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Optimizing Growth, Seed Yield and Quality of Soybean (*Glycine max* L.) Plant Using Growth Substances

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Authors' contributions

This work was carried out in collaboration between both authors. Both authors read and approved the final manuscript.

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ABSTRACT

Aims: It is become necessary to reduce the gap between the demand and supply of edible oil and protein. So, the aim of the present investigation is to study the effect of foliar application with humic acid, salicylic acid and paclobutrazol on some morphological, anatomical and seed yield characteristics, as well as on some biochemical constituents of soybean (*Glycine max* L.) seed for maximizing its growth, yield and nutritional value.
Methodology: A 2 year field trial was carried out during 2015 and 2016 at the Faculty of Agriculture Experiment Station, Benha University, Egypt . Soybean (*Glycine max* L.) cv. Giza 111 seeds were sown into plots (each plot unit about 3.5 m long and 3.0 m wide) in clay loam soil. A total of six treatments of humic acid, salyclic acid, and paclobutrazol were foliarly applied: humic acid at 2.5 and 5.0 gl^{-1}, salicylic acid at 50 and 100 mgl^{-1}, paclobutrazol at 10 and 20 mgl^{-1} and Control in a 3 randomized complete blocks.
Results: All rates of humic acid, salicylic acid and paclobutrazol had a significant effect on soybean morphological characters during the 2 years. The treatment set of 10 mgl^{-1} paclobutrazol, 5 gl^{-1} humic acid, and 100 mgl^{-1} salicylic acid resulted in relatively higher means for most vegetative

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growth characters compared to both the other 3 and the Control treatments. Despite variations among the 6 rates effects, all enhanced chlorophyll and carotenoids contents. This positive response extended to include the contents of soybean shoot endogenous phytohormones. Soybean yield components and yield were significantly improved due to applying these 3 growth substances using these current rates, this in addition to their positive effect on N P K content, crude protein, total carbohydrates and percentage total lipids in soybean seed yield. Under the conditions of this trial, either of 10 mgl^{-1} paclobutrazol, 5 gl^{-1} humic acid, or 100 mgl^{-1} salicylic acid proved useful to improve soybean growth and yield, yet more further research deem necessary to include more expanded set of rates to be assessed under other perspectives.

Keywords: Soybean; humic acid; salicylic acid; paclobutrazol; growth; anatomy; yield and chemical compositions.

1. INTRODUCTION

Soybean (*Glycin max* L.) is the widely cultivated specie of the family Fabaceae (order Fabales) [1]. It is considered one of the most important legume crops in the world. It can provide oils and protein suitable for feeding humans as well as animals. Soybean is the most important oil crop of world which contains about 18 to 22% cholesterol free oil with 85% unsaturated fatty acids , 40 to 45% protein, 24-26% carbohydrate and a good amount of vitamins in its seed [2,3,4]. Currently, soybean has gained increased attention in Egyptian agriculture, so it is become necessary to reduce the gap between the demand and supply of edible oil and protein [5].

Application of organic acids to improve the quality and quantity of agricultural products has been widely prevalent. Since these acids have hormonic compounds that positively affect plant performance. Among these acids is humic acid (HA) that forms an insoluble stable complex with the micro-nutrients [6,7,8].

Humic acid plays a major role in plant nutrients uptake and growth parameters in both vegetative and generative stages [9] on wheat and [10] on snap bean. The increment of growth parameters and crop yields due to HA application may be attributed to that it has a stable fraction of carbon, thus regulating the carbon cycle and releasing of nutrients, including nitrogen, phosphorus, and sulfur. HA stimulates plant growth by the assimilation of major and minor elements, enzyme activation and/or inhibition, changes in membrane permeability, protein synthesis and finally the activation of biomass production [9]. Moreover, [11] reported that, HA (granular and liquid forms) can reduce plant stress as well as enhances plant nutrients uptake. In addition, HA can be used as a growth regulator by regulating endogenous hormone levels [12,13]. The effects of humic acids on numerous plants such as tomato [14], strawberry [15], spinach [16] and bean [17] have been well studied.

The mechanism of HA in promoting plant growth is not completely known, but several explanations proposed [18]. Attributed the beneficial effects of HA on plant growth to increasing minerals transport during cell membrane, oxygen uptake, respiration and photosynthesis, nutrients uptake, root and cell elongation. Moreover, [19] mentioned that the positive effects of humic acid on plant growth may be due to its acting as plant growth hormone.

Salicylic acid (SA) is anther naturally occurs in plants in very low amounts and participates in the regulation of many physiological processes in plant such as nutrient uptake, chlorophyll and protein synthesis [20,21]. SA is recognized as a plant hormone [22,23]. It has a significant effect on different aspects of plant growth and development, photosynthesis, evaporation, ion transmission and absorption, and also causes changes in leaf anatomy and chloroplast structure [24]. Stimulation of growth after supplementation of SA has been reported in many plants, (wheat [21], soybean [25] and maize [26]). It was found that SA-induced growth may be related to SA-induces considerable enhancement in net photosynthetic rate [27].

[28] Mentioned that salicylic acid induces promotive effect on flowering onset, morphological characters of vegetative growth, photosynthetic pigments and yield characteristics as well as seed quality of Egyptian lupine.

Paclobutrazol is an efficient plant growth retardant listed for usage by the plant growth regulation society of America [29]. Paclobutrazol is a widely used as retardant for controlling the vegetative growth of a wide range of angiosperms [30,31]. Paclobutrazol induces a variety of morphological, physiological and biochemical responses in plants, including a reduction in stem elongation and stimulate flowering and rooting, likely as a result of a reduction in vegetative growth [32,33], yield increases [34], increasing chlorophyll and carotenoids content, altered carbohydrate status, increasing stress tolerance, delayed senescence, reducing gibberellin biosynthesis, increasing cytokinins synthesis and alterations in secondary metabolite contents [35,36,37]. The main effect of paclobutrazol takes place through the alteration of hormonal balance. Since, it inhibits gibberellin biosynthesis, reduces cell division and cell elongation and retards plant growth, whereas it promotes cytokinins [38,39,35]. Research has documented that paclobutrazol reduces plant growth of different plants: *Glycine max* L. [40], *Pelargonium zonale* L. [41], *Tabernaemontana coronaria* stapf [36], *Glycine max* L. [42], *Triticum aestivum* L [37] and *Moringa olifera* plant [43].

The objective of this research is to study the effect of foliar application with humic acid (HA), salicylic acid (SA) and paclobutrazol (PP_{333}) on some morphological, anatomical and seed yield characteristics, as well as on some biochemical constituents of soybean seed.

2. MATERIALS AND METHODS

Two field experiments were conducted at the Experimental Farm Station of the Faculty of Agriculture, Moshtohor, Benha University during 2015 and 2016.

Seeds of soybean plant (*Glycine max* L.) cv. Giza 111 were sown in 5 cm deep on 25-cm apart hills on 5 ridges in 3.0 m x 3.5 m plots. Following complete germination, seedlings were thinned into two plants per hill. Treatments were foliarly applied three times starting 45 DAP on a 15 d interval. Treatments were 2.5 and 5 gl^{-1} humic acid, 50 and 100 mgl^{-1} salicylic acid, 10 and 20 mgl^{-1} paclobutrazol, in addition to tap water as a Control. They were sprayed using a hand pump mister to the point of runoff. They were laid out in a 3 randomized complete blocks.

Common agricultural practices of growing soybean plants including fertilization, irrigation, manual weed control and so on were done as usual according to the crop requirement throughout the growing period. The following measurements were recorded:

Plant height (cm), stem diameter (cm), number of branches $plant^{-1}$, number of leaves $plant^{-1}$, total leaf area $plant^{-1}$ (cm^2), leaf area ratio $plant^{-1}$ (L.A.R) (cm^2/g) and specific leaf weight $plant^{-1}$ (mg/cm^2) were studied following the method described by [44]. Branches (including main stem) and leaves fresh and dry weights (g $plant^{-1}$) were recorded at 70 DAP during the 2 yrs. Shoots were dried in an electrical oven at 75°C till constant to determine dry weights.

Chlorophyll a, b and carotenoids were colorimetrically determined in leaves of soybean plants at 70 DAP during the 2 yrs according to the methods described by [45] and calculated as mgg^{-1} fresh weight.

Endogenous phytohormones: were quantitatively determined in *Glycine max* L. shoots at 70 DAP during season 2016 using High- Performance Liquid Chromato-graphy (HPLC) according to [46] for auxin (IAA), gibberellins, salicylic acid (SA) and abscisic acid (ABA), while cytokinins were determined according to [47] and calculated as µg g^{-1} F.wt..

Anatomical studies: Both leaf and stem samples were taken from the 4^{th} internode from top of the main stem of all treatments 70 DAP in 2016. The sample specimens were killed and fixed in FAA, washed in 50% ethyl alcohol, dehydrated in series of ethyl alcohols, infiltrated in xylene, embedded in paraffin wax with a melting point of 60-63°C, sectioned to 20 microns in thickness [48], stained with the double stain method, cleared in xylene and mounted in Canada balsam [49]. Sections were read to detect histological manifestation of noticeable responses resulted from the applied treatments.

The total number of the opened flowers per plant was recorded for each treatment.

$$\text{Abscission\%} = \frac{\text{No. of flowers plant}^{-1} - \text{No. of fruits plant}^{-1}}{\text{No. of flowers plant}^{-1}} \times 100$$

$$\text{Set pods\%} = \frac{\text{No. of pods plant}^{-1}}{\text{No. of flowers plant}^{-1}} \times 100$$

yield and yield components: Number of set pods $plant^{-1}$, pods yield $plant^{-1}$ (g), number of seeds pod^{-1}, weight of seeds pod^{-1}, weight of seeds plant^{-1} (g) and 100 seeds weight (g) were recorded in the harvest sample (i.e.,120 DAP during the 2 yrs).

Chemical analysis: In 2016, seeds at harvest were used to determine total nitrogen percentage which was determined using wet digestion according to [50], using microkjeldahl method as described by [51]. Crude protein = (total nitrogen x 5.71) [52]. Phosphorus was determined colorimetrically according to the method of [53], potassium content by flame photometer according to [54], total carbohydrates content by phenol-sulphoric acid method as described by [55] and total lipids by a Soxhlet apparatus according to [56].

2.1 Statistical Analysis

All data obtained during both seasons of study were subjected to analysis of variance and significant differences among means were determined according to [57].

3. RESULTS AND DISCUSSION

3.1 Vegetative Growth Characteristics

Data in Table 1 clearly indicate that different applied treatments significantly affected ($p<0.05$) all measured morphological parameters of soybean plant. Relatively higher increments, in the most studied vegetative growth characteristics, were obtained by applying 10 mgl^{-1} PP_{333}, 5 gl^{-1} humic acid and 100 mgl^{-1} salicylic acid. However, plant height was negatively affected by the two PP_{333} rates relative to all other applications including the Control.

Increasing number of formed branches on growing plants might be positively reflected upon many other characteristics such as number of leaves, total leaf area plant^{-1}, shoots fresh and dry weights, flowering and finally seeds yield as well.

Increasing total leaf area plant^{-1} in a similar way since this is related to accumulating more assimilates and high rates of their translocation especially toward formed seeds.

Also, increasing of stem diameter was accompanied with basic anatomical modification in different stem tissues especially phloem and xylem as mentioned later in Table 4.

At this early stage of growth great simulative effects existed with various applied treatments. Hence, this might be extended to the advanced growth stages including each of flowering and the final seed yield as well as quality of yielded seed.

The main effect of paclobutrazol (PP_{333}) takes place through the alteration of hormonal balance, since, it inhibits gibberellin biosynthesis, in turn this reduces cell division and cell elongation, whereas it promotes treated plants to create more cytokinins [35,38,39].

Such results of paclobutrazol have been previously recommended by several researchers [40] on soybean, [41] on geranium, [36] on tabernaemontana, [42] on soybean, [37] on wheat and [43] on moringa plant. [58] concluded that paclobutrazol at 200 ppm caused the highest values of fresh, dry weights and leaf area of faba bean plant.

As a plant hormone [22], salicylic acid plays diverse physiological roles in plants including plant growth, photosynthesis, and nutrient uptake [23]. Stimulation of growth after supplementation of SA has been reported Wheat [21], Soybean [25] and Maize [26]. [28] revealed that salicylic acid induces promotive effects on vegetative growth characteristics of Egyptian lupine. [24] mentioned that salicylic acid at 0.05 mM increased the level of cell division within the apical meristem of seedling roots which caused an increase in plant growth.

Humic acids plays a major role in growth parameters of plant in vegetative stage, such results are found by [9,10,18, 59, 60,61, 62].

3.2 Photosynthetic Pigments

Table 2 shows that chlorophyll a, b, Chl.(a + b) and Carotenoids content were increased. Photosynthetic pigments content were increased with 10 mgl^{-1} PP_{333}, 100 mgl^{-1}salicylic acid and 5 gl^{-1}humic acid in soybean leaves compared to the Control, respectively.

Also, PP_{333} gave relatively high in chlorophyll content, this may be partly due to the observed increase in root mass which is the major site of cytokinin biosynthesis. The high cytokinin level was associated with stimulated chlorophyll biosynthesis [38].

Table 1. Effect of different applied treatments on morphological characteristics of soybean plants at 70 DAP during 2015 and 2016 seasons

Characteristics / Treatments	Plant height (cm)	Stem diameter (cm)	Stems fresh weight g $plant^{-1}$	Number of branches $plant^{-1}$	No. of leaves $plant^{-1}$	Leaves fresh weight g $plant^{-1}$	Branches (including main stem) dry weight	Leaves dry weight g $plant^{-1}$	Total leaf area $plant^{-1}$ (cm^2)	Total dry weight g $plant^{-1}$	leaf area ratio $plant^{-1}$ (L.A.R) (cm^2 g^{-1})	Specific leaf weight $plant^{-1}$ (mg cm^{2-1})
							Season 2015					
1. Control	86.5	0..9	58. 30	2.1	61.2	92. 72	12. 88	21.68	3063.05	34.56	88.63	7.08
2. Humic acid 2.5 gl^{-1}.	82.7	1.1	61. 79	3.7	47.8	83. 75	13. 42	22. 24	4077.33	35.66	114.34	5.45
3. Humic acid 5 gl^{-1}.	88.1	1.2	107.13	5.0	76.3	151. 27	19. 63	33. 72	5141.47	53.35	96.37	6.56
4. Paclobutrazol 10 mgl^{-1}.	75.8	1.5	103.50	5.1	81.4	159. 70	20. 20	39.63	5085.85	59.83	85.01	7.79
5. Paclobutrazol 20 mgl^{-1}.	71.8	1.3	68. 30	4.7	52.9	104.13	14. 93	25. 79	3177.32	40.72	78.03	8.12
6. Salicylic acid 50 mgl^{-1}.	83.6	1.0	70. 76	4.2	69.7	108.50	14. 47	27. 24	4033.62	41.71	96.71	6.75
7. Salicylic acid 100 mgl^{-1}.	87.3	1.1	88. 09	4.9	62.2	151.20	17. 25	33. 79	4196.50	51.04	82.22	8.05
L.S.D. 0.05	6.74	0.23	11. 52	1. 41	6. 76	17. 68	1.54	4.23	321. 12	3. 29	7.03	0. 63
							Season 2016					
1. Control	94.2	0.9	58. 14	2.5	36.8	77.51	13.94	28. 36	2547.16	42.30	60.22	11.13
2. Humic acid 2.5 gl^{-1}.	101.4	0.9	74. 76	4.2	40.2	95. 26	19. 30	28.61	3570.52	47.91	74.53	8.01
3. Humic acid 5 gl^{-1}.	107.5	1.4	68.99	4.8	43.4	97. 20	18.31	31. 30	3933.88	49.61	79.30	7.96
4. Paclobutrazol 10 mgl^{-1}.	91.3	1.4	94. 47	5.4	65.3	130. 87	23. 70	37.24	4739.64	60.94	77.78	7.86
5. Paclobutrazol 20 mgl^{-1}.	80.7	1.3	55. 84	4.4	41.9	100. 98	15. 26	25. 89	3691.72	41.15	89.71	7.01
6. Salicylic acid 50 mgl^{-1}.	97.8	0.9	53. 98	3.6	39.2	71. 89	16. 56	24. 81	3259.28	41.37	78.78	7.61
7. Salicylic acid 100 mgl^{-1}.	94.2	1.1	66.46	4.7	54.4	103. 12	17.43	29. 75	3817.92	46.68	81.79	7.79
L.S.D. 0.05	8.40	0.17	13. 98	1. 07	5. 43	11.82	1. 28	3.05	295. 63	2.36	10. 13	0. 57

Table 2. Effect of different applied treatments on photosynthetic pigments content (mg g^{-1} F.W.) of soybean plants at 70 DAP during 2015 and 2016

Characteristics	Chlorophyll				Carotenoids		Chl. (A+B) / Carot.	
Treatments	(A) Mg g^{-1} F.W.	(B) Mg g^{-1} F.W.	(A + B) mg g^{-1} F.W.	(A + B) % relative to the control	Mg g^{-1} F.W.	% relative to the control	Mg g^{-1} F.W.	% relative to the control
					Season 2015			
1.Control	0.668	0.520	1.188	100.00	0.642	100.00	1.85	100.00
2.Humic acid 2.5 gl^{-1}.	0.879	0.681	1.560	131.31	0.764	119.00	2.04	110.27
3.Humic acid 5 gl^{-1}.	0.983	0.767	1.750	147.31	0.806	125.55	2.17	117.30
4.Paclobutrazol 10 mgl^{-1}.	1.074	0.884	1.958	164.81	0.871	135.67	2.25	121.62
5.Paclobutrazol 20 mgl^{-1}.	0.746	0.584	1.330	111.95	0.621	96.73	2.14	115.68
6.Salicylic acid 50 mgl^{-1}.	1.072	0.772	1.844	155.22	0.877	136.60	2.10	113.51
7.Salicylic acid 100 mgl^{-1}.	1.033	0.867	1.900	159.93	0.974	151.71	1.95	105.41

Characteristics / Treatments	Chlorophyll (A) Mg g^{-1} F.W.	Chlorophyll (B) Mg g^{-1} F.W.	Chlorophyll (A + B) mg g^{-1} F.W.	Chlorophyll (A + B) % relative to the control	Carotenoids Mg g^{-1} F.W.	Carotenoids % relative to the control	Chl. (A+B) / Carot. Mg g^{-1} F.W.	Chl. (A+B) / Carot. % relative to the control
				Season 2016				
1.Control	0.791	0.604	1.395	100.00	0.745	100.00	1.87	100.00
2.Humic acid 2.5 gl^{-1}.	0.856	0.683	1.539	110.32	0.783	105.10	1.97	105.35
3.Humic acid 5 gl^{-1}.	1.253	0.992	2.245	160.93	1.017	136.51	2.21	118.18
4.Paclobutrazol 10 mgl^{-1}.	1. 202	0.886	2.088	149.68	1.192	160.00	1.75	93.58
5.Paclobutrazol 20 mgl^{-1}.	0.981	0.738	1.719	123.23	0.916	122.95	1.88	100.53
6.Salicylic acid 50 mgl^{-1}.	0.938	0.751	1.689	121.08	0.902	121.07	1.87	100.00
7.Salicylic acid 100 mgl^{-1}.	1.273	0.793	2.066	148.10	1.039	139.46	1.99	106.42

Table 3. Effect of different applied treatments on endogenous phytohormones content of soybean shoots at 70 DAP during 2016 season

Plant hormones / Treatments	Promoters: Gibberellins µg g^{-1} F.wt.	Auxins µg g^{-1} F.wt.: 3 indole acetic acid	Auxins: Indole 3 butyric acid	Auxins: total µg g^{-1} F.wt.	Cytokinins µg g^{-1} F.wt.	Total Promoters µg g^{-1} F.wt.	% relative to the control	Inhibitors: Abscisic acid µg g^{-1} F.wt.	% relative to the control	Salicylic acid µg g^{-1} F.wt.	% relative to the control
1.Control	102.18	16.61	7.55	24.16	251.22	477.56	100.00	1.51	100.00	1.15	100.00
2.Humic acid 2.5 gl^{-1}.	248.95	13.97	15.70	29.67	241.39	556.01	116.43	1.32	87.42	2.07	180.00
3.Humic acid 5 gl^{-1}.	243.08	12.05	56.67	68.72	527.38	839.18	175.72	0.98	64.90	1.70	147.83
4.Paclobutrazol 10 mgl^{-1}.	133.93	10.95	N.D.	10.95	534.93	679.81	142.35	0.36	23.84	2.63	228.70
5.Paclobutrazol 20 mgl^{-1}.	96.90	11.63	31.18	42.81	358.59	497.67	104.21	0.43	28.48	7.22	627.83
6.Salicylic acid 50 mgl^{-1}.	117.01	10.82	N.D.	10.82	406.28	534.11	111.84	0.37	24.50	1.78	154.78
7.Salicylic acid 100 mgl^{-1}.	126.58	11.28	21.58	32.86	498.96	658.40	137.87	0.41	27.15	3.95	343.48

N.D.: Not detected

In addition, the abovementioned results of paclobutrazol are in harmony with those attained by [36,37,40,41,42]. [63] who showed that mepiquate chloride and chlormequate chloride increased photosynthetic rate by increasing leaf chlorophyll content and mesophyll cell size of cotton plant which is due to more rapid exchange of CO_2 into mesophyll cell by virtue of their large surface area.

Regarding salicylic acid (SA), it is naturally occurs in plants in very low amounts and participates in the regulation of physiological processes in plant such as chlorophyll synthesis [20,21,64]. In the same order [28] found that SA treatment at 75 mgl^{-1} promoted chlorophyll pigments in leaves of Egyptian lupine [24]. Reported that leaves treated with high concentrations of SA accumulated more Chl. and carotenoids of wheat plants.

Our results of HA are in agreement with those reported by [65] who indicated that HA supply significantly increased chlorophyll contents of common bean plants. [18] attributed the beneficial effects of HA on plant growth to increasing photosynthesis. [62] reported that chlorophyll content of faba bean significantly increased by foliar application of HA.

3.3 Endogenous Phytohormones

Applied treatments changed endogenous phytohoromones content gibberellic acids (GA_3), indole acetic acid (IAA), indole butyric acid (IBA), cytokinins, abscisic acid (ABA) and salicylic acid (μg g^{-1} F.wt.) of soybean shoots at 70 DAP in 2016. All treatment rates greatly improved the metabolic performance of soybean plant (Table 3).

Compared to the other treatments and the Control, auxins level was highly decreased in shoots with 10 mgl^{-1} paclobutrazol (PP_{333}) and 50 mgl^{-1} salicylic acid. Humic acid at 5 gl^{-1}, 50 and 100 mgl^{-1} salicylic acid and 2.5 gl^{-1} humic acid were the most relatively effective treatments on auxins content compared with the Control.

Gibberellin like-substances in soybean shoots was increased with 2.5 and 5 gl^{-1} humic acid, 10 mgl^{-1} PP_{333} and 100 mgl^{-1} salicylic acid treatments, respectively but it mostly decreased in case of 20 mgl^{-1} PP_{333} treatment. In addition, the level of cytokinins positively responded to the different assigned treatments, yet the activity was the lowest in cases of the Control and 5 gl^{-1} humic acid (Table 3).

Means of growth promoters, auxins, gibberellins, and cytokinin were relatively higher in response to application of the subset treatments of 10 mgl^{-1} PP_{333}, 5 gl^{-1} humic acid, 100 mgl^{-1} salicylic acid compared each to the performance of the other three application rates and of the Control. With increments reached 175.72, 142.35, and 137.87% relative to the Control, respectively. These positive responses may partially interpret how growth characters (Table 1), histological features (Tables 4 & 5) and yield characters (Tables 6 & 7) were all improved.

Paclobutrazol induces a variety of morphological, physiological and biochemical responses in plants [32,33], delayed senescence, reducing gibberellin biosynthesis, increasing cytokinins synthesis and alterations in secondary metabolites content [35,36,37]. The main effect of paclobutrazol takes place through the alteration of hormonal balance. Since, it inhibits gibberellin biosynthesis, reducing cell division and elongation, whereas it promotes treated plants to create more cytokinins [38,39,35,36]. This may explain the relative increases of cytokinins and other promoting hormones with some applied treatments.

Humic acid can regulate endogenous hormone levels [12,13]. [19] mentioned that the positive effects of HA on plant growth may be due to its acting as plant growth hormones.

Salicylic acid is recognized as a plant hormone [22]. Foliar application with relatively low concentrations of SA promoted and influenced the differentiation of cells and tissues of plants [66,67].

Abscisic acid level was reduced with various assigned treatments compared with the Control, but the reduction was more obvious with 10 mgl^{-1} paclobutrazol (PP_{333}) and 50 mgl^{-1} salicylic acid treatments.

Also, Table 3 shows that salicylic acid level was increased with the different treatments compared with the Control and reached relatively high values with 10 mgl^{-1} paclobutrazol, 100 mgl^{-1} salicylic acid and 20 mgl^{-1} paclobutrazol, respectively. In this respect, these results may interpret each of the obtained vigorous growth, great flowering and yield of soybean plant.

Table 4. Effect of different applied treatments on the mean counts and measurements of soybean (*Glycine max* L.) stem histological features at 70 DAP during 2016 season.

Treatments / Histological Characteristics(micron)	Control	Humic acid 2.5 gl^{-1}	Humic acid 5 gl^{-1}	Paclobutrazol 10 mgl^{-1}	Paclobutrazol 20 mgl^{-1}	Salicylic acid 50 mgl^{-1}	Salicylic acid 100 mgl^{-1}
Stem diameter	2985	3681	4740	4541	3785	3324	4647
Cuticle layer thickness	3	5	3	5	5	4	3
Epidermis thickness	10	14	15	14	12	13	13
Thickness of collenchyma layers	153	178	201	233	212	146	184
Number of collenchyma layers	5	6	6	4	6	5	6
Thickness of parenchyma layers	171	167	282	307	251	239	274
Number of parenchyma layers	7	8	8	10	7	6	9
Cambial region thickness	12	14	21	27	16	14	18
Phloem thickness	88	91	137	183	104	94	132
Xylem thickness	354	481	650	713	472	396	517
Number of xylem rows/Vascular cylinder	171	184	276	290	243	238	253
No. of xylem vessels/row	9	8	17	21	17	14	15
Diameter of the widest xylem vessel	46	61	72	74	69	65	70
Parenchymatous pith thickness	1403	1781	2122	1577	1641	1512	2365
Number of parenchymatouspith layers	24	21	19	27	19	17	25

Table 5. Effect of different applied treatments on the mean counts and measurements of soybean (*Glycine max* L.) leaves histological features at 70 DAP during 2016 season.

Treatments / Histological Characteristics(micron)	Control	Humic acid 2.5 gl^{-1}	Humic acid 5 gl^{-1}	Paclobutrazol 10 mgl^{-1}	Paclobutrazol 20 mgl^{-1}	Salicylic acid 50 mgl^{-1}	Salicylic acid 100 mgl^{-1}
Thickness of upper epidermis cuticle layer	8	6	5	9	8	7	6
Thickness of lower epidermis cuticle layer	6	5	6	7	5	4	5
Upper epidermis thickness	22	17	21	19	14	18	20
Lower epidermis thickness	14	12	13	11	14	10	13
Palisade tissue thickness	86	101	113	127	98	87	105
Spongy tissue thickness	108	116	146	151	129	122	138
Number of spongy tissue layers	5	4	5	5	6	5	5
Thickness of blade	244	257	304	324	268	248	287
Thickness of upper collenchyma layers	520	342	517	446	518	433	509
No. of upper collenchyma layers	9	8	8	7	9	6	7
Thickness of lower collenchyma layers	296	270	209	233	246	238	281
No. of lower collenchyma layers	8	6	6	7	5	6	7
Thickness of phloem	113	95	129	144	87	103	119
Thickness of xylem tissue	411	422	516	538	487	470	492
Number of xylem rows	16	18	19	21	18	19	19
thickness of widest xylem vessel	37	41	51	53	45	44	48

Histological Characteristics(micron) \ Treatments	Control	Humic acid 2.5 gl^{-1}	Humic acid 5 gl^{-1}	Paclobutrazol 10 mgl^{-1}	Paclobutrazol 20 mgl^{-1}	Salicylic acid 50 mgl^{-1}	Salicylic acid 100 mgl^{-1}
Length of midrib vascular bundle	455	471	542	580	481	505	514
Width of midrib vascular bundle	411	416	660	732	651	607	658
Thickness of leaf midrib	1443	1230	1476	1469	1434	1355	1509

Table 6. Effect of different applied treatments on flowering and seed yield components of *Glycine max* L. plant during 2015 and 2016.

Treatments \ Characteristics	Number of flowers $plant^{-1}$	Abscission%	Number of set pods $plant^{-1}$	set pods %	Pods yield $plant^{-1}$ (g)	Number of seeds pod^{-1}	Weight of seeds pod^{-1}	Weight of seeds $plant^{-1}$ (g)	100 seeds weight (g)
					Season 2015				
1.Control	231.8	50.60	114.5	49.40	58.74	2.4	0.43	48.23	16.93
2.Humic acid 2.5 gl^{-1}.	254.0	49.13	129.2	50.87	65.89	2.6	0.46	57.35	17.41
3.Humic acid 5 gl^{-1}.	281.7	44.52	156.3	55.48	83.00	2.7	0.55	88.70	20.78
4.Paclobutrazol 10 mgl^{-1}.	293.0	37.47	183.2	62.53	94.90	2.8	0.57	106.13	21.13
5.Paclobutrazol 20 mgl^{-1}.	255.2	42.16	147.6	57.84	74.83	2.6	0.48	70.25	18.22
6.Salicylic acid 50 mgl^{-1}.	247.9	46.15	133.5	53.85	75.03	2.5	0.44	61.82	17.82
7.Salicylic acid 100 mgl^{-1}.	260.3	33.85	172.2	66.15	86.44	2.6	0.53	86.91	19.54
L.S.D. 0.05	32.62	4.09	11. 67	3.96	7.13	0.12	0.03	8.16	1.09
					Season 2016				
1.Control	175.5	47.46	92.2	52.54	46.75	2.3	0.46	41.50	18.94
2.Humic acid 2.5 gl^{-1}.	236.4	51.48	114.7	48.52	59.07	2.6	0.51	56.98	19.27
3.Humic acid 5 gl^{-1}.	278.1	48.80	142.4	51.20	73.05	2.7	0.58	84.56	22.13
4.Paclobutrazol 10 mgl^{-1}.	282.6	36.91	178.3	63.09	87.01	2.9	0.61	112.17	22.18
5.Paclobutrazol 20 mgl^{-1}.	259.7	47.13	137.3	52.87	70.71	2.5	0.52	74.39	21.09
6.Salicylic acid 50 mgl^{-1}.	227.6	54.51	103.5	45.47	53.10	2.6	0.55	57. 73	20.65
7.Salicylic acid 100 mgl^{-1}.	244.0	31.48	167.2	68.52	81.59	2.7	0.58	94.20	21.25
L.S.D. 0.05	21.49	3.47	16. 03	3.72	6.47	0.10	0.04	13.70	1.28

Table 7. Effect of different applied treatments on minerals, total carbohydrates, crude protein and total lipids % of *Glycine max* L. seeds during 2016 season.

Treatments \ Characteristics	Minerals %			Crude protein %	Total carbohydrates %	Total lipids %
	N	P	K			
1.Control	5.96	0.52	1.65	34.03	26.45	18.15
2.Humic acid 2.5 gl^{-1}.	6.24	0.61	1.70	35.63	28.32	19.46
3.Humic acid 5 gl^{-1}.	6.36	0.64	1.84	36.32	31.14	21.11
4.Paclobutrazol 10 mgl^{-1}.	6.71	0.63	1.93	38.31	32.61	21.41
5.Paclobutrazol 20 mgl^{-1}.	6.46	0.58	1.78	36.89	30.72	19.90
6.Salicylic acid 50 mgl^{-1}.	6.16	0.57	1.80	35.17	27.81	18.77
7.Salicylic acid 100 mgl^{-1}.	6.50	0.53	1.96	37.12	30.30	20.87

3.4 Anatomical Studies

3.4.1 Stem anatomy

The applied treatments had positive impact on most studied histological characteristics of (*Glycine max* L.) stem i.e., cuticle thickness, epidermis thickness, cortex, thickness of phloem tissue, thickness of cambial region, xylem tissue, number of xylem vessels vascular cylinder^{-1} and diameter of the xylem vessel compared with the Control (Table 4 and Figs. 1 & 2).

Stem diameter was increased to reach 4740µ with 5 gl^{-1} humic acid followed by 4647 µ with 100 mgl^{-1} salicylic acid and 4541 µ with 10 mgl^{-1} PP_{333}, respectively compared with the Control 2985 µ.

Regarding vascular tissues, thickness of both xylem and phloem tissues reached relatively high values with 10 mgl^{-1} PP_{333} and 5 gl^{-1} humic acid treatments. Here, xylem tissue thickness was 713µ with 10 mgl^{-1} PP_{333} and 650 µ & 517 µ with 5 gl^{-1} humic acid and 100 mgl^{-1} salicylic acid, respectively compared with the Control 354 µ.

Also, phloem tissue thickness was 88µ with the Control but it increased to 183µ, 137µ and 132µ with 10 mgl^{-1} PP_{333}, 5 g/l^{-1} humic acid and 100 mgl^{-1} salicylic acid, respectively.

Cambial region thickness was 27µ with 10 mgl^{-1} PP_{333}, 21 µ for 5 gl^{-1} humic acid and 18µ for 100 mgl^{-1} salicylic acid treatments but was 12µ in case of the Control.

Increasing of stem diameter was mainly due to how different treatment rates positively impacted thickness of each cuticle layer, epidermis, cortex (collenchyma and parenchyma tissues) and pith parenchyma layers as well as the dimensions of vascular cylinder (Table 4).

In general, the stimulatory effects of applied treatments upon the anatomy features of the treated plants may be attributed to the effect upon cambium activity. Increment of cambium activity could mainly attribute to the increases of endogenous hormones level especially auxins and cytokinins [68,69] as well as to the findings of the present study.

These positive responses of different anatomical aspects to treatments especially in case of 10 mgl^{-1} paclobutrazol, 5 gl^{-1} humic acid and 100 mgl^{-1} salicylic acid were directly related to enhancing vegetative and reproductive growth. So, the present study indicated that increases of xylem tissue (the route of mineral nutrients and water translocation from roots to leaves and of the phloem tissue (the pathway of different assimilates from leaves to seeds and other plant sinks), both contributed to the improvement of translocation which in turn positively the final seed yield.

3.4.2 Leaf anatomy

Histological features of the soybean leaves were increased with different applied treatments compared with the Control (Table 5 and Figs. 3 & 4).

Blade thickness was increased with different used treatments to reach values of 324µ with10 mgl^{-1} paclobutrazol followed by 5 gl^{-1} humic acid 304 µ and 100 mgl^{-1} salicylic acid 287 µ compared with the Control 244 µ.

For mesophyll tissue, the thickness of both palisade and spongy tissues were increased with different applied treatments. Here, palisade tissue thickness was 86µ in the Control but increased to 127, 113, 105 and 101 µ with 10 mgl^{-1} PP_{333}, 5 gl^{-1} humic acid, 100 mgl^{-1} salicylic acid and 2.5 gl^{-1} humic acid, respectively. Also spongy tissues was 108µ in the Control but increased to 151, 146, 138 and 122µ with PP_{333} 10 mgl^{-1}, humic acid 5 gl^{-1} and salicylic acid 100 & 50 mgl^{-1}.

With regard to midrib anatomical features, could be noticed that increment in the midrib thickness especially, in case of 100 mgl^{-1} salicylic acid 1509 µ, 5 gl^{-1} humic acid 1476 µ and 10 mgl^{-1} PP_{333} 1469 µ treatments compared with Control 1443 µ and other treatments. The increase in midrib thickness was attributed to the increasing in many of its histological features such as thickness of both upper most and lower most collenchyma tissues, lower most parenchyma tissue and dimensions of main vascular bundle. The above mentioned results especially increment of the conductive tissues (xylem and phloem) are also of great importance because they may also be involved in interpreting about why vigorous growth and high seed yield were existed with different applied treatments especially with 10 mgl^{-1} PP_{333}, 5 gl^{-1} humic acid and 100 mgl^{-1} salicylic acid treatments compared with the Control.

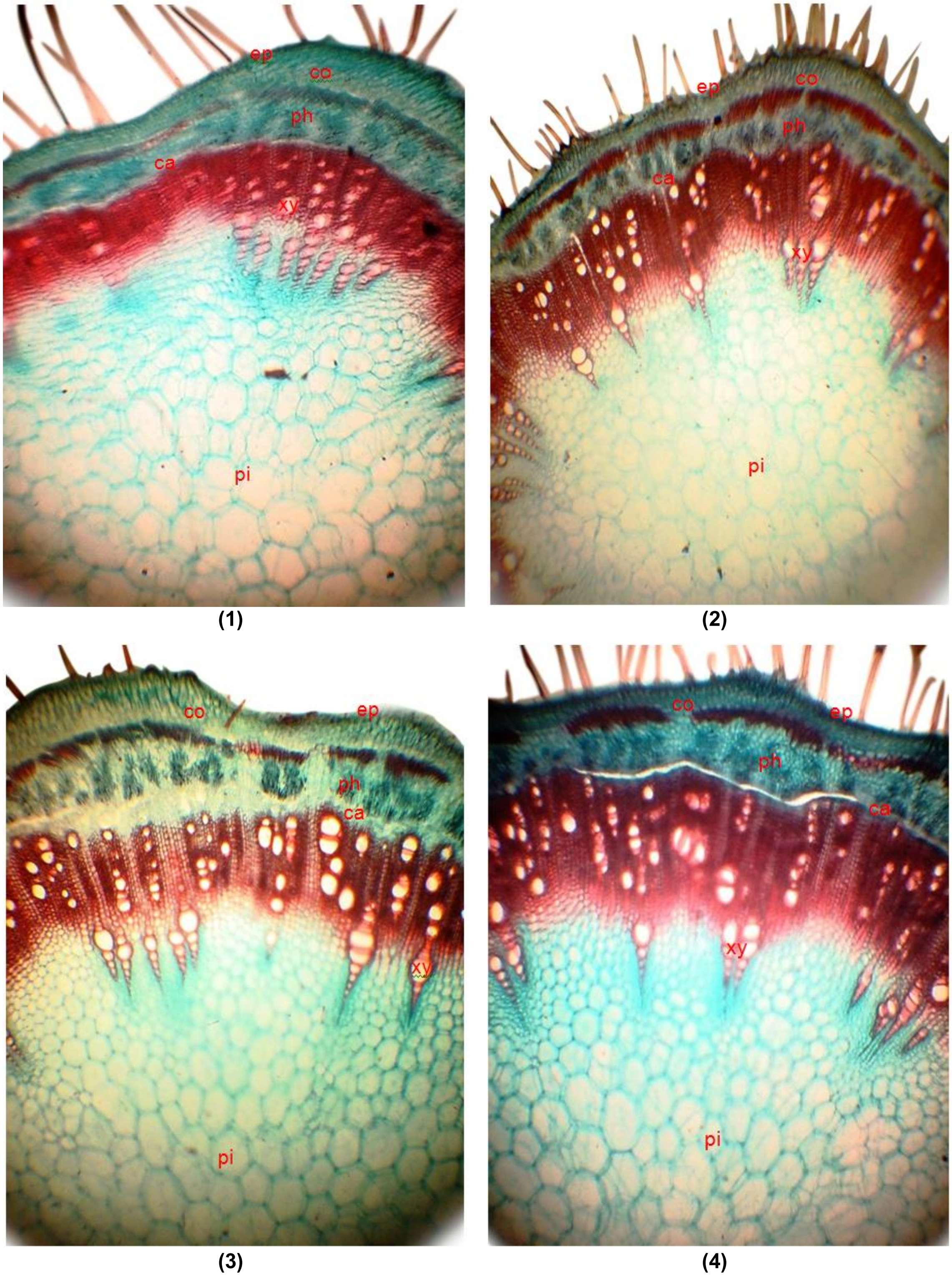
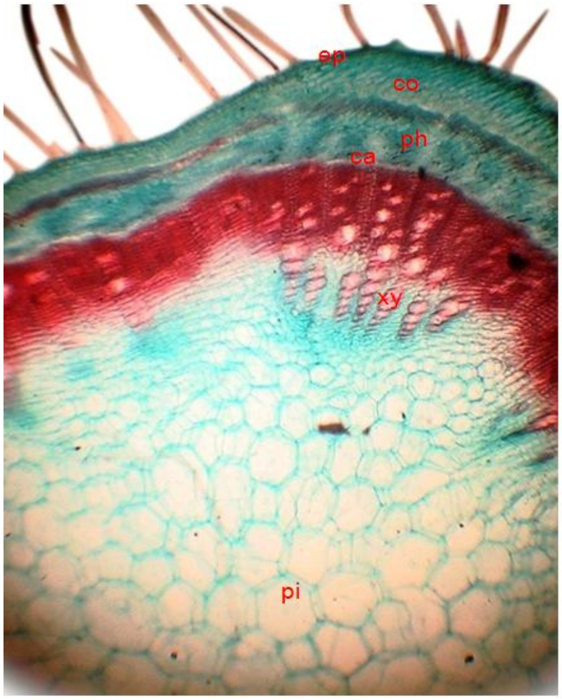


Fig. 1. Transverse sections (X = 40) through 4th internode of *Glycine max* L. main stem at 70 DAP as affected by different applied treatments.

Where: (1): Control, (2): Humic acid 2.5 gl^{-1}. (3): Humic acid 5 gl^{-1} (4): Paclobutrazol 10 mgl^{-1}

ep= epidermis co= Cortex ph= phloem tissue xy= Xylem tissue ca= cambium pi= pith



(5)

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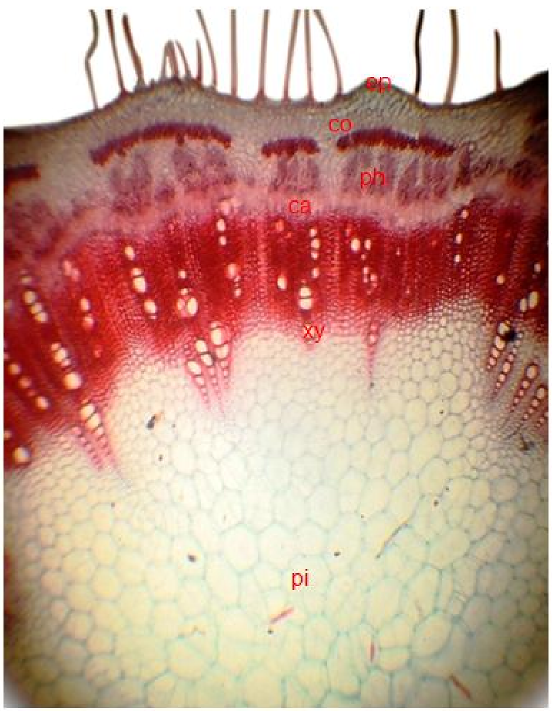

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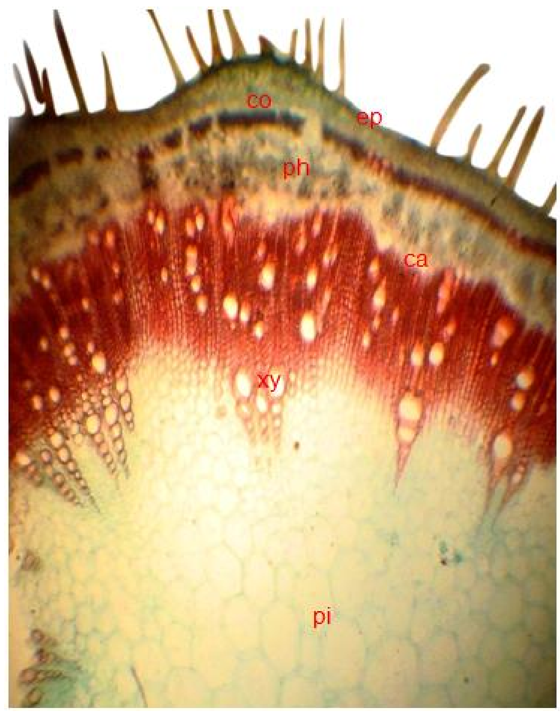

(8)

Fig. 2. Transverse sections (X = 40) through 4th internode of *Glycine max* L. main stem at 70 DAP as affected by different applied treatments.

Where: (5): Control **(6): Paclobutrazol 20 mgl^{-1}.**

(7): Salicylic acid 50 mgl^{-1}. **(8): Salicylic acid 100 mgl^{-1}.**

ep= epidermis co= Cortex ph = phloem tissue xy = Xylem tissue ca = cambium pi= pith

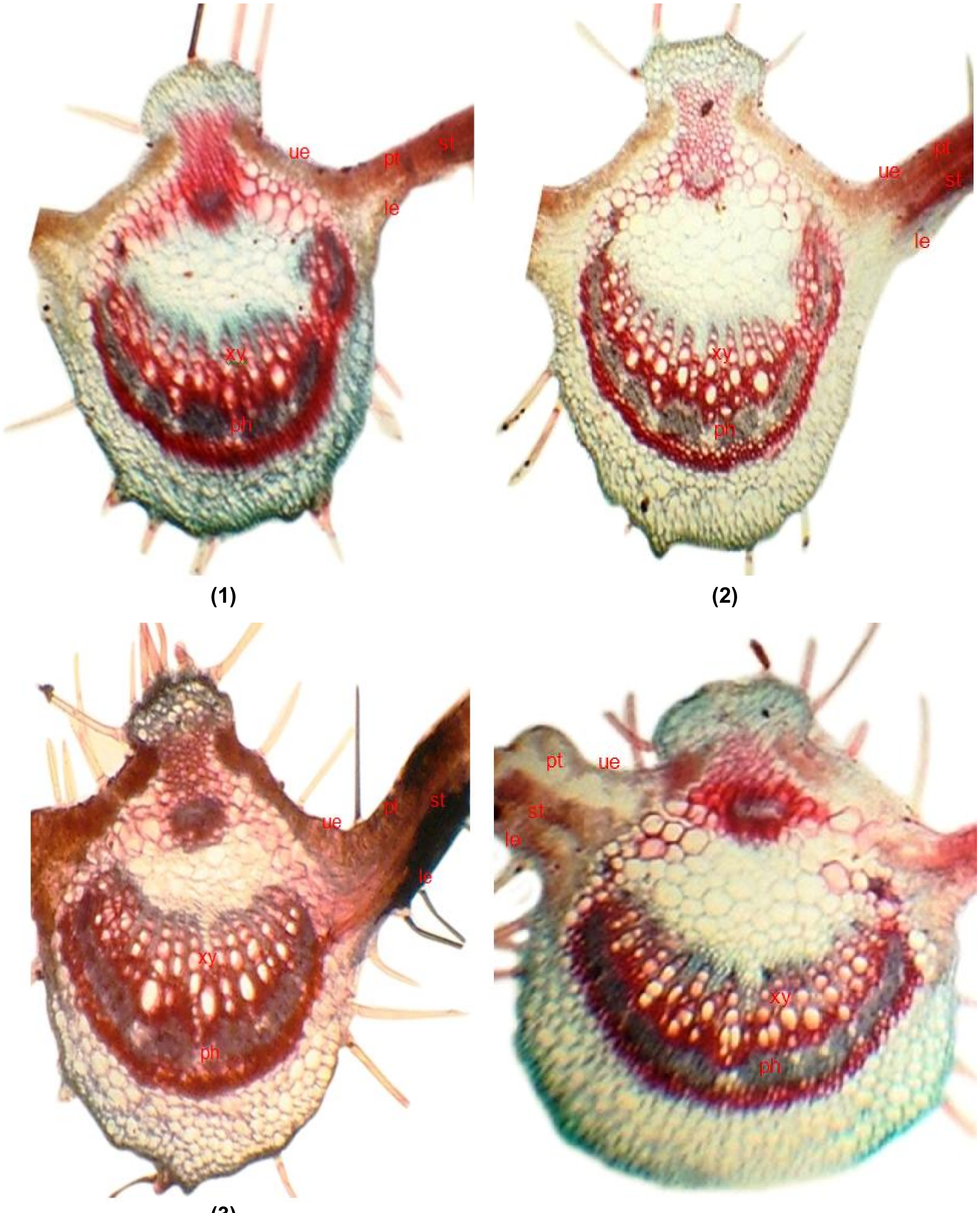

Fig. 3. Transverse sections (X = 40) through 4th apical leaf of *Glycine max* L. plant at 70 DAP as affected by different applied treatments.

Where: (1): Control (2): Humic acid 2.5 gl^{-1}.

(3): Humic acid 5 gl^{-1}. (4): Paclobutrazol 10 mgl^{-1}.

Ue = Upper epidermis le = Lower epidermis pt = Palisade tissue st = Spongy tissue ph = phloem tissue xy = Xylem tissue

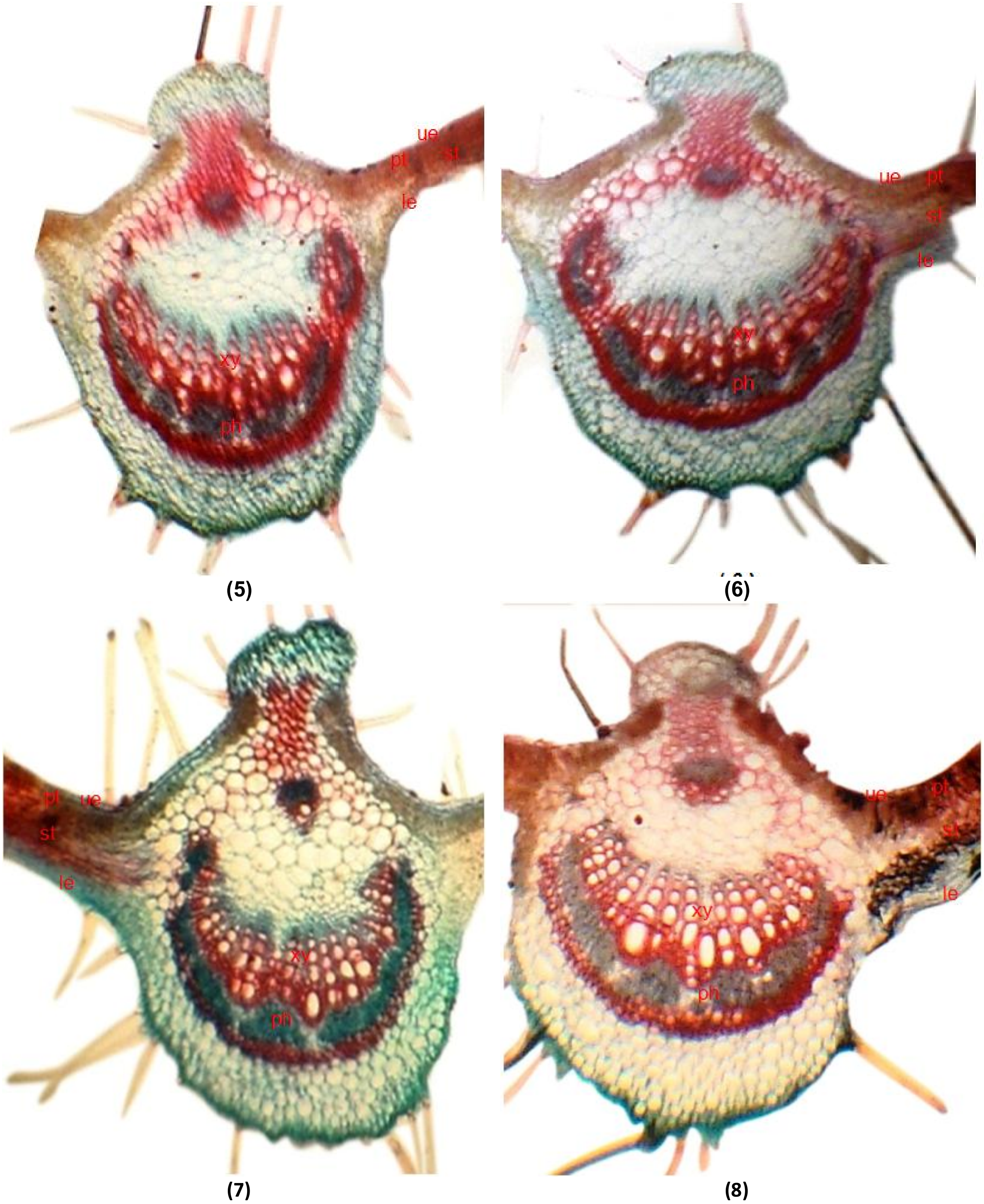

Fig. 4. Transverse sections (X = 40) through 4th apical leaf of *Glycine max* L. plant at 70 DAP as affected by different applied treatments.

Where: (5): Control **(6): Paclobutrazol 20 mgl^{-1}.**

(7): Salicylic acid 50 mgl^{-1}. **(8): Salicylic acid 100 mgl^{-1}.**

Ue = Upper epidermis le = Lower epidermis pt = Palisade tissue st = Spongy tissue ph = phloem tissue xy = Xylem tissue

Our results are in harmony with those reported by [70] who mentioned that treated *Strelitzia reginae* plants with PP_{333} at 300 and 200 mgl^{-1} resulted in increasing blade thickness, palisade thickness and phloem tissue thickness. [36] found that anatomical traits of tabernaemontana leaf i.e., thickness of leaf midrib, length and width of vascular bundle, phloem and xylem tissues and number of xylem vessels in vascular bundle, as well as the leaf blade thickness, were increased with PP_{333} 100 and 150 mgl^{-1} compared with the untreated plants. [43] concluded that moringa leaflet and stem anatomical features were increased with Paclobutrazol 20 mgl^{-1} treatment.

Salicylic acid significantly affects and causes changes in leaf anatomy and chloroplast structure [24]. Foliar application with SA promoted and influenced the development, differentiation of cells and tissues of plants [66,67]. [28] found that salicylic acid induced favorable changes in anatomical structures of Egyptian lupine plant.

There are few studies about the changes induced by the application of HA on plant structures. [18] attributed the beneficial effect of HA on plant growth to increasing cell elongation. [71] recorded that humic acid increased diameter of vascular cylinder, length, number and diameter of vessels, length and number of vascular bundles of *Calendula officinalis* L. stem. [72] stated that treated *Solanum melongena* L. with HA increased stem diameter due to the substantial increase in cortex thickness, diameter of vascular cylinder and vascular bundle and pith diameter. The increasing of leaf blade, including both palisade and spongy tissues was also documented.

3.5 Flowering and Yield Components

Table 6 shows that all applied treatments significantly enhanced flowering and yield components of soybean plant. Increments in both the number of flowers and setted pods % with applied treatments, lead to increasing of total pods number. Different applied treatments (Table 6) especially 10 mgl^{-1} PP_{333}, 5 gl^{-1} humic acid and 100 mgl^{-1} salicylic acid gave the highest values of setted pods %, pods yield $plant^{-1}$ (g), number of seeds pod^{-1}, weight of seeds pod^{-1} and weight of seeds $plant^{-1}$ (g) relative to the Control. On the other hand, different applied treatments decreased flowering abscission % of soybean plant compared with the Control. The reduction in flowers abscission percentage, means enhancement of pods setting as obtained with our studied treatments, that may be due to increasing of total carbohydrates, protein and mineral concentrations in the leaves (source) as well as the endogenous auxins, especially at full blooming and setting stages.

The obtained increases of the final seed yield may be mainly attributed to: (i) increases in growth characteristics such as number of branches $plant^{-1}$, number of leaves $plant^{-1}$, total leaves area $plant^{-1}$ and fresh as well as dry weight (Table 1) and (ii) increases in metabolical and anatomical performances, i.e., photosynthetic pigments and endogenous phytohormones contents (Tables 2 and 3).

Previous research [16,17,25,28,36,37,40,41,42, 58] had similar trends.

3.6 Chemical Analysis

The applied treatments increased the seeds content of nitrogen, phosphorus, potassium, crude protein and total carbohydrates as well as total lipids % (Table 7). PP_{333}10 mgl^{-1}, 5 gl^{-1} humic acid and 100 mg/l^{-1} salicylic acid were the most effective treatments compared with the Control.

In this respect, the increase in soybean minerals content due to applying growth substances may be the result of their roles in regulating ions and may modify the uptake movement and metabolism of nutrients with in the plant tissues. Also, the increases in total carbohydrates, as well as in total lipids in response to treatment applications, are supported by stimulating endogenous phytohormones, as well as of photosynthetic pigments and accumulating of the dry matter in the seeds of treated plants [73]. The strong positive correlation of such constituents vs. growth and seed yield confirmed and coincided such functions and roles of different applied treatments.

The aforementioned results of soybean seeds chemical compositions are in conformity with those obtained by [10,28,40,62,74].

4. CONCLUSION

All tested treatments significantly increased the morphological parameters, photosynthetic pigments, as well as endogenous phytohormones content of soybean plant. Also, they have a positive impact on histological characteristics and flowering, as well as on yield components. Furthermore, content of minerals (N, P and K), crude protein, total carbohydrates and total lipids % were increased in seeds yield. Herein, 10 mgl^{-1} paclobutrazol followed by 5 gl^{-1} humic acid and 100 mgl^{-1} salicylic acid, respectively were the most effective treatments relative to the Control.

Finally, treating soybean plants with PP_{333} 10 mgl^{-1} or humic acid 5 gl^{-1} or salicylic acid 100 mgl^{-1} as foliar application 3 times had a positive impact on maximizing its growth, seed yield and nutritional value. Based on our findings, we will intend to carry out more deep research on response of soybean plant to abiotic stress and climate changes using such treatments in the developing countries like Egypt.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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