Characters of leaf epidermis in Solanum (clade Brevantherum) species from Atlantic Forest of Northeastern Brazil

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A B S T R A C T

Solanum is the richest and most diverse genus of the Solanaceae and one of the largest among angiosperms. The high number of species in the genus and the morphological plasticity of vegetative organs make the taxonomy of the group quite complex. We carried out an anatomical study of the leaf epidermis of six species of the Brevantherum Clade occurring in the Atlantic Forest of Northeastern Brazil (Solanum asperum, Solanum maranguapense, Solanum antisochidum, Solanum rugosum, Solanum stipulaceum and Solanum swartzianum), in order to support their taxonomy. Analyses were performed by the usual techniques for leaf anatomy, followed by light and scanning electron microscopy. All species showed leaf epidermis cells with sinuous anticlinal walls, and anisocytic stomata. Anomocytic stomata were also observed in S. asperum, S. antisochidum and S. swartzianum. Except for S. stipulaceum with amphistomatic leaves, the hypostomatic pattern was predominant. A great diversity of stellate trichomes was observed, mainly porrect-stellate and multiangulate, and peltate trichomes were only observed in S. swartzianum. The characters of leaf epidermis and its annexes, mainly the morphology of trichomes, are useful in distinguishing the Solanum species (clade Brevantherum) from Northeastern Brazil.

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1. Introduction

Solanum L. is the largest and most complex genus of the Solanaceae family comprising about 1500 species (Bohs, 2005), distributed in tropical and subtropical regions of the Americas, Africa and Australia. According to Nee (1991), the American continent has the highest diversity with about 950 species belonging to three subgenera: Solanum, Bassovia and Leptostemonum. In Brazil, the genetic diversity of the genus is represented by about 268 species found in different ecosystems, from rainforests to the drier regions such as the Caatinga (Stehmann et al., 2013).

The diversity of Solanum is considered as one of the largest among the angiosperms (Frodin, 2004), as evidenced by morphological plasticity of vegetative organs (Roe, 1972) that make the taxonomy of the group rather difficult, as observed in several infrageneric treatments (Sendtner, 1846; Dunal, 1852; Bitter, 1919; Seithe, 1962; D’Arcy, 1972; Whalen, 1984; Nee, 1999). This morphological plasticity combined with the high number of species has resulted in a very complex taxonomy of the genus, which is a challenge for many taxonomists (Weese and Bohs, 2007).

Species of Solanum clade Brevantherum were placed by Nee (1991) within the subgenus Solanum. Previously species of this group were placed in Solanum subg. Brevantherum Seithe (1962) and replaced as the subgenus Minon by D’Arcy (1991). Bohs (2005) observed that some elements included in subgenus Minon are referred to other clades, including the type species for subgenus Minon. According to Bohs (2005) and Poczai et al. (2008), the sections of Solanum subg. Brevantherum do not form monophyletic groups that are placed with some elements in the clade Brevantherum.

According to Roe (1972), the subgenus Brevantherum has about 90 species with a neotropical distribution from southern United States and South America to northern Argentina that live preferentially in primary and secondary forests. Species of this subgenus are characterized as unarmed shrubs, with short, oblong anthers with large terminal pores, and indumentum with various types of trichomes: dendritic, stellate, multiangulate, and peltate; these trichomes can be sessile or stalked.

Leaf anatomical studies have provided diagnostic characters and, in general, have shown positive results for separating species of...
angiosperms, such as those recorded for the genera *Raddia* Bertoli (Oliveira et al., 2008) and *Briza* L. (Pelegrin et al., 2009), both belonging to Poaceae, and *Cissampelos* L. (Menispermaceae) by Porto et al. (2011), and *Ficus* L. belonging to Moraceae (Araújo et al., 2013), are valuable sources of information for the systematics of the genus, which contribute as diagnostic characters for specific and infrageneric delimitation due mainly to the great diversity of trichomes. Thus, leaf anatomical studies have provided a set of additional characters to the taxonomy of Solanum, particularly clarifying the specific and sectional boundaries to the sections *Brevantherum* (Rojas and Mesa, 1991), *Acanthophora* (Granada-Chacón & Rojas, 2004), *Geminata* (Rojas, 2007), *Crinitum* (Araújo et al., 2010), *Polystichum* (Nurit-Silva and Agra, 2011) and *Torva* (Nurit-Silva et al., 2012).

In this work, a study of the leaf epidermis of six species of Solanum belonging to Brevantherum Clade, occurring in the Atlantic Forest of Northeastern Brazil, was conducted in order to perform microscopic morphodiagnostics contributing to its leaf anatomical characterization, as well as to support its taxonomy.

### 2. Materials and methods

#### 2.1. Botanical collections and fieldwork

Field observations and sample collections were carried out for five species of *Solanum* (*Solanum asperum*, *Solanum mar Angus), and *Solanum stipulaceum* (*Solanum asperum* (*Solanum mar Angus), and *Solanum stipulaceum*) in areas of the Atlantic Forest, in three States of Northeastern Brazil (Alagoas, Paraíba and Pernambuco), from October 2011 to May 2012. Part of the fertile material was pressed and dried following the usual procedures described by Forman and Bridson (1989). Voucher specimens were deposited at the Herbarium Prof. Geraldo Mariz (UFP), at the Federal University of Pernambuco, with duplicates at the Herbarium Prof. Lauro Pires Xavier (JPB), of the Federal University of Paraíba, and at the herbarium of the New York Botanical Garden (NY). In addition, leaf samples from specimens of *Solanum anisocladum* of the Herbarium of the Institute of the Environment of the State of Alagoas (MAC) were also studied. The dried samples were rehydrated, according to Smith and Smith (1942). Additional leaf samples were fixed in FAA 50 (formaldehyde: acetic acid: alcohol) for 48 h and stored in alcohol 70 GL, according to Johansen (1940). A list of voucher specimens used in this study is given in Table 1.

#### 2.2. Anatomical procedures

The plant material was divided into two portions, one for analysis by light microscopy and the other for electron microscopy analysis (SEM). Epidermal impressions of the middle region of leaf laminae were removed by free hand using razor blades. Subsequently, the sections were cleared by commercial sodium hypochlorite until the complete clarification, neutralized with acetic acid (0.2%), rinsed in distilled water, stained with Safranin (1%), and mounted in glycerinated gelatin (50%). The observations and photomicrographs were performed by light microscopy (Leica DM 750) with Qwin system coupled to a video camera (Leica ICC50 HD) for image capture. For scanning electron microscopy (SEM) of the leaf epidermis portions of the leaf blade, 3.0–5.0 mm were fixed on double-sided tape and mounted on stubs and metalized with gold through sputter deposition. The classiﬁcation of stomata and trichomes was based on Wilkinson (1979) and Theobald et al. (1979), respectively.

### 3. Results and discussion

#### 3.1. Epidermis and stomata

In front view, the shape of the anticlinal walls of epidermal cells was predominantly sinusuous and thickened (Table 2), as observed on the abaxial surface of *S. stipulaceum* and on both surfaces of

### Table 1

<table>
<thead>
<tr>
<th>Species</th>
<th>Voucher specimen</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Solanum asperum</em> Rich.</td>
<td>BRASIL, Pernambuco: Jaqueira, RPPN Frei Caneca, 01-IV-2012, V.S. Sampaio et al. 80 (JPB)</td>
</tr>
<tr>
<td><em>Solanum mar Angus</em></td>
<td>BRASIL, Pernambuco: Jaqueira, RPPN Frei Caneca, 31-III-2012, L.L. Giacomini et al. 1787 (BHCB; JPB)</td>
</tr>
<tr>
<td><em>Solanum anisocladum</em> Gaioni et Stehmann</td>
<td>BRASIL, Alagoas: Murici, 16-III-2001, M.F. Agra et al. 6288 (MAC)</td>
</tr>
<tr>
<td><em>Solanum rugosum</em> Dunal</td>
<td>BRASIL, Pernambuco: Recife, Jardim Botânico de Recife, 15-II-2012, V.S. Sampaio et al. 59 (JPB)</td>
</tr>
<tr>
<td><em>Solanum stipulaceum</em></td>
<td>BRASIL, Pernambuco: Jaqueira, RPPN Frei Caneca, 01-IV-2012, V.S. Sampaio et al. 77 (JPB)</td>
</tr>
<tr>
<td><em>Solanum stipulatum</em></td>
<td>BRASIL, Alagoas: Maçaré, Parque Municipal de Maceió, 22-IX-2011, V.S. Sampaio et al. 38 (UFP)</td>
</tr>
<tr>
<td><em>Solanum swartzianum</em></td>
<td>BRASIL, Pernambuco: Paraíba, Maceió, Parque Municipal de Maceió, 22-IX-2011, V.S. Sampaio et al. 62 (JPB)</td>
</tr>
<tr>
<td><em>Solanum torvum</em></td>
<td>BRASIL: Paraíba: Areia, Mata do Paúl Ferro, 07-II-2012, V.S. Sampaio et al. 66 (UFP)</td>
</tr>
</tbody>
</table>

### Table 2

<table>
<thead>
<tr>
<th>Species</th>
<th>Anticlinal walls</th>
<th>Stomata distribution</th>
<th>Type of stomata</th>
<th>Trichomes on adaxial and abaxial surfaces</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Adaxial</td>
<td>Abaxial</td>
<td>Adaxial</td>
<td>Abaxial</td>
</tr>
<tr>
<td><em>Solanum asperum</em></td>
<td>Sinuous</td>
<td>Sinuous</td>
<td>Hypostomatic</td>
<td>Absent</td>
</tr>
<tr>
<td><em>Solanum mar Angus</em></td>
<td>Sinuous</td>
<td>Sinuous</td>
<td>Hypostomatic</td>
<td>Absent</td>
</tr>
<tr>
<td><em>Solanum anisocladum</em></td>
<td>Sinuous</td>
<td>Sinuous</td>
<td>Hypostomatic</td>
<td>Absent</td>
</tr>
<tr>
<td><em>Solanum rugosum</em></td>
<td>Sinuous</td>
<td>Sinuous</td>
<td>Hypostomatic</td>
<td>Absent</td>
</tr>
<tr>
<td><em>Solanum stipulatum</em></td>
<td>Sinuous</td>
<td>Sinuous</td>
<td>Amphistomatic</td>
<td>Absent</td>
</tr>
<tr>
<td><em>Solanum swartzianum</em></td>
<td>Sinuous</td>
<td>Sinuous</td>
<td>Hypostomatic</td>
<td>Absent</td>
</tr>
</tbody>
</table>

Legends: (+) character present; (−) character absent.
the other species: \textit{S. asperum} (Fig. 1A–C), \textit{S. maranguapense} (Table 2, Fig. 1D–E), \textit{S. anisocladum} (Table 2, Figs. 1F, 2A), \textit{S. rugosum} (Table 2, Fig. 2B), \textit{S. swartzianum} (Table 2, Fig. 2E–F). However, on the adaxial surface of \textit{S. asperum} the anticlinal cell walls, near the base of trichomes, are straight (Fig. 1B). Cells with straight to curved anticlinal walls were also observed on the adaxial epidermis of \textit{S. stipulaceum} (Fig. 2D).

The predominant pattern of epidermal cells with sinuous anticlinal walls is not distinctive for the species studied; this pattern is very common in \textit{Solanum}, and occurs mainly in the lower surface, and it was also recorded for species of other sections: \textit{Geminata} (Rojas, 2007), \textit{Acanthophora} (Granada-Chacón & Rojas, 2004), \textit{Crinitum} (Araújo et al., 2010), \textit{Polytrichum} (Nurit and Agra-Silva, 2011), and \textit{Torva} (Nurit-Silva et al., 2012), among others.

Regarding the distribution of stomata, the hypostomatic pattern was observed in the leaves of most species (Table 2): \textit{S. asperum} (Fig. 1A–C), \textit{S. maranguapense} (Fig. 1D–E), \textit{S. anisocladum} (Figs. 1F, 2A), \textit{S. rugosum} (Table 2, Fig. 2B) and \textit{S. swartzianum} (Fig. 2E–F). This is the most common pattern observed in Solanaceae by Metcalfe and Chalk (1950), and also in \textit{Solanum} (Ahmad, 1964; Rojas and Mesa, 1991; Nurit-Silva and Agra, 2011; and Nurit-Silva et al., 2012).

In this work, the amphistomatic pattern, with stomata in both surfaces of leaf epidermis, was observed only in \textit{S. stipulaceum}, which constitutes a distinctive character for this species that occurs in areas exposed to sunlight, unlike the other five species studied that occur in forest shade. However, this pattern has also been reported by Rojas and Mesa (1991) for other species of \textit{Solanum} Brevantherum Clade from Venezuela. According to Mott et al. (1982), amphistomatic pattern (stomata on both leaf surfaces) could be an adaptation that facilitates a higher photosynthetic rate in a sunlit environment.

Two different types of stomata were recognized on the leaf epidermis of the studied species: anisocytic and anomocytic. The anisocytic type was predominant and observed on the abaxial surface of all species (Table 2): \textit{S. asperum} (Fig. 1C), \textit{S. maranguapense} (Fig. 1E), \textit{S. anisocladum} (Fig. 2A), \textit{S. rugosum} and \textit{S. swartzianum} (Fig. 2F), and also on adaxial surface of \textit{S. stipulaceum} (Fig. 2D). Anomocytic and anisocytic stomata were also recorded occurring simultaneously on the abaxial surface of three species: \textit{S. asperum} (Fig. 1C), \textit{S. anisocladum}
Anisocytic and anomocytic stomata are common to *Solanum* species, and were previously reported by Metcalfe and Chalk (1950), Ahmad (1964), Rojas (2007), Nurit-Silva et al. (2007), Araújo et al. (2010), Nurit-Silva and Agra (2011), and Nurit-Silva et al. (2012), and occur also in other genera of the Solanaceae like *Datura*, *Nicotiana* and *Physalis* (Maiti et al. 2002).

### 3.2. Indumentum and trichomes

In most species studied here, the indumentum of the abaxial surface is more compact than on the adaxial side, however, both surfaces have a wide diversity of trichomes (Table 2). In *S. asperum* the indumentum is scabrous and falls to the touch mainly on adaxial surface, consisting mostly of sessile porrect-stellate trichomes, while on the abaxial surface the indumentum is tomentose with porrect-stellate, sessile and/or stalked trichomes (Table 2, Fig. 3A–B). *S. rugosum* showed an indumentum similar to *S. asperum*, from which it differs by persisting when touched and is more robust and with larger stellate trichomes, and a greater number of lateral rays (Table 2, Fig. 4A). These observations corroborate those of Roe (1971, 1972), and Rojas and Mesa (1991) for these species.

*S. stipulaceum* is a very distinctive species in the group; it is characterized by cinereous and dense velutinous indumentum on the adaxial surface, and is tomentose on the abaxial surface. It has compactly overlapping porrect-stellate and multiangulate stellate (sessile and stalked) trichomes on both sides of leaf surface (Table 2, Fig. 4B). Moreover, this species has different epidermal leaf anticlinal cell walls, straight to curved and exclusively anisocytic stomata; it is also the only species with both sessile and stalked porrect-stellate trichomes and multiangulate-stellate trichomes on both leaf surfaces.

*S. anisocladum* (Table 2, Fig. 3D) is the only species that has an indumentum comprising long finger-like trichomes on the adaxial surface, and multiseriate-stalked multiangulate trichomes on the abaxial surface (Fig. 3D), a pattern commonly found in species of *Solanum* subg. *Leptostemonum*, according to Seithe (1962). The leaf epidermis of *S. anisocladum* is very different from those of the other species due to the presence of multiseriate-stalked multiangulate trichomes on the abaxial surface, which differs from the adaxial surface that presents finger-like hairs (Seithe, 1979). The multiseriate-stalked multiangulate trichomes were recorded by Roe (1971) for *Solanum bicolor* Willd., but not recorded in other sections of *Solanum* as described by Araújo et al. (2010), Nurit-Silva and Agra (2011) and Nurit-Silva et al. (2012).

The glabrescent indumentum on the adaxial surface of *S. maranguapense* comprises porrect-stellate sessile and spaced...
trichomes with reduced central rays, which differ from the tomentose indumentum on the abaxial surface, with porrect-stellate sessile and multiangulate stalked trichomes. (Table 2, Fig. 3C), however more compact than on the adaxial surface, a pattern similar to that described by Roe (1971, 1972).

In *S. swartzianum* the indumentum of the adaxial surface is loosely lepidote, with spaced peltate trichomes, and also lepidote on the abaxial surface, with overlapping of peltate sessile and stalked trichomes, (Table 2, Fig. 4C–D). Furthermore, the presence of the peltate trichomes observed only in *S. swartzianum* is distinctive for this species in comparison with the other species investigated here, corroborating the findings of Carvalho (1996), who placed this species in *Solanum* sect. *Lepidotum* Carvalho, for whom peltate trichomes in *Solanum* are commonly present in species of this section.

Despite the superficial similarity between the stellate trichomes present in the studied species, they can be distinguished by a set of characters: the number of lateral rays, the size of the central ray (long or reduced) and sessile or stalked trichomes, as already recorded by Seithe (1979). The morphology of the trichomes is part of the main taxonomic evidence for separation between the species of clade Brevantherum in this work. Porrect-stellate trichomes (sessile or stalked) observed in four species (*S. asperum*, *S. maranguapense*, *S. rugosum* and *S. stipulaceum*), and the
multiangular trichome type were observed in most of the studied species, except in S. swartzianum, which showed only peltate trichomes on both surfaces. Porrect-stellate and multiangular trichomes were already recorded by Roe (1971, 1972) and Rojas and Mesa (1991) for species belonging to the section Brevantherum, now in clade Brevantherum. A similar trichome pattern was reported by Araújo et al. (2010) and Nurit-Silva et al. (2012) for some species belonging to the section Crinitum and Torva, respectively, but differing from those observed in species belonging to section Polytrichum by Nurit-Silva and Agra (2011).

4. Conclusions

According to this study, the indumentum of the leaf is quite diverse among the studied species of Brevantherum Clade, but both the morphology of trichomes and the type and pattern of distribution of stomata on the leaf epidermis surfaces are valuable resources that provide taxonomic evidence for interspecific delimitation of the species of Brevantherum Clade from Atlantic Forest of Northeastern Brazil.

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