

Article

Received 3 Aug 2010
Accepted 30 Dec 2010
Available online 10 Jun 2011

Keywords:

“jurubeba”
medicinal plant
morpho-anatomy
Solanaceae
Solanum subg. *Leptostemonum*

ISSN 0102-695X
doi: 10.1590/S0102-695X2011005000101

A pharmacobotanical study of vegetative organs of *Solanum torvum*

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Abstract: In this work, a morpho-anatomical study of the leaves, stems and roots of *Solanum torvum* Sw. was performed with the objective of providing a macroscopical and microscopical morphodiagnosis for its characterization. The species is popularly called “jurubeba-branca” in the Northeastern Brazil and is used in folk medicine to treat liver diseases, tuberculosis, and as anti-anemic. Anatomical studies were done by paradermic sections of the leaf blade (adaxial and abaxial surfaces), and cross sections of leaves (blade and petiole), stems and roots. The sections were clarified and stained with safranin and/or astrablue, observed and photographed on light microscope. Histochemical tests were carried out in cross sections of leaves, stems and roots and assayed for specific reagents. The leaves are chartaceous, ovate to elliptic, with lobed margins, oblique at the base. The leaf is amphistomatic with anisocytic and anomocytic stomata simultaneously, and the epidermal cells, in face view, have anticlinal walls sinuate on the upper surface and wavy on the lower surface. The mesophyll is dorsiventral; the midrib has a central bicollateral vascular bundle; and the petiole has three or four. The stem shows external phloem, xylem, internal phloem and a parenchymatic pith. The root is axial and striate longitudinally and the secondary xylem is a massive cylinder. The macroscopical and microscopical vegetative characters constitute a set of diagnostic parameters to the studied species.

Introduction

Solanum L. is the most representative of the Solanaceae family with about 1400 species and cosmopolitan distribution (Bohs, 2005). The genus is known for its economic importance having species used as food, like “potato” (*Solanum tuberosum* L.), tomato (*S. lycopersicum* L.), egg-plant (*S. melongena* (Mill.) Dunal) etc., as well as species with pharmacological interest like *S. dulcamara* (Moench) Dumort and *S. sodomium* L., for examples that showed anti-neoplastic activity (Kupchan et al., 1965; Cham et al., 1987; Cham & Meares, 1987).

Solanum torvum Sw. is native of Caribbean belonging to *Solanum* sect. *Torva* Nees, with wide distribution and found in tropical regions of the world (D’Arcy, 1973). In Brazil, it is found in various regions of the country and popularly called “jurubeba” and “jurubeba-branca” (Agra & Bhattacharyya, 1999) and its roots are used in folk medicine in liver diseases, tuberculosis, and as anti-anemic as a substitute for *S. paniculatum* L. (“jurubeba-verdadeira”).

Solanum sect. *Torva* belonging to the subgenus *Leptostemonum* has about forty species with wide distribution in Americas, Africa, Asia, New Guinea and Pacific (Nee, 1999). In Brazil, according to Agra (2007), the section comprises about eleven species and some of them have restricted distribution like *S. metrobotryon* Dunal. Species of this section are characterized by a plurifoliate sympodial units, branched inflorescence, and glabrous and juicy fruits.

Solanum torvum contains alkaloids like jurubine, paniculogenin (Schreiber & Ripperger, 1968), solasodine, solasodien, torvogenin (Dopke et al., 1975), steroids like sisalogenin, torvogenin and sisalogenone (Mendes et al., 1970), and also flavonoids, such as torvanol A and a torvoside H (Arthan et al., 2002), and sapogenin, *inter alia* (Cuervo et al., 1991).

The methanolic extracts of leaves and fruits of *S. torvum* showed biological activities such as bactericidal (Chah et al., 2000; Wiart et al., 2004), fungicidal (Chah et al., 2000) and as antiviral, against the herpes virus type I (Arthan et al., 2002). The aqueous extract from fruits showed anti-hypertensive

activity (Nguelefack et al., 2007).

In this work, a morpho-anatomical study of the vegetative organs of *S. torvum* has been carried out with the objective of providing a macroscopical and microscopical morphodiagnosis for its characterization which forms a part of a project of pharmacognostic studies of *Solanum* species used as medicinal plants in Brazil (Basilio et al., 2007; Nurit-Silva & Agra, 2005; Nurit-Silva et al., 2007a,b).

Material and Methods

The macroscopical analyses were carried out with fresh samples of *Solanum torvum* Sw. as well as specimens from the Herbarium Prof. Lauro Pires Xavier (*Agra 1477*, *Agra 2236*, *Agra & Bhattacharyya 1248*, *Agra & Góis 520*, *Agra & Góis 1295*, *Agra & Nurit 6758*, *Grisi 157*, *Grisi 236*) complemented by field observations. The study was done in the vegetative organs, helped by binocular stereomicroscope. The microscopical analyses were done by paradermic sections of leaf blades on upper and lower surfaces, and by cross sections manually supported by medulla of petiole of *Cecropia* sp. following the usual methodology. It was carried out in adult leaves (blade and petiole) from the 5th node, and fragments of stem from the apical portion, and secondary roots from median and apical portions of small caliber. The sections were cleared with sodium hypochlorite 20%, neutralized with acetic water 0.2%, washed in distilled water. The epidermal tissue was stained in a solution of safranin and the mesophyll in a solution of astrablue, and were mounted in slide with glycerin 50%. The anatomical characterizations of epidermis and mesophyll were based on Metcalfe & Chalk (1979), and the classification of stomata is according to Wilkinson (1979).

Photomicrographs of the indument of trichomes were made through scanning electron microscopy (SEM). Portions of 5.0 by 2.0 mm wide of the leaf blade were dehydrated in graded ethanol series, mounted on stubs, coated with gold and analyzed in scanning electron microscope (SEM), Jeol JSM6300.

The histochemical tests were carried out in cross sections of leaves, stems and roots by treatment with the specific reagents: Sudan III (Jensen, 1962) for cuticle and cutinized layers; acidified phloroglucinol (Johansen, 1940) for lignified and suberized elements and a solution of lugol for starch grains (Berlyn & Miksche, 1976). The anatomical structures were observed and photographed with Olympus model CX31 light microscope and Olympus PM-BP35 camera, respectively.

Results

Macroscopical morphodiagnosis

The leaf is subinerm with sparse and conical prickles, slightly recurved at the apex, 4.0-6.0 mm long, with chartaceous, ovate to ovate-elliptic and lobed or repand blades acute at apex, oblique or obtuse at the base, 8.5-25.0 x 8.0-24.0 cm, scabrous and tomentose on adaxial and abaxial surfaces, respectively, with a cylindrical and tomentose petiole. The stem and branches are cylindrical and lenticeled, green and tomentose in young plant to glabrescent and brown in adult plant. The root is axial with the main root having about 30 cm length, externally light-brown with longitudinal striations.

Microscopical morphodiagnosis

Leaf

The blade, in face view, is amphistomatic having anisocytic and anomocytic stomata. The epidermal cells have sinuous anticlinal walls on the adaxial surface (Figure 1A) and wavy on the abaxial surface (Figure 1D). In cross section, the epidermis is 1-layered with rounded cells (Figure 3A) and the stomata are located on the same level as the other epidermal cells. Porrect-stellate trichomes occur on both surfaces and they are sessile with reduced midpoint (Figures 1E, 2A) or stalked (Figures 1C, 1F, 2B).

The mesophyll is dorsiventral (Figure 3A), with palisade parenchyma uniseriate, and 4-5 layered spongy parenchyma, occupying about 60% of the mesophyll, with sparse idioblasts of sand crystals.

The midrib, in cross section, shows contour slightly biconvex at the apical portion (Figure 3B) to strongly biconvex at median and basal portions (Figure 3C). The epidermis is 1-layered and is followed by an angular collenchyma, 4-7 layered, and by the ground parenchyma. The vascularization is bicollateral and is composed of only one central bundle, in arc shape, on apical portion, which gradually becomes "U" shaped toward the median to basal portions. Idioblasts of sand crystals were observed in the external phloem. The lignified xylem was confirmed in sections of midrib (Figure 5A), and also in sections of petiole.

The petiole is circular in cross section at the median and apical portions (Figure 3D) to slightly arched at the base (Figure 3E). The epidermis is 1-layered with sparse porrect-stellate trichomes. The angular collenchyma is 7-10 layered (Figure 3D-E) followed by the ground parenchyma with sparse idioblasts of sand crystals. The vascularization is bicollateral, similar to the pattern found in the midrib, differing by the number and shape of bundles having a set of 3 to 4 central bundles in shape of "U" (Figure 3D). At the apical (Figure 3D) and median portions were observed two smaller and circular accessories

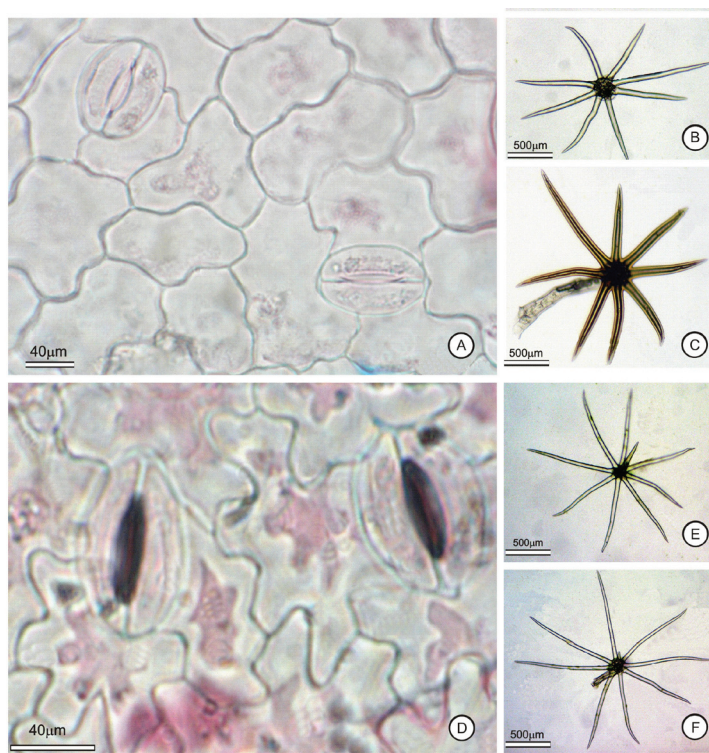


Figure 1. *Solanum torvum* Sw. (Agra & Nurit 6758). A e D. Epidermis and stomata (anisocytic and anomocytic), in face view: A. Adaxial surface with sinuous anticlinal walls; B. Sessile porrect-stellate trichome; C. stalked porrect-stellate trichome; D. Abaxial surface with waved anticlinal walls; E-F. Porrect-stellate trichomes.

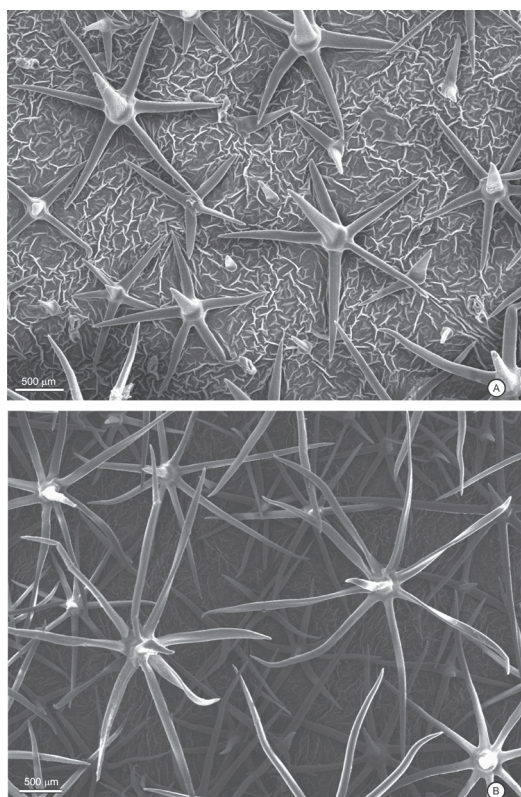


Figure 2. *Solanum torvum* Sw. (Agra & Nurit 6758). SEM photographs. A, B. Indument of the leaves: A. Adaxial surface, sessile porrect-stellate trichomes, with reduced midpoint; B. Abaxial surface, stalked porrect-stellate trichomes.

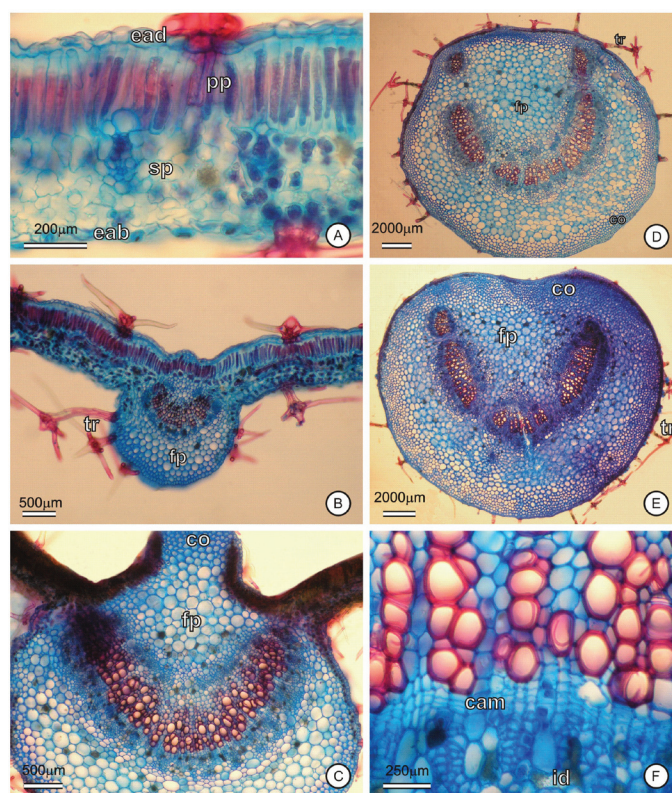


Figure 3. A-C. Leaf blade in cross section: A. Dorsiventral mesophyll; B-C. Bicolateral bundle of the midrib in shape of arc: B. Apical portion, C. Median portion. D-E. Petiole, in cross section, bicollateral vascular bundle in shape of “U”: D. Apical portion; E. Basal portion; F. Detail of a vascular bundle. Abbreviations: cam: cambium, co: collenchyma, eab: abaxial surface of the epidermis, ead: adaxial surface of the epidermis, fp: ground parenchyma, id: idioblast, pp: palisade parenchyma; sp: spongy parenchyma, tr: porrect-stellate trichome.

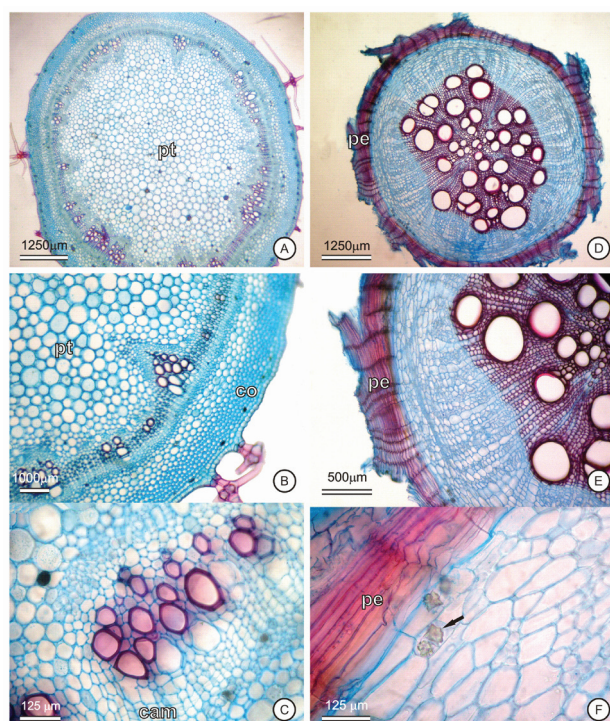


Figure 4. *Solanum torvum* Sw. (Agra & Nurit 6758). A-C. Stem, in secondary growth, in cross section: A. General view; B. Detail; C. Detail of vascular bundle. D-F. Root, in cross section: D, E. General view, in secondary growth; F. Detail of idioblasts with druses, below the periderm. Abbreviations: cam: cambium, co: collenchyma, pe: periderm, pt: pith.

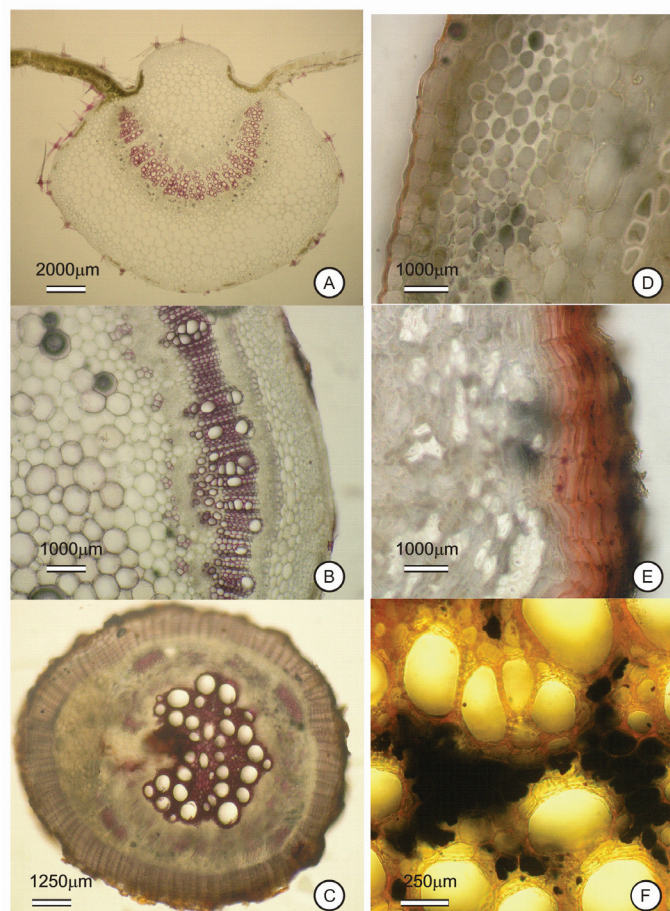


Figure 5. *Solanum torvum* Sw. (Agra & Nurit 6758). Histochemical tests in *Solanum torvum* Sw. (Agra & Nurit 6758). A-C. Lignified xylem stained by acidified phloroglucinol: A. in midrib; B. in stem; C. in root, in secondary growth, and epidermis suberized; D-E. Sudam III: D. cutinized cell walls in stem; E. periderm in root; F. Starch grains in vascular region stained by lugol.

bundles, collateral, directed to the adaxial surface. The presence of a vascular cambium is evident between the external phloem and the xylem (Figure 3F).

Stem

In cross section, the stem in secondary growth has practically circular contour and 1-layered epidermis with quadrangular cells, which are coated with a smooth and thin cuticle (Figure 5D), with sessile, subsessile and stalked porrect-stellate trichomes sparse and reduced midpoint. The collenchyma is angular forming a continuous cylinder, 5-8-layered, followed by a reduced cortical parenchyma, 4-5 layered (Figure 4B). The vascularization consists of external phloem, xylem and internal phloem, bounded by a perivascular sheath externally to the phloem. The xylem is lignified (Figure 5B) and positioned as a massive and continuous cylinder, with vessel elements in radial distribution between the lignified fibers (Figure 5B), a 3-4 layered cambial zone is evidenced adjacent to the internal phloem (Figure 4C). The medular parenchyma is formed by thin walls of rounded cells.

Root

The root, in initial secondary growth (Figure 4D), has an underdeveloped periderm (Figure 5E). The 5-6 layered parenchyma, located between the periderm and phloem, occupies about 40% of diameter. In the central cylinder is observed a pericycle and 4-5 layers of cambial zone. The secondary phloem and xylem form a massive cylinder, although a tetrarch structure can be verified.

In terminal secondary growth (Figure 4E), the root shows a developed periderm, 12-18 layered. Idioblasts bearing druses of calcium oxalate occur in the parenchyma, below the periderm (Figure 4F), as well as starch grains also occur in parenchyma and vascular region (Figure 5F).

Discussion

Morphologically, *Solanum torvum* resembles *S. paniculatum*, mainly during the juvenile phase of the plant showing large leaves with ovate to ovate-elliptic and strongly ovate or repand blades. However, in a detailed

analysis these similarities are found to be superficial, since *S. torvum* has distinctive morphological characters such as ochraceous indument which contrast with the cinereous to canescent indument, referred by Nurit-Silva et al. (2007b) to *S. paniculatum*.

In comparison with other species of *Solanum* sect. *Torva* such as *S. scuticum* Nee, *S. adpersum* Witasek and *S. guaraniticum* A. St.-Hil. (Mentz et al., 2000), *S. paniculatum* (Nurit-Silva et al., 2007b), and *S. variable* Mart. (Furlan et al., 1999), the leaf morphology including the indument is distinctive to *S. torvum*, besides the reproductive characters. In relation to the trichome morphology, the type porrect-stellate, sessile or stalked, is characteristic to the section and is referred by Mentz et al. (2000) to *S. scuticum*, *S. adpersum* and *S. guaraniticum*, and by Nurit-Silva et al. (2007b) to *S. paniculatum*, and by Furlan et al. (1999) to *S. variable*.

In many aspects, the leaf anatomy of *S. torvum* corresponds to the pattern registered to the Solanaceae family and to the genus *Solanum* by Metcalfe & Chalk (1950), such as the dorsiventral mesophyll and the angular collenchyma, characters also found in other species of *Solanum* sect. *Torva* by Nurit-Silva et al. (2007b), Furlan et al. (1999) and Lima et al. (2009).

The epidermis with wavy anticlinal walls on adaxial surface is similar to described to *S. torvum* by Ahmad (1964), for other side, is very distinctive from the type found in *S. paniculatum* by Nurit-Silva et al. (2007b) with straight anticlinal walls on the adaxial surface.

Stomata of the anisocytic type present on the epidermis of *S. torvum* were also referred to many species of *Solanum* by Ahmad (1964), but this type differs from the anomocytic type observed on the abaxial surface of *S. paniculatum* by Nurit-Silva et al. (2007b).

The leaf midrib shows similar structure to all studied species of *Solanum* sect. *Torva* such as *S. variable* (Furlan et al., 1999; Lima et al., 2009) and *S. paniculatum* (Nurit-Silva et al., 2007b). The petiole having slowly arched to circular contour differs from the type observed in other species of the section, such as *S. paniculatum* in which it is biconvex-ribbed (Nurit-Silva et al., 2007b).

The presence of idioblasts having sand crystals in mesophyll of *S. torvum* constitutes a character common to all species of Solanaceae family. It is present in different genera including *Solanum* and also in species of different infrageneric categories observed by Cosa et al. (1998), Granada-Chacón & Benítez de Rojas (2004), Maiti et al. (2002) and Nurit-Silva et al. (2007b). On the other hand, the presence of idioblasts having druses observed in *S. torvum* is rare and uncommon in *Solanum* species.

The vascular structure of stem corresponds to the pattern referred to the Solanaceae family by Metcalfe & Chalk (1950), and also observed in other species of *Solanum* sect. *Torva* such as *S. variable* (Furlan et al., 1999) and *S. paniculatum* (Nurit-Silva et al., 2007b). The

indication of eustelic structure of the stem differs from the type present in *S. variable* (Furlan et al., 1999), referred as sifonostelic amphiphloic discontinuous and resembles to the type described to *S. juvenale* (Cosa et al., 1998), *S. paniculatum* and *S. rhytidoandrum* Sendtn. (Nurit-Silva et al., 2007b).

The root organization considered tetrarch is similar to the type registered to *S. elaeagnifolium* Cav. (Cosa et al., 1998) and *S. rhytidoandrum*, and differs from the hexarch structure of *S. paniculatum* (Nurit-Silva et al., 2007b).

The results of this work as well as other already made by Cosa et al. (1998), Furlan et al. (1999) and Nurit-Silva et al. (2007b), *inter alia*, have shown the importance of the morpho-anatomical studies for identification and quality control of the herbal medicines, mainly of the species belonging to genera of great richness of diversity as *Solanum*.

Conclusion

The leaf morphology and the anatomy of epidermis and appendages of the leaf, stem and root are parameters characteristic of *Solanum torvum* and are distinctive from those found in other species of *Solanum* sect. *Torva*, mainly *Solanum paniculatum*, which has been confused and used as a substitute.

Acknowledgments

The authors thank Prof. Jnanabrata Bhattacharyya for the English revision, and Dulce G. Oliveira for her technical support. The financial support and grants to MFA (PQ), KNS (GD), VPMC and RCS (IC) were provided by the Conselho Nacional de Desenvolvimento Científico e Tecnológico.

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