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Test of the utility of apple retrotransposon insertion patterns for molecular identification of 'Jonathan' somatic mutants

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Summary: Up until today, apple sport mutants proved to be indistinguishable from each other and their progenitors at the molecular level using random amplified polymorphic DNA (RAPD), amplified fragment length polymorphism (AFLP) and simple sequence repeat (SSR) marker techniques. This is not surprising, since the genomes of these somatic mutants differ only in one or a few small regions that affect economically important characteristics, such as improved fruit colour, size, or flavour. In most cases, these genome differences are probably caused by retrotransposons which are able to convert their RNA transcripts to DNA with reverse transcriptase enzyme prior to reinsertion, but unable to leave the genome and infect other cells. Retrotransposon insertions can alter the expression of other genes and/or the structure of encoded proteins. The sequence-specific amplified polymorphism (S-SAP) technique is capable of revealing the genetic distribution of retrotransposable elements over the whole genome. The present study used this approach to try to characterize and distinguish 'Jonathan' somatic mutants via fingerprinting, which is an unsolved problem.

Key words: apple DNA fingerprinting, sequence-specific amplified polymorphism (S-SAP), bud mutation

Introduction

Apples are one of the most important fruits of the temperate zone with more than 60 million tons of annual production worldwide (http://faostat.fao.org/). Most of the apple varieties grown worldwide, such as 'Delicious', 'Jonathan', 'Gala', 'Idared', etc. are clones of bud mutants, called 'sports'. These mutations are identifiable phenotypically by fruit colour or shape, tree size or shape, branching habit, etc. However; since most sports are a single or few gene mutations, the progenitor and their mutants are identical at the genetic level, except for the mutation responsible for the difference. The knowledge of the genetic basis of bud mutations is poor. Presumably, in most cases the activities of retrotransposons are in the background.

Basically, retrotransposons can be divided into two different categories. The first group contains those elements which have flanking long terminal repeats (LTR's) and also non-LTR elements including LINEs and SINEs (long and short interspersed nuclear elements). Elements containing the flanking LTRs can be further subdivided into two major families, defined by the order of the reverse transcriptase and integrase domains in their *pol* genes. These are Ty1 or *copia*-like, and Ty3 or *gypsy*-like retrotransposons (*Friesen* et al. 2001).

Publications about transposon activity in apple are rare, but it was shown (Sunako et al. 1999, *Yao* et al. 2001) that they cause mutations in the genome. Kobayashi et al. (2004) proved in grapes that a retrotransposon-induced mutation in the *VvmybA1* gene is associated with the loss of pigmentation in white-berry cultivars.

Until now, only a few transposable elements have been described in apple. A putative SINE was identified in the promoter region of an ACC synthase gene (*Md-ACS1-2*; *Sunako* et al. 1999). It has great economical importance, because this insertion blocks the transcription of a gene that encodes the key enzyme in the ethylene biosynthesis in those cultivars which are homozygous for this allele, thus producing lower levels of ethylene and increasing the storage life of fruits. *Yao* et al. (2001) also published a complete retrotransposon from 'Granny Smith'. Recently, Ty1-copia-like retrotransposon sequences were isolated from the apple genome by chromosome walking technique (*Zhao* et al. 2007).

Several retrotransposon-based molecular marker systems have been developed in the last few years. The inter-retrotransposon amplified polymorphism (IRAP) technique (*Kalendar* et al. 1999) necessitates that the retrotransposons be close to each other in order to receive efficient amplification. This technique commonly uses LTR specific primers designed to the conserved regions within element families. The retrotransposon-microsatellite amplified polymorphism (REMAP; *Kalendar* et al. 1999) technique

uses one LTR specific primer, together with a simple sequence repeat (SSR) primer with one additional base at the 3' end. *Waugh* et al. (1997) developed the sequence-specific amplified polymorphism (S-SAP) technique to reveal the genetic distribution of the Bare–1-like retrotransposable elements in the barley genome. This technique proved to be applicable to a wide range of plant species such as *Aegilops* (*Nagy* et al. 2006), artichoke (*Acquadro* et al. 2006), barley (*Soleiman* et al. 2005, 2007), cashew (*Syed* et al. 2005), citrus (*Breto* et al. 2001), cucurbits (*Lou & Chen*, 2007), *Medicago* (*Porceddu* et al. 2002), sweetpotato (*Berenyi* et al. 2002), and also in apple (*Venturi* et al. 2006). Until now, only *Venturi* et al. were able to distinguish several clones of the apple cultivars 'Gala' and 'Braeburn' using this technique.

Jonathan clones are one of the most important polyclonal varieties in Hungary. New bud mutants are selected to get more intense and covered red fruit colour. The high similarity of the clones of bud mutants can cause problems in nursery propagation and varietals' discrimination. The present study is the first attempt to find reliable markers to characterise and distinguish 'Jonathan' mutants using S-SAP approach.

Materials and methods

Genomic DNA was extracted using a DNeasy® Plant Mini Kit (Qiagen) from young leaves of Jonathan, Jonathan M41, Szatmárcsekei Jonathan, Csány1 Jonathan, Watson Jonathan and Red Jonathan. Five different trees were sampled in all sport mutants. The total amount of DNA (500 ng) was digested with EcoRI and MseI (5 U of each) for 2 hours in the following buffer (10 mM TRIS-acetate pH 7.5, 10 mM magnesium acetate, 50 mM potassium acetate, 5 mM DTT, 5 ng/l BSA) (Vos et al. 1995) in a total volume of 20 µl. A template DNA for PCR was prepared by adding 20 µl of a ligation mix (50 pmol MseI and 50 pmol EcoRI adaptors, 2U T4 ligase and 1X ligase buffer) and the samples were incubated for overnight at 16° C.

'Ret-LTR' primers were planned (Ret-LTR1: 5' – AAA TGG AGT GAC AGA CGG GT – 3', Ret-LTR2: 5' – GGA GGG TTT TGA GGG ATG TG – 3', Ret-LTR3: 5' – CCT TCG GGA TGG GGT GTG TC – 3') for the long terminal repeat regions of the all available apple retrotransposon sequences until the start of this project (accession numbers: AJ291492, DQ898280, AM167520); these were labeled by Cy-5 and were used in S-SAP analyses with a combination of one AFLP primer.

At first, the template DNA (4 µl) was preamplified using primers homologous to the adaptor sequences (E00: 5'– GAC TGC GTA CCA ATT C – 3'; M00: 5' – GAT GAG TCC TGA GTA A – 3') in 20 µl reactions containing 15-15 ng E00 and M00 oligonucleotides, 0.2 mM dNTP's, 1.125 mM $MgCl_2$, 1X PCR buffer and 1.2 U Taq DNA polymerase (WesTeam). PCR on a Perkin Elmer 9600 instrument comprised 10 cycles of 94 °C (20 s), 6556 °C touchdown 1°C/cycle (30 s), 72 °C (1 min), and additional 25 cycles of 94 °C (20 s), 56 °C (30 s), 72 °C (1 min). PCR products were diluted to 20 X and were used (4 µl) for selective amplification where one labeled Ret-LTR primer was used in combination with an adaptor-specific primer included three additional selective nucleotides at the 3' end (MseI-48: 5' – ~ CAC – 3' or MseI-55: 5' – ~ CGA – 3' or MseI-60: 5' – ~ CTC – 3' or MseI-61: 5' – ~ CTG – 3' or EcoRI-33: 5' – ~AAG – 3' or EcoRI-36: 5' – ~ACC – 3' or EcoRI-37: 5' – ~ACG – 3' or EcoRI-44: 5' – ~ATC – 3').

Amplified fragments were visualized and evaluated in ALFexpress-II DNA analyzer (Amersham BioSciences), following standard protocols. The whole procedure can be seen in *Figure 1*.

Results and discussion

Altogether 24 primer combinations (3 Ret-LTR × 8 adaptor specific primers) were carried out; their PCR products were visualized and analyzed in the case of 'Jonathan' mutants. 10–70 multiple bands were produced (altogether 762 with the 24 primer combinations), suggesting that the copy number of these retrotransposon elements is relatively high in the apple genome (*Table 1*).

Surprisingly, only one combination (Eco-33 and Ret-LTR2) was appropriate for identifying a polymorphism between the progenitor 'Jonathan' and their bud mutants *(Fig. 2)*. In other cases, the mutants were indistinguishable from each other (see an example in *Fig. 3*), differences were not found even between different individuals within the same somatic mutants. Converting the found polymorphic band into a more reliable SCAR marker is under progress.

However, this one identifier is enough to differentiate the basic 'Jonathan' genotype from its sports. These alleles are

Table 1 Numbers of S-SAP bands produced by 24 primer combinations in 'Jonathan' mutants

Primer combination	Detected bands	Primer combination	Detected bands
Ret-LTR1 + MseI-48	21	Ret-LTR2 + EcoRI-33	35
Ret-LTR1 + MseI-55	16	Ret-LTR2 + EcoRI-36	38
Ret-LTR1 + MseI-60	30	Ret-LTR2 + EcoRI-37	45
Ret-LTR1 + MseI-61	27	Ret-LTR2 + EcoRI-44	46
Ret-LTR1 + EcoRI-33	36	Ret-LTR3 + MseI-48	32
Ret-LTR1 + EcoRI-36	24	Ret-LTR3 + MseI-55	17
Ret-LTR1 + EcoRI-37	32	Ret-LTR3 + MseI-60	31
Ret-LTR1 + EcoRI-44	27	Ret-LTR3 + MseI-61	39
Ret-LTR2 + MseI-48	19	Ret-LTR3 + EcoRI-33	71
Ret-LTR2 + MseI-55	13	Ret-LTR3 + EcoRI-36	43
Ret-LTR2 + MseI-60	23	Ret-LTR3 + EcoRI-37	33
Ret-LTR2 + MseI-61	24	Ret-LTR3 + EcoRI-44	40

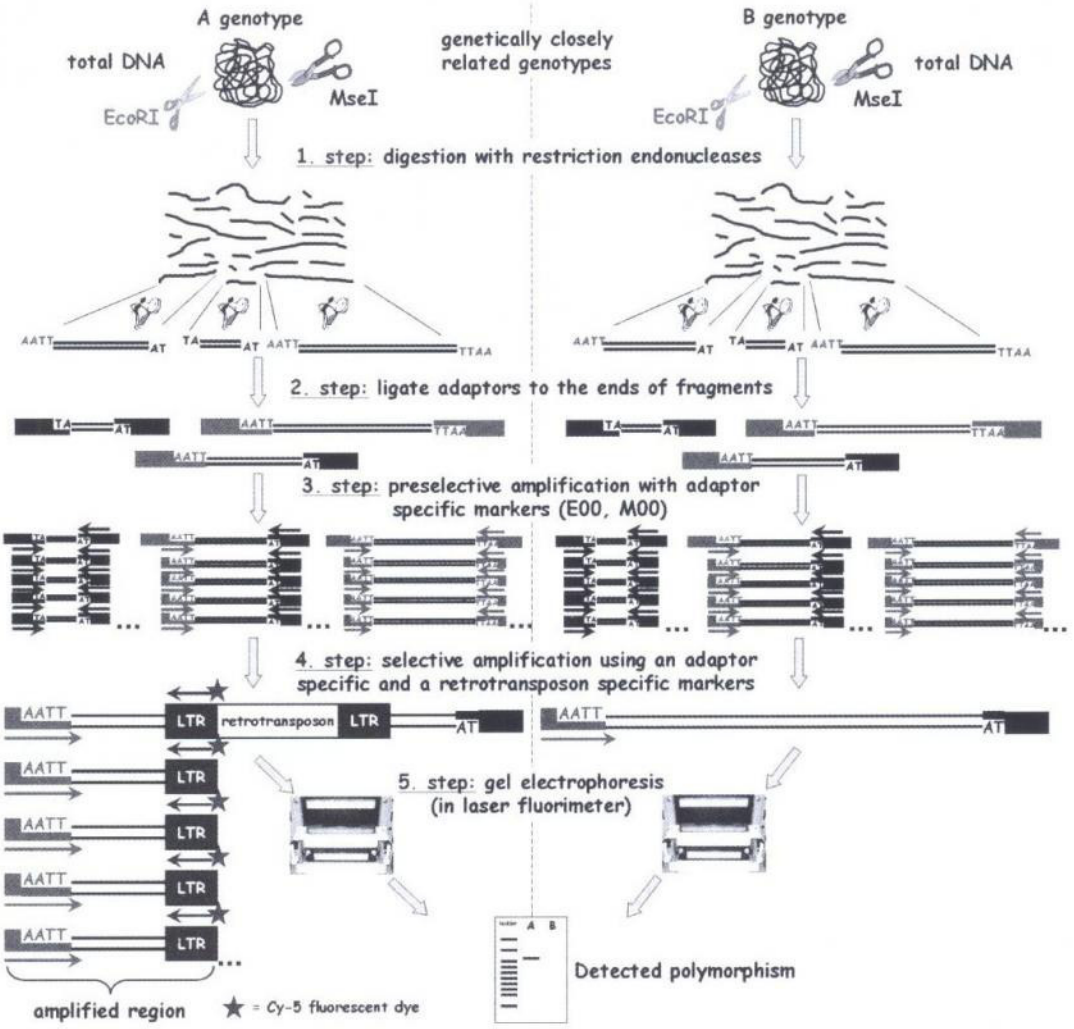

Figure 1 Schematic view of the sequence-specific amplified polymorphism (S-SAP) technique adapted to Automated Laser Fluorescence (ALF) DNA sequencer (Amersham Biosciences)

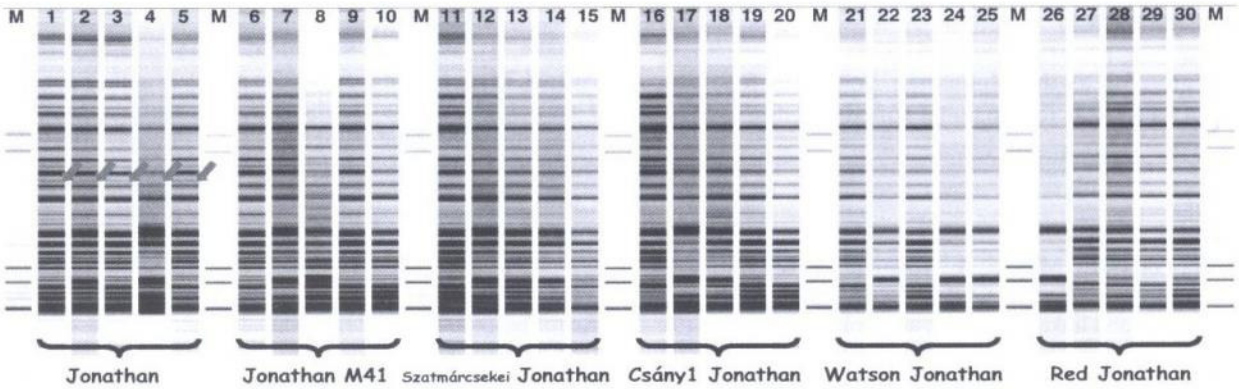

Figure 2 S-SAP patterns of 'Jonathan' and its five sport mutants using Eco-33 and Ret-LTR2 (Cy-5 labeled) primers in selective amplification. Arrows indicate a possible polymorphism. (M: molecular weight ladder)

weak, but, as can be seen, they are identical in all the 5 'Jonathan' samples and differ from the bud mutants. Unfortunately, this approach is not strong enough to differentiate the mutations from each other. Other aforementioned bud mutants are under examination to find alleles which are identical in the progenitor. This approach would presumably be quite useful in examining the genomes of other sports in order to find polymorphism in region where the combinations can identify the dissimilarities.

In literature, the S-SAP technique is – at present – the only PCR approach that can be used to discriminate different apple clones from their progenitors (*Venturi* et al., 2006). All other approaches (RFLP, RAPD, SSR, ISSR, AFLP etc.) failed to produce success. However – similarly to our results

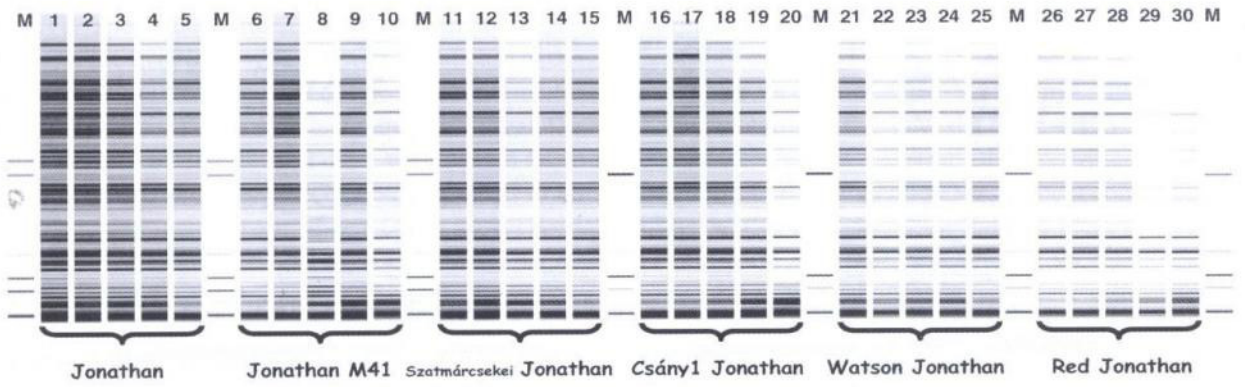

Figure 3 S-SAP patterns of 'Jonathan' and its five sport mutants using Eco-33 and Ret-LTR1 (Cy-5 labeled) primers in selective amplification. (M: molecular weight ladder)

– TRIM retrotransposons proved to be polymorphic between 12 standard apple cultivars, but not sports (*Antonius-Klemola* et al., 2006) in a recent study.

The lack of polymorphism is definitely due to the scarcity of published retrotransposon sequences in apple; or alternatively, an entirely different family of transposoble elements has been involved in mutations. It is also possible that the sport mutations in case of 'Jonathan' are SNPs (single nucleotide polymorphisms) or small insertions or deletions and thus not detected by S-SAP analysis.

To find out more information about bud mutations in species, the most used approaches are the retrotransposon origin researches. Although, in this work the investigated type of retrotransposons did distinguish bud mutations only in one case (with the Eco-33 and Ret-LTR2 combination), the approach could be useful even for that purpose for other apple bud mutants since they seem relatively high-copy number retrotransposons. The approach was not successful to differentiate the mutations from each other.

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References

Acquadro, A., Portis, E., Moglia, A., Magurno, F. & Lanteri, S. (2006): Retrotransposon-based S-SAP as a platform for the analysis of genetic variation and linkage in globe artichoke Genome. 49 (9): 1149–1159.

Antonius-Klemola, K., Kalendar, R. & Schulman, A. H. (2006): TRIM retrotransposons occur in apple and are polymorphic between varieties but not sports. Theor. Appl. Genet. 112: 999–1008.

Berenyi, M., Gichuki, S.T., Schmidt, J. & Burg, K. (2002): Ty1-copia retrotransposon-based S-SAP (sequence-specific amplified polymorphism) for genetic analysis of sweetpotato. Theor. Appl. Genet. 105 (6–7): 862–869.

Friesen, N., Brandes, A. & Heslop-Harrison, J.S. (2001): Diversity, origin and distribution of retrotransposons (*gypsy* and *copia*) in Conifers. Mol. Biol. Evol. 18: 1176–1188.

Kalendar, R., Grob, T., Regina, M.T., Suonimeni, A. & Schulman, A.H. (1999): IRAP and REMAP: two new retrotransposon-based DNA fingerprinting techniques. Theor. Appl. Genet. 98: 704–711.

Kobayashi, S., Goto-Yomomato, N. & Hirochika, H. (2004): Retrotransposon-induced mutations in grape skin color. Science. 304: 982.

Lou, Q.F. & Chen, J.F. (2007): Ty1-copia retrotransposon-based SSAP marker development and its potential in the genetic study of cucurbits. Genome. 50 (9): 802–810.

Nagy, E.D., Molnar, I., Schneider, A., Kovacs, G. & Molnar-Lang, M. (2006): Characterization of chromosome-specific S-SAP markers and their use in studying genetic diversity in *Aegilops* species. Genome. 49 (4): 289–296.

Porceddu, A., Albertini, E., Barcaccia, G., Marconi, G., Bertoli, F.B. & Veronesi, F. (2002): Development of S-SAP markers based on an LTR-like sequence from *Medicago sativa* L. Mol. Genet. Gen. 267 (1): 107–114.

Soleiman, V.D., Baum, B.R. & Johnson, D.A. (2005): Genetic diversity among barley cultivars assessed by sequence-specific amplification polymorphism. Theor. Appl. Genet. 110 (7): 1290–1300.

Soleiman, V.D., Baum, B.R. & Johnson, D.A. (2007): Analysis of genetic diversity in barley cultivars reveals incongruence between S-SAP, SNP and pedigree data. Genetic Res. and Crop Evol. 54 (1): 83–97.

Sunako, T., Wakako, S., Senda, M., Akada, S., Ishikawa, R., Niizeki, M. & Harada, T. (1999): An allele of the ripening-specific 1-aminocyclopropane-1-carboxylic acid synthase gene (ACS1) in apple fruit with a long storage life. Plant Physiol. 119: 1297–1303.

Syed, N.H., Sureshsundar, S., Wilkinson, M.J., Bhau, B.S., Cavalcanti, J.J.V. & Flavell, A.J. (2005): Ty1-copia retrotransposon-based SSAP marker development in cashew (*Anacardium occidentale* L.) Theor. Appl. Genet. 110 (7): 1195–1202.

Venturi, S., Dondini, L., Donini, P. & Sansavini, S. (2006): Retrotransposon characterisation and fingerprinting of apple clones by S-SAP markers. Theor. Appl. Genet. 112: 440–444.

Waugh, R., McLean, K., Flavell, A.J., Pearce, S.R., Kumar, A., Thomas, B.B. & Powell, W. (1997): Genetic distribution of *Bare-1-like* retrotransposable elements in the barley genome revealed by sequence-specific amplification polymorphism (S-SAP). Mol. Gen. Genet. 253: 687–694.

Yao, J.L., Dong, Y.H. & Morris, B. (2001): Parthenocarpic apple fruit production conferred by transposon insertion mutations in a MADS-box transcription factor. Proc. Natl. Acad. Sci. USA 98: 1306–1311.

Zhao, G., Zhang, Z., Sun, H., Li, H. & Dai, H. (2007): Isolation of *Ty1-copia-like* retrotransposon sequences from the apple genome by chromosome walking based on modified sitefinding-polymerase chain reaction. Acta Biochim. Biophys. Sin. 39 (9): 675–683.