Screening for the Optimum Concentration of Chitosan through Seed Priming in Mungbean Genotypes

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Authors' contributions

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

Original Research Article

ABSTRACT

Four different concentrations of chitosan and four mungbean genotypes were studied to determine the optimum concentration of chitosan seed priming. The experiment results revealed that germination percentage, shoot length, root length, fresh weight, dry weight, and seedling vigor index differed significantly among the treatments and genotypes. The greatest impact was observed at lower concentrations of 0.15% in all four genotypes showing consistent improvements in all the studied parameters. The highest concentration of 0.35% appears to be at par with the control and shows no statistical significance. However, root length was affected in IPM 2-14 and IPM 2-3 at high concentrations. In terms of seedling vigor index, IPM 2-3 showed significant reductions at 0.35%, while other genotypes recorded improvement. Based on our results, we conclude that chitosan seed priming has genotype-specific responses, and lower chitosan concentrations have significant positive effects on all parameters and provide important results for deployment in sustainable agricultural practices.

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1. INTRODUCTION

Mungean (*Vigna radiata* L.) is widely used globally as a major component of many crop systems. The plant is primarily grown in India and other Asian nations as green gram or golden gram. Due to its high protein content, it is commonly consumed as sprouts or dried seeds [1]. The plant has an amazing ability to fix atmospheric nitrogen to soil up to 251 kg/ha by maintaining a symbiosis with *Rhizobium* bacteria. It also enhances soil fertility, supporting crop production [2]. Due to its short growing period (60-70 days), mungbean is also suitable for intercropping between two cereal crops [3]. Despite the high yield potential of mungbean, its average productivity remains astonishingly low at 0.5 t/ha. Poor crop management practices, abiotic and biotic constraints and the absence of quality seeds of improved varieties contribute low productivity [2,4].

In recent years, advancements in seed technology have led to increased interest in the use of chitosan in agricultural applications. These applications should consider the nature, origin, application form, and physical characteristics of chitosan as these affect the physiological characteristics of seed [5–7].

Chitosan is a natural polysaccharide obtained from the deacetylation of chitin. Chitin is the most abundant polysaccharides found on the planet next to cellulose which is present in the exoskeleton of arthropods, such as crap, shrimp and some fungi [8]. Due to the presence of amino groups in chitosan, it can be manipulated structurally with various bioactivities for use in various agricultural systems such as defense inducers of biotic and abiotic stresses and enhancing crop growth and yield [9].

Using the priming technique, chitosan increased wheat seed germination by 18%, germination rate by 53%, and seedling vigor by 27% [10]. A significant increase in both germination rate and growth parameters was also reported for rice [11]. Additionally, chitosan seed primed seedlings in pearl millets were shown to improve germination rate by 13% and vigor by 18% [12]. Previous research findings such as in bean seeds, artichoke seed and soybean are reported to improve seedlings parameters [13–15].

A significant constraint to yield in mungbean crops is poor stand establishment [16]. In agriculture, one of the current goals is to improve the emergence and establishment of seedlings that are both physically and genetically weak. Furthermore, the advent of climate change adversely impacts crop growth, especially rainfed crops like mungbean [17].Thus the use of chitosan in seed technology is a strategy that takes advantage of its characteristics to formulate coatings that are an integral part of the management of crops; its application is intended to enhance the physiological and functional responses during the early stages of crop growth. So, the present study aimed to identify the optimum concentration of chitosan seed priming in different mungbean genotypes through observations of germination and seedling parameters.

2. MATERIALS AND METHODS

The experimental was done in Plant Stress Physiology Laboratory, Department of Plant Physiology, Banaras Hindu University located in the south-eastern of the Varanasi, between 25.18°N latitude, 83.03°E longitude and 123.93 m above mean sea level. Chitosan of low molecular weight and deacetylation ≥ 75% were obtained from Sigma-Aldrich Co (St Louis, MO, USA). Four genotypes of mungbean viz. Samrat, IPM 2-14, IPM 2-3 and HUM 2 were procured from Indian Institute of Pulse Research, Kanpur, India and genotype HUM 2 from Department of Genetics and Plant Breeding, Banaras Hindu University, Varanasi, India. All the chemicals used were analytic grade and used further used without purification. The chitosan was dissolved in 0.1% acetic acid solution under constant stirring overnight and was adjusted to pH 6 using 1N sodium hypochloride. Seeds were primed in different concentrations of chitosan viz. 0.05%, 0.15%, 0.30% and 0.45% and control with distilled water for 6 h. The soaked seeds were washed and air dried to regain the original weight. Then 20 seeds of each genotype were placed in two layers of Whatman no. 1 filtered paper placed in petri dishes moistened with distilled water and finally placed in controlled germination incubator at 28°C±1°C exposed to 16 h photoperiod under 260 µmol m^{-2} s^{-1} photon flux density (PFD). All the treatments were replicated three times. After 7 days, germination and seedlings parameters were measured. Treatments details are as follow-

T0- Control
T1- CS (0.05%)

T2- CS (0.15%)
T3- CS (0.25%)
T4- CS (0.35%)

2.1 Germination (%)

Germination percentage was observed in both the mungbean genotypes by the given formula:

$$\text{Germination \%} = \frac{\text{Total number of seed germinated}}{\text{Total number of seed sown}} \times 100$$

2.2 Seedling Vigor Index (SVI)

Seedling vigor index measured in 7-day old seedling calculated by the formula given as under: SVI= germination % × Seedling lengths (Root length + shoot length)

2.3 Measurement of Root Length and Shoot Length

Root length and seedling length in wheat seedling were measured by the help of centimeter scale.

2.4 Fresh Weight of Roots and Shoot (g plant^{-1})

The fresh and dry weight of seedlings were measured and expressed in g plant^{-1}. Roots and shoots were separated before fresh weight was assessed via a Sartorius BT-224S electronic balance. Freshly weighed seedlings were placed in the envelopes, after which they were placed in a hot air oven for one hour at 100°C. The temperature was then decreased to 65°C until the constant weight of the samples could be achieved.

2.5 Statistical Analysis

All data presented are subjected to one-way ANOVA and treatment means were compared with Duncan multiple range test (DMRT) at significant level of 5% ($p<.05$) through SPSS version 26 (IBM).

3. RESULTS

3.1 Germination %

Germination % of all the four genotypes is presented in Table 1(a). Germination % was found to increase with the increasing chitosan concentration. But there was variation in the magnitude of response between the genotypes.In Samrat and IPM 2-14, all chitosan treatments (T1-T4) showed significant increase in germination % ($p<.05$). While, in IPM 2-3, T1, T2 and T3 recorded maximum germination %. In HUM 2, T1, T2 and T3 showed maximum increase while control (T0) and T4 produced non-significant value ($p<.05$).

3.2 Shoot length

Data revealed significant differences among the treatments, as presented in Table 1 (b) and Fig. 1. In Samrat, the shoot length significantly increased ($p<.05$) in T1, T2 and T3 with maximum value obtained in T2. However, T4 resulted in significant reduction ($p<.05$). Similarly, in IPM 2-14, shoot length significant increased in all the chitosan treatments. Maximum was found in T2. In IPM 2-3, there was no significant effect ($p<.05$) between T1 and T2. In HUM-2, T2 had a significant effect as compared to other treatments. Overall, T2 recorded optimum results in all the genotypes.

3.3 Root length (cm)

Root length was measured in four genotypes of mungbean (Table 1(c) and Fig. 1). Chitosan treament induced a significant increase in root length of all the genotypes. Significant variation was observed in Samrat where highest increase was found in T1, T2 and T3, however, theyexhibited non-significant effects between them at $p<.05$. In IPM 2-14, T0, T1 and T3 showed no significant differences at $p<.05$. However, there was a significant reduction in T3 and T4. Also, in IPM 2-3, significant increase ($p<.05$) was observed in T2 and T3. The lowest value was observed in T4. In HUM-2, T1 and T2 showed significant increase ($p<.05$). However, T3, T4 and T0 showed non-significant effect between them.

3.4 Fresh Weight of the Seedlings

Fresh weight was measured in four genotypes and found that chitosan treatment in all the concentrations significantly influenced the measured parameters (Table 1d). In Samrat, maximum fresh weight was found in T2 and T1 with a non-significant value among them ($p<.05$). In IPM 2-14, there was significant ($p<.05$) increase in T2 while other treatments were found to be non-significant ($p<.05$). In IPM 2-3, T1 and T2showed maximum increase in fresh weight. While, T0, T3 and T4 observed non-significant value ($p<.05$). Lastly, in HUM 2, all chitosan treatments increased the fresh weight except T4

which was found to be non-significant with T0 (*p<.05*).

3.5 Dry Weight of the Seedlings

The dry weights of the seedlings were measured in all the genotypes showing significant variation among the genotypes and treatments (Table 1e). In Samrat, T2 was found to have maximum value while other treatments were non-significant (*p<.05*) among them. In IPM 2-14, dry weight was found to be non-signifcant in all the treatments. In IPM 2-3, maximum was found in T2. However, lowest value was found in T4 as compared to T0. In HUM 2, significant increase was found in T2 and T3 (*p<.05*).

3.6 Seedling Vigor Index

SVI increased in all the genotypes as chitosan concentrations increased (Table 1 (f)). Statistically significant differences were found in all the treatments in all the genotypes. In Samrat, the maximum vigor index was found in T2 with significant value as compared to other treatments. Similarly, in IPM 2-3, T2 was found to have maximum value while lowest was observed in T4 and T0. In IPM 2-3, T2 observed maximum increase as compared to other treatments. While maximum reduction was observed in T4. In HUM 2, T2 observed significant increase in vigor index. Overall, the optimum concentration was T2 which was 0.15%.

Table1. Effect of different concentrations of chitosan on morphological seedling parameters a) germination percentage b) shoot length c) root length d) fresh weight e) dry weight f) Seedling vigor index.Mean values of three replicates are presented. Within a column, means followed by different letters are significantly different (*P>.05*) according to the DMRT post-hoc analysis

a)

Treatment	Germination%			
	Samrat	IPM 2-14	IPM 2-3	HUM-2
T0	90.00b	91.67b	86.67b	86.67c
T1	96.67a	96.67a	96.67a	96.67ab
T2	100.00a	100.00a	98.33a	98.33a
T3	98.33a	98.33a	96.67a	95.00ab
T4	96.67a	96.67a	86.67b	90.00bc
SEm±	**1.29**	**1.49**	**1.66**	**2.23**

b)

Treatment	Shoot length (cm)			
	Samrat	IPM 2-14	IPM 2-3	HUM-2
T0	11.83c	12.33c	12.20cd	11.67c
T1	12.90b	13.17b	13.50ab	12.27c
T2	13.93a	14.20a	14.33a	13.83a
T3	12.88b	13.40b	12.93bc	12.93b
T4	11.17d	11.87c	11.41d	12.17c
SEm±	**0.14**	**0.20**	**0.29**	**0.18**

c)

Treatment	Root length (cm)			
	Samrat	IPM 2-14	IPM 2-3	HUM-2
T0	7.37c	8.47a	7.83b	7.33c
T1	8.13ab	8.80a	8.00b	8.17ab
T2	8.46a	9.17a	8.57a	8.37a
T3	8.00abc	7.10b	8.33ab	7.70bc
T4	7.67bc	7.00b	7.17c	7.27c
SEm±	**0.19**	**0.25**	**0.15**	**0.17**

d)

Treatment	Fresh weight (g)			
	Samrat	**IPM 2-14**	**IPM 2-3**	**HUM-2**
T0	0.297c	0.361b	0.317b	0.290d
T1	0.388ab	0.373b	0.393a	0.373b
T2	0.420a	0.463a	0.407a	0.39a
T3	0.350b	0.353b	0.317b	0.340c
T4	0.263c	0.327b	0.317b	0.304d
SEm±	**0.16**	**0.15**	**0.01**	**0.01**

e)

Treatment	Dry weight (g)			
	Samrat	**IPM 2-14**	**IPM 2-3**	**HUM-2**
T0	0.051b	0.056a	0.053b	0.041b
T1	0.054b	0.058a	0.055b	0.046b
T2	0.062a	0.063a	0.064a	0.058a
T3	0.054b	0.057a	0.058b	0.058a
T4	0.050b	0.057a	0.051c	0.040b
SEm±	**0.002**	**0.003**	**0.002**	**0.002**

f)

Treatment	Seedling vigor Index			
	Samrat	**IPM 2-14**	**IPM 2-3**	**HUM-2**
T0	1728.00c	1905.87cd	1735.33c	1646.67c
T1	2032.68b	2121.67b	2077.33b	1975.33b
T2	2239.00a	2336.67a	2251.00a	2183.17a
T3	2053.60b	2016.33bc	2056.17b	1960.50b
T4	1821.67c	1824.00d	1609.02d	1747.83c
SEm±	**42.49**	**40.88**	**26.96**	**46.95**

Fig.1. Photograph of seedlings primed with different concentrations of chitosan

4. DISCUSSION

As demonstrated in various crops including wheat, maize, pepper, soybean and spinach, seed priming enhanced seedling establishment with uniform seed germination and improved resistance to environmental stresses [17,18]. The present experiment aimed to screen the optimum chitosan concentration by observing germination and seedling parameters. According to our results, germination % and seedling vigor index were both increased across all chitosan treatments. Nonetheless, genotype-specific responses varied. In agreement with the previous report by Jogaiah et al. (2020) [19] seed priming with chitosan exhibited increased germination % and vigor index in cucumber seeds. Seed priming induces the expression of aquaporins, which increase the transportation of water across the membrane, facilitating tissue expansion and germination potential [17]. All chitosan treatments resulted in longer shoots and roots. However, genotypes respond differently to chitosan treatment. Higher concentrations of chitosan reduced shoot length in Samrat genotype and decreased root length in IPM 2-14 and IPM 2-3 genotypes. Based on these results, it is likely that a higher concentration of chitosan caused oxidative stress in embryo cells, which adversely affected seed germination potential and reduced antioxidative enzyme activities [20]. At the same time, a low concentration of chitosan stimulates the germination potential and increases seedling establishment. Researchers have observed similar results in seed priming with chitosan nanoparticles at 0.5% in broad bean and observed significant reductions in germination parameters, and suggested that a lower concentration of 0.05% increased the antioxidant enzymes and total phenol content [21]. Rasheed et al. (2020) [22] found that seed priming with low molecular weight of chitosan increased shoot fresh weight, root fresh weight, and shoot/root dry weight, which was consistent with our own results. As reported in rice [23] and maize [24], seed priming with chitosan also promotes early seedling establishment and synchronized growth. Furthermore, chitosan increased shoot and root lengths, fresh and dry weights of the shoot and root, and leaf area of bean plants soaked in chitosan solution [25]. Hameed et al. (2014) [10] also found that seed priming with 0.50% chitosan increased the shoot and root lengths of wheat seedlings. Additionally, chitosan at 0.50% and 0.25% reduced mean germination time, increased shoot length, shoot dry weight, root

length and root dry weight in maize genotypes as compared to controls [24]. Therefore, the present results are strongly supported by previous reports on germination and growth attributes.

5. CONCLUSION

The overall treatment that received 0.15% and 0.05% chitosan had significant effects on all genotypes. The higher concentration of chitosan negatively affected all genotypes' germination potential. The greatest impact was found in T2, 0.15% in all four genotypes, showing consistent improvements in all the parameters studied. We conclude that seed priming with chitosan at low concentrations could be beneficial to seedling establishment of mungbean plants and be used as a sustainable crop management practices. Nonetheless, the origin and characteristics of chitosan play a key role in how plants respond to growth. Hence, more research related to different origins and properties of chitosan would be needed to provide insights into the beneficial effects of chitosan on the plants, and further studies should be focused on physiological and molecular aspects related to chitosan responses.

DISCLAIMER

The products used for this research are commonly and predominantly used in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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