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Effect of B Chromosomes on Pollen Tube Growth in Diploid and Tetraploid *Impatiens balsamina* L.

SAVITA MAHAJAN and S S RAGHUVANSHI
Cytogenetics and Plant Breeding Laboratory, Department of Botany, University of Lucknow, Lucknow 226007

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In vitro pollen germination and growth of pollen tubes in noncarrier and B chromosome carrier diploid and their autotetraploids in bicolor variety of *Impatiens balsamina* were investigated. B chromosome carrier pollen grains at both the ploidy levels showed better germination and growth than non-carrier pollens. Tetraploid pollen tubes though were initially slower, grew faster later than diploids. Faster pollen tube growth rate in B chromosome carrier pollen may ensure preferential fertilization.

Key Words: **Preferential fertilization,** *Impatiens balsamina,* **B chromosome, Pollen**

Introduction

In *Impatiens balsamina* presence of two B chromosomes which were indistinguishable from chromosomes of normal complement during meiosis, have been reported (Raghuvanshi & Joshi 1968, Raghuvanshi & Mahajan 1982).

Experiments (Raghuvanshi & Singh, unpublished) have indicated preferential fertilization by B carrying gametes. Present experiment was carried out to investigate this possibility. The idea was to find out if there was actually faster growth rate of pollen tube in the B chromosome carrier pollen in diploids and tetraploids.

Materials and Methods

For the study of pollen germination, sucrose solution was prepared of 5, 10, 15 and 20% concentration in distilled water, along with the medium—H_3BO_3, 100 ppm; $Ca(NO_3)_2$, 300 ppm; $MgSO_4$, 200 ppm; KNO_3, 100 ppm were also added. Best results were obtained in 10% concentration of sucrose solution, therefore this concentration was used in the present experiment. The solution was stored in a refrigerator and only the required amount was taken when needed.

To record the time of maximum germination and to compare the pollen germination at different time during the

day in diploid and tetraploid noncarrier and carrier plants, pollen were taken from freshly open flowers for sowing. Sowing was done at 2 hr interval from 7 AM to 5 PM in double cavity slides. Ten random observations were taken for percentage germination after half an hour of sowing in diploid noncarrier and carrier and tetraploid noncarrier and carrier pollen.

For pollen tube growth, the pollen of diploid and tetraploid noncarrier and carrier were taken and sown at 9 AM in double cavity slides. In different cavity slides, noncarrier and carrier pollen were sown in three replicates. Growth of pollen tube was stopped by fixing the pollen grains by adding several drops of 1 : 3 acetic alcohol. Pollen tube length of twenty pollen tubes, taken at random, were measured in a single replicate, at an interval of fifteen minutes up to 90 min. Size of pollen tube was measured in microns with ocular micrometer, immediately after fixation. The comparison of diploid noncarrier with diploid carrier, tetraploid noncarrier with tetraploid carrier, diploid noncarrier with tetraploid noncarrier and diploid carrier with tetraploid carrier pollen was statistically carried out. The value of S Ed was calculated in each case by the following formula:

$$S\,Ed = \sqrt{(S\,E_1)^2 + (S\,E_2)^2}$$

If any difference between the actual means of the two samples is greater than S Ed × 2 then it is significant. If the difference is less than S Ed × 2 the result is nonsignificant. These experiments were performed at 28±1°C.

Results

Pollen of *I. balsamina* are four aperturate, rectangular or circular in outline (figures 1 and 2). In noncarrier plants only pollen with 7 chromosomes are produced while in the diploid carrier plants two types of pollen grains are produced one with 7 chromosomes and other with 8 chromosomes. In tetraploid noncarrier plants pollen grains with 14 chromosom es and in carrier plants two types of polle n grains, one with 14 and other with 14+1B chromosomes are produced.

The pollen grains of *I. balsamina* germinated even in distilled water and in various concentrations of sucrose solution, within five minutes of sowing. However, the maximum germination percentage and maximum tube length was found in 10% medium.

Pollen Germination

In diploid noncarrier and carrier, the maximum germination (92.7%) was found at 9 AM. Significant difference was observed at 3 PM and 5 PM.

In tetraploid noncarrier and carrier, the maximum germination (84.87%) was at 9 AM (table 1). There was no pollen germination at 5 PM in tetraploids. Significant difference was observed at 7 AM.

Table 1 *Percentage pollen germination after half an hour of sowing in noncarrier and B carrier diploid and tetraploid* Impatiens balsamina *L. at 2 hr. interval*

Time	Diploid		Tetraploid	
	Non-carrier	Carrier	Non-carrier	Carrier
7 A.M.	73.28%	74.23%	34.65%	40.63%
9 A.M.	92.04%	92.71%	84.42%	84.87%
11 A.M.	88.48%	90.76%	67.03%	72.90%
1 P.M.	60.39%	66.88%	55.51%	62.49%
3 P.M.	53.46%	66.29%	41.72%	51.07%
5 P.M.	17.51%	29.06%	No germination	No germination

Figures 1–14 (× **200**), **1–7**, Pollen from carrier diploid (2× = 14 + 2B) plants; **8–14**, Pollen from carrier tetraploid (4× = 28 + 2B) plants: **2** and **9** after 15 min, **3** & **10**, after 30 min, **4** & **11**, after 45 min, **5** & **12**, after 60 min, **6** & **13**, after 75 min, and **7** & **14**, after 90 min sowing

In diploid noncarrier and tetraploid noncarrier, there was significant difference at 7 AM, 9 AM and 11 AM. However, at 1 PM and 3 PM the difference was non-significant.

In diploid carrier and tetraploid carrier, there was significant difference except at 1 PM.

Pollen-tube Length

Maximum growth rate of pollen tube length was at 9 AM. Data and photographs were taken after 15, 30, 45, 60, 75 and 90 min of sowing (table 2, figures 2 to 7 and 9 to 14).

The pollen tube of diploid pollen grows uninterruptedly and reaches a mean length of 662.97 ± 7.38 μm in noncarrier and 713.53 ± 6.59 μm in carrier pollen (table 2).

In tetraploids the growth of pollen tube was slow up to 30 minutes and after that it grew faster. The differences between noncarrier and carrier pollen were significant (table 2).

The length of diploid noncarrier pollen tube was more than the tetraploid noncarrier pollen tubes. After 15 and 30 min the differences were significant and after 30 min it grows faster and at 45 min it was more in the tetraploid noncarrier than the diploid noncarrier (table 2). The differences were significant at 45, 60, 75 and 90 min.

The length of tetraploid carrier pollen tube was less than diploid up to 30 min. After 45 min growth rate in tetraploids was higher than diploid carrier (table 2) but statistically significant differences were observed at 60, 75 and 90 min.

Discussion

It is apparent that the pollen carrying B chromosome grow faster than normal pollen, thus it seems that gametes with Bs may fertilize the egg cell with a higher frequency. In other words, the existence of preferential fertilization for these gametes can be interpreted in terms of selective advantage for plants carrying two B chromosomes. Even in *Trigonella foenum-graecum* which has similar type of two B chromosomes, in carrier strain possibility of preferential fertilization has been clearly indicated. These B chromosomes are equally transmitted both through the male and female gametes. These results are based on properly planned reciprocal transmission experiments.

A number of workers have reported *in vitro* pollen germination (Nair & Singh 1969, Vasil 1960, Nair et al. 1973) but reports are still rare of *in vitro* germination of pollen grains with B chromosomes. Puertas and Carmona (1976) reported greater pollen tube growth in rye plants with two B chromosomes.

Table 2 *Actual mean ± standard error values (μm) of pollen tube length in noncarrier and B-carrier diploid and tetraploid* Impatiens balsamina *L.*

Minutes after sowing	Diploid		Tetraploid	
	Noncarrier	Carrier	Noncarrier	Carrier
15	51.97±1.25	69.55±1.53	30.28±1.11	37.21±1.36
30	146.40±2.48	164.90±2.78	78.23±1.68	88.03±1.97
45	318.39±6.31	371.52±8.04	333.50±2.95	378.58±7.36
60	446.60±7.99	481.50±7.67	477.46±4.31	518.21±4.76
75	536.50±6.09	593.90±5.23	572.88±5.01	624.50±4.44
90	662.97±7.38	713.53±6.59	748.50±7.78	782.44±4.68

Roman (1948) suggested that in maize at second pollen grain division, the two nuclei are different, the pollen having B chromosome has greater ability of fertilizing the egg. He further suggested that preferential fertilization is due to specific orientation of the deficient and hyperploid gametes within the pollen grain or at some time prior to fertilization, so that one of the sperms has a positional advantage in respect to fertilization. Blackwood (1956) studied the rates of directed fertilization in different stocks and found evidence that preferential fertilization varies significantly. According to Catcheside (1956) there is a strong probability that the second pollen division is polarised and it is possible that the resulting arrangement of the two gametes could determine preferential fertilization.

The present *in vitro* pollen germination studies indicate that generally the percentage germination in pollen of carrier plants is higher at different times of day. Besides this, the carrier pollen tube at both diploid and tetraploid level display a rather faster germination rate. These observations may support the possibility of preferential fertilization by carrier gametes.

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