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Influence of female to male flower crossing ratios on hybrid seed yield and quality in tomato *(Solanum lycopersicum)* cv. Arka Abhijit*

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Tomato (*Solanum lycopersicum* L) is one of the important vegetable crops in India, which occupies an area of 5,72,000 hectares with a production of 1,02,61,000 metric tonnes per hectare and productivity of 17.9 metric tonnes per hectare (Kumar, 2008). In tomato, a number of F_1 hybrids are popular due to their uniformity, resistance to pests and diseases and higher yield. The demand for hybrid seeds is also higher among the farmers as they provide high economic returns. The conventional method of hand emasculation and hand pollination is cumbersome, labour-intensive and time consuming. This results in high cost of hybrid seeds. Till date, there are no male sterile lines available for commercial hybrid seed production in tomato. The yield of hybrid seeds on artificial pollination depends on the quantity of pollen deposited on the receptive stigma of female flowers. If the load of pollen on stigma is less, seed set may be reduced. If excess pollen is used for crossing, there could be a higher than optimum competition for pollen germination and fertilization resulting in poor seed set. Published reports indicate that pollen from one flower is sufficient for pollinating 18-20 flowers (Dev, 1998) and for pollinating four female flowers (Operia *et al*. 2001, Kumar *et al*. 2008).

There has been very meager published research work in hybrid seed production of tomato. The load of pollen deposited artificially on stigma during pollination has a definite role to play on the fruitset and the resultant hybrid and seed quality (Kumar *et al*. 2008 and Nerson, 2009). Efficient pollination and successful fertilization is needed to maximize crossing efficiency and hybrid seed set. Hence, there is an acute need to standardize the optimum female to male crossing ratios in pollination specific to each hybrid combination, based on flowering habits of the seed parent. In tomato hybrid Arka Abhijit, since the seed parent is a semi-determinate type and pollen parent a determinate type, the pollen availability for pollination is important in hybrid seed production. This study is important in hybrid seed production of cv.Arka Abhijit as it decides the maximum number of seed parent plants to be maintained in a unit area for higher seed yield in relation to the male parent population.

The objective of the study was to optimize the crossing ratio requirements of female to male crossing ratios by studying the effect of pollen load deposited by hand pollination on the receptive stigma of emasculated female flowers on the per cent fruitset, seed set and seed quality in hybrid seed production of tomato cv.Arka Abhijit.

An experiment was conducted to study the effect of female and male flower ratios on fruit set (%), hybrid seed yield and quality in tomato at Indian Institute of Horticultural Research, Bangalore during *rabi* season of 2005 and 2006. The experimental material used in this study was the parental lines of the hybrid Arka Abhijit of which the seed parent is semi-determinate and pollen parent is determinate in flowering habit. The experimental design adopted was Randomised Block Design with seven replications.

The experiment consisted of three female to male crossing ratio hand pollination treatments, namely, 3:1 where three emasculated flowers of seed parent were crossed with pollen from one male flower (similarly multiples of 3:1 ratio i e 6,9,12,15 female flowers were crossed with 2,3,4 and 5 male flowers respectively), 4:1 where four emasculated flowers of seed parent were crossed with pollen from one male flower (similarly multiples of 4:1 ratio i e 8,12,16,20 female flowers were crossed with 2,3,4 and 5 male flowers respectively) and 5:1 where five emasculated flowers of seed parent were crossed with pollen from one male flower (similarly multiples of 5:1 ratio, i e 10,15,20 and 25 female flowers were crossed with 2,3,4 and 5 male flowers

*Short note

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respectively). The pollen counts in total of the six anthers of a single flower of the pollen parent were recorded in an average of five flowers. The pollen count per male flower ranged from 7 500 to 10 620 with a mean of 9 331.2. It was adequately ensured that the pollen from a single flower of pollen parent was equally deposited among the emasculated flowers of female parent by dividing the total pollen from a single flower in to three, four and five equal portions for the crossing ratios 3:1, 4:1 and 5:1 respectively. The number of pollen grains in each flower of pollen parent was counted by diluting the pollen in a drop of water placed on a glass slide and counting the total pollen grains from each anther under a microscope at 10X magnification and multiplied by the total number of anthers per flower. The load of pollen deposited on the stigma of female flower in 3:1, 4:1 and 5:1 were approximately 3 110, 2 332 and 1 866 pollen grains respectively. Emasculation of mature female flower buds was carried out in the previous day evening one day prior to flower anthesis by forceps, without injuring the stigma, covered and tagged.

Pollination was carried out in the morning on the next day of flower anthesis when the petals have turned bright yellow in colour which is also the maximum time of stigma receptivity. Three flowers in all the flower trusses on the plant were subjected to pollination treatments on the semi-determinate seed parent. For manual pollination, fresh pollen collected in the morning from male flowers, on the day of anther dehiscence (two days after flower anthesis at bright yellow colour petal stage) with highest pollen viability were filled in rings with cavities. Pollination was effected by dipping the stigma of female flower into the pollen filled cavity in the morning hours between 9 AM and 12 noon. Adequate care was taken to ensure that no selfed fruit was allowed to set on the plant and no opened flower was emasculated. Crossing was carried out for one month from start of flowering. The spacing adopted was 120 cm row-to-row and 90 cm plant-to-plant. Standard seed production practices such as recommended isolation distance of 200 m between two different varieties were followed to avoid any chance genetic contamination. Observations were recorded on fruitset (%), number of seeds/fruit and seed weight/fruit for seed yield contributing characters. Hundred seeds were counted and weighed for obtaining 100 seed weight and expressed in gram. The seed quality parameters recorded were germination (%), shoot length, root length, seedling length and vigour index.

The per cent germination (first and second count) was determined by paper towel method (ISTA 1985) after 7 days and 14 days of placing the seeds at 25°C. The total seedling length was measured by the total length of the seedling from root tip to shoot tip in 10 randomly chosen normal seedlings in each replication and treatment. Seedling vigour index was calculated by multiplying germination per cent with total seedling length.

The pooled data on seed yield attributing characters for two years were analysed by Analysis of variance method (Gomez and Gomez 1984). A completely randomized design for seed quality characters was studied for 2005 only because significant results of fruitset (%) and number of seeds per fruit were observed. The percentage data on fruitset and germination were subjected to angular transformation and used for statistical analysis (Panse and Sukhatme 1989).

The effect of female to male flower crossing ratios on hybrid seed yield contributing characters in tomato (pooled data of two years) has been presented in Table 1.

The mean fruitset (%) for combined data of two years was highest (60%) in the treatment 4:1 ratio. There was a definite increasing trend for fruitset (%) in 2005 and 2006 and in pooled data of 2005 and 2006 in 4:1 ratio although the results were significant in 2005 only. The year 2005 showed significant differences among the treatments with a maximum value in 4:1 ratio (66% fruitset).This indicated that 4:1 is the optimum ratio for crossing although no significant differences existed between the treatments for percent fruitset. Relatively higher fruitset recorded in 4:1 ratio could be attributed to the optimum availability of pollen (approximately 2 332 pollen grains on stigma of female flower) to effect fruitset without any pollen competition and higher rate of fertilization. The ratio 5:1 recorded the lowest per cent fruitset (40.51). The low fruitset observed in 3:1 ratio and 5:1 ratio might be due to higher level of pollen competition resulting in reduced pollen germination and pollen tube growth due to increased pollen load in 3:1 ratio and insufficient viable pollen grains reaching the ovules for fertilisation in the ovary due to low pollen load in 5:1 respectively. The results are in accordance with that of Kumar *et al.* (2008). Fruit set has also a direct correlation with the stigmatic pollen load in tomato influenced by pollinators (Del Sarto *et al.* 2005, Hogendoorn *et al*. 2006).

With regard to seed weight per fruit, no significant differences existed between the treatments. However, the superior treatment based on pooled mean of two years for seed weight per fruit was 4:1 ratio (0.32g). There existed no statistical differences between the treatments for seed weight per fruit in 2005, 2006 and pooled data of the years 2005 and 2006 which indicated that there was no effect of pollen load on seed weight per fruit and similar seed weights were obtained using minimal or higher pollen loads of 5:1 or 3:1 crossing ratios respectively. The present findings are in contrast to that of kumar *et al.* (2008) who reported that significant differences existed between the female to male flower crossing ratios for seed yield. This could be due to varietal differences of seed and pollen parent, relative number of ovules/fruit and pollen production ability per male flower.

The number of seeds/fruit was highest and significant in the year 2005 in the hand pollination treatment, 3:1 ratio (197.4) compared to 4:1 and 5:1 ratios. However, there were no significant differences between the crossing ratio

Table 1 Effect of female to male flower crossing ratios on hybrid seed yield contributing characters in tomato

Treatments	Fruit set (%)			Seed weight /fruit (g)			100 seed weight (g)			No. of seeds/fruit		
	2005	2006	Pooled mean	2005	2006	Pooled mean	2005	2006	Pooled mean	2005	2006	Pooled mean
3:1	54.0 (47.5)	38.06 (37.6)	46.03 (42.6)	0.32	0.27	0.30	0.18	0.26	0.22	197.4	110.5	153.9
4:1	66.0 (50.7)	54.01 (49.1)	60.00 (49.5)	0.29	0.35	0.32	0.24	0.28	0.26	114.0	95.3	104.6
5:1	30.0 (33.0)	51.02 (47.8)	40.51 (40.0)	0.27	0.30	0.29	0.26	0.39	0.33	118.8	121.9	120.4
CD (*P*=0.05)	12.68	NS	NS	NS	NS	NS	NS	NS	NS	76.16	NS	NS

Data in parentheses are angular transformed values

treatments for number of seeds/fruit in 2006. The pooled data of 2005 and 2006 also indicated highest number of seeds per fruit in 3:1 ratio although there was no definite trend observed with regard to the load of pollen and the results were statistically significant in 2005 only. The superiority for number of seeds per fruit in 3:1 ratio could be attributed to the higher load of pollen on stigma (31.10 pollen grains approximately) compared to other treatments. Similar reports of increase in the number of seeds/fruit with a linear increase in quantity of pollen placed on the stigma have been reported by Singh (2001) in tomato. In the present study, a definite linear trend was not observed and even treatment of lower pollen load (5:1) recorded relatively higher number of seeds/fruit compared to 4:1 ratio indicating that seed formation is not only dependant on pollen load but on availability of more readily distributed pollen and a greater proportion of viable pollen resulting in more pollen tubes reaching the ovary. Although, 4:1 ratio recorded higher values for per cent fruitset, the number of seeds per fruit was lowest due to individual fruit competition and dominance for the available assimilates. The fruit-set in plants is related to source–sink ratio (Kang *et al* 2011).With regard to 100 seed weight also, no significant differences existed between the treatments 3:1, 4:1 and 5:1 crossing ratios. However, relatively higher values for test weight were recorded in 5:1 ratio perhaps due to lesser pollen competition (1866 pollen grains per stigma of female flower) resulting in bolder seeds after fertilization.

The effect of female to male flower crossing ratios on hybrid seed quality characters in tomato has been presented in Table 2. Significant differences existed between the crossing ratios for hybrid seed quality in tomato. Highest germination (%) based on first count was observed in the crossing ratio of 5:1 (65.50) However, the superior germination per cent based on second count was observed in the crossing ratios of both 5:1 and 3:1. There were no significant differences between the treatments for shoot length, root length and seedling length. The seedling vigour index was highest in 5:1 ratio (1 483.6) based on first count on germination. The higher seed germination and vigour observed in the crossing ratio of 5:1 could be attributed to a higher 100-seed weight of seeds due to lesser pollen competition (1866 pollen grains on stigma approximately) observed in both the crop years (0.26g and 0.39g respectively). However,3:1 crossing ratio recorded the highest vigour index (1 747.86) based on second count on germination which was at par with 5:1 ratio. In 3:1 ratio, the pollen competition is high which has also influenced the progeny vigour. Intense competition between pollen to access the ovules often increases the progeny vigour (Windsor *et al*. 2000).

Although percent fruitset and number of seeds/fruit was higher in the crossing ratio of 4:1 in 2005, the germination (%) and seedling vigour index (based on I and II count) were less in comparison to crossing ratios of 3:1 and 5:1. Hence, it is inferred that although the differences in pollen load have influenced per cent fruitset and number of seeds/fruit, however resulted in slight decrease in seed quality in tomato in 4:1 in 2005. The results of significant differences in seed quality

Table 2 Effect of female to male flower crossing ratios on hybrid seed quality in tomato

Treatments	Germination (%) I count	Germination (%) II count	Shoot length (cm)	Root length (cm)	Seedling length (cm)	Seedling vigour Index based (on I count)	Seedling vigour index (based on II count)
3:1	54.30 (47.42)	74.88 (59.97)	12.50	10.82	23.32	1259.20	1747.86
4:1	43.20 (40.68)	60.19 (50.92)	12.90	9.58	22.48	968.7	1350.02
5:1	65.50 (54.03)	74.81 (59.90)	12.62	9.98	22.60	1483.6	1689.06
CD (*P*=0.05)	5.92	8.75	NS	NS	NS	239.5	325.45

Data in parentheses are angular transformed values

among the crossing ratios are also in concurrence with that of Kumar *et al*. (2008). Hence, in hybrid seed production of Arka Abhijit, with semi-determinate female parent and determinate pollen parent, scarcity and flexibility of the pollen availability and quantity of pollen used for crossing could be overcome by crossing even up to 5:1 ratio (female and male flower ratio) which pertains to a pollen load of approximately 1866 pollen grains on stigma without affecting seed yield contributing characters and seed quality.

SUMMARY

An experiment was conducted to study the effect of female and male flower crossing ratios on fruitset (%), seed set, seed yield per fruit and seed quality in tomato cv. Arka Abhijit at Indian Institute of Horticultural Research, Bengalury. The results indicated that there were no significant effects of female to male flower crossing ratios namely, 3:1, 4:1 and 5:1 on seed yield attributes namely, fruitset (%), number of seeds per fruit, seed weight per fruit and 100-seed weight except for higher mean percent fruitset in 4:1 ratio (66) and higher number of seeds per fruit (197.4) in 3:1 ratio in the year 2005. However, the female to male crossing ratio of 5:1 resulted in significant increase in hybrid seed quality for the characters seed germination (65.50 % and 74.81%) and seedling vigour index (based on I and II count) of 1 483 and 1689 respectively in tomato. Hence, in hybrid seed production of Arka Abhijit, pollen scarcity and quantity was not found to be a limiting factor and could accommodate crossing up to 5:1 (female to male flower ratio) which pertains to a pollen load of approximately 1866 pollen grains on stigma without compromising on seed yield and quality.

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