The effect of the ultraviolet radiation on the somaclonal variability for *solanum tuberosum*

Received for publication, May 05, 2014
Accepted, June 5, 2014

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Abstract

Among the non-ionizing radiations, the ultraviolet radiations (UV) are the only ones which can change the genetic system. The investigations related to the somaclonal variability for potato (*Solanum tuberosum*) were focused on the response to the radiation exposure UV-C (λ = 254 nm). The biological material used was represented by three types of potato: Cristian, Roclas and Rustic. For this purpose we used several RAPD markers (Random Amplified Polymorphic DNA). After the irradiation with UV-C there were some changes at molecular level by the occurrence in some cases of new DNA bands or the disappearance of DNA bands in other cases highlighted using three primers (OPF-12, OPW-11 and OPX-01).

Keywords: UV-C, somaclone, potato, genetic variability, RAPD

Introduction

*Solanum tuberosum* species is part of the *Solanaceae* family, *Solanum* type, and has two subspecies: *andigena* and *tuberosum*. The potato, a tubercular plant with high importance in the human nutrition, animal feeding and industrial processing, cultivated on all continents but mainly in Europe. In present, due to the demographic explosion (over six billion inhabitants on the globe), the potato, together with some other plants, represents a hope in insuring the human food needs competing with the most important food categories: cereals, meat and fruits.

The ultraviolet radiations (UV) currently represent a very important stress factor for plants, which can lead to the alteration of the genetic system (EHSANPOUR & al. [1]). Due to the decrease of the ozone level the negative influence of the UV-B and UV-C radiations increased.

The main purpose of this research is to study the influence of the ultraviolet radiation on the somaclonal variability from the potato. For this purpose we used several RAPD markers (Random Amplified Polymorphic DNA).

The part of the electromagnetic spectrum with shorter wave length and higher energy than the visible light is subdivided in ionized radiations and non-ionized radiations.

The ultraviolet radiations (UV) are non-ionized. The UV spectrum is subdivided in three bands according to the wave length (λ): UV-C (λ < 280 nm), UV-B (λ = 280-320 nm) and
UV-A ($\lambda = 320-400$ nm). On the genetic material act UV radiations with $\lambda = 200-300$ nm. The nucleic acids absorb the radiations with $\lambda = 240-290$ nm, the highest mutagen effect have the radiations with $\lambda = 258-260$ nm. Both the DNA and the RNA absorb most intensely the UV at 260 nm (Klug and Cummins, [2]).

The UV radiations effect (mainly UV-B and UV-C) on the plants vary according to the species and types of the same species (Danon and Gallois, [3]).

The RAPD markers were successfully used also by other researchers to evaluate the genetic variability of genotypes of the species *Solanum tuberosum* (Dehghan & al., [4]; Gherardi & al., [5]; Campbell, [6]; Chandra, [7]).

RAPD (Random Amplified Polymorphic DNA) was discovered by Williams & al. [8] in 1990. The method is fast, relatively low cost and well adapted to obtain a non-radioactive genetic mark (Welsh and McClelland, [9]). It uses a single primer (with low number of nucleotides) in an amplification reaction (Williams & al., [8]). Some problems related to the reproducibility of the method were reported (Hansen & al., [10]; Jones & al., [11]; Virk & al., [12]) and the presence of some errors which can occur when applying the evaluation software of the markers (Demeke & al., [13], Karp & al., [14]).

The amplified fragments of DNA are submitted to electrophoresis in a 2% agarose gel, separating according to the molecular weight. The staining is done with ethidium bromide and the visualization of the DNA bands is done by exposing the gel which contains the DNA to a source of UV radiations. We obtain images (photos) which can be scanned and edited by using specific software.

The RAPD technique is used mainly to identify genetic diversity: *Acacia* – Casiva & al., [15]; *Afgekia* – Prathepha and Baimal, [16]; *Astragalus* – Sanderson and Liston, [17]; *Atylosia-Cajanus* complex – Parani & al., [18]; *Lotus* – Campos & al., [19]; *Medicago* – Bena & al., [20].

**Materials and methods**

**Biological material**

As biological material we used three types of potato procured from the National Institute for Research and Development of the Potato and Sugar Cane Brasov (I.N.C.D.C.S.Z): Cristian, Roclas and Rustic. The irradiation experiments with UV-C were made on vitro plantlets obtained from scalped embryos from sleeping eyes of the mature potato tubercles.

**Working methods**

**Irradiation with UV-C**

For the irradiation of the sprouts we used UV lamp in form of tubes which emitted radiations with wavelength $\lambda = 254$ nm and approximate intensity 6, 24 $\mu$mol photon/m$^2$/s. The radiation time was 30 minutes.

**Molecular techniques for detecting the genetic variability**

In order to distinguish the genetic variability we need the following: i) DNA extraction; ii) its amplification using PCR techniques and iii) migration of the reaction products in the 2% agarose gel by electrophoresis.

The vegetal material collection for the DNA extraction was done after a week of radiation from the foliar tissue with Maxwell™ 16 Instrument from Promega. This allows obtaining a quantity of purified vegetal genomic DNA at the proper quality necessary to use it in the next amplifications. The extracted DNA was distinguished using electrophoresis and afterwards it was kept at 4°C until the amplification with RAPD markers.

**PCR amplification with RAPD markers**
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For the realization of the PCR reaction, the mix used to obtain the amplification products was composed by:

1. Kit GoTaq® Green Master Mix 2x (Promega) which consists in: GoTaq® DNA polymerase; dNTP; MgCl₂; reaction buffer, all these components can be found in optimum concentrations for an efficient amplification of the DNA samples.
2. primer: 10 pmol/µl (from Fermentas company);
3. 50-100 ng genomic DNA;
4. distilled water, free of nucleases.

For the evaluation of the genotypes variability from our study we used 6 RAPD primers (Table 1.).

**Table 1. RAPD primers used for the evaluation of the studied genotypes variability**

<table>
<thead>
<tr>
<th>Code</th>
<th>Sequence (5’-3’)</th>
<th>Code</th>
<th>Sequence (5’-3’)</th>
</tr>
</thead>
<tbody>
<tr>
<td>OPAA-18</td>
<td>TGGTCCAGCC</td>
<td>OPAA-16</td>
<td>GGAACCCACA</td>
</tr>
<tr>
<td>OPF-10</td>
<td>GCAAGCTTGG</td>
<td>OPC-16</td>
<td>CACACTCCAG</td>
</tr>
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<td>OPF-12</td>
<td>AC GGACCAGC</td>
<td>OPF-08</td>
<td>GGGATATCGG</td>
</tr>
<tr>
<td>OPW-11</td>
<td>CTGATCGCGT</td>
<td>OPW-16</td>
<td>CAGCTTACCA</td>
</tr>
<tr>
<td>OPW-13</td>
<td>CACAGCGACA</td>
<td>OPX-03</td>
<td>TTGCGCAGTG</td>
</tr>
<tr>
<td>OPX-01</td>
<td>CTGGGCACGA</td>
<td>OPA-14</td>
<td>TCTGTGCTTG</td>
</tr>
</tbody>
</table>

**Reaction conditions**

The Thermo cycler used was from type Corbett. The amplification was realized in the following conditions:

i) the initial denaturation of DNA: 4 minutes at 94°C, there were 45 cycles, each cycle had the following stages:
   - denaturation: 3 minutes, at 94°C;
   - primers attachment: 1 minute at 36°C;
   - extension: 2 minutes at 72°C.
ii) DNA synthesis: 3 minutes at 72°C

At the end the samples are cooled till 4°C in the thermo cycler and kept in the refrigerator until usage.

**The analysis of the reaction products through electrophoresis in agarose gel**

**Results and discussion**

After the amplification with the RAPD primers used for the evaluation of the genotypes variability we obtained many types which presented uniformity. The molecular marker (M) used determines bands of 1000bp, 750bp, 500bp, 300bp, 150bp and 50bp. Only 3 primers showed polymorphism: OPF-12, OPW-11 and OPX-01.

The amplification with OPF-12 primer generated bands of 730 bp, 590 bp, and 300bp. It identified differences between the radiated individuals and the non-radiated ones, fact which can be noticed in figure 1, next to number 4 individual there is a new band of approximately 400bp.
Figure 1. Analysis of gel electrophoresis for amplified products using OPF-12 primer

Legend: M - molecular marker; 1, 3, 5- non-radiated individuals; 2, 4, 6- radiated individuals;

The amplification with OPW-11 primer generated five bands (of approximately: 1100bp, 755bp, 470bp, 350bp and 220bp Figure 2.) for the non-radiated individuals (1, 3 and 5) while for the two of the radiated individuals (2 and 4) the biggest one (of 1100bp) is missing. For the last individual exposed to UV radiation it generated a band of approximately 600bp and the one of 200bp is missing.

Figure 2. Analysis of gel electrophoresis for amplified products using OPW-11 primer

Legend: M - molecular marker; 1, 3, 5- non-radiated individuals; 2, 4, 6- radiated individuals;

The amplification with OPX-01 primer generated five bands of approximately 750bp, 620bp, 500bp, 340bp and 200bp (Figure 3.). The difference for this primer can be noticed at individual number 6 where a new band of 800bp appeared.
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**Figure 3.** Analysis of gel electrophoresis for amplified products using OPX-01 primer

Legend: M - molecular marker; 1, 3, 5- non-radiated individuals; 2, 4, 6- radiated individuals;

SONIYA & al. [21] found that during plant regeneration from tomato plant, more than 90% of regenerated plants had no somaclonal variation using RAPD marker.

**Conclusions**

After irradiation with UV-C there are changes at molecular level, fact which was highlighted by the three primers (OPF-12, OPW-11 and OPX-01). We can say that the polymorphism detected by these primers is not due to the genetic instability or the in vitro culture methods because there were a low number of subcultures (two).

The ultraviolet mutation can generate mutations at the DNA level; this fact is proved by the extra DNA bands in some cases and the missing ones in other cases.

**References**