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QTL mapping for heat stress tolerance in chickpea (*Cicer arietinum* L.)

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ABSTRACT

Rising evidence of heat stress (HS) is appearing as one of the major challenges to crop performance including chickpea affecting plant growth and yield significantly. Unprecedented advancements in chickpea genomic resources have resulted in remarkable progress for genetic dissection of various complex traits including biotic and abiotic stresses. However, these genomic resources have been limitedly utilized for developing HS tolerance in chickpea. Thus, the present study was aimed to capture genetic variability and to identify HS relevant quantitative trait loci (QTL) using 206 F_2 individuals developed from DCP 92-3 × ICCV 92944 cross. Wide range of genetic variability for seventeen traits related to phenological, physiological and breeding importance was captured from the given population under HS condition by growing them in late sown condition. A total of 78 SSR markers were used for genotyping of the given F_2 individuals. Only 39 markers were fitted to Mendelian segregation and these were assigned to all linkage groups (LGs) except LG8, covering 859 cM of genome. QTL analysis revealed one QTL controlling primary branch number (PB) explaining 2% phenotypic variation (PV) on LG3 and another QTL related to chlorophyll content (CHL) on LG6 explaining 17.2% PV. In future, fine mapping of these QTL controlling genomic regions may enable uncovering the underlying candidate gene(s) contributing in HS tolerance. Thus, these genomic regions could be promisingly utilized for marker assisted breeding for developing heat tolerant chickpea genotype.

Key words: Chickpea, Genomics, Heat stress, QTL, SSR.

INTRODUCTION

Chickpea remains globally recognized important grain legume crop that plays significant role in fighting against global food insecurity by supplying 'plant based dietary protein' and vital micronutrients to the global human population (Bohra *et al.* 2014). Global production of chickpea remains 13.73 MT from 13.98 Mha (FAO, 2016). However, its productivity is severely affected by various biotic and abiotic stresses (Jha *et al.* 2014). Under the increasingly severe climatic events, HS is receiving a serious concern for cold season grain legumes including chickpea, limiting their potential yield (Jha *et al.* 2017). Significant phenological changes and detrimental impacts on pre- and post reproductive processes leading to reduction in yield have been recorded in chickpea under terminal HS (Devasirvatham *et al.* 2012; Jha *et al.* 2017). Chickpea is highly sensitive to high temperature stress (> 35°C) during reproductive stage causing significant yield reduction due to malfunctioning of various reproductive events (Devasirvatham *et al.* 2012). Reduction of 53 kg/ha yield in chickpea due to increase in 1°C "seasonal temperature" in North India during chickpea growing season has been recorded (Kalra *et al*. 2008). Thus, to breed heat tolerant chickpea genotype, significant genetic variability has been captured in chickpea under HS (Krishnamurthy *et al.* 2011; Jha and Shil, 2015; Jha *et al.* 2015, Jha *et al.* 2018a, 2018b, 2018c; Paul *et al.* 2018a). In the context, ICCV 92944, ICC 1205, and ICC 4958 chickpea genotypes have been identified as source of HS tolerance under field condition (Krishnamurthy *et al.* 2011; Gaur *et al.* 2012). In the last decade significant progress has been achieved in terms of developing chickpea genomic resources (for details see Jha, 2018), providing great opportunity to the chickpea breeding community for performing genomic assisted selection for increasing genetic gain. However, progress in genetic dissection of HS tolerance in chickpea remains limited (Thudi *et al.* 2014; Jha *et al.* 2018b; Jha, 2018; Paul *et al.* 2018b). Here, we captured wide range of genetic variability for seventeen traits of morpho-physiological and breeding importance in F_2 based mapping population developed from DCP 92-3 × ICCV 92944 cross under HS. Concurrently, we identified one QTL related to PB on LG3 and another QTL for CHL on LG6.

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MATERIALS AND METHODS

Phenotyping

DCP 92-3 a medium to late maturity chickpea variety was used as female parent and ICCV 92944 a heat tolerant

genotype (Gaur *et al.* 2012) was used as male parent for developing F_1 progenies. A total of 206 individuals were obtained from the given cross in F_2. The F_2 individuals were evaluated for various breeding and physiological traits under HS by sowing them in late condition in the first week of January in the year 2016-17 under field condition in augmented design. Data on twelve different traits of breeding importance and phenological traits such as primary branch (PB), plant height (PH), days to first flowering (FF), days to pod initiation (DPI), days to pod filling (DPF), days to maturity (DM), total pods/plant (TPP), empty pods/plant (EP), yield/ plant (YPP), 100 seed weight (100 SW), biological yield (BY), and harvest index (HI) were taken. Likewise, data on five traits of physiological interest such as nitrogen balance index (NBI), chlorophyll content (CHL), flavonoid content (FLV), anthocyanin content (ANTH) and membrane stability index (MSI) were taken under HS condition. These physiological traits were measured by leaf spectrometer instrument. General statistics for the traits were estimated by 'Analyse it' software.

Genotyping and construction of genetic linkage map

Genomic DNA was extracted from the parental genotypes and 206 F_2 individuals at seedling stage following CTAB method (Doyle and Doyle 1987). The PCR assay was carried out in a10 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, pH 8.3, 50 mM KCl), 1.00 µl dNTP mix (0.2 mM each of dATP, dGTP, dCTP and dTTP) and 0.1 µl *Taq* polymerase (5U/ µl) (Thermo Fisher Scientific Mumbai, India, Pvt. Ltd.) by using G-40402 thermo cycler (G-STORM, Somerset, UK). A touch down PCR profile was performed 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). A total of 450 SSR markers reported by (Winter *et al.* 1999; Udupa*et al.* 1999; Sethy *et al.* 2003, Sethy *et al.* 2006; Choudhary *et al.* 2009; Choudhary *et al.* 2012; Gaur *et al.* 2011) were used for checking parental polymorphism. Among the given SSRs only 78 SSRs yielded polymorphic fragments and these were used for genotyping of 206 F_2 individuals. To measure segregation pattern of each SSR marker against the expected ratio of 1:2:1 at 0.01 probability level Chi square test was performed. Linkage analysis was done by Join Map version 4.0 (Van Ooijen, 2006) at critical LOD scores of 3 between two markers. For identification of QTL, Win QTL cartographer 2.5 (Wang *et al.* 2010) was used. Composite interval mapping, with LOD score of 2.5 and Kosambi mapping function (Kosambi, 1944) was used for detection of QTLs for various breeding and physiological traits under HS condition.

RESULTS AND DISCUSSION

Genetic variability for different traits

A wide range of genetic variability for various traits of both breeding and phonological importance was recorded in the parental genotypes (Table 1) and the derived F_2 mapping population evaluated under late sown condition given in (Table 2). Importantly, considering various heat stress tolerant indicative physiological traits *viz*., NBI (Li *et al.* 2015) representing the ratio of chlorophyll to polyphenols that accurately measure canopy nitrogen concentrations, CHL, MSI, FLV and ANTH, a wide range of genetic variability was captured (Table 2). Likewise, significant genetic variability for various phenological and yield related traits in chickpea mapping population has been reported under HS (Paul *et al.* 2018a, 2018b).

QTL mapping

In order to elucidate genetic basis of heat tolerance, several mapping population segregating for various HS relevant traits have been reported in various crops including rice (Ye *et al.* 2012, Lei *et al.* 2013), wheat (Mason *et al.* 2011, Bennett *et al.* 2012, Paliwal *et al.* 2012), maize (Frova and Sari-Gorla, 1993). However, limited mapping populations have been developed for elucidating HS tolerance in chickpea (Paul *et al.* 2018a, 2018b). In the current study, a total of 78 SSR markers were assayed in the above F_2 mapping population. Out of 78 SSR markers only 39 markers showed the Mendelian segregation pattern of 1:2:1. And these SSR markers were assigned to all LG groups except LG8, covering 859 cM map distance. Two QTLs (Table 3), one minor QTL for PB flanked by TA142 and ICCM281a markers, with LOD value >3 (Fig 1 and Fig 2) explaining 2%

Table 1: Genetic variability for various traits in two parents.

Traits	DCP 92-3	ICCV 92944
Primary branch number	6	5
Plant height (cm)	26.5	35.5
Days first flowering	55	42
Days to pod initiation	63	53
Days to pod filling	76p	65
Days to maturity	112	91
Empty pod/plant	6	4
Yield/plant	4.9	7.3
Total pods/plant	26	39
100 seed wt (g)	12.6	22.1
Biological yield (g)	10.4	15.1
Harvest index (HI)	39.42	48.3
Nitrogen balance index (NBI)	12.9	28.6
Chlorophyll content (CHL)	21.2	50.6
Flavonoids content (FLV)	1.35	1.78
Anthocyanin content (ANTH)	0.12	0.23
Membrane stability index (MSI) %	39.1	56.5

Table 2: Genetic variability recorded in F_2 population (DCP 92-3 × ICC92944) under HS during 2016-2017.

Traits	Min.	Max.	Mean	SE	SD	CV %
Primary branch	3	7	4.88	0.07	0.95	19.56
Plant height (cm)	33	48	39.22	0.23	3.2	8.18
Days to first flowering (days)	35	58	47	0.39	5.67	12.06
Days to pod initiation (days)	56	76	66.86	0.24	3.46	5.16
Days to pod filling (days)	65	91	81.92	0.34	4.94	6.02
Days to maturity(days)	85	112	100.83	0.34	4.88	4.84
Total pods/plant	8	37	25	0.34	4.9	19.61
Empty pod/plant	2	12	6.65	0.16	2.36	35.44
Yield/plant (g)	1.5	9.3	4.93	0.1	1.55	31.4
100SW (g)	4.1	21.3	13.13	0.29	4.16	31.68
Biological yield(g)	4.2	17.2	11.41	0.17	2.53	22.18
HI%	14.7	62.8	43.25	0.64	9.29	21.4
NBI	4.2	46.8	16.13	0.51	7.37	35.66
CHL	11.7	69.8	26.8	0.77	11.17	41.7
FLV	1.08	2.04	1.68	0.01	0.18	10.89
ANTH	0.01	0.18	0.065	0.002	0.03	49.9
MSI%	14.3	49.7	29.18	0.6	8.69	29.8

SE= Standard Error, SD= Standard Deviation.

Table 3: List of QTLs identified in F_2 population under HS in chickpea.

Trait	LOD score	LG	Map position	Flanking marker	Additive effect	PV%
Primary branch number (PB)	4.7	3	60-76 cM	TA142 and ICCM281a	0.1193	2
Chlorophyll content (CHL)	4.9	6	89-111cM	NCPGR206 and H3G031	7.2874	17.4

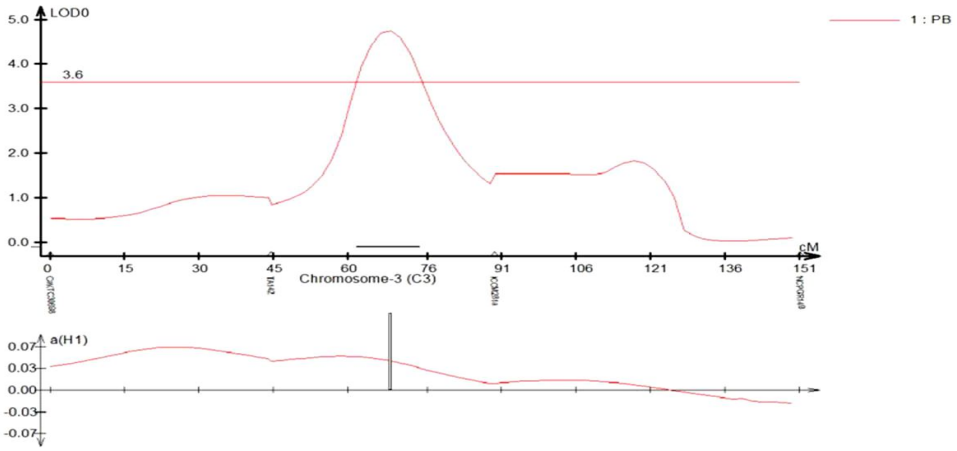

Fig 1: LOD value of primary branch number trait.

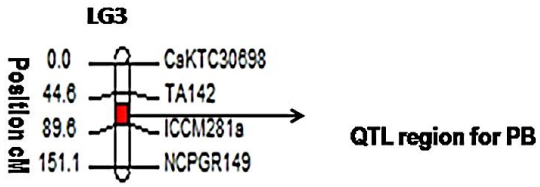

Fig 2: Primary branch number controlling QTL on LG3.

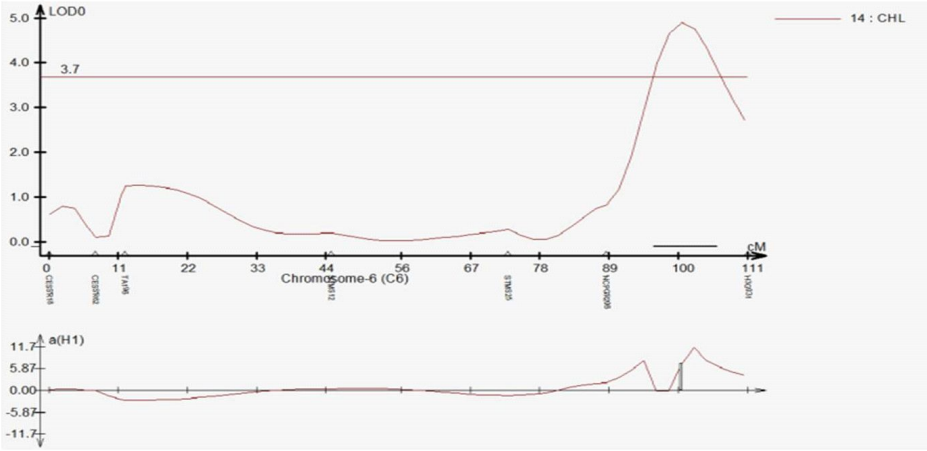

Fig 3: LOD value of chlorophyll content (CHL) trait.

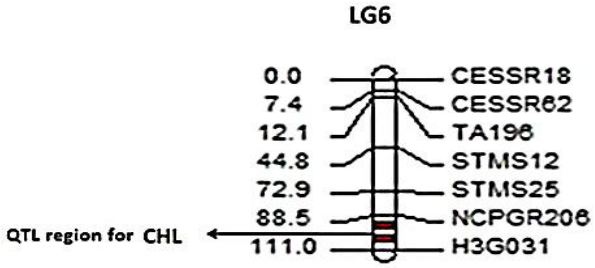

Fig 4: Chlorophyll content controlling QTL on LG6.

phenotypic variation (PV) on LG3 and the other QTL contributing in CHL content flanked by NCPGR206 and H3G031 with LOD value >3(Fig 3 and Fig 4) explaining 17.2% PV on LG6 were identified. Similarly QTLs controlling number of primary branches an important yield contributing trait have been reported on LG1 (Saxena *et al.* 2014) and on LG8 (Kale *et al.* 2015) under drought stress. While for chlorophyll content, five SRAP markers associated to chlorophyll content QTL with PV up to 53% was elucidated by Elshafei *et al.* (2013) in wheat under drought stress condition. Likewise, Talukdar *et al.* (2014) identified seven QTLs explaining PV up to 30.8% on chromosomes 6A, 7A, 1B and 1D in wheat for SCMR chlorophyll content under HS. Considering yield related traits *viz.*, filled pods/plant, Paul *et al.* (2018b) mapped *qfpod02_5* (explaining 11.57% PV) and *qfpod03_6* (explaining 6.56 % PV) QTLs on Ca LG5 and Ca LG6 respectively in chickpea by employing genotyping by sequencing in ICC15614 × ICC 4567 mapping population in chickpea under HS. They also mapped another two QTLs *qgy02_5* on CaLG05 and *qgy03_6* on CaLG06 contributing in grain yield under HS. Thus, in the present study the investigated genomic regions could be further fine mapped for underpinning the potential candidate gene(s) contributing in HS tolerance in chickpea.

CONCLUSION

The present study provides preliminary information on number of primary branches an important yield contributing trait and chlorophyll content related to HS tolerance controlling genomic regions that could be fine mapped in future. Thus these genomic regions need further detailed research for utilizing them in marker assisted breeding programme for developing heat tolerant chickpea genotype.

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