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Response of Kidney Beans (*Phaseolus vulgaris* L.) towards Salinity Stress

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ABSTRACT

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Salinity is detrimental for crop growth as it hampers agricultural productivity impacting food sustainability. The response of salt stress on germination, seedling growth and proline content was studied in kidney beans (*Phaseolus vulgaris* L.) under *in vitro* and *in vivo* conditions. Seeds were germinated *in vitro* on Murashige and Skoog's basal medium containing NaCl (0, 50 and 100 mM). Pot culture method was used for *in vivo* studies and the seedlings were irrigated by Hoagland nutrient solution supplemented with NaCl (0, 50 mM and 100 mM). Under the influence of salt stress, the time required for germination increased while germination percentage, shoot/root length and fresh weight of seedlings declined. The shoot length was more impacted compared to root length resulting in increment of root to shoot ratio. The germination stress tolerance index, seedling vigor index, shoot and root length stress tolerance index declined with increment in salt stress. Proline content doubled at higher stress levels both under *in vitro* and *in vivo* conditions. Understanding the impact of salt stress in kidney beans is helpful in selecting varieties better adapted towards saline environments for ensuring sustainable production.

Introduction

Phaseolus vulgaris L. is a nutritionally important grain legume widely consumed throughout the world. The young pods, unripe seeds (shell beans) and dried ripe seeds are utilized for human consumption (Kochar, 2009). The immature green pods named as french bean, common bean, snap bean or green bean are eaten as vegetable, cannedor frozen. The dried ripe seeds commonly termed as rajmash/rajma, kidney bean, haricot bean are consumed as pulses (Singh et al., 2014; Bhartiya et al., 2017). The seeds are endowed with the

richness of plant protein, essential vitamins (thiamine, riboflavin, niacin, vitamin B6 and folic acid), minerals (K, Ca, Mg, Zn, Cu and Fe) and phytochemicals which play a major role in providing healthy balanced diet (Rodriguez et al., 2022). With the ongoing trend of consumer preference for plant-based proteins, dry beans have immense market potential therefore it is important to enhance its production to meet the growing demands (Uebersax et al., 2023). India is one of the largest producers of dry bean still a major fraction of seeds is imported in our country presenting a demand and supply gap (FAOSTAT, 2022). Phaseolus vulgaris L. is

cultivated in marginal land with poor irrigation practises and partially saline soil resulting in lowered yields (Taibi *et al.*, 2016; Singh *et al.*, 2014).

Salinity is a severe abiotic threat hampering crop production throughout the world (Chele *et al.*, 2021). Globally around 10.7 percent of total land area (1381 million hectares) is affected by salinity (FAO, 2024) and it is predicted that nearly 50% of cultivable land will become saline by the end of 2050 (Wang *et al.*, 2003). Excessive increment in salinity reduces the soil fertility resulting in crop yield losses impacting food sustainability (FAO, 2024). Like most legumes kidney beans is sensitive to salt stress and the yield begins to decline at relatively low salinity levels of 2 ds m⁻¹ (Taibi *et al.*, 2021).

Kidney beans are produced in several states of India under diverse agroclimatic regions such as Maharashtra, Jammu and Kashmir, Himachal Pradesh, Uttarakhand, Tamil Nadu, Karnataka, Kerala, West Bengal, and Uttar Pradesh (Bhartiya *et al.*, 2017). In Maharashtra nearly 1.0 lakh ha. of area in under French bean cultivation in different districts such as Sangli, Kolhapur, Pune, Satara, Beed, Latur, Osmanabad and Jalana (Lad *et al.*, 2024).

However, some of the French bean producing districts such as Pune, Sangli and Satara in Maharashtra are affected by salinity (Mandal *et al.*, 2011). Salt stress is detrimental for plant growth because it results in (a) reduction of soil water potential in the root zone creating water deficit, (b) increment of Na⁺ and Cl⁻ ions causing phytotoxicity, and (c) nutrient imbalance either by reduced uptake or shoot translocation of micronutrients (Muhammad *et al.*, 2024).

Understanding the impact of salt stress in *Phaseolus vulgaris* L. and selecting tolerant varieties is an important step for successful cultivation of this important legume in salinity stressed environments for sustainable production. Seed germination and seedling growth are most vulnerable stages under salt stress in agriculturally important legumes including kidney beans (Cokkizgin, 2012; Cirka *et al.*, 2022). Salt stress results in osmotic changes within the plants which activate cell signalling pathways for synthesis of osmoprotectants and antioxidant enzymes (Taibi *et al.*, 2021). Accumulation of osmoprotectants such as proline is observed in response to salinity stress. Proline helps in adjusting the osmotic potential and protecting the cellular machinery (Liu *et al.*, 2024). Therefore, the present investigation

was undertaken to study the response of kidney beans towards salt stress on germination, seedling growth, and proline content under *in vitro* and *in vivo* conditions.

Materials and Methods

Seed Germination and Growth Conditions

The seeds of kidney beans (*Phaseolus vulgaris* L.) were obtained from certified seed agencies in Pune, Maharashtra, India. For *in vitro* studies, the seeds were surface sterilized with 0.1% (w/v) mercuric chloride solution for 8 min. and washed thrice with sterile distilled water. The seeds were briefly blotted and inoculated on Murashige and Skoog's basal medium (Murashige and Skoog, 1962) containing 3% (w/v) sucrose, 0.8% (w/v) agar and NaCl (50 and 100 mM) in culture tubes (25x100 mm). Medium without NaCl served as control. The pH of the medium was adjusted to 5.8±0.1 and it was autoclaved at 121°C and 1.1 Kg cm⁻²for 20 min.

The cultured seeds were kept in complete darkness for germination. The germinated seedlings were maintained under 16-h light and 8-h dark photoperiod maintained by cool white fluorescent tube light of 32 µmol m⁻² s⁻¹ intensity and at 24±2°C temperature. Seeds showing radicle protrusion were scored as germinated. The number of germinated seeds was recorded daily for two weeks. The germination percentage was calculated as follows:

Germination % Number of seeds germinated = ----- x 100 Total number of seeds kept for germination

For *in vivo* studies, pot culture method was adopted. The seeds were sown in plastic pots (8.5 x 7.5 cm) containing autoclaved soil (mixture of sandy and clay 1:1) and kept in dark for germination. On the emergence of the first two true leaves the seedlings were transferred to shade net and irrigated by Hoagland nutrient solution two to three times a week. After 15 days of growth, the seedlings were arranged into three treatment groups each containing five plants. The first treatment group served as control and was irrigated by Hoagland nutrient solution. The second and third treatment groups were irrigated by Hoagland nutrient solution supplemented with NaCl (50 mM and 100 mM) twice at 7 days interval till final concentration was achieved.

Morphological Studies

The *in vitro* grown seedlings were harvested after six weeks of culture while *in vivo* maintained seedlings were harvested after four weeks of salt treatment to study the growth parameters. The shoot length and root length of seedlings was measured using graduated ruler (cm). The seedlings were weighed on a digital balance for determination of fresh weight.

(i) Seedling Vigor Index was calculated according to Baki and Anderson (1973) as follows:

$$SVI = Germination (\%) \times Seedling length (cm)$$

(ii) Germination Stress Tolerance Index was calculated according to Tarchoun *et al.*, (2022) as follows:

(iii) Shoot Length Stress Tolerance Index was calculated according to Tarchoun *et al.*, (2022) as follows:

(iv) Root Length Stress Tolerance Index was calculated according to Tarchoun *et al.*, (2022) as follows:

Proline Estimation

Proline was estimated according to Bates *et al.*, (1973). For proline estimation, shoot cultures were harvested from *in vitro* germinated seedlings after six weeks and fresh leaf samples were taken from *in vivo* grown *Phaseolus* seedlings after four weeks (salt treated and untreated). Five independent samples of same age were procured for the estimation. The leaf or shoot samples (0.5 g) were homogenized in 10ml of 3% aqueous sulfosalicylic acid to precipitate protein. The homogenate was centrifuged at 2000 x g for 5 min. Two ml of the

supernatant was taken to which 2 ml acid ninhydrin and 2 ml glacial acetic acid were added and tubes was kept in water bath at 100°C for 1hour. The reaction was terminated in an ice bath. Four ml of toluene was added to the reaction mixture and vortexed. The toluene layer was separated and the absorbance was read at 520nm (Sysytronics 105, Ahmedabad). Proline concentration was determined from L-proline standard curve and calculated as µg proline gfwt⁻¹.

Data Analysis

The experimental layout was completely randomized design. Each experiment comprised of three treatments containing five replicates and the experiments were repeated twice. The data is presented as means of five replicates per treatment and the standard error. Statistical analysis was performed using one-way analysis of variance with replicates (p<0.05).

Results and Discussion

Germination studies

Under in vitro conditions, Phaseolus seeds required 6.4 ± 0.51 , 9.8 ± 0.58 and 13.6 ± 0.75 days to germinate on control, 50 mM and 100 mM salt stress showing an average delay of 3 days and 7 days on 50 mM and 100 mM salt stress respectively (Table 1). Similar response was observed in *Phaseolus* (Cokkizgin, 2012; Ahmadian and Bayat, 2016) and tomato (Seth and Kendurkar, 2015) where seed germination was delayed in presence salinity stress. As the osmotic potential of the medium remains high under salt stress, imbibition of water is reduced by seeds causing increment in germination period (Ucarli, 2021). The germination percentage was 82.61% on control and 53.45% on 50 mM salt stress. The LD₅₀ dose for kidney beans in presence of sodium chloride ranged at 50mM. Seeds showed limited germination percentage at 100 mM (Table 1). The germination percentage declined with increasing concentration of salinity stress exhibiting 83% decline at 100 mM. Salt stress had negative impact on Phaseolus seed germination as reported earlier (Kaymakanova, 2009; Cokkizgin, 2012; Mena et al., 2015).

The germination is hampered in presence of salinity stress in legumes mainly due to impaired water uptake, nutrient imbalance through accumulation of Na⁺ and Cl-causing ion toxicity to the embryo (Faroog *et al.*, 2017).

Salt stress had significant effect on germination percentage as shown by one-way ANOVA (Table 3). Germination stress tolerance index was 57.79 at 50 mM and 16.98 at 100 mM showing a decline of 70.6% at 100 mM salt stress (Table 1).

The seedling vigour index was 3333.84 on control and 106.34 at 100 mM stress showing a decline of 96.8% (Table 1). Seedling vigour index reduced with increasing salinity levels as reported in previous studies on *Phaseolus* (Cavusoglu, 2023) and Pumpkin (Irik and Bikmaz, 2024).

Morphological Studies

Salinity reduced the overall growth of the plants, which was evident in terms of reduced shoot and root length. The shoot length of *in vitro* maintained seedlings after six weeks of culture was 18.88 cm on control, 12.76 cm on 50 mM and 2.96 cm on 100 mM salt stress (Fig. 1). The shoot length of seedlings reduced by 32.4% and 84.3% respectively on 50 and 100 mM salinity stress.

The shoot length for *in vivo* germinated seedlings after four weeks of growth was as follows: 37.6 cm on control, 28.5 cm on 50 mM and 19.8 on 100 mM salt stress (Fig. 1). The shoot length declined with increment in salt stress and exhibited 47.34% reduction at 100 mM NaCl. The root length of kidney bean seedlings under *in vitro* conditions was 21.48 cm on control which declined to 16.5 cm on 50 mM and 4.64 cm on 100 mM NaCl (Fig. 1).

With increment in salt stress the root length declined showing 78.4% reduction at 100 mM salt stress. On control, the root length of *in vivo* seedlings was 41.8 cm which reduced to 35.8 cm and 27.6 cm at 50 mM and 100 mM respectively. The reduction in root length was 34% at 100 mM. Salt stress results in high osmotic pressure, therefore, roots are unable to absorb adequate water leading to reduction of root length and shoot length (Dikobe *et al.*, 2021).

Our results are in agreement with previous studies which reported reduction in shoot and root growth in presence of salinity stress in *Phaseolus* (Mena *et al.*, 2005; Gama *et al.*, 2007; Kaymakanova, 2009; Cirka *et al.*, 2022). Under *in vitro* conditions, the seedling length was 40.4 cm on control, 29.3 cm on 50 mM and 7.6 cm on 100 mM salinity stress (Fig. 1). The shoot and root growth were drastically reduced at 100 mM salt stress exhibiting

74% reduction in seedling length. The seedling length under *in vivo* conditions was 79.4 cm on control, it declined to 47.4 cm at 100 mM registering a decline of 40.3% on 100 mM (Fig. 1).

The root to shoot ratio on control was 1.14 (*in vitro*) and 1.11(*in vivo*) while it ranged at 1.59 (*in vitro*) and 1.39 (*in vivo*) on 100 mM NaCl stress showing an increment of 45.45% and 27.27% respectively (Fig. 2). Increment in root to shoot ratio revealed that shoot length was more impacted compared to root length in *Phaseolus* seedlings under salt stress. As the shoot growth is reduced under salinity induced water stress so more plant resources are diverted towards root thus increasing root growth (Parida and Das, 2005). Increased root growth in presence of salinity stress is helpful in retaining Na⁺ and Cl⁻ ions within the roots and control their translocation to the shoots allowing better survival under saline conditions (Acosta-Motos *et al.*, 2017).

The fresh weight of *in vitro* seedlings on control was 2.80g which reduced to 0.86g at 100 mM exhibiting a reduction of 51.7% (Table 2). On control, the fresh weight of *in vivo* seedlings was 16.83g which reduced to 8.85g on 100 mM showing a reduction of 47.4% (Table 2). Present findings are in accordance with earlier studies reporting decline in fresh weight with increment in salinity stress (Irik and Bikmaz, 2024; Jamel *et al.*, 2024; Seth, 2024).

One-way ANOVA results exhibited that growth parameters (shoot length and root length) and fresh weight were significantly affected by salinity stress under in vitro (Table 3) and in vivo (Table 4) conditions. The shoot length stress tolerance index for in vitro seedlings was 67.82 at 50 mM and 16.19 at 100 mM salt stress showing a reduction of 76.1% (Fig.3). At 50 mM salt stress, the shoot length stress tolerance index for in vivo seedlings was 75.92 which declined to 52.80 at 100 mM exhibiting a decline of 30.5% (Fig.3). The root length stress tolerance index for in vitro seedlings was 76.82 and 21.60 on 50 mM and 100 mM NaCl respectively showing decline of 71.9% at 100 mM salt stress (Fig.3). For in vivo seedlings, the root length stress tolerance index was 85.75 on 50 mM and 66.18 on 100 mM salt stress registering a decline of 22.8% (Fig.3). Our findings are similar Tarchoun et al., (2022) where the shoot length stress tolerance index and root length stress tolerance index decreased with increment in salinity stressin Tunisian Squash.

Table.1 *In vitro* response of kidney bean towards salt stress on days required for germination, germination percentage, germination stress tolerance index and seedling vigour index.

NaCl (mM)	DRG	GP	GSTI	SVI
0	6.4±0.51	82.61±0.28	-	3333.84±31.08
50	9.8±0.58	53.45±2.19	57.79±2.60	1386.67±25.29
100	13.6±0.75	14.03±0.49	16.98±0.55	106.34±2.12

DRG-Days Required for Germination, GP-Germination %, GSTI- Germination Stress Tolerance Index, SVI-Seedling Vigour Index. Data presented as mean ±SE(n=5)

Table.2 Impact of salt stress on kidney bean seedling fresh weight.

NaCl (mM)	Fresh Weight (g)			
	In vitro	In vivo		
0	2.80 ± 0.46	16.83±0.79		
50	1.78 ± 0.24	12.76±0.53		
100	0.86 ± 0.05	8.85 ± 0.48		

Data presented as mean $\pm SE(n=5)$

Table.3 One-way analysis of variance (ANOVA) for germination percentage, shoot length, root length, fresh weight and proline content in presence of NaCl under *in vitro* conditions.

	df	SS	MS	F	P-value
Germination %	2	11759.14	5879.57	688.93	0.00
Shoot length	2	644.90	322.45	355.91	0.00
Root length	2	748.41	374.20	293.88	0.00
Fresh weight	2	9.42	4.71	10.49	0.00
Proline content	2	10116.03	5058.02	517.89	0.00
df: degree of freedom;SS: sum of squares; MS: mean of squares; P < 0.05					

Table.4 One-way analysis of variance (ANOVA) for shoot length, root length, fresh weight and proline content in presence of NaCl under *in vivo* conditions.

	df	SS	MS	F	P-value
Shoot length	2	792.23	396.12	169.76	0.00
Root length	2	508.13	254.07	68.05	0.00
Fresh weight	2	159.22	79.61	42.03	0.00
Proline content	2	15736.63	7868.32	447.91	0.00
df: degree of freedom;SS: sum of squares; MS: mean of squares; P < 0.05					

Figure.1 Impact of salt stress on shoot length, root length and seedling length in *Phaseolus vulgaris* L. under *in vitro* and *in vivo* conditions. Each value represents mean (n=5) and vertical bars indicate SE of means.

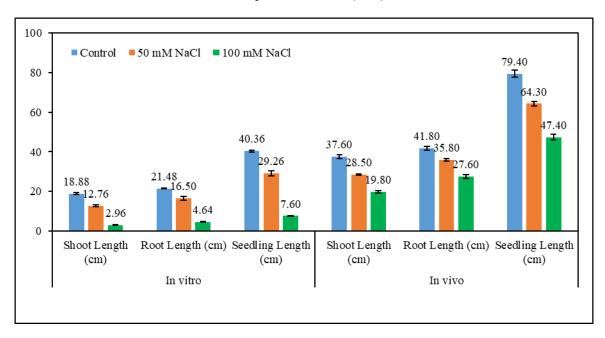


Figure.2 Impact of salt stress on root/shoot ratio in *Phaseolus vulgaris* L. seedlings under *in vitro* and *in vivo* conditions.

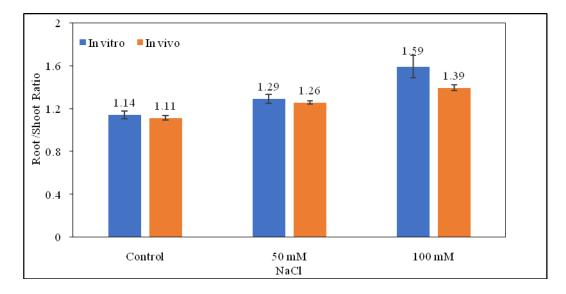
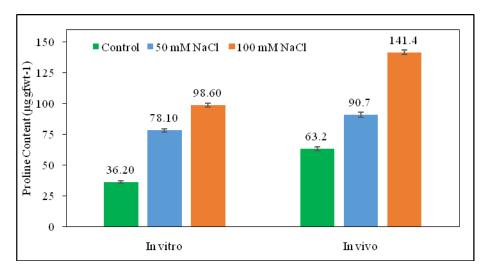


Figure.3 Impact of salt stress on shoot length stress tolerance index and root length stress tolerance index in *Phaseolus vulgaris* L. under *in vitro* and *in vivo* conditions. Each value represents mean (n=5) and vertical bars indicate SE of means.



Figure.4 Impact of salt stress on proline content in *Phaseolus vulgaris* L. seedlings under *in vitro* and *in vivo* conditions. Each value represents mean (n=5) and vertical bars indicate SE of means.



Proline Content

The proline content enhanced with increment in salinity stress. The proline content for seedlings maintained under *in vitro* conditions was 36.2 µg gfwt⁻¹, 78.1 µg gfwt⁻¹and 98.6 µg gfwt⁻¹ on control, 50 mM and 100 mM salinity stress (Fig.4). Proline content displayed enhancement of 2.7 folds at 100 mM salt stress. For *in vivo* maintained seedlings, the proline content on control was 63.2 µg gfwt⁻¹ and it enhanced to 141.4 µg gfwt⁻¹on

100 mM NaCl exhibiting 2.2 folds increment compared to control (Fig.4). The proline content doubled at higher stress levels both under *in vitro* and *in vivo* conditions. Proline acts as an osmoprotectant and helps in maintaining water balance within the plant cells under salinity stress (Parida and Das, 2005). It also helps in stabilizing subcellular structures, scavenges free radicals and reduces the redox potential (Liu *et al.*, 2024). In many plants proline functions as vital source of carbon and nitrogen during their recovery phase (Ghosh *et al.*,

2022). These findings are in agreement to earlier studies which reported increment in proline content in response to salt stress in *Phaseolus* (Jimenez-Bremont *et al.*, 2006; Taibi *et al.*, 2021). Salt stress had significant effect on proline content as demonstrated by one-way ANOVA (Table 3 and 4).

Salinity significantly enhanced the time required for germination, reduced the seed germination percentage, shoot length, root length and fresh weight of the kidney bean seedlings both under *in vitro* and *in vivo* conditions. Proline content enhanced with increment in salinity levels. Studying germination response and seedling growth in presence of salt stress is helpful for selection of kidney bean varieties that are resilient to saline stress, ensuring stable yields despite adverse conditions. Further, varieties performing better under salt stress can be incorporated into plant breeding programmes or utilized in genetic engineering studies.

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Author Contributions

Dr. Rupali Seth: Conceptualization, Formal Analysis, Investigation, Writing - Original Draft; Khopeno Khuvung: Validation, Methodology, Writing-Reviewing

Data Availability

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethical Approval Not applicable.

Consent to Participate Not applicable.

Consent to Publish Not applicable.

Conflict of Interest The authors declare no competing interests.

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