Marker-Assisted Backcrossing to Introgress Resistance to Fusarium Wilt Race 1 and Ascochyta Blight in C 214, an Elite Cultivar of Chickpea

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Abstract

Fusarium wilt (FW) and Ascochyta blight (AB) are two major constraints to chickpea (*Cicer arietinum* L.) production. Therefore, two parallel marker-assisted backcrossing (MABC) programs by targeting *foc1* locus and two quantitative trait loci (QTL) regions, ABQTL-I and ABQTL-II, were undertaken to introgress resistance to FW and AB, respectively, in C 214, an elite cultivar of chickpea. In the case of FW, foreground selection (FGS) was conducted with six markers (TR19, TA194, TAA60, GA16, TA110, and TS82) linked to *foc1* in the cross C 214 × WR 315 (FW-resistant). On the other hand, eight markers (TA194, TR58, TS82, GA16, SCY17, TA130, TA2, and GAA47) linked with ABQTL-I and ABQTL-II were used in the case of AB by deploying C 214 × ILC 3279 (AB-resistant) cross. Background selection (BGS) in both crosses was employed with evenly distributed 40 (C 214 × WR 315) to 43 (C 214 × ILC 3279) SSR markers in the chickpea genome to select plant(s) with higher recurrent parent genome (RPG) recovery. By using three backcrosses and three rounds of selfing, 22 BC_3F_4 lines were generated for C 214 × WR 315 cross and 14 MABC lines for C 214 × ILC 3279 cross. Phenotyping of these lines has identified three resistant lines (with 92.7–95.2% RPG) to race 1 of FW, and seven resistant lines (with 81.7–85.40% RPG) to AB that may be tested for yield and other agronomic traits under multilocation trials for possible release and cultivation.

Chickpea is an important cool-season food legume grown extensively by the poor farmers throughout the Indian subcontinent. India alone contributes about 67% to the global chickpea production; however, there has been little improvement in the crop productivity (0.8 t ha^{-1}) (Gaur et al., 2012). Several biotic and abiotic stresses impose adverse effects on plants at most of the growth stages, leading to low productivity. Among the biotic stresses, FW [caused by soilborne fungus *Fusarium oxysporum* f. sp. *ciceris* (*foc*)], and AB (caused by *Ascochyta rabiei*) in chickpea are the two most severe yield reducers in India, and can cause complete yield losses under favorable conditions (Navas-Cortes et al., 2000; Dubey et al., 2007; Udupa and Baum 2003). Ascochyta blight and FW are prevalent diseases across all chickpea growing regions of the world, including India. However, AB mainly occurs in northwestern plains due to favorable climatic conditions, while FW is mostly restricted to central and southern parts of India (Ghosh et al., 2013).

Till date, two pathotypes (yellowing and wilting) and eight pathogenic races (races 0, 1A, 1B/C, 2, 3, 4, 5, and 6) have been described for FW (Jiménez-Fernández et al., 2013; del Mar Jiménez-Gasco and Jiménez-Díaz, 2003). Furthermore, genetics of five races (1A, 2, 3, 4, and

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Abbreviations: AB, Ascochyta blight; APR, adult plant resistance; BGS, background selection; DAI, days after inoculation; FGS, foreground selection; FW, Fusarium wilt; PAU, Punjab Agricultural University; QTL, quantitative trait locus; MABC, marker-assisted backcrossing; RCBD, randomized complete block design; RPG, recurrent parent genome.

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5) has also been studied (Sharma et al., 2005). In context of India, the race 1 (synonymous 1A, Indian isolate) is highly virulent in Andhra Pradesh, a major chickpea-growing state of India.

To address such problems, molecular breeding strategies have been deployed in several crop species (Kulwal et al., 2011). However, availability of markers associated with trait of interest, for instance, resistance to a disease, is a prerequisite for molecular breeding. In the case of FW in chickpea, molecular markers associated with resistance to six different races (0, 1A, 2, 3, 4, and 5) have been identified (see Varshney et al., 2013). These mapping studies have located resistance genes for FW races 1 (*foc1*), 3 (*foc3*), 4 (*foc4*), and 5 (*foc5*), forming a cluster on CaLG02 (Mayer et al., 1997; Ratnaparkhe et al., 1998; Tullu et al., 1998; Winter et al., 2000; Sharma et al., 2004).

For AB resistance, high level of variability exists for the pathogenicity trait in *A. rabiei* populations, and a number of pathotypes were reported (Nene and Reddy, 1987). On the basis of aggressiveness of the pathogen, pathotypes have been classified mainly into two broad categories: Pathotype I (less aggressive) and Pathotype II (aggressive) (Chen et al., 2004).

In terms of molecular mapping, a considerable number of QTL have been identified on several linkage groups (2, 3, 4, 6, and 8) for AB resistance in many studies (see Varshney et al., 2013). However, majority of AB resistance QTL were reported mainly on two linkage groups, CaLG02 and CaLG04. For instance, AB resistance QTL *ar1* and *ar2a*, identified by Udupa and Baum (2003), and QTL_{AR3} identified by Iruela et al. (2007), are present in the same genomic region mainly flanked by GA16 and TA110 markers on CaLG02 (Supplemental Fig. S1). Quantitative trait loci present in this genomic region confer resistance to both Pathotype I and II of *A. rabiei* and contribute up to 20% phenotypic variation. Hereafter in the present study, this region is referred to as ABQTL-I. Similarly, the region ABQTL-II (hereafter labeled in this study) contains QTL *ar2b* mapped by Udupa and Baum (2003), and QTL_{AR1} and QTL_{AR2} mapped by Iruela et al. (2006) (Supplemental Fig. S2). This genomic region contributes up to 34% of the phenotypic variation.

Deployment of host plant resistance is the preferred strategy for managing the above two diseases, as it is an economical and eco-friendly approach. Marker-assisted backcrossing aims at conversion of targeted lines with respect to one or two traits without disturbing remaining all other native traits of target cultivar (Varshney et al., 2009). Marker-assisted backcrossing has been successfully employed recently to introgress AB resistance with double-podding traits in chickpea cultivars CDC Xena, CDC Leader, and FLIP98-135C (Taran et al., 2013) and a QTL-hotspot containing QTL for root traits and abiotic stress tolerance in JG 11, a leading chickpea cultivar from India (Varshney et al., 2013).

Keeping in view of above, the present study employs two parallel MABC programs that include introgression of *foc1*, resistant locus for race 1 of FW, and two QTL clusters for AB resistance, ABQTL-I (*ar1*, *ar2a*, and QTL_{AR3}) and ABQTL-II (*ar2b*, QTL_{AR1}, and QTL_{AR2}) in the genetic background of C 214, a high-yielding chickpea cultivar but susceptible to both of the devastating diseases. Phenotypic evaluation of these MABC lines identified several lines with high level of resistance to FW and AB.

Materials and Methods

Parent Materials

C 214, a well-adapted FW and AB susceptible desi cultivar suitable for rainfed conditions (Bhardwaj et al., 2010; Kaur et al., 2012) was chosen as recurrent parent for introgression of resistance to FW and AB. WR 315, a desi landrace from central India resistant to race 1A and race 3 of FW (Mayer et al., 1997; Sharma et al., 2005) was selected as donor parent for introgression of genomic segment carrying *foc1* and *foc3*. For AB resistance, ILC 3279 a *kabuli* landrace (Udupa et al., 1998) originated from former USSR was used as donor for transferring two QTL clusters, ABQTL-I and ABQTL-II, in the present MABC program.

Deoxyribonucleic Acid Extraction

Deoxyribonucleic acid was isolated from fresh leaves of 15-d-old seedlings of the parental genotypes, F_1's, and backcross progenies using the modified cetyl trimethyl ammonium bromide (CTAB) extraction method, as described in Cuc et al. (2008). Quality and quantity of DNA were checked on 0.8% agarose gel and concentration was normalized to ~5 ng μL^{-1}.

Polymerase Chain Reaction (PCR) and Marker Genotyping

Polymerase chain reaction for simple sequence repeat (SSR) markers from target genomic region (Table 1) and SSRs from complete genome (Varshney et al., 2013) for BGS 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 of Applied Biosystems, USA (Carlsbad, CA).

Backcross Breeding

Two parallel crossing programs, C 214 × WR315 and C 214 × ILC 3279, were employed for generation of F_1 seeds and the lines derived thereof designated as ICCX-100175 and ICCX-100176, respectively. Molecular markers associated with resistance to FW and AB were employed for identification of true hybrid plants in each cross and these plants were selected for generation of backcross progenies. Further, FGS for genomic regions of interest and BGS using SSR markers were employed for identification of plants for further backcrossing. After undertaking three rounds of backcrossing, selected plants were selfed three times (BC_3F_4) for making plants homozygous as well as multiplication of improved seeds of C 214 for FW and AB, separately.

Table 1. Details on molecular markers used for undertaking foreground selection in marker-assisted backcrossing programs for Fusarium wilt (FW) and Ascochyta blight (AB).

Race name	LG	Marker name	Forward primer sequence (5′–3′)	Reverse primer sequence (5′–3′)	Reference†
FW					
foc1 and *foc3*					
	2	GA16	CACCTCGTACCATGGTTTCTG	TAAATTTCATCCTCTCCGGC	1
	2	TAA60	TCATGCTTGTTGGTTAGCTAGAAA	CAAAGACATAATCGAGTTAAAGAAAA	1
	2	TA194	TTTTTGGCTTATTAGACTGACTT	TTGCCATAAAATACAAAATCC	1
	2	TS82	TCAAGATTGATATTGATTAGATAAAAGC	CTTTATTTACCACTTGCACAACACTAA	1
	2	TA110	ACACTATAGGTATAGGCATTTAGGCAA	TTCTTTATAAATATCAGACCGGAAAGA	1
	2	TR19	TCAGTATCACGTGTAATTCGT	CATGAACATCAAGTTCTCCA	2
AB					
ABQTL-I (QTL_{AR3}, *ar1*, *ar2a*)					
	2	GA16	CACCTCGTACCATGGTTTCTG	TAAATTTCATCCTCTCCGGC	3
	2	TS82	TCAAGATTGATATTGATTAGATAAAAGC	CTTTATTTACCACTTGCACAACACTAA	3
	2	TA194	TTTTTGGCTTATTAGACTGACTT	TTGCCATAAAATACAAAATCC	3
	2	TR58	CTCTATATTTGTTTGTTTTTCGTTTTG	TAAAATGTGTAGGGTGCAGAATAAATA	3
ABQTL-II (QTL_{AR1}, QTL_{AR2}, *ar2b*)					
	4	GAA47	CACTCCTCATGCCAACTCCT	AAAATGGAATAGTCGTATGGGG	4
	4	SCY17	GACGTGGTGACTATCTAGC	GACGTGGTGAAATAGATACC	4
	4	TA130	TCTTTCTTTGCTTCCAATGT	GTAAATCCCTCGAGAAATCAA	5
	4	TA2	AAATGGAAGAAGAATAAAAACGAAAC	TTCCATTCTTTATTATCCATATCACTACA	5

†References: 1, Millan et al. (2006); 2, Sharma and Muehlbauer (2005); 3, Iruela et al. (2007); 4, Iruela et al. (2006); 5, Udupa and Baum (2003).

Phenotyping for Fusarium Wilt and Ascochyta Blight

The selected BC_3F_4 families, along with their parents, were sown in controlled conditions and specific to race 1 isolates at Patancheru, India, during the off-season (July–October 2012) in two replications using randomized complete block design (RCBD) as described by Pande et al. (2012). Data on FW reaction of the entries were recorded at 60 days after inoculation (DAI) and classified as resistant (0–20%), moderately susceptible (21–50%) and susceptible (>50%) as described by Sharma et al. (2005).

Phenotyping for AB resistance for selected BC_3F_4 families along with parental lines were screened for adult plant resistance (APR). The entries were planted in RCBD with two replications at PAU, Ludhiana, during crop season 2012–2013. The experimental units were one-row plots of 2 m length, with 10-cm spacing between plants and 40 cm between rows. Another highly susceptible check, 'ICC 4991', was planted after every four-test rows to provide a constant disease pressure for the AB. The disease reaction was recorded when the susceptible check showed the maximum disease severity of 9 on a scale of 1 to 9. The lines were classified as immune (1), resistant (1.1–3.0), moderately resistant (3.1–5.0), susceptible (5.1–7.0), and highly susceptible (>7.0) as described by Kottapalli et al. (2009).

Results

Selection of Molecular Markers

For introgressing *foc1* locus conferring resistance to race 1 of FW, three SSR markers (TR19, TA194, and TAA60) present in the genomic region on linkage group CaLG02 and a few adjoining markers (GA16, TS82, and TA110) in the same region (Sharma et al., 2004; Sharma and Muehlbauer, 2005; Millan et al., 2006; Gowda et al., 2009) were targeted for deployment. However, after screening a total of 10 reported markers (nine SSRs and one allele-specific associated primer) between C 214 and WR 315, six were polymorphic between parents and were deployed for selection of target genomic region in segregating generations.

For introgressing resistance to AB, two QTL regions, ABQTL-I and ABQTL-II, conferring resistance for Pathotype I and Pathotype II of AB present on CaLG02 and CaLG04, respectively, were targeted. Genomic region ABQTL-I consisting of QTL_{AR3} (Iruela et al., 2007), *ar1* and *ar2a* (Udupa and Baum 2003), on CaLG02 contributes up to 20% phenotypic variation. Another genomic region ABQTL-II consisting of QTL_{AR1}, QTL_{AR2} (Iruela et al., 2006), and *ar2b* (Udupa and Baum, 2003) located on CaLG04 contributing up to 34% phenotypic variation was also chosen for deployment. For ABQTL-I region, out of 13 reported markers, seven were found polymorphic between parents, and only four markers were employed on the basis of differences in fragment sizes (bp) (TA194, TR58, TS82, and GA16) in the backcross generations. In the case of ABQTL-II region, out of nine, five markers were found polymorphic and four markers (SCY17, TA130, TA2, and GAA47) were used in the targeted cross C 214 × ILC 3279. It is important to note that one molecular marker SCY17 (Iruela et al., 2006) from the ABQTL-II region is a diagnostic marker and this marker has been given higher importance to select the plants. Details of these markers and sequences are given in Table 1.

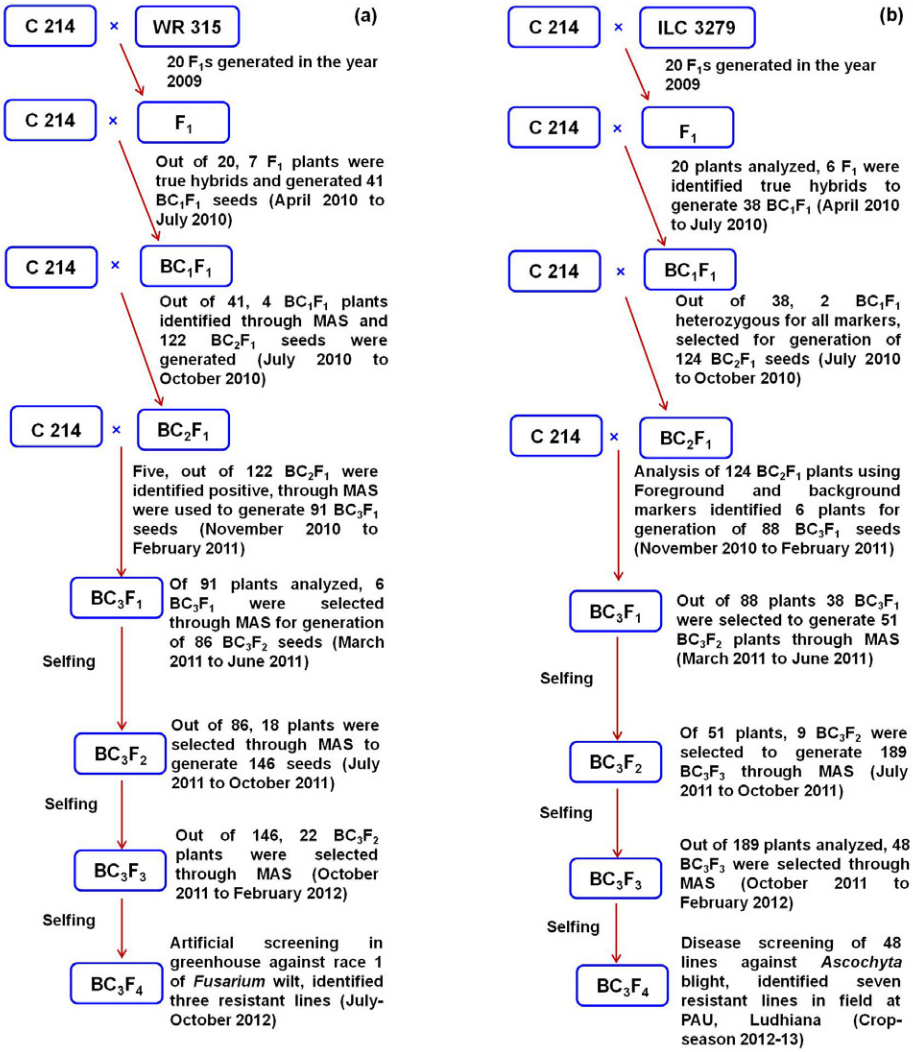

Figure 1. Scheme of marker-assisted backcrossing (MABC) deployed. To introgress *foc1* locus conferring resistance to race 1 of Fusarium wilt (FW) and two QTL regions (ABQTL-I and AB-QTL-II) conferring resistance to Aschocyta blight (AB), two different donors, WR 315 (FW-resistant) and ILC 3279 (AB resistant), were crossed separately with C 214, the recurrent parent. The F_1 seeds generated from these crosses were sown and, after marker analysis for heterozygosity, positive plants were used for making the backcrosses. Subsequently, in the backcross generation, molecular markers were used for foreground and background selection. In total, three backcrosses and three rounds of selfing were undertaken to develop homozygous introgression lines.

Marker-Assisted Backcrossing for Fusarium Wilt

Marker-assisted backcrossing scheme used to introgress FW resistance from WR 315 into C 214 is given in Fig. 1a. C 214 (recurrent parent) was used as female and crossed with WR 315 (donor parent) as male to generate 20 F_1 seeds. Out of 20 F_1 plants generated and grown in the off-season in April 2010, seven true hybrids were identified with the polymorphic markers (TAA60, TA194, and TS82) and this cross was named as ICCX-100175. All F_1's were used to make the first backcross, of which, 41 BC_1F_1 seeds were harvested in June 2010. All 41 BC_1F_1 seeds were sown in the off-season (starting July 2010). Further, DNA was isolated from 41 plants and FGS was done with four SSR markers: GA16, TAA60, TA110 and TR19. Based on FGS results, four plants having common heterozygotes for all markers were used for second cycle of backcrossing to obtain BC_2F_1 seeds. Although molecular markers were selected for undertaking BGS (as mentioned in Table 2), because of a lesser number of plants identified in FGS, BGS was not done with the BC_1F_1 plants.

Subsequently, second cycle of backcrossing was undertaken, and 122 BC_2F_1 seeds were harvested in October 2010. After growing 122 BC_2F_1 plants in November 2010, marker analysis with six markers (GA16, TAA60, TA194, TS82, TA110, and TR19), a total of 30 BC_2F_1 plants heterozygous for all the FGS markers, were selected. All 30 BC_2F_1 plants were subjected to BGS with 32 SSR markers. As a result, five BC_2F_1 plants having 89–95% genome recovery were selected and used for the third round of backcrossing. From this backcrossing, 91 BC_3F_1 seeds were harvested in February 2011. Subsequently, after growing 91 BC_3F_1 plants in March 2011, marker analysis with six markers (GA16, TAA60, TA194, TS82, TA110, and TR19), a total of 30

Table 2. Details on genotyping, selection (foreground, FGS; and background, BGS), and crossing of lines in different generations during marker-assisted backcrossing for introgressing resistance to race 1 (*foc1*) in C 214.

Markers used in FGS and plants selected during different generations	BC_1F_1			BC_2F_1			BC_3F_1			BC_3F_2			BC_3F_3		
	Analyzed	Scorable bands	Heterozygotes	Analyzed	Scorable bands	Heterozygotes	Analyzed	Scorable bands	Heterozygotes	Analyzed	Scorable bands	Homozygotes	Analyzed	Scorable bands	Homozygotes
GA16	41	38	8	122	106	38	91	85	29	86	78	21	146	106	59
TAA60	41	41	23	122	121	28	91	86	29	86	85	22	146	141	99
TA194	41	–†	–	122	121	54	91	81	18	86	85	26	146	138	99
TS82	41	–	–	122	118	55	91	84	29	86	86	32	146	130	95
TA110	41	27	13	122	86	39	91	84	25	86	78	14	146	131	62
TR19	41	40	24	122	89	42	91	88	27	86	81	19	146	139	86
Heterozygotes in the case of BC_1F_1, BC_2F_1, and BC_3F_1 for undertaking BGS; and homozygotes in the case of BC_3F_2 and BC_3F_3			4			30			30			3			22
Number of SSR markers used for BGS			*‡			32			40			*			*
Number of plants after BGS (with % RPG recovery)			*		15 (80–95)			6 (90–98)				*			*
Number of plants selected with higher background genome recovery used for generation advancement (with % RPG recovery)			4		5 (89–95)			6 (90–98)				18§			22

†Not used.

‡Symbol * indicates BGS was not done due to less number of plants.

§Plants were selected based on priority.

BC_3F_1 plants heterozygous for all the FGS markers were selected. All 30 BC_3F_1 plants were subjected to BGS with 40 SSR markers, and based on this analysis, six BC_3F_1 plants showing 90–98% genome recovery were selfed. As a result, 86 BC_3F_2 seeds were harvested in June 2011.

After foreground analysis, a total of 18 BC_3F_2 plants with 98% of the RPG along with the target regions were selfed to obtain a set of more than 150 BC_3F_3 seeds. A total of 146 plants obtained from this seed set were analyzed, and finally, a total of 22 BC_3F_4 homozygous plants with >98% genetic background recovery were selected based on marker analysis. Details about the number of plants analyzed in FGS and BGS, number of plants found heterozygous or homozygous, and plants used for next generation for crossing or for generation advancement have been provided in Table 2.

Marker-Assisted Backcrossing for Ascochyta Blight

In the MABC program for AB (Fig. 1b), C 214 (recurrent parent), was used as female and crossed with ILC 3279 (donor parent) as male to generate 20 F_1 seeds. Out of 20 F_1 plants generated and grown in the off-season (starting in April 2010), six true hybrids were confirmed with the markers (GAA47 and TA130). All six true F_1 plants were used to make the first backcross C 214//C214/ILC 3279 and 38 BC_1F_1 seeds were harvested in June 2010. All 38 BC_1F_1 seeds were sown in the off-season (starting July 2010). Genotyping of 38 BC_1F_1 plants with one SSR marker (GA16) for ABQTL-I region of CaLG02 and three SSR markers (TA130, TA2, and GAA47) for ABQTL-II region located on CaLG04 identified only 2 BC_1F_1 plants showing heterozygosity for all the markers for both ABQTL-I and ABQTL-II regions. These two plants were selected for second cycle of backcrossing.

Subsequently, a second cycle of backcrossing was undertaken using pollen from the above selected 2 BC_1F_1 plants, and 124 BC_2F_1 seeds were harvested in October 2010. After growing 124 BC_2F_1 plants in the main crop season (starting in November 2010), initially all 124 BC_2F_1 plants were screened with the diagnostic marker SCY17, and 60 BC_2F_1 plants were selected as positive for this marker. These plants were further screened with six markers: TA194, TS82, and GA16 from the ABQTL-I region, and TA130, TA2, and GAA47 from the ABQTL-II region. As a result, 46 BC_2F_1 plants heterozygous for all markers, that is, both QTL regions, were selected. All 46 BC_2F_1 plants were subjected to BGS with 29 SSR markers. On the basis of BGS, six BC_2F_1 plants with 80–87% RPG recovery were selected and used for third round of backcrossing. As a result, a total of 88 BC_3F_1 seeds were harvested in February 2011. Subsequently, after growing 88 BC_3F_1 seeds in the off-season (starting March 2011), marker analysis was initially done with the SCY17 marker, and 38 BC_3F_1 plants were found positive for the SCY17 marker. Subsequently, these 38 BC_3F_1 plants were screened with six markers (TA194, TS82, GA16 from the ABQTL-I region, and TA130, TA2, and GAA47 from the ABQTL-II region) for BGS with 43 SSR markers.

Although 23 BC_3F_1 plants showed 80 to 90% genome recovery in BGS, all 38 BC_3F_1 plants positive for the diagnostic marker (SCY17) were used for selfing to obtain more seeds. Therefore, a total of 166 BC_3F_2 seeds were harvested in the month of June 2011. After growing 51 BC_3F_2 plants in the first instance (July 2011), a total of nine BC_3F_2 plants were selected on the basis of

Table 3. Details on genotyping, selection (foreground, FGS; and background, BGS), and crossing of lines in different generations during marker-assisted backcrossing for introgressing Ascochyta blight (AB) resistance in C 214.

Markers used in FGS and plants selected during different generations	Markers	BC_1F_1			BC_2F_1			BC_3F_1			BC_3F_2			BC_3F_3		
		Analyzed	Scorable bands	Heterozygotes	Analyzed	Scorable bands	Heterozygotes	Analyzed	Scorable bands	Heterozygotes	Analyzed	Scorable bands	Homozygotes	Analyzed	Scorable bands	Homozygotes
ABQTL-I	TA194	–†	–	–	60	60	31	38	38	21	51	44	5	189	181	41
	TR58	–	–	–	–	–	–	–	–	–	51	47	3	189	177	8
	TS82	–	–	–	60	60	31	38	38	21	51	49	7	189	181	37
	GA16	38	37	14	60	60	22	38	12	8	51	47	1	189	155	23
Common heterozygotes/homozygotes for all ABQTL-I markers				NA‡	–	–	NA	–	–	NA	–	–	1	–	–	1
ABQTL-II	SCY17	–	–	–	124	61	–	88	38	–	51	50	21	189	163	89
	TA130	38	38	21	60	58	50	38	32	31	51	50	7	189	172	34
	TA2	38	28	19	60	60	53	38	37	30	51	49	7	189	179	40
	GAA47	38	29	14	60	55	29	38	33	27	51	43	8	189	172	57
Common heterozygotes/homozygotes for all ABQTL-II markers				NA	–	–	NA	–	–	NA	–	–	2	–	–	13
Heterozygotes in case of BC_1F_1, BC_2F_1, BC_3F_1 for undertaking BGS§ and homozygotes in case of BC_3F_2 and BC_3F_3				2			46			38	No common homozygotes			No common homozygotes		
Number of SSR markers used for BGS				*§			29			43			*			*
Number of plants after BGS (with % RPG¶ recovery)				*					23 (80–90%)				*			*
Number of plants selected with higher background genome recovery used for generation advancement				2		6 (80–87%)				38#			9††			48††

†Not used.

‡NA = not available.

§Symbol * indicates BGS was not done due to lesser number of plants.

¶RPG = recurrent parent genome.

#Although FGS and BGS was done, all 38 plants which were positive with SCAR marker (SCY17) for selfing to obtain more number of seeds for phenotyping.

††Plants were selected based on the priority.

phenotypic similarity to the recurrent parent to generate a BC_3F_3 generation. These plants were selfed further to generate >200 BC_3F_3 seeds. A total of 189 BC_3F_3 plants from this generation were analyzed, and finally 48 homozygous lines showing more than 85% genome recovery were selected to obtain BC_3F_4 seeds. Although 48 BC_3F_3 lines were selected, seeds from only 14 lines could be harvested (from the greenhouse in 2012) because of poor seed set. On the basis of phenotypic data (see later), seven resistant plants were identified and analyzed with foreground markers of both QTL regions, and background data with SSR markers reflected RPG recovery (ranged from 81.7 to 85.4%). Further, on the basis of FGS, it was revealed that five plants were found positive for the ABQTL-I region, and three plants were found positive for the ABQTL-II region. However, only one plant (ICCX-100176-470-2-16) was found positive for both QTL regions. Details of each activity (number of seeds generated, plants analyzed for FGS and BGS, number of plants found heterozygous and homozygous, and plants used for the next generation) have been given in Table 3.

Phenotyping of Marker-Assisted Backcrossing Lines for Resistance to Fusarium Wilt

All selected 22 BC_3F_4 progenies were grown and subjected to phenotyping against race 1 of FW under controlled conditions at ICRISAT in the off-season (July–October 2012). Of these, three progenies, ICCX-100175-349-2-2, ICCX-100175-382-4-6, and ICCX-100175-389-3-2, showed high to moderate levels of resistance (Fig. 2 and Table 4). One of these three progenies, ICCX-100175-349-2-2 has not shown any wilt symptoms, that is, complete resistance (100%), while the other two progenies have shown 95% (ICCX-100175-389-3-2) and 80% (ICCX-100175-382-4-6) resistance at 60 DAI.

Phenotyping of Marker-Assisted Backcrossing Lines for Resistance to Ascochyta Blight

Fourteen BC_3F_4 lines homozygous for ABQTL-I and ABQTL-II regions were used for AB screening under field conditions (Kaur et al., 2011) during the main crop season 2012–2013 at PAU, Ludhiana (disease hotspot region of India). Of the 14 lines tested in the field, a total

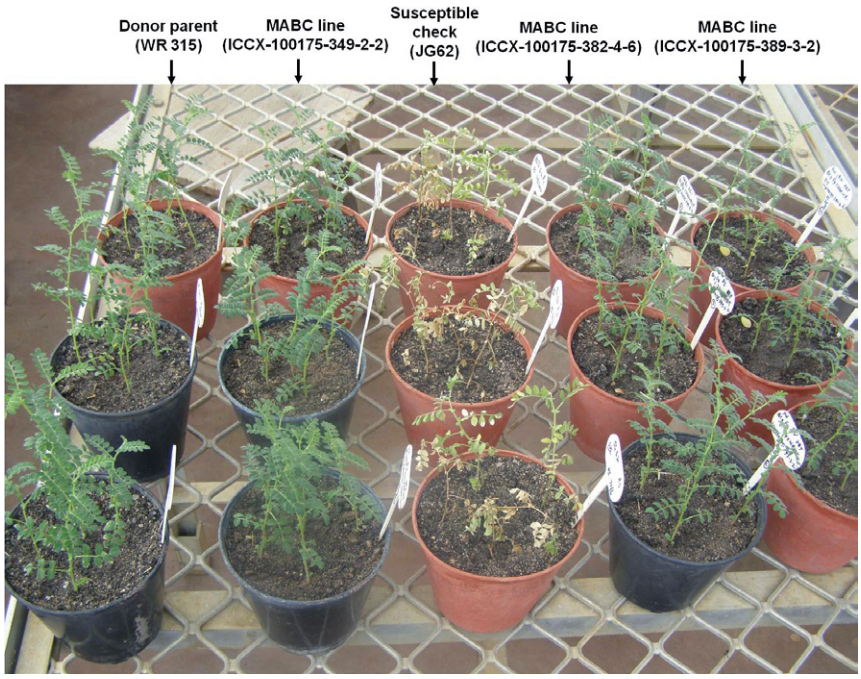

Figure 2. Screening of marker-assisted backcrossing (MABC) lines for resistance to Fusarium wilt. Phenotyping of BC_3F_4 lines for resistance to the race 1 of FW under controlled conditions identified three lines that showed resistance reaction, similar to donor parent. In the same experiment, known susceptible check showed highly susceptible reaction during artificial inoculation.

Table 4. Disease reaction of parental and BC_3F_4 lines carrying *foc1* locus conferring resistance to race 1 of *Fusarium oxysporum*.

Lines	FW incidence (%)	Disease reaction†
Parental lines		
C 214 (recurrent parent)	54.50	susceptible
WR 315 (donor parent)	6	resistant
MABC lines‡		
ICCX-100175-349-2-2	0	resistant
ICCX-100175-389-3-2	5	resistant
ICCX-100175-382-4-6	20	resistant

†FW disease reaction of each line was scored as per the scale of Sharma et al. (2005). The plants were categories as resistant (0–20%), moderately susceptible (21–50%) and susceptible (>50%).

‡MABC = marker-assisted backcrossing.

Table 5. Disease reaction of parental and BC_3F_4 lines carrying two QTL regions (ABQTL-I and ABQTL-II) conferring resistance to Ascochyta blight (AB).

Lines	ABQTL-I	ABQTL-II	AB score	Disease reaction†
Parental lines				
C 214 (recurrent parent)	–‡	–	7	susceptible
ILC 3279 (donor parent)	++	++	4	moderately resistant
MABC lines§				
ICCX-100176-421-1-11	–	++	3	resistant
ICCX-100176-421-1-12	–	++	2	resistant
ICCX-100176-470-2-5	++	–	2	resistant
ICCX-100176-470-2-7	++	–	2	resistant
ICCX-100176-470-2-16	++	++	3	resistant
ICCX-100176-470-3-1	++	–	2	resistant
ICCX-100176-470-3-3	++	–	3	resistant

†AB disease scoring of each line was on a scale of 1–9 (1 = immune, 1.1–3.0 = resistant, 3.1–5.0 = moderately resistant, 5.1–7.0 = susceptible, >7 = highly susceptible (Kottapalli et al., 2009).

‡Symbols: ++ Indicates the presence of marker alleles from donor parent at QTL region, – Indicates the presence of marker alleles from recurrent parent at QTL region.

§MABC, marker-assisted backcrossing.

of seven BC_3F_4 lines (Table 5) showed resistance reaction (Fig. 3). Out of seven lines, four lines showed a resistant score of 2, and three lines showed resistant score of 3, in comparison with a score of 7 and 4 of recurrent (C 214) and donor parent (ILC 3279), respectively. The lines possessing either of the genomic regions showed a higher level of resistance compared with the ICCX-100176-470-2-16 line possessing both QTL regions.

Molecular Analysis of Carrier Chromosomes in Marker-Assisted Backcrossing Lines

To analyze the recovery of RPG on the carrier chromosomes CaLG02 and CaLG04, SSR markers present on these chromosomes were used to analyze backcross progenies for the respective chromosomes. In the case of FW-resistant progenies, 14 additional SSR makers (other than those that were used for FGS and BGS) were used for parental polymorphism survey between C 214 and WR 315. Out of 14, only 2 SSR markers were found polymorphic between parents, and used for identification of donor parent genome. Because of the identification of a lesser number of polymorphic markers, all three improved lines showed a similar pattern with the nearest flanking marker TA103 (Fig. 4a). Interestingly, the next marker, H1F05, showed donor parent alleles in all three improved lines.

Figure 3. Screening of marker-assisted backcrossing (MABC) lines for resistance to Ascochyta blight. BC_3F_4 lines containing quantitative trait loci for AB resistance, along with parental lines, were screened in artificial epiphytotic field conditions at Punjab Agricultural University, Ludhiana, India. Under field conditions, recurrent parent C 214 completely died, and showed presence of the highly virulent isolate. Three MABC lines, however, showed resistance to the virulent isolate.

In the case of AB resistance progenies, additional 24 and 32 SSR markers corresponding to CaLG02 (for ABQTL-I) and CaLG04 (for ABQTL-II) regions were targeted for analyzing donor parent genome introgression on carrier chromosome in BC_3F_4 lines. Screening of 24 and 32 markers on parental lines showed seven and six markers polymorphic in CaLG02 and CaLG04, respectively. Genotyping of BC_3F_4 lines with these polymorphic markers identified superior recombinant lines for ABQTL-I and ABQTL-II regions. On the basis of RPG recovery on carrier chromosomes, one improved line, ICCX-100176-470-2-5, with lesser introgression of donor parent genome in both the chromosomes (CaLG02 and CaLG04) (Fig. 4b and 4c), was identified that may be used for further detailed evaluation in multilocation trials.

Discussion

Fusarium wilt and AB are two most devastating diseases of chickpea, causing severe yield losses. Conventional methods of breeding for disease resistance is a tedious and time-consuming process. However, MABC applying FGS using QTL linked markers, and BGS using genome-wide SSR markers for recovery of RPG is an environment-independent, precise, and quick approach for the development of cultivars for the trait of interest (Varshney et al., 2010). This study reports successful introgression of resistance to FW and AB in the genetic background of C 214.

Quantitative trait loci mapping identified resistance loci with flanking molecular markers for resistance to a number of races of FW: *foc0* locus for race 0 (Cobos et al., 2005), *foc1* locus for race 1 (Gowda et al., 2009), *foc2* locus for race 2 (Gowda et al., 2009), *foc3* locus for race 3 (Sharma et al., 2004; Gowda et al., 2009), *foc4* locus for race 4 (Winter et al., 2000; Sharma et al., 2004; Sharma and Muehlbauer, 2005) and *foc5* locus for race 5 (Cobos et al., 2009). Recently, two novel QTL (*FW-Q-APR-6-1* and *FW-Q-APR-6-2*) for FW for race 1 explaining 10.4 to 18.8% of phenotypic variation have also been reported (Sabbavarapu et al., 2013). Similarly, a large number of QTL for AB resistance were reported: *ar2b* (Udupa and Baum, 2003), QTL_{AR3} (Iruela et al., 2007), *ar1* (Iruela et al., 2007), *ar2* (Iruela et al., 2007). Recently, we have mapped six QTL for AB resistance, explaining up to 31.9% of phenotypic variation (Sabbavarapu et al., 2013).

In view of above, the *foc1* locus conferring resistance to race 1 of FW, and two QTL regions (ABQTL-I and ABQTL-II) for AB resistance located on two different LGs were targeted for introgression into the recurrent parent C 214. Foreground selection with QTL linked markers and BGS using genome-wide SSR markers were employed in each backcross generation to select positive plants for crossing or selfing. However, in case of FW, due to identification of only four BC_1F_1 plants positive for all foreground markers, BGS was not imposed. In the BC_2F_1 generation, five plants with RPG recovery ranging from 89 to 95% were identified based on SSR analysis in comparison with

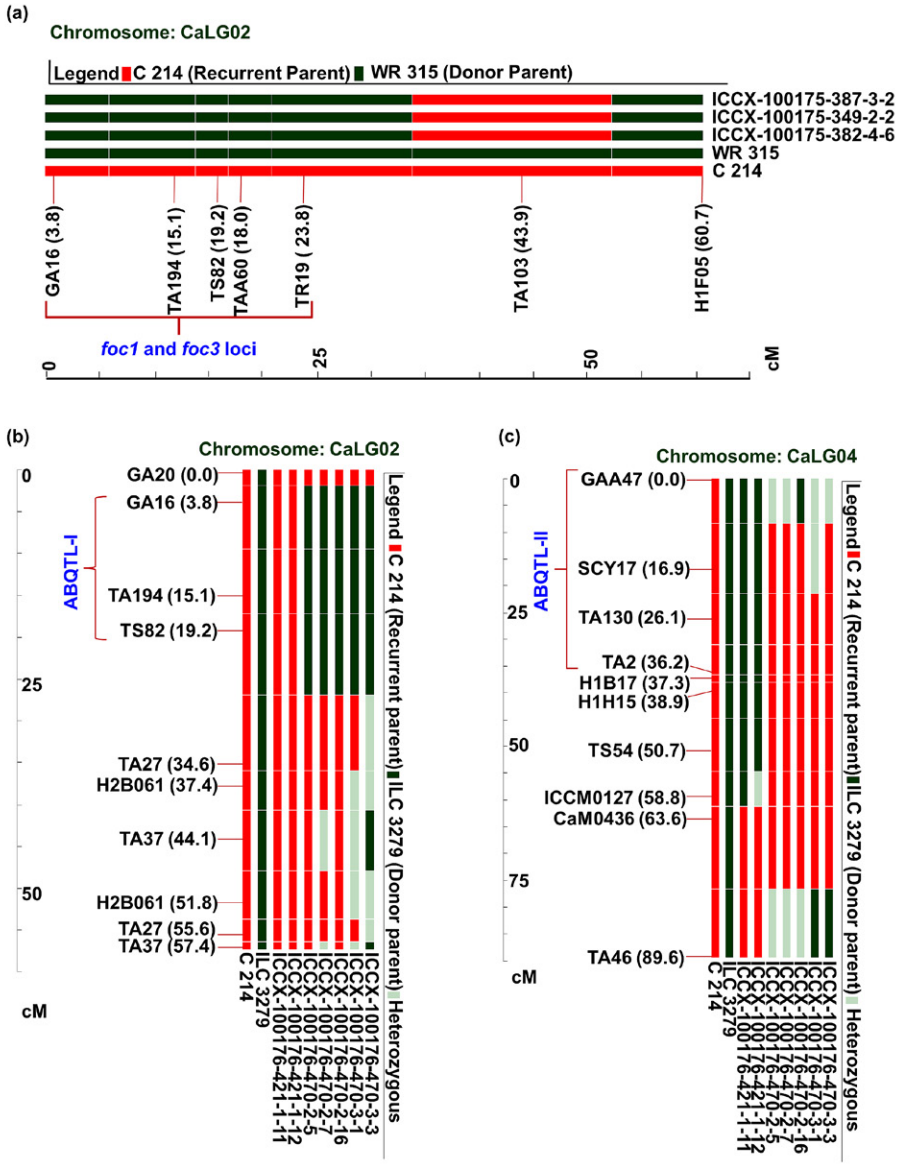

Figure 4. Graphical genotypes of selected lines using SSR markers for the carrier chromosomes for marker-assisted backcrossing lines for resistance to Fusarium wilt and Ascochyta blight. (a) Polymorphic SSR markers on the carrier chromosome (CaLG02) between parental lines (C 214 × WR 315) were used to analyze the introgression of donor parent genome associated with resistance loci *foc1*. It is evident that all three BC_3F_4 lines showed expected graphical genotypes (GGT). (b) Graphical genotypes (GGT) were generated after genotyping MABC lines for ABQTL-I with CaLG02 specific markers that showed polymorphism between C 214 and ILC 3279. (c) MABC lines for ABQTL-II region were genotyped with CaLG04 specific markers that showed polymorphism between C 214 and ILC 3279. The genotyping data was used for preparation of GGT. In each case, the GGT identified the plants with minimum amount of the donor parent genome.

87.5% of expected average similarity. Following FGS and BGS with molecular markers, several plants up to 98% of similarity were developed as early as in the BC_3F_1 generation. Similarly, in the case of AB in BC_1F_1 generation, because of identification of only two plants positive for all markers from both QTL regions, ABQTL-I and ABQTL-II, BGS was not imposed. However, the BC_2F_1 generation had plants up to 87% of RPG as expected (87.5%). However, plants with 80 to 90% RPG were identified in BC_3F_1 generation, further based on resistance reaction seven plants were selected in BC_3F_3 generation which showed the RPG recovery ranged from 81.7 to 85.4%. The lower recovery of recurrent genome of selected plants was might be due to fixations of heterozygous alleles at BC_3F_1 generation towards donor parent genome.

To analyze the recovery of recurrent parent alleles on carrier chromosomes in BC_3F_4 MABC lines, carrier chromosome specific (CaLG02 for FW cross, CaLG02 and CaLG04 for AB cross) polymorphic SSR markers were also used to identify recombinant lines with lesser donor parent segments in both crosses. However, due to lesser number of polymorphic markers identified between C 214 × WR 315, the real selection of lines in the case of MABC progenies for FW was not effective. Analyzing MABC for AB resistance lines using carrier chromosome specific markers for two LGs (CaLG02 and CaLG04) in C 214 × ILC 3279, one

line, ICCX-100176-470-2-5, was identified with lesser donor parent introgression in both the carrier chromosomes. This line with lesser donor parent introgression will be used for further evaluations for other important traits.

Phenotyping for FW resistance of MABC and parental lines showed resistance reaction in sick plot nursery. However, three MABC lines showed resistance reaction from 0 to 20% of FW resistance reaction in comparison with C 214 (recurrent parent) of 54.5 and 6% of WR 315 (donor parent). Of three, two lines have shown better resistance ICCX-100175-349-2-2 (0% of FW incidence) and ICCX-100175-389-3-2 (5% of FW incidence). It is also important to mention here that the introgressed segment in C 214 also carries the *foc3* locus, having resistance to race 3 of FW. Therefore, the MABC lines generated in this study may show resistance to race 3 of FW as well. Phenotypic evaluation, in the target region of FW race 3, however, needs to be undertaken to confirm above mentioned speculation.

Similarly, MABC lines for AB resistance showed disease reaction score <3.0 in comparison with 7 and 4 of recurrent (C 214) and donor parent (ILC 3279), respectively, on 1 to 9 scale. However, one line identified with minimum donor parent chromosome ICCX-100176-470-2-5 on both of the carrier chromosomes showed high levels of resistant reaction (score of 2), in comparison with donor parent ILC 3279 (score of 4) and recurrent parent C 214 (score of 7). Surprisingly, one line which was positive for both the QTL regions (ABQTL-I and ABQTL-II) showed resistant reaction score of 3, in comparison with lines possessing either ABQTL-I or ABQTL-II with resistant score of 2, except ICCX-100176-470-3-3, which showed resistant reaction of 3 and possesses only ABQTL-I.

In the present study, MABC lines with single genomic region (either ABQTL-I or ABQTL-II) compared with that of a line having both ABQTL-I and ABQTL-II regions, showed higher level of resistance to AB. This may be because of antagonistic epistatic interaction of two genomic regions that was also evident by the disease reaction of donor parent ILC 3214, which has comparatively low level of resistance as compared with MABC lines (Jannink, 2009). Similar observation was made by Castro et al. (2003), that during the transfer of resistant QTL for barley strip rust into the elite background, presence of single QTL alleles in lines showed higher level of resistance in comparison with presence of two QTL alleles, and they explained it may be because of double crossover between markers and disease resistance loci, undetected resistance genes, and/or incomplete penetrance. Therefore, more experiments are necessary to determine the specific role of each QTL in this study. However, based on our experiments, it can be concluded that either ABQTL-I or ABQTL-II can be targeted for development of AB-resistant breeding lines.

In summary, this study demonstrates use of MABC to develop superior lines with enhanced resistance to race 1 (and possibly race 3) of FW and AB. These lines may be used for multilocation field trials of All India Coordinated Research Project (AICRP) on Chickpea of Indian Council of Agricultural Research (ICAR) in India for possible release of the most promising MABC lines as improved cultivar for commercial cultivation. However, to add value further, intercrossing may be undertaken using superior MABC lines for FW and AB resistance, developed in the present study. Pyramided lines for resistance to FW (*foc1* and possibly *foc3*) and AB (ABQTL-I and ABQTL-II) are expected to perform better in different agroclimatic zones. Therefore, it is planned to undertake intercrossing of FW- and AB-resistant lines and then selfing of the products of intercrossing to make them homozygous. Homozygous and pyramided version of C 214 subsequently will be evaluated for yield and yield-related traits in disease hotspot locations of India. After due testing, superior lines will be tested further under AICRP on Chickpea of ICAR for release of improved cultivar with enhanced resistance to FW and AB for commercial cultivation.

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