

# QTLs identification for biochemical and physiological parameters contributing towards drought tolerance in horsegram (*Macrotyloma uniflorum*)

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## Research Article

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## Abstract

Horsegram (*Macrotyloma uniflorum* (Lam.) Verdc.) is a drought hardy legume which can be grown in varied soil and temperature regimes. Though it has numerous, nutritive and medicinal benefits. However, it still lags behind other legumes in terms of genetic improvement. This crop is mostly cultivated on marginal and drought prone area thus genetics of drought stress tolerance were understood by studying the various drought parameters. To get insight, quantitative trait loci for drought tolerant traits were identified using an intraspecific mapping population of 162 F<sub>8</sub> recombinant inbred lines derived from a cross between HPKM249 and HPK4. The linkage map already developed was used along with the phenotypic data for biochemical and physiological parameters to identify genomic regions which are linked to drought tolerance. In the study total of seven QTLs were identified for ten different drought related traits. One QTL for malondialdehyde content on linkage group 2, two QTLs for root length on linkage group 3 & 9, one QTL each for proline and chlorophyll content under drought stress on linkage group 4, one QTL each for root dry weight and root fresh weight on linkage group 5 were identified using composite interval mapping. The identified QTLs will be utilized in marker assisted breeding and increase our understanding on the physiology of drought stress tolerance.

## Introduction

Horsegram (*Macrotyloma uniflorum* (Lam.) Verdc.) is a useful pulse from prehistoric times, which is mainly cultivated for food, feed and medicine by pastoral society in African and Asian continents. It provides an inexpensive source of protein (17.9–33.0%) for the people who depends mainly on vegetarian diet. It is classified as *Macrotyloma* genus, *Phaseoleae* tribe, *Faboideae* sub-family, *Fabaceae* family [1] having diploid chromosome number  $2n = 20, 22$  [2] and its genome size is 398 Mbps [3]. The genus *Macrotyloma* comprises of 32 wild species which are distributed in African, Australian and Indian subcontinent and among them *Macrotyloma uniflorum* var. *uniflorum* grown in Indian subcontinent is regarded as the only cultivated species. *Macrotyloma uniflorum* var. *uniflorum* is indigenous to parts of Africa and Southeast Asia but Southern India is considered as its Centre of origin [4, 5]. In India it is cultivated in large area compared to other countries for the purpose of human food and as Ayurvedic medicine with the maximum area of cultivation in Andhra Pradesh, Karnataka and Tamil Nadu. It is also grown in many other states of India viz. Odisha, West Bengal, Chhattisgarh, Madhya Pradesh, Bihar, Jharkhand, Uttaranchal and Himachal Pradesh. In India its cultivation area is 0.313 million ha and production are 0.134 million tones with productivity of 430 kg/ha [6].

Horsegram is an extremely adaptive crop, which can be grown from near sea level to 1800 m above mean sea level. It can resist drought and wide range of temperature regimes and can withstand stress conditions in those areas where other crops are unable to survive [7, 8]. Being a leguminous crop, it also conditions soil with nitrogen and aids in improving the fertility of soil. Besides, it has many wide uses such as it can be used as green manure, its husks are used for its water holding capacity [9, 10] and its small height enables it to be sown under taller plant as understory crop [9]. In addition to its abundant in protein it serves as a rich source of essential amino acids, carbohydrates (51.9–60.9%), iron, molybdenum, phosphorus, fibre and various vitamins like Vitamin A, Vitamin B1, Vitamin B2, Vitamin B3 and vitamin C [11]. Different parts of horsegram plants are used in various treatments viz. seeds of horsegram are used for remedy in urinary stone and piles [12], it also acts as tonic [13], treat problems in menstrual cycle [14] and also used to cure hiccups and worms [15]. The gravy of cooked horsegram seeds produces heat, thus it is consumed to cure fever, common cold and throat infection [16]. All these valuable attributes of this legume ensure its cultivation since prehistoric times and make it a promising food source for the future [17].

Drought is one of the acute abiotic stress effecting crops and cause significant crop loss due to its devastating effect on growth and development. It is a natural phenomenon that occurred due to inadequate availability of water [18]. To tolerate drought, plants use different stress coping mechanisms. Drought tolerance is a highly complex phenotype. It is affected by many traits such as proficient water uptake through deep roots, reduced rate of transpiration via stomata and limited effect of decreased water potential on metabolites production and plant growth [19]. Plants adapt themselves under drought stress by different mechanisms like drought escape or drought tolerance [20]. The effects of climate change and global warming have increased the need for scientists to investigate how drought stress impacts crop growth and productivity. Also, it has become essential to create cultivars capable of providing maximum yield in environments with drought stress [21]. Superior genotypes of horsegram with high yield under drought stress tolerance of Kerala's south-central laterite region and the degree of diversity among the genotypes was identified [22]. Agrarian traits controlling the drought stress tolerance are polygenic in nature and are quantitatively inherited [23]. Therefore, mapping the quantitative trait locus (QTL) which are linked to drought tolerance related parameters by providing drought condition will aids in the

identification of drought- responsive QTLs or genes. These identified QTLs or gene scan further assist in molecular breeding for traits selection and introgression in breeding strategies through marker assisted selection (MAS). After the discovery of DNA markers and their utilization in the development of framework genetic linkage maps, many QTLs linked to important agronomic traits have been identified in different crops plants [24]. Although studies on identification of QTLs for drought tolerance in many leguminous crops have been done *viz.* chickpea [25, 26], cowpea [27], common bean [28], lentil [29] and mungbean [30] but very limited work has been reported in horsegram. Limited genomic resources are available in horsegram in terms of molecular markers to be utilized in its genetic improvement. SSR and ILP markers were developed in horsegram and their characterization, cross-transferability and utility for mapping were studied [31]. Also, genome-wide SSR markers were developed and analyzed for genetic diversity and cross-transferability in other legumes [32]. Further research on horsegram genetics and varietal development can be accelerated by identifying QTLs linked to drought tolerance and comprehending its mechanism. Earlier in horsegram five QTLs for drought tolerance [33] and four & seven QTLs for morphological & phenological traits, respectively [34] were identified. Also, a study on drought tolerance potential of horsegram (*Macrotyloma uniflorum* Var.) germplasm using genome wide association studies was reported by [35]. Recently, four QTLs associated with dwarfing and the determinate growth habit were identified in horsegram [36]. Though studies are very useful to get into deeper insight to various important traits in horsegram. However, there was still need to develop more saturated genetic map and identify genes/QTLs for important agronomic traits which will aid in genetic improvement of horsegram through breeding and molecular approaches. Therefore, the present study was carried out to identify the genomic position, number and magnitude of QTLs affecting genetic variation for drought related parameters by phenotyping more specific traits *i.e.*, physiological and biochemical parameters effecting drought in RILs populations derived from a cross between two horsegram genotypes (HPKM249 and HPK4) showing contrasting expression for some drought-related traits. The aim of this work is to use genetic linkage map produced by molecular markers [34] to locate the QTLs linked to horsegram drought tolerance associated traits. The QTLs discovered in this work can be used as a resource for the future genetic analysis and discovery of horsegram drought tolerance genes. It provides QTL-based marker- assisted breeding of drought tolerant varieties of horsegram for its genetic improvement.

## Materials and method

### Plant materials and experimental design

A RILs mapping population comprised of 162 lines derived from intra specific cross of HPKM249 X HPK4 was used in the study. The RILs mapping population was developed through single seed descent method from F<sub>2</sub> to F<sub>8</sub> generations. The parental lines (HPKM249 & HPK4) differ from each other in various drought related traits. HPKM249 cultivar is drought susceptible with less root length and low content of drought related metabolites. HPK4 showed tolerance to drought, long root length and high content of drought related metabolites (Fig. 1; Table 1). Parental lines along with 162 RILs were grown in a polyhouse of the department of Agricultural Biotechnology, CSK Agriculture University, Palampur, India for two summer seasons.

For recording the phenotypic data for drought related traits, one set of the RILs was planted in a polytube that were 92 cm long and 10 cm wide. The plants are watered well till initiation of flowering and watering was ceased till the appearance of drought symptoms. Another set of 162 RILs were grown in an augmented block design having two replications in a plot size of 1 m with row-to-row distance of 30 cm between plants and plant-to-plant distance of 10 cm. Four checks (Himganga, HPKC2, VLG1 and HPK4) were grown after every 20 lines in these plots. Five plants from each line were chosen randomly and the data for all the traits were recorded from them and averaged for final readings. The biochemical parameters were recorded for both non stressed and drought stressed condition. Data on days to temporary wilting (DTW), chlorophyll content (CHL), carotenoid content (CAR), proline content (PRO), malondialdehyde content (MDA), relative water content (RWC), membrane stability index (MSI), root length (RTL), root fresh weight (RFW) and root dry weight (RDW) were recorded.

### Phenotyping for drought tolerance related parameters

Phenotyping and evaluation for ten drought related traits was carried out by using the mapping population of 162 RILs for two consecutive summers at Palampur (H.P), India (32.1167° N, 76.5333° E; 1220 amsl). Data on days to temporary wilting (DTW), chlorophyll content (CHL), carotenoid content (CAR), Proline content (PRO), MDA content (MDA), relative water content (RWC), membrane stability index (MSI), root length (RTL), root fresh weight (RFW) and root dry weight (RDW) were recorded following standard procedures. Watering was stopped at the time of flowering and days were counted to the appearance of drought symptoms

which were recorded as days to temporary wilting (DTW). The biochemical parameters were recorded for both non stressed and drought stressed condition. For providing drought stress the watering was stopped at the time of flower initiation until temporary wilting occur. Five plants were chosen randomly and the data for all the traits were recorded from them and averaged for final readings. The chlorophyll content (CHL) was estimated using [37], and the amount of carotenoids (CAR) was determined according to [38], the free proline content (PRO) was estimated by the method of [39], the degree of lipid peroxidation was measured in terms of malondialdehyde (MDA) content as described by [40]. Relative water content (RWC) was measured on the second or third upper fully expanded leaf for both well-watered and stressed plants. It was calculated using the formula  $RWC (\%) = \left[ \frac{FW-DW}{TW-DW} \right] \times 100$  given by [41]. FW is the leaf fresh weight, DW is the leaf dry weight after oven drying (80°C) for 48 h, TW is the leaf turgid weight (obtained after rehydration of the leaf samples in distilled water for 4 hrs.). Membrane stability index (MSI) was estimated according to [42]. In this, 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

$$MSI (\%) = 1 - \left[ \frac{Ci}{C_{max}} \right].$$

Data from both non stressed and drought stressed plants were recorded for root traits, was done on plants planted in polytube. Root length (RL, cm), root fresh weight (RF, g) and root dry weight (RD, g) was also calculated at the time of harvesting by uprooting and removing plant and soil carefully.

## Genotypic Data and linkage map

The linkage map which was already developed in the host lab using the same mapping population [34] was utilized in the study to identify QTLs linked to drought tolerance. Katoch et al. developed a genetic linkage map using a total of 162 recombinant inbred lines created by intraspecific crossings between HPKM249 and HPK4. They have screened 2011 different molecular markers consisting of 63 Expressed Sequence Tag Simple Sequence Repeats (EST-SSRs), 403 genic Simple Sequence Repeats (genic SSRs), 387 genomic Simple Sequence Repeats (genomic SSRs), 24 drought specific Simple Sequence Repeats (DsSSRs), 300 Simple Sequence Repeats (SSRs) from other legumes, 450 Random Amplified Polymorphic DNA (RAPD) markers and 384 Conserved Ortholog Set (COS) markers on parents i.e. HPKM249 and HPK4, out of which 493 markers showed polymorphism among parents. These 493 markers were then used for genotyping of RILs. The genotypic data in the form of scoring matrix was used for construction of linkage map using JoinMap 4.1 software. 295 among 493 markers were mapped on 10 linkage groups at LOD 3.5 spanning a total distance of 1,541.7 cM with an average distance between markers of 5.20 cM.

## QTL mapping

Quantitative trait loci analysis was done on 162 RILs using phenotypic data of different drought related parameters evaluated over location and years. Distribution of each trait was inspected using skewness and kurtosis statistics using Past 3.25 software. Phenotypic differences between parents were analyzed using ANOVA. Pearson correlation coefficients and frequency distribution among different traits were calculated using the same software. QTLs were identified using Windows QTL Cartographer V2.5 software [43] by composite interval mapping (CIM) method [44, 45]. The Zmapqtl standard model 6 was used with a 10 cM of window size and walk speed of 2 cM. Cofactors were obtained using the forward regression algorithm. A LOD score threshold of a QTL at a significance level of  $P = 0.05$  was estimated at 1000-permutation of shuffling the genotypes and means of phenotypic values for a trait [46]. A LOD threshold score of  $\geq 2.5$  at 1000 permutations was 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 [47]. 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 R value from this analysis was accepted as the percent phenotypic variance explained by the locus. The figure of the QTL map was made with MapChart 2.32 software [48].

## Result & discussion

### Phenotypic Evaluation for Drought Tolerance-Related parameters

Horsegram is one of the most important legumes in terms of its medicinal, nutraceutical and drought tolerant properties but ignored by the scientific community in terms of its genetic improvement. It is mainly grown by local people as ethnical crop in some drought prone areas of developing countries [49, 50]. Drought tolerance is a complex process showing continuous variation. For accurate evaluation and phenotyping of drought related traits, RILs mapping population was taken for the study. The parents selected for mapping population differ in trait under study. Different types of mapping population are utilized for mapping *e.g.*,  $F_2$  population, backcross (BC) population, recombinant inbred lines (RILs) and double haploids (DH). Each population has its own merits and demerits. In our study we have developed 162 individuals of  $F_8$  RILs derived from intra specific cross of HPKM249 and HPK4 using single seed descent method. RILs consist of homozygous lines and different combinations of chromosomal segments from each parent were fixed after repeated cycle of meiosis and selfing.

Plants are exposed to a wide range of biotic and abiotic stresses which diminish the productivity of agricultural crops. Attempts have been made in various important crops to identify genes linked to important traits such as stress tolerance, increase yield, etc. for ideotype breeding and to increase the productivity [51, 52, 53]. Out of numerous stresses, plant suffer huge lose in its production due to drought [54]. To increase the production and ultimately the profit from legumes, drought tolerance is an important trait [55]. Therefore, to identify and locate the genes in the genome which can directly influence the drought tolerance is an important criterion to ease the selection of lines in breeding programme through MAS. Since drought tolerance is a complex trait and is controlled by many genes directly and indirectly, accurate phenotyping and analysis of several biochemical and physiological parameters, which affects it need to be investigated on the mapping population. Also study on root traits can provide better insight as roots play important role in coping the plant against drought stress and are the first organ which are exposed to drought stress in plants. In our study, the phenotypic values showed a normal frequency distribution and exhibited transgressive segregation which is a typical characteristic of quantitative traits. (Figure 2). Transgressive segregation is the formation of extreme phenotypes, or transgressive phenotypes, observed in segregated populations compared to phenotypes observed in the parental lines. These are formed due to the combinations of alleles from both parents with the same effect [56]. Descriptive statistics for drought related traits in the RIL population is presented in Table 2. Results showed that all the lines response differently to water stress for all traits under study and were significantly affected by drought stress. Phenotyping for these traits showed significant genetic variability within RILs population under normal and water stressed condition. Correlations among traits calculated within RILs revealed that root traits were found positively correlated to each other and were statistically significant ( $P<0.05$ ). Other drought traits were also positively correlated to each other and were statistically significant ( $P<0.05$ ) under different environment (Figure 3).

### QTL Mapping

For incorporating molecular markers in genetic improvement of any crop, construction of linkage maps is prerequisite, which will lead to mapping of important agronomic traits such as yield and resistance to various biotic or abiotic stresses. Availability of large number of molecular markers is essential for the construction of saturated linkage map and other plant molecular analysis. Knowledge of plant genomic research has increased manifolds due to the availability and utilization of molecular markers. These DNA molecular markers aids in increased reliability and resolution of mapping. The information of molecular markers in horsegram is very limited, however, recently few attempts have been made [31, 32, 33, 34, 35, 36, 57]. In the present study, the  $F_8$  RIL mapping population was evaluated for different ten drought related parameters for two consecutive years under drought and irrigated conditions. Identification of QTLs for the different drought related parameters were identified by QTL Cartographer V2.5 using phenotypic data of RILs under normal and water stressed condition. Total of seven QTLs were identified for six different drought related parameters namely one each for chlorophyll content under drought stress, malondialdehyde content for control, proline content under drought stress, root dry weight, root fresh weight and two for root length using composite interval mapping (Table 3).

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 MUSSR501-MUGR523 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 present on LG4 flanked by MUMST628 and 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. 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. Two QTLs were detected for root length at LOD of 3.3 and 5.0 with 8.3 and 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 (Figure 4). Further, additive effect demonstrated that allelic contribution is by both the parents resulted in increased root length. The effect of QTLs was also presented in the form of peaks in figure 5. Studies in other crops also confirm the uneven localization of QTLs across the linkage group [58, 59, 60, 61]. Most of the QTLs identified in the present study were the major QTLs as they have Phenotypic Variance Explained (PVE) more than 15%, such QTLs have practical importance in molecular breeding in this crop. Earlier [33] identified five QTLs for drought tolerance using the same mapping population however those QTLs are based on physiological parameters but the QTLs identified in our study are based on biochemical evaluation of the mapping population. Drought effects the biochemical composition of the crop by accumulation of some solutes which aids in providing tolerance. Also root traits help in differentiating tolerant genotypes as it is first organ to help plant with absorption of soil water. [34] identified 4 QTLs linked to phenological and 7 QTLs linked to morphological traits, these QTLs along with QTLs identified in our study help to create genomic resources in horsegram for its genetic improvement.

Also, we identified two QTL clusters on linkage group 4 and 5 in this study. A QTL cluster is defined as two or more traits with significant QTLs having overlapping confidence intervals. Among these clusters, one cluster harboring 20.6% Phenotypic Variance Explained (PVE with 2 traits (chlorophyll and proline content) was present on LG4 and second cluster harboring 25.8% PVE with 2 traits (root fresh weight and root dry weight) and present on LG5. This region's introduction into superior horsegram variety could anticipated to increase the horsegram tolerance to drought. These areas have enormous value for molecular marker-assisted breeding, cloning, and fine-scale gene mapping. Thus, our study could provide genomic resources in horsegram for its genetic improvement and varietal development with enhanced drought tolerance. However, addition of more molecular markers such as SNPs to the linkage map can further provide better localization and estimation of QTLs, which can be used for the MAS and genetic improvement of the horsegram.

## Conclusion

The present study reports the identification of genomic regions linked to biochemical and physiological traits controlling drought tolerance 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  $F_2$  to  $F_8$ . The RIL population was phenotyped for 10 traits linked to drought tolerance under control and drought stress condition. Polytube experiments were carried out for measurement of root traits and for biochemical traits in control and drought stress condition. The linkage map already constructed previously was used for the identification of QTLs. Total of seven QTLs ( $LOD \geq 2.5$ ) were identified for ten different drought related parameters namely one each for chlorophyll content under drought stress, malondialdehyde content for control, proline content under drought stress, root dry weight, root fresh weight and two for root length using composite interval mapping. Phenotypic variation explained (PVE) by QTLs ranged from 8.3 to 53.4 per cent. The highest phenotypic variation (53.4%) was explained by the QTLs for root length. Two QTL clusters were also identified at LG4 and LG5. Although the map used in the present study covered a significant portion of the genome, a further saturation of map with additional markers (SSRs or SNPs) is imperative for its efficient utilization. With the long-term goal of understanding the genetic basis of drought tolerance traits, it is envisaged that the present QTL map, fortified with 295 molecular markers and 7 QTLs would provide a means to breeders for further genetic enhancement of this crop species. The study would improve our genetic understanding of drought tolerance and finally provide the basis for marker assisted selection of these traits. Further, QTL validation and fine mapping is the next step towards successful application of these findings in MAS.

## Declarations

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**Competing Interests:** *The authors have no financial interest to disclose.*

**Author contribution statement:** *RKC conceptualize the idea and provide all the facility to carry out genotypic and phenotypic work and finalized the manuscript, MK performed the experiment, recorded phenotypic data, performed molecular data analysis and written the manuscript.*

### **Ethics approval**

*"This is an plant based study. Therefore, no ethical approval is required."*

### **Consent to participate**

*"Informed consent was not obtained from any individual participants as the study did not included human research."*

### **Consent to publish**

*"The authors affirm that there is no data is generated on human participants, therefore no consent is required for publication of the images"*

### **Authorship Declaration**

All authors whose names appear on the submission

- 1) made substantial contributions to the conception or design of the work; or the acquisition, analysis, or interpretation of data; or the creation of new software used in the work;
- 2) drafted the work or revised it critically for important intellectual content;
- 3) approved the version to be published; and
- 4) agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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## Tables

**Table 1** Morphological polymorphism in parents

| S.No. | Trait                      | HPK4     | HPKM249     |
|-------|----------------------------|----------|-------------|
| 1     | Drought stress             | Tolerant | Susceptible |
| 2     | Days to temporary wilting  | More     | Less        |
| 3     | Relative Water Content     | High     | Low         |
| 4     | Total carotenoids          | High     | Low         |
| 5     | Total chlorophyll contents | High     | Low         |
| 6     | Proline                    | High     | Low         |
| 7     | Malondialdehyde (MDA)      | Low      | High        |
| 8     | Root length                | More     | Less        |

**Table 2** Mean performance of parents and RILs for drought related traits in RILs (HPKM249 × HPK4) population

| Traits                   | Environment | HPKM249 | HPK4  | Range (RIL) | Mean  | SD   |
|--------------------------|-------------|---------|-------|-------------|-------|------|
| <b>Biochemical</b>       |             |         |       |             |       |      |
| Carotenoids              | Control     | 0.81    | 0.78  | 0.11-0.99   | 0.56  | 0.17 |
|                          | Stress      | 0.58    | 0.65  | 0.06-0.99   | 0.48  | 0.21 |
| Chlorophyll              | Control     | 11.46   | 12.97 | 4.07-24.64  | 13.36 | 5.09 |
|                          | Stress      | 7.91    | 10.59 | 1.69-22.58  | 11.00 | 5.01 |
| Malondialdehyde          | Control     | 16.84   | 13.47 | 10.24-19.78 | 15.22 | 2.27 |
|                          | Stress      | 29.65   | 20.83 | 17.15-32.59 | 25.23 | 3.53 |
| Proline                  | Control     | 0.21    | 0.46  | 0.07-0.97   | 0.45  | 0.22 |
|                          | Stress      | 2.03    | 3.43  | 1.14-3.65   | 2.20  | 0.59 |
| Membrane stability index | Control     | 0.94    | 1.00  | 0.80-1.00   | 0.94  | 0.05 |
|                          | Stress      | 0.86    | 0.93  | 0.69-1.00   | 0.85  | 0.07 |
| Relative water content   | Control     | 87.34   | 96.36 | 83.39-98.69 | 89.93 | 3.56 |
|                          | Stress      | 74.44   | 86.58 | 65.34-89.58 | 77.68 | 5.00 |
| <b>Root traits</b>       |             |         |       |             |       |      |
| 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 |

**Table 3** QTLs for various drought related traits identified using QTL Cartographer

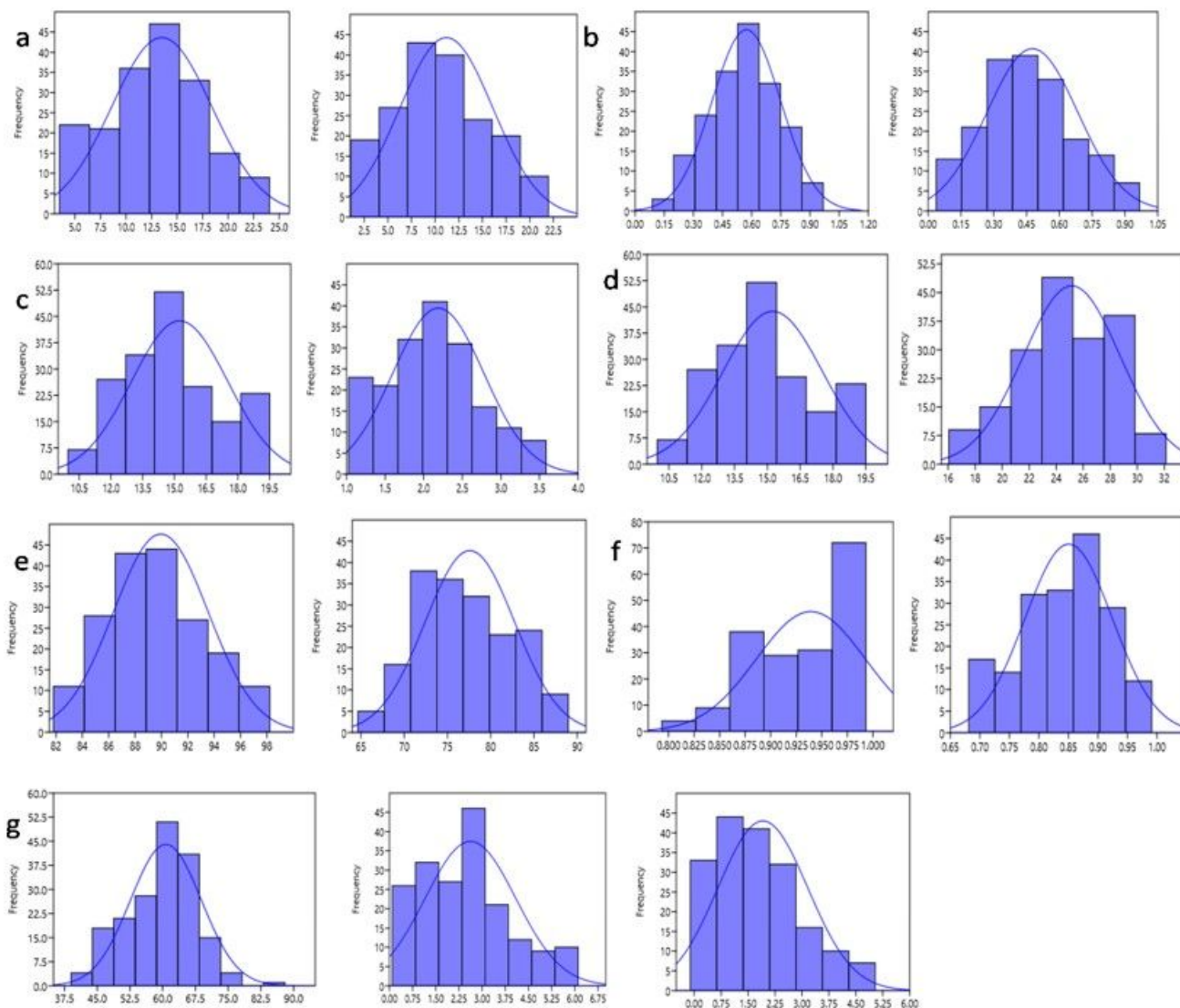
| Trait              | Year | Loc | Env | LG | QTL Name | Marker interval    | LOD Score | Additive effect | PVE (R <sup>2</sup> %) |
|--------------------|------|-----|-----|----|----------|--------------------|-----------|-----------------|------------------------|
| <b>Biochemical</b> |      |     |     |    |          |                    |           |                 |                        |
| Chlorophyll        | 2017 | PLP | S   | 4  | QCHL01   | MUSSR501- MUGR523  | 2.6       | -1.54           | 8.5                    |
| Malondialdehyde    | 2017 | PLP | C   | 2  | QMDA01   | MUMST80- OPI22     | 3.9       | 0.74            | 9.9                    |
| Proline            | 2017 | PLP | S   | 4  | QPRO01   | MUMST628-MUGSSR211 | 3.4       | -0.22           | 12.1                   |
| <b>Root</b>        |      |     |     |    |          |                    |           |                 |                        |
| Root dry weight    | 2017 | PLP | C   | 5  | qRD01    | RCS64486-MUMSD677  | 2.7       | 0.41            | 10                     |
| Root fresh weight  | 2017 | PLP | C   | 5  | qRF01    | RCS64486-MUMSD677  | 4.7       | 0.61            | 15.8                   |
| Root length        | 2017 | PLP | C   | 3  | qRL01    | MUMS41-MUMS68      | 3.3       | -2.57           | 8.3                    |
|                    | 2017 | PLP | C   | 9  | qRL02    | HUGMS3-MUGR607     | 5.0       | 6.60            | 53.4                   |

**Figures**



**Figure 1**

Parents (HPK4 is male parent, HPKM249 is female parent)



**Figure 2**

Frequency distribution curve of (a) chlorophyll content in control (left) and stress condition (right) (b) carotenoid content in control (left) and stress condition (right) (c) proline content in control (left) and stress condition (right) (d) MDA content in control (left) and stress condition (right) (e) RWC in control (left) and stress condition (right) (f) MSI in control (left) and stress condition (right) (g) Root length, Root fresh weight and Root dry weight

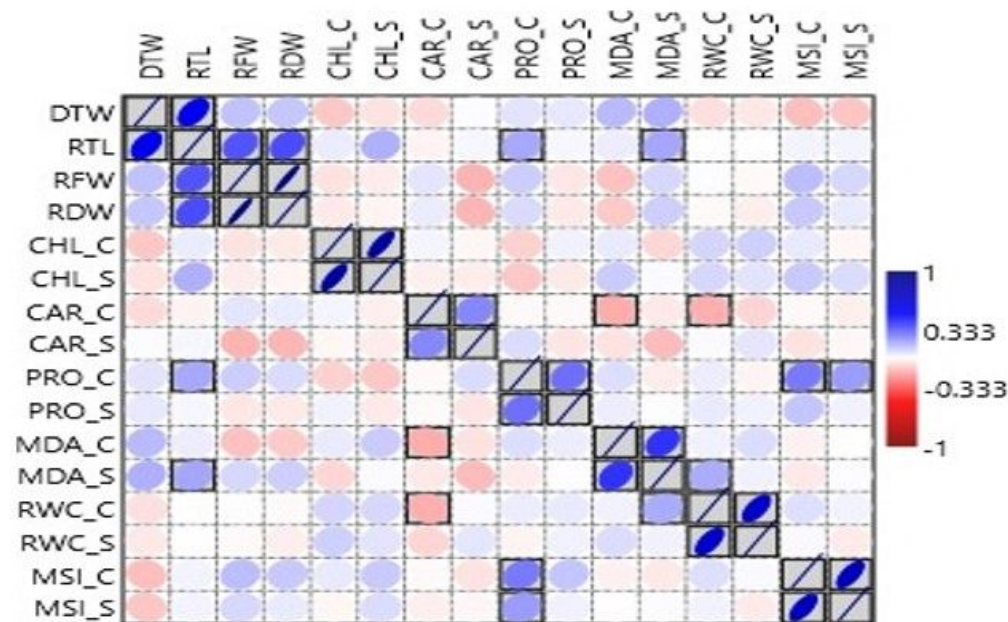


Figure 3

Pearson's correlation matrix among different traits analyzed in the HPM249 x HPK4 RILs

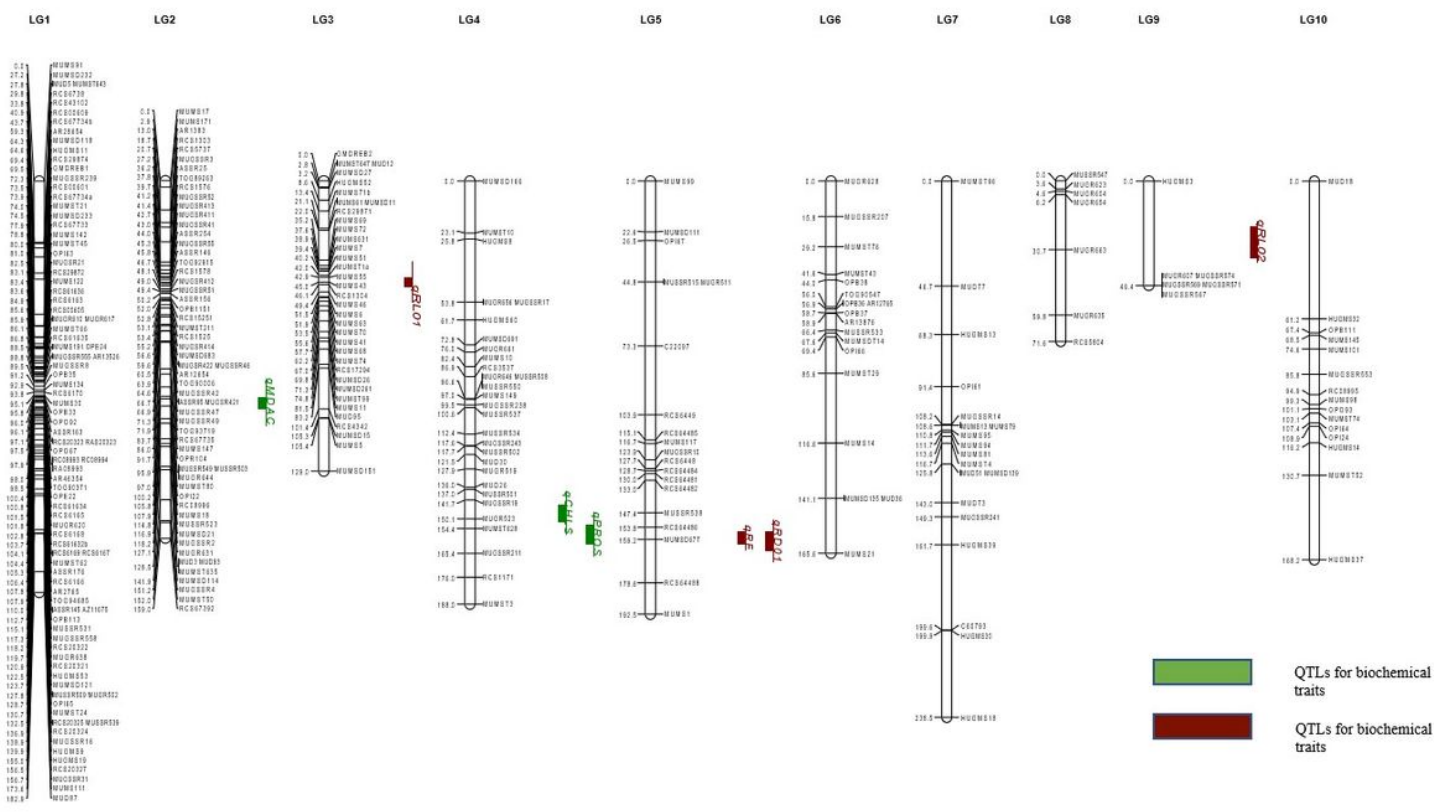
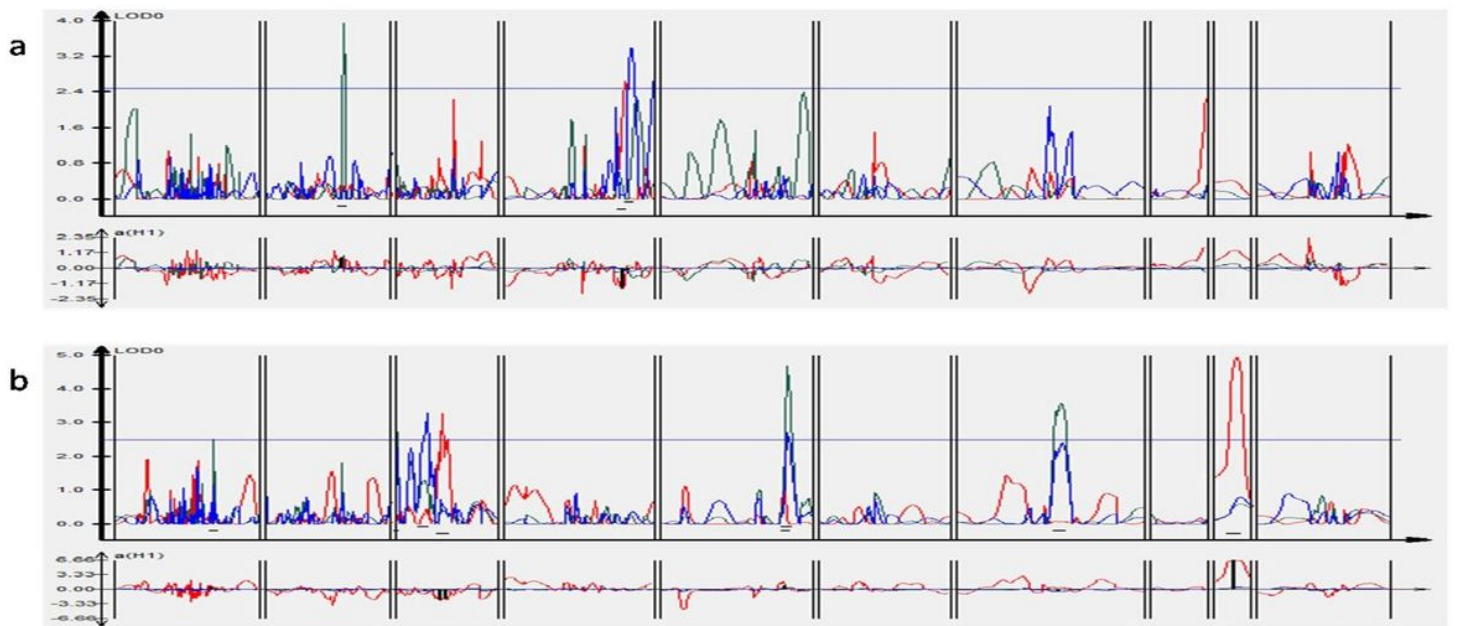


Figure 4

Locations of quantitative trait loci (QTLs) associated with drought related traits in recombinant inbred lines (RILs) mapping population



**Figure 5**

Position of QTLs for (a) biochemical traits (b) root traits on 10 linkage groups of horsegram