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ORIGINAL ARTICLE

Endophytic fungus, *Fusarium* sp. reduces alternative splicing events in rice plants under salinity stress

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Abstract Alternative splicing (AS) of transcripts is a well-recognized phenomenon in plants. Several studies have shown that the frequency of AS events are significantly enhanced in plants subjected to abiotic stresses, presumably as an adaptation to increase transcript diversity. In this study, we examine the effect of a salt tolerant endophytic fungus, *Fusarium* sp. on the AS events of IR-64 rice plants, under salinity stress. RNA seq data generated from rice plants, treated and not treated with *Fusarium* sp. respectively and subjected to 150 mM salinity stress was used to analyze the number and type of AS events and their chromosomal distribution using approriate bioinformatics pipeline. Besides, we also annotated the locus IDs of genes for gene enrichment and KEGG pathway analysis. Our results show an unequivocal decrease in the number of AS events under salinity stress as influenced by the endophyte. The average AS events per gene also decreased from 2.28 to 2.11 upon colonization by the endophyte. This is the first documented evidence of an endophyte-induced alteration in the frequency of alternative splicing event in plants subjected to salinity stress. The exact mechanisms through which the splicing activities are restrained, however need to be further investigated.

Keywords Endophyte · Alternative splicing · Salinity stress · Rice · Transcriptome

Introduction

Alternative splicing (AS), is an important modulator of gene expression that can potentially increase the proteome diversity and also regulate mRNA levels. Splicing occurs when introns are removed from the pre-mRNA transcripts by the spliceosome (Duque 2011). Based on the site at which the splicing occurs, transcripts may contain a premature stop codon or a retained intron that triggers nonsense-mediated decay or produce truncated proteins (Ottens and Gehring 2016), often referred to as 'unproductive alternative splicing' (Lewis et al. 2003). Intron retention (IR) is the predominant mode of AS in plants (Drechsel et al. 2013; Kalyna et al. 2011) and generates mostly non-sense mRNAs having premature terminal codons (PTC+). These are either degraded by the nonsense-mediated mRNA decay (NMD) pathway, or escape NMD to produce truncated proteins, thereby regulating the function and abundance of their full-length counterparts (Chaudhary et al. 2019). IR has been found to be an important regulator of gene expression affecting growth, physiology, development, biotic and abiotic stress responses (Ashwini et al. 2018; Drechsel et al. 2013; Filichkin

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et al. 2015; Kalyna et al. 2011; Syed et al. 2012). Alternatively, the RNA might be translated into a protein with a slightly different function leading to the plasticity of the genome.

In the recent past, there has been growing interest on AS events in plants in response to abiotic stresses. For example, in *Oryza longistaminata*, there was an increase of 5807 and 5256 AS events respectively in shoots and rhizomes, in response to chilling stress (Zhang et al. 2017). Compared to control, salinity stress of 150 mM caused an increase of 2059 AS events in Arabidopsis (Ding et al. 2014). In grapes, AS events increased from 23.6 to 68.2% as a result of high temperature stress (Jiang et al. 2017). The consistent patterns of increase of AS under stressful environments have lead some authors to argue that this could be an adaptive strategy of plants to diversify their response portfolios (Laloum et al. 2018; Ner-Gaon et al. 2004). Indeed, Laloum et al. (2018) reported that plant stress-related genes might be more prone to AS events, as an adaptive mechanism, by switching the ratio of active to non-active isoforms in response to abiotic stress (Laloum et al. 2018).

Here, we explore the role of endophytes in impacting AS events in plants subjected to abiotic stresses. Endophytes, both fungal and bacterial, are ubiquitously present in all plants and have been reported to play a major role in many plant growth processes and in conferring "habitat-adapted symbiotic" fitness benefits to plants (Rodriguez et al. 2008; Sangamesh et al. 2018). Studies have shown that endophytes can impart tolerance to their respective host plants against a range of stressors including drought, high temperature, salinity and also nutrient deficiency (Arshad et al. 2017; Azad and Kaminskyj 2016; Sangamesh et al. 2018). While the mechanisms leading to these effects are currently being investigated, it is believed that the endophytes might intervene in a range of stages, from stress signaling to host gene regulation to appropriately modify plant responses to the stresses. However, it is not yet known, if endophytes also influence the AS events of their host plants under stress.

In our recent study, it was observed that an endophyte, from a salt tolerant Pokkali rice variety could not only successfully colonize a salt sensitive rice variety, IR-64 but also confer the plants salt tolerance. RNA seq analysis showed that under salinity stress, the endophyte altered the host tissue gene expression. Here, using the transcriptome data thus generated, we ask if the endophyte colonization of rice plants under salinity stress has had any impact on AS events compared to plants not colonized by the endophyte. We also investigate the chromosomal distribution of the AS events ask if these have any novelty with respect to their putative functionalities. Finally we also trace the major pathways that undergo the AS events under stress as influenced by the introduced endophyte.

Materials and methods

Data sets

To mine the AS events, we used RNA seq data of the rice genotype, IR-64, enriched and unenriched with a salt tolerant endophytic fungus (*Fusarium* sp.) (GenBank Acc No. MN170565), under salinity stress. The data bearing NCBI (SRA) accession numbers, PRJNA511516 and PRJNA511618 respectively was used in the present study. The reference genome of *Oryza sativa* Nipponbare was downloaded from http://rice.plantbiology.msu.edu/pub/data/Eukaryotic_Projects/o_sativa/annotation_dbs/pseudomolecules/version_7.0/ for reference assembly.

Bioinformatic analysis

The BAM files generated after the reference assembly for the transcriptome data sets: E + S+ and E–S+ (E: Endophytic *Fusarium* sp.; S: Salinity Stress; ±: Presence/Absence) was used separately to identify the AS events and its chromosomal distribution using the R script of the SAJR package (Mazin et al. 2013) (http://storage.bioinf.fbb.msu.ru/~mazin/index.html). Novel AS events were identified following the method described by Doktor et al. (2018). Further locus Ids of the identified AS events were used for gene enrichment analysis using the online tool AgriGO v 2.0 (http://systemsbiology.cau.edu.cn/agriGOv2/) (Du et al. 2010) and visualization of the GOs were computed using REVIGO (http://revigo.irb.hr/) (Supek et al. 2011). AS genes were also annotated for the biochemical pathways using the web server KAAS v 2.1 (KEGG Automatic Annotation Server) (https://www.genome.jp/kaas-bin/kaas_main). The pipeline of the analysis has been schematically presented in Fig. 1.

Results and discussion

A total of 44,403 and 39,420 AS events, were identified from the E + S+ and E–S+ transcriptome data, respectively. In other words, there was an 11.22% reduction in the AS events under salinity stress due to colonization of IR-64 plants by the salt tolerant endophyte, *Fusarium* sp. Most of the AS events were of "Retained Intron" type as has been reported in several other studies on plants (Chaudhary et al. 2019; Li et al. 2010; Reddy et al. 2013; Sablok et al. 2011). Of the 44,403 AS events in E + S+, 39,733 were of retained intron type, while in E–S+ it was 35,285 of 39,420. The endophyte colonization caused the largest decrease in this type of AS events (Table 1). The maximum number of AS events was located on chromosome 1 (893 AS; 959 AS) and chromosome 3 (715 AS; 773 AS) in the E + S+ and E–S+ transcriptome respectively. Overall, there was a reduction in the abundance of AS events on each of the 12 chromosomes due to the endophyte *Fusarium* sp. The maximum reduction of 124 AS events due to the endophyte was on chromosome 2 (Fig. 2; Supplementary File 1).

Following the SAJR package, the novel AS events were classified into 7 classes (Fig. 3A). A total of, 46,707 and 51,944 novel AS events, were identified from the E + S+ and E–S+ transcriptome data respectively. Thus endophyte treatments lead to a 10.08% reduction in the novel AS events. The majority of the AS events mapped to the class ‘e’ (genes overlap by exons in sense) and class ‘i’ (one gene is within intron of other one (in sense)). The major reduction in the AS events was associated with class ‘i’ with about 2972 events. A Venn diagram of common and unique AS events between E + S+ and E–S+ indicated that there were 39,472 events common to both treatments while 3634 and 8300 events were unique to E + S+ and E–S+ respectively (Fig. 3B) (Supplementary File 2). The average AS events per gene, decreased from 2.28 to 2.11 upon colonization by the endophyte (Supplementary File 3).

Interestingly, most of the genes with AS events in both the E + S+ and E–S+ treatments, grouped into those responsible for cellular component and biological process and very few mapping to any molecular function group. The ReviGO representation of the GO terms indicated that there were common GO terms between the treatments which underwent the AS events but the colonization by endophyte only reduced the abundance of genes within the

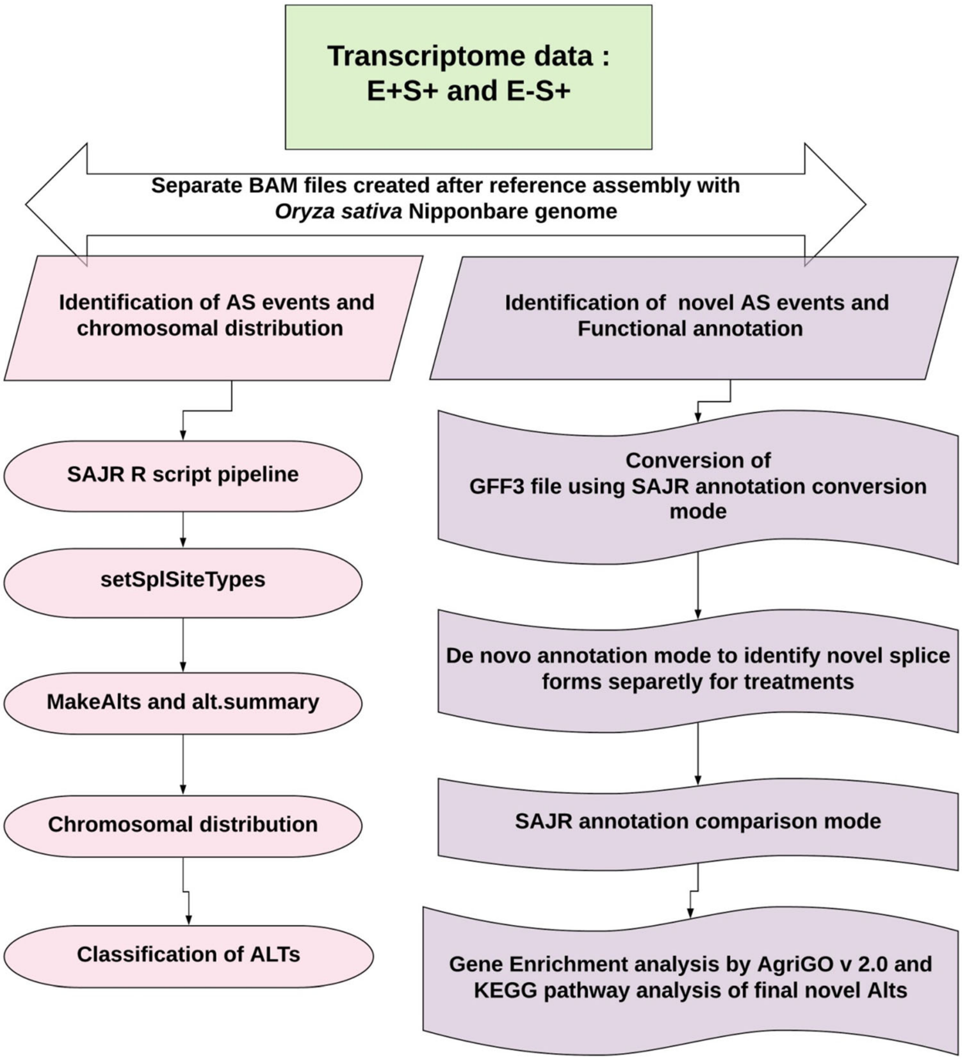


Fig. 1 Pipeline of the steps involved in the computational prediction of alternative splicing events from IR-64: E + S+ and E–S+ transcriptome data

Table 1 Total number of Alternative splicing classes and its distribution in the E + S+ and E–S+ transcriptome data

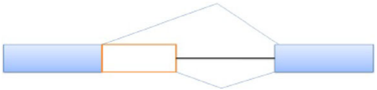
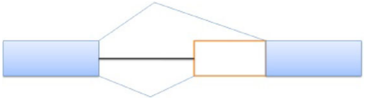
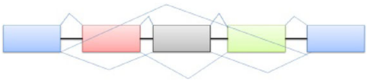
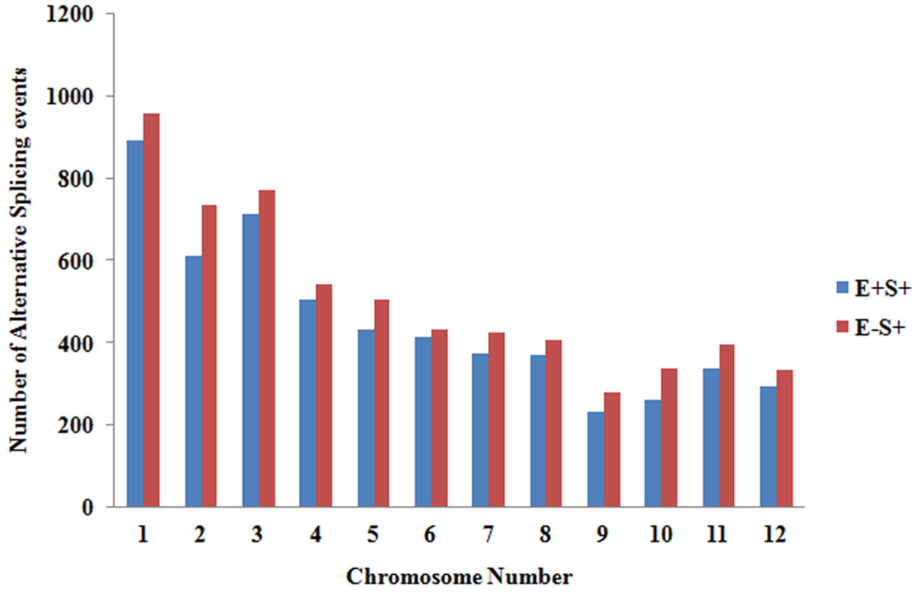
Splicing graph	Type of AS events	E + S+	E–S+
	Retained introns	35,285	39,733
	Cassette exon	135	143
	Alternative donor	375	425
	Alternative acceptor	869	945
	Complex	2756	3157
	Total	39,420	44,403

Fig. 2 Chromosomal distribution of rice AS events: distribution of AS events of E + S+ and E–S+ treatments on the *Oryza sativa* Nipponbare genome



GO terms (Fig. 4). For instance the genes coding for the 'peroxisomes' and 'intra cellular membrane bound organelle' in the cellular component decreased in its abundance in plants enriched with the endophytic *Fusarium* sp. It has been well documented that peroxisomes play a key role in the regulation of metabolic process by modulating cellular redox homeostasis in the cell (Yun et al. 2012).

The genes showing the AS events were also assigned to the biochemical pathways by KEGG pathway analysis. Majority of the genes mapped to the metabolic pathway with 728 and 893 genes in the E + S+ and E–S+ transcriptome. A reduction of 165 AS events in the genes was observed under the metabolic pathway class due to the colonization by the endophyte (Fig. 5). There was a 50% reduction in the AS events with respect to DNA replication upon colonization by the endophyte.

Since the discovery of the spliced segments in the adenovirus by Berget et al. (1977), evidence has been mounting to suggest that alternative splicing events might play an important role in transcriptional regulation and enhancing the functional diversity of eukaryotic genomes (Zhu et al. 2018). Alternative splicing events are singularly known to increase in plants exposed to abiotic stresses (Ashwini et al. 2018; Ding et al. 2014; Jiang et al. 2017; Zhang et al. 2017). Though evidence is scanty, high throughput analysis of many of such AS events under stress

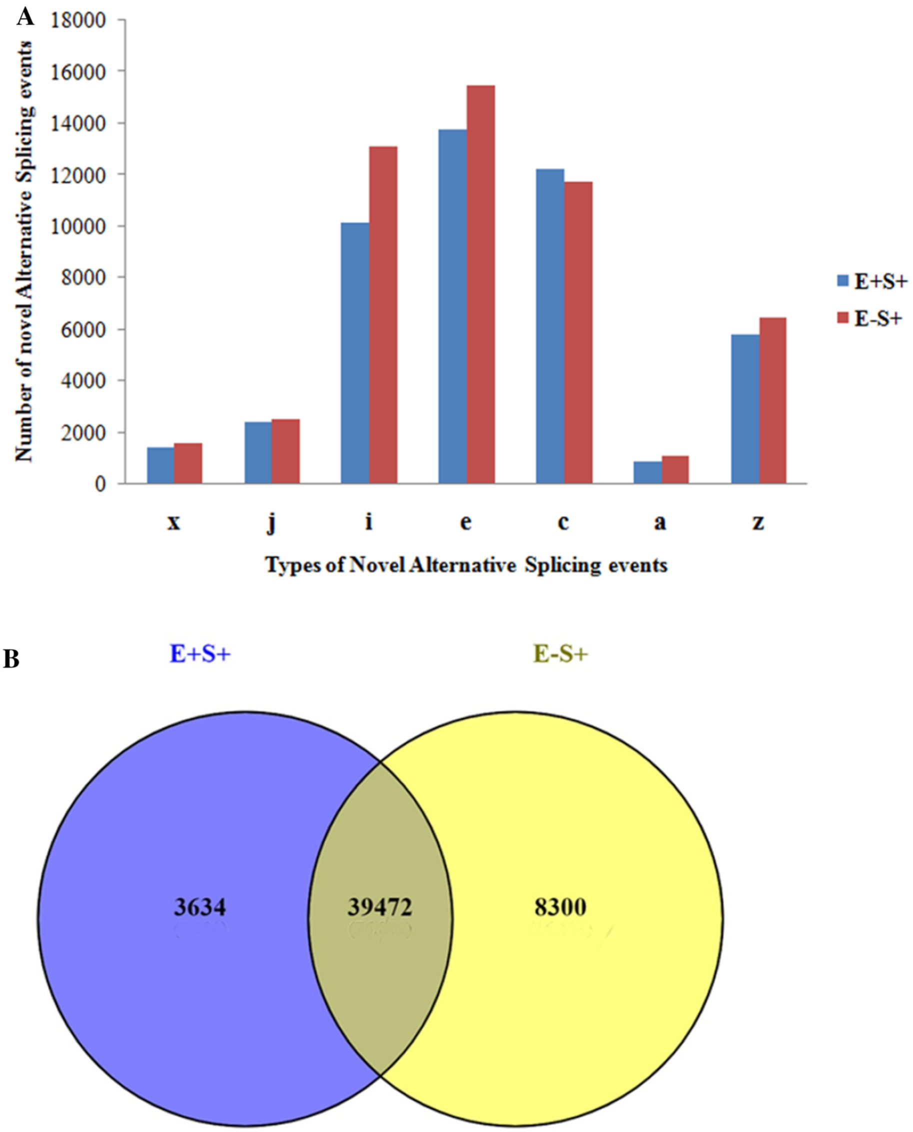


Fig. 3 Novel AS events: **A** total number of novel AS events under different classes: **x**—genes overlap by exons in antisense; **j**—genes share some junctions; **i**—one gene is within intron of other one (in sense); **e**—genes overlap by exons in sense; **c**—junctions of one gene are subset of junctions of other one; **a**—one gene is within intron of other one (in antisense); **z**—both genes have exactly the same set of junctions, **B** Venn diagram representing unique and common novel AS events between the treatments E + S+ and E–S+

show that they enable plant adaptations to the stressful environment (Laloum et al. 2018). In other words there seem to a selection for splicing variants to be generated under stressful environments (Laloum et al. 2018; Ner-Gaon et al. 2004).

Endophytes, both bacteria and fungi, have been reported in many studies to alleviate abiotic stress tolerance in plants. In a recent meta-analysis, Rho et al. (2018) showed that endophyte treatment significantly enhanced host plant growth under drought, salinity and nitrogen deficient stress conditions compared to plants not treated with endophytes. The underlying mechanisms by which endophytes are able to modulate plant responses under stress are not adequately addressed though there is some evidence to suggest that they might alter host gene expression (Afridi et al. 2019; Jan et al. 2019; Lanza et al. 2019; Pan et al. 2018). There is as yet no documented evidence to suggest that endophyte might modulate AS events in plants. In this study, we unequivocally show, for the first time, a reduction in the AS events in plants treated with the Pokkali salt tolerant endophyte compared to plants that were untreated. While the cascade leading to such decrease in AS events is currently not clear, we hypothesize that it could partly be due to a lowered perception of stress signaling by plants treated with the salt tolerant endophyte. In other words, under a given abiotic stress, such as salinity stress, the ability of an endophyte to confer stress adaptations to the plants, might restrain the stress-induced alternate splicing events. It

Fig. 4 Gene ontology (GO) analysis of the genes showing novel AS events. **A**, **B** Represents biological process of E + S+ and E–S+ transcriptome; **C**, **D** cellular components of E + S+ and E–S+ transcriptome. The scatter plot shows the cluster representatives (terms remaining after reducing redundancy) in a 2-D space derived by applying multi dimensional scaling to a matrix of GO term semantic similarities. Bubble color indicates the log10 (*p* value) for FDR derived from agriGO analysis. The bubble size indicates the frequency of the GO terms

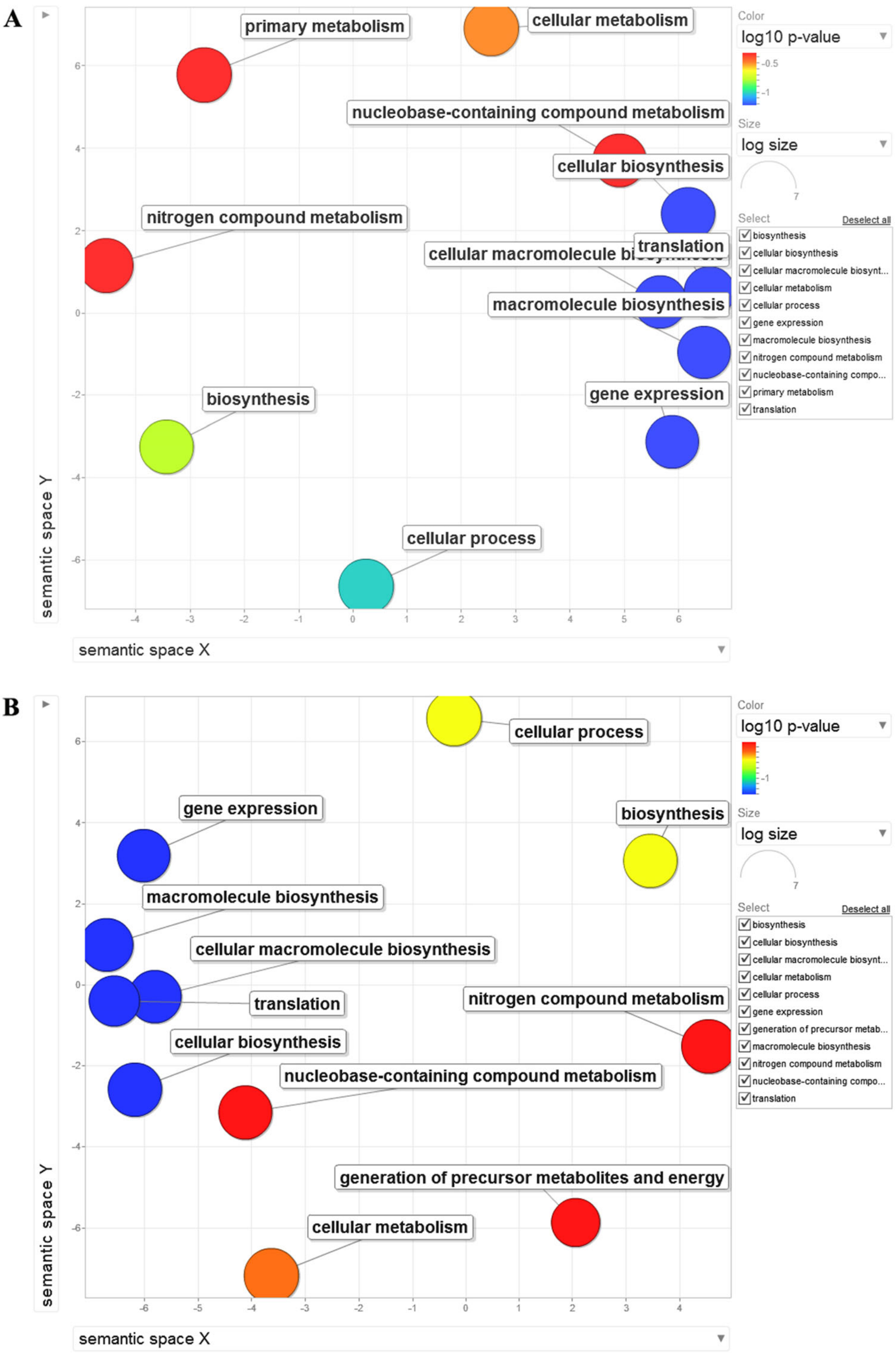
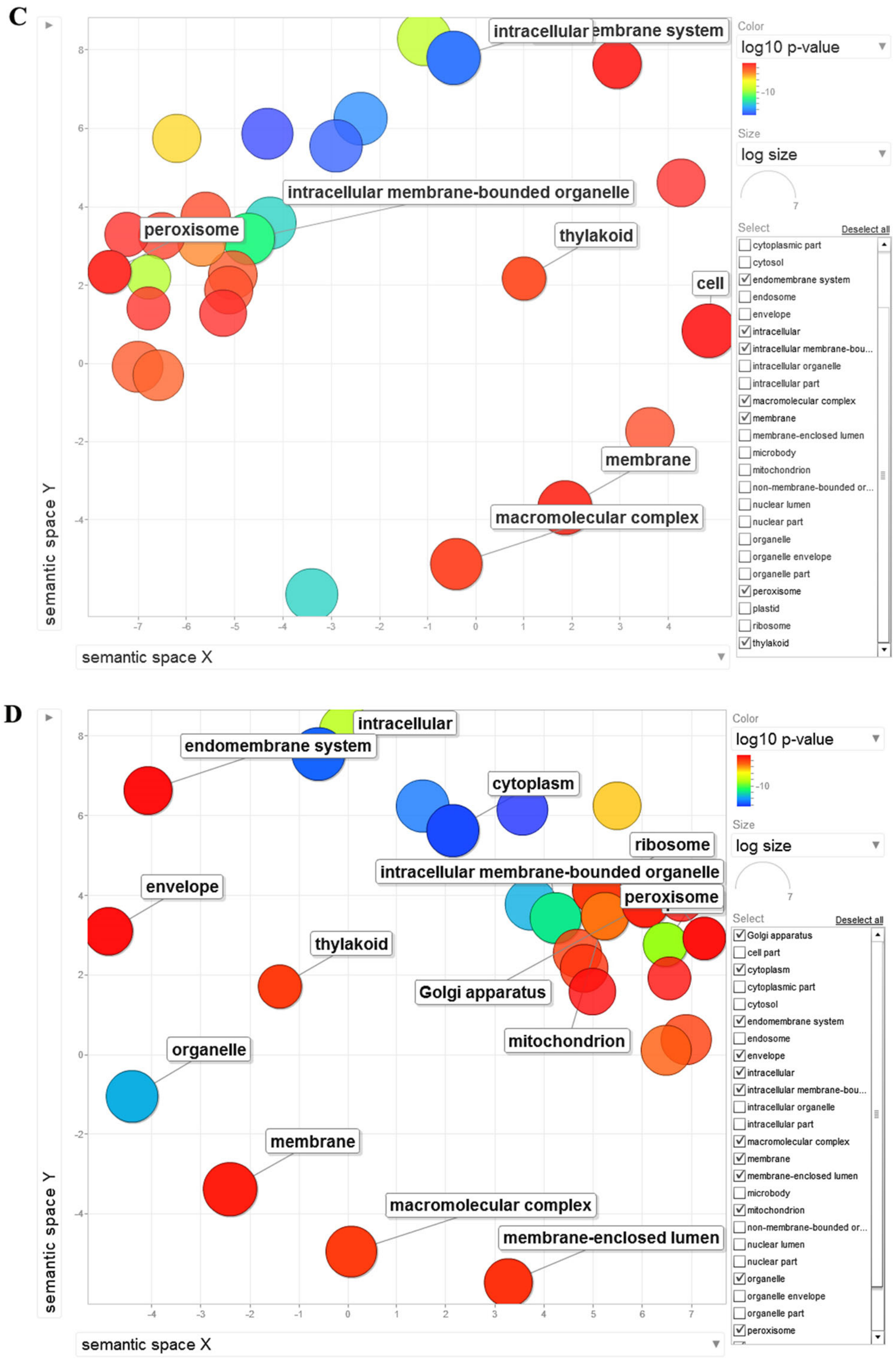


Fig. 4 continued



C

Color: log10 p-value

Size: log size

Select

- ☐ cytoplasmic part
- ☐ cytosol
- ☑ endomembrane system
- ☐ endosome
- ☐ envelope
- ☑ intracellular
- ☑ intracellular membrane-bou...
- ☐ intracellular organelle
- ☐ intracellular part
- ☑ macromolecular complex
- ☑ membrane
- ☐ membrane-enclosed lumen
- ☐ microbody
- ☐ mitochondrion
- ☐ non-membrane-bounded or...
- ☐ nuclear lumen
- ☐ nuclear part
- ☐ organelle
- ☐ organelle envelope
- ☐ organelle part
- ☑ peroxisome
- ☐ plastid
- ☐ ribosome
- ☑ thylakoid

D

Color: log10 p-value

Size: log size

Select

- ☑ Golgi apparatus
- ☐ cell part
- ☑ cytoplasm
- ☐ cytoplasmic part
- ☐ cytosol
- ☑ endomembrane system
- ☐ endosome
- ☑ envelope
- ☑ intracellular
- ☑ intracellular membrane-bou...
- ☐ intracellular organelle
- ☐ intracellular part
- ☑ macromolecular complex
- ☑ membrane
- ☑ membrane-enclosed lumen
- ☐ microbody
- ☑ mitochondrion
- ☐ non-membrane-bounded or...
- ☐ nuclear lumen
- ☐ nuclear part
- ☑ organelle
- ☐ organelle envelope
- ☐ organelle part
- ☑ peroxisome

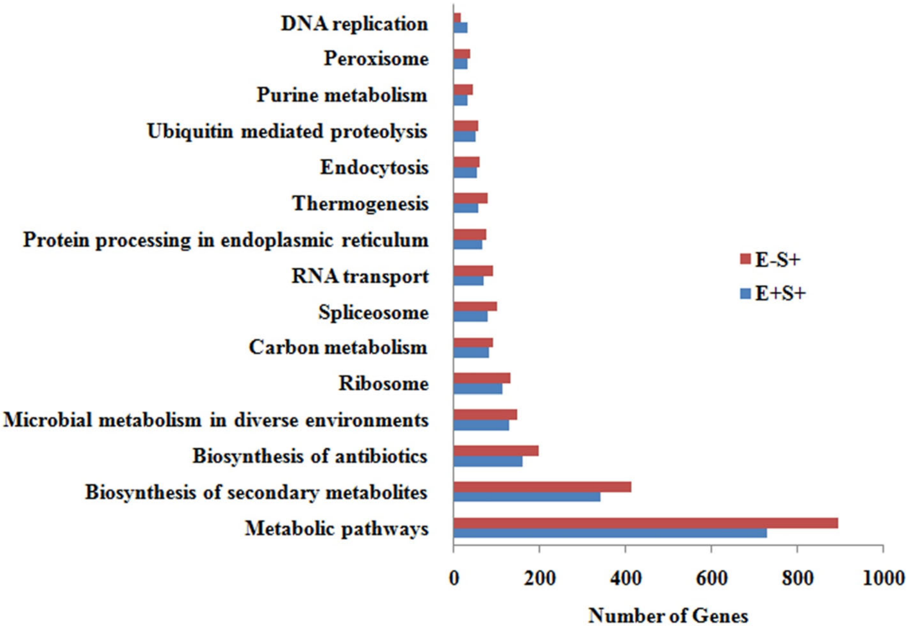


Fig. 5 KEGG pathway annotation of the novel AS events. Bar graph representing the top 15 KEGG pathways annotated between E + S+ and E–S+ AS events

would be interesting, in this context, to further examine the alterations in the splicing processes itself, such as on the spliceosome and the splicing factors, in plants treated with endophytes.

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