ABSTRACT

Pepper (*Capsicum spp*.), a member of the *Solanaceae* family, is a widely eaten vegetable and spice crop worldwide. The leaves, flowers, and fruits all have hues of red, blue, and purple. The pepper's purple hue is due to the presence of anthocyanin, delphinidin-3-pcoumaroylruntinoside-5-glucoside. It enhances the health of both people and plants. Even though it has a lot of nutritional and commercial value, unneeded anthocyanin buildup results in purple and black blotches on the fruit, Our aim was to find out the differences in DNA methylation patterns between the purple and green sections of the same pepper pod.

In this study we use the pepper pod genotype which has two distinct color sectors: green and purple, which are associated with different levels of pigmentation. The hypothesis was that the DNA methylation patterns would differ between the two-color sectors, reflecting the regulatory mechanisms underlying pigmentation variation in fruit development. To test this hypothesis, the DNA was isolated separately from the green sector and from the purple sector of the same pepper pod. The DNA was then subjected to a technique called (MSAP), which uses methylation-sensitive restriction enzymes to detect methylation differences in the DNA. The restriction enzymes used were *Hpa*II and *Msp*I, which are isoschizomers that recognize the same sequence (CCGG) but have different sensitivity to methylation. *Hpa*II can only cut the sequence when both cytosines are unmethylated, while *Msp*I can cut the sequence regardless of the methylation status of the cytosines. By comparing the fragments obtained with enzymes, it is possible to infer the methylation status of the CCGG sites in the DNA.

The MSAP technique also involves the use of 20 primer combinations that anneal to the restriction sites and amplify the fragments by selective polymerase chain reaction (PCR). The primers have selective nucleotides at their 3’ ends, which increase the specificity and resolution of the technique. The primer combinations that resulted in clearly detectable fragments were evaluated; these were mainly primers containing 4 selective nucleotides. In many cases, the use of primers with 3-3 selective nucleotides resulted in smears fragments on the gel for reliable evaluation.

The MSAP analysis revealed that out of the 36 primer combinations tested, 20 yielded an evaluable pattern, and their amplified fragments resulted in 187 distinct patterns. Of these 187, 121 were patterns that were either monomorphic or not indicative of methylation differences between the green and purple sectors. However, the patterns obtained with the

20 primer combinations differed in 11 cases in the green and purple sectors, in that both *Hpa*II and *Msp*I could cleave in the purple sector, indicating that the section was not methylated, whereas in the green sector only *Hpa*II digested the sample, indicating that the section was methylated. This suggests that there are significant differences in DNA methylation patterns between the green and purple sectors of the same pepper pod, and that these differences may be involved in the regulation of pigmentation variation in fruit development.