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Influence of Potassium Fertilizers on Stable Carbon Isotopic Ratio in Rice

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ABSTRACT

Isotope measurements are associated with critical plant resources. Thus, stable rice crop's carbon isotope composition in response to potassium (K) fertilizer was determined,since K is a primary plant nutrient that plays a major role in achieving maximum economic yields. Rice plants were grown in the field and rain shelter under five treatments, which are T1 (no-K), T2 (MOP), T3 (SOP), T4 (Polyhalite) and T5 (conventional fertilizer). The fertilizers were applied 3 days after planting (DAP), 15 DAP, 55 DAP and 75 DAP. Leaf photosynthesis and stomata conductance measurements were taken 85 DAP. The samples were then dried and reserved for carbon isotope analyses. Photosynthesis declined due to K deficiency in the no-K treatment. From this research, we can conclude that stomatal conductance is affected by K fertilizer application where it controls the carboxylation efficiency which may affect the rate of photosynthesis. Later, photosynthesis may influence the discrimination of $\delta^{13}C$ isotope value. There is discrimination against this heavier isotope of CO_2, this is because CO_2 diffuses through stomata by carbon 12 faster than carbon 13 so that is why there is discrimination against carbon 13 in the stomata.

Keywords: ***Oryza sativa L*; Potassium fertilizer; Isotope; Photosynthesis**

INTRODUCTION

Rice (*Oryza sativa L.*) is the most widely consumed staple food, consumed by more than half of the world's population. Rice is the third most widely planted crop in Malaysia, after oil palm and rubber. In the year 2014, 679,239 ha of Malaysia's land was planted with rice (DOA Crop Statistic 2017). In 2017, the area was 730,145 ha of which 558,203 ha were in Peninsular Malaysia, with the remaining in Sabah and Sarawak (DOA Crop Statistic 2017). Increasing population and preferences has caused Malaysia to be among the largest rice importer in the world. Malaysia requires more than 1000 metric tonnes of rice to fulfil its local demand.

Like most crops, rice requires Potassium (K) to improve its yield. K increases the plant's ability to resist various biotic and abiotic stress by regulating stomatal conductance to improve photosynthesis, improving osmotic adjustment, regulating enzyme functions and protein synthesis and maintaining ionic homeostasis (Wang *et al*. 2013). Potassium is a primary plant nutrient that plays a major role in achieving maximum economic yield. Furthermore, K is the nutrient that most frequently limits plant growth and crop yields. Increased application of K has been shown to enhance photosynthetic rate, plant growth, yield, and drought resistance in different crops under water stress conditions (Egilla *et al*. 2005).

Plants utilize K as the positive charged K^+ cation and a large amount of K is needed to achieve maximum yield potential. K is relatively immobile in most soils since K^+ ions are held in an exchangeable form by negatively charged clay particles. In addition, Romheld and Kirkby (2010) stated that essential roles for K are found in energy transfer and utilization, protein synthesis, carbohydrate metabolism, transport of sugars from leaves to fruits, and production and accumulation of oils.

Carbon isotope is a widely used and powerful method for looking at plant physiology. The $\delta^{13}C$ value has long been used as a standard technique for determining drought tolerance and improvement in C3 genotypes. Igamberdiev *et al*. (2004), Robinson *et al*. (2000) and Farquhar *et al*. (1989) found that drought reduces leaf $\delta^{13}C$ abundance, which is linked to stomatal aperture, photosynthetic impacts by carboxylation and changes in water use efficiency (WUE). It can be used to infer variation in the ratio of photosynthesis to stomatal conductance. Therefore, in this study, the response in carbon isotope composition ($\delta^{13}C$), stomatal conductance and photosynthesis rate were analyzed in rice grown in the field and rain shelter under different treatments of K fertilizers. This will allow us to determine whether K fertlizer application ensures improvement in rice plant's physiology.

MATERIALS & METHODS

Planting material

MR 219 rice variety was used as the planting material in this study. This is due to almost 90% of rice cultivated in Malaysia are MR 219 variety, making it the most popular rice variety in Malaysia. It was made from a cross between the MR 137 and MR 151 varieties which was released by the Malaysian Agricultural Research and Development Institute (MARDI) in 2001. MR 219 is known to have a short maturation period (105 – 111 days) and is highly resistant to blast, bacterial leaf blight and brown plant hopper (Elixon *et al.* 2012). It has a soft texture due to the low amylose content and is classified as a long grain grade. With good water management and fertilizer input, MR 219 has the potential to produce up to 10 t/ha yield.

Study area

The study was conducted in the two largest granary areas in Malaysia (KADA and MADA): 1) Alor Bakat, KADA, Kelantan on the east coast of the Peninsula (5.955354 N, 102.332283 E) and 2) PPK Sungai Limau, MADA, Kedah in the west coast of the Peninsula (5.887880 N, 100.439819 E). MADA and KADA are the largest rice granary areas in Malaysia, and the performance of rice in these areas are extremely important as it influences the overall performance of the rice industry.

Meanwhile, a rain shelter experiment was conducted since some of the parameters observed are not statistically different between treatments due to high spatial variability in the field. Thus, rice was also grown in rain shelters using soils from KADA and MADA paddy fields. The topsoil range of 0-15 cm were taken from the paddy fields. The rain shelter study site was at the Faculty of Agriculture UPM, Selangor (2.98334 N, 101.73492 E).

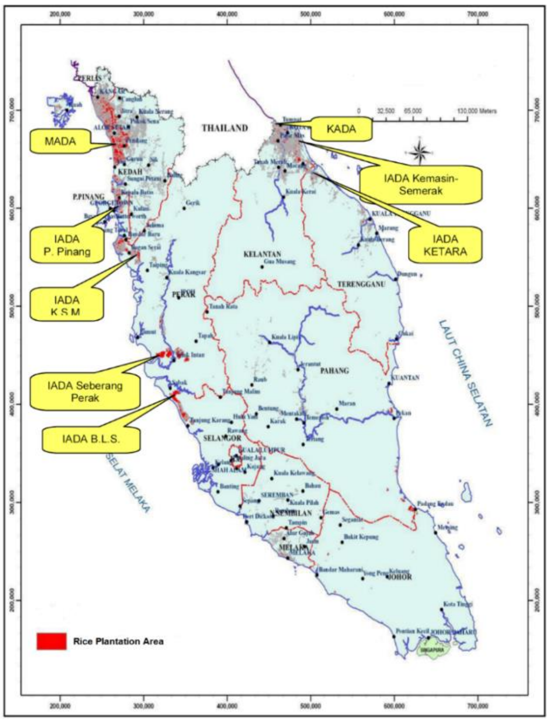

Figure 1: Distribution map of rice production areas in Peninsular Malaysia
Source: DOA Crop Statistic (2015)

Stable Isotopes Determination

Five treatments were applied which are T1) No K, T2) MOP, T3) SOP, T4) Polyhalite and T5) Conventional Fertilizer. The area for each plot in the field study is 4 m^2 (2 m x 2 m), with five replicates. The experimental layout for the field treatment was done using Randomized Complete Block Design (RCBD). The plant samples were analysed for their carbon and nitrogen isotopes using an Isotope Ratio Mass Spectrometer (IRMS) in Jabatan Kimia Malaysia. Three mg of dry samples were weighed into tin capsules and introduced using an autosampler into the elemental analyser. The elements of carbon and nitrogen in samples was combusted into CO_2 and N_2 gas, and then the CO_2 was diluted by a dilutor. After that, carrier gas was channeled into an isotope ratio mass spectrometer. The Carbon stable isotope compositions of each sample was determined by the same analysis.

Leaf Physiology

Measurements of net CO_2 assimilation (A) and stomatal conductance (g_s) were determined using the flag leaves of the representative plants using an open-top portable photosynthesis system (Li-6400XT, LI-COR, Lincoln, Nebraska – USA). Saturating red LED light (1800 μmol m^{-2} s^{-1}) with 10% blue light of the system was used during the measurements. A CO_2 cartridge was also used to supply a constant 400 ppm concentration as the reference line setting in the leaf chamber. During gas exchange measurements, the air temperature and the humidity in the leaf chamber were set to match the current environmental conditions, and the vapor pressure deficit (VPD) was set to 1.8 for consistency purposes. All the physiological parameters (A, and g_s) were taken between 9 am and 12 pm.

Data Analysis

Data obtained from this study were analyzed by one-way ANOVA for analysis of variance and Tukey test for mean comparison using SAS version 9.2 (SAS Institute, Inc., Cary, N.C., USA). Significant differences were calculated by post hoc tests across all methods for all materials using Tukey's HSD at $P < 0.05$.

RESULT AND DISCUSSION

$\delta^{13}C$ Value in Rice Plant Tissue at Different Fertilizer Applications

We evaluated rice plants' $\delta^{13}C$ trough rice leaves with different types of fertilizer under 4 separate experiments. As seen in Table 1, there were no significant differences between the $\delta^{13}C$ value in KADA field and KADA rain shelter experiment while the $\delta^{13}C$ value is significantly different in MADA field and MADA rain shelter. The result in MADA field showed a more positive $\delta^{13}C$ value on T1. This is because of low rate of photosynthesis which is 3.78 µmol CO_2 m^{-2} s^{-1} due to no K fertilizer applied. Meanwhile in MADA rain shelter T2 showed a more positive $\delta^{13}C$ value than in other treatments.

Table 1
$\delta^{13}C$ value of each research plot

Treatment	KADA FIELD (‰)	MADA FIELD (‰)	Rain shelter KADA (‰)	Rain shelterMADA (‰)
No K (T1)	$-29.78^{a} \pm 0.91$	$-28.56^{a} \pm 0.86$	$-31.27^{a} \pm 0.30$	$-29.83^{b} \pm 0.12$
MOP (T2)	$-30.02^{a} \pm 0.28$	$-30.48^{ab} \pm 0.16$	$-30.57^{a} \pm 0.56$	$-29.12^{a} \pm 0.37$
SOP (T3)	$-29.50^{a} \pm 0.25$	$-30.52^{ab} \pm 0.05$	$-30.29^{a} \pm 0.07$	$-29.83^{b} \pm 0.07$
Polyhalite (T4)	$-29.39^{a} \pm 0.11$	$-29.90^{ab} \pm 0.44$	$-30.77^{a} \pm 0.04$	$-29.96^{b} \pm 0.03$
Conventional Fertilizer (T5)	$-28.20^{a} \pm 0.69$	$-31.03^{b} \pm 0.11$	$-30.67^{a} \pm 0.04$	$-29.92^{b} \pm 0.12$

Means followed by the same letter within a column were not significantly different (Tukey's test, at $P>0.05$) ± standard error value

Discrimination against ^{13}C ($\Delta 13C$) occurs during the diffusion of CO_2 through stomata and assimilation by photosynthesis. Similarly, carbon isotope discrimination $\delta^{13}C$ increased with increasing c_a (carbon in the atmosphere). Changes in c_i (internal carbon) can also result in a change in the ^{13}C composition of a plant sample relative to the ^{12}C composition. Ci/Ca represents the balance between the rates of inward CO_2 diffusion (controlled by stomatal conductance) and CO_2 assimilation.

This ratio will differ under conditions of limiting light, poor nutrient status, or other conditions in which CO_2 uptake is enzyme or diffusion-limited (Farquhar et al., 1989). Stomatal conductance is small concerning the capacity for CO_2 fixation, c_i is small and $\delta^{13}C$ becomes less negative, indicating less discrimination against the heavier isotope. Conversely, when stomatal conductance is large, c_i approaches c_a and $\delta^{13}C$ becomes more negative, indicating increased discrimination against $^{13}CO_2$ (Farquhar *et al*., 1982).

In the 1950s, Craig (1953, 1954) measured $\delta^{13}C$ values of a variety of natural materials, including plants. They found that most plants had $\delta^{13}C$ values in the range of -25 to -35‰.

Based on the result, we did get the range of $\delta^{13}C$ value same as the theory stated. Apart from the biological impacts initiated by controlling the photosynthetic rate and stomatal openness, the physical parameters, such as aerodynamic conditions (e.g., atmospheric stability) and the conditions of the soil–atmosphere diffusion systems may also regulate $\delta^{13}C$, although they seem to act as distracters, impairing the relationship between environmental factors and $\delta^{13}C$.

Effects of Potassium fertilizer (K) on stomatal conductance

In principle, increases in stomatal conductance (g_s), which regulates gas exchange (CO_2 and water), can allow plants under well-watered growth conditions to increase their CO_2 uptake and subsequently enhance photosynthesis. However, the relationship between stomatal conductance, CO_2 uptake, and photosynthesis is not so simple. Since a large number of environmental factors affect stomatal aperture, the contribution of stomatal regulation to photosynthesis also can vary depending on the plant species.

The result in Table 2 shows that there are significant differences in MADA field and rain shelter experiments, whereby treatment with no K applied lead to low stomatal conductance among the treatment combinations tested while K fertilizer in the form of polyhalite shows high stomatal conductance compared to other treatments suggesting micronutrients presence in polyhalite could significantly improve leaf physiological performance.

Proper stomatal regulation (opening and closing) is necessary for the uninterrupted production of energy during the photosynthesis process, plant cooling, and water and nutrient transport. In the presence of K^+, stomatal guard cells are swollen by absorbing water followed by stomatal opening and the allowance of gaseous movement in between plants and the environment. Findings by Thomas (2009) highlight the vital role of K in plants provided convincing evidence that K plays a significant role in stomatal opening and closing. Stomatal number and aperture size may also be affected by K nutrition (Pirasteh *et al.* 2016) which would imply better CO_2 diffusion into the leaf and higher rates of photosynthesis.

Table 2
Stomatal conductance at each research plot

Treatment	KADA (mmol m^{-2} s^{-1})	MADA (mmol m^{-2} s^{-1})	Rain Shelter KADA (mmol m^{-2} s^{-1})	Rain shelter MADA (mmol m^{-2} s^{-1})
No K (T1)	$0.75^a \pm 0.07$	$0.05^e \pm 0$	$0.91^e \pm 0$	$1.12^e \pm 0$
MOP (T2)	$1.02^a \pm 0.34$	$0.19^b \pm 0$	$1.65^b \pm 0$	$2.01^b \pm 0$
SOP (T3)	$0.58^a \pm 0.07$	$0.12^d \pm 0$	$1.14^c \pm 0$	$1.77^c \pm 0$
Polyhalite (T4)	$1.01^a \pm 0.06$	$0.23^a \pm 0$	$2.08^a \pm 0$	$2.90^a \pm 0$
Conventional Fertilizer (T5)	$0.48^a \pm 0.11$	$0.15^c \pm 0$	$1.13^d \pm 0$	$1.62^d \pm 0$

Means followed by the same letter within a column were not significantly different (Tukey's test, at P>0.05) ± standard error value

K Fertilizer Effect on Photosynthesis Rate

The result in Table 3 shows significant differences between treatments in both fields KADA and MADA also in the rain shelter experiments of KADA and MADA soil, whereby T1 showed a low photosynthesis rate in paddy leaves respectively compared to other treatments.

This is because stomatal closure is the main limiting factor for the photosynthetic rate in response to alterations in CO_2. Potassium deficiency resulting in reduced stomatal conductance increased the mesophyll resistance and lowered the ribulose-1,5-bisphosphate carboxylase/oxygenase (RuBisCO) activity in plants, which eventually decreased the total photosynthesis rate. Other than that, the basis of the approach is that when K supply is inadequate, the root:shoot ratio will be low because the consequent low concentration of leaf K will impair photosynthate loading into the phloem and translocation to the roots.

Moreover, the result shows that the application of polyhalite resulted in the highest photosynthesis rate among the treatments. This occurs when higher stomatal conductance enhanced CO_2 diffusion into chloroplasts, the photosynthetic activity of plants increases (Kusumi *et al*. 2012). Stomatal aperture, as well as conductance, is strongly correlated with leaf photosynthesis, the decrease in stomatal conductance and the restriction of photosynthetic enzyme activity will eventually lead to an increase in plant $\delta^{13}C$ value (Jia *et al.* 2016; Lavergne et al. 2020). It is generally accepted that in photosynthesizing leaves, stomatal conductance is correlated with photosynthetic rate and coordinated with the CO_2 requirement of the mesophyll, such that the Ci/Ca ratio is maintained at a constant value.

Table 3
Photosynthesis rate of rain shelter experiments

Treatment	KADA FIELD (µmol CO_2 m^{-2} s^{-1})	MADA FIELD (µmol CO_2 m^{-2} s^{-1})	RAIN SHELTER KADA (µmol CO_2 m^{-2} s^{-1})	RAIN SHELTER MADA (µmol CO_2 m^{-2} s^{-1})
No K (T1)	$7.22^{b} \pm 4.14$	$3.78^{d} \pm 0.01$	$38.47^{e} \pm 0.03$	$41.28^{d} \pm 0.06$
MOP (T2)	$11.63^{b} \pm 2.61$	$11.52^{c} \pm 0.01$	$48.65^{b} \pm 0.17$	$53.39^{c} \pm 0.05$
SOP (T3)	$9.92^{b} \pm 3.13$	$13.57^{b} \pm 0.002$	$43.59^{c} \pm 0.05$	$54.17^{b} \pm 0.09$
Polyhalite (T4)	$18.64^{a} \pm 3.53$	$14.49^{a} \pm 0.02$	$57.62^{a} \pm 0.03$	$61.49^{a} \pm 0.26$
Conventional Fertilizer (T5)	$8.85^{b} \pm 4.23$	$11.59^{c} \pm 0.01$	$42.32^{d} \pm 0.04$	$53.50^{c} \pm 0.04$

Means followed by the same letter within a column were not significantly different (Tukey's test, at P>0.05) ± standard error value.

CONCLUSION

This study determined how rice $\delta^{13}C$ is related to whole-plant photosynthesis and stomatal conductance under conditions of different types of K fertilizer. During photosynthesis, plants discriminate against C because of small differences in chemical and physical properties imparted by the difference in mass. This discrimination can be used to assign plants to various photosynthetic groups. The isotope fractionation also reflects limitations on photosynthetic efficiency imposed by the various diffusional and chemical components of CO_2 uptake. All $\delta^{13}C$ values pointed to relatively open stomata for rice as C3 plants. Other than that, improved photosynthesis rate under different types of K fertilizer was achieved by the application of polyhalite fertilizer minimizing water loss through stomata by evapotranspiration and employing phenotypic flexibility and morphological mechanisms of drought avoidance leading to a selective biomass loss.

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