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RESEARCH ARTICLE

OBSERVATIONS ON POLLEN VIABILITY, *IN VITRO* POLLEN GERMINATION AND POLLEN TUBE GROWTH in *CHLOROPHYTUM COMOSUM* (Thunb) Jacq. and *ASPARAGUS OFFICINALIS,* L.

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ABSTRACT

The aim of the present investigation is to assess the pollen viability and *in vitro* germination in *Chlorophytum comosum* and *Asparagus officinalis.* Pollen viability was tested with 1 % acetocarmine and 0.5 % TTC stain. For *in vitro* germination different media with variable concentrations and compositions of sucrose and boric acid were used. Maximum *in vitro* pollen germination in sucrose (40%), boric acid (40 ppm), sucrose + boric acid (30 % sucrose + 50 ppm boric acid) was found to be 77.62, 76.18 and 79.56 % in *Asparagus officinalis*. In *Chlorophytum comosum* maximum in vitro germination in sucrose (30 %), boric acid (20 ppm) sucrose + boric acid (30 % sucrose +20 ppm boric acid) was found to be 86.25, 95.48, and 81.34 % respectively. The rate of growth of pollen tubes was higher in 30% of sucrose for both the species because sucrose is the best carbohydrate source for pollen germination and tube growth for most of the plants investigated. Sugar also maintains the osmotic pressure of the medium. The pollen tube length was increased with increase in the time of germination.

INTRODUCTION

The family Liliaceae represents 240 genera and 4000 species, widely distributed especially in the warm temperate and tropical regions of the world. The genus *Chlorophytum* Ker-Gawl. of family Liliaceae is perennial herb, which comprises over 200 species distributed in the tropical, subtropical regions in the world (Sheriff and Chennaveeraiah, 1972). *Chlorophytum comosum* (Thunb) Jacq. known as Ribbon plant or Spider plant. It is a grass like, clump forming, evergreen perennial plant. The leaves are linear 20.3 - 40.6 cm long. Small white flowers are borne along outward arching wiry stalks. After blooming and fruiting, little tufts of leaves-baby spider plants develop on the stalks. These little "spider" take root whenever they touch the ground. The roots and rhizomes of the spider plant are fleshy and thickened, and serve as water storage organ for dry periods. *Asparagus officinalis* L. is the member of lily family. It is a perennial vegetable that may serve as an ornamental as well as food crop. It is native to most of Europe, Southern Africa and Western Asia. The mature *Asparagus* plant has a clumping growth habit with lacy, fern like foliage about 3 feet high. Although it is considered a temperate crop. It grows best at day time. The stems are stout, leaves are in flat needle like cladodes, 6-32 mm long. The flowers are bell shaped, greenish white to yellowish. *Asparagus* rhizomes and root is used ethonomedically to treat urinary tract infections, as well as kidney and bladder stones. It is also believed to have aphrodisiac properties.

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Pollen viability

To assess the pollen viability is an essential for any Pollination Biology research. Pollen viability has an impact of environmental factors. Pollen presentation and dispersal are also affected by environmental factors. The most accurate test of pollen viability is the ability of pollen to effect fertilization and seed set (Smith-Huerta and Vasek, 1984 and Shivanna and Johri, 1985). There is a close correlation between the cytology of pollen (two or three celled) and loss of viability. Two celled pollen generally retain viability for a longer period, than three celled pollen (Brewbaker, 1957). The need for assessing viability of pollen used in artificial pollination and in breeding experiments (Stone *et al*., 1995) is also important in understanding of sterility problem and hybridization programs (Gupta and Murty, 1985), fruit breeding programmes (Oberle and Watson, 1953) and evolutionary ecology (Thomson *et al*., 1964)

Pollen germination

Many Pollen grains can germinate in water or aqueous solutions of sucrose with no additives. But pollen of some species (such as trinucleated pollen grains) needs special substrates for germination. Pollen germination rate *in vitro* may be low, but can produces satisfactory fruit set *in vivo*, and vice versa (Johri and Vasil, 1961). The optimum sucrose solution for pollen germination can be used to evaluate the maximum pollen germination rate as an indicator of pollen viability. Sucrose is the best carbohydrate source for pollen germination and tube growth for most of the plants investigated (Tupy, 1960 and Hrabetova and Tupy, 1964).

Specific growth effect of sucrose seems to be due to the presence of β-D- fructofuranose (Hrabetova and Tupy, 1963, 1964).

In vitro pollen germination is the most commonly used test for assessing pollen viability. It is rapid, reasonabally simple and fully quantitative (Shivanna and Johri, 1985). Germination is the first critical morphogenetic event in pollen to fulfilling its ultimate function of discharge of male gametes in the embryo sac. The stigma provide suitable site of pollen germination. It is possible to germinate pollen grain of a number of taxa using rather a simple nutrient medium and to achieve a reasonable length of tube growth. Our knowledge of physiology and biochemistry of pollen germination and tube growth comes largely from *in vitro* studies. Due to involvement of the pistillate tissue in the nature, physiological and biochemical investigations on pollen germination and pollen tube growth *in vivo* are rather difficult. *In vitro* germination techniques have therefore been used extensively on a variety of pollen systems.

Pollen germination and tube growth are generally divided into four phases imbibition phase, lag phase, tube initiation and rapid tube elongation phase (Linskens and Kroh, 1970).The time taken for different phases varies greatly from species to species, depending on the type of reserve food material in the pollen and the external factors. Such studies have provided considerable information on physiology and biochemistry of pollen germination and pollen tube growth (Shivanna and Johari, 1985; Heslop- Harrison, 1987 and Steer and Steer, 1989). The aim of this study is to determine the pollen viability, germination capability and pollen tube growth within two genus of Liliaceae, *Chlorophytum* and *Asparagus noital.*

MATERIAL AND METHODS

Pollen Viability

To evaluate the pollen viability, pollen grains were excised from the anther of *Chlorophytum and Asparagus* species. The viability was observed as per the method described by Shivanna and Rangaswami (1992). These pollen grains were stained on the glass slide with a drop of 1% acetocarmine : glycerin (1:1) covered with a cover slip. Number of pollen grains with colour (viable) and without colour (nonviable) was noted and accordingly viability percentage was calculated. Viability was also tested and observed by using 0.5 % TTC (Dafni, 1992). A drop of TTC (0.5%) solution was taken on the cavity slide and a small amount of pollen was suspended in the TTC drop.

After putting the cover slip, slide was transferred in humidity chamber for incubation under the laboratory temperature or at 30±2°C for 30-60 min. The slide was observed under microscope and scored the viable and nonviable pollen. Viable pollen grains i.e. the pollen grains that have turned red due to accumulation of Formazen. Mean of five reading was taken for each concentration of madia.

In vitro pollen germination

The pollen germination was observed as per the method described by Shivanna and Rangaswamy (1992). For the *in vitro* pollen germination flowers of *Chlorophytum* and *Asparagus* were collected from departmental garden at 8.00 am early in the morning for *C. comosum* during last week of June to last week of July and for *A. officinalis* during the first week of June to last week of July. Anthers were selected just before dehiscence. Pollen was collected from indehiscence anther. Germinated and nongerminated pollen grains were examined under light microscope and tube length was measured using an ocular micrometer.

RESULTS AND DISCUSSION

Pollen grain shows differences in their physiological and structural characters at the time of pollen dispersal. An assessment of pollen viability is imperative factor in the study of reproductive biology, pollen storage and hybridization. Studies of Stanley and Linskens (1974) suggest that it is the deficiency of respiratory substrates land inactivation of certain specific enzymes or growth hormones that are likely to affect the viability of pollen. It has been extremely difficult to assess the exact reasons behind the loss of viability among pollen grains within a span of short and long period.

Pollen can be shed in the binucleate or trinucleated stage, binucleate pollen germinates easily whereas trinucleate pollen (ex. Asteraceae and Poaceae) has a very short life and are difficult to germinate *in vitro* (Grayum, 1986 and Kearns and Inouye, 1993). Some antibiotics are known to have a stimulating effect on pollen germination and tube growth. Pollen grains of different species of plant have specific requirements for their germination. Sugars, nutrient elements and some growth substances and vitamins have been used in pollen germination of different species of plants.

According to Rigamoto and Tyagi (2002) pollen fertility, which can be determined using pollen viability tests *in vitro* is very important in fruit and seed production in flowering plants. Therefore, pollen fertility knowledge for any plant species is essential for plant breeders and commercial growers. The pollen viability in *A. officinalis* and *C. comosum* showed considerable variations. Pollen germination and tube length also varies amongst *Asparagus* and *Chlorophytum* species. Pollen viability may differs depending upon tests and species. The pollen viability in *Asparagus* officinalis was 84.64 % in acetocarmine and 28.37 % in TTC (Table No. 1). In *C. comosum* the viability was 98.91 % in acetocarmine and 64.38 % in TTC. The result showed that *C. comosum* have highest pollen viability in both stain. TTC gives poor response than acetocarmine for *Asparagus* and *Chlorophytum* species. Comparative study shows that, *A. racemosus* has high pollen viability and germinability than the *A. densiflorus* cv. *meyeri.* It may be due to the tetraploid genotype giant heterogeneous pollen of *A. densiflorus* cv. *meyeri.* (Dhoran *et al*., 2011). Kelen and Demirates (2003) concluded that pollen viability, germination capacity level differed depending on the varieties and the tests. Pollen viability percentage varies between 31.5 to 68.8 % in TTC test and 0.0 to 75.0% in hanging drop method with 20% sucrose medium and 1.3 to 81% in saturated petriplate method and

Table 1: Pollen viability of *A. officinalis* and *C. comosum* with 1% acetocarmine and 0.5% TTC

Sr. No.	Species	No of viable pollen grains				No of total pollen grain				% of pollen viability	
		A	Mean	B	Mean	A	Mean	B	Mean	A	B
1	*Asparagus officinalis*	24		12		27		39			
2		48		15		55		54			
3		27	38.6	09	12.2	35	45.6	21	43.0	84.64	28.37
4		75		09		85		49			
5		19		16		26		52			
1	*Chloropytum comosum*	215		180		235		222			
2		249		134		138		180			
3		120	163.6	210	142.8	110	165.4	367	221.8	98.91	64.38
4		92		90		217		150			
5		142		100		127		190			

A – 1% Acetocarmine , B - 0.5 % TTC

Table 2: *In vitro* pollen germination of *A. officinalis* in sucrose solution

Sr. No.	Media (in %)	Mean no. of germinated pollen	Mean no. of total pollen	% of pollen germination	S.D.
1	10	24.4	40.6	60.09	59.30 ± 10.6
2	20	21.4	32.4	66.04	66.72 ± 7.9
3	30	33.8	45.8	73.79	74.40 ± 4.4
4	40	34	43.8	77.62	78.62 ± 10.8
5	50	65.6	97.4	67.35	67.46 ± 3.9

Table 3: *In vitro* pollen germination of *A. officinalis* in boric acid

Sr. No.	Media (in ppm)	Mean no. of germinated pollen	Mean no. of total pollen	% of pollen germination	S.D.
1	10	21.2	33.8	63.01	62.97 ± 3.78
2	20	49.2	68.0	72.35	72.18 ± 4.02
3	30	19.6	27.6	71.01	73.42 ± 10.27
4	40	23.2	31.4	73.88	76.18 ±10.14
5	50	26.2	37.0	70.81	70.82 ± 6.54

Table 4: *In vitro* pollen germination of *A. officinalis* sucrose solution + boric acid

Sr. No.	Media	Mean no. of germinated pollen	Mean no. of total pollen	% of pollen germination	S.D.
1	30 % sucrose +10 ppm boric acid	61.2	74.6	82.03	83.14 ± 7.41
2	30 % sucrose +20 ppm boric acid	102.6	131.8	77.84	77.86 ± 7.74
3	30 % sucrose +30 ppm boric acid	84	111.6	75.26	75.02 ± 4.82
4	30 % sucrose +40 ppm boric acid	116	146.2	79.34	78.16 ± 6.85
5	30 % sucrose + 50 ppm boric acid	71	89	79.77	79.56 ± 3.46

Table 5 : *In vitro* pollen germination of *C. comosum* in sucrose solution

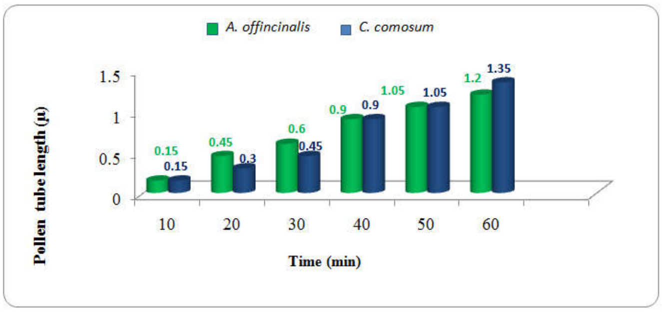
Sr. No.	Media (in %)	Mean no. of germinated pollen	Mean no. of total pollen	% of pollen germination	S.D.
1	10	152.6	180.2	84.68	81.46 ± 14.32
2	20	235.4	285.6	82.42	82.34 ± 6.22
3	30	214.6	248.8	86.25	87 .12 ± 5.28
4	40	310.0	363.8	85.21	85.17 ± 5.68
5	50	49.8	74.4	66.93	67.13 ± 10.63

Table 6 : *In vitro* pollen germination of *C. comosum* in boric acid solution

Sr. No.	Media (in ppm)	Mean no. of germinated pollen	Mean no. of total pollen	% of pollen germination	S.D.
1	10	85.2	93.4	91.22	91.07 ±3.28
2	20	101.6	106.4	95.48	94.11 ± 3.92
3	30	84.6	92.8	91.16	90.96 ± 5.02
4	40	76.2	81.2	93.84	93.29 ± 2.26
5	50	64.2	71.4	89.91	89.83 ± 4.20

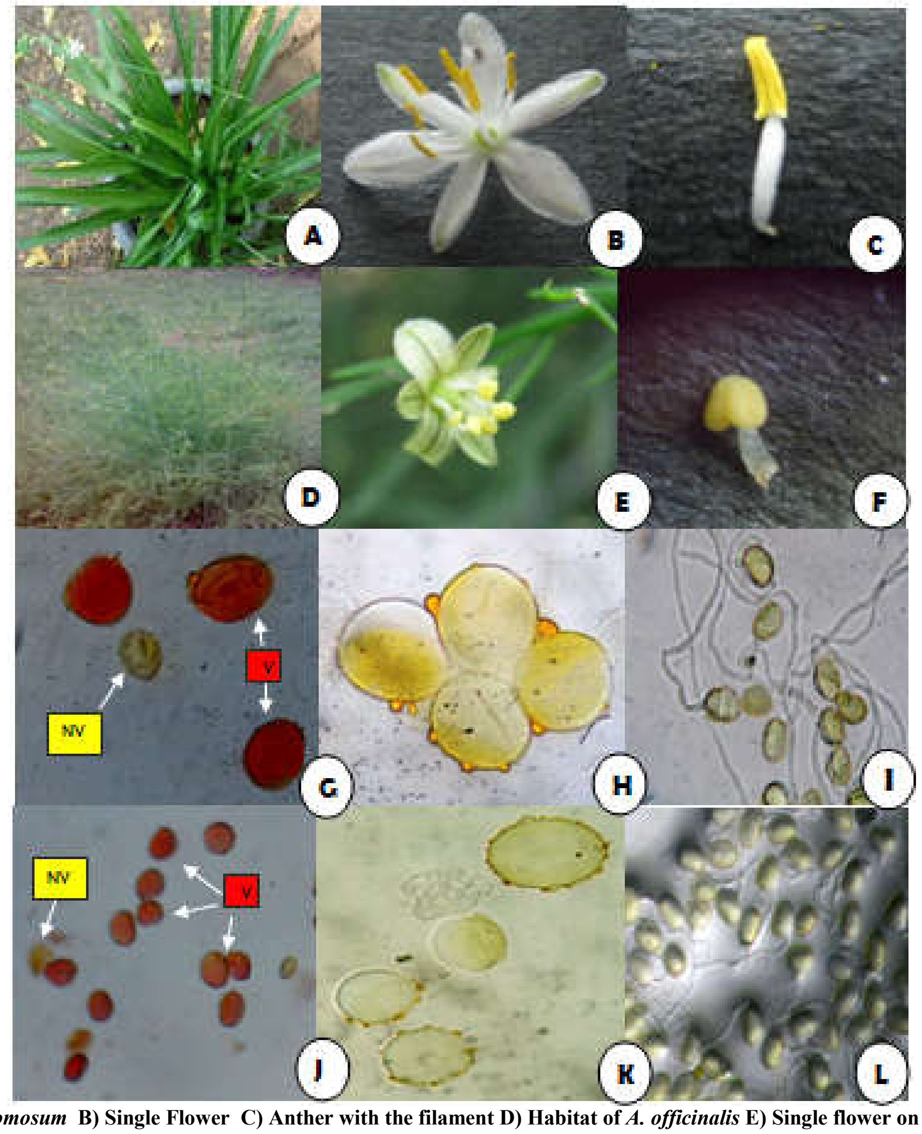
Table 7: *In vitro* pollen germination of *C. comosum* in sucrose solution+ boric acid

Sr. No.	Media	Mean no. of germinated pollen	Mean no. of total pollen	% of pollen germination	S.D.
1	30 % sucrose +10 ppm boric acid	73.6	90.8	81.05	80.94 ± 7.8
2	30 % sucrose +20 ppm boric acid	65.4	80.4	81.34	80.60 ± 13.35
3	30 % sucrose +30 ppm boric acid	35.8	53.0	67.54	68.52 ± 6.17
4	30 % sucrose +40 ppm boric acid	44.8	64.0	70.0	78.26 ± 13.13
5	30 % sucrose + 50 ppm boric acid	69.8	92.6	75.37	75.02 ± 6.3



Graph I: Pollen tube length in *A. officinalis* and *C. comosum*

essential prerequisite for artificial (*in vitro*) pollen germination. Germination of pollen is generally most successful immediately after anthesis and viability deteriorates rapidly in most species (Kearns and Inouye, 1993). The pollen germination of different families tested in basic medium and the investigated plant species, except *Malva sylvestris,* contain dextrin and among them the best pollen germination rates were in *Antirrhinum majus* and *Linaria vulgaris* (Scrophulariaceae) however, there were absence of pollen germination in *Malva sylvestris* (Malvaceae) and *Yucca filamentosa* (Liliaceae) (Dane *et al.,* 2004).



A) Plant of *C. comosum* B) Single Flower C) Anther with the filament D) Habitat of *A. officinalis* E) Single flower on a branch F) anther G) *C. comosum* pollen staning with acetocarmine H) *C. comosum* pollen staining with TTC stain I) *in vitro* pollen germination in *C. comosum* J) *A. officinalis* pollen acetocarmine staining K) *A. officinalis* TTC staining L) *A.officinalis* showing *in vitro* pollen germination

with 1.0 % agar + 15.0% sucrose medium. In *Erythronium grandiflorum* (Liliaceae), pollen viability decreases significantly within an hour of exposure to the air after dehiscence. Pollen viability for a short period after dehiscence in all plant species is not a common phenomenon. However Rosaceous and Liliaceous pollen can remain viable for 100 days (Leduce *et al.*, 1990). As the studies of pollen germination *in vivo* are difficult, our knowledge of the physiology and biochemistry of pollen germination and pollen tube growth is based on *in vitro* germination, moisture, carbohydrates, boron and calcium which are generally

A. officinalis was showed the highest pollen germination ie. 79.7 % in 50 ppm boric acid+ 30% sucrose. . The pollen germination in boric acid solution was recorded as 73.88 % in 40 ppm boric acid in *A. officinalis* and 95.48 % in 20 ppm boric acid in *C. comosum* which is found to be highest pollen germination percentage (Table no. 2,3,4). In *A. officinalis* the pollen germination percentage was 77.62 % obtained in 40% sucrose solution, in *C. comosum* the pollen germination was 86.25 % found in 30% sucrose solution. The highest pollen germination percentage was obtained in *C. comosum.* The results showed that the combination of 30 % sucrose + 50 ppm boric acid is suitable for the *A. officinalis* and 20 ppm boric acid alone is suitable for *C. comosum* (Table no 5,6,7). Sugars in culture medium serve a important function, to maintain the osmotic pressure of the medium (O' Kelley, 1955; Stanley and Linkens, 1974; Thomas and Dnyansagar, 1975 and Nygaard, 1977). Boric acid is generally used as the boron source. Pollen grains are believed to be deficient in boron, which is normally compensated by the high levels of boron present in the stigma and style. Several studies have indicated the role of boron in sugar uptake and translocation (Gauch and Dugger, 1953 and Dugger *et al*., 1975). It has been suggested that borons forms an ionisable sugar -borate complex which moves through cellular membranes more readily than non- borated sugar molecules (Gauch and Dugger, 1953).In general the medium used for pollen germination varied according to the plant species (Dane *et al*., 2004). Sucrose media was found to be best for both species of *Asparagus* but the optimum concentration for diploid was 20% and tetraploid was 30 % (Dhoran *et al.*, 2011). Pollen germination rate was increased depending on increasing sucrose concentration. The highest germination rate was found in hanging drop method with 20.0 % sucrose and saturated petriplate method with 1.0 % agar + 15.0% sucrose medium (Kelen and Demirates, 2003). Barabe *et al*. (2008) stated that among all species studied, except *Anaphyllopsis americana* and *Monstera deliociosa, in vitro* pollen germination was observed within a period of 1 hour after sowing in media. Although the pollen volume of *Montrichardia arborescence* is significantly greater than that of the other species, this did not affect the speed of germination in *A. americana,* however the smaller pollen volume seems to be linked to a lower speed of germination. Kashikar and Kalkar (2009) reported that, overall for both wild and cultivated varieties of pearl millets, 20% sucrose solution was found to be best suitable medium for germination.

The pollen tube is a cellular extrication of the pollen grain, and forms after germination of the pollen on stigma of receiving flower. The function of the pollen tube is to transmit gametes from pollen grain to the ovary, and this can occur over long distances, several centimeters in some cases also (Cresti *et al.,* 1992). Because of its fundamental importance to the process of fertilization in higher plants, the pollen tube has recently been subjected for intensive study with the aim of understanding the cell biology involved and regulating it through biotechnology (Cai *et al.,* 1997 and Stepheson *et al*., 2003). Our study reveals that when the pollen were cultured in sucrose solution by hanging drop culture, the pollen tube grews rapidly after culturing the pollen on media. *A. officinalis* showed slite increase in tube length and in *C. comosum* maximum increase within 50 to 60 min. The tube length in *A. officinalis* was 1.2 µm and *C. comosum* showed 1.35µm.

CONCLUSION

From the above observations it is concluded that both staining methods gives good response in determination of pollen viability. 1% acetocarmine stain gives best response to both *Asparagus* and *Chlorophytum* species than 0.5 % TTC stain. For 0.5% TTC *Chlorophytum* gives better result than *Asparagus*. 50 ppm boric acid in combination with 30% sucrose solution and 20 ppm boric acid also gives the best result during *in vitro* pollen germination assey. Boric acid is stimulating agent for pollen germination and pollen tube elongation. In *Asparagus officinalis* pollen tube length starts to grow slowly and then cessase at specific time. Pollen tube growth is found to be directly proportionate with time period, however, after certain period it cessase.

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