

# Bi-dimensional Image Analysis for the Phenotypic Evaluation of Russet in Asian Pear (*Pyrus* spp.)

Yumi Kim<sup>1†</sup>, Sewon Oh<sup>1†</sup> , Keumsun Kim<sup>1,2</sup>, Hae Won Jeong<sup>2</sup>, and Daeil Kim<sup>1\*</sup> 

<sup>1</sup>Department of Horticulture, Chungbuk National University, Cheongju 28644, Korea

<sup>2</sup>Pear Research Station, National Institute of Horticultural & Herbal Science, Rural Development Administration, Naju 58216, Korea

\*Corresponding author: [dkpomo@cbnu.ac.kr](mailto:dkpomo@cbnu.ac.kr)

†These authors contributed equally to the work.

## Abstract

Russet, one of the fruit skin colors of Asian pears, affects the fruit's commercial value. A precise phenotyping method is required because fruit skin colors occur irregularly in various pear cultivars. Here, we propose a bi-dimensional image analysis to evaluate the russet of pear skin accurately and effectively by comparing the results of image analysis with the Hunter a value. The fruits of 'Whangkeumbae' (non-russet), 'Minibae' (russet), and their F<sub>1</sub> individuals were used for russet evaluation. The Hunter a value was measured using a color-difference meter. The russet coverage (%) was calculated using Photoshop software. In F<sub>1</sub> individuals derived from 'Whangkeumbae' × 'Minibae', the Hunter a value represented normal distribution, and the russet coverage distributed variously. The positive correlation coefficients between the Hunter a value and russet coverage was confirmed ( $r > 0.7$ ). Although several F<sub>1</sub> individuals showed abundant russet formation, they showed a negative Hunter a value. Therefore, the bi-dimensional image analysis more accurately evaluated russet in pear fruit skin than did the Hunter a value. The bi-dimensional image analysis could be used for high-throughput phenotyping in pears.

**Additional key words:** fruit skin color, high-throughput, hunter's value, methodology, phenotype

## Introduction

Russet is a reddish-brown color on fruit skin as a result of the formation of a cork layer, which composed of suberin and lignin, and microcracks (Wang et al., 2016; Jia et al., 2021). The skin colors of Asian pears are divided into three types: russet, green, and intermediate russet and green, depending upon the occurrence of russet. Russet on fruit skin protects the fruit from external stresses, including disease, insects, and stressful weather conditions when fruits are growing (Inoue et al., 2006). A previous study reported that 'Whangkeumbae' (a non-russetting pear) developed russet on its skin during non-bagging cultivation (Hong et al., 1989). Spotted russet on pear fruit skin reduces its commercial value. Therefore, reducing russet development on pear fruit skin is one of the priorities of pear breeding programs (Shi et al., 2019).

Received: November 24, 2021

Revised: December 16, 2021

Accepted: January 3, 2022

 OPEN ACCESS



HORTICULTURAL SCIENCE and TECHNOLOGY  
40(2):192-198, 2022  
URL: <http://www.hst-j.org>

pISSN : 1226-8763  
eISSN : 2465-8588

This is an Open Access article distributed under the terms of the Creative Commons Attribution Non-Commercial License which permits unrestricted non-commercial use, distribution, and reproduction in any medium, provided the original work is properly cited.

Copyright©2022 Korean Society for Horticultural Science.

This work was carried out with the support of "Cooperative Research Program for Agriculture Science and Technology Development (No. PJ015682)" Rural Development Administration, Republic of Korea.

Asian pear skin colors are typically measured using visual estimation and a color-difference meter (Han et al., 2012; Yamamoto et al., 2014). Visual estimation is an easy and simple method, but it is subjective and poorly reproducible. Therefore, visual estimation is unsuitable for quantitative trait investigations such as russet development. A color-difference meter has also been used for phenotyping fruit color. However, Hunter's values cannot represent the whole fruit color because the russet formation is affected by environmental conditions. Thus, a precise method for russet measurement is required.

Image analysis is a technically simple method to collect accurate phenotype data (Underhill et al., 2020). In addition, image analysis could be used to phenotype numerous plants in an automated format and could increase the scale and throughput of the process (Eberius and Lima-Guerra, 2009; Ubbens and Stavness, 2017). Image analysis has become an essential method for precisely investigating the phenotypes of the trait of interest and has been used to investigate quantitative traits including berry cluster yield, drought response, and root development for quantitative trait loci (QTL) analysis and genome-wide association studies (GWASs), which require phenotype data from numerous accessions. As a result, genes controlling the expression of the target traits have been identified using accurate phenotypic data (Chen et al., 2014; Diago et al., 2014; Le Marié et al., 2014; Leitner et al., 2014). Falginella et al. (2015) used image analysis in an apple population of 'Renetta Gridia di Torriana' (russet) × 'Golden Delicious' (slight to moderate russetting) and identified two QTLs associated with russet on the apple fruit skin. Underhill et al. (2020) also applied image analysis in an investigation of the fruit skin color of six species of *Vitis* and performed QTL analysis. In addition, fruit skin color was measured through image analysis according to the maturity of tomatoes (Takahashi et al., 2013; Bello et al., 2020). However, the development of an image analysis technique for russet investigation in pear fruit skin has not been reported yet.

'Whangkeumbae' (*Pyrus pyrifolia*) has been used as a maternal parent in Korean pear breeding programs, showing slight russet formation during bagging cultivation. In contrast, 'Minibae' (*(P. pyrifolia* × *P. ussuriensis*) × *P. pyrifolia*) displays abundant russet formation on the fruit skin, and the fruits of 'Minibae' have low commercial value. Therefore, 'Whangkeumbae' × 'Minibae' is a suitable material for genetic analysis related to russet formation. To verify the accuracy and efficiency of image analysis, this study compared the russet phenotypic data obtained from a color-difference meter with the image analysis of 'Whangkeumbae' × 'Minibae'.

## Materials and Methods

### Plant Materials

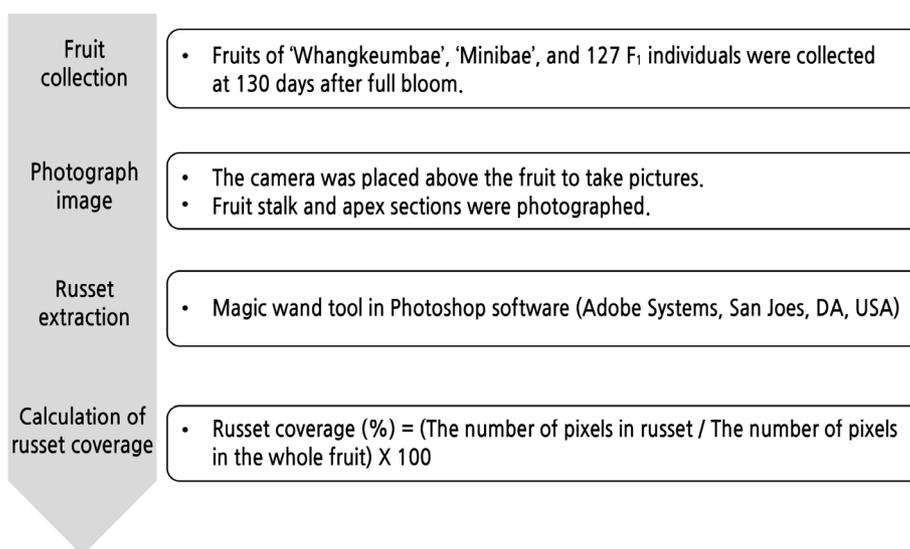
Three to four fruits of 'Whangkeumbae' (non-russet), 'Minibae' (russet), and their 127 F<sub>1</sub> individuals were randomly collected at 130 days after full bloom in 2019 and 2020 (Fig. 1). All the pear trees used in this study were planted at the Pear Research Station, National Institute of Horticultural and Herbal Science (35°01'27.9"N, 126°44'44.5"E) in Korea.

### Evaluation of Russet

Three fruits of each individual were used for russet phenotyping. To distinguish the red and green color of pear fruit skin, the Hunter *a* value was measured using a color-difference meter (CR-300, Minolta Osaka, Japan) for 2 years. Russet coverage was calculated using the fruits collected in 2019 (Fig. 2). After fixing the camera to a certain height, pear fruits



**Fig. 1.** The fruits of 'Whangkeumbae' (*Pyrus pyrifolia*) (A) and 'Minibae' (*(P. pyrifolia* × *P. ussuriensis*) × *P. pyrifolia*) (B) collected at 130 days after full bloom in 2019.

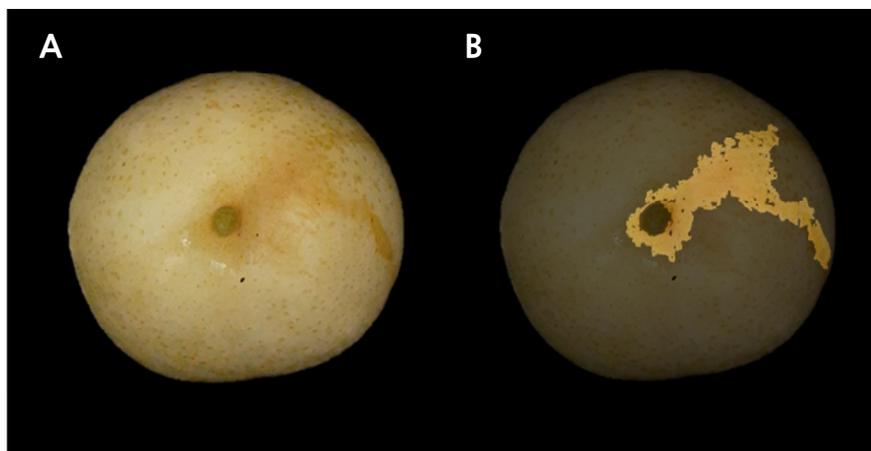


**Fig. 2.** The process of image analysis for phenotyping russet in pear fruit. The experiment was conducted in the direction of the arrow.

were placed on a black cloth, and the top (stalk) and bottom (apex) parts were photographed (Fig. 3A). The black background was removed from the photographed image, and the number of pixels in the russet region was measured using Photoshop software (Magic Wand Tool, Adobe Systems, San Joes, CA, USA). Russet coverage was expressed as a percentage of the number of pixels in the russet area to the number of pixels in the entire fruit area (Fig. 3B).

### Statistical Analysis

Kernel density estimation was performed using phenotypic data for Hunter a values and russet coverage in R-studio (RStudio, Inc., Boston MA, USA). The correlation coefficients between the Hunter a values and russet coverage were examined using Pearson's correlation coefficient in Excel (Microsoft, Seattle, WA, USA).



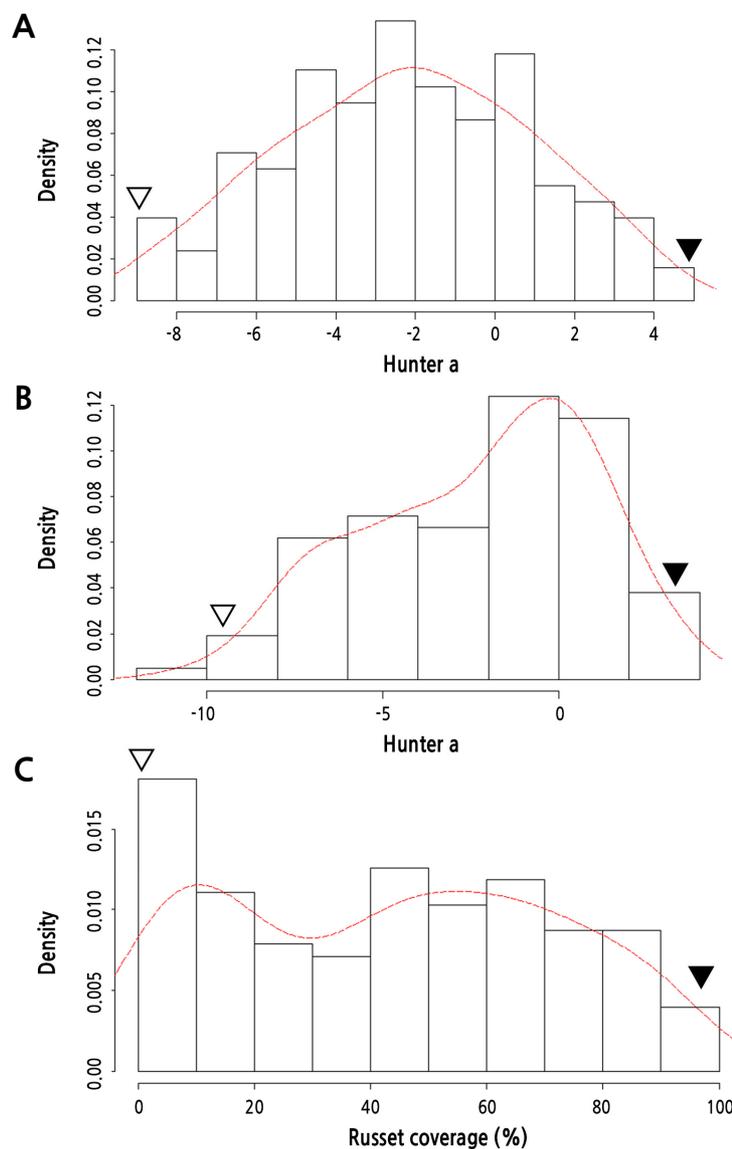
**Fig. 3.** A sample image of russet extraction from a progeny derived from ‘Whangkeumbae’ × ‘Minibae’. A, A photographed fruit stalk section; B, extracted russet image using software.

## Results and Discussion

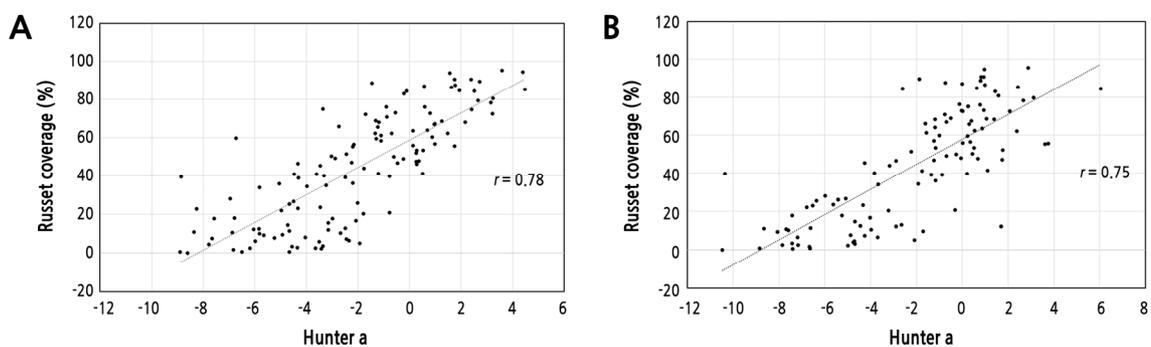
The fruit skin color of pears has been mainly evaluated by Hunter’s values, hue angle, chroma value, and visual estimation (Han et al., 2012; Yamamoto et al., 2014; Charoenchongsuk et al., 2015). Motonaga et al. (2010) constructed a color chart for investigating the fruit skin color of European pears through image analysis. In Korea, pear skin color has been assessed by visual estimation and/or using a conventional color chart. However, russet formation, which is one of the characteristics of Asian pears, varies by cultivars or species. Therefore, precise methods and criteria are required for evaluating russet.

‘Whangkeumbae’, which has a green fruit color (Fig. 1A), showed -10.1 and -9.4 Hunter a values in 2019 and 2020, respectively, whereas ‘Minibae’, displaying russet skin color (Fig. 1B), showed positive Hunter a values of 5.8 and 1.8 in 2019 and 2020, respectively. Hunter a values in  $F_1$  individuals derived from the ‘Whangkeumbae’ × ‘Minibae’ cross showed quantitative distribution in both years. The russet coverage of ‘Whangkeumbae’ × ‘Minibae’ was distributed variously (Fig. 4). The segregation of pear skin color in  $F_1$  individuals derived from ‘Nittaka’ (yellowish-brown) × ‘Suhyangri’ (yellowish-green) also displayed various phenotypes (Kim et al., 2004). Thus, the fruit skin color of pears could be used as a quantitative trait, and one or more genes could control russet formation in pear fruit skin.

Correlation analysis between Hunter a values and russet coverage was performed to determine an accurate evaluation method for russet development. The correlation coefficient between Hunter a values and russet coverage for 2 years (Fig. 5;  $r = 0.75$  to  $0.78$ ,  $p < 0.05$ ) indicated that the two phenotypes were positively correlated. Takahashi et al. (2013) and Underhill et al. (2020) confirmed a high positive correlation between Hunter a values and image analysis ( $r > 0.9$ ) in the fruit skin color of tomatoes and grapes, respectively. Russet on pear fruit skin develops informally and is affected by environmental factors. Although Hunter’s values have been used for color investigations in diverse horticultural crops, they cannot represent the color of the entire target organ. Several  $F_1$  individuals of ‘Whangkeumbae’ × ‘Minibae’ had negative Hunter a values, but the same  $F_1$  individuals developed russet on the fruit skin. For example, the  $F_1$  individual 15-91 showed  $-8.77 \pm 2.00$  Hunter a values, but its russet coverage was  $65.77 \pm 11.94\%$ . Because russet formation in pear fruit skin is not due to specific pigmentations like in tomatoes and grapes (Castellarin and Gaspero, 2007; Brar et al., 2008; Wang et al., 2014; Chattopadhyay et al., 2021), Hunter a values and russet coverage could be discordant, resulting in a



**Fig. 4.** Kernel density estimation of Hunter a values and russet coverage in 127  $F_1$  individuals derived from 'Whangkeumbae'  $\times$  'Minibae'. The normalized histogram is represented as a red line. 'Whangkeumbae' and 'Minibae' are represented as white and black inverted triangles, respectively. A, Hunter a values in 2019; B, Hunter a values in 2020; C, russet coverage.



**Fig. 5.** Correlation coefficients ( $r$ ) between Hunter a values and russet coverage (%) in 'Whangkeumbae'  $\times$  'Minibae' ( $p < 0.05$ ). The correlation between Hunter a values and russet coverage measured in 2019 (A) and 2020 (B).

relatively lower correlation coefficient than in previous studies.

In this study, russet was quantified using bi-dimensional image analysis. This process made it possible to accurately quantify russet in the whole fruit. Therefore, measuring russet coverage in fruit skin was a more accurate phenotyping method than using Hunter *a* values. To reveal the genetic basis of a quantitative trait, it is necessary to link genotypic data with phenotypic data. Thus, a precise phenotypic investigation is important. Phenotyping a large number of accessions or russet-related cross populations using an image analysis system for the russet evaluation of pear fruit skin will facilitate the identification of genes that control russet formation through GWAS and QTL analyses. Furthermore, our results can be the basis for the development of a new program that automatically evaluates the russet coverage of fruit skin.

## Literature Cited

- Bello TB, Costa AG, Silva TRD, Paes JL, de Oliveira MV (2020) Tomato quality based on colorimetric characteristics of digital images. *Rev Bras Eng Agric Ambient* 24:567-572. doi:10.1590/1807-1929/agriambi.v24n8p567-572
- Brar HS, Singh Z, Swinny E (2008) Dynamics of anthocyanin and flavonol profiles in the 'Crimson Seedless' grape berry skin during development and ripening. *Sci Hortic* 117:349-356. doi:10.1016/j.scienta.2008.05.007
- Castellarin SD, Di Gaspero G (2007) Transcriptional control of anthocyanin biosynthetic genes in extreme phenotypes for berry pigmentation of naturally occurring grapevines. *BMC Plant Biol* 7:1-10. doi:10.1186/1471-2229-7-1
- Charoenchongsuk N, Ikeda K, Itai A, Oikawa A, Murayama H (2015) Comparison of the expression of chlorophyll-degradation-related genes during ripening between stay-green and yellow-pear cultivars. *Sci Hortic* 181:89-94. doi:10.1016/j.scienta.2014.10.005
- Chattopadhyay T, Hazra P, Akhtar S, Maurya D, Mukherjee A, Roy S (2021) Skin colour, carotenogenesis and chlorophyll degradation mutant alleles: genetic orchestration behind the fruit colour variation in tomato. *Plant Cell Reports* 1-16. doi:10.1007/s00299-020-02650-9
- Chen D, Neumann K, Friedel S, Kilian B, Chen M, Altmann T, Klukas C (2014) Dissecting the phenotypic components of crop plant growth and drought responses based on high-throughput image analysis. *Plant Cell* 26:4636-4655. doi:10.1105/tpc.114.129601
- Diago MP, Tardaguila J, Aleixos N, Millan B, Prats-Montalban JM, Cubero S, Blasco J (2014) Assessment of cluster yield components by image analysis. *J Sci Food Agric* 95:1274-1282. doi:10.1002/jsfa.6819
- Eberius M, Lima-Guerra J (2009) High-throughput plant phenotyping-data acquisition, transformation, and analysis. In Edward D, Stajich J, Hansen D, eds, *Bioinformatics*. Springer, NY, USA, pp 259-178. doi:10.1007/978-0-387-92738-1\_13
- Falginella L, Cipriani G, Monte C, Gregori R, Testolin R, Velasco R, Troglio M, Tartarini S (2015) A major QTL controlling apple skin russetting maps on the linkage group 12 of 'Renetta Grigia di Torriana'. *BMC Plant Biol* 15:150. doi:10.1186/s12870-015-0507-4
- Han JH, Cho JG, Son IC, Kim SH, Lee IB, Choi IM, Kim D (2012) Effects of elevated carbon dioxide and temperature on photosynthesis and fruit characteristics of 'Nittaka' pear (*Pyrus pyrifolia* Nakai). *Hortic Environ Biotechnol* 53:357-361. doi:10.1007/s13580-012-0047-x
- Hong KH, Kim YS, Kim TC, Choi JS, Yiem MS (1989) Effect of the kinds of fruit bags and the bagging time on fruit quality of 'WhangkeumBae' pear. *J Kor Soc Hortic Sci* 37:279-284
- Inoue E, Kasumi M, Sakuma F, Anzai H, Amano K, Hara H (2006) Identification of RAPD marker linked to fruit skin color in Japanese pear (*Pyrus pyrifolia* Nakai). *Sci Hortic* 107:254-258. doi:10.1016/j.scienta.2005.07.009
- Jia B, Cheng Z, Wang Q, Zhang S, Heng W, Zhu L (2021) Characterization of the composition and gene expression involved the shikimate pathway in the exocarp of 'Dangshansuli' pear and its russet mutant. *Hortic Environ Biotechnol* 62:125-134. doi:10.1007/s13580-019-00207-8
- Kim D, Hwang JH, Lee SY, Lee DH, Shin YU, Lee HJ, Choi JK, Shin IS, Kang SJ (2004) Genetic characterization of the interspecific Asian pears (*Pyrus pyrifolia* × *P. ussuriensis*) using molecular markers linked to fruit characteristics. *Hortic Environ Biotechnol* 45:207-211
- Le Marié CA, Kirchgessner N, Marschall D, Walter A, Hund A (2014) Rhizoslides: paper-based growth system for non-destructive, high throughput phenotyping of root development by means of image analysis. *Plant Methods* 10:13-29. doi:10.1186/1746-4811-10-13
- Leitner D, Felderer B, Vontobel P, Schnepf A (2014) Recovering root system traits using image analysis exemplified by two-dimensional neutron radiography images of lupine. *Plant Physiol* 164:24-35. doi:10.1104/pp.113.227892
- Motonaga Y, Matsumoto T, Motonaga N (2010) Color chart of European Pear 'Le Lectier' based on the color image analysis. In Proceedings of SICE Annual Conference 2455-2461
- Shi CH, Wang XQ, Zhang XY, Shen LY, Luo J, Zhang YX (2019) Response of fruit bagging to lignin biosynthesis and expression of related genes in fruit peel of sand pear (*Pyrus pyrifolia* Nakai) cv. Cuiguan. *HortScience* 54:1989-1997. doi:10.21273/HORTSCI14382-19
- Takahashi N, Maki H, Nishina H, Takayama K (2013) Evaluation of tomato fruit color change with different maturity stages and storage temperatures using image analysis. *IFAC Proc* 46:147-149. doi:10.3182/20130327-3-JP-3017.00034
- Ubbens JR, Stavness I (2017) Deep plant phenomics: a deep learning platform for complex plant phenotyping tasks. *Front Plant Sci* 8:1190. doi:10.3389/fpls.2017.01190

- Underhill AN, Hirsch CD, Clark MD** (2020) Evaluating and mapping grape color using image-based phenotyping. *Plant Phenomics* 2020:1-11. doi:10.34133/2020/8086309
- Wang YZ, Dai MS, Cai DY, Zhang S, Shi ZB** (2016) A review for the molecular research of russet/semi-russet of sand pear exocarp and their genetic characters. *Sci Hortic* 210:138-142. doi:10.1016/j.scienta.2016.07.019
- Wang YZ, Dai MS, Zhang SJ, Shi ZB** (2014) Exploring candidate genes for pericarp russet pigmentation of sand pear (*Pyrus pyrifolia*) via RNA-Seq data in two genotypes contrasting for pericarp color. *PLoS ONE* 9:e83675. doi:10.1371/journal.pone.0083675
- Yamamoto T, Terakami S, Takada N, Nishio S, Onoue N, Nishitani C, Kuniyama M, Inoue E, Iwata H, et al** (2014) Identification of QTLs controlling harvest time and fruit skin color in Japanese pear (*Pyrus pyrifolia* Nakai). *Breed Sci* 64:351-361. doi:10.1270/jsbbs.64.351