Research and Reviews Journal of Agriculture and Allied Sciences

e-ISSN: E 2347-226X
p-ISSN: 2319-9857

AFLP: An Efficient Molecular Fingerprinting Technique (A review work)

Urmi Roy1 and Ushri Roy2*

[1]Department of Botany, Vijaygarh Jyotish Ray College, 8/2, Bijoygarh, Jadavpur, Kolkata, West Bengal.
[2]Department of Botany, Bhairab Ganguly College, 2, Feeder Rd, Belghoria, Kolkata, West Bengal.

Research Article

Received date: 23/10/2020

Accepted date: 16/12/2020

Published date: 26/05/2021

***For Correspondence**

Department of Botany, Bhairab Ganguly College, 2, Feeder Rd, Belghoria, Kolkata, West Bengal 700056

E-mail:

dr.ushriroy2011@gmail.com

Keywords:

AFLP, DNA fingerprinting, diversity.

ABSTRACT

The chemical structure of DNA is the same in all organisms. But the order of the base pairs differs in different organisms and even within varieties. There is a difference in sequence of the bases in DNA of a specific sample. Using these sequences, every specimen could be identified solely by the sequence of their base pairs. DNA Fingerprinting (also called DNA testing, DNA typing or DNA profiling) is a technique used to distinguish between different species and also between individuals of the same species. DNA fingerprinting therefore involves the display of a set of DNA fragments from a specific DNA sample. Most DNA fingerprinting techniques involve the use of PCR for detection of fragments. The choice of what particular fingerprinting technique to use, is dependent on some factors. These are DNA typing, DNA marker mapping and the organism under investigation e.g. prokaryotes, plants, animals, humans. AFLP technique has been extensively used with plant DNA for the development of high-resolution genetic maps. Its application is expanding for determining genetic relationships and for epidemiological typing in bacteria provides a rapid and higher eukaryotes. AFLP marker is dominant in nature and provides rapid solution. This review describes the AFLP applications in the molecular fingerprinting of DNA from different plant

e-ISSN: E 2347-226X
p-ISSN: 2319-9857

genotypes.

INTRODUCTION

For the identification and typing of prokaryotic and eukaryotic organisms at the DNA level, various methods have been developed which differ in their taxonomic range, discriminatory power, reproducibility and in standardization. The ideal genotyping method produces results that are invariable from laboratory to laboratory and allows unambiguous comparative analyses and the establishment of reliable databases.

Types of Molecular Markers:

DNA markers are fragments of DNA revealing variations or mutations to detect polymorphism between different genotypes of a gene for a particular sequence of DNA in a population or gene pool. DNA marker technology has resulted in a wealth of genetic markers (like RFLPs, RAPDs, AFLPs, microsatellites or simple sequence repeat (SSR), and single nucleotide polymorphism (SNP) with potentially widespread utility.

RFLP's (Restriction Fragment Length Polymorphisms)

In RFLP analysis, restriction enzyme-digested genomic DNA is resolved by gel electrophoresis and then blotted probes a nitrocellulose membrane. Specific banding patterns are then visualized by hybridization with labeled probe.

RAPD's (Random Amplified Polymorphic DNA)

RAPD is a molecular marker based on the differential PCR amplification of a sample of DNAs from short oligonucleotide sequences. In 1991 Welsh and McClell developed a new PCR-based genetic assay namely randomly amplified polymorphic DNA (RAPD). This method detects nucleotide sequence polymorphisms in DNA by using a single primer of arbitrary nucleotide sequence.

AFLP's (Amplified Fragment Length Polymorphisms)

AFLP is a molecular marker generated by a combination of restriction digestion and PCR amplification. This is a technique based on the detection of genomic restriction fragments by PCR amplification and can be used for DNAs of any origin or complexity. This technique thus shows an ingenious combination of RFLP and PCR techniques and is extremely useful in detection of polymorphism between closely related genotypes.

SSR markers

SSRs (or microsatellites) short tandem repeats (STRs) are PCR-based markers. They are randomly tandem repeats of short nucleotide motifs of 2-6 bp/nucleotides long. Di-, tri- and tetra-nucleotide repeats are widely distributed throughout the genomes of plants and animals. The copy numbers of these repeats are the source of polymorphism in plants.

SNP markers

An SNP is a single nucleotide base difference between two DNA sequences. Single base variants in cDNA (mRNA) are considered to be SNPs as are single base insertions and deletions in the genome. The simplest form of molecular markers as a single nucleotide base is the smallest unit of inheritance through SNPs technique and it can provide maximum markers.

About Amplified Length Polymorphism (AFLP)

e-ISSN: E 2347-226X
p-ISSN: 2319-9857

An increasing number of reports describe the use of AFLP analysis for plant and animal genetic mapping, phylogenetic studies. This review describes its applications in different plant samples. In the year 1993, Zabeau first developed the AFLP technique. Later Vos et al. (1995) described the AFLP technique as being based on the detection of restriction fragments by PCR amplification. Xu et al. (1999) suggested AFLP, as the most efficient way to generate a large number of markers that were linked to target genes.

AFLP Procedure

AFLP is a DNA fingerprinting technique that combines both PCR-based and hybridization-based fingerprinting. The analysis belongs to the selection of restriction fragment amplification techniques, which are based on the ligation of adapters (i.e., linkers) to genomic restriction fragments followed by a PCR-based amplification with adapter specific primers. A small amount of purified genomic DNA is needed which is then digested with two restriction enzymes (like EcoRI and MseI or TaqI). Double-stranded oligonucleotide adapters are designed and are allowed to re-ligated with DNA fragments. These adapter-ligated fragments are then subjected to two subsequent PCR amplifications with adapter-specific primers with an extension of 1 to 3 nucleotides running into the unknown chromosomal restriction fragment. The PCR primer which spans the average-frequency restriction site is labeled.

After polyacrylamide gel electrophoresis a highly informative pattern of bands is obtained. The patterns obtained are polymorphic due to mutations in the restriction sites, mutations in the sequences adjacent to the restriction sites and complementary to the selective primer extensions, and insertions or deletions within the amplified fragments.

Advantages of AFLP

1. Only small amounts of DNA are needed.
2. No prior sequence information is needed for amplification. Unlike randomly amplified polymorphic DNAs (RAPDs) that use multiple, arbitrary primers and lead to unreliable results, the AFLP technique uses only two primers and gives reproducible results.
3. Many restriction fragment subsets can be amplified by changing the nucleotide extensions on the adaptor sequences. It has the capability to amplify between 50 and 100 fragments at one time.
4. High resolution is obtained because of the stringent PCR conditions.
5. The AFLP technique works on a variety of genomic DNA samples.

Disadvantages of AFLP

1. High quality of DNA is required
2. Complicated methodology.
3. AFLP technique is costlier than RFLP and RAPD techniques.
4. Biologically hazardous radioactive chemical are required.
5. AFLP has become extremely beneficial in the study of taxa including bacteria, fungi, and plants, where much is still unknown about the genomic makeup of various organisms.
6. Applications in Plant Genetics

AFLPs were used in the majority of population genetics for diversity and genetic variation studies. Several studies have used AFLP markers in phylogenetic analyses. Kardolus et al. (1998) applied AFLP technique to study evolutionary trends in *Solanum* species. He concluded that the choice of primer influenced the number of bands amplified and the level of polymorphisms found, which is ultimately linked to the taxonomic level of the investigation. *Aggarwal et al*. (1999) investigated the phylogenetic relationships among *Oryza* species using AFLP markers. Onopordum represented a diverse weedy genus in Australia. O'hanlon (1999) studied genetic relationships among four naturalized species through AFLP technique. The result showed that plants of intermediate morphology are also genetically intermediate.

e-ISSN: E 2347-226X
p-ISSN: 2319-9857

Molecular data proved that commercial cotton *G. hirsutum* and *G. tomentosum* which had been originated following transoceanic dispersal were close to each other (De Joode and Wendel 1992; Wendel et al. 1995; Cronn et al. 1996; Seelanan et al. 1997). Hawkins (2005) established genetic markers to identify genomic fragments unique to each species through AFLP (Amplified Fragment Length Polymorphism) analysis.

AFLP molecular markers were used to investigate the genetic relatedness between cultivated cassava *(Manihot esculenta)* and two naturally occurring species, *Manihot flabellifolia* and *Manihot peruviana* by Colombo et al. (2000). Manihot flabellifolia and *M. peruviana* species proved to be so closely related that the markers used were unable to group them separately. From a botanical standpoint, these species were also extremely similar.

To analyze genes involved in the thermo tolerance of the legume and rhizobium symbiosis, cDNA-AFLP was carried out in cowpea nodules (Simoes-Araujoa, 2002) which were subjected to heat stress (40^0 C for up to 2 h). The cDNA-AFLP revealed a total of 600 bands of which 55 were up-regulated and 9 were down-regulated. The polymorphic bands were cloned, sequenced and were analyzed by BLAST tool which showed significant homology with known proteins, such as *Phaseolus vulgaris* low molecular weight heat shock protein, *Medicago sativa* putative wound induced protein, disease resistance protein, xylan endohydrolase isoenzyme and pherophorin protein from *Arabidopsis thaliana*.

Using AFLP analysis an attempt was made to find more markers linked to powdery mildew (obligate parasite) resistance in mung bean (*Vigna radiata var. sublobata*) by Chaitieng (2002). Among approximately 5700 fragments visualized by AFLP analysis, four polymorphic fragments were confirmed to co-segregate with powdery mildew resistance among individuals.

Sholihin (2002) used AFLP markers for mapping of drought resistance in mung bean. Among 103 AFLP markers, 70 markers were mapped to nine linkage groups which covered a total map length of 655.5 cm with an average distance of 10.7 cm between markers.

The genetic relationships among 20 species of *Arachis* were established based on AFLP technique (Gimenes, 2002). 408 fragments were detected using three pairs of primers of which majority were polymorphic. AFLP proved a very useful technique in establishing the genetic relationships among 20 different *Arachis* species which were grouped into three clusters.

The amount of genetic diversity and genetic relation among contrasting bambara groundnut [(*Vigna subterranea* (L.) Verdc] landraces from different growing regions in Africa was investigated by Massawe (2002) using fluorescence-based AFLP markers. The study demonstrated that there was considerable diversity in landraces of bambara groundnut and there were no two landraces that produced identical banding patterns. The high levels of genetic polymorphism indicate that most of these landraces are highly diverse from one other.

Ouédraogo (2002a) employing AFLP analysis in combination with Bulked Segregant Analysis (BSA) led to the identification of AFLP markers linked to genes conferring resistance to *Striga gesnerioides* in cowpea (*Vigna unguiculata*). In the same year Ouédraogo (2002b) also concluded a genetic linkage map based on the segregation of various molecular markers and biological resistance traits in cowpea (*Vigna unguiculata L. Walp*.). Amplified fragment length polymorphism (AFLP), linked biological resistance traits, resistance genes, and resistance gene analogs (RGAs) were scored for segregation within the parental and recombinant inbred lines. Coulibaly (2002) carried out AFLP analysis to study genetic variation among and within domesticated and wild annual accessions of cowpea [*Vigna unguiculata (L.) Walp*]. AFLP data showed that wild annual variety originated in East Africa and spread westward and southward. This migration was accompanied by a change towards a more annual lifespan and a more selfing breeding system.

Kaga et al. (2004) analyzed the intraspecific variation in two widely distributed taxa, *V. radiata var. sublobata (Roxb.)* and *V. trinervia* (Heyne ex Wight & Arn.) through AFLP and RAPD analysis. The results supported the taxonomic revision of *V. radiata var. sublobata* and *V. trinervia* into two distinct species.

The AFLP fingerprinting result had helped to develop specific molecular markers such as SCAR (sequence characterized amplified region). Sun et al. (2005) developed specific molecular markers (SCAR markers) by cloning and sequencing of the specific AFLP products from twenty-seven *Porphyra* lines. Their result facilitated the identification, classification and resource protection of *Porphyra* lines.

e-ISSN: E 2347-226X
p-ISSN: 2319-9857

Pafundo (2005) used fluorescent AFLPs for the characterization of olive oil DNA, to obtain highly reproducible, high quality fingerprints, testing different parameters by comparing AFLP fingerprints obtained from oil with AFLPs from the corresponding plant material.

Ipek (2006) studied the sequence homology of polymorphic AFLP markers in garlic *(Allium sativum L.)*. The AFLP amplified products were sequenced and showed a similar pattern of phylogenetic relationship among garlic clones. AFLP fragments are proved to be useful for developing simple PCR-based markers in genetic mapping and diversity assessment.

Cruz et al. (2006) conducted a study to assess molecular genetic variation and genetic relatedness among 91 populations of 31 taxa based on several molecular markers (random amplified polymorphic DNA, amplified fragment length polymorphisms and trnL sequences) in the genus *Fescuta* growing in the Iberian Peninsula. According to his analysis most populations clustered at the species level, but some subspecies and varieties mixed their populations. This study demonstrated the value in combining different molecular markers to uncover hidden genetic relationships between populations of *Festuca*.

A set of more than five hundred markers mainly based on amplified fragment length polymorphic markers and also few other genetic markers were used to generate the map intraspecific linkage map of pepper *(Capsicum annuum L.)* by Barchi (2007).

Maccaferri (2007) determined the genetic relatedness among 58 different durum wheat accessions using 234 amplified fragment length polymorphisms (AFLPs). He also observed the morphological data which largely failed to describe the pattern of genetic similarity, according to known pedigree data. But the indications were provided by AFLP based molecular markers.

Different grass pea *(Lathyrus sativus L.)* populations represented a large genetic variation at the DNA level. Tavoletti (2007) found distinct grass pea gene pools using AFLP markers with a cluster specific distribution in grass pea populations. His study showed the existence of two genetically separated groups of population.

Solis (2007) studied the level of polymorphism and the genetic relationship in different varieties of potatoes from the Chiloe Island by means of molecular markers using the amplified fragment length polymorphism (AFLP) technique as well as morphological characters. The morphologic data cluster analysis allowed the separation of two defined groups among 20 varieties studied. AFLP data defined four clusters from the cluster analysis. He observed no significant concordance between AFLP and morphology cluster analyses. This result confirmed that DNA analysis by AFLP is an efficient method for the exploration of genetic diversity in potato populations.

Production of olive oil based only on a few superior cultivars would increase yield, oil quality. But a large number of different varieties and sources of olive oil are used to produce the oil, which makes standardized oil production and marketing difficult. Murtaza (2006) had used four primer-pair combinations were used to assay 20 cotton plants from each of the 20 accessions belonging to *Gossypium hirsutum L.*, and *G. arboreum L.* from the Pakistan and US origin for AFLP based genetic diversity. But AFLP showed a narrow genetic base among these cultivars. When twenty-six landraces of black gram collected from Orissa, India were analysed using AFLP markers by Sivaprakash et al. (2004), they revealed a good polymorphism among the cultivars. The constructed dendrogram revealed three major clusters.

Ntundu (2004) selected 100 bambara groundnut [*Vigna subterranea* (L.) Verdc] landraces to study the genetic diversity using AFLP technique. Genetic diversity was found among the species based on Jaccard's variability index (ranging from 0.1 to 0.68) and all the landraces formed two major groups in cluster analysis.

Jamago et al. (2003) studied 90 accessions of *Vigna radiata* using AFLP technique which proved to be an efficient tool for analyzing genetic diversity. AFLP marker proved to be very informative to monitor the genetic diversity of *Albizia* species (Aparajita and Rout, 2010). *Senecio ovatus* subsp. *ovatus* and *S. germanicus* subsp. *germanicus* form distinct entities through AFLP fingerprint data as shown by Oberprieler (2011).

Morales et al. (2013) evaluated the genetic diversity of 20 garlic cultivars by combining morphological characters and AFLP molecular marker data. Bhattacharyya et al. (2017) used AFLP markers in gene mapping and in early selection of medicinally important orchid *Dendrobium thyrsiflorum*. Shuhua (2016) has used morphological traits

e-ISSN: E 2347-226X
p-ISSN: 2319-9857

in clustering 6-natural populations of *Rosa platyacantha* and analyzed the data with AFLP based markers. The populations were mainly grouped with similar altitudes and geographic distances. Bryan et al. (2017) showed that AFLP is highly suitable for the evaluation of within and between accession diversity in gene banks.

DNA markers have been useful in molecular systematics and molecular biology. Among the different molecular biological techniques, AFLP analysis has proven as an applicable genotyping method. It has high degrees of reproducibility and discriminatory power. AFLP markers are considered to be non-coding and are selectively natural. It has been successfully applied in different branches like breeding, taxonomy, microbiology, ecology, population genetics, evolution biology etc. AFLP based marker assisted analysis gives specific and reliable data that can be correlated or evaluated with other analytical methods. It is used in linkage mapping and in population studies. AFLP provides sufficient data for the determination of strains or variants in a short time with the highest degree of specification. Genotype databases can be created which can be used by different laboratories for a variety of purposes. AFLP is regarded as one of the best tools for the understanding and relating of the genetic relationships between and within species.

Reference

1. Aggarwal R. K., Brar D. S., Nandi S., Huang N. et al. Phylogenetic relationships among Oryza species revealed by AFLP markers. Theoretical and Applied Genetics. 1999;98: 1320-1328
2. Aparajita S. and Rout G. R. Molecular analysis of Albizia specie susing AFLP markers for conservation strategies. Journal of Genetics, 2010;89:1.
3. Barchi L., Bonnet J., Boudet C., Signoret P. et al. A high-resolution, intraspecific linkage map of pepper (Capsicum annuum L.) and selection of reduced recombinant inbred line subsets for fast mapping. Genome. 2007 Jan; 50(1):51-60
4. Bhattacharyya P., Ghosh S., Mandi S. S., Kumaria S. et al. Genetic variability and association of AFLP markers with some important biochemical traits in Dendrobium thyrsiflorum, a threatened medicinal orchid. South African Journal of Botany. 2017;109: 214-222
5. Bryan G. J., McLean K., Waugh R., Spooner D. M. Levels of Intra-specific AFLP Diversity in Tuber-Bearing Potato Species with Different Breeding Systems and Ploidy Levels. Frontiers in Genetics, 8 (119). 2017.00119
6. Chaitieng B., Kaga A., Han O.K., Wang X. W., et al. Mapping a new source of resistance to powdery mildew in mung bean. Plant Breeding. 2002;121, 521—525
7. Colombo C., Second G. and Charrier A. Genetic Relatedness between Cassava (Manihot esculenta Crantz) and M. flabellifolia and M. peruviana based on both RAPD and AFLP markers. Genetics and Molecular Biology, 2000 vol.23 No.2, p.417-423
8. Coulibaly S., Pasquet R. S., Papa R., Gepts P. AFLP analysis of the phenetic organization and genetic diversity of Vigna unguiculata L. Walp. reveals extensive gene flow between wild and domesticated types.Theor Appl Genet. 2002;104:358–366
9. Cronn R.C., Zhao X., Paterson A.H. and Wendel J.F. Polymorphism and concerted evolution in a tandemly repeated gene family: 5S ribosomal DNA in diploid and allopolyploid cottons. J. Mol. Evol. 1996;42: 685–705
10. Cruz de la, Marcelino, Monte, Juan V., et al. Genetic relationships within and among Iberian fescues (Festuca L.) based on PCR-amplified markers. Genome, 2006 Volume 49, Number 9, 1 pp. 1170-1183 (14)
11. De Joode D.R. and Wendel J.F. Genetic diversity and origin of the Hawaiian Islands cotton, Gossypium tomentosum. Amer. J. Bot. 1992;79: 1311–1319.
12. Gimenes M. A., Lopes C.R. and Valls J.F.M. Genetic relationships among Arachis species based on AFLP Genetics and Molecular Biology, 2002;25, 3, 349-353.
13. Hawkins J. S., Pleasants J. and Wendel J.F. Identification of AFLP markers that discriminate between cultivated cotton and the Hawaiian island endemic, Gossypium tomentosum Nuttall ex Seeman. Genetic Resources and Crop Evolution. 2005;52: 1069–1078.
14. Ipek M., Ipek A. and Simon P. W. Sequence homology of polymorphic AFLP markers in garlic (Allium sativum L.). Genome. 2006;49(10):1246-55.
15. Jamago J.M., Borromeo, T.H., Hautea D.M., Altoveros N.C. et al. Morpho-agronomic and molecular diversity of the Philippines mungbean (Vigna radiata (L) Wilczek) germplasm. Philippine Journal of Crop Science (Philippines) v. 2003;28 (Supplement no. 1) p. 13.
16. Kaga A., Tomooka N., Vaughan D.A, Saravanakumar P. AFLP and RAPD analyses of intra and interspecific variation in some Vigna subgenus Ceratotropis (Leguminosae)species. Australian Journal of Botany. 2004;52(3) 417 – 424.

e-ISSN: E 2347-226X
p-ISSN: 2319-9857

17. Kardolus J. P., Van Eck H. J. Van den Berg R. G. The potential of AFLPs in biosystematics: a first application in Solanum taxonomy (Solanaceae). Plant Systematics and Evolution. 1998;210: 87-103.
18. Maccaferri M., Stefanelli S., Rotondo F., Tuberosa R. et al. Relationships among durum wheat accessions. I. Comparative analysis of SSR, AFLP and phenotypic data. Genome, 2007 Volume 50, Number 4, pp. 373-384(12).
19. Massawe F. J., Dickinson M., Roberts J. A., Azam-Ali S.N. Genetic diversity in bambara groundnut (Vigna subterranea (L.) Verdc) landraces revealed by AFLP markers. Genome. 2002;45: 1175–1180.
20. Morales R.G.F., Resende J.T.V., Resende F.V., Delatorre C.A., et al. Genetic divergence among Brazilian garlic cultivars based on morphological characters and AFLP markers. Genetics and Molecular Research. 2013;12 (1): 270-281.
21. Murtaza N. Cotton genetic diversity study by AFLP markers. Electronic Journal of Biotechnology. 2006 Vol.9 No.4, Issue of July 15, 2006.
22. Ntundu W. H., Bach I. C., Christiansen J.L., Andersen S.B. Analysis of genetic diversity in bambara groundnut [Vigna subterranea (L.) Verdc] landraces using amplified fragment length polymorphism (AFLP) markers. African Journal of Biotechnology. 2004 Vol. 3 (4), pp. 220-225.
23. O'hanlon P. C., Peakall R. Briese D. T. Amplified fragment length polymorphism (AFLP) reveals introgression in weedy Onopordum. Molecular Ecology. 1999; 8, 1239–1246.
24. Oberprieler C., Hart S., Schauer K., Meister J. et al. Morphological, phytochemical and genetic variation in mixed stands and a hybrid swarm of Senecio germanicus and S. ovatus (Compositae, Senecioneae). Plant Systematics and Evolution. 2011 Vol. 293, No. 1/4, 177-191.
25. Ouédraogo J. T., Gowda B. S., Jean M., Close T. J., et al. An improved genetic linkage map for cowpea (Vigna unguiculata L.) Combining AFLP, RFLP, RAPD, biochemical markers, and biological resistance traits. Genome. 2002b;45: 175–188.
26. Ouédraogo J. T., Tignegre J.B., Timko M. P. Belzile F. J. AFLP markers linked to resistance against Striga gesnerioides race 1 in cowpea (Vigna unguiculata). Genome. 2002a;45: 787–793.
27. Pafundo S., Agrimonti C. Marmiroli N. Traceability of Plant Contribution in Olive Oil by Amplified Fragment Length Polymorphisms. J. Agric. Food Chem. 2005;53: 6995-7002.
28. Seelanan T., Schnabel A., Wendel J.F. Congruence and consensus in the cotton tribe. Syst. Bot. 1997;22: 259–290.
29. Sholihin and Hautea D.M. Molecular mapping of drought resistance in mungbean (Vigna radiata): 1. Linkage map in mungbean using AFLP markers Jurnal Bioteknologi Pertanian, 2002 Vol. 7, No. 1: 17-24.
30. Sun J. W., Jin D. M., Zhou C. H., Yang Q. K., et al. Identification of Porphyra Lines (Rhodophyta) by AFLP DNA Fingerprinting and Molecular Markers. Plant Molecular Biology Reporter. 2005;23: 251–262.
31. Shuhua Y., Ning G., Hong GE. Morphological andAFLP-Based Genetic Diversity in Rosa platyacantha Population in Eastern Tianshan Mountains of Northwestern China. Horticultural Plant Journal. 2016;2 (1): 55–60.
32. Simoes-Araujo J. L., Rodrigues R L., de A. Gerhardt L. B., Mondego J. M.C., et al. Identication of differentially expressed genes by cDNA-AFLP technique during heat stress in cowpea nodules. FEBS Letters. 2002;515: 44-50
33. Sivaprakash K. R., Prashanth S. R., Mohanty B. P., Parida A. Genetic diversity of black gram (Vigna mungo) landraces as evaluated by amplified fragment length polymorphism markers. Current Science, 2004 Vol. 86, No. 10, 1411-1416.
34. Solis J. S., Ulloa D. M., Rodríguez L. A. Molecular description and similarity relationships among native germplasm potatoes (Solanum tuberosum ssp. tuberosum L.) using morphological data and AFLP markers. Electronic Journal of Biotechnology. 2007 Vol.10 No.3.
35. Tavoletti S., Iommarini L. 2007. Molecular marker analysis of genetic variation characterizing a grasspea (Lathyrus sativus) collection from central Italy. Plant Breeding Journal compilation. 2007.
36. Vos P., Hogers R., Bleeker M., Reijans M., et al. AFLP: a new technique for DNA fingerprinting. Nucleic Acids Research.1995;23:4407-4414.
37. Welsh J. and McClelland M. Nucleic Acids Res., 1991;19, 6823–6831.
38. Wendel J.F., Schnabel A., Seelanan T. 1995. Bidirectional interlocus concerted evolution following allopolyploid speciation in cotton (Gossypium). Proc. Natl. Acad. Sci. 1995;92: 280–284.
39. Xu M. L, Melchinger A. E., Xia X. C. Lübberstedt T. High-resolution mapping of loci conferring resistance to sugarcane mosaic virus in maize using RFLP, SSR and AFLP markers. Molecular and General Genetics. 1999;261: 574-581.
40. Zabeau M. European Patent Application. 1993. Publication No. 0534858A1.