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Short Communication
Biochemical Properties of Twelve Malaysia Rice Cultivars in Relation to Yield Potential

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Abstract

Background and Objective: Yield has always been the major constraint for most crops, especially for rice. Grains, being the end product of rice plant can only be acknowledged during the grain filling period, the last stage in the life cycle of rice plants. Comparison of biochemical properties with its yield during the early stage, can act as an early selection criteria for high yielding traits. This study was conducted to evaluate the effectiveness of Rubisco activity, chlorophyll content, starch content, soluble protein content and dry weight measurement of leaves seedlings on 12 recommended local rice cultivars in comparison with its yield potential. **Materials and Methods:** The experiment was performed in a completely randomized block design. A total of 5 plants per cultivar were grown and 3 plants were randomly picked for biochemical analyses. The collected results were analyzed statistically using the analysis variance (one-way ANOVA) and Tukey's test. **Results:** The data obtained revealed that Rubisco activity, protein content and dry weight in leaves seedlings showed no correlation with yield with $p>0.05$. In contrast, both chlorophyll content and starch content showed a significant difference ($p<0.05$) that indicated variation among the cultivars. Chlorophyll content was found negatively correlated with yield in most of the studied cultivars. **Conclusion:** The potential of early detection using biochemical assessments in leaves seedlings of rice cultivars are in relation with its yield.

Key words: Biochemical, carbon metabolism, grain yield, rice seedlings, Rubisco activity

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

To meet the demand of rice in the year of 2050, current world rice production need to be doubled up to approximately 1 billion tons to feed the rising population[1]. After the green evolution, little improvement has been observed in grain yield of most cereal crops. A number of strategies have been suggested, some based on conventional breeding, others in biotechnology and perhaps some by combining both conventional and molecular techniques[2]. Although, these methods promise a remarkably results in future but the questions remain in the time/duration needed for it to be fruitful. Furthermore, the success did not guarantee an increased in grain yield. On the other hand, biochemical possessions during the early development of the plants e.g., leaves of young seedlings demonstrate some correspondence with yield, thus greater improvement of grain yield can be achieved.

Once seed is germinated, both the endogenous storage reserves and exogenous resources from the environment will be utilized to ensure the success of seedlings establishment through adaptation to developmental and metabolic programs of prevailing environmental condition[3]. Hence, early detection of biochemical properties in leaves seedlings might provide some insight on how well the seedlings behave in certain condition and thus, it might even exhibit its potential in relation with yield. Rubisco was selected as this is the key enzyme in plant photosynthesis where it incorporates carbon into the biosphere from atmospheric CO_2, giving the impression of its fanatic role in biomass accumulation. It has been reported that, Rubisco content increases rapidly during leaf expansion of cucumber but decreases once it entered the initial senescence stages[4]. Although numerous of researches have been done on Rubisco on its effect on yield, yet none of them applying it on the leaves of young growing rice cultivars that were fed exclusively from the seed's storage protein for its nitrogen source. In agricultural production, the role of nitrogen is closely related to photosynthesis. Nitrogen determines the synthesis of amino acids and therefore protein, in which ultimately all the cellular components. These synthesized components will gradually integrated into carbon metabolism, in which CO_2 assimilation requires a huge amount of components in the chloroplast, where the most important is Rubisco, plus other enzymes required for CO_2 assimilation in the stroma[5]. Therefore, in a condition where the nitrogen is limited, the activity of Rubisco in 12 local rice cultivars might exhibit differently and its correlation with yield could be observed. Other biochemical analyses such as chlorophyll content, soluble protein content, starch content and dry weight measurement were also performed as outline in addition of Rubisco to yield.

In the experiment carried out, the growth condition of the rice seedlings was completely depends on the endogenous storage proteins as the exogenous nitrogen source was completely eliminated. As nitrogen is an important and limiting nutrient in plants growth and regulation[6], hence remobilization/recycling of endogenous nitrogen was crucial for plants development and to sustain its growth. The current study demonstrates the potential of early detection of biochemical assessments in leaves seedlings of rice cultivars in relation with yield.

MATERIALS AND METHODS

Plant material and growth condition: Seeds of 12 local rice cultivars were surface sterilized, germinated and grown as previously described[7]. The nutrient solution (without external nitrogen source) used was renewed once a week and the pH was adjusted to pH 5.0 daily. Rice leaves were harvested after growing in nutrient solution for 3 weeks (Supplementary Fig. 1). One day before harvesting, the nutrient solution was renewed to maximize the nutrient uptake. The rice cultivars were grown at greenhouse and data were recorded from the period of 2014-2015. The yield data used was from the average experimental data over season during the field evaluation conducted at Seberang Prai, Penang by Malaysian Agricultural Research and Development Institute (MARDI) (Table S1). All solutions and chemicals used were of analytical grade unless otherwise stated.

Crude preparation: For crude extract preparation, leaves of individual plant were ground into powder in liquid nitrogen and extracted according to protocol described previously[8].

Table S1: Yield of 12 rice cultivars

Cultivars	Yield (kg ha^{-1})
Setanjung	5291
Muda	5954
MR 84	5196
MR 185	5160
MR 159	5115
MR 167	5573
MRQ 34	3500
MR 207	3465
MR 209	2609
MR 211	4300
MR 219	5529
MR 220	6121

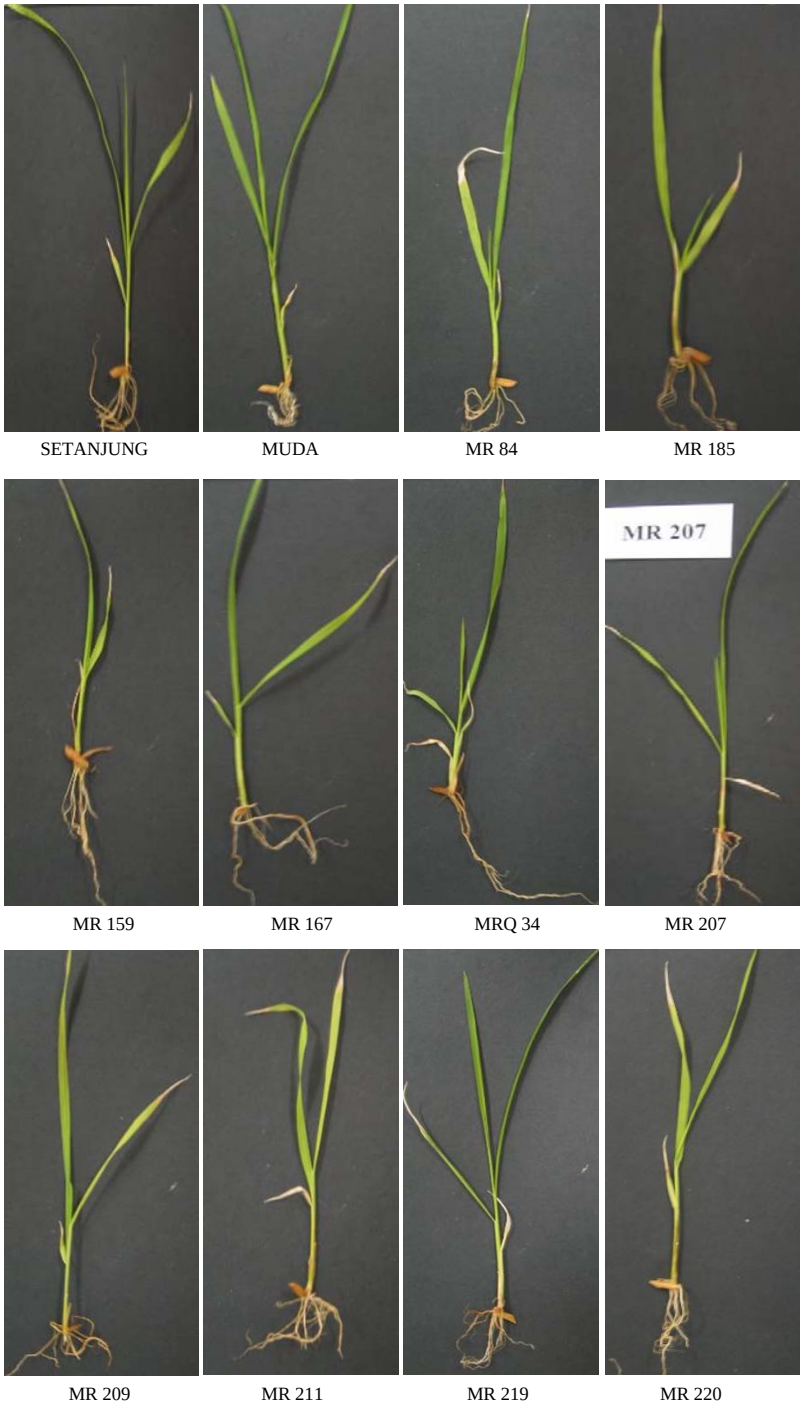

Fig. S1: Three weeks old seedlings of 12 rice cultivars. Bar: 1 cm

Rubisco activity, proteins and starch content determination: From the crude extract, supernatant was used to determine Rubisco activity and protein content while the pellet was used for starch determination. The Rubisco activity determination was a coupled or linked assay[9]. Soluble protein content was quantified using Bradford method[10], whereas, starch content was determined by Anthrone method[11].

Chlorophyll content and dry weight measurement: Chlorophyll extraction and total chlorophyll content were performed according to method described previously[12]. The

leaves dry weight (DW) of 3 weeks old seedlings (Supplementary Fig. 1) were incubated in an oven and weight were recorded using electronic balance (Sartorius, Germany) weekly until a constant weight were obtained.

Experiment design and statistical analysis: The experiment was carried out in a completely randomized block design. About 5 plants per cultivar were used and 3 plants were randomly picked for biochemical analysis determination. The data were submitted to analysis variance (one-way ANOVA) and Tukey's test. All the statistical analysis was performed at 95% confidence level which demonstrated significant ($p<0.05$) or no significant ($p>0.05$) different, unless stated otherwise. Statistical analysis with one-way ANOVA was performed using SPSS for Windows Version 24.0.

RESULTS AND DISCUSSION

In Fig. 1, Rubisco activity was the highest in MR 219 with 1046 nmol min^{-1} mg^{-1} protein, followed by MR 207 and MR 159 with 898 and 800 nmol min^{-1} mg^{-1} protein, respectively. Lower activity was found in cultivars Setanjung (483 nmol min^{-1} mg^{-1} protein), MR 185 (529 nmol min^{-1} mg^{-1} protein) and MR 211 (533 nmol min^{-1} mg^{-1} protein). Based on the results obtained, although cultivars MR 219 and MR 159 that possessed higher Rubisco activity had higher yield in field, nonetheless, MR 207 that had higher Rubisco activity had lower yield. Similarly, Setanjung and MR 185 that seized lower Rubisco activity had higher yield in field. Our result is in accordance with the finding by Hirasawa *et al.*[13], whereby no clear Rubisco activity variation was observed among rice cultivars with difference of yields.

In the set-up of the experiment, endogenous storage protein was implied as the sole nitrogen source throughout the growing phase of the rice seedlings, where the remobilization of internal nitrogen was actively triggered. In these seedlings, the nitrogen source might come from the degradation of storage protein in the seed[14] photo respiration, phenylpropanoid biosynthesis and also amino acid catabolism[15,16]. As nitrogen and carbon are integrated with a sophisticated network[17], with a different rate of metabolism, therefore, it is assume that each cultivar represent an individual itself that magnifies differently based on the available resources. However, statistical analysis of one-way ANOVA indicated no significant different of Rubisco activity was observed among the 12 cultivars analyzed, quoting that no variation of Rubisco activity was observed. Therefore, Rubisco activity in leaves of rice seedlings is inappropriate to act as an indicator of grain yield.

From Fig. 2, the chlorophyll content in most rice leaves seedlings was negatively correlated with its yield. The highest chlorophyll content was found in cultivar MR 209 (67.43 μg g^{-1}), in which this cultivar demonstrated a considerably low yield in comparison with other cultivars. Interestingly, cultivars MR 211 and MR 207 that possessed the 2nd highest batch of chlorophyll content after MR 209 also had lower yield. Nonetheless, Mia *et al.*[18] reported that, chlorophyll content of the high yielding rice cultivar is significant higher as compare to low yielding cultivar. On the other hand, cultivars MR 84, Setanjung and Muda with a noticeably high yield had a lower possession of chlorophyll content. Some cultivars (MR 185, MR 167, MRQ 34, MR 219 and MR 220) demonstrated no significant different of chlorophyll content among these cultivars.

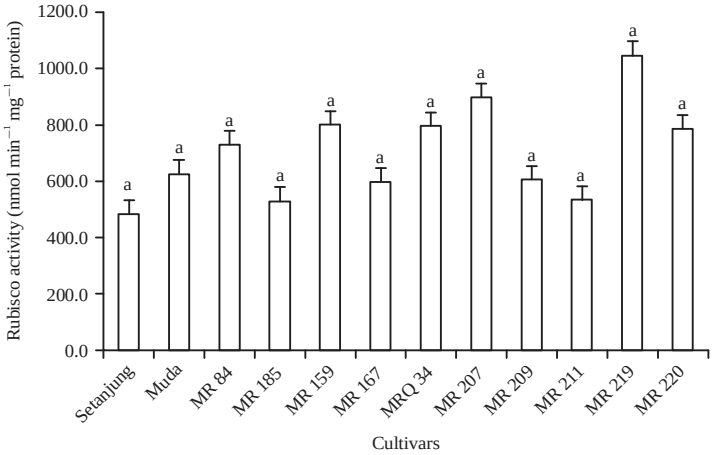

Fig. 1: Rubisco activity of twelve rice cultivars, the results are the mean±SE. (n = 3 separate plants)
Different letters indicate values are significantly different ($p<0.05$)

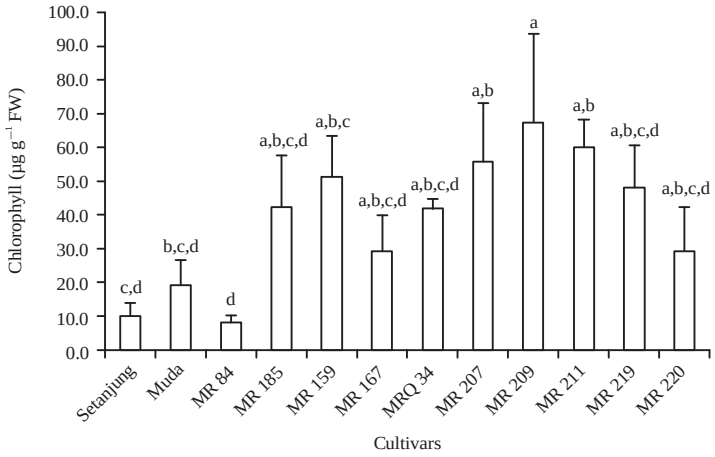

Fig. 2: Total chlorophyll content of rice cultivars. The results are the mean±SE. (n = 3 separate plants)
Different letters indicate values are significantly different ($p<0.05$)

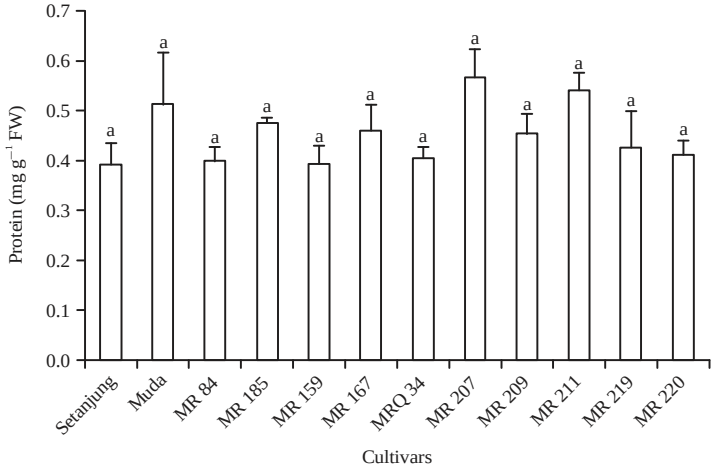

Fig. 3: Total soluble protein content of local rice cultivars. The results are the mean±SE. (n = 3 separate plants)
Different letters indicate values are significantly different ($p<0.05$)

Chlorophylls are light harvesting pigments integral to the photosynthesis activity. The concentration of chlorophylls in plants can be used to assist the potential of the plant photosynthetic activity. It has been demonstrated that leaf chlorophyll content is an important factor influencing acclimation within the leaf[19]. In present, a number of literature have stated that nitrogen nutrient is the main element in regulating the content of chloroplast, where the nitrogen supply and chlorophyll content is tightly related as chlorophyll is one of the end products integrated from nitrogen assimilation[20]. Besides, Li *et al.*[21] has mentioned that the nitrogen deficiency has great impact on chloroplasts, ranging from size, composition and function compared to those with ample nitrogen. In the experiment carried out, although no comparison was made between the deficient and ample nitrogen, nevertheless, each cultivars could act as an individual with either sufficient or deficient amount of endogenous protein that is needed for the development of chlorophyll. Thus, it can be deduced that MRQ 34, MR 207, MR 209 and MR 211 which demonstrated higher chlorophyll content had a better N assimilation, which did not attribute to grain yield in the later stage of their life cycle. This might be due to more energy is used for the assimilation of N into chlorophyll which lead to lesser energy is stored as grain.

In the result obtained in Fig. 3, cultivars MR 207, MR 211 and Muda possessed higher soluble protein content with more than 0.5 mg g^{-1} fresh weight (FW), whereas, lower soluble protein content was observed in cultivars Setanjung, MR 159 and MR 84 with the content lower than 0.4 mg g^{-1} FW. The protein content of leaves seedlings was mainly from the utilization and remobilization of internal storage protein as no nitrogen source was given in the nutrient solution. Therefore, protein content in the leaves seedlings can act as an indicator

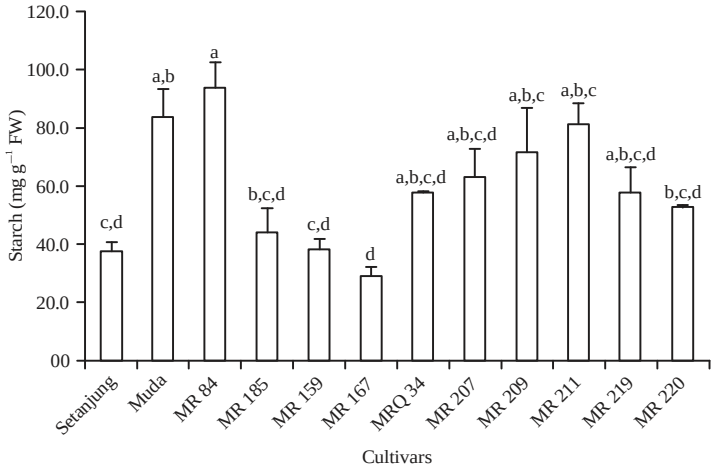

Fig. 4: Total starch content of twelve rice cultivars, the results are the mean±SE. (n = 3 separate plants)
Different letters indicate values are significantly different (p<0.05)

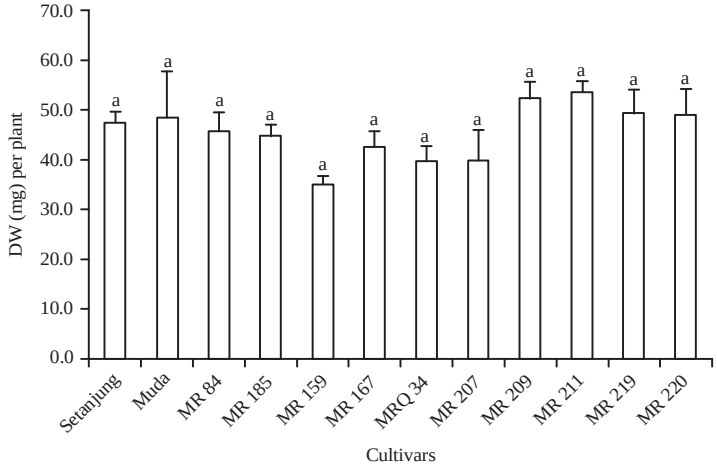

Fig. 5: Dry weight of twelve rice cultivars, the results are the mean±SE. (n = 3 separate plants)
Different letters indicate values are significantly different (p<0.05)

of the effectiveness of storage protein utilization and remobilization among the cultivars. However, our results indicated that, the soluble protein content in the leaves seedlings showed no significant different among the cultivars analyzed.

Meanwhile, in Fig. 4, cultivars MR 167, Setanjung and MR 159 that possessed lower starch content (less than 40 mg g^{-1} DW) also had higher yield with an average of 5300 kg ha^{-1} of yield. In contrast, low yield cultivars like MRQ 34, MR 207, MR 209 and MR 211 demonstrate a higher range of starch content in their leaves. The result is consistent with the previously reported study[22], whereby high yielding rice cultivar of Porsursail also showed lower starch content. Starch is the main photoassimilates from photosynthesis, in which the accumulation of starch and sucrose can be an indicator of the inefficiency of photosynthate used for growth[23]. Hence, lower content of starch can act as an indicator in which the photosynthate is being used effectively, in which these cultivars possessed efficient translocation system that been able to maximize the use of photoassimilates rather than to store it as starch. Consistently, our results indicated that, starch content showed significant different in most of the studied cultivars.

Dry weight of leaves seedlings was used as a parameter to determine the effectiveness of plant to utilize the nutrient reserved in the seed as well as the capability of its photosynthetic machinery and its translocation system that been able to convert the carbon produce to its vegetative parts for growth. Based on the results obtained, dry weight measurement for all the twelve cultivars examined showed no significant different ($p>0.05$), in which all the cultivars demonstrated the same range of variability with no significant different among themselves (Fig. 5).

CONCLUSION

Taken together, this study shows that Rubisco activity, protein content and dry weight measurement in leaves seedlings do not correlate with its yield. In contrast, chlorophyll content and starch content showed significant different in most of the studied cultivars, making it a potential early selection criteria for high yielding traits in rice.

SIGNIFICANCE STATEMENT

The present study evaluates the effectiveness of several biochemical properties on 12 rice cultivars in comparison with its yield potential. This study demonstrates the potential use of these biochemical assessments in rice seedlings as early selection criteria for high yielding traits which could benefit the plant breeder.

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