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BRIEF COMMUNICATION

The occurrence of dicotyledonar embryos in *Agave tequilana*

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Abstract

Agave tequilana Weber is a monocot plant species member of the *Asparagaceae* family. One of the characteristics of monocot species is that their embryos show only one cotyledon. In this work, the occurrence of embryos with two cotyledons and fused cotyledons in *A. tequilana* is reported for the first time. The occurrence of dicotyledonar embryos in a species that by definition should have only one cotyledon could bring an opportunity to elucidate the mechanisms that have given the origin to the only one cotyledon present in monocots. Syncotyly is considered in this work as the possible mechanism that gave rise to the only cotyledon mostly present in this species.

Additional key words: zygotic embryo, monocotyledonous species, syncotyly.

Agave tequilana Weber is a plant species of the *Asparagaceae* family (formerly *Agavaceae*) that belongs to the monocot class of angiosperms. The angiosperms are diverse in chemistry, reproductive morphology, and genome size and organization among others, but they are united by a suite of synapomorphies (Soltis and Soltis 2004). The angiosperms are divided into two major groups: monocots and dicots showing differences in the number of cotyledons in their embryos, one for monocots and two for dicots. Molecular phyllogenetic studies carried out in the 1990s and 2000s have discarded this old classification and have established a monophylletic group called monocots (which comprises all the monocot species from the previous classification), another group called eudicots (which comprises basically most of the species that were considered previously as dicots), and other clades that comprise a considerable diversity of flowering plants (Soltis and Soltis 2004). Despite that the dicot term is not considered for classification anymore, in this work, it is used for practical purposes and in order to avoid changes to the original references.

Angiosperm evolution has been a relevant topic since the angiosperms are considered as a model group for studying patterns and processes of diversification (Davies *et al*. 2004). It has been inferred that the origin of angiosperms occurred about 200 (Martin *et al*. 1993) or 340 Myr ago (Wolfe *et al*. 1989) and that monocots and dicots diverged 200 Myr ago (Wolfe *et al*. 1989, Martin *et al*. 1993). Monocots and dicots are closely related phyllogenetic groups. Vicentz *et al*. (2004) found that approximately two thirds of the transcriptome of monocot sugar cane shows similar sequences to those of dicot *Arabidopsis thaliana*. Although monocots separated from dicots, approximately two thirds of their genes have kept similarities, whereas the rest of the genes are constituted by sequences with a fast evolution (Vicentz *et al*. 2004).

In evolutionary terms, the exact position of monocots is not clear. It seems that they are inside a group of dicots (Chase 2004). Thus, monocots may be derived from some primitive dicots. However, "the distinction between monocots and dicots is most apparent when considering eudicots *vs*. monocots and less clear against the background of the highly heterogeneous 'primitive' dicots" (Chase 2004). As it was mentioned before, it is considered that monocots must have evolved from a primitive dicot. If a monocotyledon derived from a dicotyledon, it must have happened through the process known as syncotyly or heterocotyly (Bancroft 1914). Syncotyly is a concept used for the description of the fusion of two cotyledons to form one member (Bancroft 1914). It can refer to the partial or complete fusion of two cotyledons as it can be seen in the dicot species *Calphyllum*, *Swieetenia*, *Guarea*, and *Carapa* in which cotyledons are distally fused (Chandler 2008). On the

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other hand, heterocotyly is a concept used to explain the probable evolution of monocot species from a dicotyledonar ancestor. According to this last concept, the only cotyledon present in monocots must have been originated either by a process of division of functions between the original two cotyledons, or by a process where one of the two original cotyledons presented a reduction or even complete suppression, whereas the other cotyledon remained (Bancroft 1914). Sargant (1903) proposed the theory that the only cotyledon found in monocots originated primitively by the fusion of two cotyledons. This theory was reinforced by evidences shown by Haines and Lye (1979) where they showed the evidence of syncotyly in embryos belonging to the dicot families *Apiaceae* (*Umbelliferae*), *Ranunculaceae*, and *Combretaceae*. These dicotyledonous embryos can show a total or partial fusion in their cotyledons and resembled to the cotyledons found in some monocot embryos. By contrast, monocot embryos having two cotyledons have been reported in the genera *Cyrtanthus, Agapanthus*, and *Colocasia* (Haines and Lye 1979). Titova (2003) reported the percentages of embryos of the monocot species *Agapanthus praecox* showing different types of cotyledons. Some embryos showed fused-cotyledons and two cotyledons (1 and 6 %, respectively). Shuma and Raju (1991) considered the monocot species *Avena fatua* as one of the monocot species with a presence of two cotyledons considering the scutellum as one cotyledon and the first leaf primordium as second cotyledon.

The aim of this work was to study the occurrence of dicotyledonar embryos in the monocot species *A. tequilana* and to elucidate the mechanisms responsible for this phenomenon.

In this study, panicles of *Agave tequilana* Weber bearing immature fruits were randomly selected and collected around 30 - 40 d after pollination. Then, immature seeds from each fruit were extracted as well as the embryo from each seed. All the fruits were disinfected using 3 % (m/v) sodium hypochlorite for 10 min and rinsed three times with distilled water and/or submerged in ethanol (70 %, v/v) for further sterilization over a flame for 3 s. *In vitro* embryo rescue under aseptic conditions was carried out in order to study morphological characteristics and the occurrence of one cotyledon, fused cotyledons, and two cotyledons in immature embryos.

The extracted embryos were observed under a *Leica* (Wetzlar, Germany) *StereoZoom SZ-4* dissection microscope and the cotyledon morphology was registered as monocotyledonar, fused, and dicotyledonar. These immature embryos were cultured *in vitro* for maturation and germination in disposable sterile Petri dishes containing 25 cm^3 of a modified Murashige and Skoog (MS) medium without growth regulators following Santacruz-Ruvalcaba *et al.* (1998). The evolution of cultured embryos was followed during a 60-d period

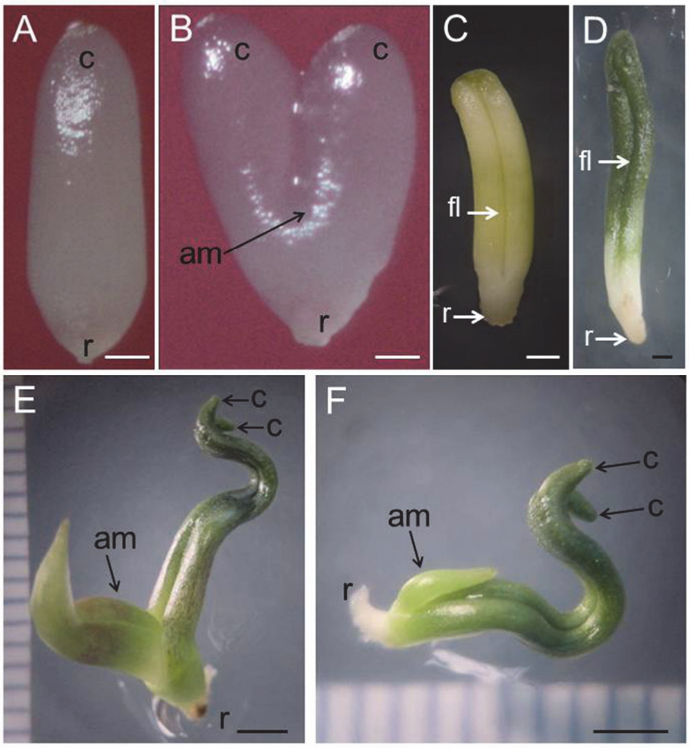

Fig. 1. Zygotic embryos of *Agave tequilana*: *A* - a monocotyledonar embryo in the torpedo stage (*bar* = 0.5 mm); *B* - a heart-shaped dicotyledonar embryo (*bar* = 0.5 mm); *C* and *D* - embryos with fused cotyledons (syncotyly) (*bars* = 0.5 mm); *E* and *F* - dicotyledonar germinating embryos (*bars* = 2 mm). c - cotyledon, r - radicle, am - apical meristem, fl - fusion line.

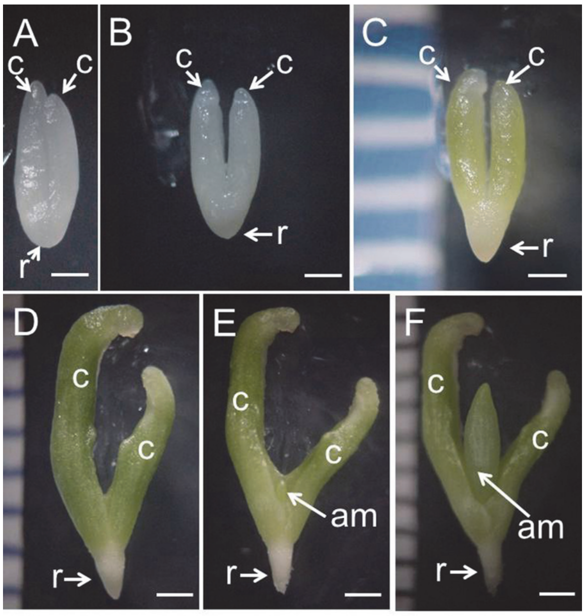

Figure 2. Germination of a dicotyledonar embryo of *A. tequilana* showing long and short cotyledons (anisocotyly): *A* - the moment of immature embryo rescue (ER); *B* - 5 h after ER; *C* - 3 d after ER;. *D* - 8 d after ER; *E* - 15 d after ER; *F* - 25 d after ER. The *bars* for *A*, *B*, and *C* = 0.5 mm, for *D*, *E*, and *F* = 1 mm. c -cotyledon, r -radicle, am -apical meristem.

under the mentioned dissection microscope.

In this work, a total of 1 164 seeds were studied. An important finding was heart-shaped embryos in *A. tequilana* (Fig. 1) which had not been reported for any other monocot species. This stage is considered to be exclusively in dicots, and by definition, it should not be present in a monocot species like *A. tequilana*. Embryos in more advanced stages showing two cotyledons were also found. Besides, embryos showing two fused cotyledons were observed as well. Embryos with only one cotyledon were also registered. *A. tequilana* showed a high percentage of embryos bearing two cotyledons and fused cotyledons.

From the total of analyzed embryos, 4 % showed two cotyledons (or dicotyledonar embryos) (Fig. 1*B*,*E*,*F*), 44 % showed two fused cotyledons (Fig. 1*C*,*D*), and 52 % showed only one cotyledon (Fig. 1*A*). Another important feature observed was a prolonged growth of one cotyledon over the other in the fused and dicot embryos (Fig. 2). Anisocotyly is the term used to describe the presence of a prolonged growth of one cotyledon over the other to give rise to a large macrocotyledon and a small microcotyledon (Chandler 2008).

The observation of the embryo stages in this species suggest that they could be considered as intermediate between the stages in the dicot species *Arabidopsis thaliana* (Park and Harada 2008) and the monocot species *Zea mays* (Feng *et al*. 2003). In the monocotyledonar embryos of *A. tequilana*, the apical meristem was located ventrally, whereas the cotyledon was developed dorsally and laterally to the apical meristem. The presence of two cotyledons and fused cotyledons in this species suggests that the only cotyledon found in *A. tequilana* could have evolved from two cotyledons. Fused, monoctyledonar, and dicotyledonar embryos germinated and gave rise to normal plantlets (Fig. 1*E*,*F*, 2*F*).

By the observation of the *A. tequilana* embryos and comparing them with the images of other monocot embryos, it was considered that this species showed an intermediate pattern of development between dicots and monocots. Leyser and Day (2002) stated that monocots do not show the heart-shape stage in their embryos. However, the monocot species *A. tequilana* has proven that this statement is not always followed. Just as it was previously mentioned, *A. tequilana* could develop embryos showing the heart-shape stage. This morphology is thought to be exclusive to dicots. The presence of dicotyledonar, fused, and monocotyledonar embryos in *A. tequilana* gave rise to an old question: does the only cotyledon found in monocots originate by the fusion of two cotyledons? The ability of monocot plants to form dicotyledonar embryos originates a question with a great theoretical significance for the origin of the monocotyledonar state (Titova 2003).

The majority of seed plants show two cotyledons, suggesting that the monocotyledonar state is derived (an apomorphy) (Chandler 2008). According to what we observed in *A. tequilana,*, it can be speculated that the only cotyledon of this species originated by the fusion of two cotyledons. The results presented here correspond to the hypothesis of syncotyly. The observations of this work show that this organ evolved from a dicotyledonar state according to hypotheses proposed by Sargant (1903), Agardh (cited in Bancroft 1914), Bancroft (1914), Haines and Lye (1979), Liu *et al*. (1993), and Titova (2003).

It is considered that *A. tequilana* presents a pattern of embryo development that could be classified as intermediate between what has been set as typical for dicot species and monocot species. The presence of embryos showing the heart-shape stage suggests that *A. tequilana* shares mechanisms related to patterns of development with dicots. These findings suggest that the typical stages assigned to monocot species (*e.g.*, *Zea mays*) do not correspond to what is shown in *A. tequilana*. Therefore, further embryo development studies that consider the morphology of the embryonary stages in other monocot species that do not belong to the *Poaceae* family should be carried out.

The necessity of studying other monocot species that do not correspond to the *Poaceae* members was highlighted in the study done by Kuhl *et al*. (2004) where they found that the genomic resources developed for the order *Poales* do not correspond to the members of the order *Asparagales*, since they discovered that *Asparagales* are more similar to *Arabidopsis* than to rice in genomic terms.

In relation to what has been shown so far, *A. tequilana* could be a key species for plant evolutionary studies. This species might be presenting several ancestral mechanisms that affect the development of cotyledons. This could represent an important genetic resource for the elucidation of plant evolutionary pathways. This, perhaps, can reveal the extension in the genes conservation through the plant kingdom and distinguish the mechanisms that have evolved divergently.

References

Bancroft, N.: A review of literature concerning the evolution of monocotyledons. - New Phytol. **13**: 285-308, 1914.

Chandler, J.W.: Cotyledon organogenesis. - J. exp. Bot. **59**: 2917-2931, 2008.

Chase, M.W.: Monocot relationships: an overview. - Amer. J. Bot. **91**: 1645-1655, 2004

Davies, T.J., Barraclough, T.G., Chase, M.W., Soltis, P.S., Soltis, D. E., Savolainen, V.: Darwin's abominable mystery: insights from a supertree of the angiosperms. - Proc. nat. Acad. Sci. USA **101**: 1904-1909, 2004.

Feng, J.H., Xu, B.X., Dong, L.X., Zhang, C.L., Liang, X.L., Wu, W.C.: Embryogenesis, germination, structure and cotyledon dimorphism of *Zea mays* embryo. - Acta bot. sin. **45**: 712-723, 2003.

Haines, R.W., Lye, K.A.: Monocotylar seedlings: a review of evidence supporting an origin by fusion. - Bot. J. Linn. Soc. **78**: 123-140, 1979.

Kuhl, J.C., Cheung, F., Yuan Q., Martin, M., Zewdie, Y., McCallum, J., Catanach, A., Rutherford, P., Sink, K.C., Jenderek, M., Prince, J.P., Town, C.D., Havey, M.J.: A unique set of 11,008 onion expressed sequence tags reveals expressed sequence and genomic differences between the monocot orders *Asparagales* and *Poales*. - Plant Cell **16**: 114-125, 2004.

Leyser, O., Day, S.: Mechanisms in Plant Development. - Blackwell, Oxford 2002.

Liu C. M., Xu, Z. H., Chua, N. H.: Auxin polar transport is essential for the establishment of bilateral symmetry during early plant embryogenesis. - Plant Cell **5**: 621-630, 1993.

Martin, W., Lydiate, D., Brinkmann, H., Forkmann, G., Saedler, H., Rüdiger, C.: Molecular phylogenies in angiosperm evolution. - Mol. Biol. Evol. **10**: 140-162, 1993.

Park, S., Harada, J.J.: Plant Embryogenesis. - In: Suárez, M.F., Bozhkov, P.V. (ed): Methods in Molecular Biology. Vol. 427. Pp 3-14. Humana Press, Totowa 2008.

Santacruz-Ruvalcaba, F., Gutiérrez-Mora, A., Rodríguez-Garay, B.: Somatic embryogenesis in some *Cactus* and *Agave* species. - J. Prof. Assoc. Cactus Dev. **3**: 15-26, 1998.

Sargant, E.: A theory of the origin of monocotyledons founded on the structure of their seedlings. - Ann. Bot. **17**: 1-92, 1903.

Soltis, P.S., Soltis, D.E.: The origin and diversification of angiosperms. - Amer. J. Bot. **91**: 1614-1626, 2004.

Shuma, J.M., Raju, M.V.S.: Is the wild oat embryo monocotylous?. - Bot. Mag. Tokyo **104**: 15-23, 1991.

Titova, G.E.: *Agapanthus praecox* Willd. (*Alliaceae*) in monocotyly, dicotyly and transitional forms. - Acta biol. cracov. Bot. **45**: 161-165, 2003.

Vicentz, M., Cara, F.A.A., Okura, V.K., Da Silva, F.R., Pedrosa, G.L., Hemerly, A.S., Capella, A.N., Marins, M., Ferreira, P.C., Franҫa, S.C., Grivet, L., Vettore, A.L., Kemper, E.L., Burnquist, W.L., Targo, M.L.P., Siqueira, W.J., Kuramae, E.E., Marino, C.L., Camargo, L.E.A., Carrer, H., Coutinho, L.L., Furlan, L.R., Lemos, M.V.F., Nunes, L.R., Gomes, S.L., Santelli, R.V., Goldman, M.H., Bacci, M., Giglioti, E.A., Thiemann, O.H., Silva, F.H., Van-Sluys, M.A., Nobrega, F.G., Arruda, P., Menck, F.M.: Evaluation of monocot and eudicot divergence using the sugarcane transcriptome. - Plant Physiol. **134**: 951-959, 2004.

Wolfe, K., Gouy, M., Yang, Y.W., Sharp, P.M., Li, W.H.: Date of monocot-dicot divergence estimated from chloroplast DNA sequence data. - Proc. nat. Acad. Sci. USA **86**: 6201-6205, 1989.