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Research Note

Genetic diversity of alfalfa (*Medicago sativa*) in response to temperature during germination

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Abstract

Temperature is one of the major factors controlling plant development, in particular seed germination. Alfalfa is a perennial pasture legume that holds an important place in cultivated grasslands. Breeding alfalfa cultivars adapted to new ranges of temperature could be necessary, requiring knowledge of the variability in response to temperature among different accessions. Six accessions of *Medicago sativa* subsp. *sativa* and one wild population of *M. sativa* subsp. *falcata* were evaluated for their germination temperature response. Seeds were tested for germination in the dark at eight constant temperatures, from 5 to 40°C. Significant differences ($P < 0.01$) were found. The optimum temperature for germination ranged between 11.7 and 21.1°C, whereas two varieties, 'Demnate' and 'Luzelle' had a very wide range of temperatures favouring maximum germination. The findings of this study suggest that the germination of alfalfa was little affected by low temperature (5°C) whereas germination at 40°C was lower and showed high variability. Our results revealed variability in the response to temperature for germination of *M. sativa* that gives room for breeding new varieties adapted to future environmental conditions induced by the global climate change.

Keywords: Alfalfa, breeding, climate change, genetic variability, germination, *Medicago sativa*, temperature

Experimental and discussion

The annual time course of temperature is one of the most important factors affected by climate change. The International Panel on Climatic Changes (IPCC) anticipates an increase in global average and extreme temperature between 3.7 to 4.8°C by 2100 (IPCC, 2014). Temperature will be subject to larger fluctuations between years, due to a higher frequency of extreme climatic events.

Alfalfa (*Medicago sativa* L.) is the oldest cultivated forage crop and one of the oldest crops in the world. It is a perennial forage species of the Fabaceae family that holds an important place in cultivated grasslands. *Medicago* is considered to have two centres of origin in Vavilov's scheme. Paleontological evidence suggests that it started to be grown around 9000 years ago in Persia and it is grown in pure stands and in mixtures, typically with grasses and other legumes. In 2006, it covered 7.1 million ha in Europe and approximately 30 million ha around the world (Mouttet *et al*., 2014).

It is well documented that germination is highly temperature dependent (Bewley and Black, 1994), and the effect of unfavourable temperature is probably more critical during germination than at any other stage of vegetative growth. However, the response to temperature during germination of alfalfa has only been described for a few varieties and a narrow range of temperatures. Modern *M. sativa* is a complex of eight diploid or autotetraploid perennial and allogamous subspecies native to different geographical zones, with contrasting annual temporal ambient temperature. Cultivated alfalfa is an autotetraploid plant showing higher vigour than diploid subspecies. Most European varieties have undergone introgression from *falcata*, which provides cold tolerance and allows breeding of varieties for Northern areas (Mouttet *et al*., 2014). Knowing the eventual relationships between responses to temperature during germination and subsequent plant functioning could be useful for breeding programmes.

On the other hand, little is known of the pattern of genetic diversity of alfalfa in response to environmental factors, particularly to temperature during germination and early growth. Despite numerous studies have explored the relation between temperature and germination in legume forages (Moot *et al*., 2000; Black *et al*., 2006; Monks *et al*., 2009; Tribouillois, 2014) and model-species *Medicago truncatula* (Brunel *et al*., 2009; Dias *et al*., 2011), only a few have focused on analysing the within-species genetic diversity. Seed germination response can be used as an early marker for selection of varieties adapted to future climatic conditions. The objective of the study presented here was to analyse the genetic variability of alfalfa in response to temperature from 5 to 40°C during germination.

Six commercial varieties of *M. sativa* subsp. *sativa* ('Barmed', 'Demnate', 'Flamande', 'Harpe', 'Luzelle' and 'Orca') and one population of *M. sativa* subsp. *falcata* (Krasnokustkaya) were studied. Seeds were obtained from INRA's Centre de Ressources Génétiques (CRG) in Lusignan, France (46°24'10''N, 0°40'48''E). They were conserved at 5°C and 30% relative humidity, until they were used. Seeds were scarified between two sheets of sandpaper (grade 180) in order to break any seed dormancy. After scarification, four sets of seeds per accession were germinated on two sheets of Whatman paper (Whatman, France) in plastic Petridish lids (90 mm-diameter). The paper was moistened with 5 ml de-ionised water. Petri-dishes were placed in the darkness in growth chambers at constant 5, 10, 15, 20, 25, 30, 35 or 40°C and watered as needed. The vapour water deficit inside the growth cabinets was kept under 1kPa. A seed was defined as germinated when the emerged radicle was at least 2 mm long. Germination counting was carried out at variable time intervals and duration that depended on temperature treatments. Here, we report data on maximum germination percentage. For each accession population, a third-degree polynomial was adjusted by the least squares method. From this fitting, optimal temperature for germination was estimated. Sequential ANOVA pair-wise comparisons were performed between the best fit of a given population and the rough data of a second one. A comparison matrix was constructed with the probabilities of *F*-calculated values greater than *F*-tabulated ones ($Pr > F$) as previously described (Ahmed, 2015).

Response-surface plots emphasise that time courses of germination vary depending on temperature (figure 1). Recording of germination was performed for as long as 1008 hours (42 days) for seeds at 5°C. At the same time, similarities between responses can be observed, for example those of varieties 'Demnate' and 'Luzelle' (figures 1 and 2). In addition, it was observed that most of the seven *M. sativa* accessions were little affected by low temperature (5°C) in their maximum germination capacity. Exceptions to this were 'Harpe' and 'Orca'.

Contrary to the report by Townsend and McGinnies (1972), we found that alfalfa is sensitive to temperature in regards to maximum germination. Furthermore, the sensitivity varied among the seven accessions. The range of temperatures over which *M. sativa* germinates is wide, but germination at 40°C was low and showed high variability in most accessions; 'Demnate' had the highest germination percentage at 40°C. For each accession, the best fit to a third degree polynomial is presented (figure 2). Moreover, estimated optimum temperatures to obtain maximum germination are within a wide range, going from 11.7°C in 'Flamande' to 21.1°C for the Krasnokutskaya seed lot. The curves were used to compare the responses of these accessions. Beyond the differences in response to particular temperatures, the shapes of the best fits were significantly different ($P < 0.01$) between some of the seven accessions. The pair-wise comparisons of normalised maximum germination percentage curves revealed that the wild population, Krasnokutskaya, and the varieties 'Flamande', 'Demnate' and 'Luzelle' responded differently to temperature. Only varieties 'Barmed', 'Harpe' and 'Orca' have exchangeable curves between them. The two varieties 'Demnate' and 'Luzelle' had a very wide range of temperatures favouring maximum germination. Further, the two varieties 'Demnate' and 'Luzelle' can be fitted linear functions ($P < 0.01$) in response to constant temperature from 5 to 40°C. The 'Demnate' variety appears to be insensitive to temperature, except at 40°C. Brar *et al.* (1991) observed that germination of alfalfa 10 days after planting was affected by temperatures between 10 and 30°C in the case of one of the two varieties they studied. For one of the entries, cumulated germination was lower at 10 and 30°C, while germination was higher in the range 15 to 25°C. Strict comparisons of our result with most data in literature are difficult because temperature regimes as well as observation periods are different (Ungar, 1967; Townsend and McGinnies, 1972; Hampton *et al.*, 1987; McDonald, 2002; Guan *et al.*, 2009; Butler *at al.*, 2014).

In conclusion, the finding of this work shows that *M. sativa* accessions differed in their response to temperature during germination. The optimum temperature for germination ranged between 11.7 and 21.1°C for those showing a peak. This prompts us to extend the analyses of the response to temperature during the early stages (germination and heterotrophic growth) to other forage species (Ahmed, 2015). Our results suggest revealed variability in the response to temperature for germination of *M. sativa* that gives room for breeding new varieties adapted to future environmental conditional induced by the global climate change.

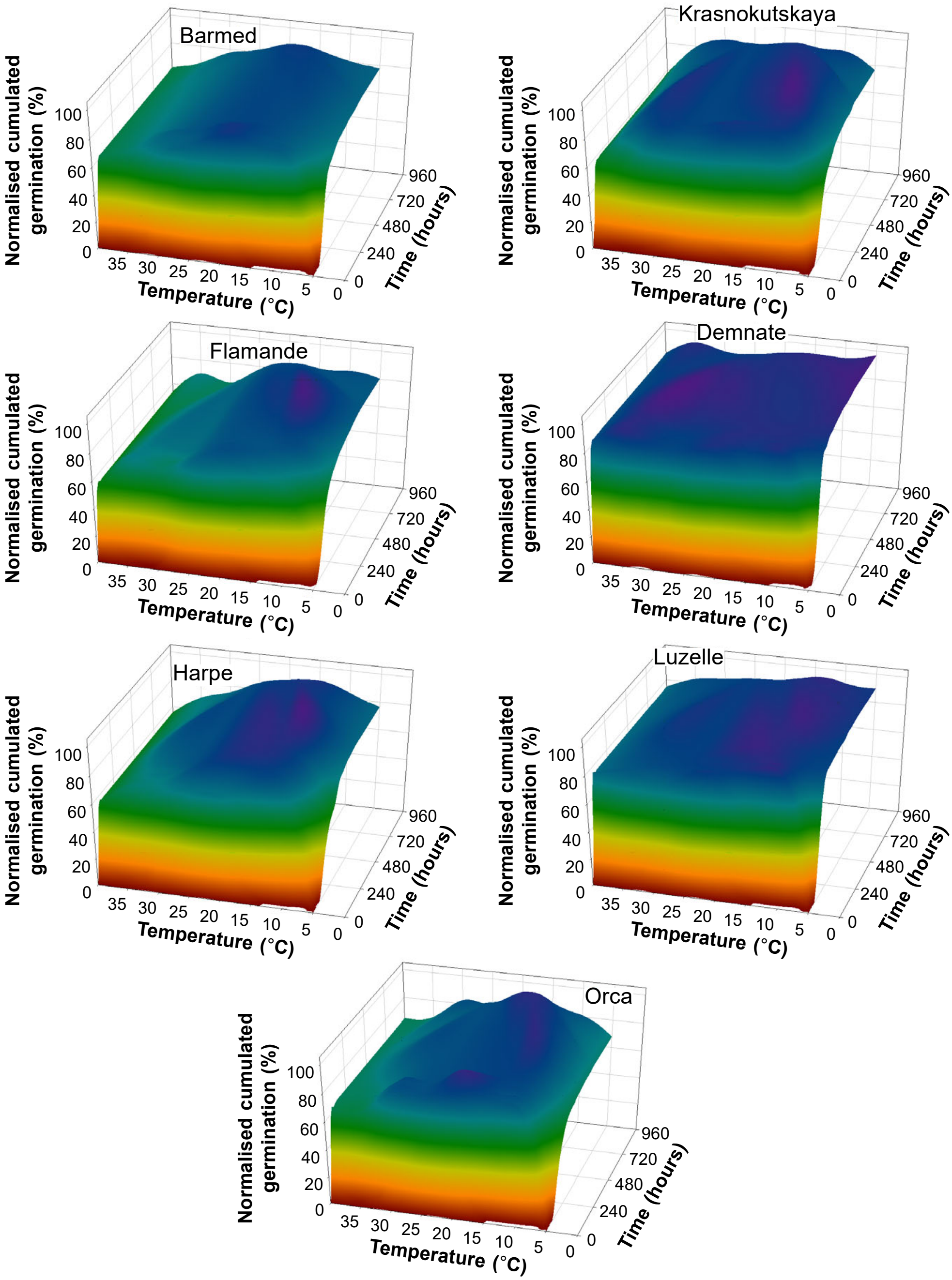

Figure 1. Temperature and time course response-surfaces of the normalised cumulative germination percentage of seven accessions of *Medicago sativa*.

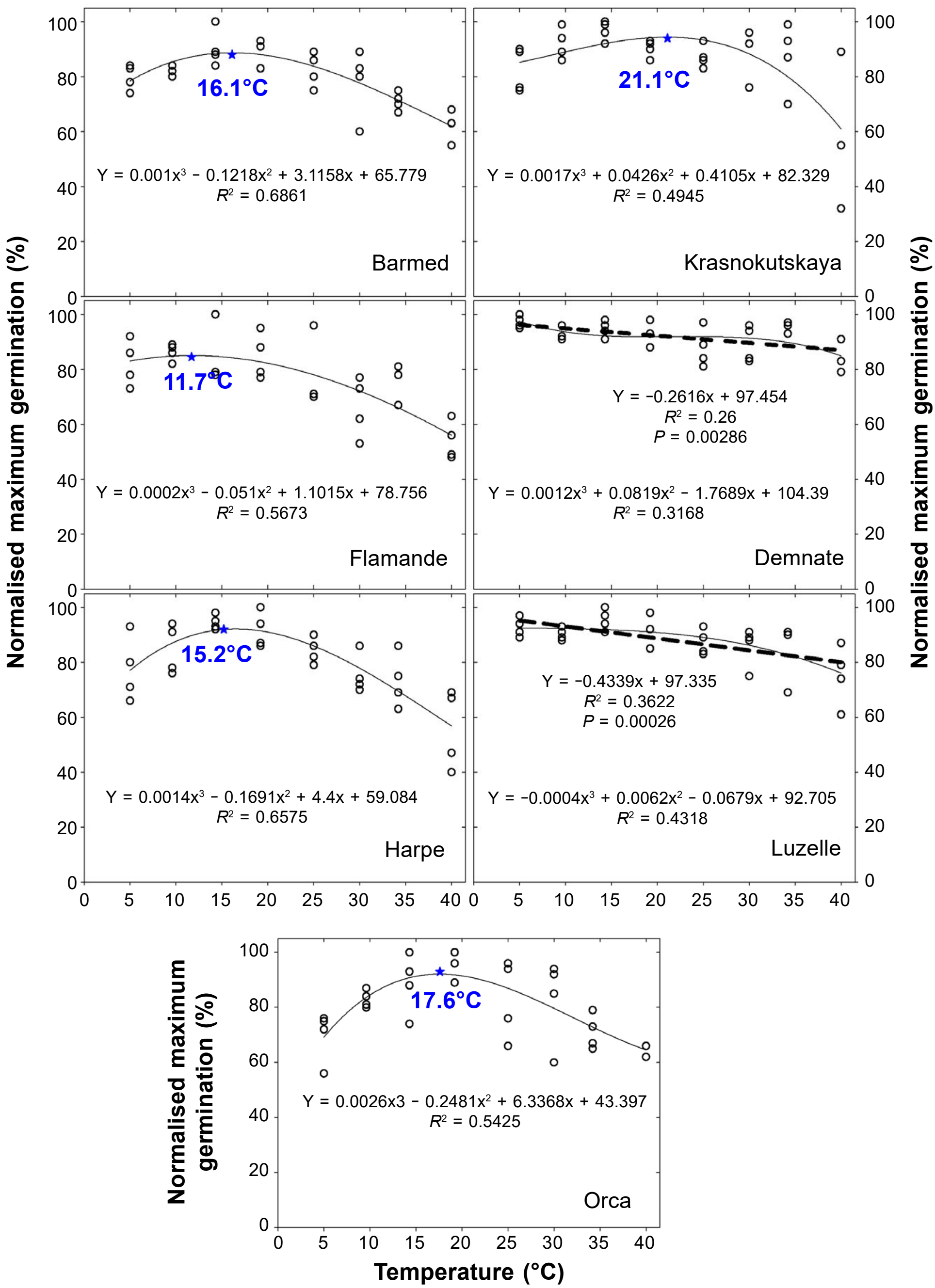

Figure 2. Normalised maximum germination percentage of seven accessions of *Medicago sativa* in response to constant temperature. Dashed lines, where presented, are the fitted straight line. Estimated optimal temperature for each seed lot is indicated (★).

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