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QTL MAPPING OF "A-GENOME" FOR INTERSPECIFIC POPULATION OF *GOSSYPIUM HIRSUTUM* AND *GOSSYPIUM ARBOREUM*

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Abstract

Cotton production in Pakistan is vital for economic development of the country. It contributes around 0.6 percent to GDP and 3.1 percent of the value added in agriculture for the year 2020. Similarly, it earned 61.5% of the foreign exchange for the country in the year 2006 and have fallen to 51% in 2018. Thus, it needs to utilize molecular tools for the enhancement of seed cototn yield and use of QTL (Quantitative trait loci) for improvemetn of crops yield is well documented. Thus the plant materials used in this study was 280 individual progenies of BC_4F_2 mapping population raised from cross between *G. hirsutum* and *G. arboreum*. These backcross progenies were manually planted in 450 cm rows for each genotype during 2013~2014 in China at Jiangpu Farm Nanjing and a total of 8488 simple sequence repeat markers (SSRs) were employed on this BC_4F_2 population. The results showed that 2056 SSRmarkers were found to be polymorphic with 83% dominant and 17 % codominant. Total 26 QTLs were detected for twelve different traits on various chromosomes. Five QTLs (Quantitative trait loci) were recognized for seed cotton yield on chromosome number 1, 5, 9, 11 and 12, showing PVE of 3.8 to 9.1%. SSR markers such as BNL3347, BNL140, GH594, GH100, NAU462, NAU650, NAU2508, NAU150, NAU3401 and NAU-400 would be helpul in Marker Assisted selection. Identified QTLs can prove to be expedient for identification of right progenies in cotton breeding program including gene mapping.

Key words: *Gossypium hirsutum* L.; *Gossypium arboreum*; QTL mapping, Intetspecific population; Within boll yield components; Marker assisted selection.

Introduction

Cotton is worldwide commercial crop for natural fiber, grown for its fibre needed for integrated industries of ginning, spinning, and textiles. The genus *Gossypium* comprised of above fifty species, encapsulating majority of diploid species and seven teraploid species (Wendel & Grover 2015). *Gossypium hirsutum* (2n=4X=52) characterized with higher yield and appropriate fibre quality is sole source of approximately 95% of world cotton production, but is prone to biotic and abiotic stresses. Its Genome (2.5 Gb) encompases 26 asymmetric chromosomes (Zhang *et al*., 2015) while diploid *G. arboreum* genome is of 13 asymetric chromosomes. Contrary to *G. hirsutum*, the *G. arboreum* experienced strict natural and artificial selection pressures in its evolution, which made it able to harbour stress tolerant traits other than tetraploid species. *Gossypium arboreum* (2n=2X=26) is characterized with muti-color, strong, coarse fibre, and lower yield but highly adaptive to drought and marginal environmental (Kantartzi *et al*., 2009) with strong resistance to the disease of cotton leaf curl virus disease (Hua *et al*., 2018). Limitations such as biotic resistance in the genomic potential of *G. hirsutum* have been tried to overcome with its interspecific crosssing with *G. arboreum*, i.e., an interspecific cross between *G. hirsutum* and *G. arboreum* was established to incorporate nematode resistance in *G. hirsutum* cultivars (Li *et al*., 2018).

History of quantitative trait loci (QTL) mapping in cotton trace back to Reinisch *et al*., (1994) and number of idenified QTLs reached above 6000 for more than 150 traits (https://www.cottongen.org/find/qtl. Lacape *et al*., 2010). QTL mapping has played a pivotal part in the studies of quantitative traits genetics and has facilitated mapping of essential traits in cotton. These loci are assessed for their unique positions on the chromosomal segments (Miles & Wayne, 2008), recognized by molecular markers. Markers known as simple sequence repeat are extensively utilized to probe important genes and construction of linkages maps (Mishra *et al*., 2013, Zhao *et al*., 2016). Cai *et al*., (2019) studied ninety nine accessions of *G. hirsutum* by the use of 97 simple sequence repeat (SSR) markers and reported 70 marker-traits association. Shi *et al*., (2019) identified 153 (QTLs) quantitative trait loci for yield and quality traits of Fibre in a population of interspecific cross between *Gossypium hirsutum* and *Gossypium barbadense*. Keerio *et al*., (2018) identified 37 quantitative trait loci for quality traits of fibre and yield in an interspecific cross between *G. hirsutum* and *G. tomentusum*.

The study was carried out for mapping of QTLs for morphological traits by using population developed which was formed by interspecific cross between *Gossypium hirsutum* and *Gossypium arboreum*. Present results might give a base for the initiation of association and MAS breeding studies in tetraploid vs artificial-teraploid mapping population of cotton. QTLs logged in present experiment could be employed in future breeding in the development of varieties have high yield, drought and CLCuD tolerant *Gossypium hirsutum* of Cotton.

Materials and Methods

Plant materials: The plant materials used in this study included *G. hirsutum* cv CRSM-38 (2n = 4X (AADD) = 52), and *G. arboreum* cv 15-Mollisoni (2n = 2X (AA) = 26). The F_1 population obtained from the cross of *G. hirsutum* and *G. arboreum*, was used for the generation of BC_4F_2 mapping population comprising 280 individual plant progenies as utilized in earlier studied by Nazeer *et al*., (2014). These backcross progenies were manually planted in 450 cm rows for each genotype during 2013~2014 in China at Jiangpu Farm Nanjing by maintaining plant to plant and row to row distance of 30 cm and 75 cm, respectively.

Phenotyping

The observations and measurements for morphological plant traits taken during the trial were:

a. Plant archetecture traits (PA): Data for plant archetecture tratis like monopod branches per plant (MPpP), fruiting braches per plant (FBpP), and number of bolls per plant (BpP) were recorded at plant maturity stage.

b. Within-boll yield components (WBYC): The 20 bolls were picked and cleaned from tagged plant of each entry. Seed cotton and lint weight of each sample was recorded by the use of electrical balance after ginning. Various within-boll yield components (WBYC) were calculated as per genetic yield model of Worley *et al*., (1976) and Basal (1996) as under;

Locule boll^{-1} (LpB)

Locule boll^{-1} of the sample is calculated by dividing total number of locules by total boll number.

Boll size(BS) (g)

Boll size of the sample was calculated by dividing total weight of seed cotton by total number of bolls.

Seeds locule^{-1} (SpL)

Seeds per locule of the sample was calculated by dividing total number of seeds by total number of locules.

Seed number boll^{-1} (SpB)

Total number of seeds boll^{-1} of the sample was calculated by dividing total number of seeds of the sample by total number of balls.

Seed cotton weight seed^{-1} (SCpS)

Seed cotton weight seed^{-1} was calculated by dividing boll size by total number of seeds boll^{-1}.

Seed cotton locule^{-1} (SCpL)

Seed cotton locule^{-1} was calculated by dividing size of the boll by locule boll^{-1}.

Seed cotton weight plant^{-1} (SCW)

Total number of seeds boll^{-1} was calculated by dividing total number of bolls plant^{-1} by size of the boll.

Lint mass per boll (LM)

Seed cotton weight plant^{-1} was calculated by dividing total lint mass of the sample by total number of bolls.

Seed mass per boll (SM)

$$\text{Seed mass per boll} = \frac{\text{(Total seed mass of the sample}}{\text{(Total number of bolls in the sample}}$$

Seed index (SI)

Ginning of the samples of seed cotton was performed first. Then 100 seeds were taken from it and its weight was calculated on electrical balance.

DNA extraction and genotyping: Tiny young leaves of cotton plants were collected in ice-box for DNA CTAB method (Zhang & Stewart, 2000) with few modifications and quantification was performed by spectrophotometer (DU800). Microsatellite (SSRs) primer pairs with BNL prefix were obtained from BNL primers Research Genetics Cp. Huntsville, AL, USA, (https://www.resgen.com), JESPR from Reddy *et al*., (2001), TM from Dr. John Tu, USDA-ARS, Crop Germplasm Reearch Unit, TE, USA, CIR from Nguyen *et al*., (2004)and NAU were synthesized at Nanjing Agricultural University (Han *et al*., 2006). 8,488 pairs of SSR (simple sequence repeat) primer were selected (Guo *et al*., 2007; Lacape *et al*., 2003; Rong *et al*., 2004; Yu *et al*., 2011) and used for the identification of polymorphic markers between *G. hirsutum* L (P_1), *G. arboreum* (P_2) and their F_1. Over all list of simple sequence repeat primers used in this research to screen parents and F_1 are given in Table 1. The SSR based PCR amplifications were performed by standard PCR procedures which are described by Zhang *et al*., (2000) by the use of a Programmable Thermal Controller (MJ Research). Separation of the Polymerase chain reaction products were performed and visulizing on agarose gel (1%) (Cregan & Quigley, 1997) as well as (30%) polyacrylamide gels (Zhang *et al*., 2002) by using the image system of SX (Sixing Biological Technology Co. Shanghai, China). Samples were run on Polyacrilamide gel and observed by silver staining of the Gel.

QTL analysis: Association analysis between markers and phenotypic values was investigated using single marker analysis (SMA) by using 2.5 version of Windows QTL Cartographer and step wise regression (RSTEP-LRT) function of IciMapping 3.0 (http://www.isbreeding.net). For the detection of effects of additive QTL of non-idealized CSILs, QTL IciMapping 3.0 (http://www.isbreeding.net) was used. For the declaration of significant additive QTL threshold of LOD 3.0 was used.

Table 1. Loci resource used for this study.

Public name	Prefix of primers	Resource	Primers
NAU747-NAU759	NAU	*G. arboreum*	13
NAU761-NAU1606	NAU	*G. arboreum*	846
NAU2000-NAU2523	NAU	*G. hirsutum*7235, Xu142	489
NAU2552-NAU4105	NAU	*G. raimondii*	1554
MUCS004-MUCS622	MUCS	*G. arboreum*	351
MUSS001-MUSS605	MUSS	*G. arboreum*	265
NAU4850-NAU5513	NAU	*G. hirsutum* acc. TM-1 ,Xu142	664
STV001-STV192	STV	*G. hirsutum*	192
NAU6093-NAU6123	NAU	*G. barbadense* cv. Hai7124	31
NAU6720-NAU6771	NAU	*G. barbadense* cv. Hai7124	52
NAU6933-NAU7229	NAU	*G. barbadense* cv. Hai7124	297
Cer0060, 63, 77, 144, 145, 148, 164	Cer	Monsanto	7
Shin0050-Shin1501	Shin	Monsanto	27
HAU0309-HAU2738	HAU	*G. barbadense* cv. Hai7124	318
w1073,w1075	W	*G. hirsutum* acc. TM-1	2
BNL0119-BNL4108	BNL	*G. hirsutum*	217
TM01, TM08, TM09-TM23	TM	Dr John Yu	110
JESPR02-JESPR311	JESPR	Reddy *et al*., (2001)	307
NAU413- NAU760	NAU	our insititue	117
CIR001-CIR418	CIR	Nguyen *et al*., (2004)	392
NAU2524-NAU2551,	NAU	BAC-SSR	28
NAU4106- NAU4111	NAU	our institute	6
BNL1015-BNL4103	BNL	*G. hirsutum*	387
NAU6124- NAU6701	NAU	*G. hirsutum* cv. Maxxa	578
Gh2-Gh697	Gh	Cotton Database	159
NAU7230-NAU7656	NAU	Our institute	427
Cgr5015-cgr6949	Cgr	Monsanto	397
cot002-cot142	Cot	Monsanto	22
dc20004-dc40407	Dc	Monsanto	54
dPL001-dPL0922	dPL	Monsanto	179
		Total	8488

On the basis of banding patterns obtained after PCR amplification of different SSR primers, scoring of the individuals of the F_2 population was performed as follows:
A = Alleles of female parent P_1
B = At this locus alleles of male parent P_2 is homozygote for the allele b from parental strain P_2
H = Heterozygous (presence of both parental alleles)
C = Not a homozygote for allele a (i.e. either B or H)
D = Not a homozygote for allele b (i.e. either A or H)
O = The data is missing at this locus for the individual

Results

Chromosome single segment substitution lines (CSSSLs) are capable of map-based cloning and QTL mapping (Frary *et al.,* 2000; Wan *et al.,* 2006). Chromosome segment substitution lines which have more than one segment of chromosome substitution makes it impossible to find a QTL on a segment of single chromosome by the comparison of the performance of trait between one CSS line and the background parent. Wang *et al.,* (2006, 2007) projected a possibility ratio test which was based on stepwise regression (RSTEP-LRT) for the detection of QTL of non-idealized Chromosome segment inbred lines. QTL IciMapping 3.0 (http://www. isbreeding.net) was used to detect the effects of additive QTL of non-idealized CSILs. The LOD threshold 3.0 was used for the declaration of significant additive QTL.

It is considered in this analysis that a key QTL will have PEV >20%, an intermediate QTL will have % PEV of 5 to 20%, and a minor QTL will have % PEV <5% (El-Feki 2010). suggestive QTL is a QTL that have value of LOD between 2.0 and 3.0 (Lander & Kruglyak, 1995), whereas the QTL which has LOD value no less than the value of threshold which is calculated by a test of permutation with 1000 times will be called as a significant QTL (Churchill & Doerge, 1994).

A total number of 8,488 SSR (simple sequence repeat) primer pairs were selected (Guo *et al.,* 2007; Lacape *et al.,* 2003; Rong *et al.,* 2004; Yu *et al.,* 2011) and were used for the identification of polymorphic markers between parents *G. hirsutum* cv CRSM-38 (2n = 4x = AADD = 52) (P_1), *G. arboreum* cv 15-Mollisoni (2n = 2x = AA = 26) (P_2), along with their F_1. Out of 8,488 only 2056 SSRs were polymorphic with 24.2% of polymorphism rate out of total 8,488 SSRs . Among 2056 simple sequence repeat polymorphic primers, 83% were dominant SSRs whereas 17% were co-dominant SSRs. There were totally 922 polymorphic primers that were dominant for P_2 and co-dominant and represented about 45% of the polymorphic primers. Figure 1 shows the screening of parents including F_1.

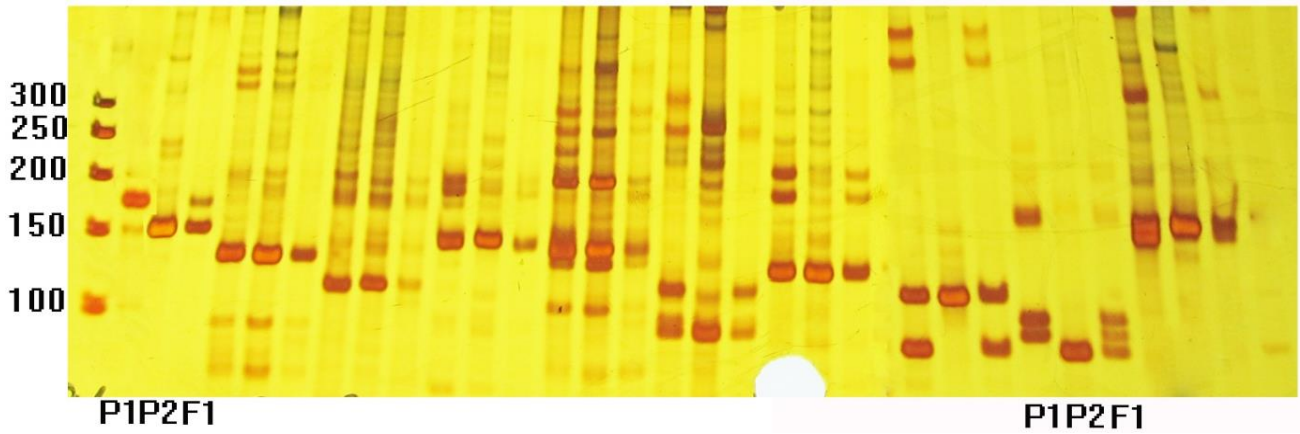

Fig. 1. Parental screening including F_1.

Quantitative traits loci: The QTLs studies were conducted for thirteen agronomic traits; Plant architecture (PA) traits, i.e. monopodial branches per plant (MPpP), fruiting braches per plant^{-1} (FBpP), number of bolls per plant (BpP); and within boll yield (WBY) components locule boll^{-1} (LpB), boll size(BS), seeds locule^{-1} (SpL), seed number boll^{-1} (SpB), seeds cotton weight seed^{-1} (SCpS), seeds cotton locule^{-1} (SCpL), seed cotton weight plant^{-1} (SCW), lint mass per boll (LM), seed mass per boll (SM), and seed index (SI). The list of the QTLs identified for morphological PA traits is shown in Table 2.

For twelve traits, 26 quantitative trait loci were identified; five QTLs for SCW, three QTLs each for FBpP, SpB, and LM; two each for LpB, BS, SpL, and SM; and one QTL each for MPpP, BpP, SpB, SCpS and SCpL. The list of all QTLs identified for these traits is presented in Table 2. Maximum and minimum phenotypic variation explained for these traits was 3.8 and 29.0 % presented by markers NAU3401-400, JESPR274-105 on chromosomes 12 and 9 for SCW trait. Whereas the LOD range for agronomic traits was 2.5 to 9.1. Chromosome 5 had maximum number of QTLs among agronomic traits, i.e., one QTL was present each for LpB, BS, SpL, SpB, SCW, LM and SM with with PVE range 9.5-24.4%. Likewise, chromosome 9 had QTLs for five traits MPpP, FBpP, LpB, BS and SCW with PVE % range 5.5-29.0%; and chromosome 12 showed QTLs for five traits FBpP, SpL, SCW, LM and SM having 3.8-21.9% PVE.

Five markers were found to be overlapped or we can call stable markers for the expression of different traits. For example, on chromosome 5, two markers BNL3347-140 for traits LpB, BS and marker Gh594-100 for five different traits i.e. SpL, SpB, SCW, LM, SM were found to be important for expression of these traits. Similarly, marker NAU462-650 actively linked for the expression of MPpP, FBpP, LpB, BS; marker NAU2508-150 for SpB, SCpL; and marker NAU3401-400 for FBpP, SpL, SCW, LM, SM.

Plant archetecture tratis (PA traits): Four QTLs (3 significant and 1 suggestive) for PA traits were detected, three for FBpP and one for MPpP located on chromosome 9, 11 and 12. The QTL qFBpP-A12-1 present on chromosome 12 exhibited major effects i.e., 21.8 % PEV for expression of FBpP with 6.7 additive value. Marker NAU3401-400 was tied up with this significant QTL. Collectively three loci for FBpP showed 38.8% PEV with LOD range 2.6 to 4.9.

Within boll yield components (WBY): For bolls plant^{-1} (BpP), one significant QTL was present on chromosome 7. Marker NAU2887-450 actively participated for expression of this trait PVE 23.3% having LOD and additive value for this QTL of 4.8 and 12.3, respectively. Two significant QTLs each for locules boll^{-1} (LpB) and boll size (BS) were detected on chromosome 5 and 9 with PVE range 6.6-24.4% and 9.2-18.4%, respectively. Markers BNL3347-140 and NAU462-650 were linked for expression of these two traits. For seeds locule^{-1}(SpL), two significant QTLs were detected on chromosome 5 and 12 with PVE range 13.4 to 17.3%. Markers Gh594-100 and NAU3401-400 were linked for expression of this trait. Similarly, three significant QTLs for seeds boll^{-1}(SpB), were detected on chromosome 5, 7 and 10 with PVE range 9.5 to 14.5%. Markers Gh594-100, NAU2108-350 and NAU2508-150 were associated with this trait. One QTL each for, seed cotton weight seed-1(SCpS), seeds cotton weight locule^{-1} (SCpL) was expressed with PVE value 14.4 and 12.6 % on chromosome 2 and 10, respectively.

The trait SCW showed maximum QTLs among studied traits. A total of five QTLs (one suggestive and 4 significant) for SCW were disbursed among chromosomes 1, 5, 9, 11, and 12. The detected quantitative trait loci described 3.8 to 29.0 % of PVE. The minimum phenotypic variations (3.8%) was observed on chromosome 12 for this trait with additive value 28.5. These five QTLs were detected with LOD range from 2.8 to 9.1. Markers NAU3401-400, NAU3022-235, Gh594-100, JESPR274-105, and Gh369-145 contributed for expression SCW.

For Lint Mass (LM), three important (QTLs) quantitative trait loci were present on chromosome 4, 5 and 12 having phenotypic variation value of 7.3 to 18.3%. These three QTLs were detected with LOD range from 3.11 to 5.43. Two significant QTLs for SM were identified on chromosome 5 and 12, with corresponding additive value -0.62 and -0.93 with phenotypic variance 17.7 and 5.2%, respectively. These two loci showed intermediate effects for expression of this trait.

Table 2. QTLs with associated marker for plant architectural and within boll yield components detected by QTL Ici Mapping 3.3.

QTL	Trait name	Chrom	Marker name	LOD	PVE (%)	Additive
qMPpP-A9-1	MPpP	9	NAU462-650	3.01	5.56	-0.93
qFBpP-A9-1	FBpP	9	NAU462-650	3.47	5.67	-9.34
qFBpP-A11-1	FBpP	11	NAU1014-185	2.61	10.97	4.31
qFBpP-A12-1	FBpP	12	NAU3401-400	4.90	21.89	6.78
qBpP-A7-1	BpP	7	NAU2887-450	4.84	23.29	12.34
qLpB-A9-1	LpB	9	NAU462-650	3.61	6.62	0.22
qLpB-A5-1	LpB	5	BNL3347-140	5.23	24.43	-0.21
qBS-A9-1	BS	9	NAU462-650	5.14	9.26	0.67
qBS-A5-1	BS	5	BNL3347-140	3.80	18.41	-0.38
qSpL-A5-1	SpL	5	Gh594-100	3.44	13.41	-0.42
qSpL-A12-1	SpL	12	NAU3401-400	4.33	17.33	0.54
qSpB-A5-1	SpB	5	Gh594-100	3.98	14.52	-2.05
qSpB-A7-1	SpB	7	NAU2108-350	3.58	12.92	-2.88
qSpB-A10-1	SpB	10	NAU2508-150	2.71	9.56	1.93
qSCpS-A2-1	SCpS	2	NAU1246-200	2.92	14.46	-0.01
qSCpL-A10-1	SCpL	10	NAU2508-150	2.52	12.64	0.07
qSCW-A12-1	SCW	12	NAU3401-400	2.82	3.88	28.50
qSCW-A1-1	SCW	1	NAU3022-235	3.51	9.56	-32.39
qSCW-A5-1	SCW	5	Gh594-100	3.50	9.51	22.45
qSCW-A9-1	SCW	9	JESPR274-105	9.10	29.01	-41.36
qSCW-A11-1	SCW	11	Gh369-145	5.75	16.65	-39.04
qLM-A4-1	LM	4	NAU6993-130	5.43	7.43	-2.59
qLM-A12-1	LM	12	NAU3401-400	4.91	7.31	-0.82
qLM-A5-1	LM	5	Gh594-100	3.11	18.29	-0.32
qSM-A12-1	SM	12	NAU3401-400	3.79	5.19	-0.93
qSM-A5-1	SM	5	Gh594-100	3.01	17.71	-0.62

MPpP, monopodial branches plant^{-1}; FBpP, fruiting braches plant^{-1}; BpP, bolls number plant^{-1}; LpB, locule boll $^{-1}$, BS, boll size; SpL, seeds locule^{-1}; SpB, seed number boll^{-1}, SCpS, seed cotton weight seed^{-1}; SCpL, seed cotton locule^{-1}; SCW, seed cotton weight plant $^{-1}$; LM, lint mass per boll; SM, seed mass boll^{-1}; SI, seed index

Discussion

Quantitative trait loci (QTL) for yield and yield related components had been widely mapped by different scientists (Gu *et al*., 2020; Ijaz *et al*., 2019: Ma *et al*., 2008; Ruixian *et al*., 2020; Saeed *et al*., 2011; Shen *et al*., 2005; Wang *et al*., 2006a; Wang *et al*., 2007). Worley *et al*., (1974) described three significant morphological traits of cotton plant which contributed for the improvement of seed cotton yield i.e., total number of bolls on unit area of the land will plays a primary role, lint mass formed by each seed is secondary and seed numbers per boll plays a tertiary roll in total lint yield production. They determined that selection should be made on the above stated three parameters for enhancement in yield. Culp & Harrell (1975) stated side by side upgrading in the yield of lint while working with important breeding lines and different cotton check cultivars. They stated that increase in the yield of lint iscaused by the increase in the total numbers of seeds in each boll, by which the total surface area of the seed is increased for better production of lint. Quantity of the lint seed^{-1} will be improved slightly with the improvement in lint percentage. Total numbers of bolls present on the unit land area had been the main factor which will contribute to yield of the lint. Because of complex genetics of yield and yield related traits , it seems to be a good idea to split the yield components into small components to minimize the effects of environment (Lacape *et al.,* 2013). Thus yield related traits were splited into plant architecture traits (MPpP, FBpP, BpP) and witinin boll yield (WBY) components (LpB, BS, SpL, SpB, SCpS, SCpL, SCW, LM, SM, Seed index).

Using likelihood ratio test which was constructed on stepwise regression (RSTEP-LRT) for detecting quantitative trait loci of non-idealized as proposed by Wang *et al*., (2006, 2007), 26 QTL were identified to be linked with 12 different quantitative traits. Shaheen *et al*., (2013) identified seven quantitative trait loci which included five for yield related traits while exploring the diploid *G. arboreum*. The trait SCW showed maximum QTLs among studied agronomic traits having minimum phenotypic variation of 3.8%. However, Wang *et al*., (2007) explained 5.12% of phenotypic variance for SCW. Yield of the Seed cotton is determined by two important yield related components, i.e., number of bolls plant^{-1} and weight of the boll. Increase in the size of boll will increase the total number of seeds present in the boll, which sequentially increase the surface area, thereby increasing the amount of lint. Two QTLs for BS were detected and linked on two chromosomes 5 and 9 representing the phenotypic variation 9.2 to 18.4% PVE with LOD score 3.8 to 5.1. Altogether these two QTLs represented 27.6 % PVE (Shaheen *et al*., 2013). The results are compatible with the studies of Ma *et al*., (2008) who suggested“A” genome for yield and yield related characters of cotton and demonstrated that most QTLs for these traits established slight effects and controlled less than 20% of the total phenotypic

variation. While 6.14% PVE had been observed in intraspecific crosses (Wang *et al*., 2007). However, FBpP, BpP, LpB, SCW, each had at least one QTL that controlled over 20% of the phenotypic variation.

Total number of bolls and fruit branches plant^{-1} directly play their role for yield improvement. Our study identified three QTLs for FBpP and one for BpP similar to that of Ma *et al*., (2008). Selection should be constructed on number of bolls per m^2 (unit land) and production of seeds in each boll, alongside with the selection for maintenance and increase in the amount of lint which is produced on each seed (Worley *et al*., 1974). Jiang (2004) observed 8.56% phenotypic variation explained for bolls per plant. Increase in locule number will simultaneously increase the seed number per boll and ultimately will increase the cotton yield. Two QTLs for LpB identified with PEV 5.6 to 24.4%. These two QTLs together showed 31.05% phenotypic variation. Similarly, three significant QTLs for seeds boll^{-1} exhibited PVE range 9.5 to 14.5%. Markers Gh594-100, NAU2108-350 and NAU2508-150 were expressed for contribution of this trait. Two QTLs for SM and SpL exhibited 5.1-17.7% and 13.4-17.3% explained variation, respectively.

Chromosome wise location of QTLs showed that chromosome 5 represented the maximum QTLs for studied traits i.e., seven QTLs were linked with six traits (LpB, BS, SpL, SpB, SCW, LM SM); followed by chromosome 9 that have five QTLs for five traits (MPpP, FBpP, LpB, BS, SCW).

Some markers can effectively be used for MAS because of close likage with more than one trait on the same chromosome. Marker BNL3347-140 on chromosome 5 was linked for BS and LpB; another maker Gh594-100 on chromosome 5 was associated for five traits SpL, SpB, SCW, LM SM. NAU3401-400 on chromosome 12 was linked with FBpP, SpL, SCW, LM, SM; marker NAU2508-150 for SpB, SCpL and marker NAU462-650 showed association with MPpP, FBpP, LpB, and BS. Traits tightly linked with the same markers also showed highly significant correlation with each other.

Conclusions

The results obtained from IcI Mapping analysis detected 26 QTLs for 12 traits; five QTLs for SCW, three QTLs each for FBpP and LM; two each for LpB, BS, SpL, SpB, and SM; and one QTL each for MPpP, BpP, SpB, SCpS and SCpL. The maximum and minimum phenotypic variation explained for these agronomic traits was 3.8 and 29.0%. Five markers were found overlapping for expression of different traits. For example, on chromosome 5, two markers BNL3347-140 for traits LpB, BS and marker Gh594-100 for five different traits i.e. SpL, SpB, SCW, LM, SM was found to be important for expression of these traits. Similarly, marker NAU462-650 actively participated for expression of MPpP, FBpP, LpB, BS; marker NAU2508-150 for SpB, SCpL; and marker NAU3401-400 for FBpP, SpL, SCW, LM, SM. The information about the associated markers can be helpful for MAS. The identified QTLs present in introgression lines will prove to be very useful in the proper identification and assortment of right progenies for improved breeding programs, which will include mapping of the gene, and eventually highlighting the importance of marker assisted selection in worldwide cotton enhancement.

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