

Screening of *Brassica juncea* Germplasm for Drought Tolerance through Seed Germination and Seedling Growth by using PEG 6000

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ABSTRACT One hundred and fifty genotypes of *Brassica juncea* Czern & Coss were evaluated for their resistance or susceptibility to drought stress on the basis of seed germination and seedling growth. The seeds of all the genotypes were germinated in Petri dishes under drought stress by PEG-6000 (-0.4 and -0.6 MPa). The data recorded on various parameters included germination percentage, shoot and root length, fresh and dry weight of ten-day-old seedlings besides seed testa colour and 100-seed weight of each genotype. Great variations among different genotypes were recorded for these parameters under control conditions, i.e. without PEG treatments. The PEG resulted in reduction of germination potential and seedling growth to varying extent in tested genotypes. On the basis of inhibitory responses for seed germination and seedling growth, due to PEG treatments, the 150 genotypes of *B. juncea* were categorized as susceptible, moderately resistant and resistant genotypes. Among the resistant genotypes, MLM-90, MLM-91, MLM-44 and MLM-32 indicated their capability to withstand water deficit conditions than other genotypes.

Key words: *Brassica juncea*, water deficit stress, seed germination, PEG-6000, germplasm

Oilseed brassicas are most sensitive to drought stress and have very low water-use efficiency [1]. Over the years, several new genotypes of *Brassica juncea* have been developed and need to be screened for drought tolerance for further use. The germination phase is critical in growth cycle of plant species since it determines the stand establishment and final yield of the crop. Selection for drought tolerance at early stage of seedling is most frequently carried out by including chemical drought inducing molecules like polyethylene glycol (PEG-6000) in the germination medium of seeds [2]. The PEG-6000 molecules are inert and non-ionic and are frequently used to induce water stress without causing physiological damage and so PEG solutions mimic dry soil more closely than solutions of other osmolytes [3].

Germination percentage and seedling growth represent one of the selection criteria for drought

resistance reported for *B. napus*, which may also prove valuable for the selection of *B. juncea* genotypes under water stress conditions and provide adequate information regarding genetic variation to enable recognition of superior genotypes [4]. Development of drought tolerant *B. juncea* cultivars is an objective of many breeding programmes. Therefore, present investigation was undertaken to screen 150 genotypes of *Brassica juncea* on the basis of germination and seedling growth under laboratory conditions using PEG-6000 and select better drought tolerant genotypes.

MATERIALS AND METHODS

One hundred and fifty genotypes of *Brassica juncea* Czern & Coss obtained from the Department of Plant Breeding and Genetics (Oilseed section), Punjab Agricultural University, Ludhiana, were used to study the effects of low

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moisture stress created by using polyethylene glycol (PEG-6000). Seeds of uniform size were surface sterilized with 0.1 per cent mercuric chloride and germinated in sterilized glass Petri dishes lined with sterilized filter paper moistened by adding fixed amount of PEG-6000 solutions (-0.4 and -0.6 MPa) [5, 6] in BOD incubator at $22^{\circ}\pm 2^{\circ}\text{C}$. Seeds germinated using distilled water only served as the control. Each treatment was investigated using three replications. Germination counts were made at 24 hr intervals until third day both in the control and PEG treated seeds. Shoot and root length of the control and PEG treated seedlings were measured on tenth day. Shoots and roots were separated and weighed to obtain their fresh weights. These samples were then dried at 70°C for 72 hr in an oven to determine their dry weights. The testa colour and 100-seed weight of all the 150 genotypes were recorded.

RESULTS

The frequency distribution showed variation in 100-seed weight and testa colour of 150 genotypes of *B. juncea* (Table 1). The 100-seed weight of tested genotypes ranged from 240 to 400 mg, with mean seed weight of 338.89 mg. The maximum seed weight was recorded in MLM-89, followed by NLM-21 genotypes, whereas the minimum seed weight was recorded in 2-6-6 CM 21-9-38 followed by 2-9-10 CSR 1020 genotypes. Frequency distribution had shown that majority of genotypes (47%) had 100-seed weight in the range of 321-360 mg (Table 1). Out of 150 genotypes (55) studied grayish-brown testa in 7

genotypes. There were 27 genotypes having dark brown testa and 21 genotypes with yellowish-brown testa.

The 150 genotypes of *B. juncea* to drought stress imposed by PEG (-0.4 and -0.6 MPa) showed a great variability in seed germination, root and shoot length of different genotypes. Decrease in water potential from 0 to -0.4 and -0.6 MPa with PEG led to a significant decrease in seed germination as well as in root and shoot length for all the genotypes.

The frequency distribution of 150 genotypes of *B. juncea* on the basis of germination percentage to drought stress after 72 hr of

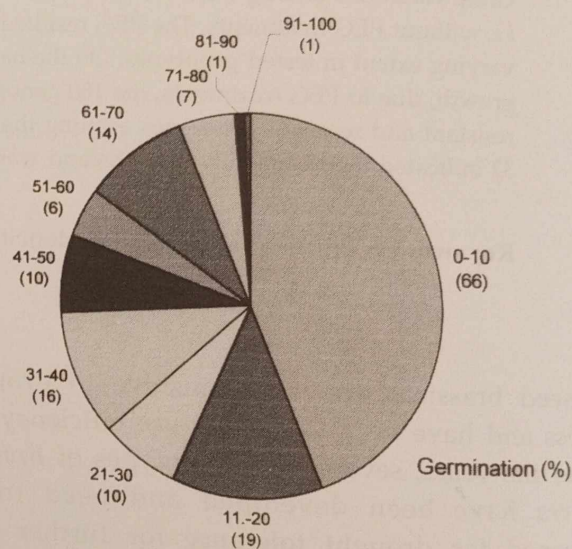


Fig. 1. Frequency distribution of *Brassica juncea* genotypes on the basis of germination percentage (values in parentheses indicate number of genotypes)

Table 1. Frequency distribution of *Brassica juncea* genotypes for 100-seed weight and testa colour

100-seed weight (mg)	Reddish-brown testa	Yellowish-brown testa	Dark brown testa	Greyish-brown testa	Total
240-280	2 (1.33)	2 (1.33)	2 (1.33)	2 (1.33)	8 (5.33)
281-320	14 (9.33)	7 (4.66)	3 (2.00)	8 (5.33)	32 (21.33)
321-360	21 (14.00)	9 (6.00)	15 (10.00)	26 (17.33)	71 (47.33)
361-400	10 (6.67)	3 (2.00)	7 (4.66)	19 (12.67)	39 (26.00)
Total	47 (31.33)	21 (14.00)	27 (18.00)	55 (36/67)	150 (100.0)

Values in parentheses are percentage

incubation had been depicted (Fig. 1). The majority of genotypes (66) showed germination range from 0-10 per cent. Only 19 genotypes showed germination from 11-20 per cent. Ten genotypes each fell into the category where germination percentage varied from 21 to 30 and 41 to 50 per cent. However, 16 genotypes showed intermediate germination range from 31 to 40 per cent. The 20 genotypes exhibited germination in the range of 51 to 70 per cent, and 7 genotypes showed germination potential ranging between 71 to 80 per cent. MLM-32 and MLM-90 genotypes at -0.6 Mpa exhibited germination above 80 and 90 per cent, respectively.

Seedling growth in terms of root and shoot length, and accumulation of fresh and dry weight of seedlings decreased consistently with increasing water deficit stress by PEG. Maximum reduction at -0.6 MPa in root and shoot length was observed in NLM-63 (90%) and NLM-42 (98%), respectively. The fresh weight of root and shoot varied 4.3 mg to 9.9 mg and 2.2 mg to 51 mg, respectively, under the control. Lowering of

water potential to -0.4 MPa led to marked reduction in fresh root weight which ranged from 52 to 72 per cent. The reduction in fresh shoot weight ranged from 22 to 84 per cent. The reduction in root and shoot fresh weight increased further at -0.6 MPa.

The dry weight of roots and shoots also decreased under conditions of water deficit stress. At -0.6 MPa, maximum reduction in root and shoot dry weight was observed in MLM-85 and NLM-44 genotypes which was 87 and 95 per cent, respectively, over the control.

Based on germination percentage and seedling growth behaviour under the control and PEG-induced drought stressed conditions (-0.6 MPa), the tested 150 genotypes of *B. juncea* were categorized as stress resistant and stress susceptible. Among these genotypes, 120 were grouped in the category of susceptible genotypes, whereas 21 in moderately tolerant and 9 as tolerant genotypes (Table 2).

Table 2. Categorization of *Brassica juncea* genotypes as drought susceptible/resistant on the basis of germination studies

SUSCEPTIBLE GENOTYPES (0-50% germination)

NLM-44, MLM-108, MLM-111, MLM-39, NLM-36, PLM-36, QLM-4, PLM-9, PLM-20, 2-9-1CSR-878, 2-5-5 CM-10-6, 2-5-3 CM-10-1, 2-9-8 CSR-960, 2-9-9CSR-1012, 2-5-9 CM-11, 2-9-4CSR-913, 2-9-6 CSR-943, 2-9-11 CSR-1025, 2-9-7 CSR-957, 2-9-12 CSR-1034, 2-5-6CM-10-7, 2-5-10 CM-11-2, 2-5-12 CM-11-6, 2-5-1 CM-9-2, 2-5-4 CM-10-2, 2-5-2 CM-10, 2-5-7 CM-10-8, 2-8-17 CSR-718, 2-8-16 CSR-717, 2-8-8 CSR-403, 2-18-15 CSR-713, 2-8-10 CSR-423, 2-8-18 CSR-729, 2-8-2 CSR-317, 2-8-4 CSR-352, 2-6-8 CM-21-9-40, 2-6-5 CM-21-9-35, 2-6-1 CM-21-5, 2-6-12 CM 21-13, 2-6-14 CM-38, 2-6-3 CM-21-8, 2-6-10 CM-21-11, 2-6-17 CM-51, 2-6-22 CSR-7, 2-6-16 CM-45, 2-6-20 CM-102-88 x CM-594275-98-1, 2-6-15 CM-39, 2-6-4 CM-21-9, 2-6-2 CM-21-7, 2-6-23 CSR-16, 2-6-6 CM 21-9-38, 2-5-20 CM-12-5, 2-5-21 CM-20-2, 2-5-18 CM-12-2, 2-5-16 CM-11-11, 2-5-17 CM-12, 2-5-22 CM-21-1, 2-5-15 CM-11-9, 2-9-5 CSR-930, 2-9-2 CSR-879, 2-9-3 CSR-901, 2-5-13 CM-11-6, 2-5-14 CM-11-8, MLM-85, MLM-96, NLM-57, MLM-97, NLM-42, NLM-52, MLM-116, NLM-68, MLM-87, MLM-43, MLM-110, NLM-30, MLM-112, MLM-113, QLM-13, PLM-18, PLM-6, NLM-58, MLM-99, NLM-60, NLM-65, MLM-104, MLM-107, MLM-114, PLM-15, 2-8-1 CSR-255, 2-8-11 CSR-579, 2-8-3 CSR-331, NLM-61, NLM-62, NLM-64, MLM-100, NLM-66, MLM-102, NLM-14, MLM-55, MLM-109, QLM-15, QLM-17, PLM-19, PLM-8, 2-8-19 CSR-740, NLM-59, NLM-63, NLM-4, MLM-115, NLM-22, NLM-27, MLM-56, QLM-12, QLM-16, PLM-21.

MODERATELY TOLERANT GENOTYPES (51-70% germination)

MLM-19, MLM-101, MLM-103, MLM-81, MLM-106, 2-8-12 CSR-590, OLM-7, MLJVI-98, OLM-8, NLM-53, MLM-69, MLM-67, NLM-8, MLM-61, NLM-11, NLM-17, NLM-52, NLM-34, ALM-774, PLM-14, PLM-17.

TOLERANT GENOTYPES (71-100% germination)

MLM-93, MLM-95, MLM-94, MLM-44, MLM-89, MLM-90, NLM-24, MLM-91, MLM-32

DISCUSSION

Solutions of glucose and polyethylene glycol (PEG) have been used widely to provide a range of water potential to stimulate osmotic stress [5, 6]. Drought conditions were induced by PEG, in order to study the effects of drought stress on seed germination. Seeds of tested genotypes showed a great variability in germination and seedling growth potential, when under normal and stressed conditions imposed by PEG (-0.4 to -0.6 MPa). Seed germination percentage decreased with increasing concentrations of PEG.

Similarly, increase in stress levels caused marked variation in seedling growth in terms of seedling length and accumulation of fresh and dry weight in tested genotypes under normal and stressed conditions. The seedlings of tolerant genotypes had greater root length as compared to susceptible genotypes under controlled conditions and maintained longer roots, when grown under conditions of water deficit. Maintenance of root growth during water deficits is an obvious benefit to maintain an adequate water supply and is under genetic control [1]. The reduction in seedling growth with increasing moisture stress were reported earlier in different plant species [7-11].

A great variability was observed for seed size (1000-seed weight) and testa colour. Seeds with greyish-brown testa were found to be highly tolerant to water deficit stress. Seeds of tolerant genotypes had higher seed weight and were comparatively large in size. Seedlings from large seeds were reported to possess high vigour and better ability to survive under conditions of stress [12, 13]. The plants of oliferous *Brassica* species were known to produce heteromorphic seeds, varying in size and germination potential.

This variation in seed size arised due to indeterminate growth habit of plant with the result that the seeds in earlier formed fruits show better growth in terms of size and weight than in those formed later [13, 14]. The variation in germination potential and seedling growth characteristics in different genotypes on *B. juncea* seems to be the result of metabolic status of seeds. According to Bewley and Black [15] low

water availability during germination stage results in decreased seed metabolism necessary for digestion of reserve substances and translocation of metabolized products.

Among the tested genotypes MLM-44, MLM-32 and MLM-90 were found highly tolerant to water deficit conditions. Further detailed studies pertaining to identification of various physio-morphological and biochemical traits for drought tolerance are needed.

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