Indian Journal of Agricultural Sciences **84** (12): 1453–6, December 2014/Article

Natural outcrossing and isolation distance requirements in exserted stigma tomato (*Solanum lycopersicum*) seed production

K PADMINI[1], A T SADASHIVA[2] and L B NAIK[3]

Indian Institute of Horticultural Research, Bangalore, Karnataka 560 089

Received: 5 June 201114; Revised accepted: 22 July 2014

ABSTRACT

Genetic purity is of utmost important in seed production of varieties and hybrids. Since exserted stigma tomato types are much prone to natural crossing the chances of genetic contamination is higher in seed production and the recommended isolation requirements for hybrid seed production is higher (200m for foundation seeds and 100m for certified seeds). Hence, studies were conducted at Indian Institute of Horticultural Research, Bangalore, during *rabi* season of three years, 2007 to 2010 with an objective of determining the extent of natural cross pollination in terms of genetic contamination and to optimize the minimum isolation distance requirements for maintaining genetic purity in seed production of exserted stigma (recessive) tomato. The natural out crossing, i.e. genetic contamination was within 2% (permissible genetic standards) at a minimum isolation of 50m in interior rows of seed plots (R2 and R3) and in all the three years of study. Hence, a safer minimum isolation of 50 m with border rows is sufficient for tomato seed production using exserted stigma Ex-3 as seed parent for obtaining seeds of permissible genetic purity standards (98 %) under IIHR, Bangalore conditions, which is lesser than the recommended isolation of 200m for foundation seeds and 100m for certified seeds in hybrid seed production of tomato.

Key words: Exserted stigma**,** Genetic purity, Hybrid seed production, Tomato

Tomato (*Solanum lycopersicum* L.) is an important vegetable crop cultivated worldwide owing to its economic significance. Tomato occupies an area of 634400 ha with a production of 12433200 mt/ha and a productivity of 19.6 mt/ha (NHB 2011). The demand for tomato hybrid seeds is ever increasing among the farmers. The maintenance of genetic purity in seed production is of critical importance as low genetic purity seed would cause serious loss for the seed growers. The extent of genetic contamination in varieties depends on the extent of natural cross pollination. Tomato is a highly self pollinated crop with more than 99% selfing (Groenewegen *et al.* 1994). The flowers of most of cultivars have a short style that places the stigma inside the anther tube assuring self pollination and virtually eliminating the opportunity for outcrossing. The impurity of the pollen source by natural crossing could lower the genetic purity of open pollinated and hybrid seeds (Liu *et al.* 2004).

Some tomato varieties have exserted stigmas which means that the stigma is positioned outside of the anther cone and it is more favourable for outcrossing. In hybrid seed production of tomato, exserted stigma types with seedling markers are used to avoid emasculation in crossing which has a definite advantage in reducing the cost of hybrid seed production. Similar reports of advantages of exserted style for tomato hybrid seed production have been reported (Atanassova 1999 and Kilcheilchevsky and Dodrodkin 2000).

In exserted stigma tomato types, higher levels of natural crossing is expected due to exposure of stigma for insect or bee pollination. Under natural conditions, cross pollination up to 5.56% has been reported in tomato (Veeraraghavathatham *et al.* 2005) and the pollinating insects are bees (Quiros and Marcias 1978). However, under highly favourable conditions the rate of natural crossing increases. The rate of out crossing is dependent on the types of insects prevalent in the area, the existence and types of inter-planted crops, the wind, the blossom structure, and the blossom timing of the varieties involved. Hence, in any seed production programme, isolation between two varieties is a pre-requisite to prevent either any cross pollination for the production of breeder, foundation or certified seeds. However, the isolation distance varies with the many factors such as crop, breeding behavior, season, adjacent crops grown, natural pollinators, wind breaks, barriers and geographical location of seed plot, etc. In the earlier studies on isolation in potato leaved forms tomato, which is often a self pollinated crop, there was no out crossing beyond 33 m and 40-50 m safe isolation was recommended for open

[1]Senior Scientist (Horticulture) (e mail: kpadmini@iihr.ernet.in), [2] Principal Scientist (Horticulture) (e mail: ats@iihr.ernet.in), [3]Principal Scientist (Agronomy) & Head, (e mail: lbnaik@iihr.ernet.in), Section of Seed Science and Technologys

pollinated seed production (Veeraraghavathatham *et al.* 2005).

Growing different tomato varieties in the vicinity would enhance the chance of contamination. Tunwar and Singh (1998) have recommended 200m and 100 m as the isolation for Foundation and Certified seed production respectively of tomato hybrid seeds. The prescribed minimum seed certification standards for open pollinated seed production of tomato, in general, is 50 m and 25 m isolation distance for foundation and certified seeds production, respectively (Tunwar and Singh 1998). Since, in exserted stigma types of tomato, natural crossing is higher due to elongated stigma outside the anther cone, standardization of minimum isolation requirements is quite relevant. In addition, systematic studies are lacking on the minimum isolation distance requirements for maintaining genetic purity of exserted stigma types of tomato under Indian conditions as tomato is a highly self pollinated crop.

The objective of the study was to determine the extent of natural cross pollination in terms of genetic contamination and to optimize the minimum isolation distance requirements for maintaining genetic purity in seed production of exserted stigma (recessive) tomato under open field conditions.

MATERIALS AND METHODS

The experimental materials used in the study for natural cross pollination were Ex-3-seed parent (exserted stigma-2 mm above the anther cone) with recessive potato leaved seedling marker and Arka Alok-contaminator parent with dominant cut leaf seedling marker. Ex-3 seed parent has not only long style and stigma exserted over the anther cone to facilitate natural crossing but also has functional pollen for setting fruits on its own (selfing). Sowing of seeds was taken up in *rabi* season (October) for all the three years of study. It was adequately ensured that there was no other tomato varieties grown until 250m isolation from the experimental materials to avoid any chance of natural crossing due to other varieties. Also, adequate care was taken to ensure that there were no tomato volunteers from previous seasons within the isolation perimeter**.** The seed parent was isolated and planted as blocks of 22.5 m^2 (6 m × 3.75 m) from the contaminator parent at six isolation distances namely, 25 m, 50 m, 100 m, 150 m, 200 m and 250 m. The field plan of the experiment is presented in Fig 1. Each block consisted of four rows of ten plants each and each row formed a replication. R1 and R4 were border rows and R2 and R3 were interior rows within the block of seed parent. The spacing between rows and plants were 75 × 60 cm. The contaminator block consisted of five rows of ten plants each. The adjacent crop grown near the contaminator and seed parent was watermelon in 2007, and chillies in the years 2008 and 2010. The contaminator parent was early to flower (30 days from planting) than the exserted stigma seed parent (37 days from planting) and they were also synchronous in flowering for 37 days.

Observations were recorded on percent fruit set and extent of genetic contamination by grow out tests (GOT).

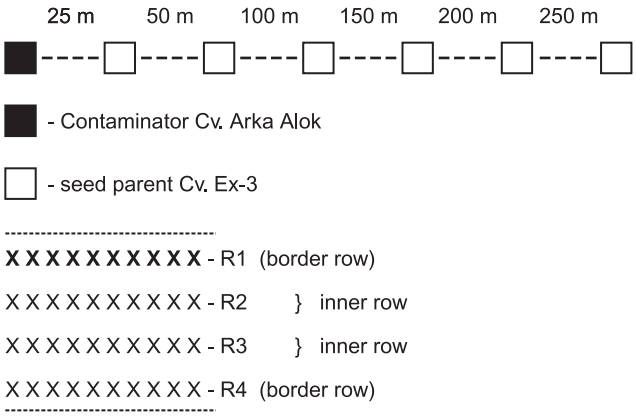

Fig 1 Field plan of the experiment

Grow out tests in nursery for assessing genetic contamination of the naturally crossed seeds were done after seed extraction from all the four rows including the border rows. The extent of genetic contamination (%) at various isolation distances (25 m, 50 m, 100 m, 150 m, 200 m and 250 m) was studied by observations on dominant cut leaf seedling marker of pollen parent (Arka Alok) and recessive potato leaf seedling marker of seed parent (Ex-3) by standard grow-out tests in nursery. A minimum of 100 plants per replication and four replications in each treatment were maintained in grow-out tests of naturally crossed progenies in nursery. The percentage of plants with cut leaf seedling marker was assessed as hybrids in the crossed progeny which determines the percentage of genetic contamination and hence the extent of natural crossing.

RESULTS AND DISCUSSION

The effect of isolation distances on genetic contamination in seed production of tomato (using exserted stigma seed parent) with respect to the years 2007, 2008 and 2010 has been presented in Table 1 and percentage of genetic contamination row wise within a seed block in Table 2. The meteorological data during flowering of tomato crop is presented in Table 3. The mean maximum temperature was favourable for anther dehiscence and the mean wind speed was 4.45 km/hour over the years.

Based on the data on natural crossing on exserted stigma seed parent Ex.3 (without emasculation) of the years showed that significant differences existed for the percentage of fruit set on exserted stigma line due to natural crossing at various isolation distances in year 2007 only and no differences in the years 2008 and 2010. The percentage fruit set ranged from 13-53% among the isolation distances over the years. Maximum fruit set was observed in 150m isolation of 43% in 2007. Ex-3 seed parent has not only stigma exserted over the anther cone to facilitate natural crossing but also has functional pollen for setting fruits on its own (selfing). Hence, the set fruits would be due to its own pollen or even naturally crossed pollen which necessitaed the grow out tests to study genetic contamination.

The extent of genetic contamination varied with the years. The differences could be mainly due to the adjacent

Table 1 Effect of isolation distances on genetic contamination in seed production of Tomato (using exserted stigma seed parent)

Treatment	Fruit set (%)			Pooled mean Genetic contamination including border rows (R1+ R2+R3+R4) (%)			Pooled mean Genetic contamination of interior rows only (R2 +R3) (%)		
	2007	2008	2010	2007	2008	2010	2007	2008	2010
25 m	28.72	18.46	53.51	8.36	0	5.38	9.86	0	4.81
	–32.36	–25.3	–47.12	–2.81	–0.71	–2.34			
50 m	25.46	15.58	49.71	0	0	0.67	0	0	1.01
	–30.25	–22.4	–44.87	–0.71	–0.71	–1.02			
100 m	13.87	18.89	49.13	6.19	0	1.33	0.6	0	1.77
	–21.81	–25.61	–44.52	–2.11	–0.71	–1.31			
150 m	43.07	14.96	39.73	9.88	0.96	0.71	0	0.6	0.64
		–40.99	–22.71	–39	–2.58	–1.18	–1.08		
200 m	25.16	14.23	40.06	0.38	0.16	1.29	0.57	0.19	1.79
	–30.06	–21	–39.17	–0.91	–0.81	–1.27			
250 m	31.13	17.72	25.61	0.85	0.36	0.19	0	0.195	0
	–28.8	–24.76	–30.23	–1.09	–0.9	–0.82			
CD = (0.05)	10.75	NS	NS	NS	0.27	0.99			

Data in parenthesis of percent fruit set are angular transformed values and of genetic contamination (%) are square root transformed values

Table 2 Percentage of genetic contamination row wise within a seed block

Treatment	Genetic contamination (%)													
	2007					2008					2010				
	R1	R2	R3	R4	Mean	R1	R2	R3	R4	Mean	R1	R2	R3	R4	Mean
25m	10	17.04	2.68	3.73	8.36 (2.81)	0	0	0	0	0 (0.71)	6.19	8.46	1.15	5.73	5.38 (2.34)
50m	0	0	0	0	0 (0.71)	0	0	0	0	0 (0.71)	0.64	0	2.02	0	0.67 (1.02)
100m	3.37	1.02	0	20.37	6.19 (2.11)	0	0	0	0	0 (0.71)	1.78	1.94	1.6	0.02	1.33 (1.31)
150m	26.13	0	0	13.39	9.88 (2.58)	1.65	1.02	0	1.18	0.96 (1.18)	0.35	0.67	0.61	1.31	0.71 (1.08)
200m	0.38	0	1.13	0	0.38 (0.91)	0	0	0.38	0.26	0.16 (0.81)	0	0.69	2.88	1.6	1.29 (1.27)
250m	2.3	0	0	1.1	0.85 (1.09)	0	0.39	0	1.06	0.36 (0.90)	0.61	0	0	0	0.19 (0.82)
CD (P = 0.05)					NS					0.27					0.99

Figures in parenthesis are square root transformed values

Table 3 Meteorological data during flowering in tomato seed production

Year	Months	Temp max (°C)	Temp min (°C)	RH (%) 7.30 hrs	RH (%) 14.00 hrs	USWB pan evaporation class A(mm)	Mean wind speed (km/hr)	Rainfall (mm)
2007	Dec	27.5	11.4	74.2	65.1	5.2	3.8	
2008	Jan	28.5	11.9	73.1	53.1	3.6	3.9	
	Feb	29.7	15.7	60.6	37.0	4.7	4.5	7.4
Mean		28.57	13	69.3	51.73	4.5	4.07	2.47
2008	Dec	26.4	14.7	68.5	49.5	4.2	4.2	3.1
2009	Jan	27.1	11	64.8	41.2	4.4	4.62	
	Feb	31.1	12.2	62.5	42.6	6.3	4.78	
Mean		28.2	12.63	65.27	44.43	4.97	4.53	1.03
2010	Dec	26.2	16.4	80	52.5	2.86	4.19	1.0
2011	Jan	28.5	13.9	71.1	41.0	4.34	4.62	
	Feb	30.0	13.2	60.7	48.2	4.73	5.47	
Mean		28.2	14.5	70.6	47.2	3.98	4.76	0.33
Mean over the years		28.32	13.38	68.39	47.79	4.48	4.45	1.28

crops grown and the relative pollinator activity over the years. The data on genetic contamination of three years, indicated that no significant differences existed between the isolation treatments during the two years 2008 and 2010. At closest isolation distance, the genetic contamination was high in 2007 and 2010. Although, the genetic contamination was highest (9.88%) at 150m in 2007, no outcrossing (0%) contamination occurred even at 50m isolation. The higher genetic contamination until an isolation of 150m was due to higher activity of honey bees adjacent to the experimental plot the adjacent crop being watermelon (a highly cross pollinated crop). If adjacent crops are cross pollinated with a higher activity of honey bees, a minimum isolation of 200 m is essential to maintain the genetic purity of exserted stigma seed parent if grown without any border rows or barrier crop. However, on closer observation, it was found that only in border row plants (R1 and R4), genetic contamination existed at 150m and 100m isolation and not on inner rows of seed blocks (R2 and R3) as presented in Table 2. The differences between the isolation treatments were also non-significant. The natural cross pollination reported in 2008 is due to pollinators from adjacent crops. Similar reports of bees and hover flies as pollinators in tomato has been made by Gladis *et al.* (1996). An isolation distance of 150m has been reported in Punjab conditions as ideal as against 25m and 100m for production of open pollinated and hybrid seeds respectively (Singh *et al.* 2012) although, there has been no mention about the pollinator activity or the adjoining crops grown. In addition, the confirmation trials for the next two consecutive years (2008 and 2010) also indicated that the results on genetic contamination (natural outcrossing) were significant for both the years and there was no outcrossing (0%) even at 25m isolation in 2008 and less than 2% from 50m isolation distances consistently in 2008 and 2010. The maximum outcrossing of 5.38% (more than permissible limits of 2%) was observed only at 25m isolation distance. The variations in genetic contamination over the years was due to the influence of adjacent crop, viz. chillies grown in the years 2008 and 2010. There were only one or two solitary wasps present in morning hours in seed blocks of tomato. From the present study, it is understood that the adjacent crop plays a major role on the extent of natural cross pollination in addition to other usual factors influencing natural cross pollination.

Although the pooled mean percentage of genetic contamination including the border rows (R1 and R4) was lower at a minimum isolation of 200 m only in 2007 (0.38%) (Table 1), interestingly, the genetic contamination was within 2% (permissible genetic standards) even at a minimum isolation of 50m in interior rows of seed plots (R2 and R3) in all the three years (Table 2). This indicates that if the recommended isolation distance of 200 m as per the standards is not achievable in exserted stigma tomato types under field conditions, block planting of seed parent at a minimum isolation of 50 m with border rows and harvesting of fruits from the interior plants for seed saving is ideal to obtain higher percentage of genetic purity in tomato seed production. This leads to economic space utilisation under practical commercial tomato seed production. This also gives a lead that since exserted stigma types themselves, require lesser isolation distances, the reduced isolation could also be practised in short styled types too as it is still lesser prone to outcrossing when it is not possible to achieve the recommended isolation. The present results are in conformity with that of Haskell and Paterson (1966) who reported that no cross pollination occurred between cut leaf cultivars and potato leaf test line. Reeve (1973) also reported lesser natural outcrossing of 0.7 % in progeny tests of tomato in line with the present findings.

CONCLUSIONS

From the present study, it is understood that the natural outcrossing (genetic contamination) was within 2% (permissible genetic standards) at a minimum isolation of 50m in interior rows of seed plots (R2 and R3) and in all the three years of study. Hence, a safer minimum isolation of 50 m with border rows is sufficient for tomato seed production using exserted stigma Ex-3 as seed parent for obtaining seeds of permissible genetic purity standards (98 %) under IIHR, Bangalore conditions, which is lesser than the recommended isolation of 200m for foundation seeds and 100m for certified seeds in hybrid seed production of tomato.

REFERENCES

Atanassova B. 1999. Functional male sterility (ps-*2*) in tomato (*Lycopersicon esculentum* Mill.) and its application. *Euphytica* **107**: 13–21.

Gladis T Hammer G K, Dathe H H and Pellmann H 1996. Insect pollination and isolation requirements in tomato collections (*Lycopersicon esculentum* Mill). *Plant Genetic Resources Newsletter* **106**: 16–9.

Groenewegen C, King G and George B F. 1994. Natural cross pollination in California commercial tomato fields. *Hort Science* **29**: 1 088.

Haskell G and Paterson E B. 1966. Genetic determination of natural self pollination in Scottish glasshouse tomatoes. *Euphytica* **15**: 128–32.

Kilcheilchevsky A and Dodrodkin M. 2000. Use of functional male sterility in seed production of tomato hybrids. *Acta physiologiae –plantarum* **22**(3): 232–4.

Liu LW, Hou X L, Gong Y Q, Zhang Y M, Wang K R and Zheng J F. 2004. Natural cross pollination in tomato. *Molecular Plant Breeding*. **2**: 563–8.

National Horticulture Board. 2010. Indian Horticulture Database. www.nhb.gov.in/.

Quiros C F and Marcias A.1978. Natural cross pollination and pollinator bees of the tomato in Celaya, Central Mexico. *Hort Science* **13** (3): 290–1.

Reeves A F I I. 1973. An observation on natural outcrossing in the tomato *Lycopersicon esculentum* L. in northwest Arkansas. *Proceedings of the Arkansas Academy of Science* **27**: 24–5.

Singh R Singh D and Kanwar J S. 2012. Studies on the extent of genetic contamination in seed production of exserted stigma tomato (*Solanum lycopersicon* L). *Hort Flora Research Spectrum* **1**(4): 306–11.

Tunwar N S and Singh SV. 1998. *Indian minimum seed certification standards*. Central Seed Certification Board. Government of India, New Delhi.