Review

Agronomics, Genomics, Breeding and Intensive Cultivation of Ciherang Rice Variety

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Abstract: Ciherang rice variety was released in year 2000 and become a superior variety in Indonesia. In a decade, due to the excellent agronomic characteristics and high productivity, Ciherang gradually replace the mega-variety IR-64 and widely cultivate in almost 40% rice production area in Indonesia. In addition to its high grain yield, short straw, early maturity, and high productive tiller number, it had superior grain quality, such as long and slender grain shape, clean yellow color, aromatic, and high milled rice percentage with excellent eating quality, matching with farmers and rice consumers demands. It has intermediate glycemic index and delicious taste. It is intolerance to flooding condition and susceptible to several diseases and pests, including rice blast, bacterial blight, brown planthopper, and yellow stem borer. Because of Ciherang has been well-characterized genetically, many quantitative trait loci (QTLs) and candidate genes which associated with certain traits have been identified. It has also been used as a parent in rice breeding program to develop mapping populations for genetic analysis. In order to increase the flooding tolerant and disease resistant in Ciherang, many valuable genes have been introduced into Ciherang through backcrossing breeding technique and transgenic approach. The optimal rice productivity in Ciherang also supported by the application of intensification system in Ciherang cultivation. Unravelling the complex genetic control of agronomic characteristics, grain yield traits, and other desirable traits which valuable for rice farmers and consumers are very important.

Keywords: Ciherang; agronomics; genetics; breeding; intensive cultivation

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Introduction

Rice (*Oryza sativa* L.) is the primary staple food in Indonesia and most of the Asian countries. Rice field areas in Indonesia are reaching 13.84 million ha with a total rice production up to 71.28 million ton and with an average rice yield 5.15 ton/ha [1]. Ciherang rice variety known as superior and high-yielding variety originating from Indonesia that release in year 2000 and cultivate widely in Indonesia with the grain yield almost 10 ton/hectare [2]. Ciherang was developed at the International Rice Research Institute (IRRI), Philippines [3,4] by multiple variety crosses, including IR-64 as the background parent. Ciherang was developed by the cross IR18349-53-1-3-1-3/IR19661-131-3-1//IR19661-131-3-1/ IR64/IR64 [4,5]. For over two decades, IR-64 was a dominant rice variety in Indonesia. Due to the similar characteristics with IR-64 rice variety and high productivity, Ciherang gradually overtook the mega-variety IR-64 to be the largest rice variety which dominantly cultivate in Indonesia up to 40% of the total irrigated rice field for decades [6].

Between IR-64 and Ciherang showed similar morphological traits, grain quality, and genetic characteristics because Ciherang was developed by using IR-64 as the background parent [5]. Ciherang also adapt easily in any field conditions, including suitable in the wet season, and relatively shorter harvest age. Since Ciherang is intolerance to flooding condition, Ciherang is more appropriate in the dry season [7]. In 2015, Ciherang-Sub1 which

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submergence tolerant was developed [5]. One of the popular characteristic from Ciherang is related to high economic yield, short straw, and the grain quality which display long and slender grain shape, clean yellow color, aromatic, and high milled rice percentage with good eating quality that meets the rice farmer and consumer demands (Rosdianti et al., 2022). Thus, Ciherang plays an important role in the economic growth of Indonesia through agricultural sector.

Ciherang established excellent agronomic characteristics, grain yield and cooking traits that have now spread over 40% rice growing areas in Indonesia. Because of its characteristics, Ciherang became a superior rice variety and was highly desired by the rice farmers and consumers. It also has a number of defects, most importantly, intolerant to flooding condition and susceptible to several diseases and insects. Since genetics characteristics of Ciherang has been well-characterized, many mapping populations were developed by using Ciherang as one of the parent. A lot of important genes has also been introduced to Ciherang to increase the flooding tolerance and resistance to diseases and insects. In this review article, the development of Ciherang and its agronomics, genomics, breeding, and intensive cultivation were described.

Major Agronomic and Quality Traits of Ciherang

Ciherang belongs to indica rice variety and popular among rice farmers and consumers due to the superior agronomic characters, including high productivity, early maturity, resistance to brown planthopper and bacterial leaf blight, and also good grain quality with delicious, aromatic, and waxy taste (Table 1 and 2) which fulfilled the farmers and rice consumer demands. Ciherang contains low amylose which influence the delicious taste and show good range of span after cooking. Ciherang rice grain has slender shape and yellow color with glutamic acid as the most abundant amino acid. The grain yield of Ciherang also influenced by seedling age and seedling number per hill with optimum age ranging 7 until 21 day-old seedlings with one seedling per hill. Planting Ciherang rice with one seedling per hill significantly increase the grain yield due to receive optimum solar radiation interception and nutrient uptake leading to increase the photosynthetic rate, growth and development of the rice plants [8,9,10].

According to Yuliani et al. [11], Ciherang moderately resistant to Xoo pathotype III in the dry season, but susceptible in the wet season. This rice variety also suitable to cultivate in the rice field with altitude below 500 m above the sea level [12]. High productivity of Ciherang also influenced by longer panicle and denser spikelet [13]. Meanwhile, Ciherang has thin epidermis layer in the grain but has harder grains, so it is not easy to be broken in the milling process. Ciherang grains also moderately resistance to stored rice insects, such as Lesser Grain Borer (*Rhyzopertha dominica*) because of the grains hardness and non-chalkiness physical characteristics and chemical composition, including phenolic content, carbohydrate, fat, protein, and ash [14,15]. *R. dominica* can decrease the grain quality in storage and made the broken grains into dust.

Table 1. Agronomic Characteristics of Ciherang [2,3,16-33].

Agronomic Traits	Ciherang
Plant height	80 cm
Plant shape	Upright
Total number of tillers	50
Productive tiller number	13
Stem color	Green
Foot color	Green
Total leaf area	825.3 cm^2/clump
Leaf number	5
Leaf surface	Rough
Flag leaf	Erect

Flag leaf length	21.86 cm
Leaf color	Green
Leaf togue color	Colorless
Chlorophyll a	27.08 mg/g
Chlorophyll b	25.81 mg/g
Chlorophyll total	52.90 mg/g
Flowering days	55 days after planting
Days to 50% heading	108 days after planting
Specific leaf weight (SLW)	0.0078 g/cm^2/week
Net assimilation rate (NAR)	0.0102 g/cm^2/week
Relative growth rate (RGR)	0.271 g/g/week
Crop growth rate (CGR)	0.0097 g/cm^2/week
Fresh weight of plants	86.96 g
Shoot dry weight	10.6 g
Root dry weight	3.2 g
Root length	36.06 cm
Total root number	127.5
Root surface area	352
Shoot root ratio	3.30
Panicle length	25 cm
Panicle number per plant	30
Total spikelet per panicle	159
Filled grain number per panicle	105
Unfilled grain number	21.2
Filled grain percentage	81%
Unfilled grain	2.84%
Productivity	10 ton/hectare
Harvest index	0.97
Harvest age	118 days after planting
Grain yield per plant	16.10 g
Bacterial leaf blight strain III and IV	Resistance
Rice brown leafhoppers biotype 2 and 3	Resistance

Table 2. Grain Characteristics of Ciherang [3,32-37].

Grain Quality Traits	**Ciherang**
Grain length	7.12 mm
Grain width	2.26 mm
Grain length/width ratio	3.15
1000-grain weight	30 g
Grain shape	Long
Grain density	546 g/liter
Grain color	Clean yellow
Grain form	Gent
Green lime grains	2.30%
Broken yellow grains	9.46%
White degree	45.10%
Translucence	2.45%
Lightness	65.97%
Chroma	12.03%

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Hue	69.37°
Gel consistency	44 mm
Texture	Medium
Alkali spreading	3
Gelatinization temperature	> 74°C
Milling recovery	69.10
Head rice	85.24
Chalkiness	2.83%
Water contents	12.68%
Glycemic index	54
Amylose contents	20%
Amylose criteria	Low
Amylopectin contents	54.44%
Ash contents	5.69%
Fat contents	2.81%
Crude fiber contents	10.03%
Carbohydrate contents	63.55%
Protein contents	5.76%
Total amino acid	7.75%
Aspartic acid	0.79%
Glutamic acid	1.68%
Serine	0.47%
Histidine	0.21%
Glycine	0.36%
Threonine	0.29%
Arginine	0.69%
Alanine	0.47%
Tyrosine	0.30%
Methionine	0.13%
Valine	0.49%
Phenylalanine	0.49%
I-leucine	0.37%
Leucine	0.72%
Lysine	0.29%

Physical and chemical characteristics of cooked Ciherang rice are influenced by cooking methods, including rice cooker machine, stovetop, boiling and steaming [36,38]. The texture, hue, chroma, lightness, and moisture content of cooked Ciherang rice were significantly affected by cooking methods. Rice cooked with boiling and steaming method showed the lowest texture value, highest chroma value, lowest lightness value, and highest moisture content. Cooking quality traits of Ciherang, such as gel consistency, cooking time, water absorption ratio and volume expansion ratio also influenced by the rice grain storage time [34,39]. Ciherang rice grains were stored in three types of packaging, including plastic bags, vacuum polypropylene plastic packaging, and non-vacuum plastic packaging under air conditioner and room temperature, respectively. After eight months storage, the gel consistency of rice had a significant reduced become harder texture under all treatment conditions. The cooking time of Ciherang rice which store in vacuum plastic packaging did not change after eight months storage [40]. Meanwhile, the water absorption ratio and volume expansion ratio displayed a higher value under room temperature compared to air conditioner temperature.

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Breeding, Parentage, Progeny, Selection, Evaluation, and Release of Ciherang

Ciherang is widely used as one of the parental lines in crossing rice breeding program to develop new rice variety with superior characteristics. In 2009, Ciherang has been crossed back to several superior rice cultivars to develop a new superior variety with improved rice yield called Inpari10 [3]. F1 hybrid was also developed with Ciherang as the background parent in order to increase the rice productivity in the dryland [41]. This F1 hybrid was derived from the crossing Jati Luhur and Dayang Rindu rice varieties, as well as high-yield rice varieties Inpago-7 and Ciherang. Based on the agronomic characteristics, including productive tiller number, plant height, panicle number per plant, spikelet number per panicle, filled grain number per panicle, and 1000-grain weight, F1 hybrids showed better performance in the dry land compared to the parents.

Flooding is one of the serious constraint in rice production especially in Indonesia. Development of a submergence-tolerant rice variety is important to improve the rice productivity in the flooding area. SUB1, a submergence tolerance gene has been introgressed to Ciherang by marker assisted backcrossing (MABC) through one backcross (BC1) with IR64-Sub1 as the donor parent. Recently, Ciherang-Sub1 was released to increase submergence tolerance [5]. Between Ciherang-Sub1 and Ciherang showed 87.5% similarity in the phenotypic characteristics, including grain yield, plant height, flowering date, maturity, tiller number, amylose content, gel consistency, texture, gelatinization temperature, protein content, brown rice percentage, milling recovery percentage, grain length, grain width, and ratio of grain length-grain width [5,42,43,44]. Ciherang-Sub1 also has an ability to germinate under anaerobic condition [45,46].

Ciherang-Sub1 was used as the recipient parent in developing rice lines which tolerant to flooding condition [47]. AG1 and AG2 quantitative trait loci (QTLs) associated with tolerance of flooding condition. Introgression of AG1 QTL from donor parent Kho Hlan On and AG2 QTL from donor parent Ma-Zhan Red and recipient parents PSB Rc82 and Ciherang-Sub1 potentially enhance the germination and seedling growth under flooding condition. Elite rice lines which carrying AG1 and AG2 QTLs showed less lipid peroxidation, higher α-amylase activity that increase the starch degradation and improve ascorbate, soluble sugars, and total phenolic content which leading to the higher germination and seedling growth under flooding condition.

Ciherang-Sub1+AG1 (CSA) was developed through gene pyramiding of two genes, including SUB1 (submergence tolerance gene) and AG1 (controlling anaerobic germination) into Ciherang [45]. The rice variety of CSA contains SUB1 and AG1 for tolerance under submergence at all rice growth stages and anaerobic germination conditions. CSA also showed highly similarity with Ciherang in genetic and morphological characteristics, including tiller number and plant height. There are five markers which associated with SUB1 locus, such as RM8300 in SUB1A, ART5, and three insertion/deletion (Indel) markers in SUB1C. Meanwhile, only four markers related to AG1 locus, including Drebdws4bp, Drebups6bp, HPP400_410_3, and TPP_GE5 [45,48].

Ciherang-Sub1+AG1+Pi9 (CSA-Pi9) rice variety consist of three genes which associated with abiotic stress tolerance (submergence and anaerobic conditions) regulated by SUB1 and AG1, and also biotic stress resistance (rice blast disease) controlled by Pi9 gene [49]. This rice variety CSA-Pi9 is suitable to be cultivated in the damp climate and flooding area. CSA-Pi9 also showed tolerance under salinity condition. CSA-Pi9 was derived by introgress the blast resistant gene, Pi9, into CSA rice variety. The CSA-Pi9 has similar agronomic traits with the recurrent parent, CSA.

A recombinant inbred lines (RILs) population, F6 generation was derived from a cross between Ciherang as a recipient parent (a popular Indonesian rice variety and bacterial blight (BB)-susceptible) and IRBB60 as a donor parent (BB-resistant) to introgress BB resistance genes, including Xa4, Xa5, Xa13, and Xa21 and identify the molecular markers associated with BB-resistant [18]. BB is caused by *Xanthomonas oryzae* pv. *oryzae* and the most destructive disease in rice which reduce the rice yield significantly. It is important to develop a BB-resistant rice variety by gene introgression approach because conventional breeding programs are not efficient and there are no methodology by using chemicals or management practices to decrease the severity of BB [50-58]. Introgression of a single BB resistance gene has not been able to protect the rice plants against BB attack. So, introgression of combinations of four BB resistance genes, including Xa4, Xa5, Xa13, and Xa21 which were pyramided in 265 RILs and confirmed by using marker-assisted selection (MAS) were more effective to develop BB-resistant rice varieties. Out of 265 RILs, 11, 34, and 45 lines contained four, three, and two BB resistance genes, respectively.

Two new rice varieties that have been developed with Ciherang background, including brown planthopper (BPH)-resistant Bioni 63 Agritan and salinity-tolerant Biosalin 1 Agritan have been released by Indonesian Center for Agricultural Biotechnology and Genetic Resources Research and Development [13,59]. BPH-resistant Bioni 63 Agritan was derived from the crossing Ciherang/Swarnalata//3*Ciherang. Moreover, salinity-tolerant Biosalin 1 Agritan was developed by gamma-irradiated Ciherang mutant. Another new rice varieties which developed with Ciherang background and right now, they are still undergoing test in multilocation prior to commercial release are bacterial leaf blight (BLB)-resistant and aromatic Ciherang. The BLB-resistant Ciherang rice variety was developed by using pyramiding gene method of three BLB resistance genes, including Xa5, Xa7, and Xa21, and also one background gene of Xa4. Aromatic Ciherang was developed by marker-assisted backcrossing method by incorporating gene controlling the aromatic characteristics BADH2, either from Mentik Wangi or Pandan Wangi [60]. Based on the preliminary data, the tested lines of aromatic Ciherang showed stable rice productivity ranging 6.28–8.83 ton/ha along with increasing aroma concentration up to 4-fold higher (0.8093 ppb) than Pandan Wangi (0.1956 ppb).

Blast disease caused by *Pyricularia oryzae* is the most destructive disease in rice plants and significantly reduce the rice productivity [61,62]. In Indonesia, Ciherang is cultivated widely and blast disease is become the main issue. One of the most affordable, effective, and more environmentally-friendly approach for controlling this disease is to develop blast disease-resistant rice varieties [11,63-65]. Developing of blast-resistant Ciherang rice variety has been done by introgression of Pita-2 gene derived from IRBLta2-Re to Ciherang and produced three backcross populations, including BC1F1, BC2F1, and BC3F1 [19]. An F1 population was developed by crossing IRBLta2-Re carrying Pita-2 gene as the male parent and Ciherang as the female parent. Then, this F1 population was backcrossed to Ciherang as the recurrent parent to produce a backcross population. BC1F1 population consist of 2074 lines and out of 148 lines showed blast resistance. A total of four lines from BC1F1 population, such as lines 2192, 2129, 1141, and 627 were backcross to Ciherang to create a BC2F1 population. About 848 lines of the BC2F1 population were screened and 59 lines showed the blast resistance. The BC3F1 population was derived by backcrossing of two lines, including 2192-3 and 627-5 lines from BC2F1 population which were blast resistance to recurrent parent Ciherang and resulting 244 lines. There were 32 lines out of 244 lines which showed blast resistance. Several lines of BC3F1 population that had blast resistance and similar agronomic characteristics to Ciherang had been identified.

Genomic Characterization of Ciherang

Ciherang belongs to indica sub-species due to detection of 32 bp insertion on open reading frame (ORF) 29-TrnC (GCA) in Ciherang genome [66]. The DNA of Ciherang was isolated from the leaves of the plants and polymerase chain reaction (PCR) amplification was performed by using primer CP2, then followed by gel electrophoresis, and the PCR products produced a band with size 32 bp. Based on the genome sequence of Ciherang as an Indonesian rice mega variety which was developed with IR-64 as the recurrent parent, the modern rice breeding footprints were revealed. Whole-genome sequencing analyses were conducted on IR-64 and Ciherang in order to study the genetic exchange during Ciherang development and the effect of these genomic changes [13,67]. About 98.6% of

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Ciherang genome sequencing data could be mapped to the rice reference genome of Nipponbare. According to the variant calling, Ciherang had 0.79% sequence difference to Nipponbare, and IR-64 showed 0.8% sequence difference to Nipponbare. Between Ciherang and IR-64 showed a little sequence difference by only 0.13% or 493,298 sequence variations which consist of 434,890 single nucleotide polymorphisms (SNPs) and 58,408 indels. These sequence differences are dominant on chromosome 8, 10, and 12 that indicate many genes introgressions from donor parents were retained on these chromosome. A total of 1,007 loci in Ciherang which are polymorphic to IR-64, such as Os01g0588900 regulating panicle trait, and Os02g0154200 controlling seed traits. Introgressed genes from the donor parent IR-64 into Ciherang is 19.60% which affected the structure, function, and gene expression of certain genes that associated with certain traits. These genomic sequence data can be useful in accelerating and increase the precision of rice breeding programs through marker-assisted selection (MAS), gene pyramiding, genetic transformation, and gene editing.

Whole genomic sequence analysis demonstrated the potential to dissect a QTL, qSub8.1 on chromosome 8 for submergence tolerance which derived from Ciherang-Sub1 [26]. The qSub8.1 was identified in the QTL mapping analysis by using a mapping population derived from the cross Ciherang-Sub1 and IR10F365. qSub1 region was analyzed by using the genome sequence information of Ciherang-Sub1 and its parents (Ciherang and IR64-Sub1) and compare to the reference genomes of Nipponbare and Minghui 63. Results from the whole genome sequence analysis showed that Ciherang-Sub1 genome is composed by 59–63% of Ciherang, 22–24% IR64-Sub1, and 15–17% of unknown sources. The genome sizes of Ciherang-Sub1, Ciherang, and IR64-Sub1 are 354.4, 343.7, and 344.7 Mb, respectively. QTL region of qSub8.1 on chromosome 8 was dissected deeply by using high-resolution SNP markers leading to analyze the potential candidate genes within qSub8.1 and to develop DNA markers associated with submergence tolerance for future rice breeding.

A total of 17 QTLs were identified from Ciherang-Sub1 rice backcross populations with 149 backcross families by using 104 molecular markers which consist of SNP and SSR markers (Table 3) [42]. These QTLs related to agronomic and yield traits, such as days to 50% flowering (DTF), plant height (PH), unfilled grain number per panicle (UFG), thousand grain weight (GW), and yield (Y). Identification of these QTLs are important in order to develop high-yield rice varieties [68]. The QTL alleles were derived from the both parents.

Table 3. QTLs associated with agronomic and yield traits of mapping population derived from Ciherang-Sub1 backcross population [42].

Traits[a]	Generation	Chromosome	QTL position (Mbp)	Flanking markers		LOD[b]	PVE[c]	Add[d]
				Left	**Right**			
	$BC_1F_{1:2}$	1	40.1645	id1023892	id1028304	3.5	9.5	1.4
DTF		6	7.6817	id6003649	id6006147	5.0	12.3	1.6
		11	2.6827	id11000515	id1000858	5.8	11.8	1.6
	$BC_1F_{1:3}$	1	36.1645	id1022408	id1023892	7.5	17.6	2.2
		3	29.9835	id3013765	id3017762	5.6	13.2	1.9
		11	3.6827	id11000858	id11002639	3.3	7.5	1.4
	$BC_1F_{1:2}$	1	36.1645	id1022408	id1023892	33.2	59.5	-15.7
PH		6	23.6817	id6012064	id6012426	2.9	4.1	4.2
	$BC_1F_{1:3}$	1	36.1645	id1022408	id1023892	28.7	58.2	-20.4
UFG	$BC_1F_{1:2}$	3	30.9835	id3013765	id3017762	2.9	8.5	4.5
	$BC_1F_{1:3}$	9	17.3273	id9005086	id9006988	3.2	10.1	-5.1
	$BC_1F_{1:2}$	2	24.8191	id2008866	id2010969	2.7	4.1	-0.5
GW		3	21.9835	id3008333	id3010345	16.7	31.3	1.4
		11	1.6827	id11000133	id11000515	4.8	7.6	0.7
	$BC_1F_{1:3}$	3	22.9835	id3010345	RM15593	6.6	19.4	1.0
Y	$BC_1F_{1:2}$	4	17.4846	id4002562	id4005120	3.4	10.9	-0.5
	$BC_1F_{1:3}$	1	35.1645	id1020828	id1022408	4.3	13.6	-0.7

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[a]DTF = days to 50% analysis (DAS); PH = plant height (cm); UFG = unfilled grain number per panicle; GW = thousand grain weight (g); Y = yield (t/ha).
[b]LOD = logarithm of the odds ratio.
[c]PVE = phenotypic variance explained.
[d]Add = additive effect.

Gamma irradiation has been used to improve Ciherang productivity by using six levels of concentration: 0, 100, 200, 300, 400, and 500 Gy [12]. The results showed that gamma irradiation with 200 Gy was the most optimum concentration to increase the vegetative growth, grain yield, and grain quality, including plant height, filled grain per panicle number, amylose content, and protein content [69].

Transgenic Lines of Ciherang

Transformation and tissue culture techniques has been conducted to Ciherang for improving the productivity and the resistance to abiotic and biotic stresses [70,72]. The results showed that Ciherang had high regeneration efficiency (33–59%) and transformation efficiency (3–12%). In 2019, 0.24% of the total rice production area in Indonesia was infected by bacterial blight (BLB) disease, and it is critical to develop Ciherang rice variety with BLB resistance [73]. Transgenic BC1F2 population was developed to increase the resistance of Ciherang to BLB disease which caused by *Xanthomonas oryzae* pv. o*ryzae* (Xoo) pathotype IV [74]. This transgenic BC1F2 population was derived from crossing between transgenic japonica rice variety 'KinLys1/3/23' that contain LYZ-C gene and indica rice variety Ciherang, then backcrossing to Ciherang. LYZ-C gene encodes Lysozyme which can degrade the *Xanthomonas oryzae* pv. o*ryzae* cell walls by hydrolyze β-1,4 glycoside bond between Nacetyglucosamine and N-acetylmuramic acid in the peptidoglycans of the bacterial cell walls [75]. Based on the PCR analysis, the genomes of F1, BC1F1, and BC1F2 had been integrated by LYZ-C gene. The transgene of LYZ-C was a dominant single-copy gene and was stably inherited followed a Mendelian pattern.

cry1Ab gene was transformed to Ciherang in order to improve the resistance to yellow stem borer (*Scirpophaga* sp) [70]. This pest is destructive to the rice plants and significantly decrease the rice yield. These transgenic lines were generated by using Agrobacterium-mediated transformation with vector p2TDNAcryIAb that carry a double T-DNA containing cry1Ab gene and hygromycin phosphotransferase (hpt) gene as a selectable marker. About 37 putative transgenic lines were generated. Based on the PCR analysis, 22 lines out of 37 lines were carrying cry1Ab. Furthermore, immunostrip assay was done to all of the Ciherang transgenic lines and resulting that all the transgenic lines expressed cry1Ab protein.

OsDREB1A gene was also transformed to Ciherang to increase the salinity tolerance [76]. Salinity is one of the abiotic stress which reduce the rice productivity in Indonesia. This transgene OsDREB1A was stable up to BC4F2 and BC5F1 generations. A total of 543 Ciherang-OsDREB1A transgenic lines of BC4F2 and BC5F1 populations were screened for salinity tolerance and stability of transgene inheritance. Screening for salinity tolerance was done in a mineral solution with electrical conductivity (EC) of 18 mS/cm for 26 days and resulted 134 putative transgenic lines. Based on the integration analysis by using PCR with hptII primer (Forward and Reverse), a total of 73 out of 134 putative transgenic lines were confirmed contained the transgene in their genomes. Then, all of the 73 transgenic lines were tested by PCR analysis with specific forward primer 35S-496 and reverse primer OsDREB1A, resulting that the transgene was integrated up to BC4F2 and BC5F1 generations.

Intensification System of Ciherang Cultivation

Ciherang has been cultivated by using three planting spacing system, including cubic, double row, and twin seed in order to find the most appropriate technique of cultivation to increase the rice productivity. Planting spacing system affect the plant growth, grain yield and grain quality. Improper planting spacing in Ciherang cultivation cause low productivity [3,77]. Most of the farmers in Indonesia use cubic planting system with space 20 cm x 20 cm which has close spacing among the rice plants and farmers assume that this panting system will produce higher yield. In fact, the cubic planting system produces low rice yield. Planting spacing correlated to the pattern of arranging the space among rice plants, and it is very crucial to the rice plants to get optimum sunlight and avoid the nutrient absorption competition [78].

Plant spacing in the rice cultivation is significantly affect the productive tiller number, panicle length and filled grain number per panicle due to the planting spacing associated with the sunlight caption, nutrient absorption, and water availability [79,80]. In the cubic planting system, the sunlight intensity is low which caused higher moisture in the rice field, leading to enhanced microorganism activity that decrease the grain quality. The unfilled grain number in the cubic planting system also higher compared to the double row and twin seed planting system. This may be because of the more dense spacing in cubic panting system cause lack of sunlight, low temperature, and higher humidity in the rice plants ecosystem resulting disrupt the fertilization process at flowering stage and reduce the photosynthetic activity leading to reduce carbohydrate formation, and produce more unfilled grain number.

Recently, it has been reported that the double row planting system in the rice cultivation is more effective to increase the rice yield due to the rice plant population is higher 33% compared to the cubic planting system. Double planting system is a pattern of rice planting which alternates between two rice lines and one free space row. The planting spacing also has to be adjusted to the soil characteristics, such as fertility and texture. In the field with the fertile soil, the optimum plant spacing are 12.5 cm within rows, 25 cm between rows, and 50 cm between double row. Meanwhile, in the less fertile soil, the plant spacing has to be wider; 15 cm within rows, 30 cm between rows, and 40 cm between double row [81]. The sunlight intensity in the double row system is higher compared to cubic planting system.

Rice productivity in the twin seeds planting system is higher 5-10% than double row system and approximately 6.70% higher compared to cubic planting system. Another advantages from the twin seed planting system are easier for weeding and the number of seeds are few. The yellow broken grains and lime green grains are lower than the double row and twin seed planting system. In the wet season, planting space among the rice plants need wider space due to the sunlight intensity is low. Meanwhile, in the dry season, the planting space is less because of maximum sunlight intensity [79].

Application of intermittent irrigation method in Ciherang enhanced the rice productivity due to this irrigation method allow the root to grow maximally which increase the absorption of water and nutrient from the soil [23,82,83]. The results showed that the intermittent irrigation has higher productive tiller number, grain yield, grain weight, harvest index, and plant biomass compared to the conventional irrigation with continuous flooding. Other advantage of intermittent irrigation is less water requirements.

Time of planting in Ciherang rice cultivation also influenced the growth and grain yield [30]. In the dry season, Ciherang has better growth and grain yield compared to wet season. Weeding frequencies in rice field also affected the growth and grain yield of Ciherang [84]. The optimal weeding frequencies for Ciherang is three times; 14, 49, and 70 days after planting which allow to have maximum productive tiller number, plant height, and grain weight.

Agronomic characteristics of Ciherang, including plant height, productive tiller number, and biomass are influenced by the soil fertility, especially from Nitrogen nutrient. Urea fertilizer is the most common chemical fertilizer that used by the farmers but recently the price of Urea is increasing. In order to decrease of using Urea, Azolla has potential to be an alternative nitrogen source for improving the biological, chemical, and physical of soil [20]. It is necessary to increase soil fertility of the rice field by application of Urea and

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Azolla with optimal combination ratio. The ratio of Urea 18 g/m^2 and Azolla 12 g/m^2 was significantly increase the plant height and productive tiller number. The biomass of the rice plants was increased by application of Urea 21 g/m^2 and Azolla 9 g/m^2. Moreover, grain weight was affected by application Urea 9 g/m^2 and Azolla 21 g/m^2. Application of Urea and Azolla combination with certain ratio can reduce the use of Urea and increased the soil fertility [85].

Another bio-fertilizer which can be applied to increase the soil fertility in Ciherang rice field is Azospirillum [86]. There are many benefit of Azospirillum to the soil, including improved nitrogen fixation, phosphor solubility, and plant growth hormone indol acetic acid (IAA) production. The use of Azospirillum in the Ciherang cultivation significantly enhanced the panicle number per plant and grain weight. Rice yield and grain carbohydrate content in Ciherang also can be increased by application of fixator bacteria, such as *Azotobacter* sp. into the soil. Macro-fertilizer like NPK also needed to increase the rice yield of Ciherang with optimal dose 656 kg/ha [28,87]. Application of bio-fertilizer that contains soil fungi, including *Penicillium* sp. and *Aspergillus* sp. also has many advantages to the growth and productivity of Ciherang [88]. These soil fungi were able to produce plant growth hormone IAA, degrade lignocellulose, and provide organic phosphate that leading to increase productive tiller number, plant height, and biomass of Ciherang [89].

Rice productivity of Ciherang can be increased by using silicate extract from the rice husk [90,91]. Micro element of silica also enhances the grain quality of Ciherang [92,93]. Indonesia as the tropical country has low amount of silica in the soil because of the desiccation process, so it is important to apply silicate element to the rice field by using the rice husk [94,95]. Based on the agronomic and grain yield characteristics, including plant height, productive tiller number, filled grain number, unfilled grain number, and thousand grain weight; the optimum concentration of silica rice husk extract is 20 ml/l. Application of Boron to the rice field soil also important in the Ciherang rice cultivation. The optimal dose of Boron that need to be applied is 3 kg/ha which increase the plant height, grain yield, straw yield, harvest index, and to decrease the unfilled grain number [31].

Conclusions

In two decades, Ciherang has been providing about hundreds of millions rice consumers in Indonesia with good-quality rice. Due to the superior agronomic and yield characteristics, Ciherang has replaced mega variety IR-64. Ciherang also has high value in rice breeding program. Conventional and molecular breeding techniques have been used to increase Ciherang characteristics. The bad characteristics of Ciherang, including flooding intolerance and susceptible to several diseases have been restored by developing Ciherang-Sub1 that flooding tolerance, and Ciherang-Sub1+AG1+Pi9 that tolerance to flooding condition and resistance to rice blast disease. The development of superior rice variety like Ciherang has provided a challenge to the rice breeders to develop further improved rice varieties which are widely-accepted by farmers and rice consumers.

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