# Wild and cultivated *Cicer* species- different evolutionary paths lead to different phenological strategies that can be exploited to broaden the adaptation of chickpea (*C. arietinum* L.)

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### Abstract

In contrast to its annual, cool-season wild relatives, the chickpea (*Cicer arietinum*) developed as a postrainy season, spring-sown crop early in its evolution. We suggest that these different life cycles imposed different selection pressures on the wild and cultivated *Cicer* species, and that as a result different phenological strategies are likely to be expressed. To test this hypothesis, diverse wild and cultivated *Cicer* species from a wide range of habitats were subjected to different cold treatments, and evaluated in the field. In terms of days to flowering, *C. arietinum, C. yamashitae*, and most *C. judaicum* accessions were unresponsive to vernalization, whereas the opposite was the case for *C. echinospermum* and *C. pinnatifidum. C. bijugum* and *C. reticulatum* were intermediate, with around 50% of accessions vernalization responsive. Since both *C. echinospermum* and *C. reticulatum* are commonly used in chickpea improvement programs this has important ramifications for breeders. The time interval between flowering and podding was significantly smaller in wild *Cicer* species (mean: 5-7 days) than in the cultigen (mean: 14 days), and this may be a reflection of reduced susceptibility to cold temperatures in the wild species, a trait urgently required in chickpea.

#### Media summary

Flowering and podding responses to cold temperatures were found to be different in wild and cultivated *Cicer* species, and these differences may help broaden the adaptability of chickpea.

# **Key Words**

Chickpea, wild Cicer, vernalization, phenology

#### Introduction

Chickpea (Cicer arietinum L.) is a member of the West Asian Neolithic crop assemblage, having been domesticated some 10,000 years ago alongside other pulses such as pea and lentil, as well as cereals such as barley, einkorn and emmer wheat (Abbo et al. 2003). Since its domestication from the narrowly distributed Eastern Anatolian wild progenitor (C. reticulatum Ladiz.), the chickpea has been disseminated widely, and now ranks second among the world's food legumes in terms of area, being grown over 9.9 million ha on all continents except Antarctica (FAO 2004). The evolutionary history of the chickpea is somewhat different from the other members of the West Asian Neolithic crop assemblage (Abbo et al. 2003), being punctuated by a series of bottlenecks which have narrowed the genetic base, and placed important limitations on where the crop can be grown. Perhaps the most significant evolutionary bottleneck was the change from autumn to spring sowing, a management technique employed by Early Bronze Age farmers to minimize the probability of Ascochyta blight (Ascochyta rabiei Pass. Labr.) epidemics. By sowing in the Mediterranean spring, cold winters were avoided, and chickpea effectively became a post-rainy season crop, facilitating its distribution to sub-tropical regions such as India (which accounts for 75% of global production), where it is grown on post-monsoonal residual soil moisture, with very little Ascochyta disease pressure, and relatively warm conditions during pod fill. However, under Mediterranean environments there is a considerable yield penalty associated with spring-sowing as the crop is forced to mature under terminal drought, and therefore there is a strong interest in returning chickpea to the autumn to early summer life cycle of its wild relatives (Singh et al. 1997). Given the

evolutionary history of the chickpea, this is a difficult task, requiring stable, economically-feasible management packages for controlling Ascochyta blight, and varieties that are less susceptible to low temperature stress at flowering (Croser et al. 2003).

Because the annual wild *Cicer* species have retained their autumn to summer life cycle, there is a high probability that the group retains important adaptive genes lost from cultivated chickpea. Indeed, there has been considerable interest recently in exploring the potential of the annual wild Cicer species with a view to widening the genetic base of cultivated chickpea (Robertson et al. 1997; Singh et al. 1998; Berger et al. 2003), and significant abiotic and abiotic stress resistance has been identified. However, to date few attempts have been made to investigate the characteristics of wild and cultivated Cicer species in light of their origin and lifecycle. The annual wild Cicer species are predominantly found in West and Central Asia, mostly above 34.5°N, but also along the coastal Eastern Mediterranean (C. judaicum Boissier and C. pinnatifidum Jaubert & Spach), and in isolated populations adjacent to the African Red Sea coast (C. cuneatum Hochst ex. Rich) (Berger et al. 2003). We suggest that both the origin and winter lifecycle will imbue the annual wild *Cicer* species with different adaptive traits than are found in cultivated chickpea. particularly with respect to phenology. This is important because when chickpea is grown as a cool season legume in Mediterranean-type environments it flowers up to 9-20 days later than other welladapted grain legumes such as narrow-leafed lupin, faba bean or field pea (Siddique et al. 1999). Recent genotype by environment studies in Australia have demonstrated that in cultivated chickpea early phenology is a requirement for both yield stability and specific adaptation to terminal drought (Berger et al. 2004). There is some indication that there are phenological differences between annual wild and cultivated Cicer species. Abbo et al. (2002) report a vernalization response in a single accession of C. reticulatum, whereas this trait appears to be absent in cultivated chickpea (Summerfield et al. 1989). Robertson et al. (1997) list a range of flowering times for the annual wild Cicer species, but make no attempt to explain these in the light of origin or life cycle.

#### Methods

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Table 1. Number and latitudinal range of wild and cultivated *Cicer* germplasm evaluated.

Species	(n)	Latitude range
C. arietinum L.	27	8.7-40.0
C. bijugum Rech.	14	32.7-38.1
echinospermum P. H. Davis	7	36.9-37.9
C. judaicum Boiss.	28	31.5-38.7
pinnatifidum Jaub. & Spach	21	31.5-38.8
C. reticulatum Ladz.	9	37.3-38.1
C. yamashitae Kitamura	3	34.6-34.7
Grand Total	109	

In order to compare phenological strategies, 82 accessions of *Cicer* species collected from the wild (comprising >70% of the world's original wild collection (Berger et al. 2004)) and 27 chickpea landraces, breeding lines and released cultivars from a wide range of habitats (Table 1) were subjected to different cold treatments, and evaluated in the field in a randomised complete block design using 5 replications of single plants.

All seeds were surface sterilized and placed on moist filter paper in sealed petri dishes. 1 ml of P-Pickle T<sup>?</sup> (a mixture of thiram and thiabendazole) was added to the filter papers to minimize the risk of fungal infection. Seeds were vernalized by storing the covered petri dishes at in a dark cool-room at 4°C for 30 days. Control seeds were germinated in the dark at room temperature two days prior to the end of vernalization to minimize developmental differences between the two treatment groups. Subsequently the seeds were scarified if unimbibed, and sown into seedling trays. *Rhizobium* inoculation was performed by irrigation. The trial was sown on the 4<sup>th</sup> July as single, spaced plants on plastic sheeting under bird netting in a deep, alkaline sand. Prior to flowering all plants were individually fertilized using a general purpose fertilizer (Garden Gold<sup>?</sup>). From October onwards the trial was watered on a weekly basis.

Phenological data recorded included emergence, 1<sup>st</sup> flowering, 1<sup>st</sup> podding, end of flowering, and maturity. ANOVA was performed with cold treatment and species as main effects. Accessions were treated as random effects, and nested within species.

#### Results

For 1<sup>st</sup> flowering, interactions were highly significant (P<0.001), reflecting different responses to vernalization between and within species. *C. arietinum, C. yamashitae*, and most *C. judaicum* accessions were unresponsive to vernalization (Fig. 1). Most *C. echinospermum* and *C. pinnatifidum* accessions responded positively to vernalization, and these species flowered 18 and 20 days earlier on average respectively, when subjected to cold treatment. Approximately half of the *C. bijugum* and *C. reticulatum* accessions responded to vernalization (Fig. 1). The effect of vernalization on podding date was very similar, differences between control and vernalized accessions being highly correlated for these two traits (r = 0.92, P<0.001). Vernalization also had a strong effect on maturity date, particularly on *C. echinospermum* and *C. pinnatifidum* (data not presented).



# Figure 1. Days to 1<sup>st</sup> flowering in control versus vernalized annual wild and cultivated *Cicer* species.

Vernalization advanced flowering in responsive wild *Cicer* genotypes to the point where there was considerable overlap with cultivated chickpea (Fig. 1), making it possible to directly compare the progress from flowering to podding. In *C. arietinum* flowering time played a very strong role in determining the flower-pod interval (r = -0.87), such that the onset of podding occurred almost 25 days after flowering in the earliest flowering accessions, but less than 10 days in the latest germplasm (Fig. 2). With the exception of *C. reticulatum*, there was no such relationship among the wild *Cicer* species, and podding generally took place within three to 10 days after 1<sup>st</sup> flowering. Importantly, relatively early accessions of *C. reticulatum, C. judaicum and C. pinnatifidum* (flowering < 95 DAS) which can switch from flowering to podding far more rapidly than *C. arietinum* of equivalent phenology, were identified (Fig. 2). In the Mediterranean context the capacity of chickpea to set pods early is very poor compared to well-adapted cool-season grain legumes (Siddique et al. 1999), and the introgression of this character should help to stabilize yield and improve productivity under terminal drought. There are only 116 original annual wild *Cicer* accessions across eight species in the entire world collection (Berger et al. 2003), so there is a pressing need for more collection, particularly of early material.

# Conclusions

- There is clear evidence of different phenological strategies between wild and cultivated *Cicer* species. In the cultigen, early phenology, the lack of a vernalization response, and the inability to set pods early under cool conditions may be a consequence of the Early Bronze Age shift from autumn to spring sowing (Abbo et al. 2003).
- In the wild *Cicer* species there is variation within and between species for vernalization responsiveness and other phenological characters which can be exploited by chickpea breeding programs.
- Inter-specific hybrids produced by breeding programs should be screened for vernalization responsiveness, as this character can have both negative and positive ramifications in the Australian chickpea growing regions.



Figure 2. The time interval between podding and flowering for vernalized wild and cultivated chickpea versus flowering date. Linear regressions for *C. arietinum* (−) and *C. reticulatum* (--) were

highly significant (P<0.001 and P<0.05, respectively). Mean daily temperatures over the flowering period are shown.

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