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Effect of Biological Priming on Metabolomic and Molecular Changes in Response to Drought Stress in *Brassica juncea*

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Indian mustard, Brassica juncea is a leading oilseed crop in India and plays a crucial role in the agricultural as well as oilseed home marketing system of the country. High oil and phytosterols, glucosinolate content make it a more valuable crop, and it is used for edible oil production, poultry feed. There, however, is always one major problem during the growth of trees and their productivity; drought. This paper discusses the use of bio-priming to compound drought resistance in Indian mustard. Bio-priming, which entails the use of various microorganisms in seed treatment is therefore modern system of farming. In the process of germination and plant growth it enhances the seed germination rate, seedling vigor and overall plant health through various metabolomic and

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molecular mechanisms. Researchers have demonstrated that bio-priming containing elements such as Pseudomonas fluorescens, Bacillus subtilis, and Trichoderma harzianum faciliates the uptake of sulfur, growth and improvement of tolerance to abiotic stresses. Furthermore, by impacting on expression of stress associated genes, bio-priming enhances the activity of the WRKY transcription factor as well as the production of abscisic acid (ABA) and jasmonic acid (JA), leading to drought tolerance. These first results based on drought tolerance genes help to priorities future breeding programs dedicated to the improvement of drought resistant cultivars. Bio-priming also stimulated the antioxidant defense pathway and improved the ability of plants to cope with oxidation pressure arising from drought stress. Overall, bio-priming is a relatively cost-effective, environmentally considerate method for enhancing drought tolerance in Indian mustard, thus being beneficial for sustainable farming and food production.

Keywords: Abiotic stress; bio-priming; Brassica juncea; drought; metabolomics.

1. INTRODUCTION

Indian mustard, *Brassica juncea*, a member of the Brassicaceae family, is an important oilseed crop in India and plays a vital part in the country's agriculture. With an oil content ranging from 38% to 50% [1], it is highly valued and accounts for about 80% of the nation's total rapeseed-mustard production [2]. This important crop during the Rabi season is produced through interspecific hybridization and has a high oil content (37–42%) [1]. India is a major producer of rapeseed-mustard, which is the third most important oilseed crop in the world and makes a considerable contribution to the oilseed economy of the nation [3]. Mustard is well known for being high in bioactive substances that have anti-inflammatory and antioxidant qualities. It also contains phytosterols, phenolic compounds, glucosinolates, and anti-carcinogenic substances [4]. India, which lies in third place behind China and Canada in the world's mustard production scenario, is an important participant. Over 6.23 million hectares of mustard are cultivated in India, producing 9.34 million tons at a productivity of 1499 kg/ha [5]. With 5.96 million hectares under cultivation and 8.32 million tonnes produced annually at a yield of 1397 kg/ha, the nation provides 28.3% of the world's rapeseed production [6]. In terms of the economy of oilseeds in India, mustard is grown in states such as Rajasthan, Uttar Pradesh, Madhya Pradesh, Punjab, Haryana, West Bengal, Assam, and Bihar [7]. It ranks second behind groundnut. Rajasthan is a prominent mustard-producing state that contributes significantly to India's overall mustard production, with Madhya Pradesh and Haryana following closely after [6]. Furthermore, while mustard is produced in other states in India, including Gujarat, Andhra Pradesh, Maharashtra, Karnataka, and Uttar Pradesh, Madhya Pradesh makes up a substantial portion of the country's GDP [8]. Drought is one of the major obstacles to Indian mustard growth and development, affecting yield and productivity. India's agriculture is highly dependent on its climate, which leaves it open to water scarcity and decreased crop growth as a result of weak monsoons [9]. Drought-proofing techniques are therefore required because of its reduced ability to grow and produce seed in drought conditions [10]. The significance of drought tolerance indices in the selection of high-yielding and drought-tolerant cultivars, such Pusa Jaikisan, which demonstrated resilience to drought stress while sustaining high yields, has been highlighted by research on a variety of mustard genotypes [11]. In rainfed regions that are vulnerable to drought, increasing mustard's tolerance to water stress through plant breeding and agronomic techniques is essential for long-term productivity [12]. Seed priming is a new generation technique that helps to resist the plants from abiotic stresses like drought.priming has various approaches, among which there is a method where we use living micro-organisms such as bacteria, fungi, called as bio-priming [13]. Biological priming improves a plant's resistance to a range of stresses by inducing particular metabolomic reactions and molecular pathways [14].

2. BIOLOGICAL PRIMING

Priming is a conventional technique that is typically employed for solid crop stands and synchronized seedling growth, but in recent years, it has become a powerful tool for sustainable agriculture [15]. There are different priming methods that effect the gemination pattern of mustard such as hydro-priming, osmo-priming, hormonal priming and bio-priming [16]. Bio-priming, a seed treatment approach which combines a physiological seed hydration and biological seed inoculation with a beneficial organism in order to protect seed component of disease management is a novel seed treatment method [17]. By improving the adherence of bacteria to seeds, seed priming with living inoculums enhances rhizosphere colonization and increases plant resistance to unfavourable environmental conditions [18]. Biopriming, a practice where seeds are treated with biocontrol agents together with priming agents, has been used to control a number of soil- and seedborne diseases [17]. Chemical fungicides are not the only option for growers to control seed and seedling diseases; biological seed treatments offer an alternative option for this and hence eco-friendly and sustainable. Various bioinoculants such as *Pseudomonas fluorescens* and *Bacillus subtilis* are used as biopriming agents to increase the sulphur Use Efficiency in *Brassica juncea* [19]. Other than these bioagent *Trichoderma asperellum* Th-14 is also use for promoting the plant growth in Indian mustard [20]. Mycorrhizal fungus-assisted biopriming of mustard plants has demonstrated encouraging outcomes in terms of improving plant growth and disease resistance. Studies have shown that the use of mustard oil cake, *Pseudomonas fluorescens*, and mycorrhizal fungi like *Glomus mosseae* and *Scutellospora erythropa* can successfully treat root-rot disorders brought on by pathogens like *Macrophomina phaseolina* [21]. To increase the rate and uniformity of seed emergence and reduce numerous seedborne diseases, seed priming has been employed either alone or in conjunction with suitable fungicides and/or biocontrol agent [22]. It has been demonstrated that biological priming, which involves soaking seeds in different substances such as gibberellic acid (GA), salicylic acid (SA), and abscisic acid (ABA), greatly increases the ability of plants such as rapeseed to withstand drought stress [23]. The processes involved in bio priming in plants help to improve seed germination, seedling vigour, and overall plant growth. When plant growth-promoting microorganisms (PGPMs) are used in the biopriming process, various mechanisms are triggered [24]. These include the production of siderophores, the solubilization or mobilisation of soil nutrients, the induction of plant growth-promoting activities, and the synthesis of defense-related enzymes, phytoalexins, and beneficial biochemicals [25]. Additionally, by promoting the synthesis of growth regulators, boosting nutrient uptake, and shielding plants from pathogens, bio priming with PGPMs like PGPR can result in increased seed quality, germination speed, vigour index, growth promotion, and disease resistance [26].

3. PROCEDURE FOR SEED BIOPRIMING

List of bio-priming agents used in mustard:

Table 1. Bio-priming agents used in mustard

SL.NO.	Bio-Priming Agents	Uses/Benefits	References
1.	*Bacillus subtilis* and *Pseudomonas fluorescens*	It enhancing sulphur use efficiency and promoting sustainable production	[19]
2.	*Bacillus amyloliquefaciens* D5 ARV	Helps in improving germination rates when combined with a static magnetic field treatment to revive white mustard seed	[27]
3.	*Trichoderma harzianum* and *Pseudomonas fluorescens*	Helps in managing pests and diseases in mustard crops and contributes increased yield and plant health	[28]
4.	*Rhizobium*	Rhizobium with vermiwash, neem leaf extract, tulsi leaf extract used. Rhizobium (10%) showed maximum growth and yield in yellow mustard	[29]
5.	*Coniothyrium minitans, Aspergilus nidulans, Trichoderma harzianum, Pseudomonas chlororaphis*	It is effective in reducing carpogenic germination in Indian mustard	[30]
6.	NSKE, Metarhizium anisopliae 2x109 CFU and Beauveria bassiana 2x109 CFU	Used as insecticide and protect against aphids in mustard	[31]

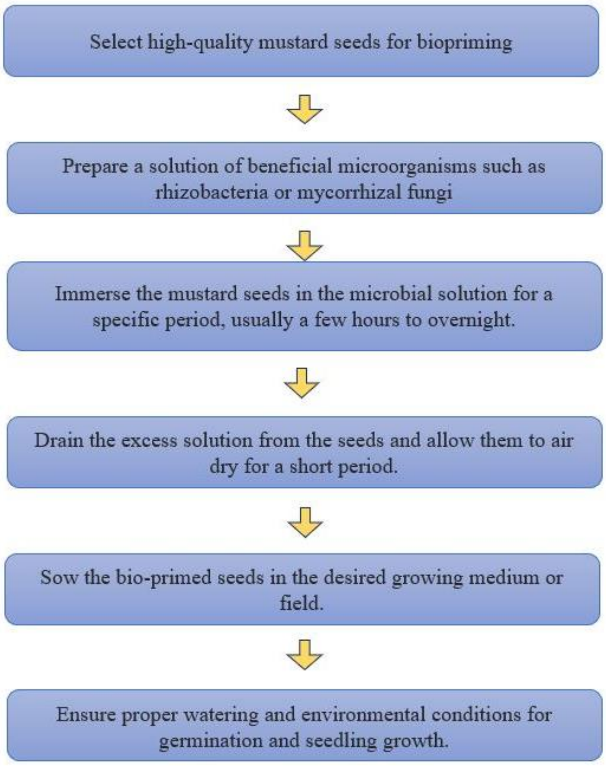

Fig. 1. Process of Biopriming of mustard seed

4. PHASES OF PRIMING AND EFFECTS

It is crucial for priming to involve a series of environmental stimuli [32]. The process is initiated with a triggering stimulus and goes up to the time when the plant is exposed to a challenging stress called priming phase [33]. During this phase, the plant is kept in a state of readiness for attack because the levels of main and secondary metabolites, phytohormones, Salicylic Acid (SA), and Jasmonic Acid (JA) fluctuate slightly [34]. Stress-hardened plants move to the second level of stress-hardening known as post-challenge primed state through a second stress which triggers defence response mechanisms promptly [35]. This includes de novo production of antimicrobial chemicals. A primed plant can revert to naïve state, however, if it inherits primed status from the parent plants, then plants can be trans generationally primed [36].

5. METABOLOMIC AND MOLECULAR CHANGES DUE TO BIO-PRIMING IN MUSTARD

Priming can be caused by natural or artificial substances such certain agrochemicals, or by interactions between the host plants and helpful microbes such as rhizobacteria, mycorrhizal fungi, diseases, or virulent/avirulent microorganisms and plants that have these kinds of interactions experience long-lasting cellular and organismal reprogramming, and they molecularly "remember" these interactions [36]. Bio-priming in Indian mustard have metabolomic changes as it involves seed priming, research reveals that the use of bioinoculants such as *Bacillus subtilis* and *Pseudomonas fluorescens* promotes sulfur usage and its uptake with increased levels of S content and enzymes responsible for S mineralization [19]. Sulphur increases the amount of glucosinolate and the proportion of oil content, which is essential for the production of oil [37] A lack of S causes an imbalance in the intake of nutrients, which in turn causes the loss of chlorophyll, stunted growth, and decreased agricultural yields [38]. Hence *B. subtilis and P. fluorescens* promotes the sulphur level that increases the oil content in mustard. Moreover, seed born plant hormones like GA3 and SA play vital role in enhancing several bio-chemical attributes of mustard crops including chlorophyll process and oil percentage in seed [39]. Studies on growth promotion, disease suppression and biochemical changes in response to bio control agents like *Trichoderma harzianum*, *Pseudomonas fluorescens*, *Bacillus subtilis* were shown to affect growth, disease and tissue biochemical composition like dry matter, phenols, sugars, lipids in the leaves of Indian mustard and alter protein quantity [40]. Treatment of tomato plants with Bacillus subtilis against Fusarium oxysporum has been shown to boost lipid content [41]. There is a demonstration after an experiment that in all cultivars of mustard, the total phenol concentration decreased after reaching a maximum at 60 DAS in healthy leaves and 80 DAS in ill leaves [42]. Furthermore, the studies on transgenic technology in *B. juncea* have also given fruitful results regarding the management of fatty acid environment in the seed, and also changing the nature of the seed itself to increase the yield of the oil [43]. The main aim of the present study involving two candidate genes was to reduce the VLCUFA (i. e. , C22: To this end, the levels of one of the undesirable fatty acids, namely, erucic acid, in the seed-oil of one Indian mustard cultivar *B. juncea* cv. PCR7, a high-yielding variety and resistant to lodging and pod-shattering were altered through genetic engineering to change the carbon flux in the metabolic pathway of fatty acid biosynthesis accompanied by the corresponding changes in the expression of the respective First, the effects of over-expression of the novel acyl-ACP thioesterase of FatB originated from the MbFatB gene from Diploknema (Madhuca) butyracea on the VLCUFA content were examined in order to shorten the acyl chain-elongation process and increase C16 and C18 FAs in the seed-oil [44]. This study concluded process to reduced erucic acid, increased beneficial fatty acids, and enhanced oil content. It has been noted that using plant growth-promoting bacteria in biopriming techniques is an eco-friendly way to increase seed germination in a variety of crops, highlighting the significance of microbial interactions in raising germination rates and seedling vigor [45]. The studies using RAPD markers established that various mustard lines and hybrid have distinct genetic patterns suggesting diversified genetic base and have prospect of utilization in breeding program [46]. In addition, polymorphic amplicons, alleles, and gene diversity among several Indian mustard genotypes have been discovered by genetic diversity assessments employing SSR markers, offering insights for the crop's genetic development [47]. The biochemical and molecular properties of Indian mustard, such as the concentration of sinigrin, the isozyme patterns of peroxidase and esterase, the intensity of seed protein bands, and the genetic diversity determined by SSR and ISSR markers, can all be affected by biopriming [48]. Furthermore, the cloning and function identification of a promoter in Indian mustard has shown strong expression activity under various stress conditions, suggesting a potential role in gene regulation for stress responses [49]. When Indian mustard genotype (RH 781) was inoculated with *Albugo candida* led to a significant increase in ascorbic acid content, and the most significant changes in metabolite levels such as ascorbic acid and glutathione were observed in the 3rd leaf of both genotypes after 3 days of inoculation, with minimal changes in the 4th leaf compared to healthy leaves and other inoculated leaves on all days of inoculation [50]. Indian mustard's molecular alterations, which impact the genotypes' genetic variability and biochemical composition, are influenced by biopriming [51].

6. EFFECT AND RESPONSE TO DROUGHT DUE TO BIO-PRIMING ON INDIAN MUSTARD

Drought modifies a number of physiological and anatomical factors that are essential for mustard crop physiology to adapt to the drought. Drought stress causes mustard plants to lose fresh weight, dry weight, and abscisic acid content [52]. Drought stress also alters the protein composition, chlorophyll content, and activity of enzymes like catalase and peroxidase in mustard, resulting in physiological alterations that impact drought resistance [53]. Moreover, osmotic adaptations to display drought tolerance preserve the turgidity of cells in Brassica species, including mustard, emphasizing the significance of physiological features in maintaining resistance to water stress [54]. Biopriming with drought-tolerant isolates such as *Trichoderma harzianum*, improves osmoregulation and induces physiological protection against oxidative damage in Indian mustard [12]. *Trichoderma* as a seed treatment before sowing can induce tolerance to water stress in plants, highlighting its potential as a sustainable approach to improving crop resilience and could thrive in water stress conditions and positively impact mustard plant growth under such stress [55]. By delaying changes in net photosynthesis, stomatal conductance, and chlorophyll fluorescence brought on by drought, this strategy eventually

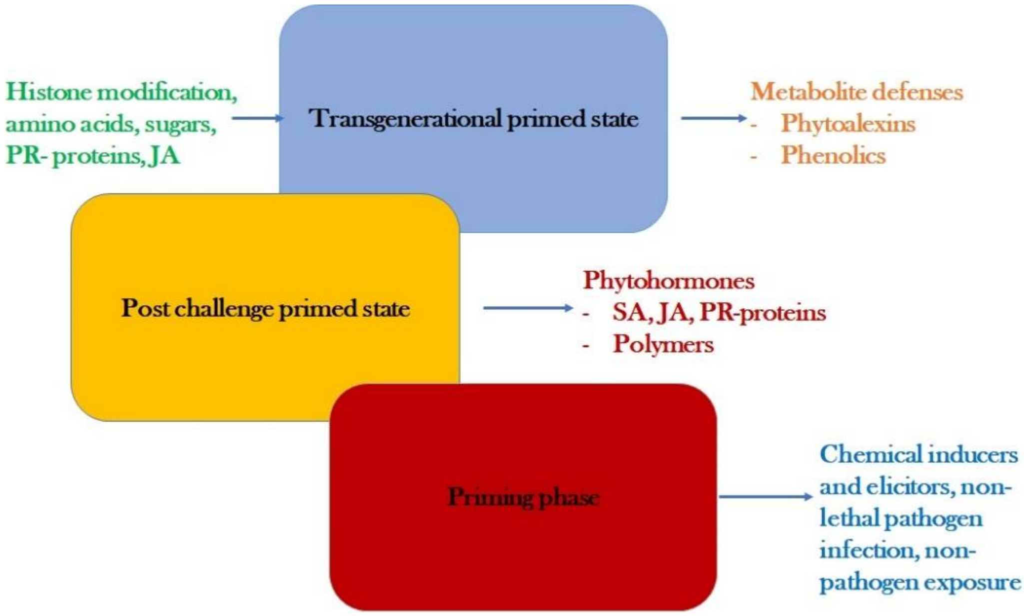

Fig. 2. Phases of priming

makes plants more resilient to water stress. It has also been discovered that using bioregulators such as glycinebetaine and salicylic acid can improve drought tolerance and growth in mustard crops by lessening the detrimental effects of water stress [56]. Moreover, it has been noted that induced drought circumstances cause mustard types to mount a defence response. This reaction results in elevated antioxidant, detoxifying enzyme, and biochemical parameter levels that improve resistance to drought stress and alternaria blight infection [57]. Furthermore, it has been researched that pre-treating mustard seedlings with hydrogen peroxide activates defence mechanisms, increasing membrane stability and resistance to drought-induced oxidative damage [58]. It has been shown that biological priming—pre-treating plants like Indian mustard with hydrogen peroxide or β-aminobutyric acid (BABA)—increases their resistance to drought. Research has indicated that the use of hydrogen peroxide priming can initiate defence mechanisms, initiate ROS and MG detoxification processes, and sustain essential enzyme functions and redox equilibrium, thereby equipping seedlings to endure drought-induced oxidative stress [59]. Similarly, BABA administration has been associated with increased accumulation of ABA, which causes stomatal closure and stress gene expression to accelerate, improving plant resistance to drought stress without causing genetic changes [60]. Indian mustard genetic expression linked to drought resistance and tolerance has been demonstrated to be positively impacted by bio priming, more especially by the use of bio stimulants such as Ascophyllum nodosum extract. It has been shown that the application of bio stimulants can suppress stress-responsive negative growth regulators, like RESPONSIVE TO DESICCATION 26 (RD26), while preserving the expression of markers of the cell cycle, such as HISTONE H4 (HIS4), to facilitate the active progression of the cell cycle under drought stress [23].

7. GENES RESPONSIBLE FOR DROUGHT TOLERANCE IN INDIAN MUSTARD

Various genes are that are either already present or incorporated in plant can result in drought tolerance.

The genes responsible for drought tolerance in Indian mustard (Brassica species) have been identified through various studies. Several unique genes involved in drought tolerance have been identified by comparative transcriptome analysis, including genes related to photosynthesis, biosynthesis of glutathione, biosynthesis of IAA signal transduction, biosynthesis of amino acids, cysteine, and methionine, and glucosinolate [11]. These genes are involved in the molecular aspects of drought stress in Brassica species and also useful in the selection of drought-resistant cultivars for the improvement of drought tolerance in breeding programs [65]. Further, genes associated with protein phosphatase 2C

Table 2. Different genes responsible for drought tolerance in mustard

Sl. No.	Gene	Importance	Reference
1.	BjRD26	A NAC transcription factor gene (NAM, ATAF1/2, and CUC2) implicated in the regulation of genes associated to stress and the response to drought stress.	[61]
2.	BjHSP17.8	This gene helps prevent damage to cellular proteins by encoding a little heat shock protein that is produced during drought stress.	[62]
3.	BjDREB2A	A transcription factor gene known as dehydration-responsive element-binding (DREB) that controls the expression of genes sensitive to stress in drought-prone environments.	[63]
4.	BjERF1	A gene that responds to ethylene and is involved in the regulation of genes that are sensitive to drought.	[64]

have been reported to be involved in drought stress and hence, it becomes important to know the genetic makeup of drought tolerance in Indian mustard [66]. IgWRKY50 and IgWRKY32, the genes of Iris germanica, are two genes which were found to be important for increasing drought resistance in Indian mustard [67]. The genes belonging to this superfamily of WRKY transcription factor are regulated by kind of stress conditions including PEG-6000, high temperature and ABA and reaches at its maximum level at 3 h in case of PEG-6000 treated plants, when overexpressed in both Arabidopsis plants, enhanced shooting with improved osmotic tolerance, higher germination rates, increased average root length, and increased stomatal closure and reduced water loss rates under drought stress conditions [67]. Moreover, the expression of the stress-responsive genes in the transgenic plants was also upregulated, proline content and soluble protein content in the plants also increased, soluble protein content in the plants also increased, together with SOD, POD and CAT activities, all of which accumulated in the transgenic plant to enhance the drought resistance of the plants [68]. Therefore, IgWRKY50 and IgWRKY32 genes could be prospective candidates for effective utilization in molecular breeding programs directed at improving DT in Indian mustard. Transgenic mustard lines demonstrated increased antioxidant enzyme activity, decreased oxidative stress markers, and improved physiological traits, leading to better survival and performance under drought conditions. Over-expression of the chickpea Metallothionein1 (MT1) gene in mustard significantly enhances drought tolerance [69].

8. EFFECT OF BIO-PRIMING ON THE EXPRESSION OF GENES INVOLVED IN DROUGHT TOLERANCE IN INDIAN MUSTARD

Research have shown that bio-priming upregulates stress-responsive genes and signalling pathways, including those involved in ABA and JA production, which in turn affects the expression of genes linked to drought tolerance [70]. Furthermore, it has been discovered that bio-priming preserves the shoot apical meristem's function and encourages active cell cycle progression during drought stress, which enhances plant resilience and growth [59]. Through the activation of stress memory systems, reinforcement of cellular defence responses, and induction of phytohormone synthesis, bio priming plays a critical role in improving drought tolerance in plants. Research has demonstrated that, in drought-stressed environments, seed biopriming with bacteria resistant to drought can greatly increase crop yields [71]. Numerous seed priming methods, such as bio-priming with bacteria that promote plant growth, have been found to be effective ways to increase crops, resistance to stress, which will boost growth, dry matter accumulation, and overall plant performance in both ideal and drought-prone environments [72]. It has been discovered that bio priming with particular bacterial strains raises antioxidant levels, including those of catalase, superoxide dismutase, and ascorbic peroxidase, all of which are essential in reducing the harmful consequences of oxidative stress brought on by dehydration [73]. Additionally, it may result in the activation of genes linked to plant hormone signalling, cell wall remodelling, and stress defence, all of which improve the plant's overall drought tolerance in Indian mustard and increase its capacity to fend off the impacts of drought [74]. CabHLH10, a bHLH transcription factor that regulates yield attributes under drought stress by altering the expression of drought-responsive genes and photosynthetic efficiency genes, is one of the specific genes bio-primed for better drought tolerance in Indian mustard [75]. The development of drought-tolerant cultivars of Brassica species has also been aided by the identification of traits such as primary branches plant-1, secondary branches plant-1, siliquae plant-1, seeds siliqua-1, seed yield plant-1, and 1000-seed weight as trustworthy selection criteria for drought tolerance [40]. Additionally, differentially expressed genes involved in photosynthesis, glutathione biosynthesis, IAA signal transduction, amino acid biosynthesis, and ABA signal transduction pathways were found in Brassica napus through comparative transcriptome analysis. These genes support drought tolerance mechanisms in rapeseed cultivars [76]. Higher seed vigour index and germination energy under drought stress processes have been observed in rapeseed cultivars that use drought-tolerant bacteria for biopriming, such as the strain SH-8, to boost drought tolerance and germination potential in wheat seeds [77]. The desirable genes could be enhanced by molecular markers like RAPD and SSRs; which can be used to investigate the genetic diversity among Indian mustard genotypes. This allows for the identification of diverse parents with high heritability and genetic advance, which can be used to improve yield and quality in early segregating generations [78]. Enhancing genes associated with plant growth and seed germination is one of the many advantages of bio priming. Based on research, biopriming entails introducing advantageous microbes, including Bacillus species, into seeds to enhance gene expression and seedling growth [79]. The processes of seed biopriming include the release of defence-related enzymes, induction of plant growth-promoting activities, and solubilization of soil nutrients, all of which support increased gene expression and plant growth [24]. Furthermore, bio priming is regarded as a sustainable method of mitigating abiotic stresses- drought in crops while guaranteeing uniform stand development under stress situations [80]. It is an environmentally benign substitute for chemical fungicides. Overall, bio priming improves seed germination and seedling establishment in difficult settings by upregulating genes involved in antioxidant defence and DNA damage repair at the molecular level [81].

9. CONCLUSION

Brassica juncea, also known as Indian mustard, is an important oilseed crop that plays a major role in the agricultural economy and food security of India. The crop's economic and health benefits are further enhanced by its high oil content and significant bioactive components. However, the crop's development and productivity are negatively impacted by the drought, which presents serious issues. Biological priming has become a viable approach to lessen these difficulties, including bio-priming with advantageous bacteria. This method induces particular metabolomic and molecular changes that improve seed germination, seedling vigour, and overall plant growth. It has been demonstrated that bio-priming increases drought tolerance through upregulating genes that respond to stress, improving physiological and biochemical characteristics, and encouraging advantageous interactions between microbes and plants. Research indicates that bio-priming can boost Indian mustard's resistance to drought stress, maintaining productivity and enhancing crop quality. These efforts are further supported by genetic and molecular breakthroughs that increase the crop's resistance to harsh conditions, such as the discovery of genes resistant to drought and the application of transgenic technology. As a whole, there is a lot of potential to increase Indian mustard's productivity and resistance to drought by combining biological priming techniques with conventional breeding and genetic methods. This integrated approach guarantees this essential crop's sustained economic and nutritional contributions to India's agricultural sector in addition to promoting sustainable agriculture.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

I, Aishmita Gantait, hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of manuscripts.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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