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Standardization of cost effective hybridization technique for hybrid seed production in okra (*Abelmoschus esculentus*)

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ABSTRACT

Due to lack of stable male sterile system in okra [*Abelmoschus esculentus* (L.) Moench], hybrid seed production is still dependant on hand emasculation and pollination which is troublesome and labour intensive. Five hybridization techniques, viz. Hand emasculation and pollination (conventional method); One day premature bud cutting and pollination next day morning; Two days premature bud cutting and pollination next day morning; Mature bud cutting and pollination immediately thereafter and One day premature bud cutting and pollination next morning just after spray of 10% sugar solution were used in the study to standardize the cost effective hybridization technique based on two marker lines, viz. 678-2 (red pigmented with five ridges) and IIVR-10 (green fruited with seven ridges) as male parent and VRO-6 (green fruited with five ridges) as female parent. Percentage of fruit set was maximum in pigment based marker (60.82%) as compared to ridge based marker (57.40%), in general, and hand emasculation and pollination method in colour based marker, in particular, whereas number of seeds/fruit was recorded maximum (44.85) in one day premature bud cutting and pollination next morning. Time requirement for 100 flower bud hybridization (emasculation and pollination) was found maximum in hand emasculation and pollination method (198 minutes) and minimum in the treatment two days premature bud cutting and pollination next morning (69.5 minutes). Maximum time saving was observed in the treatment two days premature bud cutting and pollination next morning (64.88%) over hand ema sculation and pollination method, whereas minimum time saving over hand emasculation and pollination was observed in the treatment one day premature bud cutting and pollination next morning just after 10% sugar solution spray (44.72 %). As far as the economics of the 100 hybridized fruit set is concerned, the two days premature bud cutting and pollination next morning (₹ 20.60) followed by one day premature bud cutting and pollination next morning (₹ 21.82), which were significantly at par, were the most economical methods.

Key words: Economics, Hybridization technique, Marker, Okra and Time saving

Okra [*Abelmoschus esculentus* (L.) Moench] is specially valued for its tender and delicious fruits. It has great potential as foreign exchange earner and accounts for about 60% of the export of fresh vegetables from India to middle east and European countries, excluding potato, onion and garlic. India ranks first in the world with a production of 5784.0 thousand tonnes (72% of the total world production) of okra from over 498.00 thousand ha land (FAOSTAT 2012).

The availability of quality vegetable seed is of utmost importance for increasing the vegetable production and productivity as vegetable seeds constitute the most strategic resource in the quest for world's food and nutritional security. Vegetable growers recognize quality seed of improved varieties as the most strategic resource for higher and better vegetable yields. The seeds of open pollinated varieties have been in the driver's seat, but of late the hybrids have also started getting a feel of the grip resulting in enhancement of demand of hybrid seeds as well as area under them. The hybrid seeds in India are produced mostly by private seed companies. However, there are some constraints in hybrid seed production which deter the seed producers to undertake it, viz. hybrid seed production is a very labour intensive enterprise particularly due to hand emasculation and pollination resulting in high production cost; hybrid seed production can be undertaken only by technically trained manpower for the purpose and high cost of hybrid seeds makes ordinary farmers reluctant to use it as many of them can not afford to purchase such costly seeds.

Due to lack of stable male sterile system (Dutta 1996), hybrid seed production in okra is dependant on hand emasculation and pollination which is troublesome, time consuming and cost ineffective making it difficult to achieve hybrid seed production commensurate to its demand. This necessitates working out suitable options to simplify the hybrid seed production technique of okra making the process faster and cost effective. In view of this, the present investigation was undertaken to (i) utilize the dominant marker for standardizing the crossing technique, (ii) determine the fruit set percentage on phenotypic marker

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based hybridization technique, (iii) determine the time required in hybridization and, (iv) to develop economic cost effective hybridization technique.

MATERIALS AND METHODS

The experiment was conducted at the research farm of Indian Institute of Vegetable Research, Varanasi situated at 82.52°E longitude and 25.10°N latitude, during summer season 2008-09 and 2009-10 in a factorial randomized block design with three replications based on two marker lines, viz. 678-2 (red pigmented with five ridges) and IIVR 10 (green fruited with seven ridges) as male parent and VRO 6 (green fruited with five ridges) as female parent. Five hybridization techniques were used in the study, viz. Hand emasculation and pollination (conventional method); One day premature bud cutting and pollination next day morning; Two days premature bud cutting and pollination next day morning; Mature bud cutting and pollination immediately and One day premature bud cutting and pollination next morning just after 10% sugar solution spray.

Premature bud (one or two days before anthesis) was cut with sharp knife in such a way that the cut was placed just above the stigma, which was standardized based on experiences, to make the pollination convenient. The observations were recorded on time required for 100 flower bud emasculation (minutes), time required for 100 flower bud pollination (minutes), per cent time saving over conventional methods of hand emasculation and pollination, fruit set percentage, number of seeds/fruit, seed weight/ fruit (g), seed germination percentage in seeds from F_0 fruits, pigmented plant /ridged fruits percentage in F_1's and economics of hybridized 100 fruits set. Germination potential of seeds was calculated on the basis of normal seedlings using rolled germination towel method at 25°C in a seed germinator (ISTA 1999). Statistical analysis of data was carried out by the method of analysis of variance (ANOVA) as described by Cochran and Cox (1957).

RESULTS AND DISCUSSION

The data on relationship between the marker and crossing technique used are presented in Tables 1 to 4. Plant height was found non-significant due to marker, crossing techniques and their interaction effect. Percentage of fruit set was found more in pigment based marker (60.82%) as compared to ridge based marker (57.40%), in general, and hand emasculation and pollination method in colour based marker, in particular (Table 1). This may be due to disproportionate locules in ridge based marker, whereas in colour based marker, the number of locules is same in male as well as female parent.

Minimum fruit setting was observed in hybridization method of two days premature bud cutting and pollination next morning (51.8%). It may be due to the immature stigma at the same time of pollination. It clearly indicated that conventional methods, i.e. hand emasculation and pollination has maintained its supremacy for percentage of fruit setting if considered alone and minimum fruit setting in two days premature bud cutting and pollination next morning.

The success of crossing technique depends on the expression of the dominant marker in the F_1 population. Results clearly indicate that the hand emasculation and pollination methods have more true to type F_1 (75.7%). One day premature bud cutting and pollination next morning alone and in combination with spray of 10% sugar solution gave 60.5% and 65.9% true to the type, respectively. It may probably be due to the protogyny nature of the crop. In protogyny, the stigma matures one day before stamens which gives more chance of selfing before opening of the

Table 1 Impact of pollination techniques and markers on plant height, fruit colour and fruit set in okra

Pollination technique (PT)	Markers								
	Plant height in F_1 (cm)			Colour/ridge in F_1			Fruits set (%)		
	Colour	Ridge	Mean	Colour	Ridge	Mean	Colour	Ridge	Mean
Hand emasculation and pollination (conventional method)	66.93	65.82	66.37	76.68	74.73	75.71	65.75	62.97	64.36
One day premature bud cutting and pollination next day morning	66.77	65.40	66.08	61.83	59.26	60.55	64.98	30.37	62.68
Two days premature bud cutting and pollination next day morning	63.30	66.92	65.11	51.48	52.37	51.92	51.70	51.90	51.80
Mature bud cutting and pollination immediately	65.63	66.67	66.15	45.14	49.75	47.45	59.19	56.02	57.61
One day premature bud cutting and pollination next morning just after 10% sugar solution spray	68.53	66.73	67.63	67.94	64.05	65.99	62.45	55.74	59.10
Mean	66.23	66.31	66.27	60.61	60.03	60.32	60.82	57.40	59.11
P<0.05 (Marker)		NS			1.60			0.84	
P<0.05 (Pollination techniques)		NS			2.53			1.32	
P<0.05 (Marker × Pollination techniques)		NS			3.58			1.87	

flower in okra. Pink colour and seven ridges were found dominant over green colour and five ridges surface of fruits which was expressed in F_1 plants. Both fruit pigmentation and fruit surface (ridges) were controlled by independently inherited single dominant genes. Kolhe and D'cruz (1966), Kalia and Padda (1962) and Shoran and Shridhar (1993) also reported a single dominant gene responsible for fruit pigmentation in okra.

Number of seeds/fruit was more in one day premature bud cutting and pollination next morning (44.85) closely followed by one day premature bud cutting and pollination next morning just after 10% sugar solution spray (44.35). Number of seeds/fruit was less in the treatment hand emasculation and pollination as well as two days premature bud cutting and pollination next morning (32.89) may be due to more injury to stigma during emasculation. One day premature bud cutting and pollination next morning just after 10% sugar solution spray, produced more seed weight/ fruit (2.76g) in both the ridge and colour marker based treatment (Table 2). It may be due to the proper pollination and seed development by spraying of 10% sugar solution just before the pollination. The earlier fruit set not only saves time but also adds to quality of seeds. Varis and George (1981) reported that the time available for assimilation of storage reserves for fruits that set at a later stage is less and hence the seeds from the fruits that set earlier were heavier and vigorous. Obeso (1993) however, reported that with-in fruit variation for seed quality was also evident due to changes in resolution of plant-seed conflict for nutrient availability.

The impact on seed germination percentage due to markers was found non-significant whereas it was significant

Table 2 Impact of pollination techniques and markers on seed quality in okra

Pollination technique (PT)	Markers								
	No. of seeds/fruit			Seed wt./fruit (g)			Seed germination (%)		
	Colour	Ridge	Mean	Colour	Ridge	Mean	Colour	Ridge	Mean
Hand emasculation and pollination (conventional method)	36.50	42.47	39.48	2.27	2.56	2.42	76.00	78.33	76.67
One day premature bud cutting and pollination next day morning	44.30	45.40	44.85	2.53	20.3	2.28	76.00	76.00	76.00
Two days premature bud cutting and pollination next day morning	31.68	34.10	32.89	2.15	2.29	2.22	72.00	76.00	74.00
Mature bud cutting and pollination immediately	39.13	40.13	39.63	2.33	2.41	2.37	72.00	71.67	71.83
One day premature bud cutting and pollination next morning just after 10% sugar solution spray	44.20	44.50	44.35	2.72	2.80	2.76	77.33	81.33	79.33
Mean	39.16	41.32	40.24	2.40	2.42	2.41	74.67	76.67	75.57
P<0.05 (Marker)		0.46			0.06			NS	
P<0.05 (Pollination techniques)		0.72			0.10			5.81	
P<0.05 (Marker × Pollination techniques)		1.02			0.14			8.22	

Table 3 Impact of pollination techniques and markers on time requirement in hybridization of okra.

Pollination technique (PT)	Markers								
	Time required for 100 flower bud emasculation (minutes)			Time required for 100 flower bud pollination (minutes)			Time required for 100 bud hybridization (minutes)		
	Colour	Ridge	Mean	Colour	Ridge	Mean	Colour	Ridge	Mean
Hand emasculation and pollination (conventional method)	150	152	151.0	45	49	47	195	201	198.0
One day premature bud cutting and pollination next day morning	42.0	41.0	41.50	40	36	38	82	77	79.5
Two days premature bud cutting and pollination next day morning	40.0	36.0	38.00	30	33	31.5	70	69	69.5
Mature bud cutting and pollination immediately	44.0	42.0	43.0	38	41	39.5	82	83	82.5
One day premature bud cutting and pollination next morning just after 10% sugar solution spray	40.0	42.0	41.0	65	72	68.5	105	114	109.5
Mean	63.20	42.6	62.90	56.6	46.2	44.9	106.8	108.8	107.8
P<0.05 (Marker)		1.17			1.06			1.41	
P<0.05 (Pollination techniques)		1.85			1.68			2.22	
P<0.05 (Marker × Pollination techniques)		2.61			2.38			3.14	

Table 4 Impact of pollination techniques and markers on economics of hybridization in okra

Pollination technique (PT)	Markers					
	Time consumption compared to conventional method (%)			Economics of hybridized 100 fruit set (₹)		
	Colour	Ridge	Mean	Colour	Ridge	Mean
Hand emasculation and pollination (conventional method)	100.00	100.00	100.00	52.35	55.08	53.71
One day premature bud cutting and pollination next day morning	42.05	38.30	40.18	22.14	21.50	21.82
Two days premature bud cutting and pollination next day morning	35.90	34.33	35.12	20.76	20.44	20.59
Mature bud cutting and pollination immediately	42.05	41.29	41.67	23.09	23.90	23.49
One day premature bud cutting and pollination next morning just after 10% sugar solution spray	53.85	56.71	55.28	28.80	32.89	30.86
Mean	54.77	54.13	54.45	29.43	31.03	30.10
P<0.05 (Marker)		0.57			0.82	
P<0.05 (Pollination techniques)		2.49			1.30	
P<0.05 (Marker × Pollination techniques)		3.51			1.83	

in pollination techniques and its interaction. Slightly higher germination was observed in the pollination technique one day premature bud cutting and pollination next morning just after 10% sugar solution spray (79.30%) followed by hand emasculation and pollination technique (76.60%), one day premature bud cutting pollination next morning (76.00%) , while minimum germination was observed in the treatment mature bud cutting and pollination immediately (71.80%).

Data in Table 3 indicates that time required for 100 flower bud hybridization (emasculation and pollination) indicated that the time required was maximum in hand emasculation and pollination method (152 minutes), while the minimum period was recorded in two days premature bud cutting and pollination next morning (38 minutes). During the pollination, maximum time was required in the treatment one day premature bud cutting and pollination next morning just after 10% sugar solution spray (68.5 minutes) and minimum time required in the treatment two days premature bud cutting and pollination next morning (31.5 minutes). In this way, total time required for hybridization was found maximum by hand emasculation and pollination method (198 minutes), whereas minimum time was required in the treatment two days premature bud cutting and pollination next day morning (69.5 minutes) which was statistically superior among the treatments.

Maximum time saving was observed in the treatment two days premature bud cutting and pollination next morning (64.88%) over hand emasculation and pollination method, whereas minimum time saving over hand emasculation and pollination was observed in the treatment one day premature bud cutting and pollination next morning just after 10% sugar solution spray (44.72 %). This may be due to the difference in processes involved in different hybridization techniques tried in the investigation. The minimum per cent of time consumed in hybridization was observed in the treatment two days premature bud cutting and pollination next morning (35.12 %) and one day premature bud cutting and pollination next morning (40.18 %) in comparison to hand emasculation and pollination method.

Taking the economics of the 100 hybridized fruit set, the cost was maximum with hand emasculation and pollination (₹ 53.71), whereas two days premature bud cutting and pollination next morning (₹ 20.59) followed by one day premature bud cutting and pollination next morning (₹ 21.82), which were significantly at par between themselves, were the most economical methods (Table 4).

Considering the fruit set, time required and economics of the 100 hybridized fruit set, the one day premature bud cutting and pollination next morning followed by two days premature bud cutting and pollination next morning were found economical and more than 250% cheaper than conventional method.

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