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Research Article

Impact of Plant Growth Regulators on Yield and Fiber Quality in Cotton (*Gossypium hirsutum*)

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ABSTRACT

Cotton (*Gossypium hirsutum*), usually known as 'white gold' is a perennial crop with an indeterminate growth habit and is grown as an annual crop in the agricultural system. Due to changing climatic conditions cotton has to face many stresses which can affect seed cotton yield and fiber quality. The change in climatic conditions and management practices can alter the behavior of cotton plants which is mostly due to the variations in plant hormone levels. To make cotton crops steady and get its full yield potential, the exogenous application of plant growth regulator (PGR) has imparted significant positive effects. In this study, three PGRs (auxin, cytokinin, and gibberellin at the rate of 1ppm/litter) were used to test their effect on seedlings of two established cotton varieties (FH-492 and FH-317) and for yield and fiber quality contributing traits in field conditions. Both the varieties responded differently to each PGR treatment at the flowering stage. The results revealed that PGRs do not affect seedlings to improve vigor and growth. However, yield contributing traits showed a positive response to PGRs by showing an increase in boll retention percentage up to 61%, boll weight up to 31%, seed cotton yields up to 40%, and 21% increase in ginning out turn (GOT%) compared to control. Auxin and gibberellin also increased fiber length (>5%) and decreased mic (<10%) to produce longer and finer fiber in both varieties. However, cytokinin showed a positive effect only on GOT% of both the varieties (18% in FH-492 and 11% in FH-317). Overall, the study demonstrates that exogenous application of plant growth regulators can enhance cotton yield and fiber quality, providing a promising strategy to improve crop resilience under changing climatic conditions

Keywords: Auxin, cytokinin, gibberellin, PGR, boll retention, fiber length, ginning out turn (GOT%), climatic stress, seed cotton yield

INTRODUCTION

Climate change is a major threat to agriculture production worldwide. Agricultural productivity is directly affected by climatic factors including an increase in temperature, unexpected precipitation, and flooding. By 2020, climate patterns are predicted to reduce the average global temperature by 2.9 to 5.5 °C. The temperature extremes will also adversely affect the crop phenology resulting in a decrease in crop yields (Siyal *et al.*, 2021). Cotton is a perennial woody shrub with an indeterminate growth pattern. After domestication, in a series of breeding and selection cycles by humans, it has been cultivated as an annual crop worldwide (Zhang *et al.*, 2023). The area under cotton cultivation has increased to 2.4 million hectares compared to the last year of 2.1 million hectares in 2023-24.



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Also, production has observed an immense increase resulting in 10.2 million bales due to an increase in the area of cultivation in Pakistan (Anonymous, 2024). The temperature and rain fluctuation significantly affect the cotton growth in Pakistan. These fluctuations have led to changes in the pattern of biotic stresses including insect pests and cotton leaf curl disease (CLCuD) occurrence. Thus, cotton crops have been facing several biotic and abiotic stresses that influence cotton yield negatively. All these stresses not only cause low yield but also lead to low-quality fiber being used in the textile industry (Shahzad *et al.*, 2022). The cotton growth pattern is complex compared to other major crops. It quickly responds to environmental changes and farm management practices resulting in changes in crop growth, yield, and fiber quality (Copur *et al.*, 2010). These changes are mostly due to fluctuations in plant hormones, which are critical for growth and development but are only required in small quantities by crop plants (Toungos, 2018). Some climatic factors like heat, humidity, and photoperiod are important in determining the final amount of plant hormones including cytokinin, gibberellin, and auxin (Ahammed *et al.*, 2016). These climatic factors and agronomic practices keep an optimal balance between the vegetative and reproductive growth of cotton crops (Sawan, 2017). The disturbance in the synthesis of plant hormones leads to disruption of physiological processes resulting in limited growth (Raza *et al.*, 2022). However, external application of synthetic plant growth regulators (PGR) performs a similar function as plant hormones and keeps the physiological process at its normal pace (Sabagh *et al.*, 2021). Furthermore, excessive fertilization and irrigation disturb the balance of plant development and delay plant maturation and harvesting (Ballester *et al.*, 2021).

The PGR regulates inadvertent vegetative growth and enhances the number of sympodial branches which ultimately increase the seed cotton yield (Copur *et al.*, 2010). Various combinations of plant growth regulators (PGR) have been employed in cotton to increase seed cotton yield under varying heat, drought, and salinity stress environments (Ahmad and Hasanuzzaman, 2020; Ali *et al.*, 2012). PGRs have been reported to improve germination percentage, seedling emergence, rate of photosynthesis, plant-water relationship, and fruit retention (Oosterhuis and Stewart, 2004; Sadeghi *et al.*, 2023). Studies also showed that exogenous application of PGR helps maintain plant growth and yield under insect attack (Ali *et al.*, 2012). They also play a role in relieving the plant from the damage caused by abiotic stress (Arshad *et al.*, 2023). Plant growth regulators work in different ways to regulate plant growth and development e.g. cytokinin stimulates cell division, participates in branching, and promotes bud initiation (Malik *et al.*, 2023). Gibberellins (GA) is a growth-promoting substance as it enhances cell elongation, and shoot growth and regulates dormancy (Miransari, 2016). Auxin has been reported to maintain seed dormancy and is also involved in the rapid growth of plants via the apical dominance mechanism (Beveridge *et al.*, 2023).

In summary, cotton (*Gossypium* spp.) is an important cash crop grown worldwide for its fibers, seeds, and oil. Cotton lint provides raw material for the textile industry and many other industrial and consumer manufacturers. Its growth and development are influenced by hormonal regulation which is the product of environmental and genetic factors (Shahzad *et al.*, 2022). The plant hormones can also be provided artificially; thus, growth and developmental steps can be regulated bypassing the plants' genetics and natural hormone regulations (Sabagh *et al.*, 2021). In Pakistan, the average cotton yield is declining abruptly compared to other major cotton-producing countries due to changing and unpredictable climate scenarios. Besides working on the development of climate-smart cotton genotypes, cotton production can be enhanced by the application of plant growth regulators. This study was carried out to investigate the effects of plant growth regulators on cotton growth and ultimate yield and quality and to examine which combination of the plant growth regulators gives prompt yield. The study focused on pointing out the perfect PGR combination that can be used to improve seedling vigour and cotton lint yield and quality.

MATERIALS AND METHODS

Experimental material

The present study was conducted at Cotton Research Station (CRS), Faisalabad, Pakistan during the 2023-24 growing season in two parts. The experiment used two cotton varieties developed at CRS Faisalabad, FH-492 and FH-317 to determine the suitable PGR for maximizing seed cotton yield and quality fiber. Three plant hormones (@1ppm/litter) were studied along with the control. 1-Naphthaleneacetic acid was used to test the effect of auxin, 6-benzyladenine for cytokinin, and GA3 for Gibberellic acid. The experiment had seven treatments and one control as described in Table 1.

Effect of plant growth regulators on cotton seedling development

The first part of the experiment was carried out in a completely randomized design (CRD). Cotton seeds (n=3) were planted in 16 cm wide plastic bags with a capacity of 200 g of sandy soil and shifted in the greenhouse on the shelves to provide proper sunlight and about 27-31 °C temperature was maintained via electric heaters. Later on, only one relatively vigorous plant per bag was kept to assess the effect of PGR on the growth and vigor of seedlings.

The PGRs (1ppm/litter) were applied on 7 days old seedling and their effects were observed after one week of application to check the stable changes in the seedlings. The data was collected including parameters: root weight, shoot weight, root length, shoot length, and root-to-shoot ratios. Root and shoot length were measured in centimeters (cm) via measuring tape and weight was measured via electronic balance in grams (g).

Table 1 PGR treatments used in experiment

Treatment	Treatment composition (1ppm/1litter)
T ₁	Control (no PGR)
T ₂	1-Naphthaleneacetic acid (Auxin)
T ₃	GA3 (Gibberellic Acid)
T ₄	6-benzyladenine (Cytokinin)
T ₅	Auxin + GA3 (1:1)
T ₆	Auxin + Cytokinin (1:1)
T ₇	GA3 + Cytokinin (1:1)
T ₈	Auxin+GA3+Cytokinin (1:1:1)

Impact of plant growth regulators on cotton yield and fiber quality in field conditions

To assess the effect of PGR on plant growth in field conditions, both varieties were grown in the field in factorial randomized complete block design and eight PGR treatments including one control with three replications. The plant-to-plant distance and row-to-row distance were kept 30 cm and 75 cm respectively with a net plot size of 18m × 3m. All the standard agronomic practices were implemented. The dose of each PGR was kept at 20 ppm in 20L of water. The freshly prepared solution was sprayed 60 days after sowing (DAS). While, in control treatment, no plant growth regulator was applied. Guarded plants (n=10) were tagged for data collection in each treatment. From these selected plants, average boll weight and seed cotton yield were measured through a digital electric meter. Staple length and mic were measured via USTER HVI 1000. Boll retention percentage was measured with the formula given by Sharif *et al.* (2020), while

$$\text{Boll retention} = \frac{\text{No. of intact bolls}}{\text{total No of flowers}} \times 100$$

Ginning out turn (GOT %) was calculated by employing the formula given by Singh (2004).

$$\text{GOT}\% = \frac{\text{Total weight of lint in sample}}{\text{Total cotton yield in that sample}} \times 100$$

Multiple comparison analysis was carried out for three contrasts to know the exact effect of PGRs. Three contrasts were made based on the main effect of all three PGRs (Table 2).

Table 2 Contrast comparisons in treatments based on main effects

PGR	C	T1	T2	T3	T4	T5	T6	T7
Auxin	0	X	0	0	X	X	0	X
Cytokinin	0	0	X	0	X	0	X	X
Gibberellin	0	0	0	X	0	X	X	X

Statistical analysis

The data were analyzed for variance (ANOVA) (Steel and Torrie, 1960). Later on three contrasts were made to determine the PGR individual effects in all treatments using Sheffe's multiple comparison analysis (Hulugalle *et al.*, 2004) as described in the following equation.

$$F' = \frac{(\text{mean of Group1} - \text{mean of Group2})^2}{\text{SW}^2(n_1 + n_2) / n_1 n_2}$$

Where, F' = Scheffe's Test, SW²= within mean squares, n₁ = number of samples in group 1, n₂= number of samples in group 2, These statistical analyses were done using Statistix 10. The percent change was estimated for mean values of the traits under consideration as follows and plotted in GraphPad Prism-10.

$$\% \text{ change} = \frac{\text{treatment} - \text{control}}{\text{control}} \times 100$$

RESULTS

Analysis of variance indicated no significant differences in seedling traits for FH-317 and FH-492. Similarly, auxins, gibberellin, and cytokinin showed non-significant effects in determining the final measures of root and shoot traits except for root length in T2 (Figure 1).



Figure 1: Seedling shoots (a, b) and root growth (c, d) of FH-492 and FH-317 respectively under seven PGR treatments along with control.

Morphological and yield contributing traits

In the analysis of variance for morphological and yield contributing traits, varieties showed considerable differences for boll retention and GOT% only. However, all the fiber and yield contributing traits exhibited significant differences among different PGR treatments (Table 3).

Table 3. Mean sum of squares for yield and fiber quality contributing traits.

Trait	PGR means sum of squares	Variety means sum of squares
Degree of freedom	7	1
Boll Retention	470.35**	1064.08**
Boll weight	0.31**	0.16217
Seed cotton yield	441721**	81185
GOT%	24.85**	11.06**
Staple length	2.34**	0.00775
Mike	0.10**	0.00935

Key: significant differences ($P < 0.05$) "**", highly significant results ($P < 0.001$) "***".

Boll retention

The varieties differ considerably for the boll retention percentage. Boll retention percentage is directly related to the ultimate seed cotton yield. The results revealed that the application of PGRs improved the boll retention significantly. Contrast analysis inference after ANOVA showed that auxin and gibberellin had significant effects on improving boll retention. Cytokinin did not show any significant effect on this trait. These results indicated that for improving the boll retention in any variety one can apply auxin and gibberellin, which will ultimately result in improving the cotton seed yield. The percentage change in boll retention owing to the PGR caused an increase in T2 (61 % and 40% in FH-492 and FH-317 respectively) compared to control, followed by T4 (FH-492=51% and FH-317=36%) as evident in Figure 2. Cytokinin has the least increase over the control as earlier revealed in contrast analysis. Thus, auxin appeared most effective in improving boll retention percentage.

Table 4. Sheffe's multiple comparison/contrast analysis for fiber quality and seed cotton yield.

Trait	Contrasts	Contrast
Boll retention	C1	40.83**
	C2	-11.08 ^{ns}
	C3	17.5*
Boll weight	C1	1.04**
	C2	-0.09 ^{ns}
	C3	0.84*
Seed Cotton Yield	C1	1130.3**
	C2	75.59 ^{ns}
	C3	725.41**
Ginning Out Turn (GOT%)	C1	7.39**
	C2	6.82**
	C3	5.92*
Staple Length	C1	1.89*
	C2	-1.10 ^{ns}
	C3	1.65**
Fiber Fineness (Mic)	C1	0.58 ^{ns}
	C2	0.29*
	C3	0.50 ^{ns}

Key: highly significant difference ($P < 0.001$) “***”, significant differences ($P < 0.05$) “**”, nonsignificant differences ($P > 0.05$) “^{ns}”.

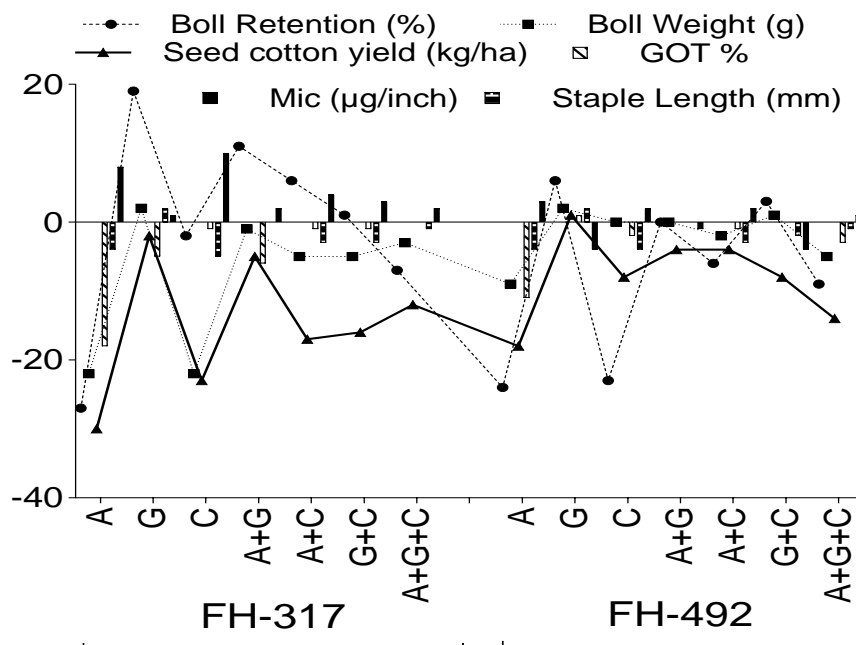


Figure 2: Percent change in all the traits due to PGR application in FH-317 and FH-492. Key: auxin “A”, Gibberellin “G”, Cytokinin “C”.

Boll weight

Varieties did not differ significantly for boll weight. Boll weight is an equally important yield contributing factor like boll retention. Auxin and gibberellin have been shown to improve the boll weight considerably compared to cytokinin, which did not affect the final weight of the boll as per contrast analysis. Applying auxin and gibberellin (20 liter @/1 ppm) after 60 days of sowing can improve boll weight of cotton crop ultimately improving overall fiber yield. Auxin improves boll weight by about 31% increase over control in FH-492 and 12% increase over control in FH-317. Combined application of auxin and gibberellin can also be effective as evident in Figure 2.

Seed cotton yield

There were no pronounced varietal effects on seed cotton yield, while auxin and gibberellin significantly affected seed cotton yield. Cytokinin has not shown any notable effects on final seed cotton yield. This may be because both these hormones had positive and significant effects on yield contributing traits i.e. boll retention and boll weight. Cytokinin did not improve any of the yield-contributing traits and ultimately did not affect the final seed cotton yield of both the cotton varieties under consideration. After plotting the percent change owing to PGR treatment it became clear that FH-492 performed best (41%) in auxin, gibberellin, and cytokinin combination (T8), while FH-317 performed best (23%) in cytokinin and gibberellin combination (T7). In general, FH-492 was more responsive to plant growth regulators than FH-317.

Analysis of variance revealed considerable differences in ginning out turn due to varietal differences and the PGR treatments. The results revealed that GOT% has increased significantly in all the PGR treatments. FH-492 came up with better GOT% compared to FH-317. However, PGR also increased the GOT% as evident in the contrast analysis. There are significant effects of auxin, gibberellin, and cytokinin on GOT% (Table 4). Furthermore, percent change over control was calculated and results indicated that T8 showed a maximum increase (21%) in GOT for FH-492 (Figure 2) and T2 had a maximum increase (14%) over control in FH-317.

Fiber quality

Fiber length had a positive influence under auxin treatment, whereas gibberellin and cytokinin did not have much effect in improving fiber length (Table 4). Percent change was also not very pronounced in both the varieties and remained at less than 10%. On the contrary, Mike had no effect of any PGR treatment under consideration. The percent decrease in mike is a sign that PGR does have some effect in making cotton fiber finer and ultimately more suitable for the textile industry (Figure 2).

DISCUSSION

Increasing temperatures and unexpected rainfall patterns under the umbrella of climate change are significantly affecting cotton crops worldwide. Behind all the crop changes there are some growth hormone level changes which are due to changes in temperature and precipitation changes. Some hormones like cytokinin and gibberellin are upregulated due to an increase in temperature like auxin and cause the plant to grow quickly while other hormones are upregulated at low temperatures. Similarly, water logging also alters the levels of hormones and enables plants to cope with adverse climatic conditions (Patel and Franklin, 2009; Zhang *et al.*, 2021).

Plant growth hormones can also be applied artificially to maintain crop productivity. For this purpose, three plant growth regulators were used to determine their effect on cotton plant seedlings and agronomic traits. The first parts of the experiment revealed that both the varieties used in the study (FH-317 and FH-492) have similar behavior to the PGR treatments for seedling traits. These findings indicate that PGR application at the seedling stage does not make any difference to the shoot and root growth habit and final expression of seedling traits. These findings are in contradiction to some previous studies where auxin seems to improve seedling growth of cotton (Sosnowski *et al.*, 2023; Zhu *et al.*, 2022). Although auxin and gibberellin have their role in initiating germination and cytokinin aids in breaking the seed dormancy (Sharma *et al.*, 2023), but they do not contribute significantly to improving the seedling vigor of already germinated seeds.

The second part of the experiment highlighted genetic differences of varieties for boll retention and ginning out turn percentage. However, the dissection of the variance for PGR treatments in contrast analysis showed that auxin and gibberellin significantly the boll retention percentage, boll weight, seed cotton yield, GOT%, and fiber length (Sawan, 2017). While cytokinin only affected micronare value and GOT% (Xiao GuangHui *et al.*, 2019). These results are in accordance with the previous studies where PGRs have been shown to improve chlorophyll, protein, and sugar contents to resist abiotic stresses like salinity heat and drought to ultimately improve seed cotton yield (Arshad *et al.*, 2023; Samad *et al.*, 2023). PGR helps in improving seed cotton yield by increasing the number of bolls due to higher boll retention and improved weight of the bolls (Gumber *et al.*, 2007; Ramesha *et al.*, 2017).

Auxin came up to play a major role in improving seed cotton yield by improving boll weight and boll retention. Exogenous auxin supplements the low indigenous auxin levels to retain bolls for a longer time and thus bolls can grow bigger meanwhile to give better fiber yield. Auxin and gibberellin also work well together for improving fiber cell development and boll retention percentage (Lee *et al.*, 2007). Auxin improves shoot and root length. It also aids in flower formation in cotton plants and play a role in fiber cell initiation and elongation (Toungos, 2018). It is also important for increasing fiber yield by improving CO₂ photosynthetic assimilation, increasing the number and weight of bolls, and improving fiber growth (Singh *et al.*, 2009). Cytokinin affects the fiber quality by slowing down the aging

process and providing more time for improving GOT and also thickens the fiber to increase its strength. It also helps in stimulating root growth initiation after encountering any stress by the plant (Liao *et al.*, 2010; Yadav *et al.*, 2022). Thus, cytokinin can be recommended for crop plants that have encountered some stress to maintain growth and yield potential by recovering from damage by producing new cells. Some previous studies came up in concluding that fiber quality parameters are not responsive to PGR treatments, while seed cotton yield decreases in response to gibberellin (Copur *et al.*, 2010). These differences in results may be due to the difference in the genetic structure of varieties used in the study. Gibberellin works in dividing the cells and stimulates the elongation of cells. An increase in the stalk length and increased flower and fruit size, staple length, and enhanced growth in cotton is its particular role (Castro-Camba *et al.*, 2022). PGR application to improve GOT and fiber traits is in contradiction to previous studies where GOT and mic did not have much influence of PGR (Gumber *et al.*, 2007). These differences are indications that the type of PGR is important in improving fiber quality. To conclude these results in relation to some previous research we can say that to get more yield and quality of cotton crop the application of exogenous PGRs is quite helpful, especially auxin and gibberellin. However, to delay the crop cytokinin can be used to get more GOT%. This somehow concludes that late-season cotton can be managed properly by using these magical PGRs to get stable yields and quality fiber (Copur *et al.*, 2010; Fang *et al.*, 2019; Hiyat *et al.*, 2020; Ramesha *et al.*, 2017).

CONCLUSION

In conclusion, while plant growth regulators (PGRs) did not significantly impact cotton plants at the seedling stage, they demonstrated substantial potential for enhancing seed cotton yield by improving boll retention, boll weight, and ginning out turn (GOT%). However, if the crop has encountered any stress at the seedling stage, the PGR sprays can be recommended according to the type of stress. Among the PGRs tested, auxin and gibberellin were particularly effective in boosting seed cotton yield and its contributing traits, with additional positive effects on fiber length and micronaire (mic), resulting in longer and finer fibers. Cytokinin, on the other hand, showed a beneficial effect only on GOT%. Therefore, for optimizing cotton growth and yield, auxin and gibberellin can be applied separately or in combination around 60 days after sowing. Cytokinin, however, is recommended specifically for enhancing GOT%. The use of auxin and gibberellin not only improves yield but also contributes to better fiber quality, thus offering a promising approach for improving both productivity and fiber characteristics in cotton cultivation.

AUTHOR CONTRIBUTIONS

Abia Younas: Writing – original draft, Writing – review & editing, Formal analysis. Iram Sharif: Writing – original draft, Writing – review & editing, Formal analysis. Ghulam Sarwar: Conceptualization, Validation, Supervision, Resources, Validation. Amna Nazir: Writing – review & editing. Jamshaid Ali Junaid: Writing – review & data analysis. Muhammad Farooq: Writing – review & editing. Jehanzeb Farooq: Validation, Writing – review & editing.

CONFLICT OF INTEREST

There is no potential conflict of interest in research and manuscript

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