Himachal Journal of Agricultural Research Vol. 33 (1): 51-54, 2007

Studies on natural out-crossing for refixing isolation distance to maintain genetic purity in okra [*Abelmoschus esculentus* (L.) Moench.] varieties

K.S. Chandel, Sanjeev Kumar, N.K. Pathania, R. K. Kataria and A.S.Gautam
Seed Production Unit
CSK Himachal Pradesh Krishi Vishvavidyalaya, Palampur–176 062.

Abstract

The studies on natural out-crossing (NOC) for refixing isolation distance to maintain genetic purity in okra [*Abelmoschus esculentus* (L.) Moench.] varieties under mid hill conditions of Himachal Pradesh viz. P-8, Pusa Makhmali, Perkin's Long Green and Punjab Padmini under different field layouts involving alternate row method and concentric ring method to refix the isolation distance requirements for okra seed production. Purple pigmentation character of varieties, P-8 and Perkin's Long Green, on both sides of petal base was used as dominant morphological marker. The varieties Pusa Makhmali and Punjab Padmini with purple colour on inside and yellow on outside of petal base served as recessive female parents. Plants of varieties Punjab Padmini were raised in alternate rows at distances with Perkin's Long Green, whereas variety Pusa Makhmali was planted at different distance of 60 cm in six circles surrounding the contaminant source 'P-8' variety in concentric ring method. Progenies of Punjab Padmini and Pusa Makhmali were observed for the occurrence of purple-pigmented plants on both sides of petal base. The extent of NOC was 5.47 per cent in alternate row method of planting, while in the concentric ring method it was 2.70 per cent. The average rate of NOC at the distance of 60, 120,180 and 360 cm was 4.16, 3.67, 1.69 and 2.12 per cent, respectively. The extent of NOC decreased with the distance; however, decline was not symmetrical *vis-à-vis* with distance of planting from the contaminant source having dominant morphological marker in concentric ring method. *Apis cerana indica, Apis mellifera, Bombus trifasciatus, Xylocopa fenestrata* were observed the most frequent pollinators in okra. Depending upon the extent of NOC which ranged from 2.70 to 5.47 per cent at Palampur, an isolation distance of 100-150m may be adopted for the multiplication of seeds of open-pollinated varieties in okra under mid hill conditions of Himachal Pradesh for maintaining genetic purity and unwanted adulteration of varieties will not cause problems to the breeders. Also the consumer's non-acceptance of variable and ungraded okra fruits implies that breeding for uniform or pure varieties is appropriate in okra. Consequently a pure line variety in okra is proposed for growing in areas where edible fruit production is envisaged in mid hills of Himachal Pradesh.

Key words: Okra [*Abelmoschus esculentus* (L.) Moench.], natural out-crossing, isolation distance and genetic purity.

Introduction

Okra [*Abelmoschus esculentus* (L.) Moench.] is the most important fruit vegetable crop of tropical and sub-tropical regions of the world. In India it is grown for its fleshy edible, immature fruits on about 3,25,500 ha with annual production of 33,80,300 metric tonnes (Anonymous 2002). In Himachal Pradesh it is grown during summer and rainy season in foot and mid hills on about 1,212 hectares with annual production of 20,157 metric tonnes (Anonymous 2004). Okra is a multipurpose crop valued for its tender and delicious pods, which are used in a variety of ways, cooked, boiled, fried, stewed in meat frozen, canned and dehydrated products (Fageria, 2003). Absence of obligate autogamy in okra continues to impede progress in both the maintenance of genetic stocks and the breeding of new varieties. The extent of natural out-crossing in okra has been reported to vary from 0-60 per cent depending upon genotype, cropping season, population of pollinators and location (Chandra and Bhatnagar, 1975, Martin, 1983 and Tanda, 1985). The contamination of genotypes during evaluation, seed multiplication or farming will result in progressive adulteration (loss of varietal distinctions). Maintaining varietal purity entails a combination of adequate field size, isolation distances and border rows that will give a product with tolerable level of contamination. The major constraint in the production of high quality seed is

the lack of standardization of seed production practices resulting in poor quality seeds.

The isolation distances have not been standardized in particular set of environmental conditions which otherwise is a major thrust in maintaining genetic purity and still we follow the borrowed standards. For seed production in okra an isolation distance distance with 400 meter for foundation seed and 200 meters for certified seeds is recommended (Anonymous. 1971). Seed production of okra in mid-hills of Himachal Pradesh offers good opportunities in view of the fact that white fly (*Bemisia tabaci*), the insect vector is not in abundance and that, it can easily be controlled, thus making the crop free from yellow vein mosaic virus. Therefore, a comprehensive information on the extent of natural out- crossing, varietal adulteration and for the breeding of pure line varieties under a given agro-climatic regions is indispensable for the varietal improvement and maintenance of genetic purity during the seed production programme in okra. Keeping in view, the above mentioned facts a field experiment was conducted with the objectives of elucidating information on percentage out- crossing, refixation of isolation distance and breeding of pure line varieties of okra under mid-hill conditions of Himachal Pradesh was envisaged.

Material and Methods

The study was conducted at the experimental farms of the Department of Vegetable Science and Floriculture, CSK Himachal Pradesh Krishi Vishvavidyalaya, Palampur during *Kharif* season (1999-2000 and 2000-2001). Two varieties viz., P-8 and Perkin's Long Green having purple colour on both sides of petal base were used as morphological dominant markers and as contaminating source. The varieties Pusa Makhmali and Punjab Padmini with purple colour on inside and yellow on outside of petal base served as recessive female parents or as seed parent. The distinguishing morphological dominant markers of the varieties used for estimating the extent of natural out-crossing has been shown in Plate 1 and 2.

Lay-out for Cross Pollination

Trial – I (Alternate Rows Method): Perkin's Long Green variety was planted in alternate rows with Punjab Padmini at a distance of 60x20 cm. The layout plan for the alternate rows method has been shown in Figure 1.

Trial – II (Concentric Ring Method): The central circular plot of the variety P-8 was surrounded by the circles of plants of Pusa Makhmali at a distance of 60 cm. Further, this central plot was surrounded by six circles with plants of Pusa Makhmali, each circle is being placed at a distance of 60 cm (Figure 1.1). Both the trials were laid out in Randomized Block Design with three replications

Planting and Harvesting Time: The seed were sown on June 15, 2000 and dried pod were

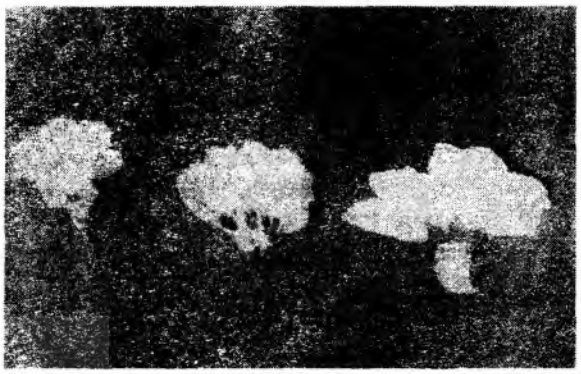


Plate 1. Dominant morphological marker (P – 8, Perkin's Long Green)

harvested between September 15 and October 7, 2000.

Determination of Extent of Natural Out- Crossing: In Trial I, seeds were harvested from the recessive parent, Punjab Padmini and a representative sample of 150 seeds from each replication was grown in the field during June 21, 2001. In Trial II, seeds were harvested from all the plants in first, second, third and upto sixth circles separately and a representative sample of 150 seeds from six circles of each replication was sown to determine the extent of natural out-crossing, the plants with dominant markers characteristics were distinguished during the flowering period and the amount of cross pollination was calculated on per cent basis after counting such plants. Depending

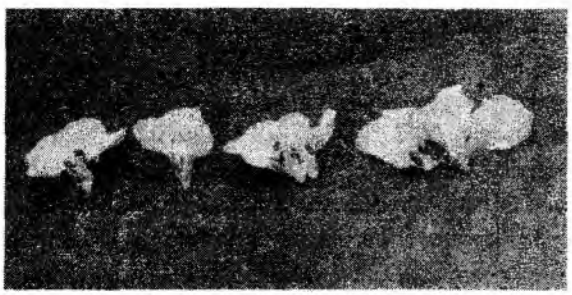


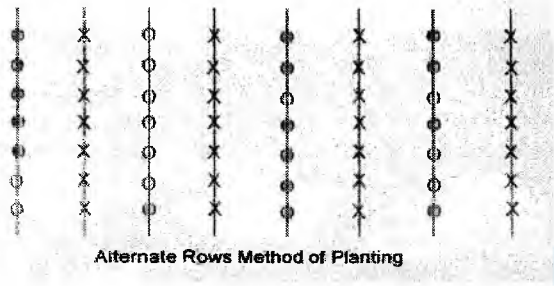
Plate 2. Recessive morphological marker (Pusa Makhmali, Punjab Padmini)

upon the extent of the natural out- crossing and varietal adulteration, the isolation distances were estimated.

Kalloo (1994) indicated that isolation distances for quality seed production or maintaining different genetic stocks may be followed on the basis of natural out-crossing as under:

0-10% = 100 – 150 m; 11-25% = 200 – 300 m;
25-50% = 300 – 500 m; 50 – 75% = 500 – 1000 m
75 – 100% = more than 1000 m.

Data were computed as per Randomised Block Design described by Panse and Sukhatme(1985).



X = Perkin's Long Green and P – 8
O = Punjab Padmini and Pusa Makhmali

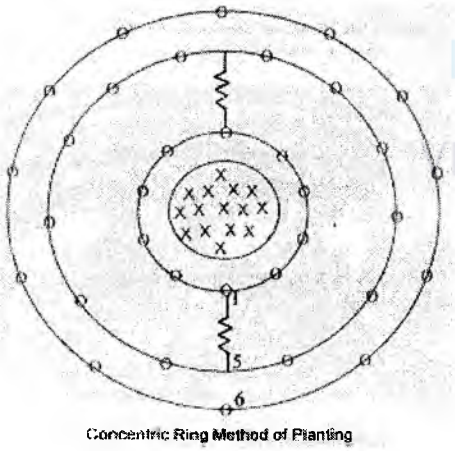


Fig 1. Field layout of different methods of planting for estimating natural out-crossing

Results and Discussion

Plants of okra variety P-8 and Perkin's Long Green is characteristics of having purple pigmentation on the both sides of petal base while the cultivars Pusa Makhmali and Punjab Padmini have flowers with purple pigmentation on inside and yellow on outside of petal base. To ascertain whether red pigmentation character can act as a morphological dominant marker crossing was done between the plants of these varieties. Monogenic control of pigmentation of petal base in okra has been reported. Purple pigmentation on both sides of petal base to purple colour on inside and yellow on outside of petal base (Thakur and Arora, 1993). In the present study all the plants from the seeds of direct and reciprocal crosses of P-8 x Pusa Makhmali and Perkin's Long Green x Punjab Padmini revealed purple pigmentation on both sides of petal base to purple colour on inside of petal base and yellow on outside, confirming that this colour can act as morphological dominant marker in an isolation experiment. In the present investigation, okra recorded natural out-crossing to the extent of 5.47 per cent, when the variety Perkin's Long Green was grown in alternate rows with Punjab Padmini, whereas in Concentric Ring Method the natural out-crossing was observed to the tune of 2.70 per cent. Analysis of variance for the effect of different layouts on the percentage of natural out- crossing indicated significant differences between Trial I and Trial II. (Table 1).

Table 1. Average percentage of natural out- crossing in okra

Trials	Natural out-crossing (%)
Trial – I (Alternate Rows Method)	5.47
Trial – II (Concentric Ring Method)	2.70
SE (d) ±	0.11
CD (P=0.05)	0.47

These results substantiate the findings of Purewal and Randhawa (1947), Choudhary and Anothai Choomsai (1970) and Gill *et al.* (1991) who had also reported out- crossing in okra under different methods of planting. The average rate of natural out-crossing in Concentric Ring Method decrease with the increase in distance from the dominant morphological marker. The decline in the rate of natural out- crossing was not symmetrical *vis-à-vis* to the distance of planting in Concentric Ring Method (Table 2).

Table 2. Average percentage of natural out-crossing (NOC) in okra at different distances from dominant per cent (P-8) in concentric ring method

Distance from the dominant parent (m)	Percentage out - crossing
60	4.16
120	3.67
180	1.69
240	2.09
300	2.46
360	2.12

The extent of natural out-crossing was 4.16, 3.67, 1.69 and 2.12 per cent at the distance 30, 60, 180 and 360 cm respectively, which could be attributed to the asymmetrical pollen dispersal. These results also corroborate with findings of Levin and Kerster (1974), who advocated that a healthy plant at a wider distance is likely to receive more pollen than a weak plant, closer to the central plot of dominant marker, because of the preferential flights of pollinators. The extent of natural out-crossing ranged from 2.70 to 5.47 per cent at Palampur conditions of Himachal Pradesh. These results substantiate the findings of Chandra and Bhatnagar (1975) and Aken' Ova and Fatokun (1984) who had also observed that the extent of natural out-crossing in a particular place is dependent on season, location and levels of insect population. On the basis of the extent of natural out- crossing, which ranged from 2.70 to 5.47 per cent, an isolation distance of 100-150m may be adopted for seed production in okra under Palampur conditions of Himachal Pradesh. Okra which is considered to be an often cross pollinated crop, the natural out- crossing may be quite high depending upon location, cropping season and the asymmetrical dispersal of pollens.

Therefore, the isolation distances for multiplication of seed in open pollinated varieties of okra may vary from place to place. Comprehensive trials are required to be conducted under different locations for different years with larger plots of pollinators and recessive parents, before a safe conclusion is drawn about the minimum isolation distance. Different kinds of insects visited okra flower, however, *Apis cerana indica, Apis mellifera, Bombus trifasciatus* and *Xylocopa fenestrata* were the most active pollinators. These insects were inadvertently responsible for natural cross-pollination in okra. Abrol (1995) also reported *Xylocopa fenestrata, Apis dorsata* and *Bombus* spp. as the most active pollinators of okra. Okra being potentially a self fertilized crop, but because of its showy corolla, the possibility of cross- pollination by insects cannot be ruled out. Therefore, the breeding of pure line varieties of okra without adulteration can efficiently be taken up either by controlled self-pollination or hand sib pollination and open pollination in isolation from other varieties of okra under mid-hill conditions of Himachal Pradesh.

References

Abrol D P 1995. Studies on abundance, diversity and behaviour of insect visitors of okra (*Hibiscus esculentus* L. Moench) blossoms and their importance as pollinators. Korean J. Apic. **10** (1): 1- 4.

Aken'Ova M E and Fatokun C A 1984. Out-crossing in okra (*Abelmoschus esculentus* L. Moench). Beitrage Zur Tropischen Landwirtischaft Und Veterinarmedizin **22**: 149 – 153.

Anonymous 1971. *Indian minimum seed certification standards*, Department of Agriculture, Ministry of Food Agriculture, Community Development and Cooperation, New Delhi.

Anonymous 2002. *Indian Horticulture Database.* National Horticulture Board, New Delhi.

Anonymous 2004. *Area and production of different vegetables in Himachal Pradesh.* Directorate of Agriculture, Shimla, Himachal Pradesh.

Chandra S and Bhatnagar S P 1975. Reproductive biology of *Abelmoschus esculentus* L. Moench, reproductive behaviour, floral morphology, anthesis and pollination mechanism. Acta Botanica **3**: 104 – 113.

Chaudhary B and Anothai Choomsai M L 1970. Natural cross-pollination in some vegetable crops. Indian J. Agric. Sci. **40**: 805 – 812.

Fageria M S, Dhaka R S and Chaudhary B R 2003. *Vegetable Production* (Vol.II), Kalyani Publishers, Rajinder Nagar, Ludhiana.

Gill B S, Gill S S, Singh Hari and Singh H 1991. Natural out-crossing and pollen dispersal studies in okra (*Abelmoschus esculentus* L. Moench.). J. Res., PAU, Ludhiana **28** (1): 24-27.

Kalloo G 1994. *Vegetable Breeding* (Combined edition). Pinima Educational Book Agency, New Delhi.

Levin D A and Kerster H W 1974. Gene flow in seed plants. Evolutionary Biol. **139**:220.

Martin F W 1983. Natural out crossing of okra in Puerto-Rico. J.Agric. University of Puerto Rico **67**(1): 50-52.

Panse V G and Sukhatme P V 1985. *Statistical Methods for Agricultural Workers.* ICAR, New Delhi, India.

Purewal S S and Randhawa G S 1947. Chromosome and pollination studies in lady's finger. Indian J. Agric. Sci. **17**:129-136.

Tanda A S 1985. Floral biology, pollen dispersal and foraging behaviour of honeybees in okra (*Abelmoschus esculentus* L. Moench.). J. Apic. Res. **24** (4): 225-227.

Thakur M R and Arora S K 1993. Okra. In: *Vegetable Crops*, eds BoseT K, Som M G and Kabir J Naya Prokash, Calcutta,India, pp 608.