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IDENTIFICATION OF SELF-INCOMPATIBLE LINE FROM AVAILABLE GERMPLASMS OF *Brassica napus*

F. Mahmud[1] and M. G. Rasul[2]

ABSTRACT

Self-incompatibility mechanism in 22 local germplasm of *Brassica napus* genotypes was investigated through seed set analysis and pollen tube growth behavior. Based on the seed set ratio, levels of self-incompatibility varied from 0.0 to 93.33 percent. The entries Nap 205, Nap 248 and Nap 2037 showed low level of self-compatibility. The genotypes Nap 0130, Nap 2013, Nap 9901, Nap 9904 and Nap 94006 showed intermediate level of self-incompatibility whereas the genotypes Nap 2001 and BARI Sarisha-13 were almost self-incompatible. Based on the number of pollen tubes in the style, the genotypes Nap 108, Nap 179, Nap 206, Nap 2012, Nap 2022, Nap 2057, Nap 2066, Nap 9905 and Nap 9908 were grouped as self-compatible; Nap 0130, Nap 2013, Nap 9901, Nap 9904, Nap 94006, BARI Sharisha-7 and BARI Sharisha-8 as intermediate. Rest of the genotypes Nap 205, Nap 248, Nap 2001, Nap 2037 and BARI Sharisha-13 were classed as self-incompatible. Both the methods provide more or less similar results. Five genotypes, namely Nap 205, Nap 248, Nap 2001, Nap 2037 and BARI Sharisha-13 showed high level of self-incompatibility along with success on bud pollination for producing SI lines.

Key words: Self - incompatibility, germplasm, *Brassica napus*

INTRODUCTION

Self-incompatibility is an out-breeding mechanism and maintains the hybridity of a population in nature. Production of hybrid seed utilizing self-incompatibility is commercially accepted, especially in radish, brussels sprouts, cabbage and kale (Kallo, 1988). In *Brassica*, self incompatability is under sporophytic gene control where the phenotype of the pollen is determined by the diploid genotypes of the pollen producer, the sporophyte. In this phenomenon, pollen rejection occurs when both the pollen and pistill exhibit the same S - phenotype, although they may have different genotypes (Bateman, 1955; Richards and Thurling, 1973). In Brassicaceae, SI is genetically controlled by a single highly polymorphic locus, termed the S locus. In contrast, self compatibility (SC) in *Brassica rapa* is due to a recessive modifier (m gene) or suppressor gene which lies outside the S locus and is epistatic to the S alleles (Nasrullah, 1989).

Self- incompatibility is widely used in the production of F_1 hybrids in *Brassica oleracea* and *Brassica rapa*, and has been considered as a mechanism of pollination control for the development of hybrid and synthetic cultivars of forage (*B. napus* var. *biennis*), swede (*B. napu* ssp. *rapifera*) and oil seed rape (*B. napu* ssp. *oleifera*) (Banks and Beversdorf, 1994; Ripley and Beversdorf, 2003). The major constrains for use of SI in *Brassica napus* breeding is its limited availability in natural *Brassica napus*. Usually if a line is self-incompatible, it could be used as a seed parent without emasculation. Considerable variations in the level of self-incompatibility are observed within the same species and even within a self-incompatible population, and there are gradations in individual plants. To exploit hybrid vigor in crucifers, the identification of self-incompatibility among selected plants and their subsequent progenies is of prime importance for the breeder (Opena *et al.*, 1988). Therefore, it is essential to estimate the extent of self-incompatibility for deciding a suitable breeding approach and genetic studies. In the present study, an attempt was made to assess the level of self-incompatibility in 22 cultivars/genotypes of *Brassica napus*.

[1]Associate Professor, Department of Genetics and Plant Breeding, Sher-e-Bangla Agricultural University, Dhaka, Bangladesh.
[2]Professor, Department of Genetics and Plant Breeding, Bangabandhu Sheikh Mujibur Rahman Agricultural University, Gazipur, Bangladesh.

MATERIALS AND METHODS

The seeds of twenty-two germplasms of *Brassica napus* were collected from Bangladesh Agricultural Research Institute, Gazipur. The genotypes were Nap 108. Nap 0130, Nap 179, Nap 205, Nap 206, Nap 248, Nap 2001,Nap 2012, Nap 2013, Nap 2022, Nap 2037, Nap 2057, Nap 2066, Nap 9901, Nap 9904, Nap 9905, Nap 9906, Nap 9908, Nap 94006, BARI Sarisha 7, BARI Sarisha 8 and BARI Sarisha 13. The research work was conducted at the experimental farm, Department of Genetics and Plant Breeding, Bangabandhu Sheikh Mujibur Rahman Agricultural University (BSMRAU), Salna, Gazipur during the period from November 2006 to March 2007. The seeds of 22 genotypes were sown in November 11, 2006 in the experimental field. Two rows of 4 m each constitute the experimental plot. The row spacing was 30 cm having plant spacing 15 cm within row. Fertilizers were applied at the rate of 270: 170: 100: 150: 5 kg/ha of Urea, TSP, MP, Gypsum and Zinc sulphate, respectively. Cowdung was applied at the rate of 10 M ton/ha. Whole amount of cowdung TSP, MP, Gypsum, Zinc sulphate and half of Urea were applied at the time of final land preparation. The remaining urea was top dressed at 30 days after seedling emergence. Necessary intercultural operation was followed during cropping period for proper growth and development of the plants.

Measurement of self-incompatibility

Two methods were used for measuring self-incompatibility: one them is seed set analysis method and another is pollen germination and pollen tube growth method.

Seed set analysis

Seed set analysis were used to identify the self incompatibility mechanism. Hand pollination method was used in this experiment. To determine the incompatibility of *Brassica napus*, selfing was done to compare the differences of fertility between flowering and bud stages. Two floral stalks from each plant were selected for this test. The stalks in which flowering started at the bottom were chosen. Then, all the bloomed flowers were removed and the buds were bagged using paper. For bud selfing about 20 buds were selfed using pollen from open flowers of the same stalks, which were bagged previously. The remaining small buds at the tip were removed. Fifteen plants of each germplasm selected for this study. Since the determination of self incompatibility is difficult, it is simplified by utilizing the 'seed set ratio' (Zuberi and Zuberi, 1981 and Hawlader and Mian, 1997). The number of seeds obtained from selfing of each 20 germplasm was recorded at harvest and the seed set ratio of the germplasm was determined as follows:

$$\text{Seed set ratio (\%)} = \frac{\text{Seeds from 20 open selfs}}{\text{Seeds from 20 bud selfs}} \times 100$$

Where, Self-incompatible: ratio is 10% or less;
Nearly self-incompatible: ratio is between 10-20 %;
Intermediate: ratio is more than 20% but less than 50%;
Self-compatible: ratio is 50% or above.

Pollen germination and pollen tube growth method

Fluorescence microscopy (Zuberi and Sarker, 1992; Hawlader and Mian, 1997; Bahrain, 1998) was employed to test the self-incompatibility mechanism by observing pollen germination on the stigma and pollen tube growth in the style of each genotype. Three types of pollination viz., bud self, bloom self and cross pollinations were done on these cultivars/genotypes. Ten flowers were pollinated in each treatment. Cross pollination was done by using pollen from alternate male parent. The pollinated flowers were removed from the plants after 24 hours of pollination. Pistils were fixed in acetic alcohol (1:3 v/v) for 24 hours and transferred into 70 per cent ethanol. The pistils were placed in 4N NaOH at room temperature (32-34° C) for about 40-45 minutes for softening the tissues, washed thoroughly and then stained for about one hour with 0.1 per cent aniline blue containing tri-potassium orthophosphate following the method described by Kho and Baer (1968). The samples were smeared on glass slide and observed under fluorescence microscope. The number of germinated pollen grains per stigma and number of pollen

tubes in the styler region were recorded following the procedure of Hawlader and Mian (1997) and Bahrain (1998). Regarding bloom self, pollinations were scored as compatible where pollen germination were abundant and more than 10 pollen tubes were observed as entering the stigmatic papilla after 24 h of pollination; as incompatible where less than four pollen tubes were seen entering the papilla, and as intermediate where 5-10 pollen tubes were observed entering the stigma. For mean number of pollen tubes calculated from bloom self and bud self, pollinations were scored as compatible where more than 16 pollen tubes were observed as entering the stigmatic papilla after 24 h of pollination; as incompatible where less than 8 pollen tubes were seen entering the stigma, and as intermediate where 8-16 pollen tubes were observed entering the stigma.

RESULTS AND DISCUSSION

Seed set analysis

The results of the seed set analysis are shown in Table 1. From the per cent seed set ratio, the genotype Nap 108, Nap 179, Nap 206, Nap 2012, Nap 2022, Nap 2057, Nap 2066, Nap 9905, Nap 9906, Nap 9908, Nap 1057, BARI Sarisa 7, BARI Sarisa 8 were classed as self-compatible while the genotypes Nap 2001 and BARI Sarisa-13 were almost self-incompatible. The genotypes Nap 0130, Nap 2013, Nap 9901, Nap 9904 and Nap 94006 showed intermediate because they had more than 20 per cent but less than 50 per cent seed set ratio. Rest of the genotypes including Nap 205, Nap 248 and Nap 2037 showed low level of self-compatibility.

Table 1. Estimation of self incompatibility in *Brassica napus* genotypes through seed set analysis

Genotypes	No. of seeds by		Percent fertility	Judgement
	Bloom self	Bud self		
Nap 108	154	165	93.33	Compatible
Nap 0130	105	263	39.92	Intermediate
Nap 179	103	183	56.28	Compatible
Nap 205	72	395	18.22	Nearly incompatible
Nap 206	192	240	80.0	Compatible
Nap 248	20	189	10.58	Nearly incompatible
Nap 2001	0	67	0.0	Self incompatible
Nap 2012	62	86	72.09	Compatible
Nap 2013	101	284	35.56	Intermediate
Nap 2022	30	33	69.69	Compatible
Nap 2037	53	366	14.48	Nearly incompatible
Nap 2057	205	384	53.38	Compatible
Nap 2066	70	85	82.35	Compatible
Nap 9901	84	190	44.21	Intermediate
Nap 9904	135	320	42.18	Intermediate
Nap 9905	183	280	65.35	Compatible
Nap 9906	170	195	87.17	Compatible
Nap 9908	76	152	50.0	Compatible
Nap 94006	98	405	24.19	Intermediate
BS 7*	156	186	80.0	Compatible
BS 8*	97	167	58.08	Compatible
BS 13*	5	50	10.0	Self incompatible

Self incompatibility = ratio is 10 % or less, Nearly incompatibility = ratio is 10-20 % , Intermediate = more than 20 % but less than 50% , Self Compatible = Ratio is 50 % or above

The number of seeds per pods was higher in bud self than that of bloom self. The results reported here indicated that out of 22 genotypes, 12 were self-compatible, five intermediate and three nearly self-incompatible and two self-incompatible. In *Brassica napus*, Gemmmel *et al.* (1989) identified self-incompatible line by seed set analysis and pollen tube counts through intra and inter-line pollinations.

They classified the lines as self-incompatible (seed set per flower from 0 to 0.40), fully cross-incompatible (seed set of 20 or more seeds per flower) and cross-compatible (seed set per flower between 10 and 20). Two out of 22 genotypes of the present study was found self-incompatible indicating that the self-incompatibility in the *Brassica napus* genotypes tested here was rather weak. Level of self-incompatibility in the genotypes studied varied from 9.9 to 89.1 per cent in *Brassica campestris* L. This indicated the different level of self - incompatibility at the varietal level. Zuberi and Zuberi (1981) and Zuberi and Sarker (1992) reported strong self-incompatibility in brown seeded local Toria. Poor seed setting might be due to lack of proper pollination and fertilization. Many of the oilseeds and vegetable crops depend largely upon pollinating insects for their seed production (McGregor, 1980). Unavailability of pollinating insects during blooming may result in poor seed yield of cauliflower (Alam *et al.*, 1987). In rape seed, inadequate pollen transfer and open pollination was found by Zuberi and Sarker (1982). Seed yield depends largely on the degree of pollen transfer, compatibility, and fertility of the gametes, successful pollination and fertilization.

Pollen germination and pollen tube growth

The traditional method of detecting self-incompatibility is through seed set analysis. However, seed set analysis takes more than a month from pollination to harvest, inadvertent cross fertilization and seed mixing at harvest are hard to eliminate completely, and environmental factors may further influence the self-incompatibility reaction. Therefore, several workers have developed possible alternatives to seed set analysis. Among those, pollen germination on the stigma and pollen tube growth in the style are important. The results of the pollen germination and pollen tube growth study are shown in Table 2. The data revealed that both the treatment and type of pollination had remarkable effect on pollen germination and pollen tube growth (Plate 1 & 2). Pollen tube screening is usually considered to be better than seed set for studying self-incompatibility in *Brassica* (Hawlader and Mian, 1997).

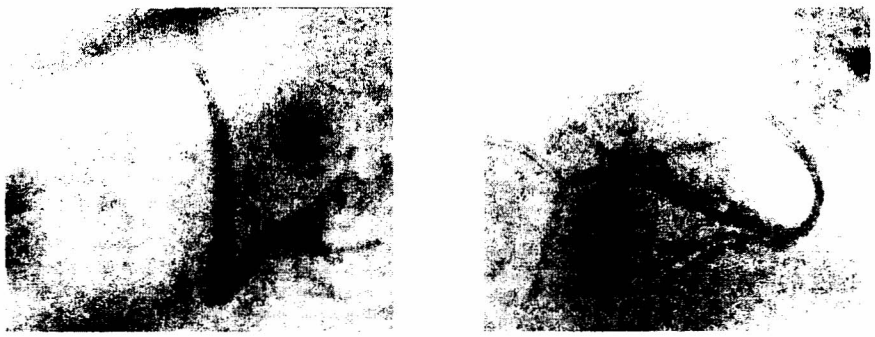
Table 2. Estimation of self-incompatability in *Brassica napus* genotypes by observing pollen tube growth in the style by using fluorescence microscope

Genotype	Number of pollen tubes per style			Compatibility reaction
	Bud self	Bloom self	Mean	
Nap 108	19.7	16.2	17.95	+ +
Nap 0130	19.7	6.2	12.95	+ -
Nap 179	17.1	16.3	16.7	+ +
Nap 205	13.5	2.2	7.85	- -
Nap 206	25	14.4	19.7	+ +
Nap 248	11.4	1.2	6.3	- -
Nap 2001	4.0	2.0	3.0	- -
Nap 2012	18.7	16.0	17.35	+ +
Nap 2013	14.0	5.4	9.7	+ -
Nap 2022	28.8	10.2	19.5	+ +
Nap 2037	9.3	3.7	6.5	- -
Nap 2057	30.0	4.5	17.25	+ +
Nap 2066	24.2	16.8	20.5	+ +
Nap 9901	15.8	2.3	9.05	+-
Nap 9904	14.4	8.0	11.2	+ -
Nap 9905	24.7	10.5	17.6	+ +
Nap 9906	24.5	14.3	19.4	+ +
Nap 9908	23.9	11.3	17.6	+ +
Nap 94006	13.1	9.2	11.15	+ -
BS 7*	17.5	13.3	15.4	+ -
BS 8*	19.5	4.1	11.8	+ -
BS 13*	5.0	1.3	3.15	- -

++: Compatable; +-: Intermediate; --: Incompatable BS= BARI Sarisha

a b

Plate 1. Fluorescent micrographs of pollen-stigma interaction in *Brassica napus*
a) Ungerminated pollen grain on stigma b) Germinated pollen grain on stigma



a b

Plate 2. Fluorescent micrographs of pollen-stigma interaction in *Brassica napus*
a) Fertilized ovules b) Abnormal pollen tube

The highest number of germinited pollen grains per stigma and pollen tubes per style were observed in the genotype Nap 2066 followed by Nap 206, Nap2022, Nap 9906, Nap108 and Nap 2012. On the contrary, the lowest number of germinated pollen grains per stigma and pollen tubes per style were counted in the genotype BARI Sarisha 13. Based on the number of pollen tubes per style the genotypes Nap108, Nap 179, Nap 206, Nap 2012, Nap 2022, Nap 2057, Nap 2066, Nap 9905 and Nap 9908 were grouped as self-compatible; Nap 0130, Nap 2013, Nap 9901, Nap 9904, Nap 94006, BARI Sarisha 7 and BARI Sarisha 8 were classed as intermediate and Nap 248, Nap 205, Nap 2001, Nap 2037 and BARI Sarisha 13 were classed as self-incompatable. Thus, these results were more or less in accordance with those obtained by seed set analysis. Zuberi and Zuberi (1981) investigated self-incompatibility in 24 collections of *Brassica campestris* L. var. *toria*.

It was clear from Table 3 that the type of pollination had a noticeable influence on the germination of pollen grains per stigma and number of pollen tube per style. The highest number of pollen tube per style was observed in Nap 2066 (20.5) due to cross pollination, but was not much different to that recorded in bud selfing. Bud- pollination has been used by many workers for selfing self-incompatible species (Pearson, 1929; Sears, 1937; Attia, 1950). Buds pollinated within one to two days from anthesis gave optimum results on overcoming self-incompatibility in the present material. Open selfing yielded the lowest number of germinated pollen grains per stigma and pollen tubes per style. In the present study, the discrepancy in the number of pollen tubes in the style and number of germinated pollen grains in the stigmas might be explained by the inhibitory action of large number of self-incompatible pollen on the stigma. Scaning electron microscope and flourescence microscope were used to study the

processes underlying pollen self-incompatible in *Brassica oleracea*. Incompatibility was manifested by the inability of the pollen tube to penetrate the stigmatic papila.

Table 3. Interaction effect of genotypes and type of pollination on the number of germinated pollen grains per stigma and number of pollen tubes per style in *Brassica napus* genotypes

Genotypes	Type of Pollination	Number of genminated pollen grains per stigma	Number of Pollen tubes per style
Nap 108	BS	86.5	19.7
	BLS	51.2	6.2
	Cross	115.6	18.0
Nap 0130	BS	48.0	19.7
	BLS	9.3	6.2
	Cross	106.5	18.0
Nap 179	BS	105.4	17.1
	BLS	54.0	16.3
	Cross	125.3	21
Nap 205	BS	75.0	13.5
	BLS	48.3	2.2
	Cross	112	20.3
Nap 206	BS	131.3	25
	BLS	15.4	14.4
	Cross	101.7	18
Nap 248	BS	37.7	11.4
	BLS	2.0	1.2
	Cross	130.5	22.6
Nap 2001	BS	58.7	4.0
	BLS	10.0	2.0
	Cross	95.5	5.0
Nap 2012	BS	89.4	18.7
	BLS	51.3	16.0
	Cross	150.5	29.3
Nap 2013	BS	60.0	14
	BLS	12.2	5.4
	Cross	130.0	13
Nap 2022	BS	144.5	28.8
	BLS	20.0	10.2
	Cross	12.6	5.6
Nap 2037	BS	102.1	9.3
	BLS	17.6	3.4
	Cross	148.2	25.6
Nap 2057	BS	157.5	30.0
	BLS	13.2	4.5
	Cross	33.3	7.6
Nap 2066	BS	69.8	24.2
	BLS	23.5	16.8
	Cross	134.0	23.0

Table 3. Contd.

Genotypes	Type of Pollination	Number of genminated pollen grains per stigma	Number of Pollen tubes per style
	BS	38.7	15.8
Nap 9901	BLS	12.4	2.3
	Cross	113.5	11.5
	BS	195.2	14.4
Nap 9904	BLS	11.0	8.0
	Cross	164.5	12.6
	BS	120.7	24.7
Nap 9905	BLS	30.0	10.5
	Cross	60.3	15.3
	BS	148.2	24.5
Nap 9906	BLS	34.0	14.4
	Cross	46.3	23.3
	BS	148.0	23.9
Nap 9908	BLS	40.0	11.3
	Cross	130	33.0
	BS	127.0	13.1
Nap 94006	BLS	35.0	9.2
	Cross	58.0	15.0
	BS	37.0	17.5
BS 7*	BLS	25.5	13.3
	Cross	150.4	26.6
	BS	76.2	19.5
BS 8*	BLS	8.2	4.1
	Cross	98.2	14.3.0
	BS	36.5	5.0
BS 13*	BLS	9.2	1.3
	Cross	99.6	7.0

BS= Bud self, BLS= Bloom self

Flourescence microscope revealed that penetration was inhibited by callus deposits at the point of contact between the papilla and the pollen tubes (Hadj, 1988). In this present study it was found that high level of self-incompatibility observed in five genotypes namely Nap 205, Nap 248, Nap 2001, Nap 2037 and BARI Sarisha-13 of *Brassica napus* along with success on bud pollination in producing self-incompatible (SI) lines.

REFERENCES

Alam, M. Z., Quadir, M. A. and Ali, M. 1987. Pollinating behaviour of honey bee, *Apis indica* F. and its influence on seed production of cauliflower. *Bangladesh Hort.* 15: 25-30.

Attia, M. S. 1950. The nature of incompatibility in cabbage. *Proc. Am. Soc. Hort. Sci.* 56: 369-371.

Bahrain, A. 1998. Investigation of self-incompatibility in mustard. MS Thesis, Dept. of Genetics and Plant Breeding, IPSA, Salna, Gazipur, pp.42

Banks, P. R. and Beversdorf, W.D. 1994. Self-incompatibility as a pollination control mechanism for spring oil seed rape, *Brassica napus. Euphytica.* 75: 27-30.

Bateman, A. J. 1955. Self-incompatibility systems in angiosperms. III. Cruciferae. *Heredity.* 9: 53-68.

Gemmell, D. J., Bradshow, J. E., Hodgkin, T. and Gowers, S. 1989. Self-incompatibility in *Brassica napus* : Seed set on crossing 19 Self-incompatible lines. *Euphytica.* 42: 71-77.

Hadj-Arab, H. 1988. Interest in reproductive biology for plant improvement-example of pollen self-incompatibility of a crucifer of the genus *Brassica. Annales del. Institute National Agro. EL Harrach, Algeria.* 12(1): 148-157.

Hawlader, M. S. H. and Mian, M. A. K. 1997. Self-incompatibility studies in local cultivars of radish (*Raphanus sativus* L.) grown in Bangladesh. *Euphytica.* 96: 311-315.

Kalloo. 1988. Vegetable Breeding. Vol. 1, CRS Press Inc. Boca Raton, Florida. p. 239.

Kho, Y. O. and Baer, J.1968. Observing pollen tubes by means of fluorescence. *Euphytica.* 17 : 298-302.

McGregor, S. E. 1980. Pollination of crops. In: Bee keeping in the United States USDA, p. 192.

Nasrallah, M.H. 1989. The genetics of self-incompatability reactions in *Brassica* and the effect of suppressor genes. *In*: H. Lord and G. Bemier (eds.). Plant reproduction: From floral induction to pollination. T*he American Soc. plant Physio. Sym. series.* 1: 146-155.

Opena, R. T., Kua, C. G. and Yoon, J. Y. 1988. Breeding and seed production of Chinese cabbage in the tropics and subtropics, Tech. Bull. No. 17, AVRDC, Shanhua, Taiwan, p.92

Pearson, O. H. 1929. Observation on the type of sterility in *Brassica oleracea* var. *capitata. Proc. Am. Soc. Hort.Sci.* 26: 34-38.

Richards, R. A. and Thurling, N. 1973. The genetics of self-incompatibility in ssp. Oleifera. Metzg. IL Genotype and environmental *Brassica campestris* modification of S-locus control. *Genetics. Princeton,* 44(3): 439-53.

Ripley, V. L., and Beversdorf, W. D. 2003. Development of self-incompatible *Brassica napus*: (III) *B. napus* genotype effects on S-allele expression. *Plant Breed.* 122(1): 12-18.

Sears, E. R. 1937. Cytological pheonomenon connected with self-sterility in the flowering plants. *Genetics.* 22: 130-181.

Zuberi, M. I. and Sarker, R. H. 1982. Preliminary studies on the level of cross pollination in self-incompatible *Brassica campestris* L. var *toria. SABRAO J.* 14(1) : 15-19.

Zuberi, M. I. and Sarker, R. H. 1992. Fluorescence microscopic study of pollen tube growth and effective pollination in *Brassica. Bangladesh. J. Bot.* 21(1): 33-38.

Zuberi, M. I. and Zuberi, S. 1981. Preliminary study of Self-incompatibility of 24 collections of *Brassica campestris* L. var. *toria* from Bangladesh. *Bangladesh J. Bot.* 10(2): 187-194.