cDNA-AFLP analysis for potato chip color quality after long term storage at 4°C

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During long term storage at low temperature (4°C), the accumulated reducing sugars glucose and fructose in tubers are the major problem for the potato processing industry resulting in bitter-tasting, dark colored and therefore unacceptable processed products (Maillard reaction or cold sweetening). The processing industry need cold chipper and up to date, „Cold sweetening" is getting one of hot issues. From 1999 to 2001, the group of Biotechnology Potato in cooperation with the group of potato breeding have evaluated chip color after long term storage at 4°C in a dihaploid population from the cultivar Artis registered as cold chipper by Bundessortenamt in 1997.

cDNA-AFLP analysis was performed in 4 selected dihaploid Artis lines producing dark chip color and 4 lines producing light chip color based on 3 years chip test results. These tubers were harvested and stored at 4°C in 2002.

The flesh tissue of each line was excised at 8 different storage times (11, 17, 21, 28, 35, 49, 98 and 207 days) during 207 days storage period. In order to confirm the chip color, two times chipping test was performed 98 days and 207 days after storage. In the first chipping test, the chip color already could be certainly distinguished. At the second chipping test, the probes clearly differentiated between dark and light color. Total RNA of 4 selected lines of each pool at 8 different sampling times (DST) were isolated with Gentra RNA isolation kit (Biozyme) and RNA pools were constituted by mixing RNA of 4 selected lines. mRNA of each pool at 8 different sampling times (DST) were isolated with OligotexR mini (Qiagen) and double stranded cDNA was synthesized with BD SMART™ PCR cDNA Synthesis kit. cDNA of each pool at 8 DST were used for AFLP assays. Two fragments were detected in cDNA-AFLP analysis, which were continuously expressed either in the dark or the light colored pool during the whole storage period with 207 days at 4°C.

These fragments were cloned and sequenced. The homologous sequence was searched in Sol Genomics Network (http://www.sgn.cornell.edu). One TDF from the dark color pool shares 99% of sequence identity with one EST (SGN-U269392) of *Solanum tuberosum*, which is annotated to encode putative vacuolar proton ATPase subunit E in tomato or Arabidopsis (At1g64200.1). The other TDF from the light color pool shares 88% of sequence identity of potato multicystatin (PMC) gene (SGN-U289637) which encodes a cysteine proteinase inhibitor or similar to cysteine proteinase inhibitor in Arabidopsis (At3g12490.1) according to blast annotation.

The sequence information from one TDF accords one of the assumed genes associated with the reducing sugar accumulation in the cytosol, which is highly correlated to chip color after long term storage at 4°C. During the storage period, it has been already reported that low temperature conditions result in an accumulation of ATP in potato tissue. The whole sequence of this gene will be obtained by using RACE (Rapid Amplification of cDNA Ends). Based on the sequence information, PCR marker will be developed for marker assisted selection in potato breeding.

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