RGAP : A TOOL FOR DIVERSITY ANALYSIS AND MARKER IDENTIFICATION FOR DISEASE RESISTANCE IN SUGARCANE

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Abstract

Sugarcane is a key cash crop of India and many other countries, which is grown in subtropical and tropical regions of the world. Around 70% of world's total sugar comes from sugarcane and its demand is constantly increasing with burgeoning world population. The production and productivity of sugarcane suffers due to various abiotic and biotic stresses. Among biotic stresses, diseases like red rot, smut, wilt and phytoplasma are major problems. Plant disease resistance (R) genes play important role in disease control. They provide resistance against various diseases across diverse plant species and they have certain conserved domains across the plant genera which include nucleotide-binding sites (NBS), leucine-rich repeat (LRR), serine/threonine protein kinases (PKs) and trans-membrane domains arranged in various combinations. These conserved sequences have led to the development of novel PCR-based approach for isolation of resistance gene analogues (RGAs) and, identification of candidate genes for disease resistance from various crop plants. Apart from isolating the resistance genes, the resistance gene analogue polymorphism (RGAP) has been found as an effective tool to identify molecular markers linked to disease resistance genes as well as gene diversity analysis in sugarcane. These RGAP markers are equally useful in development of resistant cultivars and to understand the mechanism of disease resistance and management. Such potential RGAP markers can be used to develop biotic stress related resistant genotypes *via* crosses between wild types and cultivated elite cultivars.

Key words : Disease resistance, NBS-LRR, resistance gene candidates, RGA, red rot, *Saccharum*.

Introduction

Sugarcane is grown extensively in over 120 countries across the world witha production area of 26.9 million hectares and total production of around 1.9 billion tons (FAOSTAT, 2016). At present, India is the second largest producer of sugarcane next to Brazil (FAOSTAT, 2016). Approximately 70% of the world's sugar production is derived from sugarcane, while the rest comes from sugar beet, which is cultivated in temperate regions. Sugarcane growing countries are gradually moving towards the production of bioenergy with increased interest in development of new high yielding, high sugar and resistant cultivars to meet the global demand of energy and sugar (Hoang *et al.*, 2015). Cultivation of sugarcane is severely affected by many biotic and abiotic stresses (Long and Hensley, 1987; Azevedo *et al.*, 2011). Across the word, almost 100 fungi, 10 viruses, 10 bacteria, several phytoplasma and 50 species of nematodes have been

reported as the pathogens of sugarcane, which can cause up to 120 diseases in sugarcane (Singh and Waraitch, 1981; Rott *et al.*, 2000) of which about 55 diseases have been noticed in India that have been categorized as fungal, bacterial, viral, phytoplasma and nematode diseases (Rao *et al.*, 2002). The yield loss due to fungal diseases is around 18-31% in India. Red rot caused by *Colletotrichum falcatum*, grassy shoot and yellow leaf diseases caused by phytoplasma and smut disease caused by *Sporisorium scitamineum* are major threats to sugarcane in India. Besides, wilt, leaf scald, *Pokkah boeng* and rust diseaseoutbreak have also been reported in many parts of India (Viswanathan and Rao, 2011). Various methods such as crop rotation, physical and chemical treatments and/orbiological control agents, *i.e.* fungicides alone or with biological control agents are used to manage sugarcane diseases (Viswanathan and Rao, 2011; Sharma *et al.*, 2017; Kumar *et al.*, 2017). These methods, however, do not provide adequate disease control. Therefore, development of disease resistant

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cultivars is an integral part of sugarcane improvement. Plant disease resistance (R) genes play important role in disease control across diverse plant species. The resistance (R) gene confers resistance to diverse range of plant pathogens; by recognizing the pathogen Avr gene product either directly or through intermediate product of Avr gene and provokes signalling cascade that leads to generation of various types of responses to biotic stresses (Spoel and Dong, 2012). R genes have certain conserved domains across the plant genera, which have led to the development of novel PCR-based approach for isolation of resistance gene analogues (RGAs) and identification of candidate genes for disease resistance from various crop plants. Apart from isolating the resistance genes, the resistance gene analogue polymorphism (RGAP) has been found as an effective tool to identify molecular markers linked to disease resistance genes as well as gene diversity analysis in sugarcane. Sugarcane is a complex polyploid and heterozygous crop derived from interspecific hybridization of at least three different species, hence identification of disease resistance genes in this crop is difficult as compared to other crops (D' Hont and Glaszmann, 2001). DNA markers such as RFLP, AFLP, RAPD, ISSR, SSR and SNP etc. have been proved effective in revealing the complex genetics and diversity of sugarcane.Several R genes belonging tonucleotide binding site- leucine rich repeats (NBS-LRR),serine/threonine protein kinases (PKs) and trans-membrane domains arranged in various combinationshave been isolated in sugarcane (Meyers *et al*., 2003; Glynn *et al*., 2008; Que *et al*., 2009; Hameed *et al*., 2015; Srivastava *et al*., 2016 a,b, 2017 a,b). NBS-LRR class of resistance gene family is most extensively used for isolation of resistance genes in crops and approximately 75% of the plant disease resistance genes have been cloned from this gene family alone. Resistance gene analogue polymorphism (RGAP) is an approach used to detect polymorphism linked to diseases resistance genesusing molecular markers. RGAP has been used in sugarcane (Srivastava *et al*., 2012, 2016a,b, 2017a,b; Sharma and Tamta *et al*., 2017) and various other crop such as in wheat (Chen *et al*., 2006; Xie *et al*., 2004; Yan *et al*.,2003), Barley (Yan *et al*., 2006) and Maize (Wenkai *et al*., 2006) for identification of candidate disease resistance genes and genetic diversity analysis (Srivastava *et al*., 2016 a,b; 2017 a,b). Undoubtedly, RGAP marker could be useful in the development of resistant variety with improved cane productivity and biotic stress management in sugarcane.

Identification of disease resistance genesusing RGA markers

Identification of resistance genes in sugarcane crop is always challenging owing to its polyploid complex genomic structure, while the whole genome sequencing of sugarcane is still under progress. The huge genomic size of around 10 Gband complex nature of sugarcane are due tothe combination of euploid and aneuploid chromosome sets with homologous genes present in range between 8 to 12 copies each (Souza *et al*., 2011). Plant resistance genes characterized by the conserved motifs/domains, have led to designing and synthesis of primers for resistance gene analogue (RGA) markers. The RGA markers were initially designed to map or isolate R genes in potato (Leister *et al*., 1996) and afterwards successfully used in other crops (Chen *et al*., 1998; Yan *et al*., 2003; Mutlu *et al*., 2006; Que *et al*., 2009; Hameed *et al*., 2015). Apart from other DNA markers, the resistance gene analoguemarkers require high resolution electrophoresis and visualization.Sequencing of PCR (polymerase chain reaction) amplified products based on conserved domains of R gene, is employed to isolate molecular markers supposed to be responsible for disease resistance, which is a fast and much less expensive approach. Generally, three steps are followed in development of RGA markers linked to plant disease resistance genes. First is the collection of data of resistance gene family from the databases such as NCBI, SUCEST (Sugarcane ESTs), phytozome and PRGdb. The dbEST (EST database) has the collection of 76,986,302 EST sequences from various organisms of which 284,818 ESTs belonged to sugarcane(https://www.ncbi.nlm.nih.gov/search; 12th May 2018). Next step is the designing and synthesis of primers from conserved domain of resistance genes. Third and the final step is PCR amplification of sugarcane genomic DNA with these primers and sequencing of amplicons for characterization of resistance gene like sequences subject to BLAST analysis. The primers linked to RGA gene family may be considered as potential resistance gene analogue (RGA) markers that should be employed to develop stress related resistant genotypes *via* crosses between wild type and cultivated sugarcane elite cultivars. Bioinformatics studies such as BLAST, multiple alignment search tool, Phylogenetic analysis tool, Pfam scan, motif search tool (http://www.genome.jp/tools/motif/) etc. play a significant role in the development of RGA markers linked to disease resistance genes andcharacterization and evolutionary studies of disease resistance genes in such commodity crop.

Resistance genes analogue polymorphism (RGAP)

The RGA marker has an advantage over other DNA markers because they represent potentially useful resistance genes analogues (RGAs) or genes/loci linked to diseases. Very few reports are available on RGA marker associated to diseases in sugarcaneas compared to other crops. Jayashree *et al*. (2010) designed 29 polymorphic RGA primers from conserved domain of resistance genes and found some molecular markers related to red rot resistance and susceptibility. They reported 25 amplified products generated by 14 primers that were related to resistance and 8 PCR amplified fragments generated by 8 primers that were related to susceptibility. At ICAR-IISR, Lucknow resistance gene analogue markers have been developed to provide candidate genes for disease resistance in sugarcane (Srivastava *et al.,* 2016 a, b, 2017 a, b; Yadav and Srivastava, 2016). Thirty-five primer pairs of various combinations of designed primer pairs from conserved region of P-LOOP and GLPL of NBS-LRR class resistance gene have generated 34 putative RGA sequences. These sequences have the characteristic features of the known R-genes (Srivastava *et al*., 2016 a, b, 2017 a, b; Yadav and Srivastava, 2016). Using 55 sugarcane genotypes, 18 markers were recognized as red rot resistant markers and 7 markers as red rot susceptible RGA markers (Sharma and Tamta, 2017). This is because of very close relation of specific markers to red rot resistance or susceptibility genes. Molecular marker such as AFLP, RFLP, SSR and ISSR etc. were successfully identified for different diseases in sugarcane (McIntyre *et al*., 2005a; Aljanabi *et al*., 2007; Virupakshi and Naik, 2008). The RGAP markers have another benefit over random DNA markers because they are linked to genes/loci. Few studies have reported use of RGAs against red rot, rust, mosaic and smut disease in sugarcane (Que *et al*., 2009; Hameed *et al.,* 2015; Rossi *et al*., 2003; Brune and Rutherford, 2005; McIntyre *et al*., 2005b). Que *et al*. (2009) reported 11 RGAs in a sugarcane variety (NCo376) resistant to smut. A total of 15 RGAs were identified from a red rot resistant variety of sugarcane (Hameed *et al*., 2015). Linkage analysis has revealed that RGAs are distributed in whole genome and exists in cluster form (He *et al*., 2003). Twenty eighth RGAs were reported from a clone US 01-1158, resistant to SCYLV and moderately resistant to *Puccinia melanocephala* causing rust (Glynn *et al*., 2008).

RGAP markers for genetic differentiation and diversity analysis

RGAP markers could be useful in differentiating genetic structure asthey are useful for defining genetic diversity of a group of random R genes in sugarcane and various other crops. RGAP markers could also be used in describing geographic relatedness of sugarcane germplasm. Studies using RGAP have shown differentiation in genetic structure and geographic distribution in rice (Shang *et al*., 2009; Jia *et al*., 2011, 2012), wheat (Stukenbrock *et al*., 2006), barley (Zhang *et al*., 1987) and banana (Carlier *et al*., 1996). In sugarcane, RGAP based diversity analysishas been reported by Jayashree *et al*. (2010) and Sharma and Tamta (2017). A total of 29 RGA primers were used to study the genetic diversity among the 40 sugarcane genotypes that varied in their resistance to red rot disease. The genetic similarity among them ranged from 58.4 to 90% with the mean genetic similarity of 74.2% (Jayashree *et al*., 2010). RGAP markers *viz*. as RGA 231, 169, 533 and 267 were observed on different chromosomes of *Sorghum* and *Zea mays*, which evidentlypoint outtheir close homology (Sharma and Tamta, 2017).

Other applications of RGAP markers

Apart from genetic diversity analysis among sugarcane species, other applications of RGAP marker could be to address the marker assisted breeding and to delineate the mechanism of disease resistance at genetic and molecular level. The approach using RGAs may significantly shorten the breeding time and encourage rapid development of resistant cultivars of sugarcane. RGA markers play a significant role in identification, isolation, cloning and mapping of disease resistance genes as well as genetic improvement of sugarcane. Obviously, genetic improvement programme will depend on efficiency of genetic transformation protocols, but stable gene transformations in sugarcane are still not routine.

Future perspectives

The major goal of agricultural research is to develop resistance in crops to overcome the stress. Sugarcane, a cash crop, is continuously being challenged by biotic stresses such as fungal, bacterial, viral and phytoplasma. RGAP markers have potential role in identification of R-genes, which have important role in plant defence. RGAP markers have proven themselves as excellent tool for cloning and mapping of resistance genes, identification of qualitative trait loci (QTLs) for plant disease resistance genes, genetic diversity and evolutionary studies of R genes in crops and resistance breeding. In case of sugarcane, resistance gene analogue polymorphism marker or primers based on conserved domains of R genes to identify the putative RGAs may be useful in development of resistant cultivars and tounravel the mechanism of molecular plant-pathogen interaction and to explore gene diversity. Identification of disease

resistance genes in combination with genetic transformation related studies in sugarcane would bea step forward towards the development of genetically modified disease resistant sugarcane cropon one hand and understanding the mechanism of diseases resistance on the other.

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