Rice Blast Resistance Breeding Studies in Turkey.

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ABSTRACT

The aim of this study is to develop rice blast resistance varieties and to determine the blast resistance genes under field conditions in Turkey. Since 1970, rice breeding activities have been conducting in Turkey, around 50 rice verities developed up to now. A few of them have some resistance to blast disease, however they are not accepted by the Turkish farmers due to their poor quality traits. Blast disease was not a serious and economic problem until 1995 in Turkey. The first heavy blast disease infection occurred in 1995. The yield loss was 20% in 25000 hectare area at Thrace part of the country. The infection caused yield losses again in 1997 and 2002 in the same region. Depending upon environmental conditions, the blast infection occurs sometimes in the last years. In order to control the blast, the farmers apply fungicide several times at the season depending on the disease infection starting time and intensity. To use fungicide increases the production cost and environmental pollution risk. Therefore, to develop disease resistant varieties, a Turkey-IRRI cooperative research project started to transfer *Pi40* blast resistant gene into Osamcık-97 and Halilbey elit cultivars in 2010. These susceptible varieties to blast crossed with *Pi40* gene donor in a japonica background (IR83260-1-1-1-5-B-3-1-2-B), after then, marker-assisted backcross breeding (MAS) carried out at IRRI. After reaching to advanced backcross progenies or lines, they were sent to TARI (Trakya Agricultural Research Institute) for the blast screening in the different hotspot locations and for agronomic trails. After evaluating the disease test and yield trial results, two Osmancık-97 and two Halilbey disease resistant backcross lines were nominated for registration. At the same time, breeding studies conducted at TARI, first of all, using monogenic lines, the resistance genes to blast disease, *Pik-s*, *Piz-5*, *Pib*, *Pish*, *Pi1*, *Pi7*, *Pi12*, *Pi20*, *Pik*, *Pita-2*, and *Pi40* determined at the different hotspot areas in the field conditions. And then, some of them used in the breeding program and the lines carrying these resistance genes crossed with Turkish commercial varieties. Some advanced progenies and breeding lines obtained. The selection and agronomic testing are being continued in these materials. They will be used to control blast disease in the near future.

Key Worlds: Rice (*Oryza sativa* L.), blast, monogenic line, resistance breeding, resistance gene, resistant variety.

1.Introduction

Since 1970, rice breeding activities have been conducting in Turkey, around 50 rice verities developed up to now. A few of them have some resistance to blast disease, however, they are not accepted by the Turkish farmers due to their poor agronomic and quality traits. The popular japonica rice cultivars used in the production are susceptible to blast disease.

Rice cultivated in the limited area due to shortage of available irrigation water for rice crop in temperate regions of Turkey. However, rice still is a high profitable crop in Turkey. Therefore, the farmers are very willing to grow rice, it creates monoculture rice growing systems and it also increases the disease infection risks.

All the rice diseases recorded in the other rice growing countries do not occur in Turkey. The three fungal rice diseases observed are: Blast, Brown leaf spot, Bakanea and foot rot caused by the fungus *Magnaportheoryzae*, *Helminthosporiumoryzae,* and *Fusariummoniliforme*, respectively. The blast is the most severe fungal disease in Turkey.

The earliest attempts to study pathogenic fungi in Turkey were made by Bremer and Özkan (1946) and Göbelez (1953). They reported that blast was the most important disease in Turkey. Gobelez also observed up to 25 to 75% yield losses in some rice fields in the Black Sea Region.

The most severe blast disease infection and yield losses recorded in Trakya region of Turkey in 1995. where the half of rice produced. The yield losses was 20% in 25000 hectares areas in this region and also the head rice yield was drastically decreased. Since then, the blast infection occurs depending on weather conditions, such as high temperatures, increases in rainy days, high relative humidity and lower night temperature etc. At the same time, some agricultural practices used by the farmers increase the disease infection risks. These practices are monoculture rice faming, excessive nitrogen application, nitrogen application at non recommended times, late planting, and high plant density etc.

In order to control blast disease, The Turkish rice farmers use fungicides. They apply the fungicide several times depending on the disease infection starting time and intensity. This practice increases the production cost and environmental concern**.**

Using the resistant varieties is the most economically and environmentally friendly way to control this disease. However, the majority of rice varieties used in the rice production are susceptible to the blast disease in Turkey.

Kobayashi et al., (2007) developed the first international standard differential variety set composed of monogenic lines for 24 kinds of resistance genes (*Pia, Pib, Pii, Pik, Pik-h, Pik-m, Pik-p, Pik-s, Pish, Pit, Pita, Pita-2, Piz, Piz-5, (=Pi2), Piz-t, Pi1, Pi3, Pi5(t), Pi7, Pi9, Pi11(t), Pi12(t), Pi19 (t) and Pi20*). On the other hand, Yanoria et al., (2010) developed twenty near-isogenic lines (NILs) of rice (*Oryza sativa* L.) containing 11 blast resistance genes (*Pib, Piz-5, Pi9,Pi3, Pia, Pik-s, Pik, Pik-h, Pi7, Pita, and Pita-2*) using backcross method. Beşer et al., (2015) determined that *Pi1, Pi7, Pi9, Pi12, Pi20, Pi40, Pib, Pik-h, Pik-m, Pik-p,* and *Piz-5* genes provided resistance to the blast under field conditions in the different locations in Turkey.

The *Pi40* blast resistance gene derived from a wild species, *O. australiensis*. It has durable broad-spectrum of blast resistance (Jeung et al., 2007). The *Pi40* gene confers blast resistance at seedling as well as reproductive stages.

Rice cultivars resistant to blast disease are developed by conventional breeding using the sources of resistance from land races of diverse rice varieties. Also, Marker-assisted backross breeding is a powerful tool to incorporate novel resistance genes into susceptible cultivars and todevelop improved breeding lines in a short period of time (Kush and Jena, 2009).

The objectives of this study were to develop the resistant varieties to rice blast disease and to determine the blast resistance genes under field conditions.

2. Material and method

The research activities carried out for this study in International Rice Research Institute (IRRI) in Philippines and Trakya Agricultural Research Institute (TARI) in Turkey.

1. A Turkey-IRRI cooperative research project started to transfer *Pi40* blast resistance gene into Osamcık-97 and Halilbey elit cultivars in 2010.

2. In order to determine the resistance genes, the blast disease nurseries tested in the hotspot locations and some crosses were done between near isogenic lines containing resistance genes (Kobayashi et al., 2007) and Turkish commercial varieties in Turkey.

2.1. Material

2.1.1 The material used in IRRI**.**

Two elite cultivars of Turkey, Osmanık-97 and Halilbey were used as the recurrent parents, and IR83260-1-1-1-5-B-3-1-2-B (4128) carrying the Pi40 gene was used as the donor parent.

2.1.2 The material used in TARI.

Pi40 gene donors; IR83260-1-1-1-2-1-22-B, IR83260-1-1-7-2-1-4-B, and IR83260-1-1-7-2-1-2-B, monogenic lines IRBL20-IR24 (*Pi20*), IRBLb-B (*Pib*), IRBL7-M (*Pi7*), IRBL12-M (*Pi12*), IRBL5-M (*Pi5*), IRBLta2-Re (*Pita-2*), IRBL9-W (*Pi9*), and IRBLz5-CA. Turkish varieties used in the crossing programme are Yatkın, Biga İncisi, Mis 2013, Manyas Yıldızı, Sürek M711, Kale, Paşalı, Kızıltan, Çakmak, Gala, Gönen, Halilbey, Efe, Osmancık-97, Edirne, Beşer, Durağan, Kırkpınar, and Italian varieties are Linche and 7721.

2.2 Method

2.2.1 IRRI- TARI cooperative studies

A Turkey-IRRI Cooperative Research Projects conducted to develop blast-resistant japonica rice using marker-assisted backcross breeding conducted at IRRI and TARI in Turkey. The aim of this project was to transfer the *Pi40* blast resistant gene into two Turkish elit cultivars, Osmancık-97 and Halilbey. This project started in 2010 at IRRI, two elite cultivars of Turkey (Osmancık-97 and Halilbey) were screened for their blast reaction to virulent blast races. The two elite varieties showed susceptible reaction to blast. These varieties were crossed to the *Pi40* donor in a japonica background (IR83260-1-1-1-5-B-3-1-2-B). After then, marker-assisted backcross breeding (MAS) practiced (Kush and Jena, 2009) (Figure-1) and backcross segregating materials at different generations were obtained. Also, through molecular analysis the materials or line determined with *Pi40* gene at IRRI (Figure-2). After reaching advanced backcross progenies or lines were sent to TARI for evaluating blast screening in different hotspot locations and for agronomic trials.

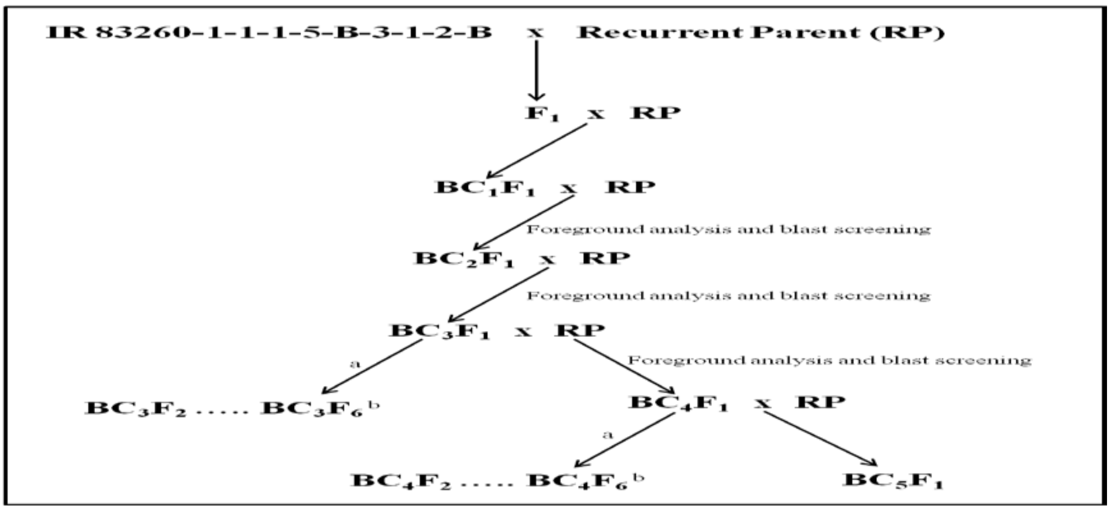

Figure 1. The development of F1 and production of early backcross generation progenies. RP= Recurrent Parent; BC=Backcross; a=selfing, foreground analysis, and phenotype selection, b=SNP genotyping.

Molecular analysis

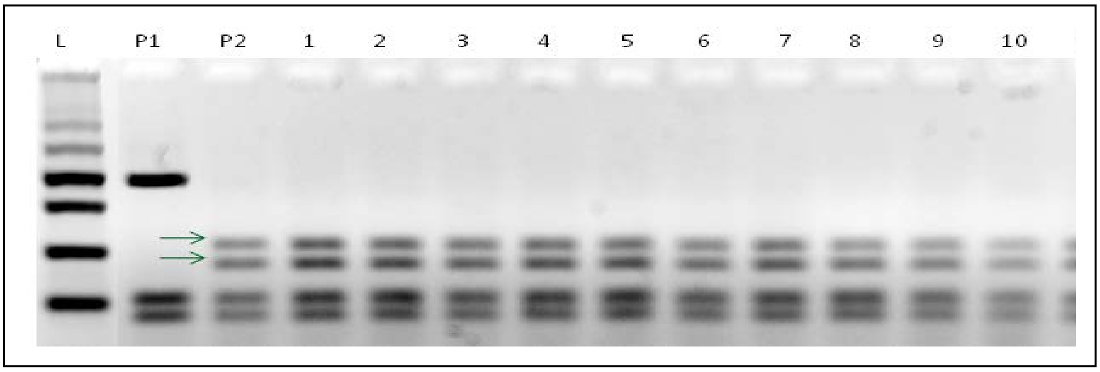

Figure 2. PCR analysis of selected advanced backcross lines; L= DNA size marker ladder; P1= recurrent parent allele; P2= donor parent with *Pi40* allele; 1–11= selected progenies with the *Pi40* gene.

The advanced bakcross lines showed good performance for agronomic traits and resistance to blast disease, tested in the yield trials. Three yield trials conducted in two locations in Edirne and İpsala town of Edirne (the main rice growing region in Turkey) in 2015. One yield trial consisted of Osmancık-97 backcross lines and the other consisted of Halilbey backcross lines in Edirne location at TARI. İpsala location consisted of both selected promising backcross lines of Osmancık-97 and Halilbey cultivars. The yield trials were carried out in a complete randomized block design with three replications. The recurrent parents were used as check in the experiments.

The data were recorded for plant height, days to flowering, the number of panicle per squarmetter, spikelet fertility, 1000 grain weight, rice and milled grain length, rough rice and head rice yield.

Rice yield data were subjected to ANOVA using MSTAT-C package programme. LSD was calculated for comparison of cultivar means.

2.2.2 The Studies at TARI

The monegenic or resistant lines to the blast crossed with main commercial varieties, and also a few backcrossing practiced in some crosses, after creating segregating populations, pedigri selection practiced. Selected lines tested in disease observations nurseries.

The disease test observation nurseries were conducted at the different hotspot locations. For these nurseries, first the seedlings were grown in the seed beds, and then 25 days old seedlings were transplanted in one meter row, distance between rows was 15 cm and 7 cm between hills. Encouraging disease infection, highly susceptible check variety's seedlings were transplanted around the plot and high rates of nitrogen fertilizer were applied. Disease evaluation was done at early vegetative and at grain repining stages, according to Standard Evaluation System for Rice (IRRI, 2014).

3. **Results**

3.1 IRRI- TARI cooperative studies

Pi40 blast resistant gene transferred into two elite Turkish rice varieties, Osmancık-97 and Halilbey, using marker assisted backcross breeding method in International Rice Research Institute. Developed backcross breeding lines were sent to Trakya Agricultural Research Institute for disease evaluation test at hotspot areas and agronomic trails. The lines were tested in disease test nurseries and yield trials. Two yield trials conducted at TARI and one regional yield trial in İpsala districts of Edirne province in 2015. The results of regional yield trail showed in table-1. After getting the disease test and yield trial results, the selected two Osmancık-97 backcross lines and two Halilbey backcross lines were nominated for registration as commercial variety at the end of 2015. The averages of their main traits were given in table-2. They were tested in National Rice Variety Registration Trial in 2016.

Table 1. The rice yield results of Ipsala regional yield trail.

Experiment Design	Randomised complete block experiment design with three replication		Planted Area	$20\ m^2$
Planting date	27 May.2015		Harvesting area	$15{,}75\ m^2$
Harvesting date	07 October.2015		Fertilizer rate	$N_{15}\ P_8$ kg/ da
Entry No	Varieties	Group Range	Rice Yield (Tonnes/ha^{-1})	Yield Range
6	TR-7-3-2-1-2	a	7,66	1
4	IR99599-1-12-B	ab	7,43	2
7	IR99586-3-7-2-B	abc	7,20	3
3	IR 99598-2-8-10-1	bcd	7,04	4
9	IR99586-3-7-14-B	cd	6,77	5
1	Osmancık-97 (Recurrent variety)	d	6,62	6
11	Halilbey (Recurrent variety)	e	5,97	7
8	Kızıltan (Susceptible check)	e	5,91	8
2	IR99586-30-2-13-B-B	e	5,67	9
10	IR101614-7-8-B	e	5,53	10
5	Sarıçeltik (Susceptible check)	f	1,08	11
	CV (%) = 5,32	$LSD_{0.05}$= 0,055		

Table 2. The averages of data obtained in the experiments for Osmancık-97 and Halilbey blast resistant backcross lines nominated for registration.

Designation	The average yield (ton /ha^{-1})	Plant height cm	Days to flow.	The num. of panicle m^{-2}	Spikelet fertility %	1000 rice grain weight (g)	Head rice yield (%)	Rice grain length mm	Milled rice grain length (mm)	Blast disease score (1-9)
IR99586-3-7-14-B	7,6	92,3	79	306	94,0	33,1	66,1	8,8	6,1	1
IR99598-2-8-10-1	8,1	91,9	81	363	91,3	31,7	61,5	8,4	6,0	1
Osmancık-97 (Recurrent parent)	7,6	98,1	81	385	89,9	33,6	61,6	8,8	6,3	7
IR99599-1-12-B	8,0	95,3	77	300	92,2	30,9	59,0	8,7	6,1	1
TR-7-3-2-1-2	7,7	89,2	83	340	76,6	32,4	64,1	8,9	6,4	1
Halilbey (Recurrent parent)	7,3	97,6	78	340	93,0	34,9	56,1	9,2	6,4	5

3.2 The Studies at TARI

3.2.1 Disease tests in hotspot locations.

In order to determine the resistance genes for the blast disease, using monogenic lines, the disease tests were carried out at the hotspot locations, in two locations in 2013 and in four locations in 2014 under filed conditions, respectively. The results of the disease test nurseries are seen in table-3 and table-4 **.** The disease test results showed that the resistance genes, *Pi40*, *Pi20*, *Pi12*, *Pita-2*, *Piz-5*, *Pik*, *Pish*, *Pi1*, *Pik-s*, *Pib*, and *Pi7* provided resistance to blast disease in all locations in both years.

3.2.2 Breeding blast resistant varieties

The cross combinations were done between the monogenic lines or varieties carrying resistances genes and Turkish commercial varieties. The monogenic lines or varieties used in the crosses are IR83260-1-1-1-2-1-22-B (Pi40), IR8360-1-1-7-1-4-B (*Pi40*), IR83260-1-1-7-2-1-2-B (*Pi40*), RBL20-IR24 (*Pi20*), IRBLb-B (*Pib*), IRBL7-M (*Pi7*), IRBL12-M (Pi*12*), IRBLta2-Re (*Pita-2*), IRBLz5-CA (*Piz-5*), IRBLks-S (*Pik-s*), IRBLb-B (Pib) and Turkish varieties are Yatkın, Kızıltan, Sürek M711, Halilbey, Çakmak, Paşalı, Gönen, Edirne, Efe, and Osmancık-97 etc. Some segregating material and breeding lines obtained in this study. Developed lines era being tested in the observation nurseries and yield trails, selection is continued in the segregating material in the different generations.

4. **Discussion**

Using monogenic lines carrying resistance genes for blast disease, the disease test nurseries conducted in hotspot locations and the resistance genes determined for main rice growing regions in Turkey. These genes are *Pi40*, *Pi20*, *Pi12*, *Pita-2*, *Piz-5*, *Pik*, *Pish*, *Pi1*, *Pik-s*, *Pib*, and *Pi7*. Beşer et al., (2015) reported similar results and the recommended to use these genes in blast resistant variety development. Therefore, these lines crossed with Turkish commercial varieties and some advanced segregating material in different generations and the promising lines developed. Some disease resistant varieties contains these genes could be possible to register in the near future. Similarly Lee et al., (2015) in Korea and Hua et al., (2015) in China, using *Pb1* and *Pi39* blast resistant genes developed blast resistant varieties, respectively.

During the past decade, nearly 50 blast resistance genes have been identified in the world. However, most of them are race specific and resistant cultivars possessing these genes break down within 2 to 3 years. However, *Pi40* gene provides durable broad-spectrum resistance to blast disease (Jeung et al., 2007). IRRI-TARI developed two Osmancık-97 and two Halilbey backcross lines having *Pi40* genes are more resistant than recurrent parents, Osmancık-97 and Halilbey to leaf and panicle blast disease. Similar results reported by Suh et al., (2009).

The averages of rice yields and agronomic traits of backcross lines and recurrent parents are seen in table-2. The developed backcross lİnes have higher rice yields and better agronomic traits than the recurrent parents. That is why, these lines were nominated for registration as a commercial variety.

The majority of rice varieties used in the rice production are susceptible to the blast disease in Turkey. One of them is Osmancık-97 which is cultivated more then at 50% of total rice growing area and its quality characteristics are well accepted by The Turkish consumers. The other one with high yield potential is Halilbey, but it is highly susceptible to the panicle blast. Sometimes a severe disease infection occurs and it reduces rice yield in Turkey.

In order to control blast disease, The Turkish rice farmers use fungicides. They apply the fungicide several times depending on the disease infection starting time and intensity. Application of fungicide increases the production cost and environmental concern**.** After developing resistant varieties, these problem will be solved.

Conclusion

The blast resistance genes determined (*Pi40*, *Pi20*, *Pi12*, *Pita-2*, *Piz-5*, *Pik*, *Pish*, *Pi1*, *Pik-s*, *Pib*, and *Pi7*) in this study and they were used for resistant breeding program. Some advanced segregating material and the promising blast resistant breeding lines developed. This material will be used to develop resistant varieties in the further studies.

Also, the resistant backcross lines of Osmancık-97 and Halilbey varieties were developed in a IRRI-TARI cooperative project. These lines nominated for registrations. Registration of these backcross lines as commercial variety will provide important contribution to solve the blast disease problem in Turkey.

The amount of fungicide applied will decrease and it will reduce the production cost and environmental concern risk.

Acknowledgments

We thank General Directorate Agricultural Researches and Policies in Turkey and International Rice Research Institute for supporting this study.

Table 3. The material tested for disease infection under field conditions in two locations in 2013.

Entry No	Designation	Locations			
		Edirne, İpsala		Balıkesir, Gönen	
		LB	PB	LB	PB
1	IR99587-2-12-15 (Halilbey) (*Pi40*)	1	1	1	1
2	IR99587-2-12-1 (Halilbey) (*Pi40*)	1	1	1	1
3	IR99586-1-2-25 (Osmancık-97) (*Pi40*)	1	1	1	1
4	IR995586-2-8-28 (Osmancık-97) (*Pi40*)	1	1	1	1
5	IR99586-5-16-10-1 (Osmancık-97) (*Pi40*)	1	1	1	1
6	IR99598-2-8 (Osmancık-97) (*Pi40*)	1	1	1	1
7	IR99598-1-5 (Halilbey) (*Pi40*)	1	1	1	1
8	IR99599-2-7 (Halilbey) (*Pi40*)	1	1	1	1
9	IR99599-5-1 (Halilbey) (*Pi40*)	1	1	1	1
10	IRBLa-A (*Pia)*	9		9	7
11	IRBLa-C (*Pia)*	5	5	7	7
12	IRBLi-F5 (*Pii*)	5	5	9	9
13	IRBLks-F5 (*Pik-s*)	7	7	5	5
14	IRBLks-S (*Pik-s*)	1	1	1	1
15	IRBLk-Ka (*Pik*)	1	1	1	1
16	IRBLkp-K60 (*Pik-p*)	1	3	1	3
17	IRBLkh-K3 (*Pikh*)	1	1	1	1
18	IRBLz-Fu (*Piz*)	1	1	1	3
19	IRBLz5-CA (*Piz-5, Pi2*)	1	1	1	1
20	IRBLzt-T (*Piz-t*)	3	1	5	1
21	IRBLta-K1 (*Pita, Pi4*)	3	3	5	5
22	IRBLta-CT2 (*Pita*)	3	3	7	7
23	IRBLb-B (*Pib*)	1	1	1	1
24	IRBLt-K59 (*Pit*)	3	3	3	3
25	IRBLsh-S (*Pish*)	3	3	1	1
26	IRBLsh-B (*Pish*)	3	3	5	7
27	IRBL1-CL (*Pi1*)	1	1	1	1
28	IRBL3-CP4 (*Pi3*)	5	5	7	3
29	IRBL5-M (*Pi5*)	5	5	9	9
30	IRBL7-M (*Pi7*)	1	1	1	1
31	IRBL9-W (*Pi9*)	3	3	3	3
32	IRBL12-M (*Pi12*)	1	1	3	1
33	IRBL19-A (*Pi19*)	5	5	5	5
34	IRBLkm-Ts (*Pik-m*)	1	3	1	5
35	IRBL20-IR24 (*Pi20*)	1	1	1	1
36	IRBLta2-Pi (*Pita-2*)	1	1	3	1
37	IRBLta-CP1 (*Pita*)	1	3	7	3
38	IRBLz5-CA® (*Piz-5=Pi2*)	1	1	1	1

LB: Leaf blast, **PB**: Neck and panicle blast.

Table 4. The material tested for disease infection under field conditions in four locations in 2014.

Entry No	Designation	Locations							
		Edirne İpsala		Tekirdağ Hayrabolu		Balıkesir Gönen		Çanakkale Biga	
		LB	PB	LB	PB	LB	PB	LB	PB
1	IR83260-1-1-1-5-B-3-1-2-B (*Pi 40*)	1	1	1	1	1	1	1	1
2	IRBL 1-CL (*Pi 1*)	3	1	3	1	3	3	1	1
3	IRBL 12-M (*Pi 12*)	1	3	1	1	1	3	1	1
4	IRBL 20-IR24 (*Pi 20*)	1	1	1	1	1	1	1	1
5	IRBL 5-M (*Pi5*)	1	1	1	1	1	1	1	1
6	IRBL 7-M (*Pi7*)	3	1	3	1	1	3	1	1
7	IRBL 9-W (*Pi9*)	3	5	3	1	1	3	1	1
8	IRBLb-B (*Pib*)	1	3	1	1	1	3	1	1
9	IRBLta-K1 (*Pita*)	1	1	1	1	1	1	1	1
10	IRBLta 2-Pi (*Pita -2*)	1	1	1	1	1	1	1	1
11	IRBLta 2-RE (*Pita- 2*)	1	1	1	1	1	1	3	5
12	IRBLz 5-CA *Piz-5*	1	1	1	1	1	3	1	1
13	IRBLz 5-CA ® *Piz-5* (*Pi2*)	1	1	1	1	1	1	1	1
14	IR 83260-1-1-1-2-1-22-B (*Pi 40*)	1	1	1	1	1	1	1	1
15	IR 83260-1-1-7-2-1-4-B (*Pi 40*)	1	1	1	1	1	1	1	1
16	IR83260-1-1-12-1-1-3-1-1-B (*Pi 40*)	1	3	1	1	1	1	1	1
17	IRBLks-S (*Pik-s*)	3	7	3	1	1	5	3	3
18	IRBLkp-K60 (*Pik-p*)	1	1	1	1	1	5	1	1
19	IRBLkp-K3 (**Pik-h**)	3	5	3	1	1	3	1	1
20	IRBLz-Fu (*Piz*)	3	5	1	1	3	7	1	1
21	IRBLzt-T (*Piz-t*)	3	3	1	1	1	3	1	1
22	IRBLkm-Ts (*Pik-m*)	1	5	1	1	1	1	1	1
23	IRBLa-C (*Pia*)	5	9	1	5	5	9	3	5
24	IRBLi-F5 (*Pii*)	1	9	1	1	5	9	5	5
25	IRBLks-F5 (*Pik-s*)	5	9	1	1	3	7	3	5
26	IRBLk-Ka (*Pik*)	1	1	1	1	1	1	1	1
27	IRBLta-CT2 (Pita)	1	7	3	1	3	7	5	3
28	IRBLt-K59 (*Pit*)	1	7	1	1	3	7	1	1
29	*IRBLsh-S (Pish)*	1	3	1	1	1	3	1	1
30	*IRBLsh-B (Pish)*	1	1	1	1	1	5	3	3
31	IRBL3-CP4 (*Pi3*)	5	9	1	1	3	5	3	3
32	IRBL19-A (*Pi19*)	3	9	3	3	1	7	5	3
33	IRBLta-CP1 (*Pita (Pi4)*)	3	9	3	3	1	5	3	3

LB: Leaf blast, **PB**: Neck and panicle blast

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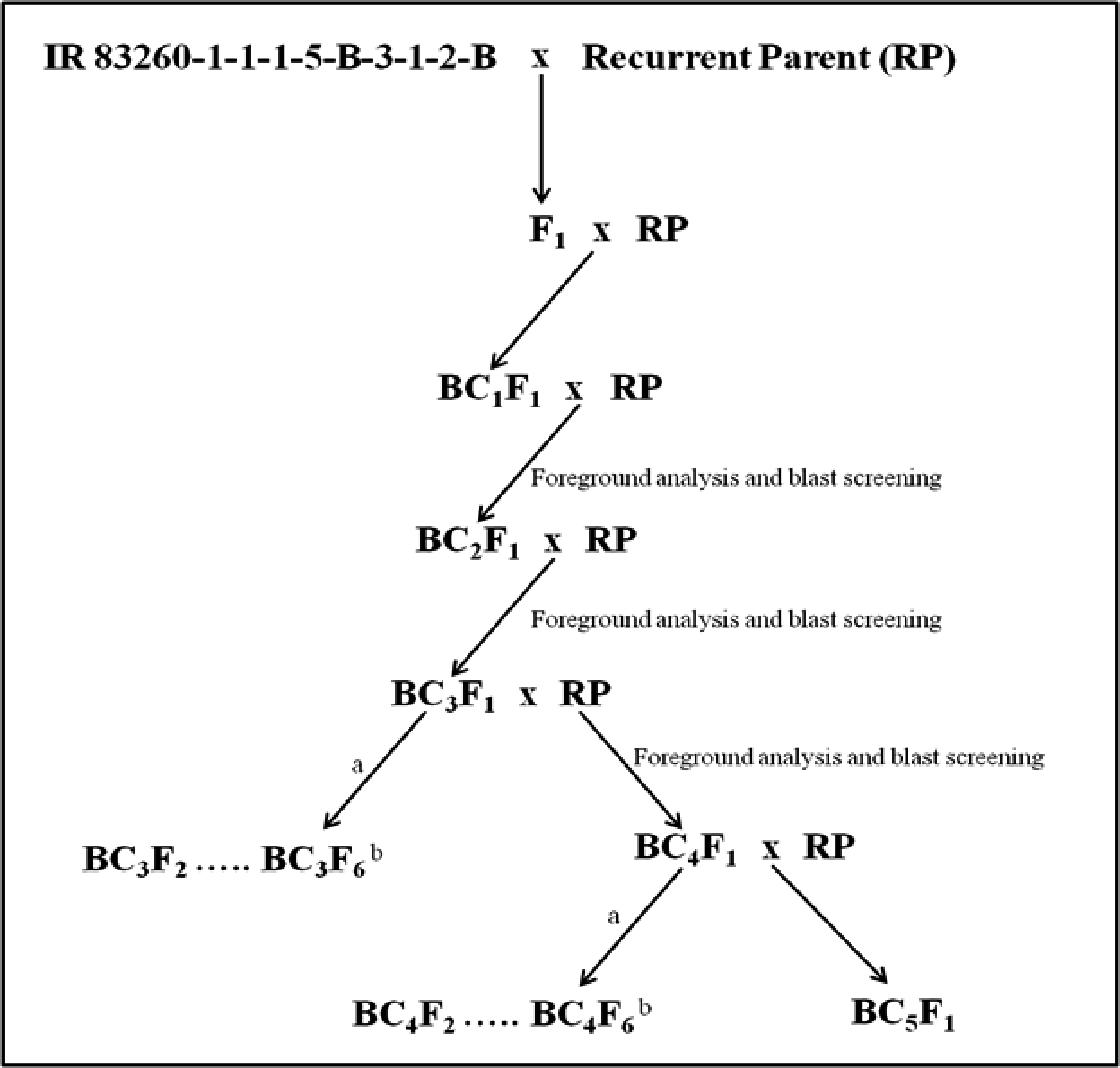
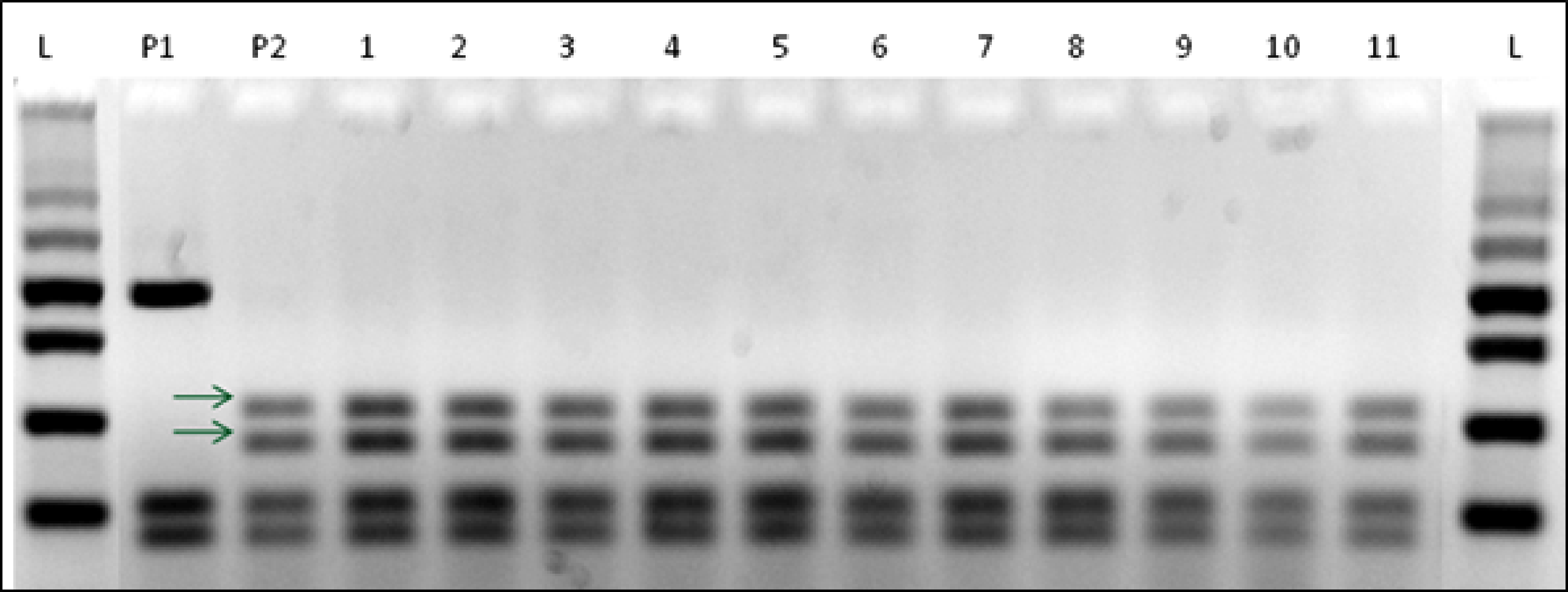

IR 83260-1-1-1-5-B-3-1-2-B x Recurrent Parent (RP)
F1 x RP
BC1F1 x RP
Foreground analysis and blast screening
BC2F1 x RP
Foreground analysis and blast screening
BC3F1 x RP
a
Foreground analysis and blast screening
BC3F2 ..... BC3F6 b
BC4F1 x RP
a
BC4F2 ..... BC4F6 b
BC5F1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P1
P2
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