Genetic Variation of *Amaranthus Retroflexus* L. and *Chenopodium Album* L. Suggests Multiple Independent Introductions

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**Genetic variation of *Amaranthus retroflexus* L. and *Chenopodium album* L.
suggests multiple independent introductions**

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Abstract

Amaranthus retroflexus L. and *Chenopodium album* L. are invasive plants that globally cause severe ecological and economic damage. In this study, we collected DNA from populations of these highly successful invaders from three countries and assessed genetic diversity using inter-simple sequence repeat (ISSR) markers. Both weed species showed low genetic diversity within a population and high genetic diversity among populations ($GST = 0.82$), and the value of gene flow among the populations was low ($Nm = 0.1$). Both UPGMA clustering and principal coordinate analysis indicated the existence of four distinct groups for *A. retroflexus* L. and *C. album* L., however these groups do not correlate with geographic origin. The high levels of genetic diversity observed among populations indicates that each population was individually introduced, probably through human activity. This knowledge helps to guide weed management and control strategies for field populations of these two prominent invasive weeds as it demonstrates each population must be considered separately and suggests they are armed with a considerable variety of genetic traits that could be used to avoid management practices.

Introduction

Bioinvasions are one of the biggest threats to biodiversity, ecosystem integrity, agriculture, fisheries and epidemiology¹. Despite the fact that the rapid spread of exotics has received appreciable attention within the international community and has galvanized vital ecological research^{2,3}, there still is a lack of understanding about what is necessary to drive a successful invasion. Redroot pigweed (*Amaranthus retroflexus* L.) and lamb's quarters (*Chenopodium album* L.) are fast-growing and highly competitive weedy annual plants in the family of Amaranthaceae. Both exhibit significant morphological and biochemical diversity⁴ and cause large yield losses throughout the world's agricultural areas^{5,6}. In many parts of the world, *A. retroflexus* is considered among the most troublesome weeds⁷ due largely to its high fecundity⁸ and long lived seeds⁹, which together allow it to maintain a constant soil seed bank. *A. retroflexus* negatively influences row crops, such as sugar beet¹⁰, soybean¹¹, potato^{12,13}, cotton¹⁴ and corn¹⁵. *C. album* is a pest of 40 crops in 47 countries⁶. These crops include wheat, barley, mustard, and gram^{16,17,18}. Like *A. retroflexus*, *C. album*'s success is driven by vigorous and highly adapted reproduction. Each *C. album* plant produces thousands to tens of thousands of seeds⁶ which remain viable for up to 4 decades¹⁹. *C. album* seeds exhibit considerable physical and biochemical polymorphisms²⁰, which alter seed dormancy and germination requirements and therefore allow plants to germinate under a range of environmental conditions²¹. These two weeds are highly successful bioinvaders and are therefore good systems to use to investigate the genetic fingerprints of effective bioinvasions.

Evolutionary genetics are a valuable tool for revealing traits that drive bioinvasion success¹. Genetic and evolutionary processes are the principal determinant of an invasive species' ability to establish and spread^{22,23}). Factors influencing a plant's potential to rapidly and efficiently colonize new habitats include: wide environmental tolerance, phenotypic flexibility, inbreeding coefficient or any form of asexual reproduction, efficient dispersal abilities, high relative growth rate, and high ability to compete²⁴. Colonization of new habitats is usually reflected in a "founder effect" and subsequent genetic drift decreases the variation among the population promoting differentiation between it and other populations²⁵. Compared to native species, alien species commonly display different genetic variation, population structure and phenotypic characteristics in response to the selective pressures of demographic transition, which may reflect earlier

evolutionary histories as well as stochastic and selection process²⁶. Bioinvasion events also provide useful opportunities for analysis of contemporary evolution²⁷.

Molecular markers are important tools for the analysis of genetic diversity and characterization of plant genotypes as they are highly reproducible and accurate. Commonly used by plant breeders, molecular phylogenetic studies discriminate among genotypes by determining the extent of variation within and between species²⁸. In the last decade, inter simple sequence repeat (ISSR) markers have been successfully utilized for diversity studies and structuring of natural populations²⁹. ISSR markers generate highly reproducible banding patterns from a single polymerase chain reaction (PCR) amplification, making them the most commonly used markers in intra-specific diversity analysis^{30,31}.

Despite the severe ecological and economic damage caused by invasive species when they successfully colonize new areas, the factors that allow invaders to become successful are not well defined. Understanding how genetic diversity correlates with distribution offers insights into the mechanisms of invasion(s). The objective of this study was to characterize the genetic diversity of Iranian compared to French and Spanish *A. retroflexus* L. and *C. album* L. populations using ISSR markers to investigate the genetic variation of these weeds. Despite being autogamous, *C. album* is the most polymorphic species of the genus *Chenopodium*; previous studies explored this taxonomic complexity through cytology³², karyotypic analysis³³, flavonoid profiling³⁴, random amplified polymorphic DNA (RAPD) profiles³⁴ and ISSR marker analysis³⁵. Conversely *A. retroflexus* is partly autogamous, but a study of the genetic structure of *A. retroflexus* in Central Europe using isoenzyme analysis showed moderate levels of genetic diversity and strong evidence for inbreeding within populations compared to other herbaceous plants³⁶. Therefore, there is a precedence for using *A. retroflexus* and *C. album* for evolutionary genetic studies. The Iranian populations investigated here show very diverse morphological and biochemical traits (^{37, 38,4}). We also included a smaller number of French and Spanish populations in our analysis to assess the effect of distance on genetic variation as it was extremely unlikely that genetic exchange between these and the Iranian populations would have occurred. Our results show similar genetic variations regardless of the distances between populations and groupings that mingled the French and Spanish populations with the Iranian ones. Our data indicates a high degree of variation among a species which suggests population isolation and multiple introduction events have occurred.

Materials and Methods

Plant Materials

The collection and cultivation of these materials are described elsewhere⁴ that comply with relevant institutional guidelines and legislation. In brief, seeds were collected from 16 *A. retroflexus* and 17 *C. album* naturally distributed populations in different provinces of Iran, Spain and France during 2016-2017 by Research Institute of Forests and Rangelands (RIFR). Each population collection point was positioned by GPS, with the location details listed in Table 1. These seeds were cultivated at distance of 20 cm in row and 30 cm between rows at the experimental field of the agriculture research of University of Mohaghegh Ardabili (38.19°N, 48.20°E) during the summer of 2018. Young leaf tissues were collected from individual plants and then stored in an -80°C freezer until required.

DNA Extraction and ISSR Analysis

Genomic DNA was isolated from the young leaves of plants according to the cetyltrimethylammonium bromide (CTAB) method described by Saghai-Marooft et al³⁹. The DNA concentration and purity were determined with a NanoDrop™ spectrophotometer and visually verified via 0.8% (w/v) agarose gel electrophoresis. ISSR primers (synthesized by CinnaGen Co., Teheran, Iran) from the University of British Columbia's UBC set no. 9 were screened for PCR amplification and thirteen primers were chosen for final analysis listed in Table 2.

Thermal cycling (T100™ Thermal Cycler) started with 4 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 2% agarose gel in 1X TBE buffer then ran at 90 voltage for 1 hours, stained with DNA-safe stain (CinnaGen, Iran) and photographed with a digital imaging system (UV tech, Germany). Molecular weights were estimated using 50 bp DNA Ladder (CinnaGen, Iran).

Data Analysis

13 of the 52 primers tested clearly and reproducibly amplified ISSR fragments. These were scored based on a binary matrix for presence (1) or absence (0) of bands. Discriminatory power of the primers was evaluated by means of resolving power (Rp), mass resolving power (MRP),

polymorphic information content (PIC), marker index (MI). Rp of each primer which is the ability of each primer to detect level of variation between individuals was calculated according to (Prevost and Wilkinson⁴⁰)

$$R_p = \sum bI \quad [1]$$

where bI (band informativeness) takes the values of: $1 - [2|0.5 - p|]$, where p is the proportion of individuals containing the band. Further, mean resolving power (MRP) for each primer was calculated via

$$MRP = \frac{1}{n} \sum bI \quad [2]$$

following Milbourne et al⁴¹. PIC value was calculated according to Roldán-Ruiz et al⁴²:

$$PIC = 2f_i(1 - f_i) \quad [3]$$

where f_i is the frequency of fragments present in that locus and $(1 - f_i)$ is the frequency of the null allele. MI, a measure of overall utility of a molecular marker technique, for each primer was calculated as a product of two functions, the polymorphic information content and effective multiplex ratio (EMR)⁴¹ i.e.,

$$MI = PIC \times EMR \quad [4]$$

The effective multiple ratio ($EMR = np\beta$) is the product of the number of polymorphic loci (np) in the population analyzed and the fraction of markers that were polymorphic (β)⁴³.

The resulting binary data matrix was analyzed using POPGENE version 1.32⁴⁴ to examine different genetic diversity parameters including number of polymorphic loci (PL), percentage of polymorphic loci (PPL), Observed number of alleles (Na), Effective number of alleles (Ne), Nei's gene diversity (H), Shannon's information index (I), gene flow based on McDermott and McDonald⁴⁵:

$$N_m = 0.5(1 - GST)/GST \quad [5]$$

To examine the genetic relationship among populations, unbiased genetic distance and genetic identity⁴⁶ were also calculated by POPGENE and a dendrogram was constructed from Nei's genetic distance with the unweighted pair-group method of averages (UPGMA) using NTSYSpc 1.02 software⁴⁷. To determine the quality of clustering⁴⁸, UPGMA cluster analysis was used to measure cophentic correlation coefficient (r) based on Rohlf and Sokal⁴⁹. Principle component analysis (PCA), and principle coordinate analysis (PCoA) to assess genetic diversity were also calculated⁵⁰. To evaluate genetic variance, analysis of molecular variance (AMOVA)⁵¹ was carried out using GenAlEx version 6.4⁵².

Results

Statistics of DNA Marker Used in Genetic Diversity Assessment

The ability to resolve genetic variation is directly related to the number of polymorphisms detected by the marker system⁵³. Table 2 indicates that the ISSR primers adequately reflect the polymorphism present in the populations and were powerful enough to differentiate between populations, indicating they were suitable for assessing genetic diversity of these populations⁵³. Against our *A. retroflexus* L. DNA, the 13 ISSR primers produced a total of 59 bands, of which 58 were polymorphic. The number of polymorphic bands ranged from 3 (UBC822, UBC829, UBC819, UBC833 and UBC817) to 13 (UBC810) (Table 2). The ISSR pattern obtained with UBC810 primer is demonstrated in Supplemental Figure 1A. The AL2 primer generated the minimum polymorphism of 80 % and primers AL1, UBC839, UBC810, UBC834, UBC829, UBC818, UBC822, UBC811, UBC819, UBC815, UBC833 and UBC817 showed 100 % polymorphism. While the highest Rp and MRP value was recorded at 7.87 and 102.31 (UBC810), the lowest was at 1.87 and 5.61 (UBC822), respectively. The EMR was the highest for UBC810 (13) and lowest for UBC822, UBC829, UBC819, UBC833 and UBC817 (3). Similarly, marker index (MI) value was highest for UBC810 (5.21) and lowest for AL2 primer with 1.1. AL2 has the lowest (1.8) observed number of alleles (Na) and other primers (2) having the highest. The effective number of allele (Ne) was invariably less than Na values showing a variation in the range of 1.44 (AL2) to 1.97 (UBC833). The Shannon index (I) estimates were low, ranging from 0.49 (AL2) to 0.68 (UBC833), as well as the estimates of Nei's genetic diversity, ranging from 0.34 (AL2) to 0.49 (UBC833) (Table 2A).

Moreover, the 13 selected primers generated 49 ISSR bands in the 17 *C. album* populations, 3 to 8 bands per primer, of which 37 were polymorphic. The number of polymorphic bands varied from 1 in AL2 and UBC811 to 7 in UBC810 (Table 2B). The ISSR pattern obtained with UBC810 primer is demonstrated in Supplemental Figure 1B. AL2 and UBC811 also provided the minimum polymorphism of 33.33% and primers UBC839, UBC829, UBC818, UBC815 and UBC817 showed 100 % polymorphism. The highest Rp and MRP value was in UBC810 primer (4.59 and 32.13 respectively), and the lowest one in AL2 (0.35 and 0.35 respectively). The EMR was the highest for UBC810 (6.12) and lowest for UBC839, UBC829, UBC818 and UBC817 (3). Similarly, marker index (MI) value was highest for UBC810 (1.86) and lowest for AL2 and

UBBC811 primers with 0.053. AL2 and UBC811 have the lowest (1.3) observed number of alleles (Na) and UBC839, UBC829, UBC818, UBC815 and UBC817 (2) having the highest. The effective number of allele (Ne) was invariably less than Na values showing a variation in the range of 1.13 (AL2) to 1.89 (UBC839). The Shannon index (I) ranging from 0.15 (AL2) to 0.66 (UBC839), as well as the estimates of Nei's genetic diversity, ranging from 0.09 (AL2) to 0.46 (UBC839 and UBC815) (Table 2B).

The PIC values ranged from 0.345 to 0.549 with the highest being for primer UBC834 and the lowest for primer AL2 for *A. retroflexus* L. (Table 2A). Furthermore, UBC834 primer with 0.09 and UBC817 primer with 0.48 showed the lowest and greatest PIC value among all primers for *C. album* populations, respectively (Table 2B). The average PIC value in both weed species indicated the selected ISSR primers was appropriate for the assessment of genetic diversity.

Genetic Diversity and Population Structure of Amaranthus retroflexus L. and Chenopodium album L.:

This paper establishes the genetic diversity of Iranian *A. retroflexus* and *C. album* populations alongside French and Spanish populations using UPGMA clustering of the ISSR data .

***Amaranthus retroflexus* L.:** The UPGMA clustering algorithm from ISSR analysis grouped the 16 *A. retroflexus* L. populations into four distinct clusters at a similarity index value of 0.48 (Figure 1A). The calculated cophenetic correlation coefficient value (r) of 0.78 indicates a high grouping efficiency. The first cluster consists of Rasht, Spain2, Ardabil and Moghan. The second group includes Rudsar, Sari, and Hamedan populations. The third cluster is populations from Shahre-e-Rey, Ilam, France, Gorgan, Spain1 and Spain3. The fourth group was formed of Yazd, Zarand and Bojnurd. Analysis of molecular variance confirmed the cut-off point of clustering (phipt=0.21) (Table 5A).

In the principle component analysis, the eigenvalues for the first three PCAs were 32.70, 18.02 and 15.45, respectively which represents 66.17% of the total variation. The PCoA analysis showed four main clusters confirming the results of the UPGMA clustering (Figure 2A). The measurements of genetic diversity are summarized in Table 3A. The number of observed alleles and number of effective alleles ranged between 1.152-1.254 (Ilam or Yazd to Ardabil) and 1.092–1.144 (Ilam or Yazd to Ardabil), respectively. The value of Nei's gene diversity ranged from 0.055 to 0.089 with the highest for Ardabil population and the lowest for Ilam and Yazd population

among the 16 populations. The average of Shannon's Information Index for the 16 populations is 0.11 which again the maximum and the minimum are respectively belonging to Ardail, Ilam or Yazd populations. The highest number of polymorphic loci (PL) and percentage of polymorphic loci (PPL) both belong to Ardail while the lowest, belongs to Ilam and Yazd. The values for total species diversity for among population (HT), within population diversity (Hs) and mean coefficient of gene differentiation (GST) were 0.429, 0.073 and 0.829, respectively. Furthermore, the level of gene flow (Nm) was estimated to be 0.102 individual per generation between populations, suggesting that genetic exchange between populations was low. The highest genetic identity is between Yazd and Zarand (0.79) with having the lowest genetic distance (0.22). The maximum genetic distance is between Rasht and Zarand, moreover Rasht and Yazd (1.08) with the minimum genetic identity of 0.33 (Table 4A).

***Chenopodium album* L.:** The UPGMA dendrogram from ISSR analysis at a similarity index value of 0.62 (Figure 1B). Cophenetic coefficient (r) of 0.71 indicates high grouping efficiency. The populations were separated into four distinct clusters. Analysis of molecular variance confirmed the cut-off point of clustering ($\phi_{\text{hpt}}=0.31$) (Table 5B): first cluster (Rudsar and Rasht), second cluster (Boyer-Ahmad, Rudan, Tehran, Dehloran, Hamedan and Kivi), third cluster (Mashhad, Spain1, Spain2 and France1), ultimately the fourth cluster is a representation of the populations from Moghan, Ardabil, Yazdabad, Shahr-e-Rey and France2.

In the PCA analysis the eigenvalues for the first three components are 32.96, 21.26 and 17.21, respectively justified 71.43 % of the total variation. The PCoA analysis showed four main clusters confirming the results of the UPGMA clustering (Figure 2B). The measurements of genetic diversity are summarized in Table 3B. The number of observed alleles and number of observed effective alleles ranged between 1.122-1.183 (Spain1 to Kivi, Ardail, Yazdabad, Shahre-Ray and Tehran) and 1.093-1.153 (Rudsar or Rudan to Yazdabad), respectively. The value of Nei's gene diversity ranged from 0.052 to 0.82 with the highest for Yazdabad population and the lowest for Spain1 population among the 17 populations. The average of Shannon's Information Index for the 17 populations is 0.094 which the maximum and the minimum are respectively belonging to Yazdabad- Spain1 populations. The highest number of polymorphic loci (PL) and percentage of polymorphic loci (PPL) both belong to Kivi, Ardail, Yazdabad, Shahre-Ray and Tehran while the lowest, belongs to Spain 1. The values for total species diversity for among population (HT), within population diversity (Hs) and mean coefficient of gene differentiation

(GST) were 0.36, 0.064 and 0.82, respectively. Furthermore, the level of gene flow (N_m) was estimated to be 0.109 individuals per generation between populations, suggesting that gene exchange between populations was low. Hamedan and Dehloran populations showed the highest genetic identity (0.91) with having the lowest genetic distance (0.08). The maximum genetic distance (0.71) and the minimum genetic identity (0.48) are between Ardabil and Rudsar along with Ardabil and Rasht populations (Table 4B).

Discussion

Analysis of *A. retroflexus* or *C. album* using ISSR markers demonstrates there is a high genetic diversity among the tested populations, suggesting that genetic exchange between populations was low and variation between populations is more than within populations. Although UPGMA clustering algorithm produced groupings, these did not correlate with geographic origin and the Spanish and French populations grouped with the Iranian populations. Our data indicate that there is little genetic exchange within populations of *A. retroflexus* or *C. album* in the populations measured and suggest that the populations have been established long enough to allow for genetic divergence to occur. Our results suggest the populations we have sampled may have been separately introduced to their current locations probably through the activity of humans but have not mixed significantly since.

The genetic analysis we performed with ISSR shows that *A. retroflexus* L. and *C. album* L. populations from different regions have high level of genetic diversity among populations compared to within populations. Mandal and Das⁵⁴ and Transue et al.⁵⁵ also reported a high genetic diversity in *Amaranthus* using RAPD markers. Together these studies show that high levels of genetic diversity may clarify the important role of geographic isolation during genetic diversification and variation⁵⁶. In principle, a high level of genetic diversity provides a varied genetic toolbox that enables adaptation to an extensive range of ecosystems⁵⁷. Conversely, low genetic diversity within populations is what is expected from a mainly autogamous invasive species²⁷, since self-fertilization reduces the proportion of heterozygous loci in individuals, causing fixation of homozygous loci⁵⁸. The genetic diversity of plant populations is mostly affected by adaptive characteristics like the breeding system, seed dispersal, genetic drift, history of introduction and therefore reflects how these have allowed plant morphology to adjust to ecological conditions. Plant morphology, phenology and breeding system significantly influences

genetic diversity where in general, long-lived and outcrossing species have higher levels of genetic diversity than selfing and/or clonal plants⁵⁹.

The genetic structure analysis (Figures 1 and 2) revealed high genetic differentiation among the sampled locations. Analysis of molecular variance results indicate that most of the genetic variation ($F_{ST} = 0.71$ in *A. retroflexus* L. and 0.7 in *C. album* L.) was found among populations, consistent with the low intra-population analysis suggesting predominant self-fertilization. The average value of F_{ST} for autogamous species using molecular markers is 0.70⁶⁰. Other autogamous species showed similar values of genetic differentiation to the measured mean value^{61, 62}. The common restriction to gene flow (N_m) among populations usually results in a high genetic divergence⁶³. Because of the small number of sampled sites in our study, it is difficult to accurately calculate genetic drift. A high number of private alleles was found in nearly all studied locations (Table 2), indicating lack of gene flow between the populations. The presence of private alleles is important because it may indicate disparate evolutionary paths⁶⁴.

Knowledge of the spatial distribution of genetic diversity is essential to better comprehend the relationships between life-history traits, stochastic effects, gene flow, selection pressures and environmental factors⁶⁵. We found no evidence of isolation by distance among the locations sampled; although our limited sample size was perhaps too small to accurately conclude a relationship between geographic and genetic distances. However, introduction and casual dispersal events by human action frequently result in a lack of correlation between genetic and geographic distances of invasive populations⁶⁶. *A. retroflexus* L. and *C. album* L. populations are most commonly found on disturbed areas⁶, and therefore dispersal driven by human activity is likely in these species. As a result, our lack of correlation between genetic and geographic distances of populations implies that seed dispersal mechanisms and colonization history have influenced the spatial distribution and genetic diversity we observed, similarly to other species⁶⁷. This result also emphasizes that the majority of these weed populations may have been established by distinct introduction events.

Innovative weed control options can be helped by gaining a better understanding of the genetic diversity of weed populations because genetic variability represents vital information about historic bottleneck effects and diversification since establishment^{68, 69}. This new knowledge provides a better understanding for how genetic variation exists and is essential to develop strategic

and effective control practices for weeds as different responses to chemical or biological control methods will be underpinned by differences in the weed genomes⁷⁰.

In conclusion, the genetic diversity analyses using ISSR molecular markers revealed that the studied populations of invasive *A. retroflexus* L. and *C. album* L. have low intra-population genetic diversity and are divergent among each other. Self-fertilization, drift events, colonization by few individuals, different selection pressures acting even within small geographic areas, and distinct multiple introductions to the region may have influenced the genetic diversity of these populations. These results are limited by the selected populations, which only cover Iran with French and Spanish outgroups, therefore further study of invasive populations covering a wider geographic distribution would be encouraged. As the chloroplast genome is maternally inherited, does not recombine, and genetic diversity of different regions of the chloroplast genome varies greatly, it would be useful for analysing phylogenetic relationships among species and evolution rates. Therefore, analysis of the plastome or the use of co-dominant markers would enhance the understanding of genetic diversity of these weed species allowing a better understanding of plant dispersal mechanisms, gene flow and expand our knowledge of the genetics of weeds.

Author Contributions

SH performed the experiments, data collection, data analysis, figure preparation, and writing of the manuscript. MA conceived the original data, formulated the research plan, oversaw the research, and writing of the manuscript. MM, DM and TT contributed to data analysis and writing of the manuscript. All authors contributed to the article and approved the submitted version.

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References

1. Lee, C. E. Evolutionary genetics of invasive species. *Trends Ecol Evol.* **17**, 386–391 (2002).
2. Ma, X., Wu, H., Jiang, W., Ma, Y. & Ma, Y. Interference between Redroot Pigweed (*Amaranthus retroflexus* L.) and Cotton (*Gossypium hirsutum* L.): Growth Analysis. *PloS one.* **10**, e0130475 (2015).
3. Bajwa, A. A., Zulfiqar, U., Sadia, S., Bhowmik, P., & Chauhan, B. S. A global perspective on the biology, impact and management of *Chenopodium album* and *Chenopodium murale*: two troublesome agricultural and environmental weeds. *Environ Sci Pollut Res Int.* **26**, 5357-5371 (2019).
4. Hamidzadeh Moghadam, S., Alebrahim, M.T., Tobeh, A., Mohebodini, M., Werck-Reichhart, D., & et al. Redroot Pigweed (*Amaranthus retroflexus* L.) and Lamb's Quarters (*Chenopodium album* L.) Populations exhibit a high degree of morphological and biochemical diversity. *Front. Plant Sci.* **12**, 39 (2021).
5. Horak, M. J. & Loughin, T. M. Growth analysis of four *Amaranthus* species. *Weed Sci.* **48**, 347–355 (2000).
6. CABI. Invasive Species Compendium. Wallingford, UK: CAB International. <https://www.cabi.org/isc> (2020).
7. Holm, L., Doll, J., Holm, E., Pancho, J. & Herberger, J. World weeds: Natural histories and distribution. (1th ed) 51–69 (John Wiley and Sons, 1997).
8. Knezevic, S. Z. & Horak, M. J. Influence of emergence time and density on redroot pigweed (*Amaranthus retrofexus*). *Weed Sci.* **46**, 665–672 (1998).
9. Telewski, F. W. & Zeevaart, J. A. D. The 120-yr period for Dr. Beal's seed viability experiment. *Am J Bot.* **89**, 1285-1288 (2002).
10. Brimhall, P. B., Chamberlain, E. W. & Alley, H. P. Competition of annual weeds and sugarbeets. *Weed Sci.* **13**: 33–35 (1965).
11. Dieleman, A., Hamil, A. S., Weise, S. F. & Swanton, C. J. Empirical models of pigweed (*Amaranthus* spp.) interference in soybean (*Glycine max*). *Weed Sci.* **43**, 612-618 (1995).
12. Weaver, S. E. Size-dependent economic thresholds for three broadleaf weed species in soybeans. *Weed Technol.* **5**, 674-679 (1991).

13. Alebrahim, M. T., Majd, R., Mohassel, M. H. R., Wilkakson, S., Baghestani, M. A. & et al. Evaluating the efficacy of pre-and post-emergence herbicides for controlling *Amaranthus retroflexus* L. and *Chenopodium album* L. in potato. *Crop Prot.* **42**, 345-350 (2012).
14. Buchanan, G. A., Crowley, R. H., Street, J. E. & McGuire, J. M. Competition of sicklepod (*Cassia obtusifolia* L.) and redroot pigweed (*Amaranthus retroflexus* L.) with cotton (*Gossypium hirsutum* L.). *Weed Sci.* **28**, 258–262 (1980).
15. Kenzevic, S. Z., Weise, S. F. & Swanton, C. J. Comparison of empirical models depicting density of redroot pigweed (*Amaranthus retroflexus* L.) and relative leaf area as predictors of yield loss in maize (*Zea mays* L.). *Weed Res.* **35**, 207-214 (1995).
16. Bhattacharjee, S. K. Handbook of medicinal plant. (3th rev.ed.) 1-2 (Pointer publishers, 2001).
17. Sarabi, V., Mahallati, M. N., Nezami, A. & Mohassel, M. H. R. Effects of common lambsquarters (*Chenopodium album* L.) emergence time and density on growth and competition of maize (*Zea mays* L.). *Aust J Crop Sci.* **7**, 532-537 (2013).
18. Jabran, K., Mahmood, K., Melander, M., Bajwa, A. A. & Kudsk, P. Weed dynamics and management in wheat. *Adv Agron.* **145**, 97-166 (2017).
19. Toole, E. H. & Brown, E. Final results of the Duval buried seed experiment. *J Agric Res.* **72**, 201-210 (1946).
20. Holm, L. G., Plucknett, D. L., Pancho, J. V. & Herberger, J. P. (1977). The world's worst weeds. Distribution and Biology. 609 (East-West Center and University Press of Hawaii, 1977)
21. Maurya, A. N. & Ambasht, R. S. Significance of seed dimorphism in *Alysicarpus monilifer* DC. *J Ecol.* **61**, 213-217 (1973).
22. Sakai, A. K., Allendorf, F. W., Holt, J. S., Lodge, D. M. Molofsky, J. & et al. The population biology of invasive species. *Annu Rev Ecol Evol Syst.* **32**, 305–332 (2001).
23. Alebrahim, M. T., Mohassel, M. H. R., Wilkakson, S., Baghestani, M. A., & Ghorbani, R. Evaluating of some preemergence herbicides for lambsquarter and redroot pigweed control in potato fields. *J. Plant Prot.* **25**: 358-367 (2012).
24. te Beest, M., Le Roux, J. J., Richardson, D. M., Brysting, A. K., Suda, J. & et al. (2012). The more the better? The role of polyploidy in facilitating plant invasions. *Ann Bot.* **109**, 19–45.
25. Ma, J. W., Geng, S. L., Wang, S. B., Zhang, G. L., Fu, W. D. & Shu, B. Genetic diversity of the newly invasive weed *Flaveria bidentis* (Asteraceae) reveals consequences of its rapid range expansion in northern China. *Weed Res.* **51**, 363–372 (2011)..

26. Keller, S. R. & Taylor, D. R. History, chance and adaptation during biological invasion: separating stochastic phenotypic evolution from response to selection. *Ecol Lett.* **11**, 852–866 (2008).
27. Barrett, S. C. H., Colautti, R. I. & Eckert, C.G. Plant reproductive systems and evolution during biological invasion. *Mol Ecol.* **17**, 373–383 (2008).
28. Parita, B., Kumar, S. N., Darshan, D. & Karen, P. Elucidation of genetic diversity among ashwagandha [*Withania somnifera* (L.) Dunal] genotypes using EST-SSR markers. *Res J Biotechnol.* **13**, 52–59 (2018).
29. Wolfe, A. D., Xiang, Q. Y. & Kephart, S. R. Assessing hybridization in natural populations of *Penstemon* (Scrophulariaceae) using hypervariable intersimple sequence repeat (ISSR) bands. *Mol Ecol.* **7**, 1107–1125 (1998).
30. Raut, V. R., Dodake, S. S. & Chimote, V. P. Evaluation of genetic diversity in grain amaranth (*Amaranthus hypochondriacus*) at molecular level using ISSR markers. *Indian J Agric Biochem.* **27**, 60–65 (2014).
31. Stefunova, V., Bezo, M., Labajová, M., & Senková, S. Genetic analysis of three Amaranth species using ISSR markers. *Emir J Food Agric.* **26**, 35–44 (2014).
32. Mukherjee, K. K. A comparative study of two cytotypes of *Chenopodium album* in West Bengal, India. *Can J Bot.* **64**: 754–759 (1986).
33. Kolano, B., Plucienniczak, A., Kwasniewski, M. & Maluszynska, J. Chromosomal localization of a novel repetitive sequence in the *Chenopodium quinoa* genome. *J Appl Genet.* **49**, 313–320 (2008).
34. Rahiminejad, M. R. & Gornall, R. J. Flavonoid, evidence for allopolyploidy in the *Chenopodium album* aggregate (Amaranthaceae). *Plant Syst Evol.* **246**, 77–87 (2004).
34. Rana, T. S., Narzary, D. & Ohri, D. Genetic diversity and relationships among some wild and cultivated species of *Chenopodium* L. (Amaranthaceae) using RAPD and DAMD methods. *Curr Sci.* **98**, 840–846 (2010).
35. Rana, T. S., Narzary, D. & Ohri, D. Molecular differentiation of *Chenopodium album* complex and some related species using ISSR profiles and ITS sequences. *Gene.* **495**, 29–35 (2012).
36. Mandák, B., Zákravský, P., Dostál, P., & Plačková, I. Population genetic structure of the noxious weed *Amaranthus retroflexus* in Central Europe. *Flora.* **206**, 697–703 (2011).
37. Alebrahim, M. T., Zangouejinejad, R. & Tseng, T.M. Biochemical and molecular knowledge about developing herbicide-resistant weeds. 101–132 (Herbicide resistance in weeds and crops, 2017).

38. Azadbakht, A., Alebrahim, M. T. & Ghavidel, A. The effect of chemical and nonchemical control methods on weeds in potato (*solanum tuberosum* L.) cultivation in Ardabil province, Iran. *Appl Ecol Environ Res.* **15**, 1359-1372 (2017).
39. Saghai-Maroofof, M. A., Soliman, K. M., Jorgensen, R. A. & Allard, R. W. Ribosomal DNA sepaer-length polymorphism in barley: mendelian inheritance, chromosomal location, and population dynamics. *Proc Natl Acad Sci.* **81**, 8014–8019 (1984).
40. Prevost, A. & Wilkinson, M. J. A new system of comparing PCR primers applied to ISSR fingerprinting of potato cultivars. *Theor Appl Genet.* **98**, 107-112 (1999).
41. Milbourne, D., Meyer, R., Bradshaw, J., Baird, E., Bonar, N. & et al. Comparisons of PCR-based marker systems for the analysis of genetic relationships in cultivated potato. *Mol Breeding.* **3**, 127–136 (1997).
42. Roldán-Ruiz, I., Dendauw, J., Bockstaele, E. V., Depicker, A. & Loose, M. D. AFLP markers reveal high polymorphic rates in ryegrasses (*Lolium* Spp). *Mol Breed.* **6**, 125–134 (2000).
43. Powell, W., Morgante, M., Andre, C., Hanafey, M., Vogel, J. & et al. The comparison of RFLP, AFLP and SSR (microsatellite) markers for germplasm analysis. *Mol Breed.* **2**, 225-238 (1996).
44. Yeh, F.C. & Boyle, T. J. B. Population genetic analysis of codominant and dominant markers and quantitative traits. *Belg J Bot.* **129**, 157-163 (1997).
45. McDermott, J. M., & McDonald, B. A. Gene flow in plant pathosystems. *Annu Rev Phytopathol.* **31**, 353–373s (1993).
46. Nei, M. Estimation of average heterozygosity and genetic distance from a small number of individuals. *Genetics.* **89**, 583–590 (1978).
47. Rohlf, F. J. NTSYS-pc. Numerical taxonomy and multivariate analysis system. Version 2.1. (Exeter Software, 2000).
48. Saracli, S., Dogan, N. & Dogan, I. Comparison of hierarchical cluster analysis methods by cophenetic correlation. *J Inequal Appl.* **203**, 1-8 (2013).
49. Rohlf, F. J. & Sokal, R. R. (1981). Comparing numerical taxonomic studies. *Syst Zool.* **30**, 459-490.
50. Mohammadi, S. A. & Prasanna, B. M. Analysis of genetic diversity in crop plants-salient statistical tools and considerations. *Crop Sci.* **43**, 1235–1248 (2003).

51. Excoffier, L., Smouse, P. & Quattro, J. Analysis of molecular variance inferred for metric distances among DNA haplotypes: application to human mitochondrial DNA restriction data. *Genetics*. **131**, 479–491 (1992).
52. Peakall, R. & Smouse, P. E. GENALEX 6: genetic analysis in excel. Population genetic software for teaching and research. *Mol Ecol Notes*. **6**, 288–295 (2006).
53. Sivaprakash, K. R., Prasanth, S. R., Mohanty, B. P. & Parida, A. Genetic diversity of black gram (*Vigna mungo*) landraces as evaluated by amplified fragment length polymorphism markers. *Curr Sci*. **86**, 1411–1415 (2004).
54. Mandal, N. & Das, P. K. Intra- and interspecific genetic diversity in grain *Amaranthus* using random amplified polymorphic DNA markers. *Plant Tissue Cult*. **12**, 49–56 (2002).
55. Transue, D. K., Fairbanks, D. J., Robison, L. R., & Andersen, W. R. Species identification by RAPD analysis of grain amaranth genetic resources. *Crop Sci*. **34**, 1385–1389 (1994).
56. Guo, H. B., Li, S. M., Peng, J. & Ke, W. D. Genetic diversity of *Nelumbo* accessions revealed by RAPD. *Genet Resour Crop Evol*. **54**, 741–748 (2007).
57. Dekker, J. Weed diversity and weed management. *Weed Sci*. **37**, 357–363 (1997).
58. Hamilton, M. B. Population genetics. (1th ed). 373–383 (Wiley- Blackwell publishing, 2009).
59. Hamrick, J. L. & Godt, M. J. W. Effects of life history traits on genetic diversity in plant species. *Philos Trans R Soc Lond B Biol Sci*. **351**, 1291–1298 (1996).
60. Nybom, H. & Bartish, I. V. (2000). Effects of life history traits and sampling strategies on genetic diversity estimates obtained with RAPD markers in plants. *Perspect. Plant Ecol Evol*. **3**, 93–114.
61. Ueno, S., Rodrigues, J. F., Alves-Pereira, A., Pansarin, E. R. & Veasey, E. A. Genetic variability within and among populations of an invasive, exotic orchid. *AoBP*. 7: plv077 (2015).
62. Aguayo, J., Adams, G. C., Halkett, F., Catal, M., Husson, C., Nagy, Z. A., Hansen, E. M., Marcais, B. & Frey, P. Strong genetic differentiation between North American and European populations of *Phytophthora alni* subsp. *uniformis*. *Phytopathology*. **103**, 190–199 (2013).
64. Yang, M., Liu, F., Han, Y., Xu, L., Juntawong, N., & Liu, Y. Genetic diversity and structure in populations of *Nelumbo* from America, Thailand and China: implications for conservation and breeding. *Aquat Bot*. **107**, 1–7 (2013).
65. Escudero, A., Iriondo, J. M. & Torres, E. Spatial analysis of genetic diversity as a tool for plant conservation. *Biol Conserv*. **113**, 351–365 (2003).

66. Guggisberg, A., Welk, E., Sforza, R., Horvath, D. P., Anderson, J. V., Foley, M. E. & Rieseberg, L.H. Invasion history of North American Canada thistle, *Cirsium arvense*. *J. Biogeogr.* **39**, 1919–1931 (2012).
67. Heywood, V. H., Brummitt, R. K., Culham, A. & Seberg, O. Flowering plant families of the world. *Curtis's bot mag.* **24**, 198-200 (2007).
68. Goolsby, J. A., De Barro, P. J., Makinson, J. R., Pemberton, R. W., Hartley, D. M. & Frohlich, D. R. Matching the origin of an invasive weed for selection of a herbivore haplotype for a biological control program. *Mol Ecol.* **15**, 287–297 (2006).
69. Slotta, T. A. B. What we know about weeds: insights from genetic markers. *Weed Sci.* **56**, 322–326 (2008).
70. Arias, R. S., Molin, W. T., Ray, J. D., Peel, M. & Scheffler, B. E. Isolation and characterisation of the first microsatellite markers for *Cyperus rotundus*. *Weed Res.* **51**: 451-460 (2011).

Table 1. The list of 16 *Amaranthus retroflexus* and 17 *Chenopodium album* populations evaluated in this study with their coordinate and origin names with their coordinate and origin names

<i>A. retroflexus</i>				
No.	Region name	Origin	Latitude (N)	Longitude (E)
1	Rasht	Iran	37°16'05 N	49°35'20 E
2	Gorgan	Iran	36°45'06 N	54°21'40 E
3	Rudsar	Iran	37°08'16 N	50°17'10 E
4	Sari	Iran	36°33'57 N	53°03'31 E
5	Shahr-e-Rey	Iran	35°34'37 N	51°27'44 E
6	Ilam	Iran	33°38'05N	46°24'54 E
7	Yazd	Iran	31°10'97 N	53°11'97 E
8	Bojnurd	Iran	37°53'74 N	57°24'96 E
9	Zarand	Iran	30°47'27 N	56°50'10 E
10	Hamedan	Iran	34°47'50 N	48°30'45 E
11	Ardabil	Iran	38°14'54 N	48°17'03 E
12	Moghan	Iran	39°13' 00 N	47°33'53 E
13	France	France	47°19'20 N	5°2'28 E
14	Spain 1	Spain	37°53'18 N	4°46'38 W
15	Spain 2	Spain	37° 53' 15 N	4° 46'35 W
16	Spain 3	Spain	37° 53' 14 N	4° 46'45 W
<i>C. album</i>				
1	Rudsar	Iran	37°08'13 N	50°16'52 E
2	Rasht	Iran	37°16'03 N	49°35'08 E
3	Boyer-Ahmad	Iran	30°53'47 N	51°24'96 E
4	Rudan	Iran	27°25'44 N	57°10'45 E
5	Moghan	Iran	39°12'03 N	47°34'24 E
6	Kivi	Iran	37°41'02 N	48°20'53 E
7	Ardabil	Iran	38°12'44 N	48°17'38 E
8	Yazdabad	Iran	32°39'41 N	51°41'21 E
9	Shahr-e-Ray	Iran	35°34'22 N	51°27' 44 E
10	Tehran	Iran	35°41'13 N	51°26'22 E
11	Dehloran	Iran	32°41'49 N	47°16'05 E
12	Hamadan	Iran	34°49'46 N	48°19' 47 E
13	Mashhad	Iran	36°16'24 N	59°38'16 E
14	Spain 1	Spain	37° 53' 15 N	4° 46'35 W
15	Spain 2	Spain	37° 53' 14 N	4° 46'45 W
16	France 1	France	47°19'20 N	5°2'28 E
17	France 2	France	47°19'29 N	5°2'22 E

Table 2. Data of ISSR primers of 13 primers in *Amaranthus retroflexus* (A) and *Chenopodium album* (B) populations

A)

Primer name	Primer seq	Tm	NT	NP	PP	β	PIC	EMR	MI	RP	MRP	Na	Ne	H	I
AL-1	(GA)6CC	43.7	6	6	100	1	0.378	6	2.26	3.25	19.5	2	1.63	0.4	0.55
AL-2	GA(GGA)2GGC	38	5	4	80	0.8	0.345	3.2	1.1	2.625	10.5	1.8	1.44	0.34	0.49
UBC839	(AC)8GA	53	4	4	100	1	0.449	4	1.79	3	12	2	1.81	0.44	0.63
UBC810	(GA)8T	52	13	13	100	1	0.401	13	5.21	7.87	102.31	2	1.71	0.37	0.58
UBC834	(AG)8YT	54	4	4	100	1	0.549	4	2.19	2.25	9	2	1.62	0.36	0.54
UBC829	(TG)8C	49	3	3	100	1	0.445	3	1.335	2.125	6.375	2	1.81	0.44	0.63
UBC818	(CA)8G	42	4	4	100	1	0.449	4	1.796	3	12	2	1.82	0.44	0.63
UBC822	(TC)8A	49	3	3	100	1	0.401	3	1.203	1.87	5.61	2	1.73	0.39	0.57
UBC811	(GA)8C	52.4	4	4	100	1	0.395	4	1.58	2.5	10	2	1.68	0.39	0.57
UBC819	(GT)8A	52.4	3	3	100	1	0.466	3	1.398	2.37	7.11	2	1.87	0.46	0.65
UBC815	(CT)8G	52	4	4	100	1	0.465	4	1.86	3	12	2	1.86	0.45	0.65
UBC833	(AT)8YG	54	3	3	100	1	0.495	3	1.485	2.75	8.25	2	1.97	0.49	0.68
UBC817	(CA)8A	49	3	3	100	1	0.458	3	1.374	2.5	7.5	2	1.86	0.45	0.64
Mean	-	-	4.54	4.46	98.46	0.98	0.44	4.4	1.89	3.01	17.09	1.98	1.75	0.42	0.6

B)

Primer name	Primer seq	Tm	NT	NP	PP	β	PIC	EMR	MI	RP	MRP	Na	Ne	H	I
AL-1	(GA)6CC	43.7	4	3	75	0.75	0.327	2.25	0.736	2	6	1.75	1.54	0.29	0.43
AL-2	GA(GGA)2GGC	38	3	1	33.33	0.33	0.162	0.33	0.053	0.35	0.35	1.33	1.13	0.09	0.15
UBC839	(AC)8GA	53	3	3	100	1	0.453	3	1.359	2.47	7.41	2	1.89	0.46	0.66
UBC810	(GA)8T	52	8	7	87.5	0.87	0.304	6.12	1.860	4.59	32.13	1.87	1.64	0.35	0.52
UBC834	(AG)8YT	54	5	3	60	0.6	0.09	1.8	0.162	0.94	2.82	1.6	1.21	0.156	0.25
UBC829	(TG)8C	49	3	3	100	1	0.266	3	0.798	1.76	5.28	2	1.68	0.39	0.58
UBC818	(CA)8G	42	3	3	100	1	0.463	3	1.389	2.24	6.72	2	1.82	0.44	0.63
UBC822	(TC)8A	49	3	2	66.66	0.66	0.267	1.33	0.355	1.06	2.12	1.66	1.41	0.24	0.6
UBC811	(GA)8C	52.4	3	1	33.33	0.33	0.161	0.33	0.053	0.71	0.71	1.3	1.28	0.15	0.21
UBC819	(GT)8A	52.4	3	2	66.66	0.66	0.24	1.33	0.319	0.94	1.88	1.66	1.37	0.23	0.36
UBC815	(CT)8G	52	4	4	100	1	0.362	4	1.448	3.06	12.24	2	1.88	0.46	0.65
UBC833	(AT)8YG	54	4	2	50	0.5	0.237	1	0.237	0.82	1.64	1.5	1.24	0.15	0.24
UBC817	(CA)8A	49	3	3	100	1	0.481	3	1.443	2	6	2	1.77	0.42	0.61
Mean	-	-	3.77	2.85	74.81	0.75	0.29	2.35	0.79	1.76	6.56	1.74	1.53	0.29	0.45

melting temperature (Tm), number of total bands (NT), number of polymorphic bands (NP), percentage of polymorphic fragment (PP), polymorphic information content (PIC), effective multiplex ratio (EMR), marker index (MI), resolving power (RP), mass resolving power (MRP), number of observed alleles (Na), number of effective alleles (Ne), Nei's gene diversity (H), Shannon's information index (I)

Table 3. Genetic diversity data of 16 *Amaranthus retroflexus* (A) and 17 *Chenopodium album* (B) populations

(A)

population	Na	Ne	H	I	PL	PPL	Ht	Hs	Gst	Nm	Fst
Rasht	1.203	1.131	0.076	0.114	12	20.34					
Gorgan	1.186	1.106	0.065	0.099	11	18.64					
Rudsar	1.203	1.122	0.073	0.11	12	20.34					
Sari	1.22	1.138	0.082	0.122	13	22.03					
Shahr-e-Rey	1.169	1.108	0.063	0.094	10	16.95					
Ilam	1.152	1.092	0.055	0.083	9	15.25					
Yazd	1.152	1.092	0.055	0.083	9	15.25					
Bojnurd	1.203	1.131	0.076	0.114	12	20.34					
Zarand	1.186	1.133	0.075	0.109	11	18.64					
Hamedan	1.237	1.137	0.083	0.127	14	23.73					
Ardabil	1.254	1.144	0.089	0.135	15	25.42					
Moghan	1.22	1.112	0.072	0.112	13	22.03					
France	1.203	1.113	0.07	0.107	12	20.34					
Spain 1	1.203	1.113	0.076	0.114	12	20.34					
Spain 2	1.22	1.138	0.082	0.122	13	22.03					
Spain 3	1.203	1.113	0.07	0.107	12	20.34					
Mean	1.201	1.12	0.073	0.11	-	-					
Total	2	1.784	0.429	0.616	59	100	0.429	0.073	0.829	0.102	0.71

(B)

population	Na	Ne	H	I	PL	PPL	Ht	Hs	Gst	Nm	Fst
Rudsar	1.142	1.093	0.054	0.08	7	14.29					
Rasht	1.142	1.104	0.058	0.084	7	14.29					
Boyer-Ahmad	1.163	1.123	0.068	0.098	8	16.33					
Rudan	1.142	1.093	0.054	0.08	7	14.29					
Moghan	1.163	1.123	0.068	0.098	8	16.33					
Kivi	1.183	1.132	0.074	0.108	9	18.37					
Ardabil	1.183	1.132	0.074	0.108	9	18.37					
Yazdabad	1.183	1.153	0.082	0.116	9	18.37					
Shahr-e-Ray	1.183	1.142	0.078	0.112	9	18.37					
Tehran	1.183	1.11	0.066	0.1	9	18.37					
Dehloran	1.163	1.112	0.064	0.09	8	16.33					
Hamadan	1.163	1.102	0.065	0.09	8	16.33					
Mashhad	1.142	1.104	0.058	0.084	7	14.29					
Spain 1	1.122	1.095	0.052	0.075	6	12.24					
Spain 2	1.163	1.112	0.064	0.094	8	16.33					
France 1	1.63	1.123	0.068	0.098	8	16.33					
France 2	1.163	1.104	0.058	0.084	7	14.29					
Mean	1.189	1.115	0.065	0.094	-	-					
Total	1.959	1.636	0.36	0.531	47	95.92	0.36	0.064	0.82	0.109	0.7

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), total population diversity for within population (Hs), among population diversity (Ht), coefficient of gene differentiation (Gst), gene flow (Nm), fixation index (Fst).

Table 4. Nei's unbiased measures of genetic identity (above diagonal) and genetic distance (below diagonal) primers in *Amaranthus retroflexus* (A) and *Chenopodium album* (B)

A)

<i>A. retroflexus</i>	Rasht	Gorgan	Rudsar	Sari	Shahr-e-Rey	Ilam	Yazd	Bojnurd	Zarand	Hamedan	Ardabil	Moghan	France	Spain 1	Spain 2	Spain 3
Rasht	1	0.72	0.71	0.64	0.5	0.49	0.33	0.47	0.33	0.59	0.71	0.55	0.47	0.47	0.59	0.59
Gorgan	0.31	1	0.71	0.61	0.61	0.42	0.37	0.5	0.44	0.52	0.67	0.52	0.54	0.47	0.62	0.62
Rudsar	0.33	0.33	1	0.72	0.45	0.47	0.35	0.45	0.38	0.61	0.66	0.5	0.52	0.49	0.61	0.54
Sari	0.44	0.49	0.31	1	0.45	0.61	0.42	0.49	0.38	0.67	0.66	0.61	0.55	0.49	0.54	0.61
Shahr-e-Rey	0.67	0.49	0.78	0.78	1	0.61	0.49	0.62	0.59	0.54	0.49	0.4	0.66	0.49	0.67	0.61
Ilam	0.71	0.85	0.74	0.49	0.49	1	0.47	0.5	0.5	0.62	0.57	0.62	0.54	0.5	0.55	0.62
Yazd	1.08	0.98	1.03	0.85	0.71	0.74	1	0.76	0.79	0.47	0.38	0.5	0.52	0.52	0.47	0.5
Bojnurd	0.74	0.67	0.78	0.71	0.46	0.67	0.27	1	0.72	0.61	0.52	0.5	0.66	0.62	0.61	0.61
Zarand	1.08	0.81	0.94	0.94	0.52	0.67	0.22	0.31	1	0.37	0.35	0.44	0.52	0.45	0.47	0.5
Hamedan	0.52	0.64	0.49	0.38	0.61	0.46	0.74	0.49	0.98	1	0.64	0.62	0.61	0.54	0.55	0.59
Ardabil	0.33	0.38	0.41	0.41	0.71	0.55	0.94	0.64	1.03	0.44	1	0.74	0.55	0.52	0.64	0.74
Moghan	0.58	0.64	0.67	0.49	0.89	0.46	0.67	0.67	0.81	0.46	0.29	1	0.47	0.5	0.49	0.59
France	0.74	0.61	0.64	0.58	0.41	0.61	0.64	0.41	0.64	0.49	0.58	0.74	1	0.55	0.67	0.67
Spain 1	0.74	0.74	0.71	0.71	0.71	0.67	0.64	0.46	0.78	0.61	0.64	0.67	0.58	1	0.67	0.61
Spain 2	0.52	0.46	0.49	0.61	0.38	0.58	0.74	0.49	0.74	0.58	0.44	0.71	0.38	0.38	1	0.76
Spain 3	0.52	0.46	0.61	0.49	0.49	0.46	0.67	0.49	0.67	0.52	0.29	0.52	0.38	0.49	0.27	1

B)

<i>C. album</i>	Rudsar	Rasht	Boyer-Ahmad	Rudan	Moghan	Kivi	Ardabil	Yazdabad	Shahr-e-Ray	Tehran	Dehloran	Hamadan	Mashhad	Spain 1	Spain 2	France 1	France 2
Rudsar	1	0.75	0.53	0.63	0.51	0.59	0.48	0.53	0.55	0.59	0.57	0.57	0.57	0.57	0.61	0.67	0.63
Rasht	0.28	1	0.61	0.67	0.51	0.55	0.48	0.57	0.55	0.63	0.65	0.61	0.65	0.69	0.77	0.79	0.63
Boyer-Ahmad	0.63	0.49	1	0.85	0.65	0.65	0.59	0.75	0.65	0.77	0.79	0.79	0.75	0.67	0.71	0.69	0.65
Rudan	0.45	0.39	0.15	1	0.63	0.67	0.53	0.65	0.59	0.79	0.73	0.77	0.65	0.61	0.69	0.63	0.55
Moghan	0.67	0.67	0.42	0.45	1	0.59	0.85	0.77	0.75	0.55	0.53	0.53	0.61	0.61	0.61	0.59	0.75
Kivi	0.52	0.59	0.42	0.39	0.52	1	0.61	0.53	0.55	0.75	0.69	0.69	0.65	0.65	0.61	0.67	0.55
Ardabil	0.71	0.71	0.52	0.63	0.15	0.49	1	0.79	0.77	0.57	0.55	0.55	0.63	0.71	0.63	0.61	0.77
Yazdabad	0.63	0.55	0.28	0.42	0.25	0.63	0.22	1	0.89	0.61	0.63	0.63	0.83	0.79	0.79	0.73	0.85
Shahr-e-Ray	0.59	0.59	0.42	0.52	0.28	0.59	0.25	0.107	1	0.67	0.57	0.57	0.81	0.73	0.77	0.71	0.83
Tehran	0.52	0.45	0.25	0.22	0.59	0.28	0.55	0.49	0.39	1	0.81	0.85	0.69	0.69	0.65	0.67	0.55
Dehloran	0.55	0.42	0.22	0.3	0.63	0.36	0.59	0.45	0.55	0.2	1	0.91	0.75	0.83	0.67	0.73	0.61
Hamadan	0.55	0.49	0.22	0.25	0.63	0.36	0.59	0.45	0.55	0.15	0.08	1	0.71	0.75	0.63	0.69	0.53
Mashhad	0.55	0.42	0.28	0.42	0.49	0.42	0.45	0.17	0.2	0.36	0.28	0.33	1	0.83	0.87	0.81	0.77
Spain 1	0.55	0.36	0.39	0.49	0.49	0.42	0.33	0.22	0.3	0.36	0.17	0.28	0.17	1	0.79	0.85	0.77
Spain 2	0.49	0.25	0.33	0.36	0.49	0.49	0.45	0.22	0.25	0.42	0.39	0.45	0.13	0.22	1	0.85	0.77
France 1	0.39	0.22	0.36	0.45	0.52	0.39	0.49	0.3	0.33	0.39	0.3	0.36	0.2	0.15	0.15	1	0.83
France 2	0.45	0.45	0.42	0.59	0.28	0.59	0.25	0.15	0.17	0.59	0.49	0.63	0.25	0.25	0.25	0.17	1

Table 5. Analysis of Molecular Variance (AMOVA) for *Amaranthus retroflexus* (A) and *Chenopodium album* (B) populations

A)

Source	df	SS	MS	PhiPT
Among populations	1	35.967	35.967	0.21**
Within populations	14	160.095	11.435	-
Total	15	196.063	-	-

B)

Source	df	SS	MS	PhiPT
Among populations	1	27.769	27.769	0.31**
Within populations	15	98.467	6.564	-
Total	16	126.235	-	-

Figure 1. UPGMA clustering of *Amaranthus retroflexus* (A) and *Chenopodium album* (B) populations based on Jaccard similarity coefficient calculated from ISSR markers

Figure 2. Principal coordinates analysis of 16 *Amaranthus retroflexus* (A) and 17 *Chenopodium album* (B) populations based on the genetic variation revealed by ISSR.

Figures

Figure 1

UPGMA clustering of *Amaranthus retroflexus* (A) and *Chenopodium album* (B) populations based on Jaccard similarity coefficient calculated from ISSR markers

Figure 2

Principal coordinates analysis of 16 *Amaranthus retroflexus* (A) and 17 *Chenopodium album* (B) populations based on the genetic variation revealed by ISSR.

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