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Full Length Research Paper

DGGE-RAPD analysis as a useful tool for cultivar identification

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Denaturing gradient gel electrophoresis- random amplified polymorphic DNA (DGGE-RAPD) was used to overcome the main drawbacks of RAPD (i.e., the low levels of reproducibility and polymorphism). As a model, six barley cultivars of known origin were tested for RAPD markers using DGGE methodology with 29 arbitrary primers. Among a total of 418 bands observed, as high as 99 were polymorphic. Comparison between agarose-RAPD and DGGE-RAPD revealed that the latter was highly reproducible and gave higher level of polymorphism and consequently more markers. The relationships among barley cultivars derived from this study based on DGGE- RAPD are consistent with the known lineage of these cultivars. In conclusion, we recommend the use of DGGE-RAPD as an alternative tool to the more costly DNA-based analysis in cultivar identification in laboratories with limited funds.

Key words: Denaturing gradient gel electrophoresis-random amplified polymorphic DNA, artificial heteroduplex, dendogram, lineage.

INTRODUCTION

Random amplified polymorphic DNA (RAPD; Williams et al., 1990) was embraced in different laboratories, especially those in the developing countries, due to its low cost compared to other DNA-based techniques, such as amplified fragment length polymorphism or AFLP (Vos et al., 1995) and simple sequence repeats or SSRs (Bruford and Wayne, 1993). Besides, RAPD protocol is fairly simple, while protocols like AFLP and SSR are technically demanding (Karp et al., 1997). More recently, RAPD has been utilized either alone or in conjunction with other DNA-based markers in plant linkage mapping and germplasm assessment (Kojima et al., 1998; McGregor et al., 2000; De La Rosa et al., 2003; Ren et al., 2003; González-Rodríguez et al., 2004; Wu et al., 2004). However, there were many drawbacks for its use as a reliable tool in cultivar identification and phylogeny reconstruction. DGGE- RAPD (denaturing gradient gel electrophoresis-random amplified polymorphic DNA) was used, in the present study, in a trial to overcome the two main drawbacks which are the low levels of reproducibility and polymorphism. The mobility of a DNA molecule in agarose-RAPD depends only on the number of base pairs. Nevertheless, the use of DGGE-RAPD detects polymorphisms not only due to the number of base pairs but also due to CG:AT ratio and consequently DNA melting properties (He et al., 1992).

MATERIALS AND METHODS

Four barley (*Hordeum vulgare*) cultivars of Egyptian origin namely Giza 123 (G123), Giza 124 (G124), CC89 and California Mariout (CM) and two cultivars namely Rihane3 (R3) and Assala introduced from ICARDA were used. The sources and pedigrees of these six cultivars and subsequent selected ancestors are shown in Table 1. DNA was extracted from fresh leaf tissues by the modified procedure of Gawel and Jarrett (1991). The minimum number of plants to be bulked for each cultivar to saturate polymorphisms within each cultivar was determined. Twenty nine arbitrary 10-mer primers (primers A01, A02, A03, A05, A12, A14, A15, A18 and A20

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Table 1. Sources and pedigrees of the six barley cultivars and some subsequent ancestors.

No	Cultivars and selected ancestors	Origin	Pedigree
1	Giza 123(G123)	Egypt	Giza 117/FAO 86
2	Giza 124(G124)	Egypt	Giza 117/Bahteem 52// Giza 118/FAO 86
3	CC89	Egypt	Selected from composite crosses
4	Rihane3 (R3)	ICARDA	As 46//Avt/Aths
5	Assala	ICARDA	Harma-03/Beecher
6	California Mariout (CM)	Egypt	Selected landrace
7	Giza 117*	Egypt	Baladi 16/Palestine 10
8	Beecher*	USA	Introduced to Egypt and named Giza 118
9	Harma-03*	Tunisia	-
As54/Tra//(Cer/Tol1)413/Avt*2/Ki//Bz/4/Vt/5/Pro			

*Ancestors of Assala, G124 and G123, respectively.

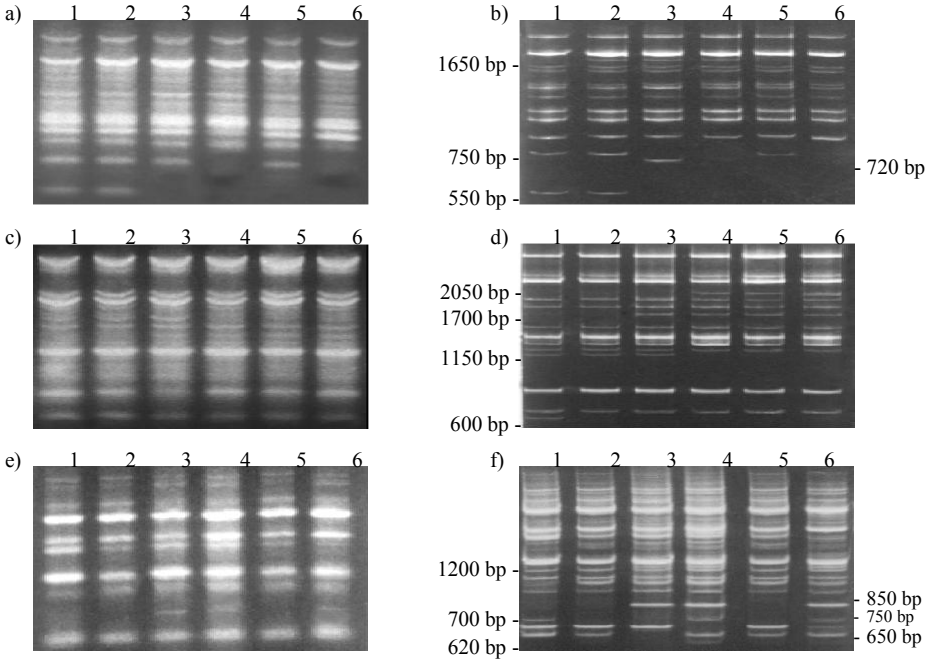

Figure 1. Comparison between agarose- RAPD (a, c and e) and DGGE-RAPD (b, d and f) generated by primers A12 (a and b), B09 (c and d) and B15 (c and f) for six barley cultivars. Refer the cultivar numbers 1 to 6 to Table 1.

and all the 20 primers of kit B, Operon Technologies Inc., Alameda, CA, 94501) were used for PCR following the protocol of Williams et al. (1990). The primers for all PCR runs were used individually. Denaturing gradient gel as well as agarose (1.2%) electrophoreses were used in this study according to Dweikat et al. (1993) . DGGE-RAPD patterns were scored visually and DICE computer package was used to draw the dendogram.

RESULTS AND DISCUSSION

Two types of polymorphisms were considered in the present study. They are missing bands from one or more cultivars (null phenotypes) and shifts in band migration.

G123 and G124 cultivars were selected and surveyed for the optimum sample size using primers B01, B04 and B13. Identical marker profiles were detected in 5-15 plant bulks for each cultivar (data not shown). A total of 418 DNA bands were detected by the 29 primers in which 319 (76%) of them were common for all the six cultivars. These DNA bands were highly reproducible using DGGE-RAPD when compared to those using agarose-RAPD. Comparison between the two matrices for identical PCR products of selected primers is shown in Figure 1. The average number of DNA bands/primer utilizing DGGE was 14, while nine utilizing agarose. Primer A12

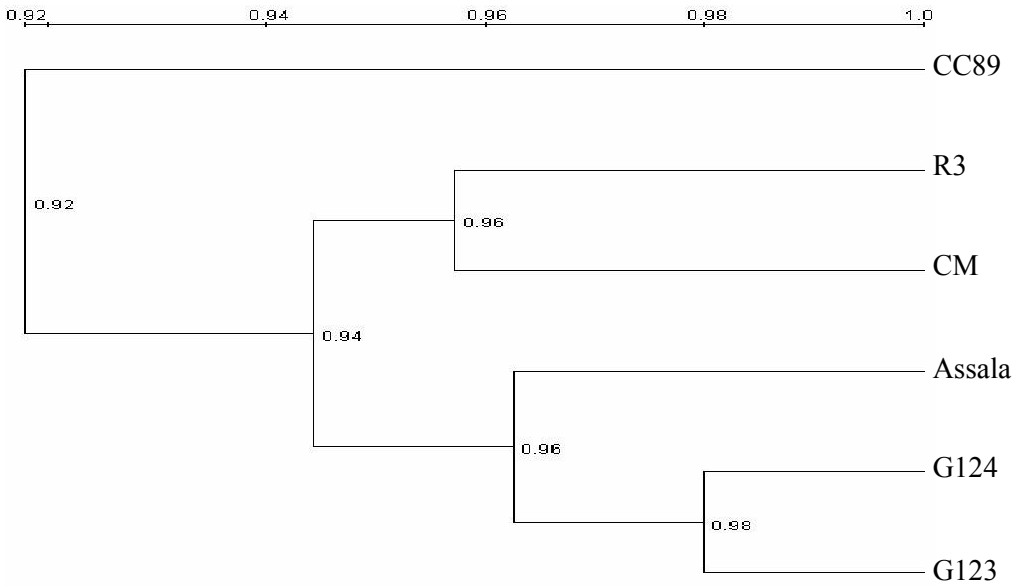

Figure 2. Dendogram demonstrating the differences among six barley cultivars based on a compiled data set recorded from DNA fragment polymorphism with DGGE-RAPD.

generated four DGGE-RAPD markers, while only two agarose- RAPD (750 and 550 bp molecular sizes) markers. One of the DGGE-RAPD markers was generated due to the subfractionation of a low-molecular-weight band of about 750 bp to two bands with molecular sizes of 750 and 720 bp. The results of primer B09 indicated that a common band in agarose gel with a molecular size of about 620 bp was sub-fractionated in denaturing gel to two bands, one of them was polymorphic (600 bp molecular size). A few unreproducible bands in agarose gel using primer B09 with molecular sizes less than 1150 bp disappeared in denaturing gel using the same primer. As a major limitation to the use of RAPD, these unstable products might represent artificial heteroduplexes between multiple amplified fragments (Halldén et al., 1996; Novy and Vorsa, 1996), or from non-specific amplification. He et al. (1992) described that these artifacts were minimized on the gradient gel, where the latter controls the consistency of PCR products by denaturing artificial heteroduplexes. The results of primer B15 indicated that a common band in agarose gel was sub-fractionated to four polymorphic bands with molecular sizes of 750, 720, 700 and 620 bp. Myers et al. *(*1987) indicated that DGGE is designed to allow the resolution of sequence differences among DNA fragments of similar or identical size in harmony with the present results.

Furthermore, the dendogram tree demonstrating the relationships among barley cultivars generated by DICE is shown in Figure 2. The six barley cultivars were readily separated by a range of differences. The relationships among barley cultivars derived from this study are consistent with the known lineage of these cultivars. In conclusion, RAPD-DGGE was shown to overcome the main two drawbacks of RAPD applications in plant (i.e., low levels of reproducibility and polymorphism) and consequently can be used as a good alternative to the other recently developed DNA-based analyses, especially in laboratories with limited funds.

The most recent methods may not always be the best or most cost-effective way of addressing a problem. In systematic studies, Harris (1999) reviewed the use of RAPD markers. Nevertheless, Schaal et al. (1998) claimed that AFLP and SSR are valuable methods for addressing population genetics and plant breeding issues, but problematic and misleading for phylogeny reconstruction and taxonomy above the species level. Sequencing and plastid RFLP methods are more rigorous of inferring phylogenies.

REFERENCES

Bruford MW, Wayne RK (1993). Microsatellites and their application to population genetic studies. Curr. Opin. Genet. Develop. 3: 939-943.

De La Rosa R, Angiolillo A, Guerrero C, Pellegrini M, Rallo L, Besnard G, Berville A, Martin, Baldoni L (2003). A first linkage map of olive (*Olea europaea* L.) cultivars using RAPD, AFLP, RFLP and SSR markers. Theo. Appl. Gene. 106: 1273-82.

Dweikat I, Mackerzie S, Levy M, Ohm H (1993). Pedigree assessment using RAPD-DGGE in cereal crop species. Theo. Appl. Gene. 85: 497-505.

Francisco-Ortega J, Newbury HJ, Ford-Lloyd BV (1993). Numerical-analyses of RAPD data highlight the origin of cultivated tagasaste (*Chamaecytisus proliferus* ssp *palmensis*) in the Canary Islands. Theo. Appl. Gene. 87: 264-270.

Gawel NJ, Jarrett RL (1991). A modified CTAB DNA -extraction procedure for *Musa* and *Ipomoea*. Plant Mol. Biol. Rep. 91: 262-266.

González-Rodríguez A, Arias DM, Valencia S, Oyama K (2004). Morphological and RAPD analysis of hybridization between *Quercus*

affinis and *Q. laurina* (fagaceae), two Mexican red oaks. Am. J. Bot. 91: 401-409.

Halldén C, Hansen M, Nilsson NO, Hjerdin A, Säll T (1996). Competition as a source of errors in RAPD analysis. Theo. Appl. Gene. 93: 1185-1192.

Harris SA (1999). RAPDs in systematics - a useful methodology? In: Hollingsworth PM, Bateman RM, Gornall RJ (eds). Molecular Systematics, Plant and Evolution. Taylor and Francis, London. pp. 221-228.

He S, Ohm H, Mackenzie S (1992). Detection of DNA sequence polymorphisms among wheat varieties. Theo. Appl. Genet. 84: 573-578.

Karp A, Kresovich S, Bhat KV, Ayand WG, Hodgkin T (1997). Molecular Tools In Plant Genetic Resources Conservation: A Guide To The Technologies, IPGRI Technical Bulletin No. 2, International Plant Genetic Resources Institute, Rome, Italy.

Kojima T, Nagaoka T, Noda K, Ogihara Y (1998). Genetic linkage map of ISSR and RAPD markers in Einkorn wheat in relation to that of RFLP markers. Theor. Appl. Genet. 96: 37-45.

McGregor CE, Lambert CA, Greyling MM, Louw JH, Warnich L (2000). A comparative assessment of DNA fingerprinting techniques (RAPD, ISSR, AFLP and SSR) in tetraploid potato (*Solanum tuberosum* L.) germplasm. Euphytica 113:135-144.

Novy RG, Vorsa N (1996). Evidence for RAPD heteroduplex formation in cranberry: implications for pedigree and genetic-relatedness

studies and a source of co-dominant RAPD markers. Theoretical and Appl. Genet. 92: 840-849.

Ren F, Lu BR, Li S, Huang J, Zhu Y (2003). A comparative study of genetic relationships among the AA -genome *Oryza* species using RAPD and SSR markers. Theo. Appl. Gene. 108:113-120.

Schaal BA, Hayworth DA, Olsen KM, Rauscher JT, Smith WA (1998). Phylogeographic studies in plants: problems and prospects. Mol. Ecol. 7: 465-474.

Vos P, Hogers R, Bleeker M, Reijans M, van de Lee T, Hornes M, Frijters A, Pot J, Peleman J, Kuiper M, Zabeau M (1995). AFLP: A new technique for DNA fingerprinting. Nucl. Acids Res. 23: 4407-4414.

Williams JGK, Kubelik AR, Livak KJ, Rafalski JA, Tingey SV (1990). DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. Nucl. Acid Res. 18: 6531-6535.

Wu SB, Collins G, Sedgley M (2004). A molecular linkage map of olive (*Olea europaea* L.) based on RAPD, microsatellite, and SCAR markers. Genome 47: 26-35.