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Effect of Some Organic Solvent on Pollen Grain Germination, Pollen Viability and Pollen Tube Length of *Manilkara Zapota* (L.) Van Royen.

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Abstract :

Current study was done with a view to unearth the effect of some organic solvent on pollen viability, pollen germination and pollen tube length of Manilkara zapota (L.) Van Royen. Study revealed that out of four organic solvent i.e Methanol, Acetone, Petroleum ether and Ethanol the most promising and superior among the four is Methanol which helped in extending the pollen viability upto 12 months.

Keywords: *Manilkara zapota* (L.) Van Royen., Pollen grain, Pollen grain germination, Pollen viability, Pollen tube length, Methanol, Acetone, Petroleum ether and Ethanol.

Introduction :

Manilkara zapota (L.) Van Royen. commonly known as chicku or sapota is an evergreen small tree developing ovoid-globular berry almost throughout the year. Fruits are sweet, edible and is in wide cultivation. Pollen grain also known as Male gametophyte are essential specialised structure responsible for pollination and later fertilization in flower. It contains important haploid gamete that eventually leads to the formation of diploid embryo after successful fusion with the female gamete egg during fertilization. Small size and large number of pollen grains production per flower increases the chances of seed formation. It is indeed a subject of curiosity which leads to the study on storage and long term effect of certain environmental and other physiological factor upon pollen grain. Pollen grain has and had been a subject of research among various research scholars. Small size of pollen grain ensures easy storage, easy transportation as well as help in studying long term effect of organic solvents on its viability and tube length formation. Similar type of studies on pollen grain of various plants were conducted with promising outcome reflecting the true potential and usefulness in various fields like plant breeding, plant tissue culture, pomology etc. (Iwanami and Nakamura, 1972; Iwanami, 1975; Kobayashi et al., 1987; Prasanna kumara Amma and Kulkarni, 1979; Yabuya *et al.* 1982; Jain and Shivanna, 1988).

Materials and Methods

- **Flower Collection**

The flowers of *Manilkara zapota* (L.) Van Royen. were collected from cultivated fields of Konkan krushi Agricultural research center, Palghar. Flowers were collected early in the morning, kept in polythene bags and then brought to research laboratory. Flower buds with stigma slightly protruding out of the buds were collected as it provides pollen grains with maximum viability as shown in **Photoplate1**. Other open flowers or under developed flowers buds were avoided. The collected flower buds were then vertically cut using sharp razor blade in two halves and later using sterozoom microscope, pollen grains were separated and kept in small glass vials.

- **Preservation**

For solvent treatment, four solvents were selected i.e Methanol, Petroleum ether, Acetone and Ethanol. The solvents were directly introduced in glass vials containing pollen grains. The glass vials were later kept in 5^0C.

- **Pre-humidification of stored pollen grains**

In order to activate the stored pollen grains, they were kept in watch glass containing water placed in moist, lined filter paper for 30 minutes before testing for viability and pollen tube growth.

- **Acetocarmine test**

1% Acetocarmine test (Singh, 2003) is followed to determine the viability of pollen grains. 1-2 drops of Acetocarmine is mixed with 2 drops of distilled water and later using dropper is placed in glass slide. Pollen grains are transferred and kept for 5 minutes. Later pollen grains were observed under compound microscope. Viable pollen grains appears bright red-pink where as non-viable appears brownish-colourless as shown in **Photoplate 2.**

- **Germination test**

For in vitro pollen germination sitting drop technique (Shivanna and Johri, 1985) were followed. In this technique, a drop of culture medium was placed on cavity slide and pollen gains were cultured in them. The slides were placed in petri plates already lined with moist filter papers to prevent unwanted evaporation. Many replicates were raised by this technique. The petri plates were kept for 48 hours as after 24 hours the pollen grains begin to germinate. For culture medium Brewbaker and Kwack method was adopted with slight modification (Brewbaker and Kwack, 1963). Maltose were used in concentration of 1-5 % with 500ppm of Boric acid. Best results for fresh pollen grains were obtained in 5 % maltose with maximum viability and good pollen tube length while for stored pollen grains 10 % were found suitable.

Results and Conclusion:

Pollen grain germination, pollen viability and pollen tube length were monitored after one month for a span of 13 months to check the effect of different organic solvent on pollen grains of *Manilkara zapota* (L.) Van Royen. Four organic solvent were selected for the process and they are Petroleum ether, Methanol, Acetone and Ethanol. Detailed germination percentage and pollen tube length in μ are given in tabular format **Table no 1, Table no 2, Table no 3 and Table no 4**. The organic solvent were kept in 5°C which also helped in retaining some of the pollen viability. Among the four organic solvent, Petroleum ether was the least effective in retaining viability as pollen grain were only able to germinate up to 10 months with 8.64 % germination and 19.22 μ tube length on 10th month. Methanol was found to be more suitable as pollen grain remain viable for 12 months. Methanol not only helped in maintaining the pollen tube length of 53.6 μ but also the germination percentage of 12.04 % was recorded on 12th month. It was clear that pollen grain remain viable only for 12 months in Methanol, Ethanol and Acetone. It is evident that pollen grain kept in the selected solvent were not able to retain their viability after 12 months.

Table no 1: Germination and tube length of pollen grain stored in Petroleum ether

Sr.no	No. of Months	Organic Solvent	
		Petroleum ether	
		Germination % Mean	Maximum tube length (μ) Mean
1	1	73.81	168
2	2	70.38	164
3	3	62.76	144
4	4	54.75	104.46
5	5	43.88	90.22
6	6	38.00	76.13
7	7	25.08	64.18
8	8	20.68	56.00

9	9	14.63	32.00
10	10	8.64	19.22
11	11	0.00	0.00

Table no 2: Germination and tube length of pollen grain stored in Ethanol

Sr.no	No. of Months	Organic Solvent	
		Ethanol	
		Germination % Mean	Maximum tube length (µ) Mean
1	1	68.23	152.2
2	2	65.66	140
3	3	63.08	134.4
4	4	56.20	123.2
5	5	43.30	112
6	6	36.15	108.8
7	7	32.12	103.2
8	8	28.63	89.6
9	9	22.11	85.6
10	10	18.44	67.2
11	11	13.21	48.8
12	12	9.16	34.4
13	13	0.00	0.00

Table no 3: Germination and tube length of pollen grain stored in Acetone

Sr.no	No. of Months	Organic Solvent	
		Acetone	
		Germination % Mean	Maximum tube length (µ) Mean
1	1	72.08	154.3
2	2	70.33	148.8
3	3	63.18	137.6
4	4	55.11	120.8
5	5	46.07	115.2
6	6	35.06	104.8
7	7	32.08	80.0
8	8	23.63	74.4
9	9	20.60	64.8
10	10	14.02	50.4
11	11	11.03	36.8
12	12	6.05	20.8
13	13	0.00	0.00

Table no 4: Germination and tube length of pollen grain stored in Methanol

Sr.no	No. of Months	Organic Solvent	
		Methanol	
		Germination % Mean	Maximum tube length (µ) Mean
1	1	78.22	184.3
2	2	74.10	172.8
3	3	69.76	165.6
4	4	62.03	156.8
5	5	54.75	147.2
6	6	45.88	137.6
7	7	36.04	130.4
8	8	32.06	114.4
9	9	25.63	100.8
10	10	22.14	83.2
11	11	17.63	58.4
12	12	12.04	53.6
13	13	0.00	0.00

Photo plate 1: Flower buds of *Manilkara zapota* (L.) Van Royen.

Photo plate 2: Acetocarmine test for pollen viability

Photo plate 3: Pollen grain germination.

Discussion

There are several factors responsible for the loss of pollen germination. Accumulation of secondary metabolic products like organic acid also inhibits the pollen tube growth (Panchaksharappa and Tirlapur, 1984). More detailed study is required to unearth other possible factor. The plant *Manilkara zapota* (L.) Van Royen. is cultivated in Palghar district particularly for its edible fruits.

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