*Journal of Stress Physiology & Biochemistry, Vol. 10 No. 1 2014, pp. 145-166 ISSN 1997-0838*


**ORIGINAL ARTICLE**

# Effect of Spermine and Spermidine on Wheat Plants Irrigated with Waste Water: Conductive Canals of Flag Leaf and Peduncle in Relation to Grain Yield

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Received September 23, 2013

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*Key words: Anatomy, spermidine, spermine, waste water, wheat, yield*

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*Key words: Anatomy, spermidine, spermine, waste water, wheat, yield*

*Abbreviations: ABA- abscisic acid; Spm – spermine; Spd – spermidine; WW- waste water*

Important sources of heavy metals in waste water are urban and industrial effluents. Heavy metals are extremely persistent in the environment and accumulate to toxic levels (Sharma *et al.,* 2007). High concentrations of heavy metals affect mobilization and balanced distribution of the elements in plant organs via the competitive uptake (Schat and Ten Bookum, 1992).

Salinity stress decreased the thickness of leaf and ground tissues in different plant species (Aldesuquy and Baka, 1991). In this respect, Moursi *et al*. (1983 a, b) reported that, anatomical structures of leaf and peduncle vascular bundles differ between maize cultivars. Furthermore, the phloem area was found to be directly proportional to the rate of assimilates import by the ears for 22 wheat lines (Evans *et al.,* 1970). The thickness of the flag leaf differed remarkably between wheat cultivars. The leaf thickness of Sakha 8, Sakha 61 and Sakha 69 was higher than those of the other varieties. On the other hand, the vascular bundle diameter of these varieties showed opposite trend (Abdel-Gawad *et al.,* 1985a).

Maruthi *et al.* (2005) demonstrated that, the Zn-treated mustard plants showed gradual changes in leaf structure with an increase in metal concentration. Also, reduction in the palisade and epidermal cells of plants under higher levels of Zn was showed in compared to the control. Moreover, the leaves of Zn-treated plants showed a breakdown of spongy and palisade parenchyma cells followed by further loss of cell shape and decrease in intercellular spaces compared to the control plants.

The Zn-treated stems showed thick black depositions along the walls of xylem and phloem vessels. In Cd-treated stems, the black depositions were not uniform, but were seen along the walls of vascular bundles. These precipitates were more intense in Zn-treated plants compared to Cd-treated plants. Moreover, the combination of Zn and Cd increased the number of vacuoles and precipitation along the cell walls in the root of mustard plants when compared to the cross sections of control (Maruthi *et al.*, 2005). Chromium caused alteration in root hair diameter, and changes in cortex and pith (Shanker *et al.,* 2005), as well as damage to the root system and unbalanced supply of nutrient and / or an alteration of their role in anabolic pathways (Valeria *et al.,* 2006).

The flag leaf of saline-treated plants showed some negative changes in conductory tissues inducing the decrease in phloem area, metaxylem area, xylem tissue area and number of vascular bundles but increasing the hairs number especially on the lower epidermis of the flag leaf (Aldesuquy and Baka, 1991). In general, irrigation of wheat plants with NaCl at 66 or 99 mM reduced the peduncle diameter in different plant species (El-Kabbia *et al.,* 1981). Moreover, Aldesuquy and Baka (1991) also reported that, salinity at the two levels (66 or 99 mM) markedly reduced the area of xylem tissue and xylem diameter but increased the number of hairs on peduncle surface of wheat plants.

Wheat yield is a function of many factors among which canopy structure and architecture are the most important (Abdel-Gawad *et al.,* 1985b). Also grain filling rate and the grain filling period are important factors in determining the final yield of wheat cultivars (Abdel-Gawad *et al.,* 1985c). The accumulation of heavy metals in plant tissues might cause reduction in physiological and biochemical activities of plants resulting lower biomass and yields. Yield thus had significant and negative relationship with the concentrations of $Ni^{++}$, $Cd^{++}$, $Cu^{++}$, $Pb^{++}$, $Zn^{++}$ and $Cr^{+3}$ in root and shoot (Anurag *et al.,* 2008; Sharma *et al.,* 2008; Aldesuquy *et al.,* 2011). The elements are Cd, Zn, Cu, Pb, Ni which, when applied to the soil in excessive amount, decreased plant yields or degrade quality of food or fiber products in different plant species (Sutapa and Bhattacharyya, 2008 ).

The main goal of this study is to find out the beneficial effect of grain priming with Spm and Spd on increasing the heavy metals tolerance of wheat sensitive variety (Sakha 94) through anatomical adaptation in relation to grain yield.

## MATERIALS AND METHODS

### Plant material and growth conditions

Homogeneous lot of wheat grains (*Triticum aestivum)* variety Sakha 94 were surface sterilized by soaking in 0.001M $HgCl_2$ solution for 3 minutes, then washed thoroughly with distilled water, and divided into four sets which were soaked in distilled water to serve as control, spermine (0.15 mM), spermidine (0.3mM) or (spermine 0.15mM + spermidine 0.3mM) respectively for about six hours. After soaking, the thoroughly washed grains were planted on 15$^{th}$ November 2005 in plastic pots (15 grains per pot; 25 cm width x 30 cm height) filled with 6 kg mixture of soil (clay and sand = 2:1, v/v). The pots were kept in greenhouse, where the plants subjected to natural day / night conditions (minimum / maximum temperature and relative humidity were: 29.2 / 33.2 ºC and 63 / 68 % respectively, at mid-day) during the experimental period. The plants in all sets were irrigated to field capacity by normal tap water. Fifteen days after planting, the plants were thinned to five / pot. On day 21 from sowing, the pots of each set were subdivided into four groups each one contained 20 pots. The pots of the first group in each set still irrigated with tap water, while 2$^{nd}$, 3$^{rd}$ and 4$^{th}$ groups in all sets were irrigated with 25%, 50% or 100% waste water respectively. The resulting sixteen treatments were marked as follows:-

| Treatments | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| WW % | 0 | 25 | 50 | 100 | 0 | 25 | 50 | 100 | 0 | 25 | 50 | 100 | 0 | 25 | 50 | 100 |
| Spm (0.15mM) | - | - | - | - | + | + | + | + | - | - | - | - | - | - | - | - |
| Spd (0.30 mM) | - | - | - | - | - | - | - | - | + | + | + | + | - | - | - | - |
| Spm + Spd | - | - | - | - | - | - | - | - | - | - | - | - | + | + | + | + |

Physicochemical characters of irrigation water revealed that, the standard fresh water contained [chemical oxygen demand (COD) = 5.0, biochemical oxygen demand (BOD) = 2.0, total suspended solids (TSS) = 4.0, total hardness = 60.0, $Cd^{++}$ = 0.05, $Pb^{++}$ = 0.05, $Cu^{++}$ = 0.04, $Ni^{++}$ = 0.07, $Zn^{++}$ = 0.08, $Na^+$ = 0.02, $K^+$ = 0.01, $Ca^{++}$ = 0.01, total phosphorus = 0.07, $Cl^-$ = 45.0, $SO^{--}_4$ = 00.0, $NO_3^-$ = 0.01, $NO_2^-$ = 0.002 ppm] while the untreated waste water contained [COD = 150.0, BOD = 60.0, total suspended solids (TSS) = 226.0, total hardness = 770.0, $Cd^{++}$ = 0.12, $Pb^{++}$ = 0.23, $Cu^{++}$ = 0.12, $Ni^{++}$ = 0.20, $Zn^{++}$ = 0.93, $Na^+$ = 0.22, $K^+$ = 0.14, $Ca^{++}$ = 0.19, total phosphorus = 0.38, $Cl^-$ = 283.6, $SO_4^-$ = 72.0, $NO_3^-$ = 50.0, $NO_2^-$ = 7.3 ppm]. These analyses were carried out according to Clescrei *et al*. (1998). The main source of untreated waste water was the main Aga drain in Dakahliya Province, Egypt.

At tillering stage (i.e. 21 days from planting) and at heading (65 days from planting), the plants received 35 kg N $ha^{-1}$ as urea and 35 kg P $ha^{-1}$ as potassium dihydrogen phosphate as fertilizers.

Measurements were carried out at ear emergence (i.e. 65 d from planting). For anatomical study, five samples from the flag leaf and peduncle of the main shoot at heading stage (65d after sowing) were used. Measurement of each anatomical parameter studied was carried out in twelve sections in the keel region of the flag leaf and in the peduncle of main shoot of wheat plants. Also, all measurements were well photographed using light microscope.

### 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. Canada Balsam was used as mounting medium. Sections were estimated by the aid of light microscope. Measurement of each anatomical parameter was calculated in twelve sections 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 (Figure 1).

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.

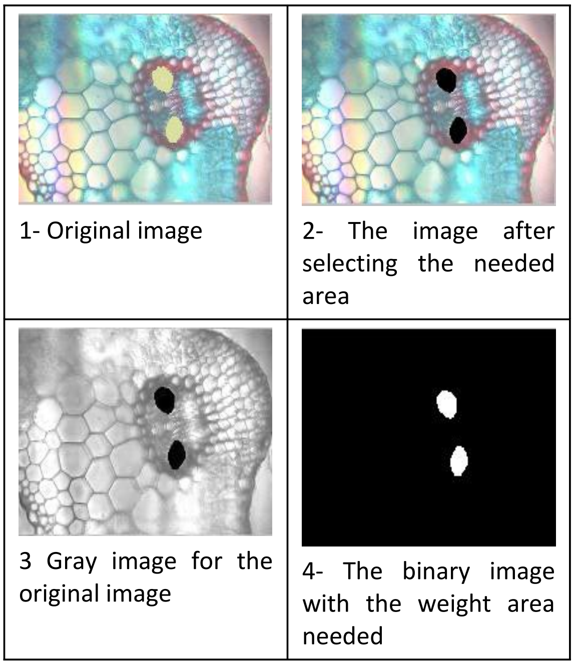


**Figure 1**. The image analysis steps for estimating the area of metaxylem (in microns) for flag leaf of wheat cultivar.

**Statistical analysis**

A test for significant differences between means at $P \leq 0.05$ was performed using least significant difference (LSD) test (Snedecor and Cochran 1970). The correlation coefficients were estimated according to SPSS programme.

## RESULTS

**Flag leaf anatomy**

Waste water markedly affected the anatomical features in flag leaves of wheat plants (Table 1 & Plates 1, 2, 3 and 4). In general, it caused significant increases ($P \leq 0.05$) in the leaf thickness, ground tissue thickness, metaxylem vessel area, xylem vessel area, vascular bundle area, number of motor cells, number of hairs, number of closed stomata on both upper and lower epidermis as well as number of fibrous layers on lower and upper surfaces of wheat flag leaf. On the other hand, leaf thickness and ground tissue thickness appeared to decrease ($P \leq 0.05$) at concentration 100% of waste water. On the other hand, waste water at all concentrations decreased ($P \leq 0.05$) phloem area and number of opened stomata on both upper and lower epidermis in flag leaves of wheat plants.

In general, grain presoaking in Spm, Spd or their interaction increased the leaf thickness, ground tissue thickness, metaxylem vessel area, xylem vessel area, phloem area, vascular bundle area, number of motor cells, number of hairs, number of closed stomata on both upper and lower epidermis as well as number of fibrous layers on lower and upper epidermis. In addition, number of opened stomata on both upper and lower epidermis appeared to be significantly decreased.

**Peduncle anatomy**

As compared to control values, waste water caused noticeable changes in the anatomical features of peduncle of wheat plants (Table 2 & Plates 5, 6, 7 and 8). Waste water at all examined concentrations caused a significant increases ($P \leq 0.05$) in metaxylem vessel area, xylem vessel area, vascular bundle area, number of vascular bundles, number of hairs, number of closed stomata. On the other hand, waste water at all concentrations decreased ($P \leq 0.05$) tracheid area, phloem area as well as number of opened stomata.

In the majority of cases, grain priming with Spm, Spd or their interaction increased tracheid area, metaxylem vessel area, xylem vessel area, phloem area, vascular bundle area, number of vascular bundles, number of hairs and number of closed stomata. On the other hand, the two chemicals alleviated the deleterious effect of waste water and decreased ($P \leq 0.05$) the number of opened stomata in peduncles of wheat plants.

**Table 1 :** Effect of spermine, spermidine and their interaction on leaf anatomy of wheat plants (at anthesis stage) irrigated with different concentrations of waste water.

| Parameters / Treatments | Leaf thickness (µm) | Ground tissue thickness (µm) | Metaxylem vessel area $(\mu m)^2$ | Xylem area $(\mu m)^2$ | Phloem area $(\mu m)^2$ | Vascular bundle area $(\mu m)^2$ | Number of motor cells | Number of hairs | No of stomata lower epidermis | | No of stomata upper epidermis | | No of fibrous layers | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | | opened | closed | opened | closed | lower | upper |
| **Cont.** | 381 | 355 | 841 | 2344 | 4877 | 12292 | 46.00 | 19.72 | 18.00 | 1.33 | 24.0 | 2.6 | 3.33 | 4.33 |
| **WW 25%** | 485 | 453 | 993 | 2747 | 4788 | 14568 | 49.00 | 22.65 | 15.71 | 3.67 | 21.7 | 5.7 | 5.67 | 7.67 |
| **WW 50%** | 546 | 514 | 1122 | 3692 | 4338 | 15330 | 52.33 | 24.71 | 15.30 | 4.33 | 19.7 | 7.7 | 7.33 | 9.00 |
| **WW 100%** | 255 | 227 | 1270 | 4023 | 4163 | 15572 | 58.00 | 27.04 | 13.01 | 6.30 | 17.0 | 10.3 | 4.33 | 6.33 |
| **Spm** | 399 | 366 | 862 | 2560 | 6407 | 12654 | 43.67 | 23.00 | 13.70 | 5.41 | 20.0 | 7.4 | 5.30 | 5.00 |
| **Spm+WW 25%** | 404 | 381 | 889 | 3361 | 5243 | 13258 | 46.33 | 25.33 | 12.33 | 7.03 | 17.6 | 9.7 | 5.00 | 5.71 |
| **Spm+WW 50%** | 416 | 389 | 1007 | 3759 | 4855 | 14155 | 49.67 | 28.02 | 10.71 | 8.66 | 16.0 | 11.3 | 6.00 | 6.67 |
| **Spm+WW 100%** | 365 | 328 | 1111 | 3835 | 4337 | 14350 | 52.03 | 29.30 | 9.33 | 9.70 | 14.7 | 12.6 | 4.67 | 7.33 |
| **Spd** | 384 | 333 | 855 | 2455 | 6064 | 12444 | 45.33 | 22.34 | 16.70 | 2.67 | 23.0 | 4.7 | 4.70 | 6.04 |
| **Spd+WW 25%** | 393 | 363 | 1095 | 3490 | 5120 | 13458 | 49.33 | 23.11 | 14.72 | 4.70 | 20.6 | 6.3 | 4.00 | 4.72 |
| **Spd+WW 50%** | 371 | 345 | 1117 | 3762 | 4679 | 14450 | 53.67 | 25.74 | 12.74 | 6.71 | 19.0 | 7.7 | 5.67 | 6.00 |
| **Spd+WW 100%** | 289 | 249 | 1186 | 3841 | 4431 | 14760 | 55.67 | 27.00 | 10.68 | 8.66 | 18.0 | 9.3 | 4.80 | 4.00 |
| **Spm+Spd** | 426 | 398 | 878 | 2620 | 7057 | 12956 | 47.67 | 24.33 | 10.00 | 9.34 | 15.6 | 12.6 | 5.33 | 6.67 |
| **Spm+Spd+WW 25%** | 432 | 391 | 911 | 2855 | 5892 | 13155 | 49.74 | 26.70 | 8.04 | 11.34 | 14.0 | 15.0 | 5.02 | 5.70 |
| **Spm+Spd +WW 50%** | 320 | 288 | 976 | 2990 | 5322 | 13648 | 51.00 | 30.30 | 5.67 | 13.70 | 11.3 | 16.6 | 5.00 | 6.01 |
| **Spm+Spd+WW 100%** | 299 | 263 | 1077 | 3361 | 4630 | 14212 | 54.00 | 34.11 | 3.00 | 16.00 | 7.7 | 20.3 | 6.33 | 7.00 |
| **LSD** | **27.76** | **21.33** | **88.69** | **324** | **126** | **672** | **1.18** | **1.83** | **2.11** | **1.12** | **0.91** | **0.62** | **0.54** | **0.39** |

**Table 2 :** Effect of spermine, spermidine and their interaction on peduncle anatomy of wheat plants (at anthesis stage) irrigated with different concentrations of waste water.

| Parameters / Treatments | Tracheids area $(\mu m)^2$ | Metaxylem vessel area $(\mu m)^2$ | Xylem area $(\mu m)^2$ | Phloem area $(\mu m)^2$ | Vascular bundle area $(\mu m)^2$ | Number of vascular bundles | Number of hairs | Number of stomata | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | opened | closed |
| Cont. | 932 | 764 | 4897 | 4360 | 11656 | 45.23 | 29.71 | 28.35 | 12.73 |
| WW 25% | 865 | 879 | 5375 | 3458 | 12970 | 48.15 | 34.70 | 23.77 | 17.22 |
| WW 50% | 777 | 1003 | 5832 | 3302 | 15735 | 53.60 | 40.05 | 21.43 | 19.74 |
| WW 100% | 642 | 1223 | 6483 | 2996 | 16234 | 58.28 | 44.00 | 18.22 | 22.78 |
| Spm | 957 | 787 | 5122 | 5734 | 11755 | 47.70 | 34.70 | 25.28 | 15.68 |
| Spm+WW 25% | 887 | 866 | 5387 | 5133 | 12050 | 48.87 | 37.70 | 22.72 | 18.33 |
| Spm+WW 50% | 824 | 995 | 5674 | 4657 | 12708 | 51.22 | 38.80 | 19.40 | 20.76 |
| Spm+WW 100% | 768 | 1115 | 6132 | 4087 | 13682 | 54.20 | 41.00 | 18.20 | 23.87 |
| Spd | 937 | 773 | 4956 | 5576 | 11542 | 46.66 | 33.33 | 26.27 | 13.88 |
| Spd+WW 25% | 866 | 880 | 5422 | 5071 | 12519 | 49.77 | 34.00 | 24.66 | 15.67 |
| Spd+WW 50% | 802 | 1114 | 5786 | 4389 | 13229 | 52.12 | 36.00 | 22.10 | 18.94 |
| Spd+WW 100% | 744 | 1189 | 6277 | 3122 | 13942 | 54.33 | 38.30 | 19.88 | 20.67 |
| Spm+Spd | 962 | 844 | 5238 | 6454 | 11814 | 48.54 | 35.00 | 21.60 | 19.33 |
| Spm+Spd+WW 25% | 901 | 854 | 5301 | 5862 | 11971 | 49.88 | 39.00 | 18.22 | 22.00 |
| Spm+Spd +WW 50% | 840 | 978 | 5588 | 5233 | 12210 | 51.23 | 41.82 | 15.67 | 24.33 |
| Spm+Spd+WW 100% | 785 | 1002 | 5892 | 4264 | 13227 | 52.67 | 42.30 | 13.40 | 26.66 |
| **LSD** | **65.3** | **89.32** | **756** | **286** | **987** | **1.79** | **1.13** | **1.24** | **1.38** |

**Table 3 :** Effect of spermine, spermidine and their interaction on grain yield of wheat plants irrigated with different concentrations of waste water.

| Parameters / Treatments | Grain yield/ plant (g) |
|---|---|
| Cont. | 2.93 |
| WW 25% | 2.70 |
| WW 50% | 2.38 |
| WW 100% | 1.92 |
| Spm | 3.59 |
| Spm+WW 25% | 3.21 |
| Spm+WW 50% | 2.82 |
| Spm+WW 100% | 2.38 |
| Spd | 3.34 |
| Spd+WW 25% | 2.92 |
| Spd+WW 50% | 2.56 |
| Spd+WW 100% | 2.23 |
| Spm+Spd | 4.16 |
| Spm+Spd+WW 25% | 3.55 |
| Spm+Spd +WW 50% | 2.98 |
| Spm+Spd+WW 100% | 2.49 |
| LSD | **0.08** |

In general, waste water decreased significantly ($P \leq 0.05$) grain yield of wheat plants as compared to the control plants (Table 3). In the majority of cases, the application of Spm, Spd or their interaction appeared to mitigate the stress imposed by waste water on all yield components of wheat plants.

For anatomical features, the economic yield (grain yield) for wheat plants appeared to be positively correlated with phloem area of flag leaf (r = 0.97) and peduncle (r = 0.94) but negatively correlated with vascular bundle area and xylem area of flag leaf and peduncle (r = -0.82, -0.75, -0.81 & -0.80) for wheat plants.

## DISCUSSION

In all over the world, treatment and reuse of waste water take the attention to face the continuous water shortage. As the wheat has about 70 – 75% carbohydrates, it considers the main source of plant carbohydrates in the food for human consumption, so wheat has up-normal importance. Thus, this research was undertaken to evaluate the possible role of polyamines (spermine, spermidine or their interaction) in mitigating the harmful effects of irrigation of wheat plants with untreated waste water polluted mainly with heavy metals from main Aga drain in Dakahliya Province, Egypt. The heavy metals were being the main problem in effluent waste water.

Most plants have developed morphological and physiological mechanisms which allow them to adapt and survive under stress conditions (Ludlow, 1989). These mechanisms mainly comprise roles for cell wall, extracellular exudates, vacuoles and plasma membrane; either by reducing the uptake of heavy metals or by stimulating the efflux pumping of metals that have entered the cytosol (Hall, 2002). In addition, the number of hairs, motor cells, fibrous layers and leaf thickness increased in wheat plants under waste water stress conditions.

Data indicated that, irrigation of wheat plants with waste water induced marked increases ($P \leq 0.05$) in the leaf thickness, ground tissue thickness, metaxylem vessel area, xylem vessel area, vascular bundle area, number of motor cells, number of hairs, number of closed stomata on both upper and lower epidermis as well as number of fibrous layers on lower and upper epidermis. The increase in leaf thickness may result from the increase in the number of fibrous layers on lower and upper surfaces of wheat flag leaf. On the other hand, leaf thickness and ground tissue thickness appeared to be decreased ($P \leq 0.05$) at concentration 100% of waste water. This is presumably due to a reduction in mesophyll tissue of the flag leaf. These results were in accordance with those obtained by Aldesuquy *et al.* (1998) with wheat plants under salinity. Moreover, Maruthi *et al.* (2005) demonstrated that, the Zn-treated mustard plants showed gradual changes in leaf structure with an increase in metal concentration. Also, reduction in the palisade and epidermal cells of plants under higher levels of Zn was showed in comparing to the control. Moreover, the leaves of Zn-treated mustard plants showed a breakdown of spongy and palisade parenchyma cells followed by further loss of cell shape and decrease in intercellular spaces compared to the control plants.

Salinity or drought stress caused marked decrease in the thickness and ground tissues of wheat flag leaf. The reduction of leaf thickness might be due to the decrease in the spongy parenchyma tissue and/or palisade parenchyma tissue (Aldesuquy and Baka, 1991; Aldesuquy *et al.*, 2013).

In general, grain presoaking with Spm, Spd or their interaction induced marked increases in thickness of both leaf and ground tissues. The increase in the leaf thickness might be due to the increase in the mesophyll tissue and number of fibrous layers on lower and upper surfaces of wheat flag leaf. 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). Waste water at all examined concentrations increased metaxylem vessel area, xylem vessel area and vascular bundle area in flag leaf and peduncle as well as number of vascular bundles in wheat peduncle. These results may explain the faster translocation of heavy metals (i.e. $Cd^{++}$, $Zn^{++}$, $Cu^{++}$, $Pb^{++}$ & $Ni^{++}$) from root to shoot. On the other hand, waste water at all examined concentrations significantly decreased phloem area. These results were in a good agreement with those obtained by Evans *et al.* (1970) using wheat plants. In this respect, Aldesuquy and Baka (1991) reported that, the flag leaf of saline-treated plants showed some negative changes in conductor tissues, where the decrease in the phloem area will lead to a slow rate of translocation of photo-assimilates towards the developing grains.

The applied chemicals induced increases in the areas of conductive canals (xylem and phloem) in flag leaf and peduncle of wheat plants as compared to the control ones. In addition, the application of these chemicals caused negative correlations between grain yield and vascular bundle area, xylem area but positive correlations between grain yield and phloem area in both leaf and peduncle of wheat plants. This furnishes better translocation of assimilates from flag leaf (as source) towards the developing grains (as sink) through the conductive canals. In this regard, Evans *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 number of hairs on the flag leaf surfaces and peduncle as a result of waste water treatment may probably be due to that hairs may increase the accumulation of heavy metals inside it to avoid the harmful effects of heavy metals stress. These increases in the hairs number of flag leaf and its peduncle in waste water irrigated-plants would be expected to increase the leaf resistance as response to heavy metals toxicity accumulating the excess of these metals inside the flag leaf and peduncle hairs. These results were in accordance with those obtained by Cho *et al.* (2003) using *Arabidopsis halleri* and *Arabidopsis thaliana* plants. In this connection, Udovenko *et al.* (1976) found that salinity treatments increased the number of hairs on peduncle surface which run in close parallelism with our results (Table 1 and 2 & Plates 1, 2, 3, 4, 5, 6, 7 and 8). This may support the idea that hairs have protective role against the accumulation and harmful effects of free heavy metals inside the plant cell. In this connection, the combination of Zn and Cd increased the number of vacuoles and precipitation along the cell walls in the root of mustard plants when compared to the cross sections of control (Maruthi *et al.*, 2005). Chromium caused alteration in root hair diameter, and changes in cortex and pith (Shanker *et al.,* 2005), as well as damage to the root system and unbalanced supply of nutrient and / or an alteration of their role in anabolic pathways (Valeria *et al.,* 2006).

Generally, the application of Spm, Spd or their interaction caused additional increases in the number of hairs in flag leaves and peduncles of wheat plants in order to increase the tolerance of waste water-treated-wheat plants. This was in accordance with Aldesuquy *et al.* (1998) with wheat plants treated with sodium salicylate under salinity.

Waste water at all examined doses resulted in additional increases in the number of closed stomata and a decrease in the number of opened ones on both upper and lower epidermis of wheat flag leaf. This modification appears to be an adaptive mechanism toward waste water stress treatments. In this respect, Fahn and Cutler (1992) 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.

As compared to control, application of Spm, Spd or their interaction decreased the number of opened stomata on both upper and lower epidermis of wheat flag leaf and peduncle. Therefore the two chemicals induced an adaptive response to waste water by keeping turgidity of leaves through stomatal closure and consequently increased the water content in leaf necessary for photosynthesis.

Waste water at all examined concentrations increased the number of motor cells on the upper epidermis of flag leaf of wheat plants. Furthermore, treatment with Spm, Spd or their interaction caused an increase in the number of motor cells in the flag leaf. This increase induced an adaptive response of wheat plants towards waste water stress, 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 turgor loss (Sibaoka, 1980). Regarding the mechanism of movement in *Mimosa*, Sibaoka (1991) reported that the motor cells contain a febrile 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. Regarding the effect of these chemicals (Spm and Spd), they appeared to be useful in improving tolerance of wheat plants towards waste water treatments.

The reduction in yield of waste water-treated wheat plants can be attributed to the decrease in total cumulative leaf area, photosynthetic pigments, carbohydrates accumulation (polysaccharides) and nitrogenous compounds (total nitrogen and protein) in leaves and consequently in wheat yielded grains. These results were in a good agreement with those obtained by Slocum *et al.* (1984); Mallan and Farrant (1998) in different plant species. The decreases in yield and yield components in different crops under similar conditions have also been reported by many workers (Mallan and Farrant, 1998; Aldesuquy *et al*., 2004). The results clearly indicated that application of Spm, Spd or their interaction was significant in alleviating the adverse effects of waste water on yield and yield components of wheat plants. The increase in yield production may be due to increase in longevity of leaves which perhaps contributed to grain filling by enhancing the duration of photosynthates supply to grains (Kaur-Sawhney *et al.,* 1982). This phenomenon was manifested particularly when we found that, there was a positive correlation between phloem area in both flag leaf and peduncle of main shoot of wheat plants which accelerate rapid translocation of photo-assimilates from source (i.e. flag leaf) to sink (i.e. grain in spike). In this respect, Polyamines play very important role in many physiological processes (related to yield quality) such as reproductive organ development, tuberization, floral initiation and fruit development and ripening (Bais and Ravishankar, 2002; Tiburcio *et al*., 2002).

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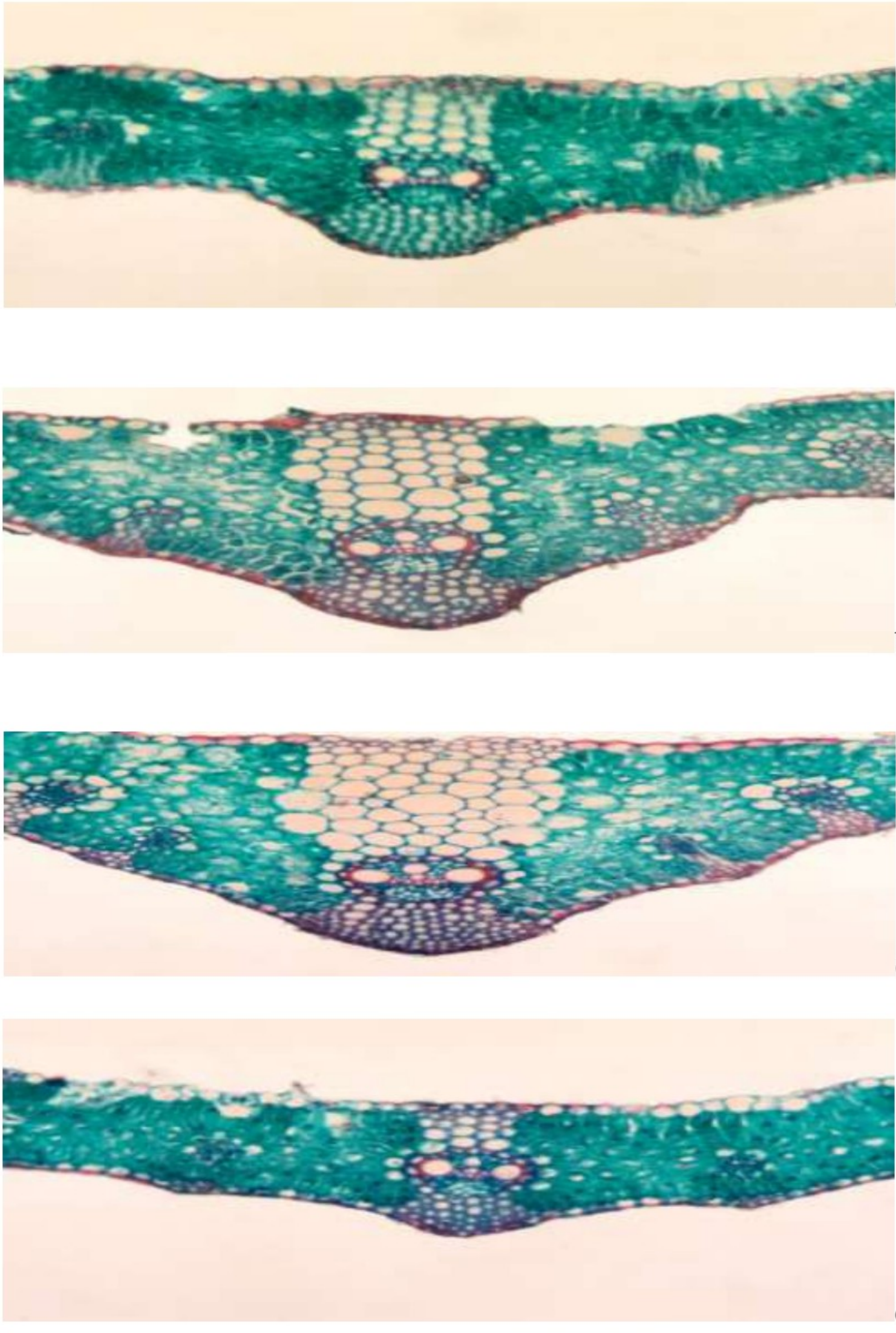
**Plates caption**



a b c d

**Plate 1.** Effect of waste water on flag leaf anatomy of wheat irrigated with different concentrations of waste water. (Cross section X = 10)

a) Control b) Waste water 25%

c) Waste water 50% d) Waste water 100%

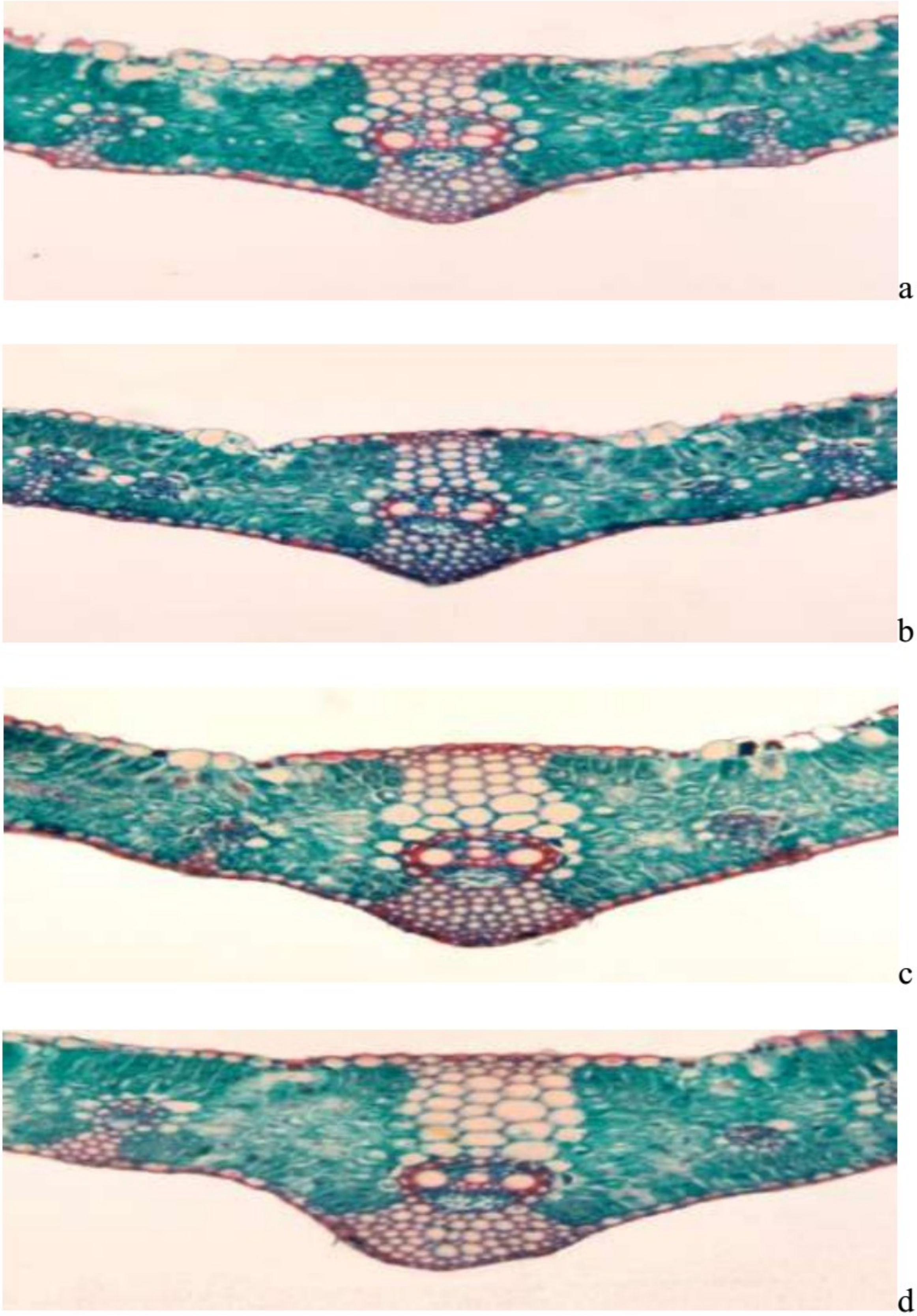
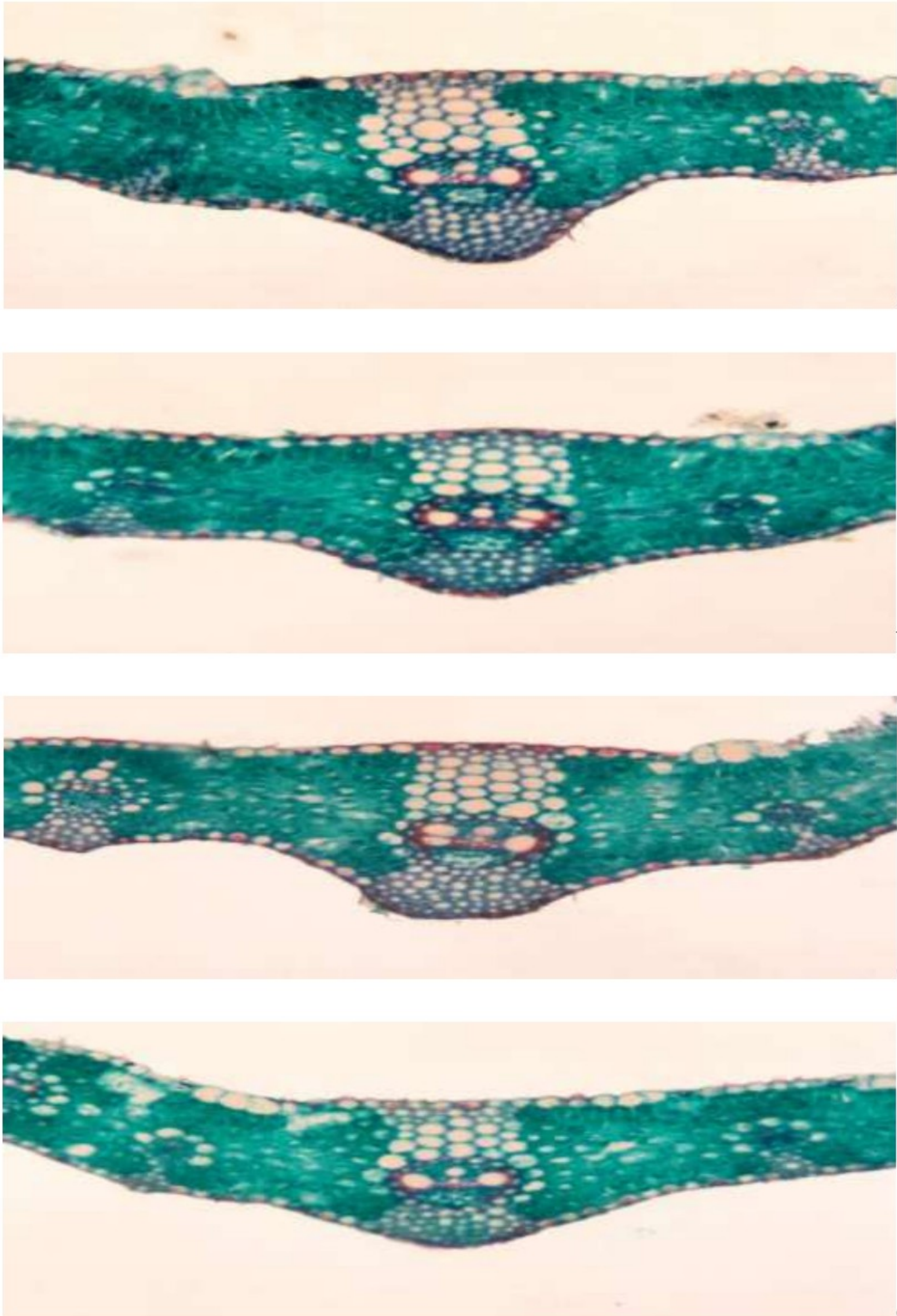



**Plate 2.** Effect of spermine on flag leaf anatomy of wheat plants irrigated with different concentrations of waste water. (Cross section X = 10)

a) Control b) Spm+waste water 25%

c) Spm+waste water 50% d) Spm+waste water 100%



a b c d

**Plate 3.** Effect of spermidine on flag leaf anatomy of wheat plants irrigated with different concentrations of waste water. (Cross section X = 10)

a) Control b) Spd+waste water 25%

c) Spd+waste water 50% d) Spd+waste water 100%

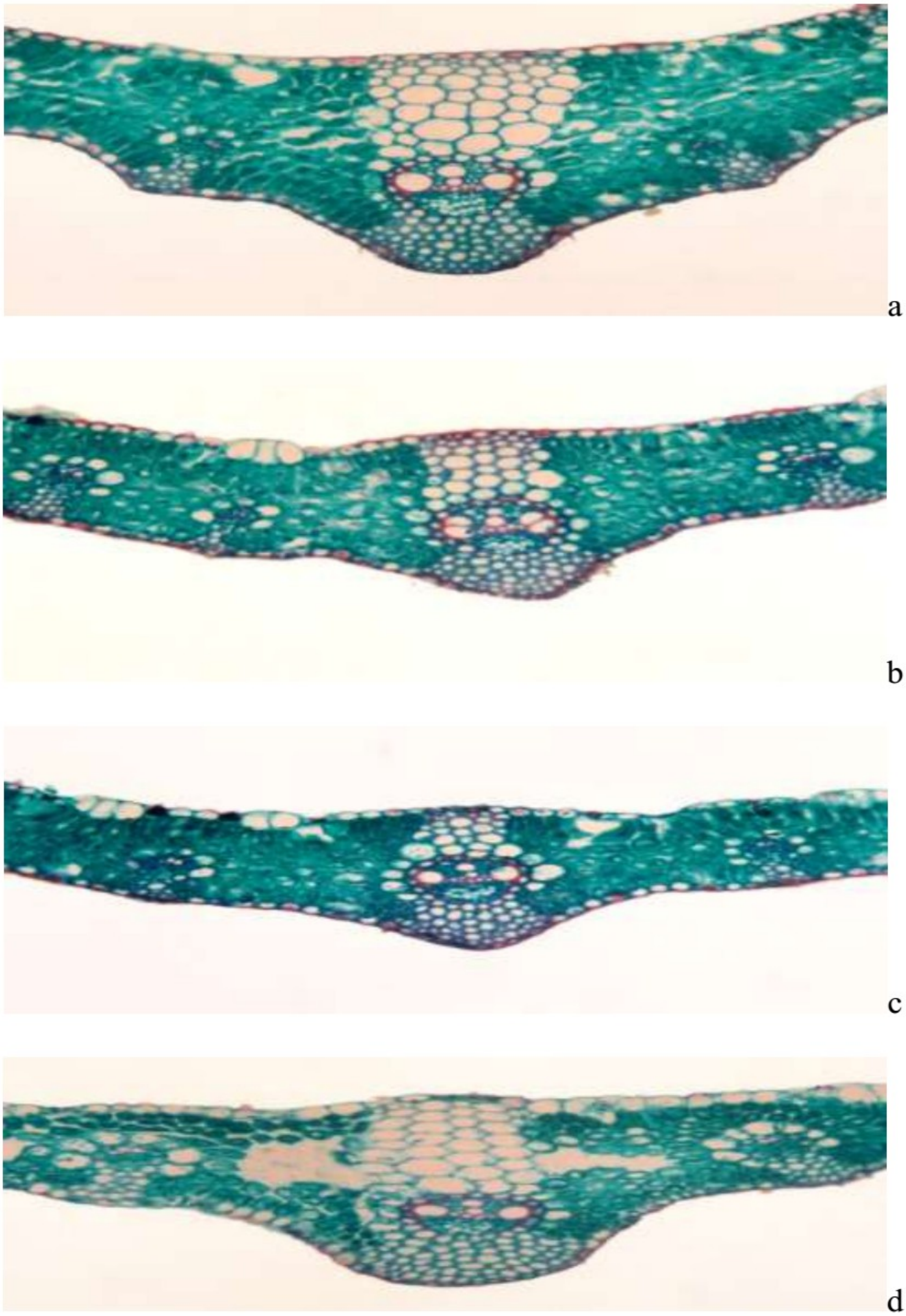



**Plate 4.** Effect of spermine and spermidine on flag leaf anatomy of wheat plants irrigated with different concentrations of waste water. (Cross section X = 10)

a) Control  b) Spm+Spd+waste water 25%

c) Spm+Spd+waste water 50%  d) Spm+Spd+waste water 100%

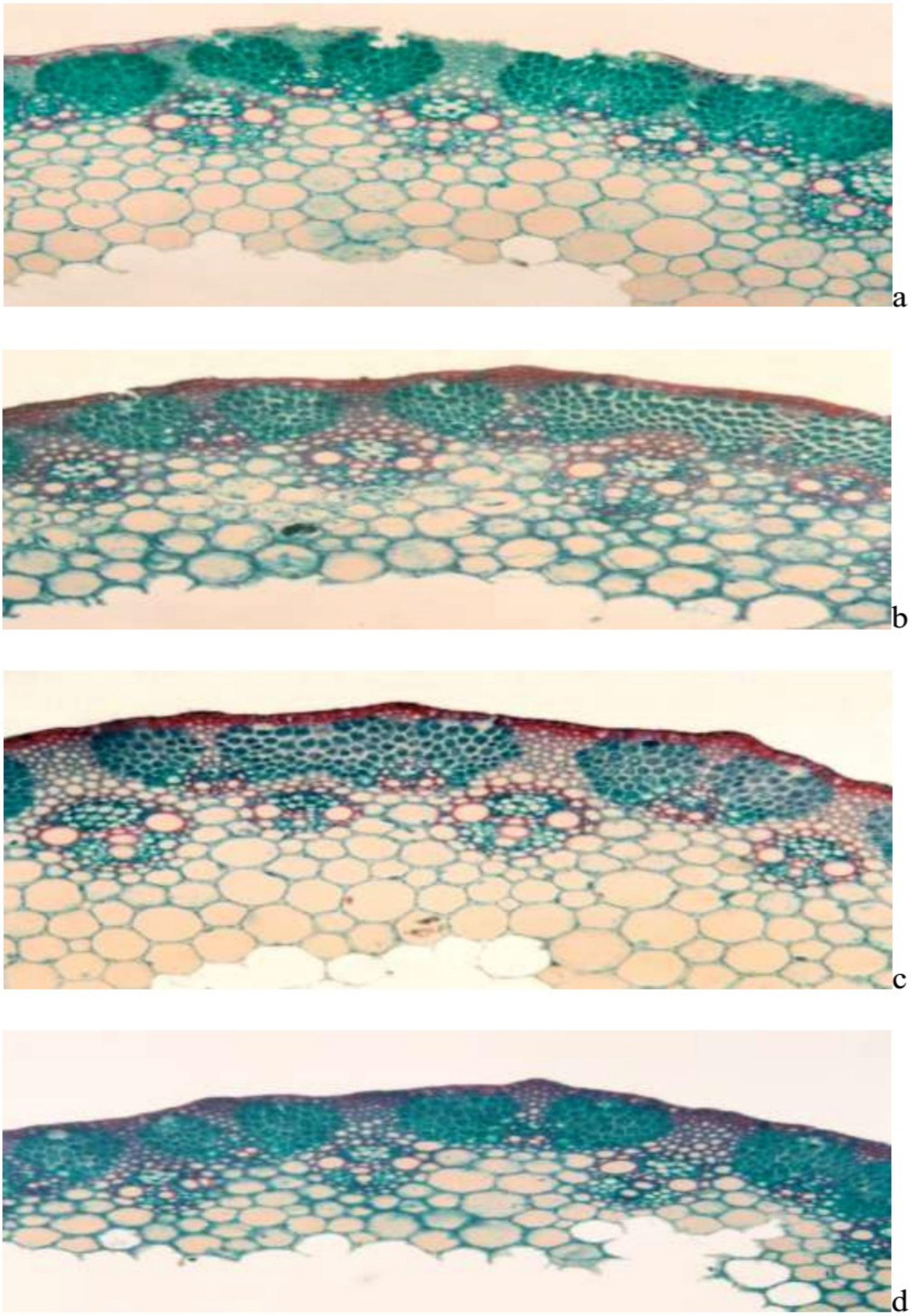



**Plate 5.** Effect of waste water on peduncle anatomy of wheat plants irrigated with different concentrations of waste water.(Cross section X = 10)

a) Control b) Waste water 25%

c) Waste water 50% d) Waste water 100%

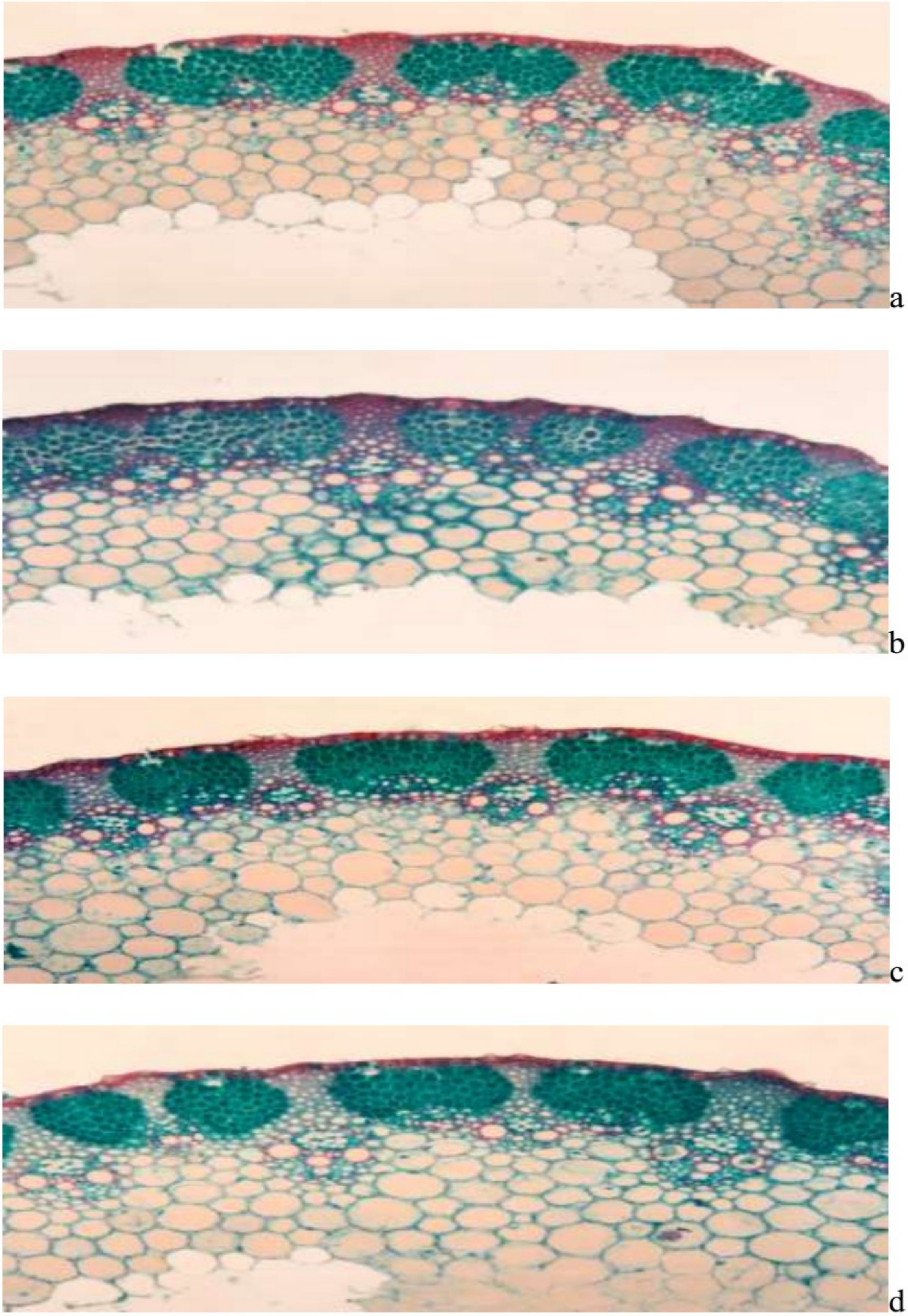



**Plate 6.** Effect of spermine on peduncle anatomy of wheat plants irrigated with different concentrations of waste water. (Cross section X = 10)

a) Control b) Spm+waste water 25%

c) Spm+waste water 50% d) Spm+waste water 100%

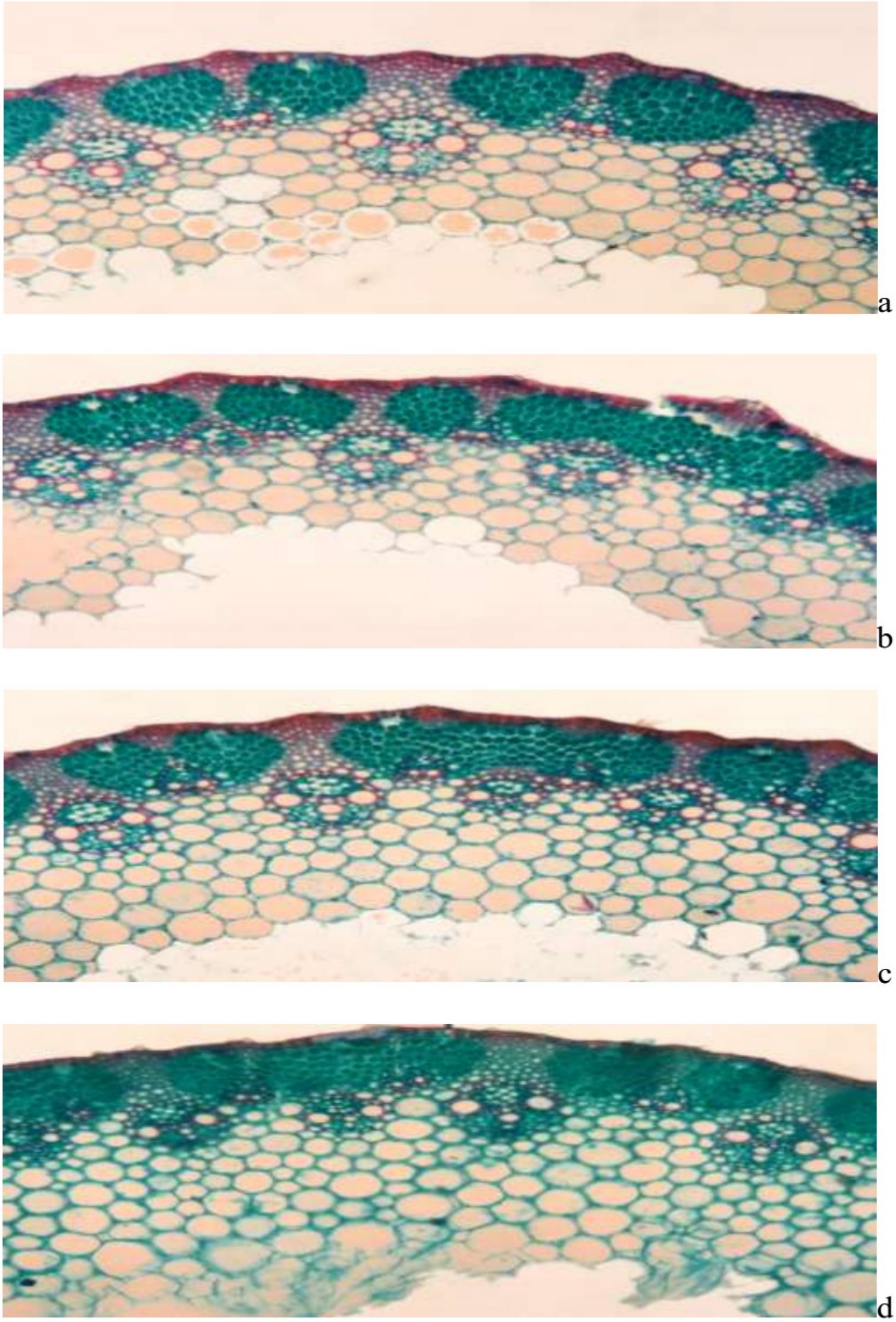



**Plate 7.** Effect of spermidine on peduncle anatomy of wheat plants irrigated with different concentrations of waste water. (Cross section X = 10)

a) Control b) Spd+waste water 25%

c) Spd+waste water 50% d) Spd+waste water 100%

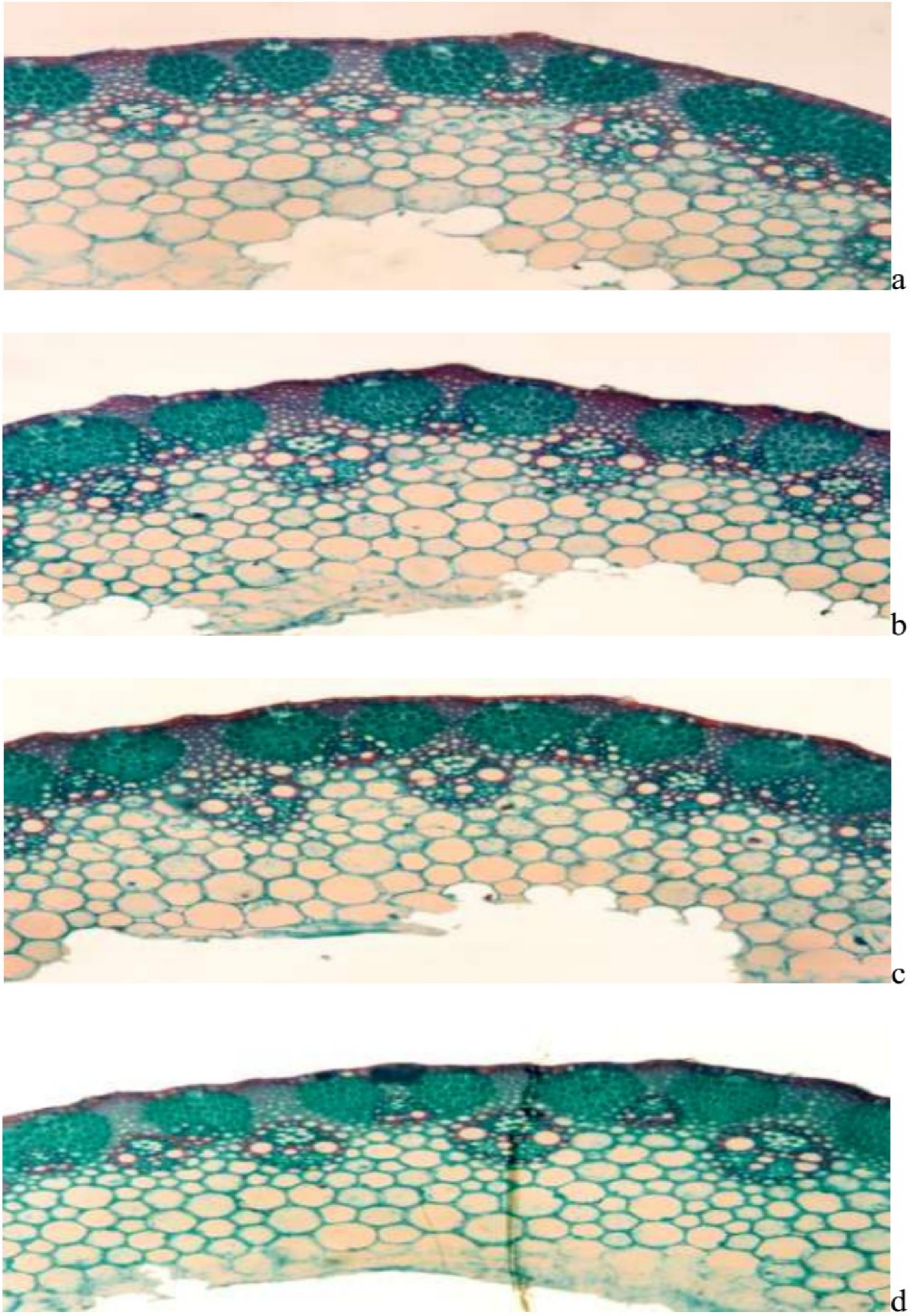


**Plate 8.** Effect of spermine and spermidine on peduncle anatomy of wheat plants irrigated with different concentrations of waste water. (Cross section X = 10)

a) Control b) Spm+Spd+waste water 25%

c) Spm+Spd+waste water 50% d) Spm+Spd+waste water 100%