Global Research journal of Natural Science & Technology (GRJNST)

Volume: 04 - Issue 2 (2026), 2050
ISSN P: 2790-7643 ISSN E: 2790-7651
www.grjnst.net
https://doi.org/10.53762/grjnst.04.02.02

Cross-Species PCR Amplification Performance of Wheat Stripe Rust Resistance Gene-Specific Primers in Common Bean (*Phaseolus Vulgaris* L.)

Received: 31 December 2025. Accepted: 30 January 2026. Published: 31 March 2026

Muhammad Mehraj Riasat (Corresponding Author)
Department of Agriculture,
Hazara University, Mansehra 21300, Pakistan
Email: mehrajhum30278@gmail.com
Muhammad Waleed
Department of Agriculture,
Hazara University, Mansehra 21300, Pakistan
Uzair Ahmad
Department of Agriculture,
Hazara University, Mansehra 21300, Pakistan
Farhan Tahir
Department of Agriculture,
Hazara University, Mansehra 21300, Pakistan
Shafaq Munir
Department of Agriculture,
Hazara University, Mansehra 21300, Pakistan
Atika Liaqat
Department of Agriculture,
Hazara University, Mansehra 21300, Pakistan
Saqib Waqar Ahmed
Department of Agriculture,
Hazara University, Mansehra 21300, Pakista

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Abstract: *PCR-based gene-specific primers are essential in crop genetics, but their cross-genome effectiveness is unclear. This study tested whether wheat (Triticum aestivum L.) stripe rust resistance markers Yr15 and Yr30 can be amplified in common bean (Phaseolus vulgaris L.). Genomic DNA from 30 bean genotypes was amplified by PCR, with wheat DNA and nuclease-free water serving as positive and negative controls. The PCR protocol used an initial denaturation at 94°C for 5 minutes, 40 cycles (94°C for 60 seconds, 55.8°C for Yr15 or 52.7°C for Yr30 for 60 seconds, 72°C for 45 seconds), and a final extension at 72°C for 10 minutes. PCR products were separated via agarose gel and visualized. Yr15 was not detected in any bean genotype, while Yr30 showed the expected 120 bp band in some genotypes with additional non-specific bands. These findings highlight that gene-specific primers may fail or yield ambiguous results in unrelated species, emphasizing the need for rigorous marker validation across species.*

Keywords: *PCR, Gene, Yr15, Yr30, Amplification, Triticum aestivum L., Phaseolus Vulgaris L.*

Introduction

Polymerase chain reaction (PCR) is a common lab method in crop breeding and genetics that helps find, copy, and study specific pieces of DNA (Mullis & Faloona, 1987; Saiki et al., 1988). PCR methods are important for selecting plants with desired traits, figuring out where genes are, and studying useful traits in crops (Collard & Mackill, 2008; Varshney et al., 2005). The success of PCR depends mostly on how well the starting pieces of DNA, called primers, match the DNA, how they attach, and the conditions used, since primers decide which parts of DNA are copied in plants (Dieffenbach et al., 1993; Kwok et al., 1990; Thornton & Basu, 2011).

Gene-specific primers amplify unique DNA regions linked to distinct genes or loci. These primers are usually highly specific within their target species (Dieffenbach et al., 1993; Thornton & Basu, 2011). However, sequence similarity, conserved motifs, primer–template mismatches, and experimental conditions can decrease specificity. These issues may cause non-specific or cross-species amplification (Kwok et al., 1990; Lefever et al., 2013). Undetected unintended amplification can lead to inaccurate or incorrect experimental interpretations, especially when primer performance is not thoroughly assessed or when primers are used outside their original genetic context (Thornton & Basu, 2011; Bustin et al., 2009).

Stripe rust (yellow rust), caused by *Puccinia striiformis f. sp. tritici*, is among the most destructive fungal diseases affecting wheat (*Triticum aestivum* L.) globally (Chen, 2005; Wellings, 2011). To address this challenge, numerous stripe rust resistance *(Yr)* genes have been identified and incorporated into breeding programs (McIntosh et al., 2010). Among these, *Yr15* confers broad-spectrum resistance, while *Yr30* provides adult plant resistance. Accordingly, gene-specific or closely linked primers for these loci are widely utilized for molecular screening and germplasm evaluation in wheat (Klymiuk et al., 2018; Kokhmetova et al., 2021).

Primers designed for wheat *Yr* genes are generally validated exclusively within wheat genetic backgrounds, and there is limited information regarding their amplification performance in non-target plant species. Therefore, assessing primer specificity beyond the target genome is crucial for identifying the limitations of PCR-based assays and for preventing misinterpretation due to non-target amplification (Bustin et al., 2009; Lefever et al., 2013).

The objective of this study was to evaluate the PCR amplification behavior of wheat stripe rust resistance gene-specific primers, *Yr15* and *Yr30,* both recognized for their roles in conferring resistance to wheat stripe rust when applied to common bean *(Phaseolus vulgaris L.)* genomic DNA. To clarify the scope, this research focuses solely on amplification patterns and primer specificity, and does not address gene function, phenotypic traits, or disease resistance.

Material and Methods

Thirty (30) common bean genotypes were collected from diverse agroecological locations in the Mansehra region of Pakistan. Genomic DNA from 30 bean genotypes was amplified by PCR, with wheat DNA and nuclease-free water serving as positive and negative controls, respectively. A standard buffer-based protocol was followed, including purification and precipitation. DNA integrity and quality were verified by agarose gel electrophoresis prior to PCR amplification. Gene-specific primers linked to wheat stripe rust resistance genes, *Yr15* and *Yr30*, were used to examine cross-species amplification behavior. PCR reactions were performed in a 10 µL volume using a commercial master mix, genomic DNA template, and primer pairs. Amplification was conducted under optimized cycling conditions. These included denaturation at 94°C for 5 minutes; 40 cycles of 94°C for 60 seconds; annealing at 55.8°C for *Yr15* and 52.7°C for *Yr30* for 60 seconds; 72°C for 45 seconds; and final extension at 72°C for 10 minutes. PCR products were resolved on 1.5% agarose gels alongside a DNA ladder for fragment size estimation. Gel electrophoresis was performed under standard conditions. Banding patterns were visualized under ultraviolet illumination and documented using a gel imaging system. Interpretation was based on direct visualization of amplification profiles in the gel images. For *Yr15*, the expected diagnostic fragment size was 390 bp. For *Yr30*, the expected fragment size was 120 bp. Only bands corresponding to these expected sizes were considered relevant for interpretation. Non-specific fragments, including lower molecular weight bands and primer dimers, were disregarded. The results are presented as gel images, which allow clear visual assessment of amplification patterns across genotypes.

Results and Discussion

Yr15 amplification profile

PCR amplification with the *Yr15* gene-specific primer failed to generate the expected 390bp diagnostic fragment in any of the tested common bean genotypes (Figure 1). No bands corresponding to the target size were detected in any sample. This lack of amplification aligns with the established species specificity of *Yr15*, which encodes the tandem kinase protein WTK1 and has been functionally characterized in wheat and closely related species (Klymiuk et al., 2018). The absence of cross-amplification in common bean, a phylogenetically distant legume, likely results from the lack of

conserved primer-binding regions necessary for specific amplification. Comparable findings have been reported for wheat rust resistance markers, which generally do not amplify in non-cereal species due to genomic divergence (Lagudah et al., 2009; McIntosh et al., 2010). The consistent absence of the 390bp fragment, therefore, constitutes a biologically meaningful result, underscoring the high specificity of the *Yr15* marker within its native genomic context.

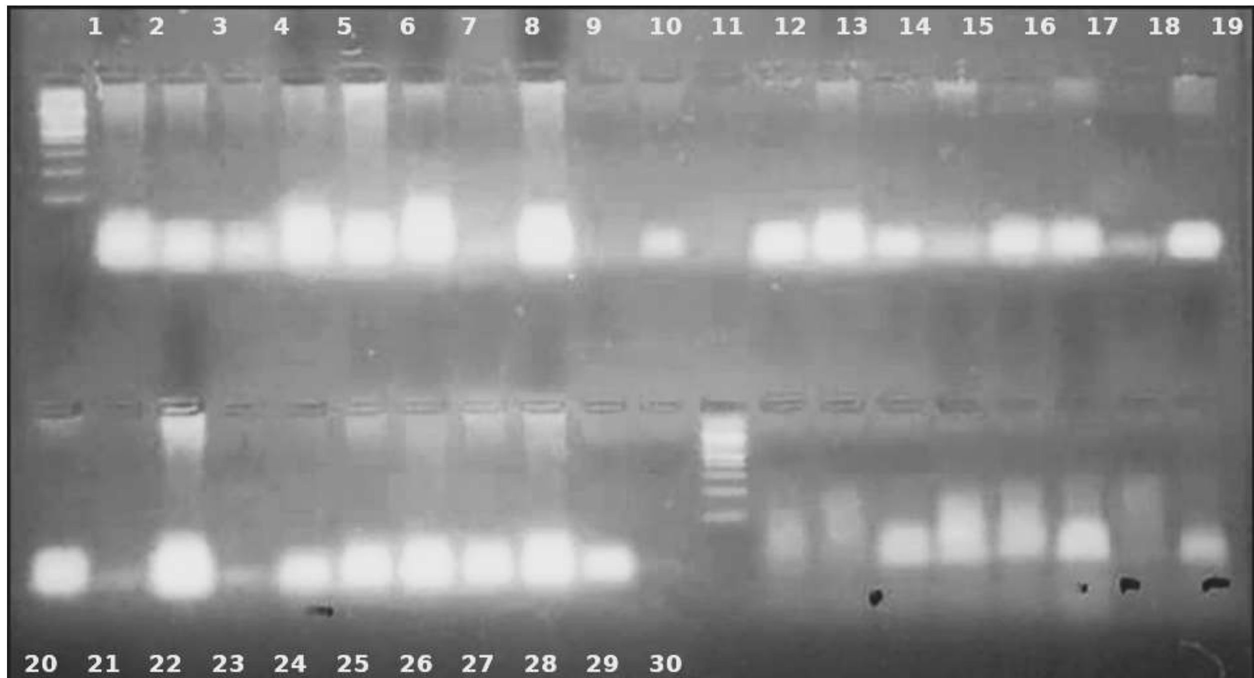

Figure 1. PCR amplification profile of *Yr15* in common bean genotypes.

Yr30 amplification profile

PCR amplification with the *Yr30* gene-specific primer produced heterogeneous banding patterns among the analysed common bean genotypes (Figure 2). Some samples had fragments close to the expected size of 120bp, while others showed bands with slightly lower molecular weight (about 90–110bp) or no amplification at all. To ensure consistency, only clearly resolved bands near 120bp were interpreted, with lower molecular weight fragments excluded from analysis. This observed variability in amplification profiles likely results from partial or non-specific primer binding, a phenomenon common when gene-specific markers are used outside their native genomic context. Such size differences have been well documented and are attributed to sequence divergence and primer template mismatches in non-target species (Kwok et al., 1990; Dieffenbach et al., 1993; Smith et al., 2007). Importantly, the presence of120bp fragments in some genotypes does not confirm that the *Yr30* resistance gene is present, but rather reflects amplification behavior under cross-species conditions. The absence of amplification for *Yr15* contrasts with these results, further highlighting differences in primer specificity and binding efficiency.

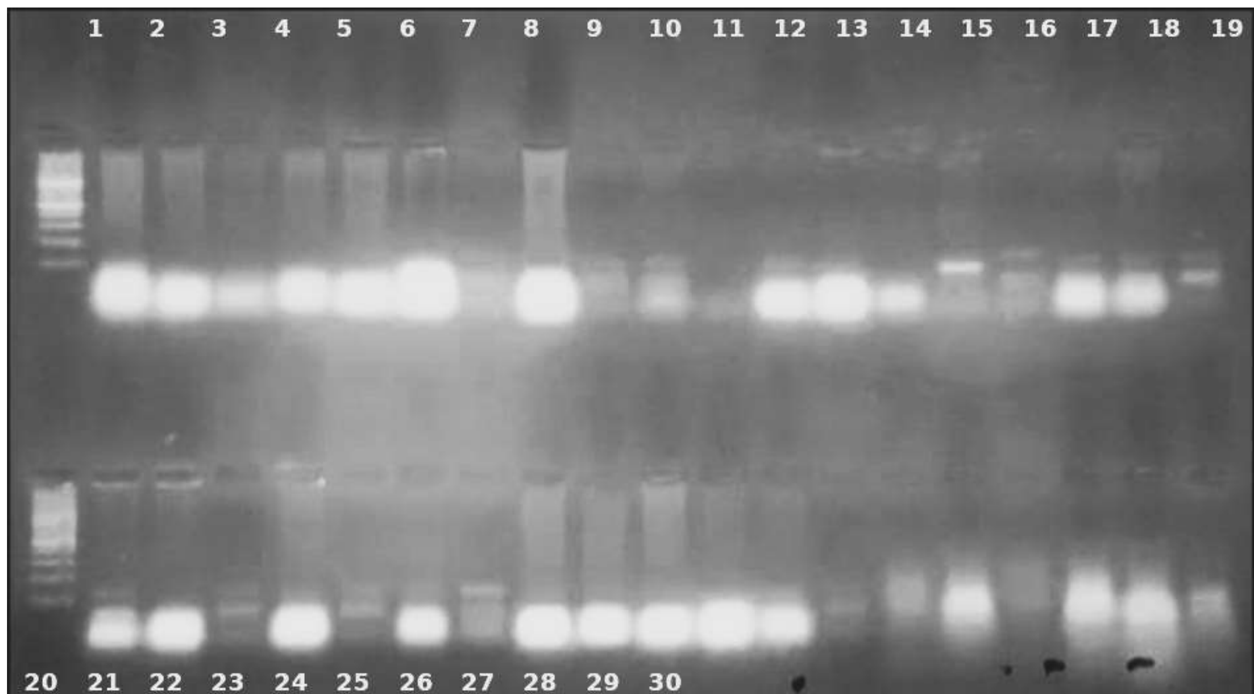

Figure 2. PCR amplification profile of *Yr30* in common bean genotypes.

Conclusion

This study shows that wheat gene-specific primers vary in amplification with non-target species. The *Yr15* primer failed to amplify target-size fragments in any common bean genotype, while the *Yr30* primer showed variable results, sometimes producing expected-size fragments. These results suggest primer specificity, not biological resistance, caused the differences. This highlights the limitation of using crop-specific molecular markers in different plant genomes and stresses the need for careful validation before applying PCR-based primers in broader molecular studies.

Author Contribution

Muhammad Mehraj Riasat: Supervise the research, Muhammad Waleed and Uzair Ahmad: Lab work, Farhan Tahir: laboratory assistance, Shafaq Munir and Atika Liaqat: Write original manuscript, Saqib Waqar Ahmed: Sample collection. All authors contributed to the review and editing of the manuscript.

Declaration of Funding

This research was supported by the Department of Agriculture, Hazara University, Mansehra, Pakistan.

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