

Genetic dissection of QTLs linked to seed yield and its contributing traits in horsegram (*Macrotyloma uniflorum*)

Megha Katoch

CSK HPKV: CSK Himachal Pradesh Krishi Vishvavidyalaya

Maneet Rana

IGFRI: Indian Grassland and Fodder Research Institute

T R Sharma

CSK HPKV: CSK Himachal Pradesh Krishi Vishvavidyalaya

Rakesh Kumar Chahota (✉ rkchahota@hillagric.ac.in)

CSK HPKV: Himachal Pradesh Agricultural University <https://orcid.org/0000-0001-9029-4345>

Research Article

Keywords: *Macrotyloma uniflorum*. Genetic linkage map. QTL map. Phenotypic variation, Seed yield

Posted Date: January 9th, 2024

DOI: <https://doi.org/10.21203/rs.3.rs-3834339/v1>

License:   This work is licensed under a Creative Commons Attribution 4.0 International License. [Read Full License](#)

Abstract

With continuous increase in world's population, the main challenge among breeders is to increase food production with endurable utilization of available natural resources to accomplish a goal of zero hunger. Cultivating the nutritious underutilized crops could be a significant approach to address the issues of nutritional insecurity. Among 39 crops identified by FAO, horsegram is also considered as an important "Future Smart Food". It has attracted interest amongst the scientific community due to its unparalleled nutritional and pharmaceutical properties. Improving seed yield and related traits are main objectives of plant breeders for enhancing productivity in the crops. Horsegram lacks the information on the genetic structure of its yield contributing traits. The present study was aimed to dissect the quantitative trait loci of seed yield and its associated traits in horsegram by utilizing linkage map already developed in our lab of intraspecific F8 RIL population of 162 individuals derived from HPKM249×HPK4 cross. Phenotypic data of different years and locations were used to identify QTLs for yield related traits by composite interval mapping (CIM). A total of six QTLs ($LOD \geq 2.5$) were detected across different environments for seed size, seeds per plant, pods per plant and yield per plant. These were major QTLs with phenotypic variation explained (PVE) greater than ten percent and one of them was stable QTL across different locations and years. Phenotypic variation explained by QTLs ranged from 10.8 to 20.16 per cent thus they are of economic importance.

Introduction

Horsegram (*Macrotyloma uniflorum*) belongs to family Fabaceae having chromosomes number $2n = 20$ (Allen and Allen 1981) and genome size of 398 Mbps (Hirakawa et al. 2017) with *M. axillare* as its probable progenitor. Despite the fact that *Macrotyloma uniflorum* is native to tropical Africa, the cultivated variety is thought to have originated in Southern India (Vavilov 1951; Zohary 1970). It is an arid crop with an inherent ability to survive in varied environmental conditions and on diverse range of soils. It is cultivated as food legume in India, Sri Lanka, Mauritius, Nepal, Malaysia and Myanmar by the poorest of the poor farmers of the marginal areas, whereas, in Africa and Australia it is being grown in arranged pastures (Asha et al. 2006). It is highly suitable for rainfed and marginal agriculture, where other food legumes aren't as effective. Other than its ability to grow under drought like situation (Reddy et al. 1990), it also possesses number of desirable traits like, tolerance to heavy metal stress (Sudhakar et al. 1992), high percentage of protein & antioxidants (Reddy et al. 2005) and various medicinal properties (Yadava and Vyas 1994; Ravishankar and Vishnupriya 2012) that make it a crop of interest and potential food source of future. The growing global population accompanied by deterioration of cultivated land has put tremendous pressure on breeders to enhance food grain production by exploring the potentiality of underutilized food sources. Horsegram is one of many such crops, which can be exploited as potential crop in the face of climate change. Low genetic potential of most of the released varieties which, are developed from narrow genetic base is the major bottleneck to enhance its productivity. Attempts have also been made in the past to improve these crops by integrating favourable QTLs for various target traits in a single plant genotype (Wu 1998). Lack of genetic variation and genetics of important traits are other roadblocks to initiate a systematic breeding programme to develop higher yielding varieties. The scarcity of genomic resources

challenged us to undertake SSR markers development programme from related well characterized legume species. We initiated horsegram marker development programme in the year 2012 and currently we have a repertoire of more than 10,000 SSRs markers including genomic and genic SSRs developed from horsegram transcriptome and genomic sequences data (Sharma et al. 2015). These genomic resources are now being employed to improve grain yield, plant architecture and important agronomic traits. In conventional breeding programme, the selection of plant is based on phenotypic variation which is not always a rewarding strategy as most of these traits are controlled by many genes hence mostly influenced by environment. In such conditions, DNA markers-based selection can be proved more efficient tools to develop cultivar, if target loci are tightly linked to the marker. Hence, mapping quantitative trait loci (QTLs) associated with genomic regions harbouring genes for these traits represent a promising selection strategy. Gene and QTLs mapping is also important for undertaking marker-aided breeding and trait improvement; however, until now only limited studies on mapping QTLs and/or genes in horsegram have been reported. QTL maps with five QTLs for drought tolerance (Chahota et al. 2020) and four & seven QTLs for morphological & phenological traits, respectively (Katoch et al. 2022) were reported earlier in horsegram. Also a study on drought tolerance potential of horsegram (*Macrotyloma uniflorum* Var.) germplasm using genome wide association studies was reported by Choudhary et al. 2022. In this study we have attempted to identify QTLs linked to yield related traits of *Macrotyloma uniflorum* using already developed linkage map based in SSR markers (Katoch et al. 2022) in our lab. SSR markers have many advantages over the other marker systems. The first advantage is their high reproducibility, The second advantage of the SSR marker system is the polymorphic genetic information contents. High allelic variations among very closely related varieties can be detected by hyper-variable nature of SSRs. The third advantage has to do with the co-dominant nature of SSR polymorphisms.

Material and methods

Plant material

Mapping population in the present study consisted of 162 RILs of F₈ generation derived from intra-specific cross of HPKM249 X HPK4 using Single Seed Descent (SSD) method. Both the parents showed contrasting characteristics for the traits under study. HPKM249 have large seed size and have a greater number of pods per plant (> 30) whereas HPKM4 have small seed size and have lesser number of pods per plant (< 10).

Phenotyping

During the crop season, F₈ and F₉ progenies were grown for field evaluations of various seed yield related traits. The mapping population along with their parents were grown at two locations (Palampur and Bajaura) over a period of two seasons (2016 and 2017) using augmented block design (ABD) with two replications and four checks, VLG-1, HPKM249, HPK4 and HPK317 repeated after 20 rows. Each one-metre line consisted of 10 plants spaced 30 cms apart.

Phenotyping was done for six yield related traits viz. seed size (SZ, mm), 100-seed weight (SW, g), seeds per pod (SP), pods per plant (PP), seeds per plant (SS) and seed yield per plant (SY, g). Data were recorded on five randomly taken plants from the central section of each row for all the trait under study. Traits distribution was studied using skewness and kurtosis statistics by Past 3.25 software. Pearson correlation coefficients and Principal Component Analysis (PCA) among different traits were calculated using the same software.

QTL analysis:

For quantitative trait analysis, genomic regions harboring loci for yield related traits were identified using composite interval mapping (CIM) method (Zeng 1993; Zeng 1994) using Windows QTL Cartographer V2.5 software (Wang et al. 2005). Walking speed selected for QTLs was 2 cM with a window size of 10 cM using the Zmapqtl standard model 6. The forward regression algorithm was used to obtain cofactors. To calculate a genome wide threshold for LOD score a 1000-permutation test at a significance level of $P = 0.05$ for shuffling genotypes with the phenotypic means was performed (Doerge and Churchill 1996). A LOD threshold score of ≥ 2.5 was selected for identification of the QTLs on the horsegram linkage groups (LGs). The location of QTLs with 95% confidence intervals were estimated by one LOD intervals around the QTL peak (Mangin et al. 1994). The estimated additive effects and the percentage of phenotypic variation explained (PVE) based on the R^2 value by each putative QTL was calculated by Zmapqtl procedure using the software with the CIM model. The QTL map was prepared with MapChart 2.32 software (Voorrips 2002).

Results

Trait analysis

Horsegram being an orphan crop, very little has been understood about genetic structure of its yield contributing traits. As indicated, we analyzed six yield-related traits. The descriptive statistics for all the traits are shown in Table 1. Parental lines, HPKM249 and HPK4 were used for developing mapping population found to have difference for all the traits studied. HPK4 has higher 100 seed weight, seed size, seed number, pod number, seed yield per plant and late maturing, whereas HPKM249 is very early maturity and having lesser number of pods, seeds, seed yield and seed size across two years. Phenotyping data for these traits showed significant genetic variation for RIL population across environments. Variations in yield related traits were observed for all six traits in different environments. The phenotypic values were distributed in a normal frequency distribution, which is typical of quantitative traits (Fig. 1). Correlations values among traits in RIL populations showed that the traits were positively correlated with each other and were statistically significant ($P < 0.05$). The maximum correlation was found between YLD and SS ($r = 0.89$) followed by SZ and SS ($r = 0.88$) (Fig. 2). YLD showed positive correlations with all traits in all the three environments.

Table 1
Mean performance of parents and RILs in different environments for yield related traits

	Year	Loc.		HPKM249	HPK4	Range (RIL)	Mean	SD
100 Seed weight (SW)	2016	PLP	-	3.12	4.02	2.14–5.21	3.52	0.53
	2017	PLP	-	4.03	5.07	3.08–6.73	4.79	0.65
	2017	BJR	-	3.47	4.30	2.71–4.33	3.46	0.31
	Combined	-	-	3.62	4.55	2.97–5.11	4.00	0.39
Seed size (SZ)	2016	PLP	-	0.57	0.63	0.51–0.66	0.57	0.03
	2017	PLP	-	0.57	0.66	0.34–0.67	0.61	0.04
	2017	BJR	-	0.58	0.67	0.51–0.62	0.57	0.02
	Combined	-	-	0.57	0.66	0.49–0.63	0.59	0.02
Seeds per pod (SP)	2016	PLP	-	4.00	4.00	2.00–6.00	4.24	0.70
	2017	PLP	-	4.00	4.50	2.50–5.50	4.19	0.64
	2017	BJR	-	4.00	4.00	3.00-5.50	4.39	0.46
	Combined	-	-	4.00	4.20	3.20–5.20	4.28	0.40
Pods per plant (PP)	2016	PLP	-	25.00	21.00	6.00–26.00	15.55	3.48
	2017	PLP	-	21.00	18.00	6.00-26.50	17.06	3.60
	2017	BJR	-	36.50	33.00	19.50–36.50	28.89	3.16
	Combined	-	-	28.00	24.60	15.00–27.00	21.49	2.37
Seeds per plant (SS)	2016	PLP	-	100.00	84.00	18.00-130.00	66.23	19.39
	2017	PLP	-	84.00	83.00	20.00-123.00	71.34	19.21

	Year	Loc.		HPKM249	HPK4	Range (RIL)	Mean	SD
	2017	BJR	-	146.00	132.00	78.00-170.50	126.69	18.31
	Combined	-	-	112.00	102.80	61.20-126.20	92.46	13.00
Seed yield per plant (YLD)	2016	PLP	-	3.12	3.38	0.39–5.31	2.34	0.77
	2017	PLP	-	3.49	4.27	0.94–7.10	3.43	1.03
	2017	BJR	-	5.05	5.67	2.64–6.32	4.38	0.73
	Combined	-	-	4.04	4.65	2.30–5.73	3.59	0.62

QTL Mapping

The QTL analysis locate 6 major QTLs ($LOD \geq 2.5$) explaining up to 20.16 per cent PVE (Fig. 3 and Table 2). Two QTLs were detected for seed size namely *qSZ01* and *qSZ02* on LG4 and LG9 flanked by MUSSR501-MUGSSR19 and HUGMS3-MUGR607 markers respectively and explaining 15.07 and 20.16 per cent of the phenotypic variation respectively. One QTL namely *qSS01* was detected for seeds per plant on LG1 at 120.91 cM position flanked by RCS20321 & HUGMS53 marker interval explaining 10.8 per cent phenotypic variation. For pods per plant, *qPP01* was detected on LG1 at 120 cM flanked by RCS20321-HUGMS53 marker with 14.36 per cent phenotypic variation explained. Seed yield per plant is considered as dependent variable as it is the final product of many other yield contributing traits. Two QTLs, namely *qYLD01* and *qYLD02* were detected on LG7 at position 56.71 cM flanked by markers MUD77-HUGMS13 and at position 101.41 cM with OPI61-MUGSSR14 flanking markers explaining 16.47 and 12.15 per cent phenotypic variation respectively. All the QTLs showed positive additive values except for *qSZ01*, which was responsible for more than 10 per cent variation for seed size (Table 2). Additive effect is the difference in the average performance of the RIL carrying yield allele of first parent with respect to those carrying the allele of second parent at the particular locus (QTL). A positive value (+) of the additive effect indicates the allele originating from HPK4 and a negative value (–) of the additive effect indicates the allele originating from HPKM249. Additive effect of each QTL is indicated in Table 2.

Table 2
QTLs for yield related traits identified using QTL Cartographer

S.No	Trait Name	QTL Name	LG	Position (cM)	Environment	Marker Interval	LOD	Additive effect	PVE
1	Seed Size	<i>qSZ01</i>	4	139.01	BJR17	MUSSR501-MUGSSR19	3.4	-0.01	15.07
2	Seed Size	<i>qSZ02</i>	9	44.01	COMBINED	HUGMS3-MUGR607	4.1	0.01	20.16
3	Seeds per plant	<i>qSS01</i>	1	120.91	PLP17	RCS20321-HUGMS53	4.5	8.06	10.8
4	Pods per plant	<i>qPP01</i>	1	120.91	PLP17	RCS20321-HUGMS53	6.2	1.75	14.36
5	Yield	<i>qYLD01</i>	7	56.71	PLP16	MUD77-HUGMS13	2.50	5.4	16.47
6	Yield	<i>qYLD02</i>	7	101.41	PLP16	OPI61-MUGSSR14	2.54	5.3	12.15

Discussion

Linkage maps and genetic information of a trait are prerequisites for using molecular markers for breeding desirable crop varieties. Well saturated linkage map is like a GPS map, which guides to reach the target loci. Various types of molecular markers are being used to construct saturated linkage maps in different crops; however SSR markers have numerous benefits over other molecular markers, such as co-dominance, multi-allelic, relatively abundant, widely dispersed across the genome, and can be easily scored (Powell et al. 1996).

In addition, microsatellites markers have been considered to be eminently proficient for linkage map construction and QTL analysis in crop plants. Since QTLs were first identified in tomato using RFLP based linkage maps by Paterson et al. (1988), however in many crops QTLs for important traits have also been identified by using different types of markers. Horsegram is an under-exploited leguminous crop with numerous medicinal and nutraceutical properties and is grown by the marginal farmers of the drought prone areas. It serves as an important source of protein for poor communities. In spite of its importance in marginal agriculture, the genetics of important traits have not yet been determined and similarly very few QTLs for important traits have been mapped (Chahota et al. 2020). This may be due to limited species variation in cultivated *Macrotyloma uniflorum* var *uniflorum* and difficulty in introgression of desirable genes from related species such as *M. axillare* (Chahota et al. unpublished work).

Identification of genomic regions controlling important yield related traits is the first step towards implementing genomic-assisted breeding in this orphan legume species. In comparison to other important legumes, the development of genetic resources in horsegram has trailed far behind. However, in recent

years efforts have been made to develop molecular markers through mining of transcriptome and genomic sequencing data (Sharma et al. 2015; Chahota et al. 2017; Kaldete et al. 2017). More recently draft genome sequencing has also been published in which 1378 SNPs have been genotyped on 214 F₂ population of HPK4 × HPKM193 (Shirasawa et al. 2021) indicated the limited variation at SNP level. In the present study the level of polymorphism in parental line is 24.52% which is comparable to polymorphism observed in other legumes such as 22.1% in chickpea (Radhika et al. 2007), 23.6% in peanut (Hong et al. 2010), 26.8% in adzuki bean (Chaitieng et al. 2006) and 27.02% in soybean (Hwang et al. 2009).

In general, the main bottleneck in the development of high yielding varieties in grain legumes is the dearth of genomic and genetic information of many important traits. However, this problem is further aggravated with narrow genetic base and presence of many weedy and wild characteristics. Yield and yield related traits are considered complex traits exhibiting quantitative inheritance because their phenotypic expression depends on many factors such as minor genes, environments and various modifiers (Kover et al. 2009). The genetic information of the important traits is revealed by dissecting the potential genomic regions harbouring QTLs controlling these traits.

Genetic improvement of this crops can also be accelerated through marker-assisted selection (MAS), which is aided by identification of major QTLs for important traits. Thus, identification of major QTLs is an essential step for the improvement of grain legumes. (Bocianowski 2013). The QTL maps for important agronomic traits aids in their mapping and in identifying closely linked markers to the specific trait for marker-assisted selection and positional cloning.

One of the concerns of the farming community for the large-scale cultivation of any crop is the the enhanced crop productivity per unit area and its yield stability. The present study allows us the identification of important genomic regions linked to yield related traits in horsegram. On the basis of this genetic map and marker-trait associations, a total of 6 QTLs for these traits were identified. Of the 6 QTLs detected in the present study, one QTLs each for seeds per plant and pods per plant were present on LG1, for seed size one QTL each was present on LG4 and LG9 and two QTLs for seed yield were present on LG7.

Since horsegram lagged behind other grain legumes in the development of genomic resources, recent efforts for the construction of linkage map and identification of important QTLs (Chahota et al. 2020; Katoch et al. 2021) aids in genetic improvement of horsegram by enhancing the availability of molecular markers. More recently SNP based linkage map has also been published (Shirasawa et al. 2021), which will further enhance the availability of genomic information in horsegram for its genetic improvement. To further strengthen the application of these QTLs in horsegram genomic-assisted breeding, saturation of linkage map with more molecular markers and locating more tightly linked markers to important genomic regions should be pursued. Construction of high-density linkage map with the inclusion of SNP markers would increase the resolution of QTLs and provide a better picture of the occurrence of these QTLs for future genetic and genomic studies.

Conclusion

To understand the genetic basis of yield related traits in horsegram, six major QTLs were identified. It was shown that yield traits are influenced by a variety of loci with varying impacts, demonstrating the complexity of these traits. These findings may increase our knowledge of genetic background of these traits, provide the basis for MAS of such traits to enhance genetic gain and facilitate the identification of genes controlling them. However, more saturated genetic linkage map with large number of robust markers like SNPs would facilitate precise and targeted identification of genomic regions which may further enhance the resolution of identified QTLs for mapping.

Declarations

Acknowledgements: Authors are grateful to WOS-A, Department of Science & Technology, Govt of India and Japan Society for the Promotion of Science, Japan.

Author Contributions: RKC conceived the idea and provided all the consumables and edited the final draft of manuscript, MK performed the experiment and recorded molecular and morphological data, MR analyzed the data and wrote the rough draft, TRS provided all the facility to carry out the molecular analysis.

Conflict of Interest: The authors declare that they have no conflict of interest.

Declaration of Funding: The proposed study was funded jointly by SERB and WOS-A, DST, Govt of India

References

1. Allen ON, Allen EK (1981) The Leguminosae: a source book of characteristics, uses, and nodulation. The Univ. of Wisconsin Press, Madison. <https://doi.org/10.1007/BF02858721>
2. Asha KI, Itha M, Abraham Z, Jayan PK, Nair MC and Mishra SK (2006) Genetic resources. In: Kumar D (ed) Horsegram in India. Scientific Publisher, Jodhpur, pp 11-28
3. Bocianowski J (2013) Epistasis interaction of QTL effects as a genetic parameter influencing estimation of the genetic additive effect. Genet Mol Biol 36(1):093–100. <https://doi.org/10.1590/S1415-47572013000100013>
4. Chahota RK, Sharma V, Rana M, Sharma R, Choudhary S, Sharma TR, Shirasawa K, Hirakawa Isobe SN (2020) Construction of a framework linkage map and genetic dissection of drought- and yield-related QTLs in horsegram (*Macrotyloma uniflorum*). Euphytica, 216, 61. doi: 10.1007/s10681-020-02583-0
5. Chahota RK, Shikha D, Rana M, Sharma V, Nag A, Sharma TR Rana JC, Hirakawa H, Isabe S (2017) Development and Characterization of SSR Markers to Study Genetic Diversity and Population Structure of Horsegram Germplasm (*Macrotyloma uniflorum*). Plant Mol Bio Rep 10:1007. <https://doi.org/10.1007/s11105-017-1045-z>
6. Chaitieng B, Kaga A, Tomooka N, Isemura T, Kuroda Y, Vaughan D A (2006) Development of a black gram [*Vigna mungo* (L.) Hepper] linkage map and its comparison with an azuki bean [*Vigna angularis*

- (Willd.) Ohwi and Ohashi] linkage map. *Theor Appl Genet* 113:1261– 1269. <https://doi.org/10.1007/s00122-006-0380-5>
7. Charu L and Manoj P (2011) Role of DREBs in regulation of abiotic stress responses in plants. *J. Exp. Bot* 62: 4731–4748
 8. Choudhary S, Isobe S, Chahota RK (2022) Elucidation of drought tolerance potential of horsegram (*Macrotyloma uniflorum* Var.) germplasm using genome wide association studies. *Gene* 819: 146241. <https://doi.org/10.1016/j.gene.2022.146241>.
 9. Doerge RW, Churchill GA (1996) Permutation tests for multiple loci affecting a quantitative character. *Genetics* 142:285-294. pmid:8770605.
 10. Eujayl I, Sledge M K, Wang L, May GD, Chekhovskiy K, Zwonitzer JC and Mian MA (2004) *Medicago truncatula* EST-SSRs reveal cross-species genetic markers for *Medicago* spp. *Theor. Appl. Genet.* 108: 414–422.
 11. Grisi MCM, Blair MW, Gepts P, Brondani C, Pereira PAA, Brondani RPV (2007) Genetic mapping of a new set of microsatellite markers in a reference common bean (*Phaseolus vulgaris* L.) population BAT93 × Jalo EEP558. *Genet Mol Res* 6:691–706. pmid: 18050090
 12. Gupta M, Verma B, Kumar N, Chahota RK, Rathour R, Sharma SK, Bhatia S and Sharma TR (2012) Construction of intersubspecific molecular genetic map of lentil based on ISSR, RAPD and SSR markers. *J. Genet* 91:279–287
 13. Hirakawa H, Chahota RK, Shirasawa K, Sharma TR, Isobe S (2017, May 29–31) Draft genome sequencing of horsegram (*Macrotyloma uniflorum*); PAG Asia, South Korea
 14. Hong Y, Chen X, Liang X, Liu H, Zhou G, Li S, Wen S, Holbrook CC, Guo B (2010) A SSR-based composite genetic linkage map for the cultivated peanut (*Arachis hypogaea* L.) genome. *BMC PlantBiol* 10:17. <https://doi.org/10.1186/1471-2229-10-17>
 15. Hwang T, Sayama T, Takahashi M, Takada Y, Nakamoto Y, Funatsuki H, Hisano H, Sasamoto S, Sato S, Tabata S, Kono I, Hoshi M, Hanawa M, Yano C, Xia Z, Harada K, Kitamura K, Ishimoto M (2009) High density integrated linkage map based on SSR markers in soybean. *DNA Res* 16:213-225. <https://doi.org/10.1093/dnares/dsp010>
 16. Kaldete R, Maneet R, Sharma V, Hirakawa H, Kumar R, Singh G, Chahota RK, Isobe SN, Sharma TR (2017) Development of genome-wide SSR markers in horsegram and their use for genetic diversity and cross-transferability analysis. *Mol Breeding* 37(8). <https://doi.org/10.1007/s11032-017-0701-1>
 17. Katoch, M., Mane, R.S. and Chahota, R.K. (2022). Identification of QTLs Linked to Phenological and Morphological Traits in RILs Population of Horsegram (*Macrotyloma uniflorum*). *Frontiers in Genetics*, 12.
 18. Kover PX, Valdar W, Trakalo J, Scarcelli N, Ehrenreich IM, Purugganan MD (2009) A multiparent advanced generation inter-cross to fine-map quantitative traits in *Arabidopsis thaliana*. *PLoS Genet* 5(7):1-15.<https://doi.org/10.1371/journal.pgen.1000551>
 19. Mangin B, Goffinet B, Rebai A (1994) Constructing confidence intervals for QTL location. *Genetics* 138 :1301-1308. pmid: 7896108

20. Murray MG, Thompson WF (1980) Rapid isolation of high molecular weight plant DNA. *Nucl Acids Res* 8:4321-4325.
21. Paterson AH, Lander ES, Hewitt JD, Peterson S, Lincoln SE, Tanksley SD (1988) Resolution of quantitative traits into Mendelian factors, using a complete linkage map of restriction fragment length polymorphisms. *Nature* 335:721–726. <https://doi.org/10.1038/335721a0>
22. Powell W, Morgante M, Andre C, Hanafey M, Vogel J, Tingey S, Rafalski A (1996) The comparison of RFLP, RAPD, AFLP and SSR (microsatellite) markers for germplasm analysis. *Mol Breed* 2:225–238. doi: 10.1007/BF00564200.
23. Radhika P, Gowda SJM, Kadoo NY, Mhase LB, Jamadagni BM, Sainani MN, Chandra S, Gupta VS (2007) Development of an integrated intraspecific map of chickpea (*Cicer arietinum* L.) using two recombinant inbred line populations. *Theor. Appl. Genet* 115:209-216. <https://doi.org/10.1007/s00122-007-0556-7>
24. Ravishankar K and VishnuPriya PS (2012) *In Vitro* antioxidant activity of ethanolic seed extracts of *Macrotyloma uniflorum* and cucumismelo for therapeutic potential. *IJPRC* 2(2): 442–445
25. Reddy AM, Kumar SG, Kumari GJ, Thimmanaik S, Sudhakar C (2005) Lead induced changes in antioxidant metabolism of horsegram (*Macrotyloma uniflorum* (Lam.) Verdc.) and bengalgram (*Cicer arietinum* L.) *Chemosphere* 60:97–104. <https://doi.org/10.1016/j.chemosphere.2004.11.092>
26. Reddy PS, Sudhakar C, Veeranjanyulu K (1990) Water stressed induced changes in enzymes of nitrogen metabolism in horsegram, (*Macrotyloma uniflorum* (Lam.)) seedlings. *Indian J Exp Biol* 28:273–276.
27. Sato S, Isobe S, Asamizu E, Ohmido N, Kataoka R, Nakamura Y, Keneko T, Sakurai N, Okumura K, Klimenko I, Sasamoto S, Wada T, Watanabe A, Kothari M, Fujishiro T and Tabata S (2005) Comprehensive structural analysis of the genome of red clover (*Trifolium pratense* L.). *DNA Res* 12: 301-364.
28. Sharma V, Rana M, Katoch M, Sharma PK, Ghani M, Rana JC, Sharma TR, Chahota RK (2015) Development of SSR and ILP markers in horsegram (*Macrotyloma uniflorum*), their characterization, cross-transferability and relevance for mapping. *Mol Breeding* 35:102. <https://doi.org/10.1007/s11032-015-0297-2>
29. Shirasawa K, Chahota RK, Hirakawa H, Nagano S, Nagasaki H, Sharma T, Isobe S (2021) A Chromosome-scale draft genome sequence of horsegram (*Macrotyloma uniflorum*). *Gigabyte*, DOI:10.46471/gigabyte.30
30. Sudhakar C, Bai LS, Veeranjanyulu K (1992) Lead tolerance of certain legume crops grown on lead ore tailings. *Agric Ecosyst. Environ* 41:253–261. [https://doi.org/10.1016/0167-8809\(92\)90114-Q](https://doi.org/10.1016/0167-8809(92)90114-Q)
31. van Ooijen JW (2006) JoinMap, software for the calculation of genetic linkage maps. Kyazma BV, Wageningen, the Netherlands, Version 4.
32. Vavilov NI (1951) Phytogeographic basis of plant breeding. The origin, variation, immunity and breeding of cultivated plants. *Chronica Botanica* 13:1-366.

33. Voorrips RE (2002) MapChart: software for the graphical presentation of linkage maps and QTLs. *J Hered* 93:77-78. <https://doi.org/10.1093/jhered/93.1.77>
34. Wang S, Basten CJ, Zeng ZB (2005) Windows QTL Cartographer 2.5 Department of Statistics, North Carolina State University, Raleigh, NC.
35. Winter P, Benko-Iseppon AM, Hüttel B, Ratnaparke M, Tullu A, Sonnante G, Paff T, Tekeoglu M, Santra D, Sant VJ, Rajesh PN, Kahl G and Muehlbauer FJ (2000) A linkage map of the chickpea (*Cicer arietinum* L.) genome based on recombinant inbred lines from a *C. arietinum* x *C. reticulatum* cross:1272 localization of resistance genes for Fusarium wilt races 4 and 5. *Theor Appl Genet* 101:1155-1163.
36. Wu RL (1998) Genetic mapping of QTLs affecting tree growth and architecture in *Populus*: implication for ideotype breeding. *Theor Appl Genet* 96:447–457. <https://doi.org/10.1007/s001220050761>
37. Yadava ND and Vyas NL (1994) Arid legumes. India: Agrobios
38. Zeng ZB (1993) Theoretical basis for separation of multiple linked gene effects in mapping quantitative trait loci. In: Proceeding of National Academy of Sciences, USA, pp 10972-10976. <https://doi.org/10.1073/pnas.90.23.10972>
39. Zeng ZB (1994) Precision mapping of quantitative trait loci. *Genetics* 136:1457-1468. pmid: 801391
40. Zohary D (1970) Centres of diversity and centres of origin. In O.H. Frankel and E. Bennett (ed) Genetic resources in plants- their exploration and conservation. Oxford, Blackwell, pp 33-42

Figures

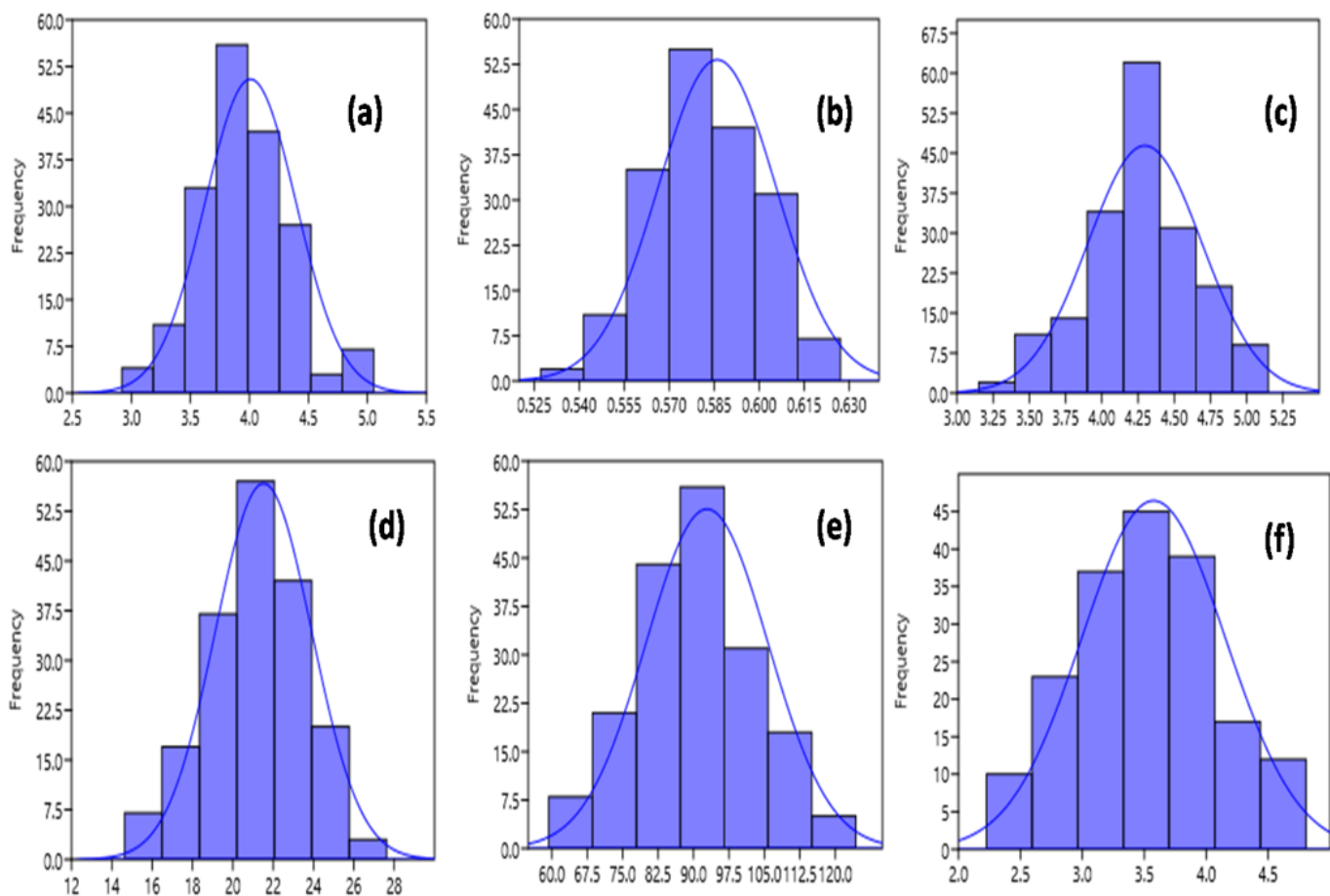


Figure 1

Frequency distribution curve **(a)** 100 Seed weight **(b)** Seed size **(c)** Seeds per pod **(d)** Pods per plant **(e)** Seeds per plant **(f)** Seed yield per plant in the F₈ RIL population

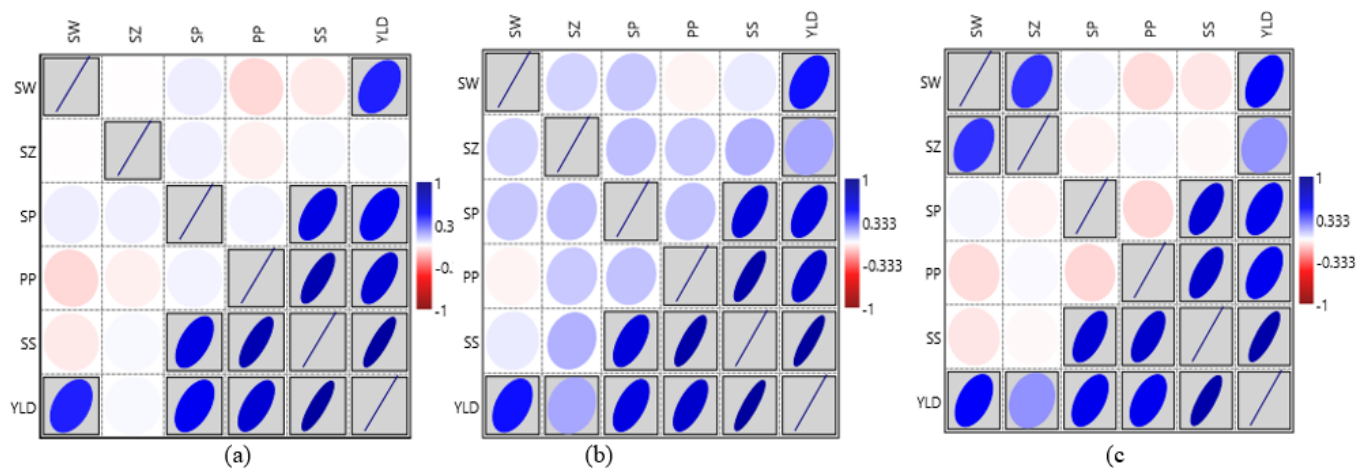


Figure 2

Pearson's correlation matrix among different yield related traits in the HPM249 × HPK4 RILs for **(a)** Palampur 2016 **(b)** Palampur 2017 **(c)** Bajaura 2017

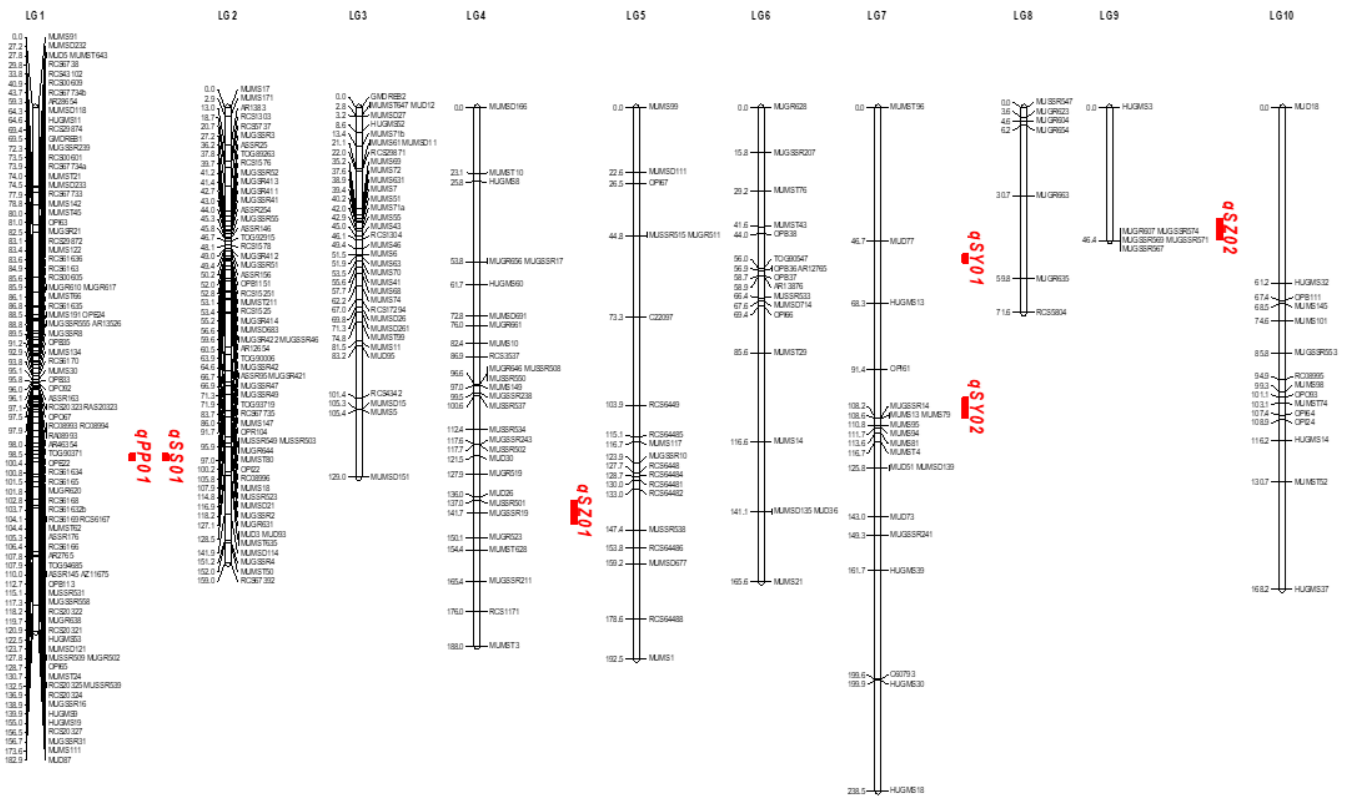


Figure 3

different LGs of horsegram linkage map **3** Quantitative trait loci (QTLs) associated with yield related traits located on

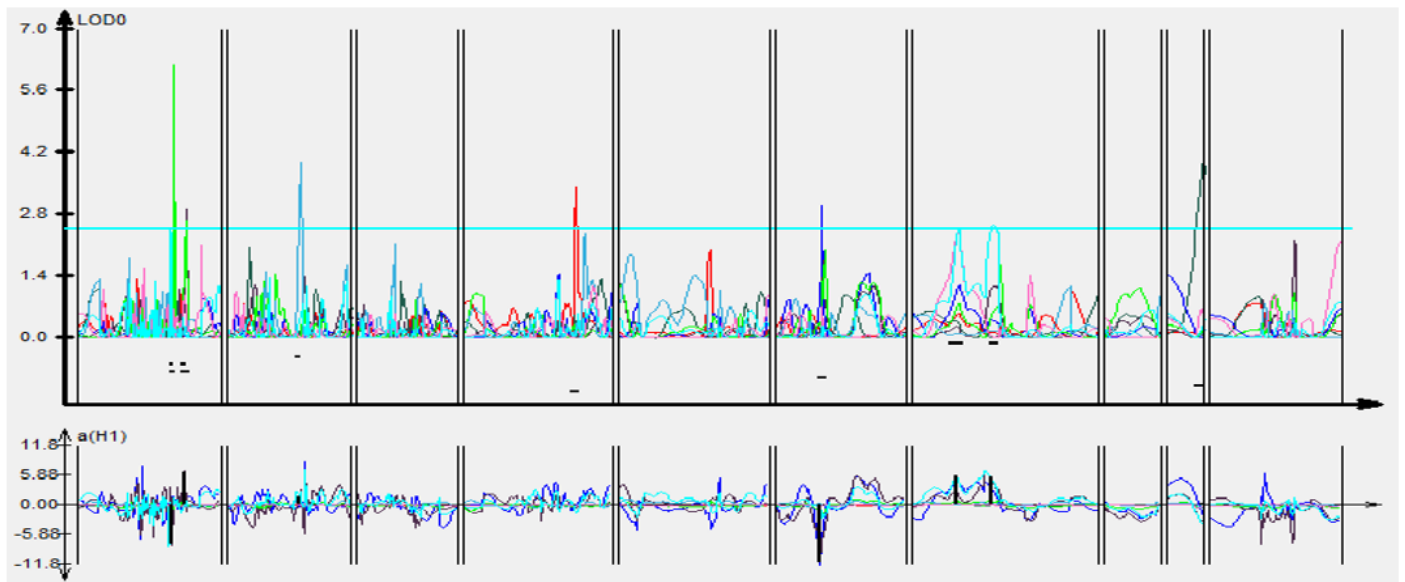


Figure 4

Position of QTLs for yield related traits on 10 linkage groups of horsegrain