Detection of quantitative trait loci for physical traits of cashew apple

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ABSTRACT - The identification of loci controlling quantitative traits (QTL) and marker-assisted selection have attracted great interest in breeding programs for fruit quality. This study aimed to identify QTLs related to the quality of cashew apple. Physical analysis were carried out in cashew fruits of 66 F1 plants from the cross of CCP 1001 x CP 96. Seven traits related to color, size and weight of the cashew apple were tested for QTLs. Candidate QTLs were identified by the methods of interval mapping and multiple QTL mapping. Thirty QTLs associated with cashew quality were identified. The lowest number of QTL (two) was detected for the trait red intensity, and the highest (seven) for cashew apple weight. The QTLs explained between 3.15% and 21.33% of the total phenotypic variation and are being evaluated for future use in marker-assisted selection.

Key words: Anacardium occidentale, QTL, breeding, cashew quality, molecular markers.

INTRODUCTION

An important goal in cashew (Anacardium occidentale L.) breeding is to develop cultivars with improved quality of the cashew apple. The consumption of cashew apple as fresh fruit has increased considerably from season to season, opening up new markets and consolidating the traditional ones. Physical traits of the peduncle such as weight, shape and color are essential for the acceptance by cashew consumers. Based on the existing genetic variability, cashew apples can be selected that meet the needs of marketing, in terms of pear shape, easy packaging, orange to red color, and large size, i.e. cashew/tray classification 4 or 5, which has the highest market value (Moura et al. 2001).

To meet the demand for new types with better fruit quality, breeders need new methods and strategies to increase efficiency and reduce the time required to establish new cultivars. Most traits of economic importance, e.g. yield, are classified as quantitative. Even traits believed to have simpler inheritance, such as disease resistance, can be considered semi-quantitative, because their expression may be controlled by several genes, one of which is a major gene, with several modifiers (Stuber et al. 1999). Conventional breeding can be accelerated by direct identification and manipulation of quantitative trait loci (QTLs) using a system based on molecular markers that co-segregate with the trait of interest (Cristofani et al. 1999).

Molecular markers are an efficient tool for indirect selection in plant breeding. They have been used to introgress monogenic traits such as disease resistance (Singh et al. 2001). For polygenic traits, several QTLs are being mapped and their individual effects estimated (Kearsey and Farquhar 1998). The great potential of

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markers for breeding lies in the fact that they are readily
detectable, perform as simply inherited traits and are not
influenced by the environment, which is useful in the
selection of genotypes (Alzate-Marin et al. 2005).

QTL mapping is the estimation of the position and
the effects of quantitative trait genes (Ribeiro et al. 2005).
A QTL analysis must begin with the construction of a
genetic linkage map with a sufficient density of molecular
markers for an adequate genome coverage (Kenis et al.
2005). Genetic maps for cashew have been developed by
Cavalcanti and Wilkinson (2007), using an F1 population
with 85 plants originated from the cross of the dwarf cashew
clones CCP 1001 with common cashew CP 96. These maps
represented a platform for an initial identification of QTLs
associated with traits of economic importance of this crop.

The markers can be used in early stages (seedling)
for the selection of plants with favorable combinations of
several traits. Marker-assisted selection is particularly
promising for cashew breeding, due to the long juvenility
of the species and the polygenic nature of the fruit quality
traits. These factors constitute a major bottleneck in
conventional breeding programs, since the progenies of a
cross must be cultivated for a long time before the fruit
quality can be assessed. In this sense, it is expected that
some specific problems can be solved by the use of
molecular markers linked to QTLs for cashew quality,
meeting consumer demands more quickly, at lower
operating costs. The objective of this study was to identify
QTLs related to the physical quality of cashew apple for a
possible application in marker-assisted selection in
breeding programs.

MATERIAL AND METHODS

The mapping population consisted of 85 plants of a
F1 full-sib progeny derived from a cross between CCP 1001
and CP 96 made in September 2000. Parental genotypes
were selected from a primary cashew population contained
in the germplasm collection of the Embrapa Agroindústria
Tropical, at the Experimental Station Pacajus, State of
Ceará, Brazil. The female parent was a commercial dwarf
clones - CCP 1001, and the male parent a giant genotype -
CP 96. Both trees belong to the same cashew species (i.e.
Anacardium occidentale L.).

The two parents were chosen to provide a maximum
contrast for plant height and resistance to the diseases
anthracnose (Colletotrichum gloeosporioides) and black
mould (Pilgeriella anacardium). They were selected on
the basis of the following contrasting features: CCP 1001
- dwarf (5.5 m), with high yields of small nuts, susceptible
to anthracnose and highly susceptible to black mould and
CP 96 - giant (12 m), with high yields of large nuts, moderate
resistance/tolerance to anthracnose and high resistance
to black mould. The field trial was conducted at the above
experimental station. Seeds of the progeny were sown in
January 2001 and transferred to the field site in March
2001.

Cashew apples were harvested by hand, disregarding
bruised fruit or with early deterioration. Since the F1
generation plants segregate for fruiting period, the cashew
apples were collected from October through November
2006, which is the peak production period. The samples
consisted of 15 cashew apples plant-1, and were harvested
at commercial maturity and transported to the Laboratory
of Physiology and Postharvest Technology of Embrapa,
in Fortaleza, CE, where the post-harvest tests were
performed. Of the originally 85 F1 plants used to construct
genetic maps of the parents, 66 plants produced a satisfactory
number of cashew apples. These were included in the
analysis to evaluate the physical traits of color, size and
weight of the cashew apple, following the methods: i) color
– obtained from a Minolta colorimeter, model CR-300, which
expresses the color in three parameters: “L” corresponds
to the brightness (0-dark/opaque and 100 white), “a”
corresponds to the red intensity (positive value tending to
red and negative to green) and “b” corresponds to the
yellow intensity (positive value tending to yellow and
negative to blue); ii) size – traits of length, basal and apical
diameter of the cashew apple were measured, using a
Starrett digital caliper 300 mm 727-12/300, according to
Almeida et al. (1987); iii) cashew apple weight – determined
after removing the nut, on a digital balance (Mark 3100),
accuracy 0.01 g.

For each trait the descriptive statistics of maximum,
minimum, mean, standard deviation, coefficient of
variation, and frequency distribution were estimated.
Pearson’s correlation coefficient was estimated to
determine the degree of association between the traits.
The analysis was performed using the software (Cruz
2001).

The QTLs were mapped using the analysis of
Multiple QTL Mapping (MQM) (Jansen 1993, Jansen 1994,
Jansen and Stam 1994), an extension of the analysis Interval
Mapping (IM) (Lander and Botstein 1989). The analyses
were performed using all markers of the genetic linkage
maps developed by Cavalcanti and Wilkinson (2007). The
maps were obtained using the methodology of pseudo-testcross for markers segregating at 1:1 ratio (Grattapaglia and Sederoff 1994). Markers with segregation of different classes (3:1, 1:2:1 and 1:1:1:1 ratios) were treated as proposed by Maliepaard et al. (1997). A total of 205 genetic markers were used, of which 194 were AFLP and 11 SSR. Two maps representing each of the parental genomes were constructed separately, i.e. CCP 1001 and CP 96. The QTLs were detected and mapped using MapQTL 5 software (Van Ooijen 2004).

The interval mapping was analyzed to identify QTLs with significant main effects. The molecular markers of these regions were used as co-factors in the subsequent analysis of multiple QTL mapping to increase the accuracy in detecting QTLs in adjacent intervals, as recommended by Van Ooijen (2004). LOD score peaks indicated the presence of QTLs in the map segment. A critical LOD score of 3.0 was used as basis for the identification of candidate QTLs. This value indicates that QTL - marker linkage is 1000 times more likely to occur than independent segregation. The QTLs detected were described by the markers closest to the region corresponding to the QTL. The QTL location was defined by confidence intervals with LOD scores of 1.0 and 2.0, corresponding to a probability of 90 and 95%, respectively. These were identified on the maps using MapChart software (Voornips 2002). The proportion of phenotypic variation explained by the trait-marker association was estimated by the formula proposed by Ukrainetz et al. (2008):

$$R^2 = \frac{SS_G}{SS_T}$$

where $R^2$ is the coefficient of determination corresponding to the proportion of variance explained by the QTL; $SS_G$ is the sum of squares of the genotype (QTL); and $SS_T$ is the total sum of squares.

The effect of phenotypic marker associated with QTL was estimated by the difference between the populations with and without the marker (Broman 2001).

RESULTS AND DISCUSSION

Physical traits of the cashew apple

The coefficient of variation and maximum and minimum values (Table 1) reflect the extent of phenotypic variation in the population. A high range of variation in all traits, indicating the importance of the population for genetic mapping studies in order to identify QTLs for traits of economic importance. With exception of the trait cashew apple weight, there was no significant deviation from normality in the traits studied (p > 0.05, Shapiro-Wilk test). In all traits evaluated continuous variation was observed, which is typical of quantitative or polygenic inheritance (Figure 1). The two parents, CCP 1001 and CP 96, differed significantly (p < 0.05) for six traits, including brightness, red intensity, yellow intensity, cashew apple length, base diameter and apical diameter. However, there was no significant difference between the two parents for cashew apple weight. Significant differences were observed (Scott and Knott 1974) between plants of the F$_1$ generation for all traits (data not shown). Transgressive segregation was observed for all traits analyzed. Based on the results, it was concluded that the degree of variability within the population for the traits under study is sufficient to identify quantitative traits loci in the mapping population.

The correlation coefficients were calculated to determine the degree of association between the traits, because QTLs of correlated traits often have similar positions in the map (Paterson et al. 1991). The correlation coefficients were generally significant for all trait combinations (Table 2). High correlation values were observed among the three traits of color. Brightness was positively correlated with yellow intensity ($r = 0.85^{**}$), brightness negatively with red intensity ($r = -0.82^{**}$) and red intensity negatively with yellow intensity ($r = -0.80^{**}$). High correlation values ($r > 0.69$) were also observed between cashew apple weight and the size traits (length, apical and basal diameter). These results indicate the possibility of the existence of genetic correlation, which can be confirmed by QTL analysis, i.e. observation of coincidences of QTLs of correlated traits.

QTL Analysis

QTL analyses of are an important source of information underlying studies in plant breeding. This study represents a QTL analysis for traits of economic importance associated with cashew quality, and is the first QTL study including the traits color, size and weight of cashew apple.

Based on the analysis of multiple QTL mapping, 30 loci were found with a peak LOD score of 3.0 or higher (Table 3). These were termed Candidate QTLs because prior to use in breeding programs they should be validated, i.e. their effectiveness of selection should be evaluated in other cashew populations, considering the trait to be improved. Two markers were identified with significant
Table 1. Maximum, minimum and mean values, standard deviations and coefficients of variation of the physical traits evaluated in cashew apples of 66 cashew plants (*Anacardium occidentale* L.) of the F₁ generation CCP 1001 x CP 96¹

| Statistics          | BR (mm) | RI (mm) | YI (mm) | CAL (mm) | BD (mm) | AD (mm) | CAW (g) |
|---------------------|---------|---------|---------|----------|---------|---------|---------|
| Maximum             | 74.83   | 37.02   | 56.91   | 96.68    | 59.73   | 47.71   | 165.76  |
| Minimum             | 51.78   | -2.24   | 23.02   | 40.01    | 31.94   | 22.46   | 24.64   |
| Mean                | 63.61   | 17.85   | 37.83   | 62.85    | 44.26   | 30.83   | 65.78   |
| Standard deviation  | 1.08    | 1.48    | 1.41    | 1.70     | 0.92    | 0.88    | 4.08    |
| CV (%)              | 6.62    | 32.13   | 14.38   | 10.45    | 8.13    | 11.05   | 24.32   |

¹ BR: brightness; RI: red intensity; YI: yellow intensity; CAL: cashew apple length; BD: base diameter; AD: apical diameter; CAW: cashew apple weight; CV: coefficient of variation.

Figure 1. Frequency distribution of 66 plants of the F₁ generation of the cross CCP 1001 x CP 96 in terms of brightness, red intensity, yellow intensity, cashew apple length, base diameter, apical diameter and cashew apple weight. Arrows indicate the phenotypes of the parents. CCP 1001 - female parent; CP 96 - male parent.
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Table 2. Results of the correlation analyses of the components of physical quality of the cashew apple

| Traits | RI   | YI   | CAL  | BD   | AD   | CAW  |
|--------|------|------|------|------|------|------|
| BR     | -0.8216** | 0.8500** | 0.2401** | 0.0137 | -0.0472 | 0.0934** |
| RI     | -0.8014** | -0.2473** | 0.0005 | 0.0892** | -0.0774* | 0.1214** |
| YI     | 0.1815** | 0.0293 | -0.0274 | 0.1214** | 0.7306** |
| CAL    | 0.5541** | 0.2394** | 0.7680** | 0.8508** |
| BD     | 0.7680** | 0.8508** |
| AD     | 0.6934** |

1 BR: brightness; RI: red intensity; YI: yellow intensity; CAL: cashew apple length; BD: base diameter; AD: apical diameter; CAW: cashew apple weight. *: significant at 5%; ** significant at 1% probability by the t test.

Table 3. Description of the candidate QTLs identified for the physical traits of the cashew apple detected in the maps of the female (F) and male (M) parents by the method of multiple QTL mapping

| Trait | QTL | Linkage group | Position (cM) | Nearest marker | Maximum LOD | R² (%) | PHE (%) |
|-------|-----|---------------|---------------|----------------|-------------|--------|---------|
| BR    | br-1f | F10 | 21.51 | Aoc23 | 4.19 | 21.33 | 10.13 |
| BR    | br-2f | F12 | 0.00 | 26CB045 | 3.00 | 16.20 | -7.84 |
| BR    | br-3m | M11 | 2.06 | 25CY020 | 3.69 | 3.15 | 4.09 |
| RI    | ri-1f | F10 | 21.51 | Aoc23 | 3.19 | 17.10 | -42.51 |
| RI    | ri-2m | M12 | 0.00 | 26CB045 | 4.13 | 11.15 | 34.54 |
| YI    | yi-1f | F10 | 25.51 | Aoc23 | 3.08 | 13.48 | 17.82 |
| YI    | yi-2m | M4 | 29.91 | 20Bq011 | 4.78 | 7.69 | 11.86 |
| CAL   | cal-1f | F1 | 85.85 | 21CB002 | 3.42 | 12.35 | 16.99 |
| CAL   | cal-2f | F3 | 64.64 | 22Ag026 | 3.74 | 11.39 | 13.95 |
| CAL   | cal-3f | F1 | 66.31 | 04CY014 | 5.55 | 10.13 | 18.08 |
| CAL   | cal-4f | F4 | 66.43 | Aoc242 | 5.19 | 10.09 | -11.22 |
| CAL   | cal-5f | F4 | 35.05 | 04Ag060 | 3.61 | 7.27 | 10.67 |
| CAL   | cal-6m | M11 | 70.34 | 03Bb087 | 3.69 | 12.28 | 13.70 |
| BD    | bd-1f | F4 | 30.29 | 22Ab121 | 6.40 | 13.76 | 9.84 |
| BD    | bd-2f | F4 | 51.43 | 05Ag114 | 4.09 | 8.69 | 7.63 |
| BD    | bd-3f | F10 | 33.86 | 04CG132 | 3.29 | 7.87 | 8.35 |
| BD    | bd-4f | F5 | 19.16 | 10Ab195 | 3.15 | 6.61 | 6.61 |
| BD    | bd-5m | M4 | 0.00 | 04CG035 | 3.03 | 7.99 | -7.24 |
| AD    | ad-1f | F5 | 24.90 | 10Ab195 | 3.48 | 8.50 | 7.44 |
| AD    | ad-2f | F9 | 33.33 | 01Ab084 | 4.50 | 7.63 | 6.89 |
| AD    | ad-3f | F4 | 32.03 | 22Ab121 | 3.65 | 4.84 | 5.60 |
| CAW   | caw-1f | F4 | 32.03 | 22Ab121 | 6.21 | 14.30 | 34.74 |
| CAW   | caw-2f | F4 | 51.43 | 05Ag114 | 6.36 | 9.06 | 26.14 |
| CAW   | caw-3f | F10 | 25.51 | 04CG131 | 3.37 | 6.18 | -20.15 |
| CAW   | caw-4f | F1 | 93.29 | 12CY005 | 4.10 | 5.49 | 27.99 |
| CAW   | caw-5m | M7 | 1.00 | 06Bb008 | 4.67 | 8.98 | 25.27 |
| CAW   | caw-6m | M1 | 83.21 | 21CB002 | 6.13 | 8.09 | 29.01 |
| CAW   | caw-7m | M11 | 69.72 | 03Bb087 | 5.18 | 7.42 | 18.82 |

1 BR: brightness; RI: red intensity; YI: yellow intensity; CAL: cashew apple length; BD: base diameter; AD: apical diameter; CAW: cashew apple weight. 2 QTL position based on the LOD peak region. 3 R²: percentage of the total phenotypic variation explained by the marker nearest to the QTL. 4 PHE: phenotypic effect.

association with QTLs for red intensity (RI), three for brightness (BR), yellow intensity (YI) and apical diameter (AD), six for cashew apple length (CAL) and basal diameter (BD) and seven for the cashew apple weight (CAW).

The percentages of phenotypic variation (R²) explained by associations between the marker locus and QTL ranged from 3.15 - 21.33% for BR, 11.15 - 17.10% for RI, 3.85 - 13.76% for BD, 4.84 - 8.50% for AD and 5.69 - 14.30% for CAW (Table 3). According to Anderson et al. (2007), the proportion of the variation of a trait explained by an individual QTL is probably the most important barrier to the implementation of marker-assisted selection. Most QTL studies revealed moderate effects (R² of 10 - 20%) in one
or few loci, while several other loci explained less than 10% of the variation. In this study, 12 QTLs with significant $R^2$ (10% - 21%) were detected: one for YI (yi-1f), BD (bd-1f) and CAW (caw-1f); two for BR (br-1f and br-2f) and RI (ri-1f and ri-2m); and five for CAL (cal-1f, cal-2f, cal-3f, cal-4f and cal-6m), considered as QTLs of moderate effect. Most QTLs identified explained less than 10% of the variation, i.e. QTLs of smaller effect. Similar results have been reported in QTL studies of fruit quality in other crops (Fanizza et al. 2005, Kenis et al. 2008). The low number of genotypes may have affected the true $R^2$ estimate, however, regardless of the magnitude, the detection of these QTLs represents relevant information for breeding programs of this crop.

The identification of alleles with smaller effects by molecular markers may increase the efficiency of the process, enhancing the gains with selection through a program of marker-assisted selection using molecular information associated with phenotypic variation (Melo et al. 2002). The phenotypic effect was estimated to determine the effect of indirect selection by means of a single marker for each QTL identified. The phenotypic effects of the markers ranged from -7.84 to 10.13% for BR, -42.51 to 34.54% for RI, -7.67 to 17.82% for YI, -11.22 to 18.08% for CAL, -7.24 to 9.84% for BD, 5.60 to 7.44% for AD and from -20.15 to 34.74% for CAW (Table 3). These results reflect the importance of the QTLs identified in the trait expression and strongly support the existence of true QTLs.

QTL clusters were identified, consistent with the results of correlation analysis. For example, the high correlation coefficient ($r > 0.80$) between color traits (BR, RI and YI) was confirmed by the fact that the most significant QTLs (major genes) of these traits are located in the same genomic region as linkage group F10 (Figure 2). QTL clusters were also observed for the traits cashew apple size and weight in the linkage groups F4 (Figure 2) and M11 (Figure 3), representing important regions for the expression of these traits. These coincidences of QTLs between correlated traits confirm the genetic nature of the associations. Based on these results we can infer that the traits are controlled by closely linked QTLs or by the action of QTLs with pleiotropic effects, as suggested by Causse et al. (2002). However, further genetic studies of the regions containing these QTL would be needed to distinguish between pleiotropy or gene linkage. Similar QTL clusters have been reported in other species, including tomato (Lecomte et al. 2004), peach (Quilot et al. 2004) and apple (Kenis et al. 2008).

The results of this study provide a basis for indirect selection by molecular markers for the physical traits studied. The identified candidate QTLs are in the process of validation that can be used in the breeding program of the cashew. These can be used to identify desirable genotypes, further increasing the efficiency of cashew breeding programs, with the possibility of selecting the most promising genotypes still in the nursery and thereby reduce the number of undesirable plants transferred to the field significantly. The information will be an orientation in future studies, increasing the chances of establishing elite genotypes with better cashew quality.

Figure 2. Localization of the QTLs identified for cashew quality on the linkage map of the female parent− CCP 1001. On the right and left hand of the linkage group is the identification of the marker and its position (cM), respectively. Vertical bar and segment of the straight line on the right hand of the linkage group define the confidence intervals at a level of 90% (LOD 1.0) and 95% (LOD 2.0), respectively. br: brightness; ri: red intensity; yi: yellow intensity; cal: cashew apple length; bd: base diameter; ad: apical diameter; caw: cashew apple weight.
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Identificação de locos de caracteres quantitativos para características físicas do pseudofruto do cajueiro

RESUMO - A identificação de locos que controlam características quantitativas (QTLs) e a seleção assistida por marcadores têm despertado grande interesse em programas de melhoramento visando à qualidade dos frutos. Esta pesquisa objetivou identificar QTLs relacionados à qualidade do pedúnculo de caju. Análises físicas foram realizadas em cajus de 66 plantas F1, originadas do cruzamento CCP 1001 x CP 96. Siete características relacionadas à coloração, tamanho e peso do pedúnculo de caju foram analisadas para detecção de QTLs. QTLs candidatos foram identificados, utilizando as metodologias de mapeamento de intervalo e mapeamento de QTLs múltiplos. Trinta QTLs foram identificados associados à qualidade do pedúnculo. A característica intensidade de vermelho apresentou o menor número de QTL (dois), enquanto peso do pedúnculo manifestou o maior número (sete). Os QTLs explicaram entre 3,15% e 21,33% da variação fenotípica total. Estes estão em processo de validação para que possam ser utilizados na seleção assistida por marcadores.

Palavras chave: Anacardium occidentale, QTL, melhoramento genético, qualidade do pedúnculo, marcadores moleculares.

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