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Enhancement of seed quality by bio-priming of cotton genotypes collected from different parts of western undulating zones of Odisha

KUMAR AVINASH BISWAL[1], NIRAKAR RANASINGH[1], RAJEEV LOCHAN MOHARANA[2] AND SIDDHARTHA DAS[3]

[1]Department of Plant Pathology, College of Agriculture, Odisha University of Agriculture and Technology, Bhubaneswar-751003, Odisha

[2]Department of Seed Science & Technology, Odisha University of Agriculture and Technology, Bhawanipatna- 766001, Odisha

[3]Department of Plant Pathology, Centurion University of Technology and Management, Paralakhemundi- 761211, Odisha

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Contemporary agriculture relies on advanced technologies to enhance crop productivity.Seed bio-priming with plant extracts and microbial agents, not only combats biotic and abiotic stresses effectively but also enhances over all plant growth and development. This integration signifies a novel approach in modern agricultural practices. The incorporation of biological control organisms into seeds may aid in pathogenic control and seedling performance. The purpose of this study was to determine the effects of biopriming cotton seeds with *Trichoderma viride, Pseudomonas fluorescens*, Biomix (*T. viride +P. fluorescens+ Acetobacter*), *T. harzianum, T. asperellun, Bacillussubtilis* and Control. Seed biopriming treatment with T3- Biomix*Trichoderma viride+ P.fluorescens + Acetobacte)* was recorded highest germination (65.0%) followed by T5(63%) -*T.asperellum*, T2(61%)- *Pseudomonas fluorescens* (SPARSHA -1.0%WP), T6(59%)- *Bacillus subtilis* and T1(58%/)- *T.viride* (Niprot-0.50%WP). Enhancement of germination by various biopriming agents ranged from 52-65%.

Keywords : Biopriming, biological control, cotton, germination

INTRODUCTION

Cotton (*Gossypium hirsutum L.*), belonging to family Malvaceae is one of the most important fiber and cash crop of India it is cultivated in an area of 131lakh hectares with production of 343.7lakh bales. In Odisha it is predominantly grown in Koraput, Balangir,Rayagada and Kalahandi districts for the availability of suitable soil and climate conditions.

As per reports in 2019, cotton is grown in an area of 169.56 thousand hectare with production of 578.50 thousand bale having productivity 580.0kg /ha (5 Decades of Odisha Agriculture Statistics., 2020. P-68).

*Correspondence: siddhartha.das10@gmail.com

The importance of cotton seed microflora in improving seed quality through bio-priming is crucial. This microflora, consisting of bacteria, fungi, and other microorganisms, supports seed germination, seedling growth, and plant health. Bio-priming, which involves soaking seeds in a suspension of beneficial microorganisms, enhances seed vigor, stress tolerance, and crop productivity. It promotes nutrient uptake, hormone production, and plant defense, leading to faster and more uniform germination, stronger seedlings, and better resistance to stresses. Additionally, bio-priming improves nutrient efficiency and reduces the need for chemical fertilizers and pesticides, aiding sustainable agriculture. Selecting the right microorganisms is essential, as different strains affect seed quality and plant performance differently. Therefore, careful screening and optimization are needed to maximize bio-priming benefits in cotton farming.Seed biopriming controls seed hydration to initiate pre-germinative metabolic activity without radical emergence (Marthandan *et al*. 2022). There are two priming systems available. Osmopriming generates osmotic potential in the primary solution by using aerated aqueous salt or polyethylene glycol (PEG) solutions (Lei Chu *et al.*2021). The use of moist, porous solid materials, such as powdered coal or peat, to generate matric potential is known as solid matrix priming (SMP) (Ling-Yun *et al*. 2022). For the control of seedling diseases, combining (SMP) with *Trichoderma spp*. has been used successfully on a variety of plants (Tyœkiewicz*et al*.2022). Seed treatment using bio-control agents and priming agents, a procedure known as “bio priming,” may be an effective way to manage a variety of soil and seed borne diseases. These priming agents, in combination with bio agents, can protect seeds from biotic and abiotic challenges, stresses such as moisture stress, and hence can protect seeds and seedlings for longer periods of time (Fiodor *et al*. 2023). Biological seed treatments for seed and seeding disease management is an alternative to chemical fungicides for the producer. When used as seed dressers, bio-control agents such as *T. harzianum* and *P. fluorescens* can colonise the rhizosphere and provide benefits to the plant beyond the seedling emergence stage (Ketta*et al*.2021).

MATERIALS AND METHODS

Selection of cotton genotypes

The selection of cotton genotypes is a crucial step in cotton biopriming investigation aimed at promoting seed vigor, enhance yields and safeguard from other protections. Breeders carefully evaluate various characteristics such as yield potential, fiber quality, disease resistance, and adaptability to specific agro-climatic conditions when selecting genotypes for further breeding efforts. Genetic diversity within cotton germplasm pools is essential to ensure the development of resilient and high-performing varieties capable of withstanding biotic and abiotic stresses. Molecular markers and genomic selection are increasingly utilized to expedite the identification and validation of superior genotypes with targeted traits. Tough under this investigation we are trying to enhance individual traits or characteristics for better seed health.Seven cotton genotypes were selected based on the greater performance and depicted in Table 1.

Statistical Analysis

For proper interpretation, the data obtained under investigation was subjected to statistical analysis. (Chaudhari*et al*., 2018) established the standard method of analysis of variance methodology relevant to the Complete Randomized Design (CRD).

Design of experiment: Completely Randomized Design (CRD)

No. of replications	:	Three
Treatments	:	Seven

Biopriming method

In a specimen tube, fresh cotton seeds were collected. Seeds were immersed in solutions of given treatmentsviz: *Pseudomonas fluorescens,* Biomix (*Trichoderma viride+ P.fluorescens + Acetobacter), T.harzianum, T.asperellum, Bacillus subtilis* for 6 hours at room temperature. After 6 hours of priming, the soaked seeds were collected and washed three times with distilled water before being dried in the shade to restore their initial moisture content. The moisture level of new seeds was evaluated before commencing the priming procedure. Seven biopriming treatments were considered under this investigation and represented through (Fig. 1 &Table 2).

RESULTS AND DISCUSSION

Germination percentage

Significant difference for germination was observed by different biopriming treatments. Seed biopriming treatment with T3 was recorded highest germination (65.0%) followed by T5, T2, T6 and T1. Enhancement of germination by various biopriming agents ranged from 52-65%. T7 recorded the lowest germination followed by T4 and T1. Enhancement of germination by bio-priming treatments by T3 increased 25.0%

percent over the control followed by T5 (21.15%), T2 (17.31%), T6-13.46%, T1 (11.54%) and T4 (5.77%). Results depicted through Fig. 2 & Table 3**.**

The bio-priming treatments by Biomix resulted highest germination percentage (65.0%) which is at per the minimum requirement for germination percentage specified for cotton crops but the other bio-priming treatments on germination was recorded higher but could not meet the IMSCS (Indian Minimum seed certification standards).

Table1 : Cotton genotypes used in the experiments

Varieties	Name of the genotypes
V1	Br .06 a (N) 410
V2	Br. 06 a (N) 409
V3	4a (Z) 2034
V4	4a (Z) 2035
V5	CZ 6 a (Z) 2051
V6	Br. 06 a (N) 411
V7	4a (Z) 2032

Table 2. Details of seed biopriming

Treatment	Priming agent	Priming agent with doses
T_1	*T.viride* (NIPROT 0.50% WP)	Hydration with *Trichoderma viride* 10 gram/ Kg seed for 6hours
T_2	T2-*Pseudomonas fluorescens* (SPARASHA 1.0% WP)	Hydration with *Pseudomonas* 10 gram/ Kg seed for 6hours
T_3	Biomix *(Trichoderma viride+ P.fluorescens + Acetobacte)*	Hydration with Biomix 10 gram/ Kg seed for 6hours
T_4	*T.harzianum*	Hydration with *Trichoderma harzianum* 10 gram/ Kg seed for 6hours
T_5	*T.asperellum*	Hydration with *Trichoderma asperellum* 10 gram/ Kg seed for 6hours
T_6	*Bacillus subtilis*	Hydration with *Bacillus subtilis* 10 gram/ Kg seed for 6hours
T_7	Control	Hydration with *water for 6hr*

Root and Shoot length

Significant difference for root length and shoot length was observed by different biopriming treatments. Seed biopriming treatment with T3 was recorded highest root & shoot length (8.35 &10.12) respectively followed by followed by T5, T2, T6 and T1. Enhancement of root & shoot length by various biopriming agents ranged from 7.92-8.35 & 9.64-10.12 cm respectively. T7 recorded the lowest germination followed by T4 and T1. Enhancement of germination by bio-priming treatments by T3 increased 5.2% over the control followed by T5 (3.5%), T2 (3.0%), T6-2.3%, T1 (1.8%) and T4 (0.6%). Longest root and shoot was recorded for the biopriming treatments by Biomix –T3 and T5- *T.asperellum*. Results depicted through Fig. 3 & Table 3.

SVI-I: Significant difference for germination was observed by different biopriming treatments. Seed biopriming treatment with T3 was recorded highest Seed vigour index-I (1255) followed by T5 (1144.71), T2 (1102.88), T6
(1060.23) and T1 (1036.46.T7) recorded the lowest SVI-I (913.12) followed by T4 (971.85) and T1 (1036.46). Highest SVI-I recorded for the biopriming treatments is Biomix –T3 followed by T5- *T. asperellum*. The similar trend in position of germination and root and shoot length is the reflection in seed vigour index (SVI-I) as expressed by germination % x (Root length & shoot length (cm). Enhancement of SVI-I by various biopriming agents for different genotypes of cotton ranged from 913.12- 1200.55 (Table 3).

Table 3: Enhancement of seed quality of apparent low germination cotton seed by bio-priming

Treatment No.	Treatments	Germination (%)	Germination % Increase	Root length (cm)	Shoot Length (cm)	Total plant height (Root + shoot length) in (cm)	% Increase seedling length	SVI-I	SVI-II	Seedling Dry weight (g)
T1	*T.viride (Niprot- 0.50%WP)*	58	11.54	8.12	9.75	17.87	1.8	1036.46	4.00	0.069
T2	*Pseudomonas fluorescens* (SPARSHA - 1.0%WP)	61	17.31	8.26	9.82	18.08	3.0	1102.88	4.82	0.079
T3	Biomix	65	25.00	8.35	10.12	18.47	5.2	1200.55	5.53	0.085
T4	*T.harzianum*	55	5.77	7.96	9.71	17.67	0.6	971.85	3.74	0.068
T5	*T.asperellum*	63	21.15	8.32	9.85	18.17	3.5	1144.71	5.17	0.082
T6	*Bacillus subtilis*	59	13.46	8.17	9.80	17.97	2.3	1060.23	4.31	0.073
T7	Control	52	-	7.92	9.64	17.56	-	913.12	3.22	0.062
	SE(m)±	0.333		0.038	0.024	-	-	-	-	0.001
	CD (P=0.05)	1.021		0.118	0.075	-	-	-	-	0.003
	CV (%)	0.976		0.809	0.427	-	-	-	-	2.074

SVI-II

Significant difference for germination was observed by different biopriming treatments. Seed biopriming treatment with T3 was recorded highest Seedling vigour index-I (5.525) followed by T5 (5.166), T2 (4.819), T6 (4.307) and T1 (4.002). T7 recorded the lowest SVI-II (3.224) followed by T4 (3.74) and T1 (4.002). The similar trend in position of SVI-II was the reflection of interaction between germination percent x (dry weight of seedling) in centimeter. Enhancement of SVI-II by various biopriming agents for different genotypes of cotton ranged from 3.224 – 5.525 (Table 3).

Dry weight of the seedling

Significant difference for dry weight was observed by different biopriming treatments. Seed biopriming by treatment T3 (Biomix) was recorded highest seedling dry (0.085) which is statistically similar to T5 (0.082) followed by treatment T2 (0.079), T6 (0.073) and T1 (0.069). T7 recorded the lowest seedling dry weight (0.062) followed by T4 (0.068) and T1 (0.085). Enhancement of dry weight by various biopriming agents for different genotypes of cotton ranged from 0.062 – 0.085. Value of seedling dry weight depends on length of both root and shoot (Table 3).

Mechanistic approach of Biopriming

The mechanistic approach of biopriming represents a sophisticated seed treatment technique that harnesses the intricate interplay between seeds and beneficial microorganisms to enhance seed quality, germination, and subsequent plant growth. One of the key mechanisms underlying biopriming is the activation of stress-responsive genes in seeds in response to microbial inoculation. Upon exposure to beneficial microorganisms, seeds perceive the presence of potential stressors and initiate a cascade of signal transduction pathways that culminate in the activation of stress-responsive genes. These genes encode various proteins, enzymes, and regulatory factors involved in mitigating stress-induced damage and promoting seedling vigor. For instance, genes

Fig. 1 : Preparation of bioprimed seeds

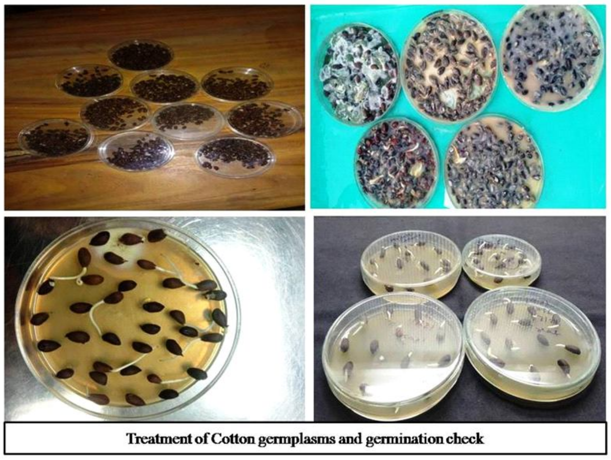

Fig. 2 : Treatment and germination check

Fig. 3 : Germination efficiency, root-shoot length other character analysis

encoding antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT), and peroxidase (POD) are upregulated in response to biopriming, leading to increased scavenging of reactive oxygen species (ROS) and protection against oxidative stress (Fig. 4).Bio priming induces changes in the expression of genes related to hormone metabolism and

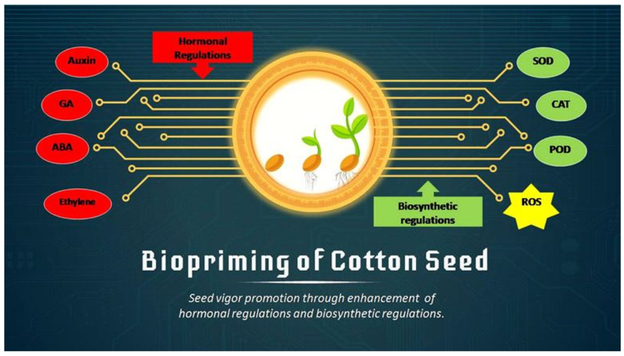

Fig. 4 : Mechanistic approach of Biopriming

signaling pathways, which play pivotal roles in regulating seed germination and early seedling growth. Beneficial microorganisms can modulate the levels of phytohormones such as abscisic acid (ABA), gibberellins (GA), cytokinins (CK), and auxins (IAA) within seeds, leading to alterations in hormone balance and signaling networks. These hormonal changes orchestrate physiological processes such as cell elongation, cell division, and nutrient mobilization, thereby promoting rapid and uniform germination and seedling emergence.

Moreover, bio priming can elicit systemic acquired resistance (SAR) and induced systemic resistance (ISR) mechanisms in plants, whereby the activation of defense-related genes confers enhanced protection against pathogen attacks. Beneficial microorganisms trigger the expression of genes encoding pathogenesis-related (PR) proteins, antimicrobial peptides, and secondary metabolites involved in plant defense responses. These defense-related genes prime plants to mount a rapid and robust defense response upon subsequent exposure to pathogenic microbes, thereby reducing disease incidence and severity.

Beneficial microorganisms stimulate the production of phenolic compounds, flavonoids, and terpenoids in seeds, which possess antioxidant properties and play roles in scavenging reactive oxygen species (ROS) generated during germination. Moreover, bioprimed seeds exhibit increased accumulation of osmolytes such as proline, glycine betaine, and sugars, which act as osmoprotectants and facilitate osmotic adjustment under stress conditions.

Seed biopriming treatment with T3- Biomix was recorded highest germination (65.0%) followed by T5 -*T.asperellum*, T2- *Pseudomonas fluorescens* (SPARSHA - 1.0%WP), T6- *Bacillus subtilis* and T1- *T.viride* (Niprot-0.50%WP). Enhancement of germination by various biopriming agents ranged from 52-65%. T7 - Control recorded 62 the lowest germination followed by T4- *T.harzianum* and T1- *T.viride* (Niprot0.50%WP). Enhancement of germination by bio-priming treatments by T3 increased 25.0% percent over the control followed by T5 - *T.asperellum* (21.15%), T2- *Pseudomonas fluorescens* (SPARSHA-1.0%WP) - 17.31%), T6- *Bacillus subtilis* - 13.46%, T1- *T.viride* (Niprot-0.50%WP)- 11.54%) and T4 (5.77%). The bio-priming treatments by Biomix resulted highest germination percentage (65%), highest Root and Shoot length (8.35 &10.12) highest seedling dry (0.085) which is at per the minimum requirement for germination percentage specified for cotton crops but the other bio-priming treatments on germination was recorded higher but could not meet the IMSCS (Indian Minimum seed certification standards). This is because of the combination effect of the Biomix which is specially designed by the combination mixture of *Trichoderma viride, Pseudomonas fluorescence* and *Acetobacter*. Their combination effect controls both fungi micoflora as well as bacterial microbes while soaking with Biomix for 6 hours. These findings were in accordance with the investigation made by Shyama*et al*(2021) who reported that Brassinosteroids (BRs) are plant steroid hormones that enhance plant growth and development as well as tolerance to a variety of environmental conditions. Cotton seeds were examined for germination and early seedling growth after being primed with BR (24-epibrassinolide) alone or in conjunction with other hormones (abscisic acid, auxin, and gibberellic acid).

Similarly Highest SVI-I & SVI-II also recorded by biopriming with Biomix – T3 followed by T5- *T.asperellum*. Enhancement of SVI-I by various biopriming agents for different genotypes of cotton ranged from 913.12- 1200.55. This is due to co-dominance by various biocontol agents like *Pesudomonas fluorescens, Bacillus subtilis, Trichoderma asperellum* and *Trichoderma viride*. These reports were also in the line of agreements with our present findings and also supported by other workers (Raghavendra *et al.*2013; Ananthand Rao, 2014) who evaluated under greenhouse and field conditions the potential of antagonistic rhizobacteria i.e *P.fluorescens* and *Bacillus subtilis* for the management of bacterial blight of cotton caused by *Xanthomonas axonopodis pv. malvacearum* (Xam) which is one of the seed borne bacterium found in our findings. These reports were also in the line of agreements with our present findings. Effect of various *Trichoderma* formulations was found in enhancing the germination as well as 63 seedling vigour of cotton seeds by biopriming. These findings were in accordance with our present investigation. These reports were also in the line of agreements with the findings of (Swain *et al*. 2021) who state that *T. harzianum* of MARS, Dharwad demonstrated 74.1 per cent inhibition of major pathogens of cotton followed by *T. koninigii* of TNAU, Coimbatore (72.55%) and *T. harzianum* of Sriganganagar (71.88%).

From this investigation it was concluded that Seed biopriming treatment with T3- Biomix was recorded highest germination (65.0%) followed by T5 -*T.asperellum*, T2- *Pseudomonas fluorescens* (SPARSHA - 1.0%WP), T6- *Bacillus subtilis* and T1- *T.viride* (Niprot-0.50%WP). Enhancement of germination and Root & shoot length of apparent low germination of cotton seed by various biopriming agents resulted from 52-65% and 5.2% respectively. This resulted the minimum seed certification standards specified for the cotton seed.Overall, hormonal and biosynthetic pathway regulations serve as key drivers of seed biopriming, mediating the physiological and biochemical changes that enhance seed quality, germination, and early seedling growth. By modulating hormone metabolism, signaling pathways, and biosynthetic processes, beneficial microorganisms confer stress tolerance, promote nutrient uptake, and activate defense mechanisms within seeds, thereby priming them for optimal performance under diverse environmental conditions. Understanding the molecular mechanisms underlying seed biopriming holds great promise for developing tailored strategies to enhance crop productivity, resilience, and sustainability in agriculture.

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DECLARATIONS

Conflict of Interest. Authors declare no conflict of interest.

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