

Effect of Salinity stress on *Vigna mungo* seed germination: Physiological and Bio-chemical parameters

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Abstract

Vigna mungo is one of the legumes with the greatest protein content. *Vigna mungo* was subjected to a pot experiment to investigate the effects of three distinct salinity levels (0, 50, 75, and 100 mM NaCl) on the germination and early seedling cycles. This investigation was implemented as a laboratory experiment with three replications for each salinity level, employing a completely randomized design. Salt stress was found to influence a variety of germination, physiological, and biochemical characteristics. The results suggest that the adverse effects were most pronounced when the seedlings were treated with 100 mM NaCl.

Key words : *Vigna mungo*, Salt stress, Germination, Biochemical test.

The consequences of stress elements, including heat, salinity, temperature, pH extremes, and drought, are diminished plant growth and harvest yield (Figure 1). Crop yields are reduced by over 50% and are the primary causes of crop failure by the most devastating abiotic stresses, such as drought and salinity. Salinity significantly diminishes agricultural yields in arid and semi-dry regions, affecting over 20% of the world's farmed land. Germinating seedlings that can tolerate salt is crucial, particularly in regions where surface soil salinity is prevalent. Kayani and Rahman¹⁷, Sharma *et al.*,²⁵ and Saboora *et al.*,²⁴ have demonstrated that seeds are unable to germinate or develop as rapidly when exposed

to elevated levels of salt. The ability of plant species to adapt to salinity during seed germination and the initial stages of seedling development is a critical factor in their establishment in saline environments. The timing and location of seedling growth are determined by the success of germination, which is essential for the survival and development of the plant as a whole. Seedlings are the most vulnerable stage of a plant's life cycle²⁰. According to Ashraf and Foolad⁷, a plethora of research has shown that salt stress causes changes in morphology, physiology, and biochemistry in crops. In plants, higher concentrations of sodium chloride reduce relative water content, growth metrics, and

germination rates. In arid-land wheat varieties, salts like NaCl and KCl have a deleterious effect on germination and growth, leading to an increase in aberrant seedling production¹. According to Wang *et al.*,³¹ treating sugar beets with NaCl inhibits their germination and the growth of young seedlings.

The United Nations Food and Agriculture Organization (FAO) expects that emerging nations will account for more than 85% of the increase in demand for agricultural commodities. That is according to Mia *et al.*²¹. Pulses, which are legumes grown for food or grain, can be promoted as part of a more diverse global cropping pattern. Leguminous plants are grouped into about 750 genera and 18,000 species^{15,23}. Due to their high protein content and usefulness as a human food source, pulses are currently in high demand. Fertilizer has long been an integral part of agriculture's green light to increase soil fertility through nitrogen fixation, which in turn increases crop yields and helps feed people the protein (24.5% of the total), carbs (59.9%), fat (0.5-4.33%), and other nutrients they need¹⁹.

Green gram thrives in warm, humid climates with temperatures between 25 and 40 degrees Celsius, moderate rainfall of 85 to 100 centimeters spread out evenly over its 100-day growth period, and low humidity. Typically, we may expect hot weather with little humidity from March to June. In northern India, green gram is grown during the hot and rainy Kharif season, while in southern India, it is farmed during the mild winter.

Legumes are a protein source that are

essential for both humans and livestock due to the presence of amino acids¹¹. A legume with a high yield and a brief growth season, green gram is a versatile variety. Its seeds and sprouts are abundant in protein. The cultivation of these legumes is difficult in dry and semi-arid regions due to the increased soil salinity and hardness, which is further exacerbated by the lack of high-quality irrigation water. Innovative agricultural methods are required to overcome the environmental constraints that restrict the development and productivity of legumes in order to produce sustainable crops.

Numerous studies have demonstrated that salinity inhibits biological reactions. Glucose, fructose, sucrose, fructus, and starch are among the carbohydrates that conduct Osmo protection, osmotic adjustment, carbon storage, and radical scavenging in response to salt stress²². *Bruguiera parviflora* leaves exhibit a decrease in carbohydrate and an increase in reducing and nonreducing sugars, as well as polyphenols, when placed under salt stress. Researchers discovered that the germination, seedling growth, relative water content, growth rate, and photosynthetic pigments of three distinct *Vigna species*—*V. mungo*, *V. radiata*, and *V. unguiculata*—declined as the concentration of sodium chloride increased. Conversely, the shoot and root exhibited an increase in metabolites, including reducing sugar, starch, protein, proline content, and peroxidase activity, in comparison to the control³.

The researcher in this study conducted the experiments at the lab of Narain College Shikohabad in Uttar Pradesh's Department of Botany. The experimental materials utilized were the seeds of the *Vigna mungo* plant.

Experimental Design :

V. mungo was the subject of an experimental design that included a control group, three different treatments, and one adjuvant. In addition, three separate experiments were conducted for each treatment and control group.

1. Distilled water was used as the control (C) to germinate the seedlings.
2. In the X-treatment, the seeds germinated in a solution of 50 mM NaCl.
3. Y, involved germinating the seeds in a solution of 75 mM NaCl.
4. Z, involved germinating seeds in a solution of 100 mM NaCl.

Germination Test :

The selected seeds were consistent in size. Germination paper was inserted into the 9 cm diameter Petri dishes after they had been disinfected with methanol. Ten seedlings of comparable dimensions were chosen and arranged in distinct Petri dishes for the purpose of germination. Five milliliters of distilled water or a 50 mM, 75 mM, or 100 mM NaCl solution were administered to each petri dish to promote seed germination on the paper. The petri dishes were permitted to chill to ambient temperature after being covered. When necessary, we implemented new germination sheets. Additionally, solutions with salinities that corresponded to the requirements were furnished. The germination percentage was ascertained by counting the number of seeds that germinated on a daily basis and on the seventh day following the sowing. The radicle length and fresh and desiccated weights of the early seedlings were also measured on day

seven. The Germination Test was conducted using these criteria.

Germination percentage (%) :

The development of radicles was assumed to be a sign of germination. We monitored the commencement and conclusion of germination on a daily basis. The following formula was employed to determine the germination percentage on a daily basis for a period of seven days⁹.

$$\text{Germination percentage} = \frac{\text{Number of germinated seeds}}{\text{Total number of seeds}} \times 100$$

Physiological parameter :

Relative water content (RWC):

$$\text{Relative water content RWC (\%)} = \frac{\text{FW}-\text{DW}}{\text{FW}} \times 100$$

The fresh weight of seedlings (7 days after sowing) is denoted as FW, while the dried weight is denoted as DW. The relative water content was determined using the following formula:²⁶.

Biochemical parameter :

The total protein estimate was conducted using Bradford's⁶ standard approach. The established procedure for calculating starch, as outlined by Chinoy⁸, was implemented. The method that was initially proposed by Somogyi²⁷ was employed to estimate the total reducing sugar.

The findings indicated that the components under investigation were

Table-1. Effect of different salinity levels on Germination % and Physiological Parameters of *Vigna mungo*

Species	Treatment	GP	RL	FW	DW	RWC
<i>Vigna mungo</i>	C (0mM)	100± 0.00	7.83±7.5	0.50± 0.01	0.14± 0.01	72.00± 0.80
	X (50mM)	95.25±2.53	6.15±0.8	0.46±0.02	0.13± 0.02	71.73± 0.84
	Y (75mM)	90.23±2.53	4.33±0.11	0.38± 0.03	0.11± 0.02	69.23±0.96
	Z (100mM)	88.71±2.53	2.23±0.09	0.32± 0.04	0.10± 0.03	68.75±0.79

Values are written as 'Mean ± S.D'

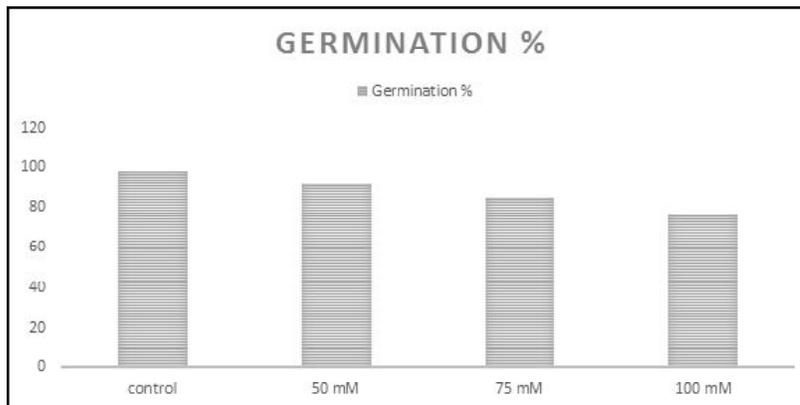
GP- Germination percentage, RL – Radicle Length; FW – Seedling Fresh Weight; DW – Seedling Dry Weight; RWC – Relative Water Content

substantially influenced by salt concentrations. On the seventh day following planting, all parameters of the investigation were assessed. (Table-1).

Germination Percentage :

As the salt content increased, the germination percentage in *Vigna mungo* decreased steadily, as indicated by the 7th DAS (Table-1). The influence of salinity level on GP is illustrated in Graph 1. *Vigna mungo* exhibited the lowest GP in Treatment Z. The maximum reported GP for *V. mungo* control seeds was 100%. Treatment Z also attained a GP of at least 88.71% for *V. mungo*. Our findings are consistent with those of Sidari *et*

*al.*²⁶, who also found that the germination percentage decreased in four distinct lentil genotypes that were subjected to salt stress as the NaCl concentration increased (50 mM, 75 mM, 100 mM). The salt tolerance of a seed is a measure of its ability to withstand germination in environments with elevated salt concentrations. Oversalted salt reduces the water potential of the germination media, resulting in a reduction in the amount of water available to the seed, which in turn slows or halts germination. El-keblawy¹² and Cokkizgin⁹ have reported comparable results for *Panicum turgidum* seeds and *Phaseolus vulgaris* seeds, respectively, when the salt concentration is elevated. (Graph-1).

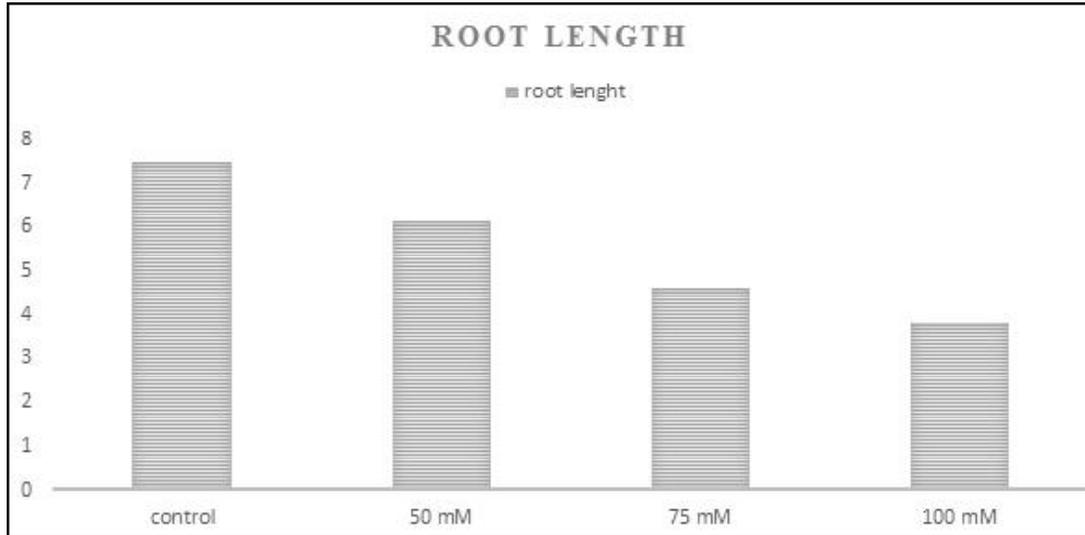


Graph 1. Effect of different salinity levels on Germination Percentage (%)

Root length (RL) :

On the seventh day following surgery (DAS), the radicle length (RL) was measured in centimeters. The longest root length (RL) in *V. mungo* seeds in the control group was 7.83 cm, as indicated by Table-1 and graph 2. This RL was observed to be decreasing

steadily as the salt content increased. The RL of Treatment Z was the shortest, measuring 2.23 cm. This study confirmed the findings of previous research, which indicated that the control group had the longest radicle length, while the group that was treated with a high concentration of NaCl had the shortest^{13,14}.



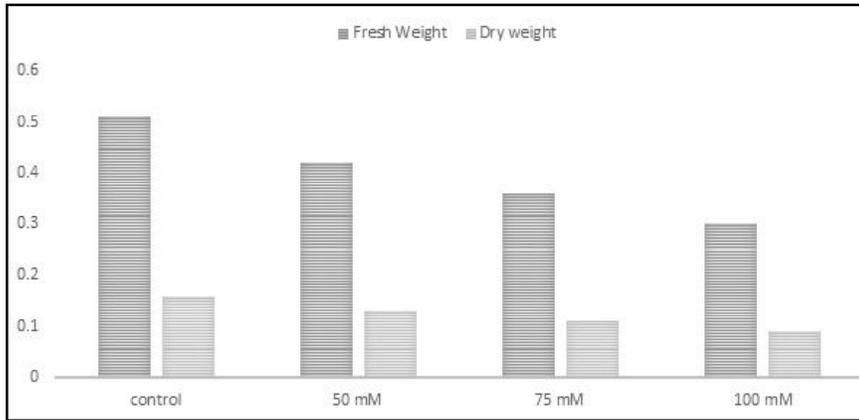
Graph 2. Effect of different salinity levels on Radicle length (cm)

Seedling fresh weight (FW) :

As the salt concentration in *Vigna mungo* increased, the seedlings' fresh weight (FW) consistently decreased, as illustrated in Table-1. Consequently, the control group in *V. mungo* has the greatest concentration of 0.50 g. Additionally, the lowest FW of 0.32g is observed in *V. mungo* seedlings from Treatment Z. According to Tunçtürk *et al.*,³⁰ plants that were exposed to salt stress had substantially lower fresh weights at harvest than those in the control condition. Their findings were consistent with this. Graph-3.

Seedling dry weight (DW) :

Table-1 shows that when the salt content increased, the seedling dry weight (DW) of *Vigna mungo* decreased. With a DW of 0.14 g, control is the most abundant in *V. mungo*. In *V. mungo*, the seedlings treated with Z had the lowest DW, which is 0.10 g. We confirm the findings of Carpycy⁷, who found that when salinity levels increased, the dry weight of maize shoots and roots dropped across all kinds. In graph 3, we can see how the salinity level affected the fresh weight and dry weight (gram).



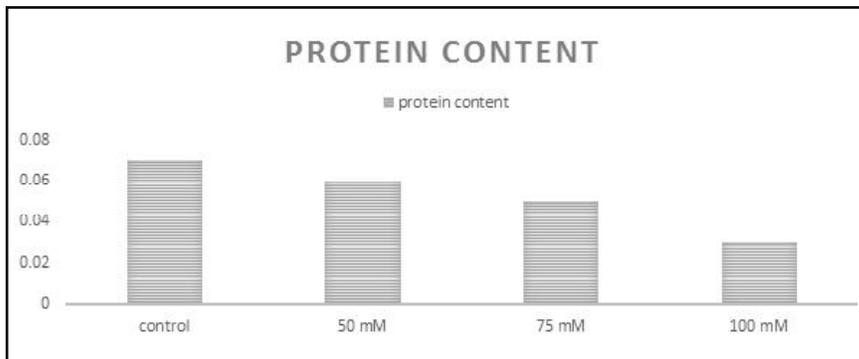
Graph 3. Effect of different salinity levels on Seedling Fresh weight (gm) & Dry weight (gm)

Relative water content (RWC) :

Both *V. mungo* species showed a decrease in relative water content (RWC) as the salt concentration increased; Treatment Z had the lowest RWC at 68.75%, while the control had the highest RWC at 72.0%. (Table-1). Our results were in agreement with those of Sidari *et al.*²⁶, who also discovered that seeds' water uptake declined as salinity stress increased and that higher concentrations of NaCl reduced germination percentage, growth parameters, and relative water content. They also found that imbibition decreased more at higher salinity concentrations.

Biochemical Parameter :

Table-2 indicates a consistent decline in the total protein content of *V. mungo* seedlings from the Control group to Treatment Z. The protein content of *Vigna mungo* varied from 0.03 mg/ml in Treatment Z to 0.07 mg/ml in the Control group. In accordance with the findings of Bohnert and Jensen⁵, our data indicate that protein synthesis is among the most adversely affected anabolic processes in stressed plants. Research conducted by Jaleel *et al.*,¹⁶ and Khosravinejad *et al.*,¹⁸ on barley (*Hordeum vulgare*) and *Catharanthus roseus* revealed that sodium chloride-treated seedlings had reduced protein levels. (Graph 4), Table-2.



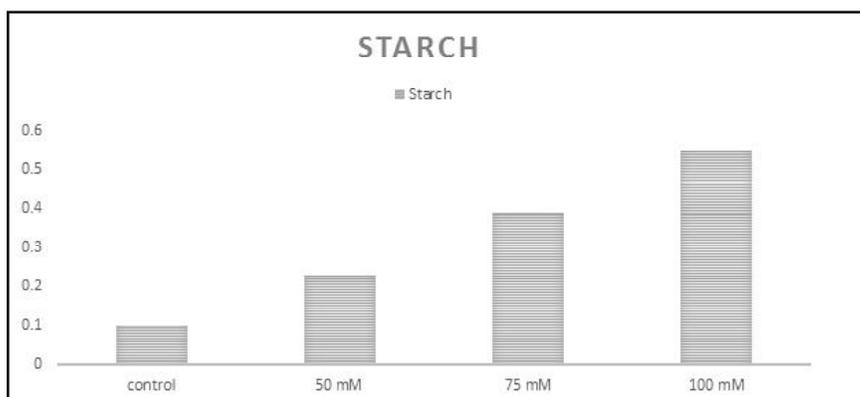
Graph 4. Effect of different salinity levels on Protein concentration (mg/ml)

Table-2 Effect of different salinity levels on Biochemical Parameters
of *Vigna mungo*

Species	Treatment	Protein (mg/ml)	Starch (mg/ml)	Reducing Sugar(mg/ml)
<i>V. mungo</i>	C (0mM)	0.07	0.10	0.03
	X (50mM)	0.06	0.23	0.03
	Y (75mM)	0.05	0.39	0.02
	Z (100m)	0.03	0.55	0.01

Starch : The starch content is shown to be increasing as the NaCl concentration treatments are increased (Graph 5, table-2). Please consult Table-2 and graph 5. In *Vigna mungo*, the starch concentration was highest in Treatment Z (0.55 mg/ml) and lowest in Control (0.10 mg/ml). Starch accumulation in

reaction to abiotic stress is consistent with our results and those of Taffouo *et al.*,²⁹. The accumulation of starch in response to salt stress could play an essential role in osmoregulation, carbon storage, osmotic protection, and radical scavenging, as suggested by Stivsev *et al.*²⁸.



Graph 5. Effect of different salinity levels on Starch concentration (mg/ml)

Reducing Sugar (RS) :

It was demonstrated that total reducing sugars (RS) in *Vigna species* reduced consistently with increasing salt concentration. According to Table-2, *Vigna mungo* had the highest RS concentration in the control group at 0.03 mg/ml and the lowest RS content in Treatment Z at 0.01 mg/ml. The results of this study contradict those of Parida *et al.*,²², who

found that reducing and nonreducing sugar levels increased in correlation with salt content in *Bruguiera parviflora* leaves.

The study reveals that salt stress negatively impacts germination, physiological and biochemical parameters in *Vigna mungo* seedlings. The study found that seedlings' fresh weight, dry weight, relative water content, protein content, starch content, and reducing

sugar levels all decreased as the saline level increased. The control group had the highest fresh weight (FW) and lowest dry weight (DW) in *V. mungo*. The relative water content decreased with increasing salt concentration, with Treatment Z having the lowest RWC (68.75%). Protein content decreased steadily from Control to Treatment Z, with the highest concentration in Treatment Z (0.55 mg/ml) and lowest in Control (0.10 mg/ml). Starch content increased with increasing NaCl concentration, with the highest concentration in the control group (0.03 mg/ml) and the lowest in Treatment Z (0.01 mg/ml). Reducing sugars also decreased with increasing salt concentration, with the highest concentration in the control group (0.03 mg/ml) and the lowest in Treatment Z (0.01 mg/ml). The study concludes that salt stress negatively impacts germination, growth parameters, and overall plant health.

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