

Abstract

The inevitable occurrence of periodic frosts has a profound impact on the performance of citrus plants. In this study, two physiological and molecular experiments were carried out during 2015-2017. For this purpose, the process of changing some physiological indices in response to four stress levels (3 ° C, 0 ° C, -3 ° C and -6 ° C) for 110 citrus genotypes in split-plot design was studied with three replications. The values of the three traits of electrolyte leakage, leaf water soaking, and lipid peroxidation increased with decreasing temperature while for tolerance indices (antioxidant capacity, proline content, total carbohydrates, the activity of superoxide dismutase and ascorbate peroxidase), a sigmoidal change was recorded. Molecular assays using 28 SSR primer pairs and 25 AFLP primer combinations showed a total of 326 and 545 polymorphic bands with mean polymorphism percentages of 97.5% and 82.68%, respectively, indicating high diversity among samples. Based on genetic diversity statistics, primers TAA41 and AC01 for SSR marker and primer combinations EACA + MCAC and ECCA + MAAC for the AFLP marker had more potential to differentiate genotypes. The results of the different statistical analyses largely confirmed each other. Based on population structure analysis and Bayesian model, it was found that in addition to the three species: citron, pomelo, mandarin, and *Poncirus trifoliata*, at least two other species or genera of citrus relatives, share different genome in studied genotype makeup. Association analysis was performed based on a mixed linear model (MLM) using the population structure matrix (Q) and kinship matrix (K). Based on this, the efficiency of MLM: K + Q model in determining false-positive relationships was more than MLM: K model. The highest marker-trait relationship was calculated at -3 ° C. The CT02, TAA1, and TAA27 markers together with the two AFLP markers: ECGC + MATG-1 and ECGC + MAAC-5 justified the most phenotypic variation for SOD activity. To our knowledge, this is the first study that reports the relationship between molecular markers located on chromosome 6 in Citrus and SOD activity. Similarly, we report the association between CT21 on chromosome 5 and GT03 on chromosome 8 with proline content (PC). Our results suggested that SOD and PC could be considered as important selective traits in screening populations for CT. Selected genotypes and markers are expected to be used to advance citrus breeding and marker-based selection programs.

Keywords: Association analysis, Citrus, Cold stress, Molecular markers, Population structure analysis.



University of Zabol

Graduate school

Faculty of Agriculture

Department of Plant Breeding and Biotechnology

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Supervisors:

Dr. M. Solouki

Dr. B. Golein

Advisors:

Dr. B. Fakhri

Dr. A. Sabouri

By:

A. Abouzari

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