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Observations on the subterranean system of *Smilax goyazana* (Smilacaceae)

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ABSTRACT: Cuttings from rhizome and root parts were obtained from adult plants of *Smilax goyazana* A. DC. (Smilacaceae) growing in the cerrado and cultivated in moist sand for three months. Seeds were left to germinate in a germination chamber under constant temperature of 25^0C and the subterranean system that formed was observed after 10 months of growth. All the cuttings had died by the end of the experiment. The subterranean system was composed of a hard tuberosity derived from the primordial node, which emitted few roots and one or more rhizomes. The rhizomes were sometimes branched; however, they did not emit adventitious roots. The roots were not branched. Thus, according to fractal geometry, the cuttings of this species were difficult to root because they were not sub-units of the adult plant.

Key words: Culm, Rhizome, Saponin, Vegetative Propagation

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INTRODUCTION

The genus *Smilax* (from the Greek, smilax, scraper, referring to foliar and caulinar prickles) occurs in tropical and temperate zones in all the continents. They are dioecious monocotyledons with petiolated leaves, reticulated nervation, growing as climbing herbs or scandent sub-shrubs (Andreata 1980).

In the past, in folk medicine, plants of the genus *Smilax* had been used extensively worldwide for the treatment of many diseases (Schroeder 1988). The interest in the genus has increased again after the discovery that the Smilacaceae family produces large amounts of steroidal saponins (Bernardo *et al*. 1996).

Due to the complexity of the steroidal nucleus and the difficulty of its laboratorial synthesis, the pharmaceutical industry extracts steroids from animal, plant and fungus sources and modifies the molecules, transforming them into a large class of medications, the steroids, used as contraceptive pills, anti-inflammatories and anabolizants, being vital to certain people suffering from autoimmune diseases, chronic inflammations and some genetically inherited disorders (Silva 1998).

Also, not only are these metabolites interesting because they are steroids, but also because they are saponins, since these substances are used for hemoglobin electrophoresis for the diagnosis of some blood diseases (Naoum 1990; Veras *et al.* 2005).

However, protocols for large scale production of such plants are still not well developed. Germination of seeds is difficult to achieve and germinability is low (Palhares *et al.* 2009a, b). In the literature, there are only a few papers on the vegetative propagation of *Smilax* through cuttings: Pogge *et al.* (1974) achieved some production of seedlings of *S. rotundifolia* and *S. glauca*, two common species from North America.

In the Cerrado of Central Brazil, although some species of *Smilax* have been found (Andreata 1997a), *Smilax goyazana* presents a great phyto-sociological dominance in the herbaceous layer, being practically the only species

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of this genus occurring in this environment (Munhoz & Felfili 2006). In spite of the great presence in the Cerrado environment, little is known about this species (Palhares & Silveira 2005; Palhares *et al.* 2009a, 2009b).

As this species may become of interest for cultivation, some initial experiments were carried out to improve our understanding of vegetable propagation related to the morphology of the subterranean system.

METHODS

Detection of saponins. Fresh material from rhizomes and leaves was immersed in boiling water for 5 minutes. After cooling, the presence of bubbles was considered to be indicative of the presence of saponins, according to Matos (1997).

Morphological description of the subterranean system. The morphological study of the subterranean system was based on plants found in the field: four flowering plants (two masculine and two feminine) and three young plants, growing naturally in the cerrado of the campus of the University of Brasilia. The surrounding soil was carefully dug until at least the beginning of the roots was seen. Two of the young plants were growing next to each other, and in this case, digging was done also to eventually disclose a subterranean connection between them. Accuracy of the sample was estimated to be 50% according to Eckblad (1991).

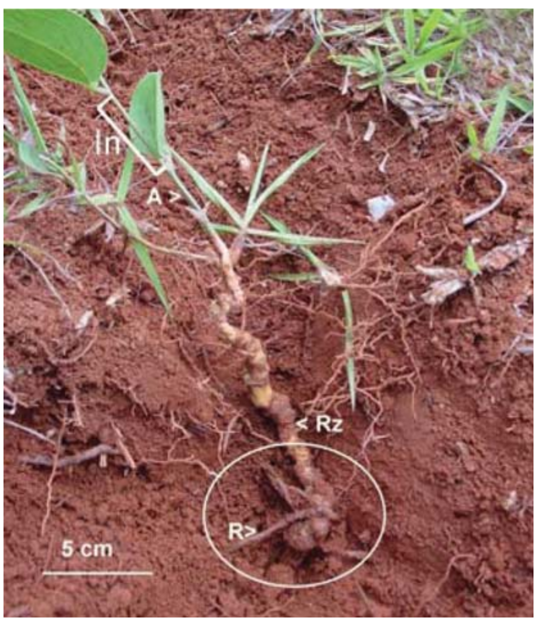

Fig. 1. The subterranean system of one sampled plant in the field. Inside the circle, the hard tuberosity producing roots (**R**) and a rhizome (**Rz**). The apical bud of the rhizome produced a culm (**A**). **In**: internode.

For observing the origin and development of the subterranean system, seeds were put to germinate at a constant temperature of 25 °C (Palhares *et al.* in prep.) and thereafter cultivated in pots in an open environment for 10 months (since this is the period that seedlings are naturally submitted to their first dry season), when the subterranean system that formed was then observed as described by Andreata & Pereira (1990) and Palhares & Silveira (2007).

Vegetative propagation. To attempt vegetative propagation, cuttings were obtained according to Pogge *et al.* (1974), in the following manner: a) cuttings of the rhizome containing at least five nodes; b) cuttings with 3 rhizome nodes and 2 aerial stem nodes; and c) cuttings from the hard tuberosity containing at least one root. The cuttings obtained (15 cuttings in total) were kept immersed in moist sandy soil (vermiculite) next to a window, thus subjected to natural variation of light and temperature, but protected from winds and rain, for three months.

RESULTS

Saponins were detected in the rhizomes, but not in the leaves, which is the most common pattern of distribution for the genus (Bernardo *et al.* 1996).

The subterranean system was composed of three well defined portions: the roots, a hard tuberosity and the rhizomes (Fig. 1). The tuberosity was a dense, very hard structure, from where the roots and the rhizomes sprout (Figures 1 and 2). It was derived from the first (primordial) node of the young plant. In the samples studied, the tuberosities were found at depths varying from 5 to 25 cm. One or more rhizomes could sprout from the tuberosity (Fig. 2).

The rhizomes presented a yellowish colour and were well-defined in nodes and internodes. Each internode was 1 to 4 cm long. In each nodal region, there was a bud, covered by a scale. The buds, when localized in the apical region of the rhizome, generally differentiated into the aerial stems. However, some buds might differentiate into a rhizome, which would confer to the subterranean system a ramified appearance (Fig. 3). The rhizomes could grow in both a horizontal or vertical direction, and in one of the plants that was growing under a small amount of earth, a bifurcated rhizome of vertical growth had reached a depth of 80 cm (Fig. 3). There were no adventitious roots in any of the rhizomes.

The aerial stems are culms (Figs. 1 and 2), clearly defined into nodes and internodes. In each node of the culms, there is a leaf, linked to the stem by an amplexicaulinar sheet, and a bud in the axil of the leaf. The bud may remain dormant or give rise to either a flower-bud or a new branch.

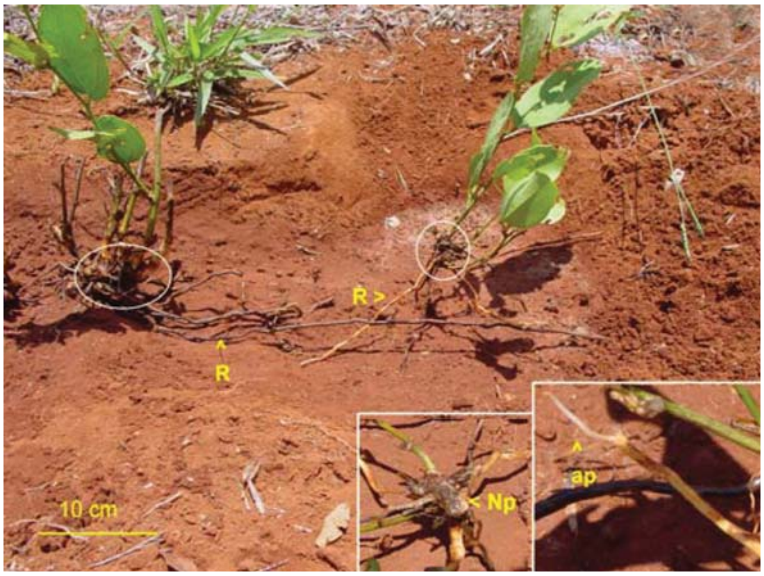

Fig. 2. Two close seedlings without connections through the subterranean systems. Inside circles, the tuberosities producing roots and rhizomes with culms **Np:** detail of the primordial node. **Ap:** detail of the radicular apex.

In such a way the complex made up by the rhizomes and culms is characterized by a periodical repetition of nodes and internodes, with eventual branching.

The roots present a soft and friable consistency and a brownish or black colour (Figs. 1 and 2). Each plant presented 3 to 10 roots that were growing to more than 50 cm, in either vertical or horizontal directions. The apex of the roots is white. No secondary roots or radicles were observed (Figs. 1 and 2). Also, there were no connections between two close seedlings, indicating that each plant had originated from one seed (Fig. 2).

After ten months, the young plants still presented remnants of the seed that by its external appearance, probably still had nutritive reservoirs. The roots of the 10-month old plants reached a depth of 15 to 20 cm. A first rhizome was differentiating from the primordial node (Fig. 4).

No cuttings produced new shoots or roots in the three months of cultivation. A test of samples immersed in tetrazolium showed no metabolic activity, confirming the death of the material (Delouche *et al.* 1962).

DISCUSSION

Many rhizomatous plants (ferns, grasses, bamboos, etc.) can be propagated through simple rhizome cuttings. Generally, these rhizomes produce adventitious roots. *Smilax glabra* produces adventitious roots and can be propagated through rhizome cuttings (Pogge *et al.* 1974).

In such cases, the subterranean system behaves as a fractal: repetitive unities where a fragment of the plant behaves like the whole plant (Oliveira 2004). The rhizome of *S. goyazana* does not produce adventitious roots, which means, a fragment of the rhizome does not possess the characteristics of the whole vegetal body. This can explain, from the fractal point of view (Souza & Buckeridge 2004), the difficulty in spontaneous rooting of rhizome cuttings. In future, treatments with plant hormones may induce differentiation of the rhizome buds into roots, similarly to what has been done with *Stevia rebaudiana* (Carvalho & Zaidan 1995).

While in the studied species the caulinar system is very ramified, the radicular system is not. Anatomical studies of the roots may eventually disclose the presence of absorbent structures, such as cortical hairs (Davis 1891). Maybe, in *S. goyazana*, all root surface is able to absorb water and minerals, and in a similar way, another cerrado species, *Brosimum gaudichaudii,* also possesses a radicular system consisting of a great unbranched root (or with just a few branches) covered by scarce absorbent radicles (Palhares *et al.* 2006).

Subterranean systems able to sprout are a way to maintain undifferentiated tissues under the soil, exposed to a lower variation of humidity and temperature compared to the soil surface (Appezzato-Da-Glória 2003).

In the Cerrado, there are basically two strategies of perennial plants to survive fire: the production of thick fire-insulator bark and/or the presence of subterranean systems able to re-sprout (Eiten 1972). Thus, the organography of subterranean systems should take into consideration the ontogeny, the caulinar or radicular structure, as well as the inherent peculiarities of each species.

There is a marked morphological variation regarding the subterranean structures of the genus *Smilax*. For example, *Smilax quinquenervia* possesses a very dense subterranean tuberosity, from where roots and aerial branches sprout (Andreata 1997b). However, *Smilax goyazana* has a typical rhizome, clearly structured in nodes, internodes,

Fig. 3. Individual growing under a small amount of earth showing a long rhizome, of 80 cm depth, bifurcated in the inferior portion.

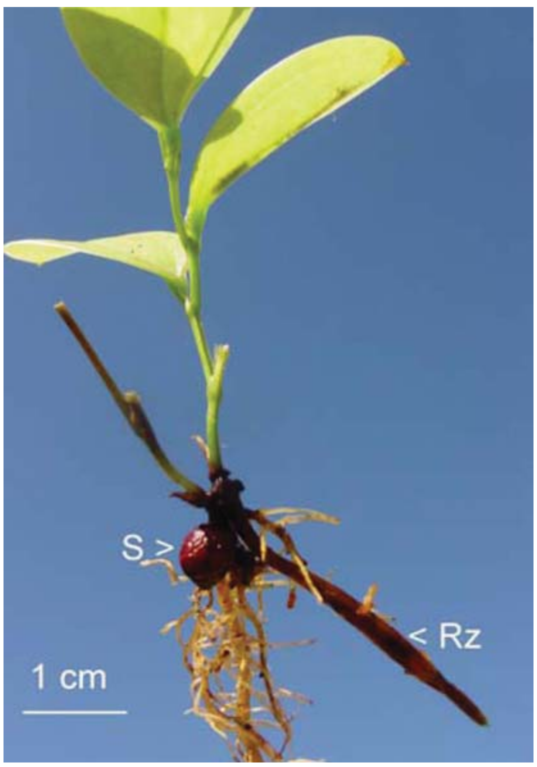

Fig. 4. 10-month old plant showing the rest of the seed (**S**), possibly still providing nutritional reservoirs. The primordial node produces roots, two small culms and a rhizome (**Rz**) in formation.

with nodal buds protected by scales (cataphyls) and whose anatomy has characteristics similar to other species from the genus, but with some peculiarities (Gattuso 1995, Palhares & Silveira 2005, Martins & Appezzato-Da-Glória 2006).

Although some germination of the seeds was achievable under controlled conditions, it is difficult to obtain germination under field conditions (Palhares *et al.* 2009b), despite the high dominance of the species in the non-grass herbaceous layer. So, from the observed subterranean system it is concluded that, in the field, germination occurs from 5 to 25 cm of soil depth.

The organography of the subterranean systems of *Smilax* has been a recent matter of discussion. Andreata & Menezes (1999) suggested the name rhizophore to refer to the subterranean system of *Smilax quinquenervia*, based on an ontogenetical study that showed the subterranean system of this species originated from cotyledonary buds. Previously, Angely (1959) had defined the rhizome, according to the international literature, as a subterranean stem with nodes, internodes and buds protected by scales, with or without adventitious roots, while rhizophore is a recent denomination initially employed for *Vernonia* spp and refers to subterranean stems originating in cotyledonary buds, producing adventitious roots or aerial stems according to light exposure and/or presence/absence of another aerial developing stem (Menezes *et al.* 1979).

Anyway, it should be considered that *Vernonia* spp. are dicotyledons with a very peculiar subterranean system, while *Smilax* spp. are monocotyledons whose subterranean systems present distinct characteristics. For example, Martins & Appezzato-Da-Glória (2006) showed that in *S. polyantha* the vegetative axis is organized into a hard tuberosity that produces both roots and rhizomes, from where the aerial culms sprout. Such structure is similar to what occurs in *S. goyazana*. Thus, as morphological studies of subterranean systems are more complex than the study of the aerial parts, due to the necessity of careful digging, a broadly and internationally validated revision of the nomenclature related to the diversity of such structures is becoming necessary.

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REZIME

Podzemni sistemi *Smilax goyazana* (Smilacaceae)

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Reznice rizoma i delova korena sa adultnih biljaka koje rastu u ceradu kultivisane su u vlažnom pesku tri meseca. Semena su isklijavana na kostantnoj temperature od 25°C i posmatran je podzemni sistem posle 10 meseci rasta. Sve reznice su na kraju eksperimenta bile mrtve. Subterestrični system se karakteriše jakom tuberoznošću koja proističe od primordijalnog nodusa koji obrazuje nekoliko korenova i jedan ili više rizoma. Rizomi se ponekad granaju, ali ne obrazuju adventivne korenove. Korenovi se ne granaju. Tako, sa stanovišta fraktalne geometrije, reznice ove vrste je teško ožiljavati jer se one ne ponašaju kao zavisni delovi adultnih biljaka.

Ključne reči: stabljičica, saponin, vegetativna propagacija