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

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SUMMARY

Multi-parent Advanced Generation Inter-Cross (MAGIC) populations are an emerging type of resource to dissect the genetic structure of traits and improve breeding populations. We developed a MAGIC population for cowpea (*Vigna unguiculata* L. Walp.) from eight founder parents which are genetically diverse and carry many abiotic and biotic stress resistance, seed quality and agronomic traits relevant to cowpea improvement in the USA 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 the population would have balanced representation from each parent, followed by single-seed descent, resulting in 305 F8 recombinant inbred lines (RILs) each carrying a mosaic of genome blocks contributed from all founders. This was confirmed by SNP genotyping with the Illumina Cowpea Consortium Array. These lines were on average 99.74% homozygous while 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.

INTRODUCTION

Cowpea (*Vigna unguiculata* L. Walp) is a highly nutritious warm-season grain legume 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 smallholder farmer cowpea grain yields are only 10-20 % of known yield potential (Widders 2012). Biotic stresses caused by insect pests, and 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 southern United States (Ehlers and Hall 1997; Huynh et al. 2013a). 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 QTL discovery and mapping (Huynh et al. 2015; Huynh et al. 2016; Lucas et al. 2012; Muchero et al. 2011; Muchero et al. 2010; Ouédraogo et al. 2012; Ouédraogo et al. 2002b; Pottorff et al. 2014; Pottorff et al. 2012). 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 (Mai-Kodomi et al. 1999a; Watanabe et al. 1997). 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 genotypes exhibiting seedling drought tolerance, two types of responses have been observed by Mai-Kodomi et al. (1999a). Type 1 response plants ceased all growth and conserved moisture in all plant tissues, thereby allowing subsequent recovery of the entire shoot upon re-hydration. In contrast, type 2 response involved plants mobilizing moisture from lower leaves to sustain 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 (Dadson et al. 2005; Hall et al. 2003; Padi 2004). On a physiological level, osmotic adjustment, carbon isotope discrimination, transpiration, assimilation rates, and stomatal conductance in cowpea have been studied (Anyia and Herzog 2004; Hussain et al. 1999; 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 (Ehlers and Hall 1997; Grantz and Hall 1982), 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 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.

Development of MAGIC populations, termed 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) each carrying a mosaic of genome blocks contributed from all founders. 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 8-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

In total 305 MAGIC F8 RILs were generated from unique 8-way crosses derived from six pedigree funnels (Fig. S1). Genotyping with the 51,128-SNP Illumina iSelect BeadArray resulted in 36,346 SNPs that were polymorphic between the 8 parents (68.26%). Among these, 11,848 SNPs were parent-unique, each of which could distinguish one parent from the other 7 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 (Fig. 1).

Recombination rate variation in MAGIC

From the 36,346 polymorphic SNPs, after removing those with MAF $\leq$ 0.05 and successful calling rate $\leq$ 90%, 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 between adjacent SNPs. The crossovers appeared to distribute throughout the MAGIC genomes, at an average of 2.14 cM/Mb, and more frequently on or near the telomeric distal regions of chromosomes (Fig. 2). Several recombination hot-spots with up to 8 cM/Mb 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) while chromosome 11 had the lowest (1.68 cM/Mb).

Phenotypic variation in MAGIC RILs and parents

The F8 lines were highly diverse in morphological traits including flowering time, growth habit, flower color, leaf shape, and seed characteristics (size, shape, color and texture) (Fig. 3). The flowering time varied widely in the population under both long-daylength condition at the UCR Citrus Experiment Station (UCR-CES) and short-daylength condition at the Coachella Valley Agricultural Research Station (CVARS) in California (Fig. 4). The genotypic differences in flowering time were quite stable across contrasting watering regimes in each daylength 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-daylength conditions, although the absolute flowering time varied considerably among lines. At UCR-CES (long daylength), the population started flowering as early as 43 days after planting, but there were many lines with delayed flowering beyond 60 days after planting (Fig. 3a and 4a). In contrast, under short daylength at CVARS, the population started flowering as early as 34 days after planting and the entire population completed flowering within another month (63 days) (Fig. 3b and 4b). Among the parents, CB27 (44 and 36 days) was the earliest to flower while 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 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-daylength conditions (Fig. S2). There was significant but moderate correlation in the growth habit scores between the two daylength conditions ($r = 0.55$, $P < 0.001$), with about 55% of the lines showing consistent growth habit between the two environments. Lines with semi-prostrate growth habit under long-daylength became

intermediate or semi-erect type when grown under short-daylength condition. Among the parents, CB27 (acute erect) and IT84-2049 (erect), IT89KD-288 (semi-erect) and Suvita-2 (semi-erect) maintained their growth habit in both short- and long-daylength 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 as 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 (Fig. S3). The plants generally produced much higher yield and developed larger seeds under full irrigation compared to 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 yielded higher than all parents under restricted irrigation conditions. Among the parents, CB27 consistently had the highest yield and largest seed across the two environments.

QTL identification in MAGIC RILs

Given the high repeatability observed for flowering time under each daylength condition, mean values for each RIL at UCR-CES and CVARS were used in QTL analysis. Four QTLs were identified under long-daylength at UCR-CES (Fig. 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 compared to the photoperiod-sensitive parent IT93K-503-1) (Table 2). The QTL with the second largest effect is located on chromosome 11, explaining approximately 15% of total phenotypic variance, with favorable alleles contributed from CB27 and IT84S-2246 (Table 2). 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 2). Under short-daylength at CVARS, four QTLs affecting flowering time were mapped on chromosomes 1, 4, 5 and 9 (Fig. 5b). Of these, QTLs on chromosomes 4 and 5 seem located in the same regions of QTLs affecting flowering time under long-daylength 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 (Fig. 5b, Table 2).

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 2). At CVARS, the QTL on chromosome 1 also was 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 the flowering time (Table 2).

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 2). 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-daylength condition at CVARS (Table 2).

One minor and one major QTL affecting seed size at CVARS were identified on chromosomes 6 and 8, respectively (Table 2). 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 x 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 2).

Validation of photoperiod QTL in biparental RILs

To verify QTL detected for photoperiod sensitivity in the MAGIC population, a biparental mapping population including 92 F8-derived F9 RILs from a cross between the non-photoperiod sensitive parent CB27 and the photoperiod sensitive IT97K-556-6 was screened under long-daylength at UCR-CES in 2016. Flowering time varied widely in the RIL population (Fig. 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 (LOD = 7.8, explaining 30% of phenotypic variance) (Fig. 7). The early flowering allele was contributed from CB27. 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-daylength condition in 2015 (Table 2 and 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 2-way crosses, plants in each F1 set were heterozygous and homogeneous because the 8 founder parents were fully inbred lines. However, the F1s derived from the 4-way crosses segregated and exhibited significant variation. To capture variation, we performed more than 300 pair-wise reciprocal 8-way crosses between different 4-way F1 individuals (Fig. 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-daylength conditions in summer, but the photoperiod 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 daylength 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 (Fig. 3, 4, S2 and 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 2-way F1 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 8-way RILs and to eliminate those that appeared to be selfed at the 4-way or 8-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 of which were replaced by sister lines that were purposely developed as backups. By removing all erroneous lines and keeping one RIL from each unique 8-way cross, we created a MAGIC core set of 305 RILs that are highly homozygous and genetically distinct from each other and eight parents (Fig. 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 populations of size 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 x 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 (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 1), and represented West Africa and southeastern Africa gene pools (Huynh et al. 2013a). By applying multiple 2-way, 4-way and 8-way intercrosses from those founders, plus seven generations of single seed descent for over 300 independent 8-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 (Fig. 2). We particularly observed that recombination was more frequent in the distal long arm than the distal short arm regions (Fig. 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 by kinship and population structure on QTL analysis 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 highly heritable (Fig. S3b). In contrast, selecting for higher yield will be more difficult given its relatively low heritability (Fig. 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 yielded better 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 1). MAGIC lines that are not photoperiod sensitive 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 more 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 1), 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 through 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), 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. The strategy would be similar to the multi-parent advanced generation recurrent selection (MAGReS) approach proposed recently by Huang et al. (2015), except that (1) prior knowledge of QTL information from cowpea bi-parental mapping will be utilized, (2) the MAGIC RILs selected for intercrosses are more advanced (F8), and (3) 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 both for genetic improvement (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 based on their high genetic diversity characterized by genotyping with 1,536 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 1). 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 University of California–Riverside (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. 2014; Pottorff et al. 2012), 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 1).

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 were rearranged to create various pedigree patterns (funnels) (Fig. S1). In spring 2010, initial

crosses were made between 4 pairs of fully inbred founder parents (IT89KD-288 x IT84S-2049, CB27 x IT82E-18, SuVita 2 x IT00K-1263, and IT84S-2246 x IT93K-503-1) to produce 2-way F1s. In spring 2011, reciprocal 4-way crosses were made between 2 pairs of the 2-way F1s to produce 4-way F1s. In fall 2011, 8-way crosses were made to produce F1s, each derived from a unique cross between different 4-way F1 individuals. Single-seed descent was then applied for each unique 8-way F1 until the F8 generation. Twenty nine crosses produced two or more F8 RILs, which were sister lines separated from earlier generations. These lines were purposely created to maintain the population size. Reciprocal crosses made at the 4-way and 8-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 F8 RIL, seeds from a single F8 plant were harvested and maintained as an original seed stock (F8:9). The F8:9 seeds were then increased in bulk to make F8:10 seeds for phenotyping.

SNP genotyping

The F1 progeny from 2-way crosses were verified by genotyping their F2 seeds (up to 21 seeds per cross) with 89 parent-unique SNPs using the kompetitive allele-specific polymerase chain reaction (KASP) cowpea assay (LGC Genomics Ltd., Hoddesdon, UK) (Semagn et al. 2014), which was converted from the 1536 SNP Illumina GoldenGate Assay developed by Muchero et al. (2009a). True F1 plants were confirmed when polymorphic markers were found segregating in the corresponding F2 progeny.

The F8 single plants derived from 8-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: (1) 3 lines carrying non-parental alleles and 5 lines with excess numbers of heterozygous and ambiguous genotypes were excluded; (2) Based on parent-unique SNPs, 15 lines that did not carry male-parent alleles (i.e., selfed) at the 4-way or 8-way crosses were excluded; (3) Among true 8-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 (4) 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 8 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 8-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. With it, 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) were calculated as the linkage distance divided by the physical distance between the first and the last SNP of each window. The recombination-rate variation 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-daylength conditions during summer, from June (14.5 hours) to September (12.8 hours), at

the UCR Citrus Experiment Station, California (UCR-CES, 33.97°N, 117.34°W). In 2015, each MAGIC RIL and parent was planted in one row of 0.76 m wide and 5.5 m long 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 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 daylength during autumn, from September (12.8 hours) to December (9.9 hours), at the Coachella Valley Agricultural Research Station, California (CVARS, 33.52°N, 116.15°W). In 2015, the population was planted in two blocks receiving different watering regimes (full irrigation and restricted irrigation) and separated by a 6-row buffer (5 m). In each block, each MAGIC RIL and parent was planted in one row of 0.76 m wide and 3.5 m long 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 two 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^{o} with the main stem, (2) Erect, branching angles between 45^{o} – 90^{o} 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, and (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)). Nonparametric correlation analysis (Spearman's rank) was used to examine the consistency in genotypic ranking of the same lines between the two daylength 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 fit in a linear model for each trait. A genomewide significance threshold of 7.56e-05 was determined empirically using the function 'sim.sigthr' with 1000 simulations

from a null distribution. 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 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 F8-derived F9 RILs from a cross between CB27 (photoperiod insensitive) and IT97K-556-6 (photoperiod sensitive) were screened under long-daylength conditions at UCR-CES in 2016. Each RIL and parent were planted in one row 0.76 m wide and 5.5 m long 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 their 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.

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CONFLICT OF INTEREST

The authors have no conflict of interest to declare.

SHORT SUPPORTING INFORMATION LEGENDS

Figure S1. MAGIC breeding design.

Figure S2. Variation in growth habit.

Figure S3. Variation in yield and seed size.

Data S1. MAGIC genotypes and phenotypes.

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Table 1. MAGIC founder parents and their traits relevant to sub-Saharan Africa and other production areas

Name	Source[a]	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	Root-knot nematode resistant
IT84S-2049	IITA	High yielding under drought in Burkina Faso; brown-eye seed; erect growth habit	Resistant to aphid, bacterial blight, viruses, 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, root-knot nematode
IT84S-2246	IITA	High yielding under drought in Burkina Faso and Mozambique; dark-brown seed	Resistant to aphid, bacterial blight, viruses, root-knot nematode

[a] 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.

Table 2. QTL estimates for flowering time and other agronomic traits measured in the 8-parent cowpea MAGIC population grown at UCR-CES (long-daylength) and CVARS (short-daylength) during 2015-2016: Chromosome (Chr), position in centiMorgans (and 1-LOD support interval), flanking markers, Wald statistics, p values, percentage of variance explained (PctVar), and founder effects (± standard errors) contributed by each founder parent relative to IT93K-503-1.

Trait	Chr	Position (SI)	Flanking markers	Wald	P value	PctVar	IT89KD-288	IT84S-2049	CB27	IT82E-18	Suvita-2	IT00K-1263	IT84S-2246
Flowering time (days) under long-daylength at UCR-CES	4	19(16, 20)	2_48582 - 2_09077	39.0	1.99E-06	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
	5	7(1, 9)	2_07393 - 2_12440	31.2	5.72E-05	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
	9	25(23, 28)	2_00738 - 2_00736	146.2	0.00E+00	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
	11	50(47, 59)	2_22669 - 2_44356	67.1	5.81E-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
Flowering time (days) under short-daylength at CVARS	1	56(54, 68)	2_20430 - 2_18422	24.9	7.91E-04	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
	4	20(15, 27)	2_31776 - 2_15171	62.1	5.88E-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
	5	8(5, 12)	2_32176 - 2_18349	39.7	1.43E-06	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
	9	10(7, 13)	2_14794 - 2_20854	57.3	5.19E-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
Growth habit at UCR-CES (1-5)	1	58(54, 61)	2_44007 - 2_24445	44.7	1.59E-07	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
	9	24(22, 28)	2_53750 - 2_33113	46.9	5.75E-08	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
Growth habit at CVARS (1-5)	1	61(59, 63)	2_18049 - 2_08603	84.9	1.33E-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
Seed size (g/100 seeds) at CVARS	6	79(74, 80)	2_14712 - 2_54463	38.7	2.27E-06	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
	8	75(73, 78)	2_32728 - 2_54221	109.5	0.00E+00	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
Maturity (days) at CVARS under normal irrigation	5	10(7, 18)	2_19540 - 2_41253	44.8	1.51E-07	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
Maturity (days) at CVARS under restricted irrigation	2	45(37, 48)	2_10022 - 2_20684	25.0	7.72E-04	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
	5	10(5, 19)	2_19540 - 2_41253	28.5	1.82E-04	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
	9	10(4, 13)	2_14794 - 2_20854	36.5	5.92E-06	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

FIGURE LEGENDS

Figure 1. Phylogenetic relationships among the 305 F8 RILs of the cowpea MAGIC core set and eight parents (in red) based on 11,848 parent-unique SNPs

Figure 2. Recombination rate (cM/Mb) variation along 11 cowpea chromosomes measured in the 8-parent cowpea MAGIC population using a sliding window of 2 Mb with 1 Mb increments.

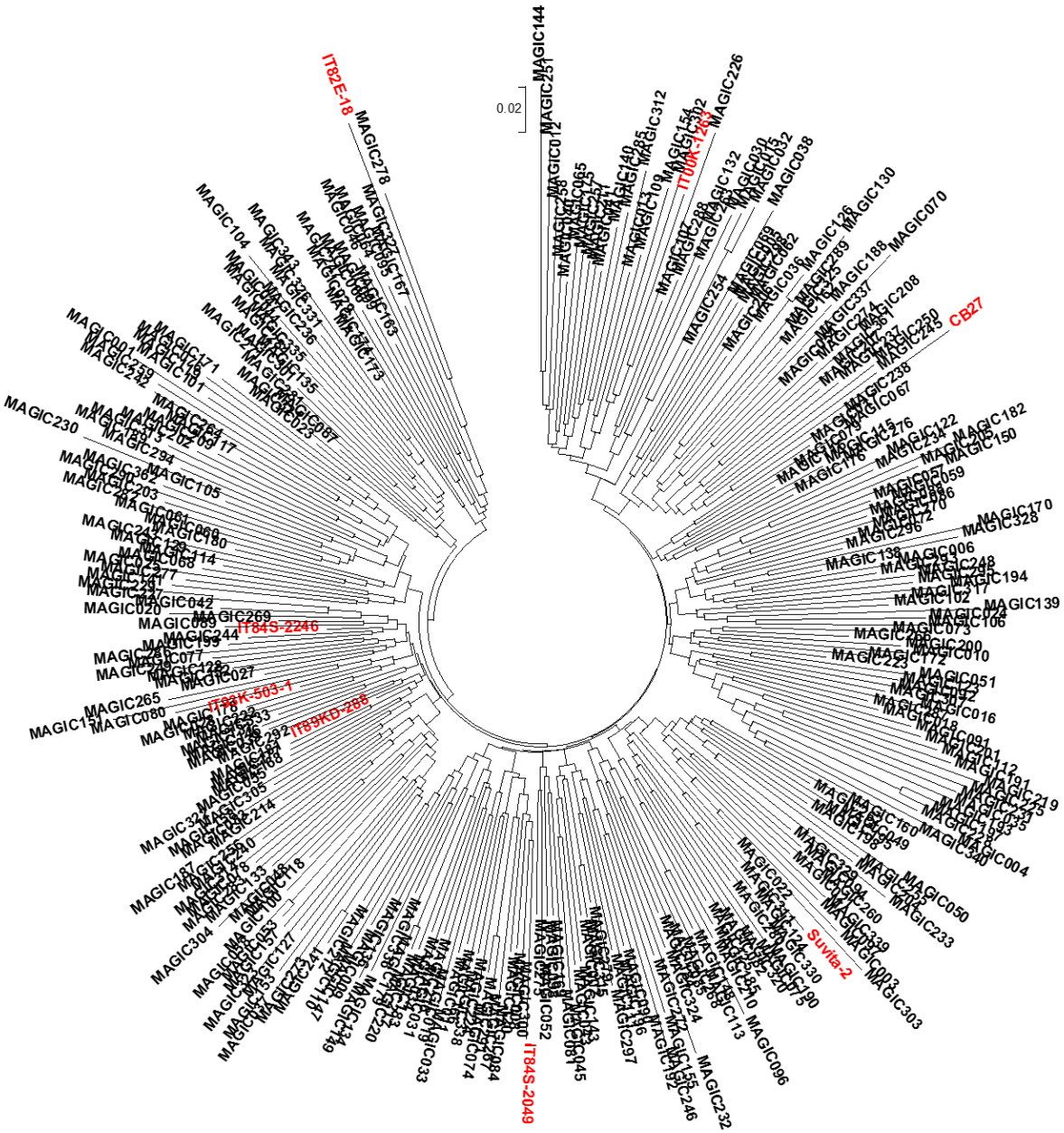
Figure 3. Morphological variation in the cowpea MAGIC population: Plant appearance at 65 days after planting under (a) long-daylength conditions at UCR-CES in 2015 and (b) short-daylength conditions at CVARS in 2016, both under full irrigation; (c) seed appearance, (d) flower color and (e) leaf shape of parents (top panel) and a representation of MAGIC F8 RILs (in lower part of 2c, each seed is from a different F8 RIL). In 2a and 2b, red arrows indicate examples of lines that matured earlier than other lines. In 2d and 2e, 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.

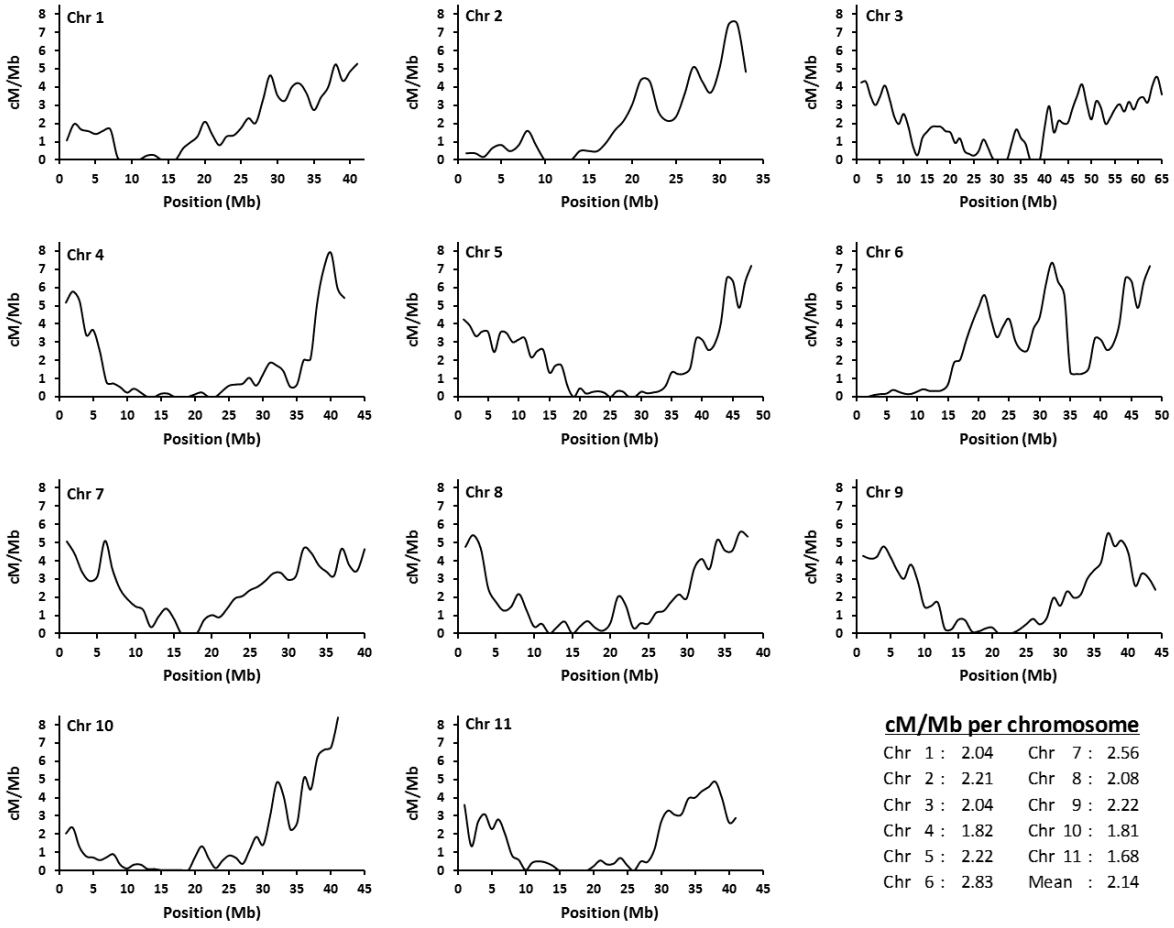
Figure 4. Variation in flowering time measured in the MAGIC core set and eight parents grown under (a) long-daylength conditions at UCR-CES and (b) short-daylength conditions at CVARS. Mean flowering time values for each line were derived from two experiments at UCR-CES and four experiments at CVARS during 2015-2016.

Figure 5. QTL profile for flowering time measured in the MAGIC population grown under (a) long daylength and (b) short daylength conditions at UCR-CES and CVARS, respectively. Green regions indicate 1-LOD support intervals of significant QTLs ($P < 0.05$) in final models. Dash lines indicate the significance threshold at 7.56E-05 using empirical null simulations (N =1000, $P = 0.05$).

Figure 6. Variation in flowering time expressed in the CB27 x IT97K-556-6 biparental RIL population grown under long-daylength conditions at UCR-CES in 2016.

Figure 7. Chromosomal regions associated with variation in flowering time measured in the CB27 x IT97K-556-6 biparental RIL population under long-daylength conditions at UCR-CES in 2016. The LOD peak on linkage group 8 is flanked by SNP markers 2_10023 and 2_04691, which are in the same region of the major QTL detected in the MAGIC population (see Fig. 5).





cM/Mb per chromosome

Chr 1 :	2.04	Chr 7 :	2.56
Chr 2 :	2.21	Chr 8 :	2.08
Chr 3 :	2.04	Chr 9 :	2.22
Chr 4 :	1.82	Chr 10 :	1.81
Chr 5 :	2.22	Chr 11 :	1.68
Chr 6 :	2.83	Mean :	2.14