Figures
Figure 1. Schematic representation of Amplified Fragment Length Polymorphism technique. Selective amplification by only one selective primer pair, E-AAC M-CAA is shown.
Figure 2. Diagrammatic representation of microsatellite analysis. Plant 1, 2 and 3 depict different genomic DNA of plant varieties or species. Arrows indicate primers designed from flanking sequences of specific microsatellite loci. The amplified products are resolved in high resolution agarose gels, polyacrylamide gels or denaturing polyacrylamide gels.
Figure 3. Schematic representation of Bulk Segregant Analysis to identify markers linked to locus conferring susceptibility/resistance to any biotic/abiotic factor. The hypothetical amplification products of sensitive parent, sensitive bulk, resistance bulk and resistant parent are shown. Band type 1 is present in all lanes hence not linked to the trait. Band type 2 and 3 are present only in parent and respective bulk lanes, hence tightly linked to the trait. Bands type 4 and 5 are present in parent and both bulk lanes, hence not very tightly linked to the trait.
Figure 4. The photoperiod, autonomous, vernalization and gibberellin promotive pathways are displayed. Each promotive pathway serves ultimately to upregulate the meristem identity genes, but the precise molecular events effecting this are not known, and therefore each is shown to feed into a pathway integration section. An asterisk indicates that there is genetic evidence for an additional role that is downstream of LFY (Figure modified from Simpson et al., 1999).
Figure 5. Geographical distribution of accessions of chickpea used in this study. Information about accessions is given in Table 1.
Figure 6. AFLP fingerprints generated by each of 30 primer combinations. Accession showing maximum size range of bands was chosen to represent band pattern of each primer combination. Lane marked T denotes standard size marker (AFLP fingerprints of tomato DNA generated using combination number 10 and pre-calibrated using pUC19 sequencing reaction).
Figure 7. AFLP fingerprints of chickpea generated using primer pair E-AAG M-CAA. Lanes marked 1-40 correspond to arbitrary accession numbers (Table 1). Lanes 1-38 represent 38 accessions of *C. arietinum*, Lane 39 represents *C. reticulatum* and Lane 40 represents *C. echinospermum*. Arrow marked M indicates loci monomorphic across all accessions. Arrow marked P indicates polymorphic loci. Arrow marked 102 denotes Russian accession specific band. The empty lane was not loaded.
Figure 8. AFLP fingerprints of chickpea generated using primer pair E-AAC M-CAA. This primer combination detects the maximum bands which are specific to *C. reticulatum* (Lane 39) and *C. echinospermum* (Lane 40) (Shown as dots). Overall the amplification products were monomorphic among 38 accessions of *C. arietinum* (Lanes 1-38). Arrow marked 1 shows polymorphic band number 1 which is amplified in accession number 33 (India) and accession number 39 (*C. reticulatum*) only.
(a)

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Figure 9. Relationship of size range of polymorphic/monomorphic bands (among C. arietinum accessions) with percentage polymorphism/monomorphism. (a) tabular representation of total polymorphic and monomorphic bands along with percentage polymorphism detected in each size range; (b) graphical representation of above data. Red line denotes percentage polymorphism and blue line denotes percentage monomorphism. Dotted red line shows expected percentage polymorphism of bands in size range 300-500 bp.
Figure 10. AFLP fingerprints of chickpea generated using various primer combinations. Arbitrary primer combination number 19 (a), number 17 (b) and number 1 (c) display loci specific to wild types, *C. reticulatum* (Lane 39) and *C. echinospermum* (Lane 40). + CE represents loci amplified only in *C. echinospermum*, – CE represents loci not amplified only in *C. echinospermum*, + CR represents loci amplified only in *C. reticulatum*, – CR represents loci not amplified only in *C. reticulatum*, + CRCE represents loci amplified only in *C. reticulatum and C. echinospermum* and – CRCE represents loci not amplified only in *C. reticulatum and C. echinospermum*. Arrow marked 126 in combination no. 17 displays loci amplified only in six Indian accessions (9, 12, 14, 15, 21, 33).
Figure 11. Graphical representation AFLP analysis of *Cicer*. 
(a) Total loci scanned and (b) Percentage polymorphism generated among three species of *Cicer* using thirty primer combinations. The graph was generated using data from Table 6.
Figure 12. Graphical representation of contribution of each species of *Cicer* to total percentage polymorphism detected. (a) Percentage polymorphism contributed by *C. arietinum* (b) Percentage polymorphism contributed by *C. reticulatum* (c) Percentage polymorphism contributed by *C. echinospermum*. The graph was generated using data from Table 6.
Figure 13. Ability of various primer combinations to differentiate between three species of *Cicer*. The graph was generated using data from Table 7.
Figure 14. Graphical representation of polymorphism detected between any two species of *Cicer*. (a) percentage polymorphism between *C. arietinum* and *C. reticulatum*; (b) percentage polymorphism between *C. arietinum* and *C. echinospermum* (c) percentage polymorphism between *C. reticulatum* and *C. echinospermum*. The graph was generated using data from Table 8.
Figure 15. Graphical representation of AFLP analysis of thirty-eight accessions of *C. arietinum* using thirty primer combinations. (a) Total number of loci detected by each primer combination. (b) Percentage polymorphism generated by each primer combination. The graph was generated using data from Table 9.
Figure 16. Graphical representation of the ability of various AFLP primer combinations to differentiate between thirty-eight accessions of *C. arietinum*. Vertical bars show percentage of accessions differentiated and dotted line shows the polymorphic loci detected by the primer combination. The graph was generated using data from Table 10.
Figure 17. Graphical representation of presence percentage of different types of polymorphic loci detected among thirty-eight accessions of *C. arietinum*. Type of polymorphic loci denotes the number of accessions (out of forty) in which the loci is detected e. g. type 2 denotes polymorphic loci present (black bar) and absent (red bar) in any two accessions. Presence percentage denotes percentage of total polymorphic loci displaying a particular type of polymorphism.
Figure 18. AFLP fingerprints of chickpea generated using primer combination 43 (a), 37 (b) and 38 (c). The numbers from 1 to 38 represent accessions of C. arietinum, 39 represents C. reticulatum and 40 represents C. echinospermum (Table 1). Arrow marked 35 and 73 display loci absent from accession number 3 (Russia). Arrow marked 94 shows loci amplified only in accession number 4 and 5 (Both Nigerian). Lane 38 in (b) contains faulty reaction which was repeated later.
Figure 19. Graphical representation of assessment of thirty AFLP primer combination for each of the thirty-eight accessions of *C. arietinum*. For each accession, the ICRISAT accession number, country and arbitrary accession number is depicted. Arbitrary primer combination numbers are as given in Table 4. Dotted green line denotes total amplified products, dotted yellow line denotes monomorphic products, black vertical bar denotes positive percentage polymorphism and red vertical bar denotes negative percentage polymorphism.
Figure 19. Graphical representation of assessment of thirty AFLP primer combination for each of the thirty-eight accessions of *C. arietinum*. For each accession, the ICRISAT accession number, country and arbitrary accession number is depicted. Arbitrary primer combination numbers are as given in Table 4. Dotted green line denotes total amplified products, dotted yellow line denotes monomorphic products, black vertical bar denotes positive percentage polymorphism and red vertical bar denotes negative percentage polymorphism.
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Figure 20. Dendrogram of forty Cicer sp. (one species each of C. reticulatum, and C. echinospermum, and thirty-eight accessions of C. arietinum) based on AFLP data using UPGMA. The numbers adjacent to country names denote arbitrary accession numbers given in Table 1. A (accessions 1 through 25), B (accessions 9 through 31), and C (accessions 20 through 37), depict three major groups displayed by dendrogram. A1, A2 and A3 represent subgroups within group A.
Figure 21. The plot of Principal Component Analysis. Thirty-eight accessions of *C. arietinum* are seen as one compact cluster. The two wild types, *C. echinospermum* and *C. reticulatum* are distinct and widely separated from each other as well as from *C. arietinum*. 
Figure 22. Bulk Segregant Analysis using AFLP primer combination number 6 (a) and number 23 (b). H and I denote *Hadas* (late flowering parent) and ICC5810 (early flowering parent) respectively. F4 and F5 denote bulks derived from F4 and F5 generations of the progeny respectively. C1 and C2 denote bulks derived from *Hadas* × ICC5810 and ICC5810 × *Hadas* respectively. Arrows marked A6 and A23a show polymorphic bands specific to *Hadas* and arrow marked A23b denotes polymorphic band specific to ICC5810.
Figure 23. AFLP analysis of individual plants using arbitrary primer combination number 6 (a), number 7 (b) and number 9 (c). H and I denote Hadas (late flowering parent) and ICC5810 (early flowering parent) respectively. Numbers of individual plants are as given in Table 3. Arrows indicate polymorphic loci.
Figure 24. AFLP analysis of individual plants using primer combination number 9 (a) and number 12 (b and c). H and I denote Hadas (late flowering parent) and ICC5810 (early flowering parent) respectively. Numbers of individual plants are as given in Table 3. Arrows indicate polymorphic loci.
Figure 25. AFLP analysis of individual plants using primer combination number 13 (a) and number 14 (b and c). H and I denote Hadas (late flowering parent) and ICC5810 (early flowering parent) respectively. Numbers of individual plants are as given in Table 3. Arrows indicate polymorphic loci.
Figure 26. AFLP analysis of individual plants using primer combination number 23 (a and b), and number 25 (c). H and I denote Hadas (late flowering parent) and ICC5810 (early flowering parent) respectively. Numbers of individual plants are as given in Table 3. Arrows indicate polymorphic loci.
Figure 27. AFLP analysis of individual plants using primer combination number 33 (a) and number 39 (b) and number 42 (c). H and I denote Hadas (late flowering parent) and ICC5810 (early flowering parent) respectively. Numbers of individual plants are as given in Table 3. Arrows indicate polymorphic loci.
Figure 28. AFLP analysis of individual plants using primer combination number 49 (a) and number 61 (b). H and I denote Hadas (late flowering parent) and ICC5810 (early flowering parent) respectively. Numbers of individual plants are as given in Table 3. Arrows indicate polymorphic loci.
Figure 29. Bulk Segregant Analysis using primers amplifying STMS loci TA2, TR29, TA43, TS45, TS53 and TS72. Numbers above lanes indicate STMS loci amplified. H, L, E and I denote amplified products of *Hadas* (late flowering parent), ICC5810 (early flowering parent), bulked DNA of ten late flowering plants and bulked DNA of ten early flowering plants respectively. Electrophoresis was performed in a 2% w/v TBE agarose gel. White arrows indicate the two alleles amplified in case of locus TS72.

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- **M**: Loading marker
- **H**: Amplified products of *Hadas* (late flowering parent)
- **L**: ICC5810 (early flowering parent)
- **E**: Bulked DNA of ten late flowering plants
- **I**: Bulked DNA of ten early flowering plants
Figure 30. STMS analysis of individual plants using primer flanking locus TA2. (a) electrophoresis of products in a 2% w/v TBE agarose gel. (b) electrophoresis of amplified products of late individual plants in a 12% PAGE. (c) electrophoresis of amplified products of early individual plants in a 12% PAGE. H, L, E and I denote late flowering parent, late bulk, early bulk and early flowering parent respectively. Numbers of individual plants are as given in Table 3. Arrow marked S2a and S2b depict different alleles amplified from the parents. Lane marked M shows 100 bp standard DNA ladder. Lane marked X is empty.
Figure 31. STMS analysis of individual plants using primer flanking locus TR29. (a) Electrophoresis of products in a 2% w/v TBE agarose gel. (b) Electrophoresis of amplified products of late individual plants in a 12% PAGE. (c) Electrophoresis of amplified products of early individual plants in a 12% PAGE. H, L, E and I denote late flowering parent, late bulk, early bulk and early flowering parent respectively. Numbers of individual plants are as given in Table 3. Arrow marked S29a and S29b depict different alleles amplified from the parents. Lane marked M shows 100 bp standard DNA ladder. Lane marked X is empty.
Figure 32. STMS analysis of individual plants using primer flanking locus TA125. (a) electrophoresis of products in a 2% w/v TBE agarose gel. (b) electrophoresis of amplified products of late individual plants in a 12% PAGE. (c) electrophoresis of amplified products of early individual plants in a 12% PAGE. H, L, E and I denote late flowering parent, late bulk, early bulk and early flowering parent respectively. Numbers of individual plants are as given in Table 3. Arrow marked S125a and S125b depict different alleles amplified from the parents. Lane marked M shows 100 bp standard DNA ladder. Lane marked X is empty.
Figure 33. STMS analysis of early and late individual plants using primers flanking loci (a) TS45, (b) TS53, (c) TA64 and (d) TS72. Electrophoresis of products was performed in a 2% w/v TBE agarose gel. H, L, E and I denote late flowering parent, late bulk, early bulk and early flowering parent respectively. Numbers of individual plants are as given in Table 3. Arrow marked S45a, S53a, S64a, S72a, S45b, S53b, S64b and S72b depict different alleles amplified from the parents. Lane marked M shows 100 bp standard DNA ladder. Lane marked X is empty.
Figure 34. Molecular marker map of polymorphic loci (AFLP and STMS) between late (*Hadas*) and early (ICC5810) flowering chickpea parents. Eighteen loci were mapped to 5 linkage groups (LG1-LG5). Six loci were unlinked. The distance between loci is indicated in centimorgan, 16.6 cM for LG1 at LOD 1.13; 2.6 cM for LG2, LG3, LG4 and LG5 at LOD 4.29. The map has been generated using MAPMAKER ver 3.0. 'C' in parenthesis indicates codominant AFLP loci. Unlinked locus A33ab (C) shows significant degree of linkage with QTL associated with time-to-flowering trait.