# Utilization of DNA markers in citrus breeding programs

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## Utilization of DNA markers in citrus breeding programs.

**Abstract — Introduction**. Various aspects of reproductive biology including sexual incompatibilities, nucellar embryony, juvenility, and the narrow genetic base of commercially important species hinders citrus breeding programs. However, once hybrids have been made, the greatest impediment is the lack of efficient, accurate, and cost-effective screening and selection procedures for most traits targeted by citrus breeders for genetic manipulation and improvement. DNA marker technology provides tools to overcome the challenge of multi-trait selection in breeding programs in the short term, and, in the long term, it holds promise to enable development of new scion cultivars with specific improvements for key traits. **Marker-assisted selection**. Marker-assisted selection (MAS) is a tool that enables plant breeders to select for a number of traits simultaneously among very young plants. Marker development takes advantage of polymerase chain reaction technology, which is a method to amplify and visualize specific DNA sequences as fragments on gels. Impacts that MAS can have on citrus breeding are projected in the paper. **Map-based cloning of genes**. A sophisticated extension of DNA marker technology called map-based cloning, in combination with methods of citrus genetic engineering, does promise in the near future to deliver some rather spectacular benefits for the improvement of cultivar groups, as oranges, grapefruit, and lemons which are not amenable to hybridization as a cultivar-improvement strategy. For example, efforts to clone the citrus tristeza virus-resistance gene are described, to illustrate the kind of genetic improvements that will be possible shortly. (© Elsevier, Paris)

***Citrus* / plant breeding / plant biotechnology / selection / genetic markers / selection criteria**

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## Utilisation des marqueurs de l'ADN pour l'amélioration des agrumes.

**Résumé — Introduction**. Divers aspects de la biologie de la reproduction dont les systèmes d'incompatibilité, l'embryonnie nucellaire, la juvénilité et l'étroitesse de la base génétique des espèces commercialement importantes gênent l'amélioration des agrumes. Cependant, une fois les hybrides créés, l'obstacle majeur est le manque de procédures de criblage efficaces, précises et rentables pour les caractères intéressant le sélectionneur. La technologie des marqueurs DNA fournit des outils qui permettent à court terme de surmonter la difficulté d'une sélection multicaractère lors de programme de sélection, et qui font espérer à long terme un éventuel développement de nouveaux cultivars greffons possédant des améliorations spécifiques pour des caractères clés. **La sélection assistée par marqueurs**. La sélection assistée par marqueurs (MAS) est un outil qui permet aux sélectionneurs d'effectuer un tri parmi de très jeunes plants à partir de la prise en compte simultanée de nombreux caractères. Le développement de marqueurs s'appuie sur la technique des PCR qui permet d'amplifier et de visualiser des séquences d'ADN spécifique en fragments sur des gels. Les impacts que peut avoir la MAS sur la sélection des agrumes sont évalués dans ce document. **Clonage de gènes sur cartographie**. Une extension sophistiquée de la technique des marqueurs ADN, appelée clonage de gènes sur cartographie (MBC), combinée avec des méthodes d'ingénierie en génétique des agrumes, promet de donner prochainement des résultats assez spectaculaires pour l'amélioration de groupes de cultivars, tels qu'orangers, pomélos et citronniers peu propices à l'hybridation en tant que stratégie d'amélioration de cultivar. Les tentative de clonage du gène de résistance au CTV (*citrus tristeza virus*) sont exposées afin d'illustrer les améliorations génétiques envisageables à court terme. (© Elsevier, Paris)

***Citrus* / amélioration des plantes / biotechnologie végétale / sélection / marqueur génétique / critère de sélection**

## 1. introduction

Historically, citrus cultivar improvements have relied on the identification of chance seedlings or bud sport mutations that possessed some characteristic trait which made them superior to previously existing cultivars. There are various biological factors that have limited progress toward improved cultivars, by excluding some potentially valuable parents from the breeding pool (such as sterility or sexual incompatibility), or by limiting the numbers of hybrids that can be recovered from controlled crosses (e.g., nucellar embryony). Furthermore, some of the most economically important citrus fruit (orange, grapefruit, and lemon) are not true species and cannot be improved by making crosses; all cultivars in these groups have originated as spontaneous mutations, in budlines or in nucellar seedlings [1]. Sexual hybridization has been somewhat useful in the development of new mandarin and rootstock cultivars, but even within these groups it should be noted that most cultivars used today are not the result of systematic breeding programs. The factors that have limited the success in breeding new rootstocks and mandarin cultivars have been the lack of information on the inheritance of important traits, and more significantly, the lack of simple, effective, and cost-efficient screening and selection procedures to identify hybrids improved for multiple traits. DNA marker technology provides tools to overcome the challenge of multitrait selection in breeding programs in the short term, and, in the long term, it holds promise to enable development of new scion cultivars with specific improvements for key traits.

## 2. marker-assisted selection

Marker-assisted selection (MAS) [2] is a tool that enables plant breeders to identify individuals from breeding families that possess genes for specific useful traits. The potential power of MAS should become obvious by consideration of the following example for citrus rootstock improvement. Breeders have made *Citrus* × *Poncirus* crosses for rootstock improvement to capture the resistance to citrus tristeza virus (CTV), citrus nematode (CN), and *Phytophthora*. If 1 000 such hybrids were produced and the frequency of hybrids with adequate resistance to CTV = 0.5, and to CN and *Phytophthora* = 0.1 each, then only five individual hybrids should possess adequate resistance to all three pathogens ($1\,000 \times 0.5 \times 0.1 \times 0.1 = 5$). If these 1 000 hybrids were to be screened for resistance to each pathogen, it could take three full-time researchers as long as 5 years to find the five best hybrids; with DNA markers proven to be linked to genes for resistance to these pathogens, the screening and selection process should take only one researcher 6 months or less to achieve the same result.

Marker development takes advantage of polymerase chain reaction (PCR) technology, which is a method to amplify and visualize specific DNA sequences as fragments on gels. The first step in marker development is to evaluate breeding families for resistance/susceptibility to the pathogen of interest, using existing methods of inoculation and testing. Once individuals in the family have been characterized, DNA is extracted from several of the most resistant and most susceptible hybrids and pooled together. These pools are subjected to PCR, and a panel of primers (which determine the nature of the banding patterns on gels) is screened to find those that produce major fragments associated uniquely with resistance. Next, the association of these markers to each other and to the gene(s) of interest are tested in several families. The markers most tightly-linked to the gene(s) can be used then on a routine basis for screening and selection in breeding programs.

Thus far in citrus, markers have been identified and are being used to select for the CTV resistance gene from *Poncirus* [3] and for a major gene conferring resistance to CN. Other markers are being pursued for rootstock-relevant traits including

tolerance of salinity and freezing temperatures, *Phytophthora* resistance, and nucellar embryony. As these new markers are developed, MAS will become a very powerful tool that can enable breeders to select for a number of traits simultaneously among very young plants. This greatly enhanced selection efficiency means that breeders will be able to select from much larger families then ever possible before, in less time, and using much less resources. Only prescreened, elite individual hybrids will need to be planted for advanced tests and field evaluation. The end result will be new cultivars that possess multitrait improvements for the benefit of producers and consumers of citrus fruit.

## 3. map-based cloning of genes

It is clear that MAS methods will have benefit for cultivar improvement programs focused on rootstocks and mandarins, both of which can be approached by controlled crosses. As indicated above though, oranges, grapefruit, and lemons are not amenable to hybridization as a cultivar-improvement strategy, and therefore MAS has no immediate benefits to provide. A sophisticated extension of DNA marker technology called map-based cloning (MBC) [4] does promise to deliver some rather spectacular benefits for the improvement of these cultivar groups in the near future, in combination with methods of citrus genetic engineering.

The principles of MBC are being applied to isolate and clone the gene for CTV resistance (designated *Ctv*) from *Poncirus trifoliata*, for subsequent transformation of CTV-susceptible citrus cultivars. Although this gene can and has been moved from *Poncirus* to *Citrus* by making crosses, it is not practical to develop scion cultivars in this manner because many other genes with adverse effects on fruit quality are likewise transmitted. Based on the genetic map of the *Ctv* region, the most closely linked markers that border the gene *Ctv* will be used to find large pieces of DNA from resistant plants that contain this target gene. These large pieces are made by partially digesting the total DNA and cloning the resulting fragments in bacteria, to produce a library that contains all of the pieces and thus, all of the genetic information from a resistant plant. Such a BAC (bacterial artificial chromosome) library [5] will consist of 15–20 000 individual bacterial colonies. To date, we have identified appropriate markers and have nearly completed construction of the library. Once fragments containing the gene have been isolated, they can be transferred into susceptible cultivars by transformation methods and tested for their effectiveness in CTV resistance. Map-based cloning is a very costly and technically demanding challenge. However, this effort should be justified by the potentially great benefit that will result by the protection of citrus scion (and rootstock) cultivars against this wide spread and damaging citrus virus.

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## references

[1] Gmitter F.G. Jr., Grosser J.W., Moore G.A., Citrus, in: Hammerschlag F.A., Litz R.E. (Eds.), Biotechnology of Perennial Fruit Crops, CAB International, Wallingford, UK, 1992, pp. 334–369.

[2] Michelmore R.W., Paran I., Kesseli R.V., Identification of markers linked to disease resistance genes by bulked segregant analysis: a rapid method to detect markers in specific genomic regions by using segregating populations, Proc. Natl. Acad. Sci. USA 88 (1991) 9828–9832.

[3] Gmitter F.G. Jr., Xiao S.Y., Huang S., Hu X.L., Garnsey S.M., Deng Z., A localized linkage map of the citrus tristeza virus resistance gene region, Theor. Appl. Genet. 92 (1996) 688–695.

[4] Martin G.B., Ganal M.W., Tanksley S.D., Construction of a yeast artificial chromosome library of tomato and identification of cloned segments linked to two disease resistance loci, Mol. Gen. Genet. 223 (1992) 25–32.

[5] Shizuya H., Birren B., Kim U.J., Mancino V., Slepak T., Tachiiri Y., Simon M., Cloning and stable maintenance of 300-kilobase-pair fragments of human DNA in *Esherichia coli* using an F-factor-based vector, Proc. Natl. Acad. Sci. USA 89 (1992) 8794–8797.

## Utilización de los marcadores del DNA para mejorar los agrios.

**Resumen — Introducción**. Varios aspectos de la biología de la reproducción entre ellos los sistemas de incompatibilidad, la embrionia nucelar, la juvenilidad y la estrechez de la base genética de las especies comercialmente importantes estorban el mejoramiento de los agrios. Sin embargo, una vez creados los híbridos, el obstáculo mayor es la falta de procedimientos de cribado eficaces, precisos y rentables para los caracteres que interesan al geneticista. La tecnología de los marcadores DNA proporciona herramientas que permiten a corto plazo superar la dificultad de una selección de varios caracteres en programa de selección, y que hacen esperar a largo plazo un posible desarrollo de los nuevos cultivares injertados que poseen mejoramientos específicos para caracteres claves. **La selección asistida por marcadores**. La selección asistida por marcadores (MAS) es una herramienta que permite a los geneticistas realizar una selección entre muy jóvenes plantas a partir de la toma en cuenta simultánea de numerosos caracteres. El desarrollo de marcadores se apoya en la técnica de los PCR que permite amplificar y visualizar secuencias de DNA específica en fragmentos sobre geles. Los impactos que puede tener la MAS en la selección de los agrios se evalúan en este documento. **Clonaje de genes en cartografía**. Una extensión sofisticada de la técnica de los marcadores DNA, llamada Clonaje de genes sobre cartografía (MBC), combinada con métodos de ingeniería en genética de los agrios, promete dar próximamente resultados bastante espectaculares para el mejoramiento de grupos de cultivares, tales como los naranjos, pomelos y limoneros poco propicios a la hibridación como estrategia de mejoramiento de cultivar. Se expone la tentativa de Clonaje del gen de resistencia al CTV (Citrus Tristeza Virus) para ilustrar los mejoramientos genéticos programados a corto plazo. 

***Citrus* / fitomejoramiento / biotecnología vegetal / selección / marcadores genéticos / criterios de selección**