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Short communication

Identification of self-incompatibility in early cauliflower using fluorescence microscopy

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In cauliflower, sporophytic incompatibility is the genetic mechanism which ensures cross pollination. F_1 hybrid culture has assumed much more importance; of late.The Production of hybrids making use of incompatibility mechanism requires the identification of self incompatible lines. The usual procedure of ascertaining the pollen–stigma compatibility relationships from the seed set data is not only laborious but also a time consuming process. Alternatively, fluorescence microscopy can be advantageously used, as the results are known within eight to ten days (Vidyasagar and Chatterjee, 8). The present study,was, therefore, undertaken to study the efficiency of seed set method and fluorescence microscopy methods to identify self-incompatible lines in early cauliflower.

The study consisted of fifteen genotypes of early cauliflower (*Brassica oleraceae* var. *botrytis* L.) namely IIHR-223, IIHR-214-1-4-6-12, IIHR-263, IIHR-73-5, IIHR-318, IIHR-351, IIHR-266-16, IIHR-250-4-1-11-28, IIHR-352, IIHR-316-17, IIHR-73-3, IIHR-305, IIHR-217-3-14, IIHR-73-24 and IIHR-272 maintained in the Division of vegetable crops, IIHR, Bangalore, India. Fifteen genotypes were planted in the field and the different kinds of pollination were taken up to identify the SI lines. All these 15 genotypes flowered profusely and set seeds under Bangalore conditions. Five plants were selected from each genotype at random during the flowering stage for pollination work. Different pollination treatments used in each plant were as follows; (1) Bud pollination: The stigmas of the flowers were exposed 1-2 days prior to anthesis and selfed with pollen from freshly opened flowers of the same plant; (2) Manual self pollination in freshly opened flowers: The well-developed buds ready to open the next day were covered with butter paper bags and selfed during the next day with pollen from the same flower; and 3. Manual cross pollination in freshly opened flowers: The well developed flower buds ready to open next day were covered with butter paper bags and crossed during the next day with pollen from other genotype.

Twenty flowers per pollination were used in each plant. IIHR- 73-24 and IIHR-217-3-14 were the pollen parents used for manual cross pollination. No. of seeds per siliqua was counted and average of 20 flowers per each pollination was worked out. Fertility index of individual selected lines was worked out as suggested by Watts (10) which is as follows.

$$FI = \frac{\text{No. of seeds/pod under cross pollination}}{\text{No. of seeds/pod under self pollination}}$$

FI <2 is self compatible
FI >2 is self incompatible

For fluorescent microscopic observation, anthesized flowers were pollinated between 9.30 to 10.00 am. Three plants per genotype were used for this study. Both self pollination and cross pollination has been done on early open flowers. Twenty flowers were used for each pollination/plant.

Pollinated pistils were collected 96 hr after pollination for fixation. For staining the pollen tubes in the pistil the procedure suggested by Alexander (1) using quadruple stain was followed. Number of pollen tubes penetrated through stigmatic surface in self as well as cross pollination was counted by fluorescence microscopy and the following score given by Wallace (9) has been followed to decide the self-incompatibility status. The scores were 1 = 1-2 pollen tubes penetration; 2 = 3-5 pollen tubes penetration; 3 = 6-9 pollen tubes penetration; 4 = 10-14 pollen tubes penetration; 5 = 15-20 pollen tubes penetration; 6 = 21-50 pollen tubes penetration; 7 = 51-100 pollen tubes penetration; 8 = > 100 pollen tubes penetration

The plants which showed score three and below were considered as self incompatible, and above three were considered as self-compatible. The data on number of seeds/siliqua is presented in Table 1. Number of seeds per siliqua was highest in self compatible lines and lowest in self incompatible lines by manual selfing of open flowers. This could be due to very high level of 'S' allele action in self incompatible lines to very low level of 'S' allele action in self compatible lines. It is evident from the mean seed set per siliqua in different kinds of pollination that number of seeds set per siliqua was highest in manual cross pollination (6.8) followed by manual selfing of open flower pollination (5.31) and bud pollination (4.10). In order to maintain high fitness, out crossing species like cauliflower can not afford to dispense with heterogeneous nature and thus, they

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must have means to encourage cross pollination. Sporophytic self incompatibility is the regular system for cruciferous plants to perpetuate through cross pollination leading to heterozygosity. So the seed set per siliqua was higher in case of cross pollination than under self pollination in this study also. Similar findings were reported by Watts (10), Singh *et al*. (6), and Sharma *et al*. (7).

The data on the fertility indices calculated using seed set data of manual selfing and crossing of open flowers is presented in Table 2. The mean data recorded through fluorescent microscopy on pollen tube penetration and their corresponding scores through self pollination and cross pollination are given in Table 3. Results from both the methods were compared to find out the most reliable and quickest method to identify self incompatible lines in early cauliflower.

Through seed set method, out of 15 genotypes, seven genotypes were found to be highly self incompatible namely IIHR-73-5 (16.6), IIHR-318 (16.4), IIHR-223 (14.4), IIHR-263 (13.6), IIHR-266-16 (9.7), IIHR-217-1-4-6-12 (4.0) and IIHR-351 (2.99) which recorded highest fertility index *i.e*., more than 2.00 (calculated using manual self pollination and manual cross pollination seed set data). This high fertility index might be due to the fact that self incompatible genotypes possess highly expressive 'S'- alleles leading to very less seed set under self pollination of open flowers, whereas other eight genotypes namely IIHR-250-4-1-11-28, IIHR-73-24, IIHR-305, IIHR-217-3-14, IIHR-272, IIHR-316-17, IIHR-352 and IIHR-73-3 were self compatible owing to their lowest fertility index *i.e*., less than 2.00. This could be due to inexpressiveness or inactiveness of 'S' alleles in self compatible genotypes leading to alleles in self-compatible genotypes leading to very good seed set on selfing of open flowers. These results are in accordance with Gangopadhyay *et al.* (3), and Sharma *et al*. (7).

Through fluorescent microscopic method the genotypes which recorded pollen penetration score of three and less than three on self pollination of open flowers were considered as self compatible (Gangopadhyay *et al*., 3). Through this method, IIHR-73-5 (2.3), IIHR-351 (3.0), IIHR-266-16 (2.0), IIHR-318 (2.3), IIHR-223 (2.0), IIHR-217-1-4-6-12 (2.6) and IIHR-263 (2.0) were found to be self incompatible as the pollen tube penetration score in self pollination of open flowers was three and less than three. However, the pollen tube penetration score in self pollination of open flowers was more than three in IIHR-73-5 (4.6), IIHR-250-4-1-11-28 (6.0), IIHR-305 (7.0), IIHR-73-24 (7.0), IIHR-272 (7.0), IIHR- 217-3-14 (6.3), IIHR-352 (6.6) and IIHR-316-17 (6.6). Thus, the above mentioned genotypes were self compatible where in

Table 1. Number of seeds per siliqua in different modes of pollination in early cauliflower genotypes.

Genotype	No. of seeds per siliqua		
	Bud pollination	Manual selfing of open flowers	Manual cross pollination
IIHR-223	4.46	1.43	8.59
IIHR-214-1-4-6-12	6.39	1.85	7.27
IIHR-263	1.47	1.75	7.89
IIHR-73-5	2.02	0.29	3.98
IIHR-318	2.82	0.66	4.22
IIHR-351	7.06	2.84	7.44
IIHR-266-16	3.36	0.75	4.82
IIHR-250-4-1-11-28	5.26	9.93	8.38
IIHR-352	5.52	7.40	5.23
IIHR-316-17	1.38	7.75	7.06
IIHR-73-3	3.69	7.12	4.84
IIHR-305	3.57	9.40	7.96
IIHR-217-3-14	3.68	8.73	7.02
IIHR-73-24	7.92	11.9	9.70
IIHR-272	2.97	9.11	6.80
Mean	4.10	5.31	6.80
SE±	0.51	1.03	0.46

Table 2. Number of seed set by manual selfing of open flowers (MSOF), manual cross pollination (MCP), fertility index (FI) and self-incompatibility (SI) status in early cauliflower by seed set method.

Line		MCP	MSOF	FI	SI status	Line		MCP	MSOF	FI	SI status	Line		MCP	MSOF	FI	SI status
IIHR-223	P1	8.6	0.6	14.77	SI	IIHR-351	P1	7.6	2.6	2.89	SI	IIHR-73-3	P1	2.6	5.9	0.43	SC
	P2	11.3	1.4	8.03	SI		P2	7.5	3.0	2.50	SI		P2	2.2	7.6	0.29	SC
	P3	8.4	2.2	3.83	SI		P3	6.7	3.3	2.05	SI		P3	6.7	7.8	0.86	SC
	P4	4.7	0.1	42.27	SI		P4	7.9	3.3	2.40	SI		P4	5.9	6.3	0.93	SC
	P5	10.0	2.9	3.51	SI		P5	7.6	1.5	5.14	SI		P5	6.8	8.0	0.84	SC
Mean		8.6	1.4	14.48		Mean		7.4	2.8	2.99		Mean		4.8	7.1	0.67	
IIHR-217-1-4-6-12	P1	4.3	1.1	3.79	SI	IIHR-266-16	P1	4.8	0.5	9.60	SI	IIHR-305	P1	7.0	9.0	0.78	SC
	P2	7.6	1.6	4.88	SI		P2	5.1	0.7	7.72	SI		P2	3.6	4.5	0.80	SC
	P3	7.3	2.4	3.05	SI		P3	4.8	0.2	23.04	SI		P3	8.8	10.1	0.86	SC
	P4	9.2	2.5	3.63	SI		P4	4.3	1.5	2.86	SI		P4	8.5	9.8	0.86	SC
	P5	8.0	1.7	4.82	SI		P5	5.1	0.9	5.74	SI		P5	7.3	10.3	0.70	SC
Mean		7.3	1.9	4.03		Mean		4.8	0.8	9.79		Mean		7.0	8.7	0.80	
IIHR-263	P1	14.4	0.3	55.38	SI	IIHR-250-1-4-11-28	P1	8.6	9.2	0.92	SC	IIHR-217-3-14	P1	8.8	9.9	0.89	SC
	P2	3.4	0.9	3.80	SI		P2	6.4	9.1	0.70	SC		P2	6.5	10.1	0.64	SC
	P3	6.2	2.2	2.79	SI		P3	8.7	9.8	0.88	SC		P3	7.0	9.2	0.76	SC
	P4	8.2	1.9	4.32	SI		P4	8.8	11.0	0.79	SC		P4	8.0	8.8	0.90	SC
	P5	7.2	3.5	2.07	SI		P5	9.5	10.5	0.90	SC		P5	8.5	9.0	0.94	SC
Mean		7.9	1.8	13.67		Mean		8.4	9.9	0.83		Mean		7.8	9.4	0.80	
IIHR-73-5	P1	5.6	0.2	35.00	SI	IIHR-352	P1	6.5	8.0	0.81	SC	IIHR-73-24	P1	10.0	11.2	0.89	SC
	P2	2.0	0.1	18.18	SI		P2	6.4	6.9	0.93	SC		P2	9.8	11.9	0.82	SC
	P3	0.8	0.1	8.30	SI		P3	4.9	7.6	0.64	SC		P3	10.3	12.4	0.83	SC
	P4	6.1	0.7	9.30	SI		P4	6.4	8.9	0.71	SC		P4	8.6	12.6	0.68	SC
	P5	5.4	0.4	12.44	SI		P5	2.0	5.7	0.35	SC		P5	9.9	11.4	0.86	SC
Mean		4.0	0.3	16.64		Mean		5.2	7.4	0.68		Mean		9.7	11.9	0.80	
IIHR-318	P1	1.0	0.1	10.00	SI	IIHR-316-17	P1	6.3	7.3	0.86	SC	IIHR-272	P1	8.9	9.6	0.93	SC
	P2	2.3	0.1	23.30	SI		P2	6.9	7.5	0.92	SC		P2	3.8	8.0	0.47	SC
	P3	6.7	1.2	5.59	SI		P3	6.3	8.9	0.71	SC		P3	6.3	9.0	0.70	SC
	P4	3.9	0.1	39.30	SI		P4	7.1	8.1	0.87	SC		P4	8.0	9.9	0.80	SC
	P5	7.2	1.8	3.90	SI		P5	7.2	7.9	0.90	SC		P5	6.9	9.1	0.75	SC
Mean		4.2	0.7	16.41		Mean		7.1	7.9	0.85		Mean		6.8	9.1	0.70	

Table 3. Mean number of pollen tubes penetrated, pollen penetration score and self-incompatibility status by fluorescence microscopic method in early cauliflower.

Genotype	Manual selfing of open flowers		Manual cross pollination		SI status
	Actual	Score	Actual	Score	
IIHR-223	4.7	2.0	72.6	7.0	SI
IIHR-214-1-4-6-12	6.4	2.6	49.8	6.3	SI
IIHR-263	7.6	2.0	50.5	6.6	SI
IIHR-73-5	7.3	3.0	53.8	6.3	SI
IIHR-318	5.3	2.3	79.4	7.0	SI
IIHR-351	8.4	3.0	84.4	7.0	SI
IIHR-266-16	4.9	2.0	24.1	5.3	SI
IIHR-250-4-1-11-28	26.1	6.0	63.0	6.6	SC
IIHR-352	64.5	6.6	50.8	6.6	SC
IIHR-316-17	49.5	6.6	49.9	6.6	SC
IIHR-73-3	16.3	4.6	68.1	7.0	SC
IIHR-305	73.6	7.0	93.9	7.0	SC
IIHR-217-3-14	47.9	6.3	59.2	6.6	SC
IIHR-73-24	77.7	7.0	76.5	7.0	SC
IIHR-272	89.1	7.0	44.9	6.3	SC

the glycoprotein induced cellulose production might not have occurred as the pollen source compatible with stigma.

The pollen penetration scores of all 15 genotypes were more than three and ranged from 5.3 to 7.0 under manual cross pollination. More over there was good seed set in bud pollination of all these lines as the mean seed set in bud pollination is 4.10 (Table 1). Thus it can be implied that all the 15 genotypes were female fertile and cross compatible in nature. Kanno and Hinata (5) reported that in case of cross pollination, pollen tubes were observed to pierce the cuticle and grow through cellulose pectin layer to the base of the papilla.

The results obtained from the fluorescent microscopic study were in accordance with the results of fertility index through seed set method (Tables 2 & 3). This confirmed the reliability of fluorescence microscopy in identification of level of self-incompatibility and self-compatibility in early cauliflower which will reduce the time required to eight to ten days after pollination through fluorescence microscopic method compared to the seed set method where one has to wait up to 90 days (days taken to seed maturity) after flowering to confirm the level of self incompatibility. The results obtained from the present study are in conformity with the results reported by Beschorner *et al*. (2) and Gangopadhyay *et al*. (3).

Out of 15 genotypes selected to identify self-incompatible lines through seed set and fluorescence microscopic methods, seven genotypes namely, IIHR-73-5, IIHR-318, IIHR-223, IIHR-263, IIHR-266-16, IIHR-217-1-4-6-12 and IIHR-351 were found to be self-incompatible and remaining eight genotypes namely, IIHR-250-4-1-11-28, IIHR-73-24, IIHR-305, IIHR-217-3-14, IIHR-272, IIHR-316-17, IIHR-352 and IIHR-73-3 were found to be self compatible under both the methods of identification.

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