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Morphological And Molecular Characterization of Locally Developed and Imported Dicoccum Wheat (Triticum Dicoccum (Schrank.) Schubl.) Germplasm Lines for Bipolaris Sorokiniana, Spot Blotch Disease Resistance

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Abstract

The importance of genetic resources lies in their pivotal role in improving the quality of crop varieties, particularly in enhancing their resilience to both biological and environmental stresses. One such challenge is Spot blotch (SB), a destructive leaf disease that primarily affects wheat, especially in warm and humid regions, particularly the eastern parts of South Asia, including the eastern Gangetic plains and the Peninsular zone. Developing wheat cultivars with resistance to SB stands out as the most effective strategy for managing this disease. Therefore, the primary objective of the current study was to validate the resistance to SB in one hundred twenty-seven, dicoccum wheat germplasm accessions. This validation process relied on both observable traits and genetic characteristics. The germplasm accessions were obtained from various sources, including the International Center for Agricultural Research in the Dry Areas in Lebanon, the International Maize and Wheat Improvement Center in Mexico, and the International Maize and Wheat Improvement Center (CIMMYT) in Mexico. The evaluation for Spot blotch resistance was conducted in the field under controlled conditions of disease prevalence during the years 2020–21 and 2021–22. The assessment involved two vulnerable control cultivars (DDK-1025 and Sonalika) and two confirmed resistant control cultivars (Chirya-3 and HI-8663). Out of the 127 germplasm accessions tested, forty displayed resistance to SB, confirmed through a specific genetic marker known as *Xgwm*120. Notably, four accessions – Acc. GPM Dicoccom-IR-76, Acc. GPM Dicoccom-IR-98, Acc. GPM Dicoccom-IR-102, and ICARDA-14-127687 – exhibited even greater resistance than the well-established Spot blotch-resistant genotype, Chirya-3. These promising lines with

resistance potential offer significant prospects for wheat breeders dedicated to developing spot blotch resistant wheat lines.

Keywords: Germplasm Lines, Host Resistance, AUDPC, Spot Blotch.

1. Introduction

Wheat (*Triticum aestivum* L.) plays a crucial role in the global cereal economy, being the most extensively cultivated crop worldwide, covering an estimated 220.94 million hectares (m. ha.) of land. In the 2021-22 season, India secured the second position with a production of 107.74 million tons from a cultivated area of 30.46 m. ha. (www.indiastat.com, 2022). Given its vital role as a fundamental food source, ensuring consistent wheat production is essential for food security and nutritional well-being. While past yield improvements focused on key traits like plant height, photoperiodism, and vernalization, meeting future demand requires tapping into novel genetic reservoirs [23]. Dicoccum wheat (*Triticum dicoccum* Schrank.), a tetraploid hulled variety, thrives in hot tropical climates and stands out for its nutritional and therapeutic superiority compared to other wheat varieties, boasting higher protein and dietary fibre contents ([26] and [3]). In warmer wheat cultivation zones worldwide, the prevalence of spot blotch (SB) or foliar blight caused by *Bipolaris sorokiniana* is a significant challenge. It affects wheat-growing regions in Bangladesh, Nepal, southeast Asia, Latin America, eastern India, southeast China, south-east Australia, sub-Saharan Africa, northern Kazakhstan, as well as the Great Plains of the USA and Canada. SB leads to substantial yield reductions, sometimes up to 70 percent under favorable climatic conditions for susceptible cultivars, concurrently compromising grain quality ([2]; [21]; [27]; [7]; [1]; [19]; [8]). With changing global climate patterns, SB is becoming a concern in new areas with irrigated and low precipitation wheat production systems, particularly in the Indo-Gangetic and Trans-Gangetic plains [10].

Research on DNA polymorphism, comparative genomics, and electrophoretic karyotypes of *B. sorokiniana* through whole-genome sequencing reveals variations in karyotypes and genome size among diverse isolates. This suggests that the differentiation observed among SB fungal isolates results from structural alterations affecting chromosomes and genomes, including translocations and deletions/duplications [9]. With evolving climatic conditions, the threat from this fungus increases. Various strategies, such as optimal planting times, chemical interventions, fertilization, tillage, and crop rotation, have been proposed to manage SB, but host resistance remains a foundational element [18]. SB resistance is quantitative, influenced by the complex interplay between genotypes and the environment ([18]; [13]; [10]).

Building on Kumar et al. (2010) findings, which identified closely linked markers, *Xgwm*120 on chromosome 2B and *Xgwm*291 on chromosome 5A, as potential markers for SB resistance, the current study assesses the susceptibility of dicoccum wheat lines from CIMMYT, Mexico, and ICARDA, Lebanon, to SB in real field conditions under controlled disease outbreak circumstances. The confirmation of resistance utilized the specific gene-linked SSR marker, *Xgwm*120.

2. Materials and methods

2.1. Site of experimentation and agricultural management procedures.

The field trials were conducted during the winter cropping seasons (Rabi) of 2020–21 and 2021–22 at the

Mainland Agricultural Research Station (MARS), University of Agricultural Sciences, Dharwad, situated in the transitional region of Karnataka state. The site is located at approximately 15º26' N latitude and 75º07' E longitude, with an elevation of 678 meters above sea level. Dharwad receives an average annual rainfall of about 675 mm, spanning seven to eight months from April to November.

To maintain consistency, standard agronomic practices for regular fertility (120 kg: 60 kg: 40 kg, N, P2O5, K2O) were strictly followed. The entire quantity of K2O and P2O5 was administered at the time of sowing. Nitrogen application was staggered, with 1/3 applied during sowing, another 1/3 at the first irrigation (21 days after sowing), and the remaining 1/3 at the second irrigation (40 days after sowing). In addition to these practices, conventional agricultural techniques were employed to ensure the cultivation of a robust crop throughout the trials.

2.2. Field-based assessment of resistance to Spot blotch

In the years 2019 and 2020, a total of one hundred twenty-seven, dicoccum wheat germplasm samples were acquired from CIMMYT and ICARDA. These samples were subjected to an assessment for Spot blotch resistance. Employing the Augmented Block Design, each germplasm entry was planted in two rows with a 20 cm gap, covering a length of 1 meter. To implement control measures, every 25th entry was paired with the planting of two resistant checks, Chirya-3 and HI-8663, as well as two susceptible checks, Sonalika and DDK-1025.

To mimic artificial epiphytotic conditions, the field environment was manipulated following the methodology outlined by [4]. For inoculation, plants were exposed to a suspension of *B. sorokiniana* isolates sourced from the ICAR-Indian Institute of Wheat and Barley Research (IIWBR) in Karnal, India. The inoculum was cultivated on sorghum seeds within the laboratory facilities at the Pathology Lab of UAS, Dharwad.

All germplasm samples underwent inoculation, involving the application of a sporidial suspension containing 104 spores/ml at three distinct stages: tillering, flag leaf emergence, and anthesis. This process was carried out in the evening, followed by irrigation to maintain elevated relative humidity levels, thereby facilitating optimal disease establishment.

2.3. Disease assessment

Disease presence was quantified using a two-digit numerical scale (00–99), adapted from Saari and Prescott's severity scale, specifically tailored for assessing foliar blight conditions in wheat. The evaluation focused on the proportion of the diseased area on both the flag (F) and the penultimate leaf (F − 1), as detailed in Table 1 [17]. The first digit (D1) denoted disease coverage on the flag leaf, while the second digit (D2) represented severity on the penultimate leaf.

Subsequently, disease severity within each set of germplasm accessions was documented at three distinct growth stages (GS): GS 63 (from the beginning of anthesis to halfway through its completion), GS 69 (completion of anthesis), and GS 77 (late milking). To provide a comprehensive assessment, the area under the disease progress curve (AUDPC) was calculated based on disease severity at GS 63, GS 69, and GS

77 over a specific time period. This practical approach for disease assessment, recognized in the field [12], was determined using the formula outlined [16].

$$AUDPC = \sum_{i=1}^{n} \left(\left\{ \frac{Yi + Y_{(i+1)}}{2} \right\} \times t_{(i+1)} - t_i \right)$$

In the context of the formula, where ti represents the time (days) between two disease scores, and t (i + 1) − ti denotes the duration between subsequent assessments. Yi signifies the disease level at time n, where n is the number of dates on which Spot Blotch (SB) was recorded. Lines exhibiting an Area Under the Disease Progress Curve (AUDPC) below 500 were categorized as resistant, while those surpassing an AUDPC of 2000 were deemed susceptible, as per the classification outlined [24].

2.4. Polymerase Chain Reaction (PCR) and electrophoretic analysis

The genetic marker analysis was conducted at the Molecular Biology Laboratory of the AICRP on Wheat and the Institute of Agri-Biotechnology, Department of Biotechnology, both situated at UAS, Dharwad. Genomic DNA isolation utilized the CTAB method, adapted from the procedure outlined by [6]. The polymerase chain reaction (PCR) was carried out using the microsatellite marker *Xgwm*120, closely linked to the QTL for Spot Blotch (SB) resistance on chromosome 2B, as detailed by [14]. The *Xgwm*120 marker yields a DNA fragment of 174 base pairs.

Table 1: A double-digit scale for scoring the Spot Blotch severity

Severities		Rating	
Flag leaf	**Flag leaf-1**	**Diseases responses**	**Range of values**
0	0–1	Immune (I)	00–01
1–2	2–4	Resistant (R)	12–24
3–4	4–6	Moderately resistant (MR)	34–46
5–6	6–8	Moderately susceptible (MS)	56–68
7–8	8–9	Susceptible (S)	78–89
9	9	Highly susceptible (HS)	99

The first and second values indicate the percentage of blighted area on the uppermost leaf (flag) and the leaf immediately below it. The numerical assignments 1 through 9 correspond to blighted areas of 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, and 90%, respectively, as outlined in reference [17].

For the PCR process, a 20 µl reaction mixture was prepared, comprising a buffer (10X) with 10mM Tris-HCl (pH 9.0), 15 mM MgCl2, 50mM KCl, and 2.5 mM of each deoxyribonucleotide (dNTP). Additionally, the mixture included 40 ng of each primer, 0.01% gelatin, 1 unit of Taq DNA polymerase from Genei Merck in Bangalore, India, and 50 ng of genomic DNA.

Amplifications were carried out using a Gradient Thermal cycler (Sigma-SVI BioSolutions Pvt. Ltd., New Delhi, India), following a program initiating at 94°C for 4 minutes. Subsequently, 40 cycles were performed at 95°C for 1 minute, the annealing temperature for 1 minute, and 72°C for 30 seconds. The final extension step was maintained at 72°C for 10 minutes. The complete PCR product was then assessed on 3.5% agarose gels stained with ethidium bromide. Visualization was accomplished using the gel documentation system. The determination of the presence or absence of the desired allele for each specific gel was conducted manually. In total, molecular characterization was applied to 127 accessions.

3. Results

In the current investigation, an integral aspect of the phenotypic assessment involved a comprehensive field screening of 127 diverse germplasm samples conducted over the consecutive years of 2020–21 and 2021–22. This screening was accomplished by introducing a mixture of potent Indian Spot Blotch (SB) isolates, resulting in a diverse range of disease severity levels and resistance patterns across germplasm with varying pathological behaviours (Fig. 1, Table 2, Table 3). To ensure the relevance of phenotypically resistant germplasm accessions, a microsatellite marker closely linked to the potential SB-resistant Quantitative Trait Locus (QTL) and the pleiotropic All-stage Pathogen Resistance (APR) gene *Lr34* was employed. The findings from both the field experiments and molecular analysis are elaborated upon in the subsequent sections.

3.1. Assessment of wheat germplasm accessions in the field under conditions conducive to disease outbreak.

During the Rabi seasons of 2020–21 and 2021–22, disease severity observations on the control entries, recorded on a double-digit scale (00–99), revealed that the Sonalika and DDK-1025 checks exhibited a consistently highly susceptible (HS) response as expected. The corresponding Area Under the Disease Progress Curve (AUDPC) values were 2072.0, 2267.0, and 2015.5, and 2130.0 for the respective years. In contrast, the HI-8663 check displayed the anticipated resistant (R) reaction, registering AUDPC values of 386.0 and 484.5 over the two years. Similarly, the Chirya-3 check demonstrated a consistently resistant response, with AUDPC values of 373.0 and 452.3 for both years (Table 2). Generally, disease pressure was lower in the first year compared to the second year, as reflected in the disease scores of the control entries.

In the field experiment conducted during the 2020–21 season, out of the total 127 germplasm accessions, 15 were categorized as highly susceptible (HS), 20 as susceptible (S), 32 as moderately susceptible (MS), 26 displayed moderate resistance (MR), 28 exhibited resistance (R), and six showed a highly resistant (HR) response (Fig. 1). Notably, germplasm accessions with an HR reaction included Acc. GPM Dicoccom-IR-98, Acc. GPM Dicoccom-IR-102, Acc. ICARDA -14- 127687, and Acc. GPM Dicoccom-IR-76, each presenting an average Area Under the Disease Progress Curve (AUDPC) value below 100 (Table 3). Additionally, Acc. ICARDA -3-127689 and Acc. ICARDA -17- 45363 exhibited an HR reaction, with average AUDPC values falling between 100 and 300.

Similarly, in the subsequent year (2021–22) of experimentation with the same set of 127 germplasm accessions (Table 3), only four demonstrated an HR reaction, 31 displayed resistance (R), 23 showed MR, 35 exhibited MS, 16 were S, and 18 exhibited an HS response (Fig. 1). It is noteworthy that three

accessions, namely, Acc. GPM Dicoccom-IR-76, Acc. GPM Dicoccom-IR-98, Acc. GPM Dicoccom-IR-102, and ICARDA-14-127687, consistently exhibited an HR reaction in both experimental years.

However, during the 2020–21 period, two accessions, namely Acc. ICARDA-17-45363 and ICARDA-3-127689, displayed a highly resistant (HR) response, while in the subsequent year, 2021–22, they exhibited a resistant (R) reaction. Interestingly, the majority of accessions, including the control entries, showcased higher Area Under the Disease Progress Curve (AUDPC) values in 2021–22 compared to the values observed in 2020–21.

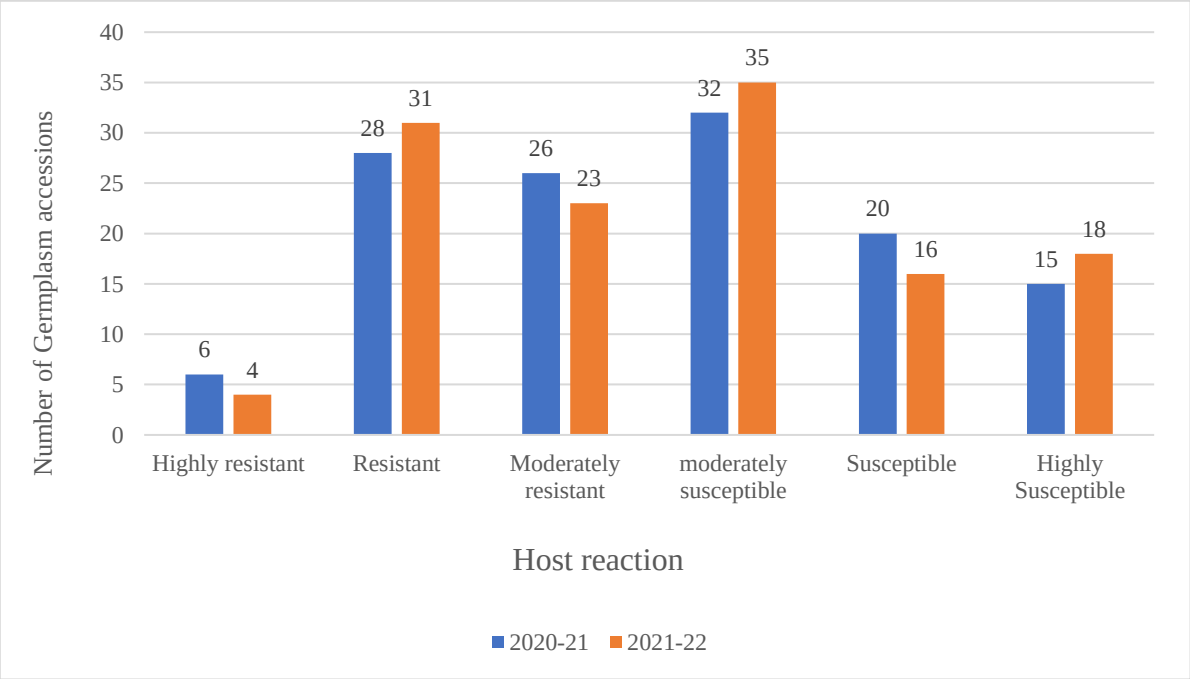

Figure 1: Illustrates the distribution pattern of pathological ratings among the 127 germplasm accessions, showcasing their response to spot blotch. This observation was made during the field-based study conducted for both the 2020–21 and 2021–22 periods.

3.2. Employing molecular techniques to validate the resistance to spot blotch (SB).

The molecular evaluation of Spot blotch resistance involved the scrutiny of 127 wheat germplasm accessions using the specific SSR marker, *Xgwm*120, associated with the resistance Quantitative Trait Locus (QTL) on chromosome 2B. The analysis also encompassed reference checks: Sonalika (C1), DDK-1025 (C2), HI-8663 (C3), and Chirya-3 (C4). The *Xgwm*120 marker produced a distinctive band of 174 bp in the cases of C3 and C4. Out of the 127 germplasm accessions, *Xgwm*120 was identified in only 40 accessions.

Notably, despite not displaying amplification for the *Xgwm*120 marker, Acc. GPM Dicoccom-IR-68 exhibited a resistant host reaction and an average Area Under the Disease Progress Curve (AUDPC) value below 100 (Table 3). The amplification of *Xgwm*120 in the resistant checks, Chirya-3 and HI-8663, as well as in germplasm lines demonstrating phenotypic resistance, is visually depicted in the illustrative gels presented in Figure 2.

Table 2: The response of the check genotypes to spot blotch severity, along with the AUDPC values for the years 2020-21 and 2021-22, and their molecular status linked to *Xgwm*120 were examined.

		Phenotypic response to disease				AUDPC value		
		2020-21		2021-22				
Sl.no	Checks	Disease severity	Resistance type[a]	Disease severity	Resistance type[a]	2020-21	2021-22	Molecular response with *Xgwm*120
1	Sonalika	98	HS	99	HS	2070.0	2072.5	-
2	DDK-1025	93	HS	96	HS	2015.5	2130.0	-
3	HI-8663	27	R	29	R	386.0	484.5	+
4	Chirya-3	22	R	23	R	373.0	452.3	+

a. The host reaction type was availed as R: resistant; HS: highly susceptible.

4. Discussion

Wheat cultivation faces numerous biotic and abiotic challenges, with rust and Spot Blotch (SB) standing out prominently. In the Indo-Gangetic Plains of India, a region primarily devoted to wheat cultivation, rust and SB pose significant threats. Spot Blotch, in particular, is a growing concern in the eastern region of South Asia and India due to its widespread occurrence and elevated severity levels. This study is specifically focused on identifying Dicoccum wheat germplasm accessions that exhibit resistance to Spot Blotch, contributing to the efforts in addressing this pressing issue.

Among the four check varieties, both Chirya-3 and HI-8663 demonstrated true resistance, evident from their low Area Under the Disease Progress Curve (AUDPC) scores, indicating a resistant host reaction. Sonalika and Chirya-3, representing susceptible and resistant genotypes, have been frequently employed in identifying Quantitative Trait Loci (QTLs) against Spot Blotch *(B. sorokiniana*), as documented in studies by [22] and [14].

The marker *Xgwm*120 failed to amplify in both susceptible benchmarks genotypes, Sonalika and DDK-1025. Notably, this marker showed no amplification in any accessions displaying highly susceptible (HS), susceptible (S), moderately susceptible (MS), and moderately resistant (MR) reactions. The consistent resistance observed in Chirya-3 and HI-8663, as well as the susceptibility demonstrated by Sonalika and DDK-1025 during both the 2020–21 and 2021–22 years, suggests highly conducive climatic conditions in Dharwad for disease development.

In a study by [5], it was noted that the genotype with the highest grain yield and weight (Altar-84/*Ae. squarrosa* (224)//Yaco) also exhibited minimal disease severity, showcasing the integration of Spot Blotch resistance and high grain yield—a previously unattained achievement. This aligns with our findings, as Acc. GPM Dicoccom-IR- 40 and Acc. GPM Dicoccom-IR- 15, within the Altar84/*Ae. squarrosa* background, were identified as having a resistant host reaction with average AUDPC values of 213.5 and 197.8, respectively (Table 3).

Over the years, diverse sources of resistance against Spot Blotch (SB) have been identified, often governed by one or multiple genes. These sources stem from three distinct categories: China, Latin America, and wild relatives of wheat or alien species [25]. Notably, *Ae. squarrosa* crosses exhibited remarkable SB resistance in Mexico. The management of SB disease in wheat has necessitated a multifaceted strategy, leading to the creation of contemporary sources of resistance that serve as donors. Consequently, numerous high-yielding lines with SB resistance have been identified and shared across different centres within India [20].

Enhancing SB resistance through breeding has been a pivotal goal in CIMMYT's wheat improvement endeavours. Large-scale screening of germplasm for Spot Blotch resistance was conducted at ICARDA and CIMMYT during the 1980s and 1990s, resulting in the widespread integration of these resistant lines into their respective wheat breeding programs. To facilitate the global adoption of SB-resistant materials by breeders and researchers, a specialized nursery named CSISA-SB was established in 2009. This nursery initially featured elite CIMMYT breeding lines with promising SB resistance, commendable agronomic traits, and high yield potential [21]. Subsequently renamed the *Helminthosporium* leaf blight screening nursery (HLBSN), this initiative expanded to several South Asian and South American countries where SB is a significant concern, as well as to other regions grappling with the disease [22].

Table 3: Germplasm accessions showing host reaction, AUDPC value <100 and between 100-200 over 2020–21 and 2021–22 and molecular status with *Xgwm*120.

	Host reaction to disease					
	2020-21		2021-22			
Accession	Disease Severity	Resistance type[a]	Disease Severity	Resistance type[a]	Average AUDPC values	Molecular status with *Xgwm*120
ICARDA -14- 127687	0	HR	0	HR	0.0	+
GPM Dicoccom-IR- 102	0	HR	01	HR	1.8	+
GPM Dicoccom-IR- 76	0	HR	0	HR	0.0	+
ICARDA -17- 45363	0	HR	12	R	176.8	+
GPM Dicoccom-IR- 82	23	R	12	R	83.8	+
ICARDA -3- 127689	01	HR	12	R	117.3	+
GPM Dicoccom-IR- 98	01	HR	01	HR	2.7	+
GPM Dicoccom -IR- 97	12	R	12	R	56.0	b
ICARDA -9-126374	12	R	12	R	52.5	+

a. The host reaction type was availed as HR: highly resistant; R: resistant
b. No amplification was observed.

The results from both experiments reveal a significant genetic diversity among the evaluated germplasm accessions. This diversity was evident in the categorization of accessions into groups representing varying levels of disease severity, including HS, MS, MR, R, and HR, based on their performance under epiphytotic conditions. Moreover, the robust molecular marker confirmed the genetic resistance of 40 accessions that exhibited phenotypic resistance in the field. Consequently, these 40 germplasm accessions,

demonstrating not only field-based phenotypic resistance under epiphytotic conditions but also validation through the *Xgwm*120 marker, stand as reservoirs of resistant genetic material. These accessions are highly suitable for integration into hybridization strategies aimed at producing Spot Blotch (SB)-resistant wheat varieties.

Noteworthy are the germplasm accessions consistently displaying an HR reaction across both years, specifically GPM Dicoccom-IR-76, Acc. GPM Dicoccom-IR-98, Acc. GPM Dicoccom-IR-102, and ICARDA-14-127687, which hold promise for the creation of mapping populations and Quantitative Trait Locus (QTL) detection. This approach can significantly accelerate the development of SB-resistant varieties using marker-assisted breeding techniques.

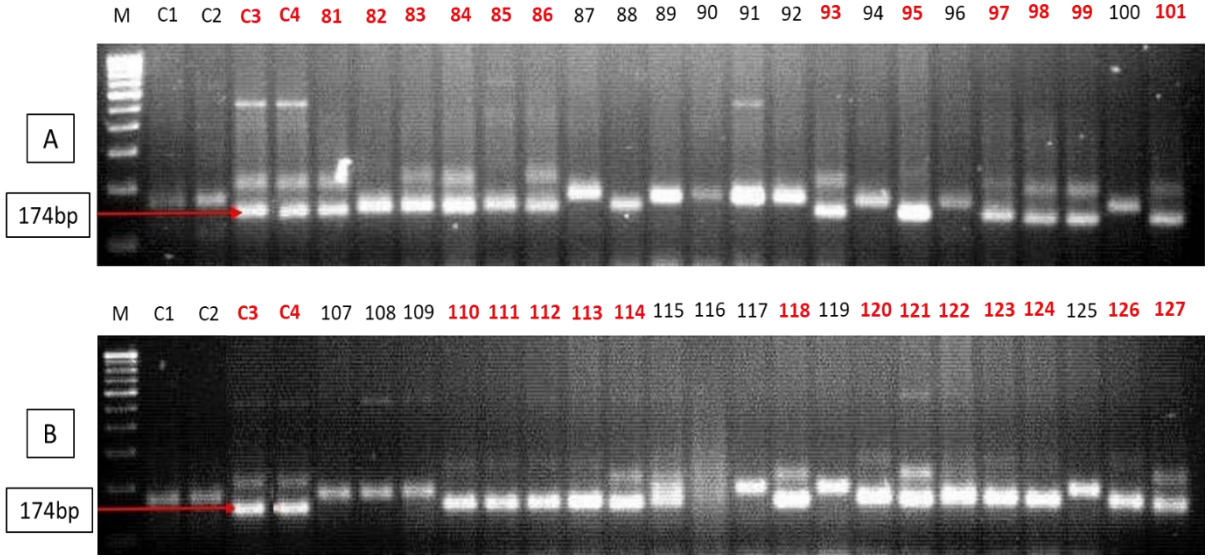

Figure 2: Representative agarose gel electrophoresis results from the screening process for spot blotch resistance across a collection of 127 germplasm accessions. In panel A, the ladder M-100bp is depicted, along with reference samples C1 (Sonalika), C2 (DDK-1025), C3 (HI-8663), and C4 (Chirya 3). Lanes 81–101 showcase the molecular screening of germplasm accessions using the SSR marker *Xgwm*120 (174bp). Accessions displaying the desired allele of 174bp are indicated by red-marked lanes. Similarly, panel B features the ladder M-100bp, along with the same reference samples C1, C2, C3, and C4. Lanes 107–127 highlight the accessions with the presence of the desirable 174bp allele, marked in red.

5. Conclusion

Elevating the quality of any crop is contingent upon the comprehensive exploration and efficient utilization of the abundant genetic diversity inherent in its cultivated varieties, indigenous strains, wild counterparts, and related genera. The importance of conserving a resource becomes particularly evident when that resource exhibits or attains recognized value [15]. In this regard, Dicoccum Wheat germplasm accessions stand out as valuable assets. The opportunity to broaden and diversify the genetic foundation for Spot Blotch (SB) resistance in cultivars can be actualized by incorporating the resistant accessions identified in the current investigation.

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