Illustrated morpho-agronomic descriptors for *Passiflora* spp.

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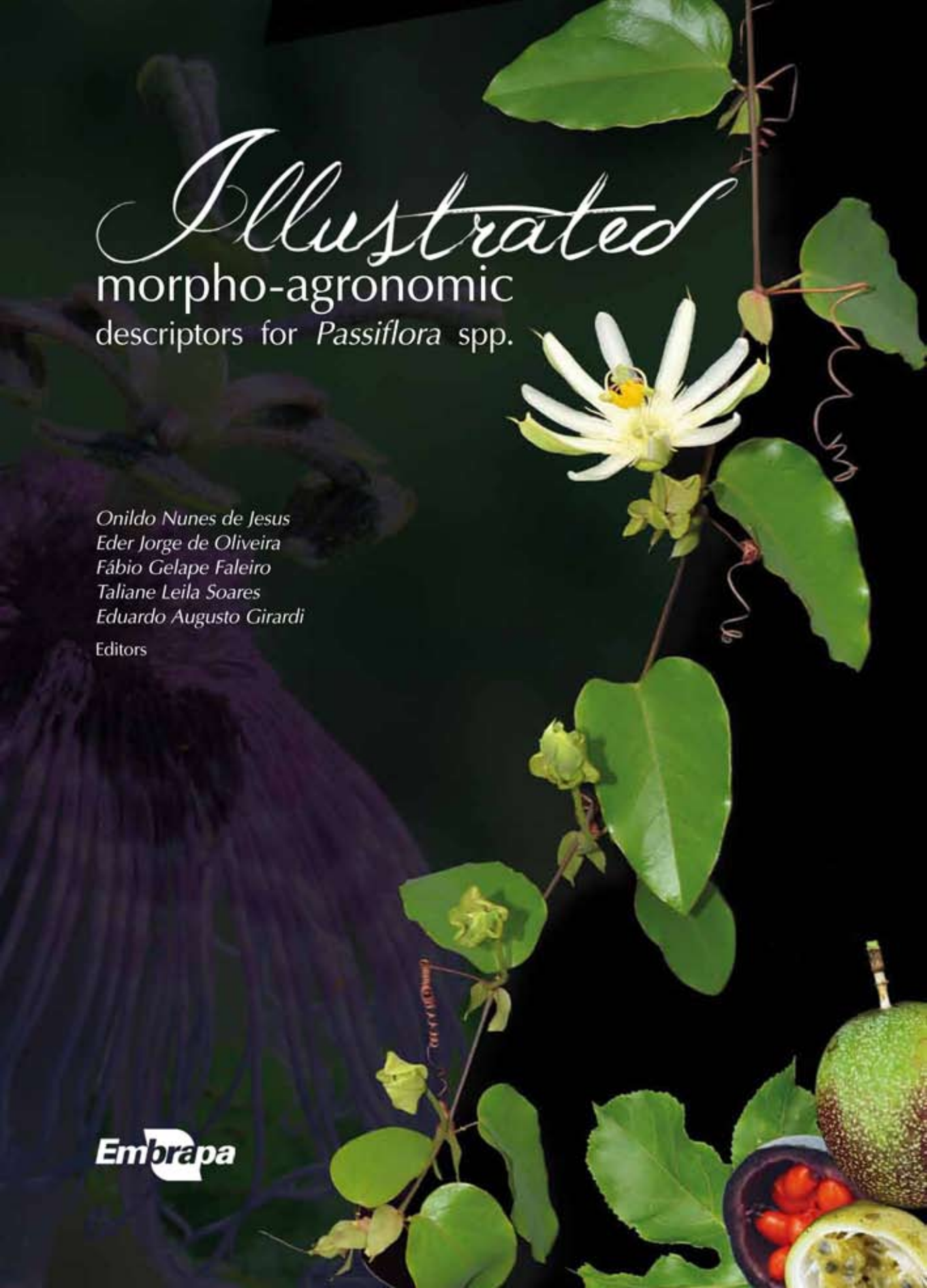
Editors



Embrapa

Brazilian Agricultural Research Corporation
Embrapa Cassava & Fruits
Embrapa Cerrados
Ministry of Agriculture, Livestock and Food Supply

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Preface

The passion fruit (*Passiflora* spp.) has high genetic variability, which can be used to diversify production systems with new varieties for fresh consumption (sweet passion fruit) and industrial processing (sour passion fruit). The ornamental, functional and medicinal values of passion fruit can also be exploited, and different varieties can be used as sources of genes for introgression into species and cultivars of commercial interest.

The wealth of genetic resources for this genus of plants is enhanced in species collections maintained at research institutions, such as germplasm banks located at Embrapa Cassava and Fruits, Embrapa Semi-Arid and Embrapa Cerrados. Such wealth also highlights the need for tools that allow proper description of the genetic resources aimed at their standardization and organization.

Morphological and agronomic descriptors are valuable means of characterizing and quantifying the existing variability, and they are important for conservation programs, for the use of germplasm, for genetic improvement of plants, and for cultivar registration and protection.

This book presents an illustrated catalog of morpho-agronomic descriptors for use in the characterization of plants and species of the genus *Passiflora*. It includes qualitaive and quantitative descriptors, many of which are already used in the process of registration and protection of cultivars. Other descriptors were established based on the practical experience of researchers dedicated to the study of this plant. It is a practical guide that aims to standardize the assessment of each characteristic by professionals from academic and research institutions engaged in the study of the genus *Passiflora*.

Domingo Haroldo Reinhardt
Director General at Embrapa Cassava & Fruits

Photo: Onildo Nunes de Jesus

Summary

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Photo: Onildo Nunes de Jesus

Importance of the characterization of genetic resources of passion fruit

The genus *Passiflora* is the largest of the family Passifloraceae, with more than 500 species. It shows extensive morpho-agronomic variation and is thus a very diverse genus. To exploit this genetic diversity, it is necessary to characterize and document the genetic resources that can be used in improvement programs or for bioprospecting.

The yellow or purple passion fruit (*Passiflora edulis* Sims.), which is planted in nearly all Brazilian states, represents most of the cultivated area in Brazil. It is popular because of the quality of its fruit and its high vigor and productivity compared with other species of the genus *Passiflora*. However, other wild species of the genus *Passiflora* have great potential to diversify production systems with new functional foods for fresh consumption, ornamental plant and medicinal plants (BERNACCI et al., 2005; FALEIRO et al., 2008a, 2008b, 2013; OLIVEIRA; RUGGIERO, 2005; SOUZA; MELETTI, 1997). In a recent survey of research demands in the cultivation of passion fruit, Faleiro et al. (2006a) indicated the characterization, domestication and development of these new species as priorities for passion fruit research.

Morphological characterization is the most accessible and most used method to characterize and quantify the genetic diversity of the Active Germplasm Bank (AGB) (DAROS et al., 2002). It contributes to the phenotypic differentiation among accessions, serving as an important tool to quantify the existing variability as well as to eliminate duplicate genotypes. This helps to minimize the use of space, labor and financial resources (JESUS et al., 2013). Morphological descriptors are important for the registration and protection of cultivars. The Brazilian Ministry of Agriculture, Livestock and Food Supply published instructions for testing distinctiveness, homogeneity and stability (DHS) of *Passiflora* cultivarsand

these instructions list the minimal descriptors used to characterize genotypes of *Passiflora* for protection (BRAZIL, 2008a, 2008b). However, many of the characteristics to be evaluated are not in common use, even for specialists in botany or crop improvement. Therefore, they need to be better described and presented to facilitate their use by students and researchers studying *Passiflora*.

Considering the importance of morphological characterization for ex situ germplasm collections, the development of a descriptive catalog for *Passiflora* was proposed with the aim of guiding and standardizing the evaluation of morphological traits among research institutions and universities. This catalog could be used in studies of genetic variability in passion fruit AGBs. Since one of the objectives of characterization is to facilitate the exchange and use of germplasm, it is crucial that morphological descriptors be as uniform as possible so that they can be applied, easily manipulated and understood by users worldwide. For the genus *Passiflora*, to date, there is no list of morphological descriptors defined by Bioversity International, which has as one of its goals to standardize the characterization of plant species through the use of descriptor lists, including species of this group. Thus, characterization studies of these species are focused on the use of descriptors commonly reported in the literature.

This germplasm catalog uses qualitative botanical descriptors that are typically controlled by a few genes and are easily visible. It also uses quantitative descriptors. It is important to note that these descriptors are part of the list used for the registration and protection of passion fruit cultivars as described by Brazil (2008a, 2008b); UPOV (2009), some listed by Gonçalves and Lorenzi (2011), and others established by experienced genetic improvement scientists. We hope that this publication will become a useful and practical guide for morphological characterization studies of *Passiflora*, and we expect that it will be accessible to institutions studying the genus *Passiflora*.

Characterization of passion fruit genetic resources

Plant genetic resources are important because they relate to the basic needs of humans, and they are needed to solve serious problems such as hunger and poverty. Wild species that are related to commercially cultivated plants, ancient varieties (landraces) and improved varieties, represent genetic resources that should be conserved and characterized, as they may be used in genetic improvement programs. Several agronomic characteristics can be obtained from genetic resources, especially those aiming at the generation of more productive varieties, with good physical and chemical quality of fruits, resistance to pests and diseases, and adaptation to different regions and production systems, among others.

The use of genetic resources is viable based on the collection, introduction, conservation and exchange of accessions of a given species or crop, as well as its characterization and evaluation. Conservation efforts help to support genetic improvement projects, enabling the exchange of germplasm and, in particular, the preservation of genetic variability. Characterization and evaluation make it possible to determine the qualities and potential of the germplasm. Much of the genetic diversity of fruit trees in Brazil is conserved in the germplasm banks of several research and academic institutions throughout the country.

The genus *Passiflora* exhibits great genetic variability with more than 500 species, of which more than 150 are

native to Brazil, offering the country a privileged position with respect to the genetic resources of these species (BERNACCI et al., 2005; FALEIRO; JUNQUEIRA, 2009; FERREIRA, 2005). This genus has wide interspecific variability and has many potential uses, including food, medicinal and ornamental. Currently, the genus is still underutilized. Quantifying this genetic variability is essential for understanding the many species and identifying valuable genetic resources, including those that can be directly introduced into production systems, as well as those that are important for genetic improvement programs or that have potential for exploitation of bioactive compounds.

Genetic improvement scientists have used morpho-agronomic descriptors to characterize the accessions and plants selected throughout the genetic improvement program. These descriptors play a key role in the genetic characterization and selection of plants, and they are decisive in the choice of new cultivars. They also allow the estimation of genetic parameters and estimation of genetic diversity in accessions conserved in germplasm banks (JESUS et al., 2013). Genetic diversity studies enable inferences to be made about the organization of the germplasm, the sampling efficiency of genotypes, the definition of artificial crosses and incorporation of native and exotic germplasm genes (VIEIRA et al., 2007). In addition to guiding crosses for genetic improvement programs, a genetic variability study can be an important tool to select essential descriptors for the characterization and identification of duplicate samples in germplasm banks (CASTRO et al., 2012; FERREIRA; GRATTAPAGLIA, 1998; FERREIRA, 2003; JESUS et al., 2013).

Embrapa has passion fruit germplasm collections obtained from the introduction of a national germplasm and collections in various regions of Brazil. The pre-improvement conducted in this collection is an essential step because it aims at the identification, characterization and subsequent use of promising genotypes in crosses with elite germplasm (FALEIRO et al., 2011). Passion fruit AGB accessions are characterized and evaluated using the descriptors

established for the crop, with the objective of identifying and documenting morphological aspects of high heritability. The molecular, cytogenetic and biochemical patterns can also be documented to generate information for the potential use of the material in genetic improvement programs. Together with these characterizations, the incidence and severity of the major pests and diseases (mites, fusarium, anthracnose, scab, bacterial and viral infections) are also evaluated both in field conditions and in greenhouses to identify sources of resistance in conserved accessions (OLIVEIRA et al., 2013; SILVA et al., 2011).

Wild passion fruit species have shown great potential for use in genetic improvement programs and as rootstocks, in addition to being alternatives to diversify production systems with new functional foods for fresh consumption (sweet passion fruit), for use as ornamental plants or for introgression of genes of interest into commercial species (FALEIRO; JUNQUEIRA, 2009; JUNQUEIRA et al., 2005). According to Cunha et al. (2002), approximately 70 species produce edible fruits, and according to Vieira and Carneiro (2004), more than 50 have commercial potential. *Passiflora* species have agronomic, functional and medicinal potential, requiring intensification of the research aimed at better understanding the germplasm of wild passion fruit (COSTA; TUPINAMBÁ, 2005; OLIVEIRA; RUGGIERO, 2005). As an ornamental plant, Peixoto (2005) describes the immense potential of the genus *Passiflora* and its use, for over a century, in countries in the Northern hemisphere, as an element of decoration and also as a source of income for producers. Thus, the diversified use of passion fruit for edible fruit, ornamental use, medicinal use and use as rootstock for trees is considered an important demand for research and development. The aim of the research and development is to meet the demands of the production, industrial and consumer sectors (FALEIRO et al., 2006a). Similarly, the use of all available molecular and quantitative genetics tools is strategic; it allows passion fruit genetic improvements to meet the demands of these sectors (FALEIRO et al., 2006b, 2012).

Use of descriptors for distinctness, uniformity and stability tests

In order for the technological products developed by genetic improvement programs to reach producers and improve the whole supply chain, validation and technology transfer actions are essential (BORGES et al., 2005). In addition, an organized system of production, sale and distribution of quality seeds and seedlings is necessary; these are important post-improvement actions (FALEIRO et al., 2008a, 2008b). The basis for this process is the registration of varieties and hybrids in the National Register of Cultivars (Registro Nacional de Cultivares - RNC) of the Ministry of Agriculture, Livestock and Food Supply (*Ministério da Agricultura, Pecuária e Abastecimento – MAPA*). This registration is required in order for accredited nurseries to purchase the seeds and sell the resulting seedlings. In addition to the legal aspect, the registration is a guarantee for producers of the continued genetic quality of the registered materials.

In addition to registration in the RNC, cultivars can now be protected in the National Cultivars Protection System (*Sistema Nacional de Proteção de Cultivares – SNPC),* which is also connected to MAPA. In December 2008, a list of 25 descriptors of *Passiflora edulis* Sims, and 33 descriptors for other wild species and interspecific hybrids, was developed and published in the Brazilian official government newspaper, *Diário Oficial da União No.* 246 (BRAZIL, 2008a, 2008b). Additionally, a set of instructions for the use of the descriptors in tests of distinctness, uniformity and stability was published. Cultivar protection safeguards the producer that the cultivar planted has the genetic potential stated by the institution or holder of the material, with assurance of place of provenance, and it deters the spread of seeds produced without proven genetic origin and without quality control.

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Morpho-agronomic descriptors

Plant descriptors

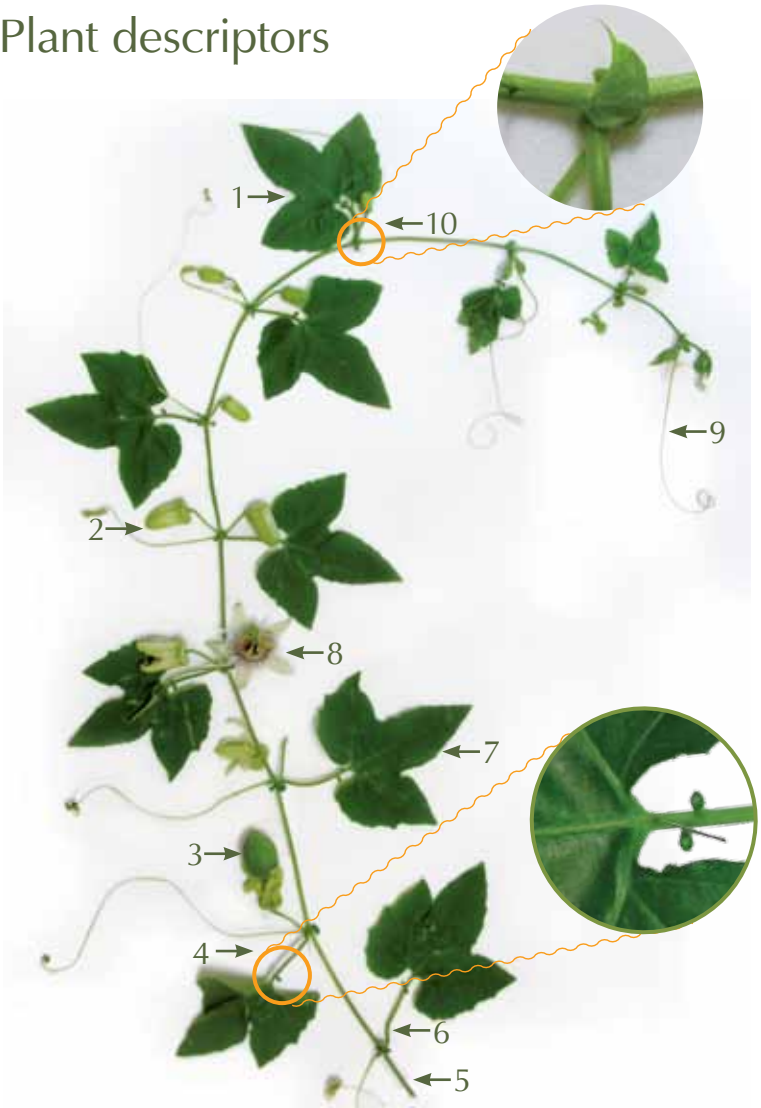

Figure 1. Parts of the *Passiflora morifolia* Mast branch.

- **Sinus** 1
- **Flower bud** 2
- **Fruit** 3
- **Nectary** 4
- **Branch** 5
- **Petiole** 6
- **Leaf** 7
- **Flower** 8
- **Tendril** 9
- **Stipule** 10

Photos: Onildo Nunes de Jesus

NFR: Number of fruits

Counting and characterizing the number of fruits should be conducted during peak crop production (suggested to be between the 10th and 12th month after field planting, depending on the growing region). Every fruit on the plant (small and large) should be counted. When counting the fruits, it is suggested to pollinate and mark some flowers in the plot with colorful ribbons to identify the next date for a new fruit count, considering the fully ripe fruits derived from this marking. Thus, the next evaluation will only occur after the development and abscission of fruits previously identified and the emergence of the new fruit crop.

YFR: Fruit yield

Fruit yield is measured by weighing and counting all the fruits of the experimental plot during part of the production period or for the whole production period. The estimated yield is expressed in tons per hectare. The total number of fruits per hectare may also complement the information on the estimated crop yield. For productivity, the number of plants in the plot and the number of plants per hectare should be considered, as well as the spacing used in the experiment (HAFLE et al., 2009; JESUS et al., 2016).

NDF: Number of days from planting to the beginning of flowering

The time from planting until the beginning of flowering may vary depending on environmental conditions and also due to differences among genotypes. Thus, this measurement is obtained by recording the number of days required from planting until the beginning of flowering, which should be recorded weekly until the end of flowering (ARAÚJO et al., 2008).

NDP: Number of days from planting to the beginning of production

Early yield is an important aspect to be evaluated in different passion fruit accessions. It is used to anticipate harvesting periods and even to increase productivity in some situations. This measure is determined by the number of days from planting in the field until the fall of the first ripe fruit.

PDE: Plant development

To evaluate this descriptor, it is important that all seedlings are sown and planted at the same time. After planting, the growth of seedlings under field conditions should be followed from the 3rd to the 4th month (Figure 2). This descriptor makes it possible to evaluate the vigor and precociousness of the genotypes (JESUS et al., 2016). This evaluation can be done in a predetermined period. For this evaluation the following classes should be adopted:

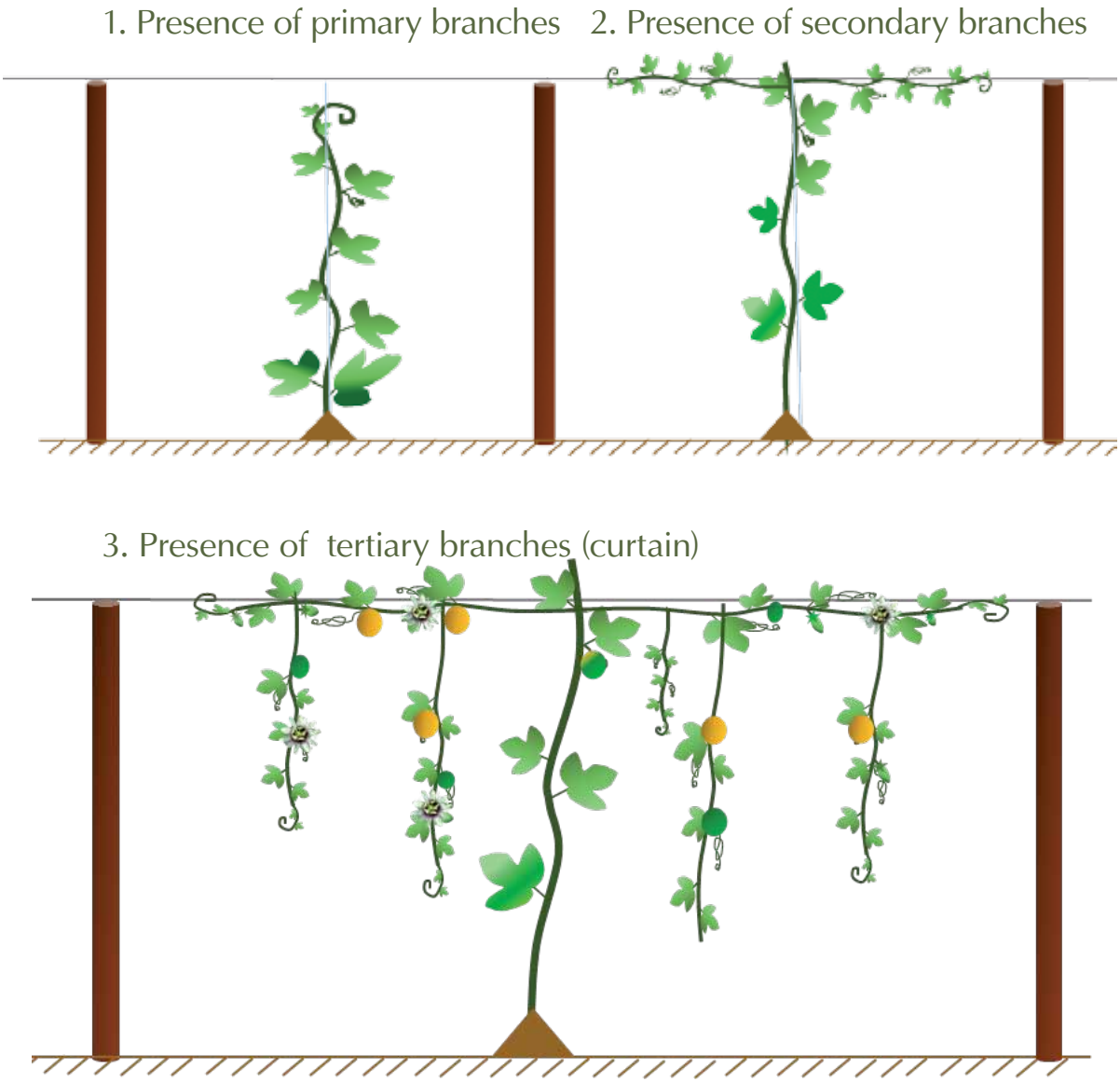

Figure 2. Plant development: Presence of primary branches (1); Presence of secondary branches (2) and Presence of tertiary branches (curtain).

PFF: Presence of flowers and fruits

After the seedlings reach the crop wire (Figure 3) and when secondary branches (lateral branches) are present, the evaluations of the presence and absence of flowers and fruits can be performed (JESUS et al., 2016):

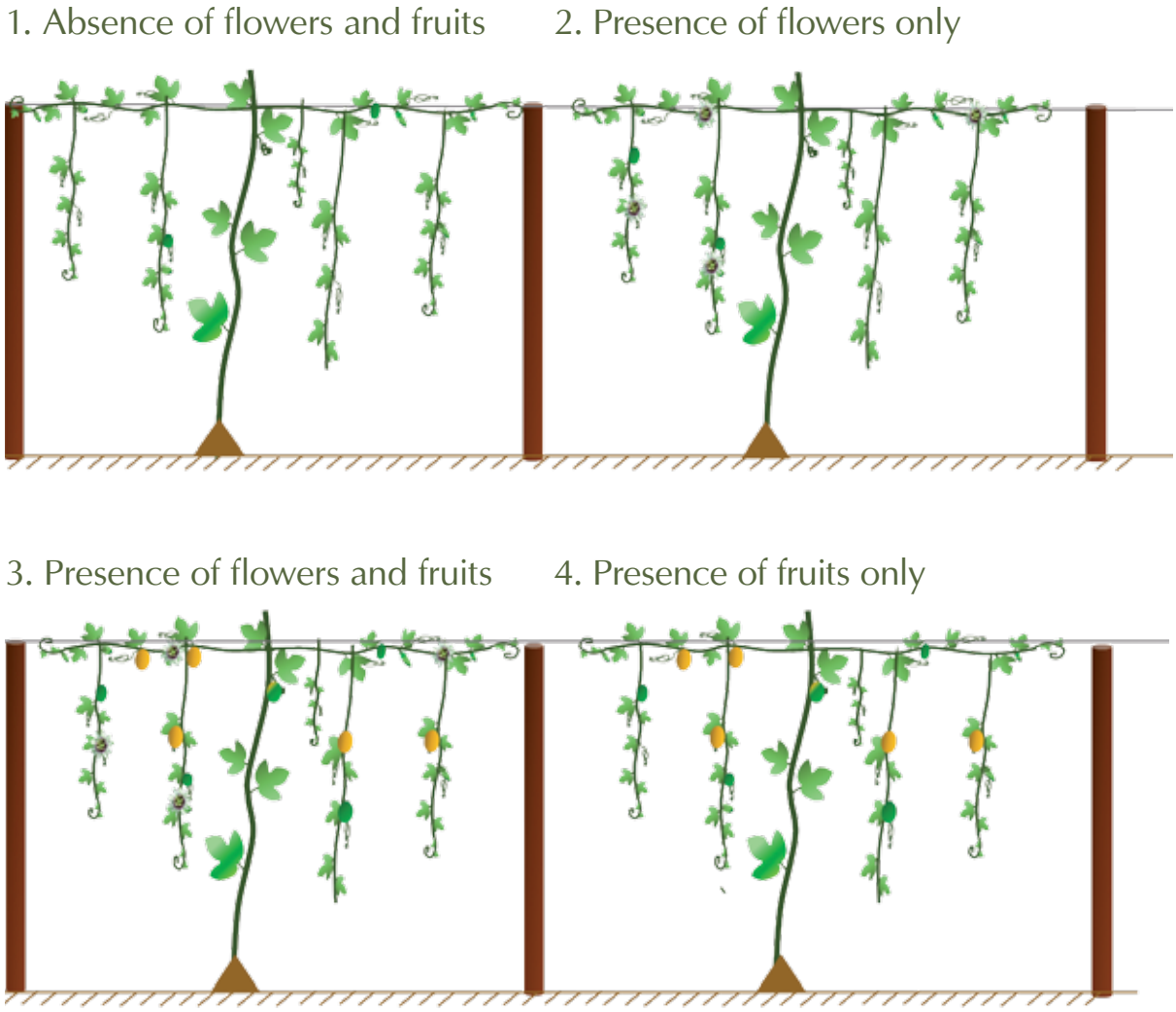

Figure 3. Flowers and fruits: Absence of flowers and fruits (1); Presence of flowers only (2); Presence of flowers and fruits (3) e Presence of fruits only (4).

Observations for the evaluation of the branch

- Branch: evaluate vigorous branches resulting from spring budding (young branches, of the year, still not fully lignified).
- If the plant does not present the characteristic under evaluation (for example absence of bracts in the flowers) assign zero to the descriptor and specify the reason.

BRC: Branch color

1. Light-green

2. Dark-green

Photos: Onildo Nunes de Jesus

3. Purplish-green

4. Reddish-purple

Source: Adapted from Brazil (2008a, 2008b).

PAB: Presence of anthocyanin on the branches

1. Absent (no anthocyanin)

2. Few (more green than purple)

Photos: Onildo Nunes de Jesus

3. Medium (more purple than green; purplish-green)

4. 4. High (completely reddish purple)

PHE: Presence of heterophylly

1. Absent

Photos: Onildo Nunes de Jesus

2. Present

PST: Presence of stipule

1. Absent

Photos: Onildo Nunes de Jesus

2. Present

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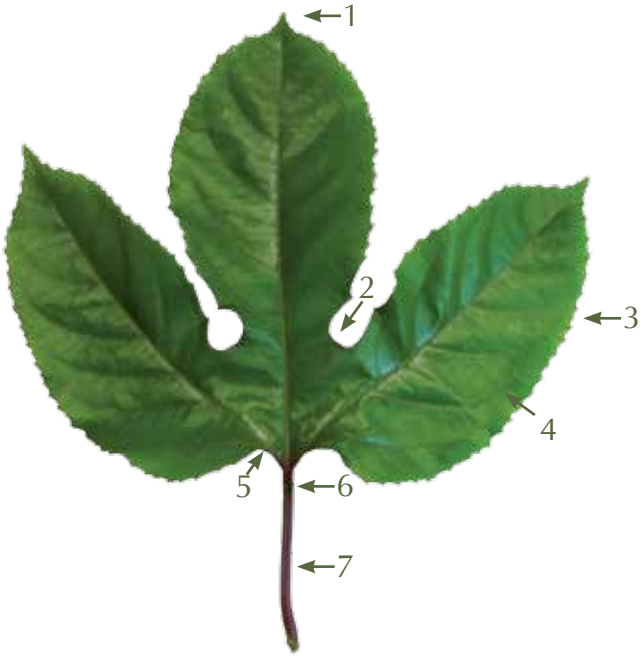
Leaf descriptors

Adaxial or Ventral



Abaxial or Dorsal

Figure 4. Parts of the *Passiflora edulis* Sims leaf.

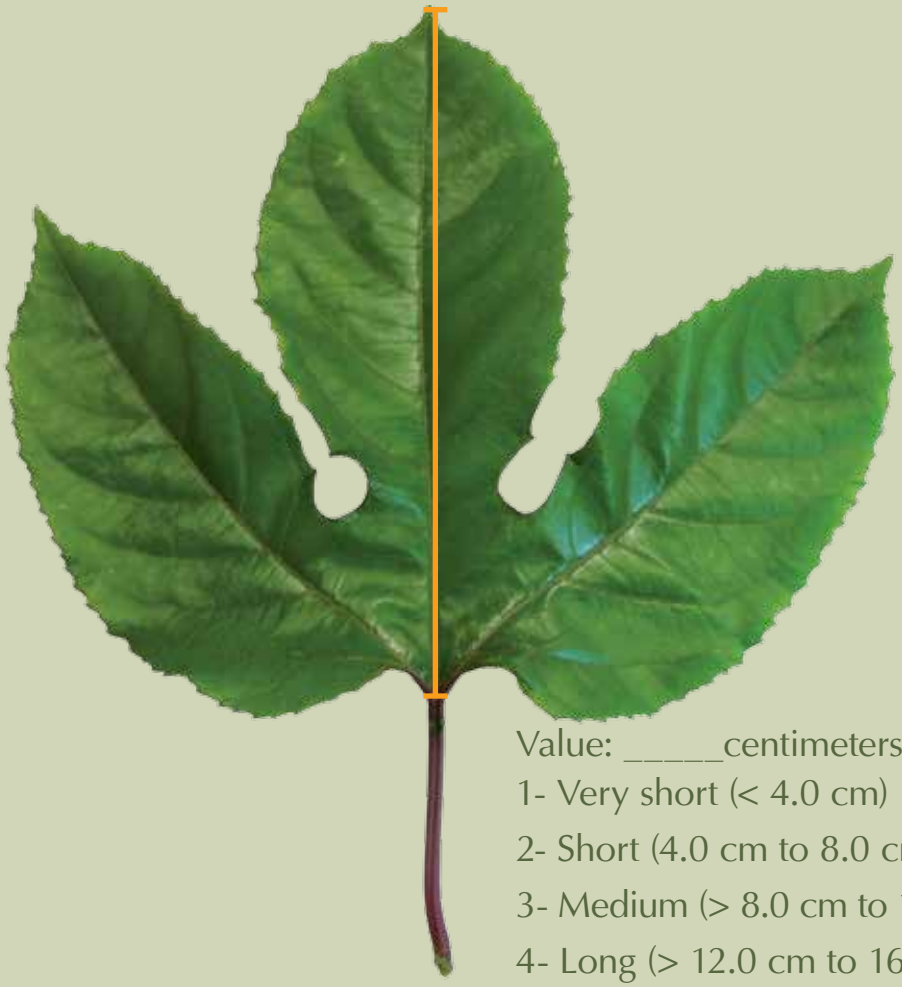
Apex 1
Sinus 2
Margin 3
Limb 4
Leaf base 5
Nectary 6
Petiole 7

Photos: Onildo Nunes de Jesus

Observations for the evaluation of the limb and petiole

- Leaf lamina or limb and petiole: evaluate fully developed leaves (basal), in the middle third of the branch, during the growing season.
- If the plant does not present the characteristic under evaluation assign zero to the descriptor and specify the reason.

LLL: Length of leaf lamina

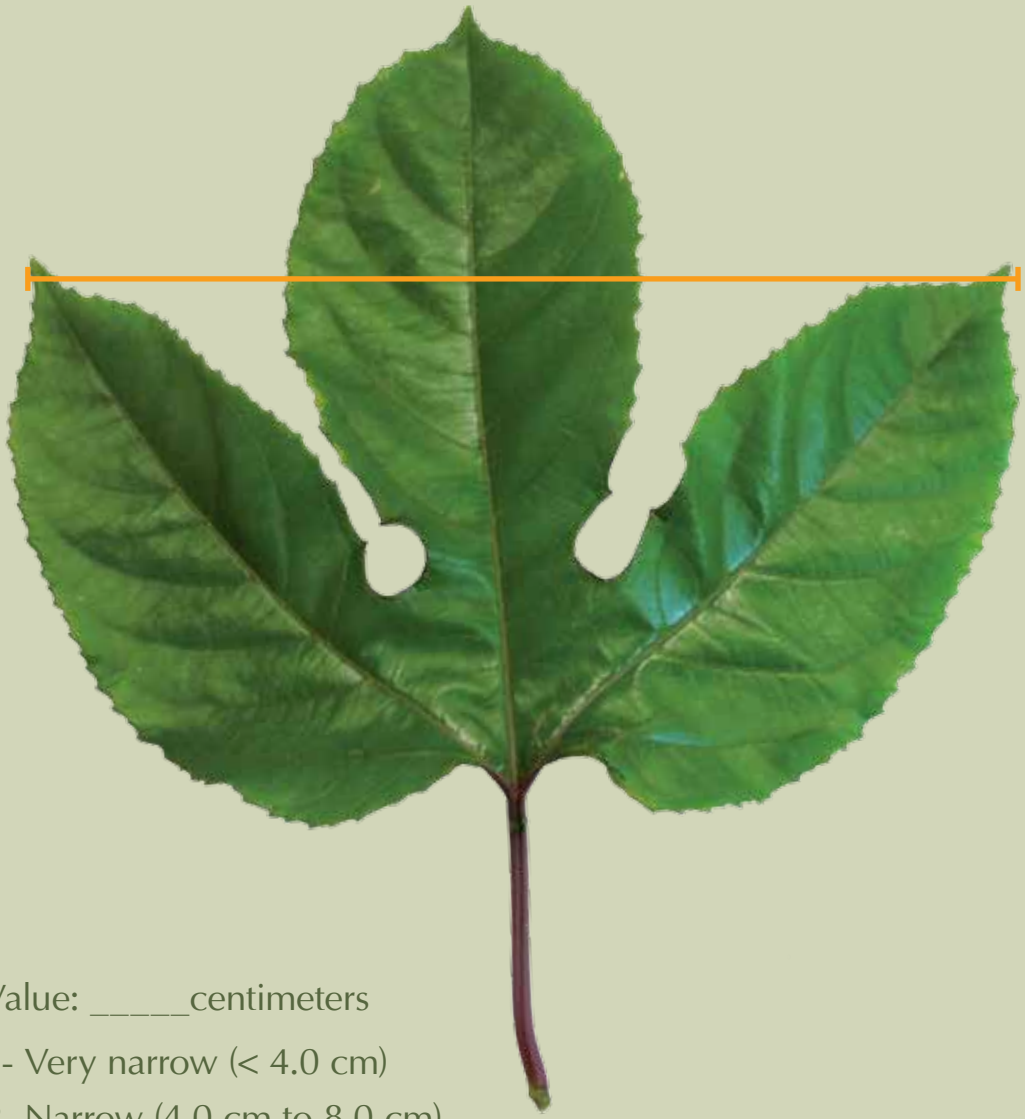


Value: _____centimeters
1- Very short (< 4.0 cm)
2- Short (4.0 cm to 8.0 cm)
3- Medium (> 8.0 cm to 12.0 cm)
4- Long (> 12.0 cm to 16.0 cm)
5- Very long (> 16.0 cm)

Source: Adapted from Brazil (2008a, 2008b).

Photo: Onildo Nunes de Jesus

MLW: Maximum leaf width



Value: _____centimeters

1- Very narrow (< 4.0 cm)

2- Narrow (4.0 cm to 8.0 cm)

3- Medium (> 8.0 cm to 12.0 cm)

4- Wide (> 12.0 cm to 16.0 cm)

5- Very Wide (> 16.0 cm)

Source: Adapted from Brazil (2008a, 2008b).

Photo: Onildo Nunes de Jesus

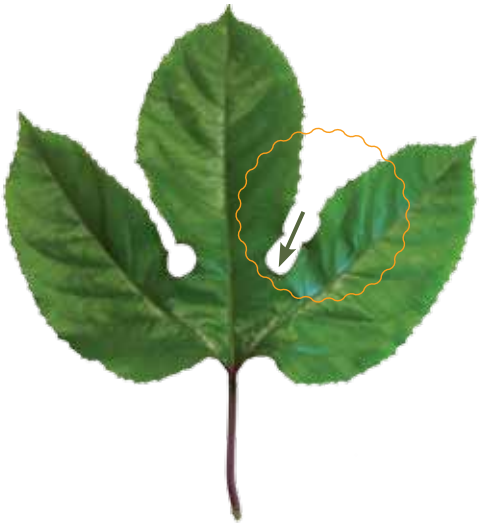
SID: Sinus depth

1. Absent

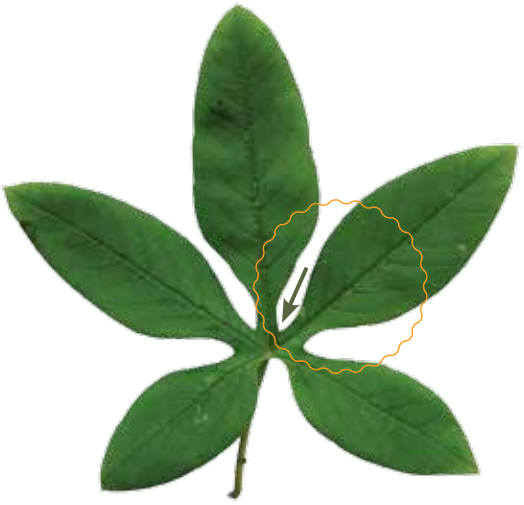
2. Shallow

Photos: Onildo Nunes de Jesus

3. Medium

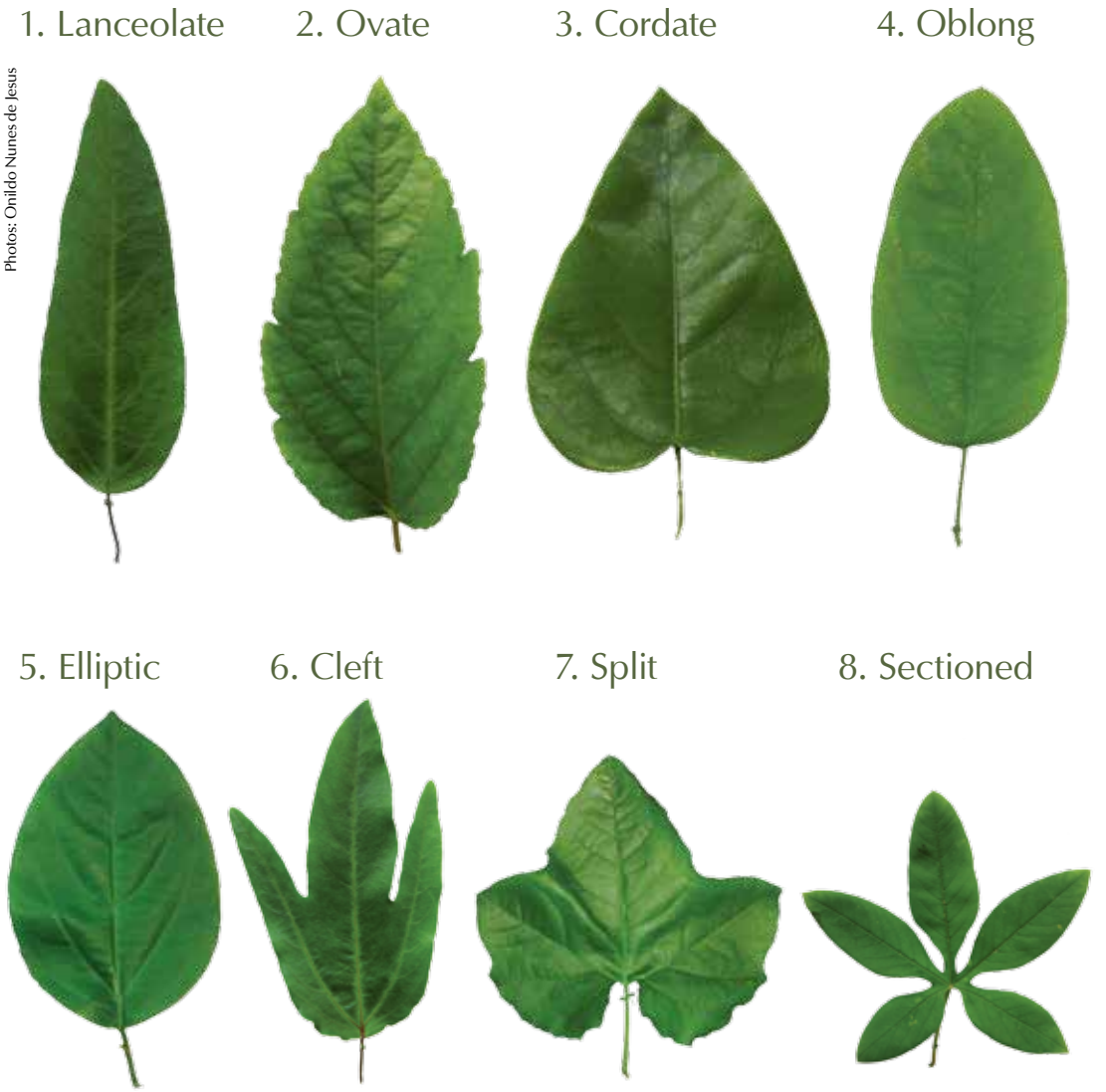


4. Deep



Source: Adapted from Brazil (2008a, 2008b).

LSH: Leaf shape

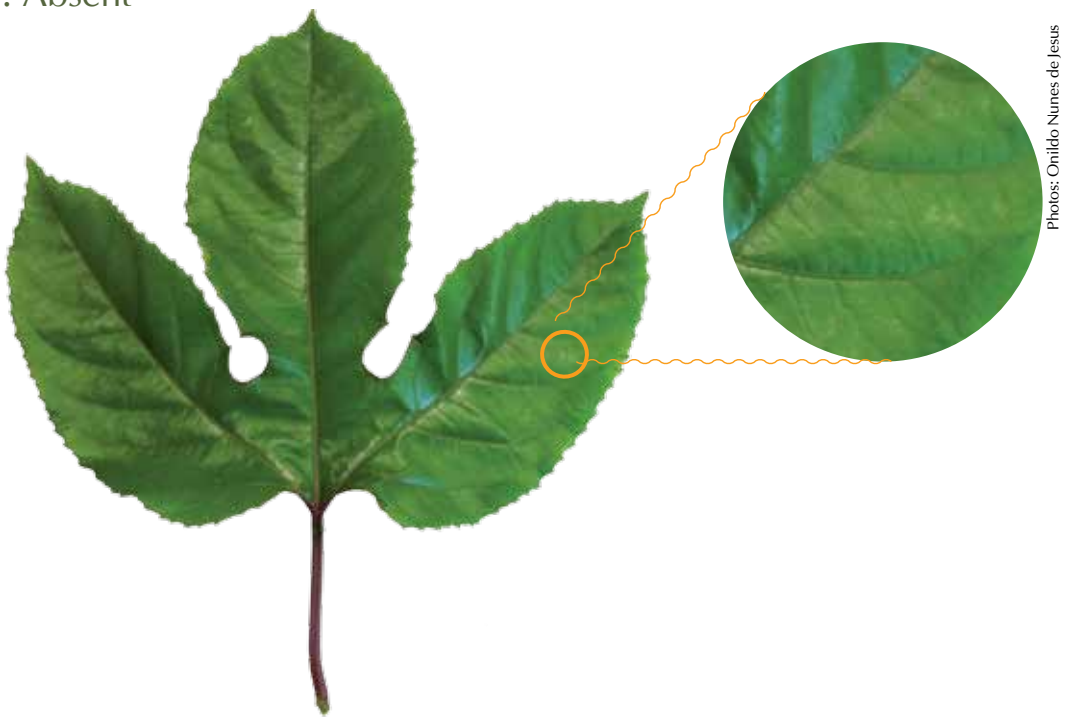


Photos: Onildo Nunes de Jesus

Source: Adapted from Brazil (2008a, 2008b).

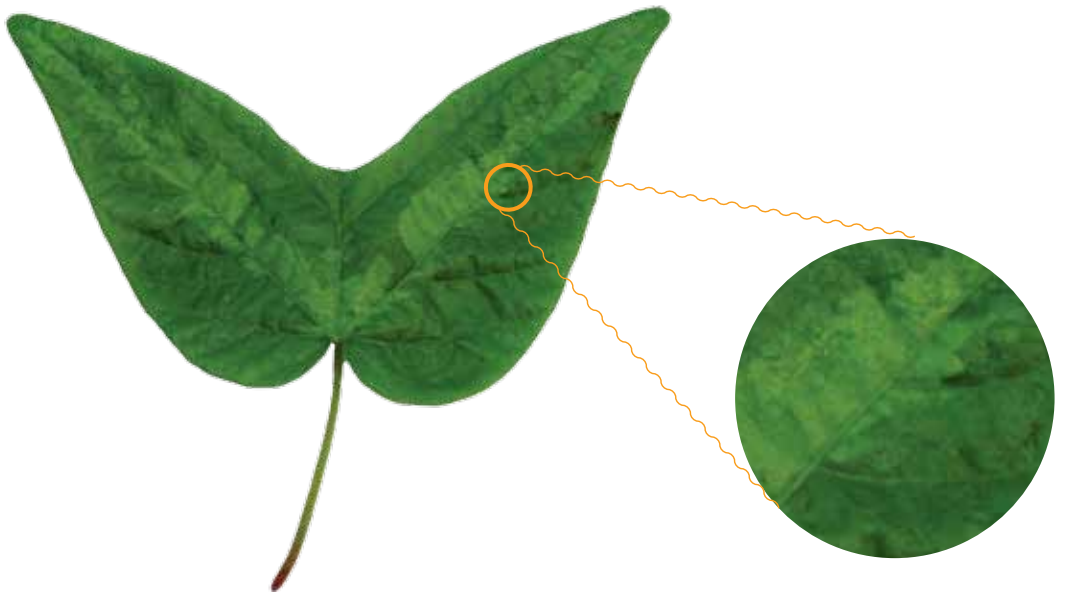
LSP: Leaf spot

1. Absent



Photos: Onildo Nunes de Jesus

2. Present



Source: Adapted from Brazil (2008a, 2008b).

LMS: Leaf margin shape

Photos: Onildo Nunes de Jesus

1. Entire

2. Undulated

3. Dentate

4. Repand

5. Serrate

6. Doubly serrate

7. Crenate

PLP: Presence of leaf pilosity

1. Absent

Photos: Onildo Nunes de Jesus

2. Present

Source: Adapted from Brazil (2008a, 2008b).

DLL: Division of the leaf lamina

Photos: Onildo Nunes de Jesus

1. Entire

2. Bilobate

3. Trilobate

4. Pentalobate

5. Hexalobate

6. Heptalobate

Source: Adapted from Brazil (2008a, 2008b).

BLL: Bullate leaf lamina

1. Absent

Photos: Onildo Nunes de Jesus

2. Present

Source: Adapted from Brazil (2008a, 2008b).

LCO: Leaf color

1. Light-green

2. Green

3. Dark-green

Photos: Onildo Nunes de Jesus

LBS: Leaf base shape

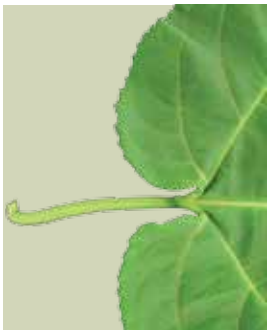
1. Round

2. Truncate

3. Attenuate

4. Subcordate

5. Cordate



6. Hastate

7. Digitiform

8. Acute

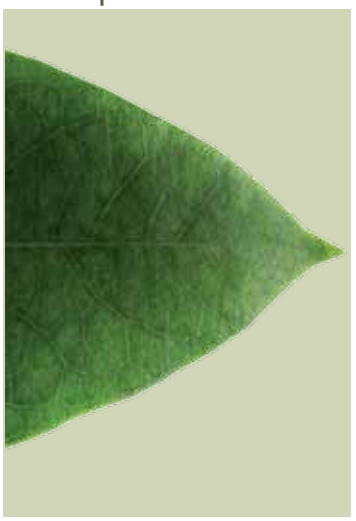
Photos: Onildo Nunes de Jesus

LAS: Leaf apex shape

1. Round

2. Attenuate

3. Cuspidate



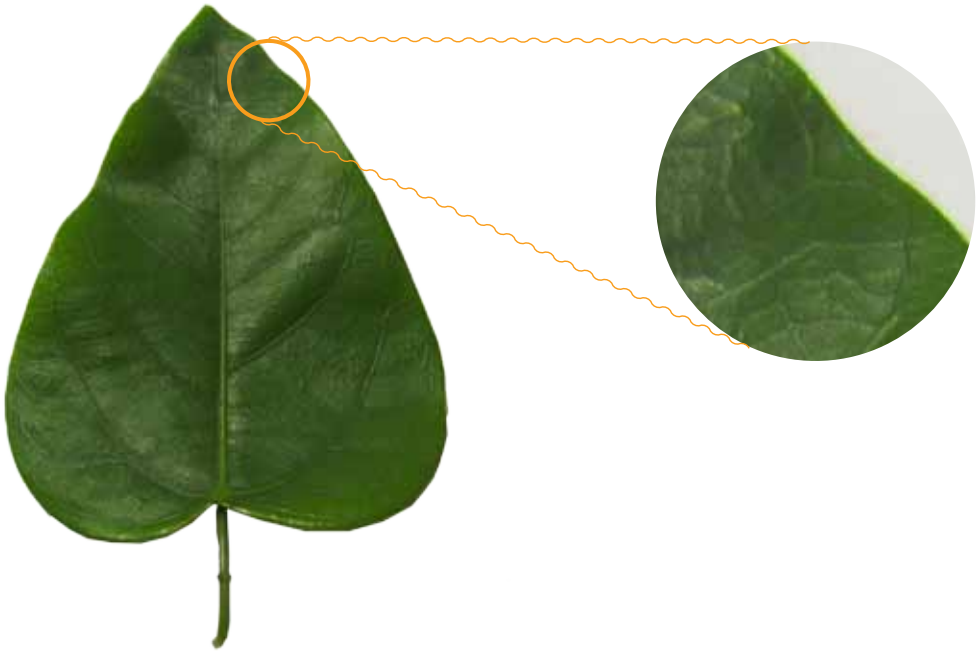
Photos: Onildo Nunes de Jesus

4. Acuminate

5. Acute

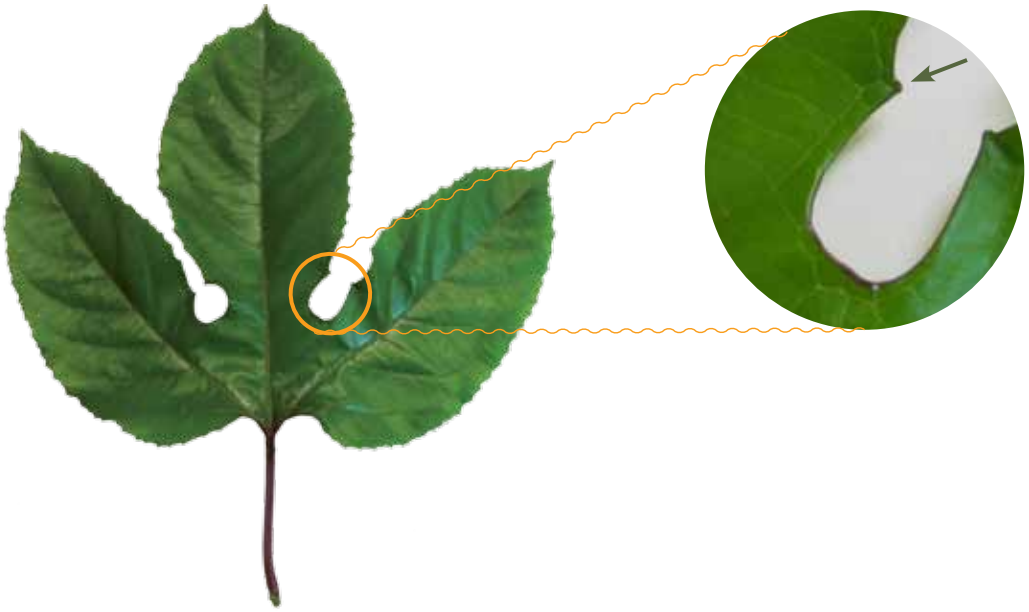
PNE: Presence of nectaries

1. Absent

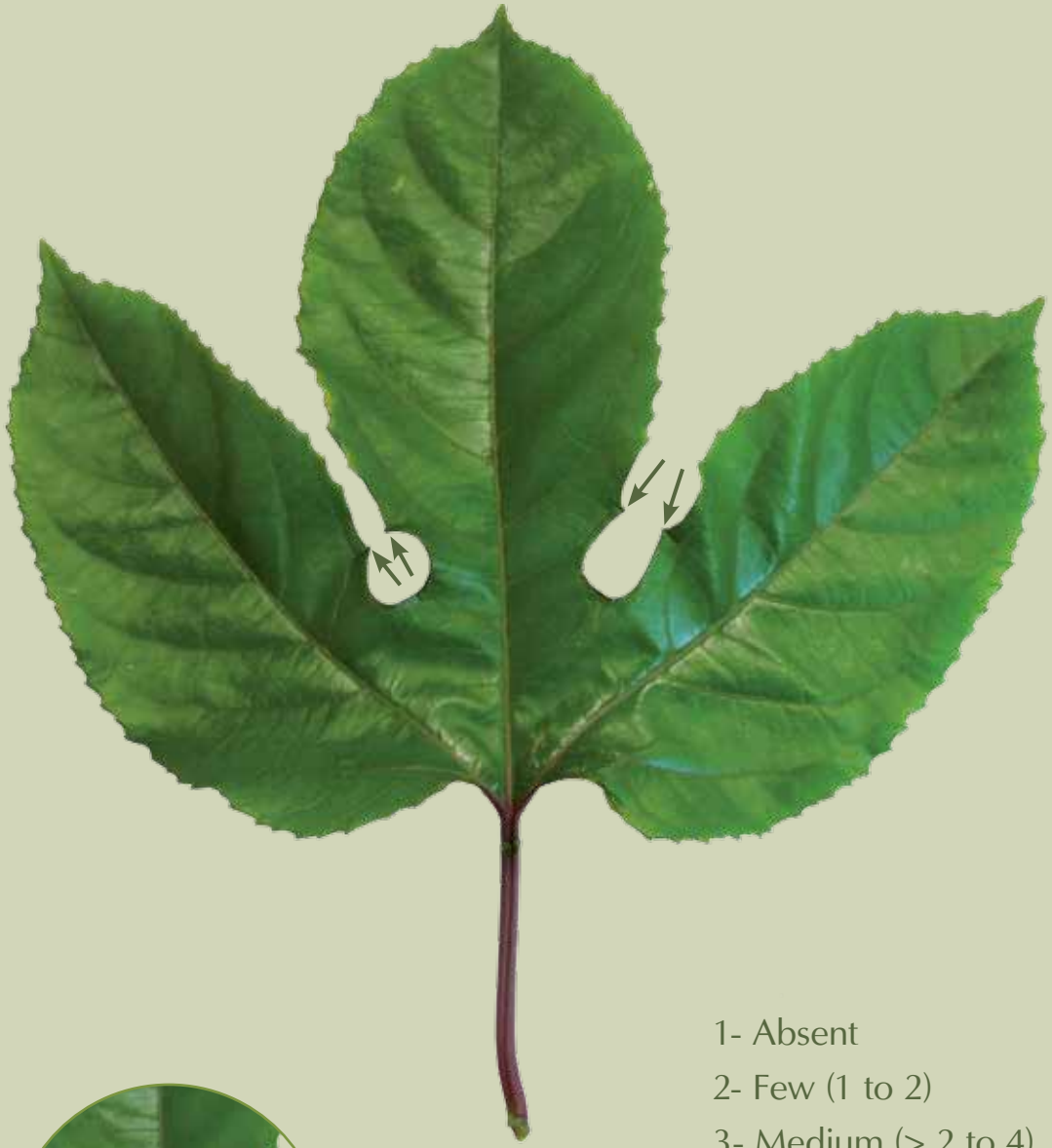


Photos: Onildo Nunes de Jesus

2. Present



NNE: Number of nectaries

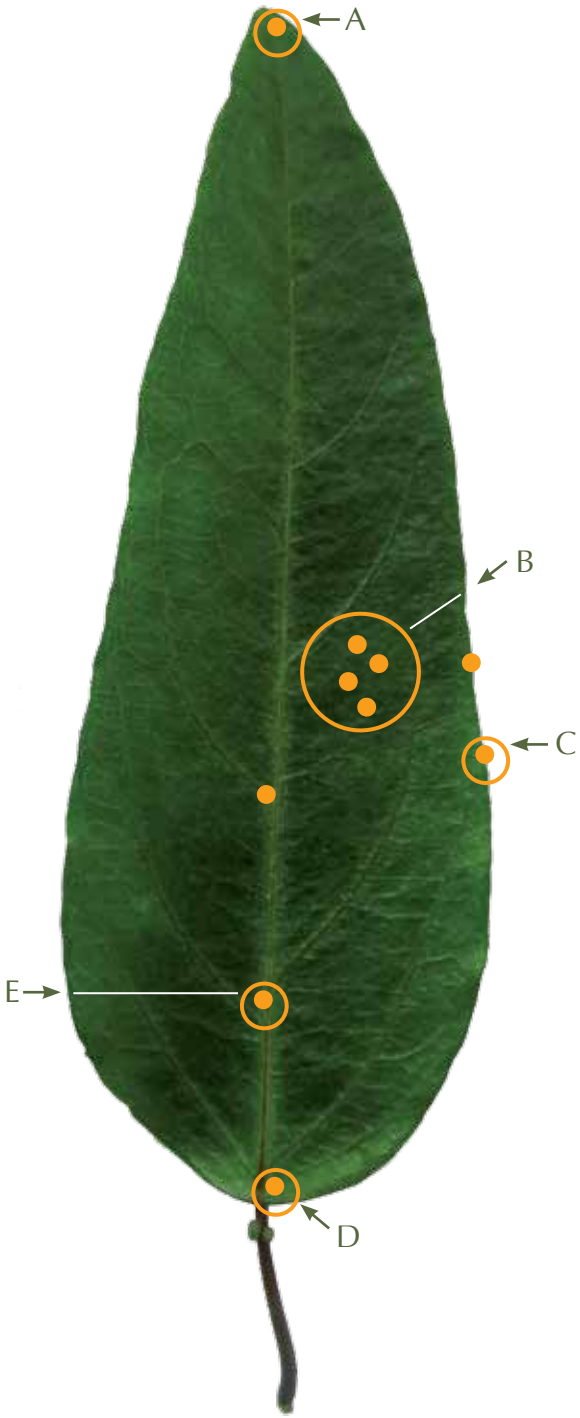


1- Absent
2- Few (1 to 2)
3- Medium (> 2 to 4)
4- High (> 4)

Photo: Onildo Nunes de Jesus

PNFL: Position of nectaries in the foliar limb

Photo: Onildo Nunes de Jesus



Apical A

Laminar B

Marginal C

Basilaminar D

Axillary nerve E

Source: Adapted from Gonçalvez and Lorenzi (2011).

1. Absent

2. Basilaminar

3. Laminar

4. Marginal

5. Axillary nerve

6. Apical

Photos: Onildo Nunes de Jesus

PTL: Petiole length

Value: _____centimeters

1- Very short (< 2.0 cm)

2- Short (2.0 cm to 3.0 cm)

3- Medium (> 3.0 cm to 4.0 cm)

4- Long (> 4.0 cm)

Source: Adapted from Brazil (2008a, 2008b).

Photo: Onildo Nunes de Jesus

NNP: Number of nectaries in the petiole

1. Absent

2. Few (1 – 2)

Photos: Onildo Nunes de Jesus

3. Medium (> 2 – 4)

4. High (> 4)

PNP: Position of nectaries on the petiole

1. Absent

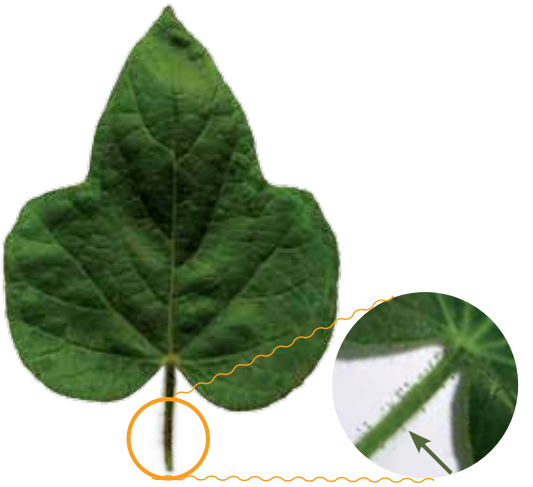
2. Adjacent to the leaf lamina

3. Near the center of the petiole

Photos: Onildo Nunes de Jesus

4. Adjacent to the insertion of the leaf on the branch

5. Distributed throughout the petiole



Source: Adapted from Brazil (2008a, 2008b).

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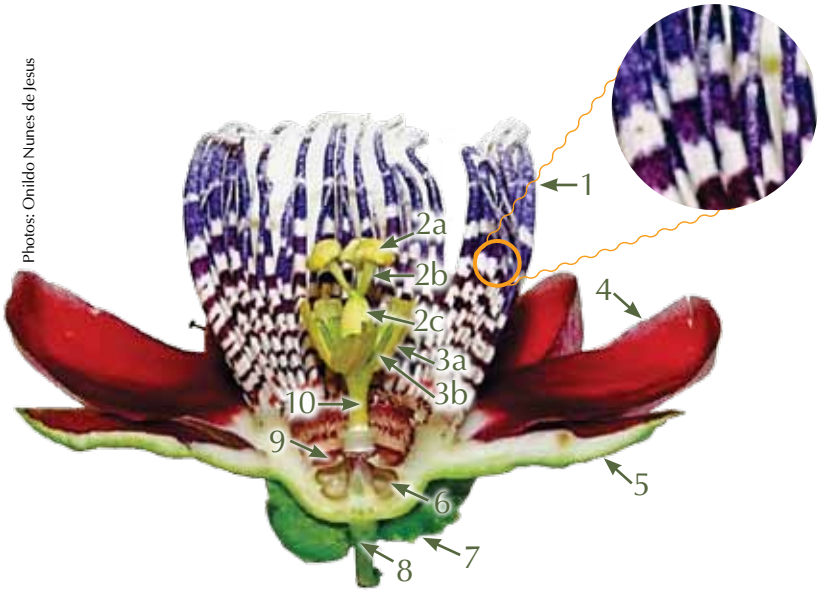
Flower descriptors

Photos: Onildo Nunes de Jesus



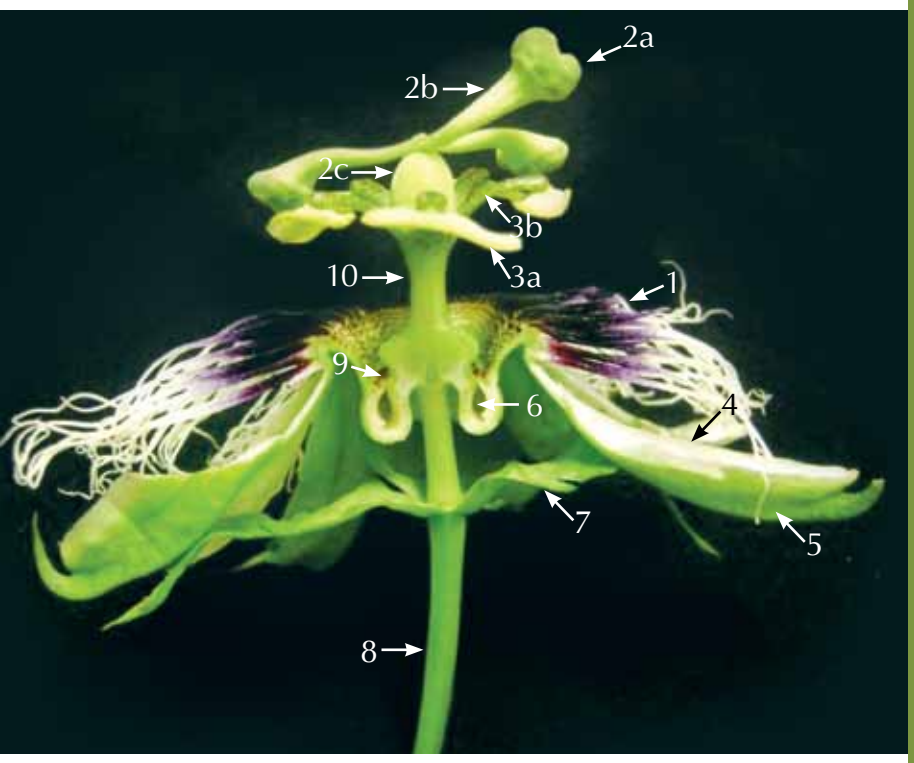


Figure 5. Longitudinal cross-sections of the flowers of *P. alata Curtis* (A) and *P. edulis* Sims (B).

Corona filaments 1

Pistil: 2
a - Stigma
b - Style
c - Ovary

Stamen: 3
a - Anther
b - Filament

Petal 4

Sepal 5

Nectary chamber 6

Bract 7

Pedicel (peduncle) 8

Operculum 9

Androgynophore 10

Observations for evaluation of the flower

The flowers have to be evaluated at anthesis (completely open), without defects resulting from pest attacks or inclement weather.

- Gynoecium (pistil) or female part: stigma + style + ovary
- Androecium (stamens) or male part: anther + filament
- Perianth: sepal (calyx- set of sepals) + petals (corolla- set of petals)
- Fimbria = corona filaments

If the plant does not present the characteristic under evaluation (for example absence of bracts in the flowers) assign zero to the descriptor and specify the reason.

PAB: Presence of anthocyanin on the bracts of the flower buds

1. Absent (no anthocyanin)

2. Few (more green than purple)

Photos: Onildo Nunes de Jesus

3. Medium (more purple than green)

4. High (completely purple)

PAS: Presence of anthocyanins on the sepals of the flower buds

1. Absent (no anthocyanin)

2. Few (few anthocyanin dots)

Photos: Onildo Nunes de Jesus

3. Medium (dispersed anthocyanin)

4. High (well distributed anthocyanin)

HYS: Hypanthium shape

1. Flattened

2. Campanulate

Photos: Onildo Nunes de Jesus

3. Cylindrical

Source: Adapted from Brazil (2008a, 2008b).

NFN: Number of flowers per node

1. Few (1 flower)

2. Medium (2 to 4 flowers)

Photos: Onildo Nunes de Jesus

3. High (> 4 flowers)

Photo: Fábio Gelape Faleiro

LEB: Length of the bract

Value: _____centimeters

1- Short (< 2.0 cm)

2- Medium (2.0 cm to 4.0 cm)

3- Long (> 4.0 cm)

Source: Adapted from Brazil (2008a, 2008b).

Photo: Onildo Nunes de Jesus

PNB: Presence of nectaries on the bract

1. Absent

Photos: Onildo Nunes de Jesus

2. Present

NNB: Number of nectaries on the bract

1- Absent
2- Few (1)
3- Medium (2 to 4)
4- High (> 4)

Photo: Onildo Nunes de Jesus

SEL: Sepal length

Value: _____centimeters

1- Short (< 3.0 cm)

2- Medium (3.0 cm to 6 cm)

3- Long (> 6.0 cm)

Source: Adapted from Brazil (2008a, 2008b).

Photo: Onildo Nunes de Jesus

SEW: Sepal width

Value: _____centimeters

1- Narrow (< 1.0 cm)

2- Medium (1.0 cm to 2.0 cm)

3- Wide (> 2.0 cm)

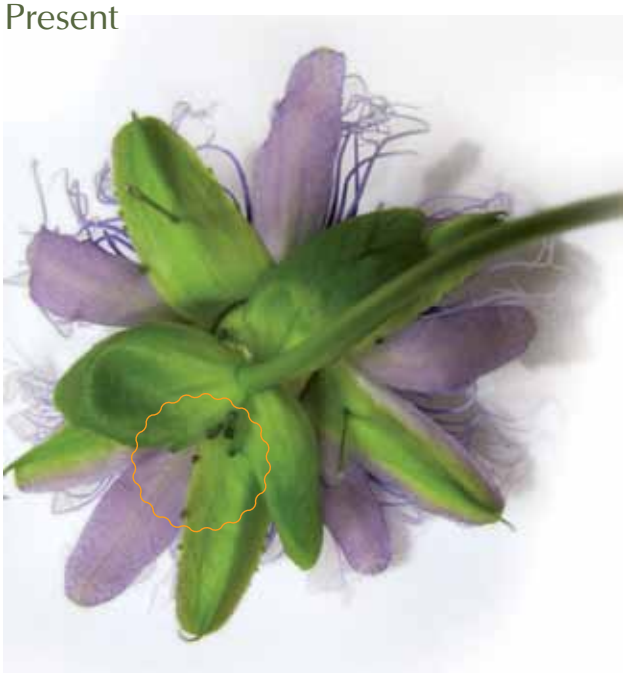
Photo: Onildo Nunes de Jesus

PNS: Presence of nectaries in the sepal

1. Absent

Photos: Onildo Nunes de Jesus

2. Present



NNS: Number of nectaries on the sepal

1- Absent
2- Few (1)
3- Medium (2 to 4)
4- High (> 4)

Photo: Onildo Nunes de Jesus

ANL: Androgynophore length

Value: _____centimeters

1- Very short (< 0.5 cm)
2- Short (0.5 cm to 1.0 cm)
3- Medium (> 1.0 cm to 2.0 cm)
4- Long (> 2.0 cm to 3.0 cm)
5- Very long (> 3.0 cm)

Photo: Onildo Nunes de Jesus

DTC: Diameter of the tip of the corona (fimbrias)

Value: _____centimeters

1- Very small (< 3.0 cm)

2- Small (3.0 cm to 6.0 cm)

3- Medium (> 6.0 cm to 9.0 cm)

4- Large (> 9.0 cm to 12.0 cm)

5- Very large (> 12.0 cm)

Source: Adapted from Brazil (2008a, 2008b).

Photo: Onildo Nunes de Jesus

BCF: Banding (rings of different colors, including white) in the longest corona filaments

1. Absent

Photos: Onildo Nunes de Jesus

2. Present

Source: Adapted from Brazil (2008a, 2008b).

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NCR: Number of colored rings (excluding white) on the corona filaments

1. Absent

Photos: Onildo Nunes de Jesus

2. One

3. More than one

Source: Adapted from Brazil (2008a, 2008b).

PCRF: Predominant color of the corona ring filaments (except white)

1. White

2. Pink

Photos: Onildo Nunes de Jesus (0 to 4 and 6) and Fabio Gelape Faleiro (5)

3. Purple

4. Green

5. Yellow

6. Red

LCR: Length of the corona filament rings

Value: _____centimeters

1- Absent (corona of only one color in all its extension)

2- Narrow (< 1.0 cm)

3- Medium (1.0 cm to 1.5 cm)

4- Large (> 1.5 cm)

Source: Adapted from Brazil (2008a, 2008b).

Photo: Onildo Nunes de Jesus

ODCC: Outer diameter of the corona cavity

Value: _____centimeters

Photo: Onildo Nunes de Jesus

IDCC: Inner diameter of the corona cavity

Value: _____centimeters

Photo: Onildo Nunes de Jesus

PEL: Petal length

Value: _____centimeters

1- Short (< 3.0 cm)

2- Medium (3.0 to 6.0 cm)

3- Long (> 6.0 cm)

Source: Adapted from Brazil (2008a, 2008b).

Photo: Onildo Nunes de Jesus

CEF: Color of the erect filament (operculum and/or corona)

1. Absent

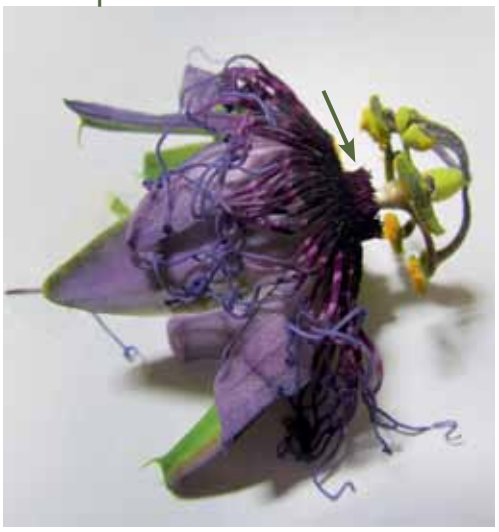
2. White

3. White + pink

Photos: Onildo Nunes de Jesus

4. White + purple

5. Purple



LCF: Longest corona filaments

1. Smooth

Photos: Onildo Nunes de Jesus

2. Wavy

Source: Adapted from Brazil (2008a, 2008b).

PAP: Predominant anthesis period

1. Morning

Photos: Onildo Nunes de Jesus

2. Afternoon

3. Night

Source: Adapted from Brazil (2008a, 2008b).

PCP: Predominant color on the perianth (petals and sepals) inner region

Photos: Onildo Nunes de Jesus (1; 3 to 8) and Fabio Gelape Faleiro (2)

1. White

2. Pinkish

3. Red

4. Purplish-red

5. Purple

6. Purplish-blue

7. Blue

8. Others

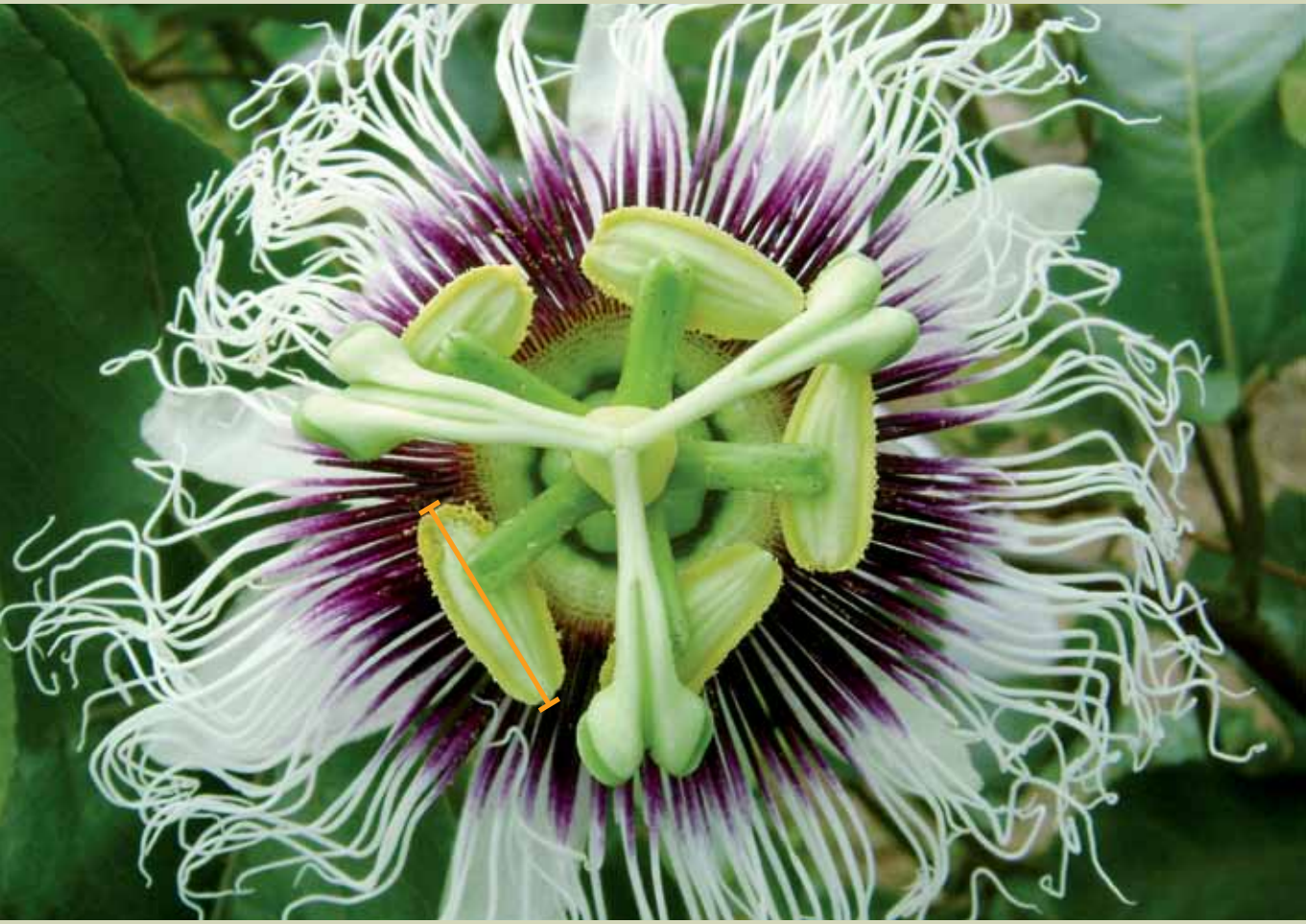
PDL: Pedicel length

Value: _____centimeters

1- Short (< 2.0 cm)
2- Medium (2.0 cm to 4.0 cm)
3- Long (> 4.0 cm)

Photo: Onildo Nunes de Jesus

ANL: Anther length

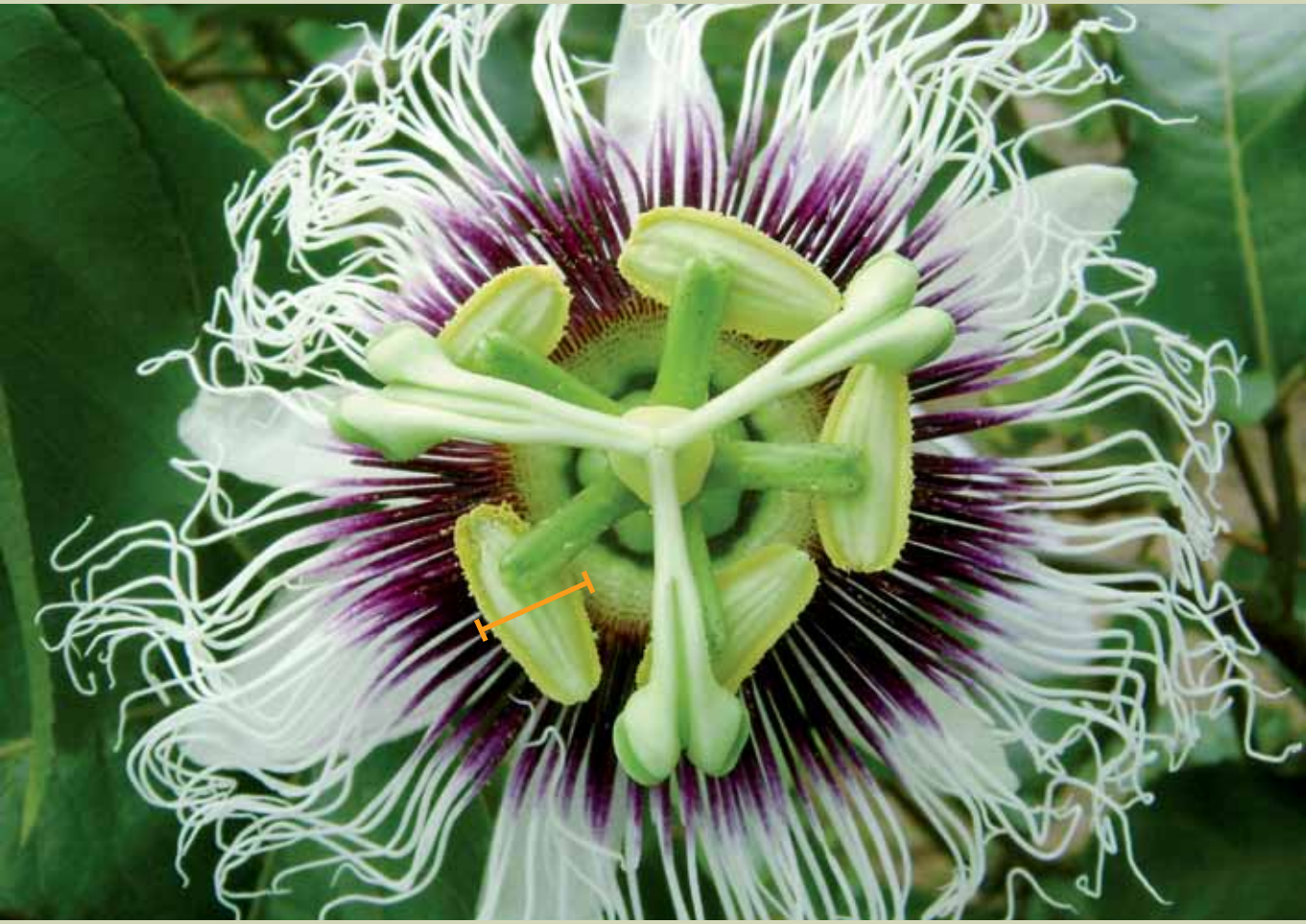


Value: _____centimeters

1- Short (< 0.5 cm)
2- Medium (0.5 cm to 1.0 cm)
3- Long (> 1.0 cm)

Photo: Onildo Nunes de Jesus

ANW: Anther width



Value: _____millimeters

1- Narrow (< 1.5 mm)
2- Medium (1.5 mm to 3.0 mm)
3- Large (> 3.0 mm)

Photo: Onildo Nunes de Jesus

OVL: Ovary length

Value: _____millimeters

1- Short (< 4.0 mm)
2- Medium (4.0 mm to 8.0 mm)
3- Long (> 8.0 mm)

Photo: Onildo Nunes de Jesus

OVD: Ovary diameter

Value: _____millimeters

1- Narrow (< 1.5 mm)
2- Medium (1.5 mm to 3.0 mm)
3- Large (> 3.0 mm)

Photo: Onildo Nunes de Jesus

PAF: Presence of anthocyanin on the fillet

1. Absent

Photos: Onildo Nunes de Jesus

2. Few dots

3. Many dots

PAS: Presence of anthocyanin on the style

1. Absent

Photos: Onildo Nunes de Jesus

2. Few dots

3. Many dots

PAA: Presence of anthocyanin on the back of the anther

1. Absent

Photos: Onildo Nunes de Jesus

2. Few dots

3. Many dots

PAG: Presence of anthocyanins on the androgynophore

1. Absent

Photos: Onildo Nunes de Jesus

2. Few dots

3. Many dots

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Onildo Nunes de Jesus
Fábio Gelape Faleiro
Taliane Leila Soares
Eder Jorge de Oliveira
Eduardo Augusto Girardi

Fruit descriptors

If the plant does not present the characteristic under evaluation assign zero to the descriptor and specify the reason.

1 **Peduncle**
2 **Epicarp (peel)**

1 **Fleshy aril**
2 **Seed**
3 **Mesocarp**
4 **Endocarp (pulp)**
5 **Epicarp (peel)**

Photos: Onildo Nunes de Jesus

Figure 6. Parts of the fruit of *Passiflora edulis* Sims.
Source: Adapted from Programa... (2009).

CPF: Color of the peel (epicarp)

Photos: Onildo Nunes de Jesus

1. Green

2. Greenish yellow

3. Yellow

4. Orange

5. Pinkish

6. Reddish-orange

7. Red

8. Purple

FSH: Fruit shape

1. Oval

2. Oblong

3. Round

Photos: Onildo Nunes de Jesus

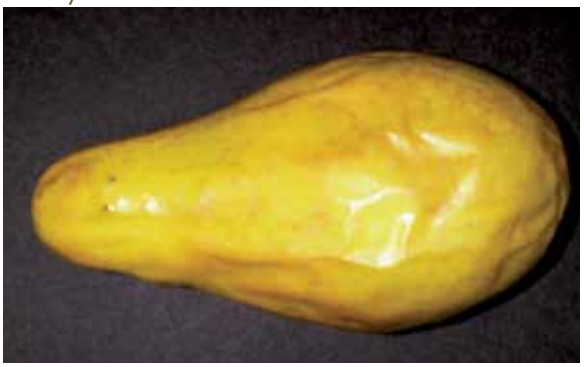
4. Oblate

5. Ellipsoid

6. Fusiform

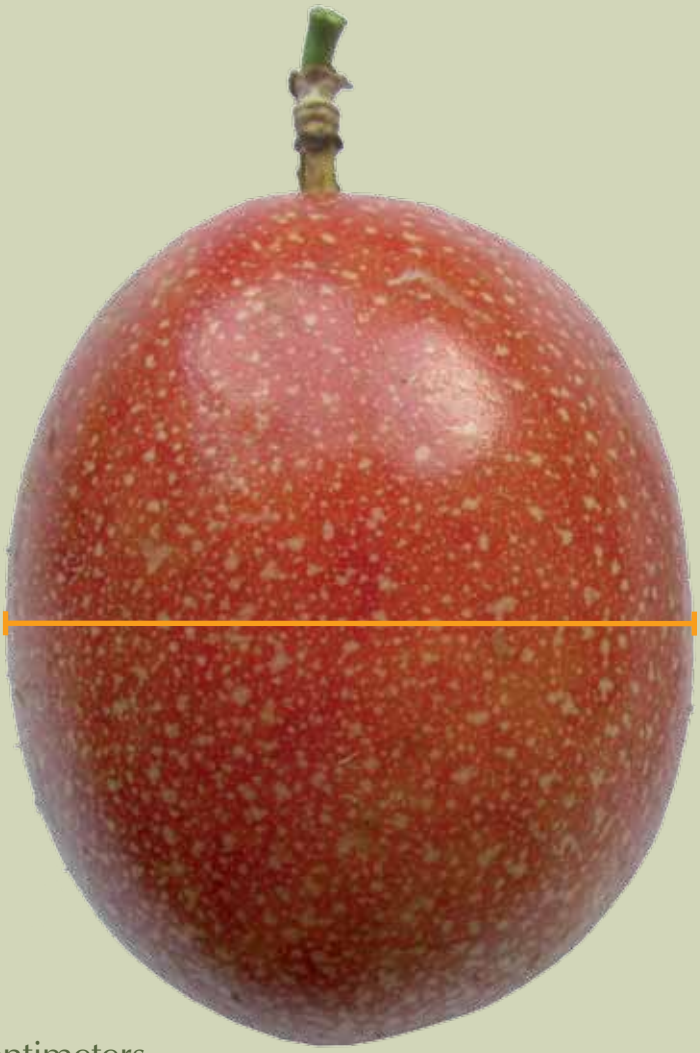
7. Obovate

8. Pyriform



Source: Adapted from Brazil (2008a, 2008b).

FDI: Fruit diameter or Transverse diameter

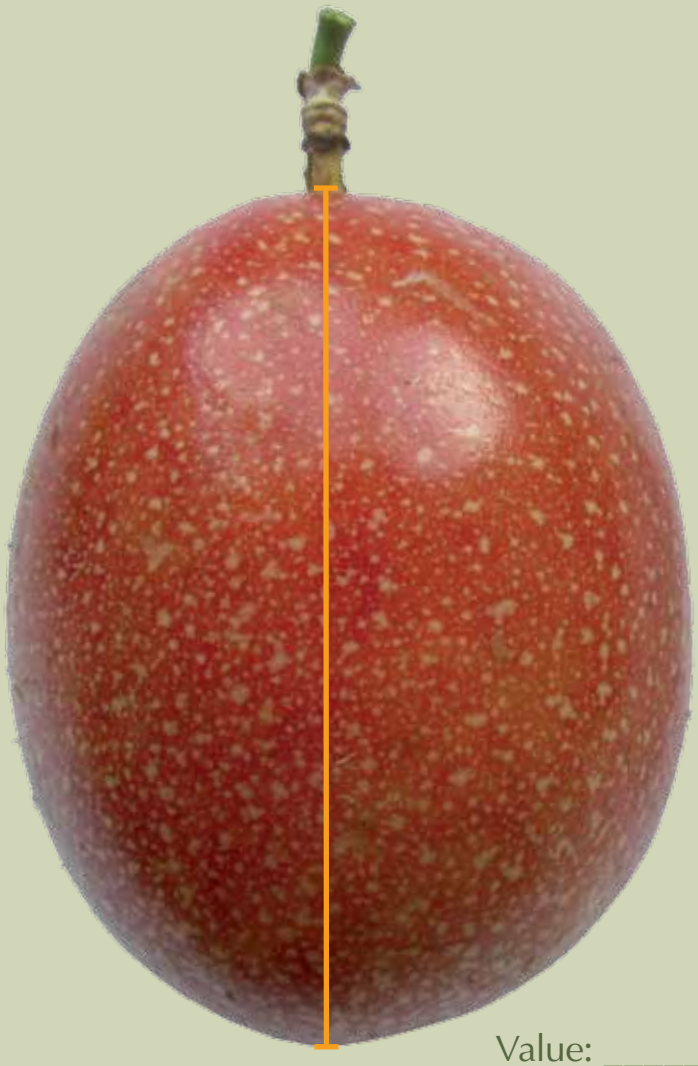


Value: _____centimeters

1- Very short (< 2.5 cm)
2- Short (2.5 cm to 5.0 cm)
3- Medium (> 5.0 cm to 10.0 cm)
4- Long (> 10.0 cm to 15.0 cm)
5- Very long (> 15.0 cm)

Photo: Onildo Nunes de Jesus

FLE: Fruit length or Longitudinal diameter



Value: _____centimeters

1- Very narrow (< 2.5 cm)
2- Narrow (2.5 cm to 5.0 cm)
3- Medium (> 5.0 cm to 10.0 cm)
4- Large (> 10.0 cm to 15.0 cm)
5- Very large (> 15.0 cm)

Photo: Onildo Nunes de Jesus

Calipers are used to measure the passion fruit length, diameter and peel thickness (Figure 7).

Figure 7. Calipers used for morphometric evaluation of passion fruit.

Photo: Onildo Nunes de Jesus

PET: Peel thickness

After cutting the fruit and removing the pulp, measure the thickness of the peel with calipers.

Value: _____centimeters

1- Very thin (< 0.3 cm)
2- Thin (0.3 to 0.6 cm)
3- Medium (> 0.6 to 1.0 cm)
4- Thick (> 1.0 to 1.5 cm)
5- Very thick (> 1.5 cm)

Source: Adapted from Brazil (2008a, 2008b).

Photo: Onildo Nunes de Jesus

RLT: Ratio of the longitudinal diameter and transverse diameter of fruits

The shape index (R) is the ratio of the longitudinal and equatorial (transverse) diameter of the fruit (SILVA JÚNIOR et al., 2010).

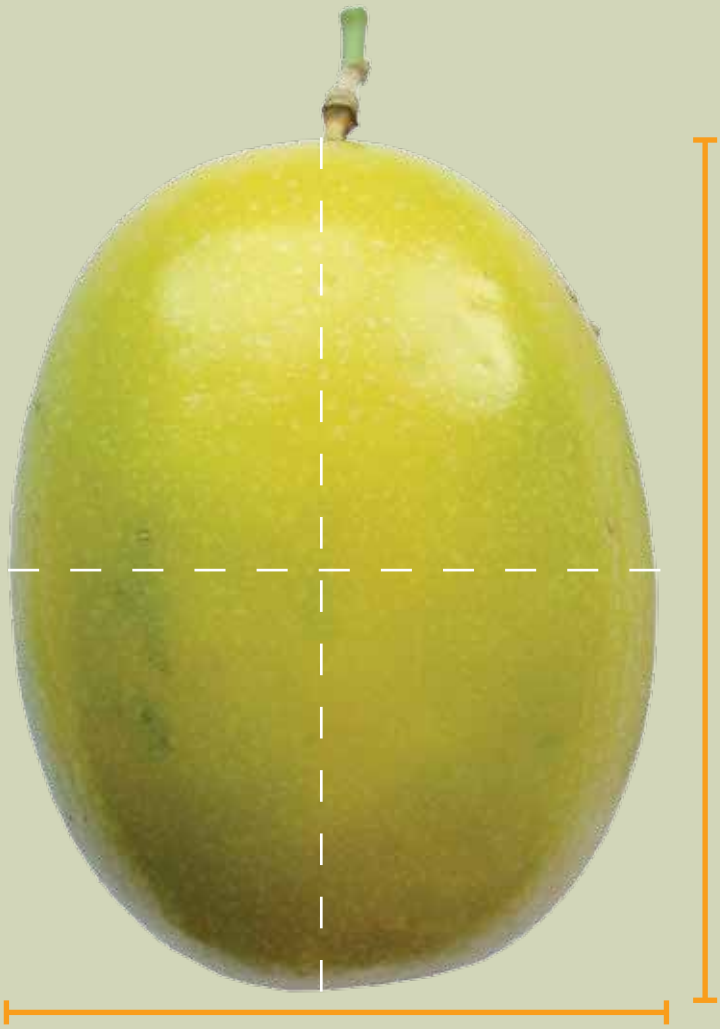
R = longitudinal diameter/transverse diameter =FLE/FDI

Photos: Onildo Nunes de Jesus

Ratio < 1.0

Ratio = 1.0

Ratio > 1.0



Value: _____centimeters

1- Very small (≤ 0.9 cm)

2- Small (> 0.9 to 1.2 cm)

3- Medium (> 1.2 to 1.5 cm)

4- Large (> 1.5 to 1.8 cm)

5- Very large (> 1.8 cm)

Source: Adapted from BRAZIL (2008a, 2008b).

Photo: Onildo Nunes de Jesus

CCF: Classification (caliber) of commercial fruits (*Passiflora edulis* Sims)

	Fruit classification	Equatorial diameter (cm)
1	First	Equal to or less than 5.5 cm
2	1B	Greater than 5.5 to 6.5 cm
3	1A	Greater than 6.5 to 7.5 cm
4	2A	Greater than 7.5 to 8.5 cm
5	3A	Greater than 8.5 cm

Source: Oliveira (2001), Programa... (2009) and Rangel (2002).

EHS: Evaluation of hybrid segregation (only for *P. edulis*)

This descriptor was prepared solely for the evaluation of *P. edulis*. It is used to assess the uniformity of the peel color in accessions of germplasm banks or in hybrids developed by genetic improvement programs. Alternatively, the number of plants per plot having purple or yellow peel can be counted.

1. 100% yellow

2. 90% yellow + 10% purple

Photos: Onildo Nunes de Jesus

3. 50% yellow + 50% purple

4. 70% yellow + 30% purple

Photos: Onildo Nunes de Jesus

5. 30% yellow + 70% purple

6. 100% purple

Photos: Onildo Nunes de Jesus

FRW: Fruit weight

Value: _____grams

1- Very low (< 50 g)
2- Low (50 g to 150 g)
3- Medium (> 150 g to 250 g)
4- High (> 250 g to 350 g)
5- Very high (> 350 g)

Photo: Onildo Nunes de Jesus

PEW: Peel weight

Photo: Onildo Nunes de Jesus

Peel weight per fruit (g)
Value: _____grams

SEW: Peel weight

Photo: Onildo Nunes de Jesus

Total weight of seeds per fruit (g)
Value: _____grams

NSF: Number of seeds per fruit

The number of seeds (Ns) is calculated based on the known weight (g) of 100 seeds (W_{100}) and the total seed weight (g) of a fruit (Wt).

$$N_s = \frac{SEW\,x\,100}{W_{100}}$$

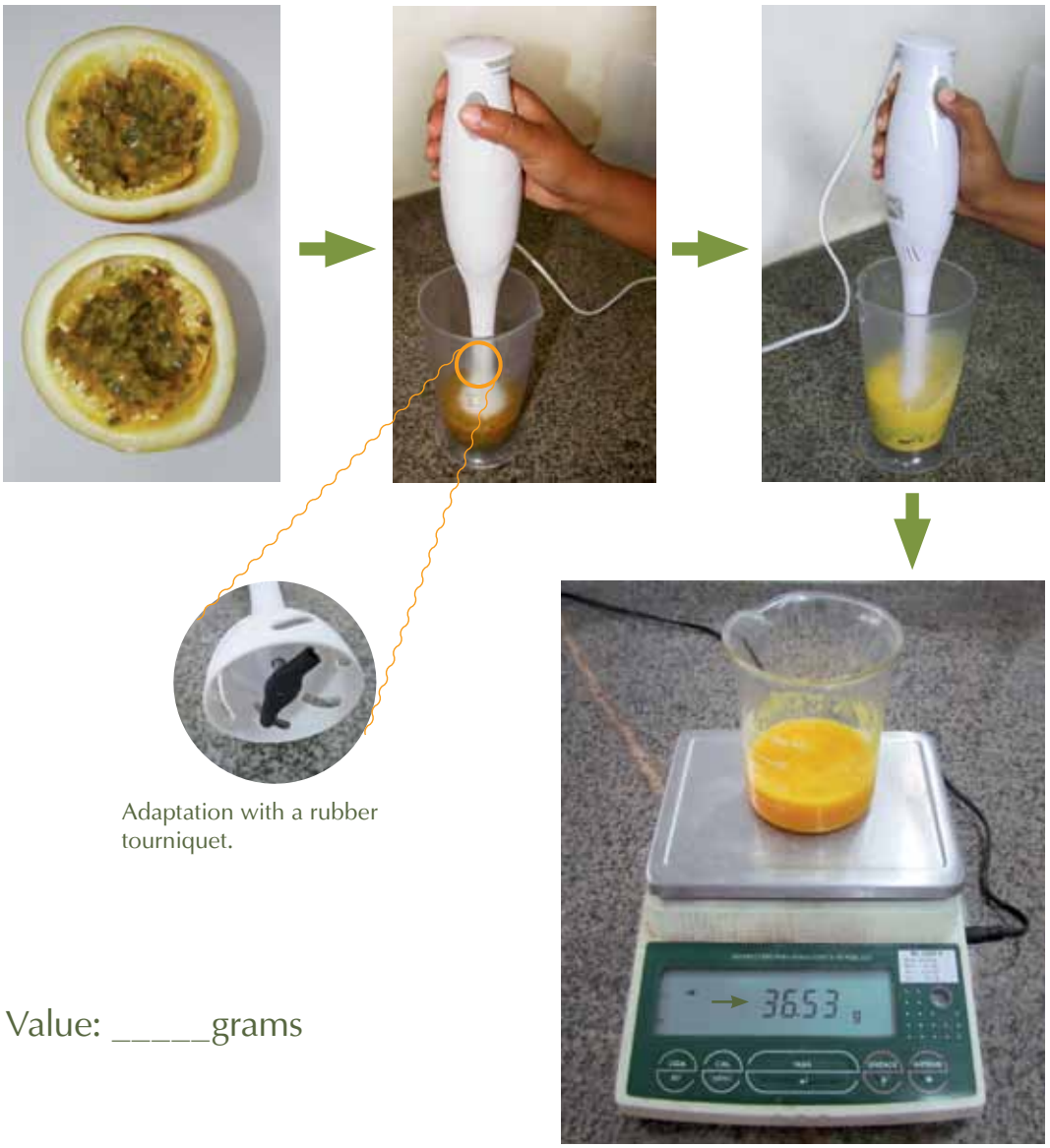
1- Very small (< 50)
2- Small (50 to 100)
3- Medium (> 100 to 200)
4- Large (> 200 to 400)
5- Very large (> 400)

Source: Brazil (2008a, 2008b).

PUW: Pulp weight

Pulp weight is based on the weight of the pulp (g) without the seeds. The pulp extraction is performed with the aid of a household mixer. The blade of the mixer is covered with a rubber tourniquet to avoid breaking the seeds. Broken seeds can release substances that alter the analysis of soluble solids (SS) and titratable acidity (TA).

Photos: Onildo Nunes de Jesus



Adaptation with a rubber tourniquet.

Value: _____grams

CPU: Color of the pulp

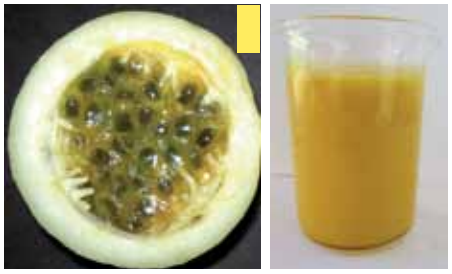
1. Whitish

Photos: Onildo Nunes de Jesus

2. Greenish-yellow

Photos: Onildo Nunes de Jesus

3. Yellow



4. Light orange

5. Dark orange

Photos: Onildo Nunes de Jesus

6. Reddish-orange

Photos: Onildo Nunes de Jesus

7. Purple

8. Other

JUY: Pulp yield

Pulp yield should be determined by the ratio of the pulp weight (g) and fruit weight (g):

$$\text{Pulp yield (\%)} = \frac{\text{Pulp weight (PUW) + seeds}}{\text{Fruit weight (FRW)}} \times 100$$

Alternatively, pulp yield can be calculated per hectare, using the formula by Hafle et al. (2009); Neves et al. (2014):

$$\text{Juice yield (t ha}^{-1}\text{)} = \text{Fruit yield (t ha}^{-1}\text{) x pulp yield (absolute number)}$$

In some articles, the yield is calculated based on the ratio of pulp weight (arils with seeds) and fruit weight. In this case, the yield is obtained by considering the seeds plus the arils:

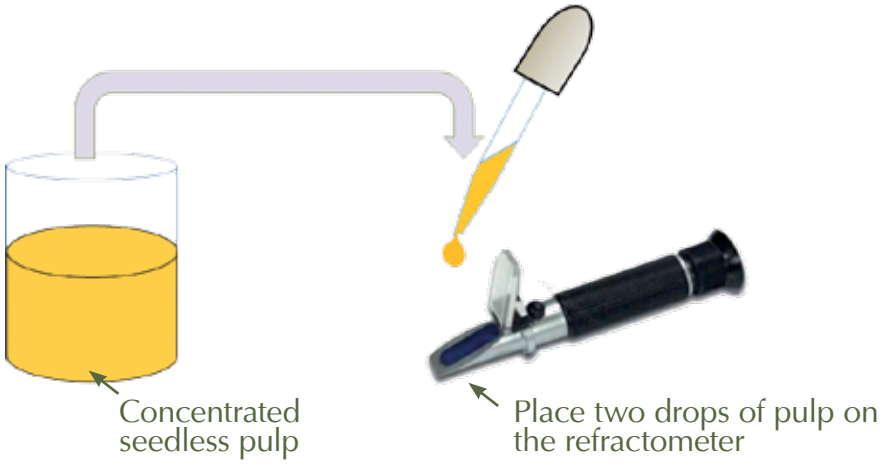
$$\text{Pulp + seed yield (\%)} = \frac{\text{Pulp weight (arils with seeds)}}{\text{Fruit weight (FRW)}} \times 100$$

SS: Soluble solids

The evaluation of soluble solids (SS) is an indicator of the quality of the fruits because it is a measure of the concentration of soluble sugars in the sample. It is an estimate that reflects the quality of fruits for industrialization. Fruits with soluble solids levels above 13°Brix are desirable (BRUCKNER et al., 2002). The content of soluble solids is obtained by direct reading on a manual refractometer, with the results expressed in °Brix.

1- Very low (< 7° Brix)
2- Low (7° Brix to 10° Brix)
3- Medium (> 10° Brix to 13° Brix)
4- High (> 13° Brix to 17° Brix)
5- Very high (> 17° Brix)

Source: Adapted from Brazil (2008a, 2008b).



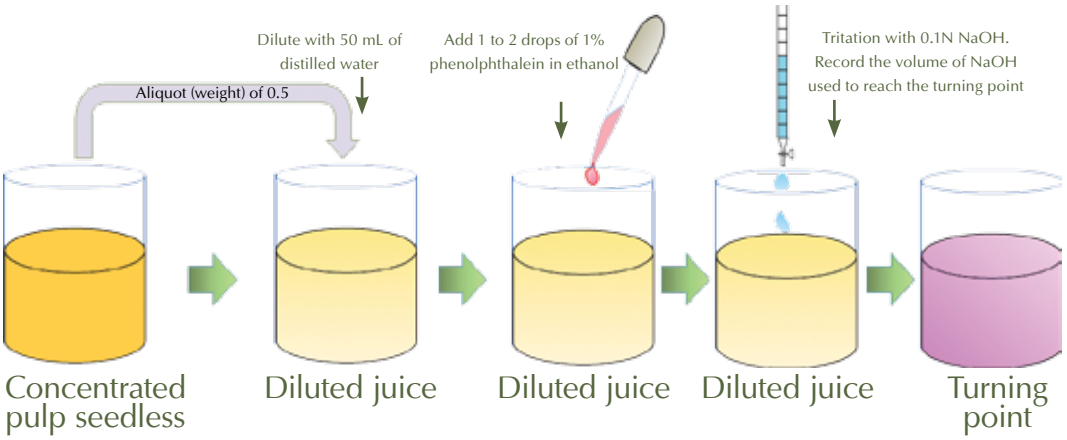
TA: Titratable acidity

Analysis of the acidity of passion fruit is of great importance. For the processing industry, fruits with higher acidity are preferred, as they avoid the addition of acidifying agents to the juice (NASCIMENTO, 1996). For fresh consumption, sweeter and less acidic fruits are more desirable (CAVICHIOLI et al., 2011).

Acidity is determined by the amount of acid in a sample that reacts with a base of known concentration. In the titration procedure, phenolphthalein solution is used to indicate the turning point. In passion fruit, there is a predominance of citric acid ($C_6H_8O_7$), and the determination of fruit acidity is based on this acid. The acidity of citric acid is due to the presence of a carboxylic group (-COOH). The titratable acidity is determined by titration with 0.1 N NaOH, and it is expressed as the percentage of citric acid.

Molar mass of citric acid: $C_6H_8O_7$ = 6 x 12 + 1 x 8 + 7 x 16 = ~192.2g mol^{-1}

General procedure



Formulas for determination of citric acid (%)

Volume (mL) of NaOH (0.1N) used; Corrected normality of the NaOH titration solution; Gram equivalent of citric acid (64.066)

$$\text{Citric acid} = \frac{\text{NaOH} \times N_c \times E_q \times 100}{w \times 1000}$$

w: Pulp mass for tritation with NaOH

$$N_c = f \times 0.1$$

Where

$$f = \frac{0.5}{0.2042 \times \overline{mL} \times 0.1}$$

$\overline{mL}$: Mean volume (mL) used to titrate (0.1N NaOH) five samples of a solution of 0.5 g of potassium biphthalate ($KHC_8H_4O_4$) in 40 mL water

$$\text{Mean citric acid (\%)} = \frac{x_1 + x_2}{2}$$

that is, the mean of the two citric acid estimates in samples 1 and 2 of the passion fruit juice analyzed.

SS/TA: Ratio

This is the ratio of soluble solids (SS) to total titratable acidity (TA). This ratio is important because the taste of the fruit is associated with the ratio of the sweet and acidic components. The value of this ratio decreases with fruit ripening because there is an accumulation of sugars in the fruit.

$$\text{Ratio} = \frac{SS}{TA}$$

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Eder Jorge de Oliveira
Fábio Gelape Faleiro
Onildo Nunes de Jesus

Descriptors for disease evaluation

EVLF: Evaluation of fruit woodiness virus infection in leaves under field conditions

The scale shown below is used to evaluate 20 leaves collected from young branches, with 10 leaves from one side of the plant and 10 from the other side. The evaluation can be made every four months, from plants with formed tertiary branches (curtain). Ideally, the evaluation is made on individual plants; however, in some situations in which a large number of plants is evaluated, leaf samples from plants in the plots will give an idea of the severity of the disease in a given accession or genotype.

1. Leaf without mosaic symptoms.

1 **R: Resistant**

Photo: Onildo Nunes de Jesus

2. Leaf showing mild mosaic and without leaf deformation.

3. Leaf with severe mosaic, blisters and leaf deformation.

4. Leaf showing severe mosaic, blisters and deformation on the leaf surface.

Sources: Novaes and Rezende et al. (1999); Junqueira et al., (2003); Fonseca (2008).

MS: Mildly resistant 2

S: Susceptible 3

HS: Highly susceptible 4

Photos: Onildo Nunes de Jesus

EVPF: Evaluation of fruit woodiness virus infection in plants under field conditions

This evaluation is based on an analysis of the plant as a whole (overview), adapted from a scale for leaves (FONSECA, 2008; JUNQUEIRA et al. 2003; NOVAES; REZENDE et al. 1999).

1. Plants with no symptoms

2. Plants with mild mosaic symptoms, without wrinkling and blistering on the leaves

Photos: Onildo Nunes de Jesus

3. Plants with mosaic symptoms, wrinkling and mild blistering

4. Plants with mosaic symptoms, severe wrinkling and blistering

1 R: Resistant **2 MS: Mildly resistant**

3 S: Susceptible **4 HS: Highly susceptible**

EVLG: Evaluation of fruit woodiness virus infection in leaves under greenhouse conditions

Inoculation of the virus (CABMV) in *Passiflora* is based on the methodology proposed by Pinto et al. (2008). The seedlings in the greenhouse must be mechanically inoculated at 120 days of age using extracts prepared from leaf samples collected from sour passion fruit plants showing symptoms of the disease (hardening of the fruits). The inoculum for the mechanical transmission of the virus should be prepared by macerating the infected leaf material at a ratio of 1 g of tissue (leaf) per 10 mL of 0.1 M sodium phosphate buffer solution, pH 7.0. Next, a small amount of "celite" (abrasive) is added to the extract. The virus must be inoculated by rubbing the upper surfaces of the leaves, with the finger wetted with the extract, five consecutive times to standardize the inoculum pressure. Three leaves per plant should be inoculated, and the youngest leaves should be selected. After 10 minutes of inoculation, the plants should be washed to avoid burning the leaves with the abrasive. After 20 days post-inoculation, 150 days after seeding, an assessment can be made of the incidence and severity of the virus by observing the symptoms in the leaves located above the inoculated leaf. The grading scale used to evaluate the virus in greenhouse conditions is the same for evaluation under field conditions.

EVFF: Evaluation of fruit woodiness virus infection in fruits under field conditions

This evaluation should be performed at the peak of passion fruit production. The fruits are evaluated on the plant, or alternatively, samples are collected from the plot (5-10 fruits) and evaluated in the laboratory according the following grading scale.

1. Normal fruit without deformation

2. Slightly deformed fruit, with or without blemishes

Photos: Onildo Nunes de Jesus

3. Totally deformed fruit with blemishes and darkening

EAFF: Evaluation of anthracnose (*Colletotrichum gloeosporioides* Penz.) in fruits under field conditions

1. Fruits without symptoms

2. Fruits with 10% of the surface covered

Photos: Eder Jorge de Oliveira

3. Fruit with 11 to 30% of the surface covered

4. Fruits with more than 30% of the surface covered

Scale adapted (with photos) from Junqueira et al., (2003).

1 **R: Resistant** **2** **MS: Mildly resistant**

3 **S: Susceptible** **4** **HS: Highly susceptible**

EALG: Evaluation of resistance to anthracnose in leaves under the controlled greenhouse conditions

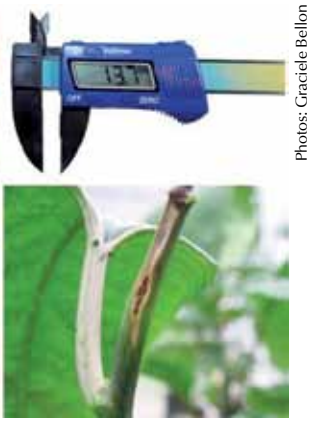
The isolates of *C. gloeosporioides* should be obtained from leaves and fruits according to the protocol established by Amorim and Salgado (1995). The isolation should be performed in Petri dishes containing PDA (Potato-Dextrose-Agar) culture medium with 250 mg L^{-1} of chloramphenicol. The dishes should be incubated in a BOD (biochemical oxygen demand) growth chamber under a photoperiod of 12 h, with an average temperature of 26°C. The conidia suspensions should be prepared from pure sporulated cultures incubated for 15 days by adding 20 mL of 0.1% Tween 80 to the dishes and the surface of the spore colony should be scraped with the aid of a soft brush. The suspension obtained should be filtered through double sterile gauze and, with the aid of a hemacytometer, the concentration of the suspensions should be adjusted to 1 x 10^6 conidia mL^{-1}. For the production of mycelial agar disks, a 4 mm diameter cork borer is used to remove disks of pure cultures of the fungus pathogen, and the disks incubated for 15 days on PDA.

Sowing should be conducted in polystyrene trays with 72 cells (120 mL per cell), and the trays should be filled with commercial substrate. After 20-30 days of germination, the seedlings should be transferred to 250 mL plastic cups or bags that are filled with commercial substrate. Statistical design should be used for the analysis of variance because the evaluations will be based on quantitative data. Two to three months after transplanting, the fungus should be inoculated at an estimated concentration of 5 x 10^6 mL^{-1}. The inoculations should be performed by drilling the first internode, located after the shoot apex of the seedlings, using 15 fine sewing needles, fixed in a rectangular bar that was previously immersed in conidia suspension. The inoculated plants should be kept in a moist chamber (> 80% RH) during the evaluation period. The evaluation of the severity of anthracnose is performed by using digital calipers to measure the length of the lesion starting on the 10th day after inoculation.

The method proposed by Dutra (2008) should be used for inoculation of *C. gloeosporioides* on fruits. First, the fruits are sterilized in 10% ethanol for

one minute. Then, they are treated with 10% sodium hypochlorite for one minute, followed by washing in sterile distilled water for one minute. After sterilization, the fruit should be punctured to a depth of 2 mm using a sterilized Philips-head screwdriver on the equatorial region at four equidistant points. Subsequently, 50 mL of conidia suspension is applied to each lesion. Inoculated fruits should be incubated for 3 days in a moist chamber, with a photoperiod of 12 h at 25°C.

Photo: Fábio Gelape Faleiro



Photos: Graciele Bellon

Inoculation of the fungus on the leaves should be performed 80 days after transplanting, when the plants have seven to nine leaves (Martins et al., 2008). Medium aged leaves should be perforated with the help of a thin steel bristle brush. Then, a suspension containing 5 x 10^6 of the isolate should be inoculated on the adaxial and abaxial surfaces of the leaf. After inoculation, the plants should be kept in a moist chamber until the end of the experiment.

ECFF: Evaluation of cladosporiosis or scab (*Cladosporium herbarum* Link.) on the fruit under field conditions

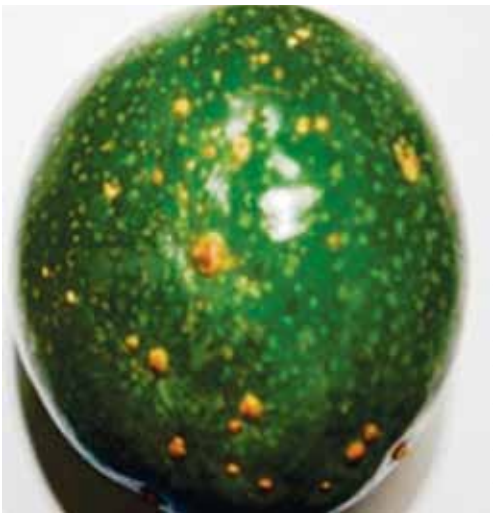
1. Fruit without symptoms

2. Fruits with 10% of the surface covered

Photos: Eder Jorge de Oliveira

3. Fruit with 11 to 30% of the surface covered

4. Fruits with more than 30% of the surface covered



Scale adapted (with photos) from Junqueira et al., (2003).

1 R: Resistant **2 MS: Mildly resistant**

3 S: Susceptible **4 HS: Highly susceptible**

ECBF: Evaluation of cladosporiosis or scab (*Cladosporium herbarum* Link.) on the branches under field conditions

This evaluation should be made using two nodes on the branches with incidence of disease (FREITAS et al., 2012; JUNQUEIRA et al., 2003; OLIVEIRA et al., 2013).

1. No symptoms

2. 0 to 10% of the branch surface covered with lesions

3. Up 10% to 30% of the branch surface covered with lesions

4. Up to 30% of the branch surface covered with lesions

Photos: Eder Jorge de Oliveira

Scale adapted (with photos) from Junqueira et al. (2003).

1 **R: Resistant** 2 **MS: Mildly resistant**

3 **S: Susceptible** 4 **HS: Highly susceptible**

ECLG: Evaluation of cladosporiosis or scab (*Cladosporium herbarum* Link.) in leaves under greenhouse conditions

Inoculation of the fungus *Cladosporium herbarum* is based on the method described by Faria et al. (2008). The fungus should be inoculated onto PDA (Poaia-de-mato-grosso) medium in Petri dishes until preparation of the inoculum and kept in a BOD chamber at 25°C. A spore suspension at a concentration of $1x10^6$ should be applied by spraying the entire plant, including the abaxial and adaxial surfaces of the leaves. After inoculation, all plants should be observed daily.

EBFF: Evaluation of bacterial blight (*Xanthomonas campestris* pv. *passiflorae*) in fruits under field conditions

Symptoms	Class
1. Fruits without symptoms	R: Resistant
2. Fruits with up to 10% of the surface covered	MR: Mildly resistant
3. Fruits with 11 to 30% of the surface covered	S: Susceptible
4. Fruits with more than 30% of the surface covered	HS: Highly susceptible

Source: Junqueira et al. (2003).

EBLF: Evaluation of resistance to bacterial blight in leaves under field conditions (adapted from Bellon, 2008)

For evaluation of bacterial infection under field conditions, six leaves per plant must be analyzed (three on each side of the trellis). The leaves are randomly selected, and the following characteristics should be evaluated: mean number of lesions per leaf; mean diameter of lesions; mean lesion area per leaf; percentage of healthy leaves and percentage of diseased leaves. The mean diameter of the lesions can be estimated based on a cm^2 diameter scale (0.5, 1.0, 1.5, 2.0, 3.0, 5.0), which is drawn on a transparency sheet and is superimposed on the lesions (figure below). The mean diseased area per leaf is calculated based on the estimated mean diameter and number of lesions.

Photo: Fábio Gelape Faleiro

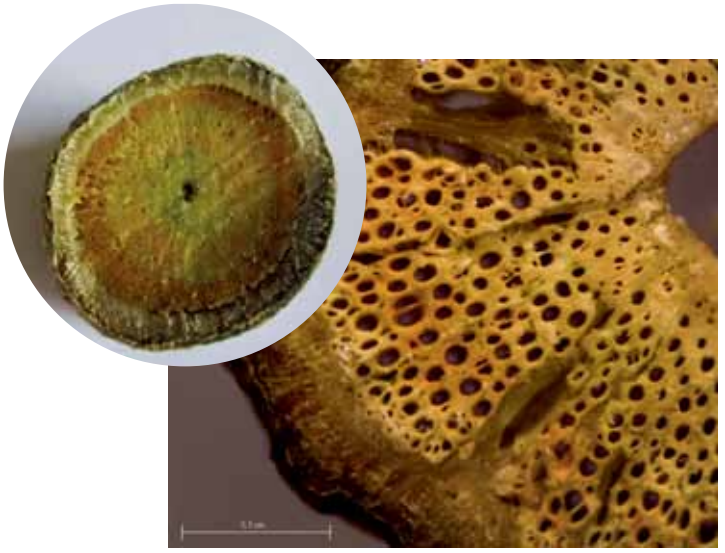
EBLG: Evaluation of resistance to bacterial blight in leaves under controlled greenhouse conditions

Sowing should be performed in polystyrene trays with 72 cells (120 mL per cell). The trays should be filled with commercial substrate. After 20-30 days of germination, the seedlings should be transferred to 250 mL plastic cups or bags filled with commercial substrate. Statistical design should be used for the analysis of variance because the evaluations will be based on quantitative data. Two to three months after transplantation, the bacteria should be inoculated at

an estimated concentration of 10^8 cfu mL^{-1}. The colonies will be obtained from pure colonies grown on nutrient agar (3 g beef extract, 8 g peptone, 15 g agar and 1,000 mL distilled water, pH 7.0) for 48 hours. Two fully developed leaves from each plant should be inoculated. In the middle of the leaf, laterally to the midrib, two holes of 3 mm in diameter should be made with a Pasteur pipette (an adapted leather puncher can be used), which must be immersed in the bacterial suspension before each punch. After inoculation, the plants should be kept in a moist chamber (> 90% RH) until the time of evaluation. Evaluate the mean longitudinal diameter, median transverse diameter, mean lesion diameter and mean lesion area, 10 to 15 days after inoculation with the aid of digital calipers (BELLON, 2008; NATAKANI et al., 2009).

EFWF: Evaluation of fusarium wilt of passion fruit under field conditions

This evaluation entails counting the number of plants in the field killed by fusariosis over time (daily or weekly assessment). Plants for which leaf wilting has begun are considered dead because once the disease starts, it cannot be stopped.



Cross-section of stem infected by *Fusarium.*

Daily record of number of plants that have started to wilt.

Photos: Onildo Nunes de Jesus

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Other descriptors used in the characterization of passion fruit

MOD: Molecular descriptors

A complementary alternative to the morphological descriptors is the development of molecular marker that can accurately identify the genetic variants present in the DNA of an organism. Several types of markers have been tested on *Passiflora*, such as markers based on microsatellites or SSR (Simple Sequence Repeats), ISSR (Inter Simple Sequence Repeats) and RAPD (Random Amplified Polymorphic DNA) (BELLON et al., 2007; BELLON et al., 2008; CERQUEIRA-SILVA et al., 2012; OLIVEIRA et al., 2005; OLIVEIRA et al., 2013; REIS et al., 2011; SANTOS et al., 2011). Amplification protocols for ISSR, SSR and RAPD markers are described in the following table:

Reaction conditions		
ISSR (Santos et al., 2011)	**SSR (Cerqueira-Silva et al., 2012)**	**RAPD (Bellon et al., 2007)**
25ng DNA	15 ng de DNA	15 ng de DNA
1× PCR buffer[1]	1× PCR buffer[1]	1× PCR buffer[1]
1.5 mM de $MgCl_2$	1.5 mM $MgCl_2$	3 mM de $MgCl_2$
0.2 mM de DNTP	0.2 µM	100 uM de cada DNTP
0.3 uM de primer	0.5 µM	0.4 uM do primer
1 U Taq Polimerase	1 U Taq Polimerase	1 U Taq Polimerase
Final volume: 25 uL	**Final volume: 15 µL**	**Final volume: 13 µL**

Standard amplification program					
4 min at 94 °C	1 cycle	5 min at 94 °C	1 cycle	15 sec at 94 °C	
40 sec at 94 °C		1 min at 95 °C		30 sec at 35 °C	40 cycles
40 sec at 48 °C or 52 °C	35 cycles	1 min at 60 °C	34 cycles	90 sec at 72 °C	
2 min at 72 °C		1 min at 72 °C		6 min a 72 °C	1 cycle
2 min at 72 °C	1 cycle	10 min at 72 °C	1 cycle	4 °C	Final
4 °C	Final	4 °C	Final		
Agarose gel 3,0 %		**Agarose gel (3.0%)/denaturing acrylamide gel (6.0%)**		**Agarose gel 1.5%**	

[1](20 mM Tris HCl [pH 8.4] and 50 mM KCl)

SED: Seed descriptors

Although seed characterization has no direct economic importance, seed size is a characteristic related to the performance of the seed and the resulting plant by means of stored reserves. The key seed descriptors are as follows:

- Seed weight: eight replicates of 100 seeds from "Pure Seed" (Brazil, 2009) should be weighed. Seeds from each replicate should be weighed on an analytical scale that is accurate to three decimal places (Figure 8).

- Seed color: seed color determination (Figure 9) should be performed using a digital colorimeter (Chroma Meter CR-400/410), which allows non-subjective evaluation of the progeny using the Munsell color chart (1976).

- Longitudinal diameter, transverse diameter and thickness of the seed: these data should be collected with digital calipers (Figure 10).

Photos: Alexandre Pio Viana

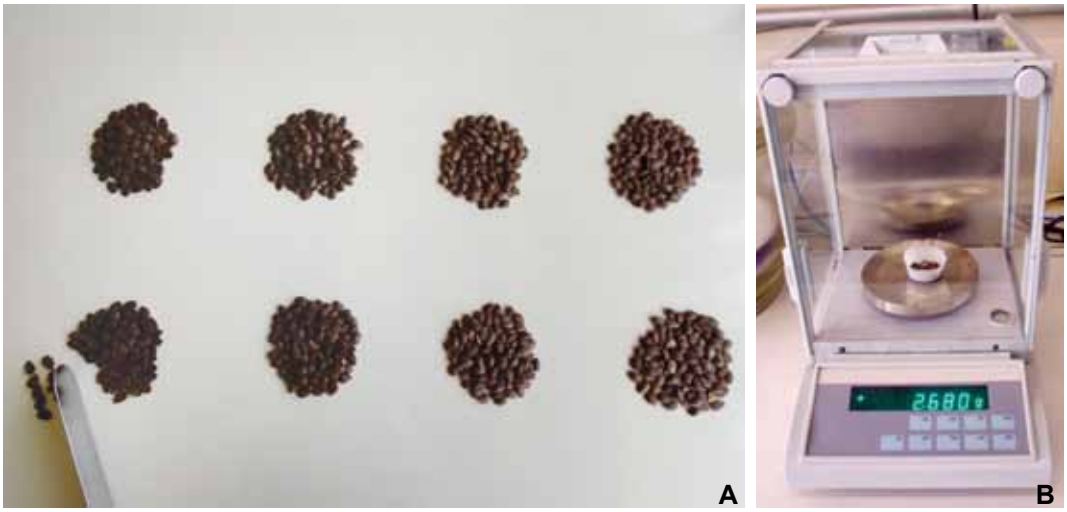

Figure 8. Determination of the weight of 100 seeds. A – seed replicates; B – weighing on an analytical scale.

Photo: Alexandre Pio Viana

Figure 9. Determination of the color of the seeds using a digital colorimeter.

Photos: Alexandre Pio Viana

Figure 10. Measurement of sour passion fruit seeds. A – longitudinal diameter; B – transverse diameter; C – thickness.

The evaluation of progeny has shown that there are significant variations in some proposed descriptors for the assessment of seeds. The differences in seed size affect the vigor test; however, these differences do not affect the final germination of the seeds.

EPV: Evaluation of pollen viability

There are several methods that may be implemented in pollen viability testing. Alexander's Triple Solution (Alexander, 1969) is one of the most used tests due to ease of distinguishing between viable from nonviable pollen grains. For this analysis, flower buds should be collected at anthesis, placed in 70% ethanol and stored in the refrigerator. During preparation of the slides, the anthers are macerated in drops of dye to release the pollen grains. Subsequently, the slides are observed under an optical microscope, and the pollen grains are counted and classified as viable (reddish-purple staining) or nonviable (bluish-green staining) (Figure 11). Typically, at least 100 pollen grains per slide should be counted, and at least three slides per genotype should be prepared. If the percentage of viable pollen grains is low, more collections and more slides should be prepared. It is expected that an accession with good pollen viability has a percentage equal to or greater than 90%.

Photo: Taliane Leila Soares

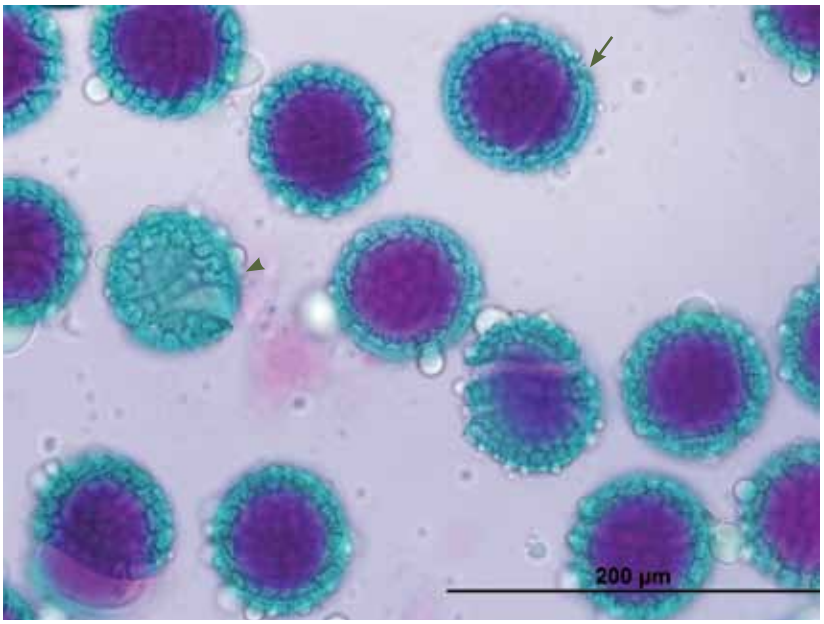

Figure 11. Pollen grains of sour passion fruit stained with Alexander's triple solution, indicating viable (arrow) and nonviable (tip) pollen.

Preparation of Alexander's Triple Solution (Alexander, 1969):

- 10 mL 35% ethanol
- 10 mL Malachite Green (1 mL of 1% solution of Malachite Green in 95% ethanol)
- 50 mL distilled water
- 25 mL glycerol
- 5 g phenol
- 5 g chloral hydrate
- 50 mL acid fuchsin (5 mL of 1% aqueous solution)
- 5 mL Orange G (0.5 mL of 1% aqueous solution)
- 1-4 mL glacial acetic acid

Mix all ingredients in the order given above and store in a dark bottle at room temperature. Replace the mixture after one month. The amount of acetic acid will depend on the pollen grain wall; in the case of *Passiflora*, which has thick pollen grain wall, up to 3 mL of acetic acid can be added.

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This book is a catalog of the main morpho-agronomic descriptors for plants of the genus *Passiflora*. The descriptors and their classes can be used to guide, facilitate and standardize the morpho-agronomic characterization of genotypes in small collections and in germplasm banks of passion fruit. It is a practical guide that can be adopted by students and professionals from universities and research institutions engaged in the study of the genus *Passiflora*.

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