Theor Appl Genet (2013) 126:1639–1647
DOI 10.1007/s00122-013-2080-2

ORIGINAL PAPER

Fast track genetic improvement of ascochyta blight resistance and double podding in chickpea by marker-assisted backcrossing

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Received: 16 November 2012 / Accepted: 23 February 2013 / Published online: 6 March 2013

Abstract Ascochyta blight (AB) caused by the fungus *Ascochyta rabiei* Pass. Lab. is one of the major diseases of chickpea worldwide and a constraint to production in western Canada. The use of varieties with high levels of resistance is considered the most economical solution for long-term ascochyta blight management in chickpea. QTL for resistance to ascochyta blight have been identified in chickpea. The availability of molecular markers associated with QTL for ascochyta blight resistant and double podding provides an opportunity to apply marker-assisted backcrossing to introgress the traits into adapted chickpea cultivars. In the present study, molecular markers that were linked to the QTL for ascochyta blight resistance and the double podding trait, and those unlinked to the resistance were used in foreground and background selection, respectively, in backcrosses between moderately resistant donors (CDC Frontier and CDC 425-14) and the adapted varieties (CDC Xena, CDC Leader and FLIP98-135C). The strategy included two backcrosses and selection for two QTL for ascochyta blight resistance and a locus associated with double podding. The fixation of the elite genetic background was monitored with 16–22 SSR markers to accelerate restoration of the genetic background at each backcross. By the BC_2F_1 generation, plants with improved ascochyta blight resistance and double podding were identified. The selected plants possessed the majority of elite parental type SSR alleles on all fragments analyzed except the segment of LG 4, LG 6 and LG 8 that possessed the target QTL. The results showed that the adapted variety could be efficiently converted into a variety with improved resistance in two backcross generations.

Communicated by I. Rajcan.

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Introduction

Chickpea (*Cicer arietinum* L.) is the third most important food legume in the world grown in over 40 countries in an area of over 11.5 million hectares with total annual production of about 10.5 million tonnes (Food and Agriculture Organization of the United Nations, FAOSTAT database (FAOSTAT 2011). Chickpea yield fluctuates highly in most countries. Drought has been the most important factor for instability of yield in major production countries in Asia and Africa, as chickpea is mainly grown as a rain fed crop on residual moisture. The other important factors contributing to instability in chickpea yield in those countries are ascochyta blight (*Ascochyta rabiei*), fusarium wilt (*Fusarium oxysporum* f. sp. *ciceri*) and pod-borer (*Helicoverpa armigera*).

Ascochyta blight is one of the major diseases of chickpea worldwide including western Canada. It reduces yield, quality and increases production costs due to the typical requirement for multiple fungicide applications (Acikgoz et al. 1994; Jimenez-Diaz et al. 1993; Nene 1984). The use of varieties with improved levels of resistance is considered the most economical solution for long-term disease management (Singh and Reddy 1996). Chickpea cultivars with improved levels of resistance to AB have been developed and commercialized (Tar'an et al. 2009, 2011; Warkentin et al. 2005). However, only partial resistance is available among the cultivated chickpea germplasm and the improved cultivars are only moderately resistant to AB. Singh and Reddy (1993) evaluated 19,343 accessions from

the ICARDA germplasm collection and found accessions with a score of 4 on a 1–9 scale for resistance. Thus, further improvement in field resistance of chickpea to AB is required to increase and stabilize production levels. One strategy being considered to increase the level of resistance to AB in chickpea is to analyze different sources of resistance within the cultivated species that potentially carry different genes and pyramid these genes to improve the levels of resistance. Combining key genes for resistance to AB may also increase the durability of resistance by giving protection against varying populations of ascochyta.

Resistance to ascochyta blight, however, is complex; many genes with minor to moderate effects control the resistance (Millán et al. 2006, 2010; Tar'an et al. 2007). The degree of infection by *A. rabiei* is also influenced by environmental conditions requiring specialized disease nurseries to enable effective selection of resistant lines making selection difficult by traditional pathological techniques. There is now extensive information available on the genetic control of ascochyta blight resistance in chickpea. Several research groups have identified a common set of ascochyta blight resistance QTL in different crosses and genetic backgrounds (Anbessa et al. 2009; Lichtenzveig et al. 2006; Millán et al. 2010; Radhika et al. 2007; Tar'an et al. 2007). Although, each QTL identified appears to explain small to medium amounts of the phenotypic variation, the effects appeared to be additive (Anbessa et al. 2009). As high as 70 % of the phenotypic variations have been reported from the cumulative effects of QTL for resistance to ascochyta blight (Cho et al. 2004). Thus, it is essential to select for multiple genes to provide sufficient resistance. The primary ascochyta blight resistance QTLs are located on Linkage Groups 2, 3, 4, 6 and 8 (Anbessa et al. 2009; Cho et al. 2004; Flandez-Galvez et al. 2003; Millán et al. 2010; Tar'an et al. 2007; Udapa and Baum 2003). This set of ascochyta blight resistance QTL has been identified in several chickpea mapping populations. In addition to increasing our understanding of QTL position for ascochyta blight resistance, more markers including microsatellite (SSR) and gene-based markers have been added to the consensus map of chickpea (Gujaria et al. 2011; Thudi et al. 2011).

Chickpea usually develops a single flower, hence also a single pod, per node, but some lines develop two flowers and pods per node or peduncle, the so called 'double podding' trait, controlled by the 's' gene (Singh and van Rheenen 1989). The double-flowered trait is well recognized in the desi marker class, but it is less common in Kabuli type chickpea. Double podding is considered to be one of the important traits for yield improvement with several reports showing a yield advantage of the double-flowered/double podding trait in chickpea. Sheldrake et al. (1978) studied the effects of converting double-podded plants to single-podded ones by cutting off one of the flowers at every double-podded node and concluded that the double-podded character may increase the yield by 6–11 %. Kumar et al. (2000) reported that the double podding trait gave a yield advantage of 18 % in the F_2 and 7 % among recombinant inbred lines. On the other hand, no yield advantage of the double podding trait was observed by Knights (1987) and Rubio et al. (1998). Singh and van Rheenen (1994) suggested that the double podding trait enhances yield only in certain environments, and thus, it can play an important role in stabilizing chickpea yield. Rubio et al. (1998) also found a positive effect of the double-pod gene on the stability of yield in chickpea. Anbessa et al. (2007) suggested that the double podding trait in chickpea increases the demand to assimilate in the sink during the pod filling period resulting in hastening maturity, especially in areas with a short growing season such as the Canadian prairies. A SSR marker TA80 linked with the double podding gene was identified (Rajesh et al. 2002).

The current research evaluated the use of the markers associated with the QTL for ascochyta blight resistance and double podding and the most recent SSR map for molecular breeding in chickpea. The strategies for molecular breeding of complex traits such as ascochyta blight resistance can be taken further than only selecting for QTL in segregating progeny as suggested by Gupta and Varshney (2000). One strategic approach is to simultaneously monitor restoration of the genetic background with QTL introgression and select progeny with recombination events in critical chromosome positions known as marker-assisted backcrossing (MAB). The basis of a MAB strategy is to transfer a specific allele at the target locus from a donor line to a recipient line while selecting against donor introgressions across the rest of the genome. The use of molecular markers which permit the genetic dissection of the progeny at each generation increases the speed of the selection process, thus increasing genetic gain per unit time (Tanksley et al. 1989; Hospital 2003). The effectiveness of MAB depends on the availability of closely linked markers and/or flanking markers for the target locus, the size of the population, the number of backcrosses and the position and number of markers for background selection (Frisch et al. 1999a; Frisch and Melchinger 2005). The other advantage of using this strategy in selecting progeny based only on genotype is the opportunity to reduce linkage drag for deleterious alleles and fix regions of the genome essential for seed quality and other agronomic characteristics in early generations.

The availability of the markers associated with QTL for ascochyta blight resistance and double podding, and the existence of donor and adapted varieties susceptible to ascochyta blight provided an opportunity to apply the

Table 1 List of parental material and their attributes used in the molecular breeding of ascochyta blight resistance and double podding in chickpea

Line	Pedigree	Status	Type (100 seed weight)	Ascochyta blight reaction[a]
CDC Xena	C188-178/ICCV 89511	Elite/recipient parent	Kabuli (46 g)	7.5
FLIP98-135C	ICARDA germplasm	Elite/recipient parent	Kabuli (44 g)	5.5
CDC Leader	FLIP95-48C/CISN-SP-99PL21117	Elite/recipient parent	Kabuli (42 g)	4.5
CDC Frontier	FLIP91-22C/ICC14912	Donor ascochyta blight resistance	Kabuli (37 g)	4.0
CDC 425-14	ICC12004/92073-40	Donor ascochyta blight resistance	Desi (20 g)	4.5
CDC Y9421-026	272-2/CDC Anna	Donor double podding	Desi (16 g)	7.0

[a] Ascochyta blight rating was based on 0–9 scale

strategy for improvement of chickpea using markers. The main objective of this research was to develop an improved version of the adapted chickpea varieties through a targeted MAB approach for the QTL associated with ascochyta blight resistance and double podding.

Materials and methodology

Plant materials

The plant materials were divided into three groups, elite parents, ascochyta blight resistance and double podding donor parents. The elite or recipient parents were the large seeded kabuli varieties CDC Xena, FLIP98-135C and CDC Leader. Each of these varieties possesses many of the desirable agronomic characteristics that are required for production in western Canadian growing conditions and good seed quality characteristics. The donor parents for ascochyta blight resistance were CDC Frontier and CDC 425-14. Each of the donor parents was a highly inbred line carrying specific ascochyta blight resistance QTL (Tar'an et al. 2007; Anbessa et al. 2009; Flandez-Galvez et al. 2003) and has demonstrated ascochyta blight resistance based on several years of field and greenhouse testing. CDC Y9421-026 was used as the donor for the 's' gene that controls double podding in chickpea. Details of the parental materials are shown in Table 1.

Introgression crossing scheme

To pyramid the resistance and the double podding genes, three sets of crosses were made to facilitate assembling the desired QTL combinations. Two sets were used for introgressing different QTL for ascochyta blight resistance and one set was for introgressing double podding into the elite parent. Figure 1 illustrates the three crossing sets to introgress the resistance to ascochyta blight and double podding into CDC Xena as the recipient variety. Initially, the parental lines were genotyped with all available

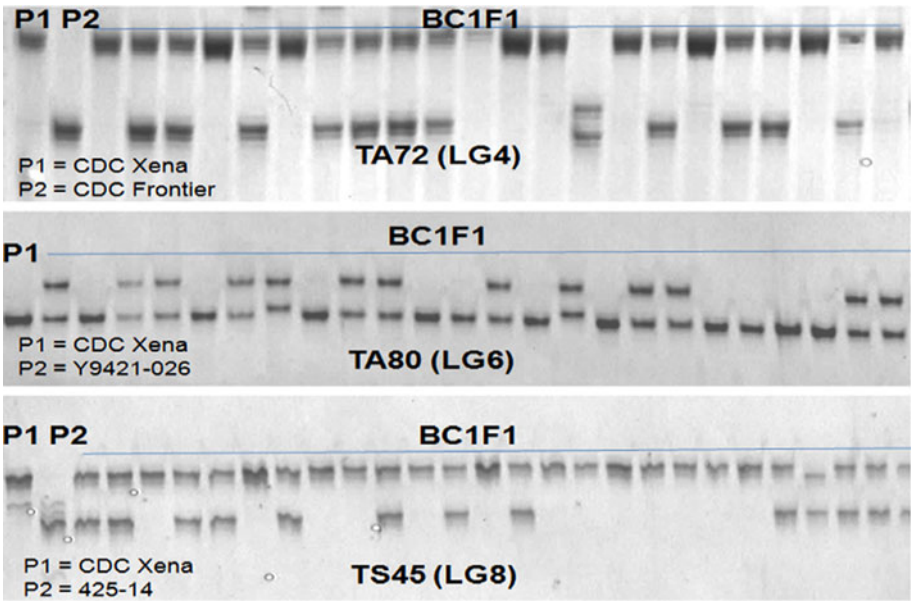

Fig. 1 SSR profiles of markers on linkage group 4 and 8 used for the selection of ascochyta blight resistance QTL in crossing set 1 and 2 and marker profile (TA80) from linkage group 6 for selection of double podding gene in crossing set 3. The heterozygous BC_1F_1 individuals were selected for further backcrossing to produce BC_2F_1

Table 2 Genomic location and linked markers of four QTL intervals for ascochyta blight resistance and one interval for double podding in each donor line

Location	QTL	SSR marker	Donor line
Ascochyta blight resistance			
LG4	*Abr QTL 3*	TA2	CDC Frontier
		TA72	CDC Frontier
		TS54	CDC Frontier
LG8	*Abr QTL 4*	TA3	CDC 425-14
		TS12	CDC 425-14
		TS45	CDC 425-14
Double podding			
LG6	*s gene*	TA80	CDC Y9421-026

microsatellite (SSR) markers in the QTL intervals associated with resistance to ascochyta blight (Table 2). This determined the parents for each cross by placing emphasis on: (1) maximizing the number of polymorphic markers in the interval; and (2) having microsatellites that were able to discriminate the allelic state of each parent. Three sets of crosses were initiated for each elite parent (Table 3). Two sets were intended to incorporate the alleles for resistance to ascochyta blight from CDC Frontier and CDC 425-14 and the other was to introduce the 's' gene for double podding from CDC Y9421-026. Each crossing set followed an identical scheme of donor to elite followed by two backcrosses to the elite parent and one selfing generation to derive BC_2F_2 plants. The selected BC_2F_2 plants from different crossing sets were then intercrossed to combine the target QTL (Fig. 2).

Initially, each parental line was screened using SSR markers at an interval of approximately 20–25 cM across the entire genome. A total of 100 SSRs selected from the published linkage map (Tar'an et al. 2007) were initially tested for polymorphism across the recipient and donor lines. Several of the SSRs that did not show clear banding patterns among the parents were excluded from further analysis. The selected SSR markers were used for recipient parent genome selection.

Each F_1, BC_1F_1 and BC_2F_1 plant was vegetatively propagated by stem cuttings to maximize the number of plants for crossing to produce sufficient seeds for marker segregation analysis and to allow simultaneous disease screening. Stimroot no. 1 (Evergro Canada Inc., Delta, British Columbia) containing the active ingredient indole-3-butyric acid (IBA) was used to induce root development. Up to ten cuttings were made from each F_1 plant and selected BC_1F_1 and BC_2F_1 plants. Initially, the cuttings were grown on a peat pellet and incubated in a high humidity chamber with fluorescent light for about 10 days. The cutting-derived plants were then transferred into individual one gallon pots (or 4 $inch^2$ pot for disease screening) filled with Sunshine mix # 4 medium (Sun Gro Horticulture Canada Ltd., Seba Beach, Alberta). Three cutting-derived plants from each BC_2F_1 plant that were relatively uniform in size were selected and used for disease evaluation. The protocol for indoor ascochyta blight disease screening was described in Tar'an et al. (2007). The disease score for each BC_2F_1 line was averaged from three cutting-derived plants. The master plants and the rest of cutting-derived plants were kept separately in the greenhouse for crossing and seed increase.

Genotyping and selection

Two weeks after sowing, fresh leaf tissues from each of F_1, BC_1F_1 and BC_2F_1 plants were collected in a 2-mL tube for DNA isolation. Collected leaf tissues were immediately freeze-dried for 24 h and ground to fine powder using a home made mixer mill. Genomic DNA was prepared using either a cetyl trimethyl ammonium bromide (CTAB) extraction method according to the protocol as described by Doyle and Doyle (1990) or DNeasy Plant DNA extraction kit (Qiagen, Mississauga, Ontario) and quantified by fluorometry using the Hoechst 33258 stain. PCR

Table 3 Number of F_1 and BC_1F_1 plants from three sets of crosses to introduce resistance for ascochyta blight from CDC Frontier and CDC 425-14 and double podding from CDC Y9421-026 into CDC Xena, CDC Leader and FLIP98-135C

Cross	Crossing set	Elite (recipient) parent	Donor parent	Number of F_1 plants	Number of BC_1F_1 plants
A	Set 1	CDC Xena	CDC Frontier	4	97
	Set 2	CDC Xena	CDC 425-14	9	119
	Set 3	CDC Xena	CDC Y9421-026	11	121
B	Set 1	CDC Leader	CDC Frontier	4	88
	Set 2	CDC Leader	CDC 425-14	13	117
	Set 3	CDC Leader	CDC Y9421-026	9	124
C	Set 1	FLIP98-135C	CDC Frontier	8	104
	Set 2	FLIP98-135C	CDC 425-14	7	116
	Set 3	FLIP98-135C	CDC Y9421-026	9	119

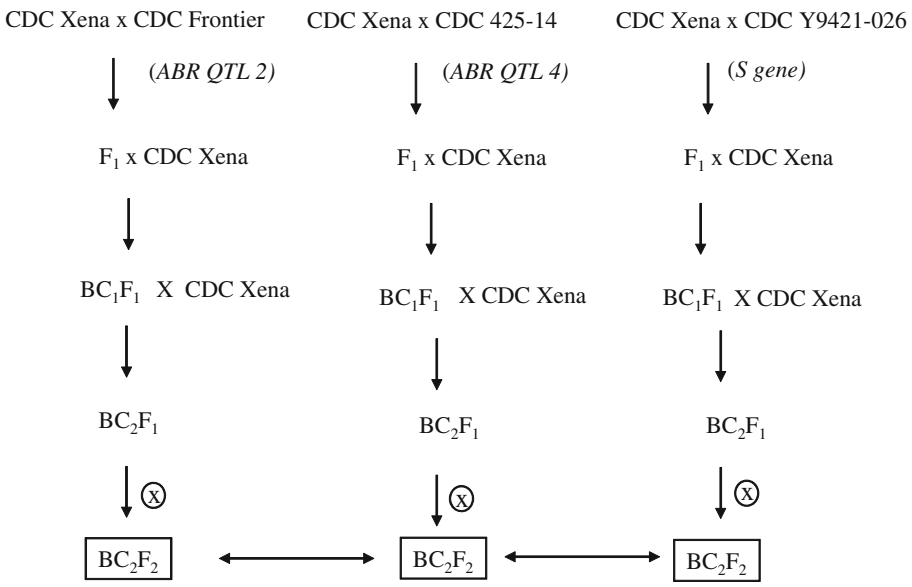

Fig. 2 Schematic of three crossing sets to introgress and combine different QTL for ascochyta blight resistance and a gene for double podding into CDC Xena

amplification was done in a 25 µL volume containing 50 ng of genomic DNA, 1 U of *Taq* DNA polymerase, 0.24 mM each of dATP, dCTP, dGTP and dTTP, 1.0 µM of each primer and buffer with a final concentration of 2.5 mM $MgCl_2$. PCR cycling conditions included an initial denaturation step at 94 °C for 3 min, followed by 35 cycles of 94 °C for 30 s, 50–58 °C (depending on the SSR primers) for 30 s and 72 °C for 2 min with a final extension step at 72 °C for 5 min. The PCR products were separated on a 6 % polyacrylamide gel followed by silver staining to visualize the DNA fragments. Both 10 and 50 base pairs DNA ladders were used as molecular weight markers for each gel. The glass plates were scanned to create electronic files for band sizing and documentation. SSR allele sizing was done using AlphaEase software (Alpha Innotech Corporation, California, USA). Alternatively, the SSR were analyzed using an ABI 3730 DNA analyzer. Amplification was carried out using the GeneAmp PCR system 9700 (Applied Biosystems). The GeneMapper ver. 3.7 software (Applied Biosystems) was used to size peak patterns using the internal Genescan-500 LIZ size standard and Genotyper 3730 (Applied Biosystems) for allele calling.

Results

On average, eight F_1 seeds from each crossing set (Table 3) were produced and grown under phytotron conditions to commence the first backcross to the respective recipient parent. The hybridity test using SSRs demonstrated that all the F_1 plants were true hybrids. Stem cuttings were made from each F_1 plant as a mean to multiply the plants to increase the number of flowers available for multiple backcrosses. On average, 112 BC_1F_1 plants were produced from each crossing set (Table 3).

Two to three polymorphic markers for each ascochyta blight resistance QTL (Table 2) were used with one being in the center of the QTL while the others were flanking markers. Each BC_1F_1 plant was genotyped with these markers. Figure 1 shows the examples of SSR profiles of markers on linkage groups (LGs) 4 and 8 used for the selection of ascochyta blight resistance QTL in crossing set 1 and 2, and the marker profile (TA80) from LG 6 for selection of double podding gene in crossing set 3. The heterozygous BC_1F_1 individuals were selected for further backcrossing to produce BC_2F_1 plants which were then screened with appropriate ascochyta blight resistance markers to select plants retaining the ascochyta blight resistance QTL and were subjected to phenotypic screening using *A. rabiei* isolate *ar68-2001* (Table 4).

The plants that were heterozygous across the interval were then genotyped with a set of background markers polymorphic for that cross. A total of 16–22 SSRs (2–4 SSRs per LG), depending on the cross, were used for background selection. Since full genetic maps were not constructed in this project, the degree of genome coverage using the background loci was based on the previous chickpea SSR map (Tar'an et al. 2007). The number of background genome loci fixed as homozygous was expressed as a percentage of the total number of background markers. Background genome loci that were fixed for elite alleles at BC_1 were not re-genotyped at BC_2, and thus, fewer markers were used in the BC_2 generation. The selected BC_1F_1 individuals that were heterozygous for the target QTL and that had the most fixation of the elite

Table 4 Percentage of fixed loci, seed weight, ascochyta blight score and percent double podding of selected BC_2F_1 plants derived from three sets of backcrosses to introgress resistance for ascochyta blight from CDC Frontier and CDC 425-14, and double podding from CDC Y9421-026 for each of recipient parents (CDC Xena, CDC Leader and FLIP98-135C

Generation	Crosses and pedigree	Number of plants	Number of selected plants	% Fixed loci[a]	100 Seed weight (g)	Mean ascochyta blight score[b]	Mean double podding (%)[c]
BC2F1	CDC Xena*3/CDC Frontier	60	13	88	44	5.8 ± 0.6	0
BC2F1	CDC Xena*3/CDC 425-14	58	5	79	41	6.4 ± 0.5	0
F1	[CDC Xena*3/CDC Frontier]/[CDC Xena*3/CDC 425-14]	121	19	NA	42	5.6 ± 0.4	0
BC2F1	CDC Xena*3/CDC Y9421-026	70	9	86	40	NA	10.4
BC2F1	CDC Leader*3/CDC Frontier	52	7	92	40	4.1 ± 0.5	0
BC2F1	CDC Leader*3/CDC 425-14	50	9	81	38	4.4 ± 0.6	0
F1	[CDC Leader*3/CDC Frontier]/[CDC Leader*3/CDC 425-14]	98	23	NA	39	4.2 ± 0.4	0
BC2F1	CDC Leader*3/CDC Y9421-026	43	7	84	39	NA	11.8
BC2F1	FLIP98-135C*3/CDC Frontier	61	11	90	42	5.2 ± 0.6	0
BC2F1	FLIP98-135C*3/CDC 425-14	86	13	87	41	5.8 ± 0.4	0
F1	[FLIP98-135C*3/CDC Frontier]/[FLIP98-135C*3/CDC 425-14]	112	21	NA	41	5.5 ± 0.5	0
BC2F1	FLIP98-135C*3/CDC Y9421-026	114	16	89	40	NA	9.6
CDC Frontier					37	4.0 ± 0.2	0
CDC 425-14					22	5.2 ± 0.3	0
CDC Xena					45	7.5 ± 0.5	0
CDC Leader					41	4.6 ± 0.4	0
FLIP 98-135C					43	6.0 ± 0.6	0
CDC Y9421-026					21	6.2 ± 0.6	39.8

NA not assessed

[a] The number of background genome loci fixed was expressed as a percentage of the total number of background markers

[b] Ascochyta blight (AB) score for each BC_2F_1 was based on the average of three cutting-derived plants on a scale of 0–9

[c] Double podding was expressed as percentage of peduncles with double pods to the total peduncles

genome were selected and multiplied by stem cutting for further use as females in the crossing scheme to produce BC_2F_1. This same process was performed at the BC_2F_1 and BC_2F_2 generations in all three sets with the exception that background genome analysis was omitted on BC_2F_2 plants. The total number of BC_2F_1 and selected plants derived from three sets of backcrosses to introgress resistance for ascochyta blight from CDC Frontier and CDC 425-14 and double podding from CDC Y9421-026 from each of elite/recipient parents (CDC Xena, CDC Leader and FLIP98-135C) are presented in Table 4. The seed size of all the introgressed lines were more or less similar to the original recipient varieties, though we noticed that the lines that received the QTL from the desi types (CDC 425-14 and CDC Y9421-026) had darker seed color. The mean percentage of fixed loci in the background genome ranged from 79 to 92 %. The ascochyta blight scores of the BC_2 derived lines of CDC Xena, CDC Leader and FLIP98-135C that had the introgression of QTL3 from CDC Frontier as donor were 5.8, 4.1 and 5.2, respectively. This was a significant improvement for CDC Xena considering that the original variety was rated as very poor with an average score of 7.5 in a 0–9 scale. The disease scores of the introgressed lines of CDC Leader and FLIP98-135C were not significantly different from the original (non-introgressed) lines. The ascochyta blight scores of CDC Xena, CDC Leader and FLIP98-135C derived from introgression using CDC 425-14 as donor for QTL4 were 6.4, 4.4 and 5.8, respectively. These scores were not significantly different from the original recipients of CDC Xena, CDC Leader and FLIP98-135C. However, knowing that the backcross lines of CDC Xena, CDC Leader and FLIP98-135C carry diverse alleles in the QTL regions for ascochyta blight resistance, we hypothesize that these alleles may contribute to the durability of the resistance. Further long-term field-testing is needed to test this hypothesis.

Mean double podding expressed as percentage of peduncles with double pods to the total peduncles of the selected BC_2 lines ranged from 9.6 to 11.8 %. These values were much lower than the percentage of double podding

(40 %) of the donor parent (CDC Y9421-026). We also noticed that many of the dual pods in the BC_2 plants which are of kabuli type actually only had one pod filled, while the other was empty or the seed was arrested at early development. The explanation for this phenomenon is unclear. We hypothesize that it may be related to seed size especially for the kabuli type, but further investigation is needed.

Discussion

Using MAS and two generations of backcrosses followed by one generation of selfing, we obtained an improved version of CDC Xena, CDC Leader and FLIP98-135C. The improved version of the recipient lines contained fragments of the donor parent, while a large proportion of the rest of the genome was similar to the recipient parent. The derived lines also showed similar seed characteristics (size and shape) and morphology fo the original CDC Xena, CDC Leader and FLIP98-135C plants. To the best of our knowledge, this is the first report of the introgression of QTL for ascochyta blight and double podding with the specific aim of reducing the size of a donor segment using linked flanking markers in chickpea.

SSR markers were used for all of the loci under selection and only markers with a clear separation of alleles by polyacrylamide electrophoresis were used (Fig. 1; Table 2). This ensured that each locus of every plant that was genotyped was classified correctly into one of the two possible allele combinations. The SSR map (Tar'an et al. 2007), which was developed from a cross between a desi and kabuli lines, provided sufficient markers for background selection. This experiment was effectively conducted and replicated in three independent crosses using CDC Xena, CDC Leader and FLIP98-135C as recipient parents and achieved relatively similar levels of recipient parent fixation each time (Table 4).

The use of SSR markers permits the selection of plants with more than one set of QTL for resistance to ascochyta blight and double podding without phenotyping. The MAB strategy has thus been shown to be an effective means of utilizing QTLs in chickpea breeding programs. The selected chickpea plants now available with up to two known resistance QTL selected on the basis of linked SSR markers exemplify the power of marker-aided backcrossing. However, the selected backcrossed lines of CDC Xena, FLIP98-135C or CDC Leader having the two QTL were not significantly more resistant than either CDC Frontier or CDC 425-14, indicating that the effects of the resistance QTL may not be additive.

These lines may provide excellent resources for studying the interaction of ascochyta blight isolates across different combinations of QTL and for testing the durability of the resistance of the lines possessing different QTL combinations. The pyramided lines may also serve as useful parental materials for integrating the QTL into other varieties from different market classes, which otherwise have poor resistance to ascochyta blight.

The average BC_1F_1 population of 112 plants was sufficient for selection of the disease resistance QTL or double podding marker. Initially, the QTL for ascochyta blight resistance and double podding were monitored by markers shown to be closely linked with the QTL as listed in Table 2. Using linked markers such as TA72 for QTL3 and flanking markers (TA2 and TS54) ensured efficient foreground and recombinant selection as suggested by Hospital and Charcosset (1997). The availability of SSR markers facilitated the selection schemes. Particular emphasis was placed on recombinant selection in this study, because of the desire to recover all the important traits of the elite varieties, and minimize the effects of linkage drag from the ascochyta blight QTL or double podding donor, especially CDC 425-14 and CDC Y9421-026, which are of desi type and possess several undesirable agronomic characters such as darker seed coat and smaller seed size. Using the linked markers in the target region, recovery of double recombinants with a relatively small donor fragment was possible. The grain quality parameters of the selected BC lines were on par with the non-introgressed varieties, except the seeds of the lines derived from introgression using CDC 425-14 and CDC Y9421-026 as donors, although they are clearly of kabuli type with white flower, they still exhibited darker seed coat color. This may illustrate the consequences of linkage drag. Young and Tanksley (1989) first proposed the idea of reducing the size of donor fragments containing target loci. They suggested that, using 1 cM flanking markers on each side of a target locus, the size of the introgressed segment could theoretically be reduced to 2 cM in two generations, in comparison with traditional backcross breeding where it would be expected to require 100 BC generations to obtain such a small segment.

By using markers for background selection, there was a great acceleration of recipient genome recovery in the present study. The general conclusion was that a few well placed markers (2–4 markers on each chromosome of 100 cM) provide adequate coverage of the genome in backcross programs (Visscher et al. 1996; Servin and Hospital 2002). In this project, an average of three markers was used per chromosome and the average distance between two markers was 25 cM. The best plant had 92 % of the recipient genome by BC_2F_1 (Table 4). Therefore, an average distance between markers of 20 cM and a minimum of four markers per chromosome as recommended from MAB simulation studies (Hospital et al. 1992; Hospital 2003; Servin et al. 2004) appears to be sufficient for the accelerated recovery of the recipient parent genome as shown in

this study. This experiment achieved a percentage close to the BC_4 level (93.75 %) of recipient parent allele fixation in only two backcrosses. Currently, we are in the process of developing a high density genetic map in chickpea using SNP markers. This map would ultimately confirm the prediction of genome recovery from this project.

In the past years, considerable research in developing and optimizing MAB schemes has been investigated using computer simulations. Particular attention has been given to reducing marker data points (MDPs) by determining minimum population sizes required for recombinant selection (Frisch et al. 1999b; Hospital 2001) and appropriate population sizes, ratios and selection strategies for background selection (Hospital et al. 1992; Visscher et al. 1996; Frisch et al. 1999a). The results in this study, which are actual experimental data, are consistent with the results from simulation studies that two or three BC generations are sufficient using markers compared to many more generations by conventional backcrossing (Frisch et al. 1999a).

The number of major QTLs that have been identified for agronomically important traits in chickpea is growing rapidly and many of them are being fine mapped, cloned or sequenced (Garg et al. 2011). With the availability of large numbers of SSR and SNP markers, high throughput genotyping and fine mapping of the major QTLs, virtually any major QTL can now be introgressed into a variety without changing the desirable agronomic characteristics. Thus, the present study also serves as a case study of a strategy for introgression of a major QTL into a set of adapted varieties.

In summary, we have developed improved ascochyta blight varieties and introduced double podding using a marker-assisted backcrossing approach. The recovery of the recipient parent genome was greatly accelerated using markers to assist selection of backcross lines. This experiment accomplished the selection of a combination of resistance QTL and gene for double podding in segregating populations and simultaneously restored the elite genetic background to an adequate level (on average 86 % after BC_2) in three separate crosses. More importantly given the agronomic characteristics of the donor parent, the size of the donor chromosomal segment containing the target locus was reduced to ensure that there were minimal changes to the genetic composition of the recipient varieties. This practical example of marker-assisted selection clearly illustrates the superiority of using MAB compared to conventional backcrossing because obtaining such a small donor region within only a few backcross generations would be impossible using conventional methods.

Acknowledgments We thank Parvaneh Hashemi and Carmen Breitkreutz for their technical assistance. Financial support from the Agricultural Development Fund of the Saskatchewan Ministry of Agriculture is gratefully acknowledged.

References

Acikgoz N, Karaca M, Er C, Meyveci K (1994) Chickpea and lentil production in Turkey. In: Muehlbauer FJ, Kaiser WJ (eds) Expanding the production and use of cool season food legumes. Kluwer Academic Publ, Dordrecht, pp 388–398

Anbessa Y, Warkentin TD, Bueckert R, Vandenberg A (2007) Short internode, double podding and early flowering effects on maturity and other agronomic characters in chickpea. Field Crop Res 102:43–50

Anbessa Y, Taran B, Warkentin TD, Tullu A, Vandenberg A (2009) Genetic analyses and conservation of QTL for ascochyta blight resistance across different populations of chickpea (*Cicer arietinum* L.). Theor Appl Genet 119:757–765

Cho S, Chen W, Muehlbauer FJ (2004) Pathotype-specific genetic factors in chickpea (*Cicer arietinum* L.) for quantitative resistance to ascochyta blight. Theor Appl Genet 109:733–739

Doyle JJ, Doyle JL (1990) Isolation of plants DNA from fresh tissue. Focus 12:13–15

Flandez-Galvez H, Ades PK, Ford R, Pang ECK, Taylor PWJ (2003) QTL analysis for ascochyta blight resistance in an intraspecific population of chickpea (*Cicer arietinum* L.). Theor Appl Genet 107:1257–1265

Food and Agriculture Organization of the United Nations, FAOSTAT database (FAOSTAT, 2011), available at http://faostat.fao.org/site/567/DesktopDefault.aspx?PageID=567#ancor

Frisch M, Melchinger AE (2005) Selection theory for marker-assisted backcrossing. Genetics 170:909–917

Frisch M, Bohn M, Melchinger AE (1999a) Comparison of selection strategies for marker-assisted backcrossing of a gene. Crop Sci 39:1295–1301

Frisch M, Bohn M, Melchinger AE (1999b) Minimum sample size and optimal positioning of flanking markers in marker-assisted backcrossing for transfer of a target gene. Crop Sci 39:967–975

Garg R, Patel RK, Jhanwar S, Priya P, Bhattacharjee A, Yadav G, Bhatia S, Chattopadhyay D, Tyagi AK, Jain M (2011) Gene discovery and tissue-specific transcriptome analysis in chickpea with massively parallel pyrosequencing and web resource development. Plant Physiol 156:1661–1678

Gujaria N, Kumar A, Dauthal P, Dubey A, Hiremath P, Bhanu Prakash A, Farmer A, Bhide M, Shah T, Gaur PM, Upadhyaya HD, Bhatia S, Cook DR, May GD, Varshney RK (2011) Development and use of genic molecular markers (GMMs) for construction of a transcript map of chickpea (*Cicer arietinum* L.). Theor Appl Genet 122:1577–1589

Gupta PK, Varshney RK (2000) The development and use of microsatellite markers for genetic analysis and plant breeding with emphasis on bread wheat. Euphytica 113:163–185

Hospital F (2001) Size of donor chromosome segments around introgressed loci and reduction of linkage drag in marker-assisted backcross programs. Genetics 158:1363–1379

Hospital F (2003) Marker-assisted breeding. In: Newbury HJ (ed) Plant molecular breeding. Blackwell Publishing, Oxford, pp 30–59

Hospital F, Charcosset A (1997) Marker-assisted introgression of quantitative trait loci. Genetics 147:1469–1485

Hospital F, Chevalet C, Mulsant P (1992) Using markers in gene introgression breeding programs. Genetics 132:1199–1210

Jimenez-Diaz RM, Crino P, Halila MH, Mosconi C, Trapero-Casas AT (1993) Screening for resistance to fusarium wilt and ascochyta blight in chickpea. In: Singh KB, Saxena MC (eds) Breeding for stress tolerance in cool-season food legumes. ICARDA, Aleppo, pp 77–95

Knights EJ (1987) The double podded gene in chickpea improvement. Int Chickpea Newslett 17:6–7

Kumar J, Shrivastava RK, Ganesh M (2000) Penetrance and expressivity of the gene for double podding in chickpea. J Hered 91:234–236

Lichtenzveig J, Bonfil DJ, Zhang HB, Shtienberg D, Abbo S (2006) Mapping quantitative trait loci in chickpea associated with time to flowering and resistance to *Didymella rabiei* the causal agent of Ascochyta blight. Theor Appl Genet 113:1357–1369

Millán T, Clarke HJ, Sidiqque KHM, Buhariwalla HK, Gaur PM, Kumar J, Gil J, Kahl G, Winter P (2006) Chickpea molecular breeding: new tools and concepts. Euphytica 147:81–103

Millán T, Winter P, Jungling R, Gil J, Rubio J, Cho S, Cobos MJ, Iruela M, Rajesh PN, Tekeoglu M, Kahl G, Muehlbauer FJ (2010) A consensus genetic map of chickpea (*Cicer arietinum* L.) based on 10 mapping populations. Euphytica 175:175–189

Nene YL (1984) A review of ascochyta blight of chickpea (*Cicer arietinum* L.). In: Saxena MC, Singh KB (eds) Ascochyta blight and winter sowing of chickpea. Martinus Nijhoff/Dr. W. Junk Publisher, The Hague, pp 17–34

Radhika P, Gowda S, Kadoo N, Mhase L, Jamadagni B, Sainani M, Chandra S, Gupta V (2007) Development of an integrated intraspecific map of chickpea (*Cicer arietinum* L) using two recombinant inbred line populations. Theor Appl Genet 115:209–216

Rajesh PN, Tullu A, Gil J, Gupta VS, Ranjekar PK, Muehlbauer FJ (2002) Identification of an STMS marker for the double-podding gene in chickpea. Theor Appl Genet 105:604–607

Rubio J, Moreno MT, Cubero JI, Gil J (1998) Effect of the gene for double pod in chickpea on yield, yield components and stability of yield. Plant Breed 117:585–587

Servin B, Hospital F (2002) Optimal positioning of markers to control genetic background in marker-assisted backcrossing. J Hered 93:214–217

Servin B, Martin OC, Mezard M, Hospital F (2004) Toward a theory of marker-assisted gene pyramiding. Genetics 168:513–523

Sheldrake AR, Saxena NP, Krishnamurthy L (1978) The expression and influence on yield of the 'double-podded' character in chickpeas (*Cicer arietinum* L.). Field Crops Res 1:243–253

Singh KB, Reddy MV (1993) Resistance to six races of *Ascochyta rabiei* in the world germplasm collection of chickpea. Crop Sci 33:186–189

Singh KB, Reddy MV (1996) Improving chickpea yield by incorporating resistance to ascochyta blight. Theor Appl Genet 92:509–515

Singh O, van Rheenen HA (1989) A possible role for the double podded character in stabilizing the yield of chickpea. Indian J Pulses Res 2:97–101

Singh O, van Rheenen HA (1994) Genetics and contribution of the multi seeded and double-podded character to grain yield of chickpea. Indian J Pulses Res 7:97–102

Tanksley SD, Young ND, Paterson AH, Bonierbale MW (1989) RFLP mapping in plant breeding: new tools for an old science. Biotechnology 7:257–264

Tar'an B, Warkentin T, Tullu A, Vandenberg A (2007) Genetic mapping of ascochyta blight resistance in chickpea (*Cicer arietinum* L.) using an SSR linkage map. Genome 50:26–34

Tar'an B, Malhotra R, Warkentin T, Banniza S, Vandenberg A (2009) CDC Luna kabuli chickpea. Can J Plant Sci 89:517–518

Tar'an B, Bandara M, Warkentin T, Vandenberg A, Banniza S (2011) CDC Orion kabuli chickpea. Can J Plant Sci 91:355–356

Thudi M, Bohra A, Nayak SN, Varghese N, Shah TM, Penmetsa RV, Thirunavukkarasu N, Gudipati S, Gaur PM, Kulwal PL, Upadhyaya HD, Kavikishor PB, Winter P, Kahl G, Town CD, Kilian A, Cook DR, Varshney RK (2011) Novel SSR markers from BAC-end sequences, DArT arrays and a comprehensive genetic map with 1,291 marker loci for chickpea (*Cicer arietinum* L.). PLoS One 6:e27275

Udapa SM, Baum M (2003) Genetic dissection of pathotype-specific resistance to ascochyta blight disease in chickpea (*Cicer arietinum* L.) using microsatellite markers. Theor Appl Genet 106:1196–1202

Visscher PM, Haley CS, Thompson R (1996) Marker-assisted introgression in backcross breeding programs. Genetics 144:1923–1932

Warkentin TD, Bannniza S, Vandenberg A (2005) CDC Frontier kabuli chickpea. Can J Plant Sci 85:909–910

Young ND, Tanksley SD (1989) RFLP analysis of the size of chromosomal segments retained around the tm-2 locus of tomato during backcross breeding. Theor Appl Genet 77:353–359