Biological Forum – An International Journal **13(3): 652-659(2021)**

ISSN No. (Print): 0975-1130
ISSN No. (Online): 2249-3239

Studies on Effect of Growing Environments and Storage Conditions on Assessment of Pollen Viability using Different Staining Techniques in Tomato

Jorrigal Laxman[1], Shiv K. Yadav[1*], Ravish Choudhary[1], Sangita Yadav[1], Zakir Hussian[2], P.K. Singh[2], Sushil Pandey[3] and Sunil Archak[4]

[1]Division of Seed Science and Technology, ICAR-Indian Agricultural Research Institute, New Delhi-110012, India.
[2]Division of Vegetable Science, ICAR-Indian Agricultural Research Institute, New Delhi-110012, India.
[3]Germplasm Conservation Division, ICAR-National Bureau of Plant Genetic Resources, New Delhi-110012, India.
[4]National Fellow, ICAR-National Bureau of Plant Genetic Resources, New Delhi-110012, India.

(Corresponding author: Shiv K. Yadav)*
(Received 16 June 2021, Accepted 21 August, 2021)
(Published by Research Trend, Website: www.researchtrend.net)

ABSTRACT: The main objective of tomato pollen storage is to preserve the genetic material for future use, to maintain their germination, vigour and genetic integrity under optimal conditions. Major challenge faced by tomato breeding program to develop new hybrids is time consuming and difficult hand emasculation and pollination for out-crossing occurs. This is because of the cleistogamous nature of flowers wherein, no cross-pollination occurs. Another challenge is of less pollen availability in tomato flowers during pollination. Multiple planting and staggered sowing can evade this constraint to some level but it is labour, time, and space intensive. Thus, accessibility of stored viable pollen for the breeding program can be of great help as it enables the easy exchange of genetic material between the researchers due to fewer stringent restrictions on the transportation. In the present study, viability of pollen produced in different growing environments and stored in variable conditions were assessed using different staining methods. The pollens were collected in the morning (8.00 am to 9.00 am) from three freshly opened flowers of genotypes; P117, P217, and P317 grown in three different environments; polyhouse, net-house, open field. The collected pollens were stored at room temperature (RT) for 5 days and put in refrigerator (4°C) up to 19 days. The pollen viability was evaluated by assessing the numbers of successful crosses (%) using the stored pollens. The pollens from RT were used up to 5 days and from refrigerated conditions the pollens were used on 5th, 12th, 19th day. Simultaneously, the pollen viability was assessed in laboratory using three different stains *viz*.; Aceto-carmine, I_2KI and Tetrazolium. The results revealed that minimum pollen viability with less fruit setting was observed in net house and open field conditions as compared to polyhouse environment. It could be because of favourable environment maintained under controlled conditions of polyhouse. During the storage at room temperature, pollens maintained viability up to 2 days and showed a decline in viability with an increase in the storage period/age of pollen. At 4°C, pollens were highly viable up to 5th day, while viability decreased after 12th and 19th day. No fruit set on flowers pollinated with pollens stored for more than 2 days at RT and more than 5 days at 4°C observed in any of the growing environments/conditions. Among the stains used for testing the viability, Acetocarmine was found highly correlated with the percent fruit set in all the three growing environments/conditions followed by the results of I_2KI and T_z stains. In conclusion this study will help the breeders to plan a hybridization program for tomato crop in future and may useful in the conservation and also exchange of the germplasm.

Keywords: Cleistogamous, environmental conditions, iodine potassium iodide, pollen viability, room temperature, storage conditions, tetrazolium, tomato, PFS= percent fruit set

INTRODUCTION

Tomato (*Solanum lycopersicum* L.), 2x = 24, belongs to a Solanaceae family, originated in the Andes region but now consists of Chile, Bolivia, Ecuador, Columbia, and Peru (Sims, 1980). The top ten tomato-producing countries worldwide are China, India, USA, Turkey, Egypt, Iran, Italy, Brazil, Spain, and Uzbekistan. Tomato commonly known as poor man's apple is the economically most significant vegetable crop. Nutritionally, it is a rich source of β-carotene, lycopene, vitamin A, vitamin C and minerals like calcium, potassium, etc. It is a natural blood purifier and also contains a large amount of organic acids like citric acid, malic acid, etc. which activates the secretion of gastric juices (Pruthi, 1993). Around 12% of the world population suffers by hunger and due to lack of nutritional food. To achieve nutritional security of people, consumption of crops like tomato may be increased (Sivakumar, 2021).

Viable pollen is important for species dispersal, fitness, and survival of the next plant generation. It is also essential for plant breeding and consequently, crop improvement (Shivanna and Rangaswamy, 1992). Pollen viability comprises different aspects of pollen performance such as fertilization ability, germinability, and stainability (Dafni and Firmage, 2000).

Successful pollination is essential for fertilization helps in seed setting in maximum plants, and thus understanding viability, pollen germination, and pollen tube growth is required for any rational approach to increase productivity (Bolat and Pirlak, 1999). There are several methods available to interpret pollen viability and the method of choice which varies from crop to crop and on correlating relationship between these staining tests and the fertility (Hanna and Towill, 1995). An easy and quick technique is very much required for differentiate the viable and non-viable pollens with high results. A conventional technique involves pollen dusting on receptive stigma monitored by evaluation of fruit and seed set (Smith-Huerta and Vasek, 1984; Shivanna and Johri, 1989). These pollination methods are indirect and time-consuming and associated with physical and physiological features of pollen with its capability to fertilize the ovule (Rodriguez-Riano and Dafni, 2000).

Common techniques to elucidate pollen viability are staining techniques, in vitro germination, seed set as well as in vivo and semi-in situ germination on the excised stigma, also termed stigmatic germination. In the last two, pollen tube growth toward or on stigmas is observed by contrasting dye and the results are assumed to give most accurate estimations of the seed set (Esser, 1955; Dionne and Spicer, 1958). However, incompatibilities, post-fertilization barriers and limited measurability may restrict the accuracy of these tests (Dafni and Firmage, 2000).

Staining method depends on cell integrity, enzyme activities, and nutrient contents (Impe *et al*., 2020). However dye test has advantages as an indicator of pollen viability because they are faster and easier compared with pollen germination, but they do tend to overestimate the viability of pollen grains. Thus it is recommended to use simultaneously several tests to reflect pollen performance. The ability to use chemical staining to discriminate aborted from non-aborted pollen grains has well-known practical applications in agriculture (Firmage, 2000).

The pollen longetivity is its ability to retain its viability during long-term storage (Vaknin and Eisikowitch, 2000.) There are several reports to show that storing pollen at low temperatures was efficient for pollen viability in mango (Dutta *et al*., 2013), date palm (Maryam *et al*., 2017), herbaceous peony (Du *et al*., 2019) and apple (Calic *et al*., 2021).

Unlike seed production in varieties, hybrids involve an effective crossing of emasculated flowers with favourable pollen. So, for success of hybrid seed setting decides by only desired and viable pollens. Therefore, it is imperative to realize about the viability of the parent pollen and its further application in hybrid seed production to obtain a higher seed yield. The pollen viability of tomato species is lost very rapidly and to date very limited systematic studies have been carried out in tomato pollen physiology. In India except for some isolated efforts in single environment condition none of the studies were carried across the different environmental conditions for pollen viability with different storage methods and staining techniques for ensuring pollen viability to be integrated in commercial hybrid seed production in tomato. Hence, the present investigation was carried out to compare different staining methods for different storage conditions in three different tomato varieties grown in different conditions and correlating them with fruit and seed set.

MATERIAL AND METHODS

Genotypes used: Seeds of different varieties namely; P117, P217, and P317 were procured from the Division of Vegetable Science, ICAR-Indian Agricultural Research Institute, New Delhi (India) and were used for pollen viability studies in the present experiment.

Collection and storage of pollens: The seeds were sown in plug trays in the month of July, 2018 with media composition of coco peat and vermicompost (1:1). The seedlings were transplanted at 2-3 leaf stage during Kharif (August, 2018-19) and grown under the three different environmental conditions *viz*., poly-house, net house and open field. The pollens were collected between 8:00-9.00 a.m. as anthesis in tomato is reported to occur in the morning when flowers are completely open (Miniraj *et al*., 1993**)**. Freshly opened male flowers in all the environments were utilized for collection of pollen and stored at room temperature (RT) up to 5 days and 4°C (refrigerated conditions) up to 19 days and analysed the pollen viability and percent fruit set studies in field crossings. The pollen viability was assessed on daily basis up to 5 days from the pollen which were stored at RT, while it was assessed on the 5th day, 12th day, and 19th days of storage at 4°C.

Pollen viability tests: Pollen viability was examined by three different staining methods, *viz*.; (i) 2.0 % acetocarmine solution (McKellar and Quesenberry, 1992), (ii) Tetrazolium test (Norton, 1966), (iii) Iodine potassium iodide test (KI) (Cheng-Yuan *et al*., 2001). After pollen staining the viable pollen became immediately dark red in acetocarmine, red colour in tetrazolium and brown colour in KI method the non-viable pollen grains remained unstained were counted using stereomicroscope. The viability percentage was calculated from the mean of five microscopic field counts. Staining percentage was determined by using the formula;

$$\text{Pollen viability (\%)} = \frac{\text{Number of stained pollen grains}}{\text{Total number of pollen grains on slide}} \times 100$$

Percent Fruit Set (PFS): Pollens were tested for viability/fertility by controlled pollination of plants in the field. Inflorescences of the desired female parents were bagged at the bud stage after the removal of male flowers. Pollen from three different genotypes was placed onto the three different receptive stigmas. Pollinated flowers were immediately covered with butter paper bags to prevent cross-pollination by insects and were removed after the fruit set. Crosses with female parents were also carried out by freshly collected pollen from male parents. Observations were recorded on the percentage of fruit formation in all the crosses after allowing it for normal development and maturity. The tests were repeated thrice (replications), a minimum of 10 crosses per replication were made.

STATISTICAL ANALYSIS

All data were taken in replications of 3. The data were laid out in the Latin square of order m, in an

arrangement of m Latin letters, in a square of m rows and m columns, such that every Latin letter occurred once in each row and once in each column, using SPSS 16.0 for Windows (SPSS Inc., Chicago, IL, USA).

RESULTS AND DISCUSSION

Assessment of Pollen viability using different staining techniques in three varieties: In case of acetocarmine method, genotype P217 showed highest viability (37.16%) followed by P317 and P117 (36.73% and 36.39%, respectively), while with Iodine potassium iodide method, the pollen viability was 29.08%, 28.95% and 28.75%, quite similar to each other with all three genotypes and showed. With Tetrazolium method, genotype P217 showed higher pollen viability (15.16%) followed by P117 and P317 (14.76% and 14.71%, respectively), while in cross pollination PFS in, P117 genotype showed higher viability (15.0%) followed by P317 and P217 (13.52% and 12.04%, respectively) (Table 1). Viability of pollen differs with in the cultivars of *Nerium* and *Passiflora* and it was in confirmation with the studies of Parashuram *et al*., (2021); Soares *et al*., (2013).

Table 1: Pollen viability of three tomato genotypes using different methods.

Genotypes	AC	KI	TZ	PFS
P117	36.39^{b}	28.96^{a}	14.76^{b}	15.00^{a}
P217	37.16^{a}	29.08^{a}	15.16^{a}	12.04^{b}
P317	36.73^{ab}	28.75^{a}	14.75^{b}	13.52^{ab}
Mean	**36.76**	**28.93**	**14.89**	**13.52**
CD (p=0.05)	0.15	0.12	0.07	0.53

Where; AC=Acetocarmine; KI=Iodine potassium iodide; TZ=Tetrazolium; PFS= Percent Fruit Set;*All the values are the average of three replicates (n = 3), and the mean with the same letter (superscript) in the columns are not significantly different ($p < 0.05$)—(Tukey's test)

Assessment of Pollen viability using different staining techniques in different environments: Overall poly-house condition showed maximum pollen viability followed by nethouse and open field (Table 2). In case of acetocarmine method, highest pollen viability was found under polyhouse condition (42.02%). Similarly, with Iodine potassium iodide and tetrazolium method, higher pollen viability (32.51%, 18.11%) respectively under polyhouse. While in percent fruit set method, among environments and pollen viability was maximum under polyhouse (17.04%) and followed by nethouse (13.33%) and open field (10.18) environments (Table 2).

Table 2: Pollen viability of tomato grown in three environments using different methods.

Environments	AC	KI	TZ	PFS
PH	42.04^{a}	32.51^{a}	18.11^{a}	17.04^{a}
NH	35.95^{b}	28.97^{b}	14.29^{b}	13.33^{b}
OF	32.29^{c}	25.32^{c}	12.27^{c}	10.18^{c}
Mean	**36.76**	**28.93**	**14.89**	**13.52**
CD (p=0.05)	0.15	0.12	0.07	0.53

Where; PH= Polyhouse; NH= Nethouse; OF= Open field; *All the values are the average of three replicates (n = 3), and the mean with the same letter (superscript) in the columns are not significantly different ($p < 0.05$) – (Tukey's test).

Assessment of Pollen viability using different staining techniques in different environments and storage conditions: Overall freshly collected pollen from the flowers showed maximum viability in all the methods. Highest pollen viability was found with fresh pollen In case of acetocarmine (90.17%), iodine potassium iodide (80.51%), and tetrazolium method (37.91) respectively. Least pollen viability was found at 5th day of storage at room temperature (4.10%, 2.61% and 2.41%, respectively). However, in PFS, it was found that pollen viability was highest in fresh pollen (37.78%) followed by storage at room temperature for 1 day (31.11%) and 4°C for 5th day (30.00%), while viability was completely lost after 3rd day storage at room temperature and storage at 12th and 19th days at 4°C (Table 3).

Table 3: Pollen viability of tomato after different storage conditions using various viability testing methods.

Storage Treat.	AC	KI	TZ	PFS
T1	90.17^{a}	80.51^{a}	37.91^{a}	37.78^{a}
T2	72.07^{c}	48.23^{c}	22.87^{c}	31.11^{b}
T3	50.39^{d}	16.10^{e}	9.70^{e}	22.78^{c}
T4	11.13^{f}	10.93^{f}	6.15^{f}	0.00
T5	6.55^{h}	5.10^{h}	4.71^{g}	0.00
T6	4.10^{i}	2.61^{i}	2.41^{h}	0.00
T7	75.17^{b}	67.36^{b}	27.62^{b}	30.00^{b}
T8	13.45^{e}	19.60^{d}	17.60^{d}	0.00
T9	7.83^{g}	9.93^{g}	5.01^{g}	0.00
Mean	**36.76**	**28.93**	**14.89**	**13.52**
CD (p=0.05)	0.25	0.21	0.11	0.92

Where; T1=Control; T2=Storage at RT for 1 Day; T3=Storage at RT for 2 Day; T4=Storage at RT for 3 Day; T5=Storage at RT for 4 Day; T6=Storage at RT for 5 Day; T7=Storage at 4ºC for 5th Day; T8= Storage at 4ºC for 12th Day; T9= Storage at 4ºC for 19th Day; *All the values are the average of three replicates (n = 3), and the mean with the same letter (superscript) in the columns are not significantly different ($p < 0.05$)—(Tukey's test).

As per the analysis of variance, all the staining tests performed in three different environments conditions and storage methods showed significant difference at 5% level of significance (Tables 4, 5, 6 and 7). Fresh pollen viability of three different genotypes was examined after different viability tests. Pollen viability using staining tests proved that fresh pollen in tomato genotypes showed greater viability as compared to the stored pollen. Number of pollen grains stained in fresh collected pollen was found maximum in the acetocarmine method compared to other staining methods in all three environments (Table 8). Relatively, greater pollen viability was witnessed using acetocarmine staining test, followed by I_2KI, PFS, and least pollen viability reported in T_z (Tables 4, 5, 6 and 7; Fig. 1).

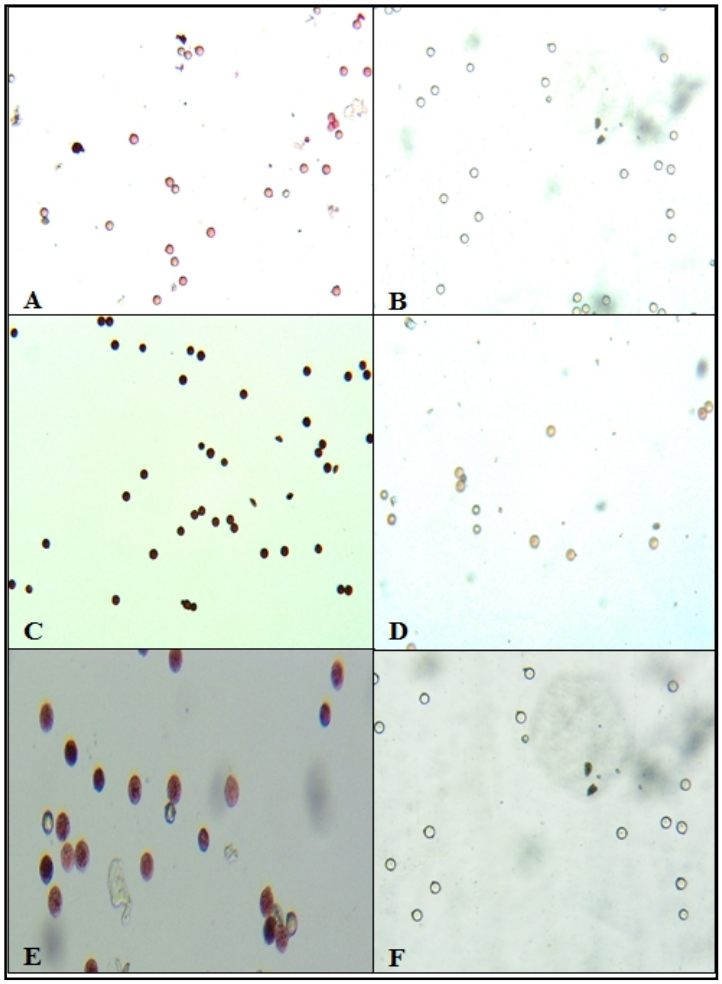

Fig. 1. Differentiation of viable and non-viable pollen of tomato pollen using different techniques A, C and E are viable pollen and B, D and F are non-viable pollen stained by aceto-carmine, I_2KI and Tetrazolium methods.

Acetocarmine was the best suitable stain for pollen viability assay in litchi crops (Gupta *et al.*, 2018). However, the other staining methods utilized in this present study, could not differentiate viable and non-viable pollens as more accurately as acetocarmine. Similarly, in *Momordica* spp. and their interspecific hybrids, five different staining techniques were used for pollen viability and to differentiate the viable and non-viable pollen grains (Rathod *et al*., 2018). The highest pollen viability was observed using acetocarmine staining test as compared to *in vitro* germination and crossability tests in cassava (Hegde *et al*., 2019).

Assessment of Pollen viability of fresh pollen using different staining techniques in three environments: Under different environment conditions it was found that viability % was maximum in polyhouse followed by other environments. In polyhouse condition, the fresh pollen viability across genotypes showed maximum staining by acetocarmine with the mean of 95.1% followed by I_2KI 85.7%, and field crossing (PFS), 48.33%, while least noted in Tz method 45.8%. In net house condition the pollen viability of all three genotypes stained by acetocarmine showed the mean value of 91.1%, followed by I_2KI 81.9%, and field crossing (PFS) 36.67%, while least was noted in Tz 35.4%. In open field condition, the pollen viability of all three genotypes stained by acetocarmine showed the mean of 84.4%, followed by I_2KI 74.0% and Tz 32.6% while it was found lowest in field crossing (PFS) (Table 4, 5, 6 and 7).

Table 4: Viability test of pollens in three tomato genotypes grown under three environments and stored in different conditions using acetocarmine staining method.

Storage Treat.	P117			Mean	P217			Mean	P317			Mean	G. Mean
	PH	NH	OF		PH	NH	OF		PH	NH	OF		
T1	98.0	91.3	82.7	**90.7**	93.8	90.8	84.5	**89.7**	93.6	91.1	85.9	**90.2**	**90.2**
T2	79.2	73.5	67.0	**73.2**	72.6	70.2	69.3	**70.7**	77.8	71.5	67.5	**72.3**	**72.1**
T3	56.5	51.2	43.3	**50.3**	58.0	48.9	43.5	**50.1**	60.8	50.3	41.1	**50.7**	**50.4**
T4	16.3	8.2	9.0	**11.2**	15.2	12.2	8.2	**11.9**	12.1	11.1	7.8	**10.4**	**11.1**
T5	9.4	5.6	7.1	**7.3**	9.1	4.5	3.9	**5.8**	8.0	6.6	5.0	**6.5**	**6.6**
T6	5.6	2.1	2.1	**3.3**	7.1	6.0	2.4	**5.2**	7.2	2.7	1.7	**3.9**	**4.1**
T7	94.1	69.3	60.9	**74.7**	89.8	71.1	67.9	**76.3**	89.1	70.4	64.1	**74.5**	**75.2**
T8	14.7	8.2	8.1	**10.3**	18.1	17.5	11.5	**15.7**	19.1	14.3	9.6	**14.3**	**13.5**
T9	8.7	5.5	5.1	**6.4**	10.2	10.1	7.4	**9.2**	11.2	6.8	5.5	**7.8**	**7.8**
Mean	**42.5**	**35.0**	**31.7**	**36.4**	**41.5**	**36.8**	**33.2**	**37.2**	**42.1**	**36.1**	**32.0**	**36.7**	**36.8**
CD (p=0.05)	Genotype (G): 0.15; Envt. (E): 0.15; Treat.(T):0.25; GXE: 0.25; GXT: .044; EXT: 0.44; GXEXT:0.76												

Table 5: Viability test of pollens in three tomato genotypes grown under three environments and stored in different conditions using potassium iodide staining method.

Storage Treat.	P117			Mean	P217			Mean	P317			Mean	G. Mean
	PH	NH	OF		PH	NH	OF		PH	NH	OF		
T1	86.7	80.5	74.3	**80.5**	82.8	82.1	72.3	**79.0**	87.5	83.2	75.3	**82.0**	**80.5**
T2	53.4	47.4	42.3	**47.7**	57.3	47.8	41.6	**48.9**	52.2	48.8	43.3	**48.1**	**48.2**
T3	19.1	13.5	12.5	**15.0**	22.5	15.4	12.6	**16.8**	20.1	16.1	13.4	**16.5**	**16.1**
T4	12.9	12.6	11.5	**12.3**	15.3	13.8	8.4	**12.5**	11.6	9.0	3.3	**8.0**	**10.9**
T5	6.8	5.6	4.8	**5.7**	7.2	4.4	4.6	**5.4**	5.0	4.3	3.2	**4.2**	**5.1**
T6	2.9	2.1	2.8	**2.6**	3.5	2.6	2.0	**2.7**	4.1	1.9	1.3	**2.5**	**2.6**
T7	68.5	65.8	63.9	**66.1**	73.1	67.2	61.8	**67.4**	74.5	70.8	60.8	**68.7**	**67.4**
T8	27.0	18.8	16.1	**20.6**	24.0	20.9	12.6	**19.2**	22.1	17.9	17.0	**19.0**	**19.6**
T9	11.9	10.3	7.9	**10.0**	12.2	10.4	6.9	**9.8**	13.8	9.0	7.0	**9.9**	**9.9**
Mean	**32.1**	**28.5**	**26.2**	**29.0**	**33.1**	**29.4**	**24.8**	**29.1**	**32.3**	**29.0**	**25.0**	**28.8**	**28.9**
CD (p=0.05)	Genotype (G): NS; Envt. (E): 0.12; Treat.(T): 0.21; GXE: 0.21; GXT: 0.37; EXT: 0.37; GXEXT:0.64												

Table 6: Viability test of pollens in three tomato genotypes grown under three environments and stored in different conditions using Tz staining method.

Storage Treat.	P117			Mean	P217			Mean	P317			Mean	G. Mean
	PH	NH	OF		PH	NH	OF		PH	NH	OF		
T1	44.0	34.6	32.0	**36.8**	47.5	36.8	32.1	**38.8**	45.8	34.9	33.6	**38.1**	**37.9**
T2	24.9	21.7	21.2	**22.6**	26.3	24.5	21.1	**24.0**	28.6	22.7	14.9	**22.1**	**22.9**
T3	11.6	10.3	8.6	**10.2**	10.7	11.0	6.6	**9.4**	11.8	8.8	7.9	**9.5**	**9.7**
T4	7.3	6.0	5.9	**6.4**	6.2	6.2	5.8	**6.1**	6.2	5.8	5.9	**6.0**	**6.1**
T5	5.1	2.7	3.2	**3.7**	7.8	5.2	3.3	**5.4**	6.6	3.8	4.7	**5.0**	**4.7**
T6	3.8	1.9	1.5	**2.4**	3.0	3.8	2.4	**3.0**	2.3	1.2	2.0	**1.8**	**2.4**
T7	34.1	27.5	21.9	**27.8**	32.5	29.0	20.2	**27.2**	33.7	27.9	21.8	**27.8**	**27.6**
T8	23.0	16.9	16.5	**18.8**	21.1	16.2	15.5	**17.6**	22.3	15.0	11.9	**16.4**	**17.6**
T9	4.7	4.0	3.7	**4.1**	7.1	4.1	3.4	**4.9**	11.1	3.6	3.5	**6.1**	**5.0**
Mean	**17.6**	**13.9**	**12.7**	**14.8**	**18.0**	**15.2**	**12.3**	**15.2**	**18.7**	**13.7**	**11.8**	**14.8**	**14.9**
CD (p=0.05)	Genotype (G): 0.07; Envt. (E): .07; Treat.(T): 0.11; GXE: 0.11; GXT: 0.20; EXT: 0.20; GXEXT:0.34												

Table 7: Viability test of pollens in three tomato genotypes grown under three environments and stored in different conditions using percent fruit set method.

Storage Treat.	P117			Mean	P217			Mean	P317			Mean	G. Mean
	PH	NH	OF		PH	NH	OF		PH	NH	OF		
T1	60.0	40.0	30.0	**43.3**	40.0	35.0	25.0	**33.3**	45.0	35.0	30.0	**36.7**	**37.8**
T2	50.0	30.0	20.0	**33.3**	35.0	30.0	25.0	**30.0**	35.0	35.0	20.0	**30.0**	**31.1**
T3	35.0	20.0	20.0	**25.0**	20.0	20.0	20.0	**20.0**	25.0	25.0	20.0	**23.3**	**22.8**
T4	0.0	0.0	0.0	**0.0**	0.0	0.0	0.0	**0.0**	0.0	0.0	0.0	**0.0**	**0.0**
T5	0.0	0.0	0.0	**0.0**	0.0	0.0	0.0	**0.0**	0.0	0.0	0.0	**0.0**	**0.0**
T6	0.0	0.0	0.0	**0.0**	0.0	0.0	0.0	**0.0**	0.0	0.0	0.0	**0.0**	**0.0**
T7	45.0	35.0	20.0	**33.3**	30.0	25.0	20.0	**25.0**	40.0	30.0	25.0	**31.7**	**30.0**
T8	0.0	0.0	0.0	**0.0**	0.0	0.0	0.0	**0.0**	0.0	0.0	0.0	**0.0**	**0.0**
T9	0.0	0.0	0.0	**0.0**	0.0	0.0	0.0	**0.0**	0.0	0.0	0.0	**0.0**	**0.0**
Mean	**21.1**	**13.9**	**10.0**	**15.0**	**13.9**	**12.2**	**10.0**	**12.0**	**16.1**	**13.9**	**10.6**	**13.5**	**13.5**
CD (p=0.05)	Genotype (G): 0.53; Envt. (E): 0.53; Treat.(T): 0.92; GXE: 0.92; GXT:NS; EXT: 1.60; GXEXT:NS												

Assessment of Pollen viability of (RT) stored pollen using different staining techniques in three environments: The decrease trend was observed in (RT) stored pollen viability (%) of net-house and open field condition as compared to polyhouse environment. The pollen collected from the polyhouse grown genotypes and stored at room temperature for 5 days, noticed that pollen viability was retained up to 2 days and it was confirmed by comparing staining methods with the Percent Fruit Set in the main field. From the 3rdday, pollen viability gradually decreased with an increase of storage period and resulted in no fruit setting in all genotypes (Table 7). With acetocarmine, I_2KI, Tz and Percent fruit set, it was observed that pollen were viable (76.5%, 54.3% 26.6% and 40.0%, respectively) after 1st day of storage at RT and it decreased on 5th day after RT stored pollen upto 6.6%, 3.6% and 3.0% respectively. In case of percent fruit set method, the pollen were viable up to 2 days and showed (40.0% and 26.67%, respectively), while from day 3 onwards pollen viability was lost completely and found no fruit setting (Table 7).

The pollen of all genotypes collected from net-house stored at room temperature for 5 days, noticed that pollen viability was retained up to 2 days as visualized by staining method with a result of percent fruit setting in field condition. From the 3rd day, pollen viability was decreased and no fruit set was observed in all three genotypes (Table 7). The acetocarmine, I_2KI and Tz method showed the maximum mean value of viability (71.7%, 48.0%, 35.4%, respectively) for 1st day of storage at RT, while least on 5th day of storage at RT (3.6%,2.2% and 2.1%, respectively). In case of crossing method of PFS, it was observed that pollen viability was retained up to 2 days with (31.67 & 21.67%), while it was completely lost from 3rd day onwards up to 5th day of storage at RT and no fruit setting was observed in field (Table 4, 5, 6, 7).

The pollen of all the genotypes collected from open field and stored at room temperature for 5 days, also noticed similar results as above. From the 3rd day pollen, viability was decreased and no fruit setting was observed in all three genotypes. The maximum pollen viability was observed using acetocarmine and showed the mean value of viability on 1st day and 5th day of storage (68.0% & 2.1%, respectively) at RT, followed by I_2KI and Tz. While assessing viability by determination of percent fruit set (PFS), it was observed that pollen were viable and resulted fruit setting on 1st day and 2nd day which was 21.67% and 20.00%, respectively. From 3rd day onwards till 5th day no fruit setting was recorded (Table 4, 5, 6 and 7).

Assessment of Pollen viability of refrigerated stored pollen using different staining techniques in three environments: The viability of stored pollen at 4°C was found to be decreasing significantly with the increase in storage period as confirmed by the pollen viability tests. The decrease trend in pollen viability percentage was observed for net-house and open field condition as compared to polyhouse environment. Overall, it was observed that pollen of all genotypes collected across environments and stored at 4°C, pollen was viable up to 5th day of storage and it was noticed that after the 5th day, as increase the pollen storage period at 4°C, viability percentage was decreased completely and no fruit setting after crossing in the main field was observed.

In case of polyhouse environment, collected pollen were stored at 4°C, Pollen were stained by acetocarmine and noticed that highest pollen viability was observed after 5th day of storage (91.0%), afterwards it was gradually decreased. Very less viability was observed on 12th and 19th day of pollen storage. Similar trend was observed for the I_2KI and Tz methods. In case of crossing method, it was found that PFS on 5th day was (38.33%), while it was completely lost after 12th and 19th day of storage and no fruit set were seen.

During the pollen collection from net-house, it was observed that pollen viability was found maximum using acetocarmine method on5th day of storage (70.3%) with decreasing trend followed in I_2KI 67.9% and Tz methods 28.1%. However, using crossing method, PFS was found on 5th day (30.00%), while after 5th day pollens were not viable.

In open field condition also gave similar trend and results revealed that highest pollen viability was observed using acetocarmine after 5th day of pollen storage at 4°C temperature (64.3%) and showed decreasing trend followed by I_2KI and Tz methods.

In case of crossing method of PFS, on the 5th day of storage (21.67%), while pollen viability was completely lost after 5th of storage period. Hence, these results indicated that pollen in the refrigerated condition (at 4°C temperature) were more preferable as compared to stored at room temperature because pollen stored at room temperature stayed viable only up to 2 days but in refrigerated (4^0c) condition it was viable up to 5days.

Percent fruit set studies in three environments and storage conditions: The freshly opened pollen of three genotypes were collected across environments and stored at RT and at refrigeration (4°C). Evaluated Pollen viability of PFS for RT and refrigerated conditions. Fresh pollen reported a higher percentage of fruit set in all genotypes and it declined gradually. The pollen stored at RT was viable up to 2 days and showed decline drastically with an increase in the age of pollen. In case of 4°C storage, pollens were viable up to 5th day, while pollen viability was very less after 5th day and observed no fruit set across environment conditions (Table 4, 5, 6 and 7).

The declining trend in the pollen viability percentage in all storage methods specifies that temperatures above 0ºC cause to decline the pollen viability percentage. Atmosphere plays an important role in the reduction of pollen viability (Quan, *et al.,* 2012). Pollen was highly sensitive to natural storage condition compared to pollen stored in a refrigerator. Pollens saved in the refrigerator for 5 days were fairly germinable with better pollen viability as compared to the pollen stored in RT. Freshly collected pollen reported a considerably higher percentage of pollen viability by one-day freezer storage of pollen percent (Yogeesha *et al.*, 1999). Pollen viability studies showed that pollens are highly sensitive to ambient storage compared to pollen in the freezer (Kivadasannavar *et al.*, 2008). Higher

crossing and fruit set% recorded in fresh pollen preceded by one-day-old freezer pollen of sunflower (Kantharaju, 2003). Similarly in earlier study, it was reported that tomato pollen can be stored for two to three days at room temperature (Kalloo, 1988). The most important part for any hybridization programme is pollen fertility and viability. The pollen viability of 4°C stored cassava was decreased significantly with the increase of storage period (Hedge *et al*., 2019). Pollen viability of pistachio pollen remained viable up to one week and decreased its viability up on storage period (Aldahadha *et al*., 2020).

There is a decrease in pollen viability% of net-house and open field when compared to polyhouse environment. In polyhouse condition, the fresh and stored pollen of all three genotypes namely, showed highly viable followed by net-house and open field. In polyhouse condition, the Percent fruit set was also observed more with crossing method but comparatively less in net house and open field conditions. This is due to more controlled conditions in polyhouse leads to more viable pollen percent and leads to more percent fruit set by crossing, but in net-house, there is partial shade so pollen viability is a little lesser than the polyhouse condition and greater than open field condition. In open field condition, the weather is uncontrolled and leads to a harsher environment and causes lesser pollen viability than the poly and net houses.

Pollination studies performed for hybrid seed development under polyhouse structure showed more fruit setting 65.1% (Sharma *et al.,* 2017). Seed production of pumpkin hybrid underneath insect-proof net-house with the open field environment and summarized that seed production of hybrid should be conducted under insect-proof net-house facilities to attain more percent fruit set by crossing (Xavier, 2010). Hybrid seed production of cucumber under naturally ventilated poly-house and insect-proof net house showed more fruit setting in polyhouse than the net house (Kaddi *et al*., 2014). Under refrigeration, the viability was slightly better than RT and almost 90% pollen were viable after 10 days of storage (Patta *et al*., 2016).

Correlation between different staining methods and percent fruit set: The Pearson's correlation coefficient was performed among the three tomato genotypes between the different staining techniques and Percent fruit set in three different environments. In a polyhouse, nethouse, open-field condition, the Pearson's correlation results presented a positive and highly significant correlation between Acetocarmine stain and PFS is (0.918) followed by I_2KI and Tz (Table 8). Overall, the present study indicated that the pollen viability in three different environments using the Acetocarmine staining method is more reliable than other methods like I_2KI and Tz staining method. Similarly, the pearson's correlation coefficient was performed among the species of *Momordica* and their intra and interspecific hybrids and between the different staining techniques and germination media and found positive and highly significant results in which they observed that aceto carmine staining technique is more reliable to the pollen viability (Rathod *et al*., 2018). Viability as determined by Tz staining tests in tomato pollen did not show good correlation with the fruitset (Aldahadha *et al.,* 2020).

Table 8: Pearson's correlation coefficient of different pollen viability methods with genotypes, environments and storage treatments.

Parameters	Genotype	Envt.	Treat.	AC	KI	TZ	PFS
Genotype	1.000						
Envt.	0.000	1.000					
Treat	0.000	0.000	1.000				
AC	0.004	-0.119*	-0.596**	1.000			
KI	-0.003	-0.106	-0.428**	0.925**	1.000		
TZ	0.000	-0.197**	-0.409**	0.878**	0.953**	1.000	
PFS	-0.035	-0.163**	-0.576**	0.918**	0.824**	0.790**	1.000
*. Correlation is significant at the 0.05 level (2-tailed); **. Correlation is significant at the 0.01 level (2-tailed).							

CONCLUSION

This is the first report on the tomato pollen collected from different environments followed by its storage at different storage conditions and evaluation of pollen viability. The results revealed that the pollen viability percentage is more in the polyhouse than the net house and open field conditions. Furthermore refrigerated (4°C) condition is more preferable storage strategy for effective preservation of nuclear genetic diversity and for pollination in the hybridization programme. Among the different staining techniques acetocarmine is more reliable and preferable than other two staining methods. These results will help the breeders to plan hybridization programme in tomato crop and further studies can be done on pollen viability using different staining techniques like FDA, in vitro germination across environments. Identifying heat stress tolerance male lines producing and maintaining viability of pollens for commercial hybrid seed production for open field conditions and studying the biochemical activity of pollen across environments could be the critical aspects for further research in this area.

Acknowledgement. The authors are thankful to the Indian Council of Agricultural Research, New Delhi, for providing the required funds for smooth conduct of the research work and the Division of Seed Science and Technology, ICAR-Indian Agricultural Research Institute, New Delhi, for providing the necessary facilities for the studies. The support and encouragement of the guide and research committee members, ICAR-IARI, ICAR-NBPGR, during the course of present investigation is gratefully acknowledged.

Conflict of Interest. The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest

REFERENCES

Aldahadha, A., Samarah, N., & Bataineh, A. (2020). Effect of storage temperature and duration on pollen viability and in vitro germination of seven pistachio cultivars. *Journal of Applied Hortulture*, *22*: 3.

Bolat, I., & Pirlak, L. (1999). An investigation on pollen viability, germination and tube growth in some stone fruits. *Turkish Journal of Agriculture*, *23*(4): 383-388.

Calic, D., Milojevic, J., Belic, M., Miletic, R., & Zdravkovic-Korac, S. (2021). Impact of storage temperature on pollen viability and germinability of four Serbian autochthon apple cultivars. *Frontiers in Plant Science*, 1480.

Cheng-Yuan, X. U., Wen-Ju, Z. H. A. N. G., Bao-Rong, L. U., & Jia-Kuan, C. H. E. N. (2001). Progress in studies on mechanisms of biological invasion. *Biodiversity Science*, *9*(4): 430.

Dafni, A., & Firmage, D. (2000). Pollen viability and logetivity. *Plant Systematics and Evolution*, *Springer*, 113-132.

Dionne, L. A., & Spicer, P. B. (1958). Staining germinating pollen and pollen tubes. *Stain Technology*, *33*: 15-17

Du, G., Xu, J., Gao, C., Lu, J., Li, Q., Du, J., & Sun, X. (2019). Effect of low storage temperature on pollen viability of fifteen herbaceous peonies. *Biotechnology Reports*, *21*.

Dutta, S. K., Srivastav, M., Chaudhary, R., Lal, K., Patil, P., Singh, S. K., & Singh, A. K. (2013). Low temperature storage of mango (*Mangifera indica* L.) pollen. *Scientia Horticulturae*, *161*: 193-197.

Esser, K. (1955). Pollen tubes in the style after chloral hydrate and aceto-carmine. *Stain Technology*, *30*: 195–197.

Gupta, A. K., Singh, M., Marboh, E. S., Nath, V., & Verma, J. P. (2018). Pollen production, viability and in vitro pollen germination of different litchi (*Litchi chinensis*) genotypes. *Indian Journal of Agricultural Sciences*, *88*(6): 884-888.

Hanna, W. W., & Towill, L E. (1995). Long-term pollen storage. *Plant Breeding Rev*iews, *13*: 179-207.

Hegde, V., Koundinya, A. V. V., Sheela, M. N., Chandra, C. V., & Mukherjee, A. (2019). Storage of cassava pollen for conservation of nuclear genetic diversity and overcoming hybridization barriers. *Indian Journal of Agricultural Sciences*,*76*(1): 104-111.

Impe, D., Reitz, J., Köpnick, C., Rolletschek, H., Börner, A., Senula, A., & Nagel, M. (2020). Assessment of pollen viability for wheat. *Frontiers in Plant Sciences*, *10*: 1588.

Kaddi, G., Tomar, B. S., Singh, B., & Kumar, S. (2014). Effect of growing conditions on seed yield and quality of cucumber (*Cucumis sativus*) hybrid. *Indian Journal of Agricultural Sciences*, *84*: 624-627.

Kalloo, G. (1988). Solanaceous crops. *Vegetable breeding*, *2*: 520-570.

Kantharaju, G. (2003). *Studies on parental vigour and pollen use efficiency on seed yield and quality of hybrid sunflower DSH-1* (Doctoral dissertation, University of Agricultural Sciences, Dharwad).

Kivadasannavar, P. (2008). *Standardization of Hybrid Seed Production Techniques in Chilli* (*Capsicum annuum* L.) (Doctoral dissertation, UAS, Dharwad).

McKellar, M. A., & Quesenberry, K. H. (1992). Chromosome pairing and pollen viability in *Desmodium ovalifolium* Wall x *Desmodium heterocarpon* (L.) DC hybrids. *Australian Journal of Botany*, *40*(2): 243-247.

Maryam, Jaskani, M. J., & Naqvi, S. A. (2017). Storage and Viability Assessment of Date Palm Pollen. In *Date Palm Biotechnology Protocols*, 3-13.

Miniraj, N., Prasanna, K. P., & Peter, K. V. (1993). Bitter gourd: Momordica spp. In *Genetic Improvement of Vegetable Crops*, pp. 239-246. Pergamon.

Norton, Y. D. (1966). Testing of plum pollen viability with tetrazolium salts. *American Society* for *Horticultural Science*, *89*: 132-134.

Parashuram, M., Pavan Kumar, P., Raghavendra Gunnaiah, S. A., Patil, B. P., Jhalegar, M. J., & Meti, S. (2021). Assessment of pollen viability and in vitro pollen germination in Nerium cultivars (*Nerium oleander* L.). *International journal of chemical studies*, *9*(2): 724-728.

Patta, S., Vari, A. K., Tomar, B. S., & Singh, B. (2016). Palynology studies to increase efficiency of tomato hybrid seed production. *Ecology, Environment and Conservation*, *22*(2): 835-838.

Pruthi, J. S. (1993). Major spices of India. Crop management and post-harvest technology. *Major spices of India. Crop management and post-harvest technology*.

Quan, Q. M., & Li, Y.X. (2012). A method for midterm storage of *Epimedium pubescens* (Berberidaceae) pollen. *Pakistan Journal of Botany*, *44*: 765-768.

Rathod, V., Behera, T. K., Munshi, A. D., Durgesh, K., Jat, G. S., Krishnan, B. G., & Sharma. N. (2018). Pollen viability and *in vitro* pollen germination studies in Mimordicaspecies and their intra and interspecific hybrids. *International Journal of Chemical Studies*, *6*(6): 32-40.

Rodriguez-Riano, T., & Dafni, A. (2000). A new procedure to assess pollen viability. *Sexual Plant Reproduction*, *12*: 241-244.

Sims, W. L. (1980). History of tomato production for industry around the world. *Acta Horticulturae*, *100*, 25-26.

Sharma, P., Kumari, M., & Kumar, P. (2017). Studies on stigma receptivity and fruit load for hybrid seed production in tomato (*Solanum lycopersicum* L.) under protected environment. *Himachal Journal of Agricultural Research*, *43*: 44-47.

Shivanna, K. R., & Johri, B. M. (1989). *The angiosperm pollen structure and function*. Wiley Eastern.

Shivanna, K. R., & Rangaswamy, N. S. (1992). Pollen Biology: A Laboratory Manual New York Springer-Verlag. 123.

Sivakumar, R. (2021). Effect of foliar application of vasicine on gas exchange parameters, proline content and SOD Activity in Tomato (*Solanum lycopersicum* L.). *Biological Forum – An International Journal*, *13*(2): 70-77.

Smith-Huerta, N. L., & Vasek, F.C. (1984). Pollen longevity and stigma-pre-emption in Clarkia. *American Journal of Botany*, *71*: 1183-1191.

Soares, T. L., Jesus, O. N. D., Santos-Serejo, J. A. D., & Oliveira, E. J. D. (2013). In vitro pollen germination and pollen viability in passion fruit (*Passiflora* spp.). *Revista Brasileira de Fruticultura*, *35*: 1116-1126.

Vaknin, Y., & Eisikowitch, D. (2000). Effects of short-term storage on germinability of pistachio pollen. *Plant breeding*, *119* (4): 347-350.

Xavier, F. (2010). Studies on hybrid seed production in pumpkin under insect proof net house and open field conditions. M.Sc. Thesis, Indian Agriculture Research Institute, New Delhi-110012).

Yogeesha, H. S., Nagaraja, A., & Sharma, S. P. (1999). Pollination studies in hybrid tomato seed production. *Seed Science and Technology*, *27*: 115-122.

How to cite this article: Laxman, J., Yadav, S.K., Choudhary, R., Yadav, S., Hussian, Z., Singh, P.K., Pandey, S. and Archak, S. (2021). Studies on Effect of Growing Environments and Storage Conditions on Assessment of Pollen Viability using Different Staining Techniques in Tomato. *Biological Forum – An International Journal*, *13*(3): 652-659.