

## Studies on hybrid seed production in tomato (*Solanum lycopersicum*) using exerted stigma line with seedling marker

K. PADMINI, A.T. SADASHIVA<sup>1</sup>, K. BHANUPRAKASH AND L.B. NAIK

Section of Seed Science and Technology, Indian Institute of Horticulture Research, Bangalore, Karnataka 560 089  
kpadmini@iihr.ernet.in

**ABSTRACT** A field experiment was conducted to adopt different pollination methods to evaluate the efficiency of a exerted stigma line (female parent) for hybrid seed production in tomato (*Solanum lycopersicum* L.). There were significant differences for hybrid seed recovery among the treatments. Highly significant and maximum values were recorded in hand-pollination without emasculation during two years of study. Among natural cross-pollination treatments, maximum per cent hybrid seed recovery was observed in alternate row planting of parental lines in a 1:1 ratio. With regard to seed quality, non-significant differences were observed among treatments for germination, seedling length and vigour index.

**Key words:** Exserted stigma, hybrid seed production, long styly, outcrossing, tomato

Tomato (*Solanum lycopersicum* L.) is an important vegetable crop grown all over the world. There is huge demand for hybrid seed of tomato for getting higher crop yield, uniformity and disease resistance. Although many hybrids are available in the market, commercial production of hybrid seed is totally dependent on hand emasculation and pollination which is labour-intensive and cumbersome. Emasculation alone contributes to 40% of the total cost of production of hybrid seeds in tomato [1]. These could be major impeding factors for popularization of hybrid seed production. Investigations on efficient hybrid seed production in tomato and use of male sterility to reduce the cost of hybrid have been reported earlier [2-8]. However, each male sterility mechanism has its own merits and demerits which should be taken into consideration for efficient and large-scale hybrid seed production.

Tikoo and Anand [9] reported that Ex-3 tomato line possessed positional sterility and bred true for exertion of stigma, with the extent of natural fruit setting under open-pollination as low as 3%

out of which 99.05% were selfs. This line was taken for the present study. Tikoo and Pal [10] reported up to 94% crossing when an exerted line Ex-1 was crossed with certain hybrid combinations (Paternal line). The use of long style-short anther seed parents crossed with short style-long anther pollinators is also suggested to obtain non-exserted hybrids [11, 12]. Similar reports of advantages of exerted style for tomato hybrid seed production were reported [5, 7, 13 and 14]. However, no systematic study has been carried out on the use of exerted stigma line for seed yield, genetic purity and seed quality for large-scale hybrid seed production.

Although tomato is a self-pollinated crop, natural crossing of up to 5% has been reported under Indian conditions [15]. Hence present study was aimed at evaluating usefulness of exerted stigma line for hybrid seed production. Besides, study included identifying pollination methods for higher seed yield, recovery of genetically pure hybrid seeds and to study the influence of pollination methods on seed quality attributes.

<sup>1</sup>Division of Vegetable Crops, IIHR, Bangalore 560 089

## MATERIALS AND METHODS

The field experiment was conducted at IIHR, Bangalore, during two consecutive *Rabi* seasons. The parental line used were Ex-3 exerted stigma line linked with recessive potato leaf marker in seedlings (seed parent) and cv. Arka Alok having normal stigma and dominant cut leaf type (Pollen parent). The plot size in each treatment was 36.46 m<sup>2</sup> (13.50 m x 2.7 m) consisting of a compact lock of rows consisting of five replications. The experiment consisted of four treatments (Fig. 1): T<sub>1</sub>-natural cross-pollination with alternate row planting of parental lines (1:1 parental ratio) with a spacing of 75 x 30 cm; T<sub>2</sub>-natural cross-pollination with alternate and adjacent planting of parental lines (1:1 parental ratio) with a spacing of 75 x 30 cm; T<sub>3</sub>-natural cross-pollination with pollen parent in centre surrounded by female line on all sides with a 1:1 parental ratio and T<sub>4</sub>-hand-pollination without emasculation of exerted line with parental lines in a 5:1 ratio of seed and pollen parent with a spacing of 150 x 60 cm. An isolation distance of 2 m and border rows of seed parent were maintained as barriers at periphery of each treatment plot. The data were recorded on four plants from each replication of seed parent selected randomly. Hand-pollination without emasculation was done in the exerted stigma seed parent in morning hours (10 AM to 12 PM) using freshly extracted pollen-grains of fresh flowers. Unopened flower buds, which were about to open the next day, having cream coloured petals, were used for crossing in the seed parent. The crop was raised with the recommended package of cultural practices for growing seed crop. The data were recorded on per cent fruit setting, seed weight fruit, number of seeds, fruit and hybrid seed recovery (%). For seed quality-attributing characters *viz.* 100-seed weight, germination (%) [16] and vigour index were calculated.

For calculating genetic purity of hybrid seed produced, the seeds extracted from ripe fruits of seed parent, in each treatment, were subjected to progeny grow-out tests in the nursery. The percentage of plants with dominant cut leaf to total plants tested (400 plants) was calculated as hybrid seed recovery. Seedling vigour index was calculated by multiplying germination (%) with total seedling length [17]. The data were

statistically analysed using analysis of variance (ANOVA) in a randomized complete block design for seed yield and a completely randomized design for seed quality. The percentage data on fruit setting, hybrid seed recovery, selfing and germination were subjected to angular transformation and used for statistical analysis. The details on the meteorological data have been presented (Table 3). The days to first flowering was 30 days from date of planting in pollen parent and 36 days in female line. Peak flowering of EX-3 and pollen parent remained for 12 days and effective synchronization of flowering was observed on 37 days.

## RESULTS AND DISCUSSION

For fruit setting, total seed weight/fruit and number of seeds/fruit, there was no significant difference between the treatments of hand-pollination and natural cross-pollination (Table 1). However, pooled mean per cent fruit setting was highest in T<sub>4</sub> - (Hand-pollination without emasculation) (49.61). This could be due to availability of adequate quantity of fresh pollen on stigma through hand-pollination. The pooled total seed weight fruit was superior in T<sub>2</sub> - alternate and adjacent planting of parental lines in a 1:1 ratio (0.57 g), followed by T<sub>4</sub> - hand-pollination without emasculation (0.55 g). The superiority of total seed weight/fruit could be due to better dispersal of pollen in alternate and adjacent planting design of parental lines with natural cross-pollination throughout the entire period of receptivity of stigma of five days [18] against pollination on the day of anthesis in T<sub>4</sub>.

There were significant differences between treatments for hybrid seed recovery (Table 1). Hand-pollination without emasculation (T<sub>4</sub>) recorded significant and highest hybrid seed recovery during both the years (80 and 72.01%). The extent of hybrid seed recovery in hand-pollination without emasculation could further be improved by choosing the right stage of flower and avoiding contamination during handling. Among natural cross-pollination treatments, alternate row close planting of parental lines in a 1:1 ratio recorded highest hybrid seed recovery (40.67 and 24.83%) during first and second years, respectively. The differences for percentage of hybrid seed recovery

Table 1. Effect of pollination methods on hybrid seed yield components and genetic purity

Treatment	Fruit setting (%)		Seed weight/fruit (g)		No. of seeds/fruit		Hybrid seed recovery (%)	
	1 <sup>st</sup> year	2 <sup>nd</sup> year	1 <sup>st</sup> year	2 <sup>nd</sup> year	1 <sup>st</sup> year	2 <sup>nd</sup> year	1 <sup>st</sup> year	2 <sup>nd</sup> year
Alternate row (T <sub>1</sub> )	39.46 (38.44)	51.28 (47.32)	0.54	0.43	259.4	201.6	40.67 (38.39)	24.83 (29.78)
Alternate and adjacent (T <sub>2</sub> )	25.31 (29.97)	53.85 (45.72)	0.72	0.41	207.8	182.4	20.48 (25.60)	21.95 (27.87)
Pollen parent in the centre surrounded by seed parent (T <sub>3</sub> )	37.33 (37.13)	53.67 (47.10)	0.47	0.33	319.4	175.7	20.78 (40.58)	16.87 (23.70)
Hand pollination without emasculation (T <sub>4</sub> )	48.99 (47.36)	50.23 (45.12)	0.82	0.27	347.6	139.3	80.00 (67.27)	72.01 (58.66)
CD(P=0.05)	NS	NS	NS	NS	NS	NS	26.99	8.28

Values in parentheses are angular transformed values

in different treatments were mainly due to adequate supply of fresh pollen-grains load on seed parent in hand-pollination, at peak stigma receptivity, compared to pollen dispersal in natural cross-pollination treatments. In the present study, up to 40.67% crossing has been observed under natural cross-pollination (T<sub>1</sub>-alternate row planting of parental lines) on exerted stigma line during 1<sup>st</sup> year, compared to only 24.83% during 2<sup>nd</sup> year. This could be attributed to lower relative humidity and higher wind speed during 1<sup>st</sup> year compared to 2<sup>nd</sup> year which would have favoured higher outcrossing.

Among natural cross-pollination treatments, higher crossing was observed in T<sub>1</sub> of alternate row planting of parental lines against T<sub>2</sub> of alternate and adjacent planting of parental lines (In spite of availability of pollen parent on all sides of seed parent) and T<sub>3</sub>. This could be due to the effect of direction of wind and overlapping of branches which could have improved the availability of pollen-grains. In the present study, relatively higher rates of natural crossing of up to 40.67% was observed by modifying the planting patterns, closer spacing and most favourable ratio of parental lines (1:1). Soost and Rick [19] reported that a large variation existed for outcrossing in

tomato, ranging from 0.4 to 98.3%, depending on the type of pollen parent used. The rate of outcrossing should be improved at least up to 50% in tomato for large-scale hybrid seed production [20]. Since rate of outcrossing has not exceeded 50% even in a 1:1 ratio of parental lines, natural crossing was found not encouraging for commercial hybrid seed production. However, there is scope for further improvement in natural crossing by appropriate choice of pollen parent having extensive growth, adjacent to seed parent, to provide sufficient flowers for adequate cross-pollination [19].

However, in natural pollination treatments, lesser recovery of hybrid seeds was observed compared to hand-pollination without emasculation treatment, due to higher natural selfing than natural crossing from pollen parent. Selfing ranging from 5-35%, when an exerted line was crossed without emasculation, was reported by Scott and George [21]. The natural selfing of 20-27.99% was observed over the two years in hand-pollination without emasculation (Table 2). The recommended minimum genetic purity standards of certified hybrid seed production of tomato under conventional methods of hand-pollination and emasculation is 90% [22]. However,

Table 2. Effect of pollination methods on seed quality

Treatment	Fruit setting (%)		Seed weight/fruit (g)		No. of seeds/fruit		Hybrid seed recovery (%)	
	1 <sup>st</sup> year	2 <sup>nd</sup> year	1 <sup>st</sup> year	2 <sup>nd</sup> year	1 <sup>st</sup> year	2 <sup>nd</sup> year	1 <sup>st</sup> year	2 <sup>nd</sup> year
Alternate row (T <sub>1</sub> )	0.25	0.20	51.33 (52.70)	75.60 (62.31)	17.88	19.70	1197.1	1632.8
Alternate and adjacent (T <sub>2</sub> )	0.24	0.22	57.44 (49.53)	83.20 (68.53)	17.03	18.68	1079.9	1417.8
Pollen parent in the centre surrounded by seed parent (T <sub>3</sub> )	0.25	0.19	53.17 (47.08)	73.20 (59.75)	17.68	18.60	1220.5	1358.1
Hand-pollination without emasculation (T <sub>4</sub> )	0.27	0.20	56.89 (49.20)	91.60 (74.58)	16.86	16.18	1383.5	1470.2
CD (P=0.05)	NS	NS	NS	NS	NS	NS	NS	NS

Values in parentheses are angular transformed values

since hand-pollination was made without emasculation on the exerted seed parent having recessive potato leaf seedling marker, the selfs could be rogued out in order to maintain the genetic purity of hybrid seedlings. In natural cross pollination, all the treatments were at par with each other for selfing during both the years.

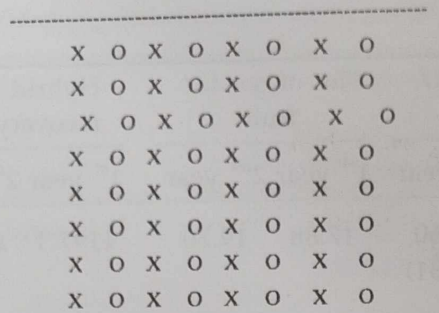
There were non-significant variation between treatments of hand-pollination and natural cross-pollination for 100-seed weight, germination (%) and vigour index (Table 2). However, higher values for germination (74.25%) and vigour index (1426.85) were recorded in hand-pollination and natural cross-pollination (Alternate row close planting arrangement), respectively. This gives ample scope to exploit natural cross-pollination as

an alternative to hand-pollination without any compromise in seed quality.

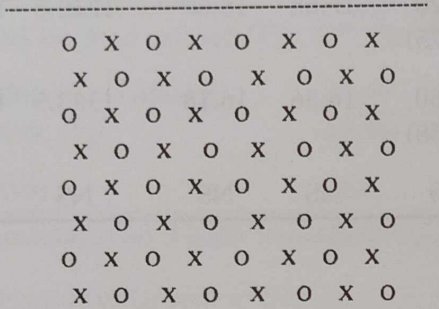
Undesirable selfing and necessity of emasculation are discussed as the two main disadvantages that limit the use of Ps 2-sterile seed parents in tomato hybrid seed production [7]. The following advantages in the performance of exerted stigma line (Ex-3) linked with seedling markers enhance the efficiency of hybrid seed production over other methods. The time and cost associated with emasculation were completely avoided in the exerted stigma male sterility, as this system eliminates the need for emasculation because of seedling markers. The selfed seed was significantly lower in hand-pollination without emasculation when using an exerted stigma line

Table 3. Meteorological data during the flowering of tomato in the two crop years

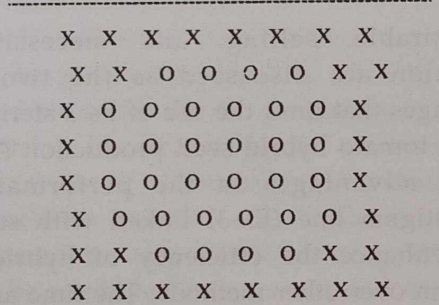
Year	Mean maximum temperature °C	Maximum temperature range °C	Mean minimum temperature °C	Minimum temperature range °C	Wind speed (km/hr)	Wind speed range (km/hr)	Mean RH (%)	RH range (%)
1 <sup>st</sup> year	29.15	27.00-32.00	13.47	9.00-18.00	4.98	2.50-9.00	66.34	50.00-89.00
2 <sup>nd</sup> year	27.66	24.00-31.50	13.65	9.00-17.00	4.86	9.00-8.50	76.06	50.00-89.00
Mean	28.41		13.56		4.92		71.20	



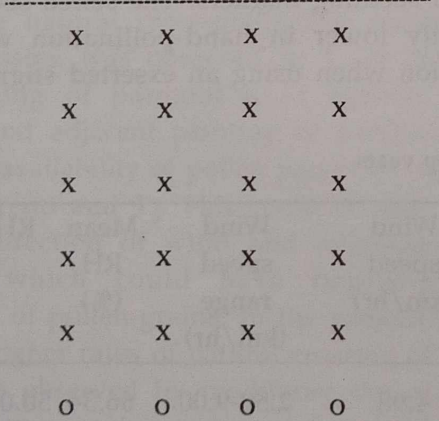
T<sub>1</sub> - NCP alternate row planting of parental lines  
(X = exserted stigma line, O = pollen parent)



T<sub>2</sub> - NCP alternate and adjacent planting of  
parents (X = exserted stigma line, O = pollen)



T<sub>3</sub> - NCP pollen parent in the centre surrounded  
by seed parent (X = exserted stigma line, O =  
pollen parent)



T<sub>4</sub> - Hand pollination without emasculatoin  
(X = exserted stigma line, O = pollen parent)

as seed parent in producing hybrid seed which could easily be rogued out using potato leaf seedling marker, to maintain the genetic purity. Thus, this male sterility is superior to conventional method of hybrid seed production in tomato where chance selfs cannot be detected easily. In male sterility system, maintenance of the parental lines is again a tedious process. Unlike these male sterility systems, the exserted stigma type of male sterility with potato leaf seedling marker is easier to maintain by artificial selfing because of the functional pollen of exserted stigma line.

To improve fruit setting in resultant F<sub>1</sub> hybrids by suitable selection of pollen parent with standard stigma positions, the results on usefulness of exserted stigma line linked with seedling marker for hybrid seed production in tomato would be quite relevant and useful. The present study would form a basis for better understanding of the use of exserted stigma line for advances in hybrid seed production of tomato. The results of hand-pollination without emasculatoin were more encouraging than natural cross-pollination for commercial hybrid seed production in tomato. Natural crossing as such was not that encouraging for commercial hybrid seed production purposes under the experimental conditions tests and require further standardization by appropriate choice of pollen parent.

REFERENCES

1. BULLARD ET AND STEVENSON EC (1953). Production of hybrid tomato seed. *Proc Am Soc Hort Sci* 61: 451-58
2. RICK CM (1945). Field identification of genetically male sterile tomato plants to use in producing F<sub>1</sub> tomato seeds. *Proc Am Soc Hor Sci* 46: 277-83
3. LAPUSHNER D AND FRANKEL R (1967). Practical aspects and the use of male sterility in the production of hybrid tomato seed. *Euphytica* 16: 300-10
4. YORDANOV M, YORDAOV M AND FRAKEL R (1983). Heterosis-Reappraisal of theory and practice. *Monographs in Theor Appl Genet* 96(6): 189-219
5. GEORGIEV H (1991). Heterosis in tomato breeding. In: Kalloo G (ed) *Genetic Improvement of Tomato*, Springer-Verlag, Berlin Heidelberg pp 83-98

Fig. 1. Planting pattern in experimental treatments

6. KALLOO G (1991). Genetic improvement of tomato. *Monographs in Theor Appl Genet* 14: 60
7. ATANASSOVA B (1999). Functional male sterility (PS-2) in tomato (*Lycopersicon esculentum* Mill) and its application in breeding and hybrid seed production. *Euphytica* 107: 13-21
8. ATANASSOVA B (2007). Genic male sterility and its application in tomato (*Lycopersicon esculentum* Mill) hybrid breeding and hybrid seed production. *Acta Horticulturae* 29: 45-51
9. TIKOO SK AND ANAND N (1980). Development of tomato genotypes with exerted stigma and a seedling marker for use as female parents to exploit heterosis. *Curr Sci* 49: 326-27
10. TIKOO SK AND PAL AB (1976). A spontaneous mutant with exerted stigma in tomato. *Annual Report of Tomato Genet Coop* 26: 18
11. OGNYANOVA A (1973). Inheritance of style and anther length and exerted stigma in two tomato crosses. *Tomato Genet Coop* 23: 25-26
12. ATANASSOVA B (1977). Inheritance of the components connected with exerted stigma in tomato F<sub>2</sub> and backcross generations. *Tomato Genet Coop* 27: 27-29
13. DASKALOV C, GEORGIEV C, DASKALOV KH, AND GEORGIEV KH (1976). *New Trends and Results of the Use of Heterosis in Tomato*. Elsevier Publishing Company, Amsterdam, Netherlands
14. KILCHEVSKY A AND DODRODKIN M (2000). Use of functional male sterility in seed production of tomato hybrids. *Acta Physiologiae-Plantarum* 22: 232-34
15. KANWAR JS (1989). Quality seed production technique of tomato. *Seeds and Farms* 15(6): 20-23
16. ANONYMOUS (1985). International rules for seed testing. *Seed Sci Technol* 13: 299-355
17. ABDUL-BAKI AA AND ADNERSON JD (1973). Vigour determination in soyabean seed by multiple criteria. *Crop Sci* 10: 31-34
18. JOLLI RB, VYAKARANAHAL BS, SHEKARAGOUDA M, DHARMATTI PR AND PATIL AA (2007). Studies on stigma receptivity of female parent in tomato hybrid seed production *Seed Res* 35(2): 151-54
19. SOOST RK AND RICK CM (1957). Effect of varieties of pollen and ovule parents on natural cross pollination of tomatoes. *Proc Am Soc Hor Sci* 70: 357-66
20. KUMAR JC AND DHALI WAL MS (1990). *Techniques of Developing Hybrids in Vegetable Crops*. Agrobotanical Publishers (India), Bikaner, pp 1-69
21. SCOTT JW AND GEORGE WL (1978). Breeding and combining ability of heterostylous genotypes for hybrid seed production in *Lycopersicon esculentum* Mill. *Euphytica* 29: 135-44
22. TUNWAR NS AND SINGH SV (1988). *Indian Minimum Seed Certification Standards*. Central Seed Certification Board, New Delhi, pp 269-72.