



STUDIES ON THE EXTENT OF GENETIC CONTAMINATION IN SEED PRODUCTION OF EXERTED STIGMA TOMATO (*Solanum lycopersicum* L)

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ABSTRACT: Studies were conducted at the experimental farm of Punjab Agricultural University, Ludhiana, during Rabi season for three years, 2006 to 2010 to standardize the minimum isolation distance required for maintaining genetic purity in hybrid seed production of exerted stigma (recessive) tomato under open field conditions. The exerted stigma seed parent with recessive potato leaved seedling marker was sown at 25 m, 50 m, 75 m, 100m, 150m, 200m and 250m distance away from normal stigma (inserted) contaminator parent cv. Castle Rock having dominant cut leaf seedling marker. The pooled data of three years indicated that the highest percentage of genetic contamination of 9.26% was recorded at a distance of 25 m from the contaminator (Castle Rock). It was also observed that there was a gradual reduction in contamination level with increasing distance at 100m isolation distance of 2.56% although it was not within the prescribed maximum permissible limit of genetic contamination (1 and 2% for foundation and certified seed, respectively). There was zero genetic contamination at the highest isolation distance of 150 m. In the present study, in the isolation distances studied until 100m, the level of contamination was well above the permissible minimum seed certification standards (98 % genetic purity for certified seed). However, based on the present study, the isolation distance required for maintenance of genetic purity of tomato using exerted stigma seed parent for hybrid seed production under open field conditions of Punjab is 150m as against the recommended isolation of 25m and 100m for production of certified seeds of open pollinated seeds and hybrid seeds, respectively.

Keywords: Tomato, genetic purity, isolation, contamination, seed production.

Tomato (*Solanum lycopersicum* L) is an important vegetable crop cultivated worldwide owing to its economic significance. In India, tomato occupies an area of 6,34,400 ha with a production of 1,24,33,200 mt and a productivity of 19.6 mt/ha (Annon., 1). The projected hybrid seed requirement for regular tomatoes for next five years is 35 t (Annon., 2). Increasing demand for hybrid seeds could stress commercial hybrid seed production abilities (Cheema and Dhaliwal, 6). The impurity of the pollen source by natural crossing could lower the genetic purity of open pollinated and hybrid seeds (Liu *et. al.*, 9). Hence, maintenance of genetic purity in seed production is of critical importance as low genetic purity seed would cause heavy loss for the seed producers. Tomato is >99% self pollinated (Groenewegen *et. al.*, 7). The flowers of most commercial cultivars have a short style that

places the stigma inside the anther tube assuring self pollination and virtually eliminating the opportunity for outcrossing. On the contrary, although tomato is a self pollinated crop, natural cross pollination of 5% has been reported (Veerarahavathatham *et al.*, 12).

An isolation distance of 100 m is required for certified seeds between two tomato varieties to prevent out crossing in hybrid seed production under open field conditions (Tunwar and Singh, 11). In any seed production programme, isolation between two varieties is a pre-requisite to prevent either mechanical mixture/cross pollination for the production of breeder/foundation/ or certified seeds.

Under normal conditions, most tomatoes have a natural cross-pollination rate of about 2 to 5%. Under some conditions though, this may be as high as 50%. The incidence depends on the types of

insects active in the area, the existence and types of inter-planted crops, the wind, the blossom structure, and the blossom timing of the varieties involved. The prescribed isolation distance for minimum seed certification standards of genetic purity in tomato is 50m and 25m for foundation and certified seed respectively for open pollinated seed. In the earlier studies on isolation in potato leaved forms tomato, which is often a self pollinated crop, there was no out crossing beyond 33m and 40-50m safe isolation was recommended for open pollinated seed production (Veeraraghavathatham *et al.*, 12). Some tomato varieties have exerted stigma which means that the stigma is positioned outside of the anther cone and it is more susceptible to foreign pollen. In hybrid seed production of tomato exerted stigma types with seedling markers are used to avoid emasculation in crossing. Similar reports of advantages of exerted style for tomato hybrid seed production were also reported (Atanassova, 5; Kilchieilchevsky and Dodrodkin, 8). However, in exerted stigma types used as seed parent for hybrid seed production, slightly higher level of cross pollination is expected due to exposure of stigma for insect or bee pollination. Under natural conditions, cross pollination up to 5.56% has been reported (Veeraraghavathatham *et al.*, 12) and the pollinating insects are bees (Quiros and Marcias, 10). Growing different tomato varieties in the vicinity would enhance the chance of contamination. The prescribed minimum seed certification standards for open pollinated seed production of tomato, in general, is 50m and 25m isolation distance for foundation and certified seeds production, respectively (Tunwar and Singh, 11). Also, if a high proportion of natural crossing occurs in exerted stigma types of tomato, it necessitates isolation between tomato varieties for maintenance of genetic purity. However, the isolation distance varies with the many factors such as crop, breeding behaviour, season, adjacent crops grown, natural pollinators, wind breaks, barriers and geographical

location of seed plot, etc., Further, there are no systematic studies on the isolation distance required for maintaining genetic purity of exerted stigma types of tomato under Indian conditions.

Hence, objective of the present study was to determine the extent of genetic contamination in exerted stigma tomato under natural crossing and to standardize the minimum isolation distance required for maintaining genetic purity in seed production of exerted stigma (recessive) tomato under open field conditions.

MATERIALS AND METHODS

Field experiments were conducted at Punjab Agricultural University, Ludhiana in the *Rabi* season of 2006, 2007 and 2008 to facilitate natural out crossing between exerted stigma seed parent (stigma exertion above anther cone of 2mm) with recessive potato leaved seedling marker and normal inserted stigma pollen parent cv. Castle Rock with dominant cut leaved seedling marker. Adequate precautionary measures were taken to ensure that only the exerted stigma type and the contaminator parent cv. Castle Rock were allowed for natural crossing and no other tomato varieties were grown adjacent until 200 m isolation from seed parent so as to avoid any other cross pollination from other varieties. The exerted stigma seed parent with recessive potato leaved seedling marker was sown at different isolation distances 25m, 50m, 75m, 100m, 150m, 200m and 250m distance away from normal stigma (inserted) contaminator cv. Castle Rock having dominant cut leaf seedling marker. The plot size of seed parent and pollen parent was 25 m² each. There were no border rows surrounding the seed parent. The spacing adopted between row to row and plant to plant was 120 cm × 30 cm. The crop was sown in a randomised block design with four replications per treatment. These maximum distances covered in the present study are even more than the prescribed isolation distance of 100m

for certified seed production of hybrid seeds. It was ensured that there were no physical barriers upto 250 m to facilitate natural outcrossing. The tomato seed crop was raised using the recommended package of practices. The adjacent crops near the experimental plot were onion and wheat during all the years. The flowering in male parent was early by five days compared to seed parent. Continuous flowering occurred during the months of March-April in all the years. Observations were recorded for per cent fruitset on natural crossing. The weather data pertaining to the months of flowering in the months of March and April of all three years have been presented (Table 1) which could also contribute to natural crossing.

The naturally crossed fruits of tomato were collected at various isolation distances from 25m, 50m, 75m, 100m, 150m, 200m and 250m from the contaminant plot of Castle Rock and seeds were extracted and evaluated for genetic purity by conducting grow-out tests (GOT) in nursery in 2008, 2009 and 2010. The presence of seedling markers facilitate easy identification of crossed and selfed seeds in each progeny after each season following standard procedures (Agarwal, 3) of 100 plants per replication and four replications in each treatment were maintained in grow out tests.

The seeds were extracted from naturally crossed fruits in various isolation distances and sown to determine the extent of genetic contamination in the progenies. The extent of genetic contamination by natural crossing in seed crop was recorded based on the number of seedlings with cut leaf marker in the progeny which were contaminated seeds and expressed as genetic contamination percentage. The remaining seedlings with potato leaf marker were selfed.

Statistical analysis of data was done using Analysis of variance (ANOVA) for various

isolation distances after data were subjected to angular transformation.

RESULTS AND DISCUSSION

The pooled data of three crops from the years 2006 to 2010 on per cent fruit set and frequency of contaminants, extent of genetic contamination in the progeny of seed crop (exerted stigma type with potato leaf seedling marker) at various isolation distances from the contaminator, Castle Rock (normal inserted stigma with cut leaf seedling marker) are given in Table 2 and Table 3, respectively.

The pooled data of effect of isolation distances on per cent fruit set in exerted stigma tomato types due to natural crossing indicated that there existed significant differences due to isolation distances. The per cent fruit set was highest at shortest isolation distances from pollen parent compared to longest isolation distances. The highest per cent fruit set was highest at shortest isolation distance. The highest per cent fruit set was recorded at 25m isolation from pollen parent of 37.32% followed by 50m isolation (34.46%). The lowest per cent fruit set was recorded at 250m isolation of 21.58%. The highest per cent fruit set at 25m could be attributed to higher natural outcrossing at shortest isolation distance from seed parent.

Results indicated that the genetic contamination in the progeny of seed crop (exerted stigma type with potato leaf seedling marker) decreased with increasing isolation distance from the contaminator. Based on pooled data of three years, highest percentage of genetic contamination/outcrossing of 9.26% occurred at an isolation of 25m from the pollen parent and decreased continuously at 6.64, 3.23 and 2.56% at larger isolation distances of 50, 75 and 100m, respectively. The lowest genetic contamination of 2.56% was recorded at the highest isolation distance of 100m isolation distance although it is not under the

Table 1: Weather data during flowering contributing to natural cross pollination in exerted stigma tomato.

Year	Months	Temp max (°C)	Temp min (°C)	RH morning (%)	RH evening (%)	SSH (hrs)	Rainfall (mm)	wind speed (km/hr)
2006	Mar	27.0	13.1	90	44	8.8	32.5	4.3
	Apr	36.0	18.2	56	17	9.8	5.1	5.2
	Mean	31.5	15.65	73	30.5	9.3	18.8	4.75
2007	Mar	26.1	12.4	91	44	9.1	41.3	5.5
	Apr	36.9	19.4	66	23	10.8	26.2	4.7
	Mean	31.5	15.9	78.5	33.5	9.95	33.75	5.1
2008	Mar	30.4	14.0	90	39	9.1	0.0	2.5
	Apr	34.1	17.7	68	28	10.2	50.2	5.6
	Mean	32.25	15.85	79	33.5	9.65	25.1	4.05
	Grand Mean	33.55	15.8	76.83	32.50	9.63	25.88	4.63

Table 2: Effect of isolation distances on per cent fruit set in exerted stigma tomato types due to natural crossing.

Isolation distance	Per cent fruit set			
	2006-07	2007 -08	2008-09	Pooled mean
25 m	31.18	42.56	38.22	37.32
50 m	30.86	35.98	36.54	34.46
75 m	25.98	25.63	31.20	27.60
100 m	21.35	24.60	19.85	21.93
150 m	25.98	19.45	20.65	22.03
200 m	31.56	22.34	23.67	25.86
250m	19.87	24.30	20.58	21.58
C.D. (P=0.05)	8.54	9.21	8.72	

Table 3: Effect of isolation distances on frequency of contaminants and percentage of genetic contamination in hybrid seed production of tomato (using exerted stigma seed parent).

Isolation distance (m)	April 2008			March 2009			March 2010			Pooled data		
	No of plants with potato leaf type	No of plants with cut leaf type	Genetic contamination (%)	No of plants with potato leaf type	No of plants with cut leaf type	Genetic contamination (%)	No of plants with potato leaf type	No of plants with cut leaf type	Genetic contamination (%)	No of plants with potato leaf type	No of plants with cut leaf type	Genetic contamination (%)
25	123	11	8.2	115	12	10.43	120	11	9.16	119.33	11.33	9.26
50	99	6	5.7	110	8	7.27	115	8	6.95	108	7.33	6.64
75	125	3	2.3	100	4	4.00	118	4	3.38	114.33	3.66	3.23
100	103	1	0.96	110	3	2.72	110	4	3.63	107.66	2.66	2.56
150	101	0	0	100	0	0	100	0	0	100.33	0.00	0.00
200	130	0	0	120	0	0	100	0	0	116.66	0.00	0.00
250	78	0	0	100	0	0	120	0	0	99.33	0.00	0.00
C.D. (P=0.05)			0.87			0.81			0.75			0.80

prescribed maximum permissible limit of genetic contamination. There was completely no genetic contamination at isolation distance of and beyond 150m.

Statistical analysis of percentage of genetic contamination revealed that there were significant differences among the various isolation distances studied for the extent of genetic contamination in the three years. The present study revealed that as the isolation distance increased from 25m to 100m, per cent contamination in the progeny of seeds crop (exerted stigma type with potato leaf seedling marker) decreased. The minimum genetic purity standards for Foundation and Certified seeds are 99 and 98 per cent, respectively. Self-pollinated vegetable seed crops exhibit lesser degree of variation as compared to cross-pollinated vegetables. However, genetic contamination even in self pollinated vegetables like tomato affects in such a way that any specific character bred into a variety is likely to be lost because of genetic contamination (Arya, 4).

In the present study, significant differences existed between the different isolation distances studied as the level of contamination is well above the permissible minimum seed certification standard, it is risky to reduce the prescribed isolation distance of certified seeds to 100m in exerted stigma potato leaved tomato forms for hybrid seed production. The higher levels of genetic contamination until 100m isolation could be attributed to a relative abundance of natural pollinators on long exerted stigma tomato types under the conditions of Punjab. The occurrence of natural pollinators i.e., honey bee species, *Apis mellifera* were observed in morning hours (7-10 am). The natural crossing would have been oured due to the foraging bees on exerted stigma from the adjacent crops near the experimental plot of onion and wheat during all the years. Also the mean maximum temperature of 33.5°C, maximum sunshine hours of

9.63 and wind speed of 4.63 km/hour would have favoured anther dehiscence and pollen dispersal by bees in the location. The present findings of genetic contamination until 100m isolation distance are in contrast to Veeraraghavathalam *et. al.* (12) who reported that the safe isolation distance in potato leaved tomato was 40-50m at Coimbatore, Tamil Nadu conditions. There was zero genetic contamination at the highest isolation distance of 150 m.

The prescribed isolation distance for certified seed production of tomato for open pollinated and hybrid seeds is 25 and 100 m, respectively. However, the prescribed isolation distance would drastically affect the genetic purity in exerted stigma tomato under conditions of Punjab Agricultural University, Ludhiana. Hence, the minimum isolation distance required to be maintained for tomato open pollinated seed and hybrid seed production using exerted stigma Ex-3 as seed parent (without emasculation) is 150 m under Punjab Agricultural University, Ludhiana conditions.

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