



Evaluating the Impact of Peanut Shell Carbon Dots Priming on Seed Longevity and Viability in Blackgram [*Vigna mungo* (L.) Hepper]

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ABSTRACT

Background: Using good quality seeds during sowing greatly determines the productivity of crops in agricultural production. Hence, adopting advanced and eco-friendly storage practices to maintain viability for a longer period becomes an hour's need. One such is a nanoprimer which uses nanomaterials as a seed invigorant to maintain the viability of seeds during storage. Hence, a study was taken up to evaluate the bio-efficacy of peanut shell carbon dots (PNS-CDs) a type of carbon nanomaterial in maintaining the seed quality of blackgram during storage.

Methods: Blackgram seeds were stored under ambient conditions in cloth and polythene bags after imposing the following treatments viz., control (untreated), hydropriming and PNS-CDs priming @ 200 ppm. Samples were drawn at monthly intervals to analyze their physiological and biochemical parameters thereby the effect of PNS-CDs priming on blackgram seed storability can be known.

Result: The results of the experiment revealed that irrespective of the containers, seeds primed with PNS-CDs @ 200 ppm has performed better and maintained its minimum standards up to 7 months. The results of higher germination (78%), speed of germination (9.7), vigour index (1979) and dehydrogenase activity (2.28 OD value/min) have proved the enhanced physiological activity by PNS-CDs priming. In addition, the antioxidant defense mechanism offered by PNS-CDs was also confirmed with maximum activity of catalase (0.77 $\mu\text{g H}_2\text{O}_2$ reduced $\text{g}^{-1} \text{min}^{-1}$), peroxidase (0.68 OD value/10 min) and superoxide dismutase (4.1 units mg^{-1} of protein). Thus, it was found that PNS-CDs priming can be given as a pre-storage treatment to maintain the storability of blackgram seeds sustainably.

Key words: Antioxidant enzymes, Black gram, Carbon dots, Storage.

INTRODUCTION

Black gram [*Vigna mungo* (L.) Hepper] a highly valuable legume, is integral to the food security landscape due to its high protein content (around 25%), rich carbohydrate reserves and vital micronutrients. Quality of the seeds during sowing highly determines the production and productivity of crops. Hence, maintaining the seed quality during storage from harvest to the next sowing becomes an integral part of agricultural practices. Proper storage techniques help in maintaining seed quality over time by reducing moisture content, preventing fungal infections and damage caused by pests (Chaitanya *et al.*, 2024). In addition, it can significantly influence germination rates, seed longevity and crop yield. To maintain the seed quality during storage, several pre-storage and mid-storage treatments were used (Islam *et al.*, 2024). The recommended moisture content for long-term storage is around 8-10% and the statistical models by Khan *et al.* (2020) indicated that for every 1% increase in seed moisture content, the longevity of blackgram seeds decreases by 4-6 months under tropical conditions. Conversely, seeds with less than 9% moisture can retain over 80% viability for more than one year when stored at temperatures below -15°C.

Seed performance can be improved manifold by applying quality enhancement technologies having

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environment-friendly nature (Weissmann *et al.*, 2023). Seed priming is one such a simple and effective technique recommended to get speedy and uniform emergence. It has potential to enable the seeds to overcome biotic and abiotic stresses (Sivasubramaniam *et al.*, 2011) and also helps in maintaining the seed quality during storage conditions (Panghal *et al.*, 2023). Similar results of enhanced seed quality during storage were also observed in French bean (Laloo and Chakraborti, 2025), Isabgol (Verma *et al.*, 2022), black gram (Dugesar *et al.*, 2025) and alfalfa (Xia *et al.*, 2021) due to seed priming. Seed priming methods include hydropriming, halopriming, osmopriming, matrixpriming and biopriming. Off late nanopriming using nano materials is picking-up. Nanotechnology has arisen as a promising technology of current era that can meet various agricultural demands (Aminzai *et al.*, 2024). But it should be adopted in safe and eco-friendly manner to avoid various nano pollution. In agriculture, nanomaterials have been adopted for improving seed germination, plant establishment, plant protection and overall crop yield (Verma Kumar *et al.*, 2020). Nanopriming is a promising emerging strategy that refers to the use of nanoprimers like metal oxide, carbon based and polymeric nanoparticles for priming seeds (Khan *et al.*, 2023), that results in improved seed germination and vigour (Hridya *et al.*, 2017). It significantly maintains the seed storability by enhancing seed resilience to abiotic, biotic stress factors and oxidative damage during the storage period and thereby maintaining seed germination and vigour (Singh *et al.*, 2024). Among the nanoprimers, carbon-based nanomaterials especially carbon dots (CDs) were found to have great potential for improving water uptake, germination and nutrient assimilation in seeds due to their structural and electrochemical properties (Shelar *et al.*, 2024). Xu *et al.* (2004) identified the CDs first during the purification of single-walled carbon nanotube and was found to have the following properties viz., <10 nm size and spherical shape. In addition, the resistance offered by carbon nanomaterials in reducing the pathogen and pest attack was also reported (Cui *et al.*, 2024). Hence, the proposed hypothesis is that, priming seeds with CDs can influence the germination, vigour and storability of the seeds. Thus, a study was framed to explore the potential of peanut shell carbon dots (PNS-CDs) synthesized by Abinaya *et al.* (2024) in enhancing the storage quality of blackgram seeds, which could have a significant impact on seed management practices and contribute to agricultural sustainability.

MATERIALS AND METHODS

Black gram cv. VBN 10 seeds were obtained from the National Pulses Research Centre, Vamban, Tamil Nadu, India for the study. A storage experiment for the period of 12 months were conducted in the Department of Seed Science and Technology, Tamil Nadu Agricultural University, Coimbatore during 2023-24. Peanut shell carbon dots (PNS-CDs) were synthesized using pyrolysis method

maintaining the temperature of 400°C for 2 h using the biochar unit available in the Department of Renewable Energy, Tamil Nadu Agricultural University, Coimbatore and the priming dosage was also selected from the previous experiment conducted in blackgram seeds using PNS-CDs (Abinaya *et al.*, 2024). The treatments imposed during storage are as follows: T₁- Unprimed control seeds stored in cloth bag, T₂- Unprimed control seeds stored in 700 gauge polythene bag, T₃- Hydroprimed seeds stored in cloth bag, T₄- Hydroprimed seeds stored in 700 gauge polythene bag, T₅- PNS-CDs (200 ppm) primed seeds stored in cloth bag and T₆- PNS-CDs (200 ppm) primed seeds stored in 700 gauge polythene bag. Approximately 30 g of seeds in each treatment were packed in each container and placed under ambient condition with the temperature of 25±3°C and relative humidity of 75% for storage. The seeds were drawn at monthly intervals for the evaluation of viability, physiological and biochemical parameters up to 12 months storage. Thus, the periods from initial to 12 months after storage were denoted as P₀, P₁, P₂, P₃, P₄, P₅, P₆, P₇, P₈, P₉, P₁₀, P₁₁ and P₁₂. The experiment was laid out in completely randomized design (CRD) with two factors and four replications for each treatment.

Physiological and biochemical assays

Speed of germination (Maguire, 1962), germination percentage (ISTA, 2023) and vigour index (Abdul-Baki and Anderson, 1973) were evaluated to assess the physiological vigour status of the seeds during storage. The biochemical changes were measured with the activity of dehydrogenase (Kittock and Law, 1968), catalase (CAT) (Aebi, 1984), peroxidase (POD) (Malik and Singh, 1980), superoxide dismutase (SOD) (Blanchamp and Fridovich, 1971), malondialdehyde content (MDA) (Heath and Packer, 1968) and electrical conductivity (EC) (Presley, 1958).

Statistical analysis

All the data were statistically analyzed using R software version 4.4.1 (Chambers *et al.*, 2008) and two-way ANOVA was performed to assess the significance between the treatments.

RESULTS AND DISCUSSION

Influence of PNS-CDs priming in seed physiological parameters during storage

Following good storage practices for maintaining seed viability from harvest to next sowing is considered as an important criterion. Pre-storage seed treatments can be done in different forms like seed coating and priming with different materials. During recent days, several reports have confirmed that nanopriming has the potential to enhance and maintain the quality of the seeds during storage through different mode of actions which was also evidenced from our results. Since, the seeds have attained its minimum standards on the 7th month itself, the results up to 8 months alone have been presented and discussed hereafter.

The physiological and biochemical parameters observed during storage showed significant differences for the imposed treatments and storage periods. The initial evaluation of the experiment revealed that irrespective of the containers, seeds primed with PNS-CDs @ 200 ppm showed better performance in all the physiological parameters. Among the treatments, T_6 registered higher speed of germination (12.9), germination (92%) and vigour index (2742) than T_1 and T_2 . During the advancement of the storage periods, on observing all the parameters, it was evidenced that T_5 and T_6 showed reduced rate of deterioration than other treatments. It was also statistically confirmed on observing the results of P_8 in which T_5 and T_6 has recorded the maximum speed of germination (9.1 and 9.3), germination (72 and 74%) and vigour index (1807 and 1865) respectively (Fig 1, 2 and 3). Whereas, the minimum was registered by T_1 for speed of germination (8.9), germination (72%) and vigour index (1670). Nanoprimered seeds retain their capacity to germinate faster and to produce more robust seedlings even after prolonged storage due to the improved mitochondrial function, which

provides energy for faster and more uniform germination (Singh *et al.*, 2024). Because of the already done priming treatments, the seed would have already completed its pre-germinative metabolism and when imbibed again, it remembers the priming event and start from the radicle emergence stage. This is one of the prime reasons for improved speed of germination and germination in the blackgram seeds. Another reason for the extended storability is mainly due to the higher resistance offered by primed seeds to desiccation (Kaur *et al.*, 2021) which is critical for reducing microbial growth and seed decay. Similar enhancement in seed germination and seedling vigour due to nanoprimering was also reported in chickpea (Khandaitaray *et al.*, 2024) and groundnut (Tamilarasan and Raja, 2024).

The observed biochemical parameters during the storage period showed significant differences for treatments, storage periods and their interactions. Electrical conductivity of the seed leachate is one of the indicators of the seed deterioration pattern. The results showed a drastic increase over the storage periods in all the treatments

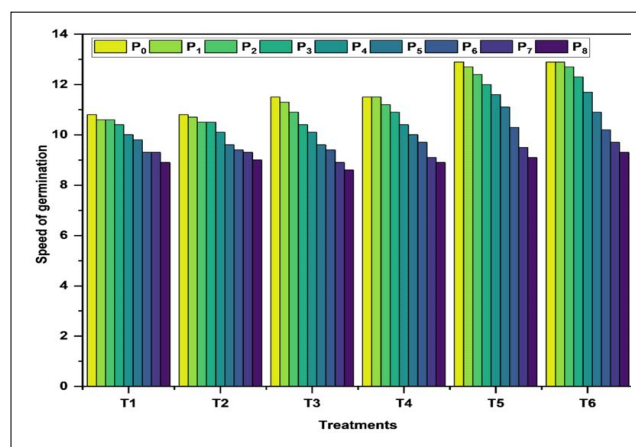


Fig 1: Effect of different seed priming treatments on speed of germination in blackgram cv. VBN 10.

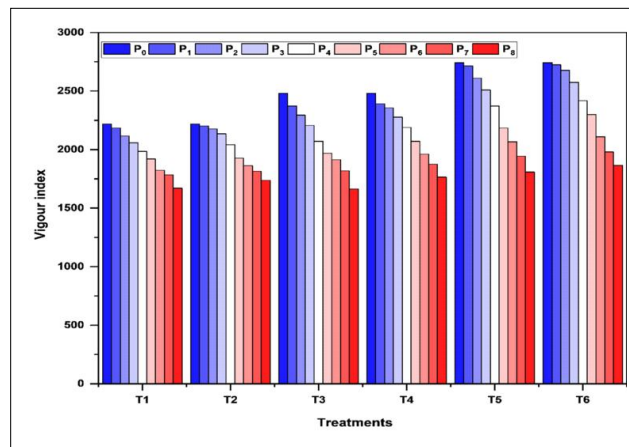


Fig 3: Effect of different seed priming treatments on vigour index of blackgram cv. VBN 10.

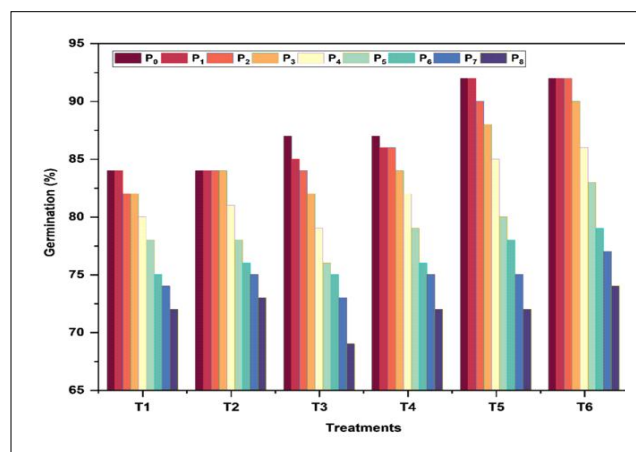


Fig 2: Effect of different seed priming treatments on seed germination of blackgram cv. VBN 10.

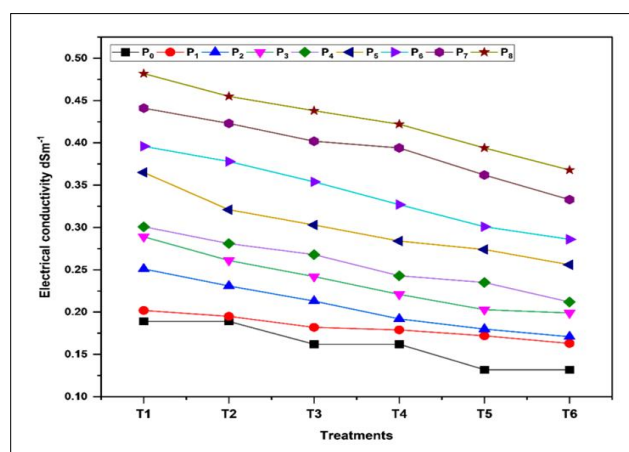


Fig 4: Effect of different seed priming treatments on electrical conductivity of blackgram cv. VBN 10.

which confirm the progress of deterioration in seeds. In which, the maximum increase between P_0 and P_8 was observed in T_1 from 0.189 to 0.482 dSm^{-1} and the minimum of 0.132 to 0.368 dSm^{-1} in T_6 , which was also on par with an increase of 0.132 to 0.394 dSm^{-1} in T_5 (Fig 4).

In our experiments, the higher and lower values of MDA in T_1 (0.53) and T_6 (0.34) confirmed the above statement and proved a higher deterioration pattern in T_1 than T_3 . During P_0 and P_8 storage, it was also observed that MDA increased from 0.53 to 1.28 $\mu\text{mole g}^{-1}$ fresh weight in T_1 and 0.34 to 1.10 $\mu\text{mole g}^{-1}$ fresh weight in T_6 (Fig 5). It was also confirmed from the report of El-Gebaly *et al.* (2024) where they stated that lipid peroxidation leads to the breakdown of unsaturated fatty acids in membranes and nanoparticles help in stabilization of cell membranes by improving the integrity of phospholipid bilayers, reducing permeability and leakage of essential ions and metabolites. Since, nanopriming induces the expression of stress-responsive genes, it plays crucial roles in seed defense mechanisms during storage. This helps in maintaining

the seed viability and integrity, avoiding the common issue of membrane disintegration in seeds during storage and reduces oxidative damage (Elkelish *et al.*, 2024). Dehydrogenase activity serves as a metabolic indicator in seeds and the higher activity suggests active mitochondrial respiration, which is crucial for producing ATP required for cellular functions and germination (Diya *et al.*, 2024). The observation on dehydrogenase activity revealed that it was higher in T_6 with the content of 2.71 OD value/min and lower in T_1 with 2.43 OD value/min (Fig 6). It was also found that T_3 seeds showed less reduction in dehydrogenase activity than T_1 during the advancement of storage period. This has proved that seeds treated with PNS-CDs have maintained its viability for a longer period than untreated seeds which is one of the reasons for the enhanced seed longevity.

Reactive oxygen species (ROS) accumulation during storage is the major damage that occurs during storage, leading to oxidative damage to lipids, proteins and DNA. One of the major biochemical mechanisms contributing to

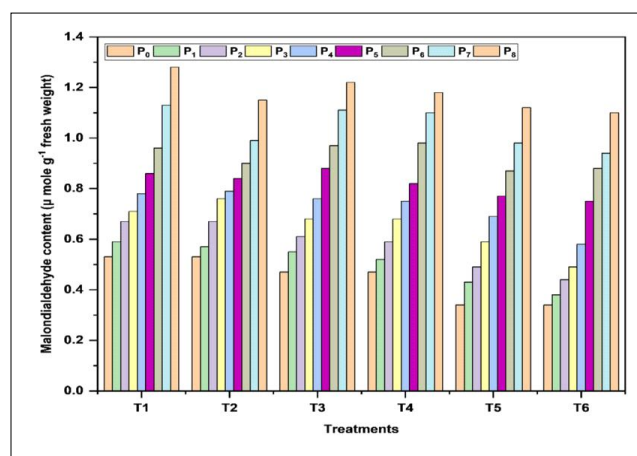


Fig 5: Effect of different seed priming treatments on malondialdehyde content of blackgram cv. VBN 10.

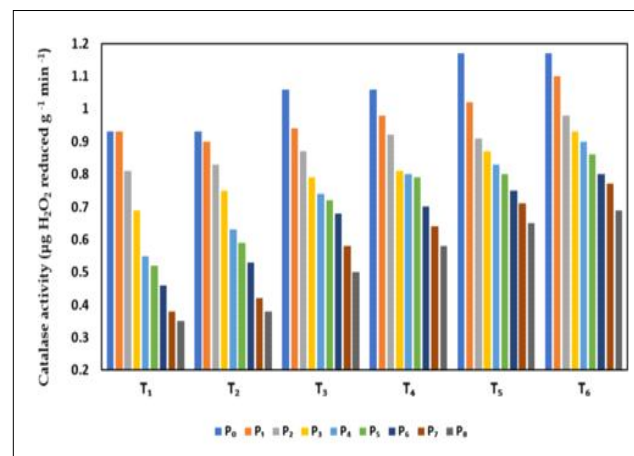


Fig 7: Effect of different seed priming treatments on catalase activity of blackgram cv. VBN 10.

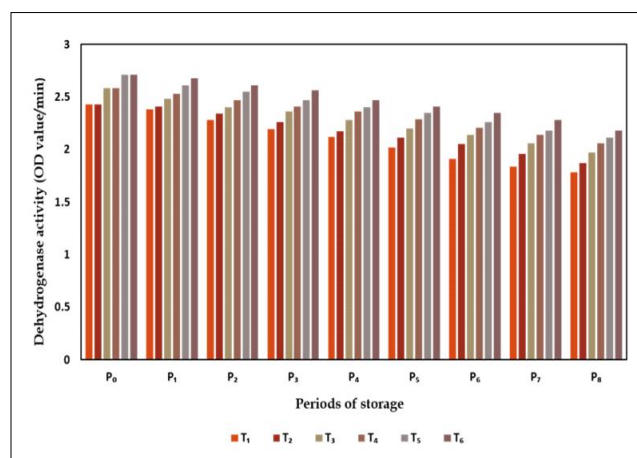


Fig 6: Effect of different seed priming treatments on dehydrogenase activity of blackgram cv. VBN 10.

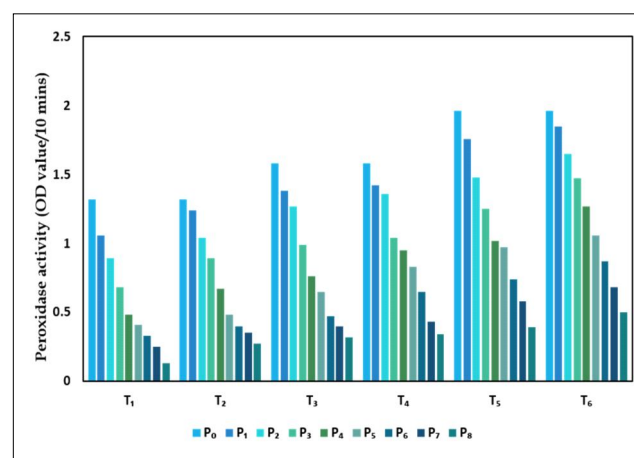


Fig 8: Effect of different seed priming treatments on peroxidase activity of blackgram cv. VBN 10.

the extended storability of nanoprimered seeds is the enhanced activity of antioxidant enzymes. The results obtained for the antioxidant enzyme activity showed highly significant difference among the treatments and storage periods. Among the treatments, T_6P_0 recorded highest CAT ($1.17 \mu\text{g H}_2\text{O}_2$ reduced $\text{g}^{-1} \text{min}^{-1}$), POX ($1.96 \text{ OD value/10 min}$) and SOD ($4.51 \text{ units mg}^{-1}$ of protein). Whereas, the lowest antioxidant activities viz., CAT ($0.93 \mu\text{g H}_2\text{O}_2$ reduced $\text{g}^{-1} \text{min}^{-1}$), POX ($1.32 \text{ OD value/10 min}$) and SOD ($4.26 \text{ units mg}^{-1}$ of protein) were noticed in T_1P_0 . With the advancement of storage periods, the amount of antioxidant enzyme activity get reduced and reaches the minimum of 0.35 and $0.69 \mu\text{g H}_2\text{O}_2$ reduced $\text{g}^{-1} \text{min}^{-1}$ for CAT, 0.13 and $0.50 \text{ OD value/10 min}$ for POX and 3.70 and $3.99 \text{ units mg}^{-1}$ of protein for SOD activities in T_1 and T_6 respectively (Fig 7, 8 and 9). Thus, it could be concluded from the results that, with the

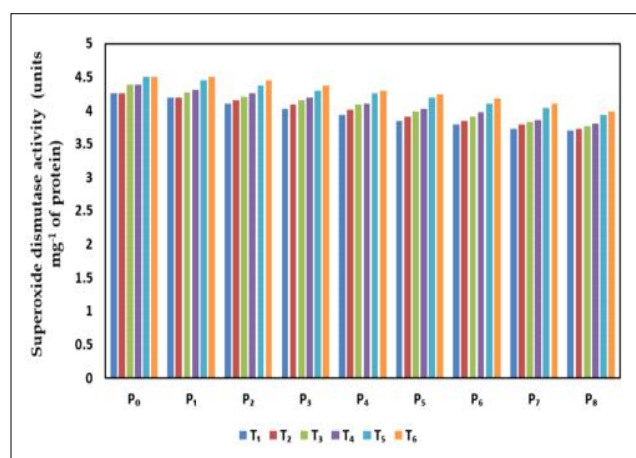


Fig 9: Effect of different seed priming treatments on superoxide dismutase activity of blackgram cv. VBN 10.

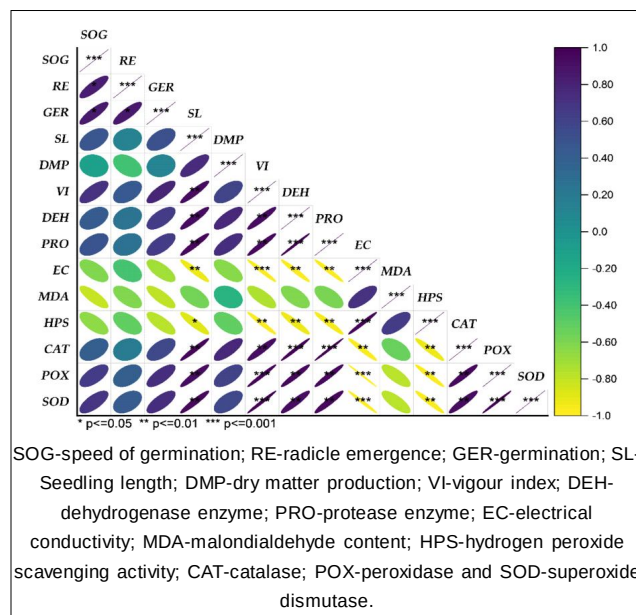


Fig 10: Correlation plot depicting the relation between observed parameters and storability of seeds.

advancement of the storage period, the physiological and antioxidant activity of the control seeds showed rapid deterioration. Whereas, it was lesser in seeds primed with PNS-CDs in both the containers and has maintained its minimum standards upto P_7 .

It was found that, nanoprimered seeds enhanced the activity of ROS scavenging enzymes and reduced the oxidative stress that occurs during storage. The activation of ROS scavenging enzymes like CAT, POX, SOD, APX and GR act as a base for enhanced antioxidant system. The mechanism behind the scavenging of ROS is that SOD converts superoxide radicals into H_2O_2 , which is further broken down into water and oxygen by CAT to prevent toxic buildup. Ascorbate peroxidase (APX) and GR work together to neutralize H_2O and maintains the antioxidant balance by utilizing ascorbate and glutathione respectively. In addition, the upregulation of genes encoding antioxidant enzymes like SOD, CAT, APX and GR in nanoprimered seeds was also considered as one of the prime reasons for enhanced storability of seeds (Islam *et al.*, 2022). This ensures a continuous supply of antioxidant molecules to neutralize ROS during storage. In addition, the better regulation of ROS signaling pathways, for maintaining cellular homeostasis and mitogen-activated protein kinase (MAPK) signaling pathways during storage helps in regulating various cellular responses to stress in nanoprimered seeds (Prasad *et al.*, 2024). Another mechanism is that it activates the expression of heat shock proteins (HSPs), which protect seed proteins from denaturation and aggregation under stress conditions. These HSPs act as molecular chaperones, ensuring proper protein folding and preventing protein degradation during long storage periods (Shelar *et al.*, 2024).

The epigenetic changes such as DNA methylation and histone modification, which can influence gene expression patterns in a way that improves seed storability was also observed after nanoprimering (Gohari *et al.*, 2024). Nanoprimered seeds exhibit higher levels of molecular chaperones and proteins involved in the repair and protection of cellular components. These chaperones, including chaperonins and HSPs, assist in the refolding of denatured proteins, reducing damage caused by protein misfolding which is common in deteriorating seeds (Sarmah *et al.*, 2024).

On the whole, the increased germination, seedling vigour, antioxidant enzymes activities and reduced lipid peroxidation, electrolyte leakage observed in T_3 during storage confirmed the optimistic role of PNS-CDs in maintaining the storability of blackgram seeds. It was also confirmed from the results of correlation study that MDA and EC showed highly negative correlation for storability. Whereas, the other parameters like physiological and antioxidant properties showed positive correlation and proved its enhancement because of the treatments (Fig 10). This has also confirmed that blackgram seeds primed with PNS-CDs @ 200 ppm can be stored up to 7 months with required germination of Indian Minimum Seed Certification Standards (IMSCS).

CONCLUSION

It is concluded that the blackgram seeds primed with PNS-CDs @ 200 ppm has maintained its viability through a combination of physiological and biochemical mechanisms. Enhanced seed vigour, viability, membrane stability and improved antioxidant defense system was an important mechanism adopted by PNS-CDs in maintaining the viability of seeds throughout the storage period. Thus, seed priming with PNS-CDs could be offered as an eco-friendly and sustainable seed quality enhancement treatment for maintaining the blackgram seed longevity and viability. The future perspectives of this study includes the application of different omics and molecular based approaches for identifying the interaction between seeds and CDs in maintaining seed storability.

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Conflict of interest

The authors declare that there are no conflicts of interest regarding the publication of this article.

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