Deciphering the genetic basis of cytoplasmic male sterility and fertility restoration in wheat

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Q&A session

Presenter: Joanna Melonek, The University of Western Australia, Australia

The webinar recording is available on the IWGSC YouTube channel at https://youtu.be/0LZHWqMC75g

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Q: Can you please share a little more information on the RFL Capture approach? Is the PPR motif used to capture RFL sequences? Would your procedure be publicly available?"

Please refer to the Materials and Methods section "RFL capture" in the Melonek et al., 2021 paper. Moreover we can say that capture probes span whole RFL sequence and that the procedure used is the one recommended by the probe manufacturer.

Q: What are your thoughts on targeting mitochondrial ORFs (using gene editing, for instance) versus only relying on nuclear restorer genes for restoration genetics? Can you please update us on the status of the patents mentioned in the recent Nature article? Also, what exactly does that patent cover?

TALEN-based approaches (e.g. MITOtalen) could be an alternative to using RFL-PPR restorer genes but currently their usage is limited by difficulties with controlling the scale of genomic rearrangements and deletions that they can cause in the mitochondrial genome. In comparison, the cleavage induced by RFL-PPR genes is much more precise. Concerning the patents. One patent covers the use of Rf1 and Rf3 sequences and the second patent covers the use of orf279.

Q: Would the bait/probe information be made available?

Please contact Pascual Perez for more information on the RFL-capture approach.

Q: Nice talk Joanna, perhaps I missed it but do you know how the orf279 causes cytoplasmic fertility at the mechanistic level?

One hypothesis could be that, Orf279 protein will be incorporated into the mitochondrial membrane system somewhere close to the ATPsynthase complex and this will cause perturbations in ATP (energy) production in these mitochondria. The lack of energy in the tapetum cells will cause abortion of pollen development and the plants will become sterile.

Q: Do you have plans to do any allele mining (targeted sequencing) for novel RF alleles across diverse wheat or relatives?

Yes, we have plans to do allele mining but most likely, we will first do some pre-selection (PCR-based screen) before we will run RFL-capture experiment on thousands of accessions and or go to targeted sequencing.

Q: did you have a closer look, when (at which dev. stage) the pollen development goes wrong with timopheevii cms cytoplasm?

No, we have not looked at that yet but we are planning to.

Q: Has T. timopheevii been integrated in the search for a strong restorer gene?

Yes, we are searching T. timopheevii for the presence of additional strong restorer genes that may have been overlooked so far.

Q: The transgenic Rfs restore almost completely. In your introduction you mention that restoration is only partial. Can you explain?

This depends how you measure fertility restoration and it probably depends on nuclear background. Rf1 and Rf3 under their natural promoters can restore at >90% in the best transgenic lines, but not all the transformants are fertile, suggesting that expression levels are important and vary with transgene position effects (and probably with environmental conditions and nuclear background)

Q: What is the original source of your timopheevi derived CMS in wheat? Where can we obtain the Timopheevi CMS lines and the restorer lines? Thanks

Fielder*CMS and other wheat lines generated in this project are available (MTA required) upon request from Pascual Perez, Limagrain.

Q: Is it possible that the CMS line still produces a small amount of viable pollen (i.e. no 100% sterile)?

No, we have not heard about that.

Q: Could you clarify if the identification of candidate genes within the repeat clusters was based solely on sequence differences between accessions?

Yes, the identification of Rf candidates was based mainly on sequence comparisons and gene copy variants between sterile and fertile genotypes.

Q: So there is no or very little additive effect by stacking Rf1 and Rf3 in restoration? Or can it be possibly depending on genetic background CMS lines?

We can't really say what the effect of stacking is because Rf3 alone works so well in Fielder*CMS. Stacking probably would help a lot under conditions when Rf3 does not restore fertility at >90%

Q: Often RFL action seems to be altered by modifiers. Any ideas as to what these are and how they fit in your model?

There are many possible scenarios how modifiers could work. They might affect expression of the restorer genes, or the accumulation of orf279 RNA, or the stability or action of the Orf279 protein, or the sensitivity of tapetal cells to mitochondrial dysfunction etc.

Q: Do the RFL genes bind alone to the orf279 or do other proteins enhance binding?

We think that RFL-PPR proteins bind their RNA targets alone without interactions with other proteins but we also think that another protein (or proteins) is required for the RNA cleavage of CMS-orf.

Q: How total number of gene family is identified from reference genome assembly, through any specific algorithm? Whether this is common for any genome or any gene family?

To identify PPR genes in a genome we use consensus HMM profiles constructed for each PPR motif variant. For more details see Experimental procedures of Cheng et al. Plant J 201685(4):532-47. doe: 10.1111/tpj.13121. For methods how to identify RFL-type PPRs please refer to Melonek et al 2016 Sci Rep 24;6:35152. doi: 10.1038/srep35152.

Q: can we map the CMS and rf genes in RNA seq data of Restorer and mainter lines and hybrid wheat

No, RNAseq data alone will not be enough to identify CMS and Rf genes in restorer or CMS lines. Genome data in form of e.g. RFL-capture will be required. Due to high sequence similarity, it is very difficult to assemble all RFL genes from RNAseq data alone.

Q: how many RF and CMS genes are there in wheat genome

On average, a bread wheat genome will have ~ 200 RFL. How many of them will have the role of an active restorer gene it will depend on a genotype. Usually, we see 1-2 restorer genes in one genotype. In majority of CMS-lines we see only one single gene that causes sterility. There may be other CMS-inducing genes present that are latent because the corresponding fertility restorer genes are fixed in the population/species.

Q: In your opinion, How many genes would lead to complete restoration in timopheevii cytoplasm in addition to Rf1 and Rf3?

We don't know that at the moment. In an ideal scenario we would need just one gene to fully restore sterility.

Q: Have you shown orf279 protein localization to a mitochondrial membrane? Seen any effect on oxphos and/or other mito functions? Does the protein product interact with any other mito proteins?

We have detected Orf279 in membrane-enriched fractions but we have not done any analyses beyond that.

Q: Is the glume still open in RFL transgenic lines?

Most of the transgenic lines carrying restorer genes had a normal phenotype.

Q: Could you please explain the possible reason that the restoration rates for RFL29a+RFL79 is not significantly higher than either one of them alone?

Please see the answer we gave above. Stacking the two genes is probably helpful under conditions (environment or nuclear background) where neither gene alone can restore ferility at > 90%