

SCREENING OF MUSTARD MUTANTS FOR SALINITY TOLERANCE IN HYDROPONIC CULTURE

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Abstract

Salinity tolerance of three gamma radiation induced mustard mutants (RM-13, RM-14, RM-17) and Binasarisha-11 was evaluated hydroponically at 0, 6, 8, 10 dSm⁻¹. Salinity reduced growth and yield traits but RM-13 and RM-14 showed superior performance, indicating moderate salt tolerance and breeding potential for saline areas of Bangladesh.

Key Words: Salinity stress, hydroponic culture, rapeseed/mustard mutants, screening, salt tolerance

Salinity stress is a significant constraint on agricultural productivity worldwide (Rahman *et al.*, 2022). Salinity is one of the most severe abiotic stresses limiting agricultural productivity, particularly in arid and semi-arid regions. It affects more than 20% of irrigated land globally (Munns & Tester, 2008). In Bangladesh, salinity intrusion from the Bay of Bengal has significantly reduced crop productivity, especially in the coastal zones (Chowhan *et al.*, 2016; Haque, 2006). Induced mutagenesis using gamma rays or chemical mutagens is a powerful approach for creating novel genetic variation and has been widely used in crop improvement programs (Ahloowalia *et al.*, 2004; Oladosu *et al.*, 2016, Hasibuzzaman *et al.*, 2025). To address these challenges, the need for research focuses on breeding high-yielding, stress-tolerant cultivars and improving management practices to enhance productivity and sustainability. Hydroponic culture provides an effective platform for early-stage evaluation of genotypes under uniform salinity stress. Hydroponic systems have emerged as effective tools for screening salinity tolerance in early plant growth stages. Aeroponic systems can provide precise stress application under controlled conditions (Arzani *et al.*, 1997). In this study, we screened gamma-induced mustard mutants for salinity tolerance using hydroponic culture to identify promising genotypes for salt-affected environments and meet the growing demand for edible oil.

Uniform seeds of four M₆ mustard mutants RM-13, RM-14, RM-17 derived from Binasarisha-9 and BARI Sarisha-18 (*Brassica napus*) and modern variety, Binasarisha-11 (*Brassica rapa*) were germinated and grown in hydroponic nutrient solutions. Salinity treatments of 6, 8 and 10 dSm⁻¹ were applied after 14 DAS when seedlings were fully established, with treatments repeated every seven days.

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Protocol of hydroponic system

The experiment was conducted in Glass house, Plant Breeding Division, Bangladesh Institute of Nuclear Agriculture (BINA), Mymensingh. Seeds were surface sterilized by immersion in 70% ethanol for 1 min, followed by treatment with 2% (v/v) NaOCl containing a few drops for 5 min, and rinsed thoroughly (3–5 times) with sterile distilled water. Sterilized seeds were germinated on moist filter paper in petri dishes under controlled conditions until radicle emergence. After 7 days, uniform seedlings were transferred to plastic trays containing modified Hoagland's nutrient solution (Hoagland and Arnon, 1950) and supported upright using sterile foam plugs, allowing roots to remain fully submerged without mechanical stress. The hydroponic system was aerated continuously to ensure adequate oxygenation of the roots, and the nutrient solution was renewed every 3–4 days to maintain optimal nutrient concentration and pH (5.8–6.0). After a 48 h acclimatization period, salinity treatments were imposed by adjusting the electrical conductivity (EC) of the nutrient solution with analytical-grade NaCl to the desired levels, with EC monitored using a calibrated meter (USDA, 2017). Control plants were maintained only in nutrient solution without added NaCl, while treatment groups were exposed to elevated salinity for the experimental period.

At the reproductive stage, data were recorded from five randomly selected plants per treatment tray for plant height, number of leaves plant⁻¹, leaf area plant⁻¹, shoot dry weight, and root dry weight. Visual injury symptoms were also scored at 7, 14, and 21 days after salinity treatment. Data were analyzed using R-studio package program for ANOVA, and treatment means were compared with the Least Significant Difference (LSD) test at the 5% significance level.

Salinity stress significantly affected the growth and biomass accumulation of mustard genotypes, as reflected in plant height, leaf number, leaf area, shoot weight, and root weight across the different salinity levels (0–10 dSm⁻¹) Table 1.

Results showed that plant height decreased progressively with increasing salinity. Under control conditions, RM-13 attained the tallest plants (42.4 cm), whereas at 10 dSm⁻¹, Binasarisha-11 recorded the shortest (26.8 cm) (Table 1). Similar reductions in plant height under salinity stress were reported in mustard and other *Brassica* species (Ashraf & McNeilly, 2004; Parida & Das, 2005). Leaf number was relatively affected by salinity were observed at higher levels. In contrast, leaf area showed a marked decline, dropping from 50–53 cm² in the control to less than 20 cm² at 10 dSm⁻¹ (Table 1). Leaf expansion is highly sensitive to osmotic imbalance and ion accumulation in tissues, which ultimately reduces the photosynthetic surface area. Earlier studies also reported that salinity inhibits leaf growth and accelerates senescence in mustard, thereby lowering biomass production (Munns and Tester, 2008; Flowers *et al.*, 2010). Shoot dry weight exhibited a clear decreasing trend, with values ranging from ~9–10 g under control to ~5 g at 10 dSm⁻¹. Root

biomass was comparatively less affected up to 8 dSm⁻¹, but declined sharply at 10 dSm⁻¹ (Table 1). This indicates that shoot tissues are more sensitive to salinity than roots in case of higher salinity level, which is likely due to ionic toxicity (particularly Na⁺ and Cl⁻ accumulation) and reduced translocation of assimilates. Parida and Das (2005) noted that the ability of plants to maintain growth, including root development, under salinity stress is a key component of salt tolerance. Another observation was reported by Munns and Tester (2008), who emphasized that salinity limits shoot growth mainly due to reduced water uptake and ionic imbalance. Shoot and root dry weights also declined due to osmotic stress and ionic toxicity, though root dry weight remained similar in range, suggesting possible adaptive mechanisms in some genotypes.

Table 1. ANOVA table of mustard genotypes and variety grown at different salinity levels

Treatment	Mutants/Variety	Plant height (cm)	Leaf Number	Leaf area (cm ²)	Shoot wt. (g)	Root wt. (g)
0 dSm ⁻¹	RM-13	42.4	14	53.0	9.3	2.2
	RM-14	41.4	15	52.5	9.9	2.2
	RM-17	40.5	14	51.3	9.6	2.1
6 dSm ⁻¹	Binasarisha-11	39.4	14	50.5	9.4	2.0
	RM-13	37.4	14	43.5	8.0	2.1
	RM-14	37.7	14	44.0	8.1	2.1
8 dSm ⁻¹	RM-17	37.1	14	42.9	7.9	2.0
	Binasarisha-11	36.6	13	41.5	7.6	1.9
	RM-13	32.5	15	29.4	6.7	2.0
10 dSm ⁻¹	RM-14	32.8	15	29.7	6.8	2.0
	RM-17	32.2	15	29.1	6.6	2.0
	Binasarisha-11	31.2	14	27.9	6.3	1.9
Combined over mean	RM-13	27.8	13	20.3	5.3	1.7
	RM-14	28.0	13	20.7	5.4	1.7
	RM-17	27.5	13	19.8	5.2	1.6
	Binasarisha-11	26.8	12	19.0	5.0	1.6
	RM-13	35.0	14.1	36.7	7.6	2.0
	RM-14	35.0	13.9	36.5	7.4	2.0
	RM-17	34.3	13.8	35.8	7.3	1.9
	Binasarisha-11	33.5	13.5	34.7	7.1	1.9
LSD (5%)		1.08	1.0	1.3	1.0	0.2
CV (%)		2.0	2.5	2.2	5.8	4.5

Genotypes exhibited different levels of visual injury under salinity stresses (Table 2). At 6 dSm⁻¹, all genotypes appeared highly tolerant (HT) and high concentration (10 dSm⁻¹), RM-13 and RM-14 maintained moderate tolerance up to the second week but showed signs of death (D) by the third week. RM-17 and Binasarisha-11 exhibited greater susceptibility, with Binasarisha-11 being highly susceptible (HS) at 14 days and completely dead by 21 days.



Fig. 1. Pictorial view of the salinity screening in hydroponic system

Table 2. Visual salt injury at vegetative stage

Mutants/ Variety	7 days after salinity imposed			14 days after salinity imposed			21 days after salinity imposed		
	6 dSm ⁻¹	8 dSm ⁻¹	10 dSm ⁻¹	6 dSm ⁻¹	8 dSm ⁻¹	10 dSm ⁻¹	6 dSm ⁻¹	8 dSm ⁻¹	10 dSm ⁻¹
RM-13	HT	HT	HT	HT	HT	HT	MT	D	D
RM-14	HT	HT	HT	HT	HT	MT	MT	D	D
RM-17	HT	HT	HT	MT	MT	MT	S	D	D
Binasarisha-11	HT	HT	MT	MT	MT	S	HS	D	D

N.B.: HT = Highly tolerant, MT = Moderately tolerant, S = Susceptible, HS = Highly Susceptible and D = Dead

The present study revealed differential responses of mustard genotypes to salinity stress at the reproductive stage, indicating significant genetic variability in tolerance levels (Table 3). It is well established that photosynthesis is markedly reduced under salinity stress; however, the mutant lines RM-13 and RM-14 showed high tolerance at 7 and 14 days after salinity imposed. At a lower salinity level (6 dSm⁻¹), all genotypes performed well, exhibiting a highly tolerant (HT) response throughout the experimental period. This suggests that mild salinity stress has little impact on the growth and survival of mustard, consistent with the findings of Ashraf and McNeilly (2004), who reported that *Brassica* species can withstand low to moderate salinity without showing severe visible injury. As salinity intensity increased, distinct varietal differences became evident. At 10 dSm⁻¹, RM-13 and RM-14 showed moderate tolerance (MT) up to 14 days, but eventually succumbed by 21 days, indicating that their tolerance capacity was only temporary. Similar results were observed by Rana *et al.* (2017), who noted that some mustard genotypes maintained initial tolerance under salinity stress but gradually declined due to cumulative ionic toxicity and osmotic stress. Mutant RM-17 showed a progressive decline in tolerance, shifting from moderate tolerance at 14 days after salinity imposed to susceptibility and death at higher stress levels. In contrast, Binasarisha-11 was found to be the most sensitive, becoming highly susceptible (HS) within 14 days after salinity imposed at 10 dSm⁻¹ and completely dying by 21 days after salinity imposed. This pronounced susceptibility may be attributed to poor ion homeostasis and impaired antioxidant defense under saline conditions, as earlier reported in susceptible *Brassica* cultivars (Kumar *et al.*, 2009; Rahman *et al.*, 2016).

The results highlight that mutants RM-13 and RM-14 as relatively more promising genotypes under salinity stress. The variability in responses among the tested genotypes suggests that physiological and biochemical mechanisms, such as Na⁺ exclusion, osmotic adjustment, and antioxidant activity, may play a role in their differential tolerance levels (Munns and Tester, 2008; Hasanuzzaman *et al.*, 2014). These findings support the potential of salt-tolerant mutants such as RM-13 and RM-14 for cultivation in saline-prone areas, as well as for use in breeding programs aimed at improving mustard tolerance to salinity stress. RM-13 consistently maintained higher plant height, shoot weight, and relatively stable root biomass, suggesting better tolerance. RM-14 and RM-17 performed moderately, while Binasarisha-11 appeared more sensitive, recording the lowest growth parameters across stress levels. These variations indicate genetic diversity in salinity tolerance, which could be exploited in breeding programs aimed at improving mustard adaptation to saline environments.

Salinity severely impairs mustard growth, but mutants RM-13 and RM-14 showed strong salt tolerance through better osmotic adjustment, photosynthetic efficiency and shoot growth under stress while Binasarisha-11 was highly susceptible. Hydroponic screening is effective for early selection and these mutants are promising candidates for saline-area cultivation and breeding.

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