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OSMOPRIMING WITH POTASSIUM NITRATE MITIGATES SALINITY STRESS DURING GERMINATION AND EARLY SEEDLING GROWTH OF FIVE BRASSICA GENOTYPES

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Abstract

Soil and irrigation-water salinity is one of the principal abiotic constraints on oilseed Brassica production, acting most severely at germination and early seedling establishment. Osmopriming, a controlled pre-sowing hydration treatment, is widely reported to buffer seeds against this early stress window, but its effectiveness varies with genotype and salt intensity. This study evaluated whether priming with a 2% potassium nitrate (KNO3) solution for 24 h could improve germination and seedling growth of five Brassica genotypes (NIFA Gold, UAF-11, Durr-e-Nifa, NIFA-Sarson T-20 and Rachna Canola) across a gradient of simulated soil salinity (0-2% NaCl). A factorial experiment under completely randomized design with three replications was conducted at the University of Agriculture, Faisalabad, recording twelve germination and seedling-growth traits. Analysis of variance showed highly significant ($P \leq 0.01$) main effects of genotype and salinity/priming treatment for all twelve traits, and a significant genotype × treatment interaction for eight of them, indicating genotype-specific priming responses. Relative to non-primed seed, KNO3 priming at zero salinity (T1) raised mean germination from 91.1% to 95.6% and seedling vigor index from 970 to 1330, while germination collapsed to 31.1% under the highest salinity level (2% NaCl). Rachna Canola, UAF-11 and NIFA Gold consistently outperformed NIFA-Sarson T-20 across traits, identifying them as comparatively salt-tolerant genetic material. Principal component analysis of the twelve traits across all genotype × treatment combinations showed that a single stress-severity axis explained 80% of total trait variance, confirming that germination capacity and seedling biomass accumulation decline in a coordinated manner under increasing salinity. These findings support KNO3 osmopriming as a low-cost, field-deployable strategy for improving Brassica stand establishment on moderately saline soils, and identify Rachna Canola and UAF-11 as

promising parental material for salt-tolerance breeding.

1. INTRODUCTION

Seed is the foundational input of crop production, and the speed and uniformity with which it germinates largely determines the productive potential of the resulting stand. Because germination and early seedling establishment are the most environmentally sensitive stages of the plant life cycle, stresses encountered during this narrow window disproportionately affect final yield relative to stresses imposed later in development (TeKrony & Egli, 1991).

Among the abiotic constraints affecting field establishment, salinity is one of the most widespread and is expanding as marginal and irrigated land is brought into cultivation. Elevated soil or irrigation-water sodium chloride lowers the osmotic potential of the soil solution, restricts water uptake by the imbibing seed, and, once ion uptake begins, imposes secondary ionic and oxidative stress that further slows radicle and plumule elongation (Zulfiqar et al., 2022). In oilseed Brassica species, salinity depresses germination percentage, seedling length and dry-matter accumulation, and can reduce final seed yield when the stress persists into vegetative growth (Gyawali et al., 2019; Ghassemi-Golezani et al., 2010).

The genus Brassica, comprising roughly 100 species within the Brassicaceae, includes some of the most economically important oilseed and vegetable crops worldwide, among them Brassica napus (canola/rapeseed) and Brassica rapa. Brassica oilseeds are a major source of edible oil and biodiesel feedstock, and demand for locally produced oilseed continues to outstrip domestic supply in oil-importing countries such as Pakistan (Government of Pakistan, 2023). Improving stand establishment on the saline soils that are increasingly common in irrigated cropping systems is therefore directly relevant to closing this production gap.

Seed priming - a controlled, sub-germinative hydration treatment - is among the most cost-effective pre-sowing interventions for improving stress tolerance at germination. During priming, seeds imbibe water slowly enough to permit the early metabolic and repair processes of germination (activation of hydrolytic enzymes, DNA repair, initiation of respiration) without allowing radicle protrusion; when the seed is subsequently dried and sown, germination resumes rapidly upon rehydration in the field (Chen & Arora, 2013). Osmopriming, in which seeds are soaked in an osmotic solution such as polyethylene glycol or an inorganic salt, is particularly effective because the osmoticum restricts water entry to a controlled rate while simultaneously habituating seed tissue to the ionic and osmotic conditions it will later encounter under stress (Chen & Arora, 2011).

Potassium nitrate (KNO3) is one of the most extensively used osmopriming agents in Brassica and other oilseed and cereal crops because it supplies both an osmotically active solute and a readily available nitrate-nitrogen source. Reported benefits of KNO3 priming include faster and more uniform germination, longer radicles and plumules, elevated antioxidant enzyme activity, and improved sodium/potassium selectivity during subsequent growth under saline conditions (Hassanpouraghdam et al., 2009; Abdollahi & Jafari, 2012; Kubala et al., 2015). At the molecular level, osmopriming of Brassica napus under salt stress has been associated with enhanced proline-synthesis gene expression (Kubala et al., 2015) and altered polyamine and ethylene metabolism that together support membrane stability and osmotic adjustment during early growth (Lechowska et al., 2021). More broadly, chemical seed priming - including with nitrate salts, polyamines and antioxidant compounds - has been shown across many crop species to lower reactive-oxygen-species accumulation and strengthen the antioxidant defense system that protects germinating tissue from salt-induced oxidative damage (Zulfiqar et al., 2022; Stassinos et al., 2021; Ellouzi et al., 2023).

A second, complementary route to improving establishment on saline land is the selection of inherently more salt-tolerant genotypes. Genotypic variation in salt tolerance at germination has been documented repeatedly in Brassica napus and related oilseed types (Gyawali et al., 2019; Bandeh et al., 2018), suggesting that priming benefits and genetic tolerance can, in principle, be combined. However, the two strategies are rarely evaluated together across a graded salinity series, and the extent to which priming benefit itself depends on genotype - a genotype × priming interaction - remains insufficiently characterized for Pakistani Brassica cultivars specifically.

The present study therefore had three objectives: (i) to quantify the effect of a graded NaCl salinity series (0-2%) on germination and seedling growth of five locally grown Brassica genotypes; (ii) to determine whether osmopriming with 2% KNO3 for 24 h improves performance relative to non-primed seed, and whether this benefit is consistent across genotypes and salinity levels; and (iii) to identify genotypes combining the strongest inherent salt tolerance with the greatest responsiveness to priming, as candidate material for salinity-focused Brassica breeding.

2. Materials and Methods

2.1 Experimental site and design

The experiment was conducted during the Rabi season in the controlled-environment facility of the Seed Physiology Laboratory, Department of Seed Science and Technology, University of Agriculture, Faisalabad, Pakistan. Treatments were arranged in a two-factor factorial layout under a completely randomized design (CRD) with three replications: Factor A was genotype (five levels) and Factor B was salinity/priming treatment (six levels), giving 90 experimental units.

2.2 Plant material

Five Brassica cultivars maintained by the national breeding program were used: NIFA Gold, NIFA-Sarson T-20 and Rachna Canola (Brassica napus L.), Durr-e-Nifa (Brassica napus L.), and UAF-11 (Brassica rapa L.), designated G1-G5, respectively, for statistical reporting.

2.3 Priming procedure

A 2% (w/v) KNO3 solution was prepared by dissolving 10 g of potassium nitrate in 500 mL distilled water. Fifty seeds of each genotype were soaked in this solution for 24 h at room temperature, then rinsed in distilled water and surface-dried on tissue paper before sowing. Non-primed seed (Control) was rinsed in distilled water only, without KNO3 exposure.

2.4 Salinity treatments

Six treatment levels were imposed through the irrigation solution: Control (non-primed, 0% NaCl), T1 (primed, 0% NaCl - the primed baseline), and T2-T5 (primed, irrigated with 0.5%, 1.0%, 1.5% and 2.0% NaCl solutions, respectively). This design isolates the priming effect (Control vs. T1) from the dose-response of primed seed to increasing salinity (T1 through T5).

2.5 Growing conditions and data recorded

Three seeds per pot were sown at approximately 1 cm depth in sand-filled pots maintained in a growth room at 20-30°C. Twelve traits were recorded following standard seed-testing procedures: germination percentage, root length, shoot length, total seedling length, seedling fresh and dry weight, root fresh and dry weight, shoot fresh and dry weight, root:shoot ratio, and seedling vigor index (vigor index = germination percentage × mean seedling length).

2.6 Statistical analysis

Data were analyzed by two-way analysis of variance (ANOVA) appropriate to the factorial CRD, testing the main effects of genotype, treatment, and their interaction (Steel et al., 1997). Where the F-test was significant, treatment and genotype means were separated using Fisher's Least Significant Difference (LSD) test at $\alpha = 0.05$. In addition to the classical ANOVA, the genotype × treatment cell means for all twelve traits were analyzed in Python (v3.11; pandas,

NumPy, SciPy, scikit-learn and Matplotlib libraries) to (i) visualize three-dimensional response surfaces of each trait across the full genotype × treatment grid, (ii) compute Pearson correlations among the twelve traits, and (iii) perform a principal component analysis (PCA) on standardized trait values to summarize the overall multivariate stress response and to position the thirty genotype × treatment combinations along the dominant axes of variation.

3. Results

Analysis of variance (Table 1) showed highly significant ($P \leq 0.01$) main effects of genotype and of salinity/priming treatment for all twelve traits measured, confirming both strong genetic variation among the five Brassica genotypes and a strong, graded response to increasing salinity. The genotype × treatment interaction was significant for eight of the twelve traits - root length, shoot length, seedling length, root fresh and dry weight, root:shoot ratio and seedling vigor index - indicating that genotypes did not all respond identically across the salinity gradient for these traits. The interaction was non-significant for germination percentage and for seedling, shoot fresh and shoot dry weight, meaning genotype ranking for these traits was consistent across salinity levels.

Table 1. Combined analysis of variance (F-values) for twelve germination and seedling-growth traits of five Brassica genotypes under six salinity/priming treatments

Trait	Genotype (G) F-value	Treatment (T) F-value	G × T F-value
Germination (%)	6.54**	44.62**	0.46ns
Root length (cm)	21.51**	118.07**	7.77**
Shoot length (cm)	124.67**	594.46**	16.75**
Seedling length (cm)	98.24**	534.82**	13.51**
Seedling fresh weight (mg)	21.79**	70.75**	0.73ns
Seedling dry weight (mg)	21.60**	71.75**	0.76ns
Root fresh weight (mg)	31.05**	478.01**	1.89*
Shoot fresh weight (mg)	21.66**	65.64**	0.77ns
Root dry weight (mg)	31.05**	478.01**	1.89*
Shoot dry weight (mg)	21.60**	65.26**	0.77ns
Root:shoot ratio	3.27*	11.50**	2.58**
Seedling vigor index	13.80*	120.32**	1.83*

Error degrees of freedom = 58 for all traits (Replications = 2, Genotype = 4, Treatment = 5, Genotype×Treatment = 20, Total = 89). ** $P \leq 0.01$, * $P \leq 0.05$, ns = non-significant.

3.1 Effect of salinity and priming (treatment means, averaged across genotypes). Non-primed seed germinated at 91.1% under stress-free conditions; priming with KNO3 (T1) raised this to 95.6%, the highest value recorded in the trial. Germination then declined steadily and steeply as NaCl concentration increased through the primed series, falling to 86.7% at 0.5% NaCl (T2), 77.8% at 1.0% (T3), 53.3% at 1.5% (T4) and only 31.1% at 2.0% NaCl (T5) - a 68-percentage-point collapse relative to the primed, salt-free baseline. The same pattern, priming benefit at low or zero salinity followed by a steep salinity-driven decline, was evident for every

growth trait measured (Table 2), and is visualized as a genotype × treatment response surface in Figure 1.

Table 2. Mean performance of twelve germination and seedling-growth traits across six salinity/priming treatments (averaged over five genotypes)

Trait	Control (0% NaCl)	T1 (0% NaCl)	T2 (0.5%)	T3 (1.0%)	T4 (1.5%)	T5 (2.0%)
Germination (%)	91.1	95.6	86.7	77.8	53.3	31.1
Root length (cm)	2.17	2.65	2.83	1.51	1.39	0.56
Shoot length (cm)	9.21	11.35	8.93	7.43	5.19	2.99
Seedling length (cm)	11.35	14	11.81	8.93	6.58	3.55
Seedling fresh weight (mg)	232.5	236.8	223.7	198.1	165.4	80.8
Seedling dry weight (mg)	69.8	71	67.1	59.4	49.6	24.2
Root fresh weight (mg)	6.99	7.5	6.39	4.73	2.34	0.56
Shoot fresh weight (mg)	225.5	229.3	217.3	193.3	163.1	80.3
Root dry weight (mg)	2.1	2.25	1.92	1.42	0.7	0.17
Shoot dry weight (mg)	67.7	68.8	65.2	58	48.9	24.1
Root:shoot ratio	0.24	0.24	0.33	0.21	0.27	0.16
Seedling vigor index	969.7	1330.2	1019.5	702.9	358.4	126.1

Control = non-primed seed; T1-T5 = KNO3-primed seed irrigated with 0, 0.5, 1.0, 1.5 and 2.0% NaCl, respectively.

3.2 Genotypic performance (genotype means, averaged across treatments). Rachna Canola (G5) recorded the highest mean germination percentage (81.5%) and the highest seedling vigor index (954), and was closely followed by UAF-11 (G2, 79.6% germination) and NIFA Gold (G1, 72.2%). NIFA-Sarson T-20 (G4) was consistently the weakest performer across almost all traits, including the lowest mean germination (59.3%) and lowest vigor index (668) (Table 3). Durr-e-Nifa (G3) combined the longest mean root length (2.37 cm) with intermediate germination, consistent with a root-foraging rather than rapid-germination strategy for coping with osmotic restriction.

Table 3. Mean performance of twelve germination and seedling-growth traits across five Brassica genotypes (averaged over six salinity/priming treatments)

Trait	G1 NIFA Gold	G2 UAF-11	G3 Durr-e-Nifa	G4 NIFA-Sarson T-20	G5 Rachna Canola
Germination (%)	72.2	79.6	70.4	59.3	81.5
Root length (cm)	1.79	1.41	2.37	1.82	1.88
Shoot length (cm)	8.13	5.52	7.92	7.19	8.82
Seedling length (cm)	9.92	6.94	10.29	9.01	10.7

Trait	G1 NIFA Gold	G2 UAF-11	G3 Durr-e-Nifa	G4 NIFA-Sarson T-20	G5 Rachna Canola
Seedling fresh weight (mg)	188.7	219.8	172.3	149.1	217.8
Seedling dry weight (mg)	56.6	65.9	51.7	44.7	65.4
Root fresh weight (mg)	4.74	4.48	5.37	3.82	5.35
Shoot fresh weight (mg)	183.9	215.3	166.9	145.3	212.5
Root dry weight (mg)	1.42	1.34	1.61	1.14	1.61
Shoot dry weight (mg)	55.2	64.6	50.1	43.6	63.8
Root:shoot ratio	0.22	0.26	0.28	0.23	0.22
Seedling vigor index	737.4	587.4	809.5	667.9	953.5

3.3 Genotype × treatment response surfaces. Three-dimensional visualization of the full genotype × treatment data grid (Figures 1-4) makes clear that the decline in performance with increasing salinity was not uniform across genotypes. For germination percentage (Figure 1), Rachna Canola and UAF-11 retained visibly higher germination than NIFA-Sarson T-20 at every salinity level, with the gap widening at 1.5-2.0% NaCl - the signature of a genotype × treatment interaction even where, as for germination, the interaction term itself narrowly missed the 5% significance threshold. For root length and shoot length (Figures 2-3), where the interaction was highly significant ($P \leq 0.01$), Durr-e-Nifa showed a distinctive root-elongation response that was most pronounced at low-to-moderate salinity (Control-T2) before collapsing at the two highest salinity levels, whereas UAF-11 maintained comparatively short but proportionally more stable roots throughout. The seedling vigor index surface (Figure 4) summarizes these patterns: vigor peaked for every genotype at T1 (primed, 0% NaCl) and fell to its lowest values for all genotypes at T5, but Rachna Canola retained the highest absolute vigor across the entire gradient.

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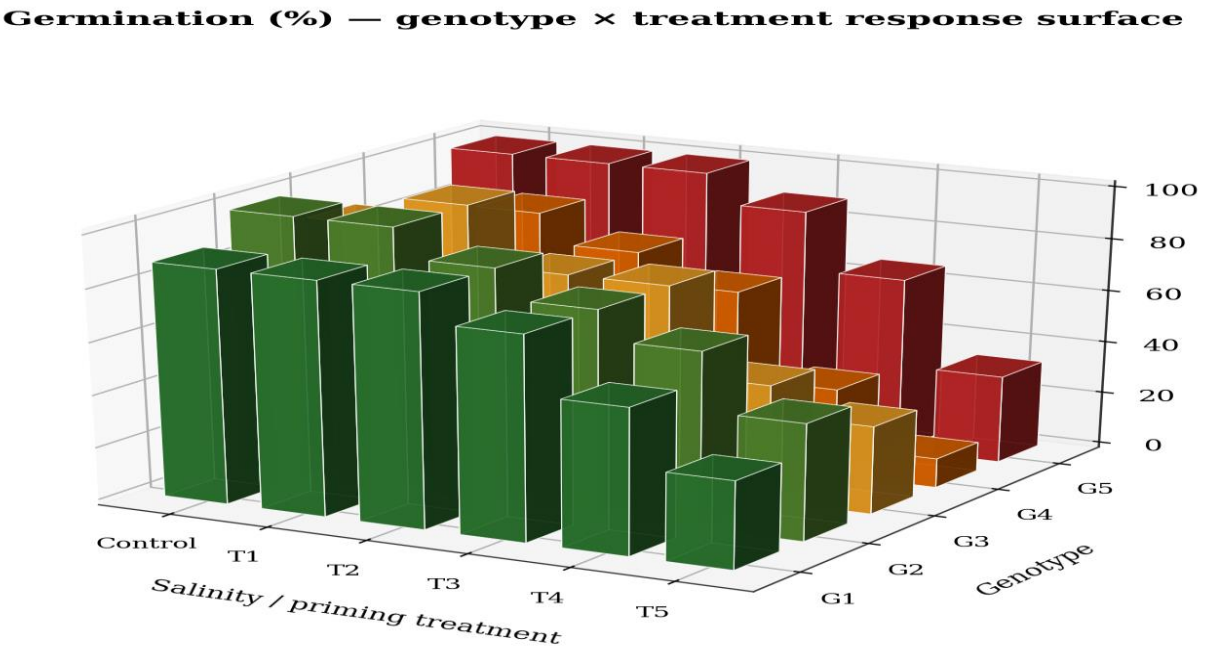


Figure 1. Germination percentage (%) of five Brassica genotypes across six salinity/priming treatments.

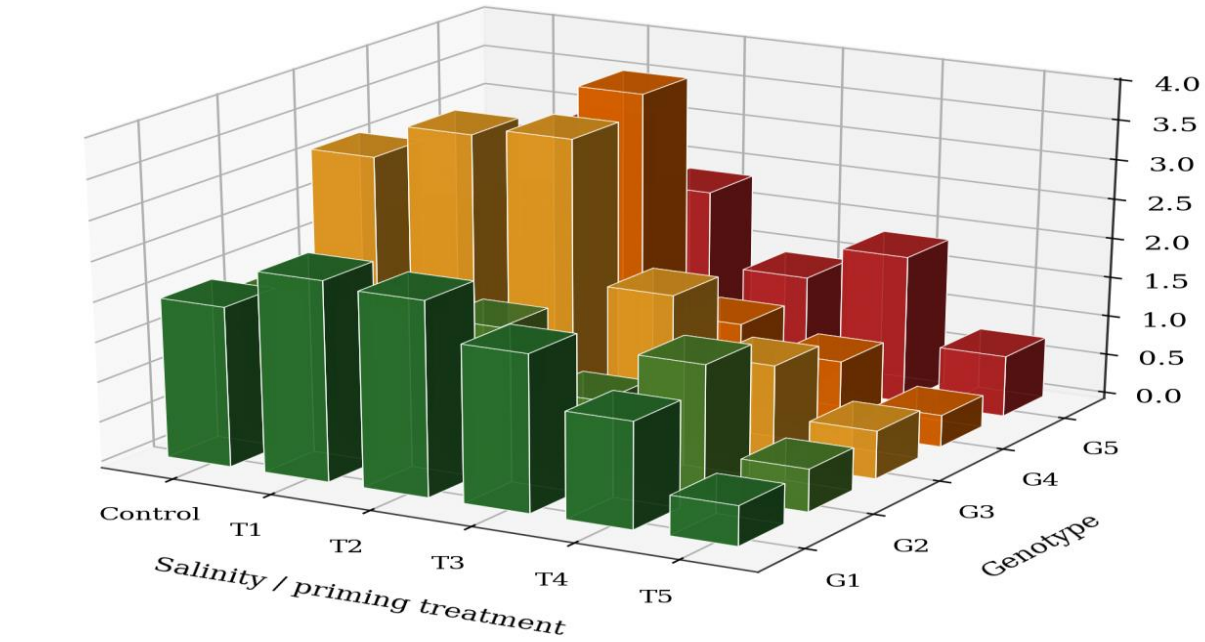


Figure 2. Root length (cm) of five Brassica genotypes across six salinity/priming treatments.

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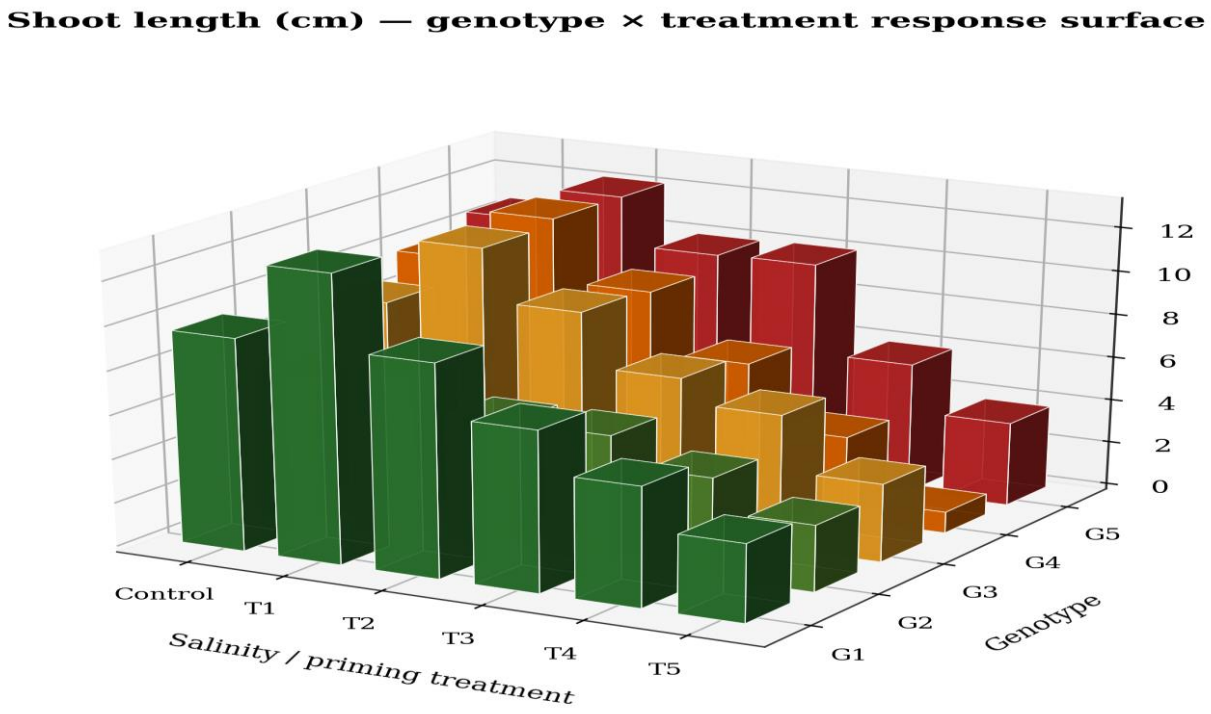


Figure 3. Shoot length (cm) of five Brassica genotypes across six salinity/priming treatments.

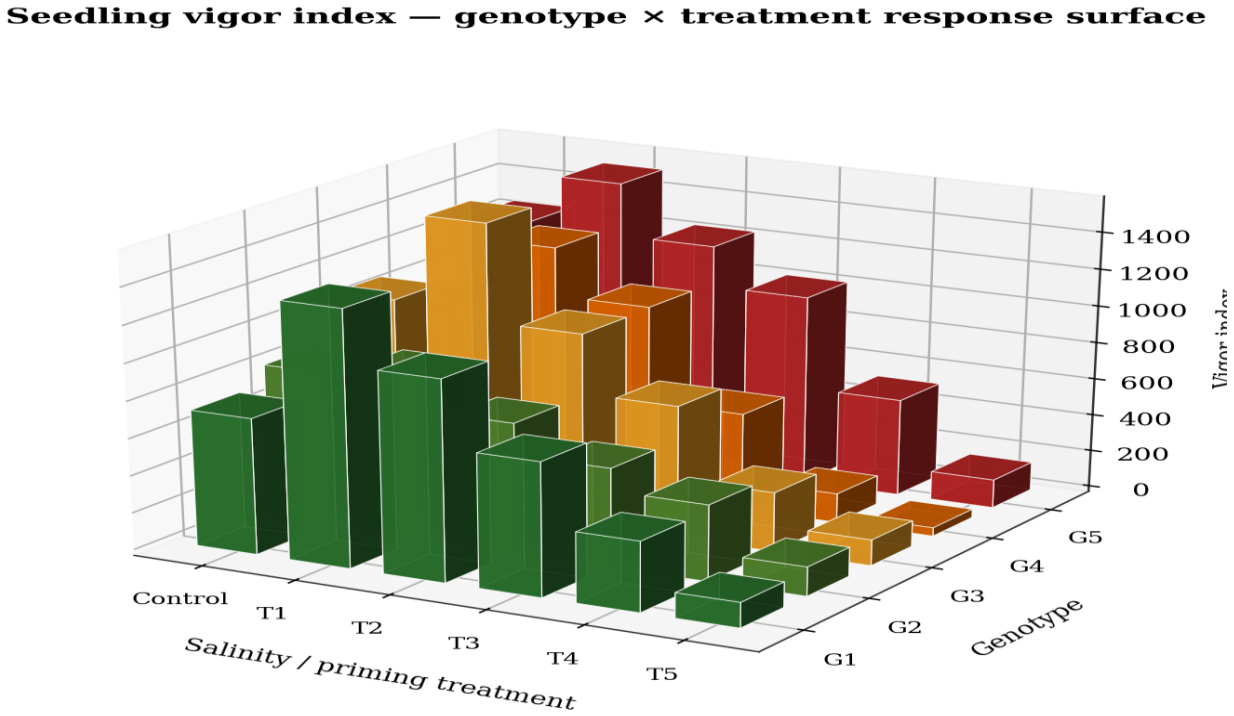


Figure 4. Seedling vigor index of five Brassica genotypes across six salinity/priming treatments.

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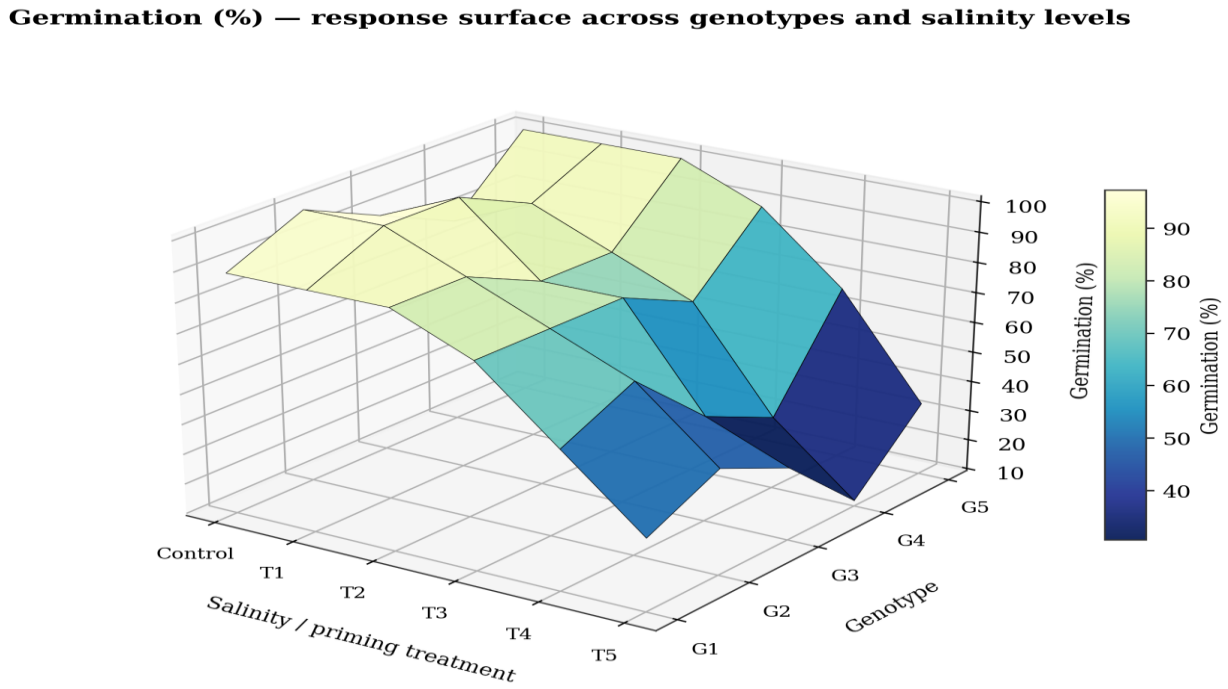


Figure 5. Response surface of germination percentage across the genotype × salinity grid, illustrating the smooth, dose-dependent decline in germination with increasing NaCl concentration and its modulation by genotype.

3.4 Correlation among traits and multivariate stress response. Pearson correlation analysis across the thirty genotype × treatment combinations (Figure 6) showed strong positive correlations among nearly all growth and germination traits (r = 0.70-1.00), confirming that salinity suppressed the entire seedling growth program in a coordinated manner rather than selectively affecting individual traits. The weakest correlations involved root:shoot ratio, which correlated only moderately with the other traits (r = 0.21-0.36), indicating that the root:shoot balance shifted somewhat independently of overall vigor - consistent with the differential root-elongation response noted for Durr-e-Nifa above. Principal component analysis (Figure 7) confirmed this structure: the first principal component (PC1) explained 80.1% of total variance among the twelve traits and loaded positively on almost every trait, representing a single dominant 'stress severity' axis along which genotype × treatment combinations at low salinity (Control, T1, T2) separated clearly from those at high salinity (T4, T5), which clustered toward negative PC1 scores. The second component (PC2, 9.6% of variance) was defined principally by root length and root:shoot ratio and separated Durr-e-Nifa from the other genotypes, reflecting its distinctive root-growth strategy. Together, PC1 and PC2 captured 89.8% of total trait variance, indicating that the twelve measured traits are highly redundant descriptors of a common underlying salt-response syndrome.

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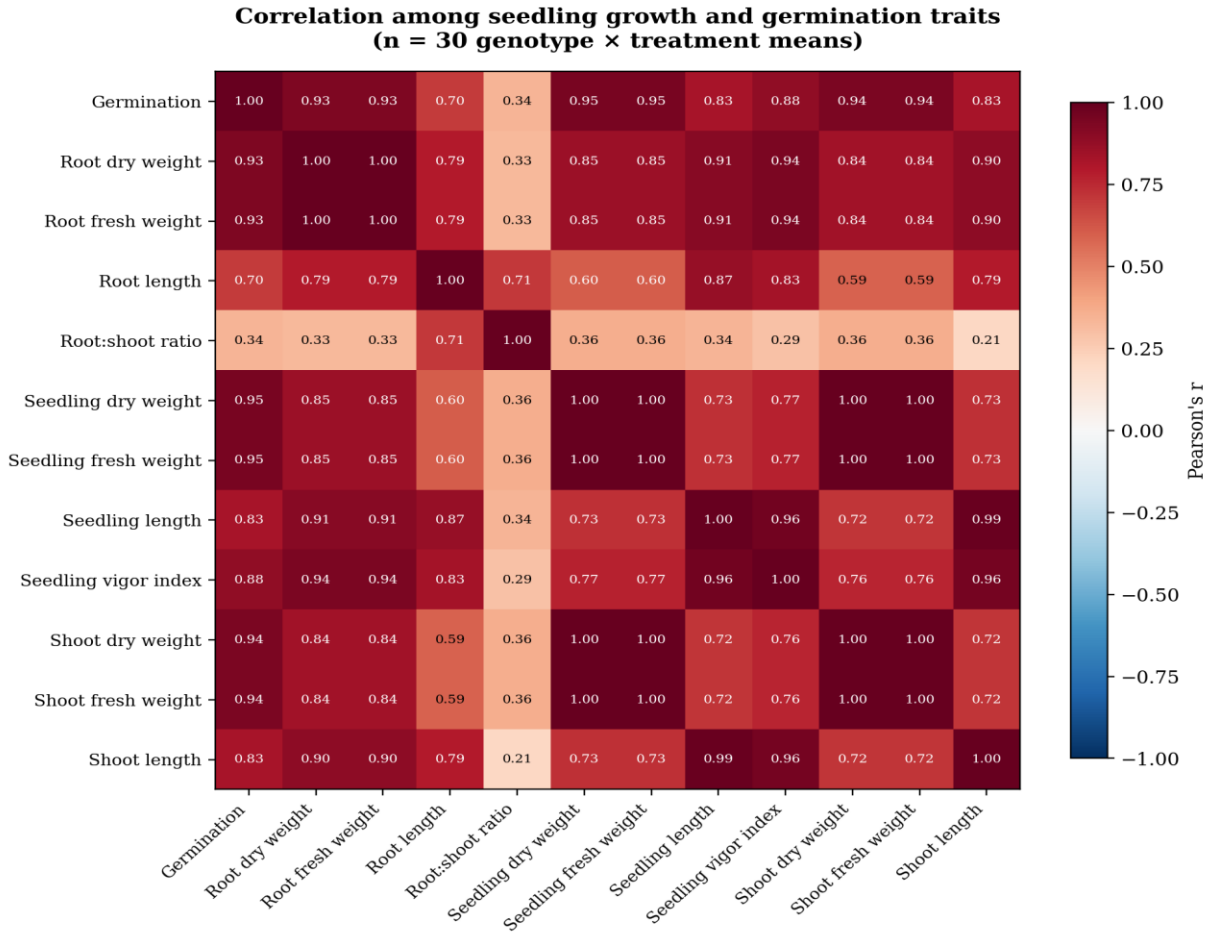


Figure 6. Pearson correlation matrix among twelve germination and seedling-growth traits (n = 30 genotype × treatment means).

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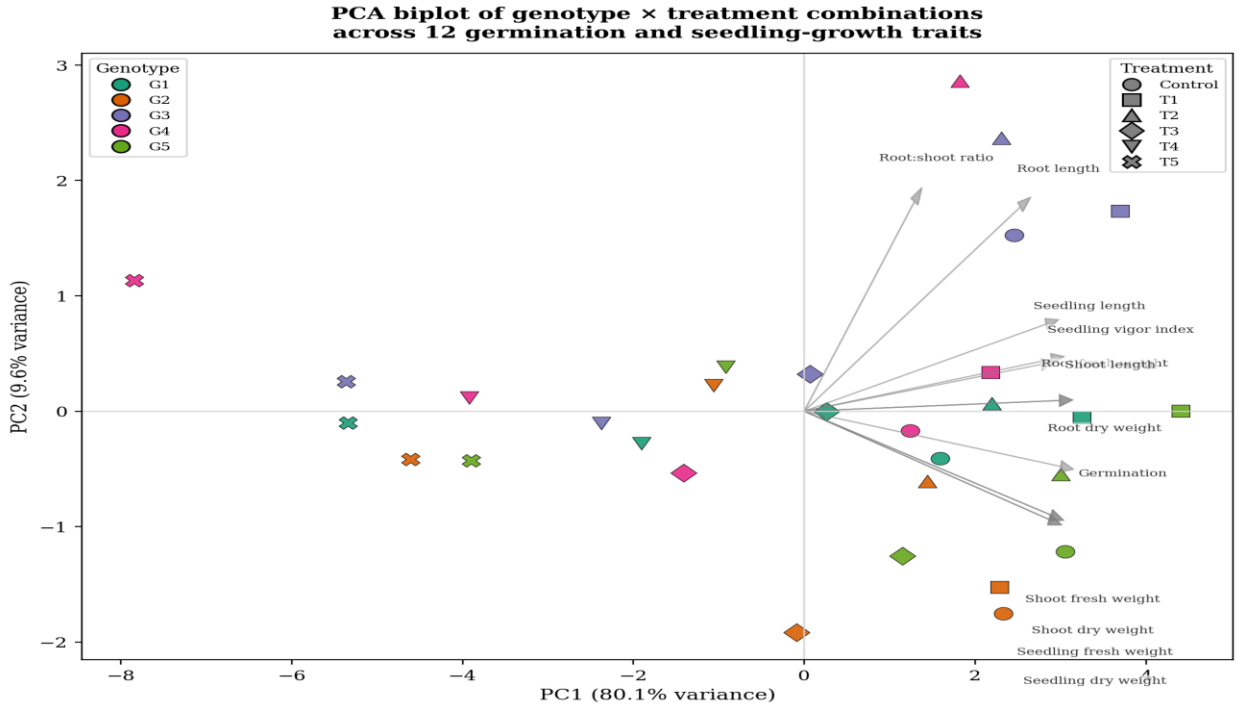


Figure 7. Principal component analysis biplot of the thirty genotype × treatment combinations across twelve traits, showing the dominant stress-severity axis (PC1) and the root-strategy axis (PC2).

4. Discussion

The steep, dose-dependent decline in germination and seedling growth observed as NaCl concentration increased from 0 to 2% is consistent with the well-established mechanism by which salinity restricts germination: falling external osmotic potential slows water uptake by the imbibing seed, delaying the activation of hydrolytic enzymes and mobilization of stored reserves, while progressive ion accumulation imposes additional ionic and oxidative stress once uptake begins (Zulfiqar et al., 2022; Gyawali et al., 2019). The magnitude of decline recorded here - germination falling from 95.6% at 0% NaCl to 31.1% at 2% NaCl in primed seed - falls within the range reported for other Brassica napus trials under comparable salinity gradients (Ghassemi-Golezani et al., 2010; Feghhenabi et al., 2020), reinforcing that salinity above roughly 1% NaCl represents a critical threshold for reliable Brassica stand establishment under the conditions tested.

The clear benefit of KNO3 priming at zero and low salinity (Control vs. T1: germination 91.1% vs. 95.6%; vigor index 970 vs. 1330) supports the broad literature identifying KNO3 as an effective osmopriming agent in Brassica napus. Hassanpouraghdam et al. (2009) and Abdollahi and Jafari (2012) both reported that KNO3-primed canola seed outperformed non-primed seed across germination and seedling-vigor traits, particularly as salinity increased, and attributed this to faster, more synchronized imbibition and to nitrate-driven activation of early metabolic processes. At the molecular level, Kubala et al. (2015) linked KNO3 osmopriming of Brassica napus to up-regulation of the P5CSA proline-biosynthesis gene, a key step in the osmotic-adjustment pathway that allows primed tissue to maintain turgor and continue growth under a lower external water potential. Complementary work by Lechowska et al. (2021) showed that osmopriming of Brassica napus altered endogenous polyamine and ethylene metabolism during germination under salt stress, both of

which contribute to membrane stabilization and stress signaling. The present data are consistent with these mechanisms operating at zero-to-moderate salinity, but also make clear that priming benefit narrows and eventually reverses at the highest salinity level tested (T5), where primed seed still germinated poorly (31.1%) - an outcome in line with reviews concluding that chemical priming shifts the salinity threshold at which growth is compromised rather than conferring unlimited tolerance (Zulfiqar et al., 2022).

The significant genotype × treatment interaction recorded for eight of the twelve traits, and the consistently superior performance of Rachna Canola and UAF-11 across the salinity gradient, indicate that genetic background modulates the practical value of priming under field saline conditions. This mirrors findings in other Brassica napus germplasm panels, where genotype-specific salt tolerance at germination has been documented repeatedly and attributed to differences in seed-coat permeability, early ion-exclusion capacity and antioxidant enzyme activity (Gyawali et al., 2019; Bandeh et al., 2018; Stassinos et al., 2021). The distinctive root-elongation response of Durr-e-Nifa, visible in both the response-surface (Figure 2) and PCA (Figure 7) analyses, is consistent with a root-foraging strategy in which cultivars invest disproportionately in root extension at moderate osmotic restriction, a pattern also reported for salt-responsive rapeseed cultivars by Stassinos et al. (2021), who found that root growth was the most sensitive - and most cultivar-dependent - trait under saline priming trials in Brassica napus.

The strong first principal component identified here (80.1% of variance, loading broadly across nearly all traits) reinforces a recurring conclusion in the priming literature: germination percentage, seedling length and biomass accumulation under salt stress are not independent traits but largely co-vary along a single physiological stress-response axis, most plausibly reflecting the shared dependence of all these processes on early water and ion status (Ellouzi et al., 2023; Zulfiqar et al., 2022). This has a practical implication for Brassica breeding and priming trials: germination percentage alone, which is by far the cheapest and fastest trait to score, can serve as a reasonably efficient proxy for overall salt-stress performance in early-stage cultivar screening, reserving the more labor-intensive biomass and root-architecture measurements for a shortlist of promising genotypes.

Compared with alternative priming agents reported for Brassica and related crops - including polyamines (Stassinos et al., 2021), salicylic acid (Ellouzi et al., 2023), and broader classes of chemical primers such as silicon, chitosan and hydrogen sulfide (Zulfiqar et al., 2022) - KNO3 remains attractive on cost and logistical grounds: it is inexpensive, widely available as agricultural-grade fertilizer, requires no specialized handling, and, as shown here, delivers a clear germination and vigor benefit at zero-to-moderate salinity. Its principal limitation, evident in the present data, is that benefit diminishes sharply above roughly 1.5% NaCl, suggesting that in highly saline fields KNO3 priming should be combined with genotype selection (favoring Rachna Canola- or UAF-11-type material) or with complementary agronomic salinity-mitigation measures rather than deployed as a stand-alone solution.

5. Conclusion

Osmopriming with 2% KNO3 for 24 h significantly improved germination percentage and seedling vigor of five Brassica genotypes relative to non-primed seed under salinity-free and low-salinity conditions, but this benefit narrowed progressively as salinity increased, with germination and seedling growth of even primed seed falling sharply above 1.5% NaCl. Genotype and salinity/priming treatment were both highly significant sources of variation for all twelve traits measured, and their interaction was significant for two-thirds of the traits, confirming that priming response is genotype-dependent. Rachna Canola and UAF-11 combined the strongest inherent salt tolerance with the greatest responsiveness to priming and are recommended as priority parental material for Brassica salt-tolerance breeding programs, while NIFA-Sarson

T-20 requires either targeted improvement or restriction to non-saline production environments. Given the coordinated, single-axis nature of the multivariate stress response identified by principal component analysis, germination percentage can serve as an efficient first-pass screening trait in future cultivar evaluation under salinity.

Conflict of Interest

The authors declare no conflict of interest.

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