African Journal of Biotechnology Vol. 8 (14), pp. 3219-3222, 20 July, 2009
Available online at http://www.academicjournals.org/AJB
ISSN 1684–5315 © 2009 Academic Journals

Full Length Research Paper

RAPD-based detection of genomic instability in cucumber plants derived from somatic embryogenesis

Khaled M. Suliman Elmeer[1]*, Thomas F. Gallagher[2] and Michael J. Hennerty[2]

[1]Plant Tissue Culture Laboratory. Department of Water Research and Agriculture Doha-Qatar.
[2]School of biology and Environmental Sciences, Belfield, Dublin 4, Ireland.

Accepted 8 May, 2009

Random amplified polymorphic DNA (RAPD) markers were used to evaluate genetic stability of regenerants of cucumber plants obtained through somatic embryogenesis. Somatic embryo plants and plants of F1 hybrids, from which they were derived, were compared during weaning, early growth, flowering, fruiting and at maturity. No differences in phenotype were observed, by simple observation. Banding patterns were scored for the presence (+) or absence (-) of a DNA band, there were no visually detectable differences between the somatic embryo derived plants compared to their F1 parents in the Random Amplified Polymorphic DNA (RAPD) test using five primers OP-C10, OP-G14, OP-H05, OP-Y03 and OP-AT01. The results indicate the usefulness of RAPD markers to detect genetic instability in cucumber primary regenerant plants derived from somatic embryogenesis, and as a certification tool for monitoring genetic stability during the generation process.

Key words: Somaclonal variation, genetic instability.

INTRODUCTION

Cucumber seed used for greenhouse production consists mainly of F1, which are preferred by growers because of superior yield, improved disease-resistance and good flavour (Ladyman, 1995). The production costs of the seed of F1 hybrids are high, which is reflected in the price of the seed and the final produce (Pellinen et al., 1997).

Somatic embryogenesis may be one solution to the problem, as soon as the initial hybrid seed is made it should be possible to clone it by generating somatic embryos (Ladyman, 1995).

The major limitation of cucumber embryogenesis so far is the high frequency of abnormal embryos (Cade et al., 1990). RAPDs have been used for cucumber genetic diversity analysis (Staub et al., 1997; Yong et al., 1998; Horejsi et al., 2000), RAPD marker are usually dominanttly inherited, technically simple, and often detect more polymorphic loci than either isozymes or RFLPs. The different properties inherent in each marker system influence their effectiveness for genetic diversity analysis of cucumber (Horejsi and Staub, 1999). Genetic identification of elite cucumber accessions is important for plant variety protection (PVP) and seed purity analysis (Staub and Meglic, 1993). Even though RAPD marker technology has proven effective for genetic analysis of diverse cucumber germplasm (Staub et al., 1997), RAPDs have not been applied to the assessment of genetic relationships in elite germplasm (Horejsi and Staub, 1999). In the literature surveyed, there are no reports on the use of RAPD markers for the characterization of somaclonal variation in cucumber. Somaclonal variation, which is a welcome source of genetic variation for crop breeding, is unwanted when direct regenerants have to be used in tissue culture mass propagation, or in the regeneration of genetically transformed plants. Random amplified polymorphic DNA (RAPD) has been used to analyse somatic embryos and plants regenerated from embryogenic cell lines in Norway spruce (*Picea abies)* (Heinze and Schmidt, 1995). Cytogenetic analysis were carried out to detect somaclonal variation in somatic embryo-derived plants from two elite genotypes of *Asparagus officinalis* cv. Argenteuil (Raimondi et al., 2001) and oak *Quercus* spp. (Wilhelm, 2000).

MATERIALS AND METHODS

Genomic DNA was isolated according to the method of Pich and

*Corresponding author. E-mail: elmeer@gmail.com.

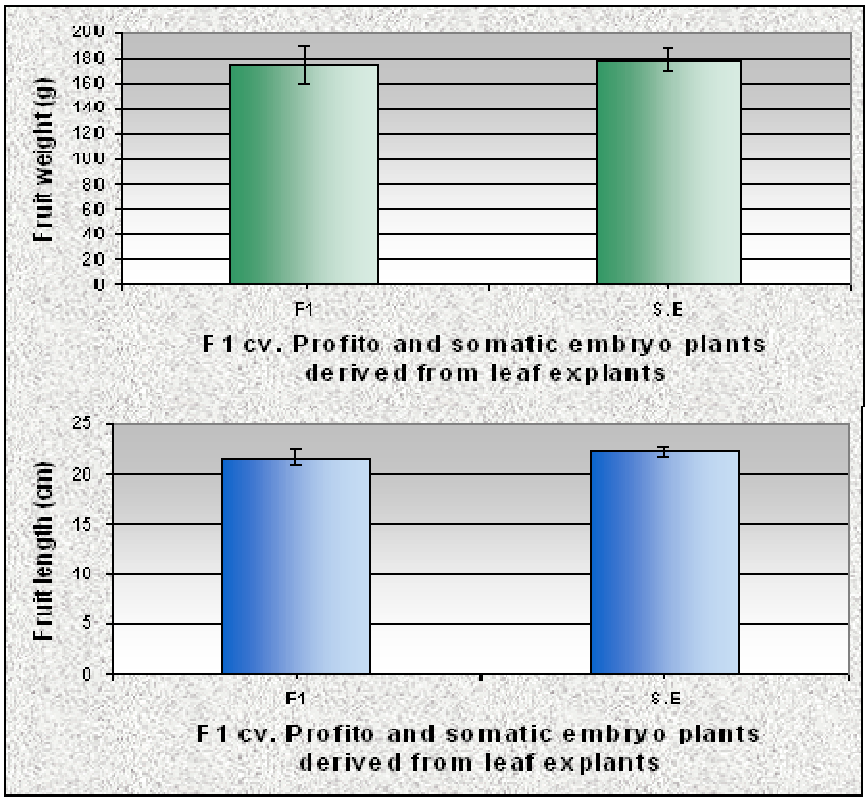

Figure 1. Comparing fruit weight and length of somatic embryo plants derived from leaf and their F1 parents.

Schubrt (1993), young leaves of F1 parent cultivars and derived somatic embryo plants were quickly frozen in liquid nitrogen and powdered with a mortar and pestle and transferred into 3 volumes of extraction buffer (500 mM NaCl, 50 mM Tris/HCl pH 8.0, 50 mM EDTA, 1% (v/v) β-mercaptoethanol (added immediately before use)). The mixture was allowed to thaw and ice-cold polyvinyl-pyrrolidone (PVP) was added to a final concentration of 6%. Solid SDS was added to a final concentration of 2% (W/V). The extract was mixed gently and incubated in a water bath at 65°C for 10 minutes. Then 0.1 volumes of 5 M potassium acetate was added, followed by 30 min incubation on ice, prior to centrifugation at 13000 xg for 10min at 4°C. The supernatant was transferred to a fresh tube and mixed with 0.6 volumes of isopropanol by gently inverting the tube three times before incubation on ice for 10 min. This was followed by another centrifugation at 13000 xg for 10 min at 4°C after which the supernatant was discarded.

DNA, thus extracted, was precipitated out of solution by adding 0.1 volumes of 3 M sodium acetate (pH 5.2) and 2.5 volumes of 100% ethanol. The solution was mixed and precipitated for the appropriate time at -20°C before centrifugation at 12000 xg for 30 min. The resultant pellet was washed three times with 70% ethanol; dried under vacuum and resuspended in a suitable volume of sterile distilled water (SDW).

Various commercially available 10-mer random primers were used for RAPD-PCR analysis. Such as the Operon Technologies commercial kits C, G, H, Y and AT. 3.0 µl of DNA from each sample to be analysed was added to a 7.0 µl PCR reaction containing 1 xPCR 1.0 µl buffer 1.2 µl $MgCl_2$ free, 1.5 mM $MgCl_2$, 1.0 µl 50 mM each of dATP, dCTP, dGTP and dTTP, 1.0 µl 0.36 µM random primer (operon Technology) and 0.13 µl 2.5 units of Taq DNA polymerase. The reaction was brought to a 10 µl total by adding appropriate amount of SDW 2.67 µl. The amplified condition were the following: 94°C for 30 s, 34°C for 30 s and 72°C for 1 min 30 s, for 45 cycles, from cycle 20 onward add 5 s per cycle finished with 10 min at 72°C. Products created during amplification were visualised by electrophoresis on 1.2 % TBE agarose gel.

RESULTS AND DISCUSSION

Somaclonal variation has to be avoided for large-scale micro propagation of diverse plant species in order to be successful and accepted by end-users. In the light of the various factors (genotype, ploidy level, *in vitro* culture age, explant and culture type, etc.) that led to somaclonal variation and divergent genetic changes at the cellular and molecular levels, genetic analysis of micro propagated plants using a multidisciplinary approach, especially at the DNA sequence level, initially and at various cultural stages are essential (Vijay and Raina, 2000).

Somatic embryo plants were grown to flower and fruit in the glasshouse to compare growth and fruit yield of seed-derived plants and somatic embryo-derived plants. The test entailed measurement of fruit length (cm) and fruit weight (g), quality and productivity parameters respectively.

As shown in Figure 1 and from the T-test, no significant differences in phenotype were observed in weight and

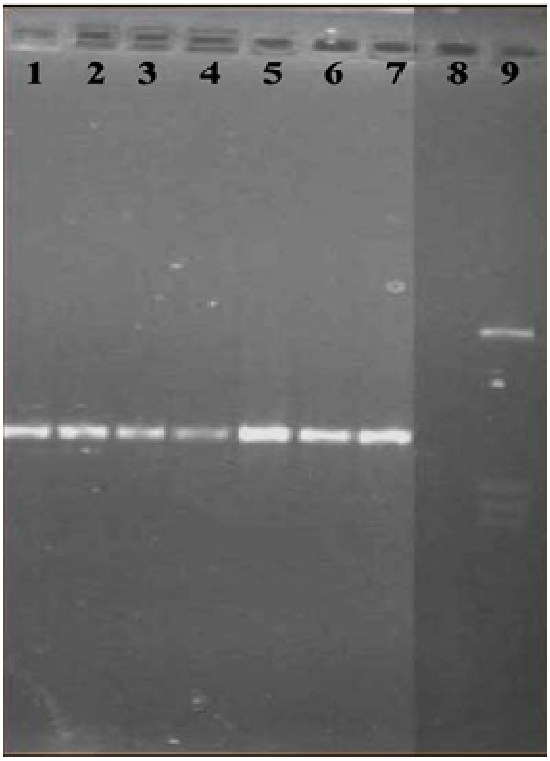

Figure 2. RAPD-PCR profiles generated using the Operon random primer OPC-10 fractionated on a 1.2% (w/v) agarose gel. Lanes 1, 2 and 3 profiles created from F1 parent genotypes. Somatic embryo derived plant genotypes of cv. Profito are represented in lanes 4,5,6 and 7; while lane 8 represents the control and lane 9 was *pGEM* restricted with *Hin*fI as references.

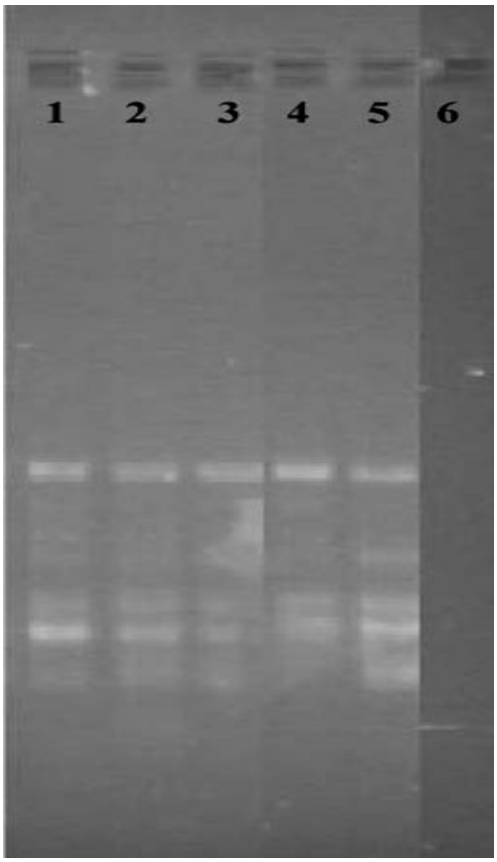

Figure 4. RAPD-PCR profiles generated using the Operon random primer OPY-03 fractionated on a 1.2% (w/v) agarose gel. Lanes 1 and 2 represent profiles created from F1 parent genotypes. Somatic embryo derived plant genotypes of cv. Profito are represented in lanes 3, 4 and 5; and lane 6 is control.

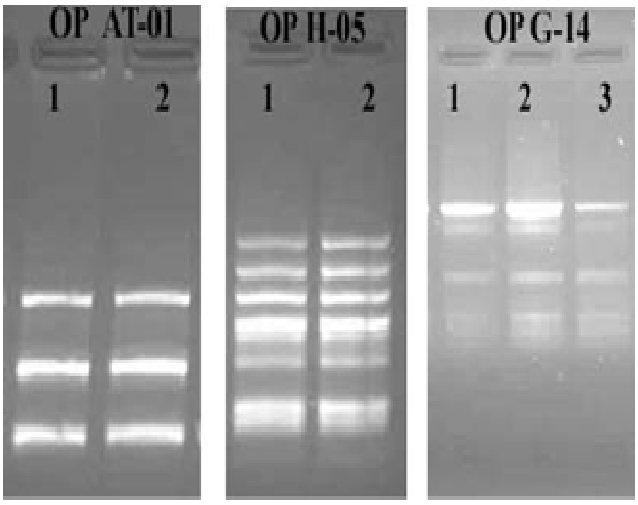

Figure 3. RAPD-PCR profiles generated using the Operon random primers OPAT-01, OPH-05 and OPG-14 fractionated on a 1.2% (w/v) agarose gel. Lane 1 represents a profile created from F1 parent genotypes; somatic embryo derived plant of cultivar Profito are represented in lanes 2 and 3 in OPG-14.

length of fruits of somatic embryo plants derived from leaf explants of cv. Profito and their F1 parents. The average fruit weight yielded by somatic embryo plants was 179.9 g compared to 175.2 g from F1 parents, while the average fruit length produced by somatic embryo plants was 22.2 cm compared to 21.7 cm from F1 parents. In addition, somatic embryo plants and plants of F1 hybrids were also compared during weaning, early growth, flowering, fruiting and at maturity. No differences in phenoltype were observed by simple observation. However in *in vitro* plant propagation, somaclonal variation is highly undesirable and should therefore be prevented (Kreuger et al., 1996).

Banding patterns were scored for the presence of (+) or absence (-) of a DNA band. There were no visually detectable differences between the somatic embryo derived plants compared to their F1 parents of Profito cultivars in the (RAPD) test using five primers OP-C10 (Figure 2), OP-AT01 , OP-G14 , OP-H05 (Figure 3), and OP- Y03 (Figure 4).

These results showed that even though the growth regulator concentrations in the medium were different, but there was no effect on somaclonal variation in somatic embryo-derived plants. This indicates that the cloning propagation of cucumber plant through somatic embryogenesis can be done without creation of somaclones.

When growth regulator concentrations, light, duration of culture and the gelling agents in the medium were changed, there was no significant difference either in weight or length of fruits of somatic embryo plants derived from leaf explants of cv. Profito, and their F1 parents. These results agree with the findings of Shoyama et al. (1997) in *Panax notoginseng* regenerated by embryogenesis.

There were no visually detectable differences between the somatic embryo plants derived from the treatment of 2.0 mgl^{-1} 2,4-D + 0.0 mgl^{-1} NAA in the dark compared to their F1 parents in the Random Amplified Polymorphic DNA (RAPD) using two primers OP-AT01 with 3 band and OP- H05 with 6 band. Jayanthi and Mandal (2001) obtained best response for callus induction on MS medium containing 2.0 mgl^{-1} 2,4-D and 0.01 mgl^{-1} kinetin, after two subcultures on the same medium, the embryogenic callus was transferred to MS medium with different concentrations of the cytokinin, 6-benzyladenine. DNA (RAPD) analysis was carried out and the amplification products were monomorphic among all the plants revealing the genetic homogeneity and true-to-type nature of the regenerants.

No visually detectable differences was noted between somatic embryo plants derived from the treatment of 0.0 mgl^{-1} cytokinin + 0.7% agar and 0.50 mgl^{-1}BAP + 0.7% agar and their F1 parents using four primers OPAT-01 with 3 band, OPH-05 with 6 band, OPG-14 with 6 band and OPC-10 with only one band. The bands were compatible with the findings of Horejsi et al. (1999).

The amplification products were monomorphic for all of the plantlets of cucumber regenerated by embryogenesis. The results obtained from these tests could help in modifying the protocols for obtaining genetically true-to-type plants and ultimate usage by entrepreneurs without any ambiguity about genotype. This suggests that somatic embryogenesis can be used for clonal micro propagation of the plant.

REFERENCES

Cade RM, Wehner TC, Blazich FA (1990). Somatic embryos derived from cotyledons of cucumber. J. Am. Soc. Hortic. Sci. 115(4): 691-696.

Heinze B, Schmidt J (1995). Monitoring genetic fidelity vs. somaclonal variation in norway spruce *(Picea abies)* somatic embryogenesis by RAPD analysis. Euphytica, 85: 341-345.

Horejsi T, Staub JE (1999). Genetic variation in cucumber (*Cucumis sativus* L.) as assessed by random amplified polymorphic DNA. Genet. Resour. Crop Evol. 46: 337-350.

Horejsi T, Box JM, Staub JE (1999). Efficiency of randomly amplified polymorphic DNA to sequence characterized amplified region marker conversion and their comparative polymerase chain reaction sensitivity in cucumber. J. Am. Soc. Hortic. Sci. 124(2): 128-135.

Horejsi T, Staub JE, Thomas C (2000). Linkage of random amplified polymorphic DNA markers to downy mildew resistance in cucumber (*Cucumis sativus* L.). Euphytica, 115: 105-113.

Jayanthi M, Mandal PK (2001). Plant regeneration through somatic embryogenesis and RAPD analysis of regenerated plants in *Tylophora indica* (Burm. f. Merrill.). *In Vitro* Cellular and Developmental Biology Plant, 37(5): 576-580.

Kreuger M, van der Meer W, Postma E, Abbestee R, Raaijmakers N, van Holst G (1996). Genetically stable cell lines of cucumber for the large-scale production of diploid somatic embryos. Physiologia Plantarum, 97: 303-310.

Ladyman JAR (1995). Somatic embryogenesis in cucumber (Cucumis sativus L.). (Bajaj YPS ed.). Biotechnol. Agric. Forest. 31: 151-163.

Pellinen TP, Sorvari S, Tahvonen R, Sewon P (1997). Somatic embryogenesis in cucumber (*Cucumis sativus* L.) callus and suspension cultures. Angewandte Botanik, 71: 116-118.

Pich U, Schubert I (1993). Midiprep method for isolation of DNA from plants with a high content of polyphenolics. Nucl. Acids Res. 21(14): p. 3328.

Raimondi JP, Camadro EL, Babinec FJ (2001). Somatic embryogenesis in A*sparagus officinalis* L. cv. Argenteuil: interaction between genotype, explant type and growth regulators on callus induction, growth and embryogenic differentiation. Biocel, 25(2): 147-154.

Shoyama Y, Zhu XX, Nakai R, Shiraishi S, Kohda H (1997). Micropropagation of *Panax notoginseng* by somatic embryogenesis and RAPD analysis of regenerated plantlets. Plant Cell Reports, 16(7): 450-453.

Staub JE, Box J, Meglic V, Horejsi TF, McCreight JD (1997). Comparison of isozyme and random amplified polymorphic DNA data for determining intraspecific variation in *Cucumis*. Genet. Res. Crop Evol. 44: 257-269.

Staub LE, Meglic V (1993). Molecular genetic markers and their legal relevance for cultivar discrimination: A case study in cucumber. Hortic. Technol. 3: 291-300.

Vijay R, Raina SN (2000). Genetic fidelity of organized meristem-derived micropropagated plants: a critical reappraisal. *In Vitro* Cellular and Developmental Biology. Plant, 36(5): 319-330.

Wilhelm E (2000). Somatic embryogenesis in oak (*Quescus* spp.). *In Vitro* Cellular and development Biology. Plant, 36: 349-357.

Yong X, Xinxing O, Haiying Z, Yongjian W (1998). Studies on the rapid and simple RAPD technique in water melon (*Citrullus lanantus* Mansf.) and cucumber (*Cucumis sativus* L.). Adv. Hortic. 2: 457-462.