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Characterization of Tomentosa cherry (*Prunus tomentosa* Thunb.) genotypes using SSR markers and morphological traits

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ABSTRACT

A collection of 44 Tomentosa cherry (*Prunus tomentosa* Thunb.) accessions from 10 eco-geographical regions was evaluated using morphological descriptors. Ten quantitative and eight qualitative traits were analyzed and significant differences among populations were found for most traits. The highest variation observed was in fruit weight, fruit width and leaf width. *P. tomentosa* is distributed in at least five northern Chinese provinces and different accessions have their own specific morphological features. It was possible to identify several highly distinct accessions based on morphological characters. Genetic variation among the 44 Tomentosa cherries and 7 accessions from 3 related species (*P. humilis*, *P. japonica* and *P. glandulosa*) was characterized by simple sequence repeat (SSR) markers developed from peach, sweet cherry, sour cherry and apricot. Forty-four out of 110 SSR markers (40%) could be transferred to Tomentosa cherry and the other 3 species. A total of 250 alleles were detected with an average of 5.68 alleles per locus and an average polymorphism information content of 0.52. The results demonstrated the cross-species transferability of SSR primers developed in cultivated species to wild species in *Prunus* for the discrimination of different genotypes. Unweighted pair group method with arithmetic average (UPGMA) analysis of SSR data clustered the *P. humilis*, *P. japonica* and *P. glandulosa* into one group and the accessions of *P. tomentosa* into another parallel group with strong bootstrap support (93–100%). Within *P. tomentosa*, the weeping accessions formed a subgroup and the rest formed several groups that generally reflected their geographical origins.

1. Introduction

Prunus tomentosa Thunb. ($2n = 16$) belongs to the *Prunus* subgenus *Lithocerasus* in the family Rosaceae (Rehder, 1940; Ingram, 1948). *P. tomentosa* is native throughout temperate regions in China and the local people have been consuming its fruits for more than 2000 years (Yü and Li, 1986). The Tomentosa cherry adapts to diverse environments, with an extensive germplasm resource for domestication and improvement. It is a very cold tolerant species and can endure temperatures as low as −40 °C. Its fruits are resistant to rain cracking and ripen synchronously. Fruits are also rich in vitamins and other antioxidative compounds, such as carotene, vitamins B_1, B_2, C, D, E and niacin (Gao et al., 2000). It has the potential to be cultivated as an ornamental shrub or for fresh fruit production in harsh and cold areas. It may also be used to improve other *Prunus* species as a gene donor because the genus is capable of wide hybridization. Seed lots were taken to America where it is known as Nanking cherry. Limited breeding has been carried out in the USA and the former Soviet Union to produce cultivars and rootstocks for various *Prunus* species (Kash, 1989). A breeding program on Tomentosa cherry in China started recently and several cultivars such as 'Jixiang', an ornamental weeping type and a white fruit type were released for commercial production, however, they are direct selections from naturally pollinated populations. Although it has been widely used as rootstock for peaches, plums and sweet cherries, the species has not been developed into a commercial fruit crop worldwide. It still remains in the realm of home gardeners in North America and local markets in Asia. The potential of this species in the breeding of new cherry cultivars for fresh production needs further investigation. The species might be a valuable germplasm to increase cultivation in

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the colder regions of the Urals and Siberia, North America, Canada, the far east region of Russia as well as northern China. An understanding of its genetic diversity among and within the wild accessions from its natural distribution is essential for the formulation of strategies for their conservation and utilization.

Simple sequence repeat (SSR) or microsatellite markers are becoming a useful tool for genotyping, germplasm characterization and fingerprinting in many plant species because they are PCR-based, highly polymorphic, codominant, abundant and highly reproducible (Powell et al., 1996). Moreover SSR markers have been developed in almost every cultivated fruit species of *Prunus*, including peach (Cipriani et al., 1999; Testolin et al., 2000; Dirlewanger et al., 2002), apricot (Lopes et al., 2002; Messina et al., 2004), sour cherry (Downey and Iezzoni, 2000), Japanese plum (Mnejja et al., 2004), and sweet cherry (Cantini et al., 2001; Clarke and Tobutt, 2003; Struss et al., 2003). By comparison of genetic linkage maps obtained from different species, the genome structure was found to be highly conserved in *Prunus* (Joobeur et al., 2000; Yamamoto et al., 2001). This provided the possibility of transferring these developed SSR markers between related species. Primers designed for peach SSR loci have been used widely in other relatives of *Prunus* (sweet and sour cherry, plum, almond, apricot and black cherry) as demonstrated by many researchers. Downey and Iezzoni (2000) used SSR markers identified from sweet cherry, peach and sour cherry to study genetic diversity in black cherry (*P. serotina*). Lambert et al. (2004) use primers developed from peach and sour cherry to construct genetic maps of apricot cultivars (*P. armeniacea*). However, there have been no reports on the use of molecular markers to study the genetic diversity among Tomentosa cherry germplasm. The biodiversity of Tomentosa cherry and its relationship to other related species remain uncertain. Therefore, it is necessary to conduct a morphological and molecular study to understand the genetic diversity among Tomentosa cherries and their relationship with other related species.

The overall objective of this study was to investigate the existing genetic resources of Tomentosa cherry, collect different accessions and evaluate the collection by morphological and DNA studies. The specific aims were to test the transferability of SSR markers developed in other related species in Tomentosa cherry and to analyze the genetic relationships within Tomentosa cherry accessions and their relationships with several other *Prunus* species. Furthermore this research was intended to offer information on the taxonomy and geographic distribution of this important wild germplasm to facilitate its development as a crop plant in the future.

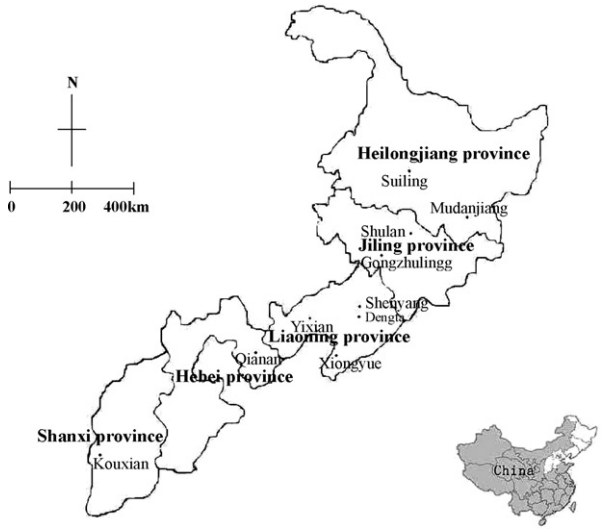

Fig. 1. Geographic location of collection sites of *P. tomentosa* accessions used in this study.

2. Materials and methods

2.1. Plant material

A total of 51 accessions (Table 1) were used in this study. All were in subgenus *Lithocerasus* of genus *Prunus* (Rehder, 1940; Ingram, 1948), including 44 accessions of *P. tomentosa*, 4 accessions of *P. humilis*, 2 accessions of *P. glandulosa* and 1 accession of *P. japonica*. The Tomentosa cherry accessions were collected from 10 locations from 5 different provinces in China. The accessions of *P. humilis* were collected from Taigu in Shanxi province. The rest were from the Arboretum of Xiongyue in Liaoning Province. The accession numbers and their geographical distributions are shown in Table 1 and Fig. 1, respectively.

2.2. Morphological characters

Characterization of vegetative material and fruits was based on almond descriptors developed by the International Plant Genetic Resources Institute (IPGRI) (Gulcan, 1985) with minor modifications. Eight qualitative traits were recorded by visual inspection of

Table 1
Plant materials used in the study and their origin

Taxa.	Collection site (County, Province)	Longitude °E	Latitude °N	Code of the accessions
P. tomentosa	Qianan, Hebei	118.73	40.01	HebeiQA(1), HebeiQB(2), HebeiQC(3), HebeiQD(4)
	Xiongyue, Liaoning	122.24	40.28	LiaoningXA(5), LiaoningXB(6), LiaoningXC(7), LiaoningXD(8), LiaoningXE(9), LiaoningXF(10)
	Dengta, Liaoning	123.34	41.43	LiaoningDA(11), LiaoningDB(12), LiaoningDC(13), LiaoningDD(14), LiaoningDE(15), LiaoningDF(16), LiaoningDG(17)
	Shenyang, Liaoning	123.57	41.82	LiaoningSA(18), LiaoningSB(19), LiaoningSC(20)
	Yixian, Liaoning	121.22	41.55	LiaoningYA(21), LiaoningYB(22), LiaoningYC(23), LiaoningYD(24)
	Gongzhuling, Jilin	124.82	43.51	JilinGA(25), JilinGB(26), JilinGC(27), JilinGD(28), JilinGE(29), JilinGF(30)
	Shulan, Jilin	126.95	44.39	Jixiang(31), JilinSA(32), JilinSB(33)
	Suiling, Heilongjiang	127.11	47.24	HeilongjiangSA(34), HeilongjiangSB(35), HeilongjiangSC(36), HeilongjiangSD(37), HeilongjiangSE(38), HeilongjiangSF(39), HeilongjiangSG(40), HeilongjiangSH(41)
	Mudanjiang, Heilongjiang	129.61	44.58	HeilongjiangMA(42)
	Kouxian, Shanxi	111.50	36.08	ShanxiKA(43), ShanxiKB(44)
P. humilis	Taigu, Shanxi	112.53	37.42	OuliA(45), OuliB(46), OuliC(47), OuliD(48)
P. japonica	Xiongyue, Liaoning	122.24	40.28	ChanggengYuli(49)
P. glandulosa	Xiongyue, Liaoning	122.24	40.28	Maili (Pink)(50)
	Xiongyue, Liaoning	122.24	40.28	Maili(White)(51)