



INTRODUCTION



Chapter - 1

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1. Introduction

1.1 Significance of studying leguminous pulse crops

In India, leguminous pulses are one of the vital food crops with high-grade dietary protein content which makes them crucial for reaching dietary sufficiency in India as they can supplement diets high in carbohydrates by supplying protein. Therefore, they have a big potential to improve people's health, preserve soil, protect the environment, and guarantee the safety of the global food supply. India has long been the largest producer, consumer, and importer of pulses in the world. Pulses contribute approximately 24% of India's total GDP in terms of global output. As the government gave away free groceries to the needy and poor during the most recent and devastating pandemic in India, demand skyrocketed.

Pulses contain 20 to 25 percent protein by weight. The protein content is astonishingly three times higher than that of rice and twice that of wheat. These factors prompt many nations or states to take charge and inform the public about the necessity of pulses for food security as well as their priceless nutritional advantages. For instance, the United Nations declared 2016 the "International Year of Pulses" (IYP) to raise public awareness of the benefits of pulses in terms of nutrition as a part of sustainable crop production to meet the rising demand for improved nutrition and food security. After the successful celebration of the IYP in 2016, the United Nations General Assembly (UNGA) declared 10 February to be World Pulses Day (WPD).

For sustainable agri-food systems, pulses help to open up economic, social, and environmental prospects. However, young people must be the focal point of any strategy if pulse-driven agriculture is to be adopted successfully. Youth can serve as a link between conventional farming methods and modern technology, making agriculture more nutrient-conscious and sustainable. Additionally, they can add value to the advertising of pulses by highlighting fresh business prospects along the value chain. As a result, in 2022, the WPD was celebrated with the theme "Pulses to empower youth in achieving sustainable agrifood

systems," emphasizing the crucial role that young people play in bringing about a better future for food and bringing testimonies and perspectives from youth organization representatives.

Although pulses have been produced and consumed in India for millennia, their contribution to total food grain production has decreased from 16.6% in 1950–51 to 7% in 2014–15, in part because of stagnation in pulse production technology and the area dedicated to pulse cultivation since the Green Revolution (Kasliwal, 2021). According to household consumption surveys, the consumption of pulses has clearly decreased, which has led to an increase in malnutrition (Shalendra *et al.* 2013). Unfortunately, India still has a record-high 24% of the world's undernourished population (Tripathi *et al.* 2019). This demonstrates the vital significance of leguminous pulse crops, particularly for improving nutrition and ensuring food security for India's enormous and alarmingly growing population.

1.2 Mung bean as one important leguminous pulse crop

Vigna radiata (L.) R. Wilczek belonging to the family Fabaceae *sensu stricto* is one crucial non-endospermic leguminous pulse crop. It is additionally referred to as green gram, mung, moong, golden gram, Oregon pea, and so on (Lambrides & Godwin, 2007). It is a self-pollinating, annual summer crop with a comparatively shorter life cycle, usually ranging from 60 to 90 days (Sandhu & Singh, 2021). It was originally domesticated in India. Later, it increased its dimensions in other tropical, and sub-tropical countries (Fuller, 2007), and is now globally cultivated in a diverse area (Nair *et al.* 2019; Aski *et al.* 2021).

India is one of the largest producers, consumers, and exporters of mung bean in the world (Ali & Gupta, 2012). From 2017 to 2018, mung bean production in India was 2.01 Mt over an area of 4.26 Mha (Nair *et al.* 2019). Being a leguminous pulse crop, mung bean is an amusing source of easily assimilated protein. Additionally, it contains vitamins, minerals, fiber, essential amino acids, and essential fatty acids (Nair *et al.* 2013; Foyer *et al.* 2016). In India, it is frequently eaten as whole grain or split daal. But, in Asian countries, it has other varied practical uses in the form of sprouts, flour, soup, porridges, and cooked mung bean seeds (Nair *et al.* 2013; Aski *et al.* 2021). Supplementing mung bean with cereals delivers a well-balanced intake of the required nutrients we need. In Western countries, it is primarily consumed as sprouts and, more recently, as processed products like meat or egg substitutes, chips, and nut-free butter (Sandhu & Singh, 2021). Moreover, due to the presence of

secondary metabolites, especially phenols, and flavonoids, with their health-promoting benefits, mung bean, specifically in the form of bean sprouts, is gaining increasing popularity day by day (Ganesan & Xu, 2018). Mung bean shows a symbiotic relationship, with *Rhizobium*, and thus enhances soil fertility by fixing atmospheric nitrogen (Allito *et al.* 2014; Fuskhah *et al.* 2019), which renders their fertilizer-free cultivation. Therefore, they not only satisfy their own nitrogen demand but also assist the succeeding crop plants in doing the same.

1.3 Origin of the problem

Although mung bean has many beneficial uses in the fields of nutrition, medicine, business, and agriculture, its overall output is capped by its vulnerability to a variety of environmental abiotic and biotic stressors, as well as the ever-increasing effects of climate change (Sehrawat *et al.* 2019; Alderfasi *et al.* 2017; Singh 2018). Among all the diverse abiotic stresses (*viz.*, heat, cold, salinity, drought, heavy metals, etc.), salinity stress is one of the most critical atrocious environmental factors in agricultural sector as it is closely associated with both ionic toxicity, and osmotic stress, and thereby significantly limits plant growth, and productivity of economically essential pulse-crops (Upadhyay & Panda, 2005), especially in arid, and semi-arid regions worldwide (Bangar *et al.* 2019; Nair *et al.* 2019; Sehrawat *et al.* 2019). Nearly 6% of the world's total land area is salt-affected (FAO, 2009), and it is predicted that by 2050 salinization could affect 50% of all the cultivated lands (Jamil *et al.* 2011). Though salinity problems are most common in arid and semi-arid regions of the world, they are also a grave problem in irrigated lands, and dry areas (Fuskhah *et al.* 2019). About 20% of the irrigated land is globally salt-affected and is constantly growing at an alarming rate of 1% to 3% per year (Qadir *et al.* 2014). In India, with approximately 6.73 million ha area is adversely affected by salinity problems (Arora, 2017). In a nutshell, throughout the world, salinity badly affects crop plant's (including mung bean) different growth parameters and reduces the overall productivity and quality (Sharifi *et al.* 2007).

Basically, increased salinization is a direct outcome of the disruption of soil-ecological balance by various anthropogenic activities and may include:

a) Speedy expansion of more extensive industrial and agricultural sectors, b) uncontrolled, and excessive use of irrigation water chiefly for cultivation purposes, c) wide-ranging transportation,

d) vigorous mining, e) extensive construction of buildings, housing complexes and concrete structures, and, lastly, f) deforestation at an alarming rate (Sharma *et al.*, 2014; Singh, 2018). By inducing osmotic stress, and ionic toxicity, salinity unfavorably affects crop plants causing several significant changes such as reduction in root's water absorption capacity, increment of evaporation rate from the leaf surface, disturbance in nutrient balance, denaturation of structural and functional proteins, and metabolic damages in growing pulse-crops. Accumulated toxic ions in plant tissues considerably hamper stomatal movement and the rate of photosynthesis by causing abnormal ionic distribution, thereby also affecting crop plants' long-term productivity (Ahmad & Umar, 2011; Hasanuzzaman *et al.* 2013).

Salinity sternly impedes water uptake by crop plants (including mung bean) through ionic toxicity (along with dehydration), and hyperosmotic stress effects (Hasanuzzaman *et al.* 2013). The detrimental effects of salinity include harmful nutrient imbalance, brutal membrane damage, deleterious alterations in growth regulator concentrations, inhibition of enzymatic activities, formation of reactive oxygen species (ROS) leading to lethal DNA damage, and the beginning of programmed cell death (Gill & Tuteja, 2010). In the cells of crop plants, ROS formation is strongly induced by higher levels of salt stress conditions. By this overproduction of ROS, serious oxidative damage may occur in membrane lipids, proteins, and nucleic acids (Pérez- López *et al.* 2009).

Another critical challenge that we usually face is the management of salt tolerance in crop plants through different innovative and valuable strategies. Salt tolerance is a complex, and polygenic trait that is directly related to plant genotype and plant development. In response to salt stress, many deleterious changes occur in plants at the molecular, biochemical, and physiological levels and thus, triggering cell signaling pathways connected with stress tolerance, and defense mechanisms (Nadeem *et al.* 2019).

Previous studies have uncovered several useful practically applicable solutions to the problem of salt stress and sustainable production. There are two categories of management strategies: (a) soil-based management and (b) plant-based management. The former entails three things: replacing scarce natural resources in the surroundings, leaching excess salt, and restoring the salty soils for plant growth. Plant-based management, on the other hand, includes: (i) osmotic homeostasis by synthesizing and accumulating different crucial osmolytes, (ii) ionic homeostasis by eradicating (or grasping) toxic Na^+ ions, (iii) enhanced free radical scavenging systems to avoid oxidation, (iv) mitigation of salt stress through seed

pre-treatments by multiple pretreating agents, (v) identification and utilization of stress-tolerant genotypes and lastly, (vi) development of stress-tolerant cultivars either by the recent and updated transgenic approach or by the so-called commonly used traditional breeding technique. When compared to the soil-based management strategy, plant-based management is the most impactful and cost-effective of the above-mentioned strategies (Hasanuzzaman *et al.* 2013; Sehwat *et al.* 2014; Nair *et al.* 2019; Sehwat *et al.* 2019).

For this purpose, seed priming, a well-established pre-sowing technique, can effectively be employed as it can mitigate the adverse effects of salinity (Gholami *et al.* 2015). There are several approaches to priming viz., solid matrix priming (SMP), hydro-priming, osmo-priming, halo-priming, thermo-priming, and bio-priming (Ashraf & Foolad, 2005).

SMP, which has garnered less academic attention and practical application than liquid priming, is a brand-new, very favourable priming approach (Sen & Mandal, 2016; Copeland and McDonald, 1995; Pill, 1995). In order to examine the physiological, biochemical, and morphological effects on mung bean seedlings grown under various salinity stress settings, the SMP approach has been chosen for the current investigation's pre-sowing seed treatment. Additionally, thanks to recent outstanding breakthroughs in nanotechnology, nano-priming is growing in popularity in the agricultural industry. Nano-chitosan and matrix conditioning have been chosen as unique strategy of seedling invigoration for the current study because there hasn't been much research on mung beans before. To the authors' knowledge, no such research works have been conducted on mung bean yet (till the publication of our papers).

The following are further justifications for choosing chitosan and nano-chitosan as the priming agents. Another benefit of the work is the relatively simple manufacture of nano-chitosan from chitosan using the faster, simpler, and Ionic gelation process, which eliminates the need for organic solvents. Additionally, chitosan, a naturally occurring biopolymer with multiple uses that is renewable and chemically inert, has a wide range of uses in biology, including agriculture. Its intriguing qualities include bioactivity, non-toxicity, biodegradability, and compatibility with living things (Cheba, 2011). In addition to promoting plant growth, chitosan, according to Zhang *et al.* (2021), also lessens the negative effects of salt stress on plants. The characteristics of nano-chitosan include small size, surface and interface effects, and the quantum size effect in addition to the qualities of chitosan (Ingle *et al.* 2008).

Studying the interactions between the genes, proteins, transcripts, and metabolites is necessary to comprehend a system like secondary metabolism. Numerous omics platforms are utilized to analyze this network to comprehend its complexity. Nevertheless, while this network permits the determination of a relationship between the genes, and metabolites, an oversized portion of data on the connection between genes, transcripts, proteome, and metabolome is still leftover as indefinable (Mounet *et al.* 2009).

1.4 Existing knowledge gaps

Although seed nano-priming has received a lot of interest recently in the world of agriculture due to its positive benefits, a survey found that far less research has been done on this topic in leguminous pulse-crops like mung bean. The possible role of nano-chitosan employing SMP in mung bean has not been exclusively studied before, to the authors' knowledge (except our published papers). In order to ascertain how the chosen elicitors, including nano-chitosan given through SMP, affected the performance and development of mung bean seedlings grown under varied salt stress conditions, that was the major goal of the current study.

On the other hand, the effect of fewer used novel priming methods, SMP in Mung bean sprouts, also demonstrates vast potential in seedling invigoration at a commercial scale that has yet to be investigated. The proposed study aims to investigate these areas in particular and highlight their promising role in modern science. There is enough room to go deeper into these topics. As a result, a deeper understanding, and applications are essential.

Evaluation of differential physiological behavior of the mung bean germplasm in a saline environment by matrix conditioning (SMP) using different selected elicitors reliably provides suitable material as a resource for agronomic traits or genes that can be further introduced by breeding into salt-sensitive leguminous pulse-crops like mung bean.

Considering the growing interest in the consumption of mung bean sprouts as a good substitute for other green vegetables used as salads or fresh dishes, the current study is intended to evaluate the effect of mung bean bioactive compounds as affected by germination under matrix conditioning (SMP) using different selected elicitors.

Further, it is vital to explore the level of antioxidants in mung bean sprouts along with the mitigation of oxidative stress induced by different levels of salinity stress through SMP treatment with selected elicitors, including nano-chitosan.

Evaluation, and exploration of antifungal activity of nano-chitosan against the mung bean seed-borne mycoflora, especially *Aspergillus flavus*, after isolating from stored mung bean seeds, and the proper identification of the most common mung bean seed-borne fungi is also an important aspect of the proposed work.

Numerous investigations have already been done on plant phenols, and their antibacterial and antifungal properties are well-established in various plants (Taiz & Zeiger, 1998). However, mung bean sprouts' phytochemical analysis and bioactivity screening have received very little attention to date. Despite the well-documented significance of the plant metabolites, there's a considerable quantity of knowledge gap concerning the genes, their regulation, accumulation, and degradation (Forster *et al.* 2003). Thus, for secondary metabolite study, it has been directed to the mapping of individual pathways, and intermediate concerns within the manufacture of those products. Such secondary metabolites of plants are actually controlled by the genome all the way to the metabolome. They work along concertedly as an established system. Thus, variations at the genomic level can result in modifications to the transcripts that eventually result in an alteration to the metabolites. These modifications end in an overall variation of different processes inside the plant body. Nevertheless, changes at every level, though connected, are not linear. Therefore, an accurate Analysis through an integrated approach of the omics technologies will undoubtedly enhance our understanding of varied phytochemicals in the model, and non- model species (Ulrich-Merzenich *et al.* 2007; Metlen *et al.* 2009; Forester and Waterhouse, 2009). However, it's vital to accumulate helpful tools that can evaluate this information at a fast pace and apply the presently obtainable information for a better understanding of the non-model plant systems (Hammami *et al.* 2009).

Quantitative study and standardization of major mung bean isoflavones through HR-LCMS of mung bean sprouts (treated, and untreated) grown under salinity stress conditions, and their in- silico analysis associated with the therapeutic implications in humans along with their correlations with salt stress-related genes using the online resources, and web-tools are also chief ideas of the current work.

1.5 Hypothesis

Being a short-duration crop, mung bean produces a large amount of biomass in the summer. As such, it can be quickly grown in newly reclaimed sandy soils and irrigated with saline

water (Radwan *et al.* 2013). It is one of the most important pulse crops both in the world as well as in India. Though it is the third most important pulse crop in India, following chickpea and pigeon pea, unfortunately, in West Bengal, compared to other states of India, its cultivation areas are not so widespread, and its production is comparatively very less. Anyway, its cultivation should be encouraged by adopting various farmer-friendly, cost-effective approaches, including the priming technique (Solid Matrix Priming) using the recent knowledge of nanotechnology (eco- friendly, and biodegradable nano-particles like nano-chitosan) in the agricultural sector to mitigate the adverse effects induced by various abiotic stresses like salinity, and biotic stresses like seed borne mycoflora prevailing in nature, responsible for decreased productivity in pulse crop like mung bean in the long run.

Mung bean sprouts can be a good choice for diversifying and nutritional food practices. Mung bean sprouts are very rich in a group of vitamin-B Complexes, especially thiamine, and also provide small amounts of many minerals, particularly copper, and zinc. They also have moderate amounts of phenolic compounds, including flavonoids. Being a legume, it contains high protein (Khalil, 2006). They have excellent nutritional values concerning methionine, and cysteine, which are usually lacking in other food crops (Tsou & Hsu, 2000). As a major legume crop in Asia, mung bean can supplement the mostly cereal-based diets of the poor. When cereals and legumes (pulses) are consumed together, people get a healthy and balanced diet. Cereals are deficient in the amino acid lysine, which is present in legumes, while legumes are deficient in sulfur-rich amino acids, which are present in grains. Not only are they high in protein content, but they are also easy to digest. Therefore, when ingested in combination with cereals, the quality of the protein in a meal is increased remarkably (Thirumaran & Seralathan, 1988). Limited cooking is good for nutritional value, while extensive cooking is harmful (Finney, 1983). Antinutritional factors can be reduced by cooking the seeds with vegetables such as tomatoes, and cabbages (Bains *et al.* 2003).

New techniques of priming are constantly being sought worldwide to improve nutraceutical properties, and yield of pulse crops. SMP is one such novel and innovative approach. The use of phenylpropanoid elicitors like chitosan in both bulk, and nano-form applied through SMP to activate mung bean seedling invigoration is also a novel approach that has not yet been implemented in mung bean. According to the literature survey, no studies on SMP using nano- chitosan in mung bean have been conducted (till the publication of our papers). In order to assess the effects of nano-chitosan on seedling vigor and antioxidant activity in mung bean seedlings under salt stress conditions, seedlings must be treated with SMP. Further,

isoflavone, one of the pharmacologically essential flavonoids, and phytoestrogen are also found in mung bean sprouts. It is helpful to prevent and treat osteoporosis. Its isolation or purification from various plants for different purposes is a recent trend. It lowers blood total and LDL cholesterol in men, the risk of various chronic diseases, and the risk of several other disorders (Taku *et al.* 2007). In this context, the present investigation was carried out with the hypothesis that nano-chitosan used through SMP will increase the nutraceutical properties in mung bean sprouts, and simultaneously improve the seedling vigor by ameliorating salinity stress in mung beans. The present study was designed as follows:

First, five common mung bean cultivars of West Bengal were chosen, and efforts were made to find out which cultivar was the most salt-sensitive and salt-tolerant (screening cultivars under salt stress). Following SMP with fifteen (15) distinct carefully chosen elicitors, the rather salt-tolerant mung bean cultivar is to be used (for screening of elicitors). Using a variety of techniques, such as X-ray diffraction analysis (XRD), transmission electron microscopy (TEM), Fourier transform infrared (FTIR) spectra analysis, scanning electron microscopy (SEM), and, dynamic light scattering (DLS) analysis, prepared nano-chitosan will be characterized. Following SMP, morphological and biochemical analyses will be conducted on mung bean seedlings under salt stress (abiotic stress) conditions using water (hydropriming), chitosan, and nano-chitosan. After that, the antifungal activity of nano-chitosan will be tested on one of the most prevalent seed-borne fungi, which will be isolated and identified from mung bean seeds. Thereafter, a study of the major mung bean isoflavones will be conducted through HR-LCMS along with their *in silico* drug study related to the therapeutic implications of the concerned isoflavones. Finally, a few chosen genes related to the implementation of salt tolerance in mung bean plants after SMP with suitable elicitor will be studied through quantitative real-time PCR.

1.6 The Objectives of Research work

The current research project's primary goals are as follows:

1. To determine the differential physiological behaviour of five different mung bean cultivars, most commonly used for cultivation in West Bengal, under different salt stress conditions, and to find out the most suitable or salt tolerant mung bean cultivar along with the most sensitive one against the given salt stress conditions, among the five selected mung bean cultivars.

2. Screening, and comparing the efficacy of fifteen (15) different selected elicitors like chitosan, gallic acid, catechol, salicylic acid, putrescine (PUT), gibberellic acid (GA₃), kinetin, naphthalene acetic acid (NAA), abscisic acid (ABA), calcium chloride, proline, CaCl₂, Lanthanum chloride (LaCl₃), ethylene glycol bis (2-aminoethyl ether) tetra-acetic acid (EGTA), and sodium nitroprusside (SNP) for seed priming for the enhancement of seed germination, and seedling growth under in three different saline solutions including control (*i.e.*, 0, 4, and 8 dS/m).
3. To assess the differential physiological behaviours of mung bean seeds after SMP using four different concentrations (0, 0.1%, 0.2%, and 0.5%) of chitosan at five (05) different salt stress (0, 4, 6, 8, and 12 dS/m) concentrations using celite as matrix under three distinct moisture levels (5%, 10%, and 20%).
4. To prepare and characterize nano-chitosan in the laboratory, through Dynamic light scattering (DLS), Zeta Potential (ZP), Fourier transform infrared (FTIR) spectra analysis, Scanning electron microscopy (SEM), Transmission electron microscopy (TEM), and X-ray diffraction analysis (XRD).
5. To analyze the ability of elicitors like chitosan, nano-chitosan, and water (hydropriming) to alleviate the detrimental effects of salt stress with an emphasis on several oxidative stress markers.
6. To evaluate the bioactive compounds, non-enzymatic and enzymatic antioxidant activity of mung bean sprouts as affected by germination under SMP using chitosan, nano-chitosan, and water (hydropriming).
7. To evaluate the antifungal activity of different concentrations of nano-chitosan on spore germination, and mycelial growth of selected mung bean seed-borne fungus (*Aspergillus flavus*) and measurement of various attributes of germination, and seedling vigor in mung bean seeds after priming (SMP) inoculated with a spore suspension of *A. flavus*.
8. To analyze on-gel isozyme patterns of native PAGE and gel electrophoresis (SDS-PAGE) of mung bean proteins obtained from treated (nano-chitosan primed, chitosan primed, hydro primed), and untreated seedlings grown under salt stress conditions.
9. To study differentially expressed mung bean proteins through Orbitrap high-resolution liquid chromatography-mass spectrometry (OHR-LCMS)

10. To evaluate the effect of SMP on mung bean sprouts grown under salinity stress for quantitative study of mung bean isoflavones studied through HR-LCMS analysis, and the metabolic shifts of major isoflavones having therapeutic implications along with the correlations with salt stress-related genes.
11. To assess the impact of SMP with nano-chitosan in the genetic expression of some selected salt stress genes of mung bean through Quantitative RT-PCR study.

