MOLECULAR MARKERS TO SELECT APPLE CULTIVARS WITH LOW ETHYLENE PRODUCTION

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Introduction

Shelf life of a fruit is an important economic aspect even after a certain period of storage, which is influenced by the genetic constitution of the fruit. Good shelf life is associated with a slow break down of firmness of mature fruit at room temperatures (Costa et al., 2005). Excessive softening is considered undesirable in apple and can impair characteristics like firmness, juiciness, crispness of fruit, as well increase mealiness (Oraguzie et al., 2004). In climacteric fruits, like apple, fruit ripening is controlled by the internal ethylene concentration. Ethylene is a gaseous hormone in higher plants and controls the ripening of climacteric fruit by coordinating the timely activation of many genes that cause changes in color, texture, aroma and flavor (Oetiker and Yang, 1995). The hormone is synthesized from S-adenosyl-L-methionine (SAM) via a short pathway catalysed by two enzymes, both encoded by two large gene families: 1-aminocyclopropane-1-carboxylic acid (ACS) synthase and 1-aminocyclopropane-1-carboxylic acid oxidase (ACO). The conversion of SAM to ACC by ACC synthase is the first step in ethylene biosynthesis and is generally considered to be the rate-limiting step (revision in Oraguzie et al., 2004; Oraguzie et al., 2007). The difference in ethylene production rate observed among apple cultivars can be related to different allelic combinations for these genes (Zhu and Barritt, 2008).

Use of marker-assisted selection in conventional apple breeding programs should significantly increase breeding efficiency since undesirable genotypes can be eliminated at the early seedling stage. Considering this scenario the aim of this study was perform a screening in apple cultivars and clones using three molecular markers to select genotypes related to low ethylene production: ACS1, ACS3a and ACO1. This screening can contribute with information for future apple breeding programs.

Material and Methods

DNA Extraction

DNA of apple cultivars and clones (names see Table 1) was extracted from leaves using the CTAB protocol, according to Romano and Brasileiro, 1999.

PCR amplifications

For the ACS1and ACO1 markers the primers sequence and amplification conditions are according to Sunako et al., 1999 and Costa et al., 2005, respectively. For the ACS3a marker the primers sequence and amplification conditions are according to Wang et al., 2009. PCR products for ACO1, ACS1 and ACS3a were visualized in 1.5% agarose gels after staining with ethidium bromide.

Results and Discussion

Genotypes for ethylene biosynthesis potential were determined for seven cultivars and seven Gala clones (names see Table 1). The Figures 1, 2 and 3 show the genotype of apple cultivar and clones for ACS1, ACS3a and ACO genes, respectively. The cultivars and clones were grouped into three classes according to ACS1 and ACO allelotype (Table 1). The alleles associated to low ethylene biosynthesis are ACS1-2 and ACO1-1. For the ACS1-2 allele was found a sequence of 162pb in the promoter region of gene, which was characterized as a transposable element, inhibiting its transcription or reduced to very slow levels (Sunako et al., 1999). The third intron of ACO1 gene presents a length polymorphism of 62-63 nucleotides among different cultivars, producing the ACO1-1 and ACO1-2 alleles. The absence of this fragment is associated to low ethylene production (Costa et al., 2005).

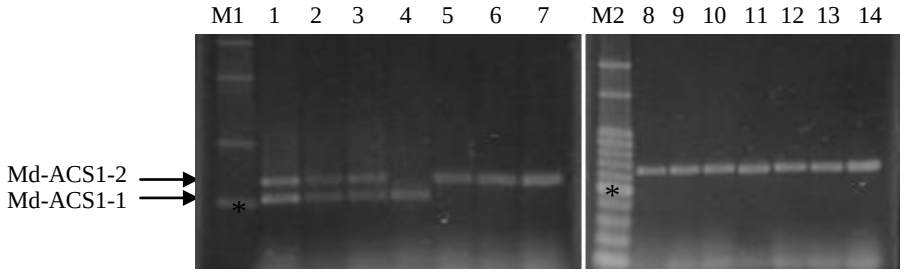
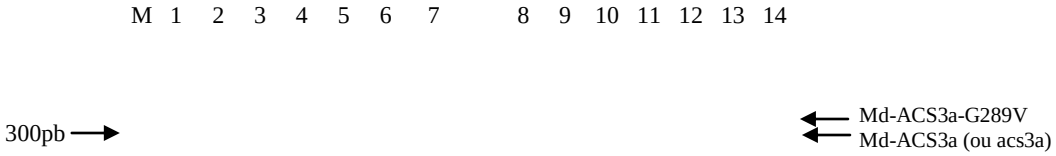


Figure 1. Genotypes of apple cultivars and clones for ACS1gene. The asterisk indicates 500pb in the molecular weight standard (M1= 1Kb DNA Ladder and M2= 100pb DNA Ladder). Length of fragments are: ACS1-1= 489pb and ACS1-2= 655pb.



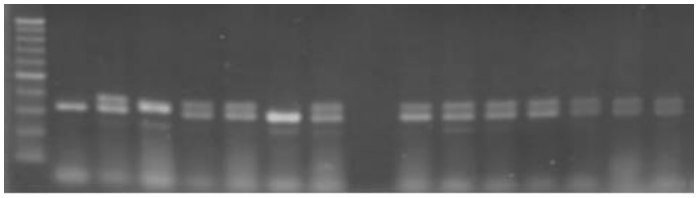


Figure 2. Genotypes of apple cultivars and clones for ACS3a gene. M= 100pb DNA Ladder, molecular weight standard. Length of fragments are: ACS3a-G289V= 351pb and ACS3a= 331pb

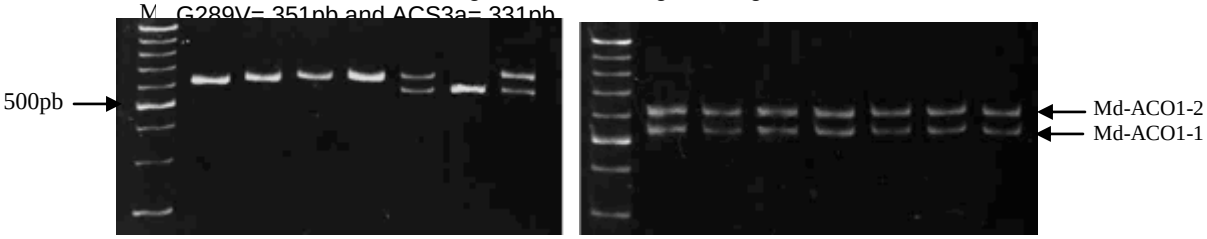


Figure 3. Genotypes of apple cultivars and clones for ACO1 gene. M= 100 pb DNA Ladder, molecular weight standard. Length of fragments are: ACO1-1= 525pb and ACO1-2= 587pb

For the ACS3 subfamily Wang et al., 2009 found three genes (*a*,*b* and *c*), but only the gene *a* is functional. The other genes possess an inserted transposon (MITE element) hindering the expression. For the ACS3a gene three allelic forms were found: ACS3a (wild type); ACS3a-G289V (mutant type) that has a substitution of glycine to valine in the active site inactivating the enzyme; acs3a (null allele) that is not transcribed. Cultivars carrying alleles Md-acs3a (null), Md-ACS3a-G289V (mutated), or a combination of both exhibit low ethylene production. No transposon or other insertion was found in the promoter region of the null gene, suggesting that the difference might occur much further upstream of the promoter than was investigated (Wang et al., 2009). Understanding the difference will lead to development of a molecular marker to differentiate between ACS3a and acs3a alleles. Until now this is possible only by expression analysis.

Table 1. Genotypes of apple cultivars and clones for ACO1, ACS1 and ACS3a genes.

	Genotypes		
Cultivars and clones	**ACO1**	**ACS1**	**ACS3a**
Pink lady	(1) 2/2	1/2	(1) ACS3a
Belgolden	(2) 2/2	1/2	(2) ACS3a/ACS3a-G289V
Joanagolden	(3) 2/2	1/2	(8) ACS3a/ACS3a-G289V
Willy Sharp	(4) 2/2	1/1	(3) ACS3a-G289V/ACS3a-G289V
Condessa	(5) 1/2	2/2	(5) ACS3a/ACS3a-G289V
Fuji Suprema	(6) 1/1	2/2	(6) ACS3a/acs3a
Daiane	(7) 1/2	2/2	(7) ACS3a/ACS3a-G289V
Maxi Gala	(8) 1/2	2/2	(9) ACS3a/ACS3a-G289V
Galaxy	(9) 1/2	2/2	(10) ACS3a/ACS3a-G289V
Royal Gala	(10) 1/2	2/2	(13) ACS3a/ACS3a-G289V
Gala Real	(11) 1/2	2/2	(4) ACS3a/ACS3a-G289V
Imperial Gala	(12) 1/2	2/2	(12) ACS3a/ACS3a-G289V
Castel Gala	(13) 1/2	2/2	(11) ACS3a/ACS3a-G289V
Baigent	(14) 1/2	2/2	(14) ACS3a/ACS3a-G289V

Red indicates the allele related to low ethylene production. The presence of null allele for ACS3a gene in the Fuji Suprema is according to Wang et al., 2009. Order of samples in the gels are indicated by numbers in parenthesis, which is the same for ACO1 and ACS1 genes.

Conclusion

Thus, the use of molecular markers in apple breeding programs enable to select genotypes associated with low ethylene production and thereby get cultivars that produce fruit that allow longer storage, which are less dependent on post-harvest environments (cold rooms) and chemical treatments (ethylene inhibitors).

Acknowledgments

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