NOTES

IN VITRO GERMINATION AND EFFECTS OF DRYING TIME ON THE PRESERVATION OF *CANARIUM SCHWEINFURTHII* POLLEN

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Canarium schweinfurthii is a dioecious woody tree of about 20 m tall from the family Burseraceae. It is found in humid tropical Africa. The flowering period ranges from January till March, but some trees can bear flowers up till April. The pulp of the fruit is rich in oil. The fruit of *C. schweinfurthii* is very much appreciated. Several works have been undertaken to study the composition of pulp oil (Fonteh 1997, Kapseu 1997). The biological characteristics were described by Njoukam (1997), but the description did not indicate the phenology of the species. The main use of stored pollen has been, until now, in plant breeding to facilitate hybridisation between plants that flower at different times and in species that flower erratically (Heywood 1995, Youmbi *et al.* 1998). In the case of trees and other woody species such as *C. schweinfurthii,* which take a long time to reach the flowering stage, e.g. eight years for the precocious plants (Njoukam 1997), stored pollen can sometimes be used to short cut the process and allow crossing to take place (Heywood 1995). When the pollen is well stored, it is possible to undertake cross-pollination for the dioecious species, which do not flower at the same time and to easily transfer pollen from one country to another (Heywood 1995). Since *C. schweinfurthii* is a dioecious plant with flowering period from January till either March or April, the male tree is easily distinguishable from the female. For better improvement of the species, *in vitro* germination and storage of pollen were undertaken.

The principal aim of this study was to determine the optimal conditions of *in vitro* germination and preservation of *C. schweinfurthii* pollen for hybridisation. The fresh pollen used were collected from the field. Two basal media, i.e. Brewbaker and Kwack (1963) as well as Heslop-Harrison (1979), to which were added 1% agarose and seven sucrose concentrations (0, 5, 10, 15, 20, 25 and 30%) to determine the basal medium and the sucrose concentration to stimulate pollen germination. The pollen were sown on solidified medium on microscope slides and placed in Petri dishes in a saturated atmosphere. After 24 hours of incubation, the slides were stained with dye (Alexander 1969). Germinated and ungerminated pollen (400–500) were scored under the microscope using a hand tabulator. Burst pollen grains were considered ungerminated. Pollen grains with tubes equal to at least twice the diameter of the pollen grain were considered germinated. The effects of temperature (20, 25, 30, 35 and 40 °C) and pH (5, 5.3, 5.6, 5.9, 6.2, 6.5, 6.8 and 7.1) on *in vitro* germination were also studied. Three replicates were carried out for each condition.

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Freshly collected pollen grains were placed in flasks numbered 0 to 4. The flasks were then put in a desiccator for 0, 1, 2, 3 and 4 weeks. After each week and considering flask No. 0 as the control, two flasks of any other number were taken out and placed under refrigeration condition of more than 4 °C and frozen condition of less than -20 °C respectively. After each week of storage, a germination test was done on each sample and for the corresponding storage condition.

For *in vitro* germination tests, the germination rate on Heslop-Harrison artificial basal medium was 40%, while on Brewbaker and Kwack medium the maximum germination was 35% (Figure 1). The lowest percentages of germination were 3 and 4 on Heslop-Harrison and, Brewbaker and Kwack artificial basal media respectively. Tests on sucrose concentration of the artificial basal medium showed that germination was not possible (0%) in the absence of sucrose. The same result was obtained in the medium made up of water gel or simply water, and the germination percentage increased with increase in sucrose concentration. On Heslop-Harrison and, Brewbaker and Kwack artificial basal media, optimum germination values (40 and 35% respectively) were obtained with 10% sucrose concentration. When the optimal concentration was exceeded, germination was inhibited. The germination percentage decreased when 30% sucrose concentration was used.

Figure 2 shows that temperature increases with an increase in germination percentage. The maximum was 40% at a temperature of 30 °C. Beyond this temperature, the germination percentage decreased and only 3% of the pollen grains germinated at 40 °C.

Germination was maximum at pH 5 (Figure 3). Nevertheless, germination remained high (16–20%) at all pH values.

Kinetic studies of pollen germination indicated a slow germination rate with 20% of

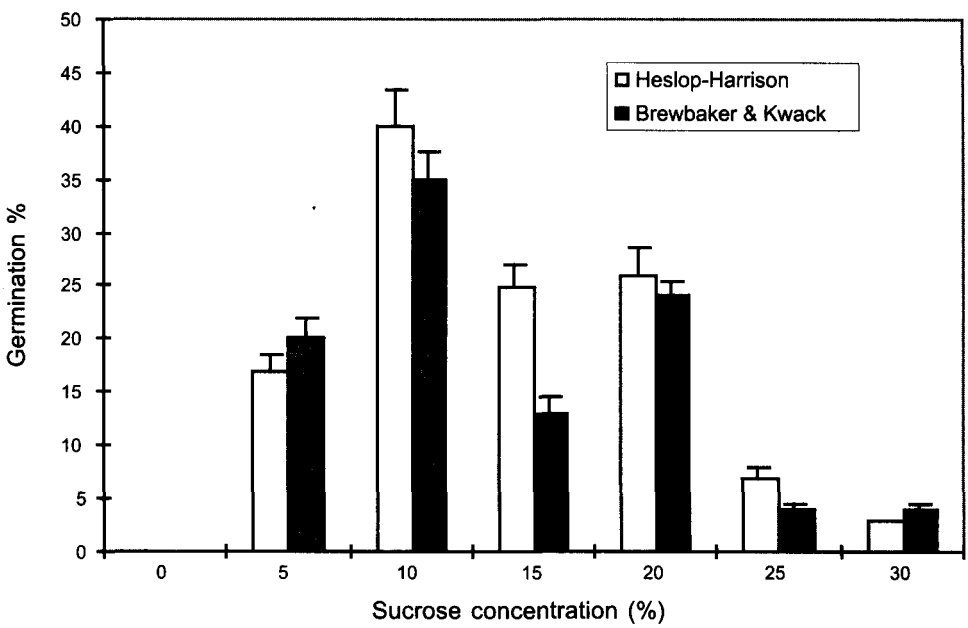

Figure 1 Effects of Brewbaker and Kwack, and Heslop-Harrison base media, and saccharose concentration on *Canarium schweinfurthii* pollen germination. Incubation for 24 hours in the dark at 30 °C.

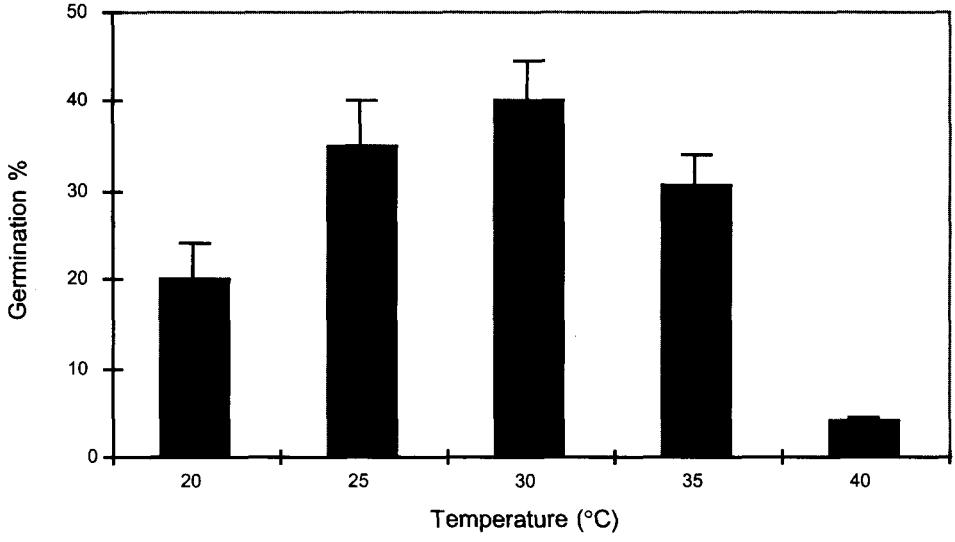

Figure 2 Effect of temperature on *Canarium schweinfurthii* pollen germination. Incubation for 24 hours in the dark.

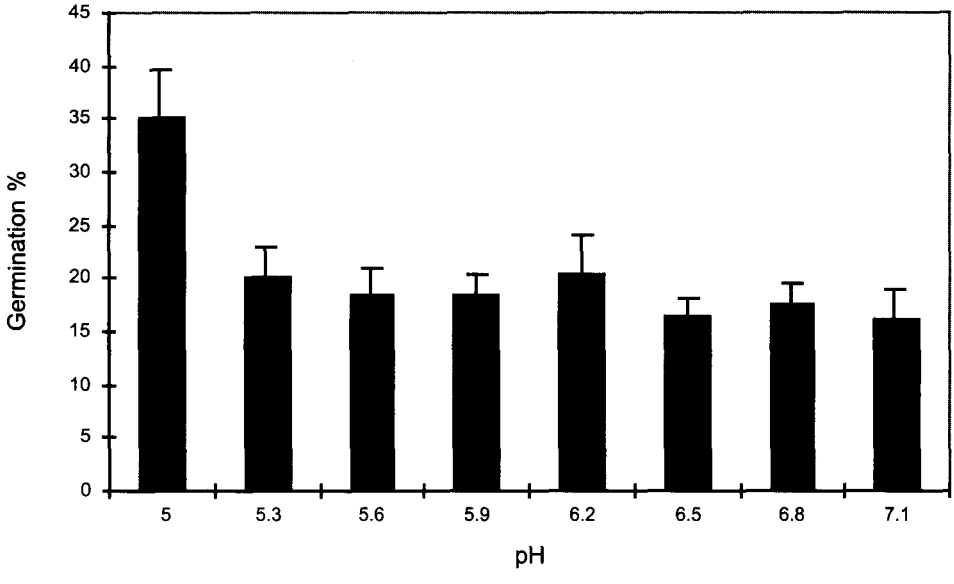

Figure 3 Effects of pH on *Canarium schweinfurthii* pollen germination. Incubation for 24 hours in the dark at 30 °C.

pollen germinating after 30 min of incubation (Figure 4). The maximum (35%) was obtained only after three hours.

Table 1 shows that after five weeks of storage at 4 °C, 20% of pollen grains were still able to germinate after one week of dehydration in a desiccator. In the case of storage at -20 °C (Table 2), 21 and 23% of the pollen grains were able to germinate after six weeks of one- and two-week dehydration respectively.

Pollen grains of *C. schweinfurthii* could germinate on both types of artificial basal media, but the optimum percentage was obtained on Heslop-Harrison's medium. According to

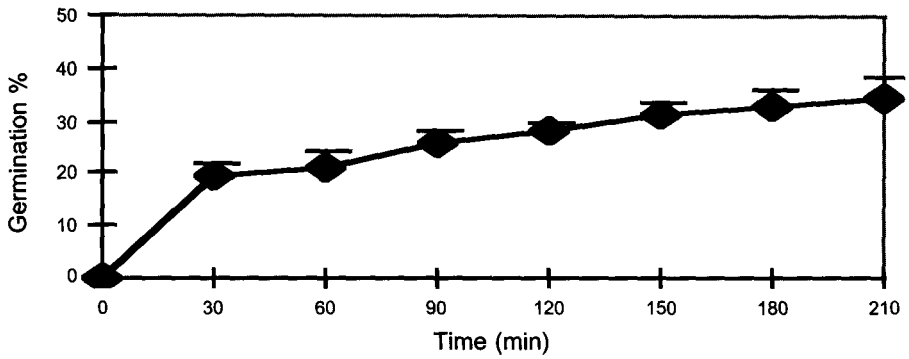

Figure 4 Kinetics of *Canarium schweinfurthii* pollen germination during three hours of incubation in the dark at 30 °C.

the classification described by Brewbaker (1967), pollen grains of *C. schweinfurthii* belong to the group which is bicellular and has thick exine. They can survive under severe dry conditions and can be stored for a long time. This was also found in other plant species by Cauneau-Pigot (1988), Charrier (1990), Youmbi (1994) and Cerceau-Larrival *et al.* (1995). The results indicated that the pollen grains of *C. schweinfurthii* could not be stored for more than two months. The results were different from those obtained for *Dacryodes edulis* which, in the same conditions of dehydration and storage, had 40% of germination (Youmbi *et al.* 1998). The 40% germinated pollen observed in this study did not agree with the findings of Dumas (1992) that binucleate pollen grains germinate easily on Brewbaker and Kwack basal medium. Nevertheless, the optimum percentage of germination (35–40%) obtained respectively on Brewbaker and Kwack, and Heslop-Harrison basal media could be shown here as one particularity of the species.

Germination of pollen is possible if the basal medium contains sucrose, as reported by Youmbi (1993) and Youmbi *et al.* (1998) on *Streptocarpus* sp. and *D. edulis* respectively. No germination of pollen was observed when the medium did not contain sucrose. The important of sucrose for pollen germination varies from species to species (Youmbi *et al.* 1998). Visser (1955) reported that sucrose does not only play the role of osmotic regulator but also serves as a nutrient for the growth of the pollen tube. This study indicates that the percentage and rate of germination as well as the development of pollen tubes are maximum when the right concentration of sucrose is used.

Germination of pollen was influenced by pH of the germination medium. For *C. schweinfurthii*, optimum germination was achieved at pH 5. The optimum pH values for tropical species, such as *Pachypodium lamerei*, *Euphobia milii* and *Streptocarpus* sp. were 6.0, 5.8 and 6.2 respectively (Youmbi 1993).

Table 1 Effects of dehydration time and storage time on pollen germination percentage of *Canarium schweinfurthii* stored at 4 °C

Dehydration (weeks)	Storage (weeks)							
	1	2	3	4	5	6	7	8
0	15.0 ± 1.5	17.0 ± 2.5	16.0 ± 2.0	21.5 ± 2.4	18.0 ± 1.2	6.6 ± 1.0	9.8 ± 1.7	5.5 ± 0.5
1	12.5 ± 0.5	15.8 ± 2.0	25.8 ± 2.8	20.5 ± 1.8	20.0 ± 2.0	13.0 ± 1.5	10.5 ± 0.5	5.1 ± 0.6
2	13.3 ± 3.0	17.1 ± 1.8	17.0 ± 1.6	16.0 ± 2.0	15.0 ± 1.4	14.7 ± 2.0	7.4 ± 0.7	2.9 ± 0.1
3	10.5 ± 1.0	11.0 ± 1.0	15.8 ± 1.8	17.0 ± 1.5	16.0 ± 2.4	10.6 ± 1.4	6.7 ± 0.9	3.8 ± 1.2
4	13.8 ± 1.5	13.2 ± 2.2	20.0 ± 2.2	19.0 ± 2.0	18.0 ± 2.1	13.0 ± 1.0	8.8 ± 1.1	3.6 ± 0.8

Table 2 Effects of dehydration time and storage time on pollen germination percentage of *Canarium schweinfurthii* stored at -20 °C

Dehydration (weeks)	Storage (weeks)							
	1	2	3	4	5	6	7	8
0	8.8 ± 1.6	5.5 ± 0.6	18.0 ± 0.8	19.3 ± 1.6	14.0 ± 2.6	3.6 ± 0.5	13.0 ± 1.5	8.5 ± 1.0
1	24.5 ± 2.5	23.5 ± 3.1	24.0 ± 2.5	21.3 ± 2.3	23.5 ± 2.1	21.6 ± 1.4	15.0 ± 2.0	11.1 ± 1.2
2	18.5 ± 2.1	23.3 ± 2.3	25.0 ± 3.5	24.8 ± 2.5	24.8 ± 1.8	23.3 ± 1.7	10.5 ± 1.0	3.1 ± 1.0
3	19.8 ± 2.0	21.6 ± 1.6	22.0 ± 1.2	22.0 ± 1.5	22.0 ± 1.2	10.6 ± 1.0	7.5 ± 0.7	6.7 ± 1.1
4	13.8 ± 1.7	13.5 ± 1.8	14.0 ± 1.0	19.5 ± 1.0	19.5 ± 1.5	13.5 ± 1.5	8.8 ± 0.6	5.1 ± 0.8

Germination of pollens was also influenced by temperature. For *C. schweinfurthii*, optimal germination occurred at 30 °C. This temperature also gives optimal germination in some tropical species such as *Eucharis, Euphorbia, Adenium* (Youmbi 1993) and *D. edulis* (Youmbi *et al.* 1998). A germination of 40% after three hours is not sufficient to indicate the germination capacity of *C. schweinfurthii*. This could be explained by the fact that pollen viability in the anemophilous species decreases more rapidly than in entomophilous ones (Bassani *et al.* 1994). *C. schweinfurthii* is dioecious with widely spaced plants. Similar results were obtained by Pacini *et al.* (1997) after studying pollen viablity in relation to the types of pollination in six angiosperm species. This result shows, otherwise, that the incubation period (24 hours) is much longer and that maximum germination (40%) can be obtained within three hours. Pacini *et al.* (1997) found that individual species may have pollen with longer or shorter viability depending on features such as life form, sex expression and the number of individuals in a certain area.

Evaluation of the germination capacity of stored pollen could be carried out on Heslop-Harrison base medium containing 10% sucrose. The pH value of the medium had to be 5 and an incubation in darkness at 30 °C was advisable. Storage was suitable within six weeks at -20 °C after a dehydration period of either one or two weeks in a desiccator.

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