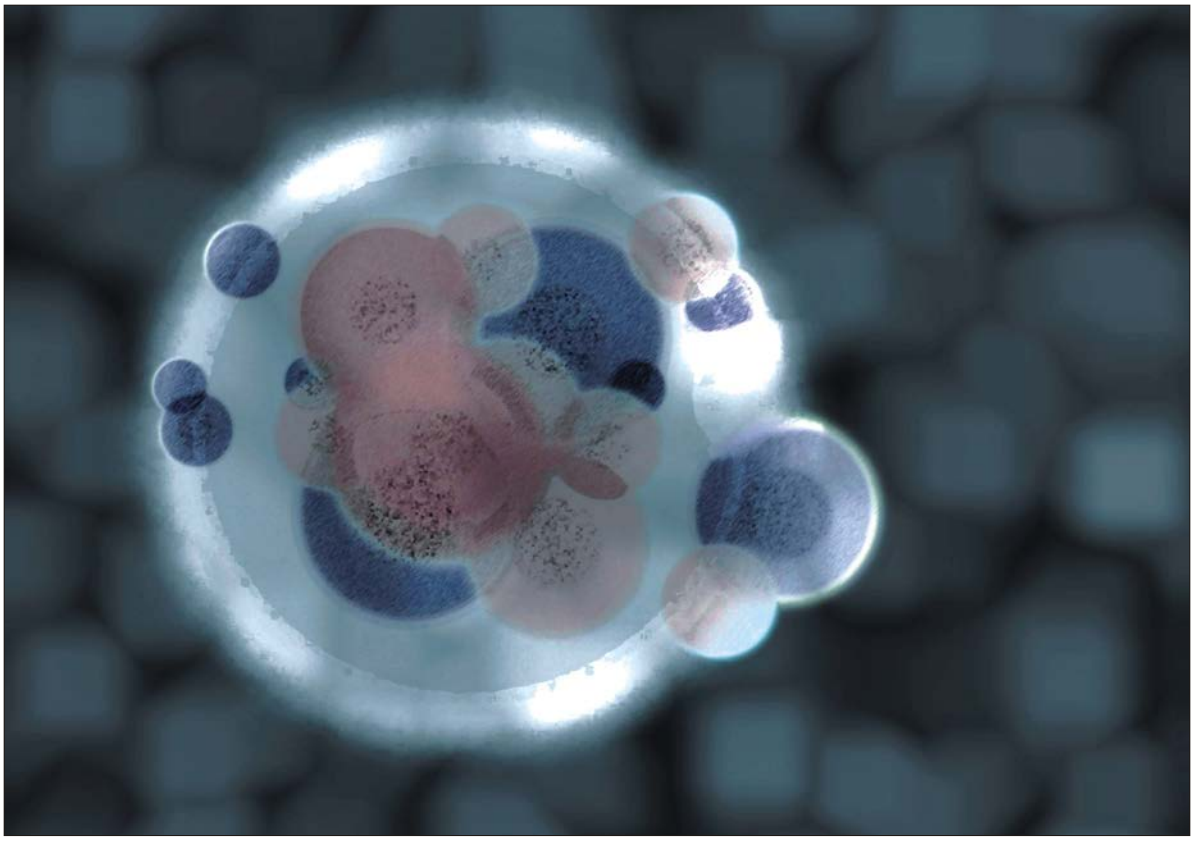

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Response of Cotton to Atonik and TIBA for Growth, Enzymes and Yield

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Abstract: A field experiment was conducted in cotton during 2002-2003, in the experimental field of Tamil Nadu Agricultural University, to study the response of cotton plant to Atonik (a nitrophenolic compound) and TIBA in combination and as individual treatment. The plants were sprayed with Atonik and TIBA at 0.25% and 100 ppm during flowering and boll set stages. The results revealed that application of Atonik increased the growth parameters viz., plant height and leaf area, while TIBA reduced it. In combination the effect of TIBA on morphological characters has been reverted by Atonik spray. Catalase, peroxidase and superoxide dismutase enzyme activity were increased by Atonik spray, whereas TIBA decreased the enzyme activity. The treatment combination of Atonik and TIBA resulted in a better performance than control. Both TIBA and Atonik increased the yield per plant over control. The yield increase was mainly due to increase in more number of bolls per plant.

Key words: TIBA, atonik, growth, antioxidant enzymes, yield

INTRODUCTION

Auxin is a class of plant hormones that regulate cell division, elongation and differentiation and therefore diverse growth and developmental processes. In developing plants, indole-3-acetic acid, the major endogenous auxin is synthesized in young tissues including apical meristems and young leaves and transported to other cells/tissues to regulate plant growth and development[1]. The transport process is unilateral and conventionally referred to polar auxin transport. Polar auxin transport inhibitor, 2, 3, 5-triiodobenzoic acid (TIBA), have various effects on physiological and developmental events. Application of TIBA results in raised auxin concentrations as it cannot be transported away from the apex[2]. TIBA is effective in releasing lateral buds of Kentucky blue grass from apical control[3]. Application of antiauxin like TIBA and other auxin analogues inhibits the auxin-directed transport of metabolites towards the shoot apex[4]. Application of TIBA 15 days after full bloom reduced fruit size and caused most severe fruit pitting in Golden Delicious apples, as TIBA interfered with the hormonal stimulus of Ca^{2+} movement into the fruit and affected cell division[5]. Dhillon *et al.*[6] reported that application of higher concentration of TIBA proved to be more effective in suppressing the plant height by inhibiting the apical dominance. The decrease in plant height in turn increased the root length because of diversion of hormones from the shoot apical meristem to other plant parts[7]. TIBA spray could produce more number of leaves during later stages of crop growth and this might be possible due to the presence of more branch number per plant[8].

MATERIALS AND METHODS

An experiment was conducted to study the interaction of Atonik with TIBA in cotton (MCU 12) during 2002-2003 in a Factorial Randomised Block Design with five replications at the experimental field of Tamil Nadu Agricultural University, Coimbatore, India. Recommended package of practices was followed from sowing to maturity. The treatment includes 2 factors, factor 1-stage of application and factor 2-Atonik or TIBA. Factor 1 was represented as S and factor 2 as T. The plants were irrigated once in five days. Atonik and TIBA were applied at flowering and boll set stage at 0.25% and 100 ppm, respectively. Unsprayed plants served as control. Observations were made in the flowers and bolls of cotton. Sampling for biochemical analysis was done after 48 h of spray. The treatments were: S_1- Foliar spray during flowering stage, S_2- Foliar spray during flowering and boll set stages, T_1-Control, T_2-Atonik 0.25%, T_3-TIBA 100 ppm and T_4-Combined spray of Atonik 0.25% and TIBA 100 ppm.

The morphological parameters like plant height and leaf area were recorded at flowering and boll set stages. Plant height was measured from the ground level to the tip

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of growing point and expressed in cm. Leaf area per plant was measured using Leaf Area Meter (LICOR, Model LI 3000) and expressed in cm^2 $plant^{-1}$.

Biochemical parameter viz., Superoxide dismutase enzyme activity was determined by using nitro blue tetrazolium (NBT) salt as described by Beau- Champ and Fridovich[9] and expressed in enzyme units g^{-1} of fresh weight of leaf sample. Catalase activity was assayed by adopting the procedure of Gopalachari[10] and expressed as mg H_2O_2 g^{-1}. Peroxidase was assayed according to Perur[11] and expressed as change in optical density per gram for min. Polyphenol oxidase was quantified and expressed as change in optical density per gram for min[12].

Yield and yield components were estimated from five random samples of each replication in each treatment at flowering and maturity stages. Number of fruiting branches produced by the plants was counted at the time of maturity and expressed in number of sympodia per plant. The number of flowers produced was recorded daily from the commencement of flowering upto 30 days prior to harvest and expressed in number of flowers per plant. Number of matured bolls was counted from boll set stage to harvest and expressed in number of boll $plant^{-1}$. Fertility co-efficient was worked out using the following formula and expressed in %.

$$\text{Fertility co-efficient} = \frac{\text{Number of bolls per plant}}{\text{Number of flowers per plant}} \times 100$$

Five well burst bolls were collected from each plant and the bolls was weighed. Seed cotton yield was recorded from five randomly selected plants of each replication and expressed as g $plant^{-1}$.

RESULTS AND DISCUSSION

Morphological attributes: At flowering stage, the maximum plant height was observed in T_2 (Atonik 0.25%), followed by T_1 (control) and T_4 (combined application of Atonik and TIBA). Both T_1 and T_4 were found to be on par with each other (Table 1). Treatment and interaction of treatment with stage was found to be significant. At boll set stage also the same treatment, T_2 recorded the highest plant height (132.48 cm) followed by T_1 (116.43 cm). Here S, T and SxT were significant. Maximum leaf area was observed in T_2 (Atonik 0.25%) followed by T_1 (control), at flowering stage. T_2 recorded an increase of 56.4% over control. T_1 and T_4 recorded a value of 612.41 and 603.84 cm, respectively at flowering. At boll set stage, the same trend was followed. The treatment, T_2 recorded an increase of 57.7% over control. In the present investigation, there was an increase in plant height over control in Atonik alone treatment (T_2) and a decrease over control in TIBA alone treatment (T_4). Atonik and TIBA, in combination produced an effect similar to control. This clearly indicated that the effect of one is nullified by the other, when they are sprayed in combination. The increase in plant height in Atonik treatment may be due to increase in the endogenous level of auxin[13]. Auxin is involved in cell division, cell differentiation and cell expansion, thereby leading to enhanced growth and development. Increase in plant height may be due to the stimulating action of auxin, which softens the cell wall by increasing its plasticity[14]. The reduction in plant height by TIBA may be due to inhibition of apical dominance and accelerated growth of lateral buds[15]. TIBA prevents the polar transport of indoleacetic acid, which results in reduced plant height. Atonik and TIBA in combination were on par with control, which reflects the antagonistic effect of Atonik and TIBA on each other.

Leaf area gives a fairly good idea of the photosynthetic capacity of the plant. Leaf area also follows the trend of plant height. The increase in leaf area in Atonik treatment might be due to the positive effect on cell division and cell elongation leading to enhanced leaf expansion[16]. TIBA also increased the leaf area. The increase might be possible due to the presence of more branches number per plant as evidenced from the study. This is in line with the results of Ravichandran and Ramaswami[8].

Biochemical and physiological parameters: Application of Atonik and TIBA at flowering stage revealed that the stage of application did not show any significant variation between them for superoxide dismutase activity (Enzyme Unit) (Table 1). Irrespective of the stage of application, T_2 (Atonik 0.25%) was found to be the best, followed by T_4. The treatments, T_1 (control) and T_4 (combined application of TIBA and Atonik) were found to be on par. At boll set stage, the treatment, stage of application and its interaction were found to be significant. The S_2 (application of Atonik and TIBA at flowering and boll set) was found to be superior over S_1 (application of Atonik and TIBA at flowering) by showing an increase of 21.63%. Here also, T_2 was the best followed by T_4. At flowering stage, only the treatment showed significant difference for peroxidase activity (ΔOD 430 nm g^{-1} min^{-1}). At flowering stage, the T_2 recorded a value of 0.282, which was a 128.69% increase over T_1. At boll set stage, the mean differences over stage, treatment and its interaction were found to be significant. At this stage, T_2 was found to be the best (0.418) followed by T_1 (0.233). T_3 and T_4 recorded a value of 0.218 and 0.209, respectively. S_2 recorded an

Table 1: Effect of Atonik and TIBA on morphological and biochemical characteristics of cotton var. MCU 12

		T_1		T_2		T_3		T_4		CD (p=0.05)		
Parameters	Stages	S_1	S_2	S_1	S_2	S_1	S_2	S_1	S_2	S	T	SxT
Morphological parameters												
Plant	Flowering	110.210	114.570	125.080	126.910	102.620	104.760	110.880	111.980	NS	4.220	4.32
height(cm)	Boll set	115.480	116.430	129.510	132.480	106.860	109.570	112.430	113.690	4.17	4.240	4.34
Leaf area	Flowering	615.380	612.410	944.080	949.630	566.420	560.920	610.630	603.840	NS	3.930	5.55
plant^{-1} (cm^2)	Boll set	615.680	619.830	963.150	986.410	578.360	591.600	624.780	632.840	2.91	4.110	5.82
Biochemical parameters												
Superoxide	Flowering	0.800	0.820	1.380	1.370	0.710	0.740	0.840	0.850	NS	0.036	0.009
dismutase (Enzyme unit)	Boll set	0.720	0.850	1.250	1.680	0.700	0.740	0.750	0.900	0.006	0.009	0.012
Peroxidase	Flowering	0.124	0.121	0.282	0.277	0.104	0.110	0.163	0.167	NS	0.005	NS
activity (ΔOD g^{-1} min^{-1})	Boll set	0.233	0.238	0.418	0.447	0.205	0.218	0.209	0.226	0.003	0.004	0.006
Catalase	Flowering	0.305	0.313	0.432	0.427	0.221	0.224	0.326	0.328	NS	0.003	0.004
activity (mg H_2O_2 g^{-1})	Boll set	0.361	0.369	0.436	0.464	0.311	0.347	0.367	0.374	0.002	0.003	0.004
IAA oxidase	Flowering	262.260	264.300	336.480	335.620	245.420	245.810	265.570	268.460	NS	2.979	3.384
(µg unoxidised auxin g^{-1} h^{-1})	Boll set	297.480	410.730	391.500	443.560	266.830	321.320	294.110	366.900	1.188	1.681	2.377
Polyphenol	Flowering	0.980	0.981	0.421	0.442	1.202	1.163	0.640	0.654	NS	0.008	0.012
oxidase (ΔOD nm g^{-1} min^{-1})	Boll set	1.481	1.361	0.874	0.602	1.242	1.133	0.935	0.746	0.006	0.008	0.012

Table 2: Effect of Atonik and TIBA on yield and yield attributes of cotton var. MCU 12

		T_1		T_2		T_3		T_4		CD (p=0.05)		
Parameters	Stages	S_1	S_2	S_1	S_2	S_1	S_2	S_1	S_2	S	T	SxT
Yield and yield components												
Number of sympodia plant^{-1}	Flowering	11.45	11.46	12.24	12.66	13.33	13.00	11.86	12.41	NS	0.182	0.258
	Boll set	11.49	11.51	13.74	14.33	14.00	14.66	11.00	11.66	0.284	0.402	0.568
Number of squares plant^{-1}	Flowering	59.25	59.66	58.21	59.57	58.62	60.00	57.40	58.33	NS	NS	NS
	Boll set	59.00	58.22	58.91	57.84	59.33	58.67	60.46	59.33	NS	NS	NS
Number of flowers plant^{-1}	Flowering	41.26	41.55	42.66	43.33	44.33	42.91	42.33	43.67	NS	0.275	0.39
	Boll set	41.00	40.33	44.28	43.82	42.75	42.66	42.56	41.66	0.251	0.355	0.503
Number of bolls plant^{-1}	Flowering	17.73	18.00	22.23	23.81	21.23	22.58	19.55	19.85	NS	0.384	0.226
	Boll set	18.12	18.33	24.53	24.38	22.89	23.00	19.32	19.28	NS	0.225	0.102
Boll weight (g)	Flowering	4.00	4.02	4.12	4.17	4.09	4.14	4.04	4.06	NS	0.012	NS
	Boll set	4.07	4.08	4.21	4.28	4.19	4.23	4.10	4.12	NS	0.013	0.011
Seed cotton yield (g plant^{-1})	Flowering	58.18	60.00	95.46	97.66	87.16	87.42	64.34	66.82	NS	5.435	5.616
	Boll set	60.16	60.14	108.73	128.33	97.84	106.64	68.72	69.53	7.489	7.692	7.979

increase of 6.01% over S_1. Stages of applications such as S_1 (application of chemical at flowering) and S (application of chemical at flowering and boll set stages) did not show any significance at flowering stage for catalase activity (mg H_2O_2 g^{-1}). Treatment and interactions were, however, found to be significant. Maximum catalase activity was noticed in T_2 (Atonik 0.25%) followed by T_4 (TIBA 100 ppm+Atonik 0.25%), which increased the activity by 41.63 and 38.8% over control , respectively. The control (T_1) recorded a value of 0.305 at flowering stage. At boll set stage, the S, T and SxT were found to be significant. Among the treatments, during flowering stage, maximum activity was observed in T_2 (0.432) followed by T_4 (0.326). T_3 recorded a decrease of 10.94% over control. Polyphenol oxidase activity (ΔOD 495 nm g^{-1} min^{-1}), at flowering stage only treatment and the interactions were significant. During flowering stage, maximum activity was observed in T_3 (1.202) followed by T_1 (0.98). The treatments T_2 and T_4 showed a decrement of 55.1 and 34.1 , respectively over control. At boll set stage, S_1 was found to be the best by showing an increase of 18.9 over S_2. Among the treatment values, T_2 had the lowest activity (0.602) followed by T_4 (0.746). Here, treatments, stages of application and their interactions were significant. IAA oxidase (µg unoxidised auxin g^{-1} h^{-1}) at flowering stage showed a significant difference in treatment and the interaction between stage and treatment. Irrespective of the stage of application, Atonik spray at 0.25% significantly reduced the activity, whereas TIBA spray increased the activity. T_4 (combined spray of Atonik and TIBA) showed a slight increase over control. At boll set stage, S_1 recorded a value of 312.48, which was a 23.4% decrease over S_2. Among the treatment, the same trend as in flowering stage was observed. The control and combined spray recorded a value of 410.73 and 366.90, respectively. Treatment, stage and its interaction was found to be significant. Catalase, peroxidase and superoxide dismutase enzyme activity were increased by Atonik spray, whereas TIBA decreased the enzyme activity. The treatment combination of Atonik and TIBA resulted in a better performance than control. The increase in peroxidase activity by Atonik may be due to the switching of peroxidase gene promoters[17]. Cationic cell-wall peroxidase is able to produce OH^-, which was

involved in the depolymerization of lignins causing cell wall loosening[18]. The increase in turgor as well as cell wall loosening triggers cell expansion[19].

Application of growth retardant reduces the peroxidase activity[20]. This decreased activity is reflected upon plant height, which is recorded in the present study. The activity was restored by the action of Atonik when sprayed along with TIBA, thereby control and Atonik+TIBA treated plants were of similar height. Catalase and superoxide dismutase activities were enhanced by the application of Atonik. The increase in activity is manifested in the maintenance of redox balance, which is a prerequisite for growth[21]. The decrease in activity of catalase and superoxide dismutase by TIBA shows its anti-auxin effect. The decrease in the enzyme activity also causes more accumulation of ROS, which is deleterious to growth. This might be the reason for reduced growth in TIBA treated plants. The finding of the experiment is in line with that of Xin and Ziv[20].

Reduced polyphenol oxidase and IAA oxidase activity was observed in Atonik treatment. The decrease may be correlated to with more IAA level in the plant tissue. The growth retardant, CCC lowered the chlorogenic acid content, which resulted in increased IAA oxidase activity[22]. The increased polyphenol oxidase activity by TIBA lowers the auxin content by decreasing the polyphenol content, which inhibits the decarboxylation of auxin[23]. As the treatment with Atonik exhibited a low PPO activity, it resulted in low IAA oxidase activity by increasing polyphenol content. The reverse was observed in treatment with TIBA. When Atonik and TIBA are applied in combination, a reduced activity was observed, showing the reversal effect of Atonik on the enzyme.

Yield and yield components: Maximum sympodia were observed at boll set stage (Table 2). Application of chemical at flowering and boll set stage (S_2) surpassed the S_1 (application of chemical at flowering) by 3.9%. The treatment, stage of application and its interaction were significant except for stage of application at flowering stage. During boll set stage, irrespective of the stage of application, maximum sympodia was observed in T_3 (TIBA 100 ppm), followed by T_2. At both stages of application, the treatment and the interaction was non-significant for number of squares plant^{-1}. Among the treatment mean values, a slightly higher value was observed in T_3 and T_4 at boll set stage, whereas at flowering stage, T_1 and T_3 showed a slightly enhanced value. During flowering stage, irrespective of the treatment, the stage of application showed a value of 40.32 and 42.66 for S_1 and S_2 , respectively, which did not show any significance for number of flowers plant^{-1}. Among the treatment mean values, T_3 produced maximum number of flowers (44.33), followed by T_4 (43.67). At boll set stage, irrespective of the stages, T_2 recorded a value of 44.28, which was 8.3% increase over control, followed by T_3 (42.75). S, T and SxT were significant. Stages of spray did not show any difference, however, treatment and its interaction showed significant differences for number of bolls plant^{-1}. At flowering stage, maximum number of bolls was observed in T_2, followed by T_3. At boll set stage, the control (T_1) and combined spray treatment (T_4) recorded a boll number of 18.33 and 19.28, respectively. The best treatment T_2 produced a boll of 24.38 followed by T_3 (23.00). Similar to square number, boll weight did not indicate any significant variation between stage of application and interaction at flowering. Maximum mean value was observed in T_2 followed by T_3 at both flowering and boll set stages. Comparing flowering stage and flowering and boll set stages of spray, the latter performed better than the former for yield plant^{-1}. At boll set stage, S_2T_2 performed better (128.33) than all the other treatments, followed by S_2T_3 (106.64). In S_1, T_2 performed better than the other treatments. Irrespective of the stage of application, T_2 recorded a value of 118.53, which was a 97.0% increase over control and 15.9% over TIBA (100 ppm) and 71.4% over combined spray of Atonik and TIBA. Treatment with Atonik increased the yield components viz., number of sympodia per plant, number of bolls per plant and boll weight. The anti-auxin, TIBA also influenced the yield components positively. When Atonik was applied with TIBA, the yield was reduced significantly over their individual treatments, but was higher than the control. The increase in yield components by Atonik may be due to enhanced peroxidase, catalase and superoxide dismutase activities and reduced polyphenol oxidase and IAA oxidase activities. Atonik increased the yield and this may be due to increase in the endogenous auxin level by external application[24]. Virizilov and Mihteleva[25] reported that higher yield in treatments with Atonik may be due to increased photosynthetic efficiency and therefore higher production of photosynthates resulting in increased translocation of organic material from source to sink.

The anti-auxin, TIBA increased the light penetration in the crop canopy by producing a vertical leaf orientation and increased the photosynthetic efficiency and yield[6]. TIBA inhibits the auxin-directed transport of metabolites towards the shoot apex[4], which results in more dry matter availability for developing sink[26]. Increase in photosynthetic activity per unit area of plant leaf by growth retardants may be due to an increase in leaf thickness[27]. The decreased yield in combination may be due to the unfavourable situation created by anti-auxin, TIBA. Atonik cannot alleviate this effect fully. Thus, from

the study, it is evident that, Atonik may increase the internal auxin pool and TIBA counteract the effect of Atonik as noticed in the yield components.

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