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Study of somaclonal variation in two rice cultivars regenerated from embryo culture, using SSR markers

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Abstract

Experiments were carried out to induce somaclonal variation in two different Iranian rice cultivars, Neda and Tarom-Jelodar. In order to induce callus, mature embryos were cultured in MS medium as explant, then the obtained calli were transferred to plants regeneration. The leaf samples were provided from regenerated plantlets of each cultivar and both of maternal as a witness, therefore DNA was extracted. Analysis of microsatellite loci was accomplished by extracted DNA from regenerated rice somaclons. In these samples Somaclonal variation were evaluated by means of Polymerase Chain Reaction with ten SSR Primers. Regardless of cultivars, 36.66% of formed bands using these primers were polymorphic, and similarity coefficient ranges, were 0.52 to 1.00.

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Introduction

Rice has a special place in molecular biology researches and turned into one of the model plants due to its small size of genome (Gao 2009). Tissue culture provides opportunity to show the extent range of genetic variety in plants that it can be used in plant breeding programs. In the past, tissue culture cycle was offered as a method for cloning a specific genotype and today this is a common method for propagating plants with commercial importance (Larkin et al, 1981, Rasheed 2005). In the past it was expected that all of regenerated plants from cell or tissue culture process, have the same genetic structure with original mother plant, therefore, it was accepted as a rule that plantlet derived from tissue culture should be exactly resembled to parental plant (Larkin 1981). But phenotypic variety was observed with high frequency among regenerated plant and recent finding showed such an opinion has been incorrect (Rasheed *et al.,* 2005). Larkin and Scowcroft (1981) suggested somaclonal common word for plants derived from any type of tissue culture and somaclonal variation as a genetic and phenotypic variety observed among the plants which were propagated as a colony of a single colon and stated that somaclonal variation is a novel source to improve the plants. Various references have introduced somaclonal variation as a novel and useful source in plant breeding (Kang-le 1989, Zong-xiu 1983). Various types of somaclonal mutations including point mutations, gene duplication, chromosome rearrangement, chromosome number variations have been reported. These changes might be naturally accumulated in somatic cells of plant and tissue culture providing opportunity for these mutations so that they emerge in plants derived from tissue culture (Larkin 2004). The mechanism of somaclonal variation includes extensive genetic drift, and epigenetic factors are also involved in complicating it. Somaclonal variation depends on plant growth regulators, variability of variety, age of variety in culture, ploidy level, explants source, genotype and other culture conditions (Karp 1995, Rasheed *et al*, 2005). The presence of some chemical material such as 2, 4-D also causes to increase this variation ratio (Rasheed *et al*, 2005). The experiments performed with numerous regenerated plants derived from protoplasts separated from a single leaf, have demonstrated that somaclonal variation occurs in some cases during the culture cycle (Larkin 2004). Somatic mutation often doesn't transfer to the next generation and the primary regenerated plants become final product while the stable gametic variation (Meiotic) is transferred to progeny. So, assaying varieties from tissue culture by using molecular markers to utilize this variety in plant breeding are more important (Rasheed *et al*, 2005). The successful use of somaclonal variation much more depends on its genetic stability, in the next generations (Mohan 2001, Rasheed *et al*, 2005). Larkin and Scowcroft (1981) have also mentioned that the importance of somaclonal variation in plants breeding, depends on the stability of genetic basis for this variation. The SSR markers are highly informative, mostly mono locus, co-dominant, easily analysed and cost effective therefore among various PCR based markers, the SSR markers are more popular in rice.

Simple sequence repeats (SSR) are abundant and well distributed throughout the rice genome. One of the most important attributes of microsatellite loci is their high level of allelic diversity, making them valuable as genetic markers. The unique sequences bordering the SSR motifs provide templates for specific primers to amplify the SSR alleles via the polymerase chain reaction (McChouch *et al*, 1977).

Khai and Lang (2005) used microsatellites to identifying allelic diversity in Somaclonal mutants. They were used 45 Somaclonal mutant lines in M_2 generation and expressed of the 16 locus have polymorphism through reviewed 20 SSR loci. Between 20 SSR primers, 16 of them showed polymorphism which demonstrated that the changing of molecular construction in the population have high rate.

Seetharam *et al*, (2009) in own study were characterized 30 rice genotypes comprising land

races, pure lines, somaclones, breeding lines and varieties specifically adapted to costal saline environments. They were used SSR markers and morphological characters. Out of 35 primers of SSR markers, 28 were found to be polymorphic.

Gao *et al*, (2009) said that Somaclonal variation can be heritate, and thus provides an opportunity for plant breedings and geneticists to generate novel variants. In order to analyze 8 somaclonal mutants which derived from 7 different cultivars, they used 120 SSR markers that distributed across all 12 chromosomes of rice. They have suggested that some SSR markers in the rice genome might detect further numbers of polymorphisms than others.

The selected varieties in this study were improved varieties that have been derived from qualitative Sang-Tarom variety. These varieties have higher harvest than the parental variety but are in low quality. So creation of diversity at them, and their renewed cross by Sang-Tarom variety presumably can increase their quality. Present study, aiming of induction and analysis of somaclonal variation in Tarom-Jelodar and Neda varieties has been done through the process of tissue culture and also the application of SSR markers in identifying this diversity.

Materials and methods

Preparation of explant

The seeds of Tarom-Jelodar and Neda varieties were provided with biotechnology laboratory from Sari Agricultural Sciences and Natural Resources University (SANRU) and numbers of healthy seeds were selected. After sterilization process, seeds were cultivated on callus induction medium including 2, 4-D hormone and maintained in incubator at 26±1 ℃. The formed calli were transferred to regeneration media and incubated in germinator at 25±1 ℃ and 3000 $^{lux}/_{m^2}$ (Bagheri *et al*, 2004). 4 weeks after incubating of calli on regeneration media, plantlets were obtained through somatic embryogenesis. The obtained plants were first transferred to pot and then to farm to adapt with farm conditions. Leaf sample was taken from regenerated plants of each variety and maternal plant as control, and transferred to lab with compliance requirements. 13 plants from regenerated plantlet of Tarom-Jelodar variety, and 4 plants from regenerated plantlet of Neda variety seeds were produced in the field. Thus due to the presence of their seed storage, rest of the experiments was only performed with these samples.

DNA extracting and PCR

Leaf samples were powdered by liquid nitrogen and DNA extraction was performed by CTAB method (Shamsun *et al*, 2007). The quality and quantity of extracted DNA were assayed and adjusted by using electrophoresis of gel and spectrophotometry. 10 primers with were used for performing polymerase chain reactions (Table 1). In this study, after surveying the references, the primers were selected and taken from Sinagene Co. The reaction mixture contained of 10 ng genomic DNA, 0.5 µl of each forward and reverse primers with concentration of 10 pmol, 0.5 µl of dNTPs with concentration of 10 mM, 2.0 of $MgCl_2$ with concentration of 50.0 mM, 2.5 µmol of PCR buffer 10X and 0.2 unit Taq polymerase, which was adjusted with sterile distill water in 25.0 µl volume. Amplification reactions were performed by BIO-RAD termocycler according to method of Gao *at el*, 2009. Products of PCR were electrophoresised on agarose gel (provided with TBE buffer) at 60 V and 90 min, and then were stained in ethidum bromide solution and were photographed by means of Geldoc set by using UV ray. After genomic fingerprinting using SSR, grouping of samples were performed in comparison to DNA fragments bonds that produced on gel, scoring based on present or absent the same fragments and determining the Jaccard similarity coefficient. Dendrograms were drawn with the Unweight Pair Group Method with Arithmetic Mean and by means of NTSYS 2.02 software.

Results and discussion

Gel analysis

Four markers out of ten that used, unable in revealed of polymorphism among regenerated population but RM302 able to distinguish between individuals of

each population (Fig. 1 and 2). Also 36.66% of formed bands using by SSR primers were polymorphic. The sizes of produced bonds by these primers were from 100 bp to 250 bp. In this study, for each line in each locus almost 2 allels were generated (Fig. 3 and 4). From 10 SSR primers used in both of varieties, just 5 primers have detected polymorphism. In Neda variety the similarity coefficient, were vary between 0.52 to 1.00. Somaclons from Neda in 87% of similarity coefficient were divided into 3 separate groups which N_1 and N_3 lines were laid in the same group along with maternal parent. Each N_2 and N_4 lines formed other group. So N_4 line would has most difference with maternal plant (Fig. 5).

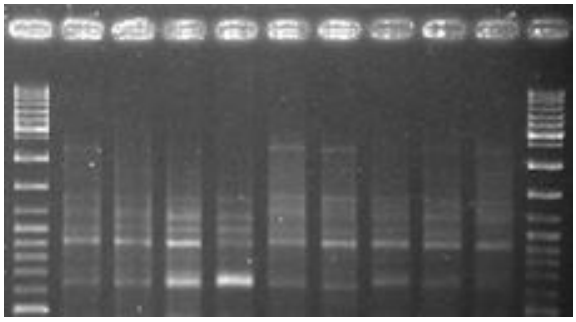

Fig. 1. PCR-amplified products from Neda variety using RM302.

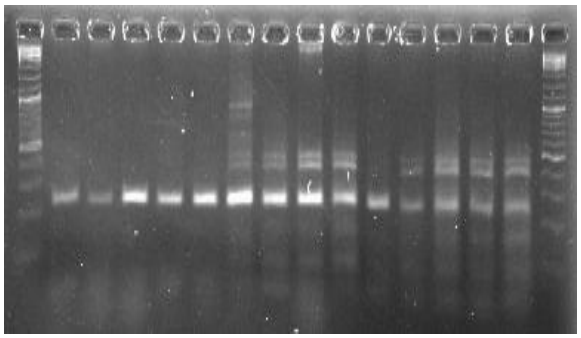

Fig. 2. PCR-amplified products from Tarom-Jelodar variety using RM302.

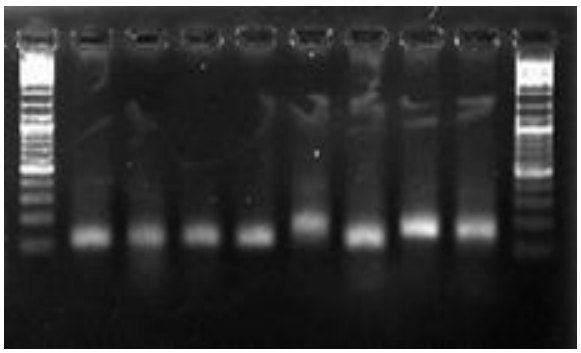

Fig. 3. PCR-amplified products from Neda variety using RM17.

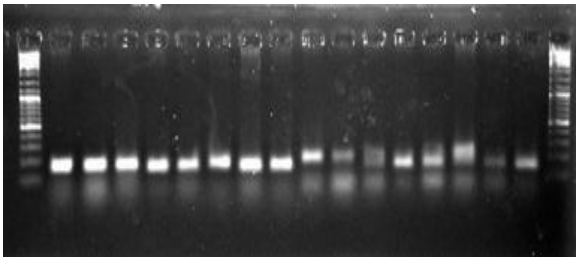

Fig. 4. PCR-amplified products from Tarom-Jelodar variety using RM 257.

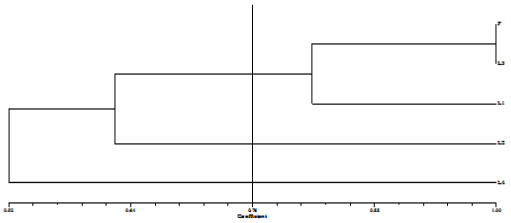

Fig. 5. Dendrogram of 4 somaclon mutants and donor plant in Neda variety based on SSR markers.
P: parental plant, L1 to L4: somaclone lines.

Also somaclons derived from Tarom-Jelodar, in 84% similarity coefficient were devided into 7 different groups that 5 somaclon lines along with mother plant located in one group. T_{10} line lonely laid in 7^{th} group that has most distance with first group which carrier mother plant (Fig. 6).

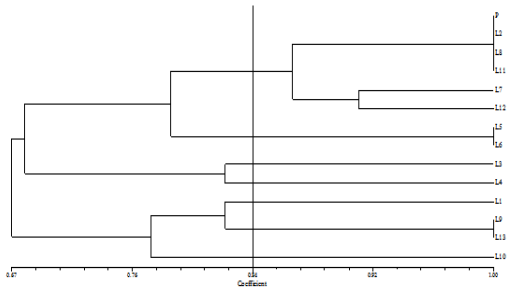

Fig. 6. Dendrogram of 13 Somaclon mutants and donor plant in Tarom-Jelodar variety based on SSR markers.
P: parental plant, L1 to L13 somaclone lines.

Gao *et al*, (2009) after investigation of DNA alterations in somaclonal mutants suggest that some SSRs detecting more polymorphisms than other markers. They also offer that polymorphic SSRs between mutants and donor cultivars might be located in gene coding regions. Simple sequence repeats (SSRs), or microsatellites are tandem repeated arrays of short (1-6 bp) sequences of DNA that occur both in coding and non-coding regions

(Temnykh *et al*, 2001). Salehian *et al*, (2014) said that tissue culture can be caused to produce of Somaclonal variation in rice and also RAPD markers are able to distinction of these variations.

Table 1. Used SSR primers and their sequences.

SSR Primers			
Name	Repeat Motif	Forward Sequence	Reverse Sequence
RM17	$(GA)_{21}$	TGCCCTGTTATTTTCTTCTCTC	GGTGATCCTTTCCCATTTCA
RM19	$(ATC)_{10}$	CAAAAACAGAGCAGATGAC	CTCAAGATGGACGCCAAGA
RM104	$(GA)_{9}$	GGAAGAGGAGAGAAAGATGTGTGTCG	TCAACAGACACACCGCCACCGC
RM110	$(GA)_{15}$	TCGAAGCCATCCACCAACGAAG	TCCGTACGCCGACGAGGTCGAG
RM257	$(CT)_{24}$	CAGTTCCGAGCAAGAGTACTC	GGATCGGACGTGGCATATG
RM274	$(GA)_{7\text{-}15}(CGC)_{5}$	CCTCGCTTATGAGAGCTTCG	CTTCTCCATCACTCCCCATGG
RM286	$(GA)_{16}$	GGCTTCATCTTTGGCGAC	CCGGATTCACGAGATAAACTC
RM302	$(GT)_{30}(AT)_{8}$	TCATGTCATCTACCATCACAC	ATGGAGAAGATGGAATACTTGC
RM304	$(GT)_{2}(AT)_{10}(GT)_{33}$	TCAAACCGGCACATATAAGAC	GATAGGGAGCTGAAGGAGATG
RM335	$(CTT)_{25}$	GTACACACCCACATAGAGAAG	GCTCTATGCGAGTATCCATGG

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