

Fast-Track Introgression of “QTL-hotspot” for Root Traits and Other Drought Tolerance Traits in JG 11, an Elite and Leading Variety of Chickpea

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Abstract

A “QTL-hotspot” containing quantitative trait loci (QTL) for several root and drought tolerance traits was transferred through marker-assisted backcrossing into JG 11, a leading variety of chickpea (*Cicer arietinum* L.) in India from the donor parent ICC 4958. Foreground selection with up to three simple sequence repeat markers, namely TAA170, ICCM0249, and STMS11, and background selection with up to 10 amplified fragment length polymorphism primer combinations was undertaken. After undertaking three backcrosses with foreground and background selection and selfing, 29 BC₃F₂ plants homozygous for two markers (ICCM0249 and TAA170) were selected and referred as introgression lines (ILs). Root trait phenotyping of these ILs showed higher rooting depth (RDp) (average 115.21 ± 2.24 cm) in all 29 ILs, better root length density (RLD) (average 0.41 ± 0.02 cm cm⁻³) in 26 ILs, and higher root dry weight (RDW) (average 1.25 ± 0.08 g per cylinder) as compared to the recurrent parent, JG 11 (111.70 cm for RDp, 0.39 cm cm⁻³ for RLD, and 1.10 g per cylinder for RDW), as well as the donor parent, ICC 4958 (114.20 cm for RDp, 0.45 cm cm⁻³ for RLD, and 1.25 g per cylinder for RDW). These ILs, developed in 3 yr, after multilocation field trials may be released as improved variety with enhanced drought tolerance.

CHICKPEA is an important food legume crop that ranks second in the world production after common bean (*Phaseolus vulgaris* L.). The crop is mostly grown in low-input conditions and on residual soil moisture especially in semiarid regions of sub-Saharan Africa and South Asia. Globally chickpea is cultivated on an area of 13.20 million ha with an annual production of 11.62 million t (FAOSTAT, 2011). It is an important source of protein, minerals, fiber, and vitamins in the diets of millions of people in Asia and Africa. The average nutritional content in chickpea are 23% protein, 64% total carbohydrates, 47% starch, 5% fat, 6% crude fiber, 6% soluble sugar, and 3% ash (Jukanti et al., 2012).

Among the various major concerns caused by different biotic stresses in rain-fed ecosystem globally, terminal drought is the major constraint for chickpea production (Kashiwagi et al., 2006). Terminal drought causes yield losses of around 3.7 million t that amounts to 40 to 50% of the average loss (Varshney et al., 2009). Drought stress occurs during the terminal growth stages, as the crop is largely grown rain fed under receding soil moisture environments in the post-rainy season. With the prediction of water shortage in the future, terminal drought will

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Abbreviations: AFLP, amplified fragment length polymorphism; IL, introgression line; JNKVV, Jawaharlal Nehru Krishi Vishwa Vidyalyaya; MABC, marker-assisted backcrossing; MAS, marker-assisted selection; PCR, polymerase chain reaction; QTL, quantitative trait loci; RDp, rooting depth; RDW, root dry weight; RIL, recombinant inbred line; RLD, root length density; RPG, recurrent parent genome; SSR, simple sequence repeat.

Published in The Plant Genome 6
doi: 10.3835/plantgenome2013.07.0022
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5585 Guilford Rd., Madison, WI 53711 USA
An open-access publication

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remain as a major constraint to chickpea production. Therefore, persuading plants to adapt to drought stress either through escape, avoidance, or tolerance mechanisms and the strategies required for enhanced adaptation are contrastingly different (Varshney et al., 2011).

The prolific root system in chickpea contributes to grain yield under terminal drought conditions (Kashiwagi et al., 2006). However, only limited efforts have been made to breed for improved root traits because screening for root traits is a destructive and labor-intensive process and difficult to use in large segregating populations. Combining different drought resistance mechanisms, for example, root traits together with other drought tolerance traits such as water-use efficiency, is a potential strategy for enhancing levels of drought resistance (Saxena, 2003). Marker-assisted selection (MAS) has been successfully applied to transfer root related quantitative trait loci (QTL) into elite background for development of near-isogenic lines or development of drought tolerant genotypes in some species such as maize (*Zea mays* L.) (Tuberosa and Salvi, 2006), wheat (*Triticum aestivum* L.) (Placido et al., 2013), and rice (*Oryza sativa* L.) (Steele et al., 2006). In chickpea, after screening mini-core collection, Kashiwagi et al. (2005) reported ICC 4958 and ICC 8261 as two germplasm accessions with prolific and deeper roots and ICC 1882 and ICC 283 as accessions with small and shallow roots. Subsequently, two recombinant inbred line (RIL) populations, namely ICC 4958 × ICC 1882 and ICC 283 × ICC 8261, were developed. Comprehensive QTL analysis based on extensive genotyping and phenotyping data for root traits and other drought tolerance traits led to the identification of a genomic region on linkage group 4 (CaLG04) that harbors several drought-related traits QTL inclusive of root traits, contributing up to 58.20% of phenotypic variation named as *QTL-hotspot* (R.K. Varshney, M. Thudi, S.N. Nayak, P.M. Gaur, J. Kashiwagi, L. Krishnamurthy, D. Jaganathan, J. Koppolu, A. Bohra, S. Tripathi, A. Rathore, A.K. Jukanti, V. Jayalakshmi, A. Vemula, S. Singh, M. Yasin, M.S. Sheshshayee, and K.P. Viswanatha, personal communication, 2013).

With an objective to enhance the drought tolerance, this study reports successful introgression of the *QTL-hotspot* from ICC 4958, which was used in the QTL mapping study into an Indian leading chickpea variety, JG 11, a leading chickpea variety in India, by marker-assisted backcrossing (MABC) approach. Several MABC lines have been developed and their root traits were not only better than JG 11 but also better than ICC 4958.

Materials and Methods

Plant Materials

To enhance the drought tolerance of high yielding variety JG 11 (ICCV 93954), a *desi* type chickpea variety that is the most widely grown variety in India, it was selected for introgression of *QTL-hotspot* from donor parent ICC 4958. ICC 4958 (*desi* type) was selected as donor parent

based on its high root related traits, namely rooting depth (RDp), root length density (RLD), and root dry weight (RDW), in this accession and it was also used in the QTL mapping study.

Deoxyribonucleic Acid Extraction, Polymerase Chain Reaction, and Marker Genotyping

Fifteen-day-old seedlings were used for DNA extraction from the parental genotypes, F₁s, and backcross progenies through modified cetyltrimethyl ammonium bromide extraction method, as described in Cuc et al. (2008). The quality and quantity of DNA were checked on 0.8% agarose gels and DNA concentration was normalized to approximately 5 ng μL⁻¹ for further genotyping work.

The polymerase chain reaction (PCR) for the targeted simple sequence repeat (SSR) markers of the QTL region was performed in 5 μL reaction volumes as mentioned in our earlier studies (Nayak et al., 2010; Thudi et al., 2011). Amplified PCR products were separated by capillary electrophoresis using an ABI Prism 3730 DNA Sequencer and analyzed using GeneMapper software (Applied Biosystems).

Amplified fragment length polymorphism (AFLP) assay was performed with IRDye fluorescent AFLP kit (LI-COR Biosciences). Genomic DNA (approximately 100 ng) was digested with *Eco*RI and *Mse*I enzyme mix (0.5 μL) for 2 h at 37°C followed by inactivation of enzymes at 70°C for 15 min. The DNA fragments were ligated to appropriate adaptors using T4 DNA ligase (0.25 μL) and 6 μL of adaptor mixture (*Eco*RI and *Mse*I adaptors) by incubating the mixture at 20°C for 2 h followed by 1:10 dilution with supplied Tris-ethylenediaminetetraacetic acid buffer of the kit. In preamplification, 1.25 μL of the 10-fold diluted ligation mixture was amplified by 20 cycles of 94°C for 30 sec, 56°C for 1 min, and 72°C for 1 min using 10 μL preamplification primer mix. For selective amplification, 50-fold diluted preamplification products (1.0 μL), IRDye labeled (either IRDye 700 or IRDye 800; LI-COR Biosciences) *Eco*RI primers with three selective nucleotides (0.75 μL), PCR buffer (0.5 μL), *Taq* polymerase (0.3 μL), and the *Mse*I (+3) primers were used. Ten primer combinations (Table 1) were examined in all foreground positive plants across all generations on a LI-COR 4300 DNA Analyzer (LI-COR Biosciences). The reaction mixture was amplified as mentioned in our earlier studies (Tatikonda et al., 2009). The resulting band patterns were analyzed as present (+) or absent (-) using SagaMX software (LI-COR Biosciences).

Backcross Breeding

JG 11 was used as the recurrent parent for three generation of backcrossing to develop improved version of JG 11 (Fig. 1). The hybridity of F₁ plants was tested using *QTL-hotspot* linked markers TAA170 and STMS11, and true F₁ plants were selected for first backcrossing using JG 11 as a female parent. Based on foreground selection with QTL linked markers (see later) and background selection with AFLP markers, the backcross progenies carrying drought

Table 1. Markers used in foreground and background selection in marker-assisted backcrossing.

Marker	Map position (cM)	Forward primer sequence (5'-3')	Reverse primer sequence (5'-3')
For foreground selection (simple sequence repeats)			
ICCM0249 (Nayak et al., 2010)	CaLG04- 54.52	TTTCTTCGCATGGGCTTAAC	GGAGATTGTGGGTAGGCTC
TAA170 (Winter et al., 1999)	CaLG04- 54.56	TATAGAGTGAGAAGAAGCAAAGAGGAG	TATTGTCATCAATGTTCTGTAGTGTTT
STMS11 (Winter et al., 1999)	CaLG04- 82.25	GTATCTACTGTAATATTCTCTCTCT	ATATCATAAACCCCCAC
For background selection (amplified fragment length polymorphisms [AFLP])			
E-AAC + M-CTG (AFLP 1)	–	GTAGACTGCGTACCAATTCAAAC	GATGAGTCCTGAGTAACCTG
E-AAC + M-CTT (AFLP 2)	–	GTAGACTGCGTACCAATTCAAAC	GATGAGTCCTGAGTAACCTT
E-AGC + M-CAA (AFLP 3)	–	GTAGACTGCGTACCAATTCAGC	GATGAGTCCTGAGTAACAA
E-AGG + M-CAC (AFLP 4)	–	GTAGACTGCGTACCAATTCAGG	GATGAGTCCTGAGTAACAC
E-AAC + M-CTA (AFLP 5)	–	GTAGACTGCGTACCAATTCAAAC	GATGAGTCCTGAGTAACCTA
E-AAG + M-CAG (AFLP 6)	–	GTAGACTGCGTACCAATTCAGG	GATGAGTCCTGAGTAACAG
E-ACG + M-CAC (AFLP 7)	–	GTAGACTGCGTACCAATTCAGC	GATGAGTCCTGAGTAACAC
E-ACT + M-CTG (AFLP 8)	–	GTAGACTGCGTACCAATTCACT	GATGAGTCCTGAGTAACCTT
E-ACA + M-CTT (AFLP 9)	–	GTAGACTGCGTACCAATTCACA	GATGAGTCCTGAGTAACCTT
E-ACC + M-CAT (AFLP 10)	–	GTAGACTGCGTACCAATTCAT	GATGAGTCCTGAGTAACAT

tolerant alleles for the target *QTL-hotspot* and with maximum recurrent parent genome (RPG) recovery were selected for undertaking another round of backcrossing. After undertaking three rounds of backcrossing, selected plants were selfed two times for making plants homozygous as well as multiplication of seeds.

Phenotyping for Root Traits of Backcross Derived Lines

Marker-assisted backcross breeding derived BC₃F₃ lines along with their recipient (JG 11) and donor (ICC 4958) parents were evaluated for root traits variation using a cylinder culture method protected from rains using a rain-out shelter when needed as mentioned in our earlier studies (Kashiwagi et al., 2006). In brief, seeds from BC₃F₃ along with the parents were planted in 160 mm diameter, 120 cm tall polyvinyl chloride cylinders in an α design (five rows \times eight blocks) with three replications. To avoid direct solar radiation incidence on the root-populated cylinders, the cylinders were placed in 1.2 m deep cement pits and the intercylinder spaces packed with sawdust in a spacing of 0.3 by 0.3 m per cylinder. The soil was an equi-mixture (mixing the equal amount of proportion) (w/w) of Vertisol soil and sand equilibrated with a soil water content of 70% field capacity to create the conditions similar to those in the field at sowing time, where the soil is not fully saturated with water. A mixture of soil and sand was used to decrease the quantity of stored soil water and facilitate root growth and easy extraction. Four seeds of each genotype were sown in the cylinder. The seed zones were then irrigated with 150 mL of water three times on alternate days to ensure uniform seedling emergence and later the seedlings were allowed to grow under receding soil water. The seed zone was wetted with a rhizobial inoculum as a water suspension. The plants were thinned to two plants per cylinder at 7 d after sowing.

The roots from the cylinders were extracted gently after 35 d by washing the soil mixture with the help of running water. After removing the soil particles,

the roots were stretched to measure their length as an estimate of RDp. The root system was then separated in portions of 30 cm segments, to measure the root length at every 30 cm depth of the root system, using an image analysis system (WinRhizo; Regent Instruments Inc.).

Root length density (RLD) in each 30 cm layer was obtained by dividing root length by the soil volume. The root dry weights (RDWs) were recorded after drying in a hot air oven at 80°C for 72 h. Root to total plant dry weight ratio was calculated as an indicator of biomass allocation to roots.

Results

Selection of Parents and Markers

JG 11 (ICCV 93954) is a *desi* chickpea variety developed through collaborative breeding efforts of ICRISAT with Jawaharlal Nehru Krishi Vishwa Vidyalaya (JNKVV), Jabalpur, Madhya Pradesh, India, and released in 1999. This is the most preferred variety by farmers in India because of its early maturity (95–100 d) and higher yield (up to 2.5 t ha⁻¹ in rain-fed and up to 3.5 t ha⁻¹ under irrigated conditions) than the other varieties with an attractive large seed (22 g per 100 seed).

ICC 4958 is a drought tolerant breeding line developed by JNKVV, Jabalpur, Madhya Pradesh, India, named as JGC 1 and assembled in ICRISAT's genebank in 1973. The drought resistance of ICC 4958 is associated with its root system that is both prolific and heavier compared to other well-adapted varieties (ICRISAT, 1992). This is also a *desi* type chickpea and parent with superior root traits of the RIL population (ICC 4958 \times ICC 1882), which was used for identification of the *QTL-hotspot*.

Based on the initial QTL mapping results, three SSR markers, ICCM0249, TAA170, and STMS11, were found associated with the *QTL-hotspot* region. All or some of these markers were used for foreground selection at different backcross generations. The markers used and

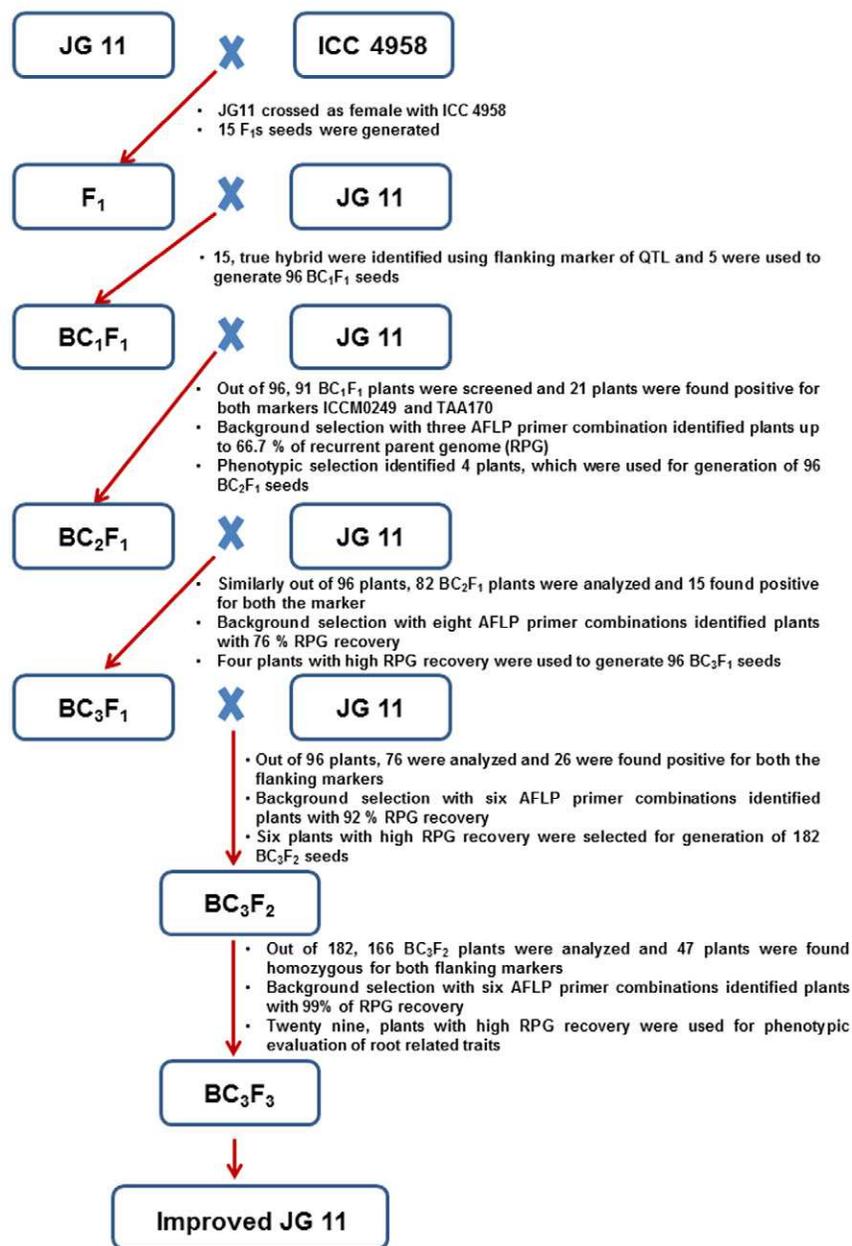


Figure 1. Scheme of marker-assisted backcrossing deployed. To introgress *QTL-hotspot*, in the first instance, the recurrent parent (JG 11) was crossed with donor (ICC 4958) to generate F₁s seeds. Subsequently, on the basis of foreground and background selections, backcrossing was undertaken. At final stage, two rounds of selfing were undertaken to make the introgression lines homozygous. QTL, quantitative trait loci; AFLP, amplified fragment length polymorphism.

their chromosomal locations, linkage distance, and primer sequences are presented in Table 1. Stepwise background analysis was performed using set of 10 different AFLP primer combinations. Details about these primer combinations are given in Table 2.

Marker-Assisted Introgression of the *QTL-hotspot* in JG 11

Marker-assisted backcrossing scheme used to introgress the *QTL-hotspot* from ICC 4958 into JG 11 is given in Fig. 1. In the first step, ICC 4958 (donor parent), as a male parent, was crossed to JG 11 (recurrent parent) and 15 F₁ seeds were obtained. Details about number of seeds generated, plants

analyzed for foreground and background selection, and number of plants found heterozygous and/or homozygous and used for next generation have been given in Table 3.

After raising these seeds, all F₁ plants were tested for hybridity using two *QTL-hotspot* linked markers TAA170 and STMS11 and all plants were found true hybrids. Subsequently, five positive F₁ plants were used for backcrossing with the recurrent parent and 96 BC₁F₁ seeds were harvested.

After sowing of 96 BC₁F₁ seeds, 91 plants were analyzed using foreground markers and 21 plants were found heterozygous for both the markers (37 for TAA170 and 48 for STMS11). Subsequently all positive

Table 2. Background selection analysis in different generations using amplified fragment length polymorphism (AFLP) markers.

Generation	Primer combinations [†]	High quality polymorphic alleles	Recurrent parent genome recovery %
BC ₁ F ₁	AFLP-1, AFLP-2, and AFLP-7	24	4 (62.5–66.7)
BC ₂ F ₁	AFLP-3, AFLP-4, AFLP-5, AFLP-6, AFLP-7, AFLP-8, AFLP-9, and AFLP-10	50	4 (74.0–76.0)
BC ₃ F ₁	AFLP-3, AFLP-5, AFLP-6, AFLP-7, AFLP-8, AFLP-9, and AFLP-10	37	6 (86.0–92.0)
BC ₃ F ₂	AFLP-3, AFLP-4, AFLP-5, AFLP-6, AFLP-8, and AFLP-10	46	29 (72.0–99.4)

[†]Name of AFLP primers are as per Table 1. The value presented in parenthesis indicated the range of recurrent parent genome recovery in selected plants during background analysis.

Table 3. Details on generation, screening, and selection of plants during different generation in marker-assisted backcrossing.

Generation	Plants generated	Plants screened	Foreground selection				Recurrent parent genome percent in background selection [†]	Plants selected after foreground and background selection
			TAA170	ICCM0249	STMS11	Common		
F ₁ [‡]	15	15	+– [§] (15) [*]	–	+–(15)	15	–	
BC ₁ F ₁ [‡]	96	91	+–(37)	–	+–(48)	21	66.7	
BC ₂ F ₁ [#]	96	82	+–(33)	+–(17)	–	15	76.0	
BC ₃ F ₁ [#]	96	76	+–(39)	+–(27)	–	26	92.0	
BC ₃ F ₂ [#]	182	166	++ ^{††} (52)	++(58)	–	47	99.4	

[†]Maximum recurrent parent genome recovery in the generation using amplified fragment length polymorphism primer combinations.

[‡]Positive plants were selected using quantitative trait loci linked markers. Generation was selected on the basis of two primer combination TAA170 and STMS11.

[§]+–, heterozygous situation of recurrent and donor parent allele at target locus

^{*}The value in parenthesis indicated the number of plants found positive during foreground analysis.

[#]Positive plants were selected using quantitative trait loci linked markers. Generation was selected on the basis of two primer combination TAA170 and ICCM0249.

^{††}++, homozygous situation of donor parent allele at target locus.

21 plants in foreground selection were analyzed with three AFLP primer combinations (Table 3). Background analysis based on 24 high-quality polymorphic AFLP alleles showed RPG recovery up to 66.7% in the BC₁F₁ plants. Subsequently, four plants with 62.5 to 66.7% RPG recovery were selected for the second backcrossing and a total of 96 BC₂F₁ seeds were harvested.

All 96 BC₂F₁ seeds were sown and 82 plants were analyzed for foreground selection with markers TAA170 and ICCM0249. As a result, 15 BC₂F₁ plants were identified heterozygous for both marker alleles (33 for TAA170 and 17 for ICCM0249). All these plants were then subjected to background analysis with eight AFLP primer combinations. Based on analysis of 50 high quality and polymorphic AFLP alleles between JG 11 and ICC 4958, four plants with 74 to 76% of RPG recovery were identified. These four plants with high RPG and similarity to recurrent parent were used to generate 96 BC₃F₁ seeds.

After sowing of 96 BC₃F₁ seeds, 76 plants were screened with two markers and 26 plants were found heterozygous for both the marker alleles, that is, 39 for TAA170 and 27 for ICCM0249. Background selection of these identified 26 plants with seven AFLP primer combinations identified plants from 62 to 92% of RPG recovery. Based on high RPG recovery 86 to 92% and similarity to the recurrent parent, six selected BC₃F₁ plants were selfed to generate BC₃F₂ population. A total of 182 BC₃F₂ seeds were harvested.

Subsequently, after sowing of all BC₃F₂ plants, 166 plants were screened with two markers and 47 were identified homozygous including 52 for TAA170 and 58

for ICCM0249. Finally, on the basis of RPG recovery and agromorphological similarity to the recurrent parent, 29 BC₃F₂ plants were selected for another round of selfing to generate BC₃F₃ families and have been referred as introgression lines (ILs).

Phenotypic Evaluation of Introgression Lines

With an objective to assess the efficacy of MABC for transferring the *QTL-hotspot*, 29 ILs were evaluated for root traits (Table 4). An average RDp (115.21 ± 2.24 cm) of all 29 ILs showed higher value in comparison to the recurrent parent; however, it ranged from 108.70 cm (IL-165) to 118.80 cm (IL-120) as compared to the recurrent parent JG 11 (111.70 cm). Similarly, RLD ranged from 0.38 cm cm⁻³ (IL-129 and IL-215) to 0.44 cm cm⁻³ (observed in eight ILs, namely IL-105, IL-150, IL-173, IL-181, IL-187, IL-216, IL-223, and IL-242). In fact, 26 out of 29 ILs showed better RLD as an average 0.41 ± 0.02 cm cm⁻³ in comparison to the recurrent parent JG 11 (0.39 cm cm⁻³).

The RDW in ILs ranged from 1.12 (IL-215) to 1.41 g per cylinder (IL-105) (Fig. 2 and 3; Table 4). The average RDW of ILs was found 1.25 ± 0.08 g per cylinder as compared to the 1.10 g per cylinder of recurrent parent JG 11.

Genome Analysis of Introgression Lines

To monitor the genetic background, genotyping of 29 ILs along with parental lines with six AFLP primer combinations showed 72.73 (IL-173) to 99.45% (IL-178) RPG recovery. The genetic relationship analysis of ILs with parental lines indicated that IL-178 as the closest

Table 4: Phenotypic evaluation of introgression lines (ILs) for root traits.

Number	ILs			
	Recurrent parent genome recovery %	Rooting depth cm	Root length density cm cm ⁻³	Root dry weight g per cylinder
JG 11	—	111.70	0.39	1.10
ICC 4958	—	114.20	0.45	1.25
IL-105	90.91	115.60	0.44	1.41
IL-120	95.45	118.80	0.41	1.29
IL-129	90.91	116.20	0.38	1.15
IL-145	86.36	118.50	0.41	1.31
IL-147	86.36	114.80	0.39	1.17
IL-150	86.36	115.30	0.44	1.36
IL-152	81.82	114.80	0.41	1.30
IL-155	95.45	118.40	0.42	1.27
IL-163	81.82	112.50	0.41	1.25
IL-165	77.27	108.70	0.41	1.26
IL-170	95.45	114.70	0.41	1.19
IL-173	72.73	113.90	0.44	1.33
IL-175	81.82	116.70	0.42	1.29
IL-177	81.82	114.00	0.40	1.20
IL-178	99.45	113.60	0.42	1.22
IL-179	90.91	114.10	0.40	1.21
IL-181	86.36	117.00	0.44	1.27
IL-187	95.45	116.90	0.44	1.36
IL-200	86.36	116.40	0.41	1.13
IL-203	81.82	115.00	0.43	1.26
IL-215	90.91	113.90	0.38	1.12
IL-216	81.82	117.50	0.44	1.30
IL-223	86.36	117.00	0.44	1.26
IL-242	77.27	116.70	0.44	1.34
IL-245	90.91	113.90	0.41	1.28
IL-248	95.45	112.90	0.39	1.15
IL-265	90.91	112.70	0.40	1.17
IL-271	86.36	115.40	0.42	1.28
IL-276	86.36	114.80	0.41	1.25
Average of ILs	87.28	115.21	0.41	1.25
SD±	6.43±1.19	2.24±0.40	0.02±0.00	0.08±0.01

and IL-173 as the farthest line from the recurrent parent (Supplemental Fig. S1). However, all ILs showed close relationship with the recurrent parent JG 11.

Furthermore, eight ILs showing better root traits in phenotypic evaluation were analyzed for the introgression of donor parent (ICC 4958) genome for the carrier chromosome using 11 polymorphic SSR markers in nontarget region at one end and two SSR markers within the target region containing the *QTL-hotspot* (Fig. 4). CaM1903 was the nearest flanking marker (1.2 cM far) to the ICCM0249 that was associated with *QTL-hotspot* and used in foreground selection. Genotyping ILs with CaM1903 marker showed homozygosity for the markers in seven ILs. Of these, five ILs carried recurrent parent alleles and two ILs carried donor parent alleles. This analysis suggested that that donor parent segment was delimited to 0.6 cM from one side. Marker analysis of



Figure 2. Root trait phenotyping of representative introgression lines (ILs) along with the parental lines. Comparative root profiling of six ILs and its recurrent (JG 11) and donor parent (ICC 4958) after 35 d of experiments under glasshouse conditions.

ILs with NCPGR127 marker, present in the *QTL-hotspot*, showed donor parent genome allele in all ILs. However, marker analysis of ILs for NCPGR21 marker present in the *QTL-hotspot* region showed recurrent parent allele in all ILs except IL-105. The ILs with least donor parent segments (IL-150, IL-173, IL-175, IL-216, and IL-271) are preferred ILs for multilocation field trials.

Discussion

Terminal drought is one of the major abiotic stresses adversely affecting crop production of chickpea especially in semiarid tropic regions and causes a 40 to 50% reduction in yield globally (Ahmad et al., 2005). Chickpea production could be improved up to 50%, equivalent to approximately US\$900 million, if drought stress can be managed (Ryan, 1997). Identification of molecular markers for drought tolerance is difficult task as it influenced by various factors such as days to flowering and maturity, early shoot growth vigor, yield, shoot biomass production, RDp, RLD, root to shoot ratio, total transpiration, and transpiration efficiency (Varshney et al., 2011). Therefore, dissection of such complex traits into components and identification of tightly linked markers for such traits can enhance the heritability of such traits and facilitate MAS for introgression of these traits into the different genetic background. As a result of several years of research, root traits such as RDp, RLD, etc., have been suggested associated with drought tolerance (Kashiwagi et al., 2006).

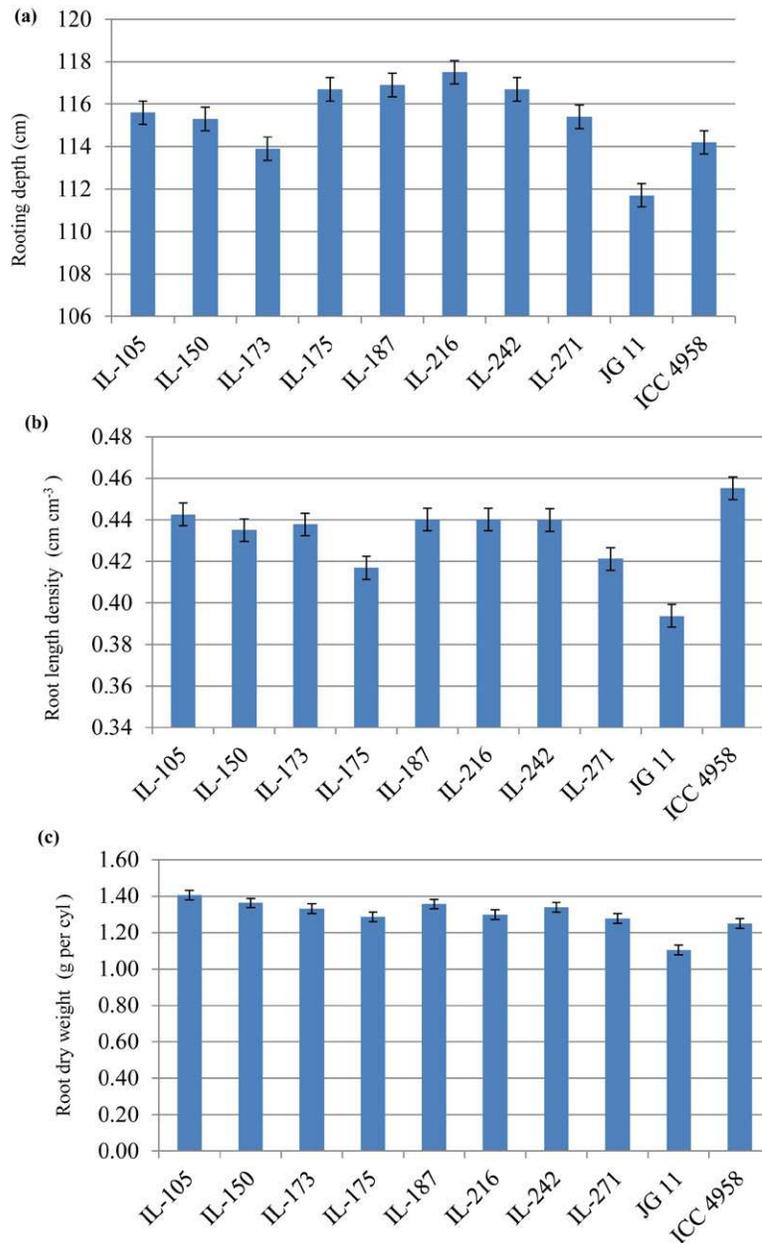


Figure 3. Phenotypic evaluation of three root traits in selected introgression lines (ILs). Analysis of phenotyping data for root traits namely root depth (a), root length density (b), and root dry weight (c) of selected ILs show better root traits as compared to the recurrent parent and in some cases to even the donor parent. cyl, cylinder.

Although ICRISAT has established high throughput and precise phenotyping techniques for root traits (Upadhyaya et al., 2012), many breeding programs in developing countries do not have these facilities. Therefore, breeding for root traits seems to be a challenging task. However, once molecular markers associated with root traits are available, root traits can be introgressed in elite varieties by using molecular breeding approaches such as MAS, MABC, etc. Molecular breeding for root traits has been successful in developing superior lines in rice (Shen et al., 2001; Steele et al., 2006, 2013), maize (Bouchez et al., 2002; Tuberosa and Salvi, 2006), and wheat (Placido et al., 2013).

In the case of chickpea, *QTL-hotspot* has been identified on CaLG04 that contains QTL for root traits

that contribute up to 58.20% phenotypic variation. Although the QTL region, at the time of initiation of MABC, had only three markers (ICCM0249, TAA170, and STMS11), to save time, MABC was started after validating these markers between ICC 4958 and JG 11, an elite variety with highest indent for breeder seed production (2010–2011 and 2012–2013) of chickpea in India. After making three backcrosses and two rounds of selfing in six crops seasons in 3 yr and following stringent criteria, 29 ILs have been developed.

Although in the MABC experiments, genomewide markers are used for background selection to monitor genome recovery and overcome the issue of linkage drag from donor, as generally donor is a wild species genotype

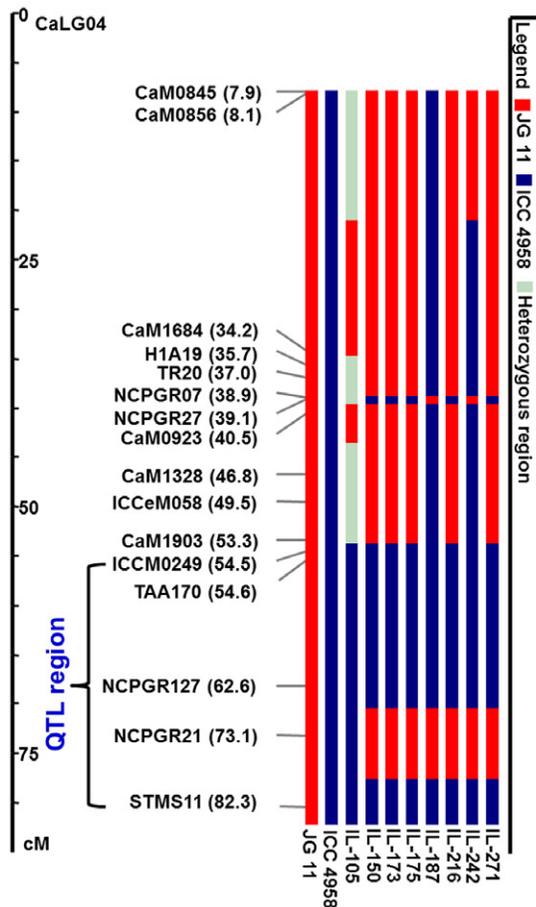


Figure 4. Graphical genotypes of selected lines using simple sequence repeat (SSR) markers for the carrier chromosome. Polymorphic SSR markers on the carrier chromosome (CaLG04) between parental lines were used to analyze the introgression of donor parent genome associated with the *QTL-hotspot*. It is evident that five introgression lines (ILs), namely IL-150, IL-173, IL-175, IL-216, and IL-271, showed expected graphical genotypes while the remaining three ILs (IL-105, IL-187, and IL-242) have higher amount of the donor parent genome. QTL, quantitative trait loci.

or unadapted germplasm. As ICC 4958 used as a donor in MABC in the present study is also an elite breeding line, not much emphasis was given on recombination selection and background selection in this study. Nevertheless, to reduce the number of plants for making crosses in ensuing backcross generations in cost-effective manner, up to 10 AFLP primer combinations, instead of SSR markers, were used for background selection. Recurrent parent genome recovery in the BC_1F_1 generation was up to 66.70%, which was far less than the expected mean recovery of 75%. The identification of plants with such less recovery was due to use of dominant markers in the background selection because it cannot differentiate the heterozygous alleles, which reduced the representation of recurrent parent alleles in the genome. In case SSR markers would have been used on the same BC_1F_1 plants, the RPG recovery might have ranged from 66.67 to 83.33% with an average of 74.21%, almost similar to the expected 75%. These observations suggest codominant markers such as SSRs or single nucleotide polymorphisms

on appropriate genotyping platforms will be the choice of markers for background selection. It is worth to mention that even by using dominant (few) AFLP markers, RPG recovery in the ILs (BC_3F_3) were found up to 99.45%. By using conventional breeding (without markers) three backcrosses might have given 93.75% RPG recovery (Allard, 1960). These analyses underline the importance of markers for better RPG recovery.

Analysis of ILs with the carrier chromosome specific markers identified ILs with less than 0.6 cM of donor parent genome at one side of the flanking marker of root trait QTL. As mentioned earlier, linkage drag was not a major problem as the donor genotypes are not an unadapted germplasm with deleterious effect. However, while analyzing the ILs with NCPGR21, marker present in the QTL region, one IL (IL-105) was identified that carried donor parent allele as compared to the other ILs and such lines can be dropped. This analysis suggests the importance of marker analysis for the carrier chromosomes before finalizing lines for multilocation field trials.

Phenotyping analysis of ILs for different root traits, namely RDp, RLD, and RDW, demonstrated better root traits as compared to the recurrent parent and in some cases even better than the donor parent. Better performance of ILs for root traits in comparison of their parents may be due to accumulation of favorable alleles from both of the parents. For instance, IL-216 showed 117.50 cm of RDp, 0.44 cm cm^{-3} RLD, and 1.30 g per cylinder of RDW in comparison to 111.70 cm of RDp, 0.39 cm cm^{-3} of RLD, and 1.10 g per cylinder of RDW in JG 11 and 114.20 cm of RDp, 0.45 cm cm^{-3} of RLD, and 1.25 g per cylinder of RDW of ICC 4958, with only RPG recovery of 81.82% although it may be underestimated because of use of dominant markers. Such observations clearly demonstrate the value of MABC to introgress root traits, otherwise difficult through conventional breeding, in the elite line although introgression of the *QTL-hotspot* does not essentially mean to enhance drought tolerance as expression of QTL introgressed depend on the genetic background in which they are introgressed as well as environments in which ILs are tested. For instance, Steele et al. (2006), after testing ILs in multiple environments in multiple years, did not find even better root traits. However, with an optimistic view, the generated ILs are expected to perform better in drought conditions as the *QTL-hotspot* contains 12 out of 25 (48%) robust main effect QTL for 12 traits (100-seed weight, RLD, days to flowering, days to maturity, biomass, plant height, pods per plant, harvest index, RDW, shoot dry weight, seeds per pod, and yield). Out of these QTL, at least one QTL each for seed weight, plant height, pods per plant, and 100-seed weight is consistent (across genetic background) and one QTL each for plant height and 100-seed weight is stable (across environments).

Above-mentioned ILs are planned for multilocation field trials in central and southern India for assessing impact of *QTL-hotspot* introgressed in the genetic background of JG 11 on yield both in irrigated and rain-fed conditions. After due testing of these ILs, superior line

will be sent for All India Coordinated Research Project on Chickpea of Indian Council of Agricultural Research in India for release of most promising IL as improved variety for commercial cultivation. As indent for “breeder seed” of JG 11, according to Department of Agriculture and Cooperation, India, ranked one during 2010 to 2011 (as 130.52 t, 13.91% of total 86 varieties) and 2011 to 2012 (as 112.30 t, 11.36% of total 83 varieties) (SeedNet India, 2013), adoption of improved variety (IL derived from JG 11) by farmers is expected to be very high.

Supplemental Information Available

Supplemental material is available at <http://www.crops.org/publications/tpg>.

Supplemental Figure S1. Genetic relationship of introgression lines with the recurrent (JG 11) and donor (ICC 4958) genotypes.

Acknowledgments

This study was undertaken as a part of Tropical Legumes I (TLI-Objective 4: Chickpea) funded by Bill & Melinda Gates Foundation (BMGF) through CGIAR Generation Challenge Programme. Thanks are also due to Mohammad Aziz, KVSS Prasad, Vinay Kumar Birapaka, and Nayakoti Swapna for extending their technical help. This work has been undertaken as part of the CGIAR Research Program on Grain Legumes. ICRIASAT is a member of CGIAR Consortium.

References

- Ahmad, F., P. Gaur, and J. Croser. 2005. Chickpea (*Cicer arietinum* L.). In: R. Singh and P. Jauhar, editors, Genetic resources, chromosome engineering and crop improvement-grain legumes. CRC Press, Boca Raton, FL. p. 185–214.
- Allard, R.W. 1960. Principles of plant breeding. 1st ed. John Wiley & Sons, New York, NY.
- Bouchez, A., F. Hospital, M. Causse, A. Gallais, and A. Charcosset. 2002. Marker-assisted introgression of favorable alleles at quantitative trait loci between maize elite lines. *Genetics* 162:1945–1959.
- Cuc, L.M., E.S. Mace, J.H. Crouch, V.D. Quang, T.D. Long, and R.K. Varshney. 2008. Isolation and characterization of novel microsatellite markers and their application for diversity assessment in cultivated groundnut (*Arachis hypogaea*). *BMC Plant Biol.* 8:55. doi:10.1186/1471-2229-8-55
- FAOSTAT. 2011. Statistical database. FAO, Rome, Italy. <http://faostat3.fao.org/home/index.html> (accessed 27 May 2008).
- ICRIASAT. 1992. ICC 4958 a drought resistant chickpea. In: Plant material description number 33. ICRIASAT, Patancheru, India.
- Jukanti, A.K., P.M. Gaur, C.L.L. Gowda, and R.N. Chibbar. 2012. Nutritional quality and health benefits of chickpea (*Cicer arietinum* L.): A review. *Br. J. Nutr.* 108:S12–S26. doi:10.1017/S0007114512000797
- Kashiwagi, J., L. Krishnamurthy, J.H. Crouch, and R. Serraj. 2006. Variability of root length density and its contributions to seed yield in chickpea (*Cicer arietinum* L) under terminal drought stress. *Field Crops Res.* 95:171–181. doi:10.1016/j.fcr.2005.02.012
- Kashiwagi, J., L. Krishnamurthy, H.D. Upadhyaya, H. Krishna, S. Chandra, V. Vadez, and R. Serraj. 2005. Genetic variability of drought-avoidance root traits in the mini-core germplasm collection of chickpea (*Cicer arietinum* L.). *Euphytica* 146:213–222. doi:10.1007/s10681-005-9007-1
- Nayak, S.N., H. Zhu, N. Varghese, S. Datta, H.K. Choi, R. Horres, R. Jüngling, J. Singh, P.B. KaviKishor, S. Sivaramakrishnan, D.A. Hoisington, G. Kahl, P. Winter, D.R. Cook, and R.K. Varshney. 2010. Integration of novel SSR and gene-based SNP marker loci in the chickpea genetic map and establishment of new anchor points with *Medicago truncatula* genome. *Theor. Appl. Genet.* 120:1415–1441. doi:10.1007/s00122-010-1265-1
- Placido, D.F., M.T. Campbell, J.J. Folsom, X. Cui, G.R. Kruger, P.S. Baenziger, and H. Walia. 2013. Introgression of novel traits from a wild wheat relative improves drought adaptation in wheat. *Plant Physiol.* 161:1806–1819. doi:10.1104/pp.113.214262
- Ryan, J.G. 1997. A global perspective on pigeonpea and chickpea sustainable production systems: Present status and future potential. In: A.N. Asthana and M. Ali, editors, Recent advances in pulses research. Indian Society of Pulses Research and Development, IIPR, Kanpur, India. p. 1–31.
- Saxena, N.P. 2003. Management of drought in chickpea—a holistic approach. In: N.P. Saxena, editor, Management of agricultural drought—agronomic and genetic options. Oxford & IBH Publishing Co. Pvt. Ltd., New Delhi, India. p. 103–122.
- SeedNet India. 2013. Indent of Breeder Seed for Rabi 2011–12. Department of Agriculture and Cooperation, New Delhi, India. <http://seednet.gov.in/> (accessed 20 May 2013).
- Shen, L., B. Courtois, K.L. McNally, S. Robin, and Z. Li. 2001. Evaluation of near-isogenic lines of rice introgressed with QTLs for root depth through marker-aided selection. *Theor. Appl. Genet.* 103:75–83. doi:10.1007/s001220100538
- Steele, K.A., A.H. Price, H.E. Shashidhar, and J.R. Witcombe. 2006. Marker-assisted selection to introgress rice QTLs controlling root traits into an Indian upland rice variety. *Theor. Appl. Genet.* 112:208–221. doi:10.1007/s00122-005-0110-4
- Steele, K.A., A.H. Price, J.R. Witcombe, R. Shrestha, B.N. Singh, J.M. Gibbons, and D.S. Virk. 2013. QTLs associated with root traits increase yield in upland rice when transferred through marker-assisted selection. *Theor. Appl. Genet.* 126:101–108. doi:10.1007/s00122-012-1963-y
- Tatikonda, L., S.P. Wani, K.S. Kannan, N. Beerelli, T.K. Sreedevi, and D.A. Hoisington. 2009. AFLP-based molecular characterization of an elite germplasm collection of *Jatropha curcas* L., a biofuel plant. *Plant Sci.* 176:505–513. doi:10.1016/j.plantsci.2009.01.006
- Thudi, M., A. Bohra, S.N. Nayak, N. Varghese, T.M. Shah, R.V. Penmetsa, N. Thirunavukkarasu, S. Gudipati, P.M. Gaur, P.L. Kulwal, H.D. Upadhyaya, P.B. KaviKishor, P. Winter, G. Kahl, C.D. Town, A. Kilian, D.R. Cook, and R.K. Varshney. 2011. Novel SSR markers from BAC-end sequences, DaRT arrays and a comprehensive genetic map with 1291 marker loci for chickpea (*Cicer arietinum* L.). *PLoS ONE* 6:e27275. doi:10.1371/journal.pone.0027275
- Tuberosa, R., and S. Salvi. 2006. Genomics-based approaches to improve drought tolerance of crops. *Trends Plant Sci.* 11:405–412. doi:10.1016/j.tplants.2006.06.003
- Upadhyaya, H.D., J. Kashiwagi, R.K. Varshney, P.M. Gaur, K.B. Saxena, L. Krishnamurthy, C.L.L. Gowda, R.P.S. Pundir, S.K. Chaturvedi, P.S. Basu, and I.P. Singh. 2012. Phenotyping chickpeas and pigeonpeas for adaptation to drought. *Front. Physiol.* 3:179. doi:10.3389/fphys.2012.00179
- Varshney, R.K., P.J. Hiremath, P. Lekha, J. Kashiwagi, J. Balaji, A.A. Deokar, V. Vadez, Y. Xiao, R. Srinivasan, P.M. Gaur, K.H.M. Siddique, C.D. Town, and D.A. Hoisington. 2009. Comprehensive resource of drought- and salinity responsive ESTs for gene discovery and marker development in chickpea (*Cicer arietinum* L.). *BMC Genomics* 10:523. doi:10.1186/1471-2164-10-523
- Varshney, R.K., L. Pazhamala, J. Kashiwagi, P.M. Gaur, L. Krishnamurthy, and D.A. Hoisington. 2011. Genomics and physiological approaches for root trait breeding to improve drought tolerance in chickpea (*Cicer arietinum* L.) In: A.D. de Oliveira and R.K. Varshney, editors, Root genomics. Springer-Verlag, Dordrecht, the Netherlands. p. 233–250.
- Winter, P., T. Pfaff, S.M. Udupa, P.C. Sharma, S. Sahi, R. Arreguin-Espinoza, F. Weigand, F.J. Muehlbauer, G. Kahl. 1999. Characterization and mapping of sequence-tagged microsatellite sites in the chickpea (*C. arietinum* L.) genome. *Mol. Gen. Genet.* 262:90–101.