Proc. Indian Acad. Sci. (Plant Sci.), Vol. 90, Number 4, August 1981, pp. 363-369.

Optimum cultural requirements for *in vitro* germination of *Amaryllis vittata* Ait (Amaryllidaceae) pollen

SAVITA SHARMA, C P MALIK and M B SINGH
Department of Botany, College of Basic Sciences and Humanities, Punjab Agricultural University, Ludhiana 141 004, India

MS received 1 December 1980; revised 25 May 1981

Abstract. The effect of various factors upon *in vitro* germination of *Amaryllis vittata* pollen was studied to find out optimum cultural requirements for obtaining high percentage of pollen germination and maximum tube growth. Temperature below —10° C was suitable for the storage of *Amaryllis* pollen. Maximum germination was observed in sucrose medium. Pollen germination and the tube growth were maximal at the culture density of 5 mg/ml of the culture medium. The optimum temperature was 28 ± 2° C. Studies on respiration suggested that optimum conditions for maximum O_2 uptake were the same as for optimum pollen germination and pollen tube growth rate.

Keywords. Pollen germination; population effect; *Amaryllis vittata*.

1. Introduction

Amaryllis vittata (Amaryllidaceae) is a perennial, bulbous herb grown for ornamental purposes and has gametophytic self-incompatibility. The pollen is unable to germinate after self-pollination. Very little information is available regarding the factors regulating pollen germination on stigma and pollen tube growth of this species during passage through the style. Similarly, information on *in vitro* germination of pollen in this species is also lacking. The present studies were undertaken to determine how *Amaryllis* pollen germination was affected by sucrose, boric acid and pentaerythritol. Attempts were also made to find out the conditions (e.g. culture density, temperature, pH) required for optimal pollen germination (%) and the tube growth. In addition, respiratory studies were also undertaken with a view to obtaining correlations, if any, between the rate of O_2 uptake and the effect of the above cultural conditions on pollen germination and the tube growth.

2. Material and methods

Amaryllis vittata Ait (Amaryllidaceae) pollen was collected from the freshly dehisced anthers and stored at different temperatures (30° C, 4° C, − 10° C). For

experimentation, the pollens stored at (− 10° C) was used because they remain viable for at least one year without any loss in their germinability. To avoid any variability due to desiccation, the pollen was equilibrated (from storage conditions at − 10° C) under laboratory conditions (30° C) for one hr before incubation. Different concentrations of sucrose and boric acid were tried at different temperatures. A suitable composition of BM (sucrose 0·1 M) and pentaerythritol medium supplemented with boric acid (10 μg/ml) was used to determine the optimal conditions for *in vitro* germination. The growth measurements were made with ocular micrometer. About 100 observations with three replicates were made for germination (%) and the tube length (μm). The respiratory activity of germinating pollen was measured using conventional Warburg constant volume monometers. Each Warburg flask contained 3 ml culture medium and 15 mg pollen was added for each set of experiment except for culture density effect. KOH (0·2 ml of 20%) was added to the central well. The O_2 uptake was measured at an interval of 30 min up to 6 hr of germination. Three replicates were run at a time for each treatment.

3. Results and discussion

3·1. *Effect of sucrose*

The optimum sucrose concentration for maximum germination (%) and tube length was 0·1M (figure 1). Our results are in variance with those of Sfakiotakis *et al* (1978) on *Ceratonia siliqua* pollen but agree closely with the observations of Calzoni *et al* (1979) on two apple cultivars. It is obvious that sugar requirements of pollen varies with different plant species. Sucrose plays a dual role both as a carbon source and as an osmotic agent. In *Lilium longiflorum*, Dickinson (1978) has shown that pollen germinating in a defined medium has calcium, boric acid and an osmoticum as basic requirement. In *Amaryllis vittata* boron and osmoticum were found to be essential but no requirement for calcium was observed.

3.2. *Effect of boric acid*

A significant increase in the germination (%) and pollen tube length was observed with an increase in boric acid concentration (0 to 10 μg/ml) (figure 2) in the sucrose medium. The effect was most pronounced on pollen tube length. Higher concentrations (100–1000 μg/ml) inhibited pollen tube growth. Sucrose medium supplemented with boron displayed increased O_2 uptake (figure 3). Boron may be helping in the translocation of sugars, stimulating synthesis of pectic material. The general view is that pollen having low boron endogenous level require low boron for tube growth.

3.3. *Effect of temperature*

Pollen grains were incubated at 15, 20, 25, 28, 35° C and maximum tube growth was observed at 28° C. Above 30° C both germination percentage and tube growth were reduced (figure 4a, b, c). No distinct correlation was observed when

Table 1. Effect of varying culture density on pollen germination percentage, tube growth and respiratory rate of *Amaryllis vittata* pollen germinated in liquid culture medium (3% sucrose containing 10 mg/l boric acid).

Sample (mg pollen/ 2 ml)	Per cent germination	Pollen tube length (μm) after 2 hr	μ O_2 uptake/ mg pollen /hr
5	65	962±19	7·4
10	72	1031±39	13·9
15	77	984±26	23·1
20	68	890±31	18·8

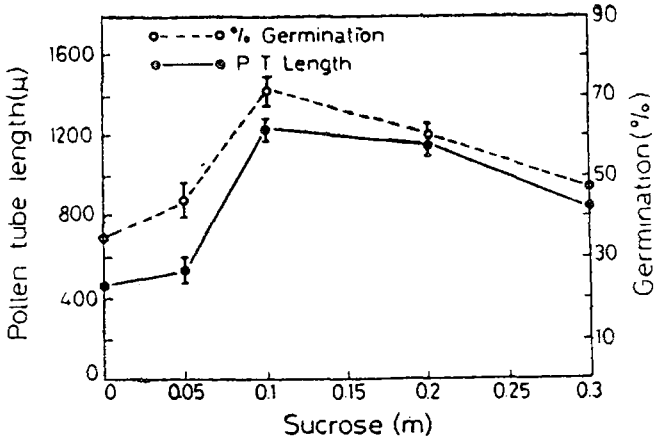

Figure 1. Effect of sucrose concentrations (0·005–0·3 M) on pollen tube length and percentage germination in *Amaryllis* pollen.

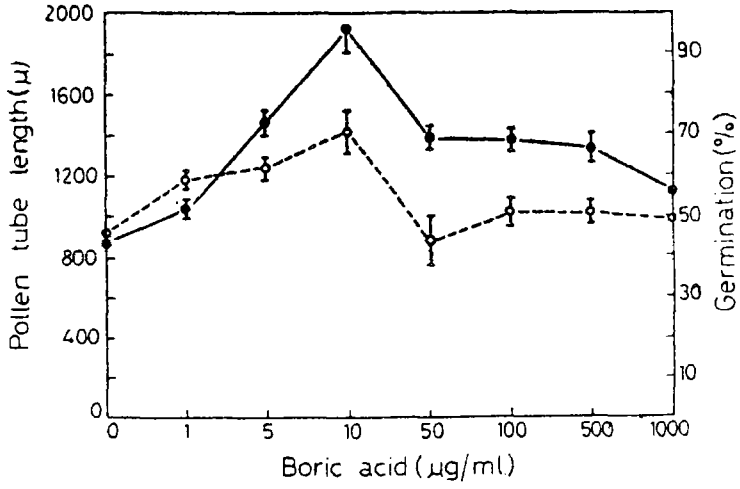

Figure 2. Influence of different concentrations of boric acid (1–1000 mg/litre) supplemented in basal medium on pollen tube length and germination percentage.

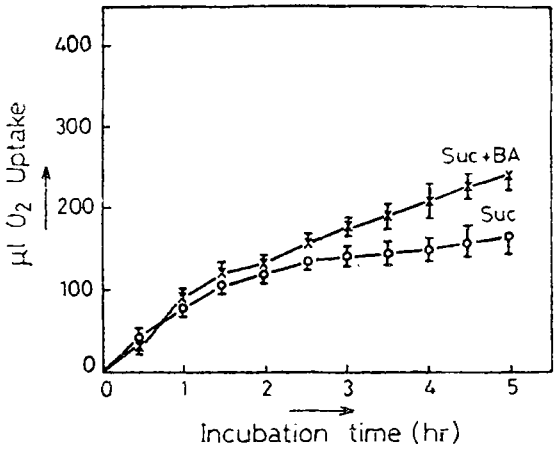

Figure 3. Effect of boric acid on the rate of respiration in germinating pollen.

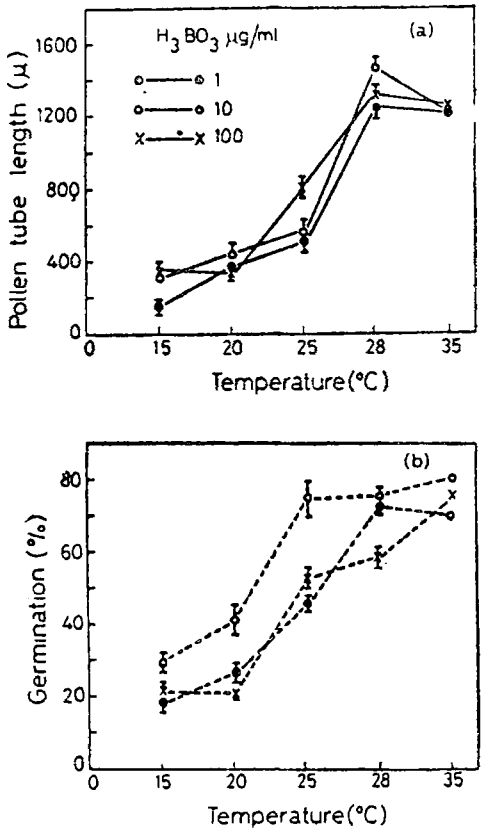

Figure 4a, b. Effect of different concentrations of boric acid (1,10,100 mg/litre) supplemented with basal medium at different temperatures (15, 20, 25, 28, 35° C) on pollen tube length and germination percentage.

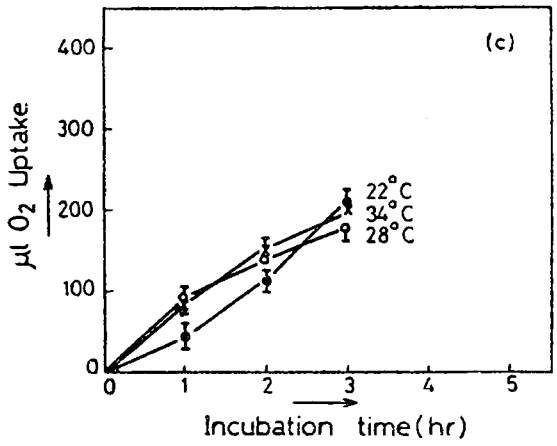

Figure 4c. Effect of temperature on respiratory rate of *Amaryllis vittata* pollen.

interaction of boric acid and temperature was studied. On the contrary, in *Ceratonia siliqua* pollen, an interaction was noticed for optimum germination since both 25° C temperature and boric acid (100 µg/ml) are required (Sfakiotakis *et al* 1978). Temperature requirement for optimal germination is reported to vary with species.

3.4. *Culture density effect*

Varying amounts of pollen (5–20 mg/ml) were incubated in sucrose medium at 28° C. Table 1 shows that optimal tube growth was observed at the culture density of 5 mg/ml ($6{\cdot}0 \times 10^4$) pollen grains approximately in one ml of culture medium. Higher concentration had inhibitory effect on pollen tube length. Nygaard (1970) reported that omission of calcium in the medium for *Pinus mugo* pollen inhibits growth to a greater extent in sparsely populated cultures than in densely populated ones. It was proposed that excretion of calcium by non-germinated pollen grains enriched the medium and caused 'population effect'. However, in the present studies, addition of calcium ions failed to give similar results. In *Acer pseudoplatanus* cell cultures Stuart and Street (1969, 1971) demonstrated the presence of a volatile factor released by an actively growing suspension for the initiation of growth in cultures at low density. The volatile factor could be absorbed in KOH, suggesting that it might be CO_2. The growth promoting effect of CO_2 has been observed in several systems including pollen tubes (Sfakiotakis *et al* 1972; Dhaliwal and Malik 1980). The removal of CO_2 from air space of pollen culture caused decrease in pollen tube growth while an increase in CO_2 level stimulated it. This stimulatory effect depended on dark CO_2 fixation by the germinating pollen (Sharma *et al* 1981).

3.5. *Effect of pH*

The germinating pollen showed distinct changes in growth response, in relation to the pH of the culture medium. The maximal tube growth was observed at

Table 2. Effect of pH on pollen germination, tube growth and respiratory rate of *Amaryllis vittata* pollen germinated in basal medium (3% sucrose supplemented with boric acid 10 mg/l).

pH in the medium	Per cent germination	PT length (μm) after 2 hr of incubation	μ O_2 uptake/ mg pollen/hr
4	No growth	No growth	1·6
5	38	336±21	4·8
6	39	592±28	7·2
7	75	1178±45	9·2
8	Initiation	Initiation	6·5
9	No growth	No growth	2·4

pH 7 (table 2). A decrease in the pH of basal medium caused decline in both these parameters. The inhibition was pronounced when the pH was changed from 7·0 to 6·0. However, between pH 5 to 6 the growth response was nearly similar. At pH 7·0, the O_2 uptake was maximum. Increasing (8·0) or decreasing (6·0) pH caused a decline in the rate of O_2 uptake. At pH 4·0 and 9·0 the respiratory rate was very low and it remained constant up to 4 hr stage. Pine pollens (e.g. *P. densiflora*, Tanaka 1955; and *P. mugo*, Nygaard 1969) show broad growth optimum in media of different pH values. However, *Amaryllis* pollen appears to have a narrow range for optimal growth responses in suspension cultures.

Acknowledgement

SS is thankful to the ICAR for financial assistance in the form of a fellowship.

References

Calzoni G L, Speranza A and Bagni N 1979 *In vitro* germination of Apple pollens; *Sci. Hort.* **10** 49–55

Dhaliwal A S and Malik C P 1980 Effect of Relative Humidity and CO_2 on the shape, volume and fresh weight of *Brassica campestris* L. pollen *in vitro* ; *Indian J. Exp. Biol.* **18** 1522–1523

Dickinson D B 1978 Influence of borate and pentaerythritol concentration on germination and tube growth of *Lilium longiflorum* pollen; *J. Amer. Soc. Hort. Sci.* **103** 413–416

Nygaard P 1969 Studies on the germination of pine pollen (*Pinus mugo*) *in vitro* I. Growth conditions and effect of pH and temperature on germination, tube growth and respiration; *Physiol. Plant* **22** 338–346

Nygaard P 1970 Studies on the germination of pine pollen *in vitro* II. Effect of different ions; *Physiol. Plant* **23** 372–384

Sfakiotakis E M, Simon D H and Dilley D R 1972 Pollen germination and tube growth dependent on CO_2 and independent of ethylene; *Plant Physiol.* **49** 963–967

Sfakiotakis E M 1978 Germination *in vitro* of Carob (*Ceratonia siliqua* L.) pollen; *Z. Pflanze Physiol.* **79** 443–48.

Sharma Savita, Singh M B and Malik C P 1981 Dark CO_2 fixation during germination of *Amaryllis vittata* pollen in suspension cultures; *Indian J. Exp. Biol.* **19** 710–714

Simons D H, Sfakiotakis E and Dilley D R 1972 Enhancement of *in vitro* germination of lily with increased pre-inoculation humidity; *Hort. Sci.* **7** 556–557

Stuart R and Street H E 1969 Studies on the gowth in culture of plant cells. IV. The initiation of division in suspension of stationary phase cells of *Acer. pseudoplatanus* L. *J. Exp. Bot.* **20** 556–71

Stuart R and Street H E 1971 Studies on the growth in culture of plant cells. X. Further studies on the conditioning of culture media by suspension of *Acer pseudoplatanus* cells ; *J. Exp. Biol.* **22** 96–106

Tanaka K 1955 The pollen germination and pollen tube development in *Pinus densiflora* Sieb; et. Zucc. III. The growth-inhibiting substances in ether extracts from *Pinus* pollen grain; *Sci. Res. Tohoku Univ.* (*Biol*)