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Analysis of Genome Differentiation Between High Toxin and Low Toxin Accessions of *Lathyrus sativus* Using RAPD Markers

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Abstract: The random amplified polymorphic DNA (RAPD) markers were used to study the nature of genome differentiation between natural populations of *L. sativus*. The degree of band sharing was used as a criterion to calculate the genetic distance and to construct phylogenetic trees. The 28 populations from Pakistan, India and Ethiopia differed in the amount of β -N-oxalyl-L- α , β -diamino propionic acid (β -ODAP) in their kernels. Irrespective of their geographical diversity high toxin and low toxin varieties clustered into genetic groups in the phylogenetic tree. The results would suggest that the variation between populations in the neurotoxin content is governed by genetic factors and has occurred for this trait during evolution

Key words: RAPD, genome, differentiation, *L. sativus*, phylogenetic tree

Introduction

Lathyrism has been known to occur in grasspea areas of the world for a long time. The disease was recorded first in the Narowal area of district Sialkot (Shah, 1939). In many parts of the world, efforts are being to develop toxin free low ODAP variation in grasspea (Campbell *et al.*, 1994). In Pakistan *Lathyrus sativus*, locally known as "matter" is grown in an area of 140 thousand hectares every year and the production is around 70 thousand metric tones per annum Khawaja *et al.*, 1996). Several species of *Lathyrus* synthesize one or more types of toxic non-protein amino acids. Ressler *et al.* (1961) identified toxic amino acid α , γ , diminobutyric acid in *L. latifolius* and *L. sylvestris* which cause neurolathyrism in animals. Since the discovery of β -ODAP as a causative agent several attempts have been made by plant breeders to eliminate the toxic substance by careful selection so far and through hybridization between low and high toxin varieties. The breeding efforts have been unsuccessful. Campbell and Briggs (1987) reported *L. sativus* variety (Var. 8246) having a low ODAP content of 0.0259 to 0.0401% w/w of dry seed. Random amplified polymorphic DNAs (RAPDs), may also have a role in molecular study of genome differentiation. The RAPD assay does not require prior knowledge of the target DNA sequence. The method has been variously termed as RAPD (Williams *et al.*, 1990), AP-PCR (Arbitrary primed polymerase chain reaction, Welsh *et al.*, 1991), DAFs (DNA amplification fingerprints, Caetano-Anolles *et al.*, 1991) and AFLPs (amplified fragments length polymorphism, Vosa *et al.*, 1995). Many opportunities now exist for the exploitation of genetic markers in crop improvement programs. Central to this challenge is the requirement to locate markers to specific regions of the genome, which are important in controlling the expression of traits of economic and biological importance. Delourme *et al.* (1994) employed bulk segregation analysis to identify the RAPD markers linked to the restorer gene (Rf_1) used in "Ogura radish cytoplasmic male sterility" of rapeseed. Chalmers *et al.* (1993), used a double haploid population of barley in combination with PCR-based polymorphic assay. Their results indicated that double haploids in combination with RAPD and bulk segregation analysis provided an efficient method for locating QTLs in barley. Yang and Quiros (1993) studied the usefulness of RAPD markers in the identification and classification of celery cultivars. Rowland and Levi (1994), Wachira *et al.* (1995), Virk *et al.* (1995), Zhu *et al.* (1998) and Khan *et al.* (2000) also made similar studies in different crop species of the genome differentiation in

evolution.

Despite the human health problems and the serious consequences of the disease in mankind, no attempts have been made at molecular level to study the nature of genome differentiation between natural populations of *L. sativus*. In this investigation we have used RAPD markers to compare the genetic polymorphism in the genomes of natural populations of *L. sativus*. Using the proportion of shared PCR amplified DNA sequences as a criterion, phylogenetic trees would be constructed. The estimated genetic distances in the phylogenetic trees between populations would tell us about the nature of phylogenetic differentiation between populations in evolution.

Materials and Methods

Plant materials, DNA isolation: The research project was carried out in the Institute of Biological Sciences, University of Wales, Aberystwyth during 1992-1996 using twenty eight accessions of *Lathyrus sativus* occurring as natural populations in Pakistan and Ethiopia. The seeds of *L. sativus* high toxin and low toxin varieties 8507 and 8246 respectively were obtained from Dr. Campbell, Agriculture Canada Research Station, Manitoba Canada. The variety 8246 was a selection from low toxin Indian variety Pusa-24.

For the extraction of nucleic acids the plants were grown under glasshouse conditions. Young leaves were collected when seedlings were about two weeks old shoots for DNA extraction. The plant material was washed thoroughly in distilled water, dried between folds of a blotting paper and frozen immediately in liquid nitrogen at -70°C. The DNA extraction method was used as described by Khan *et al.* (2000).

Polymerase chain reaction (PCR) and analysis of RAPD data:

PCR analysis was run on a Perkin Elmer 9600 PCR machine. PCR conditions were used as described by Khan *et al.* (2000). PCR products were separated in 1.2% agarose gel containing 0.5 μ g/ml ethidium bromide. Separated PCR products were visualized under UV light and photographed to examine the banding pattern of different varieties. The RAPD generated banding profiles were analyzed as described by Khan *et al.* (2000).

Results and Discussion

Genetic differentiation between varieties of *L. sativus*: The genomic DNA of 28 *L. sativus* varieties was extracted and

Khan and Majid: RAPD, genome, differentiation, *L. sativus*, phylogenetic tree

Table 1: Matrix of 'F' and 'd' values estimated for 28 varieties of *L. sativus*. (Proportional values of shared fragments (F) are on the right of the diagonal and the genetic distance values (d) on the left of the diagonal.

Var.	1	2	3	4	5	6	7	8	9	10	11	12	13	14
1	***	.8890	.7030	.7890	.5790	.8000	.7690	.7570	.7180	.7000	.8110	.7780	.7570	.6830
2	.0040	***	.8210	.8000	.6500	.7140	.7800	.8210	.7800	.7620	.8210	.8420	.8210	.7440
3	.0121	.0067	***	.7320	.6340	.8370	.7620	.8500	.8100	.7910	.7500	.8210	.8500	.7730
4	.0081	.0076	.0107	***	.7140	.7730	.7910	.7800	.07910	.7730	.8290	.7500	.7320	.6670
5	.0190	.0148	.0157	.0115	***	.6820	.6050	.6340	.6980	.6820	.6340	.6500	.6340	.5780
6	.0076	.0115	.0060	.0088	.0131	****	.7560	.8370	.8440	.8260	.7910	.8100	.7910	.7230
7	.0089	.0085	.0093	.0080	.0174	.0095	***	.8570	.8180	.8000	.8570	.8290	.8100	.7390
8	.0095	.0067	.0055	.0085	.0157	.0060	.0050	***	.8570	.8370	.8000	.9230	.8500	.7730
9	.0113	.0085	.0072	.0080	.0123	.0057	.0068	.0052	***	.8890	.8570	.8780	.8570	.7830
10	.0122	.0093	.0080	.0088	.0131	.0065	.0076	.0060	.0040	***	.8370	.8570	.8370	.7660
11	.0071	.0067	.0098	.0063	.0157	.0080	.0052	.0076	.0052	.0060	***	.8720	.8000	.7270
12	.0085	.0058	.0067	.0098	.0148	.0072	.0063	.0027	.0044	.0052	.0046	***	.8720	.7910
13	.0095	.0067	.0055	.0107	.0157	.0080	.0072	.0055	.0052	.0060	.0076	.0046	***	.8180
14	.0131	.0101	.0088	.0392	.0191	.0111	.0103	.0088	.0083	.0091	.0109	.0080	.0068	***
15	.0095	.0067	.0055	.0085	.0157	.0080	.0072	.0055	.0072	.0080	.0076	.0067	.0055	.0068
16	.0131	.0123	.0109	.0076	.0139	.0091	.0103	.0109	.0083	.0091	.0088	.0101	.0109	.0118
17	.0081	.0076	.0085	.0052	.0166	.0109	.0080	.0085	.0101	.0090	.0085	.0098	.0085	.0095
18	.0131	.0101	.0109	.0139	.0220	.0132	.0124	.0109	.0124	.0132	.0109	.0101	.0109	.0118
19	.0095	.0067	.0055	.0085	.0157	.0080	.0072	.0076	.0072	.0080	.0076	.0067	.0055	.0068
20	.0122	.0115	.0101	.0088	.0156	.0103	.0117	.0123	.0095	.0103	.0101	.0115	.0101	.011
21	.0104	.0098	.0085	.0093	.0166	.0109	.0101	.0107	.0101	.0109	.0107	.0122	.0085	.0117
22	.0130	.0098	.0085	.0093	.0166	.0088	.0101	.0085	.0080	.0088	.0085	.0122	.0085	.0095
23	.0159	.0122	.0107	.0115	.0195	.0131	.0123	.0085	.0101	.0109	.0107	.0122	.0101	.0139
24	.0139	.0104	.0113	.0122	.0209	.0139	.0131	.0089	.0131	.0115	.0113	.0130	.0113	.0148
25	.0139	.0159	.0199	.0148	.0282	.0195	.0186	.0168	.0186	.0195	.0139	.0190	.0199	.0235
26	.0129	.0121	.0130	.0139	.0234	.0157	.0177	.0159	.0148	.0157	.0130	.0149	.0159	.0195
27	.0160	.0149	.0159	.0168	.027	.0157	.0177	.0159	.0148	.0157	.0130	.0149	.0159	.0195
28	.0081	.0076	.0205	.0121	.0180	.168	.0159	.0139	.0159	.0168	.0139	.0129	.0139	.0177

Varieties code: 1. Ethopian, 2. var. 8507, 3. K241, 4. K258, 5. K256, 6. K302, 7. K333, 8. K285, 9. K247, 10. K298, 11. K295, 12. K271, 13. K290, 14. K249, 15. K482, 16. K304, 17. K27, 18. K447, 19. K554, 20. K505, 21. K397, 22. K491, 23. K434, 24. K104, 25. K655, 26. K661, 27. K663, 28. var. 8246

Table 1: (Cont)

Var.	15	16	17	18	19	20	21	22	23	24	25	26	27	28
1	.7570	.6830	.7890	.6830	.7570	.7000	.7370	.6840	.6320	.6670	.6670	.6860	.6290	.7880
2	.8210	.6980	.8000	.7440	.8210	.7140	.7500	.7500	.7000	.7370	.6320	.7030	.6490	.8000
3	.8500	.7270	.7800	.7270	.8500	.7440	.7800	.7800	.7320	.7180	.5640	.6840	.6320	.5560
4	.7800	.8000	.8570	.6670	.7800	.7730	.7620	.7620	.7140	.7000	.6500	.6670	.6150	.7030
5	.6340	.6670	.6190	.5330	.6340	.6360	.6190	.6190	.5710	.5500	.4500	.5130	.4620	.5950
6	.7910	.7660	.7270	.6810	.7910	.7390	.7270	.7730	.6820	.6670	.5710	.6340	.6340	.6150
7	.8100	.7390	.7910	.6960	.8100	.7110	.7440	.7440	.6980	.6830	.5850	.6000	.6000	.6320
8	.8500	.7270	.7800	.7270	.8000	.6980	.7320	.7800	.7800	.7690	.6150	.6320	.6320	.6670
9	.8100	.7830	.7440	.6960	.8100	.7560	.7440	.7910	.7440	.6830	.5850	.6500	.6500	.6320
10	.7910	.7660	.7270	.6810	.7910	.7390	.7270	.7730	.7270	.7140	.5710	.6340	.6340	.6150
11	.8000	.7730	.8800	.7270	.8000	.7440	.7320	.7800	.7320	.7180	.6670	.6840	.6840	.6670
12	.8210	.7440	.7500	.7440	.8210	.7140	.7000	.7000	.7000	.6840	.5790	.6490	.6490	.6860
13	.8500	.7270	.7800	.7270	.8500	.7440	.7800	.7800	.7320	.7180	.5640	.6320	.6320	.6670
14	.8180	.7080	.7560	.7080	.8180	.7230	.7110	.7560	.6670	.6510	.6120	.5710	.5710	.6000
15	***	.7730	.8780	.8180	.9500	.7910	.7800	.8780	.7800	.7690	.6150	.6840	.6840	.7220
16	.0088	***	.8440	.7080	.8180	.8090	.7560	.7560	.6670	.6510	.6510	.6190	.6190	.6000
17	.0044	.0057	***	.8000	.8780	.8640	.8570	.7620	.7620	.7000	.7000	.6670	.6670	.7030
18	.0068	.0118	.0076	***	.8640	.6810	.6670	.7560	.6670	.6510	.6050	.5710	.6190	.6000
19	.0017	.0068	.0044	.0049	***	.7910	.8290	.7800	.8290	.8210	.6670	.6840	.7370	.7220
20	.0080	.0072	.0049	.0132	.0080	**	.7730	.7270	.6820	.6670	.6190	.5850	.6830	.6150
21	.0085	.0095	.0052	.0139	.0063	.0088	***	.7140	.6670	.7000	.6500	.6150	.7180	.6490
22	.0044	.0095	.0093	.0095	.0085	.0109	.0115	***	.7620	.8000	.6500	.7180	.7180	.6190
23	.0085	.0139	.0093	.0139	.0063	.0131	.0139	.0093	***	.8000	.6500	.6150	.7180	.5950
24	.0089	.048	.0122	.0148	.0067	.0139	.0122	.0076	.0076	***	.6840	.7570	.7570	.6290
25	.0168	.0148	.122	.0174	.0139	.0166	.0148	.0148	.0148	.0130	***	.6490	.8110	.6860
26	.0130	.0166	.0139	.0195	.0130	.0186	.0168	.0113	.0168	.0095	.0149	***	.8330	.7650
27	.0130	.0166	.0139	.0166	.0104	.0131	.0113	.0113	.0113	.0095	.0071	.0062	***	.7060
28	.0111	.0177	.0121	.0177	.033	.0168	.0149	.0149	.0180	.0160	.0129	.0091	.0119	***

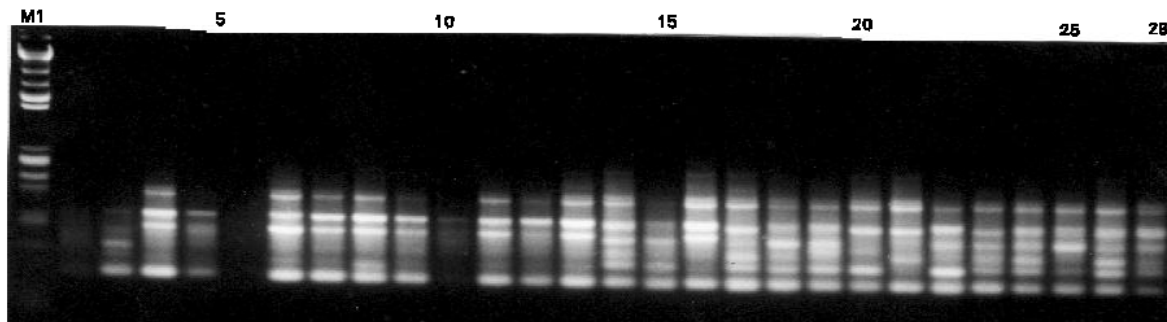


Fig. 1: PCR analysis with primer IM4 among 28 varieties. M-molecular size marker λ DNA restricted with *HindIII* and *EcoRI* indicated in kilobases. Lanes 1-28 codes listed in Table 1.

Fig. 2: Phylogenetic tree of *Lathyrus sativus* varieties. Genetic distances are indicated along each arm of the tree.

purified. The purified DNA was PCR amplified. The PCR amplified DNA sequences using RAPD primers were separated on 1.2 % agarose gel by electrophoresis. The gels were stained with ethidium bromide and photographed under UV. The banding pattern generated by IM4 (5'-GCTTAGAACC-3') primer for 28 varieties of *L. sativus* is shown in Fig. 1. The 28 varieties differed in the number of fragments amplified and in the fragment length distribution. However, most varieties showed DNA bands identical in molecular weights (Fig. 1). The amplified fragments were scored for the presence and absence of shared fragments. Similarly sequences were amplified using primer sequences IM5 (5'-GAGCTACTGT-3'), IM6 (5'-GGTACAGTCA-3') and IM2 (5'-GGTATCCTCA-3'), data not shown. For each primer sequence varieties showed several amplified DNA bands identical in length. When compared to IM4 primer sequences IM5, IM6 and IM2 produced a less level of polymorphism. The data of shared fragment lengths for *L. sativus* varieties for IM4, IM5, IM6 and IM2, primers were combined. The combined matrix was used to calculate the proportion of shared fragments " F " as described earlier. From the F ratios of the shared fragments the genetic distances were computed. In Table 1 the F value (the proportion of shared fragments between varieties) are shown on the right of the diagonal, and the d values (the

genetic distance) are shown on the left of the diagonal. The genetic distance values were put into the PHYLIP computer programme (Felsenstein, 1993). The best tree constructed using the method of Fitch and Margoliash (1967) is shown in Fig. 2. The genetic distances between different varieties were estimated by summing the genetic distance values given on the branches of the tree.

In RAPD generated dendrogram the genetic distances between varieties range from 0.00085 to 0.000794. This is substantially smaller than the genetic distances calculated for species which range between 0.01090 and 0.04396. As would be expected, the genetic distances between the two sibling species *L. sylvestris* and *L. latifolius* is greater than the genetic distance between the most widely diversified varieties of *L. sativus* (Khan *et al.*, 2000). Based on their neurotoxin contents the 28 *L. sativus* populations may be arbitrarily classified into 3 groups. They are (a) low toxin varieties (0.04 - 0.05 % β -ODAP) (b) medium toxin varieties (0.14 - 0.16 % β -ODAP) and high toxin varieties (0.20 - 0.40 % β -ODAP). The low toxin group includes varieties 8246, K663, K661 and K655. The medium toxin group includes varieties K104 and K434. The remainder (varieties from Pakistan, the Ethiopian variety and selection 8507) have high toxin content. In dendrogram the low toxin varieties cluster into one

phylogenetic group. Similarly the medium toxin varieties K104 and K434 form a phylogenetic group with very small genetic distances between them.

The major phylogenetic group in the tree is made up of high toxin varieties. This group includes 20 varieties. Within this group, there are subgroups of species that are clustered very closely. For example var. 8507 and the variety from Ethiopia, although of different geographical origin clustered together. The average ODAP value for the two varieties is 0.4%. Varieties K554, K482, K27 and K491 (ODAP values range narrowly from 0.2-0.28 %) clustered very closely together in dendrogram. K241 has a higher ODAP value of 0.4%. It is also seen in the dendrogram that the varieties K295, K333, K271, K285, K290, K298, K247 and K302 show close relationship to each other. The ODAP values for these varieties range between 0.32-0.36%. High toxin varieties K302, K258, K397, K505, K304, K447 and K. 256 show a great scatter of ODAP values ranging from 0.23 % to 0.37 %. They show a greater range of genetic distances in dendrogram. Variety K256 shows the greatest genetic divergence in phylogenetic tree.

The RAPD generated phylogenetic tree shows a close genetic relation among the 28 varieties of *L. sativus*. The estimated genetic distances reflect the degree of genome differentiation between populations from different geographic regions. The phylogenetic tree in Fig. 2 represents the best of genetic distance estimates selected from a choice of 4276 different trees. The results show that RAPD analysis may be used to study the genome differentiation at population level. It also shows that phylogenetic trees may be used for the study of genetic relatedness between varieties with different toxin contents. The 28 varieties were of different geographical origin. Low toxin variety 8246 was a selection from the Indian cultivar Pusa-24. The high toxin variety 8507 was from Canada. The Ethiopian variety is a natural population from Addis Ababa. TLC analysis and high voltage electrophoresis have confirmed that the Ethiopian variety has neurotoxin content similar to that of the high toxin variety 8507 (Getachew, 1992). The remainder varieties with high, low and medium toxin content were collected from Pakistan. Reproducible DNA fingerprints linked to neurotoxin content will be useful to screen and identify low and high toxin varieties from germplasm collections of *L. sativus*.

Several markers are tightly linked to useful genes and the method has revolutionized the gene mapping in several crop species. The RAPD phylogeny analysis as a method is applicable to only closely related groups of species. This is well exemplified in the study of *Lolium/Festuca* complex (Stammers *et al.*, 1995).

Thus the analysis of randomly amplified sequences from diverse regions of the genome will give data on the divergence of the genome as a whole. The present investigation includes studies of the nature of genome differentiation between varieties within the species of *L. sativus*. The nature of genetic differentiation between intraspecific natural populations of 28 varieties of *L. sativus* were studied by RAPD analysis. The 28 varieties indigenous to Pakistan, India and Ethiopia differed in the amount of neurotoxin (β - ODAP) in their kernels. The genetic make up differed greatly between species due to the large evolutionary divergence between them. In contrast, varieties within a species show differences of a smaller scale. The range of genetic distances between varieties (0.00085 to 0.000974) is smaller than the range of genetic distances between species (0.01090 to 0.04396) in the respective dendrograms (Khan *et al.*, 2000). The varieties within species are not reproductively separated and should therefore show

genetic similarity between them.

All four primers used in this investigation revealed polymorphism which suggests substantial genetic differentiation between 28 natural populations of *L. sativus*. Similar findings were reported in *Brassica* populations (Hu and Quiros, 1991). In the dendrogram constructed using phylogenetic distances, the 28 varieties clustered into 3 groups. The low toxin varieties clustered into a group with close genetic distances between them. The high toxin varieties show substantial genetic differentiation from low toxin varieties and they form a different genetic group. Variety 8507 and the Ethiopian accession have higher toxin content (0.4 %). Irrespective of their diverse geographical origins, they show close genetic affinity. The results would suggest that the variation in toxin content between varieties is governed by a strong genetic factor and the differentiation between varieties for this factor has occurred during evolution. The results show that RAPD analysis may be used to generate reliable molecular markers to distinguish between varieties different in toxin content. A larger selection of primer sequences, a greater number of varieties of diverse geographic origins and of different toxin content would improve significantly the resolution of experimental results.

References

- Caetano-Anolles, B. J. G., Bassab and P. M. Gresshoff, 1991. DNA amplification fingerprinting using very short arbitrary oligonucleotide primers. *Biotechnol.*, 9: 553-556.
- Campbell, C. G. and C. J. Briggs, 1987. Registration of low neurotoxic content *Lathyrus* germplasm LS 8246. *Crop. Sci.*, 27: 821.
- Campbell, C. G., R. B. Mehra, S. K. Agrawal, Y. Z. Chen, A. M. Monheim, H. I. T. Khawaja, C. R. Yadav and J. U. Tay, 1994. Current status and future strategy in breeding grasspea (*Lathyrus sativus*) in : Expanding the production and use of cool season food legumes. pp 617-630. (ed. S. J. Muehlbauer and W. J. Kaiser). kluwer Academic publishers, Dordrecht.
- Chalmers, K. J., U. M. Baraua, C. A. Hackett, W. T. B. Thomas, R. Waugh and W. Powell, 1993. Identification of RAPD markers linked to the genetic factors controlling the milling energy requirement of barley. *Theor. Appl. Genet.*, 87: 314-320.
- Delourme, R., A. Bouchereau, N. Hubert and M. Renard, 1994. Identification of RAPD markers linked to a fertility restorer gene for the Ogura radish cytoplasmic male sterility of rapeseed (*Brassica napus* L.). *Theor. Appl. Genet.*, 88: 741-748.
- Felsenstein, J., 1993. PHYLIP (Phylogenetic Inference Package) version 3.572c. University Herbarium, University of California, Berkeley, CA.
- Fitch, W. M. and E. Margoliash, 1967. Construction of phylogenetic tree. *Sciences*, 155: 279-284.
- Getachew, A., 1992. Developmental variation in the biosynthesis of neurotoxin in *L. sativus*. M. Sc Thesis, submitted to University of Wales, Aberystwyth.
- Hu, J. and C. F. Quiros, 1991. Identification of broccoli and cauliflower cultivars with RAPD markers. *Plant Cell Rep.*, 10: 505-511.
- Khan, M. Q., S. D. A. Gardezi and R. K. J. Narayan, 2000. Study of Phylogenetic Relationships Between Different Species of Genus *Lathyrus* using RAPD Markers. *Sarhad J. Agric.*, 16: 411-421.

Khan and Majid: RAPD, genome, differentiation, *L. sativus*, phylogenetic tree

- Khawaja, H. I. T., U. Ihsan and K. Iftikhar, 1996. Prevalence of Lathyrism in Pakistan in: *Lathyrus sativus* and Lathyrism in Pakistan: Proceedings of the first and second *Lathyrus* Conferences held at National Agriculture Research Center, Islamabad, in 1992 and 1993. pp.5-11.
- Ressler, C., P. A. Redstone and R. H. Esenberg, 1961. Isolation and identification of neuroactive factor from *Lathyrus latifolius*. *Science*, 134: 180-190.
- Rowland, L. J. and A. Levi, 1994. RAPD base genetic linkage map of blueberry derived from a cross between diploid species (*Vaccinium darrowium* and *V. elliotii*). *Theor. Appl. Genet.*, 87: 863-868.
- Shah, S. R. A., 1939. A note of some cases of lathyrism in Punjab village. *Ind. Med. Gaz.*, 74: 385-388.
- Stammers, M., J. Harris, G. M. Evans, M. D. Hayward and J. W. Forster, 1995. Use of random PCR (RAPD) technology to analyze phylogenetic relationships in the *Lolium/Festuca* complex. *Heredity*, 74: 19-27.
- Virk, P. S., B. V. Ford-Lloyd, M. T. Jackson and H. John, 1995. Use of RAPD for the study of diversity within plant germplasm collections. *Heredity*, 74: 170-179.
- Vosa, P., R. Hogers, M. Bleeker, M. Reijans, T. Van de Lee, M. Hornes, A. Frijters, J. Peleman, M. Kuiper and M. Zabeau. 1995. AFLP: a new technique for DNA fingerprinting. *Nucleic Acids Res.*, 23: 4407-4414.
- Wachira, F. N., R. Waugh, C. A. Hackett and W. Powell, 1995. Detection of genetic diversity in tea (*Camellia sinensis*) using RAPD markers. *Genome*, 38: 201-210.
- Welsh, J., R. J. Honeycutt, M. McClelland and B. W. S. Sobral, 1991. Parentage determination in maize hybrid using arbitrary primed polymerase chain reaction (AP-PCR). *Theor. Appl. Genet.*, 82: 473-476.
- Williams, J. G. K., A. R. Kubelik, K. J. Livak, J. A. Rafalski and S. V. Tingey, 1990. DNA polymorphism amplified by arbitrary primers are useful as genetic markers. *Nucleic Acids Res.*, 18: 6531-6535.
- Yang, X. and C Quiros, 1993. Identification and classification of celery cultivars with RAPD markers. *Theor. Appl. Genet.*, 86: 205-212.
- Zhu, J., M. D. Gale, S. Quarrie, M. T. Jackson and G. J. Bryan, 1998. AFLP marker for the study of rice biodiversity. *Theor. Appl. Genet.*, 96: 602-611.