Seed Research, Vol. 36(1): 18-23, 2008

Studies on Pollen Viability of Male Parent in Tomato Hybrid Seed Production

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ABSTRACT In order to know the pollen viability for hybrid seed production in tomato a study on pollen viability was undertaken at Agricultural Research Station, Hanumanamatti of Haveri District in Karnataka during 2002 and 2003. In this study the pollens where stored for one day, two days and three days under ambient and refrigerated condition along with fresh pollen were used for pollination in the production of hybrid seed of DTH-1. The results revealed that there was a significant variation in pollen viability due to age of the pollen and storage conditions. Pollen stored under refrigerated condition for two days found significantly superior with respect to fruit set, fruit yield, seed yield and quality as compared to ambient stored and use of fresh pollen for pollination.

Key words: Pollen viability, fruit set, seed yield, quality, hybrid seed production

The knowledge of pollen viability and stigma receptivity is very important in hybrid seed production, particularly in crops like tomato where manual emasculation, pollen collection and hand pollinations are involved. The information on pollen viability will enable the seed producer to under take emasculation and pollination for successful hybrid seed production of tomato. Particularly the knowledge on how old pollens can be used and how long the pollination can be continued will play an important role. Further, the hybrid seed production is normally organized in rural areas where controlled storage facilities do not exist. The collection of pollen and its storage for use in manual pollination is of immense importance especially under adverse weather conditions which are not favourable for pollination. In the present study pollen viability of male parent SP-2-2 was assessed, based on fruit set percentage, fruit yield, seed yield and seed quality parameters.

MATERIALS AND METHODS

The study was conducted at Agricultural Research Station, Hanumanamatti of Haveri District in Karnataka, during *rabi* season of 2002 and 2003. The material for the study consisted of two parents of Arka Alok, (Female parent) and SP-2-2 (Male parent). The seedlings were raised in raised seed bed and twenty five days old seedlings were transplanted in the crossing block. In order to get sufficient pollen, staggered planting of male parent was done. Fifty per cent of the male seedlings were transplanted one week prior to the transplanting of the female parent. The remaining fifty per cent of the seedlings of male parent was planted along with female parent after one week of transplanting of first set of male parent with a spacing of 60 x 60 cm. Five meter isolation distance was maintained between female and male parent blocks, gap filling was done seven days after transplanting. The recommended cultural practices were followed during crop growth and stalking was provided at the time of flowering.

Fully opened flowers were plucked and collected in a polythene bag during early hours of the day. Anther cones were separated and spread on a cloth and exposed to sun for three hours. Dried anthers were kept in a steel cup covered

Received July 2006 Revised November 2006 Accepted April 2008

with muslin cloth and another steel cup in an inverted position was placed over it. This was agitated rigorously. The pollens were separated from the anthers and filtered trough muslin cloth in the empty cup. Separated pollens from the anthers in this way were transferred to plastic container with the help of a camel hair brush. The plastic cup was kept in a cool place over night under ambient condition. These stored pollens were used for pollination of the emasculated flower buds.

Emasculation

Flower buds, which were expected to open on next day, were selected, emasculation was done between 2.00 to 6.00 pm using pointed forceps and emasculated flower buds were labelled and emasculation was continued up to 25 days.

The pollen stored under different conditions were collected in plastic pollen ring specially designed to carry out pollination. The stigma of the emasculated flower buds were dipped in the pollen ring to effect pollination. Two calyx of the pollinated flower buds were given half cut for identification. The pollination was carried out between 8.00 am to 12.00 noon. This experiment consisted of two factors *viz.*, the first factor-storage of pollen (M) consisted of storage of pollen in ambient condition (M_1) and storage of pollen in refrigerator (M_2), and the other factor was pollination with stored pollens (P) : Pollination with one day old pollen (P_1), pollination with two days old pollen (P_2), pollination with three days old pollen (P_3) and pollination with fresh pollen as control (P_0). The experiment was laid out in randomised block design with three replications. Out of 20 plants in net plot area five plants from each treatment were selected at random and tagged for recording the biometric observations such as number of flowers pollinated per plant, number of crossed fruits per plant, fruit yield and seed yield per plant, number of seeds per fruit and seed weight per fruit. Seed germination, field emergence percentage were recorded as per ISTA procedure [1]. While, the vigour index was computed as per the formula suggested by Abdul Baki and Anderson [2]. The data was statistically analyzed employing the randomized block design procedure [3].

RESULTS AND DISCUSSION

Fruit set percentage

The analysis of pooled data indicated that the pollen storage conditions did not influence the fruit set percentage, however the pollen stored in refrigerator (M_2) recorded higher fruit set percentage (53.28) over the ambient storage conditions (50.78). Two days old pollens (P_2) recorded significantly higher per cent of fruit set (55.69) compared to one (48.80) and three days (51.69) old pollens, used for pollination. This indicates that the two days old pollen attained full maturity and were cent per cent viable. Similar results were also reported by Chithra Devi [4]. Interaction effect of storage conditions and use of stored pollens did not show any significant difference for fruit set percentage. But when these treatment combinations were compared with control (fresh pollens), significant differences were observed. But use of fresh pollen for pollination recorded low (43.99) fruit set. (Table 1). This may be due to the use of immature pollens on fully receptive stigma resulting in failure of pollen germination and fertilization, as it was observed in case of stored pollens also. Similar results were also reported by Dev [5], who opined that the pollen of just picked flower does not have the highest fertilization ability as the pollen grains are not fully viable but on the next day greater number of pollens attained the maturity and viability.

Crossed fruit yield per plant

The crossed fruit yield per plant showed that the pollen stored in refrigerator (M_2) recorded significantly higher fruit yield of 1844.42g per plant than the ambient (M_1) one (1466.40g). This may be due to higher viable matured pollens found in the refrigerated conditions. In ambient storage conditions deterioration of pollen grains due to fluctuation in temperature and relative humidity might have affected the viability of pollens (Table 1). Two days old pollen (P_2) when used for pollination recorded significantly higher fruit yield of 1846.40g per plant compared to three days (1664.65g) and one day old pollen (1439.99g) used for pollination. This may be due to the increase in the yield components such as fruit weight, number of crossed fruits retained and fruit set percentage.

Table 1. Effect of pollen storage conditions and age of pollen on number of flowers crossed, number of crossed fruits, fruit set percentage and crossed fruit yield per plant in tomato hybrid (DTH-1)

Treatments	No. of flowers crossed/plant			No. of crossed fruits/plant			Fruit set (%)			Crossed fruit yield per plant (g)		
	2002	2003	Pooled	2002	2003	Pooled	2002	2003	Pooled	2002	2003	Pooled
Pollen storage (M) method												
M_1	75.41	77.13	76.27	37.76	39.57	38.67	50.18	51.48	50.78	1456.73	1456.07	1466.40
M_2	77.21	78.84	78.03	40.62	44.92	41.28	52.62	53.95	53.28	1837.79	1850.78	1844.42
S.Em+	1.73	1.16	1.42	1.56	1.52	1.55	0.19	2.11	1.28	19.04	18.28	18.92
CD at 5%	NS	NS	NS	NS	NS	NS	0.59	NS	NS	57.11	56.33	56.75
Age of pollen (P)												
P_1	78.14	80.51	79.33	37.71	38.84	38.28	48.29	49.31	48.80	1438.99	1440.98	1439.99
P_2	75.55	76.42	75.99	41.20	43.26	42.23	54.51	56.87	55.69	1853.90	1838.90	1846.40
P_3	75.23	77.03	76.13	38.66	40.13	39.40	51.40	51.97	51.69	1648.90	1680.39	1664.65
S.Em±	2.11	1.42	1.79	1.91	1.86	1.89	0.23	2.59	2.41	23.01	22.38	22.94
CD at 5%	NS	NS	NS	NS	NS	NS	0.72	NS	NS	69.05	68.99	68.80
Interaction (M x P)												
M_1P_1	78.73	80.67	79.70	35.31	37.36	36.34	44.84	46.26	45.55	1339.73	1341.76	1370.75
M_1P_2	74.29	75.37	74.83	40.09	41.96	41.03	53.96	56.04	55.00	1605.18	1572.83	1589.01
M_1P_3	73.20	75.34	74.27	37.88	39.38	38.63	51.74	52.13	51.80	1425.27	1453.61	1439.44
M_2P_1	77.55	80.34	78.95	40.12	40.32	40.22	51.73	52.35	52.04	1538.24	1540.98	1539.61
M_2P_2	76.82	77.47	77.15	42.30	44.56	43.43	55.06	57.70	56.38	2102.61	2104.97	2103.79
M_2P_3	77.26	78.72	77.99	39.50	40.87	40.09	51.06	51.80	51.43	1872.53	1907.17	1889.85
S.Em+	2.89	2.01	2.52	2.70	2.63	2.70	0.33	3.66	2.41	31.89	31.65	31.88
CD at 5%	NS	NS	NS	NS	NS	NS	1.01	NS	NS	95.66	97.57	95.61
Control	75.28	76.33	75.81	36.02	36.66	36.8443.85		44.12	43.99	1250.10	1258.45	1254.28
Control vs Rest of the treatments												
S.Em+	2.78	1.58	1.83	2.14	2.07	2.10	0.26	2.89	1.93	25.73	25.18	26.08
CD at 5%	NS	NS	NS	NS	NS	NS	0.80	8.90	5.80	77.09	75.50	78.21

NS - Non significant; M_1: Storage of pollen in ambient condition; M_2 : Storage of pollen in refrigerator; P_1: Pollination with one day old pollen; P_2 : Pollination with two days old pollen; P_3 : Pollination with three days old pollen

Two days old pollen showed higher seed recovery (4.58) per cent (Table 2) as compared to one (4.01) and three days old (4.30) pollen used for pollination. The interaction effect were non-significant, however, two days stored pollen in refrigerator recorded higher seed (4.72) recovery per cent and lowest (3.53) in fresh pollen as was also reported by Sidhu *et al.*, [6].

The treatment combination of two days old pollen and stored in refrigerator gave significantly highest fruit yield of 2103.79g per plant over all other combinations. The lowest fruit yield of 1370.75g was recorded with the treatment combination of pollens stored for one day under ambient condition but it was significantly higher over control or fresh pollen use (1254.28g/plant).

Seed yield per plant

Refrigerated stored pollens recorded significantly higher seed yield of 10.37g per plant over ambient method (9.79g/plant). Two days old pollen gave significantly higher seed yield of 11.46g per plant over use of three days (10.39g) and one day old pollens (8.35g) for pollination (Table 2). The significant increase in the seed yield per plant in two days stored pollens may be due to significant increase in seed weight per fruit, number of seeds per fruit and seed recovery per cent per plant. These results are in agreement with the reports of Chithra Devi [4] and Kalloo [7] in tomato hybrid seed production. Interaction effect of two days old

Table 2. Effect of pollen storage per fruit, seed recovery conditions and age of pollen on fruit weight, number of seeds and seed yield per plant of tomato hybrid (DTH-1)

Treatments	Fruit weight (g)			No. of seeds/ fruits			Seed recovery (%)			Seed yield (g) per plant		
	2002	2003	Pooled	2002	2003	Pooled	2002	2003	Pooled	2002	2003	Pooled
Pollen storage (M) method												
M_1	80.03	80.62	80.33	75.73	76.33	76.04	4.30	4.19	4.24	9.91	9.61	9.79
M_2	83.23	83.65	83.45	107.62	108.28	107.95	4.40	4.43	4.42	10.38	10.36	10.37
S.Em+	1.02	0.98	1.08	2.02	2.05	2.03	0.03	0.04	0.03	0.26	0.29	0.26
CD at 5%	3.08	2.92	NS	6.24	6.33	6.08	0.10	0.13	0.10	NS	NS	0.77
Age of pollen (P)												
P_1	78.89	79.77	79.33	88.79	89.57	89.18	3.99	4.02	4.01	8.55	8.14	8.35
P_2	84.23	84.59	84.41	99.39	99.86	99.61	4.57	4.59	4.58	11.42	11.50	11.46
P_3	81.78	82.07	81:93	86.89	87.48	87.17	4.48	4.32	4.30	10.46	10.31	10.39
S.Em+	0.91	1.25	1.29	2.48	2.51	2.50	0.04	0.05	0.05	0.31	0.36	0.34
CD at 5%	2.78	3.75	3.88	7.64	7.75	7.51	0.14	0.16	0.15	0.96	1.12	1.01
Interaction (M x P)												
M_1P_1	78.07	79.05	78.56	71.09	71.98	71.54	3.84	3.86	3.85	8.46	7.68	8.16
M_1P_2	82.00	82.70	82.35	81.06	81.15	81.11	4.43	4.45	4.44	11.02	11.08	11.05
M_1P_3	80.03	80.12	80.08	75.06	75.86	75.46	4.60	4.27	4.44	10.26	10.06	10.16
M_2P_1	79.71	80.48	80.10	106.49	107.16	106.83	4.15	4.18	4.17	8.645	8.59	8.62
M_2P_2	86.45	86.47	86.46	117.67	118.58	118.13	4.70	4.73	4.72	11.83	11.92	11.88
M_2P_3	83.53	84.02	83.78	98.71	99.09	98.90	4.35	4.37	4.36	10.66	10.57	10.62
S.Em±	3.68	3.59	4.62	3.45	3.56	3.53	0.08	0.08	0.08	0.44	0.51	0.49
CD at 5%	NS	NS	NS	NS	NS	10.57	NS	NS	NS	NS	NS	1.45
Control	69.18	70.13	69.66	69.23	69.67	69.45	3.42	3.64	3.53	7.56	7.57	7.57
Control vs Rest of the treatments												
S.Em+	3.70	3.63	3.65	2.77	2.81	2.80	0.18	0.06	0.11	0.35	0.41	0.38
CD at 5%	11.40	11.18	10.93	8.66	8.61	8.41	0.55	0.18	0.33	1.08	1.25	1.12

NS - Non significant; M_1 : Storage of pollen in ambient condition; M_2 : Storage of pollen in refrigerator; P_1 : Pollination with one day old pollen; P_2 : Pollination with two days old pollen; P_3 : Pollination with three days old pollen

Table 3. Effect of pollen storage conditions and age of pollen on germination, field emergence and vigour index of tomato hybrid (DTH-1)

Treat-ments	Germination (%)			Field emergence (%)			Vigour index		
	2002	2003	Pooled	2002	2003	Pooled	2002	2003	Pooled
Pollen storage (M) method									
M_1	77.96 (95.65)*	78.06 (95.75)	78.01 (95.65)	74.89 (94.00)*	78.39 (95.95)	75.89 (94.00)	1669	1629	1649
M_2	77.75 (95.50)	77.65 (95.75)	77.86 (95.55)	76.96 (94.95)	78.38 (96.00)	76.96 (94.95)	1750	1742	1746
S.Em±	0.78	0.68	0.68	0.30	0.45	0.30	20	15	17
CD at 5%	NS	NS	NS	0.87	NS	0.87	60	46	51
Age of pollen (P)									
P_1	76.97 (94.95)	76.39 (94.45)	76.68 (94.65)	74.29 (92.65)	77.18 (95.00)	74.29 (92.65)	1637	1621	1629
P_2	79.55 (86.75)	79.85 (96.85)	79.70 (96.85)	76.33 (94.45)	80.30 (97.10)	76.33 (94.45)	1781	1741	1771
P_3	77.04 (94.95)	77.31 (95.10)	77.18 (95.00)	74.59 (92.95)	77.79 (95.55)	74.59 (92.95)	1741	1671	1691
S.Em±	0.96	0.83	0.82	0.37	0.56	0.37	24	18	21
CD at 5%	NS	2.57	2.46	1.17	1.72	1.17	74	56	63
Interaction (M x P)									
M_1P_1	76.99 (94.95)	76.10 (94.20)	76.55 (94.55)	74.08 (92.45)	76.99 (94.95)	74.08 (92.45)	1543	1523	1533
M_1P_2	79.97 (96.95)	80.84 (97.45)	80.41 (87.25)	76.17 (94.25)	80.68 (97.35)	76.17 (94.25)	1770	1743	1757
M_1P_2	76.91 (94.95)	77.22 (95.10)	77.07 (95.00)	77.43 (95.25)	77.69 (95.45)	77.43 (95.25)	1695	1622	1658
M_2P_1	76.95 (94.90)	76.67 (94.65)	76.81 (94.75)	74.50 (92.85)	77.37 (95.25)	74.50 (92.85)	1730	1719	1725
M_2P_2	79.12 (86.45)	78.87 (96.25)	79.49 (96.65)	76.49 (94.55)	79.92 (96.95)	74.49 (94.55)	1792	1788	1790
M_2P_3	77.17 (95.00)	77.40 (95.25)	77.29 (94.15)	79.88 (96.95)	77.88 (95.55)	79.88 (96.95)	1728	1726	1724
S.Em+	1.35	1.18	1.27	0.54	0.79	0.54	34	26	30
CD at 5%	NS	NS	NS	1.61	NS	1.61	104	79	91
Control	73.57 (92.00)	71.93 (90.40)	72.15 (91.25)	69.54 (87.90)	71.93 (90.35)	69.54 (87.90)	1469	1455	1462
Control vs Rest of the treatments									
S.Em±	1.07	1.33	1.21	0.43	0.62	0.43	27	20	23
CD at 5%	3.20	4.00	3.61	1.27	1.85	1.27	82	63	72

NS - Non significant; M_1: Storage of pollen in ambient condition; M_2 : Storage of pollen in refrigerator; P_1: Pollination with one day old pollen; P_2: Pollination with two days old pollen; P_3: Pollination with three days old pollen; *Figures in the paranthesis indicate the original values

pollen in refrigerator recorded significantly higher seed yield of 11.88g per plant over all other combinations. The lowest seed yield of 8.16g per plant was recorded in ambient storage with one day old pollens but it was significantly higher over fresh pollens, control (7.57 g/plant) in tomato [8, 9].

Seed quality

Pollen stored in refrigerator recorded higher germination, field emergence percentage and vigour index as compared to ambient storage. This may be due to higher viable pollen used for pollination (refrigerated conditions) as compared to ambient storage which produced high seed quality parameters. Such high quality seeds were harvested when pollen stored in the refrigerator and used for pollination of emasculated bud in hybrid seed production was reported [4, 5, 9] in tomato. Use of two days old pollens for pollination of emasculated bud recorded significantly higher germination, field emergence and seedling vigour index over use of one day and three days old pollens. This may be due to higher seed weight and bold seeds that were harvested in the two days old pollens used for pollinations as compared to one and three days old pollens. Interaction between pollen storage conditions and use of stored pollen influenced the seed quality parameters significantly. Two days stored pollen in refrigerator recorded higher germination (96.65%), field emergence (94.55%) and vigour index (1790) as compared to other combinations (Table 3). An increase in the germination, field emergence and vigour index in this treatment (M_2P_2) may be due to higher seed weight and bolder seeds that were harvested.

From the above discussion it can be concluded that the pollen stored under controlled (refrigerator) condition for two days recorded higher fruit set, fruit yield, seed yield per plant and seed quality compared to other treatment combinations. It can be concluded that pollens stored under refrigerated condition for two days are best suited for hand emasculation and hand pollination method in hybrid seed production of tomato.

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