Detection of genetic diversity for drought tolerance in perennial ryegrass (*Lolium perenne* L.)

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Abstract

Water shortage is one of the most important constraints limiting yield in agricultural production. Global climate change will also limit yield of one of the most important grass species in Europe, perennial ryegrass (*Lolium perenne* L.). To improve drought tolerance of perennial ryegrass by breeding new varieties, natural genetic diversity for this trait was screened in field trials. 200 accessions, comprising genebank material from all over Europe and breeding material as well as some drought-tolerant grass species for comparison, were tested under natural drought conditions. Appropriate methods for an efficient selection of drought-tolerant genotypes were evaluated within the presented project. On a plot basis the visual scoring of biomass growth provided a suitable data base for selection with good correlation to drought symptoms and yield data. Broad genetic diversity for drought tolerance was observed within the material, which can be used for detailed investigation of drought-tolerance mechanisms and the development of new drought-tolerant varieties.

Keywords: *Lolium perenne* L., genetic diversity, drought stress, phenotyping

Introduction

Global climate change will have major impact on plant production in Central Europe. Perennial ryegrass (*Lolium perenne* L.) as one of the most important grass species in Central Europe, is known to be susceptible to drought stress (Sheffer *et al.*, 1987). It will be particularly affected by temporal fluctuations in soil water content. Since technical management, such as irrigation cannot be applied economically on grasslands, breeding of drought-tolerant varieties is one of the most promising approaches for securing future grassland yields. Breeding progress in drought-tolerance breeding is slow due to a lack of steady selection environments with regular drought stress and a low heritability of the trait 'yield under drought stress'. To overcome this problem, secondary selection traits are used with higher heritability's which ensure higher selection gain on the one hand and good correlation to yield performance under drought conditions on the other hand. This paper presents results from phenotyping diverse perennial ryegrass accessions under natural drought environments.

Materials and methods

Diverse accessions of perennial ryegrass (186 in total: 73 historical varieties and wild collections; 111 varieties and candidate varieties from breeding companies) supplemented by varieties from *Festulolium*, meadow fescue and tall fescue were sown in autumn 2011 at five potentially drought-prone locations (Bornhof, Kaltenhof, Malchow, Triesdorf in Germany; Les Rosiers sur Loire in France) in an alpha-lattice design with four replicates. Locations Bornhof and Malchow were omitted from data analysis due to missing drought stress at these locations in 2013. Biomass production before each cutting date was visually scored on a 1 to 9 scale with 1 for poor biomass growth and 9 as the maximum within each location. The drought-stress response (leaf rolling, wilting, and leaf senescence) was also visually scored on a 1 to 9 scale with 1 indicating no symptoms and 9 for strong expression of symptoms. Heading dates were scored at location Kaltenhof in 2012 in days after 1st of April and accessions were grouped in heading date <54 days (early to mid-heading) and >54 days (mid to very late heading) according to the official classification of German variety authorities. ANOVA, calculation of repeatability's and heritability's as well as adjusted means were conducted with the software package Plabstat version 3A (Utz, 2005). Mean comparison analysis was calculated with the software package R version 3.1.2.

Results and discussion

The testing of a total of 200 accessions for the trait biomass production and visual drought response revealed a wide genetic variation. Biomass scoring at cutting date 4 (in mid-summer 2013 after a mild drought period at the locations Kaltenhof and Triesdorf) showed a significant genotypic variance component, with a heritability of 54.6%. In contrast, the visual scoring of drought response showed no significant genotypic variance and thus, a heritability of 0% in the analysis over three locations (Kaltenhof, Triesdorf, Les Rosiers sur Loire). When considering only the location Les Rosiers sur Loire with severe drought conditions early in summer, repeatability was at 57.9% indicating the importance of a selection environment which allows good differentiation for drought tolerance. Figure 1 compares the results from the analysis of 200 accessions by visual scoring of biomass before cutting date 4 at two locations (Kaltenhof and Triesdorf; after drought period in summer) and the visual scoring of drought response during drought period at the location Les Rosiers sur Loire in 2013. Considering the different ploidy levels, the tetraploid accessions in our collection showed on average significantly fewer drought symptoms ($P<5.42\times10^{-8}$), with some diploid accessions also showing good drought performance. Visual biomass scoring after the summer drought period was significantly higher among the tetraploid accessions ($P<2.2\times10^{-16}$). One possible explanation is a generally increased tolerance to abiotic stresses in polyploid plants compared to their diploid counterparts. This relation was already described for other plant species.

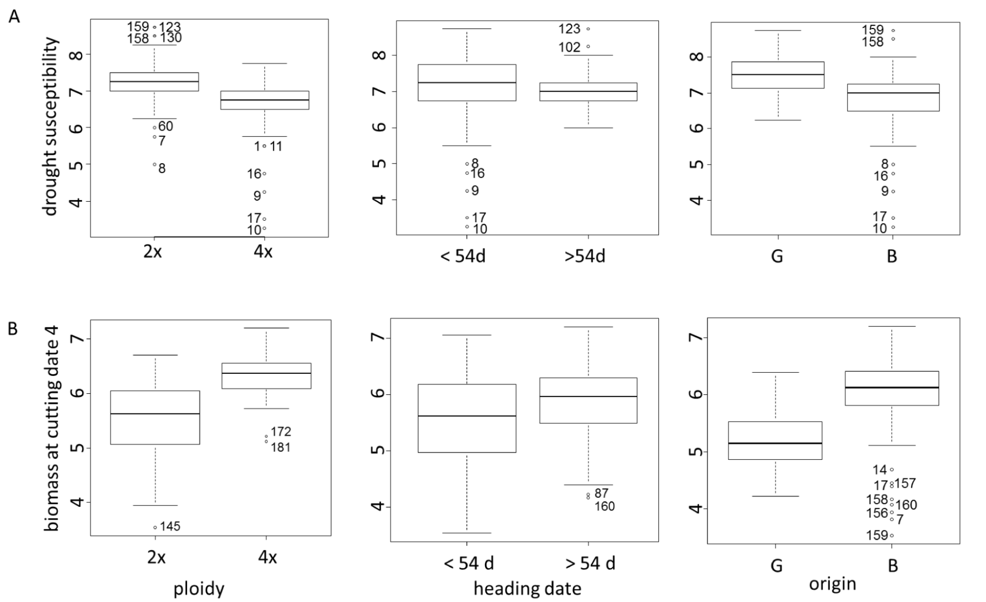

Figure 1. (a) visual scoring of drought response at location Les Rosiers sur Loire in the second cut year 2013 and (b) visual biomass scoring before cutting date 4 of second year cut 2013 (Kaltenhof and Triesdorf trials). The upper and lower edges of the boxes represent the 1st and 3rd quartiles, the black line within the boxes the median and the upper and lower whiskers the 1.5 fold interquartile distance. Diploid (2×) and tetraploid (4×) plant material, of early (<54 days) and late heading (>54 days), from gene bank (G) and breeding (B).

Polyploid plants are supposed to deplete soil moisture to a greater degree before reaching leaf water potentials that cause closure of stomata (Maherali *et al.*, 2009). Additionally, the activity of peroxidase and relative water content in tetraploid plants under drought conditions was found to be higher than in diploid plants (Liu *et al.*, 2011). There was no difference in drought tolerance observed between early and late heading accessions but significant difference in biomass production ($P<0.0009$), with slightly higher biomass production in the late flowering accessions. When considering the different origins of the plant material (breeding material vs gene bank material), a significantly higher mean drought tolerance was observed in the breeding material ($P<3.86\times10^{-12}$). As expected, breeding material showed a significantly higher biomass production ($P<1.85\times10^{-8}$), since it has been already selected for yield performance and all tetraploid accessions are in this group.

Conclusions

A wide variation of biomass production potential and drought-tolerance performance was successfully found among the 200 diverse accessions of perennial ryegrass as well as within the accessions. Tetraploid accessions in our test-set seemed to have enhanced higher stress tolerance compared to the diploid perennial ryegrass, whereas heading date had hardly any influence on these traits. Gene-bank material showed, on average, a lower biomass production than breeding material; this reflects selection work in this material in the past, which can also be seen under drought conditions.

References

Liu S., Chen S., Chen Y., Guan Z., Yin D. and Chen F. (2011) *In vitro* induced tetraploid of *Dendranthema nankingense* (Naki) Tzvel. shows an improved level of abiotic stress tolerance. *Scientia Horticulturae* 127, 411-419.

Maherali H., Walden A.E. and Husband B.C. (2009) Genome duplication and the evolution of physiological responses to water stress. *New Phytologist* 184, 721-731.

Sheffer K.M., Dunn J.H. and Minner D.D. (1987) Summer drought responses and rooting depth of three cool-season turfgrasses. *HortScience* 22, 296-297.

Utz H.F. (2005) *PLABSTAT – plant breeding statistical program version 3A*. Institut für Pflanzenzüchtung, Saatgutforschung und Populationsgenetik, Universität Hohenheim, Stuttgart, Germany.

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