

ABSTRACT

The short-duration leguminous warm-season crop, *Vigna radiata* (L.) R. Wilczek is now grown globally in a balanced manner, particularly in emerging nations. It maintains a special place in the human diet as a sprout, dahl, and in numerous other forms due to its wide nutritional range, speedy and easy preparation, excellent digestion, and anti-flatulent characteristics. Due to its high amounts of folate and iron and excellent protein content, mung bean is in high demand and commands a premium price, which makes the farmers happy. The lack of nutrient-dense food, specifically for poor vegetarians, combined with expanding population associated with increasing global food crises, and limited natural resources, made this nutritious food crop more crucial. Additionally, it has a symbiotic relationship with *Rhizobium*, which improves soil fertility by fixing atmospheric nitrogen and making it ideal for rice-based cropping systems and intercropping with other crops. Mung bean plants are occasionally broken up and buried in the soil to improve the nitrogen content of the soil. As a result, they also aid succeeding crop plants in meeting their own nitrogen needs. Despite having so many advantages, mung beans have received less attention than other pulse crops. Moreover, being a self pollinated pulse having a small sized genome, it might serve as a study model for other legumes.

But, the problem of rising salinity, one of the most serious global environmental issues, has been adversely hampering the overall mung bean productivity world-wide. An estimated 1.5 million hectares of agricultural area are lost to production annually due to high salinity levels present in the soil. More than 45 million hectares of the irrigated area have been ruined by salt globally. Alarmingly, it is anticipated that salinity will deteriorate over the next few decades. Plants are negatively impacted by salinity stress in many ways, including water stress, ion toxicity, nutritional issues, increased lipid peroxidation, metabolic process change, membrane disruption, reduced cell division, etc. These negative consequences altogether retard plant growth badly affecting its ultimate survivality. To provide humanity with a sufficient food supply and a balanced diet, scientists and researchers should show more interest in this subject. As a result, it's crucial to find and create mung bean cultivars with sustained resistance to this negative environmental pressure.

Numerous tactics were established over time to increase the quantity and quality of seedlings of various crop species. Seed priming is such a feasible, affordable, and successful method of reviving seeds. New priming techniques are constantly being developed around the world to improve the nutraceutical properties and yield of pulse crops. One such novel and innovative approach is **Solid Matrix Priming (SMP)**. According to the literature review, SMP was relatively understudied compared to the other priming approaches and is being employed for the first time in mung bean (except our published papers). Impact of this less commonly used novel priming technique (SMP) in mung bean also demonstrates vast potential in seedling invigoration at a commercial scale that has yet to be investigated. Additionally, it is, in many ways, cost-effective, advantageous and superior to liquid priming as it is convenient to any seed size, very less amount of liquid is required (thus this is economical) and the slow absorption of liquid in this method allows to repair the damages, if any, occurred during liquid uptake (very fast liquid uptake in liquid priming frequently causes injury to cotyledons). Hence, farmers should be encouraged to use it on an extensive basis.

Moreover, going with the modern approach of **seed nano-priming**, assessment of the potential role of nano-chitosan as priming agent on mung bean is not investigated yet. Synthesizing novel nanoparticles like nano-chitosan using organic, biodegradable basic materials is gaining popularity because of its prospects for economical, ecologically friendly, and mass production. In the current study, after the synthesis of nano-chitosan using biodegradable chitosan as base material through the ionic gelation method in the laboratory, it was physiochemically characterized through Dynamic Light Scattering (DLS) and zeta potential studies, Scanning Electron Microscopy (SEM), Transmission Electron Microscopy (TEM), Energy-dispersive X-ray analysis (EDX), and Fourier Transmission Infrared Spectroscopy (FTIR).

The prepared nano-chitosan was then applied through SMP in mung bean under different concentrations of salinity stress to investigate its effect on the various seedling growth parameters, their biochemical and enzymological attributes, and salt-induced oxidative stress mitigation. The antioxidant activities of the treated mung bean seedlings under the said conditions were also studied. SMP noticeably enhanced the activities of antioxidant enzymes, namely Catalase (CAT), Peroxidase (POD), Superoxide dismutase (SOD), Polyphenol Oxidase (PPO), Ascorbate peroxidase (APX) activities in treated mung bean seedlings. At the same time, significant enhancement in the nutraceutical values of mung bean sprouts were also noted. Further, it noticeably reduces oxidative damages along with noteworthy

improvement in terms of tolerance capacity in mung bean seedlings exposed to salinity stress conditions.

The efficacy of nano-chitosan was also assessed for its **antifungal activity** against *Aspergillus flavus*, the most common mung bean seed-borne mycoflora, identified by the Indian Agricultural Research Institute (IARI), Delhi, India. Fungal spore germination and mycelial growth were rigorously examined after applying nano-chitosan in a dose-dependent manner with sterilization procedure modification.

To evaluate the effect of nano-chitosan following mung bean seed priming (SMP), *Aspergillus flavus* spore suspension was added to the seeds and allowed to germinate. After seven days of germination, various growth parameters of mung bean seedlings were measured, showing the highest antifungal activities with 571 mg/ml of nano-chitosan at a 10% matrix (Celite) moisture level. Accordingly, the current study revealed that SMP with nano-chitosan is an effective seedling invigoration treatment in saline settings, especially for mung bean seedlings in their early growth stages.

Thereafter, the treated and untreated mung bean seedlings under salt stress conditions were subjected to **On-gel isozyme patterns of Native PAGE** (α -Amylase, NADPH Oxidase, Superoxide dismutase, Peroxidase) and **SDS-PAGE**.

Orbitrap High-Resolution Liquid chromatography-mass spectrometry (**OHR-LCMS**) was conducted from SAIF, IIT- Bombay, to examine mung bean seedlings' most prominent and variably expressed SDS gel band particularly obtained in lane 3 represented by nano-chitosan treated seedlings in the gel image.

The High-Resolution Liquid chromatography-mass spectrometry (**HR-LCMS**) analysis of three significant isoflavones (Genistein, Formononetin, and Biochanin A) in control and chitosan and nano-chitosan treated mung bean seedlings grown under salt-stress conditions was conducted separately from SAIF, IIT-Bombay.

Further, the major mung bean isoflavones identified by the **HR-LCMS** study, were undergone through in-silico drug study (*ADME* and druggability test, prediction of pharmacological activities through *PASS*), and toxicity analysis (**PRED SKIN**, **Pred-hERG Analysis**, **CarcinoPred-EL**, **Xenosite reactivity**) using various online web tools in connection with their associated therapeutic implications. STRING and Cytoscape analysis were also performed to realize the interaction between the isoflavones and the salt stress genes in mung bean. The

metabolic shifts of chief isoflavones having therapeutic consequences were critically detected.

Further, to evaluate the impact of SMP with nano-chitosan in the genetic expression of some selected salt stress genes (SOS1, SOS2, SOS3, and NHX1) of mung bean, **Quantitative Real Time PCR** analysis of the treated (nano-chitosan primed) and untreated (control) mung bean seedlings was conducted from Credora, Life Sciences, Bengaluru, Karnataka. In every case, distinctly significant and enhanced expression of the selected salt stress genes were remarkably noted in the nano-chitosan treated seedlings compared to the untreated ones grown under salinity stress conditions. This clearly suggests that nano-chitosan (applied through SMP), is an ideal primer for seedling invigoration and salinity induced oxidative stress mitigation.

So, mung bean sprouts can be considered as a well-known healthier alternative and because of their prospective impacts on human healthiness and the industry's rapid growth, nutritional fortification of this functional food has become an important topic of study. Moreover, this work on mung bean may shed light on the functions of some associated salt-tolerant genes and the underlying molecular mechanisms of salt tolerance in mung bean crop plants.

Thus, in modern agricultural system, application of nano-chitosan through SMP might be a promising technique in stress mitigation, along with protecting and invigorating crop plants like mung bean against abiotic stress like salinity which in turn gives a conceptual foundation for the future study on the strengthening of mung bean's salt tolerance mechanism and the genetic resources. Further, based on the valuable characteristics like, antifungal and antioxidant activity, biodegradable and biocompatible nano-chitosan might be a potential substitute in place of commonly used toxic and non-degradable agrichemicals especially for the targeted beneficiaries and would also be helpful for the society and the environment in the long run.