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Variations in Germination and Seedling Traits of Rapeseed (*Brassica napus* L) Genotypes under Salinity Stress and Salicylic Acid Treatment

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ABSTRACT

Rapeseed is an important oilseed crop in Bangladesh that's germination and seedling growth often get affected in saline-prone regions. This study focuses on assessing the variation in germination and seedling traits of rapeseed genotypes under salinity and dual stress – salinity with salicylic acid. Seeds of five parents and ten F_3 segregants of rapeseed (*Brassica napus*) were subjected to three treatments for eight days— control, 8 dSm^{-1} salt and 8 dSm^{-1} salt+0.1mM salicylic acid. The F_3 segregants were considered to evaluate the diverse inherent phenotypic expression of traits rather than only fixed genetic combination in parents. Shoot length was reduced by 52.6% and root-shoot ratio was increased by 43.7% in 8 dSm^{-1} salinity while percent germination, shoot length, root length and root-shoot ratio were reduced by 9.6%, 53.9%, 66.7%, 27.2%, respectively, in response to 8 dSm^{-1} salt+0.1mM salicylic acid, compared to control. Salinity stress with salicylic acid reduced percent germination, root length, dry mass per plant and root-shoot ratio by 9.1, 58.6, 20.0 and 18.3 %, respectively, compared to salinity stress alone. These results indicated that salicylic acid worsens the effects of salinity stress duration germination and early seedling growth. Despite, a few genotypes showed notable tolerance against both salinity and dual stress indicating their potential to select for further breeding and QTL analyses. PC1 explained 38% variation and separated the genotypes under 8 dSm^{-1} salt+0.1mM salicylic acid treatment from two other treatments for high and positive coefficient for shoot length, percent germination, root length and root-shoot ratio suggesting those are salt responsive traits. The results offer a foundation for phenotypic selection, biochemical analysis, and future molecular analysis upon dual treatment of salicylic acid and salt. The results will be also useful for early identification and development of salt-resilient rapeseed lines through high resolution genetic mapping during further studies.

1. Introduction

Rapeseed-mustard is the major provider of edible oil in Bangladesh as it covers the highest area of 74% of the total oilseed crops cultivated in the country (BBS 2023; Chakroborty et al. 2025). Salinity acts as a major abiotic stress in agricultural practices, which inhibits the growth of plants and reduces the yield of major crops by 50% (Bray 2000). Over 3600 million hectares of soil have been impacted by salinity, and the total area is growing by about 1.5 million hectares of land per year (Rahman et al. 2021; FAO 2024). About 37% of the total cultivable land in the southern coastal region of Bangladesh is presently affected by varying degrees of soil salinity because of tidal surges (Dasgupta et al. 2014).

Saline soil could be a potential hazard for rapeseed-mustard cultivation. While all the growth phases of a plant are impacted by salt stress; most plant species are particularly vulnerable during the seed germination and seedling growth stages (Stassinos et al. 2021; Zhang et al. 2022; BiBi et al. 2024). It was observed that seed germination percentage displayed an inverse relationship with salinity level in rapeseed-mustard (Batool et al. 2021). Failure in germination in saline soil is often associated with the accumulation of toxic ions at the seedling establishment zone for the upward movement of soil solution (imbibition) and subsequent evaporation at the soil surface (Sharma et al. 2013; Zhang et al. 2022).

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Two possible events e.g., either osmotic stress or ion toxicity initiated by salt stress could potentially hamper seed germination by impairing radicle growth (Sharma et al. 2013; Batool et al. 2021). The most frequent deleterious effects of salinity on *Brassica* crops include decreased plant height, growth, and yield as well as deterioration in seed quality (Ahamed et al. 2021). Salinity stress had a greater impact on the shoot & root growth, leaves, and root shoot ratio of *B. napus* (Arif et al. 2019). Salinity also induces programmed cell death (PCD) which hampers plant growth and reduces yield potential (Jalili et al. 2022).

Salicylic acid— a phenolic endogenous growth regulator, is believed to mitigate the deleterious effects of biotic and abiotic stresses in *B. napus* (Ilyas et al 2024; Raees et al 2023) while treated exogenously (seed priming or foliar spray). Salicylic acid acts as an antioxidant since it scavenges and reduces the amount of reactive oxygen species (ROS) produced in response to salt-stress (Yang et al. 2023). However, the role of salicylic acid in managing salt stress was contradictory and depended on the growth phase of plants (Afrin et al. 2025) and experimental conditions (Hayat et al. 2010). It is evident that SA regulates the root growth in a concentration dependent manner (Pour et al.; 2012, Bouallègue et al. 2017). Specific plant species and physiological stages of the plant also regulate the role of SA applied under stress (Afrin et al. 2025). Though there are several experiments which represented the role of SA in mitigating salt stress while applied through seed priming, the role of SA simultaneously applied with salt stress through the root system has not yet been understood in the germination phase of oilseed *Brassica.*

The germination rates and percentage of germinated seeds at a particular time vary considerably among species and cultivars (Mir and Somasundaram, 2021; Hasanuzzaman et al. 2010). Therefore, the treatment and genotypic variation found in rapeseed-mustard in terms of germination and early seedling traits could be exploited for introducing salt tolerant varieties (Bybordi 2010; Puppala et al.; 1999; Zheng et al. 1998).

This study investigated how salinity stress affects the germination percentage and root-shoot traits during the early germination phase in both parents and F_3 segregants of *B. napus*, as well as the combining effect of salt and salicylic acid while absorbed through the root system. Despite using the single dose of SA, this study developed a baseline understanding by selecting a physiologically relevant dose of salt (8 dsm^{-1}) and SA (0.1mM) for *B. napus* to observe the effects of dual treatments simultaneously applied in the growing media (Afrin et al. 2025). As the F_3 segregants are the very early and prominent segregating populations, presence of more genetic recombination makes them potential candidate to systematically evaluate and select for quantitative traits e.g., abiotic stress resilience (Li et al. 2013). By identifying the specific morphological responses of rapeseed during germination period, the study will facilitate the earlyselection of suitable breeding materials from parents and F_3 segregants to develop novel, salt-tolerant rapeseed genotypes.

2. Materials and Methods

2.1. Experimental site and periods with spell

The experiment was conducted in the growth room of Department of Genetics and Plant Breeding (GPB), Bangladesh Agricultural University (BAU) in Mymensingh, Bangladesh. A temperature of 22 ±2 °C, relative humidity of 70%, and a 16:8-hour day: night ratio was maintained in the growth room.

2.2. Planting materials and source

Seeds of 10 F_3 generation and five parents of advanced breeding lines of rapeseed (Table 1) were collected from the Department of GPB, BAU, Mymensingh. The hybrids were the result of the previous experiments conducted at the Dept. of GPB (Azim et al. 2024).

2.3. Experimental design and procedure

A completely randomized design (CRD) with three treatments and three replications was adopted. There were three different treatments— control (T1), 8dSm^{-1} salt stress (T2), and 8dSm^{-1} salt+0.1mM salicylic acid (T3) (Ali et al. 2023; Göre 2025; Afrin et al. 2025). To prepare 8dSm^{-1} salt solution, 39g of sodium chloride (NaCl) was mixed with distilled water and 0.138 mg of salicylic acid powder was added to create a concentration of 0.1mM of salicylic acid. Moist napkin paper was used in each sterilized petri dish as growing media and ten seeds of each rapeseed genotype were placed for germination in every petri dish (Figure 1).

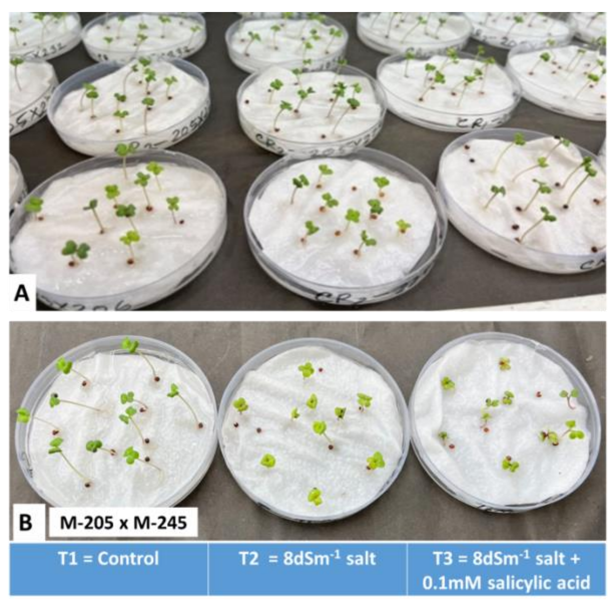

Figure 1. Germination of seeds in petri dishes in a plant culture room; (A) experimental view, (B) effect of three different level of treatments on germination and seedling growth

Table 1. Name and source of studied rapeseed genotypes

#	Code given	Genotypes	Generation	Source
1	G1	M-232×M-223	F_3 generation	Segregating generation developed by the research group of Professor Arif Hasan Khan Robin, Department of Genetics and Plant Breeding, Bangladesh Agricultural University
2	G2	M-205×M-232		
3	G3	M-205×M-223		
4	G4	M-223×M-206		
5	G5	M-223×M-205		
6	G6	M-205×M-245		
7	G7	M-206×M-223		
8	G8	M-232×M-245		
9	G9	M-206×M-232		
10	G10	M-245×M-206		
11	G11	M-205	Parents	Short duration, early flowering, high-yielding *Brassica napus* genotypes selected from previous experiments (Azim et al. 2024)
12	G12	M-206		
13	G13	M-223		
14	G14	M-232		
15	G15	M-245		

Treatment imposition started immediately after arranging the seeds on the petri-dishes and continued at regular intervals for eight days until destructive harvest. The majority of the seeds germinated after one or two days. The radicle of a seed was deemed to have germinated when it was at least 3 mm long. Dry weight per plant was recorded in a weighing machine after the plants were desiccated in an air-tight oven for 3 days in 60 ºC.

2.4. Data collection and analysis

Percent germination was measured following (n / N) × 100, where n is the number of germinated seeds at the eighth day; N is the total number of seeds. Shoot length was measured from the cotyledons to the collar region and root length was measured from the region to the root tip by using 15 cm ruler. In addition, root-shoot ratio was calculated after data collection. MINITAB 19 (Minitab Inc., State College, Pennsylvania, USA) software was used for statistical analysis. A two-way ANOVA was conducted using the General Linear Model (GLM) to examine treatment, genotype, and treatment x genotype effects on root and shoot attributes. Tukey's pairwise comparison was used for *posthoc* analysis. Additionally, a PCA biplot was presented (Rstudio) to identify associations among genotypes and traits, and Pearson correlation analysis was performed to examine correlations between selected traits.

3. Results

3.1. Effects on germination percentage

This study explored the effects of treatment and genotype on seed germination. Both factors significantly influenced germination ($P < 0.001$), though the interaction was not significant ($P = 0.232$) (Table 2). The seeds under control recorded higher germination rate of 90.9% than the seeds treated with 8 dSm^{-1} salt + 0.1 mM salicylic acid (81.3%) (Table 3). The seeds of the genotype G15 showed the highest germination rate of 98.8%, and the genotype G12 reported the lowest rate of 72.2% (Table 4).

3.2. Effects on Seedling Traits

The traits such as germination percentage, shoot length, root length, root-shoot ratio and dry weight plant^{-1} were altered in the presence of salt and combined salt and salicylic acid treatment. Analysis of variance indicated the presence of significant genotypic variation for all of the traits except for only shoot length (Table 2). Eventually, shoot length, root length and root-shoot ratio had significant against genotypic differences (Table 2). Shoot length was recorded highest 2.62 cm in control which significantly reduced when treatments were imposed i.e., 1.24 cm for 8dSm^{-1} salt and 1.21 cm for 8dSm^{-1} salt + 0.1mM salicylic acid (Table 3). That means shoot length was reduced significantly by 52.6% and 53.8% under 8 dSm^{-1} salt (T2) and 8dSm^{-1} salt + 0.1mM salicylic acid (T3), respectively, compared to control (Table 2). Notably, when the impact of salicylic acid supplementation was compared with salinity stress alone it was found that 8dSm^{-1} salt + 0.1mM salicylic acid (T3) reduced percent germination, root length, dry mass per plant and root-shoot ratio by 9.1, 58.6, 20.0 and 18.3 %, respectively, compared to salinity stress (T2) alone (Table 2).

All the genotypes except for G4 showed a clear significant reduction in shoot length in T2 and T3 compared with control (T1) (Fig. 2B). Among genotypes, significant difference was found for root length in G1, G7 within control and 8dSm^{-1} salt + 0.1mM salicylic acid (Fig. 2C). The root length of G1 was significantly reduced under 8dSm^{-1} salt (2.57 cm) and 8dSm^{-1} salt + 0.1mM salicylic acid (0.88 cm) compared to control (6.19 cm) (Fig. 2C). The genotype G7 recorded the highest root length of 4.82 cm for control but it diminished significantly to 0.91 cm under 8dSm^{-1} salt + 0.1mM salicylic acid (Fig. 2C). Plants under 8dSm^{-1} salt stress recorded the highest root-shoot ratio of 2.045 and the lowest ratio of 0.837 was recorded under 8dSm^{-1} salt + 0.1mM salicylic acid treatment (Table 3). Among genotypes, G7 recorded the highest (1.86) root-shoot ratio and G10 and G13 had recorded the lowest (0.82) root-shoot ratio (Table 4). For the genotypes G5 and G9, the root-shoot ratio was observed to be increased by 74.5% and 71% respectively, in 8dSm^{-1} salt stress compared to control (Fig. 2D). The genotype G5 recorded the highest root-shoot ratio of 2.99 in 8dSm^{-1} salt than both under control (0.76 cm) and 8dSm^{-1} salt + 0.1mM salicylic acid (0.80 cm) (Fig. 2D). Under T3, the dry weight per plant decreased significantly to 2.4 mg compared to about 4 mg in T1 and T2 (Table 3). Genotype G14 reported the highest average dry weight plant^{-1} of 4.4 mg whereas, G5 weighed the lowest 2.2 mg (Table 4).

Table 2. Analysis of variance (mean square) for germination and seedling traits of fifteen rapeseed genotypes under salt and salt + salicylic acid induced salinity stress

Source of variation	Df	PG	SL	RL	DWP-1	R/S ratio
Genotypes (G)	14	4.87***	0.141	2.54*	0.000001***	0.89*
Treatments (T)	2	13.1***	29.16***	46.16***	0.000005***	17.6***
G × T	28	0.64	0.18**	2.04**	0.000011	0.74**
Error	90	0.52	0.091	0.93	0.000010	0.37

**PG (%) =Percent germination, SL=Shoot length, RL=Root length, R/S=Root Shoot ratio, DWP^{-1}=Dry Weight Per Plant. Different letters denote Tukey's letter of significant variation among the genotypes

Table 3. Comparison of means for germination and early seedling traits across three treatments

Traits	Control	8 dSm^{-1} salt	8 dSm^{-1} salt +0.1mM salicylic acid	Mean
Percent germination	90.9±0.39^{a}	90.4±0.26^{a}	81.3±0.42^{b}	87.53
Shoot length (cm)	2.62±0.17^{a}	1.24±0.11^{b}	1.21±0.16^{b}	1.69
Root length (cm)	2.95±0.69^{a}	2.37±0.39^{a}	0.98±0.17^{b}	2.1
Root-shoot ratio	1.15±0.28^{b}	2.04±0.37^{a}	0.837±0.17^{b}	1.34
Dry weight $plant^{-1}$ (mg)	2.98±0.00^{a}	2.94±0.00^{a}	2.4±0.00^{b}	2.77

Table 4. Comparison of means for germination and seedling traits in rapeseed genotypes

Traits	PG (%)	SL (cm)	RL (cm)	R/S	DWP^{-1} (mg)
G1	87.7abc	1.64	3.2	1.7	2bc
G2	91.1a-c	1.65	2.62	1.64	3a-c
G3	86.6a-d	1.64	2.06	1.34	7a-c
G4	81.1c-e	1.65	2.15	1.34	3.3a-c
G5	84.4b-e	1.47	1.83	1.52	2.2c
G6	90a-c	1.84	1.71	0.96	3.3a-c
G7	90 a-c	1.55	2.9	1.86	2.2a-c
G8	93.3a-c	1.64	1.67	1.27	3.3a-c
G9	91.1a-c	1.55	2.22	1.67	3.3a-c
G10	95.5ab	1.71	1.3	0.82	3.3a-c
G11	90a-c	1.85	2.46	1.34	3.3a-c
G12	72.2e	1.65	1.87	1.33	3.3ab
G13	73.3de	1.88	1.38	0.82	3.3a-c
G14	87.7abc	1.73	2.21	1.25	4.4a
G15	98.8a	1.87	1.94	1.23	3.3a-c
p-value	0.001	0.998	0.215	0.285	0.001

**PG (%) =Percent germination, SL=Shoot length, RL=Root length, R/S=Root Shoot ratio, DWP^{-1}=Dry Weight Per Plant. Different letters denote Tukey's letter of significant variation among the genotypes

3.3. Principal component analysis

About 99% of the overall data variation for the influence of treatments and plant genotypes on five crucial germination and seedling traits were explained by the first four principal components (Table 4). PC1, PC2, PC3, and PC4 explained 41.8%, 27.5%, 16.1%, and 13.4% of the total data variation, respectively (Table 5). PC1 was highly significant for treatments, genotypes, and the treatment× genotype interaction (Table 5). For higher and positive coefficients of all the characteristics, the first principal component (PC1) distinguished rapeseed genotypes with higher germination percentage, root-shoot ratio and dry weight (Figure 5). PC1 clearly distinguished T3 (8dSm^{-1} salt + 0.1mM salicylic acid) from other two treatments (control and 8dSm^{-1} salt) for their lower positive coefficients for the traits e.g., percent germination, root length, shoot length and root-shoot ratio (Table 5) indicating the traits were stress-responsive (Fig. 3). Similarly, PC2 separated the genotypes under control from the genotypes treated with 8dSm^{-1} salt and 8 dSm^{-1} salt + 0.1mM salicylic acid for their higher positive PC scores and higher PC coefficients of the traits stress responsive traits (Fig. 3).

3.4. Correlation study among germination and seedling traits

Correlation study revealed that out of ten associations, four associations were highly significant, three associations were significant, and three associations were non-significant (Fig. 4). Eight associations were positively correlated, and two associations were negatively correlated (Fig. 4). Percentage germination had highly significant ($r>0.5$, $P<0.05$) positive correlation with shoot length, root length and root shoot ratio (Fig. 4). Shoot length was in highly significant ($r>0.5$, $P<0.05$) positive correlation with root length and dry weight per plant and negative significant ($r>0.5$, $P<0.05$) correlation with root-shoot ratio. Root length showed highly significant ($r>0.5$, $P<0.05$) positive correlation with root shoot ratio (Fig. 4).

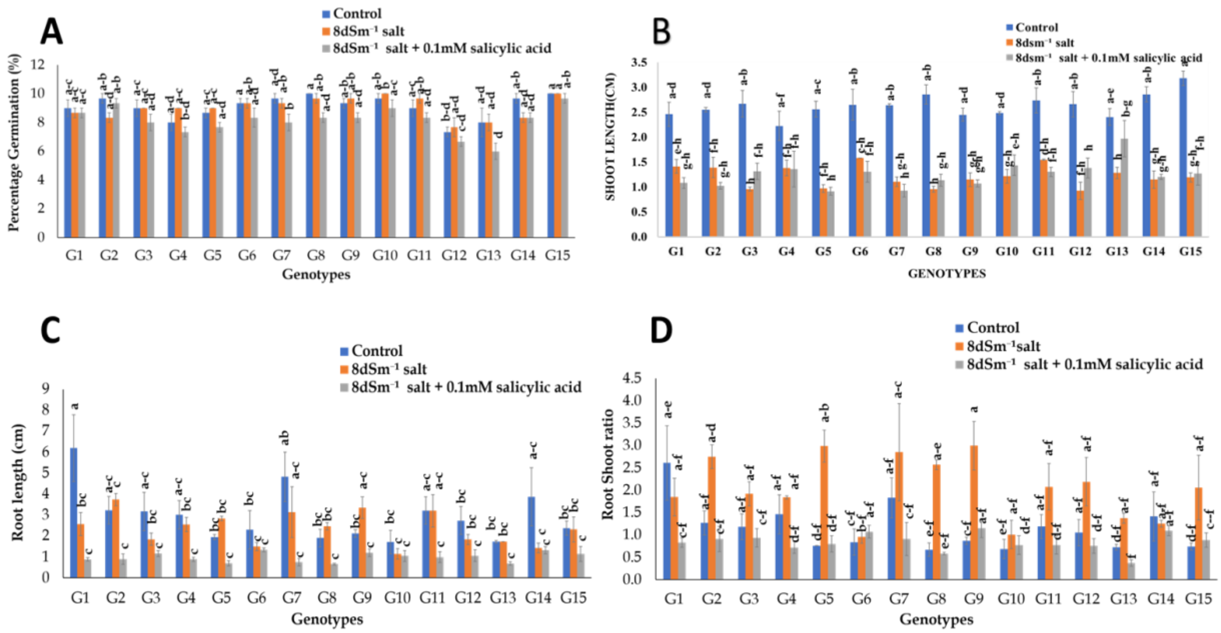

Figure 2. Treatment effect, genotypic variation and genotypic performance over environment for A) percent germination of fifteen rapeseeds genotypes under control, 8dSm-1 salt and 8dSm-1 salt + 0.1mM salicylic acid. B) shoot length of fifteen rapeseeds genotypes under control, 8dSm^{-1} salt and 8dSm^{-1} salt + 0.1mM salicylic acid. **C**) root length of fifteen rapeseeds genotypes under control, 8dSm^{-1} salt and 8dSm^{-1} salt + 0.1mM salicylic acid. **D**) root-shoot ratio of fifteen rapeseeds genotypes under control, 8dSm^{-1} salt and 8dSm^{-1} salt + 0.1mM salicylic acid. G1= M-232×M-223, G2= M-205×M-232, G3= M-205×M-223, G4=M-223×M-206, G5= M-223×M-205, G6= M-205×M-245, G7= M-206×M-223, G8= M-232×M-245, G9= M-206×M-232, G10= M-245×M-206, G11= M-205, G12= M-206, G13= M-223, G14= M-232, G15= M-245. Vertical bars indicate standard error of mean; different letters denote significant differences

Table 5. Coefficients of Principal Components for germination and seedling traits of 15 rapeseed genotypes

Variable	PC1	PC2	PC3	PC4
Percentage of Germination	0.413	0.226	-0.193	-0.860
Mean of Shoot Length	0.219	0.677	0.520	0.150
Mean of Root Length	0.676	-0.025	0.213	0.295
Dry Weight per Plant	0.076	0.519	-0.776	0.349
Root Shoot Ratio	0.564	-0.470	-0.211	0.171
Eigenvalue	1.90	1.41	0.85	0.77
% Variation explained	41.8	27.5	16.1	14.4
P-value (Genotype)	<0.001	0.075	0.258	<0.001
P-value (Treatment)	<0.001	<0.001	<0.001	0.101
P-value (Genotype× Treatment)	0.005	0.299	0.384	0.024

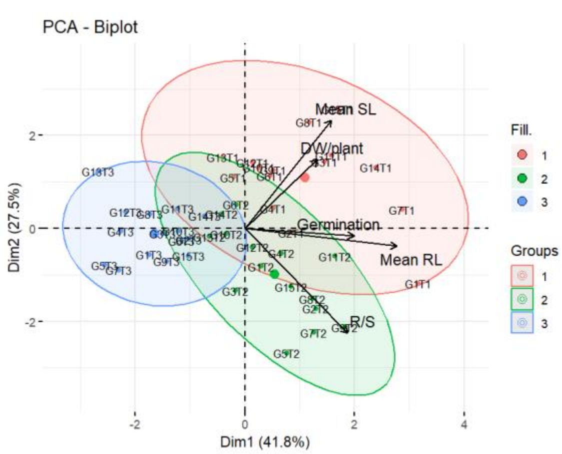

Figure 3. Biplot for germination and seedling traits of fifteen rapeseed genotypes under three treatments. G = genotype, T= Treatment, thus the combination G1T1 represent the genotype 1 under the treatement 1. Mena SL=Mean shoot length, Mean RL=Mean root length, R/S=Root Shoot ratio, DWP^{-1}=Dry Weight Per Plant. G1= M-232×M-223, G2= M- 205×M- 232, G3= M-205×M-223, G4= M-223×M-206, G5= M-223×M-205, G6= M-205×M-245, G7= M-206×M-223, G8= M-232×M-245, G9= M-206×M-232, G10= M- 245×M-206, G11= M-205, G12= M-206, G13= M-223, G14= M-232, G15= M-245. T1= control (red), T2= 8 dSm^{-1} salt (green) and T3= 8 dSm^{-1} salt + 0.1mM salicylic acid (blue)

Characters	PG	SL	RL	DWP^{-1}
SL	0.202*			
RL	0.293***	0.37***		
DWP^{-1}	0.122^{NS}	0.222**	0.019^{NS}	
R/S	0.213**	-0.272***	0.718***	-0.077^{NS}

Figure 4. Heatmap showing Pearson correlation coefficients of germination and seedling traits of rapeseed genotypes. *, **, and *** indicate significance at 5%, 1%, and 0.1% probability levels, respectively. NS= non-significant. PG= Percentage of Germination, SL= Shoot Length, RL= Root Length, DWP^{-1}= Dry Weight Per Plant, R/S= Root Shoot Ratio. Red colour represents strong positive correlation, yellow and orange colours represent weak positive correlation, green colour represent significant negative correlation which greenish colours represent non-significant correlation.

4. Conclusion

This study highlights the contrasting responses of *Brassica napus* genotypes to salinity and the combined application of salicylic acid (SA) with salt stress during the germination phase. While salinity alone significantly inhibited root and shoot growth, the addition of 0.1 mM SA further exacerbated stress by reducing germination percentage, root length, dry weight, and root-shoot ratio although the severity of stress was genotype-specific. Additionally, PCA identified the impact of the treatments across various rapeseed genotypes, demonstrating a strong association among the stress responsive traits through grouping (Fig. 5). PCA also showed that the genotypes G4 and G5 exhibited better performance under all treatments probably due to stress tolerance characteristics which needs further investigation.

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Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

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