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Marker-assisted Pyramiding Resistance Genes Against Angular Leaf Spot and Common Bacterial Blight Disease into Preferred Common Bean Cultivar ‘’REDWOLAITA”

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Abstract Angular leaf spot (ALS) caused by *Pseudocercospora griseola* and common bacterial blight (CBB) caused by *Xanthomonas campestris* pv *phaseoli X. campestris pv. phaseoli var. fuscans* are the most economically important diseases of common bean production in Ethiopia. This research aims at pyramiding the *Phg-2* R gene for angular leaf spot resistance and two CBB major resistance quantitative trait loci (RQTLs) into the background of the most popular and susceptible common bean cultivar “REDWOLAITA” (RW) with the aid of marker-assisted breeding method. Marker-assisted Parallel Back Crossing (MAPBC) breeding scheme with three separate parallel backcrossing streams were adopted for tracking three independent resistance loci linked to g796 (*Phg-2* for ALS resistance) and, SU91 and SAP6 genetic markers from two different donor parents to the REDWOLAITA recurrent parent. The two donor parental lines VAX6 (with known RQTLs tagged by the SAP6 and SU91 genetic markers on linkage groups 10 and 8, respectively) and MEX54 with the *Phg-2* R gene tagged by the g796 genetic marker at the linkage group 8 were used in the gene pyramiding program. After the BC4 generation, progenies that combined SAP6 and g796 genetic markers were created and selected from the BC4 inter-crossing of progenies. Then, further inter-crossing was made between selected progenies that combined the SAP6 and g796 genetic markers with selected progenies with the SU91 genetic marker. Finally, from this study we developed Monogenic Near Isogenic Lines (MNILs) with R genes tagged by the SAP6, g796, and SU91 molecular markers and polygenic PNILs with different gene combination includes MNIL^{SAP6}, MNIL^{SU91} & MNIL^{g796}, polygenetic PNILs $^{SAP6/g796}$, PNILs $^{SU91/g796}$, PNILs $^{SAP6/SU}$, PNILs $^{SAP6/g796/SAP6}$, with more than 97% genome recovered from the RW genetic background. Marker-assisted backcrossing facilitated selection of progenies that combined good agronomic traits with resistance loci were constructed from the RW common bean cultivar genetic background and tested under the screening house condition. The developed lines showed high level of disease resistance to the strains of CBB and ALS present under the screening conditions. They were selected to be multiplied and tested under multiple environment, before varietal release and wider production. Developed MNILs with good agronomic background will also be used as alternative donor parent for the future gene pyramiding program.

Keywords Gene pyramiding, Parallel backcrossing, RQTLs, inter-crossing, Isogenic lines

1 Introduction

Common bean (*Phaseolus vulgaris* L.) is the most important grain legume for direct human consumption and used as main food and /or food component in Latin America and eastern and southern Africa. Common bean is seed-propagated and a diploid (2n = 2x = 22) with a relatively small genome (650 Mb) (Broughton et al., 2003), originated in the Neotropics, with at least two major centers of domestication in Mesoamerica and the Andes (Gepts, 1988). Common bean is believed to have been introduced together with maize via the east coast of Africa by Portuguese and Spanish traders in the sixteenth and seventeenth century (Greenway 1945; Gentry, 1969). In Ethiopia, common bean is the principal food and nutrition security legume crop providing dietary protein and a source of cash income for resource-poor farmers. Among a number of factors that could attribute to their low yield,

diseases especially angular leaf spot (ALS) caused by *Pseudocercospora griseola* and common bacterial blight (CBB) caused by *Xanthomonas campestris* pv *phaseoli* cause the most significant harvest losses in common bean in farmer's field (Belete and Bastas, 2017). The impact of disease on crop production in Ethiopia and beyond may be worsening with the current and predicted climate change scenarios of rising temperatures and variability and changes in precipitation. These has been observed in disease incidence and severity of common beans in Ethiopia (Belete and Bastas, 2017).

Using host resistance has been proven to be the most effective and economical method to control disease in common bean and other crops. Therefore, to obtain a durable and broad-spectrum resistance variety, pyramiding multiple R genes/RQTLs into a recurrent common bean cultivar is an important and practicable breeding strategy to control angular leaf spot and common bacterial blight (de Mendonça et al., 2003). The backcrossing approach to deploy one or more genes into an elite line was proposed by Harlan and Pope (1992). Since then, backcrossing has become a widely used plant breeding approach in diverse crop species (Hasan et al., 2015; Hansan et al., 2016). This method is most commonly used to incorporate one or a few highly heritable traits into an adapted or elite variety. In most cases, the elite variety used for backcrossing has a large number of desirable attributes but is deficient in only a few characteristics. The other parent, called the 'donor parent', possesses one or more genes controlling an important trait, which is lacking in the elite variety (Hansan et al., 2016). Traditional backcrossing programs are designed on the assumption that the proportion of the recurrent parent genome is recovered at a rate of $1 - (1/2)^{t+1}$ for each of t generations of backcrossing. Thus, after four backcrosses, we expect to recover $1-(1/2)^5 = 96.9\%$ of the recurrent parent genome (Babu et al., 2004). However, any BC progeny individual will deviate from this expectation due to chance and to linkage between the gene from the donor parent being selected for and nearby genes (Muhammad et al., 2014).

Then, since the advent of molecular genetic markers, assisted backcrossing has been successfully applied in gene pyramiding programs for targeted transferring and pyramiding resistance loci to create more durable and broad specific resistance in different crops (Joshi and Nayak 2010). In the wheat cultivar "Yang", Liu et al. (2000) successfully combined three powdery mildew resistance gene combinations *pm2* + *pm4a, pm2+pm211*, and *pm4a+pm21* using restriction fragment length polymorphism (RFLP) markers. In soybean for mosaic virus disease resistance (SMV), researchers successfully pyramided three genes *Rsv1*, *Rsv3* and *Rsv4* with the aid of microsatellite markers in order to develop new soybean lines containing multiple resistance genes for soybean mosaic virus (SMV) resistance. Marker-assisted selection (MAS) and gene pyramiding have been reported in common bean research (Kelly et al., 2003, Miklas et al., 2006; Ragagnin et al., 2009); Ferreira et al., 2012; Kumar et al. 2017). Recently, Ddamulira et al, (2015) reported the efficiency and effectiveness of gene pyramiding in improving angular leaf spot resistance in susceptible common bean cultivar.

This specific research aims at cumulating the *phg-2* R gene for ALS and two major RQTL's for CBB resistance into the background of popular common bean cultivar 'REDWOLAITA' through the aid of molecular and conventional breeding techniques. The breeding strategy consisted of marker-assisted backcrossing (MABC) in transferring disease resistance in the adapted common bean cultivar 'REDWOLAITA', which belongs to the Mesoamerican gene pool.

2 Materials and Methods

2.1 Experimental location

The study was conducted in the Molecular Biotech Lab and screen house at the Southern Agricultural Research Institute (SARI), located 7° 4' N latitude and 38° 31' E longitude and an altitude of 1700 m.a.s.l in Hawassa, Ethiopia from 2015 to 2017. After the backcross pyramided progenies were generated, they were genotyped to identify those progenies with the required molecular markers; the progenies were also phenotyped for their performance and disease reaction.

2.2 Plant materials

The materials under study included three parents: 'REDWOLAITA' (RW) as the recurrent parent and VAX-6 and MEX 54 as sources of disease resistance (Table 1). REDWOLAITA (RW) common bean cultivar from the Mesoamerican gene pool was the most popular and widely grown for its colour and cooking quality and high preference by most of farmers was selected as a recurrent parent. This common bean cultivar, although, it was the most preferred by famers, for its good quality but the cultivar was the most susceptible to common bacterial blight caused by *Xanthomonas campestris* pv *phaseoli X. campestris pv. phaseoli var. fuscans* and angular leaf spot caused by *Pseudocercospora griseola* endemic to Ethiopia. Therefore, this common bean cultivar was selected as recurrent parent to be improved through marker-assisted gene pyramiding program. The other two parents including VAX-6 and MEX-54 with known sources of major sources of resistance QTL and *Phg-2* R gene for bacterial blight pathogen caused by *Xanthomonas campestris pv. phaseoli* and *X. campestris pv. phaseoli* var. *fuscans* and the angular leaf spot caused by *Pseudocercospora griseola* respectively for these disease endemics and widely distributed to common bean growing areas of Ethiopia were selected to be used as a donor parent in this specific study (Caixeta et al., 2005). For this study the best resistance donors and most reliable and polymorphic markers were used to deploy resistance into the background donor. The parents were tested for marker polymorphism and usefulness for MAS (Table 2). Crosses were made and advanced through the application of molecular markers. The resistance gene transfer was confirmed with aid molecular marker linked to the R gene /RQTL and through reliable screening techniques in screening house.

Table 1 Characteristics of Common Bean Parental Lines Which Were Used in Marker Assisted Gene Pyramiding Program (MABCP)

Parents used in MABCP	Gene pool	Seed size & colour	Growth Habit/type	Disease Reaction	
				ALS	CBB
REDWOLAITA	Mesoamerican	Small red	II	+	+
VAX-6	Mesoamerican	Small pale red	I	+	-
MEX-54	Mesoamerican	Small pink	IV	-	+

Note: CBB= common bacterial blight, ALS= angular leaf spot, += compatible(susceptible) disease reaction, - = incompatible (resistance) disease reaction

Table 2 DNA Marker Validation for selecting polymorphic markers to be used in the marker assisted gene pyramiding

DNA markers	Annealing T^0C	RW	VAX6	MEX54	Description of Marker
SAP6	58	-	+	-	[1]SCAR, Linked to CBB Resistance QTL
SU91	60	-	+	-	[2]SCAR, Linked to CBB Resistance QTL
g796	44	-	-	+	[3]STS, linked to *phg-2* ALS R gene
SN02		-	-	-	[4]STS, linked to *phg-2* ALS R gene
OPE4		-	-	+	[5]STS, linked to *phg-2* ALS R gene

Note: R= Resistance, SCAR=sequence characterized amplified region, STS= sequence tagged site (1 Miklas et al., 2000; 2 Pedraza et al., 2000; 3 Miller et al., 2018)

Molecular markers

Sequence Characterized Amplified Regions (SCAR) markers were used (Table 3) to tag angular leaf spot and common bacterial resistance genes of interest. The original oligonucleotide markers were obtained from Eurofins Genomics. A 50 / 100 bp mixed DNA molecular weight marker (Ladder) specifically designed for determining the size of double strand DNA from 25 to 300bp was used. The presence of $SU91_{700}$ (Pedraza et al., 1997) linked a resistance QTL located on B8, $SAP6_{820}$ (Miklas et al., 2000) linked to a resistance QTL on whereas the *Phg-2* resistance locus were B10, $g796_{220}$ (Miller et al., 2018) were determined using genetic markers.

2.3 Marker-assisted Selection

2.3.1 DNA extraction and amplifications

Genomic deoxyribonucleic acid (gDNA) was isolated using FTA card matrix technology following the manufacturer's procedure with minor modification from fresh leaves of 12-day-young plantlets. Common bean

progenies were sampled from each succeeding generation, i.e., BC1F1, BC2F1, BC3F1, and BC4F1, and including the progenies created through inter-crossings of BC4s and BC4F2. FTA is a paper-based technology, which was designed for the collection of nucleic acids, either in their purified form or within pressed samples of fresh tissue. Proprietary chemicals impregnated into the paper act to lyse cellular material and fix and preserve DNA within the fibre matrix. As described in the manufacturer's protocol with minor modification (www.gelifesciences.com/whatman) in which captured nucleic acids were ready for purification when taken with a punch from the FTA card, purification reagents were added, and the paper was rinsed with TE-1 (10mM Tris-HCl, 0.1mM EDTA, pH8) buffer. The DNA markers SAP6 (829bp), SU91 (700bp) and g796 (233bp) were used to select plants with linked resistance loci, which were then backcrossed to the recurrent parent. After washing the punched discs, the DNA was eluted and tested for its quality using agarose gel (0.98%) for use in PCR.

Table 3 Polymorphic molecular markers used in gene pyramiding and selection

Gene/Locus	Linked Molecular markers	Linkage group	Primer sequences	Expected Band Size/orientation	Reference
QTL	SAP6	10	F GTCACGTCTCCTTAATAGTA	806/cis	Milklas *et al.*, 2000
			R GTCACGTCTCAATAGGCAAA		
QTL	SU91	8	F CCACATCGGTTAACATGAGT	669/cis	Pedraza *et al.*, 1997
			R CCACATCGGTGTCAACGTGA		
Phg2	g796	8	F GAGAAACTACGGGCTGTTTTACCC	220	Miller *et al.*, 2018
			R AATTAAAACACCCACCCACTCCAT		
Phg2	SN02	8	F ACCAGGGGCATTATGAACAG	890/cis	Nietsche *et al.* 2000
			R ACCAGGGGCAACATACTATG		

Note: F=Forward, R= Reverse

2.3.2 Polymerase chain reaction (PCR)

Sequence characterized amplified region (SCAR) markers used in selection for CBB resistance were dominant and were scored as the presence or absence of a single band on an agarose gel. The INDEL marker used in ALS resistance selection was co-dominant. DNA amplification was performed under ABI 2720 Thermal cycler under the program for SU91, SAP6 and g796 were 34 cycles of 10s at 94 °C, 40s at 58 °C (for SAB6), 40s at 60^0C for SU91 &30s at 44^0C for g796, 2 min at 72 °C, and 5 min at 72 °C for the final extension (Table 3). PCR results were analyzed using a 1.4% agarose gel stained with ethidium bromide (0.02 μg·mL^{-1}). Bands present on the gel were compared by size to a 100-bp molecular marker.

2.3.3 Marker-assisted back crossing and gene pyramiding

The gene pyramiding and marker-assisted backcrossing breeding selection strategy used is illustrated in Figure 1. The marker-assisted backcrossing program was planned in such a way that three independent crossing streams were undertaken to track R/RQTLs loci tagged by SAB6, SU91 and g796 molecular markers on the linkage groups 10 and 08, respectively (Table 3). The resistance donor parents MEX54 with R gene (*Phg-2*) for ALS and VAX6 (with two RQTLs loci for CBB) were independently crossed with the recurrent parent 'REDWOLAITA' common bean cultivar under screening house conditions (Table 2).

The F_1 were crossed with the RP to produce the first backcross generation (BC_1F_1 or just BC_1). Markers closely linked to the resistance loci were then used to check targeted genes from each crossing streams of BC1F1 populations. Based on the plan, molecular markers, which included SAB6 in crossing stream one, g796 in crossing stream two, and SU91 in crossing stream three were used. Then, the succeeding backcross generations were made by crossing selected BC_1 F1plants (that had been screened for the targeted resistance trait (*Phg*-2 and CBB RQTL loci) from each crossing streams with the RP to produce the BC_2F1 populations. Subsequent

backcross populations were made by repeatedly crossing the selected backcross (BC) plants with the RP. That backcross progeny with the target trait were selected based on phenotype during each round of backcrossing. To further identify targeted homozygous plants at the backcross four (BC4) from each backcross streams selected plants were selfed to get targeted homozygous plants. Then, plants with homozygous genotypes for the targeted R genes were selected randomly from each segregating population. BC4F3 seeds were then harvested individually from each selected BC4F2 [RW/RW/VAX(+SAP6)], [RW/RW/MEX(+g796)] and [RW/RW/VAX(+SU91)] these created lines were monogenic near isogenic lines, constituting MNIL^{SAP6}, MNIL^{SU91} and MNIL^{g796} respectively.

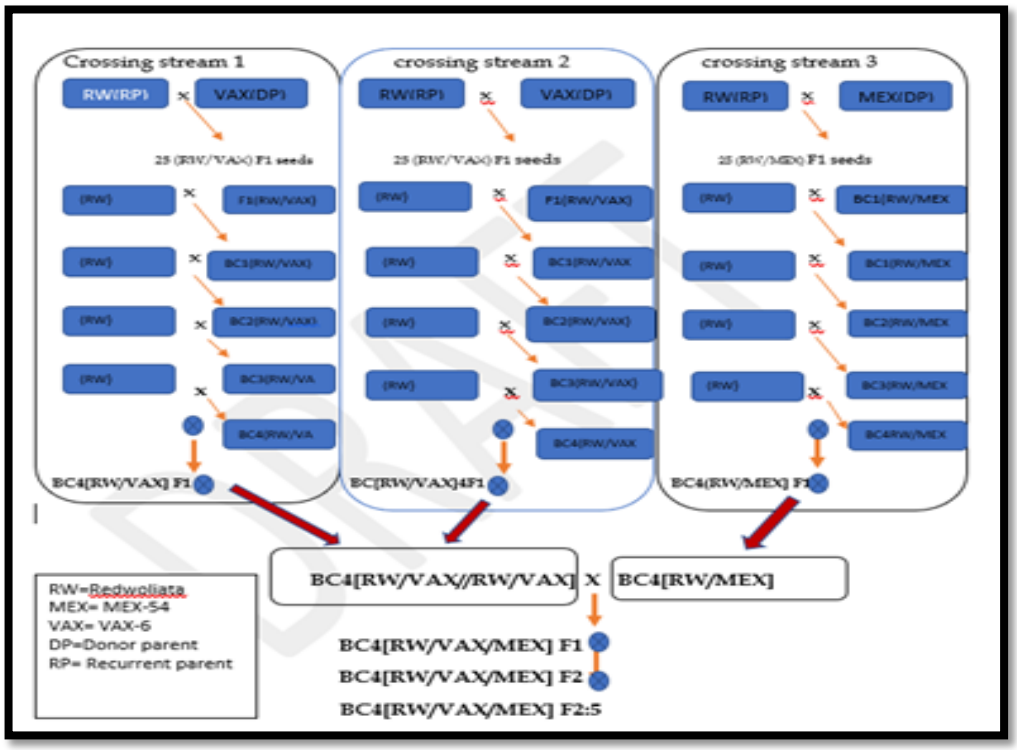

Figure 1 Breeding strategy used to develop pyramided common bean cultivar with angular leaf spot resistance and common bacterial blight disease resistance gene

Then intercrossing was made to further combine the resistance genes into a single background (Figure 1). The pyramid lines with different gene combinations were created combining SAP6 with g796 genes PNILs$^{SAP6/g796}$, PNILs $^{SAP6/SU91}$ combining genes SAP6 with SU91, PNILs $^{SAP6/g796}$ combining genes SAP6 with g796, and further crossing was made to create polygenic line with good agronomic background combining all of the R genes PNILs $^{SAP6/SU91/g796}$ polygenic lines that combined three R loci linked to SAP6, g796 & SU91 were developed with help of Marker-assisted Selection (MAS).

2.4 Selection of near isogenic and polygenic pyramided resistance lines

The isogenic and polygenic near isogenic pyramided lines were evaluated for their reaction to both common bean angular leaf spot and bacterial blight under the screening house using the most virulent pathogens collected from Ethiopia. Fourteen days old plants were inoculated with angular leaf spot suspension 10 x10^6 spore concentration and CBB which were virulent to common bean growing areas of Ethiopia. in this experiment evaluation for R genes and appropriate traits were based on the reaction of selected lines with virulent pathogen and morphological characteristics like the selection was based on the growth habit and the seed colour of the plant.

3 Results

Marker-assisted backcross breeding

Three independent and separate parallel back crossing schemes were adopted to track resistance loci from two donor parents. In our back-crossing program, three polymorphic DNA-based molecular markers were used during

marker-assisted parallel backcrossing (MABC) breeding program to deploy CBB and ALS resistance gene/QTLs into the farmer-preferred cultivar but susceptible bean variety REDWOLAITA. The molecular markers (SU 91, SAP6 and g796) allowed us to conduct early selection of bean lines with resistance to the fungal and bacterial pathogens (Figure 2; Figure 3; Figure 4).

Figure 2 Gel elctrophoresis showing amplification of loci linked to resistance loci a) g796 (233bp) for angular leaf spot resistance b) SU91 molecular marker linked to common bacterial blight resistance (669bp) & c) SAP6 molecular marker linked to resistance locus (809bp). The BC progenies with amplified regions linked to resiatnce loci were selcted and maintained for the next backcrossing

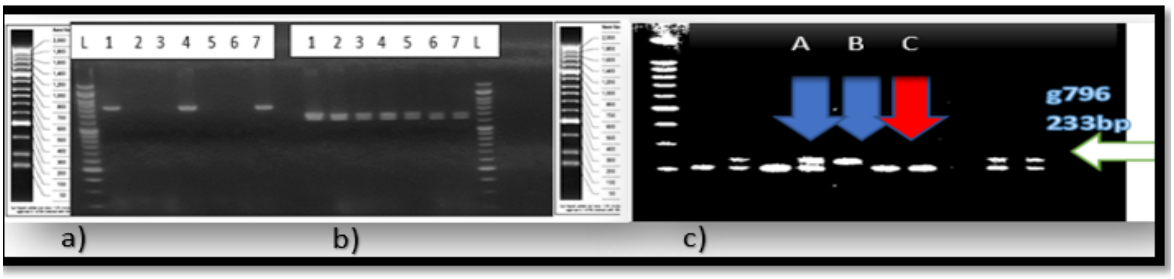

Figure 3 Polymerase chain reaction amplification (PCR) obtained using markers

Note: a) SAP6 809; b) SU91669 and identification and selection of BC progenies with loci linked to SAP6 and SU91 for major RQTL CBB resistance; Plants 1 ,4 & 7 have both SAP6 809 & SU91 669 genetic marker for common bacterial blight, whereas BC progenies; 1, 2, 3, 5 and 6 have only SU91 major RQTL marker; c) Gel amplification obtained by g796 molecular marker. BC bean progenies; A: B: C with different banding patterns, at 200bp & ~233bp: progeny; A: with co-dominant banding pattern indicating heterozygous resistance, progeny; B: with homozygous resistance, and progeny; C: with homozygous recessive susceptibility for g796 R locus

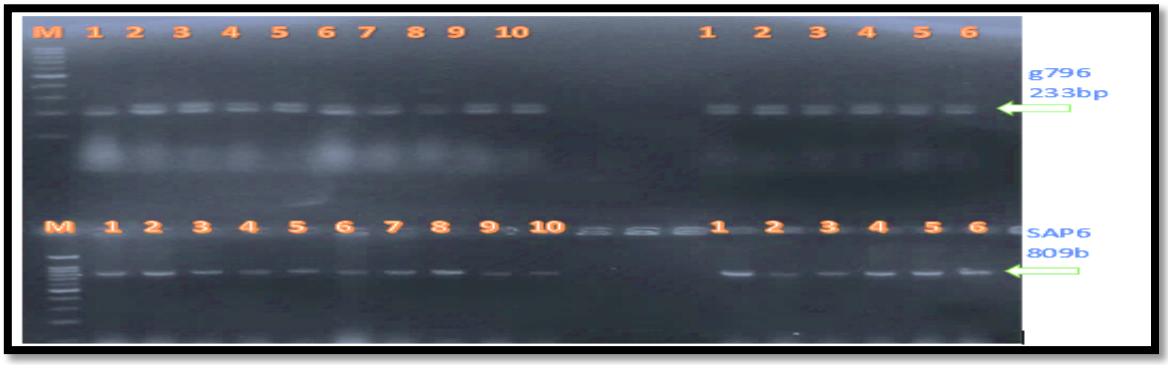

Figure 4 DNA amplification product obatined with molecular markers g796 (top band at 233bp) and SAP6 (lower band at 809bp). Lanes were the inter-cross backcrossing obtained progenies: all the progenies have both loci, hence, they were selected for further evaluation

There result revealed that the successful gene pyramiding of three R genes (*Phg-1* R gene for the angular leaf spot & two major RQTLs for CBB resistance) through DNA-based marker-assisted gene pyramiding into the popular and farmer-preferred but susceptible common bean cultivar “REDWOLAITA” (Table 4). Disease resistance

screening for the advanced lines with strains of ALS and CBB showed that single gene and poly gene pyramided lines with R genes showed effectively conferred resistance to both ALS and CBB strains (Table 5).

Table 4 Selected Progenies from the Successive Parallel Backcrossing Program in Each Generation Based on the Target Locus Linked to Molecular Marker

Generation	Progenies from Stream one MABC	Target (+SAP6)	Progenies from Stream two MABC	Target (+g796)	Progenies from Stream three MABC	Target +(SU91)
1	F1 [RW/VAX]		F1 [RW/MEX]		F1 [RW/VAX]	
2	BC1[RW/VAX] F1	3:24	BC1[RW/MEX] F1	7:24	BC1[RW/MEX] F1	2:24
3	BC2[RW/VAX] F1	5:19	BC2[RW/MEX] F1	7:20	BC2[RW/MEX] F1	11:15
4	BC3[RW/VAX] F1	5:12	BC3[RW/MEX] F1	3:7	BC3[RW/MEX] F1	5:12
5	BC4[RW/VAX] F1	5:12	BC4[RW/MEX] F1	8:17	BC4[RW/VAX] F1	5:10
	Inter-crossing isogenic lines			**Target**		
6	BC4[RW/VAX]/BC4[RW/MEX]			(+SAP6/+g796) 5/14 (2homoz, 3 heteroz)		
7	BC4[RW/VAX/MEX] F1 / BC4[RW/VAX] F1			(+SAP6/+g796/+SU91) 6:10 (g/SU/SAB) / 10:10 (g796+SAP)		
8	BC4[RW/VAX/MEX/VAX] F1			(+SAP6/+g796/+SU91)		
9	BC4[RW/VAX/MEX/VAX] F2:4			(+SAP6/+g796/+SU91)		

Note: MNILs=monogenic near isogenic lines, PNILs= polygenic near isogenic lines, R=Resistance, MR=Moderately resistance, SR=small red, SPR =small pal red, SP=small pink, HSW=hundred seed weight (gm), ALS= angular leaf spot, CBB=common bacterial blight, HSW=hundred seed weight (gm), S= susceptible

Table 5 Agronomic performance of pyramided MNILs, PNILs and parental lies under screen house

Progenies	Pedigree	ALS (1-9)	CBB (1-9)	Seed HSW (gm)	Seed colour
ETKT01	BC4 [RW/VAX(+SAP6)]	S	MR	25.7	SR
ETKT02	BC4 [RW/VAX (+SU91)]	S	MR	23.3	SR
ETKT03	BC4 [RW/MEX54(+g796)]	R	S	16.8	SR
ETKT04	BC4 [RW/VAX(+SAP6/+SU91)]	S	R	17.4	SR
ETKT05	BC4 [RW/VAX/MEX(+SAP6/+g796)]	R	MR	18.4	SR
ETKT06	BC4 [RW/VAX/MEX(+SU91/+g796)]	R	MR	11.4	SR
ETKT07	BC4[RW/VAX/MEX/VAX(+SAP6/+g796/+SU91)]	R	R	21.3	SR
P1	RW (RP)	S	S	18.8	SR
P2	MEX54 (DP)	R	S	28.2	SPP
P3	VAX6 (DP)	S	R	17.2	SR
	CV			3.3	
	LSD			1.12	

Note: MNILs=monogenic near isogenic lines, PNILs= polygenic near isogenic lines, R=Resistance, MR=Moderately resistance, SR=small red, SPR =small pal red, SP=small pink, HSW=hundred seed weight (gm), ALS= angular leaf spot, CBB=common bacterial blight, HSW=hundred seed weight (gm), S= susceptible

4 Discussion

4.1 Gene pyramiding and cultivar development

Among the pyramided polygenic NILS /pyramided lines, the lines that combined three R loci PNILs$^{SAP6/g796/SU91}$ performed best followed by pyramided lines PNILs$^{SAP6/g796}$, PNILs$^{SU91/g796}$, both with two disease resistance lines in terms of disease reaction, hundred seed weight (HSW) (gm) and seed colour. Among the developed near-isogenic pyramided polygenic line, the PNILs$^{SAP6/SU91}$ line with two R loci on chromosomes Pv08 and Pv10 for common bacterial blight, performed best under the CBB disease but showed susceptible reaction to angular leaf spot. Hence, this specific line with more than 76% with RW genetic background recovery and with good agronomic trait will be used for a future gene pyramiding program, which includes monogenic NILs, including NILs^{SAP6}, NILs^{SU91} & NILs^{g796}.

Angular leaf spot caused by *Pseudocercospora griseola* and common bacterial blight caused by *Xanthomonas campestris* pv *phaseoli* and *X. campestris pv. phaseoli var. fuscans* are the major destructive diseases of common bean (*Phaseolus vulgarise* L.) in Ethiopia. Pyramiding resistance genes/QTLs has been becoming an effective strategy to develop new variety with long-lasting and wide spectrum resistance. Marker-assisted selection (MAS) and gene pyramiding has been reported before in common bean research (Kelly *et al.,* 2003, Miklas *et al.,* 2006). Molecular markers linked to major angular leaf spot resistance loci (e.g., *Phg-2*) genes and common bacterial blight resistance QTLs have been widely reported (Namayanja et al., 2006; Miklas et al., 2006; Miller et al., 2018). The gene pyramiding approaches of this study complements that of Ragagnin et al. (2009) who succeeded using random amplified and polymorphic DNA (RAPD) and sequence-characterized amplified regions (SCAR) markers to pyramid resistance genes *Co-4, Co-6,* and *Co-10* against anthracnose, *Phg*-1 against angular leaf spot, and *Ur-ON* for rust into the susceptible 'carioca' market class cultivar Rudá. Ferreira et al. (2012) used SCAR, CAPS, and RAPD markers to successfully pyramid *Co-2, Co-3/9* anthracnose and *I* and *bc-3* common mosaic virus resistance genes into the 'fabada' market class A25 genotype. Ddamulira et al. (2015) reported the effectiveness of gene pyramiding in improving angular leaf spot resistance in susceptible common bean cultivar. Recently, Kumar et al. (2017) reported marker-assisted pyramiding of bacterial blight and gall midge resistance genes (*Gm4, Gm8, and Xa21*) into 'RPHR-1005' the restorer line of the popular rice hybrid 'DRRH-3' and the variety developed with cumulating thee genes were better yield and increased disease resistance trait.

This study also demonstrated that molecular markers can be used to successfully pyramid angular leaf spot and common bacterial blight resistance genes/QTLs into susceptible common bean varieties. This study introduced *Phg-2* and 2 RQTLs into REDWOLAITA resulting in monogenic and polygenic near isogenic lines (MNILs & PNILs) with different gene combinations for the resistance to CBB and ALS. The lines under screen house study showed significantly enhanced levels of resistance. Further inter-crossing and gene pyramiding was conducted in order to combine resistances. Pyramided NILs with R genes/RQTLs linked to SAP6, g796 & SU91 molecular markers including $MNIL^{SAP6}$, $MNIL^{SU91}$ and $MNIL^{g796}$ for the CBB and ALS disease resistance with good agronomic trait were constructed from the RW common bean cultivar genetic background and tested under screening house conditions. The resulted polygene-pyramided isogenic lines ($PNILs^{SAP6/SU91/g796}$) effectively conferred resistance to most frequently appeared pathotypes (63:59 and 19:33) of angular leaf spot and common bacterial blight pathogens that are endemic to Ethiopia. The developed pyramided lines with different gene combinations showed increased level of disease resistance compared to the parental lines. Those lines will be used for the future gene Pyramiding program

Monogenic Near Isogenic Lines (MNILs) with R genes linked to SAP6, g796 & SU91 molecular markers were developed. These include $MNIL^{SAP6}$, $MNIL^{SU91}$ & $MNIL^{g796}$ and polygenic $PNILs^{SAP6/g796}$, $PNILs^{SU91/g796}$, $PNILs^{SAP6/SU91}$, $PNILs^{SAP6/g796}$ and $PNILs^{SAP6/g796/SAP6}$, with more than 97% RW genetic background were created. The lines will be multiplied and tested under multiple environment and will be tested as a candidate variety for official varietal release.

In this particular study, we have developed seven resistance lines from the 'REDWOLAITA' common bean cultivar to both common bacterial blight and angular leaf spot diseases through marker-assisted gene pyramiding techniques (Table 5). Phenotypic background selection implemented during marker-assisted gene pyramiding accompanying molecular forward selection could be a reliable improvement strategy in the marker-assisted back cross breeding. Therefore, it could be suitable for less well-equipped breeding laboratories, as marker-mediated background selection which is costly strategy. As genetic resistance is an effective strategy for the famers to grow and reduce yield loss due to these economically important diseases and stabilize common bean production.

4.2 Conclusion and implication for the common bean improvement program

Common bean (*Phaseolus vulgaris* L.) production in Ethiopia is becoming the most and predominantly cultivated pulse crop. Although, it is traditional food and nutritional security crop, it is important as source of foreign

currency and cash as income for smallholder farmers is increasing whereas, productivity under farmer's field declining due to the frequent occurrence of the major bacterial and fungal disease. *P. griseola* with high pathogenic diversity and its seed born nature, it would be very important to change the common bean improvement strategy in Ethiopia to be able to breed for broad and durable resistance to the pathogen. Durable resistance based on the major genes has not been effective when resistance genes deployed one at a time. Therefore, cumulating complimentary resistance genes through marker-assisted gene pyramiding is a strategy that would confer a long-term resistance. In problems with multiple disease infection with different pathogens on common bean affecting its productivity and cause complete crop loss in susceptible varieties. PCR based molecular markers will be the key to success of MAS and gene pyramiding in common bean improvement. Therefore, gene pyramiding using marker-assisted breeding stagey and back crossing will provide a cost-effective controlling measure to bean diseases. The developed lines with the R genes could be evaluated under multi-location in the future to release best performing lines for the famers. The lines with good genetic background consisting R genes also will be used as parental lines in the future breeding program.

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