Development of a breeders' toolkit for drought resistance in a *Lolium/Festuca* hybrid

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Keywords: introgression-mapping, drought resistance, *Festuca glaucescens, Lolium multiflorum*

Introduction *Lolium multiflorum* (Lm) is considered an ideal grass for European agriculture. However, existing high-quality forage Lm cultivars have been bred for intensive systems in benign environments, and have proved to be insufficiently robust to meet many of the environmental challenges that face extensive agriculture in more extreme conditions. Genes for persistency, tolerance of cold, drought and poor soils, can be found in currently under-exploited native *Festuca* ecotypes. These *Festuca* ecotypes cannot however compare with Lm cultivars for productivity or quality of forage under favourable conditions. *Festuca glaucescens* (Fg) is of Mediterranean origin and as such is adapted to drought and heat stress. The object of this work was to introgress a single chromosome segment of Fg containing genes for drought resistance into a diploid Lm background. Subsequent to the introgression of a Fg chromosome segment, Fg markers were mapped and a prototype toolkit developed to follow the genes for drought resistance through a breeding programme.

Materials and methods A Fg (2n = 4x =28) genotype was hybridised onto a synthetic autotetraploid Lm cultivar Roberta (2n = 4x = 28). The F_1 hybrid as a male was backcrossed onto a diploid Lm cultivar (2n = 2x = 14) to generate triploid BC_1 plants which were further backcrossed onto diploid Lm cultivars to produce diploid BC_2 plants. Nine BC_2 populations each of 30 plants were used in a drought test carried out in polythene lined brick bins in a glasshouse. Water was withheld for 14 weeks and recovery assessed by dry matter production 4 weeks after re-irrigation. Genomic *in situ* hybridisation (GISH) analysis was used to detect the presence of introgressed Fg chromosome segments. Amplified fragment length polymorphism (AFLP) (Vos *et al.*1995) using 100 primer pair combinations and sequence tagged site (STS) markers were used to target the introgressed Fg segment. Mapping of the markers was carried out with 96 genotypes of a BC_3 population using JoinMap® 3.0 (Van Ooijen and Voorrips, 2001). The BC_3 population was then subjected to a drought test as described above.

Results BC_2 genotype P194/208/19 was selected as having the best recovery growth with a mean of 6gm dry weight. The Lm parent under the same conditions performed very poorly with a yield of 0.075gm while the Fg parent produced 1.2 gm of dry matter. GISH analysis of P194/208/19 showed 14 Lm chromosomes with a single terminal Fg segment on the satellite of chromosome 3. Nine AFLP markers and an STS marker Fg71673 discriminated clearly between the Lm and Fg derived sequences. The genetic distance of the markers within the Fg segment was estimated to be 32cM with Fg71673 at one end of the map. During the time that the BC_3 mapping population was assessed for drought resistance the stress in the glasshouse was particularly severe with maximum temperatures of >40ºC during 20 days and > 50ºC over 7 other days. Ten of the BC_3 plants survived the combination of drought and heat and were able to re-grow during the 4 week recovery period. Each of these plants contained the entire set of 10 Fg markers ($p < 0.001$) which was evidence that an intact Fg chromosome segment was necessary to enhance the drought resistance of the Lm.

Conclusion Good drought and heat tolerance but poor establishment is a characteristic of Fg, its growth rate is slow and it regularly enters quiescence during the summer months. However the Fg translocation onto Lm chromosome 3 had enhanced the drought resistance of the Lm with no compromise in its forage yield. The STS marker Fg71673 co-segregated at one end of the genetic map with the genes for drought resistance and has now been fluorescently labelled for high throughput use on the ABI 3100 Genetic Analyser. This will be used as prototype breeders' toolkit for cultivar development. Another such suitable marker will be sought at the other end of the map to bracket the Fg genes for drought resistance enabling the whole Fg segment to be followed through a breeding programme.

References

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