Agronomic Characteristics, Yield Components and Polymorphic Markers between IRBL Kp-K60 and Ciherang for Developing Rice Resistant to Blast

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Abstract. Blast is one of the most devastating diseases in rice field in Indonesia which caused by Pyricularia oryzae. Utilising rice resistant variety is one of the most effective, economical and eco-friendly solution for the disease. To develop rice resistant to blast is required resources of plant with resistance gene and popular variety as the parents. Introgressing gene pik in chromosome 11 from IRBL Kp-K60 into Ciherang was conducted using markers-assisted backcrossing. In this backcrossing program, selection process is a crucial part to gain the goal of the program. The goal of backcrossing program is to obtain plant which has all Ciherang characteristics with additional trait, resistance to blast. Some characteristics of the parents such as agronomic characteristics and yield components must be observed to assist phenotypic selection in the progeny. Moreover, applying DNA markers in backcrossing program will facilitate selection process to ensure that resistance gene is already transferred from donor into recipient. Thus, it is important to analyse polymorphic DNA markers between the parents. The aim of the study were to assess parental difference in agronomic characteristics and yields component and analyse polymorphic markers between Ciherang and IRBL Kp-K60 for developing rice resistant to blast disease. The result of study showed that several agronomic characteristics and yield component in Ciherang were more superior than IRBL Kp-K60. Flowering date of Ciherang was more longer than IRBL Kp-K60. Thirty six DNA markers were used in this study from various type of markers, namely CAPs, SSR and SNP. Some DNA markers were dominan and the others were codominan. There were four DNA markers were polymorphics between the parents and can be used in the selection process in the progeny.

1. Introduction
Declining rice production in Indonesia can be caused by biotic and abiotic stresses. For biotic stress, blast is a major disease of rice because of its wide distribution and extent of destruction under favorable conditions in rice cultivation. Pyricularia grisea is a fungus, blast pathogen, can cause severe damage usually during seedling stage. These fungus also attack rice plant at reproductive stage is called neck blast which causes the panicle falls over or grain filling is failure. The use of resistant varieties is thought to be the one of the effective, economical and eco-friendly approach to control blast disease. Introgression of rice blast resistance genes into popular variety is need to be conducted for developing rice resistant to blast. Ciherang is a popular variety in Indonesia can be inserted by blast resistance genes so that has been durable resistance to blast disease. Technique can be used for introgression or substitution of target gene from donor parent to recipient is backcrossing. The technique is also adopted as breeding strategy to improve elite varieties because of its can decline the donor genom content in the progeny [1]. The crossing back process is repeated until obtained the
Ciherang with the gene of interest from the donor parent. So in developing new variety especially through the backcrossing program, the breeder needs to compile much information about the parents to make sure that the parents are being used have an appropriate combination in agronomic, yield component characteristics and resistance gene. Some of those information are useful for breeding (ex. flowering time) and for selection process.

Selection process is a crucial part of breeding program, especially backcrossing. Selection on phenotypic resemblance to the recurrent parent (or against the donor) has long been used by breeders [2]. So phenotypic selection can be conducted to find the plant in the progeny which has similarity performance with recurrent parent, such as plant height, number of panicle etc. Then selection can also be based on molecular marker alleles typical of either parent [2]. Rice genomics will facilitate using resistance gene in breeding for developing rice resistant to disease by DNA markers assisted selection (MAS) [3]. Applying DNA marker in the selection process reduce the number of backcrossing required to recover recurrent phenotype. In addition selection using DNA markers can be done at seedling stage with small sample of DNA and produce accurate data so that diminishing laborious work and time consuming. There are several type of DNA markers have been developed for marker-assisted selection [3] in rice such as cleaved amplified polymorphic sequences (CAPS), simple sequence repeat (SSR), single-nucleotide polymorphisms (SNPs) and small insertion and deletion (InDels). SNPs and InDel are highly abundant and distributed in whole genome of rice [4]. [3] have been published the summary of resistance gene information for rice blast disease and their selection markers.

Ciherang has a potency to be a recipient’s parent in the backcrossing due to grain quality and more acceptable among farmers and consumer in Indonesia. Ciherang has good agronomic characteristic such as plant height 107-115 cm and number of productive tiller 14-17 tiller as well [5] but Ciherang has lack of resistancy to some blast races. Meanwhile IRBL-Kp-K60 is a monogenic line which has blast resistance gene derived from K60 was crossed with Koshihikari. Introgressed blast resistance gene from IRBL Kp-K60 into Ciherang expected can improve Ciherang variety to be durable resistant to several blast races.

The objective of this research were to assess parental difference in agronomic characteristics and yield component and analyse polymorphic markers between Ciherang and IRBL Kp-K60 for developing rice resistant to blast disease.

2. Methods
2.1 Agronomic characteristics and yield component
Breeders seed of popular variety, Ciherang and the seed of the donor, IRBL Kp-K60 driven from Indonesian Rice Research Center (BB Muara) were used in this research. The seeds germinated in seedling tray for 21 days and then planted in plastic pot with each genotype consisted of 5 plants. Measured parameters were plant height, number of tiller, number of productive tiller, flowering date, panicle length, number of spikelets per panicle and number of fertile spikelets per panicle. Plant height was observed at 77 days after planting while number of tiller and number of productive tiller at the time of harvest. After harvesting, yield component such as panicle length, number of spikelets per panicle and number of fertile spikelets per panicle.

2.2 Polymorphic markers between Ciherang and IRBL Kp-K60
Young green leaves from 30 days old plant were extracted by modified Cetyl-Trimethyl Ammonium Bromide (CTAB). DNA quantification and purity was estimated by measuring O.D value at 260 and 280 nm using Nanophotometer. Total volume of PCR mixture was 10 µl, consisted of 1 x Taq green mastermix, 0.5 µm forward primer, 0.5 µm reverse primer, 10-100 ng DNA (template) and Nuclease Free Water. PCR analysis was carried out in Eppendorf Thermal Cycles with program as follows: initialization in 95ºC for 5 minute to activate DNA polymerase, 35 cycles of 94ºC for 1 minute for denaturation, 50 ºC and 60ºC (depend on the markers) for 1 minute for annealing and 72ºC for elongation then followed by 72ºC for 7 minute for final elongation. PCR product run on 2 % agarose gel with 0.5 x TBE at 65 volt for 90 minutes. Agarose gel was visualized in Uvitech HD5 previously stained with editium bromide solution for 15 minutes.
A total of 39 markers were used for parental polymorphism between Ciherang and IRBL Kp-K60. All markers are located in the chromosome 11 and primer sequence of markers were obtained from [2][4][6](Table 1.)

Table 1. Primer of markers

| Marka | Forward | Reverse | Anneal Temp |
|-------|---------|---------|-------------|
| k6816 | TCGCCGATGCGGTTGATTAC | CGTATTGTTGTTTGAAGGA | 60 |
| k2167 | CGTGCTGTGCTCTGGAATCTG | CACGAAACAGAGTGCGG | 60 |
| k6438-1 | GCGACCCTGTCTTTGGACTG | GAATGATGAGGAGAAGGCT | 60 |
| k6438-2 | GCGACCCTGTCTTTGGACTG | GAATGATGAGGAGAAGGCT | 60 |
| k6415-1 | CTAATGGAATTTAACGCTG | ATCCCGATGTCTCATCGATCAC | 60 |
| k6415-2 | CTAATGGAATTTAACGCTG | ATCCCGATGTCTCATCGATCAC | 60 |
| k8823-1 | GTTGTGCGTTCTCTATACCA | GCATGACAGATGGAAGTGTAG | 60 |
| k8823-2 | GTTGTGCGTTCTCTATACCA | GCATGACAGATGGAAGTGTAG | 60 |
| k8824-1 | CCACGCTCCTAGCTACCCCG | ACAAGGAACCCAGAAGCTC | 60 |
| k8824-2 | CCACGCTCCTAGCTACCCCG | ACAAGGAACCCAGAAGCTC | 60 |
| k8824-3 | GCTTGTGCGTTCTCTATACCA | ACAAGGAACCCAGAAGCTC | 60 |
| K8824-4 | GCTTGTGCGTTCTCTATACCA | ACAAGGAACCCAGAAGCTC | 60 |
| k3951-1 | AAGTAACACATGGTCAATA | CCAGAATTCAGGCTCTTG | 60 |
| k3951-2 | GCCACATCAATGGCTACAAC | CCAGAATTCAGGCTCTTG | 60 |
| K 3951-3 | GCCACATCAATGGCTACAAC | CCAGAATTCAGGCTCTTG | 60 |
| RM206 | CCCATGCGTTTAACTATTTCT | CGTTCCATCGATCCGTATGG | 55 |
| RM27386 | TGCTTTGGTCTCTGTCTGCTTGC | TGCTACTACTAGGAGAGGAGC | 55 |
| RM1233 | TTCGTTTTCCTTTGGGTATGG | ATGGCCTCTGAAAGGA | 55 |
| RM5766 | ATGTCTGCAAAAAAGGGAGAC | ACCGAGGAGCAGTCCCACC | 55 |
| RM4069 | CTGGTGTGTTACTCCGGAAGCA | TGGCCCTTGCTATTAGTGGTC | 55 |
| RM224 | ATCAGATGATCCTTCACAGG | TGCTATAAAAGGCATTCGGG | 55 |
| RM144 | TGCCCTGGGCCAATTTGTAC | GCTAGAGGAGATGATGGTA | 55 |
| RM6293 | GCCCTGATGCGTATTGATTC | TCACTAAAACGCCCTGAA | 55 |
| RM254 | AGCCCCGATACAATCCGACCTCT | CGTCCAAGCATTGGTGATC | 55 |
| RM5926 | ATATAGTGCAGTTCCCTACCA | AGATATAGCAGTGACAGC | 55 |
| K37 | CGATTGTCTCTCTGATTTTG | CTTTTGTCGATTGCTGTG | 58 |
| K25 | TGAACATTCGATCAGAACAGTC | CAGGTTATGGTCAAGGAGAC | 62 |
| K15-2 | GATCATCCTCTTCACAGG | GCTGAGAAGAGGTTG | 60 |
| K22 | TCCTCTTCTGTTCTTCTC | GGGCACAACAGATCCACC | 60 |
| K28 | TCCCTACGACCGGATATG | GTATCGTAGAAGTGGTC | 60 |
### 3. Results and Discussion

#### 3.1 Agronomic characteristics and yield component

Agronomic characteristics both of Ciherang and IRBLKp-K60 showed a significant difference. According to table 2, plant height of IRBL Kp-K60 taller than Ciherang but number of tiller and number of reproductive tiller’s IRBL Kp-K60 more less than Ciherang. It was due to Ciherang had vegetative stage more longer than IRBL Kp-K60 so that Ciherang had more time to produce more tillers and panicles. It also was occured due to the difference time of flowering where Ciherang started flowering at 77 days after planting meanwhile IRBL Kp-K60 at 63 days after planting. Flowering time information of the parents needed to ensure they will have flowers at the same time, the practice of staggered planting is used by the some breeders to plant sets of the parents at different time [7]. In panicle length, there was no a significant difference between Ciherang and IRBL Kp-K60. Moreover Ciherang had number of spikelets per panicle and number of fertile spikelets per panicle higher than IRBL Kp-K60. The percentage of number of fertile spikelets per panicle in Ciherang was about 94% of total spikelets per panicle and 75% of total spikelets per panicle in IRBL Kp-K60. According to [8] number of reproductive tiller and number of spikelets per panicle provide useful information for rice breeders and have direct effect on yield per plant. In addition, those characteristics can be use to select the progeny that related to recurrent parent (Ciherang). Thus, the study showed that several agronomic characteristics and yield component in Ciherang were more superior than IRBL Kp-K60.

#### Table 2. Agronomic characteristics and yield component between Ciherang and IRBL Kp-K60

| Characteristics                  | Ciherang            | IRBL-Kp-K60          |
|----------------------------------|---------------------|----------------------|
| Plant height                     | 105.5 ± 5.19        | 113.12 ± 6.86        |
| Number of tiller                 | 16.25 ± 0.95        | 9.5 ± 1              |
| Number productive tiller         | 16 ± 0.81           | 9.5 ± 1              |
| Flowering date                   | 77 days after planting | 63 days after planting |
| Panicle length                   | 23.37±0.70          | 22.14±1.32           |
| Number of spikelets per panicle  | 170.22 ± 16.90      | 81.13 ± 10.64        |
| Number of fertile spikelets per panicle | 160.48±19.05       | 61.85 ± 5.29         |

#### a. Polymorphic markers between Ciherang and IRBL Kp-K60

Study of parental polymorphism is a pre requisite to begin marker assisted selection or marker assisted backcrossing. Unless the parents are polymorphic for the traits of interest, the further selection of plants carrying the traits of interest is not possible in the progenies [9].

Out of thirty nine markers were used for polymorphic study, 25 markers (65.78%) exhibited polymorphism between Ciherang and IRBL Kp-K60. Fifteen of 25 markers (60%) which polymorphic were dominant and the others were codominant. Some dominant markers were from IRBL Kp-K60 can be used to detect the presence of resistance gene in the progeny and the others can be effectively used for the speedy recovery of recurrent parent (Ciherang). Polymorphic markers between Ciherang and IRBL Kp-K60 was illustrated in table 3 and figured out on figure 1.
Table 3. Polymorphic markers between Ciherang and IRBL-Kp-K60

| No. | Marker | Ciherang | IRBL-Kp-K60 | Polymorphic | Dominan/Codominant |
|-----|--------|----------|-------------|-------------|---------------------|
| 1   | k6816  | +        | +           | √           | Codominant          |
| 2   | k2167  | +        | +           | √           | Codominant          |
| 3   | k6438-1 | +       | +           | -           |                    |
| 4   | k6438-2 | +++      | +++         | √           | Codominant          |
| 5   | k6415-1 | +        | +           | -           |                    |
| 6   | k6415-2 | +        | +           | -           |                    |
| 7   | k8823-1 | ++       | +           | √           | Codominant          |
| 8   | k8823-2 | -        | +           | √           | Dominant            |
| 9   | k8824-1 | -        | +           | √           | Dominant            |
| 10  | k8824-2 | -        | ++          | √           | Dominant            |
| 11  | k8824-3 | -        | +           | √           | Dominant            |
| 12  | k8824-4 | -        | +           | √           | Dominant            |
| 13  | k3591-1 | +        | +           | -           |                    |
| 14  | k3591-2 | -        | +           | √           | Dominant            |
| 15  | k3591-2-1 | -       | +           | √           | Dominant            |
| 16  | k3591-2-2 | +       | +           | -           |                    |
| 17  | RM 206  | +        | ++          | √           | Codominant          |
| 18  | RM 27386 | +        | +           | √           | Codominant          |
| 19  | RM 1233 | +        | +           | -           |                    |
| 20  | RM 5766 | +        | ++          | √           | Codominant          |
| 21  | RM 4069 | +        | +           | -           |                    |
| 22  | RM 224  | +        | +           | √           | Codominant          |
23 | RM 144 | + | + | √ | Codominant |
24 | RM 6293 | + | + | - |
26 | RM 254 | + | + | √ | Codominant |
27 | RM 5926 | - | + | √ | Dominant |
28 | K-37 | - | + | √ | Dominant |
29 | RM144 | - | + | √ | Dominant |
30 | K 25 | - | - | - |
31 | K 15-2 | - | + | - | Dominant |
32 | K 22 | - | - | - |
33 | K 28 | - | + | √ | Dominant |
34 | K 34 | - | + | √ | Dominant |
35 | K 39 | - | - | - |
36 | K 33 | - | + | √ | Dominant |
37 | K 40 | - | + | √ | Dominant |
38 | K41 |
39 | K42-1 |
40 | K42-2 | + | +++ | √ | Codominant |

4. Conclusion
The result of study showed that several agronomic characteristics and yield component in Ciherang were more superior than IRBL Kp-K60. Thirty six DNA markers were used in this study from various type of markers, namely CAPs, SSR and SNP. Some DNA markers were dominan and the others were codominan. There were fourthen DNA markers were polymorphics between the parents and can be used in the selection process in the progeny.

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6. References
[1] Hasan M M, Mohd Y Rafii, Mohd R Ismail, Maziah M, Harun A R, Md Amirul A, Sadegh A, Md Abdul M and Muhammad A L 2015 Biotechnology and Biotechnology Equipment 29 (2) 237-254
[2] Hospital F 2005 Philosophical Transactions of Royal Society B 360 1503–1511
[3] Koide Y, Nobuya K, Donghe X and Yoshimichi F 2009 JARQ 43 (4) 255-280
[4] Yunbi X 2010 MPG Group Books 25-43
[5] Hayashi K, H Yoshida and I Ashikawa 2006 Theor Appl Genet 113 251-260
[6] Kementerian Pertanian Republik Indonesia http://epetani.deptan.go.id 2010
[7] Wang L, Xu X, Lin F and Pan Q 2009 Phytopathology 99 (8) 900-905
[8] George A 2007 Blackwell Publishing
[9] Sadeghi SM 2011 World Applied Sci J. 13 (5) 1229-1233
[10] Kumar G S, K A Kumari, Ch V Durga Rani, R M Sundaram, S Vanisree, Md Jamaloddin and G Swathi 2013 African Journal Biotechnology 12 (40) 5833-5838