

Germplasm variation among wild populations of the southernmost highland papayas (*Vasconcellea quercifolia* and *V. glandulosa*): implications for conservation

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Abstract

Species of *Vasconcellea* are promising for the agricultural industry as a source of genes for papaya improvement because many of them have edible fruits with favorable organoleptic properties. *Vasconcellea quercifolia* and *V. glandulosa* are the southernmost species and have recently been categorized as species of high conservation priority in Argentina. Although seeds of both species can be stored in genebanks, no specific studies have been conducted on their germplasm variation, which is a key aspect to designing a conservation strategy. In this work, we assess morphological, physiological, and biochemical variability at the species and population levels and propose a conservation strategy. In four wild populations of each species located at different elevations in northwest Argentina, vegetative and reproductive material was collected from 110 individuals of *V. glandulosa* and 70 of *V. quercifolia*. Twenty-seven morphological, two biochemical and four physiological descriptors were determined to characterize each species. Then, variance decomposition, differences between populations (with ANOVA) and a principal component analysis were performed using morphological quantitative fruit and seed data, to assess variability between populations. Although both species showed a wide range of phenotypic variability, it was higher in *V. quercifolia* than in *V. glandulosa* and within each population than between them. Leaves, female flowers and physiological descriptors were the most variable, while seed morphological descriptors were the least variable. Variance analysis revealed differences between populations in the majority of morphological descriptors. We recommend collecting germplasm from the entire natural distribution range of each species and from many individuals in each population.

1. Introduction

Despite many actions to mitigate the effects of climate change and enhance food production, the increasing size of human populations and demand for food continue to result in the conversion of natural vegetation to agricultural land (FAO 2017; Mora et al. 2018; IPCC 2021). Currently, commercial agriculture accounts for two-thirds of the total deforested land area in Latin America, and projections estimate that it will reach around 80% of deforested land worldwide (FAO 2017). In this context, many wild species, especially those that are crop relatives, are threatened, and the opportunities to use their germplasm for development of new crops or plant varieties decrease as their populations are destroyed. Ideally, the genes in wild plants could lead to development of crop varieties with tolerance to pests, diseases, drought, waterlogging, and salinity, and thus they would require fewer management requirements and land-intervention practices (Khoury et al. 2015; FAO 2017). Other goals for the improvement of crops are increased nutritional values and genetic diversity (FAO 2017).

Vasconcellea species (Caricaceae), often known as 'highland papayas' or 'mountain papayas', are wild relatives of common papaya (Coppens d'Eeckenbrugge et al. 2014). They are distributed from Mexico to Argentina and from sea level to highlands at 4300 m a.s.l.. These species grow mainly in Colombia, Ecuador and Peru, and only a few of them reach Argentina (Scheldeman et al. 2007). *Vasconcellea* spp. have good potential for domestication in tropical and subtropical regions, and their edible fruits have appealing organoleptic properties (taste, aroma, color) and are locally consumed fresh in juices and salads, or cooked in stews, jellies, syrups and jams. Only two of the 21 species of *Vasconcellea* have been developed as a crop. *Vasconcellea* × *heilbornii* (Badillo) Badillo (Babaco) is cultivated in Ecuador and southern Colombia, and it has

been introduced in New Zealand, Australia, South Africa, Italy, Spain, Switzerland, the Netherlands, and Canada. *Vasconcellea pubescens* is cultivated in all Andean countries but particularly in Colombia and Chile (Carrasco et al. 2009; Coppens d'Eeckenbrugge et al. 2014). The remaining species are commercially and socially important in their local setting; thus, they could supply specific niches in national and international markets. Additionally, *Vasconcellea* spp. have many applications in folk medicine (the leaves, roots and seeds are used to remedy illness such as parasites), in the food industry (the plants secrete latex with high proteolytic activity useful in coagulating milk or making cheese), and in papaya breeding programs (Scheldeman et al. 2002, 2007, 2011; Coppens d'Eeckenbrugge et al. 2014). These wild relatives have been widely used in hybridization programs for their resistance to papaya ringspot virus, cold tolerance, and for some fertility (monoecious) and quality traits (soluble sugar) of *C. papaya* (Kyndt et al. 2005; Drew et al. 2006; Siar et al. 2011). However, crossability with *C. papaya* is highly variable and success depends on particular germplasm and eco-climatic conditions of the breeding site (Coppens d'Eeckenbrugge et al. 2014).

Nevertheless, the variability of wild populations is constantly threatened by changes in land-use and climate (Giamminola et al., 2020; Urtasun et al., in press.). In fact, six of the 21 *Vasconcellea* species are on the Red List of Threaten Species: *V. omnilingua* and *V. horovitziana* are endangered, *V. palandensis* and *V. sphaerocarpa* are vulnerable, *V. sprucei* and *V. pulchra* are near threaten (IUCN 2022). In this context, the design and implementation of *in situ* and *ex situ* conservation strategies for species of *Vasconcellea* are needed.

Only two *Vasconcellea* species (*V. quercifolia* and *V. glandulosa*) occur in Argentina (Zuloaga et al. 2008). *Vasconcellea quercifolia* grows in northern Argentina and in Brazil, Bolivia, Peru, and Paraguay (Siar et al. 2011; GBIF 2022). It is adapted to a wide range of climatic conditions and grows in the humid high and lowland forests and the drier Chaco and Monte ecoregion. It is a pioneer, heliophytic, dioecious, and xenogamy tree that can reach 12 m in height and 1 m in diameter. It produces an orange, juicy and sweet berry with numerous seeds (Fig. 1). The species has dietary potential, since its edible structures (fruits and medullar parenchyma) have high ash, protein, carbohydrate, fiber and carotenoid content (Folharini et al. 2019). The species has industry potential, since its fruits secrete latex with a higher specific activity than that of *C. papaya*. Also, it is one of the few species that has been successfully used in papaya breeding programs (Drew et al. 2007; Siar et al. 2011).

On the other hand, *V. glandulosa* is found in only two provinces of northwest Argentina and in Peru and Bolivia (GBIF 2022). In Argentina, the species grows exclusively in humid forests of the Yungas ecoregion, which is the lower slopes of the eastern side of the Andes Mountains (Zuloaga et al. 2008). The species is an umbrophilic, dioecious, little-branched shrub, with leaves clustered at the apex of the stem. It produces a green and elongated berry, with numerous seeds (Fig. 2).

Recent studies have demonstrated that both species are of high conservation priority due to wild populations being threatened by land-use and climate changes, and they are underrepresented in international and national genebanks (Urtasun et al., in press). Fortunately, the seeds of both species are orthodox; thus, they can be stored in genebanks (Urtasun et al., 2020; unpublished data). However, no specific studies have been conducted on germplasm variation of these species, which represents a key aspect to designing a conservation strategy. In this context, our overall purpose is to contribute to germplasm conservation of wild populations of *V. quercifolia* and *V. glandulosa* from northwestern Argentina. In particular, we assess

morphological, physiological, and biochemical variability at the species and population levels and propose a conservation strategy. We expected higher variability among provenances of *V. quercifolia* than *V. glandulosa*, since the former has a wider and more heterogeneous distribution than the latter.

2. Materials And Methods

2.1. Collection sites

Vegetative and reproductive material was collected during 2015 and 2018 from four wild population of each species located at different elevations in northwest Argentina (Salta, Jujuy, Tucumán and Catamarca provinces) (Table S1, Fig. S1). A total of 110 individuals of *V. glandulosa* and 70 of *V. quercifolia* were sampled during the flowering and fruiting seasons. To assess variability of each species, morphological, biochemical and physiological analyses were performed.

2.2. Germplasm characterization

Morphological characterization among accessions was determined using 21 quantitative morphological descriptors selected from the “Descriptors for papaya” list (IBPGR 1988). They were: length and width of leaves, length and width of female and male flowers, length and width of fruit and seeds; length of petiole and female and male inflorescences peduncle; number of flowers per female and male inflorescence; mass of fruit and seeds; number of seeds per fruit; mass of total seeds per fruit, and roundness index of fruit and seeds (width:length), that varies between 0 and 1 (values near 1 indicate higher roundness); and six qualitative descriptors: shape of leaves, fruits and fruit central cavity, presence/absence of latex in the leaves and fruits, and seed roughness. The shape of leaves and fruits was classified for each population of each species based on the range of variation shown in Fig. 3.

Classification of the shape of fruit central cavity is based on the classification system for *C. papaya* (Fig. 4) (IBPGR 1988).

Proteolytic activity (a biochemical character) was determined for the latex secreted by wounded leaves of *V. glandulosa* and unripe fruits of *V. quercifolia*. In all studied populations, we collected from 1 to 4 latex samples between the end of March and the beginning of April. The number of collected samples varied since the individuals of some populations secrete a low quantity of latex. The methodology to determine proteolytic activity is detailed in *supplementary materials*.

For seeds of each species from each population, we assessed the following physiological variables: moisture content (MC) with the high and constant temperature method (ISTA 2003), viability (V) with a tetrazolium chloride test, germination percentage (GP) and mean germination time (MGT) of fresh seeds. Further methodological details are explained in *supplementary material*.

For each quantitative descriptor, we calculated the mean, range (maximum - minimum value), standard deviation (SD), standard error (SE, only in experiments) and coefficient of variation (CV). For qualitative descriptors, we calculated the frequency of each one.

2.3. Variability among populations

To evaluate morphological variability among populations, we selected quantitative fruit and seed data and performed the following analyses:

Variance components.- Variance decomposition was performed with mixed linear models following the methodology proposed by Di Rienzo et al. (2018). Populations were considered as a random effect. To choose the most adequate model, we compared the AIC and BIC values between models with similar and different variance errors and selected the one with the lowest value.

Differences between populations.- One-way analysis of variance (ANOVA) was used to determine differences between populations. When significant differences were observed ($p < 0.05$), means were separated by the Di Rienzo, Guzmán and Casanoves test (DGC)(Di Rienzo et al. 2002).

Principal component.- To assess the relationship between individuals and the correlation within descriptors, a standardized Principal Component Analysis (PCA) was performed.

3. Results

3.1. Germplasm characterization

A wide range of variability was observed in the studied characters of both species. The statistical results for each descriptor are presented in Table 1. For the morphological quantitative traits, as indicated by the value of the CV, the lowest variation in both species was for seed descriptors (length and width), while the highest was for the number of male flowers per inflorescence. In addition, a high CV was obtained for peduncle length of *V. glandulosa* female inflorescences as well as total mass of *V. quercifolia* seeds per fruit. With respect to biochemical characterizations, the latex secreted by wounded leaves of *V. glandulosa* and unripe fruits of *V. quercifolia* had high proteolytic activity and protein content. The CV of proteolytic activity was higher in *V. glandulosa* than in *V. quercifolia*, while the reverse was true for the CV of protein content. For physiological characterizations, germination percentage was the most variable descriptor. In addition, mean germination time of *V. quercifolia* seeds had a high CV. Overall, *V. quercifolia* showed higher variability than *V. glandulosa*, except for eight descriptors with low variability (CV lower than 20%).

Table 1. Mean, range, standard deviation (SD), standard error (SE) and coefficient of variation (CV) for the 27 quantitative descriptors of the two studied species .of *Vasconcellea*.

| Descriptors | Code | <i>V. glandulosa</i> | | | | | <i>V. quercifolia</i> | | | | |
|--|-------|----------------------|-------|------|----|--------|-----------------------|-------|------|----|--------|
| | | Mean | Range | SD | SE | CV (%) | Mean | Range | SD | SE | CV (%) |
| Leaves | | | | | | | | | | | |
| Lenght (cm) | L_L | 21.4 | 36.0 | 6.3 | - | 29.5 | 25.5 | 40.9 | 6.3 | - | 24.6 |
| Width (cm) | L_Wi | 23.9 | 56.5 | 8.6 | - | 36.0 | 16.5 | 30.5 | 5.5 | - | 33.2 |
| Petiole length (cm) | L_PeL | 22.1 | 52.5 | 8.4 | - | 37.7 | 9.8 | 23.5 | 4.2 | - | 42.6 |
| Female flowers | | | | | | | | | | | |
| Lenght (mm) | FF_L | 29.0 | 31.0 | 5.8 | - | 20.0 | 20.7 | 17.0 | 3.1 | - | 15.1 |
| Width (mm) | FF_Wi | 8.5 | 5.9 | 1.3 | - | 15.3 | 7.3 | 7.8 | 1.7 | - | 23.1 |
| Peduncle length of female inflorescence (mm) | FI_PL | 27.9 | 57.4 | 11.7 | - | 41.9 | 33.0 | 66.6 | 13.7 | - | 41.5 |
| Number of flowers per female inflorescence | FI_NF | 5.6 | 10.0 | 1.7 | - | 29.8 | 4.4 | 8.0 | 1.5 | - | 32.9 |
| Male flowers | | | | | | | | | | | |
| Lenght (mm) | MF_L | 24.0 | 17.4 | 2.7 | - | 11.2 | 16.6 | 12.8 | 2.7 | - | 16.2 |
| Width (mm) | MF_Wi | 1.6 | 1.2 | 0.3 | - | 15.5 | 1.6 | 2.2 | 0.3 | - | 21.4 |
| Peduncle length of male inflorescence (mm) | MI_PL | 17.3 | 32.8 | 6.6 | - | 38.4 | 6.4 | 12.4 | 2.5 | - | 39.0 |
| Number of flowers per male inflorescence | MI_NF | 32.3 | 111.0 | 19.5 | - | 60.8 | 28.6 | 100.0 | 16.6 | - | 58.0 |
| Fruits | | | | | | | | | | | |
| Mass (g) | F_W | 13.0 | 45.5 | 4.4 | - | 33.6 | 19.4 | 46.1 | 8.4 | - | 43.2 |
| Length (mm) | F_L | 82.9 | 71.2 | 11.6 | - | 14.0 | 58.7 | 65.9 | 11.9 | - | 20.4 |
| Width (mm) | F_Wi | 19.8 | 13.9 | 2.2 | - | 11.2 | 25.2 | 33.1 | 5.1 | - | 20.4 |
| Roundness index | F_RI | 0.2 | 0.3 | 0.0 | - | 13.8 | 0.4 | 0.9 | 0.1 | - | 18.2 |

| Descriptors | Code | <i>V. glandulosa</i> | | | | | <i>V. quercifolia</i> | | | | |
|---|------|----------------------|-------|------|-----|--------|-----------------------|-------|------|-----|--------|
| | | Mean | Range | SD | SE | CV (%) | Mean | Range | SD | SE | CV (%) |
| Number of seeds per fruit | F_NS | 95.3 | 150.0 | 28.1 | - | 29.5 | 41.8 | 91.0 | 18.0 | - | 43.2 |
| Mass of seeds per fruit (g) | F_WS | 1.2 | 2.8 | 0.4 | - | 34.0 | 0.8 | 2.1 | 0.4 | - | 50.5 |
| Seeds | | | | | | | | | | | |
| Mass (mg) | S_W | 13.1 | 19.2 | 2.5 | - | 19.4 | 19.7 | 32.2 | 4.7 | - | 24.0 |
| Length (mm) | S_L | 5.3 | 2.9 | 0.5 | - | 8.5 | 5.9 | 6.1 | 0.6 | - | 10.0 |
| Width (mm) | S_Wi | 2.4 | 2.0 | 0.2 | - | 9.4 | 3.0 | 3.5 | 0.3 | - | 10.2 |
| Roundness index | S_RI | 0.5 | 0.4 | 0.1 | - | 11.3 | 0.5 | 0.7 | 0.1 | - | 10.5 |
| Latex | | | | | | | | | | | |
| Protein content (mg protein/g of latex) | PC | 90.2 | 64.0 | - | 4.6 | 19.1 | 93.8 | 117.2 | - | 5.9 | 36.7 |
| Proteolytic activity (Ucas/mg protein) | PA | 4.4 | 5.6 | - | 0.7 | 38.9 | 5.0 | 4.8 | - | 0.3 | 26.0 |
| Physiological descriptors of seeds | | | | | | | | | | | |
| Moisture content (%) | M_FS | 9.8 | 2.2 | - | 0.1 | 6.84 | 10.1 | 2.4 | - | 0.1 | 5.7 |
| Viability (%) | V_FS | 86.3 | 22.2 | - | 2.0 | 9.1 | 93.6 | 33.3 | - | 1.8 | 9.2 |
| Germination (%) | G_FS | 40.4 | 70.0 | - | 3.5 | 43.6 | 12.3 | 64.0 | - | 2.7 | 124.1 |
| Mean germination time (days) | T_FS | 27 | 28.2 | - | 1.4 | 25.5 | 34 | 45.8 | - | 3.0 | 39.9 |

With regard to qualitative descriptors, *V. glandulosa* leaves were more variable than those of *V. quercifolia*. In *V. glandulosa* and *V. quercifolia*, we identified six and four leaf shapes, respectively, and the most frequent shapes were the segmented (0.44) and complete (0.38), respectively (Table 2). In *V. glandulosa* and *V. quercifolia*, there were five and five fruits shapes respectively, and the most frequent shapes were straight (0.67) and oval with tip (0.44), respectively. Central cavity was slightly star in *V. glandulosa* fruits and mainly round in *V. quercifolia* fruits. Seeds were mainly smooth in both species. The leaves of both species and the

majority of *V. quercifolia* fruits secreted latex when an incision was made. Additional descriptions of morphology, phenology, and growth environment are presented in *supplementary material*.

Table 2
Relative frequency of qualitative descriptors.

| Descriptor | Category | <i>V. glandulosa</i> | <i>V. quercifolia</i> |
|----------------------|-----------------|----------------------|-----------------------|
| Leave shape | Segmented | 0.44 | 0.36 |
| | Complete | | 0.38 |
| | Heart | 0.29 | |
| | Round | 0.03 | |
| | With tips | 0.1 | |
| | Star | 0.1 | |
| | Triangular | | 0.23 |
| | Mixed | 0.04 | 0.03 |
| | | | |
| Latex in leaves | Yes | 0.76 | 0.84 |
| | No | 0.24 | 0.16 |
| Fruit shape | Straight | 0.67 | |
| | Curved | 0.21 | |
| | Coiled | 0.03 | |
| | Short | 0.04 | |
| | Mixed | 0.05 | |
| | Oval | | 0.12 |
| | Oval with tip | | 0.44 |
| | Round | | 0.14 |
| | Round with apex | | 0.03 |
| | Pear | | 0.27 |
| | | | |
| Central cavity shape | Irregular | - | - |
| | Round | | 0.63 |
| | Angular | | 0.34 |
| | Slightly star | 1 | 0.03 |
| | Star | - | - |
| Latex in fruits | Yes | | 0.72 |
| | No | 1 | 0.28 |
| Seed roughness | Smooth | 0.54 | 0.62 |

| Descriptor | Category | <i>V. glandulosa</i> | <i>V. quercifolia</i> |
|------------|----------|----------------------|-----------------------|
| | Rought | 0.46 | 0.38 |

3.2. Variability between populations

Variance decomposition. - In both species, environmental variance (error) was the component that contributed the most to explaining total variation in 19 of 21 descriptors (Table 3). In general, environmental variance was higher in *V. glandulosa* than in *V. quercifolia*, with the environmental component for *V. glandulosa* and *V. quercifolia* being higher than 80% in 12 and in eight descriptors, respectively (Table 3). The variance of the number of *V. glandulosa* flowers per female and male inflorescences was completely explained by environment (100% and 99.1%, respectively). Except for only one descriptor of *V. glandulosa* (seed mass), the difference between variance components was small (43% population vs. 57% environment). Conversely, for four *V. quercifolia* descriptors the difference between components was small: leaf petiole length, number of flowers per male inflorescence, fruit and seed length. Finally, the percentage of variation was mainly explained by the population in descriptors of *V. glandulosa* female flowers (length and width) and in descriptors of *V. quercifolia* male flowers (length and number of flowers per inflorescence).

Table 3
Percentage of morphological descriptors variance explained by population (Pop)
and environment (Env).

| Descriptors | <i>V. glandulosa</i> | | <i>V. quercifolia</i> | |
|--|----------------------|------|-----------------------|------|
| | Pop. | Env. | Pop. | Env. |
| Leaves | | | | |
| Lenght (cm) | 28.3 | 71.7 | 30.2 | 69.8 |
| Width (cm) | 20 | 80 | 33.9 | 66.1 |
| Petiole length (cm) | 13.9 | 86.1 | 41.5 | 58.5 |
| Female flowers | | | | |
| Lenght (mm) | 53.9 | 46.1 | 33.1 | 66.9 |
| Width (mm) | 51.4 | 48.6 | 6.4 | 93.6 |
| Peduncle lenght of female inflorescence (mm) | 5.9 | 94.1 | 31.3 | 68.7 |
| Number of flowers per female inflorescence | 0 | 100 | 29.1 | 70.9 |
| Male flowers | | | | |
| Lenght (mm) | 39.6 | 60.4 | 77.4 | 22.6 |
| Width (mm) | 24.2 | 75.8 | 15.9 | 84.1 |
| Peduncle lenght of male inflorescence (mm) | 16.3 | 83.7 | 31.4 | 68.6 |
| Number of flowers per male inflorescence | 0.9 | 99.1 | 51.6 | 48.4 |
| Fruits | | | | |
| Mass(g) | 8.8 | 91.2 | 28.6 | 71.4 |
| Length (mm) | 7.6 | 92.4 | 42.7 | 57.3 |
| Width (mm) | 3.7 | 96.3 | 18 | 82 |
| Roundness index | 10 | 90 | 5.9 | 94.1 |
| Number of seeds per fruit | 30.2 | 69.8 | 14.5 | 85.5 |
| Mass of seeds per fruit (g) | 22.8 | 77.2 | 2.9 | 97.1 |
| Seeds | | | | |
| Mass (mg) | 42.9 | 57.1 | 21.8 | 78.2 |
| Lenght (mm) | 32 | 68 | 42.2 | 57.8 |
| Width (mm) | 6.9 | 93.1 | 8.4 | 91.6 |
| Roundness index | 13.8 | 86.2 | 3.8 | 96.2 |

3.3. Differences between populations. - In *V. glandulosa*, there were no differences in fruit descriptors between populations. On the contrary, all seed descriptors for this species varied between populations: seed mass and length were lower in San Francisco and San Lorenzo populations, seed width was higher in Cornisa and San Francisco populations, seed roundness index was lower in Caulario population, the number of seeds per fruit was lower in Cornisa population and the total mass of seeds per fruit was higher in Caulario population (Table S2). In *V. quercifolia*, there were differences between populations in all descriptors except three (fruit roundness index, seed width, and the number of seeds per fruit). Fruits from Ancasti were smaller (minor mass, length, and width, $p < 0.001$) than those from the other populations. In addition, seeds from Ancasti were smaller, rounder, and the mass of seeds per fruit was lower than those from the other populations (Table S2).

Overall, the most variable *V. glandulosa* population (according to the CV) was Cornisa, since it had the highest CV values in six of the ten morphological descriptors. In *V. quercifolia*, the Ancasti and San Francisco populations were the most variable, since the former presented the highest CV in four descriptors (three of fruits and one of seeds) and the latter the highest CV in three seed descriptors (Table S2).

Principal component. - In *V. glandulosa*, the first four principal components had eigenvalues higher than one, and together they explained 88% of total variation. The first component accounted for 36% of total variance, and it was mainly related to fruit mass and total mass of seeds per fruit. The second component (22% of total variation) was positively correlated with seed size descriptors (mass, length and width), and the third component (17%) was positively correlated with fruit roundness index (Fig. 5, Table S3).

In *V. quercifolia*, the first three principal components had eigenvalues higher than one, and together they explained 84% of total variation. The first component accounted for 50% of variance, and it was positively correlated with fruit mass, mass of seeds per fruit and seed length. The second component (21% of total variation) was positively correlated with seed width and roundness index and negatively correlated with number of seeds per fruit, and the third component (14% of total variation) was positively correlated with fruit roundness index (Fig. 5, Table S4).

Although many correlations were identified between morphological descriptors, only a few of them had a correlation coefficient higher than 0.70 (strong correlation). In both species, a positive strong correlation was found between fruit mass (F_W) and width (F_Wi), fruit mass and total mass of seeds per fruit (F_WS), and seed mass (S_W) and length (S_L). Additionally, in *V. glandulosa* a strong positive correlation was found between number of seeds per fruit (F_NS) and mass of seeds per fruit (F_WS). In *V. quercifolia*, a strong positive correlation also was found between fruit mass and length, fruit mass and seed length, and seed length and total mass of seeds per fruit (Fig. 5).

4. Discussion

Vasconcellea quercifolia and *V. glandulosa* were recently classified as species of high conservation priority since their wild populations in northwestern Argentina are threatened by land-use and climate changes, and both species are underrepresented in the national system of protected areas and genebanks (Urtasun et al. in press). Thus, urgent conservation efforts are needed to safeguard the gene pool of these wild relatives. Here, we characterized for the first-time germplasm of wild populations of the southernmost highland papayas, and we found high variability in all the studied traits. Also, we learned that the latex of both species has high

proteolytic activity and protein content, which are important characteristics for potential uses. Recently, it has been demonstrated that *V. quercifolia* latex collected from fruits of Santiago del Estero province (Argentina) is effective as a dehairing agent for leather processing without having to use lime and sulfide products (both contaminants) (Errasti et al. 2020). Therefore, from our results, further work should be done to develop *V. quercifolia* from northwestern populations as a source of latex.

Although both species had a wide range of phenotypic variability, it was higher in *V. quercifolia* than in *V. glandulosa*, as we hypothesized. From the 27 quantitative descriptors, 21 showed a high variability (CV > 20%) in the former species and 17 in the latter. Leaves, female flowers and physiological descriptors were the most variable, while seed morphological descriptors were the least variable. The traits with no or low variation are the homogeneous and repeatable among the individuals; therefore, they may be considered as stable traits with a genetic control of the character (Khadivi et al. 2020). Conversely, variable traits are related to a strong environmental influence (Acosta-Quezada et al. 2011). For example, it has been widely demonstrated that variation in leaf size and shape is strongly correlated with climate, and therefore, with latitude, altitude and availability of resources (Li et al. 2021). In the same way, seed germination depends on many factors mainly related with the maternal environment during the flowering and fructification season (Tweddle et al. 2003; Finch-Savage and Leubner-Metzger 2006). Hence, a high CV in the above-mentioned traits is expected.

Highland papayas, as well as *C. papaya*, are characterized by having high morphological variability (Kyndt et al. 2005; Hernández-Salinas et al. 2019), which is correlated in some species with high genetic diversity and in others with medium or even low genetic diversity (Scheldeman and Damme 2002; Carrasco et al. 2009, 2014). It was reported that a wide inter and intraspecific morphological variability in *V. pubescens*, *V. stipulata* and *V. x heilbornii* of Loja province in Ecuador is related to high genetic diversity in the last two species (Scheldeman and Damme 2002). On other hand, Carrasco et al. (2014) obtained low morphological variability related to medium genetic diversity in fruits of *V. chilensis*. In all these mentioned works as well as in our work, variability was higher within each population than between populations. This pattern has been reported for dioecious angiosperms, long-lived and cross-pollinated species (Nybom 2004). Moreover, in species with an overlap in distribution (as *V. quercifolia* and *V. glandulosa*), high variability between individuals could be a product of hybridization (Kyndt et al. 2005). As mentioned before, in both species, the environmental variability (the error term calculated on the basis of replications within the same plant) was the main source of variation, which suggests that the local environment under which the plant grows is the most contributing factor to the phenotype expression of each trait (Harzé et al. 2016).

On other hand, variance analysis revealed differences between populations in the majority of morphological descriptors. For example, *V. glandulosa* seeds had less mass in the population with less precipitation (higher elevation), while seeds and fruits of *V. quercifolia* also had less mass in the population with less precipitation but with higher temperature (lower elevation). These results suggest that precipitation could be one of the environmental variables that determines fruit and seed mass and could be used as a parameter to select future collections with desirable specific fruit or seed traits.

Principal Component Analysis was congruent with the previous results, since wide variability within populations was obtained in both species. Only in the *V. quercifolia* plot, was it possible to observe differences between Ancasti and the remaining populations. In both species, fruit mass, mass of seeds per fruit, and fruit

roundness index explained most of the variability and had the highest impact on differentiation of *V. quercifolia* populations. Additionally, we found significant correlations among size-related traits of fruits and seeds, which are related in many taxa (Khadivi et al. 2020; Lamas et al. 2022) and can be used in selection programs.

5. Conclusions

Based on available information about the southernmost highland papayas, we recommend employing thorough sampling strategies in future germplasm collecting efforts. In this sense, germplasm should be collected from the entire natural distribution range of each species and from many individuals in each population. Both actions would significantly increase the genetic representativeness of *ex situ* collections and decrease the risk of losing wild germplasm potentially useful in meeting the challenges of changes in land-use or climate

Declarations

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DECLARATIONS

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Figures

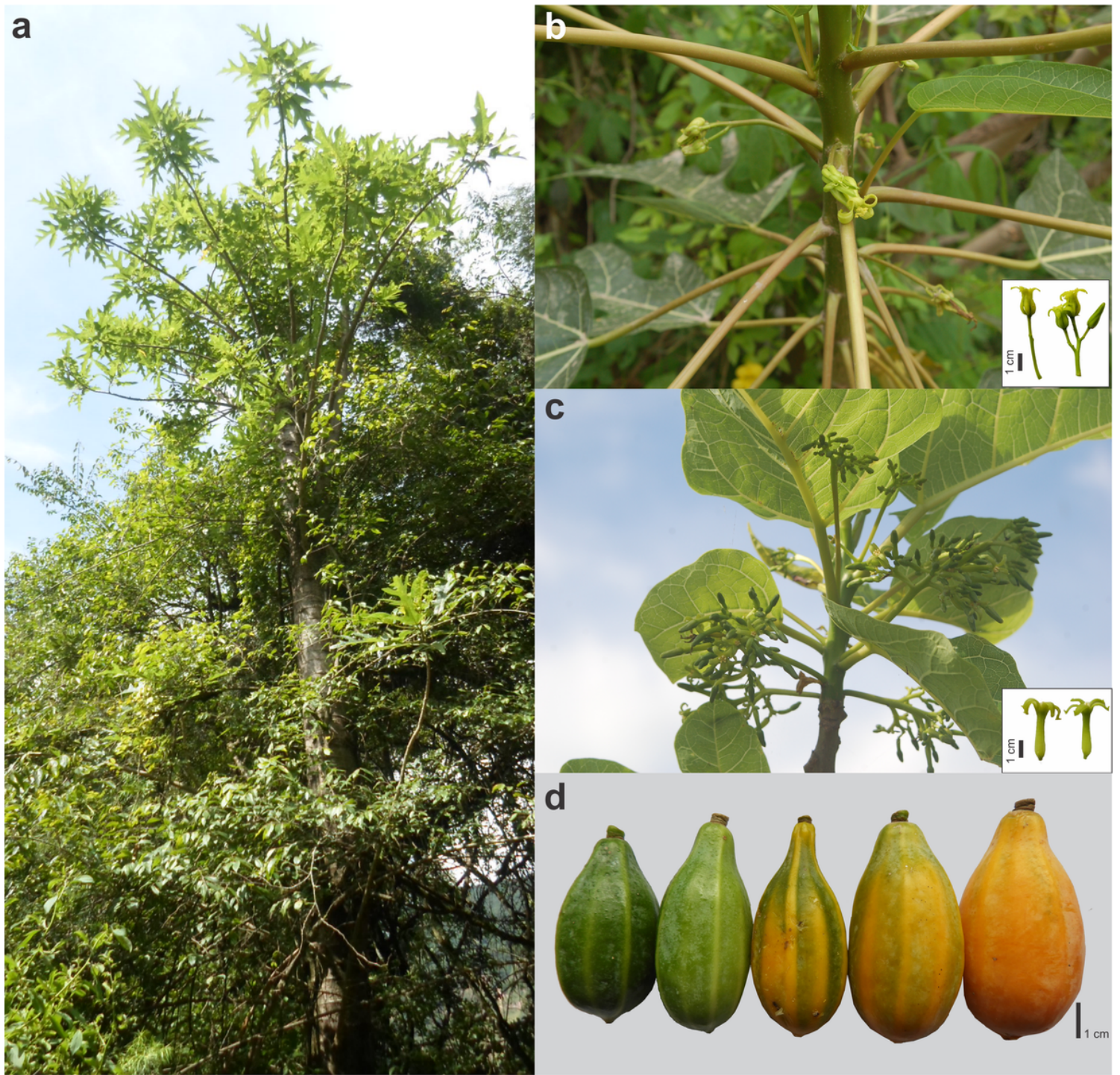


Figure 1

Vasconcellea quercifolia tree (a), female flowers (b), male flowers (c) and fruits in different stages of maturation (d).

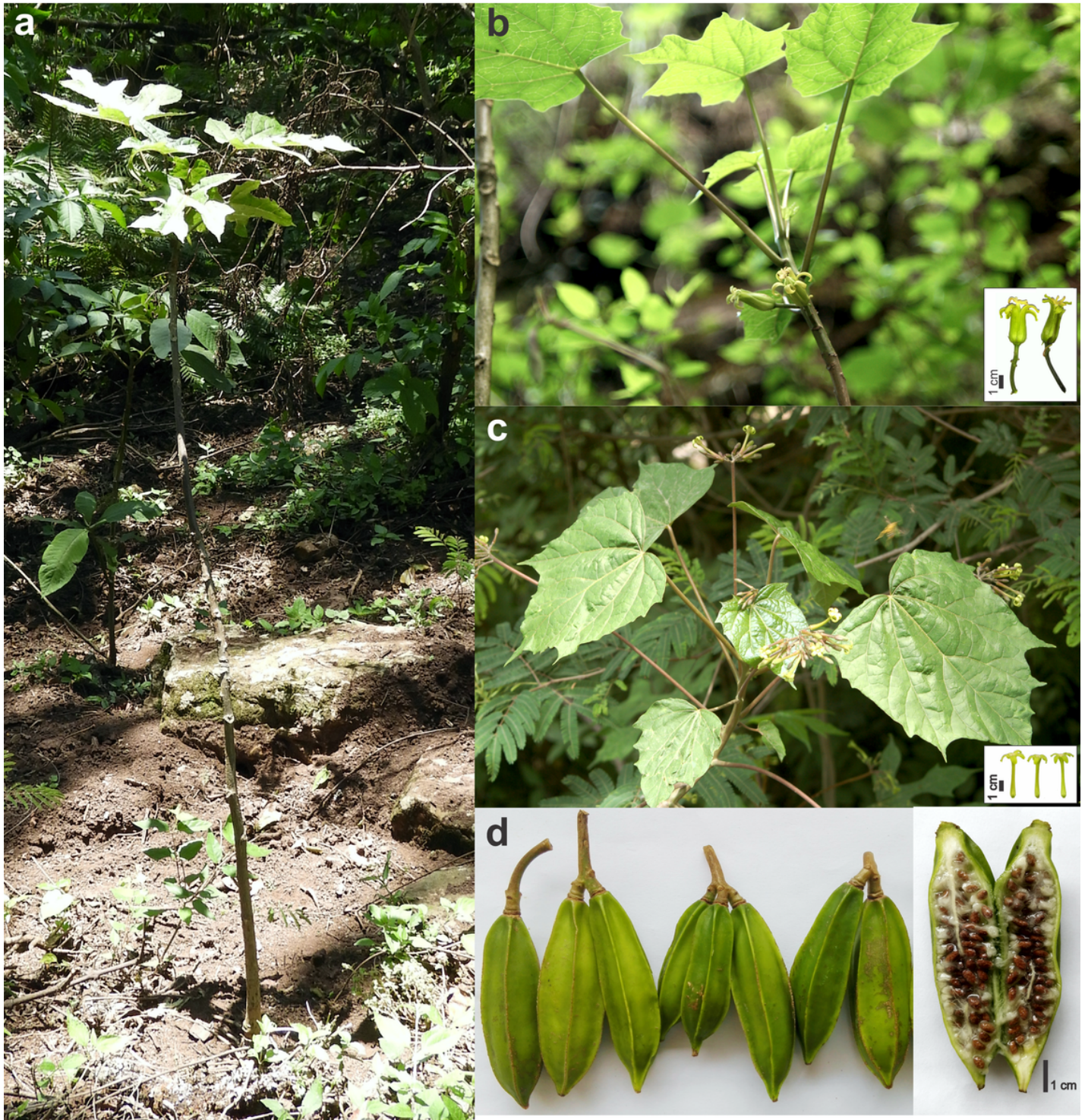
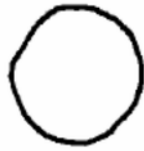


Figure 2

Vasconcellea glandulosa shrub (a), female flowers (b), male flowers (c) and mature fruits (d).



Irregular



Round



Angular



Slightly star
shaped



Star shaped

Figure 3

Range of natural variation in leaf and fruit shapes of *V. glandulosa* and *V. quercifolia*.

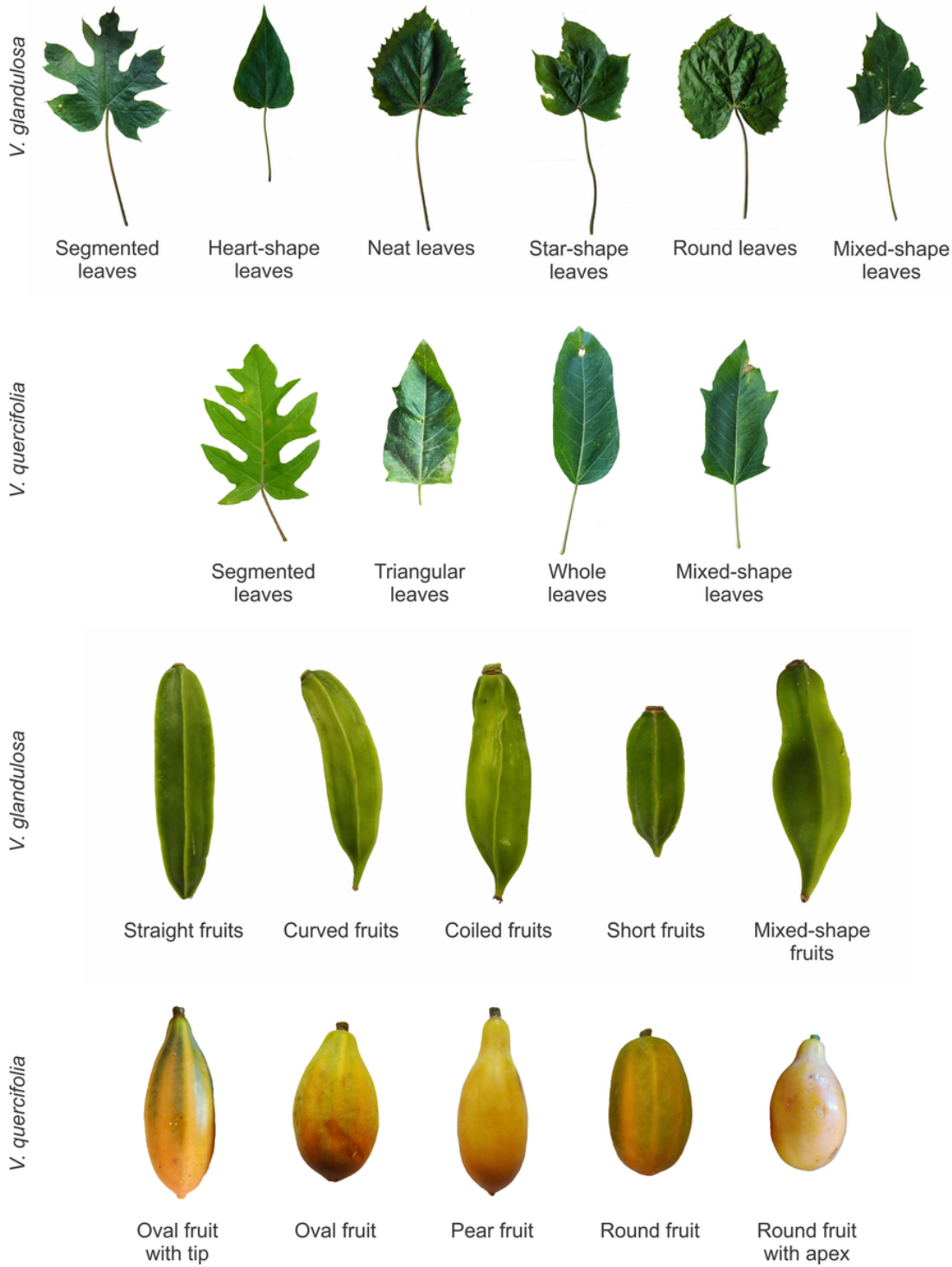


Figure 4

Shape of fruit central cavity

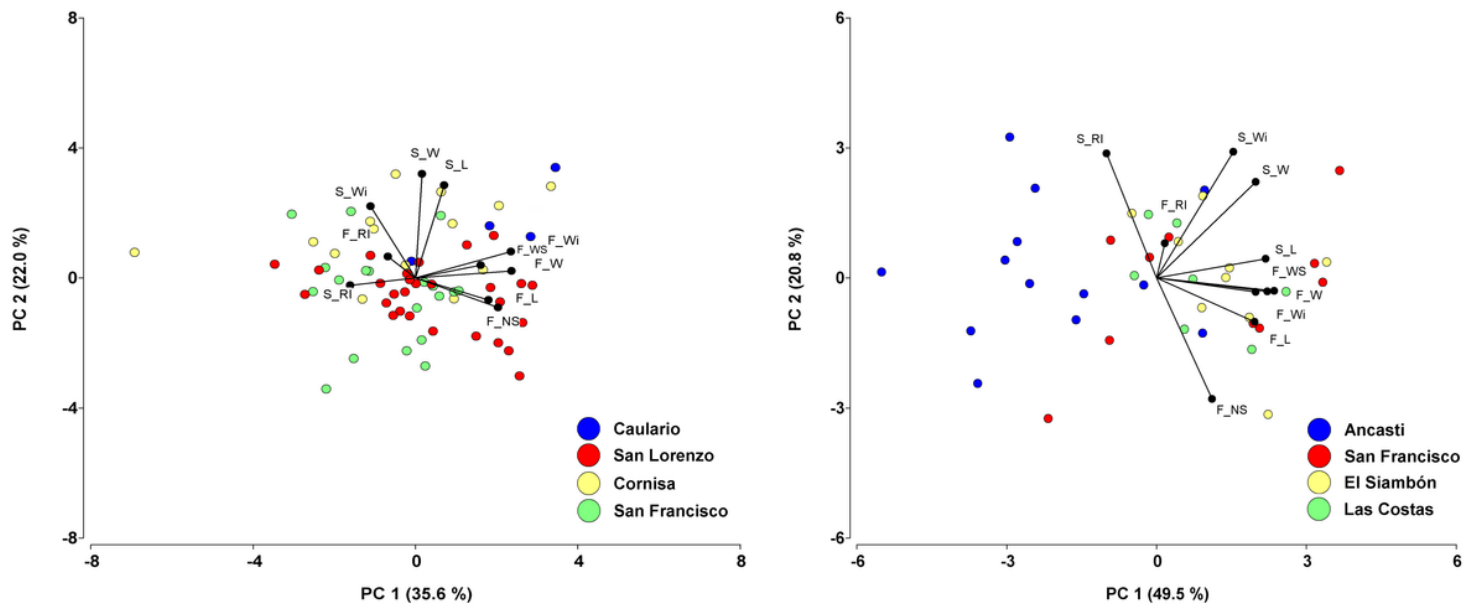


Figure 5

Scatter plot for the studied *V. glandulosa* (left) and *V. quercifolia* (right) accessions based on PC1 and PC2. *F_W*: fruit mass, *F_L*: fruit length, *F_Wi*: fruit width, *F_RI*: fruit roundness index, *F_WS*: total mass of seeds per fruit, *F_NS*: number of seeds per fruit, *S_W*: seed mass, *S_L*: seed length, *S_Wi*: seed width, *S_RI*: seed roundness index.

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