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A N I N T E R N A T I O N A L J O U R N A L

# Semi-hypogeal Germination in *Pachyrhizus ahipa* (Wedd.) Parodi (Fabaceae: Phaseoleae): Seedling and Sapling Morphology

**Camilla R. Dias Milanez, Denise M. Trombert Oliveira* and Marina A. Moraes-Dallaqua**
*Departamento de Botânica; Instituto de Biociências; Universidade Estadual Paulista - UNESP; C. P. 510; 18.618-000; dmtoliveira@icb.ufmg.br; Botucatu - SP - Brasil*

## ABSTRACT

*The aim of this work was to describe the morphology of seedlings and saplings of* Pachyrhizus ahipa*, a cultivated species of Phaseoleae (Fabaceae), analysing the seedling type and characterizing the structure of cotyledons, eophylls and metaphylls. It was observed that the seedling was semi-hypogeal, a germination type not yet recorded for the Phaseoleae tribe. It formed two opposite and unifoliated eophylls with an evident pair of stipels. Metaphylls were trifoliolate and had alternate phyllotaxis. Both eophylls and metaphylls were pinnate, camptodromous, and brochidodromous.*

**Key words:** Morphology, Phaseoleae, sapling, seedling, eophyll, metaphyll

## INTRODUCTION

Studies of the seedling morphology at first development phase can help to finding derived or primitive transitory structures which disappear during the plant development but can have major importance in understanding phylogenetic connections (Ricardi et al., 1977; Torres, 1985). The need for studies to clarify seedling structure has been emphasized since the beginning of the 20th Century (Compton, 1912); however, data are still scarce (Garwood, 1995). Today, it is generically accepted that studies of plant taxonomy should not be exclusively based on the adult specimen morphology, but should also include the juvenile phases. These studies are especially important when dealing with the large families which have many taxonomic problems, such as Fabaceae. In addition to the large number of species, the wide juvenile heterophylly of legumes has already been reported in several studies, sometimes involving a long series of eophylls (Duke and Polhill, 1981; Yeh et al., 1987; Lima, 1989-1990; Oliveira, 1999), making the study of the juvenile phases relevant.
In Fabaceae advanced tribes, seedling types tend to become fixed while basal groups more frequently display variations. Polhill (1981) reported that Sophoreae seedlings were indiscriminately hypogeal or epigeal. Also semi-hypogeal seedlings have been reported in this tribe by Cunha and Ferreira (2003) and Ressel et al. (2004). Sophoreae is considered a basal group in Faboideae, both from traditional (Polhill, 1981) and more recent molecular analyses (Doyle, 1994; Doyle et al., 1997). Despite fixation tendency of seedling types in more advanced tribes, occasionally there are reversions such as in

* Author for correspondence

Phaseoleae, where some species change from epigeal to hypogeal seedlings (Lackey, 1981; Polhill et al., 1981). For *Erythrina* (Phaseoleae), literature reports both hypogeal (Burkart, 1952) and epigeal (Flores and Rivera, 1984; Oliveira, 2001) seedlings.

The *Pachyrhizus* genus belongs to the Fabaceae, Phaseoleae tribe, Diocleinae subtribe (Lackey, 1977). It includes five species, three of which are cultivated, *P. ahipa* (Wedd.) Parodi, *P. erosus* (L.) Urban, and *P. tuberosus* (Lam.) Sprengel, and two, *P. panamensis* Clausen, and *P. ferrugineus* (Piper) Sørensen, which are wild (Sørensen et al., 1997). *Pachyrhizus ahipa* is the only species without records of wild material. It is only found cultivated in Bolivia, some areas in the Jujuy and Salta provinces of Argentina, and in Andean valleys at altitudes between 1000 and 3000m (Sørensen et al., 1997). It is well adapted to subtropical conditions and tolerates low temperatures (Grau, 1997). Due to its potential as a starch source, it was introduced into Brazil where it has been specially grown for scientific purposes.

*Pachyrhizus ahipa* is characterized by the accumulation of carbohydrates in its tuberous root and by the presence of rotenone in its seeds and leaves which are interesting to industry (Grau, 1997). It is probably the most interesting cultivated species of the genus: systematically, by the lack of the wild ancestry; morphologically, due to genotypes with erect and semi-erect habits (the others species of the genus show climbing habits); agronomically, due to its neutrality to photoperiod, short life cycle (5 months), and considerable adaptability to climatic variations (Sørensen et al., 1997). There are no reports on *Pachyrhizus* seedling and juvenile plant morphology. Only Sørensen (1988), in his taxonomic review of *Pachyrhizus*, illustrated seedlings of the five species of this genus, but without making detailed descriptions. Therefore, this study aimed to determine *P. ahipa* seed germination type, and to describe its seedling and juvenile plant morphology detailing eophyll and metaphyll structure.

## MATERIALS AND METHODS

Mature *Pachyrhizus ahipa* seeds (Weed.) Parodi were provided by CERAT (Tropical Root and Starch Centre), Lageado Experimental Farm, São Paulo State University, Botucatu, SP, Brazil. Those of medium size and homogeneous colour were selected for this study.

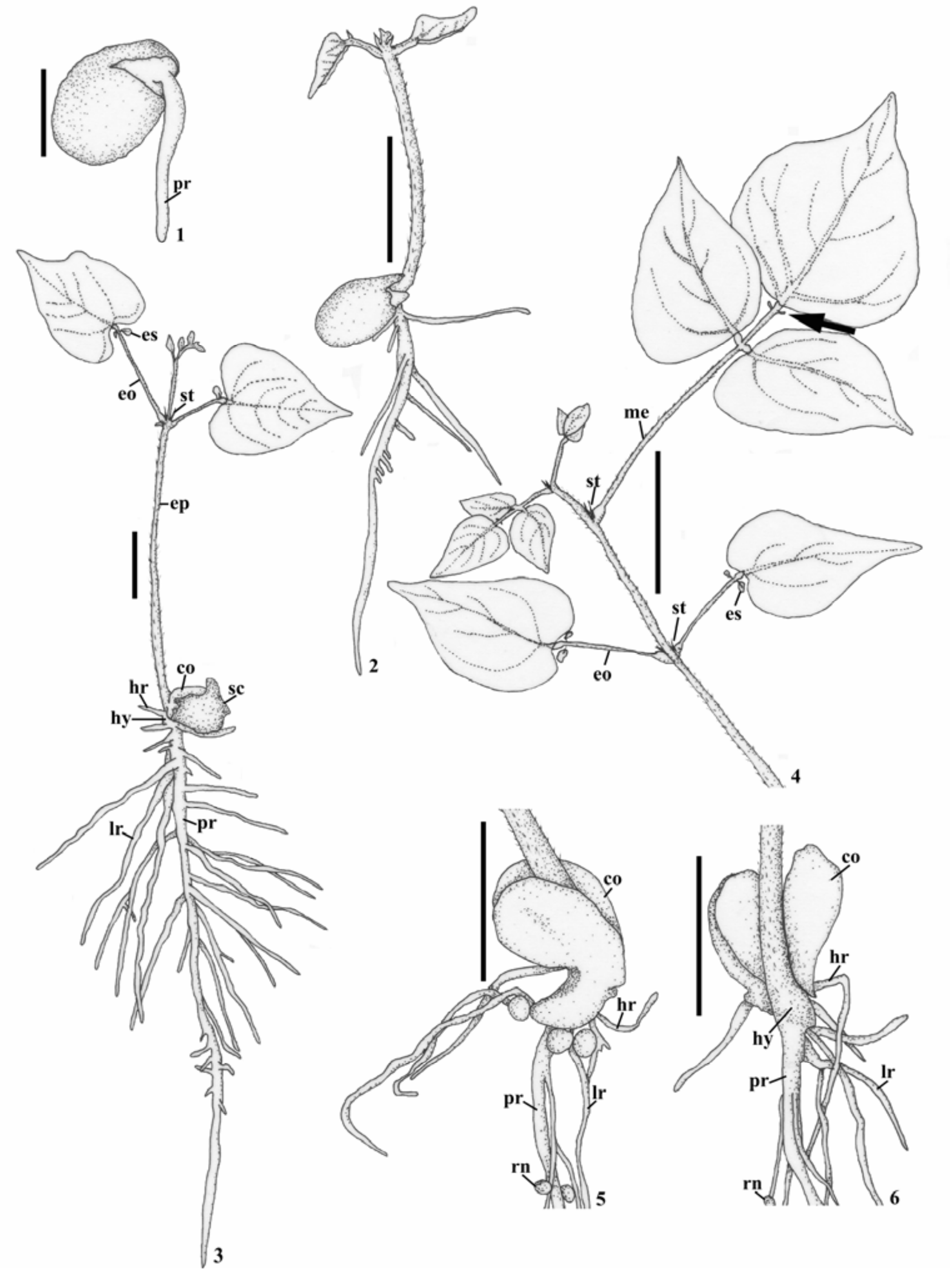
For observation of the initial post-seminal development phase, 50 seeds were first disinfected with commercial 20% sodium hypochlorate solution, washed in running water, and then placed in gerboxes under moist filter paper with distilled water and maintained in an NT 705 germinator at 25±1ºC under continuous white fluorescent light. Before seedlings reached gerbox size, they were transplanted into polyethylene bags with a 1:1 mixture of soil and sand, maintained in a 50% shaded greenhouse, and watered daily. Daily observations recorded the beginning of germination (primary root emergence) and seedling and juvenile plant development up to formation of the first metaphylls. Some seedlings and saplings were stored in 70% ethanol, although live specimens were preferentially used for morphological illustration and description.

The seedling phase was considered as the stage between primary root emergence and expansion of the first pair of eophylls, and the sapling phase from the appearance until full expansion of the first metaphyll (Oliveira, 2001). The terminology adopted for seedling description followed Miquel (1987), who considered seedling functional and morphological characteristics. Methods described by Fuchs (1963) for the clearing technique, and Hickey (1979) to descriptions of eophyll and metaphyll blades were used. Drawings were used to document results; drawing tubes adapted to a Wild stereomicroscope for seedling initial development phases, and the naked eye for juvenile plants. Venation details were illustrated using a Zeiss microscope with drawing tube.

## RESULTS

The germination process began after two days, with protrusion of a thick white primary root (Fig. 1). After seven days, lateral roots were formed and occurred the extension of light-green densely pilose epicotyls. The first pair of eophylls was outlined with a pair of stipules and stipels in each leaf (Fig. 2). In this phase, the hypocotyl was brownish and short, and the fleshy cotyledons were partially retained in the seed coat, laterally to the vegetative axis (Fig. 2).

After 17 days (Fig. 3), the eophylls were expanded, opposite to the cotyledons which were still partially covered by the seed coat. The eophylls were unifoliate, petiolate, with a pulvinus and a pair of filiform stipules. At the base of the eophyll blade, there was the pulvinule and a pair of varying sized stipels that varied from filiform to foliaceous. The petiole showed a pentagonal transverse section, forming an adaxial groove; trichomes could be seen in the entire petiole.




**Figures 1-6** - Seedling and sapling of *Pachyrhizus ahipa*. 1-3. Seedling. 1. At two days. 2. At seven days. 3. At 17 days. 4-6. Sapling. 4. Aerial part of the juvenile plant at 30 days (arrow indicates metaphyll stipel). 5-6. Detail of the cotyledon node, front and side view; note lateral and adventitious hypocotyl roots and root nodules. (co: cotyledon, eo: eophyll, ep: epycotyl, es: eophyll stipel, hr: hypocotylar root, hy: hypocotyl, lr: lateral root, me: metaphyll, pr: primary root, rn: root nodule, sc: seed-coat, st: stipule). Bars: 2cm (2-4), 1cm (1, 5-6)

The eophylls had entire symmetrical wide ovate blade. They had entire margin, acuminate apex and lobate base (Fig. 7). They showed a pinnate, camptodromous, brochidodromous venation (Fig. 7). The primary vein was moderate and have straight course; secondary veins emerge from the primary vein at acute, predominately moderate angles. Veinlets were simple or branched forming well developed and incomplete areoles, which were randomly arranged and had irregular shape (Figs. 8 and 9). Next to the margin (Fig. 9), there were more incomplete areoles.

Parallel to the eophyll expansion, there was primordia of the first metaphyll. The epicotyl and hypocotyl were as described previously. In this stage, the root system was developed and formed by several lateral and hypocotyl adventitious roots, all of small thickness (Fig. 3).

The development of the juvenile plant was fast and at around 30 days the phyllotaxis changed from opposite to alternate with complete expansion of the first pinnate-trifoliate and petiolate metaphyll, that showed an evident pulvine and a pair of filiform stipules. The petiole was similar to the eophyll. Each metaphyll had a pair of stipules at the base of the terminal foliole. All folioles were pulvinulate. The metaphylls had lateral folioles with entire asymmetrical wide ovate blade, entire margin, acuminate apex and cordate base. The terminal foliole had entire symmetrical very wide ovate blade, entire margin, acuminate apex and lobate base. It had the same venation pattern as the eophylls, but the secondary divergence angles were acute, predominantly wide (Figs. 10 and 12).

In the sapling, the cotyledons (Fig. 5) were already released from the seed coat assuming an erect position. They were green-yellow, fleshy and plain-convex. The vegetative axis was positioned between the plain faces (Fig. 6).

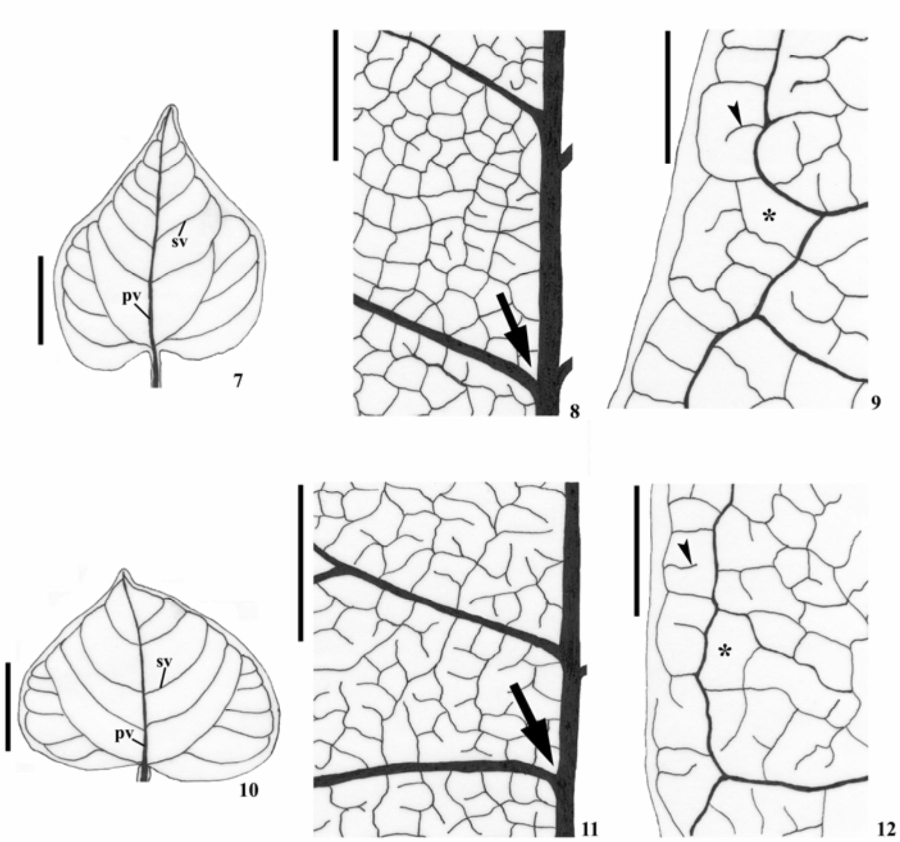
The root system of the sapling was axial with numerous branches (Figs. 5 and 6). It had determinate oblate nodules with variable size (Fig. 5), the largest in the lateral and adventitious roots. The smaller nodules occurred in the main, lateral and adventitious roots. In this stage, main root became tuberous. The above description showed that the initial morphology was semi-hypogeal, since the seed coat ruptured (phanerocotylary), although the cotyledons were kept below soil level. There were no signs of cotyledon senescence in the phases observed.

## DISCUSSION

In the dicotyledons, albuminous seeds generally tend to produce epigeal seedlings while exalbuminous seeds produce various types (Gilbert, 1939). This was also seen by Oliveira (1997), who reported epigeal seedling formation in all the albuminous species analysed. In the present study, the semi-hypogeal seedlings formation in *P. ahipa*, an exalbuminous species was noted, which agreed with Gilbert (1939). According to Lackey (1981), the *Pachyrhizus* genus is included in the 84 genera of the Phaseoleae tribe, which has both epigeal (Flores and Rivera, 1984; Oliveira, 2001) and hypogeal (Burkart, 1952) species. The *P. ahipa* semi-hypogeal seedling found in this study is a morphological type so far not reported in Phaseoleae. It is generally known that this type is a structural transition between the epigeal and hypogeal species. However, new studies with more species are needed in order to generalize for the tribe, as suggested by Oliveira (2001).

Present data agreed with Polhill et al. (1981) on the occurrence of reversions in Phaseoleae. Literature (Lackey, 1981; Yeh et al., 1987; Burtt, 1991) showed that reversions in the Phaseoleae tribe were more common in the Diocleinae subtribe (which includes *Pachyrhizus*), Erythrininae, and Phaseolinae; *Erythrina* and *Vigna* are the better studied genera.

The Phaseoleae tribe includes phanerocotylar, and more frequently, cryptocotylar seedlings with fleshy cotyledons, a well developed plumule, and almost always unifoliated eophylls with opposite phyllotaxis (Duke and Polhill, 1981). In *P. ahipa*, the seedling was initially cryptocotylar, becoming phanerocotylar as the sapling was established. In relation to other characteristics, the species was as described for the tribe. It is important to emphasize the variation in eophyll stipules that sometimes are like a distinct reduction of the trifoliate structure.




**Figures 7-12** - Venation pattern of *Pachyrhizus ahipa*. 7-9. Eophyll. 7. General view. 8. Detail of lamina central region, showing the secondary veins with acute, predominantly moderate divergence angles. 9. Detail of the margin. 10-12. Apical foliole of the metaphyll. 10. General view. 11. Detail of lamina central region; note acute, predominantly wide secondary divergence angles. 12. Detail of the margin. (pv: primary vein, sv: secondary vein, arrow: divergence angle, arrowhead: veinlet, asterisk: areole). Bars: 20mm (10), 15mm (7), 5mm (11), 2mm (8), 1mm (9, 12)

Some authors have reported the formation of simple eophylls in legume species. Yeh et al. (1987) stated that the *Dumasia*, *Pachyrhizus,* and *Psophocarpus* genera form two simple primary leaves (eophylls). This is generally due to not having observed foliar base modifications such as pulvinules and stipels. As both modifications were seen in *P. ahipa*, its eophylls must be classified as unifoliate. The pinnate brochidrodomous venation patterns seen in *P. ahipa* eophylls and metaphylls are in agreement with Hickey and Wolfe (1975) for the Fabales order.

Records of cryptocotylar seedlings for the *Pachyrhizus* genus were made by Burtt (1991), who did not report the degree of plant development. Therefore, it is possible that some of these species change to phanerocotylar over time, as seen in *P. ahipa*. Garwood (1996) emphasized that seedling establishment and development were dynamic processes and, therefore, a static classification based exclusively on one development phase tended to make the interpretation of functional morphology difficult. Studies reporting the existence of semi-hypogeal seedlings are rare. This could be due the analysis of epigeal and hypogeal seedlings which did not consider associated the character of cotyledon exposure (phanero or cryptocotylar). Thus, seedlings which maintain their cotyledons below soil level and become phanerocotylar during development tend to be described as hypogeal (that is cryptocotylar), when they should be classified as semi-hypogeal.

Oliveira and Beltrati (1992) reported semi-hypogeal seedlings in *Inga urugüensis* and *I. fagifolia*, an exception in the Mimosoideae subfamily, that has fundamentally epigeal seedlings and some hypogeal species (Compton, 1912; Duke and Polhill, 1981). Cunha and Ferreira (2003) recorded a semi-hypogeal seedling for *Amburana cearensis* (Faboideae). Ressel et al. (2004) only observed semi-hypogeal seedlings in ten of 122 arboreal species studied in an ecological station.

Yeh et al. (1987) classified *P. erosus* seedlings as hypogeal; however, they did not analyze cotyledon exposure. It is interesting to consider the structural variation of the cotyledons and their exposure to the environment. Thin foliaceous cotyledons associated with epigeal seedlings tend to abscise precociously while fleshy cotyledons, rich in reserve material, are kept on the plant for longer, especially when retained below the soil (Oliveira, 2001). Cotyledon persistence in *P. ahipa* corroborates the above. According to Vogel (1980) cryptocotylar and hypogeal seedlings are more resistant because their nutrient reserves are protected by seed-coat and are kept below the soil. Considering the few species those seedlings were studied, we do not have enough information to allow conclusions about correlations between seedling types, their possible ecological significance and species habits. Extensive studies such as Garwood (1995, 1996) have suported this position. Ressel et al. (2004) find no significant relationships between morphological and functional groups of the seedlings of tree species and dispersal syndrome, fruiting period, root/aerial part biomass, or habitat of the studied species. However, they did find a relation between the successional groups, seed weights, and sexual systems. According to the authors, climaxes species, that usually have less seeds with more reserves, present predominantly crypto-hypogeal seedlings while most pioneer species producing large quantities of small seeds, have almost exclusively phanero-epigeal seedlings.

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## RESUMO

É de conhecimento geral que existe grande escassez de estudos referentes às fases juvenis das plantas, importantes como subsídio para diversos trabalhos ecológicos, taxonômicos e filogenéticos. Com base nesse pressuposto, descreveram-se morfologicamente plântulas e plantas jovens de *Pachyrhizus ahipa*, espécie cultivada de Phaseoleae (Fabaceae), analisando-se o tipo de plântula e caracterizando estruturalmente os cotilédones, eofilos e metafilos. Verificou-se que a plântula é semi-hipógea, tipo de germinação até então não registrado para a tribo Phaseoleae. A plântula forma dois eofilos unifoliolados e opostos, acompanhados por evidente par de estipelas. Os metafilos são trifoliolados, de filotaxia alterna. Tanto eofilos quanto metafilos são pinados, camptódromos e broquidódromos.

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