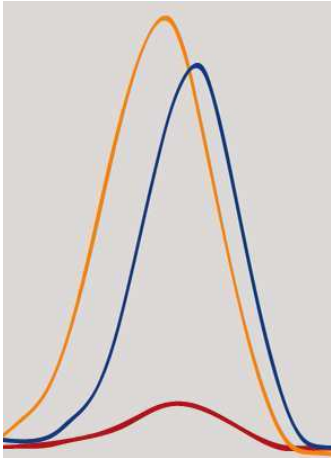
High Resolution Melting Analysis for inbred test

Li rui (Vivian)



Syngenta aims to provide high quality hybrid seeds with high germination percentage, pest and disease resistance, genetic purity, good tasty and high yield traits. The genetic purity of seed stocks is important to ensure that growers, processors, and consumers receive the crop varieties and products that they expect. With part of quality control department, the Genetic Purity lab is working on determination of the level of inbreds and off-types. Inbred and off-type tests should be done effectively, accurately as well as cost efficient. There is a strong focus on increase of sample throughput and cost-efficiency. Therefore, A research need to be done to find out the possibility of moving inbred test from the current protocol to a more automated platform, such as High Resolution Melting (HRM) Analysis.

High Resolution Melting (HRM) Analysis is a new, post-PCR analysis method used for identifying genetic variation in nucleic acid sequences. With the increase of temperature, double-stranded DNA start melting apart and the emitted fluorescence is measured on a specialized instrument to generate a characteristic curve. Melt curves that are similar in shape but that are distinguishable from each other by difference in melting temperature (Tm) of the amplicon. The melting temperature is depends on the sequence of DNA fragment, such GC content and length. Female, male and hybrid DNA can be plotted curves with different shapes and melting

temperature by HRM program. The distinguishability is relevant to the length of amplicons, dye activity and HRM machine resolution.

It is proved from the thesis research that HRM can be used for inbred test for Pepper, Melon and Squash seed samples. The current protocol of Syngenta genetic purity works better. Eva Green shows less PCR inhibitory and gives stronger signals. It can be an alternative choice. Shorter products amplify with higher efficiency. 0.3 °C/s balances the high resolution and efficiency. Precision Melt Analysis™ Software can analyze the data form melting curves and cluster the samples' genotype.

To apply HRM to the inbred is one of the ways to reach the goal of the Genetic Purity lab 2010, remove all gel-activities from the processes. It brings on a blue sky for a more automated platform of inbred test. It is simple, time efficient, environment friendly and results accurate. Additionally, it reduces laborious risk on contamination. Since HRM analysis requires no manual post-PCR processing, is performed in a closed-tube system—no need to handle samples after loading. It can save labor works, shorten the test processing time and also avoid workers dealing with a carcinogen dye, Ethidium Bromide. Moreover, it has a low reaction cost relative to other methods used to study genetic variation.