

## Genetic Variability and Marker Trait Association analysis of Various Phenological and Yield Related Traits for Heat Tolerance in Chickpea (*Cicer arietinum* L.)

Uday Chand Jha<sup>1,2\*</sup>, Paresh Chandra Kole<sup>2</sup>, Narendra Pratap Singh<sup>1</sup>

<sup>1</sup>Indian Institute of Pulses Research (IIPR), Kanpur, Uttar Pradesh (208 024), India

<sup>2</sup>Dept. of Genetics & Plant Breeding and Crop Physiology, Institute of Agriculture, Visva-Bharati, Sriniketan, Bolpur, West Bengal (731 236), India

### Corresponding Author

Uday Chand Jha  
e-mail: [uday\\_gene@yahoo.co.in](mailto:uday_gene@yahoo.co.in)

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### Abstract

Increasing incidence of heat stress (HS) is receiving serious attention as it causes significant yield reduction in various crops including chickpea worldwide. Here, we investigated the existing genetic variability for various yield related crucial traits for developing heat tolerant genotype under field condition in a panel of seventy eight chickpea genotypes under normal and HS condition via conducting augmented design analysis. Analysis of variance (ANOVA) exhibited significance difference among the checks for first flowering (FF), days to 50% flowering (50F), days to pod initiation (DPI), days to maturity (MAT), plant height (PH), empty pod (EP), yield plant<sup>-1</sup> (YPP), biological yield (BioY), harvest index (HI%), and 100 seed weight (100 SW) under normal condition. While, under HS condition significance difference among the checks for the following traits FF, 50F, PH, EP, YPP and 100SW were recorded. Additionally, to seek marker trait association (MTA), we examined MTAs for the given phenological and yield related traits under both normal and late sown condition via employing 81 simple sequence repeat (SSR) markers in the given set of genotypes. A total of 37 significant MTAs (under normal condition) and 38 significant MTAs (under HS condition) were obtained for various phenological and yield related traits. Additionally, eighteen MTAs for heat tolerance index (HTI), stress susceptibility index (SSI), yield index (YI), mean productivity (MP) and geometric mean productivity (GMP) were recorded.

**Keywords:** Genetic variability, heat stress, MTA, SSR

### 1. Introduction

Chickpea remains as an important cool season global grain legume crop, offering plant based dietary protein and essential micro nutrients to human population across the globe (Graham and Vance, 2003). Chickpea stands as the second most important global grain legume next to common bean (FAO, 2014), contributing 14.2 mt to the global food basket from 14.8 mha area across the globe with an average productivity of 0.96 t ha<sup>-1</sup> (FAO, 2014). However, chickpea yield is seriously challenged by various biotic and abiotic stresses impeding to attain its potential yield (Jha et al., 2014a). In parallel, given the current deleterious effects of global climate change, increasing event of heat stress (HS) is appearing as one of the important abiotic stresses, causing detrimental effects on various crops including cool season grain legumes (Jha et al., 2014a, 2014b; Jha et al., 2017). Significant phenological changes and detrimental effects on pre- and post reproductive processes leading to reduction in yield have been recorded in chickpea under terminal HS (Devasirvatham et al., 2013; Jha et al., 2015; Krishnamurthy et al., 2011).

Given the increase in 1 °C “seasonal temperature” in North India during chickpea growing season may lead to reduction of 53 kg ha<sup>-1</sup> yield in chickpea (Kalra et al., 2008). Therefore, breeding for heat tolerance in chickpea is urgently needed to sustain chickpea yield under the increasing incidences of HS. In the context, conventional breeding driven efforts have enabled in identification of ICC 92944, ICC 1205, ICC 4958 chickpea genotypes as source of HS tolerance under field condition (Devasirvatham et al., 2013; Krishnamurthy et al., 2011). However, progress in development in designing HS tolerant chickpea remains slow. In parallel surge of various advanced molecular markers have offered great opportunity to the breeder community to exploit them in marker assisted breeding scheme for improving various complex traits including HS in chickpea (Bajaj et al., 2015a; Thudi et al., 2014; Varshney et al., 2014; Kale et al., 2015; Jha et al., 2018). Thus, role of (MTA) analysis an approach of marker assisted molecular breeding could be of great importance for identifying genomic regions conferring HS tolerance thereby, accelerating the HS tolerance breeding in chickpea. Here, we investigated the existing genetic variability for phenological and yield related



traits in a panel of seventy eight chickpea genotypes (including historically high yielding released varieties in India, improved breeding lines and accessions) under normal and late sown condition. Additionally, we examined the MTAs for various yield related traits under both normal and late sown condition via employing 81 simple sequence repeat (SSR) markers in the 71 genotypes for facilitating marker assisted breeding for HS tolerance in chickpea.

## 2. Materials and Methods

### 2.1. Experimental material

The experimental material constituted 78 chickpea genotypes containing historically released varieties cultivated across the India, accessions from ICRI SAT, Patancheru, improved breeding lines of Indian Institute of Pulses Research (IIPR), Kanpur and JNKVV Jabalpur including three heat tolerant checks (ICC 1205, ICC 4958 and ICC 92944) (Devasirvatham et al., 2012; Devasirvatham et al., 2013). The crop was grown in the second week of November 2105 (normal sown) and the late sown crop was grown in second week of January 2016 (HS) at the main farm of Indian Institute of Pulses Research (IIPR), Kanpur. The average weekly temperature recorded during the crop growth period from 2<sup>nd</sup> week of November 2015 to April 2016 is given in (Figure 1). Each genotype was sown in two rows having 4×0.3 m<sup>2</sup> plot size. All the 75 genotypes were planted in augmented design having 5 blocks with the above given three checks replicated in each blocks. Randomly five plants of each genotype were selected. Average data of five plants for each genotype was recorded for first flowering (FF), 50% flowering (50F), days to pod initiation (DPI), days to pod filling (DPF), plant height (PH), days to maturity (MAT), primary

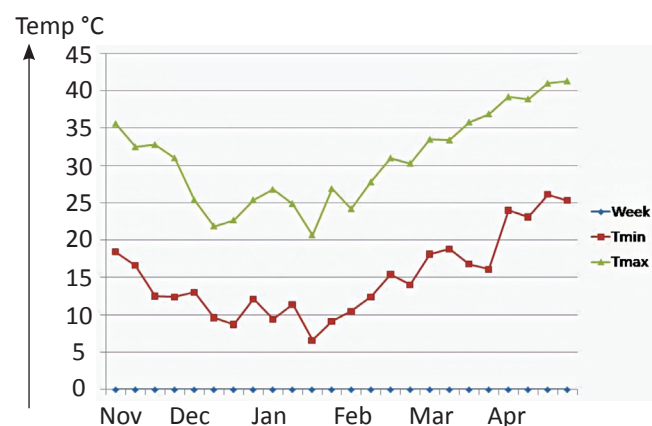


Figure 1: Mean weekly minimum and maximum day temperature recorded during the crop season in 2015-2016

branches (PB), biological yield (Bio Y), number of pods plant<sup>-1</sup> (NOPS), empty pods plant<sup>-1</sup> (EP), yield plant<sup>-1</sup> (YPP), harvest index % (HI%), 100 seed weight (100 seed Wt) and Plot Yield (PY) various traits of breeding interest under both conditions.

### 2.2. Statistical analysis

ANOVA for the given data in augmented design (Federer, 1956) was analyzed by using R software. Additionally, to seek

MTAs with the heat tolerance indices, we also estimated yield stability index HTI, YI, SSI, MP and GMP five important heat tolerance indices. The indices were calculated as per the suggested formulae below.

$$SSI = (1 - (Y_{si}/Y_{pi})) / SI \text{ (Fischer and Maurer, 1978)}$$

$$HTI = (Y_s \times Y_p) / Y_p^2 \text{ (Fernandez, 1992)}$$

$$\text{Yield index (YI)} = Y_s / Y_p \text{ (Bouslama and Schapaugh, 1984)}$$

$$\text{Mean productivity (MP)} = (Y_{pi} + Y_{si}) / 2 \text{ Hossain et al. (1990)}$$

$$\text{Geometric mean productivity (GMP)} = \sqrt{Y_{pi} \times Y_{si}} \text{ (Ramirez and Kelly, 1998)}$$

Y<sub>si</sub> and Y<sub>pi</sub> are the mean grain yield of individual genotype in HS and non HS conditions; whereas, Y<sub>s</sub> denote the mean yield of genotype under HS and Y<sub>p</sub> the mean yield of genotype under normal condition.

### 2.3. DNA extraction and SSR analysis

As per the CTAB method suggested by Doyel and Doyle (1987) genomic DNA was extracted from 71 chickpea genotypes. Given the screening of 120 SSR markers in the given set of genotypes, a total of 81 SSRs yielded polymorphic fragments. The SSR markers used here are reported previously by different research groups Winter et al. (1999, 2000); Sethy et al. (2003); Sethy et al. (2006); Gaur et al. (2011); Choudhary et al. (2009); Choudhary et al. (2012) existing across the all eight linkage groups in chickpea.

### 2.4. PCR analysis

The PCR assay was carried out in a 10 µl reaction mixture containing 5.9 µl of sterilized distilled water, 1.00 µl template DNA (25 ng), 0.5 µl of forward and 0.5 µl of reverse primer (5 µM), 1.00 µl 10×PCR buffer (10 mM Tris-HCl, 50 mM KCl, pH 8.3), 1.00 µl dNTP mix (0.2 mM each of dATP, dGTP, dCTP and dTTP) and 0.1 µl *Taq* polymerase (5U µl<sup>-1</sup>) (Thermo Fisher Scientific Mumbai, India, Pvt. Ltd.) by using G-40402 thermo cycler (G-STORM, Somerset, UK). A touch down PCR profile was used for amplifications with initial denaturation at 94 °C for 5 min followed by 10 cycles of touch down 61–51 °C, 30 s at 94 °C, annealing for 30 s at 61 °C (the annealing temperature for each cycle being reduced by 1 °C per cycle) and extension for 30 s at 72 °C. This was accompanied by 40 cycle of denaturation at 94 °C for 30 s, annealing at 51 °C for 30 s, elongation at 72 °C for 45 s, and 10 min of final extension at 72 °C. Amplified fragments were resolved in 3% agarose gel using 0.5×TBE running buffer and images were analyzed with Quantity one software (Bio-Rad, CA 94547, USA).

### 2.5. Marker-trait association analysis

The phenotypic data on fourteen traits and the genotypic data were analyzed to examine significant MTAs. Here we employed mixed linear model (MLM) based on Q+K matrix. We used TASSEL v. 3.0 (Bradbury et al., 2007; Zhang et al., 2010) to detect MTAs, and  $p=0.05$  and  $p=0.01$  were considered as a significance threshold.

### 3. Results and Discussion

#### 3.1. Genetic variability

General statistics for various traits recorded under both normal and late sown trials are given in (Table 1). Mean square for

analysis of variance (ANOVA) suggested significant difference among all the checks for most of the traits except (PB, DPF, BioY plant<sup>-1</sup> and HI %) under normal sown condition (see Table 2). While, under HS condition significant difference among the checks for the following traits FF, 50F, PH, EP, BioY/P, YPP,

Table 1: Genetic variability studied for various traits in the genotypes

Traits	FF (days)	50F (days)	DPI (days)	DPF (days)	PH (cm)	MAT (days)	PB	Bio Y (g)	NOPS	EP	YPP (g)	HI%	100 S W (g)	PY (g)
Min	37	43	52	62	38.6	98	4	8.9	31	2	6.1	11	7.1	75
Max	82	86	92	115	64.3	143	6	49.7	139	8	18.6	78.24	35.9	390
Mean	62.00	70.00	76.00	88.00	49.42	130.00	5.00	22.15	61.00	5.00	13.23	43.80	18.20	277.78
SE	2.17	2.48	3.04	4.80	3.00	1.96	0.73	0.71	9.48	0.48	0.66	3.15	1.08	9.370
CV %	3.48	3.52	4.01	5.50	6.07	1.50	14.80	3.21	15.40	8.80	5.05	7.21	5.94	3.37
Late sown trial in 2016														
Min	29	37	40	56	21.3	81	3	5.8	11	3	3.5	28.1	7.3	42
Max	59	68	78	95	39.3	112	6	13.5	39	15	6.2	78	24.8	234
Mean	48.00	55.00	63.00	76.00	30.10	98.00	4.00	9.93	22.00	7.00	4.60	49.00	14.50	141.00
SE	2.42	3.80	6.49	5.05	0.97	2.30	0.74	0.48	3.01	0.77	0.20	6.13	0.80	3.700
CV %	5.00	6.87	10.38	6.68	3.25	2.48	17.65	4.91	13.64	10.37	4.41	12.53	5.54	2.67

Table 2: Analysis of variance for normal sown trial (2015-16)

Mean squares													
Source	df	FF	50F	DPI	DPF	PH	DM	PB	Bio Y plant <sup>-1</sup>	NOPS	EP	YPP	
Blocks	4	1.23	3.23	9.56	10.06	13.23	8.266667	0.23	13.83	75.76	0.43	0.07	
Trt	77	88.34**	84.47**	61.13**	75.95*	31.86*	55.11**	0.43	36.2*	327.46*	1.43**	9.07**	
Tests	74	72.13**	73.91**	58.76**	76.37*	31.18*	50.974**	0.37	18.49	334.34*	1.38**	5.14**	
checks	2	154.4**	133.26**	122.6**	91.46	63.72*	231.2**	1.86	12.74	212.46	1.06**	4.27**	
Test vs check	1	1155.2**	768.32**	113.5**	13.86	18.52	9.38**	2	1393.56**	48.02	6.48**	309.5**	
Error	8	4.73	49.46	9.26	23.46	9.02	3.86	0.53	9.3	89.96	0.233	0.44	

Table 2: Continue...

Mean squares			
Source	HI %	100 S W	PY
Blocks	30.08	1.27	60.11
Trt	19.96	36.89**	5113.18**
Tests	19.56	29.19**	4323.14**
checks	12.68	253.21**	11557.59**
Test vs check	64.02	173.47**	50687.15**
Error	13.07	1.17	87.9

100SW and PY were recorded (Table 3). In this connection Jha et al. (2015), Jha and Shil (2015), and Krishnamurthy et al. (2011) recorded significant genetic variability for different phenological and yield related traits under HS in chickpea. Thus, genotype having high BioY, HI and 100SW under HS

could be potentially incorporated into chickpea breeding programme for transferring these traits to the HS tolerant yet low 100 SW genotypes for sustaining yield under HS. Moreover, the tested genotypes could be introduced as parent in crossing programme for developing HS tolerance in chickpea.

#### 3.2. MTA analysis

MTA study is gaining enormous attention from marker assisted breeding point of view in various crop plants including chickpea (Bajaj et al., 2015a; Thudi et al., 2014). Notable instances of MTA study for investigating genomic regions related to drought stress tolerance traits have been recorded (Jamalabadi et al., 2013; Kale et al., 2015; Thudi et al., 2014; Varshney et al., 2014). However, MTA for HS tolerance in chickpea is limitedly exploited (Thudi et al., 2014; Jha et al., 2018).

In the current study a total of 37 significant MTAs under



Table 3: Analysis of variance for late sown trial (2016)

Source	df	Mean squares										
		FF	50F	DPI	DPF	PH	DM	PB	Bio Y plant <sup>-1</sup>	NOPS	EP	YPP
Blocks	4	2.06	3.9	17.93	10.73	1.05	4.4	0.01	0.09	1.06	0.5	0.027
Trt	77	52.94**	51.39*	50.53	38.32	13.69**	34.27**	0.49	2.34**	63**	5.89**	0.42**
Tests	74	36.92**	42.25	49.89	38.26	7.5**	30.19**	0.44	1.67**	30.69*	5.59**	0.39**
checks	2	375.2**	261.66**	63.26	52.86	53.94**	16.46	0.46	1.41*	36.86	8.26**	1.26**
Test vs check	1	593.97**	307.52**	72.8	14.22	391.25**	371.73**	3.92	53.32**	2506.32**	23.12**	0.72**
Error	8	5.86	14.5	337.46	204.26	7.68	42.4	0.55	0.23	9.11	0.6	0.04

Table 3: Continue...

Source	Mean squares		
	HI %	100 S W	PY
Blocks	1.51	1.039	73.26*
Trt	20.45**	16.79**	1261.02**
Tests	16.92**	12.9**	1178.11**
checks	95.81**	49.142**	308.46**
Test vs check	131.19**	239.95**	9301.57**
Error	37.69	0.65	14.21

\* $p=0.05$  and \*\* $p=0.01$ 

normal condition (see Table 4) and 38 significant MTAs under HS condition (see Table 5) for various agronomic traits have been recorded. Concurrently, eighteen MTAs for various HS related indices have been noted (see Table 6). MTAs distribution and quantile–quantile (Q–Q) plots are depicted in Figure 2 (for normal sown), in Figure 3 (for HS sown) and in Figure 4 (for heat tolerance indices) considering MLM model. The markers witnessed significant association with the given traits by deviating from null expectation depicted in QQ plots. Phenological traits remain crucial for evaluation and selection of HS tolerance in various crops including chickpea (Devasirvatham et al., 2013). Taking note of this, three significant MTAs on LG3, LG4 and on LG7 for FF traits under normal condition and one significant MTA on LG6 under HS condition was recorded in the current study. Similarly 2QTLs for FF trait was recorded on LG3 and LG4 under drought stress (Rehman et al., 2011). In this context Jamalabadi et al. (2013) also reported one closely linked marker with FF on LG3. For 50F trait, a total of four significant MTAs on LG4 and on LG3 explaining up to 19.7 PV%, while, two significant MTAs for the same trait on LG6 was recorded under late sown condition. Considering DPI and DPF traits, significant MTA was noted on LG3, whereas two significant MTAs (for DPI) and two significant MTAs (for DPF) were recorded on LG3 and LG6, respectively under late sown condition. For MAT trait, one significant MTA recorded on LG4 (under normal condition) however, no MTA was identified for this trait under late sown condition. In this connection one QTL for MAT trait on LG7 was suggested

Table 4: MTA analysis for trial 2015-16 (Normal sown)

Trait	Marker name	LG	P value	PV%
FF	CESSR 159	LG 3	0.00007**	19
FF	CESSR 43	LG 4	0.03814*	6.3
FF	NCPGR 41	LG 7	0.04066*	6.4
50F	CESSR 159	LG 3	0.00005**	19.7
50F	CakTpSSR03637	LG 4	0.0443*	17.3
50 F	CESSR 43	LG 4	0.04981*	5.6
50 F	CESSR 45	LG 4	0.03709*	6.5
DPF	CESSR 159	LG 3	0.01706*	8
DPI	CESSR 159	LG 3	0.00935**	10.4
MAT	CESSR 45	LG 4	0.00658**	11.4
MAT	TR7	LG7	0.02*	11
PB	NCPGR231	LG3	0.039*	15.4
PB	GA105	LG5	0.02339*	7.6
PB	NCPGR12	LG6	0.03608*	7.2
BioY	GA9	LG5	0.03394*	18.4
BioY	NGPGR225	LG6	0.01952*	11.9
BioY	NCPGR56	LG1	0.046*	8
NOPS	STMS10	LG6	0.03768*	12.7
NOPS	NCPGR200	LG2	0.04*	16
EP	GA6	LG6	0.04081*	23.1
EP	NCPGR202	LG2	0.04*	17.1
EP	H2B061	LG4	0.045*	4
YPP	H1B04	LG5	0.04538*	5
YPP	NCPGR234	LG 6	0.03318*	18.5
YPP	H5A04	LG 7	0.0383*	15.4
YPP	NCPGR193	LG1	0.00784**	15.8
YPP	NCPGR232	LG3	0.048*	19
HI	CakTpSSR02719	0.04351*	13.1	
HI	CESSR45	LG 4	0.04655*	5

Table 4: Continue...





Trait	Marker name	LG	P value	PV%
HI	NCPGR 234	LG 6	0.03396*	18
HI	GA102	LG4	0.04781*	11.6
100SW	TA 18	LG 2	0.02607*	7.5
100 SW	CESSR433	LG 4	0.04346*	6.5
100 SW	TS53	LG 7	0.01654*	25
PY	CESSR114	LG 3	0.02768*	10.8
PY	CESSR159	LG 3	0.00885**	10.5
PY	GA 9	LG 5	0.00288**	24.2

\* $p=0.05$  and \*\* $p=0.01$

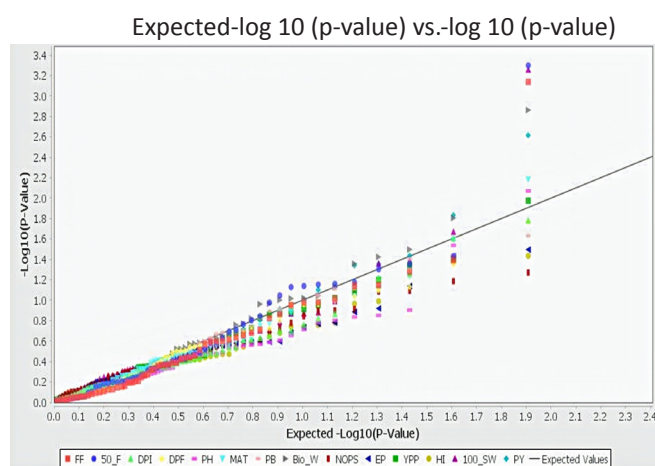


Figure 2: Quantile-Quantile (Q-Q) plots and MTAs distribution for all traits (tested by MLM) under normal sown condition

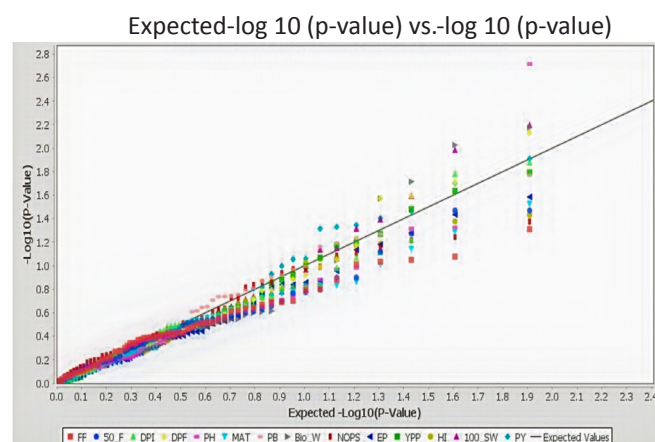


Figure 3: Quantile-Quantile (Q-Q) plots and MTAs distribution for all traits (tested by MLM) under HS sown condition

by Rehman et al. (2011). While considering PH trait three significant MTAs were found on LG1, LG2 and LG4, respectively under HS condition. Likewise two QTLs for same trait were recorded on LG1 and LG4 under drought stress (Rehman et al., 2011). In the context of PH, recently Kale et al. (2015) reported 14 QTL related to PH on LG4 under drought stress. In context of yield related trait,

Table 5: MTA analysis for trial 2016 (Late sown)

Trait	Marker name	LG	P value	PV%
FF	NCPGR 274	LG 6	0.04892*	15.4
50F	NCPCR 234	LG 6	0.03401*	18.4
50F	TA 64	LG6	0.03402*	20
DPI	NCPGR 234	LG6	0.01656*	21
DPI	NCPGR 274	LG6	0.01321*	22
DPF	GA 9	LG5	0.02674*	19
DPF	NCPGR 234	LG6	0.0257*	19.6
DPF	NCPGR 274	LG6	0.0074**	21
PH	TA106	LG1	0.04813*	24.7
PH	NCPGR 202	LG2	0.04712*	19.4
PH	C a k T p S S R 03637	LG4	0.00192**	30
PB	CESSR 43	LG 4	0.01703*	8
BioY	NCPGR13	LG1	0.01703*	8
BioY	GA105	LG 5	0.00199**	9
BioY	NCPGR46	LG7	0.048*	8
BioY	STMS	LG6	0.048*	7
NOPS	TR 7	LG 7	0.04208*	9
NOPS	TA110	LG1	0.046*	12.7
EP	TA110	LG 1	0.03679*	15
EP	TR 7	LG 7	0.02598*	11
YPP	TA 140	LG 1	0.03472*	10.7
YPP	H5G12	LG 6	0.01449*	12.8
YPP	TR7	LG7	0.04*	8
YPP	NCPGR202	LG2	0.04*	17.4
YPP	NCPGR156	-	0.04*	11.9
HI	CESSR114	LG 3	0.02806*	10.7
HI	GA105	LG 5	0.03023*	7
HI	H1B04	LG 5	0.00975**	10
HI	GA26	LG6	0.03923*	15.2
100 SW	TA18	LG 2	0.01037*	10.1
100 SW	TA8	LG 3	0.00636**	22.5
100SW	NCPGR199	LG1	0.02548*	17.8
100 SW	TA180	LG2	0.04839*	22.1
100SW	CakTpSSR03637	LG4	0.04*	17.7
PY	CESSR159	LG3	0.04111*	6
PY	NCPGR 41	LG 7	0.01627*	12.4
PY	TA110	LG1	0.01684*	18.9
PY	TR 7	LG7	0.03999*	9

\* $p=0.05$  and \*\* $p=0.01$



Table 6: MTA analysis for various HS indices

Trait	Marker	LG groups	p value	PV%
HTI	CESSR114	LG3	0.03634*	9.9
HTI	CakTpSSR03637	LG4	0.01813*	17
HTI	GA9	LG5	0.02672*	19.4
YI	TA110	LG1	0.02746*	16.7
YI	CakTpSSR03637	LG4	0.00938**	19.2
YI	NCPGR41	LG7	0.01833*	12.1
SSI	NCPCR234	LG6	0.04871*	16.9
SSI	STMS25	LG6	0.01865*	12.2
SSI	NCPGR41	LG7	0.00415**	16.9
MP	NCPGR149	-	0.03577*	20.7
MP	CESSR114	LG3	0.0095**	14.2
MP	CESSR159	LG3	0.00228**	14.7
MP	GA9	LG5	0.00264**	19.3
GMP	NCPGR149		0.01926*	17.3
GMP	CESSR114	LG3	0.01571*	12.6
GMP	CESSR159	LG3	0.01028*	10.2
GMP	GA9	LG5	0.01947*	20.8
GMP	TAAS	LG7	0.02285*	17.4

\* $p=0.05$  and \*\* $p=0.01$

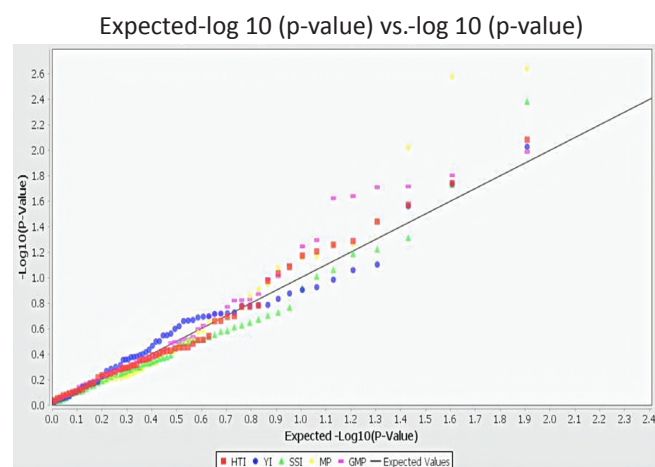


Figure 4: Quantile-Quantile (Q-Q) plots and MTAs distribution for heat tolerance indices (tested by MLM)

four significant MTAs for BioY trait on LG1, LG5, LG6 and LG7, and two significant MTA for NOPS on LG1 and LG7 were noted under late sown condition. Likewise one major QTL governing NOPS on LG7 was registered under salinity stress (Pushpavalli et al., 2015). While Bajaj et al. (2015b) reported several NOPS QTLs existing across all Ca LG (1-8). In another case, four QTLs for NOPS existing on LG1, LG2, LG6 and LG8 were reported (Verma et al., 2015). Based on HI% trait, we recorded three

significant MTAs under both normal and late sown conditions existing on LG3, LG4, LG5 and on LG6. Similarly two QTLs for the HI trait were found on LG1 and LG3 (Rehman et al., 2011). Importantly, one QTL was registered on LG06 under drought stress for HI trait (Kale et al., 2015). Additionally, a total of six QTLs related to HI residing across all the LG except LG1 and LG8 has been reported recently (Srivastava et al., 2016).

Considering YPP, five significant MTAs were recorded on LG1, LG3, LG5, LG6 and LG7 under normal condition exhibiting upto 19 % PV, and five significant MTAs on LG1, LG6 and LG7 showing up to 12.8% PV were recorded under HS condition for YPP. Similarly, six YPP related QTLs existing across all the LGs except LG1 and LG8 were reported (Srivastava et al., 2016).

For 100 SW three MTAs harboring on LG2, LG4 and LG7 under normal condition and five significant MTAs under HS located on LG1, LG2, LG3 and LG4 were recorded. Similarly seed wt related QTL located on LG1 (Abbo et al., 2005; Hossain et al., 2010; Gowda et al., 2011), on LG2 (Gowda et al., 2011), on LG4 (Cobos et al., 2007; Abbo et al., 2005; Hossain et al., 2010; Gowda et al., 2011; Jamalabadi et al. 2013; Thudi et al. 2014; Kale et al., 2015) has been reported. Additionally five significant SNPs associated with seed wt were reported on LG1, LG2, LG3 and LG4 (Bajaj et al., 2015a). Subsequently three expression QTLs (e-QTLs) related to seed wt was reported on LG2 and LG7 by Bajaj et al. (2015b). Moreover, Verma et al. (2015) showed seven seed wt QTLs residing on LG1, LG2, LG5, LG6 and LG7. Most importantly, a total of 29 QTLs related to nine different agronomic traits and drought related traits were recovered from *QTL-hotspot* region on CaLG04 (Kale et al., 2015). Taking note of PY, three significant MTAs under normal and four significant MTAs under late sown conditions lying on LG1, LG3, LG5 and LG7 were obtained in the current study. In this regard three QTLs (related to yield) each was reported to be lying on LG3, LG4 and LG7 under drought stress (Kale et al., 2015).

### 3.3. MTA analysis of heat tolerance indices

Importantly, considering heat tolerant indices viz., HTI, YI, HSI, MP, GMP several significant MTAs were recorded (see Table 5). Three significant MTAs were recorded on LG3, LG4 and on LG5. Similarly for YI; three significant MTAs were suggested residing on LG1, LG4 and LG7. Considering SSI a total of 3 MTAs were recorded on LG6 and on LG7. While for MP (4 MTAs) and for GMP (5 MTAs) were noted. Similarly a total of 4 QTLs for drought tolerance index (DTI) harboring on CaLG1, CaLG7 and CaLG8 and one QTL for DST trait on CaLG8 was reported under drought stress (Kale et al., 2015). Thus, these markers significantly associated with various traits could serve as an important repertoire for assisting marker assisted breeding for heat tolerance in chickpea.

## 4. Conclusion

Sufficient amount of genetic variability for various breeding traits was recorded under both normal and HS conditions.



Thus, the captured genetic variability for various traits under both conditions could be incorporated in breeding programme for improving yield related traits in the high yielding yet HS sensitive chickpea cultivars. Additionally, significant MTAs for various yield related traits could promisingly help facilitating conventional breeding to develop HS tolerant chickpea genotypes via marker assisted selection.

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## 6. Author's Contribution

Uday Chand Jha conducted the experiment and wrote the manuscript along with Paresch Chandra Kole and Narendra Pratap Singh. The work is part of PhD thesis of the first author. All the authors also thank, Dr. Swarup K Parida, National Institute of Plant genome Research, New Delhi for providing Chickpea SSR markers.

## 7. Conflict of Interest

The authors declare that they have no conflict of interest.

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