QTL mapping associated with rooting stem cuttings from *Citrus sunki vs. Poncirus trifoliata* hybrids

Amauri Siviero*[1]; Mariângela Cristofani [1,2] and Marcos Antonio Machado[1]
[1]Centro Avançado de Pesquisa Tecnológica do Agronegócio dos Citros 'Sylvio Moreira', Caixa Postal 04, CEP 13469-970, Cordeirópolis, SP, Brazil; [2]EMBRAPA, Empresa Brasileira de Pesquisa Agropecuária. (* Corresponding Author. E-mail: asiviero@hotmail.com)

ABSTRACT

The citrus vegetative propagation by stem cuttings depends on factors such as genetics, age, vigor, woodiness, growth regulators, environment and nutrition. Lemons (*Citrus limon)*, acid limes (*Citrus aurantifolia*) and citrons (*Citrus medica*) root very easily. Plant cuttings from the sweet orange (*Citrus sinensis*), mandarins (*Citrus reticulata*) and *Poncirus trifoliata* are more difficult to root. The objective of this study was to detect quantitative trait loci linked to the rooting of citrus stem cuttings in F_1 hybrids obtained from a crossing between *Citrus sunki* x *Poncirus trifoliata*. The semi-woody cuttings were treated with 1 g/L indole-3-butyric acid for 10 minutes and cultivated in citrus substrate under a mist environment. QTLs were identified by the composite interval mapping strategy. Two QTLs were detected for the rooting trait in plant cutting, in the *P. trifoliata* map.

KEY WORDS: Assisted selection, citrus breeding, molecular markers, propagation.

INTRODUCTION

Plant propagation through grafting and rooting of stem cuttings has the tendency to decrease the juvenile stage of the plants (Spiegel-Roy and Goldschmidt, 1996). Traditionally, citrus plants are obtained through grafting. Even though propagation through rooting of stem cuttings reduces the time of nursery plant development, keeping the original plant traits. Stem cutting is a fast and efficient method for propagating some citrus species such as 'Tahiti' lime (*C. latifolia*) (Prati et al., 1999).

Several factors are related to the rooting of citrus stem cuttings, such as age and mother plant vigor, woodiness and location of the stem cutting, growth regulators, environment, nutrition and genetic factors related to the genus or species of interest (Platt and Optiz, 1973, Ferguson et al., 1986; Ferri, 1997; Abou-Rawash et al., 1998).

Stem cuttings that are nutritionally weak and/or attacked by pests and pathogens are not easily rooted (Platt and Optiz, 1973). Studies conducted by Bester and Rabe (1996) showed that the translocation of IAA in citrus rootstock stem cuttings did not correlate with plant vigor. Rakesh-Kumar et al. (1995) reported that lemon (*C. limon*) stem cuttings treated, before planting, with indole-3-butyric acid (IBA) associated with p-hydroxybenzoic acid (pHBA) showed better rooting at the concentration of 2 g/L IBA and 2 g/L of pHBA. They also observed that stem cuttings collected during the dry season rooted better than those collected during the rainy season.

Shazly et al. (1994) studied the effect of three growth regulators at different concentrations for rooting stem cuttings of lime and acid lime. IBA promoted the best rooting percentage, higher number and longer roots for both genotypes. The benefic effect of growth regulators (IBA and NAA) was observed in rooting of acid lime 'Tahiti' and sweet orange (*C. sinensis*) (Prati et al., 1999).

Studies on citrus rooting stem cuttings deals especially with the induction of rooting by growth regulators and the differences in rooting stem cutting capacity of different species and varieties. However, little is known about the genetic basis involved in the control of the characteristics.

The construction of genetic maps is the basis for advanced genetic studies. A well- defined linkage map can describe the linkage relationships in the genetically characterized markers with traits of interest. Once verified, the trait-linked marker genotypes can serve as phenotype predictors, thus providing a timely and less costly way of identifying individuals possessing beneficial alleles without going through field testing for phenotypes. The ability to use marker-assisted selection for perennial plant crops is expected to have a profound impact on breeding schemes. The use of

DNA markers has greatly accelerated the construction of saturated linkage maps in citrus. This allows selection of more genotypes for specific traits in a shorter period of time. The selection assisted by molecular markers helps to link markers to important genetic loci for agriculture traits, facilitating the cloning of genes, thus making them very useful tools for perennial plant breeding programs such as citrus (Gmitter et al., 1998).

Great part of the citrus agronomic characteristics is controlled by quantitative loci. The study of loci that control quantitative traits (QTLs) allow the identification, mapping and quantification of the effect of them. The efficient detection of QTLs depends on the number of QTLs, magnitude of its effect, trait heritability, interactions between genes, type and size of the segregant population, genome size, recombination frequency between the QTLs and the markers, and saturation of the map (Tanksley, 1993; Young, 1996; Liu, 1998).

The mapping of QTLs is based upon linkage tests between molecular markers and phenotypic characteristics. The most commonly used methods for detecting the association between marker-QTL and phenotype are: a) single marker analysis using the following: simple *t* test, linear regression, analysis of variance, and likelihood ratio and maximum likelihood estimation, b) mapping by interval using the likelihood approach, regression and a combination of the likelihood and regression approach (Liu, 1998), and finally c) the mapping by composite intervals, a combination of simple interval mapping and multiple linear regression (Zeng, 1993, 1994). QTLs can be detected by considering the environments separately or in groups through an integrated data analysis of the different environments. The power of detection of QTLs for traits that show inheritance of complex nature and low herdability is reduced due to environmental effects (Young, 1996).

The objective of the present study was to detect associated QTLs and molecular markers for the rooting of stem cuttings in F_1 hybrids progenies obtained from the crossing between *Citrus sunki* e *Poncirus trifoliata* 'Rubidoux'.

In this study, our investigation of linkages between these molecular markers and quantitative traits related to rooting stem cuttings is presented. Associated markers will help citrus breeding by increasing our understanding of the inheritance of these characters and the prospects for marker-assisted selection.

MATERIAL AND METHODS

Greenhouse and laboratories studies were conducted at the Centro Avançado de Pesquisa Tecnológica do Agronegócio dos Citros (CAPTAC), 'Sylvio Moreira' Agronomic Institute of Campinas (IAC), in Cordeirópolis, SP, Brazil. A total of 80 F_1 hybrids obtained from the crossing between *C. sunki* vs. *P. trifoliata* 'Rubidoux' were utilized.

The *C. sunki* Hort. ex. Tanaka plant, originated from the southwest region of China, and recommended as a rootstock for sweet orange citrus varieties, tangerines and grapefruits, has several desirable characteristics, such as good canopy shape induction, tolerance to tristeza, xyloporosis, psorosis, blight, and to saline soils and droughts. The rootstock 'Sunki' has undesirable characteristics such as susceptibility to gummosis, low numbers of viable seeds per fruit (Pompeu Junior, 1991; Castle et al., 1993) and rooting difficulty (Santos et al., 1988).

P. trifoliata (L.) Rafinesque 'Rubidoux' is a rootstock widely used in Japan, Uruguay and in other countries of temperate climate. It is recommended for combinations with sweet oranges, acid limes and tangerines. *P. trifoliata* induces excellent juice quality, is ideal for cold and humid regions, produces low vigorous plant, thus reducing the canopy size, making the fruit harvesting easier and grove less dense, and is highly resistant to *Phytophthora* spp., nematodes, tristeza and xyloporosis. However, it is susceptible to exocortis, intolerant to blight, presents poor growth development in the nursery, is intolerant to drought, requires high soil fertility, and is incompatible with sweet orange 'Pêra', true limes and tangor 'Murcott' (Pompeu Junior, 1991; Castle et al., 1993).

Sixteen stem cuttings were obtained from each of the 80 hybrids analyzed out of 314 progeny plants obtained from the crossing between *C. sunki* vs. *P. trifoliata* 'Rubidoux'. The semihardwood stem cuttings were collected in the spring time with an average length of 15 cm and containing 4-5 buds. After taking all the leaves off, the stem cuttings were immersed in a solution containing 40% ethanol, 1 g/L IBA and 1 g/L of NAA for 10 minutes. They were planted in small plastic tubes filled with a commercial-type substrate 'plantimax' used to form citrus rootstock. Fertilizer pellets (18-05-09) at 40g for each 10.0 L of substrate were added. Each plant received approximately 1.0 to 2.0 g of this mixture every 15 days. The cuttings were kept in a mist chamber. Experiments were carried out according to a randomized block design. After 75 days, the percentage of the rooted cuttings for each genotype was documented.

RESULTS AND DISCUSSION

The genetic maps with linkage groups of *C. sunki* and *P. trifoliata* 'Rubidoux' utilized in this study to map the rooting stem cutting trait were built using a total of 169 RAPD markers. The linkage analysis revealed that 125 of the RAPD loci fell into 18 linkage groups (10 groups for 'Sunki' and 8 for 'Rubidoux'). The total length of the maps was 732.32 cM for 'Sunki' and 866.88 cM for 'Rubidoux' (Cristofani et al., 1999). The detection of QTLs was done through the mapping by composite intervals according to Zeng (1993, 1994), utilizing the QTL Cartographer program (Basten et al., 2000).

The distribution of the percentage values among the hybrid individuals was continuos (Figure 1). Transgressive hybrids were observed among the individuals of the progeny. The majority of the 80 hybrids had an average rooting between 50% and 100% . The parents represented points with less than 10% for *Citrus sunki* and almost 70% for *Poncirus trifoliata*. Data collected by Abou-Rawash et al. (1998) showed that rooting (percentage and number of roots) was species depended and followed the order: lemon 'Cravo' > sour orange > 'Cleopatra' mandarin.

According to Platt and Optiz (1973), the species of *Citrus* and correlated genera show differences in the rooting ability of stem cuttings. They reported that cuttings from true lemon, acid lemon and citrons root easily. On the other hand, cuttings from sweet orange, grapefruit, citranges and *P. trifoliata* are somewhat difficult to root. Villas Boas et al. (1988) studied the rooting of stem cuttings of 16 citrus species/cultivars and reported that sweet orange and tangerines showed low rooting rates; lemon 'Siciliano' and the citrons `Etrog' and 'Diamante' had the highest rooting percentage and the application of growth regulators did not induce the rooting of the cuttings from the species evaluated. Studies conducted by Sabbah et al. (1991) showed that 44 and 77% of the cuttings of tangerine 'Cleopatra' and *P. trifoliata*, respectively, rooted after being immersed in indole butiric acid solution.

The segregation in the population appeared quantitative. The percentage values distribution frequency histogram imply that the rooting stem cutting is probably confered by multiple genes.

A total of four QTLs for rooting ability (measured as % of rooting of cuttings) were detected in *Eucalyptus* (Gratapaglia et al., 1995) by combining the use of the pseudo-testcross mapping strategy using RAPD markers.

In the present work two putative QTLs controlling the percentagem of rooting stem cuttings were detected in the linkages groups of *Poncirus trifoliata.*

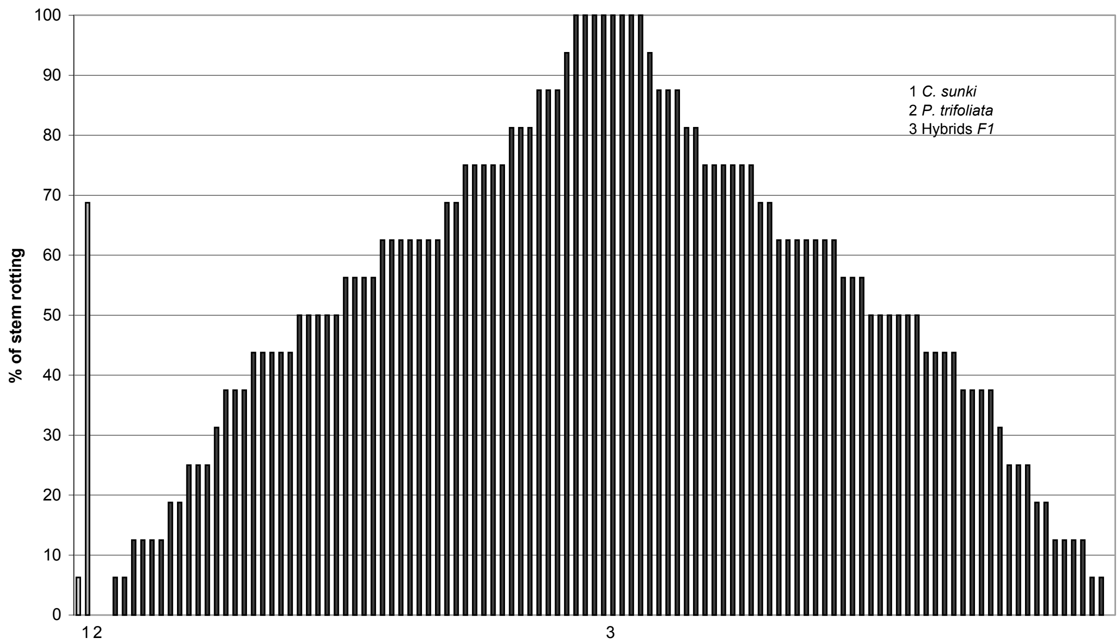

Figure 1. Percentage of rooting stem cuttings of the *P. trifoliata*, *C. sunki* and hybrids.

Significant peak values of LOD Scores, the position of these peaks, the percentage of the phenotypic variance explained and the estimated gene action based on the analysis of the QTLCartographer are shown in Table 1.

The proportion of phenotypic variation caused by the QTL was estimated by the coefficient of determination (R^2) from the program QTL Cartographer. The QTLs detected in the linkage groups II and III of *P. trifoliata* cv. Rubidoux are responsible for 20.9% and 15.8% of the total phenotypic variation for rooting stem cuttings, respectively.

The identification of QTLs associated with the rooting of stem cuttings can be observed in Figures 2 and 3. The ordinate shows the scale for LOD values for each linkage group analyzed, while the abscissa shows the representation of the linkage group and the distances in centiMorgan. The wavy line illustrates the results of the analyses done through the QTL Cartographer program. The horizontal lines indicate the LOD values and the significant statistical limit for each linkage group analyzed. The region above the LOD limit indicates a possible QTL.

The QTL with the highest LOD Score (4.4) for % Root was closely linked to the AV02_110 marker in *Poncirus trifoliata*. The detection of QTLs can be greatly improved if more markers were added to the linkage map near the regions where the QTLs are located (Liu, 1998).

This is the first QTL mapping study on rooting in Citrus and related genera. Larger progenies and more markers can be used to improve the QTL mapping. More markers can be placed in certain regions by using the bulk segregating approach.

CONCLUSIONS

The presence of molecular markers and QTLs associated to the rooting of citrus stem cuttings distributed in the linkage groups of *Poncirus trifoliata* 'Rubidoux' indicates that these regions are of interest to the selection of genotypes that root easily.

RESUMO

Mapeamento de QTLS associados ao enraizamento de estacas em híbridos de *Citrus sunki* vs. *Poncirus trifoliata*

A propagação vegetativa de citros via enraizamento de estacas depende dos fatores genético, idade, vigor, lenhosidade, reguladores de crescimento, ambiente e nutrição. Os limões (*Citrus limon)*, limas ácidas (*Citrus aurantifolia*) e cidras (*Citrus medica*) enraízam com certa facilidade. Estacas de laranjas doce (*Citrus sinensis*), tangerinas (*Citrus reticulata*) e *Poncirus trifoliata* apresentam dificuldades no enraizamento. O presente estudo teve como objetivo detectar locos controladores de características quantitativas (QTLs) associados ao enraizamento de estacas de híbridos F_1 obtidos do cruzamento entre *Citrus sunki* e *Poncirus trifoliata*. As estacas semilenhosas foram cultivadas em substrato especial para citros, sob nebulização e previamente tratadas com 1g/L de ácido indol butírico, por 10 minutos. A detecção dos QTLs foi realizada pelo método de mapeamento por intervalo composto. Foram detectados dois QTLs associados ao enraizamento de estacas no mapa genético de *P. trifoliata*.

Table 1. Linkages groups, molecular markers linked by interval map analysis, absolute position of QTL from left telomere in centiMorgans, LOD score values, phenotypic variation explained by QTL (R^2).

Linkage groups	Markers linked QTL	QTL Position cM	LOD Score	R^2
II of *P. trifoliata* 'Rubidoux'	N05_1300 AV16_1400	0.5392	3.9	0.209
III of *P. trifoliata* 'Rubidoux'	AV02_110 M12_490	0.2396	4.4	0.158

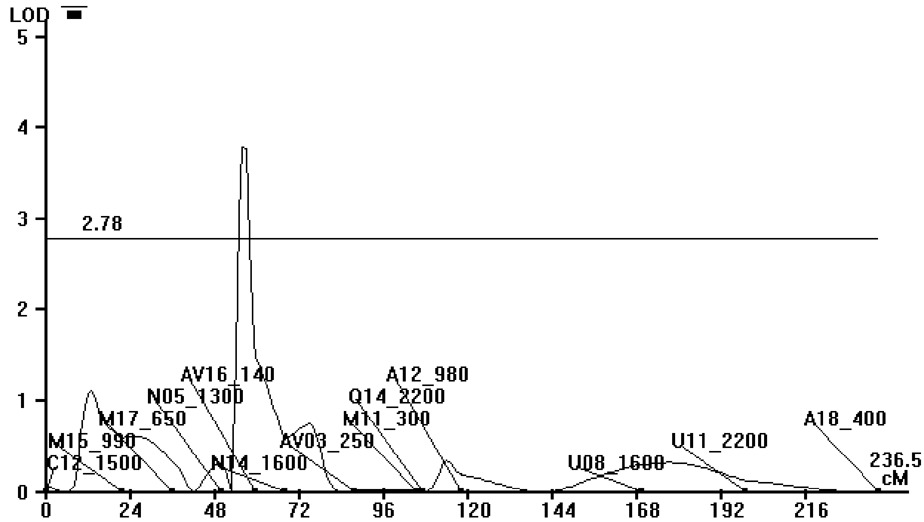

Figure 2. Analysis of linkage group II of the *Poncirus trifoliata* 'Rubidoux' map and the presence of a QTL associated with the rooting of the cuttings between the markers N05_1300 and AV03_2500. (LOD = 3.9).

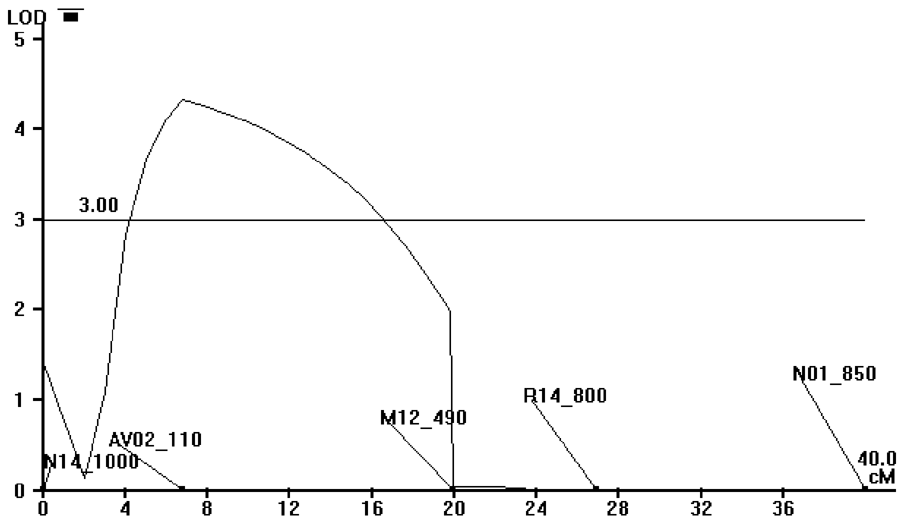

Figure 3. Analysis of the linkage group III of the *Poncirus trifoliata* 'Rubidoux' map and the presence of a QTL linked to the rooting of the cuttings associated with the RAPD markers AV02_1100 and M12_490. (LOD = 4.4).

REFERENCES

Abou-Rawash, M.; El-Wakeel, H.F.; Kassem, N.; Mohamed, E.A. 1998. Studies on the vegetative propagation of some citrus rootstocks. Annals of Agricultural Science Cairo. 43:523-537.

Basten, C.J. Weir, B.S., Zeng, Z.B. 2000. QTL Cartographer. A reference manual and tutorial for QTL mapping. Raleigh. North Caroline State University. Version 1.14. 139 p.

Bester, J.J.; Rabe, E. 1996. Auxin transport in relation to vegetative vigor in citrus rootstocks. Journal of the Southern African Society for Horticultural Sciences. 6:72-74.

Castle, W.S., Tucker, D.P.H., Krezdorn, A.H., Youtsey, C.O. 1993. Rootstocks for Florida citrus: rootstocks selection - The first step to success. Orlando. University of Florida. 92p.

Cristofani, M., Machado, M.A., Grattapaglia, D. 1999. Genetic linkage maps of *Citrus sunki* Hort. ex. Tan. and *Poncirus trifoliata* (L.) Raf. and mapping of citrus tristeza virus resistance gene. Euphytica. 109:25-32.

Ferguson, J.; Young, M.; Halvorson, J. 1986. The propagation of citrus rootstocks by stem cuttings. Proceedings of the Florida State Horticultural Society. 98:39-24.

Ferri, C. 1997. Enraizamento de estacas de citros. Revista Brasileira de Fruticultura. 19:113-121.

Gmitter, F.; Deng, Z.; Moore, G.A. 1998. Utilization of DNA markers in citrus breeding programs**.** Fruits. 53:303-306.

Grattapaglia, D., Bertolucci, F.L., Sederoff, R.R. 1995. Genetic mapping of QTLs controlling vegetative propagation in *Eucalyptus grandis* and *E. urophylla* using a pseudo-testcross strategy and RAPD markers. Theor. Appl. Genet., 90:933-947.

Liu, B.H. 1998. Statistical genomics: linkage, mapping, and QTL analysis. Boca Raton. CRC Press. 854p.

Platt, R.G.; Optiz, K.W. 1973. The propagation of citrus. In: Reuther, W. (editor). The Citrus Industry. Berkeley. University California Press. p.1-47.

Pompeu Junior, J. Porta-enxerto. 1991. In: Rodriguez, O., Viegas, F.; Pompeu Junior, J.; Amaro, A.A. (Editores). Citricultura brasileira. Campinas. Fundação Cargill. p.116-152.

Prati, P.; Mourão Filho, F.A.A.; Dias, C.T.S.; Scarpare Filho, J.A. 1999. Estaquia semi-lenhosa: um método rápido e alternativo para a produção de mudas de lima acida 'Tahiti'. Scientia Agricola. 56:1. p.185-190.

Rakesh-Kumar, R.; Gill, D.S.; Kaushik, R.A. 1995. Effect of indole-butyric acid, p-hydroxybenzoic acid and season on the propagation of lemon cv. Baramasi from cuttings. Haryana Journal of Horticultural Science. 24:13-18.

Sabbah, S.M.; Grosser, J.W.; Chandler, J.L.; Louzada, E.S. 1991. The effect of growth regulators on the rooting of stems of citrus, related genera and intergeneric somatic hybrids. Proceedings Florida State Horticultural Science.104:487-491.

Santos, R.F.A.; Villas Boas, R.M.F.; Salibe, A.A. 1988. Estudos sobre enraizamento de estacas de citros com aplicação de agentes de efeito hormonal. In: 9º. Congresso Brasileiro de Fruticultura. Campinas. Anais... Sociedade Brasileira de Fruticultura. p.387-393.

Shazly, S.M.; Sabrout, M.B.; Kassem, H.A. 1994. Root formation on the stem cuttings of Eureka lemon and El-Soukari loquat as affected by root-promoting chemicals and mist. Alexandria Journal of Agricultural Research. 39:559-569.

Spiegel-Roy, P.; Goldschmidt, E.E. 1996. Biology of citrus. Cambridge. Cambridge University. 230p.

Tanksley, S.D. 1993. Mapping poligenes. Annual Review of Genetics**.** 27:205-233.

Villas Boas, R.M.F.; Santos, R.F.A.; Salibe, A.A. 1988. Enraizamento de estacas de diferentes espécies de citros. In: 9.º Congresso Brasileiro de Fruticultura. Campinas. Anais...Sociedade Brasileira de Fruticultura. p.367-373.

Young, N.D. 1996. QTL mapping and quantitative disease resistance in plants. Annual Review Phytopathology. 34:479-501.

Zeng, Z.B. 1993. Theoretical basis of precision mapping of quantitative trait loci. Proceedings National Academy Science of United States of America. 121:10972-10976.

Zeng, Z.B. 1994. Precision mapping of quantitative trait loci. Genetics.136:1457-1468.

Received: June 21, 2002;

Accepted: October 28, 2002.