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RESEARCH ARTICLE

POLLEN VIABILITY AND *IN-VITRO* GERMINATION IN DIPLOID AND TETRAPLOID *Asparagus* L.

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ABSTRACT

Pollen germination and Pollen viability plays a vital role in flowering plants. Fertilization and seed set, is the principle outcome of pollen germination and pollen tube growth. These two aspects were worked out in a monocot plant, *Asparagus* L. family Liliaceae. Two species *Asparagus racemosus* Willd. (2n=20) and *Asparagus densiflorus* cv. *Meyeri*. (2n=40) were undertaken for comparative study. Pollen viability was evaluated in Acetocarmine Jelly and TTC, whereas *in-vitro* germination was tested in different basic medias of various concentration. It was observed that, pollen viability was high in both the species in Acetocarmine compared to TTC. It was 93% and 1.96% in diploids and 85% and 1.96% in tetraploids. *In-vitro* Pollen germination was good in sucrose media of concentration 10%-20% for diploid and 20%-30% for tetraploid. The genotype has an important role in germination.

INTRODUCTION

The genus *Asparagus* L. belonging to the family Liliaceae, has some ornamental and medicinal species with its distribution on dry land in the old world (Lee *et al.,* 1997). *Asparagus* comprises of shrubs or herbs. In India 22 species have been recorded. Among these *A. racemosus* Willd. commonly known as "Shatavari" is highly medicinal. *Asparagus densiflorus* cv. *Meyeri* is known under a variety of names Meyers is ornamental. The plant grows fairly and rapidly to attain a height of about 2 feets. The true leaves of this fern are scale like and inconspicuous. Viability has been defined as "having the capacity to grow, germinate or develop (Lincoln *et al.,*1982) but viable pollen grains may not actually germinate if the conditions are not favorable. The ability of pollen grain to germinate on stigma with specific stain and produce an effective seed set following pollination to viability. The terms viability, stainability, vigor germinability, fertility and fertilization ability are different facets of pollen potential. Pollen viability is an essential factor in pollination biology research. Studies on *in-vitro* pollen germination and pollentube growth are very useful for explaining the lack of fertility. (Pfahler *et al.,*1997). The ability of pollen to grow depends upon the inherent chemistry of the pollen (Stanley and Linskens, 1974). To minimize the effect and to standardize the experiments, pollen should be extracted and transferred directly from dehiscent anther to the experimental solution as soon as possible (Firmage and Dafni, 2001).

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Germination success in sucrose medium or in polyethylene glycol may depend on the humidity to which the Pollen grains were exposed prior to the germination test or pollen age (Heslop-Harrison, 1987). *In-vitro* germination techniques have therefore been used extensively on a variety of pollen system. These studies have provided lot of information on physiology and biochemistry of pollen germination and pollen tube growth (Johari and Vasil, 1961; Stanley and Linskens, 1974; Shivanna and Johari, 1985; Heslop-Harrison, 1987 and Steer and Steer, 1989).The present work is intended to study the pollen viability and pollen germination in two species of *Asparagus* with different ploidy level because pollen germination and pollen tube growth are significant for studying a range of biological problems like monitoring cytotoxic effects of bioactive chemicals such as herbicides, pesticides and Pollutants (Wolters and Martens, 1987; Kristen and Kappler, 1990; Pfahler, 1992) and in studies on intracellular differentiation, cytoskeleton and polarities (Jafee *et al.,*1974, Heslop-Harrison and Heslop-Harriison, 1989; Steer and steer, 1989). Both the species of *Asparagus* are evaluated comparatively with the aim to find out suitable media for *In-vitro* germination, Secondly the germination percentage and lastly the effects of different kinds of media on pollen germination.

MATERIAL AND METHODS

For the present study, the experimental samples were collected from the natural habitat of the plant. *A. racemosus* Willd. was collected from Melghat Forest Amravati and *A. densiflorus* cv.

Meyeri was collected from Gayatri Nursery Amravati. The plant species were maintained in Departmental Garden. The fresh flowers at the full blooming stage were collected for estimating the viability and *in-vitro* germination during the period October-December for *A. racemosus* Willd. and March-April for *A. densiflorus* cv. *Meyeri.*

Pollen Germination

To check the *In-vitro* pollen germination four different types of basic medias were prepared (Shivanna and Rangswamy, 1992).

Table 1. Morphological Characteristics of *Asparagus* Species

Sr.No.	Characteristics	*A. racemosus* Willd. (2n)	*A. Densiflorus* cv. *Meyeri* (4n)
1	Habit	Perennial Climber	Compact Spreading
2	Habitat	Natural	Cultivated
3	Anthesis	7.30-9.30 am.	7.00-10.00 am.
4	Flowering	Once in year	3 times in year
5	Flowering period	October-December	March-April; July-August October-November;
6	Anthers/flower	six	six
7	Anther colour	Dark red	Light orange
8	Pollen	Homogenous	Heterogeneous

Table 2. Pollen viability Acetocarmine Jelly and 0.1% TTC

Plant species	Viable Pollen grain		Non viable Pollen grain		Total Pollen grains		% of Pollen viability	
	Aceto-carmine	TTC	Aceto-carmine	TTC	Aceto-carmine	TTC	Aceto-carmine	TTC
A. racemosus Willd	210	3	5	150	215	153	97.67	1.96
A. densiflorus cv. *Meyeri*	189	4	20	220	209	224	85.00	1.78

Table 3. *In-vitro* pollen germination in various Basic medias

Sr. No.	Media Concentrations	*A. racemosus Willd*	*A. densiflorus* cv. *meyeri*
1	Sucrose 30 %	+ + + +	+ + + +
2	Boric acid (200ppm)	+	+
3	20 % Sucrose + 200ppm Boric acid	+ +	+ +
4	Boric acid (20 ppm)	_	_

Table 4. Pollen Germination in different concentrations of Sucrose solution

Sr. No	Sucrose Concentrations	*A. racemosus* Willd.	*A. densiflorus* cv. *Meyeri*
1	10 %	93.16 ± 2.27	3.66 ± 1.91
2	20 %	90.57 ± 5.68	49.45 ± 5.54
3	30 %	78.89 ± 7.93	55.51 ± 4.85
4	40 %	40.67 ± 13.04	14.72 ± 3.26
5	50 %	29.51 ± 10.11	2.45 ± 1.19

The flowering period of *A. racemosus* is from October–December, but the full blooming was during mid October to mid November. While *A. densiflorus* cv. *Meyeri* it is from March-April but full blooming was during ending of March to mid April. The pollen from the flowers were collected at morning time i.e. 7.00 to 8.00am.

Pollen Viability

To check the viability, pollen was stained with 2% acetocarmine and glycerine as (1:1). Slides were prepared and observed after 1 hour under light microscope to evaluate their viability. Darkly stained pollens were counted as viable and pollen without or very light stain were counted as nonviable ones. In another method a drop of TTC (0.1%) solution was taken on the cavity slide and in it was suspended small amount of pollen and distributed uniformly in the drop. The slide was placed as it is at room temperature for 30-60 min in dark and then observed. Pollen grains that have turned red due to accumulation of formazan are viable (Shivanna and Rangswamy, 1992). *A. racemosus* Willd. showed 2-3% pollen viability and *A. densiflorus* cv. *Meyeri* showed 1-2% viability.

- Sucrose solution (30%)
- Sucrose +Boric acid (100ppm)
- Boric acid (concentration from 100 ppm upto 500 ppm)
- Boric acid (concentration from 10 ppm upto 100 ppm)

Two drops of media was taken on cavity slide. Suitable amount of pollen was dispersed in a drop of medium, one by one. The cultures were incubated at laboratory condition at 22 ± 20°C in dark/light for 1-3 hour. After the required period the germination percentage was calculated. Pollen sample of both species were used to study the effect of various concentration of sucrose solution i.e. 10%, 20%, 30%, 40% and 50% on germination. Again slides were prepared and kept for incubation at 20°C for 2 hour under natural light in room. The observations were carried out under a light microscope and recorded (Table 3,4). More than 300 pollen grains were observed. Morphological characteristics of the two species of *Asparagus* with respect to habit, flowers were observed and recorded (Table1).

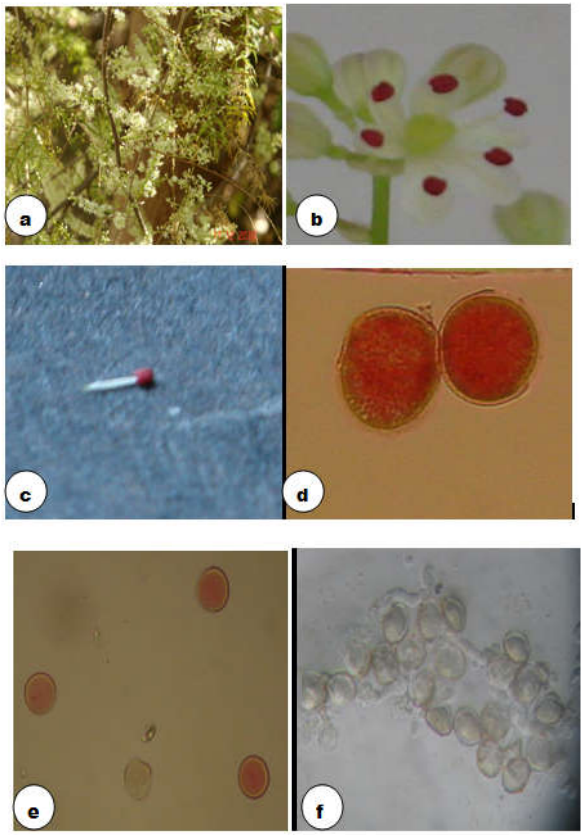

Fig (a-f): a- A twig of *Asparagus racemosus,* b-Single flower of *A racemosus,* c- Anther, d- Medium sized Pollen grains, e-viable and non-viable pollen grains tested by Acetocarmine, f- *in-vitro* pollen germination in sucrose solution

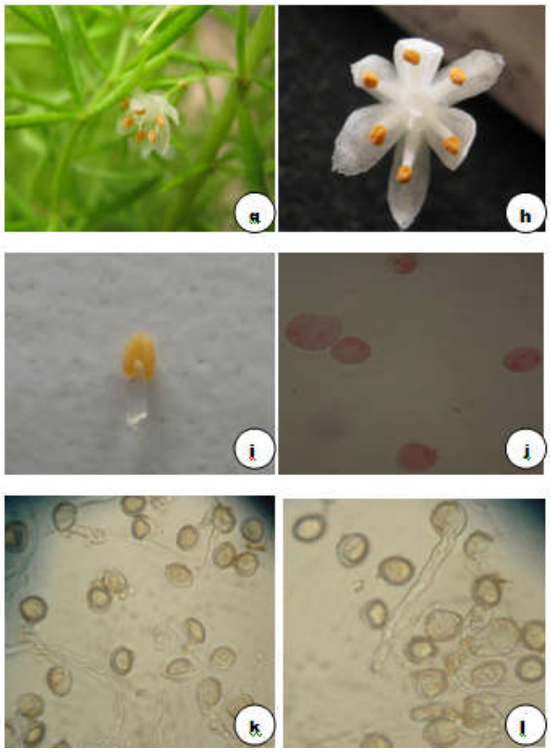

Fig (a-f): g-A twig of *Asparagus densiflorus* cv. *Meyeri,* h-Single flower of *Asparagus densiflorus* cv. *Meyeri,* i-Anther, j-Giant and small sized Pollen grains, k- *in-vitro* pollen germination in sucrose solution. l- coiled, Straight and swollen pollen tubes.

RESULT AND DISCUSSION

The result showed that pollen viability as tested by acetocarmine and 0.1% TTC was 97% and 1.96% and 85% and 1.78% respectively in diploid and tetraploid *Asparagus*. The viable pollen grains when tested with 0.1% TTC appeared red in colour due to accumulation of formazen. Whereas, non-viable remained colourless. The pollen viability was tested for the period October-December in *A. racemosus* Willd. and March-April in *A. densiflorus* cv. *Meyeri*. The pollen viability reduced due to temperature. It shows that TTC was not much effective in *Asparagus* for the study of pollen viability.

In-vitro pollen germination was good in Sucrose media compared to other media in both the species (Table 3). Further when varied concentrations of sucrose solution was prepared and pollen germination in each concentration was evaluated for the two species. It was found to be extremely high in 10% sucrose in diploid and 30% sucrose in tetraploid (Table no. 4). Sucrose solution varying from (10-30%) proved to be best for germination in *Asparagus* pollen. Boric acid 20ppm was toxic for both the species. Recently it was found to be pollen of *Helianthus annum* germinated in Boric acid deficient condition (Centin *et al*., 2000). *In-vitro* pollen germination of *Antirrhinum majus* and *Linaria vulgaris* gave best results as in *Paeonia tenufolia* in less boric acid concentration (Dane *et al.,*1994). The lowest germination was obtained in 10% sucrose for *A. densiflorus* cv. *Meyeri* i.e. 3.66 ± 1.97 and vice a versa in *A. racemosus* Willd. i.e. 93.16 ± 2.27. Generally, as the sucrose concentration increases the rate of germination percentage decreases. The pollen of *Asparagus racemosus* Willd. are of medium size and homogeneous while pollen of *A. densiflorus* cv. *Meyeri* are heterogeneous i.e. medium and large. It was found that germination started after 15 min in *A. racemosus* Willd. but in *A. densiflorus* cv. *Meyeri* it took some time for germination. The giant pollen grains germinated after one hour. Thus smaller pollen size linked to faster germination and larger size to slow germination. The optimum concentration of sucrose is good for *in-vitro* pollen germination. Comparative evaluation shows that diploid with good response than the tetraploid species. Negative effect of higher sucrose concentration on pollen germination are reported in some fruits. (Bolat *et al.,*1999). The pollen grains of different plant species require varying range of growth media like water, sugar solution inorganic salts and vitamins for their successful germination (Iwanomy, 1971; Mehan and Malik, 1975; Amma and Kulkarni, 1979). Sucrose is a best source of carbohydrates for *in-vitro* pollen germination and tube growth because it maintains the osmotic pressure and is a substrate for metabolism of pollen (Sari Gorla *et al.,*1977) During the past few years, *in-vitro* pollen tube growth have became a popular model system for cell biology studies in plant cells (Moutinko *et al.,*2001). In some species pollen tube formation is a rapid simple process. Hence is a model system in study of growth and developments in plants. Pollen hydration influences pollen viability and germination (Nepi *et al.,*2001, and Pacini *et al.,*2006). Rosaceous and Liliaceous pollen can remain viable for more than 100 days (Leduce *et al.,*1990).

Pollen grain can be shed at binucleate or trinucleate stage. Binucleate pollen germinate easily whereas trinucleate has short life period and is difficult to germinate (Grayum, 1986;

Kearns *et al;* 1993). The factors important in *in-vitro* pollen germination and viability are its genotype, temperature storage period and pollen size. *In-vitro* germination of pollen has been a powerful tool for genetical, physiological, biochemical and cytochemical studies for wide range of plant species (Heslop-Harrison, 1992). Therefore, it can be concluded that the diploid species *A. racemosus* Willd. has high pollen viability and germinability than the tetraploid *A. densiflorus* cv. *Meyeri.* It may be due to the tetraploid genotype and giant heterogeneous pollen of *A. densiflorus* cv. *Meyeri* Sucrose media was best for both but the optimum for diploid was 20% and tetraploid was 30%. Pollen viability was good in Acetocarmine.

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