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Original Research Paper

Pollen viability and *in vitro* pollen germination in chrysanthemum (*Dendranthema* x *grandiflora* Tzvelv.)

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ABSTRACT

Chrysanthemum (*Dendranthema* x *grandiflora* Tzvelv.), is one of the commercially grown flower crops worldwide, valued for its cut flower, loose flower, pot mums, bedding etc. To overcome the challenges for cross breeding in chrysanthemum, the access to viable pollen, cultivar distant in location and difference in flowering, are required. Low pollen viability along with self-incompatibility are mainly responsible for low seed set in modern chrysanthemum cultivars. In the present study, pollen viability was tested by staining pollen with Alexander staining solution and examined under light microscope (20X). *In vitro* pollen germination was investigated in 10 chrysanthemum genotypes with different concentrations of sucrose (0, 5%, 10%, and 15%), PEG4000 (0, 10%, 20% and 30%) and their combination, as basal medium. Significant differences were observed among genotypes with respect to pollen viability, which was recorded highest in Red Stone (95.02%) followed by IIHR6-26 (88.20%), IIHR9-3 (87.44%), Sweta Singar (85.59%), Kalpana (79.97%), IIHR2-7 (73.50%), and White Andaman (64.14%), while, lowest pollen viability was recorded in IIHR6-29 (49.55%). Negligible *in vitro* pollen germination was observed among the genotypes when sucrose was used as pollen germination medium. However, highest germinability of pollen was recorded at 20% PEG4000 in IIHR9-3 (43.57%) followed by Kalpana (19.43%) and IIHR6-29 (19.34%). The best media for *in vitro* pollen germination was 15% sucrose + 30% PEG. Irrespective of different media combination, the genotypes namely, IIHR9-3, IIHR2-7 and IIHR6-26 showed better *in vitro* pollen germination. These results provide a valuable background to the conventional breeding to create hybrids through cross-pollination.

Keywords: Chrysanthemum, pollen germination, pollen viability, polyethylene glycol 4000, sucrose

INTRODUCTION

Chrysanthemum, family Asteraceae, is native to East Asia and North Eastern Europe. It is recognized as a potential crop for cut, loose flower and also as pot plants in many countries. It is second most important cut flower after rose and fourth as potted plant in global trade. In India, chrysanthemum covers 32.48 thousand ha area with production of 456.99 thousand MT of loose flower and 32.64 thousand MT of cut flower, respectively during 2022-23. Karnataka is the most prominent chrysanthemum growing state with an area of 16.17 thousand ha and production of 180.43 thousand MT of loose flower in 2022-23 followed by Tamil Nadu, Andhra Pradesh, Telangana, Madhya Pradesh, Assam, Chattishgarh and Himachal Pradesh (Anon., 2022-23).

The success or failure of hybridization technique in chrysanthemum is mainly influenced by pollen viability and germination. A quick and reliable method of testing pollen viability is essential to determine the optimum time for pollination. Pollen viability can be examined by staining with nuclear and by *in vitro* germination tests, which may vary among genotypes. Chun-qing et al. (2009) investigated pollen viability of male parent, in the cross between *Dendranthema indicum* and *D. grandiflorum* and found about 12% pollen viability just before pollination. Sun et al. (2010) indicated pollen viability of three wild species ranged from 20 to 25%. Further, Xu et al. (2012) found that pollen vitality increased from 11:00 to 14:00, peaked, and then declined. It was highest on the 4th to 6th day, ranging from 35.12% to 39.89%, and dropped to 7.41% by the 15th day.

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Yang & Endo (2005) reported that sucrose was not essential for pollen germination, and PEG4000 is an effective inducer of pollen germination of five wild *Dendranthema* species and three cultivars. Chen et al. (2009) found *in vitro* pollen germinability ranged from 0.3 to 25.6% and was below 10% in 15 cultivars. However, pollens of *Dendranthemum indicum* and its three hybrids with chrysanthemum cultivars Xinhong, Saiqihong and Meiguihong (Mao Hong-yu et al., 2004). PEG4000 was shown to have the capacity of inducing germination of chrysanthemum pollens. Miao et al. (2011) studied the genetic improvement of *Dendranthema indicum* var. *aromaticum* on an improved media supplemented with sucrose and PEG. Sucrose promoted pollen germination at low concentration for pollen germination. The PEG (200 g/L) solution promoted pollen germination (6.61%). Therefore, the present study was carried out to study the pollen viability and effects of sucrose and PEG 4000 on *in vitro* pollen germination in 10 genotypes of Chrysanthemum.

MATERIALS AND METHODS

Planting material

Ten genotypes namely Kalpana, Red Stone, White Andaman, Sweta Singar, IIHR6-26, IIHR6-29, IIHR6-32, IIHR2-7, IIHR9-3 and IIHR9-12 were used for pollen viability and pollen germination studies, grown at Division of Flower & Medicinal Crops, ICAR-Indian Institute of Horticultural Research, Bengaluru, Karnataka, India (13° 582 N Latitude, 78° E Longitude at an elevation of 890 m above mean sea level) under polyhouse condition during 2016 and 2017. Pollen grains were collected from the center disc of outer/tabular disc florets just after flower opening between 9.30 am to 11.00 am during October to December.

Pollen stainability (%)

The pollen stainability was carried out by using 1% Alexander staining dye and then observed under an electronic microscope to evaluate their viability. Darkly stained pollens were counted as viable and pollens with no or light stained counted as non-viable. Pollen stainability was evaluated about 1,000 grains and 3 repetitions were performed. Pollen viability was calculated with following formula.

$$\text{Pollen viability (\%)} = \frac{\text{Number of viable pollen grain}}{\text{Number of viable pollen grains + Nuber of non-viablepollen grains}} \times 100$$

Pollen germination liquid media (hanging drop)

The basal medium for pollen germination of 10 genotypes (Kalpana, Red Stone, White Andaman, Sweta Singar, IIHR6-26, IIHR6-29, IIHR6-32, IIHR2-7, IIHR9-3 and IIHR9-12) consists of different concentrations of sucrose (0, 5%, 10%, and 15%) and PEG4000 (0, 10%, 20% and 30%) and their combination (0, 5% sucrose + 10% PEG 4000, 10% Sucrose + 15% PEG 4000, 15% sucrose + 30% PEG 4000).

In vitro pollen germination

In vitro pollen germination was tested on different liquid culture mediums. Pollen germination was determined using hanging drop method (Stanley & Linskens, 1974). A drop of germination media was placed in center of coverslip and the corners of coverslip smeared with vaseline. For each genotype, about 10 mg of pollen were collected from anthers of 5-10 flowers on the day of dehiscence during 9:30-11:00 am. The pollen grains were dipped into media then mixed it with help of needle and the cover slip, then gently inverted over the cavity region of the slide. Further, the cavity slide glass was placed into petri-dishes with a moistened piece of filter paper, later, the dishes incubated for 8 h at 20°C under natural light. Later, the pollen grains were observed under an electronic microscope. More than 700 pollen grains were observed to judge the percentage germination and 3 repetitions were performed. Pollen grains were considered to have germinated when pollen tube length was greater than or equal to pollen diameter.

Statistical analysis

Pollen viability experiment was carried out in completely randomized design (CRD) and pollen germination experiment with factorial CRD with three replications. IBM-SPSS (www.spss.com.hk/software/statistics/dataanalysis) software was used to analyse the recorded data.

RESULTS AND DISCUSSION

Pollen viability (%)

Pollen viability of ten genotypes were tested by staining pollen with Alexander staining solution and examined under light microscope (20X). There was significant differences observed among the genotypes with respect to pollen viability, which was recorded highest in Red Stone (95.02%) followed by

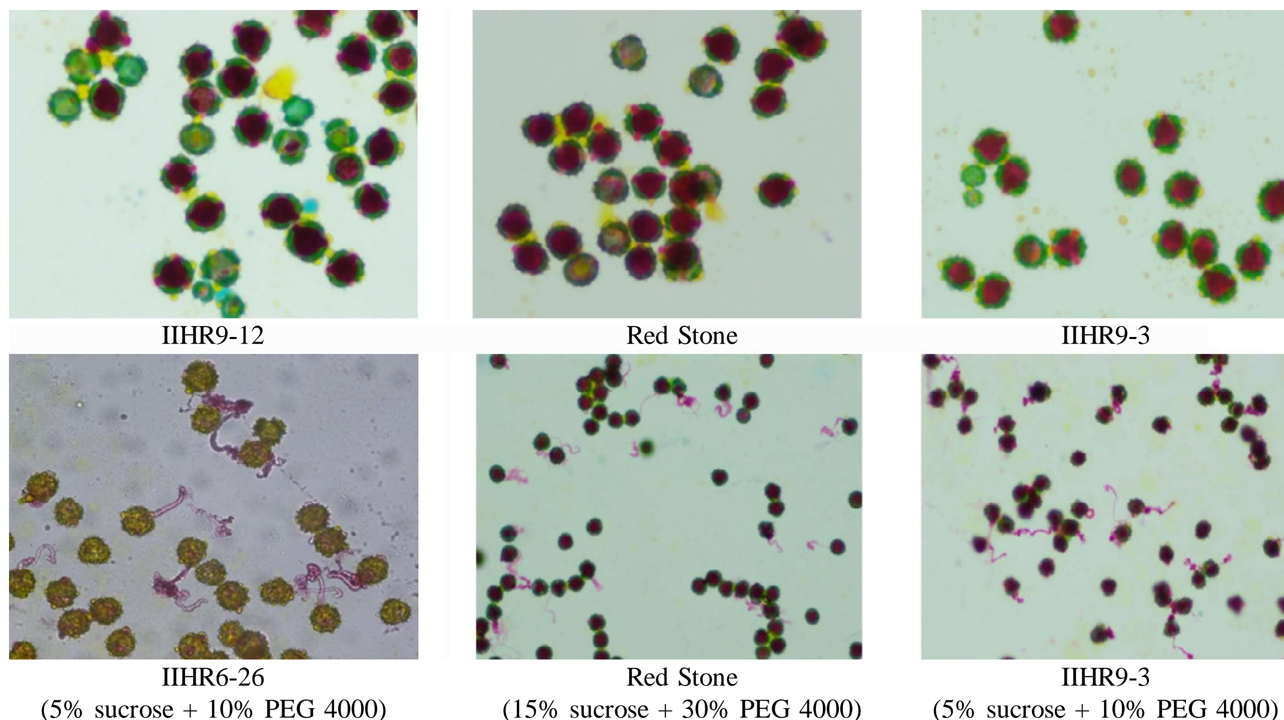

Fig. 1 : Stained pollen and pollen germination at different combination of sucrose and PEG 4000

IIHR6-26 (88.20%), IIHR9-3 (87.44%), Sweta Singar (85.59%), Kalpana (79.97%), IIHR2-7 (73.50%), and White Andaman (64.14%). However, the lowest pollen viability was recorded in IIHR6-29 (49.55%) (Table 1 and Fig. 1 & 2).

Table 1 : Pollen viability (%) of ten genotypes of chrysanthemum

Genotype	Pollen viability (%)
IIHR6-29	49.55
Swetha Singar	85.59
Red Stone	95.02
Kalpana	79.97
IIHR6-26	88.20
IIHR6-32	75.85
IIHR9-12	81.65
IIHR9-3	87.44
White Andaman	64.14
IIHR2-7	73.50
SEm±	1.50
C.D. at 5%	4.46

Pollen viability is an important parameter in deciding the pollen quality (Dafni & Firmage, 2000). Koshy & Jee (2001) reported that low pollen viability is one of the factors responsible for failure of seed set in *Bambusa vulgaris*. Poor quality pollen grains delivered on stigma may be failure in pollination, hence, reduces the seed set (Wilcock & Neiland, 2002; Huang et al., 2005). Similarly, Zhao et al. (2008) revealed that the pollen viability of 34.60%, 24.90% and 27.90% in three small flowered chrysanthemum cultivars, respectively. Chen et al. (2009) also reported pollen viability from 85.30 to 96.30%, when pollen stained with a 2% acetocarmine solution in chrysanthemum.

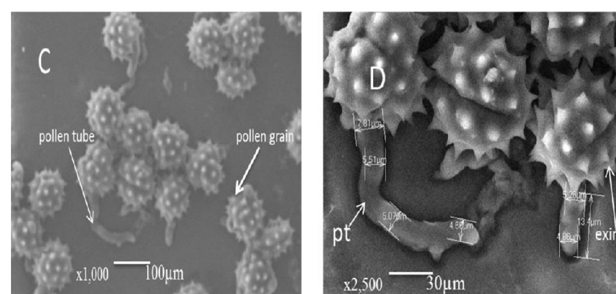

Fig. 2 : Pollen morphology, structure, germination and pollen tube growth under scanning electron microscopy

Effect of sucrose concentration on *in vitro* pollen germination

The sucrose at 0 (control), 5%, 10%, 15% was used for *in vitro* pollen germination of 10 chrysanthemum genotypes. Negligible *in vitro* germination was

observed among the genotypes when sucrose was used as pollen germination medium (Table 2). However, the significant average *in vitro* pollen germination was ranged from 0.00 (control) to 0.76% (15% sucrose). Ikeda & Numata (1998) also observed no pollen germination in liquid or agar medium with sucrose and boric acid, however, about 5% germination was recorded in agar medium in *D. grandiflora* and wild species. Yang & Endo (2005) reported that sucrose is not effective in pollen germination in three wild chrysanthemum species and one cultivar.

Effect of PEG4000 on *in vitro* pollen germination

PEG4000 at 0 (control), 10%, 20% and 30% as medium was used for *in vitro* pollen germination of 10 chrysanthemum genotypes (Table 3). The significant average *in vitro* pollen germination using PEG4000 was ranged from 0.00 (control) to 12.75% (20% PEG4000). Among the genotypes, IIHR9-3 recorded maximum pollen germination (22.63%) followed by IIHR6-29 (10.87%), White Andaman (6.08%), Kalpana (5.87%) and IIHR9-12 (5.52), whereas, it was recorded minimum in Sweta Singar (2.37%).

The pollen germination varies among the genotypes with respect to different concentrations of PEG4000. PEG4000 at 10% recorded maximum pollen germination 33.63% (IIHR9-3), while, no germination was observed in IIHR6-26, Sweta Singar and IIHR9-12. Medium PEG4000 at 20% recorded maximum pollen germination 43.57% (IIHR9-3) followed by 19.43% (Kalpana) and 19.34% (IIHR6-29), whereas, Sweta Singar (1.97%) recorded minimum germination. PEG4000 at 30% recorded maximum pollen germination of 19.84% (IIHR9-12). Among all the PEG4000 concentrations used, PEG4000 at 20% was found better for *in vitro* pollen germination in most of the chrysanthemum genotypes. Kawase & Tsukamoto (1977) elaborated that absorption of PEG by plant cells is inversely related to its molecular size. The high molecular weight of PEG possibly not observed by pollen, but worked as an osmoticum in the medium. When the osmotic pressure of a medium is lower than pollen, water is forced into the pollen grain from the medium results in enhancing pollen germination at optimal molecular weight of PEG. Yang & Endo (2005), in three wild chrysanthemum species and one cultivar, also found germination from 3.8% to 8.2% at 14% PEG4000 and reached maximum between 26.7 to 30.4 at 16% PEG4000 indicated that

Table 2 : Effect of sucrose concentration on *in vitro* pollen germination (%) in 10 genotypes of Chrysanthemum

Sucrose	Red Stone	IIHR6-26	Kalpana	IIHR6-29	Sweta Singar	IIHR6-32	IIHR9-3	White Andaman	IIHR9-12	IIHR2-7	Mean A
0 (Control)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00 (0.71)
5%	0.00	0.00	0.00	0.00	0.00	1.06	1.13	0.00	0.82	0.00	0.30 (0.86)
10%	0.00	1.31	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.13 (0.77)
15%	0.00	0.88	0.00	0.00	1.62	2.19	0.00	0.00	2.96	0.00	0.76 (1.04)
Mean B	0.00 (0.71)	0.54 (0.98)	0.00 (0.71)	0.00 (0.71)	0.40 (0.89)	0.81 (1.08)	0.28 (0.85)	0.00 (0.71)	0.94 (1.11)	0.00 (0.70)	
Factors										SEm±	C.D. at 5%
Factor (A)										0.009	0.028
Factor (B)										0.006	0.018
Factor (A x B)										0.018	0.055

Figures in parenthesis are square root transformed values

Table 3 : Effect of PEG 4000 on *in vitro* pollen germination in 10 genotypes of Chrysanthemum

PEG4000	Red Stone	IIHR6-26	Kalpana	IIHR6-29	Sweta Singar	IIHR6-32	IIHR9-3	White Andaman	IIHR9-12	IIHR2-7	Mean A
0 (Control)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00 (6.65)
10%	1.18	0.00	2.67	7.68	0.00	6.16	33.63	7.57	0	5.68	6.45 (11.10)
20%	6.03	2.22	19.43	19.34	1.97	3.69	43.57	13.86	2.26	15.14	12.75 (18.87)
30%	8.54	14.67	1.39	16.46	7.52	2.22	13.32	2.91	19.84	0.00	8.68 (15.19)
Mean B	3.93 (9.35)	4.22 (7.73)	5.87 (10.56)	10.87 (16.51)	2.37 (5.98)	3.01 (8.44)	22.63 (24.52)	6.08 (11.88)	5.52 (8.76)	5.20 (9.15)	
Factors										SEm±	C.D. at 5%
Factor (A)										0.36	0.95
Factor (B)										0.23	0.61
Factor (A x B)										0.72	1.91

Figures in parenthesis are arcsine transformed values

Table 4 : Effect of combination of sucrose and PEG4000 on *in vitro* pollen germination (%) in 10 genotypes of Chrysanthemum

Sucrose + PEG 4000	Red Stone	IIHR6-26	Kalpana	IIHR6-29	Sweta Singar	IIHR6-32	IIHR9-3	White Andaman	IIHR9-12	IIHR2-7	Mean A
Control	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00 (1.63)
5% Sucrose + 10% PEG 4000	0.00	47.45	11.20	18.07	14.54	0.00	53.27	4.11	0.00	49.76	19.84 (21.39)
10% Sucrose + 15% PEG 4000	0.00	42.24	18.20	26.19	10.85	0.71	35.02	13.34	12.20	42.27	20.10 (23.87)
15% Sucrose + 30% PEG 4000	31.66	41.76	28.18	30.34	10.56	3.11	15.72	6.04	3.36	38.64	20.93 (25.53)
Mean B	7.91 (8.53)	32.86 (31.04)	14.39 (19.02)	18.65 (22.30)	8.99 (15.11)	0.95 (3.69)	26.00 (26.62)	5.87 (11.79)	3.89 (7.73)	32.66 (30.95)	
Factors										SEm±	C.D. at 5%
Factor A)										0.72	1.92
Factor (B)										0.46	1.21
Factor (A x B)										1.45	3.84

Figures in parenthesis are arcsine transformed values

PEG4000 is an effective inducer of pollen germination in chrysanthemum. Hongbo et al. (2005) showed that the optimal culture medium for pollen germination of *D. indicum* and *D. vestitum* was modified liquid Monnior medium (ME_3) + 300 g/L PEG1500, resulted pollen germination from 54.1% and 46.3%, respectively.

Effect of sucrose and PEG 4000 on *in vitro* pollen germination

Among different sucrose and PEG combinations 15% sucrose + 30% PEG 4000 and IIHR6-26 recorded maximum *in vitro* pollen germination, 20.93% and 32.86%, respectively (Table 4, Fig. 1 and 2).

Irrespective of different media combination, the genotypes/lines namely IIHR9-3, IIHR2-7 and IIHR6-26 recorded better *in vitro* pollen germination. Qi et al. (2006) studied the effect of PEG4000, sucrose and pH on pollen germination medium of 10 mgL^{-1} H_3BO_3, 0.03% $Ca(NO_3)_2$, 15% PEG4000, 15% sucrose, and found pH 7, were optimal with a highest pollen germination rate (73.26%) in *Heptacodium miconioides*. Yong-ping et al. (2010) found that maximum marigold pollen germination (54%) sucrose (15 g/L) and polyethylene glycol (16%). Miao et al. (2011) reported that the low sucrose was favorable for pollen germination and the high sucrose inhibits it, however, PEG (200 g/L) solution promoted pollen germination (6.61%), temperature at 20^0C was the best for pollen germination, and with increasing temperature, the germination rate was reduced. Jayaprakash et al. (2018) reported 78% pollen germination and pollen tube growth in egg plant and four wild species in medium consist of 20% maltose + 250 mg/L boric acid + 300 mg/L calcium nitrate + 15% PEG 4000 + 750 mg/L EACA + 0.5–1% agar.

CONCLUSION

From the present investigation, on *in vitro* pollen viability and pollen germination in chrysanthemum, PEG4000 at 20% media recorded maximum pollen germination in IIHR9-3, followed by Kalpana and IIHR6-29. The ideal media suitable for *in vitro* pollen germination was 15% sucrose + 30% PEG. Irrespective of different media combination, the genotypes namely IIHR9-3, IIHR2-7 and IIHR6-26 recorded better *in vitro* pollen germination in all media combinations. It was also observed that, there was no correlation between pollen viability and germination in chrysanthemum.

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