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Standardized Phenotyping for Fruit Quality in Peach [*Prunus persica* (L.) Batsch]

TERRENCE J. FRETT[1], KSENIJA GASIC[1], JOHN R. CLARK[2], DAVID BYRNE[3], TOM GRADZIEL[4], AND CARLOS CRISOSTO[4]

Abstract

A standardized phenotyping protocol for peach [*Prunus persica* (L.) Batsch] has been developed by the four public peach breeding programs in California, Texas, South Carolina, and Arkansas, along with community breeders through RosBREED (www.rosbreed.org). The protocol includes productivity and fruit quality traits and facilitates collection of standardized phenotyping data. This protocol enables collaboration among researchers working with peach across various environments, institutions and countries.

Peach [*Prunus persica* (L.) Batsch] is a commercially important fruit tree species. This species is second in temperate tree fruit production only to apple (*Malus domestica* Borkh.), with 10 million tons produced on a global scale annually (7). Throughout the last century, traditional peach breeding programs have worked diligently to develop and release many new cultivars throughout the world (13). However, developing new cultivars solely through traditional techniques is very time consuming and can take 15 to 20 years before release of a new peach cultivar (6). To mitigate these problems marker-assisted breeding (MAB), a genomic approach to enhance crop improvement, holds vast potential to compliment and accelerate traditional breeding techniques (6). However, the necessary level of collaboration between geneticists and breeders to implement the use of molecular markers in peach breeding has not been examined and uniform procedures have not been established (6, 8).

Phenotyping is a crucial component for quantitative trait loci (QTL) analysis, which connects genetic variation with biological function and thus documents genetic function (2). However, quality and quantity of available phenotypic data is not keeping pace with the immense amount of available genomic information, brought forth by the Next Generation Sequencing (NGS) technologies, that is increasing dramatically. Moreover, several databases have been established for storage and retrieval of genomic and genetic data. However, storage of phenotypic data remains mainly individualistic within programs. This lack of phenotypic documentation limits QTL mapping and further gene function discovery. To address this deficiency in phenotypic data, it is recommended that protocols be standardized across different institutions, personnel, and environments to enable consistent data collection and efficient data transfer (2).

The idea of standardized phenotyping was first implemented in the mouse (*Mus musculus*) community to study human diseases (1). It has since been applied in several other studies including the identification of disease resistance genes (11), fruit quality characteristics in tomato (*Solanum lycopersicum* L.) and potato (*S.*

[1] Clemson University, School of Agricultural, Forest, and Environmental Sciences, E143 P&AS 50 Cherry Road, Clemson, SC 29634. To whom requests should be addressed: kgasic @clemson.edu
[2] University of Arkansas, Department of Horticulture, 316 Plant Science Bldg., Fayetteville, AR 72701
[3] Texas A&M University, Department of Horticultural Sciences, College Station, TX 77843
[4] University of California Davis, Department of Pomology, 1035 Wickson Hall, One Shields Avenue Davis, CA 95616.
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tuberosum L.) (14), and postharvest fruit quality traits for population comparisons (12). Additionally, standardized phenotyping has been applied to several other plant species, including certain tree fruits (9).

Demonstration peach breeders within the RosBREED project have worked together in collaboration with Rosaceae community breeders to develop and implement a standardized phenotyping protocol with emphasis on fruit quality traits. We are reporting on development and application of the standardized phenotyping protocol for fruit quality traits in peach.

Phenotyping protocol

A detailed PowerPoint presentation portraying the standardized peach phenotyping protocol, including pictures (http://www.rosbreed.org/resources/fruit-evaluation) and videos showing each step in phenotypic data collection, has been developed and is available at the RosBREED web site (http://www.rosbreed.org/resources/fruit-evaluation/phenotyping-videos/peach). The list of traits included in the protocol consists of five tree characteristic and 18 fruit quality traits (Table 1).

Tree characteristics: Phenotyping of several tree characteristics, such as 50% of bloom, bloom type, leaf gland type and fruit set have been included in the protocol (Table 1). Upon flowering, 50% bloom (the stage when 50% of flowers are open) should be recorded using Julian date (0-365). Additionally the bloom type: showy (1), non-showy (0), and the leaf gland type: reniform (1), globose (2), eglandular (3), are proposed to be used as a quick check between identically named accessions housed in different collections to make sure there were no differences. Fruit set should be estimated before thinning using a 1-9 scale (Table 1).

Fruit quality traits: Comparability and quality of phenotypic data for fruit quality depends largely on the harvest time. Consequently, peach fruit should be harvested at the tree-ripe stage to ensure uniformity of maturity. Tree ripe stage is determined at the time when a few fruit on the tree are soft/edible. Harvesting the peaches at the same stage is critical for standardization, since nearly all peach fruit quality traits are known to change with the ripening stage of the fruit (size, firmness, internal and external color, acidity, and sugar). At harvest, it is suggested that at least 10 to 20 fruits, slightly firmer than tree ripe, be picked into an open cardboard or plastic box container to allow the fruit to dry out if there is excess moisture from morning dew or rain and to equilibrate to ambient conditions. Visual estimation of the pubescence level should be performed at harvest, in the field. Accession name/ID, and harvest date in Julian days (0-365) should be recorded and attached to the container (Table 1). Collection of phenotypic data on fruit quality traits could be divided into two groups: destructive and organoleptic phenotyping.

Destructive phenotyping: Destructive phenotyping includes all of the fruit quality traits resulting in fruit damage (Table 1). From the 10 to 20 fruits harvested from each peach accession, five fruits should be selected and individually analysed for flesh color, fruit firmness, weight, size, soluble solid concentration (SSC), pH and titratable acidity (TA). Skin color and flesh color should be recorded descriptively and/or quantified using a standard Konica Minolta Chroma Meter (CR-400, Konica Minolta Chroma Meter, Tokyo, Japan; or other models; L*, a*, b*). The darkest portion of the skin on both cheeks and the flesh (with no red pigmentation) should be measured using the 'Light Protection Tube' (glass protection plate CR-A33a, 22 mm in diameter, Konica Minolta, Tokyo, Japan; or other models). Areas with a blush or red in the flesh should be avoided, since they can create a bias in Chroma Meter data (Table 1).

Fruit size is estimated by weight (g) and diameter (mm) measurements using any scale, and a micrometer caliper. Fruit firmness should be measured on two opposite

Table 1. List of traits included in the standardized phenotyping protocol for peach.

Trait	Unit of measure
Productivity traits	
50% bloom date (Julian)	0-365 days
Bloom type	Showy = 1; Non-showy = 0
Leaf gland type	Reniform = 1; Globose = 2; Eglandular = 3
Fruit set	0 = no fruit; 5 = full crop (15-20 cm spacing between fruit); 7 = 2x full crop (7.5 cm spacing between fruit); 9 = 4 x full crop (2.5 cm spacing between fruit)
Ripe date (Julian)	0-365 days
Fruit quality traits – Organoleptic phenotyping	
Pubescence	0 = glabrous or nectarine; 3 = slight ; 5 = medium ; 7 = heavy
Blush %	0 = no blush; 1 = 1-20%; 2 = 21-50%; 3 = 51-80%; 4 = 81-99%; 5 = 100%
Ground color	1 = green; 2 = cream green; 3 = cream; 4 = cream yellow; 5 = yellow green; 6 = yellow; 7 = yellow orange; 8 = orange; 9 = red
Ground color L*	L*, intensity (-L*, dark; +L*, light)
Ground color a*	a* (-a*, green; +a*, red)
Ground color b*	b* (-b* blue; +b*, yellow)
Flesh color	1 = green; 2 = cream green; 3 = cream; 4 = cream yellow; 5 = yellow green; 6 = yellow; 7 = yellow orange; 8 = orange; 9 = red
Flesh color L*	L*, intensity (-L*, dark; +L*, light)
Flesh color a*	a* (-a*, green; +a*, red)
Flesh color b*	b* (-b* blue; +b*, yellow)
Red in flesh	0 = no red overlay; 1 = 1-20%; 2 = 21-50%; 3 = 51-80%; 4 = 81-99%; 5 = 100%
Red around pit	1= red; 0 = no red
Fruit quality traits – Destructive phenotyping	
Diameter	Widest part of the fruit (mm)
Weight	Grams
Flesh firmness average	Kg/cm^2 of force
Brix %	%
pH	#
Malic acid / Titratable acidity	#
Fruit texture	Melting = 1; Non-melting = 2
Adherence to pit	Freestone = 1; Semi-freestone = 2; Semi-clingstone = 3; Clingstone = 4
Pit weight	Grams
Pit split %	Proportion of split / normal pits

sides by removing ~1-cm of the outer flesh and using either an automatic fruit texture analyzer (FTA, GUSS Manufacturing Pty. Ltd., Strand, South Africa; or other model), or a mounted or hand-held penetrometer (Table 1).

The SSC, pH and TA data should be measured in juice from all five fruits. A longitudinal slice of each of the five fruit should be cut and juiced through cheese cloth to filter the flesh debris using a hand presser. Sampling fruit in this manner is important to account for variation of sugar levels throughout the fruit, since accumulation of sugars is elevated at the stem end and decreased at the tip end. Additionally, the fruit should be kept at

room temperature (~24°C) before proceeding, since varying temperatures hinder the standardization of the subsequent phenotypic traits (as SSC, TA and pH can change with temperature fluctuations). A few drops of the composite juice sample should be placed on a hand-held (General REF113ATC Brix Refractometer) or digital refractometer (Atago USA, INC 3810 PAL-1 Digital Hand-Held Pocket Refractometer, WA, USA; or other model) to determine SSC (sugars are the most prominent SSC in fruit juice) of each accession. A solution of 6 g of the juice, diluted with 50 mL of distilled water, should be prepared for an initial pH reading and TA measurement using a pH meter, phenolphthalein indicator, or automated volumetric titrator (862 Compact Titrosampler, Metrohm AG, Herisau, Switzerland; or other model; Table 1). After the initial pH value is recorded, 0.1M NaOH is added to the solution until the end pH value of 8.2 is reached and the millilitters of NaOH used for this equilibration should be recorded. The following formula is used to calculate % of TA in the peach juice: % TA = [mLs NaOH used] x [0.1 N NaOH] x [milliequivalent factor] x [100] / grams of sample.

Flesh adherence to the pit should be recorded as: freestone (flesh easily separates from the pit), clingstone (strong adherence to the pit), semi-freestone (most of the flesh separates from the pit) or semi-clingstone (medium adherence to the pit). Flesh texture should be classified as non-melting if the flesh is firm and intact, or melting if the flesh is smooth, soft and easily falls apart. It is also recommended that fresh pit weight and tendency of the pit to split are recorded (Table 1).

Organoleptic phenotyping: Organoleptic phenotyping includes data collection for red coloration of skin or blush, skin and flesh color, and redness in flesh surrounding the pit. It should be performed on a second set of five fruits whenever possible. The percentage of blush, which develops over the background skin color, is approximated and converted using a scale from 0-5; 0 indicating no blush and 5 indicating full red over color. Skin and flesh color should also be rated using a numerical color scale (1-9). In addition, presence of redness in flesh is also recorded as a percentage and converted to a 0-5 scale where 0 indicates no red and 5 = fully red flesh. Pigmentation around the pit should be scored as present 1 or absent 0 (Table 1).

Data quality checking: Once phenotypic data have been collected and entered in the spread sheet-type document, the data needs to be checked for typing errors or incorrect outliers, using a quality checking protocol. Quality checking protocol consists of: inspecting every 30th data point for line shifts that could have occurred during data entry; calculating maximum and minimun values and / or developing histograms or box-plots to identify and remove incorrect outliers (Fig. 1). For multi-year data, differences in quality traits between and among years should be checked (for example a peach scored as white one year, cannot be yellow the next year). Considering continuous multi-year data, developing scatterplots to detect and remove incorrect outliers is suggested (Fig. 2).

Conclusions

Phenotyping is a crucial component for QTL mapping that connects genetic variation with biological function to document genetic function (2). Yet, the quality and quantity of available phenotypic data is not keeping pace with genomic data (2). The protocol developed for phenotyping a set of traits with a focus on fruit quality is a starting point in collecting phenotypic data across different institutions, environments and countries to be used for QTL documentation. With increasing interest in the peach breeding community for understanding the genetic makeup of many horticulturally important traits, this protocol will change the type and quality of data that are recorded and improve the precision and uniformity of data across different studies.

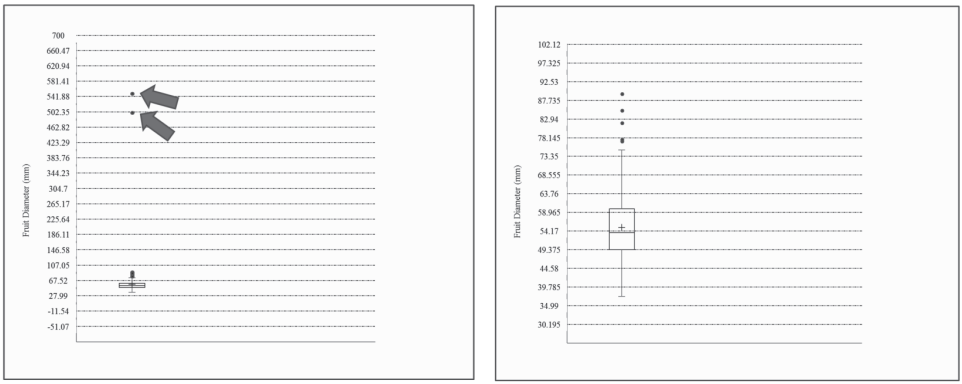

Fig. 1. Box plots of 2010 peach diameter. Left = original data, two outliers identified with arrows. Right = two outliers removed.

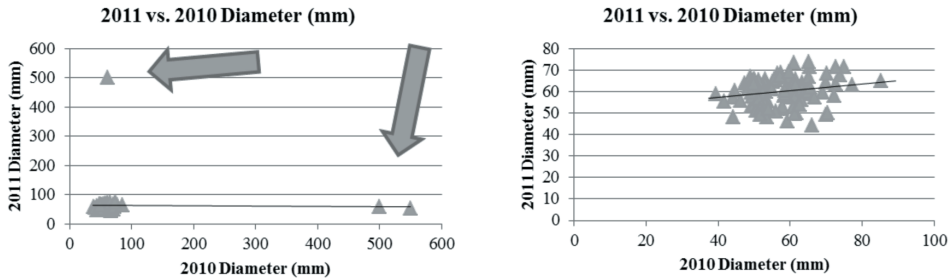

Fig. 2. Multi-year data check for additional outliers by generating scatterplots between years to determine correlations and identify data points far outside of correlations. Left = original data, three outliers identified by arrows. Right = three outliers removed.

The example of standardized phenotyping applied in RosBREED (www.rosaceae.org), and previous standardized phenotyping models (1, 2, 9, 11, 12, 14) should spur its application to other important fruit traits as well as fruit quality, tree characteristic, and biotic and abiotic stress resistance. Furthermore, all agronomically important plant species can benefit from standardized phenotyping, and enable efficient discovery of genomic regions that control important agricultural traits. Ultimately, standardized phenotyping in conjunction with genotyping and QTL analysis will enable marker assisted breeding for several vital agronomic plant traits. The developed markers will become tools to increase the efficiency of traditional breeding, leading to increased efficiency in the release of cultivars with enhanced traits.

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Literature Cited

1. Abbott, A. 1999. Database to standardize mouse phenotyping. Nature. 401:833.
2. Bassil, N.V. and G.M. Volk. 2010. Standardized phenotyping: advantages to horticulture introduction to the workshop. HortScience. 45:1306-1306.
3. Bink, M. 2004. FlexQTL software. BIOMETRIS, Wageningen UR, The Netherlands (www.flexqtl.nl).
4. Bink, M. 2005. FlexQTL software: efficient estimation of identity by descent probabilities and QTL mapping in pedigreed populations. In Plant and Animal Genomes XIII Conference, San Diego, CA, USA.
5. Bink, M., M.P. Boer, C.J.F. ter Braak, J. Jansen, R.E. Voorrips and W.E. Van de Weg. 2008. Bayesian analysis of complex traits in pedigreed plant populations. Euphytica. 161:85-96.
6. Bliss, F.A. 2010 Marker-assisted breeding in horticultural crops. Acta Hort. 859:339-350.
7. Fideghelli, C., G. Della Strada, F. Grassi and G. Morico. 1998. The peach industry in the world: present situation and trend. Acta Hort. 465:29-40.
8. Iezzoni, A., C. Weebadde, J. Luby, Y. Chengyan, E. Van De Weg, G. Fazio, D. Main, C.P. Peace, N.V. Bassil and J. McFerson. 2010. RosBREED: enabling marker-assisted breeding in Rosaceae. Acta. Hort. 859:389-394.
9. Peace, C. and J.L. Norelli. 2009. Genomics approaches to crop improvement in the Rosaceae. Genetics and Genomics of Rosaceae. 6:19-53.
10. Peace, C., J. Luby, K. Evans, S. Brown, M. Clark, Y. Guan, B. Orcheski, C. Schmitz, S. Verma, N. Bassil, E. van de Weg, and A. Iezzoni. 2011. RosBREED enables marker-assisted breeding for apple. HortScience 46(9): S384-S385.
11. Postman, J., G. Volk and H. Aldwinckle. 2010. Standardized plant disease evaluations will enhance resistance gene discovery. HortScience. 45:1317-1320.
12. Rudell, D. 2010. Standardizing postharvest quality and biochemical phenotyping for precise population comparison. HortScience. 45:1307-1309.
13. Sansavini, S., A. Gamberini and D. Bassi. 2006. Peach breeding, genetics and new cultivar trends. Acta Hort. 713:23-48.
14. Scott, J.W. 2010. Phenotyping of tomato for SolCAP and onward into the void. HortScience 45:1314-1316.