Journal of the American Pomological Society 67(4): 205-216 2013

Large-Scale Standardized Phenotyping of Strawberry in RosBREED

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Additional index words: flowering, fruit characteristics, plant characteristics, fruit chemistry

Abstract

In an effort to implement marker-assisted breeding in Rosaceae, many traits need to be characterized in diverse germplasm. The USDA-NIFA Specialty Crop Research Initiative-funded RosBREED project includes breeding programs of four Rosaceae crops (apple, peach, cherry and strawberry). Phenotyping each crop for specific horticultural and commercial traits is an important process needed to translate genomic knowledge through marker-assisted breeding into enhanced breeding efficiency. These data will directly aid in the identification of quantitative trait loci or marker-trait associations that will be used to assist breeding programs in the future. Large-scale, standardized phenotyping protocols have been set up for each crop. The standardized phenotyping protocol for strawberries was agreed upon by the breeding teams in Oregon, Michigan, New Hampshire, California and Florida and includes four trait categories: phenology and other flower-related traits, plant characteristics, fruit characteristics, and fruit chemistry traits. We describe how each of the traits in the categories was evaluated. A summary of mean values for 37 traits of the genotypes planted at the RosBREED locations in 2011 and 2012 is provided. The phenotypic data for widely used founder germplasm that has contributed to current cultivars is available through the "Breeders Toolbox" at the Genome Database for Rosaceae (http://www.rosaceae.org/breeders_toolbox).

Plant breeding programs strive to identify the ideal genotypes for the environments where the crop will be grown and that meet the ideal characteristics of the crop for the industry in that region. The plant's phenotype is the result of the interaction between its genotype and environment. A breeding program will be successful if it can reliably identify genotypes with the optimum traits needed for an individual to be released as a cultivar. Most breeding programs utilize traditional approaches to identify desirable phenotypes. Marker-assisted breeding (MAB) can facilitate and expedite the release of new cultivars.

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This technology is becoming easier to integrate into traditional breeding programs due to decreased costs, increased efficiency, and greater molecular marker availability (Bliss, 2010).

The US Department of Agriculture-National Institute of Food and Agriculture (USDA–NIFA) Specialty Crop Research Initiative-funded RosBREED project aims to 'bridge the chasm' between genomics and traditional rosaceous crop breeding programs (Iezzoni et al., 2010). Large-scale phenotyping protocols have been published for apple (Evans et al., 2011) and peach (Frett et al., 2012) and presentations of the standardized phenotyping can be viewed for all of the target crops (apple, peach, cherry and strawberry) at www.rosbreed.org.

While MAB has not been widely used in strawberry (*Fragaria ×ananassa* Duch. ex Rozier), a few breeding programs are using markers. Breeders in collaboration with Institut National de la Recherche Agronomique (INRA) in France have used molecular markers linked to *Rca2*, a single gene for resistance to *Colletotrichum acutatum* J.H. Simmonds, the causal agent of anthracnose on fruit and plant, for parent selection (Whitaker, 2011). Driscoll Strawberry Associates Inc. (Watsonville, CA) use *Rca_240* and other *Rca2* markers developed in-house to screen 20% of their seedlings (Whitaker, 2011). Other molecular markers have been discovered for red stele resistance (*Phytophthora fragariae* Hickman) (van de Weg, pers. comm.) and remontancy (Hancock, pers. comm. and Denoyes pers. comm.) and are being validated to assess their applicability across diverse germplasm.

Valuable molecular resources are increasingly becoming available in strawberry and include the *F. vesca* 815 × *F. bucharica* 601 (FV × FB) reference map for diploid strawberry (Sargent et al., 2008), a reference genome sequence of *F. vesca* L. (woodland strawberry) (Shulaev et al., 2011), and a large number of identified and mapped microsatellites for the octoploid strawberry (Isobe et al., 2013). Resources in the diploid strawberry can be used to better understand the subgenome structure and composition of the octoploid cultivated strawberry genome.

One of the issues complicating MAB in the cultivated strawberry is its octoploidy ($2n = 8x = 56$). High ploidy complicates linkage map construction. A method called Microsatellite Allele Dose and Configuration Establishment (MADCE) has been used to map the homeologs in high ploidy individuals (van Dijk et al., 2012). This method relies on quantitative analysis to identify allele dosage and assign alleles to each of the four subgenomes in strawberry (van Dijk et al., 2012).

Despite the complexity of creating octoploid linkage maps, recent advances include linkage map construction for five *F. ×ananassa* populations, all of which are included in RosBREED as part of the Breeding Pedigree Set (BPS) and Crop Reference Set (CRS). Linkage maps have been reported for populations derived from the following crosses: 'Capitola' × CF1116 (Lercerteau-Köhler et al., 2003; Rousseau-Gueutin et al., 2008), 'Tribute' × 'Honeoye' (Weebadde et al., 2008), 'Redgauntlet' × 'Hapil' (Sargent et al., 2009) and 232 × 1392 (Zorrilla-Fontanesi, et al., 2011). A linkage map is currently being constructed using the 'Holiday' × 'Korona' progeny (Thijs van Dijk, unpublished). Synteny was observed between the 'Redgauntlet' × 'Hapil' octoploid map and the FV × FB diploid reference map (Sargent et al., 2009; Sargent et al., 2012). Quantitative Trait Loci QTL were found for day-neutrality (Gaston et al., 2013; Weebadde et al., 2008) and yield, as well as important fruit quality characters such as soluble solids concentration, ascorbic acid concentration, titratable acidity, color, and firmness (Lerceteau-Köhler et al., 2012; Zorrilla-Fontanesi, et al., 2011). Furthermore, the gene underlying one QTL controlling the variation in a flavor compound has been identified (Zorrilla-Fontanesi et al., 2012). These resources are essential for enabling MAB and its use in developing new strawberry cultivars with increased quality.

RosBREED has used Pedigree-Based Analysis (PBA) to identify the favorable alleles present in a group of pedigree-linked genotypes (Iezzoni et al., 2010; van de Weg et al., 2004). Using PBA allows for the identification of QTL by analyzing populations with the same founding parents. Markers near QTL linked to traits of interest can be further used in MAB, after their validation, e.g. presence of the marker in genetic resources showing the trait of interest.

In strawberry, we used two SSR markers to confirm accession identity and further eliminate outcrosses and misidentified accessions. However, these two markers alone provided only a small amount of genomic representation. Therefore, an Axiom® 90,000 SNP chip was developed through the efforts of RosBREED and the international strawberry community and will be evaluated for genome scanning.

The lack of reliable and available phenotypic data can hinder the use of statistical methods for identifying the associations between phenotypic and genotypic data for breeders and geneticists. This shortage of phenotypic knowledge may lead to lack of understanding of the genetic basis of biological processes (Bassil and Volk, 2010). RosBREED aims to close this gap between genomics research and traditional breeding programs (Iezzoni et al., 2010). The following paper describes the standardized phenotyping protocol developed for strawberry and reports descriptive statistics for 34 traits based on evaluation of the genotypes planted at the RosBREED locations.

Materials and Methods

Strawberry germplasm

RosBREED's strawberry group identified 890 *Fragaria* individuals to include in two reference germplasm sets: the Crop Reference Set (CRS, freely distributed data through the Genome Database for Rosaceae www.rosaceae.org) and the Breeding Pedigree Set (BPS, data kept private by breeding programs). Both sets of germplasm were provided from multiple institutions including: the USDA-ARS Horticulture Crops Research Unit (HCRU), Michigan State University (MSU), the USDA-ARS National Clonal Germplasm Repository (NCGR), the University of Florida (UF), the University of New Hampshire (UNH), the Instituto Andaluz de Investigación y Formación Agraria y Pesquera (IFAPA) in Spain, East Malling Research (EMR) in the United Kingdom, the Institut National de la Recherche Agronomique (INRA) in France, and the Center for Genetic Resources (CGR) in The Netherlands. Driscoll Strawberry Associates (Watsonville, CA) provided critical support, as they imported the European material into the USA using their long-established quarantine and propagation facilities.

The BPS is made up of four European mapping populations: the INRA population comprised of 56 genotypes from 'Capitola' × CF1116 ('Pajaro' × ['Earliglow' × 'Chandler']) (B. Denoyes); the EMR population comprised of 51 genotypes from 'Red Gauntlet' × 'Hapil' (D.J. Sargent); the CGR population comprised of 29 genotypes from 'Holiday' × 'Korona' (W.E. van de Weg); and the IFAPA population comprised of 69 genotypes from 232 (Sel. 4-43 × 'Vilanova') and 1392 ('Gaviota' × 'Camarosa') (I. Amaya).

The CRS is comprised of the MSU population, 65 genotypes from 'Tribute' × 'Honeoye', as well as, 10 randomly selected genotypes from 17 populations from MSU and 19 populations from the USDA-ARS, HCRU breeding programs. The rest of the CRS included germplasm that represented important founding genotypes (165 cultivars) and 52 accessions of wild origin including individuals from the USDA-ARS-NCGR "supercore" collection (Hancock et al., 2002).

Two clones of each genotype in the form of rooted runners were planted in Michigan, Oregon, New Hampshire, California, and Florida, in August 2010 (Table 1). In June 2011, the INRA, EMR, and CGR populations were distributed and planted at each location as multi-crowned plants. The IFAPA population was distributed and planted in September 2011 as bare-root runners.

Phenology and flowering-related traits

All phenotypic traits and methods for scoring, along with the location and year, can be referenced in Table 1. Collection of flowering data began when at least one flower was open, and was recorded weekly until the end of July or the first week of August in 2011 and 2012 for the two plants of each genotype. Presence of newly opened flowers at each date was noted. The time period of the evaluation was chosen to identify short-day plants that flowered only in the spring and those that continued to flower until the days began to shorten in late summer. Day-neutrality was accounted for quantitatively by summing the number of weeks flowering after week 11 from the first bloom at all locations.

Growing degree days for first bloom and for harvest date were calculated as the mean of the maximum and minimum daily temperature minus the base 50°F (10°C). Growing degree day accumulation began 1 January, and accumulated throughout the year. Data for growing degree days was generated from weather stations in each region: Oregon (Hyslop Farm, Corvallis, OR) (Coop, 2012), Enviro-weather at Michigan (MSU Southwest MI Research & Extension Center, Benton Harbor, MI) (Andresen, 2012), and in New Hampshire (Woodman Farm, Durham, NH). Days from 1 January for bloom and harvest dates were calculated by summing the number of days from 1 January to the calendar date on which the first bloom was observed. The presence of anthers was noted when flowers of each accession were open to determine the flower's sex. In addition, the average flower number per truss from three trusses was used to determine truss size. The location of the truss in relation to the canopy (above or below) and the relative peduncle length were also noted.

Fruit were assessed once to twice weekly for when fruit color (red, yellow, white) was fully developed and 30-50% of fruit on the plant were ripe. An average of five fruit was attempted for harvested from each genotype. Fruit was harvested only once, per genotype harvesting the first available fruit. The fruit from each plant were harvested and stored in resealing freezer bags, and kept on ice until stored in a -20°C freezer for further evaluation.

Plant characteristics

Plant characteristics were evaluated in the field and scored on a scale from 1-9 based on an average of the two plants (Table 1). Traits evaluated included: estimated crop load, plant vigor, number of runners, and incidence of diseases. Crop load was estimated based on a scale from having no fruit to being over-cropped. Vigor was determined on the basis of the plant being dead to vigorously growing. The number of runners per plot was estimated from having no runners to having hundreds of runners. Disease was scored in the field at each location from having no disease symptoms to having extreme symptoms for particular diseases in each area, these included: anthracnose (*Colletotrichum* spp.), powdery mildew (*Podosphaera aphanis* [Wallr.] U. Braun & S. Takamatsu), leaf scorch (*Diplocarpon earliana* [Ell. & Ev.] Wolf), leaf spot (*Mycosphaerella fragariae* [Tul.] Landau) with a subset of genotypes screened for response to red stele root rot (*Phytophthora fragariae* Hickman) at Agriculture and Agri-Food Canada, Atlantic Food and Horticulture Research Centre, Kentville, Nova Scotia.

Fruit characteristics

Fruit characteristics were evaluated in the field and scores or measurements were based on an average from assessing two to three berries per plot (Table 1). Techniques for evaluation were based on methods breeders would typically use in the field to rate the fruit, such as tests that can be quickly and simply performed. External fruit traits evaluated included: shape, appearance, fruit deformation due to unfilled achenes, achene color, achene

Table 1. Description of the 37 strawberry phenotypic traits evaluated on 890 genotypes of strawberry (*Fragaria* sp.). Traits were evaluated in 2011 and 2012, as indicated, at locations in California (CA), Michigan (MI), New Hampshire (NH), Oregon (OR) and Nova Scotia (NS) in 2011 and 2012 as part of the USDA-funded RosBREED project.

Trait	Evaluation criteria	2011	2012
Phenology and flowering related traits			
Peduncle length	Scored 1-5; 1 = divides close to crown, 2 = divides at 25% from crown, 3 = divides 50% from crown, 4 = divides 75% from crown, 5 = divides right before flower/fruit	MI, OR, NH	MI, OR, NH
Flowering location	Determined at bloom; Scored 1-2; 1 = above, 2 = below canopy	MI, OR, NH	MI,OR, NH
Presence of anthers	Determined at bloom; Scored 1-2; 1 = yes, 2 = no	MI, NH NH	MI, OR,
Period of flowering	Yes/no, evaluated weekly	MI, OR, NH	CA, MI, OR, NH
Growing degree days for first bloom and harvest date	Mean of daily maximum and minimum temperature, minus the base 50° F (10°C). Beginning 1 January and accumulated throughout the year	MI, OR NH	MI, OR NH
Days from 1 January for first bloom and harvest date	Calculated by summing the number of days from 1 January to the calendar date the first bloom was observed or the	MI, OR NH	CA, MI OR, NH
Truss size	Number of flowers per truss	MI, OR	MI, OR, NH
Harvest date	Date fruit were harvested based on when 30-50% of the fruit were ripe	MI, OR, NH	MI, OR,
Day neutrality	Quantitative weeks of flowering after week 11 from the first week of bloom at each location	CA, MI OR, NH	OR, NH OR, NH
Plant characteristics			
Crop estimate	Amount of fruit on plant; Scored 1-9; 1 = no fruit; 9 = over-cropped	CA, MI OR	MI, OR NH
Vigor	Plant health/growth; Scored 1-9; 1 = dead; 9 = extremely vigorous	MI, OR	MI, OR, NH
Number of runners	Visual estimation of runners; Scored 1-9; 1 = none; 9 = hundreds	MI, OR, NH	MI, OR, NH
Disease	Dependent on the disease naturally present at each location, diseases scored separately when multiple diseases were present. Anthracnose (*Colletotrichum acutatum*), powdery mildew (*Podosphaera aphanis*), leaf scorch (*Diplocarpon earliana*), leaf spot (*Mycosphaerella fragariae*); Scored 1-9; 1 = severe disease; 9 = no symptoms.	MI, OR NH	MI, OR NH
Red stele root rot	A subset was screened for response to red stele root rot (*Phytophthora fragariae*) in greenhouse bench tests; Scored 0-5; 0 = death, 5 = no symptoms	NS	NS
External fruit characteristics			
Shape	Scored 1-9; 1 = long conic, 3 = globose, 5 = globose conic, 7 = cordiform, 9 = oblate	CA, MI, OR, NH	CA, MI CA, MI,

Appearance	Scored 1-9; 1 = very malformed; 9 = symmetrical and attractive	CA, MI, OR, NH	CA, MI, OR, NH
Malformation	Deformation due to unfilled achenes in 1/3 or more ripe fruit; Scored 1-2; 1 = yes, 2 = no	MI, OR NH	MI, OR NH
Achene color	Scored 1-9; 1 = dark; 9 = very light brown or green	MI, OR, NH	MI, OR, NH
Achene position	In relation to fruit surface; Scored 1-3; 1 = sunken, 2 = even, 3 = protruding	MI, OR NH	MI, OR, NH
Percent filled achenes	Percent of all achenes that were filled (10% increments)	CA, MI, NH	MI, OR, NH
External color	Scored 1-9; 1 = white; 9 = dark red	CA, MI, OR, NH	CA, MI, OR, NH
Gloss	Scored 1-9; 1 = dull; 9 = very glossy	CA, MI, OR, NH	MI, OR, NH
Skin strength	How easily fruit was abraded when thumb firmly dragged over flesh; Scored 1-9; 1 = soft; 9 = tough	MI, OR, NH	MI, OR, NH
Cap size	Calyx size in relation to fruit width; Scored 1-3; 1 = calyx smaller, 2 = calyx equal, and 3 = calyx larger	MI, OR, NH	MI, OR, NH
Calyx position	Scored 1-5; 1 = raised (necked), 3 = flat (even with shoulders), 5 = sunken	CA, MI OR, NH	CA, MI NH
Internal fruit characteristics			
Firmness	Firmness of flesh when a fully ripe fruit was compressed between thumb and forefinger Scored 1-9; 1 = very soft; 9 = very firm	MI, OR, NH	MI, OR NH
Ease of capping	Ease with which cap was removed when pulled by fingers; Scored 1-9; 1 = does not remove; 9= very easily removed	MI, OR NH	MI, OR NH
Internal color	Fruit sliced down the meridian; Scored 1-9; 1 = white; 9 = “black”	MI, OR NH	MI, OR, NH
Depth of internal color	Percentage of flesh with solid color (10% increments)	MI, OR, NH	MI, OR, NH
Flavor	Perception of sweetness and presence of off-flavors; Scored 1-9; 1 = not sweet, bad off-flavors; 9 = very sweet, no off-flavors	MI, OR NH	MI, OR NH
Fruit chemistry and weight			
Fruit weight	Average weight (g) of five primary fruit harvested when 30-50% of the fruit on each plant were ripe	MI, OR NH	CA, MI OR, NH
Drip loss	Percent weight lost when frozen berries were thawed	OR	MI, OR, NH
pH	pH of fruit pulp	MI, OR, NH	MI, OR, NH
Percent soluble solids	Percent soluble solids of fruit puree as determined using refractometer	MI, OR NH	MI, OR NH

Titratable acidity	g·L^{-1}citric acid of fruit puree; determined using auto-titrator with pH 8.1 end-point	MI, OR NH	CA, MI OR, NH
Total anthocyanins	mg·L^{-1} Pg-3-gluc equivalents; determined using pH differential method (Lee et al., 2005)	OR	OR
Total phenolics	mg·L^{-1} Gallic Acid Equivalents; determined using Folin-Ciocalteu method (Waterhouse, 2012)	OR	OR

position, percent of filled achenes, external color, gloss, skin strength, cap size, position of the calyx, and ease of cap removal. Fruit shape was scored as long conic, globose, globose conic, cordiform, or oblate. Appearance was scored ranging from very malformed to symmetrical and attractive. Fruit malformation denotes deformed fruit resulting from unset achenes and was scored for presence/absence. Achene color was scored from dark to light brown or green. Achene position was scored as protruding, even, or sunken in relation to the surface of the fruit. The percentage of filled achenes was estimated in 10% increments. External color was scored from white to dark red, and gloss was scored from dull to very shiny. Skin strength was based on how easily fruit was abraded when the evaluator's thumb was firmly dragged over surface of the fruit. Cap size was scored as greater than, less than, or equal to the width of the fruit. The position of the calyx was scored as raised, even or sunken relative to the shoulder of the fruit.

The internal fruit traits evaluated included: fruit firmness, internal color, depth of internal color, and flavor. Fruit firmness was scored by compressing fully ripe fruit between thumb and forefinger and scored from hard to mushy. Ease of "capping" was scored by removing the calyx ("cap") of the fruit. The berries were then sliced down the meridian and internal color was scored ranging from white to deep red. The estimation of depth of internal color was based on the percent of the flesh that was colored. Flavor scores were largely based on the perception of sweetness but also the presence/absence of off-flavors and aromas was considered.

Fruit chemistry

Frozen fruit samples were removed the evening before to fully thaw at room temperature. The number of fruit per bag was noted and the fruit were weighed for use in calculating average berry weight and percent drip loss. At this point fruit was put in a plastic clamshell and stored back in the plastic bag to assist in evaluating drip loss. Drip loss, pH, soluble solids concentration and titratable acidity were measured on the thawed fruit and samples were saved to measure total anthocyanins and total phenolics at a later date.

For all samples, drip loss was measured first. Clamshells were used to remove the thawed fruit from the bag, separating it from the juice without squeezing the fruit, eliminating the release of excess juice or changing the fruit's integrity. The juice was then weighed. Total drip loss was calculated as a percentage of fruit weight by the formula: W_1-W_2/W_1 where W_1 was the average berry weight before drip loss occurred and W_2 was the average berry weight after drip loss.

After measuring drip loss, the fruit in the clamshell were emptied back into the bag with the juice and homogenized by rolling a weighted glass bottle over the bag, grinding the fruit to pulp and crushing the achenes. The pH was measured on all fruit pulp samples by placing the pH probe from the DL 115 auto titrator (Mettler Toledo, Oakland, CA) in the bag of the strawberry homogenate. Percent soluble solids was measured from the pulp by placing a few drops on a refractometer (ATAGO "Pocket" Digital Refractometer PAL-S, Tokyo, Japan). Titratable acidity was measured using the DL 115 auto titrator. Titrations were performed using 5 g of fruit pulp and 45 mL of CO_2-free water. Samples were then titrated with 0.1 N NaOH to pH 8.1 (Plotto et al., 2008). After titrations, 1.5 mL of the homogenate was saved in three 2 mL tubes and stored at -80°C for later measurement of total anthocyanins and phenolics.

Total anthocyanins and total phenolics were measured simultaneously. Thirty minutes prior to testing, one vial of each of the stored samples was thawed, vortexed and centrifuged. Total anthocyanins were measured using the pH differential method as described by Lee et al. (2005) and the determination of total phenolics was performed using the Folin-Ciocalteu method as described by Waterhouse (2002). Both methods were modified for use in 96-well, flat-bottomed microplates (Greiner Bio-One, NC).

Statistical analysis

In order to illustrate the ranges in trait expression seen, trait means were calculated from the combined location of Oregon, Michigan, New Hampshire, and California data for the CRS in 2011 and 2012, and BPS in 2012, using the PROC Means procedure in SAS 9.3 (SAS Institute, Cary, NC). In addition to trait means from the combined locations, the total number of individuals used to calculate each mean and the range of means are reported (Table 2). A full analysis of variance that examines the variability available in this germplasm was conducted but will be presented separately.

Table 2. Mean, minimum and maximum values for 34 traits of the combined crop reference (CRS) and breeding pedigree sets (BRS) from Oregon, Michigan, California and New Hampshire RosBREED locations in 2011 and 2012. Mean performance of genotypes over locations varied significantly ($P \leq 0.0001$) for all traits in both years.

	CRS harvest 2011			CRS harvest 2012			BPS harvest 2012		
Variable	n^z	Mean	Range	n	Mean	Range	n	Mean	Range
Phenology									
Peduncle length	1336	3.6	1-5	2090	2.9	1-5	534	2.9	1-5
Total flowering weeks	1399	5.0	1-16	2151	7.9	1-17	538	8.0	1-17
Flowering cycles	1399	1.1	0-3	2152	1.2	0-3	538	1.1	1-2
Days from 1 January for first bloom	1399	128.5	104.0-216.0	2151	119.3	87.0-359.1	537	123.5	95-164
Growing degree days for first bloom	1399	194.8	0-1247	1508	223.9	0-790	353	252.2	90.9-570.3
Truss size	543	5.2	2-14	1466	4.6	1-17	350	7.0	1-33
Growing degree days for harvest date	937	554.3	325.0-1097.0	1067	639.9	256.0-1667.7	247	670.5	400.0-1851.2
Days from 1 January for harvest date	937	168.8	154-199	1067	157.3	138-208	247	161.9	149-200
Plant characteristics									
Crop estimate	1440	4.0	1-9	1474	3.5	1-9	292	4.9	1-9

Vigor	1244	4.6	1-9	2167	4.0	1-9	417	5.9	1-9
Number of runners	983	4.1	1-9	2142	2.9	1-9	536	3.4	1-9
External fruit characteristics									
Shape	1430	5.7	1-9	1669	5.8	1-9	377	5.6	1-9
Appearance	1430	4.6	1-9	1670	4.6	1-9	376	5.4	1-9
Achene color	906	4.8	1-9	1023	4.4	1-9	120	4.8	2-7
Achene position	907	2.0	1-3	1204	2.2	1-3	247	2.2	1-3
Percent filled achenes	1437	89.2	10-100	1200	85.0	10-100	246	85.2	40-100
External color	1467	6.6	1-9	1404	6.3	1-9	350	6.2	2-9
Gloss	1479	5.9	1-9	1146	5.5	1-9	245	6.2	2-9
Skin strength	935	4.5	1-9	881	5.4	1-9	220	4.8	1-9
Cap size	885	1.3	1-3	1202	1.5	1-3	246	1.4	1-3
Calyx position	1429	3.4	1-5	1725	3.3	1-5	375	3.3	1-5
Internal fruit characteristics									
Firmness	900	5.2	1-9	881	5.5	1-9	220	5.0	2-8
Ease of capping	938	6.0	1-9	878	4.7	1-9	221	4.7	1-9
Internal color	934	5.5	1-9	880	4.7	1-9	221	4.0	1-8
Depth of internal color %	933	72.3	10-100	881	82.7	10-100	221	89.5	20-100
Flavor	688	4.2	1-9	852	4.5	1-9	214	3.3	1-8
Fruit characteristics measured in the laboratory including weight and chemistry									
Fruit weight g	926	11.4	0.3-37.6	1582	6.7	0.2-29.3	375	13.3	1.9-34.9
Drip loss %	549	35.8	0.0-61.7	1016	19.9	0.3-60.8	239	32.2	5.8-65.8
pH	909	3.5	2.8-4.1	1555	3.5	2.9-4.2	423	3.5	3.0-4.3
Percent soluble solids	1480	10.2	3.1-19.5	1578	9.3	1-16.6	424	8.6	4.6-15.6
Titratable acidity (g·L^{-1} citric acid)	910	1.0	0.2-2.2	1557	0.9	0.3-2.2	425	0.8	0.4-2.2
Total anthocyanins (mg·L^{-1} Pg-3-gluc Equivalents)	531	328.8	5.3-1109.3	380	304.1	19.5-1631.5	125	272.0	61.7-542.2
Total phenolics (mg·L^{-1} Gallic Acid Equivalents)	537	347.4	2.0-638.8	379	380.2	1.0-742.6	125	331.6	123.2-589.8
Ratio soluble solids/titratable acidity	821	10.8	3.0-35.1	1557	10.6	3.1-37.3	424	11.5	5.1-26.6

[z] n is the total number of genotypes among the OR, MI, CA and NH locations evaluated for each trait.

Results and Discussion

The strawberry germplasm used was extremely diverse including four European mapping populations, one mapping population from MSU as well as breeding populations from both MSU and USDA-ARS Corvallis breeding programs. Germplasm also included cultivars, founders or the individuals that can be traced back to the beginning of the pedigrees of modern cultivars (Sjulin and Dale, 1987) as well as intermediate ancestors and wild species. Adding to the variation was the multi-location field sites. The diversity in germplasm and environment led to a wide range of minimum and maximum trait values. Mean trait values for locations and the total germplasm is presented in Table 1 to provide an example of how diverse this germplasm was.

A summary of the means of all traits from the RosBREED OR, MI, CA and NH locations for the CRS in 2011 and 2012, and the BPS for 2012, is reported in Table 2. Mean values for traits significantly differed among genotypes ($P \leq 0.0001$) in both the CRS and BPS in 2011 and 2012. This is seen by the trait values encompassing, in many cases, the entire measurement range for each trait. The mean phenology and flowering related traits were fairly similar between 2011 and 2012. The number of days from 1 January to the first bloom was on average 10 days earlier in 2012 for the CRS. The BPS set that bloomed in 2012 was four days earlier than the 2011 CRS bloom and approximately four days after the 2012 CRS bloom. This is similar to the days from 1 January for harvest date, which was on average 10 days earlier in 2012 across locations for the CRS. The BPS harvest was approximately seven days earlier than the 2011 CRS harvest and approximately five days later than the 2012 CRS harvest. The means for plant characteristics are, in general, similar for each year and between each reference set. However, a slightly greater runner number was detected for 2011 for the CRS and, though not exactly comparable, the BPS runner number for its first year is higher than the 2012 CRS runner number. The mean for external fruit characteristics did not differ between each year. The mean for internal fruit characteristics differed slightly such that removal of caps was easier in 2011, and the internal color was slightly darker in 2011. However, the percent of internal color was greater in 2012. The means for fruit chemistry and laboratory measured traits differed. Fruit weight, was on average 4.7 g greater in 2011 for the CRS. The BPS had a high average weight but no second year to compare too. The percent drip loss was, on average, 15.9% greater in 2011. Total phenolics and total anthocyanins differed between 2011 and 2012 for the CRS. Total anthocyanins were on average 24.7 mg·L^{-1} Pg-3-gluc Equivalents greater in 2011, and total phenolics were 32.8 mg·L^{-1} Gallic Acid Equivalents greater in 2012. Both the total anthocyanins and phenolics in the BPS were less than both years of the CRS.

A lack of reliable and available phenotypic data can hinder the use of statistical methods for identifying the associations between phenotypic and genotypic data for breeders and geneticists. Having standardized protocols in place that can be followed and used among different locations allows great power in data replication. When evaluators at separate locations follow the same phenotyping protocol, the limiting factor for combined QTL analysis comes from differences due to genotype by environment interactions.

A complete presentation on these strawberry phenotyping protocols can be viewed at http://www.rosbreed.org/resources/fruit-evaluation. RosBREED strawberry genomic and phenotypic data for all described traits from 2011 and 2012 is being integrated into the Genome Database for Rosaceae "Breeders Toolbox" at http://www.rosaceae.org/breederstoolbox; 2013 (Jung et al., 2008). This database is meant to store and integrate private (BPS) and public (CRS) phenotypic and genotypic data for apple, peach, cherry and strawberry so breeders can more accurately and efficiently assess parent and progeny performance and selection. Phenotypic data collected using this protocol will be useful to identify marker-trait associations and QTL using FlexQTL™ software (Bink et al., 2002; 2008).

Acknowledgements

"RosBREED: Enabling marker-assisted breeding in Rosaceae" is supported by the USDA-NIFA-Specialty Crop Research Initiative by a combination of federal and matching funds (grant number 2009-51181-05808).

Thank you to Ted Mackey and Mary Peterson (USDA-ARS-HCRU) and their 2010-2012 summer field crew for assistance in propagation, field establishment, packaging and shipping plants; staff at the USDA-ARS-NCGR for tissue collection and DNA extraction, and Pete Callow at MSU for phenotyping.

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