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*DKRE*1—The first full-length Ty1-*copia*-like retrotransposon in persimmon: Isolation, characteristic and potential involvement in occurrence of bud mutations

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ABSTRACT

The focus of this study revolved around persimmon (*Diospyros kaki* Thunb.) retrotransposable element 1 (*DKRE*1), a complete long terminal repeat (LTR) retrotransposon (RTN) that was isolated from persimmons. Its structure, phylogenetic classification, copy number, active characteristics and potential roles involved in the occurrence of bud mutations in persimmons were investigated. Having a length of 6, 235 bp, *DKRE*1 possessed all of the sequence features associated with Ty1-*copia*-like retrotransposons. Phylogenetic analysis showed significant, but not strong, identity in amino acid sequences with *BARE*1 (barley retrotransposable element-1), *RIRE*1 (rice retrotransposable element-1) and *Ppcrt* (*Pyrus pyrifolia copia* retrotransposon) groups. The estimated copy number of 45 copies per haploid genome suggested that *DKRE*1 possesses a low constitution in the persimmon genome. Furthermore, the transcription of *DKRE*1 could be detected in leaf tissue treated with salicylic acid (SA), methyl jasmonate (MJA), and abscisic acid (ABA), indicating its active characteristics when subjected to exogenous phytohormones. Polymorphisms were revealed between several pairs of bud mutations by way of either inter-retrotransposon amplified polymorphism (IRAP) or sequence-specific amplified polymorphism (SSAP) retrotransposon-based molecular markers, showing a potential involvement in their occurrence. To our knowledge, this is the first report of the isolation of a complete LTR retrotransposon with transcriptional activity in persimmons. This study is valuable for future research on the mechanisms of bud mutation and development of retrotransposon-based molecular markers for genetic analysis.

1. Introduction

Retrotransposons are ubiquitous in the plant kingdom and form the major component of many plant genomes (Kumar and Bennetzen, 1999). Furthermore, they disperse as interspersed repetitive sequences throughout most of the length of all host chromosomes. Retrotransposons transpose via the reverse transcription of RNA intermediates into full-length DNA copies, followed by the integration of the DNA into new sites within the host genomes. Retrotransposons possess the properties of multi-families, high copy numbers, and high heterogeneity. Moreover, they are largely quiescent during development, but activity is regulated transcriptionally and transpositionally by various factors, and biotic and abiotic stresses can enhance transposition in natural populations (Kumar and Bennetzen, 1999; Salazar et al., 2007; He et al., 2012). Retrotransposons have played an important role in the genome evolution by changing gene expression patterns. They achieve this through insertion near or within the genes or by causing alterations in the transcript processing (Henikoff and Comai, 1998; Leprince et al., 2001; Kobayashi et al., 2004; Hernández-Pinzón et al., 2009; Butellí et al., 2012). In some cases, insertions of retrotransposons have been shown to alter the spatial and temporal patterns of gene expression or the structure of the resulting polypeptides. Retrotransposons can also affect gene expression epigenetic regulations. Consequently, retrotransposons act as agents of genome restructuring and mutagenesis by promoting a wide range of mutations, representing an important natural source of plant phenotypic diversity (Grandbastien et al., 2005). The high copy number and general dispersion of retrotransposon throughout the genome, as well as the large local change caused by their insertion provides

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an excellent basis for development of marker systems. Several retrotransposon-based markers have been developed and become popular recently, and of which, IRAP and SSAP have been the most affordable and widely used for various genetic analysis in plant (Alzohairy et al., 2014a).

Retrotransposons are divided into two sub-types, the long terminal repeat (LTR) and the non-LTR retrotransposon. LTR retrotransposons are bound by two long terminal repeats and contain an internal domain encoding Gag and Pol polyproteins. The LTR sequences do not encode any known protein, but they carry certain transcription regulatory elements such as promoters, terminators, hormone-responsive elements, and polyadenylation signals. In addition, they have evolved by gaining new expression patterns predominately associated with responses to diverse stress stimuli. The Pol protein has conserved domains characteristic of protease (PR), integrase (IN), reverse transcriptase (RT) and ribonucleaseH (RH). On the basis of sequence similarity and the arrangement of these internal domains in the *Pol* gene, LTR retrotransposons are divided into Ty1-*copia* and Ty3-*gypsy* groups (Doolittle et al., 1989). The integrase domain is found at the 5′position of the reverse transcriptase domain in the *copia* group, while it is located at the 3′ position to RT in case of *gypsy* group of retrotransposons. To date, Ty1-*copia*-like retrotransposons are a well-studied group.

Bud mutations, which may be a type of somatic mutation, are vital for breeding fruit crops. They appear in established trees, occurring when a new branch bears fruit or other characteristics – leaf size, shape, color, thornlessness – that are different from the remainder of the tree. The requirements for this event are a genetic mutation of the bud meristem cell which is passed on to all of its descendent cells which, in turn, produce the new branch. For bud mutation, it is sufficient to be selected and directly utilized in production or discrimination. However, at present, our knowledge of the genetic basis of bud mutations is poor and incomplete.

A variety of factors such as environment, stress, or exogenous hormones could give rise to bud mutations (D'Amato, 1997). These factors are supposed to be the inductors to active retrotransposons. As stated in the beginning of this paper, active retrotransposons would result in multiple changes in gene expression while reshaping the genome, both structurally and functionally. Thus, in theory, there would be a correlation between active retrotransposons and bud mutation; In practice, other sources also endorse transposon activity as a source of bud mutations (Yao et al., 2001; Kobayashi et al., 2004; Butellí et al., 2012), which further give us confidence that it would be possible to explore the causes of bud mutation occurrence using retrotransposon insertion in fruit crops. Furthermore, reviewing these literatures, we see that mutations frequently correlated with the insertion of a complete full length retrotransposon at a gene locus. So, in a plant species, it is necessary to isolate a full-length retrotransposon, which would be a prerequisite for the study of mutations.

Within the genus of *Diospyros*, persimmon is the most economically important species. In persimmon, many new cultivars arise from bud mutations; for instance, 'Matsumoto-wase' and 'Uenishi-wase' were both derived from the bud mutations that occurred in 'Fuyuu' and 'Matsumoto-wase', respectively, while 'Maekawa-jirou' was derived from 'Jirou' (Yonemori et al., 2000). Though these cultivars have been widely grown in the world, there is scant information and a lack of understanding concerning their mechanisms of bud mutations. Due to the crucial role retrotransposons seem to play in bud mutations, our research team proposes to carry out a series of studies with persimmons for the purpose of elucidating not only the molecular causes of bud mutations, but also the possible inducing factors. Our end goal is to provide guidance for the purposeful creation of bud mutations in the future.

Although previous documents have identified partial sequences of Ty1-*copia*-like retrotransposons in the persimmon genome (Nakatsuka et al., 2002; Du et al., 2009a) and have shown that they were both highly heterogeneous and high in copy number, there were no reports of complete Ty1-*copia* retrotransposons apparent in persimmons. By way of molecular markers in our past investigations, we have preliminarily demonstrated that retrotransposons in persimmons may directly contribute to bud mutations (Guo et al., 2006; Du et al., 2009a). In this study, we aimed to isolate and identify a full-length Ty1-*copia* LTR retrotransposon to the bud mutations in persimmons for the first time. In addition, we sought to analyze the gene structure, phylogenetic relationships, genomic abundance, and active characteristics by way of stress-associated signaling molecules. Simultaneous, we also explored the retrotransposon's involvement in the occurrence of bud mutations.

2. Materials and methods

2.1. Plant material and DNA extraction

A total of five cultivars, belonging to the two groups of bud mutations in persimmons, were used for the assay of both IRAP and SSAP retrotransposon-base molecular markers in this study. Among them, 'Matsumoto-wase' and 'Uenishi-wase' are the bud mutations of 'Fuyuu' and 'Matsumoto-wase', respectively, while all reside within the group of 'Fuyuu'. 'Maekawa-jirou' is the bud mutation of 'Jirou', which falls in the group of 'Jirou'. All of these three bud mutations are the early ripening mutations originating from their respective stand cultivars.

'Fuyuu' was used in experiments that included the isolation of a full-length Ty1-*copia* like retrotransposon, copy number determination, and active characteristics of external phytohormones assay.

Fresh young leaves of each accession were first cleaned with damp paper tissues, and were then immediately snap-frozen with liquid nitrogen and stored at −80 °C for further use. Total genomic DNAs were extracted from the leaves following the method of Doyle and Doyle (1987).

2.2. IRAP analysis

The IRAP procedure was essentially performed as described by Du et al. (2009b). The retrotransposon primers were designed based on RT sequences of the persimmon's Ty1-*copia* like retrotransposon from Nakatsuka et al. (2002) using the software Primer Premier 5.0 (Singh et al., 1998). At least two PCR amplifications were conducted for each sample to ensure the reproducibility of the produced bands. The amplification products were resolved on 2% agarose gel at a constant 8 V/cm for 5 to 6 h using a 0.5× TBE [tris-borate-ethylenediaminetetraacetic acid (EDTA), pH 8.3] buffer. They were then stained with ethidium bromide, photographed and analyzed by SYNGENE Automated Gel Documentation System (USA).

2.3. Chromosomal-walking strategy for the isolation of full-length persimmon Ty1-copia-like retrotransposon

Isolation of the complete persimmon Ty1-*copia*-like retrotransposons was based on RT fragments, which were selected as polymorphic families with possible involvement in the bud mutations in persimmons via the analysis of IRAP retrotransposon-based molecular markers.

The sequences of step GW1 and GW2 were obtained by referring to the principal protocol of the Universal Genome Walker™ Kit (Clontech, USA). Four independent pools of 'Fuyuu' persimmon DNA (800 ng) were digested in 50 μL with 30 U *Dra*I, *Ssp*I, *Eco*RV and *Hae*III restriction enzymes (NEB, USA) at 37 °C overnight. Digested DNA was then phenol purified, precipitated with ethanol, and dissolved in 20 μL of sterile water. Each pool of digested DNA fragments was ligated to an excess adaptor (8 μM) at 16 °C