

Physiological Response of Common Bean (*Phaseolus Vulgaris* L.) to Salinity Stress in Hydroponics

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Abstract

This research aimed to assess common bean genotypes for genetic diversity, heritability, and genetic advancement under varying concentrations of sodium chloride (NaCl) in hydroponic systems. Eight common bean genotypes were grown and evaluated in a two-factor completely randomized design with three replications in three NaCl concentrations (0 mM, 150 mM, 300 mM) at the Molecular lab of the Department of Plant Breeding and Genetics, The University of Agriculture, Peshawar, during 2022. Analysis showed substantial differences among eight genotypes for all traits across three NaCl levels. Mean ranges under 0, 150, and 300 mM NaCl concentration 10.93 to 20.87 cm, 8.71 to 21.43 cm, and 11.64 to 21.58 cm for hypocotyl length, and from 13.02 to 23.63, 10.51 to 15.9, and 6.96 to 12.99 for chlorophyll content, and from 32.33 to 46.67 cm, 34.00 to 57.33 cm and 33.50 to 45.67 cm for plant height, and from 10.73 to 15.30 cm, and 11.10 to 15.30 cm and 10.00 to 15.70 cm for epicotyl length. Heritability estimates ranged from 0.62 to 0.93 for various traits of common bean genotypes in all three levels of NaCl. The highest heritability was recorded for hypocotyl length (0.93) in 150mM NaCl concentrations, while the lowest heritability was recorded for plant height (0.62) in 300mM NaCl and also for hypocotyl length in 0mM 0.62 NaCl concentrations. The highest genetic advance value was estimated for plant height (8.26) in all NaCl concentrations, i.e., 4.10 in 0 mM NaCl, 8.26 in 150 mM NaCl, and 3.96 in 300 mM NaCl, respectively. Based on the current experiment, genotypes SW-32, GL299, and GL-287 appeared to be superior, with the highest values for plant height, chlorophyll content, and hypocotyl length. These results are recommended for future breeding programs aimed at improving salt tolerance in common bean genotypes.

Keywords: Common beans, hydroponics, NaCl concentrations, heritability, genetic advance, two-factor CRD.

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INTRODUCTION

The common bean (*Phaseolus vulgaris* L.) is the most widely cultivated and consumed legume globally (Blair *et al.*, 2009; Coehlo *et al.*, 2009). This legume is a self-pollinating, annual crop that is eaten as a vegetable and dried when it reaches maturity (Ferreira *et al.*, 2000; De Luque *et al.*, 2014). Fabaceae is a family that includes peas and legumes, including common beans. Common beans were domesticated in Mexico over 6000 years ago and are believed to have originated in Latin America. It is disseminated globally from Latin America to Europe, Africa, and other regions. The common bean is a substantial genus of around 80 species, including cultivated and wild varieties (Freytag & Debouck, 2002; Porch *et al.*, 2013). The chromosomal

number is 22 ($2n = 2x = 22$). In Pakistan, it is referred to as 'Lobia'. In addition to meeting the daily protein needs of humans (Broughton *et al.*, 2003; Ulukapi *et al.*, 2014), common beans are a significant food source of dietary minerals that may supply all 15 required elements (Welch *et al.*, 2000).

The common bean is a warm-season leguminous crop that does not thrive at temperatures below 20 to 25°C. It thrives in locations with moderate rainfall, spanning from the tropics to temperate zones. In Pakistan, it grows in arid regions where conditions are conducive to maize growing (Alghamdi, 2007). Elevated temperatures disrupt seed formation, whereas reduced temperatures negatively impact growth.

This significant grain legume is globally used as a vital source of proteins, minerals (primarily zinc and iron), and vitamins in human diets (Ribeiro *et al.*, 2000), making it one of the most crucial food crops due to substantial incomes for growers in ecological variety. They possess local nomenclature based on seed color and growing region, exhibiting distinct characteristics that are well-suited to local environmental conditions, frequently distinguished by enhanced nutritional benefits and/or unique flavors highly valued by consumers (Piergiovanni and Lioi, 2010; Blair *et al.*, 2010).

Globally, common beans are grown on 34.8 million hectares, yielding 27.54 million tons, with an average productivity of 791.5 kg per hectare (FAOSTAT, 2021). In Pakistan, common beans are grown on 0.242 million hectares, yielding 0.211 million tons, resulting in an average yield of 873.9 kg ha⁻¹. In KP, the cultivated area is 0.046 million hectares, producing 0.021 million tons, with an average yield of 460.7 kg ha⁻¹.

In the developing countries of Latin America and Africa, the majority of beans are cultivated by small-scale farmers (Broughton *et al.*, 2003), and a substantial fraction of the harvest is consumed on-site, complicating precise estimations of worldwide production. The prevalent method of cultivating beans by intercropping results in an inflated assessment of the overall area cultivated and a diminished evaluation of global yields (Akibode and Maredia, 2012).

Extensive plant breeding has resulted in *Phaseolus vulgaris* possessing various cultivars that exhibit a diverse array of morphological and agronomic traits, including variations in seed size, color, and growth habit (Purseglove, 1976; Singh *et al.*, 1991). Determinate growth is a frequently chosen trait characterized by diminished branching, shorter and fewer internodes, reduced twining, insensitivity to photoperiod, and, crucially, an enhanced distribution of biomass production to reproductive development (Kwak *et al.*, 2012; Singh and Schwartz, 2010).

In Pakistan, the genetic resources of Indigenous common bean remain unexploited and underutilized, with little crop enhancement efforts. Primarily, local landraces are cultivated, exhibiting considerable genetic variability. These landraces and primitive varieties are present in blended forms. Their extensive genetic background results in significant genetic variability in seed coat color, seed shape, seed size, and growth habit (Nawaz and Farhatullah, 2020). The wild forms and landraces of *Phaseolus vulgaris* possess numerous unique and vital traits for adaptations, representing a reservoir of genetic diversity (Scarano *et al.*, 2014).

The wide range of native genetic resources is essential for breeding programs. These resources can be utilized for fundamental research, including the ability to resist both abiotic and biotic stress (Dudnik *et al.*, 2001;

Shivhare and Lata, 2017). It is recognized as being susceptible to several environmental variables, particularly salt, which significantly restricts plant development and productivity (Ghoulam *et al.*, 2002). Research indicates that elevated salinity, influenced by NaCl concentrations, impairs plant growth and development due to osmotic stress and the detrimental effects of excessive Na⁺ and Cl⁻ ions (Farissi *et al.*, 2011).

Keeping in mind the aforementioned facts, the present study aims to explore the reaction of common bean roots and leaves to salt in different growth media by determining the genetic variability among common bean accessions for salinity-related traits, identifying salinity-tolerant accessions collected from northern areas of Pakistan, and estimating broad-sense heritability and genetic advance for different traits under varying NaCl levels.

MATERIALS AND METHODS

The current experiment was conducted in the molecular lab of The University of Agriculture, Peshawar, to study saline-tolerant genotypes of common beans. The experimental material consisted of eight indigenous common bean accessions collected from the northern areas of Pakistan, including KA-70 from Kashmir, GL-287, GL-299, and GL-268 from Gilgit, SW-32 from Swat, SH-09 and SH-19 from Shangla, and MN-560 from Mansehra. A two-factor complete randomized design (CRD) was used, with seeds sown in plastic bags on October 16th, 2021, and transplanted to a hydroponic system on October 22nd, 2021, in three replications. Salinity tolerance was assessed for various traits under three NaCl levels: 0 mM, 150 mM, and 300 mM.

Table 1: Nutrients and their concentrations

Macro nutrients (250 ml)	Concentration	
	Stock/250 ml	Dilution/3L
(1M) KNO ₃	25.25g/250 ml	4.5 ml
(1M) Ca (NO ₃).4H ₂ O	59.04g/250 ml	3 ml
(0.5M) MgSO ₄ .4H ₂ O/7H ₂ O	30.8g/250 ml	1.5 ml
(0.1M) Na ₂ H ₂ PO ₄	39g/250 ml	10 ml
Micro nutrients (250 ml)	Concentration	
	Stock/250 ml	Dilution/3L
H ₃ BO ₃	0.125g/250 ml	1.5 ml
ZnSO ₄	0.125g/250 ml	1.5 ml
CuSO ₄ .5H ₂ O	0.125g/250 ml	1.5 ml
MnCl ₂	0.125g/250 ml	1.5 ml
Iron		
10% Chelated Iron	3.75g/250 ml	4.5 ml

1. **0 mM NaCl** serves as the control condition to establish baseline growth and biochemical parameters without salt stress (Hassan *et al.*, 2016; Kouam *et al.*, 2017).
2. **150 mM NaCl** represents a moderate-to-high stress level frequently used to:

- Induce measurable physiological changes (e.g., reduced shoot/root biomass, ion imbalances) while avoiding complete plant mortality. Differentiate tolerance levels among genotypes, as demonstrated in studies identifying cultivars like *P. vulgaris* "Maxidor" and KEB-CB049 as salt-tolerant under comparable concentrations (Hassan *et al.*, 2016).
3. **300 mM NaCl** tests extreme stress tolerance thresholds, helping identify:
- Genotypes with exceptional ion homeostasis mechanisms. Breakdown points for osmotic adjustment strategies like *Myo*-inositol accumulation (Hassan *et al.*, 2016).

Germination and transplantation

Common bean (*Phaseolus vulgaris* L.) seeds were gathered from different parts of the country (Pakistan). The seeds were enveloped in moist tissue paper, secured in zip-lock plastic bags, and positioned in an incubator for germination. Following germination, seedlings were placed in an incubator at 25°C with a 16/8-hour day/night photoperiod and a light intensity of 500 $\mu\text{mol m}^{-2} \text{s}^{-1}$. The seedlings were irrigated using tap water. Following seven days of seeding and the third day of germination in the chamber, the seedlings were relocated to the nutrient solution (Table 2). Subsequently, following eight days in the nutritive solution, the seedlings were subjected to saline solutions of 150 mM NaCl and 300 mM NaCl, while maintaining one replication as a control with 0 mM salinity. To achieve the target concentrations, 50 mM was incorporated into the nutrient solution every 12 hours for a duration of 48 hours. The plants were subsequently picked and preserved at -30°C awaiting further examination.

Data recorded

Data collection focused on hypocotyle length, epicotyle length, and plant height, following the guidelines set by the International Board for Plant Genetic Resources (IBPGR, 1982). This data was gathered from specific plants in each tray at different growth stages for a total of 138 common bean accessions.

Chlorophyll Analysis

One gram of leaf sample was meticulously chopped and blended with a clean mortar and pestle; to achieve homogenization of the leaf material, 20 ml of 80% acetone was used. The material was thereafter ground softly. The sample was afterward placed in a freezer at 4 degrees Celsius for 4 hours. Subsequently, the sample was centrifuged at 500 rpm for 5 minutes at ambient temperature. The supernatant was transferred to a 100 mL volumetric flask. Absorbance was measured at 147 wavelengths of 645 and 663 nm using a spectrophotometer.

Chlorophyll *a* ($\mu\text{g/mL}$):

$\text{Chl } a = 12.7A_{663} - 2.69A_{645}$

Chlorophyll *b* ($\mu\text{g/mL}$):

$\text{Chl } b = 22.9A_{645} - 4.68A_{663}$

where A_{663} and A_{645} are absorbance values at 663 nm and 645 nm, respectively (Pérez-Patricio *et al.*, 2018).

Statistical analysis:

Estimation of genetic parameters

Genotypic variance (V_g) = $\text{GMS} - \text{EMS}/r$

Where;

GMS= Genotypic variance

EMS = Environmental variance

r = number of replications

Environmental variance (V_e) = Error mean squares (EMS)

Phenotypic variance (V_p) = $V_g + V_e$

Heritability (h^2) broad sense: $h^2 = V_g/V_p$ (Mahmud and Kramer, 1951)

Expected response to selection (R_e): $R_e = k \cdot h^2 \sqrt{V_p}$ (Johnson *et al.*, (1955)

Where;

k = 1.271 at 25% selection intensity for a trait.

V_p = Phenotypic variance for a trait.

h^2 = Broad sense heritability for a trait.

RESULTS AND DISCUSSION

Hypocotyl length

Statistical analysis for hypocotyl length displayed highly significant ($p \leq 0.01$) differences among all common bean genotypes and genotype \times NaCl interaction, while it exhibited non-significant differences among NaCl levels (Table 3). Mean data for hypocotyl length ranged from 10.93 to 20.87 cm in standard (0 mM NaCl conc.) media from 8.71 to 21.43 cm in 150 mM NaCl concentration, while it ranged from 11.64 to 21.58 cm in 300 mM NaCl concentration. Genotypes GL-287 and MN-560 have the highest values for hypocotyl length at all NaCl levels (Table 3). The current results are in line with the findings of Dolatabadian and Ramin (2009), who also determined significant differences among common bean genotypes evaluated in three different NaCl levels. The genotypic variance of hypocotyl length was 2.49, while the environmental variance was 1.49, with a moderate heritability of 0.62 and a genetic advance of 1.58 in 0 mM NaCl concentration. For 150 mM NaCl concentration, the genotypic variance (18.05) NaCl concentration the environmental variance was (1.23) with a high heritability of 0.93 and genetic advance of 5.22, while in 300 mM NaCl concentration, the genotypic variance of hypocotyl length was (8.47) and the environmental variance was (1.28). Resulting in a high heritability of 0.86 and a high genetic advance of 3.44 (Table 5). Our results are in agreement with Zayed *et al.*, (2017), who also observed high heritability and genetic advancement for hypocotyl length.

Epicotyl length

Statistical analysis for epicotyl length showed highly significant ($p \leq 0.01$) differences among all common bean genotypes and genotype \times NaCl interaction, while it showed non-significant differences among NaCl levels (Table 2). Mean data for epicotyl length ranged from 10.73 to 15.30 cm in standard (0 mM NaCl conc.) media: from 11.10 to 15.30 cm in (150 mM NaCl conc) media: and from 10.00 to 15.70 cm in (300 mM NaCl conc) media; Genotype SW-32 had the highest value for epicotyl length in all NaCl levels (Table 3). The current results are in line with the findings of Dolatabadin and Ramin (2009), who also determine significant differences among common bean genotypes evaluated in three different NaCl levels. The genotypic variance of epicotyl length was 7.11, while the environment variance was 3.50, with the moderate heritability of 0.66 and genetic advance of 2.77. For 150, the genotypic variance was (2.58), and the environmental variance was (0.910) the resultant moderate heritability of 0.73 and genetic advance of (1.75). For 300, the genotypic variance was (2.13), and the environmental variance was (1.37) having a moderate heritability of 0.68 and genetic advance of 1.44 (Table 5). Our results are in agreement with Zayed *et al.*, (2017), who also observed heritability and genetic advance for epicotyl length.

Plant height

Statistical analysis for plant height showed highly significant ($p < 0.01$) differences among all common bean genotypes and genotype \times NaCl interaction. While it showed significant difference among NaCl levels (Table 2). Mean data for plant height ranged from 32.33 to 46.67 cm in standard (0 mM NaCl conc.) media and from 34.00 to 57.33 cm in 150 mM NaCl concentration, while it ranged from 33.50 to 45.67 cm in 300 mM NaCl concentration. Genotypes SW-32 showed the highest value for plant height at all NaCl levels (Table 4). The current results are in line with the findings of Ahtiyush *et al.*, (2020), who also determined significant differences among common bean genotypes evaluated in three different NaCl levels. The genotypic

variance of plant height was 15.77, while the environmental variance was 8.13 with the resultant moderate heritability of 0.65 and genetic advance of 4.19 in 0 mM NaCl concentration: For 150 mM NaCl concentration. The genotypic variance was (49.71) while the environmental variance was (8.7) resulting in high heritability of 0.85 and genetic advance of 8.26, while in 300 mM NaCl concentration, the genotypic variance of plant height was (15.42) and the environmental variation was (9.07) Resulting in moderate heritability of 0.62 and genetic advance of 3.96 (Table 5). Our results are consistent with Ahtiyush *et al.*, (2020), who also observed high heritability genetic advance and hypocotyl length.

Chlorophyll content

Statistical analysis for chlorophyll content presented highly significant ($P \leq 0.01$) differences among all common bean genotypes, genotype \times NaCl interaction, and NaCl levels (Table 2). Mean data for chlorophyll content ranged from 13.02 to 25.42 in standard (0 mM NaCl concentration). From 10.51 to 15.39 in (150 mM NaCl concentration). While it ranged from 6.96 to 12.99 in (300 mM NaCl conc). Genotype G-299 had the highest value in all NaCl levels. (Table 4). The current results are in line with the finding of Baryam *et al.*, (2014), who also determined the significant difference among common bean genotypes evaluated in three different NaCl levels. The genotypic variance of chlorophyll content was 18.6, and the environment variance was 3.05, with a high heritability of 0.85 and a genetic advance of 5.09 in 0 mM NaCl concentration. For 150, the genotypic variance was (2.33), and the environmental variance was (0.76) with a high heritability of 0.75 and genetic advance of 1.68, while the genotypic variance was (4.23) and environmental variance was (1.18) resulting in a high heritability of 0.78 and genetic advance of 2.31 in 300 mM NaCl concentration (Table 5). Our results are in agreement with Baryam *et al.*, (2014), who also observed high heritability 240 and genetic advance for chlorophyll content.

Table 2: Mean square values for various traits of eight common bean accessions, three NaCl levels, and genotype by NaCl interaction evaluated at the University of Agriculture, Peshawar in 2022

Traits	Genotypes (7)	NaCl (2)	Gen*NaCl (14)	Error (48)	CV (%)
Hypocotyl length	63.31**	2.90 ^{NS}	22.83**	2.01	9.35
Epicotyl length	17.80**	0.05 NS	3.82**	1.27	8.74
Plant height	222.93**	34.78*	22.87**	8.64	7.49
Chlorophyll content	28.187**	460.84**	26.26**	1.67	9.27

*, **= Significant at 5% and 1% level of probability, respectively

Values in () are the degree of freedoms

Table 3: Means of Hypocotyl length and Epicotyl length (cm) of eight common bean genotypes under three NaCl levels

Genotype	Hypocotyl length under NaCl Treatments			Hypocotyl mean	Epicotyl length under NaCl Treatments			Epicotyl mean
	0 mM	150 mM	300 mM		0 mM	150 mM	300 mM	
KA-70	10.93	12.97	11.64	11.85	12.97	12.00	13.23	13.21
GL-287	13.67	21.43	21.58	18.89	15.00	12.17	12.47	11.83
GL-299	15.27	8.71	14.60	12.86	11.77	11.10	12.63	12.57
GL-268	13.93	14.07	16.17	14.72	10.73	13.97	13.00	15.43
SW-32	16.17	12.67	12.50	13.78	15.30	15.30	15.70	10.79
SH-09	15.77	11.49	14.96	14.07	10.73	11.63	10.00	13.98
SH-19	16.90	17.26	14.99	16.38	13.13	15.27	13.53	12.22
MN-560	20.87	19.56	15.73	18.72	13.20	11.67	11.80	12.73
NaCl Mean	15.44	14.77	15.27		12.85	12.89	12.80	

Table 4: Means of plant height (cm) and Chlorophyll content ($\mu\text{M ml}^{-1}$) of eight common bean genotypes under three NaCl levels

Genotype	Plant height under NaCl Treatments			Plant height mean	Chlorophyll content under NaCl Treatments			Chlorophyll mean
	0 mM	150 mM	300 mM		0 mM	150 mM	300 mM	
KA-70	35.33	36.67	33.50	35.17	13.02	14.03	8.28	11.78
GL-287	39.00	40.67	41.00	40.22	15.20	12.84	8.44	12.16
GL-299	38.83	36.67	42.67	39.39	23.63	14.20	10.43	16.09
GL-268	32.33	34.00	34.20	33.51	16.31	14.33	12.82	14.49
SW-32	46.67	57.33	45.67	49.89	14.81	15.39	6.96	12.38
SH-09	37.00	37.00	36.30	36.77	19.73	10.51	9.37	13.20
SH-19	36.00	41.50	36.73	38.08	20.19	14.48	12.99	15.89
MN-560	41.00	40.73	40.37	40.70	25.42	11.83	9.23	15.49
NaCl Mean	38.27	40.57	38.80		18.54	13.45	9.81	

Table 5: Genetic (V_g), environmental (V_e), and phenotypic (V_p) variance, heritability (h^2_{BS}), and genetic advance (R_e) for various traits of eight common bean genotypes.

Traits	Treatments	V_g	V_e	V_p	h^2	R_e
Hypocotyl length (cm)	0 mM	2.49	1.49	3.99	0.62	1.58
	150 mM	18.05	1.23	19.29	0.93	5.22
	300 mM	8.47	1.28	9.76	0.86	3.44
Epicotyl length (cm)	0 mM	7.11	3.50	10.62	0.66	2.77
	150 mM	2.58	0.91	3.49	0.73	1.75
	300 mM	2.13	1.37	3.51	0.68	1.44
Plant height (cm)	0 mM	15.77	8.13	23.91	0.65	4.10
	150 mM	49.71	8.7	58.41	0.85	8.26
	300 mM	15.42	9.07	24.54	0.62	3.96
Chlorophyll content ($\mu\text{M ml}^{-1}$)	0 mM	18.66	3.05	21.72	0.85	5.09
	150 mM	2.33	0.76	3.09	0.75	1.68
	300 mM	4.23	1.18	5.41	0.78	2.31

CONCLUSIONS AND RECOMMENDATIONS

Conclusions

Analysis of variance exhibited substantial differences among the eight common bean genotypes and genotype \times NaCl interaction, while non-significant for hypocotyle length and epicotyle length in NaCl levels for the studied traits under three different NaCl concentrations (0 mM, 150 mM, and 300 mM). Increasing salinity levels negatively impacted chlorophyll content, hypocotyl length, plant height, and epicotyl length, though some genotypes showed superior tolerance. Genotype KA-70 exhibited the lowest chlorophyll content at all salinity levels, indicating poor

salt tolerance. Genotype GL-287 demonstrated the highest values for hypocotyl length across all NaCl levels, suggesting good salt tolerance for this trait. Genotype SW-32 showed superior plant height and epicotyl length across salinity treatments, making it a promising candidate for salt-tolerant breeding programs. Heritability estimates were generally high, with the highest heritability recorded for hypocotyl length (0.93) in 150 mM NaCl. The moderate heritability was recorded for plant height (0.62) in 300 mM NaCl and hypocotyl length in 0 mM NaCl. The highest genetic advance was observed for plant height under 150 mM NaCl 8.26,

indicating good potential for breeding improvements in this trait.

RECOMMENDATIONS

Genotypes SW-32, GL-299, and GL-287, exhibiting exceptional performance in hypocotyl length, plant height, and chlorophyll content, should be prioritized for forthcoming breeding projects focused on enhancing salt tolerance in common bean cultivars. Additional molecular and physiological investigations are necessary to elucidate the processes underlying salt tolerance in these genotypes. It is advisable to conduct further screening of common bean genotypes from various geographical regions to identify more salt-tolerant variants. The creation of salt-tolerant cultivars should prioritize characteristics such as hypocotyl length, plant height, and chlorophyll content, as these traits were notably influenced by salinity and shown favorable heritability and genetic progress.

Novelty Statement:

This study's novelty lies in its comprehensive evaluation of genetic diversity, heritability, and genetic advance in eight common bean genotypes under varying levels of salinity stress in a hydroponic system. This research provides new insights into the salt tolerance mechanisms of common bean genotypes and recommends specific genotypes with superior traits for future breeding programs aimed at enhancing salinity resistance in crops.

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