



# TWENTY-TWO-YEAR PAPAYA BREEDING PROGRAM: FROM BREEDING STRATEGY ESTABLISHMENT TO CULTIVAR DEVELOPMENT

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**Abstract** - The papaya crop occupies 32 thousand hectares of planted area in Brazil, with a total annual production of 1.6 million tons (12.5% of the world supply). The country stands out in this scenario as the second biggest producer of the fruit worldwide, only after India. The narrow genetic base of the crop once limited its variability, but the use of classical and molecular plant breeding techniques has enabled the development of a number of higher-yielding cultivars with different levels of resistance to fungal diseases. However, many studies still ought to be undertaken to investigate the papaya crop, given the constant search for higher-yielding cultivars with quality and flavor attributes and the wide range of pathogens affecting the crop, which has not yet shown fully resistant genotypes. Advances in the genomics of papaya provide tools that may improve cultivar production and development systems. This article describes studies conducted by the genetics and breeding group at UENF, in a partnership with Caliman Agrícola S.A., using conventional breeding, diallel cross, and topcross, among other techniques, for the development of 21 hybrids, which were registered at MAPA, in addition to studies with DNA-based markers for sex determination and for the generation of resistant and productive cultivars. This review focuses on the 22 years of conventional breeding for the most recent molecular progress in papaya growing. The information reported here is extremely useful for breeders to develop resistant, productive, and high-quality varieties through assisted selection.

**Keywords:** *Carica papaya*, integrated breeding, hybridization, topcross, resistance and genomics

## Introduction

Papaya (*Carica papaya* L.) is one of the most widely grown fruit species consumed in tropical and subtropical regions of the world. Papaya fruits are rich in phosphorus, potassium, iron, calcium, and fiber sources, besides vitamins A, B, and ascorbic acid (vitamin C), whose content increases with fruit maturity (Ming et al., 2007; Serrano and Cattaneo, 2010). Despite the great national and international economic importance of the crop, there are few breeding programs aimed at cultivar development. For this reason, until recently, 100% of the Brazilian areas cultivated with hybrids depended on the import of seeds.

Brazil is the second biggest producer and exporter of the fruit in the world, with a cultivated area of approximately 32 thousand hectares (Lucena, 2016). At present, the main papaya-producing Brazilian states are Bahia and Espírito Santo, which generate around 800 and 400 thousand tons, respectively, corresponding to 71% of the national total. These are followed by the states of Ceará, Minas Gerais, and Rio Grande do Norte, which account for 20% of production (Lucena, 2016; Treichel et al., 2016).

Considering the above facts, there is a clear need for enhancing papaya breeding programs. Through short-, medium-, and long-term goals, these programs can help to broaden the current genetic base, generating varieties with tolerance or resistance to the main diseases as well as desirable agronomic traits, consequently meeting the domestic and external market demands.

In this way, in 1996, the State University of Northern Rio de Janeiro (UENF) established, in a partnership with CALIMAN Agrícola S.A., a papaya breeding program whose main objective was the development of cultivars with great potential to contribute to the papaya agribusiness in Brazil.

For 22 years, the UENF-CALIMAN partnership has yielded at its factory results, with hybrid 'UENF/CALIMAN 01' (commercially known as 'Calimosa') standing out. The hybrid was recommended to farmers in 2003 and has been grown in different producing regions across Brazil. It should also be noted that another four hybrids were released in 2014 ('UC10', 'UC12', 'UC14' and 'UC16') and eight new hybrids were released in 2018 (UC 20', 'UC 21', 'UC 22', 'UC 23', 'UC 24', 'UC 25', 'UC 26', 'UC 27' and 'UC 28'). Together, these hybrids meet the demand of farmers for small, medium, and large fruits, providing the producer and consumer markets with new options in terms of fruit size, quality, yield, and resistance. The objective of the current paper is to present a review of the papaya breeding studies conducted at UENF over 22 years and indicate research prospects for this crop of great economic importance.

## BREEDING STRATEGIES USED

Papaya is a fruit tree with peculiar characteristics in terms of reproduction; therefore, specific strategies should be designed for its breeding. Before establishing the types of cultivar to be developed, it is necessary to know their reproductive aspects (allogamy, autogamy, cross-pollination percentage, etc.) as well as the inheritance of major agronomic attributes.

A number of studies were carried out to underpin breeding procedures, since no such information could be found in the literature. Based on the results, reproductive aspects of the species were determined, e.g. pollination rate, karyotype, and others. Essential factors pertaining to the inheritance of the main agronomic attributes were defined, making it possible to obtain both pure-line and hybrid cultivars.

### At reproductive and cytological level

The family Caricaceae consists of 35 species distributed into six genera. Papaya is the only species of commercial importance among them, although the others are also relevant, since they are considered gene repositories that might be introduced in cultivated form. For this transfer to be successful, there must be chromosomal homology between the species; hence the importance of knowing the cytological aspects of both the cultivated form and related wild species as well as reproductive aspects.

In view of the need for basic knowledge to underpin the breeding procedure, several reproductive and cytological studies were undertaken. In one of them, the authors determined the karyotype of the cultivated form (*Carica papaya*) and of related wild species *Vasconcellea monoica* and *V. Cundinamarcensis* (Damasceno Junior et al., 2009a). The three species exhibited  $2n=18$  chromosomes, with symmetric and similar karyotypes showing small and metacentric chromosomes. No heteromorphic sex chromosome was observed in any of the species, suggesting that the sex chromosome responsible for determining the sex in papaya plants is homomorphic, as described by Ming et al. (2007).

Regarding the karyotype of other species of the family, five pairs of metacentric chromosomes and four pairs of submetacentric chromosomes were observed in *V. goudotiana*. In the species *V. cauliflora* and *V. quercifolia*, however, nine pairs of metacentric chromosomes were observed. Based on the asymmetry indices, the karyotypes of the studied species were considered symmetrical, but the species *V. cauliflora* has an asymmetric karyotype when compared with *V. quercifolia* (Freitas Neto, 2010).

Addressing cytological aspects, Costa et al. (2008) employed the fluorescent *in situ* hybridization technique (FISH) in a comparative study between *C. papaya*, *V. goudotiana*, and *V. cundinamarcensis*. The authors found

greater genetic similarity between *V. goudotiana* and *V. cundinamarcensis* for number and distribution of probes. Repetitive rDNA sequences indicated that the papaya plant is similar to practically none of the *Vasconcellea* species used in the study.

The meiotic behavior of *Jacaratia spinosa*, *Vasconcellea quercifolia*, *V. goudotiana*, *V. monoica*, and *C. papaya* was also investigated to determine male gamete viability as well as meiotic stability in the species. Despite a few irregularities, meiosis was regular in all species, confirming its diploid nature. The obtained meiotic index indicates that the detected meiotic irregularities did not significantly affect the formation of post-meiotic products. Pollen viability was also high for all species, ranging from 68.0% (*V. goudotiana*) to 96.0% (*J. spinosa*), which are satisfactory values, considering that the species are wild. Considering these results, it could be inferred that the species are cytologically stable (Freitas Neto, 2010; Damasceno Junior, 2008).

Studies investigating the ‘Golden’ and ‘Tainung 01’ genotypes revealed that papaya presents variable viability and preservation of pollen grains. In the freezer ( $-18^{\circ}\text{C}$ ) and in the refrigerator ( $12^{\circ}\text{C}$ ), flower-bud pollen grain preservation in ‘Tainung 01’ and ‘Golden’ was viable for 60 and 30 days, respectively. The ‘Golden’ cultivar showed a marked reduction of viability at 60 days of storage (Damasceno Junior et al., 2008a).

Regarding reproductive aspects, the species exhibits floral polymorphism, with male, female, and hermaphrodite plants. Hermaphrodite plants are typically used for commercial cultivation, which makes it important to understand their means of reproduction. The results indicated that the preferential means of reproduction of hermaphrodite papaya is facultative autogamous with cleistogamy (Damasceno Junior et al., 2009b).

Temperature changes have a detrimental effect on the crop, especially in the reproductive stage. Two genotypes were used to determine the cardinal temperatures (minimum, optimum, and maximum) for the *in vitro* germination of pollen grains: UC JS-12 and ‘UENF/CALIMAN 01’. At low temperatures ( $15^{\circ}\text{C}$ ), the pollen grains almost did not germinate, and germination increased gradually up to a maximum value at  $30^{\circ}\text{C}$ . Higher temperatures stimulate a reduction of germination (Freitas, 2013).

The temperature also affects the papaya tree, causing floral abnormalities in hermaphrodite plants. Low temperatures favor the emergence of carpelloid and pentandric flowers. At high temperatures, however, summer sterility arises, preventing the hermaphrodite flower from developing the pistil, which leads to flower abortion and a consequent decline in final production. Damasceno Junior et al. (2008b) confirmed these facts by evaluating the floral behavior of ten hybrids from Solo and Formosa parents, during the months of March and September. The authors observed a lower number of

hermaphrodite flowers (NHF) and fewer abnormalities in March. By contrast, the number of male or sex-reversed flowers rose in that period. This phenomenon can be explained by the higher temperatures occurring in March and previous months. The genotypic coefficient of determination ( $H^2$ ) for NHF was lower in March (30.72%) than in September (85.62%), indicating strong environmental influence on the trait. On the average of both months, hybrids SS 783  $\times$  JS-12, SS 72/12  $\times$  JS-12, TJ  $\times$  JS11, SS 783  $\times$  Tailândia, and Sta. Barbara  $\times$  Tailândia showed a higher NHF, with the SS 783  $\times$  JS-12 genotype exhibiting the highest NHF at both evaluated seasons, suggesting great production potential.

Damasceno Junior (2008) evaluated the average degree of dominance (ADD) and specific combining ability (SCA) for carpelloid and pentandry and obtained over dominant inheritance, tending towards the parents of highest means. As for summer sterility, partial-dominance inheritance was observed, tending towards the parents of lowest means at the season of lower expression and parents of higher means at the season of greater expression. The Costa Rica  $\times$  SS-72/12 combination was the only one showing negative SCA values for all evaluated traits except pentandry, whose SCA value was zero. Thus, it was a very promising combination.

#### Analysis of generation means and variances

As previously mentioned, knowing the inheritance of the main agronomic attributes one aims to improve is key to defining the types of cultivar to be developed as well as the best selection strategies.

Generation analysis allows us to examine the nature and to quantify the available genetic variability in the segregating population as well as the relative importance of the gene effects constituting the means. By the generation analysis approach, it is possible to estimate genetic parameters based on the means and variances through experiments involving  $P_1$  and  $P_2$  parents,  $F_1$  and  $F_2$  generations and  $BC_{1,1}$  and  $BC_{1,2}$  backcrosses.

Results of analyses based on the means and variances suggest that the variances attributed to the additive effects were of higher magnitude than the variances attributed to the dominance effects for all studied traits. The gene effects attributed to the mean and the additive genetic effects contributed the most to explaining the available variability by the additive dominant model. The great magnitude of effects attributed to the additive genes in relation to the dominance deviations for the traits *number of fruits* and *fruit weight* suggest that intrapopulation breeding can lead to gains in these traits. In other words, the development of pure-line cultivars is a good alternative for the papaya crop. On the other hand, the higher-magnitude dominance deviations for fruit yield per plant suggest the viability of using hybridizations as a strategy to increase yield. Particularly for yield, higher

values are obtained via crossing and via hybrid production. It is thus noteworthy that both pure-line and hybrid cultivars can be developed based on the genetic analysis of the main attributes of the papaya crop.

### **Exploitation of heterotic groups**

The analyses based on means and variances (Cattaneo, 2001) indicated the viability of exploiting heterosis in papaya. In an initial phase, the authors took advantage of previous information, exploiting the Solo and Formosa heterotic groups using a partial diallel cross (Marin et al., 2006a; 2006b). This work resulted in the generation of the first nine national hybrids, named 'UENF/CALIMAN 01' to 'UENF/CALIMAN 09', the former of which was known as 'Calimosa'. Still under the aim of exploiting heterotic groups, testers were used. Genotypes of the Solo group were crossed with a Formosa tester and vice-versa (Ide, 2008; Ide et al., 2009).

Considering the existence of intragroup variability, the viability of the Solo × Solo and Formosa × Formosa crosses was also investigated using full diallel cross (Cardoso, 2012; Vivas et al., 2011; Vivas, 2012). The intragroup strategy makes it possible to produce a wider array of hybrids in terms of fruit size. In the Solo intragroup crosses, the resulting hybrids tend to bear smaller fruits weighing 300 to 600 g. The Formosa intragroup crosses, in turn, usually produce hybrids whose fruits weigh approximately 2 kg, which shows this is a viable strategy.

### **Developed and recommended hybrids**

The first national papaya hybrid was released in 2003 by the State University of Northern Rio de Janeiro - UENF, in a partnership with CALIMAN Agrícola S.A. Thereafter, several other cultivars were registered in the National Registry of Cultivated Varieties - RNC. Today, 54 records are available at RNC, including hybrids and commercial varieties.

Of those records, 21 hybrids were developed by UENF in a partnership with CALIMAN Agrícola S.A. Nine of these hybrids were cited in item 2.3, four of which were registered in 2014 (Luz et al., 2015) and termed 'UC10', 'UC12', 'UC14', and 'UC16'. Recently, in 2018, another eight hybrids were registered, namely 'UC 20', 'UC 21', 'UC 22', 'UC 23', 'UC 24', 'UC 25', 'UC 27', and 'UC 28'. The last registered genotypes share *average fruit weight* as the main trait, in addition to being the first national hybrids of the small-fruit Solo type. Given the set of hybrids registered in RNC, the program offers alternatives in terms of average fruit weight, with productive plants bearing high-quality fruits weighing from 400 g to 2000 g and thus meeting the most varied demands of the producer and consumer markets.

## **Breeding procedures**

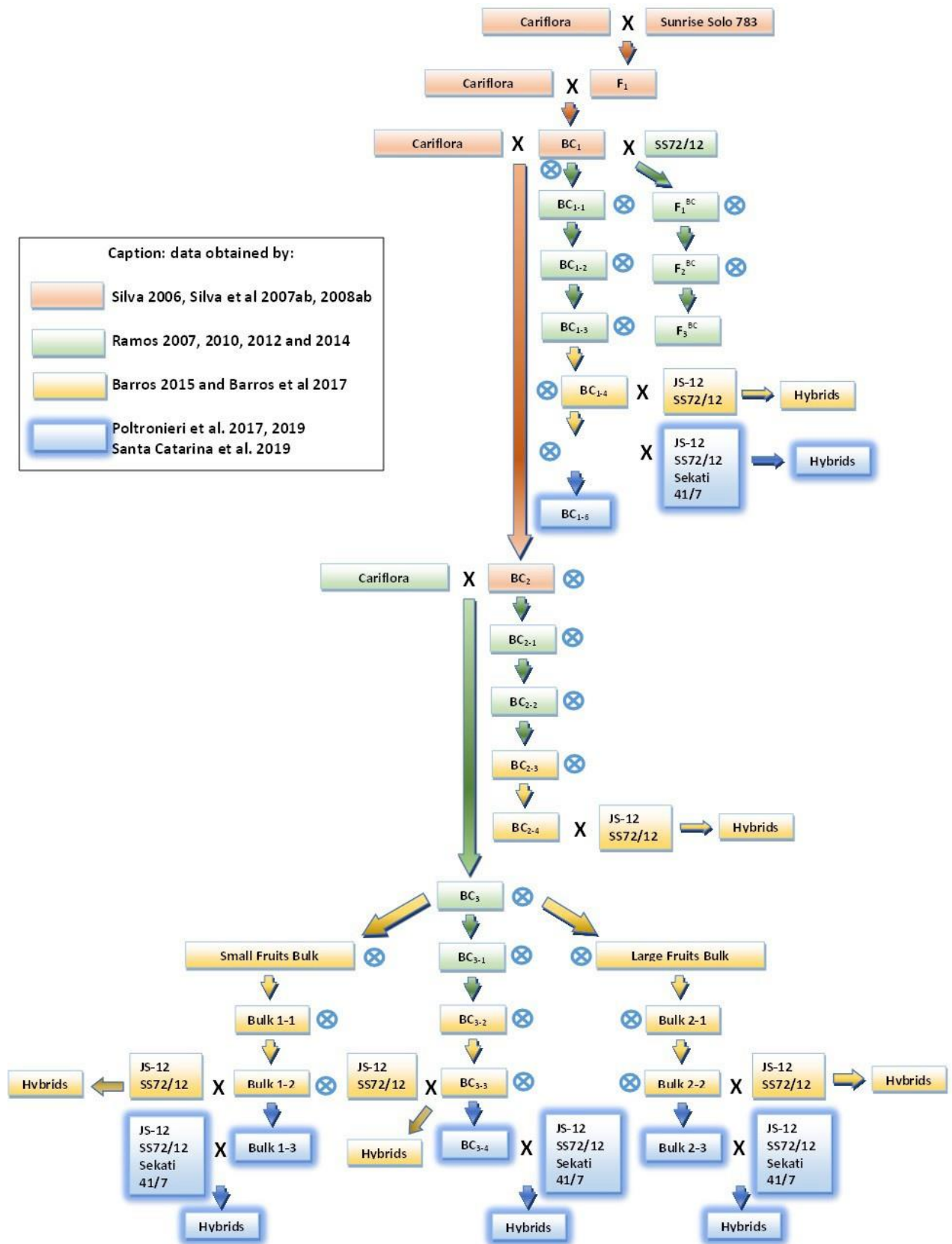
### **Development of segregating populations and generation of lines and parents**

#### **Backcrossing and generation advance**

The backcrossing method is used as a strategy to obtain segregating populations, allowing for the selection of individuals with a high number of favorable alleles for a given trait. Originally developed for the transfer of one or more genes, it can originate an initial population with considerable genetic variability, providing the development of promising segregating progeny (Lorencetti et al., 2006). In this regard, the breeding program at UENF employed this methodology for the sexual reversal of the Cariflora genotype from the dioecious state (population with female and male plants) to the gynodioecious-andromonoecious state (population with female and hermaphrodite plants) (Figure 1). Because the recurrent genotype in question is dioecious, it can be stated that its inbreeding is null, potentially generating segregating lines with a high degree of differentiation.

Studies on combining ability between the Cariflora genotype and genotypes of the Solo, Formosa, and Dioecious groups have shown excellent general and specific combining abilities for various agronomic traits (Marin, 2001). However, because it is a dioecious material, its utilization for producing inbred lines was inviable. The only possibility to exploit this genotype was by incorporating the region controlling sex determination, located on the Y chromosome (Ming et al., 2007) responsible for hermaphroditism into its genome.

Silva et al. (2007a) started the said study, using molecular markers to increase the efficiency of the process. In the study, the BC<sub>1</sub> and BC<sub>2</sub> populations were obtained from the cross between Cariflora and Sunrise Solo 783 (Figure 1) and then plants with a high degree of similarity to recurrent parent Cariflora were selected. Several studies were undertaken to associate classical breeding with molecular markers for the development, evaluation, and training of segregating populations derived from BC<sub>1</sub> and BC<sub>2</sub> (Silva et al., 2007b; Silva et al., 2007c; Silva et al., 2008a; Silva et al., 2008b; Ramos et al., 2011a; Ramos et al., 2011b). The main objective of these studies was to identify and select agronomically superior genotypes.



**Figure 1.** Flowchart depicting the backcrossing history, developed by the papaya breeding group at UENF/CALIMAN.

Ramos (2010) obtained the BC<sub>3</sub> populations (Figure 1) and Ramos et al. (2012) determined the genetic distance between lines derived from the backcross generations (BC<sub>1</sub>, BC<sub>2</sub>, and BC<sub>3</sub>) aiming at the selection of superior genotypes; generation advance by self-pollination; as well as analyzing the efficiency of morpho-agronomic and molecular data in the estimate of genetic diversity and proper distinction of the evaluated progeny. Combined selection strategies were developed for the main agronomic traits based on both the phenotypic and genetic values, via REML/BLUMP, to identify the best families (Ramos et al., 2014a). SSR markers were used to monitor the genetic proportion of the genome of the recurrent parent in progeny derived from generations BC<sub>1</sub>, BC<sub>2</sub>, and BC<sub>3</sub> and select the progeny of greatest genetic proximity to Cariflora (Ramos et al., 2014b).

In addition to lines, hybrids can also be exploited in the papaya crop. For this purpose, it is necessary to first know the genetic relationships between genotypes in crosses and then decide for the best genetic combinations, which will result in greater success. A study was thus conducted on the combining ability of papaya lines converted to hermaphroditism (Barros, 2015; Barros et al., 2017). Lines at different inbreeding levels and from different backcross generations (BC<sub>1</sub>, BC<sub>2</sub> and BC<sub>3</sub>) were selected based on fruit yield and quality attributes such as fruit and pulp firmness and soluble solids content (Barros et al., 2011). The hybrids were obtained through a top-cross using two elite testers of the Solo and Formosa heterotic groups. No hybrid showed good SCA estimates for the agronomic traits of fruit quality and production simultaneously. However, they may be selected for traits of greater economic importance such as number of marketable fruits, fruit and pulp firmness, soluble solids content, and yield.

In a more advanced study, 10 superior lineages from different testcross and self-cross generations of the Cariflora genotype were selected (Santa-Catarina et al., 2014). The backcross of the lineages with the UC-SS-72/12; UC JS-12. UC Sekati, UC 41/7 and UC 36/7 testers was evaluated by Santa-Catarina et al. (2019), who reported that it was possible to identify hybrid combinations that perfectly fit the Solo group and which, in terms of yield, surpassed cultivar 'Golden' by around 50%. Among these combinations are the hybrids registered in 2018.

### **Bi parental crosses and generation advance**

Considering the positive results for combining ability, viability was confirmed for inter- and intragroup cross of parents. Thus, the UENF/CALIMAN Program generated a segregating population derived from the cross between parents UC Sekati and UCJS-12, both belonging to the Formosa group, aiming at the development of lines and hybrids producing large fruits (Figure 2).

### **Generation and evaluation of F<sub>2,3</sub> families**

The Sekati × JS-12 cross originated the F<sub>1</sub> individuals, which were self-pollinated, generating the F<sub>2</sub> segregating population. The F<sub>2</sub> individuals were then selected and self-pollinated, generating thirty F<sub>2,3</sub> families. The objective of the present case was to examine and select superior lines *per se*, with a view to a future selection of promising lines and hybrids.

Regarding family selection, the best families had considerable genetic gains predicted for all traits (Table 1). It should be stressed that the best families in F<sub>2,3</sub> already showed higher values in the F<sub>2</sub> generation, which was also true compared to the 'Tainung 01' hybrid, used as a control.

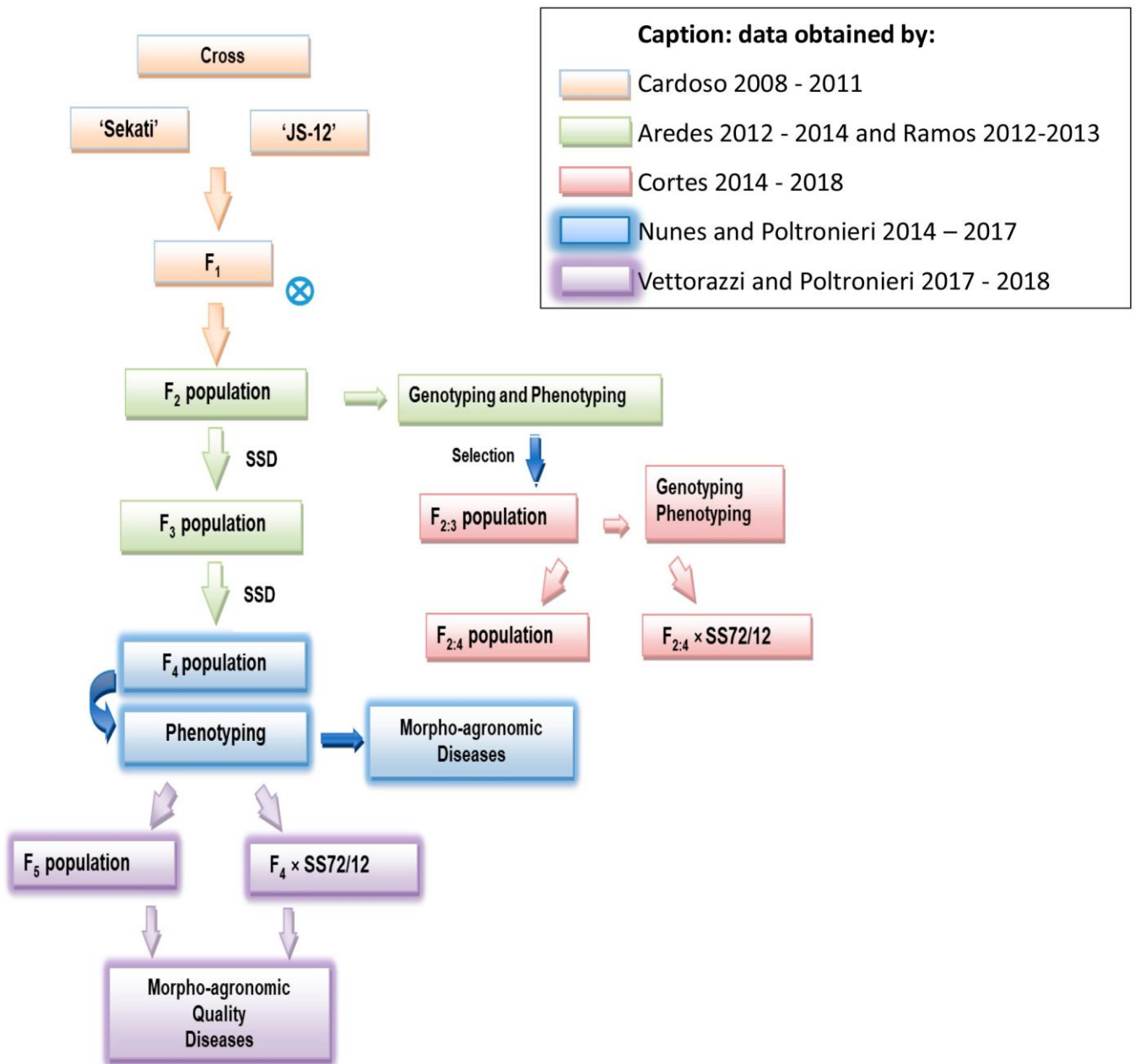
A combined selection of the families was carried out considering the traits of interest in the crop, using the additive index in Selegen software. Based on this index, the six superior families, possessing high genetic values and genetic gains, were selected. The selected families also showed satisfactory means for fruit and pulp firmness, number of marketable fruits, and deformed fruits. The presented results demonstrate the genetic potential of these families to develop superior lines.

The best F<sub>2,3</sub> families were self-pollinated, generating the F<sub>3,4</sub> families, and crossed with the UC SS-72/12 tester to evaluate the superiority of the lines based on SCA.

### **Evaluation of the F<sub>4</sub> generation**

From the F<sub>2</sub> population, two generations were advanced by the SSD (single seed descent) method up to generation F<sub>4</sub>, in which 130 lines were evaluated in a randomized-block design with six replicates, using one plant per plot (single tree plot - STP), with a view to overcoming the problems related to the demand of experimental area. The study was conducted for the selection of superior lines and analysis of experimental effectiveness using the STP methodology, which had never been used for the papaya crop.

The following traits were analyzed: plant height, stem diameter, height at the insertion of the first fruit, number of marketable fruits, number of deformed fruits, and number of fruitless nodes. These were evaluated by digital imaging, as proposed by Cortes et al. (2017). In terms of fruit quality, a study of maturation time was carried out to infer about shelf life, in addition to average fruit weight, fruit and pulp firmness, soluble solids content, and estimated yield, as described by Silva et al. (2008b).



**Figure 2.** Flowchart of the stages to obtain recombined lines and new parents from a biparental population.

**Table 1.** Components of the average of the six superior families (FA) for family production (PROD), pulp thickness (PT), and soluble solids content (SSC), mean (M), and genetic gain (GG).

Rank	PROD (kg plant <sup>-1</sup> )			PT (cm)			SSC (°Brix)		
	FA	M	GG (%)	FA	M	GG (%)	FA	M	GG (%)
1	6	58.9	43.2	22	3.2	9.7	19	8.8	10.9
2	16	47.2	32.8	2	3.1	8.5	26	8.7	10.7
3	14	47.9	28.9	15	3.1	8.0	2	8.5	10.5
4	9	44.1	26.5	8	3	7.1	22	8.6	10.2
5	5	40.4	24.7	6	3	6.5	7	8.4	9.6
6	8	40.0	22.7	23	3	6.1	24	8.3	8.9
'Tainung 01'		41.4			2.8			7.73	

The results obtained via mixed modelling enabled the selection of the best genotypes, which was based on average fruit weight. Following the study of Luz et al. (2015), the population was divided based on average weight into fruits weighing over 800 g (Formosa type - large fruit) and fruits lower than 800 g (Solo type - small fruit). In this way, it was possible to indicate cultivars capable of meeting the demands of the domestic and external markets.

Superior  $F_4$  genotypes were selected based on the Smith (1936), Hazel (1943), Pesek and Baker (1969), Mulamba and Mock (1978), and Willians (1962) selection indices; the Standardized Phenotypic Index (SFI); and the Standardized Genotypic Index (SGI) (Ramos et al., 2014). The results showed that SGI is better for ranking promising lines, given the high coincidence with other indices besides the advantages of working with the genotypic means. The 15 best Solo-type families and the 15 best Formosa-type families were ranked (Table 2).

**Table 2.** Means of families selected by the SGI index for the main agronomic traits in papaya.

SL	AFW	FF	PF	SSC	PROD	FL	AFW	FF	PF	SSC	PROD
2	736.39	123.17	88.85	13.08	23.46	4	982.28	104.80	86.15	11.49	31.90
20	555.46	95.42	83.20	12.11	21.35	14	820.09	104.22	90.31	12.20	23.00
25	742.34	126.33	103.03	10.92	19.24	15	876.99	121.97	88.56	12.49	23.92
33	583.84	127.41	94.21	13.08	17.71	18	1953.43	111.11	88.55	12.66	23.40
34	779.02	123.21	88.44	12.78	19.51	23	858.04	106.06	85.86	10.57	29.31
51	627.40	116.34	84.34	11.22	19.96	24	966.90	120.73	93.52	10.56	22.25
58	760.06	106.02	93.77	10.42	24.40	31	1095.27	130.32	89.20	9.95	30.93
70	797.07	117.81	93.21	12.13	17.65	36	1209.59	105.27	94.42	9.40	43.10
71	742.15	112.25	89.12	11.41	22.47	39	1156.81	96.65	82.26	13.44	38.65
85	620.93	134.48	77.75	13.21	20.43	56	1001.69	120.74	95.57	10.49	27.13
101	563.46	96.52	86.00	10.88	23.55	67	1380.15	120.98	92.01	10.63	22.36
107	730.07	124.67	92.81	12.05	20.19	87	849.01	116.85	91.15	13.16	20.76
121	793.10	96.82	74.14	13.12	19.75	95	980.01	114.21	90.22	11.71	26.44
127	618.06	128.25	95.09	11.89	21.55	117	920.26	111.65	88.20	11.63	22.81
128	671.07	103.90	87.25	12.17	18.79	120	853.18	129.43	95.80	10.71	23.24

SL: Solo line; AFW: average fruit weight (g); FF: fruit firmness (N); PF: pulp firmness (N); SSC: soluble solids content(°Brix); PROD: production (Kg/Planta); FL: Formosa line.

Adopting the STP design in papaya leads to great experimental plot losses, since the major field losses in this crop are due to viruses, causing experimental imbalance. For the papaya crop, it is suggested to use more than one plant per plot. However, this can be practiced in the segregating-generation advance phase, as it already allows for a mild selection throughout the generations.

Analyses for QTL mapping and identification in the studied population are being performed by associating the markers mapped in the population and the phenotyping procedure.

### Evaluation of the $F_5$ generation

The  $F_4$  lines were self-pollinated and crossed with the Solo group tester (SS-72/12), originating the  $F_5$  generation and its hybrids. The combining ability of those progeny was thus evaluated. The experiment characterized by a top-cross was composed of ninety-seven  $F_5$  lines, 62 hybrids, and 10 controls in a 13×13 Lattice design with five replicates, using two plants per plot.

An early assessment of production revealed the potential of the evaluated hybrids, whose results were superior to those of the lines and controls. This means they can positively contribute to the development of new cultivars.

### Recurrent selection

Recurrent selection is a system designed to gradually increase the frequency of favorable alleles for a quantitative trait through repeated selection cycles without there being loss of genetic variability in the population (Hull, 1945). Literature reports show the efficiency of recurrent selection in various crops like common corn, popcorn, and *Passiflora* (Rangel et al., 2011; Ribeiro et al., 2012; Freitas Júnior et al., 2013). Recently, significant gains were also observed in the papaya crop (Moreira et al., 2018).



Five progenies of dioecious papaya were used to form the base population UCP-C0, namely STA-22 (3), STA-05 (5), STA-17 (6), STA-02 (6), and STA-04 (5). These were selected and described by Vivas et al. (2012; 2013; 2014b) in terms of resistance to black spot (*Asperisporium caricae*) and to phoma spot (*Stagonosporopsis caricae*). They were recombined with each other and with five elite parents (UC SS-72/12, UC JS12, UC Sekati, UC 36/7 and UC 41/7). The elite parents were used as pollen donors to pollinate the female plants in the dioecious progeny. Thus, in addition to recombination, the dioecious progenies were sexually reversed by the incorporation of hermaphroditism. Equal amounts of seeds from the pollinated fruits were harvested and grouped, forming the UCP-C0 population.

The results show that UCP-C0 has broad genetic variability for morpho-agronomic and fruit quality traits. The fruits of the population weigh between 280 and 1890 g, encompassing both Solo and Formosa type fruits. Plants of this population also show outstanding fruit firmness, pulp firmness, and soluble solids content.

In this way, a broad-genetic-base papaya population carrying genes of resistance and with high agronomic potential for fruit yield and quality was formed. Another noteworthy factor in this population is the early elimination of the allele responsible for the male sex in plants.

Part of the seeds from the  $S_1$  progenies of the said population are currently in the field, undergoing a competition assay in a triple Lattice design with four plants per plot, for the phenotypic assessment of morpho-agronomic traits of quality and resistance aiming at future selection/recombination of superior progeny.

### Breeding aimed at disease resistance

Breeding for disease resistance is a measure of great importance for the production and sale of papaya fruit, whose market is highly demanding in terms of quality. It is also very important for the sustainability of production, which, at present, still depends on the application of agrochemicals. Additionally, breeding adds value to Brazilian papaya varieties and hybrids with higher disease resistance that have been already released/registered or are about to be released and made available worldwide.

The papaya breeding developed by UENF/CALIMAN started its works on disease resistance in 2006, when evaluations of fungal leaf diseases were then taken to the field. The evaluations and works of characterization and selection of materials resistant to fungal leaf diseases followed the different proposals and phases of the breeding program, whose main results are given next:

### Evaluation in the Germplasm bank

In evaluations using the UENF-CALIMAN germplasm, Vivas et al. (2015) reported incomplete resistance between genotypes for leaf diseases. The STZ 23 PL, Maradol, Maradol GL, JS 11, Americano, Caliman SG, Sekati, Sekati FLM, Waimanalo, Caliman AM, Papaya 46, Tailândia, and SH 12-06 genotypes are potential sources of genes with resistance to black spot, powdery mildew, and phoma spot. In another experiment, for phoma spot, they also emphasized SH 15-04 for severity in the leaf and Maradol, Maradol GL, Americano, Tailândia, and Baixinho de Santa Amália for leaf incidence (Vivas et al., 2010).

### Topcross hybrid trial

Vivas et al. (2010) evaluated tester genotypes to form hybrids resistant to black spot in leaves and fruits, phoma spot in leaves, and chocolate spot in fruits of papaya. In two experiments in an area of Caliman Agrícola S.A. (Linhares-ES), the authors evaluated hybrids obtained from crosses between 20 elite genotypes and two testers: UC SS-72/12 (Solo group) and UC JS-12 (Formosa group). In another experiment, they evaluated 38 hybrids from the cross between 23 genotypes and three testers of the Formosa group (UC Americano, UC Maradol and UC Sekati). The hybrids with the lowest incidence were Americano  $\times$  Waimanalo, Sekati  $\times$  JS-12, Maradol  $\times$  Taiwan and Maradol  $\times$  Caliman G, whereas the lowest severity was observed in Calimosa  $\times$  Tailândia, Calimosa  $\times$  Maradol, Sekati  $\times$  Caliman AM, and Sekati  $\times$  JS 12. The hybrids with negative combining ability values for all the examined traits derived from the cross of the JS-12 tester to Sunrise Solo and Kapoho Solo PV; the Americano tester to Caliman M5, Sunrise Solo, Baixinho de Santa Amália, and Waimanalo; and the Maradol tester to Caliman G, Caliman AM, and Sunrise Solo PT. These results warrant the use of breeding for disease resistance via hybridization (Vivas et al., 2010; 2011).

Poltronieri (2018) examined 10 lines derived from a backcross between the dioecious genotype Cariflora and the Sunrise Solo 783 variety (SS783), at different inbreeding levels and in different backcross generations, and another 20 hybrids derived from the cross between the 10 lines and four elite parents UC JS-12, UC Sekati, UC 41/7 and UC SS-72/12. The results pertaining to incidence and severity of black spot in the fruit and severity of black spot and phoma spot in the leaf, considering the genotypes allocated to the Solo group, included negative SCA estimates for hybrids SS-72/12  $\times$  1, SS-72/12  $\times$  4, and SS-72/12  $\times$  9. Of these three, SS-72/12  $\times$  1 and SS-72/12  $\times$  9 showed superior morpho-agronomic traits (Santa Catarina et al., 2019). For the hybrids in the Formosa group, negative SCA values for severity and incidence of

black spot in the leaf and in the fruit and for incidence of phoma spot in the leaf were observed in hybrids Sekati × 1, Sekati × 2, Sekati × 9, JS-12 × 1, and JS-12 × 17. As such, they could be used as potentially resistant material against black spot and phoma spot in the Formosa group. According to Santa Catarina et al., (2019), the Sekati × 9 hybrid is promising for number of marketable fruits, whereas the JS-12 × 17 hybrid is promising for fruit quality and traits of greater importance in the crop.

### Diallel analysis

Vivas et al. (2013) estimated GCA and SCA for resistance to phoma spot in eight papaya genotypes (four from the Solo group and four from the Formosa group) using diallel cross. The Maradol, JS12-N, Sekati, and São Mateus genotypes showed the best GCA. The Maradol × Golden, Maradol × SS-72/12, JS12-N × Golden, JS12-4 × Waimanalo, Sekati × Waimanalo, Waimanalo × Golden, Waimanalo × São Mateus, Golden × SS-72/12, and Golden × São Mateus hybrids exhibited promising results for selection for disease resistance, with negative SCA values for phoma spot severity. In the same study, the authors observed a significant dominance effect, with the possibility of exploitation of heterosis. The Waimanalo × Golden, Golden × Maradol, Golden × Waimanalo, Golden × SS-72/12, Golden × São Mateus, SS-72/12 × Waimanalo, and SS-72/12 × Golden hybrids stood out the most, with negative estimates of heterosis and heterobeltiosis for reduction of disease severity at both evaluation times. Thus, hybrids originating both from crosses within the Solo group and between heterotic groups (Solo × Formosa) could be selected for the control of phoma spot in papaya (Vivas et al., 2014a).

For resistance to black spot, the mean square values referring to GCA and SCA were significant for the traits pertaining to severity in the leaf and in the fruit, indicating that the additive and non-additive genetic effects are involved in the control of those traits (Vivas et al., 2012a). Moreover, for both studied traits, there was no reciprocal effect; i.e., the direction of the cross does not influence the result. The JS12-N, Sekati, 'Golden', and SS-72/12 genotypes exhibited the lowest GCA estimates for black spot severity in the leaf. In the fruit, however, in addition to the abovementioned genotypes, Waimanalo also presented a negative estimate for GCA. The Maradol × SS-72/12, Maradol × São Mateus, JS12-4 × Waimanalo, Sekati × Waimanalo, Sekati × São Mateus, Waimanalo × Sekati, Waimanalo × São Mateus, Golden × Sekati, Golden × SS-72/12, São Mateus × JS12-N, São Mateus × Golden, and São Mateus × SS-72/12 hybrids showed promising results for selection of hybrids with low black spot severity values, both in the leaf and in the fruit (Vivas et al., 2012a).

### Evaluation of genotypes in traditional populations for resistance to black spot and phoma spot

Vivas et al. (2012a) described a phenotypic evaluation of 24 papaya progenies derived from dioecious genotypes. For comparative purposes, one variety resistant ('Maradol') and another susceptible ('Golden') to black spot were included. Based on the estimated genetic parameters, there was sufficient genetic variability for resistance to black spot to indicate recurrent selection as the breeding method. The STA-02, STA-04, STA-08, STA-13, STA-15, and STA-16 progenies were considered promising for reducing the severity of black spot in papaya leaves. The genetic potential for resistance to black spot was evaluated via mixed modeling. The heritability and selection accuracy estimates predicted a situation very favorable to selection of resistant progenies, with STA-02, STA-04, STA-05, STA-06, STA-07, STA-11, STA-12, STA-16, STA-21 and STA-22 standing out with negative values of additive genetic effects for both disease severity and incidence in the leaves (Vivas et al., 2014b).

### Evaluation of hermaphrodite plants in a resistant dioecious population

One hermaphrodite plant with known levels of resistance to black spot and phoma spot was present in an area with the dioecious population. Considering the possibility of natural occurrence of sex reversal in male plants (XY) to hermaphroditism (XY<sup>h</sup>), Pirovani et al. (2018) investigated the origin of the hermaphrodite genotype (termed M3) to elucidate the occurrence of reversal or contamination. The authors performed analyses with molecular markers and examined morpho-agronomic and resistance attributes. The results of the analysis of microsatellite loci and of the male-specific locus discarded natural sex reversal as a cause of the origin of the M3 hermaphrodite genotype, indicating a likely contamination with pollen possibly originating from the Maradol genotype, used as a resistance control. Regarding the morpho-agronomic traits, the M3 hermaphrodite genotype exhibited good attributes for both production and fruit quality. In terms of resistance to black spot and phoma spot, it showed similar performance to that of the control used as a resistance standard (Maradol). This resistant hermaphrodite genotype can be used in new hybridizations and in the generation of stable hybrids, but more studies are needed including different seasons to confirm these results.

### Digital image-assisted phenotyping

The phenotyping of traits for breeding purposes is traditionally performed manually, which makes it a time-consuming activity that demands intensive labor, restricts the evaluation of a large number of plants, and reduces the probabilities of identifying superior genotypes (Kolukisaoglu and Thurow, 2010). The need for large-scale phenotyping in controlled environments and/or field conditions has led to a search for non-invasive methods such as image analysis and processing.

Today, the computer vision technology has been widely used to inspect the quality of agricultural and food products, replacing traditional manual operations, which are laborious, slow, and prone to human error (Pandey et al., 2013). It is also largely used to estimate the number of fruits and predict the production of fruit crops such as apple, orange, mango, grape, among others (Aggelopoulou et al., 2011; Wang et al., 2012; Payne et al., 2013; Dorj et

al., 2013; Roscher et al., 2014).

In this scenario, the team of the papaya breeding program at UENF developed studies based on digital image analysis and processing to estimate the traits of interest in the crop.

To evaluate the morpho-agronomic traits of papaya, each plant was photographed from two different positions (left and right sides). The digital images were processed using Image J 1.48 t software (Figure 3). The traits were estimated considering the same criteria adopted for the manual measurements. The data obtained via digital imaging were compared with the respective manual data. Based on the results, the means were similar between the two evaluated cultivars for all traits. The confidence intervals were also similar by both methodologies (Cortes et al., 2017).



**Figure 3.** Images used to estimate morpho-agronomic traits in plants of papaya cultivars ‘Calimosa’ and ‘THB’. (A and B) images used for the ‘UENF/Caliman 01’ hybrid; (C and D) images used for the ‘UENF/Caliman 01’ hybrid; (C and D) images used for the ‘THB’ variety; (A and C), image perpendicular to the plant, considering the line axis; (B and D), image considering the opposite side of the same plant. The orange arrows show the mark used to identify the last evaluated fruit. (Source: Cortes et al., 2017).

Quality traits were analyzed using a scanner to capture the images and obtain digital measurements. The obtained images were processed and analyzed in Image J software. The digital data were compared with the manuals to evaluate the performance of the digital methodology. The means of the traits estimated via digital imaging did not show significant differences when compared with those measured manually. Statistical data showed that this methodology can be applied to estimate those traits in a study with good precision, little labor, and reduced time (Santa-Catarina et al., 2018).

### **Early molecular sexing**

Papaya is traditionally sexed in beginning of the first flowering period, approximately three to four months after planting, when the sex can be identified by observing the flowers (Costa et al., 2003). The crop is then thinned to eliminate the female plants and leave only one hermaphrodite plant in the pit, ensuring desirable uniformity and quality of fruits for sale.

For the planting of both hybrid seeds and seeds derived from lines, not knowing the sex of a plant results in high expenses due to the higher number of seeds, seedlings, and additional services on crops in the first year of their establishment. Garcia et al. (2003) stated that planting one seedling with previously determined sex per pit would allow for a 67% economy in relation to the number of seeds produced and in services.

In view of this situation, many researchers have sought strategies to determine the sex of papaya plants as early as possible. However, no morphological, physiological, or enzymatic characteristics have been described thus far to differentiate the sex before flowering. The use of molecular markers in papaya is an interesting alternative in that it allows the identification of sex at any stage of development. A direct analysis of the genomic DNA provides a precise determination of genetic variations, thus eliminating the components of phenotypic variation and interaction between different alleles. This strategy can greatly contribute to reducing the time necessary to define the sex of the seedlings that will compose the plants of commercial interest.

Molecular markers related to the sexual character in papaya developed using the PCR technology is viewed as a reliable strategy. Some molecular markers, including RAPDs, ISSRs, and SCAR, were developed for sexual identification of papaya (Vashistha et al., 2016).

If, on the one hand, early sexing may represent economy of resources such as soil, fertilizers, water, and man labor, on the other hand, the molecular procedure of sex determination incurs relatively high laboratory costs, making these seedlings more expensive. However, when selecting a procedure, one must take into account not only the basic costs, but also the performance of the

selected plants according to each procedure. Salinas et al. (2018) worked in a protected crop and showed that the establishment of a 'BH-65' papaya crop using an SNP (single nucleotide polymorphism) molecular marker was approximately 39% more expansive than planting 'BH-65' conventionally sexed. However, the plants sexed early via SNP had a 49% higher marketable yield and produced larger fruits (in length and circumference). This finding shows that the cost of implementing molecular analyses was offset by crop yield, demonstrating the viability of using molecular markers for sexing 'BH-65' papaya. This result reflects a prospect for success also in other varieties, especially in field cultivation, which is the case in practically all commercial areas.

Despite the numerous advantages of the molecular approach, there are few literature reports about the practical use of molecular markers in the field. This is due to the relatively high costs and long time required for molecular analyses involving many plants. In this regard, the group has worked on the development of a rapid and lower-cost method (Patentable Technique), which allows for early detection of sex in a large number of seedlings, supporting producers in field works.

### **Genome analysis in papaya breeding**

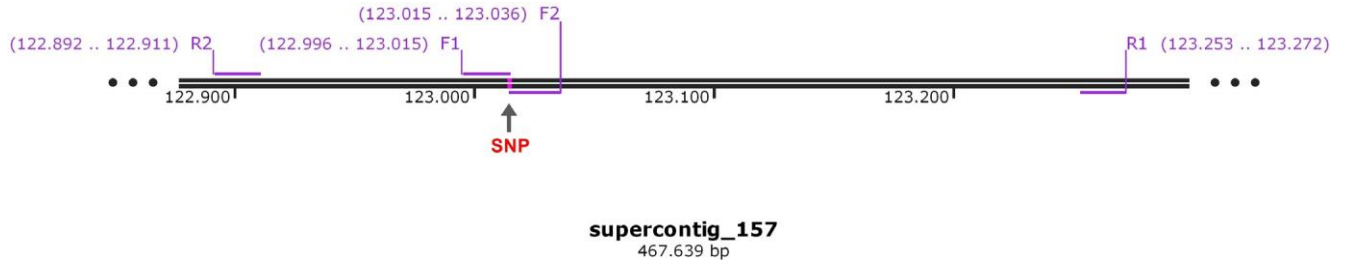
The team of researchers at UENF has used several technologies involving molecular markers, e.g., analysis of segregating populations, genetic diversity in genotypes of the UENF/CALIMAN germplasm bank, monitoring of backcross generation advance, construction of genetic maps, among others. More recently, molecular markers have been applied to understand the genetic architecture of traits of importance for the breeding of the crop as well as to identify genes involved in the expression of those traits.

The first endeavor in this sense consisted of identifying and performing a genome mapping of microsatellite (or SSR) markers, which would enable the development of an atlas of SSR markers for papaya (Vidal et al., 2014). With this information, Mendes (2017) selected SSR genetic markers (SSR-ESTs) to identify functional regions associated with the expression of pulp and fruit firmness in papaya. The results indicated eight SSR-EST loci with a polymorphic profile, making them promising for application in studies aiming at marker-assisted selection (MAS).

Another approach addressed by the team has been the identification of QTLs (quantitative trait loci) for the main morpho-agronomic and resistance traits related to phoma spot and black spot, the major diseases affecting the crop (Bohry, 2018; Poltronieri, 2018). The QTLs and QRLs are being identified based on a genetic map constructed with SNP (single nucleotide polymorphism) markers and developed by the team itself. These analyses were in the phase of adjustments and finalization; however, based on preliminary results, there has been an

advancement in the development of SNAP markers (single nucleotide amplified polymorphism), which enable the identification of single-base polymorphisms using PCR.

The papaya breeding team at UENF has worked on the development and validation of SNAP markers based on SNPs associated with the QTLs (Figure 9) identified for the above-described traits.



**Figure 4.** Schematic representation of the annealing sites of the four primers used in the genotyping of a SNP.

The validation of SNAP markers revealed efficiency in discriminating the different alleles of an SNP, possibly detecting even both alleles of a specific SNP in a single PCR reaction (Bohry, 2018). When associated with the classical papaya breeding, this primer-designing methodology will provide greater efficiency and precision in the selection of superior genotypes, using SNPs associated with QTLs that explain most part of the phenotypic variation for quantitative traits of greater importance for the breeding of the crop.

By identifying QTLs, progress can be achieved in identifying candidate genes involved in the expression of the traits of interest, elucidating the genetic architecture and expression of those traits. The base for these studies has been the papaya genome sequence, which is available for studies in two databases the National Center for Biotechnology Information (NCBI) and Phytozome, where approximately 20 thousand genes identified in the genome can be visualized. These data are highly valuable, as they make it possible to identify the genes of a given genomic region, such as those representing a QTL. In this way, the researcher can easily obtain a list of the genes present in a specific region.

The current prospect is that these studies will further elucidate the genetic structure, paving the way to a better understanding of the mechanisms of expression of important traits for papaya breeding programs. This knowledge will also enable the establishment of an effective and efficient marker-assisted breeding strategy, increasing the accuracy of the selection process and reducing the time required for the development of superior cultivars.

## Conclusions

The presented results show that the papaya breeding program at UENF in a partnership with CALIMAN Agrícola S.A. has been very effective in generating scientific knowledge, forming human resources, and yielding technological results very well represented by the developed cultivars. It is worth emphasizing the set of cultivars developed and registered at MAPA, with a total number of 21 hybrids. They encompass the entire array of average fruit weight, which ranges from the typical Solo type, with approximately 300 to 560 g (‘UC20’ to ‘UC28’) and intermediate values (700 to 900 g - ‘UC14’ and ‘UC16’), to the Formosa type, with an average fruit weight of 1 kg (‘Calimosa’) to 2 kg (‘UC10’). In the next few years, complementary information is expected to be developed regarding the regions of adaptation of this set of hybrids to better meet the demands of national farmers and, consequently, national and international consumers. It is also noteworthy that, with the continuation of the program, new cultivars are expected to be developed in the short, medium, and long terms, particularly with segregating populations and recurrent selection.

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