SEED ANATOMY AND GERMINATION OF *Phoenix roebelenii* O'Brien (ARECACEAE)[1]

EMERSON IOSSI[2], FABIOLA VITTI MORO[3], RUBENS SADER[4]

ABSTRACT - The objective of this study was to investigate the morphology, anatomy and germination behaviour of *Phoenix roebelenii* seeds. Biometric data were obtained by measuring 100 seeds extracted from recently harvested fruits and air-dried for one day. Four replications of 50 seeds each were previously treated with Vitavax-Thiran and then put to germinate in *Sphagnum* sp. in plastic trays at room temperature. Morphological details of the seeds were documented with the help of a scanning electronic microscope and then drawings were made with the help of a clear camera coupled to a stereomicroscope. Permanent lamina containing embryo sections were prepared to study its anatomy. The mean dimensions of the seeds were: length of 10.32mm, width of 5.21mm and thickness of 3.91mm. The weight of one thousand seeds was of 151.1g and the mean number of units.kg^{-1} was 6,600. Germination started between 27 and 58 days after sowing. The seeds are of the albuminous type, the endosperm is hard and the embryo (which is not clearly differentiated) occupies a lateral and peripheral position. During seed germination, seedling protrusion begins with the opening of an operculum, through which the cotyledon petiole is emitted with the embryonic axis at its tip. The portion of the cotyledon petiole that remains inside the seeds acts as a haustorium for the absorption of nutrients from the endosperm. The plumule emerges through a rift in the posterior part of the cotyledon. Secondary roots are observed to grow from the anterior part of the primary root.

Index terms: palm, pigmy date palm, pyrene, seedling.

ANATOMIA E GERMINAÇÃO DE SEMENTES DE *Phoenix roebelenii* O'Brien (ARECACEAE)

RESUMO - O objetivo desse trabalho foi estudar a morfologia, anatomia e o processo germinativo de sementes de *Phoenix roebelenii*. Para o levantamento dos dados biométricos foram utilizadas 100 sementes de frutos recém-colhidos, deixados secar ao ar por um dia. Para a germinação, quatro repetições de 50 sementes tratadas com Vitavax-Thiran foram semeadas em bandejas de plástico, contendo *Sphagnum* sp. como substrato e mantidas sob condições ambientais de laboratório. Detalhes da morfologia da semente foram documentados com o uso de microscopia eletrônica de varredura e esquematizados com auxílio de câmara clara, acoplada ao estereomicroscópio. Foram confeccionadas lâminas permanentes com cortes do embrião, para o estudo de sua anatomia. As dimensões médias das sementes foram: 10,32mm comprimento, 5,21mm largura e 3,91mm espessura. O peso de 1000 sementes foi de 151,1g e 1kg continha 6600 unidades. O início da germinação variou entre 27 e 58 dias. As sementes são albuminosas, com endosperma duro e o embrião é pouco diferenciado, lateral e periférico. A germinação inicia-se pela abertura de um opérculo, através do qual é emitido o pecíolo cotiledonar com o eixo embrionário na extremidade. O pecíolo funciona, internamente, como um haustório, digerindo gradativamente o endosperma. Na sua parte posterior, desenvolve-se a plúmula, que emerge através de uma fenda. Nota-se o aparecimento de raízes secundárias na porção anterior da raiz primária.

Termos para indexação: diásporo, palmeira, tamareira-anã, plântula.

[1] Submetido em 22/09/2005. Aceito para publicação em 24/07/2006;

[2] Doutorando em Agronomia, Departamento de Produção Vegetal, FCAV/UNESP, Via de Acesso Prof. Paulo Donato Castellane, s/n, CEP: 14884-900, Jaboticabal - SP. Bolsista Capes, iossi@fcav.unesp.br;

[3] Professora Assistente, Departamento de Biologia Aplicada à Agropecuária, fabiola@fcav.unesp.br;

[4] Professor Titular, Departamento de Produção Vegetal, rsader@fcav.unesp.br.

INTRODUCTION

The palms are a globally important family of socio-economic plants. The Arecaceae family includes palm trees of economical importance both as a source of agricultural produce and as ornamental components in landscaping projects. Its species are scattered mainly close to the equator line within the limits of 44°N and 44°18'S (Henderson et al., 1995).

Phoenix roebelenii O'Brien occurs naturally in northern Laos, Vietnam, and areas of the Yunnam, in the southern China (Barrow, 1994). It is a palm tree with very interesting characteristics, such as low height, easy cultivation and graceful crown that have made it a popular accent plant in tropical landscapes. This species is commercially important as a pot plant.

The fruit of this species are small, elliptic, violet-black in colour when mature and the seed shows a longitudinal furrow. They are very much appreciated by birds and one kilogram contains 3,663 seeds (Lorenzi et al., 2004).

P. roebelenii seeds from three different locations, harvested at different times, were studied by Matthes and Castro (1987) and they reported that depending on location, the germination started at different moments: 47 days after sowing, 60 and 120 days. This diversity of results was ascribed to the high degree of heterozygosis shown by different plants of this species. This is thought to frequently occur between different individuals of the *Phoenix* species.

According to Uhl and Dransfield (1987), the types of germination of palm seeds are: adjacent-ligular, remote-ligular, or remote-tubular. *P. roebelenii* seed germination is of the remote-tubular type. This is the type of germination in which the cotyledon petiole is considerably elongated and no ligula is visible.

Seeds of the pigmy date palm are sold by some companies but research on how to get quick information about the seed germination potential are still lacking.

Preliminary studies conducted by the authors indicated that the tetrazolium test probably has a good potential to provide quick and reliable information about *P. roebelenii* seed germination. Nonetheless, basic information about the seeds is indispensable, as pointed out by Grabe (1970), according to whom the correct interpretation of the tetrazolium test results is possible only when the analyst has a sound knowledge of the seed and the seedling structures as well as of the phases of the germination behaviour. Good knowledge concerning the procedures of the test, as well as of the possible coloration patterns resulting at the end of the test, are also important for the correct interpretation of results.

Furthermore, this is an important reason to study the anatomy of the seed embryo, which will help in future studies with the tetrazolium test to point quickly the germination percentage of the *P. roebelenii* seed lots.

Considering that in practice the propagation of palm plants is accomplished mainly by seeds, the objective of this research work was to study the morphology, anatomy and germination behaviour of pigmy date palm seeds. This basic information is important for the commercialisation of seeds and rootstocks of this species.

MATERIAL AND METHODS

The fruit of *P. roebelenii* were collected from trees located on the campus the Paulista State University at Jaboticabal (state of São Paulo, Brazil), from March to April, 2000.

The pulp of the fruit was removed by rubbing them against a sieve. Next, the pyrenes (which consisted of the endocarp and seed) were washed in running water and dried in the shade for one day. Before the germination test, the pyrenes were treated with the fungicide Vitavax-Thiran at the rate of 60mL of the active ingredient to 100 kilos of the seeds, according to the procedure described by Meerow (1994). Immediately after treatment the seeds were put to germinate.

In a sample of 100 seeds, seed length, width and thickness were determined with the help of a ruler and a digital calliper rule. Following that, the mean and the standard deviation for each one of those characteristics were calculated.

For the germination of the seeds and the description of the germination behaviour, seeds were sown in plastic trays measuring 50x25x6cm, containing a 5cm thick layer of the plant substratum *Sphagnum* sp. Four replications of 50 seeds each were used. These seeds were placed with the operculum pointing downward. The trays were left inside a laboratory, at room temperature and watered with distilled water.

Ten seeds per replication were used for the morphology studies. These seeds were halved in the transverse direction with the help of a scalpel and drawings of these halves were made as seen from a stereomicroscope to which a clear camera was coupled.

Another 10-seed sample was used for observations made from a Jeol JSM 5410 scanning electron microscope. The seeds were fixed to metal cylinders and covered with a 35nm

gold-palladium layer in a Denton Vacuum Desk II metalizer.

For the study of the embryo anatomy, permanent laminae were prepared containing either longitudinal or transversal sections of the embryo, according to procedures described by Johansen (1940). The laminae were observed and photographed in an optical microscope.

Seedlings from the several germination phases between the beginnings of germination until the moment when the second leaf was emitted were collected and drawings and a description of each phase were made. The number of days from the seed sowing until the beginning of germination was counted. The protrusion of the primary root was considered as the beginning of germination.

RESULTS AND DISCUSSION

Table 1 shows the biometric data of the *P. roebelenii* seeds. According to procedures prescribed by the Rules for Seed Testing (Brasil, 1992) the weight of 1000 seeds was found to be 151.5g. It was also verified that the number of pyrenes per kilo was 6,600. A description of the species found in Lorenzi et al. (2004) indicates a number of 3,663 pyrenes per kilo. This difference is ascribed to the fact that this is not a domesticated species. Moreover, species of the *Phoenix* genus are known to hybridise freely (Uhl and Dransfield, 1987). In addition to the genetic factor, the climatic conditions under which the plant develops, stage of fruit maturation and diapore water content are some of the factors capable of interfering on the number of seeds per kilo.

The seeds of *P. roebelenii* are elliptical, slightly flattened and with a furrow on the ventral face. On the dorsal face the operculum can be observed. They are of the albuminous type, with a very hard endosperm almost completely filling its inner part. The embryo occupies a lateral and peripheral position. It is wedge-shaped (cuneiform) and its funnelled end is oriented toward the seed periphery (Figures 1 and 2).

The results of the embryo histological sectioning are shown in Figures from 3 to 9. It can be observed that the embryo does not show a clear cut differentiation and the embryonic axis is enveloped by the cotyledon and its position is close to the angular extremity of the embryo.

In Figure 5, the transversal section of the embryo at its median position shows the presence of six procambium bundles, the cells of which can be seen in greater details in Figures 6 and 7.

In this research, the germination of *P. roebelenii* seeds began between 27 and 58 days after sowing. Matthes and Castro (1987) reported that seeds from three different locations and harvest times started to germinate 47, 60 and 120 days after sowing.

According to Fahn (1982), the cotyledon of monocotyledonous species (such as *Allium cepa*, *Phoenix* and grasses in general) in addition to storage and photosynthesis functions, is responsible for the release, absorption and transport of nutrient substances from the endosperm to the embryonic axis. During germination, the cotyledon becomes wider and pushes the radicle outside the pyrene. The other end of the cotyledon, that is, the one which stays inside the endosperm, develops haustorium properties and grows slowly as the endosperm is absorbed. The terminal bud of the embryonic stem is hidden in an intussusception of the cotyledon.

As reported by Meerow (1991), the germination of *P. roebelenii* seed begins with the opening of a circular operculum on the dorsal face of the seed, through which a bulbous structure is emitted known as the cotyledonary petiole on the end of which the embryonic axis is found (Figure 10b). This structure is the prolongation of the cotyledon which, internally, starts to function as an absorption organ, called the haustorium.

A longitudinal section of the germinating pyrene as well as the morphology of the embryo is shown in Figure 1f-g. As the cotyledon petiole elongates, a distinction of its posterior extremity can be seen. The anterior segment, which displays a smaller diameter, elongates up to 8mm. After that, a posterior portion of the cotyledon petiole with a larger diameter (around 10mm long) is verified, and it is inside this region that the embryonic axis which gives origin to the aerial part is developed. The primary root is found at the tip of the cotyledon petiole. The primary root develops with a diameter smaller than that of the posterior portion. Following that, the plumule emerges through a longitudinal rift open at the anterior segment of the cotyledon petiole. The whole seedling emerges through this rift.

Secondary roots start to develop from the anterior portion of the primary root (Figure 10f).

The plumule is composed of a sheath which dresses up

TABLE 1. Biometrics data of *Phoenix roebelenii* seeds.

Biometrics	Length	Width	Thickness
Mean (mm)	10.32	5.21	3.91
Standard deviation	0.49	0.28	0.25
CV (%)	4.76	5.40	6.35

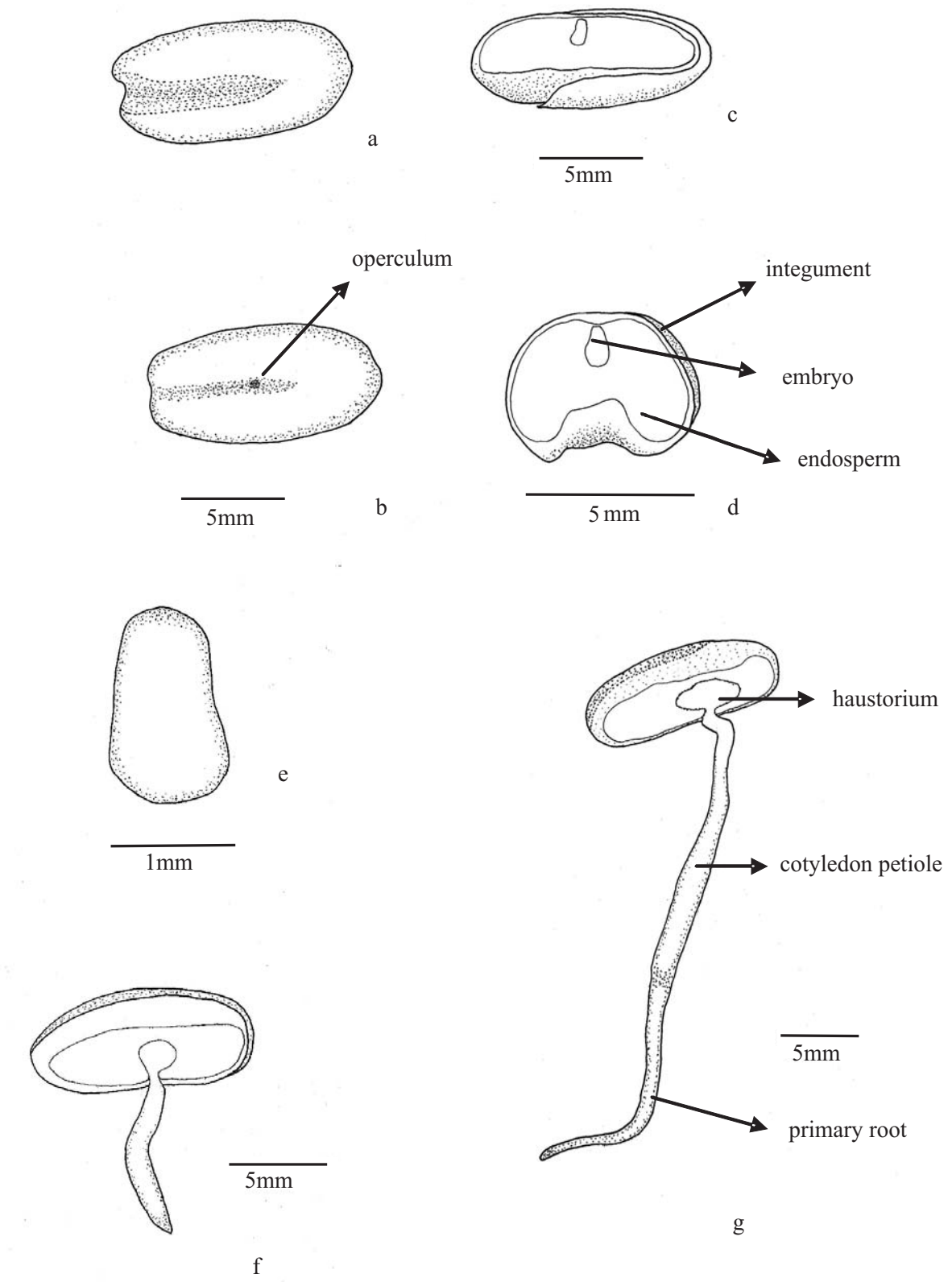

FIGURE 1. ***Phoenix roebelenii*** **pyrenes: a – ventral face; b – dorsal face; c – longitudinal section; d – transversal section; e – embryo; f–g – longitudinal section of a germinating embryo.**

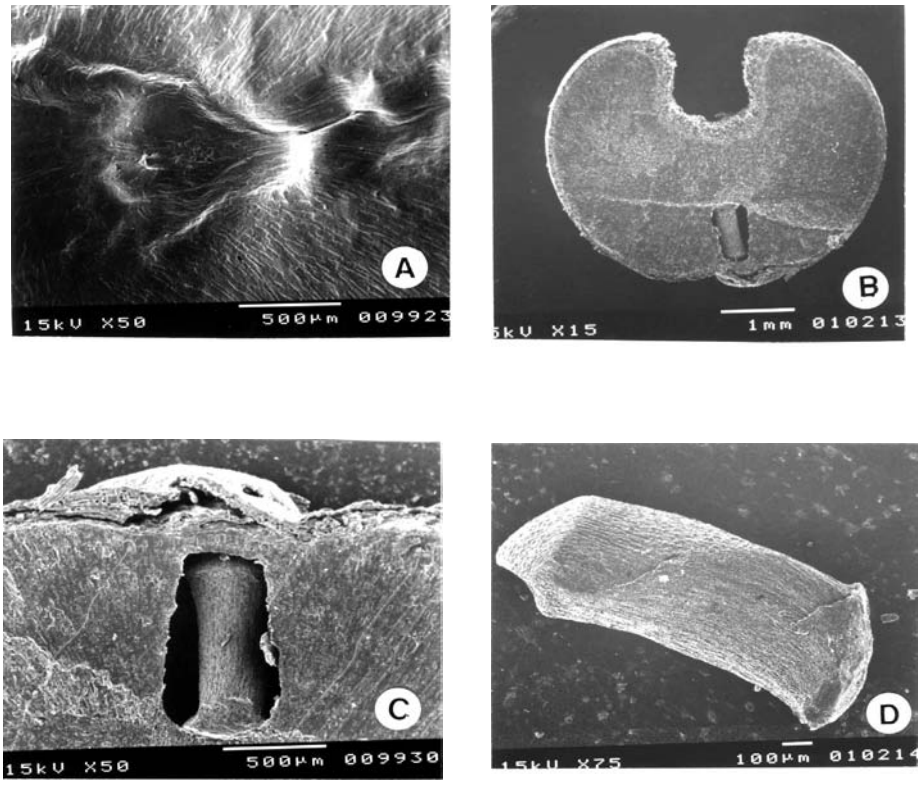

FIGURE 2. Scanning electron micrography of the pyrene of *Phoenix roebelenii*: A – detail of the operculum; B – transversal section of the pyrene; C – detail of the embryo region; D – the embryo.

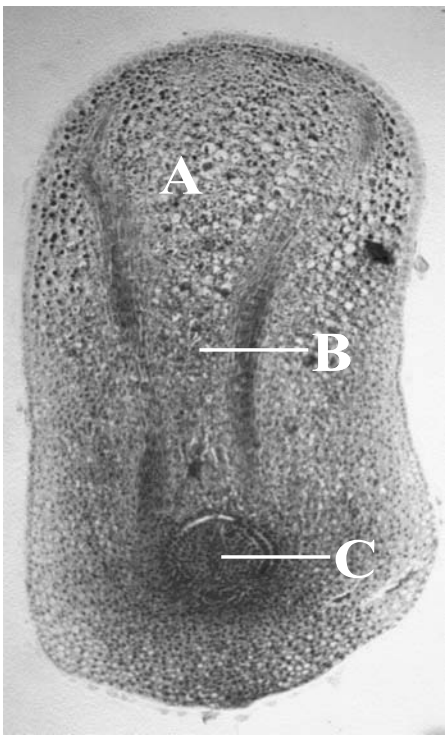

FIGURE 3. Longitudinal section of the embryo of *P. roebelenii*: A – cotyledon; B – procambium; C – embryonic axis.

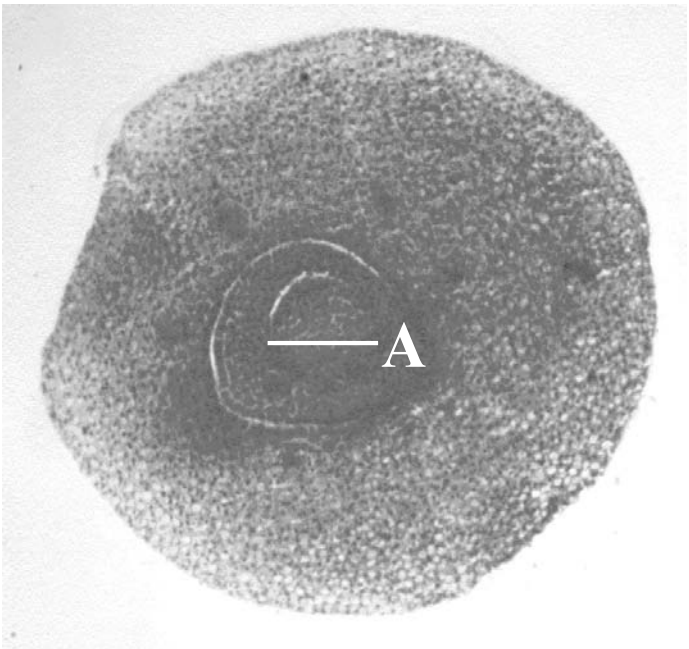

FIGURE 4. Transversal section of the embryo of *P. roebelenii* at the region of the embryonic axis: A – embryonic axis.

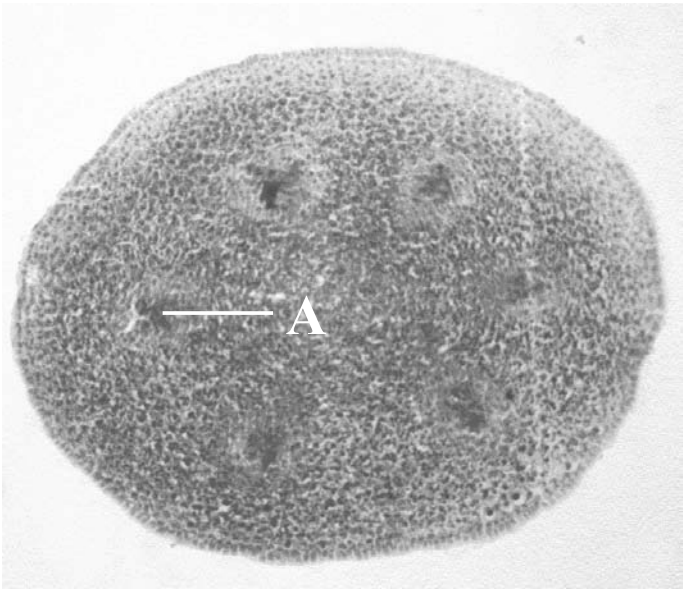

FIGURE 5. Transversal section of the embryo of *P. roebelenii*: A – procambium bundle.

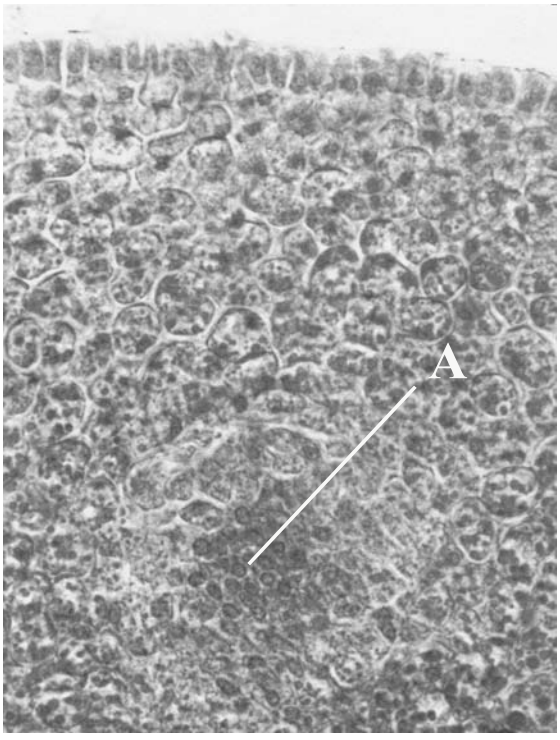

FIGURE 6. Detail: procambium bundle of the *P. roebelenii* embryo: A – procambium cells.

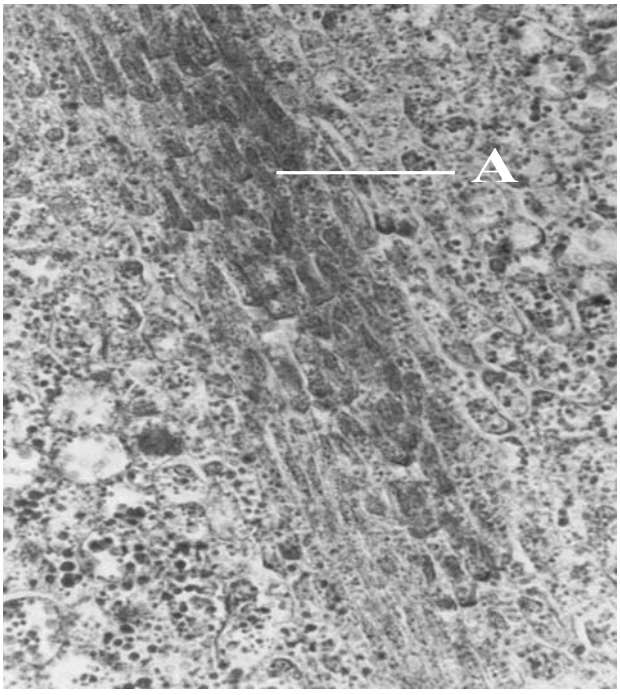

FIGURE 7. Longitudinal section of the *P. roebelenii* embryo. Detail of the procambium: A – procambium cells.

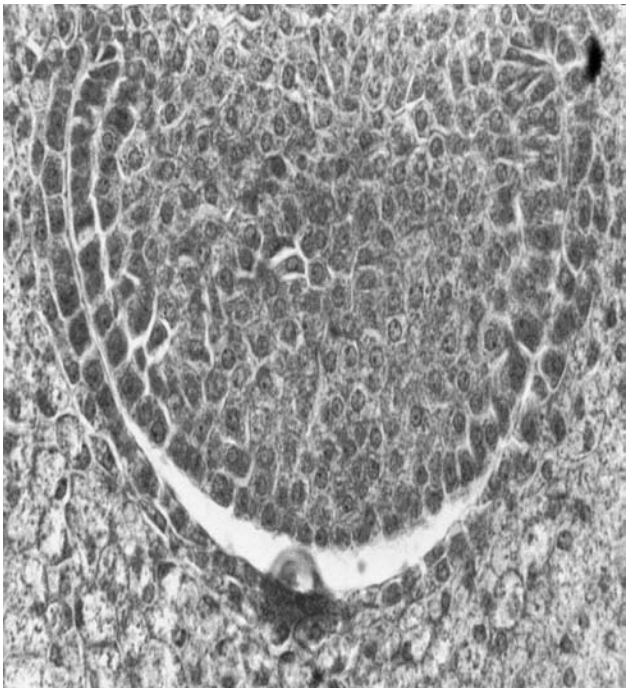

FIGURE 8. Longitudinal section of the *P. roebelenii* embryo: detail of the embryonic axis.

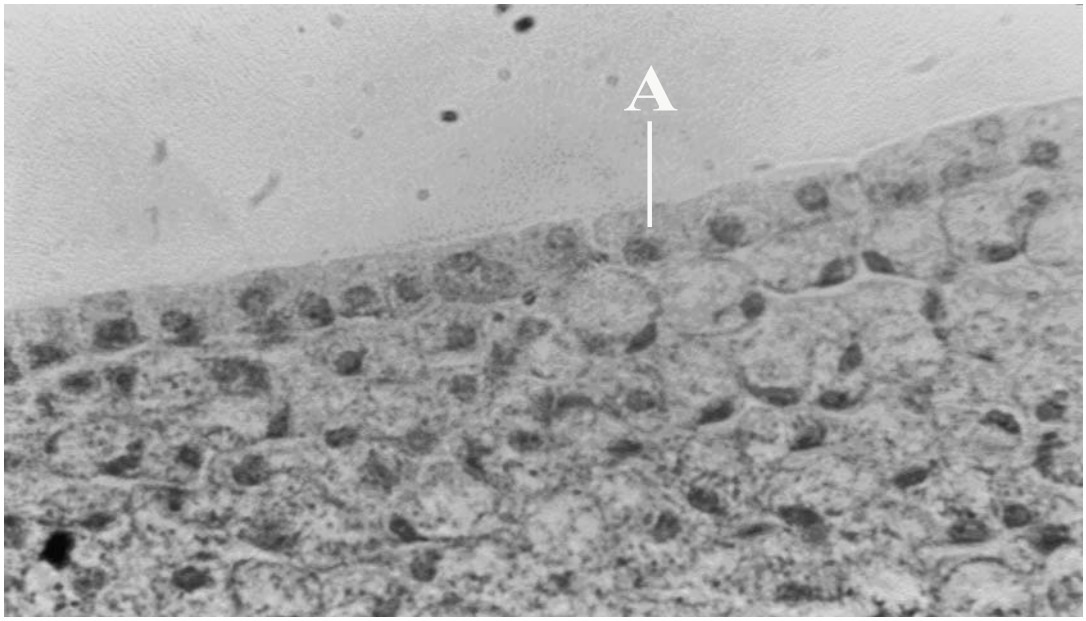

FIGURE 9. Longitudinal section of the *P. roebelenii* embryo. Detail of the protodermis: A – protodermic cells.

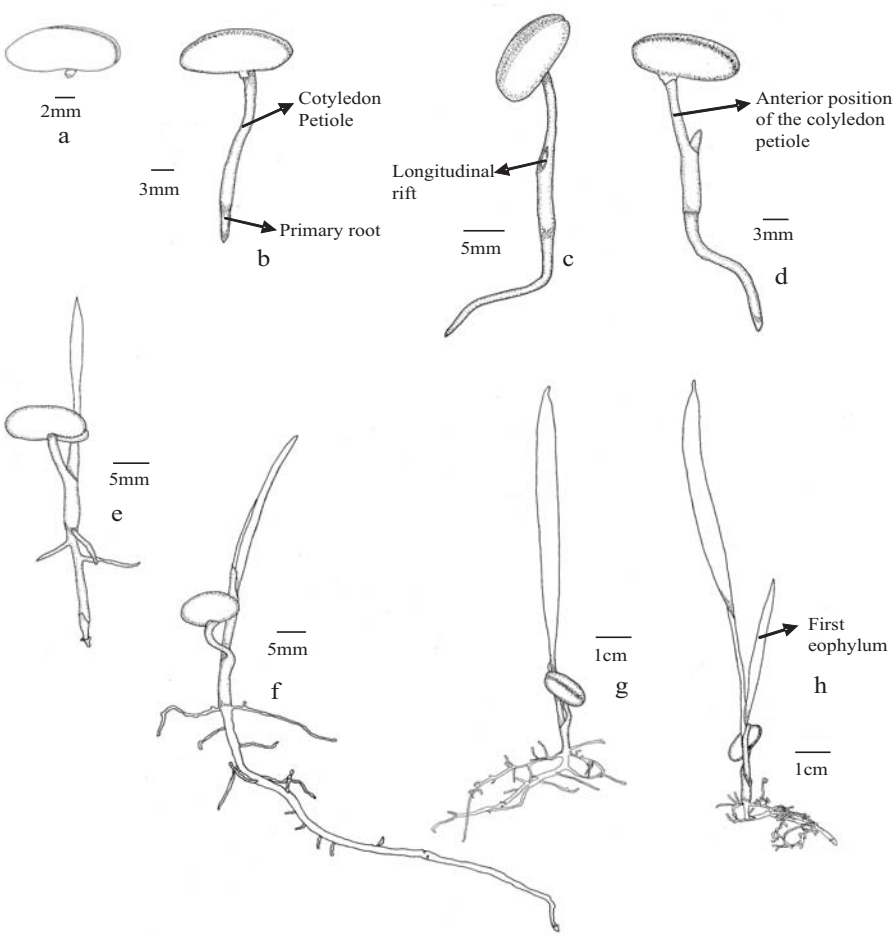

FIGURE 10. Sequential phases of the germination behaviour of *P. roebelenii* seed: a – cotyledon petiole; b – primary root; c – longitudinal rift; d – anterior position of the cotyledon petiole; e – primary root; f – secondary root; g - plumule; h – first cophylun.

the first complete juvenile leaf (first eophylum). Starting on the 42nd day after sowing, the sheath gradually opens up thus allowing the ousting of the first eophylum. The first leaves of *P. roebelenii* are simple and lanceolate. They show parallel venation with nervures of different calibres. During the process of seed germination, the endosperm is gradually used up. At the end of the germination behaviour, the haustorium occupies the whole inner space of the pyrene. The remaining integument falls off the seedling.

CONCLUSIONS

The mean dimensions of the pyrenes are: length of 10.32mm, width of 5.21mm, and thickness of 3.91mm. The 1000 pyrene weight is of 151.1g and 1 kilo contains 6,600 units. The pyrenes are albuminous, cylindrical and slightly flattened, with a hard endosperm. The embryo is not clearly differentiated and occupies a lateral and peripheral position.

The germination of the pyrenes is of the remote tubular type and it takes place between 27 and 58 days after sowing.

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