



STANDARDIZATION OF SEED PRODUCTION TECHNOLOGY IN HYBRID TOMATO (*Solanum lycopersicum* L.)

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ABSTRACT

The present study was aimed to standardize tomato hybrid seed production technology. The experiment was laid out in a randomized block design with each treatment replicated three times. The female parent stigma was pollinated once and twice and the process continued for seven days after emasculation to know stigma receptivity and to standardize best pollination method for higher seed set. Highest stigma receptivity was observed one day after emasculation (71.11%) and it was lowest on 7th day after emasculation (43.33%). Repeated pollination showed highest efficiency of fruit set (75.25%) compared to 60.47% in single pollination. Repeated pollination resulted in more fruit set (80%) with pollinations on 1st & 2nd, 2nd & 3rd and 3rd & 4th days after emasculation. Later a drastic decrease in fruit set was observed. Method of pollination and stigma receptivity showed no significant effect on seed quality and storability even after twelve months of storage.

Key words: Hybrid tomato, repeated pollination, seed set, seed quality and storability, stigma receptivity

INTRODUCTION

Tomato is the second major vegetable crop in India grown on an area of 0.882 million hectares with annual production of 18.7 million tones and average productivity of 19.5 t ha⁻¹ and the share of tomato in total vegetable production in India is 11.3% (National Horticulture Board, 2013). However, this productivity is far less than the world's productivity of 32.8 m ha⁻¹ (FAO, 2013). This huge gap could be narrowed down by following the appropriate seed production technology and through overcoming the constraints of production, including low pollination efficiency. The information on the methods of pollination efficiency in tomato hybrid is very meagre. Jolli *et al.* (2006) reported that the pollination after three days of emasculation gave significantly higher number of crossed fruits and fruit set which was followed by the pollination after 2 days of emasculation. Santosh and Malabasari (2014) also reported that pollination on the day of flower opening recorded significantly higher fruit set, fruit weight, seed weight fruit⁻¹ and seed yield plant⁻¹. The receptivity of stigma is most important in hybrid seed production particularly in tomato where manual emasculation, pollen collection and pollination are involved. Due to the non-availability of skilled labour during peak season hybrid seed production has become very expensive and has lead to the increased seed cost. Hence, it is imperative to know how long an emasculated stigma remains biologically and economically receptive without effecting seed yield and quality. In low volume

high value crops like tomato, it is also important to improve seed productivity in vertical mode by increasing the seed set per pollination. The present study on stigma receptivity and repeated method of pollination was conducted with aim to reduce the cost of cultivation of hybrid seed production and to increase seed set fruit⁻¹.

MATERIALS AND METHODS

The field experiments were carried out under greenhouse condition in the Centre for Protected Cultivation and Technology, IARI, New Delhi (India) and the laboratory experiments in the Division of Seed Science and Technology, IARI, New Delhi. The seedlings of two parents of hybrid tomato “Pusa Hybrid-4” (Pusa-120 and Chikoo) were raised in the multi-celled plastic plug trays having cell volume of 20 cm³ by using soil less media consisting of coco-peat, vermiculite and perlite in 3:1:1 ratio (v/v). Fertigation in nursery was done once a day and the concentration of NPK plus micronutrients used was from 20 to 80 ppm depending upon the growing stage of the nursery. Thirty day old tomato seedlings were transplanted in greenhouse and open field at a planting spacing of 50 x 60 cm and stacking was provided throughout the crop growth period. For emasculation, flower buds at late bud stage were selected. The corolla was opened and anthers carefully removed with the help of forceps in the afternoon (between 4 pm and 6 pm), one day before the anthers were expected to dehisce or mature and the stigma likely to become fully receptive and bud was tagged. For pollination, fully opened flowers were plucked in afternoon from pollen parents and anther cones were separated from sepals and spread over newspaper for drying overnight at ambient temperature. The pollen was then collected by putting anthers in one steel cup covered with another cup of the same size and a muslin cloth kept between them. The cups were shaken vigorously by holding tightly by hand against each other to separate pollen from the anthers. The pollen were filtered through muslin cloth, and the pollen and anther tissue collected in separate cups. This pollen was used for pollination in next morning. Collected pollen from freshly dehiscent anthers of male parent was used for dusting onto the stigmas of emasculated flower buds.

To study single method of pollination and stigma receptivity, 280 flower buds were emasculated and tagged on a single day. From the day of emasculation, on each day, 40 flower buds were pollinated and the process continued upto seven subsequent days. The method assessed were pollination on the same day of emasculation (SR0), and pollination done one, two, three, four, five, six and seven days after emasculation (SR1 to SR7). Ten days after pollination, flower buds were examined and per cent fruit set recorded. These fruits were harvested at maturity and the fruit weight and number of seeds fruit⁻¹ calculated.

To study repeated pollination, the above method was followed; in addition each bud was pollinated twice on two consecutive days. Treatments comprised of pollination on same day of emasculation and repeated next day (RP0) and pollination done 1, 2, 3, 4, 5, 6 and 7 day after emasculation and in each case repeated next day (RP1 to RP7):

For both the experiments fruit set percentage was estimated by counting the number of crosses turned into fruits in each treatment multiplied with hundred. Average weight of fruit was calculated as the weight of all the fruits divided by the total number of fruits. Seed number fruit⁻¹ was also counted. The standard germination test was conducted as per ISTA (1999). Seedling vigour index I and Seedling vigour index-II were estimated as per Abdul-Baki and Anderson (1973). The seedling length was measured from 10 randomly selected seedlings. The product of seedling length and germination percentage was tabulated for estimation of seedling vigour index I. Dry weight of 10 normal randomly selected seedlings from each replication was estimated oven drying them overnight at 60°C temperature (ISTA, 1999). Seedling vigour index II was calculated by multiplying seedling dry weight with germination percentage. The electrical conductivity was

measured by subjecting 50 seeds to 3-4 washings in distilled water, vigorously shaken followed by soaked in 25 mL distilled water at $25 \pm 1^\circ \text{C}$ temperature for 24 hr, with frequent stirring. The leachate was decanted and conductance measured using a conductivity meter and values expressed in $\mu\text{mhos cm}^{-1} 50 \text{ seeds}^{-1}$.

The seeds of each treatment replication-wise were packed in paper bags and kept at room temperature for six months. The storability of seeds was studied using germination percentage, vigour index and electrical conductivity parameters.

The field experiments were conducted using randomized complete block design and Laboratory experiments using completely randomized design. The data was statistically analyzed using analysis of variance (Gomez and Gomez, 1984). Wherever, necessary the data was transformed to angular (arc sine) values before subjecting them to statistical analysis.

RESULTS AND DISCUSSION

Effect of method of pollination on seed set

Stigma receptivity and single method of pollination: Stigma was observed to be highly receptive (71.11% fruit set and $79.0 \text{ seed fruit}^{-1}$) in treatment pollination one day after emasculation and lowest receptivity was observed in treatment pollination 7th day after emasculation (Fig. 1). With the increase in stigma age there was a gradual decrease in fruit set in both the years of study. The fruit set was more than 50% upto 5 days after emasculation. The fruit set (60.77%), fruit weight (62.69 g) and seed number fruit^{-1} (44.33) was significantly low when pollination was done on the same day of emasculation in comparison to the pollination done one day after emasculation. Stigma was highly receptive on a day after emasculation as it coincided with the day of anthesis (Sujatha *et al.*, 2015). The findings that pollination on the day of flower opening recorded significantly higher fruit set (71.43%), fruit weight (97.78 g), seed weight fruit^{-1} (4.04 g), seed yield plant^{-1} (58.48 g) are supported by Santosh and Malabasari (2014). Normally emasculation is done one or two day prior to the anthesis. It was observed that when emasculated flower bud was left open for longer period the stigma receptivity decreased, probably the stigmatic exudates might have dried due to exposure. Sometimes the stylar tissue also showed signs of drying up which appeared to have affected the growth of pollen tube to reach the ovule. Thus delayed pollination leads to reduced fruit set. The findings are in agreement with Sanjeev *et al.* (2008) who reported that fruit set, seed number fruit^{-1} ,

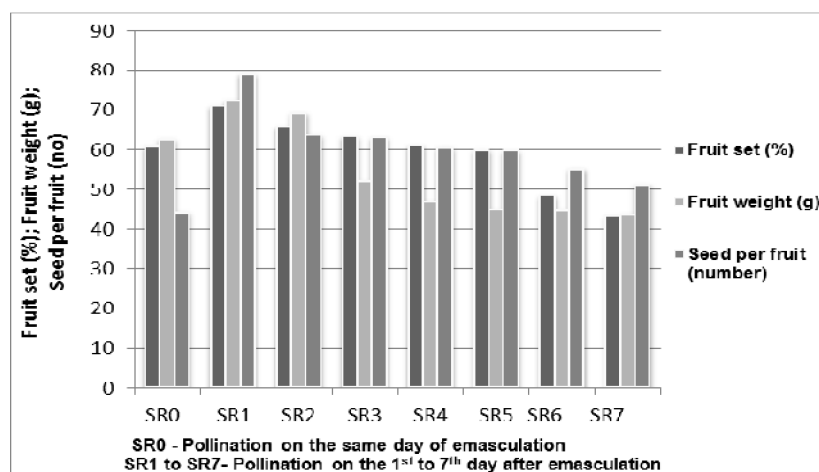


Fig. 1: Effect of stigma receptivity on the efficiency of Pusa Hybrid-4 tomato hybrid seed production

1000-seed weight, seed weight plant^{-1} and germination were significantly higher when pollination was done between 10 to 11 am one day after emasculation. Ofosu-Anim *et al.* (2006) reported that pollen grains failed to germinate on the stigma of sweet pepper on 3rd day after anthesis indicating a stigma receptivity of two days after flower opening. Our findings are in agreement with Chitra Devi (2000) who reported that under

Delhi conditions, in female parents of Pusa Hybrid 1, Pusa Hybrid 2 and Pusa Hybrid 4, the stigma was receptive for 3 days after anthesis. Valdes (1995) reported that pollination done 2 or 3 days after emasculation increased fruit set by 19 and 28%, respectively, as compared to one day after emasculation. Delayed pollination for 24 to 30 h increased seed yield and seed size by 8.6 and 10.2%, respectively in two tomato varieties (Jankulovski *et al.*, 1997). The stigma was reported to be receptive for 7 days after emasculation (Auerswald, 1978). However, Yogeesh *et al.* (1999) reported that the stigma became receptive on 2nd days after emasculation in 'Pusa Sheetal' and 'Pusa-120' and remained so for another two days and declined thereafter. Jolli *et al.* (2006) reported that the pollination after three days of emasculation gave significantly higher number of fruits and fruit set which was followed by the pollination after 2 days of emasculation. The milder conditions of greenhouse might have extended the stigma receptivity upto 7 days in the present study.

Repeated method of pollination: About 82.44% fruit set, 77.02 g fruit weight and 67.54 seeds fruit⁻¹ was observed when emasculated bud was pollinated twice on 1st and 2nd days after emasculation and the same trend was noticed for about 4 days and afterwards a drastic decrease was observed (Fig. 2). More than 60% hybrid seed production efficiency was noticed even at 7th day after emasculation. Seed number fruit⁻¹ decreased with increase in the age of emasculated bud. Higher fruit set with repeated method of pollination could be attributed to the availability of more pollen for fertilization (Sujatha *et al.*, 2013). As the stigma got pollinated two times on consecutive days the ovules whichever left without fertilization on previous day due to lack of proper amount

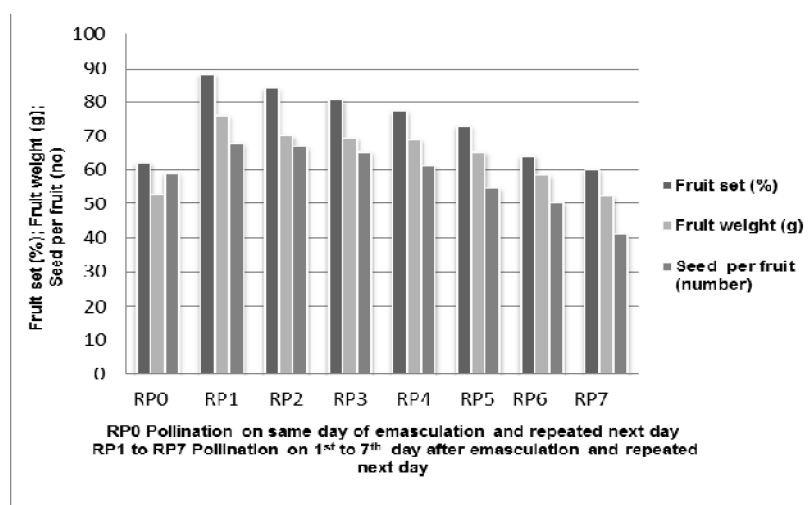


Fig. 2: Effect of repeated pollination on the efficiency of Pusa Hybrid-4 tomato hybrid seed production

of pollen got a chance to get fertilized. As the pollen was made available right in time it led to increase in fruit set. With the development of embryos the size of fruit also increased in repeated pollination as compared to single pollination. As maximum number of ovules got fertilized and were able to develop into seeds, the seed number fruit⁻¹ and ultimately the seed yield increased with repeated pollination (Sujatha *et al.*, 2015).

Effect of method of pollination on seed quality

Stigma receptivity and single method of pollination: The initial mean seed germination was 96% (Table 1). There was a significant difference in the germination of seeds produced from the pollination of one day after emasculation to 7th day after emasculation. The mean values of seedling vigour index I and II were 1627 and 1857. These values for SVI-I, SVI-II and EC varied significantly among treatments. The EC value increased with delay in pollination of stigma.

The mean seed germination and other seedling quality parameters like seedling length, seedling dry weight, seedling vigour index-I, seedling vigour index-II and electrical conductivity were better in the seeds produced from the crossings upto three days after emasculation (Table 1). Probably the pollination done when stigma was most optimum for fertilization must have helped in the proper development of embryo in the seeds produced. These finding are in agreement with Jankulovski *et al.* (1997) who reported in tomato varieties that delayed pollination (24 to 30 h after emasculation) resulted in 8.6 and 10.2% seed yield reduction, respectively. Jolli *et al.* (2006)

Table 1: Effect of single time method of pollination and stigma receptivity on seed quality

Day of pollination (after emasculation)	Seed germination (%)	Seedling length (cm)	Seedling dry wt. (mg)	SVI-I	SVI-II	EC ($\mu\text{mhos cm}^{-1}$ 50 seeds ⁻¹)
Same	99 (88)*	18.13	19.44	1806	1937	8.3
1 st	98 (82)	16.63	19.87	1635	1953	8.5
2 nd	99 (88)	15.86	19.64	1580	1957	8.6
3 rd	97 (82)	16.36	19.5	1505	1794	9.8
4 th	97 (82)	13.96	19.38	1542	1873	10.4
5 th	97 (82)	13.76	19.12	1533	1860	10.9
6 th	97 (84)	20.22	18.39	1968	1789	11.1
7 th	94 (76)	15.26	17.86	1444	1690	12.9
Mean	96 (82)	16.26	19.15	1627	1857	10.1
CD _{0.05}	4.02	0.48	1.72	46.19	73.65	0.37

SVI-I = Seedling vigour index - I; SVI-II = Seedling vigour index - II; EC = Electrical conductivity;

* Arc sine transformation values in paranthesis

reported that the pollination within 3 days of emasculation gave higher number of crossed fruits (42.91), fruit set (55.89%), seed yield plant⁻¹ (11.7 g) and higher germination (94.93%), field emergence (90.13%) and seedling vigour index (1709) as compared to the delayed pollination.

Repeated method of pollination: The mean seed germination was 99% with no significant difference between the treatments (Table 2). Other seedling characters like shoot length, root length and seedling dry weight showed significant difference between the treatments and their mean values were 7.45, 8.81 and 19.15, respectively. The value of EC increased with delay in stigma pollination after emasculation. The value of EC varied significantly between the treatments. Low EC value was recorded in seeds produced within 3 days of emasculation and in rest treatment it was comparatively high. The probable reason may be pollination done when stigma was most optimum for fertilization must have helped in the proper development of the embryo in the seeds produced. These observations are in line with Jolli *et al.* (2006) who reported that pollination within 3 days of emasculation gave higher seed germination (94.93), field emergence (90.13%) and seedling vigour index (1709) as compared to delayed pollination.

Table 2: Effect of repeated method of pollination on seed quality parameters

Day of pollination* (after emasculation)	Seed grmination (%)	Seedling length (cm)	Seedling dry wt. (mg)	SVI-I	SVI-II	EC ($\mu\text{mhos cm}^{-1}$ 50 seeds ⁻¹)
Same	99 (87)**	17.46	19.97	1734	1983	8.9
1 st	99 (87)	18.18	19.57	1818	1957	9.6
2 nd	99 (85)	16.86	19.76	1669	1956	10.5
3 rd	99 (88)	17.46	17.33	1742	1727	10.7
4 th	99 (88)	19.86	18.20	1979	1813	11.0
5 th	99 (86)	19.96	17.57	1976	1739	11.3
6 th	99 (86)	19.86	19.67	1966	1953	11.5
7 th	97 (84)	19.46	19.57	1940	1950	11.9
Mean	99 (87)	18.26	18.95	1792	1885	10.6
CD _{0.05}	4.60	0.38	1.24	53.34	78.24	0.57

*In all the treatments pollination was repeated next day.

SVI-I = Seedling vigour index-I; SVI-II = Seedling vigour index-II; EC = Electrical conductivity

** Arc sine transformation values in paranthesis

Table 3: Effect of single pollination and stigma receptivity on seed storability at six months storage

Day of pollination (after emasculation)	Seed germination (%)	Shoot length (cm)	Root length (cm)	Seedling dry wt. (mg)	SVI-I	SVI-II	EC ($\mu\text{mhos cm}^{-1}$ 50 seeds ⁻¹)
Same	94 (76)	7.37	8.60	18.56	1501	1744	9.87
1 st	88 (70)	8.63	11.23	18.13	1747	1595	10.2
2 nd	94 (76)	7.83	11.33	17.38	1801	1633	10.5
3 rd	89 (71)	9.30	10.67	16.29	1783	1455	11.9
4 th	87 (69)	7.70	8.40	15.15	1395	1313	11.0
5 th	86 (68)	7.97	9.30	15.02	1490	1296	11.0
6 th	85 (67)	9.67	8.93	17.61	1574	1491	11.7
7 th	94 (76)	7.53	8.17	18.87	1475	1773	11.9
Mean	90 (72)	8.25	9.58	17.83	1596	1537	11.0
CD _{0.05}	3.44	0.47	0.44	1.21	36.52	65.23	0.65

SVI-I = Seedling vigour index-I; SVI-II = Seedling vigour index-II; EC = Electrical conductivity

*Arc sine transformation values in paranthesis

Effect of method of pollination on seed storability

Single method of pollination: There was a slight reduction in the mean seed germination (from 96 to 90%) at 6 months of ambient storage condition (Table 3) and the variation among the treatments was non-significant. Less seed germination (85%) was observed in 6th day pollination treatment. The quality parameters like shoot length, root length and seedling dry weight were also reduced at the end of storage period and the values did not differ significantly between the treatments. There was a decrease in vigour index I and II when compared to the seed quality at initial stage. The value of EC also did not differ significantly though the mean value increased from 10.10 to 11.00 at six months storage. The EC values increased with increase in stigma age after the emasculation. The EC value increased with storage because the seed deterioration is an unavoidable process which proceeds with increase in seed age.

Repeated method of pollination: There was no drastic reduction in seed germination (99 to 94%) and other seedling quality parameters at six month storage (Table 4). The mean values of shoot length, root length, seedling dry weight, seedling vigour index I, seedling vigour index II and EC was 8.21, 9.97, 18.35, 1704.80, 1720.46 and 10.87. All the above parameters differed significantly. This difference of reduction in all the seed quality parameters is attributed to the seed deterioration which advances with the period of storage.

Table 4: Effect of repeated pollination on seed storability at six months storage

Day of pollination (after emasculation)*	Seed germination (%)	Shoot length (cm)	Root length (cm)	Seedling dry wt. (mg)	SVI-I	SVI-II	EC ($\mu\text{mhos cm}^{-1}$ 50 seeds ⁻¹)
Same	92 (74)	8.27	10.30	16.43	1714	1516	9.4
1 st	93 (75)	8.40	9.87	16.67	1705	1555	9.9
2 nd	95 (78)	8.80	9.17	16.96	1713	1616	10.5
3 rd	96 (79)	8.50	9.57	17.91	1740	1725	10.8
4 th	94 (76)	8.67	10.97	17.82	1846	1675	11.7
5 th	93 (75)	9.17	9.70	19.61	1754	1823	11.3
6 th	96 (79)	7.30	9.63	19.63	1630	1890	11.5
7 th	90 (71)	8.57	10.60	20.81	1718	1866	11.9
Mean	94 (76)	8.21	9.97	18.35	1704	1720	10.87
CD _{0.05}	4.15	0.38	0.46	1.23	51.32	68.91	0.56

*In all the treatments pollination was repeated next day. The figures in parenthesis are arc sine transformation values. SVI-I = Seedling vigour index-I; SVI-II = Seedling vigour index-II; EC = Electrical conductivity

The method of repeated pollination (pollinating the same stigma on two consecutive days) increased the fruit set, fruit weight and number of fruits seed⁻¹. Therefore, hand emasculation and pollination method is used for hybrid seed production and abundance of pollen as well as ovules are there to be fertilized, repeated pollination may be more rewarding. These findings would enable the seed producers to know when to emasculate and pollinate, for how long can we pollinate an emasculated bud, how many times an emasculated bud to be pollinated to achieve highest fruit and seed set percent. The high value commercial crop, low seed requirement and large number of seed produced per pollination makes tomato hybrid seed production commercially more viable under green house conditions.

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