

RNA-seq reveals massive down-regulation of genes in cytoplasmic male-sterile beet plants

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Introduction

In beets, hybrid seed production is based on cytoplasmic male sterility (CMS) which results from the presence of the so called sterilizing (S-) cytoplasm (carrying the mitochondrially encoded sterility determinant) as well as recessive alleles in two nuclear loci – *X/x (Rf1/rf1)* and *Z/z (Rf2/rf2)*. The corresponding dominant alleles, referred to as restorers, bring at least a certain level of pollen fertility to plants carrying the S-cytoplasm. The presented work is aimed at identification of genes showing differential expression between male-sterile plants and plants with fertility restored by the *X (Rf1)* gene.

Plant material

The analysis was performed for 30 table beet plants (*Beta vulgaris* subsp. *vulgaris*, cultivar group *conditiva*) – 15 cytoplasmic male-sterile and 15 with restored male fertility. All plants carried the sterilizing (S-) cytoplasm, in the male-fertile plants its sterilizing effect was suppressed by the presence of the nuclear restorer gene *X/Rf1*.

Methods

Flower buds were ground in liquid nitrogen and the resulting powder was suspended in TRI Reagent (Zymo Research). Further steps of RNA isolation were performed using PureLink RNA Mini Kit (Thermo Fisher Scientific). Preparation of sequencing libraries and Illumina PE100 sequencing (NovaSeq 6000) were performed by CeGaT (Tübingen, Germany).

The sequence data delivered by CeGaT were subjected to filtration with Trimmomatic (Bolger et al. 2014) to get rid of adaptor and low quality sequences. Next, using Bowtie2 (Langmead & Salzberg 2012) the reads mapping to rRNA genes were discarded. Subsequently, the filtered reads were mapped to the sugar beet reference genome (AYZS00000000.2) using STAR (Dobin et al. 2013). The mapped reads were then subjected to assembly with StringTie (Pertea et al. 2015). Analysis of gene expression was performed using programs from the Cufflinks (Trapnell et al. 2010) package. For data filtration and visualization the CummeRbund package (Goff et al. 2013) from the R software (R Core Team 2016) as well as custom Python scripts (W. Wesołowski) were used. Annotation of differentially expressed genes was performed with OmicsBox (Biobam Bioinformatics).

Using the STAR software the sequence reads were also mapped to the sugar beet mitochondrial (BA000024) and plastid (NC_059012) genomes. The results of mapping were saved in the SAM files which then were used for calculation of the mean coverage of the whole organellar genome and the mean coverage of its component coding sequences. The coverage of individual genes was subsequently normalized by the coverage of the whole organellar genome. This analysis was performed using a custom Python script (P. Gierski). Statistical significance of observed differences in the normalized coverage of individual organellar genes between male-sterile and restored plants was determined using the Student's t-test ($\alpha = 0.05$).

Results

On average for a single plant 33.5 M paired Illumina reads were obtained. The mean fraction of reads which mapped to the reference genome reached 91.2%. Differential expression between male-sterile and male-fertile plants was found for 775 nuclear genes (figs. 1 and 2). Among the genes of at least 4-fold difference in expression over 75% showed higher expression in the male-fertile plants. Among the genes showing elevated expression in male-fertile plants there was the gene coding for the OMA1 protein which corresponds to the *X/Rf1* restorer.

Analysis of Gene Ontology revealed that for the Cellular Component category the best represented differentiating nuclear genes encoded integral membrane proteins. The category of Molecular Function was dominated by genes coding for ATP binding proteins, and category of Biological Process by genes encoding proteins related to protein phosphorylation (fig. 3).

Acknowledgements

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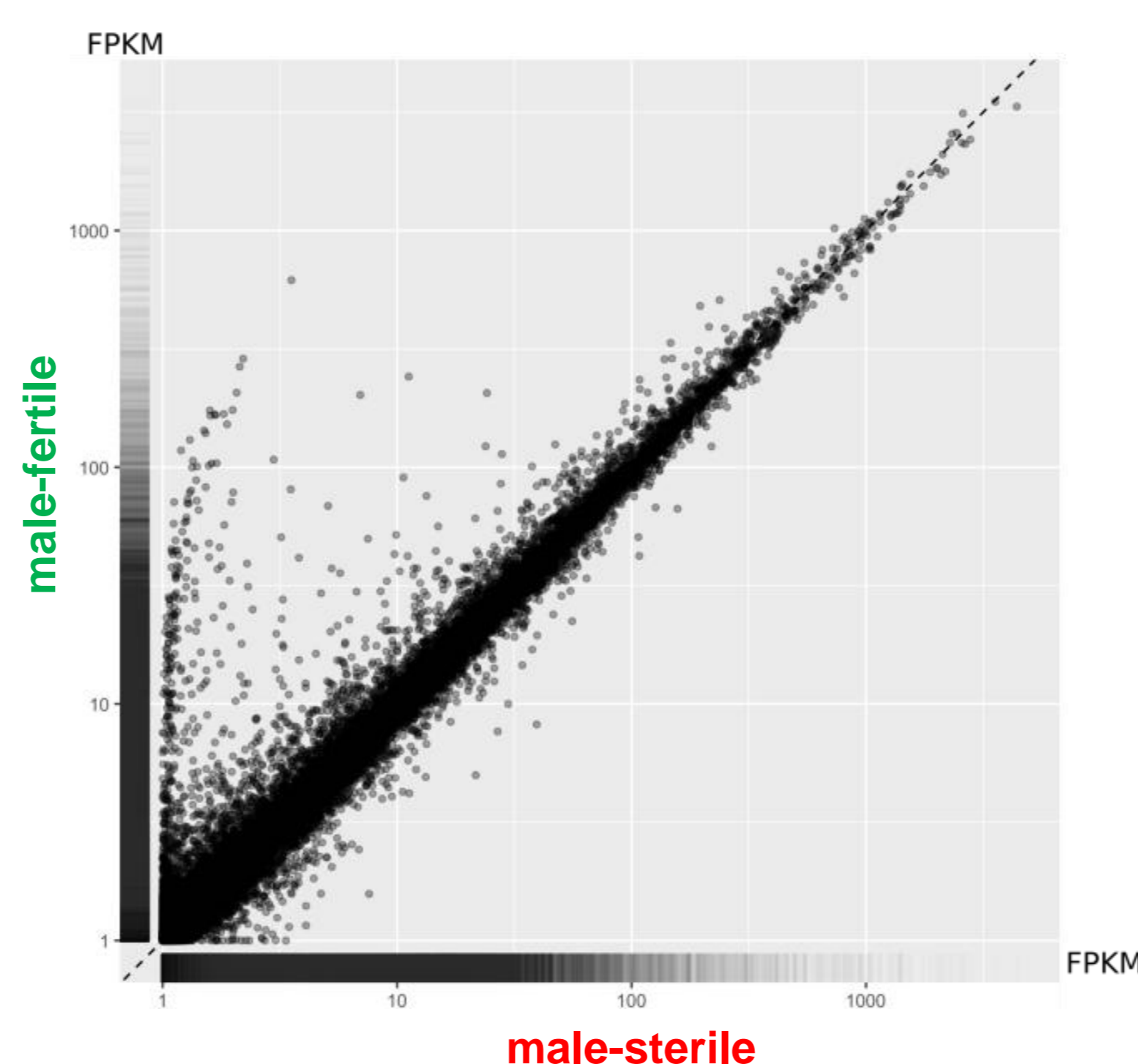


Figure 1. Scatter plot showing distribution of FPKM units in male-sterile and male-fertile (restored) plants.

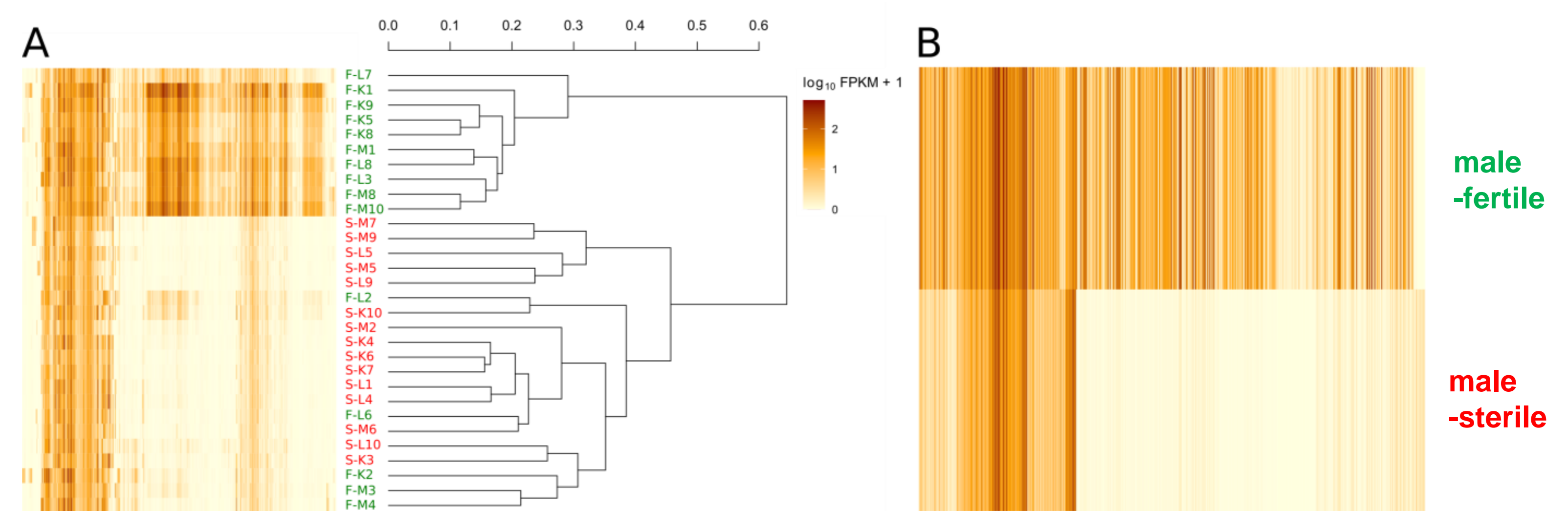


Figure 2. Heatmap plots showing expression level of genes with statistically significant difference between male-sterile and male-fertile plants. A – individual plants (red fonts – male-sterile, green fonts – male-fertile), B – phenotypic classes.

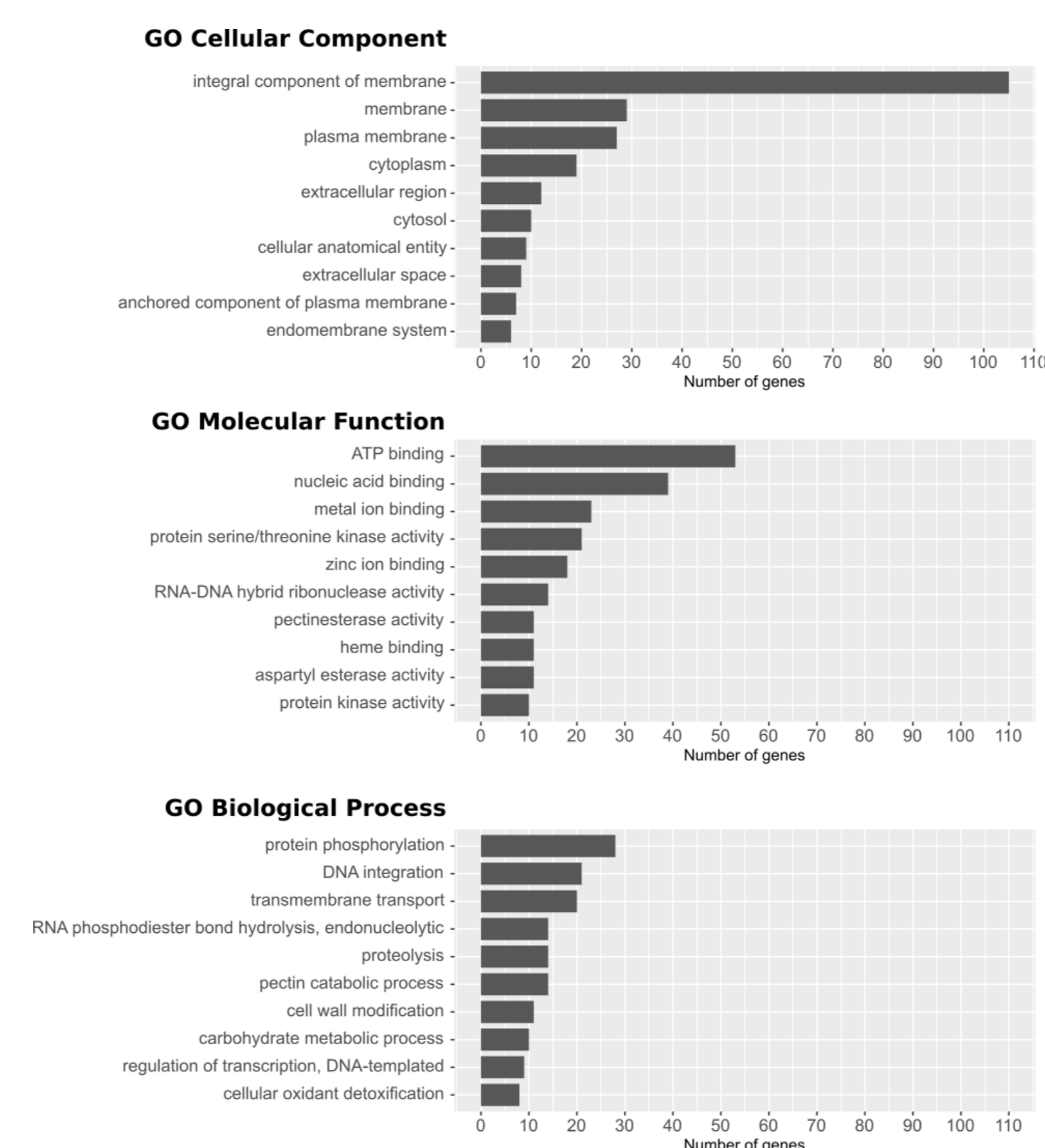


Figure 3. Fraction of differentially expressed genes in sub-categories of GO Cellular Component, Molecular Function and Biological Process.

Results – cont.

Statistically significant differences in expression were also found for 76 mitochondrial and 11 plastid sequences. The group of differentiating mitochondrial sequences was dominated by those showing increased expression in male-fertile (restored) plants (63 sequences) (fig. 4). Examples include genes *rps3*, *atp6* and some exons of *nad* genes.

Literature

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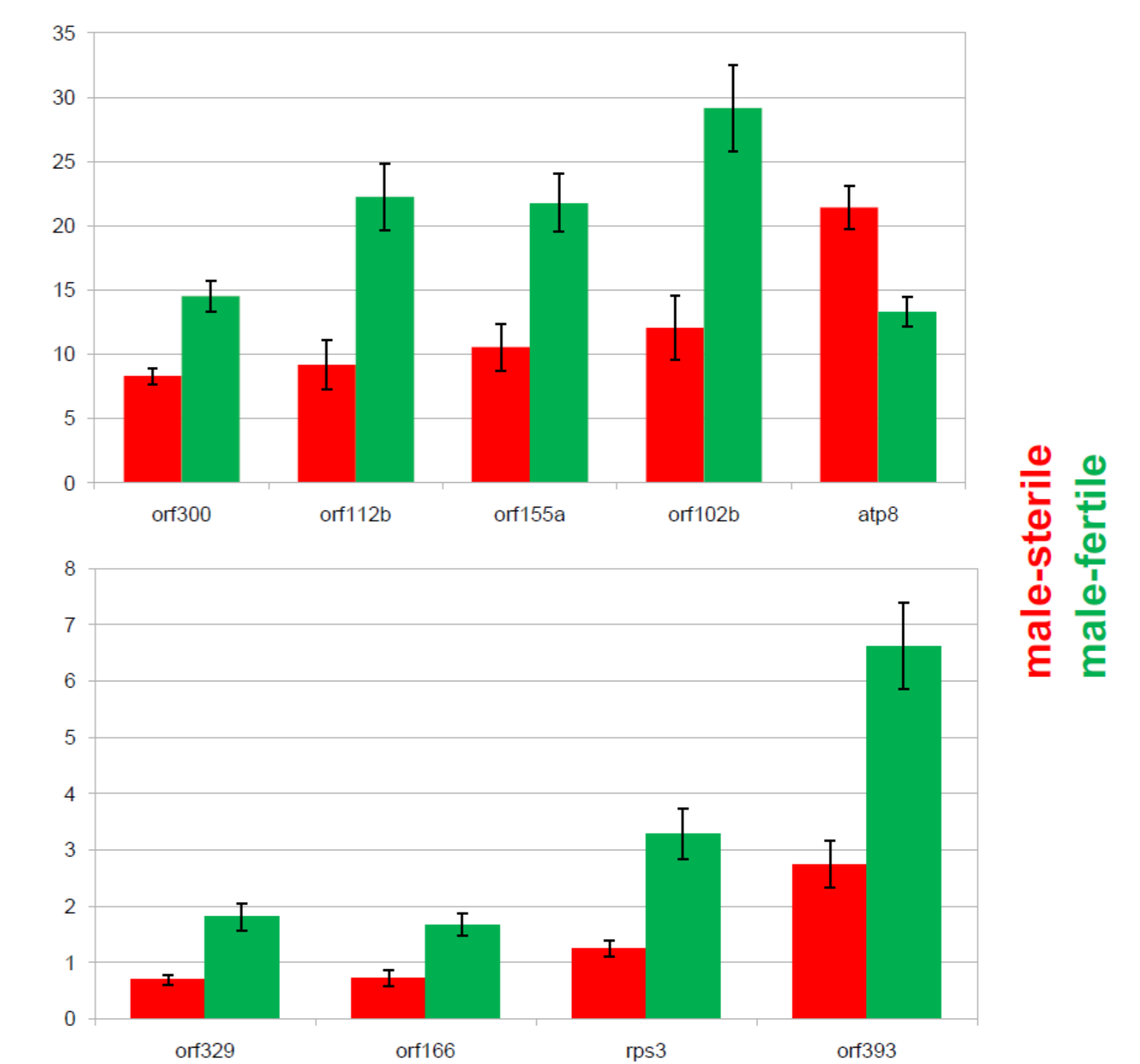


Figure 4. Normalized coverage of selected mitochondrial genes with statistically significant difference between male-sterile and male-fertile plants.