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Assessing the genetic potential of mustard genotypes for water stress through PEG-6000 treatments

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Abstract

Water stress is a primary constraint to achieve the goal of sustainable crop production. Water stress severely affects the seed production and oil yield of mustard genotypes. To overcome this problem, the development of water stress resilient mustard cultivars with potential seed and oil yield is a sustainable solution. Therefore, *in vitro* screening of mustard genotypes through PEG-6000 treatments were carried out on ten mustard genotypes. Along with control, two PEG-6000 levels were set to impose osmotic stress, such as, 6% and 10% PEG-6000. Data analyses depicted significant differences among all genotypes and between PEG-6000 treatments for all the studied traits, such as, shoot and root length, shoot and root fresh weight, shoot and root dry weight, K^+ content, Ca^{++} content and K^+/Ca^{++} ratio, demonstrating the availability of genetic differences in mustard genotypes for future stress breeding. Under PEG-6000 (6% and 10%) treatments, the genotypes like AARI-Canola, Khanpur Raya, Dhoom-1, Super Raya, Galaxy and Coral-432 exhibited high performance for seedling traits and less reduction due to PEG-6000 treatments. Hence, these mustard genotypes tend to provide useful genetic potential for water stress breeding.

Key words: Oilseed crops, genetic variations, drought stress, seed yield

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1. INTRODUCTION

Mustard (*Brassica juncea* L.) was one of the first domesticated crops and is known as the versatile oilseeds crop. *Brassica spp.* is the world's third largest source of vegetable oil, owing to its high economic and nutritional worth[1]. It is native to temperate areas of Europe and has been used as an herb for thousands of years throughout Asia, North Africa, and Europe[5]. Since centuries, the mustard has been consumed as a vegetable, and its derivatives have been utilized as sauces, as well as edible and industrial oils. The oil of this crop is often used in cooking and to give a hot and spicy flavor to food. Mustard is variously known as brown mustard, Asian mustard, oriental mustard, Chinese mustard, Indian mustard, leaf mustard, giant red, sarepta mustard, Asiatic mustard, mustard green, and wild Brazilian mustard[2]. Mustard is also considered

as one of the top oil yielding and protein rich crops[3]. The oil content of the improved mustard seeds ranges from 39 to 44 percent. Erucic acid level retention is less than 2% and is at the level of international acceptability[4]. Although Indian mustard is a naturally self-pollinated plant, however, out-crossing happens often in this crop, ranging from 5 to 30 percent, depending on climatic conditions and the random diversity of pollinating insects. Cytological studies reported that mustard is an amphidiploid (2n=36), which obtained through natural crossing between two different species including *Brassica campestris* (2n=20) and *Brassica nigra* (2n=16), followed by natural chromosome doubling[6].

Water stress has a greater adverse impact on seed yield during and after flowering than during other phases of plant development, most likely owing to the sensitivity of pollen formation, anthesis, and fertilization, resulting in decreased seed yield[7]. Water stress has different effects, depending on genotype, stress severity and duration, weather conditions, and brassica's growth and development phases[8]. Reportedly, drought-sensitive rapeseed genotypes exhibited a dramatic decline in the number of siliquae in the main stem and the number of seeds per siliquae under water stress conditions, but drought-tolerant cultivars had no significant loss[9]. Water stress induced a substantial reduction in the number of siliquae plant^{-1}, seeds siliquae^{-1}, 1000-seed weight, seed yield and oil content of five rapeseed cultivars[10].

One of the most widely used methods for identifying drought-tolerant genotypes is the screening under induced water stress conditions generated by osmotic compounds with a large molecular weight, such as polyethylene glycol (PEG)[11]. PEG is a non-penetrating inert osmoticum that can reduce water potential of nutritional solutions without being absorbed or phytotoxic[12]. It has been found that an increase in drought stress caused by PEG was accompanied by a fast decrease in tissue moisture content because PEG mimics in a way like soil drying[13]. This method has been used to simulate drought stress in plants and to select resistant genotypes in other crops[14], and it has been shown to be a successful technique for field crop selection during the early growth phases[15]. In this context, the current study was designed to evaluate the genetic potential of mustard genotypes for better adaptation under water stress condition.

2. MATERIALS AND METHODS

2.1. Plant materials and PEG preparation: In this experiment, ten mustard genotypes (Table 2) were evaluated through two PEG levels for water stress. Along with control, two other PEG-6000 levels were set to impose osmotic stress such as 6% and 10% PEG-6000. For the preparation of 6% and 10% PEG-6000 solution, 60g and 100g PEG-6000 salt (MERCK) was dissolved in 1 liter of Hoagland solution (full strength)[16] and osmotic potential of the respective solution was taken by Osmomat (Osmomat, Model-3000).

2.2. Characterization of seedling traits: The length of the root from root base to root end has been measured in centimeters (cm) then computed as average. The length of the shoot was measured in centimeters (cm) from the bottom to the top, and an average was computed. Ten fresh root and shoot were collected from each replication and measured in grams (g) using a digital electric balance before calculating an average. From each replication, ten dry root and shoot seedlings were taken and the weight of each root and shoot was measured in grams (g) using a digital electric balance. The potassium content (K^+) and calcium content (Ca^{++}) were calculated as a percentage (% g^{-1} fresh weight)[17]. Fresh grinded leaves of shoot were treated by using 0.2 mm acetic acid (CH_3 COOH) in a water bath for 1 hour pre-heated at 95^{o}C to determine K^+. The filtration was done, and an appropriate dilution was performed. The flame photometer was used to measure K^+ absorption (Jenway, Model PFP7). Ionic concentration K^+ and Ca^{++} were determined against the standard graph of K^+ and Ca^{++}. The ratio of K^+/Ca^{++} was also calculated.

2.3. Statistical analysis: The mean squares and LSD test was carried out with the help of Statistix V. 8 computer packages. However, the reduction percentage was determined as the difference between reference number and relative number by the reference number and then multiplied it by 100, which gave the percentage decrease[18].

3. RESULTS AND DISCUSSIONS

3.1. Analysis of variances

The mean squares from analysis of variances are given in Table 01. Regarding mean squares for genotypes, the parameters such as shoot length, root length, shoot fresh weight, shoot dry weight, root fresh weight,

root dry weight, K^+, C^{++} and K^+/Ca^{++} ratio were significantly differences ($P \leq 0.01\%$). Considering mean squares for treatments, all above mentioned traits were also significantly differences ($P \leq 0.01\%$). Pertaining to mean squares for genotype × treatment interactions, the traits including root length, root dry weight K^+, C^{++} and K^+/Ca^{++} ratio were differed significantly ($P \leq 0.05\%$). The mean values and reduction (%) due to PEG levels of seedling traits are given in following paragraphs.

Table 1. Mean squares of different traits of mustard genotypes

Source of variances	Replications (2)	Genotypes (9)	Treatments (2)	G × T (18)	Error (58)
Shoot length	0.304	5.733**	155.020**	0.363^{ns}	0.536
Root length	0.0041	0.8372**	97.1493**	0.7434**	0.1675
Shoot fresh weight	0.02447	0.37761**	4.72668**	0.01834^{ns}	0.03311
Shoot dry weight	0.00021	0.00157**	0.02355**	0.00010^{ns}	0.00013
Root fresh weight	0.00018	0.01035**	0.04607**	0.00030^{ns}	0.00028
Root dry weight	1.111	4.802**	3.744**	2.136**	6.858
K^+	0.0788	2.6782**	43.7742**	0.3370**	0.217
Ca^{++}	0.00349	0.09057**	1.52801**	0.00820*	0.00388
K^+/Ca^{++} ratio	0.06674	0.71571**	0.50562**	0.13545**	0.03052

**(Significant at 1%); *(Significant at 5%); ^{ns} (Non-Significant)

Shoot length (cm)

The shoot length ranged between 14.31 and 11.39 cm at control, 11.17 and 9.20 cm at 6% PEG and 9.32 to 7.13 cm at 10% PEG (Table 02). At control, the maximum shoot length of 14.30, 13.43,13.31 and 13.25 cm were produced by Coral-432, Galaxy, Dhoom-1 and Super Raya, respectively, whereas minimum shoot length was shown by JS-13 (11.39 cm), followed by Sindh Raya (11.50 cm) and S-9 (11.85 cm), with an average of 12.78 cm at control conditions. Considering the shoot length at 6% PEG level, the maximum shoot length was produced by Super Raya (11.17 cm), followed by Dhoom-1 (11.06 cm) and Coral-432 (10.87 cm), whereas minimum shoot length was observed in S-9 (9.20 cm), followed by JS-13 (9.66 cm) and Sindh Raya (9.77 cm). The average shoot length (10.37cm) was noted at 6% PEG. At 6% PEG level, the maximum shoot length was noted in Super Raya (9.32 cm), followed by Coral-432 (9.20cm) and Dhoom-1 (8.96 cm); however, the minimum shoot length was revealed by S-9 and Sindh Raya with same values of 7.13 cm, while an average shoot length of 8.23 cm was observed at 10% PEG. Nevertheless, the maximum and minimum declines at 6% PEG was shown by Bahawalpur Raya (24.17%) and Sindh Raya (15.07%), respectively. Pertaining to reduction at 10% PEG level, the highest and lowest reductions were noted in Bahawalpur Raya (43.28%) and Sindh Raya (29.64%), respectively.

Table 2. Shoot length (cm) in control and PEG levels

Genotypes	Control	PEG levels		Reduction (%) due to	
		6% PEG	10% PEG	6% PEG	10% PEG
Bahawalpur Raya	13.10	9.93	7.43	24.17	43.28
Galaxy	13.43	10.80	8.95	19.60	33.37
Coral -432	14.30	10.87	9.20	24.01	35.69
Khanpur Raya	12.72	10.73	8.18	15.62	35.67
Sindh Raya	11.50	9.77	7.13	15.07	37.97
Super Raya	13.25	11.17	9.32	15.72	29.64
AARI- Canola	12.90	10.50	8.76	18.60	32.09
JS-13	11.39	9.66	7.26	15.18	36.31
Dhoom -1	13.31	11.06	8.96	16.93	32.68
S-9	11.85	9.20	7.13	22.39	39.86
Average	12.78	10.37	8.23	18.73	35.66
Range	11.39-14.3	9.20-11.17	7.13-9.32	15.07-24.17	29.64-43.28
Genotype (LSD 5%)			0.6911		
Treatment (LSD 5%)			0.3785		
Genotype × Treatment (LSD 5%)			ns		

Root length (cm)

At control condition, the maximum root length was exhibited by Dhoom-1 (8.87 cm), followed by AARI-Canola (8.20 cm); however, the minimum root length was measured by Sindh Raya (7.20 cm), followed by S-9 (7.35 cm), showing an average root length of 7.91 cm (Table 03). Regarding root length at 6% PEG level, the maximum root length was produced by Super Raya (5.92 cm), followed by Galaxy (5.90 cm), while the minimum root length was noted in S-9 (5.18 cm), followed by Bahawalpur Raya (5.30 cm), exhibiting a mean valve of 5.54 cm at 3% PEG. At 10% PEG level, the maximum root length was demonstrated by Galaxy (4.69 cm), followed by Dhoom-1 (4.59 cm), whereas the minimum root length was produced by Bahawalpur Raya (4.04 cm), which is followed by S-9 (4.15 cm), with an average value of 4.34 cm at 10% PEG. With respect to reduction % of root length at 6% PEG, it varied from 37.90 to 24.30%, while the maximum reduction was expressed by Dhoom-1 (37.90%) and next was Coral-432 (32.39%), nonetheless, the minimum reduction of 24.30, 25.89 and 26.95% were expressed by JS-13, Sindh Raya and Super Raya, respectively. Taking reductions at 10% PEG, the maximum reduction was found in Khanpur Raya (48.41%), which is followed by Dhoom-1 (48.27%); however, the minimum reduction was recorded in JS-13 (41.30%), followed by Galaxy (42.22%).

Table 3. Root length (cm) in control and PEG levels

Genotypes	Control	PEG levels		Reduction (%) due to	
		6% PEG	10% PEG	6% PEG	10% PEG
Bahawalpur Raya	7.73	5.30	4.04	31.47	47.72
Galaxy	8.12	5.90	4.69	27.37	42.22
Coral -432	8.03	5.43	4.40	32.39	45.22
Khanpur Raya	8.08	5.50	4.17	31.96	48.41

Sindh Raya	7.20	5.33	4.16	25.89	42.15
Super Raya	8.10	5.92	4.55	26.95	43.87
AARI- Canola	8.29	5.79	4.32	30.16	47.93
JS-13	7.30	5.52	4.28	24.30	41.30
Dhoom -1	8.87	5.51	4.59	37.90	48.27
S-9	7.35	5.18	4.15	29.57	43.49
Average	7.91	5.74	4.34	27.06	45.06
Range	8.87-7.20	5.18- 5.92	4.04-4.96	24.30-37.90	48.41-41.30
Genotype (LSD 5%)			0.3862		
Treatment (LSD 5%)			0.2115		
Genotype × Treatment (LSD 5%)			0.6689		

Shoot fresh weight (g)

At control, shoot fresh weight varied from 1.75 to 2.55 g, with an average of 2.08 g. The maximum shoot fresh weight was observed in Galaxy (2.55 g), whereas the minimum shoot fresh weight was noted in S-9 (1.75 g) (Table 04). Considering the shoot fresh weight at 6% PEG, the maximum shoot fresh weight of 2.00, 1.95, 1.78 and 1.67 g were produced by Galaxy, Super Raya, Coral-432 and Dhoom-1, respectively. Whereas the minimum shoot fresh weight was produced by Sindh Raya (1.33 g), S-9 (1.37 g), Bahawalpur Raya (1.40 g) and JS-13 (1.48 g), respectively. At 10% PEG, it ranged between 1.10 to 1.60 g, showing an average of 1.29 g. The highest shoot fresh weight was produced by Galaxy (1.60 g) and the lowest shoot fresh weight was observed in Sindh Raya (1.10 g). Mentioning the reduction at 6% PEG, the average declines was 21.98% with range between 13.53 and 28.53%; yet Sindh Raya (28.53%) and Super Raya (13.53%) were high and low in reductions, respectively. At 10% PEG, the highest reduction of 44.51% was noted in Coral-432. Conversely, the lowest reduction was recorded in S-9 (35.14%).

Table 4. Shoot fresh weight (g) in control and PEG levels

Genotypes	Control	PEG levels		Reduction (%) due to	
		6% PEG	10% PEG	6% PEG	10% PEG
Bahawalpur Raya	1.96	1.41	1.20	28.34	39.03
Galaxy	2.55	2.00	1.60	21.55	37.28
Coral -432	2.38	1.78	1.32	25.21	44.51
Khanpur Raya	2.06	1.67	1.30	18.81	36.95
Sindh Raya	1.86	1.33	1.10	28.53	40.92
Super Raya	2.26	1.95	1.46	13.53	35.56
AARI- Canola	2.05	1.59	1.29	22.24	36.81
JS-13	1.90	1.49	1.22	21.61	35.48
Dhoom -1	2.06	1.67	1.31	18.60	36.39
S-9	1.75	1.38	1.14	21.33	35.14
Average	2.08	1.63	1.29	21.98	37.81
Range	1.75-2.55	1.33-2.00	1.10-1.60	13.53-28.53	35.14-44.51
Genotype (LSD 5%)			0.1717		
Treatment (LSD 5%)			0.0940		
Genotype × Treatment (LSD 5%)			ns		

Shoot dry weight (g)

The genotypes Galaxy and Khanpur Raya produced the maximum shoot dry weight with same values of 0.18 g at normal conditions, while minimum shoot dry weight was observed in S-9 (0.12 g) and JS-13 (0.13 g), with an average of 0.15 g, while ranged between 0.12 to 0.18 g (Table 05). With regards to 6% PEG, it ranged between 0.10 and 0.15 g, averaging 0.12 g of shoot dry weight. The maximum shoot dry weight was exhibited by Galaxy (0.15 g), followed by Khanpur Raya (0.14g), while the minimum shoot dry weight was shown by S-9 and JS-13 with same value (0.10 g). At 10% PEG, the maximum shoot dry weight was noted in Galaxy (0.12 g), followed by Khanpur Raya (0.11 g), however the minimum shoot dry weight was produced by Sindh Raya (0.09 g), showing an average of 0.10 g of shoot dry weight at 10% PEG. The highest reduction was shown by Super Raya with the values of 27.39% and 39.54% at 6% and 10% PEG levels, respectively, whereas the lowest reductions of 18.84% (6% PEG) and 24.90% (10% PEG) were noted in Galaxy and S-9, respectively. At 6% PEG, declines ranged between 18.89 and 27.39% with an average reduction of 22.85%. At 10% PEG, the drop in shoot dry weight was varied from 24.90 to 39.54%, showing an average decline of 35.33%.

Table 5. Shoot dry weight (g) in control and PEG levels

Genotypes	Control	PEG levels		Reduction (%) due to	
		6% PEG	10% PEG	6% PEG	10% PEG
Bahawalpur Raya	0.15	0.11	0.095	25.45	38.52
Galaxy	0.18	0.15	0.117	18.84	34.58
Coral -432	0.16	0.12	0.099	23.99	37.80
Khanpur Raya	0.18	0.14	0.109	20.46	37.88
Sindh Raya	0.15	0.11	0.091	23.26	37.17
Super Raya	0.16	0.12	0.099	27.39	39.54
AARI- Canola	0.15	0.12	0.097	23.75	36.69
JS-13	0.13	0.10	0.088	22.46	34.39
Dhoom -1	0.15	0.12	0.100	20.44	31.86
S-9	0.12	0.10	0.093	22.46	24.90
Average	0.15	0.12	0.10	22.85	35.33
Range	0.12-0.18	0.10-0.15	0.09-0.12	18.84-27.39	24.90-39.54
Genotype (LSD 5%)			0.0108		
Treatment (LSD 5%)			5.9310		
Genotype × Treatment (LSD 5%)			ns		

Root fresh weight (g)

At control, root fresh weight varied from 0.19 to 0.31 g, displaying an average of 0.24 g. The maximum root fresh weight was recorded in Super Raya (0.31 g), followed by Galaxy (0.28 g) and AARI-Canola with same mean value (0.28 g) (Table 06). However, minimum root fresh weight was produced by S-9 (0.19 g), followed by JS-13 (0.20 g). At 6% PEG level, the maximum root fresh weight was exhibited by Super Raya (0.26 g), which is followed by AARI-Canola (0.24 g); however, the minimum root fresh weight was depicted by S-9 (0.16 g), followed by Sindh Raya (0.17 g), with an average of 0.20 g. At 10% PEG level, the maximum root fresh weight of 0.22, 0.20, 0.18 and again 0.18 g were manifested by Super Raya, AARI-Canola, Coral-432 and Galaxy, respectively, whereas the bottom rank root fresh weight was shown by S-9 (0.13 g) and Bahawalpur Raya (0.14 g), displaying an average of 0.16g. At 6% and 10% PEG levels, the maximum reductions were found in Galaxy (27.58%) and Bahawalpur Raya (37.67%), respectively. Whereas the lowest

reduction was recorded in AARI-Canola 14.02% (6% PEG) and 25.99% (10% PEG). On an average, the drop of 18.74 and 31.95% were noted in root fresh weight at 6% and 10% PEG, respectively.

Table 6. Root fresh weight (g) in control and PEG levels

Genotypes	Control	PEG levels		Reduction (%) due to	
		6% PEG	10% PEG	6% PEG	10% PEG
Bahawalpur Raya	0.22	0.17	0.13	19.22	37.67
Galaxy	0.28	0.21	0.18	27.58	37.35
Coral -432	0.28	0.22	0.18	22.09	36.11
Khanpur Raya	0.26	0.21	0.17	19.14	32.72
Sindh Raya	0.21	0.17	0.14	22.81	34.12
Super Raya	0.31	0.26	0.22	16.23	29.27
AARI- Canola	0.28	0.24	0.21	14.02	25.99
JS-13	0.20	0.17	0.14	15.17	27.50
Dhoom -1	0.24	0.20	0.17	16.21	29.31
S-9	0.19	0.16	0.13	14.94	29.48
Average	0.25	0.20	0.17	18.74	31.95
Range	0.19-0.31	0.16-0.26	0.13-0.22	14.02-27.58	25.99-37.67
Genotype (LSD 5%)			0.0158		
Treatment (LSD 5%)			8.6480		
Genotype × Treatment (LSD 5%)			ns		

Root dry weight (g)

With respect to control, the maximum root dry weight was noted in Dhoom-1 (0.02 g), while Bahawalpur Raya, Sindh Raya and JS-13 were found for minimum value (0.01 g) (Table 07). Concerning root dry weight at 6% PEG, 0.014 g was the average, while it ranged from 0.01 to 0.02 g. The maximum and minimum root dry weight was produced by Dhoom-1 (0.02 g) and Bahawalpur Raya (0.011 g), respectively. At 10% PEG level, the maximum root dry weight was observed in Dhoom-1 (0.01 g) and minimum root dry weight was equally noted in Sindh Raya and JS-13 (0.010 g), with an average of 0.012 g. Referring the reduction (%) of root dry weight at 6% PEG, it ranged between 12.12 and 20.00%, whereas the maximum reduction was seen in Super Raya (20.00%), nonetheless, the minimum reduction was noted in Khanpur Raya (12.12%), giving an average of 16.23% at 6% PEG. The reduction (%) ranged from 23.88 to 32.70% with maximum in Bahawalpur Raya (32.70%) and minimum in Khanpur Raya (23.88%) due to PEG level of 6%. On an average, the reduction of 27.20% was recorded at 10% PEG.

At control conditions (Table 08), the maximum K^+ was observed in Coral-432 (10.64), followed by AARI-Canola (9.81) and JS-13 (9.56), whereas the minimum K^+ was exhibited by Dhoom-1 (8.19). An average (9.21) was recorded and a range of 8.19 to 10.64 of K^+ was noted in control condition. At 6% PEG level, the highest K^+ was shown by Coral-432 (8.75), while the lowest K^+ was noted in Super Raya (7.02), whereas the minimum K^+ was revealed by Super Raya (6.61), giving an average of 6.61- 7.59. The reduction (%) of 14.80% to 25.94% on an average in K^+ occurred at 6% and 10% PEG, respectively. However, reductions varied from 8.56 to 26.50% at 6% PEG and 18.03% to 35.57% at 10% PEG. The maximum reduction was demonstrated by Super Raya with the values of 26.50% and 35.57% at 6% and 10% PEG, respectively.

Table 7. Root dry weight (g) in control and PEG levels

Genotypes	Control	PEG levels		Reduction (%) due to	
		6% PEG	10% PEG	6% PEG	10% PEG
Bahawalpur Raya	0.014	0.011	0.009	18.77	32.70
Galaxy	0.017	0.014	0.012	15.89	31.01
Coral -432	0.017	0.014	0.013	16.44	23.96
Khanpur Raya	0.018	0.015	0.013	12.12	23.88
Sindh Raya	0.014	0.012	0.010	18.16	25.73
Super Raya	0.018	0.015	0.013	20.00	27.17
AARI- Canola	0.018	0.015	0.013	16.01	26.13
JS-13	0.014	0.012	0.010	16.62	28.82
Dhoom -1	0.019	0.016	0.014	15.51	27.97
S-9	0.015	0.013	0.011	12.75	24.59
Average	0.016	0.014	0.012	16.23	27.20
Range	0.01-0.02	0.01-0.02	0.01-0.01	12.12-20.00	23.88-32.70
Genotype (LSD 5%)			2.4710		
Treatment (LSD 5%)			1.3530		
Genotype × Treatment (LSD 5%)			4.2800		

Table 8. K^+ in control and PEG level

Genotypes	Control	PEG levels		Reduction (%) due to	
		6% PEG	10% PEG	6% PEG	10% PEG
Bahawalpur Raya	9.02	7.61	6.92	15.64	23.29
Galaxy	9.55	8.14	7.05	14.80	26.25
Coral-432	10.64	8.75	7.59	17.83	28.72
Khanpur Raya	8.55	7.66	7.01	10.41	18.03
Sindh Raya	8.35	7.64	6.19	8.56	25.86
Super Raya	9.56	7.02	6.16	26.50	35.57
AARI-Canola	9.82	8.46	7.10	13.86	27.69
JS-13	9.57	8.38	7.05	12.41	26.35
Dhoom-1	8.19	7.16	6.41	12.48	21.64
S-9	8.83	7.46	6.54	15.48	25.98
Average	9.21	7.83	6.80	14.80	25.94
Range	8.19-10.64	7.02-8.75	6.16-7.59	8.56-26.50	18.03-35.57
Genotype (LSD 5%)			0.1389		
Treatment (LSD 5%)			0.0761		
Genotype × Treatment (LSD 5%)			0.2405		

Ca^{++}

At control condition, the maximum Ca^{++} was noted in AARI-Canola (2.22), followed by Dhoom-1 (2.18) and JS-13 (2.16); however, the minimum Ca^{++} was produced in Bahawalpur Raya (1.92). Ca^{++} averaged was 2.07, while ranged between 1.92 and 2.22 (Table 09). At 6% PEG, the maximum Ca^{++} was exhibited by Galaxy (1.98), followed by AARI-Canola and Dhoom-1 (1.96), while the minimum Ca^{++} was exhibited by Sindh Raya

(1.67). At 10% PEG, the highest Ca^{++} was observed in Galaxy 1.77, followed by AARI-Canola (1.74) and Dhoom-1 (1.70), whereas the minimum Ca^{++} was shown by Sindh Raya (1.49), followed by Super Raya (1.54). Considering the averages at 6% and 10% PEG, the average values of 1.85 and 1.62 were calculated, respectively. Mentioning the reduction at 6% PEG, the average decline was 10.68%, ranging between 4.61 and 17.56%; yet Khanpur Raya (17.56%) and Bahawalpur Raya (4.61%) showed maximum and minimum reduction, respectively. At 10% PEG level, the reduction range was from 12.68 to 26.01%; however, the highest reduction (26.01%) was noted in Khanpur Raya. Conversely, the minimum reductions were recorded in Bahawalpur Raya (12.84%), with an average of 21.75% at 10% PEG.

Table 9. Ca^{++} in control and PEG levels

		PEG levels		Reduction (%) due to	
Genotypes	**Control**	**6% PEG**	**10% PEG**	**6% PEG**	**10% PEG**
Bahawalpur Raya	1.92	1.83	1.68	4.61	12.84
Galaxy	2.14	1.98	1.77	7.57	17.40
Coral-432	2.08	1.87	1.57	10.45	24.65
Khanpur Raya	2.07	1.71	1.53	17.56	26.01
Sindh Raya	1.94	1.67	1.49	13.90	23.18
Super Raya	2.07	1.87	1.54	9.35	25.68
AARI-Canola	2.22	1.96	1.74	11.88	21.51
JS-13	2.16	1.94	1.68	10.56	22.45
Dhoom-1	2.18	1.96	1.70	10.03	21.91
S-9	1.93	1.72	1.50	10.85	21.90
Average	2.07	1.85	1.62	10.68	21.75
Range	1.92-2.22	1.67-1.98	1.49-1.77	4.61-17.56	12.84-26.01
Genotype (LSD 5%)			0.0588		
Treatment (LSD 5%)			0.0322		
Genotype × Treatment (LSD 5%)			0.1018		

K^+/Ca^{++} ratio

The results regarding K^+/Ca^{++} ratio at control conditions, 6 and 10% PEG are given in Table 10. At control condition, the maximum K^+/Ca^{++} ratio was noted in Coral-432 (5.11); however, the minimum K^+/Ca^{++} ratio was produced by Dhoom-1 (3.78). With regards to 6% PEG, the maximum K^+/Ca^{++} ratio was exhibited by Coral-432 (4.69), while the minimum K^+/Ca^{++} ratio was found in Super Raya (3.75). At 10% PEG level, the maximum K^+/Ca^{++} ratio was also observed in Coral-432 (4.83), whereas the minimum K^+/Ca^{++} ratio was demonstrated by Dhoom-1 (0.50).

Mustard is a major oil-producing crop that has been found to be more heat and drought resistant than other spices, with a variety of contributing characteristics[19]. Water stress inhibits germination and seedling growth by reducing the proportion and rate of germination and seedling growth[20]. This sort of stress is one of the most significant environmental pressures influencing agricultural production globally, and it can result in significant yield losses[21]. The purpose of this study was to assess the impact of drought stress on 10 cultivars at the early seedling stage using PEG. The mean squares for shoot length, root length, shoot fresh weight, shoot dry weight, root fresh weight, root dry weight, K^+, C^{++} and K^+/Ca^{++} ratio were significantly differences ($P \leq 0.01\%$). Considering mean squares for treatments, all above mentioned traits were also significantly differences ($P \leq 0.01\%$). Pertaining to mean squares for genotype × treatment interactions, the traits including root length, root dry weight K^+, C^{++} and K^+/Ca^{++} ratio were differed significantly ($P \leq 0.05\%$). The obtained results indicate that substantial genetic variability is existed in evaluated mustard genotypes, revealing their significance in selection and hybridization program to develop new cultivars for stress breeding. The significant genetic variations for seedling traits under PEG concentrations were also reported in sorghum[22], mustard[23], rapeseed[24,25], soybean[26], millet[27], rice[28], maize[29], sugarcane[30], sesame[31], wheat[32], haricot bean[33] and cotton[24].

Table 10. K^+/Ca^{++} ratio in control and PEG levels

Genotypes	Control	PEG levels		Reduction (%) due to	
		6% PEG	10% PEG	6% PEG	10% PEG
Bahawalpur Raya	4.70	4.15	4.13	11.55	11.98
Galaxy	4.47	4.12	4.00	7.76	10.55
Coral -432	5.11	4.69	4.83	8.23	5.43
Khanpur Raya	4.57	4.48	4.13	1.89	9.71
Sindh Raya	4.58	4.30	4.16	6.05	9.19
Super Raya	4.62	3.75	4.01	18.86	13.23
AARI- Canola	4.42	4.32	4.07	2.27	7.88
JS-13	4.43	4.33	4.21	2.10	4.91
Dhoom -1	3.78	3.76	3.67	0.50	3.09
S-9	4.58	4.35	4.35	5.08	5.20
Average	4.53	4.23	4.15	6.43	8.12
Range	3.78-5.11	3.75-4.69	3.67-4.83	0.50-18.86	3.09-13.23
Genotype (LSD 5%)			0.1648		
Treatment (LSD 5%)			0.0903		
Genotype × Treatment (LSD 5%)			0.2855		

The findings showed that *in vitro* growth of a mustard genotype is influenced by osmotic stress conditions generated by varying PEG concentrations. In all genotypes, raising the PEG concentration resulted reduction in developmental characteristics for shoot and root length. However, mustard genotypes like Galaxy, Coral -432, Khanpur Raya, Super Raya, AARI- Canola, and Dhoom-1 reported greater performance and less reduction in PEG concentration for shoot and root length. Hence, these genotypes would be preferred for stress breeding to improve genetic makeup of upcoming mustard genotypes. According to a prior study[35], shoot and root length can be utilized as selection criteria for early detection of water stress-tolerant genotypes. Decrease in plant growth is a physiological reaction to water stress that causes the plant's metabolism to slow down[36]. Similarly, the authors of a prior study indicated that the ability of the roots to take water from the subsoil is crucial in plant survival under drought[37]. According to some research[35,38], an increase in root length improves the plant's ability to fetch water from deeper soil. However, elongated roots may impair shoot growth as more photosynthates migrate toward the roots[39]. Further research proposed that early root extension is a predictor of drought resistance[40].

Considering the biomass of seedling traits (fresh and dry weight of root and shoot), Galaxy, Coral-432, Khanpur Raya, Super Raya and Dhoom-1 were the promising in maintaining the higher weight and less reduction in seedling traits due to PEG concentration, providing chances to utilize in mustard stress breeding programs. The increase of biomass in mustard genotypes tested with PEG might be explained by ion processes, a rise in suitable osmolyte content, or protein and amino acid synthesis[31]. Plants acquire inorganic and organic solutes when the level of stress rises[41]. Similarly, it found in tomato genotypes that applying varying percentages of PEG enhanced dry matter content in all PEG treatments[42]. It has been also observed that dry matter increased with increasing osmotic stress in pea (*Lathyrus sativus*)[43]. Another study discovered that majority of the tolerant genotypes collected more biomass during osmotic stress with PEG in water stress-induced tomato plants[37]. The increase in dry matter during water stress can also be an indication of drought resistance[44]. In certain genotypes (Bahawalpur Raya, Sindh Raya, AARI- Canola, JS-13 and S-9), poor performance and higher reduction in different PEG concentration was noticed for biomass characters. Drought stress, in addition to root characteristics, decreases the phenotypic expression of all seedling features, including fresh and dry weight of shoot and root[45]. Drought stress decreases seedling development by reducing cell division and elongation[46,47]. A good supply of K^+ is also considered to be the key to reducing the harmful consequences of abiotic stresses[48]. As a result of their genetic composition, PEG impacted K^+ and Ca^{++} in distinct mustard genotypes in different ways. Nonetheless, the genotypes such

as Galaxy, Coral-432, AARI-Canola and JS-13 maintained higher K^+ and Ca^{++} at both PEG levels. According to the findings, genotypes with greater K^+ and Ca^{++} concentrations eventually represented higher K^+/Ca^{++} ratio, and these genotypes would be favored for water stress breeding.

4. CONCLUSIONS

It was concluded that data analyses depicted significant differences among all genotypes and between PEG treatments for all the studies traits, demonstrating the availability of genetic differences in mustard genotypes for future stress breeding. Under PEG (6% and 10%) treatments, the genotypes like AARI-Canola, Khanpur Raya, Dhoom-1, Super Raya, Galaxy and Coral-432 were on top with their high performance and less reduction due to PEG treatments.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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