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Physiological and physical quality of local *Araucaria angustifolia* seed variety

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ABSTRACT. *Araucaria angustifolia* seeds are the basis for the rural economy in southern Brazil, and the species presents different botanical varieties. The objective of this work was to evaluate the diversity of physical, physiological and biochemical characteristics of *Araucaria angustifolia* seeds from local varieties: ‘sancti josephi’, ‘angustifolia’, ‘caiova’, and ‘indehiscens’. Seeds were collected from the cities of Painel and Urubici in Santa Catarina State and tested for moisture content, 1000-seed weight, emergence, emergence speed index (ESI), root and shoot length, electrical conductivity, tetrazolium, soluble carbohydrates, and starch content. Among the varieties cited above, the angustifolia variety from Painel displayed a greater 1000-seed weight, ESI, electrical conductivity and shoot length. However, the sancti josephi variety displayed a lower emergence percentage, 1000-seed weight, ESI, root and shoot length, and carbohydrate content. In a cluster analysis, based on Euclidean distance, three groups constituting the sancti josephi, indehiscens, and caiova/angustifolia varieties were found. In conclusion, there is great diversity in *A. angustifolia* seeds. The angustifolia and caiova varieties displayed similar diversity, and their physiological quality was better than that of other varieties. Variety characterization allowed for the compilation of information regarding seed collection and use for seedling production and species conservation.

Keywords: viability, vigor, carbohydrates, caiova, indehiscens, sancti josephi.

Qualidade fisiológica e física de sementes de variedades locais de *Araucaria angustifolia*

RESUMO. Sementes de *Araucaria angustifolia* servem de base para a economia de muitas famílias rurais do sul do Brasil e apresenta diferentes variedades botânicas. Este estudo objetivou avaliar a diversidade das características físicas, fisiológicas e bioquímicas de sementes de *Araucaria angustifolia* das variedades locais sancti josephi, angustifolia, caiova e indehiscens. Sementes foram coletadas nos municípios de Painel e Urubici – Santa Catarina e submetidas aos testes: grau de umidade, massa de 1000 sementes, emergência, índice de velocidade de emergência – IVE, comprimento da raiz e parte aérea, condutividade elétrica, tetrazólio, teores de açucares solúveis e amido. A variedade angustifolia de Painel apresentou maior massa de sementes e vigor pelo IVE, condutividade elétrica e comprimento parte aérea. Enquanto, a variedade sancti josephi apresentou baixa porcentagem de emergência, massa de sementes, IVE, comprimento de raiz/parte aérea e teores de açucares. Na análise de cluster baseada na distância euclidiana, verificaram-se três grupos formados pelas variedades sancti josephi, indehiscens e caiova/angustifolia. As variedades angustifolia e caiova apresentaram similaridade e melhor qualidade fisiológica. A caracterização destas variedades possibilitou obter informações que podem auxiliar na coleta e aproveitamento das sementes para produção de mudas e conservação da espécie.

Palavras-chave: viabilidade, vigor, carboidratos, caiova, indehiscens, sancti josephi.

Introduction

Araucaria angustifolia is a native species of economic, social and environmental importance from southern Brazil (Ribeiro & Cardoso, 2012; Wendling & Brondani, 2015). There is evidence that this species has already experienced some degree of domestication as a result of pre-colonial human actions (Reis, Ladio, & Peroni, 2014). Management of this species may have favored morphological variations over time, indicating a possible domestication process related to changes in the reproductive system (Reis et al., 2014).

The first studies on botanical varieties of *A. angustifolia* were performed by Reitz and Klein (1966), who outlined the presence of nine varieties:

'elegans', sancti josephi, angustifolia, caiova, indehiscens, 'nigra', 'striata', 'semi-alba' and 'alba', mainly based on differences in coloration and ripening of the seeds. However, Mattos (2011) described five varieties: angustifolia, indehiscens, caiova, 'dependens', and 'vinacea', and one form: 'catharinensis'. In Santa Catarina, the varieties angustifolia, caiova, indehiscens and sancti josephi have also been reported in the city of Três Barras (Zechini et al., 2012).

Clearly, there is apparent morphological diversity regarding the size, color, and ripening period of *A. angustifolia* varieties. Seeds of the angustifolia variety ripen in April-May and are the most frequently observed by Santa Catarina farmers (Zechini et al., 2012); the indehiscens variety is mainly characterized by the fact that the seeds do not release female strobilus (Mattos, 2011), which remain on the tree even after seed ripening (from July/August); the caiova variety usually has larger seeds (Zechini et al., 2012) that ripen between June and July; and the sancti josephi variety presents an early ripening period (February-March) (Reitz & Klein, 1966; Zechini et al., 2012).

Characterization of these varieties can aid in the selection of superior germplasm and provide complementary information to ensure that seeds with highest physiological quality are obtained and to encourage the conservation of endangered species. Therefore, in order to ensure food security in such a fragile ecosystem, it is important to analyze the available diversity and evaluate morphological and biochemical traits (Trivedi et al., 2015).

In general, genotypes with larger seeds have better physiological qualities. Such seeds show improved survival during establishment than do smaller seeds (Moles & Westoby, 2004) because the mass of seeds is highly correlated with the amount of reserves (Kennedy, Hausmann, Wenk, & Dawson, 2004).

In *A. angustifolia* seeds, the main reserves components are carbohydrates (Rogge-Renner et al., 2013), which can serve as a carbon source during the germination process and play a role in protection against desiccation (Ferreira et al., 2009; Mello, Barbedo, Salatino, & Figueiredo-Ribeiro, 2010). Differences in the accumulation of sugars among varieties of *Theobroma cacao* have been reported (Rangel-Fajardo et al., 2011), but no studies have been performed using *A. angustifolia* varieties.

For species with recalcitrant/intermediate behavior, there are some studies evaluating the physical and/or biochemical characteristics that are linked to physiological quality, such as *Euterpe edulis* (Pizo, Von Allmen, & Morellato, 2006), *Artocarpus heterophyllus* (Khan, 2004), and *Theobroma cacao* (Rangel-Fajardo et al., 2011). However, for *A. angustifolia* seeds, there are no studies correlating the physical, physiological, and biochemical characteristics of seeds. Studies have found different rates of seedling growth among the various varieties (Coutinho & Dillenburg, 2010), and several varieties identified by farmers have been characterized (Zechini et al., 2012).

Thus, the objective of this work was to evaluate the diversity of the physical, physiological, and biochemical characteristics of *A. angustifolia* seeds from the sancti josephi, caiova, angustifolia and indehiscens varieties.

Material and methods

Seed sampling

Approximately 37 mature cones (megastrobili) were collected from 85 trees of two populations located in the municipalities of Painel and Urubici, Santa Catarina State, Brazil, in 2012 (Table 1) from March to August. Collection started when seeds presented isolated or grouped brown spots (Mattos, 2011).

Table 1. Variety, common name, site and month of collection of *Araucaria angustifolia* seeds.

Abbreviation	Variety	Common name	Site	Month of collection
P-25	sancti josephi	March 25	Painel	March
P-ang	angustifolia	Commum	Painel	April
U-ang	angustifolia	Commum	Urubici	May
P-cai	caiova	Kayuva/Caiova	Painel	June
U-cai	caiova	Kayuva/Caiova	Urubici	June
P-ind	indehiscens	Monkey	Painel	July
U-ind	indehiscens	Monkey	Urubici	August

After collection, cones were manually shelled, and the seeds from different cones were combined and randomly homogenized to obtain four replications, which were used for the biochemical, morphological, and physiological analyses. Physical and physiological analyses were given priority because the potential for seed emergence decreases after storage; however, if stored under refrigeration, the quality is maintained for up to 60 days (Garcia, Coelho, Maraschin, & Oliveira, 2014). Therefore, the seeds were stored in plastic packages in the refrigerator (8 ± 2°C) until physiological analysis, and a sample of each replication was frozen in an ultrafreezer at -80°C for subsequent biochemical analysis.

Moisture content

Two replicates of three seeds each were cut transversally and then weighed (wet weight), dried at 105 ± 3°C for 24 hours and reweighed to determine the moisture content (Brasil, 2009).

1000-seed weight

This parameter was measured using eight replications of 100 seeds, according to the Rules for Seed Analysis (Brasil, 2009).

Emergence test

Seeds were surface-decontaminated with a sodium hypochlorite solution (2%, v v^{-1}) for three minutes. Four replicates of 25 seeds each were sown in plastic trays with vermiculite and placed in a germination chamber at 25°C, with a photoperiod of 12 hours. Seeds were monitored for 70 days.

Seedling counts were performed every three days, and the emergence speed index (ESI) was calculated using the formula proposed by Maguire (1962), according to Equation 1:

$$ESI = \frac{E1}{N1} + \frac{E2}{N2} + \cdots \frac{En}{Nn} \quad (1)$$

where:

E1, E2, and En are the number of seedlings and;

N1, N2, and Nn the number of days in the test.

By the end of the emergence test, shoot and root length were measured for each seedling.

Tetrazolium test

Four replicates of 25 seeds were soaked in water for 18 hours; then, the embryo was separated from the seed coat and the nutritive tissue with a scalpel for further immersion in 0.1% tetrazolium solution at 25°C for one hour (Oliveira et al., 2014). Embryos were classified as viable or non-viable according to the color and appearance of tissues, the extent of damage, and the location of the color patches.

Electrical conductivity test

Four replicates of 10 embryos were soaked in 75 mL of distilled water for 12 hours at 25°C (Medeiros & Abreu, 2007). Then, the electrical conductivity of the solution was measured using a conductivity meter and expressed as µS cm^{-1} g^{-1} of seeds.

Total soluble carbohydrate

A sample of 0.5 g of fresh material was macerated in a mortar with liquid nitrogen and transferred to tubes. The tubes were then placed in a water bath at 100°C for 5 min, and 5 mL of 80% ethanol was added to each tube. This process was repeated three times. After each boiling, the extracts were centrifuged at 1500 g for 10 min. The extracts were then filtered through glass microfiber filters and dispensed into test tubes, and the volume was adjusted with 80% ethanol to 10 mL.

Total soluble carbohydrates were estimated through colorimetric analysis using the phenol-sulfuric method (Dubois, Gilles, Hamilton, Rebers, & Smith, 1956), and the absorbance at 490 nm was measured using a spectrophotometer. Total sugar content was calculated based on a standard curve of D-glucose.

Starch content

To the precipitate resulting from the extraction of total soluble carbohydrates, 10 mL of cold distilled water and 13 mL of 52% perchloric acid were added. The mixtures were incubated for 15 min with occasional stirring. Then, 20 mL of distilled water was added, followed by centrifugation at 1500 g for 15 min. The supernatant was discarded, and 5 mL of cold distilled water and 6.5 mL of 52% perchloric acid were added to the residue with stirring for 15 min, followed by another round of centrifugation (1,500 g, 15 min). The supernatant was decanted, and the starch was extracted in a 100 mL beaker, combining all fractions of starch. The solution was homogenized and filtered (McCready, Guggolz, Silviera, & Owens, 1950). The starch dosage followed the steps described for soluble carbohydrate content using the phenol-sulfuric method (Dubois et al., 1956).

Data analysis

The varieties were compared based on the achieved results, and two cities were selected for collection to expand the sample universe, rather than to make comparisons between varieties in the same city.

First, the data were tested for normality (Lilliefors test) and were subjected to ANOVA in a completely randomized design. Means were compared by SNK test at a 5% probability.

The exploratory techniques of multivariate statistics were applied through the Cluster and Principal Component Analysis. Variables were standardized, and analysis was performed in R software (R Core Team, 2011). The Euclidean distance between varieties was calculated based on the biochemical, morphological and physiological characteristics, and the clustering algorithm UPGMA was used.

Results and discussion

The seeds showed different behaviors for each variety, with the highest water contents in the P-25 and U-ang local varieties (53.16 and 52.44%, respectively). The other varieties displayed a moisture content of approximately 49% (Table 2). Despite this variance in moisture content, the varieties did not differ in terms of dry mass per seed.

Table 2. Moisture content (MC), 1000-seed weight (W1000), dry weight (DW), viability through emergence test (E) and tetrazolium test (TZ) of *Araucaria angustifolia* seeds. sancti josephi (P-25), angustifolia (ang), caiova (cai) and indehiscens (ind) varieties were collected in Painel (P) and Urubici (U) – Santa Catarina State – Brazil.

Variety	MC (%)	W1000 (g	DW (g seed^{-1})	E (%)	TZ (%)
P-25	53.16a	7042.0d	3.37a	43b	60a
P-ang	48.97c	9110.1a	4.27a	73a	69a
P-cai	48.65c	8113.2c	4.47a	88a	85a
P-ind	50.34b	6929.0d	3.89a	70a	71a
U-ang	52.44a	8218.9c	3.66a	75a	77a
U-cai	48.67c	8835.5b	4.25a	90a	84a
U-ind	47.96c	6864.5d	3.79a	69a	61a
CV(%)	1.55	2.68	11.10	17.82	16.67

Values followed by the same letter in a column do not differ inwardly by the SNK test at 5%.

The P-ang variety displayed the highest 1000-seed weight (9110.1 g). Moreover, the P-ind, U-ind and P-25 varieties showed the lowest weights (6929.0, 6864.5 and 7042.0 g, respectively). These values are different from the characteristics described by farmers from Três Barras – Santa Catarina State, according to whom the caiova variety has larger seeds (Zechini et al., 2012). These differences can be related to climatic conditions during seed development, as observed for *Eugenia pyriformis* seeds where hydric and thermal environmental variations during development determined the formation cycle and final seed quality (Lamarca et al., 2013).

In addition to the physical characteristics of seeds, it is essential to measure their viability and vigor and establish a link between physical and physiological aspects. Thus, emergence, tetrazolium and electric conductivity tests were carried out. Seedling emergence was low in the P-25 variety (43%), but in the other varieties, an increase from 70 (P-ind) to 90% (U-cai) was observed. The viability analysis through the tetrazolium test did not reveal differences between varieties.

Vigor tests showed higher emergence speed index - ESI (1.02) and shoot length (13.81) for the P-ang variety (Table 3). The P-25 variety showed less vigorous seeds by the ESI (0.14), root (15.92 cm) and shoot length (4.97 cm). Coutinho and Dillenburg (2010) studied *A. angustifolia* seedlings and observed the highest shoot length for the angustifolia variety and the lowest values for the indehiscens variety. In the present study, the local angustifolia (P-ang) variety showed an up to 41% greater shoot length than the indehiscens (U-ind) variety. Although the former variety had the highest shoot length, the same did not happen for root length, indicating that the seed was directing its reserves mainly to the formation of shoots. On the other hand, the caiova (P-cai and U-cai) varieties directed their reserves to the formation of roots.

Table 3. Emergence speed index (ESI), root length, shoot length and electrical conductivity (EC) of *Araucaria angustifolia* seeds. sancti josephi (P-25), angustifolia (ang), caiova (cai) and indehiscens (ind) varieties were collected in Painel (P) and Urubici (U) – Santa Catarina State – Brazil.

Variety	ESI	Root (cm)	Shoot (cm)	EC (μS cm^{-1} g^{-1})
P-25	0.14d	15.92c	4.97c	51.55ab
P-ang	1.02a	21.63b	13.81a	56.64ab
P-cai	0.80b	28.83a	10.54b	61.36bc
P-ind	0.36c	21.80b	10.83b	87.35d
U-ang	0.29c	23.48b	9.10b	47.93a
U-cai	0.32c	25.80ab	7.92b	70.31c
U-ind	0.25cd	29.14a	8.11b	84.05d
CV (%)	16.96	11.89	15.39	9.46

Values followed by the same letter in each column do not differ inwardly by the SNK test at 5%.

In the electrical conductivity test, the P-25 variety showed lower values (51.55 µS cm^{-1} g^{-1}), as did the U-ang and P-ang varieties (47.93 and 56.64 µS cm^{-1} g^{-1}, respectively) (Table 3). These lower electrical conductivity values indicate that these seeds are more vigorous than other varieties. However, the most intensive release of exudates was observed for seeds of the P-ind and U-ind local varieties. This apparent loss of membrane integrity may be associated with a longer seed maturation duration, until the months of July and August. Such seeds were more exposed to climate changes and pathogens.

For physical and physiological characteristics of *A. angustifolia* varieties, a relationship between mass and seed vigor was observed. The P-ang variety showed a higher 1000-seed weight (9110.1 g) and higher vigor by ESI (1.02), electrical conductivity (56.64 µS cm^{-1} g^{-1}) and shoot length (13.81 cm). For other recalcitrant species, such as *Euterpe edulis,* larger fruits showed the highest vigor (Pizo et al., 2006). Similarly, in *Artocarpus heterophyllus* seeds, there was a positive correlation between seed mass and germination (Khan, 2004).

In relation to biochemical characteristics, the P-25 variety showed a low soluble carbohydrate content (17.15 mg g^{-1}). However, the starch content was close to that of other varieties (370.47 mg g^{-1}), except for the U-cai variety, which showed the highest starch content (536.81 mg g^{-1}) (Table 4). Protocols for starch isolation from *A. angustifolia* are simple, produce a high yield and might be attractive for commercial-scale production (Bello-Pérez et al., 2006), mainly for the U-cai variety.

Table 4. The total soluble carbohydrate and starch content of *Araucaria angustifolia* seeds. sancti josephi (P-25), angustifolia (ang), caiova (cai) and indehiscens (ind) varieties were collected in Painel (P) and Urubici (U) – Santa Catarina State – Brazil. Values are the means ± standard error.

Variety	Carbohydrates (mg g^{-1MS})	Starch (mg g^{-1MS})
P-25	17.15 ± 1.00b	370.47 ± 13.78b
P-ang	33.31 ± 2.74a	429.04 ± 7.38b
P-cai	34.07 ± 0.80a	413.78 ± 28.54b
P-ind	35.10 ± 4.25a	445.28 ± 5.91b
U-ang	30.32 ± 2.42a	392.62 ± 49.70b
U-cai	38.53 ± 0.71a	536.81 ± 9.84a
U-ind	30.47 ± 2.45a	396.56 ± 13.29b
CV (%)	10.76	7.77

Values followed by the same letter in a column do not differ inwardly by the SNK test at 5%.

A relationship between the amount of starch and seed germination was observed in *Aesculus hippocastanum* seeds, where the genotypes with a higher starch content showed a increased germination (Čukanović et al., 2011). This relationship was not observed in this study because only the U-cai variety showed a different starch content. However, for the soluble carbohydrate content, the P-25 variety showed lower values of 17.15 mg g^{-1}, as well as lower emergence (43%), 1000-seed weight (7042.0 g) and vigor by ESI (0.14), root (15.92 cm) and shoot length (4.97 cm).

In *Theobroma cacao* seeds, differences in the accumulation of soluble sugars in genotypes of the same species were observed (Rangel-Fajardo et al., 2011). These authors linked sugar values to the desiccation tolerance of different genotypes.

A cluster analysis based on Euclidean distance using physiological and physical characteristics allowed the identification of three groups. At the end of the dendrogram, the P-25 variety (orange group) was more distant from the other varieties. Considering the Euclidean distance of 4.0 as a reference, three clear groups emerged, separated by the P-25 (orange group); U-ind and P-ind (green group); and P-cai, U-cai, P-ang and U-ang (blue group) varieties (Figure 1). The cophenetic correlation coefficient is relatively high (r = 0.83), indicating that the representation of the dendrogram is consistent with the original data and that the classification and structure are valid.

In the main components analysis, the two first components accounted for 61.9% of the original variability. In component 1, we observed a group to the left of the axis formed by the P-25 variety. For component 2, in turn, the P-ang, U-ang and P-25 varieties prevailed in the upper corner, while the P-ind and U-ind (Figure 2) varieties prevailed in the lower part.

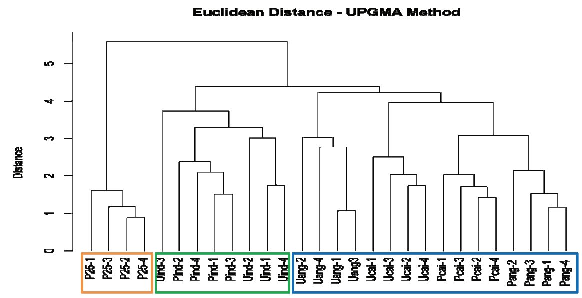

Figure 1. Dendrogram based on the Euclidean distance between 28 *Araucaria angustifolia* samples, illustrating three groups according to emergence, tetrazolium, electrical conductivity tests, emergence speed index, root and shoot length, dry weight, moisture content and 1000-seed weight of *Araucaria angustifolia* var. sancti josephi (P-25), angustifolia (ang), caiova (cai) and indehiscens (ind) collected in Painel (P) and Urubici (U) – Santa Catarina State – Brazil. UPGMA clustering method; Cophenetic correlation coefficient r = 0.83.

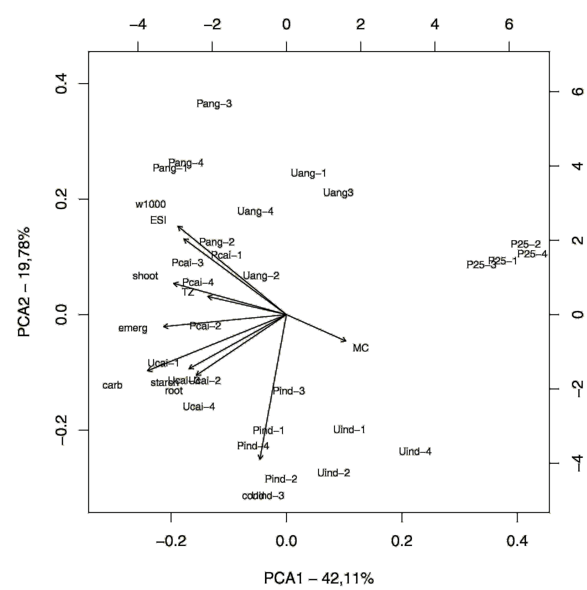

Figure 2. Ordination diagram based on axes 1 and 2 obtained through Principal Component Analysis, representing 61.9% of the original variability of *Araucaria angustifolia* seeds. Carb: carbohydrate; conduct: electric conductivity; emerg: emergence of seedlings; ESI: emergence speed index; MC: moisture content; shoot: shoot length; root: root length; w1000: 1000-seed weight; TZ: tetrazolium test; P-25: sancti josephi variety; P-ang: angustifolia variety collected in Painel and Urubici (U-ang); P-cai: caiova variety collected in Painel and Urubici (U-cai); P-ind: indehiscens variety collected in Painel and Urubici (U-ind).

The most important variables for component 1 were carbohydrates, emergence and 1000-seed weight, and the P-ang variety showed the highest values for the variables described above. For component 2, in turn, the electrical conductivity test was the most relevant between variables, and the P-ang and U-ang varieties showed better results for this variable.

The results indicate the great diversity of the physical and physiological characteristics of local varieties of *A. angustifolia,* which may reflect the high genetic diversity. The local P-25 variety showed higher diversity regarding seed characteristics than did the other varieties, and the separation of this variety was noted through cluster analysis and PCA. The lower total content of soluble carbohydrates may have been reflected in the lower physiological quality of this variety due to less availability of soluble reserves for the development of seeds and seedlings. In general, pollination of *A. angustifolia* occurs between September and October regardless of the variety (Mattos, 2011), and cone maturation occurs after 20 months (Anselmini & Zanette, 2008). Thus, the seeds of the P-25 variety ripen more quickly and therefore could accumulate lower levels of reserves during seed development. This variety and the indehiscens variety may be more vulnerable due to their low density in the landscape and potential extractive impact due to their early and late time of ripening, i.e., in the months of March and July, when large quantities of seeds are not available for trading, mirroring lower rates of regeneration of these varieties and a decrease in occurrence in the Santa Catarina region, as observed by Zechini et al. (2012).

Local angustifolia and caiova varieties showed better physiological quality, being more suitable for seedling production due to the smaller amount of seeds that are required to obtain viable and vigorous seedlings. However, it is worth mentioning the importance of encouraging the production of other varieties or morphotypes, aiming to increase the genetic diversity of the species, thus contributing to conservation and enabling production, extending the harvest season.

In recent years, many studies have been conducted to examine seed development and/or somatic/zygotic embryogenesis of *A. angustifolia* (Balbuena et al., 2009; Agapito-Tenfen, Steiner, Guerra, & Nodari, 2011; Balbuena et al., 2011; Farias-Soares, Burrieza, Steiner, Maldonado, & Guerra, 2013; Rogge-Renner et al., 2013; Shibata, Coelho, & Steiner, 2013; Farias-Soares et al., 2014; Jo, Santos, Bueno, Barbosa, & Floh, 2014; Fraga, Vieira, Puttkammer, Oliveira, & Guerra, 2015; Steiner et al., 2015), and even though knowledge of the botanical varieties of this species has been reported since 1966 by Reitz and Klein (1966), little attention has been devoted to the characterization of these varieties. In this study, the results show a great diversity of the characteristics studied, and knowledge of varieties may contribute to their maintenance and regeneration. *Araucaria angustifolia* is a socio-cultural, economic, and environmentally important native conifer; its seeds are the basis of the economy for many rural families and an important resource for the local fauna. Thus, other studies aimed at physiological, ecological, and ethnobotany characterizations are needed for its conservation.

Conclusion

The local varieties of *A. angustifolia* show great diversity in their physical and physiological characteristics, and angustifolia and caiova varieties were similar with respect to the diversity of the analyzed characteristics.

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