



## Response of NaCl and sea water salinity stress on seed germination, early seedling growth and proline content in a legume crop green gram (*Vigna radiata* L.)

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### Abstract

Recently, salinity in coastal water and soil has emerged as a major constraint to produce grain legumes; therefore, present study aims to evaluate the salinity stress effects caused by various concentrations (50mM, 100mM, and 250mM) of NaCl and sea water (5%, 10%, 15%, and 25%) on seed germination and early seedlings growth in green gram (*Vigna radiata* L.). Results reveal that after 7-days of treatments, in comparison to control response (100±1.98%), NaCl solution with high concentration (250mM) was turned out to be strongly inhibitory and treated seeds (60±0.49%) were seen partially germinated and failed to develop into complete seedlings. Furthermore, in contrast, sea water salinity treatments were turned out to be strongly inhibitory and even with very low concentration (5%) of sea water treatments, treated seeds (21±0.5%) were germinated partially and grow into shoots only. Significantly, after 10-days of NaCl (100mM) treatments, seedlings growths were found to be negatively affected and root-shoot ratio (1.75±0.2/5.31±0.31cm) was recorded as compared to control seedling (1.98±0.02/7.9±0.71cm). However, in case of sea water (10%) treatments, shoots without roots (0.0/2.05±0.46cm) were apparent. Furthermore, NaCl-stress induced seedlings on transfer to soil exhibit inhibited growth under continuous irrigation with respective NaCl-solutions. Furthermore, biochemical study indicates that endogenous proline contents gradually increase with the increase in NaCl-concentrations in treatment solutions and therefore, was found to be the maximum (203.2x10<sup>-3</sup>g<sup>-1</sup>) in the tissues that were growing with high concentration of NaCl (250mM) while it was found to be the minimum (98.6x10<sup>-3</sup>g<sup>-1</sup>) in control tissues.

**Keywords:** green gram, legume crop, proline, salinity stress, sea water

### Introduction

Pulses are treated as a primary source of protein in human diet worldwide because of high protein contents. Significantly, green gram or mung bean (*Vigna radiata* L.) is one of the important short duration pulse crops which are rich in proteins<sup>[1]</sup> and additionally, this legume is unique in terms of containing high quality of methionine proteins. However, its high yield potential has not been yet achieved because of several constraints particularly, its susceptibility to several abiotic stresses<sup>[2]</sup>.

In general, the major abiotic stresses such as drought, water logging, high salinity, heavy metals, excess heat, frost, and ultraviolet-B light irradiance (UV-B)-have been extensively studied in plants<sup>[3-11]</sup> but salt stress is known as one of the most significant environmental constraints cause to restrict legume productivity in arid and semi-arid regions<sup>[12, 13]</sup>.

Previous investigations suggest that saline soils provide unfavorable conditions for seed germination and plant growth leading to reduce agricultural production. Additionally, saline water irrigation induces an accumulation of salt at soil surface,

that causes negative impacts on germination, plant stand, plant vegetative development, productivity and yield of legume crops, and sometimes it leads to plant death<sup>[14-19]</sup>.

It is suggested that salt stress is first perceived by the root system and alters plant growth by inducing an osmotic stress due to reduced water availability and form the ion toxicity because of solute imbalance in the cytosol<sup>[20, 21]</sup>. Moreover, at lower concentrations, salt is known to suppress plant growth while at higher concentrations; salt stress can cause death<sup>[22, 23]</sup>.

Seed germination is known as a crucial phase in plant life that plays important roles in seedling establishment and subsequent growth. Previous reports reveal that germination is regulated by multiple endogenous factors, such as plant hormones, and by environmental conditions, including temperature and light<sup>[24-28]</sup>.

In general, salinity affects vegetative, and reproductive growth, pollen viability, and several physiological functions. However, some physiological, biochemical and molecular mechanisms are possibly involved to promote tolerance

against salinity stress. Recently, comparative study on salinity stress caused by NaCl and sea water during seed germination followed by early seedling growth has been reported [29] in kodo millet crop (*Paspalum scrobiculatum* L.).

In legume crops, salt stress constraints have been recorded in the several genotypes of model species *Medicago truncatula* Gaertn, *Lotus japonicas* L. and *Glycine max* L. [30-38]. Additionally, of late, impacts of osmotic stress and herbicide stress during seed germination and early seedling growth has been reported in green gram or mung bean [39].

Moreover, with the increase in salinity stress, percentage of seedling emergence and elongation in shoot/root lengths could be treated as selection traits for tolerance to salinity, however, the extensive study based on imposition of salinity stress during seed germination and early seedling growth in green gram legume is lacking.

Hence, the present study was aimed to analyze the sensitivity of green gram seeds to salinity stress caused by NaCl and sea water during seed germination and early seedling growth. Additionally, experiments were also undertaken to estimate the endogenous proline content in the tissues that were growing under NaCl-salinity stress.

## Materials and Methods

### Seed collection and sterilization

Seeds of green gram or mung bean (*V. radiata* L.) a wild cultivar were collected from PASIC, Puducherry (India). Healthy, uniform seeds were selected and washed thoroughly with teepol-20 and were further surface sterilized with ethanol (70%) for a minute followed by HgCl<sub>2</sub> (0. 1%) treatments for 7-8 minutes.

Additionally, sterilized seeds were washed 3-4 times with sterilized distilled water (SDW) and were soaked in the respective NaCl salt solutions and sea water for 3h. The soaked seeds were further transferred to sterile petridishes (9cm diameter) lined with two sterile filter papers added with either 5mL of distilled water or the respective salinity test solutions for salinity stress.

### Salinity Stress treatments

Various concentrations (50mM, 100mM and 250mM) of NaCl-salt solutions (w/v) and (5%, 10%, 15%, and 25%) concentrations of sea water (v/v) were considered to treat the seeds and responses were recorded in terms of ability of seed for germination and further early seedling growth under salinity stress conditions.

Furthermore, 10-15 seeds per petridish were considered for each treatment and two replicates in each treatment were performed. Germination tests were conducted under dark condition at normal room temperature (25-30°C). A seed was considered germinated when radicle was 2mm long. The germination percentage was determined counting the number of germinated seeds on the 3<sup>rd</sup> day for partial seed germination and 7<sup>th</sup> day of the treatments for the complete seed germination.

### Transplantation of seedlings to soil

To begin with 7-days old, germinated seedlings growing in sterile petridishes (9. 0cm diameter) lined with two sterile

filter papers supplemented with respective concentrations of NaCl-salt solutions were transferred to disposable glass contained with autoclaved soil. Further, transplanted seedlings were kept on irrigation with respective concentrations of NaCl-salt solutions and observations were recorded.

### Determination of proline content

For determination of proline accumulation in the tissues under NaCl-salinity stress, samples that were extracted from SDW-treated germinated seeds were considered as control and NaCl-treated germinated seeds as experimental. Moreover, during this study, procedure for the determination of free proline content was followed based on the protocol proposed [40].

To begin with, fresh germinated seeds (0. 5g) were homogenized with 5ml of sulfosalicylic acid (3%) using a mortar and pestle. Further, 2ml of extract was taken in test tube and 2ml of glacial acetic acid and 2ml of ninhydrin reagent were added in the extract. The reaction mixture was further boiled in a water bath at 100°C for 30 min followed by 4ml of toluene was added in it after cooling the reaction mixture

After thorough mixing, the chromophore containing toluene was separated and absorbance of developed red color was recorded at 520nm against toluene blank on UV-visible spectrophotometer. Moreover, the proline concentration was determined using calibration curve prepared from known concentrations of proline solutions and finally proline content was expressed as 10<sup>-3</sup>g<sup>-1</sup> fresh weight of tissue.

### Statistical analysis

Statistical data were recorded after first count (3<sup>rd</sup> day after treatments) for partial seed germination and final count (7<sup>th</sup> day after treatments) for full seed germination. Moreover, germination percentage (GP) and germination rate (GR) was calculated by the following formulae [41].

$$GP = \frac{\text{Number of total germinated seeds}}{\text{Total number of seeds tested}} \times 100$$

$$GR = \frac{\text{Number of Germinated seeds}}{\text{3<sup>rd</sup> Day of Count}} + \frac{\text{Number of Germinated Seeds}}{\text{7<sup>th</sup> Day of Count}}$$

All the treatments during present study were repeated three times and data are expressed as mean germination percentage with standard error (SE). Moreover, the statistical analyses were generated by applying SPSS statistical software package.

### Results

During the present study on seed germination and early seedling growth in green gram, stress responses caused by salinity stress were found to be based on concentrations and durations of NaCl and sea water treatments. Further, accumulation of proline contents in the tissues that were growing under NaCl-stress treatments were also found to be influenced due to the concentrations of NaCl- salt stress.

### Effect of NaCl-salinity stress on seed germination

To begin with, sterilized seeds of green gram for control experiment and NaCl-stress treatments were kept under

soaking conditions in SDW and respective NaCl-solutions (Fig. 1a) for three hours. Though in control experiments, seeds were found to exhibit the initiation of germination next day of the treatments but the complete emergence of radicle and plumule of the germinated seeds could be seen only at the end of 3<sup>rd</sup> day of treatments (Fig. 1b).

Also in seeds that were treated with lower concentrations of NaCl solutions (50mM and 100mM), almost similar germination symptoms were observed (Fig. 1c & d) respectively to the control treatment, however, elongations of radicle and plumule were seen as relatively shorter than control treatment. Furthermore, seeds that were treated with high concentration of NaCl (250mM), exhibited strong inhibitions in seed germination, and merely emergence of radicle or partial germination (Fig. 1e) could be apparent till the end of 3<sup>rd</sup> day of the treatments.

At the end of 7<sup>th</sup> day of treatments, the full seed germination and development of complete seedlings were observed in control experiment (Fig. 1f) whereas similar response but inhibited seedlings developments were also observed in NaCl-treated seeds. Significantly, even low concentration of NaCl (50mM) was proved to be effective to cause salinity stress inhibition and therefore, seedling elongation was found to be shorter than the control seedlings (Fig. 1g).

Moreover, such salinity stress induced inhibition in seedling elongation was more prominent in seeds that were treated with high concentration of NaCl (100mM) and therefore seedlings were seen to be relatively very short (Fig. 1h) in comparison to control seedlings (Fig. 1f). Furthermore, high concentration of NaCl (250mM) solution was proved to be lethal and even after 7-days of treatments, full seed germination with root and shoot developments was found to be completely lacking (Fig. 1i).

#### Effect of sea water- salinity stress on seed germination

During initial experiments with sea water-salinity stress treatments, green gram seeds were treated with higher

concentrations of sea water (50%, 75% and 100% data not included) but these concentrations of sea water were caused to be strongly inhibitory and therefore, complete inhibitions in green gram seed germination were recorded, results indicate that these higher concentrations of sea water (50%, 75% and 100%) caused severe salinity stress and were proved to be completely toxic and lethal.

Moreover, during additional experiments, seeds were further treated with the lower concentrations (5%, 10%, 15%, and 25%) of sea water. In comparison to control, seeds that were treated with sea water concentrations (5% and 10%) could exhibit very poor and incomplete germination (Fig. 1j & k) respectively and moreover, these poorly germinated seeds were failed to develop into complete seedlings with roots and shoots even after 7-days of treatments.

Significantly, further increase in sea water (15%) concentration was proved to be completely inhibitory and hence seed germination response was restricted (Fig. 1l). Furthermore, partially germinated seeds (seedlings without roots) that were grown with (5% and 10%) of sea water concentrations were also found to be gradually necrosed at the end of 10<sup>th</sup> day of treatments.

#### Rate of seed germination under NaCl- salt stress

In control experiments, the mean percentage of seed germination was found to be (100±1. 98%) after 7<sup>th</sup> day of treatments and similar rate of seed germination was also obtained with lower concentrations (50mM and 100mM) of NaCl treatments (Table 1) indicating that the rate of seed germination was not influenced with the increase in NaCl salt concentration (up to 100mM) in treatment solution.

Furthermore, seeds that were treated with high concentration of NaCl (250mM) solution, exhibited the partial emergence of radicles (60±0. 49%) within 3 days of treatments and moreover, these partially germinated seeds were failed to grow further into complete seedlings even after 7<sup>th</sup> day of treatments.

**Table 1:** Effects of NaCl-salinity stress on seed germination, early seedling growth and proline content in green gram (*Vigna radiata* L).

S no.	3 <sup>rd</sup> Day		7 <sup>th</sup> Day			
	Concentration of NaCl (mm)	% Seed germination (Mean± S.E.)	% Seed germination (Mean± S.E.)	Root length (cm)	Shoot length (cm)	Total proline content (10 <sup>-3</sup> g <sup>-1</sup> fresh weight)
1.	Control	100 ± 1.98	100 ± 1.98	1.98±0.02	7.9±0.71	98.6
2.	50	100 ± 1.5	100 ± 1.5	1.9±0.05	7.5±0.91	109.3
3.	100	100 ± 1.0	100 ± 1.0	1.75±0.21	5.31±0.31	146.5
4.	250	60 ± 0.49	0	0	0	203.2

#### Rate of seed germination under sea water stress

In contrast of the results obtained with salinity stress induced by NaCl salt solutions, sea water treatments were proved to be strongly inhibitory and therefore, in comparison to control (100±1. 98%) treatments, at the end of 3<sup>rd</sup> day of treatments, even very low concentrations (5% and 10%) of sea water treatments could exhibit very poor rate of mean percentage of incomplete germination (21±0. 5% and 10 ±0. 35%) respectively. Furthermore, these partially germinated seeds that were treated with sea water (5% and 10%) were failed to grow into complete seedlings even after 7-days of treatments (Table 2). Significantly, further increase in sea water

concentration (15%) was turned out to be strongly inhibitory and mean percentage of seed germination was recorded as zero.

Results based on salinity stress caused by NaCl and seawater indicating that NaCl at lower concentrations (50mM and 100mM) were proved as almost ineffective to cause significant inhibitions in seed germination while high concentration (250mM) of NaCl was turned out to be slightly inhibitory for partial seed germination but strongly suppressive for complete seed germination. Similarly, in case of sea water causing salinity stress treatments, even (5%) of sea water was proved to be toxic for full seed germination.

**Table 2:** Effects of sea water salinity stress on seed germination and early seedling growth in green gram (*Vigna radiata* L.)

S. no.	3 <sup>rd</sup> Day		7 <sup>th</sup> Day		
	Concentration of Sea water (%)	% Seed germination (Mean ± S.E.)	% Seed germination (Mean± S.E.)	Root length (cm)	Shoot length (cm)
1.	Control	100 ± 1.98	100 ± 1.98	1.98±0.02	7.9±0.71
2.	5	21 ± 0.5	0	0	2.13±0.33
3.	10	10 ± 0.35	0	0	2.05±0.46
4.	15	0	0	0	0
5.	25	0	0	0	0

### Effect of salinity stress on early seedling growth

In contrast of NaCl-salinity stress response on seed germination, inhibitory response of NaCl-induced salinity stress could be observed during early seedlings growth. In general, seedling height (shoot and root elongations) were found to decrease with increase in NaCl concentrations in the treatment solutions and therefore, with 100mM of NaCl-salt solutions treatments, seedlings (Fig. 2c) and root-shoot lengths ratio (1.75±0.21/5.31±0.31cm) was recorded in comparison to control (Fig. 2a) seedling length (1.98±0.02/7.9±0.71cm). However, seedlings that were growing with low concentration of NaCl (50mM) solution were failed to exhibit salinity inhibitions (Fig. 2b) and moreover, seedlings heights (root-shoot length ratio) were found to be almost similar (1.9±0.05/7.5±0.91cm) to the control seedlings (1.98±0.02/7.9±0.71cm).

However, in case of seeds that were treated with high concentration of NaCl (250mM), much delayed and incomplete seed germination could be observed and these poorly germinated seeds were failed to grow further into complete seedlings (Fig. 2d). Significantly, in case of sea water treatments, partially germinated seeds that were growing with lower concentrations (5% and 10%) of sea water could show much reduced seedlings without roots (Fig. 2 e & f) respectively in comparison to the control seedlings and therefore, length of root-shoot length ratio (0.0/2.05±0.46cm) was recorded with 10% of sea water treatment in comparison to control seedling length (1.98±0.02/7.9±0.71cm).

Remarkably, root formation was not seen in such partially germinated seeds growing with sea water (5% and 10%) indicating that suppression of root formation could be the primary salinity stress effects caused by sea water.

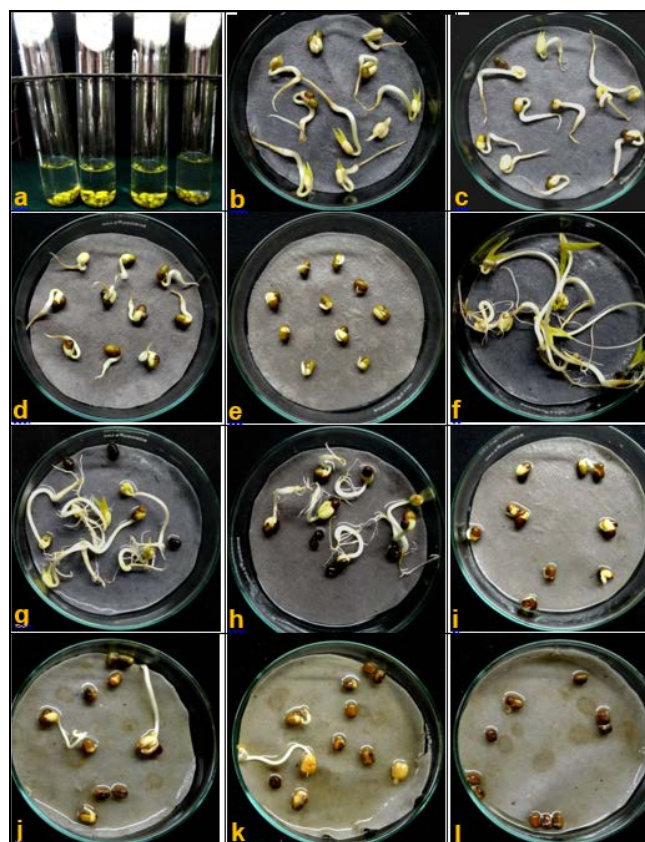
### Effect of salinity stress on seedling growth in soil

Hence, in comparison to control seedlings, there were gradual inhibitions in seedlings elongations growing with NaCl-solutions. Further, 7-day-old seedlings were transferred to soil and were given treatments with respective concentrations of NaCl-solutions. Significantly, after 14-days of transplantation, in comparison to control seedlings, gradual inhibitions in seedling growths could be observed that were growing in soil irrigated with increased concentrations of NaCl (50mM and 100mM) solutions (Fig. 2g) respectively.

### Effect of salinity stress on endogenous proline contents

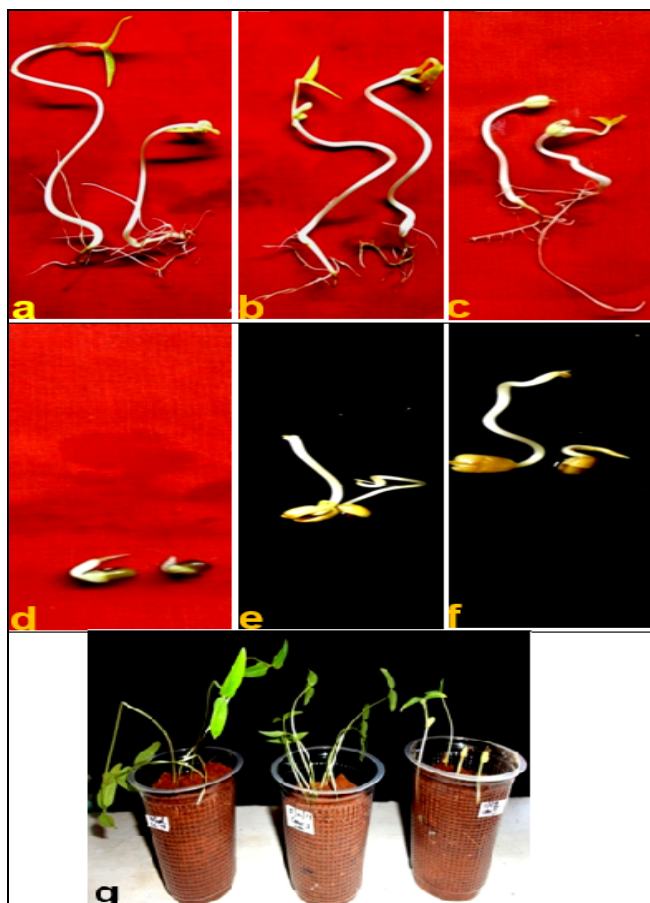
After 7-days of treatments, the accumulation of proline content was estimated from the control seedlings and seedlings that were growing with various concentrations of

NaCl (50mM, 100mM and 250mM). Results indicate that a gradual increase in proline accumulation was recorded in the tissues that were growing with the increased concentrations of NaCl solutions. In control seedlings, the proline content was found as the minimal level (98.6 × 10<sup>-3</sup>g<sup>-1</sup>) of fresh weight tissues and significantly, the amount of proline accumulation was obtained as the maximum (203.2 × 10<sup>-3</sup>g<sup>-1</sup>) in the samples that were extracted from the seeds growing with NaCl (250mM) salt solution (Table 1).



**Fig 1 (a- l):** *Vigna radiata* L., effects of salinity stress induced by NaCl-salt and Sea water treatments on seed germination in green gram legume crop

(a) Seeds under soaking treatments (Control, 50mM, 100mM, and 250mM of NaCl solutions) (b) Control (c) 50mM of NaCl (d) 100mM of NaCl (e) 250mM of NaCl solutions (Seed germination in petridish added with respective concentrations of NaCl solutions after 3-days of treatments) (f) Control (g) 50mM of NaCl (h) 100mM of NaCl (i) 250mM of NaCl solutions (j) 5% of sea water (k) 10% of sea water (l) 15% of sea water (Seed germination in petridish added with respective concentrations of NaCl solutions and Sea water after 7-days of treatments)



**Fig 2: (a-g):** *Vigna radiata* L., effects of salinity stress induced by NaCl solutions and sea water treatments on early seedling growth in green gram legume crop

(a) Control (b) 50mM of NaCl (c) 100 mM of NaCl (d) 250mM of NaCl solution (e) 5% of sea water (f) 10% of sea water (Seedlings after 7-days of treatments) (g) Control, 50mM and 100mM of NaCl solutions (Seedlings on transfer to disposable glass soil after 14-days of treatments)

### Discussion

Some of the legume crops such as common bean; chickpea (*Cicer arietinum* L.), pea (*Pisum sativum* L.) and faba bean (*Vicia faba* L.) are most cultivated and consumed worldwide as staple human food with rich source of proteins. Significantly, additional legumes, such as cowpea (*Vigna unguiculata* L.), green gram (*Vigna radiata* L.), pigeon pea (*Cajanus cajan* L.), lentil (*Lens culinaris* Medik.), and grass pea (*Lathyrus sativus* L.) also play an equally important role as staple crops for the people who inhabit mainly in most marginal and harsh regions of the world [42, 43]. Further, in addition to soybean, other model legumes like *M. truncatula*, *L. japonicus*, and *G. max* have also provided valuable approaches to understand legume responses to abiotic stresses.

### Effects of salinity stress

High salinity is considered as one of the major abiotic stresses that restrict crop yields because it causes significant reduction in rates of photosynthesis, respiration, and protein synthesis. Further, it is reported that photosynthetic reduction, membrane

denaturalization, nutrient imbalance, stomatal closure, and a dramatic increase in reactive oxygen species (ROS) production are the main physiological changes in plants under salinity stress [44-46].

Moreover, high accumulations of ROS cause serious plant toxicity, including oxidative damage in proteins, lipids, and DNA, whereas low concentrations of ROS act as signaling molecules [47-50, 11]. It is suggested that ion homeostasis is an essential factor of salt stress adaptation in salt-tolerant genotypes.

Additionally, the salt-tolerant genotype shows increase in root growth under saline conditions and accumulates more ions of both  $\text{Na}^+$  and  $\text{Cl}^-$  in shoots than in roots, in comparison to moderately salt sensitive genotype in *M. truncatula*, hence; it suggests that there is a possible link between growth and ion transport [38].

Further, salt tolerance in soybean is partially explained by the compartmentalization of  $\text{Na}^+$  into root cell vacuoles that prevents  $\text{Na}^+$  transport to the leaves [51, 52]. Furthermore, it is also suggested that  $\text{Na}^+$  is retained in roots, whereas  $\text{Cl}^-$  is transported into leaves of both salt-tolerant and salt-sensitive soybean genotypes [53], therefore, it indicates that the differential transport of  $\text{Na}^+$  and  $\text{Cl}^-$  ions in soybean roots and leaves might be due to salt stress.

During present study, results reveal that the rate of seed germination and seedling growth depend on the concentrations of salinity stressors (NaCl and sea water) and duration of treatments. Moreover, NaCl concentrations (up to 100mM) were proved to be almost ineffective to cause stress inhibition for seed germination in green gram legume crop.

However, NaCl-salinity stress could be seen as inhibitory during seedling growth and therefore, length of developing seedlings was based on the concentrations of treated NaCl solutions. Seedlings that were growing with low NaCl (50mM) solutions exhibit maximum elongations in comparison to seedlings that were growing with high concentration of NaCl (100mM) solutions while interestingly, further high concentration of NaCl (250mM) turns out to be lethal level and inhibits both seed germination and seedling growth strongly.

In similar study, salt stress decreases the percentage [54] and increases the duration of germination [55] in sweet sorghum and moreover, significant differences could be detected in the sensitivity of germination to high salinity among cultivars.

Moreover, previous study shows that salinity significantly affects germination of cowpea seeds where most cultivars were found to germinate at low concentrations of NaCl (10mM–50mM); however, high concentrations of NaCl (100mM, 150mM, and 200mM) result in significant reduction in the rate of seed germination for some cultivars and complete inhibition of seed germination for others. It is believed that inhibition in germination rate for sensitive cultivars could be because of difficulty in seed hydration due to high osmotic potential [56]. Similar inhibitory effects of NaCl-salinity stress on germination have been also reported in cowpea [14].

In additional studies, the effect of NaCl on the growth of cowpea was recorded in terms of stunting of shoots and roots but the depressive effect of salt occurs mainly in young leaves

than in roots, during the early vegetative stage of development. This difference in sensitivity between the organs of absorption and those of photosynthesis is characteristic of glycophytes.

Moreover, the poor development of these parts could be due to the increase in osmotic pressure in the medium, ionic toxicity of sodium and chlorine to the roots, and nutritional imbalance of the plant caused by a lack of absorption and/or transport of nutrients to the stem<sup>[57,56]</sup>.

Of late, germination percentage, germination index, mean germination time, seedling vigor index, seedlings' shoot and root lengths, fresh and dry seedling weight, and salinity tolerance indices have been studied in sorghum and results demonstrate that salinity was responsible for 98% of the variations in all studied parameters, whereas genotype effect contributes for only 2% of the variations<sup>[58]</sup>. Moreover, in recent study, osmotic stress (caused by mannitol and polythene glycol) and herbicide stress (induced by using dicamba and picloram) were found to be recorded inhibitory and toxic respectively during seed germination and early seedling growth in mung bean or green gram<sup>[39]</sup>.

During present study, after 7-days of NaCl solutions (100mM) treatments, seedlings growths were found to be negatively affected and root-shoot ratio (1. 75±0. 2/5. 31±0. 3cm) was recorded as compared to control seedling (1. 98±0. 02/7. 9±0. 71cm). However, in case of sea water (10%) treatments; incomplete seedlings without roots (0. 0/2. 05±0. 46cm) were obtained. Hence, this study reveals that sea water proves to be more lethal than NaCl- caused salinity stress inhibition and even (5%) of sea water treatments were proved to be inhibitory for the seed germination and seedling growth. However, in contrast, recent study in kodo millet crop suggests that sea water treatment at high concentration (50%) causes toxicity and completely inhibits seed germination<sup>[33]</sup>.

Moreover, when petridish grown seedlings were transplanted to soil and irrigated with respective concentrations of NaCl-salt solutions, inhibitory response on seedling growth was observed and such responses were found to be dependent on concentrations of NaCl- salt solutions.

### Endogenous proline status during salinity stress

Proline is a known as osmo-protectant, and plays an important role in osmotic balancing, protection of sub-cellular structures, enzymes and in increasing cellular osmolarity (turgor pressure) that provide the turgor necessary for cell expansion under stress conditions<sup>[59]</sup>. It is revealed that proline accumulates in glycophytes as well as in halophytes to restore the osmotic balance between cytoplasm and vacuole. Therefore, proline synthesis is an adaptive mechanism developed by the plant to overcome stress induction.

The determination of proline content accumulation and induction under stress is considered as one of the fast and efficient techniques for evaluating the salt tolerance in plants. During present experiment, proline content increases progressively in germinated seedlings with the increase in concentrations of NaCl-salt solutions. Moreover, proline content was recorded much higher (203. 2x10<sup>-3</sup>g<sup>-1</sup>) in seeds that were treated with high concentration (250mM) of NaCl solution as compared to control (98. 6x10<sup>-3</sup>g<sup>-1</sup>).

Moreover, present results are in conformity with previous observations where an increase in proline, glutamine, and other amino acids in maize was recorded in calli that were subjected to NaCl concentrations higher than 100mM. This study also reveals that concentration of proline increases in germinated seedlings that were treated with increased external NaCl concentration. Similar results have been also seen in rice seed germination under *in vitro* salinity stress treatments where proline accumulation increases with the increase in NaCl-concentration from low concentration to high concentration. Additionally, it is suggested that high accumulation of proline during high salt treatments might be a consequence of injury rather than being involved in the stress tolerance<sup>[60]</sup>.

Previous study further suggests that the excess of asparagine accumulation in the drought-stressed pearl millet leaves could be an indication of protein degradation under stress conditions and also could have increased partly through *de novo* synthesis<sup>[61]</sup>. Additionally, accumulation of proline results from the decrease in protein synthesis and due to conversion of glutamate to proline, and induced pH regulation.

Moreover, under field conditions, when crops are irrigated with NaCl-dominated water, salt tolerant cultivars have shown to produce greater biomass than salt sensitive cultivars indicating that rapid accumulation of free proline is a typical response to salt stress<sup>[62]</sup>.

This study therefore concludes that the salt sensitivity of green gram during seed germination and early seedling growth depends on the source of salinity either NaCl or sea water treatments. Moreover, high concentration (250mM) of NaCl proves to be toxic level for both germination and seedling growth while in contrast even very low concentration of sea water (5%) turns out to be lethal for seed germination and seedling growth both. Also, NaCl- treated seedlings could show gradual inhibitions in early seedling growth on transfer to soil under continuous irrigation with respective concentrations of NaCl-salt solutions.

Further, accumulation of endogenous proline content increases with the increase in NaCl-salt concentrations in the treatment solutions and maximum proline content was estimated in the sample that was extracted from the germinated seeds growing with high concentration (250mM) NaCl-salt solution.

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