the plant journal

SEB Society for Experimental Biology

The Plant Journal (2018) **93,** 1129–1142 doi: 10.1111/tpj.13827

RESOURCE

A multi-parent advanced generation inter-cross (MAGIC) population for genetic analysis and improvement of cowpea (*Vigna unguiculata* L. Walp.)

Bao-Lam Huynh[1,*], Jeffrey D. Ehlers[2,†], Bevan Emma Huang[3], María Muñoz-Amatriaín[2], Stefano Lonardi[4], Jansen R. P. Santos[1], Arsenio Ndeve[1], Benoit J. Batieno[5], Ousmane Boukar[6], Ndiaga Cisse[7], Issa Drabo[8,‡], Christian Fatokun[9], Francis Kusi[10], Richard Y. Agyare[10], Yi-Ning Guo[2], Ira Herniter[2], Sassoum Lo[2], Steve I. Wanamaker[2], Shizhong Xu[2], Timothy J. Close[2] and Philip A. Roberts[1,*]

[1]*Department of Nematology, University of California, 900 University Avenue, Riverside, CA, 92521, USA,*
[2]*Department of Botany and Plant Sciences, University of California, 900 University Avenue, Riverside, CA, 92521, USA,*
[3]*Discovery Sciences, Janssen R&D, 329 Oyster Point Blvd, South San Francisco, CA, 94080, USA,*
[4]*Department of Computer Science and Engineering, University of California, 900 University Avenue, Riverside, CA, 92521, USA,*
[5]*Institut de l'Environnement et de Recherches Agricoles, BP 476 Ouagadougou 01, Burkina Faso,*
[6]*International Institute of Tropical Agriculture, PMB 5320, Oyo Road, Ibadan, Nigeria,*
[7]*Institut Sénégalais de Recherches Agricoles, BP 3320, Thiès, Sénégal,*
[8]*Institut de l'Environnement et de Recherches Agricoles, 01 BP 10 Koudougou 01, Burkina Faso,*
[9]*International Institute of Tropical Agriculture, Entrance Rd, Ibadan, Nigeria, and*
[10]*Savanna Agricultural Research Institute, P. O. Box TL 52, Tamale, Ghana*

Received 16 June 2017; revised 30 November 2017; accepted 3 January 2018; published online 21 January 2018.
*For correspondence (emails baolam.huynh@ucr.edu; philip.roberts@ucr.edu).
†Present address: The Bill and Melinda Gates Foundation, Seattle, WA, USA.
‡Deceased.

SUMMARY

Multi-parent advanced generation inter-cross (MAGIC) populations are an emerging type of resource for dissecting the genetic structure of traits and improving breeding populations. We developed a MAGIC population for cowpea (*Vigna unguiculata* L. Walp.) from eight founder parents. These founders were genetically diverse and carried many abiotic and biotic stress resistance, seed quality and agronomic traits relevant to cowpea improvement in the United States and sub-Saharan Africa, where cowpea is vitally important in the human diet and local economies. The eight parents were inter-crossed using structured matings to ensure that the population would have balanced representation from each parent, followed by single-seed descent, resulting in 305 F_8 recombinant inbred lines each carrying a mosaic of genome blocks contributed by all founders. This was confirmed by single nucleotide polymorphism genotyping with the Illumina Cowpea Consortium Array. These lines were on average 99.74% homozygous but also diverse in agronomic traits across environments. Quantitative trait loci (QTLs) were identified for several parental traits. Loci with major effects on photoperiod sensitivity and seed size were also verified by biparental genetic mapping. The recombination events were concentrated in telomeric regions. Due to its broad genetic base, this cowpea MAGIC population promises breakthroughs in genetic gain, QTL and gene discovery, enhancement of breeding populations and, for some lines, direct releases as new varieties.

Keywords: Legumes, cowpea, *Vigna unguiculata*, MAGIC, QTL, recombination rate, flowering, photoperiod, genetic resources.

INTRODUCTION

Cowpea (*Vigna unguiculata* L. Walp.) is a highly nutritious warm-season grain legume that is vitally important for food security in Africa, where it provides a primary source of protein that complements cereals in the diet (Ehlers and

Hall, 1997; Kudre *et al.*, 2013) and fodder for livestock. However, in the Sudano-Sahel region of West Africa typical cowpea grain yields of smallholder farmers are only 10–20% of known yield potential (Widders, 2012). Biotic stresses caused by insect pests, diseases caused by pathogens, the parasitic weed *Striga gesnerioides* and nematodes, and abiotic stresses from heat, drought and low-fertility soils are primary constraints to cowpea grain production. Many of these problems also affect cowpea production in parts of southern Europe, Asia, Australia, Latin America and the southern United States (Ehlers and Hall, 1997; Huynh *et al.*, 2013a). The development of cowpea cultivars that tolerate or resist these constraints will increase yield and reduce chemical-based crop-protection inputs and promote human and environmental health, thus directly benefitting resource-poor farmers.

The greatest opportunity to increase cowpea grain yields lies in the genetic variation within this diploid ($2n = 22$) species, as it has numerous resistance and tolerance traits to combat biotic and abiotic stresses (Huynh *et al.*, 2013b; Muchero *et al.*, 2013). Several traits have been genetically mapped using quantitative trait locus (QTL) discovery and mapping (Ouédraogo *et al.*, 2002b, 2012; Muchero *et al.*, 2010, 2011; Lucas *et al.*, 2012; Pottorff *et al.*, 2012, 2014; Huynh *et al.*, 2015, 2016). Cowpea has the capacity to produce grain under magnitudes of water stress that render comparable crops unproductive (Ewansiha and Singh, 2006), yet significant differences in drought tolerance exist among cowpea lines at different stages of growth (Watanabe *et al.*, 1997; Mai-Kodomi *et al.*, 1999a). For example, there are significant phenotypic differences in the ability to survive vegetative-stage drought stress (Mai-Kodomi *et al.*, 1999b; Muchero *et al.*, 2013), providing opportunity for cowpea breeders to incorporate early season drought tolerance into improved varieties. Among the genotypes exhibiting seedling drought tolerance, two types of responses have been observed by Mai-Kodomi *et al.* (1999a). Plants with a Type 1 response cease all growth and conserve moisture in all plant tissues, thereby allowing subsequent recovery of the entire shoot upon re-hydration. In contrast, a Type 2 response involves plants mobilizing moisture from the lower leaves to sustain the growth of new trifoliates, with rapid senescence of unifoliates at the onset of water-stress conditions. Mid- and late-season drought stresses have received considerable attention, given their negative effects on yield parameters (Hall *et al.*, 2003; Padi, 2004; Dadson *et al.*, 2005). On a physiological level, osmotic adjustment, carbon isotope discrimination, transpiration, assimilation rates and stomatal conductance in cowpea have been studied (Hussain *et al.*, 1999; Anyia and Herzog, 2004; Odoemena, 2004). In many cases, however, results were inconclusive or no meaningful differentiation between genotypes was achieved. Morphological investigations have tended to focus on root-related parameters, where genotypes were compared for rooting depth and relative root biomass (Matsui and Singh, 2003; Ogbonnaya *et al.*, 2003). Phenologically, flowering and maturation times have been investigated for drought escape strategies (Gwathmey and Hall, 1992). Early maturing varieties may be able to complete their reproductive cycle in time to escape late-season drought (Grantz and Hall, 1982; Ehlers and Hall, 1997), but such varieties are sensitive to mid-season drought (Thiaw *et al.*, 1993). Early flowering coupled with delayed leaf senescence, which later promotes survival during mid-and late-season drought, allowing plants to produce a second flush of pods, offers great potential for managing both mid- and late-season drought conditions (Gwathmey and Hall, 1992). Association mapping has identified multiple loci with pleiotropic effects on drought-related traits in cowpea across experiments in West Africa under limited water conditions (Muchero *et al.*, 2013). Because drought tolerance is a complex trait, its genetic improvement combined with selection for biotic resistance needs a systematic breeding strategy involving multiple trait donors.

The development of multi-parent advanced generation inter-cross (MAGIC) populations, a term coined by Cavanagh *et al.* (2008), provides a state-of-the-art approach to advancing plant population resources for genetic analysis and breeding. It involves inter-mating multiple elite parents for several cycles followed by single-seed descent (SSD), resulting in recombinant inbred lines (RILs) that each carry a mosaic of genome blocks contributed by all founders. The development and analysis of MAGIC populations have been undertaken in a few crops, including wheat, barley, rice and chickpea (Huang *et al.*, 2015). The goal of the current work was to develop an eight-parent MAGIC population for cowpea using founder parents that are highly diverse and carry many key traits relevant to cowpea production in the USA and sub-Saharan Africa (SSA). Here, we report the development, genetic analysis and validation of this new genetic resource using high-density marker genotyping (Muñoz-Amatriaín *et al.*, 2017). Due to its broad genetic base, the cowpea MAGIC population provides opportunities for increasing genetic gain and QTL/gene discovery in cowpea and related species.

RESULTS

MAGIC development and genotyping

A total of 305 MAGIC F_8 RILs were generated from unique eight-way crosses derived from six pedigree funnels (Figure S1 in the online Supporting Information). Genotyping with the 51 128 single nucleotide polymorphism (SNP) Illumina iSelect BeadArray resulted in 36 346 SNPs that were polymorphic between the eight parents (68.26%). Among these, 11 848 SNPs were parent-unique, in each of which

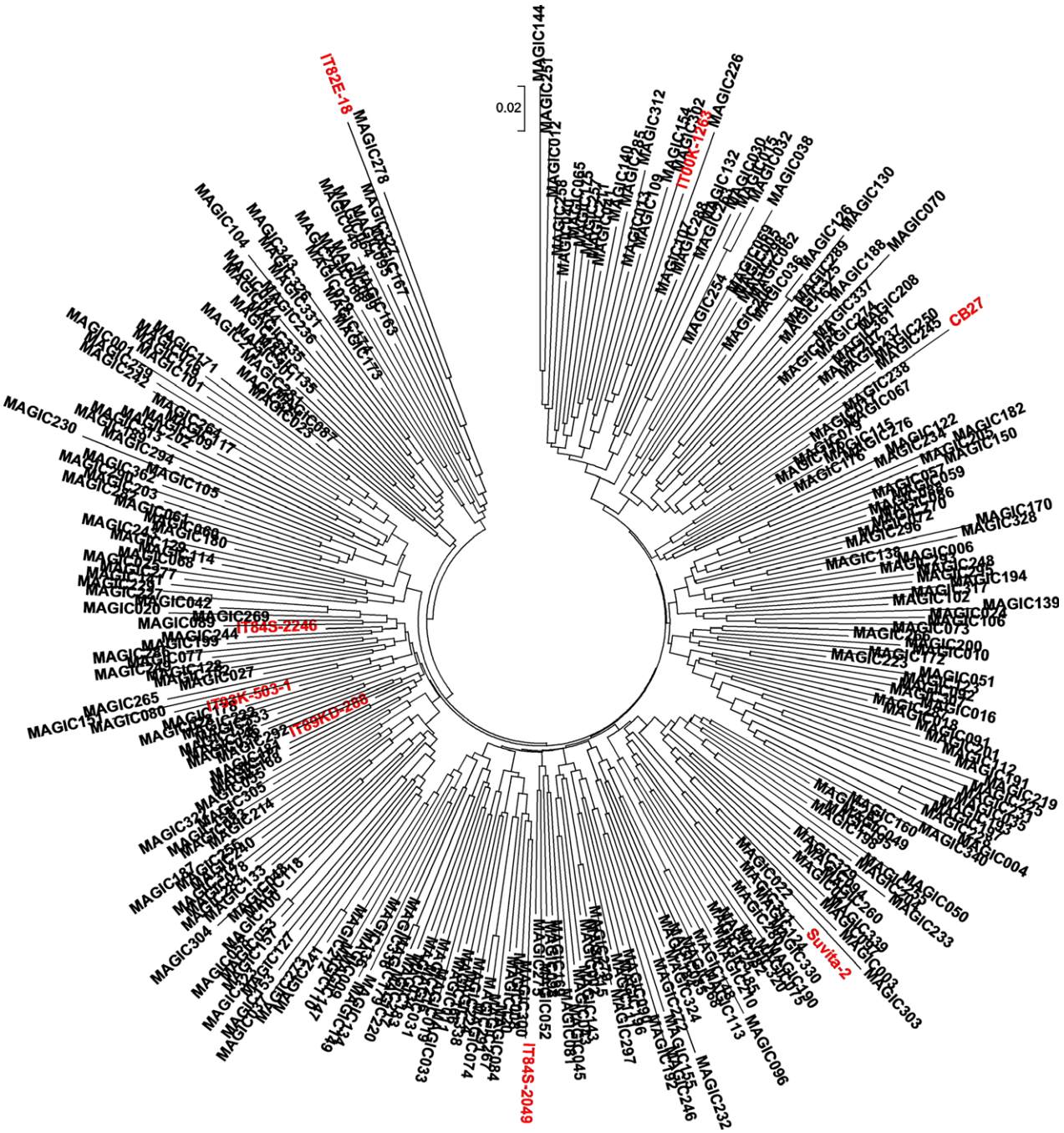

Figure 1. Phylogenetic relationships among the 305 F_8 recombinant inbred lines of the cowpea multi-parent advanced generation inter-cross (MAGIC) core set and eight parents (in red) based on 11 848 parent-unique single nucleotide polymorphisms.

one parent could be distinguished from the other seven parents. The RILs were on average 99.74% homozygous and appeared highly diverse and clustered uniformly relative to their eight parents, among which IT89KD-288, IT84S-2246 and IT97K-503-1 were closer to each other than the other parent-to-parent relationships which were dispersed throughout the population structure (Figure 1).

Variation of recombination rate in MAGIC genomes

After removing those with a minor allele frequency ≤0.05 and a successful calling rate of ≤90% from the 36 346 polymorphic SNPs, the remaining 32 130 SNPs of the 305 RILs (Data S1) with known physical positions on 11 cowpea pseudomolecules (www.phytozome.net) (Lonardi *et al.*, 2017) were used to estimate pair-wise genetic distances

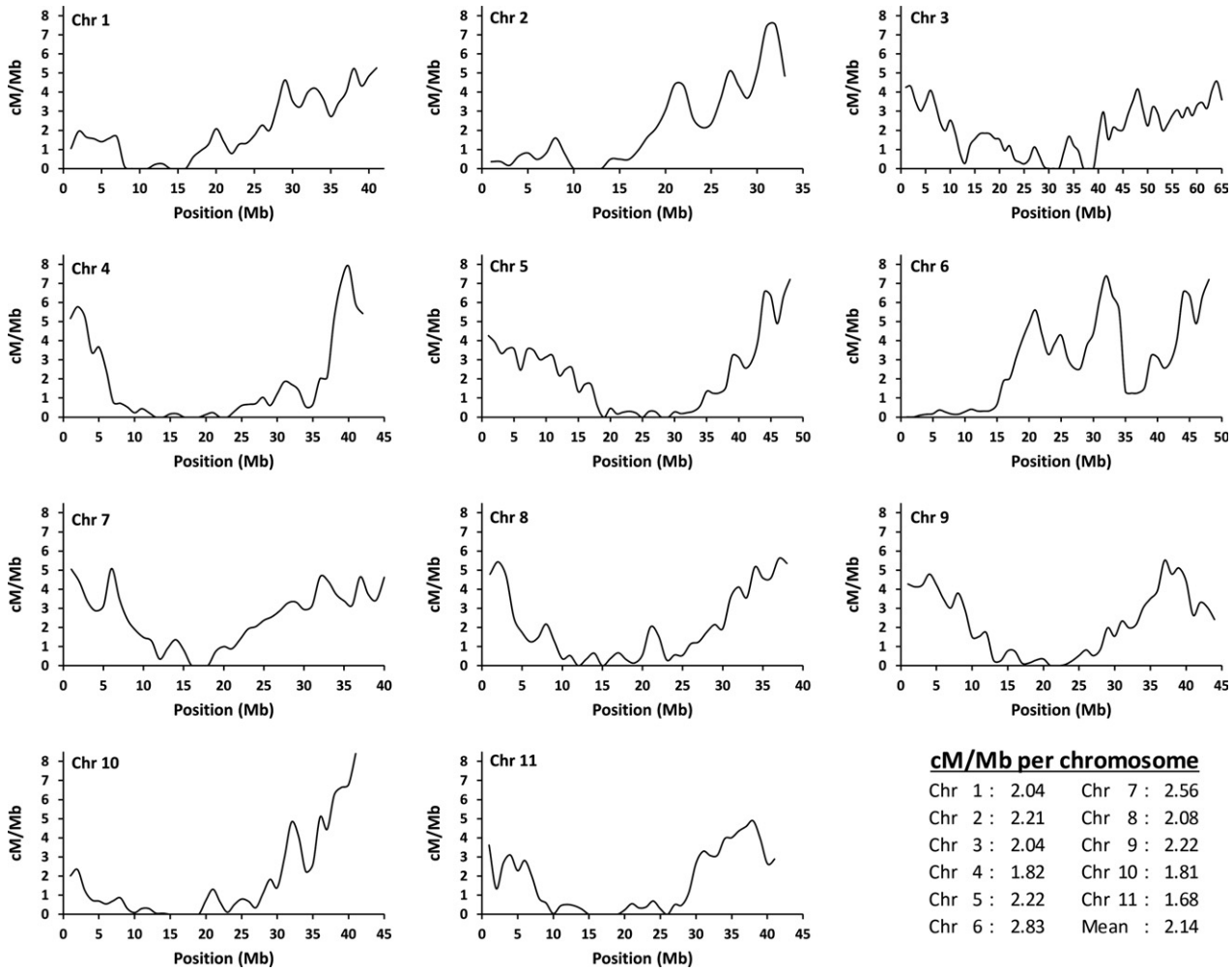

Figure 2. Recombination rate (cM Mb^{-1}) variation along 11 cowpea chromosomes measured in the eight-parent cowpea multi-parent advanced generation inter-cross population using a sliding window of 2 Mb with 1 Mb increments.

between adjacent SNPs. The crossovers appeared to be distributed throughout the MAGIC genomes at an average of 2.14 cM Mb^{-1}, and more frequently on or near the telomeric distal regions of chromosomes (Figure 2). Several recombination hot-spots with up to 8 cM Mb^{-1} were detected on the distal long arms of chromosomes 2, 4, 5, 6 and 10, while fairly large disequilibrium blocks were found on most chromosomes. On average, chromosome 6 had the highest recombination rate (2.83 cM Mb^{-1}) while chromosome 11 had the lowest (1.68 cM Mb^{-1}).

Phenotypic variation in MAGIC RILs and parents

The F_8 lines were highly diverse in morphological traits including flowering time, growth habit, flower color, leaf shape and seed characteristics (size, shape, color and texture) (Figure 3). The flowering time varied widely in the population under both long-day conditions at the University of California–Riverside (UCR) Citrus Experiment Station (UCR-CES) and short-day conditions at the Coachella Valley Agricultural Research Station (CVARS) in California (Figure 4). The genotypic differences in flowering time were quite stable across contrasting watering regimes in each day-length condition, with repeatability estimated as 0.77 and 0.71 at UCR-CES and CVARS, respectively. There was a significant correlation ($r = 0.63$, $P < 0.001$) in phenotypic ranking between the long- and short-day conditions, although the absolute flowering time varied considerably among lines. At UCR-CES (long day length), the population started flowering as early as 43 days after planting, but there were many lines with delayed flowering beyond 60 days after planting (Figures 3a and 4a). In contrast, under short day length at CVARS, the population started to flower as early as 34 days after planting and the entire population completed flowering within another month (63 days) (Figure 3b and 4b). Among the parents, CB27 (44 and 36 days) was the earliest to flower while

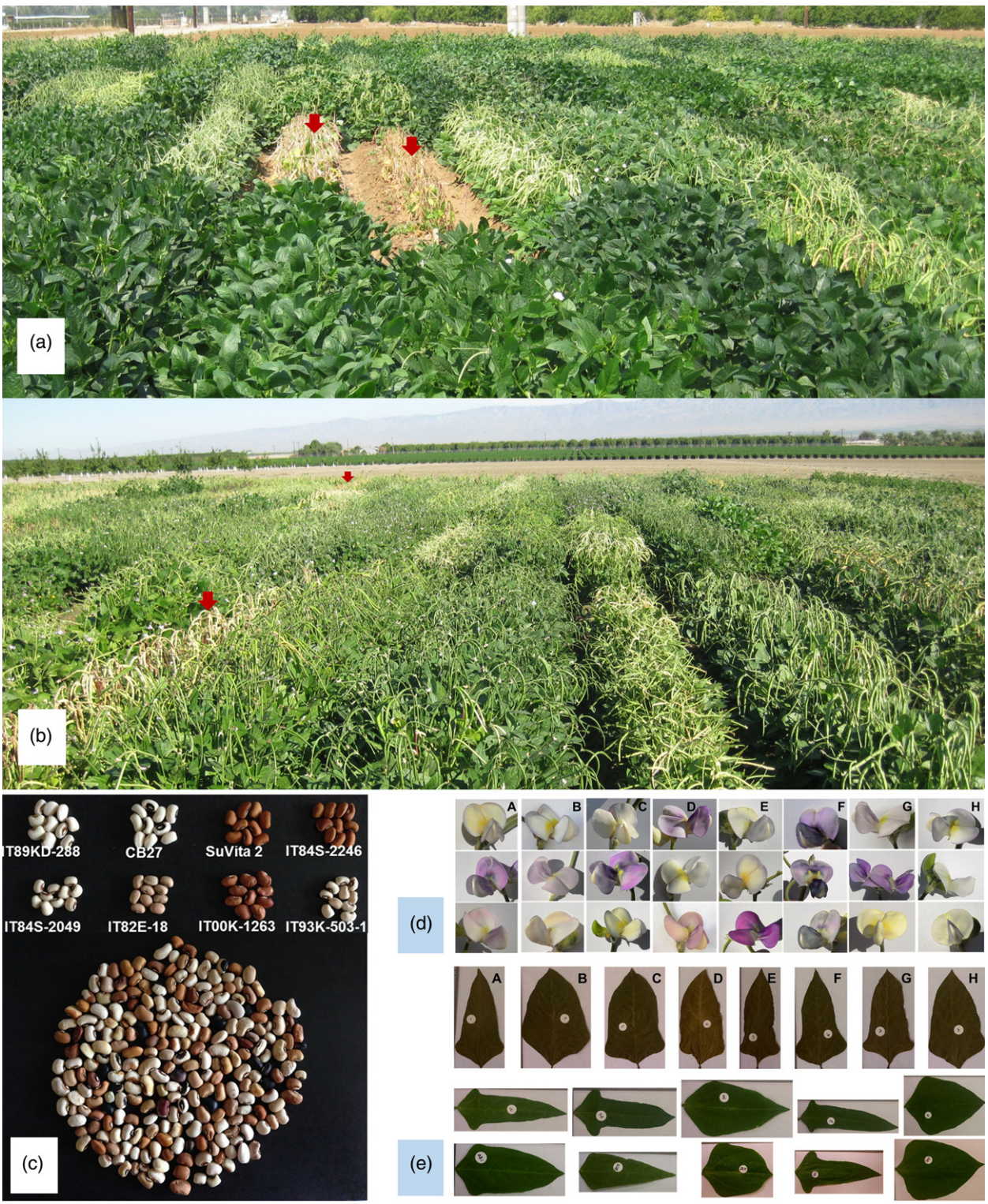

Figure 3. Morphological variation in the cowpea multi-parent advanced generation inter-cross (MAGIC) population.
Plant appearance at 65 days after planting under (a) long-day conditions at the University of California–Riverside Citrus Experiment Station in 2015 and (b) short-day conditions at the Coachella Valley Agricultural Research Station in 2016, both under full irrigation.
(c) Seed appearance, (d) flower color and (e) leaf shape of parents (top panel) and a representation of MAGIC F_8 recombinant inbred lines (RILs) (in the lower part of c, each seed is from a different F_8 RIL).
In (a) and (b) red arrows indicate examples of lines that matured earlier than other lines. In (d) and (e) parent codes are: A, IT89KD-288; B, IT84S-2049; C, CB27; D, IT82E-18; E, Suvita-2; F, IT00K-1263; G, IT84S-2246; H, IT93K-503-1.

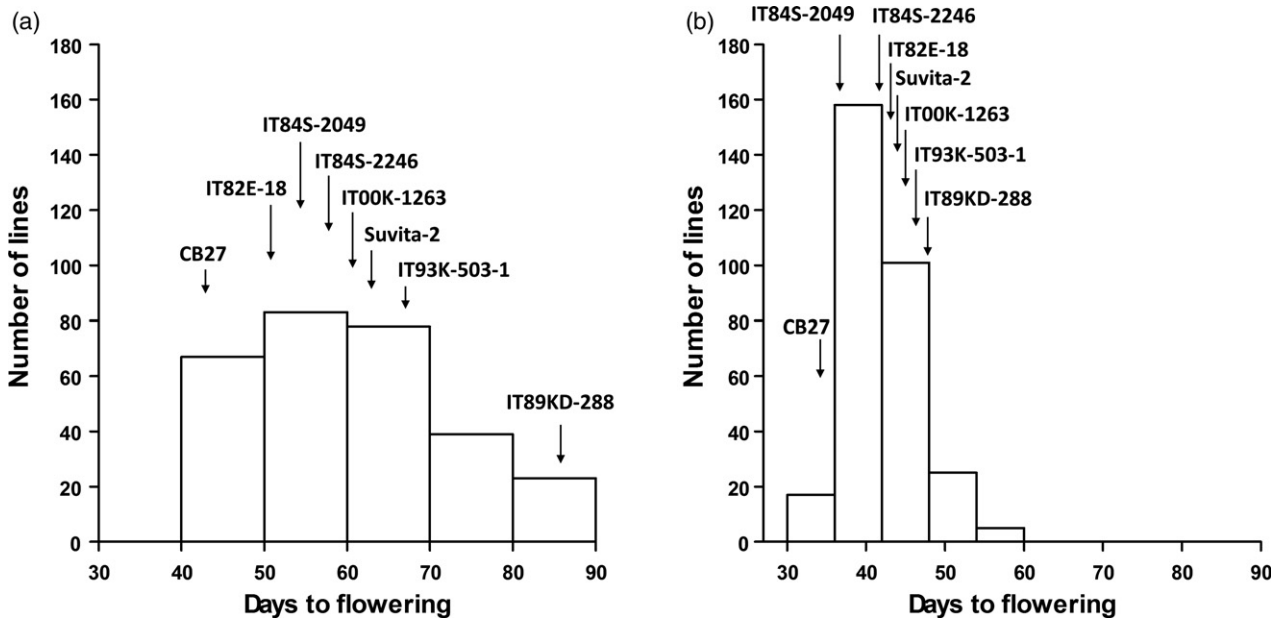

Figure 4. Variation in flowering time measured in the multi-parent advanced generation inter-cross (MAGIC) core set and eight parents.
Variation in flowering time measured in the MAGIC core set and eight parents grown under (a) long-day conditions at University of California–Riverside Citrus Experiment Station (UCR-CES) and (b) short-day conditions at the Coachella Valley Agricultural Research Station (CVARS). Mean flowering time values for each line were derived from two experiments at UCR-CES and four experiments at CVARS during 2015 and 2016.

IT89KD-288 (88 and 46 days) was the most delayed in both environments (UCR-CES and CVARS, respectively).

None of the MAGIC RILs or parents showed a prostrate growth habit. Under full irrigation, the majority of MAGIC RILs had a growth habit ranging from semi-erect to erect under both short- and long-day conditions (Figure S2). There was significant but moderate correlation in the growth habit scores between the two day-length conditions ($r = 0.55$, $P < 0.001$), with about 55% of the lines showing a consistent growth habit between the two environments. Lines with a semi-prostrate growth habit under long days became intermediate or semi-erect types when grown under short-day conditions. Among the parents, CB27 (acute erect), IT84S-2049 (erect), IT89KD-288 (semi-erect) and Suvita-2 (semi-erect) maintained their growth habit in both short- and long-day conditions under the full-irrigation regime. Under restricted irrigation, the MAGIC RILs and parents mostly showed erect or acute erect growth.

Maturity varied considerably in the MAGIC population grown under different watering regimes at CVARS in 2015 and 2016. Repeatability was estimated at 0.47, with significant but moderate correlations ($r = 0.53$, $P < 0.001$) existing in the phenotypic ranking between the two watering conditions (normal and restricted irrigation). Transgressive segregation was also observed. Some lines were fully mature as early as 60 days after planting under both watering regimes, while others were still green and kept producing pods up to 120 days under restricted irrigation in 2015, including two parents (IT00K-1263 and IT93K-503-1) and 66 MAGIC RILs (21% of the population).

Grain yield and seed size also varied considerably under both water-restricted and full-irrigation conditions at CVARS (Figure S3). The plants generally produced much higher yield and developed larger seeds under full irrigation than under water-stress conditions. Seed size appeared much more stable in the genotypic ranking than grain yield, with repeatability estimated as 0.76 and 0.30, respectively. Transgressive segregation was observed for both traits. Approximately 11% of MAGIC RILs had a higher yield than all parents under restricted irrigation conditions. Among the parents, CB27 consistently had the highest yield and largest seed across the two environments.

Identification of QTLs in MAGIC RILs

Given the high repeatability observed for flowering time under each day-length condition, mean values for each RIL at UCR-CES and CVARS were used in QTL analysis. Four QTLs were identified under long days at UCR-CES (Figure 5a). The QTL with the largest effect is located on chromosome 9, explaining approximately 31% of total phenotypic variance, with favorable (early flowering) alleles contributed from CB27 and IT82E-18 (up to 15 days earlier than the photoperiod-sensitive parent IT93K-503-1) (Table 1). The QTL with the second largest effect is located on chromosome 11, explaining approximately 15% of total phenotypic variance, with favorable alleles contributed

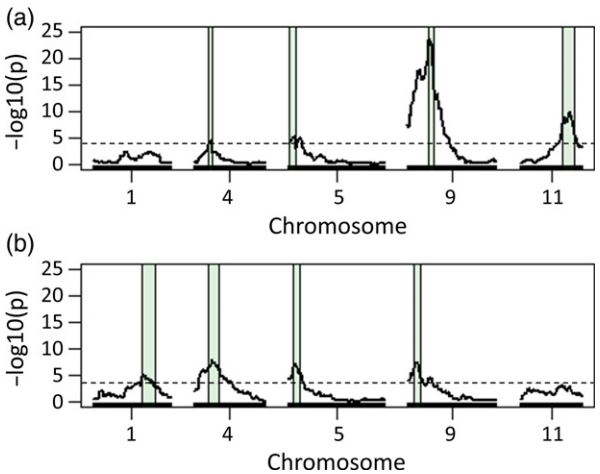

Figure 5. Quantitative trait locus (QTL) profile for flowering time measured in the multi-parent advanced generation inter-cross (MAGIC) population.
The QTL profile for flowering time measured in the MAGIC population grown under (a) long-day and (b) short-day conditions at the University of California–Riverside Citrus Experiment Station and the Coachella Valley Agricultural Research Station, respectively. Green regions indicate 1-LOD support intervals of significant QTLs ($P < 0.05$) in the final models. Dashed lines indicate the significance threshold at 7.56×10^{-5} using empirical null simulations ($n = 1000$, $P = 0.05$).

from CB27 and IT84S-2246 (Table 1). The other two QTLs with minor effects are located on chromosomes 4 and 5, explaining less than 10% of the total phenotypic variance, both with favorable alleles contributed from CB27 (Table 1). Under short days at CVARS, four QTLs affecting flowering time were mapped on chromosomes 1, 4, 5 and 9 (Figure 5b). Of these, QTLs on chromosomes 4 and 5 seem to be located in the same regions of QTLs affecting flowering time under long days at UCR-CES. All QTLs showed minor effects, each explaining less than 13% of total phenotypic variance. The parent CB27 consistently contributed early flowering alleles at every QTL (Figure 5b, Table 1).

QTLs affecting plant growth habit were identified under full irrigation. At UCR-CES, two QTLs were mapped on chromosomes 1 and 9, explaining 9% and 10% of total phenotypic variance, respectively (Table 1). At CVARS, the QTL on chromosome 1 was also expressed but with a larger effect, explaining 21.6% of total phenotypic variance, with favorable (erect-growth) alleles contributed from IT84S-2049, CB27 and IT82E-18. These QTLs seem to be collocated with those affecting flowering time (Table 1).

A QTL affecting plant maturity under full irrigation at CVARS was mapped on chromosome 5, explaining approximately 12% of total phenotypic variance, with favorable (early maturity) alleles contributed from CB27 and IT00K-1263 (Table 1). This QTL also was expressed under restricted irrigation in addition to two other QTLs on chromosomes 2 and 9, each explaining up to 10% of total phenotypic variance. The QTLs on chromosomes 5 and 9 seem to be collocated with those affecting the flowering time under short-day conditions at CVARS (Table 1).

One minor and one major QTL affecting seed size at CVARS were identified on chromosomes 6 and 8, respectively (Table 1). The major QTL explained up to 27% of total phenotypic variance, with favorable (large seed) alleles contributed from IT82E-18 and IT00K-1263. This QTL is collocated with a seed size QTL previously mapped by Lucas *et al.* (2013b) using the CB27 × IT82E-18 RIL population in which the favorable allele was also contributed from IT82E-18. The other QTL with minor effect was located on chromosome 6, explaining approximately 10% of total phenotypic variance, with the favorable allele contributed from IT89KD-288 (Table 1).

Validation of the photoperiod QTL in biparental RILs

To verify the QTL detected for photoperiod sensitivity in the MAGIC population, a biparental mapping population including 92 F_8-derived F_9 RILs from a cross between the non-photoperiod-sensitive parent CB27 and the photoperiod-sensitive IT97K-556-6 was screened under long days at UCR-CES in 2016. Flowering time varied widely in the RIL population (Figure 6). CB27 began flowering 44 days after planting, while IT97K-556-6 delayed flowering until after 70 days. A major QTL for flowering time was detected on linkage group 9 [logarithm of the odds (LOD) = 7.8, explaining 30% of phenotypic variance] (Figure 7). The early flowering allele was contributed from CB27. The SNP markers flanking this QTL (2_04691 and 2_00735) also harbor the same major QTL region detected in the MAGIC population grown under the same long-day condition in 2015 (Table 1, Data S1).

DISCUSSION

MAGIC development

The wide phenotypic variation with significant transgressive segregation observed in the cowpea MAGIC population indicates that genome regions from parents were highly recombined in the RILs. In fact, the population was developed in a way that maximized genetic variation. At the two-way crosses, plants in each F_1 set were heterozygous and homogeneous because the eight founder parents were fully inbred lines. However, the F_1s derived from the four-way crosses segregated and exhibited significant variation. To capture variation, we performed more than 300 pair-wise reciprocal eight-way crosses between different four-way F_1 individuals (Figure S1). In addition, there was no intended selection for any trait during the SSD process. The plants were grown in UCR greenhouses with optimal temperature, fertilizer, irrigation and pest and disease management. In some cases, the plants were grown during long-day conditions in summer but the photoperiod-

Table 1 Estimates of quantitative trait loci for flowering time and other agronomic traits measured in the eight-parent cowpea MAGIC population grown at UCR-CES (long day-length) and CVARS (short day-length) in 2015 and 2016. The seven right-most columns show the founder effects (± standard errors) contributed by each founder parent relative to IT93K-503-1

Trait	Chr	Position (SI)	Flanking markers	Wald	*P*-value	Pct Var	IT89KD-288	IT84S-2049	CB27	IT82E-18	Suvita-2	IT00K-1263	IT84S-2246
Flowering time	4	19 (16, 20)	2_48582–2_09077	39.0	1.99×10^{-6}	8.8	3.32 ± 6.42	7.54 ± 3.99	−1.46 ± 3.83	7.19 ± 4.01	7.61 ± 3.93	8.11 ± 3.85	4.80 ± 4.79
(days) under	5	7 (1, 9)	2_07393–2_12440	31.2	5.72×10^{-5}	9.9	0.38 ± 6.03	−4.87 ± 3.60	−6.61 ± 3.42	−1.81 ± 3.28	−4.23 ± 3.28	−4.94 ± 3.31	2.87 ± 3.53
long day	9	25 (23, 28)	2_00738–2_00736	146.2		31.1	−0.82 ± 2.89	−3.25 ± 2.70	−13.76 ± 2.66	−15.05 ± 2.60	−2.29 ± 2.76	3.96 ± 5.04	−1.81 ± 5.89
length at	11	50 (47, 59)	2_22669–2_44356	67.1	5.81×10^{-12}	15.3	11.69 ± 9.15	9.14 ± 4.70	−1.35 ± 4.78	7.12 ± 4.73	3.70 ± 4.77	2.42 ± 4.82	−4.21 ± 4.71
UCR-CES													
Flowering time	1	56 (54, 68)	2_20430–2_18422	24.9	7.91×10^{-4}	8.8	0.44 ± 0.93	−0.81 ± 0.88	−1.04 ± 0.99	−1.22 ± 0.92	0.93 ± 0.85	2.05 ± 0.86	−0.66 ± 0.95
(days) under	4	20 (15, 27)	2_31776–2_15171	62.1	5.88×10^{-11}	13.3	−6.19 ± 2.57	−1.56 ± 1.61	−6.55 ± 1.53	−1.71 ± 1.62	−3.55 ± 1.58	−1.64 ± 1.54	−4.67 ± 1.99
short day	5	8 (5, 12)	2_32176–2_18349	39.7	1.43×10^{-6}	12.3	3.20 ± 2.39	−0.81 ± 1.42	−1.35 ± 1.38	0.63 ± 1.31	−0.21 ± 1.32	−1.53 ± 1.32	2.34 ± 1.37
length at	9	10 (7, 13)	2_14794–2_20854	57.3	5.19×10^{-10}	12.5	−0.71 ± 1.52	0.73 ± 1.53	−2.54 ± 1.54	0.28 ± 1.55	−0.38 ± 1.52	4.03 ± 3.32	2.58 ± 3.83
CVARS													
Growth habit	1	58 (54, 61)	2_44007–2_24445	44.7	1.59×10^{-7}	9.4	0.81 ± 0.21	−0.16 ± 0.18	−0.04 ± 0.19	−0.32 ± 0.18	0.21 ± 0.18	0.36 ± 0.18	−0.26 ± 0.20
at UCR-CES	9	24 (22, 28)	2_53750–2_33113	46.9	5.75×10^{-8}	10.1	0.49 ± 0.25	0.10 ± 0.23	−0.50 ± 0.22	−0.36 ± 0.23	0.39 ± 0.24	−0.06 ± 0.44	−0.36 ± 0.51
(1–5)													
Growth habit	1	61 (59, 63)	2_18049–2_08603	84.9	1.33×10^{-15}	21.6	0.49 ± 0.16	−0.55 ± 0.15	−0.38 ± 0.15	−0.55 ± 0.15	0.01 ± 0.14	0.13 ± 0.14	−0.31 ± 0.15
at CVARS													
(1–5)													
Seed size	6	79 (74, 80)	2_14712–2_54463	38.7	2.27×10^{-6}	10.1	1.43 ± 1.59	−2.23 ± 1.30	−1.88 ± 1.28	−2.36 ± 1.28	−0.35 ± 1.28	−2.48 ± 1.30	−0.62 ± 1.63
(g/100 seeds)	8	75 (73, 78)	2_32728–2_54221	109.5		27.0	0.94 ± 1.96	0.54 ± 1.98	1.81 ± 1.91	5.78 ± 1.95	0.47 ± 1.97	5.32 ± 1.93	0.65 ± 3.64
at CVARS													
Maturity (days)	5	10 (7, 18)	2_19540–2_41253	44.8	1.51×10^{-7}	11.8	13.49 ± 6.89	0.91 ± 4.08	−6.26 ± 3.97	1.22 ± 3.79	0.76 ± 3.85	−2.49 ± 3.87	6.46 ± 3.95
at CVARS													
under normal													
irrigation													
Maturity (days)	2	45 (37, 48)	2_10022–2_20684	25.0	7.72×10^{-4}	9.5	−17.45 ± 9.07	0.01 ± 4.30	3.39 ± 4.18	1.60 ± 4.06	2.58 ± 4.35	−1.18 ± 4.19	−1.09 ± 9.01
at CVARS	5	10 (5, 19)	2_19540–2_41253	28.5	1.82×10^{-4}	8.9	4.71 ± 10.8	−7.83 ± 6.37	−13.37 ± 6.17	−3.84 ± 5.89	−5.21 ± 5.97	−12.44 ± 5.95	−2.22 ± 6.11
under	9	10 (4, 13)	2_14794–2_20854	36.5	5.92×10^{-6}	10.0	−19.13 ± 7.08	−14.41 ± 7.18	−17.10 ± 7.07	−6.52 ± 7.12	−18.57 ± 7.12	22.34 ± 15.7	−38.5 ± 18.3
restricted													
irrigation													

Chr, chromosome; Position, position in centimorgans (and 1-LOD support interval, SI); PctVar, percentage of variance explained; MAGIC, multi-parent advanced generation inter-cross; UCR-CES, University of California–Riverside Citrus Experiment Station; CVARS, Coachella Valley Agricultural Research Station.

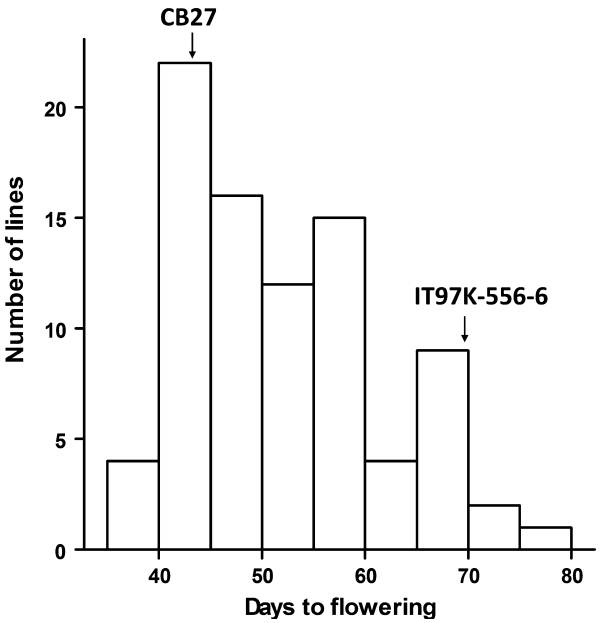

Figure 6. Variation in flowering time.
Variation in flowering time expressed in the CB27 × IT97K-556-6 biparental recombinant inbred line population grown under long-day conditions at the University of California–Riverside Citrus Experiment Station in 2016.

sensitive lines which failed to become reproductive in the summer were maintained and allowed to set flowers and pods later in the autumn when the day length shortened, to avoid selection against photoperiod sensitivity. There was also no selection for preferable seed characteristics, plant type or yield components. This blind SSD process therefore helped create the high diversity in morphological and agronomic traits in this MAGIC population (Figures 3, 4, Figure S2 and Figure S3).

The genetic integrity of the cowpea MAGIC population was confirmed by the results of high-density SNP genotyping. We used 89 parent-unique SNP markers from the Illumina GoldenGate Assay (Muchero *et al.*, 2009a) to validate true two-way F_1 crosses to avoid possible mistakes from the early stage of MAGIC development. We then used 11 848 parent-unique SNPs from the recently developed Illumina iSelect 60K SNP assay (Muñoz-Amatriaín *et al.*, 2017) to confirm true eight-way RILs and to eliminate those that appeared to be selfed at the four-way or eight-way crosses. The SNP genotyping also identified lines with non-parental alleles, identical SNP genotypes or excess heterozygosity. Fortunately, few of these unexpected lines were found (16 out of 365 lines); some were replaced by sister lines that were purposely developed as backups. By removing all erroneous lines and keeping one RIL from each unique eight-way cross, we created a MAGIC core set of 305 RILs that are highly homozygous and genetically distinct from each other and the eight parents (Figure 1). As such, they can serve as permanent genetic materials for use in replicated phenotyping trials.

The population size of 305 cowpea MAGIC RILs is relatively more compact than those reported for other crop species, such as barley (533 lines) (Sannemann *et al.*, 2015) and winter wheat (1091 lines) (Mackay *et al.*, 2014). This seems consistent with the calculations of Valdar *et al.* (2006) that argued for MAGIC population sizes of 500 or more to provide sub-centimorgan resolution in organisms with larger genomes. In our case, the eight MAGIC parents were fully inbred (i.e. one haplotype in each parent) and the 305 RILs are more than 99% homozygous (i.e. essentially one haplotype in each RIL), so a simple estimate of resolution is 0.33 cM (1/305). Further, cowpea has a relatively small diploid genome (620 Mb) (Chen *et al.*, 2007) and a genetic map of about 900 cM. So, the physical level of resolution provided by 305 cowpea MAGIC RILs is on average about 230 kb genome wide (620 × 0.33/900), though finer resolution exists in the predominantly high-recombination, gene-rich regions of the genome. A comparable level of physical resolution in an organism with a genome in the 5-Gb range, such as barley or diploid wheat, would require a MAGIC population size of about 2500 RILs.

MAGIC genetic analysis

The 36 346 markers segregating in the cowpea MAGIC population were almost double the number in any bi-parental RIL population genotyped with the same SNP array

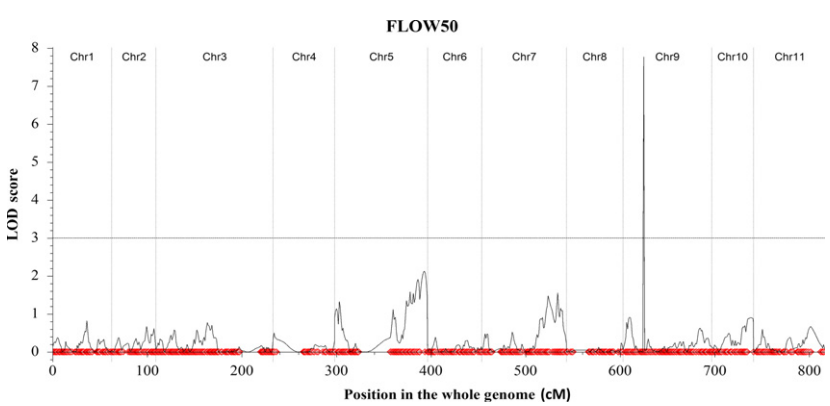

Figure 7. Chromosomal regions associated with variation in flowering time.
Chromosomal regions associated with variation in flowering time measured in the CB27 × IT97K-556-6 biparental recombinant inbred line population under long-day conditions at the University of California–Riverside Citrus Experiment Station in 2016. The LOD peak on linkage group 8 is flanked by single nucleotide polymorphism markers 2_10023 and 2_04691, which are in the same region of the major quantitative trait locus detected in the multi-parent advanced generation inter-cross (MAGIC) population (see Figure 5). [Colour figure can be viewed at wileyonlinelibrary.com].

Table 2 Multi-parent advanced generation inter-cross (MAGIC) founder parents and their traits relevant to sub-Saharan Africa and other production areas

Name	Source	Agronomic trait	Resistance or tolerance trait
SuVita 2	INERA	High yielding under drought in Senegal, Burkina Faso and Mozambique; large dark-brown seed	Drought tolerant, resistant to *Striga*, foliar thrips and *Macrophomina* disease
CB27	UCR	High yielding under drought in Mozambique; large black-eye seed; photoperiod insensitive; erect growth habit; early maturing	Heat tolerant, resistant to root-knot nematode, *Fusarium* wilt and foliar thrips
IT93K-503-1	IITA	High yielding under drought in Senegal; brown-eye seed; stay-green under drought	Drought tolerant, resistant to nematodes, *Fusarium* wilt and *Macrophomina*
IT89KD-288	IITA	High yielding under drought in Burkina Faso and Nigeria; brown-eye seed; photoperiod sensitive	Resistant to root-knot nematode
IT84S-2049	IITA	High yielding under drought in Burkina Faso; brown-eye seed; erect growth habit	Resistant to aphid, bacterial blight, viruses and root-knot nematode
IT82E-18	IITA	High yielding under drought in Mozambique; early maturing, light-brown seed; photoperiod insensitive	Broadly adapted, resistant to root-knot nematode
IT00K-1263	IITA	High yielding under drought in Mozambique and Nigeria; dark-brown seed; stay-green under drought	Resistant to *Striga*, aphid, *Fusarium* wilt and root-knot nematode
IT84S-2246	IITA	High yielding under drought in Burkina Faso and Mozambique; dark-brown seed	Resistant to aphid, bacterial blight, viruses and root-knot nematode

INERA, Institut de l'Environment et des Recherches Agricole, Burkina Faso; UCR, University of California–Riverside, United States; IITA, International Institute of Tropical Agriculture, Nigeria.

(Muñoz-Amatriaín *et al.*, 2017). This is attributable to the eight founder parents having been chosen on the basis of phenotypic and genetic diversity found in earlier studies. The parents were high yielding under drought in one or more countries, resistant to different biotic stress factors (Table 2) and represented West Africa and south-east Africa gene pools (Huynh *et al.*, 2013a). By applying multiple two-way, four-way and eight-way intercrosses from those founders, plus seven generations of SSD for over 300 independent eight-way pair crosses, one would expect more recombination events to occur in the MAGIC than in bi-parental RILs. However, it is difficult to measure accurately the number of crossovers between two SNP markers due to a lack of parent-specific alleles at every locus. At each SNP marker, one allele represents one or more parents, and the alternative allele represents the other parents, so in some cases it is impossible to identify the actual parent carrying the allele at that locus. The recombination fractions estimated in this study were based only on the recombinants that could be ascertained with certainty between two SNPs and thus may underestimate their true genetic distance.

The recombination events that are presented among the MAGIC RILs varied considerably along 11 cowpea chromosomes (Figure 2). We particularly observed that recombination was more frequent in the distal long arm than the distal short arm regions (Figure 2). This increased telomeric recombination frequency near the telomeres facilitates random association needed for QTL detection. QTLs with major effects detected for photoperiod sensitivity and seed size were verified by bi-parental genetic mapping, indicating that the MAGIC core set is effective for mapping genome regions harboring major QTLs. This MAGIC core set comprised individuals which were carefully selected based on genome-wide SNP diversity, so interference in QTL analysis by kinship and population structure would be minimal.

Perspectives for genetic improvement

The strong transgressive segregation observed for agronomic traits provides opportunities for selecting MAGIC lines that outperform the parents. Selecting for large seed size, which is preferred by consumers in SSA, would be straightforward because the trait appeared to be highly heritable (Figure S3b). In contrast, selecting for higher yield will be more difficult given its relatively low heritability (Figure S3a); based on the pattern of variation in yield under restricted versus full irrigation, it may be more effective to select for high yield under drought stress in which at least 11% of the RILs gave a better yield than the eight MAGIC parents. These lines probably carry a combination of different drought tolerance genes contributed from multiple parents, because the parents are known to yield well under drought conditions in different African countries (Table 2). MAGIC lines that are not sensitive to photoperiod could be grown widely across seasons and regions with different latitudes. Lines that flower early may escape damage by flower/pod-feeding insects and abiotic stress such as heat and terminal drought. MAGIC lines with exceptionally early or delayed crop senescence are suitable for production systems requiring single or double flushes of pods, respectively. MAGIC lines with acute erect growth could support a heavy pod load, allow greater leaf area to capture sunlight for photosynthesis and support high plant population densities to increase yield under monocropping. Since the eight parents also vary in resistance to

many major insects and diseases (Table 2), the MAGIC population will segregate for many biotic stress resistance traits and also contain lines with unique and novel combinations of defense genes. Therefore, phenotypic screening of the MAGIC population for those traits will enable genetic mapping and identification of lines carrying favorable trait combinations for selecting cultivars in target environments.

For the longer term, the cowpea MAGIC population can also benefit breeding programs by providing valuable pre-breeding resources. QTLs detected in the MAGIC population combined with existing knowledge of QTL regions and haplotypes can be applied to develop novel combinations of QTLs by intercrossing the best MAGIC RILs, providing super trait-donor lines for use in breeding programs. QTLs for many key traits were already mapped in bi-parental and diversity populations where certain MAGIC parents were used in the crosses, such as seed size (Lucas *et al.*, 2013b), heat tolerance (Lucas *et al.*, 2013a), drought tolerance (Muchero *et al.*, 2013) and root architecture (Burridge *et al.*, 2017) and resistance to foliar thrips (Lucas *et al.*, 2012), aphids (Huynh *et al.*, 2015), *Fusarium* wilt disease (Pottorff *et al.*, 2014), root-knot nematodes (Huynh *et al.*, 2016), ashy stem blight or charcoal rot disease caused by *Macrophomina phaseolina* (Muchero *et al.*, 2011), viruses (Ouédraogo *et al.*, 2002a) and the parasitic weed *Striga gesnerioides* (Ouédraogo *et al.*, 2012). It is therefore possible to track positive haplotypes contributed by different MAGIC parents in each MAGIC line and then intercross the best lines to develop ideotypes. This strategy would be similar to the multi-parent advanced generation recurrent selection (MAGReS) approach proposed recently by Huang *et al.* (2015), except that (i) prior knowledge of QTL information from cowpea bi-parental mapping will be utilized, (ii) the MAGIC RILs selected for intercrosses are more advanced (F_8), and (iii) the selection can be targeted using both QTL haplotypes and predicted breeding values based on genome-background diversity. The resulting MAGReS lines will be fixed for positive haplotypes at known QTLs and carry additional recombinations in other unknown loci conferring high grain yield. They can thus be a valuable resource for both genetic improvements (as super trait donors or new cultivars) and for detecting novel QTLs when combined with the current MAGIC RIL set.

EXPERIMENTAL PROCEDURES

Choice of parents

The eight cowpea parents used in the original crosses were elite cultivars and breeding lines selected on the basis of their high genetic diversity characterized by genotyping with 1536 genome-wide gene-based SNP markers (Muchero *et al.*, 2009a). In addition, they were chosen because collectively they carry multiple biotic and abiotic stress resistance and tolerance traits relevant to SSA (Table 2). SuVita 2, also known as 'Gorom', a local landrace in Burkina Faso, is resistant to the parasitic weed *Striga* (Ouédraogo *et al.*, 2002b) and the fungal pathogen *Macrophomina phaseolina* (Muchero *et al.*, 2011). CB27, a California blackeye cultivar bred by UCR is heat tolerant (Ehlers *et al.*, 2000) and highly resistant to root-knot nematodes (Huynh *et al.*, 2016), *Fusarium* wilt disease (Pottorff *et al.*, 2012, 2014) and foliar thrips (Lucas *et al.*, 2012). IT93K-503-1, a breeding line from the International Institute of Tropical Agriculture (IITA) breeding nursery in Nigeria, is drought tolerant (Muchero *et al.*, 2009b) and resistant to root-knot nematodes (Huynh *et al.*, 2016), *M. phaseolina* (Muchero *et al.*, 2011) and *Fusarium* wilt (Pottorff *et al.*, 2014). The other five parents (IT89KD-288, IT84S-2049, IT82E-18, IT00K-1263 and IT84S-2246) are breeding lines from IITA; they carry combinations of key traits including grain quality and resistance to root-knot nematode, *Striga*, *Fusarium*, viruses and bacterial blight (Table 2).

Population development

The eight parents were inter-mated using a strategy described in Cavanagh *et al.* (2008) with some modifications in which the order of male and female plants at each intercross cycle was rearranged to create various pedigree patterns (funnels) (Figure S1). In spring 2010, initial crosses were made between four pairs of fully inbred founder parents (IT89KD-288 × IT84S-2049, CB27 × IT82E-18, SuVita 2 × IT00K-1263 and IT84S-2246 × IT93K-503-1) to produce two-way F_1s. In spring 2011, reciprocal four-way crosses were made between two pairs of the two-way F_1s to produce four-way F_1s. In autumn 2011, eight-way crosses were made to produce F_1s, each derived from a unique cross between different four-way F_1 individuals. Single-seed descent was then applied for each unique eight-way F_1 until the F_8 generation. Twenty-nine crosses produced two or more F_8 RILs, which were sister lines separated from earlier generations. These lines were purposely created to maintain the population size. Reciprocal crosses made at the four-way and eight-way cycles resulted in RILs with different maternal parents, including CB27 (225 lines), IT89KD-288 (111 lines), Suvita 2 (9 lines) and IT84S-2246 (5 lines). There were 15 lines with illegible pedigrees on tags that were bleached by sunlight and moisture in the greenhouse. For each F_8 RIL, seeds from a single F_8 plant were harvested and maintained as an original seed stock ($F_{8:9}$). The $F_{8:9}$ seeds were then increased in bulk to make $F_{8:10}$ seeds for phenotyping.

SNP genotyping

The F_1 progeny from two-way crosses were verified by genotyping their F_2 seeds (up to 21 seeds per cross) with 89 parent-unique SNPs using the competitive allele-specific polymerase chain reaction (KASP) cowpea assay (LGC Genomics Ltd, http://www.lgcgroup.com/our-science/genomics-solutions/) (Semagn *et al.*, 2014), which was converted from the 1536-SNP Illumina GoldenGate Assay developed by Muchero *et al.* (2009a). True F_1 plants were confirmed when polymorphic markers were found segregating in the corresponding F_2 progeny.

The F_8 single plants derived from eight-way crosses were genotyped with 51 128 SNPs using the Illumina Cowpea Consortium Array (Muñoz-Amatriaín *et al.*, 2017). A core set of MAGIC RILs was selected through the following consecutive steps: (i) three lines carrying non-parental alleles and five lines with excess numbers of heterozygous and ambiguous genotypes were excluded; (ii) based on parent-unique SNPs, 15 lines that did not carry male-parent alleles (i.e. selfed) at the four-way or eight-way crosses were excluded; (iii) among true eight-way RILs and eight parents, genetic similarities were measured using the allele-sharing method (Bowcock *et al.*, 1994) with the software GGT 2.0

(van Berloo, 2008), from which phylogenetic relationships were generated using the neighbor-joining method (Saitou and Nei, 1987) and visualized using the software MEGA 5.05 (Tamura *et al.*, 2011); and (iv) for each set of genetically identical RILs (similarity 0.99 or higher), the line with the lowest number of ambiguous genotypes was retained. There were eight RILs each showing very similar SNP genotypes (more than 99%) to another RIL, and these were considered as redundant duplicates. After excluding lines with duplicates, selfing errors, non-parental alleles and excess heterozygosity, the core set of 305 MAGIC RILs derived from 305 unique eight-way crosses was selected for further analysis.

Genetic map construction

Polymorphic SNPs (success rate >90% and minor allele frequency >0.05) with known positions across 11 cowpea pseudomolecules (www.phytozome.net) were used for genetic mapping, with a new chromosome numbering convention based on synteny between cowpea and common bean. Linkage maps for all chromosomes were created using R/mpMap (Huang and George, 2011), with orders and synteny determined from the known physical positions. Recombination fractions between markers were estimated using the function 'mpestrf', and the map order refined using the R package mpMapInteractive (Shah, 2013). This interactive visualization package allows for modification of marker map order based on visual inspection of recombination fraction heatmaps. Using this we were able to quickly and easily remove markers whose distorted recombination fraction patterns might have affected the ordering within a larger region.

Based on the resulting recombination fractions, we formed bins of adjacent markers using the function 'mpcollapse', such that within each bin all markers had zero recombination fractions with each other. Similar strategies have been shown previously to be of great value with high-density map construction (van Os *et al.*, 2006; Xu, 2013). Recombination fractions between binned markers were then computed as before. We estimated map positions for the binned markers using the function 'computemap' with parameter *maxOffset* set to 20. This parameter essentially selects the number of neighboring markers to use in a nonlinear least-squares regression to compress map distance and reduce map expansion caused by variability in recombination fraction estimates. The final step was to use the function 'mpexpand' to place all markers within a bin at the same position in the map. The resulting linkage maps were presented in Data S1.

Recombination analysis

Using a sliding window of 2 Mb with 1-Mb increments along each pseudomolecule (chromosome), recombination rates (cM Mb^{-1}) were calculated as the linkage distance divided by the physical distance between the first and the last SNP of each window. The variation in recombination rate was visualized by plotting the estimated recombination rate for every 1-Mb increment along the 11 chromosomes.

MAGIC phenotyping

The MAGIC RILs and parents were screened for photoperiod sensitivity under long-day conditions during summer, from June (14.5 h) to September (12.8 h), at UCR-CES (33.97° N, 117.34° W). In 2015, each MAGIC RIL and parent was planted in one 0.76-m wide, 5.5-m long row at a density of 12 seeds per meter using a tractor-mounted planter. The field was watered to capacity before and after planting up to 100 days using furrow irrigation. The experiment was repeated in 2016 but under restricted irrigation, where the field was watered to capacity before planting, and then irrigation was withheld until the end of the trial. For each line in both trials, calendar days to flowering were determined when 50% of plants in the plot flowered.

The population was also screened under short days during autumn, from September (12.8 h) to December (9.9 h), at CVARS (33.52° N, 116.15° W). In 2015, the population was planted in two blocks that received different watering regimes (full irrigation and restricted irrigation) and were separated by a six-row buffer (5 m). In each block, each MAGIC RIL and parent was planted in one 0.76-m wide, 3.5-m long row at a density of 12 seeds per meter using a tractor-mounted planter. The field was watered to capacity before and after planting using subsurface drip irrigation. After 2 weeks when the seedlings were well established, the irrigation was withheld in the restricted-irrigation block until maturity, whereas in the full-irrigation block the rows were watered to capacity up to 100 days after planting. In 2016, the two experiments (full irrigation and restricted irrigation) were repeated on adjacent field blocks at CVARS. For each line, in four experiments, calendar days to flowering were determined when 50% of plants in the plot flowered. Plant growth habit was measured 40 days after planting using a visual rating scale from 1 to 6 based on the angles formed between primary branches and the main stem: 1, acute erect, branches form angles less than 45° with the main stem; 2, erect, branching angles between 45° and 90° with the main stem; 3, semi-erect, branches perpendicular to the main stem but not touching the ground; 4, intermediate, lower branches touching the ground; 5, semi-prostrate, lower branches flat on the ground but the main stem standing upright; 6, prostrate, the entire plant flat and spreading on the ground. Days to maturity were determined when 95% of pods in the plot had dried. At maturity, the plants in each plot were cut at the lower stems and machine-threshed for measurement of plot yield and 100-seed weight.

Each set of repeated trials at UCR-CES and CVARS was considered as a randomized complete block design, with each field site per season receiving one watering treatment corresponding to a block. Analysis of variance (ANOVA) was performed with the software GenStat version 11 (Payne *et al.*, 2008). For flowering time under each day-length condition, trait repeatability was estimated based on the variance component attributable to variation among lines (VG) and residual variation (VE) [h^2 = VG / (VG + VE)]. Non-parametric correlation analysis (Spearman's rank) was used to examine the consistency in genotypic ranking of the same lines between the two day-length conditions at UCR-CES and CVARS. For growth habit, Spearman's rank was used to examine the consistency of the trait expressed under normal irrigation at UCR-CES and CVARS. For maturity, yield and seed size, trait repeatability was estimated separately for normal and restricted irrigations at CVARS, and Spearman's rank was used to examine the relationship in the phenotypic ranking between the two conditions.

MAGIC QTL mapping

Simple interval QTL mapping was performed using R/mpMap (Huang and George, 2011) with the function 'mpIM' based on the MAGIC SNP data and linkage map (Data S1). Founder haplotype probabilities were computed at 1-cM steps across the genome (step = 1, mrkpos = *F*) and fitted in a linear model for each trait. A genome-wide significance threshold of 7.56×10^{-5} was determined empirically using the function 'sim.sigthr' with 1000 simulations from a null distribution. The QTL were initially detected as peaks on a chromosome which exceeded the significance threshold; however, as a further step we considered a full model using

the command 'fit' which incorporates all identified QTLs simultaneously. This allowed the removal of peaks which no longer met the significance threshold after accounting for all other QTLs. The final model for each trait thus consists of the full model after removal of such QTLs.

Biparental QTL mapping

The 92 F_8-derived F_9 RILs from a cross between CB27 (photoperiod insensitive) and IT97K-556-6 (photoperiod sensitive) were screened under long-day conditions at UCR-CES in 2016. Each RIL and parent was planted in one 0.76-m wide, 5.5-m long row at a density of 12 seeds per meter using a tractor-mounted planter. The planting time and conditions were similar to the MAGIC phenotyping trial in 2015. For each plot, days to flowering were determined when 50% of plants in the plot flowered. The biparental RIL population was genotyped with the 51 128 SNP Illumina iSelect BeadArray that was used to genotype the MAGIC population. Construction of genetic maps and QTL analysis were performed with the software QTL IciMapping 4.0 (Meng *et al.*, 2015) using the inclusive composite interval mapping method (Wang, 2009).

ACCESSION NUMBERS

The MAGIC core set and the eight founder parents are available on request at the cowpea gene banks of IITA (Ibadan, Nigeria) and University of California (Riverside, USA). Accession names, SNP genotypes and phenotypes are provided in Data S1.

ACKNOWLEDGEMENTS

This work was supported in large part by grants from the Generation Challenge Programme of the Consultative Group on International Agricultural Research to PAR, JDE and TJC, with additional support from the USAID Feed the Future Innovation Lab for Collaborative Research on Grain Legumes (Cooperative Agreement EDH-A-00-07-00005) to PAR and TJC, the USAID Feed the Future Innovation Lab for Climate Resilient Cowpea (Cooperative Agreement AID-OAA-A-13-00070) to TJC, PAR and SL, and the NSF-BREAD (Advancing the Cowpea Genome for Food Security) to TJC, PAR, SL, MMA and BLH. The authors thank Tra Duong, Hyun Park Kang, Eric Castillo, Jasmine Gracin-Dixon, Uriah Dixon, Mitchell Lucas and Savanah St Clair for technical assistance, and the Molecular Genomics Core Facility (University of Southern California) for iSelect SNP genotyping. The authors also thank Peggy Mauk, Vince Samons and staff at UC Riverside Agricultural Operations and Coachella Valley Agricultural Research Station for managing field trials, and two anonymous reviewers for constructive comments.

CONFLICT OF INTEREST

The authors have no conflict of interest to declare.

SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article.

Figure S1. Multi-parent advanced generation inter-cross (MAGIC) breeding design.

Figure S2. Variation in growth habit.

Figure S3. Variation in yield and seed size.

Data S1. Multi-parent advanced generation inter-cross (MAGIC) genotypes and phenotypes.

REFERENCES

Anyia, A. and Herzog, H. (2004) Genotypic variability in drought performance and recovery in cowpea under controlled environment. *J. Agron. Crop Sci.*, **190**, 151–159. https://doi.org/10.1111/j.1439-037X.2004.00096.x

Van Berloo, R. (2008) GGT 2.0: Versatile software for visualization and analysis of genetic data. *J. Hered.*, **99**, 232–236. https://doi.org/10.1093/jhered/esm109

Bowcock, A.M., Ruizlinares, A., Tomfohrde, J., Minch, E., Kidd, J.R. and Cavallisforza, L.L. (1994) High-resolution of human evolutionary trees with polymorphic microsatellites. *Nature*, **368**, 455–457. https://doi.org/10.1038/368455a0

Burridge, J.D., Schneider, H.M., Huynh, B.-L., Roberts, P.A., Bucksch, A. and Lynch, J.P. (2017) Genome-wide association mapping and agronomic impact of cowpea root architecture. *Theoretical and Applied Genetics*, **130**, 419–431. https://doi.org/10.1007/s00122-016-2823-y

Cavanagh, C., Morell, M., Mackay, I. and Powell, W. (2008) From mutations to MAGIC: resources for gene discovery, validation and delivery in crop plants. *Curr. Opin. Plant Biol.*, **11**, 215–221. https://doi.org/10.1016/j.pbi.2008.01.002

Chen, X., Laudeman, T.W., Rushton, P.J., Spraggins, T.A. and Timko, M.P. (2007) CGKB: an annotation knowledge base for cowpea (*Vigna unguiculata* L.) methylation filtered genomic genespace sequences. *BMC Bioinformatics*, **8**, 129. https://doi.org/10.1186/1471-2105-8-129

Dadson, R., Hashem, F., Javaid, I., Joshi, J., Allen, A. and Devine, T. (2005) Effect of water stress on the yield of cowpea *(Vigna unguiculata* L. Walp.) genotypes in the Delmarva region of the United States. *J. Agron. Crop Sci.*, **191**, 210–217. https://doi.org/10.1111/j.1439-037X.2005.00155.x

Ehlers, J.D. and Hall, A.E. (1997) Cowpea (*Vigna unguiculata* L. Walp.). *Field. Crop. Res.*, **53**, 187–204. https://doi.org/10.1016/S0378-4290(97)00031-2

Ehlers, J.D., Hall, A.E., Patel, P.N., Roberts, P.A. and Matthews, W.C. (2000) Registration of 'California Blackeye 27' cowpea. *Crop Sci.*, **40**, 854–855.

Ewansiha, S. and Singh, B. (2006) Relative drought tolerance of important herbaceous legumes and cereals in the moist and semi-arid regions of West Africa. *J. Food Agric. Environ.*, **4**, 188–190.

Grantz, D. and Hall, A. (1982) Earliness of an indeterminate crop, *Vigna unguiculata* (L.) Walp., as affected by drought, temperature, and plant density. *Crop and Pasture Science*, **33**, 531–540. https://doi.org/10.1071/AR9820531

Gwathmey, C.O. and Hall, A.E. (1992) Adaptation to midseason drought of cowpea genotypes with contrasting senescene traits. *Crop Sci.*, **32**, 773–778. https://doi.org/10.2135/cropsci1992.0011183X003200030039x

Hall, A.E., Cisse, N., Thiaw, S. *et al.* (2003) Development of cowpea cultivars and germplasm by the Bean/Cowpea CRSP. *Field. Crop. Res.*, **82**, 103–134. https://doi.org/10.1016/S0378-4290(03)00033-9

Huang, B.E. and George, A.W. (2011) R/mpMap: a computational platform for the genetic analysis of multiparent recombinant inbred lines. *Bioinformatics*, **27**, 727–729. https://doi.org/10.1093/bioinformatics/btq719

Huang, B.E., Verbyla, K., Verbyla, A., Raghavan, C., Singh, V., Gaur, P., Leung, H., Varshney, R. and Cavanagh, C. (2015) MAGIC populations in crops: current status and future prospects. *Theoretical and Applied Genetics*, **128**, 999–1017. https://doi.org/10.1007/s00122-015-2506-0

Hussain, I.A., Prasad, T., Wright, G., Kumar, M.U. and Rao, R.N. (1999) Variation in transpiration efficiency and carbon isotope discrimination in cowpea. *Funct. Plant Biol.*, **26**, 503–510.

Huynh, B.-L., Close, T.J., Roberts, P.A. *et al.* (2013a) Gene pools and the genetic architecture of domesticated cowpea. *The Plant Genome*, **6**, 1–8.

Huynh, B.-L., Ehlers, J.D., Close, T.J., Cissé, N., Drabo, I., Boukar, O., Lucas, M.R., Wanamaker, S., Pottorff, M. and Roberts, P.A. (2013b) *Enabling tools for modern breeding of cowpea for biotic stress resistance*. Translational Genomics for Crop Breeding. John Wiley & Sons Ltd, pp 183–199. https://doi.org/10.1002/9781118728475

Huynh, B.-L., Ehlers, J.D., Ndeve, A., Wanamaker, S., Lucas, M.R., Close, T.J. and Roberts, P.A. (2015) Genetic mapping and legume synteny of aphid resistance in African cowpea (*Vigna unguiculata* L. Walp.) grown in California. *Mol. Breeding*, **35**, 1–9.

Huynh, B.-L., Matthews, W.C., Ehlers, J.D., Lucas, M.R., Santos, J.R.P., Ndeve, A., Close, T.J. and Roberts, P.A. (2016) A major QTL corresponding to the *Rk* locus for resistance to root-knot nematodes in cowpea (*Vigna unguiculata* L. Walp.). *Theoretical and Applied Genetics*, **129**, 87–95. https://doi.org/10.1007/s00122-015-2611-0

Kudre, T.G., Benjakul, S. and Kishimura, H. (2013) Comparative study on chemical compositions and properties of protein isolates from mung bean, black bean and bambara groundnut. *J. Sci. Food Agric.*, **93**, 2429–2436. https://doi.org/10.1002/jsfa.6052

Lonardi, S., Zhu, T., Muñoz-Amatriain, M., Liang, Q., Wanamaker, S., Ounit, R., Alhakami, H., Luo, M.-C. and Close, T.J. (2017) Assembly of eleven pseudomolecules representing the cowpea genome sequence. Plant and Animal Genome XXV, January 13 - 18, San Diego, CA, USA.

Lucas, M.R., Ehlers, J.D., Roberts, P.A. and Close, T.J. (2012) Markers for quantitative inheritance of resistance to foliar thrips in cowpea. *Crop Sci.*, **52**, 2075–2081. https://doi.org/10.2135/cropsci2011.12.0684

Lucas, M.R., Ehlers, J.D., Huynh, B.-L., Diop, N.-N., Roberts, P.A. and Close, T.J. (2013a) Markers for breeding heat-tolerant cowpea. *Mol. Breeding*, **31**, 529–536. https://doi.org/10.1007/s11032-012-9810-z

Lucas, M.R., Huynh, B.-L., Vinholes, PdS., Cisse, N., Drabo, I., Ehlers, J.D., Roberts, P.A. and Close, T.J. (2013b) Association studies and legume synteny reveal haplotypes determining seed size in *Vigna unguiculata*. *Frontiers in Plant Science*. **4**, 95.

Mackay, I.J., Bansept-Basler, P., Barber, T., *et al.* (2014) An eight-parent multiparent advanced generation inter-cross population for winter-sown wheat: creation, properties, and validation. *G3: Genes|Genomes|Genetics*. **4**:1603–1610. https://doi.org/10.1534/g3.114.012963

Mai-Kodomi, Y., Singh, B., Myers, O., Yopp, J., Gibson, P. and Terao, T. (1999a) Two mechanisms of drought tolerance in cowpea. *The Indian Journal of Genetics and Plant Breeding*, **59**, 309–316.

Mai-Kodomi, Y., Singh, B., Terao, T., Myers, O., Yopp, J. and Gibson, P. (1999b) Inheritance of drought tolerance in cowpea. *The Indian Journal of Genetics and Plant Breeding*, **59**, 317–323.

Matsui, T. and Singh, B. (2003) Root characteristics in cowpea related to drought tolerance at the seedling stage. *Exp. Agric.*, **39**, 29–38. https://doi.org/10.1017/S0014479703001108

Meng, L., Li, H., Zhang, L. and Wang, J. (2015) QTL IciMapping: integrated software for genetic linkage map construction and quantitative trait locus mapping in biparental populations. *The Crop Journal*, **3**, 269–283. https://doi.org/10.1016/j.cj.2015.01.001

Muchero, W., Diop, N.N., Bhat, P.R. *et al.* (2009a) A consensus genetic map of cowpea [*Vigna unguiculata* (L) Walp.] and synteny based on EST-derived SNPs. *Proceedings of the National Academy of Sciences USA*, **106**, 18159–18164. https://doi.org/10.1073/pnas.0905886106

Muchero, W., Ehlers, J.D., Close, T.J. and Roberts, P.A. (2009b) Mapping QTL for drought stress-induced premature senescence and maturity in cowpea [*Vigna unguiculata* (L.) Walp.]. *Theoretical and Applied Genetics*, **118**, 849–863. https://doi.org/10.1007/s00122-008-0944-7

Muchero, W., Ehlers, J.D. and Roberts, P.A. (2010) QTL analysis for resistance to foliar damage caused by *Thrips tabaci* and *Frankliniella schultzei* (Thysanoptera: Thripidae) feeding in cowpea [*Vigna unguiculata* (L.) Walp.]. *Mol. Breeding*, **25**, 47–56. https://doi.org/10.1007/s11032-009-9307-6

Muchero, W., Ehlers, J.D., Close, T.J. and Roberts, P.A. (2011) Genic SNP markers and legume synteny reveal candidate genes underlying QTL for *Macrophomina phaseolina* resistance and maturity in cowpea *Vigna unguiculata* (L) Walp. *BMC Genom.*, **12**, 8. https://doi.org/10.1186/1471-2164-12-8

Muchero, W., Roberts, P.A., Diop, N.N., Drabo, I., Cisse, N., Close, T.J., Muranaka, S., Boukar, O. and Ehlers, J.D. (2013) Genetic architecture of delayed senescence, biomass, and grain yield under drought stress in cowpea. *PLoS ONE*, **8**, e70041. https://doi.org/10.1371/journal.pone.0070041

Muñoz-Amatriaín, M., Mirebrahim, H., Xu, P. *et al.* (2017) Genome resources for climate-resilient cowpea, an essential crop for food security. *Plant J.*, **89**, 1042–1054. https://doi.org/10.1111/tpj.13404

Odoemena, J. (2004) Water balance and proximate composition in cowpea (*Vigna unguiculata* (L). Walps) seedlings exposed to drought and flooding stress. *Journal of Applied Sciences and Environmental Management*, **8**, 55–57.

Ogbonnaya, C., Sarr, B., Brou, C., Diouf, O., Diop, N. and Roy-Macauley, H. (2003) Selection of cowpea genotypes in hydroponics, pots, and field for drought tolerance. *Crop Sci.*, **43**, 1114–1120. https://doi.org/10.2135/cropsci2003.1114

Van Os, H., Andrzejewski, S., Bakker, E. *et al.* (2006) Construction of a 10 000-marker ultradense genetic recombination map of potato: providing a framework for accelerated gene isolation and a genomewide physical map. *Genetics*, **173**, 1075–1087.

Ouédraogo, J.T., Gowda, B.S., Jean, M. *et al.* (2002a) An improved genetic linkage map for cowpea (*Vigna unguiculata* L.): combining AFLP, RFLP, RAPD, biochemical markers, and biological resistance traits. *Genome*, **45**, 175–188. https://doi.org/10.1139/g01-102

Ouédraogo, J.T., Tignegre, J.B., Timko, M.P. and Belzile, F.J. (2002b) AFLP markers linked to resistance against *Striga gesnerioides* race 1 in cowpea (*Vigna unguiculata*). *Genome*, **45**, 787–793. https://doi.org/10.1139/g02-043

Ouédraogo, J.T., Ouédraogo, M., Gowda, B.S. and Timko, M.P. (2012) Development of sequence characterized amplified region (SCAR) markers linked to race-specific resistance to *Striga gesnerioides* in cowpea (*Vigna unguiculata* L.). *Afr. J. Biotech.*, **11**, 12555–12562.

Padi, F. (2004) Relationship between stress tolerance and grain yield stability in cowpea. *The Journal of Agricultural Science*, **142**, 431–443. https://doi.org/10.1017/S0021859604004447

Payne, R.W., Harding, S.A., Murray, D.A., *et al.* (2008) *GENSTAT release 11 reference manual. Parts 1, 2 and 3*. Hemel Hempstead, UK: VSN International.

Pottorff, M.O., Wanamaker, S., Ma, Y.Q., Ehlers, J.D., Roberts, P.A. and Close, T.J. (2012) Genetic and physical mapping of candidate genes for resistance to *Fusarium oxysporum f.sp. tracheiphilum* Race 3 in cowpea [*Vigna unguiculata* (L.) Walp.]. *PLoS ONE*, **7**, e41600. https://doi.org/10.1371/journal.pone.0041600

Pottorff, M.O., Li, G., Ehlers, J.D., Close, T.J. and Roberts, P.A. (2014) Genetic mapping, synteny, and physical location of two loci for *Fusarium oxysporum* f. sp. *tracheiphilum* race 4 resistance in cowpea [*Vigna unguiculata* (L.) Walp]. *Mol. Breeding*, **33**, 779–791. https://doi.org/10.1007/s11032-013-9991-0

Sannemann, W., Huang, B.E., Mathew, B. and Léon, J. (2015) Multi-parent advanced generation inter-cross in barley: high-resolution quantitative trait locus mapping for flowering time as a proof of concept. *Mol. Breeding*, **35**, 86. https://doi.org/10.1007/s11032-015-0284-7

Semagn, K., Babu, R., Hearne, S. and Olsen, M. (2014) Single nucleotide polymorphism genotyping using Kompetitive Allele Specific PCR (KASP): overview of the technology and its application in crop improvement. *Mol. Breeding*, **33**, 1–14. https://doi.org/10.1007/s11032-013-9917-x

Shah, R. (2013) mpMapInteractive: interactive components for package mpMap. *R package version*, 2.

Thiaw, S., Hall, A. and Parker, D. (1993) Varietal intercropping and the yields and stability of cowpea production in semiarid Senegal. *Field. Crop. Res.*, **33**, 217–233. https://doi.org/10.1016/0378-4290(93)90081-W

Valdar, W., Flint, J. and Mott, R. (2006) Simulating the collaborative cross: power of quantitative trait loci detection and mapping resolution in large sets of recombinant inbred strains of mice. *Genetics*, **172**, 1783–1797. https://doi.org/10.1534/genetics.104.039313

Wang, J. (2009) Inclusive composite interval mapping of quantitative trait genes. *Acta Agronomica Sinica*, **35**, 239–245. https://doi.org/10.3724/SP.J.1006.2009.00239

Watanabe, I., Hakoyama, S., Terao, T. and Singh, B. (1997) Evaluation methods for drought tolerance of cowpea. In: *Advances in Cowpea Research* (Singh, B.B., Mohan Raj, D.R., Dashiell, K.E. and Jackai, L.E.N., eds). Devon, UK: Sayce Publishing, Copublication of International Institute of Tropical Agriculture (IITA) and Japan International Research Center for Agricultural Sciences (JIRCAS), pp 141–146.

Widders, I.E. (2012) Cowpea: a solution to global challenges. In: *Innovative Research along the Cowpea Value Chain Proceedings of the Fifth World Cowpea Conference on Improving Livelihoods in the Cowpea Value Chain through Advancement in Science*. (Boukar, O., Coulibaly, O., Fatokun, C.A., Lopez, K. and Tamo, M., eds). Saly, Senegal: 27 September - 1 October. International Institute of Tropical Agriculture (IITA).

Xu, S. (2013) Genetic mapping and genomic selection using recombination breakpoint data. *Genetics*, **195**, 1103–1115. https://doi.org/10.1534/genetics.113.155309