Morphology and anatomy of the diaspores and seedling of *Paspalum* (Poaceae, Poales)

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**ABSTRACT**

The knowledge regarding of the diaspore and post-seminal development of *Paspalum* L. is important for grassland biodiversity conservation, based on their representativeness and genetic improvement of forage. The morphology of the diaspore and the post-seminal development of *Paspalum dilatatum* Poir. (rhizomatous), *P. mandiocanum* Trin. var. *subaequiglume* Barreto (stoloniferous), *P. pumilum* Nees. (decumbent caespitose) and *P. urvillei* Steud. (erect caespitose) was described to distinguish species with different growth forms and to survey the characters useful for taxonomy. *P. dilatatum* differs by presenting oval diaspores larger than the others, with five prominent nerves and trichomes; *P. urvillei* presents diaspores with one central nerve that is more developed than the two lateral nerves and trichomes; *P. mandiocanum* var. *subaequiglume* presents diaspores with trichomes only in the margin; and *P. pumilum* differs by presenting glabrous diaspores. The caryopsis involves the seed that presents the differentiated embryo and disposed laterally, an elliptical hilum in all of the studied species and a rostellum in *P. dilatatum* and *P. mandiocanum* var. *subaequiglume*. The post-seminal development is similar in the four species and begins with germination that is marked by the emergence of the coleorhiza, followed by the coleoptile. These characteristics are common to other Poaceae previously studied, indicating a pattern to the family and do not distinguish the growth forms.

**Key words:** caryopsis, embryo, forage, germination, grass.

**INTRODUCTION**

Poaceae Barnhart is one of the largest angiosperm families, comprising 17% of its species (Soltis and Soltis 2004, Nardmann et al. 2007). *Paspalum* L. is included in the Panicoideae Link subfamily, tribe Paniceae R. Br., with approximately 400 species distributed in the tropics and subtropics (Pizarro 2000, Oliveira and Valls 2008). Brazil is home to approximately 202 species distributed in almost all of the grasslands (Valls and Oliveira 2010). *Paspalum* stands out by its representation and forage potential in native Brazilian grasslands and is indicated for pasture improvement in Southern Brazil (Scheffer-Basso et al. 2009).

*Paspalum* is characterized by presenting plano-convex spikelets that are unilaterally arranged on the rachis (Souza-Chies et al. 2006, Oliveira and Valls 2008, Maciel et al. 2009) and a caryopsis...
with different forms and colors that is enveloped by protective bracts. The shape of the caryopsis apex, the presence of rostellum and the trichomes and hilum shape are important characters in the taxonomy of the Poaceae (Reeder 1957, Hoshikawa 1969, Filgueiras 1986), including *Paspalum* (Oliveira and Valls 2001, 2002).

The inflorescence types and growth forms of *Paspalum* are related to the group’s taxonomy (Rua and Weberling 1995, Rua and Grottola 1997), and the morphology and anatomy of the seed and post-seminal development indicate the homogeneity in the family (Hoshikawa 1969, Morita et al. 1997, Nakamura and Scatena 2009).

The embryo of Poaceae presents characteristics that are common to other Poales, such as lateral disposition, the presence of a coleoptile, mesocotyl formation and the complete reduction of the primary root (Tillich 2007). Structures of the Poaceae embryo that are characteristics used in the taxonomy of this group include the presence or absence of the scutellum slit, the epiblast, vascularization and the overlap of the plumule margins (Reeder 1957). In *Paspalum*, studies regarding the embryos and seedlings are generally directed for pasture improvement (Pearson and Shah 1981, Marousky and West 1990, Morita et al. 1997, Espinoza et al. 2001).

As *Paspalum* is the most well-represented genus of the remnant grasslands of Brazil and has great economic potential, information about the diaspores and seedlings of its species with different growth forms are important for the taxonomy of the group and it may aid in the conservation of its biodiversity. Therefore, the morphology and anatomy of the diaspores of *Paspalum dilatatum* Poir., *P. mandiocanum* Trin. var. *subaequiglume* Barreto, *P. pumilum* Nees. and *P. urvillei* Steud. were described to survey the characters useful for its taxonomy and to verify whether differences in the post-seminal development of the species with different growth forms occur or not.

**MATERIALS AND METHODS**

The specimens studied were collected in the remnants of the native grasslands of the Plateau of Santa Catarina State, Southern Brazil. The native grasslands comprise 14% of the state area, and they risk being replaced by exotic pasture or crops due to economic pressures (Córdova et al. 2004). Such region presents livestock as one of the more traditional economic activities sustained by natural pasture, and *Paspalum* is very abundant. We have studied four species with different growth forms that are important as forage potential for Southern Brazil (Valls et al. 2009). The following vouchers have been deposited in the Herbarium of the Department of Botany (HRCB), Instituto de Biociências, Universidade Estadual Paulista (UNESP), Rio Claro: *Paspalum dilatatum* Poir. (M.T. Eichemberg 701 & N. Prestes) - rhizomatous; *P. mandiocanum* Trin. var. *subaequiglume* Barreto (M.T. Eichemberg 695 & N. Prestes) - stoloniferous; *P. pumilum* Nees. (M.T. Eichemberg 763) - decumbent caespitose and *P. urvillei* Steud. (M.T. Eichemberg 700 & N. Prestes) - erect caespitose. The specimens were identified by Dr. José Francisco Montenegro Valls.

The mature diaspores (caryopses with bracts) were placed in concentrated sulfuric acid for five minutes, then washed with water. The caryopses were placed in gerboxes (plastic boxes) on filter paper moistened with distilled water. The 25 caryopses from different individuals of each species were grown in four replicates in a completely random design and were maintained at 25°C and under uninterrupted white light. The morphological characteristics of the diaspores (n = 25) and seedlings (n = 15) for each species were surveyed.

The stages of post-development and seedlings were monitored daily. The seedlings of each species at different development stages were fixed in FAA 50 and preserved in 70% alcohol for the anatomical study (Johansen 1940). The samples were dehydrated in n-butyl alcohol (NBA), infiltrated and embedded.
in historesin (Leica Historesin Embedding Kit) (Feder and O’Brien 1968). Cross and longitudinal sections were cut with a rotative microtome, stained with periodic acid, Schiff’s reagent (PAS) and Toluidine Blue (Feder and O’Brien 1968) and mounted in Entellan. Histochemical tests were made on caryopses treated with Lugol’s solution to detect starch (Johansen 1940). Images were captured with the Leica DFC 290 digitalizing system coupled to the Leica DMLB microscope.

RESULTS

The diaspores studied are oval in *Paspalum dilatatum* (Figs. 1A, 2A) and oval-elliptical in *P. urvillei* (Figs. 1B, 2B), *P. mandiocanum* var. *subaequiglume* (Figs. 1C, 2D) and *P. pumilum* (Fig. 2C). The diaspores vary in sizes and are larger in *P. dilatatum* (\(\bar{x} = 0.24\) cm), followed by *P. mandiocanum* var. *subaequiglume* (\(\bar{x} = 0.20\) cm), *P. pumilum* (\(\bar{x} = 0.19\) cm) and *P. urvillei* (\(\bar{x} = 0.18\) cm).

The diaspores present an upper glume (Fig. 1B), a lower lemma (Fig. 1A) and a fruit-type caryopsis (Fig. 1C-D). *P. dilatatum* has five salient nerves in the glume, and trichomes are distributed over the entire surface (Figs. 1A, 2A), whereas *P. urvillei* presents one central nerve that is more developed than other two lateral, as well as the trichomes distributed over the entire surface (Figs. 1B, 2B). *P. mandiocanum* var. *subaequiglume* displays five salient nerves and trichomes are distributed over the entire surface (Figs. 1C, 2D), whereas *P. urvillei* presents one central nerve that is more developed than other two lateral, as well as the trichomes distributed over the entire surface (Figs. 1B, 2B). *P. dilatatum* has five salient nerves in the glume, and trichomes are distributed over the entire surface (Figs. 1C-D). *P. dilatatum* has five salient nerves in the glume, and trichomes are distributed over the entire surface (Figs. 1C-D). *P. dilatatum* has five salient nerves in the glume, and trichomes are distributed over the entire surface (Figs. 1C-D). *P. dilatatum* has five salient nerves in the glume, and trichomes are distributed over the entire surface (Figs. 1C-D). *P. dilatatum* has five salient nerves in the glume, and trichomes are distributed over the entire surface (Figs. 1C-D).

The adaxial surface of which is convex and encloses the embryo (Figs. 1C, 2E-arrow). The abaxial surface is flat with an elliptical hilum (Fig. 1D-arrowhead).

The caryopses display a rounded apex (Fig. 1C-D), with a rostellum in *P. dilatatum* (Fig. 1D-arrow) and *P. mandiocanum* var. *subaequiglume* (Fig. 2G-arrow), the adaxial surface of which is convex and encloses the embryo (Figs. 1C, 2E-arrow). The abaxial surface is flat with an elliptical hilum (Fig. 1D-arrowhead).

The embryos of the four species present a scutellum slit (Fig. 1F-arrowhead), and an epiblast is absent (Fig. 1E-G). The lower embryo region presents the shoot-born root with an apical meristem (am) and a cofl (cf) (Fig. 1F-H) that originates from the mesocotyl and is enveloped by the coleorhiza (Fig. 1F, 1G-H). The cotyledonary node (Fig. 1H-arrow) (mesocotyl) presents procambial cells that will vascularize the rhizome. The vascularization of the embryos is of the “panicoid” type (Fig. 1G-H), that is, a procambial vascular system that diverges for the scutellum and for the embryonic leaf in different internode spots.

The post-seminal development begins with germination, occurring approximately 24 to 48 hours after caryopsis imbibition. The germination is evidenced by the emergence of the coleorhiza (Figs. 1G - arrow, 2F-G), which presents long hairs (Fig. 2F-I). In the seedling, the mesocotyl develops and forms the rhizome of the adult plant, which establishes the vascular connection between the roots and the leaves (Fig. 1K). The growth of adventitious roots from the pericycle of the rhizome can be observed (Figs. 1J-arrow).

The coleoptile elongates and the first leaf (eophyll) emerges (Fig. 2J-K), which is long (Fig. 2L-N), with trichomes distributed throughout the blade in *P. mandiocanum* var. *subaequiglume* (Fig. 2M) and *P. pumilum* (Fig. 2L). Simultaneously, the development of new adventitious roots from the rhizome occurs (Figs. 1J, 2L-N), followed by eophyll expansion and the development of the second leaf (Fig. 2O-P).

DISCUSSION

The diaspore morphology differs among the studied *Paspalum* species and can be used for the taxonomy to distinguish the species. The size, shape, presence and localization of trichomes,
Fig. 1 - Morphology and anatomy of the diaspore, caryopsis and initial seedling stages of Paspalum. A: Upper glume of the diaspore of *P. dilatatum*. B: Lemma of the diaspore of *P. urvillei*. C-D: Caryopses of *P. mandiocanum* var. *subaequiglume* (arrow = embryonic region) and *P. dilatatum* (arrow = rostellum, arrowhead = hilum), respectively. E-J: Longitudinal sections of the caryopsis, embryo and seedling. E: Caryopsis of *P. urvillei*. F: Detail of the embryo of *P. urvillei* (arrow = aleurone layer, arrowhead = scutellum slit). G-H: Embryo of *P. dilatatum*. I: Seedling of *P. pumilum* in the stage of coleoptile expansion. J: Seedling of *P. dilatatum* with an adventitious root (arrow) and eophyll. K: Cross section of *P. pumilum* seedling. *am* = apical meristem; *cf* = coif; *co* = coleoptile; *cr* = coleorhiza; *en* = endosperm; *me* = mesocotyl; *pl* = plumule; *sc* = scutellum, *sr* = shoot-born root. Bars = 600 µm (A); 500 µm (B); 200 µm (C, D, I); 180 µm (E, G); 140 µm (J); 100 µm (F); 90 µm (H) 70 µm (K).
the number of nerves salience in the diaspore and the presence of the rostellum in the caryopses are characters that allow the identification of the studied species. These characteristics were also used in the taxonomy and phylogeny of *Paspalum* by other authors (Oliveira and Valls 2002, Rua et al. 2008, 2010, Denham et al. 2010).

The post-seminal development is similar among the studied *Paspalum* species, independent of their growth form when adults, and it constitutes a pattern for the group as observed in other Poaceae (Tillich 2007, Nakamura and Scatena 2009). Regarding the rhizomatous growth form, the stoloniferous, erect caespitose and decumbent caespitose present similar initial seedling stages, with the rhizome originating from the mesocotyl. These four growth forms are the result of rhizome development variations during the establishment of the plant, which probably occurs due to genetic differentiation, as these species inhabit the same environment.

The diaspores of the studied *Paspalum* species present only an upper glume and lemma enveloping the caryopsis, thus, corroborating the description of the genus (Filgueiras 1986, Oliveira and Valls 2001, Boldrini et al. 2005). In most Poaceae, the diaspores are composed of palea, lemma and glume or they are associated with other parts of the inflorescence (Dalhgren et al. 1985, Boldrini et al. 2005, Souza et al. 2009). For the studied species, the diaspore characteristics survey helps in their identification and corroborates the importance of its use in the taxonomy of the genus.

The presence of a rostellum in the *P. dilatatum* and *P. mandiocanum* var. *subaequiglume* caryopses was correlated with other Poaceae and can be considered an important character of the infrageneric level (Filgueiras 1986). The studied species present albuminous seeds, an embryo laterally attached to the endosperm, the presence of a coleoptile, mesocotyl and a reduction of the primary root in the seedling, as previously described for other Poaceae (Martin 1946, GPWG 2001, Tillich 2007, Nakamura and Scatena 2009). The “panicoid”-type vascularization in the embryo was verified in other representatives of the tribe Paniceae and, therefore, has taxonomic importance in Poaceae (Reeder 1957).

The elliptical hilum present in all of the studied species was correlated according to the concept of Filgueiras (1986), who defines as the placental region of the caryopsis where the ovule is inserted in the carpel wall but suggested that it is not homologous to the hilum of other angiosperms as it is a structure that involves the pericarp. The hilum shape varies in other *Paspalum* species, which has been used in taxonomic descriptions (Oliveira and Valls 2002, Rua et al. 2008, Denham et al. 2010) and in the phylogenetic analysis of the genus (Rua et al. 2010). This feature can also vary in other genera of the Poaceae family (Filgueiras 1986).

The scutellum (cotyledon) is considered a reduced leaf structure due to the presence of vascularization (Shuma and Raju 1991) or it can be considered as an embryonic leaf structure (Cocucci and Astegiano 1978). Molecular data of *Zea mays* confirm that the scutellum is an embryonic structure without any corresponding structure in the eudicot embryo (Nardmann et al. 2007) and can be considered an exclusive organ of Poaceae without homology with other Poales (Rudall et al. 2005).

In the studied species of *Paspalum*, the primary root is reduced, remaining as a vestige in the coleorhiza form, as reported also for other Poaceae (Cocucci and Astegiano 1978, Tillich 2007, Nakamura and Scatena 2009). In monocots, the reduction of the primary root has occurred throughout evolution, and its absence is a derivative character (Boyd 1931, Tillich 2007). Within the Poales, the reduction of the primary root was reported for different taxa that are considered ancestral and derivative (Ramaswamy et al. 1981, Scatena et al. 1993, Scatena et al. 1996, 2006, Tillich 2007); so, it appears to be a character without utility for the group phylogeny.
In the studied species, the germination was marked by the emergence of the coleorhiza, followed by the coleoptile. The anatomical structures of the *Paspalum* seedlings studied in this work correspond to those already described for others representatives of the genus (Hoshikawa 1969, Nakamura and Scatena 2009). Characters, such as a well-developed sheath and blade of the first leaf and the absence of an epiblast, are considered derivative in Poaceae and corroborate the intermediate position of the genus in the family (GPWG 2001).

The post-seminal development is similar in the studied species of Poaceae, and the changes that occur in the later stages of development to promote the differences in the growth forms, such as rhizomatous, stoloniferous, decumbent caespitose and erect caespitose, can be related to genetic and hormonal responses to the environment.

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