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Exogenous and Endogenous Polyamines Relation to Growth, α-cellulose Precipitation in Fibres and Productivity of Cotton Plant

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Abstract: Cotton seeds cv. G. 89 were soaked in spermidine solutions containing 0, 5, 10, 20 and 40 mg L^{-1} for one hour presowing to determine spermidine effect on germination percentage, hypocotyl and epicotyl length, mass of root, stem and leaves/plant as well as some biochemical contents at seedling, vegetative and flowering stages. In addition, boll yield/plant and yield components as well as total sugar of seed and α-cellulose precipitation in fibre were studied at completely opened boll stage. Germination percentage significantly increased at 5 mg L^{-1} spermidine. Both hypocotyl and epicotyl length of seedlings were significantly enhanced at 10 mg L^{-1} spermidine. Increasing root, stem and leaves, mass/plant (as fresh or dry) were significantly obtained at treatment 20 mg L^{-1} of spermidine at the studied stages of cotton plant growth, which were reflected on enhancement of boll number/plant, fibre mass/boll and boll yield/plant at completely opened boll stage. Leave pigments (chl. a, b and carotenoids content) were more surpassed at seedling stage by spermidine (10 mg L^{-1}). Biochemical contents (total sugars, free amino acids, phenols and indoles) of cotton plant organs were significantly differed for increase or decrease according to plant physiological stage and spermidine concentration. Polyamines (putrescine and spermidine) content in the leaves were increased at vegetative and flowering stages and only spermine at flowering stage. However, α-cellulose precipitation in the boll-fibre was significantly enhanced at 5 mg L^{-1} spermidine. Highly and positive simple correlation was obtained between free amino acids or total sugars content of stem and leaves at vegetative and flowering stages of cotton plant growth produced from presowing treatment with spermidine being negatively correlated. While, there was a highly positive correlation between leaves and seed sugar content.

Key words: Cotton · spermidine · growth · biochemical · α-cellulose

INTRODUCTION

The scope for cotton improvement productivity, using growth regulators as (spermidine) could be through with improving germination, growth physiology, cellulose precipitation in the fibre and crop productivity of cotton plant. Cotton is cultivated for ultimate uses of seed and fibre production, weave to human and feeder to animals. The value of cotton fibres is based on their high amount of nearly pure cellulose in the secondary wall. These fibres are special epidermal cells of the outer integument of the seeds [1].

Triamine spermidine is one of the most common compound of polyamines. It was suggested that they interact with macromolecules as phospholipids membranes and many types of proteins with enzymatic activities modulated the activities of the key regulatory enzymes of the cell-cycle, that were responsible for there physiological effects, ranging from promotion of cell growth differentiation to inhibition of senescence as well as capability of polyamines stabilizing protoplasts and prevent both loss of chlorophyll during senescence in protoplast and leaves [2-4]. Polyamines are low-molecular weight polycations constitute a part of the overall nitrogen metabolism and the nitrogen source in the nutrient solution determines the polyamine synthesis and accumulation in plants [5] thus ensuring the recycling of carbon and nitrogen. Their function as polycations and binding to negatively charged functional group e.g. phosphate groups of biochemical constituents [6] lead to improve sugars translocation from vegetative organs to storage site [7]. In particular, endogenous putrescine increased stimulaneously at the induction and initiation phase and might have a role in adventitious root formation. Sperimidine and spermine levels did not change significantly throughout these phases. Endogenous putrescine reduced the total duration of the initiation and expression phase [8] and increased

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the growth of both root and leave blades of barley cultivars [9]. Also, the amount of free polyamines in the homozygous transgenic plants was similar in transgenic and wild plants suggesting either a tight regulation of polyamine levels or a different compartmentalization of two recombinant proteins and the bulk amount of endogenous polyamines [10]. Hence, the present study investigated the levels of exogenously applied spermidine on endogenous polyamines, relation of germination, growth and α-cellulose precipitation in fibre of cotton plant.

MATERIALS AND METHODS

Spermidine treatments: The treatments were consisted of four concentrations (5, 10, 20, 40 mg L^{-1}) of spermidine ($NH_2(CH_2)_3$ $NH(CH_2)_4$ NH_2) supplied from Sigma co. Cotton seeds (*Gossypium barbadense* L.) cultivar G. 89 secured from Ministry of Agriculture, Cairo, Egypt were soaked in the previous concentrations of spermidine for one hour before sowing under laboratory conditions (Temperature 30 ± 2). Control seeds were soaked in distilled water for the same period. Cotton seeds were sown in pots (50 x 40 cm) filled with loamy soil at March 26 and April 6, 2002 and 2003, seasons, respectively. The plants were grown for 6 months at experimental area of Botany Department National Research Centre, Dokki, Giza under the following growth conditions: 15 h photoperiod, 32.2°/22.5° day/night temperature and 58% relative humidity. The experiments were arranged as a complete randomized design with five replications. Each pot contained 50 seeds. Germinated seed thinned to four seedlings. Phosphorus fertilizer as calcium superphosphate (15.5 P_2O_5) was added presowing at the rate of 12 g/pot. Nitrogen fertilizer as urea (46.5% N) was applied at rate 18 g/pot on three equal doses after 21, 35 and 50 days from planting.

Growth and yield measuments: Fresh and dry mass of root, stem, leaves and leaf area/plant of produced plants of both seasons were determined at seedling stage (21 days after sowing), vegetative stage (60 days after sowing) and flowering stage (90 days after sowing). Yield and its components as seed number and mass/boll, seed yield/plant, fibre mass/boll, fibre mass/plant and 100-seed mass at the end of boll opening were determined.

Biochemical constituents determination: Photosynthetic pigments (chl. a, b and carotenoids) were determined in fresh leaves according to Saric *et al.* [11]. Root, stem, leaves and seeds were dried in a ventilated oven at 70°C and then finally ground in stainless steel mill for determination total sugars [12], free amino acids [13], total phenols [14] and total indole compounds [15]. α-cellulose content of the fibres was determined as described by Updegraff [16].

Polyamine analysis: Total free polyamine levels were determined in cotton leaves. For polyamine extraction from whole leaves, 100 mg powdered was homogenized with 5% (v/v) perchloric acid. Crude extracts were clarified by centrifugation. The supernatant was used to analyse total free polyamines.

Free polyamines were quantified after derivatization with benzoyl chloride according to Flores and Galston [17]. Benzolated polyamines were separated by HPLC (Shimadzu) on a reverse-phase C_{18} column (Alex-Octadecylsilane), 5 particle diameter, 4.6 x 250 mm using methanol 64% at flow rate of 1 mL/min. Eluted peaks were detected by a spectrophotometer (Shimadzu, UV 260 nm) recorded and integrated by an attached computer. Standards and plant extracts were determined with the same method.

Statistical analysis: Analysis variance of the two year data was carried out as described by Snedecor and Cochran [18]. LSD at 5% level for significant F values was calculated to compare between means of different treatments. Simple correlation coefficients between fibre mass and biochemicals content of leave, stem and seed were determined according to Gomez and Gomez [19].

RESULTS AND DISCUSSION

Effect of spermidine on cotton seed germination: Germination percentage, hypocotyl and epicotyl were significantly increased by spermidine application as shown in Table 1. Germination percentage and seedling characters (hypocotyl and epicotyl length) were affected by spemidine at 5 or 10 mg L^{-1}. It might be due to the development stage whereas, polyamines content are contributed to plant phase. That shows polyamines important role on physiological and morphological processes of germination, root and vegetative growth. Whereas, polyamines contained in the embryogenic callus of *Medicago sativa* including putrescine, spermidine and spermine. The ratios of spermine was much less than putrescine/spermidine. Polyamine might be a prerequisite for early differentiation during the induction of embryogenesis [20] that enhanced

Table 1: Effect of spermidine on germination and root growth of cotton plant

Spermidine (mg L^{-1})	Germination (%)	Hypocotyl length (cm)	Epicotyl length (cm)	Root mass (g) Fresh See.	Fresh Veg.	Fresh Flo.	Dry See.	Dry Veg.	Dry Flo.
0	52.68	11.0	5.2	0.12	2.6	2.5	0.02	0.41	0.94
5	79.32	13.3	5.5	0.10	2.8	3.8	0.02	0.66	1.32
10	62.00	15.3	6.1	0.07	2.6	5.2	0.02	0.67	1.76
20	56.68	14.0	5.7	0.08	3.4	7.3	0.02	0.60	2.40
40	40.80	13.7	4.0	0.05	2.6	5.9	0.01	0.62	2.13
LSD at 5%	9.24	1.65	0.71	0.03	0.4	1.6	NS	NS	0.49

Table 2: Effect spermidine (mg L^{-1}) on stem and leaf growth of cotton plant

Spermidine (mg L^{-1})	Stem mass Fresh (g) See.	Fresh (g) Veg.	Fresh (g) Flo.	Stem mass Dry (g) See.	Dry (g) Veg.	Dry (g) Flo.	Leaf mass Fresh (g) See	Fresh (g) Veg.	Fresh (g) Flo.	Leaf mass Dry (g) See	Dry (g) Veg.	Dry (g) Flo.	Leaf area (cm^2) See.	Leaf area (cm^2) Veg.	Leaf area (cm^2) Flo.
0	0.59	15.2	14.0	0.06	2.13	4.6	0.93	23.6	22.0	0.14	3.5	5.07	37.5	1091.0	834.1
5	0.76	17.4	22.8	0.07	3.23	7.5	1.01	23.4	36.4	0.14	3.6	6.83	42.7	1174.7	1440.9
10	0.79	17.5	26.4	0.07	4.05	8.4	1.03	23.8	41.4	0.14	3.7	9.53	45.0	1229.1	1400.0
20	0.71	18.3	38.4	0.08	4.15	12.1	1.07	24.4	57.8	0.16	5.2	13.03	36.6	1042.0	1890.9
40	0.60	13.6	25.6	0.06	2.85	8.4	0.73	16.1	45.6	0.11	3.2	9.73	39.0	684.2	1669.2
LSD at 5%	NS	2.5	4.0	NS	0.86	2.0	NS	2.82	15.5	NS	1.01	2.45	8.4	181.9	561.7

Seedling stage = See. Vegetative stage = Veg. Flowering stage = Flo.

elongation growth and also reduced membrane damage. They show promise to harden seedlings against environmental stresses [21].

Effect of spermidine on root: Data presented in Table 1 show that cotton seeds soaked in spermidine solutions caused significant increases of root fresh and dry biomass specially at vegetative and flowering stages. The maximal values of root characters were obtained at treatment 20 mg L^{-1} spermidine. This result indicated that, the increases of root fresh and dry biomass were related to increase spermidine concentration. On the other hand, root fresh biomass was insignificantly decreased at seedling stage. These results appear that spermidine (20 mg L^{-1}) was sufficient to induce root growth of cotton plant during plant development stages and inhibit up than the previous concentration. This effect might be due to variation endogenous spermidine content at the different stages of plant development. These results are in agreement with Smith [3] who suggested that the interaction of polyamines with macromolecules in responsible for their physiological effects ranging from promotion of cell growth and differentiation of plant organs. That shows the essential role of polyamines for regulation of plant growth and development [22]. These effects might be due to the ratio of putrescine/spermidine in the plant an increase in putrescine and a high ratio are correlated with root growth.

Exogenous spermidine (1 mM) applied to *Zea mays* L. seedlings through roots caused a strong inhibition of root growth [23]. Also, spermidine levels did not change significantly the adventitious root formation of plant at the induction and initiation phase [8]. Inhibition of plant growth might be due to polyamines binding to phospholipids and affected membrane rigidity [24].

Effect of spermidine on vegetative growth: Data presented in Table 2 show that vegetative growth characters of cotton plant such as fresh and dry biomass of stem and leaves/plant significantly increased gradually at vegetative and flowering stages compared with control by spermidine application. Increasing spermidine concentration up to 20 mg L^{-1} gave the positive response of increasing biomass criteria of stem and leaves/plant, as leaf area/plant significantly responded to spermidine application. The results indicate that increasing spermidine concentration extend the leaves and made it compacted. The highest values of vegetative characteristics were obtained by spermidine (20 mg L^{-1}) at all developmental stages of cotton plant. Increasing spermidine concentration over 20 mg L^{-1} level led to inhibition of plant growth. However, the maximal values appeared at flowering stage. This effect might be due to spermidine regulation of growth or the consequence of spermidine biosynthesis at low concentration. In addition, the consequence of spermidine degradation can be the

Table 3: Effect of spermidine on yield and its components of cotton plant

Spermidine (mg L^{-1})	Boll number/plant	Seed number/boll	Seed mass/boll (g)	Fibre mass/boll (g)	Boll yield/plant (g)
0	5.0	16.0	1.33	0.70	10.2
5	7.5	21.0	1.40	0.71	15.9
10	10.5	19.0	1.36	0.72	21.9
20	10.9	18.0	1.31	0.73	21.9
40	9.0	18.0	1.37	0.73	18.6
LSD at 5%	2.2	2.2	NS	NS	3.9

Seedling stage = See. Vegetative stage = Veg. Flowering stage = Flo.

products of a precursor for other growth substances in plant. In this respect, Abd El-Wahed [7] reported that injected spermidine stimulated vegetative growth characters as plant height, leaf area, plant fresh and dry weight and net assimilation rate of maize plants. Whereas, polyamine oxidases are preferentially associated with the primary and secondary cell wall of tissues undergoing lignification, suberization and wall stiffening (such as xylem, xylem parenchyma, endodermis and epidermis) although their association to cortical parenchyma cell walls during specific development stages [25, 26]. In addition, polyamines play important roles in DNA stabilization, modulation of ion channels and protection against oxygen radicals and they are essential for cell homeostasis, cell growth and tumorigenesis [27].

Effect of spermidine on yield and its components: The data given in Table 3 show that cotton yield and its components such as boll number/plant, seed number/boll and boll yield/plant were significantly increased by spermidine application compared with control treatment. The increases of all number and fibre yield/plant were related to spermidine concentration up to (20 mg L^{-1}). Fibre mass (40 mg L^{-1}), seed number and seed mass 5.0 mg L^{-1}. The increment of ball, number/plant and boll yield/plant that resulted from spermidine treatment had been evaluated by 50 or 118% and 55.9 or 114.7%, respectively, compared with control at complete open boll stage. From these results, it could be concluded that the favourable effect of spermidine was 20 mg L^{-1}. This might be due to the reflected role of spermidine on growth and open boll/plant. These results agree with Abd El-Wahed [7] who found that injected spermidine in *Zea mays*. L. enhanced grain yield/plant and 100-grain weight. Whereas, polyamine application significantly increased α-amylase activity in the plant [28] and inducing parthenocarpic growth of tomato fruit [29]. Additionally, spermidine application led to increase flowers number and weight of chamomile plant [30].

Effect of spermidine on some biochemical constituents of cotton plant:

In root: Data presented in Fig. 1 show that spermidine had significant effect on root biochemical constituents such as total sugars, free amino acids, phenols and indoles at all developmental stages of cotton plant compared with control. Increasing spermidine level was accompanied with increasing these contents in cotton root. The maximal values of the biochemical constituents (total sugars, free amino acids and phenols) of cotton root were obtained by 10, 40 and 40 mg L^{-1} of spermidine respectively at all stages. However, spermidine (5 mg L^{-1}) was more effective on endogenous indoles content at seedling stage of cotton plant. Whereas, the interaction of spermidine with assimilated compounds was related to plant development stage. It could be conclude that spermidine plays a role on translocation processes from vegetative parts to the root. Spermine application increased hexokinase, α-amylase nitrogenase activity in root nodules of *Vigna mungo* plants [31]. Whereas, the increase in nitrate reductase activity with total organic nitrogen and a negatively with nitrate content was related to plant developmental stage [32]. Altering nitrogen metabolism in castor bean cotyledons resulted in marked changes in the allocation of carbon between carbohydrate synthesis [33].

In stem: The data given in Fig. 1 show that stem total sugars and phenols content were significantly increased at seedling and vegetative stages as well as indoles at flowering stage with spermidine application. Additionally, significant increases appeared in free amino acids content of stem at all stages of cotton development. Spermidine (10, 40 and 40 mg L^{-1}) were more effective on stem total sugars at vegetative, free amino acids at seedling, phenols at seedling and indoles at flowering stages. It appears from the results that free amino acids and indoles content of cotton stem were contrasted at seedling stage.

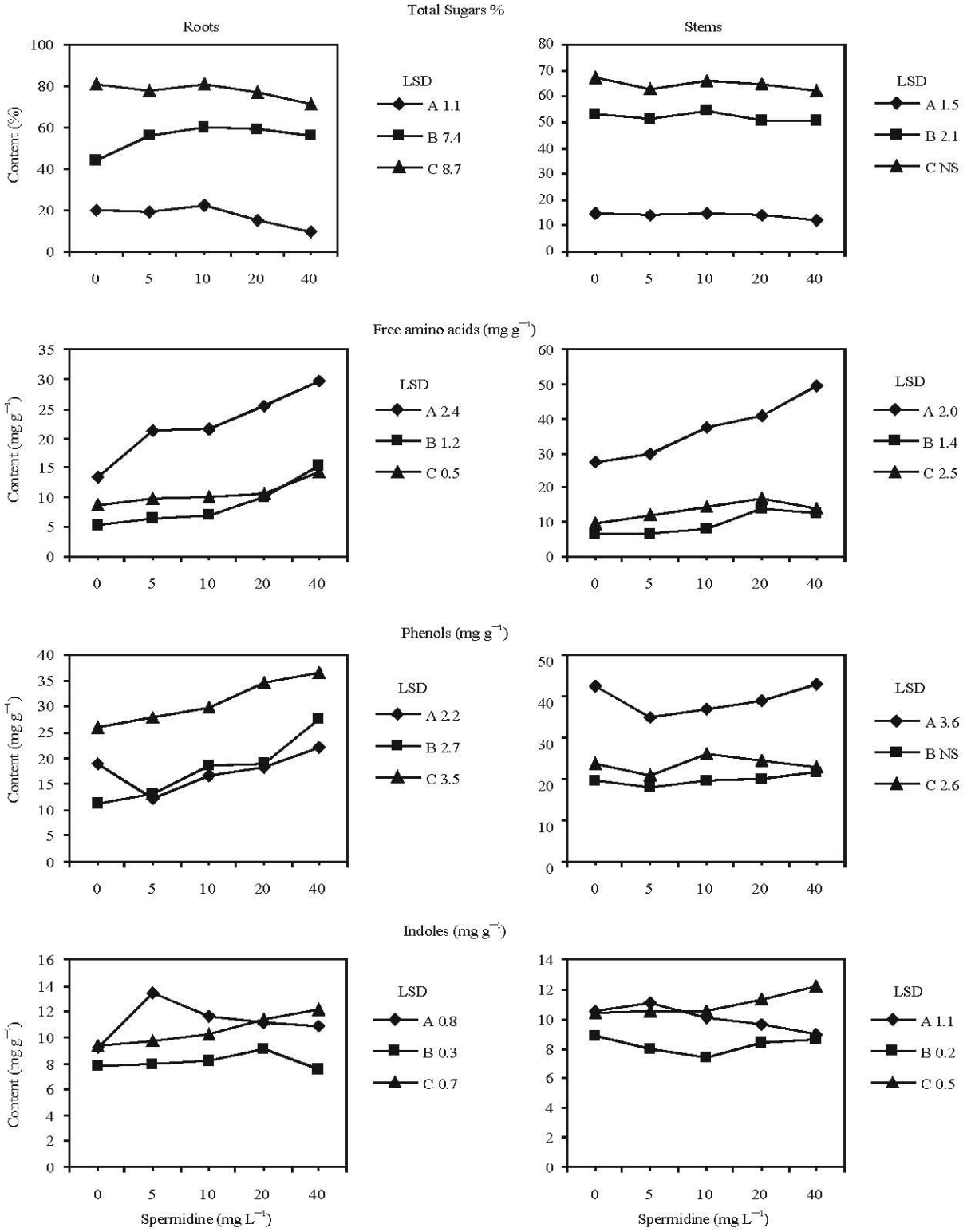

Fig. 1: Effect of spermidine on some biochemical contents of the roots and stems at seedling stage (A), Vegetative stage (B) and flowering stage © of cotton plant

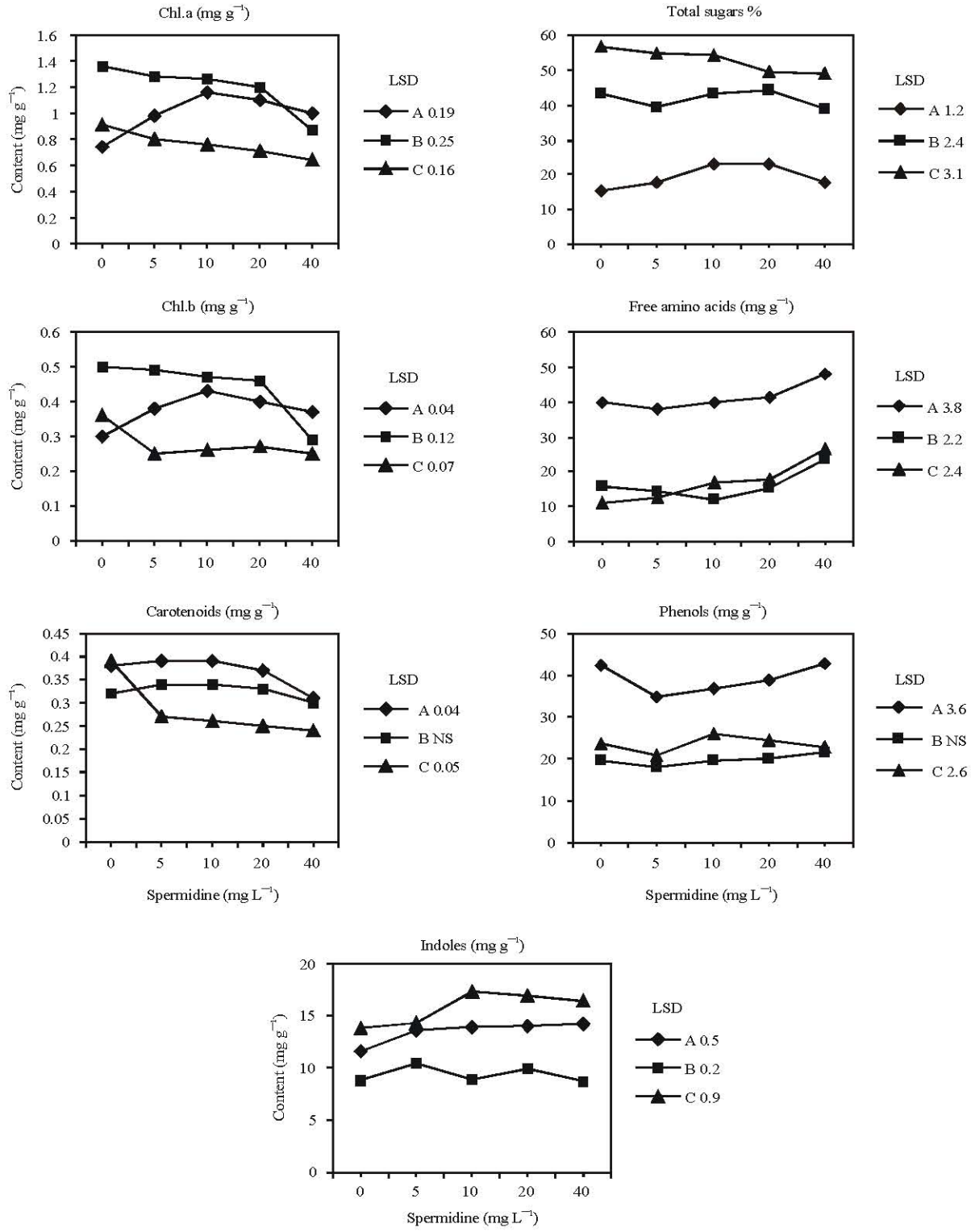

Fig. 2: Effect of spermidine on some biochemical contents of the leaves at seedling stage (A), vegetative (B) and flowering stage © of cotton plant

This shows that spermidine acts as stress initially and a source of nitrogen compounds in cotton seedlings. That might be converted to phenol compounds to make a defense against any stress. These results are in agreement with Rastagi and Davies [34] who found that pea shoots were shown to metabolize spermidine to γ-aminobutyric acid, glutamate, asparatete, sugars and organic acids. Whereas, assimilate portioning refers to the systemic distribution of sugars and amino acids from sites of primary assimilation to import-dependent tissues and organs [35].

In leaves: Data presented in Fig. 2 show that leaves, pigments (chl. a and b) content were significantly increased with increasing spermidine concentration up to 10 mg L^{-1} at seedling stage and decreased at vegetative and flowering stages compared with control treatment. Also, carotenoids content was increased by spermidine (10 mg L^{-1}) at seedling and vegetative stages. Significant increases of total sugars, free amino acids, phenols and indoles content of cotton leaves were related to enhance spermidine concentration at all stages except total sugars at flowering stage. The highest content of free amino acid and phenols were obtained by spermidine (40 mg L^{-1}) and contrasted at flowering stage. However, leave indoles content was more affective by spermidine (10 mg L^{-1}) at flowering stage. These observations suggest that high contents of biochemicals in leaves are associated with exogenous spermidine. Whereas, it inhibited RNase activity, the loss of chlorophyll and degradation of the proteins from thylakoid membranes. Spermidine was effective in the retardation of the loss of the apoprotein of the light harvesting chlorophyll a/b protein complex in primary leaves [36]. The activities of the whole chain electron transport, photosystem I and II and absorbed excitation energy distribution in favour of photosystem 1 were protected by polyamines [37]. Increasing carotenoids content may due to convert these substances to pyruvic acid that led to enhance biosynthesis of leaf carotenoids [38]. Consequently, phenolic acids conjugate with polyamines modulating the free levels of these plant growth substances [39]. Spermidine application increased leaves total sugars, soluble, nonsoluble and sucrose content of maize [7] but reduced amino acids in *Vitis vinifera* L. [40]. Additionally, exogenous spermidine increased biochemical constituents as total sugars, phenols and indoles of chamomile plant [29].

Effect of spermidine on endogenous polyamines content in cotton leaves: It appears from the presented data in Figs. 3 and 4 that spermidine application increased other

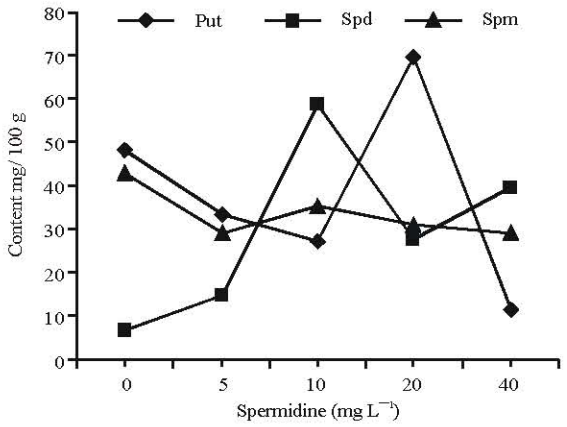

Fig. 3: Effect of spermidine on endogenous free polyamine content of cotton leaves at vegetative stage

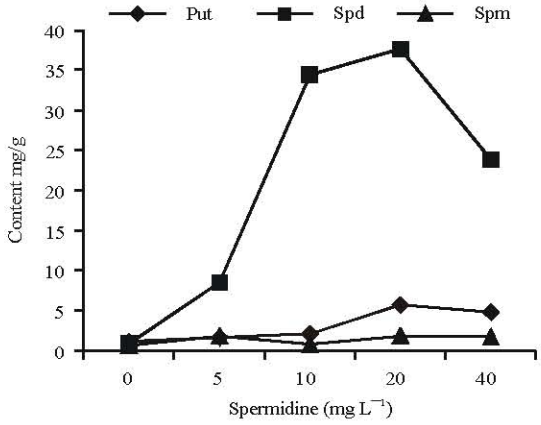

Fig. 4: Effect of spermidine on endogenous free polyamine content of cotton leaves at flowering stage

endogenous polyamines content (putrescine and spermidine) or (putrescine, spermidine and spermine) in cotton leaves at both vegetative or flowering stages. The maximal values of putrescine and spermidine were obtained at spermidine 20 and 10 mg L^{-1}, respectively at vegetative stage. Also, spermidine (20 mg L^{-1}) was the most effective level of polyamines (Putrescine, spermidine and spermine) content in the leaves at flowering stage. These results indicate that polyamines content were more enhanced in the leaves at flowering stage than vegetative stage. The increments of endogenous polyamines (putrescine and spermidine) were 1.5 and 8.6 fold of that of the control at vegetative stage and (5 and 41.8 fold) of the control at flowering stage then spermine (3 fold) at flowering stage under spermidine treatments. These results are in agreement with Torrigiani *et al.* [41] who

Table 4: Simple correlation between fibre mass/boll and some biochemical contents of leaves, stem and seed of cotton plant under spermidine treatments

		Leaves free amino acids		Stem free amino acids		Leaves total sugars		Stem total sugar		Seed total sugar	
Characters	Fibre mass/boll	Veg.	Flo.	Flo.	Veg.	Veg.	Flo.	Veg.	Flo.	25 day	50 day
1- Fibre mass/boll	1.000	-.125	.0002	.085	.247	.358	-.289	.092	.483	.009	-.172
2- Leave free amino acids at veg.		1.000	.095	.537*	.033	-.493	-.531*	-.351	-.200	.011	-.405
3- Leave free amino acids at flo.			1.000	.825**	.791**	.291	-.724**	-.635*	-.148	-.102	-.356
4- Stem free amino acids at flo.				1.000	.708*	.107	-.838**	-.649**	-.123	-.069	-.595*
5- Stem free amino acids at flo.					1.000	.240	-.644**	-.549*	-.046	-.238	-.324
6- Leave total sugar at veg..						1.000	.075	-.203	.625*	.419	.102
7- Leave total sugar at flo.							1.000	.625*	.039	.332	.641**
8- stem total sugar at veg.								1.000	-.125	-.039	.449
9- stem total sugar at flo.									1.000	.351	-.045
10- Seed total sugar after 25 days										1.000	.268
11- seed total sugar after 50 days											1.000

Seedling stage = See. Vegetative stage = Veg. Flowering stage = Flo. * Correlation is significant at 5% ** Correlation is significant at 1%

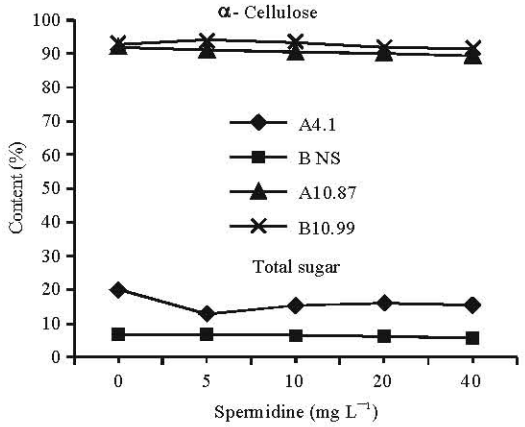

Fig. 5: Effect of spermidine on total sugars of seeds and α-cellulose of fibre after 25 (A) and 50 (B) days after flower initiating of cotton plant

found that the rates of appearance of putrescine, spermidine and spermine were slightly different between the vegetative and floral bud forming tissues of tobacco, but both showed significant increases (10-20 fold) for spermidine and putrescine levels. Thus, it could be concluded that putrescine had an increasing role in vegetative growth (Table 2 and Figs. 3 and 4) at vegetative stage. While, spermidine gave the same effect on flowering.

On the basis of the present results for endogenous polyamines, leaf growth and correlation coefficients, it could be concluded that there was a positive relation between the exogenously applied and endogenous polyamine.

Effect of spermidine on sugars and α-cellulose content of cotton seeds and fibre: Significant decrease in content of total sugars appeared in cotton seeds after 25 days of initial flowering by spermidine application as shown in Fig. 3. However, seed sugar content and α-cellulose content in cotton fibre after 50 days of initial flowering gave the maximal values by spermidine application. Spermidine (5 mg L^{-1}) was the most effective level on sugars or cellulose content in the seed or fibre. This result indicated that spermidine role on enhancing translocation of seed sugar to fibre. Which is necessary for fibre growth and development? Whereas, polyamines are involved in conformational transition of DNA [42] for cell wall related enzymes, endo-1, 4-β-glucanase and sucrose synthase [43]. These enzymes have a major role in cell wall loosening during elongation and are closely associated with the massive synthesis of cellulose [44].

Simple correlation between fibre mass/boll and biochemical content of leaves, stem and seeds: Simple correlation coefficient between fibre mass/boll, total sugars, free amino acids content of leaves and stems at vegetative and flowering stages as well as seed total sugars of cotton plant as shown in Table 4 indicated high significant positive correlation between free amino acids of leaves and stems at flowering stage, stem free amino acids at both stages and leaves total sugar content at flowering stage and seed total sugar content after 50 days from initiation flower. There are significant positive correlation between leave and stem total sugar content. However, the correlation between leaves and stems content of total sugars and free amino acids at vegetative and flowering stages were significantly negative. That refer to the systemic distribution of sugars and amino acids from sites of primary assimilation to import dependent tissues and organs [35]. Thus ensure the recycling of carbon and nitrogen.

From the above mentioned results, soaking cotton seeds in spermidine (20 mg L^{-1}) was the most effective treatment which increased root mass, stem mass, leaves mass, boll number/plant, yield/plant and fibre mass/plant of cotton cultivar G. 89.

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