



De novo transcriptome of a bast fibre crop *Crotalaria juncea* reveals T2 ribonuclease genes to investigate late-acting self-rejection of pollens

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ABSTRACT

The biology of gametophytic late-acting self-rejection of pollen in pre-zygotic ovules is relatively unknown in the Fabaceae family of plants. Our understanding of the genetic basis of late-acting self-incompatibility (LSI) is limited due to a lack of sequence data and candidate genes. *Crotalaria juncea*, a Fabaceae family member that produces commercially important phloem fiber, possesses LSI. Due to a lack of selfed seeds, it is difficult to maintain genetic purity in germplasm and develop breeding lines for improved fiber quality and yield. To investigate candidate genes for LSI in *C. juncea*, a high-quality *de novo* transcriptome was generated. It facilitated the identification of genes from the self-incompatibility-related ribonuclease (RNase) family, specifically a Class III T2/RNase gene with sequence properties similar to S-RNase proteins. Based on conserved amino acid motifs, histidine residues, and a high isoelectric point ($pI \geq 9.0$), *Cjun_RNS3.1* was identified as an S-RNase homologue (non-S-RNase) or relic S-RNase gene. Unlike typical S-RNases found in known SI plant systems, evolutionary analysis of the *Cjun_RNS3.1* protein revealed its ancestral origin. The expression of *Cjun_RNS3.1* and other T2/RNase genes revealed differential expression patterns in the pistils of LSI and self-compatible *Crotalaria* species. The upregulation of the *Cjun_RNS3.1* gene during the different stages of pollen tube development indicates that it may be involved in the LSI. In summary, the sunn hemp transcriptome is the first genomic resource reported from a Fabaceae family plant with the LSI trait. A non-S-RNase gene with an ancestral evolutionary origin and a diverse function was found, which could be related to gametophytic LSI. This could be used as a model system to investigate the molecular basis of LSI in the Fabaceae family and help develop *C. juncea* breeding lines for fibre improvement.

1. Introduction

Ribonuclease T2 (T2/RNase) gene family members are found throughout the kingdom, including the plant kingdom, and are known to play various biological roles (Green, 1994; Lv et al., 2022; MacIntosh, 2011). Based on protein sequence similarities and the presence of introns in gene sequences, plant T2/RNases are classified into three classes: Class I, II, and III (Igić and Kohn, 2001; Ramanauskas and Igić, 2017). Class II T2/RNases, also known as RNS2, are the most conserved

proteins of the three T2/RNase classes in plants, and typically function as housekeeping genes (Hillwig et al., 2011). Class I and II T2/RNases are a S-like RNases. A noteworthy biological mechanism governed by Class III T2/RNase gene family members is self-incompatibility (SI) in higher plants. Self-incompatibility is a genetic mechanism that distinguishes between self- and non-self-pollens via male- and female-specific determinants from the S-locus (Fuji et al., 2016; Takayama and Isogai, 2005). The conventional mechanism of gametophytic self-incompatibility (GSI) in the Solanaceae, Rosaceae, and

Abbreviations: ANOVA, Analysis of variance; BLAST, Basic local alignment search tool; cDNA, complementary deoxyribonucleic acid; GSI, Gametophytic self-incompatibility; LSI, Late-acting self-incompatibility; MYA, Million years ago; NCBI, National Center for Biotechnology Information; ORF, Open reading frame; PE, Paired-end; qRT-PCR, quantitative reverse transcriptase polymerase chain reaction; RNA-seq, Ribonucleic acid sequencing; SC, Self-compatibility; SFB, S-locus F-box protein; SI, Self-incompatibility; S-RNase, Self-incompatibility locus ribonuclease.

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