VOL. 10, NO. 7, JULY 2015 ISSN 1990-6145
ARPN Journal of Agricultural and Biological Science

www.arpnjournals.com

GENETIC VARIABILITY AMONG AND WITHIN WILD *Teucrium polium* L. POPULATIONS AT WADI SHUEIB AREA IN JORDAN

Ibrahim Mohammad AlRawashdeh
Department of Biology Science, Faculty of Science, Al-Hussein Bin Talal University, Ma'an, Jordan
E-Mail: irawashdeh2002@yahoo.com

ABSTRACT

Random amplified polymorphic DNA (RAPD) technique was used to determine the genetic relatedness among and within selected *Teucrium polium* populations at Wadi Shueib area in Jordan. Ten primers shown polymorphic bands were used for examining the genetic variability. A total of 1331 bands were obtained, 230 of them were polymorphic. Similarity coefficient values among the studied accessions varied from 0.00 to 0.48. High similarity values were obtained between two samples collected from middle area of Wadishueib. No similarity (value = 0.000) was detected between samples collected from West and middle area. RAPD analysis confirmed the presence of genetic diversity through tested *Teucrium polium* populations. The cluster analysis generated three groups. Genetic diversity among population was found. The information obtained through genetic diversity analysis of wild populations is necessary for conservation and exploitation of these valuable genetic resources in the future.

Keywords: *Teucrium polium* L., genetic diversity, germander, polymorphic, RAPD.

INTRODUCTION

Mountain germander or Jaadah (*Teucrium polium* L.), belongs to the Lamiaceae family, is a wild-growing flowering plant grown at different locations in Jordan. It was characterized as chamaephyte, 20-40cm, tometose-canescent or white-woolly, much branched at base. Stems and branches terete, branches ascending or erect, simple, elongate, 10-30(-40) cm, each ending in shortly paniculate or corymobes inflorescence. Leaves 1-3cm, sessile, oblong or linear, obtuse, crenate, strongly revolute, rarely flat; floral leaves linear-oblong, strongly revolute, shorter than flowers. Verticullasters forming dense, nearly globose or ovoid short-pendunculate heads. Flowers nearly sessile. Clayx tomentose or woolly, 4 mm, campanulate; teeth hidden by hairs, short-triangular, subacute or obtuse. Corolla white or pale cream-colored. Satmens slightly exserted. Flowering during April-August (Feinbrun-Dothan, 1978).

Medicinal and aromatic plants are reservoirs of curative elements used by a large population of Africans in treatment of several diseases such as malaria, diabetes, mental disorders, cancer hypertension and human immune deficiency (Okigbo *et al*., 2009). Traditional medicinal plants comprises of therapeutic practices, those have phytochemical constituents can be used in pharmaceutical purposes. *Teucrium* species have been used for many years ago and most of them still used in folk medicine. Traditionally, *T. polium* has been used for curing many pain falls such as gastrointestinal disorders, inflammations, diabetes and rheumatism (Bahramikia and Yazdanparast, 2012; Bahramikia and Yazdanparast, 2012). The chemical composition of *Teucrium polium* was analyzed and fvarious components were found such as Germacrene D, bicyclogermacrene, β-pinene and carvacol, with moderate inhibitory effects on *Bacillus cereus, Escherichia coli* and *Enterococcus faecalis* (Belmekki *et al*., 2013), and β-pinene and α-cadinol (Boulila *et al*., 2008). Alamdar *et al*. (2007) identified ten major components such as limonene, germacrene, β-bourbonene, β-caryophlene and β –elemene. Belmekki *et al*. (2013) reported that the variation in the chemical constituents obtained could be attributed to the dissimilar climatic environments. Molecular markers have been confirmed their ability for characterization and evaluation of genetic diversity within and among several wild and cultivated populations of medicinal plants. The random amplified polymorphic DNA (RAPD) (William *et al*., 1990) markers have been used for many types of genetic analyses, including mapping, genotype fingerprinting and measuring genetic similarity (Gadge and Nathar, 2014). It was used for studying the genetic diversity among and within *Teucrium polium* species (Boulila *et al*., 2010), among *Vanilla* species (Verma *et al*., 2009); genetic variability in a medicinal plant *Artemisia judaica* (Al-Rawashdeh, 2011 and Bader *et al*., 2012); exploring genetic diversity in Jordanian wheat landraces (Al-Rawashdeh, 2011); relationships among 33 accession of *Curcuma* (Zou *et al*., 2011); Molecular characterization of γ–rays induced mutants in *Jatropha curcas* (Dhillon *et al*., 2014); genetic diversity among 35 *Vigna* genotypes (Singh *et al*., 2014); genetic diversity and phylogenetic analysis of *Allium* genus (Mukherjee *et al*., 2013); barely genetic diversity (Karim *et al*., 2009); genetic variation in rhizomes lotus (Na *et al*., 2009); genetic polymorphism in bread wheat (Nimbal *et al*., 2009); assessment of genetic diversity in cashew (Thimmappaiah *et al*., 2009). Little information available on the genetic variability among and within (*Teucrium polium* L.) species. Therefore, this study was aimed to investigate the variability among and within *Teucrium polium* species at Jordan.

MATERIALS AND METHODS

Plant material

Fifty samples of *Teurcium poilum* L. species (Figure-1) collected, within paper bags and preserved in ice box, from four regions at Jordan (Table-1).

DNA isolation

Young leaves were collected from different populations (Table-1). Total cellular DNA was extracted following the procedure as described by (Doyle and Doyle, 1987; Al-Rawashdeh, 2011 and Boulila *et al*., 2010).

PCR amplification

PCR reaction was performed as described by Williams *et al*. (1990) and Al-Rawashdeh, (2011). Forty 10-mer primers (Table-2) were tested and the repeatable fragments with strong and medium intensity were used in the analysis.

Data analysis

For analysis of RAPD results the fragments were scored as present (1) or absent (0). Similarity matrix was calculated using the Jaccards' coefficient (Jaccard, 1908) and the dendrogram obtained by clustering according to the Unweighted Pair-Group Method with arithmetic averages (UPGMA) using (SPSS, 2000) (v. 11.0), software.

Table-1. Regions and coordinates of collected samples of *Teucrium polium* species at Jordan.

Region/population	Samples number	Direction	Coordinates		Altitude (m)
			Eº	Nº	
Wadishueib (1)	1-15	West-North	03543.024	3201.060	831
Wadishueib (2)	16-30	West-South	03540.088	3156.457	85
Wadishueib (3)	31-40	East	03514.936	3159.463	590
Wadishueib (4)	41-50	Middle	03544.082	3159.039	477

Figure-1. Wild *Teurium polium* species grown at Jordan.

RESULTS

For all the populations, a total of 1331 RAPD fragments were amplified (Table-2 and Figures 2, 3), with 235 polymorphic bands (17.6 %). The number of bands produced by each primer varied from 1 to 9. The percentage of polymorphic loci per primer ranged from13 (OPD14) to 17% (OPW17). The average of polymorphism for all populations was 17.6 % (Table-3). Levels of similarity between accessions ranged between 0.00 to 0.480 (with zero indicating no similarity) (Figure-4). The highest average similarity index value of 0.48 was observed between two samples of population (4) collected from middle area of Wadishueib also, high values registered for samples (23 and 24), (35 and 37), (44 and 45), (37 and 38) and (3 and 4) 0.47; 0.43; 0.42; 0.41 and 0.41; respectively (Figure-4). Similarity index of (0.00) was recorded between sample number (34) collected from east and sample collected from middle area of Wadishueib. Very low of similarity matrix was found with a range from 0.02 to the 0.11 scattered within and among population samples over all ecological sites (Figure-4). According to the dendrogram all populations were grouped into three separated clusters (Figure-4). The first cluster included nine samples of population (4) represented the middle area of Wadishueib in addition to the one sample from population (3). The second cluster has one sample from population (4). The third cluster formed two sub-clusters; the first included eleven samples of population (3) represented West-south site of wadishueib (Table-1 and Figure-4) as well samples collected from east site (31-38). The second sub-cluster of third group had two sub-sub-clusters; the first has seven samples from west north and 4 samples from west south sites (Table-1 and Figure-4). The final sub-sub-cluster had eight samples of west-north site.

Table-2. Primer name and their sequences used under this study.

Primer name	Sequence 5'-3'	Primer name	Sequence 5'-3'
1. OPA16	AGCCAGCGAA	21. OPD06	ACCTGAACGG
2. OPA18	AGGTGACCGT	22. OPD10	GGTTCACACC
3. OPA20	GTTGCGATCC	23. OPD11	AGCGCCATTG
4. OPB01	GTTTCGCTCC	24. OPD14	CTTCCCCAAG
5. OPB02	TGATCCCTGG	25. OPD16	AGGGCGTAAG
6. OPB04	AGCCAGCGAA	26. OPD18	GAGAGCCAAC
7. OPB05	TGCGCCCTTC	27 OPD20	ACCCGGTCAC
8. OPB06	TGCTCTGCCC	28. OPT03	TCCACTCCTG
9. OPB08	GTCCACACGG	29. OPT05	GGGTTTGGCA
10. OPB09	TGGGGGACTC	30. OPT10	CCTTCGGAAG
11. OPB10	CTGCTGGGAC	31. OPT13	AGGACTGCCA
12. OPB12	CCTTGACGCA	32. OPT15	GGATGCCACT
13. OPB13	TTCCCCCGCT	33. OPT16	GGTGAACGCT
14. OPB14	TCCGCTCTGG	34. OPT19	GTCCGTATGG
15. OPB19	ACCCCCGAAG	35. OPT20	ACTTTGGCGG
16. OPC09	CTCACCGTCC	36. OPZ12	TCAACGGGAC
17. OPC10	TGTCTGGGTG	37. OPZ15	CAGGGCTTTC
18. OPC12	TGTCATCCCC	38. OPZ16	TCCCCATCAC
19. OPC20	ACTTCGCCAC	39. OPW04	CAGAAGCGGA
20. OPD04	TCTGGTGAGG	40. OPW17	CTCCTGGGTT

Table-3. Total bands, percent polymorphism and range of bands per primer of most polymorphic RAPD primers used for (*Teucrium polium* L.) RAPD analysis.

Primer name	Total bands/primer	Number of polymorphic bands	% of polymorphic loci
OPB17	92	13	14
OPC12	110	15	14
OPC18	112	26	23
OPD06	94	19	20
OPD14	187	25	13
OPT15	60	11	18
OPT16	127	24	19
OPT20	211	33	16
OPW04	238	35	15
OPW17	140	34	24
Total over loci	1331	235	176
Mean per primer	133.1	23.5	17.6

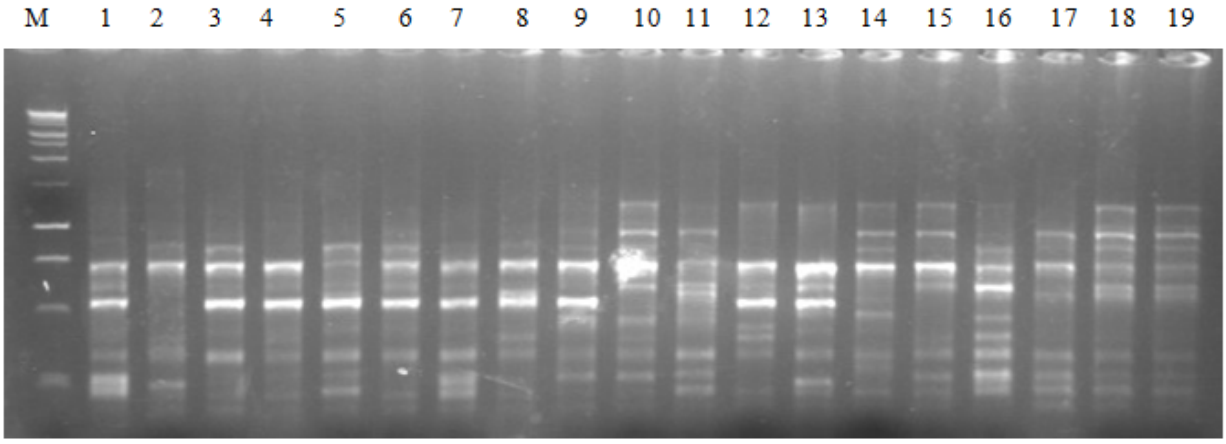

Figure-2. Representative of amplification profiling among samples of *Teucrium polium* using OPW04 RAPD marker. M: DNA ladder. Lane 1-15: West-North of Wadishueib; lane 16-19: West-South.

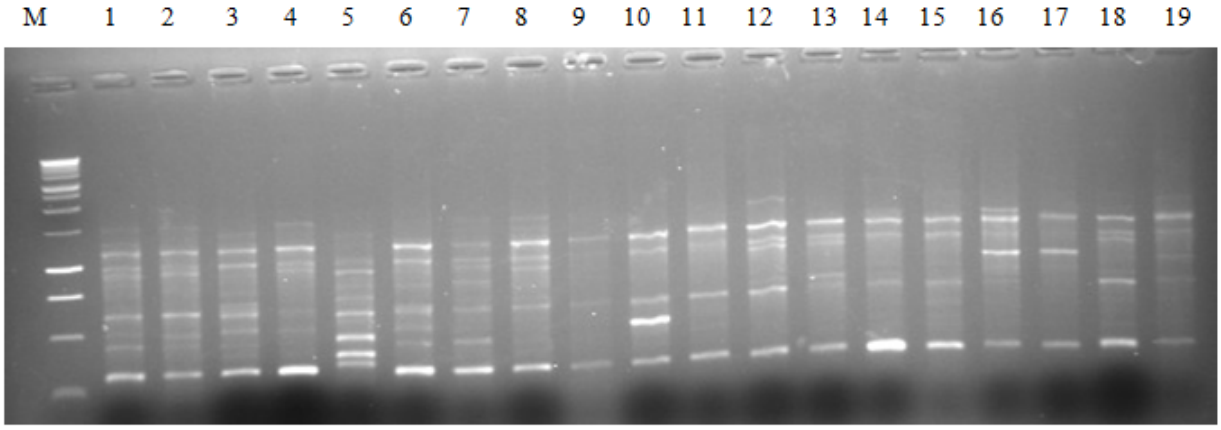

Figure-3. Representative of amplification profiling among samples of *Teucrium polium* using OPT20 RAPD marker. M: DNA ladder. Lane 1-15: West-North of Wadishueib; lane 16-19: West-South.

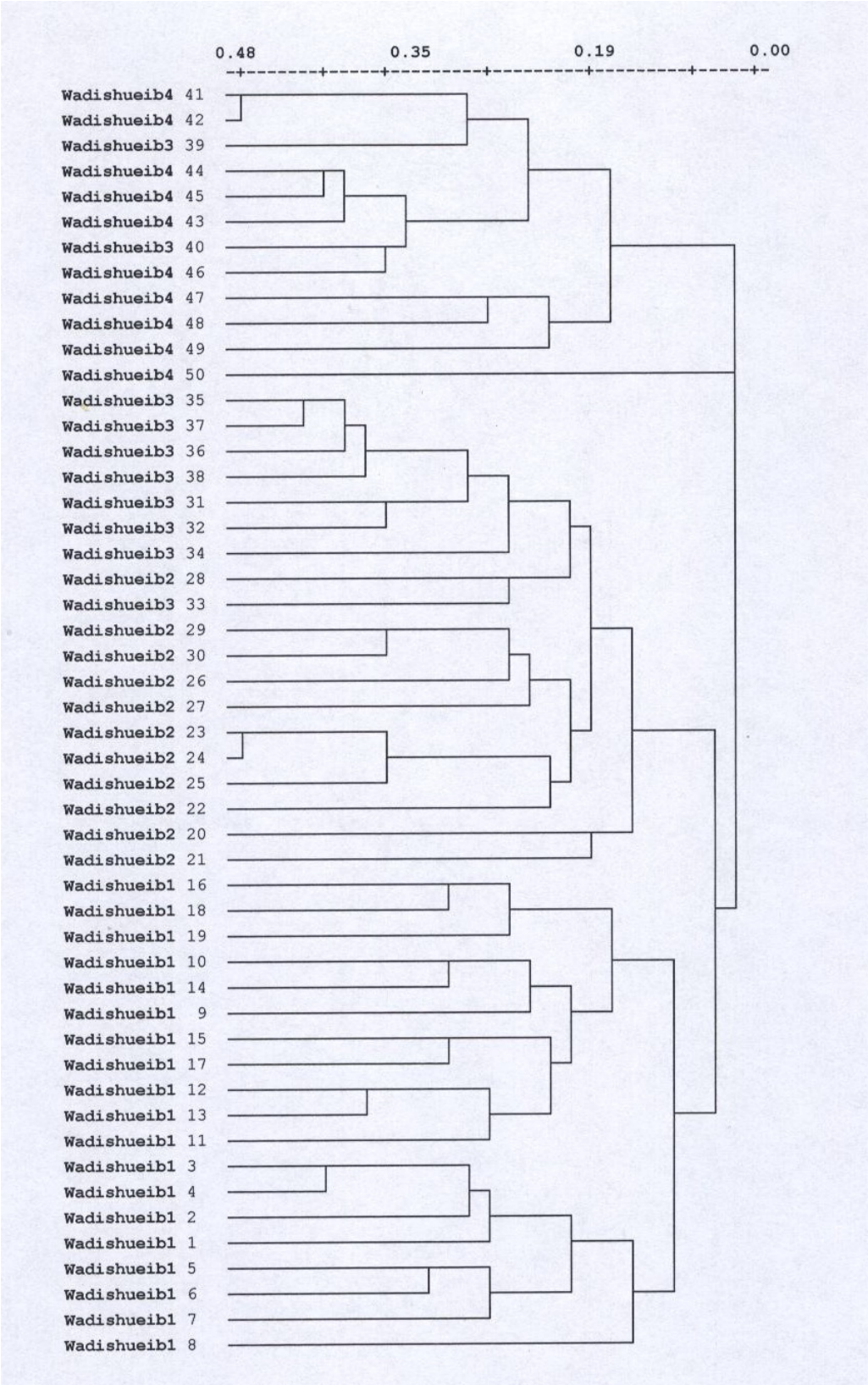

Figure-4. A dendrogram among *Teucrium polium* using ten polymorphic RAPD primers based on Jaccards´ coefficient of similarity.

DISCUSSIONS

RAPD markers were used to assess the genetic variability among and within different plant populations. In this study, the level of variation differed according to the *T. polium* population. The high level of genetic diversity related to the out crossing system in addition to the bioclimatic factors. Variability was found among and within *T. polium* populations indicates that the level of genetic diversity is high that is necessary to invest the variation for developing breeding programs. Khalighi *et al*., (2008) stressed to use strategic planning for conservation of genetic resources with highly genetic variations. Na *et al*., (2009) stated that 96% of variation, (*N. nucifera* spp. *nucifera*), was found within regions, accessions from southwest and eastern China have higher genetic diversity than those from the southern, northern and central China. Our findings revealed that high genetic variability within populations collected from South west and North West compared to East and middle sites. High variation among Jordanian durum wheat landraces was found indicating high level of diversity (Rawashdeh *et al*., 2007). Relatively high similarity values obtained between some of the wheat landraces collected from the same districts showed possible geographic linkage through natural selection, and possible gene exchange, and exchange of seeds among neighboring farmers. The results obtained in this study could be meaningful for devising strategies for conservation and domestication of *Teucrium Polium* through sampling for ex situ conservation programs. Further analysis is needed using other molecular markers techniques.

REFERENCES

Al-Rawashdeh I. M. 2011. Genetic variability in a medicinal plant *Artemisia judaica* using random amplified polymorphic DNA (RAPD) markers. International Journal of Agriculture and Biology. 13(2): 279-282.

Al-Rawashdeh I. M. and Al-Rawashdeh, N. Q. 2011. Exploring genetic diversity in Jordanian wheat landraces from different agro-ecological regions using random amplified polymorphic DNA analysis. International Journal of Agriculture and Biology. 13: 325-331

Bader A., Abo El-Khier Z., Hegazi G. A., Abd El-Kawi A. and El-Sawy A. 2012. Genetic variation in seven natural populations of *Artemisia judaica* L. in South Sinai using RAPD marker. World Applied Sciences Journal. 18(10): 1475-1480.

Bahramikia S. and Yazdanparast R. 2012. Phytochemistry and Medicinal Properties of *Teucrium polium* L. (Lamiaceae). Phytotherapy Research. 26(11): 1581-1593.

Belmekki N., Bendimerad N., Bekhechi C. Fernadez X. 2013. Chemical analysis and antimicrobial activity of *Teucrium polium* L. essential oil from western Algeria. Journal of Medicinal Plants Research. 7(14): 897-902.

Boulila A., Béjaoui A., Messaoud C. and Boussaid M. 2010. Genetic diversity and population structure of *Teucrium polium* (Lamiaceae) in Tunisia. Biochem Genet. 48: 57-70.

Boulila A., Béjaoui A., Messaoud C. and Boussaid M. 2008. Variation of volatiles in Tunisian populations of *Teucrium polium* L. (Lamiaceae). Chemistry and Biodiversity. 5: 1389-1400.

Dhillon R.S., Saharan R.P., Jattan M., Rani T. and von Wuehlisch G. 2013. Molecular characterization of γ–rays induced mutants in *Jatropha curcas* L. Indian Journal of Biotechnology. 13:67-74.

Doyle J. J. and JDoyle J. L. 1987. A rapid DNA isolation procedure for small quantities of fresh leaf tissue. Phytochem. Bull. 19: 11-15.

Feinbrun-Dothan N. 1978. Ericaceae to compositae. *In*: *Flora Palaestina*. 106, the Israel Academy of Sciences and Humanities, Academic Press, Jerusalem.

Gadge P. J. and Nathar V. N. 2014. Detection and analysis of genetic variations in species of Plumbago L. using RAPD markers. Indian Journal Biotechnology. 13: 140-143

Jaccard P. 1908. Nouvelles reserches sur la distribution florale. Bulletin Societe Vaudoise Des Sciences Naturelles. 44: 223-270.

Karim K., Rawda A., Hatem C-M. 2009. Genetic diversity in barely genetic diversity in local Tunisian barley based on RAPD and SSR analysis. Biology Diversity and Conservation. 2(1): 27-35.

Khalighi M., Arzani A. and Poursiahbidi M. A. 2008. Assessment of genetic diversity in *Triticum* spp. and *Aegilops* spp. using AFLP markers. African Journal Biotechnology. 7(5): 546-552.

Mukherjee A, Sikdar B, Ghosh B, Banerjee A, Ghosh E, Bhattacharya M, Roy SC, 2013. RAPD and ISSR analysis of some economically important species, varieties and cultivars of the genus *Allium* (Alliaceae). Turkish Journal of Biology. 37: 605- 618.

Na A., Hong-Bo G. and Wei-dong K. 2009. Genetic variation in lotus (*Nelumbo nucifera* Gaertn. ssp. nucifera) germplasms from China assessed by RAPD markers. Agricultural Science in China. 8(1): 31-39.

Nimbal S., Behl R. K. and Chhabra A. K. 2009. RAPD analysis for genetic polymorphism in bread wheat (*Triticum aestivum* L.em Thell) genotypes varying for grain protein content. The South Pacific Journal of Natural Science. 27: 49-56.

Okigbo R. N., Anuagasi C.L. and Amadi J. E. 2009. Advances in selected medicinal and Aromatic plants indigenous to Africa. Journal Medicinal Plants Research. 3(2): 086-095.

Rawashdeh N. Q., Haddad N. I., Al-Ajlouni M. M. and Turk M. A. 2007. Phenotypic diversity of durum wheat (*Triticum durum* Desf.) from Jordan. Genetic Resources Crop and Evolution. 54: 129-138.

Singh A., Dikshit H.K., Jain N., Singh D. and Yadav R. N. 2014. Efficiency of SSR, ISSR and RAPD markers in molecular characterization mungbean and other *Vigna* species. Indian Journal of Biotechnology. 13: 81-88.

SPSS. 2002. Advanced statistical analysis using SPSS (v.11.0). Chicago, Illinois.

Thimmappaiah W. G., Santhosh, Shobha D., Melwyn G .S. 2009. Assessment of genetic diversity in cashew germplasm using RAPD and ISSR markers. Scientia Horticulturae. 120: 411-417.

Verma P.C., Chakrabarty D., Jena S.N., Mishra D. K., Singh P.K., Sawant S.V. and Tuli R. 2009. The extent of genetic diversity among Vanilla species: comparative results for RAPD and ISSR. Industrial Crops and products. 29: 581-589.

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

Zou X., Dai Z., Ding C., Zhang L., Zhou Y. and Yang R. 2011. Relationships among six medicinal species of Curcuma assessed by RAPD markers. Journal of Medicinal Plants Research. 5(8): 1349-1354.