ISSN 0258-7122
Bangladesh J. Agril. Res. 35(2) : 313-322, June 2010

# MOLECULAR CHARACTERIZATION OF ONION (*Allium cepa*) USING RAPD MARKERS

MANIRUZZAMAN[1], M. E. HAQUE[2], M. M. HAQUE[3]
M. A. SAYEM[4] AND M. AL- AMIN[5]

## Abstract

A polymerase chain reaction (PCR) based approach, namely random amplified polymorphic DNA (RAPD) analysis was applied to l0 varieties of onion *(Allium cepa)* in order to assess the degree of polymorphism within the genes and to investigate if this approach was suitable for genetic studies of onion. For this study, ten cultivars of onion were evaluated for variability using a set of 15 random l0-mer primers. The polymorphisms in PCR amplification products were subjected to the unweighed pair group method for arithmetic averages (UPGMA) and plotted in a phenogram. The dendogram constructed from the similarity data showed that all the cultivars analyzed were related. Among them, 12 of the primers revealed scorable (168 bands) polymorphisms between cultivars of *A. cepa* and the rest did not show polymorphism in their genetic level. In this study, it was found that Bermis and India-2 were more dissimilar and on the other hand, Faridpuri and Bhati were the most similar in their genetic level.

Keywords: RAPD, onion, genetic diversity, polymorphism.

## Introduction

Onions *(Allium spp)* are the second most valuable vegetables in the world following only tomato. Despite their economic significance, knowledge of onion's genetic diversity and resource is limited. The qualitative and quantitative improvement of plant depends on the available gene pool and its manipulation. *Allium fistulosum* L (Japanese bunching onion) is widely cultivated from Siberia to Tropical Asia and shows the largest morphological variability in China, Korea, and Japan (Inden and Asahira, 1990, Haishima *et al.*, 1993).

*A. fistulosum* to be paraphyletic in relation to *A. altaicum* with a weak sister group relationship of both to *A. galanthum* Kar. et Kir. Van Raamsdonk, Smiech, and Sandbrink (1997) found *A. altaicum* and *A. fistulosum* to be the closest relatives in an analysis of mostly morphological characters and proposed the

[1]Scientific Officer, Biotechnology Division, Bangladesh Agricultural Research Institute (BARI), Joydebpur, Gazipur 1701, [2]Scientific Officer, Biotechnology Division, Bangladesh Agricultural Research Institute (BARI), Joydebpur, Gazipur 1701, [3]Senior Scientific Officer, Spices Research Centre, BARI, [4]Agriculture Extension Officer, DAE, [5]Principal Scientific Officer, Biotechnology Division, BARI, Joydebpur, Gazipur 1701, Bangladesh.

parphyly of A. *altaicum* with respect to *A. fistulosum* on the basis of RAPD data. A completely different hypothesis was published recently by Dubouzet *et al.* (1997), who used dot blot hybridization with RAPD probes and found *A. galanthum* to be the sister taxon of *A. fistulosum. A altaicum* in this analysis is the closest relative of both taxa. Friesen *et al.* (1998) conducted a study on RAPD and noncoding chloroplast DNA, reveal a single origin of the cultivated *A. fistulosum* from *A. altaicum*

We used RAPD analysis (Welsh and McClelland, 1990; Williams *et al.*, 1990). RAPD analyses reveal even small genetic differences, since a large part of the nuclear genome will be scanned, as can be seen by mapping studies of segregating markers in a wide range of plant families (e.g., Rieseberg *et al.*, 1993; Bachmann and Hombergen, 1996; Serquen *et al.*, 1997), and are successfully used for clarification of the phylogeographical questions (Gabrielson *et al.*, 1997; Friesen and Herrmann, 1998; Purps and Kadereit, 1998; Tollefsrud *et al.*, 1998; Friesen *et al.*, 1999). Another advantage of this method is that it is less expensive and can be performed more rapidly than most other methods (Morell *et al.*, 1995). However, RAPD techniques have some limitations, such as low reproducibility of some bands and the uncertain homology of co-migrating fragments in gel electrophoresis (Van der Zande and Bijlsma, 1994; Harris, 1995; Pillay and Kenny, 1995; Rieseberg, 1996). Most of the limitations of RAPD analysis can be overcome by carefully adjusting the reaction and detection conditions (Bachmann, 1997; Colosi and Schaal, 1997; Friesen and Klaas, 1998).

Several reasons were considered in literature which makes cladistic analysis inappropriate for RAPD data. Arguments against parsimony are founded on possible scoring of non-homologous bands, lack of genetic independence, the biased screening of only variable parts of the genome, or the lack of appropriate models of character evolution (Backeljau *et al.*, 1995). As most of the arguments also apply to morphological characters or DNA with unknown functional constraints ("noncoding" DNA, e.g., spacer and intron sequences) they could preclude the use of cladistics generally. Moreover, biased data might also influence other data analysis algorithms. We can only see one severe restriction for parsimony analysis of RAPD data. Cladistic theory and analysis relies on bi- or multifurcating lineages. It is inappropriate for the analysis of relationships within species or under inclusion of allopolyploids, where reticulate lineage relationships occur. RAPD studies are mostly used at taxonomic ranks where DNA sequences or RFLPs fail to detect differences between accessions or taxa (Bachmann, 1997; Wolfe and Liston, 1998). At this level, gene flow between the organisms under study is generally possible, thus violating a major assumption of cladistic theory. Accordingly, distance-based, phenetic algorithms are normally used to analyze RAPD data. In our experience, cladistic analyses could

nevertheless be applied, in addition to phenetic analysis methods. In cases where data contain strong reticulate structure, parsimony will fail to find resolved trees (null hypothesis), whereas phenetic methods in all cases represent data in a tree-like way. On the other side, when trees or parts of trees are stable in parsimony analyses, this can be taken as a hint that the data (sub) set contains a reliable phylogenetic signal which is not swamped by gene flow (Roelofs and Bachmann, 1997). This signal will be detected by most other methods of data analysis too, but lacking the internal control against reticulate structures (Blattner and Friesen, in preparation). Combining cladistic and phenetic analysis methods thus allows more insights into data structure than excluding one method due to theoretical considerations. In our study of *Allium* sect. *Schoenoprasum,* we show the advantages of the use of several analysis methods to understand the evolutionary history of closely related taxa.

In this study I selected nine varieties and one line (OF-5) were taken to investigate the genetic relation among the varieties. It was found that Faridpuri and Bhati were the most similar (83.87%) and Bermis and India-2 were the most dissimilar (65.71%) and the others were more or less similar in their genetic level.

## Materials and Method

The study was conducted from January 2007 to June 2008 at Biotechnology Division, Bangladesh Agricultural Research Institute, (BARI), Gazipur.

### *Plant material*

A total of 9 varieties and one line *of Allium spp.*, where some were BARI released, two of them were Indian and one of them was Bermis viz., BARI Peas-I, BARI Peas-2, BARI Peas-3, India-I, India-2, Bermis, White Onion, OF-5, Faridpuri, and Bhati.

### *Isolation of DNA*

Total DNA was isolated by CTAB method with slight modifications according to MaaB and Klass (1995). After treatment with 10 µg/ml RNase A for half hour at 3 7°C, the DNA was purified with propanol. The purified DNA was dissolved and stored in TE buffer, and the concentration was determined fluorometrically.

### *RAPD analysis*

Amplification was carried out using 15 arbitrary 10 bp primers (OPA-4, OPA-9, OPA- 15, OPA-16, OPAB-4, OPAB-18, OPG-13, OPB-6, OPC-5, OPC-7, OPC-9, OPD-1, OPD-3, OPE-17 and RE-01), obtained from Biobasic, Canada. The primers were chosen after initial screening of more than 25 different primers, based on the production of distinct and reproducible bands in PCR reactions.

Twenty µl of the isolated DNA were used for PCR (approximately 100 ng) amplification. The amplification conditions were optimized according to Friessian *et al.* (1997). Full of the reaction mixtures was separated on 1.5% agarose gel in lx TAE buffer followed by staining with ethidium bromide and were documented using UV protected gel documentation system. The DNA profiles were scored manually, directly from photographs of the gels, by assigning a value of 1 for band presence and 0 for band absence. The scores of band presence or absence were then used to calculate a pairwise genetic distance matrix using different coefficients. Finally, a phenogram based on UPGMA cluster analysis of the genetic distance matrix was prepared with help of the NTSYS-pc program (Applied Biostatistic Inc. New York, 1993). Similarity of RAPD patterns was determined by the calculation of F- values.

**Results and Discussion**

Fifteen primers were used to analyze the 10 varieties of *Allium spp.* Among them, 12 primers produced 168 interpretable polymorphic bands. Each primer produced 5 to 8 bands per individual.

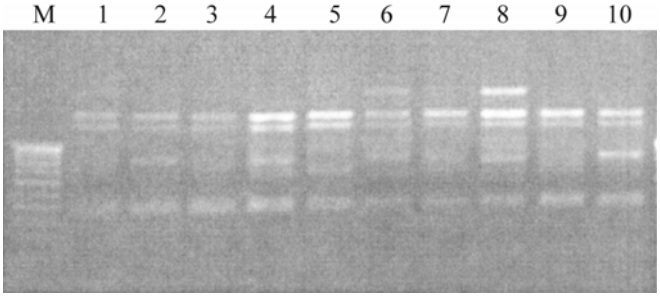


Fig.1. Gel photograph with primer OPD-01 of 10 onion cultivars; 1. BARI-1, 2. BARI-2, 3. BARI-3, 4. India-1, 5. India-2, 6. Bermis, 7. White, 8. OF-5, 9. Faridpuri, 10. Bhati and M=100 bp DNA ladder.

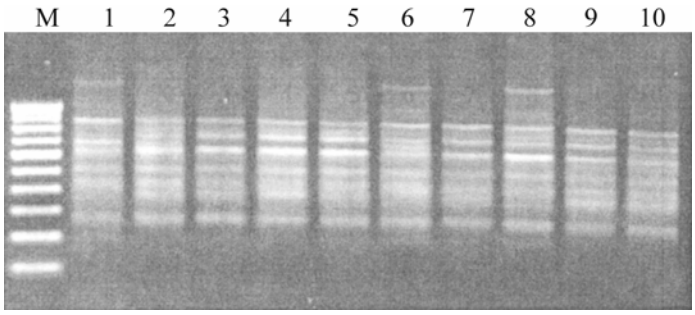


Fig.2: Gel photograph with primer OPC-O5 of 10 onion cultivars; 1. BARI-1, 2. BARI-2, 3. BARI-3, 4. lndia-1, 5. India-2, 6. Bermis, 7. White, 8. OF-5, 9. Faridpuri, 10. Bhati and M=100 bp DNA ladder.

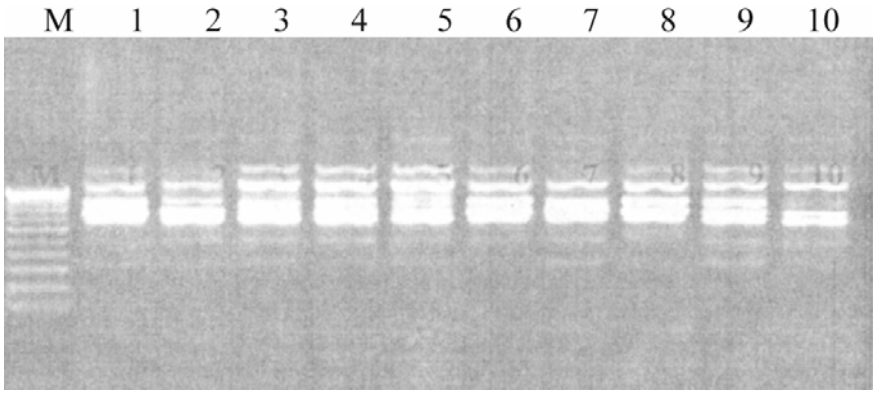


Fig.3. GeI photograph with primer OPD-03 of 10 onion cultivars; 1. BARI-1, 2. BARI-2, 3. BARI-3, 4. lndia-1, 5. India-2, 6. Bermis, 7. White, 8. OF-5, 9. Faridpuri, 10. Bhati and M=100 bp DNA ladder.

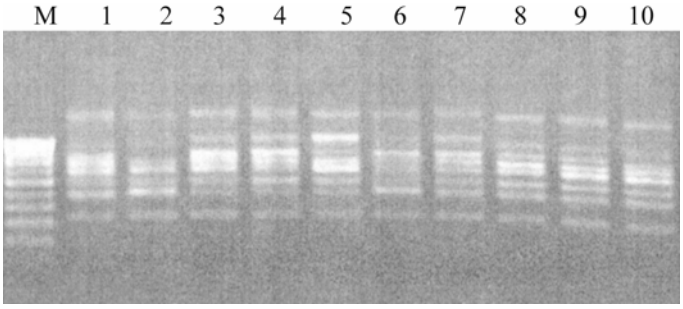


Fig.4. GeI photograph with primer OPD-7 of 10 onion cultivars; 1. BARI-1, 2. BARI-2, 3. BARI-3, 4. lndia-1, 5. India-2, 6. Bermis, 7. White, 8. OF-5, 9. Faridpuri, 10. Bhati and M=100 bp DNA ladder.

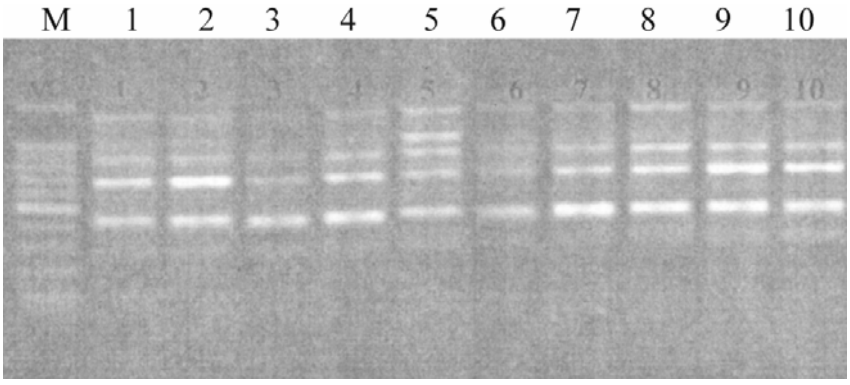


Fig.5. GeI photograph with primer OPD-9 of 10 onion cultivars; 1. BARI-1, 2. BARI-2, 3. BARI-3, 4. lndia-1, 5. India-2, 6. Bermis, 7. White, 8. OF-5, 9. Faridpuri, 10. Bhati and M=100 bp DNA ladder.

**Genetic distance-based analysis**

From the genetic similarity index (Table 2), the pair-wise genetic similarity coefficients indicated that the variety Faridpuri and Bhati are very much closest (83.87%) to each other followed by Bhati and BARI Peas-2 (81.25%), White and Faridpuri (80%).The results suggested that their genotypes are more similar to each other. So, in crop improvement programme, these genetically similar parents could not be chosen in the crossing programme to creat genetic variability. On the other hand, the lowest similarity (34.29%) was found in Bermis and India-2 as well as in Bermis and Faridpuri (40%), Bhati and Bermis (41.18%), White and BARI Peas-1 (41.18%), and Bermis and India-I (41.18%), White and Bermis (42.42%), BARI Peas-3 and Bermis (43.75%) and BARI Peas-1 and India-2 (44.44%).

It means that Bermis and India-2 are the most dissimilar in their genetic level. So, these genetically dissimilar parents should be chosen in crop improvement programme for creating genetic variability.

**Table 1. Primer used in this molecular characterization studies.**

| SI. No. | Code | No. of polymorphic bands |
|---|---|---|
| 1 | OPAB-4 | 15 |
| 2 | OPAB-18 | 24 |
| 3 | OPC-5 | 12 |
| 4 | OPC-7 | 5 |
| 5 | OPC-9 | 18 |
| 6 | OPA-9 | 22 |
| 7 | OPD-1 | 19 |
| 8 | OPD-3 | 27 |
| 9 | OPE-17 | 11 |
| 10 | RE-0l | 1 |
| 11 | OPG-13 | 12 |
| 12 | OPA-16 | 2 |
| 13 | OPA-15 | 0 |
| 14 | OPB-06 | 0 |
| 15 | OPA-4 | 0 |

**Table 2. Genetic similarity index of RAPD patterns of nine varieties and one line of onion using 15 RAPD markers.**

| | B-I | B-2 | 8-3 | India-1 | lndia-2 | Bermis | White | OF-5 | Farid-puri | Bhati |
|---|---|---|---|---|---|---|---|---|---|---|
| B-1 | 1.0000 | | | | | | | | | |
| B-2 | 0.7027 | 1.0000 | | | | | | | | |
| B-3 | 0.4848 | 0.6000 | 1.0000 | | | | | | | |
| India-1 | 0.4571 | 0.6250 | 0.7143 | 1.0000 | | | | | | |
| India-2 | 0.4444 | 0.5455 | 0.7586 | 0.6452 | 1.0000 | | | | | |
| Bermis | 0.7179 | 0.5556 | 0.4375 | 0.4118 | 0.3429 | 1.0000 | | | | |
| White | 0.4118 | 0.7097 | 0.7407 | 0.7586 | 0.7333 | 0.4242 | 1.0000 | | | |
| OF-5 | 0.7391 | 0.6047 | 0.5128 | 0.5854 | 0.5714 | 0.7111 | 0.5000 | 1.0000 | | |
| Faridpuri | 0.5556 | 0.7273 | 0.6897 | 0.6452 | 0.7500 | 0.4000 | 0.8000 | 0.6190 | 1.0000 | |
| Bhati | 0.5714 | 0.8125 | 0.5714 | 0.6667 | 0.5806 | 0.4118 | 0.6897 | 0.6341 | 0.8387 | 1.0000 |

A dendrogram based on UPGMA cluster analysis (Fig. 6) of the RAPD data showed three clearly distinct groups of the 10 varieties. BARI Peas-1, OF-5 and Bermis are on the same cluster, but BARI Peas-1 and OF-5 are closer to each other. Again BARI Peas-2, Faridpuri, and Bhati are on the same cluster, but Faridpuri and Bhati are closer to each other. On the other hand, BARI Peas-3, India-2, India-1, and white are on the same cluster group. But BARI Peas-3 and India-2 are more closer to each other. It was also same in India-1 and White onion.

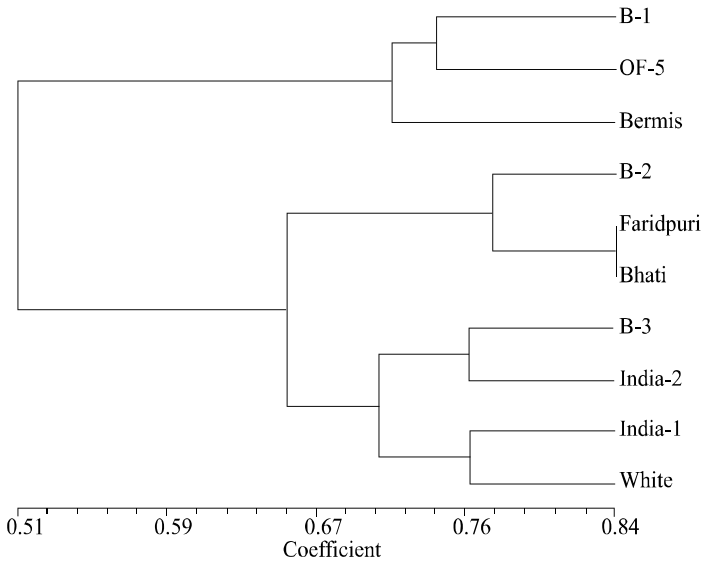


Fig 6. An UPGMA cluster dendrogram (Jaccard coefficient) showing the genetic relationships between nine varieties and one line of onion based on 15 RAPD markers.

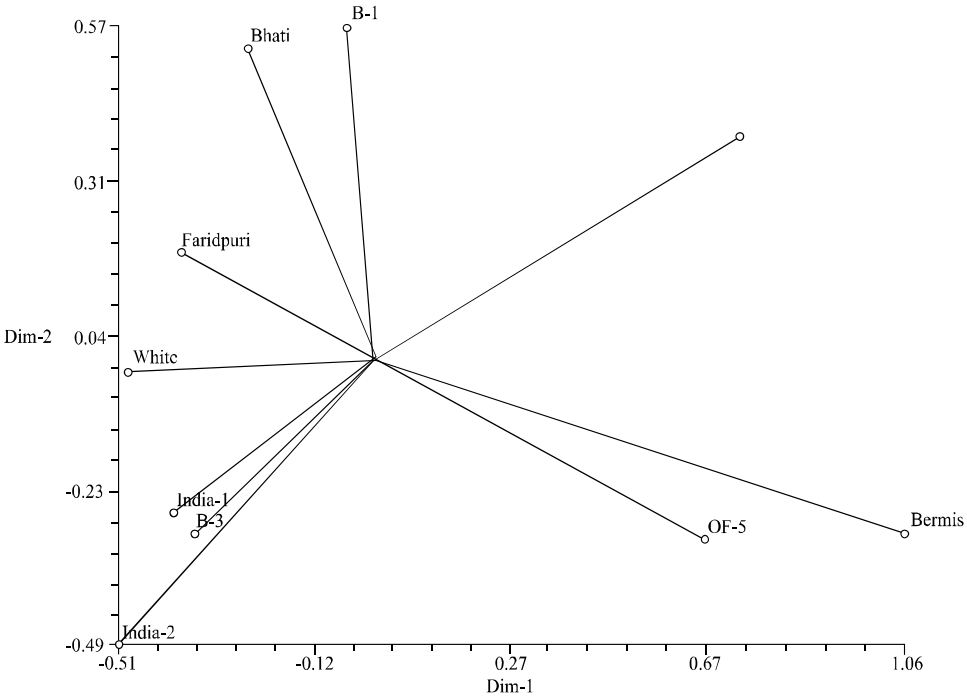


Fig. 7. Two- dimensional view of Principal Coordinate Analysis (PCoA) with 15 RAPD markers over 9 varieties and one line of onion.

The two dimensional graphical view of Principal Coordinate Analysis (PCA) showed the spatial distribution of the varieties along the two principal axes. The varieties viz., Bermis, BARI Peas-I, India-2, OF-5 and BARI Peas-2 were found placing far away from the centriod (Fig.7) of the cluster and the rest of the varieties were placed more or less around the centriod. The results indicated that the varieties placed far away from the centriod were more genetically diverse, while the varieties placed near around the centriod possessed more or less similar genetic background. However, centriod may be defined as the vector representing the middle point of the cluster which contained at least one number for each variable. The connecting line between the each variety and the centriod represented eigen vectors for the respective varieties.

## References

Bachmann, K. and E. J. Hombergen. 1996. Mapping genes for phenotypic variation in *Microseris* (Lactuceae) with molecular markers. In Compositae: Biology and Utilization, Vol. 2 (Caligari, P. D. S. and Hind, D. J. N., eds.), London: Kew Gardens, pp. 23-43.

Bachmann, K. 1997. Nuclear DNA markers in plant biosystematic research. *Opera Botanica* **132:** 137-148.

Backeljau, T., L. De Bruyn, H. DeWoif, K. Jordaens, van S. Dongen, and B. Winnepenninckx. 1995. Random amplified polymorphic DNA (RAPD) and parsimony methods. *Cladistics* **11:** 119-130.

Colosi, J. C. and B. A. Schaal. 1997. Wild proso millet *(Panicum miliaceum)* is genetically variable and distinct from crop varieties of proso millet. *Weed Science* **45:** 509-*5* 18.

Dubouzet, J. G., K. Shinoda, and N. Murata. 1997. Phylogeny of *Allium* L. subgenus Rhizirideum (G. Don ex Koch.) Wendelbo according to dot blot hybridization with randomly amplified DNA probes. *Theoritical and Applied Genetics* **95:** 1223-1228.

Friesen, N. and N. Herrmann. 1998. Taxonomy, chorology and evolution of *Allium lusitanicum*-the European *"A. senescens"*. *Linzer Biologische Beiträge* **30:** 815-830.

Friesen, N. and M. Klass. 1998. Origin of some minor vegetatively propagated *Allium* crops studied with RAPD and GISH. *Genetic Resources and Crop Evolution* **45:** 511-523

Friesen N. & F.R. Blattner. 1999. RAPD Analysis reveals geographic differentiations within *Allium schoenoprasum* L. (Alliaceae). *Plant Biol.* **2** (2000) 297-305.

Friesen, N., S. Pollner, K. Bachmann, and F. R. Blattner. 1999. RAPDs and noncoding chioroplast DNA reveal a single origin of the cultivated *Allium fistulosum* from *A. altaicum. American Journal of Botany* **86:** 554-562.

Gabrielsen, T. M. and C. Brochmann. 1998. Sex after all: high levels of diversity detected in the Arctic clonal plant *Saxfraga cernua* using RAPD markers. *Molecular Ecology* **7:** 170 1-1708.

Harris, S. A. 1995. Systematics and randomly amplified polymorphic DNA in the genus *Leucaea* (Leguminosae, Mimosoideae).

Haishima, M., J. Kato, and H. Ikehashi. 1993. Isozyme polymorphism in native varieties of Japanese bunching onion *Allium fistulosum* L. *Japanese Journal of Breeding* **42:** 497-505.

Inden, H., and T. Asahira. 1990. Japanese bunching onion *Allium fIslulosum* L. In J. L. Brewster and H. D. Rabinowich. *Onion and Allied Crops*, vol. **3:** 159-178.

Morell, M. K., R. Peakall, R. Appels, L. R. Preston and H. L. Lloyd. 1995. DNA profiling techniques for plant variety dentification. *Australian Journal of Experimental Agriculture* **35:** 807-819.

Pillay, M. and S. T. Kenny. 1995. Anomalies in direct pairwise comparisons of RAPD fragments for genetic analysis. *BioTechniques* **19:** 694-698.

Purps, D. M. L. and J.W. Kadereit. 1998. RAPD evidence for a sister group relationship of the presumed progenitor-derivate species pair *Senecio nebrodensis* and S. *viscosus* (Asteraceae). *Plant Systematics and Evolution* **211:** 57-70.

Rieseberg, L. H. 1996. Homology among RAPD fragments in interspecific comparisons. *Molecular Ecology* **5:** 99-1 05.

Rieseberg, L. H., H. Choi, R. Chan, and C. Spore. 1993. Genomic map of a diploid hybrid species. *Heredity* **70:** 285-293.

Roelofs, D. and K. Bachmann 1997. Comparison of chloroplast and nuclear phylogeny in the autogamous annual *Microseris douglasii* (Asteraceae, Lactuceae). *Plant Systematics and Evolution* **204:** 49-63.

Serquen, F. C., J. Bacher, and J. E. Straub. 1997. Mapping and QTL analysis of horticultural traits in a narrow cross in cucumber *(Cucumis sativus* L.) using random-amplified polymorphic DNA markers. *Molecular Breeding* **3:** 257-268.

Serquen, F. C., J. Bacher, and J. E. Straub. 1997. Mapping and QTL analysis of horticultural traits in a narrow cross in cucumber *(Cucumis sativus* L.) using random-amplified polymorphic DNA markers. *Molecular Breeding* **3:** 257-268.

Tollefsrud, M. M., K. Bachmann, K. S. Jakobsen, and C. Brochmann. 1998. Glacial survival does not matter -II: RAPD phylogeography of Nordic *Saxifraga cespitosa*. *Molecular Ecology* **7:** 1217-1232.

Van Raamsdonk, M. P. Smiech, and J. M. Sandbdrink. 1997. Introgression explains incongruence between nuclear and chioroplast DNA-based phylogenies in *Allium* section *Cepa. Botanical Journal of the -Linnean Society* **123:** 91-108.

Van der Zande, L. and R. Bijlsma. 1994. Limitation of the RAPD technique in phylogeny reconstruction in *Drosophila. Journal of Evolutionary Biology* **8:** 645-656.

Wolfe, A. D. and A. Liston. 1998. Contributions of PCR-based methods to plant systematics and evolutionary biology. In Molecular Systematics of Plants II: DNA Sequencing (Soltis, D. E., Soltis, P. S., and Doyle, J. J., eds.), Dordrecht: Kiuwer, pp. 43-86.

Welsh, J. and M. McClelland. 1990. Fingerprinting genomes using PCR with arbitrary primers. *Nucleic Acids Research* **18:** 7213-7218.

Williams, J. G., A. R. Kubelik, K. J. Livak, J. A. Rafaiski and S.V.Tingey. 1990. DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. *Nucleic Acids Research* **18:** 6531-6535.