QTLs identification for characteristics of the root system in upland rice through DNA microarray

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ABSTRACT. The aim of this work is the construction of a genetic map and identification of quantitative trait loci (QTLs) that control characteristics of the root system of rice. We evaluated a $F_{2:3}$ population composed of 150 families from the cross between the varieties IAC 165 × BRS Primavera. Genotyping was performed in the F_2 population using 3,742 SNP (Single Nucleotide Polymorphism) markers. The evaluation of the root system in the F_3 population was performed through a large-scale phenotyping method based on image generation with a CI-600 root scanner and on quantification through the WinRhizo® software. The experiment was arranged in a randomized block design with three replications performed under greenhouse. The variables analyzed were root length, root surface area and root volume at the depths of 5 to 25 cm and 25 to 45 cm. The SNP markers analysis allowed the construction of the genetic map with a full length of 1424 cM. The linkage group with the largest coverage area was number 3 with 270 cM (100 SNPs), followed by linkage group 1 with 249 cM (170 SNPs) and linkage group 2 with 163 cM (99 SNPs). The genetic analysis allowed the detection of QTLs for all the characteristics.

Keywords: genomic, *Oryza sativa* L., DNA chip, QTL mapping, abiotic stress.

Identificação de QTL para características do sistema radicular em arroz de terras altas por meio de microarray

RESUMO. Este trabalho objetivou a construção de um mapa genético e identificação de locos de características quantitativas (QTLs) que controlam características do sistema radicular do arroz. Foi avaliada uma população $F_{2:3}$ composta de 150 famílias a partir do cruzamento entre as variedades IAC 165 x BRS Primavera. A genotipagem foi realizada na população F_2 utilizando 3.742 marcadores SNP (Polimorfismo de um Único Nucleótido). A avaliação do sistema radicular nas populações F_3 foi realizada através de fenotipagem em larga escala baseada na geração de imagem com scanner raiz CI-600 e na quantificação através do software WinRhizo®. Foi realizado experimento em delineando blocos casualizados, em casa de vegetação com três repetições. Foram consideradas as variáveis comprimento, área de contato da raiz e volume nas profundidades de 5 a 25 cm e 25 a 45 cm. A análise dos marcadores SNP permitiu a construção de um mapa genético com comprimento total de 1424 cM. O grupo de ligação com maior área de cobertura foi o número 3 com 270 cM (100 SNPs), seguido pelo grupo de ligação 1, com 249 cM (170 SNP), e 2 com 163 cM (99 SNP). A análise genética permitiu a detecção de QTL para todas as características estudadas.

Palavras-chave: genômica, *Oryza sativa* L., chip de DNA, mapeamento de QTL, estresse abiótico.

Introduction

Upland rice is responsible for approximately 21% of Brazilian agricultural production. However, the lack of water throughout its production process has a major negative impact on the culture. According to Toescher, Righes, and Carlesso (1997) and Stone (1985), drought occurrence impacts mainly nutrient absorption by the root system, negatively affecting the crop productivity.

The understanding of the genetic mechanisms associated with the rice root system development aiming selection could help with the optimization of water absorption, and this strategy seems to be essential for breeding of the culture.

In this context, DNA markers have supported plant breeding in the orientation of crosses, formation of base populations, development of divergent populations (Li-Na, Gui-Lan, & Long-

Zhi, 2013), construction of genetic maps (Jun-Zhi, Xiao, Chao, Ke, & Yan-Jun, 2009), identification of quantitative trait loci (QTL) (Uga, Okuno, & Yano, 2011) and identification of functional markers (Andersen & Lübberstedt, 2003), as well as in marker assisted selection (MAS) and genomic selection (GS) (Steele, Virk, Kumar, Prasad, & Witcombe, 2007; Uga et al., 2013).

For such purposes, single nucleotide polymorphisms (SNPs) have been highly used in genetic analysis because they are stable, widely distributed in the genome and allow for automation, which facilitates high-throughput genotyping and QTL detection. Since 1995, QTL mapping has been conducted in rice cultivation for both temporary and permanent populations (Sabouri, Sabouri, Jafarzadeh, & Mollashahi, 2011; Liang, 2013; Liang, Zhen-Hua, & Jie-Yun, 2013).

Drought tolerance is very dependent on the root system. This dependence on the root system in turn influences other important agronomic traits in upland rice, such as efficiency in nutrient absorption and resistance to pests and root diseases (Oliveira, Freitas, Fiúza, Menezes, & Dotto, 2010; Henry, Gowda, Torres, Mcnally, & Serraj, 2011; Uga et al., 2013). For such reasons, the root system is frequently studied given the potential for the stabilization or increase of productivity under adverse conditions (Topp et al., 2013; Gamuyao et al., 2012).

DNA microarrays are a very useful tool for QTL identification, Marker Assisted Selection (MAS) and genomic selection (GS) studies because they provide greater genome coverage. Related to MAS, it is particularly important to have an adequate linkage map saturation to detect more accurate QTL positioning and its effect estimation, as it is then easier to associate it with a causative gene. Considering the importance of the root system for rice cultivation, this work sought to evaluate a segregating $F_{2:3}$ population of upland rice to construct a linkage map aiming QTL identification for root system traits by means of DNA microarrays technology.

Material and methods

Genetic material

The genetic material evaluated in this experiment was constituted by a segregating population ($F_{2:3}$) derived from a single F_1 plant obtained from the cross between the varieties of upland rice (tropical japonica subspecies) IAC 165 and BRS Primavera, composed by 148 families and its two genitors.

IAC 165 is a commercial variety of upland rice developed by the Agronomic Institute of Campinas from the cross between "Dourado Precoce" and IAC 1245. It has a moderate resistance to lodging, strong root development, tolerance to water deficit, relatively short cycle and long and thick grain. BRS Primavera is a commercial variety released for cultivation in highlands and was developed by Embrapa Rice and Beans. It is derived from the cross between IRAT 10 and LS 85-158 cultivars, has intermediate size (average of 100 cm), medium tillering, low lodging resistance and early cycle (average of 100 days). It presents average productivity, susceptibility to drought, long and thin high quality grains, and shows a very narrow harvest stage.

Root phenotyping

The root phenotyping was performed in the experimental field of Fazenda Palmital, of EMBRAPA Rice and Beans, in the municipality of Goianira, Goiás State, Brazil, from October 2012 to June 2013. The experiment was installed in a randomized block outlining with three repetitions and was performed under controlled conditions in a greenhouse. The experimental unit was constituted by a polyethylene pipe with an internal diameter of 30 cm and a height of 80 cm and was filled with a clayey red latosol that presented constitution of: 19.0 g dm^{-3} M.O., pH 5.4, 0.5 g dm^{-3} P(Mel.); 0.24 cmol$_c$ dm^{-3} K, 2.0 cmol$_c$ dm^{-3} Ca, 0.8 cmol$_c$ dm^{-3} Mg, 0.0 cmol$_c$ dm^{-3} Al, 2.5 cmol$_c$ dm^{-3} H+Al; 590.0 g kg^{-1} sand, 120.0 g kg^{-1} silt, 290.0 g kg^{-1} clay, with an added 250 grams of 5-25-15 fertilizer. Installed inside of the experimental unit was an acrylic tube of 6 cm of internal diameter with rubber covers for protection against the entry of soil, water or organic waste.

Around the acrylic tube, three rice plants were transplanted with one week of emergence. Two weeks after transplantation, the generation of the images was initiated at the depth of 5-25 cm, and three weeks after transplantation, imaging at the depth of 25-45 cm was performed. The images were digitalized using a *scanner* (CI – 600 *Cano Scan*) inserted in the acrylic tube, which scanned the surface around the tube. The evaluations were performed weekly for until the plans were eight weeks of age.

The generated images were analyzed using the image quantification software *WinRhizo*, version 2008, which makes available, among other variables,

the total length of the roots (COM) measured in centimeters (cm), the estimated contact area of the roots (ACR) measured in square centimeters (cm²) and the total volume of the roots (VOL) measured in cubic centimeters (cm³).

DNA Microarray Genotyping

For the genotyping of population $F_{2:3}$, a microarray composed of SNP-type markers was used. For the microarray construction, genetic sequencing data from eight varieties of *upland* rice (*japonica* subspecies) were employed whose characteristics related to the root system are described in Table 1.

A total of 3,742 SNP markers were developed and distributed along the 12 rice chromosomes. These markers were submitted to the DNA microarray technique (Schena, Shalon, Davis, & Brown, 1995). Any SNPs that did not present a polymorphism, had not been evaluated in at least 80% of the population, or that presented segregation distortion by the chi-square test at 5% probability level were eliminated on the basis of the genetic map's construction and the QTL identification.

Data analysis

The phenotyping process was performed through the *WinRhizo™* software (Arsenault, Pouleur, Messier, & Guay, 1995). The areas under the growth curve (AUC) were estimated for each root variable over the weeks of evaluation using the trapezoidal method. The data from the AUC were submitted to variance homoscedastic testing followed by joint variance analysis. Considering the two depths, the AUC was used to estimate a value closer to the real growth rate of the root system (Boyer, 1996) rather than using only an average calculated from the data collected along the eight weeks of this study. The variance was estimated from data of each genotype AUC related to the genotype's averages. The analysis aimed to verify significant differences within the population for the analyzed characteristics of the root system. The GENES software (version 2013) was used in this step (Cruz, 2013).

The construction of the genetic map was performed through the *IcIMapping* QTL software (version 2012 3.2), considering an LOD *Score* of 3.0 for QTL identification (Collard, Jahufer, Brouwer, & Pang, 2005). For the identification of QTLs related to the root characteristics, the simple interval mapping method was performed using the Windows QTL *Cartographer* software (version 2.5_011, 2012) (Basten, Weir, & Zeng, 2004).

Results and discussion

The phenotypic information showed variance homoscedasticity and the joint variance analysis for the two depths ($p < 0.01$), which is presented in Table 2. The results demonstrated great variability as well as significant differences for all variables and all considered factors (genotypes, depth and interaction).

Table 1. Upland rice varieties (*japonica* subspecies) and their characteristics related to the root system.

Variety	Characteristics related to the root system
Azucena	Variety from the Philippines, known in the international literature as a genotype of good root development and tolerance to the stress of drought and toxic aluminum.
Moroberekan	Variety from West Africa, also known in the international literature as a genotype of good root development, good root penetration capacity on soil, tolerant to drought and toxic aluminum.
Chorinho	Traditional variety collected in the state of Minas Gerais, Brazil, with intermediate tolerance to stress by water deficiency in the field, with tall plants.
Puteca	Traditional rice variety collected in the state of Goiás, Brazil, susceptible to stress from water deficiency, with tall plants.
IAC 165	Commercial rice variety developed by the Campinas Agronomic Institute, with high rusticity, good root development and tolerance to the stress of drought.
BRS Primavera	Commercial variety developed by Embrapa Rice and Beans, presenting susceptibility to drought stress, whose plants are of intermediate size (average of 100 cm).
Catetão	Traditional variety collected in the state of MatoGrosso, Brazil, tolerant to drought stress, with tall plants.
Ligeiro	Traditional rice variety collected in the state of Maranhão, Brazil, tolerant to drought stress, presenting a short cycle and low productivity.

Table 2. Variance analysis for the variables of the root system: length (COM), contact area (ACR) and volume (VOL) of the roots in the depths of 5-25 cm and 25-45 cm.

F. V.	GL	COM		ACR		VOL	
				Mean square			
Block	2	7943175.93		227320.49		42.24	
Genotypes(G)	149	645107.39	**	17818.13	**	3.56	**
Depths(P)	1	44593404.61	**	2080284.10	**	550.40	**
G x P	149	596237.47	**	16523.02	**	3.18	**
Residue	598	179663.06		5420.12		1.23	
Average		1657.23		275.73		3.69	
Average depth 1		1879.83		323.80		4.48	
Average depth 2		1434.64		227.65		2.91	
CV (%)		25.57		26.70		30.07	

**Significant at the level of 1% probability by the F test.

Genetic map based on the F₂ population from the cross between varieties IAC 165 and BRS Primavera

Having identified significant differences in the root system characteristics, genomic analysis was then performed by constructing a genetic map and QTLs detection for such characteristics. SNPs that did not cover 80% of the segregating population, were not polymorphic, or that presented segregation distortion evaluated through the chi-square test (p < 0.05) were eliminated from the analysis. Then, 1,061 polymorphic markers were considered for genetic analysis. The genetic maps were constructed based on these 1,061 polymorphic markers, considering an LOD *score* of 3.0 and a maximum recombination frequency of 0.3. The mapping process allowed the construction of 12 linkage groups (Figure 1), reconstituting the number of chromosomes of rice species considered, according to Cheng, Buell, Wing, Gu, and Jiang (2001).

The markers on the genetic map presented the same ordering presented in the physical map from which the SNPs were selected, based on the database available by the Gramene portal (Jaiswal et al., 2006). Linkage groups 1 and 7 presented the highest number of markers (170 SNPs on each chromosome), followed by linkage groups 11 (130 SNPs) and 3 (100 SNPs). The smaller linkage group was group 9, with only 18 SNPs markers. Many markers presented were completely connected, having been omitted from the linkage group (Figure 1). Overall, the markers presented good distribution along the chromosomes, with the exception of chromosome 9, which had a total length of 6 cM. This result is corroborated by Sabouri et al. (2011), in whose work on *Oryza sativa* L. also reported the shorter length of this chromosome in relation to others. The longest length was found in chromosome 3, with 270 cM, followed by chromosome 1 with 249 cM and by chromosome 2 with 163 cM. The total length of the genetic map constructed was 1,424 cM. The size of the genome obtained is similar to that reported by other authors in several works for the species *O. sativa* L. (Liang, 2013).

According to Uga et al. (2011), the characteristic COM is governed by a smaller quantity of genes when compared to other characteristics of the root system, such as growth in depth and the angle of growth of the roots. For the characteristic COM, QTLs with a significant effect associated with the two depths evaluated were detected in 5 to 25 cm (Figure 2) and 25 to 45 cm (Figure 3), as these are positioned at the linkage groups 3 and 1, respectively. For the depth of 5 to 25 cm, the QTL was positioned in the distal fraction of the linkage group 3, at the position 258-260 cM. This QTL was identified between the markers OsC1r3_18199829 and OsC1r3_18195592 with a LOD of 3.22. For the depth of 25 to 45 cm, the QTL was positioned in the proximal fraction of the linkage group 1, positioned between the markers OsC1r1_142021996 and OsC1r1_141152310 with a LOD of 3.37.

The characteristic ACR, in turn, is intimately associated with the capacity to absorb water and soil nutrients, a quality of great importance for the growth and development of plants. For this characteristic, at the most superficial depth (5 to 25 cm,), one QTL was detected, as well as in the distal fraction of the linkage group associated with chromosome 3, positioned at 256-260 cM, between the markers OsC1r3_18199829 and OsC1r3_18195592 with LOD = 3.02 (Figure 4). It is possible that this QTL has a pleiotropic effect for root characteristics because it is located in the same region of the QTL detected for COM characteristics. The same happens for the second QTL detected for this characteristic in the depth of 25 to 45 cm (Figure 5), detected at the beginning of the linkage group 1, at a distance of 5-8 cM between the markers OsC1r1_142021996 and OsC1r1_141152310. The LOD associated with this QTL was of 3.06.

For the characteristic VOL a QTL in linkage group 3 was detected at the depth of 5 to 25 (Figure 6). This QTL is located at the position 96-104 cM, between the markers OsC1r3_129673798 and OsC1r3_132585678, with a LOD of 2.73. Two QTLs for the VOL characteristic were located at the depth of 25 to 45 cm in linkage group 1 (Figure 7). The first QTL at the proximal fraction of the linkage group was detected at the position of 5-9 cM between the markers OsC1r1_142021996 and OsC1r1_141152310 with LOD of 2.99. The second QTL was detected at the position 67-76 cM between the markers OsC1r1_128642534 and OsC1r1_124653796 with a LOD of 3.05.

The identification of two regions controlling a quantitative characteristic suggests an inheritance of polygenic control for the VOL characteristic. This finding allows us to infer information about the existence of more genes involved in the VOL control that would allow the plant to reach the deepest regions on the soil (Uga et al., 2011). This author highlights that such genes would allow the plant to stimulate root growth in a vertical way through, for example, elongation of the root cells. Other works conducted with rice (*O. sativa* L.) also identified QTLs for characteristics, such as COM, ACR and VOL, in these same chromosomes, corroborating with the results found in the present research. Courtois, Shen, Petalcorin, Carandang, and Mauleon (2003) studied a population of endogamic lineages from the cross between the varieties IAC 165 (*O. sativa* L. subesp. *japonica*) and Co39 (*O. sativa* L. subesp. *indica*) and found the existence of a main QTL on chromosomes 1 and 4 for root system characteristics, results similar to those obtained by us. This is possibly due to the genetic pool used because the IAC 165 genetic material is a common genitor in both studies.
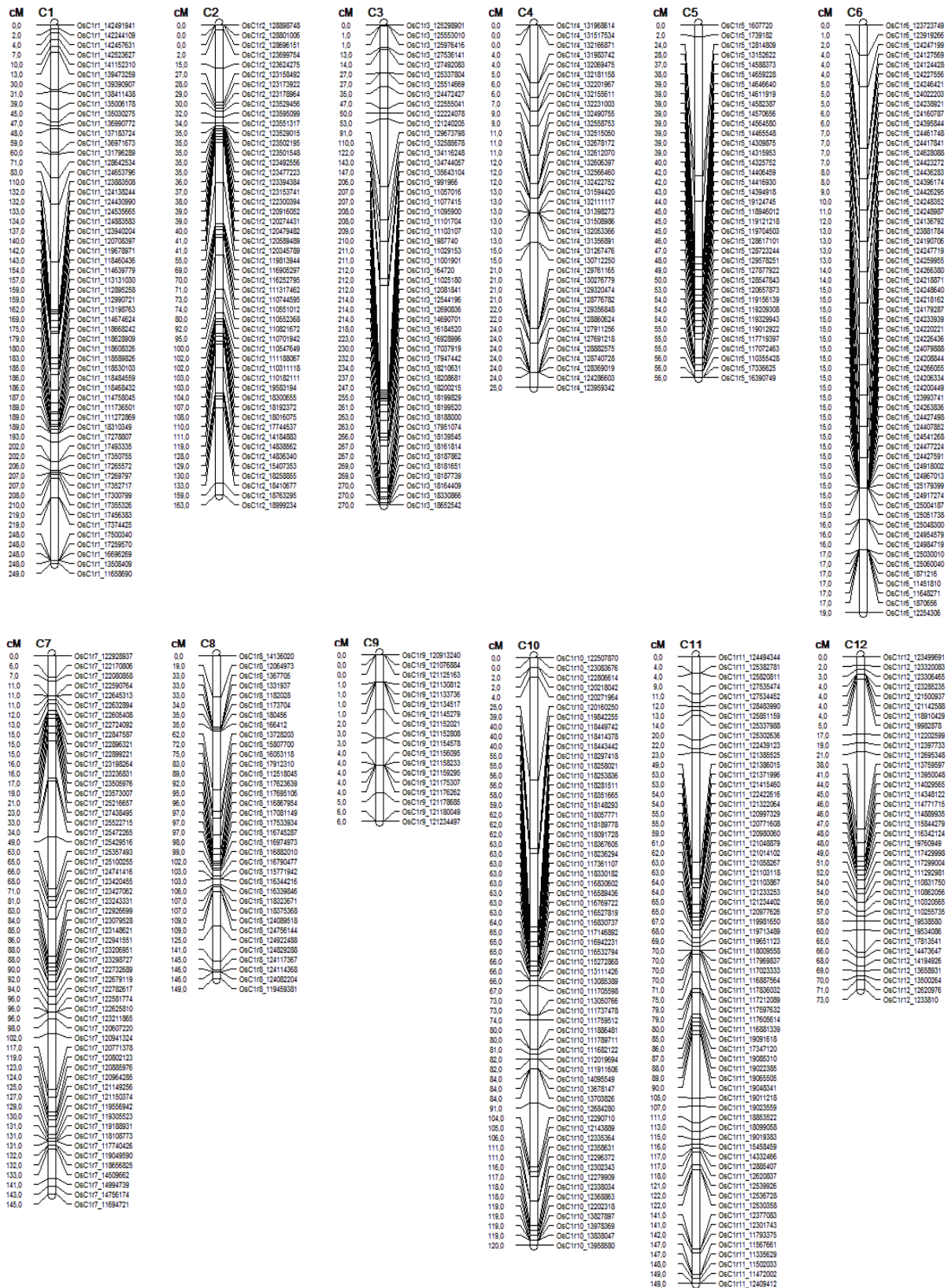
Figure 1. Genetic map of *Oryza sativa* L. based on a segregating population derived from the cross between the varieties IAC 165 × BRS Primavera, composed of 1061 SNP markers. The distance between each marker is given in cM on the left, and the name of the marker on the right, with full coverage of 1424 cM.
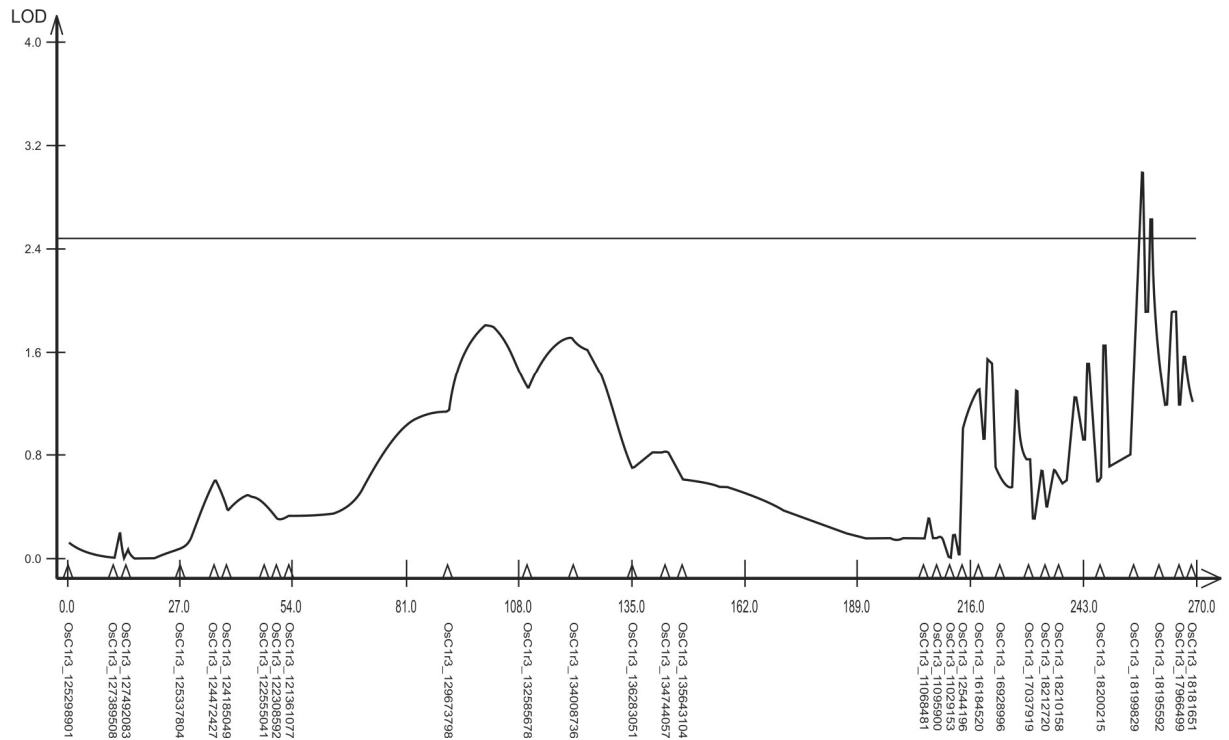
Figure 2. QTL associated to the characteristic COM at the depth of 5 to 25 cm in a $F_{2:3}$ population of rice on the chromosome 3 by the Composed Interval Mapping analysis. The critical value of LOD of 2.5 is represented by the horizontal line.
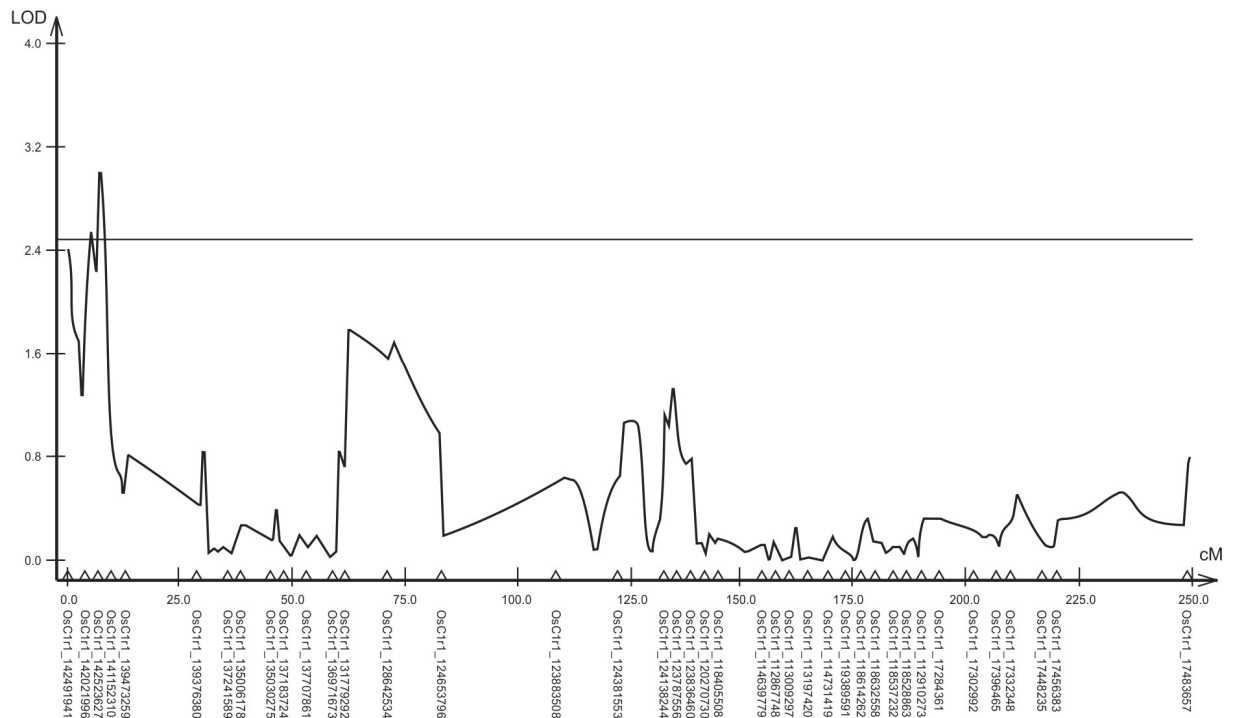

Figure 3. QTL associated to the characteristic COM at the depth of 25 to 45 cm in a $F_{2:3}$ population of rice on the chromosome 1 by the Composed Interval Mapping analysis. The critical value of LOD of 2.5 is represented by the horizontal line.

Figure 4. QTL associated to the characteristic root contact area (ACR) at a depth of 5 to 25 cm in a $F_{2:3}$ population of rice on the chromosome 3 by the Composed Interval Mapping analysis. The critical value of LOD of 2.5 is represented by the horizontal line.
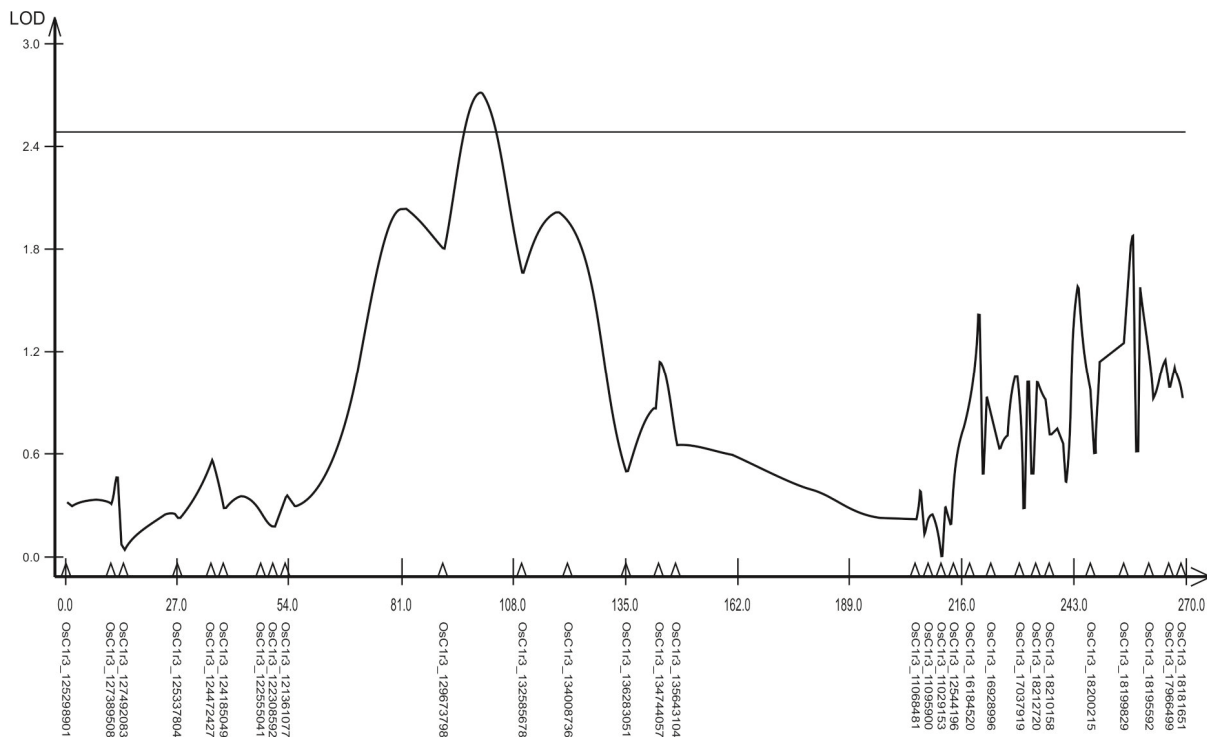

Figure 5. QTL associated to the characteristic root contact area (ACR) at the depth of 25 to 45 cm in a $F_{2:3}$ population of rice on the chromosome 1 by the Composed Interval Mapping analysis. The critical value of LOD of 2.5 is represented by the horizontal line.

Figure 6. QTL associated to Volume of the roots at the depth of 5 to 25 cm in a $F_{2:3}$ population of rice in the liaison group 3 by the Composed Interval Mapping analysis. The critical value of LOD of 2.5 is represented by the horizontal line.
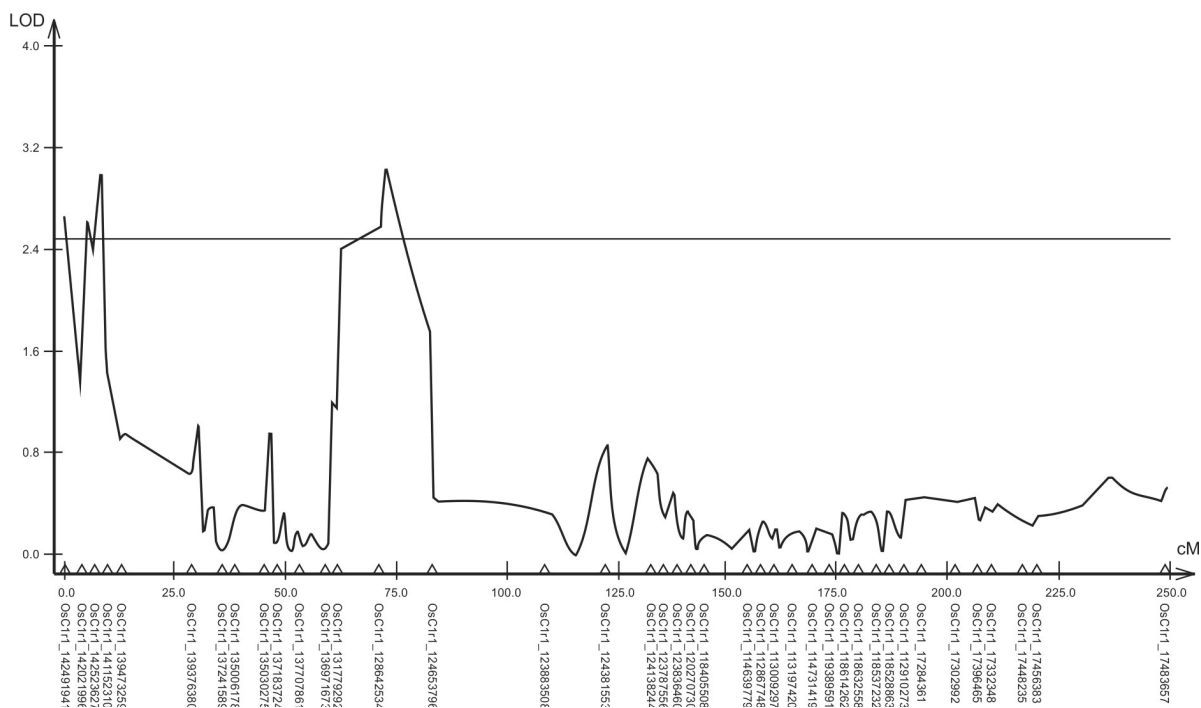

Figure 7. QTL associated to the Volume of the roots at the depth of 25 to 45 cm in a $F_{2:3}$ population of rice on the chromosome 1 by the Composed Interval Mapping analysis. The critical value of LOD of 2.5 is represented by the horizontal line.

The limited progress for drought tolerance in rice culture has been attributed, in part, to the large quantity of genes involved and the large environmental influence associated with this characteristic expression. Uga et al. (2013), however, were able to develop a rice variety that was productive even when submitted to water stress. The lineage was developed through backcrossing,

using MAS to monitor the gene *dro*1. This gene, located on chromosome 9, is reported to be responsible for the elongation of root cells.

In rice culture, many of the QTLs identification work related to root system characteristics have been performed through crossings between subspecies of genotypes *O. sativa* indica with *O. sativa* japonica (Steele et al., 2007; Uga et al., 2011; Courtois et al., 2003; Ping et al., 2003). In this type of crossing, there is a huge segregation, enabling the study and detection of QTL for certain characteristics, given the knowledge of contrasting genitors. The crossing of such genetic materials results in the segregation of smaller amounts of characteristics, which enables both the most refined molecular study and cultivar development. This study, in turn, allows for the identification of genes and their subsequent use in SAM operational procedures, such as those used by Uga et al. (2013).

Conclusion

The resulting segregating population from the cross between the varieties IAC 165 and BRS Primavera presented wide variability for the root characteristics studied.

The DNA microarray technique allowed for the construction of an upland rice genetic map with broad genome coverage and a total length of 1,424 cM, covering all chromosomes of the species.

The linkage groups 1 and 3 presented QTLs related to the characteristics COM, ACR and VOL of the root system.

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