The investigation of pellicle peelability on Japanese chestnut cultivar of ‘Yakko’ (Castanea crenata Sieb. et Zucc.)

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1. Introduction

There are four major chestnut species: Japanese chestnut (Castanea crenata Sieb. et Zucc.), Chinese chestnut (C. mollissima Bl.), European chestnut (C. sativa Mill.), and American chestnut (C. dentata Borkh.). Japanese chestnut is naturally distributed and is grown in Japan and the Korean Peninsula, and many local cultivars have been developed in Japan (Pereira-Lorenzo et al., 2012). Chinese chestnut is grown mainly in China. European chestnut is commercially grown in Europe, Asia Minor, and North Africa. American chestnut was a common species in eastern North America until the early 20th century, when it was decimated by the accidental introduction of chestnut blight (Woodroof, 1979). Japanese chestnut cultivars are believed to have been selected from wild chestnuts of Japanese origin (Kotobuki, 1994). This hypothesis is supported by the considerable genetic distance between local Japanese chestnut cultivars and Chinese chestnut accessions, as determined using amplified fragment length polymorphism markers (Yamamoto et al., 1998).

Many cultivars of Chinese chestnut and European chestnut have a pellicle that is easy to peel (hereafter, an easy-peeling pellicle: EPP). In contrast, Japanese chestnut cultivars generally have a pellicle that is difficult to peel (hereafter, a difficult-peeling pellicle: DPP), even after heating (Kikuchi, 1948; Miller et al., 1996; Pereira-Lorenzo et al., 2012; Tanaka et al., 1981). The pellicle of Japanese chestnut can be scraped away by hand using a knife, but this is laborious and costly. Thus, releasing new Japanese chestnut cultivars with EPP has been an important target for Japanese chestnut breeding, in addition to large nut size, high eating quality, and high productivity. This program started in 1947 at a national level and is currently managed by the Institute of Fruit Tree and Tea Science, National Agriculture and Food Research Organization (NIFTS). Recently, the breeding program released two Japanese chestnut cultivars with the EPP trait: ‘Porotan’ in 2006 (Saito et al., 2009) and ‘Porotan’ in 2016 (Saito et al., 2017). The area planted to ‘Porotan’ has been increasing rapidly, reaching 212 ha in 2016 (Saito et al., 2017). The EPP trait of ‘Porotan’ is controlled by a single major recessive gene: the pellicle peelability locus has been designated P/p (Tanaka et al., 2012), and a molecular marker linked to this locus was developed (Nishio et al., 2013). Today, marker-assisted selection (MAS) is
available for the EPP trait in cross-derived populations, allowing sele-
ction using large seedling populations and eliminating the need to
raise the plants until they are old enough to produce nuts, which is
laborious and time-consuming.

So far, very few genotypes (offspring, selections, or cultivars) have
been found to carry the EPP gene. This is a concern because repeated
crossing among specific genetic resources within a narrow gene pool
results in inbreeding depression, such as decreased tree vigor and
productivity, in woody fruit crops, including Japanese pear (Sato et al.,
2008) and persimmon (Yamada et al., 1994). This depression has not
yet been observed in Japanese chestnut, but based on the results for
other tree species, seems likely to develop as breeding progresses.
Outcrossing can mitigate or eliminate inbreeding depression by in-
corporating genes from accessions that are genetically distant from the
current cross parents in breeding, thus increasing genetic diversity.

Both ‘Porotan’ and ‘Porosuke’ are early-maturing cultivars, which
results in early cessation of EPP nut production in areas of cultivation
and a concentration of harvest dates within a brief period. Therefore,
the development of a mid- or late-maturing cultivar with EPP, which
would extend the season when fresh nuts are available and give farmers
more time to harvest their crops, is a current chestnut breeding target at
NIFTS. Kotoebuki et al. (1984) suggested that nut harvest time is con-
trolled by quantitative trait loci (QTLs), and Nishio et al. (2017) de-
tected QTLs for nut harvesting date. Thus, we wish to identify later-
ripening Japanese chestnut accessions with some level of EPP as cross
parents for the breeding of mid- or late-maturing cultivars.

In books published about a century ago, Nakaoka (1913), Yagioka
(1915), and Tanaka (1933) described local Japanese chestnut cultivars
having EPP on the basis of their observations, but they did not report any
test results. This suggests that some unidentified EPP genotypes
might exist among Japanese chestnut genetic resources, including the
local cultivars mentioned in those books. Our previous study suggested
the possibility of breeding novel EPP cultivars by crossing among DPP
accessions with relatively easily peeled pellicles (Takada et al., 2017).
Thus, it is necessary to identify accessions with relatively high pellicle
peelability for breeding novel EPP cultivars. The objective of this study
was to discover Japanese chestnut accessions with the EPP trait or with
relatively high pellicle peelability by surveying 59 Japanese chestnut
accessions that were not included our previous study (Takada et al.,
2017).

2. Materials and methods

2.1. Pellicle peelability of 51 local cultivars and 8 wild individuals

We tested a total of 59 Japanese chestnut accessions, consisting of
51 local cultivars and 8 wild individuals, and used ‘Porotan’ as the
standard for the EPP trait (Table 1). We grew one tree per accession
at NIFTS, in Tsukuba, Ibaraki (36°02ʹN, 140°05ʹE), Japan. The pellicle
peelability of each accession was evaluated in either 2004 or
2007 (Table 1). All trees were grown following standard cultural
techniques used in commercial production in Japan.

The harvest day for each accession was the first day that ≥10 nuts
In 2007, it ranged from 22 August for ‘Hassaku’, ‘Tanabata’, and
‘Toyotamawase’ to 17 October for ‘Choubei’ and ‘Shiomokatsugi’. Among
the 33 accessions harvested in 2004, 24 were harvested again in 2007.
The average harvest day of these 24 accessions was 18 September in
2004 and 27 September in 2007. Although there was a difference of
about 10 days in mean harvest day between the two years, the relative
maturities of the accessions were similar in each of the two years. Nuts
were harvested after the bur opened and were then stored at 5 °C for 1
month.

Ten nuts per accession were randomly used to evaluate pellicle
peelability. For accessions harvested in both 2004 and 2007, peelability
was assessed only in 2004. After the shells were removed, the nuts were
fried in canola oil at 190 °C for 2 min (the high-temperature oil peeling
[HOP] method; Shoda et al., 2006). The pellicle peelability of each nut
was then determined by means of hand-peeling with a paring knife and
was scored by visual estimation of the percentage of the surface area
that peeled away without scraping (“peeling rate”), on a scale graded in
10% increments, where “0%” represents 0%, “5%” represents
0% < and ≤10%, “15%” represents 10% < and ≤20%, ... “85%” re-
presents 80% < and ≤90%, and “95%” represents 90% < and ≤100%
(Takada et al., 2017). Pellicle peelability was quantified as the average
peeling rate of 10 nuts per genotype evaluated (APR; %). The accessions
with APR values ≥75% were classified as EPP; those with APR < 75%
were considered DPP.

2.2. Inheritance of pellicle peelability of ‘Yakko’

As described in Results, ‘Yakko’ had an exceptionally high APR
value relative to the other accessions, suggesting that it has a major EPP
gene. To test whether the mode of inheritance of pellicle peelability of
‘Yakko’ was the same as that of ‘Porotan’, we examined the segregation
ratio of pellicle peelability among F1 seedlings of crosses made using
‘Yakko’ as a parent. We crossed ‘Porotan’ (p/p) × ‘Yakko’ in 2006 and
2010, and ‘Tanzawa’ (P/p) × ‘Yakko’ in 2005 and 2006. ‘Tanzawa’ was
previously shown to be heterozygous for the p allele found in ‘Porotan’
(Takada et al., 2012; Nishio et al., 2013). Two-year-old offspring
were planted in a space of 2 m × 5 m in the NIFTS orchard. Nuts were har-
vested from each seedling of ‘Tanzawa’ × ‘Yakko’ in 2011 and of
‘Porotan’ × ‘Yakko’ in 2013 after the bur opened and stored at 5 °C for
1 month. Ten nuts from each seedling were randomly evaluated for
pellicle peelability by the HOP method as described in section 2.1. As
above, seedlings having average APR values of ≥75% were regarded as
EPP. The segregation ratio of pellicle peelability for the seedlings of
‘Tanzawa’ × ‘Yakko’ was tested by the chi-square goodness-of-fit test
for the hypotheses of a 1:1 segregation ratio.

2.3. Association between pellicle peelability and genotype estimated by
simple sequence repeat markers

Because ‘Yakko’ had an exceptionally high APR value, similar to
that of ‘Porotan’, we hypothesized that both cultivars had the same p/p
genotype. Thus, we estimated the pellicle peelability genotypes of F1
seedlings derived from ‘Tanzawa’ (P/p) × ‘Yakko’ (described in section
2.2) by determining which allele from ‘Tanzawa’ was present in each
seedling. Two simple sequence repeat (SSR) markers closely linked to
the P/p locus of ‘Tanzawa’ (PRB28 and PEB62; Nishio et al., 2013)
were used to genotype each seedling.

Genomic DNA was extracted from young leaves or young buds using
dNAeasy Plant Mini Kit (Qiagen, Hilden, Germany) according to the
manufacturer’s instructions. Polymerase chain reaction products were
separated and detected with a 3130xl Genetic Analyzer (Life
Technologies, Carlsbad, CA, USA). The size of each amplified band was
determined by comparison with a set of internal standard DNA frag-
ments (400HD-ROX, Life Technologies) in GeneMapper v. 5.0 software
(Life Technologies).

2.4. Haplotype structure around the P/p locus of ‘Yakko’ and ‘Porotan’

To determine the haplotype structure around the P/p locus of
‘Yakko’ and ‘Porotan’, we investigated an F1 population derived from
‘Porotan’ × ‘Yakko’ (described in section 2.2). Genomic DNA was ex-
tracted as described in section 2.3. The seedlings were genotyped using 10 SSR
markers associated with the P gene locus (PEA18, PEA41, PEB62,
PEBI02, PRA51, PRB25, PRB28, PRD2, PRD52, PRD58; Nishio et al.,
2013). The size of each amplified band was determined as described in
section 2.3. The order and spacing of the markers were obtained from
Nishio et al. (2013).