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Marker assisted introgression of novel spot blotch resistant QTLs into emmer wheat without background selection

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Abstract
In India, wheat is the second most important staple food crop after rice (*Oryza sativa* L.). All three species of wheat *i.e.,* bread wheat (*Triticum aestivum* L.), durum wheat (also known as Kathia or macaroni wheat) [*T. turgidum* L. subsp. Durum (Desf.) van Slageren], and emmer/dicoccum wheat (also known as Khapli wheat) [*T. dicoccon* Schrank; syn. *T. turgidum* L. subsp. *dicoccon* (Schrank) Thell] are cultivated in India. Wheat is a major staple food crop affected by several fungal diseases. Among them, spot blotch caused by *Bipolaris sorokiniana* poses a significant threat to wheat production and grain quality especially in dicoccum/emmer species in warm and humid regions of India and other South Asian countries due to its widespread prevalence and increasing severity. It's imperative to control the yield loses caused by this particular disease in order to achieve sustainability in wheat production. Marker assisted introgression will aid in developing BC_2F_1 lines which can be molecularly confirmed and heterozygous can be selfed and backcrossed simultaneously to generate BC_3F_1s and BC_2F_2s which can serve as an excellent genetic material for functional genomics and expression studies to understand the molecular mechanisms, pathways underlying the disease resistance and to develop genetic stocks and developing a resistant line in a near future.

Keywords: *Emmer* wheat, MAS, spot blotch

Introduction
In India, wheat is the second most important staple food crop after rice (*Oryza sativa* L.). All three species of wheat *i.e.,* bread wheat (*Triticum aestivum* L.), durum wheat (also known as Kathia or macaroni wheat) [*T. turgidum* L. subsp. Durum (Desf.) van Slageren], and emmer/dicoccum wheat (also known as Khapli wheat) [*T. dicoccon* Schrank; syn. *T. turgidum* L. subsp. *dicoccon* (Schrank) Thell] are cultivated in India. During 2022-2023 globally wheat is grown in an area of 220.94 million hectares (ha) with the production of 790.20 million tons. Some of the major wheat growing countries are China, India, Russia, USA, Australia, Canada, and Pakistan [4]. The total area under the crop in India is about 30.46 million hectares and production is about of 107.74 million tons with average productivity of 3537 kg/ha [3]. Major wheat growing states in India are Uttar Pradesh, Madhya Pradesh, Punjab, Haryana, Rajasthan, Bihar and Gujarat. Out of the total area bread wheat covers 95 percent of area whereas durum and dicoccum wheat covers 4 percent and 1 percent of total area respectively. In India durum wheat is largely confined to central and peninsular zones like Maharashtra, Karnataka and Tamil Nadu with the area of 1.5 million hectares and production of 3.5 million ton, whereas the dicoccum wheat is grown majorly in the regions of Northern Karnataka, Southern Maharashtra and parts of Tamil Nadu and A.P with <2 percent area of total wheat growing area of the country with the production of about 2.5 million tons [20]. In Karnataka, wheat is grown in an area of about 1.65 lakh ha with the production of 2.12 lakh tons having the productivity of 1287 kg/ha [3].
Emmer wheat/Dicoccum [*Triticum dicoccum* (Schrank.) Schübl.] a hulled wheat, is grown in typical hot tropical climate and characterized by the prevalence of high mean daily temperature during the crop growth period affecting GS1 and GS3 phase. Emmer wheat is considered to be nutritionally and therapeutically superior as compared to commercially available bread and durum wheat with high protein and dietary fibre (DF) content. Dicoccum wheat contains higher concentrations of Se, Fe, and Zn compared to other wheat species.

Supekar *et al.* (2005) compared the nutritional properties of three different species of wheat and reported that emmer wheats contain higher amount of gluten (12.4% to 12.7%) compared to other wheat species. Mundra *et al.* (2010) reported that chapati prepared with emmer wheat flour had high protein and ash contents of 12.5 percent and 2.3 percent, respectively, and low-fat content of 1.32 percent. Due to its high nutritive value, lower starch digestibility, higher content of antioxidant compounds, and high DF content, foods prepared from emmer wheat can be considered as hypoglycaemic [8].

Wheat production is limited by several biotic stresses, with diseases being a major limiting factor to wheat production worldwide. The total number of wheat diseases exceeds 200, but 50 diseases cause economic losses and are widely distributed. Each year about 20% of wheat is lost due to diseases. Some of the major wheat diseases are rusts, spot blotch, common root rot, smut, tan spot, Septoria blotch, powdery mildew, fusarium head blight, blast and a number of viral, nematode, and bacterial diseases) [1, 2, 10]. Of the many pathogenic diseases spot blotch (SB) caused by the hemibiotrophic fungus *Bipolaris sorokiniana* (Sacc.) Shoem (teleomorph *Cochliobolus sativus*) syn. *Drechslera sorokiniana*, syn. *Helminthosporium sativum* is the most devastating disease of wheat grown in warm and humid areas causes average yield loss ranging from 15 to 20 percent, but under favorable environment, yield loss may go up to 87 percent in susceptible genotypes. In Eastern Gangetic Plains (EGP) of India, Bangladesh and Nepal, *B. sorokiniana* (Sacc.) Shoem appears in a complex with *Pyrenophora tritici-repentis* (Died.) Drechs. (anamorph *Drechslera tritici-repentis* (Died.) Shoemaker) responsible for tan spot (TS) and is commonly known as *Helminthosporium* leaf blight (HLB). Occurrence of SB is more frequent in the humid and warmer wheat growing areas of South Asia (SA), Latin America and Africa [13]. Globally, the disease appears in approximately 25-million-hectare (mha) areas, out of which 10 mha areas are present in EGP.

Spot blotch is one of the major diseases that limits the cultivation of emmer wheat, as emmer wheats are highly susceptible to spot blotch, it affects all parts of the plant resulting in severe yield losses. Chemical control of the diseases alone is ineffective and not economical; hence an integrated approach is suggested involving resistant genotypes. The best way to control the disease is through development of resistant genotypes. The source of resistance to spot blotch in the cultivated emmer is not reported. The wild relative of emmer wheat *viz.*, the land races of *Triticum dicoccoides* and other related species are to be screened for resistant sources under epiphytotic conditions. However, most of the land races of *T. dicoccoides* and other species are winter types, the flowering and the seed setting is a major problem at tropical conditions like Dharwad. More over these wild land races are brittle in nature with tough glumes and transferring resistance to spot blotch alone is time consuming. The alternative would be to search the source of resistance among the cultivated species which are cross compatible to emmer wheat. Since, durum wheats are similar to emmer wheat except brittleness with tough glumes and red grain colour. It is also easy to transfer spot blotch disease resistance from these durum wheats through marker assisted breeding where the sources of resistance to this disease is identified.

Enhancing the area under cultivation of emmer wheat through genetic interventions is the best way to harness its nutritional and therapeutic value to the required level. To improve the performance of emmer wheat in peninsular zone it is necessary to cut down the yield losses caused by the major biotic constraint *i.e.*, *Bipolaris sorokiniana* (Sacc.) Shoem. The proposed research will attempt to introgress the novel QTLs conferring resistance to spot blotch from two durum varieties (*i.e.*, HI-8663 and PDW-314) into four emmer wheat lines through marker assisted introgression with the help of three recently reported markers in our lab at UAS Dharwad [15].

Materials and Methods

Materials

Plant material

1. *T. durum* genotypes HI-8663 and PDW-314 used as donor source for spot blotch resistance
2. *T. dicoccum* genotypes DDK-1025, NP-200, FT line-24 and FT line-45 were used as recurrent parents

Methodology

Crossing Technique

Emasculation and pollination

The emasculation was carried out using fine-tipped forceps. The upper and basal spikelets of *T. dicoccum* were removed. The central florets being immature were also removed leaving only primary and secondary florets for emasculation. The cut glume method for emasculation was followed. The 1/3rd part of the florets from the top was clipped to expose the anthers. Three anthers from each floret were removed carefully to avoid their crushing within the floret or injury to the ovary. The emasculated spikes were covered using glassine bags to avoid rogue pollen. The pollination was done 1-3 days after emasculation upon stigma receptivity. For pollination, only those spikes of *T. durum were* used, in which pollen extrusion was visible in upper florets. Parentage and salient features of cultivars used in the present investigation is given below in the table 1 and 2.

Development of plant material

T. dicoccum lines were crossed as female parent with two different *T. durum* genotypes during *rabi* season @ MARS, Dharwad 2021-22 which resulted in the production of F_1 (8x) seed set in different cross combinations. The F_1's so obtained were planted in *khariff* season @ MARS, Dharwad 2022 and were crossed with four different recurrent parents (DDK-1025, NP-200, FT line-24 and FT line-45) to generate BC_1F_1 (8X). In *rabi* season @ MARS, Dharwad 2022-23, single plant selection was done and selected plants were backcrossed to generate BC_2F_1's and selfed to generate BC_1F_2's. In *khariff* season @ MARS, Dharwad 2023, the resulted BC_2F_1 were sown and evaluated phenotypically and genotypically. At BC_1F_1 and BC_2F_1 marker assisted selection was done using spot blotch resistance linked SSR markers *viz., Xgwm120, Xgwm291* and *Xgwm304* identified by Kanthesh (2021) (Table 3).

Table 1: Parentage of wheat cultivars used in present study

Cultivar	Parentage
HI-8663	HI 8177/HI 8185
PDW-314	AJAIA12/F3 Local
DDK-1025	DDK 1013/DDK 1001//278-13
NP-200	Selection from local wheat of Rishi valley.
FT line-24	Mutant selection of DDK-1029
FT line-45	Mutant selection of DDK-1025

Polymerase Chain Reaction (PCR)

In order to obtain scorable and reproducible results, the following composition of PCR reaction mixture was made: The purified genomic DNA extracts (30 ng) of wheat genotypes were used as template DNA per reaction.

PCR was carried out in a 10 µl reaction volume containing 1.25X Standard *Taq* buffer (NEB), 0.25 mM dNTPs, 1pM forward and reverse primers, 2.5 U *Taq polymerase* (NEB) and 30 ng template DNA. Amplifications were performed in a thermal cycler (C1000^{TM} Thermal Cycler, Biorad, Germany) using the following temperature profile: initial denaturation step at 94 °C for 5 minutes, then 35 cycles at 94 °C for 40 s, 58 °C for 40 s and 72 °C for 50 sec, followed by a final extension step at 72 °C for 5 minutes. PCR products were separated by electrophoresis in a 3 percent Agarose gel stained with ethidium bromide along with 100 bp DNA ladder (1µg/ml) and visualized by gel documentation system (J.H. Bio).

Table 2: Salient features of the parents used in Marker assisted introgression programme

Characters	Durum parents (Donor)		Dicoccum parents (Recurrent)			
	PDW-314	HI-8663	DDK-1025	NP-200	FT LINE-24	FT LINE -45
Days to fifty percent flowering	70.0	69.00	71.00	71.00	71.00	70.00
Days to maturity	116.0	117.00	121.00	122.00	120.00	120.00
Plant height (cm)	88.50	87.47	90.32	99.80	95.42	96.32
Spike length (cm)	6.99	6.96	12.57	9.33	11.37	12.49
Numbers of productive tillers per plant	15.00	15.00	28.00	28.00	30.00	30.00
Number of spikelets per spike	19.00	18.00	23.40	19.40	24.40	25.40
Number of grains per spike	52.50	50.20	40.90	40.70	41.75	42.60
Thousand grain weight (g)	42.50	42.00	34.35	36.69	35.65	39.35
Seed yield per plant (g)	40.20	39.98	34.35	36.69	38.35	39.35
Reaction to spot blotch	Resistant	Resistant	Moderately Susceptible	Susceptible	Susceptible	Susceptible

Table 3: Details of linked SSR markers used for foreground selection in introgressed lines

Sl. No.	Primer name	Chromosome No.	Forward sequence	Reverse sequence	Amplicon Size	Reference
1	*Xgwm 120*	*2B*	*GAT CCA CCT TCC TCT CTC TC*	*GAT TAT ACT GGT GCC GAA AC*	174	Roder *et al.* (1998) [21]
2	*Xgwm 291*	*5A*	*CAT CCC TAC GCC ACT CTG C*	*AAT GGT ATC TAT TCC GAC CCG*	*158*	Roder *et al.* (1998) [21]
3	*Xgwm 304*	*5A*	*CGA GAC CTT GAG GGT CTA GA*	*GCT TGA GAC CGG CAC AGT*	*208*	Roder *et al.* (1998) [21]

Experimental Results

This research was carried out to introgress spot blotch resistance QTLs from durum resistant donor parents HI-8663 and PDW-314 to spot blotch susceptible dicoccum lines through marker assisted introgression. Precise transfer of targeted QTLs / genomic regions was monitored by foreground selection of marker loci tightly linked with the traits of interest and background selection was done through morphological observations/phenotypically. Codominant microsatellite simple sequence repeat (SSRs) markers were used in genotypic screening of the progenies in each generation for compressing the backcross breeding cycles.

QTL introgression

Strategic crossing with marker assisted foreground selection was implemented in this investigation to incorporate genomic regions governing spot blotch resistance from disease resistant donors HI-8663 and PDW-314 to disease susceptible recurrent dicoccum female parents DDK-1025, NP-200, FT line-24 and FT line-45. This study was undertaken to introgress QTLs through marker assisted introgression and confirm the transfer of QTLs with the help of tightly linked molecular markers to assist phenotypic selection and identify the genotypes to cope up in epiphytotic conditions. In each generation, MAS for foreground selection was conducted to select plants introgressed with targeted QTLs. In foreground selection, plants with QTL were selected based on presence of marker allele of donor parents HI-8663 and PDW-314 at target loci and maintained the target locus in a heterozygous state (one donor allele and one recipient allele) until the final backcross.

In BC_1F_1 and BC_2F_1 generations, desirable plants were identified based on the presence of QTLs for disease resistance. Such selections were further selected manually which corresponds to recurrent parents.

Parental polymorphism for the SSR markers

Three SSR markers tightly linked to spot blotch resistance identified earlier by Kantesh (2021) were screened to identify markers which are polymorphic between parental lines with respect to eight sets of parents independently. The markers which could clearly differentiate between alleles of two parents with respect to the particular locus on 3 percent agarose gel were found polymorphic marker in the form of 100 bp ladder was used for each gel to confirm the size difference between amplicons of two alleles of each locus for every marker. Among the three markers the marker *Xgwm120* and *Xgwm291* (Fig. 1, 2) were found to be polymorphic for eight sets of parents was selected for further genotyping studies in backcross generations.

F_1 generation hybridity testing

Recurrent parents were crossed with donor parent, maximum numbers of crosses were made to obtain sufficient amount of F_1seeds and selected F_1 were tested for hybridity using targeted QTL linked marker for the traits of interest (Fig. 3).

Maker assisted foreground selection for target traits in BC_1F_1

QTL positive 20F_1s (DDK-1025 × HI-8663), 15F_1s (NP-200 × HI-8663), 23F_1s (FT line-24 × HI-8663), 10F_1s(FT line-45 × HI 8663), 22F_1s (DDK- 1025 × PDW-314), 11F_1s (NP-200 × PDW-314), 20F_1s (FT line-24 × PDW-314), 6F_1s(FT line-45 × PDW-314) plants with QTL *i.e.* QSb.bhu-2B, QSb.5A linked

with a marker *Xgwm120* and *Xgwm291* for disease resistance were identified and back crossed with recurrent parents to generate BC_1F_1 plants, Whereas the backcross involving NP-200//NP-200 × HI-8663, FT line-45 //FT line-45 × HI 8663, NP-200//NP-200 × PDW-314 and FT line-45//FT line-45 × PDW-314 did not set required number of seeds, hence these crosses were omitted for further investigation (Fig. 4).

Out of the total 121 plants, 65 of BC_1F_1 (DDK-1025//DDK-1025 × HI-8663), Out of the total 106 plants, 50 BC_1F_1 (FT line-24//FT line-24 × HI-8663), Out of the total 93 plants, 48 BC_1F_1 (DDK- 1025//DDK- 1025 × PDW-314) and Out of the total 70 plants, 32 BC_1F_1 (FT line-24//FT line-24 × PDW-314) plants were found to be positive and heterozygous for foreground marker *Xgwm120* (Fig. 4).

Out of the total 121 plants, 64 of BC_1F_1 (DDK-1025//DDK-1025 × HI-8663), Out of the total 106 plants, 52 BC_1F_1 (FT line-24//FT line-24 × HI-8663), Out of the total 93 plants, 46 BC_1F_1 (DDK- 1025//DDK- 1025 × PDW-314) and Out of the total 70 plants, 33 BC_1F_1 (FT line-24//FT line-24 × PDW-314) plants were found to be positive for foreground marker *Xgwm291*(Table 18, 19, 20, 21) (Fig. 4).

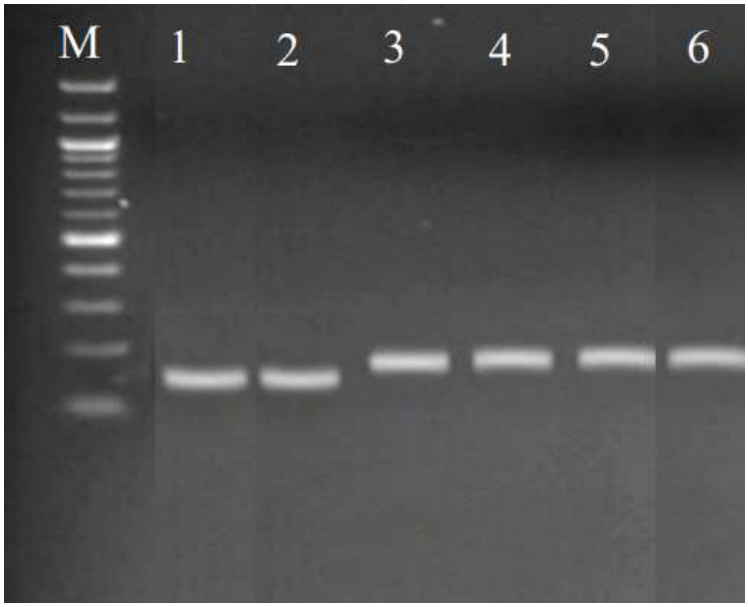
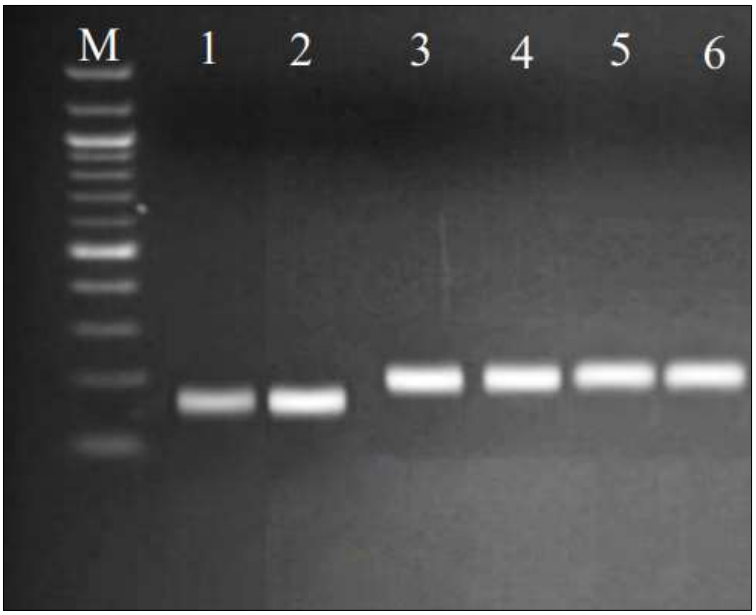


Fig 1: Band amplification pattern of SSR marker *Xgwm291* (band size= 158 bp) M= 100 bp DNA ladder



1. HI-8663 2. PDW-314 3.DDK-1025, 4. NP-200, 5. FT line-24 6. FT line-45

Fig 2: Band amplification pattern of SSR marker *Xgwm120* (band size= 174 bp) M= 100 bp DNA ladder

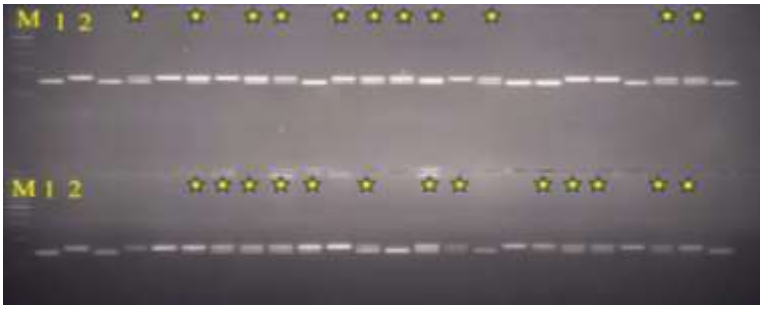


Fig 3: Representative agarose gel (3%) image of F_1 hybridity confirmation (P1- Recurrent Parent P2- Donor parent)

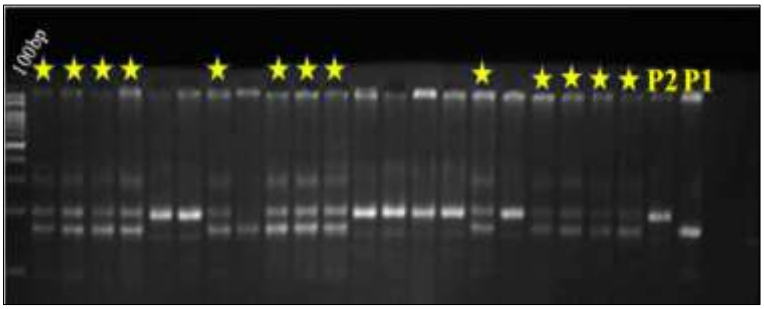


Fig 4: Representative agarose gel image of Foreground selection for QTL QSb.bhu-2B in BC_1F_1 generation using SSR marker *Xgwm120*

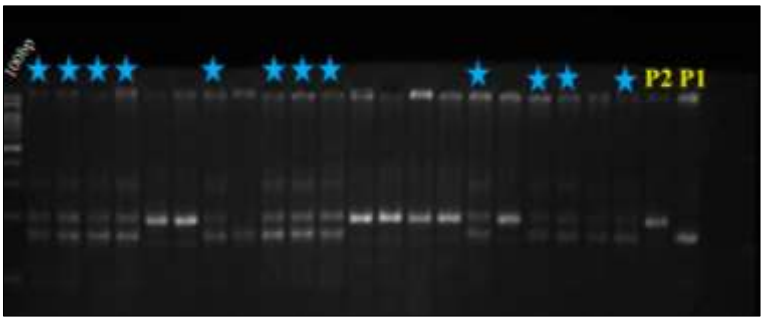


Fig 5: Representative agarose gel image of Foreground selection for SB resistance QTL in BC_2F_1 generation using SSR marker *Xgwm291*.

Among the total plants, for cross DDK-1025//DDK-1025 × HI-8663 30 plants, for FT line-24//FT line-24 × HI-8663 27 plants, for DDK- 1025//DDK- 1025 × PDW-314 23 plants and for FT line-24//FT line-24 × PDW-314 17 plants in BC_1F_1 generation found heterozygous and positive for both the markers/QTLs.

Maker assisted foreground selection for target traits in BC_2F_1

Those plants which are phenotypically similar, found positive and heterozygous for both the markers in BC_1F_1 were further crossed with a recurrent parent to generate BC_2F_1 generations

Out of 154 BC_2F_1 generation plants of a cross DDK-1025//DDK-1025 × HI-8663, 74 plants, Out of the 120 BC_2F_1 generation plants of a cross FT line-24//FT line-24 × HI-8663, 63 plants, Out of the 113 BC_2F_1 generation plants of a cross DDK- 1025//DDK- 1025 × PDW-314, 56 plants and Out of the 94 BC_2F_1 generation plants of a cross FT line-24//FT line-24 × PDW-314, 49 plants were found QTL positive for SSR marker *Xgwm120*. Targeted trait loci were confirmed with foreground markers as done in previous generation.

Out of 154 BC_2F_1 generation plants of a cross DDK-1025//DDK-1025 × HI-8663, 78 plants, Out of the 120 BC_2F_1 generation plants of a cross FT line-24//FT line-24 × HI-8663, 59 plants, Out of the 113 BC_2F_1 generation plants of a cross DDK- 1025//DDK- 1025 × PDW-314, 54 plants and Out of the 94 BC_2F_1 generation plants of a cross FT line-24//FT line-24 × PDW-314, 45 plants were found QTL positive for SSR marker *Xgwm291*. Targeted trait loci were confirmed with foreground markers as done in previous generation (Fig. 5).

Among the total plants, for cross DDK-1025//DDK-1025 × HI-8663 06 plants, for FT line-24//FT line-24 × HI-8663 05 plants, for DDK- 1025//DDK- 1025 × PDW-314 08 plants and for FT line-24//FT line-24 × PDW-314 04 plants in BC_2F_1 generation found phenotypically similar to recurrent parent, heterozygous and positive for both the markers/QTLs.

For the resulting BC_2F_1 lines observations were recorded for various traits and it is observed that the mean values for the various agronomic characters, recorded were tending towards the dicoccum recurrent parents which confirms the phenotypic similarity to the recurrent parents (Table 4).

Table 4: Mean values of different BC_2F_1 lines along with the Donor and Recurrent Parents

Sl. No	Trait	DP_1	DP_2	RP_1	RP_2	1.1	1.2	2.1	2.2
1	DFF	69.00	70.0	70.8	71.2	70.4	70.9	69.90	71.0
2	DTM	116.5	116.0	120.7	120.5	119.5	118.5	120	119.20
3	NTP	15.10	15.20	29.80	28.80	28.50	27.50	28.80	26.80
4	NPTP	15.10	15.20	27.70	27.50	26.50	25.50	27.50	26.40
5	PH	87.47	88.50	90.32	95.42	89.45	93.20	91.50	91.20
6	PL	18.85	18.60	11.59	12.20	13.82	14.50	12.05	13.30
7	SL	6.96	6.99	10.57	10.40	9.45	9.70	10.00	9.50
8	NSS	18.00	18.25	23.40	24.50	21.49	22.50	22.50	23.50
9	SYP	41.62	42.12	34.38	35.20	37.50	36.65	35.50	37.70
10	TGW	42.00	42.40	34.35	34.80	36.50	35.28	36.25	34.95
11	NGS	50.20	49.50	40.90	39.90	43.25	42.15	41.70	41.20

DFF – Days to 50 percent flowering
DTM – Days to maturity
NTP – Number of tillers per plant
NPTP – Number of productive tillers per plant

PH – Plant height (cm)
PL – Peduncle length (cm)
SL – Spike length (cm)
NSS – Number of spikelets per spike

SYP - Seed yield per plant (g)
TGW- Thousand grain weight (g)
NGS- Number of grains per spike

RP_1 - Recurent parent_1/DDK-1025; RP_2 - Recurrent parent_2/FT line 24
DP_1 - Donor parent_1/HI-8663; DP_2 - Donor parent_2/PDW-314
1.1- BC_2F_1 lines of DDK-1025//DDK-1025 × HI-8663
1.2- BC_2F_1 lines of FT line-24//FT line-24 × HI-8663
2.1- BC_2F_1 lines of DDK- 1025//DDK- 1025 × PDW-314
2.2- BC_2F_1 lines of FT line-24//FT line-24 × PDW-314

Discussions

Wheat has a large genome of about 16 Gb and spot blotch resistance is a complex trait comprising of a number of physio biochemical processes at the cellular level in different developmental stages of the plant. Hence, it has lagged behind in development of disease resistant varieties using only conventional breeding approaches.

An effective and desirable method would be combining conventional wheat breeding with MABB by introducing targeted genes or QTLs into superior wheat cultivars for overcoming such biotic and abiotic stresses [7].

Marker-assisted selection (MAS) in breeding has proven to be a straightforward, effective, sturdy, and precise approach when compared to traditional breeding methods, which are characterized by their time-consuming, labor-intensive nature and susceptibility to environmental influences. Nevertheless, it's essential to take into account key factors like the tight linkage of markers to the target trait, population size, and the presence of background markers spread throughout the entire genome when implementing MAS breeding.

MAS has been used frequently in wheat for introgression of traits such as disease resistance and quality improvement [16, 22].

In the past, the MABB (Marker-Assisted Backcrossing) technique has been effectively and successfully utilized in wheat for combining genes related to rust resistance [5, 18], as well as traits such as pre-harvest sprouting tolerance and grain protein content [25]. However, there is limited documented information regarding the transfer of identified and mapped QTLs responsible for conferring drought tolerance through MABB in wheat. Various MAS (Marker-Assisted Selection) approaches, in combination with high-throughput phenotyping methods such as MARS (Marker Assisted Recurrent Selection) and GWS (Genome-wide Selection), are now being successfully employed in wheat and other crops. These approaches are paving the way for the development of stress-resistant varieties to meet future agricultural challenges, as highlighted in the review by Jain *et al.* (2014).

From this perspective, the current study aimed to convert dicoccum lines into spot blotch-resistant variants while maintaining their original genetic makeup. The use of polymorphic markers that can genetically differentiate between the parent lines serves as a crucial tool when initiating a Marker-Assisted Backcross Breeding program. These differentiating markers provide valuable information for the identification and selection of the most promising backcross progenies during both foreground and background selection in advanced backcross generations. When implementing this Marker-Assisted Backcross Breeding (MABB) program for enhancing spot blotch resistance in dicoccum lines, several critical factors, as outlined by Frisch (2005), were taken into account. These factors include the number of target genes to be transferred, the marker map, the crossing scheme, and the selection strategy applied.

The markers *Xgwm140* and *Xgwm291* linked with spot blotch resistance QTLs explaining the phenotypic variance (R^2) of 19.27, 23.81 respectively and these markers can be used for spot blotch resistance breeding. Backcross breeding has a long history of successful use in resistance breeding in many crop species, including wheat. A particular advantage of the backcross approach is that its end-product is a variety which is very similar to that of the recurrent parent, generally chosen because it is well accepted by the farmers and consumers. With the development of usable molecular markers in wheat, the successful marker-assisted incorporation of multiple disease resistance genes is becoming more commonplace [26]. There have been only few previous reports of the successful introgression of spot blotch resistance QTLs in wheat [24].

In our current investigation, although foreground selection was conducted in the F_1, BC_1F_1, and BC_2F_1 generations, investigation did not engage in marker-assisted background selection. Instead, among the plants or progenies carrying the introgressed QTL in a heterozygous state, we visually selected superior plants or progenies in the $BC_1F_1/2$ generations, with the aim of either resembling or surpassing

the recurrent parents. These selected plants or lines, which were chosen without the use of background selection, are expected to possess a significant portion of the recurrent parent's genome, as visual selection was applied to ensure similarity with the recurrent parent. However, it should be noted that this proportion may still be lower than the 97% of the recurrent parent genome that was achieved in previous studies after only two backcross generations, following the implementation of background selection [19].

In most of Marker-Assisted Selection (MAS) programs for crop improvement, background selection is often deemed unnecessary unless there is a specific need to recover the genetic background of the recurrent parent. This need may arise in cases where a parent of a hybrid is undergoing improvement, as exemplified in various crops such as pearl millet (HHB-67-2 for HHB-67) for enhancing resistance to downy mildew at ICRISAT, India [12], maize for enhancing essential amino acids (Vivek-QPM9) at VPKAS, Almora [11], and rice for bolstering resistance to bacterial blight, blast, and brown plant hopper (Shanyou 63 and Minghui 63, Jim 23B) in China [6].

The primary rationale for omitting background selection in Marker-Assisted Backcross Breeding (MABB) is to facilitate the incorporation of additional desirable genes or QTLs, in addition to the specific target QTL, from the common parent. Another contributing factor to the decision to forego background selection was the substantial size of the wheat genome, which is approximately 40 times larger than the rice genome. This significant difference in genome size makes it challenging to fully reconstitute the genome of the recipient parent, even when employing around 100 to 200 markers for this purpose.

The confirmed heterozygous BC_2F_1 plants with both markers can be selfed and backcrossed simultaneously to generate BC_3F_1s and BC_2F_2s which can serve as an excellent genetic material for functional genomics and expression studies to understand the molecular mechanisms, pathways underlying the disease resistance and to develop genetic stocks and developing a resistant line.

Conclusion

From marker assisted introgression program 06 lines for DDK-1025//DDK-1025 × HI-8663, 05 lines for FT line-24//FT line-24 × HI-8663, 08 lines for DDK- 1025//DDK-1025 × PDW-314 and 04 lines for FT line-24//FT line-24 × PDW-314 in were identified BC_2F_1 generation which are found phenotypically similar to recurrent parent, heterozygous and positive for both the markers/QTLS. Moreover, the validated heterozygous BC_2F_1 plants, identified through both markers, can be simultaneous selfed and backcrossed to produce BC_3F_1s and BC_2F_2s. These generations can be invaluable genetic resources for conducting functional genomics and expression studies. These studies aim to unravel the molecular mechanisms and pathways involved in disease resistance, leading to the development of genetic stocks and the creation of resistant lines.

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