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IN VITRO GERMINATION OF POLLEN AND POLLEN TUBE GROWTH OF *HEVEA BRASILIENSIS* IN THE PRESENCE OF CALCIUM AND BORON

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SUMMARY

The role of calcium and boron in Hevea pollen germination and pollen tube growth was investigated. Individual significant effects of both elements on germination of pollen grains were noted upto a maximum concentration of 0.03%. When used in combination, boron appears to be more important than calcium in pollen germination. On the other hand, calcium has a clear positive effect on growth of pollen tubes in the presence of boron.

INTRODUCTION

It has been observed that over a period of twenty years, seed-set of artificial pollinations of *Hevea*, carried out at the Rubber Research Institute of Sri Lanka, has gradually decreased. Though self sterility and incompatibility can be a cause of poor seed setting, other genetic and physiological factors known or unknown, can influence pollen germination and germ tube growth in many species of plants.

Poor pollen germination may be a factor responsible for reduced fruit set in *Hevea*. The present study seeks to find out whether it could be improved with calcium as shown by Visser (1955) and Brewbaker & Kwack (1963) and boron which has also been shown to stimulate, *in vitro* germination and pollen tube growth (Schumcker, 1935).

MATERIALS AND METHODS

Pollen of clone H 440 (parentage LCB 870 x Wagga 6278) was used in this study; flowering occurs every 8 weeks in this cultivar. Mature pollen grains were collected from flowers of two trees on two days, 17 days apart, about 9.30 a.m. each day.

The basal nutrient medium used for the investigation was 15% sucrose, as preliminary tests had shown that it was the optimum concentration of sucrose for germination. Calcium nitrate and boric acid, each alone, at four levels *viz.* 0.00, 0.01, 0.03 and 0.06%, and in all possible combinations of the two, were used as test media. The pH of the media ranged from 6 to 7.

All nutrient media were prepared in de-ionised water. The glass slides and cover slips used for germination tests, were thoroughly washed in glass distilled water. Pollen was dusted with a camel hair brush on to the test media contained in the concavities of double cavity glass slides. An attempt was made to standardise the amounts of media contained in the concavities and the numbers of pollen grains used (approximately, 1.5 ml of media and 600 pollen grains). This was necessary to mitigate the 'population effect' described by Brewbaker and Majumder (1961). On each day, pollen from three flowers of the same inflorescence was dusted on duplicate slides, at each level of calcium nitrate and boric acid, and the concavities were covered and kept in a water saturated atmosphere (petri dishes lined with moistened filter paper). Germination commenced about one hour after inoculation. The slides were incubated for 18 h at room temperature (±28°C), after which no increase in germination was observed. Thereafter, the slides were stored in the refrigerator (approx. 5°C) until examined.

Three counts of 100 pollen grains, per concavity were made to estimate percentage germination. The lengths of 30 randomly selected pollen tubes per concavity were also measured, using a micrometer eye piece.

RESULTS

An analysis of variance carried out on percentage germination (transformed into inverse sines) and pollen tube length, showed highly significant responses to both calcium and boron.

However, as the interaction between calcium and boron was also highly significant in both percentage germination and pollen tube growth, the results presented here will deal with the interactions only.

Germination

TABLE 1. Mean % Germination Transformed* for Statistical Comparisons. Detransformed means in parenthesis

%B \ %Ca	0.00	0.01	0.03	0.06
0.00	0.00 (0.00)	5.01 (0.8)	8.07 (2.0)	7.85 (1.9)
0.01	22.90 (15.1)	23.36 (15.7)	23.98 (16.5)	24.75 (17.5)
0.03	27.93 (21.9)	28.92 (23.4)	30.29 (25.4)	31.32 (27.0)
0.06	27.76 (21.7)	28.14 (22.2)	27.94 (21.9)	30.10 (25.2)

*LSD (0.1%): 2.42

It was observed (Table 1) that in the absence of calcium in the medium, there is a substantial increase in the percentage germination, with increasing concentrations of boron, reaching a maximum at the concentration of 0.03%. In the absence of boron too, the percentage germination shows a significant increase at higher concentrations of calcium. Here again the optimum concentration is around 0.03%. However, the responses to calcium can be considered to be limited when compared with that of boron.

If we consider calcium and boron in combination, it appears that in connection with germination, calcium plays only a minor role. In fact, at higher concentrations of boron, the introduction of calcium into the medium does not affect the results significantly.

A response function fitted to express the percentage germination (Y) as influenced by the combined effect of calcium and boron is given below:

$$Y = 32.05 + 928.13\ Ca - 7313.07\ Ca^2 + 7861.50\ B - 95342.23\ B^2 - 4992.69\ Ca \times B.$$

From the above function, the optimum combination of calcium nitrate and boric acid in the medium for maximum germination was found to be as follows:

$Ca(NO_3)_2$ = 0.06%

H_3BO_3 = 0.04%

Tube length

TABLE 2. Mean Pollen Tube Length* (/ᵘ) of Germinated Pollen Grains

%B \ %Ca	0.00	0.01	0.03	0.06
0.00	0.00	0.08	0.18	0.18
0.01	91.77	102.70	131.83	142.87
0.03	130.60	147.50	232.63	231.13
0.06	146.03	162.40	171.73	198.03

*LSD (0.1%) : 32.39

It is observed that in the absence of boron, the germ tube length does not show a significant response to calcium (Table 2). However, in the absence of calcium the response to boron alone is highly significant.

The combined effect of calcium and boron on germ tube length, appears to be different from its effect on percentage germination. Calcium plays a minor role in germination; but it has a very clear positive effect on pollen tube length in the presence of boron.

The response equation showing germ tube length (Y) as a function of calcium and boron is given below:

$$Y = -289.92 + 48495.68\ Ca - 505236.74\ Ca^2 + 284093.80\ B - 3579895.83\ B^2 + 346289.68\ Ca \times B.$$

From the above equation we find that the optimum combination of calcium nitrate and boric acid for maximum tube growth to be:

$Ca(NO_3)_2$ = 0.06%
H_3BO_3 = 0.04%

A highly significant correlation (r = 0.9407***) was found between percentage germination and pollen tube growth, as observed in many other crops.

Occasionally, two pollen tubes were found emerging from one grain; this has been observed frequently, in other species of plants too (Pfahler, 1967).

DISCUSSION

Germination and germ tube growth of pollen were significantly affected by calcium nitrate and boric acid in the medium, boron exerting a stronger effect than calcium; there was also a highly significant Ca x B interaction. Numerous workers, including Brewbaker and Kwack (1963), have carried out deletion type experiments and shown that nitrate and other common anions have no appreciable effect on germination of pollen grains. Therefore it is concluded that the Ca ion is the operative factor in calcium nitrate.

Visser (1955), and Brewbaker and Kwack, (1963), thought that calcium probably helps in the building of pectic regions in pollen tubes, thus increasing rigidity and stability. This prevents the grains from rupturing and assists in the formation of straight tubes. Bursting of *Hevea* pollen was reported by Senanayake (personal communication) and Jayasekera (1975). Occasional bursting of grains was noticed in our tests too in the basal medium, but when calcium and/or boron was present, this was never observed.

Gauch and Duggar (1953) proposed that "Boron combines with sugar molecules to form a sugar borate complex, which is translocated with greater facility than are non-borated, non-ionised sugar molecules"; ion translocation appears to be the function of boron in pollen germination; but, its exact function does not appear to be yet fully understood.

The composition of the growth medium for *in vitro* germination of pollen is, to a large extent, specific for individual species (Johri and Vasil, 1961). It has also been observed that these requirements may even vary widely within a species (Pfahler, 1967).

Visser (1955) and Vasil (1960) have shown that in many species of plants, pollen germinates in distilled water alone, but with a lower percentage germination than in an aqueous solution of sugar. Ramaer (1932) indicated that *Hevea* pollen will not germinate in aqueous sucrose or glucose, but Majumder (1964) has shown a fairly high percentage germination in the same media. In the present study, it was observed that there is no germination when there was no calcium nitrate and boric acid in the basal sucrose medium; However, these results refer to one clone, and whether the requirements for different genotypes vary, has to be further investigated.

Todd and Bretherick (1942), and O'Kellay (1955), observed that pollen is generally deficient in boron, averaging about 0.03%. Visser (personal communication, 1976) and Antles (1951) state that pollen of pear, which failed to germinate in sugar solutions without boric acid, gave 100% germination in aqueous sucrose alone when the plants were given boron rich fertilizers for a period of four years. In our 1978 hand pollination programme, it was observed that a spray of calcium nitrate and boric acid (half the optimum concentration) on the pollinated flowers improved the fruit set considerably. However, the overall success in artificial pollination of *Hevea* has declined since the late 1950's.

It is possible that *Hevea* grows under conditions of reduced or unbalanced fertility during the third and fourth cycle of replanting, as calcium and boron have not been applied as fertilizers to rubber plantations in Sri Lanka (Yogaratnam — personal communication). It therefore appears, that deficiency of these nutrients may be a factor, in reducing fruit set in artificial pollinations in the course of the last two decades.

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REFERENCES

ANTLES, L. C. (1951). Review of commercial pollen storing shipping and research. *55th Ann. Rep. Vermont St. Hort. Sci.* 18—29.

BREWBAKER, J. L. and KWACK, B. H. (1963). The calcium ion and substances influencing pollen growth. In *Pollen physiology and fertilization*. Edited by H. F. Linskens North Holland Publ. Co. Amsterdam pp. 143—151.

BREWBAKER, J. L. and Majumder, S. K. (1961). Cultural studies of the population effect and self incompatibility inhibition. *Amer. Jour. Bot.* **48,** 457—464.

GAUCH, H. G. AND DUGGAR, W. M. JR. (1953). The role of boron in the translocation of sucrose. *Plant Physiol.* **28,** 457—466.

JAYASEKERA, N. E. M. (1975). Review of the Genetics and Plant Breeding Department. *Ann. Rev. Rubb. Res. Inst. Sri Lanka.* 1975, 37.

JOHRI, B. M. AND VASIL, I. K. (1961). Physiology of pollen. *Bot. Rev.* **27,** 325—381.

MAJUMDER, S. K. (1964). Studies on the germination of pollen of *Hevea brasiliensis in vitro* and on artificial media. *J. Rubb. Res. Inst. Malaya* **18,** 185—193.

O'KELLAY, J. C. (1955). External carbohydrates in growth and respiration of pollen tubes *in vitro. Amer. J. Bot.* **42,** 322—326.

PFAHLER, P. L. (1967). *In vitro* germination and pollen tube growth of maize (*Zea mays*) pollen I. Calcium and boron effects. *Can. J. Bot.* **45,** 839—845.

RAMAER, H. (1932). Germination of *Hevea* pollen. *Arch. Rubbercult.* **16,** 328.

SCHUMCKER, T. (1935). Uber den Einfluss. von Borsaure anf pflanzen insbesondere keimende Polleukorner. *Planta* **23,** 264—283.

TODD, F. E. AND BRETHERICK, O. (1942). The composition of pollens. *Jour. Econ. Entomol.* **35,** 312—317.

VASIL, I. K. (1960). Studies on pollen germination of certain cucurbitaceae. *Amer. Jour. Bot.* **47,** (4) 239—247.

VISSER, T. (1955). Germination and storage of pollen. *Mededeel, Land bonwboogeschool* (Wageningen) **55,** 1—68.