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The Pharma Innovation

ISSN (E): 2277-7695
ISSN (P): 2349-8242
NAAS Rating: 5.23
TPI 2023; 12(9): 116-121

www.thepharmajournal.com
Received: 21-06-2023
Accepted: 27-07-2023

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Characterization of Emmer wheat (*Triticum dicoccum* (Schrank.) Schübl.) Germplasm for spot blotch *Bipolaris sorokiniana* (SACC.) Shoemaker resistance

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Abstract
The significance of genetic resources lies in their crucial role in enhancing the quality of crop varieties, particularly in terms of their ability to withstand both biological and environmental stresses. Spot blotch (SB) is a devastating leaf disease affecting wheat, predominantly found in warm and humid regions, particularly in the eastern parts of South Asia. The most effective strategy for managing this disease is the creation of wheat cultivars that are resistant to it. As a result, the current study aimed to validate the resistance to SB in 127 emmer wheat germplasm accessions. This validation was based on both observable traits and genetic characteristics. These accessions were sourced from the International Center for Agricultural Research in the Dry Areas in Lebanon 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 took place in the field under controlled conditions of disease prevalence during the years 2020–21 and 2021–22. This evaluation included 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 exhibited resistance to SB, which was further confirmed through a specific genetic marker called Xgwm120. Among these, four accessions – Acc. GPM Dicoccom-IR-76, Acc. GPM Dicoccom-IR-98, Acc. GPM Dicoccom-IR-102, and ICARDA- 14-127687 – demonstrated even greater resistance than the widely recognized Spot blotch- resistant genotype, Chirya-3. These lines with resistance potential hold promise for wheat breeders aiming to develop SB-resistant wheat varieties.

Keywords: Dicoccum germplasm, host resistance, AUDPC, spot blotch

Introduction
Wheat (*Triticum aestivum* L.) holds a significant role within the global cereal economy. It stands as the most extensively grown crop worldwide, encompassing a projected
220.94 million hectares (mha) of land. In the 2021-22 season, India secures the second position, achieving a production of 107.74 million tons from a cultivated area of 30.46 mha (www.indiastat.com, 2022). Given its essential status as a fundamental food source, maintaining a consistent wheat production becomes imperative to ensure food security and nutritional well-being. While previous yield increments have primarily resulted from manipulating a handful of key traits like plant height, photoperiodism, and vernalization, meeting future demand for increased yields will necessitate harnessing novel genetic reservoirs [23]. Emmer wheat [*Triticum dicoccum* (Schrank.) Schübl.], a variety of hulled wheat, thrives in regions with a prevailing hot tropical climate characterized by sustained high daily temperatures during the crop's growth phase, impacting the GS1 and GS3 stages. Emmer wheat stands out for its nutritional and therapeutic superiority in comparison to commercially available bread and durum wheat, boasting higher protein and dietary fiber (DF) contents [26, 3]. Numerous diseases have an impact on the warmer zones of wheat cultivation across the world, and among these, the occurrence of spot blotch (SB) or foliar blight brought about by *Bipolaris sorokiniana* (SACC.) Shoemaker stands out as a notably troublesome challenge. Its prevalence extends across wheat-growing regions encompassing Bangladesh, Nepal, certain portions of 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. This disease results in substantial yield reductions, occasionally even reaching up to 70 percent under favorable climatic conditions in the presence of susceptible cultivars, while concurrently undermining grain quality [2, 21, 27, 7, 1, 19, 8].

In light of the evolving global climate patterns, SB is emerging as a significant concern in new areas characterized by irrigated and low precipitation wheat production systems. This encompasses specific regions within the breadbasket of South Asia, notably the Indo-Gangetic and Trans- Gangetic plains [10].

Significant discoveries emerging from investigations focused on DNA polymorphism, comparative genomics and electrophoretic karyotypes conducted via whole-genome sequencing of B. sorokiniana, reveal variations in karyotypes and genome size across diverse

isolates. This observation underscores the fact that the differentiation observed among SB fungal isolates arises from a range of structural alterations affecting chromosomes and genomes, including actions like translocations and deletions/duplications [9]. This highlights an increased potential for threats originating from this fungus within evolving climatic conditions. A variety of strategies aimed at preventing SB have been proposed and employed, encompassing actions such as optimal planting times, effective, chemical interventions, fertilization, tillage and crop rotation. However, the foundational element of disease management has consistently been the presence of host resistance [18]. The nature of SB resistance is quantitative and is influenced by the intricate interplay between genotypes and the environment [18, 13, 10].

Kumar *et al*. (2010), using two different breeding populations, HI-8663×Sonalika and Chirya-3×Sonalika (where Chirya-3 is highly resistant and Sonalika is highly susceptible for Spot blotch), validated that the closely linked markers, Xgwm120 on chromosome 2B and Xgwm291 on chromosome 5A, have the potential to serve as identifying markers for SB resistance. Building upon this insight, the current study was initiated to assess the susceptibility of emmer wheat lines sourced from the CIMMYT, Mexico, and the ICARDA, Lebanon, to SB in real field conditions under controlled disease outbreak circumstances. Subsequent confirmation of resistance utilized the specific gene-linked SSR marker, Xgwm120.

Materials and Methods

Site of experimentation and agricultural management procedures

The field trials were executed over the winter cropping seasons (Rabi) of 2020–21 and 2021–22 at the Main land Agricultural Research Station (MARS), University of Agricultural Sciences, Dharwad, part of the transitional region of Karnataka state. The geographical coordinates of the site are approximately 15º26' N latitude and 75º07' E longitude, with an elevation of 678 meters above sea level. Dharwad experiences an average annual rainfall of around 675 mm, distributed across a span of seven to eight months, from April to November. Standard agronomic practices for regular fertility (120 kg: 60 kg: 40 kg, N, P2O5, K2O,) were adhered to. The entire amount of K2O and P2O5 was administered at the time of sowing. Nitrogen was applied in increments: 1/3 during sowing, 1/3 at the first irrigation (21 days after sowing), and the remaining 1/3 at the second irrigation (40 days after sowing). Additional conventional agricultural techniques were implemented to cultivate a robust crop.

Field-based assessment of resistance to Spot blotch

A collection of 127 emmer wheat germplasm samples was sourced from CIMMYT and ICARDA in the preceding years, 2019 and 2020. These lines underwent an evaluation for resistance to Spot blotch. In the Augmented Block Design, each germplasm entry was sown in two rows with a 20 cm gap, spanning a length of 1 meter. To establish control measures, every 25th entry was followed by the planting of two resistant checks, Chirya-3 and HI-8663, as well as two susceptible checks, Sonalika and DDK-1025. The field environment was manipulated to replicate artificial epiphytotic conditions in accordance with the method outlined by [4]. For inoculation, plants received a suspension of *B. sorokiniana* isolates, derived from the ICAR- Indian Institute of Wheat and Barley Research (IIWBR), 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, achieved by spraying a sporidial suspension containing 104 spores/ml at three distinct stages: tillering, flag leaf emergence, and anthesis. This process was conducted during the evening hours, followed by irrigation to sustain elevated relative humidity levels, thus promoting optimal disease establishment.

Disease assessment

The level of disease presence was recorded using a numerical scale with two digits scale (00–99), which was devised as a modified version of Saari and Prescott's severity scale. This scale was created to assess foliar blight conditions in wheat, involving the visual evaluation of the diseased area proportion on both the flag (F) and the flag leaf minus one i.e., penultimate (F-1), as elaborated in Table 1 [17]. The initial digit (D1) indicates the extent of disease coverage on the flag leaf, while the second digit (D2) represents the severity on the penultimate leaf. Subsequently, the percentage of disease severity within each set of germplasm accessions was recorded at three different growth stages (GS): GS 63 (beginning of anthesis to halfway through anthesis completion), GS 69 (completion of anthesis), and GS 77 (late milking). The calculation of the area under the disease progress curve (AUDPC) based on disease severity at GS 63, GS 69, and GS 77 over a specific time period has been acknowledged as a practical approach for disease assessment [12]. This value was determined using the following formula [16].

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

Where ti; t (i + 1) − ti = time (days) between two disease scores; Yi = disease level at time n = number of dates on which SB was recorded. The lines that showed AUDPC (<500) were considered resistant and the lines that showed AUDPC (>2000) were considered susceptible [24].

Table 1: A double-digit scale for appraising 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

First and second values represent per cent blighted area on the top (flag) and second top leaves. Values 1, 2, 3, 4, 5, 6, 7, 8 and 9 correspond to 10, 20, 30, 40, 50, 60, 70, 80 and 90% blighted area, respectively (17).

Polymerase Chain Reaction (PCR) and electrophoretic analysis

The analysis of genetic markers was carried out 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 was executed using the CTAB method, which was adapted from the procedure described by [6]. The polymerase chain reaction (PCR) was performed using the microsatellite marker Xgwm120, which is closely linked to the QTL for SB resistance located on chromosome 2B, as detailed by [14]. The Xgwm120 marker produces a DNA fragment of 174 base pairs. For the PCR process, a reaction mixture of 20 µl was prepared, consisting of a buffer (10X) containing 10 mM Tris-HCl (pH 9.0), 15 mM MgCl2, 50 mM KCl, and 2.5 mM of each deoxyribonucleotide (dNTP); 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.

The amplifications were executed using a Gradient Thermal cycler (Sigma-SVI BioSolutions Pvt. Ltd., New Delhi, India), with the program starting at 94 °C for 4 minutes, followed by 40 cycles at 95 °C for 1 minute, at the annealing temperature for 1 minute, and 72 °C for 30 seconds. The final extension step was held at 72 °C for 10 minutes. The entire PCR product was then examined on 3.5% agarose gels that were stained with ethidium bromide. The visualization was conducted on the gel documentation system. For each specific gel, the analysis to determine the presence or absence of the desired allele was carried out manually. In total, molecular characterization was applied to 127 accessions.

Results

In the present study, as an essential component of the phenotypic assessment, a field screening investigation involving 127 diverse germplasm samples was carried out over two consecutive years, 2020–21 and 2021–22. This was achieved by inoculating a mixture of potent Indian SB isolates. Consequently, a wide spectrum of disease severity levels and resistance patterns emerged within germplasm featuring varied pathological behaviours (Fig. 1, Table 2, Table 3). To ensure the applicability of phenotypically resistant germplasm accessions, a microsatellite marker closely linked to the potential SB-resistant QTL and the pleiotropic APR gene Lr34 was employed. The outcomes from the field experiments and molecular analysis are detailed as follows.

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

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

During the field experiment carried out in the 2020–21 season, among the total of 127 germplasm accessions, 15 were highly susceptible (HS), 20 were susceptible (S), 32 were moderately susceptible (MS), 26 showed moderate resistance (MR), 28 exhibited resistances (R), and six displayed a highly resistant (HR) response (Fig. 1). The germplasm accessions indicating 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 with an average 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, among 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 showed an HS response (Fig. 1). It's worth noting that three accessions, namely, Acc. GPM Dicoccom -IR-76, Acc. GPM Dicoccom -IR-98, Acc. GPM Dicoccom -IR-102, and ICARDA-14-127687, consistently displayed an HR reaction in both experimental years Nevertheless, two accessions, specifically, Acc. ICARDA-17-45363 and ICARDA-3- 127689, exhibited an HR response in the 2020–21 period, whereas they displayed an R reaction in the subsequent year, 2021–22. The majority of accessions, encompassing the control entries, demonstrated elevated AUDPC values in 2021–22 in comparison to the values observed in 2020–21.

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

The molecular assessment of Spot blotch resistance was conducted on a collection of 127 wheat germplasm accessions, utilizing the specific SSR marker, Xgwm120, which is associated with the resistance QTL on chromosome 2B. The same analysis included the reference checks: Sonalika (C1), DDK-1025 (C2), HI-8663 (C3), and Chirya-3 (C4). The Xgwm120 marker yielded a specific band of 174 bp in the cases of C3 and C4. Out of the 127 germplasm accessions, Xgwm120 was detected in just 40 accessions. Notably, Acc. GPM Dicoccom-IR-68 displayed a resistant host reaction and an average AUDPC value below 100, despite not showing amplification for this marker (Table 3). Two illustrative gels, displayed in Figure 2, visually depict the amplification of Xgwm120 in the resistant checks, Chirya-3 and HI-8663, as well as in the germplasm lines showing phenotypic resistance.

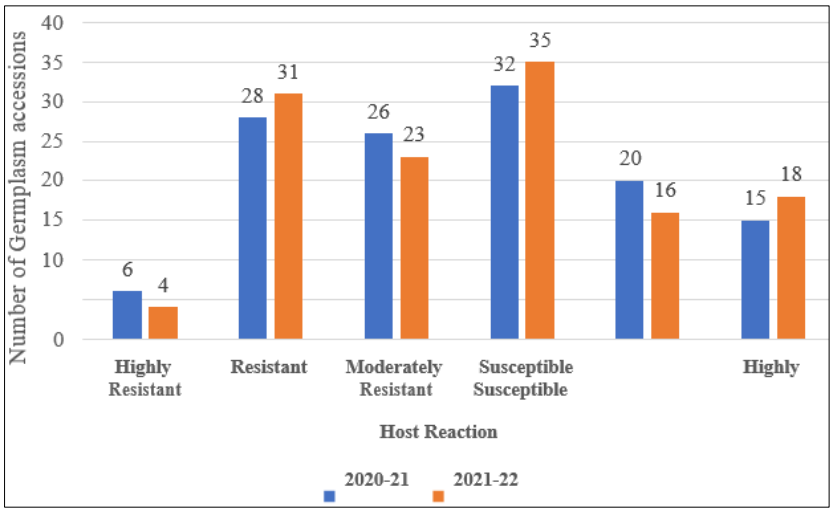

Fig 1: The distribution pattern of pathological ratings among the 127 germplasm accessions, indicating their response to spot blotch, was observed during the field-based study for both the 2020–21 and 2021–22 periods

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 Xgwm120 were examined.

		Phenotypic response to disease				AUDPC value		
		2020-21		2021-22				
Sl.no	Checks	Disease severity	Resistance typea	Disease severity	Resistance typea	2020-21	2021-22	Molecular response with Xgwm120
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	+

Discussion

Various biotic and abiotic challenges hinder wheat cultivation. Among the biotic challenges, rust and SB hold significant significance in the Indo-Gangetic Plains of India, an area that predominantly cultivates wheat. Due to its extensive occurrence and high levels of severity, SB is becoming progressively worrisome in eastern region of South Asia and the India. This study is dedicated to the identification of Emmer wheat germplasm accessions with resistant to Spot blotch

Among the four check varieties, both Chirya-3 and HI-8663 exhibited true resistance, evident from their low AUDPC scores indicative of a resistant host reaction. Sonalika and Chirya-3, which respectively represent susceptible and resistant genotypes, have been frequently utilized in identifying QTLs against Spot blotch (*B. sorokiniana*), as evidenced by studies conducted by [22, 14]. The marker Xgwm120 failed to amplify in both the susceptible benchmarks, Sonalika and DDK-1025. Notably, this marker exhibited no amplification in any of the accessions that demonstrated HS, S, MS and MR reactions. The resistance displayed by Chirya 3 and HI-8663, as well as the susceptibility shown by Sonalika and DDK-1025, during both the 2020–21 and 2021–22 years, indicated that the climatic conditions in Dharwad were highly conducive to disease development. In a study by [5], it was observed that the genotype with the highest grain yield and weight (Altar-84/*Ae. squarrosa* (224)//Yaco) also exhibited minimal disease severity. This highlights the advancement in combining Spot blotch resistance and high grain yield, an accomplishment previously unattainable. This aligns with our findings, as Acc. GPM Dicoccom -IR- 40 and Acc. GPM Dicoccom -IR- 15, within the Altar 84/*Ae. squarrosa* background, were identified as having a resistant host reaction and average AUDPC values of 213.5 and 197.8, respectively (Table 3).

Over the years, various sources of resistance against SB have been identified, often controlled by one or multiple genes. These sources originate from three distinct categories: China, Latin America and wild relatives of wheat or alien species [25]. Notably, *Ae. squarrosa* crosses demonstrated remarkable SB resistance in Mexico. Dealing with SB disease in wheat has involved a multifaceted strategy, leading to the creation of contemporary sources of resistance that serve as donors. Consequently, numerous high-yielding lines possessing SB resistance have been pinpointed and shared across different centers within India [20]. Enhancing SB resistance through breeding has been a pivotal objective within CIMMYT's wheat improvement endeavors. Sizeable-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 possessing promising SB resistance, as well as commendable agronomic traits and high yield potential [21]. Subsequently renamed the *Helminthosporium* leaf blight screening nursery (HLBSN), this initiative extended to several South Asian and South American countries where SB is of paramount 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 Xgwm120.

Accession	Host reaction to disease					
	2020-21		2021-22			
	Disease Severity	Resistance type	Disease Severity	Resistance type	Average AUDPC values	Molecular status with Xgwm120
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 findings from both experiments indicated a substantial genetic diversity present within the evaluated germplasm accessions. This observation stemmed from the categorization of these accessions into groups denoting different levels of disease severity, including HS, MS, MR, R, and HR, based on their performance under epiphytotic conditions. Furthermore, the robust molecular marker confirmed the genetic resistance of 40 accessions that displayed phenotypic resistance in the field. As a result, these 40 germplasm accessions, exhibiting not only field-based phenotypic resistance under epiphytotic conditions but also validation through the Xgwm120 marker, serve as reservoirs of resistant genetic material. These accessions are highly suitable for integration into hybridization strategies aimed at producing SB-resistant wheat varieties. Notably, the germplasm accessions consistently showing 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, hold promise for the creation of mapping populations and QTL detection. This approach can significantly expedite the development of Spot blotch-resistant varieties using marker-assisted breeding techniques.

Enhancing the quality of any crop hinges on the thorough exploration and effective utilization of the abundant genetic diversity present within its cultivated varieties, indigenous strains, wild counterparts, and related genera. The significance of conserving a resource becomes prominent when the resource demonstrates or gains acknowledged value [15]. In this context, Emmer Wheat germplasm accessions hold substantial value as valuable assets. The potential to expand and diversify the genetic foundation for SB resistance in cultivars can be realized by incorporating the resistant accessions identified in the present investigation.

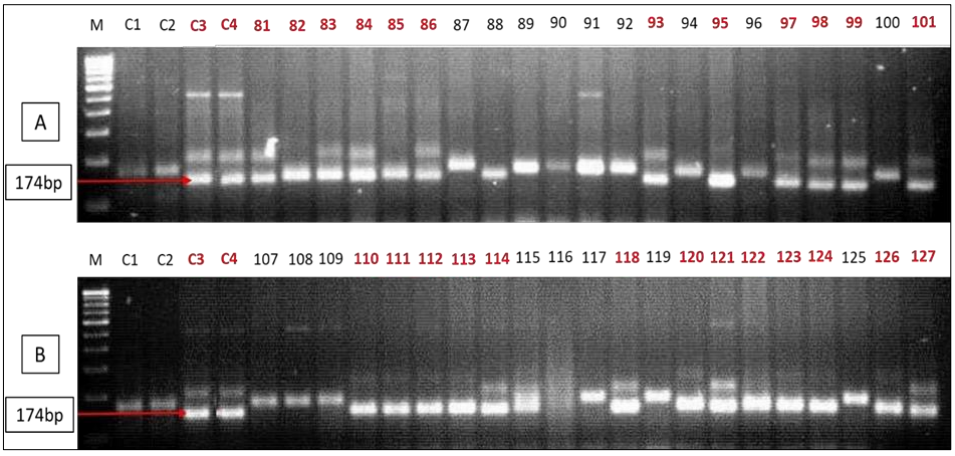

Fig 2: Representative agarose gel electrophoresis results from the screening process for spot blotch resistance across a collection of 127 germplasm accessions. In panel A, the ladder M-100bp is depicted, along with reference samples C1 (Sonalika), C2 (DDK-1025), C3 (HI-8663), and C4 (Chirya 3). Lanes 81–101 showcase the molecular screening of germplasm accessions using the SSR marker Xgwm120 (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.

Acknowledgements

The authors pose sincere thanks DST-INSPIRE for the fellowship and CIMMYT, Mexico & ICARDA, Lebanon for providing seed material and UAS, Dharwad for financial Support, field and lab facility to carrying out the investigation.

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