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ORIGINAL ARTICLE

Marker-assisted introgression of resistance to fusarium wilt race 2 in Pusa 256, an elite cultivar of *desi* chickpea

Aditya Pratap[1] · Sushil K. Chaturvedi[1] · Rakhi Tomar[1] · Neha Rajan[1,3] · Nupur Malviya[1] · Mahender Thudi[2] · P. R. Saabale[1] · Umashanker Prajapati[1] · Rajeev K. Varshney[2] · N. P. Singh[1]

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Abstract Fusarium wilt caused by *F. oxysporum* f. sp. *ciceris* causes extensive damage to chickpea (*Cicer arietinum* L.) in many parts of the world. In the central part of India, pathogen race *2* (*Foc* 2) causes severe yield losses. We initiated molecular marker-assisted backcrossing (MABC) using *desi* cultivar, Vijay, as a donor to introgress resistance to this race (*Foc2)* in Pusa 256, another elite *desi* cultivar of chickpea. To confirm introgression of resistance for this race, foreground selection was undertaken using two SSR markers (TA 37 and TA110), with background selection to observe the recovery of recurrent parent genome using 45 SSRs accommodated in 8 multiplexes. F_1 plants were confirmed with molecular markers and backcrossed with Pusa 256, followed by cycles of foreground and background selection at each stage to generate 161 plants in BC_3F_2 during the period 2009–2013. Similarly, 46 BC_3F_1 plants were also generated in another set during the same period. On the basis of foreground selection, 46 plants were found homozygotes in BC_3F_2. Among them, 17 plants recorded >91% background recovery with the highest recovery percentage of 96%. In BC_3F_1 also, 14 hybrid plants recorded a background recovery of >85% with the highest background recovery percentage of >94%. The identified plants were selfed to obtain 1341 BC_3F_3 and 2198 BC_3F_2 seeds which were screened phenotypically for resistance to fusarium wilt (race 2) besides doing marker analysis. Finally, 17 BC_3F_4 and 11 BC_3F_3 lines were obtained which led to identification of 5 highly resistant lines of Pusa 256 with *Foc* 2 gene introgressed in them. Development of these lines will help in horizontal as well as vertical expansion of chickpea in central part of India.

Keywords Backcrossing · Chickpea · BGS · FGS · MABC · Molecular breeding · SSR markers

Introduction

The genus *Cicer,* belonging to the family Fabaceae is the only genus in the tribe Cicereae. Chickpea (*Cicer arietinum* L.), is the sole cultivated *Cicer* species and is the most important cool season grain legume in terms of production and consumption. It is a self-pollinated diploid crop with genome size 740 mbp, $2n = 2x = 16$ and grown extensively by smallholder farmers throughout south Asia and several other countries. India holds the largest share of this crop and alone contributes about 73.3% of the area and 67.4% to the global chickpea production followed by Pakistan, Australia, Iran and Turkey (Jiménez-Díaz et al. 2015). During 2014–15, India recorded a total production of 7.33 million tonnes of chickpea grains with an average productivity of about 1 ton/ha (DAC 2016), with terminal drought identified as the main constraint on productivity. Among the biotic stresses, fusarium wilt is the most important and

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✉ Aditya Pratap
Aditya.Pratap@icar.gov.in

[1] Crop Improvement Division, ICAR-Indian Institute of Pulses Research, Kalyanpur, Kanpur, Uttar Pradesh 208024, India

[2] Center of Excellence in Genomics (CEG), International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Patancheru, Greater Hyderabad 502 324, India

[3] Present Address: Krishi Vigyan KendraRama Krishna Mission Ashram, Ranchi, Jharkhand 834008, India

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has a potential to cause up to 100% yield losses (Sharma et al. 2004). First reported in India in 1918, this disease is now widespread in most chickpea growing areas in Asia, Africa, southern Europe and the Americas, but has not been reported yet from Australia (Cunnington et al. 2007).

Fusarium wilt in chickpea is caused by a soil-borne, facultative, vascular wilt fungus, *Fusarium oxysporum* (Schlechtend.: Fr.) f. Sp. *ciceris* (Padwick) Matuo & K. Sato (*Foc*). Eight physiological races of the pathogen (0, 1A, 1B/C, 2, 3, 4, 5 and 6) are reported so far whereas additional races are suspected from India. The distribution pattern of these races in different parts of the world indicates regional specificity for their occurrence leading to the perception that *F. oxysporum* f. sp. *ciceris* evolved independently in different regions (Sharma and Muehlbauer 2007).

Fusarium oxysporum f. sp. *ciceris* race 2 (*foc* 2) is prevalent in central Indian states such as Uttar Pradesh, Madhya Pradesh and Chhattisgarh, causing considerable losses to chickpea every year. As the pathogen perpetuates in seed and soil, it is difficult to manage by the use of chemicals. Like other soil borne diseases, various strategies have been employed for controlling fusarium wilt in these areas, such as use of chemical fungicides, biological control strategies, etc. but these have proven ineffective or have hazardous effect. The most widely accepted, efficient and cost-effective strategy is to develop and use wilt-resistant cultivars (Haware and Nene 1982; Jalali and Chand 1992; Sharma et al. 2005).

Deployment of host plant resistance (HPR) is the most preferred strategy for managing fusarium wilt in chickpea keeping in view its long-term gains, least environmental effect and cost-effectiveness. Nevertheless, the development of resistant cultivars through a conventional breeding approach is time consuming. On the other hand molecular marker-assisted backcross breeding approach (MABC) applying foreground selection (FGS) and background selection (BGS) using genome-wide SSR markers for recovery of recurrent parent genome is an environment-independent, precise and quick approach for the development of cultivars of trait of interest (Varshney et al. 2010).

Pusa 256, an elite cultivar of chickpea, has been a very popular and widespread in central part of India, especially Uttar Pradesh, Madhya Pradesh and Chhattisgarh due to its excellent seed quality, high yield potential and better market price. Of late, it started exhibiting symptoms of fusarium wilt becoming susceptible to *F. oxysporum* race 2, affecting its area and productivity considerably. Keeping in view that farmers are still not ready to part with this cultivar even after getting reduced yield due to fusarium wilt, this experiment was undertaken with an objective to breed improved Pusa 256 having resistance to fusarium wilt race 2 (*Foc* race 2) through MABC approach. Previous genetic studies have predicted that resistance to *F. oxysporum* race 2 is controlled by single recessive gene (Sharma and Muehlbauer 2007). As reported by earlier mapping studies, gene for resistance to *foc2* is located on CaLG02 and information on markers tightly linked to it is also available (Gowda et al. 2009). Therefore, molecular breeding strategy was employed to develop HPR in this elite chickpea cultivar.

Materials and methods

Plant materials

Pusa 256, a popular and high yielding *desi* chickpea cultivar, with market-preferred grain quality but susceptibility to fusarium wilt, was chosen as the recurrent parent for introgression of fusarium resistance through MABC. The *desi* cultivar Vijay which is resistant to *foc* races races 1–5 was chosen as the donor parent. Target loci for resistance to *foc* 2 have already been mapped in resistant genotype—Vijay (Gowda et al. 2009).

DNA extraction and marker genotyping

The deoxyribonucleic acid was isolated from the fresh leaf tissues of 15- to 20-day-old seedlings of the parental genotypes as well as the F_1s and subsequent backcross populations using modified cetyl trimethyl ammonium bromide (CTAB) extraction method (Cuc et al. 2008) followed by purification of extracted DNA by removing RNA and protein impurities. Purified DNA was then analysed for quality and quantity using 0.8% agarose gel and finally normalised to concentration of 35 ng/µl.

Polymerase chain reaction for simple sequence repeat (SSR) markers analysis for the targeted genomic region linked to *foc2* locus as well as the SSRs saturating complete genome for background selection was carried out in 20 µl reaction mixture, 2 µl of 10× buffer (Genei, Banglore, India), 35 ng genomic DNA, 0.6 U of *Taq* DNA polymerase (Genei), 2 mM dNTP mix (Fermentas, Mumbai, India) and 0.8 mM each of forward and reverse primers (ILS, Gurgaon, India) in thermocycler (G-storm, Somerset, UK). PCR was programmed for an initial denaturation of 94 °C for 3 min, followed by 39 cycles of 94 °C for 1 min, annealing (specific annealing temperature) for 1 min, extension at 72 °C for 2 min and final extension at 72 °C for 5 min. The amplified PCR product was separated by Polyacrylamide gel electrophoresis using 10% PAGE and finally stained by silver staining protocol (Ji et al. 2007) to visualise intact and clear bands.

Backcross breeding

Hybridization was done between Pusa 256 × Vijay in the years 2009 and 2010 to generate sufficient quantity of F_1 seeds, derived lines were designated IIPR-VC and IIPR-VD. F_1 plants were confirmed through SSR markers, NPS1 and NPS 14 (Quadir et al. 2007), and the confirmed F_1s were retained for executing first round of backcrossing with the recurrent parent, Pusa 256. In subsequent backcross generations, foreground selection for linked markers (TA110 and TA37) was used to identify the genomic region of interest in backcross progeny; background selection using SSR markers from whole genome was used to identify plants with high recurrent parent genome retention for further backcrossing. Three rounds of backcrossing were done and the selected plants in final stage were selfed 3–4 times to generate homozygous population (BC_3F_4 and BC_3F_3) as well as to multiply sufficient quantity of seeds of Pusa 256-like plants having FW resistance introgressed in them.

Phenotypic screening for fusarium wilt

For phenotypic screening, BC_3F_4 and BC_2F_4 families were sown in controlled wilt sick micro-plots (*foc2*) as well as normal field conditions along with their parents at ICAR-IIPR, Kanpur for two consecutive crop seasons during 2015–16 and 2016–17. The wilt sick micro-plots had sufficient inoculum load as indicated by the concentration of spores (5–6 × 10^6 conidia/ml/g of soil) as well as 100% mortality of the susceptible check JG 62 in the wilt sick micro-plots. Phenotypic data for appearance of wilt symptoms were recorded after 60 days of inoculation and the progenies were designated as resistant (0–20%), moderately susceptible (21–50%) and susceptible (>50%) as per Sharma et al. (2005). The highly resistant lines were further multiplied in field to generate sufficient seed for multilocation testing.

Selection of molecular markers

Initially, four SSR markers namely H3A12, TA110, TA37 and TS47 (Gowda et al. 2009) which were present in the linkage group CaLG02 and were earlier reported to be in the genomic region conferring resistance to *Foc* 2 (Millan et al. 2006) (Table 1) were screened for selecting the closest polymorphic markers. After screening, two markers viz., TA110 and TA37 (Winter et al. 1999) were found polymorphic between both parents Pusa 256 and Vijay and were used to transfer the desirable genomic segment in the background of Pusa 256.

For short-listing of markers for background selection, a set of 371 SSR makers selected from a high density interspecific chickpea genetic linkage map (Thudi et al. 2011) were screened at ICRISAT for testing parental polymorphism among Pusa 256 and Vijay. Among these, 72 markers were identified as polymorphic, 45 polymorphic markers were used to prepare a multiplex in a way that enough molecular markers were available for each linkage group to indicate background genome recovery.

Results

Marker-assisted backcrossing

Marker-assisted backcrossing was undertaken at ICAR-IIPR, Kanpur (main season), and its Regional Station, Dharwad and University of Agricultural and Horticultural Sciences, Zonal Agricultural Research Station, Hiriyur, Karnataka (off-season) during 2010–14. The detailed scheme used to introgress FW resistance from Vijay to Pusa 256 is given in Fig. 1. Pusa 256 (recurrent parent) and Vijay (donor parent) were crossed during crop season 2010–11. As a result 89 F_1 seeds were obtained. Out of 78 F_1 plants that germinated, 49 hybrids were confirmed using polymorphic markers (NPS1 and NPS14). Using these F_1s, hybridization was undertaken to generate 112 BC_1F_{1s}

Table 1 Molecular markers used for foreground selection in the marker-assisted breeding programme to breed for fusarium wilt resistance

Foc race	Marker name	Forward primer sequence	Reverse primer sequence	References
Foc-2	H3A12	AACCTTAGACTGTGTTCGCTGA	TCAATCTTTTGTTGTTACTATGAATCTG	Lichtenzveig et al. (2005)
Foc 1, 2 & 3	TA110	ACACTATAGGTATAGGCATTTAGGCAA	TTCTTTATAAATATCAGACCGGAAAGA	Winter et al. (1999)
Foc 2	TA37	ACTTACATGAATTATCTTTCTTGGTCC	CGTATTCAAATAATCTTTCATCAGTCA	Winter et al. (1999)
Foc 2	TS47	GTTAATATTTTTCCGCTTCGT	TCAAATTGTGTTAAAAATCAAAGTGTT	Winter et al. (1999)

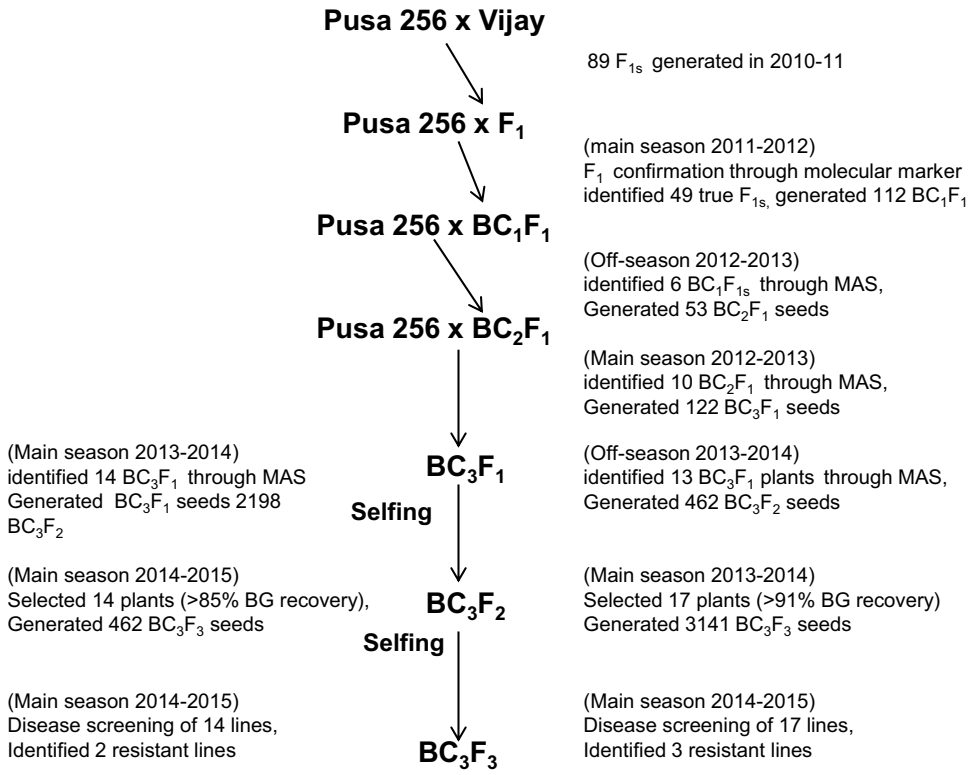

Fig. 1 Marker-assisted backcrossing scheme deployed to introgress resistance to race 1 of *Fusarium oxysporum* f. sp. *ciceris*

during crop season 2011–12. Out of 94 BC_1F_1s, 48 seeds were sown in off-season nursery (May–September 2012) at ZARS, Hiriyur, Karnataka for crossing (starting May 2012). DNA was isolated from all 48 BC_1F_1 plants for foreground and background selection. As a result of foreground selection, 13 plants were found common heterozygotes for both the markers (TA110 and TA37). These 13 BC_1F_1 plants were further subjected to background selection with 45 SSR markers (Pl. see supplementary material). One plant with 93% and 5 plants with 70–80% genome recovery were identified and used for second cycle of backcrossing to obtain 53 BC_2F_1 seeds during 2012–13.

At the start of main season October 2012–April 2013, 53 BC_2F_1 seeds of the cross Pusa 256 × Vijay were available which were sown on two different dates to ensure a longer crossing period. Of these, 51 germinated and foreground selection using the markers TA110, TA96 and TA37 revealed 16 plants as heterozygotes (Table 2). All these 16 plants were subjected to background selection with 36 markers and the background recovery estimation ranged from 71 to 91% in all the 16 plants. Among these, the top 10 plants showing genome recovery between 80 and 91% were used for the third round of backcrossing to generate BC_3F_1 seeds. As a result, 122 BC_3F_1 seeds (Table 2) were obtained.

Out of 122 BC_3F_1 seeds obtained in April 2013 at IIPR, Kanpur, 76 seeds were sown during off-season (May–August 2013) at Regional Station-cum-off-season nursery of IIPR at Dharwad, Karnataka (set I) while 46 seeds were retained as backup in off-season experiment (set II). Among these 66 germinated plants were subjected to foreground selection using TA110 and TA37 markers and 26 plants were screened as common heterozygotes for both markers. Further background selection using 45 markers from genomic background of recurrent parent resulted in the percent recovery between 61 and 93%. The top 13 plants having more than 70% background genome recovery were allowed to self and 462 BC_3F_2 seeds were generated. These 462 BC_3F_2 seeds were again sown at main farm, IIPR, Kanpur in Nov., 2013. Out of them 161 BC_3F_2 plants were subjected to foreground selection with two polymorphic markers TA110 and TA37. Foreground genome selection resulted in 46 homozygotes in BC_3F_2. Among these 17 plants recorded >91% background genome recovery with the highest 96% recovery in BC_3F_2 and these plants were selfed to obtain 1341 BC_3F_3 seeds. A total of 273 plants from this seed lot were analysed and finally 17 lines with recurrent parent genome (RPG) recovery of >96% were selected (Fig. 2).

Simultaneously, remaining 46 BC_3F_1 seeds generated during the main season (2012–13) were also sown during the main crop season 2013–14. These were subjected to foreground selection with two polymorphic markers TA110 and TA37. Foreground genome selection resulted in 39 heterozygotes. When subjected to BGS, 14 plants recorded more than 85% background recovery with the highest recovery of 94%. Top 14 plants in BC_3F_1 populations

Table 2 Details of backcrossing, genotyping, foreground and background selection in marker-assisted breeding programme to breed for fusarium wilt resistance

Markers used in foreground selection and plants selected during different generations	BC_1F_1		BC_2F_1			BC_3F_1 (set I)			BC_3F_2 (set I)			BC_3F_1 (set II)		
	Analysed	Scorable bands	Heterozygotes	Analysed	Scorable bands	Heterozygotes	Analysed	Scorable bands	Heterozygotes	Analysed	Scorable bands	Heterozygotes	Analysed	Scorable bands	Heterozygotes
TA110	48	42	18	51	41	27	66	66	29	161	144	67	46	44	42
TA37	48	45	21	51	37	20	66	65	27	161	149	66	46	43	40
Common heterozygotes for both markers for background selection			13			16			26			46			39
No. of SSR markers used for background selection			45			36			45			31			31
No. of plants after background selection (with % recurrent parent genome recovery)			13 (55–93%)			15 (61–91%)			26 (61–93%)			29 (78–89%)			29 (72–84%)
No. of plants selected with higher background genome recovery used for next backcrossing/selfing			10 (70–93%)			10 (80–91%)			13 (70–93%)			17 (91–96%)			14 (85–93%)

were selfed to obtain and 2198 BC_3F_2 seeds. Among these 222 plants were analysed using molecular markers and 11 BC_3F_3 homozygous plants with RPG recovery of >94% were selected.

Phenotyping for fusarium wilt resistance

All 17 BC_3F_4 and 11 BC_3F_3 single plant progenies were subjected to phenotypic screening against race 2 of FW under controlled conditions in wilt sick micro-plots and were also selfed for their multiplication (Fig. 3). Among the 17 BC_3F_4 plant populations, 6 lines (IIPRVD 2/23, IIPRVD 2/70, IIPRVD 2/117, IIPR VD 2/133, IIPRVD 2/145 and IIPRVD 2/161) were found highly resistant to fusarium wilt with a disease reaction between 0 and 5.0% while in BC_3F3 plant populations, 4 lines (IIPRVC 16/10-2, IIPRVC 16/10-9, IIPRVC 35/5-4 and IIPRVC 35/5-8) were highly resistant with the disease reaction ranging between 0 and 3.1 per cent (Table 3) after 60 days of sowing. The five lines viz., IIPRVD 2/116, IIPRVC 16/10-2, IIPRVD 2/145, IIPRVC 35/5-4 and IIPRVC 35/5-8, which exhibited complete (100%) resistance were further multiplied and screened for fusarium wilt during the crop season 2014–15 and 2016–17.

Discussions

Fusarium wilt is one of the most important diseases affecting chickpea production worldwide and in severe form can cause loss in yield between 70% (Halila and Strange 1996) to 100% (Sharma et al. 2004). Fusarium wilt reduces chickpea production by decreasing grain yield and grain weight (Navas-Cortés et al. 2000) as well as deteriorating seed quality. Early wilting is reported to cause more yield loss (77–94%) as compared to late wilting (24–65%) although the seeds of late wilted plants are lighter, duller and rougher than those form the healthy plants (Haware and Nene 1980), deteriorating their quality and also marketability. Although conventional practices of managing fusarium wilt such as crop rotation, use of fungicides for seed treatment and solarisation have been practised more frequently by the farmers, these are costly, less effective and time taking. On the other hand, genetic resistance to pathogens is the most practical and cost-effective individual disease control measure for management of fusarium wilt of chickpea (Jiménez-Díaz et al. 2015). HPR through conventional methods is a tedious and less precise technique while MABC applying foreground selection and background selection using genome-wide SSR markers for recovery of recurrent parent genome is an environment-independent, precise and quick approach for the development of cultivars of trait of interest (Varshney et al. 2010).

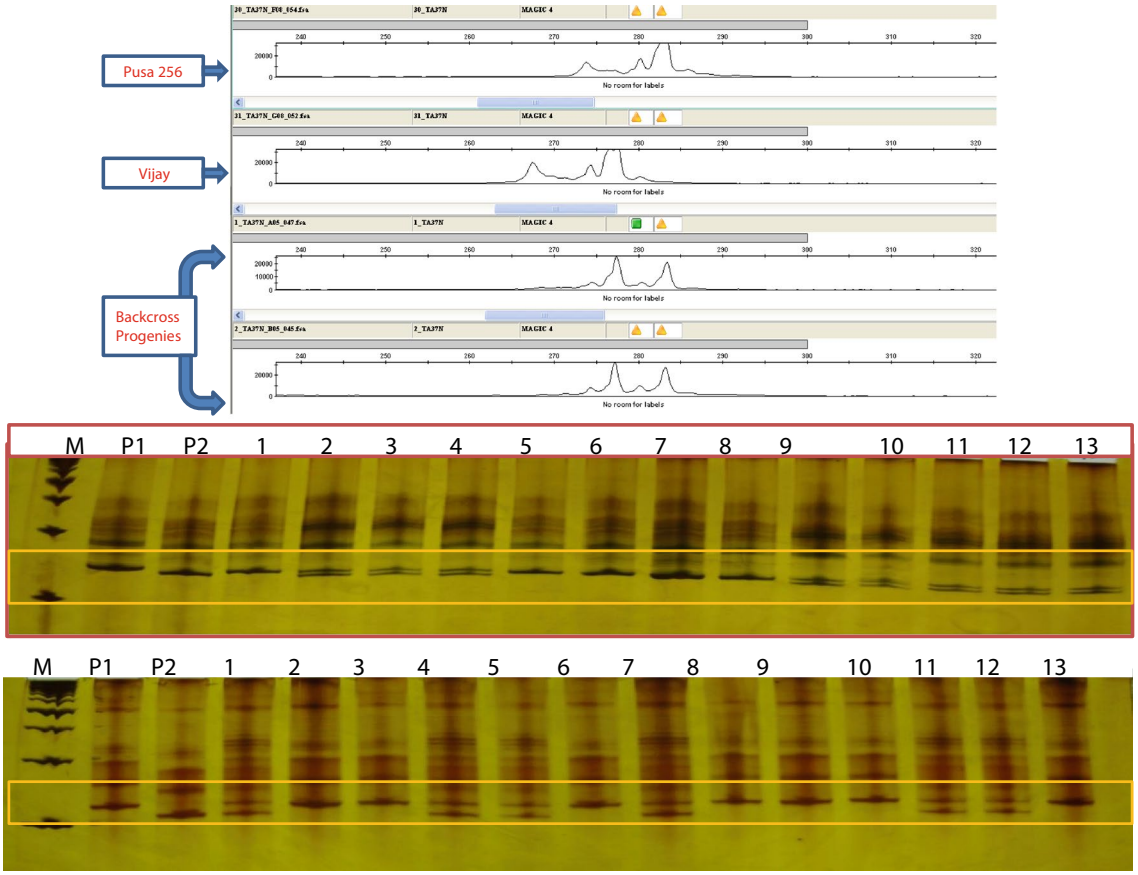

Fig. 2 **a** Foreground selection for resistance to FW (*foc 2*) in Pusa 256 × Vijay with marker TA37 showing heterozygous condition in BC_3F_1. **b** Background selection for resistance to FW (*foc 2*) in Pusa 256 × Vijay with markers TS 45 (*above*) and TA5 (*below*), M-100 bp Ladder, P1-Pusa 256, P2-Vijay, From *1* to *13*—backcross progenies

Fusarium wilt in chickpea is a monocyclic disease and the primary inoculum of the pathogen is a major driving force of spread of the disease (Jiménez-Díaz et al. 2015). Chemical control of this soil born pathogen is ineffective and has negative environmental footprints. Simultaneously, other disease management strategies such as site selection to avoid sowing in high risk soils, reduction or elimination of inoculum in the soil and seed treatment with fungicides or biocontrol agents is generally costly and is not always in the hands of farmers. In such a situation, use of resistant cultivars is the most effective and practical approach which must be promoted for efficient control of fusarium wilt. MABC approach involving foreground selection using tightly linked molecular markers from donor and background selection in the genetic background of recurrent parent genome using microsatellite markers (Varshney et al. 2013) provides breeders a practical tool to ensure precise transfer of the segment of interest in early generations besides helping in recovery of most of the recurrent parent genome in advanced backcross generations. Therefore, a plant breeder would prefer to exercise marker-assisted backcrossing (MABC) for development of superior cultivars with desired trait.

In view of above, Pusa 256, an elite cultivar of chickpea which has been highly popular among the farmers in Central and northern parts of India due to superior seed quality and high yield potential was targeted for improvement to resistance against fusarium wilt (race 2) employing MABC. Vijay, another elite cultivar of *desi* chickpea released in 1994 for Central Zone of India, being resistant to several *foc* races (races 1–5) with the target loci for resistance to *foc* 2 already mapped in it (Gowda et al. 2009) was chosen as the donor parent. While traditional backcrossing and selfing approach was used to advance the generations, foreground selection with linked SSR markers and background selection using genome-wide SSR markers was employed in each backcross generation to identify true plants for either crossing or selfing (Figs. 2 and 3). The F_1s were subjected to molecular analysis for identification of hybrids. This was useful as legumes being self-pollinated crops, have increased chances of selfing. Furthermore, differentiating between the selfed and F_1 plants is also difficult many

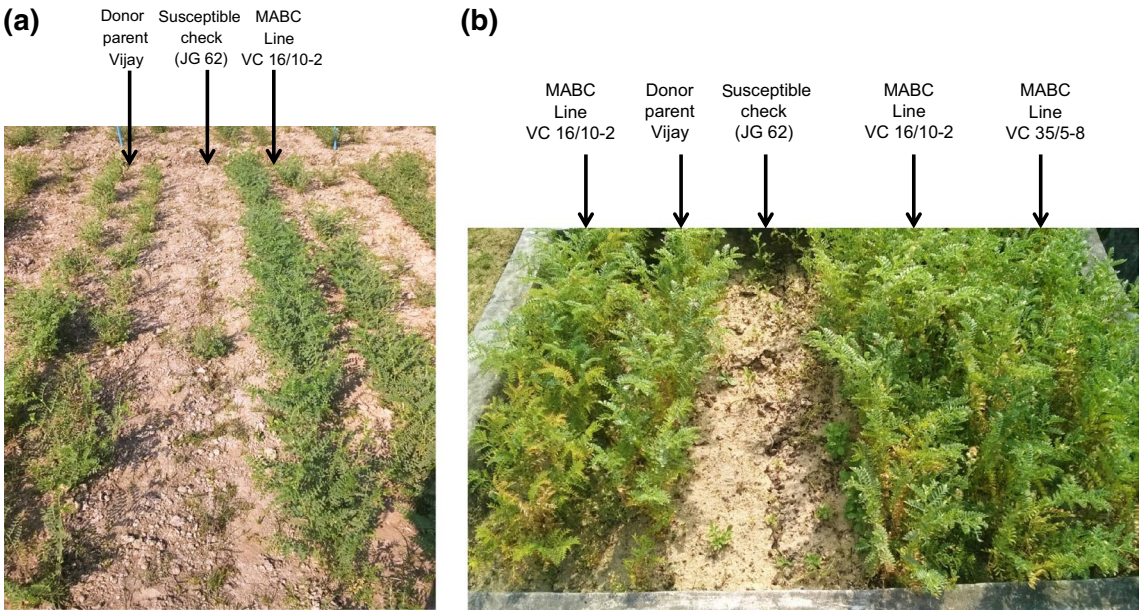

Fig. 3 Phenotypic screening of fusarium wilt-resistant marker-assisted backcrossed (MABC) lines along with parents in wilt sick nursery under natural field conditions (**a**) and wilt sick micro-plots (**b**). MABC lines showed high level of resistance to fusarium wilt while the susceptible check JG 62 was highly susceptible under both the conditions

times due to low phenological diversity between the selfed and crossed plants. Therefore, use of molecular markers has been advocated earlier also in identification of F_1s (Solanki et al. 2010).

To confirm the performance of introgressed lines in natural environment, field-level phenotyping of parental and MABC lines was done in wilt sick micro-plots as well as natural field conditions. It was interesting to note that ten advanced introgressed lines showed very high level of resistance (0–5.0%) against fusarium wilt in comparison to 30.4–41.9% disease incidence in Pusa 256 and 6.7–20.3% in Vijay. Genetics of resistance against specific races of *F. oxysporum* f. sp. *ciceri* has been extensively studied and is described as monogenic or oligogenic depending upon the race resistance source (Sharma et al. 2005; Sharma and Muehlbauer 2007). For race 2, Sharma et al. (2005) demonstrated that resistance to this race in artificial inoculation conditions was governed by a single recessive gene. Later, Sharma and Muehlbauer (2007) also demonstrated that genes *foc-01*, *-02*, *-2*, *-3*, *-4* and *-5* confer complete resistance to races 0, 2, 3, 4, and 5, respectively. This suggested that breeding for complete resistance to *Foc 2* is possible as has been demonstrated in this study also. Among highly resistant lines generated, five lines had shown complete resistance as no disease incidence was observed in these and therefore, these may be further evaluated and released as commercial cultivars in the country. Use of MABC for improvement of elite chickpea cultivars for disease resistance has been successfully demonstrated by Varshney et al. (2014). They transferred resistance to fusarium wilt Race 1 and Ascochyta blight in C 214 a *desi* chickpea cultivar in two parallel MABC programmes by targeting *Foc* 1 locus and two quantitative trait loci regions, ABQTL-1 and ABQTL-II. In both programmes FGS and BGS were

Table 3 Disease reactions of parental genotypes and BC_3F_3 and BC_3F_2 lines carrying *Foc 2* locus conferring resistance to race 2 of *F. oxysporum* f. sp. *ciceris*

Lines	FW incidence (%)	Disease reaction
Parental lines		
Pusa 256	30.4–41.9	Moderately susceptible
Vijay	6.7–20.3	Resistant
MABC lines		
VD 2/23	0	Highly resistant
VD 2/70	5.0	Highly resistant
VD 2/117	4.8	Highly resistant
VD2/133	4.0	Highly resistant
VD2/145	3.8	Highly resistant
VD2/161	4.7	Highly resistant
VC 16/10-2	0	Highly resistant
VC 16/10-9	0	Highly resistant
VC 35/5-4	0	Highly resistant
VC 35/5-8	0	Highly resistant

Scores as per Sharma et al. (2005). The plants were categorised as resistant (0–20%), moderately susceptible (21–50%) and susceptible (>50%)

employed to generate three resistant lines for Fusarium wilt and with 92.7–95.2% recurrent parent genome recovery and seven resistant lines for Ascochyta blight resistance with 81.7–85.4% recurrent parent genome recovery. In other leguminous crops, soybean is the best example where use of markers in breeding programmes has been most successfully demonstrated (for review see Pratap et al. 2012). In past several years, many improved varieties/lines for resistance to different Soybean cyst nematode (SCN) races (Arelli and Young 2009), phytophthora root rot and brown stem rot, insect resistance (Warrington et al. 2008); low linolenic acid content, yield (Concibido et al. 2003), mosaic virus resistance (Shi et al. 2009) have been developed. MAS has also been used successfully in common bean to develop several lines which are resistant to rust (Stavely, 2000; Faleiro et al. 2001), anthracnose (Alzate-Marin et al. 1999) and bean golden yellow mosaic virus (Miklas 2002). In peanut, markers linked with root knot nematode resistance were introgressed into cultivated background via amphidiploids pathway (Simpson et al. 2001).

In conclusion, this study demonstrated successful introgression of fusarium wilt resistance locus for race 2 from Vijay to a *desi* chickpea elite cultivar 'Pusa 256' through molecular marker-assisted breeding. As a result, five advanced breeding lines were developed which showed complete resistance to fusarium wilt and sufficient quantity of seeds is now available for these lines. These will now be subjected to multilocation field evaluation for disease resistance as well as yield performance under All India Coordinated Research Project (AICRP) for possible release of the most promising MABC lines as improved commercial cultivars. Further, these lines may also be used in recombination breeding through conventional breeding approaches to impart resistance to wilt susceptible chickpea genotypes. In addition, this study has opened up new avenues in our laboratory for marker aided pyramiding of multiple race resistance against fusarium wilt and other diseases as well as developing multiple stress resistant cultivars. One such experiment has already been initiated to transfer multiple race resistance against *Fusarium oxysporum* f.sp. *ciceris* (races 1–5) as well as introgress drought tolerance simultaneously in JG 16, another elite cultivar of chickpea. Success in the present study has also encouraged us to take molecular marker-assisted breeding as a routine tool in crop improvement programme in other crops like mungbean and blackgram.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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