RETROTRANSPOSON BASED MARKERS AND THEIR APPLICATIONS IN BARLEY (*Hordeum vulgare* L.cvs.) TISSUE CULTURE

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Abstract

Barley has economical value and it is an important model plant. Transposons cover more than 80% of barley genome. More than 40 retrotransposons were characterized in barley genome. This type of transposons replicate via RNA and move in the genome. As a result of these movements, mutations and genome enlargements are occurred. During the recent years, active transcripts and protein products of some retrotransposons have been determined. Somaclonal variations are spontaneously occurred variations in tissue culture conditions. These variations could be produced by genetic and/or epigenetic mechanisms and result in problems in gen transfer applications. We investigated the retrotransposon movements in barley tissue culture and regenerated plantlets using inter retrotransposon amplified polymorphism (IRAP), inter primer binding side (iPBS) and analytical techniques (DNA and RNA levels) and determined the relationship between retrotransposon movements, changes in copy number and differention in culture conditions. For these purposes *BARE1, NIKITA, BAGY2* and *SUKKULA* retrotransposons were analyzed. Our research results show that tissue culture conditions and time increase the transposon based variation and copy numbers of retrotransposons and thus, cause genome enlargements. This research will be contribute the understanding of basic mechanisms related to plant development and differentiation in cultured material and also restriction of variations in applications.

Keywords: Barley, Tissue Culture, Retrotransposon markers, Somaclonal variation

1. Introduction

Hordeum vulgare L. (barley) is an important cereal crop and is also an excellent model organism for biochemists, physiologists, geneticists and molecular biologists. In addition, barley provides a reference to the genomes of other *Triticeae* crops such as wheat, rye and some forage grasses. *H. vulgare* cvs. have been used as a model system for more than 40 years at the Istanbul University Molecular Biology and Genetics (former Biology) Department. The first studies on experimental mutagenesis were followed by tissue culture, gene transfers, DNA marker applications, DNA arrays finally epigenetic studies which, progressed further after the 1990 - 2005 when collaboration was established with the Plant Biotechnology group at the TUBITAK Research Institute for Genetic Engineering and Biotechnology in Gebze, Kocaeli-Turkey (Gozukirmizi, 2003). Since 2011, we focused on the roles of retrotransposons on tissue culture grown barley material since transposons cover more than 80% of the barley genome. More than 40 retrotransposons were characterized in barley genome (http://www.ncbi.nlm.nih.gov/). These types of transposons replicate via RNA and move in the genome. As a result of these movements, mutations and genome enlargements occur. Recently, active transcripts and protein products of some retrotransposons were determined. They use an RNA intermediate mechanism for transposition. Because of their copy–paste transposition, they cause genome expansion (Shirasu, Schulman, Lahaye, & Schulze-Lefert, 2000; Vitte & Panaud, 2005; Grzebelus, 2006). Considering their transposition mechanism and structure, they are thought to resemble retroviruses (Kalendar, Tanskanen, Immonen, Nevo, & Schulman, 2000; Sabot & Schulman, 2006; Sabot et al., 2006). Their new copies can insert themselves into near or within genes in a head-to-head, tail-to-tail or head-to-tail orientation.

Therefore, they can cause altered gene products, frame-shift mutations, reduction of transcription level or even silencing of genes (Fedoroff, 2000). Due to their dynamic feature, they are accepted as an important reason for genome evolution and speciation (Bento et al., 2008). Since retrotransposon insertions are irreversible, they are considered useful genetic elements in phylogenetic studies (Kumar, Gupta, Misra, Modi, & Pandey, 2009). Due to their variation capacity between species, retrotransposons are usually studied for detection of genetic relationships between varieties and related species (Waugh et al., 1997; Alavi-Kia, Mohammadi, Aharizad, & Moghaddam, 2008; Baumel, Ainouche, Kalendar, & Schulman, 2002; Saeidi, Rahiminejad, & Heslop-Harrison, 2008; Belyayev et al., 2010; Smykal et al., 2011;). Our group has mainly been working on *BARE1* (Evrensel, Yilmaz, Temel, & Gozukirmizi, 2011), *BAGY2* (Yilmaz, Marakli, & Gozukirmizi, 2014), *NIKITA* (Bayram, Yilmaz, Hamat-Mecbur, Kartal-Alacam, & Gozukirmizi, 2012) and *SUKKULA* (Kartal-Alacam, Yilmaz, Marakli, & Gozukirmizi, 2014, in press) retrotransposon insertion patterns in barley calli and regenerated shoots with retrotransposon-based marker techniques (IRAP and iPBS) to determine the effect of retrotransposon movements in somaclonal variations. This presentation outlines the results of retrotransposon research in barley tissue culture with the intention of contributing to barley-breeding programmes with recent biotechnological techniques.

2. Materials and Methods

2.1. Tissue Culture and Plantlet Regeneration

Mature embryos were excised from seeds after surface sterilization as described previously. Basal salts of Murashige and Skoog (MS) (Murashige & Skoog, 1962) were supplemented with 3% (w/v) sucrose, 1ml of MS vitamin mixture and 0.9% agar supplemented with 4 mg/L dicamba with a pH of 5.7. All cultures were kept in a growth chamber with standard conditions [25± ^{o}C, 16/8-h day/night photoperiod with fluorescent lights at 7000 lux] and was maintained on the same medium for different period of time. After different cultivation times, each callus was cut into three pieces and each piece was numbered with the starting embryo's number. One of the callus pieces was used for genomic DNA isolation, the second for shoot regeneration in MS medium supplemented with 0.5 mg/L zeatin, and the third was subcultured under the same callus culture conditions for aging. One of the pieces was used for shoot regeneration and the other for DNA isolation. At the end of the tissue culture, we obtained four experimental plant materials (calli with different aging times and their regenerated shoots) from one embryo; these were considered a single group. IRAP was performed with three different groups. Genomic DNA was isolated from those three groups and three control groups using Tri Reagent (Sigma T9424) according to the manufacturer's instructions. The control groups consisted of noncultured mature embryos.

2.2. IRAP

IRAP was performed with forward and reverse primers designed for LTR sequences of *BARE1* (Yilmaz & Gozukirmizi, 2013) and BAGY2 (Yilmaz et al., 2014) retrotransposon. Amplification was carried out in a 20 µL reaction volume containing 3.5 µL nuclease-free dH_2O, 0.5 µL dNTP mixture (10 mM), 2 µL of each primer (10 nmol/µL), 2 µL template genomic DNA (10 ng/µL), and 10 µL 2× Sapphire enzyme mix. PCR conditions were an initial denaturation step at 94°C for 3 min; followed by 30 cycles at 94°C for 20 s, 52°C for 20 s, and 72°C for 2 min; and a final extension step at 72°C for 10 min.

2.3. Evaluation of PCR Products

PCR products were loaded to 6% non-denature polyacrylamide gel (29:1 Acrylamide:Bis) and gel was run at 200 V for 4 h in 1X TBE buffer. A molecular weight marker (GeneRuler™ 1 kb DNA Ladder, SM0312, Fermentas) was also loaded to determine the size of amplicons. Gel was stained and photographed on a UV transilluminator. Well-resolved bands were scored with a binary value, (1) for presence and (0) for absence. The binary matrix (1/0) was used to calculate the similarity between embryo, 40 and 80 day-old calli. Jaccard's similarity index was calculated using the formula: $N_{AB} / (N_{AB} + N_B + N_A)$; where N_{AB} is the number of bands shared by 2 samples, N_A represents amplified fragments in sample A, and N_B represents amplified fragments in sample B (Jaccard, 1908).

3. Results and Discussion

Our results showed that calli which have different culturing time can have different IRAP band patterns although they originated from the same embryo (Figure 1).

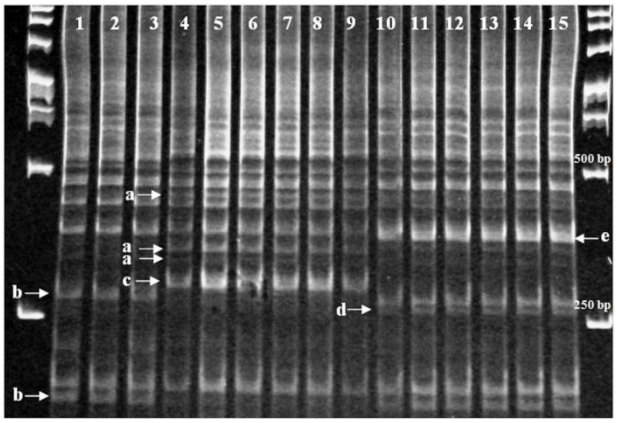

Figure 1. IRAP profiles for *BARE1*. 1-3; mature embryo (control), 4-9; first group [4, 5, 6; calli originated from the same embryo (30, 60, 90 day-old respectively), and 7, 8, 9; regenerated shoot originated from these calli respectively)], 10-15; second group [10, 11, 12; calli originated from another embryo (30, 60, 90 day-old respectively), and 13, 14, 15; regenerated shoot originated from the second group's calli)]. Arrows indicate the polymorphic bands (Yilmaz & Gozukirmizi, 2013).

We also performed studies on *NIKITA* and *SUKKULA* retrotransposons on aging calli materials (Bayram et al., 2012; Kartal-Alacam et al., 2014). We were able to observe polymorphisms in cultured materials. Finally, we studied *BAGY2* retrotransposon (Figure 2). We observed that *BARE1* and *BAGY2* are the most active retrotransposons (with polymorphism rates; up to 25% and 20% respectively) during callus culture (Evrensel et al., 2011, Yilmaz et al., 2014). We also observed *NIKITA* polymorphisms at different ages in old barley calli but the polymorphism rates were lower than *BARE1* and *BAGY2* (Bayram et al., 2012).

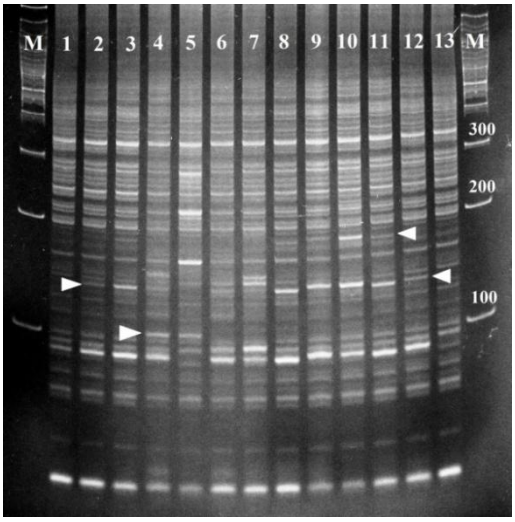

Figure 2. IRAP profiles of calli and shoots for *BAGY2*. 1, non-cultured embryo; 2-13, tissue culture materials (2, 6, 10 45-days-old calli; 3, 7, 11 shoots regenerated from 45-days-old calli; 4, 8, 12 90-days-old calli; 5, 9, 13 shoots regenerated from 90-days-old calli) (Yilmaz et al., 2014).

In addition to retrotransposon-based marker techniques, we also analyzed the copy number alterations of *BAGY2* internal domains (*GAG*, *PR*, *RT*, *RH*, *INT*) by real-time PCR (qPCR). qPCR results proved that all internal domains have copy number variations between different aged calli (Yilmaz et al., 2014). These findings show that tissue culture conditions and culturing time cause insertional activation of some barley retrotransposons.

These findings may prove that tissue culture conditions and duration of cultivation period do not cause the same effect on calli. This research will contribute to the understanding of scientific mechanisms related to plant development and differentiation and also restriction of variations in applications (Evrensel et al., 2011; Bayram et al., 2012; Yilmaz & Gozukirmizi, 2013; Yilmaz et al., 2014; Kartal-Alacam et al., 2014).

Hirochika (1993) published one of the pioneer studies showing transposon activity changes in tobacco protoplast culture. Afterward many studies were published for tissue culture effect on transposon activations. Liu et al. (2004) demonstrated that *Tos17* retrotransposon has been activated during rice tissue culture. Somaclonal variations were also studied at banana by IRAP technique (Muhammad & Othman 2005). Retrotransposon-derived polymorphisms were also reported at tissue culture of a wild barley species (*Hordeum brevisubulatum*) by various marker systems (Li et al., 2007). Campbell et al. (2011) also showed that *BARE1* retrotransposon was activated in barley tissue culture.

We need more detailed studies on transposons, and their effects on epigenetic and genetic mechanisms. Our data will be helpful for the understanding of their behavior during tissue culture. We briefly conclude that barley retrotransposons, both autonomous and non-autonomous, are very active during tissue culture procedure and we still do not have an opinion if these movements are randomly or partly directed according to the cultural development of the plants.

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