scientific reports

Check for updates

OPEN

Discovery and validation of SSR marker-based QTL governing fresh pod yield in dolichos bean (*Lablab purpureus* L. Sweet)

Basanagouda Gonal✉, Ramesh Sampangi✉, Kalpana Pundalik Mugali✉, Siddu Basavaraj Chindi✉, B. R. Chandana, H. Satish, V. Prashantha, N. Karthik, D. Sindhu, M. Kemparaju & B. V. Sinchana

Identification and validation of quantitative trait loci (QTL) governing desired phenotype of target trait is a prerequisite to implement marker-assisted selection in any crop including dolichos bean. Under this premise, we used two mapping populations (MPs) to detect and cross population validate QTL controlling fresh pod yield. One of the MPs consisted of F_2 individuals (MP1) derived from crossing two elite genotypes, the second MP consisted of random RILs (MP2) derived from a different pair of elite genotypes. The MP1 and MP2 were genotyped using polymorphic 86 and 91 SSR markers, respectively and linkage maps were constructed using QTL IciM mapping software. The MP1 and MP2 were phenotyped during 2021 and 2017 rainy and post rainy seasons, respectively for fresh pod yield plant^{-1} following two-replicated simple lattice design. QTL maps were developed in MP1 and MP2 using genotype and phenotype data. Our results indicated that the estimates of total map length, average map length per linkage group (LG) and average inter-marker distance in MP2 were greater (by at least 1.5 times) than those in MP1. While seven QTLs were detected in MP1, six were detected in MP2 with three QTL exhibiting positive and additive minor effects for fresh pod yield plant^{-1}. We also detected one common minor positive effect QTL across two seasons in MP2 and significant epistatic QTL, whose main effects were non-significant. One each of the seven and six QTL-linked SSR markers detected in MP1 and MP2, respectively were cross-population validated. The implications of these results are discussed in relation to strategies to breed dolichos bean.

Keywords Linkage map, QTL map, SSR marker, QTL epistasis

Dolichos bean var. lignosus is one of the important and ancient food legumes extensively grown in southern India[1]. It is commonly known as 'hyacinth bean', 'field bean', 'Indian bean', etc. It is a self-pollinated crop with 2n = 22 chromosomes[2] and genome size of 367 Mbp[3]. It is believed that dolichos bean is originated in India[4]. It is grown both in rainfed and irrigated eco-systems for fresh beans for use as a vegetable southern in Indian states such as Karnataka, Andhra Pradesh and Tamil Nadu. Fresh dolichos beans are one of the most important sources of protein (22–28%) to a large number of people, especially those who depend on vegetarian diets[1]. Recent research finding indicate that dolichos bean extracts impede infections of viral diseases such as influenza and SARS-CoV-2 which have been declared as world pandemics by world health organization[5]. It contributes to food security and better nutrition and increased income to rural poor[1]. While fresh pods are harvestable and marketable economic products, fresh beans are consumable economic products in dolichos bean. Enhancing on-farm productivity by developing high yielding cultivars is expected to offer competitive edge to dolichos bean production and in in turn encourage farmers for large-scale production of dolichos bean. Increased production would lead to enhanced availability of dolichos bean at price affordable to rural poor who largely depend on dolichos bean as one of the protein sources.

Being predominantly self-pollinated crop, pure-lines are the only cultivar option for dolichos bean production. Owing to the paucity of genomic resources, dolichos bean breeding has been largely based on phenotype-based selection of best homozygous genotypes for use as pure-line cultivars. However, phenotype-based selection

Department of Genetics and Plant Breeding, College of Agriculture, University of Agricultural Sciences, Bangalore, Karnataka, India. ✉email: basanagoudagonal@gmail.com; ramesh_uasb@rediffmail.com; kalpanaashu13@gmail.com; siddubc16@gmail.com

for fresh pod yield and its component traits is rather less effective due to their crop-stage specific expression, complex inheritance and significant cross-over genotype-by-environment interaction[6]. DNA markers being crop stage non-specific, simply inherited and environmentally neutral are proven to be powerful surrogates of such difficult-to-select traits. Enhancing the pace and efficiency of dolichos bean breeding for cultivars with high fresh pod yield require adoption of a well-conceived strategy that hinges on DNA marker assisted selection (MAS) and introgression of the key quantitative trait loci (QTL) controlling fresh pod yield to elite genetic background[6]. The detection and characterization of QTLs are traditionally being performed using F_2/F_3/RILs/di-haploid populations derived from parents contrasting for phenotypes of target traits and a large number of polymorphic markers.

Based on quantitative genetics theory[6], we hypothesize that detection of QTL within the very breeding populations (BPs) routinely developed and used for selection in breeding programmes is more relevant than those detected in experimental populations deliberately developed to increase the power of detection of QTL. The rationale of our hypothesis is that the QTL detected in experimental populations most often do not segregate in BPs used for selection of superior genotypes for use as cultivars[7–10]. However, BPs are generated more often from good × good parent crosses to maximize the probability of selecting recombinant inbred lines (RILs) better than the parents or the best cultivar in use. It is possible that major QTL controlling economically important traits might have fixed due to repeated selection cycles and hence do not segregate in BPs derived from them[6]. However, we hypothesize that BPs derived from good × good crosses will still segregate for a few minor QTL (if not major) that are directly relevant in practical breeding. As a prelude for implementing MAS, the objectives of the present study were to (i) detect, locate and estimate size effects of QTL controlling fresh pod yield and (ii) validate the SSR markers linked to the detected QTL in two biparental mapping populations derived from elite parents.

Material and methods

Development of mapping populations

Our study consisted of two mapping populations (MPs). One of these MPs consisted of F_2 individuals derived from crossing two elite genotypes namely HA 4[1] and HA 5[11]. These two elite genotypes are high yielding pure-line varieties released for commercial production by University of Agricultural Sciences (UAS), Bangalore, India. The F_1's from HA 4 × HA 5 was grown during 2020 post rainy season and these were selfed to obtain F_2 population. The F_2 population were selfed in 2021 summer season to obtain $F_{2:3}$ population (hereafter referred to as MP1) for detecting QTLs for fresh pod yield. The second MP consisted of random RILs ((hereafter referred to as MP2) derived from two elite genotypes, namely HA 10–8 and RIL-180[12]. While HA 10–8 is a advanced breeding line derived from HA 4 × GL 153 cross, RIL 3–180, is a RIL derived from HA 4 × CPI 31,113 cross[13]. The F_1's from HA 10–8 × RIL-180 was grown during 2015 post rainy season and these were selfed continuously to obtain F_6 RIL population which constituted MP2 for detecting QTLs for fresh pod yield. Both MP1and MP2 were developed in the experimental plots of the Department of Genetics and Plant Breeding (GPB), College of Agriculture (CoA), UAS, Bangalore, India.

Genotyping MP1 and MP2

DNA extraction

Young leaves from 21-days old seedlings were collected from 144 individual F_2 plants of MP1 and 96 F_6 RILs of MP2. The genomic DNA was isolated by Cetyl-tri-methyl ammonium bromide (CTAB) method[14].

SSR marker assay

The MP1 and MP2 were genotyped using 86 (Supplementary Table 1) and 91 SSR markers (Supplementary Table 2) polymorphic between their parents, respectively. The PCR reactions were carried-out in thermocyclers from Applied Biosystems/Veriti 96 well, in a 35-cycle program, obeying the following temperatures and time: 94 °C for one minute (initial denaturation), 94 °C for two minutes (cyclic denaturation), the specific temperature of each initiator, in °C, for one minute (annealing), 72 °C for three minutes (cyclic extension), 72 °C for 10 min (final extension), and 4 °C. The final volume was 14 µL of each sample, being: 2µL of DNA (10 ng/µL), 4 µl of Amplicon PCR Master Mix, and 8 µl of nuclease-free water. The amplicons were separated on 3.5% agarose gel, stained with ethidium bromide, and visualized through the photo- documentation system (Bio-Imaging Systems). The amplicon profiles produced by SSR markers on MP1 and MP2 were scored manually. The amplicons of SSR priming regions of genomic DNA specific to HA 4 of MP1 at defined product size range were scored as '0' and those specific to HA 5 of MP1 were scored as '2' and heterozygous genotypes as '1'. The amplicons of SSR priming regions of genomic DNA specific to HA 10–8 of MP2 at defined product size range were scored as '0' and those specific to RIL 3–180 of MP2 were scored as '2'.

Linkage map construction

The information on linkage map was extracted using QTL IciM mapping software version 4.1 based on genotypic data of 86 and 91 SSR markers in MP1 and MP2. While a threshold logarithm of odds (LOD) score of 3.0 was used to test the significance of pair-wise marker recombination fraction (RF), threshold RF of 0.30 was used for assigning markers to different linkage groups (LGs). The best order among the markers assigned to each LG was determined using the protocol "minimum sum of adjacent recombination fraction (SARF)". The map distances among the markers assigned to each LG were estimated using Kosambi (1944)[15] mapping function. Significance/otherwise of departure of frequency of alleles at each SSR marker locus from the expected frequency of 0.50 was examined using Chi-square test in both MP1 and MP2. Also, at each SSR marker locus, the segregation of F_2 individuals of MP1 and of RILs in MP2 was examined for conformity with expected segregation of 1:2:1 and 1:1,

respectively. With all these information the skeletal linkage map of MP1 and MP2 were drawn using MapChart software[16].

Marker distribution analysis

To explore if the mapped markers were randomly distributed in the genome, the LGs in MP1 and MP2 were divided into 5 cM, 10 cM and 15 cM bins. The number of markers in defined bins was counted in all the LGs. Expected number of markers per defined bins in all the LGs was predicted based on Poisson distribution function, $P(x) = e^{-\mu}\,\mu^{x}/x!$, where, 'x' is the number of markers per bin and μ is the average number of markers per defined bin[17]. The significance/otherwise of deviation of observed number of markers per bin from those expected was examined using Chi-square test. Significant and non-significant deviations were considered as evidence for non-random and random distribution of markers along LGs and genome of dolichos bean.

Phenotyping MP1 and MP2

While the seeds of 144 $F_{2:3}$ families (MP1) were planted in single rows of 3 m length with 0.60 m apart following simple lattice design during 2021 rainy and post rainy seasons, those of 96 RIL (MP2) were planted during 2017 rainy and post rainy season. The families/individuals of both MP1 and MP2 were evaluated in experimental plots of the department of GPB, CoA, UAS, Bangalore, India. The seeds of each F_3 family and RIL were planted in single row of 3 m length and 0.60 m apart. Fifteen days after sowing, seedlings were thinned to maintain 0.3 m between plants within a row. A total of 12–13 plants per $F_{2:3}$ family and RIL survived to maturity in each replication in MP1 and MP2. Ten plants in each $F_{2:3}$ family and RIL were sampled avoiding border ones. Well-filled fresh pods were harvested twice from five sampled plants. The weight of fresh pods harvested in two pickings were recorded in grams and expressed as fresh pod yield plant^{-1}. The data on fresh pod yield plant^{-1} of $F_{2:3}$ families of MP1 and RILs of MP2 were used for QTL mapping.

Statistical analysis of phenotypic data

The replication-wise mean fresh pod yield recorded from two seasons was subjected to statistical analysis. The pooled analysis of variance (ANOVA) was performed to detect genotype × season interaction. Summary statistics of phenotypic data on fresh pod yield of the two populations was estimated using MS Excel software version.

QTL mapping

The genotypic and phenotypic data of MP1 and MP2 evaluated in two seasons were integrated to detect QTL controlling fresh pod yield plant^{-1}, initially using single marker analysis (SMA). Subsequently, Inclusive Composite Interval Mapping (ICIM) was performed to detect, locate and estimate size effects of QTL and QTL × QTL interactions[18] controlling fresh pod yield plant^{-1} in each season. Under the assumption of additivity of QTL effects on the phenotype of traits of interest, the additive effect of a QTL could be completely absorbed by the two flanking marker variables and the epistatic effects between two detected QTL could be completely absorbed by the four marker-pair multiplication variables between the two pairs of markers flanking the QTL. IciM works on this property to map QTL. In IciM, marker variables were considered in a linear model for mapping QTL with additive effects and both marker variables and marker-pair multiplications were simultaneously considered for mapping QTLs with epistatic effects. Two steps were followed in IciM. In the first step, stepwise regression was applied to identify the most significant markers and marker-pair multiplications. In the second step, a one-dimensional scanning or interval mapping was conducted for mapping additive and a two-dimensional scanning was conducted for mapping di-QTL epistasis using adjusted phenotypic values based on the best regression model. The accuracy of QTL position and significance of size effect of QTL and QTL × season interaction conferring fresh pod yield plant^{-1} was determined using data-driven (empirical) estimates of threshold LOD scores obtained by 1000 permutations[19]. Similarly, significant QTL × QTL interactions controlling fresh pod yield plant^{-1} were detected at threshold LOD value of 5.0, and their size effects were estimated. All these statistical analyses were implemented using QTL ICiMapping software version 4.1[20]. The QTL maps of MP1 and MP2 were drawn using MapChart software[16].

Estimation of expected number of markers to ensure at least one marker is within defined distance from a randomly chosen QTL

The expected number (n) of markers required to achieve the probability (p) that at least one marker is within 'm' map distance from a randomly chosen QTL controlling a target trait was estimated as $\mathbf{n} = \frac{\ln(1-p)}{\ln\left(1-\frac{2m}{L}\right)}$[21,22], where, 'L' is estimated map length in a given experiment and m = inter marker distance. Say if p = 0.85; m = 16 cM; L = 1380.98 cM (as realized in MP1) (Table 1), the expected number of markers required to achieve 0.85 'p' was estimated as $\mathbf{n} = \frac{\ln(1-0.85)}{\ln\left(1-\frac{2(16)}{1380}\right)} \sim 80$ markers. Say if p = 0.85; m = 28 cM; L = 2593.95 cM (as realized in MP2) (Table 1), the expected number of markers required to achieve 0.85 'p' was estimated as $\mathbf{n} = \frac{\ln(1-0.85)}{\ln\left(1-\frac{2(28)}{2595}\right)} \sim 95$ markers.

Results

Linkage maps in MP1 and MP2

The frequency of alleles at each SSR marker locus was in conformity with expected frequency of 0.50 in both MP1 (Supplementary Table 3) and MP2 (Supplementary Table 4). Similarly, at each SSR marker locus, F_2 individuals of MP1 (Supplementary Table 3) and RILs of MP2 (Supplementary Table 4) segregated in expected ratio of 1:2:1 and 1:1, respectively. The total map length and average inter-marker distance varied with MP. While map length

Linkage group identity	Linkage map length (cM)		No. of markers		Average inter-marker distance (cM)	
	MP1	MP2	MP1	MP2	MP1	MP2
1	73.02	276.99	8	8	9.13	34.62
2	195.76	212.04	9	8	21.75	26.50
3	74.97	207.80	8	9	9.37	23.08
4	210.50	238.33	8	8	26.31	29.79
5	35.63	306.43	7	8	5.09	38.30
6	139.66	306.33	7	8	19.95	38.29
7	124.27	307.55	7	9	17.75	34.17
8	56.44	361.76	7	8	8.06	45.22
9	64.55	234.57	7	9	9.22	26.06
10	223.61	135.59	10	8	22.36	16.94
11	190.57	314.11	8	8	23.82	39.26
Total	1388.98	2593.95	86	91	177.65	313.50
Average	126.27	235.81	7.8	8.27	16.15	28.50

Table 1. Distribution of polymorphic SSR markers on 11 linkage groups in two mapping populations (MPs).

per LG varied from 35.63 cM (LG 5) to 223.61 cM (LG 10) in MP1 (Table 1; Fig. 1), it varied from 135.59 cM (LG 10) to 361.76 cM (LG 8) in MP2 (Table 1; Fig. 2). The highest and lowest number of markers per LG varied from 7 to10, respectively in MP1, while they varied from 8 to 9, respectively in MP2. The total length of the map spanned 1388.98 cM and 2593.95 cM in MP1 and MP2 with an average marker density of 16.15 cM and 28.50 cM, respectively (Table 1). These results implied that the total map length and average map length per LG in MP2 were greater (by 1.86 times) than those in MP1. Similarly, the average inter-marker distance in MP2 was greater (by 1.77 times) than that in MP1, though a number of mapped markers per LG was comparable between MP1 and MP2 (Table 1).

Distribution of markers along linkage groups

The observed number of markers deviated significantly from those expected based on Poisson distribution in 15 cM bins in each LG in MP1 (Supplementary Fig. 1a and 1b) and MP2 (Supplementary Fig. 2a and 2b). Similarly, the observed number of markers deviated significantly from those expected in 5 cM and 10 cM bins as well in both MP1 and MP2 (data not shown as number of figures will be too many). These results indicated non-random distribution of markers along LGs. The inter-marker distance on LG varied from 0.0 to 31.4 cM, with an average of 0.98 cM. Wide variation in the estimates of the inter-marker distance across LGs in both MP1 (5.09 to 36.31) (Table 1) and MP2 (16.94 to 45.22) (Table 1) provides further evidence for the non-random distribution of the markers along the LGs.

Identification of QTL

Accuracy and reliability of the QTL discovery is the function of accuracy of phenotypic data. Discovery of QTL also depends on significant variability among the individuals of the MPs for target trait (fresh pod yield in the present study) and consistency of their performance across tested environments (seasons in the present study). ANOVA (Table 2) indicated significant variability among the individuals of both MPs for fresh pod yield. Though the genotype × season interaction was statistically significant. A fairly high 'F' probability suggested that the interaction not strong enough to be considered seriously. The variability for fresh pod yield in terms of range in both MP1 and MP2 were comparable. The trait exhibited a moderate level of heritability in both the populations (Table 3). While three QTLs (*qFPWS1-2–1, qFPWS1-2–2, qFPWS1-11–3*) were detected based on 2021 rainy season data (Table 4; Fig. 3), four QTL (*qFPWS2-2–1, qFPWS2-4–2, qFPWS2-10–3, qFPWS2-11–4*) were detected based on 2021 post rainy season data in MP1 (Table 4; Fig. 3). All these seven QTL exhibited negative additive effects on fresh pod yield plant^{-1} except the one detected based on 2021 post rainy season data which showed positive additive effects. This positive effect QTL flanked by LPD 7 and LPD 100 detected in MP1 merit mention (Table 4; Fig. 3). In MP2, three QTLs each were detected based on 2017 rainy season data (*qFPWS1-2–1, qFPWS1-7–2, qFPWS1-11–3)* (Table 5; Fig. 4) and 2017 post rainy season data (*qFPWS2-2–1, qFPWS2-5–2, qFPWS2-10–3*) (Table 5; Fig. 4). Though these six QTL showed minor effects, two (*qFPWS2-1, qFPWS2-2*) (Fig. 4) of these detected at 30 cM and 60 cM on LG 2 and LG 5 and flanked by KTD 66 and KTD 76, and KTD184 and LabRRT44 (Table 5; Fig. 4), respectively merit mention.

QTL × season interaction and common QTL across two seasons

The estimates of variation explained by QTL × season interaction effects were ignorably low in both MP1 (Table 4) and MP2 (Table 5). One of the seven QTL detected based on 2021 rainy season (*qFPWS1-2–1*) (Fig. 1) and post rainy season data (*qFPWS2-2–1*) (Fig. 2) was common across two seasons in MP1 (Table 4). Similarly, one of the six QTL detected based on 2017 rainy season (*qFPWS1-1*) (Fig. 3) and post rainy season data (*qFPWS2-1*) (Fig. 4) was common across two seasons in MP2 (Table 5). The common QTL (*qFPWS1-2–1/qFPWS2-2–1*) detected in MP1 on LG 2 was flanked by same markers (LPD 190 and LPD 25) in both seasons with only

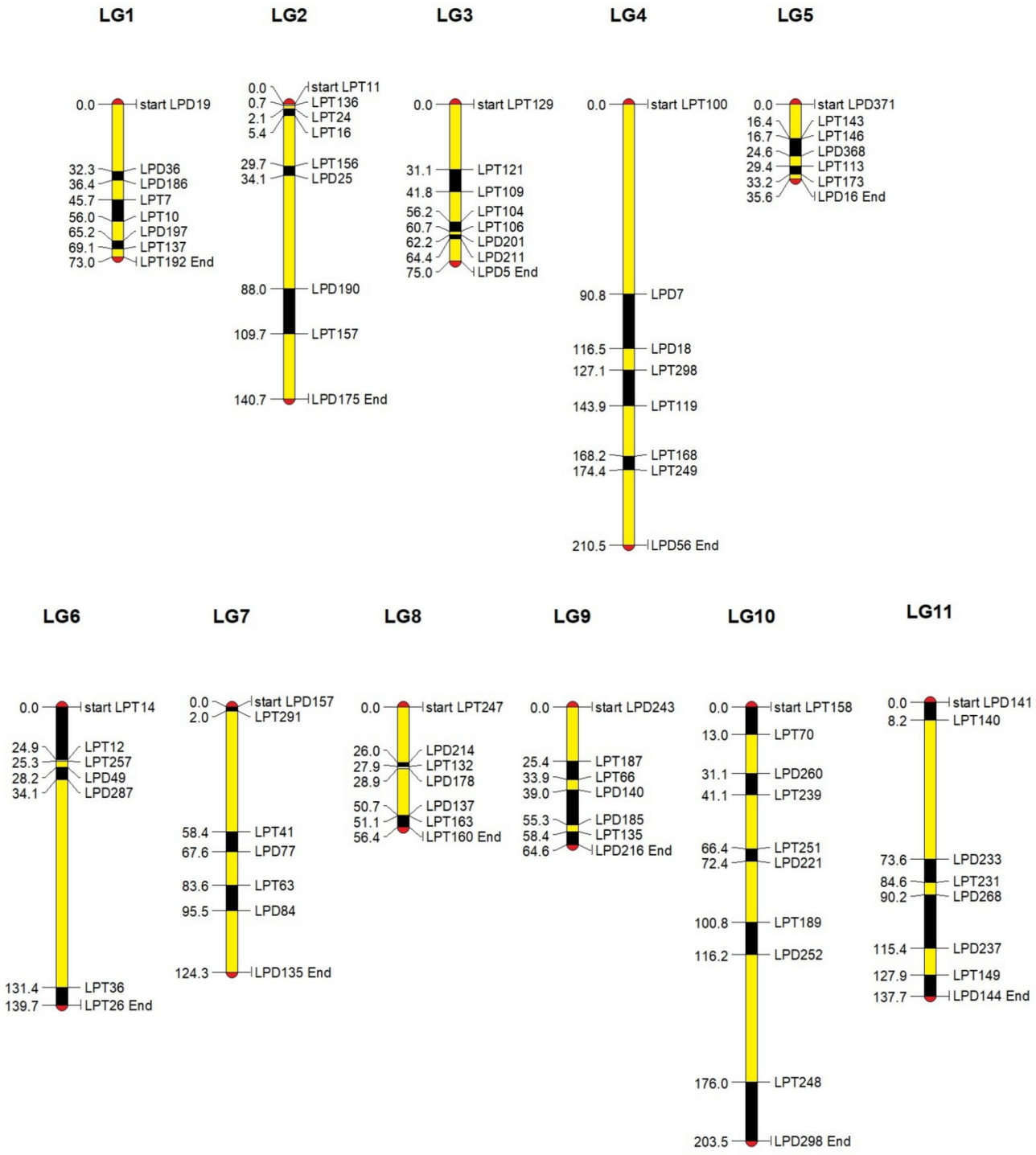

Fig. 1. SSR marker-based skeletal linkage map in F2 mapping population (MP1) derived from HA 4 × HA 5

marginal change (by 4 cM) in chromosomal location (Table 4). Similarly, the common QTL (*qFPWS1-1*/*qFPWS2-1*) detected in MP2 on LG 2 was flanked by same markers (KTD 66 and KTD 76) in both the seasons with only marginal change (by 4 cM) in chromosomal location (Table 5). However, only in MP2, the detected common QTL exhibited positive additive effects.

Di-QTL epistasis

Based on their location, two types of QTL irrespective of whether they exhibit significant/non-significant main effects interact and produce effects that could be detected. These are (i) the QTL located on same LG and (ii) the QTL located on different LG. The epistatic QTL that was detected in the present study belonged to the second category. Considering that power of detection of di-QTL epistasis is lower than that of main effect QTL[6], only

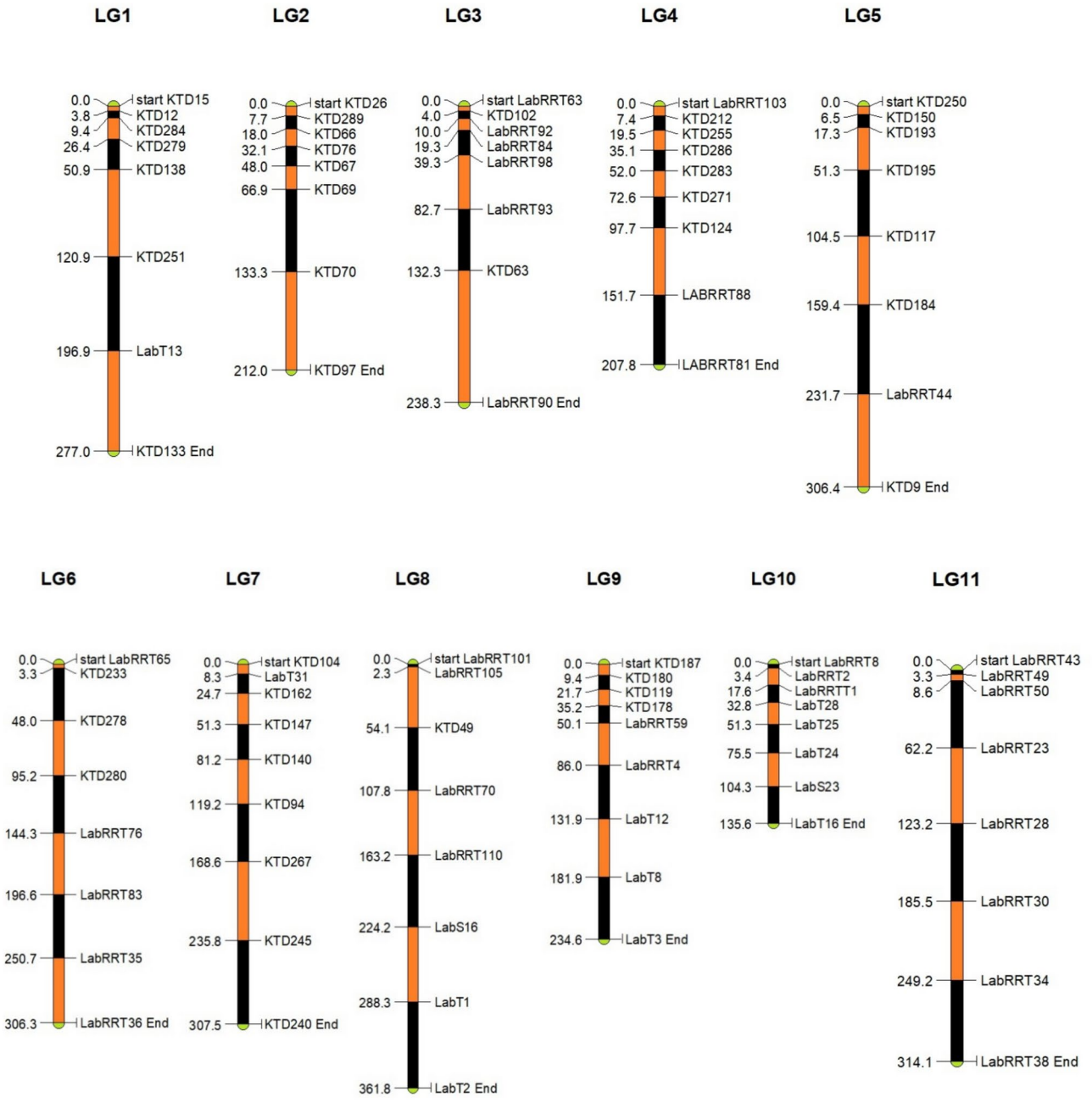

Fig. 2. SSR marker-based skeletal linkage map in RIL mapping population (MP2) derived from HA 10-8 × RIL 3-180

those epistatic QTL which were detected with LOD score of more than 4.0 are reported in this manuscript. While two pairs of QTLs interacted significantly in rainy season, three pairs of QTLs interacted significantly in post rainy season in MP1 (Table 6: Fig. 5). The QTL located on LG 11 (although detected at different positions) involved as one of the QTL in four of the five pairs of QTLs that interacted significantly in both rainy and post rainy seasons. Of these four pairs, the QTL located on LG 11 and the one located on LG 7 produced additive × additive epistatic effects of over 10.00 (Table 6). As is true in MP1, in MP2 also, while two pairs of QTLs interacted significantly in rainy season, three pairs of QTL interacted significantly in post rainy season (Table 7: Fig. 6). The QTL located on LG 11 (although detected at different positions) involved as one of the QTL in two of the five pairs of QTLs that interacted significantly in both rainy and post rainy seasons in MP 2 (Table 7). Thus, these results suggest that QTL located on LG 11 involved in di-QTL epistasis more frequently than those present on other LG in both MP1 and MP2. Further, the estimates of phenotypic variation explained by the epistatic QTLs detected in MP1 (Table 6) were, in general, greater in magnitude than those in MP2 (Table 7).

Population	Sources of variation	Degrees of freedom	Mean sum of squares
HA 4 × HA 5 ($F_{2:3}$)	Seasons	01	8279.48**
	Replications	02	103,167.2**
	$F_{2:3}$ families	143	243.87**
	$F_{2:3}$ families × seasons	143	53.68*
	Residuals	286	41.10
RIL population	Seasons	01	2484.62**
	Replications	02	7290.33**
	RIL families	95	169.23**
	RIL families × seasons	95	36.29**
	Residuals	190	26.11

Table 2. Analysis of variance for fresh pod yield in $F_{2:3}$ and RIL populations.

Population	Mean ± SE	Range		Heritability
		Min	Max	
HA 4 × HA 5 ($F_{2:3}$)	93.80 ± 1.19	66.46	117.58	0.47
RIL	88.32 ± 1.81	55.43	102.55	0.58

Table 3. Summary statistics of fresh pod yield in experimental populations.

Season	LG	Position (cM)	Flanking markers (FM)		Distance between FMs (cM)	LOD score	Phenotypic variance Q × S int. (%)	Additive effect	Phenotypic variance (PV) (%)
			Right marker	Left marker					
2021 rainy season	2	54	LPD 190	LPD 25	53.89	10.09	6.55	–	−10.00
	2	64	LPD 190	LPD 25	53.89	6.17	6.12	–	−5.22
	11	48	LPD 233	LPT 140	65.36	4.49	5.22	–	−6.51
2021 post rainy season	2	50	LPD 190	LPD 25	53.89	8.50	6.33	–	−5.20
	4	49	LPD 7	LPD 100	90.79	3.58	4.22	–	5.31
	10	134	LPT 248	LPD 252	78.18	3.04	5.56	–	−0.11
	11	40	LPD 233	LPT 140	118.19	3.70	5.22	–	−6.11
QTL × season interaction	2	00	LPT 11	LPT 136	0.72	3.71	6.42	0.04	0.23
	8	54	LPT 160	LPT 163	5.36	3.11	7.91	0.10	−2.50
	10	114	LPT 158	LPT 70	12.95	3.47	7.28	0.03	0.75

Table 4. Main effect-QTL detected using SSR markers for fresh pod yield plant^{-1} in F_2 mapping population (MP1) in two seasons.

Cross population validation of QTL-linked SSR markers

In the present study, markers found significantly linked to QTL controlling fresh pod yield plant^{-1} were examined for their linkage in MP1 and MP2. Of the eight QTL-linked SSR markers detected in MP1 (Table 4), the marker, LPD 233 was found linked to QTL in MP2 (Table 8). Similarly, of the 10 QTL-linked SSR markers detected in MP2 (Table 5), the marker, KTD 66 also was found linked to QTL in MP1 (Table 9). However, while LPD 233 was found linked to negative additive QTL, KTD 66 was found linked to common positive additive effect QTL detected across two seasons in MP2 (Table 5).

Discussion

Development of linkage map

Discovery and development of DNA-sequence based locus-specific co-dominant marker systems such as those based on SSRs accelerated the development of linkage maps in different crops. Construction of linkage map is a prerequisite for conducting strategic and applied plant breeding research activities such as (i) detection, chromosomal localization and estimation of size effects of QTL controlling economically important traits[23–25] (ii) introgression of desired QTL to elite genetic background[25] and (iii) map-based cloning of QTL[24]. As a prelude to QTL mapping and their introgression into desired genetic background, a skeletal linkage map was constructed using the genotyping data of 86 and 91 SSR markers in MP1 and MP2, respectively. The linkage map can be theoretically considered satisfactory if every point on the genome is genetically linked to at least one marker. This can be achieved if all the polymorphic markers linked and numbers of LGs are equivalent to haploid chromosome number of crop species under investigation. Based on these criteria, the linkage map that we have

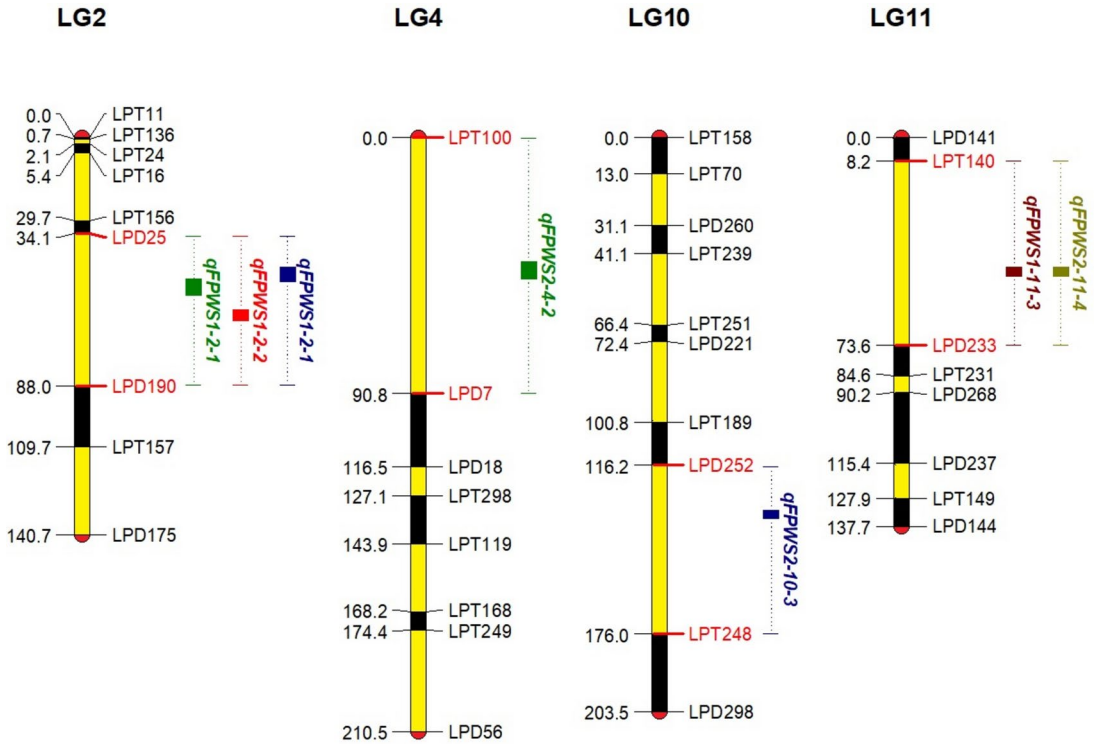

Fig. 3. Linkage map showing location of QTLs (detected in 2021 rainy and post rainy seasons) controlling fresh pod yield plant^{-1} in F_2 mapping population (MP1) derived from HA 4 × HA 5.

Season	LG	Position (cM)	Flanking markers (FM)		Distance between FMs (cM)	LOD score	Phenotypic variance (PV) (%)	Phenotypic variance Q × S int, (%)	Additive effect
			Right marker	Left marker					
2017 rainy season	2	26	KTD 66	KTD 76	14.00	4.01	7.15	–	1.25
	7	96	KTD 140	KTD 94	37.92	3.85	6.92	–	1.22
	11	140	LabRRT 28	LabRRT 30	62.32	3.52	6.56	–	−0.85
2017 post rainy season	2	30	KTD 66	KTD 76	14.00	5.22	6.25	–	2.20
	5	60	KTD 184	LabRRT 44	24.15	4.25	7.88	–	2.31
	10	180	LabT 25	LabT 24	72.26	5.16	7.49	–	−0.72
QTL × season interaction	5	60	KTD 195	KTD 117	53.17	3.02	6.58	0.14	0.33
	7	19	LabT 31	KTD 162	16.37	3.26	5.01	0.08	−1.30
	10	11	LabRRT 2	LabRRT 11	14.18	3.27	6.25	0.12	0.55

Table 5. Main effect-QTL detected using SSR markers for fresh pod yield plant^{-1} in RIL mapping population (MP2) in two seasons.

constructed, though based on fewer markers, could be regarded as fairly satisfactory as all the polymorphic markers were found linked and the number of LGs were equivalent to haploid chromosome number (n = 11) of dolichos bean[2]. Further, the expected number of markers (80 in MP1 and 95 in MP2) to achieve the estimated map length (1380 cM in MP1 and 2595 cM in MP2) are comparable to those (86 in MP1 and 91 in MP2) used in the present study. Given that linkage map length is a function of recombination frequency (RF), substantially shorter map length in F_2 population (MP1) than that in RIL population (MP2) could be attributed to lower effective RF in MP1 (r) than that in MP2 (R), where $R = 2r/(1 + 2r)$[26]. Burr et al.[27] and Beavis and Grant[28] opined that the variation of over 20% in map lengths between F_2 and RIL populations derived from the same bi-parental cross is highly frequent.

Ideally, the number of mapped markers should be evenly distributed/equally spaced throughout each LG and the genome and the average inter-marker distance should be shortest possible. However, the number of mapped markers per LG and average inter-marker distance are reflections of mean number of recombination events per meiosis within the chromosomal region between the markers[29]. A wide variation in inter-marker distance per LG in the present study, is therefore could be attributed to non-random RF all along each chromosome/LG and among the chromosomes/LGs[30–32]. A significantly reduced RF near centromeres and telomeres compared to other chromosomal regions of several crops such as tomato and potato[33], wheat[34,35], barley[36,37], rye[38,39] and

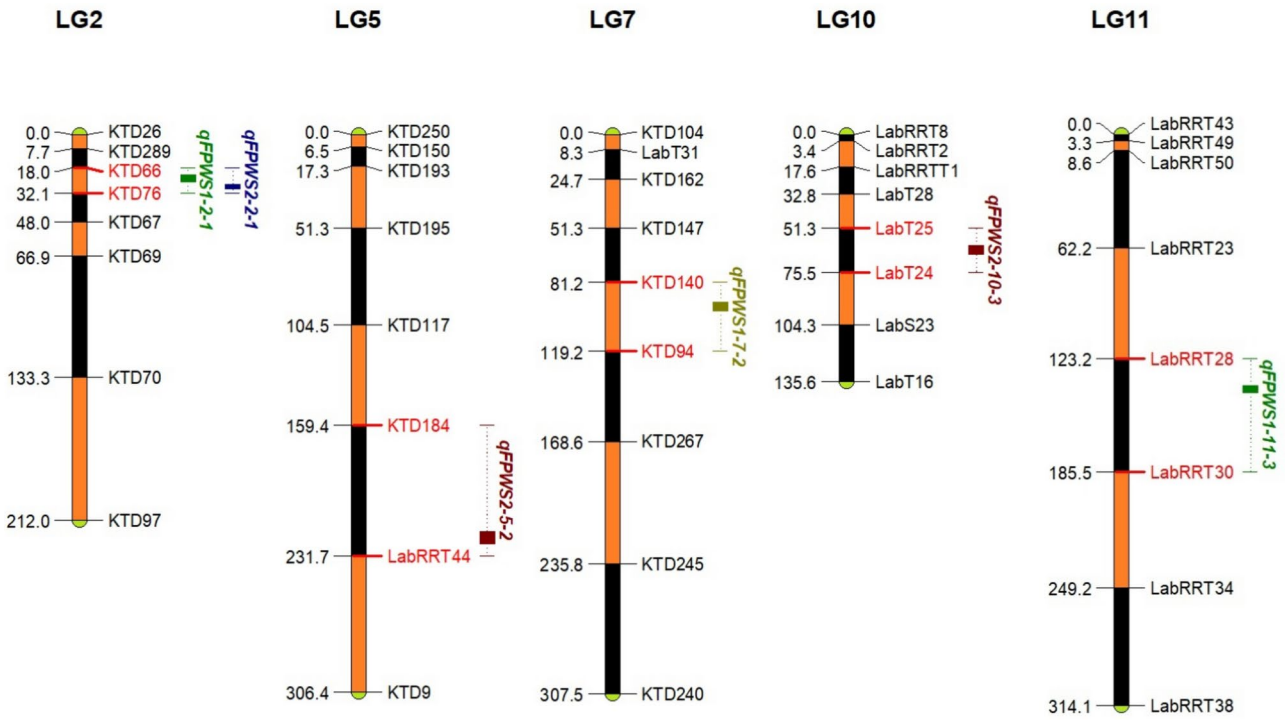

Fig. 4. Linkage map showing location of QTLs (detected in 2017 rainy and post rainy seasons) controlling fresh pod yield plant-1 in RIL mapping population (MP2) derived from HA 10-8 × RIL 3-180

Seasons	QTL A				QTLB						
	Linkage group	Position (cM)	Right marker	Left marker	Linkage group	Position (cM)	Right marker	Left marker	LOD score	Phenotypic variance (%)	Additive × Additive effect
2021 rainy season	6	110	LPT 36	LPD 287	11	80	LPD 144	LPD 141	13.49	2.01	5.41
	5	5	LPT 143	LPD 371	11	80	LPD 144	LPD 141	4.42	1.59	5.22
2021 post rainy season	3	10	LPT 121	LPT 129	4	40	LPD 7	LPD 100	14.26	2.05	2.45
	10	115	LPT 158	LPT 70	11	65	LPD 144	LPD 141	14.96	1.96	2.82
	7	30	LPT 41	LPT 291	11	110	LPD 144	LPD 141	4.98	1.97	10.82

Table 6. Epistatic QTL controlling fresh pod yield plant^{-1} across seasons in F_2 population (MP1).

lolium[40,41] provide adequate evidence for non-random RF. Non-random distribution of markers could also be possibly due to paucity of polymorphisms in a few chromosomal regions of the parents of mapping populations[17]. Therefore, the development of mapping populations from different crosses and the use of new markers, are likely to be effective to fill in gaps between makers to saturate linkage maps[17].

Even within a crop, the length of linkage map and inter-marker distances vary as average RF vary with the genotypes used as parents of MP[41]. For example, the first linkage map of dolichos bean was based on 218 (102 RAPD and 116 RFLP) markers distributed across 17 LGs covering 1610 cM in 119 F_2 population[42]. The linkage map developed by Yuan et al.[43] using 122 RAPD markers in 136 F_2 population consisted of 14 LGs spanning 1302.4 cM of dolichos bean genome. Linkage map constructed by Uday Kumar[13] based on 80 SSR markers using 136 RIL population in dolichos bean consisted of 11 LGs spanning 1067.83 cM with an average inter-marker distance of 10.59 cM. In yet another study in dolichos bean, Chandrakanth[44] constructed linkage map based on 58 polymorphic SSR markers in 109 F_{10} RIL population derived from HA 4 × CPI 60,125. The 58 polymorphic markers were mapped on to 11 LGs. The total length of the map spanned 2008.55 cM with an average density of 34.63 cM. Further, the lengths of linkage map also reported to vary between different types of MPs developed from the same cross. For instance, the total map length in RIL population was 70.5% of that in DH population derived from the same cross in rice[32].

The SSR marker-based linkage map developed in the present study could be used to anchor and orient scaffolds onto the pseudo chromosomes of yet to be sequenced dolichos bean whole genome. Markers based on SSRs are considered as efficient ones owing to their locus specificity and genome-wide distribution[45]. They are

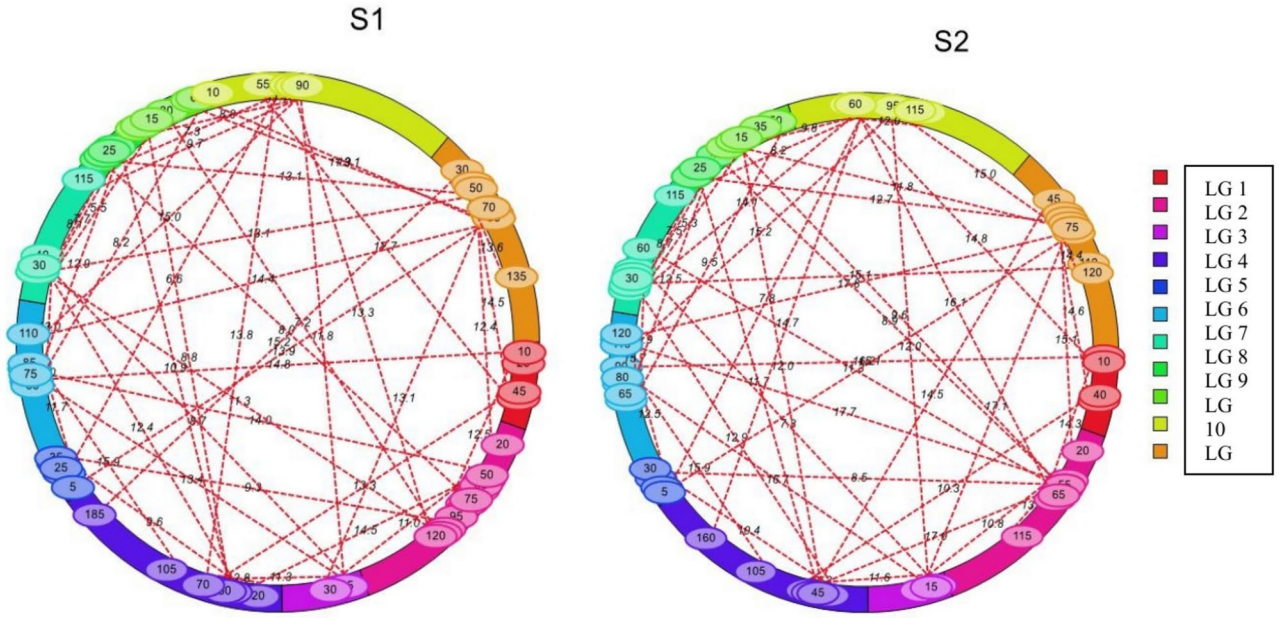

Fig. 5. Genome-wide detection of significant di-QTL interactions controlling fresh pod yield plant^{-1} in 2021 rainy and 2021 post rainy seasons in F_2 mapping population (MP1) derived from HA 4 × HA 5

Seasons	QTL A				QTLB						
	Linkage group	Position (cM)	Right marker	Left marker	Linkage group	Position (cM)	Right marker	Left marker	LOD score	Phenotypic variance (%)	Additive × Additive effect
2017 rainy season	3	40	KTD 286	KTD 283	10	85	LabT 24	LabT 23	6.89	2.22	3.69
	5	22	KTD 193	KTD 195	11	30	LabRRT 50	LabRRT 23	5.89	1.52	3.22
2017 post rainy season	2	15	KTD 289	KTD 66	5	29	KTD 193	KTD 195	10.12	2.58	1.95
	4	110	LabRRT 93	KTD 63	10	78	LabT 24	LabT 23	10.95	2.12	1.99
	6	36	KTD 233	KTD 278	11	88	LabRT 23	LabRRT 28	6.12	1.86	1.82

Table 7. Epistatic QTL controlling fresh pod yield plant^{-1} across seasons in RIL population (MP2).

also useful to integrate different linkage maps and chromosomes. SSR markers can also be used as good probes for fluorescent in situ hybridization (FISH) to integrate genetic and cytogenetic maps[45,46].

Identification of QTL

Construction of high density and high-quality marker-based linkage maps facilitated QTL mapping in a number of crop plants. This paved the way for successful introgression of adequately validated QTL, especially those with large effects into elite genetic background in highly researched crops such as rice, maize, sorghum, soybean and tomato through marker assisted selection (MAS)[6,47]. The present study to map QTL controlling fresh pod yield plant^{-1} in dolichos bean, a less researched and less genome information crop is an initial step towards replicating the success of MAS of QTL achieved in major crops.

In pursuit of enhancing the chances of detecting and mapping QTL controlling economically important traits, most researchers attempt detecting QTL in MPs derived from parents most contrasting for target traits[48]. Most often (if not always) this necessitates the use of at least one of the parents in undesirable genetic background. QTL detected in such MPs may not even segregate and hence are not directly relevant in breeding populations (BPs) routinely developed and used for selection of superior genotypes for use as cultivars[7–10]. However, QTL detected in MPs derived from elite parents (such as HA 4 and HA 5, and HA10-8 and RIL-180 in the present study) could be directly used in the selection of superior genotypes within the BPs for use as cultivars[7–10]. A potential argument against attempts to map QTL in BPs derived from elite parents is that chances and power of detection of QTL would be low and QTL, if any are mostly minor effects ones. Nevertheless, they can be directly selected using linked markers. Under these premises, the QTL controlling fresh pod yield plant^{-1} were detected and mapped by integrating genotyping and phenotyping data of two seasons separately in MP1 and MP2.

A QTL is considered as 'major' or 'minor' based on the proportion of phenotypic variation explained by the QTL[47]. The QTL which explains > 10% and < 10% of phenotypic variation are regarded as 'major' and 'minor' ones respectively. Based on this definition, the three QTL detected based on 2021 and 2017 rainy season data

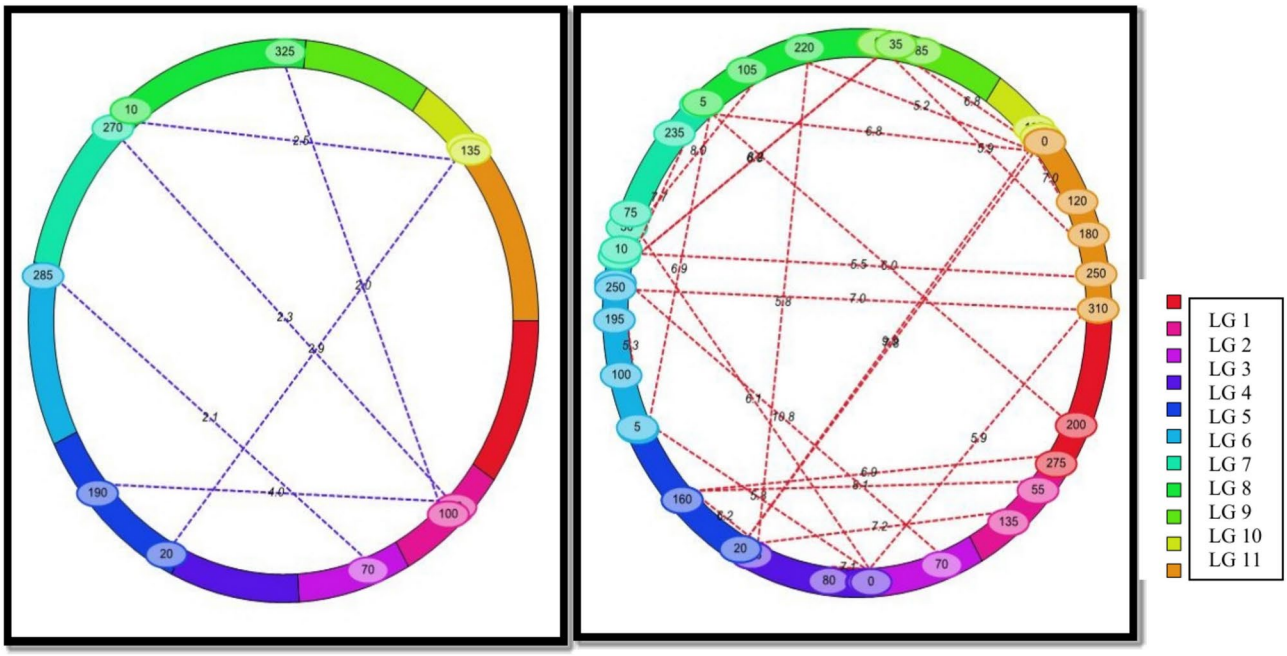

Fig. 6. Genome-wide detection of significant di-QTL interactions controlling fresh pod yield plant^{-1} in 2021 rainy and 2021 post rainy seasons in RIL mapping population (MP2) derived from HA 10-8 × RIL 3-180

Marker	Source of variation	Degrees of freedom	Mean sum of squares	F 'statistic'	P-value
LPD 233	Between marker class genotypes	01	137.43	06.17	5.4×10^{-3}
	Within marker class genotypes	96	22.27		

Table 8. Single marker ANOVA for validation of fresh pod yield plant^{-1} QTL linked SSR makers in RIL population (MP2) derived from HA 10–8 × RIL 3–180.

Marker	Source of variation	Degrees of freedom	Mean sum of squares	F 'statistic'	P-value
KTD 66	Between marker class genotypes	01	107.43	5.88	4.95×10^{-3}
	Within marker class genotypes	143	18.27		

Table 9. Single marker ANOVA for validation of fresh pod yield plant^{-1} QTL linked SSR makers in F_2 population (MP1) derived from HA 4 × HA 5.

in the present study are all 'minor' ones. The complete assembly of dolichos bean genome is expected to help identify candidate genes corresponding to these QTL governing fresh pod yield[45,46]. Fewer and small effect QTL detected in the present study could be attributed to most likely fixation of major effect QTL during the selection of the parents of MPI and MP2. In elite germplasm such as F_2/RIL MPs used in our study, no major QTL controlling fresh pod yield left segregating as they are likely to be rapidly fixed during selection[6]. Further, based on extensive review[6], documented that of the 1076 reported QTL controlling grain yield in different crops, 77% of them explained only ≤ 10% variation in the target trait, while 36% of these QTL explained 1–5%, 41% of them explained 6–10% of the variation in the target trait.

QTL × season interaction

Quantitative trait such as fresh pod yield plant^{-1} exhibits significant crossover genotype × environment interaction as a rule rather than an exception[49,50]. Such traits are also expected to exhibit significant QTL × season interaction[6]. Detection of QTL in some environments but not in others is generally used as a criterion for declaring QTL × environment interaction (QEI). However, contrary to this expectation, in the present study, variation explained by QTL × season interaction is rather very low, implying that QTL detected either in rainy or post rainy season are stable. In maize, Stuber et al.[51] also found little evidence of QEI. However, several other researchers have reported significant QEI for different traits in different crop species. To quote one, in tomato, four out of 29 QTL were detected in all three environments, 10 in two environments and 15 in only one

environment[52]. Examples of significant QEI or of QTL being detected in some environments but not in others have also been reported in different crops such as, soybean[53], sunflower[54], cotton[55], wheat[56] and oat[57].

Common QTL across two seasons

An important concept on which QTL mapping studies are performed in different environments is that certain QTL are detected in all most all test environments and are designated "consensus/common QTL", while a few others are detected in a single or a few environments and are designated as "specific QTL"[6]. In the present study, common QTL across two seasons were detected in MP1 and MP2. One of these common QTL detected in MP2 exhibited positive additive effects. Chandrakanth[44] also detected one common QTL (controlling 100-grain weight) on LG 6 at 154 cM across 2014 and 2015 rainy seasons data in dolichos bean.

Di-QTL epistasis

Mapping epistatic QTL is challenging experimentally, statistically and computationally. This is because, a large mapping population, and a large number of pair-wise interactions and multiple testing need to be evaluated. The number of interactions is of the order of the square of the number of single-locus tests for pair-wise interactions[58]. Besides these, other segregating QTL can confound the estimates of epistasis between the pair of QTL under test[58,59]. Because of these challenges, the power of detection of epistatic QTL is smaller than detection of main-effect QTL. For the same reason, the number of false-positives of significant epistatic QTL is also higher[6,58,59]. To substantially reduce the frequency of false-positives, a minimum LOD score of 4.0 was considered to detect di-QTL epistasis. None of the main effect QTL detected in either season appeared to interact with strong signal as could be inferred from their absence in di-QTL epistatic QTL detected in the present study in both MP1 and MP2. Detection of epistatic QTL with desirable additive effect whose main effects are not large enough provide evidence that the QTLs in combination can have synergistic effect on phenotype of the trait under investigation[60]. Knowledge of such epistatic QTL help understand contribution of epistasis to breeding value of interacting genes[61]. Importance of introgression of epistatic QTL via MAS has also been advocated[62].

Epistasis between QTLs detected in MPs such as F_2 has been at a frequency close to that expected by chance alone[63–65]. As a result, detection of di-QTL epistasis controlling economically important traits in crop plants is not un-common. In asparagus bean[66], reported three pairs of QTLs displaying significant epistatic interactions for days to first flowering. Liang et al.[67] in soybean reported two epistatic QTL for seed width which explained 2.05 and 1.05 *per cent* phenotypic variation. Orf et al.[68] in soybean reported significant epistasis among QTL with substantial main effects on grain yield. Li et al.[69] detected significant di-QTL epistatic effects with one or both interacting QTL with no significant additive effects for dormancy in diploid potato. On the other hand, Hu et al.[70] failed to detect epistatic QTL for kernel starch content in maize. Additive × Additive epistasis detected in the present study could be fixed in desired homozygous genotype for use as a pure line, which is the only cultivar options for commercial dolichos bean production.

Cross population validation of QTL—linked SSR markers

Most often than not, detected target traits' QTL and their linked SSR markers are genotype-specific[6]. This is because, different QTL and their linked markers are present in different genotypes. Hence, QTL and their linked markers detected in one MP may not be detected in another MP. Effective introgression of identified QTL into elite genetic background, therefore need to be confirmed/validated in diverse genetic backgrounds. Marker confirmation/validation involves testing the linkage of markers in mapping populations whose genetic background is different from those where linked markers are/were discovered[71–73]. In the present study, one each of QTL-linked SSR markers could be validated in each of the two MP. The validated marker, KTD 66 is linked to common positive additive effect QTL detected across two seasons in MP2. This validated marker could be considered as candidate surrogate in MAS for fresh pod yield in dolichos bean after further validation other genetic backgrounds.

Breeding implications

Find and introgress major QTL approach (referred to as design approach) is not likely to be effective for genetic improvement of dolichos bean for fresh pod yield which is controlled by minor effect QTL. The reasons for this are two-fold. First, pyramiding favorable OTL alleles, into a single cultivar becomes increasingly difficult as the number of OTL increases. Second, the power to detect minor QTL is often low and estimates of the minor QTL are often inconsistent[6,74]. Such inconsistency in estimate of QTL effects is evident from a comparison of location and effects of QTL detected in two MP developed from the same cross[75]. To improve traits controlled by minor effect QTL, predictive approach (rather than design approach) that do not rely on the detection of QTL is considered as most useful[6,74]. In the predictive approach, the genotyped-only breeding population (BP) individuals are selected based on their breeding values predicted using a large number of genome-wide markers effects estimated using optimized statistical model trained in both phenotyped and genotyped diverse population related to BP. This approach is now popularly called as genomic selection (GS). GS is a form of MAS that bypasses QTL mapping and validation.

Conclusion

SSR marker-based linkage maps were constructed in F_2 and RIL populations derived from bi-parental crosses. The estimates of total map length, average map length and intra marker distance in MP2 were greater than those in MP1. Only three of the 13 fresh pod yield controlling QTL detected across two MPs exhibited additive positive but minor effects. Of these, one of the QTL in each mapping population was common across two populations. The QTL whose main effects could not be detected interacted and produced significant di-QTL epistasis. One

each of QTL-linked SSR markers detected in each mapping population were cross-population validated. The validated SSR marker, KTD 66 was found linked to common positive additive effect QTL controlling fresh pod yield. To breed dolichos bean for improved fresh pod yield which is controlled by minor effect QTL, GS is likely to be more effective than MAS.

Data availability

The datasets used and/or analysed during the current study available from the corresponding author on reasonable request.

Received: 13 October 2024; Accepted: 13 February 2025
Published online: 12 March 2025

References

1. present status and future prospects. Ramesh, S. & Byregowda, M. Dolichos bean (*Lablab purpureus* (L.) Sweet var. lignosus) genetics and breeding. *Mysore J. Agric. Sci.* **50**, 481–500 (2016).
2. She, C. W. & Jiang, X. H. Karyotype analysis of *Lablab purpureus* (L.) sweet using fluorochrome banding and fluorescence in situ hybridisation with rDNA probes. *Czech J. Genet. Plant Breed.* 51, 110–116 (2015).
3. Iwata, A., Greenland, C. M. & Jackson, S. A. Cytogenetics of legumes in the pheseoloid clade. *Plant Genome* **6**, 1–8 (2013).
4. Nene, Y. L. Indian pulses through millennia. *Asian Agri. History* **10**, 179–202 (2006).
5. Liu, Y. M. et al. A carbohydrate-binding protein from the edible lablab beans effectively blocks the infections of influenza viruses and SARS-CoV-2. *Cell Rep.* **32**, 186–188. https://doi.org/10.1016/j.celrep.2020.108016 (2020).
6. Bernardo, R. *Breeding for Quantitative Traits in Plants* 3rd edn. (Stemma Press, 2020).
7. Liu, X. J. et al. Genetic analysis of segregation distortion of SSR markers in F_2 population of barley. *J. Agric. Sci.* **3**, 172–177 (2011).
8. Wurschum, T. Mapping QTL for agronomic traits in breeding populations. *Theor. Appl. Genet.* **125**, 201–210. https://doi.org/10.1007/s00122-012-1887-6 (2012).
9. Cui, F. et al. Construction of an integrative linkage map and QTL mapping of grain yield-related traits using three related wheat RIL populations. *Theor. Appl. Genet.* **127**, 659–675. https://doi.org/10.1007/s00122-013-2249-8 (2014).
10. Korontzis, G. et al. QTL detection in a pedigreed breeding population of diploid potato. *Euphytica* **216**, 145 (2020).
11. Ramesh, S. et al. HA 10–2 (HA 5), promising high yielding advanced breeding line for use in commercial production of Dolichos bean (Lablab purpureus L Sweet). *Mysore J. Agric. Sci.* **52**, 1–5 (2018).
12. Shivakumar, M. S. et al. Prediction and validation of frequency of recombinant inbred lines surpassing pre-determined standards for quantitative traits in dolichos bean (Lablab purpureus L. Sweet). *Int. J. Agric. Sci.* **8**, 1325–1328 (2016).
13. Udaykumar, H. R. Identification of SSR markers linked to genomic regions controlling yield and its components and pod fragrance in dolichos bean (*Lablab purpureus* L Sweet), *Ph. D. Thesis*, University of agricultural sciences, GKVK, Bengaluru (2016).
14. Doyle, J. J. & Doyle, J. L. Isolation of plant DNA from fresh tissue. *Focus* **12**, 13–15 (1990).
15. Kosambi, D. D. The estimation of the map distance from recombination values. *Ann. Eugen.* **12**, 172–175 (1944).
16. Voorrips, R. E. MapChart: Software for the graphical presentation of linkage maps and QTLs. *J. Heredity* **93**(1), 77–78 (2002).
17. Konduri, W. et al. Construction and analysis of a high-density genetic linkage map in cabbage (Brassica oleracea L. var. capitata). *BMC Genom.* **13**, 1–12 (2012).
18. Li, S., Wang, J. & Zhang, L. Inclusive composite interval mapping of QTL by environment interactions in bi-parental populations. *PLoS ONE* **10**, e0132414 (2015).
19. Churchill, G. A. & Doerge, R. W. Empirical threshold values for quantitative trait mapping. *Genet.* **138**, 963–971 (1994).
20. Wang, J., Li, H., Zhang, L. & Meng, L., User's manual of QTL IciMapping ver. 4.1. Quantitative Genetics group, Institute of Crop Science, Chinese Academy of Agricultural Sciences (CAAS): Beijing/Genetic Resources Program, International Maize and Wheat Improvement Center (CIMMYT): Mexico City (2016).
21. Lange, K. & Boehnke, M. How many polymorphic marker genes will it take to span the human genome?. *Am. J. Hum. Genet.* **34**, 842 (1982).
22. Beckmann, J. S. & Soller, M. Restriction fragment length polymorphisms in genetic improvement: methodologies, mapping and costs. *Theor. Appl. Genet.* **67**, 35–43 (1983).
23. Paterson, A. H. et al. Mendelian factors underlying quantitative traits in tomato: comparison across species, generations, and environments. *Genet.* **127**, 181–197 (1991).
24. Mohan, M. et al. Genome mapping, molecular markers and marker-assisted selection. *Mol. Breed.* **3**, 87–103 (1997).
25. Doerge, R. W. Mapping and analysis of quantitative trait loci in experimental populations. *Nat. Rev.* **3**, 43–52 (2002).
26 Xu, Y. B. *Molecular plant breeding* (CAB International, Cambridge, 2010).
27. Burr, E., Burr, F. A., Thompson, K. H., Albertsen, M. C. & Stuber, C. W. Gene mapping with recombinant inbreds in maize. *Genet.* **118**, 519–526 (1988).
28. Beavis, W. D. & Grant, D. A linkage map based on information from four F2 populations of maize (Zea mays L.). *Theor. Appl. Genet.* **82**, 636–644 (1991).
29. Semagn, K., Bjornstad, A. & Ndjiondjop, M. N. An overview of molecular marker methods for plants. *Afr. J. Biotechnol.* **5**, 2540–2568 (2006).
30. Liu, H. B. *Statistical Genomics, Linkage Mapping and QTL Analysis* (CRC, Boca Raton, FL, 1998).
31. Paterson, A. H. et al. Comparative genomics of plant chromosomes. *Plant Cell* **12**, 1523–1539 (2000).
32. He, P. et al. Comparison of molecular linkage maps and agronomic trait loci between DH and RIL population derived from the same rice cross. *Crop Sci.* **41**, 1240–1246 (2001).
33. Tanksley, S. D. Mendelian factors underlying quantitative traits in tomato: Comparison across species, generations, and environments. *Genet.* **127**, 181–197 (1991).
34. Gill, K. S., Gill, B. S. & Endo, T. R. A chromosome region specific mapping strategy reveals gene-rich telomeric ends in wheat. *Chromosoma* **102**, 374–381 (1993).
35. Gill, K. S. & Gill, B. S. Mapping in the realm of polyploidy: The wheat model. *BioEssays* **16**, 841–846 (1994).
36. Pedersen, C., Giese, H. & Linde-Laursen, I. Towards an integration of the physical and the genetic chromosome map of barley by in situ hybridization. *Hered.* **123**, 77–88 (1995).
37. Kunzel, G., Korzun, L. & Meister, A. Cytologically integrated physical restriction fragment length polymorphism maps for the barley genome based on translocation breakpoints. *Genet.* **154**, 397–412 (2000).
38. Heslop-Harrison, J. S. The molecular cytogenetics of plants. *J. Cell Sci.* **100**, 15–21 (1991).
39. Wang, M. L., Leitch, A. R., Schwarzacher, T., Heslop-Harrison, J. S. & Moore, G. Construction of a chromosome-enriched Hpall library from flow-sorted wheat chromosomes. *Nucleic Acids Res.* **20**, 1897–1901 (1992).
40. Hayward, M. D. et al. Genetic analysis of Lolium. Identification of linkage groups and the establishment of a genetic map. *Plant Breed.* **117**, 451–455 (1998).

41. Bert, P. F., Charmet, G., Sourdille, P. & Balfourier, F. High density molecular map for ryegrass (Lolium perenne L.) using AFLP markers. *Theor. Appl. Genet.* **99**, 445–452 (1999).
42. Konduri, V., Godwin, I. D. & Liu, C. J. Genetic mapping of the *Lablab purpureus* genome suggests the presence of *'cuckoo'* gene(s) in this species. *Theor. Appl. Genet.* **100**, 866–871 (2000).
43. Yuan, J., Wang, B. & Wu, T. L. Quantitative trait loci (QTL) mapping for inflorescence length traits in Lablab purpureus (L.) Sweet. *Afr. J. Biotech.* **10**, 3558–3566 (2011).
44. Chandrakanth. Mapping quantitative traits loci, and prediction and validation of genomic estimated breeding values for seed yield and its component traits in dolichos bean (*Lablab purpureus* L. Sweet). *Ph. D. Thesis,* Univ. Agric. Sci., Bangalore (2018).
45. Wang, W. et al. Construction and analysis of a high-density genetic linkage map in cabbage (Brassica oleracea L. var. capitata). *BMC Genom.* **13**, 1–12 (2012).
46. Cheema, J. & Dicks, J. Computational approaches and software tools for genetic linkage map estimation in plants. *Brief Bioinform.* **10**, 595–608 (2009).
47. Collard, B. C. Y., Jahufer, M. Z. Z., Brouwer, J. B. & Pang, E. C. K. An introduction to markers, quantitative trait loci (QTL) mapping and marker-assisted selection for crop improvement: the basic concepts. *Euphytica* **142**, 169–196. https://doi.org/10.1007/s10681-005-1681-5 (2005).
48. Jannink, J. L., Mcam, B. & Jansen, R. C. Using complex plant pedigrees to map valuable genes. *Trends Plant Sci.* **6**, 337–342 (2001).
49. Vaijayanthi, P. V. et al. Yield stability analysis of dolichos bean genotypes using AMMI model and GGL biplot. *Int. J. Agri. Sci.* **9**, 4800–4805 (2017).
50. Ramesh, P. S., Marappa, N., Mahadevu, P. & Kanavi, M. S. P. Adaptability of dolichos Bean (Lablab purpureus (L.) Sweet) recombinant inbred lines across locations representing eastern and southern dry zones of Karnataka. *Trop. Agric. Res.* **30**, 75–81 (2018).
51. Stuber, C. W., Lincoln, S. E., Wolff, D. W., Helentjaris, T. & Lander, E. Identification of genetic factors contributing to heterosis in a hybrid from two elite maize inbred lines using molecular markers. *Genet.* **132**, 823–839 (1992).
52. Paterson, A. H., Tanksley, S. D. & Sorrells, M. E. DNA markers in crop improvement. *Adv. Agron.* **83**, 39–90 (1991).
53. Reyna, N. & Sneller, C. H. Evaluation of marker-assisted introgression of yield QTL alleles into adapted soybean. *Crop Sci.* **41**, 1317–1321 (2001).
54. León, A. J., Lee, M. & Andrade, F. H. Quantitative trait loci for growing degree days to flowering and photoperiod response in sunflower (Helianthus annuus L.). *Theor. Appl. Genet.* **102**, 497–503 (2001).
55. Paterson, A. H., Saranga, Y., Menz, M., Jiang, C. X. & Wright, R. J. QTL analysis of genotype x environment interactions affecting cotton fiber quality. *Theor. Appl. Genet.* **106**, 384–396 (2003).
56. Campbell, B. T. et al. Identification of QTLs and environmental interactions associated with agronomic traits on chromosome 3A of wheat. *Crop Sci.* **43**, 1493–1505 (2003).
57. Zhu, S. & Kaeppler, H. F. Identification of quantitative trait loci for resistance to crown rust in oat line MAM17-5. *Crop sci.* **43**, 358–366 (2003).
58. Mackay, T. F. C. Epistasis and quantitative traits: using model organisms to study gene-gene interactions. *Nat. Rev. Genet.* **15**, 22–23 (2014).
59. Viana, J. M. S. & Souza, C. A. S. Efficiency of mapping epistatic quantitatuive trait loci. *Hered.* https://doi.org/10.1038/s41437-023-00618-5 (2023).
60. Singh, A. et al. Identification and mapping in spring wheat of genetic factors controlling stem rust resistance and the study of their epistatic interactions across multiple environments. *Theor. Appl. Genet.* **126**, 1951–1964 (2013).
61. Bokore, F. et al. Main effect and epistatic QTL affecting spike shattering and association with plant height revealed in two spring wheat (*Triticum aestivum*) populations. *Theor. Appl. Genet.* **135**, 1143–1162. https://doi.org/10.1007/s00122-021-03980-2 (2022).
62. Bocianowski, J. Epistasis interaction of QTL effects as a genetic parameter influencing estimation of the genetic additive effect. *Genet. Mol. Biol.* **36**, 93–100 (2013).
63. Doebley, J. & Stec, A. Genetic-analysis of the morphological differences between maize and teosinte. *Genet.* **129**, 285–295 (1991).
64. De-Vicente, M. C. & Tanksley, S. QTL analysis of transgressive segregation in an interspecific tomato cross. *Genet.* **134**, 585–596 (1993).
65. Xiao, J., Li, J., Yuan, L. & Tanksley, S. D. Dominance is the major genetic basis of heterosis in rice as revealed by QTL analysis using molecular markers. *Genet.* **140**, 745–754 (1995).
66. Xu, M. et al. Genetic variation in four maturity genes affects photoperiod insensitivity and PHYA-regulated post-flowering responses of soybean. *BMC Plant Biol.* **13**, 1–14 (2013).
67. Liang, H. et al. Identification of QTLs with main, epistatic and QTL by environment interaction effects for seed shape and hundred-seed weight in soybean across multiple years. *J. Genet.* **95**, 475–477. https://doi.org/10.1007/s12041-016-0648-8 (2016).
68. Orf, J. H. et al. Genetics of soybean agronomic traits: I. Comparison of three related recombinant inbred populations. *Crop Sci.* **39**, 1642–1651 (1999).
69. Li, J. et al. Additive and epistatic QTLs underlying the dormancy in a diploid potato population across seven environments. *Sci. Hortic.* **240**, 578–584 (2018).
70. Hu, S. et al. Genetic basis of kernel starch content decoded in a maize multi-parent population. *Plant Biotechnol. J.* **19**, 2192–2205 (2021).
71. Cakir, M. S. et al. Mapping and validation of the genes for resistance to Pyrenophora teres f. teres in barley (Hordeum vulgare L.). *Aust. J. Agric. Res.* **54**, 1369–1377 (2003).
72. Collins, H. M., Panozzo, J. F., Logue, S. J., Jefferies, S. P. & Barr, A. R. Mapping and validation of chromosome regions associated with high malt extract in barley (Hordeum vulgare L.). *Aust. J. Agric. Res.* **54**, 1223–1240 (2003).
73. Sharp, P. J. et al. Validation of molecular markers for wheat breeding. *Aust. J. Agric. Res.* **52**, 1357–1366 (2001).
74. Bernardo, R. *Essentials of plant breeding* 2nd edn. (Stemma Press. Woodbury, 2023).
75. Beavis, W. D. The power and deceit of QTL experiments: lessons from comparative QTL studies. In *Proceedings of the forty-ninth annual corn and sorghum industry research conference*. 250, 266 (1994).

Acknowledgements

The senior author gratefully acknowledges Council of Scientific and Industrial Research (CSIR), New Delhi, India for providing Junior Research Fellowship (JRF) vide No. 09/0271(11202)/2021-EMR-1 dated 01-07-2020 for pursuing PhD degree program at University of Agricultural Sciences, Bangalore, India.

Author contributions

Basanagouda Gonal: investigation, methodology, data curation, writing – review & editing, writing – original draft. Ramesh Sampangi: supervision, conceptualization, methodology, data curation. Kalpana Pundalik Mugali: methodology, data curation. Siddu Basavaraj Chindi: writing – review & editing. Chandana B.R.: methodology, data curation. Satish H.: Writing – review & editing. Prashantha V.: data curation. Sindhu D.: Data curation. Kemparaju M.: writing – review & editing. Sinchana B. V.: Data curation

Declarations

Competing interests
The authors declare no competing interests.

Additional information
Supplementary Information The online version contains supplementary material available at https://doi.org/10.1038/s41598-025-90558-3.

Correspondence and requests for materials should be addressed to B.G., R.S., K.P.M. or S.B.C.

Reprints and permissions information is available at www.nature.com/reprints.

Publisher's note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.