POLLEN BIOLOGY OF FOUR ENDEMIC BALSAMS FROM THE WESTERN GHATS

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ABSTRACT

*The present investigation deals with pollen biology (*in vitro *and* in vivo *pollen germination) of four endemic balsams from the Western Ghats, namely,* Impatiens diversifolia, I. fruticosa, I. trichocarpa *and* I. verticillata. *Twenty percent sucrose medium supplemented with 150ppm boric acid was found to be the best for* in vitro *pollen germination.* In vivo *pollen germination in all the selected species were studied in different time intervals after anthesis. The results indicated that in* I. diversifolia *and* I. trichocarpa*, maximum stigma receptivity of 80% and 70% were observed for up to 18 hours with pollen germination of 66% and 62%, respectively. The other two species* I. verticillata *and* I. fruticosa *have extended their stigma receptivity for up to 15 and 24 hours with 60% and 40% receptivity but having recorded a low of 36% and 23% pollen germination respectively.*

KEYWORDS

Anthesis, Balsams, pollen biology, stigma receptivity, Western Ghats.

The family of Balsams, is one of the largest groups among flowering plants and has about 900 species of which all but one belong to the genus *Impatiens*. It is essentially a sub-cosmopolitan family of old world angiosperms being distributed mainly in the montane forests of tropical Asia and Africa. However, a few are distributed in the northern temperate zone also. Balsams are handsome plants with curious and variously coloured flowers. Due to their peculiar floral structure and habits, they can be easily recognized by common man. The members are more or less succulent annuals or perennial herbs, rarely becoming shrubs or epiphytes. In India, there are 200 species of balsams growing in the wild and many have high ornamental potential (Rajalal *et al*., 1996). Only a few exotics and their varieties are widely grown as ornamentals while the indigenous are yet to be popularized. This is due to their forest dwelling nature with high degree of endemicity. Endemism gives us a clue that they are in restricted distributions either due to their reproductive syndrome or by anthropogenic pressures. To find out the exact causes of their endemicity as well as rarity, we studied the pollen biology of four species namely *Impatiens diversifolia*, *I. fruticosa*, *I. trichocarpa* and *I. verticillata* as part of an overall understanding of the reproductive capacity which directly result in the distribution of these species.

MATERIALS AND METHODS

Four horticulturally promising wild species of *Impatiens* (balsams) were selected for the present study and collected from diverse localities as follows. *Impatiens diversifolia* was collected from Palode and neighbouring places; *Impatiens trichocarpa* from Wayanad; *I. verticillata* was located from the high ranges of Idukki District; and *I. fruticosa* from the Nilgiris. All these species were multiplied and grown in the garden for experimental purposes.

For *in vitro* pollen germination studies, fresh flowers were collected from the field in the morning at 0800hr. Pollen grains were transferred to a clean slide containing different concentrations of boric acid (100, 150 and 200ppm) and sucrose (10, 15, 20, 25 and 30%). The slides were kept in petridishes lined with moist filter paper, which were microscopically investigated. For the study of pollen germination on stigma (*in vivo*), the flowers were labelled at the time of anthesis. The pistils were collected from the labelled flowers at different time intervals after anthesis. These pistils were boiled with lactic acid, stained with 10% cotton blue for 10min, mounted on clean slides and examined microscopically.

RESULTS

In vitro pollen germination

Variable responses in pollen germination and tube elongation to different concentrations of boric acid and sucrose were observed. Sucrose is the best carbohydrate source for pollen germination and tube elongation in many plants. However, the optimum concentration of sucrose varies from species to species. Higher concentration of sucrose resulted in a decrease in pollen germination and subsequent tube elongation in the balsams. However, 20% sucrose medium supplemented with 150ppm boric acid was found to be the best (Table 1). In case of *I. diversifolia* and *I. trichocarpa* the pollen germination and tube length were 86% with 420µm and 80% with 386µm respectively. *Impatiens verticillata* showed 55% pollen germination with tube length of 296µm. The lowest pollen germination was observed in *I. fruticosa* with 45% and 223µm tube length.

In vivo pollen germination

The stigmas were studied at different time intervals on the day of anthesis. The stigmas remained receptive after opening of flowers in all the cases. From the results, it was found that *I. diversifolia* and *I. trichocarpa* had extended their receptivity of 80% and 70% for up to 18 hours and pollen germination were recorded 66% and 62%, respectively. In case of *I. verticillata* only 60% of stigma receptivity with 36% of pollen germination was observed in spite of its receptivity extended for up to 15 hours. Maximum extended period of up to 24 hours with 40% receptivity was observed in *I. fruticosa*, but strikingly the pollen germination was as low as 23% (Table 2; Fig. 1).

In all the cases pollen grains adhered well on the stigmatic surface. In *I. diversifolia* and *I. trichocarpa* pollen tubes reached the ovary and successfully fertilized the ovules. The fertilized ovules developed into seeds with more than 90% viability which directly resulted in better establishment. In the case of *I. verticillata* and *I. fruticosa* pollen tubes did not penetrate the stigmatic region, 20% of them exhibited different growth abnormalities such as swollen tips, followed by bursting, and the remaining tubes grew

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Table 1. Effect of boric acid and sucrose on pollen germination and tube length of* Impatiens *spp.

Plants	Boric acid Conc. (ppm)	Sucrose concentration 10% PG(%)	TL(µm)	15% PG(%)	TL(µm)	20% PG(%)	TL(µm)	25% PG(%)	TL(µm)	30% PG(%)	TL(µm)
Impatiens diversifolia	100	68	366	69	383	80	412	72	370	65	357
	150	76	380	78	387	86	420	76	383	66	353
	200	71	377	73	382	82	416	68	362	63	346
Impatiens trichocarpa	10	58	310	60	340	75	356	62	322	60	338
	150	65	361	65	362	80	386	72	368	68	360
	200	60	344	63	356	78	362	65	360	62	320
Impatiens verticillata	100	40	246	45	260	48	288	46	264	38	228
	150	41	242	51	272	55	296	52	276	40	244
	200	38	231	43	252	46	268	43	250	37	224
Impatiens fruticosa	100	36	218	38	227	38	210	36	224	30	212
	150	37	221	40	230	45	223	38	226	33	218
	200	35	220	37	221	42	220	32	214	28	180

PG - Pollen germination; TL - tube length

in different directions other than to ovary and gradually ceased growing. Hence the seed set was totally absent. This clearly indicates that incompatibility occurs at the stigmatic region.

DISCUSSION

Successful seed set and establishing newer population generally depend upon viable pollen grains. An understanding of the factors controlling pollen germination and tube elongation is essential to facilitate interspecific and intergeneric hybridization (Vasil, 1974). The success of hybridization largely depends upon the chemical composition and physiological state of the pollen. The basic needs for the improvement of plants while undertaking breeding programme are pollen fertility, viability and its longevity. The present study suggested that the different concentrations of boric acid and sucrose enhance the pollen germination and tube elongation in *Impatiens*.

Sugar acts as a nutritive material for pollen germination (Johri & Vasil, 1961) and it helps in maintaining proper osmotic balance between the germination media and pollen cytoplasm (Mukerjee & Das, 1964). According to Shivanna and Johri (1985) the optimum concentration of sucrose varies from species to species. Beside carbohydrates, boron and calcium are other important substances required for pollen germination and tube growth.

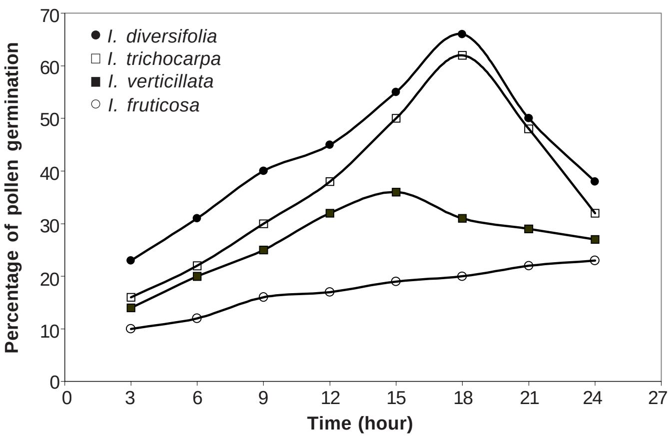

Figure 1. Peak period of* in vivo *pollen germination in* Impatiens *spp.

Boron is known to stimulate pollen tube growth and is commonly used in the form of boric acid. According to Portoni and Horovitz (1977) incorporation of boron in the medium containing different concentrations of sucrose improves pollen germination. Pollen grains are known to be deficient in boron and therefore, exogenous supply of boron enhances pollen germination and tube growth (O'Kelley, 1955). The role of boron has been confirmed in promoting pollen germination and tube elongation, especially in vascular plants (Lewis, 1980; Sidhu & Malik, 1986). In some cases, temperature plays a crucial role in both pollen germination and tube elongation apart from the basic nutrients (Kuruvilla *et al.*, 1989). In the present investigation maximum pollen germination was observed in 150ppm boric acid incorporated into the 20% sucrose and corroborated with the findings of Shivanna and Johri (1985).

Pollen germination and subsequent post pollination events depends on the receptivity of the stigma, its nature and compatibility. In the present study, stigmas remained receptive in all the cases and is usually maximum soon after anthesis. It varies from species to species depending upon temperature and humidity (Shivanna & Johri, 1985). In all the candidate species, pollen grains were well adhered to the stigma, a factor that is a primary requirement for successful pollination. After landing on the stigmatic surface, pollen grains are subjected to hydration and then pollen wall proteins are released on to the stigmatic surface (Harrison *et al.*, 1975). The stigmatic pellicle acts as a receptor of pollen wall proteins. Following this interaction, the pollen grains are recognised, which results in the activation of stigma for screening the pollen. When a pollen is accepted, a pollen tube comes out and grows towards the stigmatic pellicle by penetrating the cuticle and moves downward (Shivanna, 1977). But if the pollen is incompatible, it does not germinate

Table 2.* In vivo *pollen germination of four species of* Impatiens *spp.

Time (hrs)	*I. diversifolia* SO	SG	SR	PG	TL	*I. trichocarpa* SO	SG	SR	PG	TL	*I. verticillata* SO	SG	SR	PG	TL	*I. fruticosa* SO	SG	SR	PG	TL
3	10	3	30	23	282	10	3	30	16	178	10	2	20	14	172	10	1	10	10	88
6	10	4	40	31	298	10	4	40	22	210	10	3	30	20	220	10	2	20	12	106
9	10	5	50	40	326	10	5	50	30	235	10	5	50	25	236	10	2	20	16	112
12	10	6	60	45	338	10	5	50	38	296	10	5	50	32	248	10	3	30	17	122
15	10	7	70	55	380	10	6	60	50	332	10	6	60	36	268	10	3	30	19	128
18	10	8	80	66	396	10	7	70	62	364	10	5	50	31	230	10	3	30	20	132
21	10	6	60	50	348	10	6	60	48	290	10	4	40	29	216	10	3	30	22	136
24	10	5	50	38	322	10	5	50	32	252	10	3	30	27	198	10	4	40	23	144

SO - No. of stigmas observed; SG - No. of stigmas showing germination; SR - Stigma receptivity (%); PG - Percentage of pollen germination; TL - Average pollen tube length (mm)

and seems to be rejected (Dickinson & Lewis, 1973).

Based on this study, a maximum pollen germination on stigma was recorded in *I. diversifolia* (66%) followed by *I. trichocarpa* (62%), with 18 hours of receptivity. *Impatiens verticillata* had shown 36% pollen germination with 15 hours of receptivity. Lowest pollen germination was noted in *I. fruticosa* (23%) even after 24 hours of extended receptivity. In both the cases of *I. fruticosa* and *I. verticillata* no fruit set was observed in nature. However, they are adapted to vegetative propagation to overcome incompatibility and thus occur in small isolated populations and confined to high ranges of the Western Ghats.

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