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Evaluation and Characterization of Germination Traits in *Brassica juncea* ((L.) Czern Cross) Inbred Lines through PEG Induced Simulated Osmotic Stress

Saleem Jahangir Dar[1*], Pushp Sharma[1,2], Rajni Sharma[1] and S.S. Banga[2]
[1]Department of Botany, Punjab Agricultural University Ludhiana 141004, Punjab, India.
[2]Oilseed Section, Department of Plant Breeding and Genetics,
Punjab Agricultural University, Ludhiana 141004, Punjab, India.

(Corresponding author: Saleem Jahangir Dar)*
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ABSTRACT: Crop growth stages commencing from germination to maturity are affected by water scarcity or water deficit. Seed germination and seedling establishment are the critical phases impeded by drought stress resulting in high mortality. The present investigation was aimed to evaluate drought tolerance of 82 inbred lines at seedling stage. Polyethylene glycol was used to create osmotic potentials (-0.20, -0.40, -0.60, -0.80, -1.00 and -1.20 Mpa) and distilled water as control (0 Mpa). The experiment was conducted in completely randomised design with three replications in the growth chamber at 25 ± 1°C and RH 85%. The germination traits and their interactions with stress levels varied significantly. All the studied germination traits suffered a decline over control with increased stress levels contrary to mean germination time that increased with PEG concentrations. Root and shoot length with their respective fresh and dry weights declined in all inbred lines with increased stress levels or reduced osmotic potentials, maximum reduction was noticed at -0.6 Mpa and afterward. Furthermore, a significant and positive correlation existed among the germination traits while mean germination time was weakly associated with other studied traits. The lesser decline in germination traits along with PCA and hierarchical clustering rated three varieties RLM-619, PBR97 and Pusa Bold, five natural *B. juncea* germplasm lines DJ-101-DT-A-4, DJ-113 DT-3, DJ-124-1-DT-31, DJ-124-1-DT-62 and MCN 2-14, two exotic *B. juncea* lines EC-564647, and JM-06-0206, two introgression lines JC-1359-23-558 and MCP-12-224 and one zero erucic acid quality mustard line QM-7-196 promising for drought stress.

Keywords: Polyethylene glycol, Osmotic potential, Moisture stress, Germination traits.

INTRODUCTION

Indian mustard (*Brassica juncea* (L.) Czern and Cross) is an economically and nutritionally important oilseed crop cultivated across the Northern plains of India which covers 85-90% of the total area under the cultivation of *Brassicas* in India. *B. juncea* is cultivated in the arid and semi-arid regions of India for oil production and utilized as condiments, vegetables and fodder for cattle. These zones are under several risks like water scarcity, enormously increased population, depleted natural resource pools and above all, anthropogenic climate change. These pronounced spatial and temporal variabilities of ten threaten the survival and productivity of mustard crop. In India mustard is grown under rainfed cropping system, that limits seed germination, enhances seedling death in turn decline in yield (Rai *et al.*, 2020).

Drought stress causes a decline in both quality and quantity in *B. juncea*, as it is highly susceptible to water shortage at every growth stage commencing from germination to maturity (Khanzada *et al.*, 2020). Brassicas are negatively impacted by drought stress at several stages, including germination, seed development, grain yield and quality, as well as photosynthetic efficiency (Sujata *et al.*, 2023). A sturdy seedling is a significant indicator that describes the seed yield of the plant over a short period of time. Drought tolerant cultivars with impermeable rooting abilities at seedling stage ameliorate the drastic effects of moisture stress under open field conditions (Ahmed *et al*., 2019). Drought results in decreased plant biomass, decreased seed oil and protein content, and decreased chlorophyll content due to pigment loss and damaged thylakoid membranes. Seed germination and seedling growth are critical phases often subjected to high mortality rates. Drought and salt stress inhibit and delay germination and seedling establishment (Almansouri *et al*., 2001). Drought stress causes a significant decline in germination and seedling growth and even complete crop failure due to the severity of the stress. Cultivar selection depends on germination percentage, germination speed and seedling growth influenced by drought stress. The best performing variety at the seedling stage could increase the production in rainfed areas (Khan *et al*., 2017). Therefore, screening under drought stress is imperative to identify tolerant genotypes for better germination, seedling establishment and higher yields. Polyethylene glycol (PEG-6000) creates osmotic stress and is a drought stimulant (Ashraf *et al.*, 1996 and Ara *et al.*, 2019) which is relatively more static, non-ionic and cell impervious than lower (PEG 4000) and higher (PEG8000) concentrations. Earlier studies demonstrated the screening of *B.juncea* and *B.napus* with PEG-induced drought stress (Geetha *et al.*, 2012 and Jamil *et al.*, 2019) still, the influence of seven osmotic potentials (drought stress levels) on the 82 advanced inbred lines of *Brassica juncea* is not available.

Based on the previous studies it was hypothesized that seven osmotic potentials/stress levels would significantly impact germination traits and rigorously evaluate the inbred lines of *B.juncea*. Therefore, the study was conducted for two years (2018-20) with objectives (i) To investigate the effects of negative osmotic potentials on germination traits (ii) To screen drought tolerant *B. juncea* inbred lines over two years based on their overall performance under osmotic potentials (iii) Identify suitable germination traits as screening tools for drought stress.

MATERIALS AND METHODS

Plant material and treatments. The experiment was conducted with 82 inbred lines comprising released varieties, natural *B. juncea* germplasm, derived *B. juncea* lines, exotic Indian mustard lines, introgression material and zero erucic acid lines of *B. juncea* procured from the Oilseed section Department of Plant Breeding and Genetics, Punjab Agricultural University, Ludhiana, India, for consecutive two years (2018 –2019 and 2019-2020). Seven drought treatments consisting of distilled water control/ 0 Mpa and osmotic potentials (-0.20, -0.40, -0.60, -0.80, -1.00, and -1.20 MPa), which were prepared according to Michal and Kufmann (1973). Mature, healthy and uniform seeds were disinfected by immersion in a 0.1% mercuric chloride solution for one minute, then the seeds were washed three times with distilled water. Twenty seeds of each inbred line per replication were grown in each petri plate double-lined with filter paper placed in the bottom and top. The experiment was conducted in a completely randomized design (CRD) with three replications in each year. Germination was carried out in a growth chamber (CONVIRON CMP 6010, Model No. A1000) at 25 ±1°C and RH 85%.

Germination traits. Seedling germination was recorded daily up to 13 days after sowing (DAS). Germination percentage, germination speed and mean germination time was recorded after 7 DAS as per ISTA (2019). After the completion of experiment, seedlings were used for recording shoot length, root length, fresh weight, and dry weight and were oven-dried at 70°C until a constant weight was achieved. Formulae for germination traits are depicted in Table 1. The germplasm evaluated for drought tolerance during 2018-19 and 2019-20 Table 2.

Statistical analysis. IBM SPSS (v22) software was used to conduct statistical analysis. Data was subjected to analysis of variance (ANOVA at p<0.05), Post hoc Tukey's, to determine the statistical differences among inbred lines, drought stress levels, and their interactions. The traits were further explained through correlation and principal component analysis (PCA) for determining the linkage between traits and inbred lines under different moisture levels (Table 4 and 5). PCs with eigenvalue >1 were considered significant through PCA. The significance of the eigenvalues, established by Kaiser (1960), was used to choose the statistically significant principal components (PCs). Only the PCs that exhibited eigenvalues higher than one were significant. Principal component (PC) analysis, a multivariate statistical analysis for examining and simplifying complex and large datasets which transforms the larger number of correlated variables into smaller ones, as explained. Biplot analysis can select variables categorized into main groups and subgroups based on homogeneity and dissimilarity. The favourable germination traits and tolerant inbred lines were selected based on the overall results.

Table 1: Description of germination traits and methodology.

Traits	Symbol, unit	Methodology
Germination percentage	GP (%)	$n / N \times 100$, where n is the number of germinated seeds at the seventh day; N is the number of total seeds
Mean germination time	MGT (days)	$MGT = \Sigma ni \times di / n$, where n is the total number of seeds germinated during the 13–day experimental period; ni is the number of seeds germinated on day di; di represents the particular day during the germination period (between 1-13)
Speed of germination	SG (days)	$n_1/d_1+n_2/d_2 \ldots n_{13}/d_{13}$ where n is the number of germinated seeds at each day; d is the number of days after the start of the experiment
Root length	RL (cm)	Length of fresh root
Shoot length	SL (cm)	Length of fresh shoot
Fresh weight root	FWR (mg)	Fresh weight /10 roots
Fresh weight shoot	FWS (mg)	Fresh weight /10shoots
Dry weight root	DWR (mg)	Dry weight /10 roots
Dry weight shoot	DWS (mg)	Dry weight /10 shoots

RESULTS

PEG induced drought stress significantly affected the studied germination traits (Table 3, 4 and Fig. 1). Significant differences ($p<0.05$) among all the treatments represented diversity among the studied traits. Inbred lines and their interactions with PEG treatments were highly significant, implying differential responses of inbred lines in varied drought stress levels.

Germination Percentage. PEG induced drought stress had a negative impact on GP with the increased stress levels or decreased osmotic potential (Table 3 and 4). Inbred line DJ-1-2 showed a maximum GP (98.4%) while RL-1359 (40.0%) showed minimum under control. At -0.20Mpa germination percentage ranged from 23.4% in Pusa Bold to 90.0% in JC-1359-23-558 and MCP-12-3. At -0.40 and -0.60Mpa GP was minimum in JPM-2(10.0% and 0 or no germination) but maximum in PBR-97 (85.0%) and JC-1359-23-558 (77.5%). MCN-12-14 line showed a maximum GP of 60.8% while in seven inbred lines (CP-98, DJ-1-2, DJ-124-1-DT-70, ELM-151, GR-325, JPM-2, and K-9-108) germination was checked at -0.80Mpa. At -1.00 Mpa EC-564647 showed tolerance to PEG and exhibited a maximum of 49.2% while in sixteen inbred lines (CP-98, DJ-1-2, DJ-124-1-DT-28, DJ-124-1-DT-70, DJ-127, DJ-5, ELM-151, GR-325, JJ-210-5-4, JM-06-010, JPM-2, K-9-108 S62, M-305, MCP-12-606, NRCDR-2, and RL-1359 germination was checked. Osmotic potential of -1.20 Mpa checked the germination completely, but the eight inbred lines (A8-4-2×JR-049, JC-1359-23-558, MCP-12.224, NRCHB-101, PBR 357, PBR-91, PBR-97 and QM-7-196 germinated, with <20% GP except for JC-1359-23-558 which showed 23.8%. As compared to control reduction of GP was 10.8, 26.5, 41.7, 57.1 and 98.0% with increased stress levels corresponding to -0.20, -0.40, -0.60, -0.80, -1.00 and -1.20 Mpa respectively.

Mean Germination Time. The MGT showed an increasing trend with increased stress levels in all inbred lines except at -1.20 Mpa whereas no germination was recorded, with exception to eight inbred lines (Table 3 & 4). In control, GMCN-13 and JC-1359-23-558 took 2.9 days to germinate, while RLC-1 germinated in 5.7 days. At -0.20Mpa, GR-325 and JC-1359-23-558 took 3.0 days to germinate, whereas RL-1359 took 5.2 days. JC-1359-23-558 took 3 days, while JPM-2 took 6.2 days to germinate at -0.40Mpa. Germination of JPM-2 was checked at -0.60Mpa but DJ-1-2 showed a maximum value for MGT (6.5). Germination of seven inbred lines (CP-98, DJ-1-2, DJ-124-1-DT-70, ELM-151, GR-325, JPM-2, and K-9-108 was inhibited at -0.80Mpa but RLC-1 took 6.6 days to germinate. At -1.00Mpa, MGT ranged from 0 (CP-98, DJ-1-2, DJ-124-1-DT-28, DJ-124-1-DT-70, DJ-127, DJ-5, ELM-151, GR-325, JJ-210-5-4, JM-06-010, JPM-2, K-9-108, M-305, MCP-12-606, NRCDR-2, and RL-1359) to 7.0 days in RH-419-3.

Table 2: Germplasm evaluated for drought tolerance during 2018-19 and 2019-20.

Code	Strain	Code	Strain	Code	Strain
S1	A8-4-2×JR-049	S39	ELM-123	S75	MCP-12.224
S2	RLM-619	S41	ELM-151	S76	MCP-12-3
S3	BAUSAM-2	S42	GMCN-13	S77	MCP-12-227
S4	CP-98	S43	GR-325	S78	MCP-12-401
S5	CSR-7	S44	GR-325 (8-20-1)	S79	MCP-12-606
S6	CSR-95-7	S45	HUSM-1050	S80	MCP-12-630
S7	DIR-297	S46	IC-248988	S81	MCP-12-632
S9	DJ-1-2(DT-5)	S47	JA-1-3×RLC-1	S82	MLM-19
S10	DJ-124-1 DT-28	S49	JC-1359-23-558	S83	NLM-13
S11	DJ-57 DT-10	S51	JJ-210-5-4	S84	NRCDR-2
S12	DJ-101-DT-A-4	S53	JM-1	S85	NRCQR-376
S15	DJ-113 -DT-3	S54	JM-06006	S86	NRCHB-101
S16	DJ-124-1- DT-16	S55	JM-06-010	S87	PBR 210
S17	DJ-124-1 (DT-17)	S57	JM-06022	S88	PBR 357
S18	DJ-124-1-DT-28	S58	JM-06-0206	S89	PBR-91
S19	DJ-124-1- DT-31	S59	JPM-2	S90	PBR-97
S21	DJ-124-1-DT-62	S61	JT-152-2	S91	PLM-2
S22	DJ-124-1-DT-70	S62	K-9-108	S92	PLM-4
S23	DJ-124-1-DT-76	S63	K-106-13	S93	PRG-901
S24	DT-25(DJ-127)	S64	KLM-134	S94	PUSA BOLD
S25	DJ-127 (DT-35)	S65	KARANTI	S95	RH-419-3
S27	DJ-5	S67	M-305	S96	RL-1359
S28	DJ-13	S68	M-633	S97	RLC-1
S29	DJ-17	S69	MCN-12-33	S98	DJ-1-2(DJ-2)
S31	DJ-27	S71	MCN-12-14	S99	VARUNA
S36	EC-564647	S72	MCN-12-45	S106	QM-7-196
S37	EC-564649	S73	MCN-12-60		
S38	ELM-108	S74	MCN-12-62		

At -1.20 Mpa, since only eight inbred lines (A8-4-2×JR-049, JC-1359-23-558, MCP-12.224, NRCHB-101, PBR 357, PBR-91, PBR-97 and QM-7-196 germinated, their MGT values ranged from 6.0 in PBR-91 to 5.4 in PBR-97. MGT increased by 5 (-0.20Mpa), 12.5 (-0.40Mpa), 20.4 (-0.60 Mpa), 22.2 (-0.80Mpa), 24% (-1.00Mpa) while at -1.20 Mpa only eight lines germinated and the germination of 74 lines was checked at this stress level.

Speed of Germination. Results of means comparison (Table 3&4) showed that speed of germination (SG) decreased with decreased osmotic potential or increased stress levels. Overall speed was highest at control as compared to other osmotic potentials. At control, RL-1359 (2.3) germinated with slower speed whereas JC-1359-23-558 (6.7) germinated with higher speed. JC-1359-23-558 showed maximum SG values at 0.2 (5.9), -0.4 (5.3) and -0.6 Mpa (4.9) whereas the lowest was noticed in M-305 (1.7) at -0.2 Mpa. RL-1359 showed SG of 0.5 at -0.4 and 0.3 at -0.6Mpa. At -0.8Mpa highest SG of 3.7 was shown by EC-564647 (S36) but germination was checked in JPM-2. At -1.00 and -1.20Mpa highest SG was noticed in EC-564647(3.0) and in JC-1359-23-558 (1.5) respectively. SG got declined by 13.3 (-0.20Mpa), 33.3 (-0.40Mpa), 51.1 (-0.60Mpa), 68.9 (-0.80 Mpa, 84.4 (-1.00 Mpa) and 97.8% at (-1.20 Mpa).

Root and shoot length. The root and shoot length varied significantly among *Brassica juncea* lines under control and PEG treatments (Table 3&4). In control, root and shoots were longest. The overall reduction percentage in comparison to control were 5.4, 16, 25, 33, 51, 95% for RL and 13, 26, 37, 50, 66, 97% for SL in the increasing stress levels/decreasing osmotic potentials respectively. Root and shoot lengths ranged from 3.8 (K-9-108) to16.4 cm (PLM-2) and 2.3 (CP-98) to 8.1cm (JM-06-0206). Increased stress level reduced both root and shoot length. At -0.2Mpa RL ranged from 3.5 (JPM-2) to 14.7 (MCN-12-45) and SL from 1.6 (RH-419-3) to 6.0cm (JM-06-0206). Magnitude of root and shoot length was lower inJPM-2 (3.0cm) and in CP-98 (1.2cm) but higher in MCN-12-45 (14.6cm) and JM-06-020 (5.1cm) at -0.40Mpa. Maximum RL (12.5 cm) and SL (4.5 cm) was recorded in MCN-12-45 and JM-06-0206 at -0.6Mpa. At -0.8Mpa, MCP-12-3 and MCP-12-630 (11.2cm) possessed longer root and JC-1359-23-558 (3.0cm) longer shoot. Osmotic potential of -1.00 Mpa severely affected the seedling lengths but MCN-12-45 and MCP-12-3 (10.1cm) showed higher RL and JM-06-0206 (3.7cm) maximum shoot length. Furthermore, osmotic potential of -1.20Mpa completely checked the germination, with the exception to 8 lines (A8-4-2×JR-049, JC-1359-23-558, MCP-12.224, NRCHB-101, PBR 357, PBR-91, PBR-97 and QM-7-196). The longest root of 8.6cm was in JC-1359-23-558 while longest shoot length of 2.0 cm in PBR-91.

Fresh weights. Data recorded for the fresh weights of 10 mustard seedlings varied significantly (Table 3&4). The magnitude of reduction of fresh weights ranged from 14-99% for FWR and 15-98% for FWS in the increased PEG treatments as compared to control. The highest decline was noticed at OP -0.60Mpa *i.e.*, 51 and 48% for FWS and FWR. FWR ranged from 36.8 (ELM-123) to 285.0mg (PBR-97) and FWS from 159.1 (RH-419-3) to 529.2mg (DJ-124-1-DT-62) under control. At -0.20Mpa maximum FWR was observed in NLM-13 (252.0mg) and maximum FWS in DJ-124-1-DT-62 (481.0mg) but ELM-123 (131.2mg) showed minimum FWR and RH-419-3 (136.3mg) showed lowest FWS. At -0.40Mpa, FWR varied from 20.3 (A8-4-2×JR-049) to 228.8 (NLM-13) and FWS from 70.7 (A8-4-2×JR-049) to 412.3mg (DJ-124-1-DT-62). The declining trend of FWR and FWS continues and at -0.6Mpa germination of JPM-2 (S59) was inhibited. Higher FWR was recorded in DJ-124-1-DT-62 (149.3mg) and higher FWS in MCP-12-630 (330.1mg). At -0.8Mpa germination was checked in seven lines however PBR 357 (118.7mg) showed higher FWR and DJ-124-1-DT-62 (254.2mg) higher FWS. At the osmotic potential of-1.00Mpa, maximum FWR was recorded in RH-419-3 (77.2mg) and maximum FWS in MCP-12-227 (176.0mg). At -1.20Mpa, maximum FWR and FWS were recorded in PBR 357 (19.5mg) and PBR-91 (84.2mg) respectively.

Dry weights. The dry weight of mustard seedlings was significantly influenced by drought stress caused by the PEG 6000 treatments (Table 3 & 4). Among the germination traits, the impact of induced drought stress was higher on dry weights. Compared to control, the magnitude of reduction was in the increasing order of 23, 46, 68, 83, 94, and 99% for DWR and 18, 38, 58, 74, 88, and 94% for DWS corresponding to PEG levels. DWR at 0Mpa/control ranged from 7.7 (ELM-151) to 47.7mg (DJ-124-1- DT-31). Similarly, DWS varied from 13.8 (ELM-151) to 65.7mg (PBR-97). The higher values were exhibited by DJ-124-1- DT-31 (40.4mg DWR) and PLM-2 (50.2 mg DWS) but lower byJM-06-0206 (5.7mg DWR) and ELM-151(11.0mg DWS) at -0.2Mpa. Root and shoot growth declined with increased PEG treatments and at osmotic potential -0.4Mpa the range was 1.3 (JPM-2) to 27.7 mg (RLM-619) for DWR and 4.5 (JPM-2) to 38.9mg (RLM-619) for DWS. JPM-2 (S59) couldn't resist the severity of stress and at -0.6Mpa its germination was checked but PBR-97 exhibited higher DWR (17.7mg) and DWS (36.8 mg). Osmotic potential of -0.8Mpa extremely reduced the dry weights but PBR-97 resisted the stress and showed higher magnitudes of 11.5mg for DWR and 28.0mg for DWS. At -1.00 Mpa the mean dry matter was very less (2.7mg of DWR in PBR-97 and 13.8mg of DWS in RLM-619). As the germination was completely checked at -1.20Mpa with the exception to eight lines with higher DWR in PBR-91 and PBR-97 (1.5mg) and maximum DWS in MCP-12.224 (7.0mg).

Identification and categorization. Critical analysis of PEG induced drought stress for screening and evaluation of 82 inbred lines over the years (represented as pooled mean of two years 2018-20) under lab conditions for various germination traits led to identification and characterisation into 13 highly tolerant strains, 29 moderately tolerant,17 highly susceptible and 23 moderately susceptible strains. Further for each trait ranges and mean values are also tabulated for all highly tolerant strains and two strains for each other category (Table 3).

Table 3: Range and mean of germination traits of identified and categorised as highly tolerant, moderately susceptible and highly susceptible lines.

Osmotic potential (Mpa)		0	-0.20	-0.40	-0.60	-0.80	-1.00	-1.20
		Germination percentage						
Highly tolerant strains RLM-619(S2), DJ-101-T-A-4 (S12) DJ-113 -DT-3(S15), DJ-124-1- DT-31(S19), DJ-124-1-DT-62(S21), EC-564647(S36), JC-1359-23-58(S49), JM-06-0206(S58), MCN-12-14(S71), MCP-12.224(S75), PBR-97(S90), PUSA BOLD(S94), QM-7-96(S106)	Range	74.2-95.8	70-90	53.3-85.0	41.7-77.5	20.0-60.8	13.3-49.2	0-23.8
	Mean	86.2	79.2	69.2	58.6	45.6	31.2	4.6
		Mean germination time(days)						
	Range	3.0-3.8	3.0-4.0	3.0-4.1	3.2-4.2	3.7-5.3	4.2-6.5	0-6.3
	Mean	3.3	3.5	3.6	3.9	4.5	5.6	1.8
		Speed of germination(days)						
	Range	4.8-6.7	4.1-5.9	3.1-5.3	2.2-4.9	0.8-3.7	0.4-3.0	0-1.5
	Mean	5.3	4.7	4.0	3.2	2.3	1.3	0.2
		Root length(cm)						
	Range	4.9-13.9	4.9-13.5	4.2-12.8	3.8-12.0	3.2-11.3	1.3-10.0	0-7.4
	Mean	9.6	8.9	8.2	7.5	6.6	5.6	2.0
		Shoot length(cm)						
	Range	3.3-8.1	2.9-6.0	2.3-5.1	1.8-4.5	1.6-4.3	0.9-3.7	0-1.5
	Mean	4.4	3.8	3.3	2.8	2.4	1.8	0.3
		Fresh weight/10 roots(mg)						
	Range	92.8-285.0	83.2-219.2	68.3-175.5	36.9-139.3	24.7-106.0	18.2-73.0	0-16.6
	Mean	158.0	130.5	103.5	79.7	51.6	34.2	3.5
		Fresh weight/10 shoots(mg)						
	Range	235.9-529.2	183.1-481.0	159.2-412.3	120.4-330.1	76.0-257.4	50.2-174.4	0-33.8
	Mean	354.6	309.2	260.3	202.8	154.6	101.7	13.8
		Dry weight/10roots(mg)						
	Range	13.4-47.0	10.4-40.4	7.4-27.7	4.2-17.7	2-11.5	1.2-4.8	0-1.7
	Mean	26.7	21.5	16.9	11.5	7.2	2.6	0.4
		Dry weight/10shoots(mg)						
	Range	30.1-65.7	25.2-52.8	14.8-43.9	10.1-36.8	5.7-28.0	3.1-13.8	0-7.0
	Mean	44.1	37.0	29.3	21.2	14.7	8.2	1.4
		Germination percentage						
Moderately tolerant strains DJ-124-1-DT-16 (S16), MLM-19 (S82)	Range	70.9-75	63.3-67.5	45-55.2	36.7-42.5	31.7-34.2	21.7-22.5	-
	Mean	73	65.4	48.8	39.6	32.95	22.1	-
		Mean germination time(days)						
	Range	3.6-3.8	3.4-4.0	3.7-4.2	3.7-4.4	4.8-4.9	5.0-5.7	-
	Mean	3.7	3.7	4.0	4.1	4.9	5.4	-
		Speed of germination(days)						
	Range	4.2-4.3	3.8-4.0	3.1-3.8	2.3-3.4	1.4-2.0	1.0-1.2	-
	Mean	4.2	3.9	3.5	2.9	1.7	1.1	-
		Root length(cm)						
	Range	8.3-8.7	7.5-8.1	5.2-7.8	3.8-6.2	2.7-6.1	2.0-5.9	-
	Mean	8.5	7.8	6.5	5	4.4	3.95	-
		Shoot length(cm)						
	Range	3.4-4.2	3.2-3.3	2.7-3.1	1.8-2.6	1.2-2	1.0-2.0	-
	Mean	3.8	3.25	2.9	2.2	1.6	1.5	-
		Fresh weight/10 roots(mg)						
	Range	125.8-129.0	116.4-117.9	96.5-77.8	38.6-51.7	20.1-37.3	10.9-13	-
	Mean	127.4	117.2	87.2	45.2	28.7	12.0	-
		Fresh weight/10 shoots(mg)						
	Range	262.9-306.7	187.0-203.3	145.9-152.2	115.8-120	59.9-67.0	24.2-27.5	-
	Mean	284.8	195.15	149.05	117.9	63.45	25.85	-
		Dry weight/10roots (mg)						
	Range	12.6-12.9	10.5-10.8	6-6.5	2.5-2.5	2.2-2.3	0.5-1.0	-
	Mean	12.75	10.65	6.25	3.35	2.2	0.75	-
		Dry weight/10shoots (mg)						
	Range	28.1-31.0	22.2-23.7	14.8-18.9	8-13.9	4.1-8.2	2.1-5.5	-
	Mean	29.55	22.95	16.85	10.95	6.15	3.8	-
Osmotic potential (Mpa)		**0**	**-0.20**	**-0.40**	**-0.60**	**-0.80**	**-1.00**	**-1.20**
		Germination percentage						
Highly susceptible strains	Range	50.9-56.7	36.7-50.0	10.0-18.3	0-5.0	-	-	-
	Mean	53.8	43.35	14.15	2.5	-	-	-
		Mean germination time (days)						
	Range	3.3-3.8	3.9-4.3	5.8-6.2	0-6.5	-	-	-
	Mean	3.6	4.1	6.0	3.3	-	-	-

DJ-1-2(DT-5) (S9), JPM-2 (S59)		Speed of germination (days)						
	Range	2.8-3.6	2.0-2.5	0.3-0.6	0-0.2	-	-	-
	Mean	3.2	2.3	0.5	0.1	-	-	-
		Root length (cm)						
	Range	4.2-6.3	3.5-6.4	3.0-6.0	0-4.4	-	-	-
	Mean	5.3	5.0	4.5	2.2	-	-	-
		Shoot length (cm)						
	Range	2.2-3.7	2.1-3.2	1.8-1.9.	0-0.9	-	-	-
	Mean	3.0	2.7	1.9	0.5	-	-	-
		Fresh weight/10 roots (mg)						
	Range	123.9-132.6	114.9-115.1	71.8-102.2	0-20.5	-	-	-
	Mean	128.3	115.0	87.0	10.3	-	-	-
		Fresh weight/10 shoots (mg)						
	Range	215.3-232.9	155.2-169.1	85.9-100.8	0-21.2	-	-	-
	Mean	224.1	162.2	93.4	10.6	-	-	-
		Dry weight/10roots (mg)						
	Range	10.3-10.7	4.7-7.5	1.3-4.7	0-1.4	-	-	-
	Mean	10.5	6.1	3.0	0.7	-	-	-
		Dry weight/10shoots (mg)						
	Range	19.6-23.3	13.0-17.5	4.5-10	0-2.7	-	-	-
	Mean	21.45	15.25	7.25	1.35	-	-	-
		Germination percentage						
Moderately susceptible strains JJ-210-5-4 (S51), M-305 (S6	Range	50.0-68.3	37.5-61.7	23.3-46.7	14.2-31.7	10.0-20.9	-	-
	Mean	59.15	49.6	35	22.95	15.45	-	-
		Mean germination time (days)						
	Range	3.3-4.2	3.7-4.6	3.9-5.3	4.4-5.7	5.2-6.2	-	-
	Mean	3.8	4.2	4.6	5.1	5.7	-	-
		Speed of germination (days)						
	Range	2.4-4.2	1.7-3.5	0.9-2.5	0.5-1.5	0.3-0.8	-	-
	Mean	3.3	2.6	1.7	1.0	0.55	-	-
		Root length (cm)						
	Range	4.6-8.3	4.6-7.4	4.5-6.1	3.6-5.4	3.5-4.3	-	-
	Mean	6.5	6.0	5.3	4.5	3.9	-	-
		Shoot length (cm)						
	Range	3.3-3.4	2.2-3.2	1.8-2.6	1.1-1.2	0.9-1.2	-	-
	Mean	3.4	2.7	2.2	1.2	1.1	-	-
		Fresh weight/10 roots (mg)						
	Range	122.4-129.0	109.1-110.0	53.0-71.8	29.7-29.8	12.9-15.7	-	-
	Mean	125.7	109.6	62.4	29.8	14.3	-	-
		Fresh weight/10 shoots(mg)						-
	Range	272.5-231.0	215.5-175.8	126.4-127.8	83.4-91.8	34.7-43.2	-	-
	Mean	251.8	195.7	127.1	87.6	39.0	-	-
		Dry weight/10roots(mg)						-
	Range	10.8-11.2	8.7-8.8	5.1-5.3	2.0-2.3	0.5-1.0	-	-
	Mean	11.0	8.8	5.2	2.2	0.8	-	-
		Dry weight/10shoots (mg)						
	Range	22.0-23.8	14.0-16.7	12.3-10.7	7.7-8.8	2.3-5.2	-	-
	Mean	22.9	15.4	11.5	8.3	3.8	-	-

“0.0” in the ranges and “-” indicates no germination

Table 4: Range, mean, standard error and least significant differences of germination traits in control and PEG6000 induced six osmotic potentials.

Germination traits	**GP**		**MGT**		**SG**		**RL**		**SL**	
OP (Mpa)	**Range**	**Mean ± SE**	**Range**	**Mean ± SE**	**Range**	**Mean ± SE**	**Range**	**Mean ± SE**	**Range**	**Mean ± SE**
0/Control (DW)	40.0-98.4	$75.9^{a}\pm0.9$	2.9-5.1	$3.5^{c}\pm0.02$	2.3-6.7	$4.5^{a}\pm0.02$	3.8-16.4	$9.3^{a}\pm0.5$	2.3-8.1	$3.8^{a}\pm0.6$
-0.20	23.4-90.0	$67.7^{b}\pm1.1$	3.0-5.2	$3.7^{c}\pm0.03$	1.7-5.9	$3.9^{b}\pm0.02$	3.5-14.7	$8.8^{b}\pm0.2$	1.6-6.0	$3.3^{b}\pm0.2$
-0.40	10.0-85.0	$55.8^{c}\pm1.1$	3.0-6.2	$4.0^{b}\pm0.05$	0.5-5.3	$3.0^{c}\pm.0.1$	3.0-14.6	$7.8^{c}\pm0.3$	1.2-5.1	$2.8^{c}\pm0.5$
-0.60	0.0-77.5	$44.2^{d}\pm1.2$	0.0-6.5	$4.4^{a}\pm0.05$	0.0-4.9	$2.2^{d}\pm0.01$	0.0-12.5	$7.0^{d}\pm0.4$	0.0-4.5	$2.4^{d}\pm0.5$
-0.80	0.0-60.8	$31.8^{e}\pm0.8$	0.0-6.4	$4.5^{a}\pm0.07$	0.0-3.7	$1.4^{e}\pm0.1$	0.0-11.7	$6.2^{e}\pm0.3$	0.0-4.3	$1.9^{e}\pm0.4$
-1.00	0.0-49.2	$18.4^{f}\pm0.7$	0.0-7.0	$4.6^{a}\pm0.1$	0.0-3.0	$0.7^{f}\pm0.1$	0.0-10.1	$4.6^{f}\pm0.3$	0.0-3.3	$1.3^{f}\pm0.2$
-1.20	0.0-23.8	$1.5^{g}\pm0.1$	0.0-6.0	$0.6^{d}\pm0.01$	0.0-1.5	$0.1^{g}\pm0.01$	0.0-8.6	$0.5^{g}\pm0.01$	0.0-1.5	$0.1^{g}\pm0.01$
LSD($p\leq0.05$)	G=1.12, T=0.33, G×T=2.96		G=0.69, T=0.20, G×T=1.84		G=0.08, T=0.05, G×T=0.21		G=0.18, T=0.05, G×T=0.49		G=0.11, T=0.05, G×T=0.30	
	FWR		**FWS**		**DWR**		**DWS**			
OP	**Range**	**Mean ± SE**	**Range**	**Mean ± SE**	**Range**	**Mean ± SE**	**Range**	**Mean ± SE**		
0/Control	36.8-285.0	$152.0^{a}\pm1.2$	159.1-529.2	$314.5^{a}\pm1.9$	7.7-47.7	$18.4^{a}\pm0.4$	13.8-65.7	$31.9^{a}\pm0.4$		
-0.20	31.1-252.0	$131.2^{b}\pm1.5$	136.3-481.0	$267.0^{b}\pm1.8$	5.7-40.4	$14.1^{b}\pm0.3$	11.0-52.0	$26.1^{b}\pm0.4$		
-0.40	20.3-228.8	$101.0^{c}\pm1.6$	70.7-412.3	$216.0^{c}\pm1.6$	1.3-27.7	$9.9^{c}\pm0.3$	4.5-38.9	$19.9^{c}\pm0.4$		
-0.60	0.0-149.3	$75.1^{d}\pm1.4$	0.0-330.1	$164.1^{d}\pm1.2$	0.0-17.7	$5.9^{d}\pm0.4$	0.0-36.8	$13.5^{d}\pm0.4$		
-0.80	0.0-118.7	$46.9^{e}\pm1.3$	0.0-254.2	$115.0^{e}\pm1.7$	0.0-11.5	$3.1^{e}\pm0.3$	0.0-23.6	$8.2^{e}\pm0.3$		
-1.00	0.0-77.2	$27.2^{f}\pm1.1$	0.0-176.0	$68.4^{f}\pm1.7$	0.0-2.7	$1.1^{f}\pm0.1$	0.0-13.8	$3.7^{f}\pm0.2$		
-1.20	0.0-19.5	$1.1^{g}\pm0.1$	0.0-54.2	$4.7^{g}\pm0.1$	0.0-1.7	$0.1^{g}\pm0.01$	0.0-36.8	$0.3^{g}\pm0.4$		
LSD($p\leq0.05$)	G=6.96, T=3.99, G×T=36.11		G=5.59, T=3.21, G×T=29.1		G=2.54, T=0.74, G×T=6.71		G=0.98, T=0.29, G×T=2.58			

OP Osmotic potential, DW (Distilled water grown) GP germination percentage, MGT mean germination time, SG speed of germination, RL root length (cm), SL shoot length (cm), FWR fresh weight root (mg/10 roots), FWS fresh weight shoot (mg/10 shoots), DWR dry weight root (mg/10 roots), DWR dry weight shoot (mg/10shoots), Letters superscripted by small alphabets differ significantly in a column for a given germination trait. Significance $p\leq0.05$ (T= treatments, G= inbred lines, G×T= interactions), Value 0.0 indicates no germination

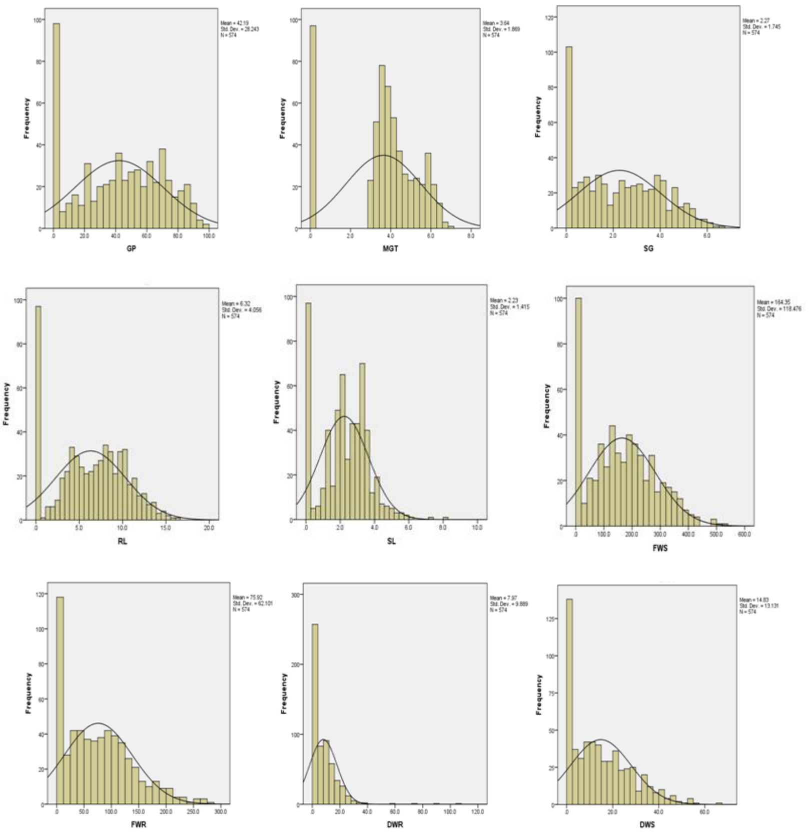
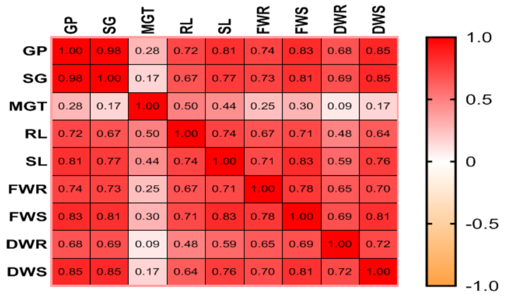


Fig. 1. Frequency distribution graphs showing cumulative germination percentage (GP), mean germination time (MGT), speed of germination (SG), root length (RL cm), shoot length (SL cm), fresh weight root (FWR mg/10roots), fresh weight shoot (FWS mg/10shoots), dry weight root (DWR mg/10roots), and dry weight shoot (DWS mg/10shoots).

Correlation analysis of germination traits under PEG induced drought stress. Highly significant and positive correlation existed among the germination traits under PEG6000 induced drought stress (Fig. 2).



	GP	SG	MGT	RL	SL	FWR	FWS	DWR	DWS
GP	1.00	0.98	0.28	0.72	0.81	0.74	0.83	0.68	0.85
SG	0.98	1.00	0.17	0.67	0.77	0.73	0.81	0.69	0.85
MGT	0.28	0.17	1.00	0.50	0.44	0.25	0.30	0.09	0.17
RL	0.72	0.67	0.50	1.00	0.74	0.67	0.71	0.48	0.64
SL	0.81	0.77	0.44	0.74	1.00	0.71	0.83	0.59	0.76
FWR	0.74	0.73	0.25	0.67	0.71	1.00	0.78	0.65	0.70
FWS	0.83	0.81	0.30	0.71	0.83	0.78	1.00	0.69	0.81
DWR	0.68	0.69	0.09	0.48	0.59	0.65	0.69	1.00	0.72
DWS	0.85	0.85	0.17	0.64	0.76	0.70	0.81	0.72	1.00

Fig. 2. Correlation heat map of germination traits under PEG 6000 induced drought stress in *Brassica juncea* advanced inbred lines. GP germination percentage, MGT mean germination time, SG speed of germination, RL root length, SL shoot length, FWR fresh weight root, FWS fresh weight shoot, DWR dry weight root, DWR dry weight shoot. Correlation is significant (p=0.01).

High positive correlation of GP with other germination traits was in the order of SG (r=0.98) > DWS (r=0.85) > FWS (r=0.83) > SL (r=0.81), FWR (r=0.74) > RL (r=0.72) > DWR (r=0.68) > MGT (r=0.28). MGT showed weak associations with all germination traits (r <0.5). RL and SL were strongly correlated with all studied traits (r ≥0.5).

Principal Component Analysis. Patterns of variation were studied for 82 *Brassica juncea* inbred lines using principal component analysis (PCA established by Kaiser 1960) based on the correlation matrix to evaluate the diversity of the germplasm and the association of germination traits under PEG 6000 concentrations. Out of 9 principal components (Table 5), the first two PCs exhibited eigenvalues higher than one (significant) under PEG 6000 concentrations. The other seven PCs exhibited non-significant variation and were not earnest of further interpretation (eigenvalues less than one). The first two PCs showed 69.9% and 82.3% cumulative proportion of variation in the studied cultivars. The PC1 accounted for 69.9% of the variance, and the PC2 accounted for 12.5%. The first PC was highly related to GP (0.94), SG (0.94), FWS (0.92) and DWS (0.91). PC2 was in strongly associated with MGT (0.94), RL (0.73) and SL (0.63) depicted in Table 6.

Table 5: Eigenvalues, variability, and cumulative of germination traits under PEG 6000 induced drought stress.

PC summary	**PC1**	**PC2**	**PC3**	**PC4**	**PC5**	**PC6**	**PC7**	**PC8**	**PC9**
Eigenvalue	6.29	1.12	0.41	0.35	0.27	0.24	0.17	0.15	0.01
Proportion of variance (%)	69.9	12.5	4.6	3.9	3.0	2.6	1.8	1.6	0.1
Cumulative proportion of variance (%)	69.7	82.3	86.9	90.8	93.9	96.4	98.3	99.9	100.0
Component selection	**S**	**S**							

PC = principal components PC1 to PC9 of studied mustard germination traits, S= selected component (PC1 and PC2)

Table 6: **Principal component analysis (loading factor) of germination traits under PEG 6000 concentrations.**

Traits / Components	GP	MGT	SG	RL	SL	FWR	FWS	DWR	DWS
PC1	0.94	0.26	0.94	0.77	0.87	0.85	0.92	0.80	0.91
PC2	0.46	0.94	0.35	0.73	0.63	0.43	0.48	0.15	0.32

GP germination percentage, MGT mean germination time, SG speed of germination, RL root length (cm), SL shoot length cm), FWR fresh weight root, FWS fresh weight shoot, DWR dry weight root, DWR dry weight shoot

PCA loading plots for germination traits under PEG 6000 induced drought stress. The smaller angles between the traits indicate the strong and positive association of germination traits, while the enlarged arches designate a negative association. The projection of traits on PC1 and PC2 under PEG 6000 induce drought stress revealed that the root length (RL) and shoot length (SL) are highly correlated and as well as with mean germination time (MGT). MGT exhibited a negative association with GP, SG, FWR, FWS, DWR and DWS. But the germination traits (GP, SG, FWR, FWS, DWR and DWS) were strongly associated with each other (Fig. 3).

PCA scores of advanced inbred lines under PEG6000 induced moisture stress. PCA score plots of 82 inbred lines of *Brassica juncea* in the first two PCA's, PCA1 and PCA2, were computed on X and Y squared distances for each inbred line. These scores were plotted on the 2D score plot (Fig. 4). A scrutiny of these results revealed that the inbred lines RLM-619, PBR97, Pusa Bold, DJ-101-DT-A-4, DJ-113 DT-3, DJ-124-1-DT-3, DJ-124-1-DT-62, MCN 2-14, EC-564647, JM-06-0206, JC-1359-23-558, MCP-12-224 and QM-7-196 fall on the same square box, indicating their diversity and high tolerance to drought stress. The inbred lines in the adjacent box showed the relatedness and moderate tolerance to drought stress were considered moderately tolerant. The lines A8-4-2×JR-049, CP-98, DJ-1-2(DT-5), DJ-124-1-DT-28, DJ-124-1-DT-70, DJ-127 (DT-35), DJ-5, ELM-151, GR-325, JM-06022, JPM-2, K-9-108, M-305, NRCDR-2, PRG-901 and RL-1359 present in the upper right-hand square boxes are highly susceptible to PEG-induced drought stress. In contrast, the square box on the upper left side was considered moderately susceptible.

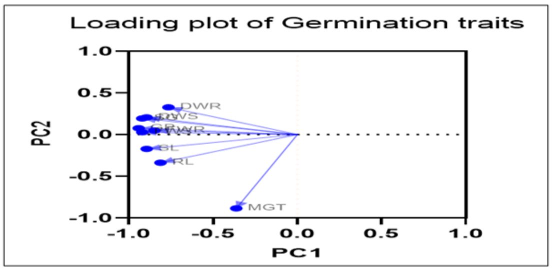


Fig. 3. Loading plot showing the germination traits of 82 inbred lines of *Brassica juncea*on PC1 and PC2.

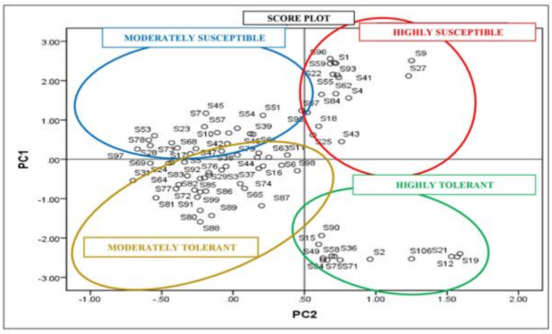


Fig. 4. Score plot of 82 inbred lines of *B.juncea* on PC1 and PC2 represented by codes (Refer Table 2 for strain name).

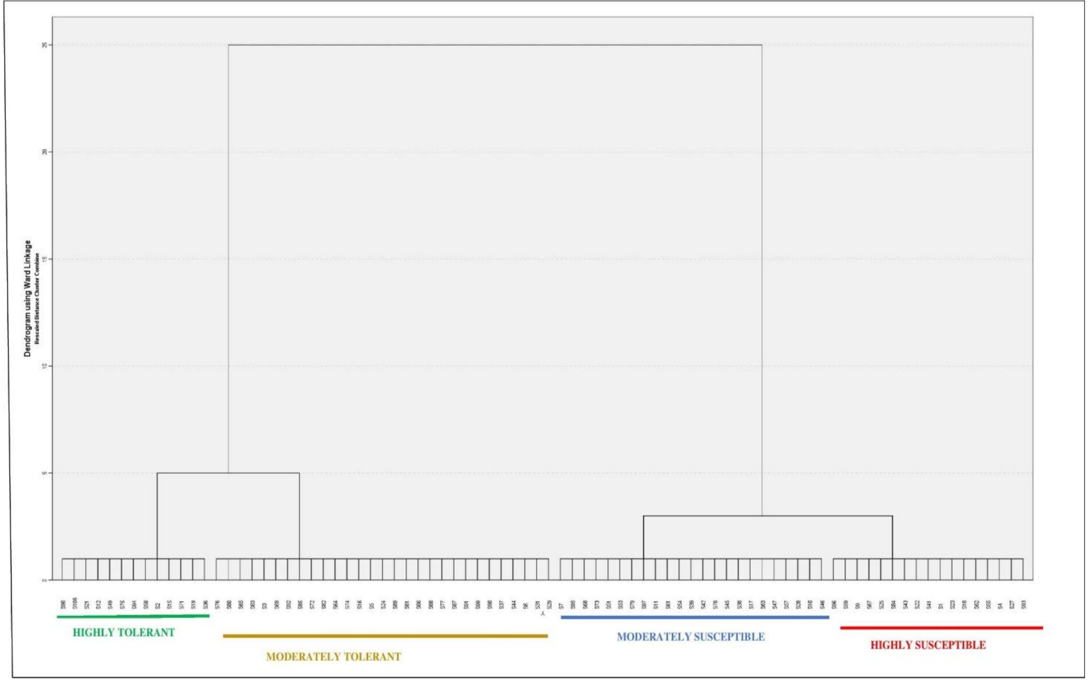


Fig. 5. Cluster dendrogram of 82 inbred lines of *B.juncea* represented by codes (Refer to table for strain name).

Hierarchical clustering. Germination traits of 82 inbred lines of mustard were subjected to hierarchical clustering using Ward's linkage (Fig. 5). The complete set was categorized into four categories (highly tolerant HT, moderately tolerant MT, moderately susceptible MS, and highly susceptible HS). There were 13 HT, 29 MT, 23 MS and 17HS inbred lines.

DISCUSSION

Seed germination and establishment are the two critical stages in the life cycle of a crop. Mortality leads to yield loss due to decreased plant density; thus, screening inbred lines at the early seedling stage is an important criterion for selecting drought stress-tolerant varieties. PEG 6000 is used to create osmotic potentials which fits well for testing seed germination (El-Deeb *et al*., 2018). Drought stress significantly affected all the studied germination traits with a significant reduction in germination and speed of germination concomitantly with the increase in the stress level, which agree with the findings of Toosi *et al*. (2014) and Channaoui *et al*. (2017) and Radhouane *et al*. (2007). Mean germination time increased with the increased stress levels. Comparable results have been reported by Toosi *et al*. (2014) and Hellal *et al*. (1999). Low hydraulic conductivity due to PEG was the probable reason for progressive decline in the GP. In the PEG induced treatments water is not available for imbibition to seeds (Radhoune *et al*., 2007) which is fundamental for germination (Sharma *et al*., 2013).

Different concentrations of PEG inhibited the developmental processes by negatively affecting all the germination traits. In the present study, lengths of root and shoot and their respective dry weight was reduced significantly at all the six stress levels with respect to control. Earlier in *Brassica juncea* (Toosi *et al*., 2014), *Brassica napus* (Jamil *et al*., 2019) and sunflower (Geeta *et al*., 2012) have reported similar reduction in lengths and dry weights. Root system has a prominent role in moisture stress tolerance (Chun *et al*., 2021) and is mainly deployed for water and mineral uptake (Jajarmi *et al*., 2014). In the current study, root and shoot growth decreased with the concomitant increase in PEG concentrations. Root length was less affected than shoot length, but the roots were relatively thin and delicate. Similarly, reduction in the dry weight of roots and shoots were observed in all inbred lines with the increase in drought stress levels/negative osmotic potentials. Our results are endorsed by Jamil *et al*. (2019) and Channaoui *et al*. (2017). Fresh and dry weights were reduced significantly at negative osmotic potentials over control. Our results are in concordance with the results of Jamil *et al*. (2019) (*Brassica napus*), Radhoune *et al*. (2007) (Pearl millet), Song *et al*. (2021) (castor bean) and Yagmur *et al*. 2008 (triticale) where PEG 6000 induced drought stress reduced fresh and dry weights of roots and shoots.

Imposed drought stress negatively and significantly impacted all the traits, but the responses varied with different stress levels. Maximum decline was observed at -0.6 Mpa and henceforth. The reduction may be attributed to the highly reduced osmotic potential (Yagmur *et al*., 2008) and the activity of α-amylase, β-amylase, and α-glucosidase enzymes involved in growth and germination (Channaoui *et al*., 2019). Accumulation of reactive oxygen species (ROS) in response to drought boosted the transcription of NADPH oxidase genes, thus affecting the developmental processes (Luo *et al*., 2021). Cross-talk between drought and ABA involved germination and seedling growth.

Principal component analysis can select variables that can be categorized into main groups and subgroups based on homogeneity and dissimilarity (Neeru *et al*., 2015 and Sisodia *et al*., 2017). The PC1 absorbed and described for maximum (69.9%) proportion of total variability in the set of all 9 PCs and the remaining ones accounted for a progressively lesser and lesser amount of variation. Our results have concordance with the findings of Zada *et al*. (2013) in *B. carinata*, Avtar *et al*. (2014) in toria and Neeru *et al*. (2015) in *B. juncea*. As per our study, inbred lines were categorised into four groups: highly tolerant (13), moderately tolerant (29), moderately susceptible (23), and highly susceptible (17). Saima *et al*. (2012) in wheat and Saleem *et al*. (2017) in Indian mustard have reported similar results.

As PEG induced drought stress affected germination traits of all *Brassica juncea* inbred lines, but the thirteen lines including three varieties (RLM-619, PBR97 and Pusa Bold, five natural *B.juncea* germplasm DJ-101-DT-A-4, DJ-113 DT-3, DJ-124-1-DT-3, DJ-124-1-DT-62, MCN 2-14, two exotic mustard lines EC-564647 and JM-06-0206, two introgression lines JC-1359-23-558 and MCP-12-224 and one zero erucic acid line QM-7-196 exhibited better performance for all germination traits *viz*., GP, SG, MGT, root and shoot lengths, fresh and dry weights of seedlings at the six osmotic potentials. Tolerance in the promising inbred lines were further confirmed by PCA and hierarchical clustering. Performance of *Brassica juncea* inbred lines under seven osmotic potentials designated that seedling germination and growth is the most reliable and efficient phase for studying drought stress. Moreover, this study also revealed that all the germination traits (GP, SG, MGT, RL, SL, FWR, FWS, DWR and DWS) can be considered as best indicators for studying moisture stress invitro. The genetic potential of elite inbred lines revealed better germination traits conferring drought tolerance, however field studies will endorse the performance of the selected lines for stress tolerance.

CONCLUSION AND FUTURE SCOPE

Screening large germplasm for drought tolerance *in-vitro* is simple, economical, and time-effective methodology. Therefore, the performance of *B. juncea* inbred lines were evaluated with PEG induced drought stress levels. Based on the lesser decline in the germination traits, confirmed through PCA and hierarchical clustering, three varieties RLM-619, PBR97 and Pusa Bold, five natural *B. juncea* germplasm lines DJ-101-DT-A-4, DJ-113 DT-3, DJ-124-1-DT-31, DJ-124-1-DT-62, MCN 2-14, two exotic

B. juncea EC-564647 and JM-06-0206, two introgression lines JC-1359-23-558 and MCP-12-224 and one zero erucic acid line QM-7-196 (S106) were found promising.

The PEG induced screening and evaluation of 82 inbred lines helped to identify and categorize the germplasm for further evaluation under field conditions for drought stress. Considering the degree and impacts of drought stress on the productivity of Brassicas, present study may have practical implications for farmers and agricultural policy makers.

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Conflict of Interest. None.

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