MOLECULAR MARKER-ASSISTED SELECTION FOR RESISTANCE TO PATHOGENS IN TOMATO

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Introduction

Tomato (*Lycopersicon esculentum* Mill.) is considered as one of the most widely grown vegetable crop in the world. It is used as a fresh vegetable and can also be processed and canned as a paste, juice sauce, powder or as a whole. World volume has increased approximately 10% since 1985, reflecting a substantial increase in dietary use of the tomato. Nutritionally, tomato is a significant dietary source of vitamin A and C. Furthermore, recent studies have shown the importance of lycopene, a major component of red tomatoes, which has antioxidant properties and may help to protect against human diseases, such as cancer and heart disease.

One of main constraint of tomato cultivation is damage caused by pathogens, including viruses, bacteria, nematodes, fungi, which cause sever losses in production. The control of pathogen spread mainly involves three strategies, which are husbandry techniques, application of agrochemicals, use of resistant varieties. Chemical control gives good results for some pathogens, but poor results against others, such as bacteria, and pratically no effects on viruses. Moreover, reduction of chemical treatements limits risks for farmers and consumers. Therefore, in order to realize a sustainable agriculture and to get high quality products in terms of health safe, the use of resistant varieties becomes a principal tool to reduce damages caused by pathogens.

Since the early days of 20th centrury classical breeding for disease resistance in plants has been a major method for controlling plant diseases. Varieties that are resistant or tolerant to one or few specific pathogens are already available for many crops. Resistant hybrids with multiple resistances to several pathogens exist and are currently used in vegetable production. For tomato, the genetic control of pathogens is a very useful pratice and most of used resistance is monogenic and dominant. So far, tomato breeding has resulted in varieties with resistance to at least 15 pathogens (Table 1), although with varying stability and level of expression. Many open-pollinated varieties presently cultivated possess genetic resistance to three or four pathogens. With the increasing use of F_1 hybrids it is possible to use varieties combining four to six resistances.

Although conventional plant breeding had a significant impact on improving tomato breeding for resistance to important diseases, the time-consuming process of making crosses and backcrosses, and the selection of the desired resistant progeny make it difficult to react adequately to the evolution of new virulent pathogens. Moreover, several interesting resistances are difficult to use because the diagnostic tests are often hard to develop due to the challenge posed by inoculum production and maintenance. In addition, where symtoms only are detectable on adult plant and/or fruits diagnostic tests could be particularly expensive and difficult to perform.

Table 1
List of pathogen resistances already present in tomato varieties obtained through conventional breeding

Virus
BCTV
TMV
TYLCV
TSWV
Bacteria
Corynebacterium michiganense
Pseudomonas solanacearum
Pseudomonas syringae pv. *tomato*
Nematodes
Meloidogyne spp
Fungi
Alternaria alternata f.sp. *lycopersici*
Cladosporium fulvum
Fusarium oxysporum f.sp. *lycopersici*
Fusarium oxysporum f.sp. *radicis-lycopersici*
Phytophthora infestans
Pyrenochaeta lycopersici
Verticillium dahliae

Modified from Laterrot (1996)

Since 1980s molecular markers are being widely used as a principal tool for the breeding of many crops, among these tomato. In particular, a great work has been realized to find molecular markers linked to disease resistance genes. Up till now, more than 40 genes (including many single genes and quantitative trait loci, QTL) that confer resistance to all major classes of plant pathogens have been mapped on the tomato molecular map (Table 2) and/or cloned from Solanaceous species, as reported by Grube *et al*. (2000). Afterwords, other resistance genes were added to the map (Bai *et al*., 2003; Chunwongse *et al*., 2002; Parrella *et al*., 2002), together with resistance gene analogues (RGAs), that are structurally related sequences based upon protein domain shared among cloned R genes (Leister *et al*., 1996). A molecular linkage map of tomato based on resistance gene analogs (RGA) was constructed where 29 RGAs were located on 9 of the 12 tomato chromsomes (Foolad *et al*., 2002; Zhang et al., 2002). Several RGA loci were found in cluster and their locations coincided with those of several known tomato R genes or quantitative resistance loci. This map provides a basis for further identification and mapping of genes and quantitative trait loci for disease resistance and will be useful for marker-assisted selection.

Independently of the type of marker used for selection, markers tightly linked to resistance genes can greatly aid disease resistance programs, by allowing to follow the gene under selection through generations rather than waiting for phenotypic expression of the resistance gene. In particular, genetic mapping of disease resistance genes has greatly improved the efficiency of plant breeding and also led to a better understanding of the molecular basis of resistance.

DNA marker technology has been used in commercial plant breeding programs since the early 1990s, and has proved helpful for the rapid and efficient transfer of useful traits into agronomically desirable varieties and hybrids (Tanksley *et al*., 1989). Markers linked to disease resistance loci can now be used for marker-assisted selection (MAS) programs, thus also allowing several resistance genes to be cumulated in the same genotype ("pyramiding" resistance genes). In addition, markers linked to resistance genes may be also useful for cloning and sequencing the genes. In tomato, several resistance genes have been so far sequenced, among which *Cf 2*, *Cf-4*, *Cf-5*, *Cf-9*, *Pto*, *Mi*, *I2*, and *Sw-5*. Cloned R genes now

provide new tools for plant breeders to improve the efficiency of plant breeding strategies, via marker assisted breeding.

Table 2
Resistance genes mapped in *Lycopersicon* genus

Gene	Pathogen	Chromosomal location
Asc	*Alternaria alternata* f.sp. *lycopersici*	3
Bw 1, Bw 3, Bw 4, Bw 5	*Ralstonia solanacearum*	6, 10, 4, 6
Cf-1, Cf-2, Cf-4, Cf-5, Cf-9	*Cladosporium fulvum*	1, 6, 1, 6, 1
Cm1.1- Cm 10.1	*Clavibacter michiganensis*	1, 6, 7, 8, 9, 10
Fr1	*Fusarium oxysporum* f.sp.*radicis- lycopersici*	9
Hero	*Globodera rostochiensis*	4
I1, I2, I3	*Fusarium oxysporum* f.sp. *lycopersici*	7, 11, 7
Lv	*Leveillula taurica*	12
Mi, Mi-3	*Meloidogyne spp.*	6, 12
Ol-1, Ol-qtl1, Ol-qtl2, Ol-qtl3	*Oidium lycopersicon*	6, 12
Ph-1, Ph-2, Ph-3	*Phytophthora infestans*	7, 10, 9
pot-1	PVY	3
Pto	*Pseudomonas syringae*	6
py-1	*Pyrenochaeta lycopersici*	3
rx-1, rx-2, rx-3	*Xanthomonas campestris* pv. *vesicatoria*	1
Sm	*Stemphylium spp.*	11
Sw-5	TSWV	9
Tm-1, Tm2a	TMV	2, 9
Ty-1, Ty-2	TYLCV	6, 11
Ve	*Verticillium dahliae*	9

However, as reported by Michelmore (2003) in a recent review, there is not a routine use of MAS for disease resistance, in spite of the deep knowledge of tomato genome and the availability of a high density molecular map for this species (Tanksley *et al*., 1992), which could both provide good opportunities to accelerate breeding through MAS.
In our laboratory, since two years we are testing the potentiality of MAS to speed up the breeding of tomato using molecular markers linked to various resistance genes. The two main goals of our research were a) to find the most suitable markers for MAS and b) to test the feasibility of MAS for pyramiding resistance genes both in fresh market and processing tomato “elite” lines.

Materials and methods

Six tomato genotypes carrying various resistance genes were available in our laboratory (Table 3). They were crossed with tomato "elite" lines, previously selected for their yield and quality performances, but which lacked resistance traits. Each resistant genotype was crossed with various "elite" tomato lines. Various backcross schemes were then carried out starting from different F_1 hybrids (from a minimum of 4 to a maximum of 8 backcross schemes were performed per each resistant genotype). At each backcross generation the screening of resistant genotypes was performed through molecular markers linked to the resistance genes tested on DNA extracted from young leaves at seedling stage. Only the resistant plants were then trasplanted and grown in greenhouse. At flowering, crosses were made with recurrent parent to get the following generations.

Table 3
Tomato genotypes used as resistant parents in the backcross breeding schemes.
For each genotype resistant genes are reported.

Genotype	Resistance gene	Pathogen
Heline	*Ph-2*	*Phytophthora infestans*
Momor	*Frl, Tm2a, Ve*	*Fusarium oxysporum* f.sp. *radicis-lycopersici,* TMV, *Verticillium dahliae*
Motelle	*I2, Mi, Ve*	*Fusarium oxysporum* f.sp *lycopersici, Meloidogyne* spp., *Verticillium dahliae*
Ontario	*Pto*	*Pseudomonas syringae*
Pyrella	*py-1*	*Pyrenochaeta lycopersici*
Stevens	*Sw-5*	TSWV

Since the efficiency of MAS is dependent on the availability of PCR-based markers highly linked to resistance gene under selection, for each resistance gene the most suitable marker system was investigated. At this purpose, three different strategies were undertaken.
The first was based on searching PCR-markers already available in the literature and on verifing their usefulness on our genetic material. The second consisted of designing PCR-primers from the sequence of cloned genes reported in database GenBank of the National Center for Biotechnology Information (http://www.ncbi.nlm.nih.gov/Genbank), whereas in the third case PCR-primers were designed from RFLP markers tightly linked to resistance genes. In the last case, this strategy was allowed since in the SolGenes database (http://probe.nalusda.gov:8000/plant/aboutsolgenes.html) sequences of many mapped tomato RFLPs are available online.

Results and discussion

Results so far obtained through these three approaches are reported in Table 4. In most cases, search of the most suitable marker was successful, leading to setting the right primer combinations, annealing temperature and restriction enzyme for each targed resistance gene. In some cases, when primers reported in the literature were used on our genetic materials, no correspondence of amplification product size or restriction fragment size was found, but we could set up a good marker by cloning and sequencing the fragments obtained. In case of gene *py-1*, we could also semplify the procedure reported in the literature, thus setting a marker system faster and cheaper to apply.

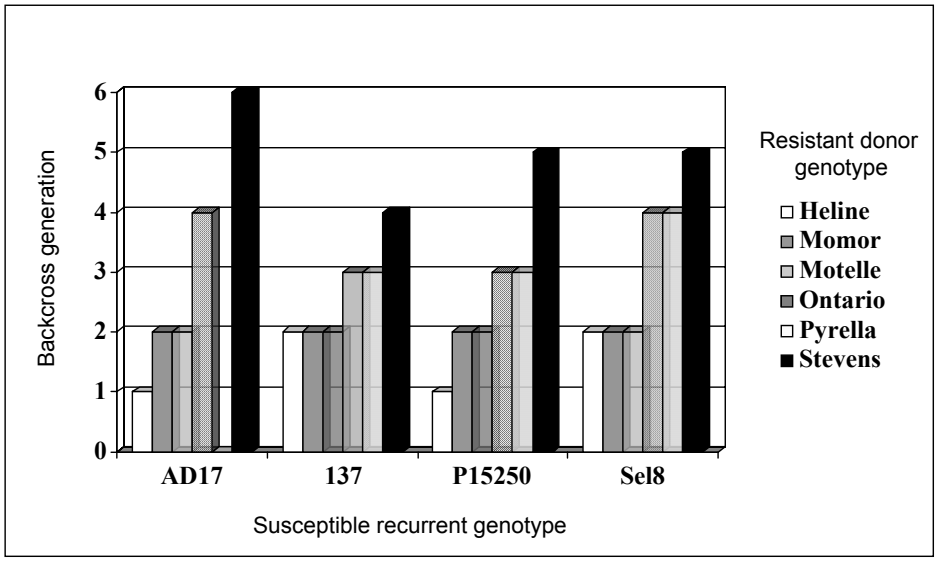
Table 4
Marker suitable for MAS for resistance traits, obtained with three different strategies

Resistance gene	Available marker	Marker type[1]	Successful strategy
Frl	+	CAPS	Linked RFLP
I2	-	-	-
Mi	+	CAPS	Literature
Ph-2	-	-	-
Pto	+	CAPS	GenBank sequence
py-1	+	SCAR	Literature
Sw-5	+	CAPS	Literature
Tm2a	+	CAPS	Literature
Ve	-	-	-

[1] CAPS= Cleaved Amplified Polmorphic Sequence; SCAR=Sequence Characterized Amplified Region

In few cases (genes *I2*, *Ph-2* and *Ve*), the search for a suitable marker is still in progress, since difficulties were met in detecting a polymorphic codominant marker. All the three strategies are being applied to reach a good result.
The markers so far found were used to select resistant genotypes in backcross breeding schemes. The molecular marker screening allowed three generation per year to be carried out. At present, for some cross combinations the BC_6 generation has been reached, for others only the BC_2-BC_3 (Figure 1). Where a BC_6 generation is already available, the breeding program is continuing by selfing BC_6 resistant genotypes, in all the other cases the backcross programm will continue up till reaching the sixth backcross generation. At the end of each backcross scheme, the resistant BC_6F_3 genotypes, selected through molecular marker analysis, will be also tested for resistance through pathogen inoculum and symtom relevance, in order to verify that no linkage breakage occurred, causing the resistance gene loss.

Figure 1
Results of backcross schemes carried out between 6 resistant genotypes and 4 susceptible tomato "elite" lines



The experience done on applying MAS for resistance breeding in tomato was positive, even though more work can be done, since many other resistance genes could be targeted with molecular markers, and therefore be introgressed into “elite” tomato lines. The hardest work

is the research of suitable markers, which often requires long time and is also expensive. In fact, this search is not so straightforward as could appear reading the literature, since many difficulties could be met when transferring techniques from one laboratory to others. However, when a marker has been set up, its use on large populations for resistance screening is then routinary. Technical facilities are today available for screening many samples contemporaneously and also costs for equipments are lowering.
The availability of PCR-based markers for many resistance genes allows the MAS for biotic resistance in tomato to be successfully applied in any laboratory withouth the need of high technologies. In addition, the rapid development of new molecular techniques, combined with the increasing knowledge on structure and function of resistance genes (Hulbert *et al*., 2001), will help getting new molecular markers for MAS. In particular, the future perspectives for pathogen resistance selection in tomato would include: mapping of other resistance genes for new pathogens which are becoming aggressive, development of PCR-based markers and design of most suitable breeding schemes, expecially for transferring QTL resistances (Ribaut and Hoisington, 1998).

Aknowledgements
This research was partially financed by MiPAF in the framework of projects PROMAR and "National Research Project for Horticulture".

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