

## Emerging opportunities for agriculture: investigating plant adaptation by characterizing germplasm collection habitats

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### Abstract

Agricultural crops and their wild progenitors are excellent candidates for ecophysiology studies because germplasm collections are often extensive and well described, and in its dissemination the crop may explore habitats not found in the natural distribution of the progenitor. The advent of high resolution elevational climate models has greatly improved our capacity to characterize plant habitats and study species' adaptive responses to different stresses encountered across the distribution range. With passport data and germplasm from the ICARDA, ICRISAT and the Australian Lupin Collection, we are applying this approach to (i) search for improved reproductive chilling tolerance in chickpea (*Cicer arietinum*); (ii) identify adaptive strategies in stress gradients across the distribution range in wild populations of narrow-leafed lupin (*Lupinus angustifolius*) and (iii) genetic improvement in yellow lupin (*L. luteus*).

### Key Words

Agro-climatic habitat characterization, phenology, vernalisation, chilling tolerance, chickpea, lupin

### Introduction

Comprehensive germplasm collections which adequately sample a species' distribution are excellent tools for studying adaptation to stresses encountered across the habitat range. Species' responses to particular stresses can be ascertained by evaluating material from habitats with contrasting selection pressures for the stress of interest. The advent of high resolution elevational climate models has greatly improved our capacity to characterize plant habitats. Altitude, and long term monthly average rainfall, minimum and maximum temperatures are available for the world's terrestrial surface in 30 second grid intervals (ca. 1 km spatial resolution) from the WORLDCLIM model (Hijmans et al. 2005). More detailed data such as relative humidity, the number of rain or frost days per month, coefficients of variation for monthly precipitation, sunshine percentage, and wind speed are available at slightly lower resolution (10 minute grid intervals-ca. 9-18 km spatial resolution) (New et al. 2002). These data can be used to describe habitats as a starting point for ecophysiological research investigating relationships between environment and plant trait expression (Berger et al. 2008), or as a screening tool to improve the chance of finding germplasm tolerant of stresses associated with climatic gradients (Mackay et al. 2008). We are using this approach to both study adaptation and screen germplasm in three grain legume species:

*Chickpea (Cicer arietinum)*

A lack of reproductive chilling tolerance delays pod set until mean temperatures >14-16°C exposing the crop to considerable terminal drought stress in Mediterranean environments of Australia. We have characterized global chickpea collections to identify habitats with low and high temperatures at flowering (Berger 2007), and are evaluating germplasm from these regions in field trials at PAU, India and ICARDA, Syria to determine whether these contrasting environments have selected for ecotypes with differential chilling tolerance.

#### *Narrow-leafed lupin (Lupinus angustifolius)*

Modern cultivars have the earliest phenology of Australian cool season grain legumes since the elimination of the vernalisation response, and are relatively unresponsive to stimuli such as daylength and temperature (Christiansen and Jørgensen 2002). GxE studies have demonstrated very little specific adaptation among Australian lupin cultivars and this has been attributed to an inability to match phenology to target environment with current advanced material. Early flowering genotypes are unlikely to perform optimally in long season environments because of early senescence and the associated source and sink limitations. We are evaluating germplasm collected along terminal drought stress gradients to understand how wild populations respond in terms of phenology, plant growth and its drivers. Lessons learnt about specific adaptation in wild populations can be applied in future breeding strategies.

#### *Yellow lupin (Lupinus luteus)*

This species has evolved as a Mediterranean winter-annual in relatively high rainfall coastal regions, but was domesticated as a summer crop in temperate central Europe (Berger et al. 2008). In developing the crop for Mediterranean southwest Australia is it more appropriate to concentrate on wild material from Mediterranean habitats, which are likely to be more similar to the target environments, or on European germplasm domesticated for temperate summer cropping?

### **Materials and methods**

A single experimental methodology was applied to the three species in the present study. Germplasm collection sites (agricultural research stations used for breeding in the case of cultivars) were mapped with DIVA-GIS (Hijmans et al. 2001) and long term monthly climate records extracted. For each site typical growing season phenology (dates of emergence, flowering and maturity) was defined using published information and feedback from local breeders. Seasons were divided into vegetative and reproductive phases, and bioclimatic variables generated for each phase. Rainfall and the number of frosty and rainy days/month were summed. Temperature, daylength, relative humidity, sunshine percentage, wind speed, and precipitation coefficients of variation were averaged. Minimum and maximum temperatures recorded in each phase were extracted directly. Habitats were characterized by performing principal components analysis and then defined by hierarchical clustering (Ward's method, SPSS Version 10) to facilitate the evaluation of contrasting ecotypes in separate common garden experiments:

#### *Chickpea.*

Germplasm from sites with cool (14.0°C) and warm (23.0°C) mean temperatures during the typical flowering month modelled after Berger (2007) were selected for field screening against local checks in northern India and Syria. At PAU (India) 619 genotypes from ICRISAT were evaluated at 2 planting dates in a split plot design (n=4), while at ICARDA 562 genotypes were screened in an alpha lattice design (n=3).

#### *Lupin.*

*L. angustifolius* (n=133) and *L. luteus* (n=73) were evaluated alongside *L. albus* (n=88) and *L. mutabilis* (n=19) in a species comparison using Maximin hierarchical clustering to generate maximally diverse habitat subsets for each species. In order to determine how vernalisation response varied between

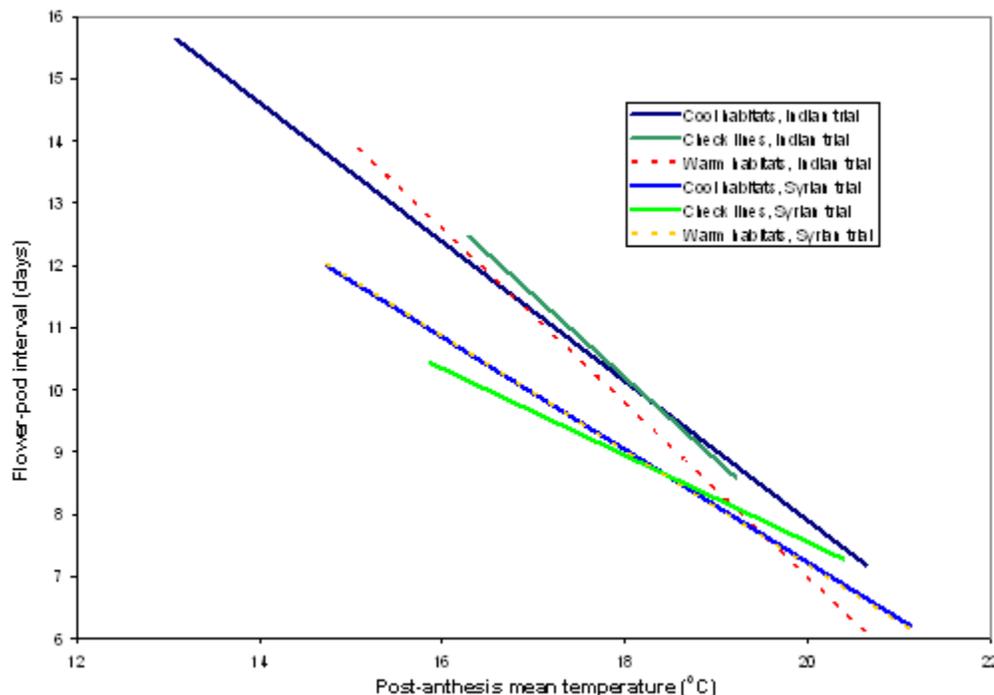
species and collection site habitats, the germplasm was exposed to low (ambient air=14.6°C meaned over the 1<sup>st</sup> 30 days), medium (8 days@10°C=13.4°C 30 day mean) and high vernalisation stimulus (16 days@8°C=12.1°C 30 day mean) prior to hand planting in a 2-way factorial RCBD (n=3).

The data recorded in all experiments covered phenology (flowering, podding, flower end, maturity), plant growth (early vigour, growth rate over time), and productivity (seed and biological yield, yield components).

## Results

### *Reproductive chilling tolerance in chickpea*

The time interval between flowering and podding regressed against mean post-anthesis temperature is a direct a measure of reproductive chilling tolerance which is independent of plant size, unlike the number of flowers or pods per plant. These results show that chickpea is even more chilling sensitive than indicated previously because the negative relationship between the flower-pod interval and temperature extends far beyond the 14-16°C upper threshold established in earlier Australian trials (Berger et al. 2004; Berger et al. 2005). Fig 1 shows significant ( $P<0.05$ ) 3-way interactions between the flower-pod interval temperature relationship, germplasm category and trial location. Despite mean post-anthesis flowering temperatures being very similar in both countries, germplasm in the Indian trial was more sensitive than that evaluated in Syria, as indicated by significantly steeper regression slopes ( $P<0.001$ ). Moreover, in the Indian evaluation, germplasm from cool flowering habitats was significantly less temperature sensitive to than that from warm environments ( $P<0.001$ ), although the rate differences were relatively small (flower-pod interval= 1.1 and 1.4 days/°C, respectively), and no outstandingly tolerant material was identified (data not presented). By



**Figure 1. Reproductive chilling sensitivity in chickpea as estimated by regressing the flower-pod interval against post-anthesis temperature meaned over the 1<sup>st</sup> 10 days after flowering in field trials at PAU, India and ICARDA, Syria. The regression accounted for 66.4% of variance, and lines represent fitted values for well-adapted local checks, and germplasm sourced from cool (14.0°C) and warm (23.0°C) flowering habitats, respectively.**

contrast, in Syria there were no differences between germplasm from cool or warm flowering habitats, whereas well-adapted local checks were significantly more tolerant than either category ( $P < 0.001$ ).

*Lupin: specific adaptation to stress gradients*

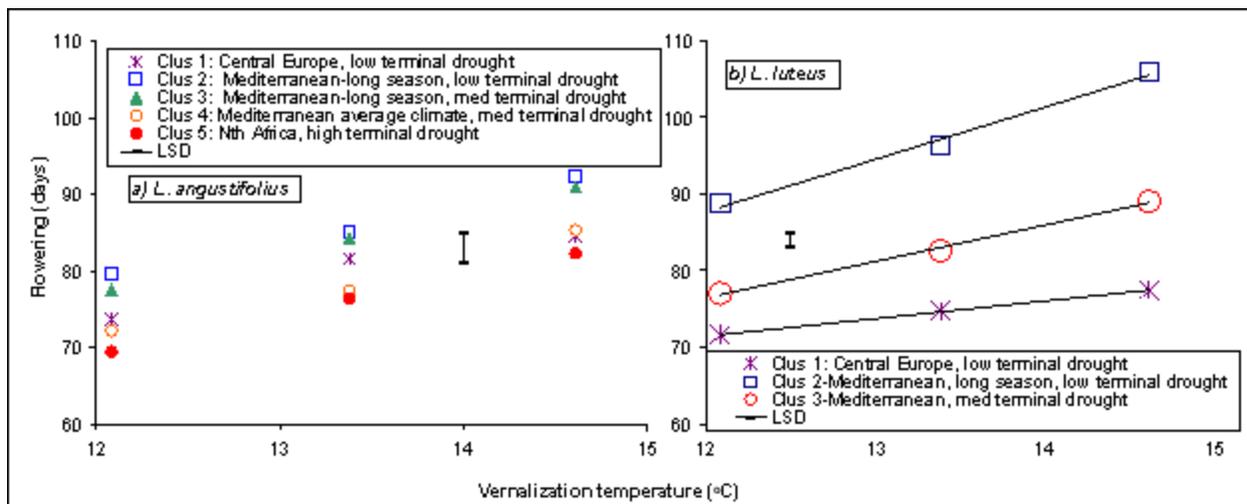
Collection sites of *L. angustifolius* and *L. luteus* clustered into discrete habitats along terminal drought stress gradients from low stress Central European habitats with consistent rainfall (281-283 pre-season; 308-321 season; coefficient of variation (CV) =51-52) and little temperature change during the reproductive phase (-0.02-0.01°C/day), to stressful Mediterranean locations with low, highly variable rainfall and rapidly rising temperatures at season's end (0.08-0.11°C/day) (Table 1).

**Table 1. Habitat types represented in the Australian Lupin Collection (*L. angustifolius*, *L. luteus*) sorted from low to high terminal drought stress.**

Cluster	Description
<i>L. angustifolius</i>	
1	Central Europe-long day, short season, cooling rep phase with high consistent rain: low terminal drought
2	Mediterranean- med elevation, long season, high consistent rainfall, cool veg phase with frost: low terminal drought
3	Mediterranean- high elevation, long season, intermediate, consistent rainfall, cold veg phase with frost: intermediate terminal drought
4	Average Mediterranean climates (clustering around origin): intermediate terminal drought
5	North Africa- high elevation, low, variable season rain, high sun: high terminal drought
<i>L. luteus</i>	
1	Central Europe –long day, short season, intermediate, consistent seasonal rainfall, high pre-season rain, rapidly warming veg phase/flat rep phase: low terminal drought
2	Mediterranean-long season, med elevation/frost, v. high rainfall: low terminal drought stress
3	Mediterranean-high max rep phase temps, intermediate, med-variable rainfall: med terminal drought stress

The phenology of wild Mediterranean lupin populations follows the predictions of Grime's (1979) triangle: as habitats become more stressed or disturbed, ruderal reproductive strategies become increasingly conservative. Thus, in *L. angustifolius* and *L. luteus*, the earliest wild germplasm came from clusters with strong terminal drought stress (Fig. 2), indicating that drought escape is an important strategy in both

species. Conversely, cool, long season habitats with consistent rainfall (Cluster 2) have selected for later flowering, avoiding cold stress during the reproductive phase, and extending the plant lifecycle to allow for competitive biomass accumulation.



**Figure 2. Vernalisation responses estimated by regressing flowering time against vernalisation temperature meaned over the 1<sup>st</sup> 30 days in ecotypes of a) *L. angustifolius* and b) *L. luteus* collected along terminal drought stress gradients from Central Europe (Cluster 1) to the Mediterranean (Clusters 2 to 5). Cluster responses in *L. angustifolius* were NS ( $P=0.358$ ) and therefore not presented. Regression slopes in *L. luteus* ecotypes were significantly different ( $P<0.001$ ) and presented as linear curves in b).**

Despite these similarities, there are some important differences between the 2 species. When comparing germplasm from broadly similar habitats (Clusters 1 to 3), *L. luteus* appeared to flower earlier than *L. angustifolius* (Fig. 1). Thus, *L. luteus* from the moderately terminal drought stressed Cluster 3 flowered even earlier than *L. angustifolius* collected from far more stressful, variable rainfall habitats in Northern Africa (Cluster 5). Similarly, domesticated European material was far earlier in *L. luteus* than *L. angustifolius* (Fig. 1). This is probably due to the disparate breeding efforts devoted to the two species in Europe. *L. luteus* was introduced to northern Germany in the 1840s (Gladstones 1998), and has been subject to considerable selection pressure for early flowering in order to allow the crop to mature in the mild, cooling seasons' end experienced in the late European summer (Berger et al. 2008). In contrast, historically *L. angustifolius* was a lower priority for European breeders, with breeding programs established in the 1920-30s (Hondelmann 1984). While domesticated European *L. luteus* germplasm has many of the requisite Mediterranean ideotype characters such as high biomass, early phenology, large seed size, high early vigour and rapid growth rates (Berger et al. 2008), it appears to be less drought tolerant than *L. angustifolius* (Palta et al. 2004). Reduced drought tolerance may explain the earlier phenology of *L. luteus* when compared to *L. angustifolius* from similar habitats, and should be tested experimentally.

The other important difference between the 2 species is their response to vernalisation. In *L. luteus* there were ecotypic differences in vernalisation response ( $P<0.001$ ), whereby germplasm from cool, relatively elevated sites (Cluster 2) was more responsive than that from terminally droughted areas (Cluster 3), which in turn was more responsive than domesticated European material (Cluster 1) (Fig. 1). These differences are likely to be the result of both natural and human selection pressure-avoiding early cold in the first instance, and selection for early maturity in the second (Berger et al. 2008). By contrast, in *L. angustifolius* there were no ecotypic differences in vernalisation response ( $P=0.36$ ), as indicated by the parallel arrangement between clusters in Fig. 2a. This does not imply that all *L. angustifolius* genotypes were vernalisation responsive, merely that the differences were averaged out between ecotypes. In fact, this study identified vernalisation responsive *and* thermoneutral genotypes in both species, in a range of

flowering time backgrounds (data not presented). Later flowering thermoneutral *L. angustifolius* genotypes are of particular interest to the Western Australian breeding program because this combination is absent among Australian cultivars, and likely to be responsible for the current lack of specific adaptation.

## Conclusions

**General:** selecting germplasm on the basis of habitat differences is a useful methodology for investigating adaptive strategies and identifying promising genotypes.

**Reproductive chilling tolerance in chickpea:** while the work has demonstrated that chickpea is even more sensitive to chilling temperatures than previously indicated, the evaluation temperatures were too high to adequately screen for chilling tolerance <14-16°C. Significant chilling sensitivities between germplasm from contrasting cool and warm flowering habitats observed in the Indian trial need to be confirmed at lower flowering temperatures.

**Lupin-specific adaptation to stress gradients:** this study has clearly demonstrated ecotypic differences in phenology which are associated with differences in vernalisation response in *L. luteus* but not in *L. angustifolius*. In order for the species' to maximise their potential as grain legume crops in Australia, cultivar phenology should similarly be matched to target environments. Currently this is not possible because later flowering varieties such as Geebung are vernalisation responsive, and grow slowly until their vernalisation requirements are met. As a consequence these genotypes do not produce more biomass even in long season locations, and therefore later phenology is no advantage. Later flowering, fast growing thermoneutral genotypes identified in the present study may add the necessary diversity to Australian breeding programs required to produce specifically adapted varieties for longer season environments.

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