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Genetic Mapping of QTLs for Agronomic Traits in Pigeonpea (*Cajanus cajan* L.)

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ABSTRACT

Background: Pigeonpea plays a crucial role in providing food and nutritional security to the people across the world. Productivity of pigeonpea has remained very low and stagnant over last five decades because of low genetic potential, poor plant type and longer crop duration of the existing cultivars. There is need to restructure the plant type through genomic tools to enhance the productivity. Therefore, the present study was done to identify quantitative trait loci (QTLs) associated with key agronomic traits, including plant height, number of primary and secondary branches, number of pods per plant, pod length and number of seed per pod.

Methods: A population of 144 $F_{2:3}$ lines derived from cross between genetically diverse genotypes ICPL84023 and ICP7035 was used to construct a linkage map using SSR markers.

Result: All phenotypic traits varied widely and skewness value for plant height, pod length, and seed per pod was less than 1.0 and for number of primary branch, secondary branch and pod per plant it was more than 1.0. Single marker analysis detected 7 SSR markers associated with 5 agronomic traits; CcGM17620 located on LG_Cc8, showed maximum phenotypic variance of 19.33% for plant height. Composite interval mapping identified 2 QTLs, qPH5.1 and qPH8.1 with PVE of 3.57% and 72.52% respectively and one QTL, qPD3.1 for number of pod per plant with PVE of 8.11%. The QTLs identified in this study provide a strong foundation for further validation and fine mapping for utilization in pigeonpea improvement program.

Key words: Linkage map, Pigeonpea, Plant type, QTL mapping, SSR marker.

INTRODUCTION

Pigeonpea [*Cajanus cajan* (L.) Millsp.] is an important grain legume cultivated mainly in tropical and subtropical regions of the world (Sarkar *et al.,* 2020). India is the largest producer with acreage of 4.90 million hectare and production of 4.22 million tons (FAOSTAT 2022). Maharashtra is the highest pigeonpea producing state followed by Karnataka, Uttar Pradesh, Madhya Pradesh, Gujarat, Jharkhand, Telangana and Andhra Pradesh. Pigeonpea is a potential source of protein, vitamins, vital amino acids and minerals. Pigeonpea is a multipurpose crop, its seeds are consumed as whole grain or as split dal, while the seed husks and leaves are used for livestock feed/fodder and the stem is used to make baskets, thatching, fencing and making houses. Hardy, drought tolerance nature and its ability to fix nitrogen and soil erosion make pigeonpea a crop of choice for small-holding marginal farmers of rainfed areas. Pigeonpea productivity has remained very low and stagnant over last few decades. Major concern in enhancing pigeonpea productivity is biotic and abiotic stresses along with low genetic potential of existing cultivars having low harvest index, poor plant type and long crop duration (Odeny *et al.,* 2007).

Productivity of pigeonpea per unit area and time can be enhanced by restructuring plant type for high harvest index and mechanical harvesting as well as earliness for increasing cropping intensity of the farming system (Saxena and Sharma 1990; Saxena 2008; Kumawat *et al.,* 2012). Ideotype breeding for traits such as plant height, number of primary and secondary branches, number of pods per plant and synchronous maturity play important role in shaping the plant architecture. It attempts to combine various component traits in a plant genotype (Dhanasekar *et al.,* 2010). Over the last five decades, breeding activities targeted at enhancing pigeonpea production have resulted in developing more than 100 improved varieties in India (Singh *et al.,* 2016). However, developing suitable plant type through conventional breeding is cumbersome task. (Varshney *et al.,* 2013). There is urgent need to strengthen pigeonpea breeding programs with new genomic technologies in order to improve the genetic gain efficacy (Bohra *et al.,* 2017). Marker assisted selection can add pace to the conventional breeding but use of MAS is restricted due to less availability of QTLs linked with traits.

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Recently a large number of SSR and SNP makers have been developed in pigeonpea creating opportunities for a large-scale mapping of genes and QTLs for important traits (Varshney *et al.,* 2012). QTL mapping studies has led to the discovery of number of QTLs associated with different plant type traits including plant height, days to flowering, growth habit, branching pattern, pod yield (Kumawat *et al.,* 2012; Geddam *et al.,* 2014; Saxena *et al.,*2017, 2018). However, the results of QTL mapping studies need to be validated across population and environment before utilizing them in marker assisted breeding. Identification of molecular markers and stable QTLs associated with important agronomic traits will help in developing cultivars with desirable plant type. Therefore, present research work was undertaken to identify markers and map QTLs associated with agronomic traits. The information generated through this study could pave the way for an efficient ideotype breeding for development of superior pigeonpea cultivars.

MATERIALS AND METHODS

Plant material

F_2 and $F_{2:3}$ population derived from an intra-specific cross between genetically diverse pigeonpea genotypes ICPL84023 and ICP7035 were used for the present study. ICPL84023 has a compact dwarf plant type, determinate growth habit, early maturity and large number of pods per plant whereas ICP7035 has tall profuse plant type, indeterminate growth habit, late maturity and less number of pods per plant. Original seed of parents were obtained from ICRISAT, Patancheru, Hyderabad.

Experimental details and trait Phenotyping

The 144 $F_{2:3}$ families and two parental lines were planted in single row with 100x35 cm spacing at the research farm of Bihar Agricultural University, Sabour in July 2022. The field was managed following standard agronomic practices. Observations were recorded for plant height (PH in cm), number of primary branch (PB), number of secondary branch (SB), number of pods per plant (PD), Pod length (PL) and number of seed per pod (SP). Five plants from middle of each row were used for trait scoring and data was recorded for each plant and mean data was used for QTL mapping. All plant type traits *viz,* PH, PB, SB, PL, SP and PD were recorded at maturity.

Genotyping and construction of linkage maps

Genomic DNA was isolated from 2 g leaf sample of each of the F_2 plants grown in previous year 2021 using cetyl trimethyl ammonium bromide (CTAB) method Doyle (1991) with minor modifications. Total 112 SSR markers selected on the basis of genome coverage and polymorphism report of Bohra *et al.,* (2017) were used for polymorphism survey between the parental lines ICPL84023 and ICP7035. The SSR markers were PCR amplified and separated by electrophoresis in high resolution 2.5% agarose gels. Genomic DNAs (100 ng per sample) were used as templates for the SSR genotyping. Allelic segregation at each of the marker loci was analysed for deviation from the expected 1:2:1 ratio in the F_2 population using χ 2 test and linkage maps were constructed using Map maker software. A cut-off recombination value of 0.35 and threshold LOD score of 5.0 was used. The Kosambi function was used for the estimation of map distances. Adjusted genetic map lengths (in cM) were calculated.

Statistical analysis and QTL mapping

Statistical analysis of the phenotypic data was performed using Microsoft excel. Pearson's correlation coefficients were calculated for pairwise trait combinations. The QTL analysis was performed using means of the phenotypic trait values. The genetic data along with phenotypic data for agronomic trait of selected progeny in $F_{2:3}$ segregating population was subjected to QTL mapping using QTL cartographer software version 2.5. QTLs were identified by using Single Marker Analysis (SMA) and Composite Interval Mapping (CIM), (Lander and Botstein. 1989) procedure of Windows QTL Cartographer v.2.5 software.

RESULTS AND DISCUSSION

Phenotyping for agronomic trait

A $F_{2:3}$ population (144) derived from an intra-specific cross between pigeonpea genetically diverse genotypes ICPL84023 and ICP7035 was used for the present study. ICPL84023 has a compact dwarf plant type, determinate growth habit, early maturity and large number of pods per plant as compared to ICP7035 having tall profuse plant type, indeterminate growth habit, late maturity and less number of pods per plant (Fig 1). Phenotypic observations of the F2:3 families and parents were recorded for plant height (PH in cm), number of primary branch (PB), number of secondary branch (SB), number of pods per plant (PD), Pod length (PL) and number of seed per pod (SP) at maturity. Five plants from middle of each row were used for trait scoring. All the traits varied widely and skewness value for PH, PL and SP was less than one whereas it was more than one for PB, SB and PD.

Plant height shows bimodal distribution patterns indicating involvement of major genes whereas PL and SP showed normal distribution pattern suggesting involvement of multiple genes. Positive skewness in PB, SB and PD suggest deviation from normal distribution as well as presence of complementary gene action for these traits (Fig 2). Transgressive segregation beyond both the parents was observed for all the traits except pod length (Table 1).

Significant variability was observed for all plant type traits in the mapping population. Similar observations were recorded for plant type and earliness trait in pigeonpea $F_{2:3}$ mapping population by Kumawat *et al.* (2012) and in F_2 population by Randive *et al.* (2018). Skewness value of less than one for PH, PL and SP and more than one for SB is in line with report of Kumawat *et al.,* (2012). Whereas deviation in skewness value was observed for PB and PD.

Plant height shows bimodal distribution patterns indicating involvement of major genes similar distribution pattern for plant height is reported by Kumawat *et al.* (2012) and Parekh *et al.* (2016). PL and SP showed normal distribution pattern suggesting involvement of multiple genes which is as per report of Lwin *et al.* (2022). Positive skewness in PB, SB and PD suggest deviation from normal distribution as well as presence of complementary gene

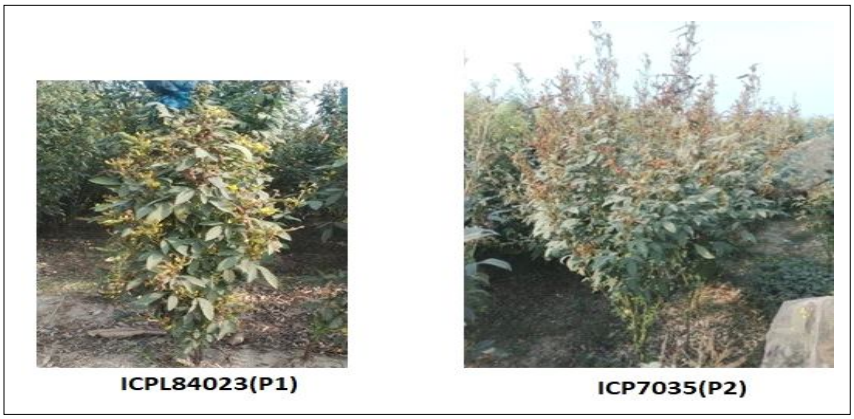


Fig 1: Field photograph of the parental genotypes.

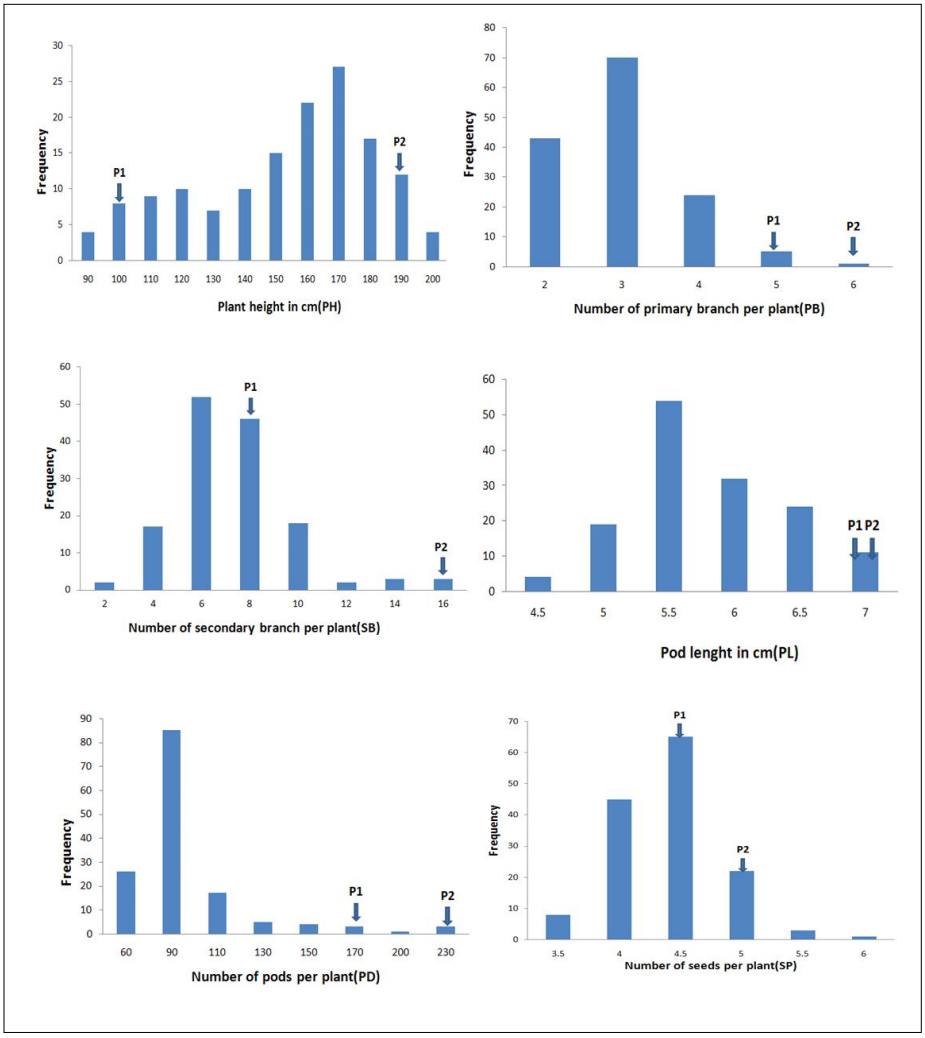


Fig 2: Frequency distribution patterns for six agronomic traits in $F_{2:3}$ population derived from cross between ICPL84023 (P1) and ICP 7035 (P2).

action for these traits. Transgressive segregation beyond both the parents was observed for all the traits except pod length which is in line with report of Kumawat *et al.* (2012).

Correlation analysis of agronomic traits

Deciphering genetic correlation between various traits gives information regarding presence of pleiotropy, linkage, prepotency and functional relationship between traits. In present study significant positive correlation was obtained between number of primary and secondary branch; plant height and pod length; pod length and number of seed per pod; remaining characters showed non-significant association (Table 2). Similar correlation pattern between traits was reported by Kumawat *et al.,* (2012), Geddam *et al.,* (2014), Sharma *et al.,* (2023) and Vanirajan *et al.,* (2023).

Positive correlation between traits showed that selection for one trait will concurrently results in changes of the other related trait and could be improved simultaneously. Whereas negatively correlated traits showed adverse effect on each other and not used to improve simultaneously (Falconer, 1960).

Construction of linkage map and QTL mapping

Linkage map was constructed using genotypic data of 36 polymorphic SSR markers generated on F_2 population through Map Maker software (Fig 3). Eleven linkage groups were generated which is equivalent to haploid chromosome number of pigeonpea (Fig 4).

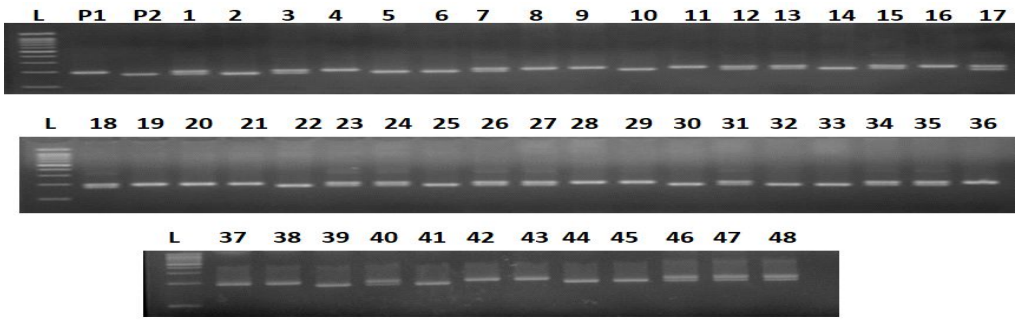
Markers associated with QTLs and flanking chromosomal regions linked with traits of interest can be identified by using single marker analysis (SMA) and composite interval mapping (CIM) respectively based on molecular markers data and phenotypic data of selected genotypes from F_2 population. Single marker analysis was done using QTL cartographer software. Seven SSR markers associated with 5 plant type traits were identified through

Table 1: Descriptive statistics of agronomic traits in parents and $F_{2:3}$ population.

Traits	Phenotypic Observations (Agronomic Traits)					
	Parent		$F_{2:3}$ population			
	ICPL84023	ICP7035	Mean	Range	Std. Dev.	Skewness
Plant height (cm; PH)	93.90	186.20	148.01	85.40-198.20	28.41	-0.49
No. of primary branch per plant (PB)	4.60	5.40	2.66	1.20-7.50	0.87	1.81
No. of secondary branch per plant (SB)	7.60	15.80	6.57	2.0-16.50	2.64	1.40
Pod length (cm; PL)	6.66	7.74	5.55	4.30-6.93	0.58	0.39
No. of pod per plant (PD)	201.20	153.80	83.47	41.20-218.60	30.37	2.12
No. of seed per pod (SP)	4.40	5.00	4.25	3.0-5.66	0.40	0.18

Table 2: Pearson correlation coefficients among agronomic traits.

	PH	PB	SB	PD	PL	SP
PH	1					
PB	0.04	1.00				
SB	0.11	0.52**	1.00			
PD	-0.05	0.28	0.20	1.00		
PL	0.41**	-0.11	0.03	0.23	1.00	
SP	0.31*	0.00	0.10	0.04	0.68**	1.00



L- Ladder 100bp, P1- Parent 1 (ICPL84023), P2- Parent 2 (ICP7035); 1 to 48-F_2 plants.

Fig 3: Genotyping of F_2 population using SSR marker.

single marker analysis (Table 3). Three markers CcGM19565, CcGM10737, CcGM17620 located on LG_Cc3, LG_Cc6 and LG_Cc8 were found to be significantly associated with plant height explaining phenotypic variance of 10.63%, 15.35% and 19.33% respectively. Marker CcGM16802 located on LG_Cc8 was found to be associated with two traits *i.e* number of primary branch and number of secondary branches simultaneously. Among all markers identified through SMA, CcGM17620 located on LG_Cc8, showed maximum phenotypic variance of 19.33% for plant height.

Similar results were reported by Randive *et al.* (2018) who have identified 9 SSR markers to be significantly associated with earliness *i.e.* days to 50 % flowering and days to maturity in F_2 population of pigeonpea. Similarly, Boranayaka *et al.* (2018) also reported two SSR markers RM518 and RM225 to be significantly associated with water use efficiency and nitrogen use efficiency in F_2 mapping population of rice through single marker analysis.

Composite interval mapping revealed one minor and one major QTL for plant height namely qPH5.1 and qPH 8.1 on the linkage group LG_Cc5 in the marker interval CcGM08129-CcGM06586 with PVE of 3.57% and on the linkage group LG_Cc8 in the marker interval CcGM19907-CcGM17620 with PVE of 72.52% respectively (Table 4; Fig 5). One minor QTL was also identified for the number of pods per plant, namely qPD3.1 on the linkage group LG_Cc3 in the marker interval CcGM19565-CcGM14521 with PVE of 8.11% (Table 4; Fig 6).

High phenotypic variance for plant height has also been reported by Kumawat *et al.* (2012). The high PVE by the QTL for this trait indicates involvement of segregating alleles of only a few critical genes leading to large change in the plant architecture of the two parents for plant height. However, this could also be due to overestimation of QTL due to small population size. Validation of QTLs for plant ideotype, earliness and growth habits reported by Kumawat *et al.* (2012) was done in RIL population by Geddam *et al.,*

Table 3: Single marker analysis.

	Single marker analysis (SMA)				
Trait	Chromosome	Marker	Additive effect	Probability	PVE (%)
Plant height	3	CcGM19565	-12.86	0.0244	10.63
	6	CcGM10737	16.23	0.010**	15.35
	8	CcGM17620	-15.91	0.001**	19.33
No. of primary branch	8	CcGM16802	-0.62	0.013*	12.57
No. of secondary branch	8	CcGM16802	-1.46	0.032*	9.96
Pod length	4	CcGM17845	0.23	0.021*	11.37
No. of seed per pod	5	CcGM12371	0.12	0.054	8.23

Table 4: Composite Interval Mapping.

	Composite interval mapping (CIM)					
Trait	Chromosome	Marker interval	Distance (cM)	LOD	Additive effect	PVE (%)
Plant height	5	CcGM08129-CcGM06586	58.7	5.08	17.22	3.57
Plant height	8	CcGM19907-CcGM17620	98.4	5.24	-22.12	72.52
Number of pod per plant	3	CcGM19565-CcGM14521	79.8	5.07	-47.83	8.11

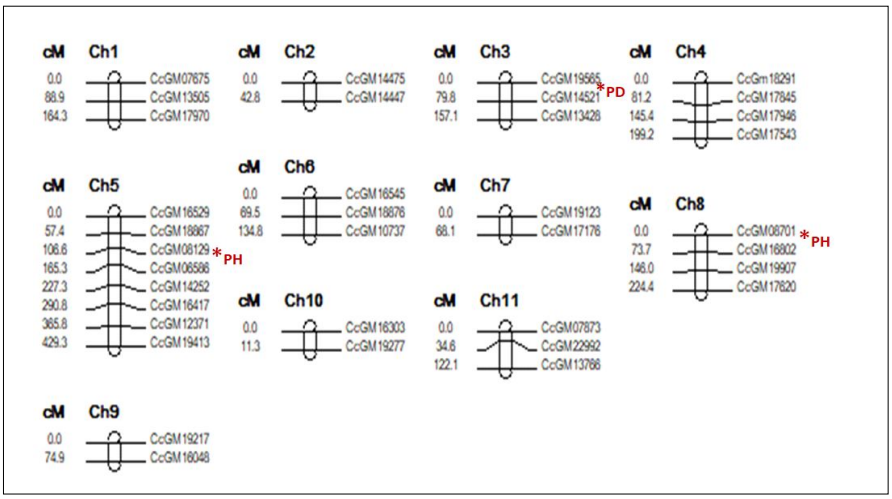


Fig 4: Linkage map.

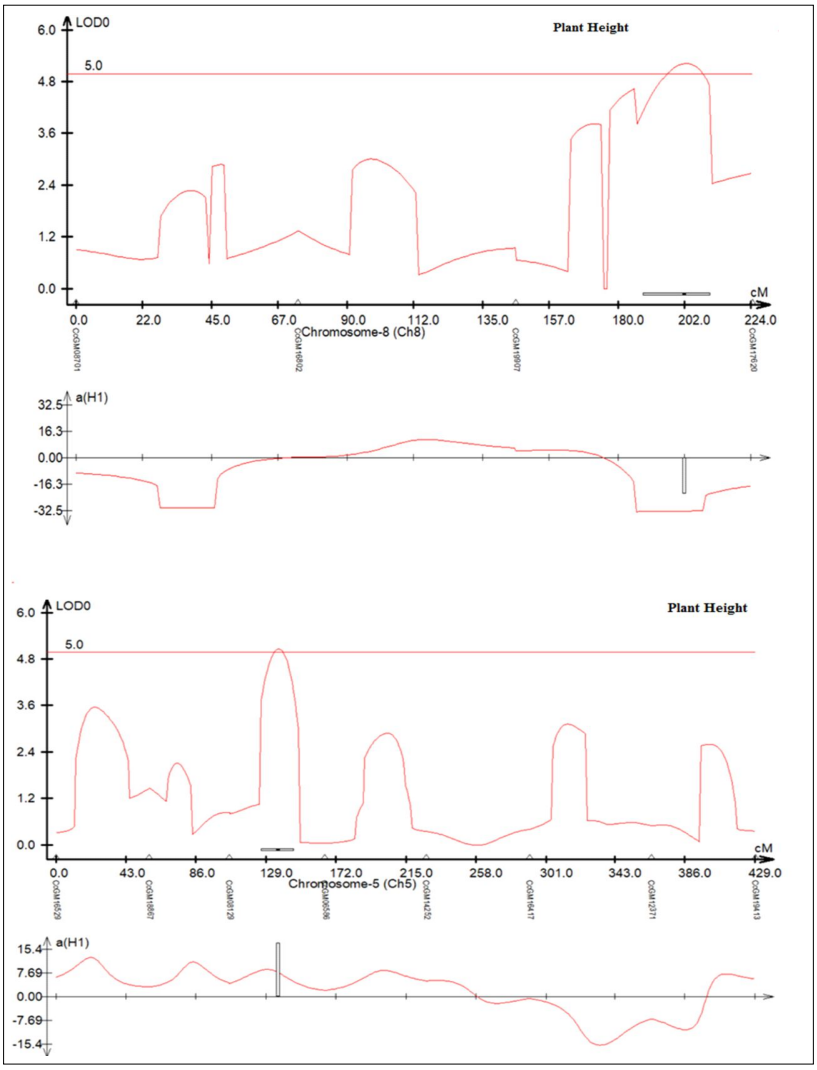


Fig 5: QTLs for plant height.

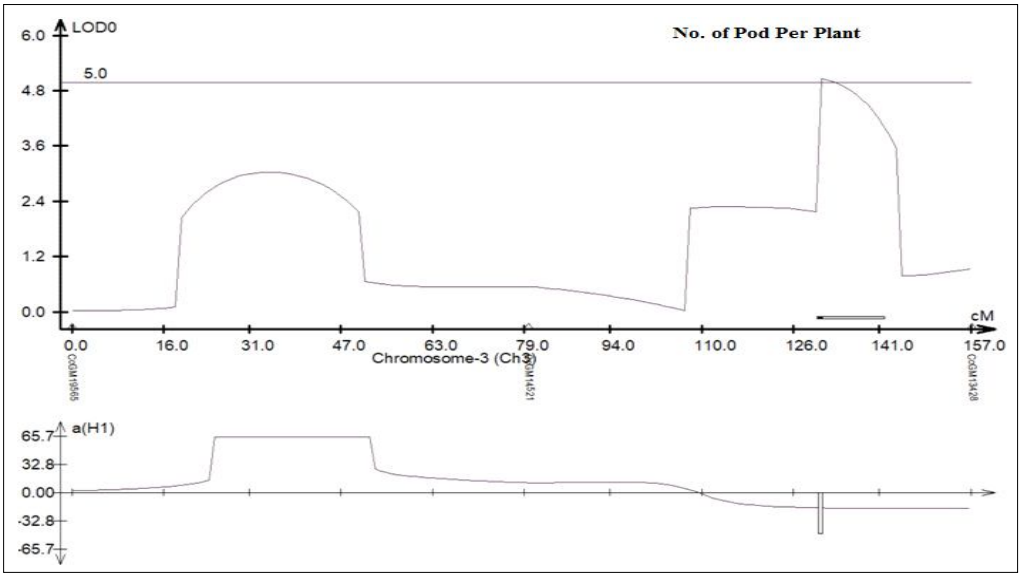


Fig 6: QTL for number of pod per plant.

in 2014 and through GWAS by Patil *et al.* (2018). Out of five QTL-flanking SSRs only one SSR could show significant association with traits under study by Patil *et al.* (2018). Therefore further validation of the QTL identified in this study is needed to be done among RIL population and different environments and genetic background before using for marker assisted selection.

CONCLUSION

Large variation was recorded in all plant type traits in the mapping population. Seven SSR markers associated with 5 plant type traits were identified through single marker analysis. Three markers CcGM19565, CcGM10737, CcGM17620 located on LG_Cc3, LG_Cc6 and LG_Cc8 were found to be associated with plant height explaining phenotypic variance of 10.63%, 15.35% and 19.33% respectively. SSR marker CcGM16802 located on LG_Cc8 was found to be associated with two traits i.e number of primary branch and number of secondary branches simultaneously. Among all markers identified through SMA, CcGM17620 located on LG_Cc8, showed maximum phenotypic variance of 19.33% for plant height.

Composite interval mapping revealed one minor and one major QTL for plant height namely qPH5.1 and qPH 8.1 on the linkage group LG_Cc5 in the marker interval CcGM08129-CcGM06586 with PVE of 3.57% and on the linkage group LG_Cc8 in the marker interval CcGM19907-CcGM17620 with PVE of 72.52% respectively. One minor QTL was also identified for the number of pod per plant, namely qPD3.1 on the linkage group LG_Cc3 in the marker interval CcGM19565-CcGM14521 with PVE of 8.11%. The QTLs identified in this study can be further validated and fine mapped before utilization in marker assisted breeding for pigeonpea improvement.

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Conflict of interest

All authors declared that there is no conflict of interest.

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