The Journal of Animal & Plant Sciences, 25(2): 2015, Page: 442-447
ISSN: 1018-7081

PHYLOGENETIC RELATIONSHIP OF SELECTED PAKISTANI WHEAT VARIETIES BASED ON A CHLOROPLAST *RPS11* GENE

S. Rehman, H. J. Chaudhary, A. Rasheed and T. Mahmood*

Department of Plant Sciences, Quaid-i-Azam University, Islamabad-45320, Pakistan
*Corresponding author e-mail: tmahmood.qau@gmail.com; tmahmood@qau.edu.pk

ABSTRACT

Wheat (*Triticum aestivum* L.) is a major crop among all cereals and consumed in major parts of the world as a staple food. The assessment of genetic diversity in wheat is of prime importance for conservation, breeding programs and to broaden the genetic makeup to develop high quality varieties. The present study aimed at the evaluation of evolutionary divergence pattern and genetic relationship among eight selected Pakistani wheat varieties based on a chloroplast gene. To achieve the main objective, *rps* 11 gene was amplified, sequenced and analyzed with the help of Bioinformatics tools. The constructed MEGA 5 phlogenetic tree revealed considerable level of genetic similarity among studied varieties with overall genetic distance of 0.05. The evolutionary divergence data based on pair wise distance analysis identified Chakwal-50 and Watan as most diverse varieties among all selected varieties. Phylogenetic relationship and genetic diversity concluded in this study based on *rps* 11 gene can be reliably used for assessing genetic kinship among wheat cultivars and to possibly change the direction of breeding programs in future.

Key words: Genetic diversity, Phylogenetic analysis, Pair wise distance

Abbreviations: MEGA 5 Molecular Evolutionary Genetic Analysis Version 5, *rps* 11 Ribosomal Protein Subunit 11, cpDNA Chloroplast DNA.

INTRODUCTION

Bread wheat (*Triticum aestivum* L.) is one of the most important cereal crops worldwide, including Pakistan. It is cultivated in all four provinces and has a central position in agricultural policies because it is a staple food and supplies 72% energy and protein in the average daily diet in Pakistan (Khalil, 2006). In the last hundred years, several studies were conducted on wheat phylogeny which provides significant insight into the evolutionary background of the definite genomes in allopolyploid species (Gu *et al.*, 2004). Data based on pedigree, morphological, agronomic performance, biochemical and molecular (DNA-based) markers can be used for genetic diversity estimation using different methods (Mohammadi and Prasanna, 2003). Different molecular markers have been compared to estimate genetic diversity in several wheat varieties and related wild species (Fufa *et al.*, 2005; Naghavi *et al.*, 2007; Mahmood *et al.*, 2011a). Direct sequencing of PCR amplified products is now becoming a rapidly expanding area of plant systematics and evolution (Clegg and Zurawski, 1992).

Chloroplast genomes are haploid; their effective population size in monoecious outcrossing plants is half of diploid nuclear genomes. The chloroplast DNA (cpDNA) has been used extensively to infer plant phylogenies at different taxonomic levels (Olmstead and Palmer, 1994; Mahmood *et al.*, 2011b,c; Saeed *et al.*, 2011; Jabeen *et al.*, 2012) and to some extent, for genetic studies within-species (Ennos *et al.*, 1999). Most of the conclusions drawn have been based on nucleotide sequence variation in a single chloroplast gene. Early studies on plant systematics were focused on using restriction site polymorphism in cpDNA (Olmstead and Palmer, 1994). Subsequently protein-coding gene sequences such as *rbc*L were designed to elucidate diversity in higher level taxa (Chase *et al.*, 1993). At higher taxonomic level, coding sequences such as *rbc*L, *rps*4, *ndh*F and *atp*B of chloroplast genomes were found more successful for evaluation of phylogenetic relationship than the noncoding sequences (*atp*B-*rbc*L intergenic spacer, *trn*L intron, *trn*L-*trn*F intergenic spacer) due to low rates of nucleotide substitutions and structural changes in coding sequences (Makarevich *et al.*, 2003; Neel *et al.*, 2004). However, phylogenetic analysis has also been conducted at the intrageneric level based on *mat*K coding gene (Young and dePamphilis, 2000; Yang *et al.*, 2004). Furthermore, in most angiosperms chloroplast DNA has shown maternal inheritance, i.e. mainly transmitted through the embryos of the seeds (Mogensen, 1996) that permit the elucidation of genetic divergent patterns and postglacial migration routes (McCauley, 1994). The present study has therefore been conducted with an aim of evaluating phylogenetic relationship among different wheat varieties based on chloroplast ribosomal protein coding gene (*rps* 11).

MATERIALS AND METHODS

Plant material collection: Seeds of eight different varieties of wheat (*Triticum aestivum* L.) were arranged from National Agriculture Research Centre (NARC) Pakistan. The names, pedigree and other information of selected wheat varieties are given in Table 1.

DNA isolation: The seeds of each variety were germinated for a week under lab condition at 25°C. The total genomic DNA from fresh and young leaves was extracted using CTAB (Cetyl Trimethyl Ammonium Bromide) method (Richards, 1997) with few modifications (Nazar and Mahmood, 2010).

Primer designing: A pair of primers that amplify *rps*11 gene was designed based on cpDNA sequences of tobacco (Accession # Z00044.2) available at NIH (National Institute of Health, USA) genetic sequence data base, GenBank. Primers were designed using online software primer 3 (version 4.0) (http//primer3. sourceforage.net/). The primer sequences are as follow:
rps 11 Forward: 5' TGGCAAAAGCTATACCGAAAA 3'
rps 11 Reverse: 5' TTCGGAGGTCTACAGCCATT 3'

PCR conditions and sequencing of rps 11 gene: PCR conditions used for amplification were pre-PCR denaturing at 94 °C for 5 minutes followed by 35 cycles of denaturing at 94 °C for 1 minute, annealing temperatures at 55 °C for 30 seconds and extension at 72 °C for 1 minute. Final cycle was the same as the previous ones, except it was extended at 72 °C for 20 minutes to complete the extension of any remaining single stranded DNA. PCR reaction contents were held at 4˚C for short term storage in Multi Gene Thermal Cycler (Labnet). About 25 µl amplification reaction mixture containing 12.5 µl PCR Master Mix (MBI Fermentas), 1µl (25 pmol) of forward and reverse primer each, 1µl template and 9.5 µl nano pure water was prepared for each sample. Successful amplification of desired gene was confirmed by running PCR products along with loading dye on 1.5 % agarose gel in 0.5 x TAE buffer. The resultant PCR products were purified with JET quick (Genomed) PCR Product Purification Kit and all samples were sent to Macrogen (Seoul, Korea) for sequencing with specific primers.

Sequence homology: Nucleotide Blast (nBlast) was used to find the similarity of sequences with already reported *rps* 11 sequences present in nucleotide databases (http://blast.ncbi.nlm.nih.gov/Blast.cgi). The sequences generated for each variety were submitted to GenBank for the allotment of accession numbers (Table 2).

Phylogeny construction: Phylogenetic relationship was analyzed using neighbor-joining (NJ) methods of molecular evolutionary genetic analysis version 5 (MEGA 5) (Tamura *et al.*, 2011). Statistical support for the tree was evaluated by bootstrapping method performed with 500 replicates. Bootstrap support values were categorized as follow: poor <50%; weak 50-74%; moderate, 75-84%; strong, 85-100%.

RESULTS AND DISCUSSION

Amplification: The *rps* 11 primers used to target DNA sequences have generated PCR amplicons of 432 bp in size. Generally, high quality amplification was observed when PCR products were fractionated through 1.5 % agarose gel and visualized by ethidium bromide staining (Fig.1).

Sequence analysis and genetic diversity estimation: The high quality of DNA sequencing results from all samples was obtained using the dye terminator sequencing method. BLAST comparisons of chloroplast *rps* 11 gene sequences from all selected varieties revealed ≥ 91% sequence identity with chloroplast genome of *Aegilops geniculata* (Accession # KF534490.1). Pair wise distance analysis was conducted to estimate genetic diversity among the sequences of *rps* 11 gene using MEGA 5 (Table 3). The results of genetic diversity estimation revealed low to moderate genetic diversity which were in the range of 0.09 to 0.75 with an average of 0.52. A high diversification was observed for Chakwal-50 and Watan (0.75); while Fareed-06 and 04FJS-26 showed less diversification (0.09) which also correspond well to their position in phylogenetic tree (Table 3, Fig. 2). Comparable to our study, Kuleung *et al.* (2006) reported moderate gene diversity (0.54) in 80 triticale lines chosen from different countries using wheat and rye SSR markers. They discussed that moderate diversity of triticale was possibly related to the relatively narrow genetic base during the initial establishment of triticale and the limited number of germplasm resources available to triticale breeders. In considering the genetic diversity identified in this study, this narrow genetic variation is an indication of lack of parental diversity that may impede further plant development. Information about the genetic relationships among accessions within and between species is important to make improvements in plant developments (Thormann *et al.*, 1994).

Phylogenetic inference based on rps 11 sequences: Two main clusters (Cluster I and Cluster II) identified by MEGA 5 suggesting that speciation occurred after gene duplication event (Fig. 2). The phylogram depicted that all the members of Cluster I and Cluster II are the orthologs with their cluster members. Cluster I includes four varieties namely 04FJS-26, Fareed-06, Iqbal-2000 and Watan respectively. In Cluster I further sub-clustering give rise to two groups. Group I comprised of Watan and Iqbal-2000 showing close affinity with 100 % bootstrap support. On the other hand, Fareed-06 and 04FJS-26 clustered together in a single group (Group II)

sharing maximum ancestry with each other (100 % bootstrap support). A bootstrap value of 100% for a certain clade actually represent that nearly all of the characters informative for this group agree that it is a group (Berry and Gascuel, 1996). Cluster II also includes four varieties namely FSD-85, Bhakkar-2000, Chakwal-50 and Lasani (Fig. 2). It was observed that all the varieties in this cluster are genetically associated with each with high bootstrap value (100 %) providing a strong support for their monophyletic origin. Among these four varieties, FSD-85 and Bhakkar appeared to be recently evolved varieties receiving 34 % bootstrap value and formed a single group. Similarly, the closely related Chakwal-50 and Lasani constituted the second group showing weak 44 % bootstrap value.

Recently, Dizkirici *et al.* (2013) phylogenetically analyzed the *Triticum* L. and *Aegilops* L. species based on *trnT-F* chloroplast DNA sequences containing three non-coding sub-regions. The results postulated a close genetic relationship between diploid *Aegilops* species containing the BB genome and polyploid *Triticum* species. Earlier, Golovnina *et al.* (2007) and Haider *et al.* (2012) conducted phylogenetic analysis for *Triticum* L. and its close relatives based on chloroplast *matK* gene and cleaved amplified polymorphic sequence (CAPS) respectively. In our study, Phylogram based on *rps*-11 gene revealed common genetic background in most of the wheat cultivars (100 % bootstrap support) that indicates close association among studied wheat varieties. This clustering of genotypes might be due to the utilization of common exotic breeding lines at different breeding stations, with similar selection pressures; the same was observed in an earlier study with other genotypes (Mukhtar *et al.*, 2002). The efficacy of *rps* 11 gene sequences in molecular typing has earlier been well reviewed in different plant species such as Artemisia, Mentha, and Apocynaceae species (Hattori *et al.*, 2006; Naciri *et al.*, 2010; Mahmood *et al*., 2011c; Saeed *et al.*, 2011; Jabeen *et al.*, 2012). The pyhogenetic estimates proposed herein are largely congruent with RAPD based relationship observed for 10 different wheat genotypes in Pakistani wheat cultivars (Siddiqui *et al.*, 2010) and with genetic diversity analysis in turkish durum and bread wheats inferred from AFLP and SAMPL markers (Altintas *et al.*, 2008). The results of our study also strengthen the earlier observation that *rps* 11 gene can be used to discriminate wheat genotypes and to access the genetic diversity. However additional molecular data from the other regions of cpDNA is also needed to further explore the genetic relationships among other wheat varieties. Overall, it has been observed that all these wheat varieties are monophyletic with medium level of genetic diversity; therefore this information generated can be exploited for genetic improvement in wheat.

Table- 1. Wheat varieties with their names, pedigree, year of release and origin.

Varieties	Pedigree	Year of release	Research Institutes*
FSD-85	MAY-MONCHO 'S' x KVZ.TRM	1985	WRI, Faisalabad
Bhakkar-2000	P102/PIMA//F3.71/TRM/3/PVN	2000	AZRI, Bhakkar
Fareed-06	PTS/3/POB/LFN//BB/HD//832/5/GV/ALD'S'//HPO'S'	2006	RARI, Bahawalpur
Iqbal-2000	BURGUS/SORT-12-13//KAL/BB/3/PAK81	2000	WRI, Faisalabad
04FJS-26	Advanced lines (Pedigree not available)	Not Available	Not Available
Chakwal-50	ATTILA/3/HUITLE/CARC//CHEN/CNTO/4/ATTILA	2008	BARI, Chakwal
Watan	LU26/HD2179	1994	WRI, Faisalabad
Lasani	LUAN/KOH97	2008	WRI, Faisalabad

* **WRI:** Wheat Research Institute, Faisalabad, **AZRI:** Arid Zone Research Institute, Bhakkar, **BARI:** Barani Agricultural Research Institute, Chakwal, **RARI:** Regional Agricultural Research Institute, Bahawalpur

Table 2. Accession numbers of sequences of chloroplast rps11 gene from eight different wheat varieties.

Wheat Varieties	Accession No.
Triticum aestivum var. FSD-85	KJ672074
Triticum aestivum var. Bhakkar-2000	KJ672075
Triticum aestivum var. Fareed-06	KJ672076
Triticum aestivum var. Iqbal-2000	KJ672077
Triticum aestivum var. 04FJS-26	KJ672078
Triticum aestivum var. Chakwal-50	KJ672079
Triticum aestivum var. Watan	KJ672080
Triticum aestivum var. Lasani	KJ672081

Table- 3. Estimation of evolutionary divergence among *rps* 11 gene sequences for eight wheat varieties.

Sr.No.	FSD-85	Bhakkar-2000	Fareed-06	Iqbal-2000	04FJS-26	Chakwal-50	Watan	Lasani
1								
2	0.12							
3	0.63	0.61						
4	0.74	0.74	0.64					
5	0.68	0.67	0.09	0.65				
6	0.13	0.13	0.65	0.74	0.68			
7	0.74	0.74	0.64	0.11	0.66	0.75		
8	0.13	0.11	0.66	0.72	0.65	0.12	0.72	

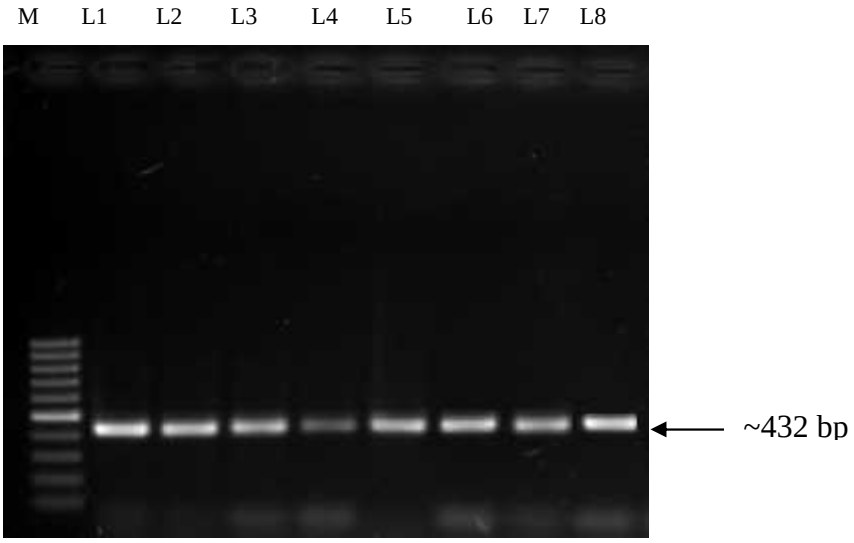

Figure 1. PCR amplification of *rps* 11 gene from selected wheat varieties. M: 100bp ladder (Fermentas), L1: FSD-85, L2: Bhakkar-2000, L3: Fareed-06, L4: Iqbal-2000, L5: 04FJS-26, L6: Chakwal-50, L7: Watan, L8: Lasani.

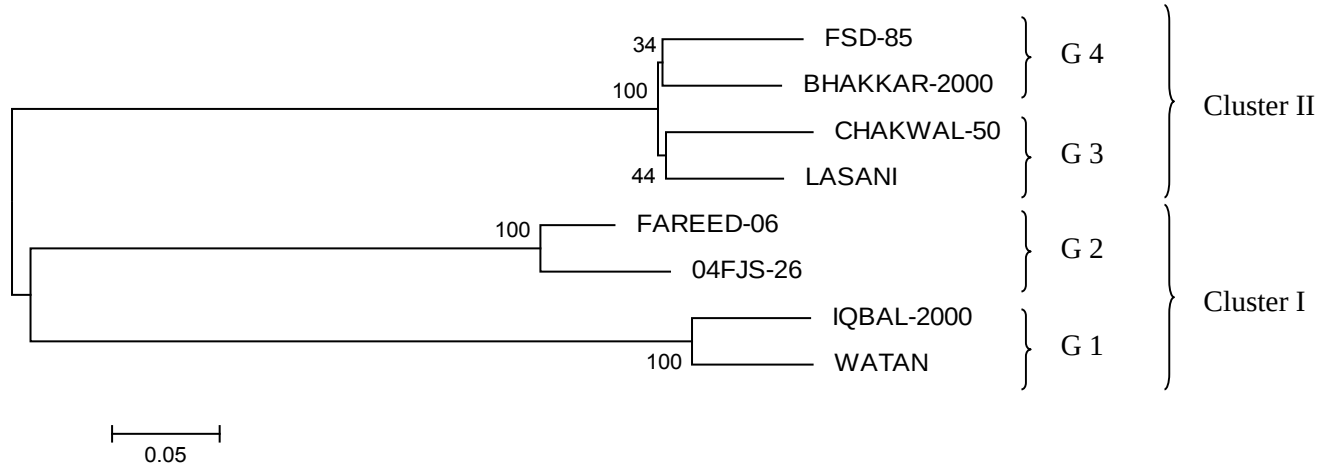

Figure 2. Phylogram produced by MEGA 5 method for *rps* 11 gene sequences indicating genetic relationship among selected wheat varieties with bootstrap values.

Conclusions: In summary the present study revealed significant genetic relatedness among different wheat varieties based on chloroplast *rps* 11 gene which clearly demonstrated the usefulness of chloroplast genes for phylogeny and diversity analysis among wheat cultivars.

Acknowledgement: This work was supported by a research grant from Higher Education Commission (HEC) of Pakistan.

REFERENCES

Altintas, S., F. Toklu, S. Kafkas, B. Kilian, A. Brandolini, and H. Özkan (2008). Estimating genetic diversity in durum and bread wheat cultivars from Turkey using AFLP and SAMPL markers. Plant Breed. 127: 9–14.

Berry V. and O. Gascuel (1996). On the interpretation of bootstrap trees: appropriate threshold of clade selection and induced gain. Mol. Biol. Evol. 13: 999-1011.

Chase, M.W., D.E. Soltis, R.G. Olmstead, D. Morgan, D.H. Les, B.D. Mishler, M.R. Duvall, R.A. Price, H.G. Hills, Y.L. Qui, K.A. Kron, J.H. Rettig, E. Conti, J.D. Palmer, J.R. Manhart, K.J. Sytsma, H.J. Michaels, W.J. Kress, K.G. Karol, W.D. Clark, M. Hedren, B.S. Gaut, R.K. Jansen, K.J. Kim, C.F. Wimpee, J.A. Smith, G.R. Furnier, S.H. Strauss, Q.Y. Xiang, G.M. Plunkett, P.S. Soltis, S.M. Swensen, S.E. Williams, P.A. Gadek, C.J. Quinn, L.E. Eguiarte, E. Golenberg, G.H. Learn, S.W. Graham, S.C.H. Barrett, S. Dayanandan, and V.A. Albert (1993). Phylogenetics of seed plants: an analysis of nucleotide sequences from the plastid gene *rbc L*. Ann. Mo. Bot. Gard. 80: 528- 580.

Clegg, M.T. and G. Zurawski (1992). Chloroplast DNA and the study of plant phylogeny: Present status and future prospects. In: Soltis, P.S., D.E. Soltis, and J.J. Doyle. (Eds.) Molecular Sytstematics of Plants. New York, Chapman and Hall, pp. 1–13.

Dizkirici, A., C. Kansu, S. Onde, M. Birsin, M. Özgen, and Z. Kaya (2013). Phylogenetic relationships among Triticum L. and Aegilops L. species as genome progenitors of bread wheat based on sequence diversity in trnT-F region of chloroplast DNA. Genet. Resour. Crop Evol. DOI:10.1007/s10722-013-9988-x

Ennos, R.A., W.T. Sinclair, X.S. Hu, and A. Langdon (1999). Using organelle markers to elucidate the history, ecology and evolution of plant populations. In: Hollingsworth, P.M., Bateman, R.M. and R.J. Gornall. (Eds.) Molecular Systematics and Plant Evolution. Taylor and Francis Ltd, London, pp. 1–19.

Fufa, H., P.S. Baenziger, B.S. Beecher, I. Dweikat, R.A. Graybosch, and K.M. Eskridge (2005). Comparison of Phenotypic and Molecular Marker-based Classifications of Hard Red Winter Wheat Cultivars. Euphytica 145:133–146.

Golovnina, K.A., S.A. Glushkov, A.G. Blinov, V.I. Mayorov, L.R. Adkison, and N.P. Goncharov (2007). Molecular phylogeny of the genus *Triticum* L. Plant Syst. Evol. 264: 195-216.

Gu, Y.Q., D. Coleman-Derr, X. Kong, and O.D. Anderson (2004). Rapid genome evolution revealed by comparative sequence analysis of orthologous regions from four Triticeae genomes. Plant Physiol. 135: 459–470.

Haider, N. (2012). Evidence for the origin of the B genome of bread wheat based on chloroplast DNA. Turk. J. Agric. For. 36: 13-25

Hattori, H., T. Iwata, Y. Nakagawa, F. Kawamoto, Y. Tomita, A. Kikuchi, and T. Kanbe (2006). Genotype analysis of Candida albicans isolates obtained from different body locations of patients with superficial candidiasis using PCRs targeting 25S rDNA and ALT repeat sequences of the RPS. J. Dermatol. Sci. 42: 31–46.

Jabeen, A., B., Guo, B.H. Abbasi, Z.K. Shinwari, and T. Mahmood (2012). Phylogenetics of selected Mentha species on the basis of rps8, rps11 and rps14 chloroplast genes. J. Med. Plant. 6: 30–36.

Khalil, S. (2006). An assessment of wheat crop, PARC, Pakistan.

Kuleung, C., P.S. Baenziger, S.D. Kachman, and I. Dweikat (2006). Evaluating the Genetic Diversity of Triticale with Wheat and Rye SSR Markers. Crop Sci. 46: 1692–1700

Mahmood, T., N. Hassan, N. Nazar, and I. Naveed (2011c). Phylogenetic Analysis of different Artemisia species based on chloroplast gene rps11. Arch. Biol. Sci. Belgrade. 63: 661–665.

Mahmood, T., F. Meer, F. Munir, N. Nazar, and I. Naveed (2011b). Genetic diversity of selected Apocynaceae species based on chloroplast gene rps11. Afr. J. Biotechnol. 8: 6561–6568.

Mahmood, T., A. Siddiqua, A. Rasheed, and N. Nazar (2011a). Evaluation Of genetic diversity in different Pakistani wheat land races. Pakistan J. Bot. 43: 1233–1239.

Makarevich, I., K. Golovnina, S. Scherbik, and A. Blinov (2003). Phylogenetic relationships of the Siberian Iris species inferred from noncoding chloroplast DNA sequences. Int. J. Plant Sci. 164: 229-237.

McCauley, D.E. (1994). Contrasting the distribution of chloroplast DNA and allozyme polymorphism among local populations of Silene alba: implications for studies of gene flow in plants. Proc. Natl. Acad. Sci. U.S.A., 8127–8131.

Mogensen, H.L. (1996). The hows and whys of cytoplasmic inheritance in seed plants. Am. J. Bot. 83: 383–404.

Mohammadi, S.A. and B.M. Prasanna (2003). Analysis of genetic diversity in crop plant salient statistical tools and considerations, review and interpretation. Crop Sci. 43: 1235-1248.

Mukhtar, M. S., M. Rahman, and Y. Zafar (2002). Assessment of genetic diversity among wheat (*T. aestivum* L.) cultivars from a range of localities across Pakistan using random amplified polymorphic DNA (RAPD) analysis. Euphytica 128: 417–425.

Naghavi, M.R., M. Mardi, S.M. Pirseyedi, M. Kazemi, P. Potki, and M.R. Ghaffari (2007). Comparison of Genetic Variation among Accessions of *Aegilops tauschii* using AFLP and SSR Markers. Genet. Res. Crop Evol. 54: 237–250.

Naciri, Y., F. Cavat, and D. Jeanmonod (2010). Silene patula (Siphonomorpha, Caryophyllaceae) in North Africa: A test of colonisation routes using chloroplast markers. Mol. Phylogenet. Evol. 54: 922-932.

Nazar, N. and T. Mahmood (2010). Morphological and molecular characterization of selected Artemisia species from Rawalakot, Azad Jammu and Kashmir. Acta Physiol. Plant. 33: 625–633.

Neel, M.C. and M.P. Cummings (2004). Section-level relationships of North American Agalinis (Orobanchaceae) based on DNA sequence analysis of three chloroplast gene regions. BMC Evol. Biol. 4: 1–12.

Olmstead, R.G. and J.D. Palmer (1994). Chloroplast DNA systematics: a review of methods and data analysis. Am. J. Bot. 81: 1205–1224.

Richard, E.J. (1997). Preparation of plant DNA using CTAB. Plant Mol. Biol. Rep. 2: 10–11.

Saeed, S., F. Munir, I. Naveed, G.K. Raja, and T. Mahmood (2011). Phylogenetics of selected Plantago species on the basis of rps-14 chloroplast gene. J. Med. Plants Res. 5: 4888-4891.

Siddiqui, M. F., S. Iqbal, S. Erum, N. Naz, and S. Khan (2010). DNA landmarks for genetic relatedness and diversity assessment in Pakistani wheat genotypes using RAPD markers. Pakistan J. Bot. 42: 1013–1020.

Tamura, K., D. Peterson, N. Peterson, G. Stecher, M. Nei, and S. Kumar (2011). MEGA5: Molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. Mol. Biol. Evol. 28: 2731–2739.

Thormann, C.E., M.E. Ferreira, L.E.A. Camargo, J.G. Tivang, and T.C. Osborn (1994). Comparison of RFLP and RAPD markers to estimating genetic relationship within and among cruciferous species. Theor. Appl. Genet. 88: 973-980.

Yang D.Y., H. Fushimi, S.Q. Cai, and K. Komatsu (2004). Molecular analysis of Rheum species used as rhei rhizoma based on the chloroplast mat K gene sequence and its application for identification. Biol. Pharm. Bull. 27: 375–383.

Young N.D. and C.W. dePamphilis (2000). Purifying selection detected in the plastid gene *mat K* and fanking ribozyme regions within a Group II intron of nonphotosynthetic plants. Mol. Biol. Evol. 17: 1933–1941.