Research Paper

Evaluation of Selected Local and Exotic Common Bean Germplasm for Genetic Resistance to Rust Pathogen *Uromyces appendiculatus* (Pers.)

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Abstract

Common bean rust caused by *Uromyces appendiculatus* is one of the major biotic constraints in the production of common bean (*Phaseolus vulgaris* L.) in Sri Lanka. The present study was conducted to identify potential sources for rust resistance by phenotypical and molecular screening of 20 local and exotic common bean germplasm lines. Six characterized rust pathogen races (15-3, 22-6, 31-1, 31-7, 5-0, and 22-52) were obtained from ARS-USDA and four rust spore samples (63-21, 23-5, 31-1, 31-11) collected from different bean growing areas in Sri Lanka were screened against selected germplasm in a protected house. In addition to phenotypic screening, Sequences Characterized Amplified Region (SCAR) markers SK 14, SI 19, and GT-2 closely associated with *Ur-3, Ur-5* and *Ur-11* were used for molecular analysis. Local bean varieties, Bandarawela green, Bandarawela Kalu (Capri type 1), and Galpalama Kalu (Capri type 2) showed resistance to rust races 15-3, 22-6, and two local isolates of rust pathogen, based on phenotypic screening. All tested local varieties were susceptible to local rust race 31-11. Molecular markers tested with local genotypes revealed none of the tested local genotypes had *Ur-3, Ur-5*, or *Ur-11* genes. Tested SCAR markers were reproducible with germplasm including relevant resistance genes, revealing their specificity. Bean germplasm enriched with *Ur-3*, *Ur-5*, and *Ur-11* resistance genes were identified as the much-needed resistant genes for bean rust disease resistance and pyramiding of those genes is important in bean crop improvement in Sri Lanka.

Keywords: Bean germplasm, Bean rust, Molecular markers, Resistant genes

Introduction

Grown in many parts of the world for its edible green pods (snap beans) or dry beans, the Common bean (*Phaseolus vulgaris* L.) is considered glaobally as one of the major pulse crops (Smith and Goenaga, 2005; Konkolongo, 2018). Snap or green bean is one of the most important leading vegetable food legumes in Sri Lanka. Snap beans have many nutritional benefits as an excellent source of protein, vitamins, dietary fiber, and minerals (Blair *et al*., 2007). In Sri Lanka, snap bean is cultivated over an area of about 7000 hectares with an annual production of 85,000 metric tons and a yield of about 11.5 mt ha^{-1} (Agstat, 2020). Among the biotic stresses, diseases contribute significantly to lowering the genetic potential of improved varieties of beans (Pastor-Corrales, 2006).

Rust caused by *Uromyces appendiculatus* (Pers.) Unger is one of the most devastating foliar diseases affecting dry and snap beans worldwide, and could cause yield losses ranging from 25 to 100% in susceptible cultivars especially in areas that experience high relative humidity and moderate temperatures (Pastor-Corrales *et al*., 2007). Rust infection is favored by temperatures between 17-27 °C and relative humidity around 95 % for 10-18 hr day^{-1} (Singh and Schwartz, 2010). The causative agent of rust (*U. appendiculatus*) has been reported as having abundant diversity of virulence (Miklas *et al*., 2002) with 90 documented races. Rust fungi isolated from many different bean growing areas in the world are maintained and stored at Beltsville, USA since 1980 by J.R. Stavely (Miklas *et al*., 2002). Differential cultivars with uniquely assigned binary values have been used to designate *U. appendiculatus* races (Pastor-Corrales *et al*., 2007). Rust resistance in the common bean is conditioned by single dominant genes, and ten such genes have been identified and mapped worldwide. Resistant genes are identified by the *Ur* symbol (Pastor-Corrales *et al*., 2007) and reported with five well-characterized genes (*Ur-3, Ur-5, Ur-7, Ur-11*, and *Ur-14*) belonging to the Middle American gene pool and another five genes (*Ur-4, Ur-6, Ur-9, Ur-12,* and *Ur-13*) belonging to the Andean gene pool (Miklas *et al*., 2002). Many of the *Ur* genes have been used extensively as the source for developing rust resistance (Pastor-Corrales *et al*., 2007). Molecular markers tightly linked to rust-resistant genes, including the Sequence Characterized Amplified Regions (SCAR), Random Amplified Polymorphic DNA (RAPD), and Single Nucleotide Polymorphic (SNP) markers, have been reported (Hurtado-Gonzales *et al*., 2017). These markers offer new opportunities for identifying genes for resistance and have been used successfully in common bean breeding programs (Miklas *et al*., 2002; Souza *et al*., 2007).

Field experiments and surveys have ranked bean rust as a major disease in snap beans in Sri Lanka (Abeysinghe, 2009; Ariyarathne and Nuwan, 2001). Farmers in Sri Lanka mainly rely on fungicides and many cultural practices to reduce disease pressure. Developing host plant resistance is the most economical and environmentally sustainable method for controlling the rust

disease in beans (Souza *et al*., 2007). The successful development of rust-resistant cultivars depends on the understanding of the levels of variability in the pathogen and the host.

The presence of these rust resistance genes can be tested phenotypically in the greenhouse and with laboratory screening techniques that involve plant evaluation for rust symptom development after inoculation with different races of the pathogen (Pastor-Corrales, 2001). Four specific races of the bean rust pathogen have been reported as phenotypic markers that effectively identify the presence of rust resistance genes; race 31-1 (53) identifies *Ur-3*, race 22-6 (49) recognizes *Ur-4*, race 15-3 (47) identifies *Ur-6* and race 31-7 (67) recognizes *Ur-11* (Pastor-Corrales, 2006; Souza *et al*., 2007). These markers have been used by many researchers for the initial identification of the presence of widely used resistance genes alone or in combination with other genes (Hurtado-Gonzales *et al*., 2017). Genetic markers also help correctly identify the presence of rust-resistant genes in the germplasm accessions. Crop improvement programs mainly focus on the identification of resistant genes for major diseases and agronomically or horticulturally important traits.

This study includes a screening of selected bean germplasm against standard rust races, determining the prevalent rust races found in major cultivation areas in Sri Lanka, and identifying potential germplasm accessions to be used as donors for future breeding programs. Selected germplasm accessions used in the study are from diverse origins, and included recommended locally cultivated snap bean varieties, differential cultivars, conserved germplasm, and improved foreign varieties to facilitate the identification of applicable genetic resources for rust resistance. Phenotypic screening method and molecular markers were used to identify the availability of genes conferring resistance while evaluating the total germplasm for morphological similarities and differences.

Materials and Methods

Plant material

A total of 20 germplasm accessions were screened to identify sources of rust resistant-genes. These included local bean germplasm which have not been previously tested for the avialbility of rust-resistant genes, and foreign snap bean cultivars Table 1).

Local germplasm lines included five recommended local varieties of snap bean, two popular farmer cultivars obtained from farmers at Ragala and Walimada, and five conserved germplasms from the Plant Genetic Resources Centre in Sri Lanka. Foreign germplasm included two improved USDA (United States Department of Agriculture) varieties and the rust differential series varieties that were obtained from the USDA, Maryland, in collaboration with Michigan State University, USA. The Improved USDA varieties and differential cultivars were used as check varieties in the study because their gene pools and resistance genes have been documented (Miklas *et al*., 2002).

Table 1. Germplasm used for resistance evaluation

Source	Germplasm	Specificity
Local varieties***	Balangoda Nil	Recommended by the Department of Agriculture
	Bandarawela Green	
	HORDI Green***	
	Keppetipola Nil***	
	Gannoruwa bil	
Loal farmer varieties****	Bandarawela Kalu (Capri type 1)	Farmer selections
	Galpalama Kalu (Capri type 2)	
PGRC accessions	Acc No 2912	Local collections
	Acc No 2915	
	Acc No.5196	
	Acc No 7817	
	Acc No 7971	
USDA variety	Pinto 114	No Rust resistant genes
Differential series varieties obtained from USDA*	Aurora	*Ur-3*
	Early Gallatín	*Ur-4*
	Mexico 309	*Ur-5*
	G.G.W.	*Ur-6*
	PI 181996	*Ur-11*
Improved varieties by USDA	BARC-RR-25	*Ur-4, Ur-5*
	BelTen-RR-1	*Ur-4, Ur-11*

* *Differential series varieties received from USDA, USA*
** *Germplasm from Plant Genetic Resources Centre, Sri Lanka*
*** *Recommended varieties to cultivate by Department of Agriculture, Sri Lanka*
**** *Farmer varieties in Sri Lanka*

The initial test for identifying rust-resistant genes in selected local bean germplasm

Controlled environment experiments were adopted considering other factors may affect results if screening was conducted in an open field. The standard disease inoculation procedure was applied as described by Stavely *et al*., 1983. A total of 15 bean germplasm lines, including five local recommended pole bean varieties (*i.e.* Balangoda nil, Bandarawela green, Gannoruwa bil, HORDI green, and Kappetipola nil), two popular farmer cultivars (*i.e*. Bandarawela Kalu, Galpalama Kalu) from Sri Lanka, two improved USDA snap bean varieties (*i.e.* BARC-RR-25 and BelTen-RR-1) and four rust differential series varieties (*i.e.* Aurora, Early Gallatín, Mexico 309, G.G.W , PI 181996) were used to analyze the reaction. Variety Pinto 114 with no rust resistant gene was uaed as susceptible check varierty.

This experiment was carried out at the Soybean Genomics and Improvement Laboratory, ARS-USDA, Beltsville, MD, the USA, from October to December 2017 under

glasshouse conditions. The experiment included 20 plants from each variety. Frozen (-80 °C) uredospore samples of selected *U. appendiculatus* races (*i.e.* 15-3, 22-6, 31-1, 31-7, 5-0, and 22-52) were obtained from stored characterized rust spore collection at pathology laboratory ARS-USDA. They were separately dissolved in 250 ml of tap water containing 0.01 % Tween-20. The spore suspensions were adjusted to 2 x 10^4 uredospores per ml. Prepared rust isolate suspensions were applied to the abaxial surface of the ten-day old bean plant leaves using a cotton bud. Inoculated plants were incubated in a moist chamber (20 ± 1°C, RH 80-95%) for 18 hrs, under dark and plants were maintained until symptoms developed in a glasshouse at USDA-ARS, Maryland, Beltsville. Rust disease occurrence of the seedling and reactions of the bean cultivars to all six rust pathogen races were examined 12-14 days after inoculation. The inoculated plants were individually rated for rust severity using the scale described by Steadman *et al*. (2002). The cultivars that had a score of three degrees or lower were classified as resistant, whereas those with the scores of four degrees or higher were considered as susceptible (Table 2).

Screening Common bean accessions for rust resistance using a collection of local races of *Uromyces appendiculatus*

This experiment was carried out at Horticultural Crops Research and Development Institute, Gannoruwa, Sri Lanka. Five locally recommended common bean varieties (*i.e.* Balangoda nil, Bandarawela green, Gannoruwa bil, HORDI green, and Kappetipola nil), five local common bean germplasm accessions from PGRC (as in Table 1), two farmer grown varieties (*i.e,* Bandarawela Kalu, Galpalama Kalu), and four exotic varieties (BARC-RR-25, BelTen-RR-1, Aurora and Mexico 235) with known resistant genes were used for this experiment. Ten days after planting, the first two leaves of the plants were inoculated with spore suspensions of the four rust spore samples collected from the major bean growing areas in Sri Lanka.

Table 2. Standard bean rust grading scale for rust pathogen (*Uromyces appendiculatus*).

Reaction grade	Definition
1	Immune, no visible symptoms.
2	Necrotic spots of different sizes and without sporulation. This type of reaction is known as the hypersensitive reaction (HR).
3	Tiny, small, sporulating pustules (uredinia) less than 0.3 mm in diameter. The reaction is considered as resistant.
4	Uredinia (Sporulating pustules), 0.3-0.5 mm in diameter. The reaction is considered as susceptible.
5	Uredinia 0.5-0.8 mm in diameter. The reaction is considered as susceptible.
6	Uredinia are larger than 0.8 mm in diameter. The reaction is considered as susceptible.

Steadman *et al*. 2002

The four rust isolates used in the experiment were identified using 57 rust isolates colleted from Badulla, Nuwara eliya, Kandy and Matale districts (Publication pending), the major bean growing areas in the country. Inoculated plants were incubated in a moist chamber (20 ± 1°C , RH 80-95%) for 18 hrs, under dark and plants were maintained in a protected house until symptoms developed.

The experiment consisted of 60 potted plants of each variety with three replicates. The experiment was repeated twice to confirm the data. The rust pathogen reaction data were collected 12- 14 days after inoculation. The inoculated plants were individually rated for rust severity using the standard scale in Table 2 and Table 3. Standard reaction type values were converted to a quantitative disease score for the data analysis. Data was analyzed using SAS 9.1 package. Any cultivar or line that had an average disease severity rating above 3.1 (Table 3) in any of the tests against a specific race was considered susceptible.

Molecular Screening for genetic resistance

Genetic markers linked to *Ur-3, Ur-5,* and *Ur-11* loci were used in this study to test the presence of these specific loci. Cultivar Aurora, Mexico 235 and PI 181996 were used as positive controls for markers associated with *Ur-3*, *Ur-5*, and *Ur-11* genes, respectively. Three SCAR markers, SK 14 for *Ur-3*, SI 19 for *Ur-5*, and *Ur 11*-GT 2 for *Ur-11*, were used to identify the presence of relevant genes and marker validation (Table 4).

Table 3. Grading scale adopted for *U. appendiculatus* survey and their corresponding quantitative disease score

Reaction type*	Description	Quantitative disease score**	Rust Reaction
1	Immune, no visible symptoms	1.1	Resistant
2	Necrotic spots without sporulation	2.1	Resistant
2,3	Reaction 2 with few type 3	2.4	Resistant
3,2	Reaction 3 with few type 2	2.7	Resistant
3	Uredinia <0.3 mm in diameter	3.1	Resistant
3,4	Reaction 3 with few type 4	3.4	Susceptible
4,3	Reaction 4 with few type 3	3.7	Susceptible
4	Uredinia 0.3 to 0.49 mm in diameter	4.1	Susceptible
4,5	Reaction 4 with few type 5	4.4	Susceptible
5,4	Reaction 5 with few type 4	4.7	Susceptible
5	Uredinia 0.5- 0.8 mm diameter	5.1	Susceptible
5,6	Reaction 5 with few type 6	5.4	Susceptible
6,5	Reaction 6 with few type 5	5.7	Susceptible
6	Uredinia 0.8- 1.2 mm in diameter	6.1	Susceptible

**According to Stavely et al. (1983)*
***According to Mmbaga et al. (1996)*

DNA was extracted from young leaves of the plants following the CTAB DNA extraction protocol (Souza *et al*., 2014). Fresh leaf tissue (5 g) were used for the DNA extraction. The PCR reaction was performed using an amplification reaction mixture of a final volume of 25 µl containing 30 ng of genomic DNA, 0.1 mM dNTPs, 2.8 mM $MgCl_2$, 10 mM/50 mM Tris HCl (pH 8.3) and 0.2 mM of each primer.

Annealing temperatures were adjusted, as shown in Table 4 for different primers. Each amplification in the PCR reaction consisted of an initial denaturation step at 94°C for 3 min, 34 cycles at 94°C for 1 min, 60 or 67 °C for 1 min 30 s, 72°C for 1 min 30s, and 72°C for 1 min 30s. The amplified products were fractionated in a 1.5% agarose gel containing Ethidium Bromide (0.2 mg mL^{-1}) immersed in the TBE buffer (90 nm Tris-borate, 1 mM EDTA, pH 8) at 100 V for 1.5 hrs. and documentation was done.

Results and Discussion

Virulence of *Uromyces appendiculatus* races on selected bean genotypes

The evaluated snap bean genotypes showed different virulence reactions to the six *U. appendiculatus* races used in the initial characterization study (Table 5 and 6). Races 31-7 and 22-52 were the most virulent across all the genotypes while races 22-6 and 5-0 were the least virulent races in the initial test. Among the tested bean cultivars, Kappetipola nil, Balangoda nil, Gannoruwa green, Gannoruwa bil were the most susceptible cultivars as it was infected by 100% of the races.

Table 4. Selected DNA markers linked to the *Ur-3* and *Ur-11* genes conferring resistance to the bean rust pathogen *Uromyces appendiculatus*

SCAR Marker	Tagged locus	Primer sequence	Chromosome	Distance	Product size (Bp)	T °C
SK 14	*Ur- 3*	F-CCC GTC ACA CAC CAA TAC CTG	Pv 11	1.0 cM (Coupling/ Cis)	620	60
		R-CCC GCT ACA CTT GAT AAA ATG TTA G				
SI 19	*Ur- 5*	F-AAT GCG GGA GAT ATT AAA AGG AAA G	Pv 4	3.31 cM Coupling/ Cis	460	67
		R-AAT GCG GGA GTT CAA TAG AAA AAC C				
UR11-GT-2	*Ur- 11*	F-CGC ACT TAG GAG CAC AAA	Pv11	0 & 5.4 cM (Coupling/ Cis)	450	60
		R-TGG TGG GTC CCA TAT TTT G				

The USDA breeding line Belten RR -1 was the most resistant variety and it showed resistance to all the tested races in the 2-3 grading scale. Differential series varieties Aurora, Early Gallatin, Mexico 309, PI 181996 and tested improved USDA lines showed reaction grades with selected known races as expected.

In the initial test, local varieties were inoculated with four races of the rust pathogen (*i.e*. 15-3, 22-6, 31-1, and 31-7) to evaluate the presence of resistant genes *Ur-6*, *Ur-4*, *Ur-3*, and *Ur-11* as explained by previous research reports (Hurtado-Gonzales *et al*., 2017).

Results obtained with phenotypic data revealed that all the tested local common bean varieties did not show resistance to race 31-7, indicating that any of the local varieties do not have the *Ur-11* gene. However, three varieties (*i.e*. Bandarawela green, Bandarawela Kalu, and Galpalama Kalu) showed resistance to races 15-3, 22-6, and 31-1, but their reactions were small pustule (with sporulation) type reactions and not HR (2, 2+) (Table 5). This observation suggested that these varieties may not have *Ur-3*, *Ur-4*, or *Ur-6* genes.

However, Bandarawela green showed the susceptible reaction to race 22-52, confirming the unavailability of the *Ur-3* gene in this bean variety as explained by Pastor Corrales, 2001. Provided all three varieties originated from the same bean growing area, they may have the same gene or genes for rust resistance. Based on the reaction to the rust races, these varieties may not have *Ur-5* gene because they did not show a susceptible reaction to race 22-6 (Table 5). As such, it could be a new gene or genes and the elucidation of this gene or genes requires crosses and further studies.

As the resistant reactions to local rust races, Bandaraewela green and Galpalama Kalu showed resistance to race 63-21 and 31-1 respectively. Bandarawela Kalu showed resistance to races 23-5 and 31-1. All the tested local varieties were susceptible to race 4 31-11), and it is revealed that there are no locally grown bean varieties that show multiple resistance to all locally identified rust races (Table 5 and 6).

Therefore, it is necessary to incorporate additional genes to improve rust resistance in locally cultivated varieties. Bandarawela green, Bandarawela Kalu, and Galpalama Kalu are popular varieties among farmers, and these varieties could be improved by pyramiding *Ur-11* or *Ur-3* and *Ur-5* rust-resistant genes. Results revealed that differential cultivars carrying these genes were more resistant. For example, the variety PI 181996 with *Ur-11* gene showed resistance to all four pathogen races identified in Sri Lanka.

The *Ur-11* locus is the most utilized in common beans for developing rust resistance, because the *Ur -11* alleles has been shown to provide broadest-based resistance compared to the other resistance alleles (Pastor-Corrales 2006). Resistant gene *Ur*-3 has been identified as one of the most effective genes which can be used to control 52 races of *U*. *appendiculatus* (Souza *et al*., 2014; Hurtado-Gonzales *et al*., 2017.). However, the combination of

two or more genes out of *Ur-3*, *Ur -4*, *Ur-5*, and *Ur-11* would be most effective in providing the broadest resistance to *U. appendiculatus* races (Table 7). Further, the study employed a better, simple, and rapid screening method to ensure the efficient selection of resistant materials. The SCAR markers tightly linked to rust resistance genes offer another means to detect specific genes and for marker-assisted selection. Results of this study presents important findings to breeding programs aiming at developing common bean cultivars with durable rust resistance with *Ur-3*, *Ur-5* and *Ur-11*, which are other Middle American originated genes previously reported as a critical component of gene pyramiding of common bean cultivars to obtain broad resistance to rust (Miklas *et al*., 2002).

Improved USDA lines also produced bands that only corresponded with the resistance genes.

Table 5. The reaction (Mean quantitative disease score) of the selected common bean cultivars to bean rust races 15-3, 22-6, 31-1, 31-7, 5-0 and 22-52 of *U. appendiculatus.*

Bean variety	Rust Resistant gene (RR)	Mean Quantitative Disease Score based on reaction type *U. appendiculatus* race					
		15-3	22-6	31-1	31-7	5-0	22-52
Pinto 114*	No RR genes	4.4	4.4	4.4	4.4	3.4	5.4
Aurora**	*Ur-3*	4.5	5.4	2.4	4.5	2.1	2.1
Early Gallatín**	*Ur-4*	4.5	2.1	4.4	4.7	6.1	2.2
Mexico 309**	*Ur-5*	3.2	5.7	3.2	5.4	2.1	5.7
G.G.W.**	*Ur-6*	2.2	4.6	4.4	4.5	2.1	5.4
PI 181996**	*Ur-11*	2.1	2.1	2.1	2.4	2.2	5.6
BARC-RR-25*	*Ur-4, Ur-5*	3.2	2.2	3.2	4.5	2.1	2.1
BelTen-RR-1*	*Ur-4, Ur-11*	2.1	2.1	2.3	2.4	2.1	2.2
Balangoda Nil***	*UN*	4.5	4.5	4.5	4.5	3.2	4.5
Bandarawela Green***	*UN*	3.2	3.1	3.2	4.5	2.4	4.5
Bandarawela Kalu (Capri type 1)****	*UN*	3.2	3.1	3.1	4.5	4.5	4.5
Galpalama Kalu (Capri type 2)****	*UN*	3.2	3.1	3.2	4.5	4.7	4.5
Gnnoruwa Bil***	*UN*	4.5	4.4	4.5	4.6	4.5	4.4
HORDI Green***	*UN*	4..5	4.6	4.7	4.5	4.7	4.7
Keppetipola Nil***	*UN*	4.5	4.7	4.4	4.5	4.5	4.5

UN Unknown
* *USDA varieties*
** *Differential series variety*
**** Recommended varieties in Sri Lanka*
**** *Farmer grown cultivars in Sri Lanka*

Table 6. The reaction of the selected common bean cultivars to 4 rust isolates of *U. appendiculatus*

Bean Variety	Rust-resistant gene	Quantitative Disease Score			
		63-21	**23-5**	**31-1**	**31-11**
Aurora*	*Ur-3*	1.1^{c}	2.1^{b}	2.1^{bc}	5.1^{a}
Mexico 235*	*Ur-3+*	2.1^{c}	1.1^{b}	2.0^{bc}	4.1^{a}
PI 181996*	*Ur-11*	2.1^{c}	2.1^{b}	1.1^{c}	1.1^{c}
Acc No 2912**	Unknown	4.5^{a}	5.1^{a}	5.4^{a}	5.1^{a}
Acc No 2915**	Unknown	2.1^{c}	5.4^{a}	$3..1^{b}$	6.1^{a}
Acc No.5196**	Unknown	3.1^{ab}	5.4^{a}	4.4^{a}	5.1^{a}
Acc No 7817**	Unknown	3.1^{ab}	4.4^{a}	4.1^{a}	4.7^{ab}
Acc No 7971**	Unknown	4.1^{a}	3.4^{a}	5.1^{a}	4.4^{ab}
Balangoda Nil***	No gene	5.7^{a}	5.4^{a}	5.4^{a}	4.4^{ab}
Bandarawela Green***	Unknown	3.1^{ab}	4.1^{a}	3.2^{b}	5.1^{a}
Bandarawela Kalu****	Unknown	3.5^{ab}	3.1^{ab}	3.1^{b}	5.4^{a}
Galpalama Kalu****	Unknown	2.1^{c}	4.1^{a}	2.4^{bc}	4.1^{ab}
Gnnoruwa Bil***	No gene	5.4^{a}	4.4^{a}	5.1^{a}	5.1^{a}
HORDI Green***	No gene	5.1^{a}	5.7^{a}	4.1^{a}	5.1^{a}
Keppetipola Nil***	No gene	3.1^{ab}	4.1^{a}	5.1^{a}	4.1^{ab}
CV %		3.4	5.2	4.8	4.2

Average values with the same letter in a column are not significantly different at the 0.05 probability level.
**Differential series varieties received from USDA, USA*
*** Germplasm from Plant Genetic Resources Centre, Sri Lanka*
**** Recommended varieties to cultivate by Department of Agriculture, Sri Lanka*
***** Farmer varieties in Sri Lanka*

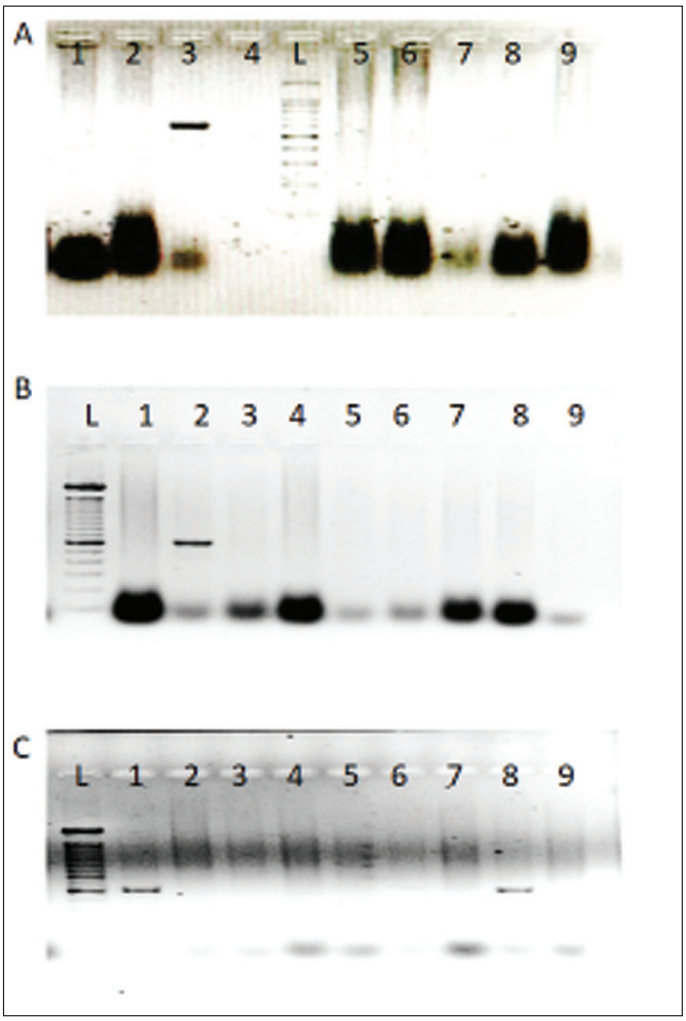

Figure 1. Amplification of bean germplasm DNA using SCAR marker, (A) SK 14 for gene *UR-3*, (B) GT-2 for *Ur-11*, (C) SI 19 for *Ur-5*

A,B,C Lane description; L 1 kb DNA Ladder (1) Mexico 309, (2) PI 181996, (3) Aurora, (4) Bandarawela green, (5) Kappetipola nil, (6) Gannoruwa bil, (7) HORDI green (8) BARC RR-25, (9) Accession No 2912

Table 7. Reaction of germplasm to SCAR markers linked to rust resistance genes

Bean variety	Rust-resistant gene	Reaction to SCAR marker		
		SK 14	GT-2	SI 19
Aurora*	*Ur-3*	1	0	0
Mexico 235*	*Ur-3+*	1	0	0
PI 181996*	*Ur-11*	0	1	0
Mexico 309*	*Ur 5*	0	0	1
BARC- RR-25*	*Ur-4, Ur -5*	0	0	1
Acc No 2912**	*Unknown*	0	0	0
Acc No 2915**	*Unknown*	0	0	0
Acc No.5196**	*Unknown*	0	0	0
Acc No 7817**	*Unknown*	0	0	0
Acc No 7971**	*Unknown*	0	0	0
Balangoda Nil***	*No gene*	0	0	0
Bandarawela Green***	*Unknown*	0	0	0
BandarawelaKalu****	*Unknown*	0	0	0
GalpalamaKalu****	*Unknown*	0	0	0
GnnoruwaBil***	*No gene*	0	0	0
HORDI Green***	*No gene*	0	0	0
Keppetipola Nil***	*No gene*	0	0	0

presence of gene 0-absence of gene
** Differential series varieties and improved lines received from USDA, USA*
*** Germplasm from Plant Genetic Resources Centre, Sri Lanka*
**** Recommended varieties to cultivate by Department of Agriculture, Sri Lanka*
***** Farmer varieties in Sri Lanka*

Conclusion

The common bean cultivars recommended for local cultivation showed a lack of resistance to prevalent local rust races. Based on the results, the local accessions (*i.e.*, Bandarawela green, Bandarawela Kalu, Galpalama Kalu) may carry an unidentified type of resistance to races15-3, 22-6, and 31-1 with susceptible reactions to race 22-52. There are no locally grown bean varieties that show multiple resistance to all locally identified rust races. The USDA germplasm accessions carrying *Ur-3*, *Ur-5*, and *Ur-11* genes could be used to pyramid resistance genes into local snap bean varieties to improve their resistance to the common bean rust disease in Sri Lanka.

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