DETECTION OF SOMACLONAL VARIATION IN POTATO CALLUS INDUCED BY UV-C RADIATION USING RAPD-PCR

A. A. Ehsanpour[1]*, S. Madani[2], M. Hoseini

[1]Department of Biology, Faculty of Science, Isfahan University, Isfahan, Iran
[2]Dept. of Health Science, Medical Science University of Isfahan, Isfahan, Iran

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Summary. In this study, calli were obtained from *in vitro* grown potato stem or leaf segments cv. *Cosima* on MS medium containing 2,4-D, NAA, kinetin and yeast extract. After three subcultures, well grown calli were exposed to UV-C radiation with approximately 6.24 μmol photon/m^2/s for 30 min three times at an interval of 2 weeks. Somaclonal variation induced by UV-C radiation was revealed by 5 out of 28 random primers and also ISSR4 primer after PCR amplification of DNA. UV-C radiation changed DNA patterns as a source of genetic variation. However, somaclonal variation can be used for selection of potato calli toward desirable traits, such as salt or drought stress.

Key words: potato, somaclonal variation, RAPD-PCR.
Abbrevations: NAA - α-naphthaleneacetic acid; CTAB - cetyltrimethylammoniumbromide; 2,4-D - 2,4-dichlorophenoxyacetic acid; BAP - 6-benzylamino purine; MS - Murashige and Skoog.

*Corresponding author, e-mail: ehsanpour@yahoo.com

INTRODUCTION

Cultivated potato (*Solanum tuberosum* L.) is one of the most important vegetable crops in the world (Solmon-Blackburn and Baker, 2001). Potato is a tetraploid species and using its botanical seeds on commercial cultivation is precluded by low germinability and large variability in the segment generations (Bordallo et al., 2004). Somatic embryogenesis and plant regeneration from callus may solve this problem. Somaclonal variation, a common phenomenon in plant cell cultures includes all types of variation among plants or cells and derives from all kinds of tissue cultures (Skirvin et al., 1993). It is also called tissue or culture-induced variation (Kaeppler et al., 2000). Somaclonal variation describes epigenetic and genetic changes in plants that become apparent either during or after *in vitro* culture of plant cells, callus or organs. During culture, changes of interest to plant breeders may be heritable and result from changes in the plastid or nuclear genome. The introduction of variation may also be either problematic or useful for horticulturists and plant breeders, and may occur in high frequency during adventitious plant regeneration or long-term callus culture (Kaeppler et al., 2000). The progress made in DNA marker technology has been tremendous and exciting. DNA marker has provided valuable tools in various analyses ranging from phylogenetic to the positional cloning of genes. Methods for detection of somaclonal variation have been explored for many years (Noval, 1980). Scoring changes in plant morphology can be useful in some studies, but there is limited diversity and trait may be affected by environmental influences. Cytological assessment is not often used and can be difficult in many species like *Proteaceae* where chromosomes are difficult to observe. Analyses of secondary metabolites and isozyme patterns have also been used, but they are limited in their sensitivity (Morell et al., 1995).

Molecular techniques such as Restriction Fragment Length Polymorphysim (RFLP) or Random Amplified Polymorphic DNA (RAPD) are often favored over traditional phenotypic or cytological measurements, and generally assess even small variations of the genome. The use of the PCR-based RAPD technique to detect somaclonal variation has been applied successfully to several monocotyledonous species, such as *Lolium* (Wang et al., 1993) and *Allium sativum* L (Al-Zahim et al., 1999) as well as

woody dicotyledonous species, such as *Picea abies* (Heinze and Schmidt, 1995). It has also been applied for tomato (Soniya et al., 2001) and potato (Bannaceur et al., 1991).

UV radiation can change many aspects of plant processes at the physiological and DNA levels (Danon and Gallois, 1998). UV radiation is divided into UV-C (below 280 nm), UV-B (280-320 nm) and UV-A (320-390 nm). UV-C radiation has often been used to study various physiologically relevant responses to DNA damages, and in particular it has been shown to induce apoptosis in animal cells. UV-C radiation has also been used to induce apoptosis-like changes in *Arabidopsis thaliana* (Danon and Gallois, 1998).

In the present study, we report for first time the induction of somaclonal variation caused by UV-C radiation in potato callus cultivar Cosima and its detection using RAPD-PCR. This variation can be used as a valuable tool for selection of callus for desirable traits in the future.

MATERIALS AND METHOD

Callus production and radiation

Segments (approx. 8-10 mm) of leaves and stems from *in vitro* grown plants on MS (Murashige and Skoog, 1962) medium were transferred on callus production medium (MS containing 2,4-D, NAA, kinetin 2mg/l each, yeast extract 1g/l). All cultures were kept in a culture room at a 16/8 light/ dark photoperiod and 25 °C. After 4-6 weeks calli were initiated and then 3 segments of calli of approximately 8-10 mm^2 in each container were sub-cultured on the same medium at an interval of 2 weeks. Four containers having 3 segments of callus were then exposed to UV-C radiation (254 nm) with approximately 6.24 μmol photon/m^2/s intensity for 30 min. The radiation treatment was repeated three times at an interval of 2 weeks. All experiments were carried out in 4 replications under a completely randomized design (CRD). After each radiation calli were then kept in the dark under the same conditions in the culture room. Unradiated calli were subcultured under the same conditions as a control. Calli were used for RAPD-PCR amplification and analysis after the 3rd subculture.

PCR analysis

For DNA extraction, approximately 1 g fully grown calli was grounded using a hand held grinder with liquid nitrogen and then extracted using cetyl trimethyl ammonium bromide (CTAB) buffer as recommended by Ehsanpour and Twell (2005). RAPD-PCR amplification was carried out using 28 randomly selected primers. PCR reactions were carried out in a total volume of 20 µl at a final concentration of 1 mM $MgCl_2$, 2 mM dNTP, *Taq* DNA polymerase enzyme (1u/20 µ), with approximately 200 ng DNA as a template and a single random primer (0.2 mM). Conditions were 94 °C for 2 min, one cycle, 94 °C for 15 sec, 35°C for 15 sec, 72 °C for 30 sec which were repeated in 40 cycles followed by 5 min-extension at 72 °C. Then 8 µl of each PCR product was revealed on 1% agarose gel subjected to electrophoresis at 80 V after staining using ethidium bromide by UV light.

Random primer sequence:

In this work 28 random primers and four ISSR primers (ISSR1, 2 3 and 4) were used. Only the following primers were able to detect variation occurred in the callus:

OPAA-03 (5’ ttagcgcccc 3’)
FPK1-05 (5’ acttggcggcct 3’)
FPK2-19 (5’ ggacggcgtt 3’)
OPAA-20 (5’ ttgccttcgg 3’)
OPAA-18 (5’ aggtgaccgt 3’)
ISSR4 (5’ gatagatagatagata 3’)

RESULTS

Evaluation of somaclonal variation by RAPD-PCR showed that at least 5 out of 28 primers could reveal some polymorphism in the amplified DNA pattern caused by UV-C radiation. The patterns of DNA amplification using different primers are shown in Figures 1-5.

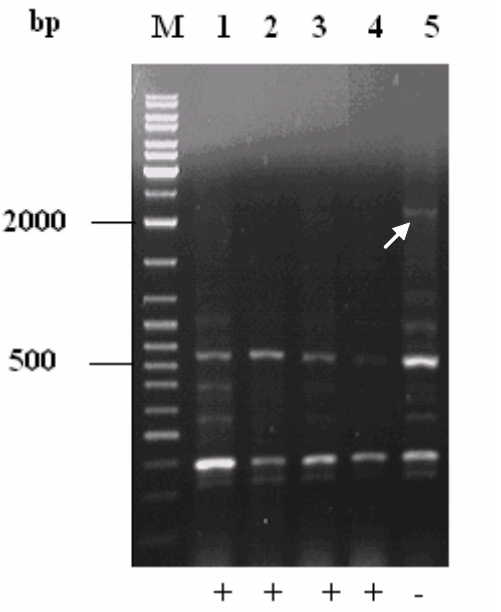

Fig. 1. DNA pattern after amplification using OPAA-03 primer. (+) UV-C radiation and (-) - no radiation (control). The arrow indicates a band present only in the unradiated calli.

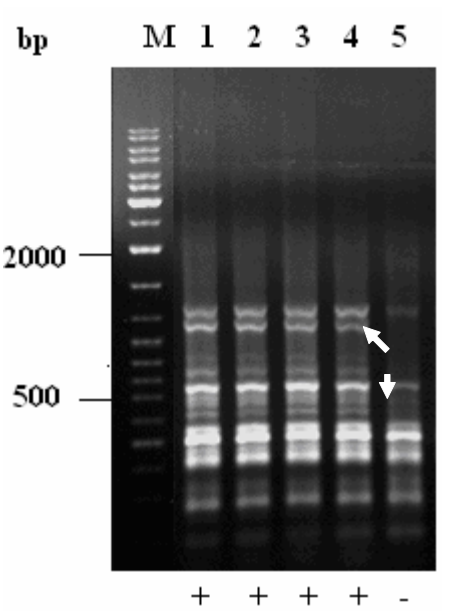

Fig. 2. DNA pattern after amplification using FPK1-05 primer. (+) UV-C radiation and (-) no radiation (control). The two arrows indicate a band present only in the radiated calli and a band missing in the control calli, respectively.

DISCUSSION

During callus culture variation occurred as a result of UV-C radiation in the cells. Variation in calli somatic cells could be classified into 3 groups: genome mutation, chromosome mutation and plasmon mutation. Callus somaclonal variation can be the result from 1) the occurrence of mutation during the tissue culture process, and in particular during plant regeneration from callus; 2) induction by mutagens like γ-ray, UV-B, UV-C and x-ray, or chemical mutagens. PCR-based polymorphisms can be random or specific depending on the type of the primer used. Inter-simple sequence repeat (ISSR) amplification polymorphism is also a powerful

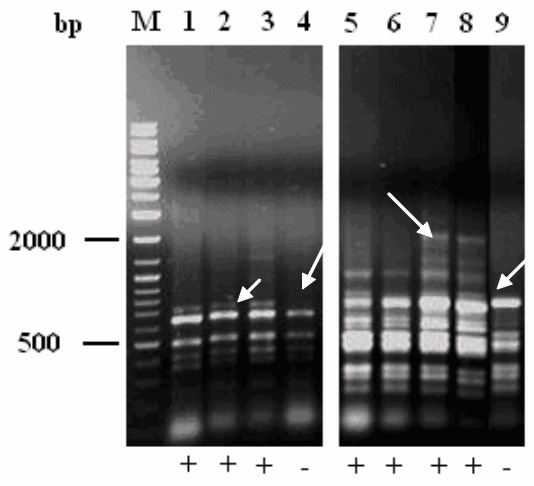

Fig. 3. DNA pattern after amplification using 1-4 OPAA-20 (only 3 replications were used), and 6-9 FPK2-19 primer. (+) UV-C radiation and (-) no radiation (control). Arrows indicate bands observed in the radiated calli and missing in the control calli.

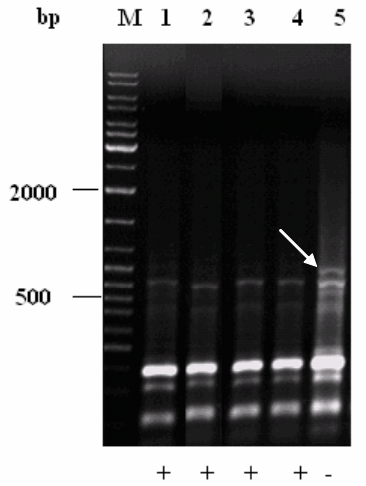

Fig. 4. DNA pattern after amplification using 1-6 OPA-18 (1-5) primer. (+) UV-C radiation and (-) no radiation (control). The arrow indicates a band in the unradiated calli which is missing in the radiated calli.

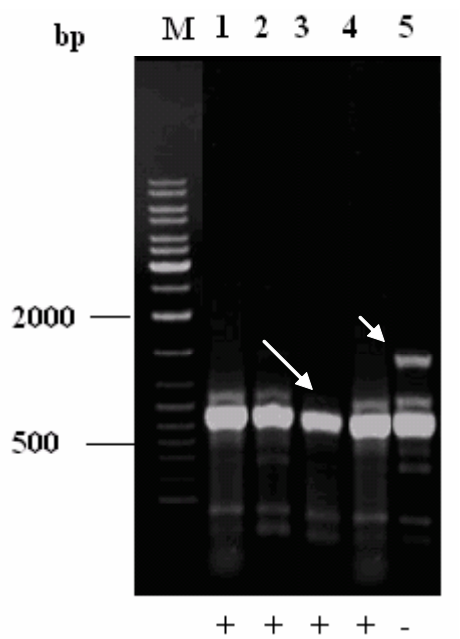

Fig. 5. DNA pattern after amplification using 1-5 ISSR4 primer. (+) UV-C radiation and (-) no radiation. The two arrows indicate a band present only in the radiated calli and a band missing in the control calli, respectively.

technique for detection of somaclonal variation. In this study we detected somaclonal variation in callus exposed to UV-C radiation by 6 primers: FPK1-05, OPAA-20, OPAA-03, FPK2-19, ISSR4 and OPA-18. Some of these primers could reveal additional DNA bands, for example FPK-05, OPAA-20, FPK2-19 were able to detect the extra DNA band after PCR amplification while OPAA-03, ISSR4 and OPA-18 detected missing DNA bands. Similar results in potato callus using RAPD-PCR have been reported by Bordallo et al. (2004). Similar to our data, they observed somaclonal variation after different treatments with plant growth regulators in potato callus based on additional or missing bands detected in the pattern of DNA using OPB-07, OPB-08, OPD-01 and OPS-11 primers.

In our study, additional or missing DNA bands were detected due to UV-C radiation and possibly consequent pyrimidin dimmer formation. It has also been documented that if the callus phase is not long enough during plant regeneration, less somaclonal variation can be expected. For example, Soniya et al. (2001) found that during plant regeneration from tomato plant, more than 90% of regenerated plants had no somaclonal variation using RAPD marker. It might be due to the long period of callus phase during optimization of plant regeneration system. In our study, the duration of callus proliferation was not too long (6 weeks at the most), but UV-C radiation of the callus could increase the occurrence of somaclonal variation.

In animal cells the nuclear changes associated with apoptosis are well defined and include nuclear fragmentation (Morell and Peakall, 1995). Similar results were reported when *Arabidopsis thalina* protoplasts were exposed to UV-C radiation. In our study, when calli were exposed to UV-C, the detected changes in DNA might be due to DNA fragmentation or unrepaired pyrimidin dimmer or other possible mutations. Another source of somaclonal variation following UV-C radiation could be the changes in DNA methylation pattern, however, changes of DNA bands after PCR amplification indicated that the chance for methylation changes in DNA was rather low.

In conclusion, we have confirmed that UV-C radiation can increase somaclonal variation which might be useful for selection of callus for desirable traits, such as salt and drought tolerance or secondary metabolytes

production. We have also developed a method based on RAPD–PCR as a molecular marker for detection of somaclonal variation caused by UV-C radiation of potato callus cv. *Cosima*.

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