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Prediction of candidate genes associated with resistance to soybean rust (*Phakopsora pachyrhizi*) in line UG-5

Hailay M. Gebremedhn[1] | Ulemu M. Msiska[2] | Miesho B. Weldekidan[3] | Thomas L. Odong[2] | Patrick Rubaihayo[2] | Phinehas Tukamuhabwa[2]

[1]Department of Biotechnology, College of Dryland Agriculture and Natural Resources, Mekelle University, Tigray, Ethiopia

[2]Department of Agricultural Production, School of Agriculture, College of Agricultural and Environmental Sciences, Makerere University, Kampala, Uganda

[3]College of Agriculture, Aksum University, Shire, Ethiopia

Correspondence
Hailay M. Gebremedhn, Department of Biotechnology, College of Dryland Agriculture and Natural Resources, Mekelle University, Tigray, Ethiopia.
Email: hailay.mehari2@mu.edu.et

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ABSTRACT

Online databases containing genetic information are crucial to extract new candidate genes from existing data and web-based resources. The objective of this study was, therefore, to predict putative candidate genes associated with resistance to SBR in line UG-5 and understand their functions using different bioinformatics tools from the online available databases. The physical positions for the flanking markers of the identified putative QTLs were searched on the SoyBase database genome browser based on Glyma 1.01 assembly. The putative candidate genes and annotated functions of the surrounding genes were discovered in the vicinity using SoyBase and Phytozome databases. A total of 18 putative candidate genes were predicted on approximately 482.7 kb region of QTL-3 (chromosome 18), among which, six putative candidate genes were found to encode leucine-rich repeat (LRR), Ser/Thr protein phosphatase, leucine-rich repeat receptor-like protein kinase (LRR-RLK) and chitinase-related proteins, which are associated with plant defence signalling pathways. Moreover, F-box and leucine-rich repeat, glycosyltransferase family member and serine/threonine-protein phosphatase 2A catalytic subunit coding genes were predicted on the novel putative QTL detected on chromosome 9. This information could, therefore, be used for further prediction and annotation of candidate genes from sequenced regions of line UG-5 as these putative candidate genes were predicted from the Glyma 1.01 assembly.

KEYWORDS

chitinase, glycotransferase, leucine-rich repeat, phytozome, SoyBase

1 | INTRODUCTION

Following challenges by biotrophic pathogens, plant genetic reaction is often expressed as either compatibility or incompatibility. Compatibility is characterized by pathogen development and reproduction with little visible cellular response by the host, while incompatibility is characterized when the pathogen is recognized by the plant and processes are triggered that hinder its further development (Sinapidou et al., 2004). Resistance responses that plants deploy in defence against pathogens are often activated following a recognition event mediated by resistance (*R*) genes. The encoded R proteins usually contain a nucleotide-binding site (NB) domain and a leucine-rich repeat (LRR) domain, which are further classified into N-terminal coiled-coil (CC) motif or a Toll interleukin receptor (TIR) domain. The nucleotide-binding site (NB) domain occurs in diverse proteins with ATP/GTP-binding activity (Bent, 1996), while the leucine-rich repeat (LRR) domain is predicted to mediate protein–protein interactions (Kobe & Deisenhofer, 1994). When these *R* genes are transferred into a susceptible plant of the same or closely related species, they usually impart full resistance capability.

Gene prediction refers to detection of genomic DNA regions that encode genes, which include protein-coding genes and/or other functional elements such as regulatory regions. It is most important in genome annotation, following sequence assembly, the filtering of non-coding regions and repeat masking (Yandell & Ence, 2012). Gene prediction is closely related to the so-called 'target search problem' investigating how DNA-binding proteins (transcription factors) locate specific binding sites within the genome (Redding & Greene, 2013). Many aspects of structural gene prediction are based on current understanding of underlying biochemical processes in the cell such as gene transcription, translation, protein–protein interactions and regulation processes, which are subject of active research in the various Omics fields such as Transcriptomics, Proteomics, Metabolomics and more generally structural and functional genomics (Hernández-Domínguez et al., 2019).

Availability of genetic information through online databases enables researchers to mine existing data and web-based resources for new candidate gene targets (Feder, 1999). The National Centre for Biotechnology Information (NCBI) (http://www.ncbi.nlm.nih.gov/), soybean breeder's databases (http://soybeanbreederstoolbox.org/) and phytozome (https://phytozome.jgi.doe.gov) are among the many, which have useful information for enhancing soybean improvement through Marker-Assisted Breeding (MAB). The information from such repositories of genomic data can be managed using the wide array of bioinformatics tools freely available online in attempt to understanding and modelling living systems (Edwards & Batley, 2010).

Large-scale development of sequencing techniques and gene expression analyses, combined with novel bioinformatics tools for data analysis, have facilitated structuring of extremely valuable databases for developing genetic engineering strategies. Several candidate genes coding for disease resistance-related proteins have been predicted and annotated from previously reported SBR resistance genes. Nucleotide-binding site leucine-rich repeat (NBS-LRR) resistance genes were predicted in *Rpp4* (Meyer et al., 2009) and *Rpp6907* (Chen et al., 2015). Moreover, 11 candidate genes were identified in *Rpp2*-mediated resistance including four soybean orthologs of known defence genes (Pandey et al., 2011; Van de Mortel et al., 2007et al., 2007).

The prediction and annotation of additional putative candidate *Rpp* genes would hence facilitate gene pyramiding and genetic transformation and generate novel molecular markers tightly linked to the resistance genes for use in future breeding programmes in the development of durable resistance to SBR and hence sustain soybean production. The aim of this study was hence to predict putative candidate genes associated with SBR resistance from the identified putative QTLs and determine their functions in UG-5 genotype.

2 | MATERIALS AND METHODS

2.1 | Mapping evaluation and SBR evaluation

A mapping population, comprising 86 F_2 segregating progenies, was developed from a cross Wondersoya × UG-5 and were evaluated for SBR resistance in screenhouse. The F_2 mapping population were evaluated for soybean rust disease under screenhouse condition and disease severity was scored at R6 reproductive stage (full-seed stage) on a 1 to 5 scale (Miles et al., 2008).

2.2 | Genotyping and statistical analysis

Genomic DNA was extracted from young leaves of the parental genotypes and 86 individual F_2 plants using CTAB (Lemos et al., 2011). After checking the purity and concentration of the DNA samples, screening was made using PCR with a total of 122 SSR markers (Hailay et al., 2018). Of the 33 polymorphic SSR markers obtained using QTL IciMapping 4.1 software, 13 SSR markers which were associated with the identified QTLs were used to genotype the F_2 progenies (Hailay et al., 2018). The phenotypic and genotypic data were deployed to map the putative QTLs using MapQTL version 4.0 software (Van Ooijen, 2009). Putative candidate genes were predicted and annotated using different bioinformatics tools from the Phytozome and SoyBase databases, from which the functions of the predicted putative candidate genes were determined.

To identify possible candidate genes underlying the mapped putative QTLs in UG-5, the soybean reference genome annotation accessible through Phytozome (http://www.phytozome.net) and SoyBase databases was exploited. The physical positions in the soybean genome for the flanking markers of the two putative QTLs detected on Chr 9 and Chr 18 of the line UG-5 were searched on the SoyBase database genome browser based on the Glyma 1.01 assembly and were used to discover the relevant putative candidate genes in their vicinity using the SoyBase and Phytozome databases. Annotated functions of the surrounding genes were investigated for their involvement in SBR resistance.

3 | RESULTS

Three putative QTLs were detected on chromosome 6, 9 and 18 from the F_2 mapping population developed from a cross Wondersoya × UG-5 using MapQTL version 4.0 software (Figure 1).

The putative QTL detected on chromosome 18 (QTL-3) was identical to a previously identified gene called *Rpp1-b* flanked by identical SSR markers, Sat_064 and Sat_372. The physical distance of the region between Sat_064 and Sat_372, which were at nucleotide positions Gm18:60,612,672 and 61,095,349, respectively, was approximately 482.7 kb.

Based on the soybean gene annotation database accessible at *G. max* genome (assembly version 1.01) (http://www.soybase.org), about 18 candidate genes were predicted on this region (Table 1). Among these predicted putative candidate genes, a cluster of three (Glyma18g51930, Glyma18g51950 and Glyma18g51960) candidate genes were leucine-rich repeat (LRR) containing proteins which are associated with disease resistance protein RPP13. These putative candidate genes undertake a biological process of reactions, triggered in

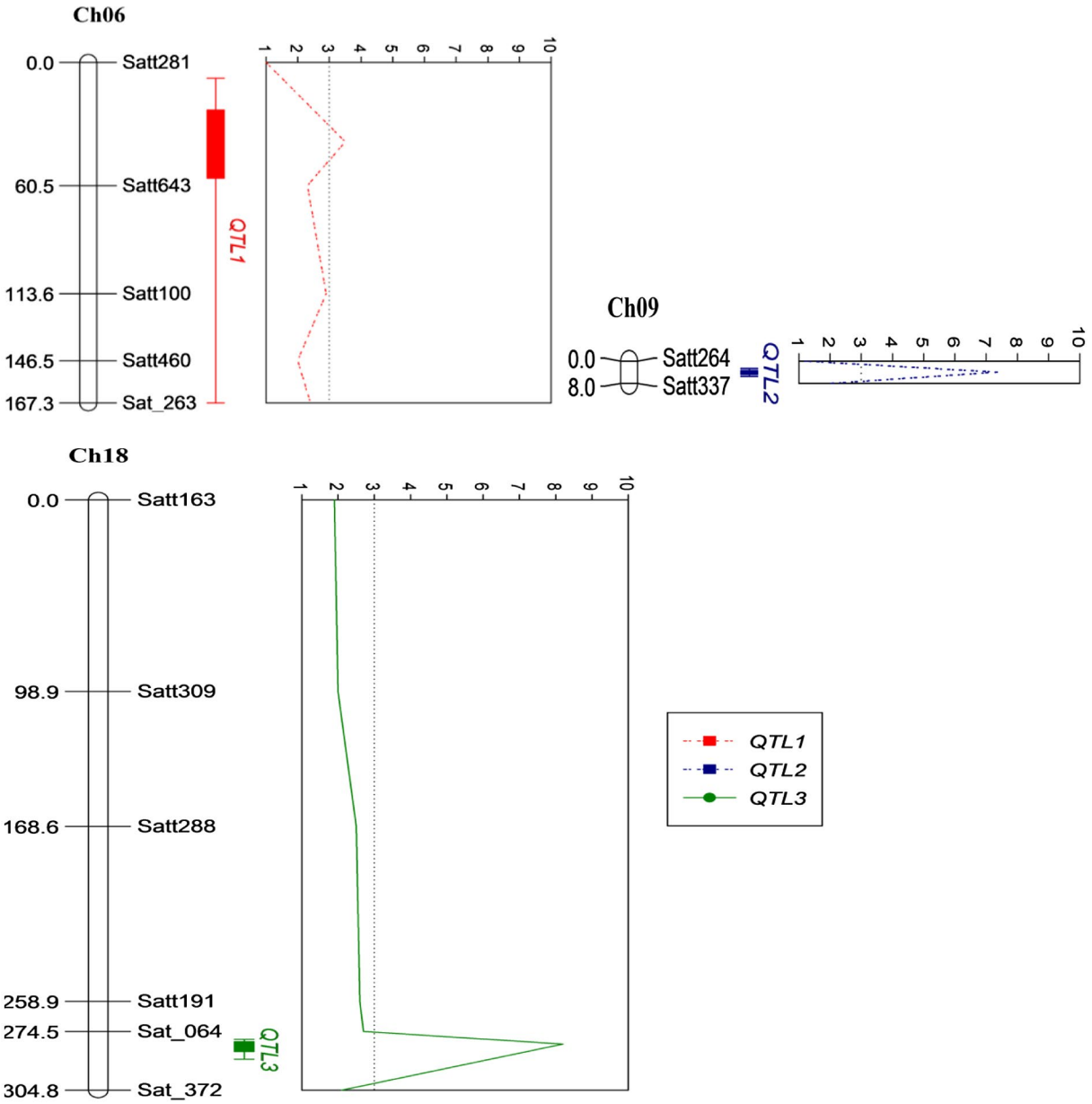

FIGURE 1 QTLs identified on chromosomes 6, 9 and 18; flanking markers along their positions in cM and the peak LOD scores.

response to the presence of a foreign body or the occurrence of an injury, which result in restriction of damage to the organism attacked or prevention/recovery from the infection caused by the attack. The molecular function of these genes is to interact selectively and non-covalently with ADP, adenosine 5'-diphosphate. Moreover, two candidate genes, designated as Glyma18g51911 and Glyma18g51970, were nucleic acid binding and Serine/threonine protein phosphatase containing proteins respectively. Glyma18g52050 and Glyma18g51980 were other predicted putative candidate genes with leucine-rich receptor-like protein kinase family and chitinase-related protein respectively.

On chromosome 9 (LG-K), the putative QTL detected between SSR markers Satt264 and Satt337 was unique as no other *Rpp* genes were previously identified elsewhere. A total of 28 candidate genes were predicted in this region (Table 2) using the phytozome v12.1 (http://www.phytozome.net). Of these, Glyma.09G105000 was serine/threonine-protein phosphatase 2A catalytic subunit, Glyma.09G105500 was F-box and leucine-rich repeat protein and Glyma.09G104700 was found to encode Glycosyltransferase 14 family member which are involved in plant defence signalling pathways.

The putative minor QTL detected on chromosome 6 was 21.5 cM from Satt643 (closest flanking marker), which is a large distance to predict genes in its vicinity.

4 | DISCUSSION

Plants protect themselves against a wide range of pathogens through resistance (*R*) genes that recognize avirulence (*Avr*) genes

TABLE 1 Predicted putative candidate genes in the mapping region of chromosome 18 near the closest SSR marker, Sat_064

Gene name	Chromosome location	Description
Glyma18g51880	Gm18:60632368..60634424	Dirigent-like protein
Glyma18g51890	Gm18:60639221..60652488	F/Y-rich N-terminus
Glyma18g51900	Gm18:60656814..60674825	protein transporter activity
Glyma18g51911	Gm18:60667870..60670284	nucleic acid binding
Glyma18g51920	Gm18:60676142..60684489	Chloroplast nucleoid DNA binding related
Glyma18g51930	Gm18:60685177..60687775	Leucine-rich repeat-containing protein
Glyma18g51950	Gm18:60693444..60696291	Leucine-rich repeat-containing protein
Glyma18g51960	Gm18:60704363..60706532	Leucine-rich repeat-containing protein
Glyma18g51970	Gm18:60708698..60714044	Serine/threonine protein phosphatase
Glyma18g51980	Gm18:60720285..60723728	Chitinase related
Glyma18g51990	Gm18:60725655..60728111	P21-Rho-binding domain
Glyma18g52000	Gm18:60733231..60734724	Acetylglucosaminyltransferase EXT1/exostosin 1
Glyma18g52010	Gm18:60738912..60740513	Acetylglucosaminyltransferase EXT1/exostosin 1
Glyma18g52020	Gm18:60742949..60749949	Protein of unknown function (DUF1664)
Glyma18g52030	Gm18:60751733..60758627	Uncharacterized conserved protein
Glyma18g52040	Gm18:60760610..60766351	GAMYB-binding protein
Glyma18g52050	Gm18:60775543..60779587	Leucine-rich receptor-like protein kinase family

TABLE 2 Predicted putative candidate genes in the mapping region of chromosome 9 (QTL-2) between SSR markers Satt264 and Satt337

Gene name	Chromosome location	Description
Glyma.09G104100	Gm09:19366455..19369117	BED zinc finger // hAT family C-terminal dimerization region
Glyma.09G104200	Gm09:19419070..19422719	Serine/arginine rich splicing factor
Glyma.09G104500	Gm09:19441235..19444166	PTHR31300:SF3 - GB
Glyma.09G104600	Gm09:19460350..19477190	SET domain-containing protein
Glyma.09G104700	Gm09:19489095..19490128	Glycosyltransferase 14 family member
Glyma.09G104800	Gm09:19494650..19495808	Hexadecanal dehydrogenase (acylating)/Fatty acyl-CoA reductase
Glyma.09G105000	Gm09:19500100..19508436	Serine/threonine-protein phosphatase 2A catalytic subunit
Glyma.09G105400	Gm09:19548840..19550770	Serine/arginine-rich splicing factor 2
Glyma.09G105500	Gm09:19575092..19580374	F-box and leucine-rich repeat protein
Glyma.09G105700	Gm09:19677273..19678420	Ubiquitin C (UBC)

in the pathogens. Proteins encoded by disease resistance (*R*) genes mediate specific molecular recognition of pathogenic microorganisms and trigger signalling cascades that activate defence reactions (Hammond-Kosack & Parker, 2003). Nucleotide binding site and leucine-rich repeat (NBS-LRR) encoding genes are members of the largest R protein. In soybean, over 400 NBS-LRR encoding genes have been predicted (Zhang et al., 2011), where NBS-LRR proteins were reported to play a role in the plant's defences against pathogens through signalling pathways (McHale et al., 2006). *R*-mediated pathogen recognition usually causes a localized hypersensitive cell death response (HR) that occurs at the site of infection by a pathogen. Presence of a large number of NB-LRRs

in plant genomes, therefore, confers resistance to many different pathogens.

Several putative candidate genes associated with resistance to soybean rust were identified in line UG-5 on chromosomes 9 and 18. A cluster of three NB-LRR candidate genes predicted on QTL-3 (chromosome 18), designated as Glyma18g51930, Glyma18g51950 and Glyma18g51960, were similar to the candidate genes predicted on *Rpp6907* within 111.9-kb region which may be involved in recognizing the presence of pathogens and ultimately conferring resistance (Chen et al., 2015). These putative candidate genes containing the NB-LRR domain were reported to involve in host–pathogen interaction and defence response via protein–protein interactions (Redekar et al., 2016). The other predicted candidate gene in this region (Glyma18g51970) contains Ser/Thr protein phosphatase, which involves prominently in a wide range of cellular processes including meiosis and cell division, apoptosis, protein synthesis, metabolism, cytoskeletal reorganization and the regulation of membrane receptors and channels (Ceulemans & Bollen, 2004). The putative candidate gene (Glyma18g52050) predicted on chromosome 18 was found to encode leucine-rich repeat receptor-like protein kinase (LRR-RLK) which was reported to represent a complex gene family in plants, mainly involved in development and stress responses (Dufayard et al., 2017; Liu, Du, Huang, Gao, & Yu, 2017). It was also reported that soybean *LRR-RLK* genes play important roles in various plant development and defence processes including leaf senescence, cell elongation and cold stress tolerance (Kim et al., 2009; Li et al., 2006; Yang et al., 2014).

Chitinolytic enzymes like chitinases are able to hydrolyze chitin, a major component of the cell wall of pathogenic fungi, and hence have been proven to be among the most promising candidates in plant disease protection (Knowles, Lehtovaara, & Teeri, 1987). Chitinases have been reported as pathogenesis-related proteins which attack directly on the fungal structural component (Sela-Buurlage et al., 1993). Moreover, many plant chitinases have been reported to possess potential antifungal activity (Kabir et al., 2016; Schlumbaum, Mauch, Vögeli, & Boller, 1986; Toufiq et al., 2017; Zhang, Kopparapu, Yan, Yang, & Jiang, 2013). Plant chitinases have also been reported to show remarkable potential for tolerating abiotic stresses, i.e. chitinases from *Hippophae rhamnoides* for cold stress (Kashyap & Deswal, 2017) and chitinases from soybean for arsenic and cadmium stress (Gálusová et al., 2015). The chitinase-related protein encoded by the candidate gene Glyma18g51890 on chromosome 18 could, therefore, potentially involve in resistance to SBR.

Glycosyltransferases (GTs) were reported to have many functions in plants, the majority of which are likely to be involved in biosynthesis of polysaccharides and glycoproteins in the plant cell wall (Hansen, Harholt, Oikawa, & Scheller, 2012). In *Arabidopsis thaliana*, most glycosyltransferases were reported to play a role in a pathogen- and stress-responsive expression where induction is dependent on the onset of the HR (Langlois-Meurinne, Gachon, & Saindrenan, 2005). The glycosyltransferase family member coding gene (Glyma.09G104700), which was predicted on chromosome 9 (QTL-2; Figure 1), could therefore be associated with and play an important role in resistance to SBR. It would increase the strength of the host's cell wall and hence reduce its direct penetration by the pathogen. Moreover, a serine/threonine-protein phosphatase 2A catalytic subunit encoding putative candidate gene (Glyma.09G105000) was predicted on chromosome 9, which was reported earlier as a crucial component that controls pathogenesis response in various plant species (Durian, Rahikainen, Alegre, Brosché, & Kangasjärvi, 2016). The protein phosphatase 2A catalytic subunit was also reported as a negative regulator of abscisic acid (ABA) signal transduction in *A. thaliana* (Pernas, García-Casado, Rojo, Solano, & Sánchez-Serrano, 2007). The use of okadaic acid has confirmed Ser/Thr protein phosphatases as negative regulators of plant defence responses against pathogens, in which the inhibitor activates anti-fungal defence responses even in the absence of infection or elicitor in several plant species, such as soybean, tobacco, tomato, potato and opium poppy (País, Téllez-Iñón, & Capiati, 2009). The other putative candidate gene (Glyma.09G10550) predicted on the novel QTL region was also found to encode the F-box and leucine-rich repeats, which are components of most of the plant disease resistance (*R*) genes reported to be large and abundant proteins involved in detection of diverse pathogens including bacteria, viruses, fungi, nematodes, insects and oomycetes (Zhang et al., 2011).

5 | CONCLUSION AND RECOMMENDATION

Results of this study showed the existence of several putative candidate genes associated with disease resistance on chromosome 9 and 18 (QTL2 and QTL3, respectively; Figure 1) of line UG-5. The identified putative candidate genes play important roles in disease resistance-related signalling pathways. These genes could, therefore, be used for further development of soybean varieties resistant to soybean rust using marker-assisted breeding and/or genetic transformation. Moreover, the information generated in this study could be used for further prediction and annotation of candidate genes from sequenced regions of line UG-5 as these putative candidate genes were predicted from the Glyma 1.01 assembly.

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CONFLICT OF INTEREST

The authors have declared no competing conflict of interest or funding disclosure.

AUTHORS' CONTRIBUTION

HMG, TLO, PR and PT designed the field experiment; HMG, UMM and MBW conducted the experiment; HMG did the statistical analyses and drafted the manuscript. All authors contributed in data interpretation and manuscript edition.

ORCID

Hailay M. Gebremedhn https://orcid.org/0000-0002-8709-4796
Miesho B. Weldekidan https://orcid.org/0000-0002-2297-6077

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