International Journal of Botany

ISSN: 1811-9700

science alert

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International Journal of Botany 5 (2): 160-165, 2009
ISSN 1811-9700

Identification of Peach Genotypes (*Prunus persica* (L.) Batsch) in the North-Central Region, Mexico

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Abstract: Twenty four peach genotypes from the Central North Region of Mexico, were characterized based on morpho-physiological traits. Fruit weights from the genotypes Roxana (135 g), San Gabriel C-167 (141.9 g) and Zacatecas landrace (162.3 g) were the higher, each in its group, since all genotypes were grouped according to their readiness to harvest as early-, middle- and late-harvest, respectively. RAPDs analysis yielded 52 monomorphic and 93 polymorphic fragments that were related to desirable characteristics from the *Prunus* genotypes. This information provide us tools for early individual identification of high-performance trees when still growing in the nursery. Therefore, growers may use this technique for assisted breeding program on their *Prunus* genotypes.

Key words: Peach (*Prunus* spp.), RAPDs, genotypes

INTRODUCTION

Peach (*Prunus* spp.) is a fruit well appreciated either fresh or canned due to its flavor, color and aroma. Additionally, this is a low-calorie fruit, useful in the human diet as a source of potassium, vitamin A and ascorbic acid (Skorza and Okie, 1990). Peaches are grown in temperate climates both sides of the Equator between parallels 30 and 40° (Joshi and Bhutani, 1995). In Mexico, Spaniards started peach cultivation in some areas and since then, only recently peach production has doubled in several states such as Chihuahua, Estado de México, Morelos, Sonora, Tlaxcala, Guanajuato, Hidalgo, Michoacán, Aguascalientes and Nayarit (Anonymous, 2008). These early and recent genotype introductions resulted into heterogeneous landraces and recently imported cultivars. As a consequence of these genotype population, characteristics of budding, flowering, ripening, vigor, quality and yield are heterogeneous among orchards (Gutiérrez-Acosta and Padilla-Ramírez, 2004). Furthermore, low yields (averaging 10.8 t ha^{-1}) and high labor required during the usually short harvest period make orchard management somewhat difficult.

Traditionally, in some Mexican regions, local identification and characterization of the genus *Prunus* spp. was mainly based on morphological, physiological and fruit traits, originating multiple names for the same genotype and scarce contribution to breeding programs. Nowadays, statistics report the presence of 52 genotypes (from USA) in Chihuahua, 12 genotypes (11 landraces and one genotype from Brazil) in Estado de Mexico and 4 genotypes in Michoacán (Martínez, 2005). Among the landraces of the last two states, Diamond is one outstanding genotype which has been used for breeding by the Colegio de Posgraduados. In Aguascalientes, the landrace San Gabriel has been present in the local commercial orchards over 30 years and has been the source of new genotypes with fruit and highly appreciated by the consumers, fruit flesh tightly adhered to the stone and yellow skin (Gutiérrez-Acosta and Padilla-Ramírez, 2004; Pérez, 2006).

Normally, for a peach three to develop completely all of its morphological features, the minimum required time is about 3-4 years. Nevertheless, it is possible to identify *Prunus* plants with good characteristics, without waiting too long, using molecular markers link to agronomic characteristics (Dirlewanger *et al*., 1998; Warburton and Becerra-Velásquez, 1996). These techniques are: RFLPs, RAPDs, AFLPs and SSRs (Martínez-Gómez and Sánchez-Pérez, 2005). From these, RAPDs (Random Amplified Polymorphic DNAs) are suitable for basic methods since is simple and does not require radioactivity

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(Bardacki, 2001). This specific technique has been applied to *Prunus* in order to identify cultivar and species (Martins and Tenreiro, 2003; MirAli and Nabulsi, 2003; Baránek *et al*., 2006) and genetic maps assembly (Warburton and Bliss, 1996; Dettori *et al*., 2001). Due to the fact that in Mexico we have not applied these techniques systematically, the objective of this study is to identify of the best peach (*Prunus persica*) genotypes through molecular markers (from RAPDs) associated to morpho-physiological descriptors.

MATERIALS AND METHODS

Morpho-physiological data was obtained from 24 peach (*Prunus persica*) genotypes (Table 1) grown under similar management (pruning, fertilization and irrigation) on a commercial orchard in Aguascalientes, Mexico. Additionally, DNA samples required to perform RAPDs analyses were taken from the leaf tissue of each genotype. Trees grown on that orchard are a heterogeneous collection of crosses derived from genotypes obtained from the North-Central region of México, Brazil, USA and Spain (Table 1). Fifty nine morphological traits (such as growing pattern, number of fruits per branch, fruit abscission and maturation pattern, color, texture, shape, °Brix and presence/absence of protuberance, etc.) were used to construct a phenogram by the simple matching coefficient procedure (Magurran, 1988). Genomic DNA was extracted from the 24 *Prunus* genotypes and the samples were run on 0.8% agarose gel. DNA concentration was measured with a spectrometer (model GBC Cintra 10e UV-visible) (Doyle and Doyle, 1990). RAPD reactions were performed in a 25 μL volume consisting of 10X buffer solution [10 mM Tris-HCl buffer (pH 8.0), 50 mM KCl_2], 2.5 mM $MgCl_2$, 2.5 unit of Taq DNA polymerase (Promega), 100 μM dNTP, 50 ng genomic ADN and 0.4 μM OPM series (Table 2) primer (Operon Technologies, Alameda, CA, USA) according to Williams *et al*. (1990). A total of 20 μL of mineral oil was placed over the reaction mixture (Williams *et al*., 1990). Amplifications were carried out in a DNA thermocycler (Model FPR0G02Y Techne Progene, England), with the following conditions: an initial denaturation step of 2 min at 94°C, followed by 35 cycles of 1 min at 94°C, 1 min at 35°C and 2 min at 72°C with a final extension step of 7 min at 72°C (Williams *et al*., 1990). Amplification products were analyzed by electrophoresis in a 1.2% agarose gel run at 100 V for 4 h and detected by staining the gel with ethidium bromide (10 ng/100 mL of agarose solution in TBE) (Williams *et al*., 1990). All visible and unambiguous fragments amplified by the primers chosen were entered under the heading of total visible fragments. Fragment data were entered on a spreadsheet to form a binary matrix, where (1) represented fragment presence and (0) absence for each fragment-accession combination. Cluster analysis was conducted by converting the data matrix into a similarity matrix using a simple matching coefficient (Nei, 1973). This coefficient was calculated by dividing the number of matches (0-0 and 1-1) by the total number of comparisons (Nei and Li, 1979). A cluster analysis was

Table 1: Morpho-physiological and productive description in the genotypes

			Fruit descriptions				
Genotypes	Origins	Codes	Maturing	°Brix	Firmness (N)	Weight (g)	Colors
NG*	Guanajuato	C-1	Early	12.3	3.2	129.0	Yellow
San Gabriel	Aguascalientes	C-3	"	11.3	7.0	68.9	"
Roxana	"	C-4	"	10.6	6.9	135.0	"
CP-9115C	Estado de México	C-5	"	13.3	4.1	103.5	"
CP-9116C	"	C-6	"	11.5	7.3	83.8	"
San Carlos	Aguascalientes	C-10	"	9.7	5.6	94.7	"
CP**	Estado de México	C-13	"	14.5	6.7	125.8	"
San Gabriel	Aguascalientes	C-163	"	12.4	7.3	118.4	"
San Gabriel	"	C-167	"	11.3	7.5	141.9	"
San Gabriel	"	C-20	Intermediate	10.0	7.4	111.4	"
San Gabriel	"	C-161	"	10.8	7.9	121.5	"
San Gabriel	"	C-162	"	12.7	7.2	82.2	"
San Gabriel	"	C-164	"	11.1	6.8	124.2	"
San Gabriel	"	C-172	"	12.4	8.9	123.5	"
San Carlos	"	C-175	"	9.9	7.0	94.3	"
San Gabriel	"	C-176	"	10.2	7.3	89.7	"
Ana	"	C-49	Late	10.1	5.9	91.3	Speckled
San Gabriel	"	C-66	"	8.4	7.6	109.8	Yellow
Landrace	Zacatecas	C-227	"	10.1	5.0	142.9	"
Landrace	"	C-229	"	10.2	5.4	130.2	"
Landrace	"	C-233	"	8.8	4.9	106.5	Speckled
Landrace	"	C-234	"	10.3	7.3	162.4	Yellow

*Norte de Guanajuato genotype, **Colegio de Postgraduados genotype

Table 2: Amplified fragments from 24 peach (*Prunus persica* L. Batsch) genotypes by means of 14 primers series OPM grown in Central North Mexico

		Amplified fragments			
Primers	Sequence 5'-3'	Total	Polymorphic	Monomorphic	Range (pb)
OPM-1	GTTGGTGGCT	12	8	4	506-3054
OPM-2	ACAACGCCTC	9	8	1	396-3054
OPM-3	GGGGGATGAG	12	5	7	298-3054
OPM-5	GGGAACGTGC	11	9	2	506-3054
OPM-6	CTGGGCAACT	13	9	4	298-3054
OPM-7	CCGTGACTCA	12	8	4	396-3054
OPM-10	TCTGGCGCAC	14	11	3	298-4072
OPM-12	GGGACGTTGG	16	9	7	298-3054
OPM-13	GGTGGTCAAG	9	5	4	506-4072
OPM-14	AGGGTCGTTC	3	1	2	1636-3054
OPM-15	GACCTACCAC	4	3	1	506-3054
OPM-16	GTAACCAGCC	14	9	5	396-4072
OPM-18	CACCATCCGT	3	3	0	1018-3054
OPM-20	AGGTCTTGGG	13	5	8	506-3054
Total		145	93	52	

then done using the unweighted pair group method, with arithmetical averages (UPGMA) process in the S-Professional Plus 2000 program.

RESULTS AND DISCUSSION

Morpho-physiological traits and productivity analysis: Table 1 shows a brief description of the used genotypes according to investigated traits such as place of origin, ripening, °Brix, fruit firmness, weight and color. Identified genotypes as early maturing were harvested between 15th of June and 15th of July; intermediate genotypes were harvested between the 16th of July and 15th of August; and late maturing genotypes were harvested after the 16th August. Comparing among the genotypes of the early maturing group, Roxana was found superior on fruit quality and higher yields (41.6 kg tree^{-1}). Likewise, San Gabriel (C-161) was the best among the genotypes from intermediate maturing group with yields of 54.67 kg tree^{-1} and good fruit weight (121.5 g). In the late maturing group, Zacatecas landrace (C-234) showed the highest fruit weight (162.3 g); although, yields were poor (13.64 kg tree^{-1}).

Two groups arose from the morphological trait analysis were obtained (data no showed). San Gabriel (C-3) was placed in Group I; nevertheless, this genotype seemed to be independent since it showed protuberance, high abscission rate, erectile plant growth, small fruits (60.7 g fruit^{-1}) and shorter maturation period as compared to other genotypes within the early maturation group. Group II was characterized by horizontal branching, higher fruits and different maturation behavior. This morphological analysis allowed finding the relationship between genotypes obtained from different sites from *Aguascalientes* and *Zacatecas*. As an example, San

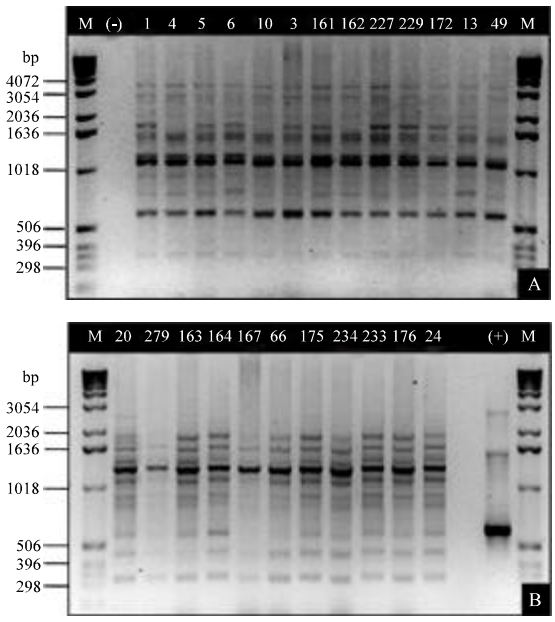

Fig. 1: RAPD analysis on peach genotypes (*Prunus persica* L. Batsch) grown by means of 14 primers OPM series: OPM-10 (A) and OPM 12 (B) in Central North Mexico region

Gabriel (C-167) and *Zacatecas landrace* C-175 are different only on the maturation period the rest of the characteristics appeared the same; furthermore, San Gabriel coded as C-175 and C-176 were very similar in both maturation and fruit firmness. Other findings were: both genotypes C-1 and C-229 (from Guanajuato and Zacatecas, respectively) showed the lowest fruit firmness. Genotypes C-164 and C-172 (San Gabriel) were slightly different on °Brix. Finally, Roxana (C-4) and San Gabriel (C-163) were similar on the maturation period.

RAPDs analysis results: Amplification of 14 fragments was obtained using primers OPM-12, OPM-10 and OPM-16, while using primers OPM-14 and OPM-18 yielded only 3 discrete bands each (Table 2). In general, amplicons ranged approximately from 300 to 4070 bp in size. Some of them were found to be genotype-specific as shown in Table 2 and Fig. 1a, b. Genotype-specific fragments shown were for genotype C-277 (3054 bp amplified with OPM-18) and genotypes C-175 and C-234 (5054, 2036 bp, respectively) amplified with OPM-13 (no showed). Fortunately, the above mentioned genotypes have good qualities for breeding (Table 1). Once the dendrogram was obtained using only the polymorphic fragments, it was evident the presence of two main groups: Group I includes only to genotype C-279 which is a *Prunus domestica* (May Bell Plum) imported from South

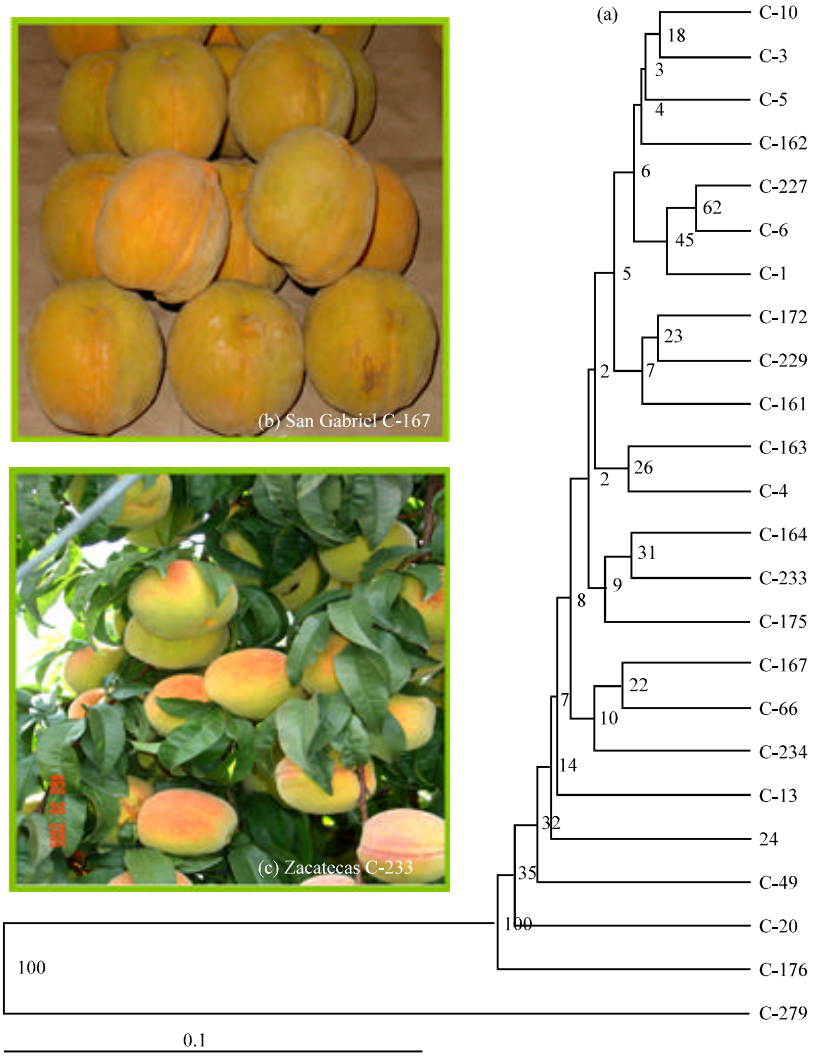

Fig. 2: (a) Dendrogram obtained from 24 peach (*Prunus persica*) genotypes grown in the Central-North region of Mexico, (b) C-167 and (c) C-233

Carolina USA, while Group II includes to the rest of the genotypes (*Prunus persica*) (Fig. 2a). Genotype C-6 (NG) and C-227 (Zacatecas landrace) showed the similarity coefficients highest (0.97349). In spite of that similarity, these genotypes differ on fruit firmness and maturation period. Low similarity coefficients (0.6091 and 0.6122) were obtained when compared genotypes C-279 to genotypes C-6 (CP-9116C) and C-227 (*Zacatecas landrace*), respectively. Among peaches, the lowest similarity values (0.8584 and 0.8622) were found when genotype C-176 was compared to C-4 and C-161, respectively. These results would be indicative of the close genetic relatedness of the peach genotypes grown in the Central-Northern Region of Mexico.

DISCUSSION

Due to the high cross pollination rate present in the genus *Prunus* spp., new genotypes can be developed with higher yields or better fruit quality. This breeding process could be accelerated if assisted by molecular markers associated with desired traits (Jun *et al*., 2002), helping in the process of selecting candidates from germinating plants. At the same time, molecular marker analyses would help on standardization for genotype naming or labeling all over Mexican peach production regions, in order to avoid confusion and promoting conservation of valuable gene sources. This study is considered the one of the first attempts in Mexico for

establishing genetic fingerprints on peach that may help on taking decisions for potential introduction of the appropriate cultivars for the specific regions. It may be a good start focusing on the genotypes with higher yields and good fruit quality such as Roxana (C-4) from the early maturing group, San Gabriel (C-172) from the intermediate maturing group and a *Zacatecas landrace* (C-234) from the late maturing group as model for peach breeding. Indeed, the remaining samples were differentiated and clustered together as a group of cultivars or genotypes without any significant relation to some botanical or agricultural traits as is indicated by Raddová *et al.* (2003) when analyzed peaches provide by different countries. Better distinguishing results were detected by means of other amplification strategy in the work of Jun *et al.* (2002) were they found RAPDS and SCAR markers linked to the flesh adhesion gene (F) in peach (*Prunus persica*) and Cheng (2007) classified the peach groups as yellow, honey, flat, red leaf, crisp, bitao and juicy. In the future, the local collection from peach (*Prunus* spp.) growers will be extended and probably will be used for further plant breeding (gene identification o genetic transformation) activities.

ACKNOWLEDGMENTS

Thanks to Eng. Leonel Quesada Parga and Aguascalientes peach growers for their support to C.M. Melgoza Villagómez while pursuing her Master´s degree at the ITEL. Our thanks to Eng. Pablo Arturo Alemán and Eng. Alberto Peralta Bringas for the help provided. Finally, we appreciate the great labor provided by Dr. Pérez y Dr. Rodríguez, since some genotypes were obtained by their peach breeding programs.

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