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Transposable Sequences in Citrus Genome: Role of Mobile Elements in the Adaptation to Stressful Environments

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ABSTRACT

Mobile DNAs make up a large proportion of the nuclear plant genome. They can rearrange genomes and other individual gene structure and regulation through a number of activities, such as transposition, insertion, excision, chromosome breakage, and ectopic recombination. Ty-like retrotransposons, a widespread class of transposable elements in the plant kingdom, have been found in the *Citrus* genome. The aim of this review is to illustrate the evolutionary relationships of Ty-like elements in *Citrus* species, as well as the genomic organization of these sequences in *Citrus* genome and their transcriptional activity. Wounding, salt and cell cultured stress produce transcriptional activation of several Ty-like elements in *C. limon.* Therefore, transcriptional activation under stress conditions of transposon sequences opens the possibility that these mobile elements have given more genetic variability to *Citrus* plants, thus facilitating adaptation to a range of stressful habitats.

Keywords: *Citrus*, transposons, retrotransposons, stress

CONTENTS

INTRODUCTION

Transposable elements are widespread within the plant kingdom and represent a main constituent of most plant genomes (Kumar and Bennetzen 1999). Transposons are sequences of DNA that can move around to different positions within the genome in a process called transposition. In the process, they can cause mutations and change the amount of DNA in the genome. Transposons were also once called "jumping genes", and are examples of mobile genetic elements. In some species, for example maize, they can represent more than 50% of all DNA in some species.

Although plant genomes expand by several mechanisms, including polyploidization, transposition, and duplication, there is additional evidence that transposable element-mediated genomic rearrangements, including insertions, deletions, inversions and duplications, are potentially associated with or subsequent to speciation events. The high proportion of mobile elements within plant genomes is a consequence of their replicative mode (copy-and-paste) of transposition, producing new copies of the element each transposition.

Transposons may play an important role in the evolution of gene function and may be involved in the restructuring of genomes due to their ability to restructure or rearrange chromosomes (Agrawal *et al*. 2001; Witte *et al*. 2001).

Retrotransposition is followed by insertion of reverse transcripts into the genome at new sites. New sites of homology for unequal crossing over arise from the new copies of transposons and retrotransposons (Lonnig and Saedler 1997). Transposons are involved in the structure of chromosomes, particularly in centromeres and telomeres and play significant roles in epigenetic regulation, such as chromatin modification and sex chromosome inactivation (Lyon 2000; Peaston *et al*. 2004; Han and Boeke 2005; Slotkin and Martienssen 2007).

Regulatory and coding sequences from transposable elements have been recruited during evolution to accomplish host functions. In humans, for example, about 4% of genes have transposable element derived sequences in their protein encoding regions, and about 25% of promoter regions contain a transposon derived sequence (Nekrutenko and Li 2001; van de Lagemaat *et al.* 2003). Moreover, numerous RNA and protein encoding genes entirely derived from transposable elements are present in eukaryotic genomes. All these observations suggest that mobile DNA has significantly contributed to the complexity of genomes.

As a result, transposition might be a major cause of plant genome expansion. Comparative genomics has revealed that plants contain quantitatively and qualitatively different populations of retrotransposable elements and DNA transposons, with significant differences between species within the same genus. This is the outcome of differential evolution of ancestral families of transposable elements, ranging from complete extinction to considerable invasion, the species-specific introduction of transposable elements by infection and horizontal transfer, as by endogenous retroviruses and the species-specific appearance of novel

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Invited Review

mobile elements. We believe that transposable elements are the major drivers of genomic and biological diversity in plants, with possible key roles in speciation and evolution.

Thus, the detailed characterization of different plant taxa with respect to the content, variability, and physical distribution of retrotransposons will invariably contribute to the understanding of host genome organization and evolution (Bennetzen 2000).

Moreover, there is speculation that certain forms of "genomic stress" may stimulate the activation of transposons (Grandbastien 1998). It has been reported that biotic and abiotic stresses are correlated to an increase of transposons activity (Hirochika *et al.* 1996). Studies performed on rice and tobacco have shown that *in vitro* growth by tissue culture, for instance, can lead to the activation and transcription of these elements (Hirochika 1993; Hirochika *et al.* 1996).

The *Citrus* genus includes the most widely producing fruit species in the world and is highly polymorphic. The lemon (*Citrus limon* (L.) Burm.) belongs to the family *Rutaceae* (*Citrinae* subtribal group C) originating from China and has been cultivated for thousands years. Lemon is one of the most economically important plants in the Mediterranean (Italy) as well as in other mid-latitude regional economies (Texas, California, Argentina and Florida), because it is utilized both as a food crop and industrial product. Important commercial species and varieties, such as sweet oranges, mandarins, lemons and grapefruits are also widely cultivated.

Some highly repetitive and dispersed DNA sequences have been isolated from the *Citrus* genus and their genomic organization has been studied (Fann *et al.* 2001; Beridze *et al.* 1994; De Felice *et al.* 2006, 2007). However, it is likely that novel repetitive sequences involving the transposable elements could increase the amount of molecular data for understanding genomic evolution and function of *Citrus limon* and related species In fact, transposons can be considered natural vectors of evolutionary force in shaping genomes. In *Citrus*, bud mutations take place often and are generally detected in brushwood of trees showing altered horticultural traits. Reported data suggests that some transposon activity might be a source of bud mutations (Bretó *et al.* 2001). Several retroelements have been isolated in *Citrus* (Asins *et al.* 1999; Bernet and Asins 2003; Tao *et al.* 2005; Rico-Cabanas and Martínez-Izquierdo 2007) including those surrounding the *Citrus tristeza virus* resistance gene (*Ctv*) locus in *Poncirus trifoliata* L. Raf (Yang *et al.* 2003). In this review, the main classes of transposable elements isolated in *Citrus* will be described, with particular emphasis to the genomic organization, evolution, transcription activity and stress activation.

Retrotransposable elements in plants genome

Retrotransposons are subdivided into three types: long terminal repeat (LTR)-retrotransposons, SINE (short interspersed)-like elements, and LINE (long interspersed)-like elements. LTR elements show striking similarities in structure to retroviruses (**Fig. 1**). The main difference is that LTR-retrotransposons lack the *env* genes, which code for the viral coat proteins in retroviruses. LTR retrotransposons are further divided, into the two groups Ty1 or *copia*, and Ty3 or *gypsy*. The major structural difference between *copia* and *gypsy* groups is in the order of the reverse transcriptase (RT) and integrase domains in their pol genes (Galun 2003); *gypsy* group elements have also similarities to retroviruses (Bennetzen 2000). Both *gypsy*-like and *copia*-like retrotransposons were soon identified in the maize genome and subsequently in many other plant genomes (Voytas *et al.* 1992; Su and Brown 1997). In contrast to terminal inverted repeats (TIRs) of class II transposons, LTRs are direct repeats, usually several hundred base pairs in length (Galun 2003).

The presence of *copia*-like retrotransposons among plants, insects, fungi and protisti suggested that this class of

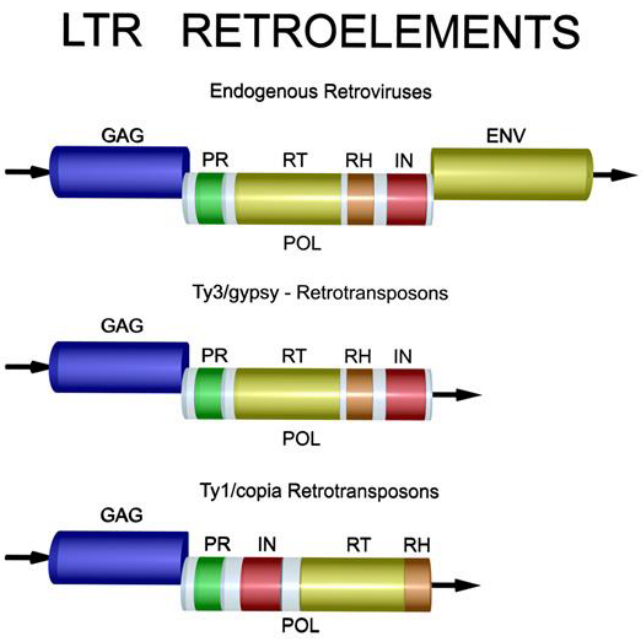

Fig. 1 Schematic representation of LTR retroelements. EN, endonuclease; ENV, envelope, which code for the viral coat proteins in retroviruses; LTR, long terminal repeat; PR, protease; gag, protein involved in maturation and packaging of retrotransposon RNA and proteins, and the Pol gene encoding a reverse transcriptase (RT), a ribonuclease H (RNase H) and an integrase (INT).

elements might be a universal component of eukaryotic genomes. The *Ty1-copia*-like retrotransposon, which has been highly researched and identified in over 100 plant species is thought to be present in all plants (Heslop-Harrison *et al.* 1997).

Ty mobile elements are RNA- or retrotransposons, very well known as class I transposons, replicating via an RNA intermediate. On the other hand, class II transposons or DNA transposons replicate via a cut-and-past system.

To date, Ty1 and TY3-like retrotransposons, a widespread class of transposable elements in the plant kingdom, have been greatly detected in *Citrus* genome.

LTRs contain the elements' promoter and enhancer (Pauls *et al.* 1994) and Ty1/*copia*-like transposons initiate transcription within the LTR (Voytas and Boeke 2002). Recombination can result in the separation of one LTR from the protein-coding sequences of the transposon it is flanking and produce so called Solo LTRs that have a key role in the genome activating neighboring genes.

Ty1-*copia*-like retrotransposons are the major group in higher plants, varying greatly in copy number over relatively short evolutionary time scales; therefore, they are one of the most important factors affecting the structural evolution of higher plant genomes (Gribbon *et al.* 1999) and have contributed much to the genetic diversity of their host species (Linares *et al.* 2001; Price *et al.* 2002).

Transposon silencing

Various mechanisms of silencing transposons and retrotransposons have been observed (Kanno *et al.* 2005; Lippman *et al.* 2003). In plants, methylation has been a frequently reported mechanism controlling transposable elements (Finnegan *et al.* 1996) that can be reactivated in methylation-defective mutants (Miura *et al.* 2001). In the rice and in maize genomes, gene islands are unmethylated and separated by a sea of methylated transposable elements (Vastenhouw *et al.* 2003). Several studies have suggested that levels of methylation are higher in repetitive DNA than in non-repetitive DNA, possibly reflecting a genome-wide defense mechanism against deleterious effects associated with transposable elements. Mobilization of transposons is

often associated with hypomethylation and transcriptional activation (Lawson *et al.* 1994; Vaucheret 2005). Indeed, the elements CAC in *Arabidopsis* mutants were hypomethylated and transcriptionally activated in *ddm1* plants (Kuhlmann *et al.* 2005), like other repeated sequences. Methylation of both DNA and histone tails appears to be intimately involved in the maintenance or formation of heterochromatin. A change in methylation either via loss of DNA or histone methylation or a rearrangment of methyl groups within the genome can cause changes in gene transcription. Beside, the loss of methylation is also correlated with transcriptional and transpositional activation of transposons, which in turn can cause gene mutations and phenotypic changes. Methyltransferases and chromatin remodelling factors appear to help in the addition and maintenance of methyl groups (Fedoroff *et al.* 1995; Jenuwein and Allis 2001).

In *Arabidopsis*, different classes of transposable elements are regulated by different, though overlapping, mechanisms that require DNA methylation, histone modification and the RNA interference (RNAi) system. Remarkably, different components contribute to various degrees to silencing specific transposable elements. In addition, transcriptional and post-transcriptional mechanisms simultaneously contribute to the silencing machinery (Lippman *et al.* 2003; Herr *et al.* 2005; Kanno *et al.* 2005).

RNAi machinery is necessary for the maintenance of heterochromatin and the silencing of DNA repeat units (Volpe *et al.* 2002; Schramke and Allshire 2003), transposons and retrotransposons. This maintenance of heterochromatin is thought to proceed via constitutive low-level transcription of sense and antisense RNAs (Volpe *et al.* 2002; Schramke and Allshire 2003). The resulting dsRNA triggers the RNAi pathway and causes silencing of homologous loci. This mechanism of heterochromatic maintenance immediately suggests a way to silence any kind of DNA repeats, especially those that might produce hairpin RNA or that are organized in opposite head-to-head arrangements or inverted repeats. In fact, RNAi failure in mutants causes the activation of transposable elements (Miura *et al.* 2001; Singer *et al.* 2001; Lippman *et al.* 2003; Miura *et al.* 2004).

Mobile elements are largely inactive during normal development, but why are they induced by stress? The activation by stress is in keeping with the genome-restructuring role predicted by McClintock for transposable elements (McClintock 1984).

Genomic stress

Species, populations and individuals have to deal with environmental alterations. Individual organisms have to adapt physiologically through reactions that are immediate and reversible. At the population and species levels, selection may lead to genetic modifications and to the evolution of the inherited traits of an organism where this long-term response becomes irreversible.

McClintock predicted large-scale genomic changes in response to unusual challenges including transposon activation and other structural modifications of the chromosomes (McClintock 1984).

Living organisms must adapt to stresses in their environment to survive; stress can originate from a change in climate, population factors, and from attacks by other organisms. It is an evolutionary force that all organisms face. In response, an organism might undergo changes in cellular physiology, gene regulation, and genome remodeling. Such stresses can generate significant activity of transposable elements within an organism. An increase in the mobility of transposable elements is tantamount to an increase in genetic variability, which is key to the organism's continued survival. In plants, it is known that retrotransposons, which are quiescent during development, can become activated by stress induced by pathogens, wounding, and cell culture (Hirochika *et al.* 1996; Grandbastien 1998; Beguiristain *et al.* 2001). Retrotransposons could be an elegant survival strategy of plant biology or stress induced generators of genomic diversity (McClintock 1984).

Stress activation may imply both vertical and horizontal transmission: vertical transmission could occur if stress-activated transposons move in lineages rising from gametes; horizontal transmission might occur if a pathogen simultaneously activates element transcription and provides a vector to retro transpose to another host (Flavell *et al.* 1992).

Negative growth and developmental effects are the measurable manifestations of stress. These can originate from internal stresses, such as aberrant cell division or spontaneous gene mutations, which might affect metabolic or genetic regulation. They can also originate from either biotic or external abiotic stresses. Biotic type stresses would include competition or attack by other organisms, such as by pathogens or the organism's place in the food chain. In contrast, abiotic stresses are related to climatic conditions of temperature, water, sunlight, and available nourishment. Mobility is one evolutionary response to stress; mobile organisms are able to avoid stress by seeking out more favorable conditions (Madlung and Comai 2004). However, plants do not enjoy this evolutionary luxury and must adapt where rooted.

Consequently, plants have developed different mechanisms for coping with stress. Plants often cope with chronic stress through alteration of their morphological features, such as desert plants developing extended roots, water retaining tissue, and thorns. Yet, plants tend to employ different mechanisms for dealing with acute stresses where changes in permanent features might not be beneficial to survival. For example, some flowers survive late spring snows by temporarily folding inward.

McClintock (1984) proposed four types of stress that could lead to genomic restructuring, which are (1) tissue culture, (2) plant pathogen attack, (3) interspecies crosses (allopolyploidization), and (4) germline separation from somatic tissues in early development. Importantly, genomic restructuring is facilitated by transcriptional transposon activation, transposition of mobile elements, and chromosome breakage fusion bridges. Modern research now allows observation of this restructuring at the molecular level and provides a better understanding of stress induced whole genome responses.

Tissue culture induces genomic changes. Indeed, it has reported the activation of a retrotransposon in rice after tissue culturing (Hirochika *et al.* 1996) and the activation of MITE, a miniature inverted repeat transposable element, subsequent to cultures of anther rice (Kikuchi *et al.* 2003).

Also wounding might contribute to transposon activetion (Grandbastien 1998), Hormones can activate promoters of transposons (Takeda *et al.* 1999). A decrease in DNA methylation has been observed (Kaeppler and Phillips 1993; Kubis *et al.* 2003) related to tissue culture and transpositional activation: perhaps, it is likely that tissue culture compromises the epigenetic homeostasis of plant genomes, resulting in genomic changes.

Pathogen stress can activate retrotransposons (Grandbastien 1998; Beguiristain *et al.* 2001). Treatment with fungal elicitors activated transcription of Tnt1 retrotransposon in tobacco (Grandbastien *et al.* 1997; Melayah *et al.* 2001). Moreover, an increase in activity of the retrotransposon Tto1 has been reported in response to wounding, treatment with methyl-jasmonate, fungal extracts and *in vitro* tissue culture (Takeda *et al.* 1999).

Beside, Bs1 retrotransposon was activated by viral infection in maize (Grandbastien 1998).

Abiotic stress can lead to genetically programmed responses as well. Transcriptional activation of multiple copies of a retrotransposon induced by cold temperatures was observed in *Medicago sativa*; however, the cold-induced response was not associated with DNA demethylation (Ivashuta *et al.* 2002). In barley, BARE1 retrotransposon activity resulted from microclimatic shifts (Kalendar *et al.* 2000).

It has also been suggested that transposition activity of

Tam3 in *Antirrhinum majus* might be correlated with the methylation state via a temperature-sensitive DNA methyltransferase (Hashida *et al.* 2003).

Allopolyploidization and hybridization may also be a source of transposon activation. *Citrus* can be an example of triploid crops in nature; these mutations or sports are often evident on a tree by their enlarged "gigas" condition. In plants, allopolyploidization has played an important role in plant evolution. The development of polyploids can also be a useful and valuable approach in plant improvement programs. In fact Grosser *et al.* (1996) developed an efficient protoplast-fusion method to produce somatic hybrid allopolyploid plants that combine *Citrus* with seven related genera, including four that are sexually incompatible. In this research, the creation of 18 new allotetraploid hybrids of *Citrus*, including ten among sexually incompatible related genera, which may have direct cultivar potential as improved citrus rootstocks, were reported.

Retrotransposon activation was observed in rice hybrids (Liu and Wendel 2000). Transcriptional activation of retrotransposons has been observed in polyploidy wheat (Kashkush *et al.* 2003). Also, the expression patterns of some genes adjacent to transposon LTRs were altered, supporting the hypothesis that hybridization can alter the silencing state of heterochromatin and thus lead to the activation of inactive genes. However, direct evidence for hybridization-induced transposition via demethylation of elements has not been provided.

How can stress induce the activity of a mobile element?

One hypothesis is that when stress is applied to an organism, this led to the release of transcription activators required for the induction of the host defence genes. The activation of the transposable elements could be due to the presence of fixation sites of transcription activators in their regulatory regions.

Epigenetic pathways are targets of programmed responses caused by stress. Relaxation of epigenetic imprints affects the genome resulting in an improper expression of sequences that should normally be silenced. If the cell survives the shock, its genome would have undergone epigenetic remodeling caused by DNA rearrangements and transposition. This induction represents a short term response. At the population level, the epigenetic and genetic arrangements that promote fitness will lead to an increase in the genetic variability retained through the action of selection (by somatic and germinal activity) in a long term response. Transposable element mediated genomic rearrangements contribute to biodiversity and evolutionary transitions.

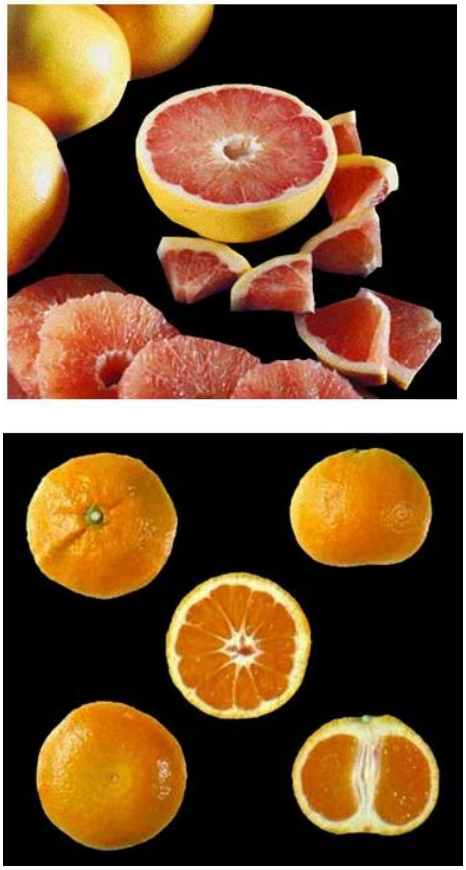

Fig. 2 *Citrus* cultivars from bud mutations. (**Top**) *Ruby Grapefruit.* An accidental discovery of red grapefruit growing on a pink grapefruit tree gave rise to the Texas Red Grapefruit Industry. In the late 1920s and early 1930s, redder bud mutations were found in numerous groves originated as sports – lower branches – growing out of 'Thompson' trees which a Texas nursery had purchased from Glen St. Mary Nursery and sold to growers in the Rio Grande Valley, and which were frozen back in 1929. All are seedless and otherwise similar to 'Thompson', but display a redder color. All the red varieties of fruit started being marketed under the name 'Ruby'. Under the name, 'Ruby Red', a member of this group and a standard commercial cultivar in Texas, was the first grapefruit to be granted a U.S. patent. (**Bottom**) *Clausellina Satsuma*, an example of a bud mutation from *Owari satsuma* selected in 1962, gave rise to a new early maturing, easy peel, seedless citrus variety.

Role of *Citrus* mobile elements in the adaptation to stressful environments

Retrotransposons are considered to be involved in citrus genetic instability and genome evolution, especially in sweet orange, where bud mutations are frequent.

Bud mutation is a main avenue for the selection of citrus cultivars, because citrus breeding has been hampered by the nucellar embryony, heterozygosity, and long juvenility of the buds (Mendel 1981). Most of the important cultivars currently available in the market have arisen through bud mutation and are propagated by grafting onto rootstocks. An accidental discovery of red grapefruit growing on a pink grapefruit tree gave rise to the 'Texas Red' grapefruit Industry. In the late 1920s and early 1930s, redder bud mutations were found in numerous groves. Each new finding was named after the grower who discovered the mutants. With several red grapefruit varieties and names being shipped commercially, keeping track of the new varieties soon became a marketing problem. All the red varieties of fruit started being marketed under the name 'Ruby' (Morton 1987). The 'Ruby' Red grapefruit was the first grapefruit to be granted a U.S. patent (**Fig. 2A**). Interestingly, one ISSR marker unique to 'Ruby' blood orange has been observed in progeny trees using inter-simple sequence repeat markers (Fang and Roose 1997). Among the *Citrus* species, sweet oranges develop spontaneous bud mutations more easily than other species do (Saunt 2000; Rodrigo *et al.* 2003). The most interesting feature of bud mutations of the citrus is parallel mutations (i.e. similar mutations occurring in different species and varieties). Both the pink grapefruit (*Citrus* × *paradisi*) and the 'Cara Cara' navel (*C. sinensis* Osbeck), a mutation of 'Washington navel' from Venezuela, exhibit a pink flesh color owing to the accumulation of lycopene and, therefore, there may be a similar mechanism involved in the color formation of these two species (Lee and Castle 2001; Xu and Deng 2002). Another example is a bud mutation from *Owari satsuma* was selected in 1962 and gave rise to a new early maturing, easy peel, seedless citrus (**Fig. 2B**) variety called *Clausellina Satsuma* (Reuther *et al.* 1967). However, the underlying mechanisms of bud mutation are intricate and it is still uncertain. Several possible hypotheses have been put forward, such as gene mutation, variations in the number and structure of chromosomes, DNA methylation and retrotransposons (Lewis and Bird 1991; Bretó *et al.* 2001).

An alternative to reveal the mechanism of bud mutation could be explained by the activity of retrotransposons.

A considerable number of transposon-like sequences and heterogeneity of reverse transcriptase sequences has been observed among *copia*-like retrotransposons in *Citrus*

are reported in NCBI database (www.ncbi.nlm.nih.gov); however, a few researches report on the functionality and transcriptional activity of mobile sequences in the *Citrus* genome.

Asins *et al*. (1999) investigated the presence of *copia*-like retrotransposons in citrus; they found that these elements were quite abundant throughout the citrus genome and very heterogeneous for the RT domain. Polymorphisms based on *copia*-like elements (RFLP and IRAP) have been found in groups of varieties within *Citrus sinensis* (Asins *et al.* 1999), *Citrus clementina* (Bretó *et al.* 2001) and *Citrus limon* (Bernet *et al.* 2003). A C-methylation phenomenon occurred in the RT sequence of navel orange, but not in that of 'Valencia', showing that retrotransposons participated in the evolution of sweet oranges (Asíns *et al.* 1999).

Gypsy-like retrotransposons sequences have been isolated in *Citrus clementina* that show homology to retroelements of the Ty3/*gypsy* group (Bernet 2003). Southern-hybridization analysis indicated that nested copies of these elements are scattered along *Citrus clementina* and *Poncirus trifoliata* genomes. IRAP markers (inter-retrotransposon amplified polymorphism) based on these *gypsy*-like sequences were also developed showing that they are less abundant than *copia*-like elements. Two of the eight clones isolated in this study showed high homology to elements within a resistance gene cluster in tomato. However, there was no data showing evidence of *gypsy*-like retroelements activation in *Citrus clementina* and *Poncirus trifoliata* genomes (Bernet 2003).

A complete retrotransposon has been identified in *Citrus sinensis* (named CIRE1), which has all the features of a typical *copia* retrotransposon (RTN) (Rico-Cabanas and Martínez-Izquierdo 2007). CIRE1 retrotransposon has around 2200 full-length copies, contributing to 2.9% of the *C. sinensis* genome. CIRE1 has root-specific expression in sweet orange plants, whereas CIRE1 transcripts were not detected in leaf tissue. It has also been determined that wounding and exogenous application of plant hormones, as methyl-jasmonate and auxin, increases the transcription level of CIRE1 in leaf tissues. In addition, CIRE1 5′LTR promoter can drive transient expression of the GUS (beta-glucuronidase) reporter gene (*uid*A) in heterologous plant systems. These findings confirm CIRE1 as one of the few transcriptionally active retrotransposons described in plants. Hence, CIRE1 LTR promoter functionality and transcriptional activity of CIRE1 in certain plant tissues and conditions suggest that CIRE1 may have the potential for increasing transposition and propagation in the sweet orange genome. It has not been reported if CIRE1 is also present in other *Citrus* genomes.

The heterogeneity, genomic distribution, and transcriptional activities *copia*-like retrotransposons, in 12 sweet orange (*Citrus sinensis* Osbeck) cultivars have been investigated using a PCR assay designed to detect partial *copia*-like RT sequences (Tao *et al.* 2005). Twelve amplification products of 300 bp from each cultivar were cloned and sequenced. The cloned sequences showed great heterogeneity, except in 'Dream' navel and 'Hamlin', both of which shared the same sequence. Frame shifting, termination, deletion, and substitution accounted for the heterogeneity of RT sequences. Southern blot hybridization using the RT1 clone from the 'Cara Cara' navel as a probe showed that multiple copies were integrated throughout the sweet orange genomes, which suggested that the retrotransposon is possibly an effective molecular marker for the detection of citrus evolution events and to reveal its relationship with bud mutation. The transcriptional activities of the *copia*-like retrotransposons in citrus have not yet been detected by RT-PCR and Northern analysis in the fruits and leaves of either 'Cara Cara' or 'Seike' navels. Ty1-*copia*-like retrotransposons are ubiquitous in the 12 sweet orange cultivars, as revealed by the results of the study (Tao *et al.* 2005). In combination with results of genomic hybridization, *copia*-like retrotransposons are presumably very involved in the evolution of sweet oranges, contribute considerably to the citrus genome, and may play a particular role in bud mutation. As described in previous studies, these RT sequences could be used as promising molecular markers to tag genes of interest and to reveal the genetic relationship between different genotypes.

A novel retrotransposon-like sequence was isolated and identified in *C. limon* as Ty1-*copia*-like reverse transcriptase domain, based on the homology to known elements (De Felice *et al*. 2009). CLCoy1 composes 3.6% of the genome, whereas transposons are mostly specific to a species, this element was identified in other *Citrus*, such as *Citrus sinensis* and *Fortunella margarita*, but undetected in *Poncirus trifoliata* (**Fig. 3**).

Moreover, it has also been determined that wounding, salt and cell cultured stress produced transcriptional activation of the novel retroelement in *C. limon.*

CLCoy1 expression in *C. limon* wounded leaves, as well as normal and salt stressed regenerated plants, was analyzed. CLCoy1 transcript was abundant in wounded leaves, in salt stressed regenerated plants and in regenerated plants from long-term cell cultures.

The novel Ty1-*copia*-like element CLCoy1 may have played a major role in shaping genome structure and size during evolution of the *Citrus* species. Southern hybridization analysis indicated that nested copies of the new elements are scattered along the *C. limon* genome and other related species such as *C. sinensis*, and *F. margarita*, but absent in *P. trifoliata*. The results suggest that this element is an ancient component of the *Citrus* genome genus, definitely introduced before the divergence of the species and conserved during evolution. This finding points toward an ancient origin of this element and leads to a common ancestral transposon emerging when the genus *Citrus* arose, separating from *P. trifoliata*. Therefore, it is possible that lineage-specific activity and evolution of transposable elements might be a source of biodiversity. This hypothesis is supported by previous reports showing that transposition events mediate genomic rearrangements are related to speciation events detected in different organisms; indeed transposons are increasingly used as markers for present phylogenic study (Ray *et al.* 2006). Several hypothetical models of speciation might be consistent with a role of transposition and transposable element-mediated rearrangements in speciation.

Moreover, the persistent localization of transposons in host genomes could be explained by the assumption that some DNA sequences present a suitable habitat for the persistence of insertions sequences.

Some transposable elements are selectively deleted from plant genomes, because some insertion regions show homogenization and an evolution rate higher than that of transposons (therefore inserted copies are lost) and/or insertion in some other regions can be lethal for the plant or cause sterility.

Other transposable elements are conserved in plant genome and are amplified by unequal crossing-over or via reverse transcription and re-insertion. Consequently, persistent localization of transposons in host genomes probably depends heavily on whether there are friendly conditions for the persistence of insertion sequences. It has been proposed that there are "ecological niches" which different types of transposons exploit (Kidwell and Lisch 2001). Stress activated transposons could give more variability to a plant, making it possible to inhabit stressful habitats, because many genes could be amplified or assembled through the action of transposable elements.

It is noteworthy that the Ty1-like RT did not show a close correspondence to the previously reported Ty1-*copia* retrotransposons in *Citrus* or to the major dispersed repeats in *Citrus* genomes, which suggests that a very complex population of such elements exists in these plants.

In conclusion, results of a novel *copia*-element in *Citrus* genome are interesting. The relative silence of the transposable element during normal development and the activation by stress is in keeping with the genome-restructuring

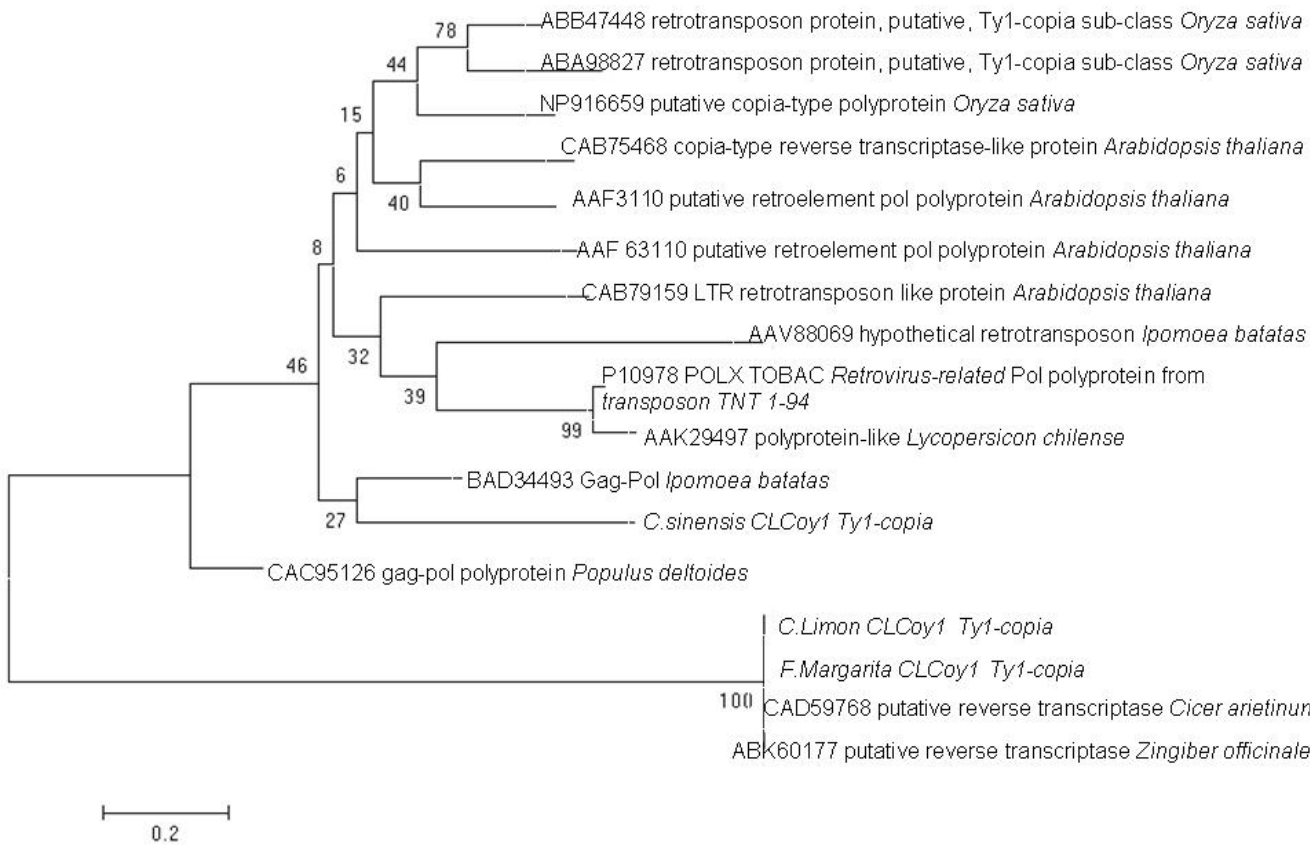

Fig. 3 Phylogenetic analysis based on the Reverse Transcriptase domain of *Citrus* sp. Ty1-*copia* like retroelements. Numbers given below the branches are frequencies (expressed as percentages) with which a branch appeared in 1000 bootstrap replicates. Branch lengths are proportional to nucleotide differences as indicated by numbers on branches. Consistency index = 0.878, retention index = 0.980, homeoplasy index = 0.122.

role predicted for transposable elements by McClintock. The finding of retrotransposon-derived sequences in the flanking regulatory regions of many normal plant genes (White *et al.* 1994) suggests that *copia*-like retrotransposons may have fulfilled this role in the past.

CONCLUSION

We can raise many new and intriguing questions regarding the interactions between transposons and *Citrus* genomes. The presence of a very complex population of transposable elements and tandem repeats in *Citrus* plants, the fact that transposons and retrotransposons are a possible target for epigenetic chromatin silencing and can be transcriptionally activated under stress, point to the need for better understanding of the regulatory roles transposable elements have in the development and disease response in *Citrus*. We could hypothesize that polymorphic retroelements have given more genetic variability to *Citrus* plants making possible the spread into stressful habitats.

Citrus is the most economically important fruit crop in the world, with million tons fruits produced for year. Cultivar improvement efforts have been disadvantaged by general characteristics of citrus biology, such as apomixis, sexual incompatibility or prolonged juvenility, that limit classical molecular biology approaches. To comprehend how genetic mobile elements can contribute to a key aspect of citrus biology will impact the advances in this economically important plant.

The Keystone Symposium "Transposition and other genome rearrangements", Santa Fe, USA, 8-14 February 2003 showed that progress has been made in all areas of transposon biology, with some stimulating advances.

We are looking for advances in this area to better understand citrus biology and develop a valuable approach in plant improvement programs.

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