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Vernalization and Photoperiod Response in Annual Wild *Cicer* Species and Cultivated Chickpea

Shivali Sharma and Hari D. Upadhyaya*

ABSTRACT

Wild *Cicer* species possess high levels of resistance to many stresses and can contribute to enhance levels of resistances besides broadening the genetic base of cultivated chickpea (*Cicer arietinum* L.). However, longer duration of wild *Cicer* species and nonsynchronization of flowering with cultigen remains a major deterrence for their use in chickpea improvement. In this study, the response to vernalization (*V*), photoperiod (*P*), and combination of both (*V* + *P*) was studied in terms of rate of progress toward flowering in cultivated and wild *Cicer* species belonging to primary, secondary, and tertiary gene pools. Both *V* and *P* treatments, alone and in combination, significantly increased the rate of progress toward flowering in wild *Cicer* species compared with control. Effect of *P* in increasing the rate of progress toward flowering was significantly higher than *V* in *C. reticulatum* Ladiz., *C. echinospermum* P. H. Davis, *C. judaicum* Boiss., *C. pinnatifidum* Jaub. & Spach, *C. bijugum* Rech. f., and *C. yamashitae* Kitam. and vice versa in *C. chorassanicum* (Bunge) Popov and *C. cuneatum* Hochst. ex A. Rich. Synergistic effects of *V* and *P* were observed in *C. bijugum*, *C. yamashitae*, *C. chorassanicum*, and *C. cuneatum*. However, both *V* and *P* treatments had minimal effect on the rate of progress toward flowering in early-maturing and greater effects on medium- and late-maturing chickpea varieties. These results would contribute significantly to enhance use of wild *Cicer* species for chickpea improvement through synchronization of flowering facilitating hybridization. Also, it would improve the regeneration efficiency of wild *Cicer* species by genebanks and offer convenient alternate methods for rapid generation turnover.

International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Patancheru, 502324, Hyderabad, India. Received 2 Sept. 2014. Accepted 2 May 2015. *Corresponding author (h.upadhyaya@cgiar.org).

Abbreviations: *C*, control; CRD, completely randomized design; *P*, photoperiod; *V* + *P*, vernalization + photoperiod; *V*, vernalization.

Chickpea is an important cool-season pulse crop that ranks second in production among food grain legumes in the world after common bean (*Phaseolus vulgaris* L.) (FAOSTAT, 2013). It is grown in a wide range of environments in over 50 countries in subtropical and temperate regions of the world but mainly in the Indian subcontinent, West Asia, North Africa, the Americas, and Australia (FAOSTAT, 2013) for its protein-rich seeds.

In spite of large breeding efforts for chickpea genetic improvement, its global production (13.12 Tg) and productivity (966.7 kg ha^{-1}) continues to be low (FAOSTAT, 2013). Chickpea yields are constrained by many biotic and abiotic stresses coupled with its cultivation in marginal lands under rainfed conditions (Kumar and van Rheenen, 2000) and has little useful variation to deal with these constraints because of limited diversity as a result of evolutionary bottlenecks (Abbo et al., 2003a).

Considerable genetic diversity exists in the genus *Cicer*, which contains 44 species classified into three gene pools following the Harlan and de Wet (1971) gene pool concept with cultivated chickpea, its landraces and wild progenitor, *C. reticulatum* in primary, *C. echinospermum* in secondary, and remaining six wild annuals and 35 perennial species in tertiary gene pools. In contrast to cultivated chickpea, wild *Cicer* species have more generic and adaptive diversity, particularly for important biotic

Published in Crop Sci. 55:1–8 (2015).
doi: 10.2135/cropsci2014.09.0598

and abiotic stresses (Stamigna et al., 2000; Collard et al., 2001; Croser et al., 2003; Rao et al., 2003; Pande et al., 2006; Sharma et al., 2005; Toker et al., 2007; Berger et al., 2012; Canci and Toker, 2009) that chickpea is exposed to. It is difficult to cross wild *Cicer* species with cultivated chickpea in India (particularly in southern India) because of inappropriate phenology, probably as a result of a vernalization requirement (Berger et al., 2005) and possibly a daylength response. In Mediterranean environments also, wild *Cicer* tends to be a little later than cultivated chickpea, particularly when grown under nonvernalizing conditions (Berger et al., 2005). This situation is particularly exacerbated in India, especially southern India, where growing season temperatures are warm and very far from satisfying the vernalization requirement. Given that India is the world's dominant producer of chickpea and that more chickpea breeding happens in India, including at ICRISAT, Patancheru than anywhere else, this becomes a real issue that prevents base broadening of the crop.

All previous evaluations of *Cicer* phenology have either measured flowering time (Robertson et al., 1997) or the effect of vernalization on flowering time (Abbo et al., 2002; Berger et al., 2005). However, none of the previous studies have looked at daylength response in wild *Cicer* species. Therefore, the current investigation was designed to study the effects of vernalization (cold treatment) and photoperiod (extended daylength) treatments alone and in combination on rate of progress toward flowering in eight annual wild *Cicer* species and cultivated chickpea.

MATERIALS AND METHODS

Plant Material

Germplasm accessions of eight annual wild *Cicer* species belonging to primary gene pool (*C. reticulatum*), secondary gene pool (*C. echinospermum*) and tertiary gene pool (*C. judaicum*, *C. bijugum*, *C. pinnatifidum*, *C. chorassanicum*, *C. cuneatum*, *C. yamashitae*) were used in this investigation. These germplasm accessions have been collected from or originated in seven countries (Table 1). Besides these, cultivated chickpea varieties belonging to desi (JG 11, ICCV 10, and G 130) and kabuli (ICCV 2, KAK 2, and L 550) types were also used in the present study (Table 1). These varieties are extensively cultivated in India. JG 11 (Dattatri et al., 2010), ICCV 10 (Gowda et al., 1995), and G 130 (Singh, 1987) are early-, medium-, and late-flowering high-yielding desi-type varieties and ICCV 2 (Kumar et al., 1985), KAK 2 (Zope et al., 2002), and L 550 (Dua et al., 2001) are early-, medium-, and late-flowering high-yielding kabuli-type varieties, respectively.

Table 1. List of eight annual wild and cultivated *Cicer* species and their accessions used in study at ICRISAT, Patancheru, India.

Species	No. of accessions	Accession identity	Country of origin or pedigree
C. reticulatum	4	ICC 17123	Turkey
		ICC 17124	Turkey
		ICC 17163	Turkey
		ICC 17164	Turkey
C. echinospermum	5	ICC 20190	Turkey
		ICC 20192	Turkey
		ICC 20218	Turkey
		ICC 20244	Turkey
		ICC 20257	Turkey
C. judaicum	7	ICC 17148	Lebanon
		ICC 17149	Israel
		ICC 17188	Syria
		ICC 17204	India
		ICC 17271	Lebanon
		ICC 17274	Syria
		ICC 17316	Ethiopia
C. bijugum	3	ICC 17156	Turkey
		ICC 17187	Syria
		ICC 17289	Turkey
C. pinnatifidum	3	ICC 17200	Syria
		ICC 17269	Turkey
		ICC 17276	Syria
C. chorassanicum	1	ICC 17141	Afghanistan
C. cuneatum	4	ICC 17162	Ethiopia
		ICC 20175	Ethiopia
		ICC 20176	Ethiopia
		ICC 20215	Ethiopia
C. yamashitae	2	ICC 17117	Afghanistan
		ICC 17281	Afghanistan
C. arietinum	6	JG 11 (Desi early)	India/(Phule G 5 × Narsinghpur bold) × ICCC 37
		ICCV 10 (Desi medium)	India/P 1231 × P 1265
		G 130 (Desi late)	India/708 × C 235
		ICCV 2 (Kabuli early)	India/F_3 [(K 850 × GW 5/7) × P 458] × F_3 (L 550 × Guamuchil)-2v
		KAK 2 (Kabuli medium)	India/(ICCV 2 × Surutato 77) × ICC 7344
		L 550 (Kabuli late)	India/Pb 7 × Rabat

Methodology

The study was performed under controlled greenhouse conditions maintained at 22°C air temperature in 2011 and 2012. In 2011, 24 accessions belonging to seven wild *Cicer* species and six chickpea varieties were evaluated under three treatments: vernalization (cold treatment at 4°C; abbreviated as *V*), photoperiod (natural daylength extended artificially up to 24 h; abbreviated as *P*), and control (without cold and under 11–12 h natural daylength at 22°C air temperature; abbreviated as *C*). In 2012, 29 accessions belonging to eight wild *Cicer* species with six chickpea varieties were evaluated under four treatments: *V*, *P*, *V* + *P*, and *C*. Overall, 2011 looked at the main effects of *V* and *P* against *C* without combination, while 2012 was a full factorial.

Seed Germination

For *V* treatment, the protocol suggested by Abbo et al. (2002) was used. To initiate the germination, the seeds of wild *Cicer* species were scarified by incising the hard seed coat. Scarified

seeds of wild *Cicer* accessions and nonscarified seeds of chickpea varieties were placed on wet filter paper in Petri dishes at room temperature to imbibe water for 36 h. After imbibition, the Petri dishes were sealed with Parafilm and aluminum foil and placed in a 4°C cold chamber for 30 d for *V* treatment. At the end of the cold treatment, the Parafilm was removed and seedlings were left to harden off at room temperature for 36 h before transplanting in pots. For *P* and *C* treatments, scarified seeds of wild *Cicer* accessions and nonscarified seeds of chickpea varieties were kept for germination at room temperature 3 d before the end of *V* treatment period so as to transplant seedlings in all treatments at the same time and at approximately the same growth stage.

Transplanting and Data Recording

All the germinated seedlings were transplanted in pots (one seedling per pot) containing a 2:1:1 mixture of black soil, farmyard manure, and sand. Each treatment was performed in separate chambers in the greenhouse maintained at 22°C air temperature. The pots were randomized within each chamber. For each treatment, three plants per accession were maintained and each plant was considered as one replication. To study the effect of *V* treatment, 30-d-old vernalized seedlings were transplanted in pots and maintained under natural daylength and 22°C temperature conditions in a greenhouse chamber till maturity. For *P* treatment, the seedlings germinated at room temperature were transplanted in pots and were exposed to 24 h daylength by using 60-W incandescent lights after 15 d of transplanting till maturity (Sethi et al., 1981). The lights were installed at a height of 1.2 m following Sethi et al. (1981) and plants were maintained at 22°C temperature. For *C*, the seedlings germinated at room temperature were transplanted in pots and were maintained under natural daylength (11–12 h) and at 22°C temperature in the greenhouse. In 2012, to study the effect of *V* + *P* treatment, 30-d-old vernalized seedlings were transplanted in pots, maintained at 22°C temperature, and were exposed to 24 h daylength after 15 d of transplanting till maturity. In both years, data were recorded for number of days to first flowering starting from the day of transplanting on individual plant in all the replications and in all treatments.

Statistical Methods

For analysis, the data on number of days to first flowering was converted into rate of progress toward flowering as 1/days to first flowering (Summerfield and Roberts, 1987). Analysis of variance (ANOVA) was performed for completely randomized design (CRD) with three replications using 30 accessions belonging to eight *Cicer* species and three treatments (*V*, *P*, and *C*) in 2011 and using 35 accessions belonging to nine *Cicer* species and four treatments (*V*, *P*, *V* + *P*, and *C*) in 2012. The replicate-wise values of rate of progress toward flowering were used for statistical analysis individually for both the years. For pooled analysis of variance (ANOVA), data on rate of progress toward flowering on 30 accessions belonging to eight *Cicer* species and three treatments (*V*, *P*, and *C*) was used. The data were analyzed as a CRD and ANOVA for various factors and their interactions was obtained using GenStat 12.1 (VSN International, 2009). The accessions were nested within species (species/accessions). The sum of squares due to treatments and the interactions treatment × species and treatment × species/accession were partitioned into their components. The significance of differences within and between species, species/accessions, treatment, and interaction means were tested by using respective least significant differences.

Table 2. Analysis of variance (ANOVA) for rate of progress toward flowering in annual wild and cultivated *Cicer* accessions under different treatments in 2012, ICRISAT, Patancheru, India.

Source of variation	Degrees of freedom	Mean squares	*F* probability
Treatment	3	1.00×10^{-2}	<0.001
Vernalization	1	1.06×10^{-2}	<0.001
Photoperiod	1	1.61×10^{-2}	<0.001
Vernalization + photoperiod	1	3.37×10^{-3}	<0.001
Species	8	9.58×10^{-4}	<0.001
Treatment × species	24	2.48×10^{-4}	<0.001
Vernalization × species	8	2.59×10^{-4}	<0.001
Photoperiod × species	8	2.80×10^{-4}	<0.001
Vernalization + photoperiod × species	8	2.05×10^{-4}	<0.001
Species/accession	26	7.75×10^{-5}	<0.001
Treatment × species/accession†	78	1.30×10^{-5}	<0.001
Vernalization × species/accession	26	9.21×10^{-6}	<0.001
Photoperiod × species/accession	26	1.95×10^{-5}	<0.001
Vernalization × photoperiod × species/accession	26	1.03×10^{-5}	<0.001
Residual	278	9.23×10^{-7}	
Total	419		

† Accessions nested within species.

RESULTS

In 2012, which is the full factorial, ANOVA showed significant differences among treatments, species, species/accession (accessions nested within species) and treatment × species, and treatment × species/accession interactions ($p \leq 0.001$). Further, partitioning of the sum of squares due to treatment, treatment × species, and treatment × species/accessions revealed greater importance of *P* (contributing ~54% variation) followed by *V* (~35% variation) among main effects of treatments (Table 2). However, in treatment × species interaction, both *P* (38%) and *V* (35%) had similar importance, whereas in the treatment × species/accession interactions, *P* again showed greater importance (50%) followed by *V* + *P* (26%) and *V* (24%). The ANOVA for 2011 and pooled data also showed similar results (data not given).

Response to Treatments and Interaction

Overall, *V* and *P* treatments alone and in combination significantly increased the rate of progress toward flowering in *Cicer* species compared with *C*. The *V* + *P* (0.034) resulted in the highest rate of progress toward flowering followed by *P* (0.030), *V* (0.028), and *C* (0.012). However, the rate of progress toward flowering in wild and cultivated

Table 3. Rate of progress toward flowering in wild and cultivated *Cicer* species under different treatments in 2012, ICRISAT, Patancheru, India.

Species	*V*†	*P*‡	*V* + *P*§	*C*¶
C. reticulatum	0.027	0.030	0.031	0.008
C. echinospermum	0.024	0.033	0.032	0.000
C. judaicum	0.023	0.030	0.030	0.010
C. pinnatifidum	0.026	0.031	0.032	0.012
C. bijugum	0.029	0.031	0.038	0.010
C. yamashitae	0.032	0.039	0.044	0.018
C. chorassanicum	0.040	0.019	0.043	0.014
C. cuneatum	0.027	0.017	0.033	0.009
C. arietinum	0.033	0.034	0.039	0.027
SE (±) with same level of treatment	0.0002			
SE (±) with different level of treatment	0.0004			
LSD (5%) with same level of treatment	0.0006			
LSD (5%) with different level of treatment	0.0012			
CV (%)	3.7			

† *V*, vernalization.

‡ *P*, photoperiod.

§ *V* + *P*, vernalization + photoperiod.

¶ *C*, control.

Cicer species varied to a great extent. In wild *Cicer* species, *V* + *P* (0.035) increased the rate of progress toward flowering to the greatest extent (250%) followed by 190% increase both under *P* (0.029) and *V* (0.029) when compared with *C* (0.010), whereas in cultivated chickpea, *V* + *P* (0.039) increased the rate of progress toward flowering only by ~40% followed by ~25% increase under *P* (0.034) and ~20% increase under *V* (0.033) when compared with *C* (0.027) (Table 3). Further, the effect of *V* + *P*, *V*, and *P* treatments on the rate of progress toward flowering varied significantly between and within *Cicer* species, and different responses in terms of increase in the rate of progress toward flowering were observed for different species or accessions within species (Table 3; Fig. 1).

Between Species Response

Both *V* + *P* and *P* were similar and resulted in the highest rate of progress toward flowering, which was significantly higher than *V* and *C* in the primary gene pool species *C. reticulatum* (0.031 under *V* + *P*, 0.030 under *P*, 0.027 under *V*, and 0.008 under C), secondary gene pool species *C echinospermum* (0.032 under *V* + *P*, 0.033 under *P*, 0.024 under *V*, and 0.000 under C), tertiary gene pool species *C. judaicum* (0.030 both under *V* + *P* and *P*, 0.023 under *V*, and 0.010 under *C*), and *C. pinnatifidum* (0.032 under *V* + *P*, 0.031 under *P*, 0.026 under *V*, and 0.012 under *C*) (Table 3). The *V* + *P* treatment resulted in significantly high rate of progress toward flowering followed by *P*, *V*, and *C* in tertiary gene pool species *C. bijugum* and *C. yamashitae* (Table 3). The *V* + *P* treatment had significantly higher effect in increasing the rate of progress toward flowering followed by *V* and *P* in the remaining two tertiary gene pool species *C. chorassanicum* and *C. cuneatum*. In cultivated chickpea, *V* + *P* (0.039) had significantly greater effect in increasing the rate of progress toward flowering followed by *V* (0.033), *P* (0.034), and *C* (0.027) (Table 3).

In 2011, when *V* + *P* was not included, both *V* and *P* treatments had similar effects in increasing the rate of progress toward flowering in *Cicer* species as observed in 2012 (data not given). Overall, in 2011 and pooled, both *V* and *P* were effective in accelerating the rate of progress toward flowering in wild and cultivated *Cicer* species. Overall, *P* had significantly higher effect in accelerating the rate of progress toward flowering followed by *V* in *C. reticulatum*, *C. judaicum*, *C. pinnatifidum*, *C. bijugum*, and *C. yamashitae* and vice versa in *C. chorassanicum* and *C. cuneatum*. Further, both *V* and *P* were similar in accelerating the rate of progress toward flowering in *C. arietinum*.

Within Species Response

Treatments *V* + *P*, *V*, and *P* increased the rate of progress toward flowering significantly compared with *C*, although to a varied extent within wild *Cicer* species except in *C. judaicum* (Fig. 1). Both *V* + *P* and *P* were similar in increasing the rate of progress toward flowering and were significantly better than *V* in all accessions of *C. judaicum*, however, the extent of increase varied between accessions (Fig. 1). Likewise, both *V* + *P* and *P* were similar but significantly better than *V* in increasing the rate of progress toward flowering in one of the three *C. reticulatum* accessions, ICC 17123, while *V* + *P* was significantly better than *V* and *P*, both having similar effects in ICC 17124 and ICC 17163. Among the five accessions of *C. echinospermum*, effects of both *V* + *P* and *P* were similar but significantly greater than *V* in increasing the rate of progress toward flowering in three accessions: ICC 20190, ICC 20192, and ICC 20218 (Fig. 1). The effect of *P* was significantly higher in increasing the rate of progress toward flowering in ICC 20244 followed by *V* + *P* and *V*, whereas *V* + *P* resulted in significantly higher rate of progress toward flowering followed by similar effects of both *V* and *P* in ICC 20257. Within *C. pinnatifidum*, both *V* + *P* and *P* were similar but significantly higher than *V* in increasing the rate of progress toward flowering in two accessions, ICC 17269 and ICC 17276, whereas *V* + *P* resulted in significantly higher rate of progress toward flowering followed by *P*, and *V* in ICC 17200 (Fig. 1). Among *C. bijugum*, *V* + *P* resulted in the highest rate of progress toward flowering in all three accessions (ICC 17187, ICC 17289, and ICC 17156), but differences among accessions were observed for their response to *P* and *V*. Treatment *P* was significantly better in increasing the rate of progress toward flowering followed by *V* in two accessions, ICC 17187 and ICC 17289, whereas both *P* and *V*

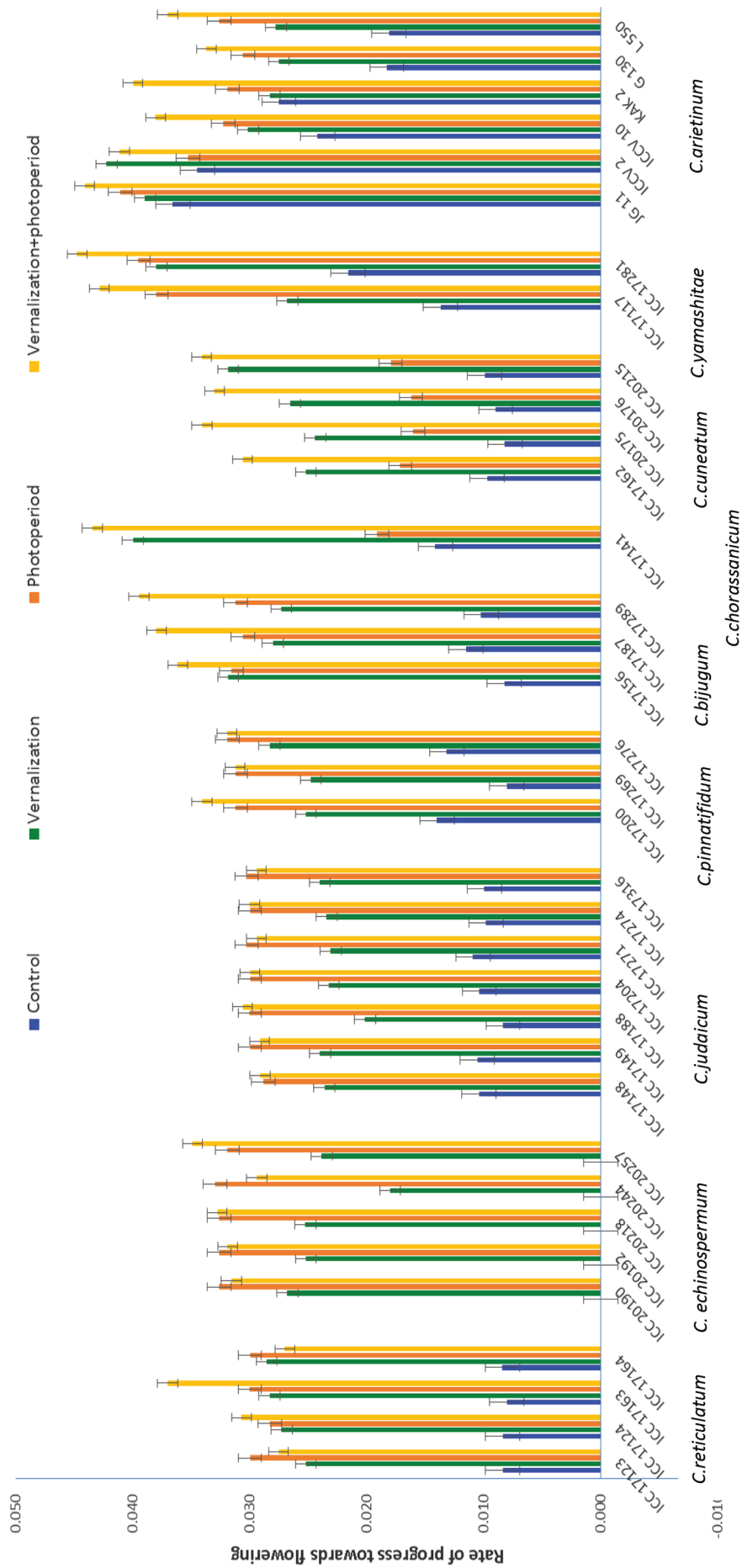

Figure 1. Rate of progress toward flowering in eight annual wild and cultivated *Cicer* species under different treatments at ICRISAT, Patancheru, India.

were similar in ICC 17156 (Fig. 1). In *C. yamashitae*, *V* + *P* was significantly better in increasing the rate of progress toward flowering in both the accessions, ICC 17117, and ICC 17281 but *P* and *V* showed the differential effects with *P* being significantly better than *V* in ICC 17117 whereas both were similar in ICC 17281 (Fig. 1). Conversely, *V* + *P* was significantly better in increasing the rate of progress toward flowering followed by *V* and *P* in three *C. cuneatum* accessions (ICC 17162, ICC 20175, and ICC 20176), whereas *V* + *P* and *V* were similar but greater than *P* in ICC 20215 (Fig. 1). Similarly, *V* + *P* was significantly better in increasing the rate of progress toward flowering followed by *V* and *P* in single accession of C. *chorassanicum* (ICC 17141). Within *C. arietinum*, *V* and *P* treatments alone and in combination had no or minimal effect in increasing the rate of progress toward flowering when compared with *C* in early-maturing chickpea varieties, JG 11 and ICCV 2, while *V* + *P* significantly increased the rate of progress toward flowering followed by equal but high response under *V* and *P* compared with *C* in ICCV 10 (Fig. 1). The *V* + *P* treatment was also effective in increasing the rate of progress toward flowering in late-maturing chickpea varieties G 130 and L 550 followed by *P* and *V* compared with *C* (Fig. 1).

DISCUSSION

Because of the narrow genetic base of most of the crop cultivars, there are considerable research efforts to exploit the potential of wild species for cultivar improvement in recent years. Wild *Cicer* species are the sources for many useful genes and hold a great potential for the genetic upgradation of cultivated chickpea. The annual wild *Cicer* species are predominantly found in western and central Asia, mostly above 34.5°N, as well as along the coastal eastern Mediterranean (*C. judaicum* and *C. pinnatifidum*) and in isolated populations adjacent to the African Red Sea coast (*C. cuneatum*) (Berger et al., 2003), whereas chickpea is cultivated in tropical, subtropical, and warm temperate zones including the Mediterranean, the Canary Islands, western and central Asia, and northeastern tropical Africa including Madagascar. Under the natural field conditions in subtropical regions such as India, the wild *Cicer* species are generally late in phenology and, therefore, cannot be used frequently in crossing program for chickpea improvement. A few studies have shown the phenological differences between annual wild and cultivated *Cicer* species (Abbo et al., 2002; Summerfield et al., 1989; Robertson et al., 1997; Berger et al., 2005). The present study is a comprehensive attempt to study the effect of *V* and *P* individually and in combination on rate of progress toward flowering in the accessions belonging to eight annual wild *Cicer* species and six cultivated chickpea varieties of different maturity durations. All the wild *Cicer* accessions responded to *V*, *P*, and *V* + *P* treatments by increasing the rate of progress toward flowering to a greater extent compared with *C*. These results show that these accessions carry vernalization- and photoperiod-responsive genes or alleles and use of these treatments alone or in combination would help in synchronizing the flowering between cultivated and wild *Cicer* species, thus enhancing the use of wild species in cultivar improvement.

In general, all the eight annual wild *Cicer* species responded to *P* and *V*, which could be linked with the geographic origin and distribution of these species. Significant effects of germplasm origin on temperature and daylength responsiveness have also been studied in chickpea genotypes of diverse origin (Berger et al., 2011). These species are primarily found in western to central Asia with average elevation ranging from 322 to 2830 m and winter mean temperature ranging from −7.5 to 17.4°C. In such locations, these species are most likely to be exposed to vernalization temperatures during winter under natural conditions (Berger et al., 2003). Particularly, *C. cuneatum* is much more responsive to *V* treatment. The ecology of its origin explains this response as this species is found in areas above 2400 m elevation with low temperature (7.4°C) during pod set and are therefore exposed to vernalization under natural conditions (Berger et al., 2003). Similarly, *C. chorassanicum* is also found over 2800 m elevation with <4°C temperature during pod set and vernalization requirement of this species is evident from the response in present study. It was also observed that species that are known to originate from lower elevations, such as *C. reticulatum* and *C. echinospermum* (both around 966 m mean elevation), *C. judaicum* (322 m mean elevation), *C. pinnatifidum* (935 m mean elevation), and *C. bijugum* (957 m mean elevation), were more responsive to *P*, which had increased the rate of progress toward flowering in these species by more than three-fold compared with *C* (Table 3; Fig. 1).

It is evident from the study that the contribution of *P*—either as a main effect (54%), in interaction with species (38%), or with species/accessions (50%)—was maximum, followed by *V* (35% as a main effect as well as in interaction with species) and then by *V* + *P* (11% as a main effect, 28% in interaction with species); however, the contribution of *V* + *P* (26%) in interaction with species/accessions was almost similar to *V* (24%). This is also reflected in higher rate of progress toward flowering in most of the *Cicer* species under *P*. From this study, it is also evident that the major differences for response to *V*, *P*, and *V* + *P* were largely between species; however, the response varied to some extent within accessions in different wild *Cicer* species (Table 2; Fig. 1). The differential response of cultivated chickpea varieties to *V*, *P*, and *V* + *P* treatments was due to their wide range of flowering. In cultivated chickpea varieties, the rate of progress toward flowering under *V*, *P*, and *V* + *P* treatments was increased to a minimal extent when compared with *C* in early-maturing chickpea varieties but

to a greater extent in medium- and late-maturing varieties that further followed the pattern of wild annuals. Among wild *Cicer* species, accessions belonging to primary gene pool species *C. reticulatum* seem to carry both vernalization- and photoperiod-responsive genes with preponderance of the latter. *C. reticulatum* is the most probable progenitor of cultivated chickpea and has also been reported to possess *V* sensitivity; however, *V* response was found absent in cultivated chickpea in earlier studies (Summerfield et al., 1989; Abbo et al., 2002; Berger et al., 2005). In these studies, the authors have argued that chickpea changed from being an autumn- to spring-sown crop early in its evolution in the eastern Mediterranean and lost its vernalization response and winter hardiness in this transition and the subsequent move to southern Asia (Abbo et al., 2002, 2003a,b; Berger, 2014). However, the present study showed that the vernalization response might still exist in late-flowering germplasm, especially from northern India, though the extent of response was lower than wild *Cicer* species, whereas, it seems to have been lost in early-flowering varieties from southern India. These results might be due to the use of chickpea varieties having a wide flowering range and are in accordance with the earlier ideas that the *V* response was lost in the transition from winter to spring, north to south, and cool to warm conditions that chickpea underwent in the move from the Mediterranean to southern India. These findings also suggest that it may be worth looking at *V* response across a wide range of Indian landraces from northern to southern India to confirm these results. The genetic variation observed for *V* and *P* response in chickpea varieties of different maturity duration and in the progenitor species, *C. reticulatum* showed that *V* and *P* response in *Cicer* species is controlled by a number of major and minor genes or alleles as in temperate cereals wheat (*Triticum aestivum* L.) and barley (*Hordeum vulgare* L.) (Allard et al., 2012; Sasani et al., 2009). In the present study, it seems that *C. reticulatum* carry genes or alleles for *V* and *P* sensitivity, and during evolution of cultivated chickpea, a few alleles might have been partly retained in late-maturing chickpea varieties and lost during further selection for early maturity. This is also reflected from the change in the pattern of chickpea cultivation in India. Chickpea is a cool-season crop and is grown in diverse climatic conditions in India, predominantly in northern India. In northern India with cooler and long-season environments, chickpea is traditionally grown as a cool-season food legume, and mostly long-duration varieties are cultivated which face low temperature of 0–5°C for about 15 to 20 d (Chaturvedi et al., 2009), whereas over the last few years, in central and southern India, with warmer and short-season environments, early-maturing chickpea varieties are grown in the postrainy season. In northern India, vernalization requirement of chickpea varieties gets fulfilled under natural conditions, while further selection for early maturity might have resulted in the loss of vernalization-responsive genes; hence, these early-maturing varieties are more suitable for cultivation in warm areas. Development of these early-maturing varieties having vernalization and photoperiod insensitivity is the most important factor responsible for major shift in chickpea area from northern India (cooler, long-season environments) to southern India (warmer, short-season environments) during the past four decades.

In secondary gene pool species *C. echinospermum*, it is evident from the study that both *V* and *P* are effective; however, the role of *P* treatment is more pronounced in reducing the vegetative phase and initiate flowering (Table 2, 3). Among the eight wild *Cicer* species, *C. reticulatum* and *C. echinospermum* are cross compatible with cultivated chickpea and hold great potential for introgression of useful genes into cultivated background. While using these species for chickpea improvement, care needs to be taken to avoid unwanted introgression of vernalization- and photoperiod-responsive genes from these wild species into cultivated varieties. Among tertiary gene pool species, *C. judaicum* and *C. pinnatifidum* responded both to *V* and *P* treatments with comparatively greater effects of *P* in reducing the vegetative phase. In *C. bijugum* and *C. yamashitae*, also, *P* treatment was more effective in reducing the vegetative phase compared with *V* but showed synergistic effects when combined together. In contrast, in *C. chorassanicum* and *C. cuneatum*, the effect of *V* treatment was more pronounced in reducing the vegetative phase and initiate flowering compared with *P*, and synergistic effects of *V* and *P* were observed in both species.

Overall, it can be concluded that both vernalization and photoperiod response are present in wild *Cicer* species as well as in medium- and late-maturing chickpea varieties and exhibit differential response in different species. Photoperiod response was predominant in *C. reticulatum*, *C. echinospermum*, *C. judaicum*, *C. pinnatifidum C. bijugum*, and *C. yamashitae* as well as in medium- and late-maturing chickpea cultivars, whereas, vernalization response was predominant in *C. chorassanicum* and *C. cuneatum*. Synergistic effects of *V* and *P* were observed in *C. bijugum*, *C. yamashitae*, *C. chorassanicum*, and *C. cuneatum*. Use of the most appropriate treatment would be effective in accelerating flowering in wild *Cicer* species and enhancing regeneration efficiency of these species for efficient conservation in genebanks. However, for using wild *Cicer* species for chickpea improvement following wide hybridization, careful monitoring is needed for introgressing useful genes or alleles with or without vernalization- and photoperiod-responsive genes from wild *Cicer* species into cultivated background. Further, the genetic variability in vernalization and photoperiod responsiveness may be the attractive traits for developing cultivars for different agroclimatic regions.

Acknowledgments

This work has been undertaken as part of the CGIAR Research Program on Grain Legumes. The help extended by Dr. Murari Singh and Mr. Sube Singh for data analysis is duly acknowledged.

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