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RESEARCH ARTICLE

PHENOTYPING AND ANALYSIS OF GENETIC DIVERSITY AMONG RUST RESISTANT SOYBEAN (*Glycine max*) (*L. Merrill*) GENOTYPES USING SIMPLE SEQUENCE REPEAT MOLECULAR MAKERS

Olasan Olalekan Joseph, Aguoru Celestine Uzoma, Ilebode-Sam Margaret Omokhio*, Ndera Ruth Msendoo, Ani Ndidiamaka Juliana

Department of Plant Science and Biotechnology, Joseph Sarwuan Tarka University, Makurdi, Benue State, Nigeria.
Corresponding Author Email: ilebode-sam.margaret@uam.edu.ng

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ABSTRACT

This study examines the phenotypic traits and genetic diversity of soybean genotypes resistant to soybean rust (*Phakopsora pachyrhizi*) using simple sequence repeat (SSR) markers. Five soybean varieties, including both resistant and susceptible types, were evaluated under controlled conditions. Molecular analysis was conducted using SSR markers (CP171/172, SSR1, RB32, and RB34) to assess genetic variation. Phenotypic assessments were performed to correlate molecular data with resistance traits, focusing on disease response and yield potential. The results showed that plant height ranged from 87.3 cm (TGx1951-3F) to 60.3 cm (TGx1835-10). Pathological analysis revealed that some varieties, such as TGx1448-2E and TGx1951-4F, exhibited resistance to rust, while TGx1904-6F had the highest disease incidence. Overall, the findings highlighted significant genetic diversity among the evaluated genotypes, with several accessions demonstrating strong resistance and high yield potential. This research enhances the understanding of the genetic basis of rust resistance in soybean and offers valuable insights for future breeding strategies to improve crop resilience against this major pathogen.

KEYWORDS

Soybean (*Glycine max*), Soybean rust (*Phakopsora pachyrhizi*), Genetic diversity, Phenotypic assessment, Simple sequence repeat (SSR) markers, Disease resistance, Molecular analysis, Breeding strategies, Yield performance, Pathological analysis

1. INTRODUCTION

1.1 Background of the study

Soybean (*Glycine max* (L.) Merrill), a member of the pea family, is a highly versatile legume cultivated for its nutrient-rich seeds. It plays a crucial role in global agriculture, providing an essential source of protein and key nutrients for both human consumption and animal feed. However, soybean's vulnerability to various diseases, such as rust, presents a major challenge to its productivity and overall yield (Mishra et al., 2024).

Soybean rust, caused by *Phakopsora pachyrhizi*, is a serious threat to soybean cultivation worldwide (Ono et al., 1992; Schneider et al., 2005). This fungal disease primarily affects the leaves, stems, and pods of the plant. Early symptoms typically manifest as small brown or yellow spots on the leaves, followed by lesions and cracks on the stems, as well as sunken patches on the pods. These symptoms often result in premature leaf drop, weakened plant structures, and significantly reduced yield and pod formation (Wikipedia). The pathogen spreads through airborne urediniospores, allowing for rapid disease progression. In regions where the disease is prevalent, yield losses can reach as high as 80% (Li et al., 2012).

The primary method for managing soybean rust involves the application of fungicides, which, although effective, significantly increase production costs and pose environmental concerns. Additionally, some *P. pachyrhizi* strains have shown increased resistance to specific fungicides (Godoy, 2009). As a result, developing and cultivating rust-resistant soybean varieties is considered the most efficient and sustainable strategy for disease control. Resistant varieties offer a cost-effective and environmentally friendly alternative that simplifies disease management.

The genetic variation responsible for resistance arises from multiple factors, including insertions, deletions, substitutions, and nucleotide rearrangements within the DNA (Redelings et al., 2024). Current research focuses on evaluating genetic diversity and phenotypic traits among rust-resistant soybean varieties using molecular markers, which play a critical role in breeding programs. Molecular markers aid in the identification and incorporation of resistance genes, enhancing breeding efficiency and the development of improved cultivars (Tabien et al., 2000).

Additionally, analyzing DNA polymorphism in soybean varieties provides valuable insights that can contribute to the development of molecular markers for rust resistance. These markers streamline the screening process, allowing breeders to efficiently identify and cultivate rust-resistant soybean varieties (Miah et al., 2013). By minimizing yield losses caused by rust, molecular marker-assisted breeding enhances soybean quality, increases farmers' income, and reduces dependence on fungicides, thereby mitigating environmental risks.

Future research in this area may focus on identifying additional DNA polymorphisms linked to rust resistance, refining molecular markers to improve accuracy and efficiency, and employing molecular breeding techniques to develop soybean varieties with enhanced traits beyond rust resistance—such as higher yield potential and improved drought tolerance (Li et al., 2023). Advancements in this research hold significant potential for strengthening soybean production, ensuring greater food security, and promoting sustainable agricultural practices.

Despite progress in developing rust-resistant soybean genotypes, genetic diversity among these genotypes remains relatively narrow. This limited

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variation poses a challenge to the long-term effectiveness of resistance, as the emergence of new rust pathogen races could overcome existing defenses. Therefore, this study utilizes molecular markers to evaluate genetic diversity and guide breeding strategies aimed at enhancing rust resistance and broadening genetic variation.

The objective of this research was to examine the phenotypic and genetic diversity of rust-resistant and susceptible soybean genotypes using Simple Sequence Repeat (SSR) markers.

2. Materials and Methods

2.1 Experimental Site and Location.

The study was carried out in the Molecular Biology Laboratory at Joseph Sarwuan Tarka University, Makurdi, Benue State. The university is situated at a latitude of 7.45°N and a longitude of 8.32°E, with an elevation ranging from 97 m to 111.2 m above sea level. The planting phase took place in the laboratory's screen house, which is positioned behind the Veterinary Medicine auditorium, South Core area.

2.2 Planting Materials

Seeds from five distinct soybean varieties were sourced from the seed store of the Molecular Biology Laboratory at Joseph Sarwuan Tarka University. The selected varieties included:

i. TG X 1835-10E
ii. TG X 1951-3F
iii. TG X 1951-4F
iv. TG X 1904-6F
v. TG X 1448-2E

To prevent any mixing, the seeds were stored in separate, labeled packets.

2.3 Planting of Soybean Seeds in the Screen House

The five soybean varieties acquired from the Molecular Biology Laboratory were sown in pots filled with topsoil. Initially, three seeds from each variety were planted per pot. After ten days, thinning was carried out, leaving two plants per pot to promote optimal growth conditions.

2.4 Collection of Leaf samples

Leaf samples were collected from young soybean plants of each variety fourteen (14) days after planting. The samples were placed in polythene zip-lock bags containing silica gel and left to dry for three days.

The equipment used for sample collection included:

i. Blade
ii. Polythene zip-lock bags
iii. 70% ethanol
iv. Paper towel

2.5 DNA Extraction using the CTAB Method

The Polymerase Chain Reaction (PCR) technique was employed to amplify a small number of copies of a specific DNA segment, thereby producing multiple replicas of that particular DNA sequence. The PCR procedure was conducted with a total reaction volume of 15µl. The components of the reaction comprised:

PuReTaqTM Ready-go-goTM PCR beads (containing PCR Buffer, MgCl2, DNTP's, and Taq Polymerase)

Distilled water

1µl of each primer and

1µl of DNA (50ng)

SSR based PCR protocol was used in carrying out PCR amplifications (Omoigui et al., 2015). 25 µl of Molecular Biology Grade water was added into 0.2 ml eppendorf tubes containing the PCR beads. The mixture was then divided into two for two PCR reaction, 1 µl primer (marker) and 1 µl DNA sample to serve as template was added into each 0.2 ml eppendorf tube. Tubes were covered and centrifuged for 15 seconds in other to assemble all components at the base of the tubes. The 0.2 ml eppendorf PCR tubes were arranged properly into the thermal cycler (PCR machine) to begin amplification.

2.6 Polymerase Chain Reaction (PCR) Mixture

The Polymerase Chain Reaction (PCR) technique was utilized to amplify specific DNA segments, generating multiple copies of the target sequence. The reaction was carried out in a total volume of 15 µL. The reaction mixture included the following components:

- PuReTaq™ Ready-To-Go™ PCR beads (containing PCR buffer, $MgCl_2$, dNTPs, and Taq polymerase)
- Distilled water
- 1 µL of each primer
- 1 µL of DNA (50 ng)

The SSR-based PCR protocol described by a group researcher was followed for amplification (Omoigui et al., 2015). Twenty-five microliters of Molecular Biology Grade water was first added to 0.2 mL Eppendorf tubes containing the PCR beads. The solution was then split into two separate tubes for two PCR reactions. One microliter of primer (marker) and 1 µL of the DNA sample were added to each tube, serving as the template for amplification.

After sealing the tubes, they were centrifuged for 15 seconds to ensure all components settled at the bottom. Finally, the 0.2 mL Eppendorf tubes were carefully placed in the thermal cycler (PCR machine), where the amplification process was initiated.

2.6.1 Polymerase chain reaction cycle

The PCR cycling protocol involved an initial denaturation step at 94°C for 4 minutes, followed by denaturation at 94°C for 30 seconds, annealing at 55°C for 1 minute, and extension at 72°C for 1 minute. The reaction was then held at 60°C indefinitely to maintain the amplified DNA.

2.7 Agarose Gel Electrophoresis

The methodology described was adopted for gel electrophoresis. A 3.5% agarose gel was prepared by weighing 3.5 g of agarose powder and dissolving it in 350 mL of 1x TAE buffer (Omoigui et al., 2015). The mixture was gently swirled and heated in a microwave until it became clear. After cooling, 30 µL of ethidium bromide (EtBr) was added and mixed thoroughly. The gel solution was then poured into a pre-prepared gel casting tray with a comb to form wells.

Once the gel solidified, it was carefully placed in the electrophoresis tank, and the comb was gently removed to prevent well damage. To prepare the DNA samples, 1 µL of DNA was mixed with 1 µL of 6x loading dye in a PCR tube and briefly spun. The prepared samples were then carefully loaded into the wells using a micropipette. Additionally, 5 µL of a DNA ladder was loaded into a separate well as a reference marker. The electrophoresis system was sealed, and the gel was run at 120V for 45 minutes.

DNA purity and quality were assessed using UV spectrophotometry. The banding patterns of the DNA samples, resolved on the agarose gel, were visualized under a UV transilluminator, and the gel image was captured for band scoring. Only distinct bands were recorded, with presence scored as (1) and absence as (0) (Omoigui et al., 2015).

2.8 Data Analysis

The MINITAB 17 software was used for statistical analysis. Phenotypic data were analyzed using descriptive statistics, and cluster analysis was performed. A dendrogram was generated using the complete linkage method to assess genetic relationships among the samples.

3. Results and Discussion

Table 1 provides information on the growth, yield and pathological characters assessed during the field work. Plant height varieties from 87.3 to 60.3(TGx-1951-3F and TGx-1835-10E) is shown in figure 1. Variety TGx-1835-10E had the highest maturity which was recorded as 109 is shown in figure 2. The data showed that variety TGx-1951-3F (723.7) had the highest seed yield/plot with variety TGx-1835-10E (555.6) as the lowest is shown in figure 3. Days to 50% flowering vary from 41 days to 45 days (TGx-1835-10E) and (TGx-1904-6F) is shown in figure 4. Pathological data shows that some of the varieties are resistant to rust disease, the variety that recorded the highest incident of rust disease was TGx-1904-6F is shown in figure 5, Frogeye leaf spot disease was 1.8 (TGx-1448-2E). Mosaic disease ranged from 1 (TGx-1904-6F) and 1.7 (TGx-1951-4F), RTNOD 1-5 ranged from 2.5 to 3.5 (TGx-1835-10E and TGx-1951-4F). Lodg 1-5 ranged from 1 to 2.2 (TGx-1951-4F and TGx-1904-6F). Pod shat late ranged from 1 to 2 (TGx-1835-10E and TGx1904-6F), 100 seed weight ranged from 11.7 to 13.7 (TGx-1448-2E and TGx-1951-4F). The variety with the Lowest Pod height was TGx-1835-10E 3.7.

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Table 1: Growth, Yield and Pathological Assessment of Soybean Varieties in the Field.

VARIETIES	DAYS TO FLOWERING	DAYS TO 50% FLOWERING	RUST 1-5	FROGEYE 1-5	B.P	MOSAIC DISEASE	RTNOD 1-5	95 % MATURITY	PLANT HEIGHT	LODG 1-5	SEED YIELD/PLOT	POD SHAT LATE 1-5	100 SEED WEIGHT	LOWEST POD HEIGHT
TGx-1835-10E	38.3	41	1	1	1	1.3	2.5	109	60.3	1.5	555.6	1	12.6	3.7
TGx-1904-6F	41.7	45	2.2	1	1.7	1	2.7	91.7	86	2.2	571.9	2	12.5	6.3
TGx-1448-2E	40.3	43.3	1	1.8	1.5	1.5	3	110	72.2	1.8	589.7	1	11.7	5.9
TGx-1951-3F	40.3	44.3	1	1.2	1.7	1	2.8	101.7	87.3	1.3	723.7	1.3	12.3	7.4
TGx-1951-4F	39.3	42.3	1	1.2	1.5	1.7	3.5	103.7	72.1	1	587.1	1.5	13.7	7.9

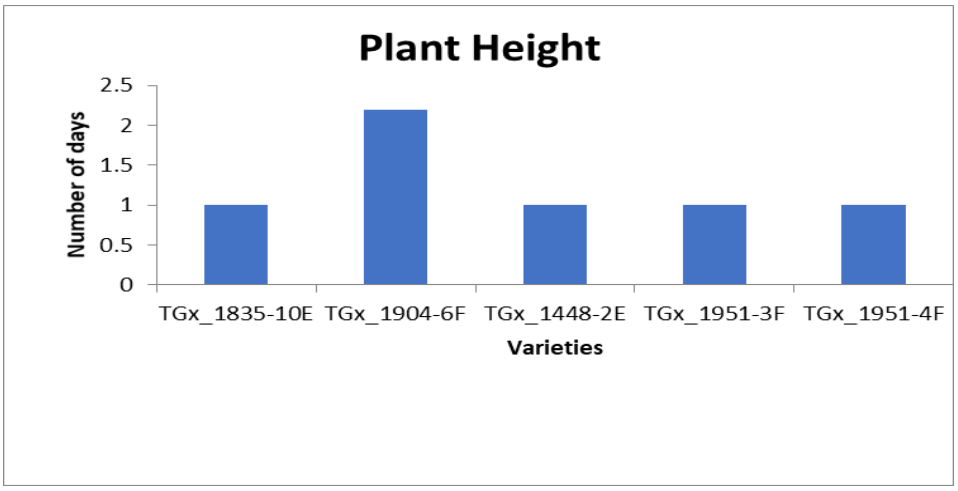

Figure 1: Plant Height of Soybean Varieties

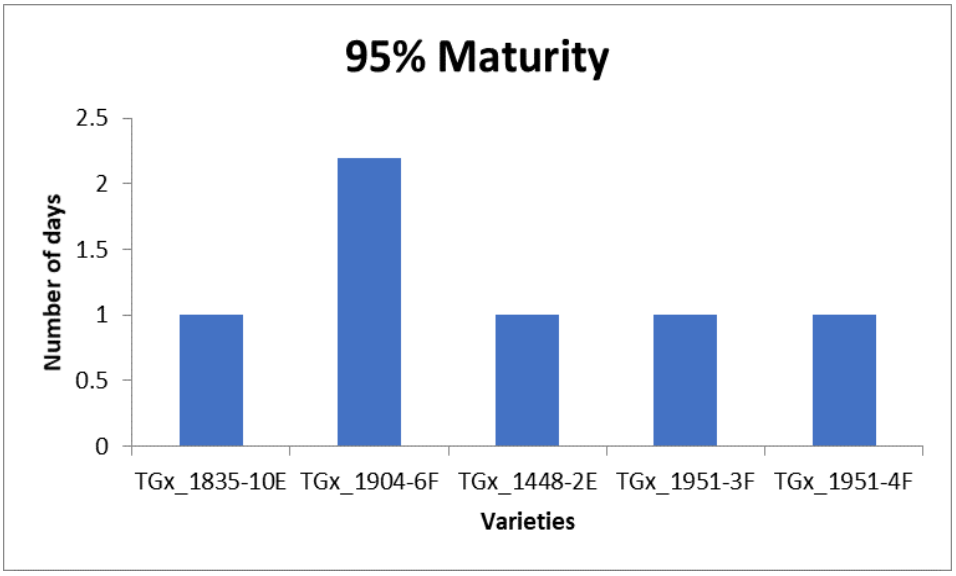

Figure 2: 95% Maturity of Soybean Varieties

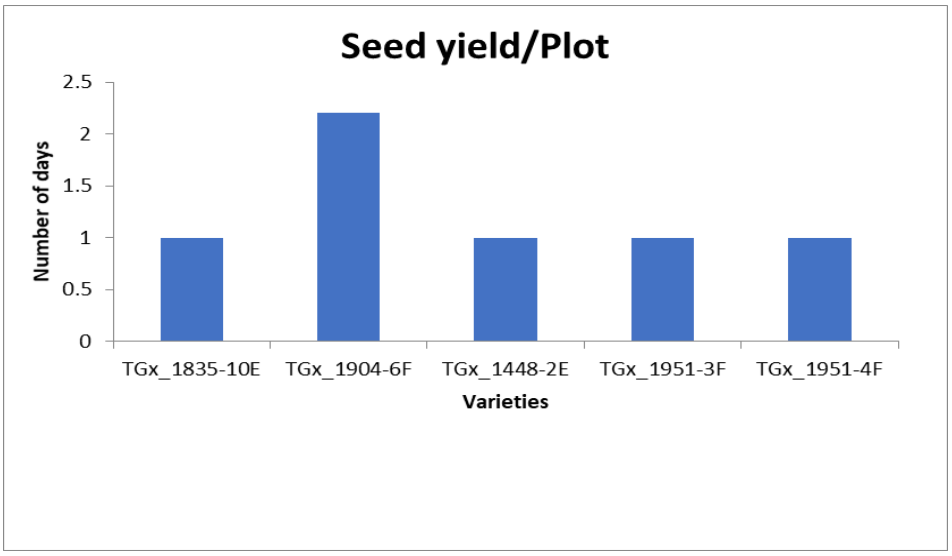

Figure 3: Seed yield/Plot of Soybean Varieties

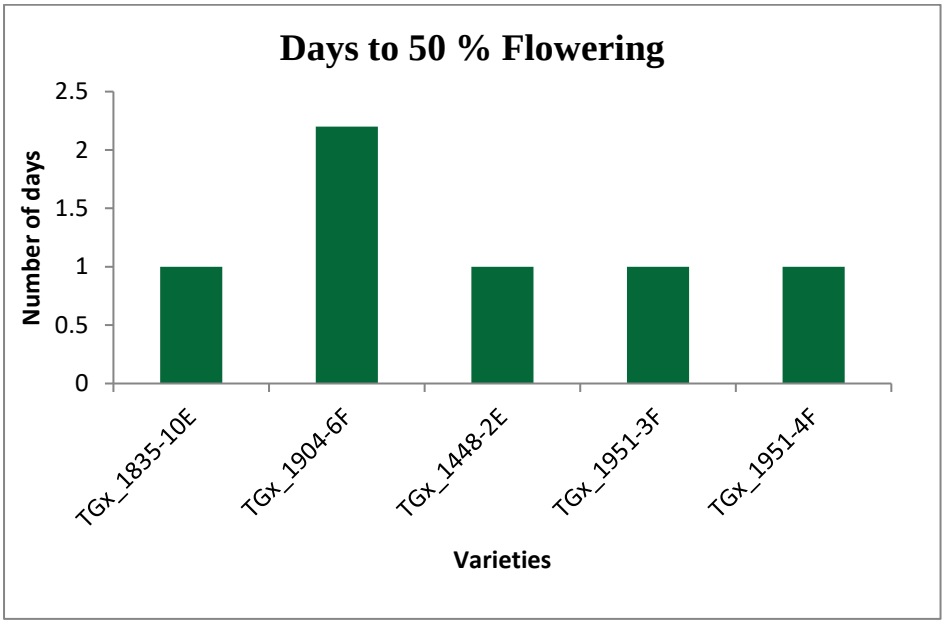

Figure 4: Days to 50% Flowering of Soybean Varieties

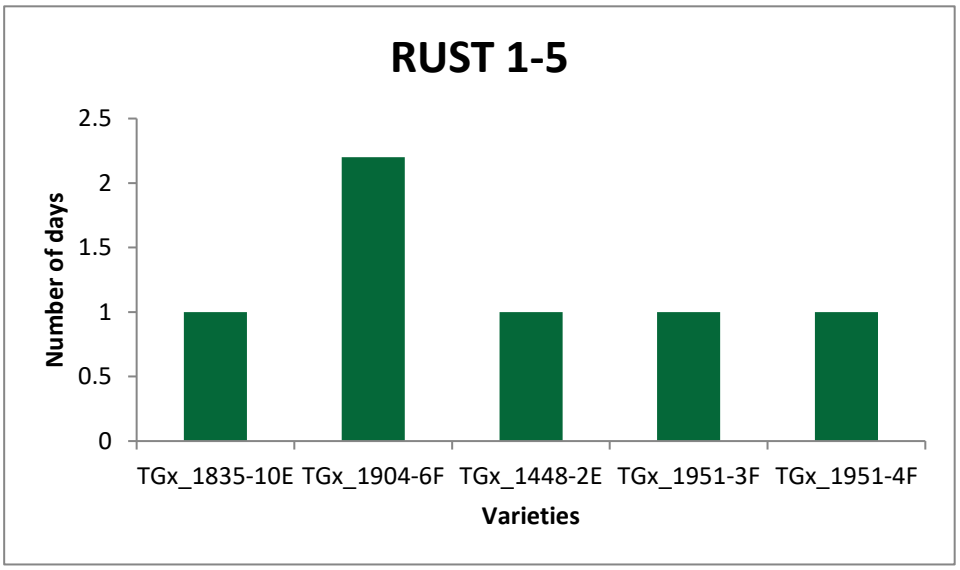

Figure 5: Rust 1-5 of Soybean Varieties

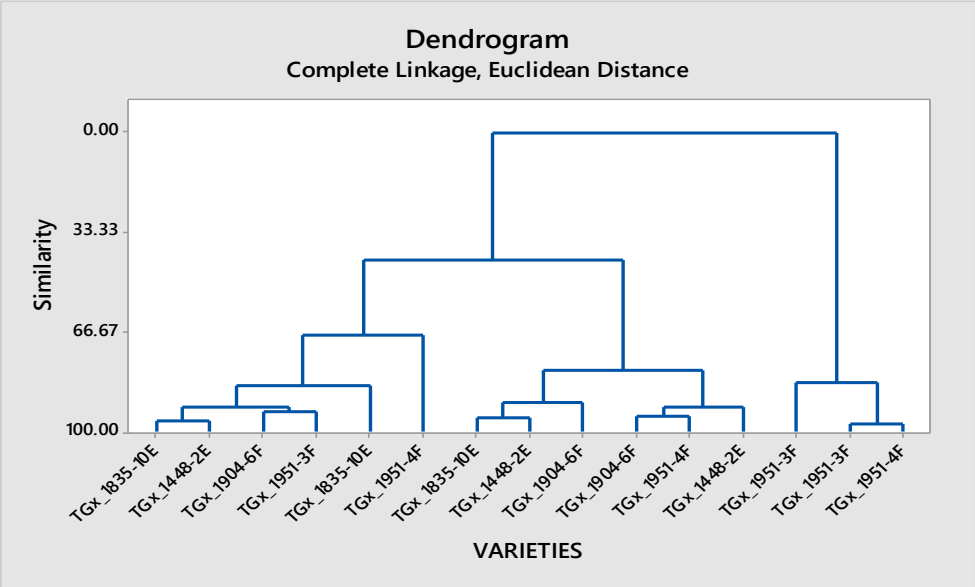

Figure 6: Dendrogram of Soybean Varieties

Plates 1 and 2 display the agarose gel images of four screened Simple Sequence Repeat (SSR) markers used in DNA amplification to assess polymorphism between rust-resistant (TGx1835-10E) and rust-susceptible (TGx1951-3F and TGx1951-4F) soybean varieties. The primers exhibited varying levels of genetic polymorphism, depending on the soybean DNA amplified and the SSR primers used. While all primers generated visible bands, SSR 1 showed no clear resolution in either rust-resistant or susceptible varieties. However, distinct bands were well resolved using primers CP 171/172, RB 32, and RB 34.

1 2 3 4 5 L 1 2 3 4 5

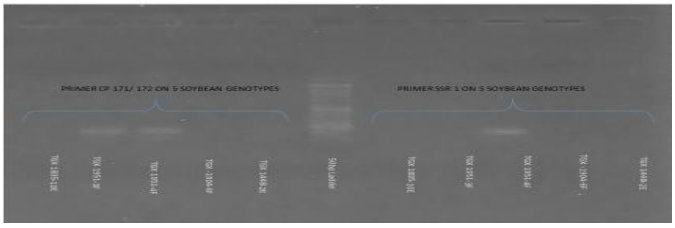

PLATE 1: Agarose Gel Image of Amplified DNA of Soybean Varieties Showing Resistance and Susceptibility to Rust Disease.

= TG X 1835-10E

= TG X 1951-3F

= TG X 1951-4F

= TG X 1904-6F

= TG X 1448-2E

L = 50bp Ladder.

Primers = CP 171/172 and SSR 1

L B 1 2 3 4 5 B 1 2 3 4 5

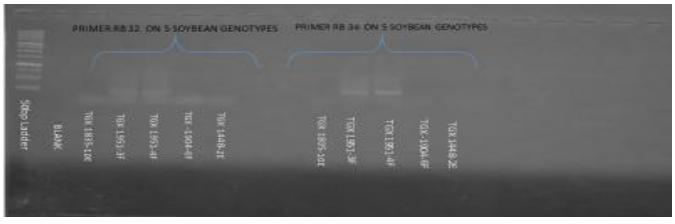

PLATE 2: Agarose Gel Image of Amplified DNA of Soybean Varieties Showing Resistance and Susceptibility to Rust Disease

L = 50bp Ladder

B = Blank

Primers = RB32 and RB34

= TG X 1835-10E

= TG X 1951-3F

= TG X 1951-4F

= TG X 1904- 6F

= TG X 1448-2E

4. Discussion

Genetic diversity among rust-resistant soybean genotypes is essential for improving breeding programs aimed at managing soybean rust, a disease caused by Phakopsora pachyrhizi. Molecular markers are valuable tools for evaluating genetic variation and identifying resistance traits by pinpointing genomic regions associated with disease resistance. Plate 1 presents the agarose gel image comparing rust-resistant and susceptible soybean varieties. The two markers used in this study were only amplified in resistant varieties, suggesting a potential link between these markers and rust resistance. This observation aligns with the findings of who identified SSR markers associated with rust resistance (Zhong et al., 2024). The application of these markers in breeding programs enables more precise selection of resistant plants, thereby accelerating the development of improved soybean varieties for farmers facing rust disease challenges.

Plate 2 shows the screening results of SSR markers for polymorphism between rust-resistant and susceptible soybean varieties. The tested markers revealed genetic variation in both groups, indicating their effectiveness in distinguishing between resistant and susceptible genotypes. Notably, RB32 was amplified only in the resistant parent, while RB34 was amplified exclusively in susceptible varieties. This suggests a strong genetic linkage between RB32 and rust resistance, and between RB34 and rust susceptibility. These findings are consistent with previous research by further supporting the potential of these markers in marker-assisted selection for rust resistance (Li et al., 2023).

A phenotypic assessment was conducted to correlate molecular data with resistance traits, particularly focusing on disease incidence and yield potential. Some varieties exhibited early maturity and demonstrated resistance to rust and other diseases. Among the genotypes analyzed, TGX-1904-6F recorded the highest rust disease incidence rate (2.2). In terms of yield performance, TGX-1951-3F had the highest seed yield per plot (723.7), while TGX-1835-10E recorded the lowest (555.6).

5. Conclusion

The results from Plate 1 and Plate 2 demonstrate that SSR markers are effective tools for identifying rust resistance in soybean varieties. The observed polymorphism across the two markers indicates their ability to distinguish between resistant and susceptible genotypes. Specifically, RB32 and RB34 showed strong associations with rust resistance and susceptibility, respectively, suggesting a close genetic link to the underlying resistance and susceptibility genes. These findings align with previous research and highlight the potential of SSR markers in breeding programs for rust-resistant soybean varieties.

Recommendations

- Explore additional markers – Further research should focus on

identifying additional molecular markers associated with rust resistance. Expanding the pool of markers will provide breeders with more precise tools for selecting resistant soybean varieties.

- Incorporate markers into breeding programs – Validated markers should be integrated into soybean breeding programs to expedite the development of rust-resistant varieties. Collaboration among researchers, breeders, and farmers will be essential for the successful implementation and adoption of marker-assisted selection.
- Increase awareness and adoption – Outreach programs and educational initiatives should be conducted to inform farmers and other stakeholders about the benefits of rust-resistant soybean varieties. Promoting awareness will encourage wider adoption and enhance the impact of these technologies on soybean production.
- Enhance breeding strategies – Molecular markers should be systematically integrated into breeding programs to develop improved soybean varieties with resistance to rust and other diseases. A multi-disciplinary approach involving geneticists, breeders, and farmers will ensure the successful deployment of these advancements in real-world agricultural settings.

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