SYSTEMATIC APPLICABILITY OF ISSR MARKERS AT INTRA-FAMILIAL LEVEL, CASE STUDY IN ASTERACEAE

Houshang NOSRATI[*], Ali MOVAFEGHI[*], MohammadAli Hosseinpour FEIZI[*], Salehe SAFFAR[*], Ahmad Razban HAGHIGHI[**]

[*]*Department of Plant Science, University of Tabriz, Tabriz, Iran*
[**]*Research Centre for Agriculture and Natural Resources, Tabriz, Iran*
Corresponding author: Houshang Nosrati, Department of Plant Science, University of Tabriz, Tabriz, East Azerbaijan, Iran, Zip code: 51666-16471, phone: (+98) 411 3356031, fax: (+98) 411 3356027, e-mail: hnosrati@tabrizu.ac.ir

Abstract. The family Asteraceae with about 25000 species is one of the two largest plant families. The systematic relationship in Asteraceae at sub-familial and tribal levels are controversial, although almost all morphological and molecular classifications have recognized two subfamilies Asteroideae and Lactucoideae. We investigated the applicability of Inter-Simple Sequence Repeats (ISSRs) in inferring the sub-familial and tribal relationship in the Asteraceae by including 17 species belonging to 12 genera representing 6 tribes, and compared the obtained results with data from morphological, nuclear DNA and cp*rbc*/L sequences data. The genetic distance between species pairs were measured based on Nei's distance and a UPGMA (Unweighted Pair-Group Method with Arithmetical Averages) dendrogram was generated based on the matrix of Nei's. Out of 10 ISSRs primers examined, 6 primers produced 107 polymorphic reproducible bands. Our results showed that there was no correlation between ISSRs-based genetic distance and taxonomic relationship between species, e.g. the two species *Achillea micrantha* and *Tanacetum polycephallum* from the same tribe showed highest genetic distance (0.50), while the taxonomically distant species *Condrilla juncea* and *Crepis sancta* showed smallest distance (0.07). Moreover, the ISSRs analysis did not separate the taxa under study into two well-known sub-families. The current work shows that due to high evolution rates, ISSRs are not reliable markers for studying the systematics of plants at higher taxonomic levels such as intra-familial and inter-tribal levels.

Key words: Asteraceae, inter-tribal relationship, intra-familial taxonomy, ISSRs

INTRODUCTION

The family Asteraceae with about 25000 species is one of the two largest plant families [23]. The systematic relationship within the Asteraceae is controversial and has been the subject of long debate [3, 5, 6, 11, 12, 41, 43] so that a symposium was long ago allocated for investigation of the family taxonomy [41]. Although the Asteraceae has been extensively studied using diverse characteristics and different taxonomic approaches, there is still huge incongruent regarding its systematics at subfamilial and tribal levels [41]. Consequently, to date, several completely different systematic classifications have been suggested for subfamilial and tribal relationship in the family [3, 6, 7, 9, 10, 22, 25, 40, 42]. Most of these classifications have recognized the two subfamilies, Asteroideae and Lactucoideae, despite disagreement in their boundaries [41]. More recently, the systematic relationship of the family was investigated using DNA sequences of different nuclear and chloroplast genomes (e.g. [3, 17, 18, 25, 29]). All these studies have confirmed the separation of the family into two subfamilies.

Inter-simple sequence repeats (ISSRs) is a PCR-based technique and involves amplifications of DNA segments between two microsatellite repeats oriented in opposite directions using a single 16-25bp primer complementary to microsatellite site [39]. ISSRs overcomes the most limitations of the other DNA markers e.g. low reproducibility of RAPDs, high cost of AFLP, and preliminary knowledge on flanking primers sites of SSRs [46]. ISSRs are stable across a wide range of PCR parameters and highly informative and reproducible [4, 31]. Since introducing of ISSRs in 1994 [46], these markers have been widely used in different fields of plant sciences [14, 39] including identification of cultivar, varieties and hybrids, and phylogenetic analysis at inter-generic, interspecific and lower taxonomic levels e.g. intra-specific and cultivars [3, 28, 35, 39, 44]. Delimitation of genera and species in several plant families were successfully carried out using ISSRs, such as *Oryza* [24], *Lolium* and *Festuca* [34], *Diplotaxis* [30] and *Dianthus* [21]. Moreover, ISSRs-based genetic variations were estimated at intra- and interspecific levels in many crop species e.g. *Triticum* [33], *Plantago* [45], *Ipomoea* [13] and *Oryza* [24].

ISSRs have been also implemented in marker-assisted selection for crop improvement due to closely linked to agricultural traits e.g. recognition of fertility restoration [2] and genic male sterility in rice [16], *Fusarium*-wilt-resistant in chickpea [36], controlling fructose/glucose ratio in tomatoes [27].

To our best knowledge, the application of ISSRs technique has not been examined at higher taxonomic levels e.g. intra-familial and inter-tribal levels. In the current study the sub-familial and tribal relationship in the Asteraceae were investigated among some representative species using ISSRs (Inter Simple Sequence Repeats) markers, and the obtained results were compared with those results based on morphological, nuclear DNA and cp*rbc*/L sequences data.

MATERIALS AND METHODS

17 species belonging to 12 genera representing 6 tribes were included in the study from the two subfamilies (Asteroideae and Lactucoideae) of the Asteraceae (Table 1). To broadly and inclusively represent each speceis, a wide range of eco-geograhically different genotypes of each speceis were included in the study.

DNA extraction, PCR profile, and ISSRs analysis

Nuclear DNA was extracted from the leaves following [32] with minor modification of using replacement of silver sand by liquid nitrogen. The DNA samples of eco-geograhically different genotypes of each speceis were mixed together in order to make the species-specific sample. PCR reaction consisted of 13µl Master Mix (consisting of PCR buffer, MgCl2, dNTP, Tag; bought from CinnaGen PCR MasterKit, Cat. No. PR8251C), 1µl of 30ng/µl template DNA, plus 1µl of 100pm/µl primer and 10µl deionized water. PCR amplifications were performed in a Biometra thermal cycler. PCR program for primer A, E and F were as follow: the initial denaturation for 5 min at 94°C, followed by 35 cycles of 30 sec at 94°C (for denaturation), annealing for 45sec at 50°C for primers A and E, and 52°C for primer F, and 45sec at 72°C (for synthesis), with final extension step of 72°C for 5min. PCR program for primers H2, H3 and H4 were as follow: initial denaturation at 94°C for 2min, followed by 39 cycles of 30 sec at 94°C (denaturation), 60 sec at 52°C (annealing), and 30sec at 72°C (synthesis), with final extension step of 72°C for 6min. The DNA concentration was measured by spectrophotometry, and adjusted at 10ng/ml. A number of 10 ISSRs primers were examined, of which 6 primers which produced the most polymorphic loci were selected for study. The ISSRs amplifications were repeated three times to insure the reproducibility of the banding patterns. The ISSRs loci were scored as 1 for present and 0 for absent. Consequently, the obtained dataset were entered in a binary matrix for cluster analysis using the NTSYS-pc (Numerical Taxonomy and Multivariate Analysis System, ver. 2.02).

Table 1. Reprehensive species of Asteraceae used for systematic relationship based on ISSRs markers

Subfamily	Species	Tribe
Asteroideae	*Tanacetum polycephallum*	Anthemideae
	Tanacetum chiliophyllum	Anthemideae
	Tanacetum parthaenium	Anthemideae
	Anthemis tinctoria	Anthemideae
	Anthemis triumfettii	Anthemideae
	Achillea micrantha	Anthemideae
	Achillea millefolium	Anthemideae
	Achillea tenuifolia	Anthemideae
	Xanthium strumarium	Heliantheae
	Inula britanica	Inuleae
Lactucoideae	*Onopordon acanthium*	Cynareae
	Cirsium haussknechtii	Cynareae
	Centaurea virgata	Cynareae
	Crepis sancta	Cichorieae
	Condrilla juncea	Cichorieae
	Cichorium intybus	Cichorieae
	Scorzonerara dicosa	Lactuceae

The levels of genetic distance were measured between pairs of species and also among tribes on the basis of Nei's distance. To study the genetic similarity among the populations, the UPGMA (Unweighted Pair-Group Method with Arithmetical Averages) dendrogram was generated based on the matrix of Nei's distance through the SHAN (sequential, hierarchical, agglomerative and nested clustering of the NTSYS-pc) with 100 bootstrap replications.

RESULTS

The ISSRs patterns were reproducible and clear for scoring (Fig. 1). Applying 6 ISSRs primers produced a number of 107 polymorphic loci (Table 2). The lowest genetic distance (0.07) was detected between *Condrilla juncea* and *Crepis sancta*, while the highest genetic distance (0.50) was revealed between *Achillea micrantha* and *Tanacetum polycephallum* (Table 3). At tribal level, the lowest genetic distance (0.137) was detected between two tribes of Cynareae and Cichorieae, while the greatest distance (0.193) revealed between Cichorieae and Anthemideae (Table 4).

In the UPGMA dendrogram all species belonging to a given genus were grouped in its own cluster. However, at tribal level, the genera of the two tribes Cichoreae and Cynareae were well grouped in their own clusters, whereas the three genera belonging to tribe Anthemideae were grouped in three different clusters. At subfamilial level, the dendrogram did no separate the taxa into two different groups representing two well-known subfamilies of the Asteraceae (Fig. 2).

DISCUSSION

In the current work ISSRs data did not infer the taxonomy of Asteraceae at intra-familial and inter-tribal levels. For example, the two species *Achillea micrantha* and *Tanacetum polycephallum* from the same tribe showed highest ISSRs-based genetic distance, while the taxonomically distant species *Condrilla juncea* and *Crepis sancta* belonging to different tribes showed smallest genetic distance. Furthermore, our data on systematic relationship in Asteraceae did not separate the taxa under study to the two well-known subfamilies of Asteroideae and Lactucoideae, whereas classification of Asteraceae into two subfamilies has been strongly supported by different markers including morphological [3, 6] and different DNA sequencing data [19, 20, 41]. The morphological-based cladistic classification showed that Asteroideae is monophyletic while Lactucoideae is paraphyletic [3, 6]. However, our data disagree with Bremer's [6] classification by indicating both subfamilies as non-monophyletic groups. Phylogenetic studies of all currently recognized tribes in Asteraceae based on 330 mutations of restriction sites showed that Asteroideae is a monophyletic group [19, 20], while, cpDNA data provided a weak support for monophyly of Asteroideae [41].

This study is one of the very rare studies which have used ISSRs markers in taxonomic investigation at intra-familial levels. Similar to the results obtained in the current study, using of ISSRs markers in taxonomic revision of Iridaceae produced results, which were inconsistent with morphological classification of this family [38]. However, investigations at inter-familial level on Chenopodiaceae and Amaranthaceae using

ISSRs markers indicated the capability of ISSRs markers in differentiating the members of these two families [37].

Application of ISSRs at lower taxonomic levels appears to be frequent. Reports on the use of ISSRs at intra- and inter-generic levels appear to be conflicting, since some studies showed successful taxonomic revision at inter-generic level, while others did not. For instance, the phylogenetic relationships among several genera in Brassicaceae inferred from ISSRs data were consistent with molecular data [13]. In addition, the application of ISSRs in the genus *Cassia* L. (*s.l.*) has strongly supported the separation of this genus into three different genera of *Chamaecrista* Moench., *Senna* P. Mill. and *Cassia* L. (*s.s.*), which later was confirmed by other data [26]. Similarly, another investigation on the genus *Cassia* using ISSRs markers has resulted in elevation of its position to a higher taxonomic level of subtribe, named as Cassiinae [1]. However, other studies cast doubt on the legitimacy of taxonomic application of ISSRs at this level. For example, Chennaoui-Kourda et al. [8] examining ISSRs markers in differentiating species *Hedysarum* from those of *Sulla*, have concluded that ISSRs are not useful markers for inter-generic taxonomic studies. Whereas application of ISSRs at higher taxonomic levels are very controversial, these markers have been widely successfully used in many different fields of studies at lower taxonomic levels including population genetic structures, cultivar recognition and interspecific relationship [15, 39].

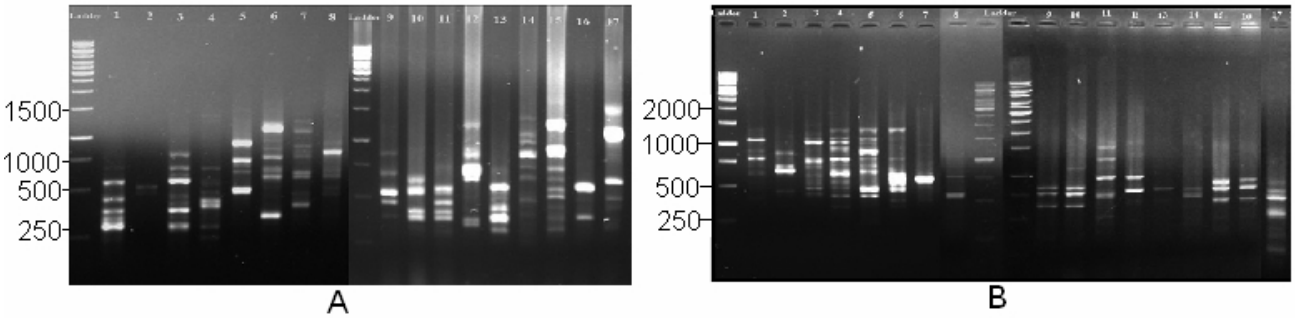

Figure 1. ISSR banding patterns obtained by primers H2 (A) and H4 (B) for 17 species representative of 12 genera of 6 tribes in Asteraceae. 1) *T. Chiliophyllum*, 2) *Tanacetum parthenium*, 3) *Tanacetum polycephallum*, 4) *Anthemis triumfetti*, 5) *Anthemistinctoria*, 6) *Achillea micrantha*, 7) *Achillea tenuifolia*, 8) *Achillea millefollium*, 9) *Xanthium strumarium*,10) *Cirsium haussknechtii*, 11) *Centaurea virgata*,12) *Onopordon acanthium*, 13) *Cichorium intybus*, 14) *Crepis sancta*, 15) *Condrilla juncea*, 16) *Inula britanica*,17) *Scorzonera radicosa*.

Table 2. Primers sequences and the numbers of polymorphic loci produced in 17 species belonging to 12 genera representing 6 tribes in Asteraceae

Primer Code	Primer sequence (5'-3')	Primer annealing time (s)	No. of Polymorphic ISSRs loci
H2	$(AC)_8$ T	52	20
H3	$(AC)_8$ G	52	16
H4	$(AC)_8$ C	52	16
A	$(AG)_{10}$ C	50	22
E	$(CA)_8$ GC	50	23
F	$(AG)_7$ C	50	10

Table 3. Matrix of Nei's genetic distance among the reprehensive species of Asteraceae based on ISSRs variations

Tanacetum chiliophyllum	0.00																	
Tanacetum parthaenium	0.22	0.00																
Tanacetum polycephallum	0.18	0.25	0.00															
Anthemis tinctoria	0.33	0.31	0.36	0.00														
Anthemis triumfettii	0.44	0.35	0.45	0.19	0.00													
Achillea micrantha	0.41	0.41	0.50	0.42	0.42	0.00												
Achillea tenuifolia	0.44	0.36	0.45	0.37	0.41	0.16	0.00											
Achillea millefolium	0.36	0.32	0.44	0.31	0.35	0.21	0.21	0.00										
Xanthium strumarium	0.34	0.28	0.42	0.29	0.35	0.43	0.36	0.30	0.00									
Cirsium haussknechtii	0.36	0.33	0.41	0.36	0.41	0.40	0.36	0.23	0.23	0.00								
Centaurea virgata	0.38	0.35	0.43	0.32	0.37	0.40	0.32	0.23	0.25	0.15	0.00							
Onopordon acanthium	0.35	0.33	0.41	0.37	0.41	0.42	0.36	0.25	0.25	0.15	0.15	0.00						
Cichorium intybus	0.35	0.31	0.39	0.28	0.36	0.40	0.36	0.25	0.20	0.21	0.21	0.24	0.00					
Crepis sancta	0.37	0.36	0.42	0.29	0.36	0.41	0.36	0.28	0.21	0.23	0.23	0.23	0.10	0.00				
Condrilla juncea	0.42	0.36	0.45	0.32	0.39	0.40	0.34	0.27	0.20	0.21	0.21	0.21	0.13	0.07	0.00			
Inula britanica	0.38	0.31	0.41	0.30	0.36	0.40	0.37	0.27	0.18	0.22	0.24	0.22	0.17	0.18	0.19	0.00		
Scorzonera radicosa	0.40	0.36	0.45	0.34	0.41	0.44	0.39	0.31	0.27	0.26	0.30	0.26	0.21	0.21	0.24	0.19	0.00	

Table 4. Nei's distance between pairs of three tribes in Asteraceae obtained from ISSRs variations

Tribes	Anthemideae	Cynareae	Cichorieae
Anthemideae	0000		
Cynareae	0.171	000	
Cichorieae	o.193	0.137	000

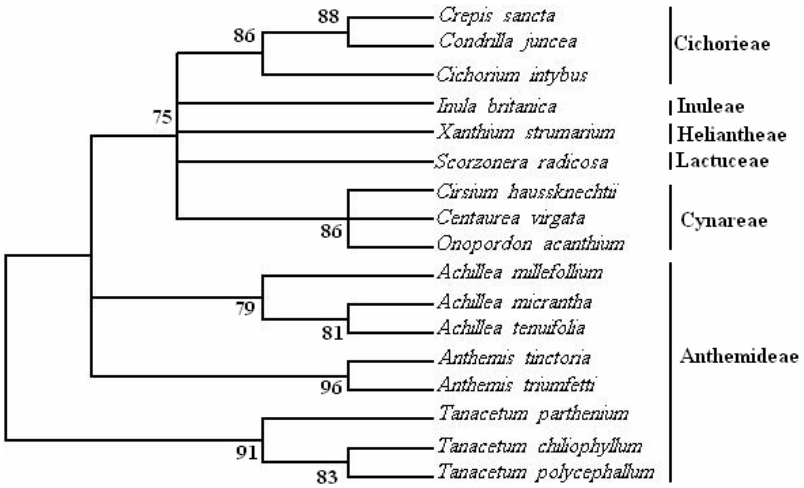

Figure 2. UPGMA dendrogram generated from matrix of Nei's distance calculated from ISSRs variations among 17 representative species of Asteraceae showing inter-subfamilial and tribal relationship (constructed by 100 bootstrap replicates)

To our best knowledge based on extensive search on database citation sites, the current study is one of two investigations on applicability of ISSRs variation at familial level. Our data indicated that these markers are not proper markers to study at higher taxonomic levelsl. This study also implies that the evolution rates of markers should correspond with the taxonomic levels of taxa under study, and that evolution rate of ISSRs is too high to apply to familial level.

REFERENCES

[1] Acharya, L., Mukherjee, A.K., Panda, P.C., (2011): Separation of the genera in the subtribe Cassiinae (Leguminosae: Caesalpinioidae) using molecular markers. Acta Botanica Brasilica 25(1): 223-233.

[2] Akagi, H., Yokozeki, Y., Inagaki, A., Nakamura A., Fujimura, T., (1996): A co-dominant DNA marker closely linked to the rice nuclear restorer gene, Rf-1, identified with inter-SSR fingerprinting. Genome 39: 1205-1209.

[3] Bayer, R.J., Starr, J. R., (1998): Tribal phylogeny of the Asteraceae based on two non-coding chloroplast sequences, the trnL intron and trnL/trnF intergenic spacer. Annals of the Missouri Botanical Garden, 85: 242-256.

[4] Bornet, B., Branchard M., (2001). Nonanchored Inter Simple Sequence Repeat (ISSR) markers: Reproducible and specific tools for genome fingerprinting. Plant Molecular Biology Reporter, 19: 209-215.

[5] Bremer, K., (1987): Tribal interrelationships of the Asteraceae. Cladistics, 3: 210-253.

[6] Bremer, K., Jansen, R.K., (1992): A new subfamily of the Asteraceae. Annals of the Missouri Botanical Garden, 79: 414– 415.

[7] Carlquist, S., (1976): Tribal interrelationships and phylogeny of the Asteraceae. Aliso, 8: 465-492.

[8] Chennaoui-Kourda, H., Marghali, S., Marrakchi, M., Trifi-Farah, N., (2007): Genetic diversity of *Sulla* genus (Hedysarea) and related species using Inter-simple Sequence Repeat (ISSR) markers. Biochemical Systematics and Ecology, 35: 682-688.

[9] Ciccareli, D., Garbari, F., Pagni, A.M., (2007): Glandular hairs of the ovary: a helpful character for Asteroideae (Asteraceae) Taxonomy. Annales Botannici Fennici, 44: 1-7.

[10] Cronquist, A., (1955): Phylogeny and taxonomy of the Compositae. American Midland Naturalist, 53: 478-511.

[11] Cronquist, A., (1977): The Compositae revisited. Brittonia, 29: 137-153.

[12] Cronquist, A., (1981): An integrated system of classification of Flowering plants. Columbia University Press, New York, pp. 268-270.

[13] Dogan, B., Unal, M., Ozgokce, F., Martine, E., Kaya, A., (2011): Phylogenetic relationships between *Malcolmia*, *Strigosella*, *Zuvanda*, and some closely related genera (Brassicaceae) from Turkey revealed by inter-simple sequence repeat amplification. Turkish Journal of Botany, 35: 17-23.

[14] Godwin, I.D., Aitken, E.A.B., Smith, L.W., (1997): Application of inter-simple sequence repeat (ISSR) markers to plant genetics. Electrophoresis, 18: 1524-1528.

[15] Huang, J., Sun, S.M., (2000): Genetic diversity and relationships of sweet potato and its wild relatives in *Ipomoea* series Batatas (Convolvulaceae) as revealed by inter-simple sequence repeat (ISSR) and restriction analysis of chloroplast DNA. Theoretical and Applied Genetics, 100: 1050–1060.

[16] Hussain, A.J., Gupta,V., Ali, J., Ranjekar, P.K., Siddiq, E.A. (2000): Physiological characterization, genetics and molecular mapping of a new source of temperature sensitive genetic male sterility in rice. Fourth International Rice Genetics Symposium, 22–27. October 2000, IRRI, Philippines, Abstracts, p. 95.

[17] Jansen, R.K., Palmer, J.D., (1987a): Chloroplast DNA from lettuce and *Barnadesia* (Asteraceae): Structure, gene localization and characterization of a large inversion. Current Genetics, 11: 553-564.

[18] Jansen, R.K., Palmer, J.D., (1987b): A chloroplast DNA inversion marks as an ancient evolutionary split in the sunflower family (Asteraceae). Proceedings National Academy science USA, 84: 5818-5822.

[19] Jansen, R.K., Holsinger, K.E., Michaels, H.J., Palmer, J.D., (1990): Phylogenetic analysis of restriction site data at higher taxonomic levels: an example from the Asteraceae. Evolution, 44: 2089-2105.

[20] Jansen, R.K., Michaels, H.J., Palmer, J.D., (1991): Phylogenetic and character evolution in the Asteraceae based on chloroplast DNA restriction site mapping. Systematic Botany, 16: 98-115.

[21] Jarda, L., Butiuc-Keul, A., Höhn, M., Pedryc, A., Cristea, V., (2014): Ex situ conservation of *Dianthus giganteus* d'Urv. subsp. banaticus (Heuff.) Tutin by *in*

vitro culture and assessment of somaclonal variability by molecular markers. Turkish Journal of Biology, 38(1): 21-30.

[22] Jeffery, C., (1978): Compositae. In Flowering Plants of the World (Ed. Heywood VH) Mayflower, New York, pp. 263-268.

[23] Jeffrey, C. (2007): Compositae: Introduction with key to tribes. pp. 61-87. In Kadereit, J.W., Jeffrey, C., (eds.): Families and Genera of Vascular Plants, vol. VIII, Flowering Plants, Eudicots, Asterales. Springer-Verlag, Berlin.

[24] Joshi, S.P., Gupta, V.S., Aggarwal, R.K., Ranjekar, P.K., Brar, D.S., (2000): Genetic diversity and phylogenetic relationship as revealed by inter-simple sequence repeat (ISSR) polymorphism in the genus *Oryza*. Theoretical and Applied Genetics, 100: 1311-1320.

[25] Kim, K.J., Jansen, R.K., Wallace, R.S., Michaels, H.J., Palmer, J.D., (1992): Phylogenetic Implications of rbcL sequence variation in the Asteraceae. Annals of the Missouri Botanical Garden, 79: 428- 445.

[26] Laxmikanta, A., Pratap, C.P., (2010): Validation of generic status of different taxa in the subtribe Cassiinae (Leguminosae: Caesalpinoidae) using RAPD, ISSR and AFLP markers. International Journal of Plant Physiology and Biochemistry, 2: 18-28.

[27] Levin, I.N., Gilboa, E., Yeselson, S., Schaffer, A.A., (2000): *Fgr*, a major locus that modulates the fructose to glucose ratio in mature tomato fruits. Theoretical and Applied Genetics, 100: 256–262.

[28] Li, H., Chen, G., (2008): Genetic relationship among species in the genus *Sonneratia* in China as revealed by inter-simple sequence repeat (ISSR) markers. Biochemical Systematics and Ecology, 36: 392-398.

[29] Lindera, C.R., Goertzen, L.R., Heuvel, B.V., Francisco-Ortega, J., Jansen, R.K., (2000): The complete external transcribed spacer of 18S-26S rDNA: Amplification and phylogenetic utility at low taxonomic levels in Asteraceae and closely allied families. Molecular Phylogenetics and Evolution, 14: 285-303.

[30] Martin, J.P., Sanchez-Yelamo, M.D., (2000): Genetic relationships among species of the genus *Diplotaxis* (Brassicaceae) using inter-simple sequence repeat markers. Theoretical and Applied Genetics, 101: 1234-1241.

[31] Matthews, D., McNicoll, J., Harding, K., Millam, S., (1999): 50-anchored simple sequence repeat primers are useful for analysing potato somatic hybrids. Plant Cell Reproduction, 19: 210-212.

[32] Miller, B., (2002): Investigating plant DNA, Student's Guide, plant science centre SAPS, pp. 6.

[33] Nagaoka, T., Ogihara, Y., (1997): Applicability of inter-simple sequence repeat polymorphisms in wheat for use as DNA markers in comparison to RFLP and RAPD markers. Theoretical and Applied Genetics, 94: 597-602.

[34] Pasakinskiene, I., Griffiths, C.M., Bettany, A.J.E., Paplauskiene, V., Humphreys, M.W., (2000): Anchored simple-sequence repeats as primers to generate species-specific DNA markers in *Lolium* and *Festuca* grasses. Theoretical and Applied Genetics, 100: 384–390.

[35] Rakoczy-Trojanowska, M., Bolibok, H., (2004): Characteristics and a comparison of three classes of microsatellite-based markers and their application in plants. Cell Molecular Biology Letter, 9: 221-238.

[36] Ratnaparkhe, M.B., Tekeoglu, M., Muehlbauer, F.J., (1998): Intersimple-sequence-repeat (ISSR) polymorphisms are useful for finding markers associated with disease resistance gene clusters. Theoretical and Applied Genetics, 97: 515-519.

[37] Ray, T., Roy, S.C., (2007): Phylogenetic relationships between members of Amaranthaceae and Chenopodiaceae of Lower Gangetic Plains using RAPD and ISSR markers. Bangladesh Journal of Botany, 36: 21-28.

[38] Raycheva, T., Stoyanov, K., Denev, I., (2011): Genetic diversity and molecular taxonomy study of three genera from Iridaceae family in the Bulgarian flora based on ISSR markers. Biotechnology and Biotechnological Equipment, 25: 2484-2488.

[39] Reddy, P.M., Sarla, N., Siddiq, E.A., (2002): Inter simple sequence repeat (ISSR) polymorphism and its application in plant breeding. Euphytica, 128: 9-17.

[40] Robinson, H., (1981): A revision of tribal and subtribal limits of the Heliantheae (Asteraceae). Smithsonian Contributions to Botany, 51: 1-102.

[41] Soltis, P.S., Soltis, D.E., Doyle, J.J., (1995): Molecular Systematics of Plants. Chapman and Hall. New York, pp. 255-258.

[42] Thorne, R., (1983): Proposed new re-alignments in the angiosperms. Nordic Journal of Botany, 3: 85-117.

[43] Wagenitz, G., (1976): Systematics and phylogeny of the Compositae (Asteraceae). Plant Systematic and Evolution, 125: 29-46.

[44] Wang, H.Z., Feng, S.G., Lu, J.J., Shi, N.N., Liu, J.J., (2009): Phylogenetic study and molecular identification of 31 *Dendrobium* species using inter-simple sequence repeat (ISSR) markers. Scientia Horticulturae, 122: 440-447.

[45] Wolff, K., Morgan-Richards, M., (1998): PCR markers distinguish *Plantago major* subspecies. Theoretical and Applied Genetics, 96: 282-286.

[46] Zietkiewitcz, E., Rafalski, A., Labuda, D., (1994): Genome fingerprinting by simple sequence repeat (SSR)-anchored polymerase chain reaction amplification. Genomics, 20: 176-183.

Received: 13 December 2013
Accepted: 27 April 2014
Published Online: 30 April 2014
Analele Universităţii din Oradea, Fascicula Biologie
http://www.bioresearch.ro/revistaen.html
Print-ISSN: 1224-5119
e-ISSN: 1844-7589
CD-ISSN: 1842-6433