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Genome-wide identification, cloning and characterization of *SNARE* genes in bread wheat (*Triticum aestivum* L.) and their response to leaf rust

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ABSTRACT

SNAREs (soluble *N*-ethylmaleimide sensitive factor adaptor protein receptors) are small polypeptides characterized by a particular domain called the SNARE motif. Compared with the genome of other eukaryotes, monocotyledonous and dicotyledonous plants have more SNAREs indicating their important roles in higher plant species. Higher plants have the capability to form SNARE complexes that are important in determining the precise process of vesicle fusion for intracellular trafficking pathways. SNAREs have been reported to be engaged in the delivery of cell wall precursors to the newly formed cell plate during cytokinesis. The role of SNARE genes in response to plant-pathogen interaction is still not well understood. We found 35 SNARE genes in the wheat genome using a Hidden Markov Model. In this study with combined usage of *in silico* and molecular cloning technologies, we identified and characterized three SNARE genes (*SNARE*3, *SNARE*5 and *SNARE*6). The deduced amino acid sequences of these SNARE genes contained two characteristic conserved domains – a SNARE motif and a transmembrane domain, and they showed a high degree of homology with other eukaryotic SNARE genes. Phylogenetic analysis and three dimensional structures built with the help of Modeller software confirmed the presence of SNARE motifs in the proteins. The spatio-temporal expression profiling studies exemplify the positive role of SNARE transcripts have in resistant and susceptible wheat plants during incompatible and compatible interaction respectively, in response to *Puccinia triticina* induced leaf-rust infection. Taken together, our study suggests a role for *SNARE* genes in vesicle mediated resistance to leaf rust in wheat.

1. Introduction

Soluble *N*-ethylmaleimide sensitive factor adaptor protein receptors (SNAREs) comprise the core membrane fusion machinery which is indispensable for vesicle trafficking in all eukaryotic cells (Heese et al., 2001; Wick et al., 2003; Jahn and Fasshauer, 2012). A typical SNARE consists of a C-terminal trans-membrane domain (TMD) followed by a SNARE domain and finally may/may not have an N-terminal domain. Some have a lipid anchor instead of the TMD while others lack both and are soluble proteins (Jahn and Scheller, 2006). Most SNAREs have a single SNARE domain, while certain SNAREs like SNAPs have two SNARE domains. The main characteristic of SNAREs is the SNARE motif, comprising heptad repeats, which forms a α- helical coil that extends all the way to the TMD (Stein et al., 2009). The specificity of the fusion events of the intracellular trafficking pathways are considered to be regulated by SNAREs (Fukuda et al., 2000), which comprises four different SNARE types: Qa, Qb, Qc and R (Fasshauer et al., 1998). During trafficking of the vesicle, three Q-SNAREs and one R-SNARE localized differentially in *trans* on the vesicle and target compartment zipper around each other to drive the fusion of their membranes leading to the formation of stable, coiled-coils referred to as the ternary, *cis*-SNARE complex (Antonin et al., 2000; Pratelli et al., 2004; Wickner and Schekman, 2008). R-SNARE types contain an N-terminal domain with a profilin-like fold (Gonzalez et al., 2001; Wen et al., 2006), often referred to as 'longin' domains (Hong, 2005) that might have originated from a common ancestor (Kloepper et al., 2007). Similarly, all Qa-SNAREs exhibit quite related domain architecture, carrying an N-terminal three-helix bundle structure (Lerman et al., 2000; Dulubova et al., 2001). Several of the Qb- and the Qc-SNAREs possess an N-terminal three-helix bundle as well (Misura et al., 2002; Fridmann-Sirkis et al., 2006). This indicates a common origin of all three main Q-SNARE groups from a prototypic Q- SNARE.

In plants, SNAREs have been implicated to play roles in plant growth, homeostasis, hormone and stress responses (Bassham and Blatt, 2008; Grefen and Blatt, 2008; Eisenach et al., 2012; El Kasmi et al., 2013). Though limited research has been performed, SNAREs have also been reported to be involved in plant immunity against various pathogens

Abbreviation: SNARE, soluble N-ethylmaleimide sensitive factor adaptor protein; Receptors TMD, Trans-membrane domain; PR, Pathogenesis-related; qRT-PCR, quantitative Real Time PCR; CDD, Conserved Domain Database; HMM, Hidden Markov Model; MSA, Multiple sequence alignment; PDB, Protein Data Bank; DOPE, Discrete Optimized Protein Energy; MOF, MODELLER Objective Function; NILs, Near-isogenic lines.

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(Inada and Ueda, 2014). In barley (*Hordeum vulgare*) SNAREs deposit callose on cell walls during non-host resistance to powdery mildew (Collins et al., 2003). The tobacco (*Nicotiana tabacum*) *NbSYP132*SNARE mediates secretion of pathogenesis-related (PR) protein 1 and thereby resists against bacterial pathogens (Kalde et al., 2007).

It is expected that plants have more number of SNAREs as compared to fungi and animals, chiefly due to the expansion in number of members in conserved SNARE subfamilies and not due to the evolution of new isoforms (di Sansebastiano et al., 2009). About 60 SNAREs have been reported in *Arabidopsis* (Sanderfoot, 2007; Lipka et al., 2007), 69 in *Populus trichocarpa* (Tuskan et al., 2006) and 57 in rice (*Oryza sativa*; International Rice Genome Sequencing Project, 2005). In contrast *Homo sapiens* encodes only 35–36 SNAREs and the yeast *Saccharomyces cerevisiae* encode 21-25 SNAREs (Bock et al., 2001; Jahn and Scheller, 2006; Sutter et al., 2006). More SNARE genes in plants might also be due to many compositionally distinct intracellular compartments that can be sorted into subsets of different sizes and shapes. The distribution of the mass of an organelle among different compartments depends on the relationship between the budding and fusion exponents (Ramadas and Thattai, 2013). Duplication of SNARE genes might have been the driving force for the emergence of new organelles (Ramadas and Thattai, 2013).

Wheat (*Triticum aestivum* L.) is one of the most significant crops globally, occupying 17% (one sixth) of crop acreage worldwide, feeding about 40% of the world population and providing 20% (one fifth) of total food calories and proteins to human nutrition (Rosegrant and Agcaoili, 2010). Wheat crop is affected by numerous biotic and abiotic stresses and is very sensitive to attack of infectious diseases caused by a large number of pathogens: fungi, viruses, bacteria, and insects like Hessian fly and Russian wheat aphids that transfer viruses to wheat. Rusts are the major diseases responsible for huge yield loss worldwide (Dean et al., 2012). Three types of rusts, namely leaf/brown rust (Caused by: *Puccinia triticina* Eriks.), stripe/yellow rust (Caused by: *Puccinia striiformis* Westend. f. sp. *tritici*) and stem/black rust (Caused by: *Puccinia graminis* Pers. f. sp. *tritici*) occur in wheat. Leaf rust occurs more regularly and in more regions world-wide than stem or stripe rust. Loss in production of wheat from *P. triticina* infections are due to decreased number of kernels per head and lower kernel weight that might reach up to 40% in susceptible cultivars (Kolmer, 2005).

So far, the physiological roles of SNARE genes in plant–pathogen interactions in general and wheat-leaf rust in particular, have not been explored in details. In this study genome wide identification of SNARE genes in wheat were carried out; of them three SNARE genes were cloned and their expression in wheat leaves in response to induced leaf rust pathogenesis was characterized using quantitative Real Time PCR (qRT-PCR) and other findings from phylogenetic and homology modeling.

2. Materials and methods

2.1. Identification and isolation of cDNA sequences

The allohexaploid *Triticum aestivum* reference genome sequences were downloaded from Ensembl Plants (http://plants.ensembl.org/Triticum_aestivum/Info/Index). A *de novo* assembled transcriptome of bread wheat with or without inoculation of *P. triticina*, prepared earlier in our laboratory (Chandra et al., 2016), were also used for understanding the role of SNARE genes in leaf rust resistance. To find the SNARE domain within the wheat genome, 10,560 sequences containing the characteristic SNARE domain were downloaded from NCBI (http://www.ncbi.nlm.nih.gov/). For the identification of wheat SNARE genes, the sequences downloaded from Ensembl Plants were translated in six reading frames using Transeq algorithm of European Molecular Biology Open Software Suite (EMBOSS version 6.6.0., ftp://emboss.open- bio.org/pub/EMBOSS/). To find and confirm the presence of SNARE domain, the sequences downloaded from NCBI were checked by Conserved Domain Database (CDD; http://www.ncbi.nlm.nih.gov/Structure/cdd/wrpsb.cgi) and aligned with Muscle (http://www.ebi.ac.uk/Tools/msa/muscle/). The Hidden Markov Model (HMM) profile was build using hmmbuild tool of the HMMER suite (HMMER 3.1, http://hmmer.janelia.org/; Finn et al., 2011) with default parameters. The HMM profile builds were then used to search the SNARE domain in the 6-frame translated reference sequence by using the hmm search tool of HMMER suite. The strategy employed is shown in Fig. 1.

To identify the SNARE genes, *de novo* transcriptome assemblies of bread wheat, with or without being challenged to *P. triticina*, were blast searched against the NCBI non- redundant protein database with an e-value of $<e^{-5}$. The sequences showing homology with SNARE genes were individually checked for the presence of a SNARE motif in the SNARE Database (http://bioinformatics.mpibpc.mpg.de/snare/snareSubmitSequence Page.jsp). The sequences having a confirmed SNARE motif were selected and subjected to BLASTN with wheat ESTs available at NCBI. The three wheat ESTs most often found were selected and primer pairs were designed using Primer-Blast (http://www.ncbi.nlm.nih.gov/tools/primer-blast/) from those particular ESTs (list of designed primers is provided in Table 1).

2.2. Comparison of SNARE containing contigs with other Poaceae members

Analysis of orthologous clusters in whole genomes is important as the identification of overlap between orthologous clusters might help understand the evolution and function of the SNARE genes across important crop species of the Poaceae family. The web platform Ortho Venn (http://probes.pw.usda.gov/OrthoVenn; Wang et al., 2015) was used for the visualization of orthologous clusters in *Hordeum vulgare*, *Zea mays*, *Oryza sativa* (indica), *Brachypodium distachyon*, *Sorghum bicolor* and predicted SNARE sequences of *T. aestivum*.

2.3. Plant materials, amplification and cloning of SNARE genes

The wheat cultivar HD2329 + *Lr28* was used for the present studies. Seeds were germinated on autoclaved composite soil (peat, sand, soil, 1: 1: 1), grown to three leaf stage (~12 days after germination) in a growth chamber under ideal conditions (22 °C, RH 80%, 16 h light at 300 lx; and 8 h of darkness).

Total RNA was extracted from leaves using TRI REAGENT (Molecular Research Center, Inc., USA) following the manufacturer's protocol. Complementary DNA was synthesized from 5 μg of total RNA using Transcriptor First Strand cDNA Synthesis Kit (Roche Diagnostics GmbH, Mannheim, Germany). DNA amplifications were conducted in 20 μl volumes containing 100 ng of cDNA and 10 pM of each primer (Table 1). Amplification reactions were performed at 95 °C for 5 min followed by 30 cycles at 94 °C for 45 s, annealing was carried out at different temperatures for different primer pairs (Table 1) for 1 min,

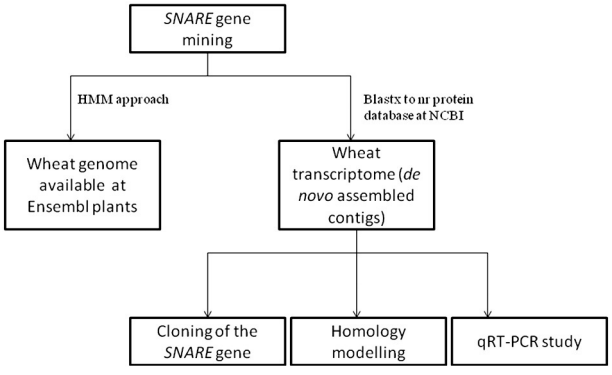

Fig. 1. Strategy employed for identification, cloning 3-D modeling and expression analysis involving SNARE genes (HMM: Hidden Markov Model; nr: non redundant; qRT-PCR: Quantitative Real Time PCR).