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Screening of oilseed Brassica varieties for drought tolerance at the early stages of plant growth

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Abstract

Drought tolerance of ten oilseed *Brassica* varieties comprising *Brassica rapa* (4), *B. juncea* (3), and *B. napus* (3) were tested *in vitro* in two separate experiments. In the first experiment seeds of the varieties were germinated on MS medium containing 0.0, 4%, 6%, 8% and 10% PEG-6000. The seedlings were grown up to 15 days. The characters germination percentage, shoot and root length, shoot and root weight both fresh and dry were studied. Highly significant variations among the varieties, drought stress levels and interactions were found for all the characters. The results revealed that impose of osmotic stress caused reduction in germination and adversely affect the seed germination and subsequent seedling growth. On the basis ranking the varieties BINA Sarisha-3, Sampad, BARI Sarisha-7, and Shambal were found to be superior in respect of drought tolerance. In the second experiment hypocotyls of the genotypes were used for callus induction capability under 8% PEG level. In the study it was observed that the varieties BINA Sarisha-3, Sampad, BARI Sarisha-7, and Shambal were more successful in callus induction. From the studies it may be concluded that varieties of *B. napus* are more drought tolerant than these of *B. rapa* and *B. juncea*.

Keywords: Oilseed *Brassica*, Drought tolerance, *In vitro* screening

Introduction

Drought provides one of the major limitations of crop production worldwide. In some parts of the world, particularly the semi-arid tropics and other locations where most of the world's poor people reside, drought is endemic. Even the most productive agricultural regions also experience short period of drought almost every year and occasionally with severe droughts. Moreover many parts of the earth's surface are not arable primarily because of severe water limitation and the amount of land with these problems grows every year. Considering ongoing climatic changes caused principally by global warming the pressure on food production in the water-limited environment should increase in the near future (Curry *et al.*, 1995).

Due to rapid and repeated changes in agro climatic situation of Bangladesh, drought has become more prevalent now-a-days than in the past. Almost every crop in the dry land farming is being affected by drought. The estimated yield reduction varies from 10 to 70 % (BARC, 1990). But unfortunately, the research approach so far taken in this line are quite insufficient and therefore, a comprehensive programme based on agronomic means accompanied with breeding methods leading to the development of drought resistant and short durational varieties should be taken.

Mustard and rapeseed is the top ranking oilseed crop of Bangladesh, covers about 60 % of the total oilseed acreage and contributes 50% of the total production (BBS, 2001). In Bangladesh almost two third of the edible oil consumed annually are imported and the foreign exchange involve in such import cost was about Tk. 800 cores (Rabbani, 1992). So the production of mustard and rapeseed should be increased to fulfill the country's need. The increase of production may be achieved by increasing area under cultivation or by increasing

the production per unit area. In Bangladesh, *Brassica* species are grown in the winter season almost without irrigation. As a result the crop inevitably suffers from drought stress resulting into diminished economic yield and quality of produce. Therefore, for economical production drought tolerant varieties to be developed. To do so the released varieties and the germplasms available should be screened first for their drought tolerance capability. But screening a large number of genotypes for drought tolerance under *in situ* condition is rather difficult as it entails a large amount of resource and space. Simultaneously determination of absolute drought tolerance under *in situ* condition also possesses difficulties because of complex interactions between the plant and different drought environments. Therefore, an attempt was taken to - screen a number of rapeseed and mustard varieties for drought tolerance at the seedling stage, identify the callus induction potentiality of the varieties under osmotic stress condition and identify the variety(s) suitable for future plant breeding program in relation to drought tolerance.

Materials and Methods

Ten oilseed *Brassica* varieties viz. Tori-7, Kallyania, Sampad, and BINA Sarisha-6 of *Brassica rapa*; Rai-5, Shambal and Daulat of *B. juncea* and BARI Sarisha-7, BINA Sarisha-3 and BINA Sarisha-5 of *B. napus* were tested *in vitro* for drought tolerance using MS (Murashige and Skoog, 1962) media containing different concentrations (0, 4, 6, 8 and 10) of polyethylene glycol (PEG-6000) in glass vials. The experiment was carried out at the Tissue Culture Laboratory of the Department of Genetics and Plant Breeding, Bangladesh Agricultural University, Mymensingh, during the period from January to April 2005. After media preparation 25 ml of warm media was poured into glass vials and were closed with plastic caps and marked into specific treatment. Surface sterilization of the seeds were done by using 15 % Chlorox solution along with 1-2 drop(s) of Tween-20 for 8-10 minutes followed by 4-5 thorough washing with sterile distilled water.

After surface sterilization, four sterilized seeds were arranged horizontally in each vial and gently pressed into the surface of the media. The vials containing seeds were placed in dark room with control temperature ($25\pm 1^{\circ}\text{C}$) for about 48-72 hrs. Then the vials were transferred to light condition. The seedlings were counted daily from 3rd day of seed inoculation for determining germination percentage and days required for germination. After 15 days of seed inoculation, all the seedlings of each vial were removed for further observation and data were recorded on shoot & root length (cm), fresh and dry shoot and root weight (mg) etc. For root fresh weight, after removing media from the root using running tap water, were kept for 10 minutes between blotting paper to remove excess water and then weighed. For dry weights, pieces of shoots and roots were kept at 90°C temp for 48 hrs in a desiccator and immediately weighed. The experimental design was randomized complete block design (RCBD) with four replications. The recorded data were statistically analyzed was done with the help of computer package MSTATC program and the mean of the different parameters were compared by the Duncan's Multiple Range Test (DMRT).

Callus induction capabilities of the varieties were performed by using hypocotyls on MS medium supplemented with 2.0 mg/l BAP and 0.1 mg/l NAA and 3 mg/l AgNO_3 . PEG 6000 was added to the induction medium at a fixed concentration of 8 %. Data for three fresh calli per replication were recorded in mg for four replications and were analyzed by proper statistical techniques.

Results and Discussion

The results of analysis of variance for the characters germination percentage, shoot and root length, fresh as well as dry shoot and root weight are presented in Table 1. The mean squares for the varieties, drought stress levels and interaction between the varieties and drought stress levels (A x B) were highly significant ($p < 0.01$) for all the characters suggesting presence of considerable variations among the varieties as well as drought stress levels.

Table 1. Mean squares of 10 *Brassica* varieties for various characters recorded in laboratory condition under control and different drought stress levels

Characters	Sources of variation				
	Factor A (Genotypes) df = 9	Factor B (Drought stress levels) df = 4	A X B (Interaction) df = 36	Error	Co-efficient of variation (CV)
Germination percentage	3178.07***	22667.06***	541.78***	1.12	1.39
Seedling length	23.69***	168.09***	2.54***	0.025	
Fresh shoot weight	4147.83***	35491.06***	383.84***	3.05	2.29
Dry shoot weight	17.00***	94.53***	3.79***	0.09	5.65
Root length	20.42***	166.05***	3.29***	0.15	7.04
Fresh root weight	177.36***	763.53***	20.33***	0.31	5.99
Dry root weight	0.86***	3.43***	0.15***	0.002	

*** Indicates significant at 0.1% level of probability

Germination percentage among the varieties, drought stress levels and their interactions were significant (Table 1). The average germination percentage of the varieties under control and different drought stress levels have been shown in Table 2. The highest germination percentage (95.97%) was found in control and the lowest (36.37%) in 10% PEG level. Among the genotypes, the highest germination percentage (93%) was found in Shambal followed by BINA Sarisha-3, and Kallyania and the lowest (46.50) was in BINA Sarisha-5. From the present study it was observed that germination percentage was decreased with an increase of PEG percentage i.e., with decrease of osmotic potential. Reduction in seed germination percentage with the increase of osmoticum (PEG 6000) has also been reported by Dhanda *et al.* (2002), Singh (2001) and Singh (2000).

Among the varieties highest seedling length was produced by BINA Sarisha-3 followed by Shambal, Sampad and Kallyania; and the lowest was in BINA Sarisha -6. Decease in seedling height with the increase of percent PEG was also reported by Archana *et al.* (2002) in *Brassica juncea* and Dhanda *et al.* (2002) in wheat.

Shoot weight both fresh and dry were significant among the varieties, drought stress levels and interactions. Over the treatments, highest shoot weight both fresh and dry were found in control and lowest at 10% PEG level. Among the varieties, highest fresh shoot weight (102.71 mg) was produced by the variety BINA sarisha-3 followed by Rai-5, Sampad and BARI Sarisha-7 and the lowest by the variety Tori-7. Similarly highest dry shoot weight was produced by the variety BINA Sarisha-3 and the lowest by BINA Sarisha-5. From the present study a reduction in fresh shoot weight was observed for all the varieties with increasing osmotic level. Similar results were also reported by Archana *et al.* (2002) and Ashraf and Mehmood (1990) in *Brassica*.

Table 2. Germination and root shoot characters of 10 Brassica varieties grown under different drought stress levels

Genotypes	BINA Sarisha-6	Tori-7	BARI Sarisha-7	Shambal	BINA Sarisha-5	Daulat	BINA Sarisha-3	Kallyania	Rai-5	Sampad	Mean
Characters	Germination percentage										
Osmotic levels											
0%PEG (control)	97.50	97.50	98.75	98.75	72.50	97.50	98.75	97.50	100.00	98.75	95.75 a
4% PEG	95.00	92.50	90.00	98.75	68.20	92.50	97.50	91.25	92.50	92.50	90.50 b
6% PEG	88.75	90.00	90.00	97.50	55.00	80.00	91.25	91.25	83.75	88.75	85.62c
8% PEG	60.00	80.00	87.50	90.00	42.50	68.75	72.50	81.25	75.00	81.25	73.87 d
10% PEG	0.00	0.00	31.25	80.00	0.00	41.25	70.00	50.00	41.25	50.00	36.37 e
Mean	68.25 h	72.00 g	79.50 d	93.00 a	46.50 i	76.00 f	86.00 b	82.25 c	78.50 e	82.25 c	
	Seedling height (cm)										
0%PEG(control)	7.19	7.72	8.17	9.32	7.42	7.56	9.60	8.59	7.59	7.84	8.10 a
4% PEG	6.76	6.66	7.57	4.47	6.12	7.20	8.89	8.00	7.13	7.24	7.41b
6% PEG	4.79	6.20	6.84	8.14	5.26	6.95	7.54	7.35	7.17	7.05	6.73c
8% PEG	4.09	4.37	5.68	6.21	4.35	6.44	7.01	5.34	5.94	6.55	5.60d
10% PEG	0.00	0.00	1.70	4.19	0.00	4.12	5.60	3.62	4.33	5.09	2.87e
Mean	4.57	4.98	5.99	7.27	4.63	6.45	7.73	6.58	6.44	6.75	
	Fresh shoot weight (mg)										
0%PEG (control)	119.89	107.42	133.20	122.67	83.95	108.54	148.63	106.48	112.35	101.36	112.42 a
4% PEG	96.45	74.93	93.27	92.61	67.51	83.08	113.20	93.70	95.07	95.74	90.55 b
6% PEG	69.69	63.26	86.22	81.21	55.20	76.28	97.52	84.05	93.14	82.33	78.89 c
8% PEG	60.63	45.26	72.64	63.56	52.41	73.81	89.46	55.01	82.00	71.97	66.68 d
10% PEG	0.00	0.00	26.54	51.65	0.00	30.56	64.74	41.33	55.12	52.38	32.23 e
Mean	69.33 h	58.11 i	78.37 e	82.34 c	51.81 j	74.45 g	102.71 a	76.12 f	87.54 b	80.77 d	
	Dry shoot weight (mg)										
0%PEG (control)	7.33	7.84	8.45	7.52	6.45	5.75	8.67	6.75	7.30	7.12	7.32 a
4% PEG	6.25	5.66	7.15	6.17	5.83	4.92	7.71	5.45	6.57	6.89	6.26 b
6% PEG	5.97	5.43	6.66	4.73	5.47	4.87	7.23	5.27	6.14	5.86	5.76 c
8% PEG	4.85	5.00	6.48	4.16	5.05	4.46	6.66	4.55	5.33	5.19	5.17 d
10% PEG	0.00	0.00	5.38	3.83	0.00	3.57	5.96	4.15	4.45	4.42	3.18 e
0%PEG(control)	4.88 e	4.78e	6.82 b	5.28 d	4.56 f	4.72 ef	7.25 a	5.24 d	5.96 c	5.89 c	
	Root length (cm)										
0% PEG(control)	7.43	7.27	9.01	7.46	6.97	7.39	7.38	7.40	6.16	9.33	7.58 a
4% PEG	7.21	6.27	8.05	7.16	4.31	7.14	6.77	6.99	6.15	8.25	6.83 b
6% PEG	6.80	5.60	7.67	6.31	3.83	6.70	5.89	5.79	5.32	6.89	6.08 c
8% PEG	3.75	4.36	6.18	5.05	1.68	4.33	5.08	5.36	4.30	6.10	4.62d
10% PEG	0.00	0.00	1.63	3.59	0.00	2.90	4.28	3.35	4.27	4.38	2.44 e
Mean	5.03 d	4.70 e	6.51 b	5.92 c	3.36 f	5.69 c	5.88 c	5.78 c	5.24 d	6.99 a	
	Fresh root weight (mg)										
0% PEG(control)	20.26	13.08	20.72	10.92	16.16	7.18	21.64	13.09	10.63	16.83	15.04 a
4% PEG	14.06	11.77	13.45	4.63	12.69	4.68	13.73	10.52	9.88	15.27	11.06 b
6% PEG	11.31	10.29	9.86	4.16	9.98	3.82	12.51	8.37	9.51	13.36	9.32 c
8% PEG	8.25	8.97	8.02	3.50	8.68	3.11	10.22	6.26	8.30	10.13	7.55 d
10% PEG	0.00	0.00	2.65	2.99	0.00	1.52	9.33	4.48	3.09	7.95	3.20 e
Mean	10.78 c	8.82 e	10.95 c	5.24 g	9.50 d	4.06 h	13.48 a	8.53 ef	8.28 f	12.71 b	
	Dry root weight (mg)										
0% PEG(control)	1.14	1.14	1.93	0.59	1.06	0.51	1.11	0.64	0.85	1.09	1.01 a
4% PEG	1.03	1.05	1.07	0.48	0.97	0.45	1.01	0.59	0.70	0.97	0.88 b
6% PEG	0.81	0.98	0.89	0.43	0.94	0.43	0.87	0.53	0.64	0.83	0.69 c
8% PEG	0.53	0.93	0.67	0.30	0.83	0.27	0.80	0.37	0.48	0.76	0.59 d
10% PEG	0.00	0.00	0.30	0.24	0.00	0.20	0.56	0.24	0.26	0.48	0.23 e
Mean	0.70 e	0.82 c	0.97 a	0.41 h	0.76 d	0.37 i	0.87 b	0.47 g	0.59 f	0.83 c	

Note: Variety means having same letter are statistically identical and those having different letter are statistically different

Considering all the treatments highest root length (7.58 cm) was observed in control and lowest at 10% PEG level. Among the varieties, highest root length was in Sampad followed by BARI Sarisha-7 and Shambal and the lowest was produced by BINA Sarisha-5. From the present study it was also observed that root length decrease with the increase of PEG percent. Similar result also reported by Archana *et al.* (2002) in *Brassica*.

Over the treatments, highest fresh root weight was observed in control and the lowest in 10% PEG level. Among the varieties highest fresh root weight was produced by the variety BINA Sarisha-3 followed by Sampad, BARI Sarisha-7 and BINA Sarisha-6 and the lowest by Daulat. Similarly highest dry root weight was produced by BINA Sarisha-3 and lowest by Daulat. Reduction in root weight both fresh and dry with an increase of osmoticum was also observed by Ashraf and Mehmood (1990) in *Brassica*.

In order to find out a clear picture of performance of the varieties for drought tolerance they were scored and ranked and presented Table 3. Scoring was done on the basis of average performance of the varieties over the PEG levels. Score 1 was given to the best performing variety and 10 to the worst. Average score and rank position of the varieties have also been determined. Similar ranking procedure was also followed by Islam (1997) in screening rapeseed and mustard varieties for salt tolerance. Considering all the characters, the varieties BINA Sarisha-3, Sampad, BARI Sarisha-7, Shambal ranked the top four positions suggesting that these are more drought tolerant among the varieties tested.

Table 3. Scoring and ranking of ten *Brassica* genotypes against drought stress

Varieties	Characters							Average Score	Rank position
	GP	SL	FSW	DSW	RL	FRW	DRW		
Tori-7	7	9	9	8	10	6	4	7.57	8
kallyania	3	5	6	6	5	7	8	5.71	5
Sampad	3	3	4	4	1	2	3	2.85	2
BINA sarisha-6	8	10	8	7	9	4	6	7.43	7
Rai-5	5	7	2	3	8	8	7	5.71	5
Shambal	1	2	3	5	3	9	9	4.57	4
Daulat	6	6	7	9	6	10	10	7.71	9
BINA sarisha-3	2	1	1	1	4	1	2	1.71	1
BARI sarisha-7	4	8	5	2	2	3	1	3.85	3
BINA sarisha-5	9	4	10	10	7	5	5	7.14	6

GP= germination percentage, SL= seedling length, FSW= Fresh shoot weight, DSW= dry shoot weight, RL= root length, FRW= fresh root weight, DRW= dry root weight.

To estimate the degree of drought tolerance the varieties we were tested in vitro for their callus induction capability under a fixed stress condition. The results of analysis of variance for days to callus induction, percent callus induction and callus weight have been presented in Table 4. According to the table days to callus induction, percent callus induction and callus weight among the varieties were significant (Table 4). The variety Tori-7 produced callus spending minimum time (6.0 days) followed by BINA Sarisha-3 (6.25 days) and BINA Sarisha-5 (7.75 days), while the variety Kallyania took longest period (9.5 days) (Table 5). On percent callus induction the variety BINA sarisha-3 was found to produce highest percentage

(95.0%) followed by BARI sarisha -7 (85.5%) and sampad (81.25%) where the variety Rai-5 produced the least (32.25%). Like percent callus induction the variety BINA sarisha-3 was also producer of the largest callus. The average callus weight of BINA sarisha-3 was 119.7 g which was followed by BARI sarisha-7 (81.93 g) and sampad (67.25g). Whereas the variety Rai-5 produced least weight (13.45g) preceded by kallyania (19.65g) and BINA Sarisha-6 (22.5g). From the first experiment it was observed that the varieties BINA Sarisha-3, Sampad, BARI Sarisha-7 and Shambal showed superiority in drought tolerance, Similarly these varieties were also found more tolerant to drought on the basis of their callus induction capabilities. Considering both the test results of *in vitro* screening the varieties BINA Sarisha-3, Sampad and BARI Sarisha-7 were found to rank first, second and third position, respectively. On the basis of the above results it can be concluded that the genotypes of *Brassica napus* are more drought tolerant than these of *Brassica juncea* and *Brassica rapa*. Similar findings were also reported by Ashraf and Mehmood (1990) while screening *Brassica* gremplasms.

Table 4. Analysis of variance (mean squares) of callus induction under drought stress condition

Sources of variation	Degrees of freedom	Days to callus induction	Percent callus induction	Callus weight
Variety	10	6.77**	1156.24**	4129.48**
Replication	3	0.33	0.42	1.01
Error	27	0.24	1.25	0.52

** indicates highly significant

Table 5. Response of the *Brassica* varieties on callus induction under drought stress condition

Varieties	Days to callus initiation	Percent callus induction	Callus weight
Tori-7	6.00b	80.00c	43.35f
Kallyania	9.50a	62.50g	19.65i
Sampad	8.50ab	81.25c	67.25c
BINA sarisha-6	8.50ab	69.00f	22.50h
Rai-5	9.75a	32.25h	13.95j
Shambal	9.25a	72.00e	55.03d
Daulat	9.25a	76.50d	39.28g
BARI sarisha-7	8.25ab	85.50b	81.93b
BINA sarisha-3	6.25b	95.00a	119.70a
BINA Sarisha-5	7.75ab	79.75c	51.38e

Note: Variety means having same letter are statistically identical and those having different letter are statistically different

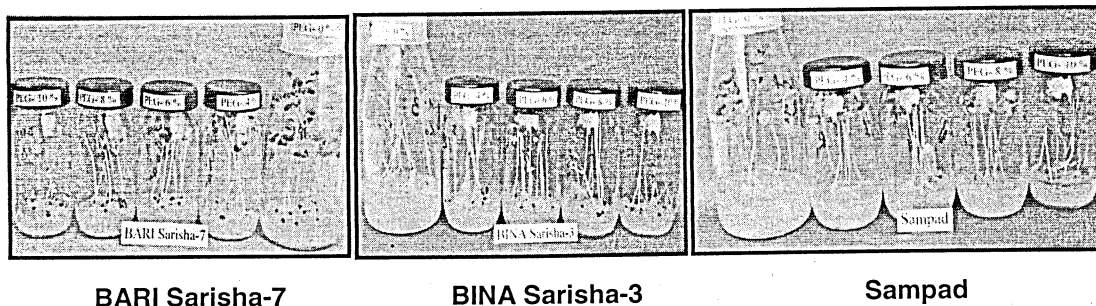


Fig. 1. Plates showing the germination of seedlings of *Brassica* under differential concentration of PEG 6000

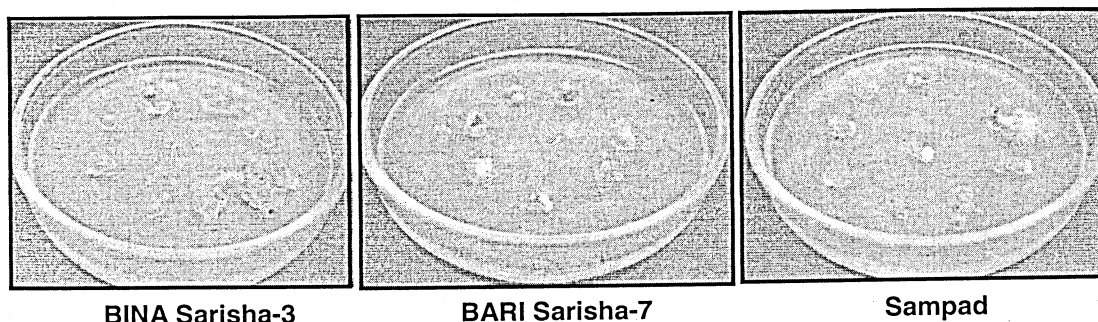


Fig. 2. Plates showing the callus induction of three varieties of *Brassica* on PEG 6000 after 15 days of explanting

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