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

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Abstract

Japanese chestnuts (*Castanea crenata* Sieb. et Zucc.) generally have difficult-peeling pellicles even after heating, making easy-peeling pellicle (EPP) an important breeding target. Recently, EPP cultivars ‘Porotan’ and ‘Porosuke’ were released by a government-funded breeding program. However, very few genotypes carry the major recessive gene responsible for the EPP trait, resulting in inbreeding within a narrow gene pool. To discover other genetic materials having the potential for EPP breeding, we evaluated the pellicle peelability of 59 accessions (51 Japanese local cultivars and 8 wild individuals) by using the high-temperature oil peeling method. We discovered that ‘Yakko’ had an exceptionally high pellicle peelability score (87%), close to that of ‘Porotan’ (94%). The results of segregation ratio analysis of pellicle peelability and genotype prediction by simple sequence repeat (SSR) markers among F_1 seedlings suggested that the EPP alleles of ‘Porotan’ and ‘Yakko’ are at the same locus. However, a haplotype structure analysis of the EPP genome region with SSR markers revealed that both haplotypes of ‘Yakko’ differed from those of ‘Porotan’, suggesting that the EPP gene of ‘Yakko’ had a different origin from that of ‘Porotan’ or was inherited from a common ancestor many generations ago.

Keywords: Japanese chestnut, Breeding, Haplotype, Inbreeding, Pellicle peelability

Highlights

- Pellicle peelability was evaluated in 59 Japanese chestnut accessions.
- The goal was to detect accessions with easily peeled pellicles.
- The pellicle peelability of ‘Yakko’ was exceptionally high.
- Peelability of ‘Yakko’ and ‘Porotan’ appears to be controlled by the same locus.

Abbreviations

APR, average peeling rate; DPP, difficult-peeling pellicle; EPP, easy-peeling pellicle; HOP, high-temperature oil peeling; MAS, marker-assisted selection; NARO, National Agriculture and Food Research Organization; NIFTS, Institute of Fruit Tree and Tea Science, NARO; QTLs, quantitative trait loci; SSR, simple sequence repeats

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 ‘Porosuke’ in 2016 (Saito et al., 2017). The area planted to ‘Porotan’ has been increasing rapidly, reaching 212 ha in 2014. The EPP trait of ‘Porotan’ is controlled by a single major recessive gene: the pellicle peelability locus has been designated *P*/*p* (Takada 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 selection 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 incorporating 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. Kotobuki et al. (1984) suggested that nut harvest time is controlled by quantitative trait loci (QTLs), and Nishio et al. (2017) detected 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°02′56″N, 140°05′56″E), 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 could be harvested. In 2004, it ranged from 25 August for ‘Yamaguchiwase’ to 6 October for ‘Daihachi’, ‘Katayama’, and ‘Kinshiu’. In 2007, it ranged from 22 August for ‘Hassaku’, ‘Tanabata’, and ‘Toyotamawase’ to 17 October for ‘Choubei’ and ‘Shimokatsugi’. 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%” represents 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 F_1 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 harvested 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 F_1 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 a DNeasy 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 fragments (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 F_1 population derived from ‘Porotan’ × ‘Yakko’ (described in section *2.2*). Genomic DNA was extracted as in section *2.3*. The seedlings were genotyped using 10 SSR markers associated with the *P* gene locus (PEA18, PEA41, PEB62, PEB102, 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).

The fragment sizes of SSR markers PRA51 and PRB25 were 19 bp and 20 bp larger, respectively, than those reported previously (Nishio et al., 2013). These size differences are explained by a change in the forward primers from M13-tailed primers (Schuelke, 2000) to fluorescently labeled primers. In addition, since the DNA sequencer was changed from a PRISM 3100 DNA sequencer (Applied Biosystems, Carlsbad, CA, USA) to a 3130xl Genetic Analyzer, a difference of 1 bp was found in some markers (PRD2, PRB28, PEB62, PRD58) relative to the results of Nishio et al. (2013).

3. Results

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

The APR of the 59 accessions and 'Porotan' ranged from 7.0% in 'Katayama' to 94.0% in 'Porotan' (Table 1). The frequency distribution of the APR values was continuous in the 58 accessions with APR < 75% (Fig. 1). 'Porotan' and 'Yakko' had exceptionally high APR values, which were discontinuous with those of the other accessions (Fig. 1). The APR value of 'Yakko' was 87.0% and close to that of 'Porotan', and only these two cultivars were classified as EPP. The mean APR value of the 58 DPP accessions was 43.3%. Among the DPP accessions, only 'Otomune' (72.0%), 'Fukunami' (71.0%), and Shibaguri-166 (71.0%) had APR values of ≥ 70% (Table 1).

3.2. Inheritance of pellicle peelability of 'Yakko'

In a population of 16 F_1 offspring of the cross of 'Porotan' (p/p) × 'Yakko', pellicle peelability segregated in a ratio of 14 EPP to 2 DPP (Fig. 2). The two DPP offspring had APR values of 59.0% and 61.0%. This segregation ratio was close to the expected ratio of 1:0 for the progeny of parents homozygous for recessive alleles at the same locus. In a population of 17 F_1 offspring from 'Tanzawa' (P/p) × 'Yakko', pellicle peelability segregated in a ratio of 6 EPP to 11 DPP (Fig. 2). The hypothesis of 1:1 segregation, as expected from a cross of a heterozygous parent by a homozygous recessive parent, was not rejected at $P < 0.05$.

3.3. Association between pellicle peelability and genotype estimated by SSR markers

On the assumption that 'Yakko' has the same *p*/*p* genotype as 'Porotan', we estimated the genotypes of 17 F_1 offspring from Tanzawa' × 'Yakko' by using SSR markers PRB28 and PEB62 and then compared the estimated genotypes with the APR scores. The segregation was estimated as 7 offspring with the *p*/*p* genotype and 10 with the *P*/*p* genotype, including one recombinant genotype that was judged to be *P*/*p* owing to its very low APR value (3%). When these data were compared to the phenotypes, 6 of the 7 seedlings estimated as having the *p*/*p* genotype had APR ≥ 75% and were classified as EPP (Fig. 3). The remaining seedling estimated as having the *p*/*p* genotype had APR = 60.0% and was classified as DPP. All 10 seedlings estimated as having the *P*/*p* genotype had APR < 29% and were classified as DPP (Fig. 3). The mean APR values were 17.4% for the estimated *P*/*p* genotypes versus 83.7% for the estimated *p*/*p* genotypes, indicating that the pellicle peelability of seedlings with the *p*/*p* genotype (as estimated by SSR) was much higher than that of seedlings with the *P*/*p* genotype.

3.4. Haplotype structure around the P/p *gene locus of 'Yakko' and 'Porotan'*

Overall, the two haplotypes of SSR markers around the *P*/*p* locus of 'Yakko' showed different structures from those of 'Porotan' (Fig. 4). In the region between markers PEB62 and PEA41, all of which lie on the same side of the *P*/*p* locus, one haplotype of 'Yakko' showed the same structure as both haplotypes of 'Porotan'. However, on the other side of the *P*/*p* locus, between markers PRD2 and PRD52, the SSR marker haplotypes differed between 'Porotan' and 'Yakko', as well as within each of the accessions.

4. Discussion

Japanese chestnut cultivars generally have DPP traits, but nearly a century ago, pellicle peelability was described in some local cultivars as EPP or relatively EPP. Cultivars ‘Shimokatsugi’ (Nakaoka, 1913), ‘Akaguri’, ‘Choubei’, ‘Imakita’, ‘Kenaga’, ‘Mikado’, ‘Shimokatsugi’, ‘Shougatsu’, ‘Wasa’ (Yagioka, 1915), ‘Akaguri’, ‘Gora’, ‘Gosha’, ‘Ideno’, ‘Terai’, ‘Teteuchi’, ‘Yakko’, and ‘Wasa’ (Tanaka, 1933) were described as EPP or relatively EPP, but these descriptions provided no supporting data. These cultivars were not grown widely, probably owing to low productivity, small nuts, or other undesirable characteristics. Ten of these cultivars (all except ‘Akaguri’, ‘Ideno’, ‘Mikado’, and ‘Wasa’) are conserved at NIFTS. At the time of the present study, ‘Imakita’ and ‘Shougatsu’ had already been classified as DPP (APR = 41.0% and 35.0%, respectively; Takada et al., 2017), so they were not retested here.

We evaluated the pellicle peelability of 7 local cultivars (‘Choubei’, ‘Gora’, ‘Gosha’, ‘Kenaga’, ‘Shimokatsugi’, ‘Teteuchi’, and ‘Yakko’) from among the 14 cultivars previously described as EPP or relatively EPP. In two cases, cultivars considered to be synonymous were used. In the first case, ‘Kenaga’, introduced by Yagioka (1915), is considered synonymous with ‘Kenagaginyose’, which is conserved at NIFTS. Similarly, ‘Teteuchi’, introduced by Tanaka (1933), seems to be synonymous with cultivar ‘Ogawateteuchi’, which is conserved at NIFTS, because both cultivars originated in Ogawa village in Hyogo prefecture. Among these cultivars, the APR values were all less than 55% except for ‘Yakko’, which was 87.0% (Table 1). Thus, only ‘Yakko’ was selected as having the EPP trait among this group of old cultivars. According to the database of NARO Genebank, this cultivar has intermediate tree vigor, intermediate nut size, and high eating quality. Since these 14 cultivars cited about a century ago included 1 EPP cultivar, another might be hidden among the 5 cultivars that were not evaluated in this study or by Takada et al. (2017). One of these, 'Terai', is included in the NIFTS collection and should be evaluated for pellicle peelability. The four cultivars that are not conserved at NIFTS will have to be acquired by exploration.

In our previous study (Takada et al., 2017), the high and continuous variation of the APR values among the DPP accessions suggested the existence of quantitative gene effects on pellicle peelability. This indicates the possibility of developing novel EPP cultivars by accumulating QTLs for more easily peeled pellicle among the DPP accessions with relatively high APR values. In a previous study, 70.0% (Shibaguri-37) was the highest APR value among the DPP accessions (Takada et al., 2017). In the present study, 'Fukunami', 'Otomune', and Shibaguri-166 had APR ≥ 70%, like Shibaguri-37. These accessions can be considered as cross parents for attempts to breed novel EPP cultivars. To develop such cultivars efficiently, it will be necessary to clarify the mode of inheritance and to perform QTL analysis on the pellicle peelability of these accessions.

The segregation of the APR values in the F_1 seedlings of 'Porotan' × 'Yakko', most of which were EPP, suggested that both 'Porotan' and 'Yakko' were homozygous for recessive alleles at the same locus (i.e., both *p*/*p*). As noted, however, there were two exceptional DPP offspring (Fig. 2). APR values have considerable environmental and non-genetic variability (Takada et al., 2017); thus, the small sample size (10 nuts) may explain the two-exceptional offspring. The distribution of the APR values in offspring from 'Tanzawa' × 'Yakko' suggests a bimodal distribution with peaks corresponding to EPP and DPP but also showing environmental and non-genetic variability. 'Tanzawa' has genotype *P/p* (Takada et al., 2012), and the approximately 1:1 segregation in the F_1

progeny of ‘Tanzawa’ × ‘Yakko’ supports the hypothesis that ‘Yakko’ has a *p/p* genotype at the same EPP locus as in ‘Porotan’ and ‘Tanzawa’ (Fig. 2).

Consistent with the assumption that the genotype of pellicle peelability in ‘Yakko’ is *p*/*p*, the seedlings from ‘Tanzawa’ × ‘Yakko’ that inherited the *p* allele of ‘Tanzawa’ were EPP, with one exception, and all of those that inherited the *P* allele of ‘Tanzawa’ were DPP (Fig. 3). The result also shows that EPP in ‘Yakko’ is controlled by the same locus as in ‘Porotan’ (which is the same as that in ‘Tanzawa’). The SSR markers closely linked to the *p* gene of ‘Porotan’ (Nishio et al., 2013) should also able to predict the *p* gene of ‘Yakko’ (Fig. 4), although the specific marker alleles would sometimes differ. In practical use, these SSR markers would be highly effective in MAS of the pellicle peelability trait in ‘Yakko’ in addition to that of ‘Porotan’ and its relatives. The APR values were also influenced by quantitative minor effects responsible for genetic variation in DPP cultivars. The low APR values in the F_1 seedlings of ‘Tanzawa’ × ‘Yakko’ with the *P*/*p* genotype (Fig. 3) may be partly due to quantitative gene effects specific to ‘Tanzawa’ and ‘Yakko’.

The crossing data indicate that it is highly possible that ‘Yakko’ has the same *p* allele at the *P*/*p* locus as ‘Porotan’. However, the two SSR marker haplotypes around the *P*/*p* locus of ‘Yakko’ showed somewhat different structure from those of ‘Porotan’ (Fig. 4). A previous study indicated that the recessive *p* allele in ‘Porotan’ was derived from ‘Higan’, a local cultivar in Kyoto Prefecture, in central Japan (Nishio et al., 2014). ‘Yakko’ is a local cultivar from the northern part of Osaka Prefecture, which is adjacent to Kyoto Prefecture. Thus, some genetic relationship possibly exists between ‘Yakko’ and ‘Higan’, although no parent–offspring relationship was detected between them (Nishio et al. 2014). If so, the *p* allele may have originated in a common ancestor many generations ago, and the haplotypes may have changed owing to recombination over time. Another possibility is that the *p* allele of 'Yakko' arose by mutation independently from that of 'Higan' and 'Porotan'. These questions may be answered by future DNA sequencing of the EPP gene. So, it is suggested that the EPP alleles of 'Porotan' and 'Yakko' are at the same locus but the EPP gene of 'Yakko' had a different origin from that of 'Porotan' or was inherited from a common ancestor many generations ago.

Nishio et al. (2014) identified 'Yakko' and 'Kanotsume' as a parent–offspring pair. The APR value of 'Kanotsume' was 57.0% in this study, which suggests that it has the *P*/*p* genotype (assuming that 'Yakko' has the *p*/*p* genotype at this locus). In addition, 'Kanotsume' and 'Dengorou' are a parent–offspring pair. 'Dengorou' had APR = 39.0%, suggesting that it might have the *P*/*p* genotype. Thus, additional Japanese accessions may carry the *p* allele as heterozygotes, and it would be impossible to identify these genotypes only from the evaluation of pellicle peelability. Thus, it is important to discover accessions with *p* alleles by genotyping linked SSR markers among a wider range of genetic resources. For this purpose, developing markers tightly linked to the *P/p* locus and identifying *p* alleles of different origins will be necessary.

Since the development of 'Porotan', only 'Higan' and its relatives have been used at NIFTS for breeding of Japanese chestnut with the EPP trait. Repeated crossing among these genetic resources will cause inbreeding, leading to depression of tree vigor and productivity. Our haplotype structure analysis revealed that both EPP haplotypes from 'Yakko' differ from those of 'Porotan', so their EPP alleles may have different origins or an old common ancestor. Additionally, no parent–offspring relationships were detected between 'Yakko' and either 'Porotan' or its ancestral cultivar 'Higan' (Nishio et al., 2014). Thus, 'Yakko' and its relatives will be effective as cross parents for avoiding inbreeding depression risks arising from repeated use of ‘Higan’ and its relatives in the Japanese chestnut breeding program at NIFTS.

Most of the accessions with the *p* gene derived from ‘Higan’ have early maturity (e.g., usually around early September). For broadening the nut harvest period of the EPP cultivars, making use of breeding materials that combine the EPP trait with later nut harvest times would be desirable. The harvest day of ‘Yakko’ was 22 September 2004 and that of its offspring ‘Kanotsume’ was 15 September 2004, both later than those of ‘Higan’ (11 September 2007) and ‘Porotan’ (4 September 2007). Also, the EPP gene region of ‘Yakko’ can be predicted by the same SSR markers as those used to detect the *p* gene of ‘Porotan’, which would enable efficient MAS of pellicle peelability in the offspring of ‘Yakko’ and its relatives. In the breeding of the EPP cultivars, ‘Yakko’ and ‘Kanotsume’ would therefore be useful as cross parents to lengthen the harvesting period while also lowering the risk of inbreeding depression.

Acknowledgements

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors. This research owes a debt to the many persons involved in Japanese chestnut breeding at NIFTS. We are sincerely grateful for their efforts.

Declaration of Interest

The authors declare no conflict of interest.

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Fig. 1. Frequency distribution of the average peeling rate (APR; %) of 10 nuts per accession evaluated by the high-temperature oil peeling method among 59 accessions and 'Porotan'. Values falling at the edges of two adjacent bins were classified into the lower bin (e.g., an APR of 15% would be classified into the "10–15" bin).

Fig. 2. Frequency distribution of the average peeling rate (APR; %) of 10 nuts per offspring evaluated by the high-temperature oil peeling method among offspring of F_1 crosses of 'Porotan' (*p*/*p*) × 'Yakko' (left) and 'Tanzawa' (*P*/*p*) × 'Yakko' (right). EPP, easy-peeling pellicle; DPP, difficult-peeling pellicle.

Fig. 3. Scatterplot of average peeling rate (APR; %) of the two genotypes estimated by SSR analysis among 17 F_1 offspring of 'Tanzawa' (*P*/*p*) × 'Yakko'. Estimation was based on the assumption that 'Yakko' has the same *p*/*p* genotype as 'Porotan'. APR was assessed in 10 nuts per offspring by the high-temperature oil peeling method.

Fig. 4. Haplotype structure around the EPP genes in 'Yakko'. That of 'Porotan' was determined by Nishio et al. (2013). Numbers indicate allele size (bp). Genetic distances from PRD2 were inferred from an integrated map of the 550-40 × 'Tanzawa' F_1 population (Nishio et al., 2013).

Table 1

Table 1. Harvest day and average peeling rate (APR)[z] of the 60 Japanese chestnut genotypes used in these experiments.

Cultivar or accession name	JP acc. No.[y]	Origin (prefecture)	Harvest day[x]	APR (%)
Local cultivars				
‘Arima’	113832	Kanagawa	8 Sep. 2004	45.0
‘Bonguri’	113834	Japan[w]	28 Aug. 2007	38.0
‘Buzen’	113836	Oita	22 Sep. 2004	32.0
‘Choubei’	113838	Kyoto	17 Oct. 2007	25.5
‘Choukouji’	113839	Hyogo	30 Sep. 2004	29.0
‘Daihachi’	113841	Kyoto	6 Oct. 2004	30.0
‘Dengorou’	113842	Akita	22 Sep. 2004	39.0
‘Enanishiki’	113843	Gifu	28 Aug. 2007	62.0
‘Fukunami’	113844	Kyoto	25 Sep. 2007	71.0
‘Fukunishi’	113845	Osaka	15 Sep. 2004	30.0
‘Ginyose’	113849	Osaka	20 Sep. 2007	48.0
‘Gora’	113850	Hyogo	30 Sep. 2004	29.0
‘Gosha’	113851	Kanagawa	22 Sep. 2004	54.0
‘Hajikami’	113852	Japan	1 Sep. 2004	19.0
‘Hassaku’	113853	Japan	22 Aug. 2007	42.0
‘Hatayaooguri’	113854	Akita	30 Sep. 2004	24.0
‘Hayadama’	113855	Wakayama	19 Sep. 2007	50.0
‘Higan’	113856	Kyoto	11 Sep. 2007	60.0
‘Hokugin’	113857	Gifu	11 Sep. 2007	57.0
‘Ichikawawase’	176782	Kanagawa	19 Sep. 2007	38.0
‘Kanotsume’	113867	Kyoto	15 Sep. 2004	57.0
‘Kasaharawase’	113868	Gifu	11 Sep. 2007	55.0
‘Katayama’	113869	Gifu	6 Oct. 2004	7.0
‘Kenagaginyose’	113870	Osaka	8 Sep. 2004	26.0
‘Kinseki’	113872	Hyogo	22 Sep. 2004	52.0
‘Kinshiu’	113873	Tokushima	6 Oct. 2004	28.0
‘Matabei’	113876	Kyoto	30 Sep. 2004	14.0
‘Ninomiya’	176780	Chiba	30 Sep. 2004	50.0
‘Obiwase’	113878	Miyazaki	28 Aug. 2007	46.0
‘Obuse 2 gou’	113879	Nagano	2 Oct. 2007	65.0
‘Obuse 3 gou’	113880	Nagano	22 Sep. 2004	20.0
‘Ogawateteuchi’	113882	Hyogo	11 Sep. 2007	26.0
‘Ookoma’	116299	Japan	15 Sep. 2004	35.0

Cultivar or accession name	JP acc. No.[y]	Origin (prefecture)	Harvest day[x]	APR (%)
‘Osaya’	113884	Kanagawa	28 Aug. 2007	64.0
‘Otomune’	113885	Hyogo	19 Sep. 2007	72.0
‘Saimyouji 1 gou’	176786	Akita	2 Oct. 2007	24.5
‘Saimyouji 2 gou’	176787	Akita	19 Sep. 2007	18.0
‘Shimokatsugi’	113894	Osaka	17 Oct. 2007	54.0
‘Shuuhouwase’	113895	Yamaguchi	11 Sep. 2007	66.0
‘Taishouwase’	113897	Kanagawa	28 Aug. 2007	55.0
‘Tajiriginyose’	113898	Osaka	15 Sep. 2004	30.0
‘Tamanishiki’	113900	Japan	11 Sep. 2007	51.0
‘Tanabata’	113901	Shizuoka	22 Aug. 2007	58.0
‘Toyotamawase’	113907	Tokyo	22 Aug. 2007	30.0
‘Tsuchidawase’	113908	Gifu	25 Sep. 2007	35.0
‘Tsunehisa’	113910	Kanagawa	4 Sep. 2007	67.0
‘Waseginzen’	113919	Japan	1 Sep. 2004	56.0
‘Yakko’	113913	Osaka	22 Sep. 2004	87.0
‘Yamaguchiwase’	113914	Hyogo	25 Aug. 2004	41.0
‘Yamaguchiwase 2 gou’	113915	Tokushima	1 Sep. 2004	16.5
‘Yourou’	113917	Gifu	9 Oct. 2007	33.0
Wild individuals				
Sandoguri Kouchi 2	113971	Kouchi	30 Sep. 2004	63.0
Shibaguri-67	113888	Hyogo	22 Sep. 2004	39.0
Shibaguri-82	176797	Hyogo	22 Sep. 2004	56.0
Shibaguri-91	113889	Hyogo	22 Sep. 2004	57.0
Shibaguri-166	113890	Hyogo	15 Sep. 2004	71.0
Shidareguri-Gifu	113892	Gifu	15 Sep. 2004	51.0
Shidareguri-Tatsuno 2	234092	Nagano	15 Sep. 2004	42.0
Shidareguri-Tochigi	113937	Tochigi	8 Sep. 2004	56.0
Cultivar				
‘Porotan’	230435	F_1 of 550-40 × ‘Tanzawa’	4 Sep. 2007	94.0

[z] Average peeling rate (APR; %) of 10 nuts evaluated by the high-temperature-oil peeling method.
[y] Accession numbers in the National Agriculture and Food Research Organization (NARO) Genebank (http://www.gene.affrc.go.jp/index_en.php).
[x] For accessions harvested in both 2004 and 2007, the 2004 harvest day is shown. For each accession listed, APR was measured in the year indicated.
[w] Prefecture is unknown.

Figure 1
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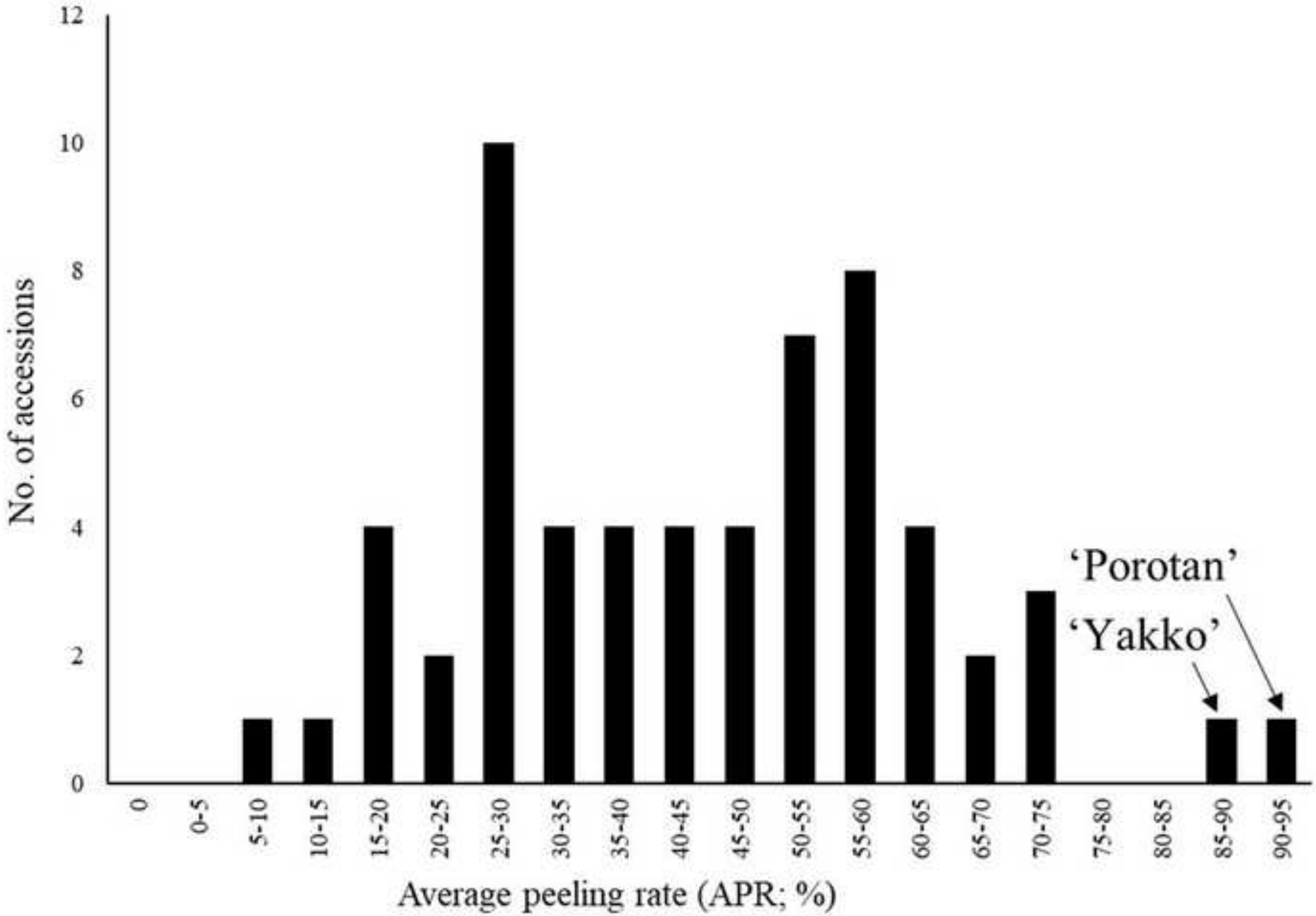

Figure 2

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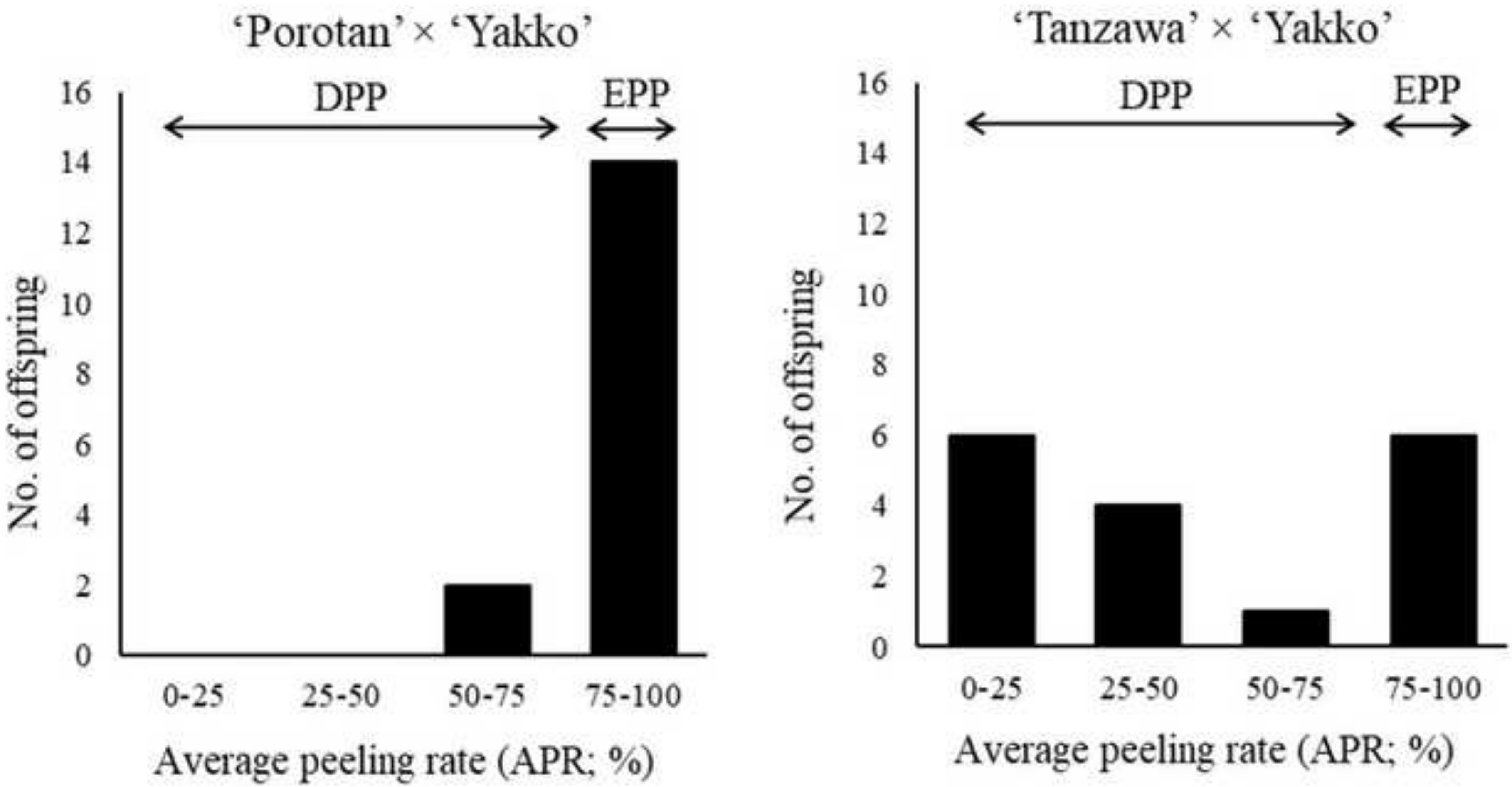

Figure 3

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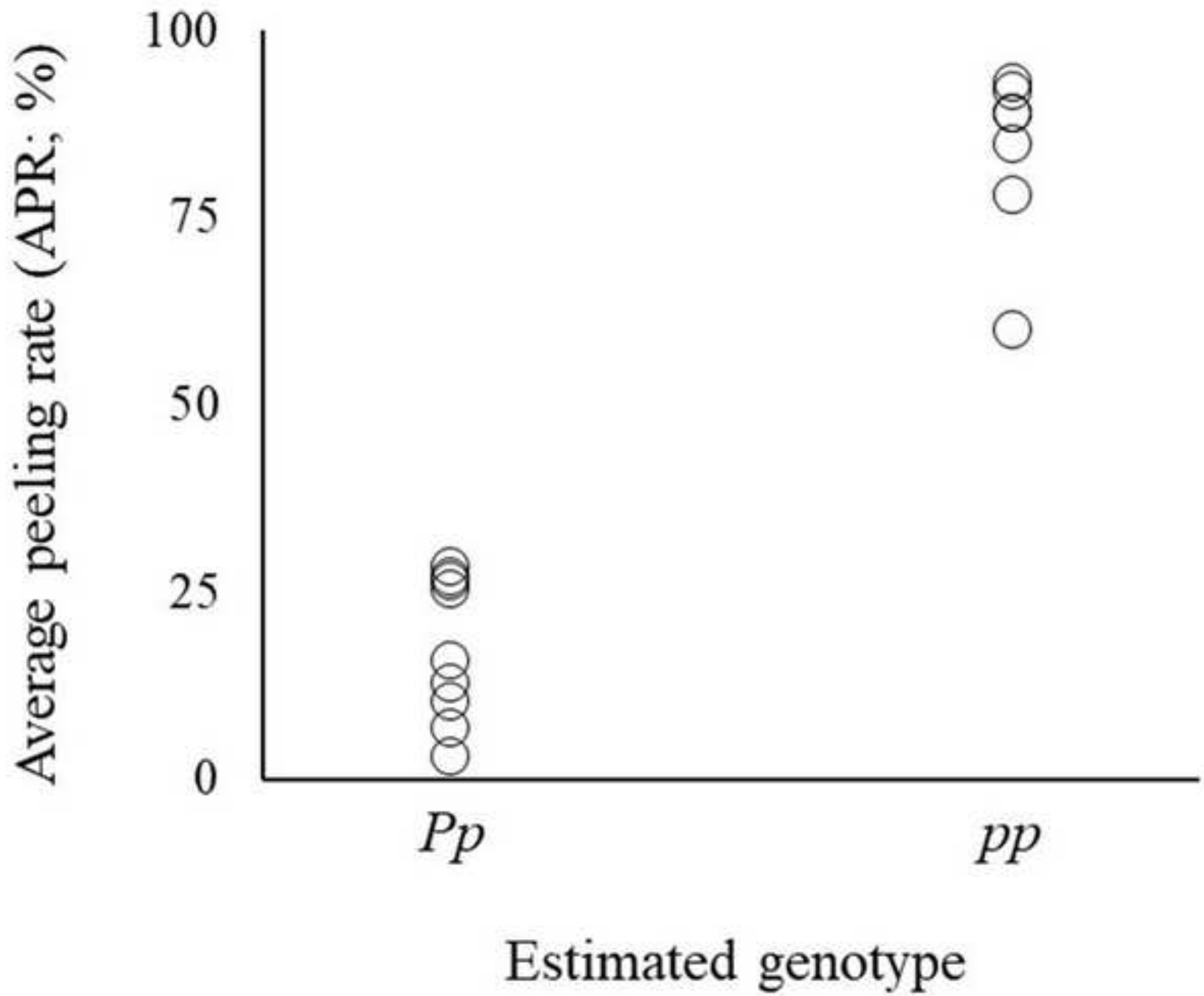

Figure 4
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	'Yakko'		'Porotan'		Genetic distance (cM)
PRD2	133	133	143	147	—
PRA51	270	270	273	268	9.6
PEA18	120	120	120	114	11.8
PEB102	171	171	176	171	11.8
PRB28	125	135	135	133	13.2
PRD52	144	144	150	150	16.6
peeling	*p*	*p*	*p*	*p*	
PEB62	169	159	169	169	19.8
PRD58	196	210	196	196	20.9
PRB25	157	157	157	157	22.3
PEA41	104	102	104	104	59.6