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ONTOGENIA DOS ORGÃOS REPRODUTIVOS E DA PLÂNTULA DE *Syagrus inajai* (Spruce) Becc. (ARECACEAE)

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Manaus, Amazonas
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SINOPSE:

Estudou-se o desenvolvimento morfoanatomico dos órgãos reprodutivos e plântula da espécie *Syagrus inajai*, palmeira ocorrente na Amazônia, com o intuito de descrever os eventos de formação dos tecidos do fruto, semente e corpo vegetal, fornecendo informações relevantes, de suma importância para avanços de estudos relacionados a família Arecaceae e as Monocotiledôneas.

Palavras-chave: Morfoanatomia, Desenvolvimento, Palmae, Pupunharana

Em memória a meu pai Luiz Gonzaga Genovese
e a meu avô Maciel Roversi

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“Tentei entender, mas não obtive resposta.
Então decidi começar pelo fim e terminar pelo início.
Só para ver se eu chegava a alguma pergunta”.
www.faceboock.com/Paçaro-da-dislexia

RESUMO

Syagrus inajai (Spruce) Becc. é uma palmeira que ocorre no Bioma Amazônico, em diferentes fisionomias vegetais, como: floresta de terra firme, vertente e baixio, além de áreas abertas, como beira de estradas. Produz grande quantidade de frutos, os quais, são comestíveis. O presente trabalho teve como objetivo realizar o estudo ontogênético dos órgãos reprodutivos e da plântula de *S. inajai*, através da morfoanatomia. As observações e coleta do material botânico foram realizados nos anos de 2009 e 2010 em área verde do *Campus* da Universidade Federal do Amazonas - UFAM, Manaus, Amazonas, Brasil, em área de baixio, ao redor das coordenadas 3 º 05 ' 45,84 " S e 59 º 58 ' 43,69 ", a partir, de 30 indivíduos de *S. inajai*. Observou-se que o número de flores por inflorescência varia entre 5.904 - 17.316 para flores estaminadas e 180 - 3.528 para as flores pistiladas. As flores estaminadas apresentam seis estames com um feixe vascular cada; pistilódio trifído e vascularizado. As flores pistiladas apresentam seis estaminódios unidos formando um círculo. O gineceu é sincárpico, tricarpelar, trilocular, um óvulo por lóculo, sin-ascidiado no ovário e plicado acima. A fusão incompleta dos flancos dos carpelos formam três cavidades septais, que vão da base do ovário a base do estígma. Estigma tripartido, apical e séssil, com epiderme composta por células papilosas alongadas, padrão de epiderme que se mantem por todo canal estilar. O ovário apresenta duas regiões meristemáticas, uma adjacente à epiderme externa e outra envolvendo a cavidade seminal. A região meristemática externa origina o mesocarpo fibroso. A região meristemática interna é responsável pelo espessamento do endocarpo, juntamente com as células provenientes de divisões periclinais da epiderme interna do ovário, formando o endocarpo *lato sensu* de origem mista. A esclerificação do endocarpo inicia-se no fruto com aproximadamente 80 dias, e ocorre centripetamente. O endosperma é do tipo coenocítico multicelular e a formação de parede das células ocorre centrifugamente. O óvulo é anátropo. A semente é paquicalazal e bitegumentada, estes restritos a região da micrópila. O opérculo é composto por células dos tegumentos externo e interno, e por células do obturador, esclerificadas. O zigoto tem sua primeira divisão celular cerca de 30 dias após o início do desenvolvimento do endosperma. O tempo de desenvolvimento do fruto é de aproximadamente 240 dias, quando se inicia a dispersão. O processo de desenvolvimento embrionário dura aproximadamente 220 dias, dividido em 4 estádios: proembrião, embrião globular, cordiforme lateral e torpedo. O embrião é pequeno, linear e derivado da célula terminal do proembrião, proveniente de divisões mitóticas na célula apical. O eixo embrionário está localizado na região proximal em ângulo reto ao maior comprimento do

embrião. O cotilédone único é formado pelo meristema fundamental, procâmbio e protoderme. O procâmbio provê o eixo embrionário e a região haustorial. A germinação de *S. inajai* inicia-se em média 101 dias após a semeadura com a formação do botão germinativo. Os eventos morfoanatômicos que se seguiram foram: alongamento do hiperfilo, intumescimento da bainha cotiledonar, emissão da raiz primária e primeiro catafilo, emissão do segundo catafilo e eofilo. O alongamento do eixo embrionário foi observado após a emissão do botão germinativo, durante o processo de alongamento do hiperfilo. A observação da raiz primária em vista desarmada foi possível após o intumescimento da bainha cotiledonar, momento em que os dois catafilos e o eofilo encontravam-se diferenciados na bainha cotiledonar. Foi observado amido no embrião antes da germinação, porém a sua quantidade aumentou nas células parenquimáticas após a formação do botão germinativo. A semente apresentou 30% de lipídios em sua concentração. Transcorre-se 270 dias, da abertura da bráctea peduncular a dispersão do fruto. A abertura da flor pistilada ocorre 20 dias após a abertura da bráctea peduncular, o fruto leva cerca de 240 dias para se formar e o embrião 220 dias.

Palavras-chave: Arecaceae, morfoanatomia, desenvolvimento, embrião, germinação.

ABSTRACT

Syagrus inajai is a palm that occurs in the Amazonian Biome, in different plant physiognomies, such lowland rain forest, slope and gallery forests, as well as in open areas such as clearings and roadsides. It produces large quantities of edible fruits. The purpose of this work is to carry out an ontogenic study of *S. inajai* from gynoecium to seedling, in terms of its its morphoanatomy. The observations and collection of botanical material were carried out in 2009 and 2010, in a green area of the Campus of the Universidade Federal do Amazonas - UFAM, Manaus, Amazonas, Brazil, which is situated in a region of baixio forest, at coordinates 3 ^{0} 05' 45.84" S and 59 ^{0} 58' 43.69 ". Thirty individual specimens of *S. inajai* were collected for analysis. The aim of the first chapter is to characterize the morphoanatomy of the inflorescence of *S. Inajai*, enabling a better knowledge of the species studied and an understanding of the processes of formation of the fruit and seed. The inflorescences are branched to one order, pedunculate, and interfoliar, measuring 62-82 cm in length, with woody bracts with longitudinal grooves on the external surface, and flowers in triads. The number of flowers to each inflorescence varies from 5,904 to 17,316 for staminate flowers, and from 180 to 3,528 for pistillate flowers. Staminate flowers with six stamens and one vascular bundle each; three-lobed pistillodium, vascularized pistillodium. Its pistillate flowers have six staminodia joined to form a circle, syncarpic, tricarpellary, trilocular gynoecium, one ovule to each locule, synascidiate in the ovary, and plicated above. Tripartite stigma, apical and sessile, with epidermis composed of elongated papillary cells, pattern of epidermis that is maintained throughout the stylar canal. Bitegmented, anatrope, pachychalazal ovule. In the second chapter, the aim was to carry out a morphoanatomical study of the development, from gynoecium to the developed fruit, and to measure the approximate time to occurrence of the separate events in this process. From the seminal primordium, the ovules are anatropous. Hypostasis with cells containing phenolic content is prolonged during development, including the entire ovule, throughout the length of the pachychalazy. The ovary has two meristemic regions: a peripheral meristem region, adjacent to the outer epidermis, which gives origin to the fibrous mesocarp; and an internal meristemic region, which involves the internal seminal cavity and is responsible for the thickening of the endocarp, forming the endocarp *sensu lato*, of mixed origin. Sclerification of the endocarp initiates in the fruit at around 80 days, occurring centripetally. The endosperm is of the multicellular coenocytic type, with formation of the cell walls occurring centrifugally. The operculum is comprised of cells of the outer and inner integuments, and by sclerified cells of the obturator. The first cell division of

the zygote occurs around 30 days after the start of development of the endosperm. The development time of the fruit is approximately 240 days. The third chapter gives a morphoanatomical characterization of the embryo of Syagrus inajai, in different phases of its development, seeking to contribute with information on the embryonic development of palms, and further our understanding of the germinative process of plants of the family Arecaceae.The development process of the embryo, until the moment of dispersion, takes approximately 220 days, and is divided into four stages: proembryo, globular embryo, lateral cordiform and torpedo. The embryo is small, linear, and derived from the terminal cell of the proembryo, arising from mitotic divisions in the apical cell. The embryonic axis is located in the proximal region, aligned parallel to the length of the embryo. The single cotyledon is formed by the ground meristem, procambium and protoderm. The procambium supplies the embryonic axis and the haustorium. The fourth chapter aims to describe the morphoanatomy of the germinative process of *S. inajai* and to determine its average duration, and also to identify the ergastric substances present in the embryo, haustorium and endosperm, and to perform centesimal analysis of the seed.The germination of *S. inajai* starts at around 101 days after seeding, with the formation of the germinative button. The morphological events that followed were: lengthening of the hyperphyll, intumescence of the cotyledonary sheath, emergence of the primary root and first cataphyll, emergence of the second cataphyll and eophyll. Lengthening of the embryo axis was observed after the emergence of the germinative button, during the process of lengthening of the hyperphyll. It was possible to see the primary root with the naked eye after the intumescence of the cotyledonary sheath, at which moment the two cataphylls and the eophyll were differentiated in the cotyledonary sheath. Starch was observed in the embryo before germination, but its quantity increased in the parenchyma cells after the formation of the germinative button. The seed presented 30% lipids in its concentration at the moment of dispersion. The time from opening of the peduncular bract to dispersion of the fruit is 240 days. The opening of the pistillate flower occurs 20 days after the opening of the peduncular bract; the fruit takes around 240 days to form, and the embryo 220 days.

Keywords: Arecaceae, morphoanatomy, development, embryo, germination.

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INTRODUÇÃO GERAL

Arecaceae é uma família monofilética, com 183 gêneros e 2361 espécies, composta por cinco subfamílias (STEVENS, 2012). Dentre elas, a subfamília Arecoideae abrange o maior número de espécies, com maior diversidade na América do Sul. São plantas geralmente monoicas, caracterizadas por apresentarem inflorescência com flores dispostas em tríades ou acérvulos (STEVENS, 2008; DRANSFIELD et al., 2008).

No ecossistema Amazônico são encontradas aproximadamente 50% dos gêneros e 30% das espécies Neotropicais de palmeiras, sendo que 8 gêneros (24%) são endêmicos da região (HENDERSON et al., 1995). Esses dados evidenciam a importância da floresta Amazônica na manutenção e expressão dessa família Botânica. De acordo com Duran & Franco (1992), a abundância e a diversidade indicam que as palmeiras são elementos importantes na estrutura e funcionamento dos ecossistemas.

De acordo com Miranda et al. (2001), a expressividade das palmeiras justifica-se pela plasticidade adaptativa a diversos tipos de habitas. A maioria das espécies de palmeiras prosperou em regiões de clima quente e úmido, mas algumas espécies habitam locais de clima árido ou com temperatura baixa (BONDAR, 1964), ou seja, indivíduos dessa família são encontrados nas mais diversas fisionomias, como florestas ombrófilas densas, florestas estacionais semideciduais com influência fluvial permanente ou não, cerradão ou cerrado *strictu sensu*.

As palmeiras possuem características morfológicas únicas, cujas dimensões variam enormemente (TOMLINSON, 2006). Exemplo disso pode ver observado nos frutos dessa família que variam de milímetros, a quase um metro (*Lodoicea maldivica* (J.F.Gmel.) Pers.). Essa grande variação morfológica também é observada na altura, hábito e diâmetro de indivíduos da família Arecaceae (BOTÂNICO, 2004). Essas variáveis observadas na morfologia externa também são encontradas na estrutura interna, o que torna evidente a importância dos estudos anatômicos, na identificação de novas características que permitem avaliar as suas inter-relações e mudanças evolutivas e classificação das palmeiras (UHL & DRANSFIELD, 1987).

A família Arecaceae está entre os grupos de plantas mais importante da região Amazônica por estar diretamente relacionado a subsistência da população local (PASSOS & MENDONÇA, 2006).

As palmeiras apresentam um grande número de espécies que contribuem no cotidiano das comunidades, de grande importância econômica, e que são exploradas comercialmente

para a produção de ceras, óleos, cremes, fibras, palmito e amido (SANTELLI et al., 2006; TOMLINSON, 1979). *Bactris gasipaes* Kunth, conhecida como pupunha, tem fruto apreciado pelas populações da Amazônia Ocidental e no sul da América Central e se tornou objeto de estudo de vários pesquisadores (CLEMENT, 1988; CLEMENT & ARKCOLLl, 1991; CLEMENT et al., 1993; YUYAMA & SILVA, 2003). *Euterpe precatória* Mart. conhecida popularmente como açaí-solitário apresenta mesocarpo comestível e é a partir do mesmo que se produz o vinho muito apreciado na região e conhecido em todo o Brasil (Castro, 1992). *Astrocaryum aculeatum* Meyer possui mesocarpo comestível, utilizado na culinária como ingrediente do popular lanche caboclinho, com grande potencial econômico e sabor inigualável (YUYAMA et al., 2008).

Algumas palmeiras têm o seu potencial medicinal conhecido popularmente, tais como *Acrocomia aculeata* (Jacq.) Lodd. ex Mart., *Allagoptera* cf. *leucocalix* (Drude) O. Kuntze e *Cocos nucifera* L. (AMOROSO, 2002).

A beleza e exuberância das folhas das espécies como *Syagrus romanzoffiana* (Cham.) Glassman e *Syagrus oleracea* (Mart.) Becc. de porte arbóreo e *Allagoptera campestris* (Mart.) Kuntze de porte herbáceo, são apreciadas por muitos e usadas na ornamentação de ambientes como pátios, parques, praças e avenidas (LORENZI, 2004; GENOVEZ, 2007; THUM & COSTA, 1999).

Pode-se dizer que os gêneros da família Arecaceae são endêmicos aos Continentes Americano, Africano ou Asiático, com algumas exceções, como a espécie *Cocos nucifera* L., que é largamente distribuída nos trópicos, mas tem sua provável origem no Pacífico Oeste (HARRIES, 1978).

Dentre as cinco subfamílias, Arecoideae, apresenta gêneros com uma ampla distribuição na América do Sul, com exceção apenas na porção sul do continente americano (STEVENS, 2008). No Brasil, ocorrem em todo o território nacional (HENDERSON et al., 1995).

O gênero *Syagrus* é composto por 31 espécies (DRANSFIELD et al., 2008), distribuídas na América do Sul. Presente no oeste da Colômbia, Guiana Francesa, sul do Uruguai, norte da Argentina, com 11 espécies endêmicas das Antilhas e Brasil, país com maior número de espécies (HENDERSON, 1995; HENDERSON et al., 1995). Ocorrem em quase todas as fisionomias vegetais brasileiras, mas são típicas de vegetação de cerrado, caatinga e solos com afloramentos rochosos, sendo a região centro-oeste sua área de concentração (HENDERSON & SCARIOT, 1993; HENDERSON et al., 1995;

DRANSFIELD et al., 2008; LEITMAN ET AL., 2012). Para o Bioma Amazônico foram relatadas seis espécies, sendo elas: *Syagrus cocoides* Mart., *S. inajai* (Spruce) Becc., *S. orinocensis* (Spruce) Burret., *S. sancona* (Kunth) H.Karst., *S. smithii* (H.E.Moore) Glassman, *S. stratincola* Wess. Boer (HENDERSON, 1995, HENDERSON et al., 1995).

Syagrus inajai (Spruce) Becc. (pupunharana) é uma palmeira nativa do Brasil, com domínio fitogeográfico na região Amazônica (LEITMAN et al., 2012), ocorrendo nos estados do Amapá, Amazonas, Maranhão e Pará, nas florestas tropicais úmidas, em áreas com floresta de platô, vertente e baixio, mas que também pode ser observada em áreas de floresta com indícios de antropização com dossel descontínuo e nas margens de estradas e capoeiras (HENDERSON & SCARIOT, 1993; HENDERSON, 1995; RIBEIRO et al., 1999; MIRANDA & RABELO, 2006). O fruto é caracterizado pela presença de endocarpo rígido e por três poros evidentes (DRANSFIELD et al., 2008) sendo conhecido popularmente como coquinho e utilizado pelas comunidades locais. Sua polpa e semente são consumidas *in natura*, o endocarpo usado em peças de artesanato e as folhas usadas na cobertura de moradias (HENDERSON et al., 1995; MIRANDA & RABELO, 2006). Mesmo sendo conhecida na sua região de ocorrência, os seus recursos são utilizados em pequena escala, ficando aquém de suas potencialidades.

Para o melhor uso, comercialização e exportação das espécies de palmeiras é necessário que ocorra a adequação às normas de mercado, o que depende de inúmeras pesquisas, estudos aprofundados nos vários ramos da Biologia, como: Ecologia, Morfologia, Anatomia, Fisiologia e Fitoquímica.

O ciclo de vida de um indivíduo consiste de uma série de estágios morfológicos perceptíveis, que se sucedem até a morte, caracterizados pela aquisição ou perda de estruturas e propriedades, além das mudanças anatômicas, fisiológicas e bioquímicas (GATSUK et al., 1980). O entendimento dos mecanismos que regem a formação e o desenvolvimento de um organismo só é possível por meio de investigações minuciosas da estrutura funcional, ou seja, através do estudo das estruturas morfológicas e anatômicas de cada órgão (ARAÚJO, 2005).

Entende-se como ontogenia a sequência de fases do desenvolvimento de um indivíduo (PORTELA & SANTOS, 2011).

Um estudo de ontogenia descreve o desenvolvimento de um organismo, definindo a sua formação. Estudos com essa abordagem possibilitam a compreensão da origem de estruturas, tecidos e órgãos, o que auxilia na percepção dos aspectos evolutivos. O estudo sequenciado, dos estágios de desenvolvimento de um individuo permite evidenciar a história

de vida de uma população, manifestada no padrão de crescimento, diferenciação, acúmulo de reservas e reprodução de seus indivíduos (BEGON et al., 1996). Portanto, para uma melhor compreensão do processo de formação, estabelecimento e propagação das palmeiras faz-se necessário a realização de estudos sobre os órgãos reprodutivos, flores, frutos e as sementes das espécies que compõem essa família.

As palmeiras, com poucas exceções são propagadas por meio de sementes, que apresentam germinação lenta e desigual, influenciada por vários fatores, como estádio de maturação, presença ou não de pericarpo, tempo entre colheita e semeadura, dormência física, temperatura do ambiente e substrato (MEEROW, 2004; NEGREIROS & PEREZ, 2004; GENTIL & FERREIRA, 2005; ELIAS et al., 2006; FERREIRA & GENTIL, 2006; PIVETTA et al., 2008).

De acordo com Orozco-Segovia (2003), das espécies de palmeiras conhecidas, poucas possuem estudo sobre a semente e seu processo germinativo. A maioria das pesquisas tem sido realizada com espécies de valor comercial (DEMASON, 1985; MARTINS et al., 1996; MENDONÇA & ARAÚJO, 1999; ARAÚJO et al., 2000; AGUIAR & MENDONÇA, 2002; AGUIAR & MENDONÇA, 2003; PANZA et al. 2004; ZONA, 2004; NEGREIROS & Perez, 2004; FERREIRA & GENTIL, 2006; LUZ et al., 2008; PIVETTA, et al. 2008; MENDONÇA et al., 2008; MENDONÇA & BIANCO, 2009; OLIVERIA et al. 2010; RIBEIRO et al., 2011).

A família Arecaceae apresenta algumas particularidades no seu processo germinativo, e sua classificação varia de acordo com os autores (MARTIUS, 1823-1850; TOMLINSON, 1960; PINHEIRO, 2002; MERROW, 2004; HENDERSON, 2006; DRANSFIELD et al., 2008). Os mecanismos de germinação e dormência são mal compreendidos para a maioria das espécies de palmeiras (OROZCO-SEGOVIA et al. 2003). Henderson (2006) observando a imprecisão na descrição dos processos germinativos baseado em poucos caracteres, constatou que só poderiam ser bem compreendidos, através de uma associação detalhada de características morfológicas e anatômicas.

De acordo com Natesh & Rau (1984), há poucos estudos sobre todos os aspectos da embriogênese, da formação do zigoto e da organização dos meristemas. Apesar dos esforços realizados até a presente data, de acordo com Ribeiro et al. (2011), não há uma descrição detalhada do eixo embrionário do embrião de palmeiras.

A carência de pesquisas sobre ontogenia das palmeiras se deve em parte as suas características morfoanatomicas como a dureza do endocarpo e a grande quantidade de fibras

presente no mesocarpo (MERROW, 2004; DRANSFIELD et al., 2008); a dificuldade em descrever o desenvolvimento do endosperma que permanece líquido durante grande parte ou até o fim do desenvolvimento da semente, além do tempo demasiadamente longo do desenvolvimento do fruto, que em várias espécies excede 200 dias (MURRAY 1973; BENASSI et al., 2007; REIS et al. 2012; SILVA et al., 2013).

Portanto, o estudo da ontogenia dos órgãos reprodutivos e da plântula de *S. inajai*, permitirá a compreensão das etapas da formação do gineceu, do pericarpo, da semente e do embrião, bem como, o processo germinativo, fornecendo informações que irão aprimorar o conhecimento das palmeiras e demais *taxa*.

O presente trabalho foi dividido em quatro capítulos, em formato de artigos, com objetivos específicos a cada um. No primeiro capítulo, fez-se a caracterização morfoanatomia da inflorescência de *S. inajai*. No segundo capítulo, estudou-se a morfoanatomia do desenvolvimento do gineceu ao fruto maduro de *S. inajai*, mensurando o tempo médio dos eventos. No terceiro capítulo, estudou-se o desenvolvimento do embrião de *S. inajai.* No quarto capítulo, estudou-se a morfoanatomia do processo germinativo de *S. inajai*, determinando o tempo médio de sua duração, identificando as substâncias ergásticas presentes no embrião, haustório e endosperma, bem como realizando a análise química da semente.

OBJETIVOS

Objetivo geral

Realizar o estudo ontogênético dos órgãos reprodutivos e da plântula da palmeira *Syagrus inajai*.

Objetivos específicos

Caracterizar morfoanatomicamente a inflorescência de *S. inajai*.

Realizar o estudo morfoanatômico do desenvolvimento, do gineceu ao fruto maduro de *Syagrus inajai*, mensurando o tempo médio dos eventos.

Descrever o desenvolvimento embrionário de *S. inajai.*

Realizar o estudo morfoanatômico do processo germinativo de *S. inajai*, determinando o tempo médio de sua duração, identificando as substâncias ergásticas presentes no embrião, haustório e endosperma, bem como realizando a análise química da semente.

Capítulo 1

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Morphoanatomy of the flower of *Syagrus inajai* (Spruce) Becc. (Arecaceae- Arecoideae-Attaleinae), Amazon

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Short title: Morphoanatomy of the flower of *Syagrus inajai*

ABSTRACT

The occurrence of *Syagrus inajai* (Spruce) Becc., popularly known as "pupunha-brava", among other names, has been registered in the Guianas and in the North of Brazil in areas of "terra firme" (non-flooding) and "baixio" (gallery forests). In order to characterize the inflorescence and further knowledge of this family, a morphoanatomical study was carried out of the palm *S. inajai* in a green area of the Campus of the Federal University of Amazonas - UFAM, Manaus, Amazonas. The inflorescences are branched to one order, pedunculate, and interfoliar, measuring 62-82 cm in length, with woody bracts with longitudinal grooves on the external surface, and flowers in triads. The number of flowers to each inflorescence varies from 5,904 to 17,316 for staminate flowers, and from 180 to 3,528 for pistillate flowers. Staminate flowers with six stamens and one vascular bundle each; three-lobed pistillodium, vascularized pistillodium. Its pistillate flowers have six staminodia joined to form a circle, syncarpic, tricarpellary, trilocular gynoecium, one ovule to each locule, synascidiate in the ovary, and plicated above. Tripartite stigma, apical and sessile, with epidermis composed of elongated papillary cells, pattern of epidermis that is maintained throughout the stylar canal. Bitegmented, anatrope, pachychalazal ovule.

Keywords: *Syagrus*, anatomy, flowers of Palmae, Cocoseae tribe, pupunharana

RESUMO

Syagrus inajai (Spruce) Becc., popularmente conhecida como pupunha-brava entre outras denominações, teve sua ocorrência registrada para Guianas e no norte do Brasil em áreas de floresta de terra firme e floresta de galeria. Com o intuito de caracterizar a inflorescência ampliando o conhecimento a respeito da família foi realizado o estudo morfoanatômico da palmeira *S. inajai,* em área verde do *Campus* da Universidade Federal do Amazonas - UFAM, Manaus, Amazonas. A inflorescência é ramificada em primeira ordem, pedunculada, interfoliar com 62-82 cm de comprimento, bráctea lenhosa com fissuras longitudinais na superfície externa e flores em tríades. O número de flores por inflorescência varia entre 5.904 - 17.316 para flores estaminadas e 180 - 3.528 para as flores pistiladas. Flores estaminadas com seis estames e um feixe vascular cada; pistilódio trífido e vascularizado. Flores pistiladas com seis estaminódios unidos formando um círculo. Gineceu sincárpico, tricarpelar, trilocular, um óvulo por lóculo, sin-ascidiado no ovário e plicado acima. Estigma tripartido, apical e séssil, com epiderme composta por células papilosas alongadas, padrão de epiderme que se mantem por todo canal estilar. Óvulo é anátropo, paquicalazal e bitegumentado.

Palavras- chave: *Syagrus,* anatomy, flores de Palmae, tribo Cocoseae, pupunharana.

1. INTRODUCTION

The genus *Syagrus* is composed of 31 species (Dransfield et al., 2008), distributed in South America, western Columbia, French Guiana, southern Uruguay, and northern Argentina. Eleven of the species are endemic to the Antilles, and Brazil, the country with the highest number of species (Henderson and Scariot, 1993; Henderson, 1995; Henderson et al., 1995). They grow predominantly in cerrado and caatinga vegetations, and soils with rocky outcrops (Henderson et al., 1995; Dransfield et al., 2008), but the occurrence of six species has also been reported for the Amazonian Biome, namely: *Syagrus cocoides* Mart., *Syagrus inajai* (Spruce) Becc., *Syagrus orinocensis* (Spruce) Burret., *Syagrus sancona* (Kunth) H. Karst., *Syagrus smithii* (H.E.Moore) Glassman, and *Syagrus stratincola* Wess. Boer (Henderson, 1995, Henderson et al., 1995).

S. inajai is a palm that is commonly found in gallery and "terra firme" forest, with many individuals in open areas where there are signs of anthropization. It is a monoic species with eye-catching inflorescences, and up to 35 rachillae (Henderson and Scariot, 1993; Henderson et al., 1995).

Syagrus like the other genera of the Cocoseae tribe, is characterized by inflorescences with large peduncular, persistent bracts, and fruits with a hard endocarp and three pores (Dransfield et al., 2008), reproductive characters that are used to distinguish taxon.

In the Arecaceae family as a whole, the evolution of floral characters has been observed, including progression from bisexual to unisexual flowers, and from monoic to dioic species (Moore and Uhl, 1982; Daher et al., 2010). Morphoanatomical study of the flower is of considerable value for delimiting monophyletic groups, and provides a better understanding of the mechanisms of speciation and co-evolution of the phylogenetic relations (Moore and Uhl, 1982; Askgaard et al., 2008). It also enables observation of structures linked to pollination and fertilization, as well as providing data to support works in Ecology and Physiology and other related areas.

With just a few exceptions, palms are propagated by seeds, which present slow, unequal germination, influenced by various factors such as state of maturation, presence or absence of a pericarpium, time between harvesting and planting, physical dormancy, environmental temperature, and substrate (Meerow, 1991; Salm, 2005; Pivetta et al., 2008). Given that the seed is the main means of propagation and establishment of palm species, it is essential to understand the morphology and anatomy of the reproductive organs of the plant.

The morphoanatomical characterization of a certain flower enables us to observe the tissues that comprise it, and that comprise the formation of the fruit and the seed.

Therefore, morphoanatomical characterization of the inflorescence of *S. inajai* will lead to a better understanding of the processes of formation of the fruit and seed, providing information that will serve as the basis for ontogenetic and ecological studies, and studies of the Floral Biology and Taxonomy.

2. MATERIAL AND METHODS

The botanical material was collected in 2009 and 2010 in a green area of the Campus of the Federal University of Amazonas - UFAM, Manaus, Amazonas, Brazil, in an area of "baixio" (gallery forest), close to coordinates 03 ^{o} 05' 45,84" S and 59 ^{o} 58' 43,69", a climate characterized as being of the Afi type (Köppen, 1931). The material was analyzed at the Agroforestry Botanical Laboratory - LABAF, of the same University. For the morphological and anatomical description, ten adult individuals of *S. inajai* were selected, from which the characterized inflorescences were removed for field and laboratory observation, according to Henderson et al. (1995) and Dransfield et al. (2008).

The flowers were measured (length and width) and quantified, estimating the average number per inflorescence and per rachilla and their positions on the rachis. Five basal, five middle and five apical rachillae were counted. Subsequently, Tukey's test was applied with alpha of 5% to calculate the level of significance.

For the anatomical study, the material collected (rachillae with staminate and pistillate flowers) was fixed in FAA 70% (Kraus and Arduin, 1997). The samples were dehydrated in ethyl series (70%- 95%), embedded in 2-hydroxyethyl-methacrylate (Historesin® Leica, prepared according to the manufacturer's instructions), sectioned at thicknesses of 4 to 7 µm in a rotary microtome, and stained with 0.5% toluidine blue in citrate buffer, pH 4.0 (O'Brien et al., 1964), and the slides were mounted in water. The semi-permanent slides were prepared from sections cut freehand using razor blades, cleared, stained with safranin and astra blue in aqueous solution (Bukatsch 1972) and mounted in glycerinated gelatin (Dop and Gautié, 1928).

For the registration of the material in a Scanning Electron Microscope (SEM), the botanical material was fixed in BNF (Buffered Neutral Formalin, Lillie, 1965) solution, dehydrated in ethyl series, and dried by the critical point method with CO_2 on a Balzers model CPD 030. The samples were collected in a metal support, covered with gold (Balzers SCD

050) and observed under a JEOL JSM 5800LV (10 kV) Scanning Electron Microscope, at the Institute of Biology/Unicamp.

Histochemical tests were performed, seeking to observe some ergastric substances, using the following dyes and reagents: Lugol to detect starch (Jensen, 1962), Ruthenium Red to identify various polysaccharides and pectins (Johansen, 1940); Xilidine Ponceau (O' Brien and McCully, 1981) to detect protein reserves; Sudan IV (Brundett et al., 1991) to detect lipophilic substances, and Ferric trichloride to determine the occurrence of general phenolic compounds (Johansen, 1940).

The results were documented in a Zeiss photo microscope, Primo Star model, with Canon photographic camera (Power Shot A650 IS). Indian ink drawings were made of the general morphological appearance and coloration of the inflorescence, characterized according to the table of Kornerup and Wanscher (1961).

3. RESULTS

The individuals of *S. inajai* (Figure 1) flower all year round, with one to three inflorescences per individual. But flowering is higher from February to June, outside the dry period.

It is a monoic palm, with staminate and pistillate flowers in the same inflorescence. However, we observed ten entirely male inflorescences, a finding not previously reported for the species.

The peduncular bract is woody, 57 to 88 cm in length. It surrounds the inflorescence during part of its development, a period lasting for 50 to 60 days after its emergence (Figure 1a-b). The external surface is waxy bronze-brown, with lanugo at the base and longitudinal grooves; the internal surface is glabrous and golden, but after opening, it oxidizes, changing in color.

The inflorescence is interfoliar, pedunculate, branched to one order, and pale yellow, with a strong, sweet odor, 62-82 cm in length (Figure 1a-b). The species is protandric. The staminate flowers open simultaneously with the peduncular bract, remaining in flower for one to two days after anthesis, thereby avoiding self-pollination. The peduncle is pale orange, cylindrical, lanuginous, and grows to 18-25 cm in length. The rachis is cream-colored and lanuginous, measuring 17-28 cm in length. The rachilla are cream-colored, with significant variations in length from the base to the apex (Table 1), ranging from 18 to 36 in number.

The flowers are arranged in cincinni forming base triads up to the middle of the rachilla. The pistillate flower is flanked by staminate flowers (Figure 1c, e), On the apex of the rachilla the staminate flowers are arranged in dyads or acervulae (Figure 1d, f).

Considering the minimum and maximum number of staminate and pistillate flowers per rachilla, and the minimum and maximum number of rachilla per inflorescence, it is inferred that the number of flowers per inflorescence varies between 5,904-17,316 and 180-3,528, respectively (Table 3).

Staminate flower

The staminate flowers have a uniform cream-yellow coloration or wine-colored base. They are elliptical, irregular, asymmetrical in shape, and sessile. The sepals are connate at the base, with three triangular apical lobes, acuminate apex, indented edges, glabrous (Figures 1f, j-m and 2a). There are three valvar, free, lanceolate, asymmetrical petals with non-indented margin, thick apex and acuminate (Figure 1f, j).

There are six free stamen, with wider filaments at the base; the anthers have asymmetric tips and are dorsifixed, with longitudinal dehiscence, and introrse (Figure 1l-m). Short, three-lobed, central pistillodium (Figure 2b).

The flowers are 6.81 to 11.44 mm in length and 2.41 to 5.24 mm in width, and are located in the apical rachillae of significantly shorter length (Table 2). The number of flowers per rachilla was between 328 and 481 (Table 3).

The sepal and petal have a thickened cuticle in the epidermis on the adaxial side, in the external periclinal and anticlinal walls (Figure 3c) and on the abaxial surface, only in the external periclinal wall (Figure 3d). They are richly vascularized, with vascular bundles surrounded totally in the sepals and partially in the petals by a sclerenchyma sheath (Figure 3a-b respectively). In the petals, the sclerenchyma cells form a calotte on the adaxial side of the vascular bundle, arranged throughout the extension of the mesophyll (Figure 3b).

Each stamen contains one vascular bundle. The anther is formed by a unistratified epidermis, and the endothecium is composed of cells, with elongated periclinal cells and irregular wall thickness (Figure 3e-f). Between each theca there are two to four layers of thin stroma cells, which break down in the anthesis, forming the longitudinal stomium, an opening through which pollen grains are released (Figure 3f). The pollen grain has a reticulate surface and cell wall of pectic nature (Figures 2c, 4c), and contains starch and protein (Figure 4a-b, respectively). The tissue of the connective is endothecium-like (Figure 3e). The filament has an epidermis with thickened external periclinal and anticlinal walls and a vascular bundle

surrounded by idioblasts with phenolic compounds (Figures 3g, 4d). The pistilodium (Figure 2b) has three vascular bundles (Figure 3i). In cross-section, vascular bundles arranged in three levels can be seen in the receptaculum: approximately 46 peripheral vascular bundles with sclerenchyma sheath, surrounding the sepals and petals (Figure 3h); three central vascular bundles that will form the pistilodium, and six intermediary vascular bundles surrounding the anthers (Figure 3i).

Pistillate flower

The pistillate flowers are cream in color, pyramidal, asymmetrical and trimerous, 4.92 to 7.24 mm in length and 4.44 to 6.09 mm in width (Table 2). The sepals are overlapping, free, pyramidal with thin margins, tomentose, and tomentose-floccose in the depressions (Figures 1g and 5a). The petals are overlapping, free, pyramidal with irregular edges, rounded bases, acute apex, thick, floccose-tomentose in the depressions, and longer than the sepals (Figures 1g-h and 5a). The stigma is tripartite, regular and apical (Figures 1h-i and 5b). The number of pistillate flowers per rachilla is 10 to 98. However, in terms the average number of flowers per rachilla in relation to their position on the rachis, there is no significant difference (Table 3). The ovary is superior, syncarpic, tricarpellary and trilocular, with one ovule to each locule and basal lateral placentation (Figure 6a). The staminods are connate a circle, and ring membranous, with six vestigial stamens (Figure 1i).

The sepal and petal present an epidermis covered by cuticle on both sides, thicker on the adaxial side; the mesophyll consists of vascular bundles surrounded by a sclerenchyma sheath, and present groups of sclerenchyma cells close to the abaxial surface, in high quantities in the sepals (Figure 7a-b).

The staminoid circle has a uniseriate epidermis, thick cuticle on the external periclincal wall, parenchyma mesophyll with a high number of idioblasts throughout its extension, and vascular bundles (Figure 7c).

The gynoecium has three distinct regions (Figure 5c): an apical region, with the stigma, characterized by epidermal cells with ornamented cuticle; a middle region with a short stylet whose epidermal cells have a high number of stomata and a basal region of the ovary, characterized by the presence of a stamen ring with thin cuticle cells, stomata and multicellular squamiform tricomas (Figure 5d-g).

The ovary is formed by three carpels linked at the base, synascidiate, but above this region the bond is not complete, constituting a single, synplicate stylar canal (Figure 6a-d).

The stigma contains three vascular bundles (Figure 6b). The inner epidermis of the stigma in continuity with the stylar canal, as far as the inner epidermis of the ovary, consists of elongated papillary cells (Figures 5b-c and 6b, f). In cross-section, the stylar canal is triradiate, with folds, and smaller invaginations entering the mesophyll, like labyrinths (Figure 6a, c, e).

The septa located between the carpels are formed by the incomplete union of the carpel flanks. However, this fusion is incomplete, forming cavities, one per septum, arranged radially from the base of the ovary to the base of the stigma, with a secretory epidermis comprised of columnar cells, and a single external opening at the base of the stigma (Figures 5c and 6c-d, g). The inner epidermis of the ovary is comprised of secretory cells, forming the obturator (Figure 7f).

The ovule is anatrope (Figure 6a) and bitegumented; the external tegument is thicker and more extensive than the inner tegument, covering it and extending beyond it, restricted to the region of the micropyle (Figure 7d, g-h). In the region of the micropyle, cells of the two teguments and a layer of nucellus with elongated cells present phenolic compounds forming the epistase (Figure 7e, g-h). In the opposite position, it is possible to see the hypostasis formed by various layers of cells with phenolic compounds (Figure 7d, g). The vascular bundle reaches the chalaza and then branches to form the pachychalaza (Figure 7g). The nucellus has a single layer of cells, terminating at the middle region of the embryo sac (Figure 7e).

4. DISCUSSION

According to Wright and Van Schaik (1994), in tropical rainforests, water is not a limiting factor and the more prolific production of flowers is related to the greater sunlight in periods of lower rainfall and higher temperatures, which was not observed in the collection sites of in individuals of *S. inajai*, fact that may be related to the size of the tree species in that there is no light restriction, since they occupy the upper strata of the forest canopy. The same is observed with *Mauritia flexuosa* L.f., which flowers from February to August (Storti, 1993).

Henderson and Scariot (1993) describe, for individuals of *S. inajai*, inflorescencis with up to 35 rachillae. In the present work, the number of rachillae varies from 18 to 36.

The arrangement of the flowers in triads characterizes the subfamily Arecoideae (Dransfield et al., 2008). This arrangement is considered a possible synamorphy for Arecoideae, lost in some *taxa*, such as *Chamaedorea,* a genus of dioic palms with solitary

flowers, or in acervulae (Askgaard et al., 2008; Dransfield et al., 2008). Asmussen et al. (2006) suggest that the origin of the triad arrangement comes from two basal staminate and one distal pistillate flower. The anatomical evidence suggests that the solitary flowers derive from a unit of the original acervulum that has suffered abortion of the distal flowers (Dransfield et al., 2008). In *S. inajai*, besides the standard triad arrangement, entirely male inflorescences were also observed. Bacelar-Lima et al., (2006) observed only staminate flowers in the apical rachillae of four of the seven inflorescences of *Astrocaryum aculeatum* studied, which is a new finding for this species. Field research enabled other patterns to be observed, often new and unlike those already reported.

It is presumed that the unisexual flowers and sexual dimorphism represent derived conditions in palms (Moore and Uhl, 1982; Tomlinson, 1990; Adam et al., 2007). In *S. inajai*, the pistillodium and the staminoidal ring of the staminate and pistillate flowers, respectively are vestigial organs. Daher et al. (2010), studying the transition from bisexual to unisexual flowers in *Phoenix dactylifera* L., observed that the non-development of the residual organs is due to paralyzation of the cell activity, and not to cell death in the tissues of these organs.

Palms belonging to the Subfamily Arecoideae present inflorescences surrounded by a penduncular bract that protects the flower during part of its development (Tomlinson, 1990; Dransfield et al., 2008). The opening of the peduncular bract in *S. inajai* occurred within 50 to 60 days, after its emergence. A study with *Astrocaryum aculeatum* (Barcelar-Lima et al., 2006) observed that the opening of the peduncular bract can take 30 to 45 days after its emergence. For *Mauritia flexuosa* this period was approximately 60 days (Storti, 1993).

The presence of protandria, in the species studied, makes self-pollination impossible. The same characteristic was observed in *Euterpe precatoria* (Kuchmeister et al., 1997), *Elaeis guineenses* (Adam et al., 2007), *Normanbya normanbyi* (W.Hill) L.H. Bailey (Inkrot et al., 2007) and *Licuala peltata* Roxb. ex Buch.-Ham. (Stauffer et al,. 2009). In *Geonoma irena* Borchs. and *Geonoma cuneata* var. s*odiroi* (Dammer ex Burret) A.J. Hend., there was overlapping of the male and female phases (Borchsenius, 1996).

In both staminate and pistillate flowers, the sepals and petals are richly vascularized, forming an almost continuous layer of vascular bundles in the mesophyll, which corroborates with Stauffer et al. (2004), who observed the same characteristic in *Pelagodoxa* and *Sommieria*.

The morphological appearance of the stamens of *S. inajai*, (six in number, with wider filaments at the base; anthers with asymmetrical tips, dorsifixed, and longitudinal in dehiscence, introrse) are confirmed with the observations in species of the genera *Pelagodoxa* and *Sommieria* (Stauffer et al., 2004), *Astrocaryum* (Bacelar-Lima et al., 2006), *Licuala* (Stauffer et al., 2009).

According to Dransfield et al. (2008) the pollen grain with finely reticulated surface (Figure 2d) is characteristic of the genus *Syagrus.* The same appearance was also observed in the palms studied by Barth and Barbosa (1971). Perera et al. (2010) observed a protein reserve in the microspore of *Cocos nucifera*, and the same was observed in *S. inajai*.

Syagrus inajai presented a secretory epidermis, in the septa (septal nectary), similar to that observed in *Euterpe precatoria*, in the Subtribe Geonomeae, *Asterogyne*, *Pelagodoxa, Sommieria* and in *Cocos nucifera* and *Licuala peltata,* which the authors termed septal nectary (Kuchmeister et al., 1997; Stauffer et al., 2002; Stauffer and Endress, 2003; Stauffer et al., 2003; Stauffer et al., 2004; Guevara and Jáuregui, 2008; Stauffer et al., 2009). The septal nectary is formed by the incomplete union of the carpel flanks (Weberling, 1992; Stauffer and Endress, 2003; Stauffer et al., 2004; Rudall et al., 2011). Uhl and Moore (1977) mention that the septal nectaries have a secretory epidermis, and interact with pollinators. Based on the morphoanatomical characteristics of the septal nectaries, possible visitors are bees and small Coleoptera. Thum and Costa (1998) report that bees are the main floral visitors of *Syagrus romazoffiana* (Cham.) Glassm. Kuchmeister et al. (1997) observed, in *Euterpe precatoria*, the presence of nectar in these septal nectary, possibly functioning as an attraction for visitation by these insects, and report bees and beetles as possible pollinators.

The anatropous ovule, a characteristic not previously reported for the genus *Syagrus* had already been reported for other genera of the Tribe Cocoseae, with *Attalea* and *Cocos* (Uhl and Moore, 1971; Guevara and Jauregui, 2008). However, *Bactris* sp and *Elaeis oleifera* (Kunth) Cortes belong to the same tribe and have an orthotropous ovule. The same occurs with *Butia capitata,* which presents a third type of ovule; hemitropous. These species were described by Uhl and Moore (1971), in that period, as belonging to the subfamily Cocosoideae (Potztal, 1964), but today they are included in the Tribe Cocoseae and *Attalea*, *Butia* and *Syagrus* the Subtribe Attaleinae (Dransfield et al., 2008). In other words, it is possible to see that in the tribe Cocoseae and the subtribe Attaleinae, there are different types of ovules, which means this characteristic cannot be used to distinguish these groups.

The description of the ovule of *S. inajai* provides new information, complementing previous research carried out with the genus (Henderson, 1995; Henderson et al., 1995, Dransfield et al., 2008).

The presence of pachychalaza in the ovule of *S. inajai* is a fact not previously reported for the genus. According to Werker (1997), the pachychalaza is formed from the enlargement of the chalaza, forming a large portion of the seed involucrum. The hypostasis is located above the vascularization of the pachychalaza, and is comprised of cells containing a phenolic compound, which form a hood around the embryo sac, protecting the ovule from the action of pathogens due to its antimicrobial and antiviral properties (Swain, 1979; Von Teichman and Van Wyk, 1994).

The occurrence of pachychalaza and hypostasis is generally associated with bitegumented. Crassinucelate ovules, with large embryos, absence of endosperm or nuclear endosperm, arboreal habit, and tropical habitat (Von Teichman & Van Wyk, 1991; Carmello-Guerreiro and Sartori Paoli 2000). These characteristics corroborate with those observed in *S. inajai,* but to characterize the nucellus, study of the development of the ovule is needed, and to characterize the endosperm, study of the development of the seed, a field as yet unexplored.

The morphoanatomical characteristics of the gynoecium, such as the pachychalazal ovule, and internal tegument restricted to the micropyle region, indicates which tissues are present in the fruit and seed of *S. inajai*.

5. ACKNOWLEDGMENTS

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Table 1. Measurements for length of the rachillae inserted in the base, middle and apex of the rachis of the inflorescence, mean (Mean), maximum (Max.) and minimum (Min.), of *S. inajai* (Spruce) Becc.

Position of the rachillae on the rachis	Length of the rachilla (cm)		
	Mean	Max	Min
Base	46.2 [a]	57	34.5
Middle	43.1 [ab]	53	33.8
Apex	38.6 [b]	47.1	31.5

Letters the same for mean in the same column represent non-significant differences, Tukey's test, α=5%

Table 2. Measurements for length and width, mean (Mean), maximum (Max.) minimum (Min.) of the pistillate and staminate flowers comparing the rachillae positioned at the base, middle and apex of the rachis of *S. inajai* (Spruce) Becc.

	Length (mm)				Width (mm)			
	Mean		Max.	Min.	Mean		Max.	Min.
Pistillate Flower								
Base	6.29	a	6.96	5.68	5.31	a	5.87	4.65
Middle	6.15	a	7.03	4.92	5.27	a	6.09	4.44
Apex	6.31	a	7.24	5.17	5.18	a	6.05	4.5
Staminate Flower								
Base	9.08	ab	10.94	7.36	4.11	a	5.24	3.16
Middle	9.56	a	11.44	8.18	3.95	a	5.02	2.41
Apex	8.96	b	10.08	6.81	4.07	a	5.24	3.02

Letters the same for mean in the same column represent non-significant differences, Tukey's test, α= 5%.

Table 3. Number of staminate and pistillate flowers per rachilla, comparing rachillae positioned at the base, middle and apex of the rachis of *S. inajai* (Spruce) Becc.

	Staminate flowers/rachilla				Pistillate flowers/rachilla			
Position	Mean		Max.	Min.	Mean		Max.	Min.
Base	383.9	ab	474	328	63.9	A	98	28
Middle	400.4	a	481	340	57.7	A	80	33
Apex	368.8	b	430	332	48.7	A	91	10

Letters the same for mean in the same column represent non-significant differences, Tukey's test, α= 5%.

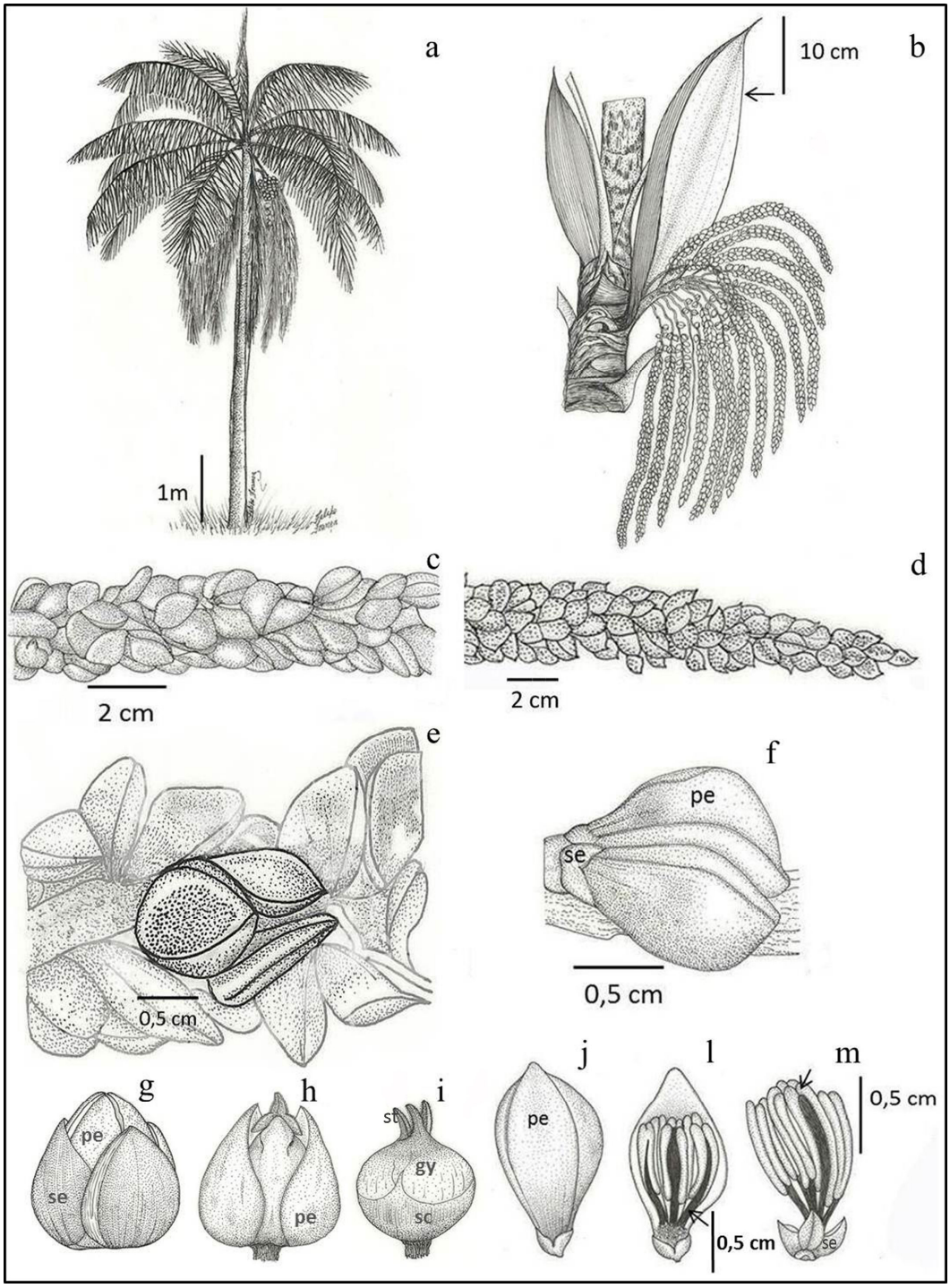
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FIGURE 1

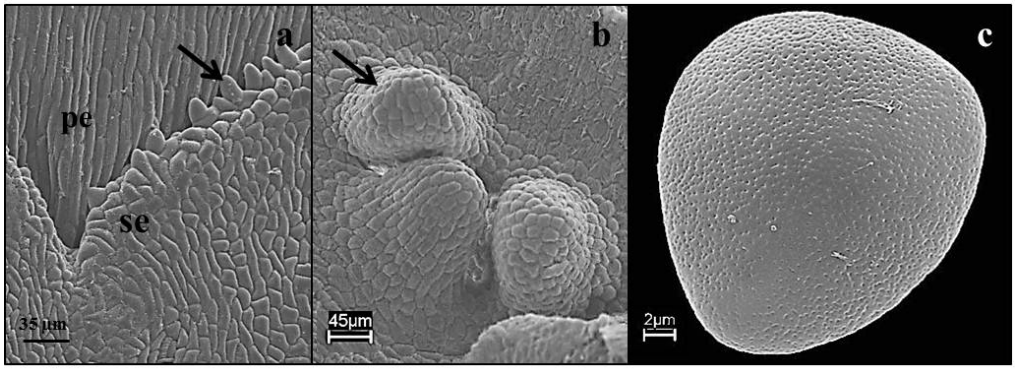

FIGURE 2

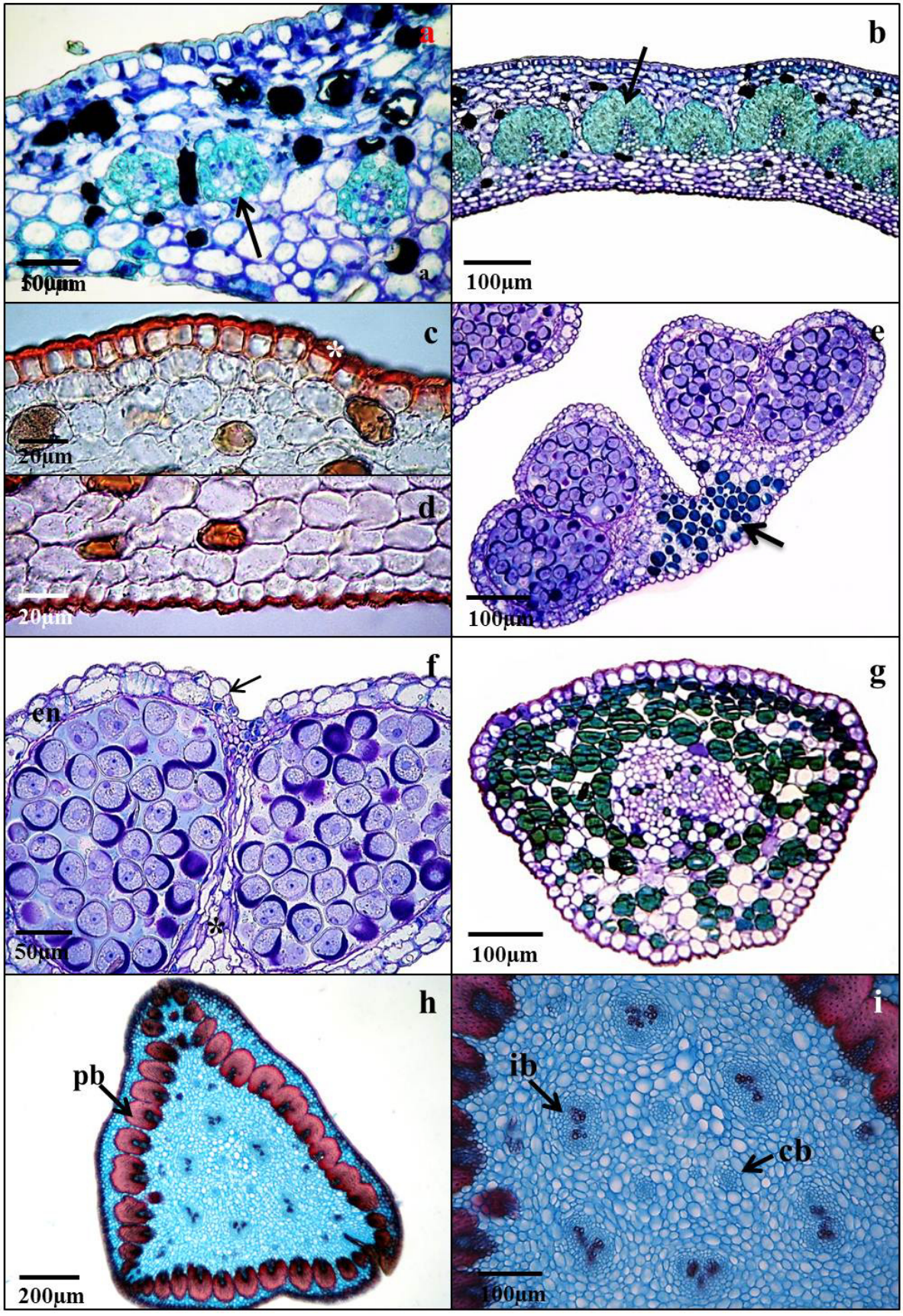
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FIGURE 3

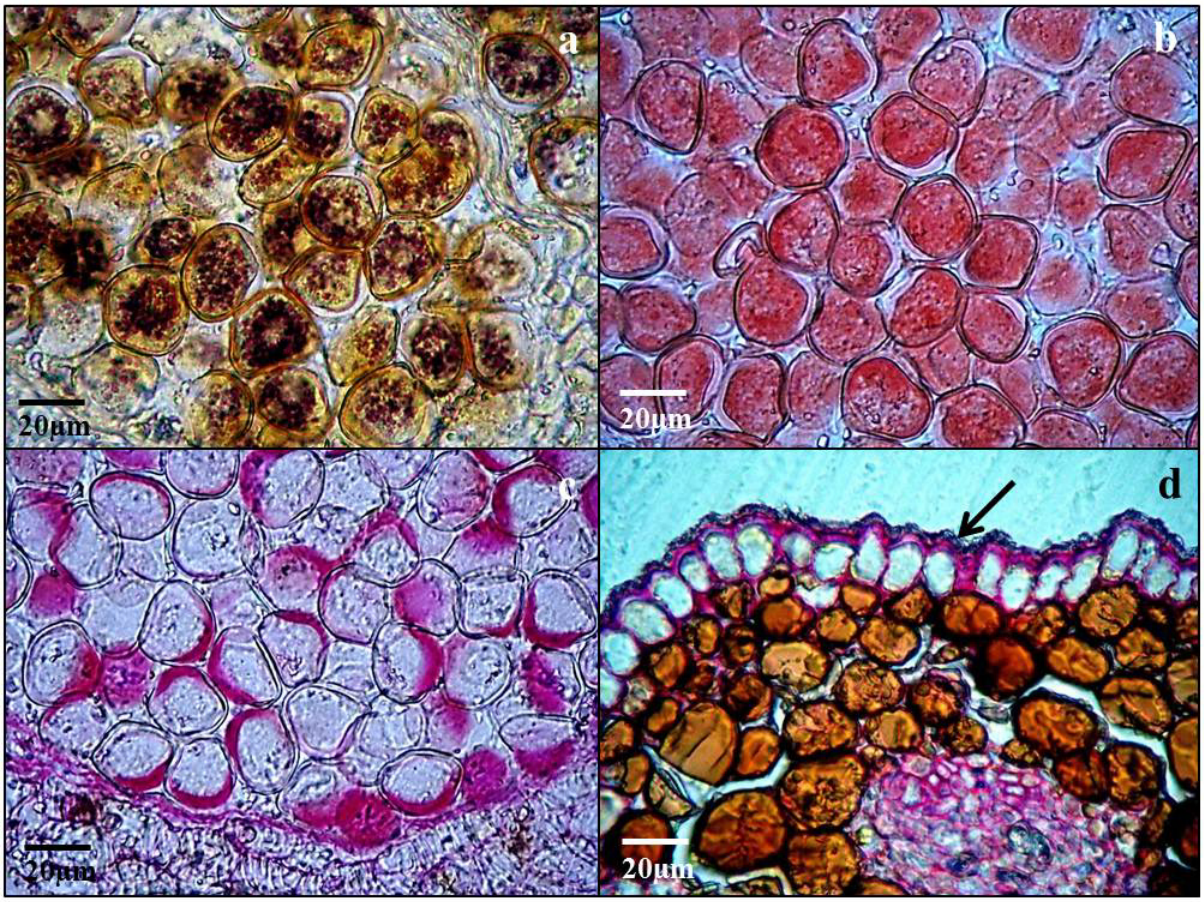

FIGURE 4

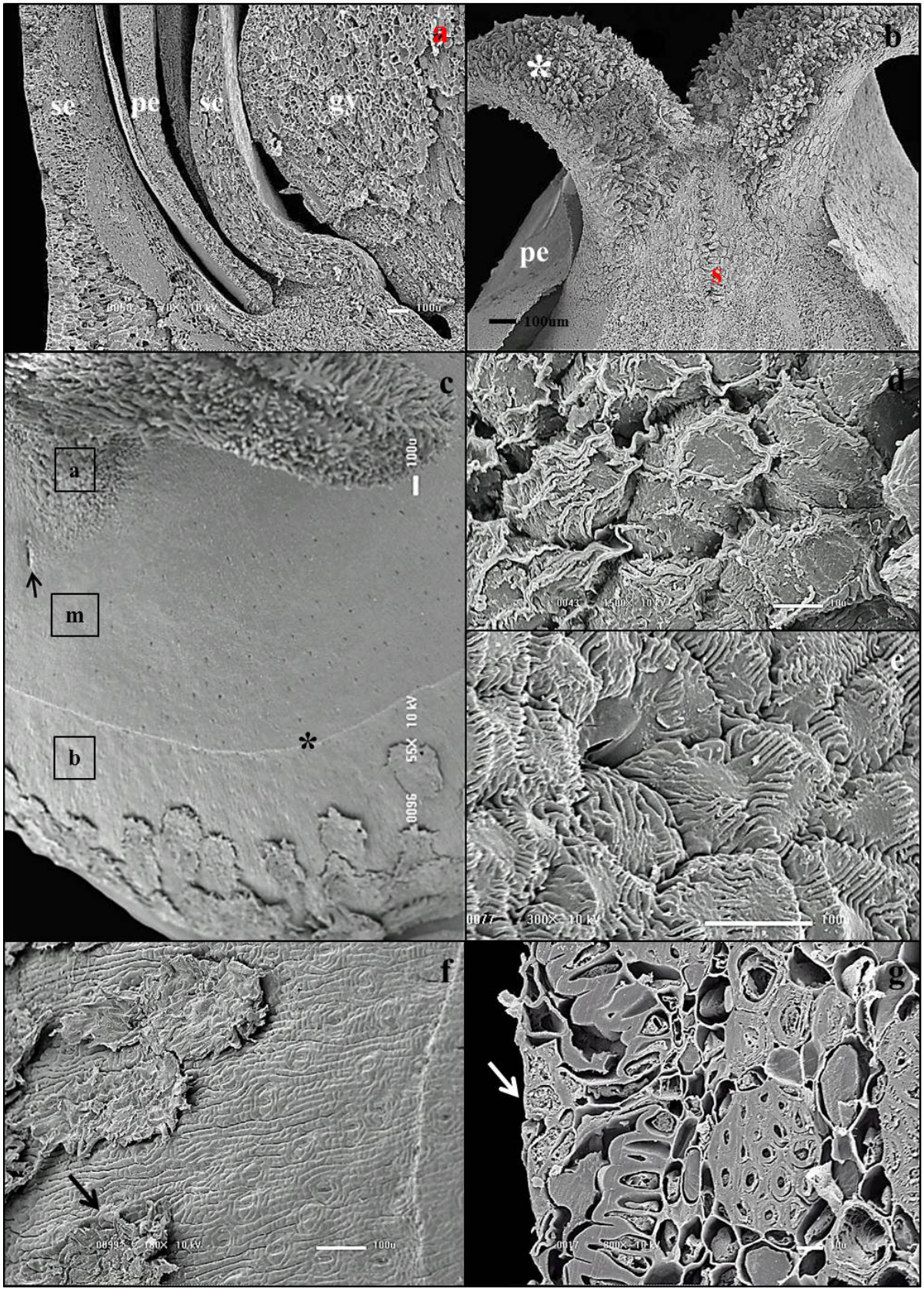

FIGURE 5

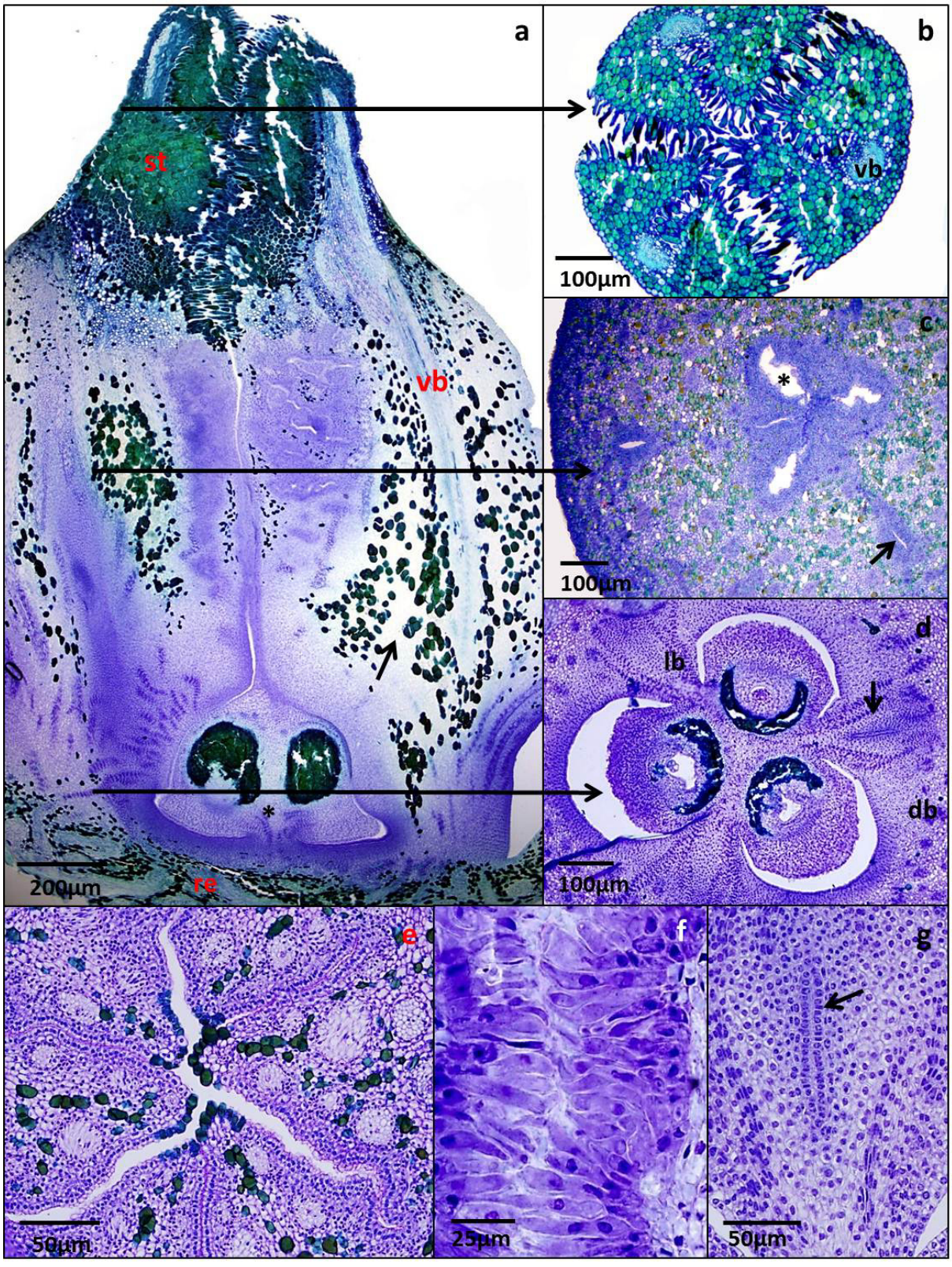
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FIGURE 6

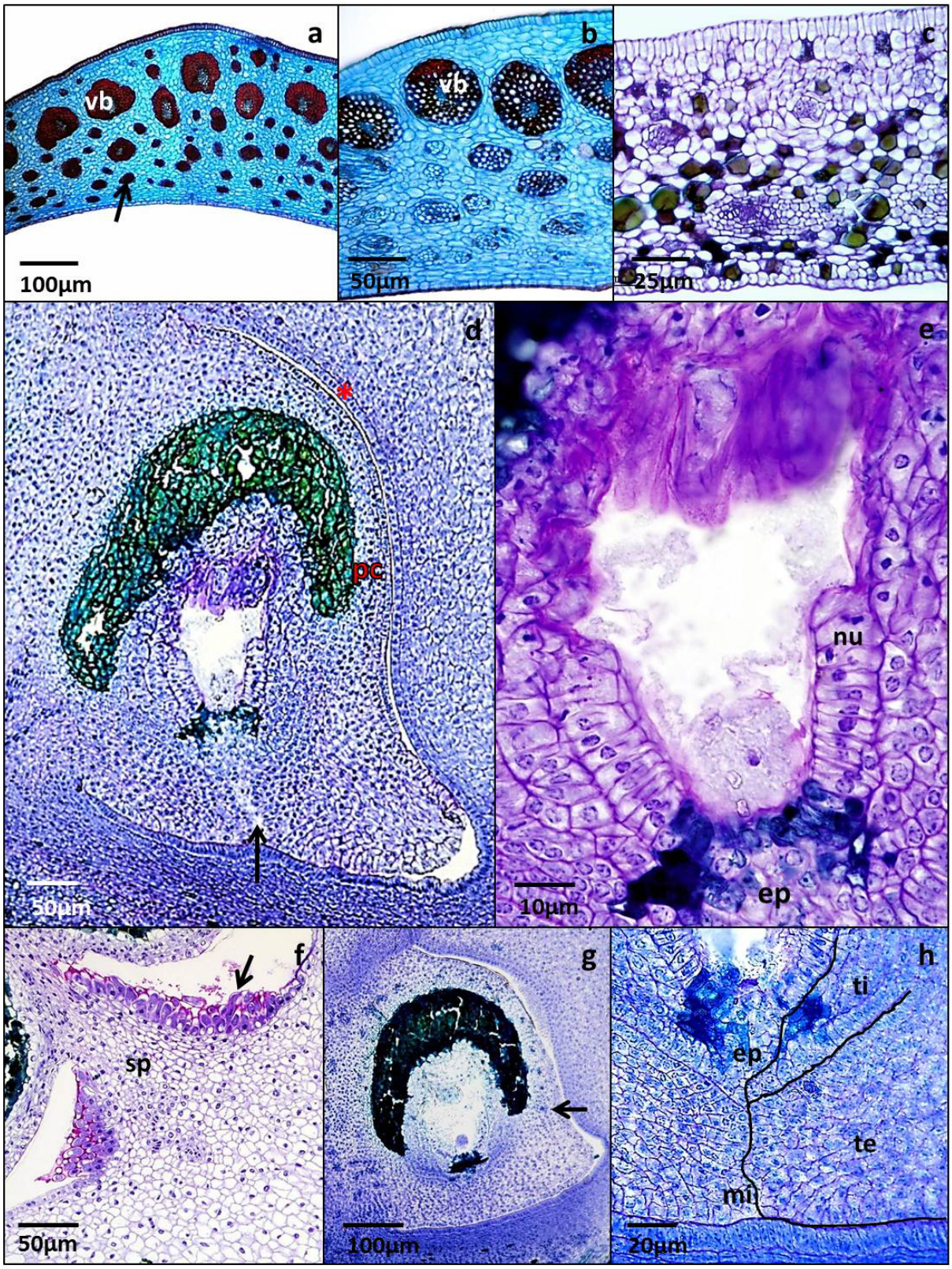
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FIGURE 7

FIGURE 1. Morphological aspects of *S. inajai*. a) General appearance of the plant; b) Inflorescence, peduncular bract (arrow); c) Base of the rachilla; d) Apex of the rachis; e) Flowers in triads; f) Staminate flowers in dyads; g) Pistillate flower; h) Pistillate flower without sepal; i) Gynoecium, detail of staminoid circle and stigma open; j) Staminate flower; l) Detail of flower, filament (arrow); and m) Arrangement of the anthers, (arrow). (gy: gynoecium, pe: petal, sc: staminoid circle, se: sepal, st: stigma).

FIGURE 2. Scanning electron micrograph of the staminate flowers of *S. inajai*. a) Indented margin of the sepal, arrow; b) Pistillodium (arrow); and c) Pollen grain. (pe: petal, se: sepal).

FIGURE 3. Photomicrograph of the staminate flower of *S. inajai*. a-i) Cross-section; a) Sepal, vascular bundle (arrow); b) Petal, vascular bundle (arrow); c) Adaxial surface of the sepal, cuticle (asterisk); d) Abaxial surface of the petal; e) Anther, connective (arrow); f) Detail of the theca, epidermis (arrow), stoma cells (asterisk); g) Filament; h) Receptaculum; and i) Region of the pistillodium. (cb: central vascular bundles, en: endothecium, ib: intermediary vascular bundles, pb: peripheral vascular bundles).

FIGURE 4. Photomicrograph with the reaction of the histochemical tests applied to the staminate flower of *S. inajai*. a) Pollen grain with starch reserve, black; b) Test for protein, protein bodies in red; c) Pectic nature of the wall of the microspore gain; and d) Pectic nature of the cell wall in the epidermis of the filament (arrow).

FIGURE 5. Scanning electron micrograph of the pistillate flower of *S. inajai*. a-b) Longitudinal section; a) Floral parts; b) Detail of the stigma (asterisk); c) Regions of the gynoecium, apical (a), middle (m) and basal (b), depression delimiting the stamen ring (asterisk), opening of the septal canal (arrow); d) Cuticle, apical region; e) Middle epidermis region; f) Squamiform trichome (arrow); and g) Longitudinal section of the squamiform trichome (arrow). (gy: gynoecium, pe: petal, s: stylar canal, sc: staminoid circle se: sepal).

FIGURE 6. Photomicrograph of the pistillate flower of *S. inajai*. a) Longitudinal section of the gynoecium, ovules (asterisk), idioblasts (arrow); b-e) Cross-section of the gynoecium; b) Stigma, vascular bundle; c) Region of the stylar canal (asterisk), septa (arrow); d) Ovary, showing the locules, septum (arrow); e) Stylar canal; f) Longitudinal section of the epidermis of the stylar canal; and g) Septum with secretory epidermis. (db: dorsal bundle, lb: lateral bundle, re: receptaculum, st: stigma, vb: vascular bundle).

FIGURE 7. Photomicrography of the pistillate flower of *S. inajai*. a-c) Cross-section; d-h) Longitudinal section; a) Sepal; b) Petal; c) Staminodes circle; d) Detail of the ovule, internal epidermis of the ovary (asterisk), micropyle (arrow); e) Embryo sac; f) Obturator (arrow); g)

Pachychalaza, vascular bundle (arrow), hypostasis with phenolic compounds; and h) Region of the micropyle. (ep: epistase, mi: micropyle, nu: nucellus, pc: pachychalaza, sp: septum, ot: outer tegument, it: inner tegument, vb: vascular bundle).

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Capítulo 2

Genovese-Marcomini, P. R.; Mendonça, M. S.; Carmello-Guerreiro, S. M. Morphoanatomy of the development of *Syagrus inajai* (Spruce) Becc. (Arecaceae, Attaleinae): from the gynoecium to the developed fruit. Manuscrito formatado para *Acta Botânica Brasilica*

Morphoanatomy of the development of *Syagrus inajai* (Spruce) Becc. (Arecaceae, Attaleinae): from the gynoecium to the developed fruit

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ABSTRACT

(Morphoanatomy of the development of *Syagrus inajai* (Spruce) Becc. (Arecaceae, Attaleinae): from the gynoecium to the developed fruit). Ontogenic study enables observation of the events responsible for the formation and characterization of an organism, as well as furthering understanding of its ecological interactions. With the aim of characterizing the morphoanatomy of the development, from the gynoecium to the developed fruit of *Syagrus inajai* ("pupunha-brava"), flowers and fruits were collected in different phases of development, and analyzed using the usual plant morphoanatomy techniques. From the seminal primordium, the ovules are anatropous. Hypostasis with cells containing phenolic content is prolonged during development, including the entire ovule, throughout the length of the pachychalazy. The ovary has two meristemic regions: a peripheral meristem region, adjacent to the outer epidermis, which gives origin to the fibrous mesocarp; and an internal meristemic region, which involves the internal seminal cavity and is responsible for the thickening of the endocarp, forming the endocarp *sensu lato*, of mixed origin. Sclerification of the endocarp initiates in the fruit at around 80 days, occurring centripetally. The endosperm is of the multicellular coenocytic type, with formation of the cell walls occurring centrifugally. The operculum is comprised of cells of the outer and inner integuments, and by sclerified cells of the obturator. The first cell division of the zygote occurs around 30 days after the start of development of the endosperm. The development time of the fruit is approximately 240 days.

Keywords: ovule, pericarp, seed, "pupunha-brava", Palmae.

RESUMO

(Morfoanatomia do desenvolvimento de *Syagrus inajai* (Spruce) Becc. (Arecaceae, Attaleinae): do gineceu ao fruto desenvolvido). O estudo ontogênico possibilita a observação dos eventos responsáveis pela formação e caracterização de um organismo, além de auxiliar na compreensão de suas interações ecológicas. Com o objetivo de caracterizar morfoanatomia do desenvolvimento do gineceu ao fruto desenvolvido de *Syagrus inajai* (Pupunha-brava), foram coletados flores e frutos em diferentes fases de desenvolvimento, os quais foram analisados por meio de técnicas usuais em morfoanatomia vegetal. Desde o primórdio seminal os óvulos são anátropos. A hipóstase com células contendo conteúdo fenólico prolonga-se durante o desenvolvimento englobando todo o óvulo, por toda a extensão da paquicalaza O ovário apresenta duas regiões meristemáticas. A região meristemática periférica, adjacente à epiderme externa origina o mesocarpo fibroso. A região meristemática interna, que envolve a

cavidade seminal externa é responsável pelo espessamento do endocarpo, formando o endocarpo *lato sensu*, de origem mista. A esclerificação do endocarpo inicia-se no fruto com aproximadamente 80 dias, e ocorre centripetamente. O endosperma é do tipo coenocítico multicelular e a formação de parede das células ocorre centrifugamente. O opérculo é composto por células dos tegumentos externo e interno, e por células do obturador esclerificadas. O zigoto tem sua primeira divisão celular cerca de 30 dias após o início do desenvolvimento do endosperma. O tempo de desenvolvimento do fruto é de aproximadamente 240 dias.
Palavras-chave: óvulo, pericarpo, semente, pupunha-brava, Palmae.

Introduction

During its development, an organism goes through various morphological, anatomical, physiological and biochemical changes (Gatsuk et al. 1980). This sequence of phases in the development of an individual is termed ontogeny (Portela & Santos 2011). Ontogenic studies are of great value for understanding the Biology of a specific species and taxa, as it enables observation of the events responsible for the formation and characterization of its tissues and organs, as well as furthering our understanding of the ecological interactions. Bernacci *et al.* (2008) observe that in *Syagrus romanzoffiana* (Cham.) Glassman, the ontogenic stages are significant events in its biology, and were not solely dependent on the age and size of the individuals.

There have been few ontogenetic studies on the family Arecaceae. This is no doubt due to the extremely long development time of the fruit, which in several of the species, exceeds 200 days (Murray 1973; Benassi *et al.* 2007; Reis *et al.* 2012; Silva *et al.* 2013). Another reason is related to the difficulty in describing the development of the endosperm, due to the difficulty of its fixation. In this context, studies like those carried out by Lloyd (1910) and Biradar & Mahabale (1968b) observing the development of the fruit, seed and embryo and the germination of the species of the genus *Phoenix* are worth highlighting, as they present a broad approach to the organism. Works like these reduce the possibility of making sweeping, incomplete and/or incorrect interpretations.

Also attempting to elucidate the development of palms, Reddy & Kulkarni (1985) carried out anatomical studies on the mature fruits and seeds of Cocosoid palms, including: *Cocos nucifera* L., and *Attalea speciosa* Mart. and the development of the fruit of *Syagrus coronata* (Mart.) Becc. Sylvestre *et al.* (1989) studied the development of the fruit of *Allagoptera arenaria* (GOMES) O. Kuntze. Romanov *et al.* (2011) carried out extensive work on the development of the pericarp of Borassoid palms, Reis *et al.* (2012) studied the development of the pericarp of *Acrocomia aculeata,* and Bobrov *et al.* (2012) studied the gynoecium, fruit histology and development of *Eugeissona*.

Syagrus is the second most numerous genus of the subtribe Attaleinae, in South America, which has a total of 31 species, according to Dransfield *et al.* (2008). Its distribution is restricted to this continent, with the highest number of species occurring in Brazil. The biological importance and representativeness of the genus demonstrates the importance of studies on the morphoanatomical development, which provide important information for extending our knowledge of the genus, the family, and the monocotyledons. *Syagrus inajai*

(Spruce) Becc., has its phytogeographic domain in the Amazon region. In Brazil, it occurs in the states of Amapá, Amazonas, Maranhão and Pará (Leitman *et al.* 2012) in areas with "terra firme" (lowland rain) and "baixio" (gallery forest), clearings, and roadsides (Miranda & Rabelo 2006), in environments with different levels of water availability. It flowers all year round, producing one to three inflorescences per individual, with 18 to 36 rachillae each (Genovese-Marcomini *et al.* 2013). It is a species that is resistant to cutting and fire while in the acaulescent phase (Miranda and Rabelo 2006). Its dispersers are attracted by the strong odor of its fruits. The pulp and nut are edible (Henderson *et al.* 1995; Miranda & Rabelo 2006). Therefore, it is a palm that adapts to different environments, and produces a high number of flowers and fruits, characteristics that have prompted studies on its ontogeny, vegetal recomposition, and floral Biology.

In view of the above, this work aims to carry out a morphoanatomical study of the development of the gynoecium to the mature fruit of *S. inajai*, seeking to further understanding of the formation of the parts that comprise it, and to measure the approximate time of each event.

Material and methods

The botanical material was collected in 2009 and 2010 in a green area of the Campus of the Federal University of Amazonas - UFAM, Manaus, Amazonas, Brazil, in an area of "baixio" (gallery forest), close to coordinates 03 ^{o} 05' 45,84" S and 59 ^{o} 58' 43,69", a climate characterized as being of the Afi type (Köppen 1931). The material was analyzed at the Agroforestry Botanical Laboratory - LABAF, of the same University. The exsiccate of the species was deposited at the INPA Herbarium, under record number 241243. For the morphoanatomical description, twenty adult individuals of *S. inajai* were selected. The collections began with the inflorescences with closed peduncular bracts in two stages: at 15 and 30 days of development, and after the opening the other collections were carried out every five days, with removal of one rachilla per inflorescence until dispersion of the fruit. The descriptions were based on the studies by Tomlinson (1990) and Dransfield *et al.* (2008).

The fruits were measured (length, width and fresh weight) every ten days. The variable length, width and mass were subjected to regression study due to the age of the fruit.

For the anatomical study, the material collected (flowers, fruit at various stages) was fixed in FAA 70% (Kraus and Arduin 1997). The samples were dehydrated in ethyl series (70%- 95%), embedded in 2-hydroxyethyl-methacrylate (Historesin® Leica, prepared

according to the manufacturer's instructions), sectioned at thicknesses of 4 to 7 µm in a rotary microtome, and stained with 0.5% toluidine blue in citrate buffer, pH 4.0 (O'Brien *et al.*1964), and the slides were mounted in water.

For the registration of the material in a Scanning electron microscopy (SEM), the botanical material was fixed in BNF (Buffered Neutral Formalin) (Lillie 1965) solution, dehydrated in ethyl series, and dried by the critical point method with CO_2 on a Balzers model CPD 030. The samples were collected in a metal support, covered with gold (Balzers SCD 050) and observed under a JEOL JSM 5800LV (10 kV) Scanning Electron Microscopy, at the Institute of Biology/Unicamp.

The histochemical tests used were: periodic acid-Schiff reagent (PAS) (Pearse 1980) to identify neutral polysaccharides and glycoproteins (Johansen 1940); Ruthenium Red to identify pectic substances (Johansen 1940); tannic acid for mucilage (Pizzolato & Lillie 1973); Sudan IV (Brundett *et al.* 1991) to detect lipophilic substances; Ferric trichloride III to determine the occurrence of phenolic compounds (Johansen 1940).

The results were documented using an Olympus (BX51) photomicroscope with Olympus (DP71) photographic camera, and a Leica (M125) stereomicroscope with Leica (DFC 490) photographic camera.

Results

Gynoecium in development

O gynoecium is cream in color, ovoid, supero, tricarpellary, and syncarpic, with one ovule per carpel (Fig. 1A-C, 2A, 4G). However, the carpels are not totally merged, a characteristic that is evidenced by the incomplete fusion of the edges of the carpels, which form three septal cavities (Fig. 2D, 4D and I).

The epidermis is uniseriate, with cells elongated on the anticlinical axis. In the stylar canal and region of the ovary, this elongation is less pronounced (Fig. 2A-C, 3A).

The mesophyll in the region of the stigma and stylar canal is comprised of parenchyma interspersed with idioblasts with phenolic compound, and in the region of the ovary, only parenchyma cells and initials of the vascular bundles are observed (Fig.2A).

After the opening of the peduncular bract, a thick cuticle is seen, covering the epidermis on the external side of the gynoecium, as well as stomata and multicellular trichomes, squamiform, with phenolic compounds (Fig. 4E-F and M). On the internal side, the epidermal cells are elongated (Fig. 4H). In the stigma, this event is accentuated, and the cells

take on the appearance of papillae (Fig. 4B-C). In the mesophyll, there is an increase in the number of idioblasts with phenolic compound, throughout the length of the gynoecium, with a higher concentration in the region of the stigma (Fig. 4A and O-P). In transversal section, at the level of the ovary, the mesophyll consists of three regions: a peripheral region, consisting of meristematic tissue, with cells arranged in rows and intense division on the periclinal axis, providing growth in thickness; a median region comprised of parenchyma cells interspersed by initials of vascular bundles and fibers at the start of differentiation of the xylem, followed by several layers of idioblasts containing phenolic compound, and another inner region, comprised predominantly of parenchyma cells and meristem tissue (Fig. 4D). It is in this region that the three septal cavities are located (Fig. 4D and I), which presents a cuticle on the external periclinal wall of its epidermal cells (Fig. 4N). The cells of the internal epidermis in the region of the ovary present periclinal and anticlinal divisions. (Fig 4D and J-L).

The ovule is anatropous from the start of its development (Fig. 2A), with micropyles formed by the two integuments, with the outer integument overlapping the inner one, and the latter limited to the micropyle region (Fig. 3D and I).

The embryo sac is octanucleated and heptacellular, comprised of three nuclei in the micropyle region, differentiated inside the large egg apparatus (two synergids and one oosphere), three cells in the chalazal region forming the antipodes, and two nuclei merged into a secondary nuclei in the center of the embryo sac (Fig. 2E-H).

Approximately one week after the opening of the peduncular bract, the appearance of idioblasts is observed, with phenolic compound in the region of the chalaza and micropyle, forming the hypostasis and the epistasis (Fig. 3B). The vascular bundles that reach the chalaza irradiate to the outer integument, forming the pachychalazy, which replaces the integument up to the region next to the micropyle, with the growth of idioblasts with phenolic compound throughout the pachychalazy (Fig. 3 D-E and G).

The nucellus is crassinucellate (Fig. 3C), comprised of two layers of cells that degenerate during megagametogenesis, with just one incomplete layer of cells remaining, in the form of an arc, covering the region of the embryo sac that comprises the egg apparatus (Fig. 3E-F).

Developed gynoecium

The opening of the pistillate flower occurs about 20 days after the opening the peduncular bract. The stigma opens, becoming moist and sticky with a yellowish appearance (Fig. 1Ao). The epidermal cells of the stylar canal elongate, becoming papillous, together with

the cells of the inner epidermis of the stigma (Fig. 5A-C), showing a reaction with to neutral polysaccharides (Fig. 5H), and secreting pectic substances (Fig. 5L) and mucilage (Fig. 5O). The cells of the mesophyll, adjacent to the stylar canal, also showed a reaction for neutral polysaccharides (Fig. 5G). The epidermal cells of the septal cavities also elongate, with reaction for neutral polysaccharides (Fig. 5I), pectic substances (Fig. 5M) and mucilage (Fig. 5P). The inner epidermal cells of the ovary at the apex and base, and in the region of the funiculus, become papillous, forming the obturator, reacting for neutral polysaccharides (Fig. 5J), and in the secretate for pectate substances (Fig. 5N) and mucilage (Fig. 5Q).

In the peripheral portion of the mesophyll, the appearance of idioblasts is observed, with phenolic compound and early differentiation of fiber bundles (Fig. 5D-E). In the middle portion, differentiation of the fiber caps initiates, partially involving the vascular bundles, and an increase in the number of idioblasts with phenolic compound (Fig. 5F). In the inner portion of the mesophyll, the parenchyma cells show intense division at different levels, followed by an increase in cell volume (Fig. 5D).

From the start of development of the gynoecium, it is observed that its differentiation is basipetal, with visible sclerification of the fibers in the region of the stigma to the base of the ovary (Figure 2A, 4A, 5A).

The developing fruit

About five days after the opening of the stigma, its darkening is observed, followed by degeneration of its cells (Fig. 1A, 7A).

The first morphological sign of the start of differentiation of the pericarp is the change in color of the epicarp, which turns greenish, an event that occurs around ten days after the opening of the pistillate flower (Fig.1A). Up until this moment, there is secretion in the stylar channel, septal cavity and obturator, with reaction for neutral polysaccharides and pectic substances, and in the cells of the obturator, with reaction for neutral polysaccharides (Fig. 7G-M).

At around ten days, the fruit begins to lengthen. At twenty days it is twice its original length, and at forty days, it has doubled in width (Fig. 1A, 6).

In the epicarp, the anticlinal and outer periclinal walls of the cells show thickening of the wall and accumulation of cuticle, taking on a bottle-like appearance (Fig. 7C-D). The peripheral region of the mesocarp shows a significant increase in fiber bundles (Fig. 8A-B). In the fruit, at around sixty days, the epicarp, peripheral region and middle mesocarp already have the appearance observed in the developed fruit (Fig. 8D-E). The next event in this region

will be the periclinal elongation of the parenchyma cells, many of which will differentiate into sclerids (Fig. 8E and H). The radial growth will show little change throughout the development, as the fibrous mesocarp is differentiated (Fig. 1, 6).

The inner region of the mesocarp along with endocarp proper, and the cells originating from its periclincal divisions, maintain intense cell division. At about 80 days, these cells begin to sclerify from the regions where fusion of the carpels occurred, next to the three septal cavities, in centrifugal form (Fig. 1, 8G).

From 130 days there is little change in the size of the fruit (Fig. 1, 6), as the endocarp *sensu lato* is in the process of sclerification, without the addition of cell layers (Fig. 1). In the mesocarp, the parenchyma cells continue to increase in volume, lengthening on the periclinal axis. At this stage, the embryo is already visible to the naked eye.

The primary endosperm cell (PEC) divides ten days after the opening of the stigma. Soon afterwards, the start of formation of the endosperm is seen; the ephemeral antipodes degenerate, and the filiform apparatus becomes large and conspicuous (Fig. 7E). The seed lengthens (Fig. 3 H and J) along with the two ovules, which atrophy after five days. Thus, a single seed continues to develop (Figure 3J-L), pushing the two ovules from the endocarp (Fig. 9A-B).

The endosperm is of the multicellular coenocytic type. The cells originating from divisions of the primary endosperm cell (PEC) first lengthen next to the chalazal region and the nucellus, and in higher quantity in the region around the zygote or embryo, depending on the phase of observation (Fig. 9C). This event is followed by the formation and thickening of the endosperm cell wall, a centripetal process (Fig. 9D-H). Anatomically, the formation of the wall can be observed ten days after the division of the PEC. In the fruit at approximately 120 days, a gelatinous endosperm can be seen with the naked eye, at the periphery of the endosperm, the remainder being in liquid form (Fig. 1B-C). At 150 days, with the fruit has a totally gelatinous endosperm. After 160 days, the peripheral cells of the endosperm already have thickened walls (Fig. 9H). The endosperm has a solid appearance after 190 days of development, filling the entire internal space of the seed (Fig. 1B-C, 9I-J).

The zygote undergoes its first cell division approximately thirty days after the start of formation of the endosperm (Fig. 7F).

In the fruit at 130 days, three morphoanatomic events that occur concomitantly should be considered: the endosperm starts to solidify, the embryo becomes visible to the naked eye,

approximately 1 mm in length, and there is intense sclerification of the cells of the endocarp *sensu lato* (Fig. 1).

Following lengthening of the seed, the pachychalazy covers almost its entire surface, except for the region of the micropyle (Fig. 2H).

The developed fruit

The start of dispersion of the fruits of *S. inajai* was observed from 240 days of development, around 270 days after the opening of the peduncular bract. At this age, the fruit is orange-yellow in color, round to elliptical in shape (Fig. 1), 37.88 cm in length, 26.49 inches in width, and with 15.69 g mass, on average (Fig. 6). The relationship between age and the variables length, width and mass followed a third-degree equation. Based on the equation, the length stabilized at 210 days, the width at 230 days, and the mass at 220 days (Fig. 6).

From the anatomical point of view, no changes were observed in the epicarp. The outer and middle mesocarp maintained the pattern observed at 130 days, but with increased volume of parenchyma cells. The endocarp *sensu lato*, brown in color (Fig. 1), was sclerified with approximately 70 cell layers, maintaining a separate epidermis with cells containing phenolic content and thickened walls (Fig. 8J, L).

The seed is pachycalazal. Associated with the vascular bundles that comprise it, there is a high number of cells with phenolic compound, which are responsible for the dark color of the seed (Fig. 1). The outer (testa) and inner (tegmen) teguments are restricted to the micropyle, forming the operculum, together with cells of the obturator that have become sclerified (Fig. 9L-O).

The endosperm is white, with thick-walled cells (Fig. 9I-J), giving a stony consistency to the tissue, and occupies almost the entire space inside the seed. The embryo is cream in color, small, straight, in contact with the lid operculum, and oblique to the longitudinal axis of seed, measuring approximately 8.5 mm in length (Fig. 1B-C).

Discussion

In *S. Inajai*, the two seeds lengthen along with the developing seed for approximately ten days. In *Arecastrum romanzoffianum* (Chamisso) Beccari, two of the three ovules present are aborted and crushed against the inside of the endocarp (Murray, 1973), as observed in *S. inajai*. Reis *et al.* (2012) noted that in *Acrocomia aculeata* (Jacq.) Lodd., at only two days of development of the fruit, there was atrophy of the two carpels, characterizing it as a kind of pseudo monomer. For Rudall et al. (2003) the term pseudomonomeric gynoecia describes the moment when only a single carpel is fertile; the other carpels have begun to develop, but are

subsequently aborted. Stauffer and Endress (2003) argue that the term pseudo monomer is generally used to refer to gynoecium with the appearance of monomer, but which are comprised of more than one carpel, only one of which is well-developed. In the *Geonoma* species studied by the authors, the two carpels atrophy and only a third continues to develop. In the species studied, the pericarp continues to develop, and only the two ovules atrophy. Therefore, the gynoecium is not a pseudo monomer, as it presents three well-developed carpels up until the fruit stage. According Font Quer (1977) the term monomeric applies to whorls, but also to the other organs. Thus, the developed fruit, and not the gynoecium of *S. Inajai*, has the appearance of a pseudo monomer, as only one seed is observed inside the pericarp.

According to Spujt (1994), drupe is a fleshy indehiscent fruit with a woody endocarp. In this type of fruit, the mature pericarp is differentiated into a thin, soft epicarp, a fleshy mesocarp, and a disk (the woody endocarp), and this pericarp is often formed by a single piece containing a single seed, which is also observed in the fruit of *S. inajai*, characterizing it as a drupe.

Septal nectaries are the result of incomplete fusion of the edges of the carpels, with a secretory epidermis of elongated, uninucleate cells (Endress & Stauffer 2003; Stauffer *et al.* 2009). In more derivative palms, with syncarpic gynoecium, the nectaries are located in the spaces between the incompletely fused carpels (Uhl & Moore 1971, 1977). These may be present in palm trees of different sizes. Their presence or absence and position may characterize the genera of some groups (Uhl & Moore 1971). Also according to the authors, the main divisions within Cocosoids can be automatically separated by the presence or absence of septal nectaries in combination with various histological criteria. *S. inajai* has three septal cavities, with secretory epidermis, secreting neutral polysaccharides, glycoproteins, pectic substances and mucilage. However, considering the nature of the secretion, this information does not allow us to affirm that this is a septal nectary, as no nectar secretion was observed. However, its origin and anatomical description corroborate the description of septal nectary observed in other palms (Uhl & Moore 1971; Stauffer & Endress 2003; Rudall *et al.* 2003; Stauffer *et al.* 2009), many of which have not been tested for glucose, and their presence is linked visitation of the flower by insects (Rudall *et al.* 2003). For Silberbauer-Gottsberger (1989), the secretion of nectar in external nectaries present in the gynoecium, with free carpels, must have its origin in palms, the septal nectaries being apparently

derivative. The occurrence and location of septal nectaries are useful markers of evolution of the gynoecium in monocotyledons (Rudall 2002; Rudall *et al.* 2003; Rudall *et al.* 2011).

In *S. inajai* the pericarp begins to differentiate around ten days after opening of the stigma. It is morphologically observed by the change in its color, turning from cream to green, and anatomically, by the start of differentiation of fiber bundles of the peripheral mesocarp. Romanov *et al.* (2011), in an anatomical study of the fruits of Coryphoideae-Borasseae, observed the start of differentiation of the pericarp after fertilization. The same authors state that the differentiation and growth of the pyrene begins soon after fertilization. The species studied, this event only occurs in the fruit at 70 days. However, in both species, the end of differentiation occurs well before the end of development of the seed.

The differentiation of the pericarp tissues is basipetal, as also observed for Cocosoid (Murray 1973) and for Eugeissona species (Bobrov *et al.* 2012). According to Uhl (1976), basipetal maturation can guarantee protection, as the distal parts of the floral organs are the first to be exposed, corresponding to what occurs in *S. inajai*. As observed in the present work, the phenolic compounds first appear at the apex of the gynoecium. However, when the observation plane is changed to transversal, it is seen that in *S. Inajai*, the epicarp is the first to differentiate, followed by the mesocarp and finally, the endocarp *sensu lato*, the maturation of the pericarp occurring in centripetal form. However, the sclerification of the endocarp is centrifugal.

Some phenolic compounds are known to have antimicrobial effects against food-borne pathogens (Asolini *et al.* 2006; Ahn *et al.* 2007). The occurrence of cells with phenolic content in specific areas of the gynoecium and fruit of *S. Inajai*, such as phenolic compounds present in the cells of the stigma in the developing gynoecium, which is a gateway for the entry of organisms, the zone of cells with phenolic compound around the embryo sac forming the hypostasis and epistasis, and the presence of idioblasts in the peripheral mesophyll, corroborate this characteristic.

During the development of the pericarp, both the epicarp and the endocarp *sensu strictu* differentiate, acquiring conspicuous characteristics that enable them to be easily observed, with a cell wall and thick cuticle, and cells with thickened wall and phenolic content, respectively, as seen in species of Coryphoideae-Borasseae and *Acrocomia aculetata*, which have differentiated epicarp (Romanov *et al.* 2011; Reis *et al.* 2012). This differs from the species studied by Bobrov *et al.* (2012), who found little specialization in the epicarp and endocarp.

The mesophyll of the developing gynoecium, up until the mesocarp in the developed fruit, presented three anatomically distinct regions. The peripheral region, which is meristematic in the flower, is comprised, in the fruit, of parenchyma and fiber bundles. The middle region of the initials of fibrovascular bundles undergo differentiation. The inner region, initially comprised of parenchyma, undergoes sclerification, forming the endocarp *sensu lato*. In *Acrocomia aculetata* the mesophyll also presents three regions (Reis *et al.* 2012), which corresponds to the same regions observed in *S. inajai*. In species of *Eugeissona,* four regions were observed (Bobrov *et al.* 2012). In Borassoids palms, this number varies, with up six regions, as observed in *Lodoicea maldivica* (J.F.Gmel.) Pers. (Romanov *et al.* 2011).

In palms, the fruits are characterized by a specialized layer, usually sclerotic, known as the endocarp in the inner portion of fruit, next to the locule. Its formation may involve the locular epidermis or, more commonly, several layers of cells on the inner wall of the gynoecium. However, when cited, usually no mention is made of its histological nature or development (Murray 1973). In *S. inajai,* the cells of the inner epidermis of the ovary undergo periclinal division, providing several layers of cells, together with the inner region of the mesocarp, consisting of parenchyma, from the formation of the endocarp of mixed origin. Mendonca *et al.* (2008) and Reis *et al.* (2012), with the endocarp being considered to be of mixed origin, formed by cells of the endocarp and mesocarp. According to Roth (1977), the endocarp, as a stony portion of mixed origin, is referred to by the term *sensu lato*, differing from endocarp *sensu stricto* which is comprised only of cells originating from the inner wall of the ovary. Romanov *et al.* (2011) do not use the term to refer to the hard portion of the pericarp, but rather, pyrene. The authors state that in the palm Borassoid, the endocarp is formed by a single, unspecialized epidermal layer, which does not play a role in the formation of pyrene.

Murray (1973) defined three types of endocarp, with type III, corresponding to that of Cocosoids and Arecoids. According to Murray, this type of endocarp consists of three tissues: the sclerified locular epidermis; sclerids which are sometimes confluent with the sheath of the internal vascular bundles, and an extensive parenchymal zone, also sclerified. The endocarp observed in *S. inajai* corroborates with this description, except in relation to the tissue of sclerids confluent with sheaths of vascular bundles, as the vascular bundles are few in number, and are restricted to the region of the three septal cavities.

At 130 days, the fruit of *S. inajai* showed small variations in length and width. From this stage, the vascular bundles and fibers of the mesocarp are differentiated, the cells of the parenchyma are elongated, and the endocarp *sensu lato* is sclerified. In *Acrocomia aculeata* the final volume of the fruit is defined by sclerification of the exocarp and endocarp approximately 70 days after anthesis, with mature pericarp at 380 days (Reis *et al.* 2012), well before that observed in *S. inajai*. In the Arecoid and Cocosoid species examined by Murray (1973), cell divisions were observed in the basal portions of the ovary until the fruit reached around half of its final length. After that, lengthening occurred by an increase in cell size.

The degeneration of the inconspicuous and ephemeral antipodes of *S. inajai*, after fertilization, corroborates that observed in *Livistona chinensis* R. BR. (Kulkarni & Mahabalé 1974) and *Johannesteijsmannia lanceolata* J (Chan & Lim 2011).

The multicellular coenocytic-type endosperm was also reported in *Phoenix sylvestris* Roxb. (Mahabalé & Biradar 1967), and *Livistona chinensis* R. BR. (Mahabalé & Kulkarni, 1974). The formation of the wall in cells of the endosperm of *S. inajai* is a centripetal process that was also observed in the Cocosoid palm species studied by Reddy & Kulkarni (1985). The authors observed that in *Syagrus coronata* (Mart.) Becc. the endosperm remains liquid in the center of the seed, unlike what occurs in *S. inajai*.

As observed in *S. inajai* the degeneration of the nucellus in the chalazal region also occurs in other palms, such as *Phoenix pusilla* Gaertn., *Phoenix acaulis* Buch., *Phoenix reclinata* Jacq. (Biradar 1967) and *Phoenix robusta* Hook (Biradar & Mahabale 1968).

A pachychalazal seed has a network of vascular bundles originating from the chalaza which, by interspersed growth below the fixation point of the integument(s), replaces the seed integument either partially or entirely (Boesewinkel & Bouman 1984). The vascular bundles that comprise the pachychalazy are associated with idioblastos with phenolic compound. The pachychalazal seeds are always richly vascularized and generally present tanniferous hypostasis (Carmella-Warrior & Paoli 1999). Mahabale & Biradar (1967), studying *Phoenix sylvestris,* observed a zone of tanniferous cells around the embryo sac, and affirm that the pronounced development of the chalazal region indicates a tendency towards formation of the hypostasis. The presence of the pachychalazy in the seed of *S. inajai* may be related to its size and family. The extent of vascularization of the seed is closely related to the size of the ovules and the seeds. Seeds of the more basal families, in general, are relatively large, and have a more extensively developed vascular system (Boesewinkel & Bouman 1984). In the palm

Hyphaene indica Becc., 16-18 vascular bundles were observed, extending two-thirds of the length of the integuments (Mahabale & Chennaveeraiah 1957).

The operculum in *S. inajai* is composed cells of the epistasis, the inner and outer integument of the micropyle region, and the sclerified cells of the obturator. In *Jubaeopsis caffra* Becc. endocarp at the 'eye' consists of an epidermis of palisade cells along with the inner and outer integument of the micropyle region (Robertson 1977). In *Acrocomia aculeata*, meanwhile, the germinative pore of the endocarp consists of parenchyma cells (Reis *et al.* 2012).

Conclusions

The opening of the flower and stigma are indicators of the presence of secretion in the stylar cavity, obturator, and septal cavity, events that precede the start of development of the fruit by around ten days.

During the development of the gynoecium, the cells of the inner epidermis of the ovary divide on the periclinal plane, promoting a growth in diameter.

The endocarp, region of the pericarp with stony consistency, is comprised of cells of the inner mesocarp and cells of the inner epidermis of the ovary (endocarp *sensu stricto*) and its derivatives, therefore it is of mixed origin, characterized as endocarp *sensu lato*.

The two carpels, whose seeds atrophy, continue to develop, participating in the formation of the pericarp. The developing seed, pushes the two atrophied ovules against the endocarp, giving the developed fruit the appearance of pseudo monomer.

The formation of the endosperm of *S. inajai* is of the multicellular coenocytic type.

The late development of the embryo may be related to sclerification of the endocarp and the solidification of the endosperm, structures that give protection to the embryo.

The seed coats have two distinct regions, one pachychalazal and the other integumentary, limited to the region of the operculum.

The average time of development of the fruit is 240 days, after darkening of the stigma.

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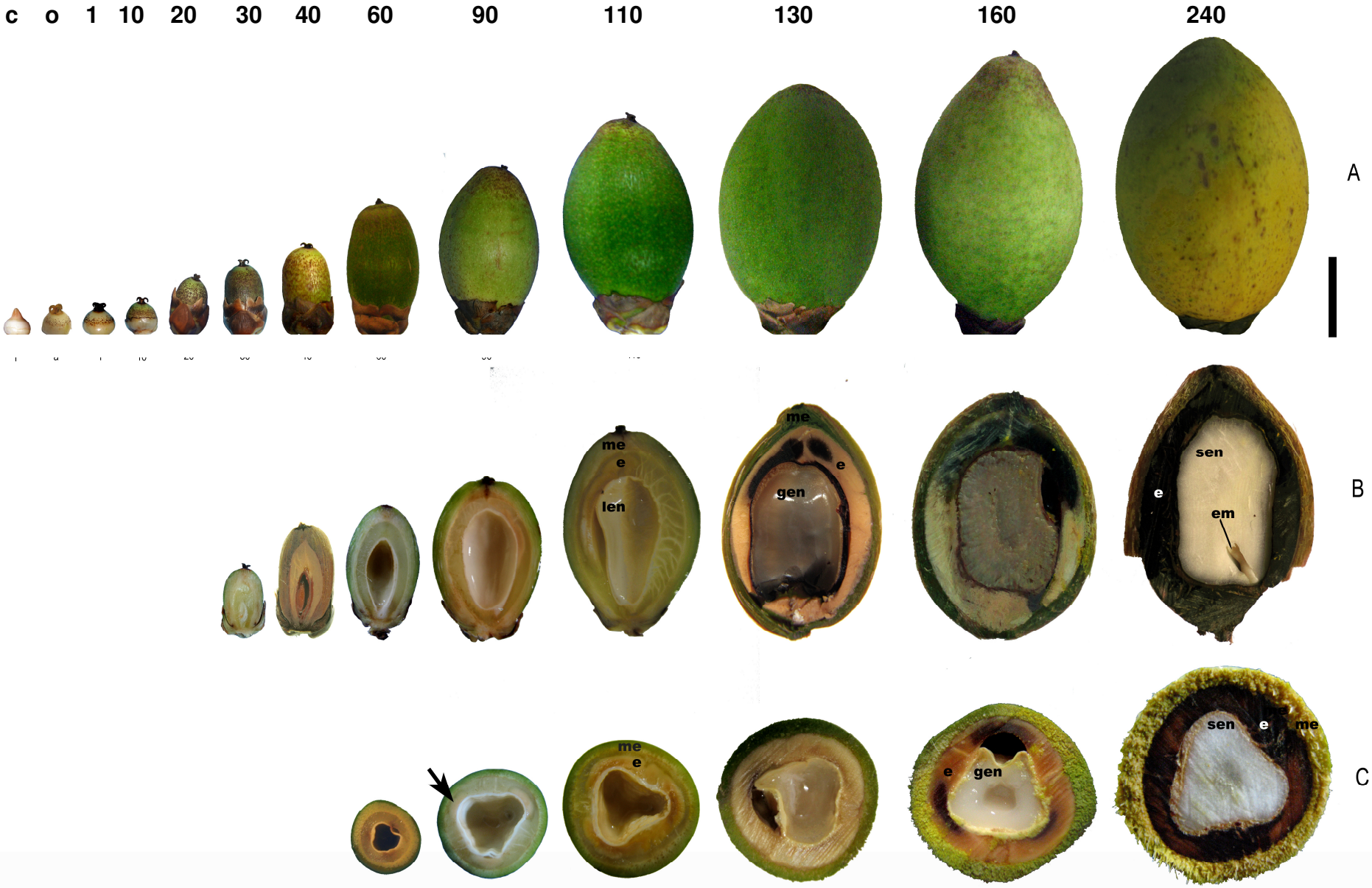
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Figure 1. Development of the gynoecium and fruit of *Syagrus inajai*, in days. A. General appearance; B. Longitudinal section ; C. Transversal section, start of sclerification of the endcarp (arrow). Bar= 1cm. c, gynoecium with stigma closed. e, endocarp; em, embryo; gen, gelatinous endosperm; len, liquid endosperm; me, mesocarp; sen, solid endosperm; o, gynoecium with stigma open.

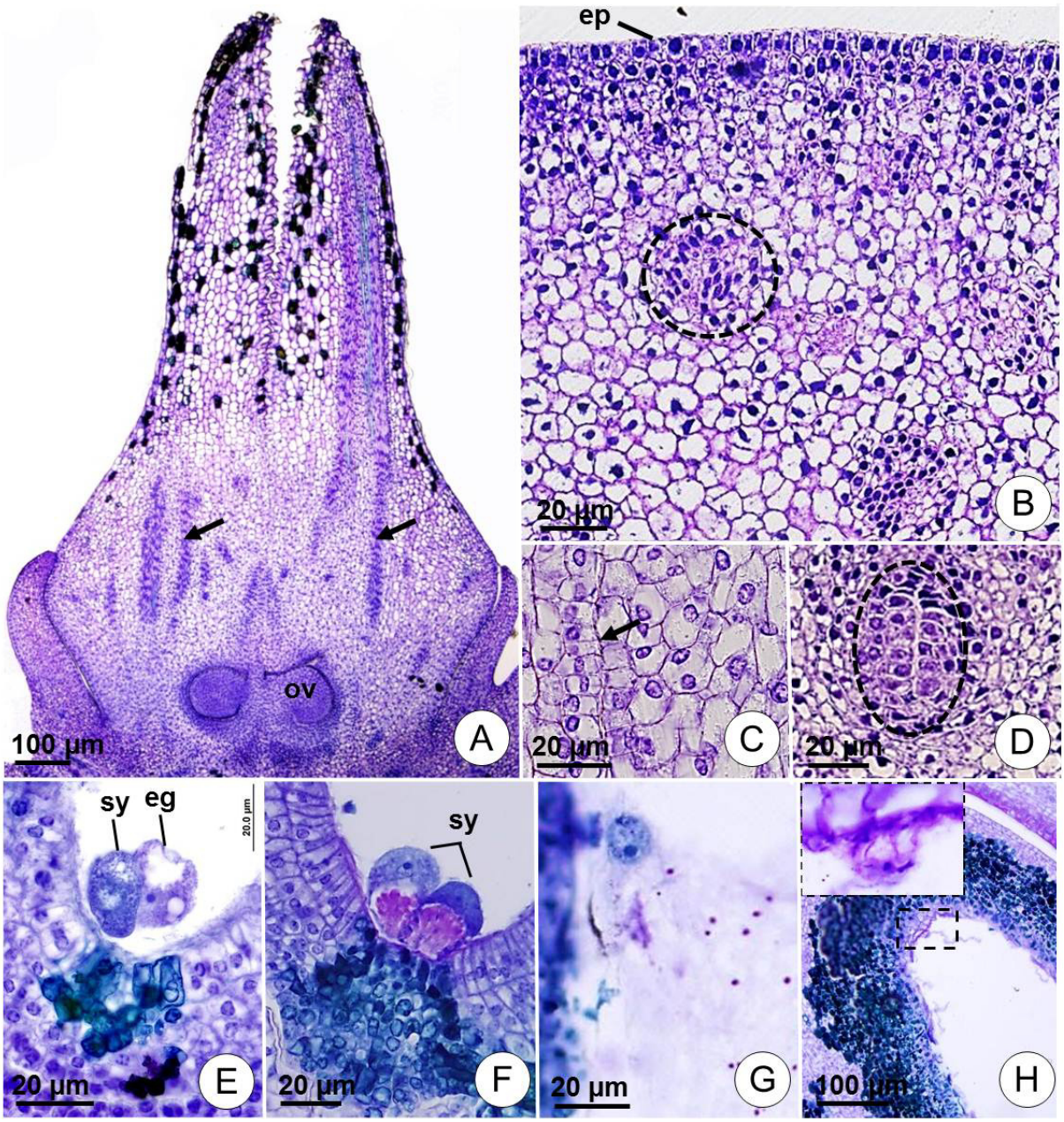

Figure 2. Gynoecium of the flower with closed peduncular bract and gynophyte of *Syagrus inajai*. A-D. Gynoecium. E-H. Longitudinal section of parts of gynophyte. A. Longitudinal section, general appearance of the gynoecium, procambium (arrow). B-D. Transversal section. B. The appearance of the epidermis and mesophyll in the middle region of the gynoecium, procambium (dotted line). C. Details of the stylar canal (arrow). D. Septal cavity (dotted line). E-F. Longitudinal section of gynophyte. E. Egg cell and synergid. F. Synergids G. Nucleus of the middle cell. H. Antipodes, detail (dotted line). eg, egg cell, ep, epidermis; ov, ovule, sy, synergid.

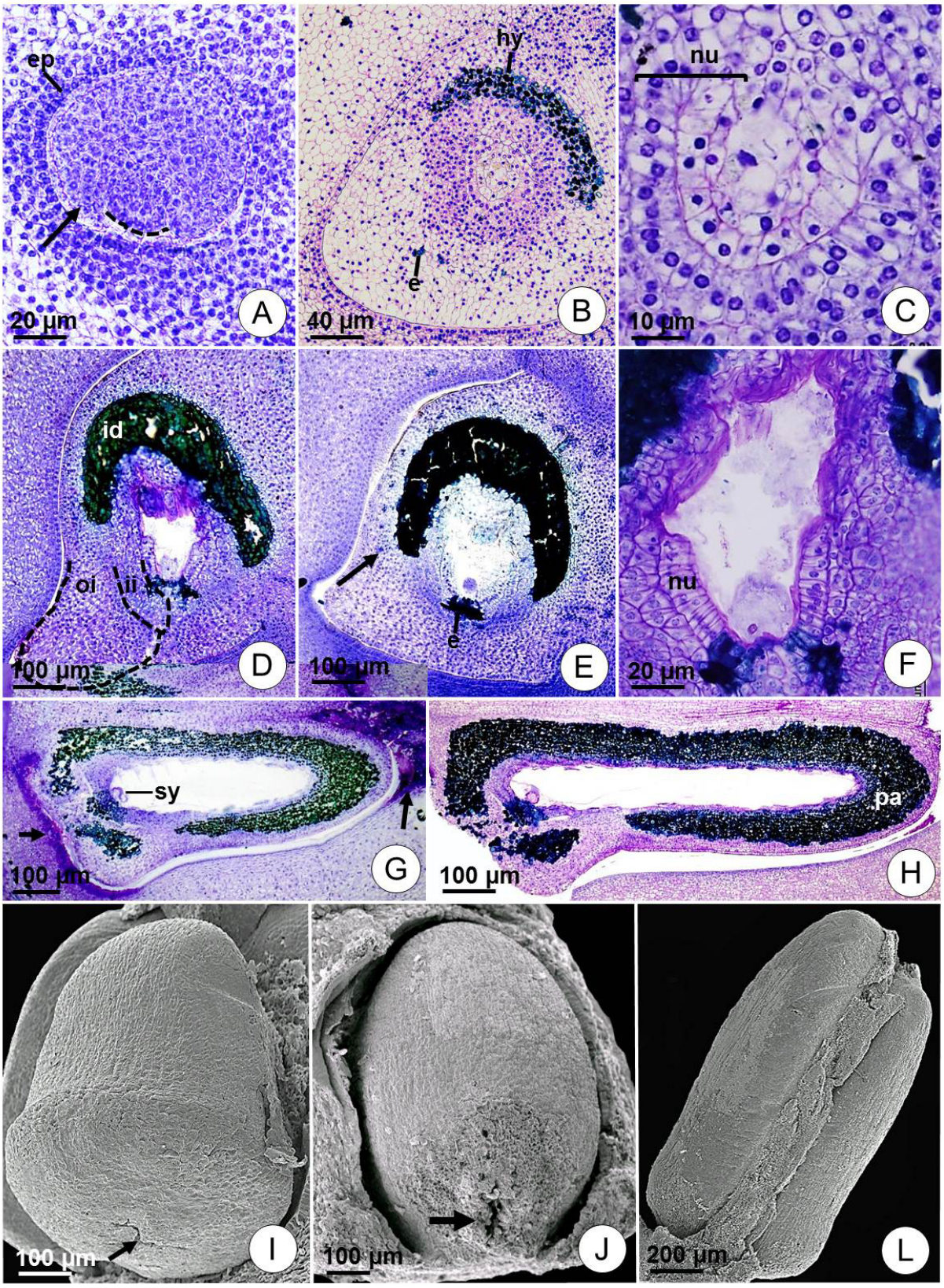

Figure 3. Development of the ovule and seed of *Syagrus inajai.* A-H. Longitudinal section. I-L. Electronmicrograph. A-C. Ovules of flowers with closed peduncular bracts. D-G. Ovules after opening of the peduncular bracts. A. Start of formation of the ovule (seminal

primordium), integument (arrow). B. Ovule in megasporogenesis, start of formation of the hypostasis and pachychalazy. C. Details of the crassinucleate nucellus. D. Pachychalaza around the embryo sac. E. Ovule showing the vascular bundles (arrow) and cells with phenolic content forming the pachychalazy. F. Degenerated nucellus. G. Appearance of the ovule and obturator cells (arrows). H. Seed at the start of development. I. General appearance of the ovule, the micropyle (arrow). J. Seed at the start of development, micropyle (arrow), L. Seed at around 20 days of development; e, epistasis, ep, epidermis; hy, hypostasis; id, idioblasts, ii, inner integument; nu, nucellus; oi, outer integument, pa, pachychalazy, sy, synergid.

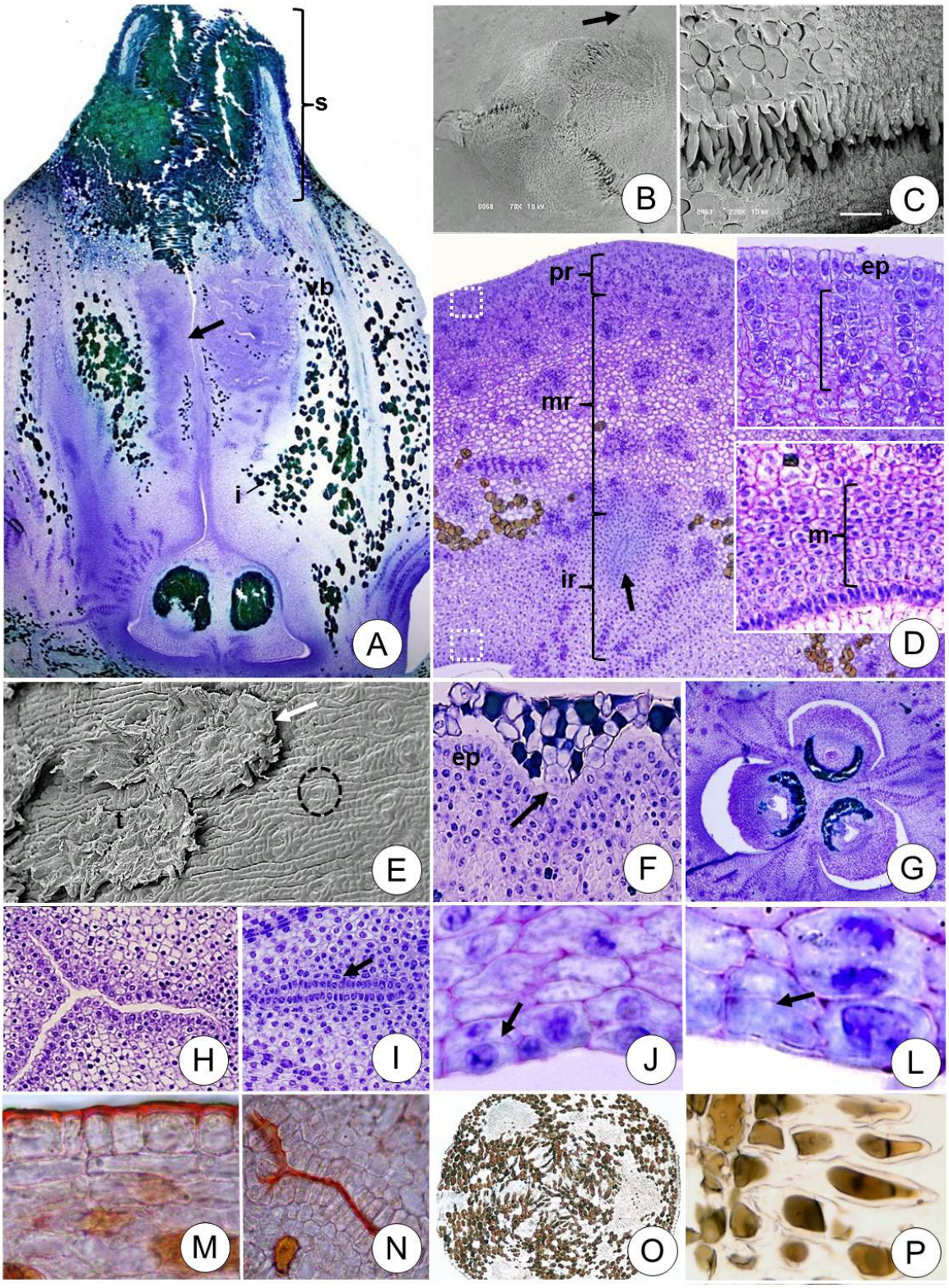

Figure 4. Gynoecium of *Syagrus inajai* after opening of the peduncular bract. A. Longitudinal section, general appearance of the gynoecium, stylar canal (arrow). B-C. Electronmicrograph. B. Closed stigma, opening of the septal cavity (arrow). C. Papillary cells of the stigma. D. Transversal section, median region of the gynoecium, septal cavity (arrow), detail of the peripheral meristematic region (dotted line), detail of the inner meristem (dotted

line). E Electromicography, detail of the trichoma (arrow) and stomata (dotted line). F-P. Transversal section. F. Trichoma with phenolic content (arrow). G. Tricarpellate gynoecium. H. Stylar channel. I. Septal cavity, epidermal cells (arrow). J. Cells of the inner epidermis of the ovary in the periclinal division plane (arrow). L. Detail of the inner epidermis of the ovary, cell division (arrow). M. Epidermal cells on the outer surface with cuticle. N. Cuticle in the epidermal cells of the septal cavity. O. Stigma, cells with phenolic content, dark brown. P. Detail of the papillary cells of the stigma with phenolic content, dark brown. ep, epidermis; ir, inner region, m, meristem; mr. middle region, pr, peripheral region.

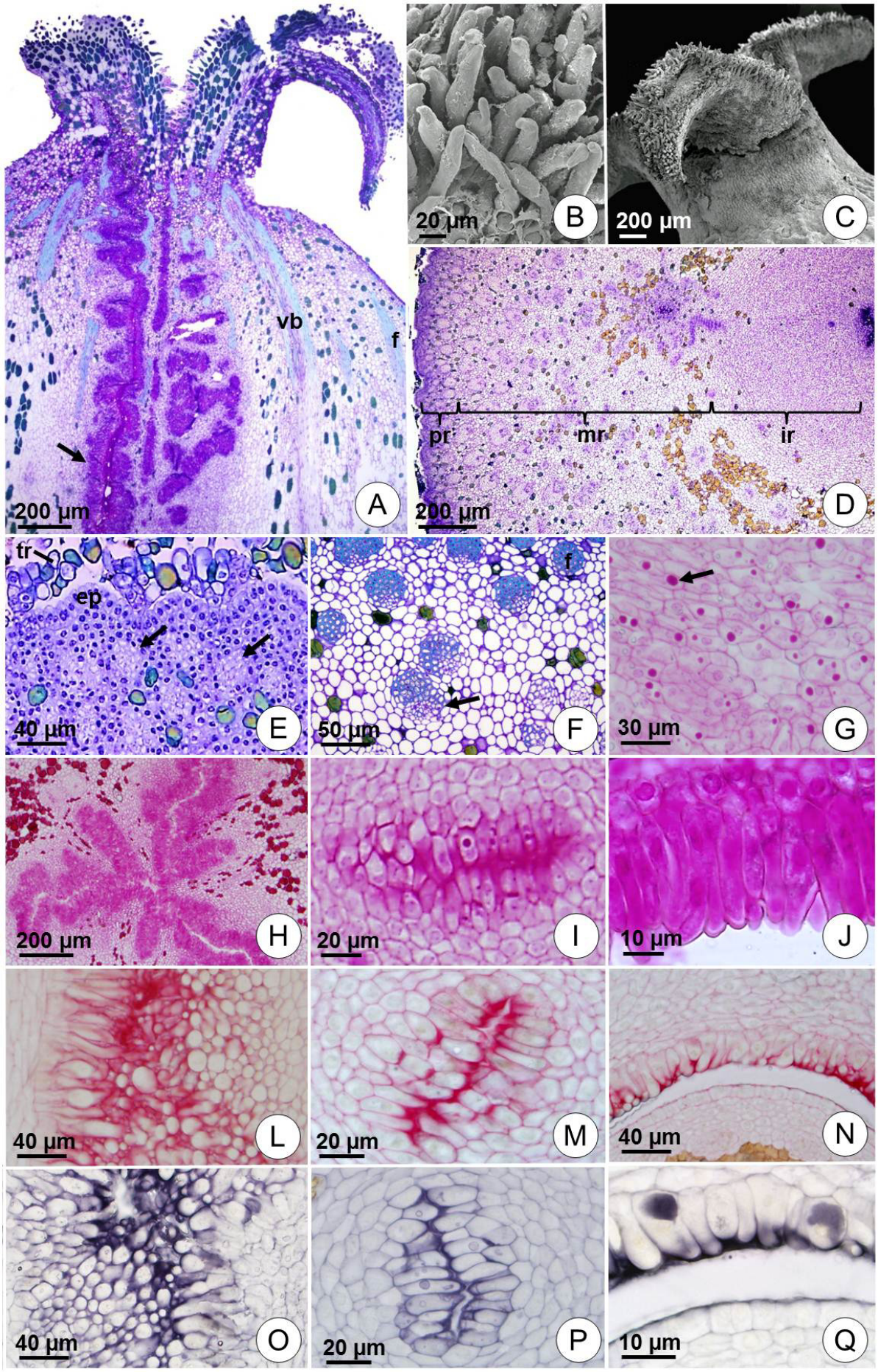
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Figure 5. Gynoecium of *Syagrus inajai* with stigma open. A. Longitudinal section, general appearance of the gynoecium, from the stigma to the stylar canal (arrow). B-C. Electronmicrograph. B. Papillary cells of the stigma. C. Stigma open. D-Q. Transversal section. D. Middle region of the gynoecium. E. Detail of the epidermis and peripheral region of the mesophyll, fiber bundles in differentiation (arrow). F. Detail of the middle mesophyll, vascular bundle with sheath of fibers in differentiation (arrow). G-Q. Histochemical tests. G-J. Detection of polysaccharides with PAS, deep pink positive staining. G. Reaction in the cytoplasm of the mesophyll cells near the stylar canal (arrow). H. Epidermal cells of the stylar canal showing polysaccharides. I. Reaction in the epidermal cells of the septal cavity and secretate. J. Reaction cells of the obturator. L-C. Detection of pectic substances with Ruthenium red, red positive staining. L. Secretion of the stylar canal with reaction. M. Secretion of the septal cavity with reaction. N. Secretion of the obturator with reaction. O-Q. Detection of mucilage with Tannic acid, gray positive staining. O. Secretion of the stylar canal with reaction. P. Cells of the septal cavity and secretate with reaction. Q. Cells of the obturator and secretate with reaction. ep, epidermis; f, fibers; ir, inner region; mr: middle region, pr, peripheral region, tr, trichoma, vb, vascular bundles.

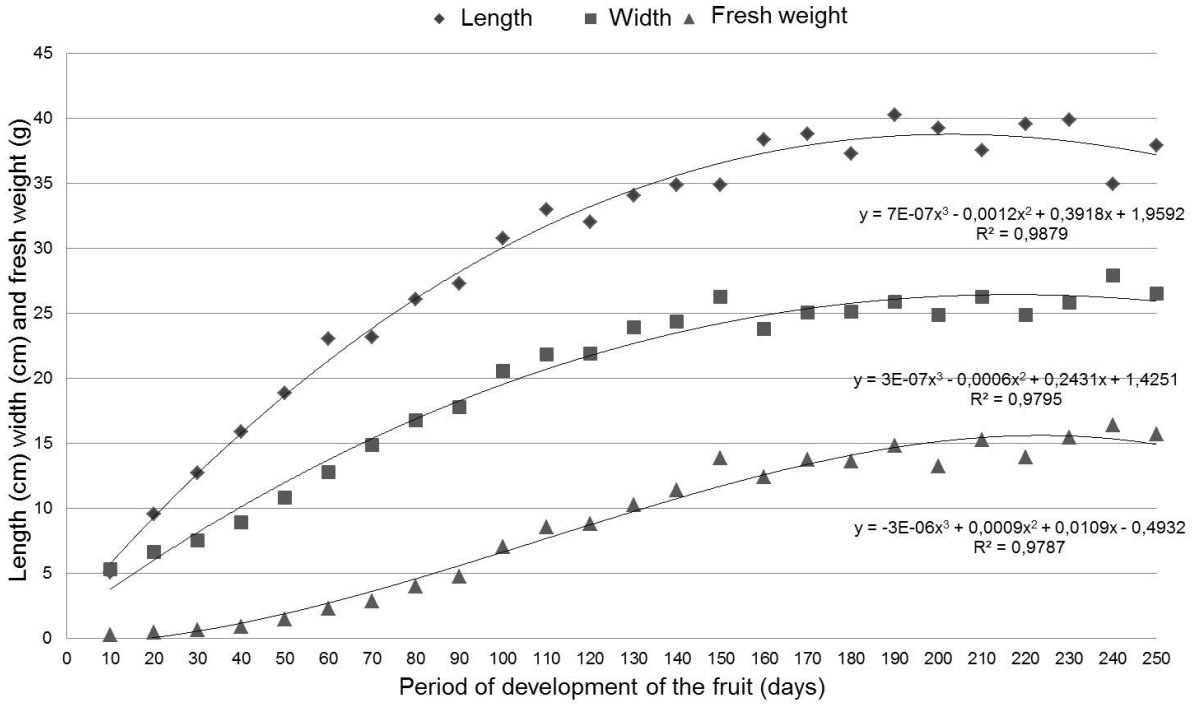

Figure 6. Biometry of the fruit of *Syagrus inajai*, based on development in 10-day intervals, length (cm) width (cm) and fresh weight (g).

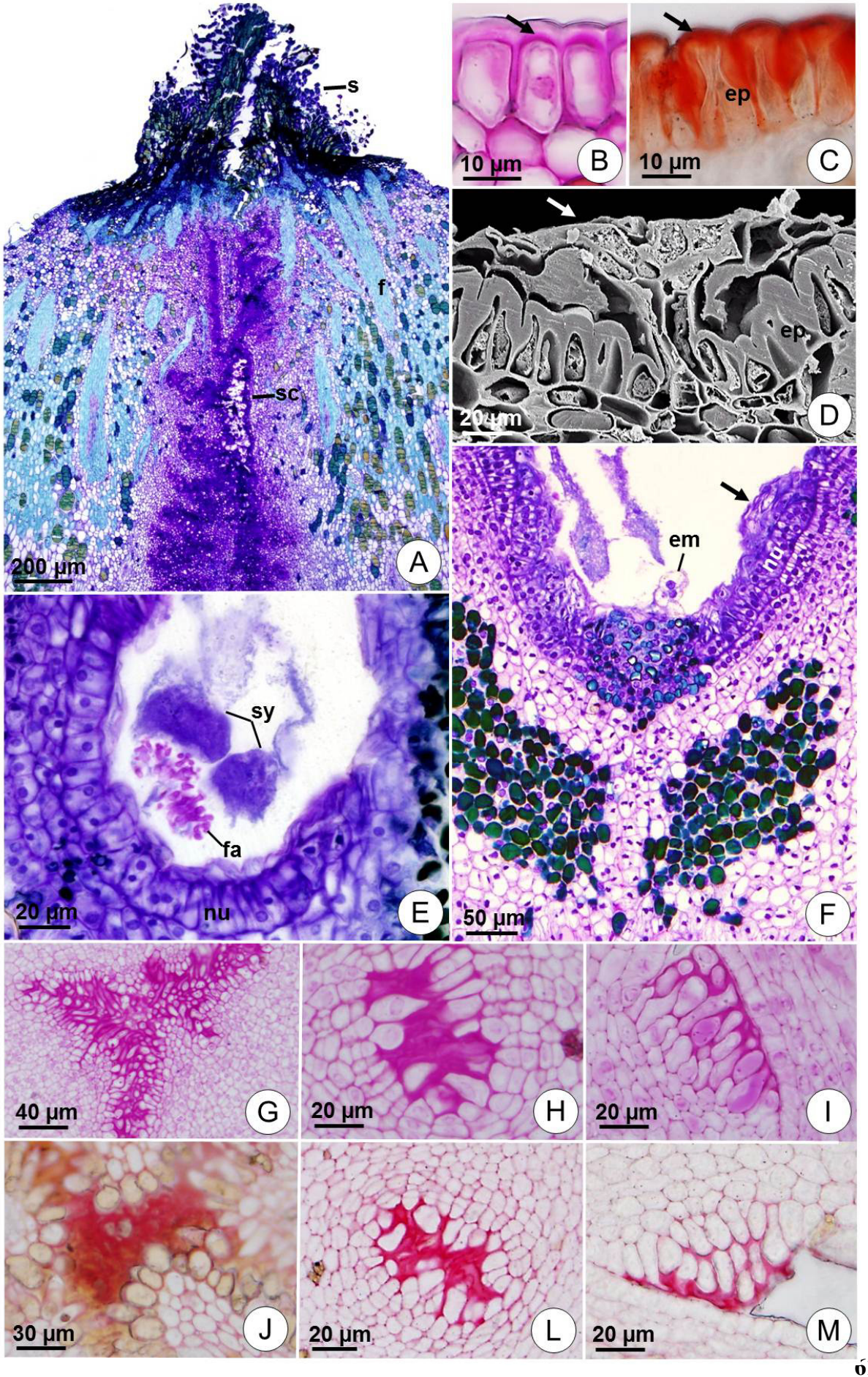
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Figure 7. Fruit of *Syagrus inajai* in early development. A and E-F. Longitudinal section. B-D. Transversal section. B-C and G-M, histochemical tests. A general appearance of the fruit, from the stigma to the stylar channel. B. Epicarp with thickening of the wall (arrow), reaction to Sudan IV. C. Epicarp with thickened cuticle (arrow), reaction to PAS. D. Electronmicrograph of the epicarp with thickened cuticle and multicellular trichoma (arrow). E. Longitudinal section, conspicuous filiform apparatus. F. Seed with the first divisions of the embryo and endosperm in formation. G-I. Detection of polysaccharides with PAS, deep pink staining reaction. G. Reaction in the stylar channel. G. Reaction in the septal cavity. H. Reaction in the cells of the obturator and secretate. J-M. Detection of pectic substances with Ruthenium red, red staining reaction. J. Reaction in the secretion the stylar canal. L. Reaction in the secretion of the septal cavity. M. Reaction in the secretion of the obturator. em, embryo, ep, epicarp f, fibers, fa: filiform apparatus; nu, nucellus; s. stigma, sc, stylar canal, sy, synergid

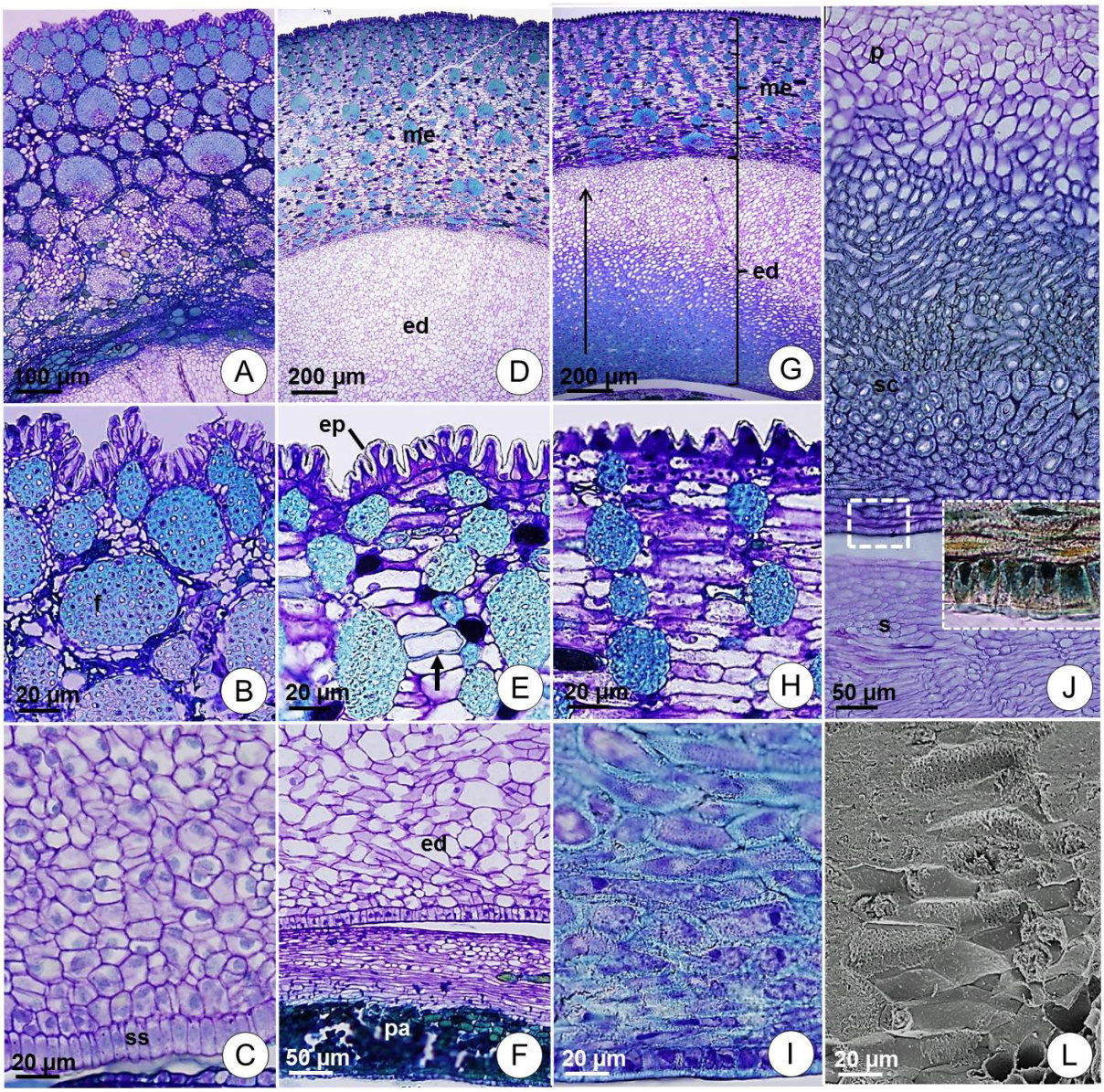

Figure 8. Development of the pericarp of *Syagrus inajai*. Transversal sections. A-C. Fruit at 20 days. D-F. Fruit at 60 days. G-I. Fruit at 110 days. J-L. Fruit at 140 days. A. General appearance of the epicarp, peripheral and median mesocarp. B. Epicarp and peripheral region of the mesocarp. C. Inner region of the mesocarp and endocarp *sensu stricto*. D. General appearance of the pericarp. E. Detail of the peripheral region of the mesocarp, sclerid (arrow). F. Detail of the inner region of the mesocarp and endocarp *sensu stricto* and pachychalazy of the seed. G. General appearance of the pericarp, cells of the inner region of the mesocarp and endocarp *sensu stricto* sclerifying, direction of tissue differentiation (arrow). H. Detail of the epicarp and peripheral region of the mesocarp. I. Endocarp *sensu lato* sclerified. J. Endocarp , endocarp *sensu stricto* (dotted line). L. Electromicography of the endocarp *sensu lato*. ed,

endocarp; ep, epicarp; f, fibers; me, mesocarp; p, parenchyma; pa, pachychalazy, seed, sc, sclerified cells; ss, endocarp *sensu stricto*.

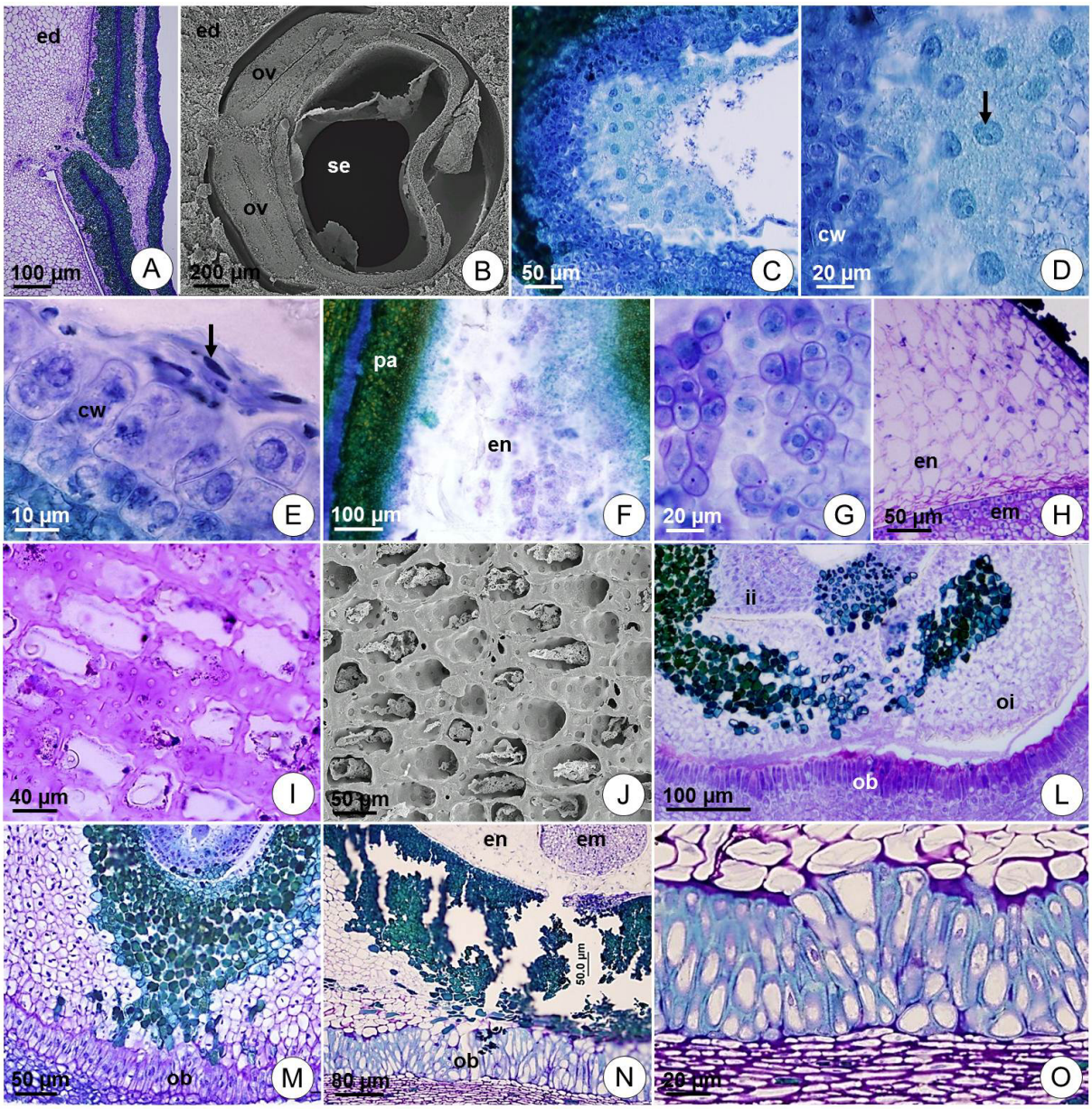

Figure 9. Seed of *Syagrus inajai*. A and B. Transversal sections. C-O. Longitudinal section. A. Collapsed ovules. B. Seed developing and pushing the ovules against the endocarp. C. Seed at 25 days, endosperm, lengthened endosperm in the region of the chalazy. D. Formation of the endosperm, cells with formed wall, followed by free nuclei (arrow). E. Deposition of nuclei on cells of the endosperm with wall formed. F. Appearance of the endosperm at 35 days, cells occupying almost all the space of the seed. G. Detail of the cells of the endosperm. H. Seed at 160 days, thickening of the wall of the endosperm. I. Cells of the endosperm with thickened walls. J. Electromicography of the endosperm, seed at 190 days. L-O. Sclerification

of the cells of the obturator. L. Cells of the obturator in the ovule. M. Cells of the obturator elongating during development of the fruit. N. Seed at 130 days, sclerified cells. O. Detail of sclerified cells. cw, cells of the endosperm with wall; ed, endocarp; em, embryo; en, endosperm; ii, inner integument; ob, obturator; oi, outer integument; ov, ovule; pa, pachychalazy; se, seed.

Capítulo 3

Genovese-Marcomini, P. R. G.; Mendonça, M. S. Carmello-Guerreiro, S. M. Embryonic development of *Syagrus inajai* (Spruce) Becc. (Arecaceae, Arecoideae), an Amazonian palm Submetido a *Australian Journal of Botan*

Embryonic development of *Syagrus inajai* (Spruce) Becc. (Arecaceae, Arecoideae), an Amazonian palm

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Short title: Embryonic development of *Syagrus inajai*

Summary text for the Table of Contents: The study of the embryonic development of palms is essential for understanding the establishment and evolutionary success of these plants. The aim of this study was to carry out a morphoanatomical analysis of the embryo of *Syagrus inajai,* in different phases of its development, culminating in the establishment of four specific development stages. These states are observed in other species of palms. The existence of an embryonic development pattern in this family is therefore proposed.

ABSTRACT

Syagrus inajai (“pupunharana”) is a native palm of Brazil, with phytogeographic prevalence in the Amazon region. A morpho-anatomical analysis was undertaken in order to gain a better knowledge on the embryonic development and germinative process of the *S. inajai*. Plant material was collected from the Campus of the Universidade Federal do Amazonas - UFAM, Manaus, Amazonas, Brazil, and processed using standard morphological and anatomical techniques. The development process of the embryo takes approximately 220 days, and is divided into four stages: proembryo, globular embryo, lateral cordiform and torpedo. The embryo is small, linear, and derived from the terminal cell of the proembryo, arising from mitotic divisions in the apical cell. The embryonic axis is straight, located in the proximal region, aligned parallel to the length of the embryo. The single cotyledon is formed by the ground meristem, procambium and protoderm. The procambium supplies the embryonic axis and the haustorium.

Keywords: Palms, Embryology, Monocotyledons.

Introduction

The Amazonian ecosystem hosts approximately 50% of the Neotropical genera and 30% of the species that comprise the Arecaceae family. Of these, eight genera (24%) are endemic to the Amazon region (Henderson 1995; Henderson *et al.* 1995).

The abundance and diversity of palms indicate that they play an important role the structure and functioning of the ecosystems (Duran and Franco, 1992), and the importance of the Amazon rainforest in the maintenance and expression of this botanical family is evident. According to Miranda *et al.* (2001), the abundance of palms can be explained by their ability to adapt to various types of habitat.

Syagrus inajai ("pupunharana") is a native palm of Brazil, with phytogeographic prevalence in the Amazon region (Leitman *et al.* 2012). Belonging to the Cocoseae Mart. in Endl. tribe, its fruit is characterized by the presence of a hard endocarp with three visible pores (Dransfield *et al.* 2008). The fruits are popularly known as "coquinho" and are widely used by the local communities. The pulp and seeds are consumed *in natura* and the endocarp is utilized in local handicrafts (Henderson *et al.* 1995; Miranda and Rabelo 2006).

However, there is a lack of research into the reproduction process of the species that make up this tribe. This shortfall is partly due to the morphoanatomical characteristics of the fruits and seeds, including: the hardness of the endocarp, the slow development of the pericarp, the large quantity of fibers present in the mesocarp (Merrow 2004; Dransfield *et al.* 2008), the liquid endosperm in which much or all of the development of the seed occurs, and finally, the small size of the embryo.

In order to gain a better understanding of the germinative process and the establishment and propagation of palms, further studies are needed on the reproductive organs, fruits and seeds. Haccius and Philip (1979) consider that the palm embryo appears to have more uniform development, without large variations, as cited in the literature in general. However, due to the lack of research in this area, further studies are needed to corroborate this claim. The majority of works carried out with zygotic embryos of palms were developed in the second half of the last century, e.g. those of: *Areca catechu* L.; tribe Sabaleae; *Chamaerops humilis* L.; *Phoenix; Livistona chinensis* (Jacq.) R.Br. ex Mart. and *Jubaeopsis caffra* Becc. (Rao 1955, 1958; Guignard 1961; Biradar 1968; Mahabale and Biradar 1967; Biradar and

Mahabale 1968; Kulkarni and Mahabale 1974; Robertson 1976). However, a growing number of works are being carried out with somatic embryos, cultivated *in vitro*, with the aim of enabling the production and commercialization of clones (Karunaratne and Periyapperuma 1989; Sane *et al.* 2006) as *Cocos nucifera* L. (Nunez *et al.* 2006) and *Phoenix dactylifera* L. (Othmani *et al.* 2009). The methods used in these studies have managed to overcome some of the difficulties imposed by the nature of the tissues of the plant.

Despite the efforts made so far, according to Ribeiro *et al.* (2011), we still do not have a detailed description of the embryonic axis of the embryo in palms. According to Natesh and Rau (1984) there are few studies on all the aspects of embryogenesis, zygote formation, and organization of the meristems.

Therefore using *Syagrus inajai*, we seek to contribute information on the embryonic development of palms which will further enhance our understanding of the germinative process of plants of the Arecaceae family.

Therefore, seeking to contribute information on the embryonic development of palms which will further understanding of the germinative process of plants of the Arecaceae family, we decided to undertake the study of embryonic development of *S. inajai*.

Material and methods

Plant materials were collected in 2009 and 2010 from a forest area the Campus of the Universidade Federal do Amazonas - UFAM, Manaus, Amazonas, Brazil, in an area of baixio (gallery forest), around coordinates 03 º 05' 45.84" S and 59 º 58' 43.69", under Afi climatic conditions (Köppen 1931). The material was analyzed at the Laboratório de Botânica Agroflorestal [Agroforestry Botanical Laboratory] - LABAF, in the same University. For the morphoanatomical description, adult individuals of *S. inajai* were selected, from which one rachilla was removed every ten days, beginning 30 days after the opening of the spathe, when the pistillate flowers also opened.

The embryo length was measured every ten days. Using these measurements, the ratio between the developmental periods and the embryo length was calculated by means of a regression study using Origin 8.6 software.

For the anatomical study, the collected material (whole fruit in various stages) was fixed in FAA $_{70\%}$ (formol: acetic acid: ethylic alcohol 70%) and conserved in 70%

ethylic alcohol (Kraus and Arduin 1997). The samples were dehydrated in ethyl series (70%- 95%), embedded in 2-hydroxyethyl-methacrylate (Historesin® Leica, prepared according to the manufacturer's instructions), sectioned to thicknesses of 4 to 7 µm in a rotary microtome, and stained with 0.5% toluidine blue in citrate buffer, pH 4.0 (O'Brien *et al.* 1964). The slides were mounted in water.

Samples were fixed in BNF (Buffered Neutral Formalin) (Lillie 1965) solution, dehydrated in ethyl series, and dried using the critical point technique with CO_2 on a Balzers dryer (model CPD 030). The samples were collected in a metal support, covered with gold (Balzers SCD 050) and observed under a JEOL JSM 5800LV (10 kV) Scanning Electron Microscope (SEM), at the Institute of Biology/Unicamp.

The results were documented using an Olympus (BX51) photomicroscope with Olympus (DP71) photographic camera, and a Leica (M125) stereomicroscope with Leica (DFC 490) photographic camera. Indian ink drawings were also made.

Results

The growth of the embryo of *S. inajai* follows the sigmoid function model of Boltzmann (Fig 1).

Proembryo stage

The start of embryogenesis was observed at around thirty days after the start of endosperm formation. The zygote is approximately the same size as the egg cell (Fig. 2a) and is located in the micropilar region. Its first mitotic division occurs on the periclinal or oblique plane, resulting in a larger basal cell and smaller apical cell than those that comprise the proembryo (Fig. 2a). Next division occurs on the anticlinal plane, which may be initiated with the basal cell or the apical cell (Fig. 2b-d). The apical cell may also divide primarily in the periclinal plane (Fig. 2e). The two cells originating from the apical cell subsequently divide in the periclinal, oblique or anticlinal planes, with no particular pattern being observed (Fig. 2f-j). When the cells derived from the apical cell reach nine in number, it is possible to see a larger polarized terminal cell, precursor of the embryo, which is marked by a densely stained nucleus and dense cytoplasm (Fig. 2l). The other cells derived from basal and apical cell form the small suspensor, comprised of approximately eight cells (Fig. 2m-n). The two conspicuous synergids persist until the start of the globular stage of the embryo (Fig. 2i).

Globular stage

Approximately 30 days after mitosis of the zygote, the terminal cell, precursor of the embryo, derived from apical cell division, undergoes successive divisions, in different planes, until it forms the globular body of the embryo, a process that takes around 50 days. Concomitantly with these divisions the formation of the protoderm occurs, which is established by anticlinical divisions. With the end of this stage comes the formation of the embryo, with globular formation and radial symmetry (Fig. 2m).

Lateral cordiform stage

It was possible to observe, in the lateral terminal region of the embryo at around 90 days, the start of differentiation of the stem promeristem, which is characterized by a group of cells with dense cytoplasm and large, densely stained nuclei (Figs. 2m and 3a,a`). The position of the shoot apical promeristem, dislocated from the center, shows asymmetry, with uneven distribution of the tissues surrounding it, the larger side corresponding to the developed cotyledon (Fig. 2o-r) and the smaller side to the undeveloped cotyledon (Fig. 3a). The presence of this vestigial tissue relates to the second cotyledon of the dicotyledons (Fig. 3a). The differentiation of the promeristem marks the end of the globular phase; the radial symmetry is replaced with bilateral symmetry, which will continue until the end of its development (Fig. 3a').

Alongside with the development of the shoot apical promeristem, the cotyledonary primordium surrounding it begins to grow over the promeristem (Fig. 2n) up to the junction of its borders (Fig. 3a-e'), a process that takes around 30 days and culminates with the intrusion of the promeristem and the formation of the cotyledonary cavity (Fig. 3 a-e). The basal region of the cotyledon grows around the shoot apical promeristem, and shifts towards the center, repositioning it so that it becomes central, with an inclination of 90 °C, parallel to the body of the embryo (Fig. 3a-e). The upper region of the cotyledon includes the terminal portion of the shoot apical promeristem (Fig. 3c-d).

The primary cells of the procambium are observed in the embryo at around 100 days, when they begin to differentiate around the shoot apical promeristem, within the confines of the cotyledon (Figs. 4a-b). These cells present dense cytoplasm and large, densely stained nuclei (Fig. 6a).

Torpedo stage

At 110 days, an increase in embryo length is observed (Fig. 5), with the maximum value at between 190 and 220 days (Figs. 1 and 5).

At the end of the process of intrusion of the shoot apical promeristem, intense cell division is observed in the upper region of the cotyledon. This gives rise to lengthening of the embryo, which expands in the opposite direction to that of the micropile by cell division, leading to the development of the distal region of the embryo and exerting a haustorial function (Fig. 2n and 3c-d, c'-e'). With the lengthening of the cotyledon, the embryo becomes conical in shape (Figs. 2q and 3d-e). The protoderm accompanies the lengthening by anticlinal divisions, and is continuous in the cotyledonary cavity surrounding the shoot apical promeristem.

At around 120 days, the shoot apical promeristem is internally located (Fig. 4d,h), forming the cotyledonary cleft (Figs. 3c-e and 4h-i), which is imperceptible externally in the developed embryo and can only be visualized in cross-section (Figs. 5 and 6m). The presence of the cotyledonary cleft shows the point of meeting of the borders of the basal and upper regions of the growing cotyledon (Fig. 3a-e'). Concomitantly with the lengthening of the upper portion of the cotyledon, the formation of the primary leaf primordium is observed, its differentiation occurring subsequently to the intrusion of the shoot apical promeristem (Figs. 3d; 4d and 6b). In this phase, two morphoanatomical regions are distinguished in the embryo, namely, the distal and the proximal (Fig. 4d). From the cotyledonary node, the procambium emits eight traces to the proximal region, which will irrigate the leaf primordia and the radicle, and eight traces to the distal region, which will irrigate the haustorium (Figs. 3c-e and 4a-j).

At around 130 days, it is possible to discern, on the opposite border of the shoot apical promeristem of the embryo, the initial cells of the second leaf primordium (Fig. 6c). Next to the micropile, in the opposite direction to the plumule, we observed that the cells of the procambium are organized in a semicircle, with a rudimentary embryo axis clearly visible (Fig. 6c).

The cells of the procambium, arranged in a semicircle, are elongated (Fig. 6b). At the apex there is a set of juxtaposed, tiny cells, comprising the radicular promeristem (Fig. 6d-f).

The first cells of the third leaf primordium are visible in the embryo at around 140 days.

During the formation of the distal region of the embryo, the cells of the ground meristem located inside the procambial strands increase in number and size. At the end of the lengthening, these cells are characterized by the presence of large vacuoles (Fig. 4d).

Embryo stage

At the moment of dehiscence of the fruit, around 270 days after the opening of the spathe, the embryo is at around 220 days, approximately 8 mm in length, straight, lateral, oblique and small (Figs. 1 and 5).

The proximal region of the embryo is cylindrical, with the funnel-shaped apex in contact with the micropile. Its surface is smooth (Fig. 7b), except for a protuberance on the apex, which is a vestige of the suspensor (Fig. 7c). It is composed of the cotyledon sheath, leaf primordia and embryo axis (Fig. 6g). The protoderm presents cuboid cells and one two stomata are also visible (Fig. 6o). The cells of the ground meristem are heterodimensional (Fig. 7d) with idioblasts containing raphides (Fig. 6q). The embryo axis is short, and parallel to the length of the embryo (Fig. 6g). The hypocotyl-radicle is short, with a radicle presenting a procambium, ground meristem and radicular promeristem, characterized by small, juxtaposed cells, without a differentiated protoderm (Figs. 6e-f). The three leaf primordia and shoot apical promeristem form the plumule, located in the cotyledonary cavity and formed by three leaf primordia and the shoot apical promeristem with a protoderm of isodiametric cells, procambial strands and ground meristem (Figs. 6e,j-l and 7d).

The distal region of the embryo has laminar appearance (Fig. 5 and 7a). It has a sinuous surface, forming elevations that increase the contact surface with the endosperm and can be observed along the embryo (Figs. 5 and 7a). The protodermal cells are radially elongated and larger than those of the proximal region (Fig. 6p). The cells of the ground meristem have large vacuoles (Figs. 6 g,n,p). The procambial strands become gradually more peripheral as they distribute approximately 18-26 strands to the outer edges of the distal region, and parallel to the length of the embryo (Figs. 4d,i-j; 5 and 6g).

The procambium is arranged along its length, forming two loops, emerging from the cotyledonary node (Figs. 3e and 4d). A larger loop with eight traces branches into 18-26 strands (Fig. 4h-j and 6n) peripherally providing with vascular

bundles in the distal region of the cotyledon, which are responsible for the translocation of substances from the endosperm to the embryo; and another smaller loop in the proximal region (Fig. 6f) comprising the radicular procambium, with eight traces issued to the plumule (Figs. 4e-f and 6h-j).

Discussion

The sigmoid growth curve observed for the embryo of *S. inajai* has also been reported for *Elaeis guineensis* Jacq. (Fernandino *et al.* 1985), and is similar to the typical growth curve of dicotyledons.

The embryo of *S. inajai* is comprised only of cells derived from the apical cell, characterizing it as the Onagrad type, according to Johansen (1950). The same characteristic was observed in *Areca catechu, Sabaleae* and *Cocos nucifera* (Rao 1955; Haccius and Philip 1979). In studies carried out with *Phoenix and Livistona chinensis* (Biradar 1968; Mahabale and Biradar 1967; Biradar and Mahabale 1968; Kulkarne and Mahabale 1974), it was found that the embryo was of cells derived from apical cell and basal cell, classified as the Asterad type.

The shoot apical promeristem was described as lateral terminal in *S. inajai*, as although its origin is terminal, it does not occur in the center of the embryo axis, but it is displaced. Haccius and Philip (1979) describe the origin of the shoot apical promeristem as terminal in *Cocos nucifera*, although they report that the subsequent development of the cotyledon causes the shoot apical promeristem to become lateral. Guignard (1984) argues that there is a transition from dicotyledons to monocotyledons in relation to the position of the shoot apical promeristem, and sees this character as transitory in palms. This author believes that in *Cocos nucifera*, the quiescent central axial zone is positioned between the two meristem zones, but only one of them continues its development. In other monocotyledons, the position of the shoot apical promeristem becomes progressively more lateral, until it is entirely lateral, as observed in *Cyperus fuscus* L.. Therefore, the other meristematic zone of *Syagrus inajai* does not develop, because it is a vestigial cotyledon.

The lateral cordiform stage is characterized by the formation of the shoot apical promeristem in the lateral terminal region of the embryo, and by the growth of the edges of the cotyledon around it. The longitudinal section of this region reveals the embryo, with cordiform appearance similar to the cordiform stage in dicotyledons. However, what we see in *S. inajai* is the edges of a single cotyledon that grows

around the shoot apical promeristem. According to Guignard (1984) and Cronk (2009), the embryos of monocotyledons have a single cotyledon, which initiates its development early. Mahabale and Biradar (1968), studying the embryo of *Phoenix sylvestris* (L.) Roxb. in longitudinal plane, describe two cotyledons. However, Haccius and Philip (1979), studying the embryo of *Cocos nucifera* in the frontal longitudinal and sagittal longitudinal planes, observed that this was an error caused by the section plane.

In *S. inajai* the growth of the cotyledonary regions is not uniform in space and time. The basal portion plays a more active role in the intrusion of the shoot apical promeristem, with intense cell division at the start of embryo development, while the upper region elongates in the opposite direction to the micropile, forming the haustorial region, with intense cell division after the intrusion of the shoot apical promeristem. The same sequence of events was observed in *Cocos nucifera* (Haccius and Philip 1979).

The position of the embryo axis parallel to the length of the embryo, as observed in *S. inajai*, *Borassus flabellifer* L. and *Jubaea chilensis* (Molina) Baill., *Phoenix roebelenii* O'Brien and *Allagoptera leucocalyx* (Mart.) Kuntze (Dassanayake and Sivakadachchan 1973; Iossi *et al.* 2003; Henderson 2006), is mainly due to the growth of cells close to the shoot apical meristem, in the basal region of the cotyledon. The embryo axis, arranged obliquely to the length of the embryo, as observed in species of *Euterpe precatoria* Mart., *Oenocarpus minor* Mart., *Acrocomia aculeata* (Jacq.) Lodd. ex. Mart. (Aguiar and Mendonça 2003; Oliveira *et al.* 2010; Ribeiro *et al.* 2011) may be the result of a greater participation of the upper region of the cotyledon in the process of intrusion of the shoot apical promeristem, with a smaller addition of cells in the basal region, as observed in *Cocos nucifera* (Haccius and Philip 1979).

The development of the shoot apical promeristem in *S. inajai* occurs at the start of embryogenesis, after the formation of the globular stage of the embryo. The same sequence of events was observed in the zygotic embryo of *Cocos nucifera* and *Elaeis guineensis* (Haccius and Philip 1979; Kanchanapoom and Domyoas 1999) and in the somatic embryo of *Phoenix dactylifera* L. (Sane *et al.* 2006). The development of the radicular promeristem occurs after the intrusion of the shoot apical promeristem and start of differentiation of the primary leaf primordium, in

accordance with Haccius and Philip (1979). The radicle can be seen visualized in sections 3 to 5 µm thick, due to the small size of the cells that comprise it. It is characteristic of cells of the procambium, arranged to form a cap in longitudinal plane, by cells of the ground meristem and by the radicular promeristem, comprised of small, densely blue stained cells. The presence of the radicle was also reported in embryos of *Allagoptera arenaria* (Gomes) Kuntze, *Acrocomia aculeata, Borassus flabellifer, Euterpe edulis* and *Livistona chinensis* (Dassanayake and Sivakadachchan 1973; Kulkarni and Mahabali 1974; Sylvestre *et al.* 1989; Panza *et al.* 2004; Ribeiro *et al.* 2011). However, in *Euterpe precatoria* Mart. and *Oenocarpus minor* (Aguiar and Mendonça 2003; Oliveira *et al.* 2010), the presence of a radicle pole was described. The fact that the plumule is located in a cotyledonary cavity, being covered by protoderm makes it easier to observe than the radicular hypocotyl axis, which is inserted in the ground meristem without a protoderm, which can make it difficult to distinguish.

In the embryo at 140 days, approximately 80 days before the dispersal of the fruit of *S. inajai* it is possible to observe their distal and proximal regions and its embryonic axis. According to Tomlinson (1990), it is possible to see two morphoanatomical regions in the embryo of palms; the proximal region, known as the cotyledonary petiole, and the distal region, with haustorial function. These two regions were observed in mature embryos of various palm species, such as *Attalea maripa* (Aubl.) Mart., *Euterpe precatoria*, *Archontophoenix alexandrae* (F.Muell.) H.Wendl. & Drude*, Acrocomia aculeata* and *Oenocarpus minor* (Araujo *et al.* 2000; Aguiar and Mendonça 2003; Charlo *et al.* 2006; Moura *et al.* 2008; Oliveira *et al.* 2010, Ribeiro *et al.* 2011).

According to Orozco-Segovia *et al.* (2003) anatomical immaturity is common in the embryo of palms during dispersion of the fruit, attributing morphological dormancy to the seed. Baskin and Baskin (1998) suggested that morphological dormancy occurs when the embryo is undifferentiated, rudimentary, or in the torpedo stage at the moment of dispersion. However, the embryo of *S. inajai* does not present these characteristics, as its embryo axis differentiates around 50 days before dispersion of the fruit. At approximately 190 days, the embryo is around 8 mm in length, close to the size observed in dispersion. Haccius and Philip (1979) report that the plumule in *Cocos nucifera* initiates its development before the cotyledon reaches

its full length. Ribeiro *et al.* (2011) Hemanthakumar *et al.* (2012) managed to produce germination of the zygotic embryo of *Calamus thwaitesii* Becc.; by removing the fruit months before dispersion, they promoted the extraction of young embryos of the fruit for cultivation *in vitro*, when the tissue damage is reduced and the embryo is viable.

In the protoderm of *S. inajai*, the presence of apparently inactive stomata was observed, and the same has been reported for *Acrocomia aculeata* (Ribeiro *et al.* 2011). However, the authors believe that its presence may be related to the oxygen demand in the initial phases of germination. The presence of idioblasts with raphides in the ground meristem was also reported by Zona (2004) and Oliveira *et al.* (2010). According to Zona (2004), the presence of raphides is common in the Areceae tribe. However, in the species of the *Syagrus* genus studied by the author, these were not observed, and the author reports the need for additional sampling. For the author, the probable function of the raphide crystals in the embryos is the storage of calcium oxalate and or hydrogen peroxide. Moura *et al.* (2010) and Ribeiro *et al.* (2011) observe raphides in *Acrocomia aculetata* only after germination.

The arrangement of the procambium in the embryo resembles two loops, the larger with traces issued to the distal region, and the smaller with the traces to the plumule and embryo axis. The same was also observed in *Cocos nucifera* and *Acrocomia aculetata*. However, in these two species, the occurrence of a procambium around the plumule was observed, which was not observed in *S. inajai* (Haccius and Philip 1979; Moura *et al.* 2008; Ribeiro *et al.* 2011).

Conclusion

The embryo of *S. inajai* presents four specific stages of development: the proembryo stage, the globular stage, the lateral heart stage, and the torpedo stage. These stages are also observed in other species of palms. We therefore propose the existence of a pattern of embryo development in this family.

The greater participation of the basal region of the cotyledon in the process of intrusion of the shoot promeristem and formation of the cotyledonary cavity results in the central positioning of the embryo axis, parallel to the length of the embryo. Therefore, the final position of the embryo axis, whether parallel or oblique, is related to the growth dynamic of the basal and upper regions of the cotyledon during the process of intrusion of the shoot promeristem.

Finally, we found that the embryo of *S. inajai* does not present morphoanatomical immaturity.

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Fig. 1. Regression analysis of the growth of the embryo of *Syagrus inajai*. Measurements of embryo length at 10-day intervals.

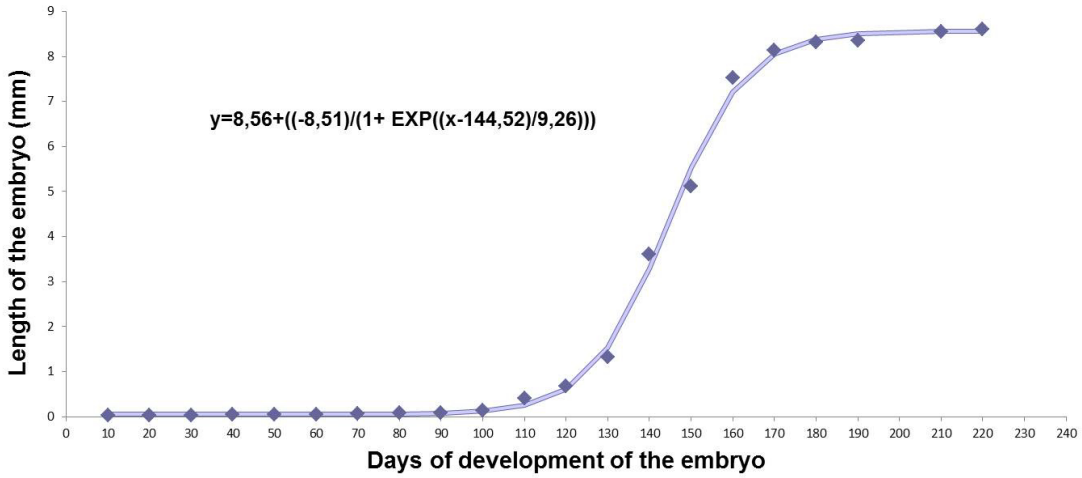
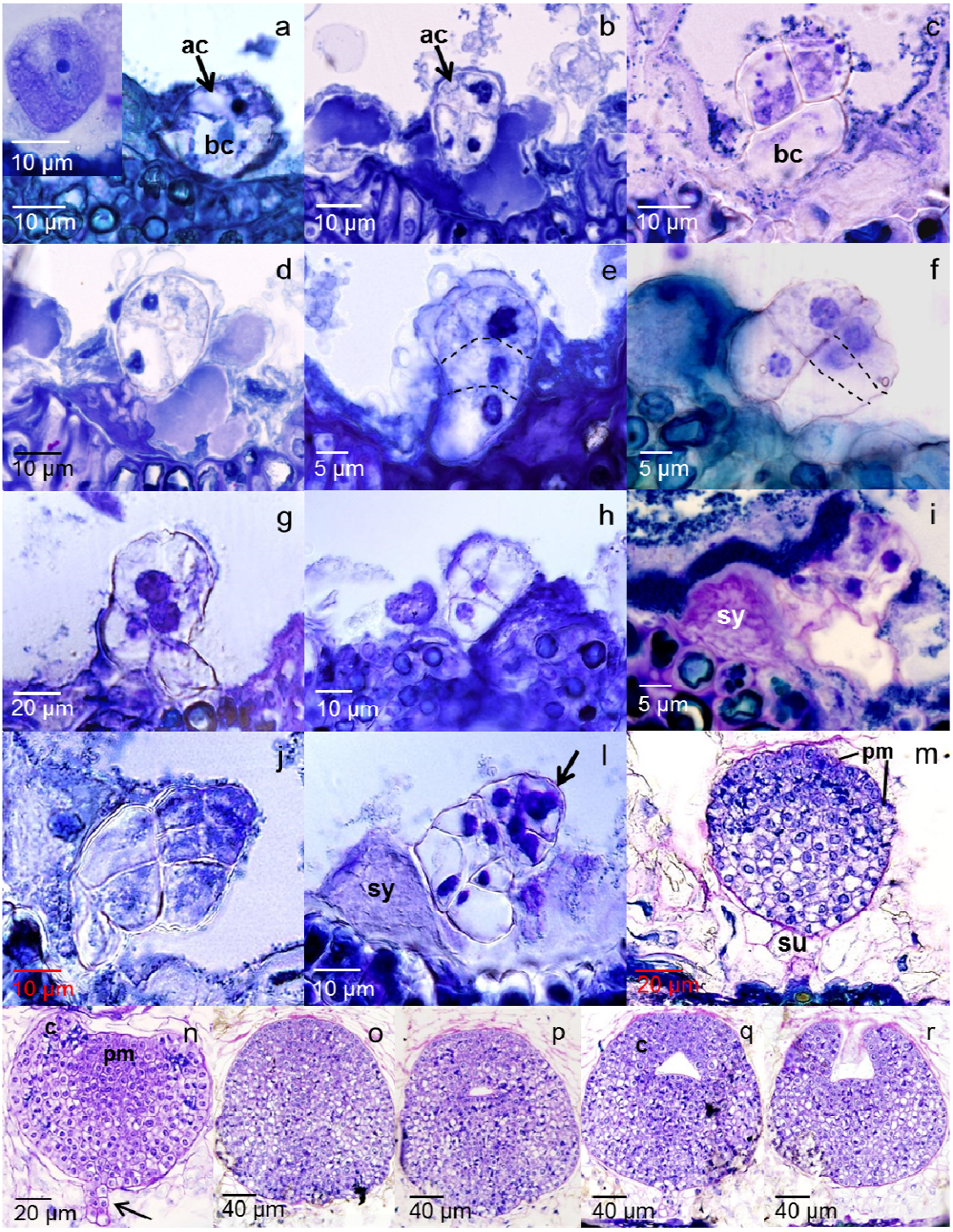


Fig. 2. Photomicrograph of longitudinal section of the development of the proembryo and zygotic embryo of *Syagrus inajai*. a) Zygote, first division, detail of the basal cell, apical cell (arrow); b) Second basal cell division, apical cell (arrow); c) Second apical cell division; d) Quadratic proembryo; e) Periclinal division in the apical cell; f-g) Second division, cells derived from apical cell; h) Third division, cells derived from apical cell; i) Proembryo with six cells; j) Proembryo with nine cells; l) Proembryo with polarized cell (arrow); m) Globular stage; embryo at 80 days; n) Longitudinal sagittal section of the embryo at 90 days, suspensor arrow; o-r) Sequence of sections on the longitudinal sagittal plan of the embryo at around 95 days; o) Central region of the embryo; p) Start of the cotyledonary cavity and shoot apical promeristem; q) Edges of the cotyledon; and r) Lateral with free borders. Initials: ac, apical cell; bc, basal cell; co, cotyledon; pm, promeristem; su, suspensor; sy, syndergids.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40 µm

Fig. 3. Development and internalization of the shoot apical promeristem and formation of the procambium in the embryo of *Syagrus inajai*. a-e) View in longitudinal section; a'-e) View of the surface; a) Start of shoot promeristem; b) Start of cotyledonary growth; c) Cotyledon growing around the shoot promeristem; d) Elongation of the cotyledon; and e) Internalization of the plumule. Initials: bc, cotyledonary base; ca, cotyledonary cavity; ec, cotyledonary edge; p, plumule; pm, promeristem; pr, procambium; ps, procambial strand; uc, upper cotyledon.

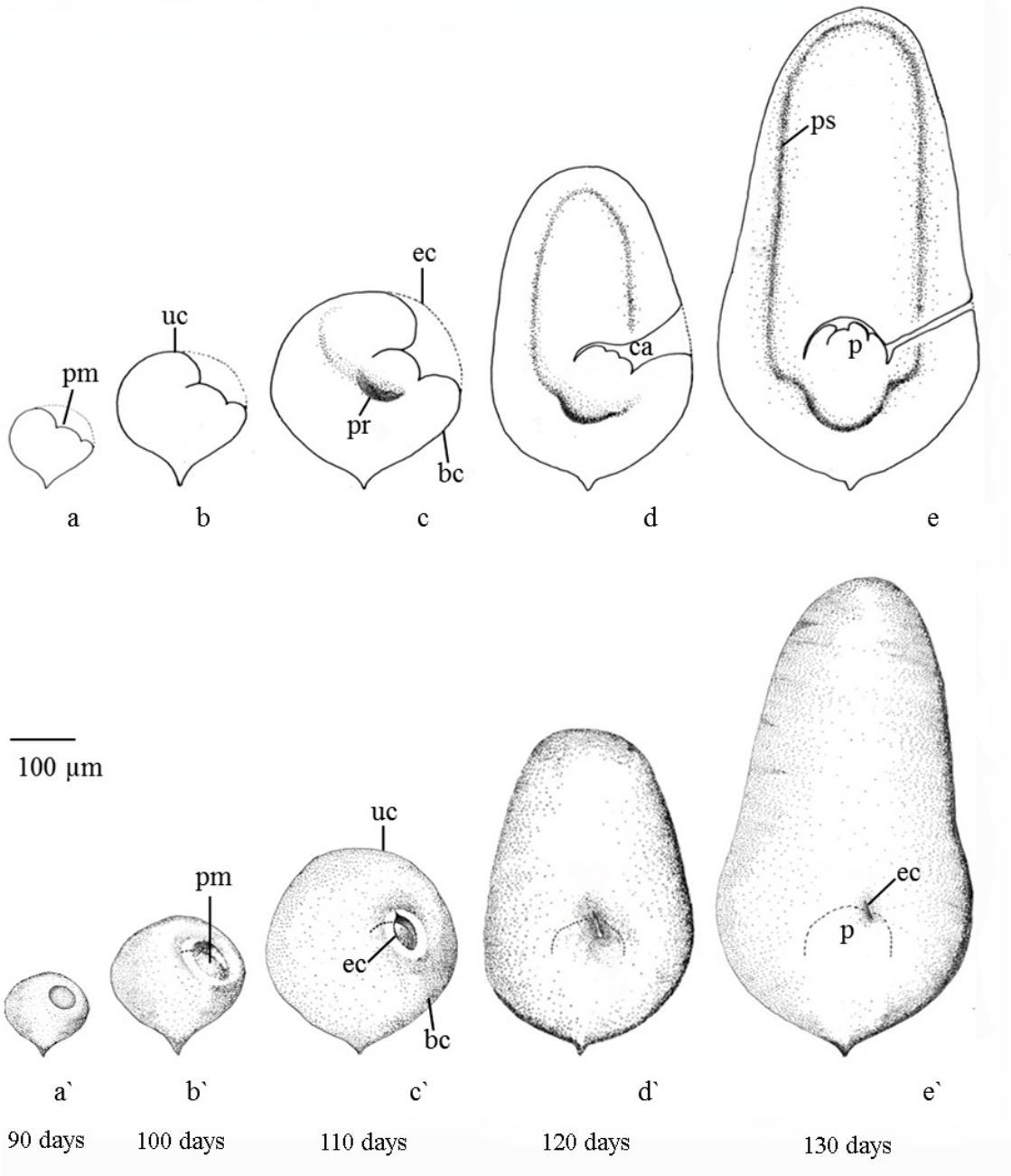

Fig. 4. Photomicrograph of the embryo of *Syagrus inajai.* a-c) Embryo at 110 days; a) Longitudinal sagittal section, procambium (arrow); b-c) Frontal longitudinal section; b) Growth of the border around the shoot apical promeristem, procambium (arrow); c) Region of the embryo with shoot apical promeristem; d-j) Embryo of *S. inajai* at approximately 120 days. a) General appearance of the embryo in longitudinal section, line indicating position of the transversal sections; e-j) Sequence in transversal section of the body of the embryo; e) Procambium of the radicle; f) Procambial strands of the hypocotyl; g) Base of the plumule; h) Apex of the plumule; i) Cotyledonary gap (arrow); and g) Distal region of the embryo. Initials: p, plumule; pm, promeristem; ps, procambial strand.

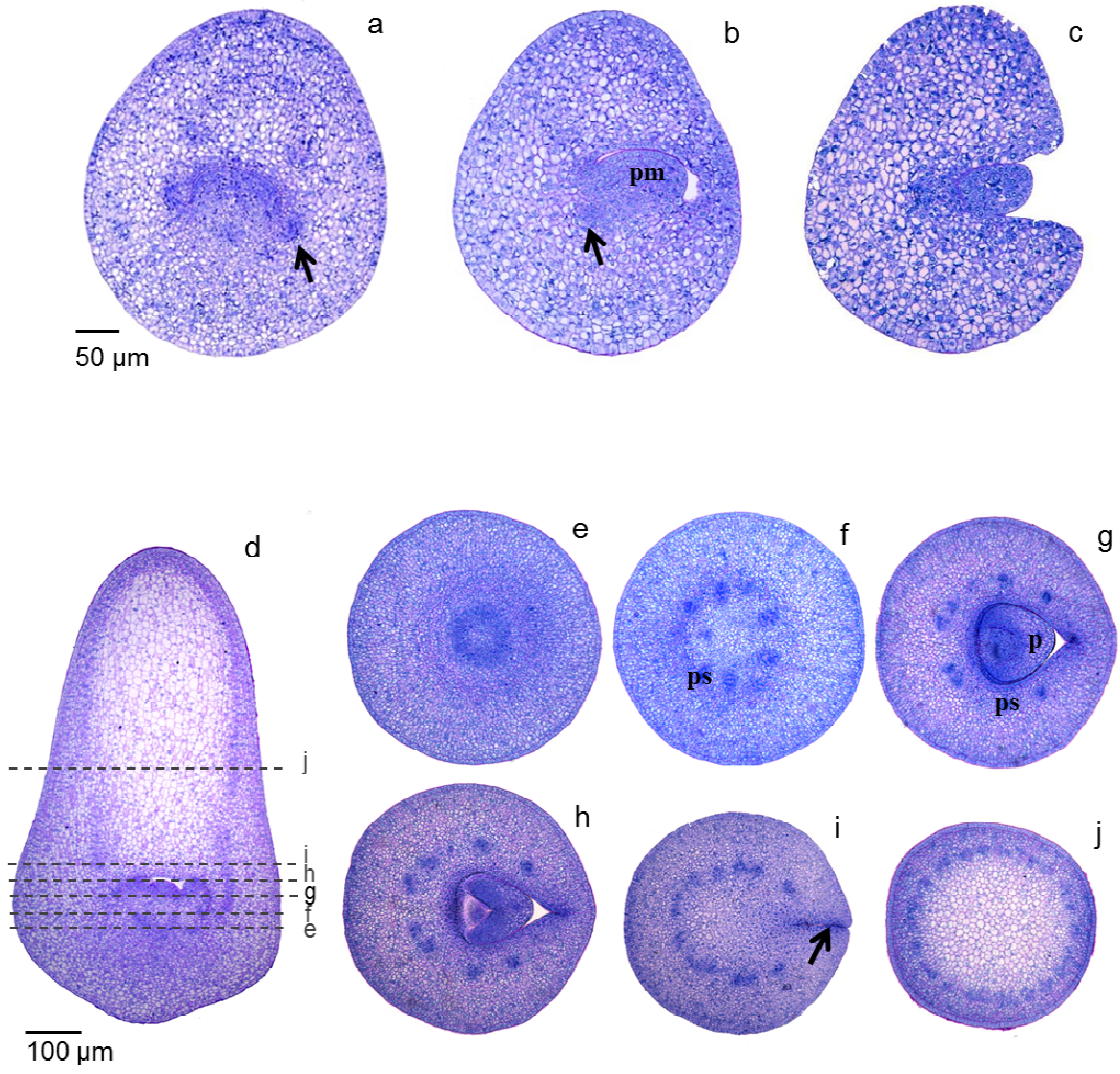

Fig. 5. Morphological appearance of the development of the embryo of *Syagrus inajai*, days after fertilization, relief formed by the procambial strands (arrow). Initials: dr, distal region; pr, proximal region; bar 3mm.

Fig. 6. Photomicrograph of the embryo of *Syagrus inajai*. a-f) Development of the embryo axis in longitudinal section; g-q) Fully-formed embryo; a) Shoot apical promeristem (arrow); b) First leaf primordium; c) Start of formation of the second leaf primordium; d) Visible procambium; e) Fully-formed embryo axis; f) Detail of the radicle); g) General appearance of the embryo at 210 days; h) Procambium of the radicle; i) Procambial strand; j) Start of the plumule; l) Plumule; m) Cotyledonary border forming the cotyledonary gap (arrow); n) Region of the haustorium; o) Protoderm of the proximal region, stomata (arrow); p) Epidermis in the distal region; and q) Idioblasts with raphides. Initials: ca, cotyledonary cavities; dr, distal region; l1, first leaf primordium; l2; second leaf primordium; l3, third leaf primordium; gm, ground meristem; p, plumule; pc, procambium; pm, promeristem; pr, proximal region; ps, procambial strand.

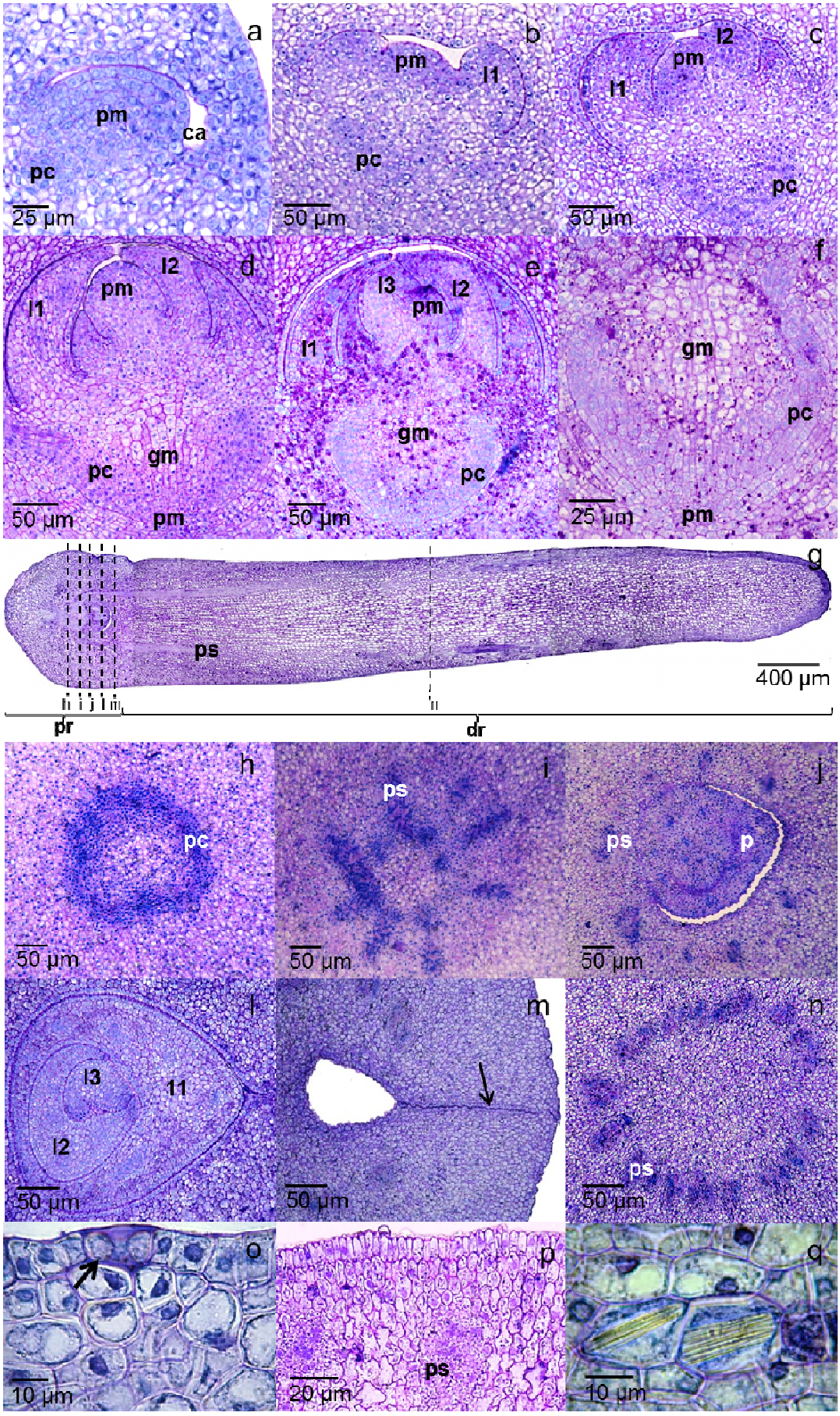
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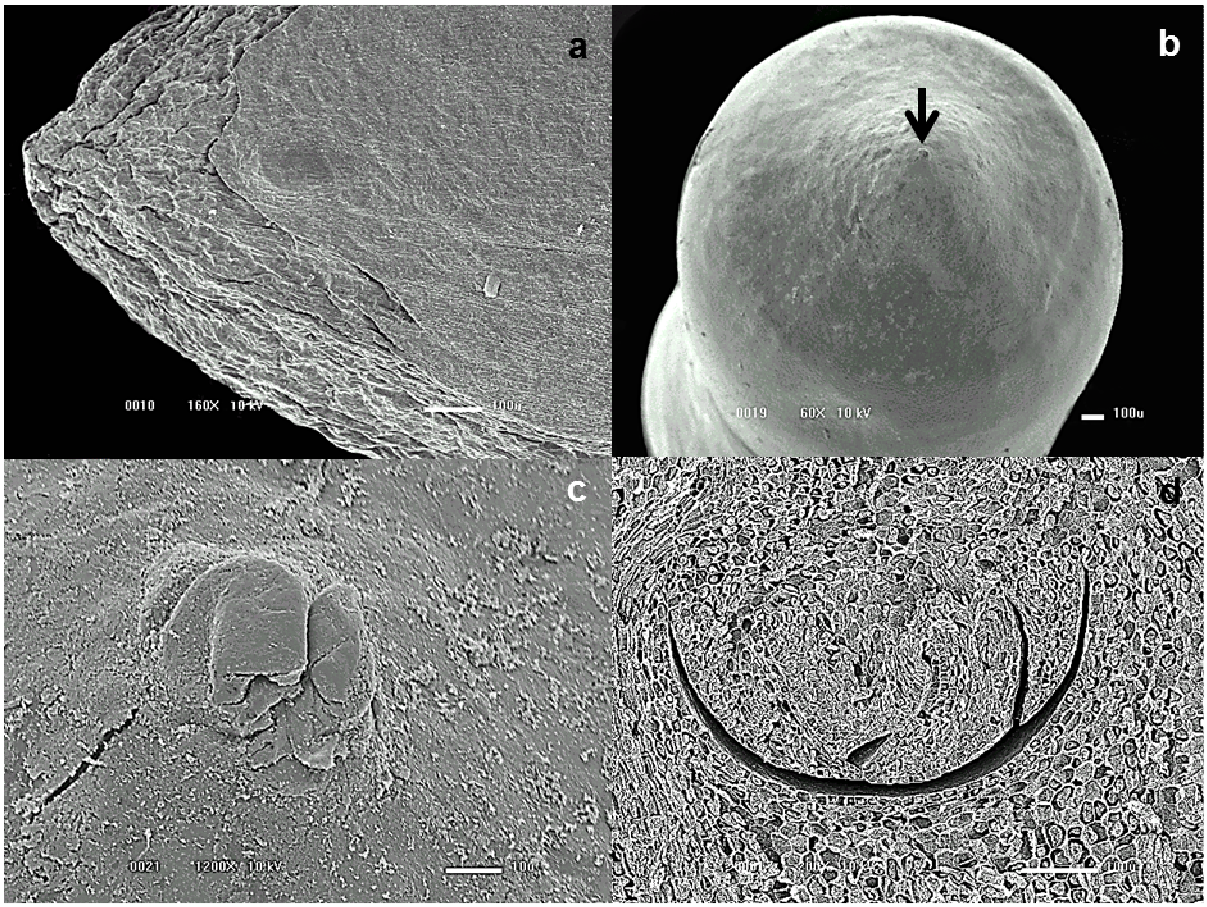
Fig. 7. The micrographs from the scanning electron microscope of the appearance of the embryo of *Syagrus inajai* at the moment of dispersion. a) Haustorial region; b) Proximal region, suspensor (arrow); c) Vestige of the suspensor; and d) Appearance of the plumule.



Capítulo 4

Genovese-Marcomini, P. R. G.; Mendonça, M. S. Carmello-Guerreiro, S. M. Germination process of the palm *Syagrus inajai* (Spruce) Becc. (Arecaceae): a morphoanatomical and histochemical approach. Submetido a *Australian Journal of Botany*

Germination process of the palm *Syagrus inajai* (Spruce) Becc. (Arecaceae): a morphoanatomical and histochemical approach

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Short title: Germination process of the palm *Syagrus inajai*

Summary text for the Table of Contents: Study of the germination process of *Syagrus inajai* enables an understanding of the events responsible for the establishment of the seedling, and that guarantee its reproductive success. Through this study, five distinct phases were recognized in the germination process of the species, based on which the average times were estimated. The results obtained add to the knowledge of palms, and enable better use of this natural resource, as well as providing useful information for the preservation of the species, and their planting.

Abstract

Syagrus inajai (Spruce) Becc. popularly known as “pupunha-brava”, grows in the municipality of Manaus, in forest of the “baixio” (gallery), secondary, and “terra firme” types, as well as in natural clearings. This work aims to describe the morphoanatomy of the germination process of *S. inajai*, and to determine its average duration; to identify the ergastric substances present in the embryo, haustorium and endosperm; and to perform a centesimal analysis of the seed composition. The germination process of 300 seeds, collected during dispersion and put to germinate, was monitored on a weekly basis. The anatomical study followed the usual light microscopy procedures, with the performance of histochemical tests. The germination of *S. inajai* starts at around 101 days after seeding, with the formation of the germinative button. The morphological events that followed were: lengthening of the hyperphyll, intumescence of the cotyledonary sheath, emergence of the primary root and first cataphyll, emergence of the second cataphyll and eophyll. Lengthening of the embryo axis was observed after the emergence of the germinative button, during the process of lengthening of the hyperphyll. It was possible to see the primary root with the naked eye after the intumescence of the cotyledonary sheath, at which moment the two cataphylls and the eophyll were differentiated in the cotyledonary sheath. Starch was observed in the embryo before germination, but its quantity increased in the parenchyma cells after the formation of the germinative button. The seed presented 30% lipids in its concentration at the moment of dispersion.

Keywords: Attaleinae, palms, embryo, seedling, ontogenesis.

Resumo

Syagrus inajai (Spruce) Becc. conhecida popularmente como pupunha-brava ocorre no município de Manaus, em floresta de baixio, floresta secundária e floresta de terra firme, além de áreas de clareiras naturais. O presente trabalho objetivou descrever a morfoanatomia do processo germinativo de *S. inajai* e determinar o seu tempo médio de duração, além de identificar as substâncias ergásticas presentes no embrião, haustório e endosperma e realizar análise centesimal da semente. Para tanto, foi realizado o acompanhamento semanal da germinação de 300 sementes, coletadas durante a dispersão e colocadas para germinar. O estudo anatômico seguiu as práticas usuais de microscopia de luz com a realização de testes

histoquímicos. A germinação de *S. inajai* inicia-se em média 101 dias após a semeadura com a formação do botão germinativo. Os eventos morfológicos que se seguiram foram: alongamento do hiperfilo, intumescimento da bainha cotiledonar, emissão da raiz primária e primeiro catafilo, emissão do segundo catafilo e eofilo. O alongamento do eixo embrionário foi observado após a emissão do botão germinativo, durante o processo de alongamento do hiperfilo. A observação da raiz primária a vista desarmada foi possível após o intumescimento da bainha cotiledonar, momento em que os dois catafilos e o eofilo encontravam-se diferenciados na bainha cotiledonar. Foi observado amido no embrião antes da germinação, porém a sua quantidade aumentou nas células parenquimáticas após a formação do botão germinativo. A semente apresentou 30% de lipídios em sua concentração no momento da dispersão.
Palavras-chave: Attaleinae, palmeira, embrião, plântula, ontogenia.

Introduction

Syagrus is an endemic species to South America. The majority of the species grow in “cerrado” and “caatinga” (arid scrubland) environments, in sandy and rocky soils, with an area of concentration in the Central West region (Henderson and Scariot 1993; Henderson *et al.* 1995; Dransfield *et al.* 2008; Leitman *et al.* 2012). *Syagrus inajai* (Spruce) Becc., popularly known as “pupunha-brava”, has strong leaves which are used as a roofing material, and edible pulp and nut (Henderson *et al.* 1995; Miranda and Rabelo 2006). Although well-known in its region of occurrence, its resources are used on a small scale, and its potential is under-utilized. It occurs in areas of “platô” (plateau), “vertente” (slope) and *de “*baixio” (galerry) forest, but it has also been observed in natural clearings, associated with degraded environments, with secondary vegetation, in full sunlight (Henderson and Scariot 1993; Ribeiro *et al.* 1999; Miranda and Rabelo 2006).

According to Orozco-Segovia (2003), of the known species of palms, few have been studied for the seed and their germination process. The majority of studies carried out involve species of commercial value (DeMason 1985; Martins *et al.* 1996; Mendonça and Araújo 1999; Araújo *et al.* 2000; Aguiar and Mendonça 2002; Aguiar and Mendonça 2003; Panza *et al.* 2004; Zona 2004; Negreiros and Perez, 2004; Ferreira and Gentil 2006; Luz *et al.* 2008; Pivetta *et al.* 2008; Mendonça *et al.* 2008; Mendonça and Bianco 2009; Oliveria *et al.* 2010; Ribeiro *et al.* 2011).

The Arecaceae family presents some peculiarities in its germination process, and its classification varies with different authors (Martius 1823-1850; Tomlinson 1960; Pinheiro 2002; Merrow 2004; Henderson 2006; Dransfield *et al.* 2008). The authors have divergent opinions in relation to which structures are relevant. However, there is no consensus in relation to the development of parts of the cotyledon as the basis for this classification. Merrow (2004) considers the development of the cotyledonary petiole, and whether it’s lengthening, or not, leads to distancing from the embryo axis of the seed. Other authors consider, besides this lengthening, the formation or non-formation of the ligule (Martius 1823-1850; Tomlinson 1990; Pinheiro 2002; Dransfield *et al.* 2008), and there is also a divergence of opinion regarding the inclusion of the ligule in the classification.

The mechanisms of germination and dormancy are little understood for the majority of palm species (Orozco-Segovia *et al.* 2003). Henderson (2006), observing

the imprecision in the description of the germination processes based on just a few characters, observed that they could only be better understood, through a detailed association of the morphological and anatomical characters. The author proposed an organization of the seedlings of palms into groups, based on the orientation of the primary radicle in relation to the principal axis of the seedling, and other distinctive characteristics.

Considering the difficulty in establishing the start and end of germination, and the steps involved in the germination process, there is a clear need for studies that will contribute to the knowledge of the germination process of palms.

In view of the above, this work sought to describe the morphoanatomy of the germination process of *S. inajai*; to determine its average duration; to identify the ergastric substances present in the embryo, haustorium and endosperm; and to carry out a chemical analysis of the seed, which will contribute to the knowledge of the biological cycle of the species. Because this is a species that is adapted to various environments, which facilitates its cultivation, and in view of its potential for oil extraction, the results will bring information that could further works on its natural regeneration and management.

Material and methods

The botanical material was collected in 2009 in a green area of the Campus of the Federal University of Amazonas - UFAM, Manaus, Amazonas, Brazil, in an area of baixio (gallery forest), close to coordinates 3 º 05 ' 45,84 " S e 59 º 58 ' 43,69 ", a climate characterized as being of the Afi type (Köppen 1931). The material was analyzed at the Agroforestry Botanical Laboratory - LABAF, of the same University. The seeds were collected from five individual specimens of *S. inajai* at the moment of dispersion.

Of the 300 fruits collected, the pericarps were extracted with the aid of a clamp and saw, and the seeds were put to germinate. Of this sample, 100 seeds were set aside for observation of the germination process (20 seeds per morphological event), 100 for morphoanatomical characterization, and 100 for analysis of the chemical composition of the seed. The morphoanatomical descriptions were based on the terminologies used by Tomlinson (1990), Henderson (2006) and Tillich (2007).

All the seeds were soaked in water for five days, changing the water daily. They were then planted in a greenhouse, covered with 150-micra agrofilm, with minimum mean temperature of 26 °C and maximum temperature of 34 °C, in plastic boxes (53 cm X 34 cm X 9 cm), with operculum parallel to the level of the substrate, at a depth of 2 cm, in substrate of sawdust and washed sand at a proportion of 2:1. The observations were carried out at 7-day intervals, starting on the first day of seeding. After emergence of the germinative button, five seeds of each of the stages to be described were removed from the substrate.

For the anatomical study, the material collected (seeds) was fixed in FAA 70% (Kraus and Arduin 1997). The samples were dehydrated in ethyl series (70%-95%), embedded in 2-hydroxyethyl-methacrylate (Historesin® Leica, prepared according to the manufacturer's instructions), sectioned at thicknesses of 4 to 7 µm in a rotary microtome, and stained with 0.5% toluidine blue in citrate buffer, pH 4.0 (O'Brien *et al.* 1964), and the slides were mounted in water. The semi-permanent slides were prepared from sections cut freehand using razor blades, cleared, stained with safranin and astra blue in aqueous solution (Bukatsch 1972) and mounted in glycerinated gelatin (Dop and Gautié 1928).

For the registration of the material in a Scanning Electron Microscopy(SEM), the botanical material was fixed in BNF (Buffered Neutral Formalin) (Lillie 1965) solution, dehydrated in ethyl series, and dried by the critical point method with CO2 on a Balzers model CPD 030. The samples were collected in a metal support, covered with gold (Balzers SCD 050) and observed under a JEOL JSM 5800LV (10 kV) Scanning Electron Microscopy, at the Institute of Biology/Unicamp.

The histochemical tests used were: periodic acid-Schiff reagent (PAS) stain (Feder and O'Brien 1968) for polysaccharides with vicinal glycol groups; Ruthenium red to identify pectic substances (Johansen 1940); Xilidine Ponceau (O' Brien and McCully 1981) to detect protein reserves; Sudan IV (Brundett *et al.* 1991) to detect neutral lipids substances, Nile blue sulphate (Cain 1947) for acid and neutral lipids, and Lugol (Jensen 1962) to detect starch and Ferric trichloride to verify the occurrence of phenolic compounds (Johansen 1940).

The approximate centesimal composition of the seeds, in terms of lipid, protein and carbohydrate concentrations, was determined according to the analytical norms of the AOAC (1998) and the Instituto Adolfo Lutz" (1985), at the Fishing

technology laboratory of the Universidade Federal do Amazonas, in three phases: seed dispersion of the seed; emergence of the primary radicle and first cataphyll; emergence of the second cataphyll and eophyll. The samples, 50 g from each phase, were selected and titrated and homogenized in a domestic multiprocessor, for analysis. For the calculation of crude protein, a correction factor of 6.25 was used; the total carbohydrates were calculated by difference, according to the equation FG = 100% - (% moisture + % lipids +% crude protein + % crude fiber +% ashes). Each determination was performed using 3g of sample, with three repetitions, and the results are expressed as percentages.

The results were documented using an Olympus (BX51) photomicroscope with Olympus (DP71) photographic camera. Indian ink drawings were also made.

Results

Embryo

The embryo of *S. inajai* small, positioned in the operculum, and oblique to the longitudinal axis of the seed (Figs. 1a-b, 2a). It has two morphoanatomical regions; proximal and distal, with milky and grey coloration respectively, the homogenous milky coloration being more common (Fig. 2a-b).

The proximal region is cylindrical, comprised of the cotyledonary sheath, plumule and hypercotyle-radicle (Fig. 2b-c). The cotyledonary sheath surrounds the plumule and presents a ground meristem and protoderm (Fig. 2c-d, f). The embryo axis parallel to the longitudinal axis of the embryo has a short hypocotyle, with procambial strands that emit rays to the plumule, which is located in the cotyledonary cavity, covered by the protoderm with three leaf primordia, two cataphylls and one eophyll (Fig. 2c-d). The radicle is comprised of the promeristem, procambium and ground meristem, originating from a group of common initial cells (Fig. 2d-e).

The distal region is aphyllous**,** with a sinuous surface, which exerts a haustorial function after the start of germination (Fig. 2b, h). Immersed in the ground meristem, peripheral vascular strands are observed (Fig. 2c, h).

The endosperm is solid and white, filling almost the entire seed cavity, except for where the embryo is situated (Figs. 1b, 2a). The walls of its cells are thickened (Fig. 2g) and store carbohydrates, mannans, in the form of crystals that reflect with polarized light of the optical microscope (Fig. 3 h-i). Between the endosperm and the distal region of the embryo, there is a space, the translocation zone (Fig. 2h).

Lipid, protein and pectic substances bodies were present both in the cytoplasm of the cells of the embryo, and those of the endosperm (Fig. 2 l-q). Only some starch bodies were observed in the embryo (Fig. 2i). The epidermis of the proximal region of the embryo is covered by a cuticle (Fig. 2j).

The lipid content in the seed corresponds to 30.55 %, while proteins make up 4.49% and carbohydrates 55.25% of the composition (Table 2).

Germinative button

The protrusion of the germinative button is observed after the rupture of the operculum (Figs. 1c, 3a-b) at around 101 days after seeding (Table 1). This event characterizes the start of the germination process. It occurs through imbibition of the seed and intense mitotic division of the cells of the promeristem, located adjacent to the cotyledonary sheath, in the region characterized as the hyperphyll (Fig. 3c-d). The growth of cells in the hyperphyll leads the hypocotyl-radicle axis to the exterior environment, forming the germinative button (Fig. 3a). In this stage, it is possible to differentiate the start of differentiation of the epidermis and the increase of starch bodies in some cells of the root cap (Fig. 3e-i).

With the start of germination, the distal region of the cotyledon exerts a haustorial function, and it is possible to see a zone of cells of the endosperm, with collapsed walls, close to the epidermis of the haustorium, with reduced or absent cytoplasmatic content, followed by a region with cells with degeneration of the cytoplasm (Fig. 3g).

Lengthening of the hyperphyll

In the region of the hyperphyll, the ground meristem maintains intense cell division. The cells resulting from this process differentiate into parenchyma and lengthen, leading to an increase in their length resulting in lengthening of the hyperphyll, bringing the leaf primordial and primary root approximately 5 cm below the seeding point (Figs. 1d-e and 4a). The average total lengthening time of the hyperphyll is 16 days (Table 1). The hyperphyll presents a single epidermis, followed by two layers of parenchyma cells with thickening of the wall and idioblasts with phenolic compounds, followed by layers of parenchyma cells with intercellular spaces, and 18-20 concentric bicollateral vascular bundles, surrounded by a sheath of sclerenchyma fibers, the largest in the region surrounding the center (Fig. 4 e-g).

Throughout this stage, the cataphylls and eophyll present progressive lengthening by intense divisions, and increased cell volume (Fig. 4 i-l). In the germinative button stage the leaf primordial measured approximately 200 µm, and at the end of lengthening of the hyperphyll were approximately 1 cm, remaining in the cotyledonary cavity up until the protrusion of the primary cataphyll (Fig. 5 d). There is an increase in the number of idioblasts with raphides in the cells around the plumule and radicle (Fig. 4h, l). The root meristem showed intense cell division, adding cells to the procambium, cortex, medulla and root cap (Fig. 4i-l) with the occurrence of idioblasts with phenolic compounds in their peripheral region (Fig. 5e). The epidermis differentiates and evidences the structure of the primary root (Fig. 4 h-l).

In the haustorial region, the invaginations of the epidermis become more prominent through intense anticlinal cell division and cell divisions in the adjacent ground meristem (Fig. 4b-d). The cells of the procambial strands differentiate into the phloem and xylem, forming the vascular bundles (Fig. 4c). Internally there is an increase in intercellular spaces, and differentiation of the cells of the parenchyma, which an increase in volume (Fig. 4c-d). At the end of this phase, the haustorium doubles in size (Fig. 1e). Simultaneously with these events, breakdown of the endosperm occurs in centrifugal form, with the cells passing from the haustorium to the seed tegument, forming the dissolution zone comprised of three distinct regions: the first with cells of the collapsed walls next to the haustorium, the second by cells with breakdown and degeneration of the cytoplasm and wall reduction (Fig. 3 g) and finally, the third with cells with dense cytoplasm and thickened walls (hard endosperm) (Fig. 3h).

Intumescence of the cotyledonary sheath

After the end of lengthening of the hyperphyll, intumescence of the region comprising the cotyledonary sheath, epicotyl, hypocotyl and primary root is observed (Fig. 1f, 5a). This intumescence is caused by a division and increase in volume of the parenchyma cells of the leaf primordial, and an increase in the number of idioblasts containing raphides, which also merge to form large areas (Fig. 5d). This event lasts about three days and precedes the emergence of the primary root of the first cataphyll (Table 1).

At this stage there is an increase of starch grains in the parenchyma cells of the haustorium (Fig. 5f), with a concentration of protein bodies in the epidermal cells

and adjacent parenchyma (Fig. 5g), and polysaccharides in the cytoplasm of the parenchyma cells and intercellular spaces (Fig. 5i, l). In the endosperm, it was possible to observe breakdown of the protein bodies and degeneration of the cytoplasm (Fig. 5d, h), polysaccharides in the cytoplasm and cell walls of the endosperm, which become thinner, indicating the use of these polysaccharides in the wall in the germination process (Fig 5b-c, j, m), and neutral lipids in the cytoplasm (Fig. 5n). At this stage the lipid concentration increased to 14.04%, less than half of that of the seed at the moment of dispersion. Meanwhile, carbohydrate concentration increased, while that of protein was slightly reduced (Table 2).

Emergence of the primary root and first cataphyll

The primary root, as yet imperceptible to the naked eye, becomes evident at the apex of the cotyledonary sheath, opposite the region of the hyperphyll, with darker coloration and smaller diameter than that of the cotyledonary sheath (Figs. 1f and 5a), at around three days after intumescence of the cotyledonary sheath (table 1). This is due to intense cell division of the root apical meristem, and elongation of most of the cells resulting from these divisions. The root cap becomes more pronounced, with approximately twenty layers of cells (Fig. 5d).

After the emergence of the primary root, the tip of the first cataphyll is observed, emerging from the cotyledonary cavity (Fig. 1g), at around five days after protrusion of the primary root (Table 1). However, individuals have been observed in which the two events occur simultaneously, an acceptable fact given that the sheath is separate and lengthens inside the cotyledonary cavity, so that when it emerges it is approximately four times the size of the primary root.

At this stage the haustorium occupies almost half the space initially filled by the endosperm (Fig. 1g).

From the beginning, at the end of this phase, an increase is observed in in cells of the starch bodies in the cells of the cataphylls and roots (Fig. 7a-b).

Emergence of the second cataphyll and eophyll

Germination of *S. inajai* is hypogeal and cryptocotyle, as its cotyledon is submerged and does not expand in the way a green, photosynthesizing blade does (Fig. 1a-i).

At around six days after the emergence of the first cataphyll, emergence of second is observed, and ten days after that, the emergence of this eophyll (Table 1).

The two cataphylls not develop a leaf blade, only a sheath, and contain large quantities of starch grains in their cells (Fig. 7d). The eophyll, on the other hand, presents an entire plicate leaf blade (Fig. 6e). In this phase, the second and third eophyll are formed (Fig. 6b).

The short hypocotyl did not present emergence of roots, but only rays from the vascular bundles to the cataphylls and eophylls (Fig. 6f).

The primary root is persistent, consisting of a rhizodermis of large cells, elongated on the periclinal plane, with thin walls. The cortex presents exodermis consisting of about five layers of cells with thickened walls. After that, it presents layers of juxtaposed parenchyma cells, followed by eleven layers with large intercellular spaces forming the aerenchyma (Fig. 6h-i). The endoderm is conspicuous, with U-shaped thickening of the wall (Fig. 6m). The vascular cylinder has a uniseriate pericycle. The vascular bundles are peripheral, with a polyarch arrangement. In the medullary region, at the periphery of the vascular tissue, the cells are sclerenchymatous in the central parenchymatous region. Moving further away from the hypocotyl region (Fig. 6j), the sclerenchymatous cells have progressively thicker walls (Fig. 6l). The secondary roots originate from the pericycle in the primary root, following the same anatomical pattern (Fig. 6g n-o).

In this stage, even with apparent eophyll apparent on the soil surface, detailed analysis indicated the presence of lipids, proteins and carbohydrates in the seed (Table 2). The haustorium almost completely occupies the internal space of the seed, and presents large, intercellular spaces (Fig. 1i and 7c), with voluminous parenchymal cells, starch grains and protein bodies, and the presence of few vascular bundles (Fig. 7c, e-f). The epidermis and translocation zone reacted to pectic substances and protein (Fig. 7 d-e). The dissolution zone becomes evident in the region of degeneration of the breakdown of the wall reserves (Fig. 7g-h), corresponding to the largest portion of existing endosperm (Fig. 7 g-h). In the region of cells with collapsed walls, reaction to polysaccharides is observed, which does not occur in the region of degeneration and in the hard endosperm (Fig. 7i). At the end of the germination process, the endosperm is almost totally consumed, remaining thick in the region next to the operculum (Fig. 1 h-i).

Discussion

Germination of *S. inajai* of the hypogeal type is cryptocotyle and is characteristic of the family Arecaceae (Tomlinson 1960, Moore and Uhl 1973).

Formation of the germinative button was the first morphological change observed in the seed of *S. inajai* after seeding, an event that has also been observed in other palm species, such as *Acrocomia aculeata* (Jacq.) Lodd. (Ribeiro *et al.* 2011), *Euterpe edulis* Mart. (Queiroz 1986), and *Oenocarpus minor* Mart. (Oliveira *et al.* 2010). We consider this to be the start of germination of *S. inajai,* resulting from rupture of the operculum by intense mitotic activity and increased volume of the cells in the ground meristem in the region of the hyperphyll. In *Butia capitata* (Mart.) Becc., protrusion was due to lengthening of the parenchyma cells in the proximal region of the cotyledon petiole (Magalhães *et al.* 2013). According to Labouriau (1983), germination occurs when one of the parts of the embryo emerges from inside the layers surrounding the seed, along with a signal of active metabolism. Orozco-Segovia *et al.* (2003) state that the protrusion of the embryo may be a result of development of the radicle or plumule. In *S. inajai* the elongation of the embryo axis starts during the process of lengthening of the hyperphyll, when it is approximately 2 cm in length. For *Acrocomia aculeata*, Ribeiro *et al.* (2011) considered lengthening of the embryo axis observed on the second day in culture, and lengthening of the cotyledonary petiole on the fifth day, as morphological indicators of germination. For Bewley and Black (1994), germination starts with seed imbibition and ends with the end of lengthening of the embryo axis. Queiroz (1986), analyzing the germination of seeds of *Euterpe edulis* in two distinct stages: protrusion of the germinative button (cotyledonary growth) and germination proper (growth of radicle and plumule) considered the two stages quantitatively equivalent, and noted that the two processes are the same.

For Tomlinson (1960), the species of palms may present different types of germination, according to variations in length of the different parts of the cotyledon. Martius (1823-1850) based on the lengthening of the cotyledon, classified the germination family Arecaceae as *remotive* and *admotive*. The same author divided *remotive* germination into “tubulosa” and “ocreata”. Subsequent authors maintained this classification system, adopting the terms remote, adjacent and ligule as synonymous for *remotive*, *admotive* and ocrea (Tomlinson 1960; Pinheiro 2002;

Orozco-Segovia 2003; Dransfield *et al.* 2008). Meerow (2004) considered only the development of the cotyledonary petiole, admitting remote and adjacent germination without emphasizing the presence or absence of the ligule. Tillich (2007) emphasizes that the coleoptile is often confused with the ligule, and is used as a synonym. However, according to the same author, the ligule occurs only in mature leaves of monocotyledons and other angiosperms, while the coleoptile is a tubular structure originating from the edge of the cotyledonary sheath, developing in the cotyledon of monocotyledons in individuals with a closed sheath and unifacial hyperphyll. According to Tillich (2007), the hyperphyll is understood as the terminal region of the haustorium, which connects it to the cotyledonary sheath. There are cases where the hyperphyll lengthens, receiving the name of apocole or cotyledonary petiole (Tomlinson 1960, Tomlinson 1961). The continuous lengthening of the hyperphyll distances the embryo axis from the haustorial region. If the hyperphyll is inserted into the base or directly on the plumule-radicle node, formation of a distinct coleoptile occurs. In some taxa, the hyperphyll is inserted in the center of the hypocotyl, leaving a short, more visible cotyledonary sheath and a short coleoptile. If the hyperphyll is connected to the distal end of the sheath, the coleoptile does not develop (Henderson 2006), as observed in *S. inajai*. Therefore, it is the lengthening of the hyperphyll, and not the development of the coleoptile, that characterizes its germination as tubulated remote.

Henderson and Stevenson (2006) affirm that the seedlings have few, but consistent morphological and anatomical characters for phylogenetic study. The authors studied in this area used, as a diagnostic character, the angle of the embryo axis. It was found that a straight embryo axis occurs in more basal palms belonging to the subfamily Coryphoideae. However, this character also occurs in *Syagrus* and *Allagoptera*, a characteristic that is not shared by other species of the Cocoseae tribe, subfamily Arecoideae, thus characterizing a reversal. The two genera have remote germination (Henderson 2006). According to Tomlinson (1960), this type of germination is characteristic of the subfamily Coryphoidea, like the characteristic previously mentioned, and may have importance in adaptation to drought. Tillich (1998) considers the lengthening of the hyperphyll as a primitive character. The adaptation of palms to the dry habitat was suggested as a primary selection pressure of the types of germination (Uhl and Dransfield 1987). The placement of the plant

embryo under dry soil, where moisture is more favorable for its development, would be an ecological advantage for development, protecting its growth tip from disturbances on the soil surface, such as a fire or cutting (Pinheiro 2002), and affording giving greater protection from mechanical damage and predators.

The genus *Syagrus* is typical of areas with “cerrado”, “caatinga” and “campos rupestres” vegetation, and the central region of Brazil is its center of occurrence (Henderson *et al.* 1995), accounting for 50% of the species. Some species occur in the Amazon biome, as is the case of *S. inajai* where they occupy areas with sandy soil, anthropized forests, and clearings, given their resistance to strong sunlight. This characteristic is highly reminiscent of the environmental conditions of the cerrado. On the other hand, it also occurs in gallery forest, along with the palm *Mauritia flexuosa* L.f., which demonstrates its adaptability to various environments, and may be related to the type of germination and persistent primary root.

The first two leaf primordia of the plumule in *S. inajai* do not develop a leaf blade, only the sheath portion, with a large quantity of starch bodies. Henderson (2006) describes the leaf primordia, with the exception of the eophyll, as cataphylls. For Cronk (2009) cataphylls are small leaves that are usually morphologically equivalent to the basal parts of the full developed leaves. According to Tillich (2007), specifically in the seedling, the first leaves to form after the cotyledon are cataphylls. Therefore, this terminology is used to designate the first two primordial present in the seedling of *S. inajai,* between the cotyledon and the eophyll. The third leaf primordium gives rise to the eophyll.

The three leaf primordia seen in the embryo during the dispersion corresponds, therefore, to two cataphylls and the eophyll. This two-to-one ratio also occurs in *Acrocomia aculeata* (Ribeiro *et al.* 2011), *Elaeis guineensis* Jacq. (Jouannic *et al.* 2011) *Euterpe precatoria* Mart. (Aguiar and Mendonca 2002) and *Oenocarpus minor* Mart. (Oliveira *et al.* 2010). However, in palms there is variation in the number of primordia, with respect to the number of cataphylls (Henderson 2006). The author believes that the number of cataphylls is different for each tribe and is related to the type of eophyll. If the seedling presents only one cataphyll, the eophyll will be entire, and if it has more than one it will be palmate, bifid or pinnate. However, *S. inajai* presents two cataphylls and an undivided eophyll.

Syagrus inajai has a persistent primary root, a characterized shared by other genera of the Cocoseae tribe, such as *Allagoptera*, *Astrocaryum* and *Bactris*, and is present in the basal groups Calamoids and Ceroxyloids and intermediaries Chamaedoreoids and Geonomoids (Henderson and Stevenson 2006). Noting the position of the secondary roots of the seedling of *S. inajai* would give the observer the false impression that they come from the hypocotyl, and are adventitious roots; however, their origin is in the pericycle of the primary root, as also observed in seedlings of *Euterpe precatoria* (Aguiar and Mendonça 2002) and *Acrocomia aculeata* (Ribeiro *et al.* 2011). In individual adults, adventitious roots are commonly reported (Corner 1966; Tomlinson 1990; Dransfield *et al.* 2008).

After germination, the distal region of the embryo of *S. inajai* assumes the function of haustorium, as seen in *Cocos nucifera* L. (Sugimuma and Murakami 1990). According to these authors, the epidermis of the haustorium and adjacent cells play a key role in the absorption of lipids reserves from endosperm degradation and conversion into sugars. Such an event would help explain the reduction in lipid concentration and increased carbohydrate present in the seed after the start of germination. The surface layers of the haustorium have enzymes that convert inert materials of the endosperm into soluble substances that pass through the cotyledon and thus feed the seedling until it is capable of independent growth (Tomlinson 1960).

Tillich (1998) states that there are species in the family Arecacae with assimilating cotyledon, lengthened, and vertical hyperphylls, as observed in *S. inajai*. The sinuosity of the epidermis in the region of the haustorium, forming invaginations that increase the contact area with the endosperm, favoring this assimilation, a characteristic shared by other palms such as *Acrocomia aculeata* (Moura *et al.* 2010; Ribeiro *et al.* 2011), *Cocos nucifera* (Sugimuma and Murakami 1990), and *Phoenix* (Tomlinson 1990). This characteristic facilitates the assimilation of the nutrients present in the endosperm and secretion and/or absorption by the haustorium (DeMason 1985). During the germination process, the cotyledon of *S. inajai* expands, appropriating the space formerly occupied by the endosperm, as occurs in *Phoenix* (Tomlinson 1990), except in the region of the endosperm around the tissues that connect the haustorium to the rest of the cotyledon, as observed by DeMason *et al.* (1985).

According to Balick (1979), the literature cites the existence in Brazil of 25 species of oil palms, among them, *Syagrus cocoides* Mart.. In this study, a concentration of 30% lipids was observed in the concentration of the seed at the time of the dispersion of the fruit, characterizing the species as oleaginous, with potential benefit for the cosmetics and biofuel industries. The same is true of *Syagrus coronata* (Mart.) Becc. known as licuri, with a concentration of 49.2% lipids in its nut (Crepaldi *et al.* 2001) and *Syagrus oleracea* Mart. With 40% (Bora and Moreira 2003).

Lipid and protein have also been reported in the cells of the embryo of *Acrocomia aculeata* (Moura *et al.* 2010; Ribeiro *et al.* 2011), *Euterpe edulis* Mart. (Panza *et al.* 2004) and *Phoenix dactylifera* L. (DeMason *et al.* 1985).

According to Lersten (2004), the main function of all types of endosperm is to provide nutrients to the developing embryo.

No starch was observed in the endosperm cells at the time of seed dispersal, but a large quantity of carbohydrate was identified in the detailed analysis (55.25%), located in the thick walls of the endosperm, as a reserve in the form of mannans, a characteristic that has also been reported in *Phoenix dactylifera* (DeMason *et al.* 1985; Buckeridge *et al.* 2000b) and *Euterpe edulis* (Panza *et al.* 2004). Mannans correspond to 90% of the reserves of the palm seed in the wall of the endosperm (Tomlinson 1990), in the form of crystals, which also give hardness to the tissue, a characteristic that may be related to the system of protection of the embryo against mechanical damage (Buckeridge *et al.* 2000). DeMason *et al.* (1985) suggest that although the haustorium probably regulates the breakdown of mannan in the endosperm, it does not appear to secrete the hydrolytic enzymes responsible for this action. In *S. inajai* this breakdown is seen occurring in cells near the haustorium up to 15 layers into the endosperm.

Conclusion

The first morphological sign of germination is the formation of the germinal button; the anatomical sign is the resumption of cellular activity in the region of the hyperphyll.

Lengthening of the embryonic axis occurs only after the start of lengthening of the hyperphyll, and is not the first anatomical change observed in the germination process.

The development of the primary root and leaf primordia occur simultaneously; however, the root is usually observed first due to the fact that the cataphylls and eophyll are protected by the cotyledonary sheath.

The germination of *S. inajai* is classified as remote tubulated.

The germination process takes around 43 days (protrusion of the button to emergence of the eophyll) due to morphoanatomical stages: lengthening of the hyperphyll; intumescence of the cotyledonary sheath; protrusion of the primary root and primary cataphyll; protrusion and the second cataphyll and eophyll. Therefore, for the cultivation of this species, is necessary to consider the time between the start of germination and emergence of the eophyll.

The seed of *S. inajai* at the moment of dispersion has a concentration of 30% lipids, which may show its potential for oil extraction.

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Table 1. Morphological events observed during the germination process of *Syagrus inajai.*

Morphological events	Interval between morphological events (days)		
	Mean	Minimum	Maximum
Germinative button	101	20	218
Lengthening of the hyperphyll	16	17	18
Intumescence of the cotyledonary sheath	3	2	4
Emergence of the primary root	3	3	5
Emergence of the first cataphyll	5	7	8
Emergence of the second cataphyll	6	5	9
Emergence of the eophyll	10	7	14
Total	144	61	276

Table 2. Mean content of the seed substances of *Syagrus inajai* during their dispersal and in two phases of the germination process.

Sample phases	Lipid %	Protein %	Carbohydrate %
Dispersion of the seed	30.55	4.49	55.25
Emergence of the primary root and primary cataphyll	14.04	4.32	69.49
Emergence of the eophyll	9.3	2.78	48.12

Fig 1. Germination process of *Syagrus inajai*. a) External appearance of the seed, b-i) Longitudinal section showing the embryo and the development of the haustorial region; b) Seed at the moment of dispersion, embryo (arrow); c) Emergence of the germinative button; d) Lengthening of the hyperphyll, e) End of hyperphyll lengthening; f) Intumescence of the cotyledonary sheath (arrow); g) Emergence of

the primary root (arrow) and first cataphyll; h) Emergence of the second cataphyll; i) Emergence of the eophyll. (en: endosperm, e1: first eophyll, ha: haustorium, hy: hyperphyll, pr: primary root, c1: first cataphyll, c2: second cataphyll, sr: secondary root).

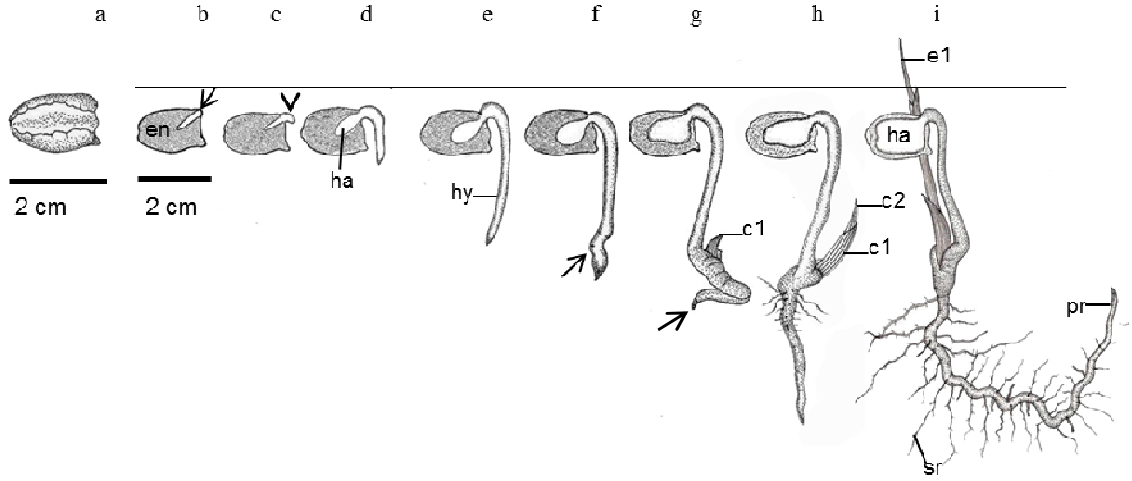

Fig. 2. Embryo and endosperm of *Syagrus inajai* from a mature seed. a-g) Longitudinal sections; h) Transversal sections; i-q) Longitudinal sections submitted to different Histochemical tests: a) Embryo embedded in the seed; b) General appearance of the embryo, sinuosity of the distal region (arrow); c) General appearance of the embryo; d) Detail of the plumule, hypocotyl and radicle; e) Detail of the radicle; f) Protoderm (arrow); g) Cells of the endosperm h) Endosperm and distal region of the cotyledon; i) Parenchyma cells with starch grain, black; j) Cuticle in the proximal region of the embryo, red, l) Cells of the endosperm with lipids, orange; m) Cells of the embryo with lipid, orange; n) Red protein bodies in the endosperm; o) Red protein bodies in the embryo; p) Pectic substances in the cytoplasm of the endosperm cells, pink; and q) Pectic substances in the embryo cells, pink. Initials: cs, cotyledonary sheath; dr: distal region; em, embryo; en, endosperm; h, hypocotyl; ha, haustorium, hy: hiperphyll; l1, first leaf primordium; l2, second leaf primordium; l3, third leaf primordium; pc, procambium; pl, plumule; pm, promeristem; pr, proximal region; ps, procambial strand; qc, quiescent center; r, radicle; s, seed coat; tz, translocation zone.

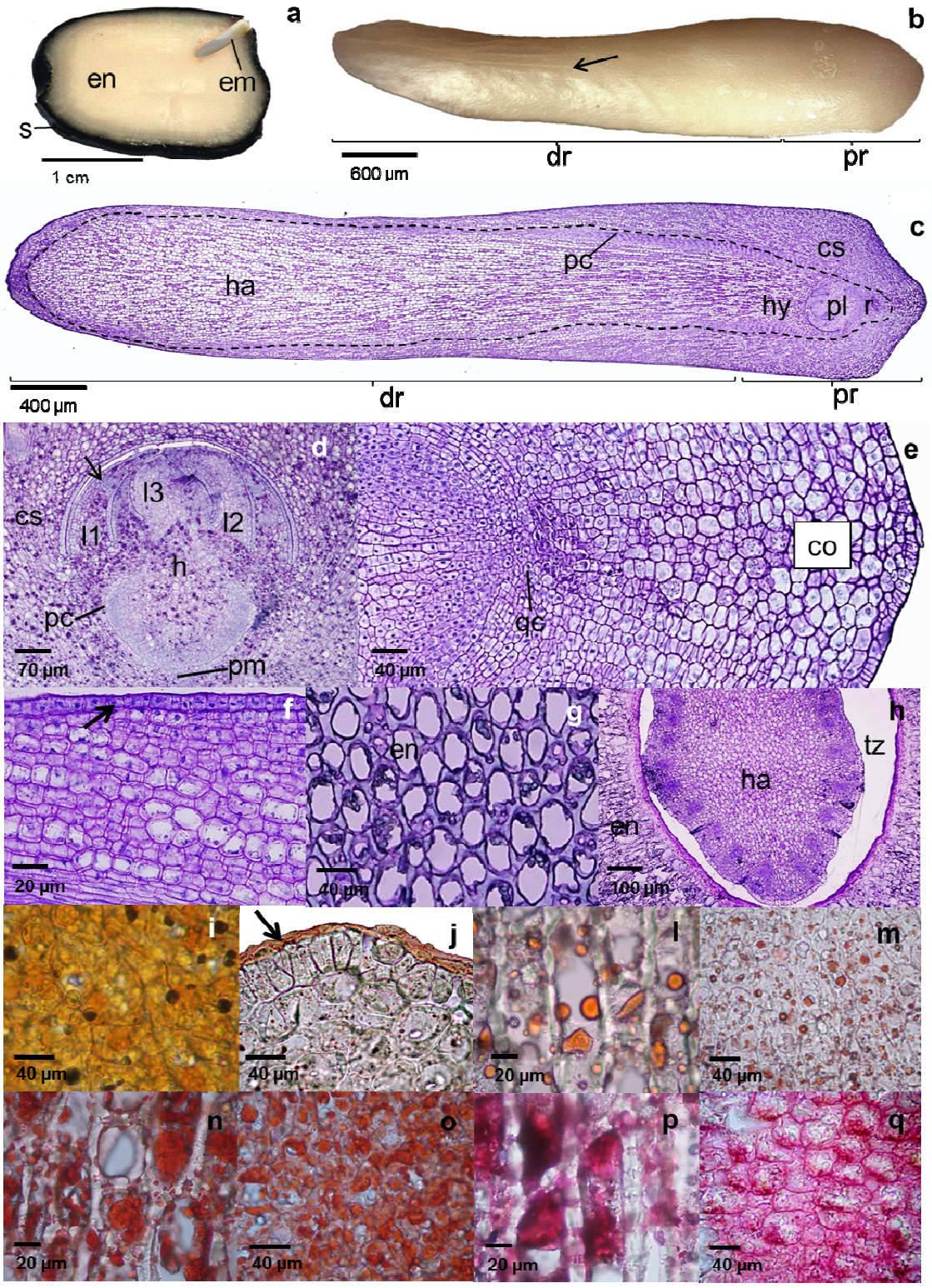

Fig. 3. Early germination *Syagrus inajai.* a) germinating seed with germinative button (arrow); b-i) Longitudinal sections b) Germinative button with radicle promeristem in intense cell division (arrow); c) Hyperphyll, dividing cells; d) Detail of dividing cells; e) Differentiated epidermis; f) Parenchyma cells with starch grains, purple; g) Endosperm showing cell region with collapsed walls next to the haustorium and

region of degeneration; h) Endosperm with cells of thickened wall, and; i) Cell wall of endosperm cells with mannans, crystals forming Maltese cross in polarized light. Initials: ep, epidermis; d, region of degeneration; se, seed; tz, translocation zone; cw: collapsed walls.

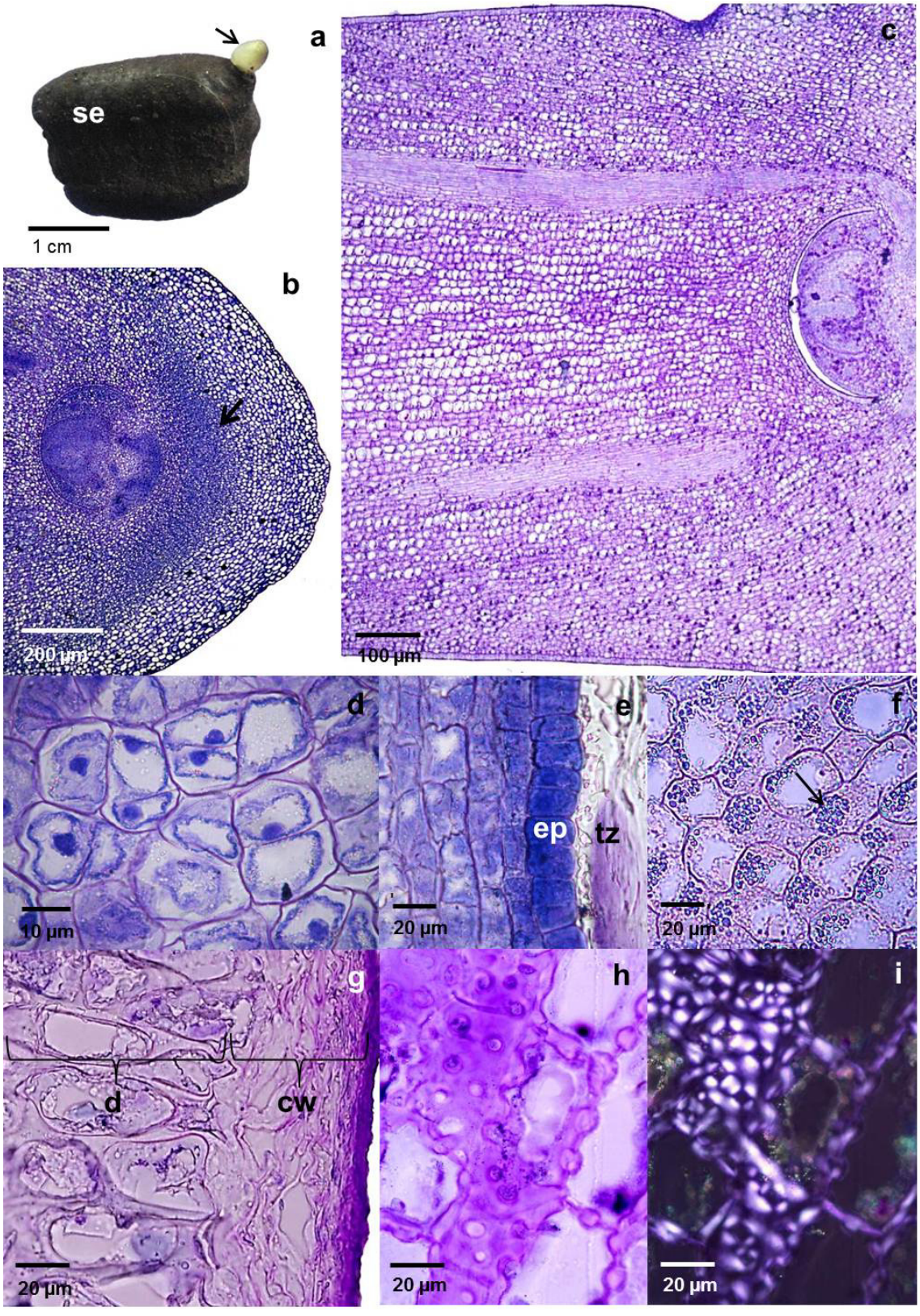

Fig. 4. lengthening of the hyperphyll of *Syagrus inajai*. a) Seed of *Syagrus inajai* with lengthened hyperphyll; b-g) Transverse sections; hl) Longitudinal sections b) General appearance of the region of the haustorium; c) Detail of the sinuosity of the surface of the haustorium; d) Increased sinuosity of the surface of the haustorium; e) hyperphyll; f) Detail of epidermis of the hyperphyll; g) Vascular bundles of the hyperphyll; h) Detail of the epidermis of the primary root (arrow), detail of idioblasts with raphides; i) Hyperphyll at the start of its lengthening, cells in intense division (dotted line); j) Hyperphyll approximately 3 cm in length; and l) Hyperphyll elongate. Initials: c, cortex; c1, first cataphyll; c2, second cataphyll; co, root cap; cs, cotyledonary sheath; e, eophyll; f, fibers; m, medulla; me, promeristem; ph, phloem; h, hypocotyl; hy, hyperphyll; pc, procambium; pd, protoderm; qc, quiescent region; se, seed; vb, vascular bundle; xy: xylem.

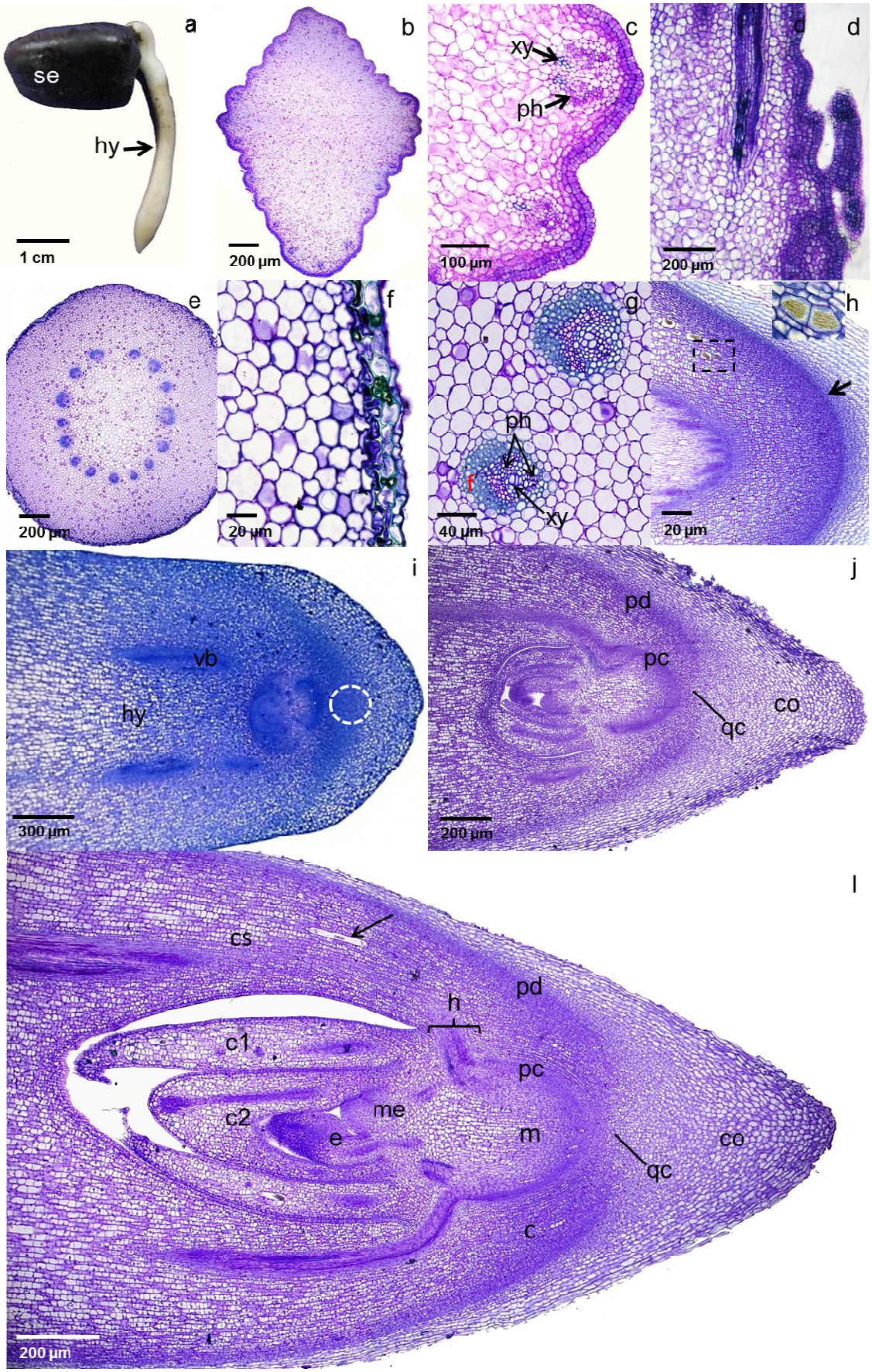
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Fig. 5. Seedling of *Syagrus inajai* with cotyledon with intumescence, emergence of the primary radicle and first cataphyll a) General appearance of the seed with protrusion of the primary root; b-i) Longitudinal sections; j) transversal section; l-n) Longitudinal sections; b) Detail of the endosperm cells with thickened walls; c) Detail of the endosperm cells with the reserves of the cell wall being used; d) General appearance of the cotyledonary sheath with intumescence, and primary roots evident, detail of the idioblasts with raphides; e) Region of the root cap idioblasts with phenolic compound (arrow), dark green (arrow); f) Parenchyma cells of the haustorium with starch, purple; g) Haustorium with protein bodies, red; h) Endosperm with protein bodies, red; i) Positive for polysaccharides in the intracellular medium of the cells of the haustorium, pink; j) Presence of cell wall polysaccharides in the endosperm, pink; j) Pectic substances in the haustorium cells, pink; l) Pectic substances in the cytoplasm of the endosperm cells, pink; and m) Cells of the endosperm with neutral lipids; pink Initials: ca, cotyledonary cavity; c1, first cataphyll; c2, second cataphyll; co, root cap; cs, cotyledonary sheath; e, eophyll; hy, hyperphyll; pc, procambium; pd, protoderm; pr, primary root; se, seed.

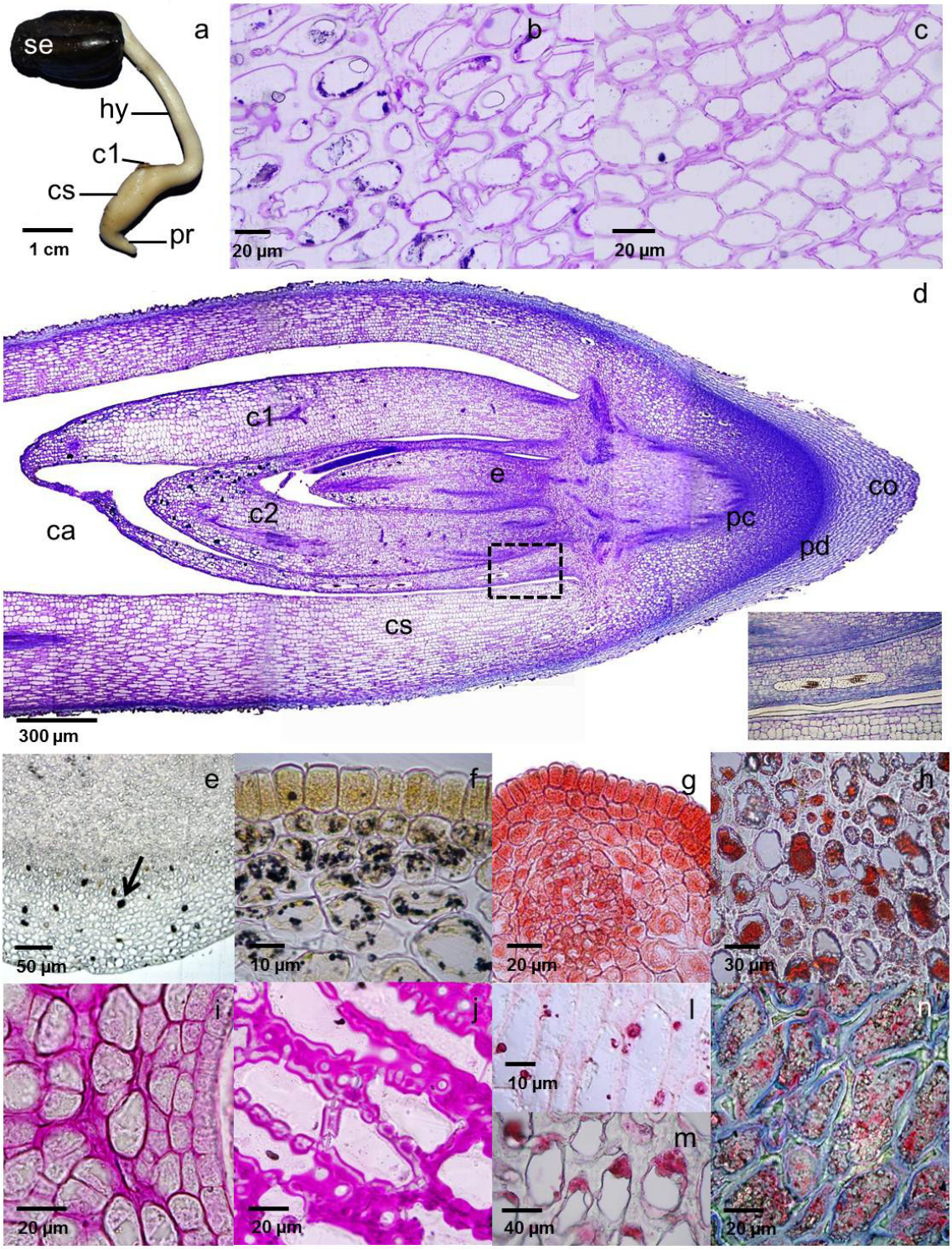


Fig. 6. Seedling of *Syagrus inajai* with emission of the eophyll. a) General appearance of the seedling; b) Longitudinal section; c-f) Transversal section; g) Longitudinal section; h-n) Transversal section b) General appearance of the eophylls c) Detail of the cataphylls and first eophyll; d) Detail of the first cataphyll; e) Detail of the eophyll; f) General appearance of the hypocotyl, vascular bundles (arrow); g) Secondary root; h) General appearance of the primary root, i) Rhizodermis; j) primary

root next to the hypocotyl; l) Primary root distant from the hypocotyl; m) Detail of wall thickening U of endoderm (arrow); n) General appearance of the secondary and tertiary roots, and o) Detail of surface of the rhizodermis. Initials: a, aerenchyma; c, cortex; c1, first cataphyll; c2, second cataphyll; cs, cotyledonary sheath; e1, first eophyll; e2, second eophyll; e3, third eophyll; h, hypocotyl; rh, rhizodermis; hy: hyperphyll; pa, parenchyma; ex, exodermis; pc, procambium; ph, phloem; pr, primary root; s, sclerenchyma; se, seed; sr, secondary root; tr, tertiary root; xy: xylem.

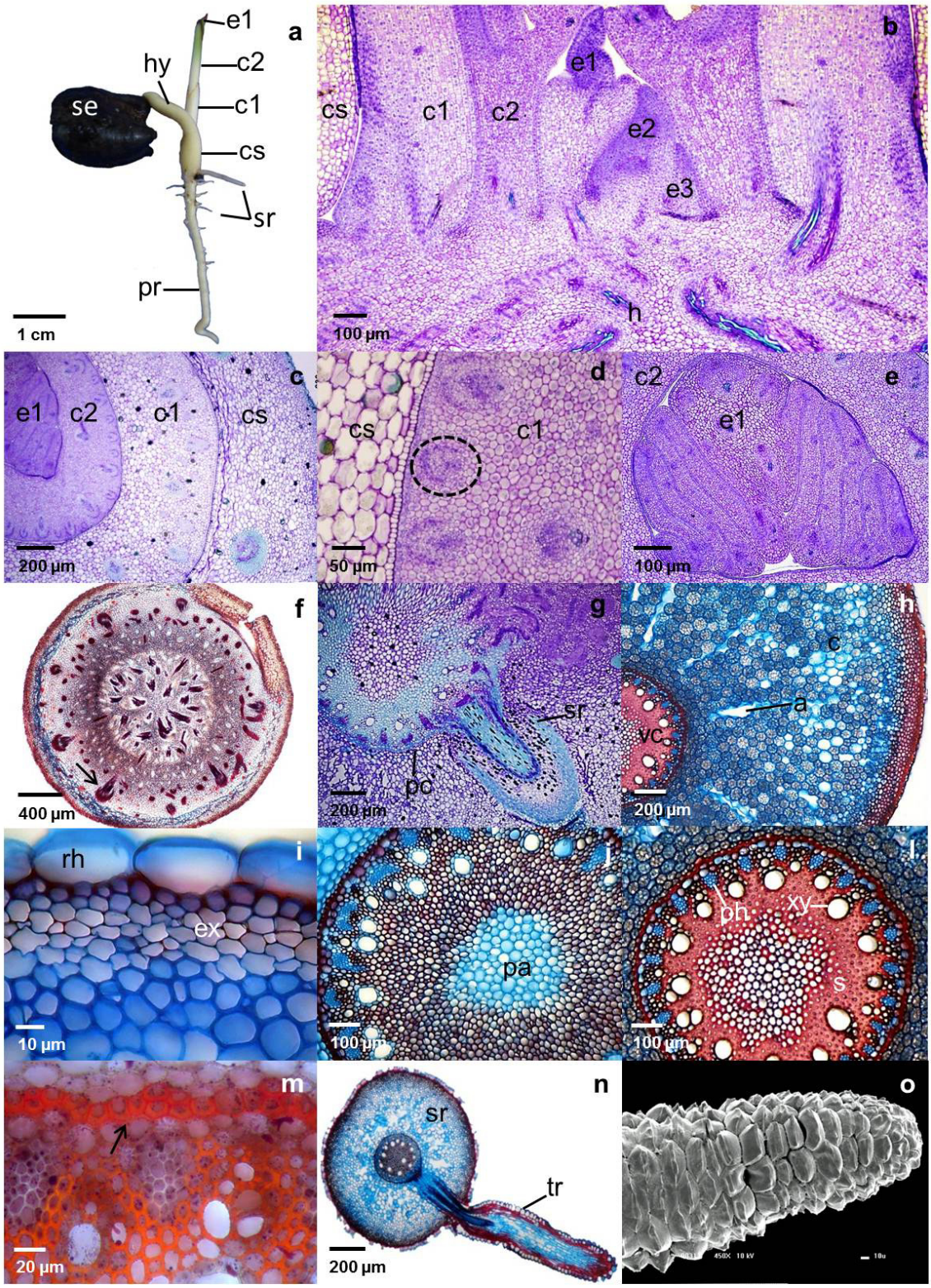
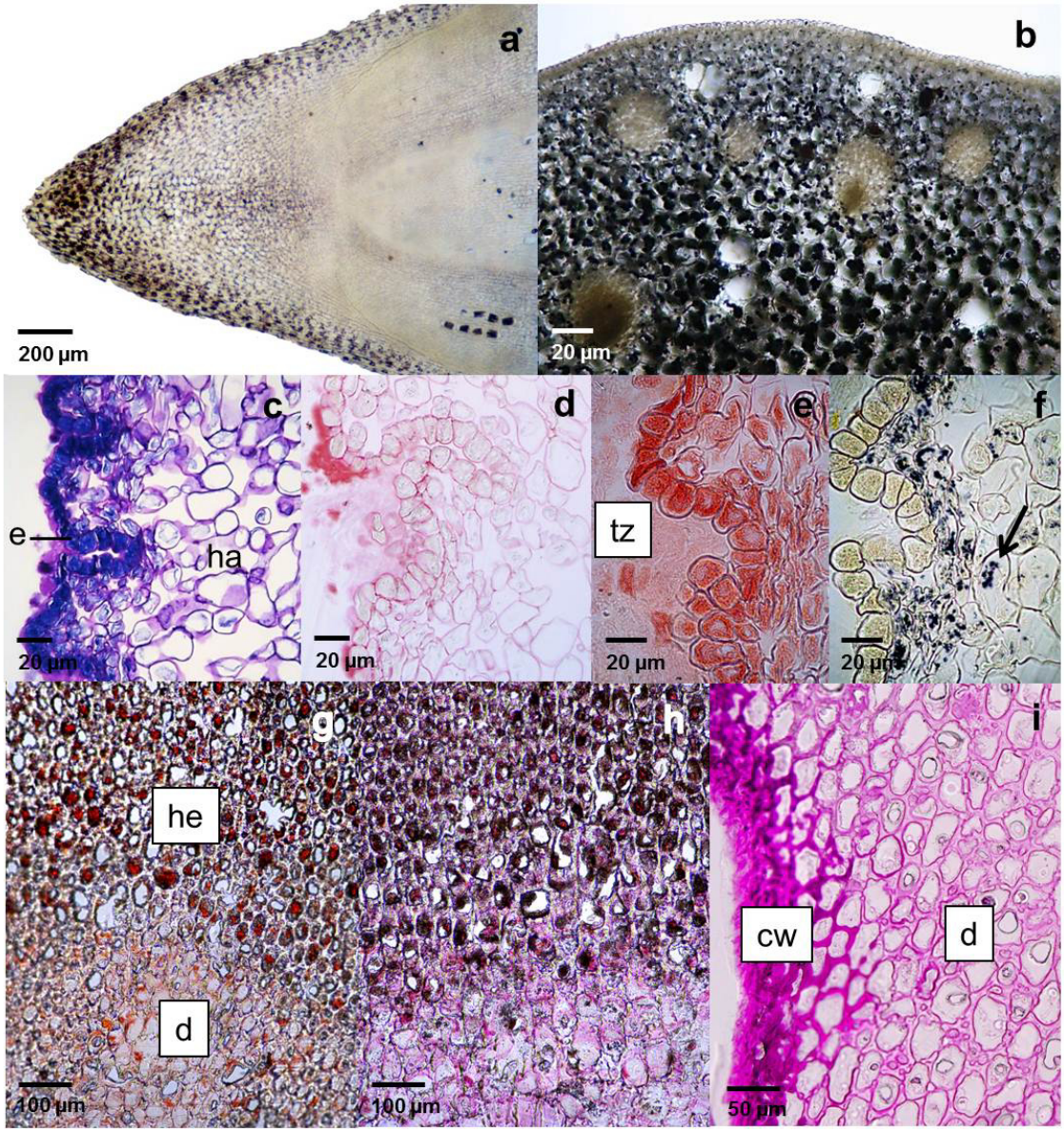


Fig. 7. Anatomy and histochemical tests performed on seedling of *Syagrus inajai* after emergence of the eophyll. a) Longitudinal section; b-i) Transversal section; a-d) Detection of starch grains, purple. a) Starch grains in the primary root; b) Starch grains in the cataphyll; c) General appearance of the haustorium, d-f) Histochemical tests applied to the haustorium; g-i) Histochemical tests applied to the endosperm; d)

Pectic substances in the translocation zone, red; e) Protein in the translocation zone and protein bodies in the cells of haustorium, red; f) Starch grains, purple, in the parenchymal cells (arrow); g) Protein Bodies, red; h) Pectic substances, pink; and i) Polysaccharides in the cell wall, pink. Initials: d, region of degeneration; e: epidermis; ha, haustorium; he, region of the hard endosperm; cw, region of cells with collapsed walls.



SÍNTESE

O presente trabalho permitiu descrever a ontogenia de *Syagrus inajai* do gineceu à plântula através de observações de campo e técnicas morfoanatomicas.

As observações da flor estaminada e pistilada permitiram constatar que a floração da espécie ocorre durante todo ano. O número de flores por inflorescência varia entre 5.904 - 17.316 para flores estaminadas e 180 - 3.528 para as flores pistiladas. As flores estaminadas abrem-se logo em seguida a abertura da bráctea peduncular, enquanto as flores pistiladas, só se abrem 20 dias depois. As flores estaminadas apresentam seis estames e um feixe vascular cada; pistilódio trífido e vascularizado. As flores pistiladas têm seis estaminódios unidos formando um círculo. O gineceu é sincárpico, tricarpelar, trilocular, um óvulo por lóculo. Estigma tripartido, apical e séssil, com epiderme composta por células papilosas alongadas, padrão de epiderme que se mantem por todo canal estilar. O óvulo é anátropo, paquicalazal e bitegumentado.

O gineceu apresenta óvulo anátropo desde o primordio seminal. O tegumento externo é progressivamente substituido pela paquicalaza que engloba todo o óvulo, exceto na região micrópilar. Os tegumentos externo e interno, ficam portanto, restritos a essa região. O ovário apresenta duas regiões meristemáticas, uma adjacente à epiderme externa e outra envolvendo a cavidade seminal. A região meristemática externa origina o mesocarpo fibroso do fruto. A região meristemática interna é responsável pelo espessamento do endocarpo, juntamente com as células provenientes de divisões periclinais da epiderme interna do ovário, formando o endocarpo de origem mista (*lato sensu*). A esclerificação do endocarpo inicia-se no fruto com aproximadamente 80 dias, sendo um processo centripeto. O endosperma é do tipo coenocítico multicelular e a formação de parede das células ocorre centrifugamente. O opérculo é composto por células dos tegumentos externo e interno, e do obturador.

O tempo de desenvolvimento do fruto é de aproximadamente 240 dias, quando se inicia a dispersão.

A primeira divisão do zigoto ocorre cerca de trinta dias após o início do desenvolvimento do endosperma. O tempo de desenvolvimento do embrião é de aproximadamente 220 dias. O embrião é do tipo onagrado, reto, com duas regiões distintas. Uma região proximal arredondada, que compreende o eixo embrionário e bainha cotiledonar e uma região distal, afilada que corresponde ao haustório durante o processo germinativo. O embrião *S. inajai* apresenta quatro estádios de desenvolvimento específicos: estádio proembrião, estádio globular, estádio coração lateral, estádio torpedo. Tais estádios são

observados em outras espécies de palmeiras. Portanto, propõe-se a existência de um padrão no desenvolvimento embrionário dessa família.

A maior participação da região basal do cotilédone, no processo de intrusão do promeristema caulinar e formação da cavidade cotiledonar, resulta no posicionamento central do eixo embrionário, paralelo ao comprimento do embrião. Portanto, a posição final do eixo embrionário, paralelo ou oblíquo, está relacionado a dinâmica de crescimento da região basal e superior do cotilédone durante o processo de intrusão do promeristema caulinar.

A germinação de *S. inajai* é do tipo remota tubulada e inicia-se em média 101 dias após a semeadura, com a formação do botão germinativo. Constatou-se que o primeiro sinal morfológico da germinação é o desenvolvimento do botão germinativo, que juntamente com o alongamento do hiperfilo, intumescimento da bainha cotiledonar, emissão da raiz primária e primeiro catafilo, emissão do segundo catafilo e eofilo compõem os eventos do processo germinativo. O alongamento do eixo embrionário foi observado após a emissão do botão germinativo, durante o processo de alongamento do hiperfilo. A observação da raiz primária em vista desarmada, foi possível após o intumescimento da bainha cotiledonar, momento em que os dois catafilos e o eofilo encontravam-se diferenciados na bainha cotiledonar.

A quantidade de amido aumentou nas células parenquimáticas após a formação do botão germinativo. A semente apresentou 30% de lipídios em sua composição.

Os resultados obtidos no presente trabalho são esclarecedores, uma vez que, elucidaram o processo de formação do óvulo, do pericarpo, da semente e do embrião, além de descrever o processo germinativo de *S. inajai*, fornecendo informações úteis para estudos relacionados a Agronomia, Fisiologia, Ecologia, Taxonomia, em programas de produção e recomposição vegetal, entre outros.

Por conseguinte, a melhor compreensão da espécie e a estimativa do tempo médio de cada estádio, possibilitarão certamente a melhor utilização desse recurso natural.

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BRASIL
PAÍS RICO É PAÍS SEM POBREZA

Curso de Pós-Graduação em Botânica
INPA

ATA DA DEFESA PÚBLICA DA TESE DE DOUTORADO DE DISCENTE DO PROGRAMA DE PÓS-GRADUAÇÃO EM BOTÂNICA DO INSTITUTO NACIONAL DE PESQUISAS DA AMAZÔNIA

Aos vinte oito dias do mês de junho 2013, às 09h00min, na sala de seminários da biblioteca do INPA, reuniu-se a Comissão Examinadora da Defesa Pública, composta pelos seguintes membros: **Dra. Isolde D. Kossmann Ferraz,** do Instituto Nacional de Pesquisas da Amazônia, **Dra. Maria Gracimar Pacheco de Araújo,** da Universidade Federal do Amazonas, **Dr. Sidney Alberto Ferreira**, do Instituto Nacional de Pesquisas da Amazônia, **Dra. Andréia Barroncas de Oliveira,** da Universidade Federal do Amazonas e **Dra. Aristéa Alves Azevedo** da Universidade Federal de Viçosa - MG, tendo como suplente a **Dra. Flor M. Henderson** de Hostos Com. College – NY e Dra. Zilvanda Lourenço Oliveira Melo, do Instituto Nacional de Pesquisas da Amazônia, sob a presidência do primeiro, a fim de proceder a argüição pública da **TESE DOUTORADO** da discente **POLIANA ROVERSI GENOVESE-MARCOMINI**, intitulada **"ONTOGENIA DA PALMEIRA *Syagrus inajai* (Spruce) Becc. (ARECACAE): do gineceu à plântula"** sob a orientação Dra. Maria Silvia de Mendonca e Co-orientação Dra. Sandra Maria Carmello-Guerreiro Swenson.

Após a exposição, dentro do tempo regulamentar, o (a) discente foi argüido (a) oralmente pelos membros da Comissão Examinadora, tendo recebido o conceito final:

EXAMINADORES	PARECER		ASSINATURA
ISOLDE D. KOSSMANN FERRAZ	(X) Aprovado	( ) Reprovado	
MARIA GRACIMAR PACHECO DE ARAÚJO	(X) Aprovado	( ) Reprovado	
SIDNEY ALBERTO FERREIRA	(X) Aprovado	( ) Reprovado	
ANDRÉIA BARRONCAS DE OLIVEIRA	(X) Aprovado	( ) Reprovado	
ARISTÉA ALVES AZEVEDO	(X) Aprovado	( ) Reprovado	

08/07/2013 08:28

OBS:

Nada mais havendo, foi lavrado a presente ata, que, após lida e aprovada, foi assinada pelos membros da Comissão Examinadora.

Ministério da Ciência e Tecnologia

AULA DE QUALIFICAÇÃO

PARECER

Aluno(a): **POLIANA ROSERVI GENOVEZ**
Curso: BOTÂNICA
Nível: Doutorado
Orientador(a): Maria Silvia Mendonça (INPA)

Título:

"ONTOGENESE DE *SYAGRUS INAJAI* (SPRUCE) BECC. (ARECACEAE): DA FECUNDAÇÃO A FASE PLANTULAR"

BANCA JULGADORA

TITULARES:	**SUPLENTES:**
MARIA GRACIMAR PACHECO DE ARAÚJO (UFAM)	
ZILVANDA L. OLIVEIRA MELO (INPA)	ANTONIO CARLOS WEBBER (UFAM)
DANIEL FELIPE DE OLIVEIRA GENTIL (UFAM)	SIDNEY ALBERTO DO NASCIMENTO FERREIRA (INPA)
VERIDIANA VIZONI SCUDELLER (UEA)	
CHARLES ROLAND CLEMENT (INPA)	

EXAMINADORES	PARECER		ASSINATURA
MARIA GRACIMAR P. DE ARAÚJO	(X) Aprovado	( ) Reprovado	
ZILVANDA L. OLIVEIRA MELO	(X) Aprovado	( ) Reprovado	
DANIEL FELIPE DE OLIVEIRA GENTIL	(X) Aprovado	( ) Reprovado	
VERIDIANA VIZONI SCUDELLER	(X) Aprovado	( ) Reprovado	
ANTONIO CARLOS WEBBER	(X) Aprovado	( ) Reprovado	

Manaus(AM), 17 de dezembro de 2008.

OBS: A aula, do ponto de vista didático foi satisfatório, entretanto a banca considera que os conhecimentos teóricos sobre o tema precisam ser lecionados e aprofundados devido a falta de domínio da metodologia proposta. Sugere que o delineamento experimental e os métodos sejam revistos e ajustados.

PROGRAMA DE PÓS-GRADUAÇÃO EM BIOLOGIA TROPICAL E RECURSOS NATURAIS
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