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MICROSPORE CULTURE AND THE PERFORMANCE OF MICROSPORE DERIVED DOUBLED HAPLOID IN *Brassica juncea* (L.)

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Abstract

The experiment was conducted at the Tissue Culture Laboratory as well as in the net house of Oilseed Research Centre, Bangladesh Agricultural Research Institute (BARI), Joydebpur, Gazipur on microspore culture and the performance of microspore derived doubled haploid (DH) in *Brassica juncea* (L.). The variety BARI Sarisha II of *B. junccea* was used for microspore culture and 8 DH genotypes viz., BJDH 01, BJDH *05*, BJDH ii, BJDH 12, BJDH 14, BJDH 17, BJDH 18, and BJDH 20 along with their parent Rajat collected from the Plant Research International, the Netherlands were used for performance evaluation. Four-day cold treatment at 6 °C along with bud size 3.0-3.1 mm of BARI Sarisha II showed better response in the embryonic development. Significant variation was observed for the different yield contributing characters among the DH genotypes along with their parent Rajat. All the DH genotypes were late both for days to flower and days to maturity compared to the parent. Most of the DH genotypes produced higher seed yield than the parent. The genotypes BJDII 14 (7 g) and BJDH 01(7 g) were bold seeded and almost double or more in 1000-seed weight compared to the parent. Total oil content of the DH genotypes (40.64-42.28) were significantly different from the parent (3 g). Oil content of BJDH 01 was almost 3% higher than the parent could be selected to incorporate in the future breeding program.

Key Words: Microspore culture, doubled haploid, *Brassica juncea*.

Introduction

Rapeseed and mustard are the major sources of edible oil among the oilcrops grown in Bangladesh. Acute shortage of edible oil in Bangladesh has been prevailing during the last several decades. The country produces only one-fourth of our national requirement. This gap is increasing day by day. To address this challenge, it is necessary to develop high yielding variety with short duration to fit into the existing cropping pattern. For a better breeding programme, it should have broad genetic base. The existing genetic base we have is not enough to meet the challenge. To create variability and for their utilization, it is necessary to go for hybridization and selection of desirable types from the succeeding generation to ensure homozygosity of a material during breeding programme. To achieve

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this, it needs at least 4-5 generation of selfing, which is time consuming, the this regard, haploid technique through microspore embryogenesis along with DH technique can play a significant role to reach homozygsity in shorter period of time. Microspore culture has a number of practical uses (Dunwell, 1985; Keller *et al.,* 1987; Kasha *et al.,* 1990). The main advantages are the reduction in time to develop new varieties, rapidly fix traits in the homozygous condition and can increase selection efficiency. Thus, the present research work was undertaken to develop a suitable protocol for microspore embryogenesis in *B. juncea* and to comparative study between amphidiploid Rajat and its microspore derived DH generation for different agronomic traits.

Materials and Method

The experiment was conducted in the Tissue Culture Laboratory and the net house of Oilseed Research Centre, Bangladesh Agricultural Research Institute. The genotypes BARI Sarisha 11 *of B. juncea* was used for microspore embryogenesis, while the DH genotypes collected from Business unit, Plant Research International, The Netherlands under the project International Co-operation for the Developing Countries (FNCO-DC) were used for performance evaluation.

a) Microspore culture: For microspore culture, inflorescences of BARI Sarisha 11 having 1-3 buds flowering were collected from the field and exposed to 1-6 days at 6°C in refrigerator taking in a beaker with water. For microspore isolation 6-8 inflorescences were taken from the beaker. Desired buds (mostly 2.8- 3.2 mm) were picked with a fine tweezer and classified in 3 lengths group by using a measuring gauge. Ten buds per length group were selected. The laminar flow hood was switched on 30 minutes in advance and disinfected with 70% Ethyl alcohol (EtOH). Hands were also sterilized with EtOH. The buds were transferred in to tea-sieves for sterilization 10 mm in 2% NaOC1 making sure there were no air bubbles left. The buds were rinsed in sterile tap water for 1, 4 and 10 minutes. Adding 2 ml of sterile NLN 13 medium in 50 ml beaker, the buds were homogenized with injection piston by a turning pressure movement. Rinsing the piston on the 50 ml beaker, the suspension was poured over the sterile filter funnel with 10 ml centrifuge tubes. The volume of all the tubes adjusted upto 10 ml. The suspensions with microspores were centrifuged 3 mm at 800 rpm in 4°C adjusted. Supernatant was poured off and resuspended the pellet with loml medium. Centrifugation and resuspension were repeated twice. After the last washing,, microspores were resuspended in I ml NLN 13. Taking a sample of 15 µl microspores suspensions were counted by FuchsRosenthal counting chamber. The number (X) of microspores in *5* sub cells multiplied by 16000 were the density of microspore per ml. NLN 13 medium was added in the tube until the right density for cultivation (4x104 microspore/mi). The petridishes

(TC quality) of 3 cm in diameter (for I ml culture) were filled out and tapped with Parafilm. The cultures were placed 35°C for 2 days and then transferred to *25°C* continuously in dark. The embryo development was observed by an inverted microscope regularly. After 14 days globular, embryo development was found. Finally, after 21 days, the embryos were counted.

b) Performance evaluation of DH of *B. juncea:* The seeds of eight DH genotypes viz., BJDH 01, BJDH *05,* BJDH 11, BJDH 12, BJDH 14, BJDH 17, BJDH 18, BJDH 20 and along with the parent Rajat were sown in the pots (8″ × 12″) on November 2004. The pots were arranged in a randomized block design with 3 replications. Ten seeds were sown in each pot. The seedlings emerged three to four days after sowing. After 15 days only, 4 plants were allowed to grow well in each pot. The data days to flower, days to maturity, plant height, primary branches per plant, secondary branches per plant, siliquae per plant, siliqua length, seeds per siiiqua, 1000-seed weight, yield per plant were recorded from selected four plants. Total oil content and fatty acid composition were estimated using by Soxhelt's extraction apparatus and Gas Liquid Chromatograph (GLC), respectively. Analysis of variance and mean separation was done by Duncan's Multiple Range test (DMRT) following the LStat program.

Results and Discussion

a) Microspore culture: Bud size 3.0-3.1 mm have better response in the embryonic development and produced 7 in number per dishes by 4th day (Table 1). Bud size 2.8-2.9 mm did not show any positive response in 2, 4 and 6 days cold treatment, whereas the bud size 3.2-3.3 mm produced one embryo in 4-day treatment (Table 1 and Fig. 1). One-day treatment of all the bud sizes produced the globular embryos. After 2-3 weeks, the further development of globular embryo seemed to be stopped. Ultimately the treatment did not produce any coteledonary embryos. Hirarnatsu *et al.* (1995) reported the 76% globular embryo produced from the anther culture of 'H sueb Hung' variety of *B. juncea.*

Table 1. Effect of bud size and cold treatment in the microspore embryogenesis of BARI Sarisha 11.

Bud size (mm)	No. of embryo development per 3 cm. dish			
	1 day	2-day	4-day	6-day
2.8-2.9 *5*	(microscopic)	0	0	0
3.0-3.1 13	(microscopic)	2	7	2
3.2-3.3 3	(microscopic)	0	1	0

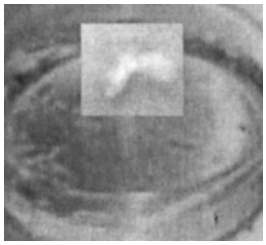

Fig. 1. Embryo development of BARI Sarisha-7

BJDH 01

Rajat

Fig. 2. Flowering difference between the DH genotype BJDH 14 with the parent Rajat

b) Performance evaluation of DH of *B. juncea:* Highly significant difference was observed among the DH genotypes and the parent for days to flower (Table 2). All the DH genotype were found late in flowering compared to their parents. Among the DH genotypes, BJDH 14 took the maximum duration of 46 days, while the genotype BJDH 01 required minimum of 36 days for flowering (Fig. 2). Significant difference was also observed among the DH genotypes and the parent for days to maturity. The DH genotypes required 5-18 days more to mature than the parent. The genotype B.JDII 14 took the maximum duration of 109 days for maturity and was similar with the BJDH 05 and BJDH 18. The parent Rajat matured in minimum of 91 days for harvest. Palmer *et al.* (1996) demonstrated similar result in the DH materials obtained from microspore in broccoli of *Brassica.* High variation for plant height was observed among the DH genotypes and were significantly different from their parents. The highest plant height was observed in BJDH 12 of 200 cm, which was almost double than the parent (104 cm) (Fig. 3). Highest number of primary branches were produced by the genotypes BJDH 12 (8.58) and BJDH 14 (7.67) were significantly different with the parent (5.22). The tallest genotype BJDH 12 produced the highest number of secondary branches (20), which was similar with the genotypes BJDH 20, BJDH 18, BJDH 17, BJDH 14 and BJDH 05.

Table 2. Mean performance of different characters of DH accessions along with parent Rajat

Genotypes	Days to flower (day)	Days to maturity (day)	Plant height (cm)	Primary branches/ plant	Secondary branches/ plant
BJDH 01	36 d	101 c	136 e	*3.75* d	7.25 b
BJDHO5	44b	l07ab	150d	7.08b	17.08a
BJDH 11	37d	103c	144d	4.58cd	9.67h
BJDH 12	*45* ab	106 b	200 a	8.58 a	20.00 a
BJDH 14	46 a	109 a	163 c	7.67 ab	*15.58* a
BJDH 17	41 c	96 d	166 be	6.90 b	15.33 a
BJDH 18	43 b	107 ab	172 b	*6.85* b	16.71 a
BJDH 20	44 b	106b	152d	5.27c	18.67a
Rajat (Parent)	32 e	91 e	104 f	5.22 c	8.83 h
CV (%)	*2.53*	1.13	3.13	10.47	17.35
Level of significance	**	**	**	**	**

** significantly different at P=0.01

The highest number of siliquae per plant produced by the genotype BJDH 12 (610) was significantly different from the parent Rajat along with other DH genotypes (Table 3). The parent produced the least number of siliqaue, which was statistically similar with the genotypes BJDH 01 and BJPH 11. Significant difference was observed for siliqua length among the DH genotypes and the parent Rajat. The DH genotype BJDH 17 produced maximum siliqua length (5.14 cm) which was statistically different from the remaining accessions along with the parent, which produced the minimum length (3.96 cm). Most of the DH genotypes produced similar number of seeds per siliqua with the parent except thc genotypes BJDH 05, BJDH 11 and BJDH 14. The 1000-seed weight of all the DH gcnotypcs were much higher than the parent The highest 1000-seed weight was produced by thc genotype BJDH 14 (7.06g), which was similar with DH genotypes BJDH 01, BJDH 11, BJDH 12, BJDH 17 and BJDH 20 and were significantly different from the parent Rajat (3.39 g). Morden *et al.* (1989) repoted similar results in DH breeding genotypes of barmy. The DH genotype

BJDH 12 produced the highest seed yield per plant of 12.44 g which was significantly different from the parent Rajat (8.38 g).

Table 3. Mean performance of DH accessions and parent for different yield contributing characters.

Genotypes	Siliquae/ plant	Siliqua length	Seeds/ siliqua	1000-seed wt (g)	Plant(g)
BJDH 01	289 d	*4.52* b	11 a	7.03 a	10.83 ab
BJDH 05	410 c	4.07 cd	8 b	5.93 b	11.78 a
BJDH 11	291 d	4.25 b-d	10 b	6.85 a	8.72 c
BJDH 12	610a	4.47bc	12a	6.88 a	12.44 a
BJDH 14	438 c	4.11 b-d	9 b	7.06 a	7.58 d
BJDH17	140e	5.14a	12a	6.46ab	8.49cd
BJDH 18	5,08 b	4.47 be	12 a	5.71 b	9.92 b
BJDH 20	395 c	4.44 be	11 a	6.56 a	7.93 cd
Rajat (Parent)	241 d	3.96 d	12 a	3.39 c	8.38 cd
CV (%)	9.29	4.94	6.79	6.52	7.24
Level of significance	*	**	**	**	**

** significantly different at p=0.01

BJDH 12 Rajat BJDH 17

Fig. 3: Difference in plant height between the DH genotypes BJDH 12 and BJDH 17 with the parent Rajat

Table 4. Oil content and fatty acid composition of DR accessions along with parent Rajat.

Genotypes	Total oil content (%)	Fatty acid content (%)					
		Palmitic $C_{16:1}$	Oleic $C_{18:1}$	Linoleic $C_{18:2}$	Linoleic $C_{18:3}$	Ecosenoic $C_{20:1}$	Erucic $C_{22:1}$
BJDH 01	42.28 a	2.43	10.36	16.65	13.88	4.95	49.41
BJDH 05	41.37 be	2.38	8.21	15.33	13.31	4.77	53.07
BJDH 11	41.28 be	2.40	5.91	15.60	20.16	11.93	43.84
BJDH 12	40.64 c	2.33	7.36	15.70	13.64	4.95	53.33
BJDR 14	41.62 b	2.55	5.83	16.59	20.11	10.93	42.95
BJDH 17	40.98 c	3.12	8.64	20.36	18.59	5.14	43.82
BJDH 18	40.79 c	2.43	7.85	15.32	13.76	4.52	53.57
BJDH 20	41.39 be	2.54	6.47	16.56	17.53	7.04	47.83
Rajat (Parent)	39.49 d	3.69	8.26	22.28	18.07	5.52	41.80
CV (%)	1.4	-	-	-	-	-	-
Level of significance	*	-	-	-	-	-	-

* significantly different at p=0.05

There was a significant variation observed for the character oil content among the DII genotypes along with their parent Rajat (Table 4). Total oil content of the DH genotype BJDH 01(42.28%) was the maximum followed by the genotype BJDH 14 (41.62%) and were statistically different from the remaining genotypes along with the parent, which contained 39.49%. Oil content of the DH genotypes BJDH 12, BJDH 17 and BJDH 18 were similar. A range of variation was observed for fatty acid composition in oil of DH genotypes along with their parent. The variation for palmitic (C16 2.33 - 3.69%) acid content for all the genotypes along with parent was low. On the contrary, the ranges of other fatty acids $C_{18:1}$ (5.83-10.36%), $C_{18:2}$ (15.3222.28%) C18:3 (13.3120.16%), C201 (4.52-11.93%), and C22.1 (41 .89-53.57%) were found high. Though the erucic acid content was high in all the DH genotypes including parent, minimum of 41.89% was found in the parent Rajat, while the maximum in the DH genotype BJDH 18 of 53.57% followed by BJDH 05 of 53.07%. The DH genotype BJDH 01 could be exploited in future breeding programme for its higher oil content in the seed.

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