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RESEARCH

Development of Local Breeding Lines for Rust Resistance in the Common Bean (*Phaseolus vulgaris*) through the Incorporation of Rust-Resistant Genes

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ABSTRACT

Bean rust is one of the major diseases of the common bean (*Phaseolus vulgaris*) reported in Sri Lanka and at the global level. This study aimed to develop rust-resistant snap bean breeding lines via gene pyramiding assisted by molecular markers. Resistant sources; PI 181996, BelMiNeb-RMR-8, and BelDakMi-RMR-19, enriched with the rust-resistant genes *Ur-3* and *Ur-11*, were selected as donor parents to obtain a wide range of resistance to the rust pathogen. Resistant genotypes were crossed with popular local varieties *Kappetipola nil* and *Galpalama Kalu* (Capri) to introgress *Ur-3* and *Ur-11* resistant genes. Successive F_1, F_2, and BC (backcross) generations were obtained with the self-pollination and backcrossing processes. Standard phenotypic disease screening methods were applied to identify resistant lines. Phenotypically resistant plants obtained from these crosses were tested with sequence-characterized amplified region (SCAR) markers linked to two rust-resistant genes: SK 14 (linked to *Ur-3*) and SI 19 (linked to *Ur-11*). Molecular marker SI-19 showed higher reproducibility (50% to 80%) with the availability of relevant banding patterns for phenotypically resistant F_1, F_2, and BC_1 progenies. However, SK 14 showed lower reproducibility (30–60%) for the same progenies. Approximately 450 genotypes introgressed with rust-resistant genes (*Ur-3* and *Ur-11*)were produced. Among them, four advanced resistant lines obtained from the different cross combinations (*Kappetipola nil* x BelDakMi-RMR-19, *Galpalama Kalu* x BelMiNeb RMR-8, *Kappetipola nil* x PI 181996, and *Kappetipola nil* x BelMiNeb RMR-8) with preferred agronomic characters were selected for further variety development. All new genotypes will be important for future bean-resistant breeding programs in Sri Lanka.

INTRODUCTION

The common bean (*Phaseolus vulgaris L.*) is an economically and nutritionally important vegetable crop in Sri Lanka. It originated in Ecuador and Peru (Kelly, 2010). The domestication process resulted in two gene pools: Andean and Middle American. The Andean gene pool originated in the Andean region in the south of Ecuador, while the Middle American gene pool was domesticated in Colombia and northward (Kelly, 2010). Bean production is often compromised by destructive diseases, with rust caused by the fungus *Uromyces appendiculatus* being one of the major diseases affecting beans (*P. vulgaris*) worldwide, leading to substantial yield and quality losses (Kelly, 2010). A 1% increase in rust severity can result in a yield loss of approximately 19 kg/ha (Lindgren *et al*., 1995). Breeding crops for pathogen resistance is an environmentally sound and cost-effective method to manage diseases and minimize crop losses. Scientists have identified about 90 different rust races and more than ten resistant genes for these virulent types (Miklas *et al*., 2002; Hurtado-Gonzales *et al*., 2017). The distribution of resistance to *U. appendiculatus* across host populations in different regions affects the influence of host diversity on the evolution of pathogen virulence (Acevedo *et al*., 2008). Recent work in Sri Lanka has identified four rust races (Kumari *et al*., 2023).

Over the past two decades, scientists have extensively utilized rust-resistant genes for resistance breeding. Five resistant genes (*Ur-3*, *Ur-5*, *Ur-7*, *Ur-11*, and *Ur-14*) belong to the Middle American gene pool, while another five genes (*Ur-4*, *Ur-6*, *Ur-9*, *Ur-12*, and *Ur-13*) belong to the Andean gene pool (Stavely, 2000). Pyramiding multiple genes in the same plant can enhance overall resistance and durability (Pastor-Corrales, 2001). Phenotypic and molecular screening techniques have been developed to identify resistant gene components from the Andean and Middle American bean gene pools (Miklas *et al*., 2002; Miklas *et al*., 2006). Rust resistance breeding has utilized various genotypes to produce different resistant breeding lines (Pastor-Corrales, 2004). However, some of the selected resistant germplasm sources may contain undesirable characteristics that are commercially unacceptable (Kelly, 2010). Therefore, popular cultivated varieties with desirable traits can serve as suitable sources for incorporating resistance genes (Souza *et al*., 2005).

In breeding for disease resistance, pyramiding several disease resistance genes has proven to be effective and durable. However, conventional breeding methods alone may not be effective due to the challenges of selecting genotypes with different resistant genes (Hurtado-Gonzales *et al*., 2017). Molecular markers are used to assist in gene pyramiding, helping to overcome difficulties related to multiple or sequential inoculations and epistatic interactions among different resistant genes. Molecular marker-assisted selection (MAS) is a helpful approach in overcoming challenges in the introgression of single or multiple genes and shortening breeding cycles (Miklas *et al*., 2002; Pastor-Corrales, 2005). SCAR markers SK 14, GT 2, and SI 19 have been successfully used to tag the *Ur-3*, *Ur-11*, and *Ur-5* genes, respectively (Miklas *et al*., 2006; Acevedo *et al*., 2008).

The overall aim of this study was to initiate a rust-resistant breeding program and develop new breeding lines enriched with rust-resistant genes using known genetic sources to combat the identified rust pathogen races in Sri Lanka. This process involved the introgression of single and multiple genes, utilizing phenotypic and genetic markers. The Middle American common bean accession PI 181996 (*Ur-11*) and improved USDA-released varieties, BelDakMi-RMR-19 (*Ur-3*, *Ur-4*, *Ur-6*, and *Ur-11*) and BelMiNeb-RMR-8 (*Ur-3*, *Ur-4*, *Ur-6*, and *Ur-11*), were utilized as the rust-resistant donor parents (Miklas *et al*., 2002; Acevedo *et al*., 2008). These genotypes exhibit significant resistance to rust disease in Sri Lanka and worldwide (Kelly *et al.,* 2010; Kumari *et al.,* 2022). Furthermore, the Beldade and BelMiNeb common bean cultivars have been developed through the combination of Middle American and Andean genotypes, resulting in resistance to all reported rust isolates to date (Pastor-

Corrales, 2005). However, limited published reports are available on the utilization of these crucial genetic resources in resistance breeding attempts in Sri Lanka (Kumari *et al*., 2022), and this research aims to fill this information gap.

METHODOLOGY

Plant genetic material for breeding

New cross combinations were made using selected local germplasm and foreign germplasm as parental sources. We chose local varieties, namely *Kappetipola nil* and *Galpalama Kalu* (Capri), which possess characteristics accepted by farmers and consumers and have suitable adaptations. However, both of these varieties are susceptible to rust disease, and no resistance has been reported (Ariyarathne and Nuwan, 2001). To introduce rust resistance, we utilized germplasm obtained from the United States Department of Agriculture (USDA), which carries the *Ur-11*, *Ur-3*, *Ur-4*, and *Ur-6* genes, as donor parents for rust-resistant breeding. The rust-resistant germplasm was obtained from USDA, ARS in collaboration with Michigan State University, USA, following the plant quarantine regulations of the Department of Agriculture.

The rust-resistant donor parents used in this study were the Middle American common bean accession PI 181996 (*Ur-11*), as well as the improved USDA-released varieties, BelDakMi-RMR-19 (*Ur-3*, *Ur-4*, *Ur-6*, and *Ur-11*), and BelMiNeb RMR-8 (*Ur-3*, *Ur-4*, *Ur-6*, and *Ur-11*).

Development of new cross combinations

Five different new cross combinations were created, involving the following crosses: *Kappetipola nil* x PI 181996, *Kappetipola nil* x BelDakMi-RMR-19, *Galpalama Kalu* x BelDakMi-RMR-19, *Kappetipola nil* x BelMiNebRMR-8, and *Galpalama Kalu* x BelMiNeb RMR-8 (refer to Table 1). Hand pollination techniques were employed to generate an F1 population. Subsequent self-pollination led to the production of F_2 populations. Additionally, backcrossed generations were created using *Kappetipola nil* and *Galpalama Kalu* as the recurrent parents in the initial crosses. All crosses were conducted within protected house conditions at the Horticultural Crops Research and Development Institute (HORDI) in Gannoruwa (GPS 7.8731° N, 80.7718° E).

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Table 1.New cross combinations for resistance breeding

	Cross combination Recurrent x Donor parent	**Genes present in donor parents**
1	*Kappetipola nil* x PI 181996	*Ur-11*
2	*Kappetipola nil* x BelDakMi-RMR-19	*Ur-3*, *Ur-6*, *Ur-4*, *Ur-11*
3	*Galpalama Kalu* x BelDakMi-RMR-19	*Ur-3*, *Ur-6*, *Ur-4*, *Ur-11*
4	*Kappetipola nil* x BelMiNeb RMR -8	*Ur-3*, *Ur-6*, *Ur-4*, *Ur-11*
5	*Galpalam aKalu* x BelMiNeb RMR -8	Ur-3, Ur-6, Ur-4, Ur-11

Backcross breeding and gene pyramiding process

Ten F_1 plants from each combination listed in Table 1 were crossed with the corresponding recurrent parents, resulting in the creation of backcrossed (BC_1) populations. From these five crosses, selected BC_1F_1 plants were utilized to initiate the subsequent BC cycle (Figure 1). Separate backcross (BC) programs were conducted for each cross, extending until the BC_2 generation by employing the initial relevant recurrent parents. Only resistant plants displaying higher yield performance (>20t/ha) and possessing round pod characteristics similar to locally desired plant types were chosen and advanced beyond the F_2 and BC_1F_1 stages. Resistant plants obtained from the BC_2F_1 population underwent self-pollination to obtain BC_2F_2, followed by successive generations of BC_2F_3, BC_2F_4, and BC_2F_5 using the same methodology.

Breeding line screening and selection for rust disease resistance

Phenotypic screening

All newly generated populations, including up to the BC_1F_1 plants, underwent phenotypic screening using the artificial inoculation method. Rust virulence tests were conducted using a mixture of local rust races (65-23, 23-5, 31-11, and 31-1). The rust screening trials took place in greenhouse conditions at the Horticultural Crops Research and Development Institute in Gannoruwa, Sri Lanka, in 2019.

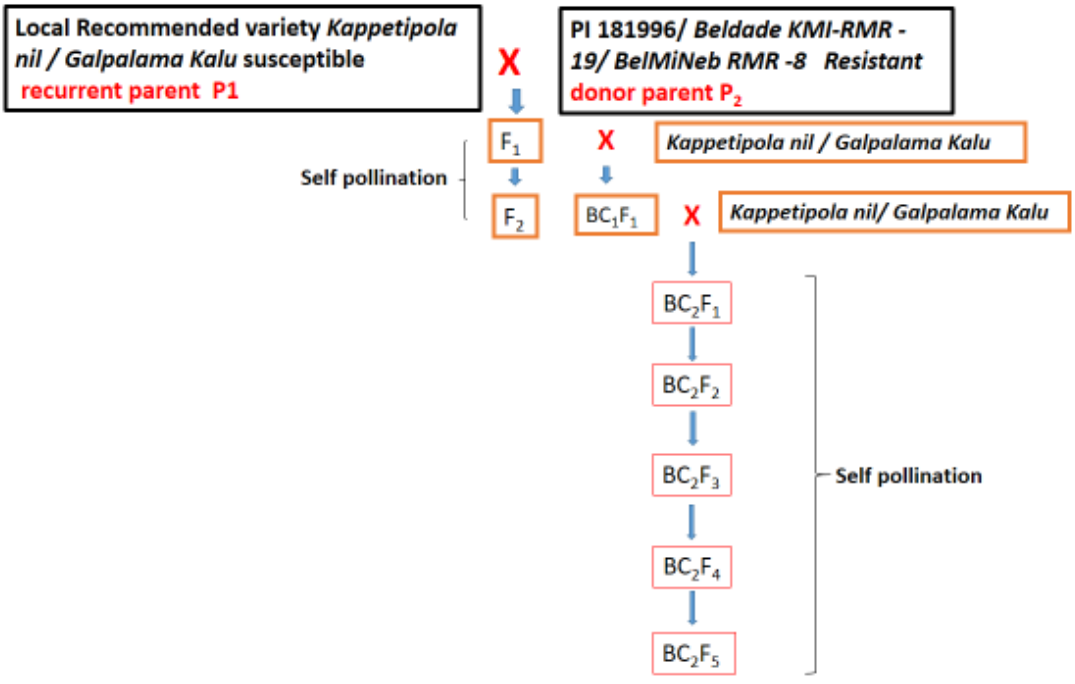

Figure 1. Schematic diagram of back cross breeding procedure

To prepare the inoculum, a concentration of 2.0 × 10^4uredospores/mL of distilled water with Tween-20 (0.05% v/v) was created using a hemocytometer. This standard concentration was used to inoculate the primary leaves of the plants, as previously tested (Kumari *et al*., 2022). Prior to inoculation, the plants were kept in greenhouse conditions at 20°C for 10 days. The inoculum was applied to the lower surface of the leaves using a paintbrush. Following inoculation, the plants were transferred to a mist chamber with conditions of 20 ± 1°C and a relative humidity exceeding 95%, where they remained in the dark for approximately 24 hours. After this period, the plants were moved to a greenhouse with a temperature of 20°C, where they were monitored for the appearance of symptoms.

The reaction of the breeding lines to the mixture of rust pathogen races was evaluated using the scale developed by Steadman *et al*. (2002) (Table 2). Gene introgression was assessed using both phenotypic and molecular markers. Rust-resistant plants from each generation were identified through the single plant selection method. Additionally, the consistency of other improved agronomic characteristics, such as plant growth and pod traits, was considered during the selection and advancement of each population.

Molecular screening

The detection of gene introgression was performed using DNA SCAR markers SK 14 and GT-2, which are linked to the rust resistance genes *Ur-3* and *Ur-11* (Table 3). A molecular marker-assisted selection method was employed alongside phenotypic screening for each population. Resistant plants obtained from different populations underwent testing for the presence of the *Ur-3* and *Ur-11* genes. Only plants that were genetically confirmed to possess these genes were selected as truly resistant lines.

Table 2. Bean rust grading scale for scoring the reaction of rust pathogen (*Uromyces appendiculatus*) on common bean plants (Steadman *et al*., 2002)

Reaction grade	Definition	Symbol
1	Immune, no visible symptoms.	I
2, 2+, 2 ++, 2 +++	Necrotic spots of different sizes and without sporulation. This type of reaction is known as the hypersensitive reaction (HR). The reactions are considered resistant. 2 = Necrotic spots less than 0.3 mm in diameter. 2+ = Necrotic spots between 0.3-1.0 mm in diameter. 2 ++= Necrotic spots between 1.0-3.0mm in diameter. 2+++= Necrotic spots greater than 3.0 mm in diameter	HR
3	3 = Tiny, small, sporulating pustules (uredinia) less than 0.3mm in diameter. The reaction is considered resistant.	R
f2	Faint and tiny chlorotic spots are known that usually accompany the tiny pustules less than 0.3mm. The reaction is considered resistant.	R
4	Uredinia (Sporulating pustules), 0.3-0.5mm in diameter. The reaction is considered susceptible.	MR
5	Uredinia 0.5-0.8 mm in diameter. The reaction is considered susceptible.	MS
6	Uredinia are larger than 0.8mm in diameter. The reaction is considered susceptible.	S

Table 3. Selected DNA marker details for genes conferring resistance to bean rust pathogen *Uromyces appendiculatus*(Acevedo *et al.*,2008)

SCARMarker	Tagged locus	Primer sequence	Chromosome	Distance	Product size (Bp)	Tm °C
SK 14	*Ur- 3*	F-CCC GTC ACA CAC CAA TAC CTG R-CCC GCT ACA CTT GAT AAA ATG TTA G	Pv11	1.0 cM (Coupling /Cis)	620	60
UR11-GT-2	*Ur- 11*	F-CGC ACT TAG GAG CAC AAA R-TGG TGG GTC CCA TAT TTT G	Pv11	0 & 5.4 cM (Coupling /Cis)	450	60

DNA extraction was carried out using the Cetyl-Trimethyl Ammonium Bromide (CTAB) method (Doyle and Doyle, 1987), using two grams of fresh leaf tissue from selected local and foreign germplasm. The PCR reaction was performed with an amplification reaction mixture of a final volume of 25 µl, including 30 ng of genomic DNA (measured using Nanodrop), 0.1 mM dNTPs, 2.8 mM MgCl2, and 0.2 mM of each primer. The annealing temperatures for the different primers were adjusted as shown in Table 3. The PCR program consisted of an initial denaturation step at 94°C for 3 minutes, followed by 34 cycles at 94°C for 1 minute, annealing at 60/67°C for 1 minute and 30 seconds, extension at 72°C for 1 minute and 30 seconds, and a final extension at 72°C for 10 minutes. The amplified products were separated on a 1.5% agarose gel containing Ethidium Bromide (0.2 mg/mL) at 100 V for 1.5 hours and photographed using a Gel documentation system

Evaluation of advance snap bean lines for yield parameters and rust resistance under field conditions

A field experiment was conducted at the Regional Agricultural Research and Development Centre (RARDC) in Bandarawela using $BC_2F_{5;4}$ plants obtained from five cross combinations. This location was chosen due to its favorable environmental conditions, including temperature and humidity, for the development of rust disease at the field level as well as appropriate conditions for bean cultivation. Four advanced bean lines, along with a newly recommended local variety (*Kekulu*) and the former variety *Kappetipola nil,* were tested in a replicated trial using a Randomized Complete Block Design (RCBD). The locally identified resistant USDA variety Beldade 1 was also included as a resistant control variety. The field trials took place during the Yala 2020 (June 2020–September 2020) and Maha 2020/21 (October 2020-January 2021) cultivation seasons on reddish-brown soil with a pH range of 6.5–7.5. Each genotype consisted of 40 plants in a plot, grown in four rows measuring 4 meters in length, with a spacing of 50 cm between rows and 40 cm between plants. The recommended crop management practices of the Department of Agriculture were followed.

Crop performance data, including important parameters such as days to 50% flowering, plant height at maturity, number of pods per plant, and pod yield, were collected. The days to 50% flowering of the plants in each plot were recorded. Ten randomly selected plants from each plot were measured for plant height and the number of pods per plant. The entire plot was harvested, and the pod yield per hectare was calculated. Rust disease occurrence at the field level was observed without the application of fungicides. Disease severity was assessed

using a 1-6 disease rating scale (Table 2). The data were analyzed using the SAS 9.1 statistical package, and means were compared using Duncan's Multiple Range Test at a significance level of $p<0.05$. Random individuals showing resistance were tested using SCAR molecular markers to confirm the introgression and pyramiding of the Ur-3 and Ur-11 genes.

RESULTS AND DISCUSSION

Crossing, selection, and development of breeding lines with rust resistance

New cross combinations, including *Kappetipola nil* x PI 181996, *Kappetipola nil* x BeDakMi-RMR-19, *Galpalama Kalu* x BelDakMi-RMR-19, *Kappetipola nil* x BelMiNeb RMR-8, and *Galpalama Kalu* x BelMiNeb RMR-8, resulted in the successful production of F_1 plants. These F_1 plants further gave rise to phenotypically distinct breeding lines in the F_2 and BC generations, as indicated in Table 4. The breeding population was improved up to the BC_2F_5 generation using the phenotypic screening method outlined earlier.

Resistant lines from each generation were then verified for the presence of *Ur-3* and *Ur-11* genes using the molecular markers SK14 and GT-2 SCAR, respectively (Figure 2). Only the lines that tested positive for these genes were chosen for further advancement, as indicated in Table 5.

Table 4. Breeding lines generatedfrom each population

Cross combinations	F_1	F_2	BC_1F_1	BC_2F_1
Kappetipola nil x PI 181996	20	280	25	28
Kappetipola nil x BelDakMi-RMR-19	24	220	20	26
Galpalama Kalu x BelMiNeb RMR -8	22	250	20	25
Kappetipola nil x BelMiNeb RMR -8	25	200	20	20
Galpalama Kalu x BeDakMi-RMR-19	25	230	20	25

Table 5. No of resistant progenies used for marker assisted selection and reproducibility percentage (in parenthesis) for *Ur-11*gene introgression

Cross combinations	F_1	F_2	BC_1	BC_2F_1	BC_2F_2	BC_2F_3	BC_2F_4	BC_2F_5
Kappetipola nil x PI 181996	12 (80%)	50 (72%)	11 (55%)	18 (75%)	18 (75%)	17 (66%)	16 (50%)	14 (50%)
Kappetipola nil x BelDakMi-RMR-19	10 (60%)	35 (60%)	10 (75%)	11 (60%)	14 (50%)	16 (66%)	14 (50%)	16 (40%)
Galpalama Kalu x BelMiNeb RMR -8	5 (80%)	60 (60%)	8 (64%)	10 (50%)	18 (50%)	12 (50%)	14 (50%)	18 (45%)
Kappetipola nil x BelMiNeb RMR -8	4 (50%)	60 (72%)	10 (60%)	12 (50%)	16 (50%)	18 (75%)	12 (50%)	16 (50%)
Galpalama Kalu x BelDakMi-RMR-19	5 (75%)	63 (68%)	18 (64%)	18 (50 %)	12 (50%)	18 (50%)	16 (66%)	18 (30%)

(% value calculated as No of plants positive for *Ur-11* linked marker/ No of Phenotypically resistant plants x 100)

The results of the study confirmed the successful incorporation of the rust resistance gene *Ur-11* from PI 181996 into the F_2 and BC populations of the *Kappetipola nil* x PI 181996 cross. This gene was introgressed as a single gene in 50–80% of the resistant lines. However, the SCAR marker Ur 11-GT-2 (450 bp), which is closely linked to the *Ur-11* locus, was only detected in a limited number of plants in the segregating resistant backcrossed (BC_2) progenies, ranging from 30 to 50%. This proportion is lower than expected. The parental lines or progenies that were susceptible to different rust races of the bean rust pathogen did not exhibit the markers associated with the tested rust resistance loci.

Phenotypic rust resistance was observed even with a single *Ur-11* gene, thanks to its broad-spectrum resistance, as documented in previous studies (Kumari *et al*., 2022). However, the introgression of both *Ur-3* and *Ur-11* genes was rarely observed in progenies resulting from other cross combinations, such as *Kappetipola nil* x BelDakMi-RMR-19, *Galpalama Kalu* x BeDakMi-RMR-19, *Kappetipola nil* x BelMiNeb RMR-8, and *Galpalama Kalu* x BelMiNeb RMR-8. In most cases, only single-gene (*Ur-11*) introgressions were observed in the progenies. The molecular markers demonstrated higher reproducibility in the initial populations of F_1, F_2, and BC_1, ranging from 50% to 80% (Figure 2). However, in later generations, a smaller proportion of progenies (30–50%) showed marker availability for *Ur-11* gene introgression (Table 5).

On the other hand, SK 14 exhibited lower reproducibility, even in the initial populations (42–60%). Several factors contribute to false positive and false negative results with the SK 14 molecular marker, as it is positioned at a distance of 2.23 cM from the gene (Araya et al., 2004; Arunga *et al*., 2012; Hurtado-Gonzales *et al*., 2017). The proximity between the *Ur-3* and *Ur-11* genes poses a challenge for differentiation. This observation could be explained by imperfect linkage between the marker and the target resistant gene or recombination events. Recombination frequencies may be higher due to different genotype segregation patterns and events in the development of BC populations. Moreover, the tracking and pyramiding of genes using the aforementioned molecular markers have been reported in many previous studies (Miklas *et al*., 2002; Miklas *et al*., 2006; Arunga and Odikara, 2020).

New local snap bean lines, enriched with the resistant allele, were obtained from backcrossed progenies and successfully identified at an early stage using conventional and molecular marker tools (Figure 2). However, the unexpected low positive marker responses (30–50%) for resistant types obtained from the application of SCAR markers with BC_2 populations highlight the need for the development of more precise and effective molecular marker-assisted selection tools for common breeding, aiming to develop a broader range of disease resistance. The lines developed through the introgression of resistant genes were further selected based on important morphological characteristics. However, some of the considered morphological traits, such as pod shape and flower color, did not exhibit consistency up to BC_2F_4. The utilization of recurrent selection and marker-assisted selection in this process has facilitated the development of new advanced lines. Combining different target alleles with other desirable agronomic traits in progeny remains an important breeding strategy.

Field evaluation of new rust-resistant breeding lines

Rust-resistant plants that were selected from BC_2F_5 and exhibited morphological similarities to local plant types were chosen for field evaluation. Among them, four selected lines were further used for seed multiplication. The $BC_2F_{5:4}$ seeds from the selected progenies were then planted in the field for yield assessment at the Regional Agriculture Research and Development Centre (RARDC) in Bandarawela. The performance of these lines was evaluated over two consecutive seasons: *Yala* 2020 and Maha 2020/21 at RARDC, Bandarawela. The yield performance of the new breeding lines was compared with that of the recommended variety, *Kekulu,* and the USDA improved line

BelDade RR-1. The results showed that the yield performance of the new breeding lines was comparable to that of these established varieties (Table 6).

The tested advanced lines exhibited various improved agronomic characteristics, including round pods (Figure 3), early flowering traits, and higher yield, which are desirable traits in recently accepted varieties. Among them, one improved new line from the *Kappetipola nil* x BelDakMi-RMR-19 cross showed superior yield performance compared to the control variety. Other new lines also demonstrated comparable yield performance to the newly released variety, *Kakulu*. In the field trial, segregation for rust disease resistance was observed in the plants of the new line obtained from the *Kappetipola nil* x PI 181996 combination, with 5% of plants showing signs of the disease (Table 6).

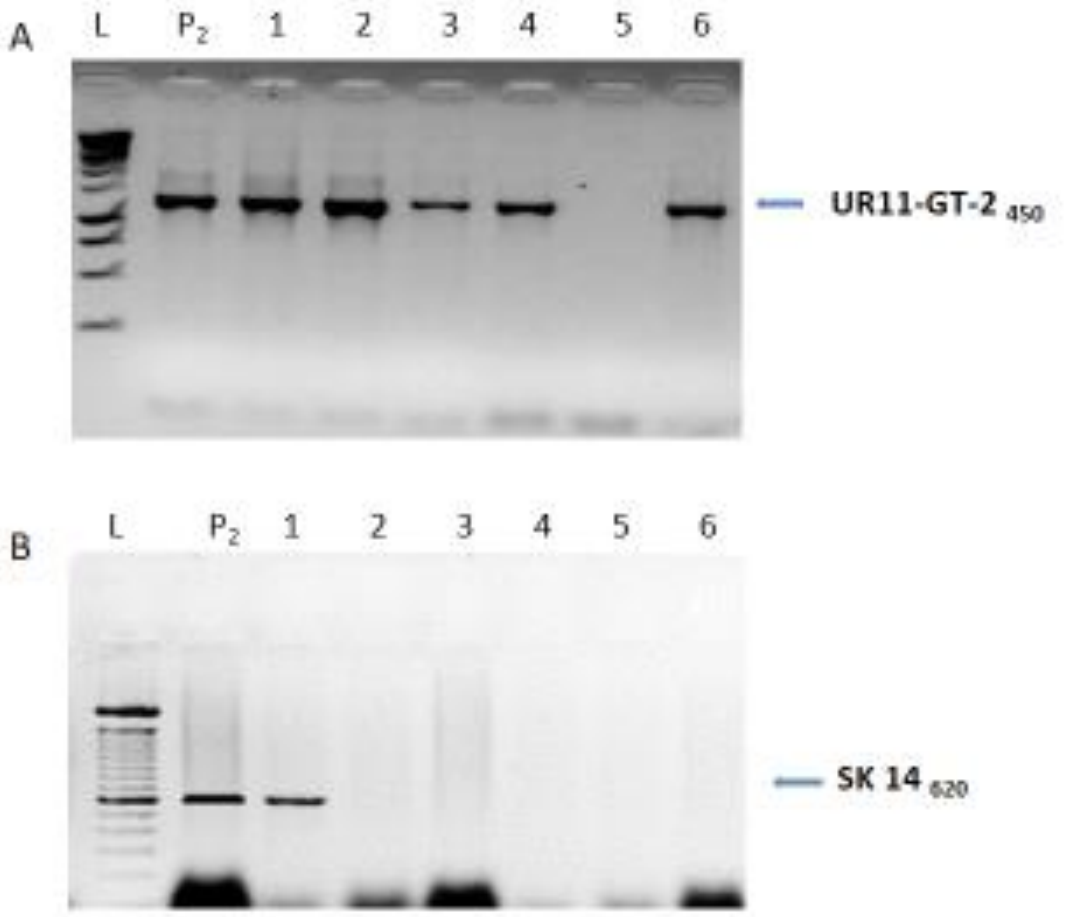

Figure 2.Amplification DNA products obtained with SCAR markers in 1% agarose gel after electrophoresis. Lanes are as follows: L lambda DNA cut with *Eco*RI, *Bam*HI and *Hind*III (size marker), P_2 resistant parent, 1-6 BC_1F_1 plants obtained with each SCAR markerUR11 GT 2 (A) and SK 14 (B) BC_1 F_1 plants obtained by the cross of *Kappetipola nil* x BelDakMi-RMR-19

Figure 3. Pod characteristic of four new bean lines: A (*Kappetipola nil* x BeDakMi-RMR-19, B (*Galpalama Kalu* x BelMiNeb RMR -8), C (*Kappetipola nil* x PI 181996) and D *Kappetipola nil* x BelMiNeb RMR-8). Control varieties E (*Kekulu*).

The selected new lines consistently exhibited black seed color, vine growth type, early flowering, and round pod shape. Considering their overall performance, these lines have the potential to be released as new varieties. The development of numerous rust-resistant lines (1200) enriched with multiple resistant genes through this research will be valuable for further crop improvement. The new lines selected from crosses such as *Kappetipola nil* x BelDakMi-RMR-19, *Galpalama Kalu* x BelMiNeb RMR-8, *Kappetipola nil* x PI 181996, and *Kappetipola nil* x BelMiNeb RMR-8 are currently undergoing further national-level trials. This research has also resulted in the production of a wide range of genotypes, which can serve as a base population for gene pyramiding with desirable plant and pod characteristics (Table 6). Furthermore, this has contributed to the development of other superior progenies for the national bean breeding program in Sri Lanka.

This research marks an important milestone in Sri Lanka as it supports the development of genetically improved new breeding lines with known resistant genes, which is the first achievement of its kind in the country's efforts to develop rust-resistant breeding lines. Additionally, this research represents the first successful utilization of molecular markers, specifically in the context of rust resistance breeding, in Sri Lanka, particularly with the use of *Galpalama Kalu*.

CONCLUSIONS

Rust-resistant genes for bean rust disease were successfully incorporated into local germplasm, specifically *Galpalama Kalu*(Capri) and *Kappetipola nil*, by utilizing foreign resistance sources such as PI 181996, BelDakMi-RMR-19, and BelMiNeb RMR-8. The breeding approach involved recurrent selection combined with molecular markers to introduce both *Ur-3* and *Ur-11* rust-resistant genes into progenies that also exhibited other desirable agronomic traits. Phenotypically resistant lines were evaluated for the presence of introgressed resistant genes using molecular markers, specifically SI 19 for *Ur-11* and SK 14 for *Ur*-3 genes. Among the evaluated lines, four new lines (*Kappetipola nil* x BelDakMi-RMR-19, *Kappetipola nil* x BelMiNeb RMR-8, *Kappetipola nil* x PI 181996, and *Kappetipola nil* x BelMiNeb RMR-8) were selected as they carried both resistant genes and were deemed suitable for advanced field trials. The progenies developed through this research, which possess the introgressed rust-resistant genes, hold significant value for the ongoing efforts in rust disease-resistant breeding programs in Sri Lanka.

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