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Research Article

Screening of pear genotypes cultivated in Azerbaijan for resistance genes to fungal disease (*Venturia pyrina)*

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Abstract

Pear scab (*Venturia pyrina*) is one of the most dangerous pear diseases in pear orchards in Azerbaijan. Therefore, it is very important to breed scab-resistant pear varieties to minimize the use of fungicides and develop resistance to fungicides. Molecular identification could considerably upgrade pear breeding. Our study aimed to evaluate the resistance of some local pear genotypes using molecular markers against the pathogen *Venturia pyrina* cultivated in Azerbaijan. Samples of 26 pear genotypes distributed in different regions of Azerbaijan were collected. The 12 molecular markers used have proven useful in identifying resistance genes against *Venturia pyrina* in pear genotypes. As a result of our research with the help of molecular markers, it was learned that Sarchabudu, Nar pear and Zanciraband pear varieties have more resistance genes against *Venturia pyrina* pathogen. In addition, Jir pear and Uzunboghaz pear cultivars were found to have no scab resistance gene.

Keywords: Genotypes, markers, EF-PCR analysis, *Venturia pyrina*

Introduction

Pear (*Pyrus communis*) occupies a modest place (less than 36%) among the main crops envisaged in the area expansion plans for orchards despite being a highly profitable crop (2-3 times higher than the purchase price). This situation is explained by the fact that in recent decades, pear culture has been intensively affected by the pathogens of a number of diseases, including the blight caused by the fungus *Venturia pyrina* Aderh, especially the harmful

anamorph of *Fusicladium pirinum* (Sib.) Fokl (Cam et al. 2019).

Venturia pyrina Aderh., caused by the European pear scab, is one of the main diseases of pears in pear-growing areas around the world. Breeding of resistant varieties is very important for long-term control of the disease. Research on pear resistance and pathogen virulence is still limited (Sokolova et al. 2021). Pear scab caused by V. pyrina is one of the most important economic diseases of pear. This pathogen overwinters in the form of spherical pseudothecia on the remains of infected leaves. However, in areas with mild winters, they persist as mycelium or conidia in buds and branches. Symptoms of the disease on the leaves are black spots with a velvety surface. In severe and early infections, deformation and cracks are formed in the fruits. Humidity and temperature are important and influencing factors in the spread and severity of this disease. The disease will be severe in areas where the weather is cool and humid in spring and early summer. (Ebrahimi 2020). The symptoms of the lesion on the fruits are manifested by small necrosis on the lid, which merge to form a large spot. Epiphytotic infection occurs when the vegetative tissue of the plant has condensed moisture for at least 48 hours (Balykina et al. 2021).

Sustainable genes current in savage pear varieties have been included into commercial species among the genes responsible for resistance to pear scab by the traditional breeding method. Studies in various countries around the world have shown that many resistance (R) genes can be associated with resistance genes against the pathogen *Venturia pyrina* in the pear plant, and the genetic characteristics of the R gene can vary depending on the resistant host. Rvn3 was described in linkage group 6 of species relationship hybrid pear, and co-linearity was established between Rvn3 and Rvi14. A set of resistant genes against *V. pyrina* have been determined in diverse pear genotypes. The *Rvp1* gene was identified in *Pyrus communis*, the *Vnk* gene was identified in *Pyrus pyrifolia* Nakai, and the *Rvn2* gene was identified in interspecific hybrid pear [(*P. pyrifolia* × *P. ussuriensis*) × *P. communis*] (Iketani et al. 2001; Sewon et al. 2021).

In general, efforts should be made to manufacture durable varieties with a market in breeding programs. It is necessary to screen for the presence of resistance in the pear genotypes available in different regions of the country to successfully develop the breeding program in this direction. The aim of this study was to determine the presence of scab resistance in different pear genotypes grown in Azerbaijan and to identify precious cultivars for future breeding goals. It used 12 molecular markers for different scab resistance genes in the above-mentioned screening.

Materials and methods

Sampling

In this study, twenty-six (26) different pear genotypes were used for molecular screening. As shown in Table 1, 26 pear genotypes were selected in our experiment from orchards in the Guba, Gabala, Ganja, Tovuz, and Masalli regions of Azerbaijan (Babayeva, 2021a).

Table 1. Names, regions, and locations of pear accessions used in the study

No	Accessions	Region/ location	No	Accessions	Region/ location
1	Sarchabudu	Guba,Azerbaijan	14	Zanciraband	Guba, Azerbaijan
2	Yemish armud	Guba, Azerbaijan	15	Ispiya	Guba, Azerbaijan
3	Garpiz armud	Guba, Azerbaijan	16	Jir armud	Guba, Azerbaijan
4	Gorkhmazi	Guba, Azerbaijan	17	Qush armudu	Tovuz,Azerbaijan
5	Khanim armudu	Guba, Azerbaijan	18	Meshe armudu	Tovuz,Azerbaijan
6	Khirda nargila	Guba, Azerbaijan	19	Khirda sarchabudu	Tovuz,Azerbaijan
7	Nar armudu	Guba, Azerbaijan	20	Ghand armudu	Ganja, Azerbaijan
8	Jir Nadiri	Guba, Azerbaijan	21	Shushe armud	Ganja, Azerbaijan
9	Gara armud	Guba, Azerbaijan	22	Sini armud	Gabala, Azerbaijan
10	Tikani armud	Guba, Azerbaijan	23	Bal armud	Gabala, Azerbaijan
11	Gov armudu	Guba, Azerbaijan	24	Dash armud	Masalli, Azerbaijan
12	Abasbayi	Guba, Azerbaijan	25	Uzunboghaz armud	Masalli, Azerbaijan
13	Ahmadgazi	Guba, Azerbaijan	26	Boyuk uzunboghaz	Masalli, Azerbaijan

Nuclear DNA extraction and Elongation factor Polymerase Chain Reactions (EF-PCR)

Isolation of genomic DNA was performed according to the CTAB (cetylmethylammonium bromide) protocol developed by Rogers (Rogers, 1985). DNA was extracted from young leaves collected from different pear genotypes according to the CTAB protocol. DNA evaluation was performed with the help of gel electrophoresis. To determine quality and concentration, a 1% agarose gel prepared from 1xTBE (18 mM Tris-HCl, 100 mM EDTA, pH 8.0, 18 mM Boric acid), stained with 0.5 µg/ml ethidium bromide, was run at 120 V for about 20-25 minutes, and the gel images were taken in "Bio-Rad Gel Documentation" program.

For each sample used for PCR analysis, a 23 µl volume of PCR mix was prepared using 17,375 µl ddH2O, 2.5 µl 10xDream Taq Buffer (10 mM Tris-HCl, pH 8.0, 50 mM KCl, 1.5 mM MgCl2), 2 µl dNTP, 1 Using µl Forward EF Primer, 1 µl Reverse EF Primer, 0.125 µl Dream Taq Polymerase. Afterwards, 2 µl of diluted genomic DNA taken from different samples is added to the obtained PCR mixture, and the total volume is equalized to 25 µl. We put 25 µl of the mixture into a PCR device (Thermo Fisher, AB Applied Biosystem, Veriti TM 96 - Well Fast Thermal Cycler) to multiply DNA. 6 SSR primers of 20-25 bp nucleotide length and 6 RAPD primers of 10 bp nucleotide length were used for EF-PCR analysis. They are given in Table 2.

The PCR mixture placed in the PCR machine consists of 3 parts connected, starting with the initial denaturation of DNA at a temperature of 94°C for 45 seconds. The second part itself is composed of 3 stages of 94°C temperature for 30 seconds, 1 minute at the primer's temperature (50-60°C for SSR primers, 32-34°C for RAPD primers), 1 minute at 72°C temperature, and repeated for 35 cycles. Part 3 concludes with an 8-minute incubation at 72°C and cooling to 4°C. A 1.8% agarose gel prepared from 1xTBE (18 mM Tris-HCl, 18 mM Boric acid, 100 mM EDTA, Ph 8.0) solution is used to check the quality of the obtained PCR product. 10 µl of each PCR product from the PCR machine was transferred to the gel, and electrophoresis was started at 90 V for 120 minutes. The evaluation was performed using Bio-Rad software, and the quality of nuclear DNA was studied based on the samples obtained.

Table 2. Characteristics of molecular markers used for amplification of pear scab resistance genes

Marker Name	Resistance Genes	Marker Type	Size of Allele (bp)	Primer Sequence (5′→3′)	Linkage group	References
CH02b10	*Rvi2* *Rvi4* *Rvi15*	SSR	113-159	F: CAAGGAAATCATCAAAGATTCAAG R: CAAGTGGCTTCGGATAGTTG	2	Liebhard et al. 2002
CH03d10	*Rvi4* *Rvi15*	SSR	166-182	F: CTCCCTTACCAAAAACACCAAA R: GTGATTAAGAGAGTGATCGGGG'	2	Liebhard et al. 2002 Yamamoto et al. 2002
CH05d08	*Rvi5*	SSR	91-143	F: TCATGGATGGGAAAAAGAGG R: TGATTGCCACATGTCAGTGTT	17	Liebhard et. al. 2002 Patocchi et al. 2005
CH05e03	*Rvi2* *Rvi4* *Rvi9* *Rvi11*	SSR	160-173	F: CGAATATTTTCACTCTGACTGGG R: CAAGTGTTGTACTGCTCCGAC	2	Bus et al. 2005a Gygax et al. 2004 Patocchi et al. 2009
Hi02c07	*Rvi12*	SSR	108-182	F: AGAGCTACGGGGATCCAAAT R: GTTTAAGCATCCCGATTGAAAGG	12	Silfverberg-Dilworth et.al. 2006 Patocchi et al. 2009
Hi07h02	*Rvi5*	SSR	227	F: CAAATTGGCAACTGGGTCTG R: GTTTAGGTGGAGGTGAAGGGATG	17	Patocchi et al. 2005
OPAQ-11	*Vnk*	RAPD	112	F: CTTGGCCATCATGCATCTGT R: GAATTTTCCTTTTCGCAGGT	1	Iketani et al. 2001
OPB-18	*Rvi*8 *Vr*	RAPD	620-799	F: CCACAGCAGTCATTGGGA R: CCACAGCAGTGCATAAAC	2	Patocchi et al. 2004 Bus et al. 2005
OPL-19	*Rvi2* *Rvi8*	RAPD	430	F: ACCTGCACTACAATCTTCACTAATC R: ACTCGTTTCCACTGAGGATATTTG	2	Bus et al. 2005a Patocchi et al. 2009
OPL-20	*Vnk*	RAPD	600-1300	F: TGGTGGACCA	1	Patocchi et al. 2004
OPO-09	*Vnk*	RAPD	790	F: AAGCACCAAGACAGCACAAC R: CATGTATCAGGCACACGAAC	1	Iketani et al. 2001
OPW-02	*Vnk*	RAPD	803	F: TTGGTAGGGGACCATGACTC R: CCAAAGAAGAGGCAGAGTGG	1	Iketani et al. 2001

Results

In our experiment, pear scab resistance genes were determined in 26 pear genotypes using molecular markers. The microsatellite marker CH02B10 was made to identify gene *Rvi2* in 15 connections of pear genotypes. Then, the molecular marker CH03d10 of the scab gene *Rvi4* was found in 23 scab-resistant genotypes (Table 3).

Table 3. Results of the identification of scab resistance genes of pear genotypes.

№	Name of genotypes	Scab resistance marker											
		CH02b10	CH03d10	CH05d08	CH05e03	Hi02c07	Hi07h02	OPAQ-11	OPB-18	OPL-19	OPL-20	OPO-09	OPW-02
1	Sarchabudu	+	+		+	+	+	+	+	+	+		+
2	Yemish pear	+	+		+	+	+	+	+				+
3	Garpiz pear	+	+			+	+	+	+				+
4	Gorkhmazi	+	+		+	+	+	+	+				
5	Khanim pear	+	+		+	+	+						
6	Khirda nargila	+	+		+	+	+						
7	Nar pear	+	+	+	+	+	+	+	+		+		+
8	Jir Nadiri	+	+		+	+	+						
9	Gara pear	+	+		+				+				
10	Tikani pear	+	+		+	+	+	+	+			+	
11	Gov pear	+	+		+	+	+						
12	Abasbayi		+					+		+	+		+
13	Ahmadgazi	+	+		+	+	+	+					
14	Zanciraband	+	+	+		+	+		+	+		+	+
15	Ispiya	+	+		+	+		+					
16	Jir pear												
17	Qush pear		+					+	+				+
18	Meshe pear		+					+	+	+			+
19	Dash pear		+					+	+				+
20	Khirda sarchabudu		+			+	+						+
21	Ghand pear		+					+	+	+			+
22	Shushe pear	+	+			+		+		+			+
23	Sini pear		+			+				+			
24	Bal pear		+										
25	Uzunboghaz armud												
26	Boyuk uzunboghaz								+				

The CH05d08 SSR molecular markers of the scab disease resistance gene *Rvi5* were detected in 2 pear cultivars (Nar pear, Zanciraband). The CH05e03 molecular marker was found in 12 pear genotypes. Furthermore, the SSR molecular markers Hi02c07 and Hi07h02 were detected in 16 and 13 pear genotypes, respectively. Accordingly, the OPAQ-11 and OPB-18 RAPD molecular markers were determined in 14 and 13 pear genotypes. The molecular marker OPL-19 was defined in Sarchabudu, Abasbayi, Zanciraband, Meshe pear, Ghand pear, Shushe pear, and Sini pear pear genotypes. The OPL-20 RAPD molecular marker of the scab resistance gene *Vnk* was determined in 3 pear cultivars. During the experiment, the OPO-09 RAPD molecular marker of the scab resistance gene *Vnk* was identified in only two pear cultivars (Tikani pear and Zanciraband). However, OPW-02 RAPD molecular marker of the scab resistance gene *Vnk* was found in 12 pear cultivars. In addition, the molecular markers CH02b10, CH03d10, CH05d08, CH05e03, Hi02c07, Hi07h02, OPAQ-11, OPB-18, OPL-19, OPL-20, OPO-09, and OPW-02 of scab resistance genes *Rvi2, Rvi4, Rvi15, Rvi5, Rvi9, Rvi11, Rvi12, Rvi5, Vnk, Rvi8, Vr, Rvi8, Vnk* genes were not detected in 2 pear cultivars in Azerbaijan (Jir pear and Uzunboghaz armud).

Discussion

Liebhard et al. (2002) developed the molecular marker CH05d08 for *Rvi5*. These markers have been charted on pear linkage group 17 and are positioned 0.12 cM from *Rvi1*. These scab genes were detected in two Azerbaijan pear genotypes (Nar pear and Zangiraband). The results of molecular screening by Madenova et al. (2024) revealed that resistance to *Rvi* (*Rvi14, Rvi8, Rvi6, Rvi4, Rvi15, Rvi11, Rvi9, Rvi5, Rvi2*) was detected in 38 out of 45 studied varieties, including 11 Kazakh and 34 foreign varieties. This study performed molecular screening using eight molecular markers (OPL19, AM19, CH02f06, Hi07f02, CH05e03, AL07, K08, and HB09).

Some of the 680 apple varieties obtained from the Fruit Gene Bank in Dresden Pillnitz were examined for scab disease for two years. Höfer et al. (2021) described 35 varieties expressing *Rvi2*, *Rvi4*, *Rvi6, Rvi13*, *Rvi14* or *Rvi17* related alleles or allelic combinations. Eleven cultivars were identified to be highly low susceptible to scab. Oh et al (2021) investigated to identify a scab-resistance gene using an interspecific hybrid population ((*Pyrus pyrifolia*×*P. communis*) ×*P. pyrifolia*). Kruskal-Wallis test and range mapping were performed on two-year phenotypic data. As a result, 12 common markers were found to be significantly associated with scab resistance. The *Rvn3* gene was described in linkage group 6 of interspecific hybrid pear, and

co-linearity was confirmed between *Rvn3* and *Rvi14*. Uzun et al. (2023) have identified more than 20 major scab-resistance genes in different cultivars and some wild relatives. Using 12 molecular markers expressing 8 genes (*Rvi1, Rvi4, Rvi5, Rvi6, Rvi8, Rvi11, Rvi12,* and *Rvi15*) associated with scab resistance, four apple cultivars found in Kyrgyzstan at the time of this study were tested for scab resistance.

The CH05e03 marker for *Rvi11* gene was developed by Gygax et al. (2004). This marker was mapped to pear linkage group 2 (Patocchi et al., 2005). The specific molecular marker CH05e03 (SSR) is positioned 0.6 cM from *Rvi11*. The results of the molecular screening revealed that *Rvi 2, Rvi 4, Rvi 9,* and *Rvi 11* scab resistance genes were amplified in 12 of the 26 pear genotypes examined. The *Rvi12* gene was revealed by the Hi02c07 marker, and it was discovered in16 genotypes. The Hi07h02 marker is made for *Rvi5* by Patocchi et al. (2009). This SSR marker was discovered in 13 genotypes. The RAPD marker OPAQ-11 is made for *Vnk* by Iketani et al. (2001). This marker is charted to pear linkage group 10 (Patocchi et al., 2005). As shown in Table 4, the RAPD marker of *Vnk* scab gene was discovered in 14 genotypes. The OPB-18 marker is made for *Rvi8* by Bus et al. (2005a). This gene was charted to linkage group 2. This RAPD marker was discovered in 13 genotypes. The RAPD marker OPL19 is made by Bus et al. (2005b). *Rvi2* and *Rvi8* scab genes have been nearly charted on LG2 (Bus, 2005a). This OPL19 marker of the *Rvi2* and *Rvi8* genes was identified in 7 Azerbaijan pear genotypes. This marker is charted to pear linkage group 10 (Patocchi et al., 2005). The molecular markers of the gene *Vnk* were discovered in six scab-resistant genotypes. Marker OPL-20 amplified fragment 600–1300 bp was observed in scab-resistant genotypes Sarchabudu, Abasbayi, and Nar Pear. The OPO-09 marker is made for *Vnk* by Iketani et al. (2001). This molecular marker of *Vnk* gene was discovered in two pear genotypes (Tikani and Zangiraband). The OPW-02 is made for *Vnk* by Iketani et al. (2001). The *Vnk* gene was discovered in 12 scab resistance genotypes.

In our research, for the first time, the genetic diversity of the local, selected, and introduced pear varieties cultivated in our country was studied using the SSR and RAPD markers. It was determined that the selected pear varieties are suitable for use in modern breeding programs (MAS) in the future (Babayeva, 2021b). Furthermore, the resistance of local selection and introduced pear genotypes cultivated in Azerbaijan to scab disease (Venturia pyrina Aderh.) was assessed under greenhouse conditions (Babayeva, 2024). We believe that the resistant genotypes we have studied can easily be used to breed scab-resistant cultivars using marker backcrossing.

Conclusion

Our country occupies a significant place due to its genetic opportunities. The results show that the Azerbaijani introduction genotypes are the variability of gene-specific markers to the pathogen. Taking into account the present worth, the cultivation of high-brand pear cultivars convenient for manufacturing territories, ripening at various times, and durable to total diseases such as scab will bring superior cost to the country's farm and profit the surroundings and health. Therefore, modern sources of durability may be used in future durable cultivation programs.

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References

Babayeva N.S. (2021a). Assessment of pear (*Pyrus communis* L.) genotypes based on pomological and biochemical parameters in Azerbaijan. Advances of modern natural science, 1: 5–12.

Babayeva N.S. (2021b). Assessment of genetic diversity of common pear (*Pyrus communis*) genotypes in Azerbaijan using SSR and RAPD markers. Bulletin of Michurinsky State Agrarian University, 4 (67): 71-78.

Babayeva, N.S. (2024). Assessment for resistance genes against pathogen *Venturia pyrina* Aderh. in local, selected and introduced varieties of pear cultivated in Azerbaijan. Advances in Biology & Earth Sciences, 9(2), 253-258.

Balykina, E., Ivanova, O., Yagodinskaya, L. et al. (2021). Susceptibility of collection pear cultivars to the agent of scab pathogen *Venturia pirina* Aderh. BIO Web of Conferences, 34, 04014, 1-6.

Bus, V.G.M., Laurens, F.N.D., W.E. van de Weg et al. (2005a). The Vh8 locus of a new gene-for-gene interaction between *Venturia inaequalis* and the wild apple *Malus sieversii* is closely linked to the *Vh2* locus in *Malus pumila* R12740-7A. New Phytologist, 166(3): 1035–1049.

Bus, V.G.M., Rikkerink, E.H.A., W.E. van de Weg et al. (2005b). The *Vh2* and *Vh4* scab resistance genes in two differential hosts derived from Russian apple R12740-7A map to the same linkage group of apples. Molecular Breeding, 15(1): 103–116.

Cam, Le B., Sargent, D., Gouzy J. (2019): Population Genome Sequencing of the Scab Fungal Species *Venturia inaequalis, Venturia pirina, Venturia aucupariae* and *Venturia asperata.* Genes, genomes, genetics, 9(8): 2405-2414.

Ebrahimi, L. (2020). Scab diseases of apple and pear. Plant Pathology Science, 9(1): 57-67.

Gygax M., Gianfranceschi L., Liebhard R., et al. (2004). Molecular markers linked to the apple scab resistance gene *Vbj* derived from *Malus baccata* jackii. Theoretical and Applied Genetics, 109(8): 1702–1709.

Höfe,r M., Flachowsky, H., Schröpfer S., Peil, A. (2021). Evaluation of Scab and Mildew Resistance in the Gene Bank Collection of Apples in Dresden-Pillnitz // Plants, 10(6): 1227.

Iketani, H., Abe K., Yamamoto T., Kotobuki K., Sato Y., Saito T., Terai O., Matsuta N., and Hayashi T. (2001). Mapping of disease-related genes in Japanese pear using a molecular linkage map with RAPD markers. Breed. Science, 51: 179–184.

Liebhard, R., Gianfranceschi L., Koller B., Ryder C.D., Tarchini R., Van De Weg E., and Gessler C. (2002). Development and characterization of 140 new microsatellites in apple (*Malus x domestica* Borkh.). Molecular Breeding, 10(4): 217–241.

Madenova, A., Aitymbet Z., Bolat M., Kaldybayeva D., Galymbek K., Kuan A., Kabylbekova B., Irkitbay A., Yeszhanov T., Bakirov S., Sapakhova Z. (2024). Screening of Apple Cultivars for Scab Resistance in Kazakhstan // Horticulturae, 10: 184.

Oh S., Han H., & Kim D. A. (2021). Novel Pear Scab (Venturia nashicola) Resistance Gene, Rvn3, from Interspecific Hybrid Pear (*Pyrus pyrifolia _ P. communis*), Plants, 10: 2632.

Patocchi, A., Walser M., Tartarini S., Broggini G.A., Gennari F., Sansavini S., et al. (2005). Identification by genome scanning approach (GSA) of a microsatellite tightly associated with the apple scab resistance gene *Vm.* Genom, 48(4): 630–636.

Patocchi, A., Fernandez-Fernandez F., Evans K., et al. (2009). Development and testing of 21 multiplex PCRs composed of SSRs spanning most of the apple genome. Tree Genetics & Genomes, 5: 211–223.

Rogers, S.O., Bendich A.J. (1985). Extraction of DNA from milligram amounts of fresh, herbarium and mummified plant tissues. Plant molecular biology, 5: 69-76.

Sewon, Oh, Hyeondae Han, & Daeil Kim (2021). A Novel Pear Scab (*Venturia nashicola*) Resistance Gene, *Rvn3*, from Interspecific Hybrid Pear (*Pyrus pyrifolia* × *P.communis*). Plants (Basel), 10(12): 2632.

Sokolova, O., Morocko-Bicevska I. (2021). Evaluation of *Venturia pyrina* virulence on European pear (*Pyrus communis*) cultivars by an in vitro methodology. Journal of Phytopathology, 169 (7-8): 461–470.

Silverberg-Dilworth E., Matasci C.L., W.E. van de Weg, M.P.W. van Kaauwen, Walser M., Kodde L.P., Soglio V., Gianfranceschi L., Durel C.E., Costa F., Yamamoto T., Koller B., Gessler, C., Patocchi, A. (2006). Microsatellite markers spanning the apple (*Malus x domestica* Borkh.) genome. Tree Genetics and Genomes, 2(4): 202-224.

Uzun, A., Koçyiğit Ş., Pinar H., Turgunbaev K., and Kaymak S. (2023). Selection of Central Asian apple species for scab resistance genes using molecular markers // Zemdirbyste-Agriculture, 110(3): 245–254.

Yamamoto, T., Kimura, T., Shoda M., Band, Y., Hayashi, T., Matsuta N. (2002). Development of microsatellite markers in Japanese pear (*Pyrus pyrifolia* Nakai). Molecular Ecology Notes, 2(1): 4–16.