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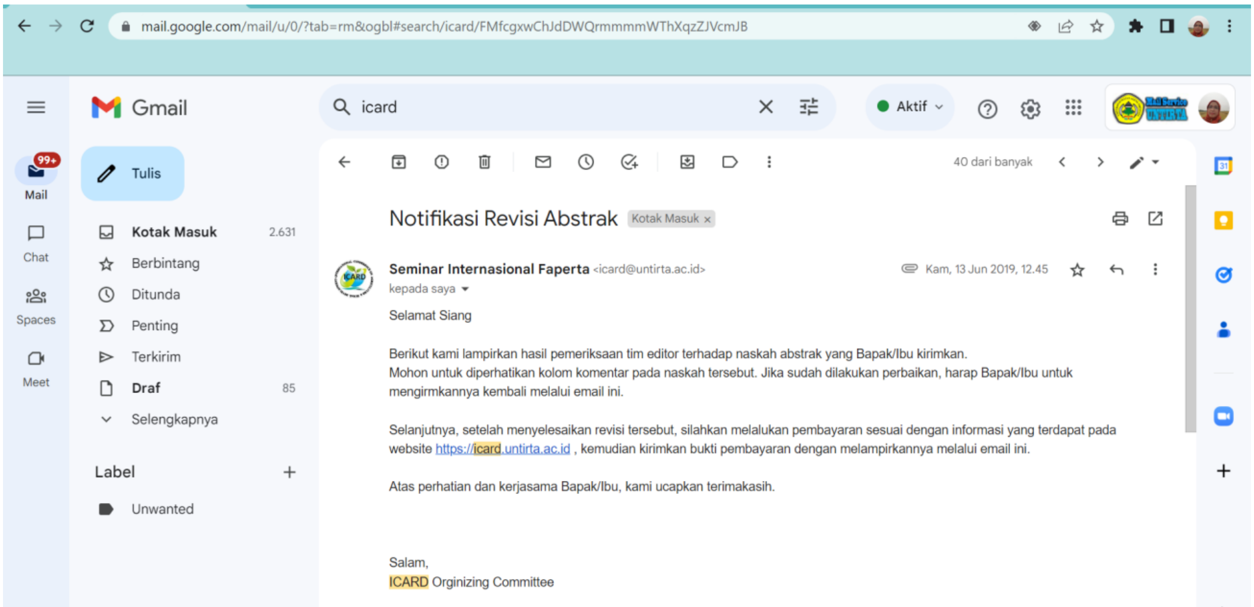

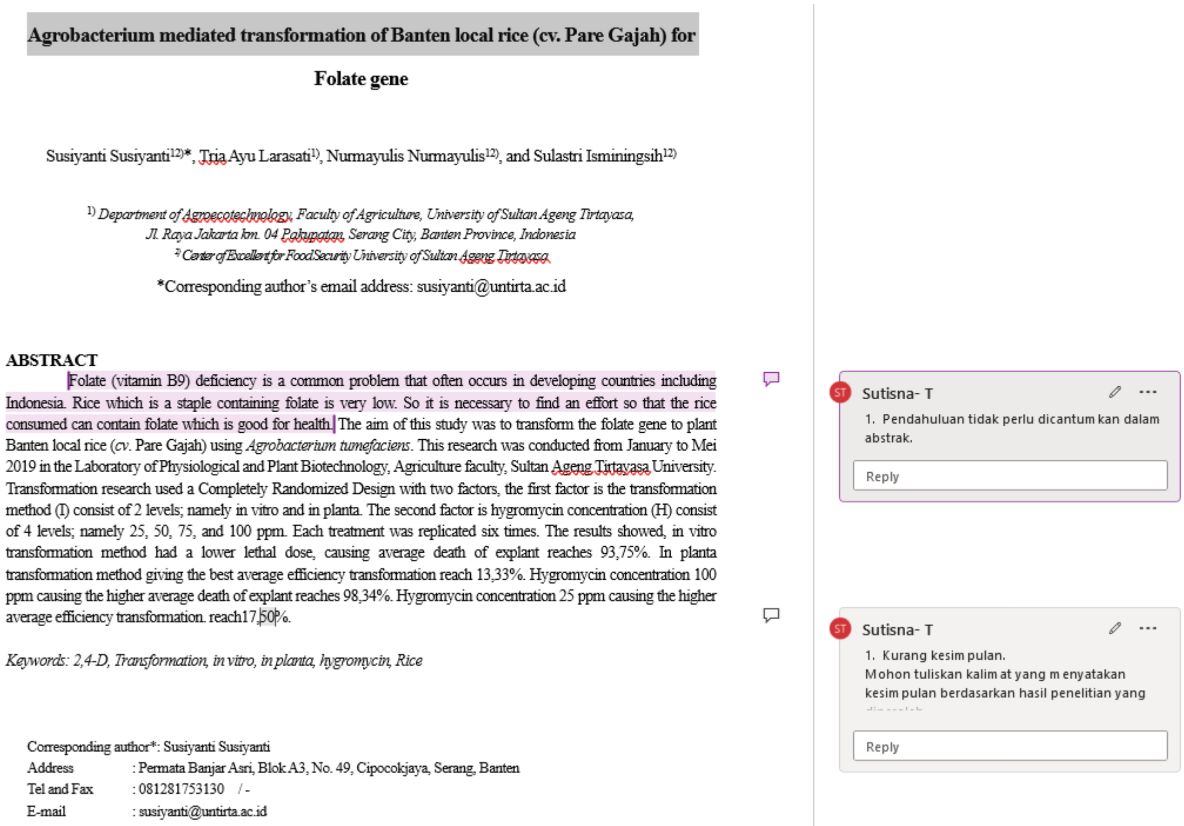

Agrobacterium mediated transformation of Banten local rice (cv. Pare Gajah) for Folate gene

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ABSTRACT

Folate (vitamin B9) deficiency is a common problem that often occurs in developing countries including Indonesia. Rice which is a staple containing folate is very low. So it is necessary to find an effort so that the rice consumed can contain folate which is good for health. The aim of this study was to transform the folate gene to plant Banten local rice (*cv.* Pare Gajah) using *Agrobacterium tumefaciens*. This research was conducted from January to Mei 2019 in the Laboratory of Physiological and Plant Biotechnology, Agriculture faculty, Sultan Ageng Tirtayasa University. Transformation research used a Completely Randomized Design with two factors, the first factor is the transformation method (I) consist of 2 levels; namely in vitro and in planta. The second factor is hygromycin concentration (H) consist of 4 levels; namely 25, 50, 75, and 100 ppm. Each treatment was replicated six times. The results showed, in vitro transformation method had a lower lethal dose, causing average death of explant reaches 93,75%. In planta transformation method giving the best average efficiency transformation reach 13,33%. Hygromycin concentration 100 ppm causing the higher average death of explant reaches 98,34%. Hygromycin concentration 25 ppm causing the higher average efficiency transformation. reach17,50%.

Keywords: 2,4-D, Transformation, in vitro, in planta, hygromycin, Rice

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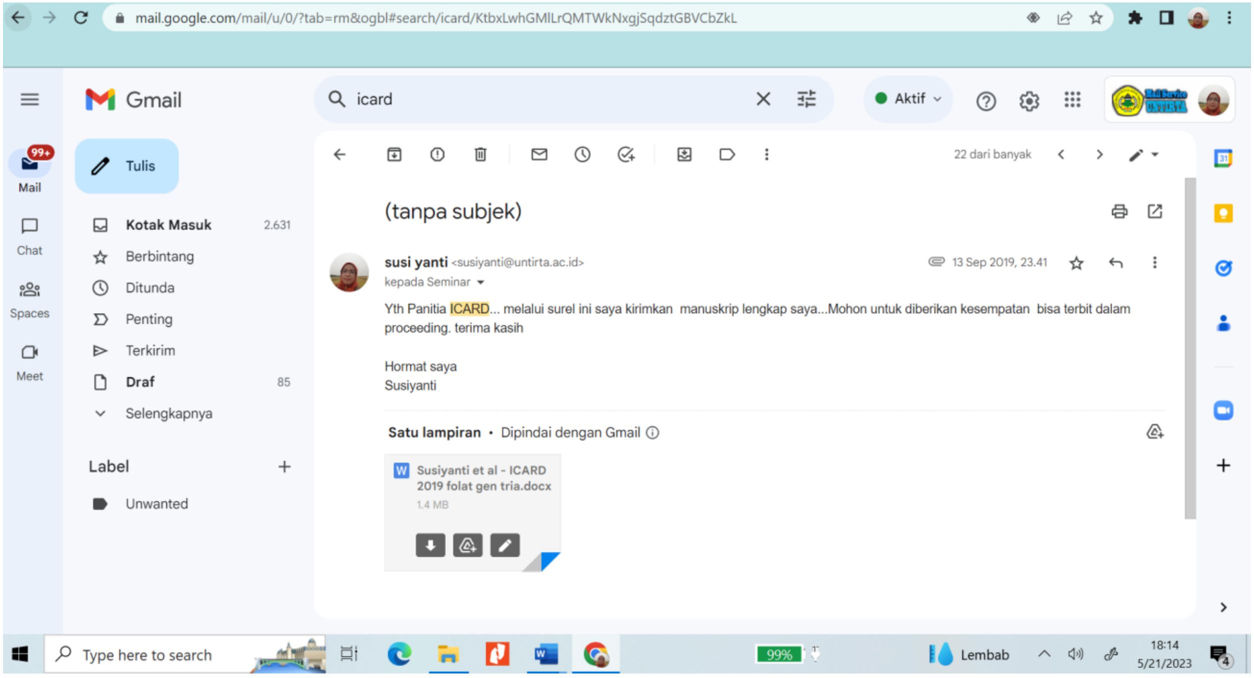
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Agrobacterium mediated transformation of Banten local rice (cv. Pare Gajah) for Folate gene

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Abstract. Folate (vitamin B9) deficiency is a common problem that often occurs in developing countries including Indonesia. Rice which is a staple containing folate is very low. So it is necessary to find an effort so that the rice consumed can contain folate which is good for health.The aim of this study was to transform the folate gene to plant Banten local rice (*cv.* Pare Gajah) using *Agrobacterium tumefaciens*. This research was conducted from January to Mei 2019 in the Laboratory of Physiological and Plant Biotechnology, Agriculture faculty, Sultan Ageng Tirtayasa University. Transformation research used a Completely Randomized Design with two factors, the first factor is the transformation method (I) consist of 2 levels; namely in vitro and in planta. The second factor is hygromycin concentration (H) consist of 4 levels; namely 25, 50, 75, and 100 ppm. Each treatment was replicated six times. The results showed, in vitro transformation method had a lower lethal dose, causing average death of explant reaches 93,75%. In planta transformation method giving the best average efficiency transformation reach 13,33%. Hygromycin concentration 100 ppm causing the higher average death of explant reaches 98,34%. Hygromycin concentration 25 ppm causing the higher average efficiency transformation reach 17,50%.

Keywords: 2,4-D, Transformation, in vitro, in planta, hygromycin, Rice, folate, CGH1 gene.

Introduction

Folic acid (vitamin B9) is needed by children and adults to produce red blood cells and prevent anemia. Folic acid plays a major role in cell growth and development, and tissue formation. Folic acid deficiency, the body will be susceptible to diseases such as depression, anxiety, fatigue, insomnia, difficulty remembering, red tongue and sores to digestive disorders. Folic acid deficiency in pregnant women increases the risk of premature delivery, babies with low birth weight or with neural tube defects [1], [2] said that someone who lacks folate is more at risk of getting cancer because folate deficiency can increase chromosomal damage and incorporation of uracil into DNA.

According to [3] this development effort can be carried out with plant breeding, one of which is biotechnology. Assembling rice varieties using genetic transformation techniques is expected to produce superior rice varieties with desired characteristics.

The use of local varieties of rice in breeding programs has often been advocated with the aim of broadening the genetic background of the superior varieties to be produced [4]. According to [5] Pare Gajah variety is one of the local varieties of Banten which is cultivated by the Kasepuhan community of Cisungsang Village because it is known for its long and rather large grains. By doing the breeding process to improve the weaknesses of local varieties of rice without changing other traits that have been favored such as the taste of rice and good adaptation in areas where rice is widely cultivated, local varieties can be obtained which are more beneficial while adding to its economic value.

Agrobacterium tumefaciens is one of the transformation techniques that many researchers have done and has been successful. *Agrobacterium* is a bacterium that plays a role in helping to insert genes into plant genomes. Rice is one of the agricultural crops that has been carried out a lot of genetic transformation to get superior varieties of rice. Some genes that have been successfully inserted in rice plants and are being evaluated for expression and function are genes for resistance to yellow stem borer pests, fungal disease, and drought-tolerant genes **[6].**

Transformation with *Agrobacterium* is divided into 2 methods, namely in vitro and *in planta*. *In vitro* transformation is a transformation method that uses tissue culture techniques, namely by callus culture or callus induction. While the *in planta* transformation method is a new method that is simpler than in vitro because it is done in ex vitro (outside the lab).

Based on the description above, it is necessary to do research on the Transformation of Folate Gene in the Banten Local Rice Varieties of Pare Gajah (*Oryza sativa* L.). The purpose of this study was to carry out the transformation of the folate gene into the genome of rice plants of the Banten local variety Pare Gajah (*Oryza sativa* L.).

1. Materials and Methods

This type of research is experimental research. This research was conducted at the Biotechnology Laboratory, Faculty of Agriculture, Sultan Ageng Tirtayasa University from November 2018 to January 2019

Treatment:

a. Callus Induction

The design used in the manufacture of callus as a preparation for transformant material consisted of one factor, namely the concentration of 2,4 Dichlorophenoxyacetate (D). The 2,4-D concentration factor consists of 3 levels, namely:

D_1 : 1 ppm
D_2 : 2 ppm
D_3 : 3 ppm

Each treatment was repeated 9 times to obtain 27 experimental units. Each experimental unit contained 6 seed explants. Then the total explants needed are 162 seed explants.

a = The smallest dose that causes the highest death of explants in one dose group.
b = Total number of multiplications between different doses and the average mortality at the same interval
c = The number of explants in one group
([9] [with modification)

2) Efficiency of Putative Transgenic Transformation (%)
Calculation of transformation efficiency *in vitro* and *in planta* is done using the following formula:

$$\text{Transformation efficiency (\%)} = \frac{\text{The amount of hygromycin - resistant explants}}{\text{The number of initial explants of transformation}} \times 100\ \%$$

Source: [6] with modification.

Research Implementation

a) *In Vitro* Transformation

1) Making Embryogenic Callus

Rice seeds are washed clean and soaked for 30 minutes in distilled water. After that the seeds are peeled and sterilized with 2 $g.L^{-1}$ detergent solution, 2 g^{-1} agrymicin, and 2 g^{-1} dithane for 15 minutes, then the seeds are rinsed with distilled water and sterilized again with 30% chlorine solution for 15 minutes in LAF. After that the seeds are rinsed with sterile distilled water 6 times, and then soaked in betadine solution for 5 minutes ([8] with modification). Then the seeds are planted on 2,4-D callus induction media. Media that has been planted with explants is then placed on a culture rack at 25-28°C. Every 2 days the culture bottle is sprayed with 70% alcohol to avoid contamination. Callus began to form 2-3 weeks after planting explants in the media. The resulting callus was transferred to the same callus induction medium by cutting buds and endosperm. The first 2-week embryogenic callus can be used as an explant for transformation.

2) *Agrobacterium tumefaciens* Culture

Agrobacterium strain LBA4404 which carries the construction of the folate gene is grown on solid LB media containing 100 ppm kanamycin antibiotic and 50 ppm hygromycin antibiotic. Bacteria culture that has been distreaked on solid LB media is stored in an incubator of 28°C for about 3 days or until the bacteria grows.[6], [7] with modification).

3) Transformation of *Agrobacterium tumefaciens* to Embryogenic Callus

The infection solution to be used was inserted into corning as much as 15 mL, *Agrobacterium tumefaciens* culture was added to OD (optical density) 0.03-0.05 at 600 nm wave, acetosiringone was also added and allowed to stand for 15 minutes before use. The embryogenic callus that has been prepared is soaked with an infusion solution that has been added with acetosiringon first for 15 minutes for adaptation, then soaked in a bacterial suspension infectious solution for 15 minutes, after which the suspension is discarded and the callus is drained on filter paper for 10 minutes ([11]with modifications).

4) Co-cultivation

Co-cultivation media were made with 2 sheets of filter paper that were given liquid infection and acetosiringone that were previously made as much as 2 mL. Callus that has been infected by *Agrobacterium tumefaciens* was carried out co-cultivation in the dark for 3 days on the previously created co-cultivation media ([11] with modification).

5) Selection of Transgenic Plants

Co-cultivation callus was subsequently grown on selection media with modified hygromycin concentrations of 25 ppm, 50 ppm, 75 ppm, and 100 ppm, then grown in dark conditions for 2 weeks (until the callus grows with callus enlarged, callus colorless black or necrosis) ([11] with modification).

b) *In planta* Transformation

1) Rice Sprouts Preparation

Rice seeds used as explants are washed and soaked for 30 minutes in distilled water. After that the seeds are peeled and sterilized with 2 g/L detergent solution, 2 $g.L^{-1}$ agrymicin, and 2 $g.L^{-1}$ dithane for 15 minutes, then the seeds are rinsed with distilled water and sterilized again with 30% chlorine solution for 15 minutes in LAF. After that the seeds are rinsed with sterile distilled water 6 times, and then soaked in betadine solution for 5 minutes ([8] et al., 2014 with modification). Furthermore, the seeds are soaked in sterile water for 2 days at 20°C. Water is replaced once during the soaking process. After 2 days of immersion, the embryo will turn white (Supartana et al., 2014).

2) *Agrobacterium tumefaciens* Culture

Agrobacterium strain LBA4404 which carries the construction of the folate gene is grown on solid LB media containing 100 ppm kanamycin antibiotic and 50 ppm hygromycin antibiotic. Bacteria culture that has been distreaked on solid LB media is stored in an incubator of 28°C for about 3 days or until the bacteria grows. [6] [7] with modification).

3) *Agrobacterium tumefaciens* inoculation to rice shoot

The infection solution to be used was inserted into corning as much as 15 mL, added *Agrobacterium tumefaciens* culture to OD 0,3-0,6 at 600 nm, also added acetosiringone and allowed to stand for 15 minutes before use. For bacterial inoculation of rice shoots is done by pricking the embryo (1-1,5 mm depth) with a sterile needle tip and then dipping in bacterial suspension for 15 minutes, after that the suspension is removed and rice sprouts are drained on filter paper for 10 minutes ([10] with modifications).

4) Co-cultivation

Co-cultivation media were made with 2 sheets of filter paper that were given liquid infection and acetosiringone that were previously made as much as 2 mL. Rice sprouts that have been infected by *Agrobacterium tumefaciens* were co-cultivated in the dark for 5 days on previously cultivated co-cultivation media ([11] with modification).

5) Transgenic Plants Selection

Rice seeds were grown under sterile conditions in water (2-3 mm depth) containing hygromycin antibiotics with concentrations of 25 ppm, 50 ppm, 75 ppm and 100 ppm at 28°C. Resistance to hygromycin antibiotics was assessed after 7 days later ([10]., 2014 with modification).

6) DNA Isolation.

Leaves of 23 F1 from cross-bred Kewal Bulu Hideung X Mira-1 and the two parents (21 day after planting) were used to isolate the DNA with CTAB method. DNA from each samples was amplified with specific CGH1 SSR primer. The PCR product was separated using 2% agarose and visualized with Chemidoc gel system.

2. Results and Discussion

3. Callus Induction

The results of the variance recapitulation in Table 1. show the 2,4-D concentration significantly affected the time callus parameter (HST) and had a very significant effect on the number of explants forming callus (%), but gave no significant effect on the callus diameter parameter (mm) and callus area (mm2).

Table 1. Recapitulation of Variance Effects of Growth Regulatory Substances 2,4-D Concentration on Banten Local Callus Pare Gajah (*Oryza sativa* L.)

No.	Observation Parameters	Concentration 2,4-D	KK (%)
1.	Time when Callus Formed (HST)	*	6,52
2.	Number of Explants Forming Callus (%)	**	13,83
3.	Callus Diameter (mm)	tn	5,20[a]
4.	Callus Area (mm^2)	tn	5,82[a]

Notes : tn : Have no influential effect
* : Significant influential effect at the 5% level
** : Very significant influential effect at 1% level
KK : Coefficient of Diversity
HST : Days After Planting (*Hari Setelah Tanam*)
a : Transformed data $\sqrt{x + 0,5}$ once

Time of Callus Formation (HST)

Based on the data presented in Table 2, it is known that the 2,4-D concentration significantly affects the time parameters of callus formation (HST). The 2,4-D concentration gives an average callus formation time at 13,37 HST (days after planting), and the best 2,4-D concentration is produced at the D2 level (2 ppm) which is 12.67 HST. The process of callus formation in Pare Gajah rice embryos induced using 2,4-D can be seen in Figure 1.

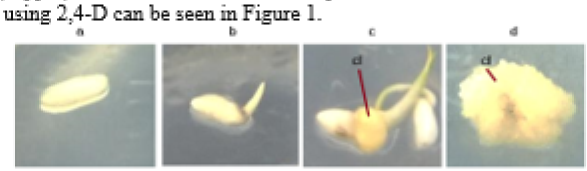

Figure 1. Process of Callus Formation in Pare Gajah Rice Embryo Induced using 2,4-D. Notes: a. Rice seeds were planted on N6 media with the addition of 2,4-D on day 0. b. Shoots on the 7th day after planting. c. Callus formed at the base of the bud of Pare Gajah rice embryo on the 12th day after planting. d. Callus shape on the 35th day. Information: (bj) seeds, (tn) shoots, and (kl) callus.

Table 2. The Effects of Growth Regulatory Substances 2,4-D Concentration towards Time when Callus Formed (HST)

2,4-D Concentration	Time when Callus Formed (HST)
D1 (1 ppm)	14,00c
D2 (2 ppm)	12,67a
D3 (3 ppm)	13,44b
Average	13,37

Note : the numbers that followed by the same letters in the same row or column show no significant difference according to the DMRT test of 5% level.

Number of Explants Forming Callus (%)

Based on the data presented in Table 3, the 2,4-D concentration at D2 level (2 ppm) is the best concentration with the percentage of explants forming callus that is 94.44%.

Based on the data presented in Table 4. it is known that the three concentrations of growth regulators 2,4-D have no significant effect on the callus texture, because all three concentrations of 2,4-D produce a callus that is crumbly (friable)

Table 4. The Effects of Growth Regulatory Substances 2,4-D Concentration towards Callus Texture

2.4 D concentration	(1)	(2)	(3)
		%	
D1 (1 ppm)	0	0	100
D2 (2 ppm)	0	0	100
D3 (3 ppm)	0	0	100

Note: Scoring of callus texture : (1) solid, (2) intermediate, dan (3) friable.

Callus Colour

From the data in Table 5. it is known that administration of different 2,4-D growth regulators produces different callus colors. At the beginning of the formation of the callus, the resulting callus is yellowish white. But over time there are even calluses that change color to white, yellow, and even brownish white at the end of the observation (Figure 2). In the treatment D1 (1 ppm), the color of the callus is dominated by yellowish white and brownish white. Whereas the treatment of D2 (2 ppm) and D3 (3 ppm) was dominated by a yellowish white color.

Table 5. The Effects of Growth Regulatory Substances 2,4-D Concentration towards Callus Colour

2.4 D concentration	(1)	(2)	(3)	(4)	(5)	(6)	(7)
				%			
D1 (1 ppm)	22,22	11,11	0	0	33,33	0	33,33
D2 (2 ppm)	33,33	0	0	0	66,67	0	0
D3 (3 ppm)	22,22	0	0	0	44,44	0	33,33

Notes: scoring of callus colour : (1) white, (2) yellow, (3) green, (4) brown, (5) yellowish white, (6) greenish white, dan (7) brownish yellow.

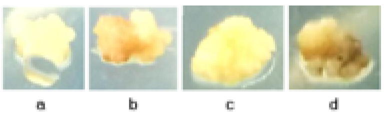

Figure 2. Callus Colour of Pare Gajah Rice on 35 HST (days after planting). Notes: (a) White, (b) Yellow, (c) Yellowish White, and (d) Brownish Yellow

Callus Diameter (mm)

Table 6. Shows the average callus diameter decreases with increasing 2,4-D concentrations given. The average callus diameter tends to be longer to be found at the D1 level (1 ppm) which is 0.49 mm and which tends to be smaller at the D3 level (3 ppm) which is 0.37 mm.

Table 6. The Effects of Growth Regulatory Substances 2,4-D Concentration towards Callus Diameter (mm)

2.4 D concentration	Callus diameter (mm)
D1 (1 ppm)	0,49

D2 (2 ppm)	0,44
D3 (3 ppm)	0,37
Average	0,44

Callus Area (mm^2)

Table 7. The Effects of Growth Regulatory Substances 2,4-D Concentration towards Callus Area (mm^2)

2.4 D concentration	Callus area (mm^2)
D1 (1 ppm)	43,32
D2 (2 ppm)	38,14
D3 (3 ppm)	30,25
Average	37,24

The diameter of the callus is directly proportional to the area of the callus, the greater the diameter of the callus produced, the greater the callus area. Based on Table 7., it is known that the 2,4-D concentration gives an average callus area of 37.24 mm2. Callus tends to be more widely produced at the D1 level (1 ppm) which is 43.32 mm2 and callus tends to be smaller at the D3 level (3 ppm) which is 30.25 mm2. Calculation of callus diameter and callus area using millimeter block can be seen in Figure 3.

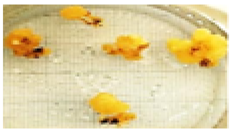

Figure 3. Calculation of Callus Diameter and Area Using Millimetar Block.

4. Folate Gene Transformation

The results of the variance recapitulation in Table 8. show that the transformation method treatment significantly affected the hygromycin lethal dose parameters (LD50) and the transgenic putative transformation efficiency (%), while the hygromycin concentration treatment had a very significant effect on the parameters of lethal hygromycin dose (LD50) observations and the efficiency of putative transformation transgenic (%). transgenic putative transformation efficiency (%), but there is no interaction between the transformation method treatment and hygromycin concentration.

Table 8. Recapitulation of the Variance effects of the transformation method and Hygromycin concentration towards the results of the transformation of folate genes in local Banten rice explants Pare Gajah

No.	Observation parameters	Treatment			cv (%)
		Transformation Method (I)	Hygromycin concentration (H)	Interaction (I*H)	
1.	Lethal Hygromycin Dose (LD_{50})	*	**	ns	10,97
2.	Efficiency of Transformation (%)	*	**	ns	10,08[a]

Notes: ns : Not significant
* : Significant at 5%
** : Very significant at 1%

a : Transformed data $\sqrt{x+0,5}$ twice

Lethal Hygromycin Dose

Table 9. Percentage of dead Pare Gajah Rice Explants in Hygromycin Lethal Antibiotic Test Trials on Selection Media

Hygromycin Concentration	Transformation Method		Average
	In vitro (I1)	*In planta* (I2)	
H1 (25 ppm)	83,33	81,67	82,50[a]
H2 (50 ppm)	95,00	83,33	89,16[b]
H3 (75 ppm)	96,67	85,00	90,84[b]
H4 (100 ppm)	100,00	96,67	98,34[c]
Average	93,75[b]	86,67[a]	

Notes : the numbers that followed by the same letters in the same row or column show no significant difference according to the DMRT test of 5% level.

The table shows the percentage of dead callus and dead or non-growing sprouts increasing with increasing concentrations of hygromycin antibiotics. In the in vitro transformation the lowest percentage of callus was 25 ppm which was 83,33%, while the largest percentage of callus died was 100% at 100 ppm hygromycin concentration. *In planta* transformation data obtained dead sprouts tend to be lower at 25 ppm hygromycin concentration that is 81,67% and tend to be higher 96,67% at 100 ppm.

From the table shows LD50 hygromycin using the Karber Method formula for in vitro transformation which is 29,17 ppm. This figure is smaller than the LD50 transformation *in planta* which is 35,63 ppm.

The results of transformation carried out *in vitro* and *in planta* using Agrobacterium tumefaciens were tested based on the effectiveness of hygromycin in inhibiting the growth of explants that failed to be transformed. Explants that have successfully grown and which have died in the selection media can be seen in Figure 4. and Figure 5.

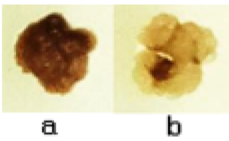

Figure 4. Appearance of a callus that is not resistant to hygromycin (a); kalus resisten terhadap higromisin (b).

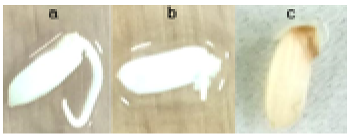

Figure 5. (a) Transgenic putative rice seeds are still able to germinate in hygromycin-containing media, (b) Non-transgenic rice seeds are germinated in hygromycin-containing media, (c) Non-transgenic rice seeds blackened after selection on hygromycin media.

Efficiency of Putative Transgenic Transformation (%)

Table 10. The Effect of Transformation Method and Concentration of Hygromycin towards the Efficiency of Putative Transgenic Transformation of Pare Gajah Rice Explants (%)

Hygromycin Concentration	Average Number of Callus Resistant	Average Number of Sprouts Resistant	Efficiency of *In Vitro* Transformation (%)	Efficiency of *In Planta* Transformation (%)	Average Transformation Efficiency (%)
25 ppm	1,67	1,83	16,67	18,33	17,50^{c}
50 ppm	0,50	1,67	5,00	16,67	10,84^{b}
75 ppm	0,33	1,50	3,33	15,00	9,16^{b}
100 ppm	0,00	0,33	0,00	3,33	1,66^{a}
Average			6,25^{a}	13,33^{b}	

Notes : the numbers that followed by the same letters in the same row or column show no significant difference according to the DMRT test of 5% level.

Based on Table 10. it is known that the transformation efficiency of putative transgenic *in planta* method is greater than *in vitro* method, which is 18.33% and the percentage of transformation efficiency of putative transgenic *in vitro* and *in planta* decreases with increasing hygromycin concentration in the selection media. Putative transgenic that pass in hygromycin media are then maintained and PCR tested with a phen-specific CG CG1 primeer. The PCR results can be seen in Figure

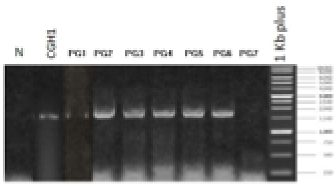

Figure 6. PCR result of putative transgenic rice folate gene
Notes:

N = Pare Gajah (Non-transgenegenic)
PG1,2,3,4,5,6,7 = Pare Gajah putative transgenic folate gene
CGH1 = *Agrobacterium tumefaciens* which contains the folate gene construct
1 kb plus: Ladder

Figure 7. Appearance of Pare Gajah transgenic rice in vegetative phase that has been inserted with the folate gene (a); starts flowering (b); starts the grain filling phase (c)

Positive rice has a 1200 bp folate gene band, treated and implanted in soil media. Based on information from PCR results it is known that putative GMOs PG1, PG2, PG3, PG4, PG5, PG 6 have a DNA band size of 1200 bp (the same as the control which is *Agrobacterium tumefaciens* which carries 'construct folate gene'). Putative transgenic samples of PG7 did not have a 1200 bp DNA band (the same as the negative control which is Pare Gajah rice that is not transformed). The appearance of the transgenic plant can be seen in Figure 7.

5. Conclusions

Callus induction, namely: 2,4-D concentration gives a significant effect on the time parameters of callus formed (HST) and significantly affects the number of explants forming callus (%), but not significantly on callus texture parameters, callus diameter (mm) and callus area (mm^2). Of the three concentration levels used, a 2,4-D concentration of 1 ppm produced the best callus diameter (0.49 mm) and callus area (43,32 mm^2). 2,4-D concentrations of 2 ppm for the time the callus formed (12,67 days), the number of explants formed a callus (94,44%) and the best callus color (yellowish white).

Transformation of folate genes, they are:

1. The transformation method significantly affects the lethal parameters of hygromycin dose and the efficiency of transgenic putative transformation (%). Of the two transformation methods used, the *in vitro* transformation method has a lower lethal dose (more toxic), causing an average explant mortality of 93.75% with an average transgenic putative transformation efficiency of 6,25%. In the in planta lethal method the dose is higher with an average explant mortality of 86,67%, and the best efficiency of transgenic putative transformation is 13,33%.
2. Hygromycin concentration has a very significant effect on lethal hygromycin dose and the efficiency of transgenic putative transformation (%). Of the four levels of hygromycin concentration used, the 100 ppm hygromycin concentration had the most toxic lethal dose which caused the highest mortality rate (98,34%) with the lowest average level of transgenic putative transformation efficiency (1,66%). Hygromycin concentrations of 25 ppm had lethal doses causing the lowest explant death rates (82,50%), with the highest average transgenic putative transformation efficiency (17,50%).
3. There is no interaction between the treatments of transformation method with the treatment of hygromycin concentration on the results of the transformation of folate genes in the Banten Pare Gajah local rice.
4. PCR results show DNA samples of Pare Gajah rice PG1, PG2, PG3, PG4, PG5, PG 6 have a folate gene encoding DNA band with a size of 1200 bp.

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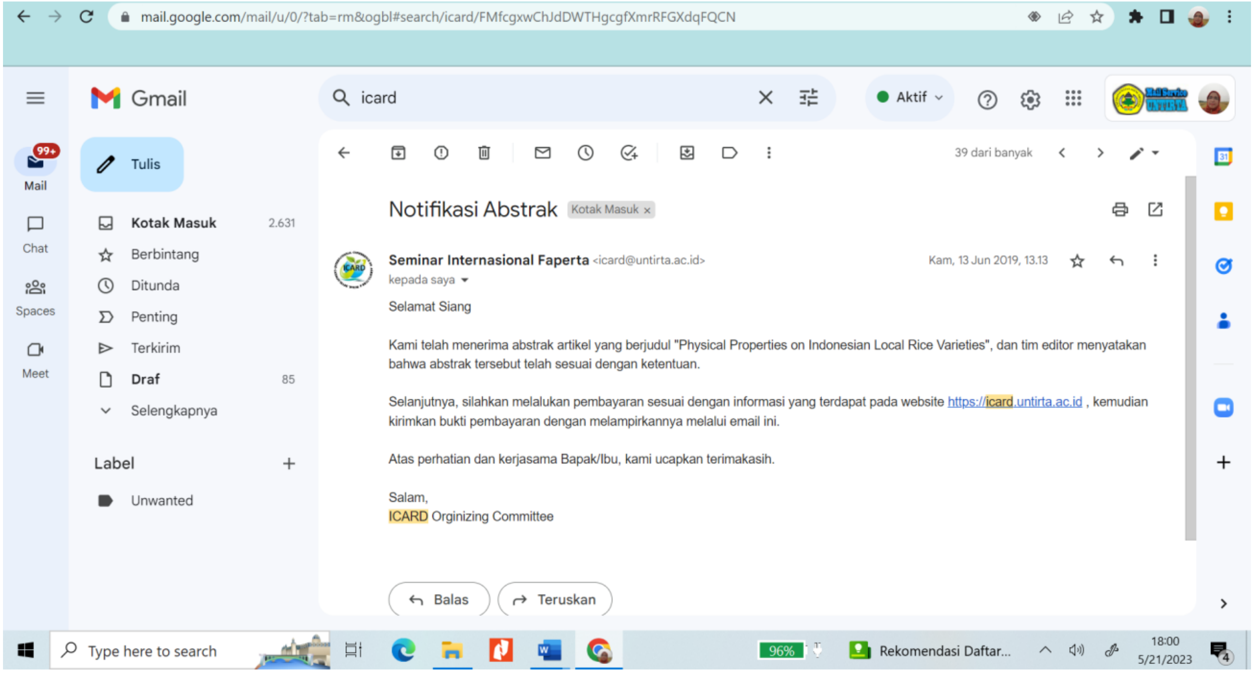
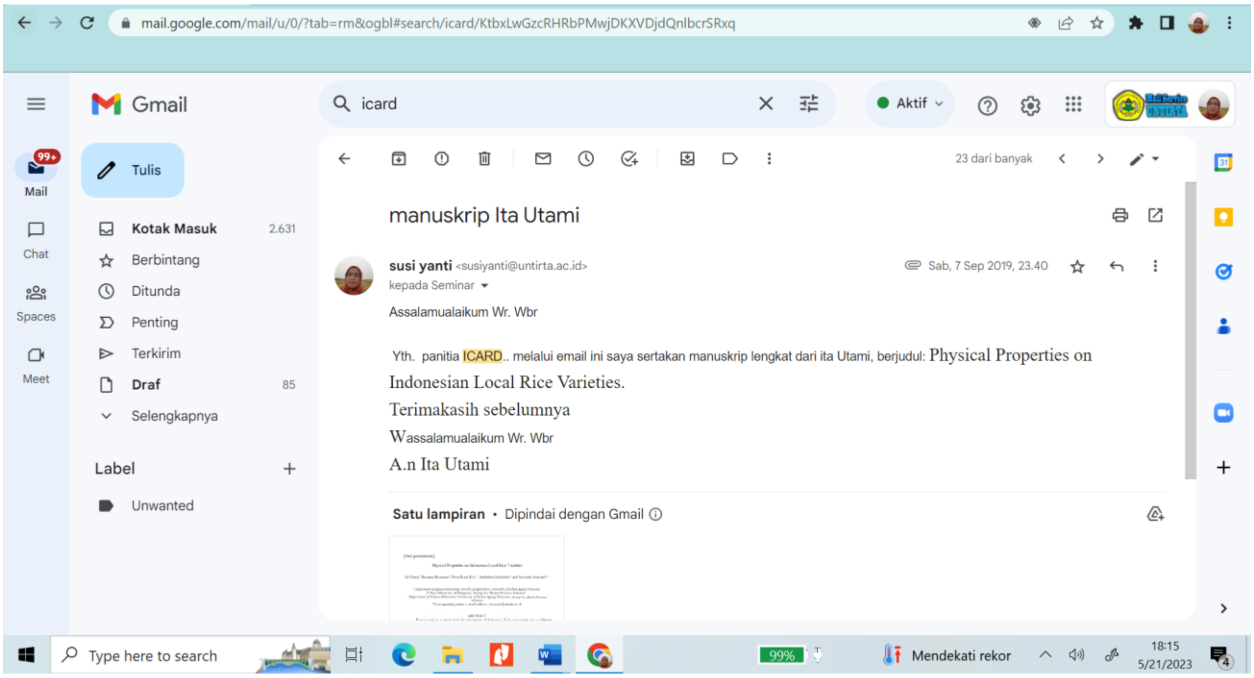
Dokumentasi korespondensi artikel: Physical Properties on Indonesian Local Rice Varieties





[Oral presentation]

Physical Properties on Indonesian Local Rice Varieties

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ABSTRACT

Rice is used as a staple food for the people of Indonesia. Each rice variety has a different physical shape and size in the grain. This study aims to determine the physical properties of several local Indonesian rice varieties. Research on the physical properties of grain was carried out on nine Indonesian local rice varieties from Banten, West Sumatra, and East Java provinces. Physical properties parameters observed in grain include length, width, shape, 1000 grain weight, bulk density and chalkiness percentage of rice. The results showed that the grain shape of Tunggul Hideung and Genjah Nganjuk was slender and the other varieties were oval. While the type of Kewal Bulu Hideung includes round rice, the Kewal Bulu Hideung is slim in shape and other varieties are oval. The highest 1000 grain weight in grain and rice was owned by Kewal Gudril (28.23 g and 22.44 g) and the lowest was in Kuriak Kusuik (20.62 g and 14.09 g). The highest density of unhulled bulk density is Kuriak Kusuik (0.62 g..mL^{-1}) and the lowest is Kewal Bulu Hideung (0.3 g.mL^{-1}), while the highest rice bulk density is Susu Putih (0.89 g .mL^{-1}) and the lowest is the Genjah Nganjuk (0.79 g.mL^{-1}). Based on the percentage of calcareous rice, Kewal Benur and Genjah Nganjuk including premium quality rice, Kewal Bulu Hideung, Tunggul Hideung and Kuriak Kusuik rice including medium quality class 1 rice, and other varieties do not meet the quality requirements set by SNI (> 5%).

Keywords: Rice, Grain, Local Rice, Physical Properties

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INTRODUCTION

Rice is one of the main food crops of almost half the world's population (Childs, 2004). About 90% of the world's rice is grown and consumed in Asia (Tyagi *et al.*, 2004). Indonesian society has become the largest nation that consumes rice as a staple food and makes it a major source of carbohydrates because it is easy to obtain, tastes good and can be combined with other food ingredients. According to Widjayanti (2004), rice as a staple food contributes around 40-80% of energy and 45-55% of protein in the average menu of the Indonesian people.

Rice (Oryza sativa) is the most important cereal crop in the developing world and is the staple food of over half the world's population. It is generally considered a semi-aquatic annual grass plant. About 20 species of the genus Oryza are recognized, but nearly all cultivated rice is O. sativa L. A small amount of Oryza glaberrima, a perennial species, is grown in Africa. So-called "wild rice" (Zizania aquatica), grown in the Great Lakes region of the United States, is more closely related to rice than to rice (Juliano, 1993).

Since 2011 to 2017 rice production has continued to increase at 65.75 million tons in 2011 and 81.38 million tons in 2017. The achievements in 2017 have actually exceeded the rice production target set at 79 million tons (Indonesia Ministry of Agriculture, 2019). National rice production in the period 2010-2017 continues to increase. In 2017 rice production is estimated to reach 81,382,451 tons, growing 2.56 percent from the previous year (BPS, 2018).

Indonesia actually has a great genetic diversity of rice, because the archipelago was formerly united with the Asian continent which is the center of origin *(center of origin*) rice (Abdullah, 2006). Rice germplasm in the form of varieties is very diverse, ranging from national varieties and local varieties. Ariani *et al.,* (2011) reported that some rice-producing farmers in several regions in the Indonesian province still planted local varieties hereditary. According to Yadav et al. (2007) and Susiyanti et al. (2020), differences in varieties show significant differences in morphology, physicochemistry, and cooking properties.

The marketing values of rice as an agricultural product depend on its physical qualities after the harvesting. Information on nutritional content and physical properties of rice is generally only found in superior varieties of rice. While rice on the market is very diverse in types and varieties. So far, there is still little information related to the physicochemical nature of Indonesia's local rice germplasm. So it is deemed necessary to obtain basic data regarding the physical properties of Indonesian local rice for various purposes, especially for plant breeding activities. Based on this, it is necessary to conduct research on "The Physical Properties of Indonesian Local Rice (*Oryza sativa* L.) Varieties ".

MATERIALS AND METHODS

This type of research is descriptive quantitative research. The research has been carried out in the Laboratory of Biotechnology Faculty of Agriculture, University of Sultan Ageng Tirtayasa and Analysis Laboratory Faculty of Agriculture, University of Jember from October 2017 until February 2018.

The tools used in this study are the meter, calipers, analytical scales, measuring cups, stirring rods, and *mini rice mills.*

The research began with the collection and identification of local rice variety from several regions in Indonesia. The local rice varieties used were Kewal Bulu Hideung, Kewal Gudril, Kewal Benur, Kewal Sampai Putih and Tunggul Hideung from the Banten region; Kusuik Kuriak from West Sumatra and MS Pendek, Susu Putih and Genjah Nganjuk from East Java. After collecting and searching for data on local rice varieties used including the date of harvest and the method of storing the local rice, the physical characteristics were analyzed, namely length, width, size, shape, weight of 1000 grains, density and chalkiness.

RESULTS AND DISCUSSION

Length, Width and Shape on Grain and Rice

Physical properties that are closely related to the quality of grain and rice and the level of consumer acceptance include grain size (length and width) and length and width ratio that reflects the shape of grain and rice. Classification of grain size and shape criteria is based on the SNI scale (1987) and rice is based on the FAO scale (1980).

Table 1. Average of length (L), width (W), the ratio of L/W, size, and shape of grain

No	Varieties	Length (mm)	Width (mm)	Ratio L/W	Size Grain	Form Grain
1	Kewal Bulu Hideung	8.01	3.38	2.37	Very long	Oval
2	Kewal Gudril	8.42	3.22	2.62	Very long	Oval
3	Kewal Benur	8.56	3.00	2.85	Very long	Oval
4	Kewal Sampai Putih	8,12	3.37	2.41	Very long	Oval
5	Tunggul Hideung	9.68	2.60	3.72	Very long	Slim
6	Kuriak Kusuik	8.05	2.75	2.93	Very long	Oval
7	MS Pendek	8.44	2.92	2.89	Very long	Oval
8	Susu Putih	9.44	3.18	2.97	Very long	Oval
9	Genjah Nganjuk	9.54	2.88	3.31	Very long	Slim

All rice varieties studied have very long grain size with a length of more than 7, 5 mm (Table 1). The length and shape of the grain are largely determined by the layer of the husk, which is the longer and the larger size of the grain shows the thicker layer of the husk that wraps the rice grains on the rice.

Table 2. Average of length (L), width (W), the ratio of L/W, size, and shape of rice

No	Varieties	Long (mm)	Wide (mm)	Ratio L/W	Size Rice	Form Rice
1	Kewal Bulu Hideung	5.61	2.81	1.99	Medium	Round
2	Kewal Gudril	5.67	2.74	2.07	Medium	Oval
3	Kewal Benur	6.08	2.45	2.48	Medium	Oval
4	Kewal Sampai Putih	5.76	2.70	2.13	Medium	Oval
5	Tunggul Hideung	6.50	2.10	3.10	Medium	Slim
6	Kuriak Kusuik	5.57	2.07	2.69	Medium	Oval
7	MS Pendek	5.71	2.21	2.58	Medium	Oval
8	Susu Putih	6.12	2.42	2.53	Medium	Oval
9	Genjah Nganjuk	6.79	2.33	2.91	Long	Oval

The size of the local rice studied was obtained in length ranging from 5.57 to 6.79 mm (Table 2). In general, rice in Indonesia is of medium to long size. Long sized rice is preferred on the international market compared to medium and short sized rice. However, in certain countries short-sized rice is preferred as in Japan, Korea and Taiwan.

The width of rice obtained ranged from 2.07 to 2.81 mm. The widest rice is Kewal Bulu Hideung rice from Banten, while the rice with the smallest width is Kuriak Kusuik rice from West Sumatra. The length and width ratio of rice obtained ranged from 1.99 - 3.10. Based on the ratio of length/width, the shape of ground rice according to FAO is divided into three types namely slim, oval and round. Kewal Bulu Hideung Variety from Banten has a round shape with a length / width ratio of 1.99, and Tunggul Hideung variety has a slim shape with a length / width ratio of 3.10, while other varieties have an oval shape with a long/wide ratio between 2.00-3.00. According to Setyono and Wibowo (2008), the size and shape of rice is the dominant character derived from the genetic characteristics of the parent and can be used as a parameter for determining the purity of a variety. The length and shape of rice are influenced by genetic factors, agroecosystems and land fertility.

All rice varieties studied have a variety of sizes, shapes and colors as well as the appearance of grain and rice as seen in Figure 1 and Figure 2. The grain color is generally yellow, but the yellow Kuriak Kusuik grain is mixed with brown. The variety of Kewal Bulu Hideung and Kewal Sampai Putih also have fur on their grain. The color of rice is not only white, but also yellowish as on the Kewal Gudril variety, red on the MS Pendek variety and a pale white color on the Susu Putih glutinous rice variety.

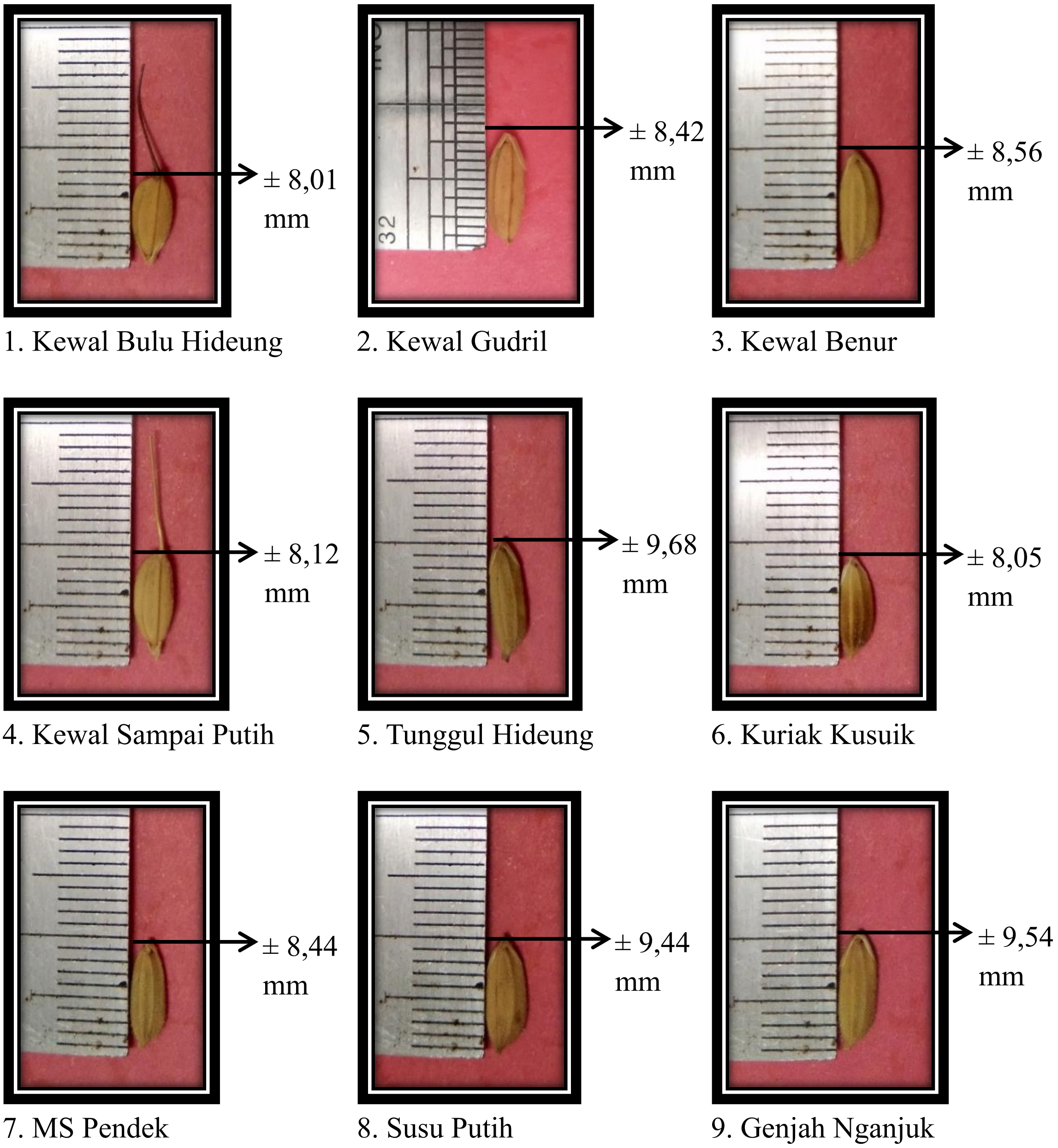

Figure 1. Diversity of shape, size and color of grain in 9 local Indonesian rice varieties

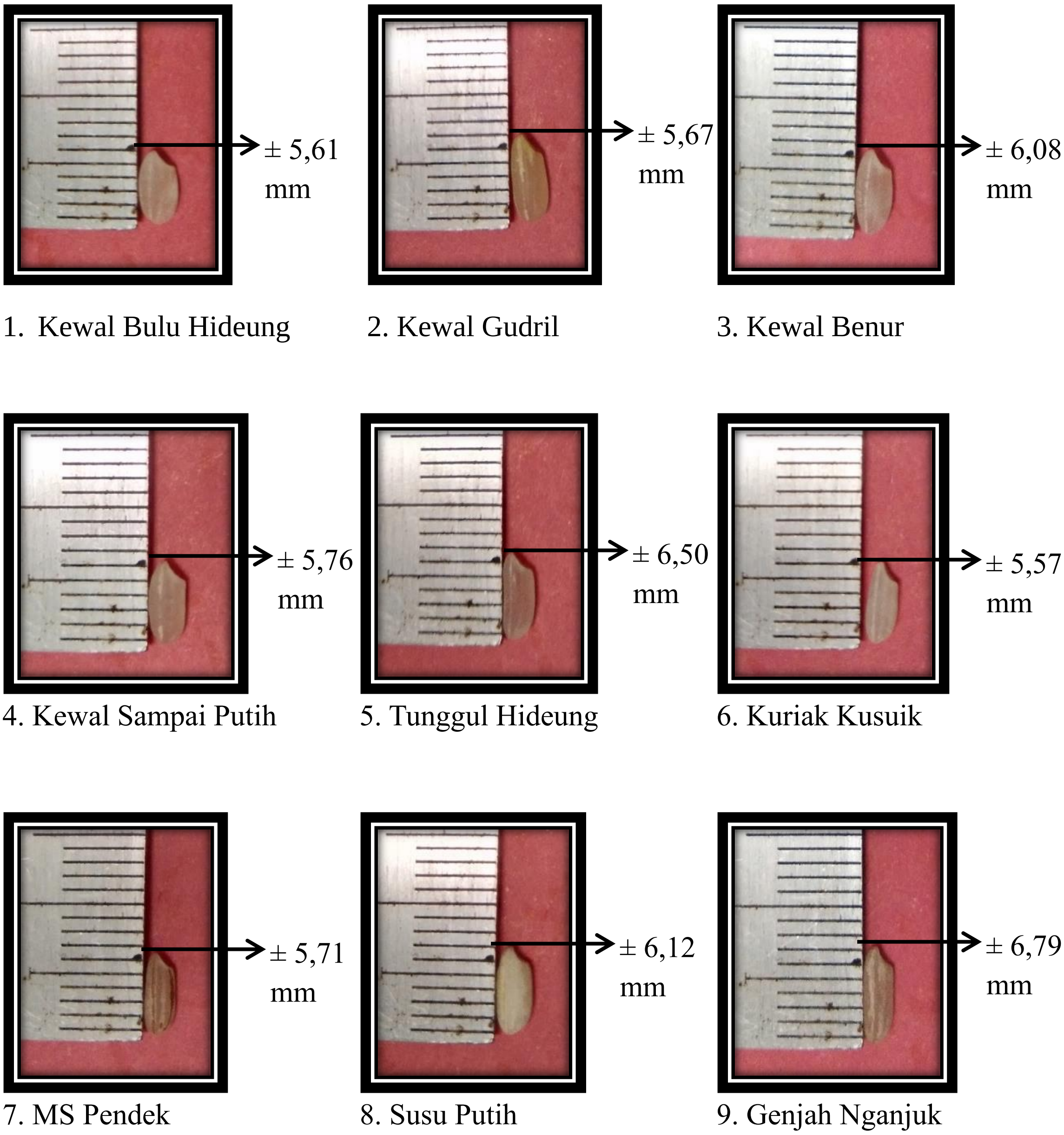

Figure 2. Diversity of shape, size and color of rice in 9 local Indonesian rice varieties

1000 Grain Weight in Grain and Rice

The weight of 1000 points indicates the weight of each grain which determines the yield of production. 1000 grain weight grain also determines the yield of milled rice. This value can be used to determine whether there is a mixture in a rice sample on the market. It also can be used to determine the purity of a rice variety. The results of an analysis of the measurement of 1000 grain weights on the grain and rice studied are presented in Figure 3.

The weight value of one thousand grains analyzed ranged between 20.62 - 28.23 grams, while the weight value of one thousand grains of rice ranged between 14.09-22.44 grams. According to Hernawan (2016), one thousand grains are affected by the availability of nutrients in the soil during rice planting. Nutrient deficiencies at the time of planting will result in the weight of a thousand grains produced lower than they should

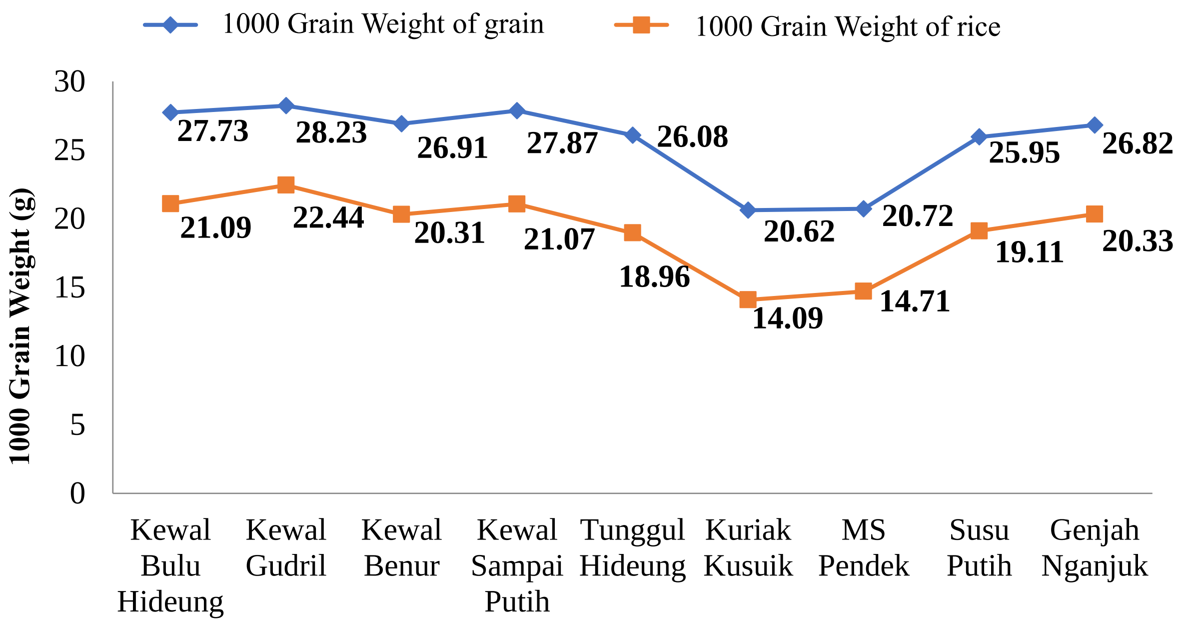

Figure 3. 1000 Grain Weight in Grain and Rice

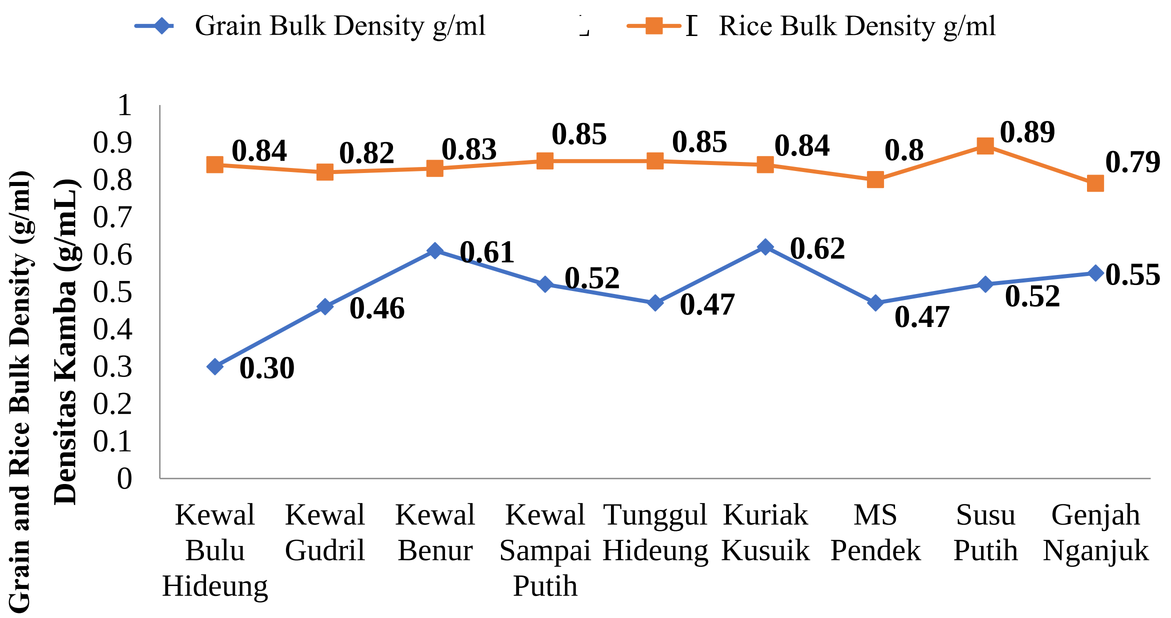

Figure 4. Grain and rice bulk density

Table 3. Percentage of chalkiness rice

No	Varieties	*White-based Rice* (%)	*White-belly Rice* (%)	*White-core Rice (%)*	*Milky-white Rice (%)*	Rice Total Whitewashing (%)	*Perfect Rice* (%)
1	Kewal Bulu Hideung	1,4	44,4	5,2	1,0	52,0	48,0
2	Kewal Gudril	0,0	73,4	4,6	5,4	83,4	16,6
3	Kewal Benur	0,0	59,6	9,6	0,0	69,2	30,8
4	Kewal Sampai Putih	0,0	83,0	9,2	5,8	98,0	2,0
5	Tunggul Hideung	0,0	4,6	9,2	1,2	15,0	85,0
6	Kuriak Kusuik	0,6	1,4	32,2	1,8	36,0	64,0
7	MS Pendek	0,8	24,4	23,0	7,0	55,2	44,8
8	Susu Putih	-	-	-	-	-	-
9	Genjah Nganjuk	1,4	0,4	9,0	0,0	10,8	89,2

Note: Total Whitewashing Rice (%) = *White-based Rice* + *White-belly Rice* + *White-core Rice* + *Milky-white Rice*
Total observed rice (%) = total whitewashing rice+ *Perfect Rice* = 100%

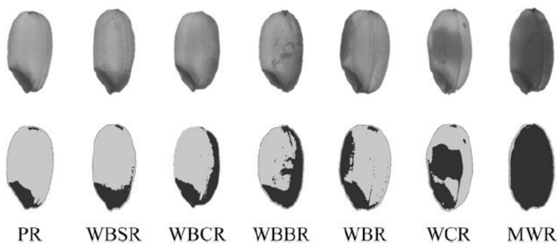

Figure 5. Grouping chalkiness: perfect rice (PR), white-based rice (WBSR), white-back rice (WBCR), white-back and -based rice (WBBR), White- belly rice (WBR), white-core rice (WCR) and milky-white rice (MWR) (Yoshioka, 2007

Grain and Rice Bulk Density

Density of grain or rice is a measure that describes the weight of grain or rice per unit volume expressed in units of g/ml. 1000 grain weight grain also determines the yield of milled rice. According to Suismono *et al.* (2003), the greater the density and weight of 1000 grain the greater the yield of ground rice.

The size and shape of the grain and rice also directly affect the density. The rough rice of each variety show shows the least value of true density, which agrees with Araullo, De Padua, and Graham (1976), who stated that the specific gravity of paddy is lower than that of brown rice. In addition, Correa *et al*. (2007) reported similar results in the case of true density of rice by processing.

The bulk density of rice varieties in Indonesia ranges from 0,45 - 0.58 g/mL. Thus all grain samples are within the bulk density range, except for the Kewal Bulu Hideung grain variety which has a bulk density below the range of only 0.30 g/mL. This is because the Kewal Bulu Hideung variety has fur at the tip of its grain, thereby reducing its density in storage. The highest bulk density of rice is owned by Kuriak Kusuik and the lowest is owned by the Kewal Bulu Hideung grain. This means that the Kewal Bulu Hideung grain needs 2 times more storage space than the Kuriak Kusuik grain. Whereas the densest rice is owned by Susu Putih variety. That is, for the same weight of Genjah Nganjuk rice requires 1.13 times more storage space than Susu Putih rice.

Percentage of Number of Chalking Rice (Chalkiness)

Whitewashing grains are white broken rice skin such as chalk and soft texture caused by physiological factors (SNI, 2015). This white area is the result of very little starch development in the peripheral area of the endosperm. This is caused by insufficient carbohydrate transport during rice ripening (Juliano, 1973)

When referring to the SNI standard for rice (2015) which requires a maximum content of limestone grains of 5%, then the level of whitewashing rice in varieties of Kewal Gudril (5,4 %), Kewal Sampai Putih (5.8%) and MS Pendek (7.0%) does not meet these requirements . Whereas the Susu Putih variety is glutinous rice so it cannot be observed its whitewashing grain because the color of the rice is indeed all murky white like glutinous rice in general. *Chalkiness* grouping based on Figure 5. Below

CONCLUSIONS AND SUGGESTIONS

Conclusions

The grain size indicates that all grain is very long. The grain form of the Tunggul Hideung and Genjah Nganjuk varieties is slim and the other varieties are oval shaped. Whereas in the size of rice, the Genjah Nganjuk variety are long and the other varieties are medium. Rice forms of Kewal Bulu Hideung are round, Tunggul Hideung is slim and other varieties are oval shaped.

The weight value of one thousand grains analyzed ranged between 20.62 - 28.23 gams, while the weight value of one thousand grains of rice ranged from 14.09 - 22.44 grams. The grain samples studied have densities in the range 0.3 - 0.55 g / mL. Kewal Bulu Hideung variety has the smallest density (0.3 g / ml) because there are long black feathers in each grain of grain.

The grain samples studied have densities in the range 0.3 - 0.55 g / mL. The weight value of one thousand grains analyzed ranged between 20.62 - 28.23 grams, while the weight value of one thousand grains of rice ranged between 14.09 - 22.44 grams. Samples of grain y ang studied has a density k amba range of 0.3 to 0.55 g / m L. G abah varieties Fur Kewal has a density Hideung smallest (0.3 g / ml) because there is a long black hairs in every grain gabahnya .

The level of whitewashing rice in the studied rice fulfills SNI requirements (maximum 5%), except varieties of Kewal Gudril (5.4%), Kewal Sampai Putih (5.8%) and MS Pendek (7.0%).

Suggestion

The rice varieties on the market are very diverse. Therefore, the characteristics of physical characteristics need to be known by the community in order to know the right steps or processing of rice and rice that is processed to get the desired characteristics according to their respective preferences.

Varieties -varietas rice found in the market are very diverse. Therefore, the characteristics of the physical characteristics need to be known by the community in order to know the steps or the proper processing of the rice they process to get the desired characteristics according to their individual prefe

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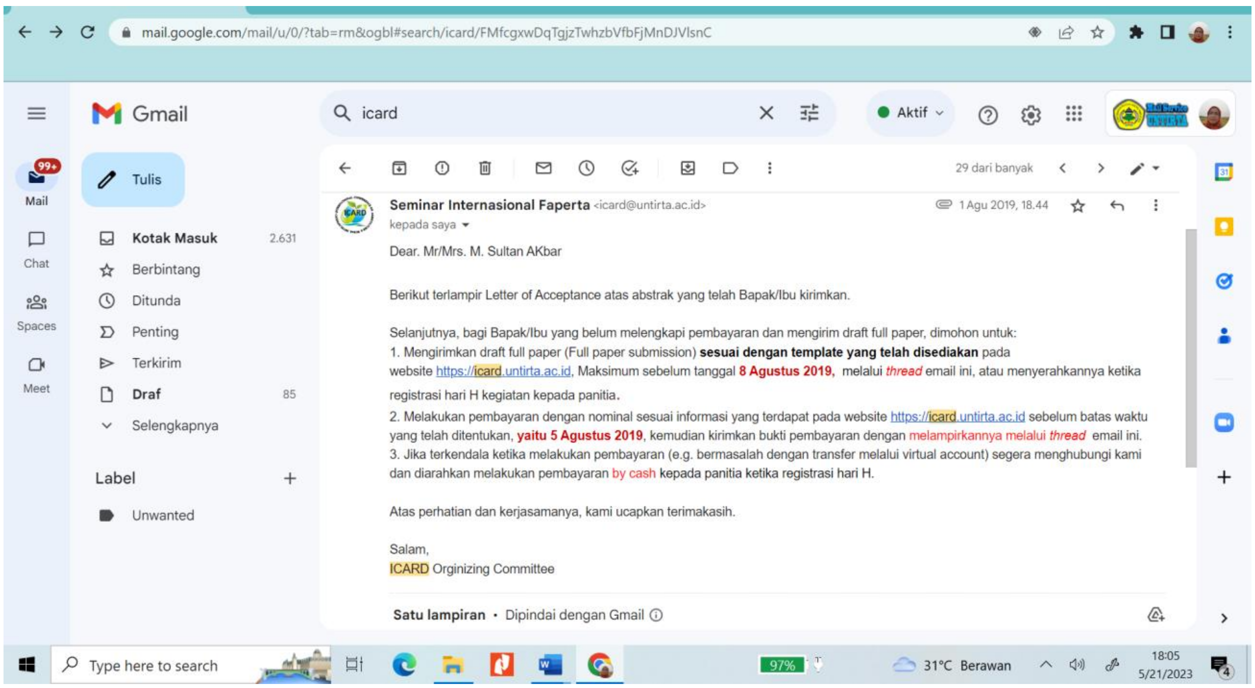
Dokumentasi korespondensi artikel:

Title : Molecular Identification of Early Age and Taste of Rice from F1 Segregation Results of Kewal Bulu Hideung local Varieties with Mira-1 National Varieties through Simple Sequence Repeat Method.

Authors : Muhammad Sultan Akbar, Rusmana Rusmana, Nurmayulis Nurmayulis, Sjaifuddin Sjaifuddin, and, Susiyanti Susiyanti



Seminar Internasional Faperta <icard@untirta.ac.id>

kepada saya

1 Agu 2019, 18.44

Dear. Mr/Mrs. M. Sultan AKbar

Berikut terlampir Letter of Acceptance atas abstrak yang telah Bapak/Ibu kirimkan.

Selanjutnya, bagi Bapak/Ibu yang belum melengkapi pembayaran dan mengirim draft full paper, dimohon untuk:
1. Mengirimkan draft full paper (Full paper submission) **sesuai dengan template yang telah disediakan** pada website https://icard.untirta.ac.id, Maksimum sebelum tanggal **8 Agustus 2019**, melalui *thread* email ini, atau menyerahkannya ketika registrasi hari H kegiatan kepada panitia.
2. Melakukan pembayaran dengan nominal sesuai informasi yang terdapat pada website https://icard.untirta.ac.id sebelum batas waktu yang telah ditentukan, **yaitu 5 Agustus 2019**, kemudian kirimkan bukti pembayaran dengan melampirkannya melalui *thread* email ini.
3. Jika terkendala ketika melakukan pembayaran (e.g. bermasalah dengan transfer melalui virtual account) segera menghubungi kami dan diarahkan melakukan pembayaran by cash kepada panitia ketika registrasi hari H.

Atas perhatian dan kerjasamanya, kami ucapkan terimakasih.

Salam,
ICARD Orginizing Committee

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The 1st International Conference on Agriculture and Rural Development (ICARD)

Acceptance of Abstract Submission

Dear Mr/Mrs. Muhammad Sultan Akbar

On behalf of the editors, I am pleased to advise that your abstract submission to the The 1st International Conference on Agriculture and Rural Development (ICARD) 2019 has been accepted. Congratulation.

Detail of your abstract are outlined below:

Title : **Molecular Identification of Early Age and Taste of Rice from F1 Segregation Results of Kewal Bulu Hideung local Varieties with Mira-1 National Varieties through Simple Sequence Repeat Method.**

Authors : **Muhammad Sultan Akbar, Rusmana Rusmana, Nurmayulis Nurmayulis, Sjaifuddin Sjaifuddin, and, Susiyanti Susiyanti**

Serang, July 25th, 2019

Chief of Editor

Tatang Sutisna