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GENETIC ANALYSIS OF QTLs CONTROLLING CARPEL NUMBER IN CITRUS

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Abstract

Purpose of this research is to determine the markers controlling carpel number in mandarin. Progenies obtained by hybridization between the Clementine mandarins (C. clementina Hort. ex Tan.) and Orlando tangelos (C. reticulata Blanco x C. Paradise Macf.) have been used in this research. Morphological and molecular marker data were analyzed in SAS software by using GLM and REGRESSION module. Population includes variation in respect to carpel number, which transgressive segregation was observed and distribution was positively skewed. Based onvariance analysis made by using GLM option in SAS software, ten markers were associated with carpel number All loci explained 100% of the variation for carpel number. OPW19.25, a RAPD marker explained 43% of total variation and OPM20.23 explained 22% of total variation. These results revealed that two loci had major effect in respect to carpel number and other loci had a minor effect. This research revealed significant clues about genetic mechanism of carpel number in mandarin fruit. These markers should be further investigated for applicability and conversion to more specific markers such as SCAR and CAP. This was the first report of the genetic mechanism and molecular markers associated with carpel number in citrus.

Key words*: regression analysis, inheritance, QTL.*

INTRODUCTION

Citrus is the fruit group with higher production worldwide, approximately 120 million tons annually (FAO, 2012). It exists in diploid forms generally, but occasionally exists in triploid and tetraploid forms (2n = 2x = 18). Improving citrus types in respect to important fruit characteristics requires new techniques due to high level of heterozygosity and apomixis. Mutation breeding and somatic hybridization are mostly used in current improvement programs, but these methods rarely contributed to improve the fruit character (DAVIES and ALBRIGO, 1994). Molecular genetic technology can provide new methods which will remove/reduce the obstacles mentioned above.
To date, genetic mapping studies have been focused only on some rootstock features which allows farming under several stress conditions: apomixes (GARCIA et al., 2000), salt resistance (TOZLU et al., 1999) and tristeza virus resistance (ROOSE, 2000). Many characters are controlled by quantitative trait loci (QTL) and genetic maps were usually based on quantitative traits (CHEN et al. 2007). Reports of mapping efforts on fruit characters are scarce (GULSEN etal., 2011). Inheritance of commerciality important fruit characters of citrus fruits is unknown. Data about the characteristics of other fruits, for example easy peeling, flesh color, puffing, granulation, pipiness, and aroma and carpel membrane thickness have not been reported. The purpose of this study was to investigate the association between molecular markers and carpel number in a segregating population derived from a cross between mandarin and tangelo.

MATERIALS AND METHODS

164 progenies derived from the hybridization between Clementine mandarin (*C. clementina* Hort. ex Tan.) and Orlando tangelos (*C. reticulata* Blanco x *C. paradise* Macf.) available in mandarin collection of Alata Horticultural Research Station as described by GULSENet al. (2010). Data file including fruit characteristics and molecular DNA markers were analyzed by using GLM and REGRESSION module in SAS software. Variance analysis has been implemented by GLM module first in respect to all DNA markers. All markers detected to be significant at 5% alpha level were subjected to advanced regression analysis by using REGRESSION module.

RESULTS AND DISCUSSIONS

The population used in this study indicated transgressive segregation between 9 and 14 carpels where parents Clementine and Orlando had 10 and 11 carpels, respectively. Distribution of hybrids to carpel number was as follows: 17 individuals with 9 carpels, 20 with 10 carpels, 18 with 11 carpels, 6 hybrids with 12 carpels and one with 13 and 14 carpels (Figure 1). Positive skewness were observed among the hybrids and segregation was transgressive meaning progenies indicated more or lower values for the trait. Distribution is bell-shaped also indicating quantitative control.

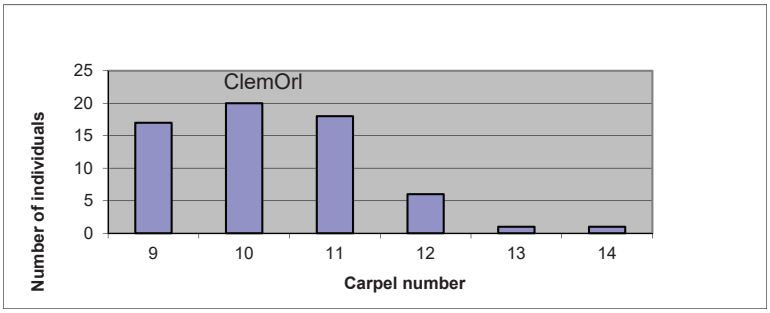

Figure 1. Distribution of F1 hybrids based on carpel number.
Abbreviations: Clementine, Cle; Orlando tangelo, Orl

In GLM analysis in SAS software detected 10 molecular markers that contributed 1% or more to carpel number in the population studied (Table 1). The first 5 loci in Table 1 explained 80% of the total variation, which was very significant. The markers explaining 5% or more of variation: OPW19.250 explained 43%, OPM20.230 22% and ISSRHVH $(CA)_7$ 5%. These 10 markers were subjected to regression analysis. The regression model explained 90% (R^2) of the total variation for carpel number and intercept value calculated was 0.69102. This was consistent with our observation from the Figure 1.

Table 1. SAS Abstracts obtained with the slice number analyze and SAS software 602 markers and 63 F1 hybrid slice number data have been shown with the explained variation ratios under partial R^2

Marker	R^2	F-value	Pr> F
OPW19.250	0.4321	15.98	0.0007
OPM20.230	0.2233	12.96	0.0018
ISSR HVH(CA)7	0.0532	3.47	0.0782
SRAPEM14ME7a	0.0459	4.42	0.0516
SSRAG14.150	0.0417	3.34	0.0851
SRAPEM2ME7b	0.0378	2.68	0.1188
SRAPEM10ME5a	0.0325	4.84	0.0465
SRAPEM7ME12a	0.0279	3.25	0.0928
SRAPEM16ME1a	0.0184	1.87	0.1919
SSRGA01.200	0.0153	2.55	0.1361

Hybrids derived from Clementine mandarin and Orlando tangelo were used in this research. F1 hybrids were characterized for carpel number by simply visually counting them. Molecular marker data were available from GULSEN et al. (2010). They have been used in SAS software in order to determine molecular markers associated with the carpel number. It was concluded that carpel number was controlled by quantitative loci (several genes). By using these molecular markers regression model explained more than 90% of the total variation. This was the first report related to genetic mechanism of carpel number in citrus. Thus this provided valuable insight into possible genetic mechanism of a fruit trait in

addition to fruit acidity previously reported by Fang et al. (1997). Developing genetic maps and molecular markers can provide important tools for citrus breeding programs. As we emphasized, in citrus which have long juvenility period and apomixes, the tools allowing early selection could play an important role in speeding improvement programs.

There is lack of studies of morphological characters which are quantitatively controlled in citrus. FANG et al. (1997) revealed that the population showed binary distribution but it is not significant in inter-class difference in the research which was carried out in order to find related markers for citrus acid level. It could be understood that the genes which has fewer effects played role besides the major gens. OPW19.250 marker determined in this study explained 43% of total variation alone. The second important locus, OPM20.230, explained 22% alone. Other 8 loci explained low level of variations. It could be said that loci in respect to carpel number have major effects and other loci have minor effects. This situation resembles the situation in acid accumulation. The obtained results could be used in order to increase our understanding of the genetic mechanism of important traits and speed up the breeding programs. Genetic studies about carpel number in citrus have not been reported yet. As mentioned above carpel number varied between 9 and 14. Carpel number of mandarins should not neither so few nor so many. When compared with orange, mandarins have fewer carpels in their fruits.

CONCLUSIONS

In this study the statistical analyses revealed significant findings on possible genetic mechanism of carpel number in citrus. First it has been showed that the segregation for the carpel number was transgressive in citrus, in which the progenies exceed their parents. Secondly, we detected two very significant loci that explained 44 and 22% of the total variation. Carpel number varied between 9 and 14 while parents had 10 and 11 carpels in their fruits. The population indicated positive skewness toward higher carpel number. The regression analysis indicated that 10 markers were placed in the regression model and explained more than 90% of the total variation for carpel number in citrus. This information may provide an important base for further research on this trait or other similar traits. Applicability of these markers for early selection of progenies of citrus with long juvenility should be further investigated. In addition, conversion of these markers to more locus specific markers such as SCAR and CAP) is necessary.

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