

Genetic Linkage Studies in F₂ Cowpea (*Vigna Unguiculata* (L.) Walp) Mapping Populations Using Qualitative Morphological and SNP Markers

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Abstract

The research was conducted to study genetic linkage in 45 F₂ cowpea mapping populations derived from a cross between two inbred lines Golinga (cultivated variety) and a Wild relative. The data used for the study were based on 13 polymorphic SNPs and 17 morphological markers. The segregation ratios were analyzed revealed that five traits (growth habit, growth pattern, leaf shape, plant hairiness and pod hairiness) segregated significantly according to 3:1 mendelian classical ratio for inheritance whilst the remaining 13 showed 9:7 ratios. Genetic linkage mapping was performed by using the software QTLiCIMapping version 4.2. All the markers used were polymorphic. Eleven linkage groups were detected which spanned a total map length of 93.8217 cM at a LOD score of 4. Variation in marker distribution on all the 11 linkage groups was observed. Fourteen markers were distributed on Chromosome 1. In all cases, segregation distortion was observed for all the markers in each of the linkage groups. Segregation distortion in Chromosome 1, Chromosome 5, Chromosome 8 and Chromosome 11 were highly skewed to the wild parental type. This work provides one of the few integrated morphological–SNP linkage maps for cowpea and highlights genomic regions associated with non-Mendelian inheritance. These findings supply foundational genetic information for QTL discovery, marker-assisted selection, and breeding strategies aimed at improving yield, nutrition, and climate resilience in cowpea.

Author Summary

In this study, we set out to understand how important physical traits in cowpea are passed from one generation to the next, and to build a simple genetic map that can guide future crop improvement. Cowpea is a major source of food and nutrition for millions of people, especially in Africa, yet progress in improving the crop has been slowed by limited genetic information.

By crossing a cultivated cowpea variety with its wild relative, we examined how traits such as growth form, leaf shape, pod appearance, and seed characteristics segregate in their offspring. We also combined these observations with DNA markers to create a genetic map showing how these traits and markers are arranged along the chromosomes. Our results show that some traits follow the classic patterns of inheritance, while others are influenced by interactions between pairs of genes. We also discovered regions of the genome where inheritance was skewed toward one parent, offering clues about underlying biological processes. By providing one of the few combined morphological and DNA maps for cowpea, our work creates a foundation that breeders and researchers can use to develop improved, desired, and climate-resilient cowpea varieties.

Introduction

Genetic mapping (also known as genetic linkage mapping or meiotic mapping) is one of the various applications of molecular markers in any species. It refers to the determination of the relative positions of genes on a DNA molecule (Chromosome or plasmid) and the distance between them [1] The genetic map indicates the position and relative genetic distances between markers along chromosomes [2]

The construction of detailed genetic maps with high levels of genome coverage is the first step for some of the applications of molecular markers in plant breeding [3] and serves five purposes: allow a detailed genetic analysis of qualitative and quantitative traits that enable localization of genes or quantitative trait loci [4]; facilitate the introgression of desirable genes or QTLs through marker-assisted selection; allow comparative mapping between different species to evaluate the similarity between genes orders and function in the expression of a phenotype [5,6]; provide a framework for anchoring with physical maps based on Chromosome

translocations, DNA sequence, or other direct measures [4], constitute the first step towards positional or map-based cloning of genes responsible for economically important traits [7]. Genetic-map construction is a critically important tool for further genomic studies, as well as for genetic breeding of economically important species such as cowpea[1].

Cowpea (*Vigna unguiculata* L. Walp) is one of the most ancient crops known to man. Cowpea is also a widely grown legume in tropical and subtropical Africa where it is mostly cultivated by small-scale farmers, usually intercropped with maize or sorghum. The seeds are an excellent source of carbohydrate (50–60%) and an important source of protein (18–35%) [8] They also contain an appreciable quantity of micronutrients such as vitamin A, iron and calcium [9]. It contains significant amounts of polyphenolic compounds that are beneficial to human and animal health including phenols, flavonoids and tannins [10,11]. Cowpeas contain bioactive antioxidants such as vitamin C, carotenoids and phenolic compounds [10].

Highly saturated genetic linkage maps are extremely helpful to breeders and are an essential prerequisite for many biological applications such as the identification of marker-trait associations, mapping Quantitative Trait Loci (QTL), candidate gene identification, development of molecular markers for Marker-Assisted Selection (MAS) and comparative genetic studies [1]. With the rapid development of biotechnology, dominant DNA markers were gradually replaced by co-dominant markers in genetic mapping including Single Nucleotide Polymorphisms (SNPs) and simple-sequence repeats (SSRs) OR microsatellites. Thus, this research work aimed at constructing a genetic linkage map of cowpea using morphological and SNP makers to facilitate the development of improved cowpea varieties to ensure food security and combat malnutrition globally.

Results and Discussion

Segregation pattern of makers

Five traits , namely: growth habit [*GH*], growth pattern [*GP*], leaf shape [*LSH*], plant hairiness [*PLH*] and pod hairiness [*PDHN*] segregated significantly according to 3:1 mendelian classical ratio for inheritance whilst the remaining 13 showed 9:7 ratios (Table 1). Chi-square segregation was calculated at a *P-value* of 0.05 and one and two degrees of freedom. Since all the recorded chi-square values were below that of the expected value, the null hypothesis was failed to be rejected *i.e.*, there were significance difference between the observed and expected ratios.

The results of the current study support monogenic control for growth habit, growth pattern, leaf shape, plant hairiness and pod hairiness. [12] working on cowpea pod shape reported 9:7 ratios for straight pod to curved pods. This agrees with the results obtained in the current study where F₂ ratio of 9:7 was obtained for straight pods to curved pods. This signifies the presence of epistatic interaction of duplicate recessive genes for straight-shaped pods.

[13] also reported digenic control for pod colour in cowpea with a segregation ratio of 9:7 implying the presence of epistatic gene interaction. Similarly, this present study observed a ratio 9:7 ratio in F₂ progenies. This shows the dominance of pigmentation over non-pigmentation, which is in agreement with the work of [14] , who reported that wholly purple pods are dominant over green pods.

Furthermore, in the study of unripped pod tip pigmentation by [13], the F₂ segregated 9:7 ratios. This corroborates with the finding of this study where pod tip colour the F₂ segregated fitted closely to a 9:7 ratio. [13] noted that two pairs of genes appeared to control the trait in a complementary manner.

Table 1: Segregation pattern for qualitative markers

Marker	Trait	Ratio	χ^2
<i>GH</i>	Growth habit	3:1	0.1773*
<i>GP</i>	Growth pattern	3:1	3.1277*
<i>TT</i>	Twinning tendency	9:7	0.5677*
<i>PP</i>	Plant pigmentation	9:7	0.0165*
<i>LSH</i>	Leaf shape	3:1	3.1277*
<i>LSZ</i>	Leaf size	9:7	0.5677*
<i>PLH</i>	Plant hairiness	3:1	2.0496*
<i>LC</i>	Leaf colour	9:7	0.5137*
<i>LT</i>	Leaf texture	9:7	0.0274*
<i>LM</i>	Leaf markings	9:7	2.5562*
<i>PTH</i>	Petiole thickness	9:7	0.5137*
<i>RP</i>	Raceme position	9:7	0.5137*
<i>PDAT</i>	Pod attachment	9:7	0.5137*
<i>PDC</i>	Pod curvature	9:7	0.5137*
<i>PDTH</i>	Pod thickness	9:7	0.5137*
<i>PDCL</i>	Pod colour	9:7	0.5677*
<i>PDHN</i>	Pod hairiness	3:1	2.0496*
<i>PTC</i>	Pod tip colour	9:7	0.0274*

*= significant at 0.05 or 5% level

Construction of the genetic linkage map

Thirteen polymorphic SNP markers and eighteen main morphological markers were used to generate eleven (11) linkage groups at a LOD score of 4.0 (Figure 1). The map contained 31 markers spanning a Chromosome length of 93.82 cM at an average marker length of 3.13 cM (Table 2). Chromosome 1 contained 14 markers spanning a Chromosome length of 57.94 cM at an average marker length of 4.13 cM. Six of the eleven Chromosomes had 1 marker each. Chromosomes 4 and 7 had two markers each spanning total length of 25.19 cM and 10.69 cM, respectively. The long arm of Chromosome 1 had 10 markers while the short arm had 4 markers (Figure 1).

The eleven linkage groups generated in the present study is at variance with the results of; [15] using 125 polymorphic SNP markers generated 12 linkage groups at a LOD score of

3.5. The findings of the current study were in disagreement with [16] who developed the first genetic map using a mapping population of 58 F₂ plants derived from a biparental cross

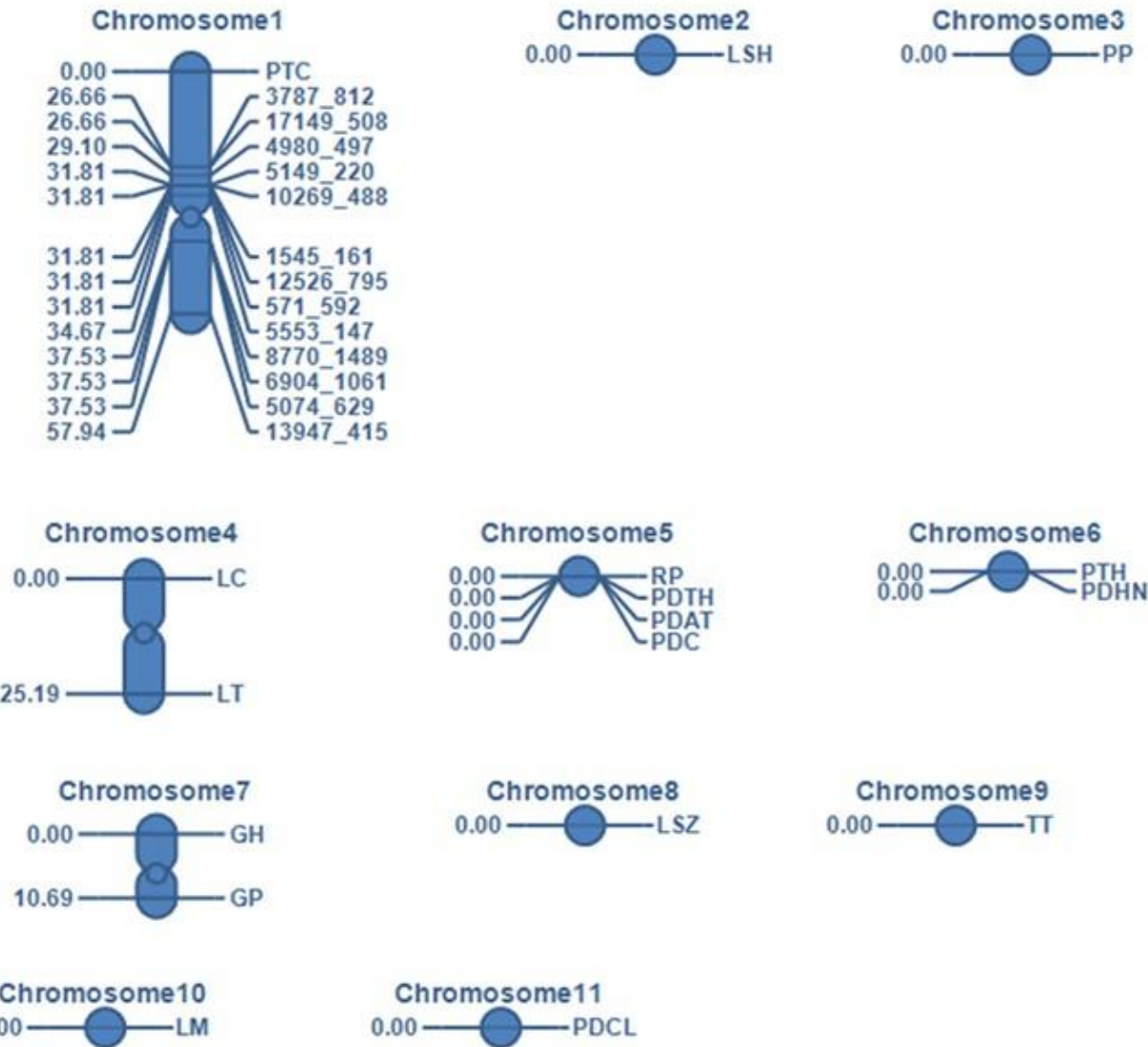


Figure 1: Linkage groups and their associated main morphological markers and SNPs

markersKey: PTC=Pod tip colour, GH=Growth habit, GP=Growth pattern, TT=Twining tendency, PP=Plant pigment, LSZ=Leaf size, LSH=Leaf shape, PTH=Petiole thickness, LC=Leaf colour, LT=Leaf texture, LM=Leaf markings, RP=Raceme position, PDAT=Pod attachment, PDC=Pod curvature, PDTH=Pod thickness, PDCL=Pod colour, PDHN=Pod hairiness

Table 2: Marker information and test of segregation distortion for morphological and SNPs markers

Chr	Marker Name	Position (cM)	RF	IF	Sample size				X ²	Pr>χ ²
					F ₂	Golanga	Wild	Missing		
1	PTC	0.00	0.50	0.00	0.00	27.00	20.00	0.00	49.09	0.0000
	3787_812	26.66	0.76	0.00	0.00	13.00	28.00	6.00	51.98	0.0000
	17149_508	26.66	0.00	0.00	0.00	14.00	28.00	5.00	51.33	0.0000
	4980_497	29.10	0.02	1.92	0.00	15.00	26.00	6.00	46.90	0.0000
	5149_220	31.81	0.03	0.00	0.00	13.00	26.00	8.00	47.67	0.0000
	10269_488	31.81	0.00	0.00	0.00	14.00	26.00	7.00	47.20	0.0000
	1545_161	31.81	0.00	0.00	0.00	14.00	26.00	7.00	47.20	0.0000
	12526_795	31.81	0.00	0.00	0.00	14.00	25.00	8.00	45.21	0.0000
	571_592	31.81	0.00	0.00	0.00	13.00	24.00	10.00	43.54	0.0000
	5553_147	34.67	0.03	1.00	0.00	12.00	26.00	9.00	48.32	0.0000
	8770_1489	37.53	0.03	0.00	0.00	12.00	24.00	11.00	44.00	0.0000
	6904_1061	37.53	0.00	0.00	0.00	13.00	25.00	9.00	45.58	0.0000
	5074_629	37.53	0.00	0.00	0.00	11.00	25.00	11.00	46.89	0.0000
	13947_415	57.94	0.81	0.00	0.00	26.00	13.00	8.00	47.67	0.0000
2	LSH	0.00	0.50	0.00	0.00	30.00	17.00	0.00	54.19	0.0000
3	PP	0.00	0.50	0.00	0.00	17.00	6.00	24.00	33.52	0.0000
4	LC	0.00	0.50	0.00	0.00	23.00	23.00	1.00	46.00	0.0000
	LT	25.19	0.23	0.00	0.00	24.00	20.00	3.00	44.73	0.0000
5	RP	0.00	0.50	0.00	0.00	23.00	24.00	0.00	47.04	0.0000
	PDTH	0.00	0.00	0.00	0.00	18.00	23.00	6.00	42.22	0.0000
	PDAT	0.00	0.00	0.00	0.00	18.00	23.00	6.00	42.22	0.0000
	PDC	0.00	0.00	0.00	0.00	18.00	23.00	6.00	42.22	0.0000
6	PTH	0.00	0.50	0.00	0.00	23.00	16.00	8.00	41.51	0.0000
	PDHN	0.00	0.00	0.00	0.00	23.00	16.00	8.00	41.51	0.0000
7	GH	0.00	0.50	0.00	0.00	13.00	6.00	28.00	24.16	0.0000
	GP	10.69	0.11	0.00	0.00	30.00	17.00	0.00	54.19	0.0000
8	LSZ	0.00	0.50	0.00	0.00	15.00	28.00	4.00	50.86	0.0000
9	TT	0.00	0.50	0.00	0.00	15.00	7.00	25.00	27.82	0.0000
10	LM	0.00	0.50	0.00	0.00	26.00	21.00	0.00	48.06	0.0000
11	PDCL	0.00	0.50	0.00	0.00	18.00	29.00	0.00	52.15	0.0000

PTC=Podtip colour, GH=Growth habit, GP=Growth pattern, TT=Twinning tendency, PP=Plant pigment, LSZ=Leaf size, LSH=Leaf shape, PTH=Petiole thickness, LC=Leaf colour, LT=Leaf texture, LM=Leaf markings, RP=Raceme position, PDAT=Pod attachment, PDC=Pod curvature, PDTH=Pod thickness, PDCL=Pod colour, PDHN=Pod hairiness

generated 10 linkage groups which spanned 680 cM of the cowpea genome. This present study diverged with that of [17] who generated the second cowpea genetic linkage map using 94 F8 recombinant inbred lines (RILs) derived from a cross between two cultivated genotypes *IT84S-2049* and *524B*. A total of 181 loci, comprising 133 RAPDs, 19 RFLPs, 25 AFLPs and three each of morphological and biochemical markers were assigned to 12 LGs spanning 972 cM

with an average distance of 6.4 cM between markers. This second map was improved with the addition of 242 new AFLP markers generated 11 LGs spanning a total of 2670 cM, with an average distance of 6.43 cM between markers [18] similar to the number LGs formed in the current study. Comparable results were observed in the study by [19] , who used AFLP and 5 cowpeas derived microsatellites to construct a linkage map using a set of RILs to generate 11 linkage groups with an average distance between markers of 9.6 cM. These variances in reports may be due to the sample size, differences in genotypes, the differences in marker types and the number of markers used.

In addition, maps developed from crosses between cultivars are most useful for breeding applications as they identify markers that are polymorphic within the cultivated gene pool and are therefore more likely to be present in other cultivated X cultivated crosses used by breeders.

Segregation distortion linkage

There was significant segregation distortion ($P=0.0000$) at all the thirty gene loci. Segregation distortion in Chromosome 1, Chromosome 5, Chromosome 8 and Chromosome 11 were highly skewed to the wild parental type whereas Chromosome 2, Chromosome 3, Chromosome 6 and Chromosome 10 skewed to the Golinga parental type (Table 2).

In a study by [20] , they constructed genetic linkage map and conducted genetic analysis of domestication related traits in mung bean noted segregation distortion ($P=0.05$) was observed for 61 markers. Most of the markers were on Linkage group (LG) 4 showed a high level of segregation distortion ($P=0.0000$) toward a higher-than-expected frequency of the Wild-parent allele. In contrast with this study which reported segregation distortion ($P=0.0000$) was observed in 30 markers with most of the markers observed on Chromosome 1 towards a lower than expected frequency of the wild-parent allele which suggests the association of these

loci in the preferential transmission of the paternal line. Deviation from normal assortment may be due to linkage of some traits and this type of distorted ratios have been observed in studies by [21] in chickpea; [22] in *Phaseolus* and [23] in blackgram.

All the markers located on Chromosome 1, Chromosome 5, Chromosome 8 and Chromosome 11 exhibited strongly distorted segregation with respect to the expected Mendelian inheritance, towards the male parental line (wild). This skewed segregation was also observed in a second F₂ population of 45 plants derived from the biparental cross, confirming the presence of a region of distorted segregation on this linkage group and its heritability. The most skewed markers which were tightly linked to each other, indicated that they may both be closely associated with the genetic factor responsible for segregation distortion in cowpea. According to [24,25], the distorted segregation could be caused by gametophytic factors that affect either male or female gametes. Pollen fertility and meiosis can be analysed to determine their relationship with segregation distortion.

Materials And Methods

Development of mapping population

Forty - five F₂ mapping populations (accR1-accR45) of cowpea were developed from a biparental cross between Golinga (accR46) and a Wild relative (accR47). These accessions were obtained from the Genetics Lab in the Department of Plant and Environmental Biology (DPEB). A single row plot design was used. Two seeds from each F₂ population and the two parental checks (Golinga and Wild) were planted in a pot. A distance of 1m x 1m was maintained between the pots. The 5-row plots consisted of 10 pots.

Segregation pattern of morphological markers

Chi-square test of significance was used to study gene interactions for the F₂ cowpea mapping populations. The segregation ratios were analyzed using the formula:

$$\chi^2 = \sum \frac{(O-E)^2}{E} \quad (1)$$

χ^2 = Test statistic, Σ = the sum of, O = Observed frequency, E = Expected frequency

Genotyping with single nucleotide polymorphism (SNPs) markers

Four leaf discs of 6 mm diameter were punched from 15-day old leaves and collected into 96 deep well PCR plates. Before sampling, leaves were dried at 50°C for 2 days to prevent possible mould development during shipment to the outsourced laboratory. DNA extraction and genotyping were done by sequencing in the United Kingdom hosted by the Laboratory of the Government Chemist, LGC. LGC genomics used a unique proprietary in-house technology (oKtopure™ protocol) to extract the total DNA. Isolated DNA was analyzed using UV spectrophotometry to estimate both the quality and quantity of the DNA, while preliminary PCR at a serial dilution was carried out and the results produced were duplicated to identify the best dilution to run the samples. The SNP genotyping was done using KASP genotyping reactions. KASP™ is also proprietary genotyping technology of LGC™. It consists of three components namely the sample DNA, KASP assay mix and KASP master mix. KASP master mix contains two universal (FRET) fluorescent resonance energy transfer cassettes (FAM and HEX), ROX™ passive reference dye, Taq polymerase, free nucleotides and MgCl₂ in an optimized buffer solution, while the KASP assay mix is specific to the targeted SNP and consists of two competitive, allele-specific forward primers and one common reverse primer. Each forward primer incorporates an additional tail sequence that corresponds to one of two universal FRET cassettes present in the KASP master mix.

Linkage mapping construction

Screening was done to select polymorphic morphological traits and SNPs markers within the mapping populations. Out of 43 SNPs markers that were screened thirteen showed polymorphisms (Table 3). Eighteen polymorphic morphological markers were scored (

Segregation distortion linkage

Segregation distortion linkage was performed by using the software QTL IciMapping version 4.2. The Interval Mapping Method was used for the analysis. A segregation distortion linkage map was constructed using the eighteen morphological and the 13 SNPs markers. (Table 4, S1). The QTL IciMapping software version 4.2 [26] was used for the linkage map construction. The ‘Group’ command was used to identify linkage grouped and the ‘Order’ command was used to establish the most likely order within each linkage group. The orders were confirmed by permuting all adjacent markers by the ‘Ripple’ command. LOD score of 4.0 was used as the threshold for detection of linkage. The analysis was performed by using the Inclusive Composite Mapping with additive and dominance ICIM ADD method.

Table 3: SNPs markers used for Linkage map construction

SNP ID	SNP Num	AlleleY	AlleleX	Sequence
10269_488	12650013	C	A	GATTTCTATAGGATT[A/C]GATTGCCGTCCAAT
12526_795	12650165	C	T	GTAAAGGTTACTATA[T/C]ATTGCATGGAGATC
13947_415	12650273	T	A	CTTGTTACCTGTCAC[A/T]GAGACCTTGTTCTC
1545_161	12650374	C	T	TGGCAAGTACACCCT[T/C]GGTTACAAGACCGT
17149_508	12650484	C	T	GTAACTGATATACC[T/C]CCCTTG TAGACATG
3787_812	12650665	C	T	TCTTACTCATGTCAT[T/C]ACCAAAGCCATCAC
5074_629	12650788	C	T	ATAATAAGATGTTTC[T/C]CATTTTAATGTTCA
5149_220	12650795	G	C	TCTTCAAGTGGAAGA[C/G]AAAATCGAATACTA
5553_147	12650834	C	T	TCTTGTTTGTGACAA[T/C]GGAACAGGAATGGT
571_592	12650847	G	A	TCAGCAAGCTTGAAA[A/G]ATTGCTCTCTTTTG
6796_936	12650924	C	T	TGAATTACTTACATG[T/C]GCTGAGCAGGTTCC
6904_1061	12650932	G	C	AGACTGTGTTGTATT[C/G]CYTGTTTCATTCCG
8770_1480	12651062	G	T	AAATAATAGAAGCTT[T/G]TACAATGTGTCCCA

Table 4: Morphological markers for linkage map construction

Morphological marker	Main marker genetic symbol
Growth habit	<i>GH</i>
Growth pattern	<i>GP</i>
Twinning tendency	<i>TT</i>
Plant pigmentation	<i>PP</i>
Leaf size	<i>LSZ</i>
Leaf shape	<i>LSH</i>
Plant hairiness	<i>PLH</i>
Leaf colour	<i>LC</i>
Leaf texture	<i>LT</i>
Leaf markings	<i>LM</i>
Petiole thickness	<i>PTH</i>
Raceme position	<i>RP</i>
Pod attachment	<i>PDAT</i>
Pod curvature	<i>PDC</i>
Pod thickness	<i>PDTH</i>
Pod colour	<i>PDCL</i>
Pod hairiness	<i>PDHN</i>
Pod tip colour	<i>PTC</i>

Conclusion

All morphological traits studied segregated significantly according to either 3:1 or 9:7 mendelian classical ratio for inheritance. Eleven chromosomes of cowpea (*Vigna unguiculata*) were detected which spanned a total map length of 93.8217 cM at a LOD score of 4. Markers located on Chromosomes 1, 5, 8 and 11 exhibited strongly distorted segregation towards the wild parental types which was observed in the F₂ population. The study shows that that the set of SNPs markers used in this study provides baseline information to study cowpea genome with the aim of facilitating its improvement in the breeding programmes. Therefore, SNP markers could be used to construct genetic linkage map of cowpea genome which can provide basic information towards genetic improvement of cowpea in breeding programmes.

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References

1. Begna T, Yesuf H. Genetic mapping in crop plants. Open Journal of Plant Science [Internet]. 2021;6(1):19–26. Available from: <https://www.agriscigroup.us/articles/OJPS-6-128.php>
2. Collard BC, Jahufer MZ, Brouwer JB, Pang EC. An introduction to markers, quantitative trait loci (QTL) mapping and marker-assisted selection for crop improvement: the basic concepts. Euphytica. 2005;142(1):169–96.
3. Tanksley SD, Young ND, Paterson AH, Bonierbale MW. RFLP mapping in plant breeding: new tools for an old science. Bio/Technology [Internet]. 1989;7:257–63. Available from: <https://go.nature.com/3sXCWng>
4. Yim YS, Davis GL, Duru NA, Musket TA, Linton EW. Characterization of maize BAC libraries for anchoring physical to genetic maps. Plant Physiol [Internet]. 2002;130:1686–96. Available from: <https://bit.ly/3rsX3ty>
5. Ahn S, Tanksley SD. Comparative linkage maps of the rice and maize genomes. Proceedings of the National Academy of Sciences USA [Internet]. 1993;90:7980–4. Available from: <http://bit.ly/3bhyN7U>
6. Paterson AH, Bowers JE, Burow MD, Draye X, Elisk CG. Comparative genomics of plant chromosomes. Plant Cell [Internet]. 2000;12:1523–39. Available from: <http://bit.ly/3uWENut>
7. Vuysteke M, Mank R, Antonise R, Bastiaans E, Senior ML. Two high-density AFLP linkage maps of Zea mays L. and distribution of AFLP markers. Theoretical and Applied Genetics [Internet]. 1999;99:921–35. Available from: <https://bit.ly/3bluf0n>
8. Stancheva IK, Geneva M, Radka M, Donkova M, Hristozkova I, Perfanova M, et al. Nutritional value of cowpea grain grown under different moisture regimes with dual inoculation. Bulgarian Journal of Soil Science. 2016;1:112–21.
9. Prinyawiwatkul W, McWatters K, Beuchat L, Phillips R. Cowpea flour: A potential ingredient in food products. Crit Rev Food Sci Nutr. 1996;36:413–36.
10. Cai R, Hettiarachchy NS, Jalaluddin M. High-performance liquid chromatography determination of phenolic constituents in 17 varieties of cowpeas. J Agric Food Chem. 2003;51:1623–7.

- 305 11. Adjei-Fremah S, Jackai LE, Worku M. Analysis of phenolic content and antioxidant
306 properties of selected cowpea varieties tested in bovine peripheral blood. *Am J Anim*
307 *Vet Sci.* 2015;10(4):235–45.
- 308 12. Amusa OD, Ogunkanmi LA, Adetumbi JA, Akinyosoye ST, Ogundipe OT. Morpho-
309 genetic variability in F2 progeny cowpea genotypes tolerant to bruchid
310 (*Callosobruchus maculatus*). *Unspecified Journal.* 2019;
- 311 13. Mustapha A, Singh B. Inheritance of pod colour in cowpea. *Science World Journal.*
312 2008;3(2):39–42.
- 313 14. Harland SC. Inheritance of certain characters in the cowpea (*Vigna sinensis*). *J Genet.*
314 1920;10:193–205.
- 315 15. Adetumbi JA, Akinyosoye ST, Olowolafe MO, Oloyede-Kamiyo QO, Agbeleye OA.
316 Genetic linkage map of cowpea (*Vigna unguiculata* (L.) Walp) using SNP markers.
317 *Afr J Biotechnol.* 2016;15(20):830–4.
- 318 16. Fatokun CA, Danesh D, Young ND. Molecular taxonomic relationships in the genus
319 *Vigna* based on the RFLP analysis. *Theoretical and Applied Genetics.* 1993;86:97–
320 104.
- 321 17. Menéndez CM, Hall AE, Gepts P. A genetic linkage map of cowpea (*Vigna*
322 *unguiculata*) from a cross between two domesticated lines. *Theoretical and Applied*
323 *Genetics.* 1997;95(8):1210–7.
- 324 18. Ouédraogo JT, Gowda BS, Jean M, Close TJ, Ehlers JD, Hall AE, et al. An improved
325 genetic linkage map for cowpea combining AFLP, RFLP, RAPD, biochemical markers
326 and resistance traits. *Genome.* 2002;
- 327 19. Omo-Ikerodah EE, Fawole I, Fatokun CA. Genetic mapping of QTLs for resistance to
328 flower bud thrips in recombinant inbred cowpea lines. *Afr J Biotechnol.*
329 2007;7(3):263–70.
- 330 20. Isemura T, Kaga A, Tabata S, Somta P, Srinives P, others. Construction of a genetic
331 linkage map and genetic analysis of domestication-related traits in mungbean (*Vigna*
332 *radiata*). *PLoS One.* 2012;7(8):e41304.
- 333 21. Kazan K, Muehlbauer FJ, Weeden NE, Ladizinsky G. Inheritance and linkage
334 relationships of morphological and isozyme loci in chickpea (*Cicer arietinum* L.).
335 *Theoretical and Applied Genetics.* 1993;86(4):417–26.
- 336 22. Koenig R, Gepts P. Segregation and linkage of genes for seed proteins, isozymes, and
337 morphological traits in common bean (*Phaseolus vulgaris*). *Journal of Heredity.*
338 1989;80(6):455–6.
- 339 23. Ghafoor A, Ahmad Z, Munir M, Qureshi AS. Inheritance and linkage among
340 morphological markers in diverse hybrids of *Vigna mungo* (L.) Hepper. *SAARC*
341 *Journal of Agriculture.* 2003;1:181–92.

24. Flandez-Galvez H, Ford R, Pang ECK, Taylor PWJ. An intraspecific linkage map of the chickpea (*Cicer arietinum* L.) genome based on STMS and RGA markers. *Theoretical and Applied Genetics*. 2003;106(8):1447–56.
25. Flandez-Galvez H, Ades PK, Ford R, Pang ECK, Taylor PWJ. QTL analysis for ascochyta blight resistance in an intraspecific population of chickpea (*Cicer arietinum* L.). *Theoretical and Applied Genetics*. 2003;107:1257–65.
26. Wang J, Li H, Zhang L, Meng L. Users' Manual of QTL IciMapping. Beijing, China; Mexico City, Mexico; 2014.

Supporting Information

S1 Table: Morphological traits and sources of cowpea accessions

ID	Plant type	Growth habit	Growth pattern	Twinning tendency	Plant pigment
R1	F ₂ (G X W)	Erect	Indeterminate	None	At the leaf base
R2	F ₂ (G X W)	Acute erect	Determinate	None	None
R3	F ₂ (G X W)	Semi-erect	Indeterminate	Pronounced	Slightly at the nodes
R4	F ₂ (G X W)	Climbing	Determinate	None	None
R5	F ₂ (G X W)	Climbing	Determinate	None	None
R6	F ₂ (G X W)	Erect	Determinate	None	None
R7	F ₂ (G X W)	Erect	Determinate	None	None
R8	F ₂ (G X W)	Climbing	Determinate	None	Slightly at the nodes
R9	F ₂ (G X W)	Erect	Determinate	None	None
R10	F ₂ (G X W)	Prostrate	Indeterminate	Pronounced	None
R11	F ₂ (G X W)	Climbing	Indeterminate	Pronounced	None
R12	F ₂ (G X W)	Prostrate	Indeterminate	Pronounced	Slightly at the nodes
R13	F ₂ (G X W)	Semi-prostrate	Indeterminate	Pronounced	Slightly at the nodes
R14	F ₂ (G X W)	Semi-prostrate	Indeterminate	Pronounced	Slightly at the nodes
R15	F ₂ (G X W)	Prostrate	Indeterminate	Pronounced	Slightly at the nodes
R16	F ₂ (G X W)	Semi-erect	Determinate	Pronounced	Slightly at the nodes
R17	F ₂ (G X W)	Semi-prostrate	Indeterminate	Pronounced	Slightly at the nodes
R18	F ₂ (G X W)	Acute erect	Determinate	None	None
R19	F ₂ (G X W)	Climbing	Determinate	None	None
R20	F ₂ (G X W)	Climbing	Determinate	None	None
R21	F ₂ (G X W)	Erect	Determinate	None	None
R22	F ₂ (G X W)	Erect	Determinate	None	None
R23	F ₂ (G X W)	Semi-erect	Determinate	None	None
R24	F ₂ (G X W)	Erect	Determinate	None	Slightly at the nodes
R25	F ₂ (G X W)	Semi-erect	Determinate	Pronounced	At the leaf base
R26	F ₂ (G X W)	Erect	Determinate	Pronounced	At the leaf base
R27	F ₂ (G X W)	Climbing	Determinate	Slightly pronounced	None
R28	F ₂ (G X W)	Semi-erect	Determinate	None	Slightly at the nodes
R29	F ₂ (G X W)	Erect	Determinate	Slightly pronounced	Intermediate
R30	F ₂ (G X W)	Prostrate	Indeterminate	Slightly pronounced	At the leaf base
R31	F ₂ (G X W)	Semi-prostrate	Indeterminate	Intermediate	Intermediate
R32	F ₂ (G X W)	Semi-prostrate	Indeterminate	Slightly pronounced	Intermediate
R33	F ₂ (G X W)	Semi-erect	Determinate	None	None
R34	F ₂ (G X W)	Climbing	Determinate	Slightly pronounced	Slightly at the nodes
R35	F ₂ (G X W)	Prostrate	Indeterminate	Pronounced	At the leaf base
R36	F ₂ (G X W)	Climbing	Determinate	None	None
R37	F ₂ (G X W)	Erect	Determinate	None	Intermediate
R38	F ₂ (G X W)	Semi-prostrate	Indeterminate	Intermediate	None
R39	F ₂ (G X W)	Erect	Determinate	None	None
R40	F ₂ (G X W)	Semi-erect	Determinate	Pronounced	Slightly at the nodes
R41	F ₂ (G X W)	Semi-prostrate	Indeterminate	Pronounced	Slightly at the nodes
R42	F ₂ (G X W)	Climbing	Determinate	Slightly pronounced	Slightly at the nodes
R43	F ₂ (G X W)	Climbing	Indeterminate	Intermediate	Slightly at the nodes
R44	F ₂ (G X W)	Climbing	Determinate	None	Slightly at the nodes
R45	F ₂ (G X W)	Erect	Determinate	None	None
R46	GOLINGA	Prostrate	Determinate	Pronounced	Slightly at the nodes
R47	WILD	Climbing	Indeterminate	Slightly pronounced	At the leaf base

353 **S1 Table: Morphological traits and sources of cowpea accessions (Continued)**

ID	Plant type	Leaf size	Leaf shape	Plant hairiness	Leaf colour
R1	F ₂ (G X W)	Big	Hastate	Glabrescent	Light green
R2	F ₂ (G X W)	Medium	Sub-globose	Pubescent	Dark green
R3	F ₂ (G X W)	Big	Globose	Short appressed hairs	Dark green
R4	F ₂ (G X W)	Medium	Sub-globose	Glabrescent	Light green
R5	F ₂ (G X W)	Medium	Sub-globose	Glabrescent	Light green
R6	F ₂ (G X W)	Medium	Sub-globose	Glabrescent	Light green
R7	F ₂ (G X W)	Medium	Sub-globose	Glabrescent	Light green
R8	F ₂ (G X W)	Medium	Sub-globose	Pubescent	Dark green
R9	F ₂ (G X W)	Medium	Sub-globose	Glabrescent	Light green
R10	F ₂ (G X W)	Big	Globose	Pubescent	Dark green
R11	F ₂ (G X W)	Big	Globose	Pubescent	Dark green
R12	F ₂ (G X W)	Big	Globose	Pubescent	Light green
R13	F ₂ (G X W)	Big	Sub-globose	Glabrescent	Dark green
R14	F ₂ (G X W)	Big	Globose	Pubescent	Light green
R15	F ₂ (G X W)	Big	Globose	Pubescent	Dark green
R16	F ₂ (G X W)	Big	Sub-hastate	Glabrescent	Dark green
R17	F ₂ (G X W)	Small	Sub-hastate	Glabrescent	Dark green
R18	F ₂ (G X W)	Medium	Sub-globose	Short appressed hairs	Light green
R19	F ₂ (G X W)	Medium	Sub-globose	Glabrescent	Dark green
R20	F ₂ (G X W)	Medium	Sub-globose	Glabrescent	Dark green
R21	F ₂ (G X W)	Small	Sub-globose	Glabrescent	Dark green
R22	F ₂ (G X W)	Medium	Sub-globose	Glabrescent	Dark green
R23	F ₂ (G X W)	Big	Globose	Pubescent	Dark green
R24	F ₂ (G X W)	Medium	Sub-globose	Glabrescent	Light green
R25	F ₂ (G X W)	Big	Hastate	Glabrescent	Dark green
R26	F ₂ (G X W)	Big	Sub-hastate	Glabrescent	Light green
R27	F ₂ (G X W)	Big	Globose	Glabrescent	Light green
R28	F ₂ (G X W)	Medium	Sub-globose	Short appressed hairs	Light green
R29	F ₂ (G X W)	Narrow	Hastate	Pubescent	Variegated
R30	F ₂ (G X W)	Narrow	Hastate	Short appressed hairs	Dark green
R31	F ₂ (G X W)	Narrow	Hastate	Pubescent	Light green
R32	F ₂ (G X W)	Medium	Sub-hastate	Glabrescent	Light green
R33	F ₂ (G X W)	Big	Globose	Short appressed hairs	Dark green
R34	F ₂ (G X W)	Medium	Sub-globose	Glabrescent	Light green
R35	F ₂ (G X W)	Narrow	Hastate	Pubescent	Dark green
R36	F ₂ (G X W)	Medium	Sub-globose	Short appressed hairs	Light green
R37	F ₂ (G X W)	Medium	Hastate	Pubescent	Dark green
R38	F ₂ (G X W)	Medium	Sub-globose	Pubescent	Dark green
R39	F ₂ (G X W)	Medium	Sub-hastate	Glabrescent	Light green
R40	F ₂ (G X W)	Medium	Sub-hastate	Glabrescent	Light green
R41	F ₂ (G X W)	Medium	Sub-hastate	Pubescent	Dark green
R42	F ₂ (G X W)	Medium	Sub-hastate	Short appressed hairs	Dark green
R43	F ₂ (G X W)	Medium	Sub-globose	Glabrescent	Light green
R44	F ₂ (G X W)	Medium	Sub-hastate	Pubescent	Light green
R45	F ₂ (G X W)	Medium	Sub-globose	Short appressed hairs	Light green
R46	GOLINGA	Big	Globose	Glabrescent	Light green
R47	WILD	Medium	Hastate	Pubescent	Dark green

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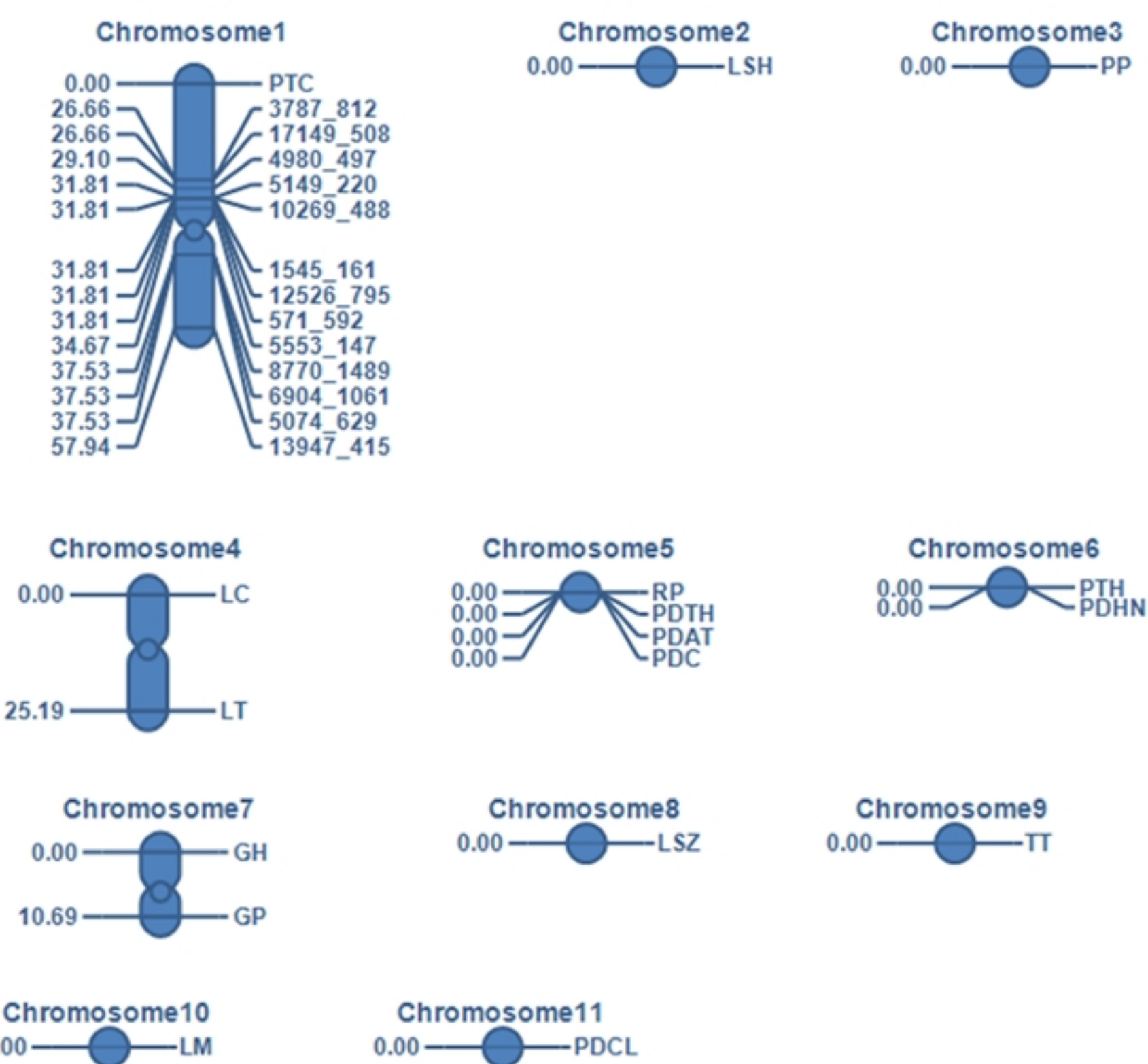
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356 **S1 Table: Morphological traits and sources of cowpea accessions (Continued)**

ID	Plant type	Leaf texture	Leaf markings	Petiole thickness	Raceme position
R1	F ₂ (G X W)	Cariaceous	Present	Thick	Uppermost canopy
R2	F ₂ (G X W)	Cariaceous	Absent	Intermediate	Throughtout canopy
R3	F ₂ (G X W)	Cariaceous	Present	Thick	Uppermost canopy
R4	F ₂ (G X W)	Cariaceous	Absent	Thick	Uppermost canopy
R5	F ₂ (G X W)	Cariaceous	Absent	Thin	Throughtout canopy
R6	F ₂ (G X W)	Cariaceous	Absent	Thin	Throughtout canopy
R7	F ₂ (G X W)	Intermediate	Present	Intermediate	Uppermost canopy
R8	F ₂ (G X W)	Cariaceous	Present	Thick	Uppermost canopy
R9	F ₂ (G X W)	Intermediate	Present	Thick	Uppermost canopy
R10	F ₂ (G X W)	Membranous	Absent	Thin	Throughtout canopy
R11	F ₂ (G X W)	Membranous	Present	Thick	Uppermost canopy
R12	F ₂ (G X W)	Cariaceous	Present	Thin	Throughtout canopy
R13	F ₂ (G X W)	Membranous	Absent	Thick	Uppermost canopy
R14	F ₂ (G X W)	Cariaceous	Present	Thin	Throughtout canopy
R15	F ₂ (G X W)	Membranous	Present	Thin	Throughtout canopy
R16	F ₂ (G X W)	Membranous	Present	Thin	Throughtout canopy
R17	F ₂ (G X W)	Cariaceous	Absent	Thick	Uppermost canopy
R18	F ₂ (G X W)	Cariaceous	Present	Thick	Uppermost canopy
R19	F ₂ (G X W)	Membranous	Absent	Thick	Uppermost canopy
R20	F ₂ (G X W)	Membranous	Absent	Thick	Uppermost canopy
R21	F ₂ (G X W)	Cariaceous	Absent	Thick	Uppermost canopy
R22	F ₂ (G X W)	Cariaceous	Absent	Thick	Uppermost canopy
R23	F ₂ (G X W)	Membranous	Present	Intermediate	Throughtout canopy
R24	F ₂ (G X W)	Cariaceous	Absent	Thin	Throughtout canopy
R25	F ₂ (G X W)	Membranous	Absent	Thick	Uppermost canopy
R26	F ₂ (G X W)	Membranous	Absent	Thick	Uppermost canopy
R27	F ₂ (G X W)	Cariaceous	Absent	Thick	Uppermost canopy
R28	F ₂ (G X W)	Cariaceous	Absent	Thin	Throughtout canopy
R29	F ₂ (G X W)	Cariaceous	Present	Thin	Throughtout canopy
R30	F ₂ (G X W)	Membranous	Present	Thick	Uppermost canopy
R31	F ₂ (G X W)	Cariaceous	Present	Thin	Throughtout canopy
R32	F ₂ (G X W)	Cariaceous	Absent	Thin	Throughtout canopy
R33	F ₂ (G X W)	Membranous	Present	Thin	Throughtout canopy
R34	F ₂ (G X W)	Cariaceous	Absent	Intermediate	Throughtout canopy
R35	F ₂ (G X W)	Membranous	Present	Thick	Uppermost canopy
R36	F ₂ (G X W)	Cariaceous	Absent	Thick	Uppermost canopy
R37	F ₂ (G X W)	Membranous	Present	Thick	Uppermost canopy
R38	F ₂ (G X W)	Intermediate	Present	Thin	Throughtout canopy
R39	F ₂ (G X W)	Membranous	Present	Thin	Throughtout canopy
R40	F ₂ (G X W)	Membranous	Present	Intermediate	Throughtout canopy
R41	F ₂ (G X W)	Membranous	Present	Thick	Uppermost canopy
R42	F ₂ (G X W)	Membranous	Present	Thin	Throughtout canopy
R43	F ₂ (G X W)	Cariaceous	Present	Thick	Uppermost canopy
R44	F ₂ (G X W)	Membranous	Absent	Thin	Throughtout canopy
R45	F ₂ (G X W)	Cariaceous	Present	Intermediate	Throughtout canopy
R46	GOLINGA	Cariaceous	Present	Thin	Throughtout canopy
R47	WILD	Membranous	Absent	Thick	Uppermost canopy

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Figure