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Climate change adaptation in forage grasses: from phenotype to genotype

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Abstract. Precise, non-invasive and high-throughput phenotyping methods are a crucial part for the identification of genes underlying plant adaptivity to abiotic stresses and for transferring this knowledge to practical breeding. Baltic and Nordic regions of Europe are facing an increased frequency of mild summer droughts. Therefore, securing stable forage production by improving grass tolerance to water deprivation is a pressing issue. Dissecting biomass accumulation by precise phenotyping of leaf growth under optimal and drought stress conditions, associating adaptive traits with candidate genes and subsequently validating their functions by employing CRISPR-Cas based genome editing, can be an efficient strategy to increase sustainability in agriculture through improvement of perennial ryegrass adaptation to current and future climates.

Keywords: perennial ryegrass, growth, drought

1 Introduction

Permanent grassland and meadows make up one-third of the agricultural land in the EU [1], forming an instrumental input for livestock farming. The growing number of beef cattle farms demands increasing amounts of high-quality pastures, but at the same time need to adapt to new environmental and economical requirements and restrictions. These require maximizing production per unit area with less input of water, fertilizers and pesticides leading to lower environmental footprints including GHG emissions and resulting in a fair, healthy and environmentally friendly food system. Global warming offers a possibility to increase productivity in the Baltic region as the higher average temperatures, extended growing seasons and longer frost-free periods may result in improved yield and reduced carbon footprint due to higher yields and better feed efficiency [2]. On the other hand, the predicted climate shifts increase frequency of dry periods which may impose on grassland productivity leading to huge yield losses and thus escalated fodder demand [3,4]. Mild drought, which is typical for temperate environments, does not threaten survival of the crops, however, it significantly reduces yield. Therefore, applied research that addresses future demands of forages in response to climate change and supports sustainable agriculture is receiving much attention. In this paper we summarized our recent year achievements in perennial ryegrass breeding for biomass formation under drought using novel phenotyping and genotyping methods.

2 Phenotyping a Dynamic Trait

Superior feed quality and productivity makes perennial ryegrass (*Lolium perenne* L.) the predominant forage grass species in Western Europe. However, the main limitation of perennial ryegrass wider distribution further north and east is its poor performance under unfavourable conditions. Water limitation is one of the major factors reducing the yield of crops in temperate regions and worldwide [5]. Despite increasing interest in research on mechanisms causing yield losses under water deficit, such studies proved to be difficult and not very successful [6]. A limitation is that yield often is measured destructively at the end of the experiment and in this way the dynamic process of growth is overlooked. As the biomass accumulation is largely determined by leaf growth, phenotyping leaf elongation in response to water deficit might give a hint in what directions we should breed. Using a non-destructive, high-throughput and non-labor intensive method to monitor real time leaf growth under adverse environmental conditions [7] we have revealed that leaf elongation under stress conditions is not linear but can be described by three phases: the first phase describes growth in response to temperature, in the second phase the plant decelerates leaf elongation proportionally to soil water potential, and finally, leaf growth terminates at the third phase [8]] Additionally, the Tri-Phase model enabled us to determine points which demarcate the phases thus they can be used to describe the response of plants to water deficit. For accurate quantification of plant tolerance to water deficit, we have used this dynamic phenotyping method in a perennial ryegrass association panel and revealed high variation of leaf elongation traits with no apparent clustering of genotypes representing turf or forage type cultivars and natural ecotypes ([9] (Fig. 1). The most desirable trait combination for a forage cultivar is fast growth under optimal conditions, paired with moderately early slow down under drought stress, but late growth arrest, i.e., high tolerance, allowing the crop to produce biomass regardless of unfavourable weather conditions. Forage-type genotypes tended to grow faster under optimal conditions, especially compared to turf-type genotypes [9]. However, some of the forage-type genotypes were located at the negative coordinates of PC1 and PC2 and were thus associated with low values of leaf elongation under optimal and stressed conditions (Fig. 1). This indicates, that there is still plenty of room for improvement in forage breeding.

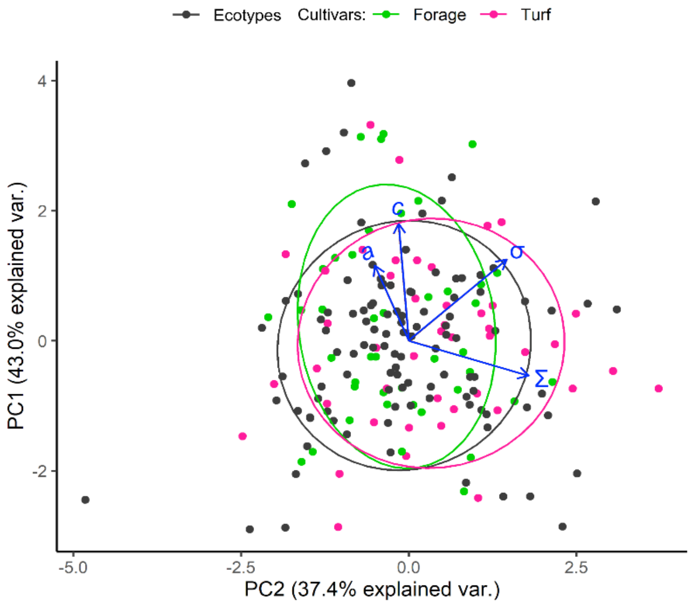

Fig. 1. Principal component analysis (PCA) biplot of 197 perennial ryegrass cultivars and ecotypes based on leaf growth traits: thermal leaf growth (*a*), leaf growth reduction point (Σ), leaf growth arrest point (σ), and water deprivation tolerance (*c*).

3 Phenotype to Genotype

The breathtaking progress in genomic technologies allowed fast and relatively cheap accumulation of vast amounts of genotypic data, while plant phenomics lagged behind. Recent development of imaging, sensor technologies and data analysis provide endless opportunities for in-depth studies of molecular mechanisms behind various plant physiological processes and to accelerate breeding for stress-tolerant crops [10]. Agriculture is traditionally considered an important part of economy in Baltic countries, however, the breeders rely mainly on the classical approach which is a time-consuming process. Lacking the implementation of state-of-art phenotyping and genotyping methods and strategies will result in lagging behind the economies/companies which apply modern breeding methods. In addition, the scarcity of functional genomics studies in perennial ryegrass hampers the understanding of the role of specific genes which can be utilized to develop cultivars with improved adaptation. Applications in breeding require linking genes to specific traits and validating their role and recently emerged genome editing technology offers the approach to achieve this. Drought, as well as other abiotic stress resistance is governed by a complex network of genes, however, the major players are relatively well established and proposed as possible targets for CRISPR/Cas9 editing [12]. The two candidate genes with well-known roles in stress resistance, *phytochrome B* and *MYB* transcription factor, were associated with leaf growth under water deficit in perennial ryegrass, making them primary candidates as well [9]. A first report on genome editing in perennial ryegrass was published recently [13]; however, transformation efficiency was relatively low and still far from routine. The newly launched project EditGrass4Food aims at performing functional studies of gene variants using gene editing (CRISPR) technologies. We intend to utilize unique pre-breeding material and CRISPR-based editing to validate candidate genes involved in northern adaptation of perennial ryegrass. We will focus on genes involved in the mechanisms of freezing tolerance and biomass growth under water deficit. Moreover, we will investigate changes during abiotic stress periods at the transcriptome level to reveal gene regulatory pathways and networks. This will enable us to utilize the gained information in future genomic selection programs to develop ryegrass cultivars with improved freezing and drought tolerance and persistence. It will also help breeders and agriculture in general in the Nordic/Baltic region to prepare for meeting new demands due to climate change and changing societal demands.

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