

## Hybrid breeding in cereals: lessons from rye

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In rye (*Secale cereale* L.), hybrid breeding enables the systematic exploitation of heterosis since almost four decades and serves as a model to establish this cutting edge technology for the genetic improvement of barley and wheat. Breeding and commercial seed production of hybrid rye is mainly based on the cytoplasmic male sterility (CMS)-inducing Pampa (P) cytoplasm, which originated from an Argentinian landrace. Breeding of CMS-based cereal hybrids requires the use of effective restorer-of-fertility (*Rf*) genes, which are of central importance to minimize ergot contamination of the harvest. *Rf* genes from non-adapted rye populations have been mapped to a small segment on the long arm of chromosome 4R using conserved ortholog set (COS) markers (Hackauf *et al.* 2009, 2012). COS markers provide efficient tools for marker-assisted backcrossing (MABC) of *Rf* genes in the pollinator gene pool of rye. We report on the introgression of small donor chromosome segments (DCS) carrying *Rf* genes from three unadapted genetic resources of rye in two elite pollinator genotypes. The established genotypes were evaluated with respect to possible linkage drag effects associated with individual *Rf* genes using a highly diagnostic phenotyping platform.

Initial crosses were executed in 2011 and MABC of the restorer genes *Rfp1*, originating from a weedy rye population, as well as *Rfp2* and *Rfp4*, both originating from forage rye populations, has been conducted using the previously established flanking markers TC176835 and TC363404 (syn. TC135788), respectively (Hackauf *et al.* 2012). BC<sub>3</sub>S<sub>1</sub> single plants carrying either the elite (E) non-restorer allele or the donor (D) restorer allele from *Rfp1* ( $n = 3$  BC<sub>3</sub> lines), *Rfp2* ( $n = 4$ ) or *Rfp4* ( $n = 4$ ) have been selected to establish near isogenic homozygous BC<sub>3</sub>S<sub>2</sub> bulks (NIB), that were used as pollinator for crossing with two male sterile single-cross non-restorer genotypes between isolation walls. Evaluation of the established three-way cross hybrids in the field was done in 2016 and 2017 in 12 environments (locations  $\times$  year combinations). Entries were grown on drilled plots of 5 to 6 m<sup>2</sup> size. The experiment was laid out in a partially balanced incomplete block design (alpha design) with two replications per entry. All plots have been assessed for agronomic traits including grain yield (GYD, dt ha<sup>-1</sup>), thousand grain weight (TGW, g), and plant height (PHT, cm). Male fertility scores were visually assessed directly in the field on plot basis and used to calculate a restorer index (RI) as described by Miedaner *et al.* (2017). All statistical computations were performed with *R* software packages in a two-step procedure. Adjusted entry means were calculated for all traits in each environment separately and were used in a second step to estimate variance

components based on the following linear mixed model:  $y = G + E + G \times E$ , where *G* and *E* denote genotype and environment, respectively. Both factors as well as the interaction term were treated as random effects. Broad sense heritability ( $h^2$ ) on an entry-mean basis was estimated from the variance components as the ratio of genotypic to phenotypic variance. Linkage drag effects were calculated as the difference ( $\Delta E-D$ ) between the means of individual NIB partners, which either carry the E or the D at the 4RL DCS. Comparisons between individual NIB partners were performed with Welch's *t*-test.

All traits revealed significant genotypic and genotype by environment interaction variances. The estimates of broad-sense heritability were high ( $h^2 > 0.88$ ) for all traits. Non of the agronomic traits deviated from a normal distribution. On average, hybrids carrying *Rfp1* or *Rfp4* were significantly ( $p < 0.05$ ) taller compared to their near isogenic hybrids carrying the elite non-restorer allele. Likewise, *Rfp1*, *Rfp2* and *Rfp4* resulted in significantly ( $p < 0.05$ ) lower grain yield. Interestingly, the linkage drag effect of *Rfp2* (-3,73 dt ha<sup>-1</sup>) and *Rfp4* (-4,68 dt ha<sup>-1</sup>) were less pronounced compared to *Rfp1* (-8,41 dt ha<sup>-1</sup>). A significantly negative effect on TGW could only be observed for *Rfp2*. Hybrids carrying the restorer allele of the *Rfp1* and *Rfp4* gene revealed a high degree of pollen fertility restoration, while those with the corresponding non-restorer allele had a low pollen fertility. This could be observed for *Rfp2* as well except of one replicate in each genetic background, which both were characterized by a comparable low RI.

The described results demonstrate, that MABC of *Rfp* genes is superior to conventional backcrossing (Miedaner *et al.* 2017) in precision and efficiency. Furthermore, the yield penalty associated with *Rfp2* in the present study was less pronounced compared to a backcross programme, that was solely based on phenotypic selection (Miedaner *et al.* 2017). This observation may be attributed to the marker-assisted foreground selection on the short DCS in the present study.

A shared ancestry has recently been described between an Argentinian forage rye population and the population variety 'Carokurz' (Parat *et al.* 2016), the latter of which served as founder population of the pollinator gene pool in hybrid rye breeding. Thus, the 50% lesser linkage drag effect of *Rfp2* and *Rfp4* may be attributed to alleles located on the 4R DCS, that are more favourable for grain production in central European target environments than those alleles associated with *Rfp1* from weedy rye. The observed costs of restoration of *Rfp* genes can be counterbalanced *e.g.* by

using recently identified major QTL for grain yield (Hackauf *et al.* 2017). The comparable low RI in genotypes carrying the 4RL DCS from 'Pico Gentario' can be explained by epistatic interaction of *Rfp2* with modifier genes on chromosomes 1R and 6R (Hackauf *et al.* 2009). This obvious difference between *Rfp2* and *Rfp1* challenges the assumption of a single origin of the *Rf* mutation (Miedaner *et al.* 2017), as *Rfp1* is a gene acting independently from other genes (Hackauf *et al.* 2012).

In conclusion, nuclear-cytoplasmic gynodioecy in rye offers a natural, reliable, environmentally friendly and cost-effective production of hybrid seed. COS markers linked to *Rfp* genes increase the efficiency of hybrid rye breeding and provide efficient means to evaluate valuable gene variants from diverse genetic resources of rye. The genetic diversity at the *Rfp* locus opens novel options for the breeding of ergot resistant and toxin free rye hybrids.

### Keywords

Costs of restoration · cytoplasmic male sterility · marker assisted selection · restorer of fertility gene · *Secale cereale*

### References

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