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# Phylogeny relationship among commercial and wild pear species based on morphological characteristics and SCoT molecular markers

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ABSTRACT

Pear is an important temperate fruit worldwide containing different species, but there is controversial classification regarding different species and cultivars in this genus. Start Codon Targeted (SCoT) molecular markers and morphological attributes were used to evaluate phylogenetic relationship and population structure of 30 accessions from four species of *Pyrus* in Iran. Morphological attributes showed high variation among different studied samples and classification based on these traits clearly separated different species. Twelve SCoT primers were used to assess genetic relationship of pear accessions. Eighty-three fragments were produced, of which 78 (95.90%) were polymorphic. High level of genetic diversity was observed among the studied samples. The mean values of PIC and RP indices were 0.58 and 2.85, respectively, indicating usefulness of these markers for genetic analysis in *Pyrus* spp. UPGMA clustering based on SCoT markers separated the accessions into two major clusters with four main sub-clusters mainly according to the species' sources. Similar to results of principal coordinate analysis (PCoA), STRUCTURE analysis showed K = 3 as the optimum number of groups and completely separated the members of *P. communis* and *P. pyrifolia*, while wild samples from two species of *P. syriaca* and *P. glabra* grouped together. The results indicated that *syriaca* and *glabra* species were the most closely related, whereas *pyrifolia* was relatively distant from other studied species. Some private alleles were identified in *P. pyrifolia* and *P. communis* species that will be important for identification of these species and marker based selection studies. In addition, high level of association was observed between some of the produced fragments and fruit traits that would be of great importance for sequence characterized amplification regions (SCAR) development and markers assistant selection. Altogether, our results indicated SCoT markers are good source for *Pyrus* genetic studies and are useful tools for more precise classification of different members in this genus and can shed light to assign controversial species and cultivars in the relevant species.

## 1. Introduction

Pear (*Pyrus* spp.), one of the most important commercial fruit crop available worldwide, is a member of Rosaceae family. According to recent phylogenic studies, *Pyrus* along with other pome bearing genera (*Malus* and *Cydonia*) are classified in Maloideae subfamily (Silva et al., 2014). The genus *Pyrus* contains at least 22 widely recognized species, all indigenous to Asia, Europe and the mountainous area of North America, (Wu et al., 2013). The origin and domestication centers of the genus *Pyrus* spanned from China, Central Asia, the Middle East and Asia Minor (Vavilov, 1992). It has been though that inter and intra specific hybridization is the main fashion of pear evolution and species identification is somehow problematic due to the high cross-ability among different samples (Ferradini et al., 2017). As a result, there are various types of wild samples that are intermediate and species allocation is problematic and controversial. Therefore, in some cases, it is very difficult to estimate genetic relationships of pear accessions by using phenotypic markers solely. Moreover, although implementation of morphological characterization is easier than molecular studies and does not require complicated technologies, large land areas are often required for these experiments, making it possibly more expensive than molecular evaluation. In addition, morphological attributes are often susceptible to phenotypic plasticity; conversely, this allows evaluation of diversity in the presence of environmental variation (Mondini et al., 2009).

DNA markers served as versatile tools in plant molecular studies such as characterization of germplasm, identification of origins of different cultivars, detection of duplications and assessing genetic

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diversity, cultivar discrimination as well as phylogenetic studies (Zamani et al., 2010; Wolko et al., 2015; Guo et al., 2016; Zarei et al., 2017). Genetic variability of *Pyrus* and several other related species has been assessed using different traditional DNA markers such as RAPD, ISSR, AFLP, RBIP and SSR (Wunsch and Hormaza, 2007; Erfani et al., 2012; Kim et al., 2012; Wu et al., 2014; Zarei et al., 2017). Among different molecular markers, SSRs have been used more frequently, mainly due to the high polymorphism, reproducibility and its codominant nature. However, high mutation rate, stutter bands, and low association with coding region somehow limit the utilization of this type of markers for specific purposes (Weber and Wong, 1993). Start Codon Targeted (SCoT) polymorphisms are among the promising molecular markers newly emerged as a result of availability of EST sequencing data in the recent years (Collard and Mackill, 2009). These types of markers are based on the short conserved region surrounding the start codon (ATG) of genes and using long primers with high annealing temperature (50 °C) that enhance its reproducibility. Simplicity of this approach makes it suitable for utilization in most molecular biology labs. SCoT markers proved their usefulness in different aspects of plant studies including cultivar identification, evaluation of population structure, quantitative trait loci (QTL) mapping, association analysis of marker/trait and DNA fingerprinting in various plants (Gorji et al., 2012; Mulpuri et al., 2013; Cabo et al., 2014; Zhang et al., 2015; 2016;Yan et al., 2016).

Due to the situation in the range of pear domestication areas, Iran contains different species of *Pyrus,* hence contains a rich gene pool of native *Pyrus*. In addition, there are many pear introductions from other regions, some of which were cultivated in the vicinity of native accessions and hybridized with them; resulted in many naturally seed propagated genotypes. The objectives of this investigation were to characterize commercial and wild pears germplasm through morphological and SCoT markers, and to evaluate the efficiency of SCoT markers for providing insights into phylogenetic relationships among different *Pyrus* species and to clarify the genetic divergence between Iranian native and introduced accessions.

## 2. Materials and methods

### 2.1. Plant materials and morphological study

Thirty accessions including four Japanese pears (*P. pyrifolia*), 12 European pears (*P. communis*), seven wild samples from *P. syriaca* and seven wild accessions from *P. glabra* were evaluated based on morphological characteristics and SCoT molecular markers (Table 1, Fig. 1). The sampling distance between the wild pear accessions and Khoj samples (seedling from *P. communis*) was at least 1000 – 1500 m. Leaf and fruit samples of each accession were collected randomly from various parts of trees at full ripening. Thirty-five qualitative and quantitative characteristics were recorded for 30 mature fruits and leaf per accessions. Moreover, most attributes were evaluated based on UPOV descriptor (2000) for *P. communis* (Table 2). SPSS software ver. 17 (SPSS Inc., Chicago, United States, Norusis, 1998) was used to statistically analyze morphological attributes. For this purpose, the mean values of morphological traits for each sample were used for descriptive analysis and the respective cluster was constructed using Ward's method based on the distances between the individuals estimated by Euclidean coefficients.

### 2.2. DNA extraction and SCoT analysis

Genomic DNA was extracted from fresh leaves according to the protocol described by Doyle and Doyle (1987). Fifteen Start Codon Targeted (SCoT) primers were tested on three different accessions and 12 of them produced clear and reproducible polymorphic bands (Table 3). The PCR was carried out using 12 SCoT primers in a final volume of 20 µl containing 4 µl DNA template (10 ng/µl), 10 µl PCR Master Mix (SinaClon BioScience Co. Iran) containing (0.2 Units/µl Taq, 1.6 mM dNTPs, 3 mM $MgCl_2$), 1 µL primers (10 pM), and 5 µl sterile distilled water. PCR reaction was programmed as: one cycle of 5 min at 94 °C as initial denaturation, followed by 35 cycles including a denaturation step at 94 °C for 1 min, an annealing step at 53 °C for

**Table 1**
Name, code and information about sampling sites of different *Pyrus* spp.

| No. | Species | Accession type | Collection area | Latitude (N) | Longitude (E) | Altitude (m) | Fruit weight (gr) |
|---|---|---|---|---|---|---|---|
| 1 | *P. communis* | Shahak | Babol | 36° 32' 25" | 52° 40' 50" | 3.5 | 110 |
| 2 | *P. communis* | Khoj 1 | Babol | 36° 32' 25" | 52° 40' 50" | 3.5 | 129.8 |
| 3 | *P. pyrifolia* | Chojuro | Babol | 36° 32' 25" | 52° 40' 50" | 3.5 | 168 |
| 4 | *P. communis* | Shahmive | Babol | 36° 32' 25" | 52° 40' 50" | 3.5 | 144 |
| 5 | *P. communis* | Dare-Gazi | Babol | 36° 32' 25" | 52° 40' 50" | 3.5 | 156 |
| 6 | *P. communis* | Bartlett | Babol | 36° 32' 25" | 52° 40' 50" | 3.5 | 123.2 |
| 7 | *P. pyrifolia* | Shinko | Babol | 36° 32' 25" | 52° 40' 50" | 3.5 | 166.8 |
| 8 | *P. communis* | Duchess | Babol | 36° 32' 25" | 52° 40' 50" | 3.5 | 164 |
| 9 | *P. pyrifolia* | Olympic | Babol | 36° 32' 25" | 52° 40' 50" | 3.5 | 188 |
| 10 | *P. pyrifolia* | Nijisseiki | Babol | 36° 32' 25" | 52° 40' 50" | 3.5 | 162 |
| 11 | *P. communis* | Khoj 2 | Eslam-Abad | 34° 7' 39" | 46° 31' 32" | 1402 | 42 |
| 12 | *P. glabra* | Wild pear | Gahvare | 34° 21' 17" | 46° 25' 16" | 1446 | 4.8 |
| 13 | *P. syriaca* | Wild pear | Gahvare | 34° 21' 17" | 46° 25' 16" | 1446 | 14.4 |
| 14 | *P. communis* | Khoj 3 | Gahvare | 34° 21' 17" | 46° 25' 16" | 1446 | 94 |
| 15 | *P. syriaca* | Wild pear | Karand | 34° 17' 36" | 46° 13' 34" | 1860 | 32 |
| 16 | *P. communis* | Khoj 4 | Gahvare | 34° 21' 17" | 46° 25' 16" | 1446 | 78 |
| 17 | *P. communis* | Khoj 5 | Gahvare | 34° 21' 17" | 46° 25' 16" | 1446 | 87 |
| 18 | *P. syriaca* | Wild pear | Karand | 34° 17' 36" | 46° 13' 34" | 1860 | 27 |
| 19 | *P. syriaca* | Wild pear | Karand | 34° 17' 36" | 46° 13' 34" | 1860 | 18 |
| 20 | *P. syriaca* | Wild pear | Dalaho | 34° 16' 47" | 46° 14' 23" | 1545 | 21 |
| 21 | *P. glabra* | Wild pear | Gahvare | 34° 21' 17" | 46° 25' 16" | 1446 | 5 |
| 22 | *P. syriaca* | Wild pear | Ilam | 33° 38' 61" | 46° 25' 16" | 1388 | 18 |
| 23 | *P. glabra* | Wild pear | Dalaho | 34° 16' 47" | 46° 14' 23" | 1545 | 6.87 |
| 24 | *P. communis* | Beiroti | Eslam-Abad | 34° 7' 39" | 46° 31' 32" | 1402 | 84 |
| 25 | *P. glabra* | Wild pear | Gahvare | 34° 21' 17" | 46° 25' 16" | 1446 | 6 |
| 26 | *P. syriaca* | Wild pear | Ilam | 33° 38' 61" | 46° 25' 16" | 1388 | 19.5 |
| 27 | *P. glabra* | Wild pear | Ilam | 33° 38' 61" | 46° 25' 16" | 1388 | 10 |
| 28 | *P. glabra* | Wild pear | Ilam | 33° 38' 61" | 46° 25' 16" | 1388 | 8.5 |
| 29 | *P. glabra* | Wild pear | Ilam | 33° 38' 61" | 46° 25' 16" | 1388 | 12 |
| 30 | *P. communis* | Khoj 6 | Ilam | 33° 38' 61" | 46° 25' 16" | 1388 | 89 |