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SCREENING FOR WATER DEFICIT STRESS TOLERANCE IN *Brassica napus* L. USING PEG-6000

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The present *in vitro* study was conducted to screen 60 genotypes of *Brassica napus* L. at seedling stage in Polyethylene glycol (PEG-6000) mediated drought stress. The trial was laid out in a completely randomized design with factorial structured treatments having three replications. Three levels of stress, i.e. 0 (control), -0.35 and -0.60 MPa were developed by dissolving 0, 5, 10g of PEG-6000/100 ml de-ionized water respectively. After 14 days data was recorded for shoot length (cm), root length (cm), fresh root and shoot weight (g) and dry root and shoot weight (g). The seedlings growth decreased with increasing concentration of PEG-6000 due to an increase in water stress. The genotypes which showed better performance in both treatments were selected as drought tolerant genotypes. Eight genotypes that are Faisal sarson, Dunkled, RBN, Punjab sarson, Rainbow, Chakwal sarson, Shiralee and Legand were selected as drought tolerant genotypes. These drought tolerant genotypes will be helpful for genetic improvement in *Brassica napus* L. crop in the future breeding programmes. The current research will be helpful for the selection of tolerant genotypes in water deficit conditions at early seedling stage.
Keywords: Rapseed, edible oil, drought tolerance, osmotic stress, polyethylene glycol, *in vitro*.

INTRODUCTION

Rapeseed is the chief source of edible oil in the world. Local production of edible oil is provisionally estimated to be 0.446 million tonnes. Total accessibility of edible oil from all sources is likely to be 2.426 million tons. There is shortage of edible oil in Pakistan therefore; enormous quantity of edible oil is imported to fulfill its consumption requirements (Economic survey of Pakistan, 2016-17). There is need to improve our varieties and introduce new oilseed crops, so the imports may be lessened and different oil seed crops could be improved to be more profitable (Gangapur *et al*., 2009).The quality and quantity of rapeseed is continuously decreasing due to spontaneous fluctuation in the environmental stresses (Wan *et al*., 2009). Among those stresses, water deficit stress is the main cause to reduce crop production. Drought stress affects plants at all developmental stages and functional characteristics of the plant. Plant survival becomes impossible during strict water deficit environment (Khayatnezhad *et al*., 2010). The effect of drought depends on time and extent of water shortage (Pandey *et al*., 2001). Drought incidence time affects more than its intensity (Abbasian and Rad, 2011). Mostly oilseed rape crops are grown in autumn and are affected by drought stress. Typically drought is an imperative limiting factor for seedling development and survival reducing the population dynamics (Macar, 2008). Hence, drought stress often results decline in yield which is an essential agricultural research topic (Zhang *et al*., 2008).

We need to grow such varieties that could sustain optimal production under drought stress and are drought tolerant. Drought resistance breeding is difficult due to the lack of efficient screening techniques and due to the failure to make repeated water stress environment regularly while a lot of genotypes are needed to be evaluated competently (Akcura *et al*., 2011). Polyethylene glycol (PEG-6000) creates osmotic stress and is utilized as a drought stimulant (Ashraf *et al*., 1996; Turhan, 1997). Polyethylene glycol molecules with a molecular weight (PEG 6000) are static, non-ionic and cell impervious. They are small enough to affect the osmotic pressure, but are large enough which are not absorbed by the plants. Therefore, they are frequently used.
In the current study, PEG-6000 is used to develop osmotic stress at seedling stage. Previous researchers mostly used this compound to develop water deficit conditions in their screening studies (Smok *et al*., 1993; Hu and Jones, 2004). It is need of the time to use an efficient way of screening against drought stress in *Brassica napus* L. advanced genotypes/lines. Therefore, the current research was planned with the objective to identify best performing drought tolerant genotypes at early seedling stage under water deficit conditions.

MATERIALS AND METHODS

***Experimental conditions*:** The current experiment was carried out in the green house of the Department of Plant

Table 1. Genotypes used in screening.

S. No.	Genotypes	S. No.	Genotypes	S. No.	Genotypes	S. No.	Genotypes
1	DGL	16	Shiralee	31	ZRM-12	46	KN-256
2	Long	17	Dunkled	32	FH-23	47	Mat-RBN
3	B-56	18	Zm-R3	33	64-A	48	Zn-R1
4	ZMR-2	19	D-265	34	Faisal sarson	49	Zm-R4
5	Zm-Rn	20	Zm-21	35	FH-10	50	DK
6	G-96	21	Zm-R6	36	Zm-M5	51	Zurr-e-nifa
7	Toria	22	Zn-R8	37	Punjab sarson	52	Star
8	FSB	23	8-LBN	38	ZNR	53	Zn-R3
9	RBJ-8007	24	Nifa raya	39	R-5	54	F-2
10	Zm-R10	25	Zm-M9	40	R-7	55	RBN-4016
11	R-6	26	ZNR-7	41	Lambi sarson	56	G-46
12	Eycal	27	Zm-M6	42	74	57	Anmol
13	Zm-8	28	RBN	43	Zm-R7	58	Kn-258
14	Rainbow	29	023627	44	Layya-15	59	Legand
15	R-1	30	Cyclone	45	Zm-R12	60	Chakwal sarson

Breeding and Genetics, University of Agriculture, Faisalabad, Pakistan. Seedling traits were studied in September, 2013.

***Collection of germplasm*:** Sixty different genotypes (table 1) of *Brassica napus* L. were collected from Department of Plant Breeding and Genetics, University of Agriculture, Faisalabad, Pakistan.

***Screening at seedling stage using PEG-6000 mediated drought stress*:** The contemporary research was conducted in greenhouse of the Department of Plant Breeding and Genetics, University of Agriculture, Faisalabad, Pakistan. Sixty *Brassica napus* L. genotypes / advanced lines were evaluated against drought stress at germination and seedling stage. Five seeds of each genotype/ advanced lines per replication were grown in each Petri dish (9cm) with moist filter paper placed in the bottom of each Petri plate. The experiment comprised of a completely randomized design (CRD) with factorial structured treatments and three replications. Three levels of stress, i.e. zero (control), -0.35 and -0.60 MPa were prepared after dissolving 0, 5 and 10 g of PEG-6000/ 100 ml deionized water, respectively (Hadi *et al*., 2014; Razaji *et al*., 2014). After 14 days of sowing, data of root and shoot fresh and dry weight (g) and their length (cm) was recorded. Shoot and root length were measured by measuring tape and weight was taken with an electrical weighing balance for each entry. Average values of three replications from each treatment were calculated separately for all the traits.

***Statistical analysis*:** The arithmetic means of fresh and dry root weight, fresh and dry shoot weight, root and shoot lengths was calculated by using Minitab Statistical software. Fisher's Least Significant Difference (LSD) test was subjected to statistical analysis of variance as indicated by Steel *et al*. (1997).

RESULTS

Analysis of Variance accessible in Table 2 indicated significant differences (P=0.05-0.001) among all the treatments representing that seedling growth under all treatments was reasonably diverse. Genotypes and their interactions with treatments also revealed highly significant results indicating that genotypes reacted differently to the drought levels.

***Mean performance of seedlings in Control 0% PEG-6000 treatment (T_0)*:** Mean comparisons of seedling traits i.e. shoot length, root length, fresh and dry shoot and root weight are given in Table 3. The longest shoot and root length was observed in Shiralee (code 16 in the table) under normal conditions. Fresh shoot weight, fresh root weight and dry root weight had good performance for the Shiralee genotype. Rainbow (code 14 in the table) genotype showed good performance for shoot and root length; fresh root weight and dry shoot weight; and dry root weight. RBN (code 28 in the table) revealed good performance for shoot and root length;

Table 2. Analysis of variance for the seedling traits.

SOV	DF	SL	RL	FSW	FRW	DSW	DRW
Treatment	2	220.180**	13.073**	0.003**	0.00256**	5.853**	1.097**
Genotype	59	3.778**	1.240**	7.822**	0.00064**	3.157**	1.188**
T × G	118	0.748**	0.267**	5.486**	0.00048**	1.279**	7.227**
Error	358	0.011	0.003	8.623	0.00011	1.235	8.552

*= significance at 0.05 level **= significance at 0.01 level; SL = Shoot Length, RL = Root Length, SFW = Fresh Shoot Weight, FRW = Fresh Root Weight, DSW = Dry Shoot Weight, DRW = Dry Root Weight

fresh shoot and root weight. Dry shoot and root weight values were also high for RBN. Chakwal sarson (code 60 in the table) showed good performance for shoot and root length; fresh root and shoot weight; and dry root weight. Dunkled (code 17 in the table) showed good performance in shoot and root length; fresh shoot and root weight; and dry root weight. Punjab sarson (code 37 in the table) revealed good performance for all traits except fresh shoot weight. Faisal sarson (code 34 in the table) showed good values for all traits except for dry root weight. For Legand (code 59 in the table) showed good results except for dry shoot weight.

Table 3. Mean performance of seedlings under 0% PEG-6000 treatment (T_0).

Sr.	Shoot length (cm)	Root length (cm)	Fresh shoot wt (g)	Fresh root wt (g)	Dry shoot wt (g)	Dry root wt (g)
1	16 6.8667 A	16 2.9300 A	30 0.0323 A	60 0.1403 A	11 0.0052 A	25 0.0032 A
2	14 5.8733 B	28 2.8767 B	34 0.0317 A	59 0.1263 A	4 0.005 B	18 0.0031 A
3	28 5.8500 B	60 2.8767 B	60 0.0317 A	17 0.0247 B	47 0.005 B	10 0.003 AB
4	60 5.7100 C	14 2.8667 BC	16 0.0313 AB	16 0.0227 B	50 0.005 B	13 0.003 B
5	17 5.6300 D	59 2.8567 C	28 0.0313 AB	28 0.0217 B	53 0.005 B	29 0.003 B
6	37 5.6233 D	17 2.8367 D	59 0.0313 AB	57 0.0217 B	42 0.0045 C	16 0.0024 C
7	34 5.6167 DE	12 2.8300 DE	50 0.0303 BC	14 0.0207 B	14 0.0044 D	37 0.0023 CD
8	59 5.5933 E	37 2.8233 DE	7 0.0297 CD	37 0.0207 B	20 0.0043 D	60 0.0023 CD
9	31 5.5567 F	57 2.8167 E	17 0.0297 CD	33 0.0197 B	30 0.0043 D	5 0.0022 CD
10	57 5.5467 F	19 2.7633 F	25 0.0297 CD	12 0.0183 B	31 0.0043 D	8 0.0022 CDE
11	43 5.4333 G	13 2.5733 G	20 0.0293 CDE	29 0.0183 B	37 0.0043 DE	22 0.0022 DEF
12	38 5.1667 H	18 2.5700 G	58 0.0293 CDE	13 0.0177 B	55 0.0043 DE	23 0.0022 DEF
13	50 5.0667 I	29 2.5267 H	11 0.0287 DEF	25 0.0163 B	12 0.0042 DE	26 0.0022 DEF
14	30 4.9867 J	25 2.5233 H	12 0.0287 DEF	18 0.0157 B	17 0.0042 DE	27 0.0022 DEF
15	45 4.9867 J	10 2.5033 I	19 0.0287 DEF	50 0.0157 B	27 0.0042 DE	35 0.0022 DEF
16	9 4.9733 JK	7 2.4367 J	31 0.0287 DEF	52 0.0157 B	43 0.0042 EF	40 0.0022 DEF
17	11 4.9733 JK	11 2.3267 K	57 0.0287 DEF	34 0.0153 B	32 0.0041 EF	50 0.0022 DEF
18	39 4.9533 K	31 2.2533 L	5 0.0283 EFG	1 0.0149 B	34 0.0041 EF	59 0.0022 DEF
19	19 4.8933 L	34 2.2167 M	38 0.0283 EFG	31 0.0147 B	57 0.0041 EF	1 0.0021 EFG
20	52 4.8867 L	9 2.1533 N	52 0.0283 EFG	54 0.0147 B	58 0.0041 EF	4 0.0021 EFG
21	32 4.8467 M	32 2.1233 O	39 0.0277 FGH	5 0.0133 B	1 0.004 EF	11 0.0021 FG
22	47 4.8467 M	33 2.1233 O	45 0.0277 FGH	19 0.0127 B	5 0.004 EF	14 0.0021 FG
23	12 4.8267 M	43 2.1167 OP	56 0.0277 FGH	32 0.0127 B	6 0.004 EF	28 0.0021 FG
24	10 4.7933 N	50 2.1167 OP	40 0.0273 GHI	43 0.0127 B	7 0.004 EF	38 0.0021 FG
25	53 4.7767 NO	5 2.1033 P	54 0.0273 GHI	7 0.0123 B	13 0.004 EF	41 0.0021 FG
26	46 4.7533 O	47 2.0567 Q	2 0.0267 HIJ	21 0.0123 B	18 0.004 EF	3 0.002 FG
27	40 4.7267 P	4 2.0533 Q	1 0.0267 HIJ	23 0.0123 B	19 0.004 F	7 0.002 FG
28	54 4.7267 P	6 2.0467 Q	46 0.0267 HIJ	24 0.0123 B	25 0.004 F	9 0.002 FG
29	1 4.7167 P	23 1.9567 R	47 0.0267 HIJ	26 0.0123 B	28 0.004 F	12 0.002 FG
30	20 4.6733 Q	21 1.9533 R	15 0.0263 IJK	35 0.0123 B	39 0.004 F	15 0.002 FG
31	58 4.6733 Q	52 1.9433 RS	32 0.0263 IJK	47 0.0123 B	45 0.004 F	19 0.002 FG
32	44 4.6467 R	1 1.9367 ST	43 0.0263 IJK	10 0.0117 B	51 0.004 F	21 0.002 FG
33	13 4.5533 S	40 1.9367 ST	53 0.0263 IJK	15 0.0117 B	54 0.004 F	33 0.002 G
34	29 4.5467 ST	54 1.9367 ST	14 0.0257 JKL	22 0.0117 B	56 0.004 F	43 0.002 G
35	25 4.5400 STU	15 1.9333 ST	27 0.0257 JKL	27 0.0117 B	15 0.0039 G	44 0.002 G
36	49 4.5333 TUV	22 1.9267 T	42 0.0257 JKL	41 0.0117 B	16 0.0039 G	45 0.002 G
37	24 4.5267 TUV	24 1.9033 U	49 0.0257 JKL	46 0.0117 B	26 0.0039 G	52 0.002 G
38	7 4.5233 TUV	49 1.8767 V	51 0.0257 JKL	49 0.0117 B	38 0.0039 G	54 0.002 G
39	42 4.5167 VW	26 1.8567 W	55 0.0257 JKL	4 0.0113 B	46 0.0039 G	6 0.0019 H
40	55 4.5100 VWX	27 1.8567 W	4 0.0257 JKL	11 0.0113 B	59 0.0039 G	17 0.0019 H
41	8 4.4933 WX	41 1.8567 W	18 0.0253 KLM	20 0.0113 B	22 0.0038 G	32 0.0019 H
42	18 4.4900 XY	3 1.8533 W	24 0.0253 KLM	38 0.0113 B	60 0.0038 G	42 0.0019 H
43	15 4.4867 XY	35 1.7700 X	26 0.0253 KLM	42 0.0107 B	8 0.0032 H	46 0.0019 H
44	26 4.4667 YZ	46 1.7367 Y	22 0.0247 LMN	53 0.0103 B	24 0.0031 HI	57 0.0019 H
45	35 4.4533 Z	8 1.7267 Y	9 0.0247 LMN	56 0.0103 B	48 0.0031 HI	24 0.0018 H
46	48 4.4467 Za	53 1.6633 Z	29 0.0247 LMN	36 0.0097 B	2 0.003 HI	39 0.0018 H
47	27 4.4233 ab	45 1.6433 a	37 0.0247 LMN	51 0.0097 B	3 0.003 HI	47 0.0018 H
48	2 4.4067 b	38 1.6367 a	48 0.0247 LMN	55 0.0097 B	9 0.003 HI	49 0.0018 H
49	23 4.3467 c	42 1.6167 b	3 0.0243 MNO	58 0.0097 B	10 0.003 HI	20 0.0012 I
50	33 4.3467 c	44 1.5767 c	35 0.0237 NOP	3 0.0087 B	21 0.003 IJ	36 0.0012 I
51	21 4.3333 cd	2 1.5367 d	13 0.0233 OPQ	30 0.0087 B	29 0.003 IJ	51 0.0011 IJ
52	5 4.3300 cd	55 1.5233 de	21 0.0233 OPQ	48 0.0087 B	35 0.003 IJ	53 0.0011 IJ
53	4 4.3233 cd	39 1.5167 e	23 0.0233 OPQ	9 0.0083 B	41 0.003 IJ	2 0.001 JK
54	6 4.3100 d	51 1.4833 f	41 0.0233 OPQ	2 0.0081 B	44 0.003 IJ	31 0.001 JK
55	36 4.2533 e	20 1.4267 g	6 0.0227 PQ	6 0.0077 B	49 0.003 JK	34 0.001 JK
56	41 4.0333 f	58 1.4167 g	44 0.0227 PQ	8 0.0077 B	36 0.0029 K	48 0.001 JK
57	3 4.0133 f	36 1.3767 h	8 0.0223 Q	45 0.0043 B	33 0.0028 K	55 0.001 K
58	56 3.9667 g	30 1.3433 i	10 0.0223 Q	44 0.0037 B	40 0.0028 K	56 0.001 K
59	22 3.8833 h	56 1.3167 j	36 0.0223 Q	40 0.0027 B	23 0.0027 K	58 0.001 K
60	51 3.8667 h	48 1.2633 k	33 0.0117 R	39 0.0013 B	52 0.002 L	30 0.0009 K

Table 4. Mean performance of seedlings under 5% PEG-6000 treatment (T_1).

Sr.	Shoot length (cm)	Root length (cm)	Fresh shoot wt (g)	Fresh root wt (g)	Dry shoot wt (g)	Dry root wt (g)
1	16 5.1267 A	17 2.6633 A	49 0.0333 A	53 0.0267 A	4 0.0052 A	59 0.0025 A
2	28 5.1067 B	14 2.6233 B	28 0.0323 B	14 0.0183 B	28 0.0052 B	28 0.0024 AB
3	14 5.1033 B	37 2.6233 B	56 0.0323 B	28 0.0183 B	60 0.0045 C	34 0.0024 AB
4	60 5.0767 C	16 2.5833 C	53 0.0317 BC	34 0.0160 C	17 0.0044 C	39 0.0024 BC
5	48 5.0633 D	59 2.5833 C	58 0.0313 C	16 0.0153 C	34 0.0044 C	7 0.0023 BCD
6	34 5.0300 E	60 2.5767 C	14 0.0283 D	59 0.0143 D	14 0.0043 CD	9 0.0023 BCD
7	17 5.0233 E	34 2.5500 D	37 0.0283 D	37 0.0137 D	11 0.0042 DE	37 0.0023 CDE
8	37 5.0233 E	57 2.5233 E	59 0.0283 D	17 0.0127 E	13 0.0042 DE	47 0.0023 DEF
9	59 5.0233 E	28 2.4633 F	4 0.0277 DE	48 0.0127 E	47 0.0042 DE	60 0.0023 DEF
10	57 4.9133 F	48 2.4433 G	24 0.0273 EF	57 0.0127 E	56 0.0042 DE	14 0.0022 EF
11	49 4.7833 G	49 2.2633 H	47 0.0273 EF	60 0.0127 E	58 0.0042 DE	16 0.0022 DEF
12	11 4.7367 H	27 2.2233 I	48 0.0273 EF	27 0.0123 E	59 0.0042 EF	46 0.0022 DEF
13	47 4.6433 I	7 2.2133 J	5 0.0267 FG	40 0.0123 E	2 0.0041 EF	2 0.0021 DEFG
14	38 4.6367 I	26 2.1233 K	11 0.0267 FG	26 0.0120 EF	9 0.0041 FG	6 0.0021 DEFG
15	39 4.3133 J	5 2.1133 L	7 0.0263 G	4 0.0113 F	18 0.0041 FG	11 0.0021 DEFG
16	33 4.2633 K	33 2.0633 M	18 0.0263 G	13 0.0113 F	37 0.0041 FG	17 0.0021 DEFG
17	15 4.1567 L	47 2.0433 N	57 0.0263 G	38 0.0113 F	48 0.0041 FG	38 0.0021 EFG
18	26 4.1433 M	38 1.9767 O	60 0.0263 G	39 0.0113 F	57 0.0041 GH	42 0.0021 FG
19	4 4.1033 N	39 1.9767 O	2 0.0253 H	46 0.0113 F	16 0.004 H	49 0.0021 G
20	5 4.1033 N	24 1.9633 P	51 0.0253 H	47 0.0113 F	49 0.004 H	26 0.002 G
21	13 4.0767 O	46 1.9633 P	55 0.0253 H	49 0.0113 F	5 0.0039 I	57 0.002 G
22	9 4.0567 P	19 1.8833 Q	9 0.0243 I	19 0.0103 G	15 0.0038 I	5 0.0018 H
23	41 4.0433 Q	13 1.8767 Q	13 0.0243 I	31 0.0103 G	21 0.0038 I	27 0.0018 H
24	18 4.0233 R	18 1.8767 Q	19 0.0243 I	33 0.0103 G	26 0.0038 I	48 0.0018 H
25	19 4.0133 S	11 1.8633 R	29 0.0243 I	55 0.0103 G	33 0.0033 J	53 0.0014 I
26	7 3.8567 T	4 1.8233 S	38 0.0243 I	58 0.0103 G	42 0.0033 JK	31 0.0013 I
27	29 3.8367 U	58 1.8233 S	39 0.0243 I	22 0.0097 GH	50 0.0033 JK	32 0.0013 IJ
28	46 3.8367 U	15 1.7733 T	26 0.0237 IJ	52 0.0097 GH	54 0.0033 JK	4 0.0012 IJK
29	42 3.8133 V	2 1.7433 U	6 0.0233 JK	12 0.0093 HI	3 0.0032 KL	8 0.0012 IJK
30	45 3.8133 V	9 1.7433 U	44 0.0233 JK	36 0.0093 HI	8 0.0032 LM	15 0.0012 JKL
31	2 3.7767 W	42 1.7233 V	41 0.0227 KL	54 0.0093 HI	12 0.0032 LM	19 0.0012 JKL
32	24 3.7767 W	3 1.7133 W	3 0.0223 LM	3 0.0087 IJ	19 0.0032 LM	20 0.0012 JKL
33	56 3.7367 W	10 1.7133 W	8 0.0223 LM	11 0.0087 IJ	22 0.0032 LM	23 0.0012 JKL
34	40 3.7133 Y	40 1.7133 W	12 0.0223 LM	18 0.0087 IJ	27 0.0032 LM	30 0.0012 JKL
35	44 3.7133 Y	8 1.6767 X	33 0.0223 LM	21 0.0087 IJ	51 0.0032 MN	33 0.0012 JKL
36	58 3.6767 Z	51 1.6567 Y	36 0.0223 LM	25 0.0087 IJ	55 0.0032 MN	35 0.0012 JKL
37	51 3.6367 a	45 1.6433 Z	40 0.0223 LM	29 0.0087 IJ	6 0.0031 MN	36 0.0012 JKL
38	10 3.5767 b	41 1.6233 a	42 0.0223 LM	35 0.0087 IJ	46 0.0031 N	40 0.0012 KLM
39	12 3.5133 c	6 1.5767 b	45 0.0223 LM	50 0.0087 IJ	29 0.003 O	41 0.0012 KLM
40	54 3.5133 c	22 1.5767 b	46 0.0223 LM	51 0.0087 IJ	36 0.003 O	43 0.0012 KLM
41	27 3.4767 d	54 1.5633 c	50 0.0223 LM	56 0.0087 IJ	40 0.003 O	52 0.0012 KLM
42	31 3.4433 e	31 1.5433 d	43 0.0217 MN	5 0.0083 JK	44 0.003 O	55 0.0012 KLM
43	3 3.4367 e	29 1.5367 d	15 0.0213 N	7 0.0083 JK	53 0.0029 P	56 0.0012 KLM
44	53 3.4233 f	55 1.5367 d	27 0.0213 N	9 0.0083 JK	7 0.0028 P	3 0.0011 LMN
45	32 3.3767 g	56 1.4767 e	10 0.0187 O	20 0.0083 JK	10 0.0028 P	12 0.0011 LMN
46	8 3.3233 h	32 1.4633 f	31 0.0187 O	24 0.0083 JK	24 0.0028 P	22 0.0011 LMN
47	55 3.3133 i	35 1.4633 f	32 0.0187 O	32 0.0083 JK	31 0.0028 P	44 0.0011 LMN
48	36 3.2167 j	44 1.4633 f	1 0.0183 OP	43 0.0083 JK	45 0.0028 P	54 0.0011 MN
49	50 3.1567 k	12 1.4433 g	35 0.0183 OP	2 0.0080 JK	25 0.0023 Q	18 0.001 N
50	35 3.1233 l	21 1.4133 h	23 0.0177 PQ	6 0.0077 KL	23 0.0022 R	25 0.001 N
51	6 3.1000 m	25 1.3433 i	30 0.0177 PQ	15 0.0077 KL	32 0.0022 RS	29 0.001 N
52	21 3.0167 n	50 1.3167 j	54 0.0173 QR	41 0.0077 KL	38 0.0022 ST	51 0.001 O
53	22 2.6633 o	36 1.3133 j	21 0.0167 RS	10 0.0076 KL	1 0.0021 ST	1 0.0009 O
54	25 2.5367 p	53 1.1833 k	22 0.0163 S	8 0.0070 L	20 0.0021 TU	10 0.0009 O
55	43 2.4767 q	52 1.1433 l	25 0.0163 S	42 0.0053 M	30 0.002 UV	21 0.0009 O
56	20 2.3433 r	23 1.1233 m	34 0.0160 ST	44 0.0043 N	39 0.002 VW	24 0.0009 O
57	1 2.1567 s	30 1.1133 n	16 0.0153 T	45 0.0043 N	41 0.002 W	58 0.0009 O
58	52 2.1567 s	43 1.0833 o	20 0.0143 U	1 0.0031 O	35 0.0018 X	13 0.0008 O
59	23 2.1133 t	1 1.0433 p	52 0.0133 V	23 0.0023 O	43 0.0018 X	45 0.0008 O
60	30 2.0433 u	20 1.0233 q	17 0.0127 V	30 0.0023 O	52 0.0018 X	50 0.0008 O

1. DGL 2. Long 3. B-56 4. ZMR-2 5. ZM-RN 6. G-96 7. Toria 8. FSB 9. RBJ-8007 10. ZMR-10 11. R-6 12. Eycal 13. ZM-8 14. Rainbow 15. R-1 16. Shiralee 17. Dunkled 18. Zm-R3 19. D-265 20. Zm-21 21. Zm-R6 22. Zn-R8 23. 8-LBN 24. Nifa raya 25. Zm-M9 26. ZNR-7 27. Zm-m6 28. RBN 29. 023627 30. Cyclone 31. ZRM-12 32. FH-23 33. 64-A 34. Faisal sarson 35. FH-10 36. Zm-m5 37. Punjab sarson 38. ZNR 39. R-5 40. R-7 41. Lambi sarson 42. 74 43. Zm-r7 44. Layya-15 45. Zm-R12 46. KN-256 47. Mat-RBN 48. ZN-R1 49. Zm-R4 50. DK 51. Zurre nifa 52. Star 53. Zn-R3 54. F-2 55. RBN-4016 56. G-46 57. Anmol 58. Kn-258 59. Legand 60. Chakwal sarson

Table 5. Mean performance of seedlings under 10% PEG-6000 treatment (T_2).

Sr.	Shoot length (cm)	Root length (cm)	Fresh shoot wt (g)	Fresh root wt (g)	Dry shoot wt (g)	Dry root wt (g)
1	60 4.4533 A	28 2.3333 A	2 0.0363 A	17 0.021 A	14 0.0043 A	14 0.0024 A
2	28 4.4533 A	37 2.1833 B	8 0.0267 B	29 0.019 B	17 0.0043 B	17 0.0024 AB
3	37 4.4467 A	17 2.1767 B	38 0.0267 B	37 0.018 BC	28 0.0043 C	34 0.0024 AB
4	16 4.3733 B	38 2.1767 B	28 0.0257 BC	28 0.017 C	38 0.0043 C	37 0.0024 BC
5	14 4.3467 C	60 2.1767 B	3 0.0257 BC	44 0.017 C	59 0.0043 C	51 0.0024 BCD
6	17 4.2667 D	16 2.1633 BC	18 0.0253 BCD	16 0.016 D	16 0.0042 CD	59 0.0024 BCD
7	38 4.2467 DE	14 2.1467 BC	14 0.0247 CDE	14 0.015 DE	34 0.0042 DE	60 0.0024 CDE
8	59 4.2467 DE	59 2.1233 BCD	60 0.0247 CDE	34 0.015 DE	37 0.0042 DE	28 0.0023 DEF
9	34 4.233 E	34 2.1167 BCD	5 0.0243 CDEF	18 0.014 DE	60 0.0042 DE	13 0.0022 DEF
10	32 3.5267 F	18 2.0833 CD	34 0.0237 DEFG	19 0.014 DEF	18 0.0041 DE	15 0.0022 DEF
11	33 3.4733 G	3 2.0467 D	59 0.0237 DEFG	43 0.014 EFG	58 0.0034 DE	16 0.0022 DEF
12	13 3.0167 H	13 1.9133 E	37 0.0233 EFGH	50 0.014 FGH	5 0.0033 EF	26 0.0022 DEF
13	35 2.9667 I	15 1.8467 EF	50 0.0227 FGHI	15 0.013 GHI	26 0.0033 EF	33 0.0022 DEFG
14	49 2.9567 I	29 1.8433 EF	44 0.0223 GHI	33 0.013 GHI	57 0.0033 FG	39 0.0022 DEFG
15	18 2.9467 I	58 1.8333 EFG	47 0.0223 GHI	42 0.013 GHI	7 0.0032 FG	49 0.0022 DEFG
16	50 2.9233 J	19 1.8167 FG	49 0.0223 GHI	51 0.013 GHI	13 0.0032 FG	50 0.0022 DEFG
17	19 2.7667 K	50 1.7467 GH	57 0.0223 GHI	5 0.012 GHI	33 0.0032 FG	38 0.0021 EFG
18	46 2.5833 L	33 1.7233 HI	19 0.0217 HI	8 0.012 GHI	48 0.0032 GH	40 0.0021 FG
19	26 2.5667 L	7 1.6767 HIJ	33 0.0217 HI	26 0.012 GHI	50 0.0032 H	7 0.002 G
20	9 2.5633 L	26 1.6733 HIJK	7 0.0213 I	32 0.012 HI	51 0.0032 H	4 0.0019 G
21	11 2.5333 M	39 1.6667 HIJK	17 0.0213 I	39 0.012 IJ	4 0.0031 I	29 0.0019 G
22	44 2.5267 M	4 1.6567 HIJK	32 0.0187 J	49 0.012 IJ	8 0.0031 I	11 0.0018 H
23	4 2.5033 N	51 1.6433 IJK	4 0.0183 J	52 0.012 IJ	11 0.0031 I	18 0.0018 H
24	7 2.4833 N	49 1.6233 JKL	26 0.0183 J	56 0.012 IJ	49 0.0031 I	19 0.0018 H
25	29 2.4567 O	9 1.5833 KLM	39 0.0183 J	60 0.012 IJ	6 0.0029 J	48 0.0018 I
26	48 2.4467 OP	6 1.5467 LMN	27 0.0177 JK	3 0.011 IJ	9 0.0028 JK	36 0.0013 I
27	58 2.4467 OP	5 1.5333 LMNO	9 0.0173 JKL	11 0.011 IJ	15 0.0023 JK	45 0.0013 IJ
28	5 2.4267 PQ	11 1.5167 MNOP	35 0.0173 JKL	38 0.011 IJ	24 0.0023 JK	5 0.0012 IJK
29	51 2.4167 Q	46 1.4767 NOP	46 0.0173 JKL	47 0.011 J	35 0.0023 KL	21 0.0012 IJK
30	39 2.3767 R	40 1.4633 NOPQ	54 0.0173 JKL	53 0.011 J	40 0.0023 LM	23 0.0012 JKL
31	47 2.3667 RS	44 1.4633 NOPQ	11 0.0163 KLM	4 0.009 K	44 0.0023 LM	25 0.0012 JKL
32	56 2.3633 RS	45 1.4567 NOPQ	36 0.0163 KLM	21 0.009 K	45 0.0023 LM	27 0.0012 JKL
33	15 2.3467 ST	32 1.4433 OPQ	45 0.0163 KLM	25 0.009 K	52 0.0023 LM	41 0.0012 JKL
34	45 2.3333 T	31 1.4333 PQR	48 0.0163 KLM	27 0.009 K	54 0.0023 LM	46 0.0012 JKL
35	57 2.3333 T	48 1.3767 QRS	51 0.0163 KLM	41 0.009 K	10 0.0022 MN	54 0.0012 JKL
36	6 2.2900 U	54 1.3433 RST	56 0.0157 LMN	55 0.009 K	19 0.0022 MN	58 0.0012 JKL
37	40 2.2833 UV	2 1.3267 ST	6 0.0153 MNO	59 0.009 K	23 0.0022 MN	3 0.0011 JKL
38	36 2.2667 V	12 1.3233 STU	22 0.0153 MNO	2 0.008 K	32 0.0022 N	9 0.0011 KLM
39	31 2.2067 W	47 1.3233 STU	24 0.0153 MNO	7 0.008 K	36 0.0022 O	12 0.0011 KLM
40	10 2.1733 X	56 1.3167 STU	29 0.0153 MNO	9 0.008 K	43 0.0022 O	30 0.0011 KLM
41	42 2.1267 Y	8 1.2833 TUV	53 0.0153 MNO	12 0.008 K	56 0.0022 O	32 0.0011 KLM
42	55 2.1233 Y	35 1.2733 TUVW	58 0.0153 MNO	13 0.008 K	20 0.0021 O	35 0.0011 KLM
43	3 2.0733 Z	57 1.2733 TUVW	31 0.0143 NOP	22 0.008 K	22 0.0021 P	44 0.0011 KLM
44	25 2.0667 Z	36 1.2633 TUVWX	52 0.0143 NOP	24 0.008 K	25 0.0021 P	52 0.0011 KLM
45	52 2.0633 Z	21 1.2533 TUVWXY	55 0.0143 NOP	31 0.008 KL	27 0.0021 P	8 0.001 LMN
46	54 1.9533 a	10 1.2333 UVWXY	40 0.0137 OPQ	35 0.008 KL	31 0.0021 P	10 0.001 LMN
47	24 1.9467 a	53 1.2133 VWXY	16 0.0133 PQR	36 0.008 KL	39 0.0021 P	20 0.001 LMN
48	41 1.9467 a	42 1.1833 WXY	23 0.0127 PQRS	40 0.008 KL	42 0.0021 P	24 0.001 LMN
49	53 1.9167 b	52 1.1833 WXY	43 0.0127 PQRS	45 0.008 KL	46 0.0021 Q	47 0.001 MN
50	8 1.8733 c	25 1.1767 XY	10 0.0123 QRS	48 0.008 KL	53 0.0021 R	57 0.001 N
51	43 1.8267 d	55 1.1667 YZ	21 0.0123 QRS	54 0.008 LM	47 0.002 RS	6 0.0009 N
52	2 1.7667 e	24 1.0833 Za	42 0.0123 QRS	57 0.008 LM	12 0.0019 ST	22 0.0009 N
53	12 1.6733 f	43 1.0667 ab	12 0.0123 QRS	58 0.008 LM	21 0.0019 ST	31 0.0009 O
54	21 1.6267 g	1 1.0633 ab	1 0.0117 RST	6 0.007 M	30 0.0019 TU	42 0.0009 O
55	22 1.6233 g	27 1.0233 abc	15 0.0117 RST	10 0.007 M	41 0.0019 UV	43 0.0009 O
56	1 1.6167 g	41 0.9833 bc	20 0.0117 RST	30 0.004 N	55 0.0019 VW	53 0.0009 O
57	30 1.5467 h	22 0.9667 c	25 0.0117 RST	46 0.004 N	29 0.0018 W	55 0.0009 O
58	27 1.5433 hi	30 0.9633 c	41 0.0117 RST	1 0.003 N	3 0.0013 X	1 0.0008 O
59	23 1.5233 i	20 0.9433 c	30 0.0113 ST	20 0.003 N	1 0.0012 X	2 0.0008 O
60	20 1.3267 j	23 0.5813 d	13 0.0112 T	23 0.003 N	2 0.0011 X	56 0.0008 O

1. DGL 2. Long 3. B-56 4. ZMR-2 5. ZM-RN 6. G-96 7. Toria 8. FSB 9. RBJ-8007 10. ZMR-10 11. R-6 12. Eycal 13. ZM-8 14. Rainbow 15. R-1 16. Shiralee 17. Dunkled 18. Zm-R3 19. D-265 20. Zm-21 21. Zm-R6 22. Zn-R8 23. 8-LBN 24. Nifa raya 25. Zm-M9 26. ZNR-7 27. Zm-m6 28. RBN 29. 023627 30. Cyclone 31. ZRM-12 32. FH-23 33. 64-A 34. Faisal sarson 35. FH-10 36. Zm-m5 37. Punjab sarson 38. ZNR 39. R-5 40. R-7 41 . Lambi sarson 42. 74 43. Zm-r7 44. Layya-15 45. Zm-R12 46. KN-256 47. Mat-RBN 48. ZN-R1 49. Zm-R4 50. DK 51. Zurre nifa 52. Star 53. Zn-R3 54. F-2 55. RBN-4016 56. G-46 57. Anmol 58. Kn-258 59. Legand 60. Chakwal sarson

***Mean performance of seedlings at 5% PEG-6000 treatment (T_1)*:** Mean performance of seedlings at 5% PEG-6000 treatment (T_1) is shown in Table 4. Genotypes showed decrease in performance with increase in drought level. Decline in root length was less in maximum genotypes as compared to all other parameters. The longest shoot length was observed in Shiralee (code 16 in the table). Root length, dry shoot and root weight had good performance in Shiralee. Rainbow (code 14 in the table) showed good values for all the traits. RBN (code 28 in the table) revealed good performance for all the traits. Chakwal sarson (code 60 in the table) showed good performance for all the traits except for fresh shoot weight. Dunkled (code 17 in the table) showed good performance for all traits except for fresh shoot weight. Punjab sarson (code 37 in the table) revealed good performance for all traits except dry shoot weight. Faisal sarson (code 34 in the table) showed good values for all traits except for fresh shoot weight. For Legand (code 59 in the table) showed good results except for dry shoot weight.

***Performance of seedlings traits at 10% PEG-6000 treatment (T_2)*:** Mean performance of seedlings for screening (for selection of parents) at 10% PEG-6000 stress (T_2) is mentioned in Table 5. Genotypes showed decrease in performance in 10% PEG-6000 treatment than 5% PEG-6000 treatment with an increase in drought stress. The shoots were thin and roots were delicate due increased concentration of PEG-6000. Shiralee (code 16 in the table) had good performance except for fresh shoot weight. Rainbow (code 14 in the table) showed good performance for all the traits. RBN (code 28 in the table) revealed good performance for root length and for all the other traits. Chakwal sarson (code 60 in the table) showed good performance for shoot length and all other traits had good performance except for fresh root weight. Dunkled (code 17 in the table) showed good performance in root weight and all other traits had good values except fresh shoot weight. Punjab sarson (code 37 in the table) revealed good performance for all traits except fresh shoot weight. Faisal sarson (code 34 in the table) showed good values for all traits. For Legand (code 59 in the table) showed good results except for fresh root weight.

***Mean comparison of all treatments (T_0, $T_1$and T_2)*:** Pooled mean comparisons of six seedling traits i.e. shoot length, root length; fresh and dry shoot and root weight are revealed in the Table 6. Rainbow, Shiralee, Dunkled, RBN, Faisal sarson, Punjab sarson, Legand and Chakwal sarson expressed statistical closeness to the highest values for all these traits.

***Selection of tolerant genotypes on the basis of their performance*:** On the basis of overall performance, genotypes having values near to maximum for most of the t raits in all the treatments were selected namely Rainbow, Shiralee, Dunkled, RBN, Faisal sarson, Punjab sarson, Legand and Chakwal sarson as drought tolerant genotypes.

DISCUSSION

Early seedling growth is a critical stage for plant establishment (Ahmad *et al*., 2009; Li *et al*., 2011) and plants are prone to drought stress during this stage (Qu *et al*., 2007; Sidari *et al*., 2008). Drought stress depends on type of species how they respond to such type of stress. Good adaptability at early seedling stage maximizes the establishment of the whole plant population (Qu *et al*., 2007). Germplasm collection is a primary requirement to develop best quality and higher yielding genotypes of Brassica (Khan and Khan, 2003). PEG-6000 is used at germination stage for the evaluation of drought resistance and to create different water potential levels. PEG-6000 has high molecular weight so it cannot penetrate the cell wall and is used in germination tests to regulate water potential (Hellal *et al*., 2018). So, PEG-6000 is the most appropriate for an external osmoticum to analyze osmotic preservation in the plants (Roy *et al*., 2009).

In the current study, all the seedling parameters measured were affected by drought stress created by PEG-6000 and there was significant reduction in shoot and root length, fresh and dry weight of the seedling's shoots and roots of *Brassica napus* (Mohammadi and Amiri, 2010; Gheeta *et al*., 2012; Toosi *et al*., 2014; Jajarmi *et al*., 2014). Same results were also reported in *Brassica juncea* (Toosi *et al*., 2014). There are many reports on various crops that are in accordance with this finding (Zraibi *et al*., 2011; Toosi *et al*., 2014; Harfi *et al*., 2015). However, we found that the genotypes showed different performances under all stress levels. Root growth decreased when PEG-6000 concentration was increased. Root length was less affected with the increase in stress levels as compared to the shoot but they were very thin and delicate**.** Dry weight of shoots and roots was reduced in all genotypes by raising the PEG-6000 concentration. Same results were reported by Murillo-Amador *et al*. (2002) in cowpea, Radhouane (2007) in pearl millet and Yagmur and Kaydan (2008) in triticale. If there is less effect on root length by drought stress as compared to other traits, it indicates the presence of tolerance for water stress (Roy *et al*., 2009).

Hence, drought stress affected the seedling growth of all *Brassica napus* genotypes. This study revealed that, for all drought stress levels combined, the genotypes *viz.* Rainbow, Shiralee, Dunkled, RBN, Faisal sarson, Punjab sarson, Legand and Chakwal sarson were the most tolerant, exhibiting maximum performance for shoot and root length, fresh and dry shoot and root weight. The variation in *Brassica napus* genotypes performance determined by the seedling growth parameters indicated that seedling growth is a reliable and efficient phase for the study of *Brassica napus* genotypes response to drought stress. The genotypes having genetic potential to sustain good seedling development under drought stress environment are drought tolerant in this particular stage, and their tolerance should be confirmed at fully developed plant stages.

***Conclusion*:** Polyethylene glycol of high molecular weight is non-penetrating and is safe to simulate water deficit stress in plants. The use of PEG-6000 is simple and easy. So, its use for the screening of large number of genotypes for drought tolerance studies *in-vitro* is preferable. Selection of tolerant genotypes at early seedling stage saves time and the selected genotypes in this research would be helpful in future drought tolerance breeding programs.

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