



Genetic diversity and phylogenetic analysis in Asian and European *Asparagus* subgenus species

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Abstract Garden asparagus (*Asparagus officinalis* L.) is a diploid ($2n = 2x = 20$), perennial and dioecious species belonging to *Asparagus* subgenus and worldwide cultivated as a vegetable crop. A narrow genetic base has been pointed out for the current cultivars. Crop wild related species (CWR) could be a valuable genetic resource in this crop but they have been underused up to now. To investigate the phylogenetic relationships between CWR asparagus species from different origin and *A. officinalis* L., 12 EST-SSR markers were used to assess the genetic variability of 20 accessions. These accessions belong to 10 *Asparagus* spp. from *Asparagus*

subgenus including wild and naturalized *A. officinalis* L. (2x, 4x, 8x, 10x) and CWR species with European (*A. tenuifolius* Lam. (2x), *A. pseudoscaberr* Greco. (6x), *A. macrorrhizus* Pedrol & al. (12x), *A. prostratus* Dumort (4x), *A. brachyphyllus* Turcz. (6x), *A. maritimus* (L.) Mill. (6x)) and Asian distribution (*A. verticillatus* L. (2x), *A. persicus* Baker (2x), *A. breslerianus* Schult. & Schult. (8x)). *A. albus* L. (2x) from the *Protasparagus* subgenus was used as outgroup. As a result, a total of 248 alleles were obtained and specific alleles of accessions were detected among them. After cluster analysis the accessions did not group by their geographical origin. All wild polyploid accessions with European and Asian distributions were grouped together with *A. officinalis* L. Hence, that cluster could be considered as the ‘*officinalis* group’ suggesting a monophyletic origin. The diploid accessions of *A. verticillatus* L. and *A. persicus* Baker clustered together and were the most genetically distant respect to ‘*officinalis* group’. The results obtained in this study may provide useful information to design new crosses among accessions aimed to develop new asparagus germplasm or pre-breeding populations.

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Introduction

Asparagus is a large genus comprising about 200 species of herbaceous perennials, tender woody shrubs and vines (Bailey 1942; Bozzini 1959; The plant List 2013). This genus is classified into three subgenera: *Asparagus*, *Protasparagus* and *Myrsiphyllum* (Obermeyer 1983, 1984; Clifford and Conran 1987). The species of the *Asparagus* subgenus are dioecious and mostly distributed in the temperate climate regions of Europe and Asia whereas the ones belonging to *Protasparagus* and *Myrsiphyllum* subgenera are hermaphroditic, and their distribution range encompass different regions of Africa, Oceania and Southern Asia and Europe (Kubota et al. 2012). Garden asparagus (*Asparagus officinalis* L.) is a dioecious and perennial species cultivated worldwide as a vegetable crop and is considered the most economically important species of the three subgenera. Other species of the *Asparagus* genus are cultivated or collected from their natural habitats for different uses such as food (*A. acutifolius* L., *A. albus* L., *A. maritimus* Mill.), ornamental (e.g., *A. scandens* Thunb., *A. plumosus* Baker, *A. densiflorus* (Kunth) Jessop, *A. falcatus* L.) or for their medicinal properties (e.g., *A. cochinchinensis* (Lour.) Merr., *A. racemosus* Willd.). The *Asparagus* genus has a wide range of ploidy levels (2x, 4x, 6x, 8x, 10x and 12x) and a basic chromosome number of $x = 10$. Also, intraspecific variation in ploidy levels has been detected in some species of this genus (Moreno et al. 2006; Kanno and Yokoyama 2011; Mousavizadeh et al. 2016). Thus, polyploidization seems to have played an important role in the evolution of the *Asparagus* genus (Castro et al. 2013).

Nearly all current cultivars of garden asparagus are diploid and derive from the Dutch population ‘Violet Dutch’ (eighteenth century) (Knaflewski 1996). Different studies employing molecular markers have revealed the narrow genetic base of this crop (Brettin and Sink 1992; Geoffriau et al. 1992; Khandka et al. 1996; Moreno et al. 2006) supporting its common origin. In the current climate change scenario, the development of new cultivars adapted to new environmental conditions and increasing the genetic diversity available in breeding programs are issues of the utmost importance to meet future environmental challenges (Galluzzi et al. 2020). Landraces and wild species may play a key role in broadening the genetic base of cultivated species (Zeven 1998; Dempewolf

et al. 2014). Recently, a new approach called ‘introgressomics’ has been proposed (Prohens et al. 2017). It consists of mass scale development of plant materials and populations with introgressions from crop wild relatives (CWR) into the genetic background of crops. The development of new germplasm is a major goal in asparagus breeding, and several studies have been performed to that end in the last years. For instance, Castro et al. (2013) developed a diploid population carrying introgressions from ‘Morado de Huétor’, which is a tetraploid landrace with an interspecific (*A. officinalis* L. x *A. maritimus* (L.) Mill.) origin (Moreno et al. 2008). Riccardi et al. (2011) used tetraploid plants derived from interspecific crosses between two wild species (*A. acutifolius*, *A. maritimus*) and one landrace (‘Violetto d’Albenga’) to obtain diploid plants by in vitro anther culture. However, the use of CWR in the development of new asparagus germplasm has largely remained unexploited. In this sense, wild relative species of the *Asparagus* subgenus could be a source of new alleles for the genetic improvement of yield, nutritional quality, adaptability, and resistance or tolerance to biotic or abiotic stresses affecting the asparagus crop (Kanno and Yokoyama 2011; Nothnagel et al. 2017; Jaramillo-Carmona et al. 2017). Extensive knowledge of the phylogenetic relationships between the wild species related to *A. officinalis* can be useful to guide the choice of the most suitable species to include in a breeding program. To date, the phylogenetic studies employing genetic resources of this crop have been mainly focused on landraces cultivated in the south of Europe (‘Morado de Huétor’, ‘Violetto d’Albenga’) and CWR species with European or Eurasian distribution such as *A. prostratus* Dumort, *A. maritimus* Mill., *A. pseudoscaberr* Grec., *A. tenuifolius* Lam., *A. macrorrhizus* Pedrol and al. or *A. brachyphyllus* Turcz. (Stajner et al. 2002; Moreno et al. 2006, 2008; Castro et al. 2013).

The proposed centre of origin of garden asparagus includes eastern Europe, Caucasus, and Siberia (Sturtevant 1919; Ellison 1986). In these regions, other CWR species such as *A. persicus* Baker, *A. breslerianus* Schult. & Schult. or *A. verticillatus* L. and wild populations of *A. officinalis* L. (Mousavizadeh et al. 2015) have been also described. Some Iranian populations of these species have been employed in genetic variability studies based on morphological traits and molecular markers (Sarabi

et al. 2010; Mousavizadeh et al. 2015, 2018). Phylogenetic studies using wild populations of *A. officinalis* and European and Asian wild species closely related to *A. officinalis* will provide new information useful for asparagus breeding and knowledge of the evolutionary pathway of *Asparagus* genus.

Simple sequence repeats (SSR) markers derived from genomic DNA are widely used for assessing genetic diversity and inferring phylogenetic relationships between closely related species or different populations from the same species (Simpson 2006). In *Asparagus* genus, SSR markers have been employed in different studies to assess the genetic variability and phylogenetic relationships of diverse genetic stocks such as current cultivars, landraces, wild species and introgressed populations (Caruso et al. 2008; Li et al. 2016; Castro et al. 2013, 2014).

In previous studies, we analyzed the genetic variability of *Asparagus* spp. with European distribution (Castro et al. 2013) and *Asparagus* spp. from Iran (Mousavizadeh et al. 2018) using EST-SSR markers (Expressed Sequence Tag-derived Simple Sequence Repeats). To have a more comprehensive picture of the genetic variability and the relationships between the wild asparagus species, in this study, we have analyzed together the whole collection of wild asparagus species (European plus Near East accessions) using a higher number of EST-SSR markers than in previous studies.

Materials and methods

Plant materials

Nineteen accessions from 10 *Asparagus* species of *Asparagus* subgenus and one accession (*A. albus* L.) from the *Protasparagus* subgenus were employed in this study. Those species include *A. officinalis* L. (eight accessions), *A. verticillatus* L. (three accessions) and one accession of each *A. brachyphyllus* Turcz., *A. macrorrhizus* Pedrol & al., *A. maritimus* (L.) Mill., *A. tenuifolius* Lam., *A. pseudoscaber* Grec., *A. prostratus* Dumort, *A. persicus* Baker and *A. breslerianus* Schult. & Schult. (Table 1). *A. albus* L. was included as outgroup. The origin, ploidy level and number of plants studied per accession are detailed in Table 1. All the accessions except *A. albus* L. (ABB) and *A. tenuifolius* Lam. (ATU) are growing in an

experimental field trial which contains the collection of genetic resources of the asparagus breeding program developed at the University of Cordoba.

EST-SSR markers analysis

Total genomic DNA of individual plants was isolated from 1 g of tips from young spears following a modified CTAB extraction protocol (Torres et al. 1993). The DNA was quantified by spectrophotometric absorbance at 260 nm (NanoDrop ND-1000; Thermo SCIENTIFIC, Waltham, MA). Two DNA bulks each composed of 4–5 individuals (depending on the number of plants available per accession) were made for all accessions but *A. tenuifolius* Lam. and *A. officinalis* (AOB). Each bulk was generated by pooling equal amount of DNA from each individual. There were 37 bulks total. Bulks were used for PCR amplification. A set of 12 EST-SSR markers developed in *A. officinalis* (Caruso et al. 2008; Mercati et al. 2013) were employed to analyze the genetic variability and relationships between accessions. The markers are distributed in 9 out of the 10 chromosomes of the basic chromosome number of this species (Moreno et al. 2018). The forward primers of the markers (AAT1, AG2, AG7, AG8, TC1, TC3, TC7, TC9) developed by Caruso et al. (2008) were synthesized with fluorescent dyes (6-FAM or HEX) at the 5'-ends whereas the forward primers of the markers (asp_c12534, asp_c1367, asp_c17476 and asp_c5587) developed by Mercati et al. (2013) were synthesized with a 19 bp long M13 tail (5'-CACGACGTTG-TAAAACGAC-3') at the 5' ends for fluorescent labeling PCR fragments following Schuelke (2000). The PCR amplifications carried out with the markers developed by Mercati et al. (2013) were performed in a 10- μ l reaction mixture containing 20 ng of bulked genomic DNA, 5X polymerase buffer, 2 mM MgCl₂, 0.8 mM of dNTP, 0.125 μ M of forward primer, 0.25 μ M of reverse primer, 0.25 μ M of the fluorescent labeled M13 primer (HEX or 6-FAM) and 1U of Taq polymerase (Promega). No fluorochrome M13 and equal amounts of both forward and reverse primers was added in the PCR reaction mix performed with the SSR markers developed by Caruso et al. (2008).

Amplification cycles were performed in a Perkin Elmer Cetus DNA Thermal Cycler (9600) programmed as follows: (A) 1 min at 94 °C for DNA denaturation, (B) 35 cycles with the following

Table 1 Characteristics of the asparagus accessions of *Asparagus* subgenus and outgroup taxa used in the study

Code	Origin	No plants	Ploidy ^a	Type	Species
<i>Asian accessions</i>					
AOM	Mahmood abad (Iran)	10	4x	Wild	<i>A. officinalis</i> L
AOB	Balade (Iran)	10	8x	Wild	<i>A. officinalis</i> L
AOG	Gazanak (Iran)	10	8x	Wild	<i>A. officinalis</i> L
AOT	Taleghan (Iran)	10	4x	Wild	<i>A. officinalis</i> L
AOI	Bayanloo (Iran)	2	10x	Wild	<i>A. officinalis</i> L
AOK	Kerman (Iran)	10	2x	Wild	<i>A. officinalis</i> L
AOD	Karaj (Iran)	10	2x	Naturalized	<i>A. officinalis</i> L
AVH	Chalaki (Iran)	10	2x	Wild	<i>A. verticillatus</i> L
AVP	Shal (Iran)	10	2x	Wild	<i>A. verticillatus</i> L
AVC	Klarood (Iran)	4	2x	Wild	<i>A. verticillatus</i> L
APM	Manjil (Iran)	10	2x	Wild	<i>A. persicus</i> Baker
ABY	Yazdoo (Iran)	10	8x	Wild	<i>A. breslerianus</i> Schult
<i>European accessions</i>					
AOW	Yorshkar-Ola (Russia)	8	2x	Naturalized	<i>A. officinalis</i> L
ABR	Yorshkar-Ola (Russia)	10	6x	Wild	<i>A. brachyphyllus</i> Turcz
APB	Bares (Spain)	9	4x	Wild	<i>A. prostratus</i> Dumort
AMA	Albania	10	6x	Wild	<i>A. maritimus</i> L. Mill
AMC	Cartagena (Spain)	10	12x	Wild	<i>A. macrorrhizus</i> Pedrol & al
ATU	Padova (Italy)	2	2x	Wild	<i>A. tenuifolius</i> Lam
APS	Brno (Czech Rep.)	10	6x	Wild	<i>A. pseudoscaberr</i> Grec
<i>Outgroup</i>					
AAB	Córdoba (Spain)	10	2x	Wild	<i>A. albus</i> L

^aData of ploidy level were obtained from Castro et al. (2013) and Mousavizadeh et al. (2016) for European and Iranian accessions, respectively

temperature profile: 94 °C for 1 min, 55 °C for 1 min and 72 °C for 1.5 min; (C) followed by a final extension at 72 °C for 10 min.

The PCR products were separated using an automated capillary sequencer (ABI 3130 Genetic Analyzer: Applied Biosystems/HITACHI, Madrid, Spain) in the Unit of Genomics of the Central Research Support Service at the University of Córdoba. The size of the amplified bands was calculated based on an internal DNA standard (400HD-ROX) with GeneScan software (version 3.x) and the results were interpreted using the Genotyper program (version 3.7) all from Applied Biosystems.

Phylogenetic and genetic diversity analyses

A large number of the accessions used in this study are polyploid, hence the difficulty to infer allele

frequency. Therefore, for each marker the bands (putative alleles) obtained in each accession were scored as 1 (presence) or 0 (absence). The number of different alleles, specific accession alleles and polymorphic information content (PIC) were also computed. The PIC value of each marker was calculated as $PIC = 1 - \sum p_j^2$, where p_j is the band frequency of the j th allele in the examined bulk of plants. The marker data were also used to create a binary data matrix for the phylogenetic analysis. The Dice similarity index was used to calculate the genetic distance (1-Dice) among accessions. All the species analyzed are members of the *Asparagus* subgenus. Therefore, the assumption of a molecular clock was acceptable and the unweighted pair group method with arithmetic mean (UPGMA) was used to group the accessions. A dendrogram was obtained using the software NTSYS (Rohlf 2000). The correlation

coefficient between the similarity matrix and the cophenetic value matrix was computed to test the goodness of fit of the cluster analysis.

Results and discussion

A total of 248 alleles were detected across all 20 asparagus accessions analyzed in this study using 12 EST-SSR markers. The allele sizes ranged from 118 (Asp_c5587) to 256 bp (AG8). The number of different alleles per marker varied from 4 (Asp_c5587) to 41 (TC9), with an average of 20.7 alleles per marker (Table 2). The total number of alleles per accession varied from 17 (*A. verticillatus* L. (AVP)) to 88 (*A. maritimus* (L.) Mill.). The markers TC9, TC1 and AG7 did not show amplification in *A. persicus* Baker, *A. verticillatus* L. (AVP, AVH, AVP) and the wild *A. officinalis* accession (AOK), respectively. Two markers (TC7 and Asp_c12534) did not amplify in *A. albus* L. These results suggest that the transferability of the markers is high between *Asparagus* species. Sixty-three of the 248 alleles obtained were specific from accession. The number of specific alleles per marker ranged from 1 (Asp_c17476) to 16 (TC9). Specific alleles were found in 13 of the 20 accessions ranging from 1 to 8 alleles. *A. albus* L. and *A. maritimus* (L.) Mill. had the highest number of specific alleles whereas *A. officinalis* (AOT) had the lowest number (Table 2). The specific alleles could be useful to verify the hybrid origin of plants derived from crosses among accessions included in this study. All EST-SSR except Asp_c5587, were considered informative markers (PIC > 0.8) (Table 2).

The phylogenetic relationship among the species was investigated by cluster analysis. A cophenetic correlation of 0.89 was obtained, indicating a good fit between the tree and the similarity matrix. According to the dendrogram results, there are five different groups (Fig. 1). The first group is separated from all the others and contains just *A. albus* L. (2x), which belongs to *Protasparagus* subgenus and is considered as an outgroup. The remaining groups cluster together and are formed by the species of the *Asparagus* subgenus, showing a monophyletic origin like it has been reported by other authors (Fukuda et al. 2005; Kubota et al. 2012). All wild polyploid accessions with European or Asian distribution are grouped together with *A. officinalis* in the same cluster (group

V), indicating that they are genetically related. Hence, that cluster could be considered as the ‘*officinalis* group’ previously reported by Castro et al. (2013). Within the ‘*officinalis* group’ the accessions did not group according to their geographical origin and seem to have a monophyletic origin within *Asparagus* subgenus. High crossability among hexaploid and tetraploid European accessions of this ‘*officinalis* group’, together with a regular meiotic behavior and high pollen and seed fertility was reported by these authors. The dodecaploid *A. macrorrhizus* Pedrol & al., an endemic species from the southeast of Spain growing near to the seaside, was previously classified as *A. maritimus* (L.) Mill. species (Sánchez-Gómez et al. 2007). Recent botanical studies catalogued this accession as a new species within the *Asparagus* subgenus (Pedrol et al. 2013; Regalado et al. 2017). Our study provides several results supporting that *A. macrorrhizus* Pedrol & al. might have recently evolved from *A. maritimus* (L.) Mill.; i) both species grouped together in the same cluster, and ii) *A. macrorrhizus* Pedrol & al. (12x) had lower number of alleles than *A. maritimus* (L.) Mill. (6x) accession. Successful crosses between *A. macrorrhizus* Pedrol & al. and the tetraploid landrace ‘Morado de Huétor’ have been obtained (Amian et al. 2018), proving its close phylogenetic relationship with *A. officinalis*. Another polyploid species included in this group, the octoploid *A. breslerianus* Schult. & Schult. from Iran, grows in a warm and dry climate with a high salinity soil (Mousavizadeh et al. 2016). This species has been also successfully crossed with the landrace ‘Morado de Huétor’ (unpublished data). All these results support the hypothesis that the species clustered in group V could be in a recent process of speciation. As it has been suggested in previous work, polyploidization could be playing an important role in the evolutionary process of *Asparagus* subgenus (Castro et al. 2013). Unreduced gametes could explain the different polyploidy levels. This type of gametes has been described in *A. officinalis* (2x) (Camadro et al. 1992) and in the landrace ‘Morado de Huétor’ (4x) (Regalado et al. 2015). ‘Morado de Huétor’ has an interspecific origin and derives from diploid *A. officinalis* x hexaploid *A. maritimus* (L.) Mill. (Moreno et al. 2008). Also, introgression of this landrace into current diploid cultivars has been reported (Castro et al. 2014).

Table 2 Number of alleles and PIC values for 12 EST-SSR loci in the *Asparagus* accessions analyzed in this study

Accession (code)	Ploidy level	TC1	TC3	TC7	TC9	AG7	AG8	AG2	AAT1	Asp_c1367	Asp_c12534	Asp_c17476	Asp_c5587	Total	Mean
<i>A. breslerianus</i> (ABY)	8x	5	10	6 (1)	9 (2)	10	3	6 (1)	5	5	4 (1)	3	1	67 (5)	5.6
<i>A. officinalis</i> (AOB)	8x	8	11	10 (3)	11	9	12 (1)	11	3 (1)	3	4	5	1	88 (5)	7.3
<i>A. officinalis</i> (AOI)	10x	7	9	6 (1)	9 (2)	4	10	6 (1)	2	4	4	6	1	68 (4)	5.7
<i>A. officinalis</i> (AOD)	2x	3	3	4	2	2	3	7	2	1	2	2	1	32	2.7
<i>A. officinalis</i> (AOG)	8x	6	10	9 (1)	11 (2)	6	15 (3)	11	3	5	4	5	1	86 (6)	7.2
<i>A. officinalis</i> (AOK)	2x	3	4	1	10 (5)	–	3	3	2	4	1	3	1	35 (5)	3.2
<i>A. officinalis</i> (AOM)	4x	5	2	4	5	3	4	3	2	3	1	2	1	35	2.9
<i>A. officinalis</i> (AOT)	4x	6	5	2	6 (1)	3	2	4	2	3	1	2	2	38 (1)	3.2
<i>A. persicus</i> (APM)	2x	3	3	1	–	2	6	2 (2)	1 (1)	4	1	1	1	25 (3)	2.3
<i>A. verticillatus</i> (AVC)	2x	–	3	2	5	1	6	2	2	2	1	2	1	30	2.7
<i>A. verticillatus</i> (AVH)	2x	–	1	1	1	3	4	4	2	2	1	1	1	23	2.1
<i>A. verticillatus</i> (AVP)	2x	–	1	1	4	1	3	1	1	1	1	2	1	17	1.5
<i>A. brachyphyllus</i> (ABR)	6x	9	9	7 (1)	4	4 (1)	9 (2)	9	3	2	3	5	1	65 (4)	5.4
<i>A. officinalis</i> (AOW)	2x	4	3	6	4	2	2	6	3	3	2	3	1	39	3.3
<i>A. prostratus</i> (APB)	4x	6	8 (2)	7 (1)	6	3	3	6	3	3	3	3	1	52 (3)	4.3
<i>A. maritimus</i> (AMA)	6x	12 (3)	12	8 (1)	7	8 (2)	11 (1)	8	4 (1)	5	2	5	2	86 (8)	7.2
<i>A. machrorrhizus</i> (AMC)	12x	13	1	10	3	5 (1)	9 (1)	5 (2)	3 (1)	3	1 (1)	4 (1)	1	69 (7)	5.8
<i>A. pseudoscaberr</i> (APS)	6x	11	8	5	6	6	6	8	3	1	3	3	1	61	5.1
<i>A. tenuifolius</i> (ATU)	2x	2	3	2	3 (3)	1	2	2	1	2 (1)	1	1	1	21 (4)	1.8
<i>A. albus</i> (AAB)	2x	2 (1)	3 (2)	–	7 (1)	3	4	4 (2)	5	3	–	1	2 (2)	34 (8)	3.4
Total^a		22 (4)	32 (4)	31 (4)	41 (16)	19 (4)	26 (8)	31 (8)	14 (4)	10 (1)	8 (2)	10 (1)	4 (2)	248 (63)	
PIC		0.930	0.960	0.948	0.959	0.886	0.936	0.951	0.829	0.853	0.798	0.845	0.324		

The number of specific alleles is shown in brackets

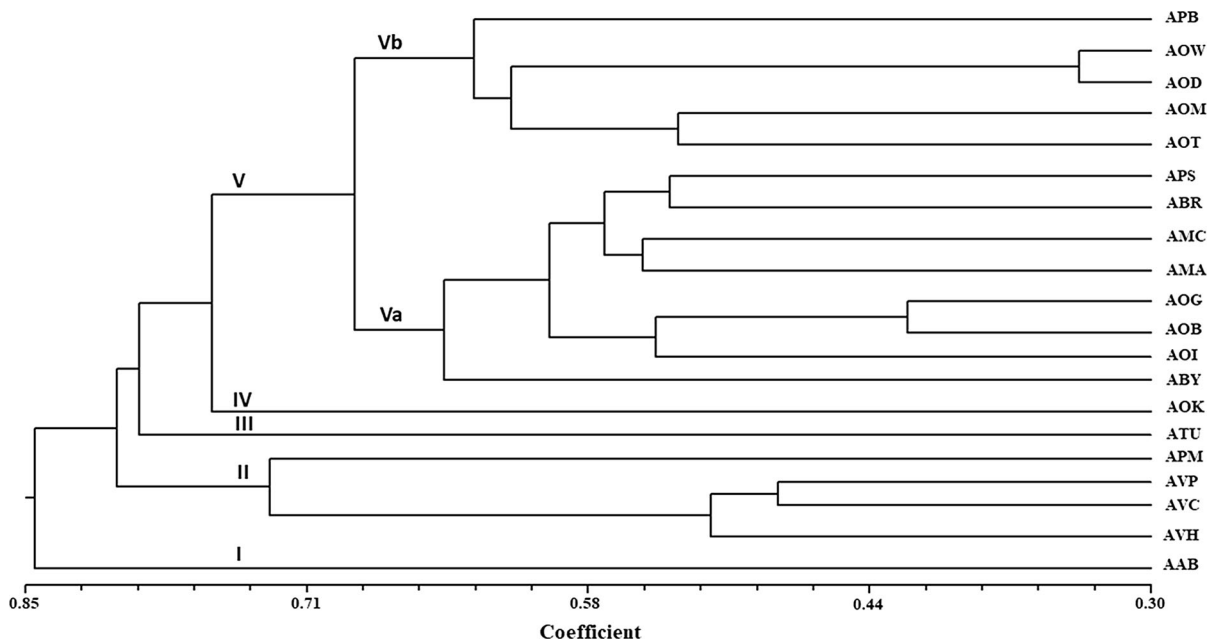


Fig. 1 UPGMA dendrogram obtained from cluster analysis of 20 asparagus accessions (37 bulks of plants) based on Dice dissimilarity index matrix obtained using the alleles from 12 EST-SSR markers

The group formed by *A. verticillatus* L. and *A. persicus* Baker (group III) was the most genetically distant from the ‘*officinalis* group’. This result could explain the unsuccessful crosses between *A. verticillatus* L. and *A. officinalis* reported up to now (Ito et al. 2008). To our knowledge, there is no information regarding the crossability between *A. persicus* Baker and *A. officinalis*. Resistance to *Stemphylium vesicarium* Wallr. has been reported in *A. verticillatus* L. (Kanno and Yokoyama 2011). Hence, a crossability study including *A. persicus*, *A. verticillatus* and diploid *A. officinalis* could be interesting in order to find out whether it is possible to transfer the resistance genes into the diploid current cultivars.

According to our results, *A. tenuifolius* Lam. (group III) seems to be closer to the ‘*officinalis* group’ in terms of evolution. Successful crosses between *A. tenuifolius* Lam. and *A. officinalis* were early reported by Bozzini (1963). Group IV is the closest to the ‘*officinalis* group’ and is formed by *A. officinalis* wild population (AOK). This accession shows a vining growth habit and has a different morphology compared with the rest of the group V (Mousavizadeh et al. 2018). Successful crosses between wild *A. officinalis* (AOK) and cultivated asparagus have been obtained by our research group (unpublished data).

The genetic relationships among accessions obtained in this study agree with previous genetic variability studies that used rDNA or cpDNA markers to analyze *A. officinalis*, *A. verticillatus* L., *A. maritimus* (L.) Mill. or *A. pseudoscaberr* Grec. species, among other *Asparagus* spp. (Stajner et al. 2002; Ito et al. 2008; Fukuda et al. 2005; Kubota et al. 2012).

Several studies have pointed out the existence of wild and naturalized populations of *A. officinalis* (Stutervant 1919; Geoffriau et al. 1992; Mousavizadeh et al. 2015; Sarabi et al. 2010; Melyan et al. 2016). Besides, different species closely related to the crop distributed across different parts of Europe or Asia have been also described (Valdes et al. 1980; Kay et al. 2001; Komarov et al. 1935; Xinqi and Tamanian 2000). To date, there is no general agreement in the taxonomic classifications of the CWR species of *A. officinalis*. Some of them have been considered like subspecies of *A. officinalis*. In breeding, it is important to know the possibility of transferring genes from CWR to the crop. In this sense, Harlan and de Wet (1971) proposed a classification system for cultivated plants and its related species assigning taxa to primary, secondary, and tertiary pools. Because the polyploid nature of some accessions in the ‘*officinalis* group’, these could be classified in the secondary pools.

Our findings support the results from previous studies performed by our group in which the European and Asian accessions were analyzed separately (Castro et al. 2013; Mousavizadeh et al. 2018). In this study, we have used both groups of accessions and a higher number of markers providing a clearest insight into the genetic relationships within the *Asparagus* subgenus. Most accessions are currently growing in a field ex-situ collection of asparagus genetic resources that is being used in our asparagus breeding program at the University of Cordoba (Spain). To create highly diverse introgressed populations, as proposed by Prohens et al. (2017), the specific alleles found in our study could be helpful to verify the hybrid origin of plants derived from crosses between wild relatives and the crop. The results obtained in the current study may provide useful information to design new crosses aimed to develop new asparagus germplasm or pre-breeding populations.

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Declarations

Conflict of interest The authors declare no conflict of interest.

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