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Short Communication

Detection of somaclonal variations using RAPD fingerprinting in *Silybum marianum* (L.)

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Random amplification of polymorphic DNA (RAPD) markers was used to detect somaclonal genetic variations between *in vitro* and *in vivo* grown tissues of *Silybum "S. marianum".* Ten primers (OPC1-OPC10) of RAPD OPC were used. All the primers gave reproducible banding pattern except OPC3. Monomorphic bands were observed in case of all primers, whereas only OPC10 generated different banding pattern among the samples of *S. marianum*. OPC 10 produced ten unique bands in each sample ranging in size from 200 - 1000 bp. On the basis of the results obtained, it was observed that genetic variation is present in different samples of *S. marianum* and RAPD technique can be used to detect genetic similarities and dissimilarities between *in vitro*- and *in vivo*-grown tissues of *S.* (*S.*) *marianum*.

Key words: RAPD, regeneration, *Silybum*, somaclonal variation, thistle.

INTRODUCTION

Silybum (*S.*) *marianum* (L.) Gaertn is used for oral treatment of toxic liver damage and for therapy of chronic inflammatory liver diseases and liver cirrhosis (Morazzoni and Bombardelli, 1995). This activity of *S. marianum* is due to a mixture of flavonolignans, known as silymarin (Kurkin et al., 2001). At present, all of the commercially available *Silybum* is obtained from wild. The increasing worldwide demand for *Silybum* is endangering the sparse populations. Thus, to fulfill the increasing demand, developing an efficient *in vitro* regeneration protocol of genetically similar *S. marianum* plants with desirable agronomic and chemical traits is needed. In this respect different studies related to the standardization of its tissue culture conditions and antioxidants activity has been reported (Abbasi et al., 2010). Nonetheless, somaclonal variation frequently occurs as a consequence of the propagation process in different plant species (Chuang et al., 2009). Somaclonal variation reduces the commercial value of plants (Oropeza et al., 1995). Thus it is very important to detect somaclonal variation at earlier stage of plant growth to avoid economic loss (Chuang et al., 2009).

Genetic variation analyses can be observed from morphological characters and other markers as protein or deoxyribonucleic acid (DNA)-based markers. However, the morphological characters evaluation requires the plants to grow to full maturity prior to identification. Subsequently genetic resources characterization also needed to establish development of genetic markers for valuable traits (Paterson et al., 1991).

RAPD is the simplest, sensitive and useful technique for the analysis of genetic fidelity of *in vitro* propagated plants (Chuang et al., 2009; Williams et al., 1993). Because RAPD polymorphism results from either a nucleotide base change that alter the primer binding site, or from an insertion or deletion within amplified region. Polymorphism usually results in the presence or absence of amplification products from a single locus (Tingey and del Tufo, 1993).

In this study, RAPD was employed to evaluate the genetic reliability of *S. marianum* plantlets regenerated by leaf organogenesis. To the best of our knowledge, this is

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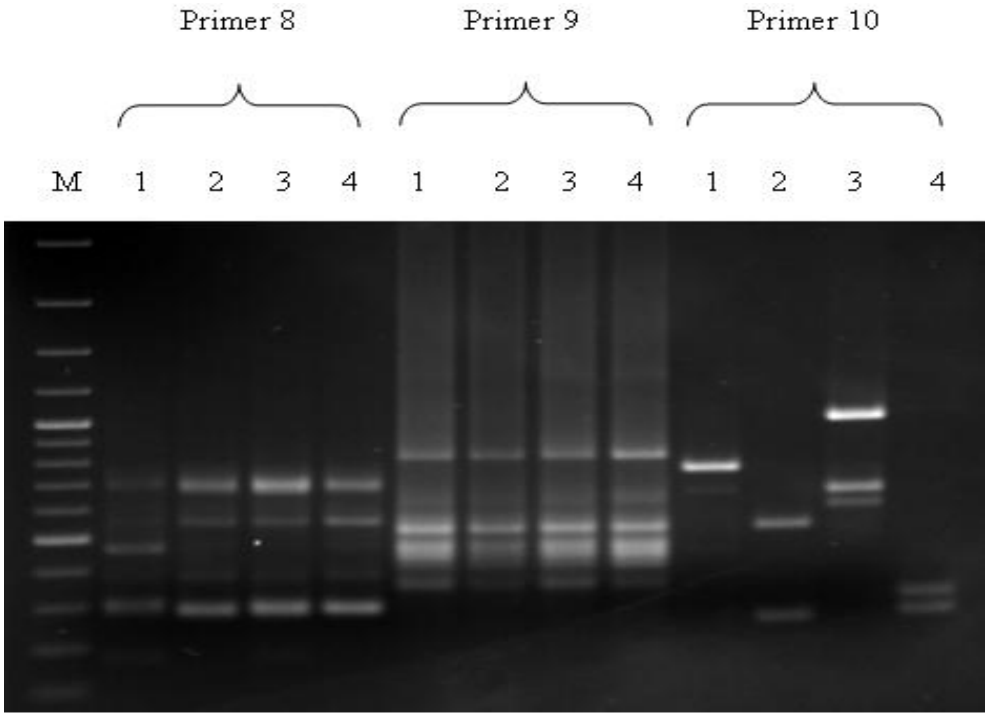

Figure 1. RAPD banding profiles generated by primers OPC8, OPC9 and OPC10. 1: wild-grown plantlets 2: seed derived plantlets 3: callus tissues and 4: regenerated plantlets of *Silybum marianum*.

the very first assessment of somaclonal variation using RAPD analyses in the *in vitro* propagated *S. marianum* regenerants. The data would be helpful in developing a protocol to minimize occurrence of somaclonal variation so as to make *in vitro* regeneration a feasible alternative to conventional propagation techniques in commercial production of *S. marianum* plants.

MATERIALS AND METHODS

Isolation of genomic DNA

Genomic DNA was isolated from different plant sources of *Silybum marianum* (callus tissues, leaves of regenerated plants, seed derived plantlets and plantlets collected from wild environment; data not shown). Cetyl trimethyl ethyl ammonium bromide (CTAB) method by Richards et al. (1997) was used for DNA extraction. DNA quality and quantity was estimated by gel electrophoresis (1% Agarose in 0.5 X TAE buffer).

Primers screening and PCR amplification

Ten primers of RAPD OPC series (OPC1-OPC10) were used to analyze the genetic variations among the samples. The genomic DNA was used for amplification reactions by preparing 25µl reaction mixture containing 25 - 50 ng/µl DNA, 25 pmol primers, 12.5 µl 2 x PCR master mixtures and 10.5 µl of PCR water (Ferementas). DNA amplification was performed in thermal cycler (Labnet, Multigene Gradient) with the conditions including, an initial denaturation at 94°C for 1 min followed by 44 cycles of denaturation at 94°C for 30 s, annealing at 40°C for 1 min and extension at 72°C for 2 min. One additional cycle of 7 min at 72°C was used for final extension. Amplification products were resolved by electrophoresis on 1.5% agarose gel run in 0.5% X TAE. The amplified products were observed under UV transilluminator (Wealtech Dolphin Doc^{Plus}) after staining with ethidium bromide (0.5 mg/ml).

RESULTS AND DISCUSSION

RAPD markers were used to find out the somaclonal variations among the four samples (seed derived plantlets, callus tissue, regenerated plant and wild-grown plantlets). Out of ten primers of OPC series, all the primers except OPC3 have given reproducible results. Only OPC10 showed genetic variations whereas all other primers gave monomorphic bands. A total of 110 bands were generated by nine primers ranging from 200 - 1000 bp in size. OPC10 generated 10 unique bands and differentiated each sample of *S. marianum* on gel (Figure 1). Two bands of 650 and 700 bp were observed in wild sample, whereas two bands of 200 and 500 bp were shown by the plant generated from seed. DNA from callus tissues has given three amplified bands with molecular weights of 500, 550 and 1000 bp in case of OPC10. Regenerated sample from same callus tissue gave different banding pattern by OPC10 and generated two bands of 300 and 400 bp. Hence, OPC10 has revealed high level of variation between generated and regenerated plants. Similarly, genetic variation was also detected among *in vitro* regenerated garlic plants and

revealed 20% polymorphiosm as described by Saker and Sawahel (1998).

Similar findings have already been reported earlier regarding the use of RAPD technique to detect the level of polymorphism of somaclonal variants of *Triticum* spp. (Brown et al., 1993) *Saccharum* spp. (Oropeza et al., 1995; Taylor et al., 1995a; 1995b), *Picea glauca* (Isabel et al. 1995), sugar beet (Munthali et al., 1996), and *Asparagus officinalis* L. (Dan and Stephens, 1997). In another related report, it was observed that variability associated with *in vitro* regeneration after a phase of callus formation was greater than direct regeneration or propagation from meristem and buds (Skirvin and Janick, 1976).

RAPD technology has also been utilized to analyze genetic stability of micropropagated plants. Mohamed (2007) reported genetic stability among the regenerated plants of strawberry. Similar results were observed in *Festuca pratensis* (Vallés et al., 1993) and *Achillea* spp. (Wallner et al., 1996). On the basis of these results, it may be concluded that RAPD is a useful technology to detect variation in micopropagated plants of *S. marianum.*

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