

Detection of Genetic Diversity and Phylogenetic Relationship of *Tanacetum parthenium* L. Germplasm: Implications for Conservation and Breeding

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ABSTRACT

Feverfew (*Tanacetum parthenium* L.), belonging to the family Asteraceae, is a valuable medicinal plant containing effective anti-cancer compounds. The current study was conducted to investigate the diversity of feverfew ecotypes. Ecotypes were collected from different geographical areas of Iran in 2016 and 2017. The experiment was conducted in a completely randomized design with four replications. The analysis of variance showed significant phenotypic differences among the studied ecotypes. The plant height ranged from 55.75 to 124.50 cm and shoot and capitule diameters varied from 3.81 to 8.18 and 7.51 to 15.42 mm, respectively. The aerial biomass weight ranged from 36.62 to 136.40 g, root weight ranged from 9.81 to 36.65 g, and total biomass ranged from 46.44 to 173.05 g. In addition, flower weight varied from 3.46 to 6.21 g, while flower yield ranged from 1.48 to 13.90 g per plant. The obtained results regarding the phenotypic correlation demonstrated that plant height had the highest positive correlation with functional traits. Furthermore, the aerial biomass weight was positively correlated with flower weight, flower number, and flower yield, suggesting that vegetative growth had a positive correlation with reproductive growth. In general, tall plants produced more flowers. We also found some correlations between the geographical distances of ecotypes and phenotypic diversity. The dendrogram obtained based on the measured traits revealed that the classification of ecotypes in close groups can show their kinship and genetic relationships between them.

Keywords: Breeding, Correlation, Diversity, Feverfew, Medicinal plant

INTRODUCTION

Tanacetum parthenium (L.), commonly known as feverfew, is a species belonging to the Asteraceae family [1, 2]. The term feverfew derives from the Latin word febrifugia, meaning “fever reducer” [3, 4, 5]. In addition, because of its feathery leaves, it is called “featherfew” [6, 7, 8]. Feverfew is a small and aromatic perennial herb with a bushy form, which grows about 0.3–1 m. Its leaves are alternate and its small daisy-like yellow flowers arrange in a dense flat-topped cluster that bloom from July to October [1]. Leaves have bright green color and blade shape as egg and their surface is covered with crack. Flowers are seen at the end of main stem and branches. They are hermaphrodite and self-fertile. The mentioned species is a diploid ($2n=2x=18$) plant [1, 2, 5]. Feverfew contains many secondary metabolites including phenols, essential oils, bitter compounds and sesquiterpene lactones, which are of high value in chemotaxonomy and medicine [9, 10]. Traditionally, it has been consumed for treating migraine headaches, rheumatism, inflammation, stomach pains, and fever [11, 12].

Plant relationships are a major part of the coexistence mechanisms of species and this type of relationship includes a wide range of positive and negative relationships [13, 14, 15, 16]. Study of plant populations indicates that plants change their phenotypic and physiological traits in response to different ecological conditions [17]. Genetic diversity of species is a crucial way for adaptation and survival of populations to changing environments [18, 19, 20, 21]. Genetic diversity assessment in crops also provides an opportunity for plant breeders to develop new improved cultivars with desirable characteristics, which could be the first step toward domestication [22, 23, 24,

25]. Hence, in case of exploitation and introduction of medicinal crops into the cultivation and industry, study of genetic diversity will be very important [26, 27].

In the last few decades, genetic variation of medicinal plant species has been mainly assessed based on phenotypic, phytochemical, and recently molecular markers. Due to the few studies on the diversity of medicinal plants and because of their wide distribution, phenotypic markers are highly valued and preferred [28, 29]. Assessment of diversity using phenotypic characteristics is a direct, inexpensive, and easy method for exploring genetic diversity and phenotypic differences within and between populations [30, 31, 32]. Moreover, diversity studies provide important insights into the efficient use of crop germplasm and significant information for breeding programs [33, 34, 35]. Thus, the objective of the present study was to characterize the phenotypic diversity of different feverfew ecotypes from different natural habitats in Iran.

MATERIALS AND METHODS

Plant Materials

In the current study, ecotypes of *T. parthenium* were collected from different natural habitats of Iran in 2016 and 2017. Based on the sources of flora, distribution areas were identified and populations were studied. Distribution of feverfew was very limited in many areas. Therefore, in order to prevent the destruction of the limited ecotypes, sampling was performed carefully. The details of the studied natural habitats are available in Table 1. A herbarium sample was prepared from each habitat and matched with the herbarium of the Plant Sciences Research Institute of Ferdowsi University of Mashhad with the herbarium code (FUMSH/21321) and related references.

The Characters Recorded

The feverfew plants from each ecotype were dried in the shade and away from direct sunlight conditions. Then, the plants were investigated in terms of 15 phenotypic traits and yield components including plant height, shoot number, shoot color, shoot diameter, leaf color, aerial biomass weight, root weight, total biomass, aerial biomass weight/root weight, capitule diameter, flower color, flower number, flower weight, flower yield, and flower harvest index.

Statistical Analysis

The experiment was conducted in a completely randomized design with four replications. Statistical analysis was performed using ANOVA procedure of SAS v. 9.4 (Statistical Analysis System, SAS, Cary NC, U.S.). Duncan's Multiple Range Test was performed to determine significant differences between the evaluated traits. Cluster analysis was performed using UPGMA method, and Euclidian distance was done using SPSS and XLSTAT software.

RESULTS AND DISCUSSION

Diversity Index and Traits Description

In the present study, phenotypic markers were used to evaluate the diversity among the ecotypes and to examine their relationships (Table 2). The analysis of variance (Table 3) indicated that the ecotypes exhibited significant differences in all the phenotypic traits (not shown). High significant differences could demonstrate the presence of high diversity among the ecotypes. These observations were confirmed by the relatively high CV values (Table 2). The highest levels of variation were found for shoot number (CV=8.06%), shoot diameter (CV=8.66%), aerial biomass weight (CV=6.44%), root weight (CV=7.28%), total biomass (CV=6.30%), flower yield (CV=7.15%), and flower harvest index (CV=9.72%). Considering that the main metabolites of feverfew plants exist in shoots and flowers, these indicators provide opportunities for breeding programs. Other traits showed low CVs (<6%). The traits with lower CVs can be more consistent among the ecotypes. CV is a parameter that is not related to a unit of measure and is often used to measure and compare the variation of the evaluated traits. CV value is an indicator of the ability to distinguish among the ecotypes based on phenotype. Phenotypic traits with low CV values are more homogeneous and relatively stable, and are slightly affected by the environment, while traits with high CV values are more discriminating and can be reliable markers for the characterization of ecotypes [36].

According to the results, significant differences were found among the studied ecotypes regarding plant height. The lowest height was observed in (Es/Sem-Esfahan), (Es/Aby-Esfahan) and (Ch/Kou-Chaharmahal) ecotypes, while the highest height was seen in (Al/Gac-Alborz), (Al/Nes-Alborz) and (Gi/Esh-Gilan) ecotypes. Regarding the shoot number, ecotypes (Ya/Ash-Yazd) and (Lo/Kah-Lorestan) had the minimum values, while (Qa/And-Qazvin) and (Ha/Mol-Hamedan) ecotypes had the maximum values. The minimum stem diameter was found in (Se/Jan) and (Kh/Gal-Khorasan) ecotypes and the maximum in (Kh/Diz-Khorasan) ecotype. Significant differences were also found among the ecotypes in qualitative traits such as shoot color, leaf color, and flower color, so that (Es/Sem-Esfahan), (Es/Aby-Esfahan), (Ch/Kou-Chaharmahal), (Ch/Vas-Chaharmahal), (Ch/Ban-Chaharmahal), (Ya/Taz-Yazd) and (Ya/Ash-Yazd) ecotypes had the minimum values and (Ko/Sis-Kohgilyuyeh) and (Ma/Jav-Mazandaran) ecotypes had the maximum values. The highest and lowest root weights were related to (Es/Sem-Kohgilyuyeh) and (Ko/Sis-Kohgilyuyeh) ecotypes, respectively. A similar trend was observed for total biomass and aerial biomass weight. The minimum and maximum values of aerial biomass weight/root weight were observed in (Ch/Vas-Chaharmahal) and (Te/Fir-Tehran) ecotypes, respectively.

Compared to other quantitative parameters, aerial biomass weight/root weight had less variation and the ecotypes were scattered close to the mean. Low diversity in aerial biomass weight/root weight ratio trait could indicate self-regulation of plants in response to environmental changes in order to create a balance between different organs [37, 38]. Regarding capitule diameter, the lowest rate was related to (Ya/Deh-Yazd) and (Kh/Gal-Khorasan) ecotypes, whereas the highest was related to (Gi/Ole-Gilan), (Ma/Jav-Mazandaran), (Ko/Bah-Kohgilyuyeh), (Ko/Yas-Kohgilyuyeh), (Ko/Sis-Kohgilyuyeh) and (Gi/Mas-Gilan) ecotypes. Capitule diameter, flower number, flower weight, and flower yield are the effective parameters in the final function of reproductive organs and can be considered in breeding programs and superior ecotypes selection. For flower number, (Ch/Che-Chaharmahal) ecotype had the minimum and (Ma/Jav-Mazandaran) and (Gi/Jav-Gilan) ecotypes had the maximum numbers. Regarding flower weight, minimum and maximum values were observed in (Al/Zid-Alborz) and (Qa/Por-Qazvin) ecotypes, respectively. The minimum values of flower yield were related to (Ch/Che-Chaharmahal), (Ya/Ash-Yazd) and (Ch/Vas-Chaharmahal) ecotypes, and the maximum values were related to (Ma/Jav-Mazandaran) and (Gi/Jav-Gilan) ecotypes. The ratio of flower yield to the total aerial biomass was considered as the harvest index. Since one of the components of calculating the harvest index is flower yield, changes in harvest index are highly dependent on the changes in flower yield. In the breeding of medicinal plants, we need to select the traits that improve dry biomass, metabolites, and propagation methods. The aerial biomass and flowers of feverfew plants are the main organs producing metabolites that are used in traditional medicine and pharmaceutical industries. Therefore, some traits such as dry matter yield and flower components (flower number, flower weight, and flower yield) can be highly considered useful characteristics for producing product and desired metabolites. In this regard, (Ko/Sis-Kohgilyuyeh), (Ko/Kak-Kohgilyuyeh), (Ko/Yas-Kohgilyuyeh), (Ko/Lou-Kohgilyuyeh), (Ko/Meh-Kohgilyuyeh), (Ma/Jav-Mazandaran), (Gi/Esh-Gilan), (Gi/Jav-Gilan), (Ma/Kel-Mazandaran), (Qa/Via-Qazvin), and (Qa/Gar-Qazvin) ecotypes are of high importance and can be the most suitable choices for breeding goals. In addition, among the evaluated ecotypes of feverfew, the ecotypes (Ma/Jav-Mazandaran), (Gi/Jav-Gilan), (Gi/Mas-Gilan), (Ma/Jan-Mazandaran), (Qa/Por-Qazvin), (Ha/Mor), and (Ko/Sis-Kohgilyuyeh) had the highest flower production potential and are the most important choices for *T. parthenium* breeding. Since more flowers and more flower yield lead to an increase in seed production, the above-mentioned ecotypes could also be a good choice for production purposes.

Phenotypic trait diversity could be due to the differences in climatic and habitat conditions, and low variation of the traits might be owing to the low effect of environmental conditions [39, 40]. Richards *et al.*, [41] found a correlation between environmental conditions and the phenotypic diversity of plant species. Species that were located in a wide range of habitats showed more phenotypic diversity. Some researchers believed that diversity in phenotypic and reproductive traits is also affected by the difference in ploidy level of different populations [42, 43, 44, 45, 46]. The phenotypic diversity of a given species indicates the adaptation of the species to different habitats. This issue is very important in domestication, breeding, and germplasm protection programs [47].

Correlation among the Phenotypic Traits

Simple correlation coefficient analysis showed significant positive and negative correlations among the measured traits (Table 4). The positive genetic correlation between traits means that the two traits tend to change in the

same direction, and both are under the control of a dominant gene; however, this relationship is largely unknown. The negative correlation means that as one trait increases, the other decreases. In addition, genetic independence indicates that each of the two traits acts independently of the other. The plant height was highly positively correlated with aerial biomass weight ($r = 0.81$), root weight ($r = 0.81$) and total biomass ($r = 0.81$), followed by flower number ($r = 0.71$), shoot number ($r = 0.68$) and flower yield ($r = 0.67$). In addition, the results of correlation analysis between traits indicated that shoot diameter and plant height showed positive correlations with reproductive traits including flower number and flower diameter. Aerial biomass weight was positively correlated with flower weight, flower number, and flower yield. These findings suggested that vegetative growth has a positive correlation with reproductive growth. It can be concluded that the taller plants produce more flowers or in some cases produce fewer but larger flowers that in turn increased flowering efficiency. Furthermore, in small plants, which produce fewer flowers, the flower yield can be increased by increasing the number of plants per unit area. Crossing two populations, tall and small plants, can also lead to the production of small plants with larger flowers, and eventually increase flower yield per unit area. These results can play a valuable role in the selection of superior ecotypes. The genetic correlation indicates only the statistical relationship between the traits, and without complete knowledge of the biosynthetic pathway and genes involved in controlling traits, the meaning of correlations is not entirely clear [48].

Cluster Analysis

Dendrogram was used to represent the similarities and dissimilarities among the ecotypes based on all the measured traits. The ecotypes were classified into two main clusters and five sub-clusters based on the traits data (Fig. 1). The first cluster (I) was divided into two sub-clusters and contained 27 ecotypes of feverfew including (Gi/Mas-Gilan), (Gi/Ole-Gilan), (Gi/Esh-Gilan), (Gi/Jav-Gilan), (Ma/Jan-Mazandaran), (Ma/Kel-Mazandaran), (Ma/Jav-Mazandaran), (Go/Ziy-Golestan), (Go/Der-Golestan), (Kh/Zos-Khorasan), (Kh/Kan-Khorasan), (Kh/Bar-Khorasan), (Kh/Fer-Khorasan), (Ha/Mol-Hamedan), (Ha/Gan-Hamedan), (Ha/Mor-Hamedan), (Qa/Hos-Qazvin), (Qa/Via-Qazvin), (Qa/Gar-Qazvin), (Qa/And-Qazvin), (Qa/Por-Qazvin), (Ko/Kak-Kohgilyuyeh), (Ko/Lou-Kohgilyuyeh), (Ko/Yas-Kohgilyuyeh), (Ko/Meh-Kohgilyuyeh), (Ko/Sis-Kohgilyuyeh), and (Ko/Bah-Kohgilyuyeh). The second cluster (II) included three sub-clusters (III, IV and V) which consisted of 45 ecotypes including (Kh/diz-Khorasan), (Kh/Qas-Khorasan), (Kh/Gal-Khorasan), (Ya/Deh-Yazd), (Ya/Taz-Yazd), (Ya/Ash-Yazd), (Se/Jan-Semnan), (Se/Par-Semnan), (Ar/Kha-Ardabi), (Ar/Sey-Ardabi), (Ar/Nou-Ardabi), (Ae/Tor-East Azerbaijan), (Ae/Var-East Azerbaijan), (Ae/Bol-East Azerbaijan), (Ae/Che-East Azerbaijan), (Ae/Sho-East Azerbaijan), (Ae/Kal-East Azerbaijan), (Ae/Qal-West Azerbaijan), (Aw/Pir-West Azerbaijan), (Aw/Qas-West Azerbaijan), (Aw/Qey-West Azerbaijan), (Te/She-Tehran), (Te/Mey-Tehran), (Te/Kal-Tehran), (Te/Osh-Tehran), (Te/Lav-Tehran), (Te/Dam-Tehran), (Te/Hes-Tehran), (Te/Fir-Tehran), (Te/Pey-Tehran), (Al/Tal-Alborz), (Al/Zid-Alborz), (Al/Sha-Alborz), (Al/Gac-Alborz), (Al/Nes-Alborz), (Al/Ebr-Alborz), (Lo/Kah-Lorestan), (Lo/Dor-Lorestan), (Es/Sem-Esfahan), (Es/Aby-Esfahan), (Ch/Tan-Chaharmahal), (Ch/Ban-Chaharmahal), (Ch/Kou-Chaharmahal), (Ch/Vas-Chaharmahal), and (Ch/Che-Chaharmahal).

In the first cluster (I), sub-cluster (I) mainly included Mazandaran, Hamedan, Qazvin, and Mazandaran ecotypes, and second cluster (II) consisted mostly of Golestan and Khorasan ecotypes. In the second cluster (II), sub-clusters III and V were completely heterogeneous and included ecotypes of different regions. Subgroup IV contained Yazd, Esfahan, and Chaharmahal ecotypes. In general, in terms of functional parameters such as aerial biomass weight, total biomass, flower number, flower weight, and flower yield, subgroups I and II can be considered for using in domestication, selection, and hybridization programs in order to produce the desired product and metabolites. The obtained findings indicated that the highest genetic distance was related to (Ko/Bah-Kohgilyuyeh), (Ae/Tor-East Azerbaijan) and (Aw/Qas-West Azerbaijan) ecotypes. In addition, (Go/Ziy-Golestan), (Go/Der-Golestan), (Se/Par-Semnan), and (Aw/Pir-West Azerbaijan) ecotypes showed the lowest euclidean distance and the highest phenotypic similarity.

In the current study, the results of cluster analysis showed that there was high phenotypic diversity between the ecotypes of feverfew. Classification of the genotypes according to the evaluated traits is one of the appropriate methods for determining the degree of genetic relationship. It was also able to show the differences between the ecotypes of different geographical origins based on their phenotypic characteristics. In addition, in some

ecotypes, there was no correlation between geographical origin and phenotypic classification. In other words, the ecotypes of different regions were located in one group, indicating that geographical diversity does not follow genetic diversity. The lack of relationship between geographical origin and phenotypic classification could be due to the sexual reproduction and recombination, mutation, genetic repulsion, and natural selection that lead to high genetic diversity [49]. Selection along with generation testing is one of the methods used in plant breeding. Success in selection depends on diversity through genetic recombination and heterosis. The probability of heterosis in cross-breeding programs enhances with increasing genetic distance [50]. In breeding programs, the classification of genotypes based on genetic distance is effective if several traits are evaluated simultaneously [51]. Since one of the most important goals in genetic diversity programs for medicinal plants is the selection of superior ecotypes in order to produce maximum biomass, yield, metabolites, and their interactions, therefore choosing parents with long distances is necessary. In addition, parents with high genetic diversity that were placed in different habitats and geographic locations lead to the production of plants with more heterosis in comparison with their parents. Thus, according to the obtained results, could be suggested to establish a poly-cross nursery with the first and fifth subgroups of the ecotypes. The results of this study also showed some correlations between geographical diversity and phenotypic diversity. The classification of the ecotypes in close groups indicated the genetic and kinship similarities between them. The ecotypes classification of close habitats in separate groups as well as the ecotypes of the different geographical origins in the same groups may be due to the displacement of germplasm and also wide distribution in different areas with relatively similar environmental, edaphic, and microclimate conditions. The high diversity among the studied ecotypes of close habitats could be attributed to sexual reproduction and some issues such as displacement, crossbreeding, and recombination. The high genetic diversity allows the species to be more adaptable to different environments, so that, in addition to effective use in the management and protection of the germplasm, it can be a useful tool in breeding programs.

CONCLUSION

This is the first study to evaluate and compare the phenotypic diversity of feverfew populations. We observed significant phenotypic differences in the evaluated ecotypes. The results of this study revealed that there were some correlations between geographical diversity and phenotypic diversity. The classification of the ecotypes in one group indicated kinship and genetic relationships between them.

Conflict of Interest

The authors declare that there is no conflict of interest.

REFERENCES

1. Pareek A., Suthar M., Rathore G.S., Bansal V. Feverfew (*Tanacetum parthenium* L.): A systematic review. *Pharmacogn Rev.* 2011; 5: 103-110. <http://dx.doi.org/10.4103/0973-7847.79105>
2. Freund R.R., Gobrecht P., Fischer D., Arndt H.D. Advances in chemistry and bioactivity of parthenolide. *Nat Prod Rep.* 2019; 37: 541-565. <https://doi.org/10.1039/c9np00049f>
3. Kaye A.D., Sabar R., Clarke R.C., Vig S., Hofbauer R. Nutraceuticals-current concepts and the role of the anesthesiologist (part 2). *Am J Anesth.* 2000; 467-471.
4. Zhang F., Zhou J., Shi Y., Karaisz K. Identification of antioxidative ingredients from feverfew (*Tanacetum Parthenium*) extract substantially free of parthenolide and other alpha-unsaturated gamma-lactones. *Open J Anall Bioanal Chem.* 2019; 3: 076-082. <https://doi.org/10.17352/OJABC.000015>
5. Alenzi K.A., Alharbi F.H., Tawhari F.M., Fradees G.S. Alteration of coagulation test results and vaginal bleeding associated with the use of feverfew (*Tanacetum parthenium*). *J Med Cases.* 2021; 12: 9. <https://doi.org/10.14740%2Fjmc3601>
6. Duke JA. *CRC Handbook of Medicinal Herbs.* Boca Raton, FL: CRC Press.1985.
7. Jackson B, McDonald RL. In: *Dobelis IN, editor. Magic and Medicine of Plants.* Pleasantville, NY: Reader's Digest Assoc. 1986.
8. Meyer J.E. *The Herbalist.* Hammond, IN: Hammond Book Co. 1934.
9. Majdi M., Charnikhova T., Bouwmeester H. Genetical, developmental and spatial factors influencing parthenolide and its precursor costunolide in feverfew (*Tanacetum parthenium* L. Schulz Bip.). *Ind Crops Prod.* 2013; 47: 270-276. <https://doi.org/10.1016/j.indcrop.2013.03.021>

10. Maxia A., Sanna C., Piras A., Porcedda S., Falconieri D., Goncalves M.J., Salgueiro L. Chemical composition and biological activity of *Tanacetum audibertii* (Req.) D.C. (Asteraceae), an endemic species of Sardinia Island, Italy. *Ind Crops Prod.* 2015; 65: 472-476. <https://doi.org/10.1016/j.indcrop.2014.10.039>
11. Maggi F. Feverfew (*Tanacetum parthenium* (L.) Sch. Bip.). In *Nonvitamin and nonmineral nutritional supplements*. Academic Press. 2019; 223-225. <https://doi.org/10.1016/B978-0-12-812491-8.00032-1>
12. Sadat-Hosseini M., Farajpour M., Boroomand N., Solaimani-Sardou F. Ethnopharmacological studies of indigenous medicinal plants in the south of Kerman, Iran. *J Ethnopharmacol.* 2017; 199: 194-204. <https://doi.org/10.1016/j.jep.2017.02.006>
13. Connell J.H., Slatyer R.O. Mechanisms of succession in natural communities and their role in community stability and organization. *Am Natur.* 1977; 111: 1119-1144. <https://doi.org/10.1086/283241>
14. Hacker S.D., Gaines S.D. Some implications of direct positive interactions for community species diversity. *Ecol.* 1997; 78: 1990-2003. [https://doi.org/10.1890/0012-9658\(1997\)078\[1990:SIODPI\]2.0.CO;2](https://doi.org/10.1890/0012-9658(1997)078[1990:SIODPI]2.0.CO;2)
15. Cavieres L.A., Badano, E.I. Do facilitative interactions increase species richness at the entire community level? *J Ecol.* 2009; 97: 1181-1191. <https://doi.org/10.1111/j.1365-2745.2009.01579.x>
16. Maestre F.T., Callaway R.M., Valladares F., Lortie C.J. Refining the stress-gradient hypothesis for competition and facilitation in plant communities. *J Ecol.* 2009; 97: 199-205. <https://doi.org/10.1111/j.1365-2745.2008.01476.x>
17. Bartels S.F., Chen, H.Y. Interactions between overstorey and understorey vegetation along an overstorey compositional gradient. *J Veg Sci.* 2012; 24: 543-552. <https://doi.org/10.1111/j.1654-1103.2012.01479.x>
18. Falk D.A., Knapp E.E., Guerrant E.O. An introduction to restoration genetics. Plant conservation alliance, bureau of land management. US Department of Interior, US Environmental Protection Authority. 2001.
19. Frankham R. Genetics and extinction. *Biol Conserv.* 2005; 126: 131-140. <https://doi.org/10.1016/j.biocon.2005.05.002>
20. Baricevic D., Mathe A., Bartol T. Conservation of wild crafted medicinal and aromatic plants and their habitats. In *Medicinal and aromatic plants of the world*. Springer, Dordrecht. 2015; 131-144.
21. Hoban S., Archer F.I., Bertola L.D., Bragg J.G., Breed M.F., Bruford M.W., Hunter M.E. Global genetic diversity status and trends: towards a suite of essential biodiversity variables (EBVs) for genetic composition. *Biol Rev.* 2022; 97: 1511-1538. <https://doi.org/10.1111/brv.12852>
22. Bhandari H.R., Bhanu A.N., Srivastava K., Singh M.N., Shreya H.A. Assessment of genetic diversity in crop plants-an overview. *Adv Plants Agric Res.* 2017; 7: 279-286. <https://doi.org/10.15406/apar.2017.07.00255>
23. Helmstetter A.J., Oztolan-Erol N., Lucas S.J., Buggs R.J. Genetic diversity and domestication of hazelnut (*Corylus avellana* L.) in Turkey. *Plants People Planet.* 2020; 2: 326-339. <https://doi.org/10.1002/ppp3.10078>
24. Mezaka I., Kronberga A., Nakurte I., Taskova I., Jakovels D., Primavera, A. Genetic, chemical and morphological variability of chamomile (*Chamomilla recutita* L.) populations of Latvia. *Ind Crops Prod.* 2020; 154: 112614. <https://doi.org/10.1016/j.indcrop.2020.112614>
25. Begna, T. Role and economic importance of crop genetic diversity in food security. *Inter J Agric Sci Food Technol.* 2021; 7: 164-169. <https://doi.org/10.17352/2455-815X.000104>
26. Mathe A. An ecological approach to medicinal plant introduction. *Herbs, Spices and Medicinal Plants: Recent Advances in Botany, Horticulture, and Pharmacology*. Oryx Press. 1988; 3: 175-205.
27. Tetenyi P. Chemical variation (chemo differentiation) in medicinal and aromatic plant. *Acta Hort.* 2002; 576: 15-23. <http://dx.doi.org/10.17660/ActaHortic.2002.576.1>
28. Joshi K., Chavan P., Warude D., Patwardhan B. Molecular markers in herbal drug technology. *Curr Sci.* 2004; 87: 159-165.
29. Hadi N., Shojaeiyan A., Sefidkon F., Jafari A.A., Misic D., Banjanac T., Siler B. Assessment of infraspecific genetic diversity in *Nepeta kotschyi* Boiss., a native Iranian medicinal plant. *J Agric Sci Technol.* 2020; 22: 1327-1342. <http://jast.modares.ac.ir/article-23-33509-en.html>
30. Cirak C., Radusiene J., Karabuk B., Janulis V., Ivanauskas L. Variation of bioactive compounds in *Hypericum perforatum* growing in Turkey during its phenological cycle. *J Integr Plant Biol.* 2007; 49: 615-620. <https://doi.org/10.1111/j.1744-7909.2007.00479.x>
31. Swarup S., Cargill E.J., Crosby K., Flagel L., Kniskern J., Glenn K.C. Genetic diversity is indispensable for plant breeding to improve crops. *Crop Sci.* 2021; 61: 839-852. <https://doi.org/10.1002/csc2.20377>
32. Cruz V.M.V., Dierig D.A., Lynch A., Hunnicutt K., Sullivan T.R., Wang G., Zhu J. Assessment of phenotypic diversity in the USDA, National Plant Germplasm System (NPGS) guayule germplasm collection. *Ind Crops Prod.* 2022; 175: 114303. <https://doi.org/10.1016/j.indcrop.2021.114303>
33. Heidari P., Rezaei M., Sahebi M., Khadivi, A. Phenotypic variability of *Pyrus boissieriana* Buhse: Implications for conservation and breeding. *Sci Hort.* 2019; 247: 1-8. <https://doi.org/10.1016/j.scienta.2018.11.075>
34. Khadivi A., Mirheidari F., Moradi Y., Paryan S. Phenotypic variability of oleaster (*Elaeagnus angustifolia* L.) as revealed by morphological characteristics. *Ind Crops Prod.* 2020; 149: 112322. <https://doi.org/10.1016/j.indcrop.2020.112322>

35. Samsampour D., Kazemzadeh-Beneh H., Damizadeh G.R., Mirzaei Z. Morphological and biochemical classification of Iranian mango germplasm collection by multivariate analysis: implications for breeding. *Adv Horti Sci* 2020; 34: 381-395. <https://doi.org/10.13128/ahsc-9005>
36. Khadivi-Khub A., Etemadi-Khah A. Phenotypic diversity and relationships between morphological traits in selected almond (*Prunus amygdalus*) germplasm. *Agrofor Syst.* 2015; 89: 205-216. <https://doi.org/10.1007/s10457-014-9754-x>
37. Hibberd J.M., Quick W.P. Characteristics of C₄ photosynthesis in stems and petioles of C₃ flowering plants. *Nature.* 2002; 415: 451-454. <https://doi.org/10.1038/415451a>
38. Qi Y., Wei W., Chen C., Chen L. Plant root-shoot biomass allocation over diverse biomes: A global synthesis. *Global Ecol Conserv.* 2019; 18: e00606. <https://doi.org/10.1016/j.gecco.2019.e00606>
39. Foust C.M., Preite V., Schrey, A.W., Alvarez, M., Robertson M.H., Verhoeven K.J.F., Richards C.L. 2016. Genetic and epigenetic differences associated with environmental gradients in replicate populations of two salt marsh perennials. *Molecular Ecol.* 2016; 25: 1639-1652. <https://doi.org/10.1111/mec.13522>
40. Xiong F., Nie X., Yang L., Wang L., Li J., Zhou G. Non-target metabolomics revealed the differences between *Rh. tanguticum* plants growing under canopy and open habitats. *BMC Plant Biol.* 2021; 21: 1-13. <https://doi.org/10.1186/s12870-021-02897-8>
41. Richards C.L., Pennings S.C., Donovan L.A. Habitat range and phenotypic variation in salt marsh plants. *Plant Ecol.* 2005; 176: 263-273. <https://doi.org/10.1007/s11258-004-0841-3>
42. Otto S.P., Whitton J. Polyploid incidence and evolution. *Annu Rev Genet.* 2000; 34: 401-437. <https://doi.org/10.1146/annurev.genet.34.1.401>
43. Beaulieu J.M., Leitch I.J., Patel S., Pendharkar A., Knight C.A. Genome size is a strong predictor of cell size and stomatal density in angiosperms. *New Phytol.* 2008; 179: 975-986. <https://doi.org/10.1111/j.1469-8137.2008.02528.x>
44. Stahlberg D. Habitat differentiation, hybridization and gene flow patterns in mixed populations of diploid and autotetraploid *Dactylorhiza maculata* s.l. (Orchidaceae). *Evol Ecol.* 2009; 23: 295-328. <https://doi.org/10.1007/s10682-007-9241-1>
45. Kim S., Rayburn A.L., Boe A., Lee D.K. Neopolyploidy in *Spartina pectinata* Link: 1. Morphological analysis of tetraploid and hexaploid plants in a mixed natural population. *Plant Syst Evol.* 2012; 298: 1073-1083. <https://doi.org/10.1007/s00606-012-0617-5>
46. Laport R.G., Ramsey J. Morphometric analysis of the North American creosote bush (*Larrea tridentata*, Zygophyllaceae) and the microspatial distribution of its chromosome races. *Plant Syst Evol.* 2015; 301: 1581-1599. <https://doi.org/10.1007/s00606-014-1179-5>
47. Pastorino M.J., Marchelli P. Genetic resources: The base material for managing nature. *Conserv.* 2021; 77-87. <https://doi.org/10.1016/B978-0-12-821139-7.00192-6>
48. Babazadeh Darjazi, B. Comparison of vitamin C in mandarin (*Citrus Blanco.*) cultivars. *Eco-phyto J Med Plant.* 2013; 3: 82-93.
49. Zarabiyani M., Majidi M.M., Bahrami, F. Relationship of morphological and agronomic traits in Iranian and exotic sainfoin populations using multivariate statistical analysis. *Iran J Rang For Plant Breed Genet Res.* 2014; 22: 278-290. <https://dx.doi.org/10.22092/ijrfpbgr.2015.12233>
50. Birchler J.A., Yao H., Chudalayandi S. Unraveling the genetic basis of hybrid vigor. *Proc Natl Acad Sci.* 2006; 103: 12957-12958. <https://doi.org/10.1073/pnas.0605627103>
51. Pank F. Adaptation of medicinal and aromatic plants to contemporary quality and technological demands by breeding: aims, methods and trends. *Rev Bras Plant Med, Botucatu.* 2006; 8: 39-42.