The Pharma Innovation Journal 2022; 11(8): 620-624

www.ThePharmaJournal.com

The Pharma Innovation

ISSN (E): 2277-7695
ISSN (P): 2349-8242
NAAS Rating: 5.23
TPI 2022; 11(8): 620-624

www.thepharmajournal.com
Received: 07-06-2022
Accepted: 20-07-2022

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Palynological studies in *Carica papaya* L., *Vasconcellea cauliflora* and an intergeneric hybrid, as an intrinsic approach to understand the breeding potential

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Abstract
Carica papaya L. (papaya) is an economically important fruit crop with huge benefits. Pollen quality is an important tool for plant breeders for carrying out breeding programmes. The present study was conducted to compare three different staining methods and three different composition of germination medium for assessing pollen viability and in-vitro pollen germination studies in three papaya genotypes *viz.,* TNAU Papaya CO.8 (variety), *Vasconcellea cauliflora* (wild relative) and intergeneric hybrid (3-8-22-84). Unopened flower buds of three papaya genotypes were collected at 7:30-8:30 am for evaluating pollen viability and *in-vitro* pollen germination. Pollen viability was assessed using three different stains *viz.,* acetocarmine (V1), potassium iodide (V2) and aniline blue (V3). *In vitro* pollen germination was assessed using three pollen germinating media *viz.,* 10% sucrose (T1), 10% sucrose + 300mg calcium nitrate + 100mg boric acid + 200mg magnesium sulphate +100mg potassium nitrate (T2), 20% sucrose + 300mg calcium nitrate + 100mg boric acid + 200mg magnesium sulphate +100mg potassium nitrate (T3). Among all the three papaya genotypes, maximum pollen viability was recorded in
TNAU papaya CO.8 whereas maximum pollen germination recorded in *Vasconcellea* cauliflora. Among three stains, Acetocarmine (V1) stain effectively differentiated viable pollen grains from non-viable pollen grains compared to other stains in all three papaya genotypes. Among three *in vitro* germination medium, 10% sucrose (T1) media composition resulted in maximum percentage of pollen germination compared to other media composition in all three papaya genotypes.

Keywords: *Carica papaya*, *Vasconcellea cauliflora*, intergeneric hybrid, pollen viability, pollen germination

Introduction
Papaya (*Carica papaya* L.) is one of the most important fruit crops grown in tropical regions of the world. It belongs to the family Caricaceae. The papaya originated in tropical America and spread to many Caribbean and Asian countries in the 16th century. During 15th century, papaya was initially introduced to panama and then spread to West Indies. Through Malacca, it was introduced by the Portuguese to India in 1598. It later became an Indian plant in China (J. Auxcilia *et al.*, 2020) [13]. The family caricaceae consist of six genera and 35 species. *Carica*, *Vasconcellea*, *Jacaratia*, *Jarilla*, *Cylicomorpha and Horovitzia* are the six genera of Caricaceae family. Among them, edible papaya belongs to monotypic genus *Carica* and the wild papaya belongs to *Vasconcellea* genera. With a genome size of 372 Mb, papaya is a dicotyledonous, diploid species (Arumuganathan and Earle, 1991). Papaya crop is otherwise known as melon tree, backyard fruit crop. Papaya fruit is the richest source of Vitamin A (2020 IU). The entire portion of the plant such as fruits, leaves, seed, root, bark, juice and latex are used for nutritional, medicinal and other purposes.
Papaya is polygamous plant with three sex forms *i.e.,* male, female and hermaphrodite. Male flowers are tubular in shape, whereas female flowers are bell shaped and hermaphrodite flowers are long with tubular base that bulges into goblet shape. Only female flowers are stable in nature whereas male and hermaphrodite flowers are unstable because under different environmental conditions these flowers vary in sex expression (Storey, 1953) [14].
Papaya can be classified as both self and cross pollinated crop (Nakasone and Paull, 1998; Louw, 2000; Teixeirada Silva *et al.*, 2007) [11, 7, 15]. Storey (1958) [14] classified papaya flowers into eight groups on the basis of modification of sex expression. The eight groups are staminate, pistillate, teratological staminate, elongate, reduced elongate, carpelloid elongate, Pendantria and Carpelloid Pendantria (J. Auxcilia *et al.*, 2014) [2]. High quantities and qualities

of pollen are passed to stigma during high receptivity for successful pollination (Sharafi *et al.,* 2011) [17]. There are some factors affecting pollination in papaya *viz.*, flower types, environmental factors, pollinator efficiency and pollen abundance (Teixeirada Silva *et al.,* 2007) [15]. Pollen grains of papaya are spheroidal in shape. During pollen germination, the pollen tube arises from three colporate apertures present in the pollen grains. Some histological studies revealed that pollen was rich in lipids and starch (Phuangrat *et al.,* 2013) [12]. Pollen can transmit the genetic makeup of male gametes to the subsequent new generations.

Quality pollen is essential for breeding, incompatibility, crop improvement, pollen vigor and fertility studies (Shivanna and Rangaswamy., 1992) [13]. Pollen viability and pollen vigour can be used to evaluate the quality of pollen. Pollen vigour describes the rate of pollen germination and pollen tube growth. *In vitro* pollen germination test indicates the viability of pollen. Some linear relationship was found between pollen viability and pollen germinability in many fruit species (Sulusoglu *et al.,* 2014) [10].

Pollen viability and germination capacity plays an important role in breeding program. In general, papaya being an commercial crop, only limited researches have been conducted in papaya pollen. The main objective of this study is to evaluate pollen viability and pollen germination studies in three papaya genotypes *viz., Carica papaya* (TNAU Papaya CO-8), *Vasconcellea cauliflora* and intergeneric hybrid (3-8-22-84) using different staining methods and different composition of germination medium.

Materials and Methods

The study was conducted at Department of Fruit Science and Cytogenetics laboratory of the Department of Genetics and Plant Breeding, Tamil Nadu Agricultural University, Coimbatore, during the year 2021-2022, with three papaya genotypes *viz., Carica papaya* (TNAU papaya CO.8), *Vasconcellea cauliflora* and intergeneric hybrid (3-8-22-84) developed by crossing CP 50 and *Vasconcellea cauliflora* maintained in Fruit Orchard, Horticultural College and Research Institute, Tamil Nadu Agricultural University, Coimbatore. Unopened flower buds of *Carica papaya* (TNAU papaya CO.8), *Vasconcellea cauliflora* and intergeneric hybrid (3-8-22-84) were collected from the field. The flower buds were collected at 7:30 to 8:30 am for pollen viability and pollen germination.

Pollen viability

Pollen viability was assessed using three different colorimetric tests *viz.*, acetocarmine (V1), potassium iodide (V2) and aniline blue (V3). The sepals and petals of papaya flower buds were separated and anthers were extracted. Pollen grains were dusted on glass slides and three different stains were subsequently added to the glass slide. The glass slides were covered with cover slips. A 10 x magnification light microscope was used to analyse the pollen grains. The viability percentage was recorded for five slides and the average was calculated for working out the pollen viability percentage. Pollen viability was assessed as percentage of total number of stained pollen grains divided by sum of total number of stained and unstained pollen grains.

Acetocarmine test: Carmine indicates the presence of cytoplasm. The pollen nucleus is densely packed with chromatin, and viable pollen stains pink to deep red with acetocarmine, whereas sterile pollen does not absorb any stain and thus remains almost white and transparent (McKellar and Quesenberry, 1992; Marutani *et al*., 1993) [9, 8].

Procedure

Carmine stain was dissolved in acetic acid to prepare acetocarmine solution. For this, 45 percent acetic acid (45ml glacial acetic acid + 55ml distilled water) were gently boiled. At about boiling point, one gram of carmine powder was added to 45 percent acetic acid and allowed to boil for a few minutes. The solution was removed from the flame and allowed to cool to room temperature after boiling. The solution was then filtered through a Whatman No.1 filter paper into a clear bottle. The filterate was red in colour. Viable pollen grains were normal in shape and red in colour, whereas non-viable pollen grains were irregular in shape and colourless

Aniline blue test

Callose in pollen walls and pollen tubes were assessed using Aniline blue stain test (Hauser and Morrison, 1964) [4]. Dark blue stained pollen grains were considered to be viable and light blue and shriveled pollen grains were considered to be non-viable.

Procedure

Aniline blue stain was prepared by dissolving 200mg/lit of aniline blue in a mixture of 10 ml each of phenol, lactic acid, glycerol and distilled water.

Potassium iodide test

This method determines the viability and starch content of pollen grains. Iodine was broken down in a watery arrangement of potassium iodide, the tri-iodide-anion structures with starch, resulting in a blue-black colour.

Procedure

1.0 gram of potassium iodide and 0.5 gram of iodine were dissolved in 100 ml of distilled water. One or two drops of the dye was added into pollen and thoroughly mixed. The cover slip was placed over the slide and the pollen grains were counted after 5-10 minutes. Viable pollen grains were stained and non-viable pollen grains were unstained.

Pollen germinability

Pollen germination of papaya genotypes was evaluated under *in vitro* conditions. Pollen grain was considered as geminated, only when the pollen tube length was greater than pollen diameter. Pollen germination study was done using hanging drop technique (Shivanna and Rangaswamy, 1992) [13] with three different germination media. Three different germination media composition was reported by Brewbaker and Kwack (1964) [3]. Three different germination medium *viz.*,10% sucrose (T1), 10% sucrose + 300mg calcium nitrate + 100mg boric acid +200mg magnesium sulphate + 100mg potassium nitrate (T2), 20% sucrose + 300mg calcium nitrate + 100mg boric acid + 200mg magnesium sulphate +100mg potassium nitrate (T3).

Pollen grains were dusted over the cavity slide and 1-2 drops of two different composition of Brewbaker and Kwack liquid media (1964) [3] and control treatment media were placed over it. The cavity slides were covered with cover slips. The

prepared slides were incubated in humid environment to avoid evaporation. The slides were examined under light microscope at 10x magnification and the germination percentage was calculated. The number of germinated pollen grains per field view was divided by the total number of pollen grains per field view and the results were expressed as percentage (Kearns and Inouye, 1993) [5].

Statistical analysis

The present study was conducted using Completely Randomized Design (CRD) with three treatments and five replications. Data were analyzed using ANOVA at 5% level of significance.

Results and Discussion

The results of staining test in three different papaya genotypes *viz.,* TNAU papaya CO.8, *Vasconcellea cauliflora* and intergeneric hybrid (3-8-22-84) revealed that TNAU papaya CO.8 recorded maximum pollen viability percentage in acetocarmine stain test (95.48%) followed by potassium iodide stain test (92.64%) and aniline blue stain test (89.54%). In Intergeneric hybrid (3-8-22-84), maximum pollen viability percentage was observed in acetocarmine stain test (88.50%) followed by potassium iodide stain test (85.78%) and aniline blue stain test (85.32%). In *Vasconcellea cauliflora,* maximum pollen viability percentage was observed in acetocarmine stain test (79.06%) followed by potassium iodide stain test (76.48%) and aniline blue stain test (73.80%). Based on analysis of variance, all the staining tests recorded significant difference at 5% among three genotypes. Among all the three papaya genotypes, maximum pollen viability percentage was observed in TNAU papaya CO.8 followed by intergeneric hybrid (3-8-22-84) and *Vasconcellea cauliflora.* Comparison of all the three staining tests indicated that maximum pollen was stained in acetocarmine test (V1) followed by potassium iodide (V2) and aniline blue staining tests (V3)

The results of *in vitro* germination test in three different papaya genotypes *viz.,*TNAU papaya CO.8, *Vasconcellea cauliflora* and intergeneric hybrid (3-8-22-84) revealed that, TNAU papaya CO.8 showed maximum percent germination of pollen grains in T1 (32.34%) followed by T2 (22.62%) and T3 (17.66%). In intergeneric hybrid (3-8-22-84), maximum pollen percent germination of pollen grains was observed in T1 (43.44%) followed by T2 (30.98%) and T3 (27.52%). In *Vasconcellea cauliflora,* maxiumn pollen percent germination of pollen grains was observed in T1 (59.06%) followed by T2 (53.54%) and T3 (41.04%).Based on the analysis of variance, all the three different composition of germination medium recorded significance difference at 5% level of significance. Among all the three papaya genotypes, the highest pollen germination rate was observed in *Vasconcellea* cauliflora followed by intergeneric hybrid (3-8- 22-84) and TNAU papaya CO.8. Comparison of all the three different composition of germination medium indicated that maximum number of pollen germinated in medium with composition of 10% sucrose (T1) followed by 10% sucrose + 300mg calcium nitrate + 100mg boric acid + 200mg magnesium sulphate +100mg potassium nitrate (T2) and 20% sucrose + 300mg calcium nitrate + 100mg boric acid + 200mg magnesium sulphate +100mg potassium nitrate (T3).

In this study, among all the three staining methods, acetocarmine staining method effectively distinguished viable pollen grains from non viable pollen grains. In acetocarmine staining method, dark red stained pollen grains were viable whereas colorless pollen grains were non-viable. Using acetocarmine stain, highest number of viable pollen grains were observed compared to other stains. Among all the three different composition of germination medium, media composition of 10% sucrose resulted in higher germination rate. Highest pollen viability was recorded in V1 whereas highest pollen germination rate was recorded in T1. There is no quantitative relationship between pollen germination results and pollen viability assessed with different methods, so different staining techniques could be useful in the estimation of pollen viability accomplished using *in vitro* pollen germination methods (Rathod *et al.,* 2018) [16].

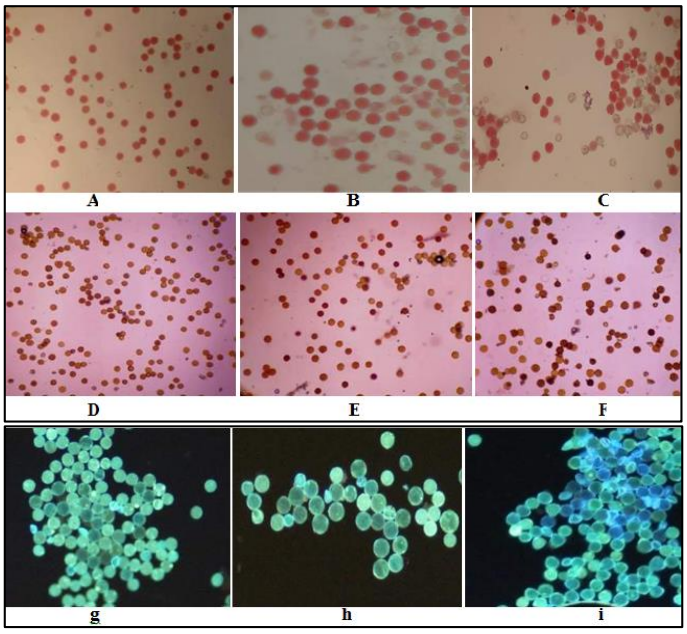

Fig 1: Viable and non-viable pollen grains of three papaya genotypes *viz.,* TNAU papaya CO.8, *Vasconcellea cauliflora* and an intergeneric hybrid a) TNAU papaya CO.8 - V1 b) intergeneric hybrid - V1 c) *Vasconcellea cauliflora* – V1 d)TNAU papaya CO.8 - V2 e) intergeneric hybrid - V2 f) *Vasconcellea cauliflora* - V2 g) TNAU papaya CO.8 - V3 h) intergeneric hybrid -V3 i) *Vasconcellea cauliflora* -V3

Table 1: Pollen Viability of three papaya genotypes *viz.*, TNAU papaya CO.8, *Vasconcellea cauliflora* and an intergeneric hybrid using three different staining methods

Treatments	TNAU papaya CO.8	Intergeneric hybrid	*Vasconcellea cauliflora*
V1	95.48	88.50	79.06
V2	92.64	85.78	76.48
V3	89.54	85.32	73.80
SE d	0.77	0.66	0.40
CD (P=0.05)	1.68*	1.45*	0.89*
CV (%)	1.32	1.21	0.82

V1 - Acetocarmine, V2 – Potassium iodide and V3 – Aniline blue

Table 2: *In-vitro* pollen germination of three papaya genotypes *viz.*, TNAU papaya CO.8, *Vasconcellea cauliflora* and an intergeneric hybrid using three different germination medium

Treatments	TNAU papaya CO.8	Intergeneric hybrid	*Vasconcellea cauliflora*
T1	32.34	43.44	59.06
T2	22.62	30.98	53.54
T3	17.66	27.52	41.04
SE d	1.09	1.43	1.40
CD (P=0.05)	2.37*	3.11*	3.05*
CV (%)	7.03	6.63	4.33

T1 - 10% sucrose, T2 - 10% sucrose + 300mg calcium nitrate + 100mg boric acid + 200mg magnesium sulphate +100mg potassium nitrate, T3 - 20% sucrose + 300mg calcium nitrate + 100mg boric acid + 200mg magnesium sulphate +100mg potassium nitrate

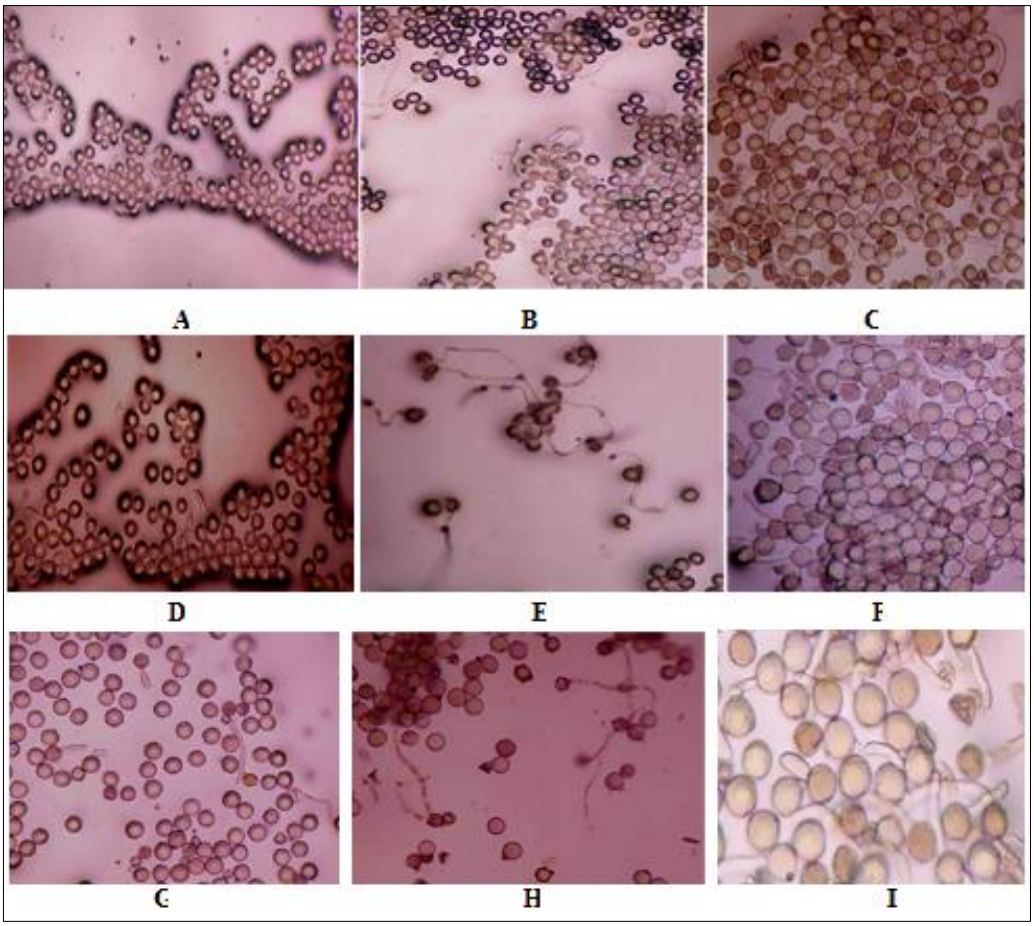

Fig 2: *In-vitro* pollen germination of three papaya genotypes *viz.*, TNAU papaya CO.8, *Vasconcellea cauliflora* and an intergeneric hybrid a. TNAU papaya CO.8 - T1 b) intergeneric hybrid - T1 c) *Vasconcellea cauliflora* - T1 d) TNAU papaya CO.8 - T2 e) intergeneric hybrid - T2 f) *Vasconcellea cauliflora* - T2 g) TNAU papaya CO.8 - T3 h) intergeneric hybrid - T3 i) *Vasconcellea cauliflora* - T3

Conclusion

Acetocarmine stain was found to be more effective for differentiating viable pollen grains from non-viable pollen grains in three papaya genotypes *viz.*, TNAU papaya CO-8 (cultivated variety), *Vasconcellea cauliflora* (wild relative) and an intergeneric hybrid (3-8-22-84) followed by Potassium iodide and Aniline blue. Maximum pollen germination percentage was recorded in medium with 10% sucrose followed by medium with 10% sucrose + 300mg calcium nitrate + 100mg boric acid + 200mg magnesium sulphate

+100mg potassium nitrate and medium with 20% sucrose + 300mg calcium nitrate + 100mg boric acid + 200mg magnesium sulphate +100mg potassium nitrate.

Acknowledgement
The authors are thankful to all the staffs in the Department of Fruit Science, Horticultural College and Research Institute and the Department of Genetics and Plant Breeding, Tamil Nadu Agricultural University, Coimbatore for providing facilities to conduct the research work.

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