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ORIGINAL ARTICLE

Effect of Glycine Betaine and Salicylic Acid on Growth and Productivity of Droughted Wheat Cultivars: Image Analysis for Measuring the Anatomical Features in Flag Leaf and Peduncle of the Main Shoot

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The present study was planned to investigate the possible role displayed by water stress and application of GB, SA or their interaction on some anatomical features in flag leaf at anthesis (after 95 days from sowing) by measuring leaf thickness, ground tissue thickness, number of hairs, metaxylem vessel area, xylem vessel area, phloem tissue area, vascular bundle tissues area, number of motor cells as well as number of opened and closed stomata on both upper and lower epidermis and some anatomical features of peduncle (peduncle diameter, tracheids area, metaxylem vessel area, xylem area, phloem area, vascular area, number of vascular bundle as well as opened and closed stomata) of the two wheat cultivars. Water stress markedly affected the anatomical features in flag leaves of both wheat cultivars. It caused massive decreases ($P< 0.05$) in the leaf thickness, ground tissue thickness, number of hairs, metaxylem vessel area, xylem vessel area, phloem tissue area, vascular bundle area, number of motor cells as well as number of opened stomata on both upper and lower epidermis. On the other hand, water stress increased ($P< 0.05$) the number of hairs and closed stomata on both upper and lower epidermis in flag leaves of the two wheat cultivars. The magnitude of decrease in all anatomical features in flag leaf was more pronounced with the sensitive cultivar. Furthermore, water stress led to a marked decrease ($P< 0.05$) in peduncle diameter, tracheids area, metaxylem vessel area, xylem tissue area, phloem tissue area, vascular tissue area, number of vascular bundles as well as opened stomata but increased the number of hairs and closed stomata on the peduncle surface of the two wheat cultivars. In relation to wheat cultivar, the sensitive was more affected by water stress than the resistant one. The application of GB, SA or their interaction induced some modifications in the anatomical features of the flag leaf and peduncle of main shoot which appeared to be an adaptive response to drought stress

Key words: Anatomy, Glycine betaine, Drought, Wheat, Salicylic acid

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Key words: Anatomy, Glycine betaine, Drought, Wheat, Salicylic acid

Abbreviations: Glycine betaine= GB, Salicylic acid= SA, water Stress= SW

Conductive canals between source and sink in wheat cultivars play a prominent role in translocating the photosynthates to the developing grains (Abd El-Gawad *et al*., 1985). Anatomical changes induced by water deficits in higher plants were better-observed indicators, which can be directly applied to agriculture and handled. To aim at exploring efficient anatomical indices, much information has been documented, but more attention should be paid to link them with physiological and molecular one (Shao *et al*., 2008).

In principal, the production of cereal grains is directly correlated to the growth of shoot as the main factory in which assimilates are produced. However, the photosynthetic ability of these shoots depends mainly on their pigments content (Aldesuquy and Baka, 1991). Considering the conclusion of Egeli *et al.* (1985) that the accumulation of dry matter in the grains requires the production of assimilates in the leaves, their translocation to the rachilla of the spikelets, movement into the endosperm and embryo of the grain and synthesis of materials to be stored. Leaf structure seems to be of great importance in the regard. Thus conductive canals between source and sink in wheat cultivars have been reported to the developing grains (Evans *et al*., 1970).

In water-stressed barley, wheat and horse bean plants the diameter of xylem vessels decreased noticeably. In addition the thickness of the walls of the conduction path in leaves, stems and roots increased resulting in inhibition of further transport (Udovenko *et al*., 1976).

Soil drench with sodium salicylate at 4000 and 8000 ppm increased the thickness of wheat leaf, leaf ground tissue and number of fibrous layers at the upper epidermis, whereas, the xylem thickness increased only with higher concentrations (Abo-Hamed *et al*., 1987). Furthermore, irrigation of wheat plants with NaCl, particularly 99mM induced noticeable increases in flag leaf blade, mesophyll and xylem tissue thickness, phloem tissue area and number of motor cells as well as number of hairs on lower epidermis. On the other hand, NaCl at 66 mM reduced all the pervious anatomical features. Treatment with NaCl generally reduced the peduncle diameter, xylem tissue thickness number of hairs as well as number of stomata of the peduncle of the main shoot (Aldesuquy *et al*., 1998).

MATERIALS AND METHODS

Plant Material and Growth Conditions

Two wheat cultivars (*Triticum aestivum* L.) Sakha 94 (sensitive var.) and Sakha 93 (tolerant var.) were used in this study. The variety Sakha 93 is known to be more drought tolerant than Sakha 94. These two varieties are common in Egypt.

A homogenous lot of wheat grains (i.e. either sensitive or tolerant var.) were separately surface sterilized by soaking in 0.01 % $HgCl_2$ for 3 minutes, followed by thoroughly rinsing in sterile water. The sterilized grains from each variety were divided into two sets (≈ 500 g per set for each var.). Grains of 1st set and 2nd set were separately soaked in distilled water or salicylic acid (0.05 M) respectively. In 20 November 2005, grains of each set were planted in plastic pots (fifteen grains per pot; 25cm width X 30cm height) filled with 6 kg mixed soil (clay and sand = 2:1, v/v). The pots were kept in a greenhouse and the plants were subjected to natural day/ night conditions (minimum /maximum air temperature and relative humidity were 29.2 / 33.2 ºC and 63/68 % respectively). Irrigation to field capacity was carried out when soil water content had fallen to 60% of its initial value. Twenty days after planting, thinning to five uniform seedlings per

pot took place.

On the day 65 after planting (at the beginning of heading) the pots of the 1st set was allocated to four groups (20 pots per each group) as follows: control (cont.), water stress (WS), glycine betaine control (GB.), glycine betaine + water stress (GB + WS). The 2nd set group was allocated to four groups as follows: salicylic acid control (SA), salicylic acid + water stress (SA+WS), control glycine betaine + salicylic acid (GB + SA) and glycine betaine + salicylic acid + water stress (GB+SA+WS). For glycine betaine (10 mM) treatment, the plants were sprayed by glycine betaine 48 hrs before starting the stress period and weekly during the stress period.

Water deficit was imposed by withholding water at the reproductive stage for 30 days within two periods: on the day 65 from planting (heading stage) and the day 80 from planting (anthesis stage). Each droughted pot received 500 ml water at the end of 1st stress period. At the end of stress periods, rewatering to the field capacity was carried out. The undroughted (control) plants were irrigated to the field capacity during the stress period, and all plants were left to grow until grain maturation under normal irrigation with tap water.

At the bud stage, 20 days from planting (tillering stage), and before heading (at ear emergence) the plants received 35 kg N ha^{-1} urea and 35 kg P ha^{-1} potassium dihydrogen phosphate as fertilizers.

Anatomical Studies:

For anatomical studies, samples from fresh plant materials were used. Samples were killed and fixed in Formalin- Acetic acid –Alcohol (FAA) for at least 48 hours. Dehydration, sectioning staining and mounting procedures was followed according to the method described by Sass (1951). Sections were cut at thickness 15 µm, and then stained with safranin and light green combination. Canda Balsam was used as mounting medium. Sections were estimated by the aid of light microscope. Measurements of all anatomical parameters were calculated in keel region to µm.

Measurements of conductive canals area in flag leaf and peduncle:

A new technique developed using the image analysis for measuring the anatomical features of leaf and peduncle of the wheat plants was performed using the following steps:

1. Image acquisition-obtaining precise microscopic images (transmitted) of the leaf and peduncle to determine (the areas of metaxylem, tracheids, xylem, phloem, and vascular bundle of the leaf and peduncle).

2. Color planes HSL extraction- this steps aims at extraction of saturation plane from HSL images. (Note: Because each color plane is made up of 8 bits, the color plane extracted will appear as an 8-bit grayscale image).

3. Bright points filtering out- In this step bright points in the image that are associated with the periodic structure of the web are filtered out.

These bright and relatively small points could be confused with pores if they were not removed. The Gaussian model for the background is applied locally to the image to establish the threshold for each area of the image. The result is a binary image where objects pores are segmented from the background are converted into black segments.

Images are two-dimensional computer arrays of numbers. Each point in the image has x and y coordinates so that pixels are often specified by (x, y). Images can be of several types but in this analysis only 2 types are considered: integer or grey level images, and binary images. Integer or grey

level images are typically the most common type. Each of the pixels has an integer value which might be between 0 and 255 or possibly something larger. Usually each possible value is associated with a shade of grey between black (0) and white (the maximum value). Binary images contain only 0's and 1's and are the same as 1 bit integer images. Binary images are usually created by the image analysis technique. Very often we want to identify some parts of the image and, for example, measure their geometrical features. The way this is done is to create a binary image with 1's in the feature area and 0's everywhere else. This removes all the information from the image except the part we want, which is where the features are and then makes the measurements on the binary image (Gonzalez and Woods, 1992).

It was also important to implement techniques of image segmentation to measure and count special features contained in the image. Image segmentation implies separating the parts of the image which are of interest from the rest.

In order to segment the image, a threshold of darkness was established using image processing of the grey levels. In other words, all grey levels darker than some value G were considered as ink and everything else as a background. The image was then converted to binary, which gives us a binary image containing 1's in the places where the original image was < G and 0's where the original image was > G. In other words, measures and counts of clusters of 1's in this binary image (representing the needed areas) were made.

The raw data from each measurement was in pixels. These were converted into real area units by calibration. This was done by measuring the size in pixels and calculating a scale factor in mm (or µm) per pixel. In this case, the lengths are multiplied by this value and the areas are multiplied by its square. Sometimes the scaling is different in the x and y directions and two scale factors have to be used.

RESULTS

Flag leaf anatomy:

Water stress markedly affected the anatomical features in flag leaves of both wheat cultivars (Table 1 & Plates 1-8). It caused massive decreases ($P< 0.05$) in the leaf thickness, ground tissue thickness, number of hairs, metaxylem vessel area, xylem vessel area, phloem tissue area, vascular bundle area, number of motor cells as well as number of opened stomata on both upper and lower epidermis. On the other hand, water stress increased ($P< 0.05$) the number of hairs and closed stomata on both upper and lower epidermis in flag leaves of the two wheat cultivars. The magnitude of decrease in all anatomical features in flag leaf was more pronounced with the sensitive cultivar.

Foliar application with GB or presoaking in SA mitigated the adverse effect of water stress on the anatomical features in flag leaves of the both wheat cultivars. Treatment with GB+SA appeared to be the most effective in the recovery of the adverse effect of water stress on anatomical features of flag leaves.

Peduncle Anatomy:

As compared to control values, water stress caused noticeable changes in the anatomical features of the two wheat cultivars peduncle (Table 2 & Plates 9-16). Water stress led to a marked decrease ($P< 0.05$) in peduncle diameter, tracheids area, metaxylem vessel area, xylem tissue area, phloem tissue area, vascular tissue area, number of vascular bundle as well as opened stomata but increased the number of hairs and closed stomata on the peduncle surface of the two wheat cultivars.

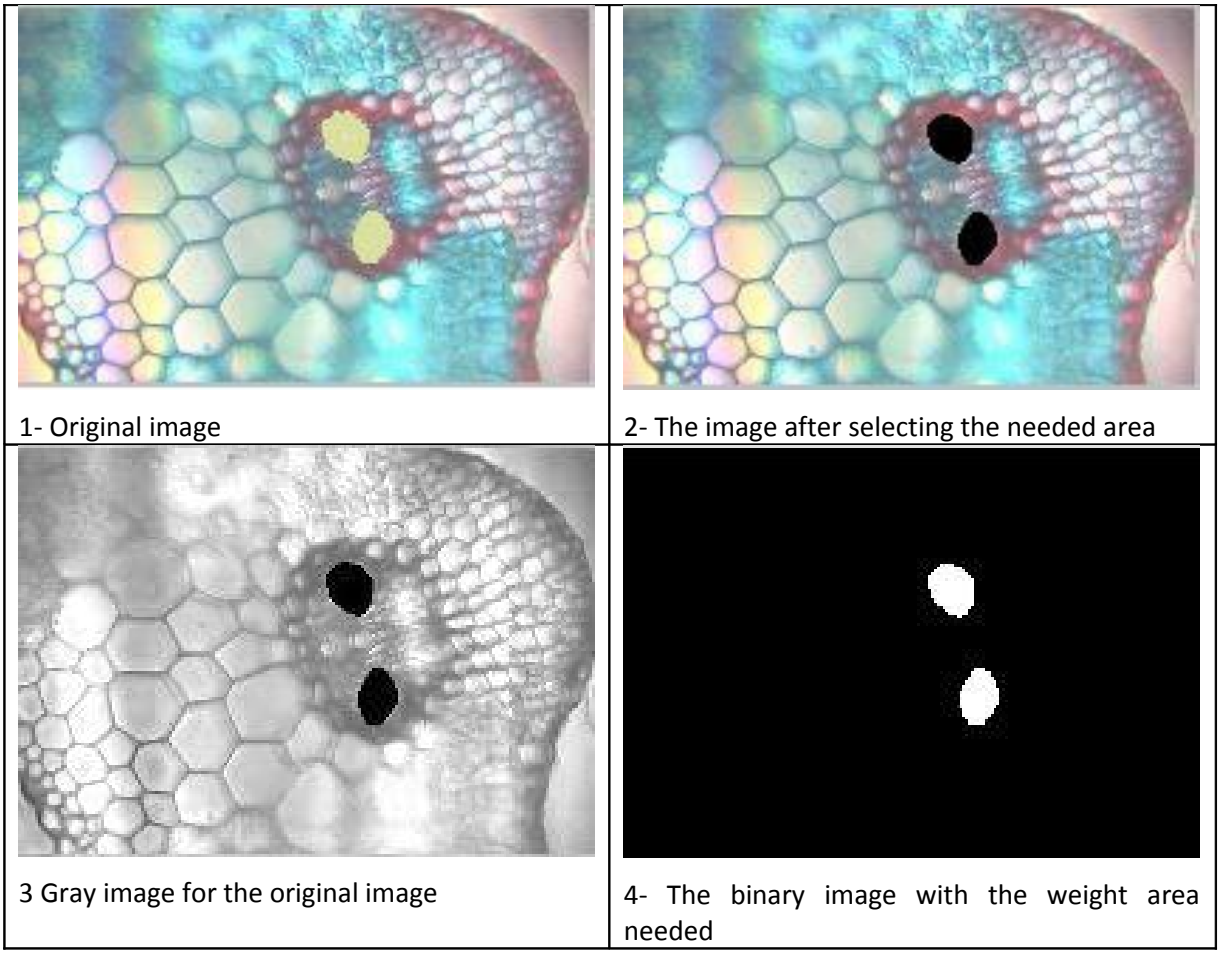
In relation to wheat cultivar, the sensitive was more affected by water stress than the tolerant. In general, the anatomical features in droughted plants were stimulated by GB, SA or GB+SA in both wheat cultivars. The magnitude of response was more pronounced with SA + GB treatment.

The results in tables 1 & 2 revealed that, there was positive correlation between phloem tissue area in leaf and peduncle of both wheat cultivars and assimilates (polysaccharides, protein and total nitrogen) of both flag leaves (source) and yielded grains (sink).

Changes in grain yield

Water stress reduced ($P<0.05$) the grain yield of both wheat cultivars. This effect was more pronounced with sensitive plants. The used chemicals improved the grain yield of both cultivars. Glycine betaine + salicylic acid treatments appeared to mitigate the effect of water stress on wheat plants more than the other treatments under control and stress conditions (Fig. 2).

The economic yield (grain yield) for sensitive cultivar appeared to be positively correlated with the vascular bundle area (r = 0.74, 0.78), xylem area (r = 0.92, 0.78), and phloem area (r = 0.83, 0.86) for leaf and peduncle respectively (Table 3). In addition, for the tolerant cultivar, the relation coefficient values were (r = 0.88, 0.82) with vascular bundle area, (r = 0.85, 0.94), with xylem area and (r = 0.90, 0.93) with phloem area for leaf and peduncle respectively (Table 3).



1- Original image

2- The image after selecting the needed area

3 Gray image for the original image

4- The binary image with the weight area needed

Figure 1. The image analysis steps for estimating the area of metaxylem (in microns) for flag leaf of droughted wheat cultivars.

Table 1. Effect of glycine betaine, salicylic acid and their interaction on flag leaf anatomy of droughted wheat cultivars at anthesis stage.

Variety Wheat	Parameters / Treatments	Leaf thickness (µm)	Ground tissue thickness (µm)	Metaxylem vessel area (µm²)	Xylem area (µm²)	Phloem area (µm²)	Vascular bundle area (µm²)	Number of motor cells	Number of hairs	No. of stomata lower epidermis		No. of stomata upper epidermis	
										Opened	Closed	Opened	Closed
Sensitive	Cont	390	366	755	4415	2256	14502	48.00	20.30	20.0	0.00	28.20	0.00
	W S	334	312	496	3411	1842	11438	52.17	24.00	3.80	15.20	6.00	21.50
	GB	406	389	761	4465	2740	16714	47.83	24.30	8.00	11.80	14.00	14.30
	GB+WS	367	332	712	4288	2393	12570	54.33	29.70	3.20	17.20	5.20	23.20
	SA	411	391	758	4809	2531	17069	48.60	24.20	5.70	14.50	8.50	18.70
	SA+WS	372	345	740	4576	2177	12743	54.67	32.80	0.00	20.20	0.00	27.80
	GB+SA	425	398	831	5339	2609	17960	49.00	26.00	5.20	14.80	7.80	18.30
	GB+SA+WS	378	359	790	4869	2263	14805	55.50	34.50	0.00	20.50	0.00	27.20
	LSD 0.05	**29.72**	**22.54**	**91.42**	**587**	**348**	**655**	**1.12**	**2.77**	**1.10**	**1.21**	**1.19**	**1.40**
Resistant	Cont.	413	382	814	5252	2312	18439	45.17	25.20	12.2	4.5	18.0	5.30
	WS	375	344	761	4248	1867	15274	51.17	28.50	2.20	14.0	2.00	23.0
	GB	417	393	1318	5664	2917	18562	45.00	28.50	6.80	9.70	11.0	13.0
	GB+ WS	389	362	787	5142	2586	17257	52.50	28.30	2.20	14.20	2.30	22.2
	SA	431	419	1171	5642	2726	20821	46.67	24.80	4.70	12.00	6.30	17.7
	SA+ WS	408	374	822	4885	2575	18354	51.86	30.50	0.00	16.20	0.00	24.5
	GB + SA	444	422	1211	5789	2851	21146	47.50	26.00	4.20	12.7	6.00	16.3
	GB +SA+WS	411	380	854	5476	2564	18680	52.79	35.80	0.00	16.50	0.00	24.2
	LSD 0.05	**12.97**	**13.27**	**190.6**	**709**	**252**	**778**	**1.31**	**2.61**	**0.72**	**1.31**	**1.11**	**1.29**

Table 2. Effect of glycine betaine, salicylic acid and their interaction on peduncle anatomy of droughted wheat cultivars at anthesis stage.

Wheat Variety	Parameters / Treatments	Peduncle diameter (mm)	Tracheids area (µm²)	Metaxylem vessel area (µm²)	Xylem area (µm²)	Phloem area (µm²)	Vascular bundle area (µm²)	Number of vascular bundles	Number of hairs	Number of stomata	
										opened	closed
Sensitive	Cont	2.20	540	570	7420	3016	12078	50.70	28.80	32.00	13.00
	W S	2.01	355	478	4942	2394	10005	48.20	37.50	4.00	40.70
	GB	2.27	548	581	7935	3190	12700	53.20	31.00	22.80	21.00
	GB+WS	2.24	435	491	5517	2704	11274	51.00	43.80	9.20	36.70
	SA	2.29	593	624	7766	3120	13505	50.30	32.70	10.70	32.80
	SA+WS	2.24	499	520	6155	2821	12110	46.70	42.50	1.50	43.00
	GB+SA	2.31	606	653	8059	3373	16028	49.50	35.30	8.00	35.20
	GB+SA+WS	2.26	493	630	7096	2933	13847	61.30	44.80	1.00	43.70
	LSD 0.05	**0.11**	**69.00**	**85.10**	**900**	**391**	**1250**	**1.95**	**2.71**	**1.88**	**2.42**
Resistant	Cont.	2.33	629	627	8086	3161	16806	46.00	32.30	19.30	19.30
	WS	2.09	379	512	6064	2556	14448	54.70	40.20	2.30	36.70
	GB	2.37	694	739	8559	3560	17503	55.20	35.30	12.70	26.00
	GB+ WS	2.26	493	674	6624	2972	15636	57.80	45.20	2.20	36.80
	SA	2.34	742	763	8130	3390	18478	52.80	30.80	7.50	30.20
	SA+ WS	2.29	547	647	6754	3116	15814	53.20	48.30	1.00	37.30
	GB + SA	2.46	747	789	8714	3529	18516	55.20	37.80	5.50	32.70
	GB +SA+WS	2.30	552	676	7690	3237	15913	53.50	51.80	0.00	38.00
	LSD 0.05	**0.10**	**86.40**	**98.10**	**763**	**622**	**988**	**1.30**	**2.90**	**1.80**	**01.02.30**

Table 3. Correlation coefficient between phloem area of leaf and peduncle of wheat cultivars and assimilates of flag leaf and yielded grains.

Phloem area of flag leaf			
Wheat cultivar **Variables**		**Sensitive**	**Resistant**
Polysaccharides	Flag leaf	0.87	0.98
	Grains	0.85	0.94
Protein	Flag leaf	0.90	0.93
	Grains	0.83	0.92
Total nitrogen	Flag leaf	0.94	0.96
	Grains	0.91	0.90
Phloem area of peduncle			
Wheat cultivar **Variables**		**Sensitive**	**Resistant**
Polysaccharides	Flag leaf	0.94	0.95
	Grains	0.87	0.95
Protein	Flag leaf	0.92	0.96
	Grains	0.84	0.95
Total nitrogen	Flag leaf	0.93	0.97
	Grains	0.92	0.94

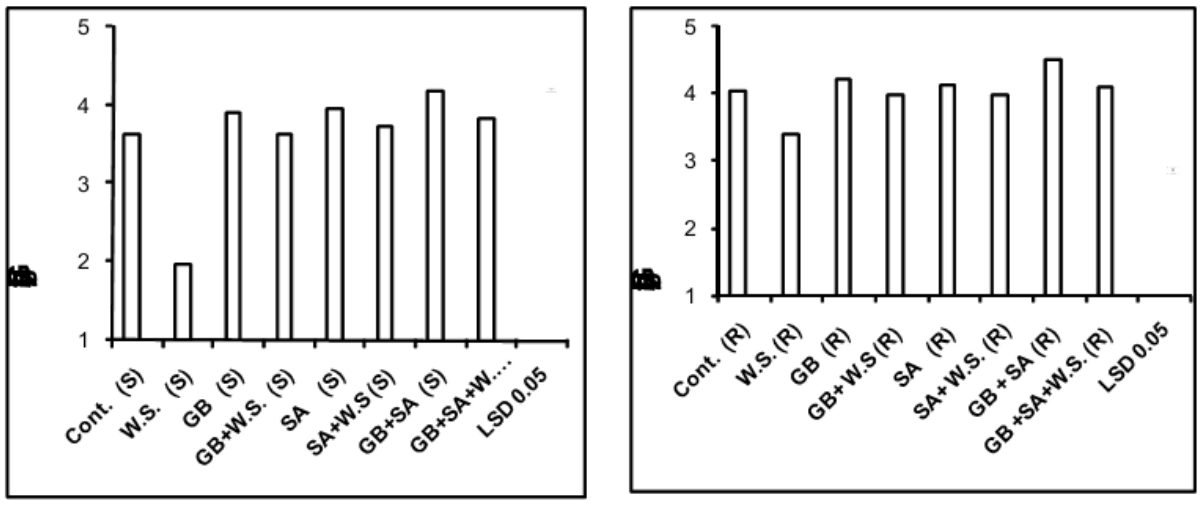


Figure 2. Effect of glycine betaine, salicylic acid and their interaction on grain yield of wheat cultivars grown under water stress condition. Vertical bars represent LSD values at P< 0.05.

DISCUSSION

Most plants have developed morphological and physiological mechanisms which allow them to adapt and survive under stress conditions (Ludlow,

1989). These mechanisms mainly comprise a reduction of the leaf size, leaf rolling, dense leaf pubescence, deeply developing stomata, accumulation of mucilage and other secondary metabolites in the mesophyll cells, increase of mesophyll compactness (Bosabalidis and Kofidis, 2002).

Results in table 1 and plates 1, 5 indicated that, water stress induced marked decreases ($P< 0.05$) in thickness of the leaf and ground tissue in both wheat cultivars and this was obvious with the sensitive cultivar. This is presumably due to a reduction in mesophyll tissue of the flag leaf. These results are in accordance with those obtained by Aldesuquy *et al.* (1998) with wheat plants under salinity. According to Cutler *et al.* (1977) reduction in cell size appears to be a major response of cells to water deficiency. Furthermore, the reduction in cell size under water stress conditions may be considered as drought adaptation mechanism (Steudle *et al*., 1977).

Drought stress application with two olive cultivars (Mastoidis and Koroneiki) resulted in a decrease of the size of the epidermal and mesophyll cells with a parallel increase of the cell density. These changes are more characteristic in cv. 'Mastoidis'. Stomata became more numerous and smaller, while non-glandular hairs (scales) greatly increased in number particularly in cv. Koroneiki (Bosabalidis and Kofidis, 2002).

Treatment with GB, SA or their interaction induced marked increases in thickness of both leaf and ground tissues. This was clear in table 1 and plate 2, 3, 4, 6, 7, 8. The increase in the leaf thickness might be due to the increase in the mesophyll tissue. These tissues (mesophyll) are characterized by high concentration of chloroplast. As the leaf thickness could be considered as a good indicator to specific leaf weight which is a reliable index to photosynthetic efficiency. It could be concluded that, the tolerant cultivar was more efficient in synthesizing metabolites than the sensitive one. These results are in accordance with Planchon (1969) who showed that high yielding ability is strongly associated with longer leaves.

The conductive canals between source and sink in wheat cultivars have been reported to play a prominent role in the translocation of photosynthates to the developing grains (Evans *et al.,* 1970). Water stress reduced the peduncle diameter (Table 2 and Plates 9- 16). This reduction may be as a result of decreased cell enlargement and cell division (Nieman, 1965), or inhibition of meristematic division (El-Kabbia *et al*., 1981).

The aforementioned data of vascular bundle area of flag leaf, smaller area of vascular bundle of both wheat cultivars may be associated with higher number in the peduncle section. So the total area of conductive tissue of the peduncle of the tolerant cultivar may exceed that of sensitive one. As shown by Evans (1970), the area of the phloem tissue of the peduncle of wheat from all stages of evolution of the crop, varies over tenfold range from wild diploid Aeglops to modern hexaploid.

The obtained results revealed that, drought caused reduction in metaxylem vessel and xylem tissue areas in flag leaf and peduncle of both wheat cultivars. This may probably be due to the decrease in the number of additive divisions in the cambium (El-Shami, 1987). Furthermore, this would result in a lower rate in translocation of water necessary for photosynthesis. These results are in accordance with those obtained by Aldesuquy *et al*. (1998) with wheat plants under salinity.

The applied chemicals induced additional

increases in the areas of conductive canals (xylem and phloem) in flag leaf and peduncle of both cultivars. In addition, the application of these chemicals caused positive correlations between grain yield and vascular bundle area, xylem area and phloem area in both leaf and peduncle of the two cultivars. This furnishes better translocation of assimilates from flag leaf (as source) towards the developing grains (as sink) through the conductive canals, where there was a good source-sink relationship particularly there was positive correlation between phloem tissue area in leaf and peduncle of both wheat cultivars and assimilates (polysaccharides, protein and total nitrogen). In this regard, Evan *et al*. (1970) mentioned that the phloem area was found to be directly proportional to the calculated maximum rate of assimilate import by the ears for the 22 lines of wheat examined and expressed as rate per cm^2 of the phloem area was similar to rates calculated for import into other rapidly growing organs.

The highly numbers of hairs on the flag leaf surfaces as a result of water stress treatment may probably due to that trichomes (hairs) may increase the leaf boundary layer resistance and this may improve water use efficiency. These results are in a good agreement with (Quarrie and Jones, 1977). Also number of hairs on the peduncle surface increased as response to drought in both wheat cultivars. In this connection, Udovenko *et al.* (1976) found that salinity treatments increased the number of hairs on peduncle surface which coincided with the results of this investigation (Table 2 and Plate 9-13). This may support the idea that hairs have protective action against the reduction of water loss from peduncle surface. On the other hand, application of GB, SA or their interaction caused additional increases in the number of hairs. This was in accordance with Aldesuquy *et al*. (1998) with wheat plants treated with sodium salicylate under salinity.

Water stress resulted in an increase in the number of closed stomata and a decrease in the number of opened ones on the flag leaf of both wheat cultivars particularly the tolerant ones. This modification appeares to be an adaptive mechanism toward drought conditions. In this respect, Fahn and Cutler (1992) reported that, many xerophytes in order to save internal water, develop their stomata in local leaf epidermal depressions or in crypts. Furthermore, they reported that, a major process of water economy is the reduction of transpiration by closure of stomata. This process entails in parallel a reduction of the rate of photosynthesis, since CO_2 is prevented to enter the mesophyll. Decline of photosynthesis in water stressed plants was found, however, not to be exclusively due to closure of stomata.

As compared to control, application of GB (osmoprotectant), SA (antitranspirant) or their interaction decrease the number of opened stomata on both upper and lower epidermis in flag leaf and peduncle, therefore increase the water content in leaf necessary for photosynthesis. Salicylic acid application induced an adaptive response to water stress by keeping turgidity of leaves through stomata closure.

Water stress increased the number of motor cells on the upper epidermis of flag leaf of both wheat cultivars. Furthermore, treatment with GB, SA or their interaction added more increase in the number of motor cells in the fag leaf (Table 1 and Plate 2, 3, 4, 6, 7, 8). This increase induced an adaptive response of wheat plants towards drought, where the role of motor cells is important

in leaf rolling. Like muscle cells which have excitation-contraction coupling, the motor cells in plants exhibit rapid movement resulting from excitation tugor loss (Sibaoka, 1980). Regarding the mechanism of movement in Mimosa, Sibaoka (1991) reported that the motor cells contain a fibrillar structure, contraction of these fibrils may open pores in the membrane of the motor cells upon activation. Outward bulk flow of the vacuolar sap through these pores, due to the pressure inside the cell, results in turgor loss of the motor cells and then the bending of the organ. In fact, the susceptible cultivar contained higher number of motor cells as a result of GB, SA or their interaction which facilitated the rolling of leaves in that cultivar than the resistant one. These chemicals appeared to be useful in improving tolerance of wheat plants towards water stress.

REFERENCES

Abd El-Gawad, A.A., Abd El-Gawad, M.A., Edris, S.A. and Abo-Shetaia, A.M. (1985) Positional productivity of wheat in Egypt V. Conductive canals of flag leaf and peduncle of certain wheat cultivars. *Annals Agric. Sci., Fac. Agic., Ain Shams Univ., Cairo, Egypt*. **30**: 883-892.

Abo-Hamed, S.A., Mansour, F.A. and Eldesuquy, H.S. (1987) Yield and yield attributes of wheat as affected by sodium salicylates, alar, kinetin and asulam. *Mansoura, Univ. Conf. of Agric. Sci. On food Deficiency, 2nd June*, 1987.

Aldesuquy, H. S., Mankarios, A. T. and Awad, H. A. (1998) Effect of some antitranspirants on growth, metabolism and productivity of saline-treated wheat plants. III. Anatomical changes in flag leaf and peduncle. *Mans. Sci. Bull*., **25**: 1-22

Aldesuquy, H.S. and Baka, Z.A.M. (1991) Interactive effects of NaCl salinity and growth subctances on the anatomy of wheat flag leaf. *Mans. Sci. Bull*., **18**:148.

Bosabalidis, A. M. and Kofidis, G. (2002) Comparative effects of drought stress on leaf anatomy of two olive cultivars. *Plant Science,* **163**: 375 - 379.

Cutler, J. M., Rains, D.W. and Loomis, R. S. (1977) The importance of cell size in the water relations of plants. *Physiol. Plant*., **40**: 255–260.

Egeli, D.B., Guffy, R.D., Meckel, L.W. and Leggett, J.E. (1985) The effect of source-sink alterations on soybean seed growth. *Ann. Bot*., **55**: 395.

El-Kabbia, T., Habib, S.A. and Abdel-Aziz (1981) Effect of salinity on the internal structure of sunflower and cotton. *Res. Bull. 1562, Fac. Of Agric. Ain Shams Univ., Cairo, Egypt*.

El-Shami, I. Z. M. (1987) The role of endogenous growth hormones during water stress and salinity stress. M.Sc. Fac. Agric. Ain Shams Univ. Cairo, Egypt.

Evans, L. T., Dunston, R.L., Rawson, H.M.R. and Williams, A.F. (1970) The phloem of the wheat stem in relation to requirements for assimilates by the ear. *Aust. J. Biol. Sci*., **23**: 723-752.

Fahn, A. and Cutler, D.F. (1992) Xerophytes, *Gebrueder Borntraeger, Berlin*, p. 167.

Gonzalez, R. C. and Woods, R. E. (1992) Digital Image Processing, Addison-Wesley

Ludlow, M. M. (1989) Strategies of response to water stress. In: K.H. Kreeb, H. Richter, T.M. Hinckley (Eds.), Structural and Functional Responses to Environmental Stresses: Water Shortage, SPB Academic Publishing, London, pp. 269 -282.

Nieman, R.H. (1965) Expansion of bean leaves and its suppression by salinity. *Plant Physiol*.**40**:156-

169.

Planchon, C. (1969) Photosynthetic activity and yield of soft wheat (*Triticum aestivum*). *Genet. Agr.*, **23**: 485-490.

Quarrie, S.A. and Jones, H.G. (1977) Effect of ascisic acid and water stress on development and morphology of wheat. *J. Exp. Bot*., **28**: 192- 197.

Sass, J.E. (1951) Botanical microtechnique. The Iowa State College Press, Am. Iowa, pp.130.

Shao, H. B., Chu, L. Y., Jaleel, A. C. and Zhao, C. X. (2008) Water-deficit stress-induced anatomical changes in higher plants. *C. R. Biologies*, **331**: 215–225.

Sibaoka, T. (1980) Action potentials and rapid plant movement. In F. Shoog, ed, Plant Growth Substances 1979, pp. 462-469. Springer-Verlag, Berlin.

Sibaoka, T. (1991): Rapid plant movements irrigated by action potentials. *Bot. Mag. Tokyo*. **140**: 73.

Steudle, E.; Zimmermann, U. and Luttge, U. (1977) Effect of turgor pressure and cell size on the wall elasticity of plant cells. *Plant Physiol*., **59**: 285–289.

Udovenko, G.V., Gradchaninova, O.D., Gudkova, G.N., Semushina, L.A. and Morozova, A.G. (1976) Strucural and anatomical changes in plant grown under salinity and their physiological importance. *Fiziol. Biokhimiya Kulťurnykh Rastenii,* **8**: 228-235.

Additional material

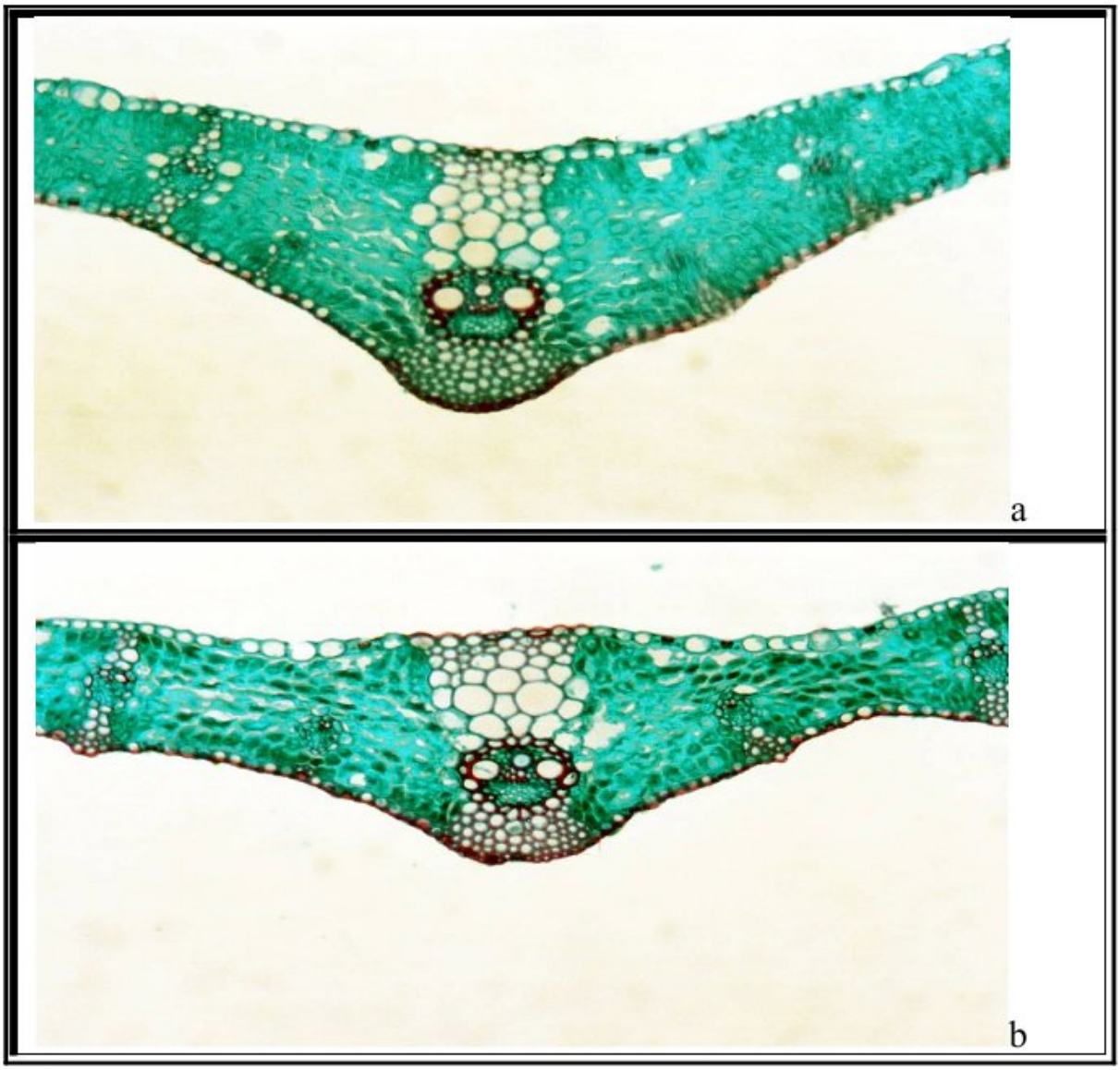


Plate 1. Effect of water stress on flag leaf anatomy of sensitive cultivar. (Cross section X = 10)

a) Control b) WS

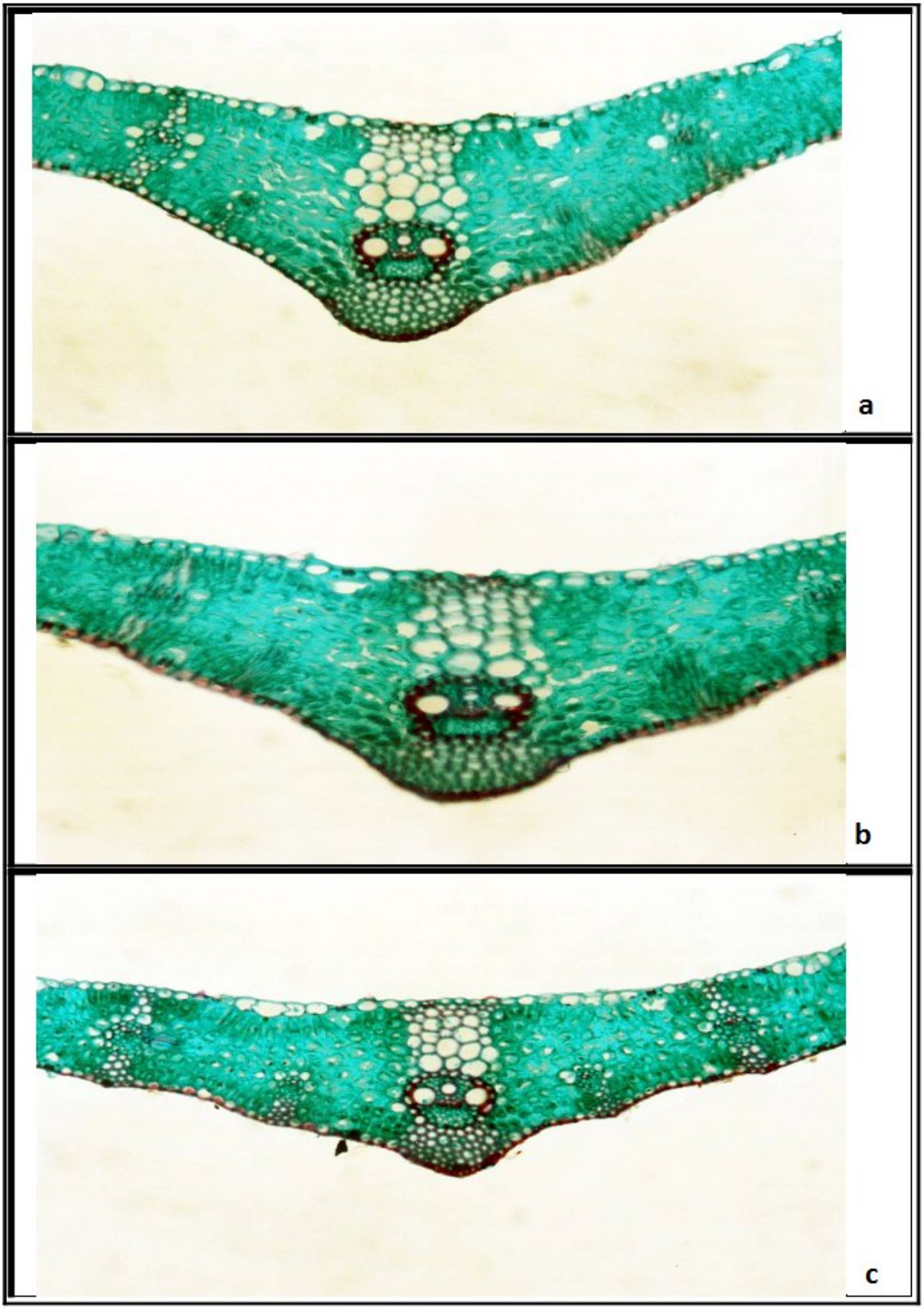


Plate 2. Effect of glycine betaine on flag leaf anatomy of droughted sensitive cultivar. (Cross section X = 10)

a) Control b) GB c) GB+WS

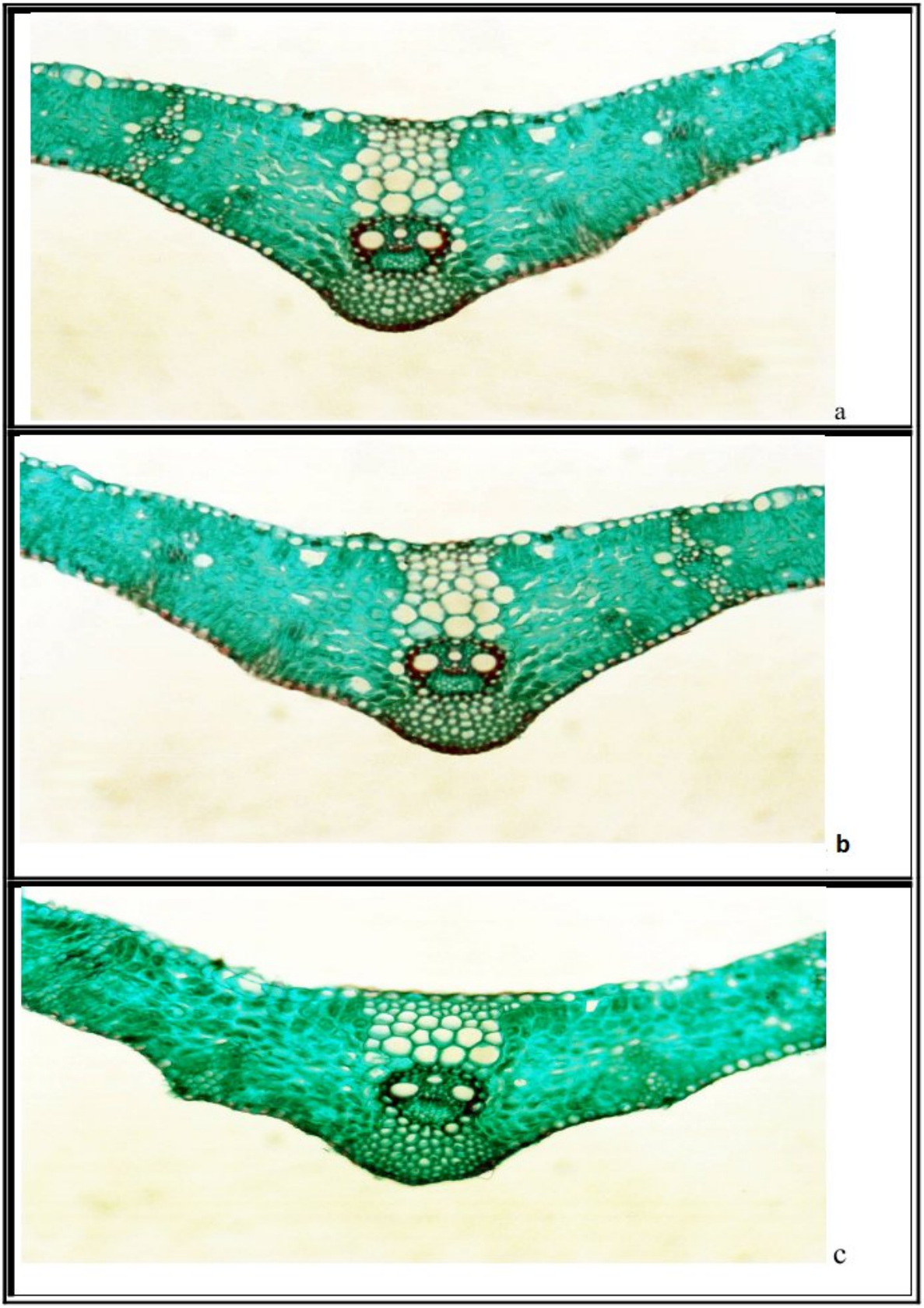


Plate 3. Effect of salicylic acid on flag leaf anatomy of droughted sensitive cultivar. (Cross section X = 10)

a) Control b) SA c) SA+WS

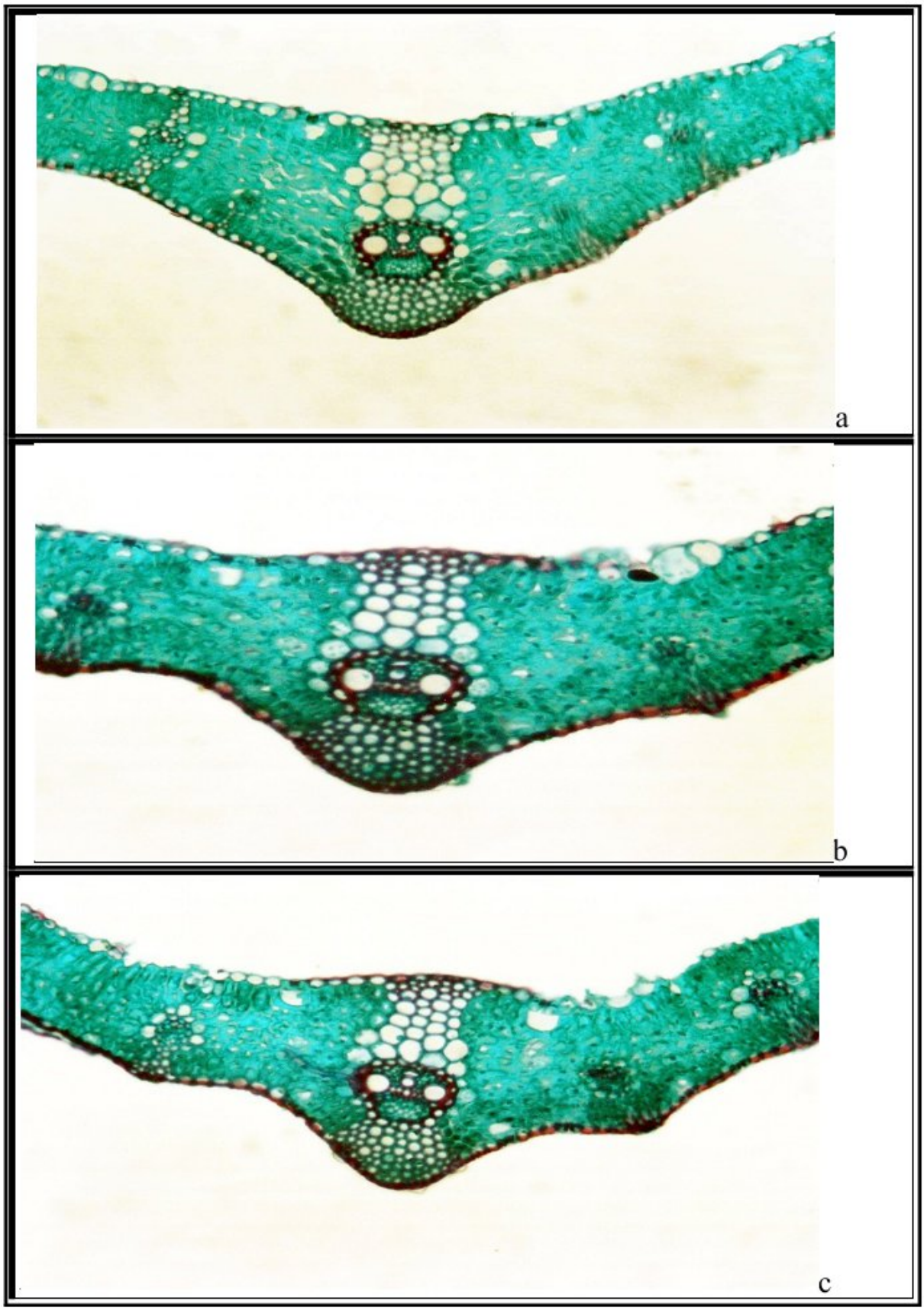


Plate 4. Effect of glycine betaine +salicylic acid on flag leaf anatomy of droughted sensitive cultivar. (Cross section X = 10)

a) Control b) GB+SA c) GB+SA+WS

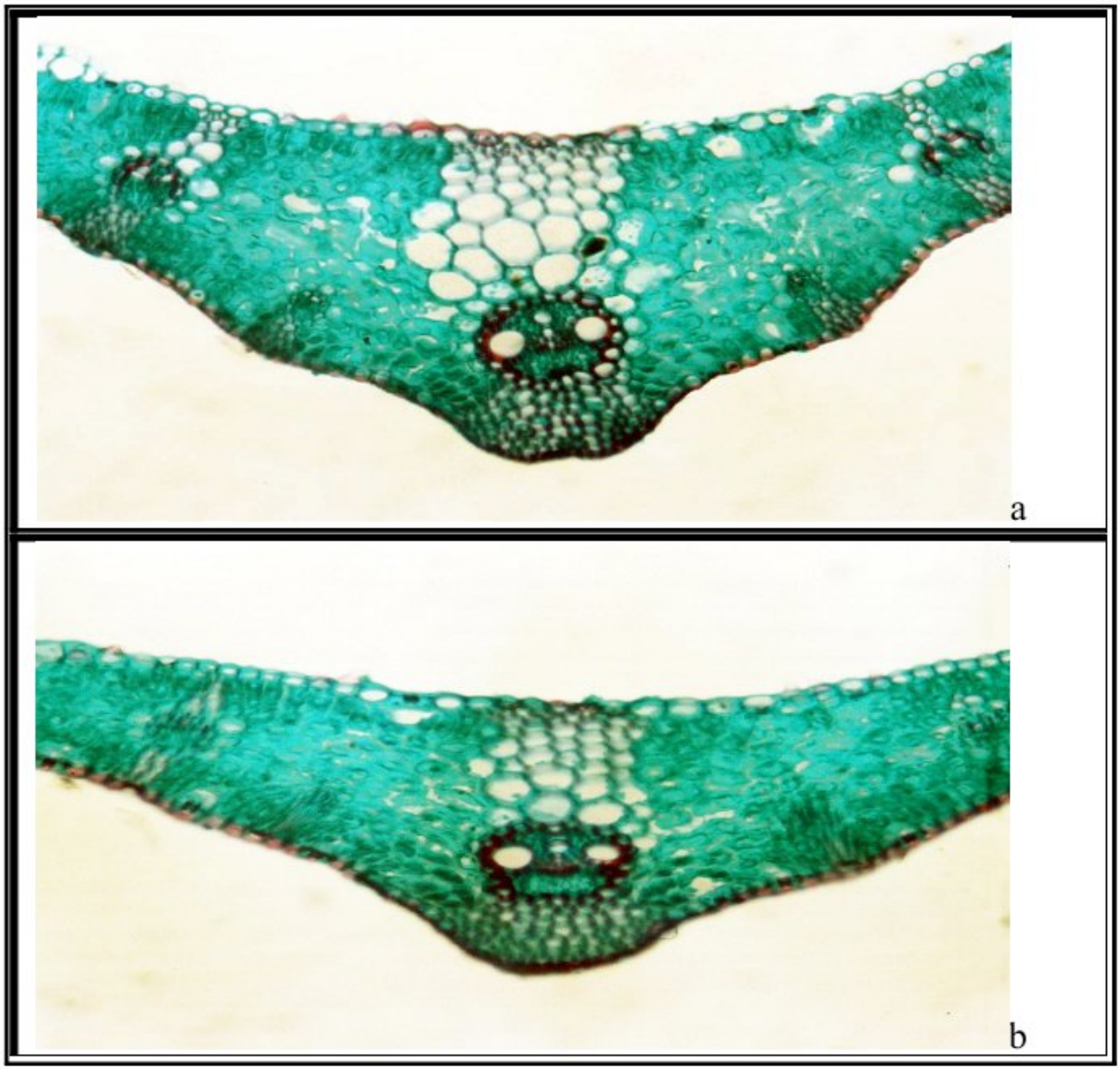


Plate 5. Effect of water stress on flag leaf anatomy of tolerant cultivar. (cross section X = 10)

a) Control b) WS

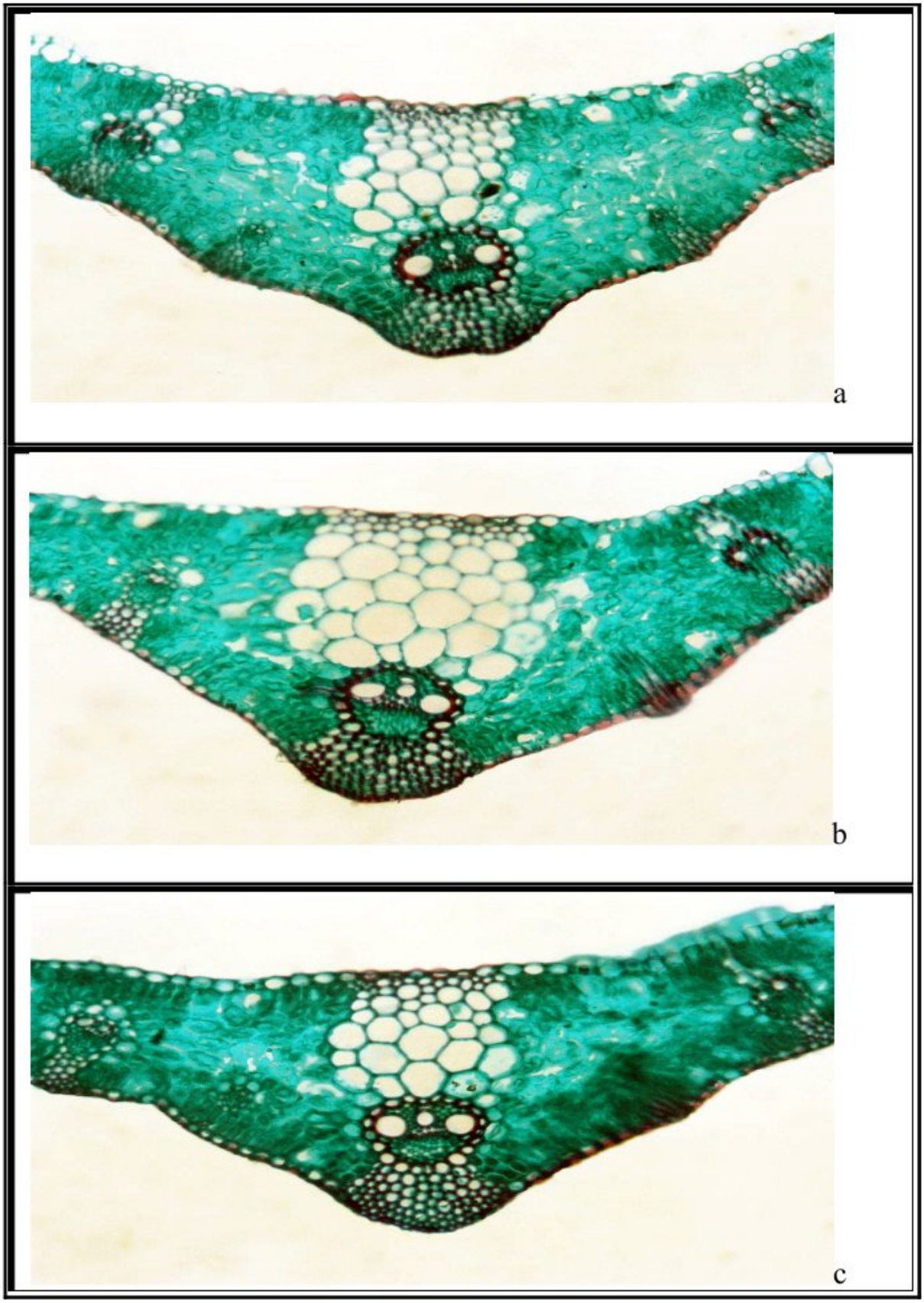


Plate 6. Effect of glycine betaine on flag leaf anatomy of droughted tolerant cultivar. (cross section X = 10)

a) Control b) GB c) GB+WS

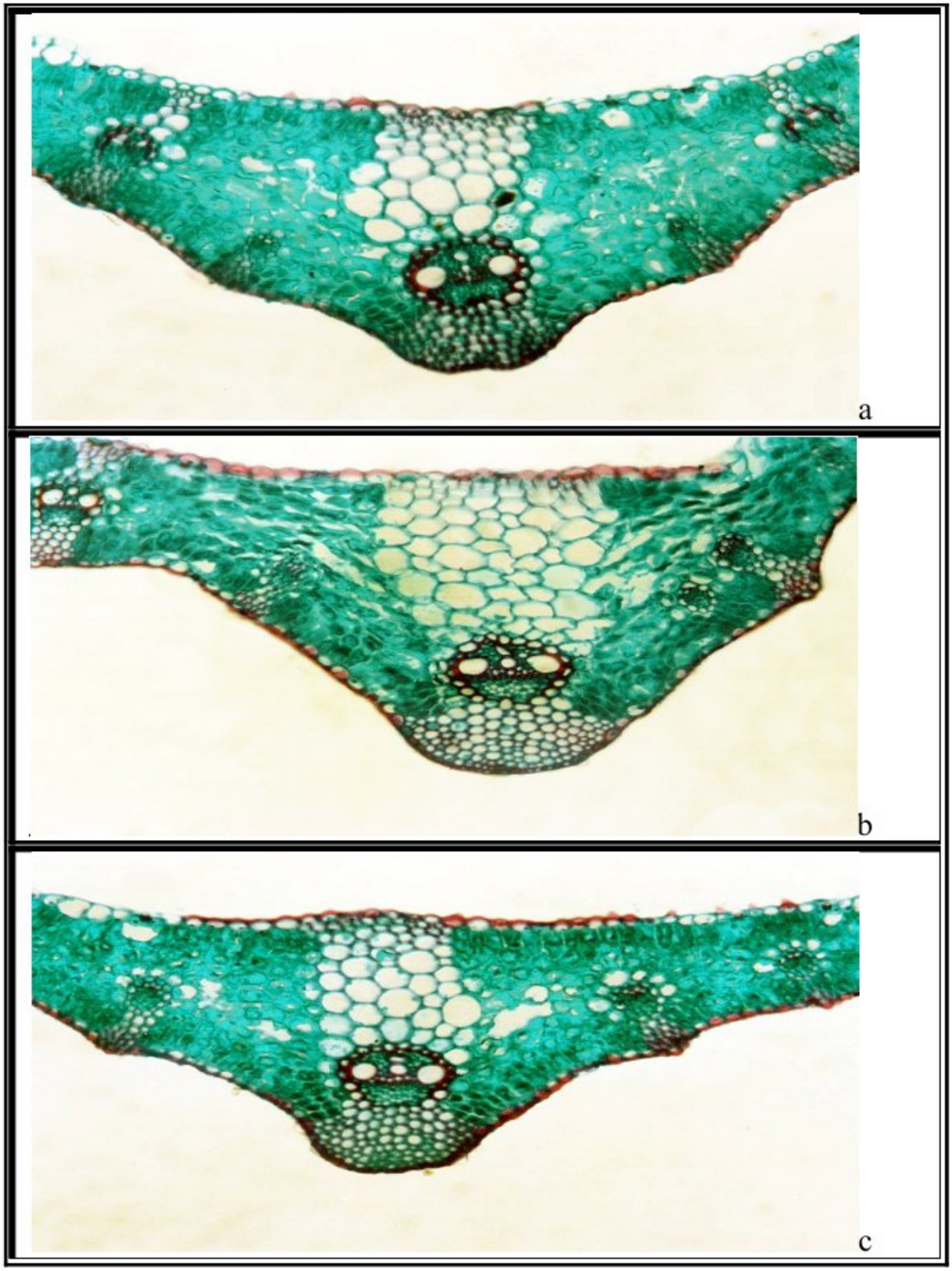


Plate 7. Effect of salicylic acid on flag leaf anatomy of droughted resistant cultivar. (cross section X = 10

a) Control b) SA c) SA+WS

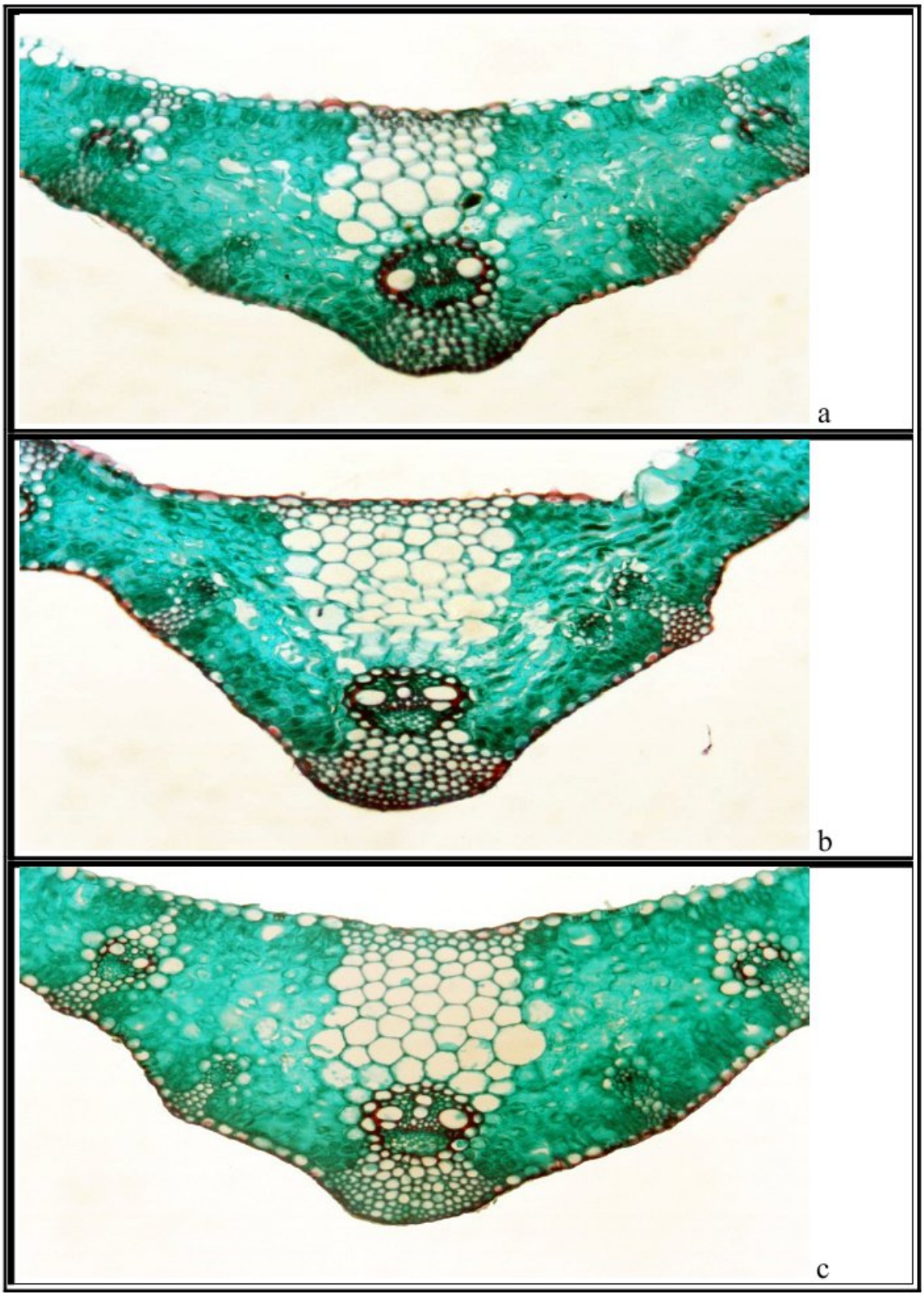


Plate 8. Effect of glycine betaine + salicylic acid on flag leaf anatomy of droughted resistant cultivar. (Cross section X = 10)

a) Control b) GB+SA c) GB+SA+WS

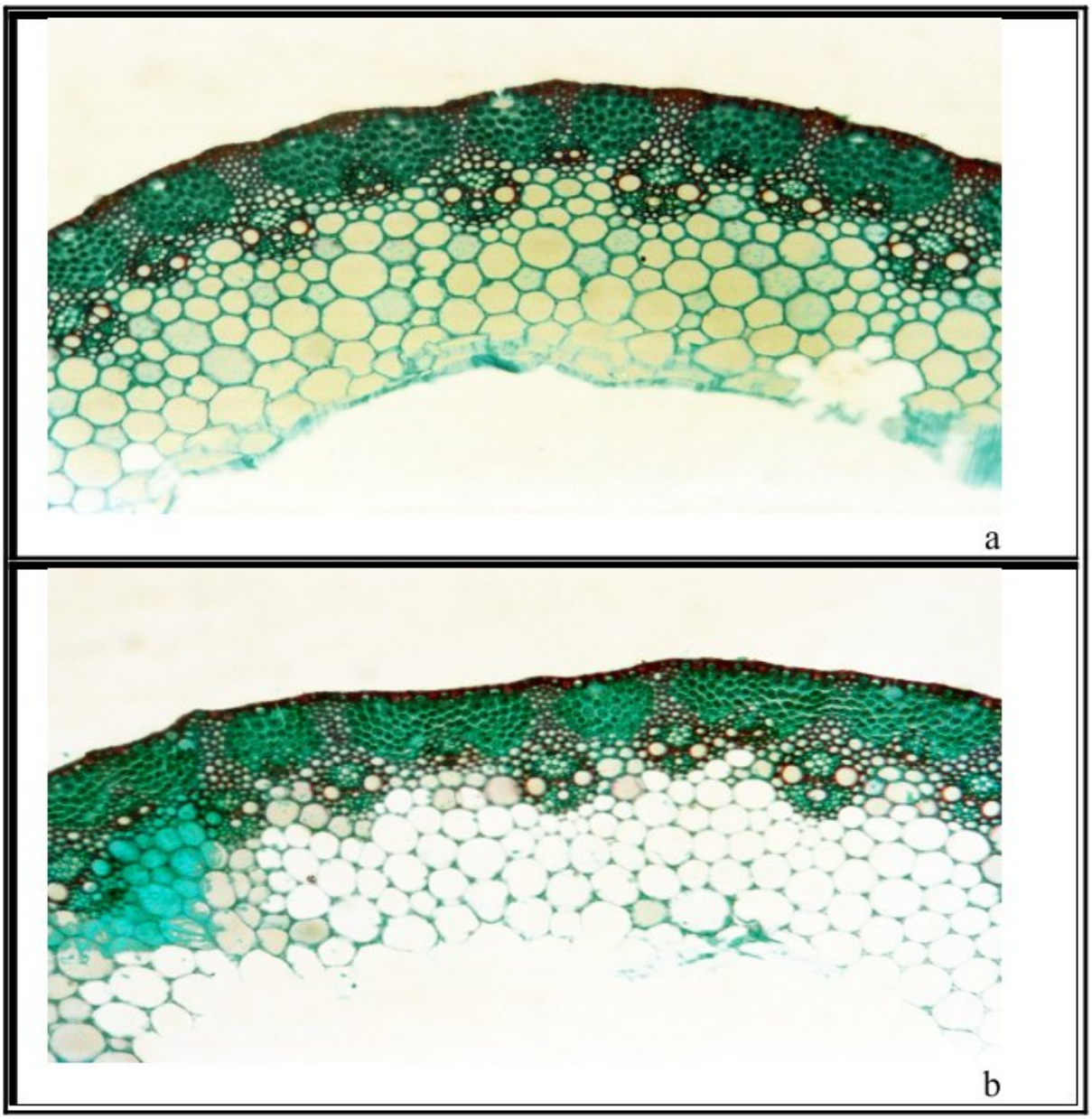


Plate 9. Effect of water stress on peduncle anatomy of sensitive cultivar. (Cross section X = 10)

a) Control b) WS

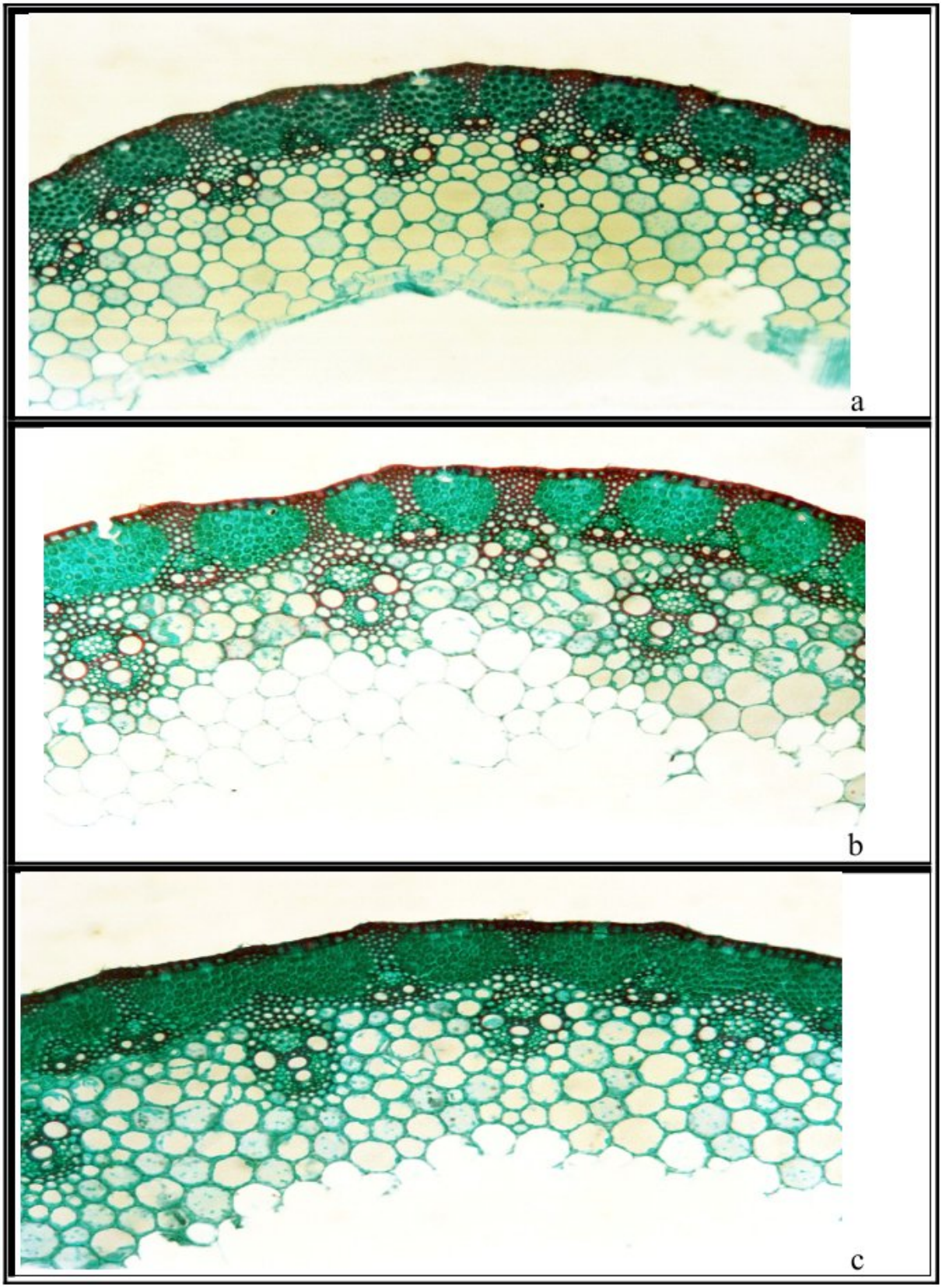


Plate 10. Effect of glycine betaine on peduncle anatomy of droughted sensitive cultivar. (Cross section X = 10)

a) Control b) GB c) GB+WS

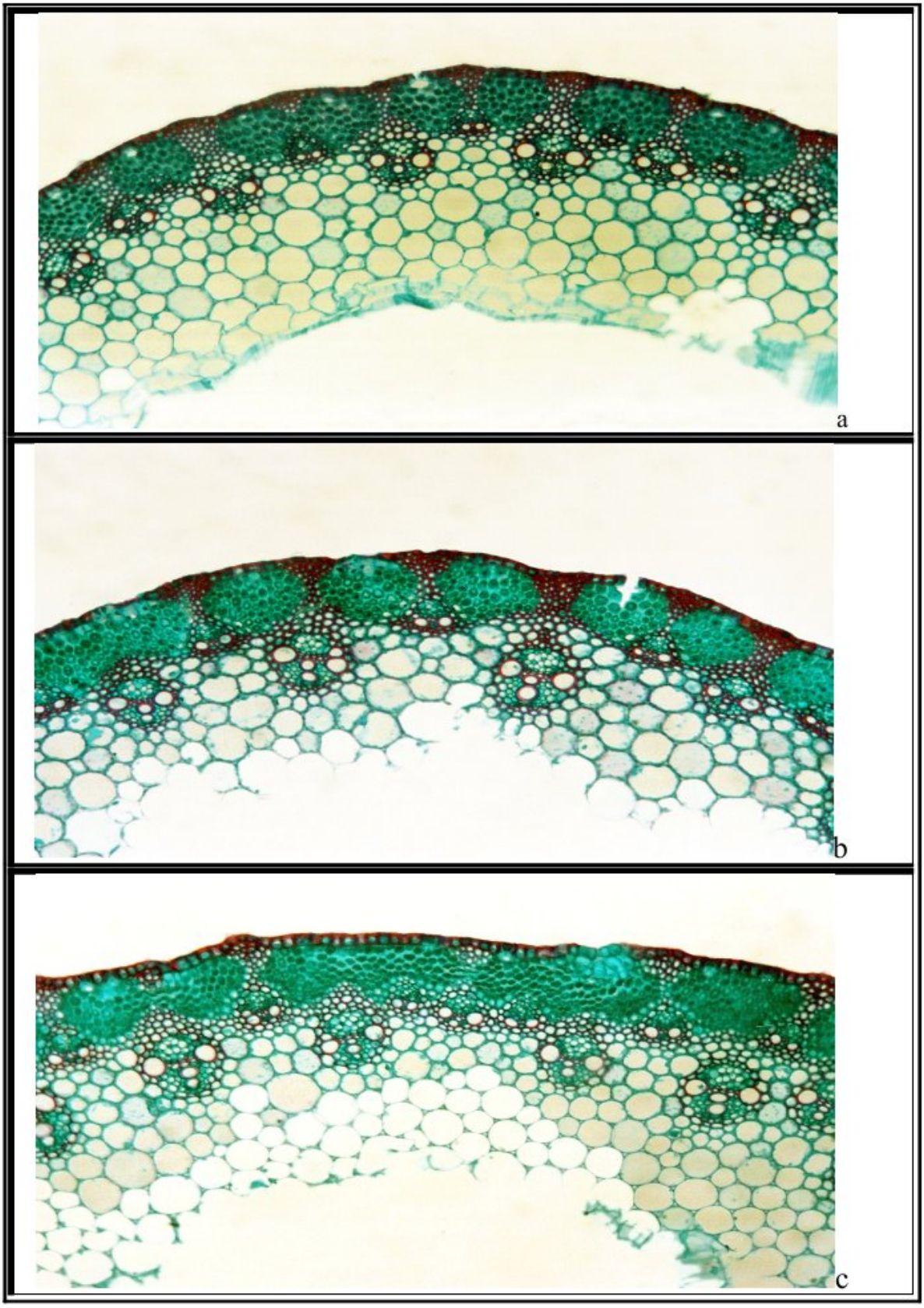


Plate 11. Effect of salicylic acid on peduncle anatomy of droughted sensitive cultivar. (Cross section X = 10)

a) Control b) SA c) SA+WS

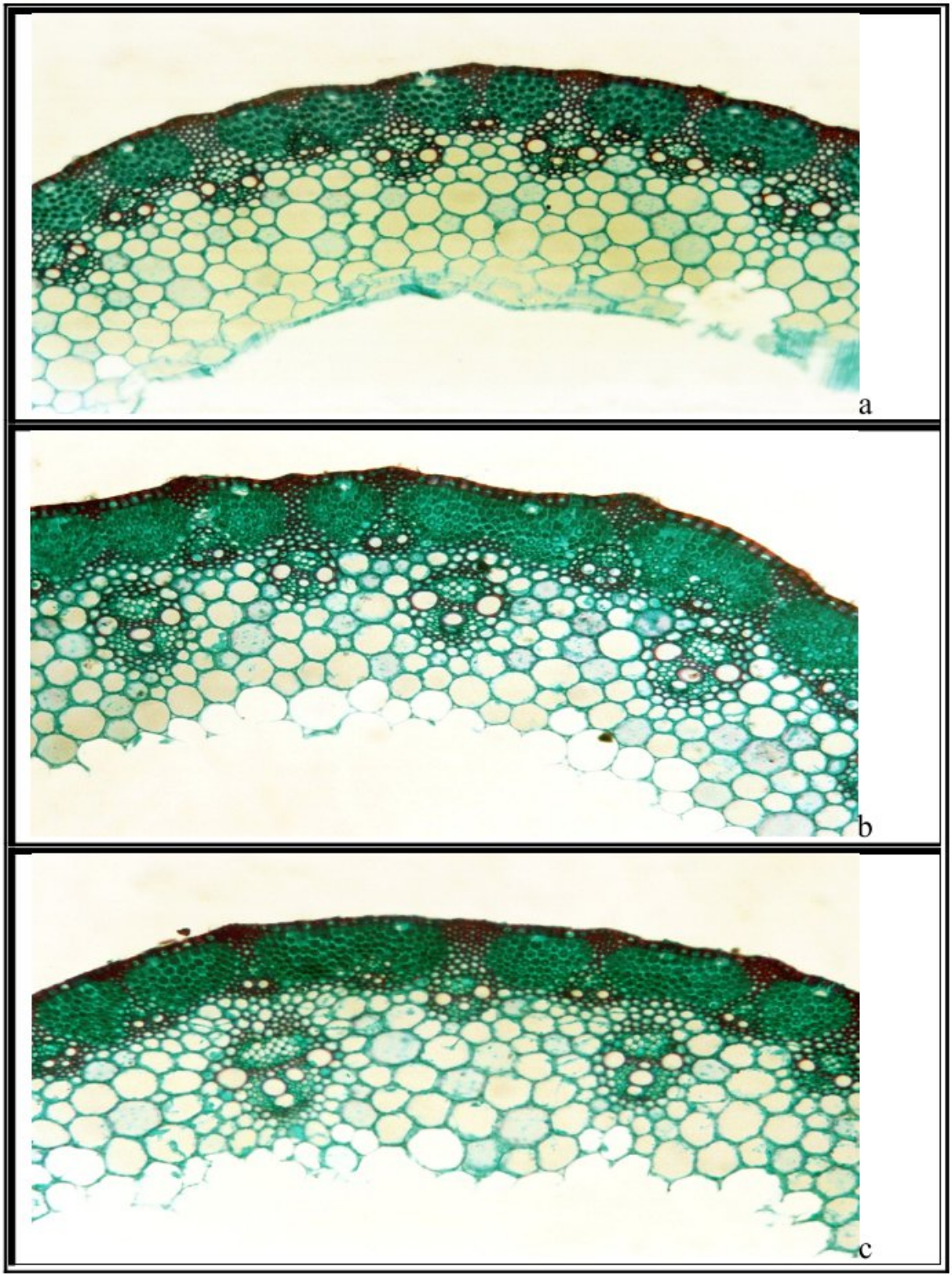


Plate 12. Effect of glycine betaine + salicylic acid on peduncle anatomy of droughted sensitive cultivar. (Cross section X = 10)

a) Control b) GB+SA c) GB+SA+WS

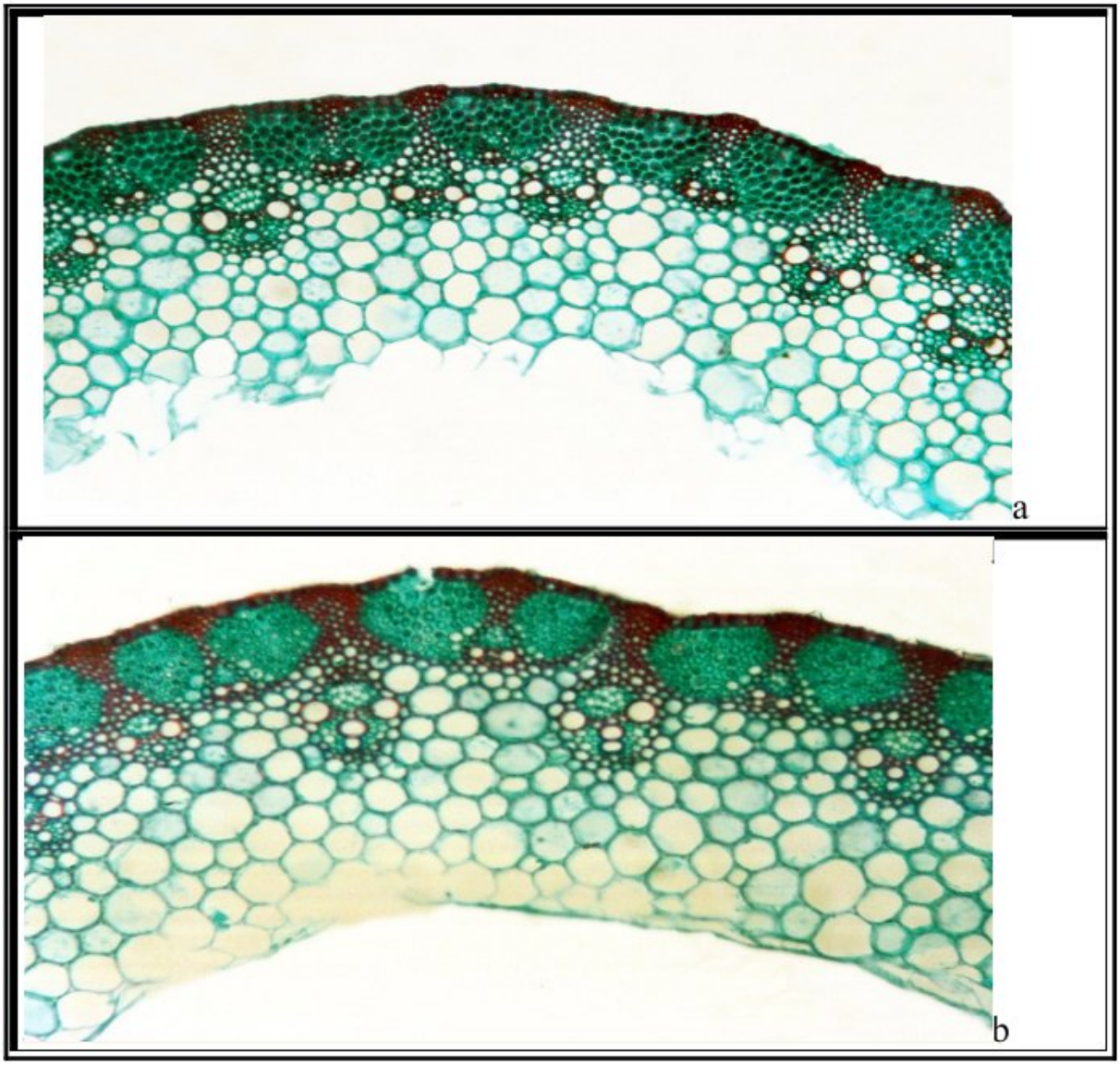


Plate 13. Effect of water stress on peduncle anatomy of tolerant cultivar. (Cross section X = 10)

a) Control b) WS

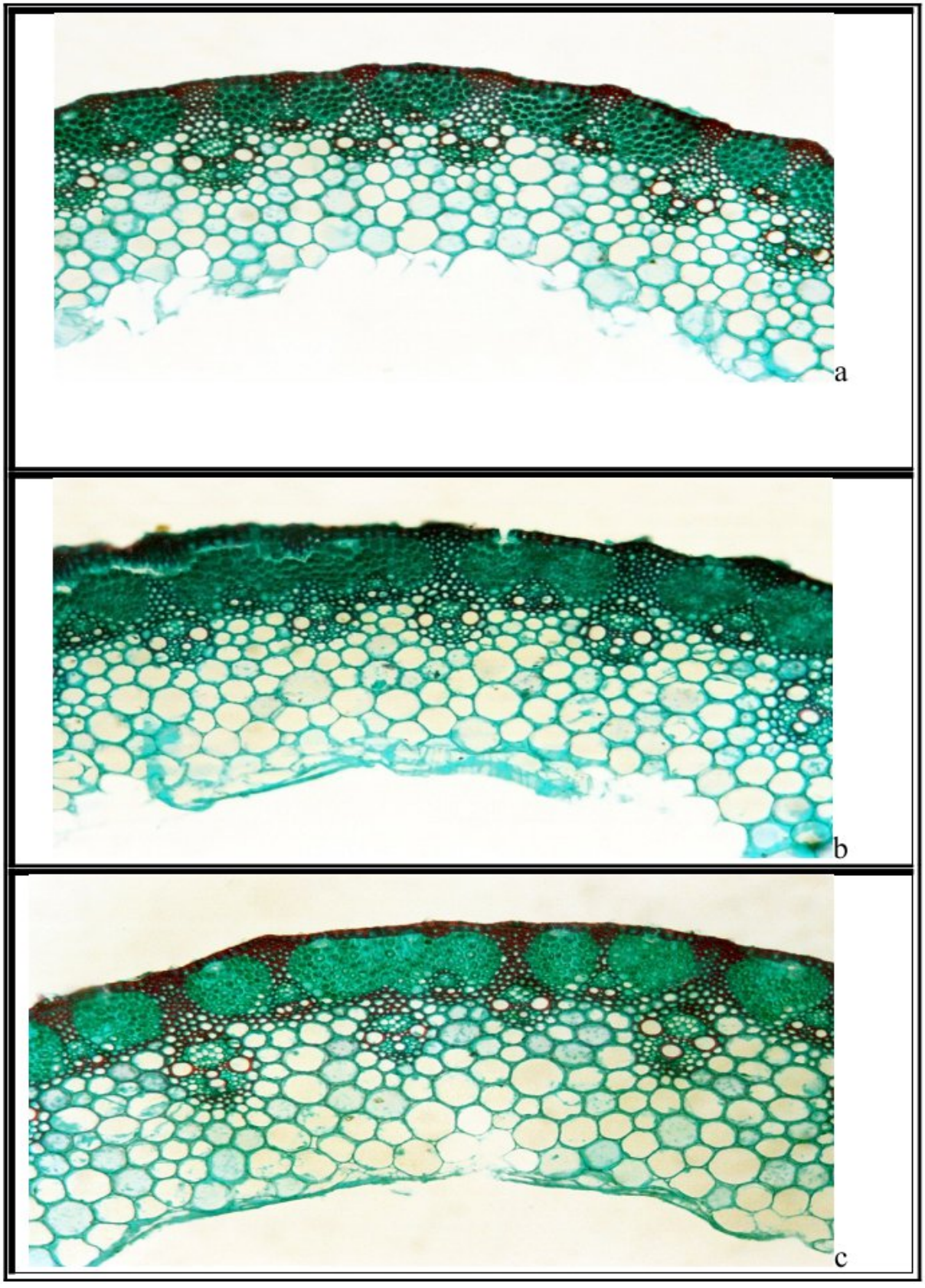


Plate 14. Effect of glycine betaine on peduncle anatomy of droughted tolerant cultivar. (Cross section X = 10)

a) Control b) GB c) GB+WS

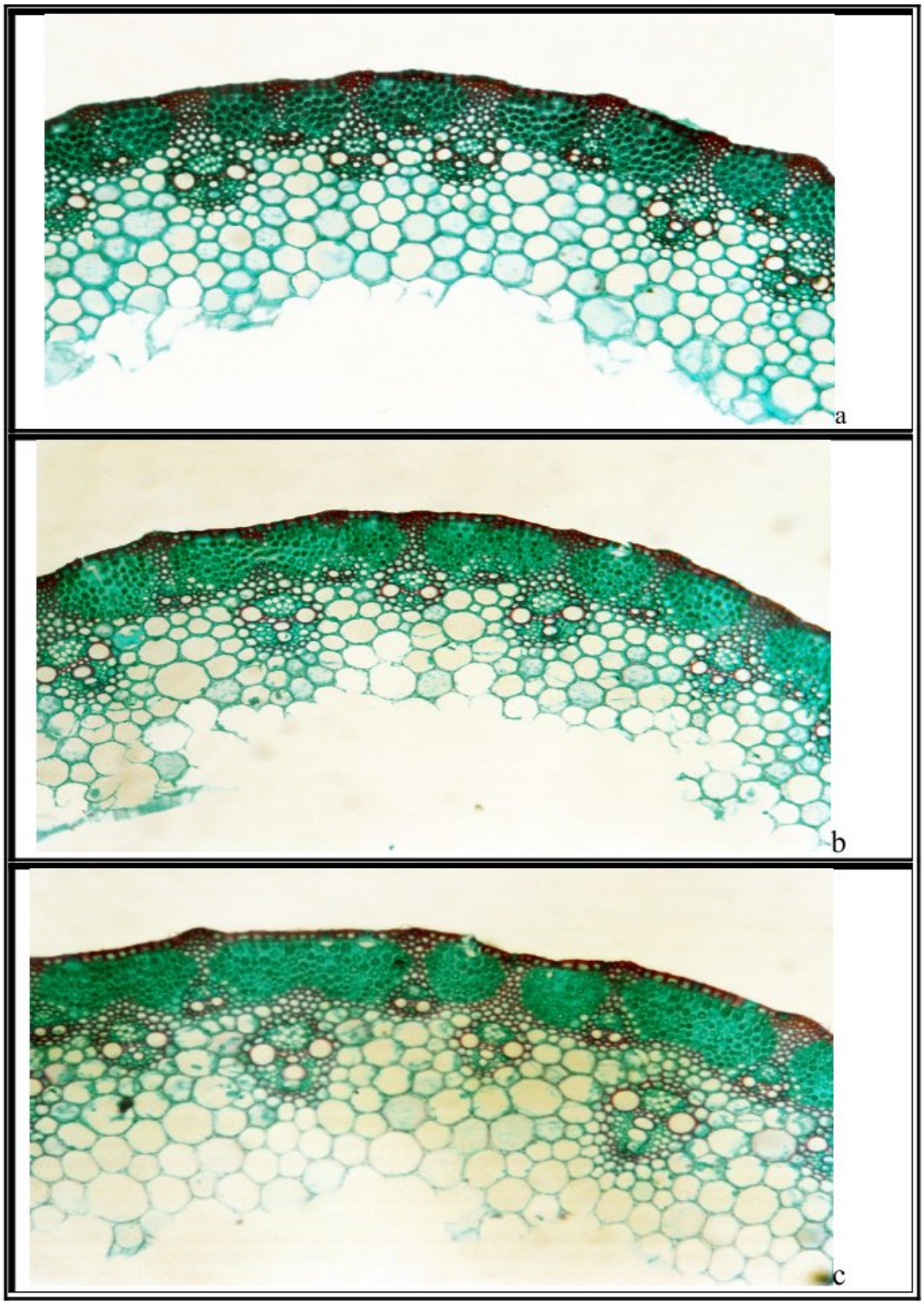


Plate 15. Effect of salicylic acid on peduncle anatomy of droughted tolerant cultivar. (Cross section X = 10)

Control b) SA c) SA+WS

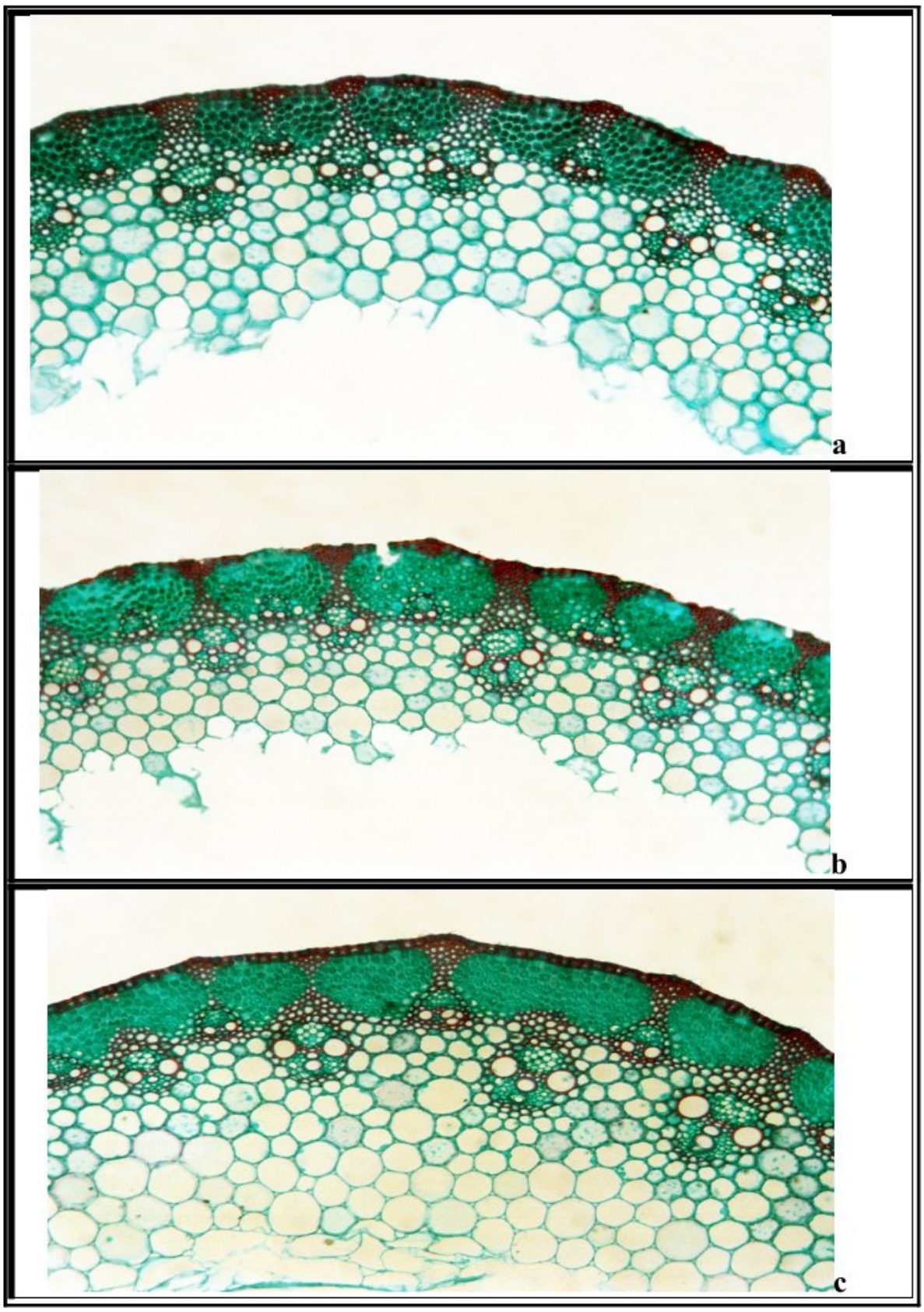


Plate 16. Effect of glycine betaine + salicylic acid on peduncle anatomy of droughted tolerant cultivar. (Cross section X = 10)

a) Control b) GB+SA c) GB+SA+WS