Germination and Seedlings Heterotrophic Growth of Cocksfoot (*Dactylis glomerata* L.) in Response to Temperature

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Abstract. In the context of climate change, grasslands are considered, similar to forest, as an important sink for atmospheric CO_2. However, environmental change seems to go faster than species adaptation to survive on site. Germination and heterotrophic growth are key phases for plant, and consequently, communities' establishment and structure. They are under genetic control and affected by temperature. The objective of this study was to analyze the intra-specific variability of six accessions of *Dactylis glomerata* in their responses to eight constant temperatures (5 to 40°C) during germination and initial heterotrophic growth. The novelty of this work comes from the non-linear modeling of germination and growth velocities and the estimation of cardinal temperatures. High variability within temperatures and significant differences between accessions were observed for germination speed. No germination was observed at 40°C for any accession. Further, seed germinated at 25°C died soon after they were transferred to 40°C for heterotrophic growth. The growth of the axes, whenever it existed, was negligible at 40°C. The speed of heterotrophic growth of the roots and shoots showed asymmetric bell-shaped response curves to temperature. Base temperature for germination was fixed to 0°C. After curve-fitting, optimum temperatures for germination were estimated to be between 21.5 and 26.3°C. Those for heterotrophic growth were, up to 5°C, higher. Contrariwise, the upper limits, for both processes, appeared between 34 and 40°C. Overall, this study demonstrates that genetic variability does exist between accessions. For each accession, the response of the germination rate was different from the response of heterotrophic growth rate.

Introduction

Grasslands cover more that 40% of earth's surface and at least 30% of the 160 Mha Agricultural Surface Area of Europe. They are among the largest ecosystems on earth and one of the major sources of forage. Further, in the context of climate change, grasslands are considered, similar to forest, as an important sink for atmospheric CO_2. The agricultural use-value of grasslands depends on their floristic composition and the structure of their canopy. These two intertwined features directly determine the quality and the quantity of the biomass harvested by grazing or mowing. Cocksfoot (*D. glomerata* L.) is one of the major perennial grass species in temperate and Mediterranean regions. However, during the lifespan of grassland, both floristic and genetic compositions as well as canopy structure evolve under the influences of environmental factors and the competitive interplay between individuals. Floristic and genetic composition evolves because of individuals' mortality and recruitment of new species and genotypes from the soil seed-bank or natural sowing. Temperature is one of the major factors controlling plant development (i.e. plant phenology, organogenesis and expansive growth). It is important in controlling seed germination. Indeed, higher plant species, as well as populations, varieties and cultivars within species, respond differently to temperature during the critical period of germination and seedling establishment in the field. In the context of global change, breeding grasses adapted to new ranges of temperature could be necessary. Knowing the variability of responses to temperature by different accession of germplasm is an unavoidable first step towards such breading. Thus, the objective of the work presented here was to analyse the genetic variability of *D. glomerata* in response to temperature during heterotrophic growth.

Methods and Study Site

Six accessions of *D. glomerata* were evaluated. Four of them were wild accessions collected in Continental France (507 and 566), in Corsica (387) or in Algeria (438). All seed lots had tetraploid genome (2n = 4x = 28). The other two accessions were commercial varieties sold in France (Ludac)

or in Australia (Kasbah). The genetic background of Kasbah came from Morocco and it was collected as seed by Mr CA NealSmith of the FAO-CSIRO Plant Exploration Mission in 1954 (Norton, 2015). Seeds were obtained from "Centre de Ressources Génétiques des Espèces Fourragères", INRAE-UR4 P3F, Lusignan, France. They were conserved at 5°C and 30% Relative Humidity (RH). Seed germination and heterotrophic growth: for each accession, one thousand seeds, placed in polypropylene boxes (55 mm × 120 mm × 180 mm, GEVES trademark, Loire Plastic, France, called GEVES-type box), were cold stratified in the dark for 7 days at 5°C and 30% RH, to break any physiological seed dormancy. Seeds were placed in a germination chamber at 25°C in the dark. Germination rate (α) was estimated at each tested temperature on four replicates of 100 seeds, as described by Ahmed and Escobar-Gutiérrez (2022a). When the embryo axes protruded over 1 mm out of the kernel, three replicates of 10 seedlings were placed in GEVES boxes over one sheet of blotter blue paper and transferred into growth chambers at constant temperature from 5 to 40°C. For heterotrophic growth, seedlings were pictured routinely using a digital camera. Radicle and shoot growth were measured by image analysis using Image J software version 1.47(http://imagej.nih.gov/ij/).

Here, we report data on relative growth rates of roots and shoots. The kinetics of growth of the root, and the shoot were fitted the Schnute's non-linear model (Eq. 1) (Schnute, 1981):

$$y = \left[c^b [d^b - c^b] \cdot \frac{1-exp^{[-g[t-v]]}}{1-exp^{[-g[w-v]]}} \right]^{\frac{1}{b}} \text{ Eq. 1}$$

where the dependent variable is y (mm); unitless parameters g and b implicitly define the shape of the curve; parameters c (mm) and d (mm) are the lower and upper values of y at v and w respectively; t (hours) is time while v (hour) and w (hour) are respectively the initial and last time of the fitted growth period.

First and second derivatives of Schnute's adjustments were used to estimate the maximum growth rates ($MGR_{temp(x)}$) of the two axes for each seedling at each growing temperature. For each seedling, the $MGR_{temp(x)}$ of each axes was used to estimate the maximum relative growth rate of root (*RGR-Root*) and shoot (*RGR-Shoot*). The overall relative growth rate of axes in response to temperature were fitted a five parameters Beta model (Eq.2).

$$RGR = RGRmax \cdot \left[\left[\frac{[Tmax-T]}{[Tmax-To]} \cdot \frac{[T-Tmin]}{[To-Tmin]} \right]^{\frac{To-Tmin}{Tmax-To}} \right]^{\delta} \text{ Eq. 2}$$

with the three cardinal temperatures, T_{min}, T_o, and T_{max}; *RGRmax* being the relative growth rate at optimal temperature, T_o, and a shape parameter δ (Yin et al. 1995). From this fitting, optimal temperature for germination and heterotrophic growth were estimated. Sequential ANOVA pair-wise comparisons were performed between the best fit of a given population and the rough data of a second one. The probability of a calculated F value greater that a tabular F ($Pr>F$) was calculated and a comparison matrix was constructed as previously described (Ahmed and Escobar-Gutiérrez, 2022a).

Results and Discussion

Data on germination rate (α) and heterotrophic growth (*RGR*) as a function of temperature were analysed. In general, a five-parameter Beta model (Eq. 2), with a low boundary fixed to 0°C, was adjusted to the relative growth rates for each accession (Fig. 1). Germination rate (α) and relative growth rate of the axes (*RGR-Root* and *RGR-Shoot*) increased as temperature increased from low to optimal temperature and decline afterwards. Germination was not observed for any accession within-specie at 40°C and, thus, it was excluded for the curve fitting. Further, high variability of the estimated values was observed. This variability was higher for germination rate (α) and *RGR-Root*, than for *RGR-Shoot*. Response curves of germination rate were asymmetric bell-shaped well fitted by the Beta model (Fig. 1-left). This contrasts with the reports of Qiu (2005) and Stanisavljevic et al (2011), who observed that under constant temperatures, germination rates and final germination percentage for *D. glomerata*, followed linear responses to temperature from 5 to 25°C and a decline afterwards. Furthermore, Trudgill et al (2000) showed that the base temperature (at which no growth

or development is observed) of a natural population of *D. glomerata* was 6.7°C. Nevertheless, our wild populations and varieties were less sensitive to temperature and significant growth rates were recorded even at 5°C. Optimum temperature for α was estimated to be in the range from 20 to 30°C (Ahmed and Escobar-Gutiérrez 2022b). The maximum constant temperature for germination of *D. glomerata* could be expected between 35 and 40°C (Ahmed and Escobar-Gutiérrez 2022b). In some cases, followed seedlings revealed abnormal, showing no growth of the root or the shoot. Thus, estimated parameters in both situations are not presented. A Beta model used to estimate accessions' cardinal temperatures and maximum velocity of growth revealed that wild population 507 had the fastest *RGR-Root* (0.039 mm.h^{-1}.mm^{-1}) whereas wild population 387 had the slowest one (0.022 mm.h^{-1}.mm^{-1}) (Fig. 1-center). Optimum temperature for *RGR-Root* was estimated to be in the range 27.3 to 31.08°C. *RGR-Root* curves were used to compare the responses of populations to temperature. Wild population 387 was significantly different (*P < 0.01*) from all other accessions (data not shown). Wild populations 438, varieties Kasbah and Ludac had exchangeable curves. Likewise, the response curves of accessions 438, 577, Kasbah and Ludac fitted the data of population 507. Wild population 387 had the highest *RGR-Shoot* (0.023 mm.h^{-1}.mm^{-1}) whereas, variety Kasbah had the lowest (0.021 mm.h^{-1}.mm^{-1}) (Fig. 1- right). Moreover, the range for T$_{min}$ (0 to 1.33°C) was narrower than for T$_{max}$ (35- 40°C). The responses *RGR-Shoot* curves showed differences (*P < 0.01*). Indeed, the response of some accessions had exchangeable curves. The response of variety Ludac adjusted on data of population 566 (data not shown). For accessions 387, 507 and Ludac, *RGR-Root* and *RGR-Shoot* curves showed significant differences (*P < 0.01*). On the other hand, the relative growth rates of root and shoot of population 438 were not significant different. Further, the *RGR-Shoot* for the accessions 566 and Kasbah adjusted on the *RGR-Root*. These could reflect the variation in *RGR-Root* response within *RGR-Shoot*. In this regard, Ahmed and Escobar-Gutiérrez, (2022a) showed that in ryegrass cardinal temperatures for germination were different from those for growth of both root and shoot seedling axes. Further, the most favorable temperatures to reach the maximum RGR of the root and shoot were between 25 and 31°C (Ahmed and Escobar-Gutiérrez, 2022a).

Conclusions

Our results demonstrate that high genetic variability does exist within *D. glomerata* in response to constant temperature during germination and heterotrophic growth. Even with the small sample of germplasm in this study, it was possible to reveal new information about the germination rate and the initial growth of root and shoot. The response curve to temperature of germination rate for each accession was different from the curves of *RGR-Root* and *RGR-Shoot*. This should prompt physiologist to extend the analyses of response to temperature to other processes (Zaka et al., 2017). In addition, this variability could be used to breed varieties adapted to the conditions induced by the ongoing climate change.

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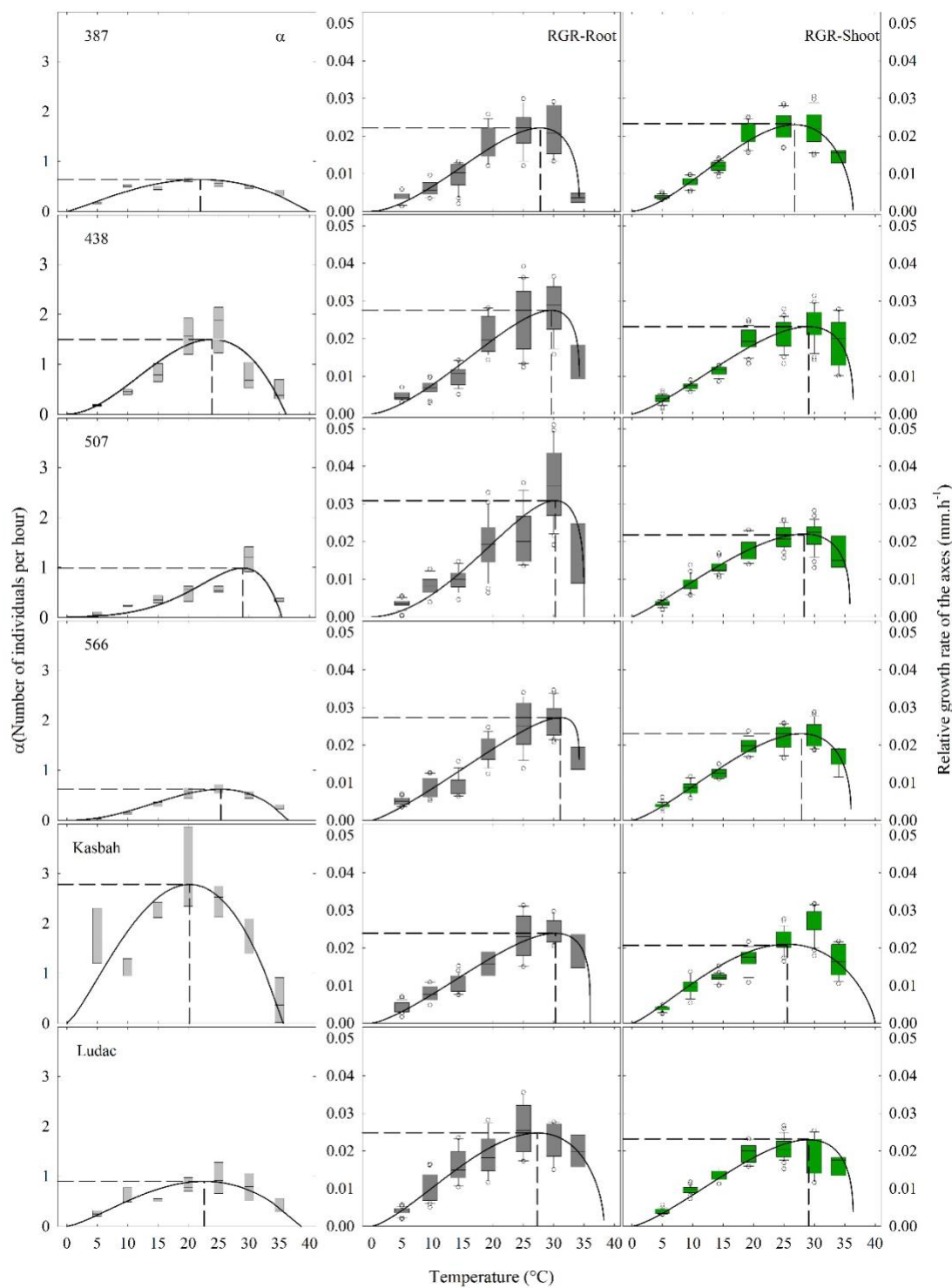
FIGURE 1. Box-plots for germination rate (α) and relative growth rates of root and shoot during heterotrophic growth, observed for six accessions of *D. glomerata* in response to constant temperature. Box-plots responses to temperature were fitted with the Beta model, between 5 and 34.2˚C, n = 30. For 40°C, 0 ≤ n ≤ 30 because of seedling mortality.