# Hybrid Rye Breeding and Production

Subjects: Genetics & Heredity Contributor: Thomas Miedaner,

Hybrid rye breeding leads to considerably higher grain yield and a higher revenue to the farmer. The basis of hybrid seed production is the CMS-inducing Pampa (P) cytoplasm derived from an Argentinean landrace and restorer-tofertility (Rf) genes. Breeding is based on inbred line development and intensive testing for line per se performance and general combining ability (GCA). The finally selected inbred lines are used to compose a topcross hybrid with two genetically different seed parent lines (A-P, B-N) and a restorer synthetic composed of two S<sub>2</sub> lines (Syn<sub>Rf</sub>). European restorer sources show low-to-moderate pollen-fertility levels. This results in higher susceptibility to ergot (Claviceps purpurea) because rye pollen and ergot spores are in strong competition for the unfertilized stigma. Rf genes from non-adapted Iranian primitive rye and old Argentinean cultivars proved to be most effective. The major Rf gene in these sources was localized on chromosome 4RL, which is also a hotspot of restoration in other Triticeae. Commercial hybrids with these Rf genes showed a similar low ergot infection when compared with population cultivars. The great future challenges of climate change, such as increased drought stress tolerance, improved lodging tolerance, as well as the increasing need for resistant varieties can effectively only be met by hybrid breeding.

Claviceps purpurea

CMS

hybrid breeding pollen

restorer genes vield

## 1. Introduction

Rye (Secale cereale L.) originates from an area extending from central and eastern Turkey through nortwesten Iran to Transcaucasia. It was probably not cultivated continuously in its area of origin. When rye came to northern Europe as a weed, it was able to exploit its advantages of superior winter hardiness and modest nutrient requirements and became the dominant cereal from the early Middle Ages<sup>[1]</sup>. Initially, rye was grown in a vast region from northern France to eastern Siberia. Nowadays, rye-growing is still centered in Europe, where 86% of grains are produced, with Germany, Poland, the Russian Federation, Denmark, and Belarus as the main producers in 2019<sup>[2]</sup>. In rye as a cross-pollinating crop, population and hybrid cultivars are available. Hybrid breeding started in the 1970s at the University of Hohenheim, Germany, and in 1985 the world's first three hybrid cultivars were released<sup>3</sup>. This breeding method revolutionized rye-growing, because hybrid breeding provided about 20% higher grain yield and a threefold higher gain from selection, compared to population rye, over a 30-year period<sup>[4]</sup>. Nowadays, about 80% of the German rye-growing area is planted with hybrid rye cultivars, and hybrid rye is available in all other main production countries, as well as in Austria, the USA, and Canada.

## 2. Basics of hybrid rye breeding

Hybrid rye breeding leads to considerably higher grain yield and a higher revenue to the farmer. The basis of hybrid seed production is the cytoplasmic-male sterility (CMS)-inducing Pampa (P) cytoplasm derived from an Argentinean landrace<sup>[5]</sup> and restorer-to-fertility (*Rf*) genes<sup>[6]</sup>. To ensure a maximal heterosis, two gene pools are used, that are in Central Europe the Petkus (seed parent) and the Carsten (pollen parent) gene pool. The pools were derived from two breeding companies that used different landraces for starting their selection work in the 19<sup>th</sup> and the beginning 20<sup>th</sup> century. The basis of hybrid breeding are inbred lines that are produced in rye by subsequent selfing with the use of a dominant single-gene mutation leading to full self-fertility (*sf*).

Breeding starts with crossing of parents (**Figure 1**), followed by two selfings of the progenies and subsequent per se performance test of  $S_2$  lines for highly heritable traits (e.g., vitality, heading date, plant height, disease resistances, lodging tolerance, 1000-grain weight, falling number). The selected fraction is crossed in the seed parent pool to a CMS donor to start the backcrossing procedure that is necessary to result at the end in near-isogenic lines with the CMS-inducing P cytoplasm and the normal cytoplasm. During backcrossing selection for non-restorer is performed. In contrast, in the pollen parent pool a strict selection for *Rf* genes has to practised. Here, only two selfings are done, because afterwards the best selected lines are crossed to a synthetic. In parallel to selfing and backcrossing two stages of yield tests (YT) are made by crossing the inbred lines with testers from the opposite pool. These testcrosses (TC) are planted in 3-5 locations in two replicates to select for general combining ability (GCA) for grain yield and the other mentioned traits. The finally selected inbred lines are used to compose a topcross hybrid with two genetically different seed parent lines (A-P, B-N) and a restorer synthetic composed of two S<sub>2</sub> lines (Syn<sub>*Rf*</sub>). This complex hybrid is necessary due to the high inbreeding depression that rye is suffering as an obligate cross-pollinating crop.



Figure 1. Scheme for hybrid rye breeding

Hybrid rye production requires strict logistics for seed multiplication, because rye is an effective wind pollinator. All stands of seed production must be strictly isolated and checked for outcrossings several times. Mostly the line multiplications (A-P x A-N, B-N) and the production of basic seed (A-P x B-N) are done in foreign countries with no commercial rye growing (France, Italy, Hungary). The lines are planted in stripes with a 3:1 proportion for females:males. The multiplication of the synthetics and the production of certified seed (A-P . B-N x Syn<sub>Rf</sub>) is done in the area of marketing of the rye hybrid cultivars. The certified seed is produced in a technical mixture of 90-95% CMS seed parent and 5-10% restorer synthetic. This approach cheapens production, but currently requires the presence of synthetics to escape the strong inbreeding depression of lines.

#### 3. Ergot as a challenge for hybrid rye production

Since Medieval times, the results of ergot infections have been documented. Rye and ergot were so closely interwoven that botanical drawings of rye in the 17<sup>th</sup> century included ergot. Ergot refers to the blackish purple sclerotia of the fungus *Claviceps purpurea* (Fries ex Fries) Tulasne that extend far beyond the husks of rye (**Figure 2**a). They contain more than 80 ergot alkaloids, of which six alkaloids and their corresponding epimers were found

to be the most important ones in Europe. Prolonged consumption of ergot-containing flour, which was common among the peasant population in the Middle Ages, leads to a serious disease, ergotism, that is characterized either by nervous disorders (fever, muscle spasms, tremor, paralysis, hallucinations) or by vasoconstriction effects (gangrene, violent burning, shooting pain in the fingers and toes, called St. Anthony's fire).



(a)



(b)

Figure 2. (a) Ergot sclerotia in a ripening rye head; (b) Full pollen-fertile rye during flowering.

All mammalian livestock are affected by ergot contamination of feed. Epidemic outbreaks in the Middle Ages resulted in a death rate of between 10% and 20% and a reduction in the birth rate due to the premature onset of labor and miscarriages<sup>[Z]</sup>. The main factor for ergot epidemics is rainy weather during rye flowering. High humidity stimulates fungal growth and reduces the availability of rye pollen. The fungus cannot grow through intact glumes and the spores must be brought into the open florets by insects or by aerosols induced by rain and wind. Therefore, cross-pollinating grasses, such as rye, are a primary target of the fungus. The infection success of the fungus is reduced when the florets are already pollinated and the then-closed glumes prevent access to the stigma. Ergot spores mimic the fertilization process of rye. After landing on the stigma, the germ tubes grow through the pistil, like the pollen tubes, and infect the ovary exclusively. Therefore, the pollination has a considerable effect on infection frequency and disease development<sup>[3]</sup>. The combination of less available pollen, poor pollen flight properties, and an extended opening time of the unfertilized florets in rainy weather gives the fungus a competitive advantage in relation to pollen. This is less pronounced as the cultivar sheds more pollen (**Figure 2**b). The close association between pollen availability and ergot susceptibility poses a problem for hybrid breeding when full pollen

shedding cannot be guaranteed due to lack of superior restoration. In the early hybrid cultivars, pollen-fertility restoration was inferior, leading to high ergot contamination when the weather was conducive for the disease. From 1993 to 2004, the mean percentage of natural ergot incidence in the German rye harvest was 0.04% for population cultivars and 0.18% for hybrid cultivars according to results from the Max-Rubner Institute, Detmold, Germany. This was not acceptable, as the EU tolerance limit for ergot sclerotia in commercial grain lots for food at that time was 0.05%, and for feed it was 0.1%. The high susceptibility of the early hybrids to ergot stimulated research for better restorer (*Rf*) genes.

European restorer sources show an oligogenic inheritance, with major and minor *Rf* genes, and mostly result in low-to-moderate pollen-fertility levels. This results in higher susceptibility to ergot because rye pollen and ergot spores are in strong competition for the unfertilized stigma. *Rf* genes from non-adapted Iranian primitive rye and old Argentinean cultivars proved to be most effective. The major *Rf* genes in these sources were all localized on chromosome 4RL, which is also a hotspot of restoration in other *Triticeae*. Marker-based introgression into elite rye materials led to a yield penalty and taller progenies. The *Rfp1* gene from the primitive rye IRAN IX was fine-mapped, and two linked genes of equal effects were detected.

#### 4. How to Proceed in Future Breeding

Hybrid breeding is the future of rye! The higher yield performance allows the crop to compete with wheat, which usually fetches a higher price on the market. In view of climate change, with drier and warmer conditions in the future, hybrid rye is superior in all growing regions. It also requires less pesticide and nitrogen fertilization, in line with the European Green Deal, which calls for higher environmental protection standards in agriculture. The EU has significantly tightened the limits for ergot since 1 January 2022. In addition to the previous limit of a maximum of 0.5g/kg sclerotia and sclerotial fragments on unprocessed rye, harvested grain may not contain more than 500 µg/kg total alkaloids on rye milling products for human consumption. From 1 July 2024, the limits will be reduced to 0.2g/kg sclerotia and a maximum of 250µg/kg alkaloids according to the EU Commission Regulation 2021/1399. Therefore, the high pollen-shedding of hybrid varieties must be the top priority to avoid ergot.

Besides maximizing pollen-shedding by using non-adapted *Rfp* genes, the question of resistance of rye to the pathogen *Claviceps purpurea*, arises. It was previously taken for granted that ergot infection does not cause any defense reactions in rye. However, recent transcriptomic studies showed that in both wheat and rye, the host plant responds to the infection by upregulating defense-related genes, precisely when the germ tube reaches the ovary<sup>[9]</sup>. Thus, the host obviously recognizes the *Claviceps* infection. Indeed, it has been shown several times that diverse sets of both fully pollen-fertile rye and fully male-sterile rye, when grown under pollen-isolated conditions, showed small but significant differences in their ergot reaction<sup>[10]</sup>. These quantitative maternal differences could be exploited by targeted selection, as shown by some commercial hybrids without *Rfp1* gene and with a low restorer index, but also lower ergot severity after artificial infection (**Figure 3**). The best-restored hybrids with *Rfp1* have a similar low ergot severity like population cultivars. In Germany, Poland, and Austria, the official cultivar registration procedure includes multi-locational testing for ergot severity over two years, with artificial inoculation in a special experimental setting.



**Figure 3.** Ergot severity and restorer index of the entries of the Official VCU trials from Germany in 2021 after artificial ergot infection across four locations (cv./cvs = cultivar/s, G=G cytoplasm, P=P cytoplasm).

The high effectiveness of non-adapted *Rfp* genes will play a prominent role in future hybrid breeding. The main task is to reduce the obvious yield penalty and other negative agronomic features. Even if the introgressed genomic section is significantly shortened, a significant yield loss remains that could not be further reduced using more closely linked markers. However, the effects of the same *Rfp* gene on different genotypes significantly differs. Therefore, a consequent selection in the elite gene pools for mitigating undesired side effects of *Rfp* genes seems feasible in the long run. This would replicate the coevolution that has occurred in adapted CMS/*Rf* systems over evolutionary time periods.

This coevolution could be enhanced in a breeding context, when environmentally stable adaptation genes exist in the elite gene pools that lower the negative agronomic effects of non-adapted *Rf* genes. Research activities should be started to identify and to exploit these genes. In conclusion, the development of commercial hybrid cultivars in P cytoplasm, with a similarly low or an even lower ergot severity than population cultivars, appears to be promising.

In hybrid rye breeding, modern genomic techniques are practised to increase the performance of hybrids and ensure a steady selection gain in future (**Table 1**). Marker-assisted selection is useful for all monogenically inherited traits. Marker-assisted backcrossing helps to shorten the backcrossing procedure in the seed-parent pool (rf. to Figure 1) by selecting against the CMS donor background. Using a low-density marker array, in BC<sub>3</sub> already the same low donor background could be achieved like normally in BC<sub>6</sub>. QTL mapping is an important tool when lines with special alleles are searched that are not available in the current breeding populations. With this tool, the genetic architecture of the trait can be analysed with high power, because even rare alleles are pushed to an allele frequency of 50% in bi-parental crosses.

Technique	Useful for	Requirement	
Marker-assisted selection (MAS)	Introgression of mono-genic resistances, <i>Rf</i> genes, dwarfing genes	Closely linked diagnostic KASP markers	botany
Marker-assisted backcrossing (MAB)	Conversion of N lines: Selection against CMS donor background	Low-density marker array	009; pp.
Quantitative trait loci (QTL) mapping	Detection of rare alleles with large effects	Low-density marker array	lye
Genome wide association mapping (GWAS)	Detection of common alleles with smaller effects	T Medium-density marker array	Science net.
Genomic selection (GS)	Selection of genome-wide marker effects for all traits	High-density marker array	to 1 <b>5</b> , 60,

Table 1. Genome-based techniques and their use in hybrid rye breeding (KASP=kompetitive allele-specific PCR)

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line per se performance (rf. to Fig. 1). Based on previous cycles that are used as training population, superior lines 9. Birgitt Oeser, Sabine Kino, Selma Schurack; Thomas Schmutzer, Paul Tudzynski, Janine Hinsch; in GCA for grain yield and other traits are predicted solely on their genomic composition and only a reduced set of Cross-talk of the Diotrophic Pathogen Claviceps purpured and its host Secale cereale... *BMC* positively predicted lines enters the costly 2-year testcross procedure<sup>[11]</sup>. After the yield test, a final selection can *Genomics* **2017**, *18*, 273, 10.1186/s12864-017-3619-4. take place based on an index of the phenotypically measured and genomically predicted GCA values. 10. Anna-Kodisch: Brigitta Schmiedeben: Jakob Eifler: Andres Cardillo: Dörthe Siekmann Brigitta Teasing selection biotrophic Pathogen Clavice across procedure; Maternal differences for the reaction

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