FRUIT CROPS
SCIENCE JOURNAL

Functional Papaya Breeding Program: Strategies, Achievements and Challenges

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Abstract: The functional papaya breeding program at UENF, in partnership with CALIMAN Agrícola S.A. company, was established in 1996 to develop superior cultivars, generate scientific knowledge, and forming papaya breeders. Brazil, the fourth-largest global papaya producer, relies on papaya breeding program to reduce dependence on imported seeds and enhance disease resistances. The program employs advanced and classical breeding strategies, including mating designs, backcrossing for sexual conversion, and recurrent selection. Significant achievements include the development of 21 registered hybrids, addressing the demand for different fruit sizes. Genetic studies identified key heterotic groups, demonstrating the viability of intragroup and intergroup hybridizations. Molecular approaches, such as QTL mapping and marker-assisted selection, contribute to papaya breeding. Additionally, digital phenotyping enhances precision in trait evaluation. Despite progress, challenges remain, including resistance to viral and fungal diseases, genetic purity maintenance, and the need for improved breeding efficiency. Future strategies involve genomic tools like CRISPR, participatory breeding, and recurrent selection to accelerate genetic gains. The program plays a crucial role in strengthening Brazil's papaya industry, ensuring high-yield, disease resistant cultivars that meet market demands while advancing genetic research and breeding methodologies.

Index terms: *Carica papaya* L.; breeding strategies; cultivars development; fruit quality; fruit yield; disease resistance.

Melhoramento Genético Funcional do Mamoeiro: Estratégias, Conquistas e Desafios

Resumo: O programa de melhoramento funcional do mamoeiro da UENF, em parceria com a empresa CALIMAN Agrícola S.A., foi estabelecido em 1996 para desenvolver cultivares superiores, gerar conhecimento científico e formar melhoristas. O Brasil, o quarto maior produtor mundial de mamão, depende do programa de melhoramento para reduzir a dependência de sementes importadas e buscar a resistência a doenças. O programa emprega estratégias de melhoramento avançadas e clássicas, incluindo delineamentos genéticos, retrocruzamento para conversão sexual e seleção recorrente. Conquistas significativas incluem o desenvolvimento de 21 híbridos registrados, atendendo a demanda por diferentes tamanhos de frutos. Estudos genéticos identificaram aspectos importantes sobre grupos heteróticos, demonstrando a viabilidade de hibridizações intra e intergrupo. Abordagens moleculares, como mapeamento de QTL e seleção assistida por marcadores, contribuem para maiores avanços no melhoramento do mamoeiro. Além disso, a fenotipagem digital aumenta a precisão na avaliação de características. Apesar do progresso, os desafios permanecem, incluindo resistência a doenças virais e fúngicas, manutenção da pureza genética e a necessidade de maior eficiência de melhoramento. Estratégias futuras envolvem ferramentas genômicas, como CRISPR, melhoramento participativo e seleção recorrente para acelerar ganhos genéticos. O programa desempenha papel crucial no fortalecimento da indústria de mamão do Brasil, garantindo cultivares de alto rendimento e resistentes a doenças que atendam às demandas do mercado, ao mesmo tempo em que avançam a pesquisa genética e as metodologias de melhoramento.

Termos para indexação: *Carica papaya* L.; Estratégias de melhoramento; desenvolvimento de cultivares; qualidade de frutos; produtividade; resistência a doenças.

Introduction

The papaya tree (*Carica papaya* L.) is one of the most economically important fruit trees for tropical and subtropical regions of the world, and is widely known for its nutritional and medicinal benefits. The fruits are rich in vitamin A, vitamin C, potassium, folate, niacin, thiamine, riboflavin, iron, calcium and fiber (HUERTA-OCAMPO et al., 2012).

Brazil stands out on the international scene as the fourth largest producer, with a total annual production of 1.2 million tons in 2022, corresponding to 8.9% of world production (FAOSTAT, 2024). In the country, the states of Espírito Santo and Bahia stand out, representing approximately 67% of national production (IBGE, 2024). Despite the great national and international crop importance, there are few breeding programs aimed at developing cultivars. Therefore, until recently, 100% of Brazilian areas cultivated with hybrids depended on seed imports.

In accordance with the above considerations, it is clear that there is a need to strengthen papaya breeding programs, which, with short, medium and long-term aims, contribute to expanding the current genetic base, generating cultivars with tolerance or resistance to the main diseases, in addition to presenting desirable agronomic traits, aiming to meet the demands of the national and international markets.

Therefore, the Universidade Estadual do Norte Fluminense Darcy Ribeiro - UENF established, in 1996, a papaya breeding program in partnership with the company CALIMAN Agrícola S.A., one of the largest producers and exporters of this fruit. The program was established with the aim of developing new cultivars, generating scientific knowledge and training human resources capable of contributing substantially to the papaya production chain.

The paper aim is to provide a history of this program, highlighting the strategies used in terms of breeding procedures, the main results obtained and the challenges to be overcome.

Material and Methods

When this program was established, it was found that there was no basic information in the literature on the main attributes inheritance, reproductive species aspects, the combining ability of the available germplasm, among other information that are essentials to obtain the desired results in the short, medium and long term.

Papaya is a fruit species with peculiar aspects in terms of reproduction; therefore, specific strategies must be designed for its breeding. Before establishing the types of cultivars to be developed, it is necessary to know their reproductive characteristics such as allogamy, autogamy, the percentage of cross-pollination, etc., as well as the inheritance of the main agronomic traits. In this sense, several studies were carried out to support of papaya breeding strategies. The karyotype and other reproductive aspects were characterized according to methodologies described by Damasceno Júnior et al. (2008a; 2009a; 2009b) and Freitas Neto (2010).

With regard to the inheritance of the main agronomic traits, the breeding design of generational means analysis involving the parents Maradol and Baiximo de Santa Amalia with the respective generations F_1, F_2, $RC_{1.1}$ and $RC_{1.2}$ was used (CATTANEO, 2001).

As for the studies of combining ability, as well as the definition of heterotic groups, several classical breeding strategies were used. Considering information from the literature that there are two heterotic groups in papaya: the Solo group consisting of genotypes with smaller fruits, approximately 400 to 800 g, and the Formosa group consisting of genotypes with large fruits, approximately above 1000 g, the first study of combining ability was based on the partial diallel mating design. In this sense, eight genotypes from each heterotic group were used, totaling 64 hybrid combinations as described by Marin et al. (2006a; 2006b).

Still on the subject of combining ability, considering the scarcity of information, aiming to better investigate whether the Solo and Formosa groups actually constitute heterotic groups or whether it is possible to obtain superior hybrids in intragroup crossings, a complete diallel mating design was used, consisting of eight genotypes, four from the Solo group and four from the Formosa group. Thus, 28 F_1 hybrids and their respective reciprocals were generated, including the Solo versus Solo, Solo versus Formosa and Formosa versus Formosa combinations, in addition to the respective self-fertilizations, as described by Vivas et al. (2012a) and Cardoso et al. (2014).

As for the development of superior hybrids, in addition to the partial and complete diallel mating designs, top cross trials were also used using unrelated and narrow-base testers as described by Ide et al. (2009); Barros et al. (2017); Cortes et al. (2019); Santa Catarina et al. (2019); Poltronieri et al. (2020); Miranda et al. (2021) and Vettorazzi et al. (2021).

In order to continue the generation of new parents, classical strategies for developing pure lines were also sought, with biparental crosses followed by generation advancement via the genealogical method (CORTEZ et. al., 2019; MIRANDA et al., 2021; VETTORAZZI et al., 2021a; 2021b; SANTANA et al., 2021). Backcrossing followed by the genealogical method was also adopted (SILVA et al., 2007a; 2007b; 2007c; 2008a; 2008b; RAMOS et al., 2011a; 2011b; 2014). Another classic strategy was population improvement, using recurrent selection of S_1 progenies, as described by Santa Catarina et al. (2020a; 2020b), and by Pereira and Santa Catarina et al. (2021).

Another aspect implemented in this breeding program was genomic and DNA marker analyses as auxiliary tools in selection procedures. In this sense, an Atlas of SSR markers (VIDAL et al., 2014) was constructed and QTLs (quantitative trait loci) were identified for the main morpho agronomic and resistance traits related to phoma spot and black spot, the main diseases that affect papaya crops (BOHRY et al., 2021). The QTLs and QRLs are being identified from a genetic map constructed with SNP (single nucleotide polymorphism) markers and developed by the team itself (BOHRY et al., 2021).

Furthermore, considering that, over the years, the hybrids developed tend to lose genetic purity, a work of purification and production of genetic seeds of the main hybrids developed and used by producers was also implemented. This research not only aimed to increase the level of homozygosity of the respective parents, but also sought to take advantage of their possible genetic variability to select and fix the individuals with the highest breeding values in relation to the agronomic traits of greatest importance to meet the demands of producers and consumers. Plants were selected at field and laboratory levels using DNA markers, followed by progeny and combining ability testing as described by Pirovani et al. (2022) and Rodrigues et al. (2023).

In line with the program, Image-based phenotyping methodologies were developed by Cortes et al. (2017) and Santa Catarina et al. (2018) and are being used to support data collection at field and laboratory levels, contributing to rapid and accurate assessments.

Results and Discussion

The cultivated form (*Carica papaya* L) and the related wild species *Vasconcellea monoica* and *V. Cundinamarcensis* exhibited 2n = 18 chromosomes, with symmetrical and similar karyotypes, presenting small and metacentric chromosomes (DAMASCENO JUNIOR et al., 2009a). It was also observed that the sex chromosomes in the three studied species are homomorphic, as described by MING et al. (2007). Regarding meiotic behavior, it was regular in all species, confirming their diploid nature, and it can be inferred that the species are cytologically stable. The results indicated that the preferred reproductive system of the hermaphrodite papaya plant is facultative autogamy with cleistogamy (DAMASCENO JUNIOR et al., 2008b; 2009b; FREITAS NETO, 2010).

The analyses results based on means and variances suggest that the variances attributed to additive effects were of greater magnitude than the variances attributed to dominance effects for all the evaluated traits. The gene effects attributed to the mean and the additive genetic effects were the ones that contributed most to explaining the variability available by the additive dominant model. The large magnitude of the gene's additive effects in relation to the dominance deviations for the traits number of fruits and fruit weight suggest that intrapopulation breeding, i.e., development of pure lines, can lead to gains in these traits. On the other hand, the dominance deviations of greater magnitude for fruit production per plant suggest the viability of using hybridizations as a strategy to increase productivity. Particularly for yield, higher values are obtained through crossing and through the production of hybrids. Therefore, based on the genetic analysis of the main traits, both pure line and hybrid cultivars can be explored and developed in papaya breeding programs.

Based on the heterotic groups Solo and Formosa, Marin et al. (2006a; 2006b) explored heterosis in papaya through hybrids obtained in partial diallel crossing, resulting in the generation of the first nine registered Brazilian papaya hybrids, with the names UENF/CALIMAN 01 to UENF/CALIMAN 09. This work constitutes a milestone in papaya cultivation in Brazil, as it is a pioneer in terms of exploring hybrid vigor and developing commercial hybrids.

Regarding the complete diallel results that allowed the generation of intragroup and intergroup hybrids, the results were quite interesting. The existence and feasibility of exploiting heterosis were demonstrated in all types of crosses: intragroup Solo, intragroup Formosa and intergroup Solo versus Formosa. It was therefore shown that the Solo and Formosa groups do not typically constitute distinct heterotic groups. As a consequence, associating this versatility of crosses and the predominantly additive gene action for fruit weight, it is possible to produce a greater variety of hybrids in terms of fruit size. In the intragroup Solo crosses, the resulting hybrids tend to produce smaller fruits, from 300 to 600 g. Intragroup Formosa crosses, in turn, generally produce hybrids whose fruits weigh approximately 2000 g. Intergroup crosses generate intermediate to large fruits, producing fruits predominantly from 800 to 1200 g. Such information is crucial since the consumer market has good acceptance for both large and smaller fruits, thus making it possible to fully meet the consumer market's needs, always exploring hybrid vigor, with significant advantages in relation to traditional pure-line varieties, notably in the Solo group (VIVAS et al., 2011; 2012a; CARDOSO et al., 2014).

In addition to fruit weight, Vivas et al. (2013) found that some hybrids showed promising results for selection aiming at disease resistance, with negative values of combining ability for severity of phoma spot. In the same study, the authors observed a significant dominance effect, with the possibility of exploiting heterosis. Vivas et al. (2014b) found that hybrids originating from both intragroup and intergroup crosses could be selected for the control of phoma spot in papaya. For resistance to black spot, Vivas et al. (2012c) found that additive and non-additive genetic effects are involved in the control of these traits. Furthermore, for both studied traits, there was no reciprocal effect; that is, the cross direction does not influence the result. Some hybrid combinations showed promising results with low values of black spot severity, both in the leaf and in the fruit (VIVAS et al., 2012c).

The backcrossing method was used to sexual conversion of the dioecious genotype Cariflora, by transferring the region responsible for hermaphroditism (Sunrise Solo 783 used as donor parent), located on the Y^h chromosome (MING et al., 2007), obtaining gynoecious-andromonoecious lines. It is worth mentioning that the Cariflora genotype was used in the partial diallel study conducted by MARIN et al. (2006a; 2006b), standing out in terms of combinatory ability, especially for yield. However, being a dioecious genotype, it does not allow the generation of pure line-type parents, hence the need for the sexual conversion described here, through the backcrossing procedure.

As described by Silva et al. (2007a), backcrossing for sexual conversion of the Cariflora parent was assisted by molecular markers in the RC_1 and RC_2 generations, allowing the selection of plants with a high degree of similarity to the recurrent Cariflora parent. Several studies have been carried out to associate classical breeding with molecular markers for the development, evaluation and selection of segregating populations derived from RC_1 and RC_2 (SILVA et al., 2007b; 2007c; 2008a; 2008b; RAMOS et al., 2011a; 2011b). Ramos et al. (2012) determined the genetic distance between lines derived from the RC_1, RC_2 and RC_3 generations aiming at the selection of genotypes via combined selection and molecular markers, and generation advancement by self-fertilization (RAMOS et al., 2014a; 2014b).

The obtained lines were tested for combining ability by Poltronieri et al. (2020) and found estimates of negative combining ability for the incidence and severity of black spot on the fruit and severity of black spot and phoma spot on the leaf, for the hybrids SS-72/12×UCLC01-01, SS-72/12×UCLC01-04, SS-72/12×UCLC01-09, Sekati×UCLC01-01, Sekati×UCLC01-02, Sekati×UCLC01-09, JS-12×UCLC01-01 and JS-12×UCLC01-17. According to Santa Catarina et al. (2019) the same hybrids SS-72/12×UCLC01-01, SS-72/12×UCLC01-09, Sekati×UCLC01-09 and JS-12×UCLC01-17 showed good estimates of combining ability for morphoagronomic traits related to fruit quality and production.

As a result of the use of complete diallel mating design, topcross trials with the use of testers and backcrossing for sexual conversion of the Cariflora parent, four more hybrids were released in 2014: UC10, UC12, UC14 and UC16 (LUZ et al., 2015), and in 2018, eight more hybrids were released, named UC 20, UC 21, UC 22, UC 23, UC 24, UC 25, UC 27 and UC 28, totaling 21 hybrids. Together, these hybrids can meet the demand of farmers who prefer to grow Solo standard fruits, fruits weighing smaller than 300 to 600 grams, intermediate fruits weighing between 700 and 900 grams and Formosa standard, fruits weighing between 1000 and 2000 g. Such hybrids will certainly bring new options to the producer and consumer market in terms of size, quality and fruit yield.

In terms of results associated with advances in generation and development of lines, from the biparental cross between the parents UC-JSF × UC-SKF, generations F_1 to F_5 were obtained (Figure 1). Cortes et al. (2019) selected six superior $F_{2:3}$ lines using a genealogical method with high breeding value and genetic gains with satisfactory averages and higher than the average of the Tainung01 hybrid, for fruit production, pulp thickness and soluble solids content. The selected progenies presented production ranging from 40 to approximately 59 kg plant^{-1}. The selected fruits have a thicker pulp (3.0 to 3.2 cm) and a higher soluble solids content (8.3 to 8.9 °Brix), higher averages when compared to the Tainung 01 hybrid with 2.8 cm and 7.7 °Brix. The genetic gain with the progeny's selection was on average 10%. The F_2 population was advanced two generations using the Single Seed Descent (SSD) method, up to the F_4 generation. In this generation, 130 F_4 lines were evaluated in a Single Tree Plot (STP) design, and the 15 best Solo type families and the 15 best Formosa type families were selected via mixed model. The STP design in papaya resulted in large losses of experimental plots, since the greatest field losses in this crop are due to viruses, causing experimental imbalance. For papaya crops, it is suggested to use more than one plant per plot, with the ideal minimum number of four plants per plot. However, this method can be used in the advanced generation phase of segregating populations, as it allows for smooth selection over generations.

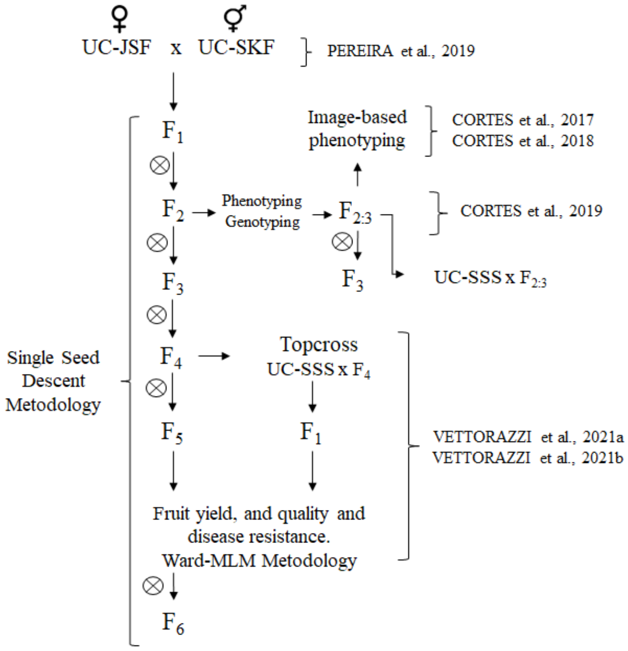

Figure 1. Bi-parental crossing applied in the UENF-CALIMAN papaya breeding program. Flowchart of the steps to obtain recombined lines and new parents.

Individuals from the F_4 population were self-fertilized and crossed with the tester UC-SS7212 from the Solo group (VETTORAZZI et al., 2021a; 2021b), giving rise to the F_5 generation with 97 lines and 62 hybrids. Vettorazzi et al. (2021a) carried out a genetic diversity study of the 97 lines using the Ward-MLM methodology and obtained the formation of three distinct groups mainly for traits related to fruit size. Groups I and III were the closest (13.25), while groups II and III were the most distant (21.31). In the same work, Santana et al. (2021) identified lines 96, 61, 39, 1, and 88 as sources of favorable alleles for the traits of average fruit weight, pulp volume, external and internal fruit firmness, pulp yield, and soluble solids; and lines 129

107, 19, 61, 63 are described as ideotypes for use per se. Vettorazzi et al. (2021b) carried out a study of the combining ability in 62 hybrids for traits related to fruit production and identified a hybrid with good combining ability for plant height, fruit insertion height, and fruit production per plant, which is promising for papaya breeding. Miranda et al. (2021) evaluated the 62 hybrids potential for fruit quality and production, selecting eight hybrids with satisfactory gains in pulp and fruit firmness, soluble solids content, and pulp percentage. Among the eight hybrids selected by Miranda et al. (2021), one of them corresponds to the one selected by Vettorazzi et al. (2021b).

In papaya breeding, the development of segregating populations with broad genetic variability is essential for the selection of new cultivars. In this sense, Santa Catarina et al. (2020a) developed the UCP-C0 population, a base population for recurrent selection in papaya, with genetic potential for fruit production and quality. UCP-C0 was obtained through the recombination of five dioecious papaya progenies with resistance to black spot (*Asperisporium caricae*) and phoma spot (*Stagonosporopsis carica*) (VIVAS et al., 2012b; 2013; 2014a), with five elite parents used in commercial hybrids, as source of genes for fruit production and quality.

UCP-C0 population showed high genetic variability among the evaluated individuals. Studies carried out by Santa Catarina et al. (2020a; 2020b) identified individuals from this population with genes associated with traits of greater agronomic relevance. The plants produced fruits with an average weight ranging from 305 g to 1780 g, and the bunches showed differences in traits such as number of commercial fruits, fruit firmness, fruit weight, soluble solids content, and fruit yield. For selection purposes, individuals belonging to clusters II, III, and XI stand out as the most suitable, as they represent sources of alleles of interest for the most important crop traits. These individuals can be used in long-term recurrent selection programs, aiming to increase the allele frequency for these desirable traits. In the short term, however, these same individuals can be selected early, allowing generations to advance with the aim of developing lines per se and/or parents for the development of hybrids (SANTA CATARINA et al., 2020a; 2020b).

Aiming to gradually increase the alleles frequency of interest in UCP-C0, it was subjected to recurrent selection (Figure 2).

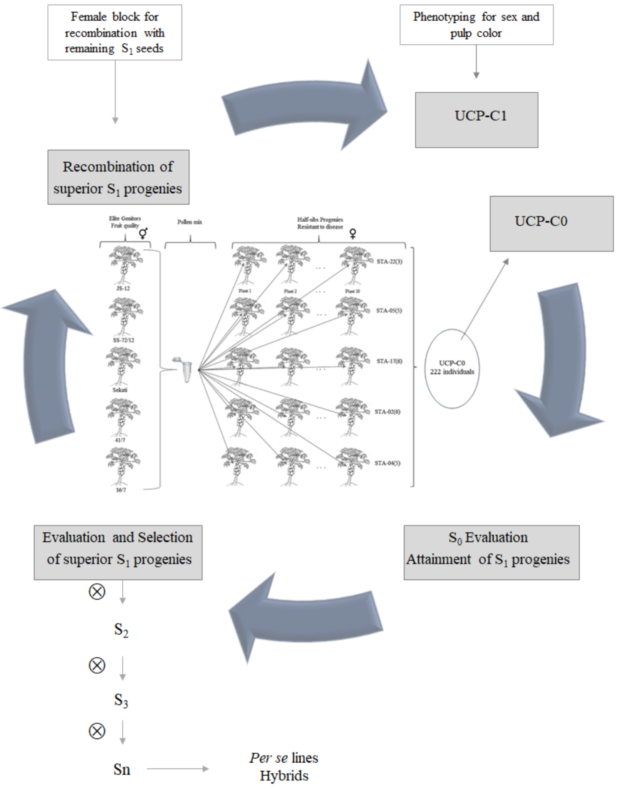

Figure 2. Recurrent selection program in papaya. Recombination to form the UCP-C0 population, using elite parents and dioecious progenies, obtaining and evaluating S_1 progenies, selecting the superior S_1 and, finally, their recombination. In parallel with the recurrent selection, the S_1 lines advance to the Sn generations, generating superior lines and/or parents of new hybrids, (PEREIRA & SANTA CATARINA, 2021).

A total of 196 S_1 progenies were obtained and evaluated in terms of plant development, fruit production and quality, and the 40 best S_1 progenies were selected via combined selection. According to Pereira and Santa Catarina (2021), the selected progenies have high genetic potential for fruit production per axil. Some progenies produce two to three fruits per axil, producing up to four or five fruits. Another interesting trait highlighted by Pereira and Santa Catarina (2021) is the presence of genotypes with a phenotype of fruits with light skin. This trait is important because the fruits tend to have fewer physiological spots. The 40 selected progenies were recombined to obtain UCP-C1, the base population for the second cycle of recurrent selection. Concomitantly with the recombination stage, the 40 superior S_1 progenies were evaluated and eight $S_{1:2}$ progenies were selected for generation advancement to S_n.

Regarding the purification of hybrid parents, Pirovani et al. (2022) and Rodrigues et al. (2023) carried out the mass selection of 400 plants from some parents of commercial hybrids. In the analysis of genetic diversity, it was possible to verify the existence of variability in the parents, enabling the selection of lines with potential for adjustment in the fruit weight of commercial hybrids (PIROVANI et al., 2022). At the molecular level, Rodrigues et al. (2023), observed the existence of genetic variability in two of the three analyzed parents, with a satisfactory result regarding the fixation of alleles, where 73.25% (293) of the genotypes analyzed presented the maximum value (F=1). The existence of genetic variability observed is probably due to management during the seed multiplication stages, where contamination of pollen grains from other genotypes may occur. Based on the observed variability, 80 progenies were obtained and evaluated in progeny testing. Twenty lines were selected from the best individuals within the best progenies, based on agronomic data associated with allelic fixation level data. These lines will be used as parents in crosses aimed at obtaining and subsequently evaluating and selecting hybrids with fruit size most suited to the consumer market.

Genomic mapping of microsatellite markers allowed the development of an atlas of SSR markers for papaya (VIDAL et al., 2014). The SSRs were distributed into four categories of genomic locations, Exon with 5344 (3.33%), Exon-intron with 73 (0.05%), Intron 15814 (9.86%) and Intergenic 139,087 (86.76%) (VIDAL et al., 2014). With this information, Mendes (2017) indicated eight SSR-EST loci with a polymorphic profile, making them promising for application in studies aiming at marker-assisted selection for traits associated with fruit and pulp firmness.

Another approach taken by the team was the identification of QTLs (quantitative trait loci) for the main morpho agronomic and resistance traits related to phoma spot and early blight, the main diseases that affect papaya crops (BOHRY et al., 2021). The QTLs and QRLs are being identified from a genetic map constructed with SNP (single nucleotide polymorphism) markers and developed by the team itself (BOHRY et al., 2021). A total of 12,790,090 sequence reads were obtained, with lengths ranging from 31 to 251 bp. From these results, 28,451 SNPs and 1,982 InDels were identified, of which 1,061 were insertions and 921 were deletions (BOHRY et al., 2021). SNPs were identified in all nine papaya chromosomes, with the highest concentration on chromosome 4, which has 3,375 SNPs, and the lowest on chromosome 5, with 1,751 SNPs. In addition, 8,079 SNPs, corresponding to 28.4% of the total, were found in contigs and scaffolds that have not yet been associated with any known papaya linkage group, being attributed to unidentified chromosomes. The validation of SNAP markers revealed efficiency in discriminating the different alleles of a SNP, possibly even detecting both alleles of a specific SNP in a single PCR reaction (BOHRY et al., 2021). When associated with classical papaya breeding, this primer design methodology will provide greater efficiency and accuracy in the selection of superior genotypes, using SNAPs associated with QTLs that explain most of the phenotypic variation for quantitative traits of greatest importance for papaya breeding.

Digital phenotyping through digital image analysis and processing developed by Cortes et al. (2017) and Santa Catarina et al. (2018) has helped in the papaya breeding program, increasing the efficiency in the selection of superior genotypes. For the phenotyping of morpho-agronomic traits of papaya at the field level, the methodology developed and validated by Cortes et al. (2017) is used. In this methodology, the traits were estimated considering the same criteria adopted for manual measurements. The means obtained do not present a significant difference in relation to traditional phenotyping and the confidence intervals were similar for both methodologies. Cortes et al. (2017) emphasize that in commercial cultivars with uniform fruiting along the plant stem, it is enough to simply count the fruits on one side of the plant (a single image) and multiply by two, while in segregating genotypes that do not present uniform fruiting, it is necessary to add the two sides of the plant (two images – Side A and Side B).

For laboratory phenotyping, the methodology developed and validated by Santa Catarina et al. (2018) is used. In this methodology, the quality traits were analyzed using a scanner to capture the images and obtain digital measurements. The images obtained were processed and analyzed in the ImageJ software. The digital data were compared with the manual ones to evaluate the performance of the digital methodology. The traits mean estimated by digital image showed no significant differences when compared with the manual measurements. The statistical data showed that this methodology can be applied to estimate these traits in a study with good precision, little work and reduced time, assisting the program in the identification and selection of superior genotypes.

Conclusion

UENF-CALIMAN papaya breeding program presents outstanding achievements for significant advances in productivity, fruit quality and disease resistance. The main advances include the development of the first national papaya hybrids, a pioneering development in Brazil. In total, 21 hybrids were developed and registered, meeting the full range of preferences of producers and consumers, both for the domestic and foreign markets, with hybrids from small fruits (400 g) to large fruits (2000 g). The scientific knowledge generated in this program is also noteworthy, from the inheritance of the main agronomic attributes, methodological aspects such as plot size (minimum of four plants per plot), as well as a better understanding of heterotic groups, showing that the denomination of Solo groups (small fruits) and Formosa heterotic group (large fruits) is inappropriate since intra and inter-group hybrid vigor is observed, with clear possibilities of obtaining hybrids in the entire range of fruit sizes demanded by the production chain of this fruit tree. Also noteworthy is the training of human resources, with dozens of thesis and dissertations, contributing to the breeders trained in this program being able to work in both private and public companies, continuing the work of functional papaya breeding program, with a clear focus on the development of new cultivars. Despite the advances already achieved, there are still many challenges to be overcome in the context of papaya breeding, which can be highlighted in the following areas:

a) Cultivars resistant to phoma spot and black spot diseases: Despite progress in the development of moderately resistant hybrids and genotypes, controlling these diseases remains challenging. With the evolution of pathogens, new races emerge that can reduce the effectiveness of developed resistances, requiring constant monitoring and the introduction of new sources of resistance.

b) Cultivars resistant to viral diseases: Viral diseases are the main causes of early losses in papaya crops. Often, before the crop completes the cycle, more than 30% of the plants have already been eliminated, if not 100% of the crop. Considering the difficulties of resistance sources, papaya breeding program should prioritize all possible strategies, such as identification of more resistant/tolerant genotypes, interspecific crossings and gene editing. Viral diseases also compromise the response capacity and success of breeding programs. Experimental losses caused by viruses often compromise the consistency of experiments and make it difficult to accurately evaluate lines and hybrids. These losses can lead to inconsistent results, reducing the selection process efficiency and delaying the development of superior cultivars. Experimental strategies, such as using more plants per plot to compensate for these losses, significantly increase management costs and make logistics difficult, especially in large-scale genetic breeding programs. In addition, controlling vectors of viruses in experimental areas is challenging due to high infestation and the need for intensive practices, such as frequent spraying and constant monitoring. The most efficient alternative to minimize the impact of viruses is to implement integrated management of experimental and commercial areas. This management should include the use of physical barriers, chemical and biological control, agricultural zoning, crop rotation, and sanitary management.

c) Genetic purity: Reducing genetic variability in hybrid parents is a significant challenge for papaya breeding programs, as it compromises progress in obtaining new genotypes with desirable traits, such as productivity, disease resistance and adaptation to different environments. Genetic contamination by pollen during seed multiplication can compromise the genetic purity of parents, requiring greater rigor in management, such as strict isolation of seed production areas, control of pollination practices, constant genetic monitoring, team training and improved technical management:

d) Maintenance of germplasm banks: The conservation of genetic resources in germplasm banks is essential to ensure the availability of genetic variability for future programs. These banks must be maintained under rigorous conditions and protected against genetic losses.

e) Digital phenotyping and automation: The expanded use of digital phenotyping in the field and in the laboratory will increase the efficiency and accuracy in the collection of phenotypic data, especially in segregating genotypes. The incorporation of image processing and artificial intelligence tools can further refine selection criteria.

f) Breeding efficiency: The search for greater efficiency in papaya breeding program faces significant challenges related to precise selection in segregating populations and the balance between multiple desirable traits. These aspects require the application of more integrated and innovative strategies to accelerate genetic progress, and meet the needs of the producer and consumer markets. To this end, some approaches can be adopted: i) Selection assisted by molecular markers that allows the identification and selection of individuals with alleles associated with disease resistance in the initial generations, reducing the number of susceptible plants in the process. ii) Implementation of efficient methods for evaluating disease resistance in the first generations, using greenhouses or fields under high inoculum pressure, improves the accuracy of selection. iii) Techniques such as CRISPR-Cas9 can be used to develop resistant genotypes by editing directly the elite genitors, accelerating population breeding procedures and obtaining resistant hybrids. iv) Simultaneously improving complex traits, such as disease resistance, productivity and fruit quality, is one of the greatest challenges in plant breeding. These traits often present antagonistic genetic correlations, which makes it necessary to strike a balance between program aims. The use of weighted selection indices allows combining multiple criteria into a single metric, prioritizing genotypes with balanced performance across different traits. Genomic prediction models use information from genetic markers to predict the performance of genotypes in relation to several traits simultaneously, increasing selection efficiency. v) Participatory breeding, seeking information from producers and consumers, can help define clear priorities for specific traits during the selection process, ensuring that the program meets real market demands.

Success in breeding depends on an integrated approach that combines biotechnological tools, advanced statistical and phenotyping methods, and practical management strategies. In this way, it is possible to achieve genotypes that meet the standards required by the market, while optimizing the use of available resources and increasing the sustainability of the program.

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