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Advances in Biotechnology: Tomato as a Plant Model System

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ABSTRACT

This review reports some aspects of advances in biotechnology such as tissue culture, genetic engineering, molecular markers, and the new approach of the "omics" in tomato. *In vitro* regeneration has been a subject of research because of the commercial value of the crop and its amenability for further improvement via genetic manipulation. Numerous studies on plant regeneration from a wide range of tissues of wild and cultivated tomato germplasm have been conducted. Several genes involved in fruit ripening and others traits have been characterized and genetically engineered plants were successful obtained. One of the main uses of molecular markers has been the construction of linkage maps. Linkage maps have been utilized to identify chromosomal regions that contained genes controlling simple trait and QTL. DNA markers that are tightly linked to important genes are used as molecular tools for marker-assisted-selection (MAS) in tomato breeding. The latest trend is to combine QTL mapping with methods in functional genomics. There are wide collections of ESTs. The DNA microarrays analysis is also used to study the expression of many genes. Nowadays the proteomics and metabolomics allow identifying biochemical factors underlying important traits for tomato breeding programs.

Keywords: *in vitro* culture, genetic engineering, *Lycopersicon*, molecular markers, omics

Abbreviations: AFLP, amplified fragment length polymorphism; **EST**, expressed sequence tag; **PCR**, polymerase chain reaction; **QTL**, quantitative trait locus; **RAPD**, random amplified polymorphic DNA; **RFLP**, restriction fragment length polymorphism; **SCAR**, sequence characterized amplified region; **SNP**, single nucleotide polymorphism; **SSR**, simple sequence repeat

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INTRODUCTION

The cultivated tomato (*Lycopersicon esculentum* Mill., 2n = 2X = 24) is a dicotyledonous plant belonging to the Solanaceae family. It is one of the most popular and extensively consumed vegetable crops. Tomatoes are used either as fresh fruits or in the form of various processed products such as paste, whole peeled tomatoes, diced products, and

Received: 27 February, 2007. Accepted: 9 April, 2007.

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various forms of juices and soups. It is not a very energetic food, since approximately 95% of its weight is water and nearly 4% are carbohydrates. However, for its high consumption level it is an important source of mineral salts such as potassium and magnesium as well as vitamins (B_1, B_2, B_5 and C) and carotenoides like the lycopene. In recent studies, Blum *et al*. (2005) have reported that a dietary intake of tomatoes and tomato products containing lycopene is associated with a decreased risk of chronic diseases such as cancer and cardiovascular disease.

The *Lycopersicon* genus comprises nine species cross compatible to varying degrees, of which only *L. esculentum* was domesticated. All wild tomato species are native to western South America. They are distributed along the coast in the Andes, from Ecuador to northern Chile, and there are also two endemic species in the Galápagos Islands (Rick 1973). The small fruit cherry tomato *L. esculentum* var. *cerasiforme* (Dun.) Gray, the putative ancestor of the cultivated tomato, spread from Mexico to Europe and through the process of selection eventually led to the large fruited varieties. Tomato under selection has led to increased productivity, but at the same time it has arrowed its genetic basis. According to Miller and Tanksley (1990) less than 5% of the available genetic variation exists in tomato cultivars and the remainder is found in wild species of the genus. Related wild species of *Lycopersicon*, which show a high degree of homosequentiality in their chromosomes, were extensively used in tomato breeding for modifying traits such as insect and pathogen resistance, adverse weather conditions tolerance and fruit quality (Rick 1982; Zamir *et al*. 1994; Zhang *et al*. 2002; Rodríguez *et al*. 2006).

There are great controversy about the number of species in the group, their interrelationship and their treatment in the genus *Solanum* or *Lycopersicon* (for a review, see Spooner *et al*. 1993). Although the majority of taxonomists are adopting *Solanum* as the genus for tomatoes (Spooner *et al*. 2005) we will retain the old nomenclature for consistency with our papers and other reviewed ones.

The tomato fruit is a fleshy berry composed of an epidermis, a thick pericarp, and placental tissues surrounding the seeds. Tomato has emerged as the primary model for climacteric fruit ripening because this unique aspect of research can not be afforded by others plant models plant as *Arabidopsis thaliana* o rice. The importance of tomato as an agricultural commodity has resulted in decades of public and private breeding efforts that have yielded numerous spontaneous and induced mutations, including many that affect fruit development and ripening. Moreover, simple diploid genetics, small genome size, short cycle of crop, routine *in vitro* regeneration and transformation technology, and availability of genetic and genomic resources render tomato among the most effective model crop systems (Giovannoni *et al*. 2004).

This review reports some aspects of advances in biotechnology applied in tomato such as tissue culture, genetic engineering, molecular markers, and the new approach of the "omics".

IN VITRO CULTURE APPROACHES

Like other Solanaceae, such as *Nicotiana* and petunia, tomato is relatively favourable for *in vitro* culture from various types of tissues and organs.

Several empirical parameters are known to be crucial for successful tissue culture and *in vitro* plant regeneration. They include plant species and genotype, age and size of the explants, and an appropriate definition of culture conditions (e.g., composition of culture medium, type and relative amounts of growth regulators, temperature, light intensity and photoperiod).

The first report about regeneration of tomato shoots in cell culture was noticed by Norton and Boll (1954). They obtained shoots from callus induced from roots cultures of *L. peruvianum* (L.) Mill.

Genotypic differences in organogenic competence among *Lycopersicon* species and genotypes have always been characterized by growing cultures under defined media composition including plant growth regulator. Kartha *et al*. (1976) studied growth and morphogenetic responses of leaf sections of the *L. esculentum* variety Starfire to various individual phytohormones and combinations of phytohormones. Also, Zorzoli *et al*. (1988) reported the capacity to form shoots in five Argentinean cultivars of tomato from leaf explants. These results demonstrated that the *in vitro* propagation of these cultivars depended such as the state in which the explant was removed, the time of seedling and the concentration of the growth regulators. Shoot and roots from leaf explants are shown in **Fig. 1A**.

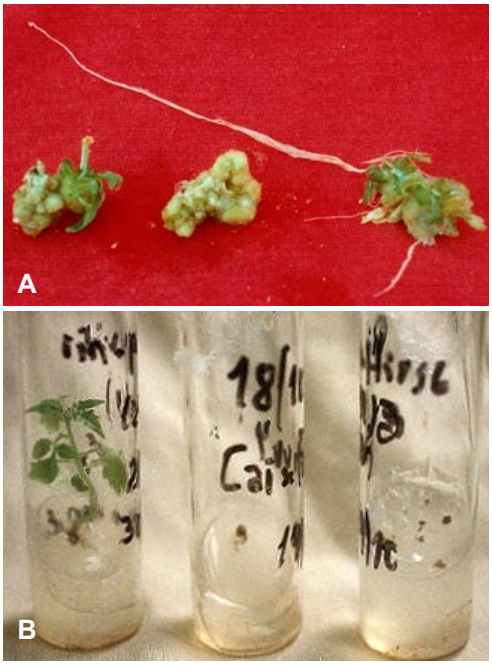

Fig. 1 ***In vitro*** **culture of tomato.** (**A**) Different types of response in tomato from leaf explants: callus, shoots and roots. (**B**) Rescue of immature embryo from hybrids with unilateral incompatibility.

Kut and Evans (1982) investigated the regeneration potential of leaf explants of eight *Lycopersicon* species and two closely related *Solanum* species as compared to cultivated *L. esculetum* on a medium supplemented with eight different hormone combinations. They concluded that all wild species studied with the exception of *L. hirsutum* Humb & Bonpl. and *L. pimpinellifolium* (Jusl.) Mill. regenerated more efficiently than *L. esculentum*. In addition to this, Pratta *et al*. (1997) utilizing cultivars of *L. esculentum* and accessions of *L. esculentum* var. *cerasiforme*, *L. pimpinellifolium* and *L. peruvianum* found highly significant differences among genotypes for regeneration capacity.

Recently, Bathia *et al*. (2005) demonstrated that the abaxial orientation of the explants and entire cotyledons induce better shoot regeneration and produce phenotypically normal shoots in 10 commercially important cultivars.

Regarding the culture conditions, light quality is one of the most important environmental signals that might modify the regeneration response in tissue culture. In these sense, Pugliese *et al*. (1999) reported that light is absolutely essential for regeneration of tomato shoots, as they found no regeneration in the absence of light. Lercari and Bertram (2004) compared different genotypes for the *in vitro* shoot formation capability of different parts of tomato hypocotyls obtained from donor seedlings initially grown in the dark or under continuous red or far-red light and subsequently cultured *in vitro* under different light qualities. They reported that the culture of competent hypocotyl segments under red, far-red or blue light reduced the frequency of explants forming shoots compared to those cultured under white light.

Composition of culture media (basal medium, type and concentration of phytohormones), culture condition and the

Table 1 Comparison of different protocols for *in vitro* culture and their response used by some authors.

Explant	Basal medium	Phytohormone (μM)	Light conditions (Photoperiod: hrs)	Response	Somaclonal variation	Reference
Leaf	MS salts + B5 vitamins	0.1-10 IAA + 10 BA	3000 lux (16)	C - S (+++) - R	-	Kartha *et al*. 1976
		0.1-1 IAA + 1 ZEA		C - S (+++) - R		
		5 BA		C - S (+++) - R		
		5 KIN		C - S (+) - R		
		1 NAA + 1 BA		C		
		1 NAA + 1 ZEA		C - R		
		0.1 KIN		C		
Leaf	MS	1 IAA + 10 BA	50 μmol m^{-2} s^{-1} (16)	C - S (+++) - R	-	Zorzoli *et al*. 1988
		5 BA		C - S (++)		
Cotyledon	B5	15 ZEA	38 μmol m^{-2} s^{-1} (16)	C -S (+++)	-	Bhatia *et al*. 2005
Hypocotyl	MS	Free	Darkness	No shoot	-	Pugliese *et al*. 1999
			50 μmol m^{-2} s^{-1} (16)	S (+++)		
Hypocotyl	MS	Free	White – 50 μmol m^{-2} s^{-1} (24)	S (+++)	-	Lercari and Bertram 2004
			Red, far-red or blue – 5 μmol m^{-2} s^{-1} (16)	No shoot or S (+)		
Leaf	MS	22.8 IAA + 18.6 KIN	Not reported	C - S (+++)	13 nuclear mutations	Evans and Sharp 1983

MS medium, Murashige and Skoog (1962); B5 medium, Gamborg *et al*. (1968).

IAA, indole-3-acetic acid; BA, benzylaminopurine; ZEA, zeatin; KIN, kinetin.

Calli (C), shoot (S), root (R). Poor response (+), moderate response (++), good response (+++)

in vitro response (calli, shoots and roots) are summarized in **Table 1**.

In vitro culture methods have opened the possibilities for various applications. These can be divided into two types: those in which stability should be maintained, and those in which the aim is to achieve some kind of genetic changes resulting from *in vitro* culture itself, or from manipulations *in vitro*.

In vitro culture applications in tomato breeding

Haploid plants from anther or pollen culture

The production of haploid plants from anther or pollen culture would allow the fixation and analysis of new gene combinations from hybrid plants in less time than the required in conventional breeding programs, as well as allowing the establishment of homozygous true. Haploid plants were induced from anther callus by Gresshoff and Doy (1972). Pollen culture is another means of obtaining haploid plants. Sharp *et al*. (1972) were the firsts to report successful cultivation of isolated tomato pollen cells by nurse culture. No report was made on shoot regeneration, but haploid callus colonies were obtained. Ziv *et al*. (1984) succeeded in regenerating and confirming the identity of dihaploid homozygous plants employing the *ms* 10^{35} mutant in a culture system described by Zamir *et al*. (1980). Anther or pollen culture in tomato has been frustrated by an extremely low efficiency of haploid/diploid recovery. On the other hand, these methodologies have an excessive cost when is compared to the conventional plant breeding methods.

Protoplasts and somatic hybridization

Several authors have shown that tomato mesophyll protoplast can be regenerated into plants (Koblitz and Koblitz 1982; Wijbrandi *et al*. 1990). The induced fusion of isolated protoplast followed by the regeneration of plants constitutes a way of circumventing sexual crossing barriers and provides approaches to genetic manipulation. The first somatic hybrid including tomato was described by Melchers *et al*. (1978), and it was made between tomato and potato. This success has demonstrated that somatic hybridization could be of great value for tomato plant breeding. Later, somatic hybridization between tomato and its wild relative *L. peruvianum* was reported (Kinsara *et al*. 1986). Asymetric somatic hybrids between *L. esculentum* and *L. peruvianum* have been obtained by the fusion of leaf protoplasts after irradiation of protoplasts of leaf tissue of *L. peruvianum* (Wijbrandi *et al*. 1990). Lefrançois *et al*. (1993) reported that the wild *Lycopersicon* species are much easier to regenerate from protoplast than the cultivated tomato. The large effort put into somatic hybridization experiments with tomato started with the first publication on the production of "pomatoes" as early as 1978, by Melchers and his associates. Then, the generation of protoplast culture and its *in vitro* regeneration have been improved considerably, which has led to the production of a large number of both symmetric and asymmetric hybrids and cybrids between tomato and other *Lycopersicon*, *Solanum* and even *Nicotiana* species. The limitation in the use of somatic hybridization for tomato breeding has been the difficulty to use the hybrids in backcrossing with diploid tomatoes (Wolters *et al*. 1993). The difficulty to obtain hybrids and cybrids between tomato and *Solanum* or *Nicotiana* species, together with the sterility and poor vigour of these hybrids, indicate the occurrence of strong somatic incongruity. Today, genetic engineering has displaced the interest in the use of somatic hybridization for tomato breeding as it will be detailed next.

Rescue of immature embryo

Embryo culture is used to rescue hybrid plants from sexual crosses blocked by postzygotic barriers. This method is especially important to achieving interspecific hybridization which is used for the introduction of new and desirable traits from wild species into cultivated tomato.

This technique was first studied by Smith (1944), who crossed *L. esculentum* var. Michigan State with *L. peruvianum* PI 128657 with the aim of transferring the wild nematode resistance *Mi* gene into commercial tomato cultivars. In interspecific crosses, hybrids of *L. esculentum* × *L. peruvianum* have been obtained via embryonic calli at 35-40 days after pollination (Thomas and Pratt 1981). *L. esculentum* × *L. chilense* Dun. and *L. esculentum* × *L. peruvianum* hybrids were obtained from embryos rescue and *in vitro* propagation (Chen and Adachi 1996). *In vitro* embryo culture, for tomato, is a useful tool for overcoming the problems in obtaining hybrids when wild species with unilateral incompatibility are incorporated to breeding programs (**Fig. 1B**).

Somaclonal variation - in vitro selection

The exploitation of tissue culture induced variation, also know as somaclonal variation, as a potential source of agronomically important traits has been studied in many crops. In very general terms, the variability detected in regenerated plants is due to either preexisting genetic variability in the donor tissue used for regeneration or induced by any of

the components of the culture medium during *in vitro* growth and plant regeneration. For tomato, several regenerated plants contain variability that is morphologically visible, a second commonly observed variation in tomato is tetraploidy and the third frequent variation among regenerated plants is sterility (Evans and Sharp 1983). The genetic variation has been detected both directly in regenerated plants and more importantly in the self-fertilized progenies of regenerated plants. For tomato, somaclonal variation has been studied extensively. For example, Evans and Sharp (1983) studied the progeny of 230 plants derived from leaf explants and found 13 nuclear mutations. *In vitro* selection has been used effectively for the isolation of mutants that are resistant to fungal toxins, salt or herbicides. A large majority of the mutant cell lines described have been obtained by resistance selection, in which a bulk population is subjected to a selection pressure (e.g., toxic drug, microbial, salt), and resistant cell lines recovered from surviving cells. Successful selection of disease resistant somaclone has been reported for fungal, viral and bacterial diseases (Barden *et al*. 1986; Shahin and Spivey 1986). Generally, the frequencies of disease resistant variants were very high. For instance, Barden *et al*. (1986) reported the selection of 6 out of 370 tomato somaclones, about 2%, with resistance to tobacco mosaic virus. In addition, van den Bulk *et al*. (1990) evaluated somaclones and their progenies for heritable phenotypic traits, e.g., lethality, reduced chlorophyll content, altered leaf morphology, absence of anthocyanin, variegation and dwarfing, and the frequency of somaclones that showed segregation for a particular variant trait was approximately 1%. A positive correlation between the salt responses at the cellular and whole plant levels was found when calli of the first subculture were used for evaluating the salt tolerance at the cell level (Rus *et al*. 2000). Through this technique, a somaclonal variant with high dried weight fruit content was commercialized by DNA Plant Technology of New Jersey. In very general terms, the somaclonal variation were used to development new breeding lines, however there are problems associated with these variants, e.g., variant plant cell are often found difficult to regenerate and chromosomal instability can occur in the regenerated plants.

Genetic analysis of in vitro culture

Studies on the genetics of regeneration in tomatoes have demonstrated that is highly heritable with a dominance effect (Wijbrandi *et al*. 1988). Model of qualitative inheritance of plant regeneration have been described (Koornneff *et al*. 1987; Faria and Illg 1996). It has been know that explants of some of the other *Lycopersicon* species, such as *L. peruvianum*, have higher regeneration ability than *L. esculentum*. Koornneef *et al*. (1987) introduced the superior regeneration capacity of *L. peruvianum* into the cultivated tomato by backcrossing (BC). The trait was apparently controlled by two loci, named *Rg*-1 and *Rg*-2 and the locus necessary for shoot formation (Rg-1) was mapped on chromosome 3 near the *r* locus that confers a yellow color to the fruit (Koornneef *et al*. 1993). Takashina *et al*. 1998 reported that the shoot regeneration capacity of *L. chilense*, which is a species of the "peruvianum complex", was dominant against *L. esculentum*, similar to that of *L. peruvianum*. They suggested that at least 2 loci or more may contribute to this capacity of *L. chilense* PI128644. Faria and Illg (1996) established a model for the inheritance of the plant regeneration trait on the basis of the F_1, F_2 and BC_1 generations obtained from crosses between the wild species *L. pimpinelllifolium* and five cultivars of *L. esculentum*. They found an interaction between two dominant genes determined the trait, indicating that the transfer of high plant regeneration ability from wild to commercial varieties would be relatively easy since a qualitatively inheritance pattern was involved. Approaches from quantitative genetics allow to segregate the genetic and environmental factors affecting the *in vitro* response, so that the analysis of some aspects of gene transmission and regulation becomes possible (Koornneef *et al*. 1987; Pratta *et al*. 2006). Using quantitative approaches Koornneef *et al*. (1987) found in a segregating generation of a hybrid between *L. esculentum* and *L. peruvianum* high values of heritability for callus growth and for the regeneration capacity of plants. Faria *et al*. (2002) were able to increase *in vitro* regeneration by artificial selection practiced on interspecific crosses. They obtained genotypes of high and low regeneration capacity using a genotype of *L. pimpinellifolium* as source of increasing alleles. Moreover, Pratta *et al*. (2003) found additive variation for callus percentage and regeneration percentage, as well as non-additive variation for productivity rate when evaluating different intra- and inter-specific tomato hybrids. Besides, Pratta *et al*. (2006) evaluated the *in vitro* behavior of 16 recombinant lines and these authors found an important value of narrow sense heritability for callus percentage as well as for regeneration percentage. Recently, it were evaluated the components of the genetic mean values and variances of the *in vitro* culture response in a cross between "Caimanta" of *L. esculentum* as the high regeneration capacity genotype and accession LA722 of the exotic *L. pimpinellifiolium* as the low regeneration capacity genotype (Marchionni Basté *et al*. 2007). The results demonstrated that *in vitro* regeneration capacity was controlled by gene action of complete dominance of the cultivated genome over the exotic one. Finally, the knowledge about genetic variation for the *in vitro* traits facilitates their genetic improvement to increase the regeneration capacity. The different composition of the genetic variation determines the different improvement strategies to be followed.

ABOUT TOMATO TRANSGENIC PLANTS

Transgenic plants and Genetically Modified Organisms (GMOs) are organism with a segment of foreign DNA incorporated into their genome or with any modifications introduced artificially in their genome sequence. The GMOs have been studied for more than 30 years and a dramatic increase in plant transgenic science is denoted in Asia, during the past decade, a sustained expansion in North America and, recently, a slow down in the rest of the world. Publications focusing on the development of transgenic technology have been slowing down worldwide, since the early mid-1990s, a trend that contrasts with the increase in studies related to crop molecular genetics. Nevertheless, since the pioneer experience of Smith *et al*. (1988), a remarkable progress has been made to obtain tomato transgenic plants. These authors obtained a long shelf life tomato, the first commercialized GMO in the world, by incorporating through *Agrobacterium*-mediated transformation the polygalacturonase antisense construct. However, the earliest reports of *Agrobacterium*-mediated transformation in tomato were by Horsch *et al*. (1985) and McCormick *et al*. (1986).

This review will focus on recent advances in tomato transgenesis for improving commercial traits and for studying metabolic pathways involved in fruit development. For a selection of some summarized results, see **Table 2**.

Improving commercial traits by genetic engineering

Diseases and pests resistance

The defense responses, which serve to limit growth and spread of pathogens within plants, include hypersensitive programmed cell death. Li and Steffens (2002) have produced transgenic tomato plants constitutively overexpressing a potato PPO (Polyphenol Oxidase) cDNA under control of the cauliflower mosaic virus (CaMV) 35S promoter. The transgenic plants were infected with the bacterial pathogen *Pseudomonas syringae* pv. tomato and an increased plant disease resistance was obtained. Chan *et al*. (2005) used systemic acquired resistance related genes to enhance resistance to multiple diseases in tomato. A successful result

Table 2 Experimental data about genetic transformation in tomato.

Trait	Inserted gene	Vector	Selector gene	Method	Reference
Fungal and bacterial diseases	Arabidopsis *NPR1*	Ti plasmid	Kanamycin	*Agrobacterium*	Lin *et al*. 2004
Carotenoids	*Erwinia uredovora CrtB*	pRN_2	Kanamycin	*Agrobacterium*	Fraser *et al*. 2002
Delayed leaf senescence	*ipt*	pSG516	Kanamycin	*Agrobacterium*	Luo *et al*. 2005
Drought tolerance	*Populus bsp*A	pB1G-HyG	GUS	*Agrobacterium*	Roy *et al*. 2006a, 2006b
Antibiotic resistance	*npt*II	pBI121	GUS	Biolistics	van Eck *et al*. 1995

was the experiment of Lin *et al*. (2004) to enhanced resistance to a spectrum of fungal and bacterial diseases. The *Arabidopsis* NPR1 (nonexpresser of PR genes) gene was introduced into a tomato cultivar, which possesses heat-tolerance and resistance to tomato mosaic virus (ToMV). The transgenic lines expressing NPR1 were normal as regards overall morphology and horticultural traits for at least four generations. Additionally, these plants showed resistance to several diseases such as bacterial wilt and *Fusarium* wilt. Recently transgenic tomato plants showing enhanced tolerance to the oomycete pathogen *Phytophthora* was produced (Sarowar *et al*. 2006).

An experiment was undertaken to develop tomato plants with broad resistance to tospoviruses, which are a major limiting factor to tomato production worldwide. Six transgenic lines expressing the nucleocapsid protein gene of the lettuce isolate of tomato spotted wilt virus showed high levels of resistance when inoculated with the viruses (Gubba *et al*. 2002). Plants showing resistance to tomato leaf curl disease were obtained by antisense methodology (Praveen *et al*. 2005). Progeny analysis of these plants showed classical Mendelian pattern of inheritance in two of the six transgenic lines having single transgene insertion.

The goal of the study of Goggin *et al*. (2004) was to assess the susceptibility of the nematode resistance gene, *Mi-1.2* by transgene inactivation. The stability of *Mi*-mediated nematode resistance and *Mi-1.2* transcripts levels were observed in two independently transformed tomato lines carrying *Mi-1.2*. In both lines a reduction in resistance was noted in the T2 generation, and was more pronounced in the T3 generation. The decrease in resistance varied among cuttings that were clonally propagated, which suggests that epigenetic effects influenced resistance levels. However, the transgenic plants did not show the reduced transcript levels characteristic of gene silencing or negative position effects.

Also transgenic methodology could be appropriated for generating plants resistant to insect attack. The transgenic tomato plants expressing a Cry1A(b) protein of *Bacillus thuringiensis* suffered significantly lower damage by *Helicoverpa armigera* than the non-transgenic control plants in the laboratory, greenhouse and field (Kumar and Kumar 2004).

Abiotic stress

Plant biotechnology techniques have been successfully applied for developing biotic stress resistence plants through genetic transformation but there have not been great advances for abiotic stress resistance such as drought, salinity, chemical toxicity and oxidative stress.

Transgenic tomato plants were generated by introduction of the yeast trehalose-6-phosphate synthase gene. These plants under drought, salt and oxidative stress improved tolerance with respect to wild type (Cortina and Culiañez Macià 2005). Roy *et al*. (2006a, 2006b) obtained transgenic tomato plants showing slightly increased drought tolerance by incorporating the *bspA* gene from *Populus*.

Nutraceutical foods

The trend to view many foods not only as sustenance but also as medicine, so called functional foods, is nowaday increasing. It is a well known fact that fruits and vegetables contain an array of phytochemicals that contribute to good health. The elevation of carotenoid biosynthesis in plants, especially the tomato, by genetic manipulation should increase the lycopene and carotene levels and hence improve the nutritional quality of the crop. Several efforts were made to improve these traits through the transgenic technology. Fraser *et al*. (2002) have shown that the ripening specific expression of a bacterial phytoene synthase can overcome previous unintended pleiotropic effects and elevate nutritionally important carotenoids in ripe tomato fruit. The changes in flux coefficients have revealed a shift in the regulatory step of carotenogenesis, which has important implications on future metabolic engineering strategies. Although the carotenoids and polyphenols and their bioactive products have been the main areas of focus others such as tocopherols, glucosinolates and ascorbate acid for example, have also attracted considerable attention. Carotenoids, among the terpenoids, have accessory pigment functions in plants as well as roles as protectants. D'Ambrosio *et al*. (2004) have also demonstrated the feasibility of engeneering tomato to divert carotenoid metabolic flux toward a desired useful product.

Flavonoids are a group of polyphenolic plant secondary metabolites important for plant biology and human nutrition. In particular, flavonoids are potent antioxidants and their dietary intake is correlated with reduced risk cardiovascular diseases. Muir *et al*. (2001) have upregulated flavonol biosynthesis in tomato in order to generate fruit with increased antioxidant capacity and a wider range of potential health benefit properties. This approach involved transformation of tomato with the Petunia *chi-a* gene encoding chalcone isomerase. This experiment resulted in transgenic tomato lines producing an increase of up to 78-fold in fruit peel flavonols without any gross differences between high flavonol transgenic and control lines. Also, Bovy *et al*. (2002) have demonstrated that it could be obtained transgenic tomatoes with a considerable increase of the level of flavonoides. On the other hand, Enfissi *et al*. (2006) concluded that the elevation of carotenoids levels in tomato fruit or the formation of those not normally found in fruit by genetics engineering clearly showed that this approach is successful although the increases are relatively modest and rarely above 2-3–fold.

The chlorogenic acid (CGA) acts as an antioxidant in plants and protects against degenerative, age-related diseases in animals when supplied in their diet. Overexpression of hydroxycinnamoyl transferasa in tomato caused plants accumulating higher levels of CGA with no side-effects on the levels of other soluble phenolics (Niggeweg *et al*. 2004). Another experiment (Ralley *et al*. 2004) allowed discussing in terms of metabolic engineering of carotenoids and their sequestration in higher plant tissues. Recently Long *et al*. (2006) showed that a panel of transgenic and mutant tomato lines has been subjected to metabolite profiling in compareson with wild type Ailsa Craig for both carotenoids and phenolics. A range of mutants and transgenic lines were selected showing a range of phenotypes varying from down-regulation through to increased levels of lycopene and β-carotene. These lines can act as the hosts for further genetic manipulation for increased antioxidant content.

Studying metabolic paths involved in fruit development

Tomato is an appropriate model to study metabolic paths involved in the ripening and the shape of fruit through the transgenic technology.

The *OVATE* gene, conditioning pear shaped tomato, was recently cloned (Liu *et al*. 2002). It is expressed early in flower and fruit development and encodes a previously uncharacterized hydrophilic protein with a putative bipartite nuclear localization signal. Moreover, ectopic transgenic expression of *OVATE* unevenly reduces the size of floral organs and leaflets, suggesting that *OVATE* represents a previously uncharacterized class of negative regulatory proteins important in plant development.

Another experiments using transgenic plant have produced a highlight approach about fruit ripening. Climateric fruit ripening is regulated by the phytohormone ethylene. Ethylene Insensitive 3 (*EIN3*) is a transcription factor that function downstream from the ethylene receptors in the Arabidopsis ethylene signal transduction pathway. Three homologous of the *Arabidopsis EIN3* gene have been identified in tomato. Chen *et al*. (2004) demonstrated that expressing candidate genes in the Nr (*Never ripe*) ethylene insensitive background was a valuable approach for testing the role of putative downstream components in the ethylene signaling pathway.

Other ripening related facts were investigated by Wang *et al*. (2005). They found that antisense suppression of deoxyhypusine synthetase delays softening and produces changes in fruit growth and development. Though polygalacturonase-antisense methodology was developed to obtain long shelf life tomatoes, recently it has been used in some experiment in order to investigate changes in other traits (Orozco-Cárdenas and Ryan 2003; Luo *et al*. 2005), demonstrating pleiotropic effects on morphological characteristics.

Kim and Grierson (2005) have use microprojectile bombardment in order to learn more about the function of tomato ripening-associated membrane protein (TRAMP). In order to learn more about the function of TRAM, the cDNA was fused to the green fluorescent protein (GFP) coding region and used to bombard onion cells or generate stably transformed tomato plants to allow visualisation of the TRAMP protein within the plant cell. Plants that contained multiple copies of the transgene frequently exhibited silencing of the endogenous gene and transgene, whereas plants with low copy number transgenes tended to show overexpression of tomato ripening-associated membrane protein. It was detected predominantly in the plasma membrane when stably expressed in transgenic tomato fruit.

Another interesting results to study fruit quality were obtained by Liu *et al*. (2004) manipulating light signal transduction and Giliberto *et al*. (2005) altering the blue light photoreceptor criptochrome 2 (*cry2*). The *cry2* is a central player in tomato development. Its manipulation through transgenic overexpression results in an alteration of a large set of development and biochemical process responses.

MOLECULAR MARKERS APPLIED TO TOMATO

Molecular markers in basic and applied studies have been used in the tomato as model species. All kind of molecular markers were developed for analyzing the tomato genome, and many saturated maps were obtained in past years (Haanstra *et al*. 1999; Zhang *et al*. 2003). Even map-based cloning of agronomic valuable genes was attained, while transformation of genotypes lacking the respective trait allowed verify the cloned gene (Martin *et al*. 1993; Frary *et al*. 2000).

Isozymes as molecular markers

Isozymes were the first type of molecular markers applied to tomato. They were early used by to quantify and study genetic variation both within and between populations of cultivated and wild tomato species (Rick 1973). The set of treatises produced during this period (1970s and early 1980s) is now the definitive source of taxonomy and genetic variation for species in this genus. This resulted in the first molecular linkage map and was the predecessor of the DNA linkage maps that are so commonly used in plant genetic, molecular, and breeding studies today.

Rick and Fobes (1974) were ones of the first people to recognize the potential of molecular breeding. During one of the systematic studies of genetic variation in cultivated tomato accessions, highly significant linkage disequilibrium was discovered between a rare isozyme allele and resistance to nematodes. These authors went on to show that the nematode resistance gene was tightly linked to the isozyme gene and that breeders had introduced the rare allele together with the resistance gene from a wild species. It was concluded that the rare isozyme allele would provide a faster and more accurate screen for resistance than nematode inoculations.

Also, the I-3 gene for *Fusarium* wilt resistance was introgressed from *L. pennelli* LA716 in assisted breeding programs using the Got-2 (glutamate oxaloacetate transaminase-2) isozyme marker. Nowadays, efforts were made to develop PCR-markers from sequences of this isozyme annotated in the SOL Genomics Network unigenes database as a means of revitalizing old isozymes markers and recruiting new ones (Wang *et al*. 2007). The development of PCR-based markers from multigene families, exemplified by this Got marker, serves to anchor their associated unigene sequences to specific subregions of the tomato genome and these in turn can be used to underpin the development of new markers tailor-made for marker-assisted breeding of traits of interest in these subregions.

DNA markers

DNA markers offer great potential uses in plant breeding and pre-breeding. As shown in **Table 3**, the tomato genome was characterized by a wide kind of molecular markers, including RFLP, RAPD, SSR, AFLP, SCAR, SNP, and differential display (Kardolus *et al*. 1998; Frary *et al*. 2005; Primieri Carelli *et al*. 2006). Additionally, all kind of plant populations (homozygous lines, hybrids, F_2 and backcross generation with different degree of homozygocity, near isogenic lines, recombinant inbred lines, wild accessions, etc.) were well characterized by molecular markers. In general, these studies pointed that genetic variation is low among the cultivated varieties, but it could be highly widen by incurporating wild species to breeding program. As mentioned in the Introduction, less than the 5% of the total variation is present within the cultivated tomato, while the rest is spread in the wild germplasm (Miller and Tanksley 1990).

Application in taxonomic studies

In studies of the Solanaceae taxonomy, Kardolus *et al*. (1998) were able to distinguish *L. esculentum* from other *Solanum* species by applying AFLP markers. In spite of the low genetic variation within the cultivated tomato, cultivars were differentiated by using SSR markers by He *et al*. (2003).

Application in tomato breeding

Identification of genes associated to agronomic traits (Foolad 2004; Lecomte *et al*. 2004; Rousseaux *et al*. 2005), marker assisted selection (Robert *et al*. 2001; Chaib *et al*. 2006), supporting in choosing the adequate parent and determination of heterosis (Monforte *et al*. 2001; Semel *et al*. 2006), among other application, were achieved in tomato.

Anatomical traits such as stem morphology and vascular development were associated to a QTL in a cross among *L. esculentum* and *L. hirsutum* by Coaker *et al*. (2002). Genotypes carrying the LA407 (*L. hirsutum*) markers on chromosome 2 had larger primary vascular bundles, more developed secondary vascular tissue, and a triangular vascular shape.

Fruit traits including soluble solids content, weight, shape, size, acidity, antioxidants content, firmness, ripening

Table 3 Selection of DNA marker protocols reported in this review

DNA marker	Primer sequence (5′ - 3′)	Amplification conditions	Reference
TG58	*F* TGTGATACGGAACTTTGAACCTCC	One cycle of 94ºC for 5 min at 94°C; 35 cycles of 94°C for 30	Frary *et al*. 2005
	R CCTGGATTTGGTCAGCTCCTTAG	s, 50°C or 55°C (according to the primer composition) for 45 s	
TG83	*F* TTATGGCACTCAGGATGGTG	and 72°C for 45 s; and one cycle of 72°C for 5 min	
	R AGGCATGAAAACCACAAAGG		
SSR117	*F* AATTCACCTTTCTTCCGTCG		
	R GCCCTCGAATCTGGTAGCTT		
SSR156	*F* CACGCCTATGCACCTTTCTT		
	R CTTCAAGGCTAAACCTCCGA		
cLEC7P21	*F* TGAACAGAAAGCACGAGTGG		
	R GACAGTTCTTCGAAGCGTTTG		
GOT-A	*F* AGGATAAAGAGTGTGAGACAGAAGC	One cycle of 94°C for 5 min; 35 cycles of 94°C for 1 min, 55ºC	Wang *et al*. 2007
	R AGAAATTGTCAAAATCTGCTTCATAC	for 1 min and 72°C for 1 min; and one cycle of 72°C for 5 min	
GOT-B	*F* AGTGGCAGTGAAAAGTCAGTTG		
	R CCAAGTAACCAACATTTCCAGTAG		
GOT-D	*F* AGAGATGATAAAGGAAAACCAGTAAC		
	R TATGAGCGCAAGGATGAAGTAAG		
OPX-01	CTGGGCACGA	One cycle of 94ºC for 1 min; 45 cycles of 94ºC for 1 min, 40ºC	Primieri Carelli *et al*.
OPX-03	TGGCGCAGTG	for 1 min and 72ºC for 2 min; and one cycle of 72ºC for 3 min	2006
OPX-12	TCGCCAGCCA		
OPX-14	TGGCGCAGTG		
OPX-17	GACACGGACC		
OPX-18	GACTAGGTGG		
OPX-19	TGGCAAGGCA		
A8	*Mse*I +3 GATGAGTCCTGAGTAACTA	*Preamplification*: 30 cycles of 94ºC for 30 s, 56ºC for 1 min	Pratta *et al*. 2006
	*Eco*RI +3 GACTGCGTACCAATTCAGA	and 72ºC for 1 min. *Selective Amplification*: PCR touch down,	
N14	*Mse*I +3 GATGAGTCCTGAGTAACAT	denaturation: 94ºC for 30 s, annealing starts at 62ºC for 30 s	
	*Eco*RI +3 GACTGCGTACCAATTCATC	with temperature diminishing in 0.5ºC during 12 cycles,	
B16	*Mse*I +3: GATGAGTCCTGAGTAACAT	extension: 72ºC for 1 min; then 20 cycles of 94ºC for 30 s,	
	*Eco*RI +3: GACTGCGTACCAATTCAGC	56ºC for 1 min and 72ºC for 1 min	
Z15141	*F*: CCAAATACTGCAGCGGAAAG	One cycle of 94°C for 2 min; 35 cycles of 94°C for 25 s, 45 or	He *et al*. 2002
	R: TTCTAAATGGGCATACAGAATC	50ºC (according to the primer composition) for 25 s and 68ºC	
AW032445	*F* TGATTCAAGGTACAAGTAGTAGTGC	for 25 s	
	R GGAGGAGGGTGAATAATCG		
AW033091	*F* TTCTCACACCTGCACACACC		
	R AGCGGGATGATTACAGAAATG		
AW037257	*F* CCGGTGAAGGTGAGTCTGAG		
	R TTTATGCACCGCGACTCG		

F: forward primer *R*: reverse primer

time and vitamins content were currently associated to molecular markers (Monforte *et al*. 2001; Lecomte *et al*. 2004; Rosseaux *et al*. 2005). All of these researches pointed out that wild germplasm contributes to an increase in internal fruit quality (as measured by an increment in soluble solids, vitamins and antioxidants content) but they reduce fruit weight. In an effort to identify the chromosomal regions that affect the last trait, the *fw2.2* QTL was identified on tomato chromosome 2 (Frary *et al*. 2000). This QTL was conserved in a wide range of wild species, whose alleles cause a reduction in fruit weight. It was the first plant QTL to be cloned by map-based approaches, and plant transformed with different artificial dosage the *L. esculentum* allele showed a corresponding increase in fruit weight (Liu *et al*. 2003). The cloned gene appears to encode for an ovary early expressed protein that affects the final number of fruit locules. Other conclusions of these studies suggest that it is frequent that wild germplasm could increase a given trait even that it is not expressed, since recombination of wild and cultivated genes in the hybrid genotypes could create new phenotypes that did not appear in the parents, a phenomenon known as transgression. Particular molecular markers were associated to transgressive variation for many traits (de Vicente and Tanksley 1993). Recombinant inbred lines (RILs) were characterize by AFLP with the goal of detecting molecular markers associated to morphological and fruit quality traits (**Fig. 2**). Such RILs were derived from a cross among the Argentinean cultivar 'Caimanta' (*L. esculentum*) and LA722 (*L. pimpinellifolium*). AFLP associated to the number of flower per cluster, fruit weight, and soluble solid contents were detected. Also, it was demonstrated that the wild accession carry genes that prolong fruit shelf life. Phenotypic observations had pointed out a transgres-

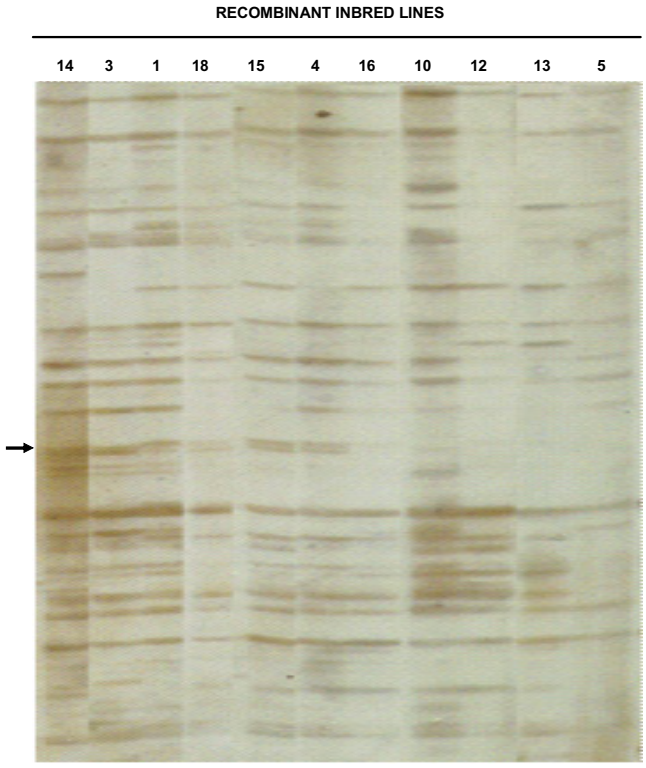

Fig. 2 Each line shows the AFLP profile for recombinant inbred lines (RILs). Arrow indicates an associated fragment to fruit weight.

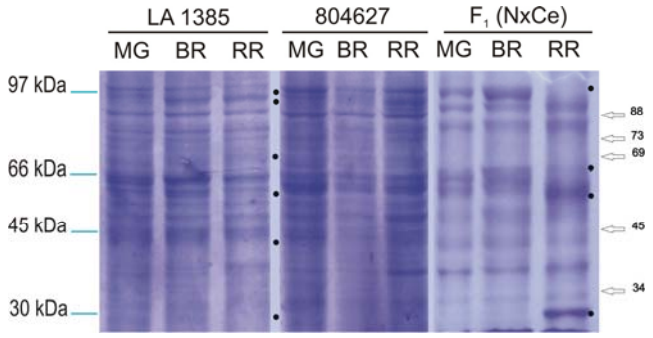

Fig. 2 Protein profiles for the accession LA1385 of *L. esculentum* var. *cerasiforme*, the accession 804627 of *L. esculentum* (a homozygous genotype for the *nor* mutant) and the F_1 between them at mature green (MG), breaker (BR) and red ripe (RR) ripening stage. Circles and arrows indicate polypeptide differences among stages and between genotypes at each ripening stages, respectively.

sive variation for the shelf life among some RILs. Interestingly, a fragment carried by the shorter shelf life parent also accounted for the transgressive variation. This results indicate that recombination among genes from the two parents broadened the genetic variation available in the crop. The association between this AFLP marker and the shelf life was conserved across years of evaluation (Pratta *et al*. unpublished results). Molecular characterization of the ripening stages mature green, breaker and red vine of the tomato fruit were carried out by Rodríguez *et al*. (2007) in F_2 population derived from crosses among a mutant for ripening genotype (*nor*/*nor*), a normal for ripening genotype (*nor*+/*nor*+) and LA1385 of *L. esculentum* var. *cerasiforme* (**Fig. 3**). It was possible to identify each one of these stages by means of the total protein of the pericarp tissue. Interestingly, the protein profile of the mutant genotype did not change along ripening. Association of polymorphic proteins with fruit traits such as weight, soluble solid content, firmness, color, pH and shelf life could be established.

Resistances to biological and environment factor were identified in wild tomato germplasm and transferred to the cultivated species by marker assisted approaches. Many nematode resistance genes (*Mi-1*, *Mi-3*) were identified in *L. peruvianum*. *Mi-1* was associated to an inverted segment in chromosome 6 (Seah *et al*. 2004), while *Mi-3* was located on chromosome 12. It was finely mapped, and a *L. esculentum* DNA contig spanning this locus was constructed in an effort to clone the gene (Yaghoobi *et al*. 2005). It is worthy to note that the first plant disease resistance gene that was cloned by a map-based approach, *Pto* that confers resistance to the bacteria *Pseudomonas*, was isolated in tomato (Martin *et al*. 1993). Marker-assisted introgression of blackmold resistance QTL alleles from wild *L. cheesmanii* Riley to cultivated tomato and evaluation of QTL phenotypic effects were achieved by Robert *et al*. (2001). A molecular linkage map of tomato displaying chromosomal locations of resistance gene analogs based on a *L. esculentum* × *L. hirsutum* cross was developed by Zhang *et al*. (2002). These authors were able to map some QTLs conferring early blight (*Alter-naria solani*) resistance in a similar cross by selective geno-typing (Zhang *et al*. 2003). QTLs for *Ralstonia solanacearum* race 3-phylotype II resistance were identified in crosses to *L. pimpinellifolium* by Carmeille *et al*. (2006).

On the other hand, the inheritance of several tolerance-related traits has been determined and QTLs associated with tolerance at individual developmental stages have been identified and characterized. It has been determined that at each stage salt tolerance is largely controlled by a few QTLs with major effects and several QTLs with smaller effects. Different QTLs have been identified at different developmental stages, suggesting the absence of genetic relationships among stages in tolerance to salinity. Furthermore, it has been determined that in addition to QTLs which are population-specific, several QTLs for salt tolerance are conserved across populations and species. Research is currently underway to develop tomatoes with improved salt tolerance throughout the ontogeny of the plant by pyramiding QTLs through marker-assisted selection (for a review, see Foolad 2003).

Others traits of direct or indirect interest in plant breeding were characterized by molecular markers in tomato. For instance, the nuclear male sterile locus, ms-10, was reported to be linked with two enzyme marker loci (Est-1 and Prx-2) located on chromosome 2 (Tanksley *et al*. 1983). The levels and synthesis of proteins during the ontogeny of normal and male sterile stamenless-2 (*sl-2/sl-2*) mutant stamens of tomato were examined by Bhadula and Sawhney (1991). The mutant stamens contained low levels of soluble protein which were related to reduction in protein synthesis. Stoeva-Popova *et al*. (2007) found PCR products with primer combination *atp9*/*rps12* that are suitable as a marker for early selection of plants with different phenotypes: cytoplasm male sterile plants or plants with restored fertility in the hybrid progenies.

Reports on molecular markers associated to the tomato *in vitro* culture responses are scarcer. Koornneef *et al*. (1993) mapped a high regeneration QTL to chromosome III in an interspecific tomato cross by RFLP analysis. Torelli *et al*. (1996) detected by the differential display technique, some specific mRNA transcripts expressed during the earlier incubation period of tomato explants that were associated to the shoot formation capacity, while Takashina *et al*. (1998) found at least two RAPD and one isoenzymatic markers linked to the high regeneration capacity of the wild *L. chilense*. AFLP markers associated to *in vitro* regeneration capacity were also detected in a set of tomato genotypes by Pratta *et al*. (2006).

Cytoplasmic DNA markers

Molecular analysis at the mithocondrial and chloroplastic DNA were made (McClean and Hanson 1986; Daniell *et al*. 2006). These studies evidenced about the taxonomic status of the tomato among the Solanaceae family, and have important implications on transplastomic engineering.

Construction of genomic and cDNA libraries were currently achieved in tomato (Alpert and Tanksley 1996; Budiman *et al*. 2000) and large collections of ESTs are currently available, which will be pointed out in next sections.

GENOMIC AND POST-GENOMIC ADVANCES IN TOMATO

Following analysis of complete genome sequences in several organisms, much of the attention in the genome sciences is shifting towards functional studies of the gene products. In this way, the post-genomic focuses not only on the study of the genome but also on their products, and it essentially follows the central dogma of molecular biology proposed by Watson and Crick 50 years ago, with the addition of enzymes and metabolism (**Fig. 4**). Thus, the genome (all DNA) gives rise to the transcriptome (complete transcripts produced by an organism), the proteome (protein complement expressed by a genome) and the metabolome (entire complement of all the low molecular weight metabolites). Each step in the flow of the genetic information is accompanied by recent technological innovations also called "omics" technologies that allow genome-wide analysis. These technologies, as a part of functional genomics approaches, can be used to dissect biological systems. According to Fukusaki and Kobayashi (2005), proteomic and transcriptomic are both considered to be a flow of media concerning genetic information. In contrast, metabolomic should be thought as being concerned with phenotype. In the following section, this paper reviews the advances made by these technological innovations in tomato.

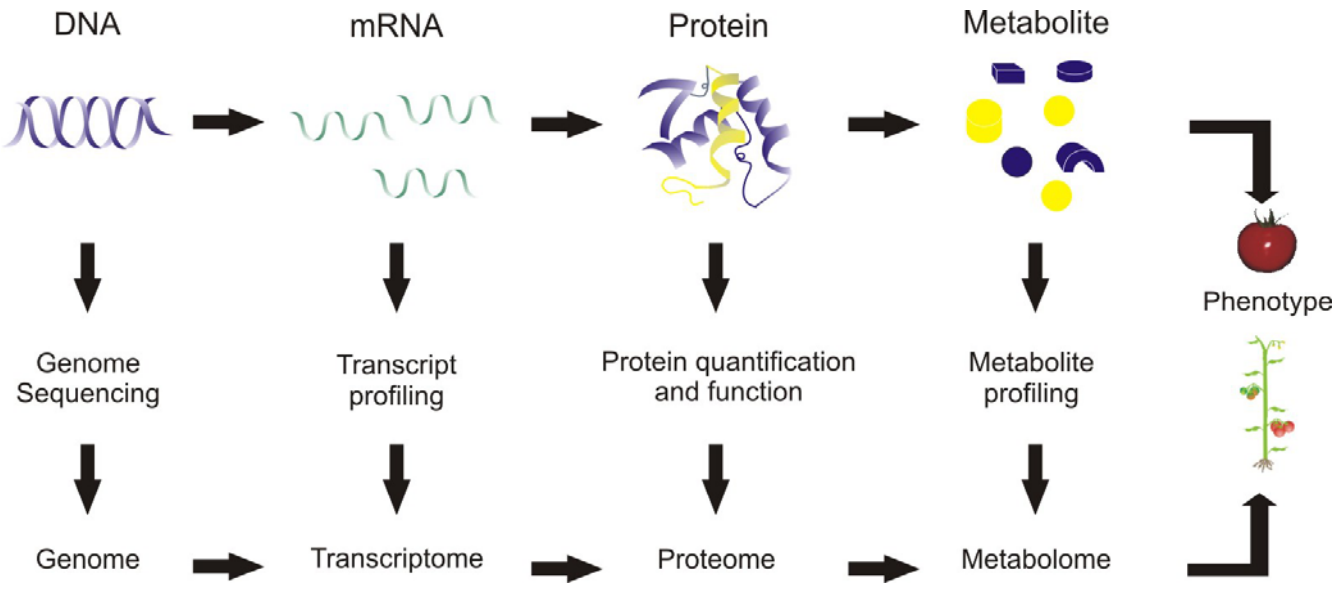

Fig. 4 The modern "omics" technologies follow the pattern established by the central dogma of biology, with the addition of active enzymes and metabolities, which taken together reflect tomato phenotypes.

Tomato genome sequencing

As mentioned in the introduction, although genome sequencing of *Arabidopsis thaliana* and rice has been completed, several aspects of plant biology cannot be studied in those model plants; e.g., climacteric fruit ripening. On the other hand, tomato will provide necessary diversity to reinforce findings based on the most informative model plant, *Arabidopsis*, because these two plants diverged from their common ancestor early in the radiation of dicots. An international initiative entitled the "SOL - International Solanaceae Genome Project" which has as one of the main goals to obtain high quality sequence of the tomato genome as a reference for solanaceous plants as well as plants from other related taxa has begun in 2003. The International Tomato Genome Sequencing Project (http://www.sgn.cornell.edu/about/tomato_sequencing.pl) aims to sequence the gene-rich euchromatic portions of the tomato chromosomes. The tomato genome, comprising 12 chromosomes, contains 950 Mb of DNA (Arumuganathan and Earle 1991). The strategy of the SOL consortium is to sequence the approximately 220 Mb of euchromatin that contains the majority of the genes (~35,000), rather than to sequence the entire tomato genome. As part of the Tomato Genome Sequencing project the tomato FISH map (Fluorescence *In-Situ* Hybridization) is being generated. The aim is to verify the genetic and physical maps and to explore the extent of the euchromatin, and to more precisely locate the euchromatin/heterochromatin boundary on tomato chromosomes.

Analysis of a collection of ESTs (expressed sequence tag), derived from 26 different tomato cDNA libraries revealed that 70% of the unigenes have identifiable homologs in the *Arabidopsis* genome and the majority of the about 30% of the tomato genes that did not significantly match any *Arabidopsis* genes have unknown functions (van der Hoeven *et al*. 2002). According to this, reverse genetics approaches have been conduced to identified some gene functions (for a review, see Shibata 2005).

Transcriptomics

Several methods have been developed for quantifying mRNA abundance in plant tissues ranging from traditional RNA gel-blot analysis to those providing a more global view that include differential display, cDNA-AFLP and microarrays.

Differential display has important advantages when compared with scale-limited approaches such as RNA gel-blot analysis. However, the main disadvantage of this technique is that the quantity of individual amplification products is not only a function of the initial concentration of that cDNA, but also is dependent upon the quality of a particular match between primer and template. Despite this disadvantage some genes differentially expressed, involved in synthesis and ethylene response, ripening, chilling injury response and regeneration capacity has been reported (Barry *et al*. 1996; Kadyrzhanova *et al*. 1998; Zegzouti *et al*. 1999; Torelli *et al*. 2004). The cDNA-AFLP method relies on the selective amplification of a subset of DNA molecules from a more complex pool. The main advantage that offers this technique is that poorly characterized genomes can be investigated in a high-throughput manner. In addition, it allows much greater confidence in acquired data and differences in the intensities of amplified products. As with the other profiling methods described here, the sensitivity of cDNA-AFLP is only limited by the ability of cDNA libraries to capture low-abundance transcripts. The technique has been mainly used to study tomato defense to biotic stress (Rowland *et al*. 2005; Li *et al*. 2006).

Microarray techniques are based on the capacity to bind either DNA fragments or previously characterized oligonucleotides on a microscope slide. The plates are built by depositing specific DNA fragments at indexed positions using a computer controlled high speed robot with the specific DNA fragment sequences determined from any database originated including: cDNA clones, EST clones, anonymous genomic clones or DNA amplified from open reading frames (ORFs). This technique for gene expression profiling has important advantages when compared with the previously detailed ones. According to Alba *et al*. (2004) the most important advantage in this technology is that it can measure tens-of-thousands of different mRNA transcripts in parallel, it is semi-quantitative, and it is sensitive to low-abundance transcripts that are represented on a given array. A tomato microarray with 12,899 ESTs clones (TOM1 microarray) representing 8500 independent tomato genes has been developed from a large collection of tomato ESTs (>150,000 entries). These unigenes have been selected at random from different cDNA libraries made from a range of tissue including leaf, root, fruit and flowers (van der Hoeven *et al*. 2002). Transcriptome profiling via TOM1 microarray has been focused in studying the development and ripening tomato fruit as well as response to environmental and biotic stress. Fei *et al*. (2004) studying different ripening stages of tomato fruit have reported a total of 333 ripening induced genes (greater expression at breaker stage) and 185 ripening

repressed genes (greater expression at mature green stage). Interestingly, they have found 36 genes related to ripening that encode putative transcription factors including three MADS box (a highly conserved sequence motif). In addition, when EST collections from ripening grape and tomato fruits were compared three common transcription factors, including a MADS box were found. These results have suggested that a conserved mechanism of ripening control transcending climacteric or non-climacteric distinctions. Climacteric fruits are distinguished from non climacteric by their increased respiration and ethylene biosynthesis rates during ripening. According to this, it was demonstrated that the mutation of an ethylene receptor (Nr, never ripe) alters the expression of many genes involved in fruit morphology, seed number, ascorbate accumulation, carotenoid synthesis and ethylene evolution indicating that ethylene governs multiple aspects of development both prior and during fruit ripening in tomato (Alba *et al*. 2005). Besides, there are others works in which TOM1 microarray has been used to analyze the plant response to environmental and biotic stress (Gibly *et al*. 2004; Sagi *et al*. 2004; Bonshtein *et al*. 2005; Carbone *et al*. 2005).

Proteomics

Proteomic is the large-scale study of proteins from a given cell or organism. It can be divided into three main areas: (1) protein micro-characterization for large-scale identification of proteins and their post-translational modifications; (2) 'differential display' proteomic for comparison of protein levels such as two genotypes, environmental conditions, etc. and (3) studies of protein-protein interactions (Pandey and Mann 2000). Various approaches are possible, including 1-D dimensional polyacrilamide gels. Pratta *et al*. (2004) have found different pattern of expression for GS (glutamine synthetase) and GDH (glutamate dehydrogenase) at the green mature and the ripe stages of the pericarp of fruit from different genotypes of tomato (**Fig. 5**). However, there is still no widely available technology that surpasses 2-D dimensional polyacrilamide gels for quantitative comparative proteomic analysis. Once a set of differential expressed polypeptide has been found from, a series of 1-D or 2-D gels, the next step is identified the proteins and genes. Since the 1990s, biological mass spectrometry emerged as a powerful analytical method that removed most of the limitations of protein analysis. There are two main approaches to mass spectrometric protein identification: MALDI-TOF (matrix-assisted laser desorption-ionization-time of flight) and ESI-MS/MS (electrospray ionization tandem mass spectrometry). For details, see Zivy and de Vienne (2000).

Proteomic offers the opportunity to examine simultaneous changes and to classify temporal patterns of protein accumulation occurring in complex developmental processes. However, not many data on fruit development proteomic are yet available in tomato. As an example, fruit of cv. 'Cervil' (*L. esculentum* var. *cerasiforme*) were evaluated during fruit development by Faurobert *et al*. (2007). They found a total of 148 spots differentially expressed which are mainly involved in stress response, carbon compounds and carbohydrate, amino acid metabolism, electron transport, photosynthesis and respiration. The developmental stage effect was the main factor to explain spot variation when a cluster analysis was performed. Similar results were found by Rocco *et al*. (2006) when compared two tomato genotypes regard to the role of differentially expressed protein. Between genotypes, almost 57% of protein presented overlapping gel coordinates.

Some discrepancies between proteins and their specific mRNA can exist since post-transcriptional modifications of proteins can be result in a dramatic increase in protein complexity without a concomitant increase in gene expression (Rose *et al*. 2004). Thus, coupling transcriptomic with proteomic studies will lead to a better knowledge of gene networks (Faurobert *et al*. 2007). Moreover, it is necessary to include the metabolome level to achieve an overall comprehensive of complex biological systems.

Metabolomics

Numerous analyses of tomato metabolites, especially of ripening and matured fruits have been reported. For example, Pratta *et al*. (2004) evaluated free amino acids composition and glutamate dehydrogenase and glutamine synthetase levels in fruits of genotypes differing in fruit shelf life. They concluded that, the relative glutamate content of ripe mature fruits differed considerably among lines and negative correlation between the relative glutamate content and fruit shelf life was found. However, metabolomic approaches to analyze metabolites comprehensively have begun for tomato. As a first experiment in this direction, an established metabolic profiling method using GS-MS (gas chromatography coupled to mass spectrometry) was optimized for tomato tissues (Roessner *et al*. 2001). Then, new analytical technologies were developed such as LC-MS (liquid chromatography coupled to mass spectrometry) and NMR (nuclear magnetic resonance). Significant advances describing complex biological systems have been made by metabolomic. Through the analysis of metabolite profile in leaves and fruits from six tomato species it was determined that a tremendous variance it has not been exploited yet from the wild species (Schauer *et al*. 2005). This methodology also has been used to analyze the effects of modifications introduced by transgenesis upon the fruit metabolic profiles (Carrari *et al*. 2003; Roessner *et al*. 2003). Besides, modifications in fruit metabolite composition were found when an *hp* (light-hyperresponsive high-pigment) tomato mutant and isogenic non-mutant plants were compared. The *hp* mutant fruits were characterized by overproduction of many metabolites, several of which are known for their antioxidant or photoprotective activities (Bino *et al*. 2005). An area where metabolomic approaches will likely prove indispensable in the coming year's concerns the need both to define better plant genotypes and to relate observations to phenotype. Recently, it was phenotyped a tomato near isogenic

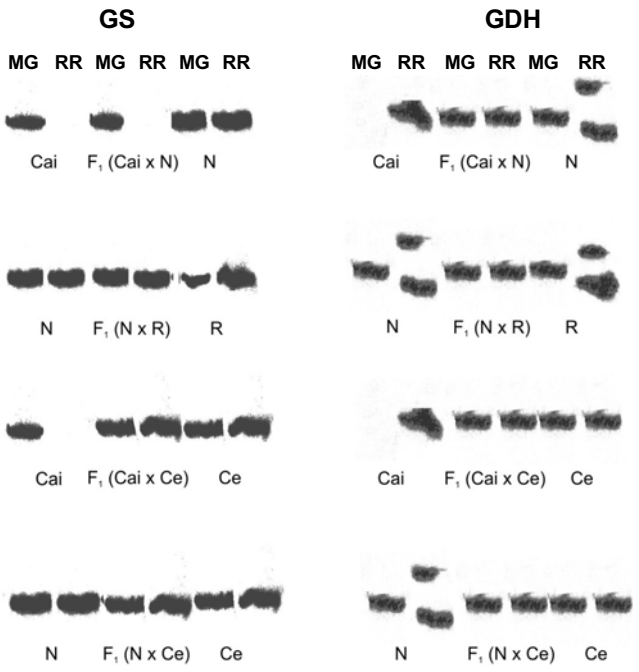

Fig. 5 Changes in polypeptide pattern of glutamine synthetase (GS) and glutamate dehydrogenase (GDH) in fruits at the mature-green (MG) and red ripe (RR) stages in different groups of tomato genotypes formed by a given hybrid and its parents (the pistillate parent is placed at the left). Parental genotypes: Cai: standard ripening cultivar of *L. esculentum*, N: homozygote for the mutant *nor* (*L. esculentum*), R: homozygote for the mutant *rin* (*L. esculentum*), Ce: sampled accession of *L. esculentum* var. *cerasiforme*.

lines population in which genomic regions of the wild species *L. pennelli* were replaced with homologous intervals of a cultivated variety to explore the genetic basis underlying of tomato fruit biochemistry (Schauer *et al*. 2006). They identified both metabolite associate and independent to whole plant phenotype and concluded that plant morphology is a major factor affecting fruit metabolic profiles. A significant number of these studies have, been dedicated to metabolic profiling specifically of the nonvolatile compounds involved in primary plant metabolism using GC-MS. Another significant part of the plant metabolome, comprising the volatile metabolites, is of a particular interest, since they play an important role in fundamental processes such as signaling mechanisms and interorganism interactions. Solid phase microextraction (SPME-GC-MS) is an analytical approach that is suitable for metabolomic studies of volatiles. This methodology was used for a comparative multivariate analysis of a set of 94 contrasting tomato genotypes (including cherry, round and beef tomatoes) covering the variation in the germplasm of commercial tomato varieties (Tikunov *et al*. 2005). The analysis revealed a total of 322 different compounds in the entire genotype set covering approximately 80% of the more than 400 tomato volatile compounds. Cherry tomatoes could be distinguished from round and beef by a relatively high accumulation of phenolic-derived volatiles. On the other hand, the NMR technology has been used to determine metabolic changes in transgenic tomatoes (Le Gall *et al*. 2003) as well as to compare the content of soluble substances in the pulp and juice of tomato (Sobolev *et al*. 2003). Recently, a total of 92 metabolites comprising sugars, sugar alcohols, organic acids, amino acids, vitamins, and a select few secondary metabolites in addition to pigments and the monosaccharide composition of the cell wall, in parallel to transcript levels were analyzed (Carrari *et al*. 2006) resulting in a integrated analysis of metabolite and transcript levels via TOM1 microarray. These experiments revealed several aspects of the regulation of metabolism during fruit ripening: transcript abundance was less strictly coordinated by functional group than metabolite abundance, there were some correlations between specific transcripts and metabolites and there was a strong relationship between ripening associated transcripts and specific metabolite groups.

BIOINFORMATICS

Bioinformatic is a necessary complement of "omics". The enormous quantity of biological data that has become available sine the early 1990s has made computational methodologies in the life sciences increasingly important in research. Scientists with interdisciplinary skills in computational science and biology have led to the emergence of bioinformatics as a distinct field. In this direction, the long term goal of the SOL Consortium (http://www.sgn.cornell.edu) is to build a network of resources and information dedicated to the biology of the Solanaceae family. Today, there are various software and tools available for making searches, algorithms analysis, modeling and the computer graphics of the databases to analyze genomic and post-genomic data (for a review, see Edwards and Batley 2004). However, a dare of this field is to be able to integrate the data generated by the expanding "omics" technologies to elucidate the functional relationships between genotype and observed phenotype.

FUTURE PROSPECTS

It has been widely demonstrated in this review that tomato is a successful plant model system to apply different biotechnologies. Trends in *in vitro* culture are focused to elucidate the genetic basis of the tomato regeneration ability and to manipulate it in order to more efficiently apply other biotechnologies. Regarding to genetic engineering a great effort must be done to choose the appropriate combinations of gene(s) of interest with efficient promoters. Besides, future studies will focus upon signaling mechanism and transcriptional regulators to modify metabolic pathways involved in fruit quality and other physiological traits. In addition, tomato has been an appropriate model to use molecular markers in basic and applied studies. Present studies are currently being made to clone and characterize the candidate genes that were identified by means of molecular markers. A broad stage is nowadays focusing in the "omics". These exciting technologies need to reach a higher integrative level of analysis to elucidate the functional relationship between genotype and observed phenotype. The "omics" have been mainly used for physiological studies in inbreed genotypes but its application in genetic population will allow develop a map of expressed genes. Another important step will be the knowledge about the genome sequence that will allow both develop molecular marker directly correspondding to the gene and understand their functions. Moreover, RNA interference (RNAi) methodology will be a powerful tool to search for genetic and physiological pathways in tomato.

ACKNOWLEDGEMENTS

Financial support was received from CONICET (Consejo Nacional de Investigaciones Científicas y Técnicas), CIUNR (Consejo de Investigaciones de la Universidad Nacional de Rosario) and ANPCyT (Agencia Nacional de Promoción Científica y Tecnológica).

REFERENCES

Alba R, Fei Z, Payton P, Liu Y, Moore SL, Debbie P, Cohn J, D'Ascenzo M, Gordon JS, Rose JK, Martin G, Tanksley SD, Bouzayen M, Jahn MM, Giovannoni J (2004) ESTs, cDNA microarrays, and gene expression profiling: tools for dissecting plant physiology and development. *The Plant Journal* **39**, 697-714

Alba R, Payton P, Fei Z, McQuinn R, Debbie P, Martin GB, Tanksley SD, Giovannoni JJ (2005) Transcriptome and selected metabolite analyses reveal multiple points of ethylene control during tomato fruit development. *The Plant Journal* **17**, 2954-2965

Alpert KB, Tanksley SD (1996) High-resolution mapping and isolation of a yeast artificial chromosome contig containing *fw2.2*: A major fruit weight quantitative trait locus in tomato. *Proceedings of the National Academy of Sciences USA* **93**, 15503-15507

Arumuganathan K, Earle E (1991) Estimation of nuclear DNA content of plants by flow cytometry. *Plant Molecular Biology Reporter* **9**, 208-218

Barden KA, Smith SS, Murakishi HH (1986) Regeneration and screening of tomato somaclones for resistance to tobacco mosaic virus. *Plant Science* **45**, 209-213

Barry CS, Blume B, Bouzaye M, Cooper W, Hamilton AJ, Grierson D (1996) Differential expression of the 1-aminocyclopropane-1-carboxylate oxidase gene family of tomato. *The Plant Journal* **9**, 525-535

Bathia P, Ashwath N, Midmore DJ (2005) Effects of genotype, explants orientation, and wounding on shoot regeneration in tomato. *In Vitro Cellular and Developmental Biology – Plant* **41**, 457-464

Bhadula SK, Sawhney VK (1991) Protein analysis during the ontogeny of normal and male sterile stamenless-2 mutant stamens of tomato (*Lycopersicon esculentum* Mill.). *Biochemical Genetics* **29**, 29-41

Bino RJ, Ric de Vos CH, Lieberman M, Hall RD, Bovy A, Jonker HH, Tikunov Y, Lommen A, Moco S, Levin I (2005) The light-hyperresponsive high pigment-2dg mutation of tomato: alterations in the fruit metabolome. *New Phytologist* **166**, 427-438

Blum A, Merei M, Wirsansky I, Ben-Arzi S (2005) The beneficial effects of tomatoes. *European Journal of Internal Medicine* **16**, 402-404

Bonshtien A, Lev A, Gibly A, Debbie P, Avni A, Sessa G (2005) Molecular properties of the *Xanthomonas* AvrRxv effector and global transcriptional changes determined by its expression in resistant tomato plants. *Molecular Plant-Microbe Interactions* **18**, 300-310

Bovy A, de Voss R, Kemper M, Schylen E, Pertejo MA, Muir S, Collins G, Robinson S, Verhoeyen M, Hughes S, Santos-Buelga C, van Tunen A (2002) High flavonol tomatoes resulting from the heterologous expresion of maize transcription factor genes LC and C1. *The Plant Cell* **14**, 2509-2526

Budiman MA, Mao L, Wood TC, Wing RA (2000) A deep- coverage tomato BAC library and prospects toward development of an STC framework for genome sequencing. *Genome Research* **10**, 129-136

Carbone F, Pizzichini D, Giuliano G, Rosati C, Perrotta G (2005) Comparative profiling of tomato fruits and leaves evidences a complex modulation of global transcript profiles. *Plant Science* **94**, 165-175

Carmeille A, Caranta C, Dintinger J, Prior P, Luisetti J, Besse P (2006) Identification of QTLs for *Ralstonia solanacearum* race 3-phylotype II resistance in tomato. *Theoretical and Applied Genetics* **113**, 110-121

Carrari F, Baxter C, Usadel B, Urbanczyk-Wochniak E, Zanor MI, Nunes-Nesi A, Nikiforova V, Centero D, Ratzka A, Pauly M, Sweetlove LJ, Fernie AR (2006) Integrated analysis of metabolite and transcript levels reveals the metabolic shifts that underlie tomato fruit development and highlight regulatory aspects of metabolic network behavior. *Plant Physiology* **142**, 1380-1396

Carrari F, Nunes-Nesi A, Gibon Y, Lytovchenko A, Ehlers Loureiro M, Fernie A (2003) Reduced expression of aconitase results in an enhanced rate of photosynthesis and marked shifts in carbon partitioning in illuminated leaves of *Lycopersicon pennellii*. *Plant Physiology* **133**, 1322-1335

Chaïb J, Lecomte L, Buret M, Causse M (2006) Stability over genetic backgrounds, generations and years of quantitative trait locus (QTLs) for organoleptic quality in tomato. *Theoretical and Applied Genetics* **10**, 1-11

Chan Y, Prasad V, Chen SK, Liu PC, Chan M, Cheng C (2005) Transgenic tomato plants expressing *Arabidosis* thionin (*Th2.1)* driven by fruit-inactive promoter battle against phytopathogenic attack. *Planta* **221**, 386-393

Chen G, Alexander L, Grierson D (2004) Constitutive expression of EIL-like transcription factor partially restores ripening in the ethylene-insensitive Nr tomato mutant. *Journal of Experimental Botany* **55**, 1491-1497

Chen LZ, Adachi T (1996) Efficient hybridization between *Lycopersicon esculentum* and *L. peruvianum* via "embryo recue" and *in vitro* propagation. *Plant Breeding* **115**, 251-256

Coaker GL, Meulia T, Kabelka EA, Jones AK, Francis DM (2002) A QTL controlling stem morphology and vascular development in *Lycopersicon esculentum* x *Lycopersicon hirsutum* (Solanaceae) crosses is located on chromosome 2. *American Journal of Botany* **89**, 1859-1866

Cortina C, Culiañez-Macià FA (2005) Tomato abiotic stress-enhaced tolerance by trehalose byosynthesis. *Plant Science* **169**, 75-82

D'Ambrosio C, Giorno G, Marino J, Merendino A, Petrozza A, Salfi L, Stigliani A, Cellini F (2004) Virtually complete conversion of lycopene into β-carotene in fruits of tomato plants transformed with the tomato *lycopene β-cylase(tley)* cDNA. *Plant Science* **166**, 207-214

Daniell H, Lee SB, Grevich J, Saski C, Quesada-Vargas T, Guda C, Tomkins J, Jansen RK (2006) Complete chloroplast genome sequences of *Solanum bulboscastanum*, *Solanum lycopersicum* and comparative analyses with other Solanaceae genomes. *Theoretical and Applied Genetics* **112**, 1503-1518

de Vicente MC, Tanksley SD (1993) QTL analysis of transgressive segregation in an. interspecific tomato cross. *Genetics* **134**, 585-596

Edwards D, Batley J (2004) Plant bioinformatics: from genome to phenome. *Trends in Biotechnology* **22**, 232-237

Enfissi EMA, Fraser PD, Bramley PM (2006) Genetics engineering of carotenoid formation in tomato. *Phytochemical Review* **5**, 59-65

Evans DA, Sharp WR (1983) Single gene mutation in tomato plants regenerated from tissue culture. *Science* **221**, 949-951

Faria R, Destro D, Bespalhok Filho JC, Illg RD (2002) Introgression of *in vitro* regeneration capability of *Lycopersicon pimpinellifolium* Mill. into recalcitrant tomato cultivars. *Euphytica* **124**, 59-63

Faria R, Illg RD (1996) Inheritance of *in vitro* plant regeneration ability in the tomato. *Genetics and Molecular Biology* **19**, 113-116

Faurobert M, Mihr C, Bertin N, Pawlowski T, Negroni L, Sommer N, Causse M (2007) Major proteome variations associated with cherry tomato pericarp development and ripening. *Plant Physiology* **143**, 1327-1346

Fei Z, Tang X, Alba RM, White JA, Ronning CM, Martin GB, Tanksley SD, Giovannoni JJ (2004) Comprehensive EST analysis of tomato and comparative genomics of fruit ripening. *The Plant Journal* **40**, 47-59

Foolad MR (2004) Recent advances in genetics of salt tolerance in tomato. *Plant Cell*, *Tissue and Organ Culture* **76**, 101-119

Frary A, Nesbitt TC, Frary A, Grandillo S, van der Knaap E, Cong B, Liu J, Meller J, Elber R, Alpert K, Tanksley S (2000) Cloning and transgenic expression of *fw2.2*: a quantitative trait locus key to the evolution of tomato fruit. *Science* **289**, 85-87

Frary A, Xu Y, Liu J, Mitchell S, Tedeschi E, Tanksley SD (2005) Development of a set of PCR-based anchor markers encompassing the tomato genome and evaluation of their usefulness for genetics and breeding experiments. *Theoretical and Applied Genetics* **111**, 291-312

Fraser PD, Romers, Shipton CA, Mills PB, Kiano JW, Misawa N, Drake RG, Schuch W, Bramley PM (2002) Evaluation of transgenic tomato plants expressing an additional phytoene synthase in a fruit-specific manner. *Proceedings of the National Academy of Sciences USA* **99**, 1092-1097

Fukusaki E, Kobayashi A (2005) Plant metabolomics: Potencial for practical operation. *Journal of Bioscience and Bioengineering* **100**, 347-354

Gamborg O, Miller R, Ojima K (1968) Nutrient requirements of suspension cultures of soybean root cells. *Experimental Cell Research* **50**, 151-168

Gibly A, Bonshtien A, Balaji V, Debbie P, Martin GB, Sessa G (2004) Identification and expression profiling of tomato genes differentially regulated during a resistance response to *Xanthomonas campestris* pv. *vesicatoria*. *Molecular Plant-Microbe Interactions* **17**, 1212-1222

Giliberto L, Perrotta G, Pallara P, Weller JL, Fraser PD, Bramley PM, Fiore A, Tavazza M, Giovanni G (2005) Manipulation of the blue light photoreceptor cryptochrome 2 in tomato affects vegetative development, flowering time, and fruit antioxidant content. *Plant Physiology* **137**, 199-208

Giovannoni JJ (2004) Genetic regulation of fruit development and ripening. *The Plant Cell* **16 (Suppl)**, 170-180

Goggin FL, Shah G, Williamson VM, Ullman DE (2004) Instability of *Mi*-mediated nematode resistance in transgenic tomato plants. *Molecular Breeding* **13**, 357-364

Greeshoff PM, Doy CH (1972) Development and differentiation of haploid *Lycopersicon esculentum* (tomato). *Planta* **107**, 161-170

Gubba A, Gonsalves C, Stevens MR, Tricoli DM, Gonsalves D (2002) Combining transgenic and natural resistance to obtain broad resistance to tospovirus infection in tomato (*Lycopersicon esculentum* Mill). *Molecular Breeding* **9**, 13-23

Haanstra JPW, Wye C, Verbakel H, Meijer-Dekens F, van den Berg P, Odinot P, van Heusden AW, Tanksley S, Lindhout P, Peleman J (1999) An integrated high-density RFLP-AFLP map of tomato based on two *Lycopersicon esculentum* × *L. pennellii* F_2 populations. *Theoretical and Applied Genetics* **99**, 254-271

He C, Poysa V, Yu K (2003) Development and characterization of simple sequence repeat (SSR) markers and their use in determining relationships among *Lycopersicon esculentum* cultivars. *Theoretical and Applied Genetics* **106**, 363-373

Horsch RB, Fry JB, Hoffmann NL (1985) A simple and general method for transferring genes into plants. *Science* **227**, 1229-1231

Kadyrzhanova DK, Vlachonasios KE, Ververidis P, Dilley DR (1998) Molecular cloning of a novel heat induced/chilling tolerance related cDNA in tomato fruit by use of mRNA differential display. *Plant Molecular Biology* **36**, 885-895

Kardolus JPH, van Eck J, van den Berg RG (1998) The potential of AFLPs in biosystematics: a first application in *Solanum* taxonomy (Solanaceae). *Plant Systematics and Evolution* **210**, 87-103

Kartha KK, Gamborg OL, Shyluk JP, Constabel F (1976) Morphogenic investigations on *in vitro* leaf culture of tomato (*Lycopersicon esculentum* Mill. cv. Starfire) and high frecuency plant regeneration. *Zeitschrift für Pflanzenphysiologie* **77**, 292-301

Kim SH, Grierson D (2005) Subcellular localisation and silencing of ripening-associated membrane protein (TRAMP) in tomato *Lycopersicon esculentum* Mill. *Plant Science* **169**, 1022-1029

Kinsara A, Patnaik SN, Cocking EC, Power JB (1986) Somatic hybrid plants of *Lycopersicon esculentum* Mill. and *Lycopersicon peruvianum* MilI. *Journal of Plant Physiology* **125**, 225-234

Koblitz H, Koblitz D (1982) Experiments on tissue culture in the genus *Lycopersicon* Miller. *Plant Cell Reports* **1**, 143-146

Koornneef M, Bade J, Hanhart C, Horsman K, Schel J, Soppe W, Verkerk R, Zabel P (1993) Characterization and mapping of a gene controlling shoot regeneration in tomato. *The Plant Journal* **3**, 131-141

Koornneef M, Hanhart CJ, Martinelli L (1987) A genetic analysis of cell culture traits in tomato. *Theoretical and Applied Genetics* **74**, 633-641

Kumar H, Kumar V (2004) Tomato expressing Cry1A(b) insecticidal protein from *Bacillus thuringiensis* protected against tomato fruit borer, *Helicoverpa armigera* (Hubner) (*Lepidoptera: Noctuidae*) damage in the laboratory, greenhouse and field. *Crop Protection* **23**, 135-139

Kut SA, Evans DA (1982) Plant regeneration from cultured leaf explants of eight wild tomato species and two related *Solanum* species. *In Vitro* **18**, 593-598

Le Gall G, Colquhoun IJ, Davis A, Collins GJ, Verhoeyen ME (2003) Metabolite profiling of tomato (*Lycopersicon esculentum*) using ^{1}H NMR spectroscopy as a tool to detect potential unintended effects following a genetic modification. *Journal of Agricultural and Food Chemistry* **51**, 2447-2456

Lecomte L, Saliba-Colombani V, Gautier A, Gomez-Jimenez MC, Duffé P, Buret M, Causse M (2004) Fine mapping of QTLs of chromosome 2 affecting the fruit architecture and composition of tomato. *Molecular Breeding* **13**, 1-14

Lefrançois C, Chupeau Y, Bourgin JP (1993) Sexual and somatic hybridization in the genus *Lycopersicon*. *Theoretical and Applied Genetics* **86**, 533-546

Lercari B, Bertram L (2004) Interactions of phytochromes A, B1 and B2 in light-induced competence for adventitious shoot formation in hipocotil of tomato (*Solanum lycopersicum* L.). *Plant Cell Reports* **22**, 523-531

Li C, Bai Y, Jacobsen E, Visser R, Lindhout P, Bonnema G (2006) Tomato defense to the powdery mildew fungus: differences in expression of genes in susceptible, monogenic- and polygenic resistance responses are mainly in timing. *Plant Molecular Biology* **62**, 127-140

Li L, Steffens JS (2002) Overexpression of polyphenol oxidase in transgenic tomato plants results in enhanced bacterial disease resistance. *Planta* **215**, 239-247

Lin W, Lu C, Wu J, Cheng M, Lin Y, Yang N, Black L, Green SK, Wang J, Cheng C (2004) Transgenic tomato plants expressing the Arabidopsis NPR1 gene display enhanced resistance to a spectrum of fungal and bacterial diseases. *Transgenic Research* **13**, 567-581

Liu J, Cong B, Tanksley SD (2003) Generation and analysis of an artificial gene dosage series in tomato to study the mechanisms by which the cloned quantitative trait locus *fw2.2* controls fruit size. *Plant Physiology* **132**, 292-299

Liu J, van Eck, J, Cong B, Tanksley SD (2002) A new class of regulatory genes underlying the cause of pear-shaped tomato fruit. *Proceedings of the National Academy of Sciences USA* **99**, 13302-13306

Liu Y, Roof S, Ye Z, Barry C, van Tuinen AJ, Vrebalov J, Bowler C, Giovannoni J (2004) Manipulation of light signal transduction as a means of modifying fruit nutritional quality in tomato. *Proceedings of the National Academy of Sciences USA* **101**, 9897-9902

Long M, Millar DJ, Kimura Y, Donovan G, Rees J, Fraser PD, Bramley PM, Bolwell GP (2006) Metabolite profiling of carotenoid and phenolic pathways in mutant and transgenic lines of tomato: Identification of a high antioxidant fruit line. *Phytochemistry* **67**, 1750-1757

Luo YY, Gianfagna TJ, Janes HW, Huang B, Wang Z, Xing J (2005) Expression of the *ipt* gene with the AGPase S1 promoter in tomato results in unbranched roots and delayed leaf senescence. *Plant Growth Regulation* **47**, 47-57

Marchionni Basté E, Pratta GR, Zorzoli R (2007) Genetic analysis of the *in vitro* culture response in tomato. *Plant Cell, Tissue and Organ Culture* **88**, 233-239

Martin GB, Brommonschenkel S, Chunwongse J, Frary A, Ganal MW, Spivey R, Wu T, Earle ED, Tanksley SD (1993) Map-based cloning of a protein kinase gene conferring disease resistance in tomato. *Science* **262**, 1432-1436

McClean PE, Hanson MR (1986) Mitochondrial DNA sequence divergence among *Lycopersicon* and related *Solanum* species. *Genetics* **112**, 649

McCormick S, Niedermeyer J, Fry J, Barnason A, Horsch R, Fraley R (1986) Leaf disc transformation of cultivated tomato (*L. esculentum*) using *Agrobacterium tumefaciens*. *Plant Cell Reports* **5**, 81-84

Melchers G, Sacristan MD, Holder AA (1978) Somatic hybrid plants of' potato and tomato regenerated from used protoplasts. *Carlsberg Research Communications* **43**, 203-218

Miller JC, Tanksley SD (1990) RFLP analysis of phylogenetic relationships and genetic variation in the genus *Lycopersicon*. *Theoretical and Applied Genetics* **80**, 437-448

Monforte J, Friedman E, Zamir D, Tanksley SD (2001) Comparison of a set of allelic QTL-NILs for chromosome 4 of tomato: Deductions about natural variation and implications for germplasm utilization. *Theoretical and Applied Genetics* **102**, 572-590

Muir SR, Collins GJ, Robinson S, Hughes S, Bovy A, de Vos CHR, van Tunen AJ, Verhoeyen ME (2001) Overexpression of petunia chalcone isomerase in tomato results in fruit containing increased levels of flavonols. *Nature Biotechnology* **19**, 470-474

Murashige T, Skoog F (1962) A revised medium for rapid growth and bio-assays with tobacco tissue cultures. *Physiologia Plantarum* **15**, 473-497

Niggeweg R, Michael AJ, Martin C (2004) Engineering plants with increased levels of the antioxidant chlorogenic acid. *Nature Biotechnology* **22**, 746-754

Norton JP, Boll WG (1954) Callus and shoot formation from tomato roots *in vitro*. *Science* **119**, 220-221

Orozco-Cárdenas ML, Ryan CA (2003) Polygalacturonase subunit antisense gene expression in tomato plants leads to a progressive enhanced wound response and necrosis in leaves and abscission of developing flowers. *Plant Physiology* **133**, 693-701

Pandey A, Mann M (2000) Proteomics to study genes and genomes. *Nature* **405**, 837-846

Pratta G, Cánepa LN, Zorzoli R, Picardi LA (2003) Diallel analysis of *in vitro* culture traits in the genus *Lycopersicon*. *HortScience* **38**,110-112

Pratta G, Zorzoli R, Boggio SB, Picardi LA, Valle EM (2004) Glutamine and glutamate levels and related metabolizing enzymes in tomato fruits with different shelf-life. *Scientia Horticulturae* **100**, 341-347

Pratta G, Zorzoli R, Picardi LA (1997) Intra and interspecific variability of *in vitro* culture response in *Lycopersicon* (tomatoes). *Brazilian Journal of Genetics* **20**, 75-78

Pratta G, Zorzoli R, Picardi LA, Valle EM (2006) Variability for the *in vitro* culture response in a set of tomato genotypes. *Biologia Plantarum* **50**, 421-424

Praveen S, Kushwaha CM, Mishra AK, Singh V, Jain RK, Varma A (2005) Engineering tomato for resistance to tomato leaf curl disease using viral rep gene sequences. *Plant Cell, Tissue and Organ Culture* **83**, 311-318

Primieri Carelli B, Gerald LTS, Gobbi Grazziotin F, Echeverrigaray S (2006) Genetic diversity among Brazilian cultivars and landraces of tomato (*Lycopersicon esculentum* Mill.) revealed by RAPD markers. *Genetic Resources and Crop Evolution* **53**, 395-400

Pugliesi C, Cionini G, Bertram L, Lercari B (1999) A histological study of light-dependent shoot regeneration in hypocotyl explants of tomato cultured *in vitro*. *Advances in Horticulture Science* **13**, 168-172

Ralley L, Enfissi EMA, Misawa N, Schuch W, Bramley PM, Fraser PD (2004) Metabolic engineering of ketocarotenoid formation in higher plants. *The Plant Journal* **39**, 477-486

Rick CM (1973) Potential genetic resources in tomato species: clones from observations in native habitats. In: Srb A (Ed) *Genes*, *Enzymes and Populations*, Plenum, New York, pp 255-269

Rick CM (1982) The potential of exotic germplasm for tomato improvement. In: Vasil IK, Scowcroft WR, Freys KJ (Eds) *Plant Improvement and Somatic Cell Genetics*, Academic Press, New York, pp 1-28

Rick CM, Fobes JF (1974) Association of an allozyme with nematode resistance. *Reports of the Tomato Genetics Cooperative* **24**, 25

Robert VJM, West MAL, Inai S, Caines A, Arntzen L, Smith JK, St. Clair DA (2001) Marker-assisted introgression of blackmold resistance QTL alleles from wild *Lycopersicon cheesmanii* to cultivated tomato (*L. esculentum*) and evaluation of QTL phenotypic effects. *Molecular Breeding* **8**, 217-233

Rocco M, D'Ambrosio C, Arena S, Faurobert M, Scaloni A, Marra M (2006) Proteomic analysis of tomato fruits from two ecotypes during ripening. *Proteomics* **6**, 3781-3791

Rodríguez GR, Pratta GR, Zorzoli R, Picardi LA (2006) Recombinant lines obtained from an interspecific cross between *Lycopersicon* species selected by fruit weight and fruit shelf life. *Journal of the American Society for Horticultural Science* **131**, 651-656

Rodríguez GR. Sequin L, Pratta GR, Zorzoli R, Picardi LA (2007) Protein profiling in F_1 and F_2 generations of two tomato genotypes differing in ripening time. *Biologia Plantarum*, in press

Roessner U, Willmitzer L, Fernie AR (2001) High-resolution metabolic phenotyping of genetically and environmentally diverse potato tuber systems. Identification of phenocopies. *Plant Physiology* **127**, 749-764

Roessner-Tunali U, Hegemann B, Lytovchenko A, Carrari F, Bruedigam C, Granot D, Fernie AR (2003) Metabolic profiling of transgenic tomato plants overexpressing hexokinase reveals that the influence of hexose phosphorylation diminishes during fruit development. *Plant Physiology* **133**, 84-89

Rose JKC, Bashir S, Giovannoni JJ, Jahn MM, Saravanan RS (2004) Tackling the plant proteome: practical approaches, hurdles and experimental tools. *The Plant Journal* **39**, 715-733

Rousseaux MC, Jones CM, Adams D, Chetelat R, Bennett A, Powell A (2005) QTL analysis of fruit antioxidants in tomato using *Lycopersicon pennellii* introgression lines. *Theoretical and Applied Genetics* **111**, 1396-1408

Rowland O, Ludwig AA, Merrick CJ, Baillieul F, Tracy FE, Durrant WE, Fritz-Laylin L, Nekrasov V, Sjölander K, Yoshioka H, Jones JDG (2005) Functional analysis of *Avr9*/*Cf-9* rapidly elicited genes identifies a protein kinase, *ACIK1*, that is essential for full *Cf-9*-dependent disease resistance in tomato. *The Plant Cell* **17**, 295-310

Roy R, Purty RS, Agraval V, Gupta SC (2006a) Promoterless *gus* gene shows leaky β-glucuronidase activity during transformation of tomato *bspA* gene for drought tolerance. *Biologia Plantarum* **50**, 352-358

Roy R, Purty RS, Agraval V, Gupta SC (2006b) Transformation of tomato cultivar "Pusa Ruby" with *bspA* gene from *Populus tremula* for drought tolerance. *Plant Cell*, *Tissue and Organ Culture* **84**, 55-67

Rus AM, Ríos S, Olmos E, Santa-Cruz A, Bolarin MC (2000) Long-term culture modifies the salt responses of callus lines of salt-tolerant and salt-sensitive tomato species. *Journal of Plant Physiology* **157**, 413-420

Sagi M, Davydov O, Orazova S, Yesbergenova Z, Ophir R, Stratmann JW, Fluhrb R (2004) Plant respiratory burst oxidase homologs impinge on wound responsiveness and development in *Lycopersicon esculentum*. *The Plant Cell* **16**, 616-628

Sarowar S, Kim Y, Kim E, Kim KD, Choi JY, Hyung NI, Shin JS (2006) Constitutive expression of two pathogenesis-related genes in tomato plants enhanced resistance to oomycete pathogen *Phytophthora capsici*. *Plant Cell, Tissue and Organ Culture* **86**, 7-14

Schauer N, Semel Y, Roessner U, Gur A, Balbo I, Carrari F, Pleban T, Perez-Melis A, Bruedigam C, Kopka J, Willmitzer L, Zamir D, Fernie AR (2006) Profiling of tomato interspecific introgression lines facilitated the identification of pathway and network quantitative trait loci revealing that plant morphological traits are major determinants of fruit metabolic networks. *Nature Biotechnology* **24**, 447-454

Schauer N, Zamir D, Fernie AR (2005) Metabolic profiling of leaves and fruit of wild species tomato: a survey of the *Solanum lycopersicum* complex. *Journal of Experimental Botany* **56**, 297-307

Seah S, Yaghoobi J, Rossi M, Gleason CA, Williamson VM (2004) The nematode-resistance gene, Mi-1, is associated with an inverted chromosomal segment in susceptible compared to resistant tomato. *Theoretical and Applied Genetics* **108**, 1635-1642

Semel Y, **Nissenbaum J, Menda N, Zinder M, Krieger U, Issman N, Pleban T, Lippman Z, Gur A, Zamir D** (2006) Overdominant quantitative trait loci for yield and fitness in tomato. *Proceedings of the National Academy of Sciences USA* **103**, 12981-12986

Shahin EA, Spivey R (1986) A single dominant gene for Fusarium wilt resistance in protoplast-derived tomato plants. *Theoretical and Applied Genetics* **73**, 164-169

Sharp WR, Raskin RS, Sommer HE (1972) The use of nurse culture in the development of haploid clones in tomato. *Planta* **104**, 357-361

Shibata D (2005) Genome sequencing and functional genomics approaches in tomato. *Journal of General Plant Pathology* **71**, 1-7

Smith CJS, Watson CF, Ray CR, Morris PC, Schuch W, Grierson D (1988) Antisense RNA inhibition of polygalacturonase gene expression in transgenic tomatoes. *Nature* **334**, 724-726

Smith PG (1944) Embryo culture of a tomato species hybrid. *Proceedings of the American Society of Horticultural Science* **44**, 413-416

Sobolev AP, Segre A, Lamanna R (2003) Proton high-field NMR study of tomato juice. *Magnetic Resonance in Chemistry* **41**, 237-245

Spooner DM, Andersen GJ, Jansen RK (1993) Chloroplast DNA evidence for the interrelationships of tomatoes, potatoes and pepinos (*Solanaceae*). *American Journal of Botany* **80**, 676-688

Spooner DM, Peralta IE, Knapp S (2005) Comparison of AFLPs with other

markers for phylogenetic inference in wild tomatoes [*Solanum L.* section *Lycopersicon* (Mill.) Wettst.]. *Taxon* **54**, 43-61

Stoeva-Popova PK, Dimaculangan D, Radkova M, Vulkova Z (2007) Towards cytoplasmic male sterility in cultivated tomato. *Journal of Agricultural*, *Food and Environmental Sciences* **1**

Takashina T, Suzuki T, Egashira H, Imanishi S (1998) New molecular markers linked with the high shoot regeneration capacity of th wild tomato species *Lycopersicon chilense*. *Breeding Science* **48**, 109-113

Tanksley SD, Rick CM, Vallejos CE (1983) Tight linkage between a nuclear male-sterile locus and an enzyme marker in tomato. *Theoretical and Applied Genetics* **68**, 109-113

Thomas BR, Pratt D (1981) Efficient hybridization between *Lycopersicon esculentum* and *L. peruvianum* via embryo callus. *Theoretical and Applied Genetics* **59**, 215-219

Tikunov Y, Lommen A, Ric de Vos CH, Verhoeven HA, Bino RJ, Hall RD, Bovy AG (2005) A novel approach for nontargeted data analysis for metabolomics. Large-scale profiling of tomato fruit volatiles. *Plant Physiology* **139**, 1125-1137

Torelli A, Borinato M, Soragni E, Bolpagni R, Bottura C, Branca C (2004) The delay in hormonal treatment modulates the expression of *LESK1*, a gene encoding a putative serine-threonine kinase, marker of *in vitro* caulogenesis in tomato (*Lycopersicon esculentum* Mill.). *Plant Science* **167**, 607-620

Torelli A, Soragni E, Bolchi A, Petrucco S, Ottonello S, Branca C (1996) New potential markers of *in vitro* tomato morphogenesis identified by mRNA differential display. *Plant Molecular Biology* **32**, 891-900

van den Bulk RW, Lindhout WH, Koornneef M (1990) Somaclonal variation in tomato: effect of explant source and a comparison with chemical mutagenesis. *Theoretical and Applied Genetics* **80**, 817-825

van der Hoeven R, Ronning C, Giovannoni J, Martin G, Tanksley S (2002) Deductions about the number, organization, and evolution of genes in the tomato genome based on analysis of a large expressed sequence tag collection and selective genomic sequencing. *The Plant Cell* **14**, 1441-1456

van Eck JM, Blowers AD, Earle ED (1995) Stable transformation of tomato cell cultures after bombardment with plasmid and YAC DNA. *Plant Cell Reports* **14**, 299-304

Wang GP, Lim GTT, Jones DA (2007) Development of PCR-based markers from the tomato glutamate oxaloacetate transaminase isozyme gene family as a means of revitalising old isozyme markers and recruiting new ones. *Molecular Breeding* **19**, 209-214

Wang T, Zhang C, Wu W, Nowack LM, Madey E, Thompson JE (2005) Antisense suppression of deoxihypresine synthase in tomato delays fruit softening and alters growth and development. *Plant Physiology* **138**, 1372-1382

Wijbrandi J, Posthuma A, Kok JM, Rijken R, Vos JGM, Koornneef M (1990) Assymetric somatic hybrids between *Lycopersicon esculentun* and irradiated *Lycopersicon peruvianum*. 1. Cytogenetics and morphology. *Theoretical and Applied Genetics* **80**, 305-312

Wijbrandi J, Vos JGM, Koornneef M (1988) Transfer of regeneration capacity from *Lycopersicon peruvianum* to *L. esculentum* by protoplast fusion. *Plant Cell, Tissue Organ Culture* **12**, 193-196

Wolters AMA, Vergunst AC, van der Weff F, Koornneef M (1993) Analysis of nuclear and organellar DNA of somatic hybrid calli and plants between *Lycopersicon* spp. and *Nicotiana* spp. *Molecular Genetics and Genomics* **241**, 707-718

Yaghoobi J, Yates JL, Williamson VM (2005) Fine mapping of the nematode resistance gene Mi-3 in *Solanum peruvianum* and construction of a *S. lycopersicum* DNA contig spanning the locus. *Molecular Genetics and Genomics* **274**, 60-69

Zamir D, Ekstein-Michelson I, Zakay U, Navot N, Zeidan M, Sarfatti M, Eshed Y, Harel E, Pleban T, van-Oss H, Kedar N, Rabinsowitch HD, Czosnek H (1994) Mapping and introgression of a tomato yellow leaf curl virus tolerance gene, *Ty-1*. *Theoretical and Applied Genetics* **88**, 141-146

Zamir D, Jones RA, Kednar N (1980) Anther culture of male-sterile tomato (*Lycopersicon esculentum* Mill.) mutants. *Plant Science Letters* **17**, 353-361

Zegzouti H, Jones B, Frasse P, Marty C, Maitre B, Latché A, Pech JC, Bouzayen M (1999) Ethylene-regulated gene expression in tomato fruit: characterization of novel ethylene-responsive and ripening-related genes isolated by differential display. *The Plant Journal* **18**, 589-600

Zhang L, Lin GY, Niño-Liu D, Foolad MR (2003) Mapping QTLs conferring early blight (*Alternaria solani*) resistance in a *Lycopersicon esculentum* x *L. hirsutum* cross by selective genotyping. *Molecular Breeding* **12**, 3-19

Zhang LP, Khan A, Niño-Liu D, Foolad MR (2002) A molecular linkage map of tomato displaying chromosomal locations of resistance gene analogs based on a *Lycopersicon esculentum* × *Lycopersicon hirsutum* cross. *Genome* **45**, 133-146

Ziv M, Hadary D, Kedar M, Ladizinsky G (1984) *Lycopersicon esculetum*: Trifoliate plants recovered from other cultures of heterozygous plants. *Plant Cell Reports* **3**, 10-13

Zivy M, de Vienne D (2000) Proteomics: a link between genomics, genetics and physiology. *Plant Molecular Biology* **44**, 575-580

Zorzoli R, Cointry E, Prado E, Mroginski L, Picardi LA (1988) Regeneración de plantas de tomate (*Lycopersicon esculentum*) por cultivo *in vitro* de folíolos. *Turrialba* **38**, 332-336