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Evaluating growth stimulants for improving different morpho-physiological and seed quality parameters in American cotton

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Abstract
Cotton is a crucial fiber and oilseed crop, significantly contributing to the global textile industry and agricultural economy. Enhancing cotton yield and seed development through agronomic interventions, such as the application of growth stimulants, has gained importance in optimizing productivity under varying environmental conditions. In this experimental study, effects of different growth stimulants-spermidine, potassium nitrate (KNO_3), bavistin and a combination of KNO_3 + bavistin were evaluated on key physiological traits, yield components, and seedling quality traits of American cotton variety H 1098i. The experiment was conducted during the *Kharif* 2018 in randomized block design with three replications. Treatments were applied at 90 and 120 days after sowing and key morpho-physiological and seed quality traits were measured. Our results revealed that KNO_3 treatments significantly improved chlorophyll content and seedling dry weight, while spermidine-treated plants showed enhanced photosynthetic rate and stomatal conductance. Additionally, KNO_3 and its combination with Bavistin significantly increased the number of bolls per plant, with the highest boll production observed in KNO_3-treated plants. Yield traits such as biomass, seed weight, and lint weight were also positively affected by spermidine, with the highest seed weight. Furthermore, seedling quality traits, including seedling vigour and dry weight, were significantly improved by KNO_3 treatments, highlighting its role in enhancing early plant growth. This study underscores the potential of growth stimulants, particularly KNO_3 and spermidine, in improving physiological efficiency, seed development and yield in American cotton.

Keywords: American cotton, bavistin, chlorophyll content, photosynthesis, potassium nitrate, stimulants, yield

Introduction

Cotton is one of the most important fiber crops globally, contributing significantly to the textile industry and the livelihoods of millions of farmers (Beniwal *et al*., 2024) [4]. It is an important cash crop which is grown in arid and semi-arid areas of our country (Cetin and Basbag, 2010) [7]. Cotton is also known as "White Gold" and "King of Fibre" (Jogender *et al*., 2023a) [10]. Cotton plant is a member of family "Malvaceae" and genus "Gossypium" (Jogender *et al*., 2023b) [11]. More than 50 species belonging to the genus Gossypium. Out of which, four are cultivated due to their spinnable lint, while 44 are wild diploids and remaining are wild tetraploids (Percival and Kohel, 1990) [17]. The cultivated species of cotton are *G. hirsutum* and *G. barbadense* which are tetraploids (2n=4x=52; AADD genome); and *G. arboreum* and *G. herbaceum* are diploid (2n=2x=26; AA genome) which are also known as new world and old-world species respectively (Ulloa *et al.,* 2006) [22]. In North India, only *G. hirsutum* and *G. arboreum* are cultivated commercially (Kumar *et al.*, 2023) [12]. Although *G. hirsutum* cultivars generate superior quality fibre with greater lint yield and contributed over 95% of the current global production, yet these are vulnerable to several biotic and abiotic stresses including pest infestations, water availability, and nutrient management. Enhancing the yield and quality of cotton seeds through agronomic interventions, such as growth stimulants, has become a critical focus for researchers to optimize cotton production (Ghaffar *et al*., 2020) [8]. In this context, the American cotton variety H 1098i is widely grown in India, known for its fiber quality and adaptability, making it an ideal candidate for evaluating the effects of different growth stimulants on seed development and overall plant performance.

The use of growth stimulants, including plant growth regulators (PGRs), fungicides and fertilizers, is a well-known agronomic practice to enhance plant growth, stress tolerance and yield attributes. Growth regulators like spermidine, a polyamine involved in various physiological processes such as cell division, differentiation and stress response, have shown promise in improving photosynthetic efficiency and plant vigor (Bibi, 2008; Alobaidy, 2013, Noreen *et al.*, 2020) [1, 5, 16]. Similarly, potassium nitrate (KNO_3), a balanced source of potassium and nitrogen, plays a crucial role in enhancing plant metabolism, photosynthesis and yield (Sawan *et al.*, 2007; Sawan, 2013) [19, 20]. Additionally, Bavistin, a fungicide widely used to control fungal pathogens, may also contribute to plant health by reducing stress caused by diseases (Metwally *et al.*, 2022) [15].

Keeping in mind above facts, the present experiment was planned to evaluate different growth stimulants and their impact on various morpho-physiological and seed quality traits of the American cotton variety H 1098i. This study could provide a foundation for future research aimed at optimizing growth stimulant applications to enhance cotton yield and quality under varying environmental conditions.

Materials and Methods

The present experiment was conducted at Research Area of Cotton Section, Department of Genetics and Plant Breeding, CCS Haryana Agricultural University, Hisar, Haryana during *Kharif* 2018. American cotton variety, namely, H 1098i was used as experimental material which was sown in two rows of row length 6.0 m in Randomized Block Design with three replications. Row to row spacing was 67.5 cm and plant to plant spacing was maintained about 30 cm. The treatments included the application of spermidine, Bavistin, potassium nitrate (KNO_3), a combination of potassium nitrate and Bavistin, water spray and a control (no spray). These treatments were applied at critical growth stages, *i.e.*, 90 and 120 days after sowing (DAS) to assess their effects on key physiological traits such as photosynthetic rate, chlorophyll content, chlorophyll fluorescence, transpiration rate, stomatal conductance and yield attributes like number of bolls per plant, biomass per plant, lint weight per plant, seed weight per plant. After ginning, seeds were tested for germination in laboratory conditions by taking different observations like seed germination, seedling length, seedling dry weight, seedling vigor and root: shoot ratio.

Table 1: Different Spray Treatments used in present experiment

T_1	Spermidine (1mm)
T_2	Bavistin (0.2%)
T_3	KNO_3= 13:0:45 (4.5ml/5l)
T_4	KNO_3= 13:0:45 (4.5ml/5l)+ Bavistin (0.2%)
T_5	Water spray
T_6	Control (No spray)

Results and Discussion

Effect of different spray treatments on various physiological parameters

The table 2 presents the effects of six different treatments on physiological traits, *viz.*, chlorophyll content, chlorophyll fluorescence, photosynthetic rate, transpiration rate and stomatal conductance in the American cotton variety H 1098i.

Table 2: Effect of different spray treatments on various physiological parameters in American cotton variety H 1098i

Treatments	CC	CFL	PR	TR	SC
Spermidine (1mm)	38.9	0.761	15.2	6.09	0.250
Bavistin (0.2%)	42.8	0.772	14.5	5.51	0.210
KNO_3 = 13:0:45 (4.5ml/5l)	45.3	0.751	15.2	5.64	0.223
KNO_3 = 13:0:45 (4.5ml/5l)+ Bavistin (0.2%)	44.1	0.749	14.6	5.99	0.217
Water spray	38.2	0.772	14.1	6.13	0.209
Control (No Spray)	38.3	0.742	13.2	5.27	0.191
C.D. at 5 %	2.32	NS	NS	0.61	0.027
C.V.	5.87	6.978	9.24	8.82	10.574

CC: Chlorophyll content (SPAD Units); CFL: Chlorophyll fluorescence (Fv/Fm); PR: Photosynthetic rate (μmol m^{-2} s^{-1}); TR: Transpiration rate (mmol m^{-2} s^{-1}); SC: Stomatal conductance (mol m^{-2} s^{-1})

- **Chlorophyll Content (SPAD Units):** Chlorophyll content was significantly influenced by the treatments. The highest chlorophyll content (45.3 SPAD units) was observed in the plants treated with 13:0:45 (KNO_3), followed by the combination treatment of KNO_3 =13:0:45 + Bavistin (44.1 SPAD units). Spermidine-treated plants showed lower chlorophyll content (38.9 SPAD units), similar to the water spray and control treatments.
- **Chlorophyll Fluorescence (Fv/Fm):** Bavistin and water spray treatments had the highest CFL values (0.772), indicating optimal photochemical efficiency of photosystem II. The lowest value was observed in the control treatment (0.742). However, chlorophyll fluorescence did not show significant differences between treatments.
- **Photosynthetic Rate (μmol m^{-2} s^{-1}):** The photosynthetic rate was highest in spermidine-treated and KNO_3 =13:0:45-treated plants (15.2 μmol m^{-2} s^{-1}). The control plants had the lowest rate (13.2 μmol m^{-2} s^{-1}). Despite these numerical differences, the photosynthetic rate was not significantly affected by the treatments.
- **Transpiration Rate (mmol m^{-2} s^{-1}):** The water spray treatment resulted in the highest transpiration rate (6.13 mmol m^{-2} s^{-1}), closely followed by the spermidine treatment (6.09 mmol m^{-2} s^{-1}). The control plants exhibited the lowest transpiration rate (5.27 mmol m^{-2} s^{-1}). Result indicated that there was a significant difference in transpiration rates between treatments.
- **Stomatal Conductance (mol m^{-2} s^{-1}):** The highest stomatal conductance was observed in spermidine-treated plants (0.250 mol m^{-2} s^{-1}), followed by 13:0:45-treated plants (0.223 mol m^{-2} s^{-1}). The control treatment had the lowest stomatal conductance (0.191 mol m^{-2} s^{-1}).

Chlorophyll content, transpiration rate, and stomatal conductance showed significant variation across treatments, indicating that specific treatments, such as KNO_3 and its combination with Bavistin, improved plant physiological traits. In particular, the KNO_3 treatment led to the highest chlorophyll content, while spermidine enhanced photosynthetic rate and stomatal conductance. The lack of significant variation in CFL and photosynthetic rate across treatments suggests that these parameters were relatively stable, with only minor treatment-related differences. On the other hand, transpiration rate and stomatal conductance, both related to water-use efficiency, were significantly affected by growth stimulants, highlighting the role of these treatments in modulating plant water relations and gas exchange processes. Similar findings were observed by Aslam *et al.*, 2021 [2], Waraich *et al.*, 2011 [23], Loka *et al.*, 2013 [14] and Yousefi *et al.*, 2021 [25]. Overall, these results suggest that treatments like KNO_3 and spermidine can improve physiological traits in cotton, which may contribute to better growth and yield outcomes under field conditions.

Effect of different spray treatments on various morphological parameters

The table 3 presents the effects of six different spray treatments on various yield-related traits in the American cotton variety H 1098i.

Table 3: Effect of different spray treatments on various morphological parameters in American cotton variety H 1098i

Treatments	Biomass / plant (g)	Lint weight / plant (g)	Seed weight / plant (g)	No. of bolls/ plant
Spermidine (1 mm)	223.6	79.6	136.8	68.3
Bavistin (0.2%)	218.7	78.9	12 5.0	70.0
KNO_3= 13:0:45 (4.5 ml/5l)	211.7	77.8	124.2	79.0
KNO_3= 13:0:45 (4.5ml/5l) + Bavistin (0.2%)	219.8	79.2	122.7	68.3
Water spray	218.3	76.0	124.5	69.3
Control (No Spray)	206.8	74.6	119.6	64.3
C.D. at 5 %	NS	NS	9.68	7.38
C.V.	9.28	8.21	10.07	5.73

- **Biomass per plant (g):** The highest biomass (223.6 g) was observed in spermidine-treated plants, followed by the combination treatment of 13:0:45 + Bavistin (219.8 g). The lowest biomass was recorded in the control plants (206.8 g). Despite the variation in biomass, the results were statistically non-significant, indicating that no treatment had a significant effect on biomass.
- **Lint weight per plant (g):** Lint weight was highest in spermidine-treated plants (79.6 g) and lowest in the control plants (74.6 g). However, the variation in lint weight among the treatments was not significant.
- **Seed weight per plant (g):** Spermidine-treated plants had the highest seed weight (136.8 g), significantly higher than the control treatment (119.6 g). Bavistin-treated plants had the second-highest seed weight (125.0 g), while 13:0:45 alone and its combination with Bavistin showed intermediate seed weights. Results indicated that there was a significant difference in seed weight across the treatments.
- **Number of bolls per plant:** The number of bolls per plant was highest in the 13:0:45-treated plants (79.0), followed by Bavistin-treated plants (70.0). The lowest number of bolls was recorded in control plants (64.3). The variation in the number of bolls was significant showing that the treatments significantly influenced boll production.

Spermidine treatment led to the highest biomass and seed weight, suggesting that it enhances overall plant growth and seed development. While the difference in lint weight across treatments was not statistically significant, spermidine-treated plants also had the highest lint weight, indicating its positive but not substantial influence on lint production. Potassium nitrate (13:0:45) treatments, particularly when combined with Bavistin, showed significant improvements in the number of bolls per plant, with the KNO_3= 13:0:45 treatment alone resulting in the highest number of bolls. This suggests that potassium nitrate can positively impact fruiting, potentially contributing to higher cotton yields. The control plants consistently showed the lowest values across most traits, underscoring the beneficial effects of growth stimulants. The significant differences in seed weight and the number of bolls indicate that certain treatments, especially spermidine and KNO_3, play a critical role in improving seed yield and boll production, both of which are key indicators of cotton crop performance. Our results coincide with results of Basavanneppa *et al.*, 2015 [3], Kumar *et al.*, 2011 [13], Pothiraj *et al.*, 1995 [18], Sharma and Singh, 2007 [21], Weir, 1999 [24], Waraich *et al.*, 2011 [23], Brar and Brar, 2004 [6] and Yousefi *et al.*, 2021 [25].

Effect of different spray treatments on various seedling quality traits

The table 4 provides data on seedling quality traits in harvested seeds collected from different spray treated American cotton variety H 1098i.

Table 4: Effect of different spray treatments on various seedling quality traits in American cotton variety H 1098i during germination test under lab conditions

Treatments	Germination (%)	Seedling Length (cm)	Seedling dry weight (g)	Root: Shoot ratio	Seedling Vigour
Spermidine (1mm)	46.8	31.7	0.386	0.236	1.8
Bavistin (0.2%)	46.5	31.4	0.434	0.220	2.0
KNO_3= 13:0:45 (4.5ml/5l)	48.0	32.0	0.496	0.223	2.4
KNO_3= 13:0:45 (4.5ml/5l)+ Bavistin (0.2%)	47.8	29.7	0.449	0.214	2.2
Water spray	46.9	29.1	0.384	0.252	1.8
Control (No Spray)	43.3	29.5	0.334	0.215	1.5
C.D. at 5 % level of significance	NS	NS	0.063	NS	NS
C.V.	9.38	9.29	8.435	13.29	15.54

- **Germination (%):** The highest germination percentage (48.0%) was observed in the 13:0:45 (KNO_3)-treated plants, followed by 13:0:45 + Bavistin (47.8%). The lowest germination was in the control plants (43.3%). However, germination differences were statistically non-significant, indicating that the treatments did not have a significant impact on seed germination.

- **Seedling length (cm):** The longest seedlings were recorded in the KNO_3=13:0:45 treatment (32.0 cm), with a close second being spermidine-treated plants (31.7 cm). Control plants had shorter seedlings (29.5 cm). Seedling length was also not significantly affected by the treatments.
- **Seedling dry weight (g):** The highest seedling dry weight was observed in the 13:0:45-treated plants (0.496 g), followed by the combination treatment of KNO_3= 13:0:45 + Bavistin (0.449 g). The control treatment had the lowest seedling dry weight (0.334 g). There was a significant difference in seedling dry weight between treatments. This suggests that KNO_3, especially in its pure form, significantly increases seedling biomass.
- **Root: shoot ratio:** The water spray treatment had the highest root: shoot ratio (0.252), while spermidine-treated plants had a lower ratio (0.236). The control plants showed an intermediate value (0.215). Despite these differences, the root: shoot ratio did not show significant variation across treatments.
- **Seedling vigour:** The highest seedling vigour (2.4) was recorded in the 13:0:45-treated plants, indicating better overall seedling quality and robustness. The control plants had the lowest seedling vigour (1.5). Similar to germination and seedling length, seedling vigour differences were not statistically significant across treatments.
- While germination percentage, seedling length, root: shoot ratio, and seedling vigour did not show significant differences across treatments, seedling dry weight was significantly influenced, with the highest dry weights observed in plants treated with KNO_3. This suggests that KNO_3 enhances seedling biomass, which is a crucial factor for early plant establishment and growth. The KNO_3= 13:0:45 treatment emerged as the most beneficial across several traits, including germination percentage, seedling length, seedling dry weight, and seedling vigour, suggesting that potassium nitrate positively influences early seedling development. The combination of KNO_3= 13:0:45 with Bavistin also produced good results but was slightly inferior to 13:0:45 alone in terms of seedling vigour and dry weight. Control plants consistently showed lower values across all parameters, reinforcing the importance of growth stimulant treatments in enhancing seedling traits. The non-significant differences for many parameters suggest that while treatments may have visible effects, these differences were not strong enough to be statistically conclusive for germination, length, or vigour. Our results were similar as reported by Ghurde *et al.*, 2021 [9], Kumar *et al.*, 2011 [13] and Sharma and Singh, 2007 [21]. Overall, the results suggest that potassium nitrate (13:0:45), either alone or in combination with Bavistin, can improve seedling quality and vigour, making it a valuable treatment for improving early growth in cotton crops.

Conclusion

The present study on the American cotton variety H 1098i revealed that growth stimulants such as spermidine, potassium nitrate (KNO_3), and Bavistin have impact on enhancing key physiological traits, yield components, and seed development. The treatments led to improvements in chlorophyll content, photosynthetic rate, and seed weight, with potassium nitrate showing a notable increase in the number of bolls and seedling vigour, while spermidine enhanced biomass and photosynthetic efficiency. The combination treatment of KNO_3 and Bavistin also showed promising results, particularly in germination percentage and boll number. The use of KNO_3, either alone or in combination with Bavistin, was particularly effective in promoting seedling vigour and enhancing seed development. This study highlights the potential of growth stimulants to boost cotton yield, offering a promising agronomic intervention for enhancing crop performance under both optimal and stressed conditions. In conclusion, this study of plant hormones and their effects on seed germination in cotton offers a promising avenue for enhancing agricultural productivity and sustainability. By delving into the intricate hormonal regulation of germination, this research aims to provide practical solutions for improving cotton crop yields and resilience. The knowledge gained from this study has the potential to benefit farmers, the agricultural industry, and society at large by contributing to food security, economic growth, and environmental conservation.

Future prospects

Further studies can refine the concentration and application timing of growth stimulants like spermidine and potassium nitrate to maximize their benefits in improving cotton yield and quality. Investigating the molecular mechanisms by which these stimulants influence cotton growth and stress tolerance could lead to the development of cotton varieties with enhanced responsiveness to growth regulators. Combining growth stimulant treatments with other agronomic practices such as drip irrigation, nutrient management, and pest control could provide a holistic approach to improving cotton production sustainably. By exploring these future directions, the potential of growth stimulants in optimizing cotton production and resilience to stresses can be further unlocked, contributing to sustainable cotton cultivation globally.

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