ASSESSMENT OF GENETIC DIVERSITY IN YEAST AND BARLEY BY RETROTRANSPOSON-BASED MOLECULAR MARKERS

MARWA M. SHEHATA, LAMYAA M. SAYED, FATMA M. BADAWY AND EMAN M. FAHMY

Department of Genetics, Faculty of Agriculture, Ain-Shams University

Survival of a species and its adaptability to the environment depends on genetic diversity which is so important for representing the heritable variation between and within populations of organisms (Rao and Hodgkin, 2002). The presence of different alleles in the gene pool is reflected by genetic diversity among individuals and hence different genotypes within populations (Templeton, 1991 & 1993). Genetic diversity has been the major focus of evolutionary biology since the beginning of the 20th century.

Assessment of genetic diversity has become more advanced, whether through the utilization of variation in enzymes (allozymes) or through PCR-based marker systems, allowing direct examination of DNA sequence variation (Mondini *et al.*, 2009; Poczai *et al.*, 2012). Molecular markers have a great influence in the characterization and evaluation of genetic diversity within and between species and populations (Russel *et al.*, 1997). DNA markers as RAPD, ISSRs, SSRs and AFLP and RFLP-PCR are considered the most suitable means for estimating genetic diversity at the DNA level because of their abundant polymorphisms and the fact that they are independently of environmental conditions (Zietkiewicz *et al.*, 1994; Esselman *et al.*, 1999; Holderegger *et al.*, 2006).

Retrotransposons are a subclass of mobile genetic elements and are abundant components of the DNA in many eukaryotic organisms (Kumar and Bennetzen, 1999; Fedoroff, 2000; Zou *et al.*, 2009). They replicate through an mRNA intermediate (Boeke and Sandmever, 1991; Havecker *et al.*, 2004). One of the powerful molecular tools; Retrotransposon-based molecular markers, although these markers are not readily available due to the difficulty in obtaining species-specific retrotransposon primers (Du *et al.*, 2013). Three retrotransposon-based techniques; Inter retrotransposon amplified polymorphism (IRAP), Retrotransposon Microsatellite Amplified Polymorphism (REMAP) and RAPD-retrotransposon amplified polymorphism (RRAP) were used to study the genetic diversity in different organisms; in rice (Branco *et al.*, 2007), in fungus (Santana *et al.*, 2012) and in tomato (Mansour *et al.*, 2010). IRAP technique was carried out between two retrotransposons-Long Terminal Repeats (LTRs) to measure the distance between them using PCR (Kalendar and Schulman,

Egypt. J. Genet. Cytol., 44:371-385, July, 2015
Web Site (*www.esg.net.eg*)

2006), while PCR-amplification for the REMAP was carried out between primers matching an LTR sequence and a microsatellites domain (SSRs) to measure the distance between LTR and the nearest microsatellites locus (Kalendar and Schulman, 2006). On the other hand, RRAP technique uses a combination of primers for RAPD and LTR-retrotransposon to amplify the distance between LTR and the binding site of the RAPD primer (Aalami *et al.*, 2012).

The aim of this study was to evaluate the efficiency of RAPD, ISSRs and IRAP-PCR as molecular markers and the new developed RRAP & REMAP-PCR techniques in assessment of genomic diversity using two eukaryotes; yeast and barley as model genomes.

MATERIALS AND METHODS

Yeast strains and barley cultivars

Five yeast (*Saccharomyces cerevisiae* L.) strains; (UQM-49, NRRLY-17008 LBC-1208, LBC-254 and ATCC-58523) and six barley (*Hordeum vulgare* L.) cultivars; (Giza-123, Giza-126, Giza-129, Giza-130, Giza-131 and Giza-2000) were used to measure the genetic diversity among them and to evaluate the efficiency of retrotransposon-based molecular markers. All yeast strains were kindly provided by the Microbiological Resources Centre (Cairo MIRCEN). The barley cultivars were kindly provided by the Field Crop Research Institute, Agricultural Research Center (ARC), Giza, Egypt.

DNA extraction and PCR-based molecular markers

Pure cultures of the yeast strains were grown in a liquid medium on a rotary shaker (150 rpm) at 30°C for 24 h for the isolation of genomic DNA using the method described by Curran and Bugeja (2006). The method of Kang *et al.* (1998) was used to extract DNA from barley grains. Ten RAPD primers (OPA-08, OPA-15, OPA-20, OPB-08, OPB-10, OPB-15, OPB-17, OPD-15 and OPO-10, OPO-14) and nine ISSRs primers (814A, 844A, 17898B, 17899B, 844B, HB12, HB13, HB14 and HB15) detected PCR-based molecular markers. Two newly designed IRAP primers; (ScM1; 5'GCTGTCATCGAAGTTAGAGG3' and ScM2; 5'AGAAGATGACGCAAATGA TGAG3') were used to study the genetic diversity of the five yeast strains, while three retrotransposon-based primers (Aalami *et al.*, 2012) 5'LTR; 5'ATCATTGCCTCTAGGGCA TAATTC3', Sukkula; 5'GATAGGGTCGCATCTTGGGCGTGA C3' and Wltr2105; 5'ACTCCATAGATGGATCTTGGTGA3') were used to study the genetic diversity for the six barley cultivars. The combination between RAPD and IRAP primers (RRAP-PCR) and combination between ISSRs and IRAP primers (REMAP-PCR) were applied, also, to assess genetic diversity.

PCR reaction was performed in a gradient thermal cycler (Eppendorf, Germany). The reaction mixture of 10 µl con-

sisted of 50 ng of genomic DNA, 0.5 U of Taq DNA polymerase, 1 µl of 10 X PCR amplification buffer, 0.2 mM dNTP, 10 p moles each of the primers and 1.5 mM $MgCl_2$. Amplification for the RAPD and ISSR-PCR was done by initial denaturation at 94°C for 4 minutes, followed by 30 cycles of denaturation at 94°C for 45 second, annealing temperature of the primers was 37-44°C for 45 second, extension at 72°C for 2 minutes and the final extension was conducted at 72°C for 10 minutes. For the IRAP, RRAP and REMAP-PCR, amplification was done by initial denaturation at 94°C for 2 minutes, followed by 30 cycles of denaturation at 94°C for 45 second, annealing temperature of primers was 50-61°C for 1 minute, extension at 72°C for 2 minutes and the final extension was conducted at 72°C for 10 minutes. Amplification production was analyzed by submarine gel electrophoresis using 1.2% agarose and ethidium bromide at 8V/cm and the reaction product was visualized under Gel doc/UV trans-illuminator.

Primer design and gel analysis

Freeware Clustal X 2.1 was used to align LTR sequences to get the conserved regions which were used to design primers. The aligned sequences were formatted by CLC-Free-Workbench 3.2.3 to be more readable to the human eye. PerlPrimer 1.1.21 freeware was used to design the IRAP primers for yeast genome. To confirm that the designed primers are appropriate to match only LTR positions of the yeast retrotransposons, the PrimerSelect program in the Lasergene7 software package was applied. Gel images were analyzed using the freeware (GelAnalyzer 3) to determine molecular sizes of the amplified fragments scoring for their polymorphic type either monomorphic or polymorphic.

RESULTS AND DISCUSSION

Retrotransposons consist of a conserved domain encoding products required for transposition, bounded by direct repeats (long terminal repeats, LTRs). The domain order in the polyprotein encoded between the LTRs defines retrotransposons as ‘‘copia -like’’ or ‘‘gypsy -like’’, after the type elements of *Drosophila melanogaster*, and defines two ancient lineages shared by plants, animals and yeasts (Xiong and Eickbush, 1990). The LTRs are unique to each retrotransposon family, and this specificity has been exploited in genetic analyses by using primers within the LTRs to amplify flanking regions. In barley there are six retrotransposon families: *BARE -1*, *Sukkula*, *Sabrina*, *Nikita*, *BAGY-1* and BAGY-2 (Leigh *et al.*, 2003). Whereas there are five retrotransposon families in yeast: Ty1, Ty2, Ty3, Ty4 and Ty5 (Kim *et al.*, 1998). In this work, PAPD and ISSRs-based PCR together with IRAP, R-RAP and REMAP retrotransposons were used to study the genetic diversity of the five strains of yeast (*Saccharomyces cerevisiae*) and the six cultivars of barley (*Hordeum vulgare* L.).

Tagging new genomic regions using IRAP retrotransposons molecular marker

IRAP analysis can be carried out with a single primer matching either 5´or 3´ end of the LTR but oriented away from the LTR itself, or with two LTR primers from the same or a different family of retrotransposons (Kalendar and Schulman, 2006). Two newly designed primers; ScM1 and ScM2, which carried out through PearlPrimer freeware parameters depending on the conserved regions of ten Ty1-retrotransposon sequences retrieved from NCBI database, were used with the five strains of yeast and showed moderate polymorphism percentages of 46.15% with ScM1 and 63.16% with ScM2 as a single primer, whereas the combination between primers gave 81.82% polymorphism (Table 1). The total number of bands ranged from 11 to 19 bands with molecular sizes from 207 to 2586 bp. While for the six cultivars of barley, three primers, i.e., Wltr2105, Sukkula and 5' LTR were used and showed different percentages of polymorphisms; 89.47%, 85% and 12.5% with 5' LTR, Sukkula and Wltr2105, respectively as a single primer. Whereas the combination between the primers gave less percentages of polymorphisms; 24% and 54.55% with 5'LTR+Sukkula and 5'LTR+Wltr2105, respectively. The total number of bands using IRAP method ranged from 8 to 25 with molecular sizes ranged from 134 to 2632 bp. (Table 1).

Efficiency of RAPD and R-RAP-PCR for genetic diversity detection

The ten RAPD primers showed high percentages of polymorphisms almost 100% either with yeast strains or barley cultivars. Total number of bands ranged from 8 to 31 with molecular sizes from 109 to 2718 bp in yeast, while it ranged from 6 to 26 with molecular sizes from 81 to 2399 bp in barley (Table 2). In yeast, seven RAPD primers were selected with the highest percentages of polymorphisms to be combined with the two newly designed IRAP primers, so they gave different percentages of polymorphisms between 31.25% with ScM1+OPB08 and 100% with ScM1+OPA20. The total number of bands ranged from 15 to 30 with molecular sizes from 125 to 2941 bp as shown in Table (3). At the same time in barley six RAPD primers were chosen to be combined with the three IRAP primers and they also gave different percentages of polymorphisms between 12.5% with 5'LTR+OPO14 and 72.73% with 5'LTR+OPO10. The total number of bands ranged from 11 to 27 with molecular sizes from 64 to 1963 bp as shown in Table (4).

Efficiency of ISSRs and REMAP for genetic diversity detection

Nine ISSRs primers were examined and gave high percentages of polymorphisms which reached 100% with yeast strains, and about 75% with barley cultivars (Table 5). Total number of bands; ranged from 10 to 24 with molecu-

lar sizes from 159 to 2221 bp in yeast, while it ranged from 6 to 22 with molecular sizes from 127 to 2014 bp in barley. In yeast, six ISSR primers with the highest percentages of polymorphisms were selected to be combined with the two newly designed IRAP primers to make REMAP, they showed different percentages of polymorphisms between 33.33% with ScM2+17898B primers and 88.46% with ScM2+HB14 primers. The total number of bands ranged from 16 to 33 with molecular sizes from 113 to 3876 bp as shown in Table (6). Likewise in barley, six ISSRs primers were chosen to be combined with the three IRAP primers to obtain REMAP and gave different percentages of polymorphisms between the smallest 11.11% with 5'LTR+HB13 primers to the highest 61.90% with Wltr2105+ HB13 primers. The total number of bands ranged from 9 to 226 with molecular from size 97 to 2341 bp as shown in Table (7).

Comparative assessment of genetic diversity in yeast strains and barley cultivars using RAPD, ISSRs, IRAP, REMAP and RRAP molecular markers

Data of both the five yeast strains and the six barley cultivars were arrayed together to compare between the five used techniques; RAPD, ISSRs, IRAP, RRAP and REMAP in the assessment of genetic diversity. Depending on the marker technique used, the resulted banding patterns varied dramatically. However, each of them targeted the genome differently and thus results in a different banding pattern. For instance, RAPD primers targeted random homologous genomic regions (Williams *et al*., 1990) while ISSRs primers amplify the highly repetitive inter-simple sequence repeats of the microsatellite region in the genome (Zietkiewicz *et al*., 1994). In contrast, IRAP primers amplify specific genomic retrotransposons inside the middle repetitive region (Kalendar *et al*., 1999). The comparison among the data obtained from the five techniques was made using three parameters: polymorphism percentages, number of bands for each technique and developing specific bands for each genotype (Table 8) as following:

• ***Polymorphism percentages***

Table (8) and Fig. (1) show the comparison between the five techniques based on their ability to detect polymorphism among the studied genotypes. RAPD technique exhibited the highest mean of polymorphism percentages per primer comparing with the other four techniques in both yeast strains and barley cultivars. ISSRs primers represented polymorphism percentages more than IRAP primers in yeast, but they exhibited vice versa in barley. Both REMAP and RRAP showed the lowest polymorphism percentage in barley, while were higher than IRAP in yeast.

In a comparative study among RAPD, ISSRs, IRAP and REMAP markers to assess the genetic diversity and relationship among the *Citrus* and its relative genotypes, Biswas *et al.* (2010) reported that RAPD technique generated the highest number of polymorphic bands and the

average number of polymorphic bands per primer. Bublyk *et al.* (2013) investigated in formativeness and effectiveness of different marker types including ISSRs, IRAP and REMAP, and found that ISSRs showed the highest polymorphism among the other techniques. In a study on ten *Ficus sycomorus* L. genotypes, Saleh (2013) reported that RAPD markers displayed polymorphism percentages lower than those of IRAP markers (71.59% and 84.83%, respectively).

- ***Number of bands for each technique***

Retrotransposon-based techniques showed higher number of bands than those of non-retrotransposon based-techniques which may confirm the genetic diversity. As illustrated in Table (8) and Fig. (2), yeast REMAP-PCR technique produced the highest band numbers (24) whereas RAPD and RRAP-PCR technique showed equal number of bands (20). In barley, RRAP-PCR technique revealed the highest band numbers (20), while the lowest number of bands (12) was scored for ISSRs-PCR technique. Bublyk *et al.* (2013) studied the genetic diversity of *Iris pumila* L. depending on different marker types including RAPD, ISSRs, IRAP and REMAP. They found that ISSRs were the best markers among the other techniques in amplifying different loci on the genome of *Iris pumila* L.

- ***Developing specific bands for each genotype***

In yeast, a total of 178 unique bands were observed; each of them appeared only in a specific yeast strain (unique band). RAPD-PCR showed the highest number of strain-specific bands (71). RRAP, ISSRs and REMAP showed about 36, 33 and 29 unique bands, respectively. IRAP revealed only nine unique bands which may be due to the small number of the used IRAP primers. Barley cultivars displayed a total of 74 cultivar-specific bands. More than the half of barley unique bands was obtained by RAPD-PCR technique. IRAP-PCR technique displayed ten unique bands, the other three techniques showed equal number of 8 unique bands (Table 8). It is evident that RAPD technique excelled all others used here in this respect.

However some investigators advocate the notion that, many features of retrotransposons make them appealing as the basis of molecular marker systems. They are ubiquitous, abundant, dispersed components of eukaryotic genomes. Their activity simultaneously leads to genome diversification and provides means of their detection. Retrotransposons are long and produce a large genetic change at the point of insertion, thereby providing conserved sequences that can be used to detect their own integration. This event is not linked to removal of the transposable element from another locus, as it is for DNA transposons (Schulman, 2007). An ideal molecular marker technique should have the following criteria: 1) be polymorphic and evenly distributed throughout the genome, 2) provides adequate resolution of genetic differences, 3) generates multiple, independent and reliable markers, 4) be sim-

ple, quick and inexpensive, 5) needs small amount of DNA sample, 6) requires no prior information about the genome for an organism and 7) has linkage to distinct phenotype (Agrawal *et al.*, 2008). As yet, no molecular marker has been known to have all of these aspects together, but scientists should select a suitable marker according to the aim of the research. Retrotransposon transcriptional activation will lead to an increase in copy number and genome size if the newly transposed copies survive selection. They claimed that, all retrotransposon-based methods create high polymorphism between and within species and can meet most of the above mentioned criteria (Kalendar *et al.*, 2011).

Retrotransposon-based technique can be used to obtain molecular markers, due to many features and advantages, where IRAP and REMAP techniques have more advantages, over other techniques already used; versatility, as they allow for the combination of various primers that anneal to conserved regions of retrotransposons (IRAP) or microsatellites (REMAP), easy handling because the techniques involve straight forward PCR, lower cost and less labour than the AFLP and the microsatellite techniques, IRAP and REMAP use agarose gels and inexpensive reagents but generate several different polymorphic markers to be used in studies of genetic variability and high reproducibility by using specific primers (Kalendar *et al.*, 1999; Leigh *et al.*, 2003; Le *et al.*, 2008; Santana *et al.*, 2012).

Saleh (2013) studied the accuracy, repeatability and reproducibility of the RAPD and IRAP techniques for determining the genetic variability and found that the IRAP marker was more efficient than the RAPD assay. Usually, the REMAP pattern was considerably more variable than the corresponding ISSRs pattern; and often, but not always, depending on LTR sequence.

SUMMARY

Five Molecular genetics techniques (RAPD, ISSRs, IRAP, REMAP and RRAP) were used to study the genetic diversity in five yeast strains and six barley cultivars. New two retrotransposon-based primers (ScM1 and ScM2) were designed. RAPD technique represented the highest polymorphism percentages per primer compared with the other four techniques. ISSR primers represented polymorphism percentages more than IRAP primers in yeast, but lower in barley. Both REMAP and RRAP combinations showed similar results either in yeast or barley. Retrotransposon-based techniques (IRAP, RAP and REMAP) showed more number of bands more than those non-retrotransposon (RAPD and ISSRs) based techniques which make them a useful approach as molecular markers.

REFERENCES

Aalami, A., Samira Safiyar and B. A. Mandoulakani (2012). R-RAP: a retrotransposon-based DNA fingerprinting technique in plants. Plant Omics J., 5: 359-364.

Agrawal, A., A. Chhatre and R. Hardin (2008). Changing governance of the world's forests. Science, 320: 1460.

Biswas, M. K., Q. Xu and X. Deng (2010). Utility of RAPD, ISSR, IRAP and REMAP markers for the genetic analysis of *Citrus* spp. Scientia Horticulturae, 124: 254-261.

Boeke, J. D. and S. B. Sandmeyer (1991). In The molecular and cellular biology of the yeast *Saccharomyces*, pp. Yeast transposable elements. Broach, J., E. Jones and J. Pringle. 193-261. Cold Spring Harbor, NY.

Branco, C. J. S., E. A. Vieira, G. Malone, M. M. Kopp, E. Malone, A. Bernardes, C. C. Mistura, F. I. F. Carvalho and C. A. Oliveira (2007). IRAP and REMAP assessments of genetic similarity in rice. J. Appl. Genet., 48: 107-113.

Bublyk, O. M., I. O. Andreev, R. N. Kalendar, Kateryna V. Spiridonova and V. A. Kunakh (2013). Efficiency of different PCR-based marker systems for assessment of *Iris pumila* genetic diversity. Biologia, 68: 613-620.

Curran, B. P. G. and Virginia Bugeja (2006). In Yeast protocols basic investigations in *Saccharomyces cerevisiae*., Xiao, W. 2nd ed., Methods in Mol. Biol., (313). Humana Press Inc. Totowa, New Jersey, USA. p. 1-13.

Du, X. Y., Q. N. Hu, Q. L. Zhang, Y. B. Wang and Z. R. Luo (2013). Transferability of retrotransposon primers derived from Persimmon (*Diospyros kaki* Thunb.) across other plant species. Genet. Mol. Res., 12: 1781-1795.

Esselman, E. J., J. Q. Li, D. J. Crawford, J. L. Winduss and A. D. Wolfe (1999). Clonal diversity in the rare *Calamagrostis porteri* ssp. *Insperata* (*Poaceae*): comparative results for allozymes and random amplified polymorphic DNA (RAPD) and inter-simple sequence repeat (ISSR) markers. Mol. Ecol., 8: 443-451.

Fedoroff, N. (2000). Transposons and genome evolution in plants. Proc. Natl. Acad. Sci. USA, 97: 7002-7007.

Havecker, E. R., X. Gao and D. F. Voytas (2004). The diversity of LTR retrotransposons. Genome Biol., 5: 225.

Holderegger, R., U. Kamm and F. Gugerli (2006). Adaptive *vs*. neutral genetic diversity: Implications for landscape genetics. Landscape Ecol., 21: 797-807.

Kalendar, R. and A. H. Schulman (2006). IRAP and REMAP for retrotransposon-based genotyping

and fingerprinting. Nature Protocols, 1: 2478-2484.

Kalendar, R., A. J. Flavell, T. H. N. Ellis, T. Sjakste, C. Moisy and A. H. Schulman (2011). Analysis of plant diversity with retrotransposon-based molecular markers. Heredity, 106: 520-530.

Kalendar, R., T. Grob, M. Regina, A. Suoniemi and A. Schulman (1999). IRAP and REMAP: Two new retrotransposon-based DNA fingerprinting techniques. Theor. Appl. Genet., 98: 704-711.

Kang, H. W., Y. G. Cho, U. H. Yoon and M. Y. Eun (1998). A rapid DNA extraction method for RFLP and PCR analysis from a single dry seed. Plant Mol. Rep., 16: 1-9.

Kim, J. M., S. Vanguri, J. D. Boeke, A. Gabriel and D. F. Voytas (1998). Transposable elements and genome organization: A comprehensive survey of retrotransposons revealed by the complete *Saccharomyces cerevisiae* genome sequence. Genome Res., 8: 464-478.

Kumar, A. and J. L. Bennetzen (1999). Plant retrotransposon. Annu. Rev. Genet., 33: 479-532.

Le, Q. V., H. K. Won, T. S. Lee, C. Y. Lee, H. S. Lee and H. S. Ro (2008). Retrotransposon microsatellite amplified polymorphism strain fingerprinting markers applicable to various mushroom species. Mycobiology, 36: 161-166.

Leigh, F., R. Kalendar, V. Lea, D. Lee, P. Donini and A. H. Schulman (2003). Comparison of the utility of barley retrotransposon families for genetic analysis by molecular marker techniques. Mol. Genet. Genomics, 269: 464-474.

Mansour, A., J. A. T. da Silva, S. Edris and Rania A. A. Younis (2010). Comparative assessment of genetic diversity in tomoto cultivars using IRAP, ISSR and RAPD molecular markers. Genes, Genomes and Genomics, 4: 41-47.

Mondini, L., A. Noorani and M. A. Pagnotta (2009). Assessing plant genetic diversity by molecular tools. Diversity, 1: 19-35.

Poczai, P., I. Varga, N. E. Bell and J. Hyvonen (2012). In Genomics meets biodiversity: Advances in molecular marker development and their applications in plant genetic diversity assessment. Caliskan, M., Agric. Biol. Sci., Publisher: Tech. (DOI: 10.5772/2639).

Rao, V. R. and T. Hodgkin (2002). Genetic diversity and conservation and utilization of plant genetic resources. Plant Cell Tiss. Org. Cult., 68: 1-19.

Russel, J. R., J. D. Fuller, M. Macaulay, B. G. Hatz, A. Jahoor, W. Powell

and R. Waugh (1997). Direct comparison of levels of genetic variation among barley accessions detected by RFLPs, AFLPs, SSRs and RAPDs. Theor. Appl. Genet., 95: 714-722.

Saleh, B. (2013). Genetic diversity in *Ficus sycomorus* L. species (*Moraceae*) using RAPD and IRAP markers. Agriculture, 59: 120-130.

Santana, M. F., Elza F. de Araújo, J. T. de Souza, E. S. G. Mizubuti and M. V. de Queiroz (2012). Development of molecular markers based on retrotransposons for the analysis of genetic variability in *Moniliophthora perniciosa*. Eur. J. Plant Pathol., 134: 497-507.

Schulman, A. H. (2007). Molecular markers to assess genetic diversity. Euphytica, 158: 313-321.

Templeton, A. R. (1991). In Genetics and conservation biology. Species conservation a population-biological approach. Seitz, A. and V., Loeschche Basel, Birkhauser Verlag., 15-29.

Templeton, A. R. (1993). In Translocation as conservation tool. Biodiversity in mangrove landscapes, theory and practice. Szaro R., Oxford Univ. Press.

Williams, J. G., A. R. Kubelik, K. J. Livak, J. A. Rafalski and S. V. Tingey (1990). DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. Nuc. Acids Res., 18: 6531-6535.

Xiong Y. and T. H. Eickbush (1990). Origin and evolution of retroelements based upon their reverse transcriptase sequences. EMBO J., 9: 3353-3362.

Zietkiewicz, E., A. Rafalski and D. Labuda (1994). Genome fingerprinting by simple sequence repeat (SSR) anchored polymerase chain reaction amplification. Genomics, 20: 176-183.

Zou, J., H. Gong, T. J. Yang and J. Meng (2009). Retrotransposons - a major driving force in plant genome evolution and a useful tool for genome analysis. J. Crop Sci. Biotech., 12: 1-8.

Table (1): IRAP-PCR banding patterns for the five strains of yeast and the six cultivars of barley using IRAP primers and their combinations.

Organism	Primer	Total number of bands	Molecular sizes range (bp)		Polymorphism %
			Min	Max	
Yeast	ScM1	13	207	2470	46.15
	ScM2	19	281	2586	63.16
	ScM1+ScM2	11	270	2157	81.82
Barley	5'LTR	19	192	2632	89.47
	Sukkula	20	165	1665	85.00
	Wltr2105	8	201	767	12.50
	5'LTR+Sukkula	25	134	1422	24.00
	5'LTR+Wltr2105	22	226	1959	54.55

Table (2): Results of the Ten RAPD based PCR primers with the five strains of yeast and the six cultivars of barley.

Primer	Yeast				Barley			
	Total number of bands	Molecular sizes range (bp)		Polymorphism %	Total number of bands	Molecular sizes range (bp)		Polymorphism %
		Min	Max			Min	Max	
A08	17	149	2657	88.24	21	173	2023	71.43
A15	16	155	2718	100.00	21	81	2399	100.00
A20	20	229	2241	85.00	26	125	2393	57.69
B08	16	366	2639	100.00	8	301	1822	87.50
B10	22	226	2769	86.36	12	123	1777	16.67
B15	8	222	2204	87.50	13	358	953	69.23
B17	23	224	2464	82.61	6	502	1726	50.00
O10	31	185	2569	93.55	26	162	1930	38.46
D15	18	109	1696	77.78	17	181	1499	82.35
O14	28	138	1714	100.00	15	320	1935	73.33

Table (3): R-RAP-PCR banding patterns for the five strains of yeast (*Saccharomyces cerevisiae* L.) using primers combinations.

Primer combinations	Total number of bands	Molecular sizes range (bp)		Polymorphism %
		Min	Max	
ScM1+OP-A08	16	149	3696	50.00
ScM1+ OP-A15	15	233	2434	46.67
ScM1+ OP-A20	21	208	2941	100.00
ScM1+ OP-B08	16	318	2548	31.25
ScM1+ OP-B17	15	328	2574	66.67
ScM1+ OP-O14	19	253	2183	52.63
ScM2+ OP-A15	24	213	3254	87.50
ScM2+ OP-A20	16	187	1931	68.75
ScM2+ OP-B08	24	125	1935	70.83
ScM2+ OP-O10	27	171	2128	88.89
ScM2+ OP-O14	30	158	1972	83.33

Table (4): R-RAP-PCR banding patterns for the six cultivars of barley (*Hordeum vulgare* L.) using primers combinations.

Primer Combinations	Total number of bands	Molecular sizes range (bp)		Polymorphism %
		Min	Max	
5'LTR+OP-A15	19	159	1112	31.58
5'LTR+ OP-A20	19	117	1176	26.32
5'LTR+ OP-B08	27	208	1675	48.15
5'LTR+ OP-B15	19	290	1952	36.84
LTR+ OP-O10	11	142	822	72.73
LTR+ OP-O14	24	138	1759	12.50
Sukkula+ OP-A15	20	176	1648	30.00
Sukkula+ OP-B15	23	169	1536	26.09
Wltr2105+ OP-A15	22	64	1462	18.18
Wltr2105+ OP-A20	18	174	1569	55.56
Wltr2105+ OP-B08	17	157	1538	29.41
Wltr2105+ OP-B15	17	121	1963	17.65
Wltr2105+ OP-O10	21	142	1288	14.29

Table (5): Results of the Nine ISSR based PCR primers with the five strains of yeast and the six cultivars of barley.

Primer	Yeast				Barley			
	Total number of bands	Molecular sizes range (bp)		Polymorphism %	Total number of bands	Molecular sizes range (bp)		Polymorphism %
		Min	Max			Min	Max	
814A	13	213	1311	46.15	14	237	1143	57.14
844A	12	199	1821	83.33	13	304	2014	38.46
844B	10	251	1041	100.00	14	439	1520	35.71
17898B	14	213	1550	71.43	12	429	1741	66.67
17899B	16	159	1819	93.75	22	127	1914	68.18
HB12	14	229	2206	64.29	10	259	874	20.00
HB13	20	200	2221	85.00	8	259	1151	75.00
HB14	14	217	2003	85.71	13	190	1668	0.00
HB15	24	190	2195	95.83	6	356	975	66.67

Table (6): REMAP-PCR banding patterns for the five strains of yeast (*Saccharomyces cerevisiae* L.) using primers combinations.

Primer Combinations	Total number of bands	Molecular sizes range (bp)		Polymorphism %
		Min	Max	
ScM1+17898B	17	138	3876	41.18
ScM1+17899B	16	141	3019	62.50
ScM1+HB12	23	172	3588	78.26
ScM1+HB13	33	113	1946	72.73
ScM1+HB14	17	149	2402	64.71
ScM1+HB15	24	210	2146	83.33
ScM2+17898B	27	125	2192	33.33
ScM2+HB12	32	179	2583	81.25
ScM2+HB13	24	125	1811	70.83
ScM2+HB14	26	167	3404	88.46
ScM2+HB15	26	208	2087	65.38

Table (7): REMAP-PCR banding patterns for the six cultivars of barley (*Hordeum vulgare* L.) using primers combinations.

Primer Combinations	Total number of bands	Molecular sizes range (bp)		Polymorphism %
		Min	Max	
5'LTR+814A	20	104	1356	30.00
5'LTR+844A	22	107	1564	40.91
5'LTR+844B	17	140	1060	29.41
5'LTR+17898B	18	183	1131	38.89
5'LTR+17899B	21	97	2086	28.57
5'LTR+HB13	9	308	867	11.11
Sukkula+844B	12	166	984	58.33
Sukkula+17898B	16	126	1328	31.25
Sukkula+17899B	14	182	1306	35.71
Sukkula+HB13	14	220	1088	21.43
Wltr2105+814A	14	1000	1000	42.86
Wltr2105+844A	16	100	2341	37.50
Wltr2105+844B	9	195	1735	44.44
Wltr2105+17898B	26	126	1787	15.38
Wltr2105+17899B	23	178	2338	47.83
Wltr2105+HB13	21	112	1375	61.90

Table (8): Comparative assessment of different molecular markers in the yeast strains and barley cultivars.

Molecular techniques	5 Yeast strains					6 Barley cultivars				
	RAPD	ISSRs	IRAP	REMAP	RRAP	RAPD	ISSRs	IRAP	REMAP	RRAP
Number of primers	10	9	3	11	11	10	9	5	16	13
Total number of bands	20	15	14	24	20	17	12	19	17	20
Polymorphism %	90.10	80.61	63.71	67.45	67.87	64.67	47.54	53.10	35.97	32.25
Unique (specific) bands	71	33	9	29	36	40	8	10	8	8

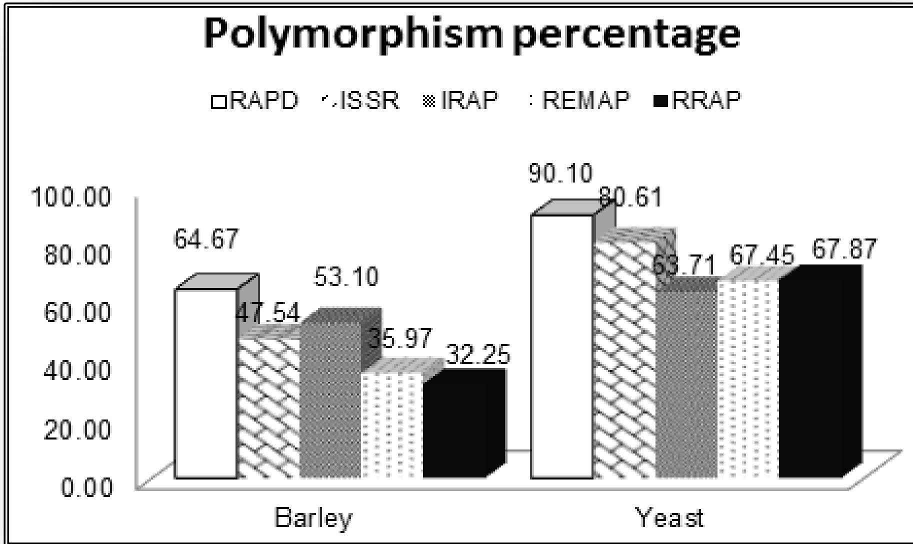

Fig. (1): Histogram of polymorphism percentages using the five techniques in yeast and barley.

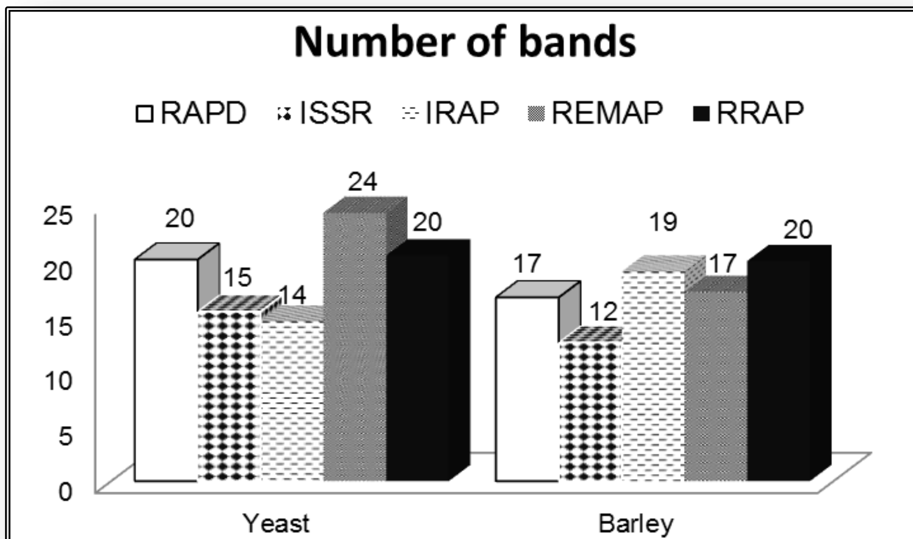

Fig. (2): Histogram of comparison of bands number in yeast and barley using the five techniques.