

**Identification of QTLs linked to early maturity
and yield-related traits in horsegram
(*Macrotyloma uniflorum*)**

THESIS

By

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(A-2014-40-001)**

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CERTIFICATE – I

This is to certify that the thesis entitled, “**Identification of QTLs linked to early maturity and yield-related traits in horsegram (*Macrotyloma uniflorum*)**” submitted in partial fulfilment of the requirements for the award of the degree of **Doctor of Philosophy (Agriculture)** in the discipline of **Agricultural Biotechnology** of Chaudhary Sarwan Kumar Himachal Pradesh Krishi Vishvavidyalaya, Palampur is a bonafide research work carried out by **Ms. Megha Katoch** daughter of Mr. Ramesh Katoch under my supervision and that no part of this thesis has been submitted for any other degree or diploma.

The assistance and help received during the course of this investigation have been fully acknowledged.

Place: Palampur
Dated: 24th December 2019

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CERTIFICATE- II

This is to certify that the thesis entitled, “**Identification of QTLs linked to early maturity and yield-related traits in horsegram (*Macrotyloma uniflorum*)**” submitted by **Ms. Megha Katoch (Admission No. A-2014-40-001)** daughter of Mr. Ramesh Katoch to the Chaudhary Sarwan Kumar Himachal Pradesh Krishi Vishvavidyalaya, Palampur in partial fulfilment of the requirements for the degree of **Doctor of Philosophy (Agriculture)** in the discipline of **Agricultural Biotechnology** has been approved by the Advisory Committee after an oral examination of the student in collaboration with an External Examiner.

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LIST OF ABBREVIATIONS USED

Sr. No.	Abbreviation	Meaning
1	SW	100-seed Weight
2	APS	Ammonium per sulphate
3	AFLP	Amplified Fragment Length Polymorphism
4	ANOVA	Analysis of variance
5	et al	and co-workers
6	Bp	Base pair
7	CAR	Carotenoids
8	Cm	Centi meter
9	cM	Centi Morgan
10	CTAB	Cetyl Trimethyl Ammonium Bromide
11	CHL	Chlorophyll
12	CIM	Composite Interval Mapping
13	Cv	Cultivar
14	FL	Days to 50% flowering
15	MT	Days to maturity
16	°C	Degree Celsius
17	dATP	Deoxyadenosine triphosphate
18	dCTP	Deoxycytosine triphosphate
19	dGTP	Deoxyguanosine triphosphate
20	dNTP	Deoxynucleotide triphosphate
21	DNA	Deoxyribonucleic Acid
22	dTTP	Deoxythymidine triphosphate
23	EtBr	Ethidium Bromide
24	EDTA	Ethylenediamine Tetra Acetic Acid
25	EST-SSR	Expressed Sequence Tagged-Simple Sequence Repeats
26	Fig	Figure(s)
27	G	Gram
28	GH	Growth habit
29	GP	Growth type
30	Ha	Hectare
31	H	Hour
32	HCl	Hydrochloric acid
33	ISSR	Inter Simple Sequence Repeats
34	Kb	Kilobase
35	Kg	Kilogram

Sr. No.	Abbreviation	Meaning
36	LG	Linkage Group
37	MgCl ₂	Magnesium Chloride
38	MDA	Malondialdehyde
39	MAS	Marker Assisted Selection
40	MtSSRs	<i>Medicago truncatula</i> Simple Sequence Repeats
41	MSI	Membrane Stability Index
42	µg	Microgram
43	µl	Microlitre
44	µM	Micromolar
45	MS	MicroSatellites
46	Mg	Milligram
47	ml	Millilitre
48	Mm	Millimeter
49	mM	Millimolar
50	Mbp	Million base pairs
51	Min	Minute(s)
52	M	Molar
53	Ng	Nanogram
54	nm	Nanometer
55	NGS	Next Generation Sequencing
56	OD	Optical Density
57	ppm	Parts per million
58	%	Percent
59	PVE	Phenotypic Variance Explained
60	PGM	Pigmentation
61	PH	Plant height
62	PP	Pods per plant
63	PEG	Poly Ethylene Glycol
64	PAGE	Polyacrylamide gel electrophoresis
65	PCR	Polymerase Chain Reaction
66	PVP	Polyvinylpyrrolidone
67	PB	Primary branches
68	PCA	Principal Component Analysis
69	P	Probability
70	PRO	Proline
71	pH	Puissance de hydrogen (ion conc.)
72	QTL	Quantitative Trait Loci
73	RAPD	Random Amplified Polymorphic DNA
74	RIL(s)	Recombinant Inbred Line(s)
75	RcSSRs	Red clover Simple Sequence Repeats
76	RWC	Relative Water Content
77	RP	Reproductive Period
78	RFLP	Restriction Fragment Length Polymorphism

Sr. No.	Abbreviation	Meaning
79	Rpm	Revolutions per minute
80	RNase	Ribonuclease
81	RNA	Ribonucleic acid
82	RD	Root dry weight
83	RF	Root fresh weight
84	RL	Root length
85	Sec	Second(s)
86	SB	Secondary Branches
87	SZ	Seed size
88	SY	Seed yield per plant
89	SS	Seeds per plant
90	SP	Seeds per pod
91	SNPs	Single Nucleotide Polymorphisms
92	NaCl	Sodium Chloride
93	SDS	Sodium dodecyl Sulphate

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ABSTRACT

Macrotyloma uniflorum is an important, self pollinated diploid ($2n=2x=20$) food legume with probable genome size of 400Mbps. Limited genomic resources and lack of genetic variation are major constraints in its genetic improvement. Further, horsegram production is hampered due to twining growth habit, longer days to maturity, photosensitivity and indeterminate growth habit. The present study was aimed to construct linkage map of an intraspecific F_8 RILs population of 162 individuals derived from HPKM249×HPK4 of horsegram and identification of genomic regions linked to early maturity and yield related traits. Two thousand and eleven molecular markers were screened for parental polymorphism and 493 (25.42 %) were found to be polymorphic among the parents. Of these, 295 were mapped on ten linkage groups at LOD 3.5 spanning 1541.7 cM with an average marker density of 5.20 cM.

Analysis of variance of 162 RILs revealed significant differences for all the measured traits. Phenotypic data from the RILs were used to identify QTLs for early maturity and yield related traits by composite interval mapping (CIM). A total of 27 QTLs ($LOD \geq 2.5$) were detected across the three environments (Palampur 2016, Palampur 2017, Bajaura 2017) and combined data) for 24 traits. Among these, 15 were major QTLs with PVE greater than ten per cent and five were stable QTLs across locations and years. Phenotypic variation explained (PVE) by QTLs ranged from 6.4 to 53.4 per cent. The highest phenotypic variation (53.4 %) was explained by the QTLs for root length.

In conclusion, it is envisaged that the present linkage map, fortified with 295 SSR markers and 27 QTLs for early maturity and yield-related traits would provide genomics tools to breeders for further genetic enhancement of this crop species. Thus, the current study will serve as a strong foundation for further validation and fine mapping of QTLs for utilization in horsegram breeding programs.

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1. INTRODUCTION

The genus *Macrotyloma* belongs to the family Fabaceae and consists of about 32 wild species having chromosome number $2n = 20, 22, 24$ (Allen and Allen 1981). All the wild species of genus *Macrotyloma* are distributed in African, Australian and Indian subcontinent and *Macrotyloma uniflorum* is the only cultivated species grown in Indian subcontinent. *Macrotyloma uniflorum* has a probable genome size of 400 Mbps (Bhardwaj et al. 2013) and *M. axillare* is the probable progenitor. *Macrotyloma uniflorum* (Lam.) Verdc. commonly known as horsegram, kulthi, kulth, gahet and madrasgram is an important legume crop of India. It is potential self-pollinated warm season food legume with sparse genetic and genomic information available. It is an arid food legume grown in diverse environmental conditions of the country (Duke and Reed 1981), ranging from tropical climate of Southern India to wet temperate regions of North Western Himalayas (Himachal Pradesh, Jammu & Kashmir and Uttarakhand). The species is native to Southeast Asia and tropical Africa, but the centre of origin of cultivated species is considered to be Southern India (Vavilov 1951; Zohary 1970). Horsegram is cultivated in India, Myanmar, Nepal, Malaysia, Mauritius and Sri Lanka for food purpose whereas in Australia and Africa it is being grown as a fodder crop (Asha et al. 2006). In India it is cultivated over an area of 3.126 Lakh ha with an estimated production of 1.343 Lakh tonnes and yield of 430 Kg/ha (Directorate of Economics and Statistics (DES), 2016–2017). Horsegram possessed number of desirable traits like drought tolerance (Reddy et al. 1990), heavy metal stress tolerance (Sudhakar et al. 1992), high protein content, antioxidant activity (Reddy et al. 2005), antimicrobial activity and various medicinal properties that make it a crop of interest and potential food source of future. It is highly suitable for rainfed and marginal agriculture and thus, has a potential to cover the risk of dry land agriculture.

Despite the presence of such significant properties, the area and production under this crop could not be increased due to the presence of many undesirable traits such as twining growth habit, longer days to maturity accompanied by asynchrony, photosensitivity and indeterminate growth habit. The distribution of desirable traits in

different Indian germplasm lines further aggravated the problem to initiate a successful breeding programme (Chahota et al. 2005). Owing to biotic and abiotic stresses and the fact that horsegram is grown in low-input and risk prone marginal environments, there is very low productivity observed in this crop. Besides, limited genomic resources and low level of genetic diversity accompanied by narrow genetic base in the primary gene pool have constrained genetic improvement of horsegram.

A steady increase in global land degradation over the past 50 years as a result of agricultural activities and increasing population has put a pressure on agriculture for enhanced food production. With predicted climate change scenario and continuous population explosion, there is a great need to develop high yielding, early maturing and climate smart horsegram varieties. A major constraint to horsegram productivity is the low genetic potential of horsegram varieties that have low harvest index, poor plant type, long crop duration and susceptibility to a host of biotic and abiotic stresses, besides socio-economic factors leading to poor crop management. Exploitation of hybrid vigor, restructuring of plant type and early maturity are potential targets for increasing horsegram productivity per unit area and time (Saxena and Sharma 1990; Saxena 2008). Ideotype breeding is crucial for the suitability of a crop plant for modern farming practices, including traits for high harvest index and mechanical harvesting. It attempts to combine favorable QTLs for various component traits in a plant genotype (Wu 1998). Component traits of plant ideotype including plant height, number of branches, number of pods per plant and synchronous maturity play important role in shaping the plant architecture for high harvest index and mechanical harvesting. Early maturity is also needed for increasing cropping efficiency of the farming system. Early maturity traits play crucial roles in economic crop production. Yield is also an important and complex trait and many morphological characteristics and physiological processes contribute to seed yield. Yield-related traits may also directly influence yield by affecting the yield-component traits (Chapman et al. 2003).

Efforts for remodelling of horsegram plant type using genetic variability in the landraces and wild relatives with the help of modern biotechnological tools has not yet started. Further, improving crop production in stressed environments is feasible with new technologies and knowledge. A viable solution for yield improvement in such environments is the understanding of its biochemical, physiological and

molecular basis. Hence, biochemical, physiological and molecular based plant breeding could be crucial for further progress in improving yield potential and yield stability.

The lack of information about the genetics of various important traits and unavailability of variation for such traits in the horsegram germplasm are some of the major bottlenecks to initiate a systematic breeding programme. This is the reason that information on horsegram genomic resources is also scarce as compared to other plant species. As of now there are only 1,025 Expressed Sequence Tags (ESTs) available in the NCBI as compared to other legumes like *Glycine max* (1,461,624), *Cicer arietinum* (44,982), *Medicago truncatula* (269,501), *Lotus japonicus* (242,432) and *Pisum sativum* (18,576) (Bhardwaj et al. 2013). Similarly, no Genome Survey Sequences (GSS) is available for horsegram as compared to the above mentioned legumes.

Breeding efforts to improve early maturity and grain yield have proven to be difficult. Early maturity and grain yield are controlled by multiple genes (Gueguen and Barbot 1988) and are strongly influenced by the environment (Santalla et al. 2001). Thus, the use of molecular markers will improve our understanding of the genetic factors conditioning grain yield and maturity in horsegram. Since these factors can be localized to specific regions of the genome and their effects can be estimated individually and is expected to assist in selection of superior genotypes. Furthermore, the use of molecular markers has potential to assist in early selection of horsegram breeding lines that carry the genes for improved yield and early maturity.

Within the last two decades, many types of markers have been developed and used for crop breeding (Paux et al. 2012). Of these markers, simple sequence repeats (SSRs) are widely used due to their co-dominant inheritance, multi-allelic nature, high reproducibility and transferability, extensive genome coverage and simple detection methods (Varshney et al. 2005a; Agarwal et al. 2008). Application of SSR markers is a robust, reliable and cost-effective approach to characterize and analyse the germplasm of non-model species. These markers have been widely used for genetic mapping, marker-assisted selection, genetic diversity analysis and population genetics.

In order to develop cultivars with optimum flowering time, early maturity and improved yield, mapping quantitative trait loci (QTLs) associated with genomic regions harbouring genes for these traits represent a promising selection tool. However, the genetic control of agronomic traits in the horsegram remains poorly understood. Fine mapping of quantitative trait loci (QTLs) and qualitative trait genes plays an important role in gene cloning, molecular-marker-assisted selection (MAS) and trait improvement. Gene and QTL mapping is very important for gene cloning, MAS breeding and trait improvement; however, until now no such study on mapping the QTL and the qualitative trait genes in the horsegram has been reported.

Thus, the purpose of this study is to ascertain the genomic position, number and magnitude of QTLs affecting genetic variation for a number of physiological, biochemical and yield-related traits in RILs populations derived from a cross between two horsegram genotypes, HPKM249 and HPK4 showing contrasting expression for some maturity-related and grain yield parameters. The study thus provides valuable information on the feasibility of using QTLs in a marker-assisted selection scheme to improve maturity time and stacking favourable QTLs contributing to grain yield in horsegram. Keeping in view the above considerations, the present investigation was carried out with the following objectives:

- i. To construct linkage map of *Macrotyloma uniflorum* using morphological and DNA markers
- ii. To identify genomic regions linked to early maturity and yield related traits

2. REVIEW OF LITERATURE

The literature pertaining to different aspects of the present investigation has been reviewed under the following heads:

2.1 Taxonomy, botanical description and origin of horsegram

2.2 Genomic resources in horsegram

2.3 Various approaches to study early maturity and yield trait

- i. Phenotypic approaches
- ii. Biochemical approaches
- iii. Molecular approaches

2.4 Construction of linkage map using PCR based markers

2.5 Mapping of quantitative traits

2.1 Taxonomy, botanical description and origin of horsegram

Macrotyloma uniflorum, commonly known as horsegram (formerly known as *Dolichos biflorus*) is an unexplored (Reddy et al. 2008) and underutilized (Aiyer 1990) pulse crop. Previously Linnaeus classified horsegram to the genus *Dolichos* but Verdcourt (1980) reclassified horsegram to genus *Macrotyloma*. The style, standard and pollen characteristics distinguish *Macrotyloma* from *Dolichos* (Verdcourt 1970). The name *Macrotyloma* is derived from the Greek words *macros* meaning large, *tylos* meaning knob and *loma* meaning margin, in reference to knobby statures on the pods (Blumenthal and Staples 1993). It belongs to the Kingdom *Plantae* (Plants); Subkingdom *Tracheobionta* (Vascular plants); Superdivision *Spermatophyta* (Seed plants); Division *Magnoliophyta* (Flowering plants); Class *Magnoliopsida* (Dicotyledons); Subclass *Rosidae*; Order *Fabales*, Family *Fabaceae* (Pea family) and Genus *Macrotyloma*. *Macrotyloma* is a member of clade phaseoloids which also contain important warm-season legumes such as *Glycine*, *Phaseolus*, *Vigna* and *Cajanus* species (Doyle and Luckow 2003). The genus *Macrotyloma* consists of about 32 wild species having chromosome numbers $2n=2x=20$, $2n=2x=22$ and $2n=2x=24$ (Allen and Allen 1981) with probable genome size of 400 Mbps.

The origin of horsegram is still ambiguous. The wild members of *M. uniflorum* prevailed in both Africa and India (Verdcourt 1971), but the centre of origin of cultivated plant is regarded as India (Purseglove 1974; Smartt 1985; Vavilov 1951; Zohary 1970). Arora and Chandel (1972) specifically stated that cultivated plants of *M. uniflorum* var. *uniflorum* originated and used in south-western India. Mehra and Magoon (1974), on the other hand, suggested that *M. uniflorum* has both African and Indian gene centres. The region of maximum genetic diversity is considered to be in the Old World tropics, especially the southern part of India and the Himalayas (Zeven and de Wet 1982). It was probably domesticated in India, where its cultivation is known since prehistoric times and it is still an important cultivated crop. Now a days horsegram is cultivated as a low-grade pulse crop in many Southeast Asian countries, such as India, Bangladesh, Myanmar, Sri Lanka and Bhutan. It is also grown as a forage and green manure in many tropical countries, especially in Australia and Africa. The wild relatives of horsegram are reported mainly in Australia, Papua New Guinea, Africa and India. There is no report that horsegram is cultivated as a pulse crop in Central, Eastern and Southern Africa where most of its wild forms occur (Blumenthal and Staples 1993). Archaeological investigations revealed that horsegram was used as food around 2000 BC (Mehra 2000). India is the only country cultivating horsegram on a large acreage, where it is used as human food, the maximum area being in Andhra Pradesh, Karnataka and Tamil Nadu. It is also grown in Odisha, Madhya Pradesh, Chhattisgarh, Bihar, West Bengal, Jharkhand and in foot hills of Uttaranchal and Himachal Pradesh. Horsegram is cultivated in India over an area of 3.126 Lakh ha, with an estimated production of 1.343 Lakh tonnes and yield of 430 Kg/ha (Directorate of Economics and Statistics (DES), 2016–2017). It is grown mainly to furnish feed and fodder for cattle and horse.

Horsegram is a versatile crop and can be grown from near sea level to 1800 m above mean sea level. It is highly suitable for rainfed and marginal agriculture but does not tolerate frost and waterlogging. It is a drought-tolerant plant and can be grown with rainfall as low as 380 mm. Being a leguminous crop, it adds nitrogen to the soils where it grows, thus improving the soil fertility. It is grown under low soil fertility status with few inputs (Witcombe et al. 2008). It is adapted to wide range of temperature regimes (Smartt 1985) where other crops invariably fail to survive. In

India, it is generally sown late in the rainy season by resource-poor farmers in marginal and drought-prone condition. Along with horsegram's catholic growing conditions its main agrarian value lies in its multiple usages such as green manure, its husks have excellent water retaining capacities (Nezamuddin 1970; Zaman and Mallick 1991), its short height allows it to be used as an understory crop and can be grown under taller crops such as sorghum or pearl millet (Nezamuddin 1970). All these beneficial traits in this pulse would have secured its place in cultivation since ancient times.

It is an excellent source of protein (17.9 – 25.3%), carbohydrates (51.9 – 60.9%), essential amino acids, energy, low content of lipid (0.58 – 2.06%), iron (Bravo et al. 1999; Sodani et al. 2004), molybdenum (Bravo et al. 1999), phosphorus, iron and vitamins such as carotene, thiamine, riboflavin, niacin and vitamin C (Sodani et al. 2004). In Ayurveda it is considered as an important medicinal crop, seeds of horsegram are used for treatment of urinary stones (Yadava and Vyas 1994; Ravishankar and Vishnupriya 2012), urinary diseases and piles (Yadava and Vyas 1994), act as astringent, tonic (Brink 2006), regulate the abnormal menstrual cycle in women (Neelam 2007), and also used to treat calculus afflictions, corpulence, hiccups, and worms (Chunekar and Pandey 1998). Also, the cooked liquor of the horsegram seeds generates heat and is used to cure common cold, throat infection and fever (Perumal and Sellamuthu 2007). Different parts of the horsegram plants are used for the treatment of heart conditions, asthma, bronchitis, leucoderma, urinary discharges and for treatment of kidney stones (Ghani 2003). The extracts from *M. uniflorum* seeds had significant activity against *Bacillus subtilis*, *Staphylococcus aureus*, *Escherichia coli* and *Pseudomonas aeruginosa* (Gupta et al. 2005). It contains polyphenols and free radical scavengers that have high antioxidant properties, molybdenum that regulates calcium intake and iron that helps in transporting oxygen to cells and forms part of haemoglobin in blood (Murthy et al. 2012; Ramesh et al. 2011). Owing to their nutritional and medicinal value and its capability to thrive under drought-like conditions, the US National Academy of Sciences has identified this legume as a potential food source for the future (National Academy of Sciences 1978). Thus, there is an urgent need to explore this legume (ChelGuerrero et al. 2002; Arinathan et al. 2003) for further utilization as nutraceutical forage and food for malnourished areas of the world (Morris 2008).

2.2 Genomic resources in horsegram

The world's population is increasing explosively and estimated to reach from 7.2 billion to 9.6 billion by 2050 (Gerland et al. 2014). To feed this increasing population, there is a need to produce about 70% more food. Since legumes are important dietary source for protein and other nutrients, there is a constant effort to increase the quality and quantity of legumes. Conventional approaches are being used from long time to increase legume production but global production during the last 50 yrs has only increased marginally. Thus the use of genomic assisted breeding (GAB), which combines conventional breeding with genomic tools has been now widely employed to develop improved varieties (Varshney et al. 2009). For implementing genomic assisted breeding in legumes, the availability and easy accessibility of genomic resources is a pre-requisite which provide the starting point for understanding the unique traits present in the given crop. Additionally, availability of genomic resources provides better opportunities for characterization, utilization and bio-prospecting of targeted plant species in future.

Molecular markers are widely used for evaluation of genetic diversity, construction of linkage maps, cultivar identification, quantitative trait loci (QTLs) analysis and many other purposes in molecular breeding and conservation studies (Henry 1997; Jahufer et al. 2003; Weising et al. 2005). DNA markers are particularly useful if they can uniquely distinguish the closely related individuals of the same or different species. Such markers are called polymorphic markers, whereas markers that failed to discriminate between genotypes are categorized as monomorphic markers. DNA markers, which reveal variable sites in DNA, are the most widely used marker types predominantly due to their abundance, precision and reproducibility irrespective of changing environment and the developmental stage of the plant (Jones et al. 1997). These variations arise from different types of mutations at the DNA level, which include point mutations, insertions or deletions and errors in replication of tandemly repeated DNA regions (Paterson 1996). Considering multiple advantages, molecular markers are preferred against morphological and biochemical markers, which are often influenced by environment and stage specific expression (Winter and Kahl 1995). The advantage of this technique is that genetic variations can be recorded without a prior knowledge of the primer sequences in the target species.

Among the methods targeting known sites in the genome, an important one to emerge in the last decade of 20th century was the detection of simple sequence repeats (SSRs) or microsatellites in plants (Tautz and Renz 1984). These are tandemly repeated sequences of two to six base pairs of DNA. Primers designed flanking to these repeated regions represent one of the best co-dominant marker systems and are exploited in genome diversity, genome mapping and conservation studies in crops. Microsatellites mutate much more rapidly than most other types of sequences and the high mutation rates of microsatellites allow a more detailed analysis of the mutation patterns (Winter and Kahl 1995; Jones et al. 1997; Joshi et al. 2000; Kump et al. 2011; Kilian and Graner 2012). During the last two decades, these have arguably become the most important and versatile source of polymorphic genetic markers for the construction of linkage maps, parentage testing, population and conservation genetics, management of biological resources and other related fields (Sunnucks 2000; Weising et al. 2005).

Traditionally, development of microsatellite markers was a cumbersome job due to some laborious and costly protocols for marker development (Wright and Bentzen 1995; Gardner et al. 1999). The number of markers produced was also low, but with the advent of high-throughput Next Generation Sequencing (NGS) platforms, the development of these markers has become easier and cost effective. One of the ways of generating marker data through sequencing is via transcriptome sequencing approach. Transcriptome or Expressed Sequence Tag (EST) sequencing is a resourceful means to generate functional genomics data for non-model organisms (Bouck and Vision 2007). Huge collections of EST sequences are priceless for gene annotation and discovery (Emrich et al. 2007), comparative genomics, development of molecular markers (Novaes et al. 2008) and population genomics studies of genetic variation associated with adaptive traits (Namroud et al. 2008). Recent years have witnessed a large number of studies including marker development through transcriptome analysis (Guichoux et al. 2011) and an increasing number of EST datasets have become available for model and non-model organisms which have been exploited for marker development (Emrich et al. 2007; Namroud et al. 2008; Novaes et al. 2008; Parchman et al. 2010; Zhang et al. 2010; Dutta et al. 2011; Garg et al. 2011; Guichoux et al. 2011).

There are only 1025 EST sequences of *M. uniflorum* available in National Centre for Biotechnology Information (NCBI) indicating lack of genomic information in this crop. As a first step towards characterization of genes that contribute to combating abiotic stresses, 1050 ESTs were isolated and sequenced (Reddy et al. 2008). Bhardwaj et al. (2013) conducted transcriptome analysis for eight shoot and root tissues of a drought sensitive and tolerant genotype of horsegram under controlled and drought stress conditions using Illumina GAIIX. A total of 229,297,896 paired end reads were generated and utilized for *de novo* assembly of horsegram. Significant BLAST hits were obtained for 26,045 transcripts while 3,558 transcripts had no hits but contained important conserved domains. A total of 21,887 unigenes were identified. SSRs containing sequences covered 16.25 per cent of the transcriptome with predominant tri- and mono-nucleotides (43%). The total GC content of the transcriptome was found to be 43.44 per cent. The genes and pathways identified suggested efficient regulation leading to active adaptation as a basal defense response against drought stress by horsegram.

Sharma et al. (2015a) studied genetic diversity present in available horsegram germplasm using 45 randomly amplified polymorphic DNA (RAPD) and 30 inter simple sequence repeat (ISSR) markers. They also assessed genetic inter-relationship among two wild species of genus *Macrotyloma* namely *M. axillare* and *M. sar-gharwalensis*. A total of 25 polymorphic primers amplified 156 fragments ranging in size from 300 to 3000bp. STRUCTURE analysis clustered accessions on the basis of their geographic origin and showed the presence of two distinct gene pools which were later confirmed by PCA and dendrogram based on Jaccards similarity coefficient.

Sharma et al. (2015b) developed and characterized simple sequence repeat (SSR) and intron length polymorphism (ILP) from public sequence data in horsegram. They retrieved and checked these 1025 EST sequences, out of which 33 contaminant sequenced were rejected and remaining 992 sequences were assembled into unigenes/contigs and of these 617 unigenes were searched for the presence of SSRs. Of these 617 unigenes/contigs, only 84 contained SSR sequences with di, tri, tetra, penta and hexa repeat motifs. Of these 84 SSR containing sequences, they designed 63 EST-SSR (HorsegramUniGeneMicroSatellite, HUGMS) and 13 ILP (Horsegram

Intron Length Polymorphism, HILP) primer pairs. They also mined SSR sequences from transcriptomic data given by Bhardwaj et al. (2013). They found 3337 sequences from this transcriptomic data, identified 2847 SSR primers containing di, tri, tetra, penta and hexa repeat motifs and 169 primers were synthesized. In total, out of 245 (169+63+13) primers pair synthesized and validated in twenty lines of horsegram, 115 primers amplified the specific product and were polymorphic. These newly developed markers were also assessed for their transferability across different legume species, viz., *Macrotyloma axillare*, *M. sar-gharwalensis*, *Trifolium pratense*, *Phaseolus vulgaris*, *Vigna umbellata*, *Vigna radiata*, *Cicer arietinum*, *Pisum sativum*, *Lens culinaris*, *Vigna mungo*, *Glycine max* and *Vigna unguiculata*. The cross-transferability that ranged from 25.5% (*G. max*) to 68.0% (*V. umbellata*) revealed the extent of syntenic relationships across different legumes. Also, dendrogram and principal component analysis using these SSR and ILP distinguish 20 horsegram accessions into two groups, one from north western Himalayan region and other from different geographical locations. A sufficient number of genic SSRs from transcriptome sequence data from two horsegram lines M-191 and M-249 and SSRs were designated as *M. uniflorum* micro-satellite (MUMS) and these SSR-containing sequences covered 16.25% of the total transcriptome.

Chahota et al. (2017) identified and developed large number of new SSRs in horsegram using next generation sequencing technology (NGS) and used these SSRs for the evaluation of genetic diversity and population structure of horsegram germplasm from different locations of the country. They used two horsegram lines, HPK4 and HPKM193 for generation of genomic libraries and sequencing using Illumina HiSeq 2000 platform. Out of 23,505 potential SSRs motifs that were identified on HPK4 scaffold, 5755 primer pairs were designed containing di, tri, tetra, penta and hexa repeat motifs. 30 polymorphic SSR primers and 24 morphological traits in 360 horsegram accessions were then utilized to detect genetic diversity and population structure. Dendrogram based on Jaccard's similarity coefficient grouped these horsegram accessions into seven clusters which formed two major clusters, namely Himalayan origin and Southern India. The intergenetic distance among the accessions from Southern India is less in comparison to accessions from Himalayan region.

Kaldate et al. (2017) also used next generation sequencing (NGS) technology for genome wide development and characterization of novel SSR markers in horsegram and used these for genetic diversity and cross transferability analysis. They generated sequence data using Illumina sequencing comprised of 186,445 scaffolds and found 86,498 sequences containing SSRs having di, tri, tetra, penta and hexa repeat motifs. Of these, 2458 primer pairs were designed and randomly selected 117 primers were synthesized and validated on 48 diverse horsegram lines. The neighbour joining tree of 48 accessions showed two major clusters, one from Himachal Pradesh and other from southern states of India. Further analysis of SSR primers on nine related legume species showed variable extent of cross transferability.

2.3 Various approaches to study early maturity and yield trait

i. Phenotypic approaches

Phenotyping of important trait related to early maturity and yield can lead to a better understanding of a particular plant mechanism. Physiology-based phenotyping for traits of specific interests is significant in crop improvement programs of the 21st century. To enhance crop yields, screening for the stability of traits across wider environments, crop architecture, physiology, phenology and source to sink relationship in partitioning the resources available is of paramount importance (Malcolm et al. 2013). An efficiently planned and careful phenotyping, backed up by relevant experimental designs, will narrow the gap between genotype and phenotype (Tuberosa 2012). The yielding ability of crops cannot be directly determined by an individual physiological or morphological mechanism (Turner 2001). Only with a thorough physiological understanding of these yield attributes and their negative relationship will guide us towards manipulating them either through conventional or gene editing assisted breeding strategies (Slafer 2003).

Horsegram is an arid food crop and is grown in diverse environmental conditions. Early phenology (time to flowering, podding and maturity) has been found as an important trait to study early maturity and adaption of plants to different environments (Kumar and Abbo 2001; Berger et al. 2007; Gaur et al. 2008). Early maturity helps the crop escape end-of-season stresses, such as drought (Subbarao et al. 1995) and frost (Anbessa et al. 2006) and thus an important factor in increasing the

yield of the crop. The correlation between several morphological, physiological and phenological parameters results in the duration of crop maturity. Consequently, breeding for early maturity has been one of the major breeding objectives in recent years to increase yield and to overcome stresses. Proper phenotyping is thus an important step to screen new crop ideotypes generated from diversified genetic resources and significantly improve crop genetic gains (Reynolds and Langridge 2016).

a. Phenological traits

With predicted climate change scenario and continuous population explosion, there is a great need to develop high yielding, early maturing and climate smart horsegram varieties. Horsegram has a large variation in the flowering and maturity time therefore genetic information of these traits has direct implications for the development of short duration high yielding horsegram varieties. Breeders generally used days to flowering as a key indicator of maturity duration since this trait provides a good indication of subsequent phenological traits, such as time of podding and maturity (Gaur et al. 2015). Kong et al. (2018) suggested that maturity consists of flowering time and reproductive period, and a balance between appropriate flowering time and reproductive period is critical to maximize the maturity and yield productivity. In addition, different environmental conditions also influence maturity. Genetic and environmental interactions should thus be taken into the consideration to elucidate the underlying mechanism of flowering time and maturity.

b. Morphological traits

Morphological traits like plant height, growth type, number of primary and secondary branches are important plant architectural trait for crop yield (Jyotirmaya et al. 2016). Plant breeders have extensively modulated plant architectural traits; in particular plant height, branching and canopy features for optimizing crop performance and yield (Horton 2000; Peng et al. 1999). Jain (1975) suggested that improving yield in chickpea would likely to be associated with determinate and compact growth habit. Bahl and Jain (1977) included erect growth habit, many primary and secondary branches and few tertiary and lateral branches in chickpea ideotype as this plant type would intercept more sunlight and permit large population

per unit area. Sedgley et al. (1990) also emphasized that an ideotype for high input environments should have erect growth habit and limited branching.

c. Leaf–water relations

Relative Water Content (RWC) is the measure of the health and sturdiness of a plant and is lowered in the state of stress. Plants showed better maintenance of higher RWC ensuring better hydration and more favorable internal water retention of tissue with a possibly higher pressure potential and showed better drought tolerance capacity (Chavan et al. 2010). Nezami et al. (2008) stated that drought tolerance is important trait for plant grown in arid conditions as it showed plant ability to preserve vegetative growth and crop yield under drought conditions. Since, horsegram is mostly grown under arid environment its yield is directly related to drought tolerance capability of the plant.

d. Membrane Stability index

Stress injury leads to oxidative damage from active oxygen species and alterations in structure and function of cell membranes. Membrane stability of plant tissues, mostly leaves, is often determined by electrolyte leakage measured as electrical conductivity. Cell membrane stability has been widely used to express stress tolerance in plants and higher membrane stability is correlated with stress tolerance by Premachandra et al. (1992). It is well known that a functional cell-membrane system is central to crop yield productivity and adaptation of plants (Raison et al. 1980). Membrane thermal stability was positively associated with yield performance in wheat (*Triticum esculentum* L.) under stressed conditions (Reynolds et al. 1994). It is also a suitable screening technique for drought-tolerance rating in legume (Grzesiak et al. 1996; Gupta et al. 2000; Deshmukh and Kushwaha 2002).

e. Root characteristics

Deep and extensive root system helps plant to uptake soil water more efficiently. Water uptake is considered to be crucial factor during key stages like flowering and grain filling (Westgate and Boyer 1984) and small differences in water uptake at these stages can bring large yield benefits (Boote et al. 1982). In several crops, adaptation to drought is closely associated to root development, which provides

a better water extraction ability to plants (Jongrunklang et al. 2011). Many workers suggested that in chickpea under terminal drought condition plants with deep root and high root density adapted better (Kashiwagi et al. 2006). Similarly, Dhanda et al. (2004) and Nazari (2005) suggested that root length, root dry weight and seedling dry weight are the major traits to select for studying tolerance of genotypes under water stress conditions.

ii. Biochemical approaches

Biochemical studies are important to know tolerance and sensitivity of crops towards different stresses. Abiotic stress imposed by drought, salinity and extreme temperatures acts as major impediment and pose serious threat to the growth and productivity of crop plants. On a global basis, drought, in conjugation with coincident temperature and radiation, pose the most important environmental constraints to plant survival and to crop productivity (Boyer 1982). Rao et al. (2013) reported drought tolerance of early-maturing genotypes, given their lower net water requirement throughout their plant life cycle compared with late-maturing genotypes. Horsegram (*Macrotyloma uniflorum*) is cultivated as a pulse crop in semi-arid regions of peninsular India. This crop comes up reasonably well in drought prone areas in very poor soils where other crops invariably fail. Horsegram is considered as one of the important dry land crops especially in drought prone areas. There are few reports in literature, concerning physiological and biochemical responses of horsegram to abiotic stress and very little is known about the genetic mechanism of stress tolerance in horsegram. Biochemical analysis has long been proposed to be useful strategy for selection of stress tolerant genotypes in plant breeding (Abebe et al. 2003; Bowne et al. 2012; Mwadzingeni et al. 2016). Different parameters like chlorophyll content, carotenoids content, proline, MDA content and some antioxidant enzymes activities have been considered as markers of stress. These have been associated with the different tolerance levels of plants towards stress (Unyayar and Cekic 2005; Hura et al. 2007; Gajewska and Sklodowska 2008; Azooz et al. 2009; Bhardwaj and Yadav 2012).

a. Chlorophyll content

Chlorophyll is an extremely important biomolecule, critical in photosynthesis, which allows plants to absorb energy from light. Drought stress decreases the rate of photosynthesis (Kawamitsu et al. 2000). Severe drought stress also inhibits the photosynthesis of plants by causing changes in chlorophyll content, by affecting chlorophyll components and by damaging the photosynthetic apparatus resulting in less assimilate production for growth and yield of plants (Iturbe Ormaetxe et al. 1998). Ommen et al. (1999) reported that leaf chlorophyll content decreases as a result of drought stress. Kumar et al. (2011) found that in pigeonpea PEG-induced drought stress significantly decreased chlorophyll a, chlorophyll b and total chlorophyll content both at the stress level.

b. Carotenoids content

Carotenoids (carotens and xanthophylls) which are lipid soluble antioxidants are yellow, orange, and red pigments present in many plants. Several of them are precursors of vitamin A (i.e. β -carotene, γ -carotene, and β -cryptoxanthin) and they are both radical scavengers and quenchers of singlet oxygen due to conjugated double bonds (Podsdek 2005). Carotenoids have critical roles as photoprotective compounds by quenching triplet chlorophyll and singlet oxygen derived from excess light energy. With this, they limit membrane damage (Howitt and Pogson 2006).

c. Osmolytes

Under environmental stress conditions, plants accumulate some kind of compatible solutes such as proline, glutamate, betaine and polyols in the cytosol to increase osmotic pressure and thereby maintain turgor and the driving gradient for water uptake (Rhodes and Samaras, 1994) and to protect membranes and proteins. Proline is one of the most common compatible osmolytes in stressed plants. It is responsible for osmotic adjustment, protection of plasma membrane integrity and free radical scavenger. Proline does not interfere with normal biochemical reactions but allows the plants to survive under stress (Stewart 1981). Bhardwaj and Yadav (2012) found that increase in proline content was higher in drought tolerant horsegram variety as compared to drought sensitive horsegram variety.

d. Malondialdehyde content

Accumulation of malondialdehyde (MDA), a product of fatty acid peroxidation has been used as an indicator for abiotic stress including drought, salt and cold stress conditions suggesting serious membrane damage and disturbed plants status. Measuring the end products of lipid peroxidation such as MDA, a good marker for stress injury, is one of the most widely accepted assays for oxidative damage (McKersie 1996). MDA has been widely used to assess abiotic stress injury as criterion in various plants (Katsuhara et al. 2005; Jaleel et al. 2007) including lentil (Oktem et al. 2008). Bhardwaj and Yadav (2012) reported that the increase in MDA content was more in drought sensitive horsegram variety as compared to tolerant variety.

iii. Molecular approaches

The main objective of any crop breeding program is the development of elite breeding lines with important agronomic traits and increase in yield. Identification of quantitative trait loci (QTLs) and candidate genes involved in early maturity and yield related traits may be used to produce transgenic lines or can be applied to breeding programs e.g. marker assisted selection (MAS). Once a series of candidate genes to improve a particular trait has been identified in legumes, a number of options are possible for exploiting this information in legume crops breeding. The involved steps are: (1) confirmation of candidate gene functions either directly or indirectly at the biochemical and physiological level (2) identification of favourable alleles for selection (3) variety improvement by MAS.

In the last few decades, innovations in genomics- based techniques and platforms have provided a wealth of genetic and genomics resources (Varshney et al. 2005b) that revolutionized research in both model and non model legumes crop. The increased application of molecular markers and reference genome sequences has had a substantial impact in accelerating progress in plant breeding. Legume research has benefited widely from molecular markers of different types. For example, hybridization based markers, such as restriction fragment length polymorphism, were applied to develop linkage maps in many legumes e.g soybean (Keim et al. 1990) and common bean (Nodari et al. 1993). These methods were subsequently replaced with

polymerase chain reaction based markers, including both non- specific markers [random amplified polymorphic DNA (RAPD) and amplified fragment length polymorphism (AFLP) markers] and locus specific markers [simple sequence repeats (SSR) and single nucleotide polymorphism (SNP) markers]. DNA sequencing technology has made major advances over the last decade, making many of the previous marker based systems redundant and genome sequences are now available for many legume species including cultivated soybean (Schmutz et al. 2009), common bean (Schmutz et al. 2014; Vlasova et al. 2016), pigeonpea (Varshney et al. 2012), etc. The availability of these resources provides an unprecedented opportunity for trait improvement through marker assisted evaluation of plant material, identification of QTLs and gene discovery, marker assisted selection, and genomic selection.

Currently, there are two general methods to identify genes and mechanisms related to important agronomic traits in plant species, known as “top- down” and “bottom up.” The top- down approach begins with a phenotype of interest followed by forward genetic analysis to identify candidate genes. Two popular genetic analyses used in the top- down method are QTL and association or linkage disequilibrium (LD) mapping. QTL mapping is the more traditional approach and has been successful in identifying genomic regions associated with adaptive traits. Contrastingly, bottom up approaches use population genetic analyses to identify signatures of adaptation in a set of potentially adaptive genes and then apply bioinformatics and reverse genetic tools to associate selected genes to a phenotype (Ross- Ibarra et al. 2007). Molecular population genetics, which forms the basis of bottom- up approaches, appears to be promising for advancing our knowledge of the molecular signature of adaptation (Wright and Gaut 2005). It has great potential for identifying candidate genes harbouring adaptive mutations. However, careful consideration must be taken to exclude demographic effects such as population size and structure which could bias the results by increasing the statistical variance applied to detect the selection signature.

2.4 Construction of linkage map using PCR based markers

Genetic linkage maps have become an important tool in basic genetic analysis as well as in applied plant breeding. It refers to the determination of the relative

positions of genes on a DNA molecule (chromosome or plasmid) and their distance among them. Obtaining a large number of genetic markers and conducting cost effective genotyping in populations are essential prerequisites for construction of a high-density linkage map.

Linkage maps have assisted in the identification of DNA markers linked to single genes of major agronomic importance and have permitted the identification of tightly linked DNA tags for use as diagnostic tools in plant breeding. As a tool for genetic research and breeding, genetic linkage maps have been widely used to discover the position and to clone genes controlling biotic and abiotic stress resistance, agronomic and seed quality traits and to facilitate marker-assisted selection of the traits with low heritability and/or high phenotyping cost. Linkage mapping enables identification of associations between traits and markers for both simple Mendelian traits and quantitatively inherited traits (QTLs) (IbarraPerez et al. 1997; Gepts et al. 2008; De Ron et al. 2015). It also allows for characterization of recombination hotspots along individual chromosomes (Kujur et al. 2015). High-density genetic map provides a powerful tool for analysing the heredity of target gene, monitoring specific genes or genomic regions transmitted from parent to next generation, as well as map-based cloning.

Linkage maps based on molecular markers also have the potential to bridge the gap between understanding of phenotype based on genetics and of organismal biochemistry and physiology (Gilpin et al. 1997). Once major QTLs have been unraveled, tightly linked markers may be validated for use in marker-assisted selection (MAS) and potentially even as a starting point for the positional cloning of the underlying functional resistance gene(s).

Since horse gram is considered as a pulse of poor tribal people, it has not attracted much research efforts like other major pulses and very limited work has been carried out for its improvement. Further, little genetic information of major agronomic traits has restricted its genetic improvement and posed a hurdle in systematic breeding of this legume. Also no efforts have been done for construction of its genetic linkage map using molecular markers. Therefore, horsegram lack genetic linkage map till now. Whereas other legumes of same clade i.e. phaseoloid/millettioid like *Glycine*

max (soybean), *Phaseolus* (garden bean and runner bean), *Vigna* (cowpea and mungbean), *Cajanus cajan* (pigeon pea), etc (Bruneau and Doyle 1990) has fine linkage map of molecular markers. Therefore linkage map construction of these legumes will be discussed in this review.

In soybean, Keim et al. (1990) reported the first molecular genetic linkage. The map contained 150 restriction fragment length polymorphism (RFLP) markers that were mapped using an interspecific F₂ population with 60 progeny derived from a cross of A81-356022 (*G. max*) × PI468916 (*G. soja*). The early genetic linkage maps were primarily based on RFLP or AFLP markers and due to the lack of polymorphism or the complexity of the multiple banding patterns with these markers, simple sequence repeat (SSR) or microsatellite markers were proposed and then evaluated for the construction of genetic linkage maps (Akkaya et al. 1992; Akkaya et al. 1995). Cregan et al. (1999) developed three separate linkage maps containing a total of 1421 markers including 606 SSRs, 689 RFLPs, 79 RAPDs and 47 other markers. These markers were mapped using three RIL populations: the Minsoy × Noir 1 population with 240 RILs, the A81-356022 × PI468916 population with 57 F₂ plants, and the Clark × Harosoy population with 59 F₂ plants and resulted in 20 linkage groups which were assumed to correspond to the 20 pairs of soybean chromosomes. As large numbers of expressed sequence tags (ESTs) and genomic sequence became available in later years, Choi et al. (2007) discovered >5500 single nucleotide polymorphism (SNP) markers by comparing DNA sequences acquired from a set of diverse genotypes after PCR amplification and sequence analysis of the EST or genomic sequences. A total of 1141 of the 5500 SNPs were mapped using three mapping populations including the Minsoy × Noir 1 with 164 RILs, Minsoy × Archer with 89 RILs as well as the Evans × PI 209332 with 75 RILs.

In common bean, the first widely used genetic map was developed from a backcross (BC) mapping population between Mesoamerican line ‘XR-235-1-1’ and ‘Calima’ (Andean cultivar) (Vallejos et al. 1992). This linkage map included 9 seed proteins, 9 isozymes, 224 RFLP and seed and flower color markers. These molecular markers were placed on 11 linkage groups, spanning 960 centimorgans (cM). SSR markers were first reported in bean by Yu et al. (1999; 2000) with 15 different microsatellite markers included in a molecular linkage map constructed primarily

using RAPD and RFLP markers. An important recent SNP map is the high resolution Mesoamerican $\times$ Andean cross of Stampede $\times$ Red Hawk produced by Song et al. (2015) which utilized 7276 SNP markers in an F₂ mapping population of 267 RILs. Numerous subsequent maps have been generated using a succession of marker types (Gonzalez et al. 2016).

In cowpea, the first attempt to build a genetic map was based mainly on the segregation of RFLP markers in the progeny of a cross between an improved cultivar and a putative wild progenitor type (*Vigna unguiculata* subsp. *dekindtiana*) (Fatokun et al. 1992). The map consisted of 92 markers placed in eight linkage groups that spanned a total genetic distance of 684 cM. Andargie et al. (2011) constructed a genetic linkage map using SSR markers and RILs of 159 individuals derived from a cross between the breeding line 524B and 219-01. 202 polymorphic SSRs were used to construct a genetic map consisting of 11 linkage groups spanning 677 cM. Lucas et al. (2011) also reported that 941 of 1107 total SNP markers i.e., 85 per cent that mapped in cowpea show homologs with soybean (*Glycine max*). The markers also showed synteny and co-linearity in the soybean genome.

Genomics research in pigeon pea has gained momentum recently (Varshney et al. 2010), the limited availability of genomics tools in the past has impeded progress in this important crop. Recent efforts towards building a genetic map of pigeonpea have led to the development of several interspecific and intraspecific maps. The first interspecific map of pigeonpea was developed on F₂ mapping population of *C. cajan* acc. ICP-28 and *C. scarabaeoides* acc. ICPW-94 using 554 diversity arrays technology (DArT) markers covering a total map distance of 451.6 cM (Yang et al. 2011) and the same mapping population was used to develop another map wherein SSR markers were used (Bohra et al. 2011). Sheetal et al. (2017) reported a large SNP-based, high-density, intraspecific consensus linkage map of the pigeonpea genome, which included 932 loci that cover a high genome length of 1,411 cM with an average marker interval of 1.51 cM. These maps have helped QTL mapping of agronomically useful traits and anchoring of the pigeonpea draft genome.

A high-density linkage map is crucial for the identification of quantitative trait loci (QTLs), positional cloning, and physical map assembly. Due to dearth of linkage

map, horsegram lag behind development of elite lines using MAS and in identification of genomic regions linked to various important agronomic traits. Therefore the present study was aimed to construct first genetic linkage map of horsegram using molecular markers which will provide a foundation to future genomic research, enable the discovery of useful genes and accelerate the breeding of horsegram.

2.5 Mapping of quantitative trait

The identification and localization of genes in the genome which control variation for quantitative traits can greatly facilitate their selection in breeding programmes. Thoday (1961) demonstrated that simply inherited gene markers can be used as tags to locate quantitative trait loci (QTLs). The technique for identification of QTL by gene markers became more efficient with the availability of molecular markers. The identification of QTLs allows the analysis and selection of complex quantitative traits as a set of single-gene traits (Tanksley et al. 1993). Quantitative traits have been studied in legumes since Mendel.

Improvement of crop yield and quality has become the major interest of plant breeders. Development of early maturing lines with optimum days to flowering combined with high and stable yield is an important breeding goal. Earliness is an adaptive trait and is one of the major factors of agronomic variation (Worland 1996). The term “*earliness genes*” was first used by Ford et al. (1981), and it was proposed to be different from genes controlling photoperiod response in wheat (*Triticum aestivum* L.). Early maturity and yield-related traits are usually complex quantitative traits influenced by multiple QTLs. With the advent of molecular markers like RFLP, AFLP, RAPD and SSR, together with the convenience of the advanced analytical techniques, the molecular study of quantitative traits becomes facility in many plant species (Wang et al. 1999). The application of molecular markers to plant breeding using modern statistical methods (Malosetti et al. 2013) has allowed breeders to accurately estimate the positions and effects of genomic regions associated with variation in quantitative traits (Perseguini et al. 2016).

Flowering time is known to be an important reproductive characteristic of agronomic interest and plays a principal role in the geographical adaptation. Time of

flower opening and mainly days to flower or the duration from sowing or planting to flowering in annual crops is an important component of adaptation of a variety to a particular agro-ecological zone as days to flowering determines when crops will ripen to harvest (Roberts et al. 1993). QTL studies using linkage mapping are abundant in nearly all crop species. But horsegram lack any studies related to identification of QTLs linked to important agronomic traits. Therefore like in linkage map construction, the review for QTL mapping will be discussed for other legumes belonging to same clade i.e. phaseoloid/millettioid like *Glycine max* (soybean), *Phaseolus* (garden bean and runner bean), *Vigna* (cowpea and mungbean) and *Cajanus cajan* (pigeon pea).

Soybean (*Glycine max*) is a major legume crop that is mainly distributed in temperate regions, and days to flowering and maturity are key factors for developing soybean cultivars with a wider geographical adaptation (Lu et al. 2017). Flowering time and reproductive period (RP) greatly impact soybean maturity however reproductive period is also an important soybean trait that is closely related to yield, seed quality, and tolerance to various environmental stresses (Xu et al. 2013). Both time of flowering and maturity in soybean are quantitative traits that are controlled by multiple genes. 12 major genes/loci related to time of flowering and maturity [E1, E2 and E3 (Buzzell 1971), E4 (Buzzell and Voldeng 1980), E5 (McBlain and Bernard 1987), E6 (Bonato and Vello 1999), E7 (Cober and Voldeng 2001), E8 (Cober et al. 2010), E9 (Kong et al. 2014; Zhao et al. 2016), E10 (Samanfar et al. 2017), J (Ray et al. 1995), and Dt1 (Liu et al. 2010; Tian et al. 2010)] have been reported in soybean. Hundreds of QTLs for yield related traits were detected across the whole genome of soybean and many were simultaneously detected in multiple populations (Orf et al. 1999; Funatsuki et al. 2005; Palomeque et al. 2009; Kim et al. 2010; Liu et al. 2011; Han et al. 2012). Furthermore, multiple research groups have searched for QTLs related to flowering time and maturity dates that could influence soybean yield (Tasma et al. 2003; Watanabe et al. 2004; Zhang et al. 2015).

In cowpea, many researchers have utilized different genetic maps based on molecular markers to locate many QTLs associated with yield. The genetic map developed by Ubi et al. (2000) positioned QTLs for several agronomic and morphological traits including days to flowering, days to maturity, pod length,

seeds/pod, leaf length, leaf width, primary leaf length, primary leaf width and derived traits such as leaf area and primary leaf area. Muchero et al. (2009) reported the mapping of 12 QTL associated with seedling drought tolerance and maturity in a cowpea recombinant inbred (RILs) population. Muchero et al. (2011) also identified the QTLs for maturity in cowpea with SNP markers. For heat stress, Pottorff et al. (2014) identified three QTLs, Hbs-1, Hbs-2, and Hbs-3 associated with heat-induced browning of seed coats using the cowpea RIL populations derived from IT93K-503-1 $\times$ CB46 and IT84S-2246 $\times$ TVu 14676. The identification of SNP markers co-segregating with the heat induced browning of seed coats phenotype in the Hbs-1 and Hbs3 loci will help indirect selection in breeding cowpea with better quality grain.

There are many reports on the identification of QTLs controlling agronomic traits in common bean. Jung et al. (1996) were the first to report markers associated with architecture. They identified two and three QTLs, respectively, for two general measures of architecture, plant uprightness and branch density. Recently, Taran et al. (2002) located a number of QTLs responsible for plant architecture. One QTL was detected for both hypocotyl diameter and pod distribution whereas two QTLs were identified for both branch angle and plant height. Multiple researchers worked to find different QTLs linked to early maturity and yield traits in common bean e.g. days to flowering, days to maturity or harvest (Blair et al. 2006; Perez-Vega et al. 2010; Gonzalez et al. 2016; Bhakta et al. 2017), plant architecture (Blair et al. 2006), seed (Park et al. 2000; Melo et al. 2002; Cichy et al. 2009; Yuste et al. 2014) and yield-related traits (Blair et al. 2006; 2012; Leite et al. 2011; Galeano et al. 2012).

In case of pigeonpea, QTL mapping is in its infancy with few successful efforts in recent years. Bohra et al. (2012) reported four different QTLs for fertility restoration (QTL-RF-1, QTL-RF-2, QTL-RF-3 and QTL-RF-4) in pigeonpea using three different F_2 mapping populations (ICPA-2039 $\times$ ICPR-2447, ICPA2043 $\times$ ICPR-2671 and ICPA-2043 $\times$ ICPR-3467) based intraspecific genetic maps. Kumawat et al. (2012) constructed an intraspecific genetic map involving a F_2 population to identify 13 QTLs for the six agronomic traits. Two major additive effect QTLs were identified for plant height, two major QTLs were identified for the number of primary branches per plant, another major additive effect QTL for number of secondary branches per plant. Three QTLs were detected for the number of pods

per plant, one major and one minor QTL were detected for days to flowering, two major additive effect QTLs and one minor QTL were also identified for days to maturity. In addition to the main effects, significant epistatic interaction effects were detected between the QTLs for number of pods per plant.

Fine mapping of quantitative trait loci (QTL) and qualitative trait genes plays an important role in gene cloning, molecular-marker-assisted selection (MAS) and trait improvement. However, there is no information on genetic control of important agronomic traits in horsegram. Therefore the present study was aimed to identify QTLs linked to early maturity and yield related traits which will elucidate genetic control of these traits, expedite MAS breeding and the improvement of horsegram.

3. MATERIALS AND METHODS

The present investigation was carried out in the Department of Agricultural Biotechnology, College of Agriculture, CSK Himachal Pradesh Krishi Vishvavidyalaya, Palampur, Himachal Pradesh. The material used and the methodology adopted to achieve the objectives of the investigation is given here:

3.1 Plant material

An F_8 recombinant inbred lines (RILs) population of 162 individuals derived from an intraspecific cross of HPKM249 and HPKV4 was used for the construction of genetic linkage map. For RILs development F_2 seeds from a single F_1 plant were harvested and advanced to F_8 recombinant inbred lines (RILs) by single seed descent method with no bias. The parents differed from each other with respect to various agro-morphological traits under study as shown in Table 3.1. Standard agronomic practices were followed to raise the crop.

Table 3.1 Morphological variations in parents

S.No.	Trait	HPK4	HPKM249
1	Growth habit	Twining	Bush type
2	Flowering time (days)	60-65	30
3	Growth	Indeterminate	Determinate
4	Maturity (days)	120-124	80-82
5	Photosensitivity	Photosensitive	Photoinsensitive
6	Plant height (cm)	100.0	35.0-40.0
7	Maturity	Asynchronous	Synchronous
8	Seed characteristics	Bold seed size	Medium seed size
10	Drought stress	Tolerant	Susceptible
12	Stem pigmentation	Dark brown	Absent
13	Number of pods/plant	>30	<10
14	Relative Water Content	High	Low
15	Total carotenoids	High	Low
16	Total chlorophyll contents	High	Low



Fig. 3.1 Morphology of the two contrasting parents

All RILs along with parents were evaluated for different agro-morphological traits at two locations, Palampur and Bajaura (KVK).

3.2 Methodology

i. Extraction of plant genomic DNA

Genomic DNA was isolated from young leaf tissues (0.5-1 g) of the parents and F₈ RILs individuals using modified CTAB method (Murray and Thompson 1980). The leaf tissues were rinsed in deionized water, dried on tissue paper discs and ground to fine powder in liquid nitrogen in autoclaved pre-cooled pestles and mortars. The ground tissue was transferred to a separate 2 ml eppendorf tubes containing 800 µl of extraction buffer (2% CTAB, 100 mM Tris, 20 mM EDTA, 1.4 mM NaCl and 1% PVP, pH 8.0) maintained at 60°C in water bath and mixed vigorously. The mixture was incubated at 60°C for 1 h with occasional mixing. An equal volume of chloroform-isoamyl alcohol (24:1) was added to the tubes followed by gentle mixing. The mixture was centrifuged at 10,000 rpm for 10 minutes at 4°C. The aqueous phase was transferred to fresh tube, followed by addition of 500 µl of pre-chilled isopropanol. The contents of the tubes were mixed gently and the mixture was incubated at -20°C for 1 h. DNA was precipitated by centrifugation at 10,000 rpm for 10 minutes.

The supernatant was drained and the resulting pellet was washed twice with 1 ml of 70 per cent chilled ethanol. The pellet was dried in a stream of sterile air in a laminar air flow cabinet for 3-4 h. Dried DNA pellet was dissolved in 500 µl TE buffer (10 mM Tris-HCl, 0.1 mM EDTA, pH 8.0). This was treated with RNase A (final concentration of 10 µg/ml) by incubating at 37°C for 30 min. The enzyme was removed using an equal amount of chloroform: iso amylalcohol (24:1) and the DNA was precipitated by adding 2 volumes of ice-cold ethanol, washed with 70 per cent ethanol, dried and dissolved in 200 µl of TE buffer (pH 8.0) and stored at -20°C.

ii. Quantification of genomic DNA

DNA concentration was checked by agarose gel electrophoresis. All the DNA samples were electrophoresed on 0.8 per cent agarose gels in 1X TAE buffer (pH 8.0) with known concentration of uncut λ DNA. The gel was stained in ethidium bromide

solution in a final concentration of 10 µg/ml and scanned in a gel documentation system (ENDURO™ GDS Gel Documentation System, USA). The concentrations of DNA samples were compared with the uncut λ DNA (13 ng/µl) and diluted accordingly. The DNA samples were also quantified on microvolume spectrophotometer (Biospec-nano, Shimadzu Biotech, USA) using Tris EDTA as blank and DNA concentration was recorded in ng/µl. The DNA samples were then diluted with TE to make the final working concentration of 13 ng/µl.

iii. Primers used for mapping in this study

Different types of SSR markers were used for parental polymorphism survey. A summary of the polymorphic markers is presented in Table 3.2

Table 3.2 Markers utilized for construction of the intra-specific linkage map of horsegram

S. No.	Type of primers	Source	Number
1.	HUGMS	EST SSRs (Sharma et al. 2015b)	63
2.	MUMS	Genic SSRs (Sharma et al. 2015b)	200
3.	MUMST	Genic trirepeats (Sharma et al. 2015b)	100
4.	MUMSD	Genic Direpeats (Sharma et al. 2015b)	103
5.	MUGSSR	Genomic SSRs (Chahota et al. 2017)	99
6.	MUSSR	Genomic SSRs (Chahota et al. 2017)	50
7.	MUGR	Genomic SSRs (Chahota et al. 2017)	94
8.	MUD	Genomic SSRs (Kaldate et al. 2017)	96
9.	MUGSR	Genomic SSRs (Chahota et al. 2017)	48
10.	RAPD	Operon Tech, USA and Fred Muehlbaue, USA	450
11.	Drought specific primers	Charu and Manoj 2011	24
12.	RcSSRs	Sato et al. 2005	196
13.	MtSSRs	Eujayl et al. 2004	104
14.	COS	Douglas R. Cook, UC, Davis, USA	384
TOTAL			2011

iv. PCR amplification

The primers as shown in Table 3.2 were used for polymorphism survey in two parental lines namely HPKM249 and HPK4. Each primer was tested for parent polymorphism at different annealing temperatures. The polymorphic primers were used for genotyping of F₈ RILs mapping population. For amplification of genomic DNA, a reaction mixture of 10.0 µl volume was prepared using 4.80 µl of sterilized distilled water, 2.0 µl template DNA (13 ng/µl), 0.5 µl of forward and 0.5 µl of reverse primer (5 µM), 0.5 µl MgCl₂ (25 mM), 1.0 µl 10X PCR buffer (10 mM Tris-Hcl, 50 mM KCl, pH 8.3), 0.5 µl dNTP mix (0.2 mM each of dATP, dGTP, dCTP and dTTP) and 0.2 µl *Taq* polymerase (5U/µl). The amplifications were carried out in Veriti 384[®] (Applied Biosystems, CA, USA) and 2720 Thermal Cycler (Applied Biosystems, CA, USA) using PCR protocol as given in Table 3.3.

The amplification products were electrophoresed in either 6 per cent PAGE or 3 per cent metaphore agarose gel (Lonza) depending on the resolution pattern, along with size markers. Gels were prepared and run in 1X TAE buffer (3% metaphore agarose gel) or in 1X TBE (6% PAGE) and visualization of fragments was done using Gel-Documentation Unit (ENDURO[™] GDS Gel Documentation System, USA) or silver-staining procedure depending upon the requirement. Size of alleles was noted with the help of 100-bp DNA ladder (Fermentas, Lithuania).

Table 3.3 PCR conditions used for amplification of horsegram genomic DNA

Primers	Steps	Temperature and time	Cycles
Horsegram SSRs	Initial denaturation	94 ⁰ C for 3 Minutes	35
	Denaturation	94 ⁰ C for 1 minute	
	Annealing	40-60 ⁰ C for 1 minute	
	Extension	72 ⁰ C for 1 minute	
	Final extension	72 ⁰ C for 5 Minutes	
	Storage	4 ⁰ C / 16 ⁰ C for ∞	
	Initial denaturation	94 ⁰ C for 3 Minutes	
	Denaturation	94 ⁰ C for 1 minute	
	Annealing	43-54 ⁰ C for 1 minute	

Red clover SSRs	Extension	72 ⁰ C for 1 minute	35
	Final extension	72 ⁰ C for 5 Minutes	
	Storage	4 ⁰ C/16 ⁰ C for ∞	
<i>M.truncatula</i> SSRs	Initial denaturation	94 ⁰ C for 3 Minutes	35
	Denaturation	94 ⁰ C for 1 minute	
	Annealing	43-54 ⁰ C for 1 minute	
	Extension	72 ⁰ C for 1 minute	
	Final extension	72 ⁰ C for 5 Minutes	
	Storage	4 ⁰ C/16 ⁰ C for ∞	
RAPD	Initial Denaturation	94 ⁰ C for 5 Minutes	39
	Denaturation	94 ⁰ C for 1 Minute	
	Annealing	37 ⁰ C for 1 Minute	
	Extension	72 ⁰ C for 2Minutes	
	Final extension	72 ⁰ C for 5 Minutes	
	Storage	4 ⁰ C/16 ⁰ C for ∞	
COS	Initial Denaturation	94 ⁰ C for 3 Minutes	35
	Denaturation	94 ⁰ C for 1 minute	
	Annealing	55-60 ⁰ C for 1 minute	
	Extension	72 ⁰ C for 1 minute	
	Final extension	72 ⁰ C for 5 Minutes	
	Storage	4 ⁰ C/16 ⁰ C for ∞	

v. Preparation of gel and running conditions

a. Metaphor agarose gel

Three per cent Metaphor (Cambrex, East Rutherford, N.J) agarose gel containing 0.5µg ethidium bromide/ml was used to separate PCR amplification products. The gel was prepared according to manufacturer's instructions with slight modifications. Briefly, for 3 per cent metaphor agarose gel, 3gm of metaphor agarose was added to pre-chilled 1X TAE buffer. Care was taken to avoid the formation of agarose clumps in the buffer and mixed well. After the addition, the metaphor agarose

was allowed to swell by incubating the mixture at 4°C for 1-1.5 h. The resulting solution was weighed and boiled in a microwave for 2 min. The conical flask was swirled in order to dissolve the agarose properly. After complete dissolution the flask was weighed again and the distilled water was added to make up the weight loss. The solution was cooled down to 55°C and gel was cast after adding the EtBr (0.5 µg/ml). The PCR products were mixed with the tracking dye, loaded on gel and electrophoresed at 60W for 2 h in 1X TAE.

b. Polyacrylamide gel

Six per cent polyacrylamide gels were prepared with acrylamide: bisacrylamide (19:1) dissolved in autoclaved double distilled water. To make 6 per cent PAGE gels, following mix of 100 ml was prepared: 45g urea, 30 ml of acrylamide: bisacrylamide (19:1) solution, 20 ml of 5X TBE, 44 µl of TEMED, 750 µl of 10 per cent (w/v) ammonium persulfate and 20 ml of double distilled water was added to make up the final volume. The resulting solution was mixed well and poured into assembled glass plates. After insertion of comb, the gel was allowed to polymerize for 30-60 min. and fitted onto the electrophoresis tank. Both the lower and upper tank was filled with 1X TBE buffer. The amplified products were loaded on the gel at a constant power supply of 60W at room temperature for 90 min. Gels were prepared and run in 1X TBE buffer and visualization of fragments was done using silver-staining procedure. Firstly, the gel plate was put in 10 per cent glacial acetic acid (fixing solution) for ten minutes and was then washed twice with distilled water. Staining was then done using staining solution (2 g Silver Nitrate in 2l distilled water and 2 ml 37% formaldehyde) for 30 minutes with continuous shaking. After staining, the gel plate was again washed with distilled water and bands were developed using pre chilled developing solution (30 g Sodium carbonate in 1l distilled water, 200µl of sodium thiosulphate and 1.5 ml 37% formaldehyde). The developed gel plate was then washed with distilled water and dried.

vi. Phenotyping of recombinant inbred lines (RILs)

A population of 162 RILs along with parents was phenotyped for 24 early maturity, drought tolerance and yield traits. The RILs were evaluated for two consecutive years (2016-2017 and 2017-2018) at Palampur (Fig. 3.2 a, b). The geographic coordinates for Palampur was 32.1167°N, 76.5333°E. The plants were grown in pots and in 1-meter rows having row to row distance of 30 cm and plant to plant distance of 5 cm in a Augmented Block design (ABD) with four checks namely VLG-1, HPKM249, HPK4 and HPK317 using two replications. Cylinder culture experiments were also carried for measurement of root traits (Fig. 3.2 c). The recommended agronomic practices were followed during the cropping season. Further for some agro-morphological traits the RILs were also evaluated at Bajaura in 2017 (Fig. 3.2 d).



Fig. 3.2(a)



Fig. 3.2(b)



Fig. 3.2(c)



Fig. 3.2(d)

Fig. 3.2 Horsegram RILs grown in (a) pots under polyhouse condition at Palampur (b) in one meter rows in ABD at Palampur (c) in polytubes at Palampur (d) in one meter rows in ABD at Bajaura

vii. Measurements for various traits

a. Measurement of biochemical and physiological traits

Chlorophyll content (CHL): The chlorophyll content was estimated using Yoshida et al. (1976). Prior to extraction, fresh leaf samples were cleaned with deionized water to remove surface contamination. Chlorophyll extraction was carried out on fresh, fully expanded leaf material. 200 mg of leaf material from control as well as drought stressed plants was ground in 80 per cent acetone using a pestle and mortar. The absorbance was measured with a spectrophotometer at 663 and 645 nm wavelength, respectively.

$$\text{Chlorophyll 'a' (mg/g FW)} = \frac{12.7 \times A_{663} - 2.69 \times A_{645} \times \text{Volume made up (1ml)}}{1000 \times \text{wt. of sample (200mg)}}$$

$$\text{Chlorophyll 'b' (mg/g FW)} = \frac{22.9 \times A_{645} - 4.68 \times A_{663} \times \text{Volume made up (1ml)}}{1000 \times \text{wt. of sample (200mg)}}$$

Carotenoid content (CAR): The amount of carotenoids was determined according to Lichtenthaler and Wellburn (1983). Leaf tissues (200 mg) from the control and drought stressed plant were homogenized in acetone (80%). Extract was centrifuged at 3,000 x g and absorbance was recorded at 480 nm by spectrophotometer.

$$\text{Carotenoids (mg/g FW)} = \frac{A_{480} + 0.114 \times A_{663} - 0.638 \times A_{645} \times \text{Volume made up (1ml)}}{1000 \times \text{wt. of sample (200mg)}}$$

Proline content (PRO): The free proline content was estimated by the method of Bates et al. (1973). Leaf samples (200mg) from control and drought stressed plants were homogenized in 1 ml of 3 per cent sulphosalicylic acid. The homogenate was centrifuged at 18,000 x g for 5 minutes at 4°C. Following this, in 100 µl of the supernatant 100 µl of 3 per cent sulfosalicylic acid, 200ul of glacial acetic acid and 200 µl of acid ninhydrin were added. The resulting mixture was heated for 1 hour at 100°C in a water bath and the reaction was terminated by placing the tubes on an ice bath. The mixture was extracted with 1 ml toluene and the absorbance of the fraction with the toluene aspirated from the liquid phase was measured at 520 nm using a UV-Vis Light spectrophotometer. Proline content was determined using a calibration curve and expressed as µ mole proline per gram fresh weight (µmoles/g FW).

Malondialdehyde content (MDA): The degree of lipid peroxidation was measured in terms of MDA content as described by Heath and Packer (1968). Leaf samples (200mg) from control and drought stressed plants were homogenized in 1 ml of 5 per cent trichloroacetic acid (TCA) solution and centrifuged at 13,500 x g for 10 minutes at room temperature. The supernatant of tissue extract was mixed with an equal volume of 20 per cent (v/v) TCA containing 0.5 per cent (v/v) thiobarbituric acid (TBA). The resulting mixture was heated at 96°C for 30 minutes, cooled in ice and centrifuged at 9,500 x g for 10 minutes. The content of MDA was calculated from the absorbance at 532 nm using 0.5 per cent TBA in 20 per cent TCA solution as blank. The value for the non-specific absorption at 600 nm was subtracted from 532 nm value. The concentration of MDA was calculated using the extinction coefficient of 155 mM⁻¹cm⁻¹. The results were expressed as nmoles MDA per gram fresh weight (nmoles/g FW).

Relative water content (RWC): Relative water content was measured on the second or third upper fully expanded leaf for both well-watered and stressed plants. A leaf was placed in a weighed vial immediately after excision and its fresh weight was recorded. Then the leaf petiole was immersed in deionized water at room temperature (22°C) in low light for 7 h to attain full turgidity. The turgid leaf weight was measured and the sample oven-dried at 65°C for 48 h for dry weight determination. RWC was calculated using the formula (Shrestha et al. 2006):

$$\text{RWC} = 100 \times (\text{fresh weight} - \text{dry weight}) / (\text{turgid weight} - \text{dry weight})$$

Membrane stability index (MSI): MSI was estimated according to Sairam (1994). Two sets of leaf tissues (0.1 g) were placed in 10 ml of double distilled water. One set was kept at 25°C for 24 h, kept on shaking, initial conductivity (Ci) of the bathing solution was measured with the conductivity meter. Second set of tissue was autoclaved at 121°C for 30 min and cooled down to 25°C before final conductivity (Cmax) was measured as:

$$\text{MSI (\%)} = 1 - \text{electrical conductivity before incubation} / \text{electrical conductivity after incubation}$$

b. Measurement of root traits

Root traits were studied in polytubes/ cylinder culture. Cylinders (0.6 m length x 0.15 m diameter) were created using polyvinyl chloride (PVC) drain pipes to provide enough space for root growth for a single plant. Cylinders were filled with 1:1 mixture of potting mix (contains mixture of soil, sand and FYM) so as to facilitate root recovery. Three seeds were sown in each cylinder and thinned to one after emergence and seedling establishment. Stress was employed by withholding water at 50% flowering stage. Traits measured were root length (RL, cm), root fresh weight (RF, g) and root dry weight (RD, g) (Table 3.4).

c. Measurement of morphological traits

Plant height (PH, cm), number of primary branches (PB), number of secondary branches (SB), growth habit (GH), growth type (GP) and pigmentation of stem (PGM) were important morphological traits to characterize genotypes. Plant height was measured just prior to physiological maturity by taking five readings on each RIL and averaging before analysis. The detailed description of other traits has been provided in Table 3.4.

d. Measurement of phenological traits

Days to 50% flowering (FL), days to maturity (MT) and reproductive period (RP) are the important phenological traits to be measured. Data were collected on days from sowing to flowering by calculating the difference of days from date of sowing to the date when 50% of the plants in a line showed the first fully open flower. Days from sowing to physiological maturity were recorded by calculating the difference of days from date of sowing to the date when 90% of the plants had turned brown. The reproductive growth period was calculated as the days between the start of flowering and physiological maturity.

e. Measurement of yield and related traits

Grain yield and associated yield components are important in determining the performance of genotypes. The five middle plants from each row were harvested

individually for subsequent measurements. All the measurements were made on an individual plant basis and the means of five plants were used for analysis for yield and related traits. At maturity, plants were harvested by cutting at ground level and placing each plant in separate bags. Traits measured were 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) (Table 3.4).

Table 3.4 List of traits evaluated along with their description

S. No.	Trait	Trait wise description
BIOCHEMICAL AND PHYSIOLOGICAL TRAITS		
1.	Chlorophyll content (CHL, mg/g FW)	Estimated using standardized protocol of Yoshida et al. (1976)
2.	Carotenoid content (CAR, mg/g FW)	Estimated using standardized protocol of Lichtenthaler and Wellburn (1983)
3.	Proline content (PRO, μ moles/g FW)	Estimated using standardized protocol of Bates et al. (1973)
4.	Malondialdehyde content (MDA, nmoles/g FW)	Estimated using standardized protocol of Heath and Packer (1968)
5.	Relative Water Content (RWC)	Measured on the second or third upper fully expanded leaf
6.	Membrane stability index (MSI)	Estimated using standardized protocol of Sairam (1994)
ROOT TRAITS		
7.	Root length (RL, cm)	Measured at the time of harvesting by uprooting and removing plant and soil carefully
8.	Root Fresh Weight (RF, g)	Fresh weight of root was measured using weighing balance
9.	Root Dry Weight (RD, g)	Weighed after complete drying of roots in oven at 60 ⁰ C for 24 hrs
MORPHOLOGICAL TRAITS		
10.	Plant height (PH, cm)	Measured from the base to the tip of main shoot at maturity

11.	Primary branches (PB)	Branches originated from the main shoot were counted at maturity
12.	Secondary branches (SB)	Branches originated from the primary branches were counted at maturity
13.	Growth habit (GH)	Twining or Bushy growth habit of plants were noted at maturity
14.	Growth type (GP)	Determinate or indeterminate growth type of plants were noted at maturity
15.	Pigmentation of stem (PM)	Presence or absence of purple pigmentation of stem was noted at maturity
PHENOLOGICAL TRAITS		
16.	Days to 50 per cent flowering (FL)	Difference of days from sowing to the date when 50% of the plants showed first fully open flower
17.	Reproductive period (RP)	Days between the start of flowering and physiological maturity
18.	Days to maturity (MT)	The difference of days from date of sowing to the date when 90% of the plants had turned colour
YIELD AND YIELD RELATED TRAITS		
19.	Seed size (SZ, cm)	Length of 10 seeds was measured (cm)
20.	100-seed weight (SW, g)	One hundred randomly counted seeds of each entry in each replication were weighed
21.	Seeds per pod (SP)	Total number of seeds in a pod counted after harvesting
22.	Pods per plant (PP)	Total number of pods counted after harvesting
23.	Seeds per plant (SS)	Total number of seeds counted after harvesting
24.	Seed yield per plant (SY, g)	All the plants were hand thrashed and seed yield was recorded in grams

3.3 Data analysis

i. Genotyping

All the primers used in this study were utilized for the polymorphism analysis between the parents of the mapping population derived from HPKM 249 × HPKV4. The markers which exhibited polymorphism were selected for genotyping of 162 RILs mapping population. The PCR amplification was carried out using the protocol and conditions mentioned in Table 3.3. The PCR products were electrophoresed on either 3 per cent metaphor/agarose gel or 6 per cent denaturing polyacrylamide gels depending upon the resolution of bands obtained along with size markers and stained with ethidium bromide/silver nitrate. The gels were analyzed in gel documentation unit (ENDURO™ GDS Gel Documentation System, USA)

ii. Generation of data

The amplified banding patterns were scored manually as ‘A’ for HPKM249 type banding pattern, ‘B’ for HPKV4 type banding pattern and H for heterozygous loci if any. The data matrix was used as an input files for map construction using JOINMAP® 4.1 program (van Ooijen 2006).

iii. Linkage analysis and map construction

To identify linkage groups, grouping of markers were done using the minimum independence LOD threshold of 3.0 and a maximum of 8.0 with a step up of 0.5. The groups showing maximum number of markers and highest linkage at the variable LODs were selected.

iv. QTL mapping

a. Phenotypic data evaluation

The phenotypic data of the RILs mapping population derived from HPKM 249 × HPKV4 was obtained from evaluated traits at Palampur and Bajaura. Statistical analysis of the data such as ANOVA, frequency distribution, correlation coefficient analysis and principal component analysis was done using Past 3.25 software. The phenotypic correlations between each pair of traits were obtained using the Pearson’s

correlations coefficient applied on the individual phenotypic values. These correlations were tested assuming global significance level of 0.05.

b. Statistical analysis and QTL mapping

Quantitative trait loci analysis was carried out on the set of 162 F₈ individuals with phenotypic data for early maturity and yield traits and the genotypic data consisted of 295 mapped markers in ten linkage groups of horsegram. QTLs were detected with the Windows QTL Cartographer V2.5 software (Wang et al. 2005) by composite interval mapping (CIM) method (Zeng 1993; 1994) using the Zmapqtl standard model 6 with a window size of 10 cM and a 2 cM walk speed. The forward regression algorithm was used to obtain cofactors. A 1000-permutation test of shuffling the phenotypes means with the genotypes was performed to estimate a genome-wide LOD score threshold for a QTL at a significance level of P=0.05 (Doerge and Churchill 1996). An LOD threshold score of ≥ 2.5 at 1000 permutations were significantly considered (5% level of significance) to identify and to map the QTLs on the horsegram LGs. The 95% confidence intervals of the QTL locations were determined by one LOD intervals surrounding the QTL peak (Mangin et al. 1994). The estimated additive effect and the percentage of phenotypic variation explained by each putative QTL were obtained using the software with the CIM model by the Zmapqtl procedure. The R² value from this analysis was accepted as the percent phenotypic variance explained by the locus.

4. RESULTS AND DISCUSSION

The present investigation entitled “Identification of QTLs linked to early maturity and yield-related traits in horsegram (*Macrotyloma uniflorum*)” was carried out with the objectives to construct a horsegram linkage map using molecular markers and to identify quantitative traits loci linked to early maturity and yield-related traits.

Many agriculturally important traits such as early maturity, yield, quality and resistance to abiotic stresses are controlled by many genes and are known as quantitative traits (also polygenic, multifactorial or complex traits). The regions within genomes that contain genes associated with a particular quantitative trait are known as quantitative trait loci (QTLs). The identification of QTLs based only on conventional phenotypic evaluation is not possible. A major breakthrough in the characterization of quantitative traits that created opportunities to select for QTLs was initiated by the development of DNA (or molecular) markers in the 1980s. One of the main uses of DNA markers in agricultural research has been in the construction of linkage maps for diverse crop species. Linkage maps have been utilised for identifying chromosomal regions that contain genes controlling simple traits (controlled by a single gene) and quantitative traits using QTL analysis (Mohan et al. 1997). The process of constructing linkage maps and conducting QTL analysis to identify genomic regions associated with traits is known as QTL mapping (McCouch & Doerge 1995; Mohan et al. 1997). QTL mapping is based on the principle that genes and markers segregate via chromosome recombination (called crossing-over) during meiosis (i.e. sexual reproduction), thus allowing their analysis in the progeny (Paterson, 1996). The frequency of recombinant genotypes can be used to calculate recombination fractions, which may be used to infer the genetic distance between markers. Markers that have a recombination frequency of 50 per cent are described as ‘unlinked’ and assumed to be located far apart on the same chromosome or on different chromosomes. DNA markers that are tightly linked to agronomically important genes may be used as molecular tools for marker-assisted selection (MAS) in plant breeding (Ribaut and Hoisington 1998). MAS involves using the

presence/absence of a marker as a substitute for or to assist in phenotypic selection, in a way which may make it more efficient, effective, reliable and cost-effective compared to the more conventional plant breeding methodology. It is expected that the development of high resolution QTL maps will also facilitate the identification of actual genes (rather than markers) via map based cloning (also known as positional cloning). Map based cloning involves the use of tightly linked markers to isolate target genes by using the marker as a probe to screen a genomic library. The identification of genes controlling important traits will enable plant scientists to predict gene function, isolate homologues and conduct transgenic experiments. Therefore keeping in view the immense importance of linkage and QTL maps and lack of any such map in horsegram for further improvement of the species, the present work was designed to construct linkage map of horsegram using molecular markers and to identify QTLs linked to early maturity and yield related in horsegram.

The results obtained on different aspects of present study have been presented and discussed under the following heads:

4.1 Linkage map construction using PCR-based markers

- i. Genotyping of mapping population**
- ii. Construction of an intraspecific linkage map of horsegram**

4.2 Identification of quantitative trait locus

- i. Analysis of morphological traits**
 - (a) Analysis of variance**
 - (b) Phenotypic trait variation**
 - (c) Trait correlation analysis**
 - (d) Principal Component Analysis**
- ii. QTLs analysis and trait dissection for early maturity and yield related traits**
- iii. Candidate genomic regions for molecular breeding**

4.1 Linkage map construction using PCR-based markers

i. Genotyping of mapping population

Different types of SSR markers viz. *Macrotyloma uniflorum* EST SSRs (HUGMS), *Macrotyloma uniflorum* genomic SSRs (MUGSSR, MUGR, MUGSR, MUSSR, MUD) *Macrotyloma uniflorum* genic SSRs (MUMS, MUMST, MUMSD), drought specific SSRs, red clover SSRs (RcSSRs), *Medicago truncatula* SSRs (MtSSRs) along with RAPDs and COS markers were used for parental polymorphism survey. A summary of polymorphic markers identified is presented in Table 4.1.

Table 4.1 Markers used for construction of intra-specific linkage map of horsegram

S. No.	Markers	Source	Markers screened	Polymorphic Markers	Percent Polymorphism	Markers Mapped
1	HUGMS	EST SSRs	63	36	57.14	15
2	MUMS	Genic SSRs	200	55	27.50	45
3	MUMST	Genic SSRs	100	37	37.0	22
4	MUMSD	Genic SSRs	103	44	42.72	20
5	MUGSSR	Genomic SSRs	99	42	42.42	31
6	MUSSR	Genomic SSRs	50	24	48.0	16
7	MUGR	Genomic SSRs	94	30	31.91	20
8	MUD	Genomic SSRs	96	28	29.17	13
9	MUGSR	Genomic SSRs	48	8	16.67	7
10	RAPD	Operon Tech, USA and Fred Muehlbaue, USA	450	55	12.22	22
11	Drought specific primers	Charu and Manoj 2011	24	5	20.83	4
12	RcSSRs	Sato et al. 2005	196	88	44.90	56
13	MtSSRs	Eujayl et al. 2004	104	33	31.73	17
14	COS(conserved orthologous sequences)	Douglas R. Cook	384	8	2.08	7
TOTAL			2011	493	24.52	295

In the present study, genic and genomic SSRs of *Macrotyloma* along with SSRs of red clover (*Trifolium pratense*) and *Medicago truncatula*, drought specific primers of *Glycine max*, RAPD and COS primers were used for screening of parental DNA to detect polymorphism. Two thousand and eleven primers yielded 493 clear and scorable polymorphic markers. Of all the scored SSR markers, 295 (59.84%) were mapped on ten linkage groups, whereas 199 markers were found unlinked. The amplicons generated using SSR primers have been shown in Figures 4.1- 4.2.

For implementing genomic-assisted breeding in legumes, the availability and easy accessibility of genomic resources is a pre-requisite. Molecular breeding by marker-assisted selection relies on DNA markers closely linked to the trait of interest (Collard et al. 2005). Thus in marker-assisted selection, high-resolution mapping of a trait should be conducted to identify markers closely linked to the target trait. Co-dominant markers are effective in identifying desirable homozygous genotypes at early stages of selection (Hamwieh et al. 2005). Microsatellite markers are useful for genetic studies because they are co-dominant, multi-allelic, widely distributed across the genome, polymerase chain reaction (PCR)-based, and transferable between different genotypes. Information generated by these markers allows comparisons and information exchange between different studies, especially for comparative genetic mapping (Grattapaglia 2000). Recently, several research groups have made advances in the development of microsatellite markers for various species of the leguminosae family (Song et al. 2004). However there are very few reports of molecular marker development in horsegram. Efforts have been made to develop SSR markers in horsegram through development of enriched genomic library (Chahota et al. 2017; Kaldate et al. 2017). However, the total number of currently available SSR markers is insufficient for genetic analysis in horsegram. With the ever-increasing number of DNA sequences available in public databases, genomic sequences provide a more rapid and economic method for developing SSR markers. Based on SSRs developed from the genome sequences, high-density genetic linkage maps can be constructed in crop plants (Li et al. 2011; Thudi et al. 2011). Genomic resources in horsegram lagged considerably behind major pulses. Sharma et al. (2015a) developed SSR and ILP markers from expressed sequence tag (EST) sequences and transcriptome data of horsegram available in public domain. A set of 2847 genic SSRs from transcriptome sequence data from two horsegram lines HPKM191 and HPKM249 were designated

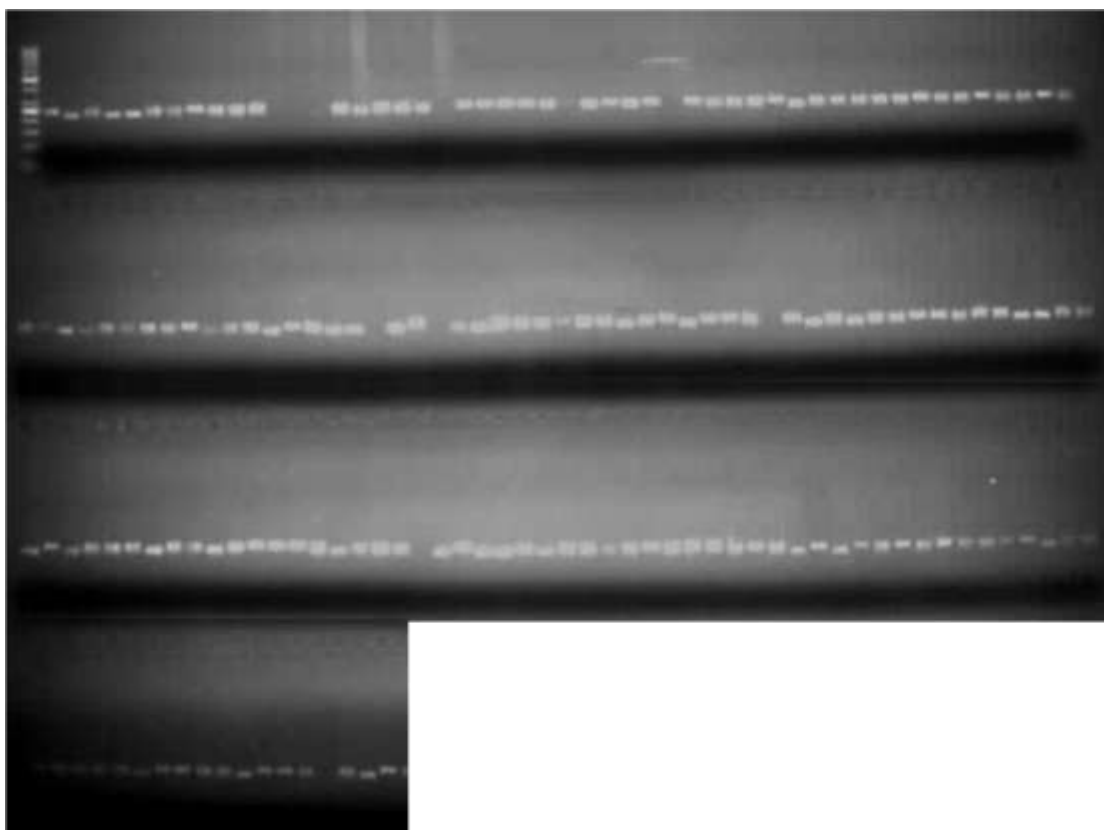


Fig. 4.1 SSR banding profile using HUGMS7 primer on P1 (HPKM249), P2 (HPK4) and 162 F₈ RILs

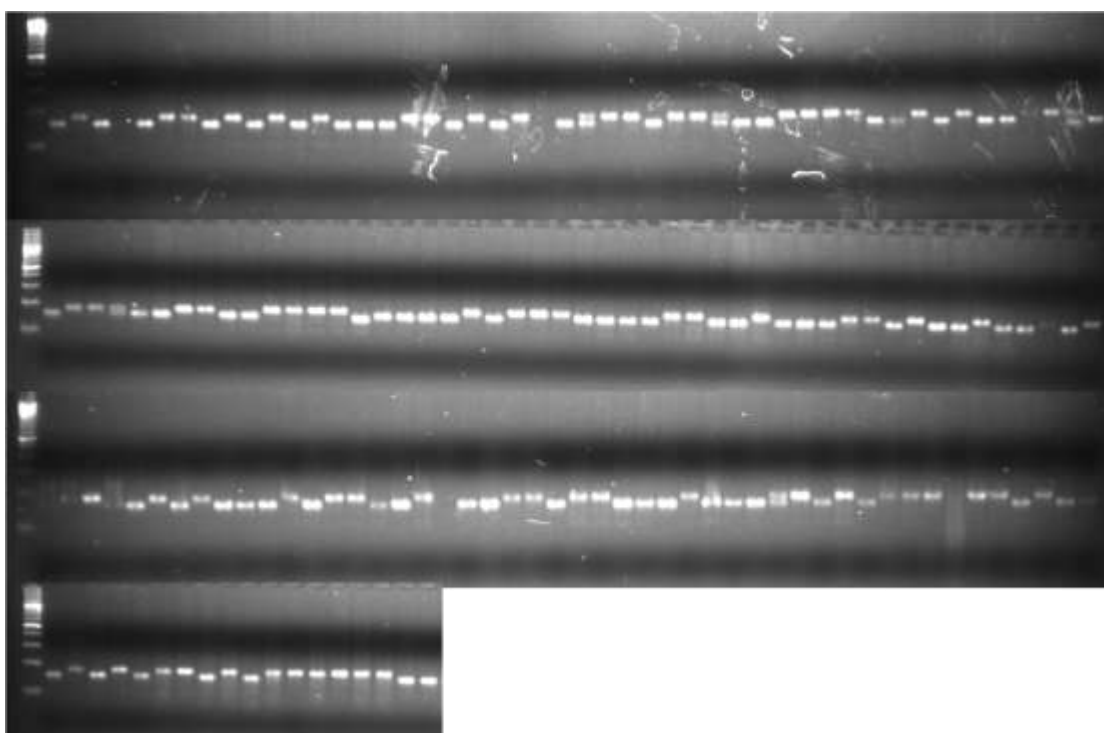


Fig. 4.2 SSR banding profile using MUD27 primer on P1 (HPKM249), P2 (HPK4) and 162 F₈ RILs

as *M. uniflorum* micro-satellite (MUMS) and these SSR-containing sequences covered 16.25% of the total transcriptome. Sharma et al. (2015b) validated 245 primer pairs in 20 horsegram accessions. Given the estimated ~ 400 Mbps size of the horsegram genome, the SSR density was 58 per Mb in the DNA sequence of horsegram (Chahota et al. 2017) lower than those reported for other plant species, viz., *Arabidopsis* (370 SSRs/Mb), rice (529 SSRs/Mb), poplar (508 SSRs/ Mb) and grapevine (506 SSRs/Mb). Kaldate et al. 2017 designed 2458 SSR primer pairs and 117 SSRs were characterized in 48 diverse lines of horse gram and the di-nucleotide and tri-nucleotide accounted for 47% of all of the SSR identified and the remaining 53% consisted of tetra, penta and hexa-nucleotide repeats SSRs. It has been noted that the SSR in different locations within the gene might play different functional roles in organism development, adaptation, survival and evolution were never ending. The markers developed could be useful for linkage and QTL mapping involving interspecific mapping population (Aditya et al. 2019).

In the present study, 2011 markers [63 (Horsegram EST SSRs) + 403 (Horsegram genic SSRs) + 387 (Horsegram genomic SSRs) + 24 (drought specific SSRs) + 300 (SSRs from other legumes viz. red clover and *Medicago*) + 450 (RAPD) + 384 (COS)] were used for polymorphism survey in parental lines for the construction of horsegram linkage map. Of these 493 polymorphic markers, 36 were horsegram EST SSRs, 136 were horsegram genic SSRs, 132 were horsegram genomic SSRs, 5 were drought specific SSRs, 121 were SSRs from other legumes species, 55 were RAPD primers and 8 were COS primers. The level of polymorphism observed in our study was in agreement with varied levels of 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), 27.02% in soybean (Hwang et al. 2009) and 37.0% in lotus (Yang et al. 2012). It has been documented that different molecular tools for genomic analysis and improvement could not be extended in legumes to a certain level due to their narrow genetic base (Gupta et al. 2012). However, the polymorphism detected in this study was also compared to other plants which varied from as low as 6.5% in tomato (Shirasawa et al. 2010), 23.2% in cucumber (Zhang et al. 2012) to as high as 32.8% in *Catharanthus* (Shokeen et al. 2011) and 50% in *Vitis* (Riaz et al. 2004). A number of factors affect the level of polymorphism exhibited by the parents of the mapping population such as type of

marker, type of cross (self- or cross-pollinated, inter- or intraspecific cross), type of population (F_2 /BC/RIL) etc. Crosses involving parents from different domestication centers with variation in different traits are desirable for genetic mapping, since the possibility of detecting polymorphism among parents is high due to higher number of segregating loci (Grisi et al. 2007).

ii. Construction of an intraspecific linkage map of horsegram

A framework linkage map was constructed using the genotyping data of 295 polymorphic markers using JoinMap software, version 4.0 [van Ooijen 2006 (as described in Chapter 3, Material and Methods, Section 3.3.3)]. A total of 295 SSR markers were assigned positions on ten linkage groups (LGs) at LOD 3.5, based on the number of chromosomes of *Macrotyloma uniflorum* ($2n=20$, $n=10$). The generated linkage map of horsegram using these markers spanned 1541.7 cM distance with an average marker density of 5.20 cM (Fig. 4.3). Of the 295 mapped markers include 15 EST SSRs, 87 genomic SSRs, 87 genic SSRs, 22 RAPDs, 73 SSRs from other species, 4 drought specific markers and 7 COS SSRs. All the above depicted SSR markers were distributed across ten linkage groups and have been shown in Fig. 4.3.

Grain legumes, particularly horsegram has lagged behind in the development of high yielding cultivars due to lack of genetic and genomic information of various genes associated with important traits. In the present study, a molecular linkage map of horsegram was constructed using DNA markers to identify the QTLs or genomic regions associated with the early maturity and yield related traits.

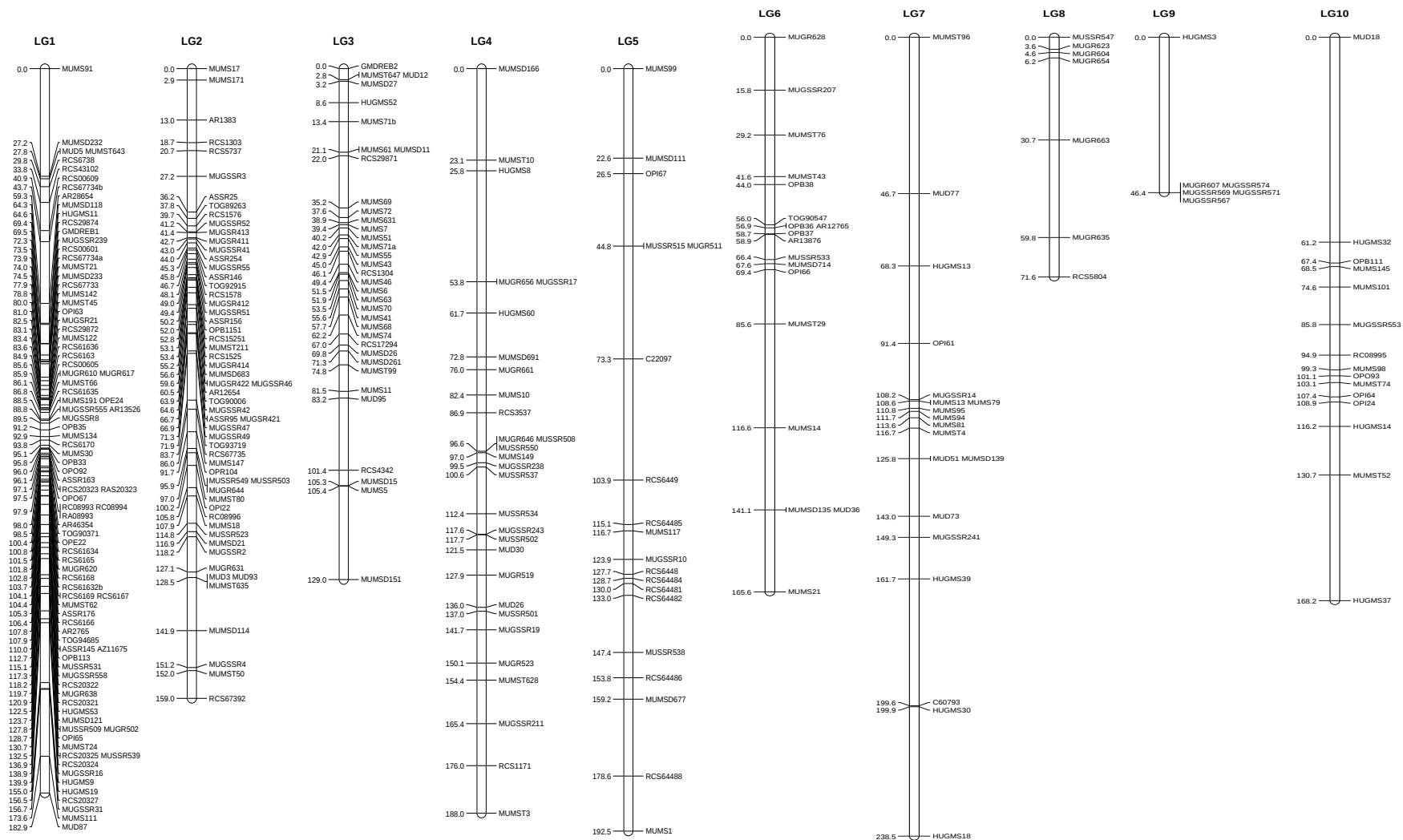


Fig 4.3 An intraspecific linkage map of *M. uniflorum* based on RILs mapping population generated by crossing HPKM249×HPK4. The map was generated with 295 polymorphic molecular markers using JoinMap version 4.0 at a LOD value of 3.5, with Kosambi mapping function.

The present map is the first intra-specific molecular linkage map of horsegram based on DNA markers and covers a much higher genome map length of 1541.7 cM. This may be due to a larger population size and better pairing and crossing over between the chromosomes of two varieties of the same species. Furthermore, the present map represents expressed regions of the horsegram genome due to mapping of genic SSRs therefore it will be highly useful for comparative genomics and synteny studies. Since till now there is no earlier information on construction of linkage map in horsegram, the map length (1541.7 cM) of the horsegram linkage map in the study was compared to intraspecific linkage maps of other legumes present in clade phaseoloid/milletioid with the map length of 2458.0 cM in soybean (Kong et al. 2018), 1079.21 cM in common bean (Blair et al. 2018), 1588.7 cM in cow pea (Somta et al. 2019) and 1,411 cM in pigeon pea (Sheetal et al. 2017). The details of number of markers mapped in a linkage group, region they spanned and the average marker density exhibited by them have been summarized in Table 4.2. The length of LGs varied from 46.4 cM in LG9 to 238.5 cM in LG7. The average marker density varied from 2.0 cM to 12.5 cM, with an average of 5.2 cM indicating differing degrees of saturation of LGs. The average marker density on each linkage group revealed that the markers were randomly distributed. The maximum number of markers was mapped on LG1, which harboured 89 markers with the average marker density of 2.0 cM and minimum on LG9 which harboured 6 markers with the average marker density of 7.7 cM (Fig. 4.3). Such discrepancies could probably be eliminated either by increasing the population size or by further saturating the map with more SSRs and SNPs markers (Grisi et al. 2007).

Among the ten linkage groups, each group differed from one another with respect to length and marker distribution. As a result random distribution of markers in the present study was noticed e.g. some groups were densely packed (LG1 and LG2), whereas LG8 and LG9 contained only seven and six markers, respectively, which can be explained by the fact that SSRs are ubiquitously and randomly distributed in the plant genomes (Ramsay et al. 1999; Elsik and Williams 2001). This may be the reason that most of the markers were located on the centromeric region resulted in the lower recombination in these regions (Areshechenkova and Ganai 1999; Ramsay et al. 2000). The genomic origin of DNA sequences used for the SSR identification is also responsible for their unequal distribution on the groups and thus lead to less genome coverage (Tanksley et al. 1992).

Table 4.2 Distribution of 295 markers on ten linkage groups of an intra-specific linkage map of horsegram.

LGs	Markers Mapped	Map length (cM)	Average marker density (cM)
LG1	89	182.9	2.0
LG2	58	159.0	2.7
LG3	35	129.0	3.7
LG4	29	188.0	6.5
LG5	19	192.5	10.1
LG6	18	165.6	9.2
LG7	19	238.5	12.5
LG8	7	71.6	10.2
LG9	6	46.4	7.7
LG10	15	168.2	11.2
Total	295	1541.7	5.2

The markers were unevenly distributed on all the LGs, except for LG1 and LG4, which showed cluster of four markers within 1 cM distance. A single cluster with seven markers within 1 cM distance was identified on LG1. However, 50 gaps were observed across all the LGs. Sixteen large gaps ranging from 20-40 cM on LGs 1, 3, 4, 5, 6, 7 & 8 and twenty six small gaps ranging from 11-20 cM on all LGs were observed. Most of them were located near distal ends. The largest gap of 61.2 cM was observed on LG10 followed by 46.71 cM and 46.36 cM on LG7 and LG 9, respectively (Fig. 4.3). This may be due to the occurrence of fewer marker polymorphisms in these gaps or regions thus resulting in lower marker density. As homozygous regions possess lower recombination frequency and thus may be a possible explanation of low density of markers in these distal regions (Souza et al. 2013). Strategies that could help to fill the gaps are use of BAC libraries to anchor markers on the physical map and to identify and develop specific markers for the under-represented genomic region. This strategy has been shown to be an effective way to target for low-density marker regions on the soybean genome (Song et al. 2004). Another approach to fill the gaps in linkage maps is to make an assessment of the correspondence between the physical and genetic distances, in order to estimate

the sizes of the gaps, helping to elucidate aspects related to recombination in the genome, as performed for the entire rice genome (Chen et al. 2002). The consolidation of linkage information will also provide the possibility to obtain a more consistent genetic map by increasing the number of markers well distributed along the genome. In this regard, knowing that the distribution of the SSRs across the genome is not random and that the SSRs frequency, of mostly trinucleotide repeats, is higher in transcribed regions, especially non-translated portions (Morgante et al. 2002), the use of SSRs derived from expressed sequences could help to reduce the large intervals between markers and increase the representation of markers across specific regions of the genome.

The maximum and the minimum distance between markers was 61.2 cM (LG7) and 0.003 cM (LG2), respectively. The distribution of markers between linkage groups was unequal. There were four large groups having 12-19 markers within length of 10 cM and five groups having 28-31 markers within length of 30 cM. The distance between markers on the current map also varied greatly across different linkage groups, and the size of the LG did not necessarily reflect the number of linked markers. For instance, LG1 with 89 markers covered 182.9 cM with an average marker spacing of 2.0 cM, whereas LG4 spanning a distance of 188.0 cM was covered by 29 markers with average spacing of 6.5 cM and LG5 spanning a distance of 192.5 cM was covered by 19 markers with average spacing of 10.0 cM. Similar results have been reported by Winter et al. (2000) in *Cicer* and Gupta et al. (2012) in lentil. The list of markers linked on different linkage groups of horsegram along with their locus name, map position and distance between markers are given in Table 4.3(a-j).

The differences in the crossing-over frequency can influence marker density in a linkage group. Tanksley et al. (1992) explained the uneven marker distribution with the reasoning that centromeres and centromeric heterochromatin and in some instances telomeres experience up to tenfold less recombination. Heterogeneity in recombination along the genome has implications on the development of high resolution linkage maps as the latter are much easier to develop for regions of higher recombination. On the other hand, mapping of recombination suppressed regions requires much larger progeny sizes in order to allow the rare recombination events to occur, which is necessary for the construction of fine maps.

A non-random distribution of markers due to centrally located clusters has been reported in soybean (Cregan et al. 1999), common bean (Yu et al. 2000) and chickpea (Winter et al. 2000). Sometimes, the apparently random marker distribution is due to a low number of markers; when more markers were added to the map, clusters became evident, such as SSRs in soybean (Song et al. 2004), common bean (Gonzalez et al. 2016) and cowpea (Muchero et al. 2009). In cereals where cytogenetic markers are available, the crossing over frequency in the distal regions of the chromosomes has been shown to be higher than in the regions proximal to the centromere (Lukaszewski 1992; Alonso-Blanco et al. 1993).

Table 4.3 List of markers used in the present study for the linkage map construction.

(a) List of markers linked on LG1 of horsegram. The locus name, map position and distance between markers are mentioned.

S.No.	Locus	Marker Position (cM)	Distance between markers (cM)
1	MUMS91	0	-
2	MUMSD232	27.203	27.203
3	MUD5	27.765	0.562
4	MUMST643	27.765	0
5	RCS6738	29.792	2.027
6	RCS43102	33.751	3.959
7	RCS00609	40.901	7.15
8	RCS67734b	43.719	2.818
9	AR28654	59.256	15.537
10	MUMSD118	64.289	5.033
11	HUGMS11	64.572	0.283
12	RCS29874	69.385	4.813
13	GMDREB1	69.543	0.158
14	MUGSSR239	72.288	2.745
15	RCS00601	73.484	1.196
16	RCS67734a	73.862	0.378
17	MUMST21	74.005	0.143
18	MUMSD233	74.523	0.518
19	RCS67733	77.92	3.397
20	MUMS142	78.794	0.874
21	MUMST45	80.045	1.251
22	OPI63	81.028	0.983
23	MUGSR21	82.515	1.487
24	RCS29872	83.112	0.597
25	MUMS122	83.4	0.288

26	RCS61636	83.616	0.216
27	RCS6163	84.893	1.277
28	RCS00605	85.594	0.701
29	MUGR610	85.938	0.344
30	MUGR617	85.938	0
31	MUMST66	86.075	0.137
32	RCS61635	86.769	0.694
33	MUMS191	88.51	1.741
34	OPE24	88.526	0.016
35	MUGSSR555	88.808	0.282
36	AR13526	88.817	0.009
37	MUGSSR8	89.498	0.681
38	OPB35	91.195	1.697
39	MUMS134	92.867	1.672
40	RCS6170	93.835	0.968
41	MUMS30	95.142	1.307
42	OPB33	95.767	0.625
43	OPO92	96.014	0.247
44	ASSR163	96.134	0.12
45	RCS20323	97.142	1.008
46	RAS20323	97.142	0
47	OPO67	97.548	0.406
48	RC08993	97.941	0.393
49	RC08994	97.941	0
50	RA08993	97.941	0
51	AR46354	98.009	0.068
52	TOG90371	98.526	0.517
53	OPE22	100.422	1.896
54	RCS61634	100.845	0.423
55	RCS6165	101.493	0.648
56	MUGR620	101.825	0.332
57	RCS6168	102.805	0.98
58	RCS61632b	103.711	0.906
59	RCS6169	104.149	0.438
60	RCS6167	104.149	0
61	MUMST62	104.366	0.217
62	ASSR176	105.297	0.931
63	RCS6166	106.445	1.148
64	AR2765	107.799	1.354
65	TOG94685	107.874	0.075
66	ASSR145	110.038	2.164
67	AZ11675	110.047	0.009

68	OPB113	112.697	2.65
69	MUSSR531	115.117	2.42
70	MUGSSR558	117.312	2.195
71	RCS20322	118.175	0.863
72	MUGR638	119.706	1.531
73	RCS20321	120.858	1.152
74	HUGMS53	122.527	1.669
75	MUMSD121	123.658	1.131
76	MUSSR509	127.819	4.161
77	MUGR502	127.819	0
78	OPI65	128.654	0.835
79	MUMST24	130.704	2.05
80	RCS20325	132.463	1.759
81	MUSSR539	132.49	0.027
82	RCS20324	136.913	4.423
83	MUGSSR16	138.894	1.981
84	HUGMS9	139.922	1.028
85	HUGMS19	155.002	15.08
86	RCS20327	156.459	1.457
87	MUGSSR31	156.675	0.216
88	MUMS111	173.614	16.939
89	MUD87	182.898	9.284

(b) List of markers linked on LG2 of horsegram. The locus name, map position and distance between markers are mentioned.

S.No.	Locus	Marker Position (cM)	Distance between markers (cM)
1	MUMS17	0	-
2	MUMS171	2.907	2.907
3	AR1383	13.039	10.132
4	RCS1303	18.72	5.681
5	RCS5737	20.656	1.936
6	MUGSSR3	27.178	6.522
7	ASSR25	36.229	9.051
8	TOG89263	37.803	1.574
9	RCS1576	39.687	1.884
10	MUGSSR52	41.212	1.525
11	MUGSR413	41.388	0.176
12	MUGSR411	42.665	1.277
13	MUGSSR41	43.011	0.346
14	ASSR254	43.985	0.974
15	MUGSSR55	45.318	1.333
16	ASSR146	45.784	0.466

17	TOG92915	46.693	0.909
18	RCS1578	48.076	1.383
19	MUGSR412	49.039	0.963
20	MUGSSR51	49.352	0.313
21	ASSR156	50.216	0.864
22	OPB1151	51.99	1.774
23	RCS15251	52.761	0.771
24	MUMST211	53.112	0.351
25	RCS1525	53.415	0.303
26	MUGSR414	55.214	1.799
27	MUMSD683	56.624	1.41
28	MUGSR422	59.62	2.996
29	MUGSSR46	59.623	0.003
30	AR12654	60.492	0.869
31	TOG90006	63.896	3.404
32	MUGSSR42	64.56	0.664
33	ASSR95	66.733	2.173
34	MUGSR421	66.738	0.005
35	MUGSSR47	66.863	0.125
36	MUGSSR49	71.271	4.408
37	TOG93719	71.882	0.611
38	RCS67735	83.68	11.798
39	MUMS147	85.966	2.286
40	OPR104	91.696	5.73
41	MUSSR549	95.855	4.159
42	MUSSR503	95.855	0
43	MUGR644	95.862	0.007
44	MUMST80	97.041	1.179
45	OPI22	100.237	3.196
46	RC08996	105.842	5.605
47	MUMS18	107.907	2.065
48	MUSSR523	114.816	6.909
49	MUMSD21	116.905	2.089
50	MUGSSR2	118.235	1.33
51	MUGR631	127.144	8.909
52	MUD3	128.544	1.4
53	MUD93	128.544	0
54	MUMST635	128.544	0
55	MUMSD114	141.871	13.327
56	MUGSSR4	151.235	9.364
57	MUMST50	152.042	0.807
58	RCS67392	159.041	6.999

(c) List of markers linked on LG3 of horsegram. The locus name, map position and distance between markers are mentioned.

S.No.	Locus	Marker Position (cM)	Distance between markers (cM)
1	GMDREB2	0	-
2	MUMST647	2.761	2.761
3	MUD12	2.761	0
4	MUMSD27	3.178	0.417
5	HUGMS52	8.575	5.397
6	MUMS71b	13.353	4.778
7	MUMS61	21.107	7.754
8	MUMSD11	21.146	0.039
9	RCS29871	22.013	0.867
10	MUMS69	35.22	13.207
11	MUMS72	37.556	2.336
12	MUMS631	38.865	1.309
13	MUMS7	39.372	0.507
14	MUMS51	40.19	0.818
15	MUMS71a	41.99	1.8
16	MUMS55	42.922	0.932
17	MUMS43	45.004	2.082
18	RCS1304	46.128	1.124
19	MUMS46	49.444	3.316
20	MUMS6	51.487	2.043
21	MUMS63	51.871	0.384
22	MUMS70	53.524	1.653
23	MUMS41	55.572	2.048
24	MUMS68	57.666	2.094
25	MUMS74	62.211	4.545
26	RCS17294	66.964	4.753
27	MUMSD26	69.805	2.841
28	MUMSD261	71.267	1.462
29	MUMST99	74.794	3.527
30	MUMS11	81.468	6.674
31	MUD95	83.238	1.77
32	RCS4342	101.445	18.207
33	MUMSD15	105.308	3.863
34	MUMS5	105.393	0.085
35	MUMSD151	129.029	23.636

(d) List of markers linked on LG4 of horsegram. The locus name, map position and distance between markers are mentioned.

S.No.	Locus	Marker Position (cM)	Distance between markers (cM)
1	MUMSD166	0	-
2	MUMST10	23.117	23.117
3	HUGMS8	25.828	2.711
4	MUGR656	53.769	27.941
5	MUGSSR17	53.769	0
6	HUGMS60	61.658	7.889
7	MUMSD691	72.765	11.107
8	MUGR661	76.018	3.253
9	MUMS10	82.385	6.367
10	RCS3537	86.857	4.472
11	MUGR646	96.576	9.719
12	MUSSR508	96.576	0
13	MUSSR550	96.576	0
14	MUMS149	96.97	0.394
15	MUGSSR238	99.46	2.49
16	MUSSR537	100.608	1.148
17	MUSSR534	112.365	11.757
18	MUGSSR243	117.599	5.234
19	MUSSR502	117.7	0.101
20	MUD30	121.503	3.803
21	MUGR519	127.85	6.347
22	MUD26	135.97	8.12
23	MUSSR501	136.976	1.006
24	MUGSSR19	141.737	4.761
25	MUGR523	150.086	8.349
26	MUMST628	154.366	4.28
27	MUGSSR211	165.442	11.076
28	RCS1171	176.04	10.598
29	MUMST3	187.974	11.934

(e) List of markers linked on LG5 of horsegram. The locus name, map position and distance between markers are mentioned.

S.No.	Locus	Marker Position (cM)	Distance between markers (cM)
1	MUMS99	0	-
2	MUMSD111	22.585	22.585
3	OPI67	26.475	3.89
4	MUSSR515	44.826	18.351
5	MUGR511	44.826	0
6	C22097	73.297	28.471

7	RCS6449	103.871	30.574
8	RCS64485	115.087	11.216
9	MUMS117	116.7	1.613
10	MUGSSR10	123.939	7.239
11	RCS6448	127.742	3.803
12	RCS64484	128.666	0.924
13	RCS64481	130.044	1.378
14	RCS64482	133.03	2.986
15	MUSSR538	147.351	14.321
16	RCS64486	153.809	6.458
17	MUMSD677	159.189	5.38
18	RCS64488	178.551	19.362
19	MUMS1	192.523	13.972

(f) List of markers linked on LG6 of horsegram. The locus name, map position and distance between markers are mentioned.

S.No.	Locus	Marker Position (cM)	Distance between markers (cM)
1	MUGR628	0	-
2	MUGSSR207	15.849	15.849
3	MUMST76	29.245	13.396
4	MUMST43	41.609	12.364
5	OPB38	44.035	2.426
6	TOG90547	55.986	11.951
7	OPB36	56.881	0.895
8	AR12765	56.881	0
9	OPB37	58.722	1.841
10	AR13876	58.939	0.217
11	MUSSR533	66.389	7.45
12	MUMSD714	67.589	1.2
13	OPI66	69.448	1.859
14	MUMST29	85.618	16.17
15	MUMS14	116.638	31.02
16	MUMSD135	141.103	24.465
17	MUD36	141.103	0
18	MUMS21	165.604	24.501

(g) List of markers linked on LG7 of horsegram. The locus name, map position and distance between markers are mentioned.

S.No.	Locus	Marker Position (cM)	Distance between markers (cM)
1	MUMST96	0	-
2	MUD77	46.713	46.713
3	HUGMS13	68.319	21.606
4	OPI61	91.392	23.073
5	MUGSSR14	108.191	16.799
6	MUMS13	108.555	0.364
7	MUMS79	108.611	0.056
8	MUMS95	110.827	2.216
9	MUMS94	111.749	0.922
10	MUMS81	113.606	1.857
11	MUMST4	116.686	3.08
12	MUD51	125.842	9.156
13	MUMSD139	125.842	0
14	MUD73	143.035	17.193
15	MUGSSR241	149.302	6.267
16	HUGMS39	161.689	12.387
17	C60793	199.603	37.914
18	HUGMS30	199.851	0.248
19	HUGMS18	238.518	38.667

(h) List of markers linked on LG8 of horsegram. The locus name, map position and distance between markers are mentioned.

S.No.	Locus	Marker Position (cM)	Distance between markers (cM)
1	MUSSR547	0	-
2	MUGR623	3.558	3.558
3	MUGR604	4.617	1.059
4	MUGR654	6.247	1.63
5	MUGR663	30.747	24.5
6	MUGR635	59.826	29.079
7	RCS5804	71.619	11.793

(i) List of markers linked on LG9 of horsegram. The locus name, map position and distance between markers are mentioned.

S.No.	Locus	Marker Position (cM)	Distance between markers (cM)
1	HUGMS3	0	-
2	MUGR607	46.36	46.36
3	MUGSSR574	46.36	0
4	MUGSSR569	46.36	0
5	MUGSSR571	46.36	0
6	MUGSSR567	46.36	0

(j) List of markers linked on LG10 of horsegram. The locus name, map position and distance between markers are mentioned.

S.No.	Locus	Marker Position (cM)	Distance between markers (cM)
1	MUD18	0	-
2	HUGMS32	61.242	61.242
3	OPB111	67.431	6.189
4	MUMS145	68.516	1.085
5	MUMS101	74.585	6.069
6	MUGSSR553	85.817	11.232
7	RC08995	94.888	9.071
8	MUMS98	99.304	4.416
9	OPO93	101.118	1.814
10	MUMST74	103.114	1.996
11	OPI64	107.371	4.257
12	OPI24	108.918	1.547
13	HUGMS14	116.192	7.274
14	MUMST52	130.705	14.513
15	HUGMS37	168.248	37.543

4.2 Identification of quantitative trait locus

The availability of genetic maps allows the localization and mapping of different agronomically important traits with the help of phenotypic data of segregating populations and identification of markers closely linked to the particular trait for marker-assisted selection and positional cloning. Early maturity and yield are related traits for enhancing crop productivity per unit area and time by harnessing high harvest index and cropping efficiency. The information on genetics of different traits is lacking in horsegram. Conventional selection of these traits is not always fruitful as these traits are influenced by the environment. Mapping of QTLs related to early maturity and yield traits can enable dissection of their genetic control and molecular mechanism which can lead to the development of early maturing varieties coupled with higher yield. This can be achieved by transferring the genes/QTLs responsible for these complex traits using marker-aided strategy. There are several reports on QTL analysis of early maturity and yield related traits in soybean (Han et al. 2012; Zhang et al. 2015), cowpea (Ubi et al. 2000; Muchero et al. 2011), common bean (Bhakta et al. 2017; Galeano et al. 2012) and pigeon pea (Kumawat et al. 2012). As morphological and yield related traits have been identified and mapped in a number of plants including the major crop species (rice, tomato, soybean, maize, barley and wheat) relatively very few loci have been mapped for early maturity and yield traits compared to other quantitative traits.

QTL studies using linkage mapping are abundant in nearly all crop species. But horsegram lack any such studies related to identification of QTLs linked to important agronomic traits due to the unavailability of molecular maps required for selecting stable QTLs for fine mapping. Horsegram has a large variation in the flowering and maturity time therefore genetic mapping of these traits has direct implications for the development of short duration high yielding horsegram varieties. Synchronous maturity play important role in shaping the plant architecture and for increasing cropping efficiency of the farming system. Yield is also an important and complex trait and many morphological characteristics and physiological processes contribute to seed yield. Early maturity with good yield is also important as early maturing variety can escape terminal drought stress than late maturing types.

Therefore, the present study was undertaken to develop linkage map of horsegram using intraspecific RILs mapping population and to identify genomic regions associated with early maturity and yield related traits. The identification of QTLs controlling agronomically important traits would enable to analyze association between the mapped loci and traits and provide the basis for horsegram genomics and breeding.

i. Analysis of morphological traits

(a) Analysis of variance

The results for the different locations and environments showed significant differences between the two parents, HPKM249 and HPK4. HPK4 has higher values for root length, root fresh weight, root dry weight, plant height, days to flowering, reproductive period, days to maturity, 100 seed weight, seed size and seed yield per plant whereas HPKM249 has more primary branches and secondary branches across two years. Also proline accumulation, chlorophyll content and relative water content of HPK4 is higher under drought stress condition indicating HPK4 as drought tolerant than HPKM249 (Table 4.6). The ANOVA of 162 RILs for the different locations and environments revealed significant differences for almost all the traits except for primary branches evaluated at Palampur in 2016 (Table 4.4 a-c).

Table 4.4 Analysis of variance (ANOVA) of the phenotypic data across multiple environments

(a) ANOVA table for Palampur 2016

S.No.	Trait	Sum of square	Mean sum of square	F value
1	Plant height	94321.7911	585.8496*	1142.95
2	Primary branches	2596.4306	16.1269	0.94
3	Secondary branches	1107.3965	6.8782*	2009.22
4	Days to 50% flowering	17358.7358	107.8182*	583.27
5	Days to maturity	52981.3354	329.0766*	314.77
6	Reproductive period	54020.9619	335.5339*	1169.81
7	100 seed weight	136.6864	0.8490*	672.59
8	Seed size	0.3263	0.0020*	12.85
9	No. of seeds per pod	247.2454	1.5357*	7.78
10	No. of pods per plant	5858.7446	36.3897*	1174.51
11	No. of seeds per plant	201138.3970	1249.3068*	15.86
12	Seed yield	288.8928	1.7944*	2536.27

(b) ANOVA table for Palampur 2017

S.No.	Trait	Sum of square	Mean sum of square	F value
1	Plant height	171612.3750	1065.9154*	13.72
2	Primary branches	1005.9120	6.2479*	3.83
3	Secondary branches	2907.1343	18.0567*	5.35
4	Days to 50% flowering	11793.7037	73.2528*	28.19
5	Days to maturity	40897.7037	254.0230*	45.43
6	Reproductive period	29634.3148	184.0641*	25.83
7	100 seed weight	47.8586	0.2973*	9.17
8	Seed size	0.2493	0.0015*	5.70
9	No. of seeds per pod	100.5000	0.6242*	3.89
10	No. of pods per plant	4836.4120	30.0398*	3.75
11	No. of seeds per plant	161891.8333	1005.5393*	3.32
12	Seed yield	258.5309	1.6058*	4.36

(c) ANOVA table for Bajaura 2017

S.No.	Trait	Sum of square	Mean sum of square	F value
1	Plant height	171612.3750	1065.9154*	13.72
2	Primary branches	1005.9120	6.2479*	3.83
3	Secondary branches	2907.1343	18.0567*	5.35
4	Days to 50% flowering	11793.7037	73.2528*	28.19
5	Days to maturity	40897.7037	254.0230*	45.43
6	Reproductive period	29634.3148	184.0641*	25.83
7	100 seed weight	47.8586	0.2973*	9.17
8	Seed size	0.2493	0.0015*	5.70
9	No. of seeds per pod	100.5000	0.6242*	3.89
10	No. of pods per plant	4836.4120	30.0398*	3.75
11	No. of seeds per plant	161891.8333	1005.5393*	3.32
12	Seed yield	258.5309	1.6058*	4.36

(b) Phenotypic trait variation

The intraspecific RILs mapping population was phenotyped for a total of 24 traits for two years at two locations. The component traits, their codes, units of measurement, locations, seasons and environments have been listed in Table 3.4. The mean value of these traits along with their frequency distribution is presented in Annexure I to V. The key features of extensive phenotyping are given and detailed analysis such as mean performance, range of trait values and SD of traits at different locations and years on RILs are provided in Table 4.5.

Morphological traits

RIL population was phenotyped for various morphological traits like plant height (PH), number of primary branches (PB), number of secondary branches (SB), growth habit (GH), growth type (GT) and pigmentation on stem (PGM). In 2016 (Palampur), PH varied from 34-98 cm while in 2017 (Palampur) it varied from 48-106 cm. Significant difference was observed for PH in 2017 at Bajaura (60-145 cm) as compared to Palampur location. Similarly, PB varied from one to six branches both in 2016 (Palampur) and 2017 (Palampur), but a significant difference was observed for PB in 2017 at Bajaura which varied from three to ten. Further, significant differences for PH and PB among RILs were observed in both seasons and locations. Similar result was observed for SB with significant differences among RILs in both years and locations (Table 4.5 a).

Phenological traits

Three phenological traits, namely days to 50% flowering (FL), reproductive period (RP) and days to maturity (MT) are important indicators of maturity and were used for phenotyping of RILs population. Phenotyping of FL showed significant genetic variability for RILs in different years and locations. HPKM249 flowered in 36 days as compared to 54 days of HPK4 during 2016 at Palampur and similar results were observed at Palampur during 2017, whereas at Bajaura during 2017, HPKM249 flowered in 32 days as compared to 57 days of HPK4. The range for days to flowering among RILs varied from 30-58 days in 2016 at Palampur, 32-52 days in 2017 at

Palampur and 31-57 days in 2017 at Bajaura location. Further, no significant difference was found among RILs in different years and locations. Similar was the trend for RP and MT with no significant difference among RILs in different years and locations (Table 4.5a).

Yield and yield related traits

RILs population was phenotyped for yield and yield related traits like 100-seed weight (SW), seed size (SZ), seeds per pod (SP), pods per plant (PP), seeds per plant (SS) and seed yield per plant (YLD) under different locations and years. Phenotyping for SW showed significant genetic variability within RILs for Palampur (2016) which varied from 2.14-5.21 g, Palampur (2017) which varied from 3.08-6.73 g and for Bajaura (2017) which varied from 2.71-4.33 g. However no significant difference was found among RILs *w.r.t.* different years and locations. Phenotyping for SS showed no significant genetic variability within RILs at Palampur during 2016 which ranged from 0.51-0.66 cm, at Palampur during 2017 which ranged from 0.54-0.67 cm and at Bajaura during 2017 which ranged from 0.51-0.62 cm. The genetic variability for SS among RILs was also found to be similar during both the years and locations. SP showed no significant difference within and among RILs in different years and locations. However PP showed high genetic variability among RILs in Palampur (2017) and Bajaura (2017), which ranged from 6.0-26.50 and 19.50-36.50, respectively. Also significant difference was observed within RILs *w.r.t.* different years and locations. SS also showed high variability among RILs in Palampur (2017) and Bajaura (2017) which ranged from 18.0-130.0 and 78-170.50, respectively. Also significant difference was observed within RILs *w.r.t.* different years and locations. Phenotyping for YLD showed significant genetic variability within RILs for Palampur location during 2016 which ranged from 0.39-5.31 g, during 2017 it ranged from 0.94-7.10 g whereas at Bajaura during 2017 it ranged from 2.64-6.32 g. (Table 4.5 a).

Table 4.5 Mean performance of parents and RILs across seasons and locations for different traits in RILs

(a) Mean performance of parents and RILs across years and locations for morphological, phenological and yield related traits

Traits	Year	Loc. ^a	HPKM249	HPK4	Range (RIL)	Mean	SD ^b
Morphological							
Plant height (PH)	2016	PLP	38	101.00	34.00-98.00	68.26	13.97
	2017	PLP	41	99.00	48.00-106.00	72.89	12.02
	2017	BJR	39	99.00	60.00-145.00	91.69	18.85
	Combined	-	39.83	99.33	52.17-116.83	78.38	13.32
Primary branches (PB)	2016	PLP	6.67	2.6	1.00-6.00	2.50	0.86
	2017	PLP	6.44	2.50	1.61-5.33	2.97	0.69
	2017	BJR	10.00	3.00	2.50-9.50	6.05	1.44
	Combined	-	7.67	2.68	1.89-5.75	3.92	0.71
Secondary branches (SB)	2016	PLP	8.00	5.2	1.80-14.00	5.04	1.51
	2017	PLP	12.20	3.60	3.73-10.73	6.48	1.37
	2017	BJR	14.00	5.00	5.00-18.00	10.85	2.45
	Combined	-	12.10	4.33	5.03-11.03	7.70	1.25
Phenological							
Days to 50% flowering (FL)	2016	PLP	36.00	54.00	30.00-58.00	41.25	5.94
	2017	PLP	36.00	54.33	32.67-52.67	41.58	4.74
	2017	BJR	32.00	57.50	31.00-57.00	40.86	4.94
	Combined	-	34.67	55.33	33.50-52.17	41.28	4.61
Reproductive period (RP)	2016	PLP	39.00	64.00	19.00-77.00	50.38	10.62
	2017	PLP	46.67	62.67	32.33-74.33	52.83	8.72
	2017	BJR	48.00	56.50	37.00-73.50	55.45	7.83
	Combined	-	45.83	60.83	34.67-73.67	53.30	7.85
Days to maturity (MT)	2016	PLP	75.00	118.00	71.00-115.00	91.64	10.43
	2017	PLP	82.67	117.00	71.67-114.00	94.41	9.70
	2017	BJR	80.00	114.00	78.50-112.50	96.31	9.20
	Combined	-	80.50	116.17	74.17-111.00	94.58	9.05

Yield and yield related traits							
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 (SS)	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
	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

^aLoc. – Location, PLP - Palampur; BJR - Bajaura; ^bStandard deviation

(b) Mean performance of parents and RILs at Palampur 2017 for biochemical and root traits in RIL (HPKM249 × HPK4) population

Traits	Env. ^a	HPKM249	HPK4	Range (RIL)	Mean	SD ^b
Biochemical						
Carotenoids	C	0.81	0.78	0.11-0.99	0.56	0.17
	S	0.58	0.65	0.06-0.99	0.48	0.21
Chlorophyll	C	11.46	12.97	4.07-24.64	13.36	5.09
	S	7.91	10.59	1.69-22.58	11.00	5.01
Malondialdehyde	C	16.84	13.47	10.24-19.78	15.22	2.27
	S	29.65	20.83	17.15-32.59	25.23	3.53
Proline	C	0.21	0.46	0.07-0.97	0.45	0.22
	S	2.03	3.43	1.14-3.65	2.20	0.59
Membrane stability index	C	0.94	1.00	0.80-1.00	0.94	0.05
	S	0.86	0.93	0.69-1.00	0.85	0.07
Relative water content	C	87.34	96.36	83.39-98.69	89.93	3.56
	S	74.44	86.58	65.34-89.58	77.68	5.00
Root traits						
Root length	-	51.00	63.00	40.00-89.00	60.75	8.26
Root fresh weight	-	0.42	1.34	0.24-6.26	2.66	1.47
Root dry weight	-	0.08	0.97	0.04-5.19	1.96	1.27

^aEnv. – Environment, C - Control; S - Stress; CC - Cylinder culture; ^bStandard deviation

Root traits

Phenotypic data of RILs population were recorded for three root traits, namely root length (RL), root fresh weight (RF) and root dry weight (RD) in polytubes during 2017 at Palampur location. The genetic variability for RL among RILs was high which ranged from 40.0-89.0 cm. The variation among RILs for RF and RD was also high which ranged from 0.24-6.26 g and 0.04-5.19 g, respectively (Table 4.5 b). High genetic variations has been reported for root and shoot traits such as stem length, stem weight, taproot length, lateral root number, total root length and total root weight for different grain legumes (Serraj et al. 2004; Kashiwagi et al. 2005; Vadez et al. 2008; Aswaf and Blair 2012).

Biochemical and physiological traits

Early maturity has been considered as an important trait for the adaption of plants to drought condition (Gaur et al. 2018). Early maturity helps the crop to escape end-of-season stresses, such as drought (Subbarao et al. 1995) and thus an important factor in increasing the yield of the crop. Biochemical analysis has long been proposed to be useful strategy for selection of stress tolerant genotypes in plant breeding (Abebe et al. 2003; Bowne et al. 2012; Mwadzingeni et al. 2016). Several biochemical and physiological traits, namely total carotenoid content (CAR), total chlorophyll content (CHL), relative water content (RWC), malondialdehyde (MDA), proline (PRO) and membrane stability index (MSI) were used for phenotyping of RIL population. The genetic variability for CAR was high in drought stress environment (0.06-0.99 mg/g) as compared to control (0.11-0.89 mg/g). Also, genetic variability for CHL ranged from 4.07-24.64 mg/g in drought stress environment and 1.69-22.58 mg/g under control condition. There was a significant decrease in the RWC of the susceptible lines as compared to tolerant RILs under drought stress environment. MDA content was increased under drought stress environment in susceptible RILs. Its range was low (10.24-19.78 nmoles/g FW) in control and high (17.15-32.59 nmoles/g FW) in drought stress environment. PRO also showed variation among RILs which ranged from 0.07-0.97 μ moles/g and 1.14-3.65 μ moles/g in control and drought stress environments, respectively (Table 4.5 b). Bhardwaj et al. (2012) found higher level of

proline, RWC and phenols in drought tolerant and higher level of MDA in drought sensitive variety of horsegram.

In general, the mean values of RILs were intermediate to that of parents. The values of RILs outside the parental range were also observed for the traits under study. This indicated that the alleles that increased phenotypic values were dispersed in both parental lines, even when their values differed markedly thus indicating transgressive segregation for the traits. Significant effect of environment was also observed for all the traits measured. The standard deviations (SDs) and coefficient of variations (CVs) indicated abundant population variation. The existence of genetic variability among the RILs for the evaluated traits justified the QTLs detection for these traits. The population distribution histogram showed continuous variation with a normal distribution of traits for 162 RILs, which is typical characteristic of quantitative traits (Annexure-VI). Therefore, this population was suitable for the construction of the genetic linkage map and detection of QTLs.

(c) Trait correlation analysis

Correlation analysis of the RILs in each specific environment and location showed that the early maturity traits were highly positive correlated to each other and were statistically significant ($P < 0.05$). Similarly the yield related traits were also found to highly positive correlated to each other and were statistically significant ($P < 0.05$). Plant height is positively correlated to days to flowering, days to maturity, reproductive period, growth habit and growth type in all environments and to the number of primary branches, number of secondary branches and seed weight in Bajaura 2017 and combined data. Number of primary branches is positively and significantly correlated to SB in all environments and to FL in all environments except in Palampur 2016. Number of secondary branches is positively and significantly correlated to FL, MT, RP and SW in Bajaura 2017 and combined data. FL and MT are positively correlated to each other and to GH and GT in all environments. YLD is positively and significantly correlated to SW, SZ, SP, PP and SS in all environments. GH and GT are positively and significantly correlated to each other and to PH, MT and RP in all environments (Fig. 4.4). Information about the correlations among traits is important for defining ideotypes for selection. Kong et al

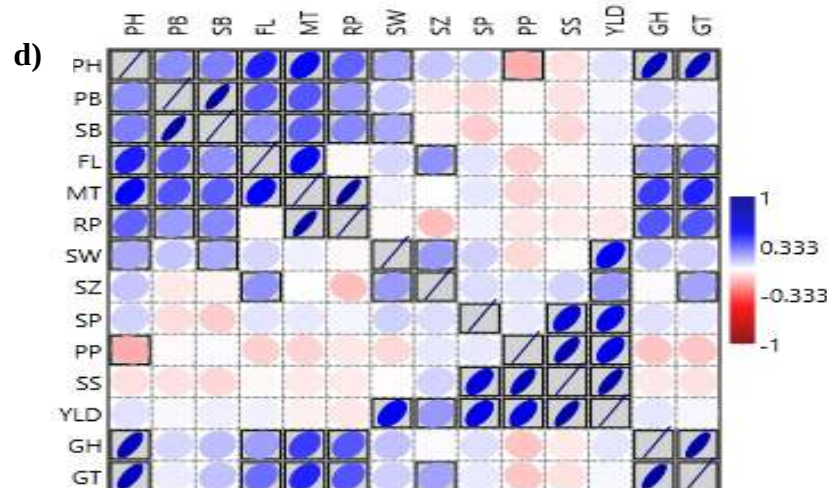
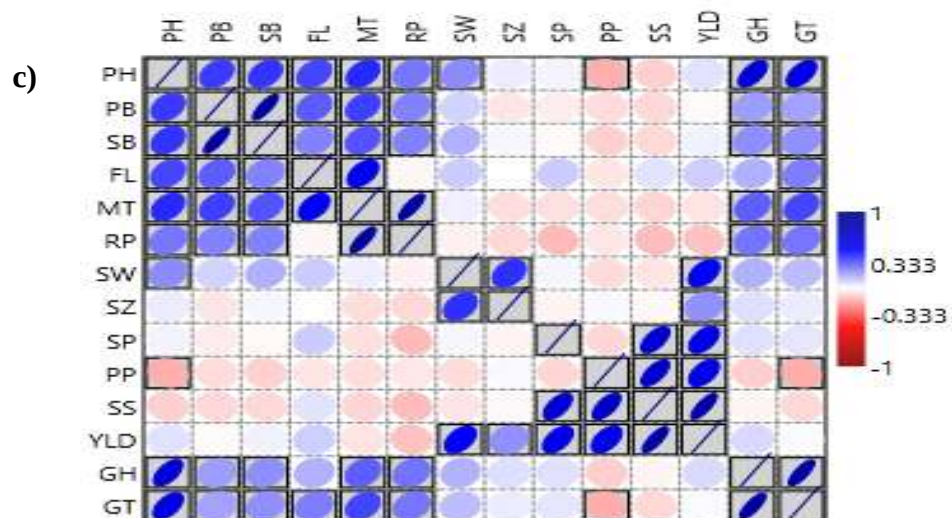
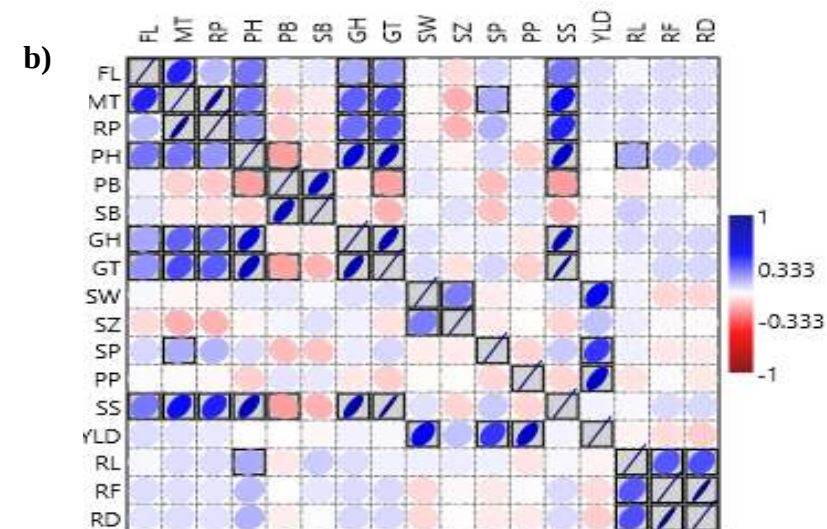
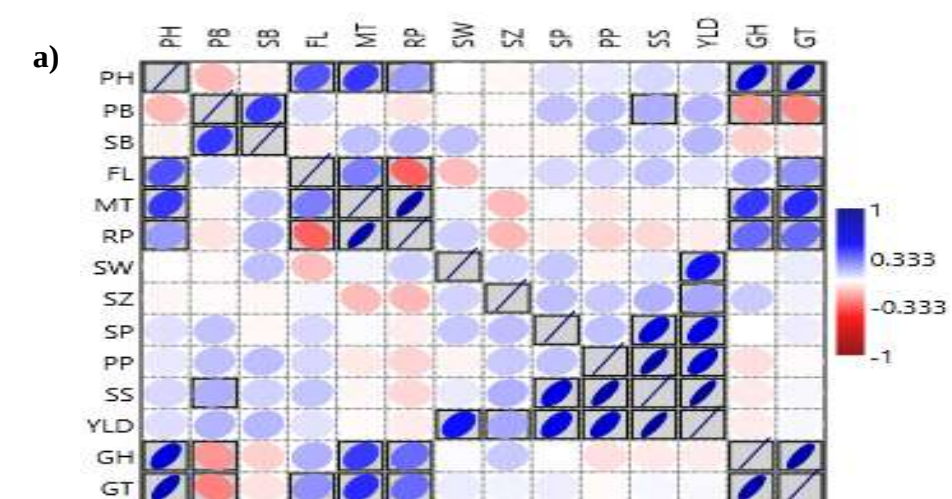


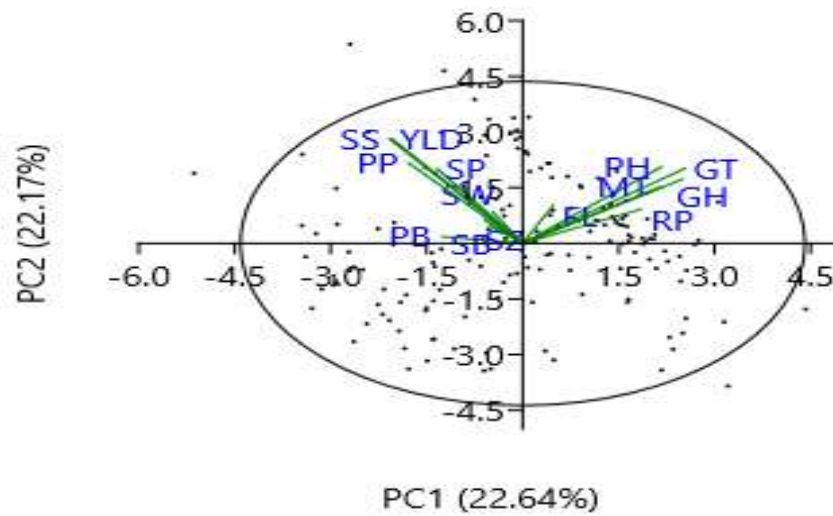
Fig. 4.4 Pearson's correlation matrix among different traits analyzed in the HPM249 $\times$ HPK4 RILs for (a) Palampur 2016 (b) Palampur 2017 (c) Bajaura 2017 (d) Combined data

(2018) suggested that maturity consists of flowering time and reproductive period and significant correlation between them is critical. Positive correlations among the components of architecture and yield would be desirable. However, negative relationship known as compensation phenomenon among yield-related traits have often been observed (Adams 1967) and can hinder progress to improve yield. His work and others (Nienhuis and Singh 1988; Scully et al. 1991) have shown good correlations between yield and pods per square meter, seeds per pod and seed weight.

(d) Principal Component Analysis

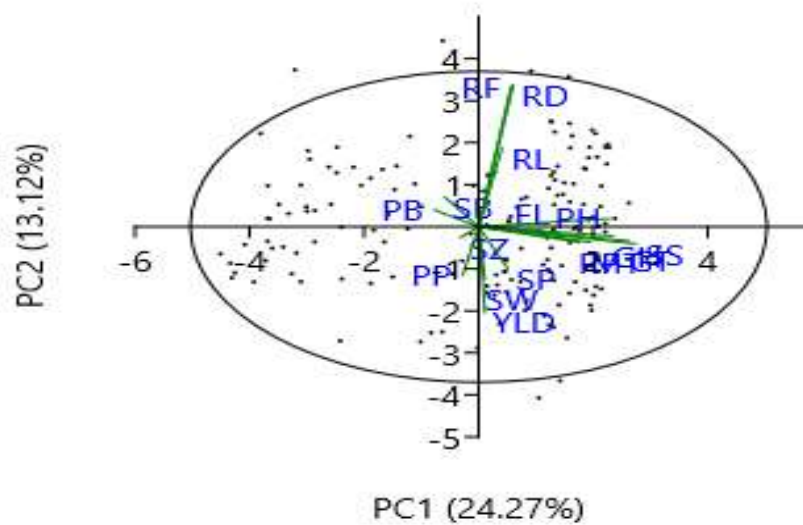
For Palampur location during 2016, the principal component analysis (PCA) of 162 RIL populations was extracted in two major principal components (eigenvalues > 1) that accounted collectively for 44.81 per cent of the variance (Fig.4.5a). Principal component 1 (PC1, X-axis, Fig. 4.5a) explained 22.64 per cent of the data set variation and was loaded positively and negatively for different measured traits. PC2 (Y-axis, Fig. 4.5a) explained 22.17 per cent of the data set variation and was positively loaded with all the measured traits. On PC 1 axis, growth type (0.70) and growth habit (0.69) had the maximum contribution towards variation in the RILs followed by plant height (0.59), days to maturity (0.59) and reproductive period (0.51). On PC 2 axis, seed yield (0.77), no. of seeds per plant (0.76) and no. of pods per plant (0.59) were the most distinctive characteristics effecting the variation among the RIL. Similarly for Palampur location during 2017, PCA extracted two major principal components (eigenvalues > 1) that accounted collectively for 37.39 per cent of the variation. Principal component 1 (PC1, X-axis, Fig. 4.5b) explained 24.27 per cent of the data set variation, and was loaded positively and negatively with different measured traits. PC2 (Y-axis, Fig. 4.5b) explained 13.12 per cent of the data set variation, and was positively and negatively loaded with different measured traits. On PC 1 axis, seed size (0.46) and growth type (0.44) had the maximum contribution towards variation in the RILs followed by growth habit (0.39), plant height (0.38) and days to maturity (0.33). On PC 2 axis, root dry weight (0.57) and root fresh weight (0.56) had the maximum contribution towards variation in the RILs followed by seed yield per plant (0.34) and root length (0.31). For Bajaura location during 2017, the PCA of 162 RIL populations extracted two major principal components (eigenvalues > 1) that

accounted collectively for 46.37 per cent of the variance. (Fig. 4.5c). Principal component 1 (PC1, X-axis, Fig. 4.5c) explained 26.54 per cent of the data set variation, and was loaded positively and negatively with different measured traits. PC2 (Y-axis, Fig. 4.5c) explained 19.83 per cent of the data set variation, and was positively and negatively loaded with different measured traits. On PC 1 axis, plant height (0.40), growth type (0.37), growth habit (0.36) had the maximum contribution towards variation in the RILs followed by number of primary and secondary branches (0.33). On PC 2 axis, seed yield (0.58), no. of seeds per plant (0.54) and no. of seeds per pod (0.39) were the most distinctive characteristics effecting the variation among the RILs followed by pods per plant (0.35) and 100-seed weight (0.21). Similarly for combined data, Principal component 1 (PC1, X-axis, Fig. 4.5d) explained 26.47 per cent of the data set variation, and was loaded positively with almost all measured traits. PC2 (Y-axis, Fig. 4.5d) explained 20.57 per cent of the data set variation, and was positively loaded with almost all the measured traits. On PC 1 axis, plant height (0.43), growth type (0.40) and growth habit (0.39) had the maximum contribution towards variation in the RILs followed by days to maturity (0.41) and reproductive period (0.32). On PC 2 axis, seed yield (0.56), no. of seeds per plant (0.55) and no. of pods per plant (0.41) were the most distinctive characteristics effecting the variation among the RILs.



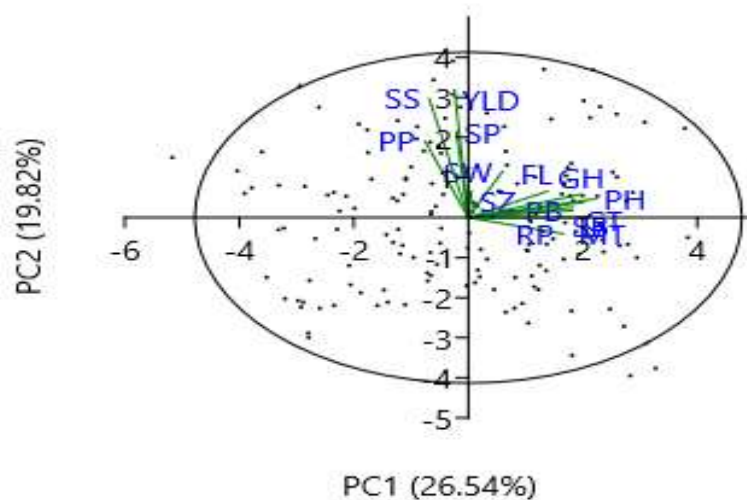
PC	1	2	3	4	5
Eigenvalue	3.1698	3.10433	1.70454	1.339	1.10221
% variance	22.641	22.174	12.175	9.5643	7.873
Cum %	22.641	44.815	56.99	66.5543	74.4273

Fig. 4.5 (a) Principal Component Analysis (PCA) of different measured traits in horsegram RIL for Palampur 2016



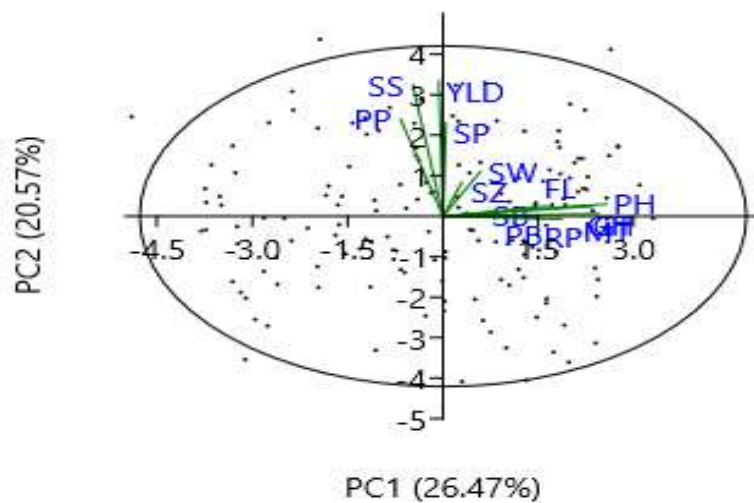
PC	1	2	3	4	5	6
Eigenvalue	4.12594	2.23002	1.90183	1.7169	1.64919	1.13201
% variance	24.27	13.118	11.187	10.099	9.7011	6.6589
Cum %	24.27	37.388	48.575	58.674	68.3751	75.034

Fig. 4.5 (b) Principal Component Analysis (PCA) of different measured traits in horsegram RIL for Palampur 2017



PC	1	2	3	4	5
Eigenvalue	3.71601	2.77654	1.62581	1.45838	1.22744
% variance	26.543	19.822	11.613	10.417	8.7674
Cum %	26.543	46.375	57.988	68.405	77.1724

Fig. 4.5 (c) Principal Component Analysis (PCA) of different measured traits in horsegram RIL for Bajaura 2017



PC	1	2	3	4	5
Eigenvalue	3.70604	2.87984	1.81742	1.46335	1.05238
% variance	26.472	20.57	12.982	10.452	7.517
Cum %	26.472	47.042	60.024	70.476	77.993

Fig. 4.5 (d) Principal Component Analysis (PCA) of different measured traits in horsegram RIL for combined data

ii. QTLs analysis and trait dissection for early maturity and yield related traits

To understand the genetics and molecular basis of early maturity and yield related traits, developed genetic map and phenotyping data generated on RIL population were analyzed in detail for identification of QTLs linked to these traits.

A total of 27 QTLs ($LOD \geq 2.5$) were detected across different environments [Palampur (2016), Palampur (2017), Bajaura (2017) and combined data] and LG 10 (Table 4.6, Fig. 4.6). Out of these 27 QTLs, 15 QTLs were major with PVE of greater than 10 per cent. Also five stable QTLs were found which were associated with combined data of traits across different years and locations. In total, 3 QTLs were detected for biochemical and physiological traits, 4 for root traits, 7 for morphological traits, 4 for phenological traits, and 9 for yield related traits. In case, flanking markers were common in more than one QTL, that region was considered as only one genomic region. By following this criterion, 27 QTLs identified were present in 18 genomic regions. Of the 27 QTLs detected, nearly 23 per cent were located on LG7 harbouring 6 QTLs followed by LG4 and LG9 harbouring 4 QTLs each.

Trait dissection

LG1 contained a total of 4 QTLs (one each for plant height and pods per plant, while two for seeds per plant); LG2 contained 3 QTLs (one each for MDA(C), days to 50% flowering and pods per plant); LG3 contained only 1 QTL (for root length); LG4 had a total of 3 QTLs (one each for Chlorophyll (S), Proline (S) and seed size); LG5 contained a total of 3 QTLs (one each for root dry weight, root fresh weight and reproductive period); LG6 contained 3 QTLs (two for number of primary branches and one for no of seeds per plant); LG7 contained 6 QTLs (three for secondary branches, two for seed yield and one for days to maturity) and LG9 contained 4 QTLs (one each for root length, primary branches, days to maturity and seed size) (Fig. 4.6). This uneven distribution of QTLs across LGs is in agreement with Zhang et al. (2015) in soybean, Pottorff et al. (2014) in cowpea, Leite et al. (2011) in common bean and Fratini et al. (2007) in lentil.

Comprehensive QTLs analysis provided an opportunity to analyze early maturity and yield related traits in depth. As QTLs analysis was undertaken based on phenotypic data for 24 traits, collected during two years (2016 and 2017) at two locations (Palampur and Bajaura). Phenotypic variation explained (PVE) by QTLs ranged from 6.4 to 53.4 per cent (Table 4.6). The highest phenotypic variation (53.4 %) was explained by the QTLs for root length.

Table 4.6 QTLs for various early maturity and yield related traits identified using QTL Cartographer

RIL (HPKM249 × HPK4)									
Trait	Year	Loc. ^a	Env. ^b	L G	QTL Name	Marker interval	LOD Score	Additive effect ^c	PVE (R ² %) ^d
Biochemical									
Chlorophyll	2017	PLP	S	4	<i>qCHL01</i>	MUSSR501- MUGR523	2.6	-1.54	8.5
Malondialdehyde	2017	PLP	C	2	<i>qMDA01</i>	MUMST80- OPI22	3.9	0.74	9.9
	2017	PLP	S	4	<i>qPRO01</i>	MUMST628-MUGSSR211	3.4	-0.22	12.1
Proline									
Root									
Root dry weight	2017	PLP	CC	5	<i>qRD01</i>	RCS64486-MUMSD677	2.7	0.41	10
Root fresh weight	2017	PLP	CC	5	<i>qRF01</i>	RCS64486-MUMSD677	4.7	0.61	15.8
Root length	2017	PLP	CC	3	<i>qRL01</i>	MUMS41-MUMS68	3.3	-2.57	8.3
	2017	PLP	CC	9	<i>qRL02</i>	HUGMS3-MUGR607	5.0	6.60	53.4
Morphological									
Plant height	2016- 2017	COMBINE D	-	1	<i>qPHT01</i>	RCS6168-RCS6169	2.7	3.96	6.6
Primary branches	2017	PLP	-	6	<i>qPB02</i>	OPI66-MUMST29	4.2	0.37	22.0
	2017	PLP	-	9	<i>qPB01</i>	HUGMS3-MUGR607	5.4	-0.63	32.4
	2016- 2017	COMBINE D	-	6	<i>qPB03</i>	OPI66-MUMST29	3.8	0.34	17.0
Secondary branches	2017	BJR	-	7	<i>qSB01</i>	MUD77-HUGMS13	4.9	1.21	23.6
	2017	BJR	-	7	<i>qSB02</i>	MUMS13-MUMS95	3.3	-0.75	7.5
	2016- 2017	COMBINE D	-	7	<i>qSB03</i>	MUD77-HUGMS13	3.7	0.50	15.5

Phenological									
Days to 50% flowering	2016-2017	COMBINE D	-	2	<i>qFL01</i>	MUGR644-MUMST80	2.8	1.19	6.62
Reproductive Period	2017	BJR	-	5	<i>qRP01</i>	MUGSSR10-RCS6448	2.7	3.87	6.36
Days to Maturity	2016	PLP	-	7	<i>qMT01</i>	MUGSSR241-HUGMS39	2.6	2.86	7.25
	2017	PLP	-	9	<i>qMT02</i>	HUGMS3-MUGR607	2.9	7.82	47.53
Yield and yield related traits									
Seed Size	2017	BJR	-	4	<i>qSZ01</i>	MUSSR501-MUGSSR19	3.4	-0.01	15.07
	2016-2017	COMBINE D	-	9	<i>qSZ02</i>	HUGMS3-MUGR607	4.1	0.01	20.16
Seeds per plant	2016	PLP	-	6	<i>qSS01</i>	AR12765-OPB37	3.0	-11.75	7.3
	2017	PLP	-	1	<i>qSS02</i>	RCS20321-HUGMS53	4.5	8.06	10.8
	2017	PLP	-	1	<i>qSS03</i>	MUSSR539-MUGSSR16	2.9	6.23	6.6
Pods per plant	2017	PLP	-	1	<i>qPP01</i>	RCS20321-HUGMS53	6.2	1.75	14.36
	2017	BJR	-	2	<i>qPP02</i>	OPR104-MUSSR549	4.0	1.31	8.97
Yield	2016	PLP	-	7	<i>qYLD01</i>	MUD77-HUGMS13	2.50	5.4	16.47
	2016	PLP	-	7	<i>qYLD02</i>	OPI61-MUGSSR14	2.54	5.3	12.15

^aLoc. – Location, PLP - Palampur; BJR - Bajaura; ^bEnv. – Environment, C - Control; S - Stress; CC - Cylinder culture; ^cThe estimated additive effect; ^dPhenotypic variation explained

(a) Biochemical and Physiological traits

Of the four biochemical traits analyzed, QTLs were identified for three traits viz. one for chlorophyll content under drought stress, one for malondialdehyde content for control and one for proline content under drought stress. Thus, three QTLs were detected for biochemical traits using composite interval mapping (Table 4.6 and Fig. 4.6). One drought specific QTL for chlorophyll content was present on LG4 with phenotypic variation explained of 8.5 per cent at LOD value of 2.6 flanked by MUSSR 501-MUGR 523 marker interval. Similarly, one drought specific QTL was also detected for proline content at LOD of 3.4 with 12.1 per cent of phenotypic variation explained. This QTL was also present on LG4 flanked by MUMST628-MUGSSR211 marker interval. One QTL for malondialdehyde content was present on LG3 with 9.9 per cent of phenotypic variation explained at LOD 3.4 flanked by MUMST80- OPI22 marker interval. Additive effect demonstrated that HPK4 contributed alleles for chlorophyll, proline and HPKM249 contributed alleles for malondialdehyde content. Position of QTLs for different biochemical traits on ten linkage groups of horsegram were shown in Fig. 4.7a.

(b) Root traits

Of the three root traits analyzed, QTLs were identified for all three traits, one for root dry weight and root fresh weight and two for root length (Table 4.6, Fig. 4.6). QTL for root dry weight was present on LG5 with 10 per cent of phenotypic variation explained at LOD value of 2.7, flanked by RCS64486-MUMSD677 marker interval while QTL for root fresh weight was also present on LG5 with 15.8 per cent of phenotypic variation explained at the LOD value of 4.7, also flanked by RCS64486-MUMSD677 marker interval. Both these QTLs were contributed by the alleles from the parent HPKM249 which resulted in increased root dry weight and root fresh weight by 0.41g and 0.61g, respectively. Two QTLs were detected for root length at LOD of 3.3-5.0 with 8.3-53.4 per cent of phenotypic variation. These QTLs were present on LG3 and LG9 flanked by MUMS41-MUMS68 and HUGMS3-MUGR607 marker interval, respectively. Further, additive effect demonstrated that allelic contribution is by both the parents resulted in increased root length by 2.57cm (qRL01) and 6.60cm (qRL02). Position of QTLs for different root traits on 10 linkage groups of horsegram (HPKM249 × HPK4) were shown in Fig. 4.7b.

(c) Morphological traits

Of the six morphological traits analyzed, 7 QTLs (one for plant height and three each for primary branches and secondary branches) with up to 32.4 per cent PVE were identified (Table 4.6, Fig. 4.6). QTL for plant height was present on LG1 with 6.6 per cent of phenotypic variation explained at LOD value of 2.7 flanked by RCS6168-RCS6169 marker interval. This QTL had an additive effect of 3.96cm and contributed by the allele from HPKM249. A total of three QTLs were detected for primary branches with two on LG6 both flanked by OPI66-MUMST29 marker interval and one on LG9 flanked by HUGMS3-MUGR607 marker interval with a LOD score range of 3.8-5.4, explaining 17.0-32.4 per cent of the phenotypic variation (Table 4.6). These QTLs in combination explained 71.4 per cent of total phenotypic variation for primary branches. Additive effect demonstrated allelic contribution from both the parents. Further, total of three QTLs were detected for secondary branches all on LG7, two of which were flanked by MUD77-HUGMS13 marker interval and one flanked by MUMS13-MUMS95 marker interval with a LOD score range of 3.3-4.9, explaining 7.5-23.6 per cent of total phenotypic variation. Additive effect demonstrated allelic contribution from both the parents. Position of QTLs for different morphological traits on 10 linkage groups of horsegram (HPKM249 × HPK4) was shown in Fig 4.7c.

(d) Phenological traits

A total of four QTLs (one for days to 50 per cent flowering, one for reproductive period and two for days to maturity) with up to 47.53 per cent of PVE were detected during the study (Table 4.6, Fig. 4.6). One QTL for days to 50 per cent flowering was detected on LG3 and with a LOD score of 2.8, explaining 6.62 per cent of the phenotypic variation with allelic contribution by HPKM249 resulted in reduced flowering time by about >2 days. Quantitative trait loci showing reduced days to flowering has been reported in pea by Pilet-Nayel et al. (2002), common bean (Tarán et al. 2002) and in lentil (Tullu et al. 2008) and thus could be desirable for marker-assisted selection programmes (Veldhoom and Lee 1996; Pilet-Nayel et al. 2002; Kahraman et al. 2004). One QTL for reproductive period was detected on LG5 flanked by MUGSSR10-RCS6448 with a LOD score range of 2.7, explaining 6.36 per cent of the phenotypic variation (Table 4.6). Similarly, for days to maturity a total of

two QTLs were detected with one on LG7 flanked by MUGSSR241-HUGMS39 marker interval and one on LG9 flanked by HUGMS3-MUGR607 marker interval with a LOD score range of 2.6-2.9, explaining 7.25-47.53 per cent of the phenotypic variation (Table 4.6). Additive effect demonstrated that HPKM249 contributed alleles for reproductive period and days to maturity with QTL named *qMT01* resulted in reduced days to maturity by >3d and *qMT02* resulted in reduced days to maturity by >8d (Table 4.6). Position of QTLs for different phonological traits on 10 linkage groups of horsegram (HPKM249 × HPK4) were shown in Fig. 4.7d.

(e) Yield and yield related traits

The QTL analysis of six yield-related traits detected a total of nine QTLs (two each for seed size, pods per plant and seed yield per plant and three for seeds per plant) which explained up to 20.16 per cent PVE (Table 4.6, Fig. 4.6). A total of two QTLs were detected for seed size with one each on LG4 and LG9 flanked by MUSSR501-MUGSSR19 and HUGMS3-MUGR607 marker interval with LOD score ranging from 3.4-4.1, explaining 15.07-20.16 per cent of the phenotypic variation. These QTLs in combination explained almost 35.23 per cent of phenotypic variation for seed size. Similarly, three QTLs were detected for seeds per plant with one on LG6 flanked by AR12765-OPB37 marker interval and two on LG1 flanked by RCS20321-HUGMS53 and MUSSR539-MUGSSR16 marker interval respectively, with a LOD score range of 2.9-4.5, explaining 6.6-10.8 per cent of the phenotypic variation. These QTLs in combination explained almost 24.7 per cent of the total phenotypic variation for seeds per plant. Further, for pods per plant two QTLs were detected with one on LG1 flanked by RCS20321-HUGMS53 marker interval and one on LG2 flanked by OPR104-MUSSR549 marker interval with a LOD score range of 4.0-6.2, explaining 8.9-14.36 per cent of the phenotypic variation. These QTLs in combination explained 23.26 per cent of total phenotypic variation for pods per plant.. Finally, for seed yield per plant two QTLs were detected on LG7 flanked by MUD77-HUGMS13 and OPI61-MUGSSR14 marker interval with a LOD score 2.5, explaining 12.15-16.47 per cent of the phenotypic variation. These QTLs in combination explained 28.62 per cent of total phenotypic variation for yield. Position of QTLs for different biochemical traits on 10 linkage groups of horsegram (HPKM249×HPK4) were shown in Fig 4.7e.

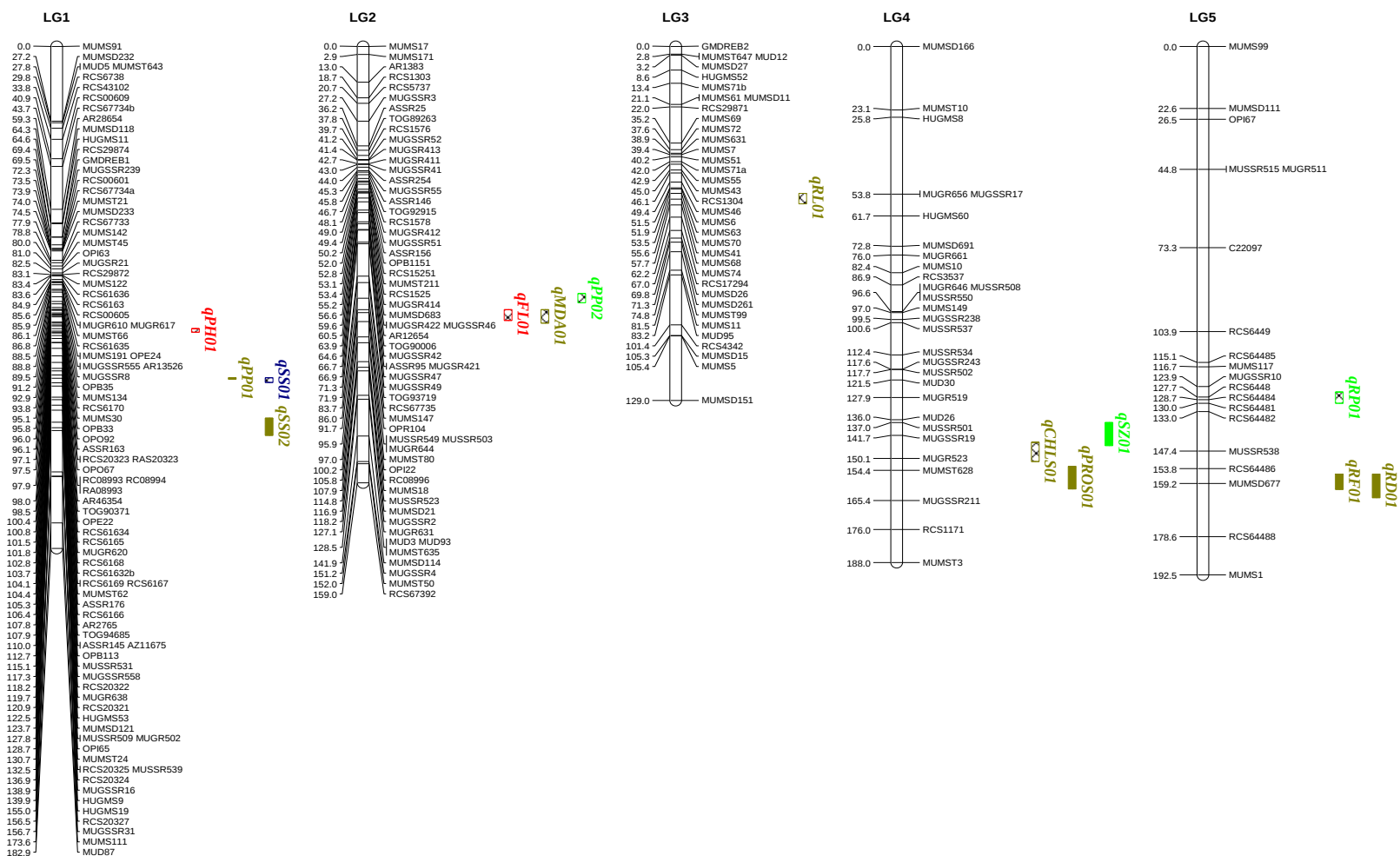


Fig. 4.6 Likelihood intervals for quantitative trait loci (QTLs) associated with early maturity and yield related traits in recombinant inbred lines (RILs) mapping population.

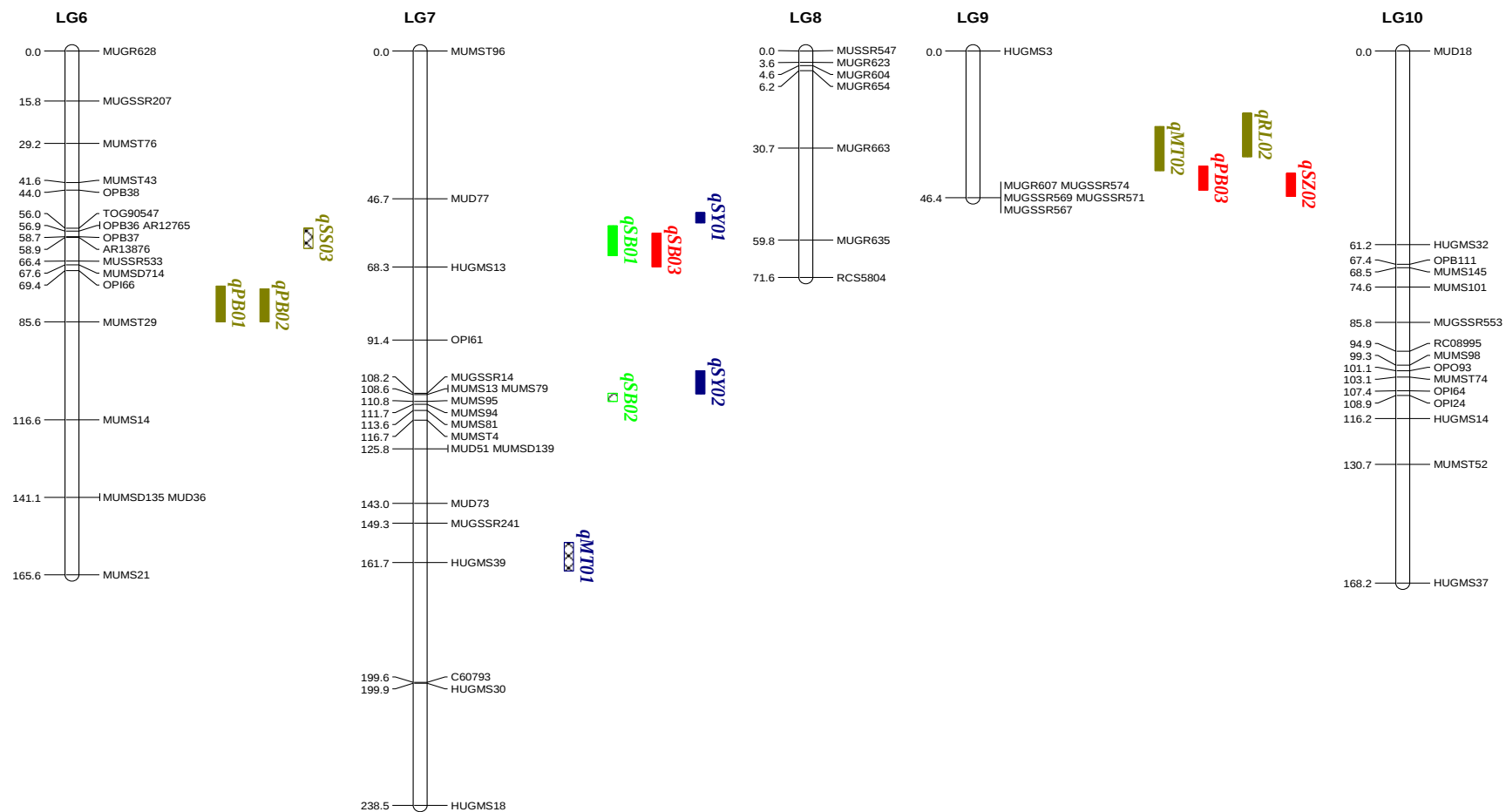


Fig. 4.6 Likelihood intervals for quantitative trait loci (QTLs) associated with early maturity and yield related traits in recombinant inbred lines (RILs) mapping population.

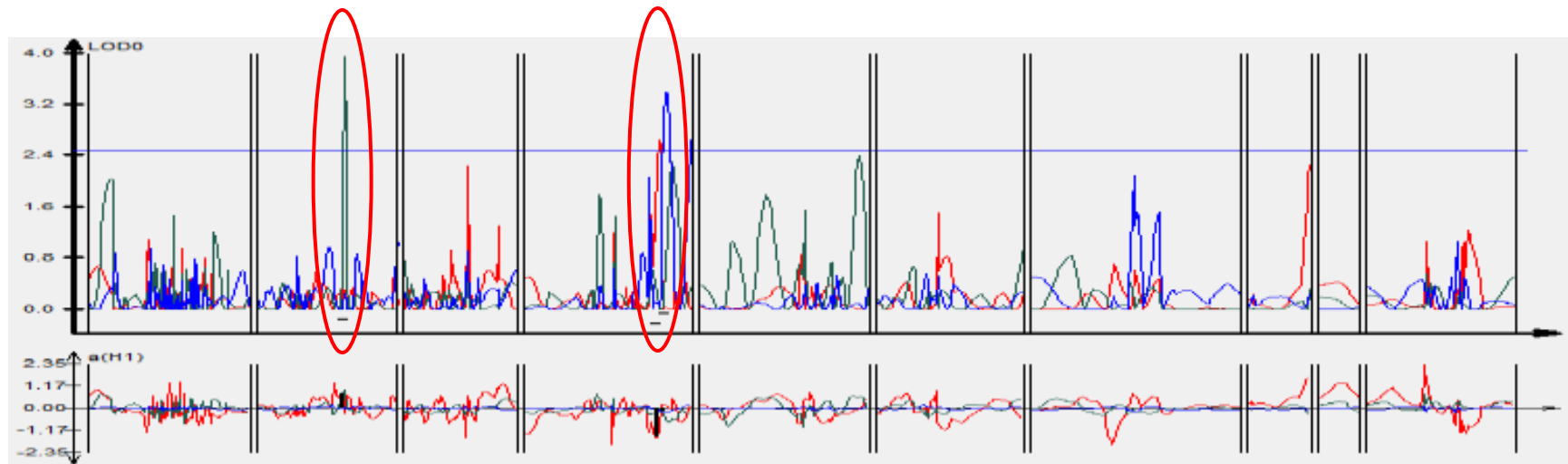


Fig 4.7(a) Position of QTLs for biochemical traits on 10 linkage groups of horsegram

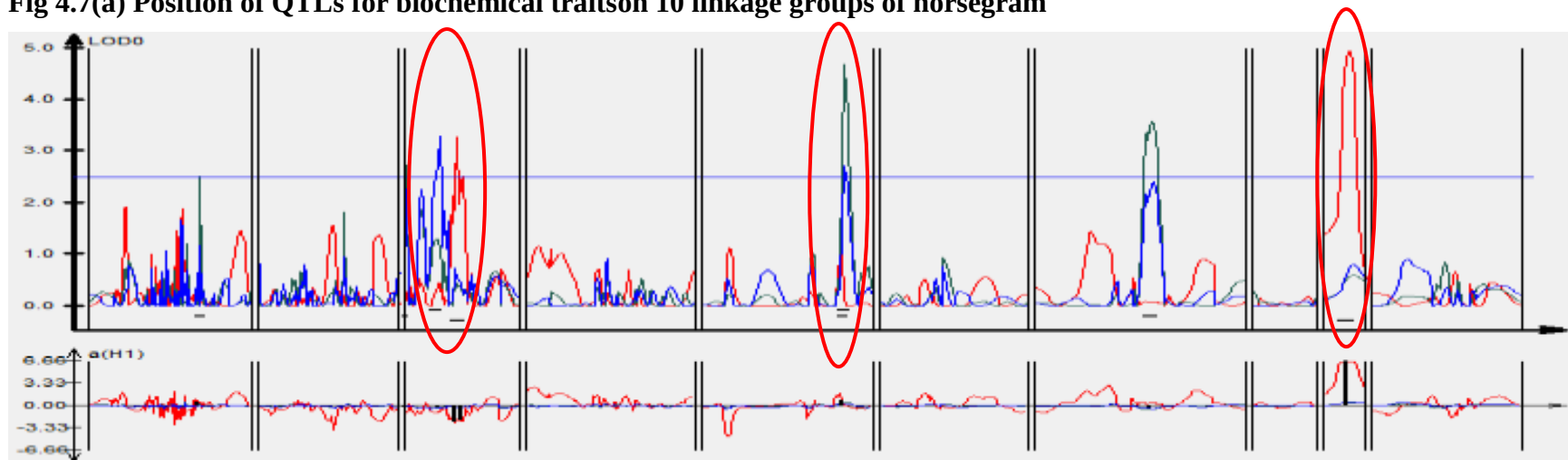


Fig 4.7 (b) Position of QTLs for root traits on 10 linkage groups of horsegram

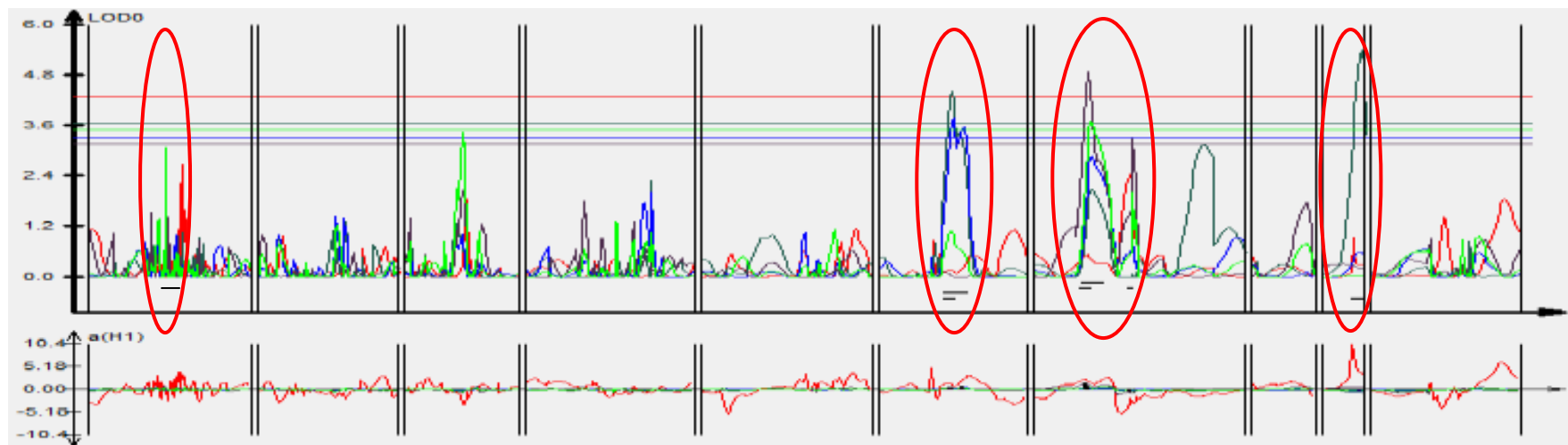


Fig 4.7 (c) Position of QTLs for morphological traits of on 10 linkage groups of horsegram

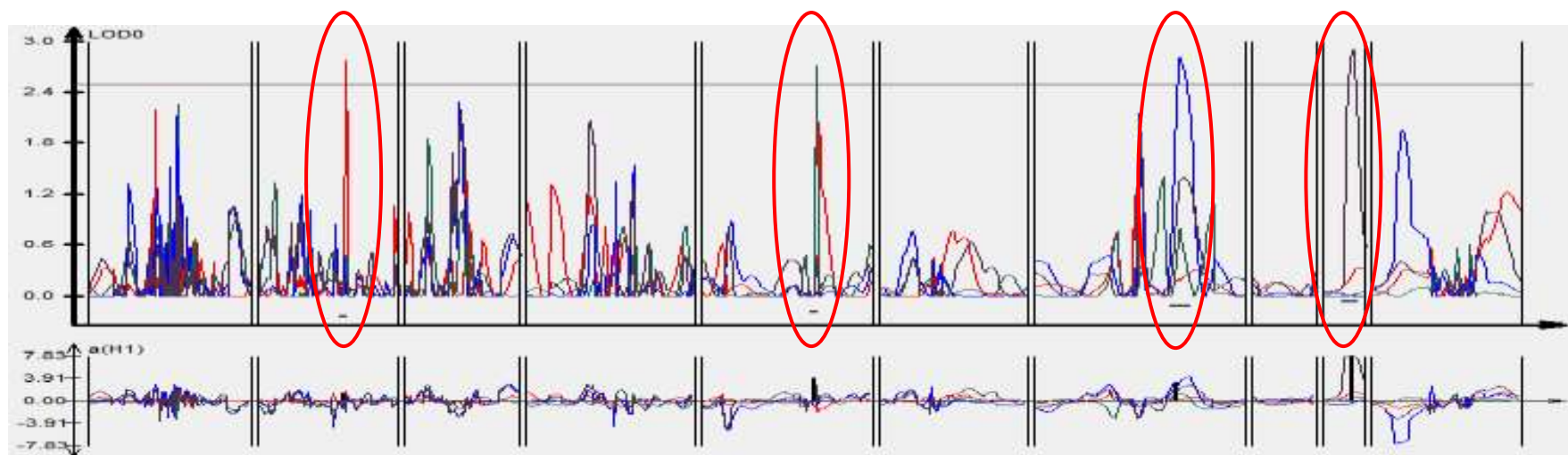


Fig 4.7 (d) Position of QTLs for phenological traits of on 10 linkage groups of horsegram

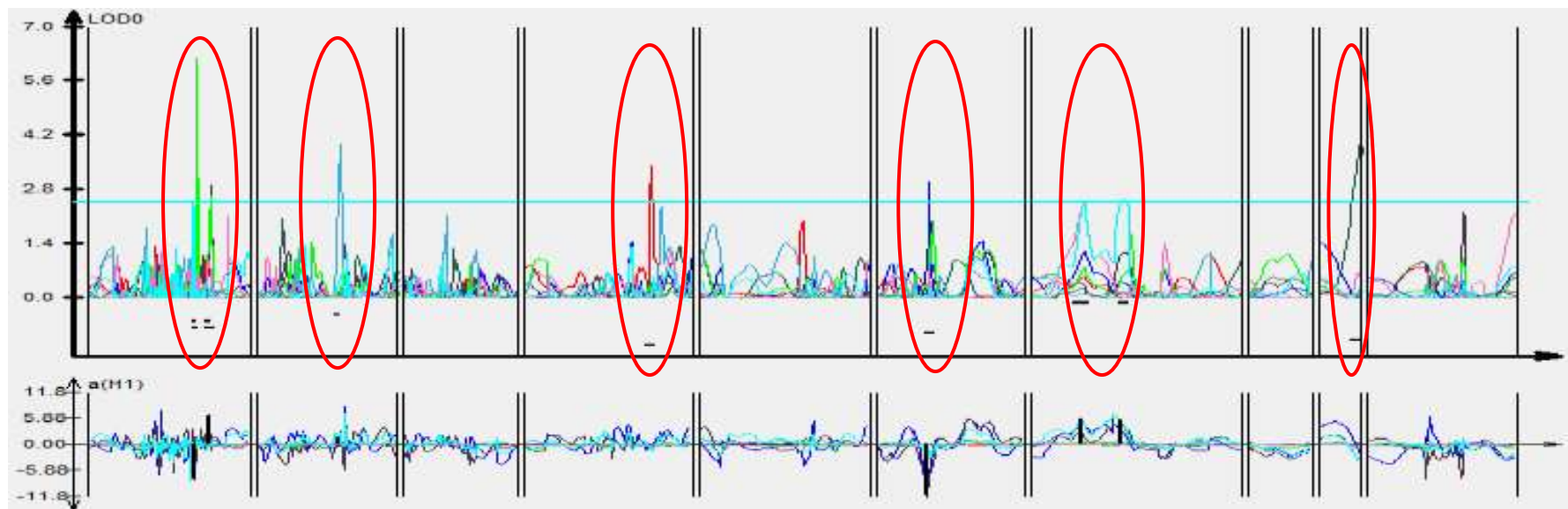


Fig 4.7 (e) Position of QTLs for yield related traits of on 10 linkage groups of horsegram

Figure 4.7 Position of QTLs for (a) biochemical traits (b) root traits (c) morphological traits (d) phenological traits (e) yield related traits on ten linkage groups developed from 295 PCR-based markers on 162 F₈ RILs

QTL analysis in genetically fixed population e.g. recombinant inbred lines, facilitates the dissection of the genetic basis of early maturity and yield related traits. Successful marker identification would facilitate integration of MAS procedures in breeding programs enabling the pyramiding of favourable alleles and target loci. The development of a dense linkage map for horsegram containing a large number of molecular markers is required in order to identify more genomic regions and to effectively use the identified markers in marker-assisted breeding programmes.

iii. Candidate genomic regions for molecular breeding

In our study, we identified total of 27 QTLs associated with different traits. 15 QTLs were major QTLs with PVE greater than 10 per cent. Also 5 stable QTLs were identified which were associated with combined data of different years and locations. Summary of QTLs identified for different traits in horsegram were shown in Table 4.7.

In any breeding program, the traits to be considered as potential selection targets for early maturity must be genetically correlated with yield. Genomic regions containing QTLs for several traits are much valued by breeders. In this context, the detected QTLs were analyzed and considered QTL cluster/co-localized QTLs if they represent for more than two traits. Overall, five QTL clusters were identified. Among these, QTL Cluster 1 was located on LG1; QTL Cluster 2 was located on LG2; QTL Cluster 3 was located on LG4; QTL Cluster 4 was located on LG7 and QTL Cluster 5 was located on LG9.

QTL Cluster 1 located on LG1 contained QTLs for yield related traits i.e one for pods per plant (14.37% PVE) and two for seeds per plant (10.80% and 6.65% PVE). Overall, the region harbored 3 QTLs with high PVE (6.65-14.37%) for two different traits therefore introgression of this region will increase yield in horsegram. Similarly, QTL Cluster 2 located on LG2 contained genetic loci for biochemical traits (MDA 9.94% PVE), yield related trait (PP 8.96% PVE) and phenological trait (FL 6.62% PVE). Overall, this region harbored 3 QTLs for three different traits that contributed to 6.62-9.94% PVE. Therefore, introgression of this region will also

improve early maturity and horsegram yield. Onziga et al (2019) also found QTL cluster for maturity and yield thus demonstrating the significant effect of these QTL on phenology and seed yield in common bean. Similarly, QTL Cluster 3 with three QTLs present on LG4 also appears to be an interesting target for molecular breeding as it contained two QTLs for biochemical traits (PRO 12.14% PVE; CHL 8.50% PVE) and yield related trait (SZ 15.07% PVE). Hence introgression of this region will not only improve the component traits but also likely to increase yield with high PVE.

Table 4.7 Summary of QTLs identified for early maturity and yield related traits in horsegram

Traits	Total QTLs	Environments				PVE (%)
		Palampur 2016	Palampur 2017	Bajaura 2017	Stable QTLs	
Biochemical						
Chlorophyll (Stress)	1	-	1	-	-	8.49
Malondialdehyde (Control)	1	-	1	-	-	9.94
Proline (Stress)	1	-	1	-	-	12.13
Root						
Root dry weight	1	-	1	-	-	9.97
Root fresh weight	1	-	1	-	-	15.85
Root length	2	-	2	-	-	8.34-53.42
Morphological						
Plant height	1	-	-	-	1	6.60
Primary branches	3	-	2	-	1	17.00-22.00
Secondary branches	3	-	-	2	1	7.50-23.60
Phenological						
Days to 50% flowering	1	-	-	-	1	6.62
Reproductive Period	1	-	-	1	-	6.36
Days to Maturity	2	1	1	-	-	7.25-47.53
Yield and yield related traits						
Seed Size	2	-	-	1	1	15.07-20.16
Seeds per plant	3	1	2	-	-	6.60-10.80
Pods per plant	2		1	1	-	8.97-14.36
Yield	2	2	-	-	-	12.15-16.47
Total	27	4	13	5	5	

Further, QTL Cluster 4 with three QTLs present on LG7 contained one QTL for yield trait (YLD 16.47% PVE) and two QTLs for morphological trait (SB 23.65% and 15.55% PVE). Overall, this region harboured 3 QTLs for two different traits explaining 15.55-23.65 % phenotypic variation. Similarly, QTL Cluster 5 present on LG9 contained a total of four QTLs with QTL for different traits. It contained one QTL for morphological trait (PB 32.40% PVE), one QTL for yield related trait (SS 20.24% PVE), one QTL for phenological trait (MT 47.53% PVE) and one QTL for root trait (RL 53.42% PVE). Interestingly, this region contained maximum QTLs with higher PVE. Therefore, this region seems to be of utmost importance for introgression in elite varieties for improving early maturity, yield and other component traits.

Earliness is an adaptive trait and is one of the major factors of agronomic variation. Development of early maturing lines with optimum DTF combined with high and stable yield is a major breeding goal in horsegram research. The term “earliness genes” was first used by Ford et al. (1981) and it was proposed to be different from genes controlling photoperiod response in wheat (*Triticum aestivum* L.). In this study, all the QTLs detected were found to be clustered across maximum linkage groups. Clustering of QTLs for various agronomic traits has been reported in many agriculturally important crops like sorghum (Lin et al. 1995), common bean (Blair et al. 2006), wheat (Quarrie et al. 2006), cotton (Qin et al. 2008), soybean (Xu et al. 2011), rice (Wang et al. 2012) etc. QTL clusters having more than one traits may have multiple effects on each other as they belong to the same genomic regions, like in this study QTLs for several traits were identified on a very small region on linkage groups. These QTLs clustered for several traits revealed that these regions were directly associated with grain yield. The clustering of QTLs can arise due to pleiotropic effect of a single regulatory gene (Aastveit and Aastveit 1993). The occurrence of pleiotropy could be explained in a way that certain traits are phenotypically correlated with each other due to the presence of certain genes coexisting in these QTLs. Fine mapping of these identified QTLs is the next step to understand whether linkage or pleiotropic effects are responsible for their clustering. As molecular markers are still limited in horsegram, construction of second generation 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.

Overall, the linkage maps are extremely useful for plant breeding and crop improvement programs, wherein they have profound applications. Evidently the map generated in the present study although covered a significant portion of the genome, a further saturation of this map with additional markers (SSRs or SNPs) is imperative for its efficient utilization. With the long-term goal of understanding the genetic basis of early maturity and yield related traits, the present study was focused on identification of major QTLs for 24 traits in horsegram. In conclusion, it is envisaged that the present linkage map, fortified with 295 SSR and RAPD markers and 27 QTLs for early maturity, drought tolerance and yield-related traits would provide a means to breeders for further genetic enhancement of the crop species. However, as discussed earlier a denser genetic linkage maps with large number of markers by the inclusion of SNPs would facilitate the identification of more resolved and fine QTL positions which can significantly improve the resolution of identified QTLs for mapping. The knowledge of marker-trait association may also lead to the identification of genes influencing agronomic traits

5. SUMMARY AND CONCLUSIONS

The present investigation entitled, “Identification of QTLs linked to early maturity and yield-related traits in horsegram (*Macrotyloma uniflorum*)” was undertaken to identify markers for construction of intraspecific map and to identify genomic regions linked with early maturity and yield traits in horsegram.

The study was carried out using recombinant inbred lines (RILs) population of 162 individuals derived from an intraspecific cross of horsegram HPKM249 X HPK4 using single seed descent method for the advancement of generations from F₂ to F₈. The RIL population was phenotyped for 24 early maturity, drought tolerance and yield-related traits in two locations at Palampur and Bajaura for two consecutive years (2016 and 2017). Field phenotyping were carried out in Augmented Block Design (ABD) with four checks namely; VLG-1, HKP-4, HPKM249 and HPKM317 using two replications and plot size of one meter long having row to row distance of 30cm and plant to plant distance of 5-6cm. Polytube experiments were also carried out for measurement of root traits and for biochemical traits in control and drought stress condition.

For construction of linkage map JOINMAP® 4.1 program (van Ooijen 2006) was used. To identify linkage groups, grouping of markers were done using the minimum independence LOD threshold of 3.0 and a maximum of 8.0 with a step up of 0.5. The groups showing maximum number of markers and highest linkage at the variable LODs were selected. QTLs were detected with the Windows QTL Cartographer V2.5 software (Wang et al. 2005) by composite interval mapping (CIM) method (Zeng 1993; 1994) using the Zmapqtl standard model 6 with a window size of 10 cM and a 2 cM walk speed. An LOD threshold score of ≥ 2.5 at 1000 permutations were significantly considered (5% level of significance) to identify and to map the QTLs on the horsegram LGs.

A linkage map was constructed using different sets of PCR based markers. A total of 2011 PCR based markers [[63 (Horsegram EST SSRs) + 403 (Horsegram

genic SSRs) + 387 (Horsegram genomic SSRs) + 24 (drought specific SSRs) + 300 (SSRs from other legumes viz. red clover and *Medicago*) + 450 (RAPD) + 384 (COS)] were used to identify polymorphic primers between HPKM249 and HPK4, the parental lines of the mapping population. Of the 2011 primer pairs, 493 (24.52%) primer pairs were found polymorphic and were further used for genotyping of 162 individuals of the RILs mapping population for map construction. A total of 295 markers were assigned map positions at LOD 3.5 on ten linkage groups, spanning 1541.7 cM distance of the horsegram genome with an average marker density of 5.2 cM. These markers exhibited a non-random distribution varying in density from 2.0 cM/locus to 12.5 cM/locus on ten LGs.

Further, the linkage map constructed was used for the identification of QTLs related to early maturity and yield related traits. The results for the different locations and environments showed significant differences between the two parents, HPKM249 and HPK4. Except primary branches and secondary branches, the parent, HPK4, had higher values in root length, root fresh weight, root dry weight, plant height, days to flowering, reproductive period, days to maturity, 100 seed weight, seed size and yield than those of HPKM249 across two years. Also proline accumulation, chlorophyll content and relative water content of HPK4 is higher under drought stress condition indicating HPK4 is drought tolerant than HPKM249. Analysis of variance revealed significant differences for all the 24 measured traits between the early maturing 'HPKM249' and the late maturing cultivar 'HPK4'. The ANOVA of 162 RILs for the different locations and environments revealed significant differences for almost all the traits except for primary branches evaluated at Palampur in 2016. The phenotypic value correlation analysis of the RILs in each specific environment across the year showed that the early maturity traits and yield related traits were positively correlated to each other.

The PCA of 162 RIL populations for extracted two major principal components (eigen values > 1) that accounted collectively for 44.81, 37.39, 46.37 and 47.04 per cent of the variance for the Palampur 2016, Palampur 2017, Bajaura 2017

trials and combined data, respectively. On PC 1 axis for Palampur 2016, growth habit (0.70) and growth type (0.69) had the maximum contribution towards variation in the RILs followed plant height (0.59), days to maturity (0.59) and reproductive period (0.51). Similarly, On PC 1 axis for Palampur 2017, seed size (0.46) and growth type (0.44) had the maximum contribution towards variation in the RILs followed by growth habit (0.39), plant height (0.38) and days to maturity (0.33). Similarly for Bajaura 2017, on PC 1 axis, plant height (0.40), growth type (0.37), growth habit (0.36) had the maximum contribution towards variation in the RILs followed by number of primary and secondary branches (0.33). Also for combined data, on PC 1 axis, plant height (0.43), growth type (0.40) and growth habit (0.39) had the maximum contribution towards variation in the RILs followed by days to maturity (0.41) and reproductive period (0.32).

A total of 27 QTLs ($LOD \geq 2.5$) were detected across the three environments [Palampur (2016), Palampur (2017), Bajaura (2017)] and combined data for 24 traits analyzed and QTLs detected on all the linkage groups except on LG8 and LG 10. Out of these 27 QTLs, 15 QTLs were major with PVE of greater than 10 per cent. Also 5 Stable QTLs were found which were associated with combined data of traits across different years and locations. In total, 3 QTLs were detected for biochemical and physiological traits, 4 for root traits, 7 for morphological traits, 4 each phenological traits, and 9 for yield related traits. In case, flanking markers were common in more than one QTL, that region was considered as only one genomic region. By following this criterion, 27 QTLs identified were present in 18 genomic regions. Of the 27 QTLs detected, nearly 23 per cent (6 QTLs) were located on LG7 followed by LG4 and LG9 (4 QTLs). Phenotypic variation explained (PVE) by QTLs ranged from 6.4 to 53.4 per cent. The highest phenotypic variation (53.4%) was explained by the QTLs for root length.

LG1 contained a total of four QTLs (one each for plant height and pods per plant, while two for seeds per plant); LG2 contained three QTLs (one each for

MDA(C), days to 50% flowering and pods per plant); LG3 contained only one QTL (for root length); LG4 had a total of three QTLs (one each for Chlorophyll (S), Proline (S) and seed size), LG5 contained a total of three QTLs (one each for root dry weight, root fresh weight and reproductive period); LG6 contained three QTLs (two for primary branches and one for no of seeds per plant); LG7 contained six QTLs (three each for secondary branches, two each for seed yield and one for days to maturity) and LG9 contained four QTLs (one each for root length, primary branches, days to maturity and seed size. QTLs were detected on eight linkage groups, except for on LG8 and LG10. Overall, five QTL clusters were identified. Among these, QTL Cluster 1 was located on LG1; QTL Cluster 2 was located on LG2; QTL Cluster 3 was located on LG4; QTL Cluster 4 was located on LG7 and QTL Cluster 5 was located on LG9.

Evidently, the map generated in the present study although covered a significant portion of the genome, a further saturation of this map with additional markers (SSRs or SNPs) is imperative for its efficient utilization. With the long-term goal of understanding the genetic basis of early maturity and yield related traits, the present study was focused on identification of major QTLs for 24 traits in horsegram. In conclusion, it is envisaged that the present linkage map, fortified with 295 molecular markers and 27 QTLs for early maturity and yield-related traits would provide a means to breeders for further genetic enhancement of this crop species. However, a denser genetic linkage maps with large number of markers with the inclusion of SNPs would facilitate the identification of more resolved and fine QTL positions which can significantly improve the resolution of identified QTLs for mapping. The identification of QTLs controlling agronomically important traits would improve our genetic understanding of these traits and finally provide the basis for MAS of these traits. Therefore, QTLs validation and fine mapping is the next step towards successful application of these findings in MAS.

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Appendix I: Values of RILs for morphological traits

RIL	PLANT HEIGHT (cm)							PRIMARY BRANCHES							SECONDARY BRANCHES						
	PLP 2016	PLP 2017			BJR 2017		COMBINED	PLP 2016	PLP 2016			BJR 2017		COMBINED	PLP 2016	PLP 2017			BJR 2017		COMBINED
		R1	R2	R3	R1	R2			R1	R2	R3	R1	R2			R1	R2	R3	R1	R2	
1	72	78	63	63	74	66	69.33	2.33	3	3.83	3.67	5	2	3.31	5.8	6	4.8	6.4	9	6	6.33
2	71	111	65	77	100	66	81.67	2.67	2	2.33	3.33	5	6	3.56	5.67	5	4.6	5.8	9	9	6.51
3	74	77	84	98	103	123	93.17	1.17	1	1	3	6	6	3.03	4	3	4.8	4.8	12	11	6.60
4	66	68	49	74	92	91	73.33	1.67	1	1.67	2.67	3	8	3.00	4.22	3	4.2	5.2	5	12	5.60
5	67	71.5	73	70	70	78	71.58	1.2	2	2.33	3.15	4	4	2.78	4.83	3	6.2	6.2	9	7	6.04
6	74	80	71	81	84	83	78.83	1.67	1	3.83	4.33	4	5	3.31	5	3	6.8	8.6	7	9	6.57
7	98	87	90	94	94	98	93.50	1.5	3	6.33	6.67	6	5	4.75	4.2	5	10.8	12	12	13	9.50
8	78	71	83	86	82	109	84.83	2	2	2.17	4.33	6	3	3.25	4	6	5.8	8.6	8	6	6.40
9	56	77	40	72	88	79	68.67	1.67	2	2	3.5	5	4	3.03	4.8	3	6	6.8	9	8	6.27
10	55	59	52	89	130	114	83.17	2.67	6	3.61	5	7	10	5.71	5.6	7	7	12.6	9	16	9.53
11	81	82	73	88	105	110	89.83	2	2	3.33	6.67	8	6	4.67	7	5	6.8	12.5	10	18	9.88
12	35	59	42	60	96	60	58.67	2.5	3	4	4.33	7	5	4.31	14	7	8.6	8.6	12	15	10.87
13	62	68.5	66	71	70	73	68.42	2	3	4.33	4.33	6	4	3.94	4	5	8.6	8.2	9	12	7.80
14	62	65	51	58	73	77	64.33	1.33	1	1.33	2.67	3	2	1.89	4	3	3	5.2	8	9	5.37
15	75	82	87	81	85	102	85.33	1.33	3	1.33	3.33	7	5	3.50	4.2	6	3	7	12	12	7.37
16	52	57.5	47	82	70	108	69.42	2.5	3	3.33	4	5	7	4.14	5.8	5	6.8	8.6	7	10	7.20
17	82	50	94	92	100	116	89.00	1	2	2.67	5.67	8	8	4.56	3	6	6.4	12.2	12	14	8.93
18	54	71	46	78	110	81	73.33	3.67	5	2.33	5.33	9	5	5.06	5.2	8	7	12.2	14	12	9.73
19	68	81	64	87	108	94	83.67	1.33	2	1.17	3.67	6	4	3.03	5	5	8.2	8.4	8	7	6.93
20	48	41.5	66	58	63	68	57.42	2.83	2	4.67	4	5	5	3.92	4.8	5	9.6	9	12	10	8.40
21	34	79	60	79	105	90	74.50	1.4	3	2	2.17	6	5	3.26	5.6	8	6.8	4.4	11	11	7.80
22	77	97	84	89	83	90	86.67	1.67	1	2	3.33	4	5	2.83	5.8	3	5	6.8	8	9	6.27
23	43	61	82	77	87	84	72.33	4	2	2.17	3.67	5	5	3.64	3	3	6.2	8	11	9	6.70
24	79	73	115	82	76	84	84.83	1.67	2	4	4.67	4	6	3.72	6.6	5	8.6	9.2	7	10	7.73
25	72	79	65	75	80	79	75.00	1.4	3	1.67	2.17	10	3	3.54	4.8	6	5.2	6.8	17	7	7.80
26	42	76	68	86	114	78	77.33	2.17	4	4.33	5.67	11	7	5.70	4.8	9	8.6	9.8	19	15	11.03
27	36	68	59	79	94	107	73.83	1.4	2	1	3.33	7	5	3.29	5.2	5	8.8	8.4	12	9	8.07
28	66	65	69	74	87	112	78.83	3	3	3	4	9	7	4.83	5.4	5	6.8	9.8	17	13	9.50
29	45	51	49	52	62	66	54.17	1.67	2	3.83	3	8	5	3.92	7.8	4	7.4	6.8	15	11	8.67
30	48	66	55	82	74	96	70.17	3.83	5	1.67	4.33	7	5	4.47	6.6	7	4.8	5.2	12	11	7.77
31	70	72	66	87	122	90	84.50	2.5	2	1.33	2.67	8	2	3.08	5.8	4	5	3.6	12	6	6.07
32	78	71	89	86	112	138	95.67	2.5	2	3.67	4	9	4	4.20	5.8	5	7.4	8.6	15	9	8.47
33	92	116	97	105	122	169	116.83	1.4	1	1.5	2.67	7	4	2.93	4.4	3	4.6	5.6	11	9	6.27
34	58	71	72	66	72	79	69.67	3.83	1	3.67	2.17	4	6	3.45	5.8	3	6.2	6.8	7	10	6.47

35	57	51	51	62	64	67	58.67	2.67	3	2.5	3.67	3	3	2.97	5.4	6	5.6	4.2	5	5	5.20
36	50	51	49	63	67	64	57.33	3.5	4	3.67	3.33	4	7	4.25	5.6	7	7	6.5	6	10	7.02
37	68	70	77	86	95	101	82.83	2.5	1	2.83	3.83	3	7	3.36	5.8	2	4.6	8.6	6	10	6.17
38	72	97	78	75	82	78	80.33	1.83	4	1.67	4.33	7	6	4.14	4	11	7.8	8.6	11	13	9.23
39	70	68	76	72	79	105	78.33	3	1	1.75	3.83	5	7	3.60	6.6	2	7	7.4	9	10	7.00
40	58	72	53	56	59	65	60.50	2.8	2	1.83	2.67	3	6	3.05	5.6	4	7	4.6	5	7	5.53
41	72	71	58	74	106	88	78.17	2.4	2	0.67	2.17	5	2	2.37	5.8	5	4.6	5.8	7	5	5.53
42	74	75	79	86	122	122	93.00	2.67	4	2.33	4.33	9	8	5.06	5	9	6	7.8	15	11	8.97
43	55	57	62	63	67	63	61.17	3.5	3	2.33	3	6	10	4.64	5	5	6.4	5.2	13	16	8.43
44	74	71	65	68	66	78	70.33	2.5	3	2.33	3.33	5	6	3.69	5.8	6	5	7	12	11	7.80
45	48	49	44	51	62	59	52.17	4	2	1	3	8	5	3.83	5	4	6.4	7	14	10	7.73
46	72	70	74	77	108	88	81.50	1.33	1	1.67	3	5	7	3.17	2	3	4.8	5.2	11	13	6.50
47	72	86	79	85	126	103	91.83	2.6	2	1.33	3.83	7	4	3.46	4.4	3	5.6	7.2	13	9	7.03
48	62	73	79	71	83	114	80.33	3.67	3	2.8	2.67	4	7	3.86	6	4	6.8	4.8	7	11	6.60
49	89	74	86	83	110	114	92.67	2.67	3	2.6	4.5	11	5	4.80	5.8	7	5.2	14.8	18	12	10.47
50	81	76	82	82	108	110	89.83	1.33	2	1.5	3.4	6	5	3.21	4	4	4.6	8.4	10	9	6.67
51	74	67	64	71	87	77	73.33	2.6	2	2.33	3.33	7	5	3.71	6.6	4	5.2	6.8	10	10	7.10
52	72	70	83	84	98	91	83.00	1	4	1.67	3.61	8	9	4.55	2.4	10	6.2	8.2	11	15	8.80
53	58	72	71	87	91	112	81.83	3.33	3	3.67	4.33	6	6	4.39	5.8	5	7.8	11.6	8	11	8.20
54	78	65	81	84	106	99	85.50	2.67	2	2.8	4.33	6	9	4.47	5.6	6	7	12.2	9	14	8.97
55	52	58	73	72	102	82	73.17	2.4	3	2.67	4	8	6	4.35	5	5	6.4	9.2	14	12	8.60
56	80	82	79	84	93	92	85.00	2	2	2.67	4.33	8	5	4.00	5	7	5.8	7.8	12	11	8.10
57	58	65	54	62	64	66	61.50	2.2	2	2.67	5.33	5	6	3.87	4	3	4.6	11.8	8	10	6.90
58	58	74	79	74	121	116	87.00	3.5	4	1.83	3.67	6	4	3.83	5.8	5	4.2	8.6	12	9	7.43
59	58	66	61	62	65	68	63.33	2.6	2	4.33	2.17	4	8	3.85	6	4	9.6	6.2	9	13	7.97
60	73	70	79	84	108	116	88.33	1.33	1	1.4	3.33	6	8	3.51	4.8	3	4.2	6.7	13	12	7.28
61	52	58	74	72	110	96	77.00	3	3	1.67	3.67	7	3	3.56	2.8	5	4.8	5.8	14	7	6.57
62	63	65	76	73	86	79	73.67	2.17	4	3.67	3	9	4	4.31	4.8	6	8.6	7	14	10	8.40
63	82	77	74	78	95	105	85.17	1	1	1.5	3.33	8	6	3.47	2.2	2	4.8	7.4	13	11	6.73
64	59	55	47	58	64	62	57.50	3.4	3	2.33	2.4	7	6	4.02	2.5	5	6.8	3	11	10	6.38
65	82	73	85	86	132	81	89.83	2.33	2	3.67	2.17	5	5	3.36	6.8	4	8.6	4.8	8	10	7.03
66	38	38	61	64	61	65	54.50	3.67	4	1.5	2.67	5	6	3.81	4.2	10	7	5.2	10	15	8.57
67	74	88	95	85	112	92	91.00	1.5	1	1.67	4.33	4	6	3.08	2	3	4.2	8.6	7	15	6.63
68	38	51	43	58	65	66	53.50	2.5	3	1.33	3.67	4	5	3.25	1.8	8	5	6.8	9	9	6.60
69	87	88	86	78	112	96	91.17	2.5	1	1.67	4.4	6	5	3.43	5.4	5	4.2	8.6	12	11	7.70
70	89	75	74	83	151	128	100.00	3.33	2	4.33	4.33	11	7	5.33	5.8	4	9.6	8.4	18	15	10.13
71	85	82	79	88	103	102	89.83	3.2	2	3.67	3.61	6	9	4.58	7.6	5	8.6	7	10	13	8.53
72	85	90	82	94	111	102	94.00	1.33	1	1.83	2.67	7	6	3.31	5.4	2	4.5	5.8	12	14	7.28

73	72	86	81	82	103	104	88.00	2.83	1	3	3.33	7	8	4.19	5	3	7	6.2	10	13	7.37
74	82	68	71	78	106	112	86.17	2.5	2	3	3.67	6	4	3.53	6.8	6	6.8	7	11	9	7.77
75	86	97	96	94	133	92	99.67	1.67	3	2.33	4.4	10	4	4.23	4.2	10	7	5.8	17	11	9.17
76	72	74	79	86	93	91	82.50	1.67	1	1.4	4.33	8	6	3.73	2.6	3	6.8	8.6	16	13	8.33
77	79	72	87	74	93	117	87.00	2.5	1	2.5	3.67	8	11	4.78	4.6	2	7.2	6.8	15	18	8.93
78	48	54	40	51	62	58	52.17	3.15	3	2.4	2.4	3	2	2.66	6.8	6	5.8	5.2	8	6	6.30
79	48	45	47	56	67	62	54.17	2	2	2.8	2.83	5	4	3.11	3	6	5.8	7	12	9	7.13
80	82	92	89	86	117	94	93.33	6	4	1.67	2.33	6	4	4.00	3.5	12	4.8	4.8	12	7	7.35
81	88	91	84	82	103	114	93.67	2.67	2	3.61	3	8	5	4.05	5.2	3	7.4	5.4	14	9	7.33
82	83	86	77	79	97	112	89.00	1.33	1	3.11	2.67	4	7	3.19	2.4	3	6.8	5	9	16	7.03
83	51	51	52	58	62	75	58.17	2.17	3	2	2.5	5	7	3.61	4.8	8	5.6	6.8	7	11	7.20
84	76	72	64	77	82	75	74.33	3.67	3	3	2.6	6	6	4.05	4.4	11	6.8	4.8	14	10	8.50
85	84	81	72	86	102	97	87.00	1.67	2	3	3.33	7	6	3.83	7	5	6.8	7	12	13	8.47
86	71	74	68	84	108	104	84.83	1	2	2.67	2.5	6	6	3.36	4.8	6	6.2	5.2	12	12	7.70
87	58	59	56	62	71	67	62.17	2.6	2	3.67	2.67	6	6	3.82	5.2	4	7.4	5.8	11	12	7.57
88	60	62	53	64	62	64	60.83	1.67	3	1.8	2.67	7	3	3.19	4.8	10	8	4.8	12	7	7.77
89	78	79	82	88	127	91	90.83	2.2	2	3.67	3.33	10	7	4.70	4	6	7	6	21	15	9.83
90	93	98	89	92	103	95	95.00	4	2	3	3	9	6	4.50	7.4	9	6.8	5.8	15	13	9.50
91	78	73	65	72	90	88	77.67	3.4	2	3	3.83	5	5	3.71	2.8	7	4.8	6.8	7	9	6.23
92	77	70	86	88	132	132	97.50	2.67	4	1.67	4.83	11	8	5.36	2.8	7	4.6	8.4	18	15	9.30
93	72	77	80	75	88	115	84.50	1.4	2	2.83	4.33	9	5	4.09	2.8	5	4.8	7.4	16	12	8.00
94	89	96	99	94	102	114	99.00	2.4	3	2.83	4	5	6	3.87	5	3	6.2	8.2	8	13	7.23
95	53	61	50	66	65	64	59.83	2.83	2	2.67	3.67	5	4	3.36	5.2	4	6	6.8	11	10	7.17
96	71	74	68	78	103	82	79.33	2.5	2	4.33	4.17	7	8	4.67	5.4	4	8.6	8.4	12	14	8.73
97	83	77	88	84	120	91	90.50	2.2	3	6.67	3.67	11	6	5.42	7.2	7	10.6	7	17	13	10.30
98	55	68	73	68	82	89	72.50	2	3	4.33	4.83	4	7	4.19	3	6	8.4	8.6	5	11	7.00
99	70	76	71	66	105	85	78.83	1.67	3	1.67	4	8	5	3.89	2.8	6	4.4	8.2	14	9	7.40
100	71	76	59	71	78	72	71.17	2.5	4	2	3.67	8	3	3.86	3	9	6.4	7	13	8	7.73
101	88	94	93	89	105	116	97.50	2	2	2.6	3.33	9	6	4.16	5.2	5	7.2	6.8	17	13	9.03
102	68	71	73	69	87	76	74.00	1.4	1	1.67	4.67	5	5	3.12	2.8	3	4.8	8.4	8	11	6.33
103	79	74	82	86	115	96	88.67	2.5	2	4.33	4.17	6	7	4.33	5.2	5	7.6	8.6	11	12	8.23
104	55	38	52	54	66	66	55.17	2.4	2	2.67	2.67	4	2	2.62	4.8	5	8	4.8	9	5	6.10
105	62	64	68	74	65	67	66.67	3.61	2	4.33	5	7	6	4.66	6.8	6	8.6	10.8	12	12	9.37
106	82	87	91	98	112	84	92.33	2	2	5	3.67	9	6	4.61	4.8	5	9.8	7	14	12	8.77
107	79	83	87	84	115	87	89.17	2	3	2.67	4.33	7	7	4.33	4.2	7	6.8	8.6	11	12	8.27
108	86	87	81	94	122	114	97.33	1.67	2	1.67	3.33	8	8	4.11	2.8	3	6.2	7.4	13	14	7.73
109	79	85	82	86	102	90	87.33	2.5	2	4.33	4.17	8	8	4.83	5.2	4	8.6	7.8	12	15	8.77
110	49	55	52	57	70	75	59.67	3.83	2	4	3.67	5	4	3.75	5.4	5	7.6	7.2	9	7	6.87

111	62	66	66	68	77	79	69.67	2.2	2	2.83	4.5	6	6	3.92	5.2	5	6	8.6	10	10	7.47
112	77	74	81	79	104	105	86.67	2.83	2	3.11	4.33	6	7	4.21	5.2	4	6.8	9.6	13	14	8.77
113	84	80	92	86	128	108	96.33	2.5	5	3.67	3.67	8	7	4.97	5	13	8.6	7.4	14	12	10.00
114	52	64	72	68	79	62	66.17	2.17	2	2.83	4.67	7	6	4.11	5.2	4	6.2	8.4	9	12	7.47
115	62	41	76	71	87	102	73.17	2.4	3	1.17	2.83	5	5	3.23	4.8	7	6.4	6.8	6	9	6.67
116	74	71	89	82	120	98	89.00	2.4	2	1.17	3.33	8	5	3.65	5.2	7	3	5.4	12	11	7.27
117	41	58	58	61	65	62	57.50	2.4	2	2.83	3.67	4	9	3.98	3	6	7	6.8	7	14	7.30
118	54	56	67	62	64	67	61.67	2.5	4	4.33	5.17	7	10	5.50	4.8	14	8.6	9.6	11	17	10.83
119	82	80	96	94	118	109	96.50	3.33	2	3	3.33	2	7	3.44	6.8	7	5.4	9.8	4	10	7.17
120	68	71	64	79	87	131	83.33	3.15	2	4	4.67	7	5	4.30	5.8	5	8.2	8.6	11	10	8.10
121	51	49	57	59	62	72	58.33	4.25	3	2.67	4.4	8	5	4.55	7.5	5	6.8	7	12	10	8.05
122	49	50	42	62	93	67	60.50	2.17	2	1.67	3.5	8	6	3.89	5.2	6	5.2	6.2	13	10	7.60
123	69	75	80	73	106	104	84.50	3.83	2	3.33	3.67	10	8	5.14	4.6	6	7	6.8	14	12	8.40
124	88	75	95	96	102	92	91.33	3.33	5	4.67	4.5	8	9	5.75	6.8	10	12.6	9.6	12	14	10.83
125	86	71	88	85	100	100	88.33	3.67	5	4	4	10	6	5.45	6.8	13	8.6	8.4	11	13	10.13
126	68	75	62	78	105	94	80.33	1.4	2	2.5	3.33	8	3	3.37	2.8	6	3.6	6.8	13	8	6.70
127	58	52	55	50	64	85	60.67	1	3	1.4	3.67	8	4	3.51	2.8	7	8.4	6.2	12	10	7.73
128	87	81	90	96	126	127	101.17	2.5	2	3.67	3.33	7	7	4.25	5.2	4	7.4	7	11	13	7.93
129	48	44	50	52	67	96	59.50	2.4	3	1.33	3	8	6	3.96	5	6	5.2	5.4	12	8	6.93
130	77	79	88	83	124	88	89.83	2.83	2	3.33	3.67	5	9	4.31	4.6	8	7	6.8	10	13	8.23
131	83	50	47	62	67	63	62.00	3.5	2	2.83	2.5	8	4	3.81	6.8	6	4.8	5	11	9	7.10
132	78	89	81	82	102	118	91.67	2.67	2	3.83	2.4	6	6	3.82	5.2	6	7.8	5.6	8	14	7.77
133	57	51	65	63	72	89	66.17	1.33	3	2.17	3.67	7	5	3.70	2.8	5	7.2	7.2	10	8	6.70
134	83	83	85	82	98	106	89.50	2.6	5	2.5	3.67	7	3	3.96	5.2	12	4.8	7	11	6	7.67
135	63	53	58	68	89	98	71.50	3.15	2	5.67	3.83	3	4	3.61	5.8	6	10.4	7.2	5	7	6.90
136	78	61	76	83	135	112	90.83	2.5	5	3.83	3	6	5	4.22	5	14	8.2	6.2	14	7	9.07
137	71	70	87	79	137	108	92.00	2.4	2	2.17	2.67	7	5	3.54	4.8	2	5	5.2	15	11	7.17
138	75	76	79	81	112	80	83.83	2	1	2.67	4.5	6	7	3.86	3	3	5.8	8.6	9	13	7.07
139	83	89	80	91	134	118	99.17	2	2	4.33	3.67	5	7	4.00	4.2	6	7.4	6.8	11	12	7.90
140	73	71	77	84	118	76	83.17	2.83	2	5.67	3	6	3	3.75	6.2	4	9.6	7	10	8	7.47
141	54	66	63	67	88	84	70.33	2.67	3	2.67	2.17	6	6	3.75	5.2	2	5.6	5.2	11	7	6.00
142	79	72	86	75	116	80	84.67	2	2	2.67	3.83	7	5	3.75	3	5	6	6.8	14	9	7.30
143	83	70	88	86	129	95	91.83	2.5	2	2.33	3.83	9	7	4.44	5	4	5.8	7.2	15	13	8.33
144	68	72	75	78	105	81	79.83	3.2	4	3.11	3.11	9	5	4.57	6.2	12	7	6.2	13	12	9.40
145	75	75	79	77	116	83	84.17	2	2	3.67	3.67	3	7	3.56	5.2	3	7.2	6.8	6	10	6.37
146	51	58	54	61	64	69	59.50	2.6	2	3.83	3	4	6	3.57	5.2	6	7.8	7	9	10	7.50
147	53	50	60	62	65	66	59.33	3.33	2	4.33	3.83	2	6	3.58	6.8	6	9.6	8	6	11	7.90
148	65	64	74	60	63	60	64.33	3.11	2	4.33	3	4	5	3.57	5.4	6	7.8	4.6	9	9	6.97

149	83	95	89	87	100	102	92.67	2.67	2	4	3.83	4	4	3.42	5.2	6	8.6	6.2	7	9	7.00
150	38	56	64	67	79	95	66.50	3.11	2	4	3.11	8	7	4.54	4.6	5	7.4	4.6	13	13	7.93
151	68	72	76	80	64	63	70.50	2	1	3.67	2.67	3	4	2.72	6.8	2	5.2	4.2	8	4	5.03
152	83	82	78	77	102	87	84.83	3.33	2	5.5	4.33	6	7	4.69	5.8	5	7.2	4.8	9	11	7.13
153	64	52	38	69	66	65	59.00	5.33	4	6.67	4.33	5	5	5.06	8.8	10	10.8	7.4	10	6	8.83
154	78	79	82	80	87	101	84.50	4	2	5	4.4	5	5	4.23	7	6	9.6	8.6	12	8	8.53
155	72	81	80	84	66	62	74.17	3.83	2	3.33	2.67	7	9	4.64	7	6	6.8	5.2	9	10	7.33
156	67	65	56	72	78	92	71.67	3.17	2	3	2.5	4	6	3.45	4.8	5	4.8	5.6	5	13	6.37
157	75	76	72	85	92	119	86.50	3.67	2	3	2.4	5	11	4.51	7	5	6.8	5	7	16	7.80
158	77	85	89	75	102	101	88.17	2	2	3.11	2.67	2	3	2.46	4.8	4	5.4	5.2	6	7	5.40
159	69	77	75	70	72	76	73.17	2.33	2	2.67	3.67	5	3	3.11	5.2	5	6.8	6.8	8	2	5.63
160	55	57	65	63	62	67	61.50	3.61	2	3.61	2.83	6	5	3.84	4.6	6	7	5.2	10	7	6.63
161	52	57	55	61	65	64	59.00	2	2	2.17	3.83	7	6	3.83	5	5	5.2	6.8	11	11	7.33
162	72	86	81	84	124	94	90.17	3	2	5.33	3.33	8	10	5.28	6.2	6	9.6	7	12	18	9.80
163	101	97	102	98	100	98	99.33	2.6	2	2.67	2.83	3	3	2.68	5.2	3	4.2	3.6	5	5	4.33
164	38	40	42	41	38	40	39.83	6.67	6	5.5	7.83	9	11	7.67	8	13	10.8	12.8	16	12	12.10

Appendix II: Values of RILs for phenological traits

RIL	DAYS TO FLOWERING							DAYS TO MATURITY							REPRODUCTIVE PERIOD						
	PLP 2016	PLP 2017			BJR 2017		COMBINED	PLP 2016	PALAMPUR 2017			BJR 2017		COMBINED	PLP 2016	PALAMPUR 2017			BJR 2017		COMBINED
		R1	R2	R3	R1	R2			R1	R2	R3	R1	R2			R1	R2	R3	R1	R2	
1	30	33	38	36	30	35	33.67	82	85	86	89	92	98	88.67	52	52	48	53	62	63	55.00
2	34	39	35	38	35	38	36.50	99	102	95	102	97	98	98.83	65	63	60	64	62	60	62.33
3	40	37	40	42	45	43	41.17	107	109	115	118	106	103	109.67	67	72	75	76	61	60	68.50
4	38	36	40	36	35	40	37.50	86	87	88	90	94	92	89.50	48	51	48	54	59	52	52.00
5	33	40	35	38	30	32	34.67	82	85	98	92	90	90	89.50	49	45	63	54	60	58	54.83
6	35	38	33	35	30	32	33.83	75	88	85	90	92	95	87.50	40	50	52	55	62	63	53.67
7	42	42	43	42	35	40	40.67	110	114	103	93	95	98	102.17	68	72	60	51	60	58	61.50
8	42	41	45	45	44	42	43.17	98	92	98	102	110	111	101.83	56	51	53	57	66	69	58.67
9	42	41	39	37	38	38	39.17	85	105	96	112	95	92	97.50	43	64	57	75	57	54	58.33
10	48	42	46	45	45	42	44.67	92	87	94	102	95	97	94.50	44	45	48	57	50	55	49.83
11	44	43	48	52	48	45	46.67	104	102	103	108	106	112	105.83	60	59	55	56	58	67	59.17
12	35	40	38	38	43	36	38.33	94	90	92	97	95	98	94.33	59	50	54	59	52	62	56.00
13	44	41	38	31	32	33	36.50	86	94	72	81	81	88	83.67	42	53	34	50	49	55	47.17
14	44	41	42	44	40	41	42.00	80	85	87	92	96	92	88.67	36	44	45	48	56	51	46.67
15	38	40	36	42	40	35	38.50	83	85	85	82	88	90	85.50	45	45	49	40	48	55	47.00
16	52	45	55	53	50	46	50.17	84	85	88	102	97	95	91.83	32	40	33	49	47	49	41.67
17	40	38	33	36	32	35	35.67	105	116	110	103	99	102	105.83	65	78	77	67	67	67	70.17
18	30	36	35	36	32	33	33.67	89	104	94	82	87	85	90.17	59	68	59	46	55	52	56.50
19	33	36	35	35	33	35	34.50	81	99	78	78	85	88	84.83	48	63	43	43	52	53	50.33
20	40	39	38	36	35	36	37.33	115	114	112	107	108	110	111.00	75	75	74	71	73	74	73.67
21	38	36	32	43	39	37	37.50	94	93	97	95	97	97	95.50	56	57	65	52	58	60	58.00
22	38	36	35	32	31	35	34.50	100	103	85	82	88	86	90.67	62	67	50	50	57	51	56.17
23	30	32	35	39	32	33	33.50	82	81	76	82	81	78	80.00	52	49	41	43	49	45	46.50
24	38	37	33	38	36	36	36.33	86	85	82	86	87	87	85.50	48	48	49	48	51	51	49.17
25	32	35	35	40	33	33	34.67	95	103	85	94	85	88	91.67	63	68	50	54	52	55	57.00
26	35	35	35	33	33	34	34.17	83	101	84	85	100	100	92.17	48	66	49	52	67	66	58.00
27	36	38	30	31	35	35	34.17	98	95	98	95	90	94	95.00	62	57	68	64	55	59	60.83
28	31	42	48	44	42	40	41.17	108	114	102	108	95	99	104.33	77	72	54	64	53	59	63.17
29	38	33	32	39	35	35	35.33	86	94	89	85	99	94	91.17	48	61	57	46	64	59	55.83
30	32	42	36	38	39	40	37.83	88	85	83	87	90	94	87.83	56	43	47	49	51	54	50.00
31	45	36	38	38	42	35	39.00	87	95	92	91	98	95	93.00	42	59	54	53	56	60	54.00
32	47	44	45	38	44	42	43.33	105	114	108	111	105	111	109.00	58	70	63	73	61	69	65.67
33	43	43	52	56	48	45	47.83	104	95	88	100	106	95	98.00	61	52	36	44	58	50	50.17
34	41	38	35	41	40	39	39.00	110	103	104	108	104	108	106.17	69	65	69	67	64	69	67.17

35	46	46	44	45	58	51	48.33	98	92	98	90	100	95	95.50	52	46	54	45	42	44	47.17
36	41	39	42	43	43	42	41.67	87	92	88	91	106	95	93.17	46	53	46	48	63	53	51.50
37	41	45	47	41	41	40	42.50	92	110	95	101	100	102	100.00	51	65	48	60	59	62	57.50
38	45	45	42	42	47	45	44.33	101	110	98	100	106	98	102.17	56	65	56	58	59	53	57.83
39	31	36	38	35	32	33	34.17	92	114	94	84	102	98	97.33	61	78	56	49	70	65	63.17
40	38	45	35	35	35	42	38.33	88	110	101	100	87	89	95.83	50	65	66	65	52	47	57.50
41	38	36	38	36	33	35	36.00	82	99	79	82	85	88	85.83	44	63	41	46	52	53	49.83
42	43	45	44	44	40	44	43.33	103	114	100	103	102	107	104.83	60	69	56	59	62	63	61.50
43	42	44	40	38	38	42	40.67	102	114	87	82	105	100	98.33	60	70	47	44	67	58	57.67
44	42	41	41	42	41	40	41.17	101	103	79	81	88	86	89.67	59	62	38	39	47	46	48.50
45	38	39	36	35	35	36	36.50	93	99	95	96	87	88	93.00	55	60	59	61	52	52	56.50
46	44	42	42	43	40	41	42.00	82	99	84	86	91	90	88.67	38	57	42	43	51	49	46.67
47	48	43	43	43	40	42	43.17	84	99	83	78	87	92	87.17	36	56	40	35	47	50	44.00
48	36	41	38	38	39	39	38.50	91	103	100	84	84	86	91.33	55	62	62	46	45	47	52.83
49	52	41	48	56	54	60	51.83	98	100	101	108	100	102	101.50	46	59	53	52	46	42	49.67
50	38	40	36	38	42	38	38.67	93	99	89	105	112	108	101.00	55	59	53	67	70	70	62.33
51	42	42	40	44	39	40	41.17	105	114	103	91	97	94	100.67	63	72	63	47	58	54	59.50
52	33	33	35	32	40	36	34.83	82	85	101	92	112	99	95.17	49	52	66	60	72	63	60.33
53	48	44	46	44	42	42	44.33	105	120	112	100	110	108	109.17	57	76	66	56	68	66	64.83
54	48	44	44	42	40	42	43.33	100	114	98	97	95	95	99.83	52	70	54	55	55	53	56.50
55	45	43	44	44	38	42	42.67	77	103	102	87	87	96	92.00	32	60	58	43	49	54	49.33
56	42	45	47	43	40	42	43.17	108	123	78	99	92	93	98.83	66	78	31	56	52	51	55.67
57	44	42	45	46	39	42	43.00	100	104	92	96	94	98	97.33	56	62	47	50	55	56	54.33
58	46	38	42	44	42	42	42.33	87	95	92	88	95	86	90.50	41	57	50	44	53	44	48.17
59	38	36	38	37	35	35	36.50	74	72	71	72	79	78	74.33	36	36	33	35	44	43	37.83
60	30	38	30	33	36	35	33.67	98	100	93	90	95	91	94.50	68	62	63	57	59	56	60.83
61	52	40	44	44	43	35	43.00	71	100	77	72	88	88	82.67	19	60	33	28	45	53	39.67
62	42	44	46	48	48	42	45.00	78	100	85	91	100	105	93.17	36	56	39	43	52	63	48.17
63	48	44	46	48	52	50	48.00	85	89	110	92	112	100	98.00	37	45	64	44	60	50	50.00
64	36	36	36	38	32	35	35.50	81	114	84	80	88	85	88.67	45	78	48	42	56	50	53.17
65	36	39	38	33	39	38	37.17	101	108	100	82	99	102	98.67	65	69	62	49	60	64	61.50
66	38	47	52	49	49	45	46.67	92	103	89	101	99	98	97.00	54	56	37	52	50	53	50.33
67	42	39	42	44	43	36	41.00	72	100	77	88	95	92	87.33	30	61	35	44	52	56	46.33
68	35	36	36	33	33	34	34.50	92	99	92	90	98	98	94.83	57	63	56	57	65	64	60.33
69	40	38	33	35	42	36	37.33	78	85	82	81	92	90	84.67	38	47	49	46	50	54	47.33
70	51	43	46	46	48	44	46.33	100	104	98	101	106	102	101.83	49	61	52	55	58	58	55.50
71	48	43	55	52	50	48	49.33	101	103	105	100	106	105	103.33	53	60	50	48	56	57	54.00
72	44	44	58	46	50	44	47.67	91	95	83	88	98	98	92.17	47	51	25	42	48	54	44.50

73	44	43	44	48	48	45	45.33	100	92	101	98	94	99	97.33	56	49	57	50	46	54	52.00
74	44	43	44	36	40	42	41.50	95	108	100	96	99	102	100.00	51	65	56	60	59	60	58.50
75	42	40	44	42	40	38	41.00	93	100	97	102	98	100	98.33	51	60	53	60	58	62	57.33
76	44	42	46	44	46	42	44.00	106	108	100	108	113	112	107.83	62	66	54	64	67	70	63.83
77	38	42	43	38	45	42	41.33	108	108	106	111	112	112	109.50	70	66	63	73	67	70	68.17
78	35	36	33	32	32	35	33.83	81	75	79	72	79	78	77.33	46	39	46	40	47	43	43.50
79	45	42	45	33	43	42	41.67	77	84	74	87	86	85	82.17	32	42	29	54	43	43	40.50
80	50	43	44	48	45	42	45.33	84	100	95	94	97	95	94.17	34	57	51	46	52	53	48.83
81	53	44	48	46	42	42	45.83	97	108	104	105	103	105	103.67	44	64	56	59	61	63	57.83
82	44	42	46	44	38	42	42.67	92	115	98	100	100	101	101.00	48	73	52	56	62	59	58.33
83	35	37	33	32	35	37	34.83	79	104	80	71	79	78	81.83	44	67	47	39	44	41	47.00
84	36	34	33	33	34	36	34.33	78	73	77	75	82	80	77.50	42	39	44	42	48	44	43.17
85	40	38	39	39	36	36	38.00	94	104	92	97	99	98	97.33	54	66	53	58	63	62	59.33
86	42	40	42	48	45	40	42.83	100	104	101	94	110	105	102.33	58	64	59	46	65	65	59.50
87	44	41	44	44	40	38	41.83	71	78	70	78	86	88	78.50	27	37	26	34	46	50	36.67
88	32	36	35	45	33	35	36.00	71	80	81	75	79	78	77.33	39	44	46	30	46	43	41.33
89	43	42	42	44	39	42	42.00	82	87	90	91	95	98	90.50	39	45	48	47	56	56	48.50
90	44	45	43	43	49	45	44.83	100	103	108	101	100	105	102.83	56	58	65	58	51	60	58.00
91	45	45	48	44	43	42	44.50	107	114	100	91	95	101	101.33	62	69	52	47	52	59	56.83
92	43	40	38	58	48	40	44.50	92	103	87	98	90	97	94.50	49	63	49	40	42	57	50.00
93	44	43	45	48	50	42	45.33	99	103	108	111	100	106	104.50	55	60	63	63	50	64	59.17
94	38	44	52	50	50	45	46.50	91	94	91	100	95	98	94.83	53	50	39	50	45	53	48.33
95	35	39	35	39	35	35	36.33	71	72	70	75	79	78	74.17	36	33	35	36	44	43	37.83
96	44	41	44	48	48	45	45.00	100	103	102	98	97	100	100.00	56	62	58	50	49	55	55.00
97	44	45	45	48	48	45	45.83	100	103	102	100	100	108	102.17	56	58	57	52	52	63	56.33
98	44	43	42	41	39	40	41.50	98	103	101	100	82	109	98.83	54	60	59	59	43	69	57.33
99	42	42	42	42	42	40	41.67	91	91	92	98	95	105	95.33	49	49	50	56	53	65	53.67
100	31	36	36	45	38	36	37.00	78	77	84	84	85	84	82.00	47	41	48	39	47	48	45.00
101	52	43	43	44	46	42	45.00	87	99	93	94	103	109	97.50	35	56	50	50	57	67	52.50
102	42	39	48	42	40	40	41.83	80	99	81	80	79	84	83.83	38	60	33	38	39	44	42.00
103	45	43	57	44	45	43	46.17	100	102	104	101	107	109	103.83	55	59	47	57	62	66	57.67
104	35	39	36	42	41	42	39.17	81	81	82	85	88	92	84.83	46	42	46	43	47	50	45.67
105	36	42	38	38	45	42	40.17	100	108	101	102	109	108	104.67	64	66	63	64	64	66	64.50
106	36	46	35	39	44	44	40.67	100	114	108	104	100	107	105.50	64	68	73	65	56	63	64.83
107	48	42	52	47	45	42	46.00	100	107	97	92	94	98	98.00	52	65	45	45	49	56	52.00
108	48	42	44	60	45	42	46.83	105	107	107	98	97	98	102.00	57	65	63	38	52	56	55.17
109	48	46	49	58	52	50	50.50	101	103	100	107	104	99	102.33	53	57	51	49	52	49	51.83
110	38	36	30	32	34	34	34.00	71	79	72	72	80	78	75.33	33	43	42	40	46	44	41.33

111	32	44	48	48	48	45	44.17	108	107	102	100	106	105	104.67	76	63	54	52	58	60	60.50
112	38	46	45	48	42	45	44.00	100	103	98	102	97	95	99.17	62	57	53	54	55	50	55.17
113	44	45	43	42	48	45	44.50	91	103	107	101	100	108	101.67	47	58	64	59	52	63	57.17
114	33	39	35	42	35	38	37.00	92	77	81	81	82	82	82.50	59	38	46	39	47	44	45.50
115	38	36	36	34	35	35	35.67	78	83	82	86	90	88	84.50	40	47	46	52	55	53	48.83
116	41	44	43	43	42	42	42.50	85	86	74	80	82	82	81.50	44	42	31	37	40	40	39.00
117	41	41	42	42	44	41	41.83	91	99	92	93	112	102	98.17	50	58	50	51	68	61	56.33
118	38	42	45	44	43	42	42.33	94	99	100	91	95	99	96.33	56	57	55	47	52	57	54.00
119	38	46	44	58	53	46	47.50	87	95	91	92	90	86	90.17	49	49	47	34	37	40	42.67
120	52	44	46	48	42	42	45.67	101	103	100	102	107	109	103.67	49	59	54	54	65	67	58.00
121	38	36	36	35	36	35	36.00	71	79	73	74	87	82	77.67	33	43	37	39	51	47	41.67
122	34	33	38	38	35	35	35.50	74	81	73	75	87	92	80.33	40	48	35	37	52	57	44.83
123	37	38	34	34	37	35	35.83	91	102	100	89	97	96	95.83	54	64	66	55	60	61	60.00
124	44	44	48	52	50	48	47.67	95	92	98	97	98	102	97.00	51	48	50	45	48	54	49.33
125	44	45	48	48	48	44	46.17	100	103	104	107	112	112	106.33	56	58	56	59	64	68	60.17
126	47	43	45	58	44	44	46.83	82	99	87	91	95	92	91.00	35	56	42	33	51	48	44.17
127	30	36	38	38	35	35	35.33	78	99	79	78	79	78	81.83	48	63	41	40	44	43	46.50
128	48	43	44	42	38	42	42.83	91	100	99	111	107	112	103.33	43	57	55	69	69	70	60.50
129	38	43	43	44	38	43	41.50	74	79	75	74	86	84	78.67	36	36	32	30	48	41	37.17
130	44	43	47	52	45	43	45.67	100	114	104	102	113	112	107.50	56	71	57	50	68	69	61.83
131	41	41	44	53	45	41	44.17	103	108	102	111	112	108	107.33	62	67	58	58	67	67	63.17
132	44	41	48	42	45	41	43.50	100	91	95	98	103	98	97.50	56	50	47	56	58	57	54.00
133	42	36	48	33	45	42	41.00	81	82	80	79	79	86	81.17	39	46	32	46	34	44	40.17
134	38	42	44	45	45	42	42.67	98	99	102	100	106	100	100.83	60	57	58	55	61	58	58.17
135	47	42	44	48	45	42	44.67	84	100	101	89	100	100	95.67	37	58	57	41	55	58	51.00
136	42	44	32	35	38	42	38.83	100	103	104	104	107	110	104.67	58	59	72	69	69	68	65.83
137	38	43	42	41	40	43	41.17	105	109	100	111	113	109	107.83	67	66	58	70	73	66	66.67
138	42	36	41	42	42	42	40.83	102	103	100	97	99	105	101.00	60	67	59	55	57	63	60.17
139	48	43	49	42	42	42	44.33	100	103	94	98	107	104	101.00	52	60	45	56	65	62	56.67
140	37	39	41	38	42	42	39.83	99	103	92	97	98	100	98.17	62	64	51	59	56	58	58.33
141	38	37	38	40	40	38	38.50	81	75	79	79	84	82	80.00	43	38	41	39	44	44	41.50
142	42	42	40	38	39	42	40.50	100	101	107	104	105	106	103.83	58	59	67	66	66	64	63.33
143	52	45	48	50	50	42	47.83	98	101	102	100	104	102	101.17	46	56	54	50	54	60	53.33
144	52	43	44	48	48	42	46.17	97	104	98	95	94	99	97.83	45	61	54	47	46	57	51.67
145	58	51	45	48	50	45	49.50	100	101	98	99	96	98	98.67	42	50	53	51	46	53	49.17
146	38	39	44	43	40	39	40.50	78	82	72	81	80	84	79.50	40	43	28	38	40	45	39.00
147	39	36	38	33	38	38	37.00	80	72	80	77	84	85	79.67	41	36	42	44	46	47	42.67
148	39	36	37	38	38	39	37.83	77	80	75	74	80	87	78.83	38	44	38	36	42	48	41.00

149	48	47	49	53	44	50	48.50	98	104	104	100	100	105	101.83	50	57	55	47	56	55	53.33
150	45	46	38	43	50	44	44.33	91	103	98	104	107	105	101.33	46	57	60	61	57	61	57.00
151	43	43	45	44	45	43	43.83	92	103	97	100	99	105	99.33	49	60	52	56	54	62	55.50
152	52	44	50	58	50	45	49.83	100	105	98	97	98	102	100.00	48	61	48	39	48	57	50.17
153	40	40	46	48	42	40	42.67	80	79	77	79	84	84	80.50	40	39	31	31	42	44	37.83
154	32	41	39	42	42	41	39.50	99	85	89	78	97	105	92.17	67	44	50	36	55	64	52.67
155	45	44	48	47	48	48	46.67	99	103	100	104	107	111	104.00	54	59	52	57	59	63	57.33
156	52	43	47	45	42	41	45.00	74	78	87	82	79	78	79.67	22	35	40	37	37	37	34.67
157	58	47	54	57	50	47	52.17	101	103	104	100	110	105	103.83	43	56	50	43	60	58	51.67
158	43	43	38	38	39	42	40.50	100	103	100	101	100	108	102.00	57	60	62	63	61	66	61.50
159	33	38	35	33	34	35	34.67	81	75	75	79	79	78	77.83	48	37	40	46	45	43	43.17
160	32	39	33	33	32	38	34.50	78	89	82	90	90	94	87.17	46	50	49	57	58	56	52.67
161	48	39	36	33	36	38	38.33	98	103	100	99	109	112	103.50	50	64	64	66	73	74	65.17
162	44	47	52	56	53	50	50.33	100	106	102	103	113	112	106.00	56	59	50	47	60	62	55.67
HPK4	54	50	58	55	58	57	55.33	118	120	116	115	112	116	116.17	64	70	58	60	54	59	60.83
M249	36	37	36	35	32	32	34.67	75	85	83	80	81	79	80.50	39	48	47	45	49	47	45.83

Appendix III (a): Values of RILs for yield traits

RIL	100 SEED WT (g)						SEED SIZE (cm)						NO OF SEEDS/PODS					
	PLP 2016	PLP 2017	BJR 2017		COMBINED		PLP 2016	PLP 2017	BJR 2017		COMBINED		PLP 2016	PLP 2017	BJR 2017		COMBINED	
		R1	R2	R1	R2			R1	R2	R1	R2			R1	R2	R1	R2	
1	3.46	4.25	3.82	3.37	3.66	3.712	0.56	0.67	0.62	0.58	0.61	0.61	4	4	4	4	4	4
2	3.12	3.66	3.11	3.13	3.32	3.268	0.51	0.62	0.63	0.58	0.59	0.59	4	4	4	4	4	4
3	3.68	3.56	3.08	3.4	3.82	3.508	0.61	0.63	0.67	0.6	0.61	0.62	4	4	4	4	4	4
4	3.56	4.42	5.23	3.11	3.65	3.994	0.56	0.59	0.68	0.56	0.58	0.59	5	5	4	5	4	4.6
5	3.11	3.75	3.45	2.54	3.11	3.192	0.54	0.54	0.57	0.54	0.54	0.55	3	3	4	5	4	3.8
6	3.78	4.91	4.94	2.86	3.84	4.066	0.61	0.61	0.68	0.57	0.6	0.61	5	5	4	5	4	4.6
7	3.96	4.61	3.68	3.02	3.64	3.782	0.54	0.63	0.61	0.53	0.58	0.58	4	4	5	4	4	4.2
8	3.78	4.61	4.75	3.55	3	3.938	0.59	0.62	0.64	0.57	0.56	0.60	6	6	5	4	5	5.2
9	4.11	4.91	4.46	3.94	3.13	4.11	0.57	0.63	0.6	0.6	0.58	0.60	3	3	3	4	5	3.6
10	3.93	4.91	4.48	3.38	3.1	3.96	0.56	0.59	0.66	0.54	0.54	0.58	3	3	4	4	5	3.8
11	3.45	4.88	4.42	3.6	3.53	3.976	0.61	0.62	0.64	0.63	0.6	0.62	4	4	4	4	4	4
12	4.87	5.93	5.87	4.03	4.62	5.064	0.6	0.59	0.63	0.6	0.61	0.61	4	5	4	5	4	4.4
13	3.68	4.97	5.39	3.35	3.05	4.088	0.51	0.56	0.63	0.54	0.55	0.56	4	3	4	5	5	4.2
14	3.48	3.84	3.91	3.97	3.43	3.726	0.58	0.58	0.69	0.56	0.59	0.60	5	5	5	5	4	4.8
15	4.21	5.08	4.56	3.8	3.93	4.316	0.58	0.6	0.65	0.54	0.58	0.59	4	6	4	4	4	4.4
16	4.25	5.38	4.74	3.67	3.39	4.286	0.57	0.65	0.63	0.57	0.59	0.60	5	3	5	5	5	4.6

17	3.95	4.73	5.02	3.63	3.72	4.21	0.58	0.59	0.59	0.6	0.54	0.58	5	3	5	5	5	4.6
18	3.64	4.43	4.89	3.23	3.95	4.028	0.56	0.6	0.56	0.54	0.52	0.56	4	4	4	5	4	4.2
19	3.54	4.69	4.47	3.47	3.33	3.9	0.53	0.59	0.58	0.54	0.53	0.55	4	4	5	4	4	4.2
20	2.98	3.91	3.23	2.82	2.79	3.146	0.55	0.59	0.6	0.52	0.56	0.56	4	4	4	4	4	4
21	3.65	6.45	5.87	3.99	3.82	4.756	0.58	0.58	0.59	0.54	0.58	0.57	4	4	4	4	4	4
22	2.87	4.87	4.47	3.38	3.78	3.874	0.61	0.58	0.68	0.68	0.55	0.62	5	5	4	5	5	4.8
23	3.15	5.39	5.6	3.75	3.59	4.296	0.58	0.64	0.6	0.56	0.58	0.59	5	5	4	5	5	4.8
24	3.54	5.06	4.91	3.62	3.3	4.086	0.56	0.58	0.64	0.55	0.55	0.58	5	5	3	3	5	4.2
25	3.69	5.22	5.47	3.86	3.41	4.33	0.6	0.61	0.61	0.58	0.58	0.60	5	5	3	3	5	4.2
26	3.65	5.08	5.8	3.6	4.16	4.458	0.6	0.061	0.62	0.57	0.66	0.50	4	4	3	4	4	3.8
27	2.96	5.47	5.02	3.35	3.7	4.1	0.59	0.058	0.63	0.58	0.58	0.49	4	4	5	4	4	4.2
28	4.65	5.77	5.46	3.14	3.81	4.566	0.61	0.66	0.65	0.55	0.6	0.61	3	3	4	4	4	3.6
29	3.96	4.81	5.12	3.3	3.64	4.166	0.6	0.6	0.64	0.59	0.57	0.60	4	4	4	5	3	4
30	5.11	6	5.83	3.83	3.79	4.912	0.64	0.061	0.7	0.6	0.63	0.53	5	5	5	5	4	4.8
31	3.65	4.84	4.92	3.08	2.89	3.876	0.56	0.58	0.6	0.53	0.55	0.56	4	4	4	4	4	4
32	4.59	6.89	6.08	4.36	3.62	5.108	0.55	0.63	0.64	0.55	0.53	0.58	5	5	4	5	5	4.8
33	3.65	5.48	4.75	3.52	3.37	4.154	0.55	0.62	0.58	0.54	0.53	0.56	3	3	3	5	5	3.8
34	2.15	4.52	4.94	3.34	3.49	3.688	0.55	0.58	0.61	0.55	0.55	0.57	3	3	4	4	5	3.8
35	2.36	5.51	4.33	2.52	3.24	3.592	0.56	0.6	0.62	0.52	0.57	0.57	3	3	4	4	4	3.6
36	3.56	4.56	4.56	3.32	3.58	3.916	0.54	0.59	0.6	0.54	0.55	0.56	6	6	5	4	4	5
37	4.11	4.55	4.06	2.74	2.88	3.668	0.57	0.59	0.59	0.5	0.57	0.56	6	6	5	5	4	5.2
38	4.32	4.38	4.61	2.99	3.74	4.008	0.59	0.61	0.59	0.55	0.6	0.59	3	3	4	5	4	3.8
39	4.29	4.57	4.86	3.07	2.92	3.942	0.58	0.61	0.62	0.57	0.54	0.58	4	4	5	4	4	4.2
40	4.12	5.25	4.27	2.94	3.22	3.96	0.58	0.62	0.6	0.55	0.58	0.59	5	5	4	4	5	4.6
41	3.15	4.97	4.33	3.12	4.18	3.95	0.56	0.59	0.58	0.55	0.61	0.58	4	4	4	5	5	4.4
42	3.25	5.29	4.84	2.96	3.44	3.956	0.58	0.64	0.61	0.55	0.58	0.59	4	4	4	4	4	4
43	3.96	4.56	4.64	3.26	3.4	3.964	0.58	0.67	0.62	0.59	0.58	0.61	5	4	5	4	4	4.4
44	3.54	5.05	4.7	3.21	3.4	3.98	0.53	0.62	0.68	0.52	0.55	0.58	5	4	5	5	5	4.8
45	2.57	5.63	5.7	3.2	3.76	4.172	0.57	0.6	0.61	0.57	0.52	0.57	5	5	5	5	4	4.8
46	2.56	4.19	4.1	2.92	3.32	3.418	0.56	0.57	0.6	0.53	0.56	0.56	4	5	4	4	5	4.4
47	5.21	5.237	5.25	4.37	3.8	4.7734	0.59	0.6	0.62	0.64	0.6	0.61	4	3	4	5	4	4
48	3.67	7.02	6.44	3.61	3.96	4.94	0.58	0.64	0.6	0.6	0.6	0.60	4	4	4	5	5	4.4
49	3.58	6.04	5.88	3.41	3.85	4.552	0.58	0.63	0.67	0.57	0.58	0.61	5	6	5	4	5	5
50	2.17	3.37	3.64	2.76	2.92	2.972	0.56	0.55	0.55	0.54	0.58	0.56	4	2	4	5	4	3.8
51	2.14	4.06	3.77	3.13	2.94	3.208	0.56	0.6	0.58	0.58	0.54	0.57	4	4	4	5	4	4.2
52	3.19	4.88	4.21	3.48	3.51	3.854	0.58	0.6	0.62	0.52	0.58	0.58	3	3	3	4	3	3.2
53	2.51	5.96	2.55	3.66	4.08	3.752	0.6	0.65	0.6	0.6	0.61	0.61	5	6	5	4	5	5
54	3.65	5.44	5.12	3.84	3.65	4.34	0.59	0.62	0.61	0.6	0.58	0.60	5	5	5	4	4	4.6

55	3.56	5.57	5.88	3.56	3.94	4.502	0.58	0.61	0.61	0.58	0.6	0.60	5	5	5	4	4	4.6
56	4.26	5.38	4.64	3.08	3.22	4.116	0.54	0.6	0.6	0.55	0.53	0.56	5	5	5	4	4	4.6
57	3.48	4.34	3.6	3.17	3.14	3.546	0.58	0.61	0.63	0.58	0.54	0.59	5	4	5	5	5	4.8
58	3.24	4.56	4.94	3.35	3.5	3.918	0.58	0.6	0.66	0.58	0.6	0.60	4	4	4	4	5	4.2
59	3.98	4.2	4.06	3.62	3.13	3.798	0.56	0.67	0.62	0.65	0.56	0.61	4	6	4	4	4	4.4
60	5.02	4.57	5.34	3.02	3.47	4.284	0.58	0.58	0.68	0.58	0.55	0.59	4	3	4	4	4	3.8
61	3.48	4.27	4.77	3.04	3.92	3.896	0.66	0.6	0.61	0.54	0.65	0.61	4	6	4	4	4	4.4
62	3.11	4.88	4	3.36	3.17	3.704	0.57	0.63	0.62	0.6	0.57	0.60	5	5	5	5	4	4.8
63	2.84	3.32	3.32	3.7	3.55	3.346	0.58	0.58	0.6	0.54	0.56	0.57	5	4	5	5	4	4.6
64	3.87	4.18	3.88	3.49	3.4	3.764	0.52	0.58	0.61	0.54	0.5	0.55	5	5	5	4	5	4.8
65	2.91	2.81	3.35	3.71	3.5	3.256	0.58	0.61	0.58	0.62	0.58	0.59	4	6	4	4	4	4.4
66	4.25	5.2	4.25	3.51	3.34	4.11	0.54	0.64	0.6	0.54	0.58	0.58	3	3	3	4	5	3.6
67	3.25	5.28	5.1	3.3	3.57	4.1	0.58	0.62	0.61	0.59	0.58	0.60	4	4	4	4	6	4.4
68	3.65	3.98	4.28	2.92	2.82	3.53	0.56	0.6	0.61	0.57	0.53	0.57	5	5	5	4	4	4.6
69	3.78	3.86	4.47	2.82	3.28	3.642	0.58	0.58	0.59	0.57	0.57	0.58	5	2	5	5	5	4.4
70	3.56	5.87	5.1	3.85	4.46	4.568	0.58	0.63	0.6	0.57	0.6	0.60	4	4	5	5	4	4.4
71	3.78	5.64	4.96	3.6	3.56	4.308	0.54	0.55	0.65	0.53	0.53	0.56	4	5	5	5	4	4.6
72	3.56	5.21	4.7	3.94	4.43	4.368	0.56	0.56	0.66	0.55	0.61	0.59	4	6	5	5	4	4.8
73	3.71	4.71	4.1	3.47	3.39	3.876	0.6	0.61	0.62	0.61	0.59	0.61	4	2	5	5	4	4
74	3.61	5.461	4.7	3.54	3.32	4.1262	0.55	0.64	0.63	0.57	0.55	0.59	4	6	5	4	4	4.6
75	3.26	4.85	5.24	3.65	4.15	4.23	0.57	0.63	0.68	0.59	0.57	0.61	4	5	4	4	4	4.2
76	2.63	6.06	4.93	4.15	3.84	4.322	0.54	0.62	0.62	0.53	0.58	0.58	5	3	4	6	5	4.6
77	3.48	5.56	4.93	3.49	3.44	4.18	0.58	0.61	0.62	0.54	0.58	0.59	4	5	5	5	4	4.6
78	2.96	4.45	3.69	2.6	3.38	3.416	0.59	0.53	0.61	0.54	0.6	0.57	5	4	5	6	5	5
79	3.11	5.89	5.24	3.52	3.17	4.186	0.55	0.61	0.63	0.57	0.55	0.58	4	4	4	4	4	4
80	3.21	4.41	4.1	3.44	3.52	3.736	0.6	0.63	0.62	0.58	0.6	0.61	5	4	5	5	5	4.8
81	3.24	5.03	4.72	3.26	3.66	3.982	0.59	0.62	0.62	0.6	0.59	0.60	5	4	5	5	5	4.8
82	3.26	4.39	5.84	3.22	3.19	3.98	0.58	0.62	0.64	0.58	0.56	0.60	4	4	5	4	4	4.2
83	3.87	4.74	4.8	3.74	3.72	4.174	0.6	0.6	0.66	0.56	0.55	0.59	4	4	5	6	4	4.6
84	3.45	4.14	3.92	2.94	3.07	3.504	0.58	0.58	0.62	0.57	0.6	0.59	4	3	5	5	4	4.2
85	3.21	5.64	4.94	3.18	3.29	4.052	0.54	0.6	0.58	0.51	0.55	0.56	4	3	4	4	4	3.8
86	3.62	5.41	4.68	3.89	4.03	4.326	0.57	0.61	0.66	0.57	0.58	0.60	4	3	4	5	4	4
87	3.11	4.66	4.17	3.55	3.26	3.75	0.58	0.62	0.58	0.58	0.56	0.58	4	4	4	4	4	4
88	2.45	4.47	4.52	3.32	3.37	3.626	0.63	0.61	0.64	0.58	0.58	0.61	3	3	4	5	4	3.8
89	3.25	5.94	2.86	3.84	3.97	3.972	0.63	0.6	0.63	0.54	0.57	0.59	5	5	5	6	5	5.2
90	3.95	5.04	4.92	3.22	3.29	4.084	0.57	0.6	0.65	0.54	0.58	0.59	5	5	5	4	5	4.8
91	4.12	4.28	4.32	3.56	3.2	3.896	0.54	0.6	0.62	0.52	0.53	0.56	5	5	5	5	4	4.8
92	3.84	5.15	5.26	3.65	3.39	4.258	0.66	0.6	0.64	0.57	0.55	0.60	5	5	3	6	4	4.6

93	3.62	6.15	5.08	3.85	3.59	4.458	0.6	0.62	0.65	0.61	0.59	0.61	5	5	5	5	5
94	3.18	5.2	4.37	3.89	3.05	3.938	0.57	0.6	0.58	0.55	0.53	0.57	3	3	5	5	4
95	3.52	6	5.86	3.67	3.66	4.542	0.61	0.63	0.62	0.58	0.61	0.61	3	3	4	5	6
96	3.44	4.58	4.15	2.68	2.78	3.526	0.54	0.58	0.6	0.5	0.54	0.55	2	2	4	5	6
97	3.25	5.7	4.26	3.57	3.87	4.13	0.56	0.59	0.57	0.54	0.57	0.57	3	3	4	3	5
98	3.26	5.07	4.88	3.25	3.43	3.978	0.56	0.62	0.57	0.51	0.56	0.56	3	3	5	5	4
99	3.25	4.7	3.98	3.75	3.55	3.846	0.6	0.62	0.64	0.54	0.6	0.60	4	4	3	4	5
100	3.58	5.5	4.56	3.91	3.96	4.302	0.58	0.6	0.68	0.58	0.58	0.60	3	4	3	4	4
101	4.25	6.14	5.01	3.38	3.59	4.474	0.6	0.64	0.63	0.58	0.57	0.60	5	4	4	4	4
102	3.18	4.35	4.67	3.22	3.01	3.686	0.61	0.6	0.61	0.58	0.6	0.60	5	4	4	5	5
103	3.89	4.99	4.53	3.25	3.28	3.988	0.52	0.62	0.59	0.54	0.53	0.56	4	3	4	5	4
104	3.55	4.99	3.93	3.66	3.01	3.828	0.56	0.66	0.67	0.58	0.57	0.61	4	4	4	3	5
105	3.32	4.62	5.44	3.24	3.36	3.996	0.61	0.59	0.65	0.59	0.6	0.61	4	3	4	5	4
106	3.84	4.74	3.98	3.25	3.5	3.862	0.52	0.62	0.61	0.51	0.51	0.55	5	5	5	4	5
107	3.22	4.7	4.1	3.82	3.73	3.914	0.56	0.62	0.63	0.58	0.57	0.59	3	4	4	4	4
108	3.56	4.75	4.04	3.45	3.39	3.838	0.6	0.63	0.6	0.57	0.58	0.60	3	5	5	4	4
109	4.12	6.13	4.93	3.68	3.52	4.476	0.57	0.63	0.63	0.57	0.56	0.59	4	3	4	4	3
110	3.85	4.6	4.88	3.57	3.47	4.074	0.56	0.6	0.66	0.58	0.56	0.59	4	4	4	4	4
111	3.89	4.5	5.24	3.56	3.12	4.062	0.58	0.59	0.65	0.52	0.57	0.58	4	4	4	5	5
112	3.67	5.43	5.24	3.81	3.76	4.382	0.57	0.62	0.65	0.57	0.56	0.59	4	4	4	5	4
113	3.26	4.53	4.74	3.59	3.35	3.894	0.54	0.64	0.63	0.54	0.53	0.58	4	5	5	3	3
114	3.25	4.14	3.7	3.24	3.11	3.488	0.57	0.54	0.63	0.58	0.55	0.57	4	4	4	3	4
115	3.45	5.55	4.57	3.48	3.71	4.152	0.58	0.63	0.64	0.57	0.6	0.60	5	4	4	4	5
116	3.95	5.16	4.84	3.51	3.82	4.256	0.6	0.64	0.67	0.58	0.57	0.61	5	4	4	5	4
117	3.26	4.98	3.94	3.87	3.45	3.9	0.57	0.65	0.62	0.6	0.57	0.60	5	3	3	4	5
118	3.65	4.4	4.88	3.45	3.68	4.012	0.57	0.64	0.64	0.58	0.59	0.60	4	2	3	3	4
119	2.59	6.03	4.6	3.58	3.67	4.094	0.57	0.6	0.66	0.57	0.58	0.60	4	5	4	5	5
120	3.64	5.35	3.37	2.85	3.42	3.726	0.56	0.62	0.56	0.53	0.6	0.57	4	4	5	4	4
121	3.65	3.94	4.21	2.44	2.97	3.442	0.53	0.58	0.62	0.52	0.54	0.56	4	4	4	5	4
122	3.15	4.73	4.33	2.74	3.37	3.664	0.57	0.57	0.65	0.52	0.58	0.58	5	4	5	4	4
123	4.1	4.37	4.06	3.16	3.64	3.866	0.55	0.61	0.64	0.55	0.55	0.58	5	6	4	5	4
124	3.95	4.75	4.32	3.65	2.84	3.902	0.58	0.59	0.64	0.57	0.58	0.59	5	4	4	5	5
125	3.48	5.15	3.98	3.26	3.38	3.85	0.55	0.6	0.62	0.55	0.57	0.58	5	4	4	5	5
126	3.59	4.88	4.71	3.14	3.19	3.902	0.56	0.6	0.58	0.56	0.55	0.57	4	4	5	4	5
127	3.65	4.26	2.63	2.85	3.32	3.342	0.56	0.58	0.57	0.56	0.54	0.56	3	4	5	4	4
128	3.26	4.84	4.16	3.1	3.08	3.688	0.6	0.65	0.6	0.54	0.6	0.60	4	5	5	4	4
129	3.65	5.83	4.56	3.7	3.78	4.304	0.58	0.63	0.59	0.56	0.57	0.59	5	5	4	4	5
130	3.45	4.96	4.77	3.02	3.27	3.894	0.6	0.61	0.63	0.53	0.57	0.59	5	5	5	4	4

131	3.36	4.37	4.6	3.27	3.44	3.808	0.58	0.61	0.65	0.58	0.6	0.60	5	4	4	4	5	4.4
132	3.25	6	4.75	4.02	4.28	4.46	0.6	0.62	0.61	0.58	0.55	0.59	5	4	5	4	4	4.4
133	3.95	5.05	5.41	3.13	3.48	4.204	0.57	0.63	0.62	0.57	0.51	0.58	4	3	4	4	5	4
134	3.75	5.34	5.31	3.76	3.63	4.358	0.6	0.62	0.62	0.57	0.61	0.60	5	3	5	5	4	4.4
135	3.71	5.24	5.02	3.64	3.82	4.286	0.56	0.6	0.65	0.56	0.61	0.60	4	4	4	4	4	4
136	3.65	5.11	4.81	3.43	3.7	4.14	0.54	0.65	0.61	0.54	0.56	0.58	4	4	4	4	4	4
137	3.26	4.86	3.84	3.43	3.6	3.798	0.57	0.63	0.64	0.56	0.56	0.59	5	4	5	5	5	4.8
138	3.25	4.7	4.56	3.29	3.51	3.862	0.54	0.59	0.59	0.54	0.55	0.56	4	4	6	4	4	4.4
139	3.36	6.23	5.45	3.79	3.73	4.512	0.56	0.61	0.58	0.56	0.57	0.58	4	3	6	4	4	4.2
140	4.02	4.72	4.26	3.19	3.14	3.866	0.56	0.59	0.63	0.57	0.55	0.58	4	3	4	6	4	4.2
141	3.26	4.42	5.52	3.82	3.94	4.192	0.6	0.58	0.65	0.6	0.62	0.61	4	2	4	4	4	3.6
142	3.25	4.7	3.97	3.54	3.46	3.784	0.58	0.63	0.64	0.6	0.57	0.60	5	4	4	4	4	4.2
143	3.98	5.02	4.25	3.56	3.29	4.02	0.57	0.61	0.58	0.58	0.56	0.58	4	4	4	4	4	4
144	2.57	4.82	4.35	3.35	3.44	3.706	0.57	0.61	0.6	0.58	0.59	0.59	4	5	4	4	4	4.2
145	3.21	5.94	6.03	3.87	3.6	4.53	0.58	0.6	0.6	0.57	0.58	0.59	4	3	4	4	5	4
146	3.26	4.12	3.7	3.54	3.13	3.55	0.57	0.58	0.63	0.57	0.54	0.58	4	2	4	4	4	3.6
147	3.25	3.99	4	3.89	3.11	3.648	0.56	0.59	0.61	0.55	0.56	0.57	4	5	4	4	5	4.4
148	2.98	4.94	4.8	3.27	3.58	3.914	0.54	0.59	0.64	0.53	0.53	0.57	4	4	5	5	5	4.6
149	3.26	3.44	4.7	3.81	3.64	3.77	0.59	0.6	0.62	0.59	0.57	0.59	4	3	4	4	3	3.6
150	3.15	4.03	3.5	3.79	3.3	3.554	0.54	0.6	0.53	0.57	0.55	0.56	4	5	3	5	5	4.4
151	3.95	5.25	5.76	3.85	3.96	4.554	0.56	0.62	0.64	0.56	0.56	0.59	5	4	4	4	5	4.4
152	3.25	4.51	4.16	3.44	3.01	3.674	0.6	0.64	0.64	0.59	0.6	0.61	4	4	3	5	5	4.2
153	3.15	4.56	5.19	3.01	2.94	3.77	0.6	0.6	0.66	0.6	0.59	0.61	4	4	4	4	4	4
154	3.29	5.44	5.64	3.18	3.19	4.148	0.55	0.63	0.56	0.54	0.54	0.56	4	4	4	4	4	4
155	3.35	4.87	4.3	3.58	3.45	3.91	0.55	0.61	0.6	0.56	0.55	0.57	4	3	3	5	4	3.8
156	2.64	3.84	3.6	3.24	3.06	3.276	0.56	0.61	0.61	0.59	0.55	0.58	4	4	4	4	4	4
157	3.24	5.43	6.08	3.45	3.65	4.37	0.6	0.65	0.68	0.6	0.61	0.63	4	4	4	5	5	4.4
158	4.35	6.02	6.4	3.99	4.04	4.96	0.57	0.62	0.66	0.57	0.58	0.60	4	5	5	4	4	4.4
159	4.26	6.57	6.12	4.08	4.23	5.052	0.59	0.62	0.62	0.57	0.59	0.60	4	5	5	4	5	4.6
160	4.26	5.82	6.04	3.98	4.16	4.852	0.56	0.6	0.6	0.56	0.6	0.58	4	4	4	4	4	4
161	2.95	4.72	4.8	2.95	3.1	3.704	0.52	0.6	0.65	0.53	0.54	0.57	4	4	5	4	4	4.2
162	3.36	4.45	4.81	3.15	3.26	3.806	0.56	0.59	0.66	0.54	0.58	0.59	5	4	4	5	5	4.6
HPK4	4.02	5.29	4.85	4.36	4.23	4.55	0.63	0.63	0.69	0.67	0.67	0.66	4	5	4	4	4	4.2
M249	3.12	4.56	3.5	3.28	3.65	3.622	0.57	0.56	0.57	0.58	0.57	0.57	4	4	4	4	4	4.2

Appendix III (b): Values of RILs for yield traits

RIL	NO OF PODS/PLANT						NO OF SEEDS /PLANT						SEED YIELD/PLANT (g)					
	PLP 2016	PLP 2017	BJR 2017		COMBINED		PLP 2016	PLP 2017	BJR 2017		COMBINED		PLP 2016	PLP 2017	BJR 2017		COMBINED	
		R1	R2	R1	R2			R1	R2	R1	R2			R1	R2	R1	R2	
1	17	18	24	28	32	23.8	68	72	96	112	128	95.2	2.35	3.06	3.67	3.77	4.68	3.51
2	15	16	15	24	32	20.4	60	64	60	96	128	81.6	1.87	2.34	1.87	3.00	4.25	2.67
3	12	17	15	32	32	21.6	48	68	60	128	128	86.4	1.77	2.42	1.85	4.35	4.89	3.06
4	8	10	10	25	36	17.8	40	50	40	125	144	79.8	1.42	2.21	2.09	3.89	5.26	2.97
5	11	22	9	26	29	19.4	33	66	36	130	116	76.2	1.03	2.48	1.24	3.30	3.61	2.33
6	22	28	18	24	30	24.4	110	140	72	120	120	112.4	4.16	6.87	3.56	3.43	4.61	4.53
7	14	15	15	30	26	20	56	60	75	120	104	83	2.22	2.77	2.76	3.62	3.79	3.03
8	15	25	16	24	26	21.2	90	150	80	96	130	109.2	3.40	6.92	3.80	3.41	3.90	4.29
9	15	22	14	26	28	21	45	66	42	104	140	79.4	1.85	3.24	1.87	4.10	4.38	3.09
10	20	25	13	28	29	23	60	75	52	112	145	88.8	2.36	3.68	2.33	3.79	4.50	3.33
11	14	26	16	24	29	21.8	56	104	64	96	116	87.2	1.93	5.08	2.83	3.46	4.09	3.48
12	15	21	9	26	28	19.8	60	105	36	130	112	88.6	2.92	6.23	2.11	5.24	5.17	4.34
13	16	12	19	23	26	19.2	64	36	76	115	130	84.2	2.36	1.79	4.10	3.85	3.97	3.21
14	13	16	16	29	28	20.4	65	80	80	145	112	96.4	2.26	3.07	3.13	5.76	3.84	3.61
15	6	16	9	32	29	18.4	24	96	36	128	116	80	1.01	4.88	1.64	4.86	4.56	3.39
16	17	14	8	25	27	18.2	85	42	40	125	135	85.4	3.61	2.26	1.90	4.59	4.58	3.39
17	13	16	14	29	26	19.6	65	48	70	145	130	91.6	2.57	2.27	3.51	5.26	4.84	3.69
18	14	18	11	28	30	20.2	56	72	44	140	120	86.4	2.04	3.19	2.15	4.52	4.74	3.33
19	15	22	13	26	30	21.2	60	88	65	104	120	87.4	2.12	4.13	2.91	3.61	4.00	3.35
20	17	26	15	30	32	24	68	104	60	120	128	96	2.03	4.07	1.94	3.38	3.57	3.00
21	18	14	14	32	31	21.8	72	56	56	128	124	87.2	2.63	3.61	3.29	5.11	4.74	3.87
22	16	24	21	21	32	22.8	80	120	84	105	160	109.8	2.30	5.84	3.75	3.55	6.05	4.30
23	13	25	18	35	28	23.8	65	125	72	175	140	115.4	2.05	6.74	4.03	6.56	5.03	4.88
24	14	26	21	35	29	25	70	130	63	105	145	102.6	2.48	6.58	3.09	3.80	4.79	4.15
25	17	24	22	36	36	27	85	120	66	108	180	111.8	3.14	6.26	3.61	4.17	6.14	4.66
26	16	24	16	32	30	23.6	64	96	48	128	120	91.2	2.34	4.88	2.78	4.61	4.99	3.92
27	17	25	15	35	29	24.2	68	100	75	140	116	99.8	2.01	5.47	3.77	4.69	4.29	4.05
28	14	18	13	28	28	20.2	42	54	52	112	112	74.4	1.95	3.12	2.84	3.52	4.27	3.14
29	15	19	15	29	36	22.8	60	76	60	145	108	89.8	2.38	3.66	3.07	4.79	3.93	3.56
30	16	15	15	32	32	22	80	75	75	160	128	103.6	4.09	4.50	4.37	6.13	4.85	4.79
31	14	15	9	24	36	19.6	56	60	36	96	144	78.4	2.04	2.90	1.77	2.96	4.16	2.77
32	11	12	8	24	35	18	55	60	32	120	175	88.4	2.52	4.13	1.95	5.23	6.34	4.03
33	8	9	5	26	35	16.6	24	27	15	130	175	74.2	0.88	1.48	0.71	4.58	5.90	2.71
34	6	8	4	28	32	15.6	18	24	16	112	160	66	0.39	1.08	0.79	3.74	5.58	2.32

35	14	19	14	35	34	23.2	42	57	56	140	136	86.2	0.99	3.14	2.42	3.53	4.41	2.90
36	15	20	18	39	31	24.6	90	120	90	156	124	116	3.20	5.47	4.10	5.18	4.44	4.48
37	16	26	18	32	29	24.2	96	156	90	160	116	123.6	3.95	7.10	3.65	4.38	3.34	4.48
38	14	27	15	36	24	23.2	42	81	60	180	96	91.8	1.81	3.55	2.77	5.38	3.59	3.42
39	15	22	16	35	30	23.6	60	88	80	140	120	97.6	2.57	4.02	3.89	4.30	3.50	3.66
40	17	24	14	39	31	25	85	120	56	156	155	114.4	3.50	6.30	2.39	4.59	4.99	4.35
41	16	24	15	32	28	23	64	96	60	160	140	104	2.02	4.77	2.60	4.99	5.85	4.05
42	14	18	18	31	29	22	56	72	72	124	116	88	1.82	3.81	3.48	3.67	3.99	3.35
43	16	14	16	25	31	20.4	80	56	80	100	124	88	3.17	2.55	3.71	3.26	4.22	3.38
44	12	17	14	26	32	20.2	60	68	70	130	160	97.6	2.12	3.43	3.29	4.17	5.44	3.69
45	9	10	15	24	29	17.4	45	50	75	120	116	81.2	1.16	2.82	4.28	3.84	4.36	3.29
46	9	9	10	32	28	17.6	36	45	40	128	140	77.8	0.92	1.89	1.64	3.74	4.65	2.57
47	15	22	16	32	29	22.8	60	66	64	160	116	93.2	3.13	3.46	3.36	6.99	4.41	4.27
48	18	26	15	25	30	22.8	72	104	60	125	150	102.2	2.64	7.30	3.86	4.51	5.94	4.85
49	21	27	15	26	26	23	105	162	75	104	130	115.2	3.76	9.78	4.41	3.55	5.01	5.30
50	16	24	16	24	25	21	64	48	64	120	100	79.2	1.39	1.62	2.33	3.31	2.92	2.31
51	25	28	14	28	28	24.6	100	112	56	140	112	104	2.14	4.55	2.11	4.38	3.29	3.29
52	16	19	13	21	26	19	48	57	39	84	78	61.2	1.53	2.78	1.64	2.92	2.74	2.32
53	14	17	15	25	28	19.8	70	102	75	100	140	97.4	1.76	6.08	1.91	3.66	5.71	3.82
54	17	19	14	26	29	21	85	95	70	104	116	94	3.10	5.17	3.58	3.99	4.23	4.02
55	15	18	19	38	29	23.8	75	90	95	152	116	105.6	2.67	5.01	5.59	5.41	4.57	4.65
56	14	24	18	24	28	21.6	70	120	90	96	112	97.6	2.98	6.46	4.18	2.96	3.61	4.04
57	15	22	16	26	30	21.8	75	88	80	130	150	104.6	2.61	3.82	2.88	4.12	4.71	3.63
58	14	24	12	25	29	20.8	56	96	48	100	145	89	1.81	4.38	2.37	3.35	5.08	3.40
59	18	24	14	29	35	24	72	144	56	116	140	105.6	2.87	6.05	2.27	4.20	4.38	3.95
60	14	22	16	28	32	22.4	56	66	64	112	128	85.2	2.81	3.02	3.42	3.38	4.44	3.41
61	18	20	15	26	28	21.4	72	120	60	104	112	93.6	2.51	5.12	2.86	3.16	4.39	3.61
62	15	19	14	32	32	22.4	75	95	70	160	128	105.6	2.33	4.64	2.80	5.38	4.06	3.84
63	16	27	15	35	38	26.2	80	108	75	175	152	118	2.27	3.59	2.49	6.48	5.40	4.04
64	14	16	9	25	39	20.6	70	80	45	100	195	98	2.71	3.34	1.75	3.49	6.63	3.58
65	18	24	17	29	36	24.8	72	144	68	116	144	108.8	2.10	4.05	2.28	4.30	5.04	3.55
66	15	22	19	35	32	24.6	45	66	57	140	160	93.6	1.91	3.43	2.42	4.91	5.34	3.61
67	16	16	9	34	31	21.2	64	64	36	136	186	97.2	2.08	3.38	1.84	4.49	6.64	3.68
68	14	18	10	32	29	20.6	70	90	50	128	116	90.8	2.56	3.58	2.14	3.74	3.27	3.06
69	15	14	9	36	30	20.8	75	28	45	180	150	95.6	2.84	1.08	2.01	5.08	4.92	3.18
70	10	15	11	31	27	18.8	40	60	55	155	108	83.6	1.42	3.52	2.81	5.97	4.82	3.71
71	22	16	9	29	28	20.8	88	80	45	145	112	94	3.33	4.51	2.23	5.22	3.99	3.86
72	10	12	8	28	26	16.8	40	72	40	140	104	79.2	1.42	3.75	1.88	5.52	4.61	3.44

73	15	13	18	29	30	21	60	26	90	145	120	88.2	2.23	1.22	3.69	5.03	4.07	3.25
74	15	15	9	29	24	18.4	60	90	45	116	96	81.4	2.17	4.91	2.12	4.11	3.19	3.30
75	22	22	14	25	30	22.6	88	110	56	100	120	94.8	2.87	5.34	2.93	3.65	4.98	3.95
76	24	24	15	24	28	23	120	72	60	144	140	107.2	3.16	4.36	2.96	5.98	5.38	4.37
77	25	26	8	26	26	22.2	100	130	40	130	104	100.8	3.48	7.23	1.97	4.54	3.58	4.16
78	26	24	15	35	24	24.8	130	96	75	210	120	126.2	3.85	4.27	2.77	5.46	4.06	4.08
79	21	28	14	31	26	24	84	112	56	124	104	96	2.61	6.60	2.93	4.36	3.30	3.96
80	18	26	14	36	32	25.2	90	104	70	180	160	120.8	2.89	4.59	2.87	6.19	5.63	4.43
81	19	24	16	25	26	22	95	96	80	125	130	105.2	3.08	4.83	3.78	4.08	4.76	4.10
82	15	26	14	26	32	22.6	60	104	70	104	128	93.2	1.96	4.57	4.09	3.35	4.08	3.61
83	16	24	15	24	31	22	64	96	75	144	124	100.6	2.48	4.55	3.60	5.39	4.61	4.13
84	18	22	16	28	26	22	72	66	80	140	104	92.4	2.48	2.73	3.14	4.12	3.19	3.13
85	15	26	18	26	30	23	60	78	72	104	120	86.8	1.93	4.40	3.56	3.31	3.95	3.43
86	14	22	14	24	26	20	56	66	56	120	104	80.4	2.03	3.57	2.62	4.67	4.19	3.42
87	16	26	15	26	30	22.6	64	104	60	104	120	90.4	1.99	4.85	2.50	3.69	3.91	3.39
88	18	24	16	32	31	24.2	54	72	64	160	124	94.8	1.32	3.22	2.89	5.31	4.18	3.39
89	13	18	14	25	32	20.4	65	90	70	150	160	107	2.11	5.35	2.00	5.76	6.35	4.31
90	22	19	15	31	31	23.6	110	95	75	124	155	111.8	4.35	4.79	3.69	3.99	5.10	4.38
91	14	16	14	36	31	22.2	70	80	70	180	124	104.8	2.88	3.42	3.02	6.41	3.97	3.94
92	17	18	15	24	26	20	85	90	45	144	104	93.6	3.26	4.64	2.37	5.26	3.53	3.81
93	18	15	14	24	24	19	90	75	70	120	120	95	3.26	4.61	3.56	4.62	4.31	4.07
94	16	14	9	25	25	17.8	48	42	45	125	100	72	1.53	2.18	1.97	4.86	3.05	2.72
95	15	22	19	26	26	21.6	45	66	76	130	156	94.6	1.58	3.96	4.45	4.77	5.71	4.10
96	14	25	15	25	36	23	28	50	60	125	216	95.8	0.96	2.29	2.49	3.35	6.00	3.02
97	18	26	14	29	35	24.4	54	78	56	87	175	90	1.76	4.45	2.39	3.11	6.77	3.69
98	9	10	11	27	35	18.4	27	30	55	135	140	77.4	0.88	1.52	2.68	4.39	4.80	2.85
99	8	9	10	26	26	15.8	32	36	30	104	130	66.4	1.04	1.69	1.19	3.90	4.62	2.49
100	21	26	11	28	24	22	63	104	33	112	96	81.6	2.26	5.72	1.50	4.38	3.80	3.53
101	25	22	14	29	28	23.6	125	88	56	116	112	99.4	5.31	5.40	2.81	3.92	4.02	4.29
102	20	25	15	34	30	24.8	100	100	60	170	150	116	3.18	4.35	2.80	5.47	4.52	4.06
103	25	24	16	35	27	25.4	100	72	64	175	108	103.8	3.89	3.59	2.90	5.69	3.54	3.92
104	10	26	14	36	36	24.4	40	104	56	108	180	97.6	1.42	5.19	2.20	3.95	5.42	3.64
105	18	21	15	34	36	24.8	72	63	60	170	144	101.8	2.39	2.91	3.26	5.51	4.84	3.78
106	14	23	19	32	24	22.4	70	115	95	128	120	105.6	2.69	5.45	3.78	4.16	4.20	4.06
107	15	25	14	35	24	22.6	45	100	56	140	96	87.4	1.45	4.70	2.30	5.35	3.58	3.47
108	16	21	15	36	25	22.6	48	105	75	144	100	94.4	1.71	4.99	3.03	4.97	3.39	3.62
109	17	22	15	37	26	23.4	68	66	60	148	78	84	2.80	4.05	2.96	5.45	2.75	3.60
110	17	23	16	32	25	22.6	68	92	64	128	100	90.4	2.62	4.23	3.12	4.57	3.47	3.60

111	18	12	11	36	29	21.2	72	48	44	180	145	97.8	2.80	2.16	2.31	6.41	4.52	3.64
112	13	16	18	31	27	21	52	64	72	155	108	90.2	1.91	3.48	3.77	5.91	4.06	3.82
113	15	22	14	32	29	22.4	60	110	70	96	87	84.6	1.96	4.98	3.32	3.45	2.91	3.32
114	14	20	15	34	30	22.6	56	80	60	102	120	83.6	1.82	3.31	2.22	3.30	3.73	2.88
115	14	21	12	32	29	21.6	70	84	48	128	145	95	2.42	4.66	2.19	4.45	5.38	3.82
116	14	18	13	32	39	23.2	70	72	52	160	156	102	2.77	3.72	2.52	5.62	5.96	4.11
117	18	22	14	28	25	21.4	90	66	42	112	125	87	2.93	3.29	1.65	4.33	4.31	3.30
118	15	16	15	27	36	21.8	60	32	45	81	144	72.4	2.19	1.41	2.20	2.79	5.30	2.78
119	15	14	11	29	25	18.8	60	70	44	145	125	88.8	1.55	4.22	2.02	5.19	4.59	3.52
120	16	19	11	24	36	21.2	64	76	55	96	144	87	2.33	4.07	1.85	2.74	4.92	3.18
121	14	20	14	29	25	20.4	56	80	56	145	100	87.4	2.04	3.15	2.36	3.54	2.97	2.81
122	11	28	15	25	32	22.2	55	112	75	100	128	94	1.73	5.30	3.25	2.74	4.31	3.47
123	16	23	13	24	25	20.2	80	138	52	120	100	98	3.28	6.03	2.11	3.79	3.64	3.77
124	14	21	14	26	28	20.6	70	84	56	130	140	96	2.77	3.99	2.42	4.75	3.98	3.58
125	15	23	15	27	36	23.2	75	92	60	135	180	108.4	2.61	4.74	2.39	4.40	6.08	4.04
126	14	20	13	26	30	20.6	56	80	65	104	150	91	2.01	3.90	3.06	3.27	4.79	3.41
127	15	25	14	25	32	22.2	45	100	70	100	128	88.6	1.64	4.26	1.84	2.85	4.25	2.97
128	17	27	19	24	23	22	68	135	95	96	92	97.2	2.22	6.53	3.95	2.98	2.83	3.70
129	15	26	15	28	28	22.4	75	130	60	112	140	103.4	2.74	7.58	2.74	4.14	5.29	4.50
130	18	24	12	29	32	23	90	120	60	116	128	102.8	3.11	5.95	2.86	3.50	4.19	3.92
131	19	25	14	25	28	22.2	95	100	56	100	140	98.2	3.19	4.37	2.58	3.27	4.82	3.64
132	12	23	12	26	29	20.4	60	92	60	104	116	86.4	1.95	5.52	2.85	4.18	4.96	3.89
133	15	14	15	27	29	20	60	42	60	108	145	83	2.37	2.12	3.25	3.38	5.05	3.23
134	16	19	13	29	31	21.6	80	57	65	145	124	94.2	3.00	3.04	3.45	5.45	4.50	3.89
135	14	16	14	24	26	18.8	56	64	56	96	104	75.2	2.08	3.35	2.81	3.49	3.97	3.14
136	15	10	19	24	25	18.6	60	40	76	96	100	74.4	2.19	2.04	3.66	3.29	3.70	2.98
137	13	13	18	28	26	19.6	65	52	90	140	130	95.4	2.12	2.53	3.46	4.80	4.68	3.52
138	15	15	11	29	27	19.4	60	60	66	116	108	82	1.95	2.82	3.01	3.82	3.79	3.08
139	14	16	14	24	32	20	56	48	84	96	128	82.4	1.88	2.99	4.58	3.64	4.77	3.57
140	15	14	9	28	26	18.4	60	42	36	168	104	82	2.41	1.98	1.53	5.36	3.27	2.91
141	13	16	8	26	32	19	52	32	32	104	128	69.6	1.70	1.41	1.77	3.97	5.04	2.78
142	14	12	9	24	32	18.2	70	48	36	96	128	75.6	2.28	2.26	1.43	3.40	4.43	2.76
143	15	18	9	14	25	16.2	60	72	36	56	100	64.8	2.39	3.61	1.53	1.99	3.29	2.56
144	13	14	7	15	26	15	52	70	28	60	104	62.8	1.34	3.37	1.22	2.01	3.58	2.30
145	14	16	20	16	24	18	56	48	80	64	120	73.6	1.80	2.85	4.82	2.48	4.32	3.25
146	15	17	14	17	33	19.2	60	34	56	68	132	70	1.96	1.40	2.07	2.41	4.13	2.39
147	16	18	15	16	26	18.2	64	90	60	64	130	81.6	2.08	3.59	2.40	2.49	4.04	2.92
148	14	13	10	18	24	15.8	56	52	50	90	120	73.6	1.67	2.57	2.40	2.94	4.30	2.78

149	15	15	10	25	26	18.2	60	45	40	100	78	64.6	1.96	1.55	1.88	3.81	2.84	2.41
150	21	26	19	29	35	26	84	130	57	145	175	118.2	2.65	5.24	2.00	5.50	5.78	4.23
151	22	25	14	27	31	23.8	110	100	56	108	155	105.8	4.35	5.25	3.23	4.16	6.14	4.62
152	14	24	14	32	26	22	56	96	42	160	130	96.8	1.82	4.33	1.75	5.50	3.91	3.46
153	20	21	15	25	32	22.6	80	84	60	100	128	90.4	2.52	3.83	3.11	3.01	3.76	3.25
154	14	12	20	27	26	19.8	56	48	80	108	104	79.2	1.84	2.61	4.51	3.43	3.32	3.14
155	15	16	14	28	24	19.4	60	48	42	140	96	77.2	2.01	2.34	1.81	5.01	3.31	2.90
156	21	29	24	24	31	25.8	84	116	96	96	124	103.2	2.22	4.45	3.46	3.11	3.79	3.41
157	15	25	19	35	26	24	60	100	76	175	130	108.2	1.94	5.43	4.62	6.04	4.75	4.56
158	14	24	14	32	28	22.4	56	120	70	128	112	97.2	2.44	7.22	4.48	5.11	4.52	4.75
159	15	27	15	36	32	25	60	135	75	144	160	114.8	2.56	8.87	4.59	5.88	6.77	5.73
160	14	18	12	34	30	21.6	56	72	48	136	120	86.4	2.39	4.19	2.90	5.41	4.99	3.98
161	11	19	16	38	26	22	44	76	80	152	104	91.2	1.30	3.59	3.84	4.48	3.22	3.29
162	20	20	14	32	30	23.2	100	80	56	160	150	109.2	3.36	3.56	2.69	5.04	4.89	3.91
HPK4	21	22	14	34	32	24.6	84	110	56	136	128	102.8	3.38	5.82	2.72	5.93	5.41	4.65
M249	25	26	16	38	35	28	100	104	64	152	140	112	3.12	4.74	2.24	4.99	5.11	4.04

Appendix IV: Values of RILs for root traits

RIL	ROOTS		
	root length (cm)	root fresh weight (g)	root dry weight (g)
1	53	3.031	0.83
2	48	2.623	2.08
3	59	1.747	1.36
4	66	3.971	2.97
5	46	1.343	1.11
6	58	1.522	1.26
7	53	1.24	0.96
8	54	2.775	2.35
9	49	1.964	1.454
10	57	3.517	2.76
11	54	2.8	2.35
12	48	1.485	1.12
13	44	0.992	0.563
14	63	2.56	2.128
15	89	1.47	1.14
16	64	1.9	1.48
17	55	0.975	0.58
18	45	1.14	0.83
19	69	1.47	0.98
20	55	3.75	2.49
21	64	3.52	2.11
22	64	0.35	0.09
23	50	0.75	0.48
24	66	3.96	3.18
25	69	4.65	3.94
26	66	2.56	1.81
27	62	0.78	0.19
28	66	0.424	0.28
29	56	3.36	2.87
30	64	0.462	0.13
31	46	1.1	0.25
32	54	1.93	1.162
33	60	3.03	2.72
34	58	3.77	3.28
35	57	2.87	1.47
36	55	2.44	1.058
37	68	1.94	1.11
38	66	4.29	2.87
39	46	2.6	1.86
40	54	5.8	4.67
41	66	6.01	4.089
42	61	6	3.86
43	52	2.24	1.72
44	50	5.5	4.21
45	60	2.23	1.85
46	57	3.68	2.11
47	64	6.26	5.189
48	63	2.54	1.84
49	50	2.6	1.35
50	65	2.09	1.14
51	65	4.87	3.44
52	61	1.55	0.97
53	42	1.09	0.47
54	64	1.72	1.17
55	72	2.42	1.97
56	70	2.5	2.18
57	64	3.65	2.87
58	68	1.86	1.55
59	66	2.4	1.97

60	59	1.55	1.14
61	59	1.54	0.89
62	61	3.48	2.82
63	62	1.86	1.27
64	75	2.73	2.17
65	67	0.3	0.15
66	66	3.3	2.82
67	67	4.7	3.94
68	55	1.45	0.98
69	60	0.81	0.17
70	63	3.5	3.08
71	69	2.1	1.68
72	69	4.56	3.48
73	47	4.03	3.61
74	60	4.22	3.58
75	64	1.35	0.87
76	71	5.45	4.15
77	63	3.17	2.6
78	47	1.16	0.67
79	46	2.25	1.35
80	66	5.1	4.28
81	58	4.72	3.97
82	69	3.17	2.53
83	64	2.98	2.17
84	65	3.22	2.74
85	58	2.45	1.83
86	66	3.04	2.57
87	49	1.7	1.14
88	70	6.22	5.09
89	73	5.98	4.85
90	73	2.99	2.13
91	61	3.38	2.45
92	65	2.9	1.87
93	76	2.94	2.18
94	74	2.53	2.13
95	62	3.02	2.48
96	66	3.56	2.97
97	63	2.8	2.48
98	53	2.65	2.19
99	52	2.75	2.28
100	73	5.89	4.94
101	66	4.23	3.58
102	63	2.68	2.13
103	79	3	2.27
104	48	1.89	0.09
105	71	5.12	4.47
106	58	3.2	2.15
107	50	3.43	2.81
108	66	2.76	2.17
109	64	2.87	2.11
110	64	2.62	1.98
111	63	1.97	0.84
112	54	1.54	0.97
113	63	1.06	0.28
114	66	3.2	2.17
115	64	2.6	2.19
116	64	3.27	2.57
117	73	1.25	0.68
118	67	3.2	2.48
119	56	0.63	0.14
120	66	1.87	0.99
121	51	0.5	0.15
122	68	2.55	1.81
123	69	1.66	1.17

124	47	1.35	0.97
125	49	0.44	0.08
126	50.5	1.37	0.92
127	69	3.17	2.54
128	70	5.87	4.21
129	58	1.47	0.98
130	59	0.91	0.18
131	61	2.24	1.89
132	66	0.52	0.17
133	55	1.01	0.87
134	71	5.89	4.28
135	63	4.12	3.45
136	60	2.71	1.58
137	55.5	2.22	1.87
138	66	3.25	2.84
139	73	4.25	3.15
140	62	3.27	2.74
141	49	4.29	3.15
142	64	5.89	4.98
143	63	3.25	2.74
144	60	1.69	1.11
145	40	0.65	0.09
146	54	2.11	1.96
147	48	1.33	0.84
148	50	1.98	1.14
149	63	3.11	2.37
150	66	2.55	1.76
151	64	1.56	0.85
152	61	0.44	0.15
153	71	4.82	3.23
154	58	3.22	2.62
155	64	4.57	3.33
156	61	3.25	2.19
157	58	1.94	1.15
158	49	0.64	0.09
159	67	0.85	0.04
160	69	0.35	0.08
161	54	0.24	0.05
162	60	0.99	0.25
HPK4	63	1.34	0.97
M249	51	0.42	0.08

Appendix V: Values of RILs for biochemical traits

RIL	CHLOROPHYLL (mg/g)		CAROTENOID (mg/g)		PROLINE (μmoles/g)		MDA (nmoles/g)		RWC (%)		MSI (%)	
	CH_C17	CH_S17	CAR_C17	CAR_S17	PRO_C17	PRO_S17	MDA_C17	MDA_S17	RWC_C17	RWC_S17	MSI_C17	MSI_S17
1	12.81	10.41	0.26	0.15	0.48	1.4	14.96	20.25	94.26	82.39	0.98	0.87
2	23.75	14.45	0.24	0.19	0.131	2.5	12.36	18.59	88.52	75.27	0.94	0.82
3	16.2	7.79	0.84	0.57	0.439	2.3	18.44	24.15	96.38	80.29	0.96	0.74
4	24.63	22.12	0.78	0.57	0.07	1.5	14.57	19.51	89.32	79.61	0.95	0.88
5	14.43	6.5	0.84	0.64	0.64	2.3	10.24	17.26	87.27	80.28	0.88	0.81
6	13.08	5.67	0.73	0.42	0.43	2.4	15.64	28.26	90.11	77.53	1	0.94
7	19.99	12.33	0.57	0.66	0.11	2.5	13.22	29.32	89.18	82.69	0.9	0.87
8	13.79	7.96	0.64	0.06	0.45	1.9	15.21	24.26	92.54	75.96	1	0.93
9	11.33	5.32	0.39	0.48	0.63	1.6	15.32	24.12	91.27	80.28	1	0.93
10	15.25	18.25	0.25	0.3	0.68	2.4	14.21	26.58	90.64	75.39	1	0.89
11	15.61	19.82	0.38	0.41	0.47	2.4	10.57	19.15	87.32	69.58	1	0.88
12	15.56	13.05	0.49	0.61	0.41	3.5	14.21	30.15	96.89	82.63	0.97	0.9
13	15.83	12.55	0.23	0.07	0.51	2.8	16.22	29.59	90.32	69.09	1	0.93
14	18.47	12.65	0.55	0.46	0.12	2.5	14.29	26.16	94.52	80.27	0.88	0.77
15	18.33	17.57	0.51	0.4	0.59	1.6	15.36	26.57	88.59	76.59	0.87	0.83
16	18.46	13.25	0.53	0.34	0.58	3.4	14.97	30.24	91.28	81.23	0.89	0.79
17	18.56	17.26	0.59	0.36	0.49	1.3	19.78	32.12	94.38	71.29	0.9	0.82
18	15.02	13.04	0.22	0.19	0.38	1.9	16.57	24.16	96.58	84.23	0.97	0.93
19	14.39	13.68	0.45	0.37	0.52	1.7	17.69	29.34	95.25	83.67	0.92	0.88
20	13.92	13.58	0.36	0.19	0.48	1.8	15.87	28.11	89.32	72.88	0.96	0.86
21	17.53	15.53	0.95	0.32	0.39	3	14.97	29.15	87.59	74.21	0.96	0.94
22	9.91	11.76	0.72	0.81	0.12	2.2	15.38	26.15	84.39	69.11	0.88	0.73
23	14.36	9.43	0.57	0.41	0.12	2.5	12.25	19.15	87.26	77.23	0.89	0.81
24	14.36	11.35	0.32	0.67	0.51	2.8	14.92	19.57	92.19	84.27	0.97	0.85
25	24.64	16.14	0.75	0.68	0.59	1.8	13.62	20.58	93.67	81.22	1	0.96
26	13.52	8.68	0.11	0.36	0.64	2.4	15.23	24.56	92.57	83.98	0.96	0.89
27	20.26	14.47	0.57	0.48	0.33	2.6	12.33	25.36	88.27	70.29	0.88	0.85
28	17.63	15.16	0.57	0.24	0.69	1.93	18.65	22.56	84.89	72.91	0.87	0.84
29	10.23	8.69	0.98	0.76	0.56	2.1	12.54	28.45	90.98	73.59	1	0.91
30	13.33	8.88	0.63	0.52	0.38	2	14.62	29.51	92.37	82.96	0.89	0.82
31	7.34	8.13	0.38	0.47	0.69	1.9	15.68	21.29	91.27	84	1	0.87
32	14.09	18.14	0.76	0.41	0.2	2.33	15.55	23.26	84.29	77.53	0.97	0.92
33	14.81	6.92	0.56	0.77	0.22	1.44	16.57	24.36	83.39	74.29	0.92	0.78
34	12.48	15.54	0.69	0.46	0.11	1.19	13.29	25.56	91.27	84.97	0.89	0.73
35	17.25	12.02	0.99	0.45	0.68	2.44	14.77	23.78	90.26	80.19	1	0.86

36	12.99	7.44	0.66	0.54	0.74	1.83	12.87	25.36	87.62	77.52	0.97	0.87
37	14.76	12.64	0.57	0.49	0.54	2.44	16.92	32.59	86.27	71.39	0.97	0.87
38	10.82	7.98	0.37	0.13	0.48	2.68	14.56	31.58	89.57	73.98	0.87	0.78
39	14.66	10.53	0.8	0.84	0.51	1.29	12.35	23.53	92.69	80.52	0.84	0.71
40	18.71	13.7	0.75	0.46	0.54	1.93	12.28	21.48	89.32	75.54	0.87	0.74
41	16.58	14.78	0.81	0.31	0.37	2.31	14.59	22.98	87.59	77.59	0.92	0.85
42	9.24	8.12	0.55	0.53	0.56	1.3	15.54	26.98	92.35	84.89	0.96	0.95
43	19.98	16.84	0.32	0.85	0.14	2.22	16.38	29.54	93.35	86.69	0.84	0.79
44	10.39	7.19	0.84	0.14	0.11	1.98	15.25	24.59	87.36	74.52	0.85	0.81
45	12.52	12.31	0.62	0.51	0.07	1.8	18.56	29.59	86.35	72.83	0.83	0.82
46	10.25	2.38	0.49	0.92	0.26	1.38	14.25	20.48	91.28	78.64	0.92	0.89
47	11.58	6.01	0.17	0.37	0.14	1.76	15.26	29.54	89.37	73.33	0.94	0.82
48	13.54	8.76	0.64	0.37	0.27	1.79	12.56	21.26	87.59	72.28	1	0.83
49	8.48	3.08	0.69	0.34	0.61	3.51	11.29	26.51	87.31	71.25	1	1
50	9.65	7.28	0.76	0.19	0.63	2.35	14.22	29.12	90.69	76.59	0.92	0.79
51	8.52	8.11	0.77	0.27	0.67	2.31	12.89	24.39	90.3	84.69	0.96	0.83
52	12.84	9.1	0.33	0.87	0.55	2.4	16.69	28.61	88.37	74.59	0.92	0.71
53	10.94	3.13	0.68	0.41	0.38	1.65	14.55	26.27	90.53	78.26	0.89	0.74
54	20.56	18.56	0.36	0.95	0.6	1.43	13.78	22.29	92.57	79.28	1	0.89
55	24.11	22.58	0.58	0.29	0.51	1.9	16.44	26.35	88.54	79.29	1	0.93
56	13.17	5.05	0.36	0.35	0.95	3.09	11.28	21.36	85.64	78.42	0.98	0.77
57	13.35	11.44	0.28	0.81	0.37	2.91	12.26	24.61	91.29	79.25	1	1
58	10.06	8.18	0.48	0.39	0.47	2.78	15.64	26.48	94.29	81.22	0.95	0.72
59	10.46	12.51	0.89	0.74	0.49	1.54	16.45	30.59	93.61	80.66	1	0.88
60	19.28	14.13	0.79	0.49	0.25	2.26	12.89	18.15	92.27	85.39	1	0.83
61	15.26	14.61	0.37	0.4	0.46	2.53	18.35	25.64	88.37	76.28	0.99	0.97
62	19.66	13.63	0.63	0.52	0.38	2.97	19.65	26.51	84.29	75.98	0.97	0.82
63	23.58	20.65	0.81	0.32	0.91	3.13	14.85	17.15	88.62	74.52	0.97	0.87
64	10.36	8.16	0.41	0.91	0.89	2.88	14.56	19.15	87.36	78.25	0.96	0.87
65	11.64	14.69	0.67	0.47	0.58	2.28	19.36	29.15	87.29	71.29	0.89	0.69
66	13	10.66	0.53	0.19	0.56	1.97	16.35	24.15	95.31	84.78	1	0.84
67	18.34	16.91	0.63	0.11	0.23	2.2	16.39	29.15	92.39	82.29	0.87	0.7
68	16.1	9.06	0.88	0.64	0.27	2.19	15.62	24.81	94.29	86.93	0.95	0.7
69	14.59	12.21	0.73	0.8	0.49	1.36	14.36	19.13	86.32	78.91	0.96	0.84
70	13.41	7.31	0.78	0.74	0.72	3.65	13.31	21.97	88.94	74.59	0.93	0.73
71	12.38	8.14	0.68	0.66	0.56	2.97	17.55	28.15	87.25	72.28	0.92	0.89
72	18.62	7.83	0.42	0.56	0.7	3.14	19.57	27.15	87.29	78.25	0.93	0.82
73	10.55	8.73	0.87	0.37	0.63	2.21	17.52	26.19	84.32	72.59	0.97	0.85
74	10.35	6.52	0.26	0.17	0.76	1.77	15.84	28.49	91.59	80.22	1	0.96

75	12.93	9.53	0.29	0.53	0.58	1.75	18.67	26.59	87.26	82.28	0.95	0.89
76	12.49	9.6	0.34	0.23	0.52	2.26	18.62	27.25	93.67	83.6	0.84	0.71
77	23.98	21.56	0.32	0.62	0.43	2.69	19.62	26.52	93.27	85.28	0.89	0.75
78	11.1	9.6	0.68	0.99	0.38	1.38	14.22	27.31	89.14	74.52	0.95	0.93
79	13.36	7.05	0.61	0.54	0.87	2.87	16.32	25.42	86.31	79.21	0.94	0.84
80	18.67	14.25	0.37	0.42	0.25	2.03	18.39	26.65	88.97	76.26	0.93	0.74
81	12.56	10.58	0.2	0.15	0.43	1.86	14.13	19.59	95.27	83.66	1	0.95
82	7.23	10.51	0.34	0.41	0.68	2.37	18.56	26.65	93.26	85.22	1	0.96
83	20.56	18.98	0.37	0.25	0.66	1.52	19.65	29.54	97.59	85.21	0.92	0.86
84	12.29	10.28	0.62	0.72	0.82	1.98	13.33	29.58	90.32	73.98	0.95	0.92
85	17.12	11.64	0.54	0.78	0.93	2.5	19.54	26.24	96.67	83.67	0.9	0.89
86	8.56	7.64	0.44	0.32	0.42	1.42	18.22	29.61	85.91	72.99	0.96	0.86
87	17.04	17.62	0.55	0.23	0.84	2.33	18.56	24.63	85.29	79.11	1	0.87
88	5.86	4.75	0.43	0.33	0.33	2.58	17.12	32.59	85.39	72.69	1	0.84
89	7.15	6.58	0.55	0.27	0.87	3.02	12.25	21.26	89.59	75.28	0.93	0.89
90	9.75	6.37	0.9	0.62	0.52	1.62	13.65	24.56	90.27	81.29	0.9	0.83
91	7.72	4.58	0.67	0.15	0.25	3.06	19.25	30.25	89.17	74.48	0.87	0.72
92	17.78	18.34	0.66	0.64	0.64	2.12	16.34	27.51	90.28	75.28	0.92	0.84
93	10.56	12.05	0.52	0.53	0.49	2.25	14.16	26.36	94.26	82.81	0.86	0.71
94	5.09	6.57	0.51	0.48	0.86	2.28	16.98	28.62	93.27	84.32	0.91	0.91
95	13.45	14.17	0.32	0.26	0.23	2.22	15.69	29.61	96.27	83.29	0.92	0.77
96	17.95	14.34	0.68	0.58	0.36	3.21	10.26	24.26	89.67	76.68	0.97	0.79
97	6.69	10.51	0.6	0.51	0.21	3.38	16.38	29.25	90.11	72.28	1	1
98	7.17	5.34	0.52	0.68	0.25	2.25	15.39	26.24	89.67	76.27	0.94	0.84
99	16.21	13.58	0.59	0.32	0.24	2.11	14.26	23.39	84.29	68.28	0.92	0.8
100	21.26	20.65	0.51	0.71	0.22	1.22	15.31	26.36	86.37	77.27	1	0.88
101	6.98	6.75	0.71	0.45	0.21	1.55	15.23	23.61	88.94	77.29	0.92	0.87
102	12.58	9.78	0.8	0.41	0.51	2.35	15.15	26.68	85.96	74.91	1	0.92
103	10.59	12.27	0.69	0.77	0.27	2.94	17.63	25.62	92.51	78.28	1	0.98
104	13.52	9.68	0.77	0.97	0.31	2.36	16.96	21.36	93.57	83.81	1	0.94
105	20.56	22.56	0.63	0.34	0.56	2.56	12.37	24.68	89.27	80.87	0.99	0.89
106	15.91	12.65	0.57	0.66	0.39	2.67	15.36	23.61	92.34	80.15	0.87	0.71
107	8.36	4.54	0.74	0.62	0.6	3.02	13.74	24.63	95.68	88.29	0.95	0.83
108	18.45	16.87	0.64	0.62	0.42	1.64	14.29	25.39	97.39	88.77	0.92	0.89
109	8.56	5.78	0.53	0.26	0.42	2.02	15.36	21.39	87.25	74.51	1	0.95
110	21.56	20.11	0.53	0.27	0.46	2.21	17.26	25.68	86.92	74.01	1	0.96
111	13.52	8.13	0.65	0.32	0.9	3.52	15.39	21.39	87.25	75.41	0.94	0.92
112	15.25	12.81	0.57	0.61	0.33	1.38	12.29	23.39	83.39	71.27	0.87	0.75
113	18.42	15.76	0.44	0.74	0.82	3.25	17.29	28.61	95.28	83.29	0.97	0.91

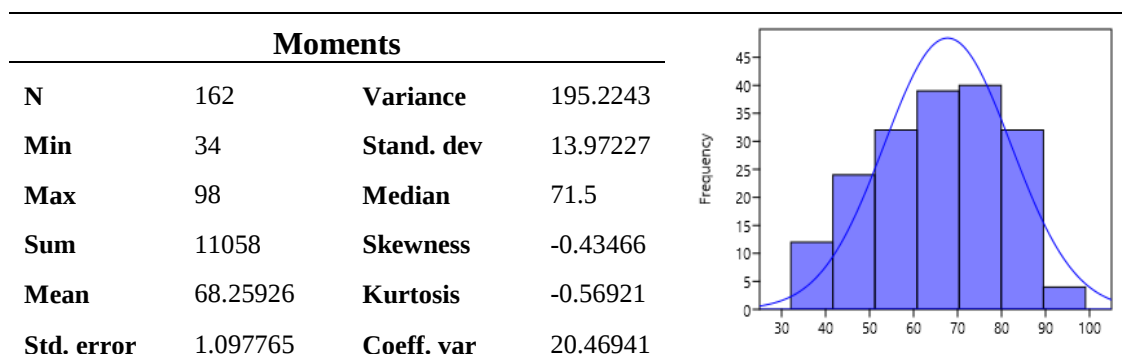
114	9.61	7.59	0.67	0.43	0.25	2.75	12.61	20.69	88.29	71.92	0.92	0.89
115	7.08	7.07	0.72	0.58	0.15	1.21	14.29	29.15	90.27	82.29	0.84	0.79
116	4.58	5.17	0.48	0.28	0.25	1.59	17.38	28.61	90.31	81.22	1	0.92
117	5.27	2.52	0.52	0.78	0.58	1.22	13.32	22.31	89.34	71.08	1	0.89
118	13.39	13.88	0.5	0.42	0.71	2.53	14.29	24.62	92.91	77.45	1	0.91
119	13.42	12.81	0.22	0.29	0.51	2.83	15.31	24.31	95.37	80.17	0.89	0.74
120	16.59	18.36	0.45	0.3	0.1	3.03	12.35	25.68	96.28	88.25	0.87	0.71
121	4.78	1.69	0.46	0.32	0.13	2.43	13.35	25.15	98.69	89.58	0.93	0.86
122	14.5	13.12	0.53	0.47	0.32	1.45	14.35	29.31	90.39	70.29	0.89	0.81
123	20.69	13.12	0.48	0.44	0.37	2.88	15.59	26.11	90.02	76.81	0.89	0.81
124	6.03	2.23	0.51	0.53	0.38	2.05	17.25	25.67	85.27	76.27	0.87	0.79
125	15.69	18.87	0.6	0.59	0.52	2.37	19.25	21.26	84.59	72.59	0.96	0.85
126	15.96	12.02	0.55	0.38	0.38	1.89	16.54	20.16	87.52	76.81	0.87	0.8
127	14.58	12.72	0.58	0.22	0.23	1.69	12.35	29.15	92.36	75.72	1	0.92
128	9.13	8.78	0.83	0.56	0.71	1.83	10.37	27.29	90.07	74.28	0.98	0.88
129	5.85	7.04	0.56	0.74	0.84	3.25	19.45	28.15	98.28	83.58	1	0.89
130	5.05	2.34	0.52	0.61	0.53	1.65	12.48	18.16	89.29	74.28	0.95	0.85
131	5.34	5.93	0.5	0.91	0.73	2.26	13.59	17.15	84.31	69.85	0.88	0.81
132	24.11	20.58	0.58	0.34	0.33	2.14	15.24	32.15	85.06	76.29	1	0.93
133	5.07	9.27	0.49	0.69	0.29	1.35	13.26	23.15	86.22	74.21	0.87	0.8
134	6.43	5.72	0.62	0.38	0.97	2.77	14.26	23.59	85.74	73.54	0.97	0.92
135	5.81	2.34	0.62	0.55	0.57	1.33	13.15	26.15	92.51	79.67	0.91	0.83
136	16.76	13.87	0.69	0.58	0.28	1.24	15.62	29.15	95.27	83.59	1	0.94
137	20.24	18.96	0.39	0.48	0.68	2.28	14.61	21.29	96.38	65.34	1	0.91
138	8.29	6.97	0.71	0.41	0.32	1.23	13.97	25.69	89.34	72.82	0.93	0.86
139	4.48	3.89	0.42	0.39	0.92	2.37	12.54	26.21	86.98	73.28	0.97	0.89
140	13.59	14.58	0.59	0.46	0.32	2.02	17.56	25.15	85.39	71.29	0.96	0.89
141	6.71	3.22	0.67	0.71	0.88	2.05	19.65	31.26	89.41	77.29	0.97	0.92
142	5.68	4.11	0.58	0.49	0.41	2	12.15	25.69	90.09	78.21	0.85	0.75
143	6.23	3.91	0.58	0.32	0.13	2.38	15.65	24.36	89.28	74.2	0.8	0.71
144	18.65	13.54	0.72	0.66	0.23	2.36	15.41	25.15	92.34	85.25	0.9	0.79
145	4.14	3.09	0.47	0.27	0.41	2.49	14	28.65	94.27	80.67	0.94	0.82
146	19.61	13.26	0.59	0.55	0.19	1.36	15.65	21.62	90.36	72.58	0.88	0.87
147	9.12	10.68	0.63	0.67	0.18	2.55	14.65	23.15	88.96	80.29	1	0.91
148	4.92	3.02	0.54	0.4	0.13	2.34	13.65	21.15	86.27	74.29	1	0.96
149	12.35	13.39	0.53	0.47	0.2	2.29	16.65	23.78	86.91	74.28	0.88	0.84
150	12.56	11.25	0.59	0.6	0.65	3.02	19.42	25.31	92.29	81.31	0.94	0.88
151	4.07	2.07	0.4	0.23	0.34	1.51	15.54	29.65	86.12	71.29	0.86	0.8
152	4.33	4.14	0.43	0.79	0.47	2.08	19.54	26.51	85.28	73.44	0.84	0.75

153	18.59	16.68	0.56	0.83	0.55	1.25	10.52	21.29	89.61	71.39	0.89	0.78
154	14.62	10.47	0.43	0.09	0.19	1.51	15.51	26.61	88.23	77.19	1	0.92
155	16.26	18.52	0.66	0.57	0.52	2.08	13.65	28.15	87.29	71.29	1	0.94
156	14.53	14.02	0.55	0.39	0.31	1.76	16.84	23.02	85.29	72.28	1	0.89
157	9.56	8.7	0.34	0.18	0.29	1.52	17.65	23.15	85.3	73.75	0.88	0.72
158	24.25	19.57	0.48	0.31	0.1	1.14	13.51	24.69	90.27	82.64	0.87	0.83
159	9.59	10.21	0.41	0.39	0.19	2.19	12.55	20.08	91.29	87.28	0.95	0.89
160	16.01	12.23	0.69	0.55	0.59	2.35	14.56	25.31	85.23	74.81	0.89	0.8
161	11.98	11.26	0.54	0.61	0.11	2	15.65	21.62	85.48	72.59	0.91	0.86
162	13.28	12.03	0.48	0.54	0.13	2.38	15.65	23.15	92.28	80.67	0.89	0.83
HPK4	12.97	10.59	0.78	0.65	0.46	3.43	13.47	20.83	94.36	86.58	1	0.93
M249	11.46	7.91	0.81	0.58	0.21	2.03	16.84	29.65	87.34	74.44	0.94	0.86

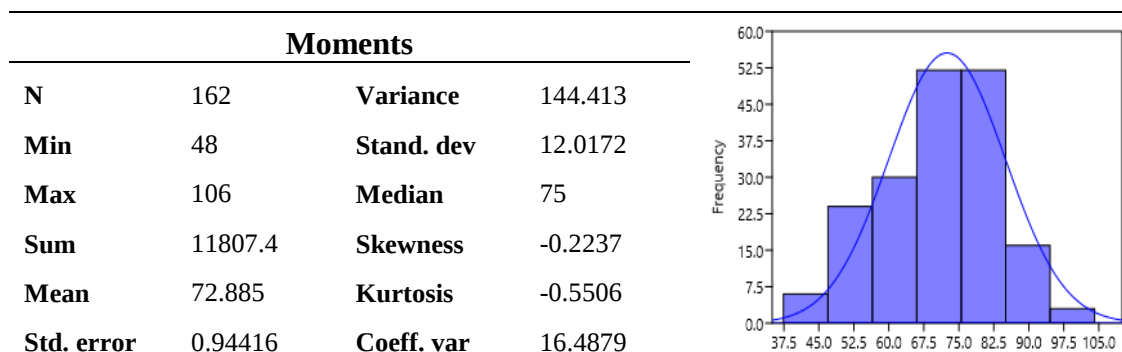
Appendix VI

1. Descriptive statistics and frequency distribution of Plant height under different seasons

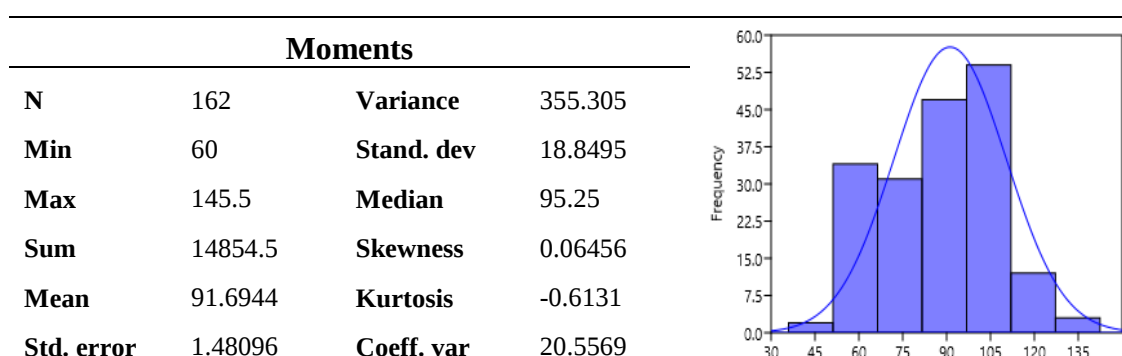
(a) Variable : Plant height (Palampur 2016)



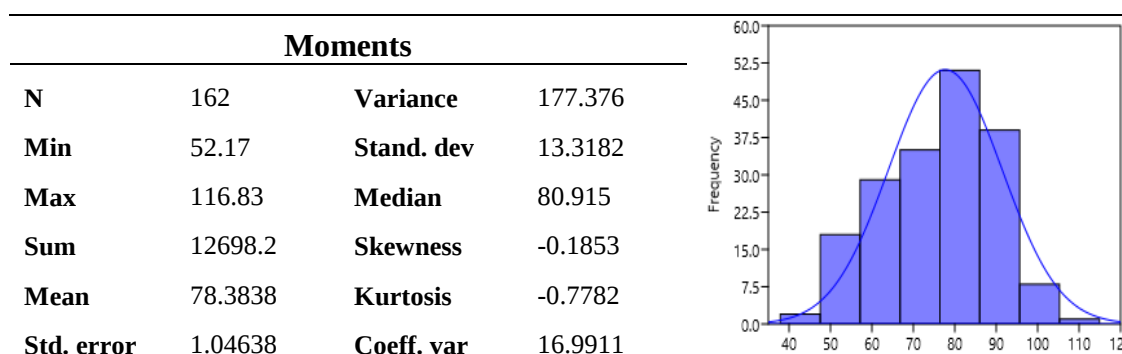
(b) Variable : Plant height (Palampur 2017)



(c) Variable : Plant height (Bajaura 2017)

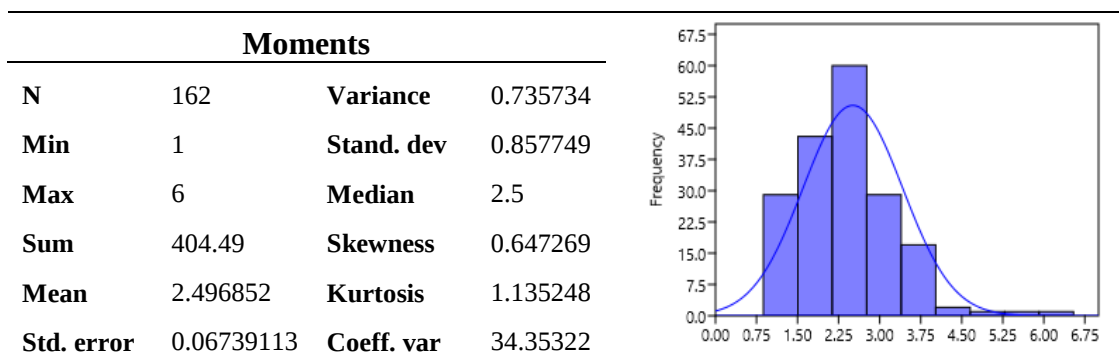


(d) Variable : Plant height (Combined)

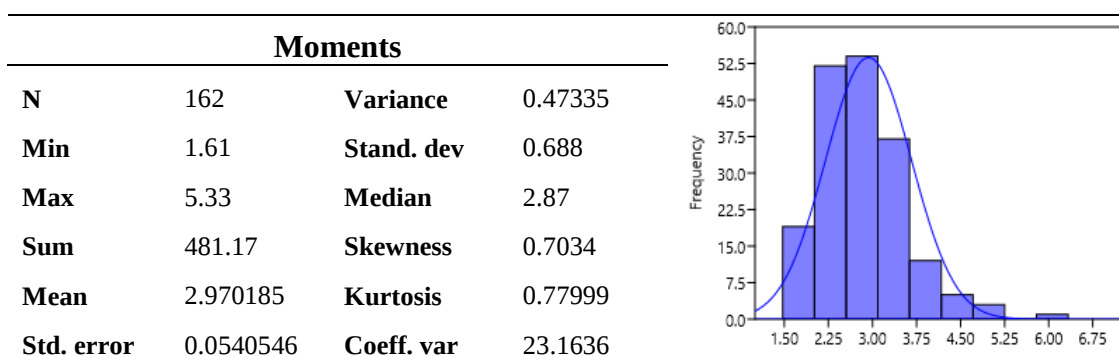


2. Descriptive statistics and frequency distribution of Primary branches under different seasons

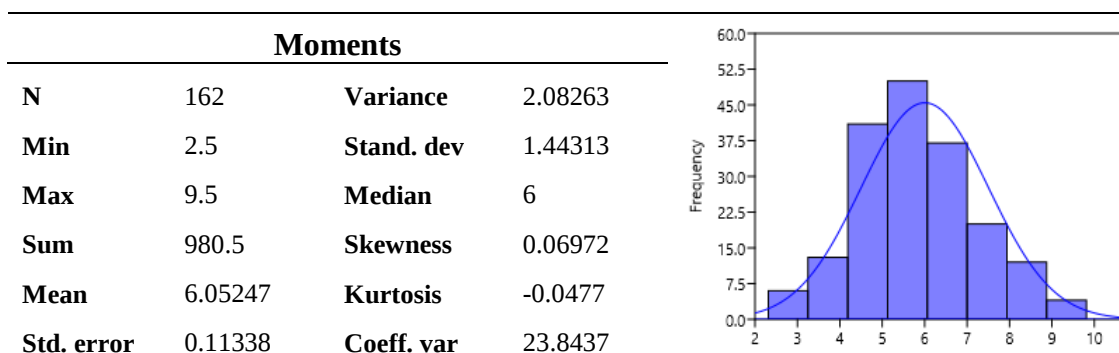
(a) Variable : Primary branches (Palampur 2016)



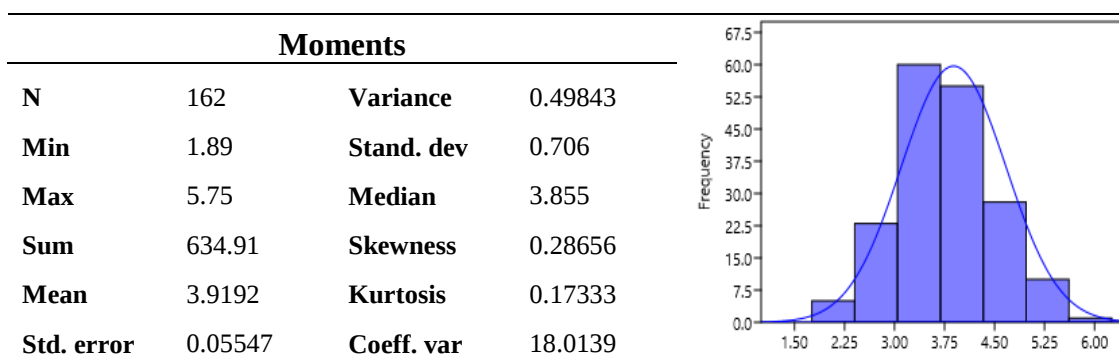
(b) Variable : Primary branches (Palampur 2017)



(c) Variable : Primary branches (Bajaura 2017)

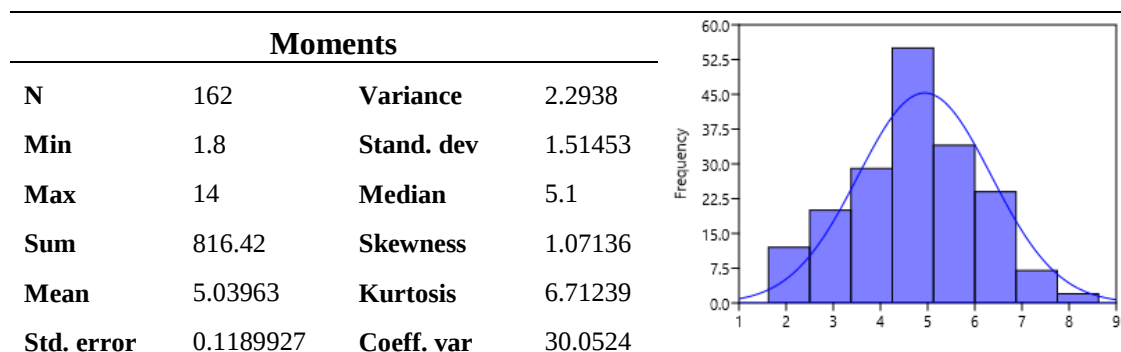


(d) Variable : Primary branches (Combined)

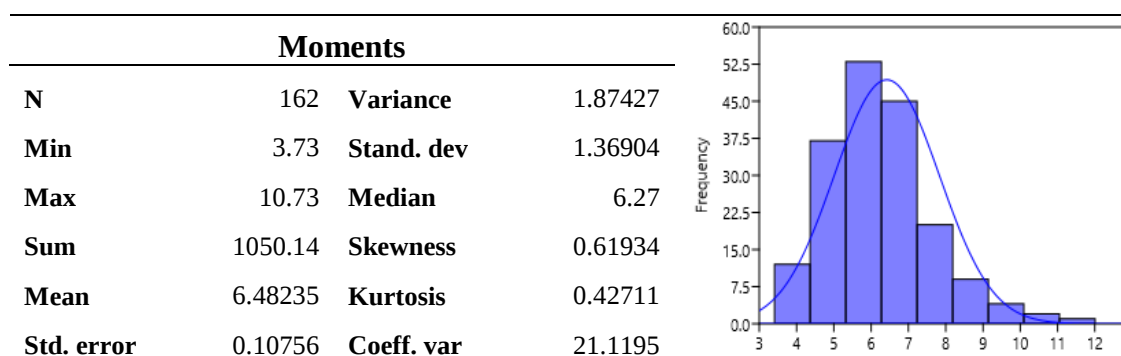


3. Descriptive statistics and frequency distribution of Secondary branches under different seasons

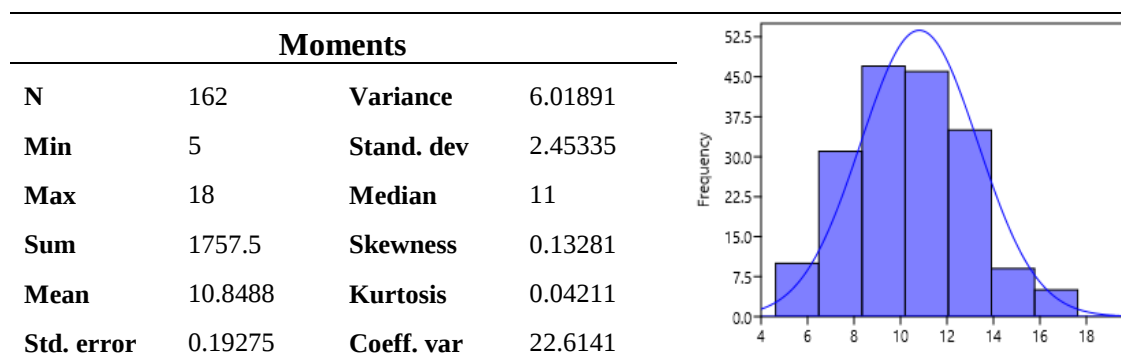
(a) Variable : Secondary branches (Palampur 2016)



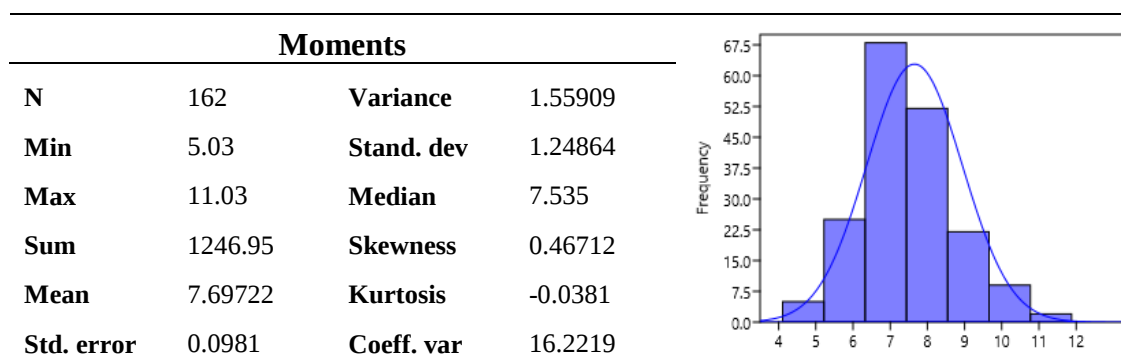
(b) Variable : Secondary branches (Palampur 2017)



(c) Variable : Secondary branches (Bajaura 2017)

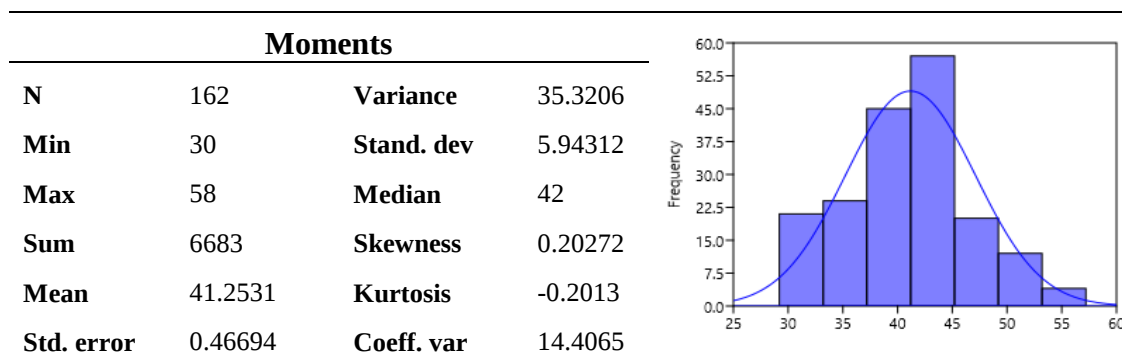


(d) Variable : Secondary branches (Combined)

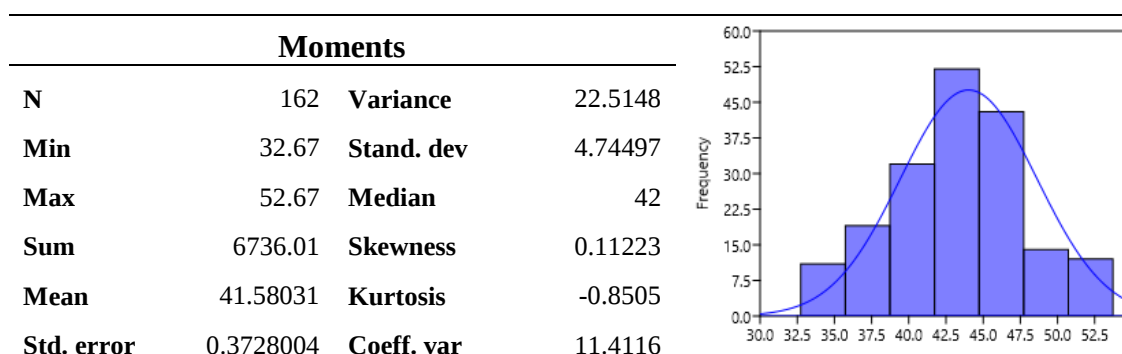


4. Descriptive statistics and frequency distribution of Days to 50% flowering under different seasons

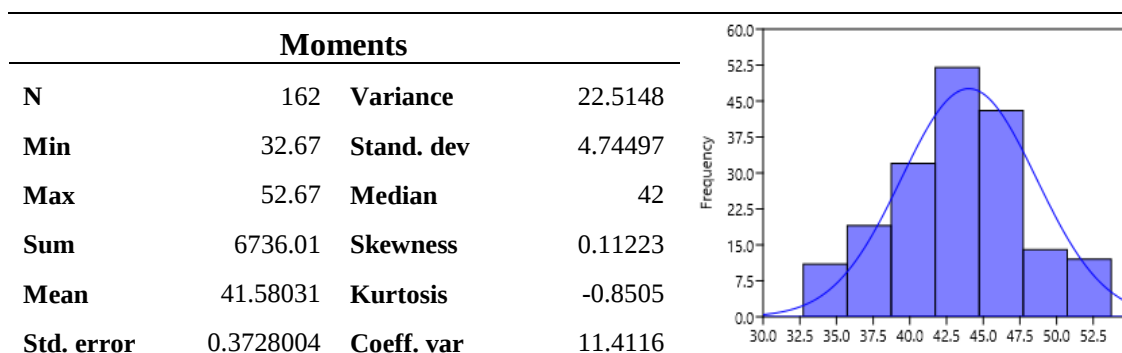
(a) Variable : Days to 50% flowering (Palampur 2016)



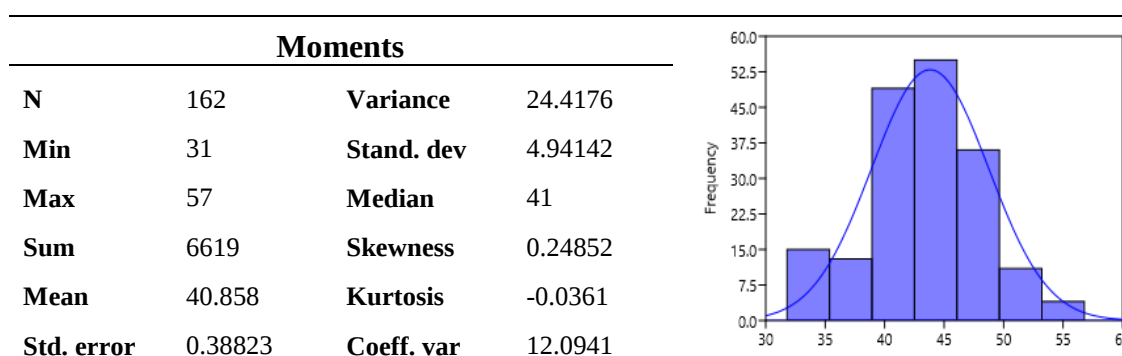
(b) Variable : Days to 50% flowering (Palampur 2017)



(c) Variable : Days to 50% flowering (Bajaura 2017)

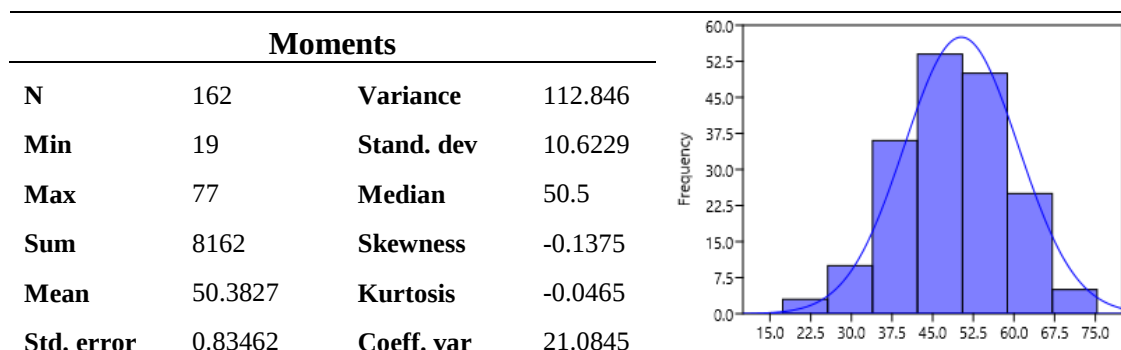


(d) Variable : Days to 50% flowering (Combined)

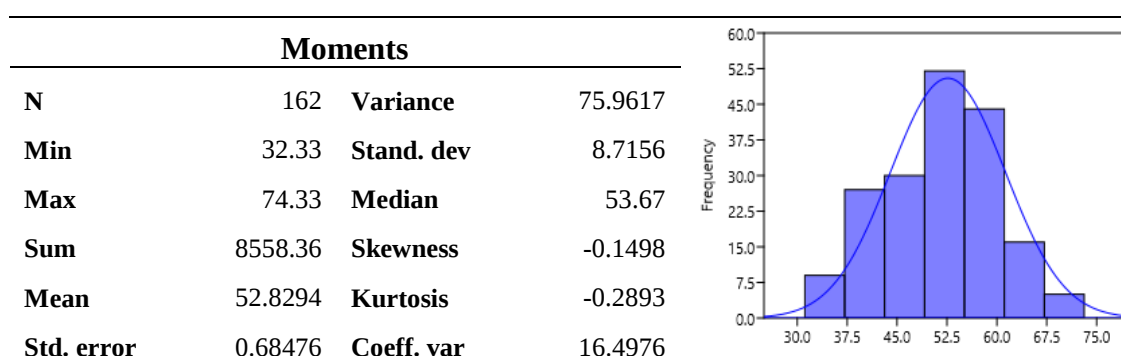


5. Descriptive statistics and frequency distribution of Reproductive period under different seasons

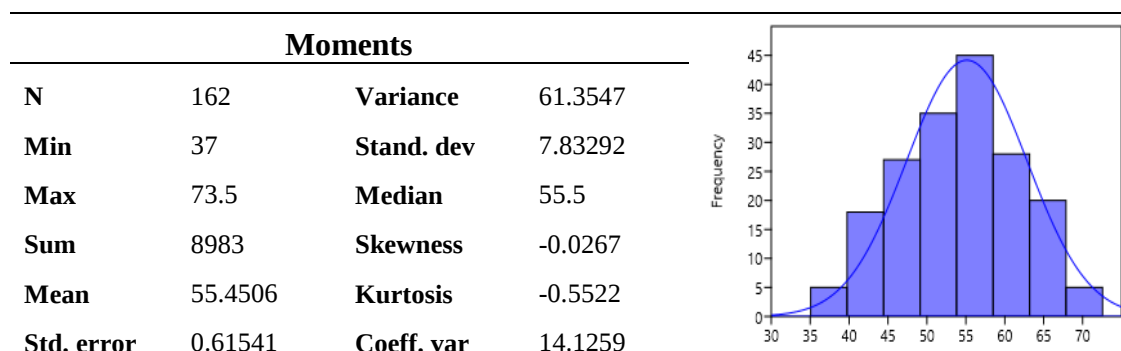
(a) Variable : Reproductive period (Palampur 2016)



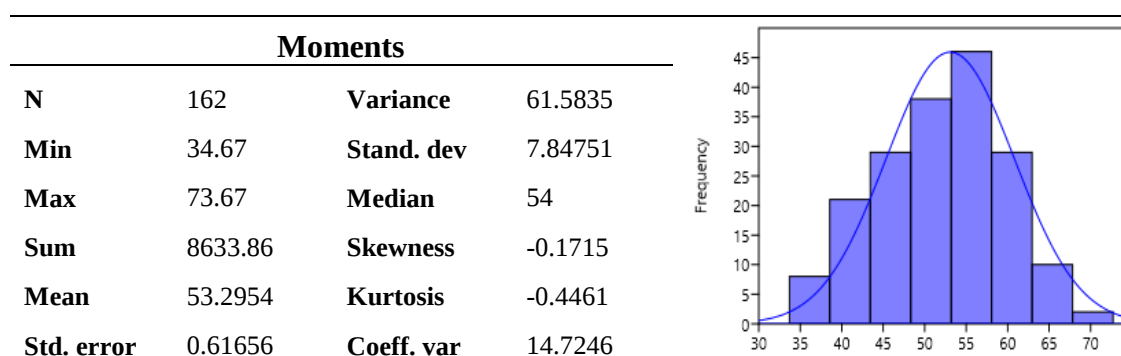
(b) Variable : Reproductive period (Palampur 2017)



(c) Variable : Reproductive period (Bajaura 2017)

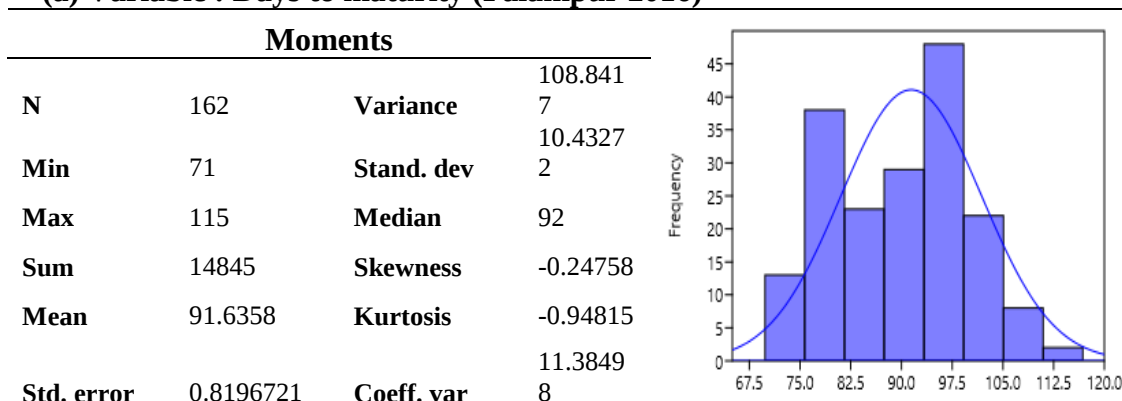


(d) Variable : Reproductive period (Combined)

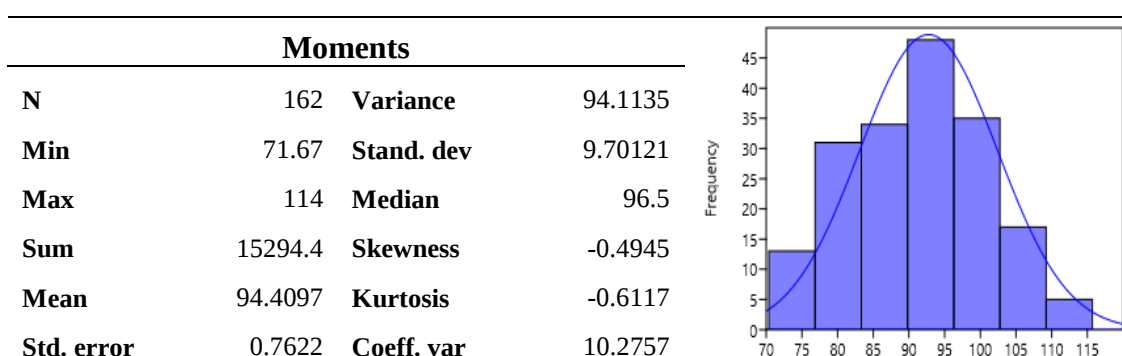


6. Descriptive statistics and frequency distribution of Days to maturity under different seasons

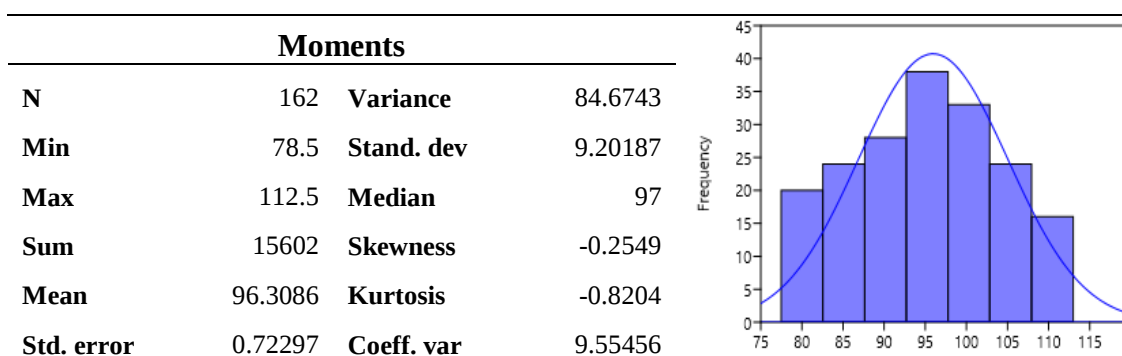
(a) Variable : Days to maturity (Palampur 2016)



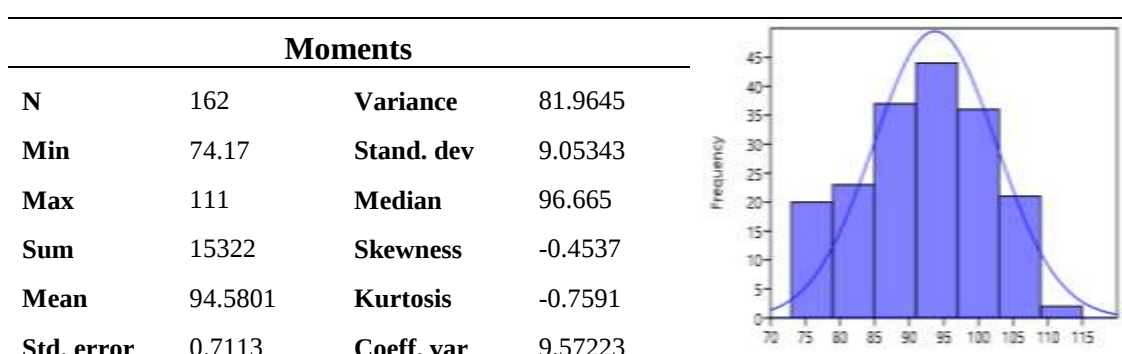
(b) Variable : Days to maturity (Palampur 2017)



(c) Variable : Days to maturity (Bajaura 2017)

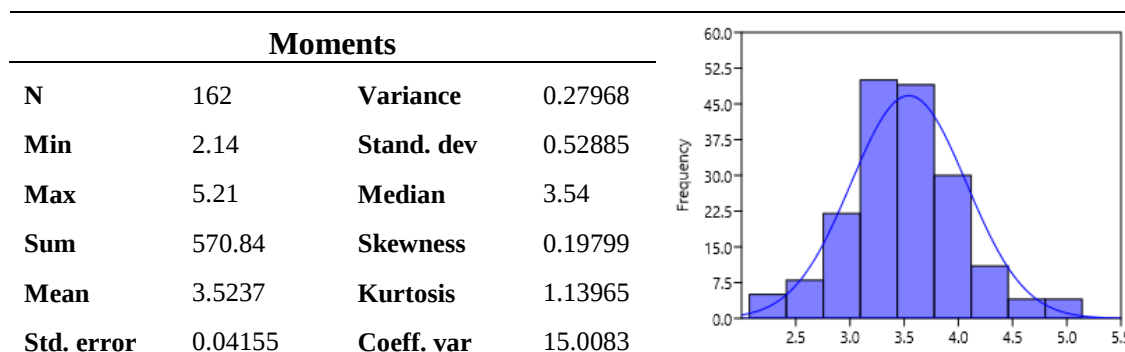


(d) Variable : Days to maturity (Combined)

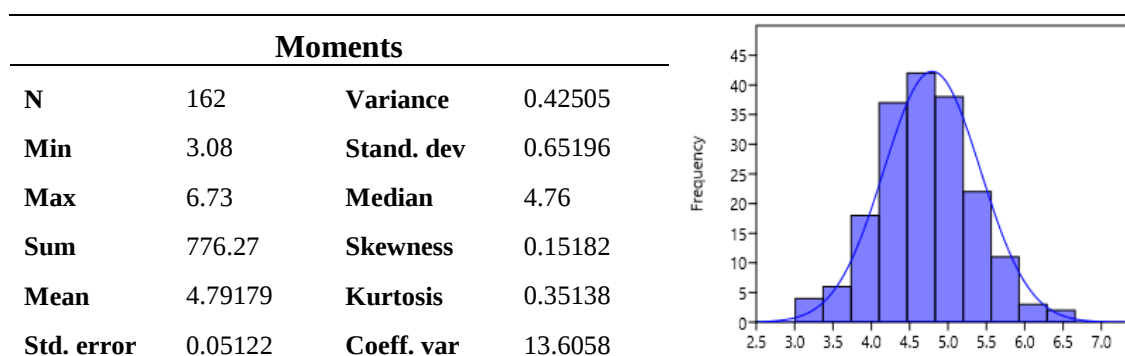


7. Descriptive statistics and frequency distribution of 100 seed weight under different seasons

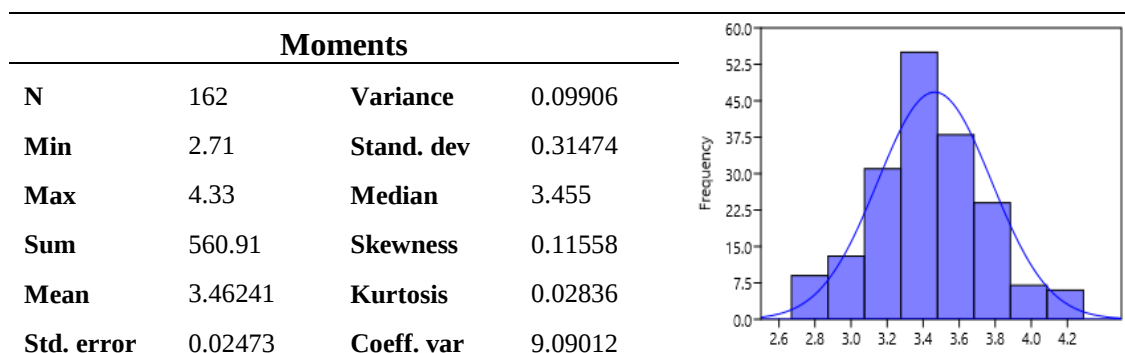
(a) Variable : 100 seed weight (Palampur 2016)



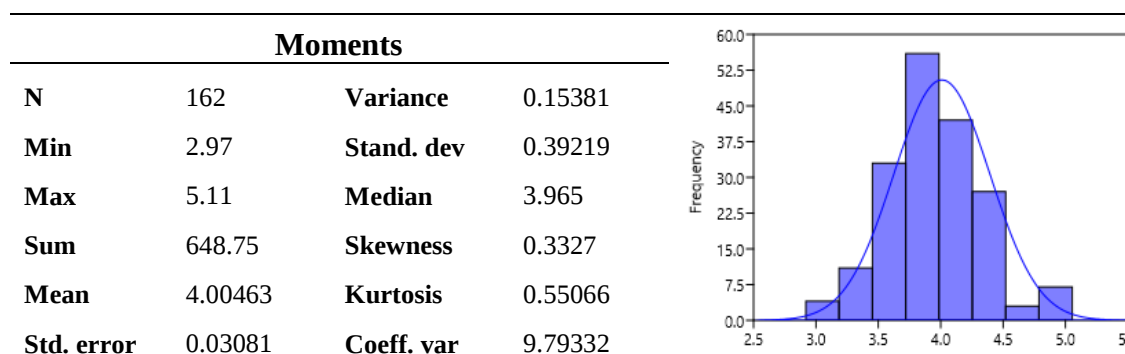
(b) Variable : 100 seed weight (Palampur 2017)



(c) Variable : 100 seed weight (Bajaura 2017)

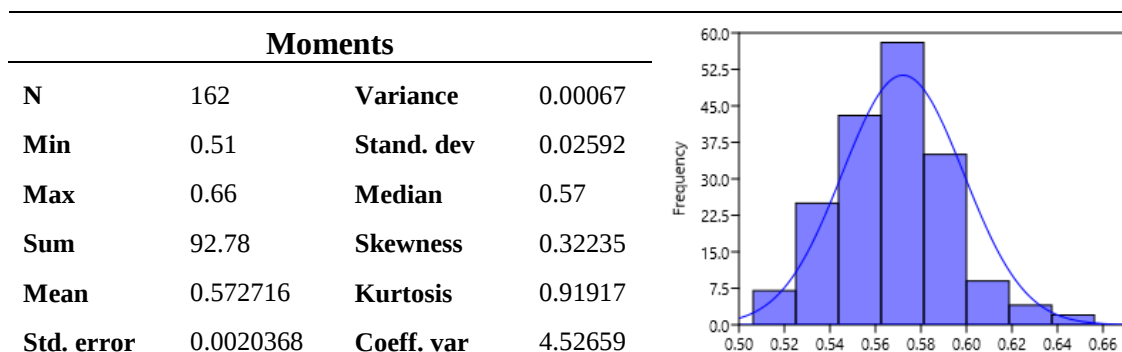


(d) Variable : 100 seed weight (Combined)

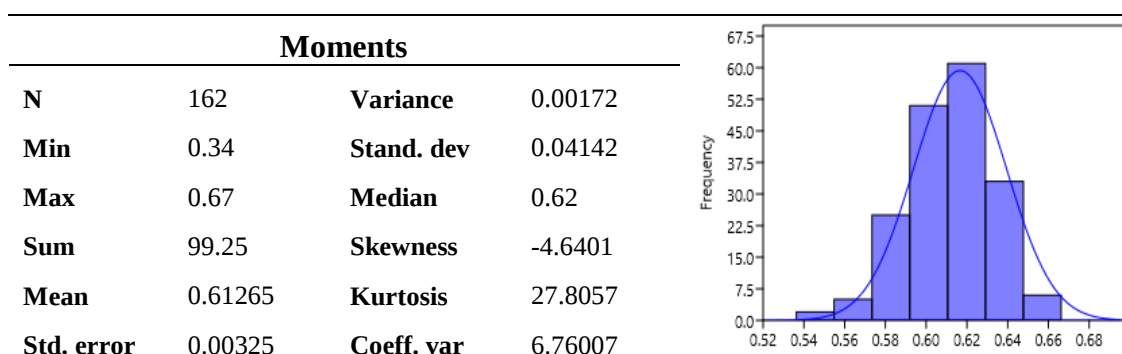


8. Descriptive statistics and frequency distribution of Seed size under different seasons

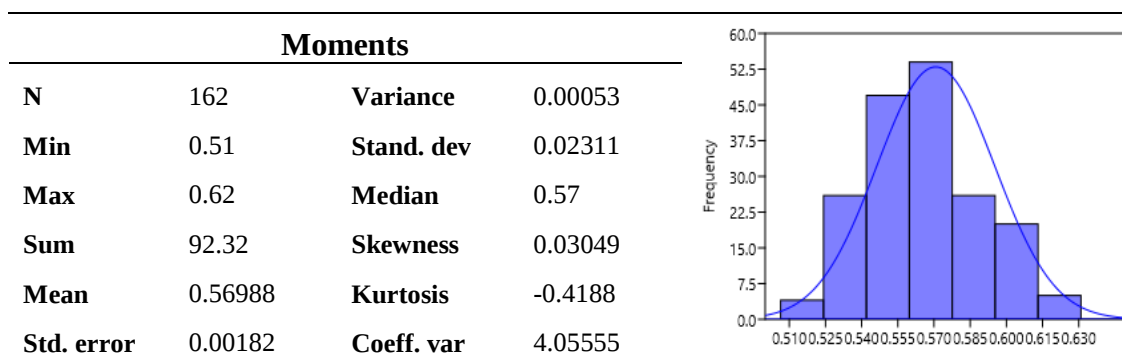
(a) Variable : Seed size (Palampur 2016)



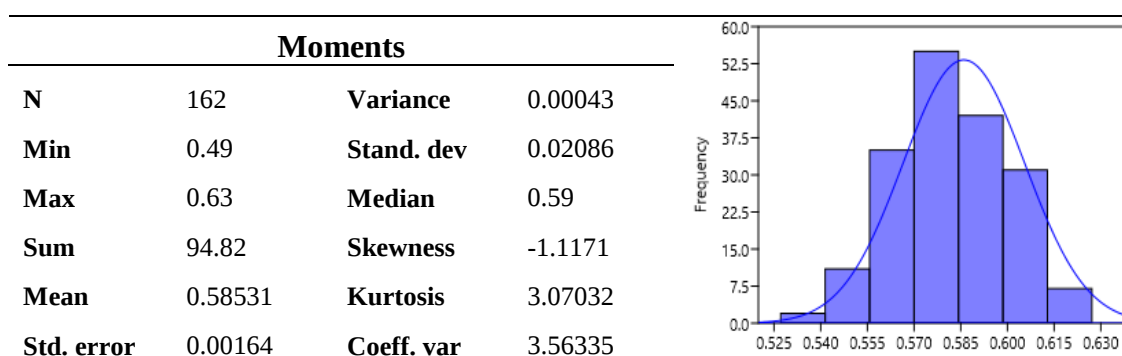
(b) Variable : Seed size (Palampur 2017)



(c) Variable : Seed size (Bajaura 2017)

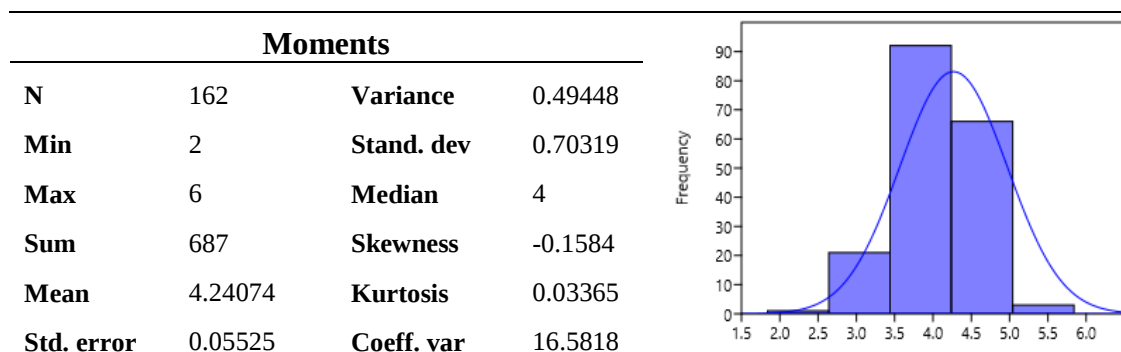


(d) Variable : Seed size (Combined)

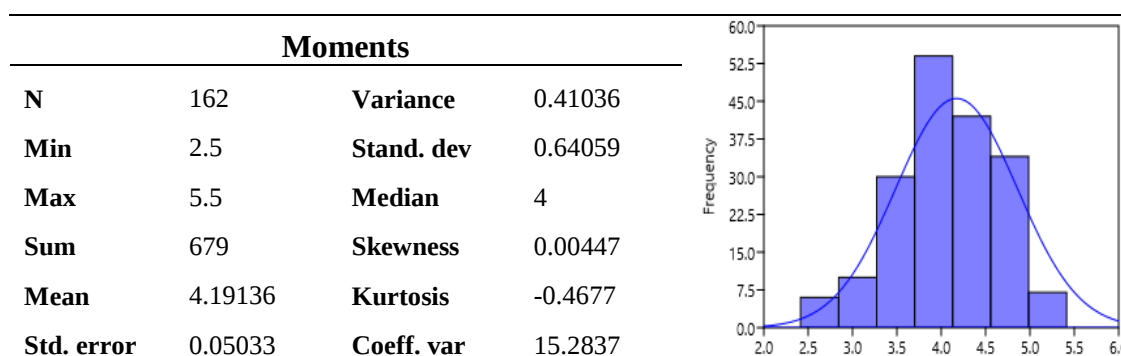


9. Descriptive statistics and frequency distribution of Seeds per pod under different seasons

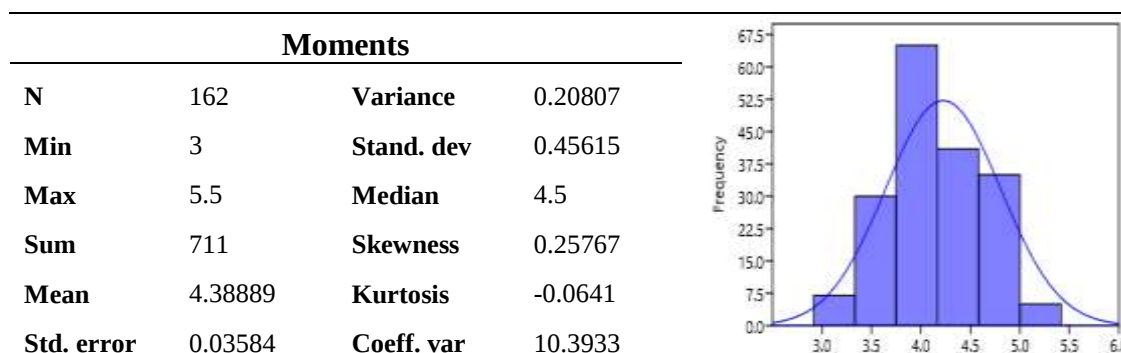
(a) Variable : Seeds per pod (Palampur 2016)



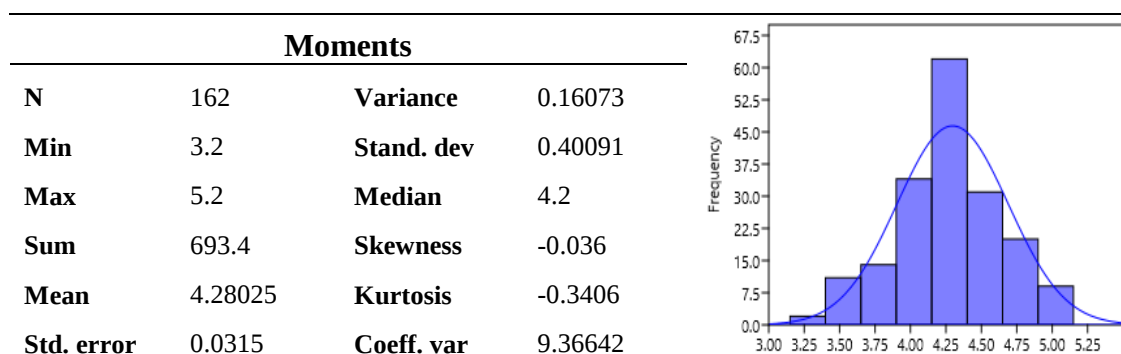
(b) Variable : Seeds per pod (Palampur 2017)



(c) Variable : Seeds per pod (Bajaura 2017)

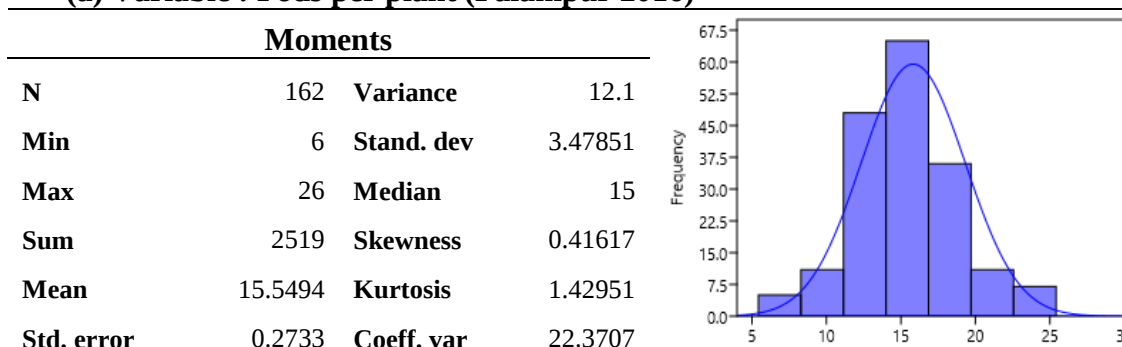


(d) Variable : Seeds per pod (Combined)

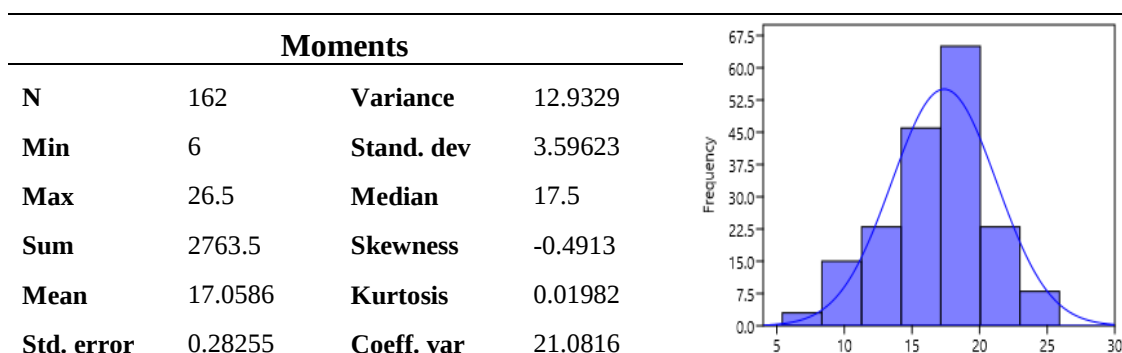


10. Descriptive statistics and frequency distribution of Pods per plant under different seasons

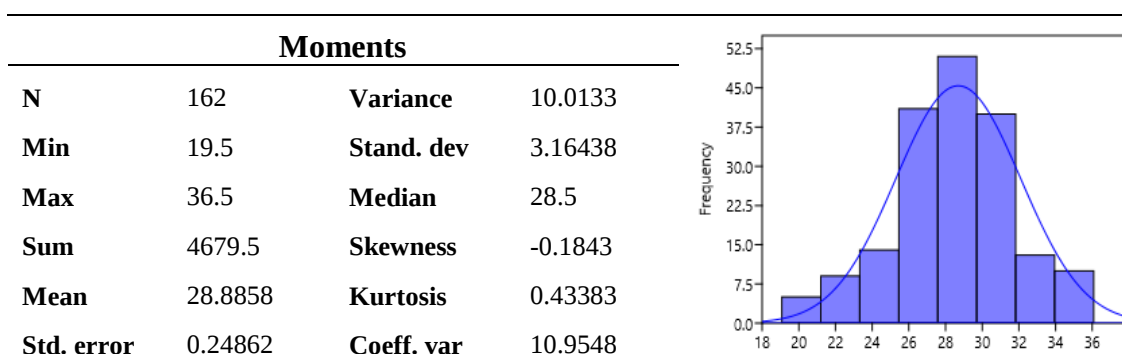
(a) Variable : Pods per plant (Palampur 2016)



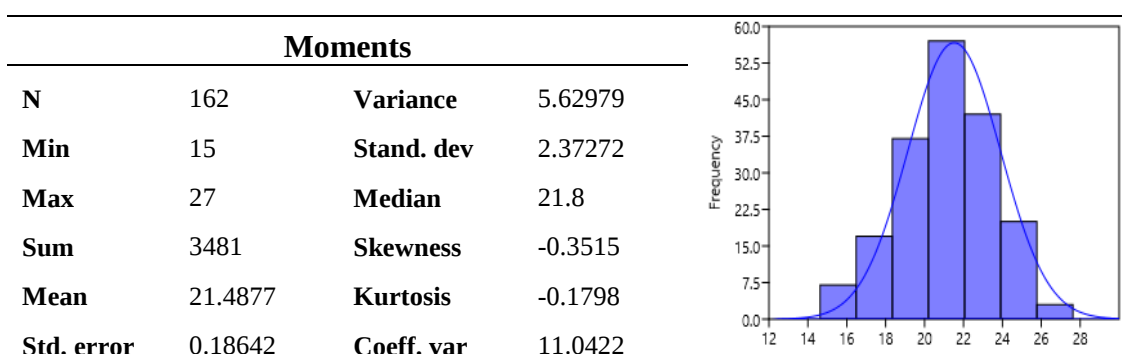
(b) Variable : Pods per plant (Palampur 2017)



(c) Variable : Pods per plant (Bajaura 2017)

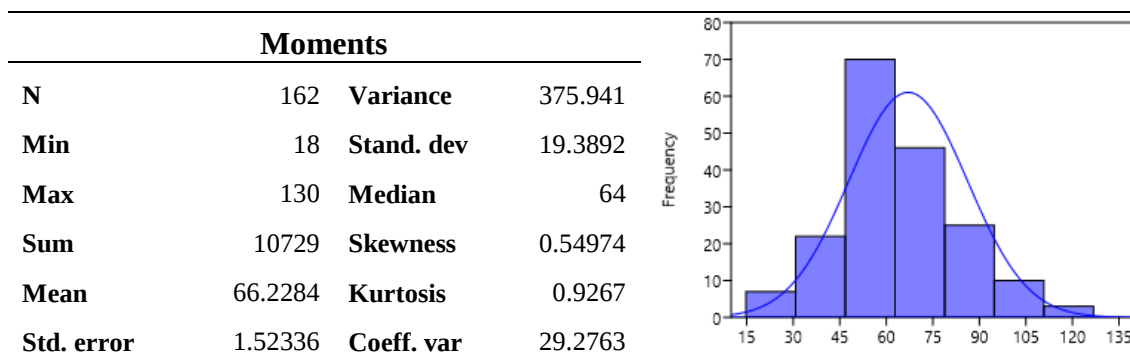


(d) Variable : Pods per plant (Combined)

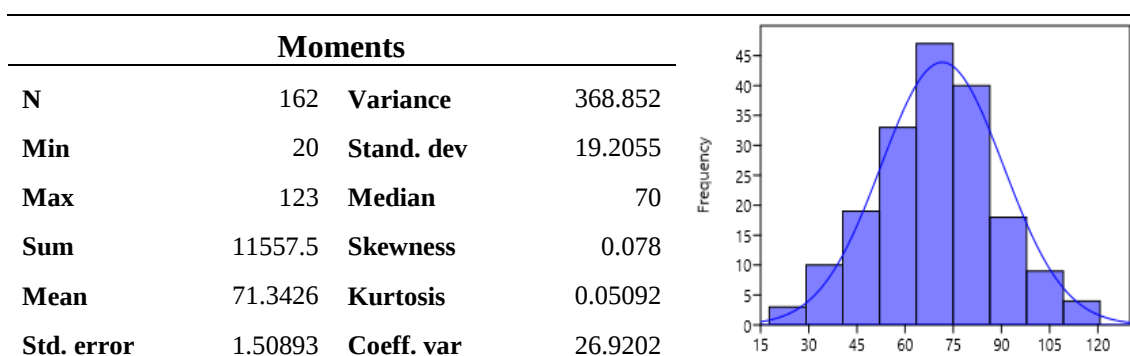


11. Descriptive statistics and frequency distribution of Seeds per plant under different seasons

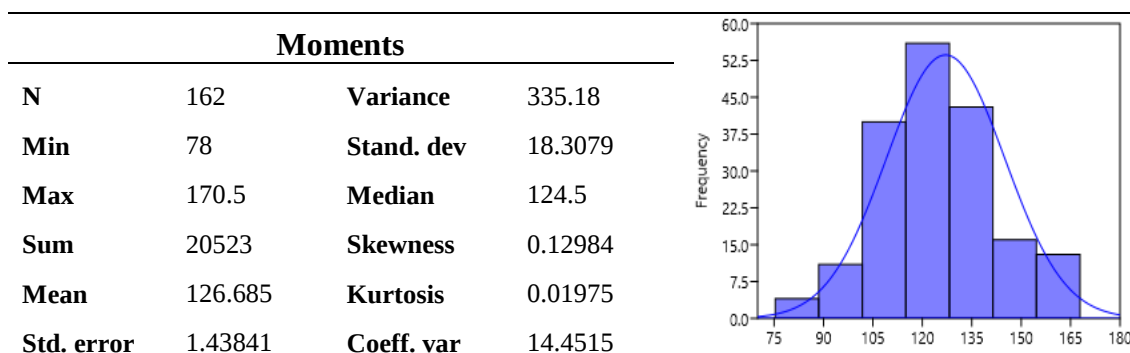
(a) Variable : Seeds per plant (Palampur 2016)



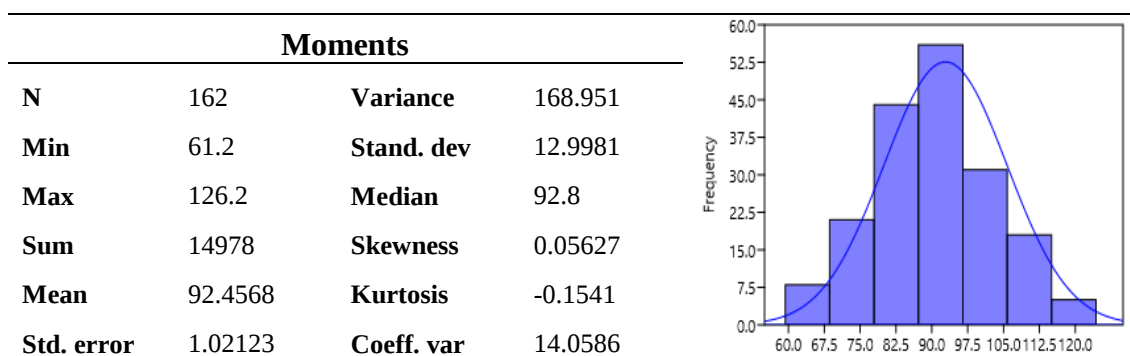
(b) Variable : Seeds per plant (Palampur 2017)



(c) Variable : Seeds per plant (Bajaura 2017)

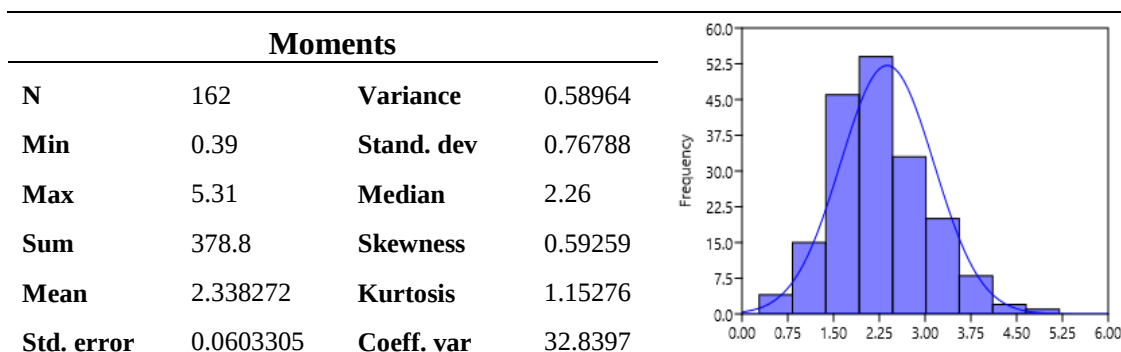


(d) Variable : Seeds per plant (Combined)

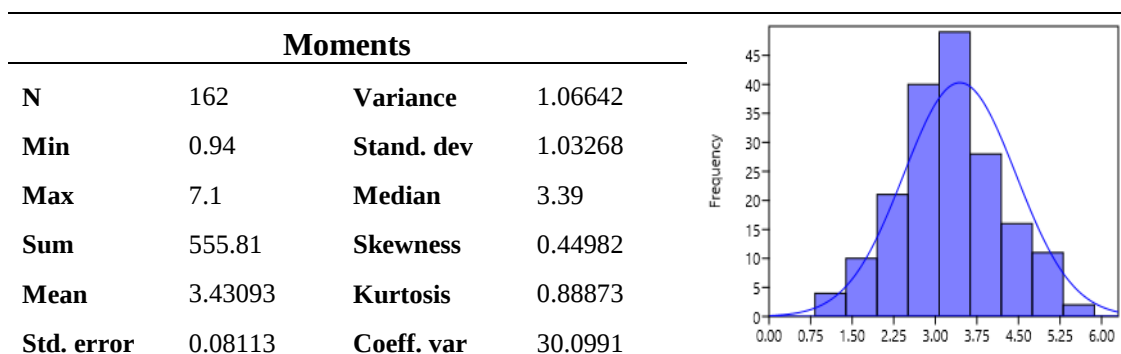


12. Descriptive statistics and frequency distribution of Seed yield per plant under different seasons

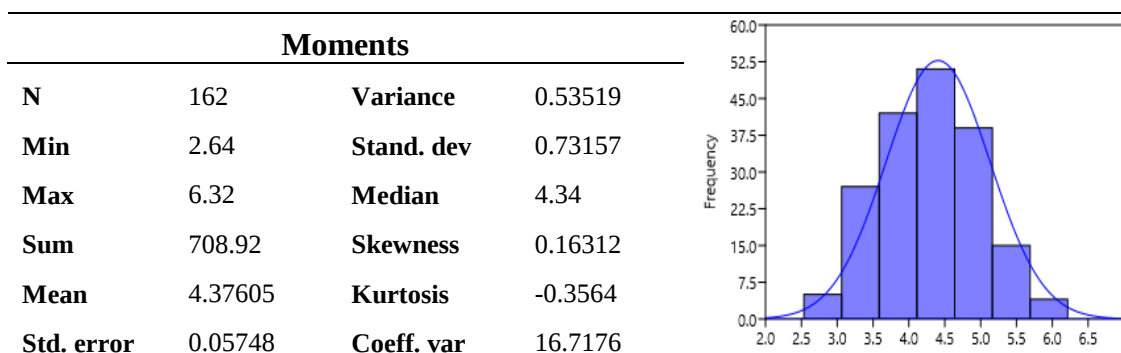
(a) Variable : Seed yield per plant (Palampur 2016)



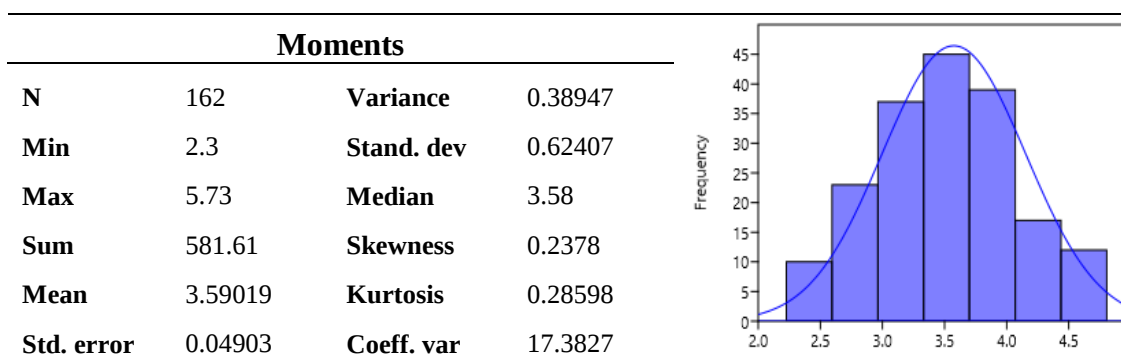
(b) Variable : Seed yield per plant (Palampur 2017)



(c) Variable : Seed yield per plant (Bajaura 2017)

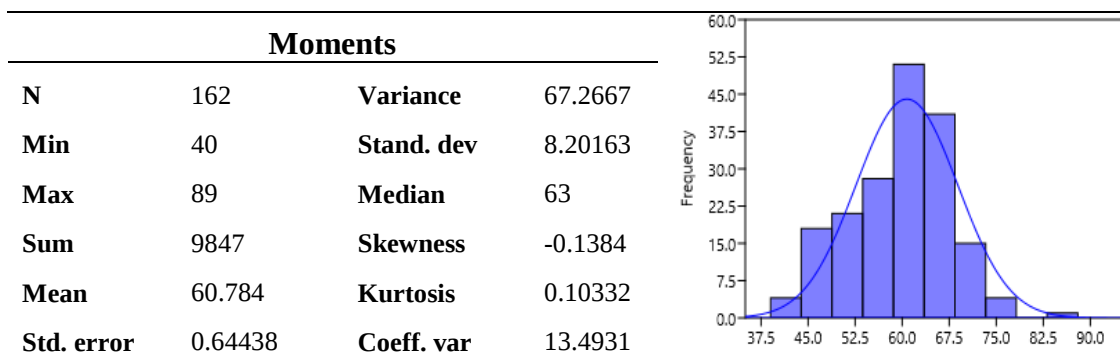


(d) Variable : Seed yield per plant (Combined)

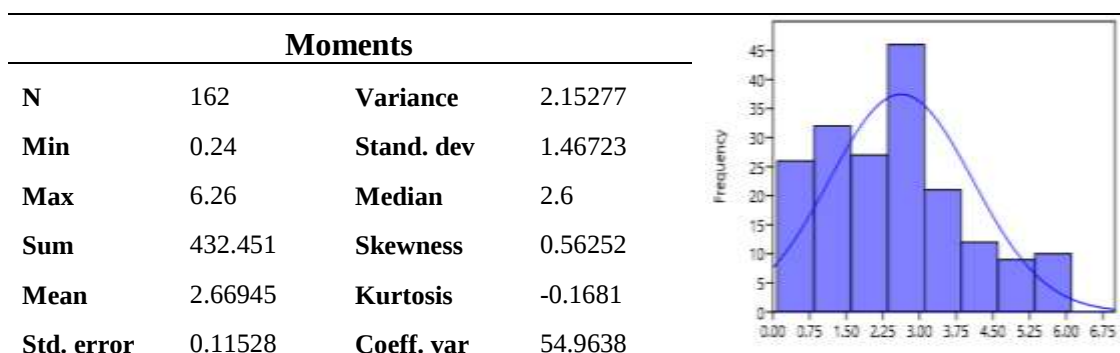


13. Descriptive statistics and frequency distribution of root traits

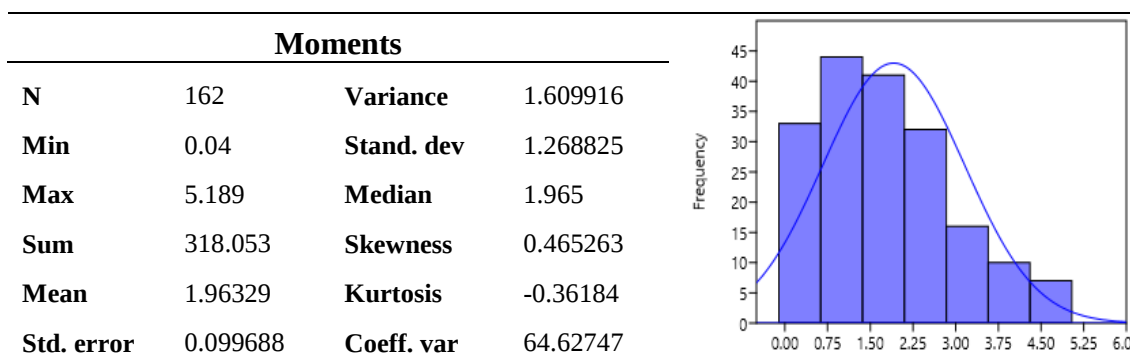
(a) Variable : Root length (Palampur 2017)



(b) Variable : Root fresh weight (Palampur 2017)

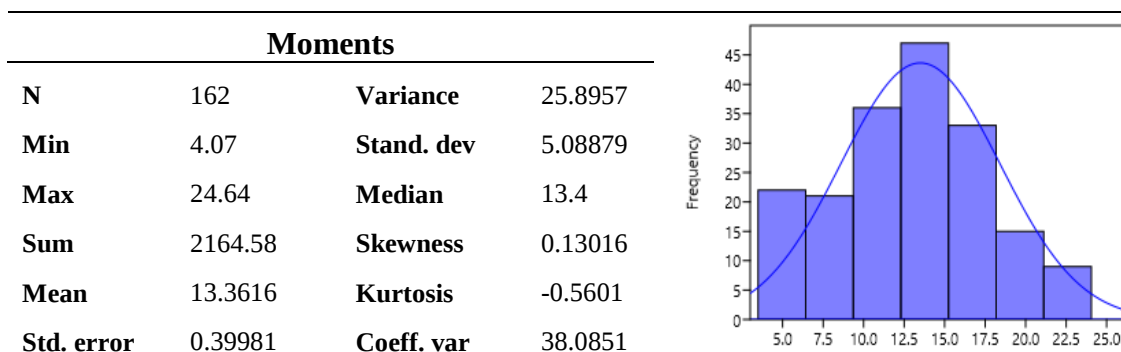


(c) Variable : Root dry weight (Palampur 2017)

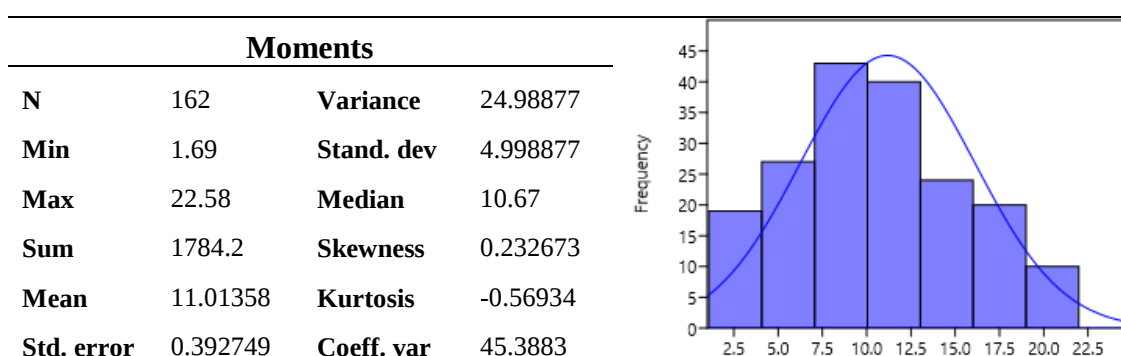


14. Descriptive statistics and frequency distribution of Chlorophyll and Carotenoid content under control and drought stress environment

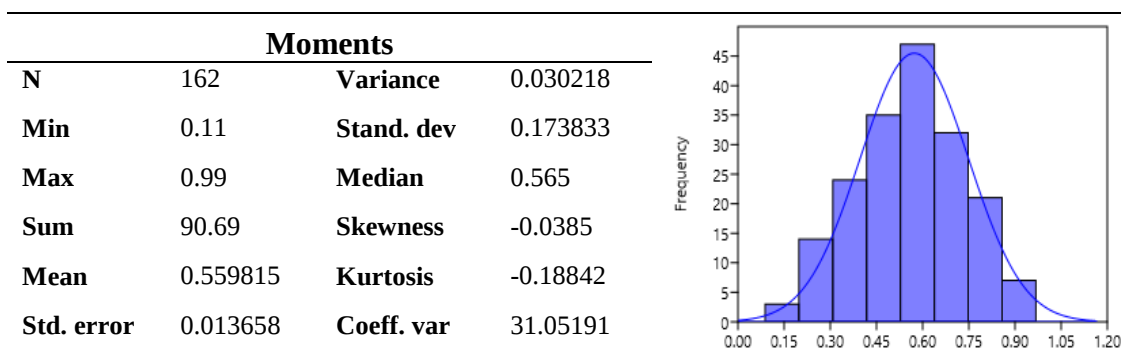
(a) Variable: Chlorophyll content (Control)



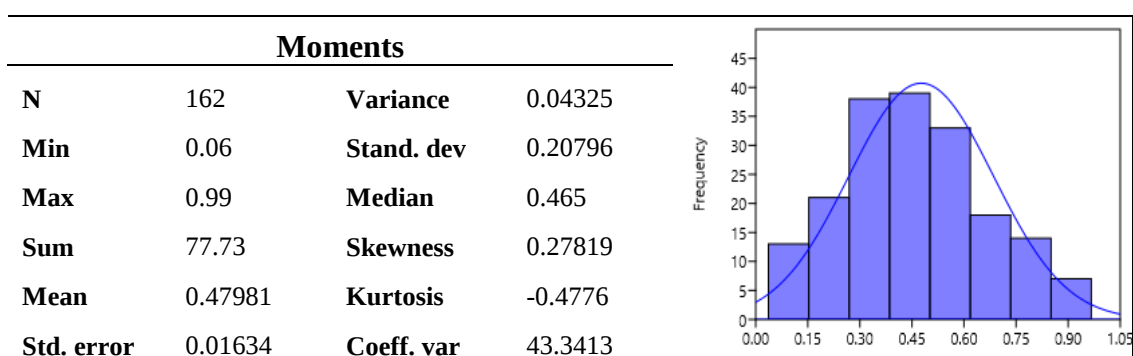
(b) Variable : Chlorophyll content (Stress)



(a) Variable: Carotenoid content (Control)



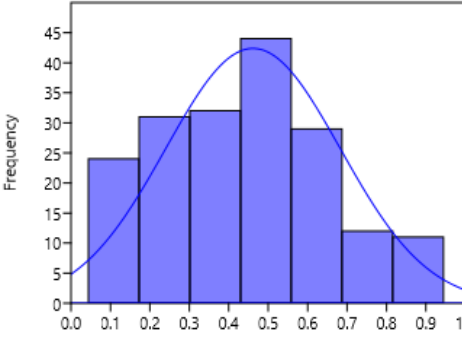
(b) Variable: Carotenoid content (Stress)



15. Descriptive statistics and frequency distribution of Proline and Malondialdehyde (MDA) under control and drought stress environment

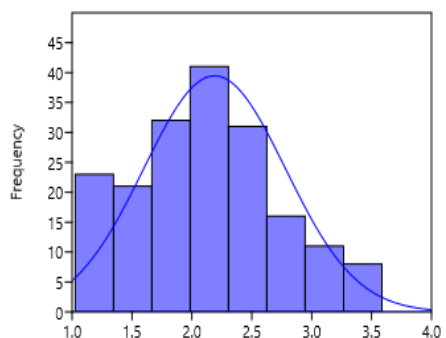
(a) Variable : Proline (Control)

Moments			
N	162	Variance	0.05027
Min	0.07	Stand. dev	0.22422
Max	0.97	Median	0.455
Sum	73.24	Skewness	0.29146
Mean	0.4521	Kurtosis	-0.6313
Std. error	0.01762	Coeff. var	49.5949



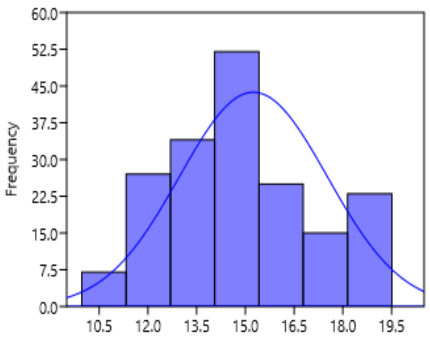
(b) Variable : Proline (Stress)

Moments			
N	162	Variance	0.34466
Min	1.14	Stand. dev	0.58708
Max	3.65	Median	2.22
Sum	354.42	Skewness	0.24338
Mean	2.18778	Kurtosis	-0.5256
Std. error	0.04613	Coeff. var	26.8346



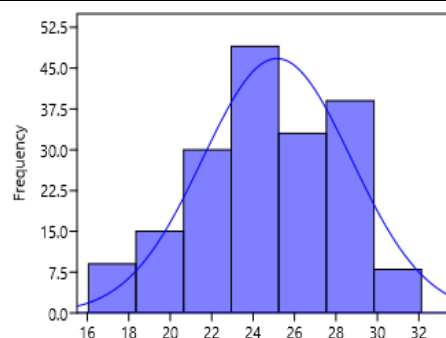
(a) Variable : MDA (Control)

Moments			
N	162	Variance	5.09466
Min	10.24	Stand. dev	2.25713
Max	19.78	Median	15.245
Sum	2468.67	Skewness	0.14718
Mean	15.2387	Kurtosis	-0.4153
Std. error	0.17734	Coeff. var	14.8119



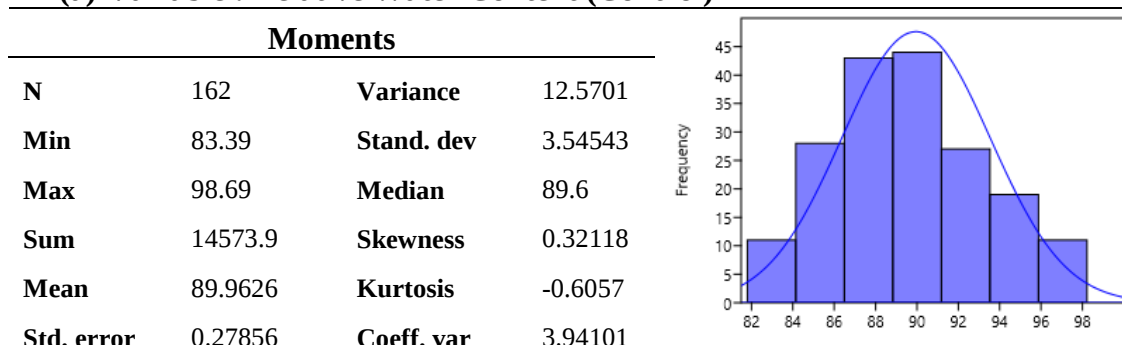
(b) Variable : MDA (Stress)

Moments			
N	162	Variance	12.1956
Min	17.15	Stand. dev	3.49222
Max	32.59	Median	25.49
Sum	4092.89	Skewness	-0.2201
Mean	25.2648	Kurtosis	-0.4934
Std. error	0.27437	Coeff. var	13.8225

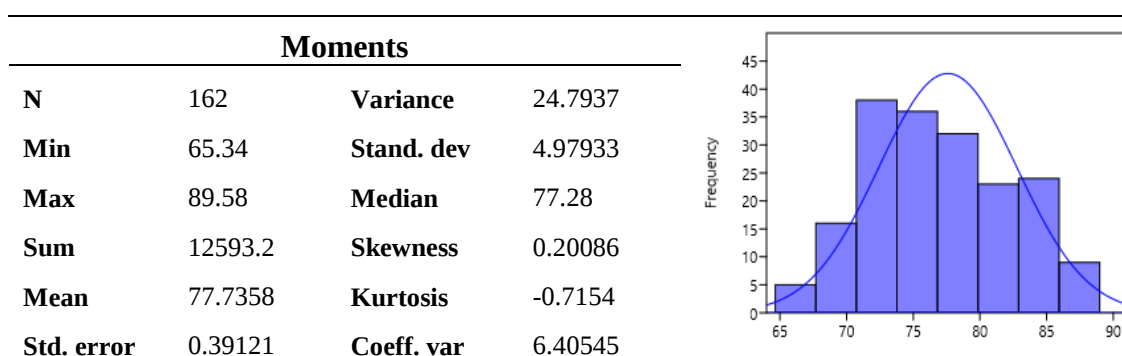


16. Descriptive statistics and frequency distribution of Relative Water Content and Membrane Stability Index (MSI) under control and drought stress environment

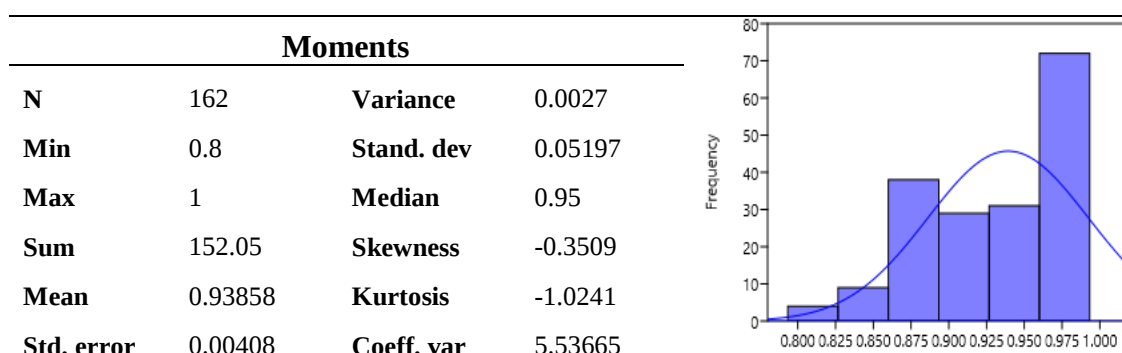
(a) Variable : Relative Water Content (Control)



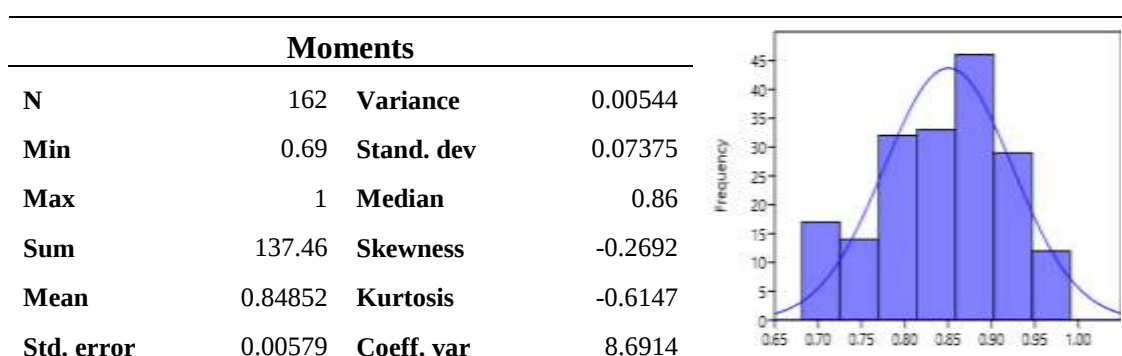
(b) Variable : Relative Water Content (Stress)



(a) Variable : MSI (Control)



(b) Variable : MSI (Stress)



Brief Biodata of student

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Palampur, Distt. Kangra (H.P.) - 176061

Academic Qualifications:

Examination Passed	Year	Board/University	Marks (%)	Division
Matriculation	2004	ICSE, New Delhi	65.5	I st
Higher Secondary	2006	HPBSE, Dharamshala (H.P.)	73.0	I st
B. Sc. (Hons. Biotechnology)	2009	HPU, Shimla (H.P.)	73.5	I st
M. Sc. (Biotechnology.)	2011	PU, Chandigarh	73.9	I st
Ph. D. (Agri. Biotechnology)	2019	CSK HPKV, Palampur (H.P.)	82.0	I st

Fellowships – Woman Scientist (WOS-A)

Publications:

Research Articles: 4 (Published: 2; Communicated: 2)

Abstracts: 3

Qualified ICAR SRF-2014, ASRB NET-2014, ASRB NET-2015, CSIR NET-2016, ASRB NET-2018