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Phenotypic variability in the response to salinity during common bean germination

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Abstract. Common bean (*Phaseolus vulgaris* L.) is an important crop whose establishment can be affected by salinity. The present study evaluated five local populations collected from Caras - Severin County, Romania: Comoraste, Sacu, Bocsa Romana, Carasova, and Iablanita, with respect to seed morphology, germination capacity, and early seedling growth under saline conditions. Salinity was simulated using NaCl solutions corresponding to osmotic pressures of 1, 3, and 5 atm, with distilled water used as the control. Germination, radicle and cotyledon lengths, and dry matter content were evaluated after 96 and 192 hours. The results showed a general reduction in germination and seedling growth as salinity increased, although the magnitude of the response differed among local populations. After 192 hours, the "Comoraste" cultivar maintained the greatest radicle length in variant V3 (1.91 cm), while "Carasova" recorded the lowest value (0.70 cm). Iablanita maintained comparatively high germination at V_1 and V_2 but showed a marked decline at V_3. The mean ± standard deviation values demonstrate substantial biological variability for several growth traits. Dry matter content also varied among populations and treatments. These results indicate variability among the studied local populations in their responses to saline conditions and provide a basis for further evaluation of common bean genetic resources.

Introduction

Common bean (*Phaseolus vulgaris* L.) is a major grain legume and an important source of dietary protein and minerals. Salinity can impair water uptake, disturb ionic balance, and inhibit cell expansion, with effects becoming evident in roots and shoots. Experimental studies in common bean have shown that increasing NaCl concentrations delay germination and reduce seedling growth, while the magnitude of the response varies among genotypes [3,4,5]. Bayuelo-Jiménez et al. demonstrated substantial genetic variation in salinity responses during germination and early seedling development across *Phaseolus* accessions. Studies of *Phaseolus vulgaris* have likewise reported reductions in root and shoot growth under saline conditions and genotype-dependent differences in salt responses [4,5,22]. More recent genetic studies confirm that salt tolerance in common bean is a complex trait with substantial genetic variation. Raggi et al. identified genomic regions associated with salt responses at the early vegetative stage, reinforcing the value of phenotypic screening of diverse germoplasm. Germination depends on the activation of hydrolytic enzymes (amylases, proteases, and lipases) responsible for mobilising nutrient reserves [17]. Saline stress inhibits enzyme synthesis and activity, causes denaturation of enzyme proteins due to ionic imbalances, and reduces the conversion of starch into simple sugars [12,13]. Consequently, the embryo receives less energy and substrate for growth, which may explain the reduced germination percentage and delayed germination [16,25]. Cellular respiration is essential for supplying the ATP required for embryonic growth [9]. Under saline conditions, excess Na^+ and Cl^- induce oxidative stress, reducing the efficiency of the mitochondrial respiratory chain and lowering ATP production [10]. This energy deficit limits radicle elongation and cell division [24]. High concentrations of Na^+ and Cl^- ions cause direct toxicity to embryonic cells [14], disrupt the K^+/Na^+ ratio, which is essential for cellular functions [21], and impair membrane permeability [6]. These effects lead to loss of cell membrane integrity, electrolyte leakage, and irreversible damage to some seeds [18]. The objective of this study was to evaluate five local common bean populations from Caraș-Severin County, Romania, using germination capacity, radicle and cotyledon growth, and dry matter content as indicators of their response to NaCl-induced salinity.

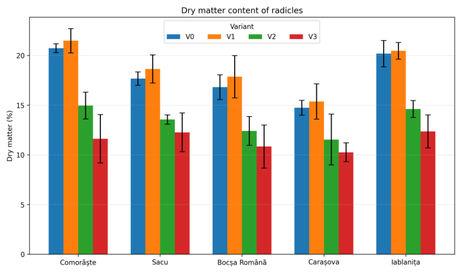
Material and method

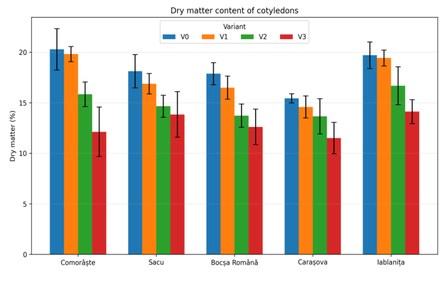
The biological material consisted of five local populations of *Phaseolus vulgaris* L. obtained during collecting expeditions in Caraș-Severin County, Romania. The local population names correspond to the localities of origin: Comoraste, Sacu, Bocsa Romana, Carasova, and Iablanita. The experiments were conducted to investigate genetic variability in salinity tolerance within *Phaseolus* by assessing seed germination performance and seedling growth. NaCl solutions corresponding to osmotic pressures of 1, 3, and 5 atm were used to simulate saline conditions. A series of tests was used to study salinity tolerance: seed germination capacity in a saline medium (measured as a percentage over the experimental period); root and cotyledon growth in seedlings cultivated in a saline medium (cm); and dry matter content in the radicle and cotyledon of seedlings grown in a saline medium (%). Germination capacity is an index of a seed's ability to germinate, expressed as the percentage of pure seeds that germinate normally under optimal conditions within a timeframe specific to each species. For beans, the NaCl tolerance of the five local populations under study was evaluated by cultivating sterilized seeds treated with various salt concentrations. The bean seeds were sterilized using a 0.1% mercuric chloride solution for 20 minutes and subsequently rinsed repeatedly with distilled water. They were placed in 10-cm-diameter Petri dishes between two filter paper discs moistened with either distilled water (for the control variant, V0) or NaCl solutions at various osmotic pressures. The Petri dishes containing the bean seeds were placed in an incubator at 22°C for germination.Four experimental variants were used: V0 (control), treated with distilled water; V_1, NaCl at 1 atm; V_2, NaCl at 3 atm; and V_3, NaCl at 5 atm. The first measurement was taken after 96 hours, and the germination rate for the various NaCl treatments was calculated using the following formula:

Germination rate (%) = (number of germinated seeds/number of sown seeds) × 100

Germination tolerance in the presence of NaCl was assessed by comparison with the control treatment. A second measurement was performed after 192 hours to calculate the percentage of germinated seeds. In addition to the seed germination test, measuring root and cotyledon growth provides a complementary method for evaluating plant tolerance to salt stress, as plant roots in contact with the saline medium (solution or soil) are directly exposed to the stress factor. Radicle and cotyledon growth in the saline medium were measured for all experimental variants at 96 and 192 hours after germination (cm). Dry matter content was determined in fresh material using a Kern MLS 50 thermobalance. The following plant materials were used for these determinations: radicle and cotyledon. Statistical analysis was performed on the collected data, and the results are expressed as the mean ± standard error of the mean.

Dry matter generally increased slightly from V_0 to V_1 in the radicles of all five populations but declined strongly at V_2 and V_3. For example, Comoraste radicle dry matter was 20.73% in V_0, 21.48% in V_1, 14.96% in V_2, and 11.62% in V_3; Iablanita followed a similar pattern (20.18, 20.47, 14.61, and 12.35%). Cotyledon dry matter also tended to decline at the higher salinity treatments. Comoraste decreased from 20.28 ± 2.05% in V_0 to 12.12 ± 2.45% in V_3, whereas Iablanita decreased from 19.70 ± 1.32% to 14.12 ± 1.19%. The magnitude of the decrease differed among populations. The marked decline at stages V_2 and V_3 is correlated with reduced seedling elongation and is consistent with growth inhibition under saline conditions





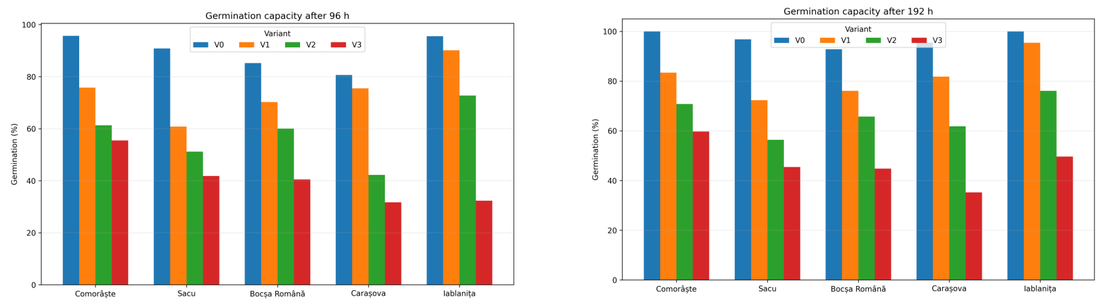
Results and discussions

The five populations showed clear morphological differentiation. Comoraste, Bocsa Romana, Carasova and Iablanita were described as reniform, whereas Sacu was round. Seed weight and dimensions also varied among populations, indicating phenotypic heterogeneity in the collected material (Table 1).

Table 1. Morphological characteristics of seeds from the five local common bean populations

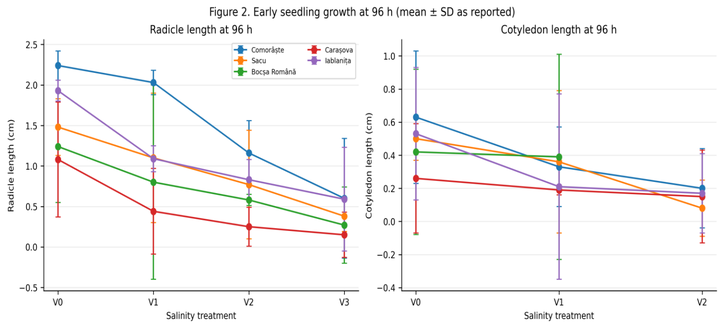
Population	Shape	Colour		Weight (g)	Length (cm)	Diameter (cm)
Comoraste	reniform	white	matte	0.27–0.53	0.9–1.3	0.4–0.5
Sacu	round	white-grey	shiny	0.40–0.66	1.2–1.4	0.6–0.8
Bocsa Romana	reniform	slightly grey	shiny	0.29–0.56	1.4–1.6	0.5–0.6
Carasova	reniform	white	matte	0.29–0.46	1.4–1.6	0.4–0.6
Iablanita	reniform	very white	transparent	0.36–0.49	1.0–1.2	0.5–0.6

Seed germination begins with the process of imbibition, which depends on the osmotic potential gradient between the seed and the external environment. In the experimental variants involving NaCl (V1–V3), the increase in osmotic pressure leads to reduced water absorption by embryonic tissues, delaying macromolecule hydration and inhibiting the initiation of early metabolic processes. The decline in germination observed particularly in variants V_2 and V_3 reflects a physiological water deficit, even though the seeds are placed in an apparently moist environment. This also accounts for the lower values recorded at 96 hours, as imbibition remains insufficient at high saline concentrations. Germination decreased with increasing salinity in all five local populations. After 96 h, germination under the control treatment ranged from 80.6% in Carasova to 95.7% in Comoraste, while under 5 atm NaCl it ranged from 31.7% in Carasova to 55.5% in Comoraste. Iablanita maintained 90.1% germination at 1 atm and 72.7% at 3 atm after 96 h, indicating a relatively small reduction under moderate salinity. After 192 h, germination increased in all populations relative to 96 h, but the same dose-related reduction remained. Iablanita reached 95.4% at V_1 and 76.1% at V_2 after 192 h, but only 49.6% at V_3 (Fig.1).



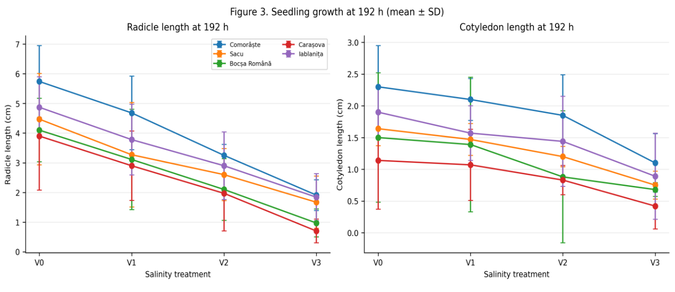
- At 96 h, radicle length declined as salinity increased. For example, in Comoraste, radicle length decreased from 2.24 ± 0.18 cm in V0 to 0.60 ± 0.74 cm in V3. A similar reduction was observed in Sacu (1.48 ± 0.35 to 0.38 ± 0.19 cm), Bocsa Romana (1.24 ± 0.69 to 0.27 ± 0.47 cm), Carasova (1.08 ± 0.71 to 0.15 ± 0.28 cm), and Iablanita (1.93 ± 0.13 to 0.59 ± 0.64 cm). These data show a consistent inhibitory effect of increasing salinity on early radicle development. After 96 hours from germination, the cotyledons have less development than the radicle (under 1 cm in most cases). In the V_2 and V_3 variants, the formation of cotyledons is very weak or even absent. The results highlight a reduced development of the cotyledons in the early phase, compared to the radicle. The Comoraste genotype presents the highest values (0.63 cm), followed by Iablanita (0.53) and Sacu (0.50 cm), in V_0 (the non-saline-stressed variant). Carasova has the lowest values , indicating a high sensitivity – 0.26 cm in V_0 (Fig. 2).

Figure 2. Early seedling growth at 96 h (mean ± SD as reported)



At 192 h, the data provide the clearest quantitative evidence of a dose-dependent reduction in radicles and cotyledons. For radicles, Comoraste decreased from 5.74 ± 1.21 cm in V_0 to 1.91 ± 0.52 cm in V_3, while Carasova decreased from 3.90 ± 1.82 to 0.70 ± 0.40 cm. Bocsa Romana decreased from 4.10 ± 1.07 to 0.97 ± 0.47 cm, and Iablanita from 4.87 ± 1.03 to 1.84 ± 0.79 cm. Comoraste maintained the longest radicles at V_1, V_2 and V_3 among the five populations, whereas Carasova had the shortest radicles at V_2 and V_3. The relatively large standard deviations in some treatments indicate substantial biological variation among seedlings (Fig. 3).

Figure 3. Seedling growth at 192 h (mean ± SD)



Conclusions. The reduction in germination capacity under salt stress conditions results from the cumulative effects of water deficit (osmotic stress), inhibition of enzymatic activity, reduced respiration, and ionic toxicity. At the same time, the increase in germination observed at 192 hours demonstrates the existence of physiological adaptation mechanisms, varying in intensity according to genotype, that may form a basis for selection for salinity tolerance.

Saline stress causes significant inhibition of radicle and cotyledon growth, with more severe impairment of aerial organs and significant differences among genotypes. The Comoraste and Iablanita populations stand out by maintaining relatively high values and exhibiting better physiological stress tolerance. In contrast, Carasova displays marked sensitivity, evidenced by severe growth inhibition.

Dry matter content in the radicle is a key physiological indicator of seedling vigour, closely linked to growth processes. Its reduction under salinity stress highlights the sensitivity of primary metabolism and root development to osmotic and ionic stress conditions.

The five local populations of *Phaseolus vulgaris* L. showed differences in germination and early seedling responses to NaCl - induced salinity. Increasing salinity generally reduced germination and inhibited radicle and cotyledon development. The strongest effects were observed at 5 atm NaCl. Iablanita and Comoraste maintained comparatively high germination under moderate salinity, while Carasova showed marked reductions in several measured traits. Dry matter content also varied among populations and salinity treatments. The results support the existence of phenotypic variability among the studied local populations and justify further replicated studies aimed at characterising salinity tolerance in common bean genetic resources.

Considering germination, radicle growth, cotyledons and dry matter together, the five populations show a common dose-response pattern, but differ in the magnitude and timing of the response. Comoraste combined high germination with comparatively strong radicle growth under severe treatment, particularly at 192 h. Iablanita maintained very high germination at V_1 and relatively high germination at V_2, although V_3 caused a marked decline. Carasova had lower control germination and the strongest reduction in radicle length at the higher salinity treatments. Sacu and Bocșa Romana showed intermediate responses. Published research indicates that salinity tolerance in beans involves osmotic stress, ion accumulation, water relations, genotype, and phenotypes at early stages can help identify germoplasm.