Evaluation of genetic diversity amongst *Descurainia sophia* L. genotypes by inter-simple sequence repeat (ISSR) marker

Sahar Saki¹ · Hedayat Bagheri¹ · Ali Deljou¹ · Mehrshad Zeinalabedini²

Received: 20 June 2015 / Accepted: 27 October 2015 / Published online: 14 November 2015
© Prof. H.S. Srivastava Foundation for Science and Society 2015

Abstract *Descurainia sophia* is a valuable medicinal plant in family of Brassicaceae. To determine the range of diversity amongst *D. sophia* in Iran, 32 naturally distributed plants belonging to six natural populations of the Iranian plateau were investigated by inter-simple sequence repeat (ISSR) markers. The average percentage of polymorphism produced by 12 ISSR primers was 86 %. The PIC values for primers ranged from 0.22 to 0.40 and Rp values ranged between 6.5 and 19.9. The relative genetic diversity of the populations was not high ($G_{st} = 0.32$). However, the value of gene flow revealed by the ISSR marker was high ($N_m = 1.03$). UPGMA clustering method based on Jaccard similarity coefficient grouped the genotypes into two major clusters. Graph results from Neighbor-Net Network generated after a 1000 bootstrap test using Jaccard coefficient, and STRUCTURE analysis confirmed the UPGMA clustering. The first three PCAs represented 57.31 % of the total variation. The high levels of genetic diversity were observed within populations, which is useful in breeding and conservation programs. ISSR is found

to be an eligible marker to study genetic diversity of *D. sophia*.

Keywords *Descurainia sophia* · Genetic diversity · Iranian plateau · ISSR marker

Introduction

The global herbal product market has an annual growth rate of 5 to 15 %. It is expected that the world herbal drug market will reach a total of 5 trillion US dollars by 2050 (Joshi et al. 2004). *Descurainia sophia* L. Webb ex Prantl belongs to Brassicaceae family and is a self-compatible, dicotyledonous plant that grows extensively in forage, rangelands, cultivated crops, disturbed areas, roadsides and waste sites (Mitich 1996; Baskin et al. 2004; Blackshaw et al. 2005; Hernandez Plaza et al. 2011; Li et al. 2011). *D. sophia* originated in South Europe and North Africa (Zhang 2003). The seeds are widely used for medicinal, food and industrial purposes (Mitich 1996; Peng et al. 1997; Bekker et al. 2005; Sun et al. 2005; Mohamed and Mahrous 2009; Li et al. 2010; Mosaddegh et al. 2012; Khan and Wang 2012). *D. sophia* seeds contain 3.5–4 % ash, 7.6 % fiber, 22–44 % oil and 25 % protein (Tkachuk and Mellish 1977; Duke and Ayensu 1985; Peng et al. 1997; Bekker et al. 2005). They are expectorant, restorative and stimulant and traditionally used to make a sweet Iranian drink that detoxifies the liver (Pasalar et al. 2013; Khodarahmi et al. 2015). The highest amount of essential un-saturated fatty acid (oleic, linolic, linoleic) in the oil seed of *D. sophia* is on the average of 71.4 % (Gruzdienė and Bagdonaitė 2003) and the content of essential linoleic fatty acid is as great as 44 % (Maršalkienė et al. 2009).

The role of genetic variation in facilitating plant breeding and preservation strategies has long been acknowledged

✉ Hedayat Bagheri
bagheri.hedayat@gmail.com

Sahar Saki
sakisahar@gmail.com

Ali Deljou
alideljou@yahoo.com

Mehrshad Zeinalabedini
mzeinolabedini@abrii.ac.ir

¹ Faculty of Agriculture, Department of Biotechnology, Bu Ali-Sina University, Hamedan 6517838695, Iran

² Agriculture Biotechnology Research Institute of Iran (ABRII), Seed and Plant Improvement Institutes Campus, Mahdasht Road, Karaj 315351897, Iran

(Sehgal and Raina 2008; Rivers et al. 2014; Govindaraj et al. 2015). This variation is needed to improve favorite traits for future challenges such as climate change and disease evolution (Kisha and Johnson 2012; Alberto et al. 2013). In Iran, the majority of medicinal plants are still harvested from natural fields and no cultivar have been developed. This will eventually lead to gradual extinction of favorable germplasms. A prerequisite to the identification of species is the analysis of genetic diversity (Chawla 2003). Useful tools such as molecular markers assess genetic variation and provide an efficient mean to link phenotypic and genotypic variation (Varsheny et al. 2005) and to characterize plants (Chawla 2003; Kalia et al. 2011).

The ISSR technique is applied to investigate genetic diversity in a wide range of plant species (Reddy et al. 2002; Liu et al. 2013; Singh et al. 2014). Here, we used ISSR markers to investigate and analyze the genetic diversity of 32 *D. sophia* plants collected from six locations in Iranian plateau.

Materials and methods

Plant materials

A total of 32 naturally distributed genotypes were collected from six different populations in Iran (Hamedan, Lorestan, Central, Fars, Yazd, Kerman). From Hamedan 12, Lorestan 6, Central 5, Fars 4, Yazd 3 and Kerman 2 individuals were collected (Table 1, Fig. 1).

DNA extraction and ISSR analysis

Genomic DNA was isolated from the young leaves of plants according to the modified CTAB method of Doyle and Doyle (1987). Inter simple sequence repeat markers were amplified through PCR by twelve primers listed in Table 2. Thermal cycling (BIO-RAD T100) started with 5 min at 94 °C, and 40 cycles of 1 min at 94 °C, 75 s at each primer's annealing temperature (Table 2) and 2 min at 72 °C ended by an extension for 10 min at 72 °C. The PCR products were separated on 1.2 % agarose gels, stained with ethidium bromide and documented using a UV transilluminator system (Liu et al. 2007a, 2007b).

Data analysis

The amplified ISSR fragments were scored for presence (1) or absence (0) of bands. Only clear and reproducible bands were scored. Resolving power (Rp) of ISSR primers which indicates the discriminatory potential of the applied primers was calculated based on Prevost and Wilkinsin (1999) formula: $Rp = \sum Ib$, where Ib is the band informativeness. Ib is computed via $Ib = 1 - [2 \times (0.5 - p)]$, where p is the proportion of the

Table 1 The list of 32 *D. sophia* genotypes evaluated in this study with their latitude, longitude and location names

No.	Genotypes	Latitude and Longitude	Location
1	H1	35°23.12 N 49°02.02E	Hamedan
2	H2	34°32.53 N 48°26.48E	Hamedan
3	H5	35°12.29 N 48°43.26E	Hamedan
4	H6	34°48.06 N 48°28.49E	Hamedan
5	H22	34°41.57 N 48°37.03E	Hamedan
6	H23	34°45.11 N 48°36.22E	Hamedan
7	H24	34°44.22 N 48°36.26E	Hamedan
8	H29	34°45.23 N 48°36.08E	Hamedan
9	H40	34°15.13 N 48°57.46E	Hamedan
10	H48	34°36.07 N 48°27.02E	Hamedan
11	H52	34°43.14 N 48°23.04E	Hamedan
12	H57	34°12.33 N 48°42.21E	Hamedan
13	L7	33°29.34 N 49°02.47E	Lorestan
14	L4	33°30.09 N 49°02.34E	Lorestan
15	L10	33°27.21 N 49°27.20E	Lorestan
16	L1	33°53.39 N 48°46.01E	Lorestan
17	L12	33°55.01 N 48°43.28E	Lorestan
18	L9	34°01.01 N 48°38.29E	Lorestan
19	CI1	35°41.46 N 51°25.23E	Central
20	CI2	34°38.23 N 50°52.33E	Central
21	CI3	36°16.00 N 50°00.00E	Central
22	CI4	33°55.39 N 49°24.42E	Central
23	CI5	35°34.22 N 53°23.50E	Central
24	F2	29°06.15 N 53°02.45E	Fars
25	F3	29°35.30 N 52°35.01E	Fars
26	F4	28°56.18 N 53°38.54E	Fars
27	F5	30°53.56 N 52°41.12E	Fars
28	Y2	32°01.48 N 53°32.60E	Yazd
29	Y14	31°44.50 N 54°12.32E	Yazd
30	Y17	31°07.49 N 53°15.01E	Yazd
31	K1	30°24.24 N 55°59.38E	Kerman
32	K3	29°13.59 N 56°36.08E	Kerman

32 genotypes containing the band. PIC value for each polymorphic locus was calculated according to Roldán-Ruiz et al. (2000): $PIC_i = 2f_i(1 - f_i)$, where f_i is frequency of fragments present in that locus. To examine different genetic diversity parameters PopGene 32 (Yeh and Boyle 1997) software was used. Number of observed alleles (N_a), number of effective alleles (N_e), Nei's gene diversity (H) and Shannon's information index (I) were calculated. The Nei's unbiased measures of genetic identity and genetic distance among populations were obtained by PopGene 32 software. Mantel's test (1967) for Jaccard and Dice coefficients were performed. Other genetic diversity parameters including the number of polymorphic loci (PL), the percentage of polymorphic loci (PPL), gene flow (N_m) based on $N_m = 0.5 \times (1 - G_{st}) \div G_{st}$ were

Fig. 1 Schematic map of Iran and few places where the *D.sophia* plant materials were collected from. Letters on map are genotype names based on Table 1

calculated (McDermott and McDonald 1993). Similarity coefficient of Jaccard (1908) was used to calculate the pairwise genetic similarities among individuals. To determine the quality of clustering, cophentic correlation coefficient (r) was measured based on the UPGMA cluster analysis (Rohlf and Sokal 1981). These calculations were carried out by NTSYS 2.02e software package (Rohlf 1992). A dendrogram was developed by the Neighbor-Net Network method, carried out with

SplitsTree4 software (Huson and Bryant 2006) after a 1000 bootstrap test using Jaccard coefficient. Nei's average number of pairwise differences in six populations of *D. sophia* was accomplished by Arlequin 3.5.1.2 (Excoffier and Lischer 2010). Differences among samples were calculated by use of Structure software version 2.3.3 (Hubisz et al. 2009). Two other multivariate methods such as principle component analysis (PCA) and principle coordinate analysis (PCoA)

Table 2 Data of ISSR primers used in the present study including primer name, primer sequence, annealing temperature (T_a), size range of amplified fragments (SR), number of total bands (NT), number of

polymorphic bands (NP), percentage of polymorphic fragment (PP), polymorphic information content (PIC_{avg}) and resolving power (R_p) of 12 primers are presented. ($Y^* = C$ or T , $R^* = G$ or A)

Primer name	Primer seq.	T_a	SR	NT	NP	PP (%)	PIC_{avg}	R_p
UBC 807	(AG)8 T	54	220–1850	106	74	69	0.22	6.54
UBC 834	(AG)8Y*T	53	150–2250	230	230	100	0.30	14.18
UBC 855	(AC)8YT	54	300–2525	214	182	85	0.26	13.24
UBC 818	(CA)8G	53	400–3000	205	205	100	0.36	12.68
UBC 844	(CT)8R*C	54	350–1350	175	143	81	0.40	10.86
UBC 848	(CT)8RG	42.5	300–3100	260	228	87	0.29	14.28
UBC 811	(GA)8C	51	300–2500	257	225	87	0.34	15.9
UBC 810	(GA)8 T	52	250–2500	244	212	86	0.29	15.08
UBC 841	(GA)8YC	53	150–2250	320	160	50	0.33	19.9
UBC 823	(TC)8C	54	300–2000	156	156	100	0.31	9.64
UBC 830	(TG)8G	52	370–1875	163	163	100	0.33	9.36
M2	GGGC(GA)8	56	425–2500	283	251	88	0.31	17.56
Mean	—	—	—	217.75	185.75	86	0.31	13.26
Total	—	—	—	2613	2229	—	—	—

Fig. 2 ISSR patterns generated by UBC 848 primer on 32 genotypes of *D. sophia* DNA. The ladder is a VC 100 bp Plus DNA Ladder (Vivantis). Lanes designate 32 genotypes based on Table 1

(Mohammadi and Prasanna 2003) were also calculated to assess genetic diversity. The partitioning of genetic variance was carried out by analysis of molecular variance (AMOVA) (Excoffier et al. 1992) in GenAlEx version 6.4 (Peakall and Smouse 2006).

Results

The twelve ISSR primers produced a total of 2613 reliable fragments, scored in 202 loci, of which 190 were polymorphic. Each primer produced an average of 217.75 bands. The length of amplified bands ranged from 150 bp in primers UBC 834 and UBC 841 to 3100 bp in primer UBC 848. The highest number of fragments (283) was produced by primer M2. M2 also provided the maximum

number of polymorphic bands (251). The UBC807 primer developed the lowest number of fragments (106) of which 74 were polymorphic. The ISSR pattern obtained with UBC 848 primer is shown in Fig. 2 (Fig. 2). The mean of polymorphism percentage for all 12 primers was 86 %. The UBC841 primer generated the minimum polymorphism of 50 %. Primers UBC834, UBC818, UBC823 and UBC 830 showed 100 % polymorphism. UBC807 primer with 0.22 and UBC844 primer with 0.40 showed the lowest and the greatest PIC value among all primers, respectively. The mean of Rp for the 12 primers was 13.26, with the highest value in UBC 841 primer (19.9) and the lowest one in UBC 807 primer (6.54) (Table 2).

The similarity value of the 32 genotypes based on Jaccard coefficient varied between 0.265 and 0.806. The lowest

Fig. 3 UPGMA clustering of *D. sophia* genotypes based on Jaccard similarity coefficient calculated from ISSR markers

Fig. 4 The constructed dendrogram based on the binary matrix of the scored ISSR marker data in 32 individuals of *D. sophia*

similarity was between L9 and Y17 genotypes (0.265) which correspond to their different origins, whereas the highest similarity (0.806) was observed between two genotypes of Lorestan (L4 and L7) (Data not shown).

The UPGMA clustering algorithm from ISSR analysis grouped the 32 genotypes into two clusters at a similarity index value of 0.36 (Fig. 3). The top cluster is smaller and consists of Hamedan, Fars and Lorestan populations. The

second cluster embraces populations from Hamedan, Lorestan, Central, Fars, Kerman and Yazd. At similarity index of 0.41 Yazd genotypes (Y14, Y17) have been separated in one cluster. This partitioning of genotypes does not show a precise correlation with the location classification of genotypes. Cophenetic value (r) of 0.90 indicates that cluster analysis strongly represents the similarity matrix (Wang et al. 2011).

Fig. 5 Nei's average number of pairwise differences in six populations of *D. sophia*

Fig. 6 Bar plots of *D. sophia* genotypes generated by Structure software 2.3.3. The groups are represented by different colors. Each bar which is divided into segments indicates its genetic composition. The longer the segment the more a sample resembles one of the groups. The labels below

the bar plots are the corresponding numbers for each individual, based on Table 1, including the number of their location in the bracket (1: Hamedan, 2: Lorestan, 3: Central, 4: Fars, 5: Yazd, 6: Kerman)

A dendrogram was constructed based on the binary matrix of the scored ISSR marker data (Fig. 4). The displayed clustering corresponds to the two major groups resulted by UPGMA dendrogram. The genotypes in green and red accommodate with the smaller and the second cluster of the UPGMA dendrogram, respectively. The three populations of Yazd could be considered as a separate group.

In Fig. 5 the average number of pairwise differences between each population in the upper half of the matrix can be observed (green). The average number of pairwise differences within each population is shown in the diagonal (orange), and the lower half of the matrix (blue) shows the corrected average pairwise differences of the populations. Nei's average numbers of pairwise differences are observed within Hamedan and Fars populations and between populations of Lorestan and Fars.

Bar plots of the 32 genotypes generated by Structure software confirmed the constructed dendrogram on the binary matrix in Fig. 4. In Fig. 6 individuals such as 28, 29 and 30 corresponding to genotypes Y2, Y14 and Y17 respectively are between the two other groups according to the dendrogram based on the binary matrix. Individuals placed in the green bar plots (11, 10, 27, 18, 26, 6, 1, 5 and 8 represent genotypes H52, H48, F5, L9, F4, H23, H1, H22 and H29, respectively)

are the genotypes that were positioned at the smaller group in UPGMA clustering (Fig. 3). They are also corresponding with genotypes in the green group generated by the Neighbor-Net Network method (Fig. 4).

In the principle component analysis the eigenvalues for the first three PCAs are 14.89, 2.40, and 1.04, respectively that represent 57.31 % of the total variation. This shows a proper distribution of ISSR markers through the entire genome. The cumulative arrangement of the 32 individuals, using genetic similarity based on ISSR markers, is shown in Fig. 7. The graph of PCoA analysis shows two main associations which confirms the partitioning results of the UPGMA clustering.

The PopGene calculations (Table 3) showed that the number of observed alleles and effective alleles ranged between 1.19–1.75 (Kerman- Hamedan) and 1.13–1.37 (Kerman- Lorestan), respectively. Hamedan and Kerman have the highest and the lowest H value among the six populations (0.22 and 0.08, respectively). The average of Shannon's Information Index (I) for the six populations is 0.26 which again the maximum and the minimum are respectively belonging to Hamedan and Kerman populations. The highest number of polymorphic loci (PL) and percentage of polymorphic loci (PPL) both belong to Hamedan while the lowest, pertains to Kerman. The diversity among populations (G_{st}) and gene flow (N_m) is 0.32 and 1.03 respectively.

Fig. 7 Distribution of the 32 *D. sophia* genotypes revealed by PCoA analysis based on genetic similarity estimates calculated from ISSR data

Table 3 Genetic diversity data and differentiation parameters for six natural populations of *D.sophia* in Iran. Sample Size (SS), Number of observed alleles (Na), Number of effective alleles (Ne), Nei's gene

diversity (H), Shannon's information index (I), Number of polymorphic loci (PL), Percentage of polymorphic loci (PPL), Diversity among populations (Gst), Gene flow (Nm), Fixation Index (Fst)

Population	SS	Na	Ne	H	I	PL	PPL	Gst	Nm	Fst
Hamedan	12	1.7574	1.3584	0.2238	0.3473	153	75.74			
Lorestan	6	1.6337	1.3722	0.2148	0.3230	128	63.37			
Central	5	1.5099	1.3045	0.1786	0.2684	103	50.99			
Fars	4	1.5297	1.3203	0.1900	0.2851	107	52.97			
Yazd	3	1.3960	1.2605	0.1513	0.2242	80	39.60			
Kerman	2	1.1931	1.1365	0.0800	0.1168	39	19.31			
Mean	—	1.5033	1.2920	0.1730	0.2608	—	—			
Total	32	1.9356	1.4166	0.2588	0.4045	189	93.56	0.3248	1.0394	0.141

The highest genetic identity is between Fars and Hamedan (0.93) with having the lowest genetic distance (0.06) (Table 4). The maximum genetic distance is between Yazd and Kerman (0.20) with the minimum genetic identity of 0.81.

AMOVA analysis shows 86 % and 14 % of genetic variation accounted for within and among *D. sophia* populations, respectively (Table 5).

Discussion

The Brassicaceae family is an economically important family containing well-known crop species such as *Brassica napus* and weedy species like *Arabidopsis thaliana*. Many genera of this family are distributed in Iran including *D. sophia*. The economic value of *D. sophia* is mainly based on the attainability of seed production (Peng et al. 1997; Mokhtassi-Bidgoli et al. 2013). Seeds are well known for their medicinal uses in Iran (Pasalar et al. 2013; Khodarahmi et al. 2015). The aim of this study was to investigate the genetic diversity of this medicinal plant in part of Iran. The chief origin of *D. sophia* in Iran includes the northern part of the country, such as mountains and elevated areas of Alborz and Zagros (Khezri 2001). The habitats of the 32 natural *D. sophia* plants collected for this study are diverse, such as from farmland, valley, desert or from the mountain. Different analysis methods of genetic

relatedness (UPGMA clustering, binary matrix dendrogram and STRUCTURE analysis) showed two separated groups but, the observed genetic variation was not completely consistent with lifestyle. Hamedan and Fars genotypes divided in two symmetric groups. This is confirmed by Nei's average number of pairwise differences within Hamedan and Fars populations (Fig. 5). Results of analysis of molecular variance reveal a higher distribution of genetic variation within *D. sophia* populations (86 %) as compared to between populations (14 %). This is also supported by Gst value of 0.32. Nei's average number of pairwise differences data. Therefore there is a significant difference between Fars and Lorestan genotypes while there is a high pairwise difference within Fars and Hamedan genotypes. The AMOVA results confirm the absence of an efficient clustering of genotypes coincident to their geographical locations. However collected plants from dry area (Y2, Y14, Y17) can be considered as a different group according to the constructed dendrogram. The outcome is similar to the results of Stenoien et al. (2005); Bakker et al. (2006) and Jorgensen and Mauricio (2004) on *A. thaliana* that found no clear association between geographical origin and genetic similarity in populations distributed in different regions. In the present study the gene flow (Nm = 1.03) indicates low genetic differentiation among populations which is corresponding to low Fixation index of 0.141. When gene flow occurs at a high level, the isolated populations will not diverge genetically. Since *D. sophia* produces many seeds, wind could help in its dispersal. Of course there is a chance of germplasm exchange between regions by human-aided dispersal as seeds commonly used in a sweet Iranian drink. This

Table 4 Nei's unbiased measures of genetic identity (above diagonal) and genetic distance (below diagonal)

Population	Hamedan	Lorestan	Central	Fars	Yazd	Kerman
Hamedan	*****	0.9162	0.9165	0.9371	0.9077	0.8221
Lorestan	0.0876	*****	0.9191	0.8782	0.8628	0.8697
Central	0.0872	0.0844	*****	0.9001	0.9050	0.8820
Fars	0.0650	0.1299	0.1053	*****	0.8669	0.8222
Yazd	0.0968	0.1476	0.0998	0.1429	*****	0.8108
Kerman	0.1959	0.1396	0.1255	0.1958	0.2097	*****

Table 5 Analysis of Molecular Variance (AMOVA) for six populations of *D. sophia* from Iran by ISSR marker

Source	df	SS	MS	Est. Var.	%
Among populations	5	255.381	51.076	4.643	14 %
Within populations	26	731.900	28.150	28.150	86 %
Total	31	987.281	-	32.793	

high genetic diversity will allow them to easily adapt to environmental changes.

The estimated Nei's gene diversity value of 0.26 also confirms high genetic diversity level within germplasms. Understanding such diversity is useful in the germplasm management. Conservation programs should aim to preserve all the extant populations as genetic differentiation among populations is low. Considering the low correlation between genetic distance and geographical locations in this study on limited populations, a more intricate survey must be investigated for *D. sophia*, which could include samples from regions that have not been covered in this study. As population sizes could make positive effects on genetic variation within population (Leimu et al. 2006) therefore collection of more individuals will make a better view of genetic variation. Another important implication of the results is selection of diverse parents for plant population construction. This study also shows ISSR technique is an eligible tool to detect the genetic diversity and genetic relatedness of *D. sophia* germplasm as it revealed 94 % of loci to be polymorphic and the effective number of alleles to be about 1.4 which corresponded with the findings in other medicinal plants (Liu et al. 2013; Bhattacharyya and Kumaria 2015).

In conclusion, the calculated percentage of polymorphic fragments reveals a high degree of genetic variability of this species in Iran.

Acknowledgments This work was financially supported by a research grant (No. 90322651) from Bu Ali-Sina University, Hamedan, Iran.

Compliance with ethical standards

Author contribution All authors made substantial contributions to the work presented in this paper.

Conflict of interest The authors declare that they have no conflict of interest.

References

- Alberto FJ, Aitken SN, Alía R, González-Martínez SC, Hänninen H, Kremer A, Lefèvre F, Lenormand T, Yeaman S, Whetten R, Savolainen O (2013) Potential for evolutionary responses to climate change-evidence from tree populations. *Glob Chang Biol* 19:1645–1661
- Baskin CC, Milberg P, Andersson L, Baskin JM (2004) Germination ecology of seeds of the annual weeds *capsella bursa-pastoris* and *Descurainia sophia* originating from high northern latitudes. *Weed Res* 44:60–68
- Bakker EG, Toomajian C, Kreitman M, Bergelson J (2006) A genome-wide survey of R gene polymorphisms in *Arabidopsis*. *Plant Cell* 18:1803–1818
- Bekker NP, Ulchenko NT, Glushenkova AI (2005) Lipids from *Descurainia sophia* seeds. *Chem Nat Compd* 41:346–347
- Bhattacharyya P, Kumaria S (2015) Molecular characterization of *Dendrobium nobile* Lindl., an endangered medicinal orchid, based on randomly amplified polymorphic DNA. *Plant Syst Evol* 301: 201–210
- Blackshaw RE, Molnar LJ, Larney FJ (2005) Fertilizer, manure and compost effects on weed growth and competition with winter wheat in western Canada. *Crop Prot* 24:971–980
- Chawla HS (2003) Introduction to plant biotechnology. Oxford and IBH pub, 2nd edn. Delhi, p. 538
- Doyle JJ, Doyle JL (1987) A rapid DNA isolation procedure for small quantities of fresh tissue. *Phytochem Bull* 19:11–15
- Duke JA, Ayensu ES (1985) Medicinal plants of China. Reference Pub Inc, ISBN 0917256204:705
- Excoffier L, Lischer HEL (2010) Arlequin suite ver 3.5: a new series of programs to perform genetics analyses under Linux and windows. *Mol Ecol Resour* 10:564–567
- Excoffier L, Smouse P, Quattro J (1992) Analysis of molecular variance inferred for metric distances among DNA haplotypes: application to human mitochondrial DNA restriction data. *Genetics* 131:479–491
- Govindaraj M, Vetriventhan M, Srinivasan M (2015) Importance of genetic diversity assessment in crop plants and its recent advances: an overview of its analytical perspectives. *Genet Res Int* 2015:431487. doi:10.1155/2015/431487
- Gruzdienė D, Bagdonaitė K (2003) Traditional and nontraditional oil plants grown in Lithuania: chemical characteristics and stability of edible oil. In: Šeškevičienė J, Mikulionienė S, Klimas E, Jeroch H (eds) The 11th Lithuania-Germany oily plants day. Lithuanian Academy of Veterinary, Kaunas, pp. 31–37 [In Lithuanian]
- Hernandez Plaza E, Kozak M, Navarrete L, Gonzalez-Andujar JL (2011) Tillage system did not affect weed diversity in a 23-year experiment in Mediterranean dryland. *Agric Ecosyst Environ* 140:102–105
- Hubisz MJ, Falush D, Stephens M, Pritchard JK (2009) Inferring weak population structure with the assistance of sample group information. *Mol Ecol Resour* 9:1322–1332
- Huson DH, Bryant D (2006) Application of phylogenetic networks in evolutionary studies. *Mol Biol Evol* 23(2):254–267
- Jaccard P (1908) *Nouvelles recherches Sur la distribution florale*. *Bull Soc Vaud Sci Nat* 44:223–270
- Jorgensen S, Mauricio R (2004) Neutral genetic variation among wild north American populations of the weedy plant *Arabidopsis thaliana* is not geographically structured. *Mol Ecol* 13:3403–3413
- Joshi K, Chavan P, Warude D, Patwardhan B (2004) Molecular markers in herbal drug technology. *Curr Sci* 87(2):159–165
- Kalia RK, Rai MK, Kalia S, Singh R, Dhawan AK (2011) Microsatellite markers: an overview of the recent progress in plants. *Euphytica* 177:309–334
- Khan M, Wang N (2012) *Descurainia sophia* (L.): a weed with multiple medicinal uses. *Punjab Univ J Zool* 27(1):45–51
- Khezri SH (2001) Culture of herbal plants. Shahab Khezri Pub, Sanandaj. ISBN:9640622672, pp 572 [In Persian]
- Khodarahmi E, Asghari GH, Hassanzadeh F, Mirian M, Khodarahmi GA (2015) Cytotoxic evaluation of volatile oil from *Descurainia sophia* seeds on MCF-7 and HeLa cell lines. *Res Pharm Sci* 10(2):169–176
- Kisha TJ, Johnson RC (2012) Technological innovations in major world oil crops, chapter 6: safflower, SK Gupta (ed), Volume 1, Chapter 6: <http://www.springer.com/us/book/9781461403555#>
- Leimu R, Mutikainen PIA, Koricheva J, Fischer M (2006) How general are positive relationships between plant population size, fitness and genetic variation? *J Ecol* 94:942–952
- Li J, Liu X, Dong F, Xu J, Li Y, Chen W, Zheng YI (2011) Potential allelopathic effects of volatile oils from *Descurainia sophia* (L.) Webb ex prantl on wheat. *Biochem Syst Ecol* 39:56–63
- Li J, Liu X, Dong F, Xu J, Zheng Y, Shan W (2010) Determination of the volatile composition in essential oil of *Descurainia sophia* (L.) Webb ex prantl (flixweed) by gas chromatography/mass spectrometry (GC/MS). *Molecules* 15:233–240

- Liu G, Liu L, Gong Y, Wang Y, Yu F, Shen H, Gui W (2007a) Seed genetic purity testing of F1 hybrid cabbage (*Brassica oleracea* var. capitata) with molecular marker analysis. *Seed Sci Technol* 35:476–485
- Liu J, Shi S, Chang E, Yang W, Jiang Z (2013) Genetic diversity of the critically endangered *Thuja sutchuenensis* revealed by ISSR markers and the implications for conservation. *Int J Mol Sci* 14: 14860–14871
- Liu L, Zhu X, Gong Y, Song X, Wang Y, Zhao L, Wang L (2007b) Genetic diversity analysis of radish germplasm with RAPD, AFLP and SRAP markers. *Acta Horticult* 760:125–130
- Mantel N (1967) The detection of disease clustering and a generalized regression approach. *Cancer Res* 27:209–220
- McDermott JM, McDonald BA (1993) Gene flow in plant pathosystems. *Annu Rev Phytopathol* 31:353–373
- Mitich LW (1996) Flixweed (*Descurainia sophia*). *Weed Technol* 10: 974–977
- Mohamed NH, Mahrous AE (2009) Chemical constituents of *Descurainia sophia* L. and its biological activity. *Rec Nat Prod* 3 (1):58–67
- Mohammadi SA, Prasanna BM (2003) Analysis of genetic diversity in crop plants-salient statistical tools and considerations. *Crop Sci* 43: 1235–1248
- Mokhtassi-Bidgoli A, Agha Alikhani M, Nassiri-Mahallati M, Zand E, Gonzalez-Andujar JL, Azari A (2013) Agronomic performance, seed quality and nitrogen uptake of *Descurainia sophia* in response to different nitrogen rates and water regimes. *Ind Crop Prod* 44:583–592
- Mosaddegh M, Naghibi F, Moazzeni H, Pirani A, Esmaili S (2012) Ethnobotanical survey of herbal remedies traditionally used in kohgiluyeh va Boyer Ahmad province of Iran. *J Ethnopharmacol* 141(1):80–95
- Maršalkienė N, Sliesaravičius A, Karpavičienė B, Dastikaitė A (2009) Oil content and fatty acids composition of seeds of some Lithuanian wild crucifer species. *Agron Res* 7(II):654–661
- Pasalar M, Bagheri Lankarani K, Mehrabani D, Tolide-i HR, Naseri M (2013) The effect of *Descurainia sophia* and *Prunus domestica* in prevention of constipation among Iranian hajj pilgrims, Saudi Arabia. *Res J Pharm Biol Chem Sci* 4(2):1195–1204
- Peakall R, Smouse PE (2006) GENALEX 6: genetic analysis in excel. Population genetic software for teaching and research. *Mol Ecol Notes* 6:288–295
- Peng L, Yi Y, Fu-li G, Ze-qū L (1997) A preliminary study on the introduction of *Descurainia sophia*, an oil plant species for industrial uses. *Acta Bot Sin* 39:477–479
- Prevost A, Wilkinsin MJ (1999) A new system of comparing PCR primers applied to ISSR fingerprinting of potato cultivars. *Theor Appl Genet* 98:107–112
- Reddy M, Pradeep Sarla N, Siddiq EA (2002) Inter simple sequence repeat (ISSR) polymorphism and its application in plant breeding. *Euphytica* 128:9–17
- Roldán-Ruiz I, Dendauw J, Bockstaele EV, Depicker A, Loose MD (2000) AFLP markers reveal high polymorphic rates in ryegrasses (*Lolium Spp*). *Mol Breed* 6:125–134
- Rohlf FJ (1992) NTSYS-pc. Numerical taxonomy and multivariate analysis system. Setauket, NY, Exeter Software
- Rohlf FJ, Sokal RR (1981) Comparing numerical taxonomic studies. *Syst Zool* 30:459–490
- Sehgal D, Raina SN (2008) DNA markers and germplasm resource diagnostics: new perspectives in crop improvement and conservation strategies. In: Arya ID, Arya S (eds) Utilization of biotechnology in plant sciences. Rastogi Press, Meerut, India, pp. 39–54
- Singh P, Sharma H, Srivastava N, Bhagyawant S (2014) Analysis of genetic diversity among wild and cultivated chickpea genotypes employing ISSR and RAPD markers. *Am J Plant Sci* 5:676–682
- Stenoien HK, Fenster CB, Tonteri A, Savolainen O (2005) Genetic variability in natural populations of *Arabidopsis thaliana* in northern Europe. *Mol Ecol* 14:137–148
- Sun K, Li X, Liu JM, Wang JH, Li W, Sha Y (2005) A novel Sulphur glycoside from the seeds of *Descurainia sophia* (L.). *J Asian Nat Prod Res* 7:853–856
- Rivers MC, Brummitt NA, Nic Lughadha E, Meagher TR (2014) Do species conservation assessments capture genetic diversity? *Glob Ecol Conserv* 2:81–87
- Tkachuk R, Mellish VJ (1977) Amino acid and proximate analysis of weed seeds. *Can J Plant Sci* 57:243–249
- Varsheny RK, Graner A, Sorrells ME (2005) Genic microsatellite markers in plants: features and applications. *Trends Biotechnol* 23: 48–55
- Wang S, Yang W, Shen H (2011) Genetic diversity in *Apium graveolens* and related species revealed by SRAP and SSR markers. *Sci Hortic* 129:1–8
- Yeh FC, Boyle TJB (1997) Population genetic analysis of codominant and dominant markers and quantitative traits. *Belg J Bot* 129:157
- Zhang ZP (2003) Development of chemical weed control and integrated weed management in China. *Weed Biol Manag* 3(4):197–203