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Genetic distance study for improved hybrid breeding in perennial ryegrass (*Lolium perenne*)

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Introduction: Currently, the development of varieties in perennial ryegrass (*Lolium perenne* L.) relies heavily on synthetic breeding. This leads to breeding material not favourable for hybrid breeding as it is closely related and as heterotic groups within the breeding material are difficult to identify (Pembleton *et al.*, 2015). The objective of our study is to assist the exploitation of heterosis by using an amplicon-sequencing (AmpSeq) based approach to improve the initial selection of heterotic groups. AmpSeq allows the calculation of the pairwise genetic distance of parental components, which can be used to develop a yield prediction model. The method enables breeders to target specific genes or entire pathways to retrieve haplotype structures of parental components and to establish a genotyping platform.

Materials and methods: Parental components of cytoplasmic male sterility (CMS)-based tetraploid hybrid breeding programmes were selected for this analysis. Hybrid combinations of 20 pollinators and twelve CMS parents, resulting in 26 hybrids, were tested in yield trials in the years 2014 to 2017. Complete randomized yield trials were analysed using the Agrobase® software. Amplicons of genes linked to agronomically important traits were used for the analysis. AmpSeq was performed with an in-house protocol modified on pools of 60 individual tetraploid plants of each parental component per sample. Pairwise genetic distance was calculated using CLC Genomics Workbench (Version 11.0), Shapiro-Wilk test and Pearson correlations were calculated using R.

Results: Fresh yield of the 26 tested hybrids showed wide ranges in total yield performance ranging from 108.6 dt ha⁻¹ to 154.6 dt ha⁻¹. Crown rust (scored in France) reached values from 4.0 to 8.3 (susceptible to resistant: 1-9). Sequence information used for calculation of genetic distance between parents of the hybrids showed a negative correlation for crown-rust resistance ($P < 0.001$) and a positive correlation to relative yield ($P < 0.05$). Diverse genes were more suitable for correlation than conserved genes.

Conclusion: AmpSeq, to describe the haplotype structure of tetraploid parental components for hybrid breeding in perennial ryegrass, can be used as a tool to identify heterotic groups within the breeding material. Correlation of sequence information to performance in yield and quality-related traits is possible. This will allow the implementation of a prediction model for the performance of hybrids in modern forage grass breeding.

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Pembleton L.W., Shinozuka H., Wang J., Spangenberg G.C., Forster J.W. and Cogan N.O. (2015) Design of an F1 hybrid breeding strategy for ryegrasses based on selection of self-incompatibility locus-specific alleles. *Frontiers in Plant Science* 6, 764.