Morphological, Molecular, and Self-(In) Compatibility Characteristics of New Promising Apricot Genotypes

H. Pinar1, S. Ercisli2, M. Bircan3, M. Unlu3, A. Uzun1, K. U. Yilmaz1, and M. Yaman1

ABSTRACT

World apricot (Prunus armeniaca L.) production is increasing steadily due to breeding of new high yielding cultivars in different countries. More recently, breeding programs have been modified according to consumers’ demands and also improvement in resistance to diseases (Sharka, Monilinia etc.), frost damages, and determination of self-(in)compatibility. In this study, fourteen apricot breeding progenies and six of their parents were evaluated by using both morphological and molecular markers. As morphological markers, fruit weight, width, length, height, total soluble solids, acidity, and fruit firmness were used. In molecular analysis, to determine genetic relationships, Sequence-Related Amplified Polymorphism (SRAP), Inter-Simple Sequence Repeat (ISSR) and Damage-Associated Molecular Patterns (DAMP) markers were used. In addition, SRc-F/R markers were used to determine S allele profile. The results showed that, although there were no earlier genotypes than Ninfa and Priana, Ay × P3 cross was a promising genotype with regard to earliness and fruit characteristics. A total of 224 scorable bands obtained with 8 SRAP primer combinations (25 bands), 8 DAMP primers (81 band) and 16 ISSR primers (118 bands) showing high diversity among crosses and cultivars. A total of 4 S-RNase alleles (S_C, S_2, S_3, S_6) were identified in this study and the most widely identified alleles were S_C and S_3 alleles.

Keywords: Cross breeding, Hybridization, New variety, Prunus armeniaca.

INTRODUCTION

Turkey dominates world apricot production with 795,000 tons yearly production, followed by Iran (465,000 tons) and Italy (247,000 tons) (FAO, 2012). Apricot production in the world is increasing steadily year-by-year and such increase is closely related to breeding activities carried out in different apricot producing countries. Apricot breeding programs are mostly modified to meet consumers’ demands and preferences and also based on certain traits such as resistance to diseases (Sharka, Monilinia etc.), frost damages, and self-compatibility (Hormaza et al., 2007; Ercisli, 2009; Yilmaz and Gurcan, 2012).

The primary goal in fruit tree breeding programs is to develop new cultivars with desired characteristics in an economical way. Despite the rich diversity in common apricot germplasm, high degree of heterozygosis within the species, most of the time, slow down the breeding processes. Production in many countries comes from chance seedlings and local cultivars (Bassi, 1999). Limited market value of local cultivars and poor adaptability of cultivars out of their native area are considered among the primary factors limiting the expansion of apricot growing sites in the world (Badenes et al., 1998). Although the objectives in apricot breeding programs differ based on the country and on intent of

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use (dry, fresh, or canned), there are certain selection criteria commonly applied to most apricot breeding programs (Hormaza et al., 2007).

More recently, climatic adaptation is considered one of the basic targets in the majority of apricot breeding programs. Apricot cultivars have highly specific ecological requirements. Thus, commercial production is commonly limited to certain locations where generally a couple of cultivars account for large portion of the production. Then, there is a need to evaluate apricot cultivars for high and reliable yield in each production site. Adaptation works are performed based on location and such adaptation works commonly involve breeding for late blooming cultivars to avoid frost damages, breeding for early blooming (low chilling) in frost-free regions, breeding to develop early ripening cultivars, or breeding for greater mid-winter cold hardiness in colder regions. Local adaptations are mostly expressed in terms of productivity and regularity of production and such adaptations are directly related to specific environmental conditions (Hormaza et al., 2007).

Apricot cultivars are mostly evaluated by morphological, molecular, and self-(in) compatibility characteristics (Ercisli, 2009). Morphological characteristics are among the most significant quality attributes affecting consumers’ preferences. For apricots, large size (more than 60 g), attractive appearance (a bright blush over bright orange or cream), firmness, freestone, uniform ripening, and resistance to skin cracking are the primary quality attributes. Good orange skin and flesh colors, as well as uniform medium size, regular shape, good texture, high sugar content, small pit, and a good balance of acid and sugar are preferred for canned apricots. On the other hand, high soluble solids, medium-large size and good texture are required for dried apricots (Ercisli, 2009).

The molecular marker technology, with a rapid development during the last 20 years, offered various new approaches in identification of genetic diversity among the cultivars and, today, molecular markers are efficiently used in plant systematics, breeding, and assessment of gene sources (Kaczmarska et al., 2015; Mishra et al., 2015; Nemli et al., 2015; Wojnicka-Poltorak et al., 2015). So far, different DNA-based marker techniques such as Restriction Fragment Length Polymorphism (RFLP), Random Amplification of Polymorphic DNA (RAPD), Amplified Fragment Length Polymorphism (AFLP), Simple Sequence Repeats (SSR) have been applied to assess the genetic diversity and relationships between apricot cultivars.

Species of the family Rosaceae show Gametophytic Self-Incompatibility (GSI) to prevent inbreeding depression. This intercellular reaction is controlled by a single multi-allelic locus, the S-locus (de Nettancourt, 1977). The S-gene product in styles is a Ribonuclease enzyme (S-RNase) (McClure et al., 1989), while the pollen product is an F-box protein (Romero et al., 2004). Knowledge of the self-compatibility of commercial cultivars and selections from breeding programs is imperative for any apricot breeder hoping to design compatible combinations (Burgos et al., 1998). In apricot, 7 S-alleles (S$_1$,S$_7$) were described in North American and Spanish apricot cultivars for self-incompatibility, while the SC-allele was identified as responsible for self-compatibility (Alburquerque et al., 2002). Later, 9 additional alleles (S$_{10}$–S$_{16}$) were found using Non-Equilibrium pH Gradient Electro focusing (NEpHGE) and Polymerase Chain Reaction (PCR) analyses (Halász et al., 2005). From Chinese cultivars, more than 50 S-alleles have been identified based on S-RNase activity staining, PCR, and sequencing (Jie et al., 2005; Zhang et al., 2008; Wu et al., 2009; Halász et al., 2012). In total, 17 Cross Incompatibility Groups (CIG) are known, including North American, European, Turkish, and Tunisian apricot cultivars (Szabó and Nyéki, 1991; Egea and Burgos, 1996; Halász et al., 2010; Milatovic et al., 2010; Lachkar et al., 2013).
In Turkey, the first planned apricot-breeding program was initiated in 1989 to develop new table apricot cultivars; and with this program, 5 apricot cultivars (Dr. Kaska, Çağataybey, Çagribey, Sahinbey and Alata Yıldızı were released (Bircan et al., 2010). In addition, a cross breeding study between Turkish (Alata Yıldızı, Çagribey, Çağataybey and Sakit-6) and foreign (Priana, Feriana and Precocce de Colomer) parents were initiated. Thus, in the present study, the aim was to select 14 crossbreed genotypes with regard to earliness, tree structure, fruit yield, fruit characteristics and development status and, for the first time, assess them with regard to pomological characteristics, self-compatibility, and genetic similarity.

**MATERIALS AND METHODS**

In this study, a total of 20 apricot genotypes (14 of them are new hybrid and 6 of them are their Turkish and foreign parents) were used. The 14 new hybrids were obtained in cross breeding study within the table apricot breeding program initiated at Alata Horticultural Research Institute. This breeding program was started in 1989 by using Alata Yildizi, Cagribey and Çağataybey as Turkish parents and Priana, Feriana and Precocce de Colomer as foreign parents. The 14 crossbreed genotypes were selected with regard to earliness, tree structure, fruit yield, fruit characteristics, and development status. For pomological characteristics, fruit analyses on crosses were performed in 2013, 2014, and 2015 and average of 3 years were provided for all traits, except for harvest dates. Average fruit weight, width, length, height, seed weight, fruit firmness, Total Soluble Solids (TSS%), titratable acidity (%), TSS/acid ratio, and pH were determined. Samples were taken from 3 trees in 3 replications with 25 fruits in each replication. Data gathered through quality analyses on fruit samples were subjected to statistical analyses with JMP software and means were compared with Tukey test. Sequence-Related Amplified Polymorphism (SRAP), Inter-Simple Sequence Repeat (ISSR) and Damage-Associated Molecular Patterns (DAMP) marker systems were used to identify genetic similarities among the crosses and parents. S allele analysis was also performed on crosses and parents. In molecular study, total genomic DNA was extracted from fresh leaf tissues by using the CTAB method described by Doyle and Doyle (1990). A microplate spectrophotometer (BioTek Instruments Inc. Vinoskii, USA) was used to measure DNA concentrations and 10 ng μL⁻¹ DNA templates were prepared by using TE (10 mM Tris-HCl, 0.1 mM EDTA, pH 8.0). Eight DAMP primers (URP1F, URP2F, URP2R, URP4R, URP6R, URP9F, URP13R and URP17R), eight combinations of 14 forward and 16 reverse SRAP primers and sixteen ISSR primers (CA8R, HVHTGT7, GACA4, DBDACA7, GT8YA, AGC6G, HVHCA7T, AG7Yc, CT8T6, TCC5RY, BDCCA7C, HVHTCC7, GA8YG, CAA6, AG8T, GT6GG) were used to target the amplification of marker sequences from genomic DNA of genotypes. For SRAP (Li and Quiros, 2001), ISSR (Uzun et al. 2015) and DAMP (Karaca and Ince, 2008), PCR conditions were used. Polymerase chain reaction (PCR) products were separated on 2.5% agarose gel at 90V for 4 or 5 hours. Amplified DNA bands were visualized with ethidium bromide staining. The fragment patterns were photographed under UV light for further analysis. A 100 bp standard DNA ladder was used as the molecular standard in order to confirm the appropriate markers.

Each band was scored as present (1) or absent (0) and data were analyzed with Numerical Taxonomy Multivariate Analysis System (NTSYS-pc) software (Rohlf, 2000). A similarity matrix was constructed by using SRAP, DAMP and ISSR data based on Simple Matching (SM) coefficient. Then, the similarity matrix was used to construct a dendrogram with UPGMA (Unweighted-Pair Group Method Arithmetic average) to determine genetic relationships among the cultivars studied. The genetic similarity
matrix and ultrametric distance matrix produced from UPGMA-based dendrogram with COPH module nested in the same software was compared using Mantel’s matrix correspondence test (Mantel, 1967).

SRc-R (5’-GGC CATTGT TGC ACA AAT TG-3’) (Vilanova et al., 2005) and SRc-F (5’-CTC GCT TTC CTT GTT CTT GC-3’) (Romero et al., 2004) primer pairs were used to determine S allele composition of apricot genotypes (Vilanova et al., 2005). The PCR products were electrophoresed in 2% (w/v) metaphor agarose stained with ethidium bromide (0.5 lg mL⁻¹) using 1X TAE buffer at 110V for 2 hours and visualized under UV light. Molecular size of the amplified fragment was estimated using a 100-bp ladder (Thermo).

RESULTS AND DISCUSSION

New apricot cultivars are mostly characterized with their high fruit quality attributes satisfying consumers’ preferences (Ruiz and Egea, 2008). The pomological characteristics of new apricot crosses and parents are shown in Table 1. The differences in fruit quality attributes of the crosses and cultivars were found to be significant at 5% level (Table 1).

Earliness is the main desired attribute in fresh apricots grown in the Mediterranean region of Turkey (Polat and Caliskan, 2013). Feriana was the earliest harvested cultivar in 2013 (May 9) followed by Ninfa (May 14), Ay×P3 (May 20) and Fer×Col9 (May 25), respectively. The latest harvest was performed on June 7 in Fer×Col12 genotype. In 2014, the earliest harvest was performed on May 8 in Feriana cultivar, on May 9 in Ninfa cultivar, on May 17 in Ay×P3, Fer×Col12 and Fer×Col15 genotypes. In 2015, the earliest harvest was performed on May 5 in Feriana cultivar, on May 9 in Ninfa cultivar, on May 16 in Fer×Col12 genotype and May 12 in Fer×Col15 genotype. Polat and Caliskan (2013) reported Beliana and Feriana (May 20) as the earliest and Precoce de Colomer as the latest (June 7) ripening apricot cultivars in eastern Mediterranean in Turkey. In previous researches, ripening dates of apricot cultivars were reported as between May 14-June 26 in Spain (Ruiz and Egea, 2008), between June 11-September 10 in Hungary (Hegedus et al., 2010) and between May 26 - June 25 in Italy (Lo Bianco et al., 2010).

Fruit size is another significant quality attribute in apricot affecting consumers’ preferences. The greatest fruit weight was observed in Ay×P3 genotype (65.10 g) and the lowest value was seen in Fer×Col5 (25.52 g) genotype and Priana (24.95 g) cultivar. The genotypes Ay×P5 (64.98 g) and Ay×P7 (64.17 g) had the greatest fruit weights after Ay×P3 genotype. The greatest fruit width was again observed in Ay×P3 (47.76 mm) genotype, and it was followed by Ay×P5 (47.27 mm) and Ay×P7 (46.85 mm) genotypes. The lowest fruit width was observed in Priana (32.87 mm) cultivar. Fruit length was the highest in Ay×P3 (50.13 mm) genotype and the lowest in Priana (34.41 mm) cultivar. With regard to fruit height, the greatest value was observed in Ay×P5 (50.13 mm) genotype and the lowest in Fer×Col10 (33.26 mm) genotype. With regard to seed weight, the highest value was seen in Fer×Col7 (5.03 g) genotype and the lowest in Fer×Col15 (2.52 g) genotype. Fruit flesh firmness is an important quality attribute for export and shelf life in local markets. Although Ninfa is an early cultivar, it is negatively affected in export because of low flesh firmness. The greatest flesh firmness was observed in Cgr×Col1 (3.44 kg cm⁻²) and the lowest in Fer×Col5 (0.87 kg cm⁻²). The TSS content is an important quality parameter with significant impacts on fruit taste (Polat and Caliskan, 2013). Total soluble solid (TSS) content was the highest in Cgr×Col1 (15.11%) and the lowest in Fer×Col9 (9.58%) genotype. The greatest titratable acidity was observed in Fer×Col10 (3.18%) genotype and the lowest in the Ninfa (1.22%) cultivar. The highest TSS/acid ratio was seen in the Cagataybey (11.08%)
### Table 1. Harvest dates and some fruit quality parameters of apricot genotypes and their parents from 2013, 2014, and 2015 growing seasons.

<table>
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<tr>
<td>20</td>
<td>Priana</td>
<td>May 09</td>
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</table>

*Fruit weight; *Fruit weighty; *Fruit height; *Fruit size; *Seed weight; *Fruit firmness; *Fruit acidity; *Total Soluble Solids.
cultivar, the lowest TSS/acid ratio was observed in Ay×P5 (2.95%) genotype. Polat and Caliskan (2013) assessed the phenological and pomological characteristics (flowering period, average fruit weight, yield, fruit dimensions, flesh/seed ratio, acidity, and total soluble solids) of seven apricot cultivars (Beliana, Canino, Precoce de Colomer, Feriana, Rouge de Sernhac, Tokaloğlu, and Macar) in Hatay, Turkey. In that study, the highest average fruit weight was obtained from Rouge de Sernhac (37.9 g), while Feriana had the lowest (30.9 g) value; flesh/seed ratio was the highest in the Canino (16.8), which had the highest TSS content with 14.5%, whereas Beliana had the lowest TSS value with 10.6% and researchers recommended Beliana and Feriana cultivars for early production in Hatay. In the present study, Cgr×Col1 hybrid was prominent with TSS value.

In a previous breeding program, Bircan et al. (2010) used Alyanak, Sakit-1, Sakit-2, Sakit-6, 07-K-11 as local and Cafona, Canino, Fracasso, Joubert Foulon and Precoce de Colomer as foreign apricot cultivars and reported that 370 of 4,173 crosses contained desirable traits. Among them, 5 new apricot cultivars were registered as Dr. Kaska, Çağataybey, Çağribey, Sahinbey and Alata Yildizi. Dr. Kaska has juicy and freestone fruits. Its fruits are sweet and aromatic. Çağataybey is freestone and fruits are juicy, aromatic, and more colored. Çağribey is freestone, juicy, and aromatic. Alata Yildizi is also freestone and has good fruit quality. In that study, Alata Yildizi had the greatest fruit weight and Çağataybey had the highest TSS (%) value. In the present study, the greatest fruit weight was observed in Ay×Pr-3 crosses and the highest TSS content was observed in Cgr×Col-1 crosses. Pinar et al. (2008) determined that 2-89 genotype had the highest yield, Harcot had the largest fruit and Bebeco had the firmest fruits. While Ninfa, Priana, Precoce de Tyrinthe was found as the earliest cultivars, 15-90, Fracasso, Sahinbey were determined as the latest genotypes in the Turkey from the same breeding program.

Yilmaz and Gurcan (2012) indicated that a precise characterization and discrimination of the cultivars were pre request for breeding of promising apricot cultivars. Different markers including morphological, molecular, and biochemical markers were employed in apricot. ISSR markers were used by Chenjing et al. (2005) and Yilmaz et al. (2012) and SRAP markers were used by Uzun et al. (2010). In the current study, a total of 224 scorable bands were obtained with 8 SRAP primer combinations (25 bands), 8 DAMP primers (81 band) and 16 ISSR primers (118 bands). A dendrogram was constructed by using the UPGMA method. There was a high cophenetic correlation (r = 0.86; P<0.01) between ultrametric similarities of tree and similarity matrix. Such a correlation indicated that constructed dendrogram strongly represented the similarity matrix. Current accessions had similarity values ranging from 0.65 to 0.87, indicating a high level of variation (Figure 1). Priana was identified as the most distinct cultivar with a similarity value of 0.65. Çağribey and Cgr×Col4 were identified as the second distinct ones with a similarity value of 0.69. Çağribey and Cgr×Col4 nested at the same cluster that Çağribey is progeny of Cgr×Col4. Also, Ay×P7 and Ay×P5 were placed at the same cluster with Alata Yildizi. Notably, Ay×P3 nested away from Ay×P5, while and Ay×P7 nested close to Priana because AyXP progenies were obtained from Alata Yildizi x Priana crossing. There were nine Fer×Col progenies obtained from Feriana×Colomer crossing and they nested among Feriana and Colomer. There was one Çağataybey×Colomer progeny (Cgt×Col18) nested in the same cluster with Çağataybey cultivar. Progeny of Çağribey (Cgr×Col14) also nested in the same cluster with Çağribey. Uzun et al. (2010) carried out a study and determined genetic diversity and relationships among Turkish and some foreign apricot cultivars by using SRAP markers. The researchers obtained 87 bands.
Figure 1. Dendogram of 20 apricot genotypes (14 of them are new hybrid and 6 of them are their parents) obtained from SRAP, ISSR and DAMP markers.

and 63 of them (73%) were found to be polymorphic. Similarity values among the apricot cultivars were identified as between 0.77-0.97.

According to S allele results, some genotypes have only amplification of just one allele-S (Table 2). This could be a problem with the primers used or some alleles have few differences in molecular weight and also with the methods used. Vilanova et al. (2005) determined S-genotypes of total 20 apricot genotypes by using SRc-F and SRc-R consensus primers and compared the sizes of PCR products with previously published ones. A total of 4 S-RNase alleles (SC, S2, S3, S6) were identified in this study (Table 2). The most widely identified alleles were SC and S1 (occurred in 10) and they were followed by, respectively, S6 (9) and S2 (3) alleles. Group of Fer×Col progenies had mostly SC allele like Colomer, except Fer×Col4 which carried S3S6 coming from Colomer and Feriana. Besides, Ay×P5 and Ay×P7 had the same alleles (S2S3), but Ay×P3 had S6 allele. Cagataybey yielded 355 bp bands and had SC allele. Also, Cgt×Col8 had SC and S6 alleles coming from Cagataybey and Colomer. Cagribey had S3 and S6 alleles like Cgr×Col14. There were no inconsistent results among parents and their progenies. Burgos et al. (1997) identified self-incompatibility to Priana carrying S2 and S7 alleles. In the present study, S2 allele was identified in Priana and its two crosses (Ay×P5 and Ay×P7). Similar to other fruits, self-incompatibility is also a significant problem in apricots. As in other Prunus species, there is a gametophytic incompatibility in apricots usually controlled by a pair of S alleles (de Nettancourt, 2001). Similarly, Sonneveld et al. (2001) indicated that self-incompatibility in Rosaceae family was gametophytically observed with multi-allelic S loci. Faust (1989) carried out a study with inbred apricot genotypes under field conditions and indicated that “the individuals with a fruit set ratio below 5%” could be defined as self-incompatible; “the ones with 5-10% fruit set” could be defined as semi-compatible, and “the ones with more than 10% fruit set” could be defined as self-compatible. In previous molecular studies carried out until 2007, 20 different S-incompatibility alleles and a Self-Compatibility (SC) allele were identified in European apricots (Burgos et al., 1998; Halasz et al., 2005; Halasz et al., 2007a; Halasz et al., 2007b). Gulcan et al. (2006)
Apricot (Prunus armeniaca L.) is particularly prone to erratic productions and species are adapted to narrow spaces (Layne et al., 1996). Therefore, the majority of apricot cultivars have highly specific ecological requirements and relatively lower yields are often experienced when the cultivars are grown in other regions. Climatic adaptation has become the subject matter of several apricot breeding programs (Hormaza et al., 2007). However, the reasons for such low adaptability levels have not been clarified, yet (Julian et al., 2007). In recent years, apricot breeding programs were carried out in several countries and various new cultivars were developed. Bellini et al. (2008) implemented long-term breeding works since 2000 and selected 13 genotypes with ripening dates seven days before and three days after Aurora. Ruiz et al. (2010) indicated that the works to develop new apricot cultivars along with consumers' preferences have been initiated since the early periods of 1900s. The researchers reported that they had developed 5 new cultivars, namely, Toni, Estrella, Sublime, Maravilla, and Rosa. Topor et al. (2010) initiated apricot breeding programs in Romania for late-ripening cultivars and developed three new apricot cultivars (Euxin, Histria and Augustin). Pennone et al. (2010) carried out cultivar development researches to meet the needs of producers and processing industry and registered two

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Table 2. S Allele profiles of 20 apricot genotypes.

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</tr>
<tr>
<td>18</td>
<td>Cgr×Col4</td>
<td>270</td>
<td>420</td>
<td>S₃</td>
</tr>
<tr>
<td>19</td>
<td>Priana</td>
<td>332</td>
<td>-</td>
<td>S₃</td>
</tr>
<tr>
<td>20</td>
<td>Feriana</td>
<td>270</td>
<td>-</td>
<td>S₃</td>
</tr>
</tbody>
</table>
early-ripening cultivars (Ischia and Procida). Bircan et al. (2010) initiated apricot breeding works in 1989 to develop new table apricot cultivars through hybridizations of local and foreign cultivars and developed and registered 5 new apricot cultivars (Dr. Kaska, Çagataybey, Çagribey, Sahinbey, and Alata Yildizi). Egea et al. (2010) developed 9 new apricot cultivars (Rojo Pasion, Selene, Murciana, Dorada, Toni, Estrella, Sublime, Maravilla and Rosa) complying with market demands. Demirtas et al. (2010) carried out an apricot breeding program to develop new apricot cultivars resistant to spring early freezes. In another multi-purpose apricot breeding carried out in Malatya (Turkey), an early-ripening apricot cultivar “Dilbay” was developed and it was indicated that this cultivar ripened 5 days later than the earliest-ripening Ninfa cultivar, but the cultivar was found to be superior with regard to other fruit characteristics (Asma, 2013).

In conclusion, the Mediterranean region of Turkey has an especially great potential for table apricot. Currently, productions are performed with Ninfa, P. de Tyrinthe and Aurora cultivars. Although these cultivars are early ones, quality problems experienced in Ninfa and P. de Tyrinthe and self-incompatibility problem in Aurora limit the productions. The promising cultivar candidates developed in the present breeding program may prevent such problems and may significantly improve apricot culture both in Turkey and in other Mediterranean countries.

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بخش زردآلو

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چکیده

تولید زرد آلو در جهان به طور بیشتری ای رو به افزایش است و علت آن اصلاح نژاد کولنی‌ها یا جدید پرمحصول است. این‌جواب می‌تواند ب‌کمک ابزار (مانند کولنی‌های Monilinia و Sharka) به‌وجود مقاومت به امراض، اصلاح نژاد برای مطمئن‌کردن ویژگی‌های مورد نیاز خود (نا)‌اسوگاری نگه‌داری کرده است. در این پژوهش، 14 فرزنده (ناتوان) از زردآلو و 6 عد از والدین آن‌ها از استفاده نشان‌گری مشخصات مولکولی و مورفولوژیکی از وزن میوه، طول و عرض و ارتفاع آن، کل جامدات محلول، اسیدیت، و سختی میوه استفاده شدند. تجربه SRAP (نمونه‌گیری از Lifener)، ISSR (توالی تکرار توالی نماد (SRR)، و اسیره‌های مولکولی همراه خاتمه (DAMP) استفاده شد. افزون بر این، برای تعیین مشخصات آلی S، نشانگر های SRc-F/R، SRc-I/F و نشانگر های Priana و Ninfa وجود نداشت، حاصل تلاقی Ay x S. نشان داد که هر چند هیچ‌یک از زنوبیپ‌های زودرس تر از P3 زنوبیپ ایندی‌سری در مورد زوردرس و یک‌زینوبیپ های میوه بود. در کل، 244 باند قابل تبادل‌دهی به S1 تا 8 باند (188 باند) و دست آمد 8 گونه آغازگر (DAMP) (58 باند)، و 8 غیر ارقایش (S) که نشان داده نتوان زیاد بین دورگه‌ها و کولنی‌های بود. در این پژوهش، کلا 4 آلی S-2 مشابه و به طور گسترده ای (S2) رنگ کردن S. شناسایی شد که در میان نژاد های SC، S2، S3، S6 RNase حضور داشتند.