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Molecular Markers Characterization of Transgenic Crop

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ABSTRACT

Prior to commercialization, the development of detection and identification techniques, traceability compliance, and labelling requirements can all benefit from the molecular characterization of genetically modified plants. A difficult phase in the safety assessment used to be the detailed description of the genetic change because it called for the use of time-consuming and arduous techniques. The molecular characterization of genetically modified (GM) plants was created in this study using an accurate, straight forward, and quick method that adheres to a user-friendly workflow for researchers with basic bioinformatic skills. Perennial ryegrass, white clover and canola were utilized as three GM events to evaluate a technique that makes use of long-read sequencing by the Oxford Nanopore Technologies MinlON device. The method delivered a higher degree of resolution of the transgenic events within the host genome than has previously been possible with standard lllumina short-range sequencing strategies. Each of the plant genomes' flanking sequences, copy quantity, presence of backbone sequences and overall transgene insertion structure were identified, along with some moderately sized secondary insertions that would have gone unnoticed in the past. Since the suggested workflow makes the process quicker, easier and more affordable, it will complement the current methodologies for molecular characterization of GM plants. The proposed workflow takes just about 1 week from DNA extraction to analysis result.

Keywords Molecular marker, Transgenic crop, GM crop, Crop improvement.

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INTRODUCTION

Officials from the United States Department of Agriculture's Animal and Plant Health Inspection Service (USDA-APHIS), Health Canada and the Canadian Food Inspection Agency (CFIA) met in July 1998 to discuss aspects of molecular characterization that are included in their review processes for transgenic plants and to compare and, where possible, harmonise them. Regulators from the US, Canada and Mexico have been meeting often since the early 1990s to try to harmonise their respective countries' biotechnology regulatory frameworks. One result of these conversations was the modification of the document, which was completed in September 2012 and updated to incorporate modern scientific theories and methods for molecular characterization as well as the standards and methods shared by the three nations.

When transgenic plants are evaluated before being sold, information other than their molecular

makeup is taken into account. Developers requesting regulatory approval to use these plants in agricultural production or commerce in any of the three countries will find it easier to submit the necessary supporting data if there is agreement on common requirements and acceptable analytical approaches for molecular characterization. Through genetic transformation, specific modifications of agricultural attributes are now possible thanks to the application of plant biotechnology. For the purpose of identifying potential risks to people, animals, and the environment, safety evaluations of new transgenic plants are necessary. The molecular characterization of the transgene is the first stage in any risk assessment of genetically modified (GM) plants before their approval for distribution onto the market (s).

Identification of the genetic modification's locus or loci, a description of the surrounding genomic regions, the number of copies of the transgene expression cassette inserted, and endogenous host gene disruptions caused by the transgenic DNA are all required as part of this characterization . The cultivation of biotech (GM) crops is growing quickly, creating a demand for precise and affordable molecular characterization techniques (Liu *et al*. 2017). Since no transformation approach can completely control the quantity of transgene insertions into the host genome, copy number determination of the transgenic event is the first parameter to be taken into account . DNA blot analysis and polymerase chain reaction have been the most widely used methods for molecularly analyzing novel transgenic events (PCR) (Liu *et al*. 2017). These techniques can make an attempt to count the transgene copies, but they are unable to give an accurate description of the insertion's structure. The insertion site, flanking areas and any potential endogenous gene interruption brought on by the transgenic insert have all been identified using DNA sequencing techniques. Sanger sequencing has been the tried-and-true approach for this purpose. Sanger sequencing is a reliable technique, but because of the numerous processes involved in its execution, which add time and expense, it might be difficult to precisely sequence complicated portions of the genomes.

The size of DNA fragments can be limited by the use of primer walking methods to target specific regions, although this method may miss aspects like copy number, deletions, or unintentional insertions. Due to the decrease in sequencing costs and gradual improvements in data yields and accuracy of the ONT flowcells, low coverage whole-genome shotgun sequencing may be the quickest method to detect transgenic insertions. This strategy works especially well when the transgene integrates into intricately repetitive regions of the genome or when the transgene is cisgenic and the target genome already contains an endogenous copy of the same sequence, making walking strategies extremely difficult. The error rate (5–15%) in nanopore sequencing is the main difficulty. In this work, we show how a single ONT Min ION flowcell can be used to quickly and affordably accomplish the comprehensive molecular characterization of a transgenic event. To demonstrate the effectiveness of this methodology, three transgenic crops representing mono- and dicotyledonous species, with varying genome sizes, complexity, and accessibility of reference genomes, were utilized as examples. The current shotgun (random) whole genome sequencing's objective is to find reads that span the genome-transgene boundary and can provide information on the transgene's integration site and copy number. Owing to the ONT technology's uniform genome coverage, as long as the DNA extracted is of sufficient length and provides long enough sequence reads to provide the resolution necessary, an overall lower coverage (5-10x) might be adequate to obtain satisfactory resolution of the transgene. This method's main contribution is its user-friendly data processing workflow, which is especially beneficial for researchers with little access to bioinformatics tools.ONT has recently been employed in several research to sequence transgenic events using various methods.

DNA extraction, concentration and purity

A modified version of the high molecular weight genomic DNA extraction from plant species was used to extract the DNA (Vaillancourt and Buell 2019). In conclusion, after disruption, plant samples were digested and genomic DNA was purified in three different phases using the enzymes RNase, proteinase K and G2 lysis buffer (QIAGEN, Hilden, Germany). Following the manufacturer's recommendations, the samples were then put into equilibrated QIAGEN

Genomic-tip 100/g columns, washed, and the DNA was eluted. After being cleaned with isopropanol and ethanol, the DNA was redissolved in TE buffer. According to the manufacturer's instructions, the Qubitds DNA HS assay kits and a Qubit 3.0 Fluor meter (InvitrogenTM, Thermo Fisher Brand, CA, United States) were used to determine the concentration of each sample. According to the manufacturer's recommendations, DNA size was determined using the Genomic DNA Screen Tape, through the Agilent 2200 Tape Station system (Agilent Technologies, Santa Clara, CA, United States) and DNA quality was assessed using the Nano Drop 1000 UV-vis spectrophotometer (Thermo Fisher), using the A260/A280 and A260/A230 ratios. For sequencing purposes, samples containing a minimum of 1.5 g of genomic DNA in 47 l of distilled water, an A260/A280 ratio of 1.8, 0.1, an A260/A230 ratio of 2.0 0.1 and DNA length more than 60 kbp were employed.

DNA fingerprinting for varietal and hybrid identification

This directly affects the differentiation of varietal traits and the accuracy of identification in numerous crop plants viz., tomato, beans, pepper and potato. As well as in entire live organisms. Although RFLP was the pioneer of DNA profiling, a wide number of molecular markers are currently being utilized in a variety of vegetable crops to use DNA fingerprinting to identify cultivars and breeding lines.

Detection of QTLs

The creation of a linkage map provides a precise depiction of the physical location of a gene on a chromosome. It shows that minor genes (polygenes), in addition to oligogenes, play a part in character inheritance. Observed a relationship between the color of the seed coat (a qualitative property) and seed size (a quantitative trait) in common beans, which led to the beginning of polygene mapping (*Phaseolus vulgaris*). As a result, the mapping of polygenes was started based on the idea that a genetic marker and a quantitative trait phenotypic are related. Due to their enormous quantity, dispersed location within the genome, and cumulative impact on character expression, such an approach was unable to pinpoint the precise site of polygenes. Later, other gene clusters in the genomic region that were linked to the expression of quantitative features were discovered (Huang *et al*. 2000). Quantitative trait locus refers to each of these genomic regions that contains a group of minor genes on several chromosomes (QTL). The segregation patterns of the specific genes that make up a QTL can be used to characterize and map that QTL. Numerous vegetable crops have undergone extensive QTL mapping in order to identify QTL. A requirement for MAS is the creation of connected molecular markers to a specific gene or QTL. However, because there are relatively few recombination events, using biparental mapping populations (F2, Backcross, DH, RIL) results in low mapping resolution.

Breeding lines and accession identification

Identification of accessions and breeding lines is crucial in a breeding effort. Large numbers of lines are handled during breeding trials, and as a result, they are susceptible to contamination from field cross-contamination and seed sample mixing (Gaikwad 2001). Mislabeling of the seeds and plants could result from this. These accessions are challenging to distinguish since they have minimal morphological differences. However, such closely related genotypes can be easily distinguished by molecular markers. Waycott and Fort effectively distinguished essentially identical germplasm lines of bitter head lettuce using RAPD markers. The purity of breeding lines and F1 progeny tests were assessed using microsatellite probes to identify the fingerprints of different tomato accessions. Identified variation across and among artichoke breeding populations using RAPD markers. In order to differentiate F1 from F2 seeds and assess the quality of the seeds, RAPD and RFLP markers were applied to asparagus. Fisher and Bachmann separated 83 onion accessions using microsatellite markers Doganlar *et al*. (2002).

Sex identification

By quickly distinguishing between male and female plants, breeding program for dioecious species can be made considerably more effective. Jiang and Sink created the SCAR markers that can be found in asparagus. Reamon Buttner and Jung created STS markers,

which were connected to the sex locus at a distance of 1.6 cm to make it easier to distinguish XY from YY males in asparagus (Devran *et al*. 2018).

Identification of cultivar

With the development of microsatellites, cultivars of crops like potato, tomato, cucurbit, pepper, lettuce, and spinach can now be identified with a high degree of reliability (Demir *et al*. 2010). The comparative evaluation of various DNA fingerprinting methods in tetraploid potatoes revealed that the AFLP had the highest level of discrimination power, followed in decreasing order by multilocus SSR, RAPD, ASSR, and single locus SSR. Gaikwad (2001), found that ISSR markers were the best in spotting polymorphism in pepper. However, due to the extremely high number of markers generated per assay by AFLP, the marker index of AFLP markers was noticeably greater than that of ISSR and RAPD (Cruz *et al.* 2013). Under The Indian Plant Variety Protection and Farmers' Rights (PVPFR) Act 2001, the creators of novel crop varieties are given intellectual property rights in the form of plant breeders' rights (Brekketet *et al*. 2019). In order to be eligible for registration and protection under this legislation, a candidate variety must satisfy the distinctness, uniformity, and staibility (DUS) requirements. The morphological information serves as the foundation for DUS testing. A variety's distinctness is assessed by comparing it to the existing varieties for a number of characters (Bennewitz *et al*. 2018). There is a provision for the Essentially Derived Varieties in the PPVFR (EDV). According to this clause, breeders of the variety from which the EDV has been developed are to receive a portion of the protection advantages. The DNA profiles of the variety by themselves are now insufficient as evidence of a plant's distinct identification. However, molecular profiles may support plant breeders' claims that new varieties should be protected in order to prove the uniqueness of their cultivars. If a new variety and an extinct one only have small phenotypic variations, molecular profiles may be highly significant in cases of biotechnologically generated variants Ansari and Singh (2013).

CONCLUSION

Molecular markers added to conventional plant breeding has been shown to be an effective strategy for improving vegetable crop yields. In order to ensure that QTL are located with greater accuracy, new genomic techniques and next-generation sequencing technology will aid in the production of saturated linkage maps. Additionally, the creation of closely related molecular markers can make marker assisted selection possible with great selection efficiency. Such molecular methods are extremely useful for utilizing a variety of genetic resources to create superior crop cultivars. Additionally, upcoming and existing genetic developments will trigger the next green revolution.

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