**Breeding for ergot avoidance via Self-compatibility and Cleistogamy in Rye**

**Introduction**

Rye (*Secale cereale*) is a recently domesticated diploid crop (2n=14) It is a spring and winter planted annual crop. Rye is grown as a grain, cover, or as a forage crop. It is closely related to barley and wheat in the Triticeae tribe. Rye is highly susceptible to ergot fungus and consumption of ergot infected rye causes a serious health condition known as ergotism (Need a reference for the facts in the above sentences). Ergot in rye is caused by *Claviceps purpurea* which can infect more than 400 grasses including wheat, barley etc. It is a soil born pathogen and requires moisture and moderate temperatures for sporulation. This fungus is morphologically highly variable in terms of sclerotial length, color of stromata, shape and size of conidia and spectra of alkaloids produced by different isolates ([Esser K](http://www.ncbi.nlm.nih.gov/pubmed/?term=Esser%20K%5BAuthor%5D&cauthor=true&cauthor_uid=24309592)& [Tudzynski P](http://www.ncbi.nlm.nih.gov/pubmed/?term=Tudzynski%20P%5BAuthor%5D&cauthor=true&cauthor_uid=24309592), 1978). Breeding for ergot resistance in rye is still not advanced due to the lack of sufficient genotypic variation for this trait (Mirdita & Miedaner, 2009).

**Rye Reproduction and Ergot infection**

Rye has perfect flowers but possesses gametophytic self-incompatibility. Therefore, it is always a wind-pollinated outcrosser. This is an advantage for the ergot fungus. Ergot mostly infects the ovary via stigma as soon as flower opens for pollination. Generally, ovaries are infected before pollination when the flowers are open and stigmas are exposed. After landing on a stigma, ergfotinfects ovaries, and forms sclerotia. Usually, the infection is higher in exposed stigma which are not pollinated (Miedaner & Geiger, 2015). Reducing the duration between the time between flowers opening and flower closing which occurs after pollination is a key to success for ergot avoidance. Therefore, a useful approach to reduce ergot infection is to perform selection for closed flowers also termed cleistogamy in rye which would prevent stigma exposure and ergot entry into ovary. Since rye has self-incompatibility system, breeding has to be done to select for self-compatibility before cleistogamy.

It has been documented that the Z locus on the 2RL chromosome of rye is responsible for self-incompatibility where TC116908 (a STS marker) co-segregated with the Z locus (and self-incompatibility). The Z locus gene likely encodes a ubiquitin-specific protease (UBP) which has a ubiquitination function and is generally found in self-incompatibility reactions in plants(Hackauf & Wehling, 2005). The orthologue of this Z-locus has also been widely studied in rice and barley. Therefore Z locus could be promishing target for gene silencing.

**Research Outline:** The main idea to get rid of ergot is to perform selection for cleistogamy in rye which prevents stigma exposure. This will also restrict cross pollination. Therefore, before selecting for cleistogamy, breeding for self-compatibility has to be done. For transforming rye into a self compatible plant, RNAi will be used to turn off the Z gene.

**Method:**

**Roshan,**

**Instead of this level of detail, it would be preferable to describe RNAi as an approach and the then the Z gene in particular. Assuming the Z gene could be cloned from RNA, that would be the first step. You would need only a 100-200 bp section of the gene and then make a vector. The RNAi gene itself would consist of ~150 bp of Z gene coding in sense orientation, followed by an intron spacer, followed by the same 150 bp of Z gene coding in reverse orientation. Note though that the Hackauf paper you cite did not prove that they had theZ gene, they state that it was a promising target.**

**So… things you might describe in more general terms are**

1. **Making the RNAi vector: The first step would be cloning the Z gene from RNA and making a vector where a few hundred base pairs of Z-gene, coding in sense orientation, followed by an intron spacer and again the same gene coding in reverse orientation will be used.**
2. **Transforming rye.**
3. **Testing for self compatibility**
4. **Describe hunt for cleistogamous mutants or genetic variation for degree of flowering opening in rye. I didn’t search to see if they exist in rye, but perhaps lots is known.**
5. **RNAi**

* Use the forward primer GCTCAACAACCTCGGCAAC to engineer double stranded RNA.
* Introduce the double stranded RNA to the explants of the already selected two parental lines and regenerate number of plants via tissue culture. No, vector is introduced that is DNA, then the segment containing the sense/intron/antisense sequence integrates into a chromosome and is then expressed in the transgenic plant.
* Since the plants regenerated from the explants of each parents may differ only in the loss of self-incompatibility function, depending upon the success or failure of transformation.

1. **Breeding after Transformation**

* Select successfully transformed line from each group which is exactly similar to its parent except it has loss of self-incompatibility using the marker TC116908.
* Let them self to attain large number of homozygous lines.
* Select best homozygous line as a parent from each group which differs in degree of flower opening. Generate enough seeds.
* Make crosses and get F1 and self again to have homozygous lines.
* Select few lines which have closed flowering (cleistogamy) and high biomass yield.
* Perform PYT and AYT
* Select the best line
* Release.

CONCLUSION

**Breeding technique**

Parent 1

Parent 2

RNAi GENE SILENCING Normally, would only bother to transform one line and then integrate same transgene into other parental genotypes by crossing.





Select Self-compatible line which is nearly identical to its parent except for self-incompatibility

Selfing of the selected line from both groups to achieve homozygosity and to increase seed

Select best yielding line which differ in degree of flower opening

I think you could introduce open versus closed flowers better, quite a few papers on this and I think the gene was cloned in barley.

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**X**





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Select for few best lines having cleistogamy

Perform PYT, AYT and Release Variety

Reference

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