Germination of Jubaeopsis caffra Seeds

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Abstract

Preliminary work indicated that fresh, viable seeds of *Jubaeopsis caffra* failed to germinate promptly under normal incubation conditions. Subsequent studies showed that none of the covering structures (including the thick, hard endocarp) are impermeable to oxygen and water, but that an increased oxygen tension markedly improved germination. The addition of endosperm to the nutrient medium of excised embryos in culture inhibited the growth of these embryos. It is considered possible that a factor inhibiting germination, and which can be overcome by a high oxygen tension, might be present in the endosperm. Maximum germination was achieved by sowing seeds in coarse sand with a 14 percent (oven-dry basis) moisture content at 25°C in pure oxygen.

According to Good (1964), more than 90 percent of all palm species enjoy very limited distribution and occur naturally only in small areas. *Jubaeopsis caffra* Becc. is no exception. It is perhaps one of the palm species with the most restricted distribution in that it is confined to the northern banks of the Mtentu and Msikaba estuaries in Pondoland on the east coast of South Africa (Robertson and Visagie, 1975). These authors ascribe the rarity of *J. caffra* largely to the lack of viable seeds or, at least, to the apparently very specific requirements of the seeds for successful germination.

A preliminary study of the germination pattern indicated that under normal conditions seeds fail to germinate, and further research was conducted, firstly, to try and establish the possible cause of this failure, and secondly, to ascertain the optimum requirements for successful germination.

Procedure

The detailed procedure for each experiment will be given together with the results to avoid confusion. However, a few general procedures were used throughout and are presented here.

All germination experiments were conducted in Controlled Environments model E 7H growth cabinets. Temperatures were maintained within 0.5°C of the programmed conditions and incubation was conducted in total darkness.

For the culturing of excised embryos, a modified White's nutrient solution for plant tissues (White, 1963) was used as culture medium. Each liter of the culture medium contained the following compounds:

Sucrose	20.0 g
$Ca(NO_3)_2 \cdot H_2O$	0.3 g
KNO_3	0.08 g
KCl	0.065 g
$MgSO_4 \cdot 7H_2O$	0.75 g
Na_2SO_4	0.2 g
$NaH_2PO_4 \cdot H_2O$	19.0 mg
$Fe(SO_4)3$	2.5 mg
$MnSO_4 \cdot 4H_2O$	5.0 mg
$ZnSO_4 \cdot 7H_2O$	3.0 mg
H_3BO_3	1.5 mg
KI	0.75 mg
$CuSO_4 \cdot 5H_2O$	0.01 mg
MoO_4	0.001 mg

The fruit of *J. caffra* is a drupe with a thick, hard endocarp covered by a fibrous mesocarp. In all the experiments, the mesocarp was removed prior

to use, while the endocarp was left intact.

Results

(a) *Preliminary experiments*

Preliminary experiments were conducted to establish whether seeds would germinate under normal conditions and to ascertain whether the fresh seeds were in fact viable. In the first of these experiments, dehusked seeds were half-buried in coarse sand, watered daily, and incubated in the dark at 25°C.

Concurrently, excised embryos from the same batch of seeds were cultured in White's medium. Thirty milliliters of White's medium were placed in 100 ml conical flasks, stoppered with nonabsorbent cotton wool and sterilized by autoclaving for 30 minutes at a pressure of 1 kg/cm^2. Seeds were surface-sterilized by immersing them in 0.2 percent $HgCl_2$ for five minutes and subsequently washing them very thoroughly with autoclaved water. The endocarp was then cracked in a vise and the embryo excised aseptically. One excised embryo was placed into each flask and incubated on a Kotterman orbital shaker in an E 7H growth cabinet at 25°C.

None of the sown seeds had germinated at the end of a three-month incubation period. However, the excised embryos showed immediate signs of growth and increased their mean length from 5.16 mm to 29.0 mm in 32 days (see also Fig. 2). From these results it is clear that fresh seeds were viable but remained dormant under the experimental conditions.

Because *J. caffra* seeds are enclosed within a hard, thick endocarp (Robertson, 1977), moisture and oxygen uptake of the seeds was subsequently investigated. Following these experiments, the possible presence of a germination inhibitor in the endosperm was investigated.

(b) *Moisture absorption by the nut, kernel and endocarp*

This experiment involved the establishment of the rate of water absorption by the seed with and without the endocarp and by the endocarp itself. (The term "nut" used in this section refers to a seed enclosed within an intact endocarp.)

The seeds used had been air-dried at ambient temperature (approximately 20°C) for 25 days after harvesting. At this stage they were 14.58 percent lighter than fresh material. This loss of mass constitutes 79.4 percent of the moisture that can be lost by *J. caffra* seeds through air-drying and consequently the seeds used in the experiment were considered to be dry. Five intact nuts (i.e., kernels with unbroken endocarp), five kernels (endosperm plus embryo with endocarp removed), and the endocarp shells of five fruits were weighed. The three groups of material were then soaked in water at 25°C. The water was changed daily and the mass of each category was recorded at various times in the 16 days during which the experiment was conducted. The mass increase of each category is expressed as a percentage of the air-dried mass of that category. The results are shown in Figure 1A, B.

From these figures it can be seen that the absorption rate of the intact nut was fairly high during the first six days of imbibition. Absorption of water continued after this time, but at a very much reduced rate. Sixteen days after commencement of the experiment, the mass of the seeds had increased by 22.5 percent. This represented an absorption of 2.4 g of water per nut (Fig. 1B).

The endocarp fragments or shells ab-

sorbed water extremely rapidly and were fully saturated within two days. At this stage the percentage increase in mass was 10.5 percent. The endocarp of a single nut absorbed only 0.75 g of water. Relative to the other two categories, the absorption of the kernel was very high and after imbibing for ten days, the mass of these kernels increased by 79 percent. After sixteen days the increase in mass rose to 85 percent. This however, represented an absorption of only 1.92 g water per kernel.

Although the endocarp absorbed water very rapidly during the first two days of imbibition, it became just as rapidly saturated and it was incapable of absorbing more than 10.5 percent of its own mass in moisture (Fig. 1A). The intact seed however, absorbed moisture at a rate only slightly slower than that of the endocarp, but over a much longer period and as is shown in Figure 1A, the mass of the nut was still increasing after 16 days imbibition. The amount of water absorbed by the seed was therefore somewhat larger than that absorbed by the endocarp alone, viz. 12.2 g for five seeds as opposed to 4.0 g for the endocarp of five nuts (Fig. 1B).

The difference in the mass of water absorbed by the intact nut and that absorbed by the endocarp alone must be attributed to absorption by the kernel. This is confirmed by the data presented in Figures 1A, B. The great absorptive power of the endosperm is demonstrated not only by the fact that the mass of the kernel increases by 85 percent during 16 days of imbibition (Fig. 1A) but also that between 66 and 75 percent of the total moisture absorbed by the intact nut is absorbed by the kernel (Fig. 1B).

It is interesting to note too that during the first day of imbibition, the rate of absorption of the endocarp is precisely the same as that of the intact nut (Fig. 1B). This suggests that during this initial period, all the moisture absorbed by the nut is retained by the endocarp and that the kernel receives moisture only from the second day onwards, as the endocarp nears saturation point. It is evident from this experiment that the endocarp is permeable to water.

To establish whether absorbed moisture was reaching the embryo, a further experiment on 35 air-dried kernels was conducted. Five of these were dissected and the excised embryos weighed while the other 30 kernels were soaked in

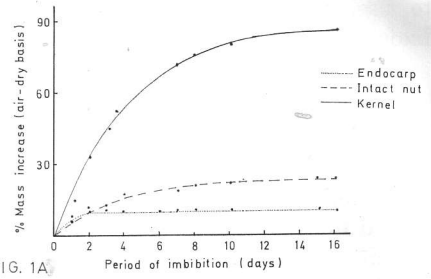


FIG. 1A

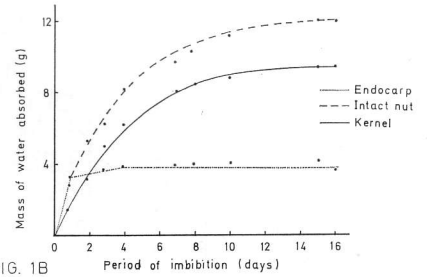


FIG. 1B

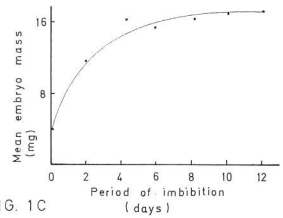


FIG. 1C

1. The absorption of moisture (A); the amount of water absorbed by five air-dried nuts, kernels, and endocarp of *J. caffra* (B); and absorption of moisture by the embryo while still in the endosperm kernel (C).

water, which was changed daily. After every two days of soaking, five kernels were removed and their embryos excised and weighed. This pattern was continued for a total of 12 days. In this way the absorption rate of the actual embryo was ascertained. These results are reflected in Figure 1C. It is evident that the embryo within the intact seed increased in mass. This is evidently due to moisture uptake.

An experiment in which embryos were soaked in water and weighed at the same intervals as above, showed that the initial rate of water uptake was greater than in the case of embryos in intact seeds. The excised embryos were fully imbibed within two days. However, the final mass was approximately the same as that of embryos in intact seeds.

From the above results it is concluded that the seeds are permeable to water.

(c) *Effect of oxygen on respiration of the seed*

The aim of this experiment was to determine whether an increased oxygen tension would affect oxygen uptake by the seed. Although indirect, this procedure would give some indication of the permeability of the covering structures to oxygen.

Fresh nuts were immersed in water for eight days, with a daily change of water. Thereafter the nuts were air-dried in the laboratory for one day and their respiration determined on a Gilson Differential Respirometer at a constant temperature of 25°C. Owing to the large size of *J. caffra* seeds, standard respirometer flasks could not be used and special large flasks, which could each hold three seeds, were constructed.

Oxygen uptake in air and in pure oxygen did not differ significantly (Table 1) indicating that the covering structures are sufficiently permeable to permit oxygen uptake from the air.

Table 1. Oxygen uptake of J. caffra *seeds in air and pure oxygen*

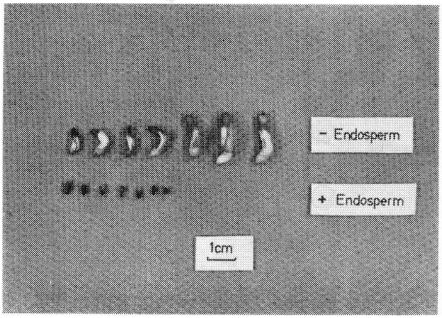
Treatment	O_2 uptake (μl/hr/g (oven-dry basis)).
Air	9.69
Pure O_2	10.96

(d) *Inhibitory effect of endosperm on growth of excised embryos*

Wareing (1969), Egley (1972) and van de Venter (1974) all state that, in certain cases, germination inhibitors which induce and maintain dormancy are present in the tissues of the seed surrounding the embryo. In view of the fact that excised *J. caffra* embryos grew satisfactorily in culture and the covering structures apparently did not inhibit the uptake of moisture and oxygen, an experiment was performed to test the effect of endosperm on the growth of excised embryos.

Twenty embryos were excised from five-week-old seeds, sterilized as described previously, and placed in White's modified medium in conical flasks. Whole kernels were sterilized by immersing them in 0.2 percent $HgCl_2$ for five minutes and subsequently washing them very thoroughly with autoclaved water. The kernels were then cut in half, their embryos discarded, and half a kernel added to each of ten flasks while the remaining ten flasks served as controls for the experiment. Incubation took place in the dark at 25°C and the trial was terminated after 17 days.

During the course of the experiment, 30 percent of the control embryos and 10 percent of the embryos in the endo-



2. Excised embryos of *J. caffra* cultured for 17 days in a liquid nutrient medium with and without endosperm.

sperm treatment became infected with microorganisms and were discarded.

At the end of the experiment the mean embryo mass of the control embryos was 118.17 mg while that of the embryos cultured with endosperm was only 6.1 mg. Mean embryo lengths were 1.43 cm and 0.36 cm respectively (Fig. 2).

These results indicate that the endosperm might contain a factor which inhibits the growth of excised embryos. Whether this factor functions to induce dormancy in intact seeds requires further study.

(e) *Experiments to establish optimum conditions for seed germination*

In oil palm seeds, oxygen tension, temperature, and moisture content were shown by Hussey (1958) to be of prime importance for germination. By manipulation of these three factors, germination of *Elaeis guineensis* seeds could be increased from five percent in eight weeks to over 80 percent during the same period. Since both *J. caffra* and *E. guineensis* are members of the subfamily Cocosoideae, and are the only two cocosoid palms that occur naturally on the African mainland, it was decided to base this investigation largely on Hussey's (1958) work.

Three experiments were conducted, and because the only seed available at that stage was already two months old, the experiments were conducted concurrently.

In the first of these, the effect of oxygen and temperature on germination was investigated. The other two experiments were aimed at establishing the optimum moisture level for maximum seed germination. In one of the experiments the optimum moisture content of the seed itself was studied, while in the other, an attempt was made to ascertain the optimum moisture content of the sand in which the seeds were sown.

(i) *Effect of temperature and oxygen tension on germination*

The procedure followed in this study was similar to that described by Rees (1963). In this method the seeds are presoaked in water, thereafter air-dried for a day and then placed in a beaker or flask without a medium. The moisture content of the seed is subsequently kept constant by weighing the flask daily and supplementing any loss in mass with water.

Seeds were soaked in water which was changed daily. After eight days they were removed from the water and air-dried in the laboratory until their moisture content was equal to two-thirds of that amount which is required to saturate air-dried nuts. This particular level was found by Hussey (1958) to be optimal for *Elaeis* seeds.

The seeds were then sterilized in 0.1 percent $HgCl_2$ in 10 percent ethyl alcohol for one minute and thereafter washed very thoroughly three times with autoclaved water.

Twenty seeds were placed into each of four one-liter Erlenmeyer flasks which had been previously autoclaved. Two of these flasks were stoppered with non-absorbent cotton-wool while the other

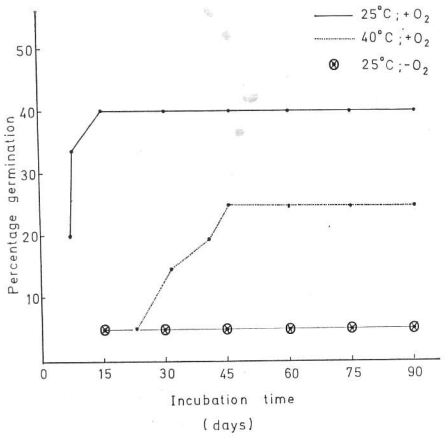


FIG. 3

3. The effect of oxygen on the germination of *J. caffra* seeds at 25° and 40°C.

two were sealed with vaccine caps. The latter two flasks were flushed daily for five minutes with pure oxygen. The two flasks with cotton-wool tops served as the control treatment.

Hussey (1958) found that a response to the increased oxygen level was only evident at 40°C. Consequently two temperatures were used in this experiment and incubation took place at 25°C and 40°C. A control flask and one oxygenated flask were incubated at each of these temperatures. The mass of each flask was recorded at the start of the experiment and maintained throughout by the addition of water when necessary. Germinated seeds were removed regularly and the change in mass taken into account. The experiment was continued for four and a half months.

The results of this experiment are shown in Figure 3. At both temperatures, germination was stimulated by the increased oxygen tension, while virtually no germination occurred in the control flasks. Germination also proved to be better at 25°C than at 40°C, not only in terms of actual number of seeds germinated, but also with respect to the rate at which germination occurred. While the oxygen treatment at 25°C resulted in 40 percent germination within 15 days, pure oxygen at 40°C produced 25 percent in 45 days, the first seed germinating only after 23 days (Fig. 3).

(ii) *Effect of moisture on seed germination*

Although Hussey (1958) gives very precise figures with respect to optimum moisture content of both seeds and germination medium (sand) for the oil palm, Rees (1963), in describing his method of germination for palm seeds in general, states rather vaguely that the seed's moisture content must be maintained "as wet as possible with no superficial moisture." An attempt was therefore made to try and establish the optimum moisture level for *J. caffra.*

Two experiments were conducted. In the first one the seeds were germinated in a beaker without a medium and the effect of the moisture content on germination of the seeds themselves was studied. The second experiment, in which the seeds were germinated in sand, was conducted to ascertain the effect of the moisture content of the medium on seed germination.

In the first trial, air-dried seeds, which had previously been weighed, were soaked in water for eight days with daily changes of water. Thereafter the seeds were again weighed and divided into three groups comprising 20 seeds each. The first group of seeds was air-dried to a moisture content of seven percent (based on the mass of air-dried seeds); the second group was air-dried to 14 percent, while the third group was not dried at all. The seeds in this latter group were very nearly saturated and contained 21 percent moisture.

After sterilizing all the seeds as in the previous experiment, the three cate-

gories of seeds were placed into separate beakers and covered with a thin sheet of polythene held in place by an elastic band. (This cover minimized evaporation of moisture from the seeds but permitted the free exchange of gases.)

The mass of each beaker was recorded and maintained at this level by briefly immersing the seeds in water every third or fourth day, depending on the rate of loss of moisture. Germinated seeds were removed when observed and the differences in mass taken into account. The three beakers were placed in a large desiccator (without desiccant) which was flushed daily with oxygen for five minutes. The desiccator was in turn placed in a growth cabinet and the seeds incubated at 40°C for 150 days.

In the second experiment, three 15 cm petri dishes were filled with coarse sand and varying amounts of water added so that three different moisture contents could be obtained. In the first one, 21 g of water was added, resulting in a moisture content of seven percent (on oven-dried basis); the second dish received 42 g of water and contained 14 percent moisture, while the sand in the third dish was saturated (21 percent) by adding 63 g of water. (It is purely coincidental that these three levels were numerically similar to the ones in the previous experiment.)

Six seeds were then half-buried in the sand in each dish and the mass of each dish recorded and maintained by the addition of water to the sand when necessary. The petri dishes were placed, without lids, into a desiccator, flushed daily with oxygen for five minutes and the seeds incubated at 40°C for 150 days. Germinated seeds were removed and the change in mass taken into account.

It was not known at this stage that 25°C was more conducive to germination of *J. caffra* seeds than 40°C. The

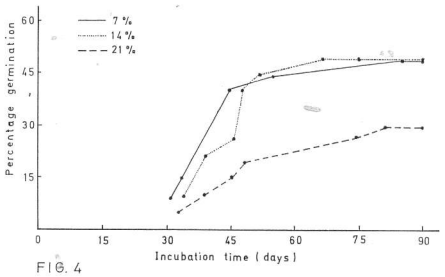


FIG. 4

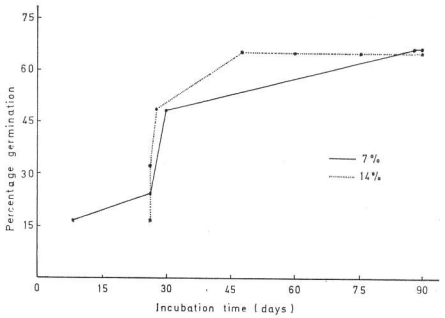


FIG. 5

4. Effect of moisture contents of the nuts of *J. caffra* on germination at 40°C in oxygen.
5. Germination of *J. caffra* seeds (nuts) in oxygen at 40°C half-buried in sand at two levels.

latter temperature, as well as the increased oxygen tension were used in both these experiments purely because of their effect on the germination of *Elaeis* seeds as described by Hussey (1958). The results of the experiments are presented in Figures 4 and 5. The overall germination percentages obtained in these two experiments were very good, especially in the second experiment, where 66.6 percent of the seeds germinated. In this same experiment, however, the seeds in water-saturated sand failed to germinate. So too, the germination of the water-saturated seeds in the first experiment was poorer than the other two treatments.

The results of these two experiments indicate that *J. caffra* seeds tend to ger-

minate better in sand than without a medium, provided that the moisture content of the sand does not exceed 14 percent, i.e., a moisture level that is equivalent to two-thirds of the moisture content at saturation point.

When no medium was used the best results were obtained when the moisture content of the seed was 14 percent because, although the percentage germination (50 percent) did not exceed that of the seven percent treatment, germination occurred sooner in the former case (Fig. 4). The saturated seeds germinated relatively poorly. Similarly, where seeds were sown in saturated sand, no germination occurred.

When seeds are sown in the conventional manner, i.e., in sand, it would appear that 14 percent moisture is optimal because, although the seven percent treatment also resulted in 66.6 percent germination (Fig. 5), this figure was attained within 48 days by the 14 percent treatment, and only after 89 days in the seven percent treatment (Fig. 5).

Discussion

In view of the thick and hard nature of the endocarp it was expected that this structure might prevent the absorption of sufficient moisture for germination. However, the results indicate that neither the endocarp nor any of the other covering structures are impermeable to water.

In the event of an oxygen deficiency, germination is very much reduced. This would explain the complete inhibition of germination during the preliminary trials in which the seeds were totally saturated as the result of overwatering. Further confirmation of this is to be found in the later experiments in which the interaction of moisture, temperature, and oxygen were studied. From these results it is clear that irrespective of the temperature and oxygen level, germination is severely inhibited when the moisture level is too high.

Under normal moisture conditions, i.e., not total saturation, the covering structures of the seed are apparently sufficiently permeable to oxygen. This is substantiated by the results of the respiration experiment in which the uptake of oxygen was studied. No significant increase in oxygen uptake occurs when the seeds are placed in pure oxygen.

Clearly, though, an increased oxygen tension stimulates germination of *J. caffra* seeds, but there is also an interaction between oxygen and moisture and temperature. As the moisture content of the covering structures increases, so the uptake of oxygen will naturally decrease. Further, according to Hussey (1958), the diffusion of oxygen into compact tissues may be considerably restricted at high temperatures.

While the optimum moisture level in the seeds of *J. caffra* seems to be the same as that for *Elaeis guineensis*, viz. two-thirds of the moisture required to saturate the seed (14 percent in *J. caffra*), the optimum temperature for germination of *E. guineensis*, viz. 40°C, proved to be too high for *J. caffra* and inhibited the germination of this species' seeds. This difference in response of these species to temperature might have an ecological explanation in that while *Elaeis* is essentially a tropical palm, occurring mainly in the warmer latitudes, *Jubaeopsis* is one of the most southerly occurring palm species and is probably adapted to lower temperatures.

To obtain the maximum percentage germination in the shortest possible time, an optimum combination of temperature, moisture, and oxygen is necessary. From the results obtained in this study, it appears that this optimum combination would be the incubation of

seeds in sand with a 14 percent moisture content at 25°C in pure oxygen.

Concerning the positive effects of an increased oxygen tension on the germination of *J. caffra* seeds, and the negative effects of endosperm on the growth of excised embryos, the following might be relevant.

In a number of species, slow seed germination has been associated with a lack of oxygen (Crocker, 1948 cited by Hussey, 1958). Originally it was thought that the covering structures of certain seeds restrict the uptake of oxygen and consequently also respiration, which in turn inhibits germination.

It was demonstrated in the respiration experiments, though, that the oxygen uptake did not increase when *J. caffra* seeds were placed in pure oxygen. Consequently, it would seem that a higher oxygen tension affects not the respiration of *J. caffra* seeds, but rather some other physiological process.

Roberts (1969) states that endogenous germination inhibitors increase the oxygen requirements of some seeds, while in others, it appears as if the function of the oxygen is to oxidize the inhibitor into an inactive form. Whether or not an inhibitor of this nature is present in the seed of *J. caffra*, is at this stage still not clear. However, in view of the fact that the addition of endosperm to the nutrient medium of excised embryos severely inhibits the growth of those embryos, it seems possible that an endogenous inhibitor might be present in the endosperm. It is tentatively suggested that the stimulation of germination of *J. caffra* seeds by oxygen may be due to the effect of oxygen on the inhibiting factor itself. This would be in accordance with the hypothesis proposed by Roberts (1969) for other plant species.

Acknowledgements

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