



## Phenotyping Protocol for Sweet Cherry (*Prunus avium* L.) to Enable an Understanding of Trait Inheritance



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### Introduction

The USDA Specialty Crop Research Initiative-funded RosBREED project has the objective of enabling marker-assisted breeding (MAB) in the economically important agricultural family of Rosaceae. To standardize and increase the accuracy of MAB in Rosaceae, it is necessary to characterize many horticultural and fruit quality traits in representative germplasm. A well-developed comprehensive phenotyping protocol for productivity, fruit quality and horticulturally objectionable traits, developed for use in the Washington State University (WSU) Sweet Cherry Breeding Program, is described and correlations among selected traits presented.

### Materials and Methods

This study was carried out at Washington State University, Irrigated Agriculture Research and Extension Center, Prosser. Trees were maintained at the Roza farm under standard orchard conditions. Data collected (see Table 1 for protocols) from a sweet cherry 'Crop Reference Set' and 'Breeding Pedigree Set' between 2010 and 2012 were subjected to ANOVA and phenotypic correlations among selected traits were also calculated.

### Results

A strong positive correlation (Figure 1) was observed between harvest date and fruit weight and fruit firmness (+0.26,  $p < 0.0001$  and +0.39,  $p < 0.0001$ , respectively), with later-maturing varieties tending towards larger and firmer fruit. Also, fruit weight has a positive and significant association with pedicel-fruit retention force with a value of 0.43 ( $p < 0.0001$ ). On the other hand, soluble solids content showed a negative relationship with fruit weight and firmness (-0.34 and -0.20, respectively). The box plot for representative traits is presented in Figure 2. There were significant ( $p < 0.05$ ) yearly differences in pedicel fruit retention force, soluble solids content, bloom time and harvest time while titratable acidity was fairly consistent across years.

Figure 1: Phenotypic correlations among 20 sweet cherry traits at WSU Prosser IAREC, WA based on 3 years data combined<sup>1,2,3</sup>

SSC	TA	FL	FW2	FWt	PL	PW2	PWt	FS	CMS	CBS	CFI	ShF	Fir	FirB	HD	HT	BD	BT	
ns	ns	<u>0.38</u>	<u>0.41</u>	<u>0.43</u>	<u>0.24</u>	<u>0.19</u>	<u>0.27</u>	ns	ns	ns	ns	ns	<u>0.31</u>	<u>0.20</u>	<u>0.16</u>	<u>0.16</u>	ns	ns	PFRF
<u>0.33</u>	<u>-0.41</u>	<u>-0.38</u>	<u>-0.34</u>	<u>-0.25</u>	-0.15	-0.15	-0.18	<u>0.29</u>	ns	ns	ns	ns	<u>-0.20</u>	<u>-0.13</u>	<u>-0.18</u>	<u>-0.17</u>	ns	ns	SSC
	-0.14	ns	ns	-0.17	ns	ns	ns	ns	ns	ns	ns	ns	<u>0.23</u>	<u>0.23</u>	<u>0.23</u>	<u>0.23</u>	ns	ns	TA
		<u>0.90</u>	<u>0.89</u>	<u>0.71</u>	<u>0.38</u>	<u>0.49</u>	<u>0.24</u>	-0.15	ns	0.14	<u>0.25</u>	<u>0.41</u>	<u>0.30</u>	<u>0.24</u>	<u>0.23</u>	<u>0.23</u>	ns	ns	FL
			<u>0.95</u>	<u>0.57</u>	<u>0.45</u>	<u>0.50</u>	<u>0.23</u>	ns	-0.29	ns	<u>0.18</u>	<u>0.43</u>	<u>0.31</u>	<u>0.24</u>	<u>0.23</u>	<u>0.23</u>	ns	ns	FW2
				<u>0.61</u>	<u>0.44</u>	<u>0.53</u>	<u>0.15</u>	ns	-0.31	ns	<u>0.19</u>	<u>0.39</u>	<u>0.21</u>	<u>0.26</u>	<u>0.25</u>	<u>0.25</u>	ns	ns	FWt
					<u>0.29</u>	<u>0.60</u>	<u>0.13</u>	ns	ns	ns	<u>0.14</u>	<u>0.21</u>	<u>0.28</u>	<u>0.20</u>	<u>0.20</u>	<u>0.20</u>	ns	ns	PL
						<u>0.43</u>	ns	ns	ns	ns	ns	ns	<u>0.18</u>	<u>0.18</u>	<u>0.16</u>	<u>0.15</u>	ns	ns	PW2
							ns	ns	ns	ns	ns	ns	<u>0.27</u>	<u>0.18</u>	<u>0.26</u>	<u>0.26</u>	ns	ns	PWt
							ns	ns	ns	ns	ns	ns	<u>0.19</u>	<u>0.21</u>	<u>0.19</u>	<u>0.19</u>	ns	ns	FS
							ns	ns	ns	ns	ns	ns	<u>0.19</u>	<u>0.21</u>	<u>0.19</u>	<u>0.19</u>	ns	ns	CMS
							ns	ns	ns	ns	ns	ns	<u>0.19</u>	<u>0.21</u>	<u>0.19</u>	<u>0.19</u>	ns	ns	CBS
							ns	ns	ns	ns	ns	ns	<u>0.19</u>	<u>0.21</u>	<u>0.19</u>	<u>0.19</u>	ns	ns	CFI
							ns	ns	ns	ns	ns	ns	<u>0.19</u>	<u>0.21</u>	<u>0.19</u>	<u>0.19</u>	ns	ns	ShF
							ns	ns	ns	ns	ns	ns	<u>0.19</u>	<u>0.21</u>	<u>0.19</u>	<u>0.19</u>	ns	ns	Fir
							ns	ns	ns	ns	ns	ns	<u>0.19</u>	<u>0.21</u>	<u>0.19</u>	<u>0.19</u>	ns	ns	FirB
							ns	ns	ns	ns	ns	ns	<u>0.19</u>	<u>0.21</u>	<u>0.19</u>	<u>0.19</u>	ns	ns	HD
							ns	ns	ns	ns	ns	ns	<u>0.19</u>	<u>0.21</u>	<u>0.19</u>	<u>0.19</u>	ns	ns	HT
							ns	ns	ns	ns	ns	ns	<u>0.19</u>	<u>0.21</u>	<u>0.19</u>	<u>0.19</u>	ns	ns	BD

<sup>1</sup> Correlation values significantly different at  $p < 0.0001$  level are underlined,  $p < 0.001$  level in italics, and all remaining values are at  $p < 0.01$  level. Values not significant at any of these levels are identified as non-significant (ns).

<sup>2</sup> PFRF=pedicel-fruit retention force, SSC=soluble solids content, TA= titratable acidity, FL=fruit length, FW2=fruit width, FWt=fruit weight, PL=pit length, PW2=pit width, PWt=pit weight, FS=freestone, CMS=mahogany skin color, CBS=blush skin color, CFI=flesh color, ShF=fruit shape, Fir=Firmness, FirB=bulked fruit firmness, HD=harvest date, HT=harvest time, BD=bloom date, BT=bloom time.

<sup>3</sup> Correlations were calculated with the corr procedure in SAS version 9.2 (SAS Institute Inc., Cary, NC).

Figure 2: Box plots of pedicel fruit retention force, firmness, fruit weight, soluble solids content, harvest date, bloom date and titratable acidity based on three years data.

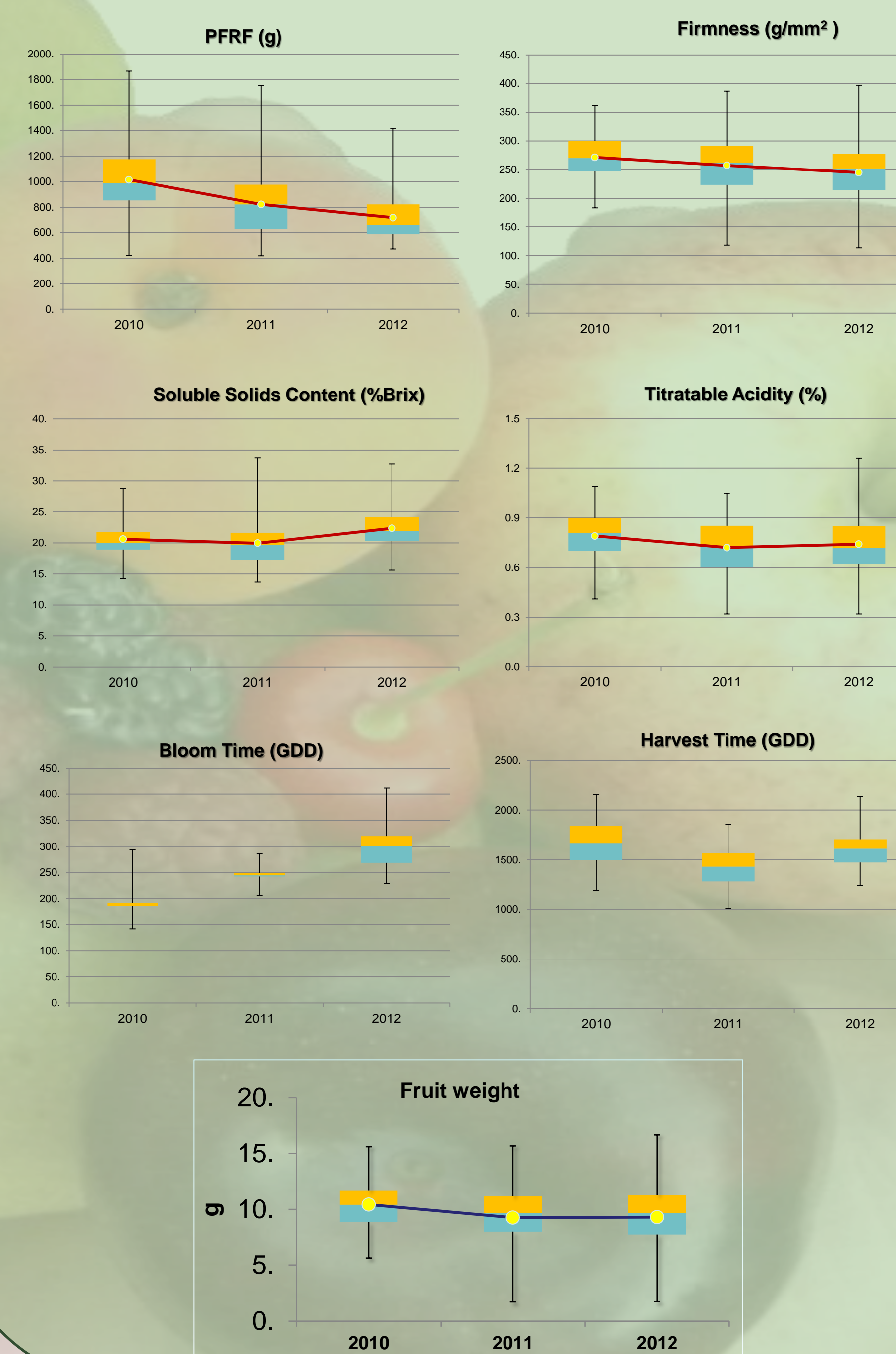


Table 1: Phenotypic traits, trait code, unit of measure and description used to categorize sweet cherry accessions

Trait	Trait code	Unit of measure	Description
Productivity traits			
Bloom Date	BD	Julian day	The number of days from January 1 to date of 50% bloom
Bloom Time	BT	Growing Degree Days	GDD accumulated from January 1 to date of 50% bloom calculated with a base temperature of 4.4 °C
Harvest Date	HD	Julian day	The number of days from January 1 to harvest
Harvest Time	HT	Growing Degree Days	GDD accumulated from January 1 to harvest calculated with a base temperature of 4.4 °C
Fruit quality traits			
Fruit Firmness	Fir	g/mm <sup>2</sup>	Measured by compression (cheek to cheek) on a Firmtech (BioWorks, Kansas USA) using 5 TF <sup>1</sup>
Bulked Fruit Firmness	FirB	g/mm <sup>2</sup>	Measured by compression (cheek to cheek) on a Firmtech (BioWorks, Kansas USA) using 20 BF <sup>2</sup>
Pedicel-Fruit Retention Force	PFRF	gr	This is the force required to pull the pedicel off the fruit measured with a pull force gauge (Imada DS-2). 5 TF samples are used.
Soluble Solids Content	SSC	Brix%	Juice from 5 TF samples pulled together is used and Brix% is recorded on a refractometer (Atago PAL-1)
Bulked Fruit SSC	SSCB	Brix%	Juice from BF samples is used
Titratable Acidity	TA	mg/L	Measured with a 10 ml strained juice from BF on an autotitrator (Metrohm, Florida, USA) titrated to pH endpoint 8.2
pH	pH	pH	Same as above
Fruit Weight	FWt	gr	Average weight of 5 TF
Bulk Fruit Weight	FWtB	gr	Average weight of 20 BF t
Stem Length	SL	mm	Average of 5 TF
Fruit Length	FL	mm	Measured from stem to blossom scar, average of 5 TF
Fruit Width 1	FW1	mm	Measured from the suture to opposite cheek, average of 5 TF
Fruit Width 2	FW2	mm	Measured from cheek to cheek, average of 5 TF
Mahogany Skin Color	CMS	#	Using the CTFILcolor chart and recording 1 to 7
Blush Skin Color	CBS	#	1=0-25%, 2=26-50%, 3=51-75% and 4=76-100%
Fruit Shape	ShF	#	Ratio of Fruit Length and Width; 1=kidney, 2=flat-round, 3=round, 4=elongate, 5=cordate (heart-shaped)
Flesh Color	CFI	#	Visual Flesh Color Card rating 1-5, 1 = cream, 2 = pink, 3 = red, 4 = red purple and 5 = mahogany
Free Stone	FS	#	Pit cling: 1= no cling, 2=slight cling, 3=semi cling, 4=cling, 5=total cling, average of 5 TF
Pit Weight	PWt	gr	Average pit weight of 5 TF
Pit Length	PL	mm	Pit length from point to point, average of 5 TF
Pit Width 1	PW1	mm	Pit width from suture to back, average of 5 TF
Pit Width 2	PW2	mm	Pit width perpendicular to width 1, average of 5 TF
Fruit objectionable traits			
Pitting incidence	PI	%	Calculated as the percentage of fruit with pitting out of a total of 20 fruit sampled
Rain cracking incidence	Cr	%	Calculated as the percentage of fruit with splits out of a total of 20 fruit sampled
Powdery Mildew incidence in field	PM	Scored	0=no visible symptoms, 1=very slight infection on leaves, 2=slight infection on leaves, occasionally on shoots, 3=moderate infection on leaves and/or shoots, 4=heavy infection on leaves and/or shoots and 5=very high infection on leaves, most shoots damaged
Bacterial Canker incidence based on a detached leaf assay	BC	Scored	0=no necrosis, 1=line of infection, 2= ≤50% of the leaf, 3= >50% of the leaf and 4=total necrosis

### CONCLUSIONS

Correlations revealed through statistical analysis of data collected in multiple harvest seasons can be used to elucidate trends in trait associations. Measuring either bulked fruit firmness or firmness based on five fruit only, as well as, either bloom/harvest time in julian days or growing degree days, will streamline the phenotyping protocol and improve cost effectiveness. The significant and positive correlations between SSC and TA as well as between harvest date, fruit size and firmness may suggest a genetic linkage of genes controlling these traits. Use of this standardized phenotyping protocol will enable comparisons of data between years and programs and aid in identifying sweet cherry regions that control trait variation.