Screening of Indian Mustard (*Brassica juncea* L. Czern and Coss) Genotypes with Respect to Seedling Growth Physiology under Salinity and High-Temperature Stress

Satya Narayan Prasad¹*, Kavita¹, Kiran¹ and Trisha Sinha¹

¹Department of Botany, Plant Physiology and Biochemistry, Dr. Rajendra Prasad Central Agricultural University, Pusa-848125, Bihar, India.

Authors’ contributions

This work was carried out in collaboration among all authors. Authors SNP and Kavita designed the study, wrote the protocol and wrote the first draft of the manuscript. Authors SNP and TS managed the analyses of the study. Authors SNP, Kiran and TS managed the literature searches. All authors read and approved the final manuscript.

ABSTRACT

The experiment was carried out to screen mustard genotypes under individual and combined salinity and high-temperature stress at seedling stage. Seeds after being sown in soil-filled trays were subjected to two levels of salinity stress i.e. 4.0 dSm⁻¹ and 6.0 dSm⁻¹, and high-temperature (40°C), and their performances were also compared with control (1.2 dSm⁻¹). Contrasting sets of genotypes were selected on the basis of seedling growth parameters such as germination percentage, seedling length, dry weight of seedlings, vigour index-I and vigour index-II, recorded in 15-day-old seedlings. With consideration to the genotypic variations observed under all the treatments, genotypes CS2009-347 and CS-52 were identified as tolerant, and genotypes CS2009-256 and CS2009-145 were identified as susceptible under salinity and high-temperature stress conditions. The results also revealed that the impact of salinity and high-temperature in...
combination on mustard at seedling stage was more detrimental than that of their effects under individual conditions. These findings of genotypic variations in terms of tolerance in seedling stage of Indian mustard might be helpful in selection of genotypes with improved tolerance to salinity and high-temperature.

Keywords: Indian mustard crop; combined salinity and high-temperature stress; seedling growth parameters; genotypic variations; tolerant and susceptible genotypes.

1. INTRODUCTION

Rapeseed-mustard is considered to be the second-largest edible oilseed crop in the world after soybean [1]. Indian mustard (Brassica juncea L. Czern and Coss) is grown across the North Indian plains where the majority of ground water resources are having high salinity and sodicity problems. Environmental stresses like drought, salt, cold, and high-temperature affect plants independently, and also in combination [2]. The germination stage or seedling stage of crop is the first stage to be affected severely for more sensitivity of this stage to abiotic stresses than other growth and developmental stages of crop [3]. Salt and osmotic stresses for plants are responsible for inhibition of germination, delayed germination and also seedling establishment [4], reduced root/shoot elongation and lowered dry matter accumulation [5]. Air temperature above 32-35°C acts as high-temperature stress for most of the sub-tropical and tropical crops [6]; however, a daily maximum temperature above 25°C is considered the upper threshold for high-temperature stress for rabi crops [7].

Indian mustard is widely grown as rabi crop, thus, it gets affected greatly due to higher temperature than its optimum range of 6-27°C [8]. High-temperature stress affects mustard plants through developmental, biochemical and physiological changes [9]; and the response depends on several factors such as stress intensity, stress duration and genotype [10]. Salinity stress is also very harmful for this crop; although seedling stage is highly sensitive to salinity stress as it suppresses germination and early crop growth [11]. Salinity-induced negative impacts at initial growth stage of Indian mustard have also been reported earlier by Mitlimbanya et al. [12].

Many studies are now available regarding the effect of individual stress of salinity and high-temperature on seedling growth of plants, but very few findings had revealed their combined effects on plants and responses of plants to the combined stress. So, the selection of tolerant genotypes would be helpful to counteract the adverse effects of these two stresses in combination at the early growth stage. Hence, the present experimental planning was designed to study the performance of Indian mustard genotypes under salinity and high-temperature stress conditions in terms of seedling growth parameters.

2. MATERIALS AND METHODS

Twenty-one Indian mustard genotypes viz., CS-52, CS-56, CS2002-61, CS2002-189, CS2002-195, CS2004-105, CS2004-106, CS2004-114, CS2004-191, CS2005-124, CS2005-125, CS2009-105, CS2009-145, CS2009-256, CS2009-261, CS2009-321, CS2009-347, CS2013-10, CS2013-19, CS2013-27 and CS1013-41 were procured from CSSRI, Karnal. The investigation was carried out in rabi season of 2016-17 at the laboratory, Department of Botany, Plant Physiology and Biochemistry, Dr. Rajendra Prasad Central Agricultural University, Pusa, Bihar. Seeds of Indian mustard genotypes were surface sterilized with 0.1% HgCl₂ solution for two minutes and then thoroughly washed with distilled water before sowing. Two salt solutions of 4.0 and 6.0 dSm⁻¹ were prepared by using NaCl: CaCl₂ in the ratio of 7:2 (w/v) by the method given by Hardie and Doyle [13]. Twenty-five seeds of each genotype were sown with three replications in seedling trays each filled with 5 kg of normal soil with EC 1.2 dSm⁻¹ for control (T₀). For high-temperature stress (HT), seeds were at first sown in trays with 5 kg of normal soil at 25°C. After 4-5 days (when seedlings were 2.5 cm tall), the seedlings were shifted to the germination chamber at 40°C (T₁) for 5-6 days with four long hours daily. Then the rest normal soil was divided in two sets for preparation of two different saline soils which included the though mixing of the normal soil with salt solutions of 4.0 dSm⁻¹ and 6.0 dSm⁻¹ until it (the normal soil) obtained the EC of 4.0 dSm⁻¹ and 6.0 dSm⁻¹ for the first and second set respectively. Equal number of seeds was sown in trays filled with saline soils viz. 4.0 dSm⁻¹ and 6.0 dSm⁻¹, prepared earlier, for exposing the seeds.
to two salinity stress treatments ($T_2$ and $T_3$ respectively). Similarly, salt-treated germinated seeds for 4-5 days at saline soils of 4.0 dSm$^{-1}$ and 6.0 dSm$^{-1}$ were kept in germination chamber for 5-6 days with the same duration for inducing combined salinity and high-temperature stress ($T_4$ and $T_5$ respectively). Contrasting sets of genotypes were selected following observations recorded in 15-day-old seedlings on the basis of germination percentage by the method given by Rau et al. [14], seedling length, seedling dry weight, and vigour index-I and vigour index-II by the method of Abdul-Baki and Anderson [15]. The data collected from the experiments were subjected to analysis of variance following the statistical design of complete randomized design (CRD).

3. RESULTS AND DISCUSSION

3.1 Germination Percentage

Seed germination is a phenomenon comprising of different physiological and biochemical processes involved in it. In this present experiment, germination percentage decreased under several stress treatments in comparison to control for all the genotypes (Table 1). The analysis of variance indicated that mean germination percentage recorded under control condition ($T_0$) for all the Indian mustard genotypes was 88.38, which was the highest mean among other treatments. Mean germination percentage further decreased to 73.91 at individual HT, followed by 67.62 at individual salinity stress treatment of 4.0 dSm$^{-1}$ ($T_2$), 56.64 at individual salinity stress treatment of 6.0 dSm$^{-1}$ ($T_3$), 50.10 at combined stress treatment of HT+4.0 dSm$^{-1}$ ($T_4$) and 48.79 at combined stress treatment of HT+6.0 dSm$^{-1}$ ($T_5$). So, the detrimental effects of both salinity and high-temperature on germination percentage of Indian mustard genotypes were clearly observed. Treatment-wise, the highest mean germination percentage of 76.22 was recorded in the genotype CS2009-347 followed by the genotype CS2009-347 and CS2009-347 were kept in germination chamber for 5-6 days with the same duration for inducing combined salinity and high-temperature stress ($T_4$ and $T_5$ respectively). Contrasting sets of genotypes were selected following observations recorded in 15-day-old seedlings on the basis of 

3.2 Seedling Length

The root and shoot provide an effective channel for the movement of water from soil to the whole plant, and thus offer an important clue to the response of plants to stress [20]. Results from our study revealed a decline in the seedling length of Indian mustard genotypes under stress (Table 2). However, maximum average seedling length was observed in the genotypes CS2009-347 and CS-52 which was 11.66 cm and 11.53 cm respectively; and minimum average seedling length of 9.59 cm and 9.49 cm was recorded by the genotypes CS2009-256 and CS2009-145 respectively over the treatments. The percentage decrease in seedling length in the highest seedling length recording genotype viz. CS2009-347 was 8.11 at $T_1$, 12.20 at $T_3$, 17.94 at $T_3$, 28.42 at $T_5$, and 31.29 at $T_5$ over control ($T_0$); whereas the reduction percentage in seedling length was 8.17, 12.51, 18.08, 28.63 and 32.03 for the genotype CS-52 (second highest recorder in seedling length) at $T_1$, $T_2$, $T_3$, $T_4$, and $T_5$ respectively over control. The maximum percentage reduction in seedling length was recorded in the genotype CS2009-145 under all the treatments which was 10.78, 15.74, 22.94, 35.62 and 42.83 at $T_1$, $T_2$, $T_3$, $T_4$, and $T_5$ respectively over control. Next to that, the genotype CS2009-256 recorded the decrease of 10.68%, 15.62%, 22.74%, 35.34% and 41.18% in seedling length at $T_1$, $T_2$, $T_3$, $T_4$, and $T_5$ respectively over control. In mungbean crop also, it was observed that retardation of seedling growth enhanced drastically with the progressive increase in salt concentrations as reported by Ghosh et al. [21]. Results by the study of Rahman et al. [22] exhibited that high-temperature (30°C) gave positive results on seedling length of Swietenia.
*macrophylla*, whereas combined effect of high-temperature and salinity of various concentrations drastically reduced its seedling length.

3.3 Seedling Dry Weight

Dry matter production is a potent indicator of plant’s performance under stress [5]. Dry weight of seedlings in this present experiment exhibited reducing pattern in all the Indian mustard genotypes as described in Table 3. Seedling dry weight under both salinity and high-temperature stress varied significantly with genotypes, from the highest recorded mean value of 95.22 mg to the lowest recorded mean value of 64.22 mg by the genotype CS2009-347 and CS2009-145 respectively. The genotype CS-52 obtained seedling dry weight of 117.33 mg at control from which it further decreased to 108.00 mg, 98.67 mg, 89.33 mg, 76.00 mg and 72.00 mg at the respective rate of 7.95%, 15.90%, 23.87%, 35.22% and 38.63% under T1, T2, T3, T4 and T5. The minimum percentage decrease in seedling dry weight varied from its lowest of 7.34 and 7.95 at T1 to its highest of 37.29 and 38.63 at T5 for the genotype CS2009-347 and CS-52 respectively. The maximum percentage reduction in seedling dry weight was recorded in the genotypes CS2009-145 and CS2009-256 for all the treatments, which varied from the lowest of 18.33 and 17.22 at T1 to the highest of 64.67 and 62.91 at T5 respectively over control condition (T0).

Severe reduction in dry weight of salinity-sensitive rice cultivars for exposure to salinity stress was reported by Senadheera et al. [23]. Results of declined seedling dry weight were reported by Akasha et al. [24] under high-temperature stress in rice seedlings. High-temperature and salinity stress in French bean caused inhibition of shoot, and root growth and reduction in the biomass of seedlings [25].

3.4 Vigour Index-I and Vigour Index-II

Seed vigour is defined as the sum total of all those properties of a seed that determine the level of activity and performance of the seed during germination and seedling emergence. All the twenty-one genotypes expressed gradual decrease in the value of vigour index-I (Table 4) under stress treatments from control. The genotype CS2009-347 recorded highest vigour index-I of 1127.07 under T1 (HT), 978.73 under T2 (4.0 dSm⁻¹), 777.58 under T3 (6.0 dSm⁻¹), 625.15 under T4 (4.0 dSm⁻¹+HT) and 574.87 under T5 (6.0 dSm⁻¹+HT) with the respective percentage decrease of 17.96, 29.70, 43.36, 54.55 and 58.28 over control (T0) in which the recorded vigour index-I was 1375.87 followed by the genotype CS-52 that recorded vigour index-I of 1346.74 at T0 which progressively decreased to 1101.60, 952.24, 755.70, 592.53 and 552.17 under T1, T2, T3, T4 and T5 respectively, with the respective percentage decrease of 18.21, 29.29, 43.89, 56.00 and 60.00 over control. Minimum recorded values of vigour index-I were 966.27 and 974.27 respectively in genotype CS2009-145 and CS2009-256 at control condition from which these values decreased tremendously to 646.87, 556.32, 409.53, 290.39 and 249.07 in CS2009-145; and 667.32, 589.11, 426.43, 304.67 and 261.98 in CS2009-256 under T1, T2, T3, T4 and T5 respectively.

Similar pattern of results in terms of vigour index-II for the Indian mustard genotypes under various treatments was obtained in this present experiment (Table 5). The genotype CS2009-347 recorded the highest vigour index-II which was 11643.73 at control (T0), 9621.71 at high-temperature (T1), 8053.27 at 4.0 dSm⁻¹ (T2), 6210.11 at 6.0 dSm⁻¹ (T3), 4887.93 at 4.0 dSm⁻¹+HT (T4) and 4439.33 at 6.0 dSm⁻¹+HT (T5). Percentage reduction in vigour index-II for CS-52 was 18.03, 32.03, 47.86, 60.08 and 63.01 respectively at T1, T2, T3, T4 and T5 from T0. The maximum reduction in vigour-index-II was observed under T5 for all the genotypes with the lowest of 61.88% in CS2009-347 to the highest of 84.11% in CS2009-145. The lowest recorded vigour index-II as obtained by the genotype CS2009-145 was 8000.67, 4900.87, 3535.18, 2434.19, 1803.83 and 1271.21 at T0, T1, T2, T3, T4 and T5 respectively. The genotype CS2009-256 recorded nearly similar range of vigour index-II which varied from the maximum of 8054.27 at control to the minimum of 1392.86 at T5 with the percentage reduction of 36.53, 52.54, 67.54, 76.31 and 82.70 at T1, T2, T3, T4 and T5 respectively over control condition (T0). Mean value of vigour index-II for all genotypes over the treatments varied from the lowest of 3657.66 in CS2009-145 to the highest of 7476.01 in CS2009-347.
Table 1. Effect of individual and combined stress of salinity and high-temperature on germination percentage of Indian mustard genotypes

| Genotypes (G) | T0  | T1  | T2  | T3  | T4  | T5  | Mean |
|---------------|-----|-----|-----|-----|-----|-----|------|
| CS-52         | 97.33 | 86.67 (-10.96) | 78.67 (-19.17) | 66.67 (-31.50) | 60.00 (-38.35) | 58.67 (-39.72) | 74.67 |
| CS-56         | 90.67 | 77.33 (-14.71) | 70.67 (-22.06) | 60.00 (-33.82) | 53.33 (-41.18) | 52.00 (-42.65) | 67.33 |
| CS2002-61     | 93.33 | 80.00 (-14.28) | 73.33 (-21.43) | 62.67 (-32.85) | 56.00 (-39.99) | 54.67 (-41.43) | 70.00 |
| CS2002-189    | 94.67 | 81.33 (-14.09) | 76.00 (-19.72) | 64.00 (-32.39) | 57.33 (-39.44) | 56.00 (-40.85) | 71.56 |
| CS2002-195    | 92.00 | 78.67 (-14.49) | 72.00 (-21.74) | 61.33 (-33.33) | 54.67 (-40.58) | 53.33 (-42.03) | 68.67 |
| CS2004-106    | 85.33 | 70.67 (-17.18) | 64.00 (-24.99) | 53.33 (-37.50) | 46.67 (-45.31) | 45.33 (-46.88) | 60.89 |
| CS2004-105    | 86.67 | 72.00 (-16.92) | 65.33 (-24.62) | 54.67 (-36.92) | 48.00 (-44.62) | 46.67 (-46.15) | 62.22 |
| CS2004-114    | 94.67 | 81.33 (-14.09) | 74.67 (-21.13) | 64.00 (-32.39) | 57.33 (-39.44) | 56.00 (-40.85) | 71.33 |
| CS2004-191    | 88.00 | 74.67 (-15.15) | 68.00 (-22.73) | 57.33 (-34.85) | 52.00 (-40.91) | 52.67 (-40.15) | 65.44 |
| CS2005-124    | 82.67 | 65.33 (-20.97) | 60.00 (-27.42) | 49.33 (-40.32) | 42.67 (-48.39) | 41.33 (-50.00) | 56.89 |
| CS2005-125    | 83.33 | 73.33 (-12.00) | 66.67 (-19.99) | 56.00 (-32.80) | 49.33 (-40.80) | 48.00 (-42.40) | 62.78 |
| CS2009-105    | 84.00 | 69.33 (-17.46) | 62.67 (-25.39) | 52.00 (-38.10) | 45.33 (-46.03) | 44.00 (-47.62) | 59.56 |
| CS2009-145    | 80.00 | 60.00 (-25.00) | 54.67 (-31.67) | 44.00 (-45.00) | 37.33 (-53.33) | 36.00 (-55.00) | 52.00 |
| CS2009-256    | 80.00 | 61.33 (-23.33) | 57.33 (-28.33) | 45.33 (-43.33) | 38.67 (-51.67) | 37.33 (-53.33) | 53.33 |
| CS2009-261    | 96.00 | 85.33 (-11.11) | 77.33 (-19.44) | 65.33 (-31.94) | 58.67 (-38.89) | 57.33 (-40.28) | 73.33 |
| CS2009-332    | 81.33 | 62.67 (-22.95) | 57.33 (-29.51) | 46.67 (-42.62) | 40.00 (-50.82) | 38.67 (-52.46) | 54.44 |
| CS2009-347    | 98.67 | 88.00 (-10.81) | 80.00 (-18.92) | 68.00 (-31.08) | 62.67 (-36.49) | 60.00 (-39.19) | 76.22 |
| CS2013-10     | 92.00 | 77.33 (-15.94) | 72.00 (-21.74) | 61.33 (-33.33) | 54.67 (-40.58) | 53.33 (-42.03) | 68.44 |
| CS2013-19     | 82.00 | 64.00 (-21.95) | 58.67 (-28.45) | 48.00 (-41.46) | 41.33 (-49.59) | 40.00 (-51.22) | 55.67 |
| CS2013-27     | 84.00 | 66.67 (-20.63) | 61.33 (-26.99) | 50.67 (-39.68) | 44.00 (-47.62) | 42.67 (-49.20) | 58.22 |
| CS2013-41     | 89.33 | 76.00 (-14.92) | 69.33 (-22.39) | 58.67 (-34.32) | 52.00 (-41.79) | 50.67 (-43.28) | 66.00 |
| Mean          | 88.38 | 73.91 | 67.62 | 56.64 | 50.10 | 48.79 |

Factors: C.D. at 5% = SEM
Genotypes: (G) = T0 = Control, T1 = High-temperature (40°C), T2 = Salinity (4.0 dSm⁻¹), T3 = Salinity (6.0 dSm⁻¹),
T = Salinity (4.0 dSm⁻¹) + high-temperature (40°C)
Treatment (T) = T4 = Salinity (6.0 dSm⁻¹)+ high-
Interaction (G x T) = Non-significant
Table 2. Effect of individual and combined stress of salinity and high-temperature on seedling length (cm) of 15-day-old Indian mustard genotypes

| Genotypes (G) | Treatments (T) | Mean |
|---------------|---------------|------|
|               | T0   | T1   | T2   | T3   | T4   | T5   |
| CS-52         | 13.83| 12.70 (8.17) | 12.10 (12.51) | 11.33 (18.08) | 9.87 (28.63) | 9.40 (32.03) | 11.53 |
| CS-56         | 13.17| 12.00 (8.89) | 11.37 (13.67) | 10.60 (19.51) | 9.20 (30.14) | 8.30 (36.98) | 10.78 |
| CS2002-61     | 13.47| 12.30 (8.69) | 11.67 (13.37) | 10.93 (18.86) | 9.47 (29.70) | 8.80 (34.67) | 11.10 |
| CS2002-189    | 13.63| 12.50 (8.29) | 11.87 (12.91) | 11.13 (18.34) | 9.63 (29.34) | 7.60 (44.24) | 11.06 |
| CS2002-195    | 13.37| 12.20 (8.76) | 11.53 (13.77) | 10.80 (19.22) | 9.37 (29.91) | 9.13 (31.71) | 11.07 |
| CS2004-106    | 12.83| 11.60 (9.59) | 10.97 (14.49) | 10.13 (21.04) | 8.70 (32.19) | 8.63 (32.73) | 10.48 |
| CS2004-105    | 12.73| 11.50 (9.67) | 10.87 (14.61) | 10.03 (21.20) | 8.60 (32.44) | 7.97 (37.39) | 10.29 |
| CS2004-114    | 13.57| 12.40 (8.62) | 11.80 (13.04) | 11.03 (18.71) | 9.57 (29.48) | 7.70 (43.25) | 11.01 |
| CS2004-191    | 13.03| 11.80 (9.43) | 11.17 (14.28) | 10.37 (20.41) | 8.93 (31.47) | 8.97 (31.15) | 10.71 |
| CS2005-124    | 12.47| 11.17 (10.42) | 10.57 (15.23) | 9.70 (22.21) | 8.23 (34.00) | 7.30 (41.46) | 9.91 |
| CS2005-125    | 12.93| 11.70 (9.51) | 11.07 (14.39) | 10.23 (20.89) | 8.80 (31.94) | 7.80 (39.68) | 10.42 |
| CS2009-105    | 12.63| 11.37 (9.98) | 10.77 (14.72) | 9.90 (21.61) | 8.47 (32.93) | 7.50 (40.61) | 10.11 |
| CS2009-145    | 12.07| 10.77 (10.78) | 10.17 (15.74) | 9.30 (22.94) | 7.77 (35.62) | 6.90 (42.83) | 9.49 |
| CS2009-256    | 12.17| 10.87 (10.68) | 10.27 (15.61) | 9.40 (22.77) | 7.87 (35.33) | 7.00 (42.48) | 9.59 |
| CS2009-261    | 13.73| 12.60 (8.23) | 12.00 (16.60) | 11.23 (18.20) | 9.77 (28.84) | 9.23 (32.78) | 11.43 |
| CS2009-332    | 12.27| 10.97 (10.60) | 10.3 (16.06) | 9.50 (22.58) | 8.03 (34.56) | 7.10 (42.13) | 9.69 |
| CS2009-347    | 13.93| 12.80 (8.11) | 12.23 (12.20) | 11.43 (17.94) | 9.97 (28.42) | 9.57 (31.29) | 11.66 |
| CS2013-10     | 13.33| 12.10 (9.22) | 11.50 (13.73) | 10.70 (19.73) | 9.33 (30.00) | 8.47 (36.46) | 10.91 |
| CS2013-19     | 12.37| 11.07 (10.51) | 10.47 (15.36) | 9.60 (22.39) | 8.13 (34.28) | 7.20 (41.79) | 9.81 |
| CS2013-27     | 12.57| 11.27 (10.34) | 10.67 (15.11) | 9.80 (22.03) | 8.33 (33.73) | 7.40 (41.12) | 10.01 |
| CS2013-41     | 13.13| 11.90 (9.37) | 11.27 (14.17) | 10.47 (20.26) | 9.10 (30.70) | 8.13 (38.09) | 10.67 |

Mean 13.01 11.79 11.18 10.37 8.91 8.10

Factors C.D. at 5% SEM T0 = Control, T1 = High-temperature (40°C), T2 = Salinity (4.0 dSm⁻¹), T3 = Salinity (6.0 dSm⁻¹), T4 = Salinity (4.0 dSm⁻¹) + high-temperature (40°C), T5 = Salinity (6.0 dSm⁻¹) + high-temperature (40°C)

Genotype (G) 0.54 0.20 dSm⁻¹, T4 = Salinity (4.0 dSm⁻¹) + high-temperature (40°C), T5 = Salinity (6.0 dSm⁻¹) + high-temperature (40°C)

Treatment (T) 0.29 0.10

Interaction (G x T) N/S 0.48 Figures in parentheses indicate percent decrease over control

N/S = Non-significant
Table 3. Effect of individual and combined stress of salinity and high-temperature on seedling dry weight (mg) of 15-day-old Indian mustard genotypes

| Genotypes (G) | T0 | T1 (-7.95) | T2 (-15.90) | T3 (-35.22) | T4 (-45.17) | T5 (-56.63) | Mean |
|---------------|----|------------|-------------|-------------|-------------|-------------|------|
| CS-52         | 117.33 | 108.00     | 98.67       | 89.33       | 76.00       | 72.00       | 93.56|
| CS-56         | 110.67 | 99.00      | 88.67       | 79.67       | 64.67       | 58.67       | 83.56|
| CS2002-61     | 113.00 | 102.33     | 92.67       | 83.00       | 68.67       | 64.00       | 87.28|
| CS2002-189    | 114.33 | 105.33     | 94.67       | 85.67       | 72.00       | 68.00       | 90.00|
| CS2002-195    | 112.33 | 101.67     | 90.67       | 81.67       | 67.33       | 62.00       | 85.94|
| CS2004-106    | 106.33 | 94.00      | 80.67       | 71.67       | 59.33       | 50.67       | 77.11|
| CS2004-105    | 105.67 | 93.33      | 78.67       | 69.67       | 57.33       | 48.67       | 75.56|
| CS2004-114    | 114.33 | 104.00     | 94.67       | 85.00       | 70.67       | 66.00       | 89.11|
| CS2004-191    | 108.67 | 96.33      | 84.67       | 75.67       | 62.33       | 54.67       | 80.39|
| CS2005-124    | 102.67 | 87.00      | 72.67       | 63.67       | 52.00       | 42.67       | 70.11|
| CS2005-125    | 107.00 | 95.33      | 82.67       | 73.67       | 60.67       | 52.67       | 78.67|
| CS2009-105    | 104.00 | 91.33      | 76.67       | 67.67       | 55.33       | 46.67       | 73.61|
| CS2009-145    | 100.00 | 81.67      | 64.67       | 55.33       | 48.33       | 35.33       | 64.22|
| CS2009-256    | 100.67 | 83.33      | 66.67       | 57.67       | 49.33       | 37.33       | 65.83|
| CS2009-261    | 116.33 | 106.67     | 96.67       | 87.33       | 74.00       | 70.00       | 91.83|
| CS2009-332    | 101.33 | 85.00      | 68.67       | 59.67       | 50.33       | 38.67       | 67.28|
| CS2009-347    | 118.00 | 109.33     | 100.67      | 91.33       | 78.00       | 74.00       | 95.22|
| CS2013-10     | 112.00 | 100.33     | 89.67       | 81.33       | 66.67       | 60.33       | 85.06|
| CS2013-19     | 102.00 | 86.33      | 70.67       | 61.00       | 51.00       | 40.67       | 68.61|
| CS2013-27     | 103.00 | 89.33      | 74.67       | 66.33       | 53.33       | 44.67       | 71.89|
| CS2013-41     | 112.33 | 103.33     | 94.00       | 81.33       | 67.93       | 56.67       | 85.93|
| Mean          | 108.67 | 96.33      | 83.92       | 74.66       | 62.15       | 54.49       |      |

T0 = Control, T1 = High-temperature (40°C), T2 = Salinity (4.0 dSm⁻¹), T3 = Salinity (6.0 dSm⁻¹), T4 = Salinity (4.0 dSm⁻¹) + high-temperature (40°C), T5 = Salinity (6.0 dSm⁻¹) + high-temperature (40°C)

Figures in parentheses indicate percent decrease over control

NS= Non-significant

Factors C.D. at 5% SEM Genotype (G) 3.24 1.16 Treatment (T) 1.73 0.62 Interaction (G × T) N/S 2.85
Table 4. Effect of individual and combined stress of salinity and high-temperature on vigour index-I of 15-day-old Indian mustard genotypes

| Genotypes (G) | T0  | T1  | T2  | T3  | T4  | T5  | Mean |
|---------------|-----|-----|-----|-----|-----|-----|------|
| CS-52         | 1346.74 | 1101.38 | 952.24 | 755.70 | 592.53 | 552.17 | 883.46 |
| CS-56         | 1194.79 | 928.62 | 803.85 | 636.33 | 490.97 | 432.27 | 747.80 |
| CS2002-61     | 1257.82 | 984.67 | 856.10 | 685.31 | 530.66 | 481.76 | 799.39 |
| CS2002-189    | 1291.02 | 1017.29 | 902.46 | 712.65 | 552.42 | 426.27 | 817.01 |
| CS2002-195    | 1230.71 | 960.44 | 830.50 | 662.70 | 512.60 | 406.37 | 780.75 |
| CS2004-106    | 1095.45 | 820.43 | 702.41 | 540.57 | 406.37 | 391.87 | 659.51 |
| CS2004-105    | 1103.98 | 828.67 | 710.48 | 548.68 | 413.13 | 372.62 | 662.92 |
| CS2004-114    | 1285.34 | 1009.16 | 881.43 | 706.25 | 548.99 | 431.87 | 810.50 |
| CS2004-191    | 1147.31 | 881.78 | 759.89 | 594.84 | 464.69 | 473.11 | 720.28 |
| CS2005-124    | 1031.56 | 730.40 | 634.53 | 478.83 | 351.50 | 302.38 | 586.20 |
| CS2005-125    | 1078.12 | 858.62 | 738.37 | 573.21 | 434.43 | 375.07 | 676.30 |
| CS2009-105    | 1061.59 | 788.49 | 675.29 | 515.13 | 384.28 | 330.67 | 625.99 |
| CS2009-145    | 966.27 | 646.87 | 556.32 | 409.53 | 290.39 | 249.07 | 519.74 |
| CS2009-256    | 974.27 | 667.32 | 589.11 | 426.43 | 304.67 | 261.98 | 537.30 |
| CS2009-261    | 1318.75 | 1075.83 | 928.29 | 733.99 | 573.53 | 529.82 | 860.03 |
| CS2009-332    | 998.59 | 688.15 | 590.83 | 443.70 | 321.53 | 275.22 | 553.00 |
| CS2009-347    | 1375.14 | 1127.07 | 978.73 | 777.58 | 574.87 | 507.73 | 909.76 |
| CS2013-10     | 1227.03 | 936.36 | 828.33 | 656.57 | 510.40 | 452.37 | 768.51 |
| CS2013-19     | 1015.01 | 709.14 | 614.60 | 461.13 | 336.34 | 288.67 | 570.81 |
| CS2013-27     | 1056.55 | 752.03 | 654.72 | 496.90 | 366.86 | 316.42 | 607.24 |
| CS2013-41     | 1173.57 | 905.07 | 781.68 | 614.60 | 473.53 | 412.61 | 726.84 |
| Mean          | 1153.79 | 877.06 | 760.49 | 591.93 | 451.67 | 400.89 | |

Factors: C.D. at 5% SEm

Genotype (G): 49.13 17.63

Treatment (T): 26.27 9.42

Interaction (G × T): N/S 43.19

Notes: Figures in parentheses indicate percent decrease over control
N/S = Non-significant

1) T2 = Salinity 6.0 dS m⁻¹, T3 = Salinity 4.0 dS m⁻¹ + high-temperature (40°C), T4 = Salinity 6.0 dS m⁻¹ + high-temperature (40°C), T5 = Salinity 4.0 dS m⁻¹ + high-temperature (40°C).
Table 5. Effect of individual and combined stress of salinity and high-temperature on vigour index-II of 15-day-old Indian mustard genotypes

| Genotypes (G) | \( T_0 \) | \( T_1 \) | \( T_2 \) | \( T_3 \) | \( T_4 \) | \( T_5 \) | Mean |
|---------------|---------|---------|---------|---------|---------|---------|-------|
| CS-52         | 11,420.40 | 9,361.03 (-18.03) | 7,762.04 (-32.03) | 5,955.30 (-47.86) | 4,559.67 (-60.08) | 4,223.57 (-63.01) | 7,213.67 |
| CS-56         | 10,035.12 | 7,656.34 (-23.70) | 6,265.98 (37.56) | 4,779.87 (52.37) | 3,448.52 (-65.63) | 3,050.17 (-69.60) | 5,872.66 |
| CS2002-61     | 10,546.96 | 8,187.07 (-22.38) | 6,795.16 (-35.58) | 5,201.28 (-50.69) | 3,845.19 (-63.54) | 3,498.21 (-66.83) | 6,345.64 |
| CS2002-189    | 10,824.29 | 8,567.16 (-20.86) | 7,194.59 (-33.53) | 5,482.55 (-49.34) | 4,127.43 (-61.87) | 3,807.33 (-64.82) | 6,667.22 |
| CS2002-195    | 10,335.03 | 7,999.05 (-22.60) | 6,527.91 (-36.83) | 5,008.49 (-51.53) | 3,680.60 (-64.39) | 3,305.79 (-68.01) | 6,142.81 |
| CS2004-106    | 9,073.81  | 6,643.65 (-26.79) | 5,162.55 (-43.10) | 3,821.83 (-57.89) | 2,768.60 (-69.49) | 2,296.20 (-74.70) | 4,961.10 |
| CS2004-105    | 9,159.09  | 6,720.43 (-26.62) | 5,139.18 (-43.89) | 3,808.53 (-58.41) | 2,751.51 (-70.00) | 2,270.76 (-75.20) | 4,974.92 |
| CS2004-114    | 10,824.29 | 8,458.99 (-21.86) | 7,068.68 (-34.70) | 5,439.67 (-49.74) | 4,051.18 (-62.58) | 3,695.33 (-65.87) | 6,589.69 |
| CS2004-191    | 9,563.63  | 7,193.63 (-24.79) | 5,757.23 (-39.80) | 4,337.83 (-54.64) | 3,240.83 (-66.11) | 2,878.80 (-69.90) | 5,495.32 |
| CS2005-124    | 8,488.40  | 5,684.38 (-33.03) | 4,359.87 (-48.63) | 3,140.51 (-63.00) | 2,185.51 (-73.87) | 1,762.88 (-79.23) | 4,275.76 |
| CS2005-125    | 8,916.98  | 6,991.22 (-21.60) | 5,511.28 (-38.19) | 4,125.19 (53.73)  | 2,992.52 (-66.44) | 2,527.49 (-71.66) | 5,177.44 |
| CS2009-105    | 8,736.67  | 6,332.58 (-27.51) | 4,804.58 (-45.00) | 3,518.51 (-59.72) | 2,507.78 (-71.29) | 2,052.81 (-76.50) | 4,658.82 |
| CS2009-145    | 8,000.67  | 4,902.87 (-38.74) | 3,535.18 (-55.81) | 2,434.19 (-69.58) | 1,803.83 (-77.46) | 1,271.21 (-84.11) | 3,657.66 |
| CS2009-256    | 8,054.27  | 5,111.30 (-36.53) | 3,821.86 (-52.54) | 2,613.85 (-67.54) | 1,907.26 (-76.31) | 1,392.86 (-82.70) | 3,816.90 |
| CS2009-261    | 11,168.35 | 9,102.82 (-18.50) | 7,475.16 (-33.07) | 5,704.94 (-48.91) | 4,341.25 (-61.12) | 4,012.43 (-64.08) | 6,967.49 |
| CS2009-332    | 8,241.84  | 5,327.62 (-35.36) | 3,936.52 (-52.23) | 2,784.47 (-66.21) | 2,012.87 (-75.58) | 1,494.70 (-81.87) | 3,966.34 |
| CS2009-347    | 11,643.73 | 9,621.71 (-17.37) | 8,053.27 (-30.83) | 6,210.11 (-46.67) | 4,887.93 (-58.02) | 4,439.33 (-61.88) | 7,476.01 |
| CS2013-10     | 10,304.67 | 7,759.19 (-24.70) | 6,455.91 (-37.34) | 4,987.64 (-51.60) | 3,644.52 (-64.63) | 3,216.73 (-68.79) | 6,061.44 |
| CS2013-19     | 8,364.67  | 5,525.79 (-33.93) | 4,145.88 (-50.43) | 2,927.67 (-65.00) | 2,107.50 (-74.80) | 1,626.13 (-80.56) | 4,116.27 |
| CS2013-27     | 8,652.67  | 5,956.30 (-31.16) | 4,579.18 (-47.08) | 3,360.61 (-61.16) | 2,346.19 (-72.89) | 1,905.40 (-77.98) | 4,466.72 |
| CS2013-41     | 10,035.11 | 7,853.75 (-21.73) | 6,516.69 (-35.07) | 4,771.30 (-52.46) | 3,532.03 (-64.80) | 2,870.80 (-71.39) | 5,929.94 |
| Mean          | 9,637.65  | 7,188.32 | 5,755.65 | 4,305.44 | 3,179.79 | 2,742.81 |

Factors: C.D. at 5% SEM
Genotype (G) 363.88 130.57
Treatment (T) 194.50 69.79
Interaction (G × T) N/S 319.82
T0 = Control, T1 = High-temperature (40°C), T2 = Salinity (4.0 dSm⁻¹), T3 = Salinity (6.0 dSm⁻¹), T4 = Salinity (4.0 dSm⁻¹) + high-temperature (40°C), T5 = Salinity (6.0 dSm⁻¹) + high-temperature (40°C)
Figures in parentheses indicate percent decrease over control
N/S = Non-significant
Decrease in seedling vigour under salinity stress is due to the reduced ability of imbibitions resulting in limited hydrolysis of food reserves from storage tissues. Such depression in seedling vigour under saline stress is attributed to the reduced ability of water uptake or imbibitions, as well as due to impaired translocation of food reserves from storage tissues to the developing embryo [26]. Similar results were obtained by Bina and Bostani [27] in three medicinal plant species viz. Plantago ovata, Cucurbita pepo and Caryophyllus aromaticus L. under different salt concentrations. Ghosh et al. [20] also reported reduced root and shoot length in 10-day old mungbean seedling when treated with salt. Reduced germination percentage, seedling emergence, poor vigour, reduced radicle and plumule growth were major impacts of high-temperature stress as reported in black gram by Piramila et al. [28]. Seedling traits such as germination percentage; root and shoot length; root and shoot dry weight; and vigour index were observed to be decreasing with the increasing salt concentration as reported by Mtilimbanya et al. [12].

4. CONCLUSION

This study showed that increasing salt concentration, as well as elevated temperature significantly affected the seedling growth of Indian mustard genotypes. The severity of effects depended on the type of stress; from the individual high-temperature stress being the least affecting factor followed by individual salinity of 4.0 dSm⁻¹ to the combined salinity and high-temperature stress of 6.0 dSm⁻¹+40°C being the most detrimental one. Prominent genotypic variations in performances were observed. In consideration to the parameters studied in this present experiment, CS2009-347 and CS-52 were identified as the most tolerant genotypes; while CS2009-145 and CS2009-256 as the most susceptible genotypes as a response to both individual and combined salinity and high-temperature stress conditions. Therefore from these results, it can be concluded that the genotypic variations among Indian mustard genotypes for these parameters at the germination stage or seedling growth stage might be good criteria for the selection of tolerant genotypes under salinity and high-temperature stress, individually and also when combined.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Kumrawat M, Yadav M. Trends in area, production, and yield of mustard crop in Bharatpur region of Rajasthan. International Journal of Engineering Development and Research. 2018;6(1): 315-321.
2. Serrano R, Mulet JM, Rios G, Marquez JAFI, Leube MP. et al. A glimpse of the mechanisms of ion homeostasis during salt stress. Journal of Experimental Botany. 1999;50:1023-1036.
3. Luan Z, Xiao M, Zhou D, Zhang H, Tian Y. et al. Effects of salinity, temperature, and polyethylene glycol on the seed germination of sunflower (Helianthus annus L.). The Scientific World Journal; 2014. Available: https://doi.org/10.1155/2014/170418
4. Almansouri M, Kiner JM, Lutta S. Effect of salt and osmotic stresses on germination in durum wheat (Triticum durum). Plant and Soil. 2001;231(2):243-254.
5. Singh D, Singh CK, Kumari S, Singh Tomar RS, Karwa S. et al. Discerning morpho-anatomical, physiological and molecular multiformity in cultivated and wild genotypes of lentil with reconciliation to salinity stress. PLoS One; 2017. Available: https://doi.org/10.1371/journal.pone.0177465.
6. Bitz CE, Gerats T. Plant tolerance to high temperature in a changing environment: Scientific fundamentals and production of heat tolerance crops. Frontiers in Plant Science. 2013;4:273.
7. Wahid A, Gelani S, Ashraf M, Foolad M. Heat tolerance in plants: An overview. Environmental and Experimental Botany. 2007;61:199-223.
8. Shekawat K, Rathore SS, Premi OP, Kandpal BK, Chauhan JS. Advances in agronomic management of Indian mustard [Brassica juncea (L.) Czernj,Cosson]: an overview. International Journal of Agronomy; 2012. Available: https://doi.org/10.1155/2012/408284
9. Sanchez B, Rasmussen A, Porter JR. Temperatures and the growth and development of maize and rice: A review.
Global Change Biology. 2014;20:408-417.
10. Moradshahi A, Eskandari BS, Kholdebarin B. Some physiological responses of canola (Brassica napus L.). Iranian Journal of Science and Technology, Trans, A. 2004; 28:43-50.
11. Singh J, Sharma PC, Sharma SK, Rai M. Assessing the effect of salinity on the oil quality parameters of Indian mustard using Fourier transform near-infrared reflectance (FT-NIR) spectroscopy. Grasas Aceites. 2014;65:e009.
12. Milimibanya KY, Jakhar ML, Ram M, Rankunwar, Ahmad S. et al. Assessment of variability parameters for germination and seedling traits in mustard (Brassica juncea L.) under salinity stress condition. Journal of Pharmacognosy and Phytochemistry. 2020;9(3):2151-2154.
13. Hardie M, Doyle R. Measuring soil salinity. Methods in Molecular Biology. 2012;913: 415-425.
14. Ruan S, Xue X and Tylkowska K. The influence of priming on germination of rice (Oryza sativa L.) seeds and seedling emergence and performance in flooded soil. Seed Science and Technology. 2002; 30:61-67.
15. Abdul-Baki AA, Anderson JD. Vigor determination in soybean seed by multiple criteria. Crop Science. 1973;13: 630-633.
16. Dubey SR. Effect of sodium chloride salinity on enzyme activity and biochemical constituents in germinating salt tolerant rice seeds. Oryza. 1984;21:213-217.
17. Hanumantha Rao B, Nair RM, Nayyar H. Salinity and high temperature tolerance in mungbean [Vigna radiata (L.) Wilczek] from a physiological perspective. Frontiers in Plant Science. 2016;7. Available:https://doi.org/10.3389/fpls.2016.00957
18. Rai AN, Saini N, Yadav R, Suprasanna P. A potential seedling-stage evaluation method for heat tolerance in Indian mustard (Brassica juncea L. Czern and Coss). Biotech-3. 2020;10:114. Available:https://doi.org/10.1007/s13205-020-2106-9
19. Khan MA, Gulzar S. Light, salinity and temperature effects on the seed germination of perennial grasses. American Journal of Botany. 2003;90(1):131-134.
20. Pandey M, Penna S. Time course of physiological, biochemical and gene expression changes under short-term salt stress in Brassica juncea L. The Crop Journal. 2017;5:219-230.
21. Ghosh S, Mitra S, Paul A. Physiochemical studies of sodium chloride on mungbean (Vigna radiata L. Wilczek) and its possible recovery with spermine and gibberellic acid. Scientific World Journal. 2015;Article ID 8580168.
22. Rahman MS, Akter S, Al-Amin M. Temperature and saline stress on seedlings of Swietenia macrophylla: A comparative study. Pakistan Journal of Biological Science. 2013;16(23):1765-1770.
23. Senadheera P, Tirimanne S and Maathuis FJM. Long term salinity stress reveals variety specific differences in root oxidative stress response. Rice Science. 2012;19(1): 36-43.
24. Akasha A, Ashraf M, Shereen A, Mahboob W, Faisal S. Heat tolerance screening studies and evaluating salicylic acid efficacy against high temperature in rice (Oryza sativa L.) genotypes. Journal of Plant Biochemistry and Physiology. 2019; 7:235. Available:https://doi.org/10.35248/2329-9029.19.7.235
25. Nagesh Babu R, Devaraj VR. High temperature and salt stress response in French bean (Phaseolus vulgaris). Australian Journal of Crop Science. 2008; 2(2):40-48.
26. Roychowdhury A, Ghosh A. Physiological and biochemical responses of mungbean to varying concentrations of cadmium chloride or sodium chloride. Unique Journal of Pharmaceutical and Biological Science. 2013;1:11-21.
27. Bina F, Bostani A. Effect of Salinity (NaCl) stress on germination and early seedling growth of three medicinal plant species. Advancements in Life Sciences. 2017;4(3): 77-83.
28. Piramila HMB, Prabha LA, Nandagopalan V, Leo AS. Effect of heat treatment on germination, seedling growth and some biochemical parameters of dry Seeds of black gram. International Journal of Pharmaceutical and Phytopharmaceutical Research. 2012;1(4):194-202.