efficient on leaf area and anatomical structure with variable degree Agron at exposure time 7.5 min.,followed by gamma rays 150 rad was more efficient in these respect and the combined treatment between He-Ne + progesterone showed a promotive effect. Anatomical structure of leaf was affected with variable degree with the provious mentioned treatments, (single and combined treatments) where progesterone 30 ppm combined with helium neon at exposure time 1 min.increased dimention of bundles, number of xylem rows and number of xylem vessels and had no or slight decreasing effect on number of bundles.

Key words: Gerbera, leaf area, anatomical structure, Gamma irradiation, laser rays, progesterone.

Introduction

Gerbera jamsonii flowers are one of the most attractive cut flowers. It has economic important for exportation. From the ornamental plants of view gerbera needed extensive efforts to improve the product quality. Plant growth promoting, irradiation treatments (gamma and laser) have attained much interest at different parts of world. The growth promoting Progesterone (Steroidal se hormone) has been reported to be present in apple seeds and in pea (*Pisum sativum*) as mentioned by Gawienowski and Gibbs, 1968 and Lino *et al*. (2007).

The effect of progesterone in inducing flowers or generation development in wheat was recorded by Janeczka and Filek, (2002) and confirmed by Braun and Wild (1984 a, b) on wheat and Ebtihal (2008) on *Triticum aestivum.*

The increase in leaf area of plants is expected to be may reflected on anatomical structure of leaves, though Gerard and Csaba (2002) mentioned that BRs promated vascular differention, where as Harb *et al*.(2005) mentioned that gamma rays had stimulative effect on anatomical parameters.However,there are scare in the literature devoted to the effect of laser on anatomical strucucture of plant organs. The present experiment was designed to investigate the response of leaf structure to irradiation treatments, progesterone and some of combined treatments.

Materials and Methods

Apot experiment was conducted during the seasons of 2006 and 2007 at greenhouse of National Research Center, Dokki, Egypt.For cultivation uniform small offshoots of *Gerbera jamesoni*i cv.superb were obtained from Zohria Garden 10-15 cm length with 2-3 leaves. The experiments were 40 treatments included control which were progesterone concentrations (10, 20 and 30 ppm), gamma rays doses (100,150 and 250 rad), helium neon laser (He-Ne) exposure time (1, 5.5 and 11.5 min), Argon laser rays (Ar) exposure time (1, 7.5 and 15.5 min) and the interaction between different progesterone concentrations and the ether treatments. In both seasons off shoots subjected to gamma rays using Co60 source and laser irradiation from the standard device was generally characterized in terms of power [in units of Watts (W) and milli watts (Mw)]. The power levels vary from laser to laser (Alawacy, 1988).The energy in Jouls is defined as the power multiply, with time intervals during which its emitted.i, e Energy (Joules) = Power x time (second). As for our experiment helium-neon (He-Ne) laser of power 20 Mw and argon (Ar) laser of power 15 Mw were used for the shoots of *Gerbera Jamesoii* cv.superba for red and green light irradiation respectively. The progesterone (10,20 and 30 ppm) concentration

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sprayed twice; the first was 15 days after transplanting and the other four weeks later. Besides progesterone concentrations were mad single or combinations with every one of the other irradiation treatments taking all possible combination i.e. irradiation treatments followed by progesterone. The experiment layout was arranged in completely randomized block design where each treatment replicated 3 times and each replicates represented by three pots. The plants were left under natural daylight in the greenhouse, the fertilizers and water supply were added as recommended by Agriculture Research Center.

At flowering stage 120 days from transplanting, samples were collected from treatments to calculate the leaf area (in both seasons) and studying the anatomical structure of some treatments (data of one growing season was tabulated) 14 treatments were choosed randomly and prepared for anatomical structure Table (2).

The preparation of leaves was carried out according the methods described by Johansen (1940) and (Corgen and Widmayer, (1971).and sections were mounted in Canada balsam then examined microscopically and microphotography.

Results:

Effect of progesterone, irradiation treatments and their combination on leaf area and its anatomical structure:-

Table 1: Effect of progesterone, gamma radiation, helium neon, argon, and some of their interaction between progesterone and irradiation treatments on leaf area of *Gerbera jamasonii* plants during the two seasons of 2006 and 2007

	Leaf area (cm^2)	
Treatments	1st season	2nd season
Cont	64.21 m-o	66.60 kl
$prog_1$	66.63 l-n	94.09 b
$Prog_2$	54.48 p	56.72 mn
$Prog_3$	74.42k	75.48gh
γ_1	76.27 jk	71.98 ij
γ_2	80.66 hi	95.39 ab
γ_3	67.18 l-n	54.32 n
Ar_1	67.42 lm	69.09jk
Ar_2	103.5 b	75.84 gh
Ar_3	69.23 l	81.67 f
$He\text{-}Ne_1$	95.85 d	56.13 mn
$He\text{-}Ne_2$	60.97 o	53.39n
$He\text{-}Ne_3$	85.21 fg	57.70 m
γ_1+ $Prog_1$	68.45 l	75.77gh
γ_2+ $Prog_1$	63.7 no	69.48 j
γ_3 + $Prog_1$	83.88 fg	76.69 g
Ar_1+ $Prog_1$	95.10 de	85.41de
Ar_2+ $Prog_1$	106.5 b	88.34 c
Ar_3+$Prog_1$	93.37 de	97.36 a
$He\text{-}Ne_1$+ $Prog_1$	73.73 k	82.43 f
$He\text{-}Ne_2$+ $Prog_1$	99.33 c	87.64 cd
$He\text{-}Ne_3$+ $Prog_1$	92.12 e	83.57ef
γ_1+ $Prog_2$	75.62jk	75.40 gh
γ_2+ $Prog_2$	74.92 i-k	71.54 ij
γ_3+ $Prog_2$	83.92 f-h	88.14 cd
Ar_1+ $prog_2$	86.25 f	70.14 j
Ar_2+ $Prog_2$	73.82 k	64.77 l
Ar_3+ $Prog_2$	87.19 f	86.95 cd
$He\text{-}Ne_1$+ $Prog_2$	103.4 b	92.89 b
$He\text{-}Ne_2$+ $Prog_2$	83.95 f-h	83.39 ef
$He\text{-}Ne_3$+ $Prog_2$	124.7 a	97.80 a
γ_1+ $Prog_3$	68.69 l	71.45 ij
γ_2+ $Prog_3$	69.01 l	69.44 j
γ_3+ $Prog_3$	78.08 ij	77.80 g
Ar_1+ $Prog_3$	80.63 hi	86.97 cd
Ar_2+ $Prog_3$	66.33 l-n	73.66 hi
Ar_3+ $Prog_3$	57.00 p	64.00 l
$He\text{-}Ne_1$+ $Prog_3$	86.88 f	86.00 c-e
$He\text{-}Ne_2$+ $Prog_3$	84.85 fg	85.33 de
$He\text{-}Ne_3$+ $Prog_3$	82.08 gh	81.91 f

Prog.: Progesterone , $prog_{.1}$:10 ppm , $prog_2$:20 ppm , $prog_3$:30 ppm, γ: Gamma radiation , γ_1:100 rad , γ_2:150 rad , γ_3:250 rad , He-Ne : helium-neon , $He\text{-}Ne_1$:1 min $He\text{-}Ne_2$:5.5 min , $He\text{-}Ne_3$: 11.5 min , Ar : Argon , Ar_1:1 min, Ar_2:7.5 min , Ar_3: 15.5 min Data with the same letter vertically are not significant according to Duncan's multiple range test at 5 % level.

1- Leaf area:

Leaf area of gerbera offshoots was greatly affected with variable degree with progesterone, irradiation supplied alone or in combination .Table (1). Offshoots irradiated with Argon laser at exposure time 7.5 min.followed by gamma rays at 150 rad induced additional increase in leaf area two seasons compared with the control, through offshoots treated with 30-ppm progesterone induced increases in leaf area, reached 15.90 and 13.33% over the control plants. In the first and second seasons respectively. In combined treatments, data reveal that, all of the combined treatments proved to be more efficient on leaf area compared with control and single treatments in most cases. Combination of progesterone with irradiation treatments had generally resulted substantial improvement in leaf area, such effect explained in term of synergistic effect.

The highest leaf area in the first season i.e 124,127.7 cm2 was recorded with the combined treatment of (He-Ne) at exposure time 11.5 min.combined with 20 ppm progesterone. In the second season the previous mentioned treatment showed the highest increasing effect compared with the control.

2- Anatomical structure of the leaf:

a- Number of vascular bundles:

Table (2) reveals that the chosen single treatments, progesterone 30ppm, gamma ray150 rad, Helium-neon at exposure time 1min. and Argon at exposure time 7.5 min.increased number of vascular bundles compared with the control fig (1) A.The highest number of vascular bundles were recorded by treated plants with gamma rays at 150 rad, followed by Argon rays treatment at exposure time 7.5 min. Fig1 (C, E). In combined treatments, progesterone at 20 ppm combined with He-Ne rays at 11.5 min or Ar. rays 15.5 min.exposure time proved to be more efficient in increasing number of vascular bundles compared with all treatments including control, whears He-Ne laser at 11.5 min. and /or Ar. laser at 7.5 min exposure time combined with progesterone at 10 ppm reduced number of vascular than the control, Table (2) or Fig (3)

B-Dimension of bundles (length/width µm):

The same table (2) shows that the chosen single treatments increased the length of bundles compared with the control. While the lowest bundles width was recorded from plants treated with He-Ne at 1 min.exposure time compared with control.

The combined treatments, generally increased length of bundles with some exception. The highest dimension value 525 µ was recorded when offshoots treated with 10 ppm progesterone combined with gamma rays 250 rad.

C- Thickness of midvein (µm):

In general, the chosen single treatments increased thickness of midvein. The treatment of progesterone 30 ppm and He-Ne at exposure time 1 min. proved to be more efficient in this respect Fig (1,B.D).

The combined treatments interact in different ways.Progestrone 20 ppm treatment combined with He-Ne at exposure time 11. min. was efficient ,though 10 ppm progesterone combined with Ar. at exposure time 7.5 min. had very low efficiency compared with all treatments including control.

d- Thickness of lamina(µm):

The same table shows that most of the chosen treatments caused a depressive effect on thickness of lamina. The promotive effect on thickness of lamina were recorded for the single treatments of He-Ne rays at exposure time 1 min.,while 20 ppm progesterone combined with Ar, laser at exposure time 15.5 min showed the higher response in this respect.

E- Number of xylem rows:

Examination of data in Table (2) and Fig (2, B, D) indicate that, the single treatment of 30 ppm progesterone and He-Ne at exposure time 1 min. greatly increased number of xylem row compared with the control.

Comparing the interaction treatments, it is evident that the highest increments in number of xylem row were recorded for the treatments of progesterone 30 ppm compared with He-Ne rays at exposure time 1 min., compared with the other treatments and control plants.Fig (4,I).

f- Number of vessels:

Table (2) shows that , progesterone 30 ppm treatment was more efficient among the single treatments in increasing number of vessels though both treatments gamma rays 150 rad and He-Ne at exposure time 1 min. decreased number of vessels compared with the control plants. In combined treatments, the higher average numbers of vessels were produced by the treatment of 30 ppm progesterone combined with He-Ne at exposure time 1 min.

Discussion:

Our results presented in this paper demonstrated that, single treatments of progesterone (steroidal sex hormone), gamma rays, laser and their combination showed stimulatory effect with variable degree in leaf area and leaf structure.

Progesterone 30 ppm was more efficient than other treatments and control on increasing number of vessels, number of xylem row and thickness of midvein,while it has decreasing effect on thickness of lamina.

The effect of progesterone may be due to, it is a new group of plant hormone affect on cell elongation or cell division or may be act as brasinostroids compound which have a role in the control of RNA synthesis (Mandava and Thomson 1983) and Hamada (1986), or may be due to its effect on stimulating same endogenous hormones such as cytokinin and GA biosyntheses (Metwally 2010).

This results hold true with the finding of Ebtihal (2008) on *Triticum aestivum,* However,Gerard and Csaba (2002) mentioned that brasinosteroid compounds promoted vascular differentiation.Also the effect of steroidal compound on growth ,elongation and regulating gene exparasion was recorded by Zurek and Clouse (1994)

Our results also show that gamma and laser treatments increased leaf area and changed anatomical structure, and these depended on irradiation dose or time exposure,where gamma rays 150 rad was more efficient than other treatments in increasing number of vascular bundles and number of vessels.

Thus the effect of gamma rays in these respect may be due to that it belongs to ionizing radiation and interact to atoms or molecular to produce free radical in the cell,these radicals can damage or modify important compounds in plant cell or may be affected differentially the morphology and anatomy which depended on irradiation dose.(Kin *et al.*2004,Kovacs and Kereszies 2002 and Wi *et al.*2005),or may be due to its promotive effect on POD which essentially for different cellular function such as cell elongation,lignification,suberization and regulation of cell wall (Chendo and Singh,1997,Espelie *et al*.1986 and Lagrimmi *et al* 1987).

The finding of Seung Gon *et al* (2007) emphasized the effect of gamma irradiation on anatomical structure of some organs such as thylekoid and mitochondria of plant.

Our results also show that, laser irradiation He-Ne at exposure time 1 min and Argon at exposure time at 7.5 min. which differ in wave length and exposure time show apparent effect on leaf structure and leaf area, and the extent of effect differ according to exposure time, where He-Ne laser at exposure time 5.5 min.was more effective in increasing leaf area, length of vascular bundles, thickness of midvein,thickness of lamina and number of xylem row, while Ar. at exposure time 7.5 min was more efficient on number vascular bundles and number of vessels.

Table 2: Effect of progesterone, gamma, helium neon and argon laser irradiation as well as the interaction between progesterone with irradiation treatments on leaves anatomical structure of *Gerbera jamesonii* plants, (Date of one growing season 2006).

Parameters / Treatment	Number of bundles	Dimension of bundle		Thickness of midvein µm	Thickness of lamina µm	Number of xylem Rows	Number of vessels
		Length µm	Wide µm				
Cont	3	330	300	1200	315	6	71
$Prog_3$	5	450	255	1500	300	10	100
γ_2	7	330	195	1425	255	6	65
$He\text{-}Ne_1$	4	450	285	1500	360	9	65
Ar_2	6	360	225	1470	255	5	75
γ_3+Prog_1	3	525	450	1455	285	8	46
$He\text{-}Ne_3+Prog_1$	2	375	360	1080	240	6	53
Ar_2+Prog_1	1	390	330	945	240	8	130
γ_3+Prog_2	2	345	330	975	225	6	58
$He\text{-}Ne_3+Prog_2$	7	420	375	1500	270	7	52
Ar_3+Prog_2	5	420	270	1380	390	5	36
γ_3+Prog_3	4	360	210	1305	240	8	67
$He\text{-}Ne_1+Prog_3$	2	450	420	1380	270	11	136
Ar_1+Prog_3	3	300	165	1260	285	6	42

Prog. : Progesterone	$prog._1$:10ppm	$Prog._2$:20ppm	$Prog._3$:30ppm
γ: Gamma radiation	γ_1:100 rad	γ_2:150 rad	γ_3:250 rad
He-Ne: helium-neon	$He\text{-}Ne_1$: 1 min.	$He\text{-}Ne_2$:5.5 min.	$He\text{-}Ne_3$:11 min.
Ar: Argon	Ar_1:1 min.	Ar_2:7.5 min.	Ar_3 15.5 min.

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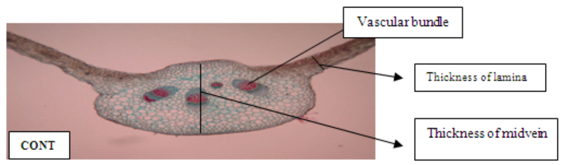
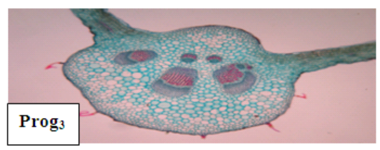
The promotive effect of laser on leaf area were emphasized by Angelov (1987) on maize plants, Yi Ping Chen *et al.* (2005) on *Isatis indogotica* Zong Boqiu *et al* (2008).The results could be explained that may be laser effect on cell division of the exposure shoots and this effect continues to the cell division of plants specially leaves, or may the endogenious content of GA is promoted by red light treatments (Kamiya *et al* 1999) or may be GA cause cell elongation by induction of enzymes that weaken the cell wall of the leaves (Macleod *et al* 1962) However, the effect of laser on anatomical structure of allium was recorded by Bariglio (1989).

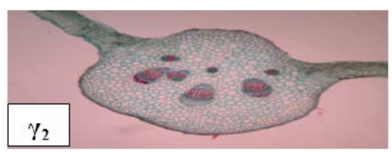
Our results also show that the combined treatment between progesterone 30 ppm and He-Ne at exposure time 1 min.proved to be more efficient on most of structure of leaves. These results may be due to the stimulatory effect of progesterone is activated by irradiation treatments or may be prasinosteroids represented by progesterone have synergistic effect with irradiation treatments.However,Scanty information was devoted to the combined treatments between irradiation and most of biological steroidal sex hormone on plant structure.



(A)-Untreated plant (control)

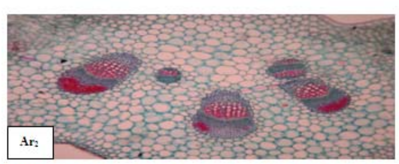


(B)- Plant treated with 30 ppm progesterone.



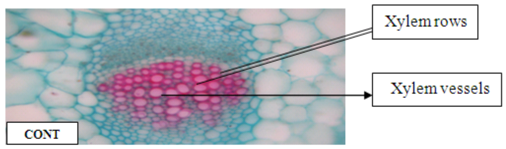
(C)- Plant treated with gamma rays (γ_2) 150 rad

(D)- Plant treated with helium-neon laser ($He\text{-}Ne_1$) laser rays 1 min. exposure.

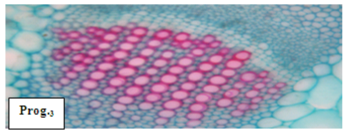


(E)- Plant treated with argon (Ar_2) laser rays 7.5 min.exposure.

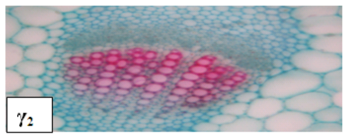
Fig. 1: Transverse section through the blade of the fourth leaf developed on the main stem of *Gerbera jamesonii* cv.superba plant. (x = 100).



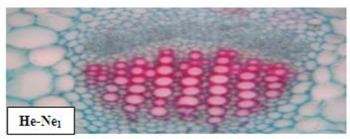
(A)- Untreated plant (control)



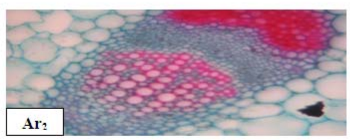
(B)- Plant treated with 30 ppm progesterone.



(C) – plant treated with gamma rays (γ_2) 150 rad.

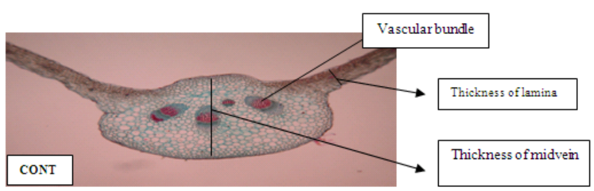


(D)- Plant treated with helium-neon (He-Ne$_1$) laser rays 1 min.exposure

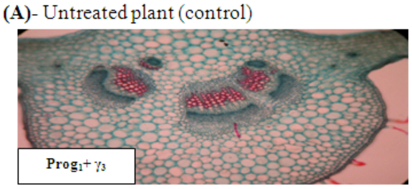


(E)- Plant treated with argon (Ar$_2$) laser rays 7.5 min. exposure.

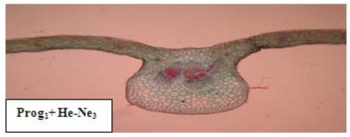
Fig. 2: Transverse section through the blade of the fourth leaf developed on the main stem of *Gerbera jamesonii* cv. superba plant. The section shows vascular bundle (vessels and number of xylem rows). (x = 400).



(A)- Untreated plant (control)

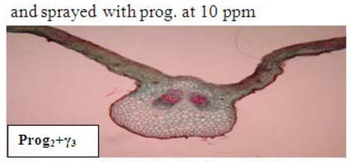


(B)- Plant treated with gamma rays (γ_3) 250 rad and sprayed with prog. at 10 ppm

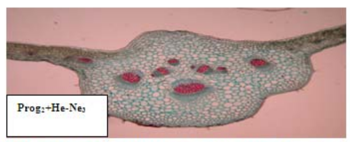


(C)- Plant treated with He-Ne_3 laser 11.5 min.exposure and sprayed with prog. at 10 ppm

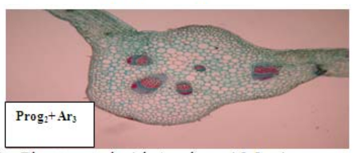
(D)-Plant treated with argon laser (Ar_2) 7.5 min.exposure and sprayed with prog. at 10 ppm



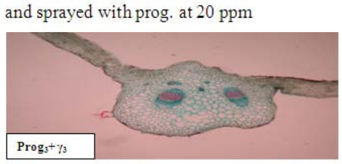
(E)- Plant treated with He-Ne_3 laser 11.5 min. exposure and sprayed with prog. at 20 ppm.



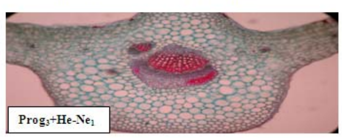
(F)- Plant treated with gamma rays (γ_3) 250 rad and sprayed with prog. at 20 ppm



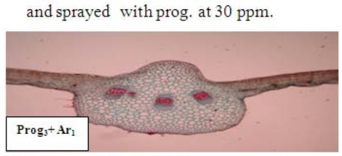
(G)- Plant treated with Ar_3 laser 15.5 min. exposure and sprayed with prog. at 20 ppm



(H)- Plant treated with gamma rays (γ_3) 250 rad and sprayed with prog. at 30 ppm

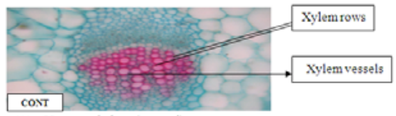


(I)- Plant treated with He-Ne_1 laser 1min.exposure and sprayed with prog. at 30 ppm.

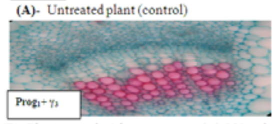


(J)- Plant treated with Ar_1 laser 1 min. exposure and sprayed with prog. at 30 ppm.

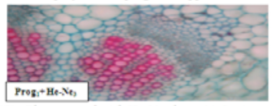
Fig. 3: Transverse section through the blade of the fourth leaf developed on the main stem of *Gerbera jamesonii* cv. superba plant. (x = 100).



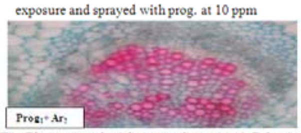
(A)- Untreated plant (control)



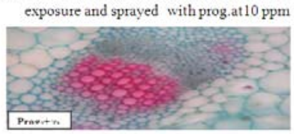
(B)- Plant treated with gamma rays (γ_3) 250 rad and sprayed with prog. at 10 ppm

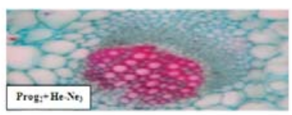


(C)- Plant treated with He-Ne$_3$ laser 11.5 min. exposure and sprayed with prog. at 10 ppm

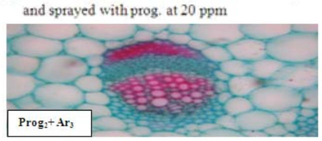


(D)- Plant treated with Argon laser (Ar$_2$) 7.5 min. exposure and sprayed with prog.at10 ppm

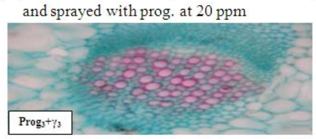

(E)- Plant treated with gamma rays (γ_3) 250 rad and sprayed with prog.at 20 ppm



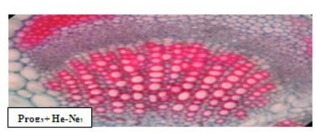
(F)- Plant treated with He-Ne$_3$ laser 11.5 min. exposure and sprayed with prog. at 20 ppm



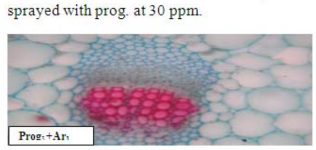
(G)- Plant treated with Ar$_3$ laser at 15.5 min. exposure and sprayed with prog. at 20 ppm



(H)- Plant treated with gamma rays (γ_3) 250 rad and sprayed with prog. at 30 ppm.



(I)- Plant treated with He-Ne$_1$ laser 1min.exposure and sprayed with prog. at 30 ppm.



(J) - Plant treated with Ar$_1$ laser 1 min. exposure and sprayed with prog. at 30 ppm.

Fig. 4: Transverse section through the blade of the fourth leaf developed on the main stem of *Gerbera jamesonii* cv. superba plant. The sections show vascular bundle (vessels and number of xylem rows). (x = 400).

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