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Research Article

Marker assisted introgression for brown planthopper resistance genes *Bph20* and *Bph21* in CO43*Sub1* variety of rice

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Abstract
BPH is the most destructive pest of rice. Host plant resistance is the most desirable way to control the BPH damage. The single gene resistance is easily broken by the evolution of new BPH biotypes, it can be addressed by the gene pyramiding. In order to improve CO43*Sub1* with BPH resistance, the foreground selection was carried out in BC_2F_2 lines derived from the cross CO43*Sub1* and donor IR71033-121-15. In this, 44 plants were homozygous and 13 plants were heterozygous for *Bph20*. Seven plants were homozygous and 31 plants were found heterozygous for *Bph21*. Only four plants were found to be homozygous for both the genes. Fifteen lines were selected for the phenotypic screening of BPH resistance. The lines 32-4-34 and 32-4-35 were resistance to BPH and six lines were observed with moderate resistance.

Key words
Rice, CO43*sub1*, gene pyramiding, BPH resistance, *Bph20*, *Bph21*

Introduction

Rice productivity is affected by various biotic and abiotic factors. Almost 52% of world's total production is lost due to biotic factors, of which 21% is contributed by the insect pest attack (Brookes and Barfoot, 2003). Among insects, Brown planthopper (*Nilaparavata lugens* stal.) is the most destructive monophagous pest of rice in Asian countries. It is seen as the severe pest because of its monophagy and migration ability. BPH sucks the phloem sap from the plants resulting in "hopper burn" (Watanabe and Kitagawa, 2000) and severe infestation causes the lodging of crops results in yield loss upto 10-70 % and also it acts as a vector for transmitting viruses namely grassy stunt virus and rugged stunt virus causes further yield loss (Brar *et al.*, 2009; Cha *et al.*, 2008) One of the common practice to control BPH is spraying insecticide (eg. Imidacloprid) which is expensive in terms of labour and hazardous to the health and environment. In addition, it makes the BPH population resistance to insecticide results in resurgence and development of new biotypes (Tanaka *et al.*, 2000) creates the demand for the development of resistant varieties. Rice is the only crop available with numerous genomics and bioinformatics resources, which would help in speed up the breeding processes (Swamy *et al.*, 2013).The complete rice genome sequence will provide an enormous pool of markers and genes for the improvement of rice (Khush, 2001). To date, 33 BPH resistance loci have been identified in *indica* and other wild species of rice (Hu *et al.*, 2018) most of them were mapped on the chromosome 2,3,4,6 and 12 (Jena and Kim, 2010). Among 33, 22 genes or QTLs have been fine mapped (Jairin *et al.*, 2007; Rahman *et al.*, 2009) 8 genes were isolated by map based cloning (Du *et al.*, 2009). Most of genes are documented as dominant and few were reported as recessive (*bph4, bph5, bph7, bph8, bph19, bph25 and bph29*). The durable and stable resistance can only achieved by introgression of resistance genes into the popular background. Gene pyramiding through marker assisted selection is the valuable approach to develop the elite resistant cultivar.

O. minuta (2n=48, BBCC genome) belonging to the *O. officinalis* complex acts as the resistant sources for BLB, blast and BPH. Amante-Bordeos *et al.* (1992) developed the introgression line IR71033-121-15-B derived from the cross, *O. sativa/ O. minuta* containing the gene *Bph20* and *Bph21* showed high resistance to BPH biotypes. The present study was conducted to evaluate BC_2F_3 introgressed lines with *Bph20* and *Bph21* in the background of CO43*Sub1* for BPH resistance.

Materials and Methods

The rice variety CO43*Sub1* was released by TNAU during 2016 was used as the recurrent parent. The variety CO43*Sub1*, which has multiple resistance namely salinity, gall midge, blast and submergence with good yield was crossed with the donor

IR71033-121-15-B which carries the genes *Bph20* and *Bph21*.The BC_2F_1 population was developed earlier through marker assisted backcross breeding in Department of Rice, TNAU under DBT project. The genotyping was done in BC_2F_1 to identify the plants with heterozygous loci by using SSR markers and these plants were selfed and advanced to BC_2F_2 generation. Genotyping was carried out in nearly 600 plants to identify the plants homozygous for the loci. Among them 15 progenies were selected and evaluated for BPH resistance along with recurrent parent, donor, resistant check PTB 33 and susceptible check TN1.

Genomic DNA was isolated from the individual plants by using modified hexadecyl trimethyl ammonium bromide protocol given by Doyle and Doyle (1990). DNA was quantified by using NanoDrop 2000 (gentex). SSR analysis was carried out according to the procedures described by Mccouch *et al.* (2002). For PCR amplification, the final concentration of components were 8 µL of Master Mix (2X) (contains DreamTaq DNA Polymerase, 2X DreamTaq Green buffer, dNTPs, and 4 mM MgCl2), 0.5 µM of forward and reverse primers, and 50 ng of genomic DNA. PCR amplification was carried out on a Bio-Rad T100 Thermal Cycler with the program of denaturation of 95 °C for 5 min; 94 °C for 30s; annealing of 55°C for both *Bph20* and *Bph21* for 45s ; extension of 72°C for 10 min followed by rapid cooling at 4°C. Primer sequence of the markers used in the study is given in Table1.

Brown planthopper used for infestations were collected from paddy fields of Department of Rice, TNAU, Coimbatore and maintained on the susceptible *indica* cultivar TN1. The experiment was conducted at the ambient temperature of 28-30°C and relative humidity of 70-80% by standard seed box screening technique proposed at IRRI by Heinrichs (1985).The seeds were presoaked and sown in rows along with the resistant and susceptible checks in the seed box of size 60 × 45 × 10 cm, 15-20 seedlings were maintained per lines. Seven days old seedlings were infested with the first instar nymphs at the rate of 8-10 per seedlings, a week after infestation 'hopperburn" symptoms were observed on the seedlings. When more than 90% of the susceptible check shows wilting and drying, each plant were scored individually based on the scoring system proposed by International Rice Research Institute (IRRI, 1996).(Table2.)

Results and Discussion

It has been suggested that some varieties bearing single BPH resistance were broken down quickly because of the rapid evolution and adaptation of BPH (Jena and Kim, 2010). *Bph1* single resistance gene varieties were developed in 1973 for biotype 1 and their resistance were broken down in 1976 due to the development of new biotypes i.e., biotype2. Varieties with *Bph2* with conferred resistance were developed and effectively grown, but again they are conquered by the new BPH population (biotype 3). So, it was proposed that Pyramiding of multiple genes for BPH resistance is the effective strategy to develop most durable and stable resistant varieties against BPH (Wang *et al.*, 2017). The CO43*Sub1*, an elite cultivar with the good yield is famous for its salinity and submergence tolerance and cultivated in the coastal areas of Tamil Nadu. Unfortunately, the elite variety is highly susceptible to BPH. To improve CO43*Sub1*, backcross inbreed lines were developed for BPH resistance using the donor IR71033-121-15-B and the recurrent parent CO43*Sub1*. For foreground selection, 17 linked markers were used to identify *Bph20* such as RM435, RM540, RM589, RM586, RM588, RM190, R127, RM261, RM273, RM280, RM335, RM349, RM401, RM537, RM551, RM5953 and RM8213 from which RM8213 is found polymorphic between the parents and used as foreground marker for further genotyping. Similarly for *Bph21*, 8 linked markers were analyzed namely RM131, RM124, RM6487, RM185, RM222, RM222, RM244, RM5348 and RM311 from which RM5348 clearly distinguish the CO43*Sub1* from the donor, which is further used for the identification of positive plants in various generation. In BC_2F_1 population, 17 and 6 plants were heterozygous for *Bph20* and *Bph21* alone respectively and 12 plants were found with both *Bph20* and *Bph21* alleles. From which, three plants were forwarded to BC_2F_2 generation, the genotyping results suggested that 44 and seven plants were found homozygous for *Bph20* and *Bph21* respectively, 13 and 31 plants were identified as heterozygous for *Bph20 and Bph21* respectively. Only four plants were found homozygous for both the genes (Fig 2a, Fig 2b).

To test whether the segregants derive from the cross CO43*Sub1* / IR71033-121-15-B could improve the resistance to BPH. The lines are evaluated for BPH resistance at seedling stage under greenhouse condition. While the results suggested that recurrent parent CO43*Sub1* and the susceptible control TN1 were died completely. Two lines 32-4-34 and 32-4-35 are found resistant with the recorded score of 3. Six lines 32-4-15, 32-4-18, 32-4-61, 32-4-65, 32-4-69 and 32-4-98 and the donor parent IR71033-121-1-B shows moderate resistance to BPH with the score of 5. Four lines recorded the score 7 falls in the category of moderately susceptible and three lines were susceptible with the score of 9 (Fig. 1). The dissimilarities between genotypic and phenotypic study could probably due to incomplete linkage between the selected markers and the target genes or because of the effect of genetic background. The similar response was observed by Hu *et al.* (2012) while pyramiding *Bph14* and *Bph15* genes, the pyramided lines showed higher resistance to BPH than the single gene introgression lines. Similarly, the *Bph6* and *Bph9* pyramided lines in the background of 93-11 conferred enhanced resistance to BPH (Wang *et al.*, 2017). This results is also in agreement with Liu *et al.* (2016) while introgressed the genes *Bph27* and *Bph3* into japonica variety Ningjing3 (NJ3) and *indica* variety 93-11. The results were in accordance with the findings of Fan *et al.* (2017) who developed the BPH resistant restorer line by pyramiding big-panicle gene *Gn8.1*, BPH resistance genes *Bph6* and *Bph9*, fertility restorer genes *Rf3*, *Rf4*, *Rf5* and *Rf6* through marker assisted selection. Thamarai and Soundararajan (2017) reported that entries which are known for resistant to BPH with specific genes had shown varied reaction to Coimbatore (India) population of brown planthopper. The entries Rathuheenathi, Ptb-33 which possess *Bph* 3 gene and T-12 which has *bph* 7 gene recorded resistant and moderately resistant reaction in seed box screening methods. However, the other entries Swarnalatha (*Bph* 6), Chinsaba (*bph* 8), Pokkali (*Bph* 9) observed as moderately resistant in SSST and moderately susceptible in MSST. The entry Babawee which has *Bph* 4 gene showed susceptible reaction for the BPH population. The donor parent used in the present study, IR71033-121-15 was recorded as moderate resistant reaction to *N. lugens* population of Andhra Pradesh (Bhanu *et al.*, 2014).

In the present investigation two introgressed liness 32-4-34 and 32-4-35 showed it's resistant to brown planthopper population. These lines will provide the durable BPH resistance than the donors and recurrent parent. This results illustrated that marker assisted selection is precise and efficient to conventional breeding for improvement of CO43*Sub1* with resistance against BPH. Use of marker assisted selection along with phenotypic screening against biotic stresses will serve as an excellent alternative for quick development of resistant varieties.

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References

Amante-Bordeos, A., Sitch, L., Nelson, R., Dalmacio, R., Oliva, N., Aswidinnoor, H., and Leung, H. (1992). Transfer of bacterial blight and blast resistance from the tetraploid wild rice Oryza minuta to cultivated rice, *Oryza sativa*. *Theor. Appl. Genet.*, **84**(3-4), 345-354.

Bhanu, K,V., V. Jhansilakshmi, G. Katti, A. Vishnuvardhan Reddy (2014). Antibiosis and tolerance mechanism of resistance in rice varieties carrying brown plant hopper resistance gene. *Asian J.Biol.Life Sci.,* **5** (1): 32-37.

Brar, D., Virk, P., Jena, K., and Khush, G. (2009). Breeding for resistance to planthoppers in rice, Planthoppers: new threats to the sustainability of intensive rice production systems in Asia. 401-409.

Brookes, G., and Barfoot, P. (2003). *GM Rice:* Will This Lead the Way for Global Acceptance of GM Crop Technology? ISAAA Los Banos, Philippines: ISAAA Los Banos, Philippines.

Cha, Y., Ji, H., Yun, D., Ahn, B., Lee, M., Suh, S., Lee, C., Ahn, E., Jeon, Y., and Jin, I. (2008). Fine mapping of the rice Bph1 gene which confers resistance to brown planthopper and development of EST markers for marker assisted selection. **26(**Mol. Cell), 146-151.

Doyle, J. J., and Doyle, J. L. (1990). Isolation ofplant DNA from fresh tissue. *Focus*, **12**(13), 39-40.

Du, B., Zhang, W., Liu, B., Hu, J., Wei, Z., Shi, Z., He, R., Zhu, L., Chen, R., and Han. (2009). Identification and characterization of Bph14, a gene conferring resistance to brown planthopper in rice. *Proc. Natl. Acad. Sci.,* **106**(52), 22163-22168.

Fan, F., Li, N., Chen, Y., Liu, X., Sun, H., Wang, J., He, G., Zhu, Y., and Li, S. (2017). Development of elite BPH-resistant wide-spectrum restorer lines for three and two line hybrid rice. *Front. Plant sci., 8*, 986.

Heinrichs, E. (1985). Genetic evaluation for insect resistance in rice. Los Baños, the Philippines: International Rice Research Institute: Los Baños, the Philippines: International Rice Research Institute:.pp 71-170

Hu, J., Chang, X., Zou, L., Tang, W., and Wu, W. (2018). Identification and fine mapping of Bph33, a new brown planthopper resistance gene in rice (*Oryza sativa* L.). *Rice,* **11**(1), 55.

Hu, J., Li, X., Wu, C., Yang, C., Hua, H., Gao, G., Xiao, J., and He, Y. (2012). Pyramiding and evaluation of the brown planthopper resistance genes Bph14 and Bph15 in hybrid rice. *Mol. Breed.,* **29(**1), 61-69.

Jairin, J., Teangdeerith, S., Leelagud, P., Phengrat, K., Vanavichit, A., and Toojinda, T. (2007). Detection of brown planthopper resistance genes from different rice mapping populations in the same genomic location. *Sci. Asia* **33,** 347-352.

Jena, K. K., and Kim, S.-M. (2010). Current status of brown planthopper (BPH) resistance and genetics. *Rice,* **3(**2-3), 161-171.

Khush, G. S. (2001). Green revolution: the way forward. *Nat. Rev. Genet.,* **2(**10), 815-822. doi: 10.1038/35093585

Liu, Y., Chen, L., Liu, Y., Dai, H., He, J., Kang, H., Pan, G., Huang, J., Qiu, Z., and Wang, Q. (2016). Marker assisted pyramiding of two brown planthopper resistance genes, Bph3 and Bph27 (t), into elite rice cultivars. *Rice,* **9**(1), 27.

Mccouch, S. R., Teytelman, L., Xu, Y., Lobos, K. B., Clare, K., Walton, M., Fu, B., Maghirang, R., Li, Z., and Xing, Y. J. D. R. (2002). Development and mapping of 2240 new SSR markers for rice (*Oryza sativa* L.). *DNA Res.,* **9(**6), 199-207.

Rahman, M. L., Jiang, W., Chu, S. H., Qiao, Y., Ham, T.-H., Woo, M.-O., Lee, J., Khanam, M. S., Chin, J.-H., and Jeung, J.-U. (2009). High-resolution mapping of two rice brown planthopper resistance genes, Bph20 (t) and Bph21 (t), originating from *Oryza minuta*. *Theor. Appl. Genet.,* **119**(7), 1237-1246.

Swamy, B. P. M., Ahmed, H. U., Henry, A., Mauleon, R., Dixit, S., Vikram, P., Tilatto, R., Verulkar, S. B., Perraju, P., Mandal, N. P., Variar, M., Robin, S., Chandrababu, R., Singh, O. N., Dwivedi, J. L., Das, S. P., Mishra, K. K., Yadaw, R. B., Aditya, T. L., Karmakar, B., Satoh, K., Moumeni, A., Kikuchi, S., Leung, H., and Kumar, A. (2013). Genetic, physiological, and gene expression analyses reveal that multiple QTL enhance yield of rice mega-variety IR64 under drought. *PloS one,* **8(**5), e62795-e62795. doi: 10.1371/journal.pone.0062795

Thamarai, M. and Soundararajan, R.P. (2017). Reaction of rice genotypes against specific population of brown planthopper, *Nilaparvata lugens* (Stal). *Annals of Plants Protection sciences* **25**(1): 74-77.

Tanaka, K., Endo, S., and Kazano, H. (2000). Toxicity of insecticides to predators of rice planthoppers: spiders, the mirid bug and the dryinid wasp. *Appl. Entomol. Zool.,* **35**(1), 177-187.

Wang, Y., Jiang, W., Liu, H., Zeng, Y., Du, B., Zhu, L., He, G., and Chen, R. (2017). Marker assisted pyramiding of Bph6 and Bph9 into elite restorer line 93–11 and development of functional marker for Bph9. *Rice,* **10**(1), 51.

Watanabe, T., and Kitagawa, H. (2000). Photosynthesis and translocation of assimilates in rice plants following phloem feeding by the planthopper *Nilaparvata lugens* (Homoptera: Delphacidae). *J. Econ. Entomol.,* **93**(4), 1192-1198.

Table 1. Primer sequence of markers used in the study

S.No.	Genes	Marker	F: Forward primer	R: Reverse primer
1	*Bph20*	RM8213	AGCCCAGTGATACAAAGATG	GCGAGGAGATACCAAGAAAG
2	*Bph21*	RM5348	AATCCGATAGGAGTACCGCC	AAGTGTATGGGCTGGAATGG

Table 2. Standard evaluation system for BPH resistance

Scale	Damage	Resistance level
0	No damage	Immune
1	Very slight damage	Highly resistant
3	First and 2nd leaves of most plants partially yellowing	Resistant
5	Pronounced yellowing and stunting or about 10 to 25% of the Plants wilting or dead and remaining plants severely stunted or dying	Moderately resistant
7	More than half of the plants dead	Moderately susceptible
9	All plants dead	Susceptible

Table 3. Phenotypic and genotypic analysis of 15 BC_2F_3 lines along with parents

S.No.	Pyramided lines	Phenotypic analysis	Genotypic analysis		Category
		Score	RM8213	RM5348	
1	32-4-15	5	B	H	MR
2	32-4-18	5	B	B	MR
3	32-4-20	9	B	B	S
4	32-4-27	9	B	H	S
5	32-4-34	3	B	H	R
6	32-4-35	3	B	H	R
7	32-4-43	7	B	H	MS
8	32-4-50	7	B	H	MS
9	32-4-52	9	B	A	S
10	32-4-57	7	B	H	MS
11	32-4-61	5	B	B	MR
12	32-4-63	7	B	H	MS
13	32-4-65	5	B	B	MR
14	32-4-69	5	B	B	MR
15	32-4-98	5	H	B	MR
16	CO43*Sub1*	9	A	A	S
17	IR71033-121-15-B	5	B	B	MR
18	TN 1	9	-	-	S
19	PTB 33	3	-	-	R

A: Homozygous recipient allele; B: Homozygous donor allele; H: Heterozygous allele
A - CO43*Sub1*B-IR71033-121-15-B

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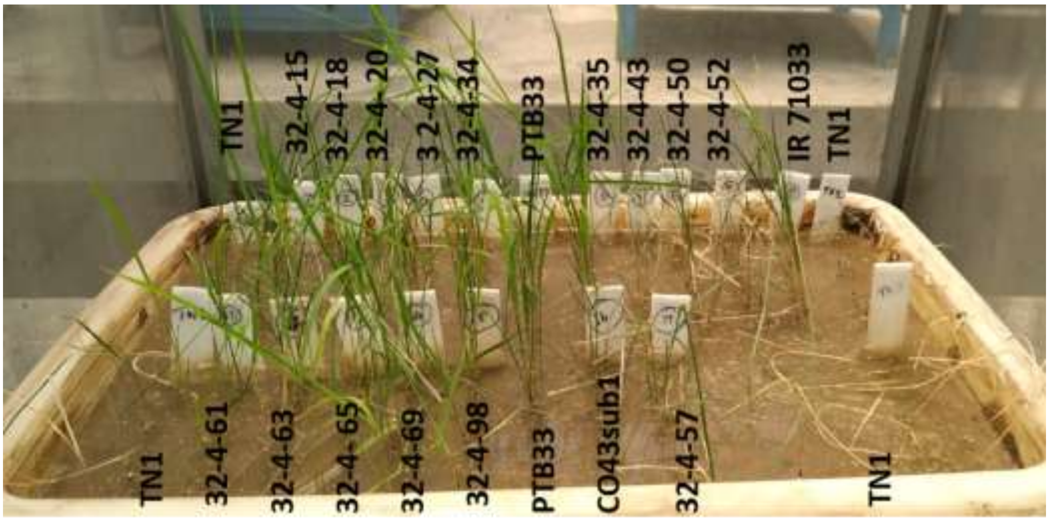

Fig. 1. Standard seed box screening in pyramided lines of CO43Sub1 with *Bph20* and *Bph21* resistance gene

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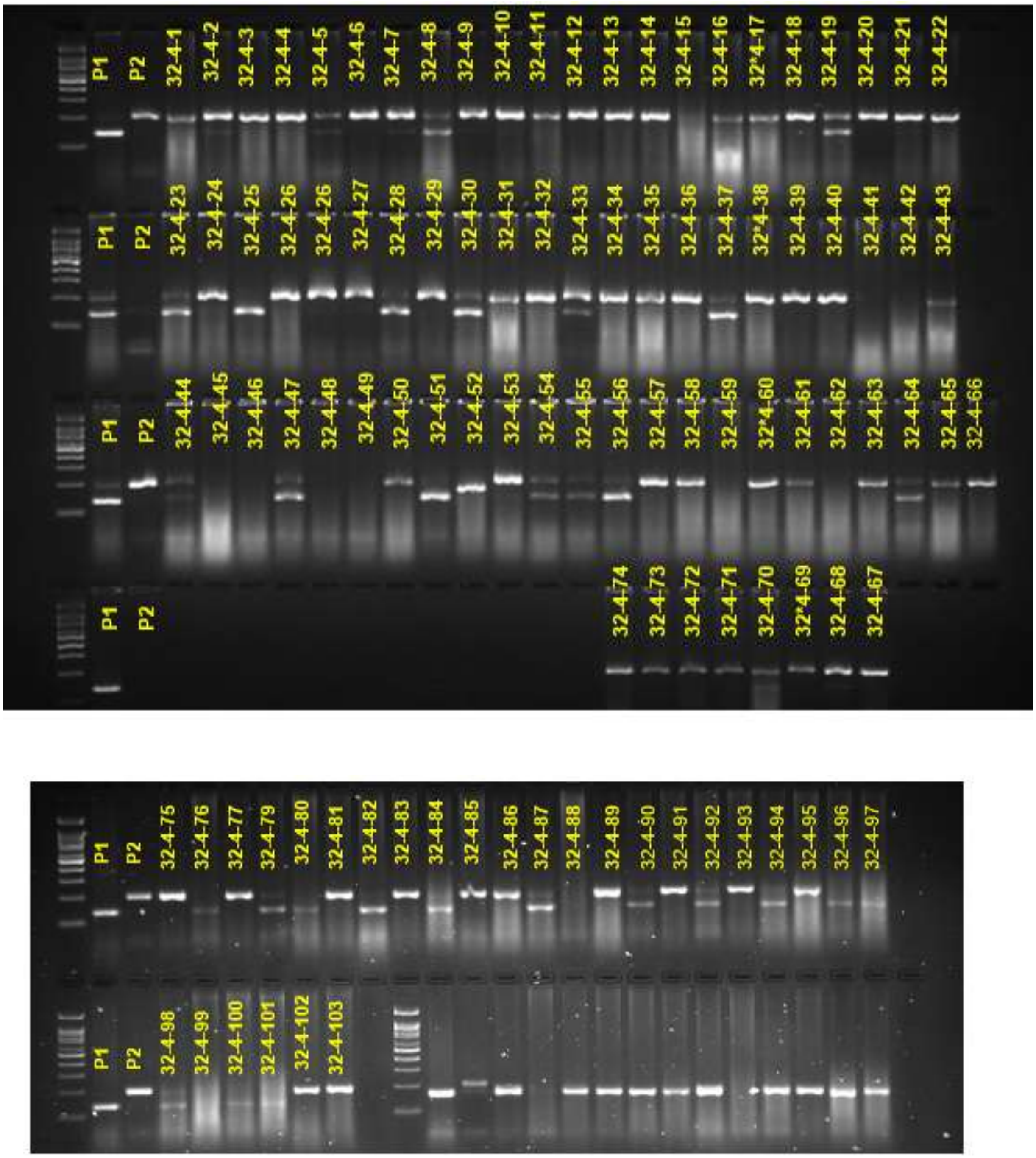

Fig. 2a. Foreground selection for *Bph20* using RM8213

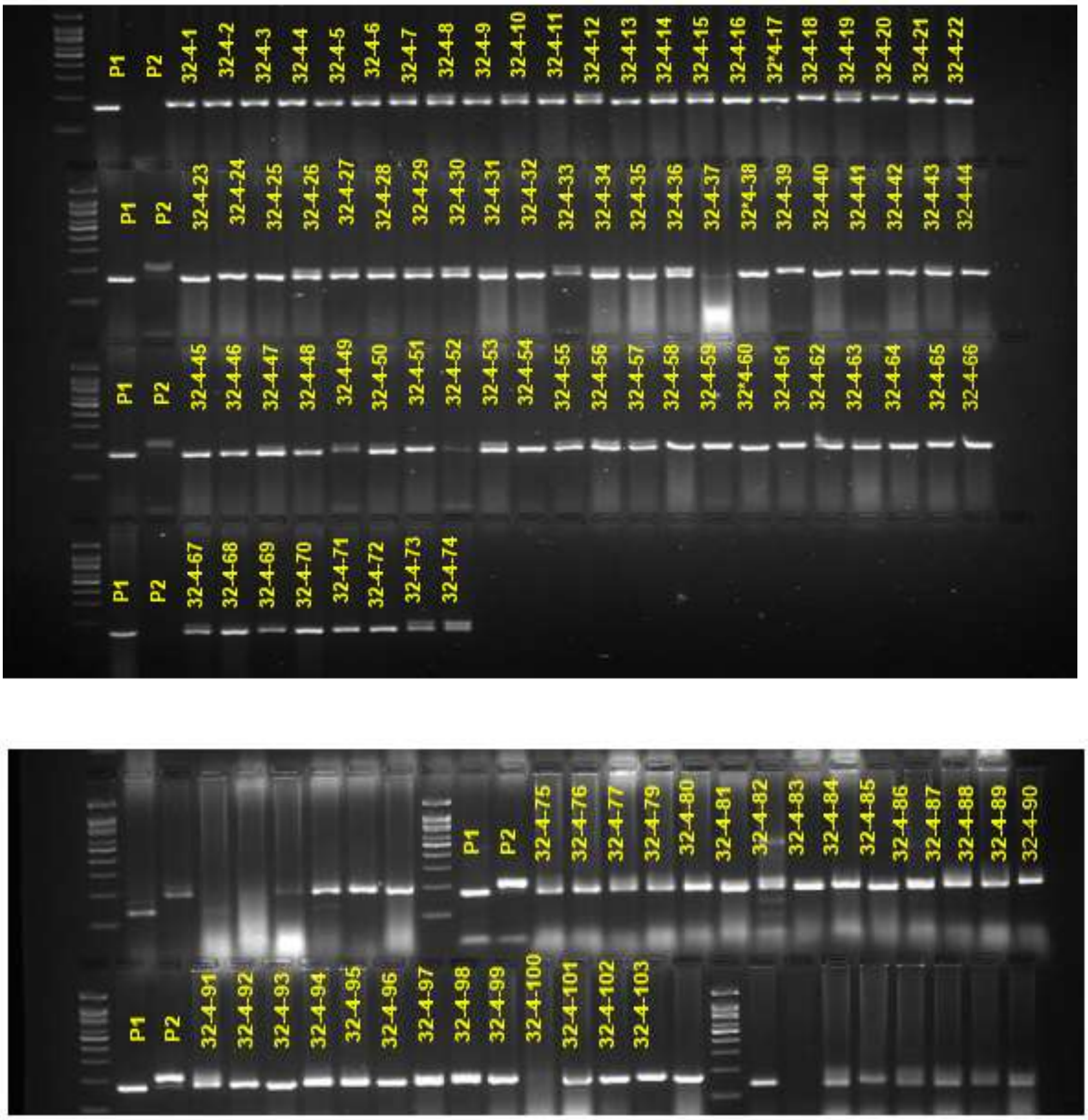

Fig. 2b. Foreground selection for *Bph21* using RM5348

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