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Anthesis and Pollen Germination in *Hevea brasiliensis* Muell. Arg.¹

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ABSTRACT

Anthesis, pollen germination and tube growth of RR11 33, a clone of *Hevea brasiliensis*, were investigated. Anthesis of male flowers takes place between 1:30 and 1:45 pm and that of female flowers between 3:15 and 4:00 pm. Pollen stainability was 94.7% and pollen size ranged from 35 μ m to 45 μ m. Pollen grains recorded high germination and tube growth in the medium comprising 20% sucrose with 100 ppm each of calcium, boron, magnesium and potassium.

INTRODUCTION

Mature trees of *Hevea brasiliensis* undergo wintering, refoliation and flowering during the period December-February. In some trees there is occasionally off-seasonal flowering during September-October. A knowledge of anthesis and germination potential of pollen grains is an essential pre-requisite in breeding programmes. However, work on this aspect is only fragmentary. Dijkman (2) gave a

COMPENDIO

Se estudió la anthesis, la germinación del polen y el crecimiento del tubo germinativo del clon RR11 33 de *Hevea brasiliensis*. La anthesis de las flores masculinas se lleva a cabo entre las 13:30 y 13:45 horas, mientras que las flores femeninas lo hacen entre las 15:15 y las 16:00 horas. La tinción del polen fue de 94.7% y el tamaño varió de 35 μ m a 45 μ m. La germinación del polen y el crecimiento del tubo germinativo fueron altas en medio conteniendo 20% de sacarosa con 100 ppm de cada uno de los elementos calcio, boro, magnesio y potasio.

general account of floral biology and generative selection in *Hevea*. Germination of pollen grains and anthesis of a few clones of *Hevea* were also reported (4, 6, 7, 8). In the present investigation an attempt is made to study the anthesis, germination of pollen grains and effect of calcium, boron, magnesium and potassium on pollen germination and tube growth with respect to one clone of this species.

MATERIALS AND METHODS

The clone used for the study was RR11 33, an ortet selection of *Hevea brasiliensis* Muell. Arg, made by the Rubber Research Institute of India. The clone has been found to be a modest seeder. Five trees of the clone were selected, and from each tree five branches were selected at random for regular observation of

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anthesis. Male flowers just prior to anthesis were collected for pollen studies. Pollen grains were dusted in 1:1 acetocarmine-glycerol mixture and stainability was assessed after one hour. Pollen morphology was studied by acetolysis (3). Measurements of 50 pollen grains were taken for the observations. Germination was studied by dusting pollen to a drop of the medium on a glass slide and the slides were inserted in a Petri dish containing two glass rods. The Petri dishes were lined with moist filter paper. Different concentrations of sucrose (10, 15, 20 and 25%) were employed for a preliminary assessment of pollen germination. Based on the observations, a 20% sucrose medium was chosen for further study, with the following treatments:

1. 20% sucrose with 100 ppm of Ca
2. 20% sucrose with 100 ppm of B
3. 20% sucrose with 100 ppm each of B and Ca
4. 20% sucrose with 100 ppm of Mg.
5. 20% sucrose with 100 ppm of K.
6. 20% sucrose with 100 ppm each of Ca, B, Mg, and K.

Each treatment consisted of a minimum of five replicates and the average values were calculated. The data were subjected to statistical analysis. The growth of the pollen tube could be studied only with treatments one, two, three and six as the percentage of germination was very poor in treatment four and five. Fifty germinated pollen grains were chosen randomly to ascertain the tube length, which was measured after 3 h.

RESULTS

The clone RRII 33 is a complete wintering type whose refoliation starts after wintering by the middle of January. Anthesis of the male flower takes place between 1:15 pm and 1:45 pm. The anthesis of the female flower takes place between 3:15 pm and 4:00 pm. The stainability of pollen grains in acetocarmine-glycerol mixture was 94.7%. The pollen grains are 3-zonicolporate. They showed a size ranging from 35-45 μm . Among the four treatments of different concentrations of sucrose alone, the medium containing 20% recorded the maximum pollen germination (35%), whereas the medium containing 25% sucrose showed 15% germination, and 15% sucrose produced 10% germination. There was no pollen germination in the 10% sucrose solution. Based on this, Ca, B, Mg, and K alone and in combinations were incorporated into a 20% sucrose basal medium. The results are summarized in Table 1. There is significant variation in the germination percentage between the treatments. The highest percentage of pollen germination (86.44%) was recorded in the

Table 1. Germinación of pollen grains.

| Treatment No. | Medium | Percentage pollen germination. Mean |
|---------------|--|-------------------------------------|
| 1. | 20% sucrose with 100 ppm of Ca. | 39.95 (41.26) |
| 2. | 20% sucrose with 100 ppm each of B + Ca | 60.33 (75.28) |
| 3. | 20% sucrose with 100 ppm of B | 63.06 (79.28) |
| 4. | 20% sucrose with 100 ppm of Mg. | 13.98 (5.88) |
| 5. | 20% sucrose with 100 ppm of K. | 11.71 (4.38) |
| 6. | 20% sucrose with 100 ppm each of Ca, B, Mg and K | 68.41 (86.44) |

S.E. = 1.41

C.D. = 4.12

Note: 1. S.E. and C.D. are for angles obtained using arc sine transformation

2. The figures within brackets indicate respective germination percentage.

medium containing 100 ppm each of Ca, B, Mg and K in addition to 20% sucrose. This was followed, in order, by 20% sucrose with 100 ppm boron alone (79.28%) and 20% sucrose with 100 ppm each of boron and calcium (75.28%)

Observations on the growth of the pollen tube, measured in terms of tube length after three hours of treatment, are given in Table 2. Among the five treatments, highest tube growth (294 μm) was recorded in the treatment containing 100 ppm each of Ca, B, Mg and K in addition to sucrose. The medium with 100 ppm Ca alone, in addition to sucrose, recorded the lowest tube growth (138 μm), while the other two treatments were in between.

DISCUSSION

Among the different concentrations of sucrose solution tried, 20% solution was found to be the optimum for pollen grain germination. The germination of pollen grains as well as the growth of pollen tubes were high in the medium comprising 20% sucrose with 100 ppm of Ca, B, Mg and K. In

the presence of boron, the germination was accelerated, whereas the tube growth was greater in the presence of Ca and B compared to B alone. Media with K and Mg showed only low percentages of germination. But traces of these chemicals along with B and Ca in the medium appeared to enhance germination and tube growth. Some chemicals stimulating pollen germination as well as tube growth, e.g. boron, calcium and magnesium, were first noted in the stigmatic fluid in which the pollen naturally germinates (11). Importance of Ca and B for germination and tube growth had been investigated by Vasil (12) and Johri and Vasil (5). The role of calcium ions in pollen tube growth was reported by Brewbaker and Kwack (1). Ground nut pollen showed high germination and tube growth in a basal medium comprising Ca, Mg, K and B (10).

Pollen grains are considered to constitute one of the central units in the biological cycle of plants. Information pertaining to pollen viability is essential to attempt a generative improvement programme in any crop. Ramaer (9) indicated that *Hevea* pollen will not germinate in aqueous sucrose or glucose, but Majumder (6) had shown a fairly high percentage of germination in the same medium. Majumder (6) and Samaranyake *et al.* (8) showed that the percentage of sucrose solution required for germination was 15%. Markose and Bhaskaran Nair (7) reported that a 20% sucrose solution gave better germination. In

the present investigation, it was observed that very good germination and tube growth was obtained in a 20% sucrose solution. However, it appears probable that the optimum requirement of sucrose concentration in the medium may vary depending on the genotype. This has also been indicated by Markose and Bhaskaran Nair (7). The pollen collected late in the season as well as that from flowers infected by *Cidium* gave poor germination and also showed short tube length compared to those from healthy flowers. Though the clone studied is a modest seeder, the present studies indicate that pollen sterility is not the cause.

Table 2. Pollen tube growth.

| Treatment No. | Medium | Mean tube length (μm) |
|---------------|--|------------------------------------|
| 1 | 20% sucrose with 100 ppm of Ca | 138.20 \pm 8.86 |
| 2 | 20% sucrose with 100 ppm of B | 208.60 \pm 9.01 |
| 3 | 20% sucrose with 100 ppm each of B and Ca | 234.30 \pm 8.14 |
| 4 | 20% sucrose with 100 ppm each of Ca, B, Mg and K | 294.17 \pm 12.56 |

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