Moroccan Journal of Agricultural Sciences
Vol 5 No 2 (2024): (June 2024), 77-84
Crop Production and Environment

Analysis of genetic diversity of the critically endangered Onobrychis conferta subsp. conferta using cross-genera transferability of SSR markers developed from related legume species

Anis SAKHRAOUI	Departamento de Biología Vegetal y Ecología, Universidad de Sevilla, Sevilla41004, Spain
Hela BELHAJ LTAIEF	Department of Agricultural Production, Higher School of Agriculture of Mograne, University of Carthage, Mograne, Zaghouan, Tunisia
Yoser FERCHICHI	Department of Agricultural Production, Higher School of Agriculture of Mograne, University of Carthage, Mograne, Zaghouan, Tunisia
Sahari KHOUFI	National Institute of Agronomy of Tunis, Carthage University, Tunis, Tunisia
Jesús M. CASTILLO	Departamento de Biología Vegetal y Ecología, Universidad de Sevilla, Spain
Slim ROUZ	Department of Agricultural Production, Higher School of Agriculture of Mograne, University of Carthage, Mograne, Zaghouan, Tunisia

The use of transferable cross-species/genera SSR markers is an alternative strategy to ensure availability of markers in genomic resources-limited crops, such as critically endangered species. Eighteen microsatellite markers derived from the genomes of *Medicago truncatula* Gaertn., *Phaseolus vulgaris* L. and *Onobrychis viciifolia* Scop. were tested for transferability and used to study the genetic diversity of the three remaining populations of the critically endangered species *Onobrychis conferta* subsp. *conferta*, collected from their natural habitats. All pairs of primers tested were found to be polymorphic and reproducible. A total of 257 alleles were obtained from 134 loci, resulting in an average of 1.93 alleles per locus. The average number of alleles per accession was 51.4, yielding an average of 14.27 alleles per SSR marker and accession. The lowest number of alleles was recorded in *O. conferta* from Aïn Dyssa, with 37 alleles, while the highest was observed in *O. viciifolia*, with 63 alleles. Each SSR amplified 3-16 alleles. The MTIC343 primer yielded the highest number of loci (16 loci). The mean Polymorphism Information Content (PIC), Marker Index (MI), and Resolving Power (Rp) were 0.36, 2.22, and 4.58, respectively, indicating a high level of polymorphism in the studied SSR markers. UPGMA cluster analysis grouped genotypes into two main clusters in corroboration with the morphological distinction of sections. Our study demonstrated that *O. conferta* subsp. *conferta* genomes could be successfully examined using other legume SSR markers, providing a valuable tool to detect polymorphism for future genetic studies, breeding programs, and conservation strategies, addressing the lack of available SSR markers in this genus. These microsatellite loci may help to further survey the adaptive evolution and genetic variation of *Onobrychis conferta* conservation.

INTRODUCTION

The genus Onobrychis Miller belongs to the tribe Hedysareae (Fabaceae) and comprises nearly 170

species distributed worldwide (Sakhraoui et al., 2023a). Onobrychis species are mainly found in the north temperate regions, with the most important diversity centres in the eastern Mediterranean and West Asia. The genus includes annual and perennial species, predominantly caulescent herbs (rarely spiny shrubs) with an indumentum of simple hairs or glabrous (Lock and Simpson, 1991, Yakovlev et al., 1996, Mabberley, 1997).

Wild species of the genus Onobrychis have some interesting agronomic and ecological features such as perenniality, a deep root system and high-stress tolerance, which make them suitable for future cultivation under climate change (Sakhraoui et al., 2023a). Wild Onobrychis species can be considered advantageous in crop rotation, as they positively influence weed control by limiting the growth and reproduction of herbaceous species through their procumbent and compact canopy (Erfanzadeh et al., 2020). They are multipurpose species that can be utilized for fodder and honey production even under harsh environmental conditions. Onobrychis species provide valuable browse for livestock and are essential wildlife grazing sources throughout the Mediterranean Basin. In arid and semiarid areas, especially during the dry season, they can serve as an alternative food source for livestock when other forage yields are insufficient (Le Houérou, 1995). Moreover, these species play a crucial role in the conservation and recovery of degraded areas (Niknam et al., 2018). Additionally, extracts from wild Onobrychis species exhibit a wide range of pharmacological effects, including antioxidant, antidiabetic, anti-inflammatory, antitumor, antimicrobial, and anti-stressor properties (Sakhraoui et al., 2023a).

In comparison to cultivated forage legumes, wild Onobrychis species remain underexploited although they have the potential to address forage and food insecurity, either through domestication or by contributing novel alleles for the breeding of the cultivated species Onobrychis viciifolia (Sakhraoui et al., 2023a). Wild Onobrychis species possess a very rich genetic diversity to adapt to high temperatures, drought, salinity, disease and pests, and can be integrated into breeding programs to create resistant varieties (Hart, 2001). Therefore, targeted breeding activities are needed to select individuals better adapted to a broad range of environmental conditions. However, breeding activities for Onobrychis species have been rather scarce and the species has only recently gained broader interest (Bhattarai et al., 2016). For targeted improvement of Onobrychis species, diverse and well characterised genetic resources are needed. Novel breeding approaches must be developed based on knowledge of the mode of inheritance of key breeding targets and the development of molecular genetics tools to assist phenotypic selection.

Although novel approaches such as genotyping by sequencing (Elshire et al., 2011) may be readily applied to any species, most approaches require the availability of molecular markers or genome sequence information. Such resources are scarce for Onobrychis species and most of genetic studies have been largely based on universal marker systems such as internal transcribed spacer regions (ITS) (Hayot-Carbonero et al., 2012), inter simple sequence repeats (ISSR) (Bhattarai et al., 2016), sequence-related amplified polymorphism (SRAP) markers (Kempf et al., 2015; Kempf et al., 2017) or sequence specific simple sequence repeats (SSR) markers derived from other species (Demdoum et al., 2012). The development of highly informative, specific markers for Onobrychis species is indispensable to create a genetic knowledge base and assist breeding by marker assisted selection (MAS) (Sorrells and Wilson, 1997).

SSR markers show codominance of alleles and are randomly distributed along the genome, particularly in low-copy regions (Morgante et al., 2002; Kumar et al., 2009). Considering the complex Onobrychis species genome, and especially the lack of knowledge about its genetic information and maps, SSRs are the markers of choice. These markers are multi-allelic in contrast to next generation high-throughput sequencing (NGS) derived single-nucleotide polymorphism (SNP) marker that are bi-allelic. This makes SSR markers highly variable and useful for distinguishing even between closely related populations or varieties (Smith and Devey, 1994). Furthermore, SSR are easily detected using standard PCR methods and are transferable to closely related taxa (Chen et al., 2015; Ziya, 2016). Transferability is an important property for further

studies that have been hampered by the limited availability of species-specific molecular markers (Avci et al., 2014).

Onobrychis conferta subsp. conferta (Desf.) desv. is a Critically Endangered species native to arid and semi-arid Pinus halepensis forests in North Africa and has only three remaining populations in Tunisia, facing an extremely high risk of extinction in the wild (Sakhraoui et al., 2023b). This endemic species is currently in sharp decline on the calcareous skeletal soils of the Western Tunisian Ridge and the Saharan Atlas (Le Houérou, 1995). Consequently, it is very urgent to understand the genetic diversity and develop a conservation strategy for O. conferta subsp. conferta.

To develop a set of O. conferta specific SSRs markers which reliably detected polymorphism among the three remaining populations of the critically endangered species Onobrychis conferta subsp. conferta, we explored cross species/genera transferability of 18 SSR markers from M. truncatula, P. vulgaris and O. viciifolia genomes. We hypothesized that these microsatellite markers derived from related legume species would represent a valuable tool to successfully differentiate O. conferta populations and to study their genetic diversity.

MATERIALS AND METHODS

Plant material

Based on data published by Pottier-Alapetite (1979) on the range of Tunisian wild species of the Onobrychis genus, prospecting missions were carried out during May and June 2018 in North-western Tunisia. Three populations of the critically endangered species Onobrychis conferta subsp. conferta were found and leaf samples were collected from each population. The geographic locations of the collection sites were recorded using a GPS (Garmin 72H receiver, Olathe, Kansas, USA) (Table 1). Herbarium specimens of the sampled O. conferta subsp. conferta populations were preserved at the Herbarium of Higher School of Agriculture of Mograne.

Genomic DNA extraction

Thirty plants per population were randomly selected and young fresh healthy leaves from each plant were collected for DNA extraction as described by Reyes-Valdés et al. (2013). Genomic DNA extraction was carried out according to Saghai-Maroof et al. (1984) with slight modifications. DNA quality and quantity were assessed using gel electrophoresis, spectrophotometry and diluted to 50 ng/µl before PCR amplification.

SSR genotyping

Eighteen microsatellites' loci originally developed for M. truncatula (15) O. viciifolia (2) and P. vulgaris (1) were tested for transferability and to identify genetic diversity in O. conferta (Table 2). PCR amplification reactions were performed in a final volume of 25 µl containing 2.5 µl of genomic DNA (50 ng), 2.5 µl each of primer, 1.5 µl of MgCl2 (25 mM), 0.2 µl of Taq DNA polymerase (5 U/µl), 2.5 µl each dNTP (0.2 mM), 5 µl of 10X Taq buffer and 8.3 µl of sterilized distilled water. PCR was carried out using an Applied Biosystems™ 2720 Thermal Cycler (Thermo Fisher Scientific Inc., Illkirch, France). The PCR conditions were optimized with a 4 min initial denaturation at +94 °C followed by 35 cycles of +94 °C for 1 min, with the annealing temperature optimized for each SSR locus for 1 min and an extension at +72 °C for 2 min and final extension step at +72 °C for 3 min before cooling to +4 °C. PCR products were separated by electrophoresis using 3% PhileKorea agarose gels in 1x TBE buffer at 5 V/cm, stained with Cleaver Scientific Runsafe Stain CSL-RUNSAFE and visualized under UV light using the Gel-Documentation Unit (Consort Bvba, Turnhout, Belgium) and photographed. Band scoring was carried out using DNA size standards (100 bp plus DNA Ladder) depending on the SSR amplification range. Bands of a particular

molecular weight were scored manually as presence (1) or absence (0).

Data analysis

The percentage of transferability of the markers was calculated by determining the presence of target SSR loci to the total number of the SSR loci analysed. POPGENE 1.32 software (Yeh et al., 1999) was used to generate the pairwise genetic distance (GD) matrix and to calculate the effective number of alleles (ne), observed number of alleles per locus (na), Nei's (1973) gene diversity (h) and Shannon's information measure (I) (Lewontin, 1972). A similarity coefficient among species was calculated according to Nei (1972). Jaccard's coefficient of similarity (Jaccard, 1908) was measured, and a dendrogram was constructed based on the similarity coefficient generated by Unweighted Pair Group Method using Arithmetic average (UPGMA) according to Nei and Li (1979) using statistical package NTSYS-pc v2.02 (Applied Biostatistics Inc., New York, USA) (Sneath and Sokal, 1973; Rolf, 2002). To characterize genetic variation, the information content (PIC), resolving power (RP), and marker index (MI) of SSR markers were calculated as follow: PICi = (2fi × (1 – fi)) (Roldán-Ruiz et al., 2000), Rpi = ∑ [1 – (2 × □ 0.5 – fi □)] (Prevost and Wilkinson, 1999), MIi = PICi × Ni × βi (Powell et al., 1996), where the subscript i represents the ith primer, fi is the frequency of the amplified allele, (1 – fi) is the frequency of the null allele, PICi is the information content of the ith primer, Ni is the total band for the ith primer, and βi is the percentage of the ith primer's polymorphic band.

RESULTS

The eighteen pairs of SSR primers were polymorphic, reproducible, and showed 100% transferability despite the various genetic origin (Table 3). A total of 257 alleles were obtained from the 134 loci with an average of 1.93 alleles per locus. Each SSR amplified 3-16 alleles with an average number of alleles per SSR marker of 14.3. The average number of alleles per taxa was 51.4 over all SSR markers, leading to an average of 2.85 alleles per SSR marker and taxa. The lowest number of alleles was recorded for population OC3 with 37 alleles and the highest for OV1 with 63 alleles.

The PIC values in this study varied from 0.32 to 0.48, with an average of 0.36 ± 0.09 (Table 3). The highest PIC value was recorded for MTIC84 marker. Marker index mean value was 2.22 ± 0.52. The highest value was recorded for MTIC432 and MITC451 (3.52), while the lowest was recorded for MTIC432 (1.4). The 18 markers used in the study exhibited a Rp value ≥ 1.0, ranging from 1.6 for MTIC343 and 10 for OVM130 (average of 4.58 ± 1.08) (Table 3).

In general, the populations included in this study showed a relatively high level of genetic diversity. O. conferta populations showed a slightly lower level of genetic variation than O. viciifolia. Within O. conferta populations, OC2 had the highest level of effective alleles, Shannon's Information index (I) and Nei's gene diversity (h), whereas population OC1 exhibited the lowest value for these parameters. The observed number of alleles (na) for all loci was 2.0000 and the effective number of alleles (ne) ranged between 1.1637-1.2863 (Table 4). Similarly, I and h ranged between 0.2692-0.3817 and 0.1407-0.2226, with a mean value of 0.3358 ± 0.044 and 0.1885 ± 0.032, respectively.

Nei's genetic distance between pairs of populations (GD) ranged from 0.0292 to 0.2513, with an average number of 0.138 ± 0.043. The GD estimation revealed that the most genetically related pair of populations (excluding the outgroup) were OC1 and OC2 (GD = 0.0349). The accessions of OC3 and OV1 were the most distant (GD = 0.1224) (Table 5). The UPGMA dendrogram displayed two primary clusters with a similarity coefficient of 0.89, exhibiting various degrees of sub-clustering that aligned with the morphological distinctions of sections (Figure 1). The three O. conferta populations were closely grouped, sharing a 0.94 similarity. OC1 and OC2 appeared particularly related, sharing a 0.97 similarity. O. viciifolia presented 0.91 and 0.79 similarity

coefficient with the three O. conferta populations and with M. truncatula, respectively.

DISCUSSION

As we hypothesized, the microsatellite markers derived from related legume species (M. truncatula, P. vulgaris and O. viciifolia) successfully differentiated O. conferta populations, indicating their usefulness for molecular studies. This implies that genomes of related legumes could be sources of SSR markers useful for dissecting O. conferta genomes that have nil SSR markers developed so far. Amplified markers from the donor species yielded banding patterns in the genome of the three studied O. conferta populations, suggesting that they originated from the same loci and that these allelic regions of the primer binding sites were conserved (Saini et al., 2023).

Several attempts have been made to transfer markers among legume species such as M. truncatula, Pisum sativum L. and Trifolium pratense L. to Lens culinaris Medik. (Reddy et al., 2010), and M. truncatula to six Mediterranean Hedysarum species (Zitouna et al., 2013). However, there has been a limited attempt to transfer SSR markers to Onobrychis species (Băcilă et al., 2023). In this study, the proportion of transferable microsatellites between the three genera Medicago, Phaseolus and Onobrychis to O. conferta was higher than previously reported. Intra-genera amplification was usually about 50% (Peakall et al., 1998; Eujayl et al., 2004) and it decreased rapidly when it became inter-genera. However, Demdoum et al. (2012) reported 81% of SSR markers transferability from Medicago and Glycine to several Onobrychis taxa. In addition, cross genera transferability from Iris to Gladiolus was 48% (Hiremath et al., 2023). Only 19% of transferability from M. truncatula and Glycine max (L.) Merr. to Onobrychis taxa genome was reported (Avci et al., 2014; Zhang et al., 2007) reported a transferability rate of 18-22% from Medicago to Trifolium, while Peakall et al. (1998) found only 1 to 3% transferability from Glycine to other legume genera.

The success of cross-transferability depends upon the evolutionary distance between the source and the target species. The higher the genomic homology, the greater the conservation of SSR-flanking regions and, hence, the transferability of SSR markers (FitzSimmons et al., 1995; Saini et al., 2023). Moreover, it is also worth considering that the degree of transferability and the level of polymorphism of a given SSR marker could be influenced by the level of ploidy, as well as by mutational events (Dirlewanger et al., 2002). Differences in amplification success for SSR markers observed among species are due to the genetic variability between the different species in the Fabaceae Family, where the SSRs were developed. It has been shown that closely related species are more likely to share SSR priming sites than more distantly related ones, but it is possible to transfer functional SSR primers from more distantly related species in some cases (Lorieux et al., 2000). In this sense, these results indicate that O. conferta is genetically close to M. truncatula, P. vulgaris and O. viciifolia. The main reason for the high-level transferability of SSR markers taken from the above-mentioned species to O. conferta may be attributed to reshuffling of regions in genomes and variations incorporated through mutation and crossing during evolution, where some part of genome might have got modified and resulted in speciation (Singh et al., 2014).

In the attempt of Demdoum et al. (2012) to transfer microsatellites from M. truncatula and Glycine max, a total of 35 alleles have been detected using the six SSRs among 25 Onobrychis populations. However, Avci et al. (2014) found a higher allele number than the previous study by amplifying 725 alleles from 18 SSR markers in diverse Onobrychis subspecies using markers from P. vulgaris and M. truncatula. The higher number found by this author compared to the reported results could be explained by the larger diversity of germplasm used and collected from different subspecies. The number of polymorphic bands ranged from 6 to 14, which is higher than previously reported (5 to 7) by Demdoum et al., (2012) in several Onobrychis taxa using a bulk of 10 individual for DNA extraction, Avci et al., (2014) using only one plant per population (2 to 7 polymorphic bands), and Falahati-Anbaran et al., (2004) in Medicago sativa L. (4 to 14 polymorphic bands). This superiority is probably because the studied O. conferta populations were analysed in a bulk of 30 individuals. Indeed, the number of detected alleles can be underestimated. This could be caused by various

reasons. First, the detection method used (agarose gel electrophoresis) is not sensitive enough to differentiate alleles with small size differences and may not detect all the alleles that have been amplified. More sensitive detection methods, such as capillary electrophoresis, would provide even more difficult-to-label fragment motifs due to stuttering of the markers. On the other hand, the swelling of DNA samples and their co-amplification by PCR are responsible for the loss of rare alleles and only detect the most common alleles. This may result in the non-detection of several present alleles, leading to an underestimation of detected number of alleles per locus in each population. The generation of multiple products during interspecific amplification could occur by mutation, rearrangements, and duplications in the flanking region and / or changes in the number of repeats (Peakall et al., 1998; Gutierrez et al., 2005).

The PIC value provides an estimate of the discriminatory power of a locus or loci, by taking into consideration of number of alleles (Saini et al., 2023). The PIC value will be almost zero if there is no allelic variation and it can reach a max of 0.5 if a genotype has only new alleles, which is a rare phenomenon. In our study, the PIC values varied from 0.32 to 0.48 with an average of 0.36, which indicated a high level of polymorphism in the studied SSR markers. Average PIC value was found higher than obtained in 32 O. viciifolia genotypes (Kempf et al., 2016). Nevertheless, PIC mean value was comparable to the values detected in other genera such as Catharanthus, Cuminum and Rheum (Shaw et al., 2009; Rostami-Ahmadvandi et al., 2013; Tabin et al., 2016), indicating that the microsatellite flanking regions were conserved and highly useful in inferring the phylogenetic relationship between several species. High PIC values outlined the high discriminatory power of these applied SSR and their considerable molecular polymorphism (Ben Romdhane et al., 2018)

The resolving power of a marker is an indication of the utility of the markers in assessing genetic differentiation and diversity. A resolving power ≥ 1.0 is considered more informative (Azevedo et al., 2012). All the markers used in this study, exhibited a Rp ranging from 1.6 to 10 (average of 4.58), which indicates that they are useful for genetic discrimination and diversity studies in O. conferta. The high genetic diversity and polymorphism observed in the present study are in concordance to results of Demdoum et al., (2012) and Avci et al., (2014). Marker index elucidates the discriminatory power of a marker and therefore it was calculated for all the markers. Marker index values ranged between 1.4 and 3.52, showing that transferred markers are a valuable tool in revealing polymorphism in O. conferta.

The average number of observed alleles (na), effective alleles (ne), Shannon’s Information index (I) and Nei’s gene diversity (h) were used to demonstrate the level of population genetic diversity. In general, the populations included in this study showed a relatively high level of genetic diversity. O. conferta populations showed a slightly lower level of genetic variation than O. viciifolia. This observation could be due to populations decline due to bottleneck effect generated by several faceted threats linked to habitat deterioration and overgrazing as recently discussed by Sakhraoui et al., (2023b). Based on the genetic diversity indexes, the set of transferable SSRs from M. truncatula, P. vulgaris and O. viciifolia to O. conferta genomes showed an untapped potential in the discrimination and analysis of genetic diversity of O. conferta populations, forming a promising molecular tool, which could be used in further genetic studies.

All the studied taxa shared a similarity coefficient of 0.80, which is equal to the findings of Demdoum et al., (2012) using SSR markers and slightly lower than those obtained by Sardaro et al., (2003) using Amplified Fragment Length Polymorphism (AFLP) technique who reported a similarity of 0.73 between several Onobrychis accessions. The shortest genetic distance was between OC1 and OC2 (0.0349). These two populations are close situated. The high similarity indicates that even having cross-pollination and seed dispersal mechanisms, somehow this species is maintaining homozygosity in its populations, which requires further investigations. According to the clustering analysis, O. conferta was well separated from the other taxa. The studied taxa could be divided into three subgroups based on Nei’s original GD. The pattern of clustering of O. conferta populations could correspond to their geographic distribution (Zhao et al., 2011). The two collections from Dyr el Kef (OC1) and Fedj el Hdoum (OC2) clustered separately from accessions collected from Ain

Dyssa (OC3), which reflected the geographic separation by mountains.

CONCLUSION

In conclusion, the selected genetically mapped SSR markers were conserved and allowed successful cross-amplification between the three genera Medicago, Phaseolus and Onobrychis belonging to the same family (Fabaceae). The high level of transferability detected indicates that SSR selection for transferability to new species is a good strategy for identifying SSR markers. The transferable markers detected a considerable level of inter/intraspecific genetic diversity among the studied taxa. Although additional SSRs could be used, the highly informative markers studied here could be considered for assessing accession identification in O. conferta. The results undertaken for the first time on the critically endangered species O. conferta subsp. conferta would be of great interest to establish strategies aiming at rational management and conservation strategies, genetic diversity evaluation, gene mapping and molecular breeding facing up to environmental changes.

REFERENCES

Avci S., Ilhan E., Erayman M., Sancak C. (2014). Analysis of Onobrychis genetic diversity using SSR markers from related legume species. J. Anim. Plant Sci., 24: 556-566.

Azevedo A.L.S., Costa P.P., Machado J.C., Machado M.A., Pereira A.V., da Silva Le ´do F.J. (2012). Cross species amplification of Pennisetum glaucum microsatellite markers in Pennisetum purpureum and genetic diversity of napier grass accessions. Crop Sci., 52: 1776-1785.

Băcilă I., Şuteu D., Coste A., Balázs Z.R., Coldea G. (2023). Evaluation of cross-genus transferability of SSR markers from other legumes to two closely related Onobrychis (Fabaceae) taxa. Not. Sci. Biol., 15: 11549.

Ben Romdhane M., Riahi L., Jardak R., Ghorbel A., Zoghlami N. (2018). Fingerprinting and genetic purity assessment of F1 barley hybrids and their salt-tolerant parental lines using nSSR molecular markers. 3 Biotech., 8: 57.

Bhattarai S., Coulman B., Biligetu B. (2016). Sainfoin (Onobrychis viciifolia Scop.): renewed interest as a forage legume for western Canada. Can. J. Plant Sci., 96: 748-756.

Blair M.W., Diaz L.M., Buendia H.F., Duque M.C. (2009). Genetic diversity, seed size associations and populations structure of a core collection of common beans (Phaseolus vulgaris L). Theor. Appl. Genet., 119: 955-972.

Chen H., Liu L., Wang L., Wang S., Somta P., Cheng X. (2015). Development and Validation of EST-SSR Markers from the Transcriptome of Adzuki Bean (Vigna angularis). PLoS One, 10: e0131939.

Demdoum S., Muñoz F., Delgado I., Valderrábano J., Wünsch A. (2012). EST-SSR cross-amplification and genetic similarity in Onobrychis genus. Genet. Resour. Crop. Evol., 59: 253-260.

Dirlewanger E., Cosson P., Tavaud M., Aranzana J., Poizat C., Zanetto A., Arús P., Laigret F. (2002). Development of microsatellite markers in peach [Prunus persica (L.) Batsch] and their use in genetic diversity analysis in peach and sweet cherry (Prunus avium L.). Theor. Appl. Genet., 105: 127-138.

Dirlewanger E., Cosson P., Tavaud M., Aranzana J., Poizat C., Zanetto A., Arús P., Laigret F. (2002). Development of microsatellite markers in peach [Prunus persica (L.) Batsch] and their use in genetic diversity analysis in peach and sweet cherry (Prunus avium L.). Theor. Appl. Genet., 105:

127-138.

Elshire R.J., Glaubitz J.C., Sun Q., Poland J.A., Kawamoto K., Buckler E.S., Mitchell S.E. (2011). A robust, simple genotyping-by-sequencing (GBS) approach for high diversity species. PLoS One, 6: e19379.

Emberger L. (1976). Carte bioclimatique de la Tunisie selon la classification d'Emberger, Étages et variantes. République Tunisienne. Institut National de Recherches Forestières.

Erfanzadeh R., Shayesteh Shayesteh A.S., Ghelichnia A., Ghelichnia H. (2020). Shrub effects on germinable soil seed bank in overgrazed rangelands. Int. J. Acarol., 13: 1-10.

Eujayl I., Sledge M.K., Wang L., Chekhoskiy K., Zwonitzer J.C., Mian M.A.R. (2004). Medicago truncatula EST-SSRs reveal cross-species genetic markers for Medicago spp. Theor. Appl. Genet., 108: 414-422.

Falahati-Anbaran M., Habashi A.A., Esfahany M., Mohammadi S.A., Ghareyazie B. (2004). Population genetic structure based on SSR markers in alfalfa (Medicago sativa L.) from various regions contiguous to the centers of origin of the species. J. Genet., 86: 59-63.

FitzSimmons N.N., Moritz C., Moore S. (1995). Conservation and dynamics of microsatellite loci over 300 million years of marine turtle evolution. Mol. Biol. Evol., 12: 432-440.

Gutierrez M.V., Vaz-Patto M.C., Huguet T., Cubero J.I., Moreno M.T., Torres A.M. (2005). Cross-species amplification of Medicago truncatula microsatellites across three major pulse crops. Theor. Appl. Genet., 110: 1210-1217.

Hart G.E. (2001). Molecular-marker maps of the cultivated wheats and other Triticum species. DNA-based markers in plants. Phillips RL and Vasil IK (ed). Second Edition. Kluwer Academic Publication. London. pp. 421.

Hayot-Carbonero C., Carbonero F., Smith L.J., Brown T. (2012). Phylogenetic characterisation of Onobrychis species with special focus on the forage crop Onobrychis viciifolia Scop. Genet. Resour. Crop. Evol., 1-12.

Hiremath V., Singh K.P., Jain N., Swaroop K., Jain P.K., Panwar S., Sinha N. (2023). Cross species/genera transferability of simple sequence repeat markers, genetic diversity and population structure analysis in gladiolus (Gladiolus × grandiflorus L.) genotypes. PeerJ. 11: e15820.

Jaccard P. (1908). Nouvelles recherches sur la distribution forale. Bull. la Société Vaudoise des Sci. Nat., 44: 223.

Kempf K., Grieder C., Walter A., Widmer F., Reinhard S., Kölliker R. (2015). Evidence and consequences of self-fertilization in the predominantly out breeding forage legume Onobrychis viciifolia. BMC Genet., 16: 117.

Kempf K., Malisch C.S., Grieder C., Widmer F., Kölliker R. (2017). Marker-trait association analysis for agronomic and compositional traits in sainfoin (Onobrychis viciifolia). Genet. Mol. Res., 16.

Kempf K., Mora-Ortiz M., Smith L.M., Kölliker R., Skøt L. (2016). Characterization of novel SSR markers in diverse sainfoin (Onobrychis viciifolia) germplasm. BMC Genet., 17: 124.

Kimura M., Crow J.F. (1964). The number of allels that can be maintained in a finite population. Genetics, 49: 725-738

Kumar P., Gupta V.K., Misra A.K., Modi D.R., Pandey B.K. (2009). Potential of Molecular Markers in Plant Biotechnology. Plant Omics, 2: 141-62.

Le Houérou H.N. (1995). Bioclimatologie et biogéographie des steppes arides du Nord de l'Afrique: diversité biologique, développement durable et désertisation. In: Le Houérou H.-N. (ed.). Bioclimatologie et biogéographie des steppes arides du Nord de l'Afrique: diversité biologique, développement durable et désertisation. Montpellier: CIHEAM, 1995. p. 1-396 (Options Méditerranéennes: Série B. Etudes et Recherches; n. 10)

Lewontin R.C. (1972). The Apportionment of Human Diversity. In: Dobzhansky, T., Hecht, M.K., Steere, W.C. (eds) Evolutionary Biology. Springer, New York, NY.

Li X., Liu X., Wei J., Li Y., Tigabu, M., Zhao X. (2020). Development and Transferability of EST-SSR Markers for Pinus koraiensis from Cold-Stressed Transcriptome through Illumina Sequencing. Genes, 11: 500.

Lock J.M., Simpson K. (1991). Legumes of West Asia, a check-list. Royal Botanic Gardens, Kew.

Lorieux M., Ndjiondjop M.-N., Ghesquière A. (2000). A first interspecific Oryza sativa and Oryza glaberrima microsatellite-based genetic linkage map. Theor. Appl. Genet., 100: 593-601.

Mabberley D.J. (1997). The plant book. A portable dictionary of the vascular plants, 2nd ed. Cambridge University Press.

Majourhat K., Jabbar Y., Hafidi A., Martinez-Gomez P. (2008). Molecular characterization and genetic relationships among most common identified morphotypes of critically endangered rare Moroccan species Argania spinosa (Sapotaceae) using RAPD and SSR markers. Ann. For. Sci., 65: 805.

Malisch C.S., Lüscher A., Baert N., Engström M.T., Studer B., Fryganas C., Mueller-Harvey I., Salminen J.P. (2015). Large variability of proanthocyanidin content and composition in sainfoin (Onobrychis viciifolia). J. Agric. Food Chem., 63: 10234-10242.

Morgante M., Hanafey M., Powell W. (2002). Microsatellites are preferentially associated with non repetitive DNA in plant genomes. Nat. Genet., 30: 194-200.

Nei M. (1972). Genetic Distance between Populations. Am. Nat., 106: 283-292.

Nei M. (1973). Analysis of gene diversity in subdivided populations. Proc. Natl. Acad. Sci. USA, 70: 3321-3323.

Nei M., Li W. (1979). Mathematical model for studying genetic variation in terms of restriction endonucleases. Proc. Natl. Acad. Sci. USA, 76: 5269-5273.

Niknam P., Erfanzadeh R., Ghelichnia H., Cerdà A. (2018). Spatial variation of soil seed bank under cushion plants in a subalpine degraded grassland. Land Degrad. Dev., 29: 4-14.

Peakall R., Gilmore S., Keys W., Morgante M., Rafalski A. (1998). Cross-species amplification of soybean (Glycine max) simple sequence repeats (SSRs) within the genus and other legume genera: Implications for the transferability of SSRs in plants. Mol. Biol. Evol., 15: 1275-87.

Pottier-Alapetite G. (1979.) Flore de la Tunisie: Angiospermes-Dicotylédones: Gamopétales. Publications Scientifiques Tunisiennes, Programme Flore et Végétation Tunisiennes, Première Partie.

Powell W., Machray G.C., Provan J. (1996). Polymorphism revealed by simple sequence repeats. Trends Plant Sci., 1: 215-222.

Prevost A., Wilkinson M.J. (1999). A new system of comparing PCR primers applied to ISSR fingerprinting of potato cultivars. Theor. Appl. Genet., 98: 107-112.

Reddy M.R., Rathour R., Kumar N., Katoch P., Sharma T.R. (2010). Cross-genera Legume SSR markers for analysis of genetic diversity in Lens Species. Plant Breed., 129: 514-518.

Reyes-Valdés M.H., Santacruz-Varela A., Martinez O., Simpson J., Hayano-Kanashiro C., Cortés-Romero C. (2013). Analysis and optimization of bulk DNA sampling with binary scoring for germplasm characterization. PLoS ONE, 8: e79936.

Roldán-Ruiz I., Dendauw J., Van Bockstaele E., Depicker A., De Loose M. (2000). AFLP markers reveal high polymorphic rates in ryegrasses (Lolium spp.). Mol. Plant Breed., 6: 125-34.

Rolf F.J. (2002). Numerical Taxonomy and Multivariate Analysis System. Version 2.02i. Exeter Software. New York. NY

Rostami-Ahmadvandi H., Cheghamirza K., Kahrizi D., Bahraminejad S. (2013). Comparison of morpho-agronomic traits versus RAPD and ISSR markers in order to evaluate genetic diversity among Cuminum cyminum L. accessions. Aust. J. Crop Sci., 7: 361-367.

Saghai-Maroof M.A., Soliman K.M., Jorgensen R.A., Allard R.W. (1984). Ribosomal DNA spacer-length polymorphism in barley: mendelian inheritance chromosomal location and population dynamics. Proc. Natl. Acad. Sci., 81: 8014-8019.

Saini P., Bains, T.S., Sirari A., Yadav I.S., Mandahal K.S., Patel S.A.H., Akhatar J., Ludhar N., Saini P., Nagpal S., Pratap A. (2023). Validation and cross-species transferability of SSR markers developed from whole genome sequence (WGS) of Mungbean (Vigna radiata L. Wilczek) and their application of population genetics in Vigna species. Preprints: 2023080658.

Sakhraoui A., Ltaeif H.B., Castillo, J.M., Rouz S. (2023). Habitat characterization and decline of Critically Endangered Onobrychis conferta subsp. conferta. J. Nat. Conserv., 79: 126603.

Sakhraoui A., Ltaeif H.B., Sakhraoui A., Rouz S., Castillo J.M. (2023a). Potential use of wild Onobrychis species for climate change mitigation and adaptation. Crop Sci., 63: 3153-3174.

Sardaro S., Molinari L., Albertini E., Reosellini D., Negri V., Falcinelli M. (2003). Distinguibilità molecolare di poplazioni spontanee di Poa pratensis. Lolium perenne e Onobrychis viciifolia. Sementi Elette, 6: 47-9.

Shaw R.K., Acharya L., Mukherjee A.K. (2009). Assessment of genetic diversity in a highly valuable medicinal plant Catharanthus roseus using molecular markers. Crop Breed Appl Biotechnol., 9: 52-59.

Singh R., Narzary D., Bhardwaj J., Singh A.K., Kumar S., Kumar A. (2014). Molecular diversity and SSR transferability studies in Vetiver grass (Vetiveria zizanioides L. Nash). Ind. Crops. Prod., 53: 187-198.

Smith D.N., Devey M.E. (1994). Occurrence and inheritance of microsatellites in Pinus radiata. Genome, 37: 977-83.

Sneath P.H.A., Sokal R.R. (1973). Numerical taxonomy: the principles and practice of numerical classification. W. H. Freeman and Co.

Sorrells M.E., Wilson W.A. (1997). Direct classification and selection of superior alleles for crop improvement. Crop Sci., 37: 691-7.

Tabin S., Kamili A.N., Ganie S.A., Zargar O., Sharma V., Gupta R.C. (2016). Genetic diversity and population structure of Rheum species in Kashmir Himalaya based on ISSR markers. Flora, 121-128

Yakovlev G.P., Sytin A.K., Roskov J.R. (1996). Legumes of Northern Eurasia, a check-list. Royal Botanic Gardens, Kew.

Yeh C.F., Yang R.C., Boyle T. (1999). POPGENE: Microsoft Window-Based Freeware for population genetic analysis.

Zhang Y., Sledge M.K., Bouton J.H. (2007). Genome mapping of white clover (Trifolium repens L.) and comparative analysis within the Trifolieae using cross-species SSR markers. Theor. Appl. Genet., 114: 1367-1378.

Zhao H, Yu J et al (2011). Transferability of microsatellite markers from Brachypodium distachyon to Miscanthus sinensis, a potential biomass crop. J. Integr. Plant Biol., 53: 232-245.

Zitouna N., Marghali S., Gharbi M., Chennaoui-Kourda H., Haddioui A., Trifi-Farah N. (2013). Mediterranean Hedysarum phylogeny by transferable microsatellites from Medicago. Biochem. Syst. Ecol., 50: 129-135.

Ziya M.E., Kafkas S., Khodaeiaminjan M.N.Ç., Gözel H. (2016). Genome survey of pistachio (Pistacia vera L.) by next generation sequencing: Development of novel SSR markers and genetic diversity in Pistacia species. BMC Genomics., 17: 998.

References