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The Screening of EST-SSR Markers Associated with Peel Color in Tomato Fruit

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Authors' contributions

This work was carried out in collaboration between all authors. Authors NC and HF designed the study. Authors JY, YS and TZ performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors SP and YY managed the analyses of the study and the literature searches. All authors read and approved the final manuscript.

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Short Research Article

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ABSTRACT

Aims: The color of tomato fruit is an important property in improving of tomato breed. So we screened the differences of yellow tomato peel and transparent tomato peel using EST-SSR markers, and it is in favour of the research of the tomato fruit color and tomato breeding.
Study Design: Primers were designed from ESTs in which the repeat bases of SSR were more than 18bp, then tested in yellow peel and transparent peel of gene pools built by F_2 from E-95F8(♀) and Zhonghuajufeng8(♂).
Place and Duration of Study: College of Biological Science and Technology, between February 2015 and March 2016.
Methodology: The yellow peel gene pool and the transparent peel gene pool were built according to the trait segregation group of F_2, and then screened the differential bands by the primers. In the end, the differential bands were tested in the segregation group of F_2.
Results: 250 primer pairs were designed. One (HML52) of 250 primer pairs had diversity between yellow peel and transparent peel of gene pools built by F_2 from E-95F8(♀) and

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Zhonghuajufeng8(♂). In the end, the primer pair of HML52 passed test, and genetic distance was 7.04 cM.

Conclusion: The primer pair of HML52 of EST-SSR marker associated with peel color in tomato was found in this study, and the genetic distance of the primer pair was 7.04 cM.

Keywords: Tomato; EST-SSR; transferability; peel color.

1. INTRODUCTION

The color of tomato fruit is an important property in improving of tomato breed. Dark color and high content of pigment are welcomed by customer because there is a great quantity of lycopenes in these fruits. Lycopene is a natural antioxidant, and its activity of oxidation resistance is more than Beta-carotene and Vitamin-E [1]. Lycopene also enhances the antioxidant activity of enzymes, thus, it can improve antioxidant ability of organism, maintain normal cell metabolism and eventual senility [2-4]. Furthermore, lycopene can prevent LDL (low density lipoprotein) to be oxidized, adjust concentration of plasma cholesterol, repair oxidized cells, promote formation of colloid between cells, reinforce flexibility degrees of blood vessel, and slow down development of Cardiovascular Disease [5-7].

It is generally acknowledged that the higher content of lycopene leads to the deeper color of tomato fruit. Many studies report the relation between tomato fruit color and lycopene, and they make tomato fruit as the whole objective to study [8,9]. Thus, it's important to explore and regulate the genes controlling the red fruit color in tomato in order to cultivate the fruit with dark red color and high content of pigment.

The color of tomato fruit is controlled by many genes. These genes' functions are unclear in detail which control tomato peel color. So the study of the related genes on controlling the fruit peel color is important for improving tomato genetic traits and genetic breeding. In this research, we screened the differences of yellow tomato peel and transparent tomato peel using EST-SSR markers, and it is in favour of the research of the tomato fruit color and tomato breeding.

2. MATERIALS AND METHODS

2.1 Plant Materials

All plant materials were provided by Institute of Vegetable Research, Shandong Academy of Agricultural Sciences.

The materials used for screening the difference of yellow peel and transparent peel were F_1 and F_2 plants of E-95F8(♀), Zhonghuajufeng8(♂). Zhonghuajufeng8(♂) and E-95F8(♀) all were the stable-genetic and homozygous inbred lines. F_1 came from a cross combination of parents, and F_2 was from F_1's inbred lines. According to the result of chi-square test, F_2 segregation population accorded with Mendel's rule.

2.2 Primer Design

The total number of repeat bases of SSR was at least 18bp, which was used for primer design. The primers were designed from unique sequences flanking regions of the SSR using the Primer Premier 5.0 program with length of 17-24 bp, annealing temperature of 50-60℃, and product sizes ranging from 100 to 400 bp.

2.3 PCR Reaction

Polymerase chain reactions (PCR) were performed in a total volume of 20 μL, including 45 ng of tomato DNA, 10 μM of each primer, 10 μL reaction mix and 7 μL ddH_2O.

PCR reactions were performed on MyCycler Thermal Cycler (Bio-Rad, Laboratories, USA) with an initial 5 min of denaturation at 94℃, followed by 35 cycles of 94℃ for 30s, appropriate annealing temperature for 45s, 72℃ for 1 min, and a final extension at 72℃ for 10 min.

PCR products were separated on 8% polyacrylamide gel with TBE buffer according to the standard protocol.

2.4 Screening the Difference of Trait

The yellow peel gene pool and the transparent peel gene pool were built according to the trait segregation group of F_2. In the end, the differential bands were tested in the segregation group of F_2.

3. RESULTS

3.1 Screening of Primers

Primers were designed according to the conserved sequence of both ends in SSR locus, naming HML1-HML250 [10]. We designed 250 primer pairs and screened for the yellow peel gene and the transparent peel gene. In the 250 primer pairs, primer of HML52 showed difference and stability (Fig. 1).

3.2 Screening the Difference of Trait

There was one pair of primer, HML52 (upstream 5'-3': CTTATGTGAAAACACCTCGCTC; downstream 5'-3': TTCAAAATTCCCCAAAGACG), which produced differential band in all of 250 primer pairs. HML52 was fit for prospective result after testing in the segregation group of F_2 (Figs. 2, 3 and 4), and genetic distance is 7.04 cM.

4. DISCUSSION

EST-SSR marker is an effective functional molecular marker and has been used in study of many plants, such as wheat [11], soybean [12], common bean [13], cabbage [14], and so on. Those sequences, in which the total number of repeat bases of SSR was at least 18 bp, were selected from 2039 ESTs for primer design (http://www.ncbi.nlm.nih.gov/). At last, we got 526 ESTs that met the requirements, occupying 25.8% in total ESTs containing EST-SSRs. We founded that some ESTs can not be used for primer design, because SSRs were in the either ends of the ESTs. In this research, some ESTs could not be used for primer design because its SSRs were located in head or tail.

In our study, HML52 could produce different bands. HML52 came from gb|AC239575.2| which was sequence of *Solanum lycopersicum* strain Heinz 1706 clone hba-17d14. The result of testing in F_2 group proved that this primer might be valuable in the process of tomato breeding. What's more, in materials, the different traits between female parent and male parent decide the peel color, that is, the peel color of female parent is yellow, and the peel color of male parent is transparent. Their pulp colors are carmine, so the fruit color of female parent is red, and the fruit color of male parent is pink.

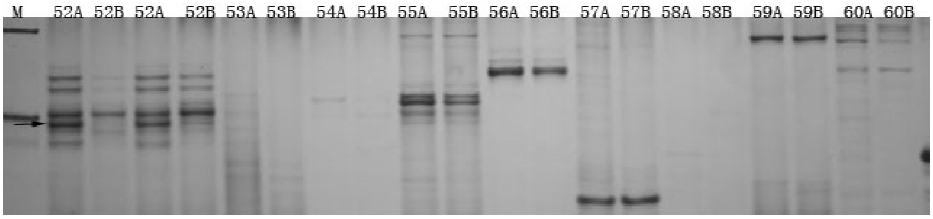

Fig. 1. PCR amplification of HML52 primer in gene pools of yellow and transparent fruits, marker: 50 bp

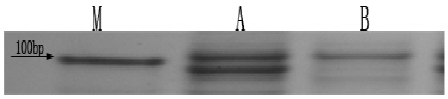

Fig. 2. HML52 was selected in yellow fruit peel pool, marker: 50 bp

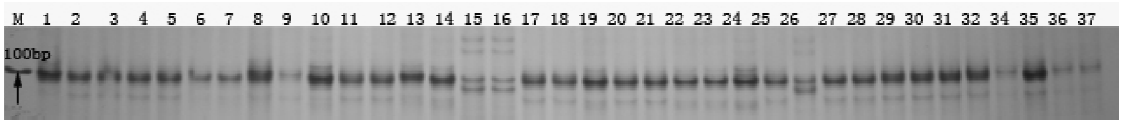

Fig. 3. HML52 was tested in transparent fruit peel of F2 group, marker: 50 bp

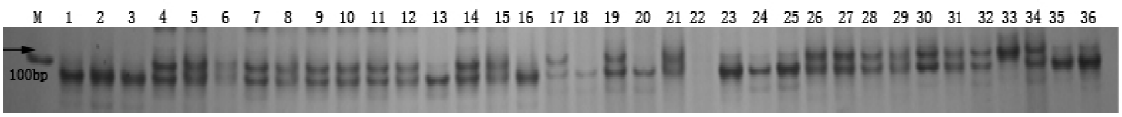

Fig. 4. HML52 was tested in yellow fruit peel of F2 group, marker: 50 bp

In terms of physiology, carotenoids and flavonoids play important roles in determining the color of tomato fruit [15,16]. Carotenoids are lipid-soluble 40-carbon isoprenoids, and more than 700 naturally occurring carotenoids have been identified [17]. Carotenoids also accumulate in chromoplasts of flowers and fruit, where they function as yellow to red-colored pigments [18,19]. The red color of ripe tomato fruit is due mainly to the accumulation of the carotenoid all-trans-lycopene, which is produced during fruit ripening [20]. What's more, one of the most abundant flavonoids in tomato fruit peel is the yellow-colored naringenin chalcone. It accumulates in the cuticle upon ripening and is responsible for the yellow color that develops in the peel at breaker stage, preceding the production of lycopene [21]. But metabolic analysis shows that pink fruits lack the ripening-dependent accumulation of the yellow-colored flavonoid naringenin chalcone in the fruit peel, while carotenoid levels are not affected [22]. Thus, the function of metabolic mechanism about secondary substances in the regulation of peel color needs further research.

5. CONCLUSION

The EST-SSR marker of HML52 associated with peel color in tomato was found in this study, and the genetic distance of this primer pair was 7.04 cM.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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