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DETERMINATION OF PURITY AND QUANTIFICATION OF VARIETAL COMPONENTS THROUGH NGS (NEXT GENERATION SEQUENCING)

Document prepared by an expert from Seed Association of the Americas (SAA)

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1. Agricultural systems requires the verification of the genetic identity and purity in seeds, leaves, and industrial products. Often this process is too long to await the identification using phenotypic descriptors. Therefore, molecular markers will increase the identification of varieties making it easier and more accurate.

2. Short Tandem Repeats (STRs) have been a powerful tool for varieties identification, but not for the determination of varietal purity and quantification of different components in a mixture sample. Because they have many issues at analysis time.

3. Two major problems are presented when STRs are used to quantify varietal components: 1- single seed genotification, 2- and intensities of different alleles in a seed pool. At the first case, the number of seeds to be analyzed should be too high to reach the needed representatively. Secondly, fluorescence of each allele is not accurate at quantification time. When q PCR is used to quantify different varieties is more accurate but the number of SNPs genotyped is not sufficient to discriminate close related varieties.

4. We developed a method based on NGS by genotyping around 350 SNPs. For each sample we analyzed the flour of 10,000 seeds. Also we have created a database of 43 and 46 varieties of barley and soybean respectively.

5. This method is able to determine varietal purity with an error lower than 1% (99% confidence) and a 95-99% of purity, and the system can detect 0.8% of contaminant varieties. Which has a great power in varieties discrimination, by a reliable statistic that can determine a varieties with high precision and accuracy. This method was validated by mixtures of known composition. At the moment more than 600 samples of industrial barley have been analyzed and we are analyzing the first Soybean samples.

6. These methods can also by applied in other kind of samples, or genetics improvement programs.

Carlos Azambuja, Director, GENIA - Laboratory of molecular biology, Zonamerica, Ruta 8, km 17,500, Biotec local 012, Montevideo, Uruguay

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