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# Stomatal Morphology, Conductance and Transpiration of *Musa* sp. cv. Rastali in Relation to Magnesium, Boron and Silicon Availability

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## ABSTRACT
A field experiment was conducted to determine the influence of magnesium (Mg), boron (B) and silicon (Si) availabilities on stomatal morphology, stomatal conductance and transpiration rate of Rastali. The experiment was done at Universiti Putra Malaysia under field condition arranged in split-split plot design with four blocks. The main factor was NPK fertilizer recommended by Department of Agriculture Malaysia (DOA) and United Plantation Berhad (UPB). The sub-factors were applications and non-application (as control) of kieserite (Mg), boric acid (B) and sodium silicate (Si). The sub-sub factors were the four Rastali accessions, namely, R08, R62, R34 and R12. In particular, the Berangan accession was selected as the control treatment. The observations were carried out on the stomatal morphology, stomatal conductance and transpiration rate. Data were analyzed with Analysis of Variance (ANOVA) at 5% level and continued with the Least Significant Differences (LSD) if significant. The relationship patterns among the parameters were determined using regression analysis. The findings showed that high-dose applications of NPK fertilizer enhanced stomatal length and width (abaxial and adaxial), stomatal conductance and transpiration rate, with or without Mg, B and Si. Similarly, in low doses, NPK fertilizers added with Mg, B and Si significantly enhanced stomatal length and width, stomatal conductance and transpiration rate. On the contrary, stomatal density (abaxial and adaxial) and ratio were not influenced by NPK fertilizer, Mg-B-Si or their combinations. Moreover, stomatal density and ratio were found to be different among the cultivars (Berangan and Rastali), but similar within the Rastali accessions. In particular, Berangan had a high stomatal density (abaxial and adaxial) compared Rastali, so stomatal ratio became smaller. There were linear relationships between stomatal width, stomatal conductance and transpiration rate, whereby wider stomata were shown to have resulted in higher conductance and transpiration rate.

**Key words:** Stomatal length, width, conductance, transpiration, fertilizers

## INTRODUCTION
During the growing stage of the plants, stomata play an important role in the productivity and quality of crops (Singh *et al*., 2006; Ismail *et al*., 2004). This is related to the role of stomata during photosynthesis (Guo *et al*., 2011; Ilgin and Caglar, 2009). The accumulation of biomass is dependent on the rate and duration of the assimilation of $CO_2$. The stomata mechanism effectively

regulates crop productivity through regulation of water loss and $CO_2$ absorption (Xuan *et al.*, 2011; Kosobryukhov, 2009; Dhir *et al.*, 2001; Allen and Pearcy, 2000). Indeed, the limited activity of stomata is the main factor of crop productivity limitation (Diaz-Espejo *et al.*, 2007; Flexas *et al.*, 2006; Grassi and Magnani, 2005). Stomata have several characteristics which control or determine the rate of photosynthesis, i.e., density, size and stomata conductance (Khazaei *et al.*, 2010). In general, the influence of each character to the photosynthesis rate is indirect, including the roles of stomata in water loss, as well as absorption and sequestering $CO_2$ (Khazaei *et al.*, 2010; Silim *et al.*, 2009; Keel *et al.*, 2007). For example, the frequency and size of stomata have a positive correlation with the rate of water loss (Khazaei *et al.*, 2010). In addition, stomatal density and size have a very close relationship with the efficiency of water use through its influence on stomatal conductance. The large density and size of stomata, together with photosynthesis, produce higher efficiency of water use compared to the small one (Guo *et al.*, 2011; Khazaei *et al.*, 2010; Zhang *et al.*, 2007; Hardy *et al.*, 1995).

Transpiration is controlled by stomatal opening and closing, while water loss and absorption were maintained by transpiration (Ilgin and Caglar, 2009; McDowell *et al.*, 2008; Tricker *et al.*, 2005; Yu *et al.*, 2004; Jones, 1998). It is important to note that there are two environmental factors which are important for stomatal conductance, namely relative humidity and $CO_2$ concentration on the leaf surfaces. Stomatal conductance increases with decreasing relative humidity and $CO_2$ concentration on the leaves surface (Ainsworth and Rogers, 2007; Leakey *et al.*, 2006; Yu *et al.*, 2004; Long *et al.*, 2004; Bunce, 2000). Gas circulation, especially the absorption of $CO_2$, is determined by stomatal density, size and conductance (Xuan *et al.*, 2011; Ilgin and Caglar, 2009). Among the three factors, stomatal conductance is the most dominant. Melgar *et al.* (2008) stated that the decrease in the net photosynthetic rate was caused by low stomatal conductance. The influence of stomatal conductance on the decrease of net photosynthesis rate was found to be related to the disturbance of $CO_2$ absorption (Silim *et al.*, 2009). Meanwhile, low relative humidity is a key factor for the high $CO_2$ absorption (Ichihashi *et al.*, 2008).

Most enzymes are activated by Magnesium (Mg) compared to other mineral nutrients (Epstein and Bloom, 2004). Mg involved in a number of physiological and biochemical processes which affected the growth and development of plant. Mg is also a central atom in chlorophyll structures, therefore, Mg is associated with the development of leaf chlorophyll. One of the initial reactions of Mg deficiency is the imbalance distribution of dry material between roots and shoots that leads to the accumulation of carbohydrates in the leaves. The accumulation of these carbohydrates inhibits the rate of photosynthesis. This phenomenon is known as the feedback effect (Cakmak and Kirkby, 2008; Hermans *et al.*, 2006). The decrease in photosynthetic capacity during Mg deficiency is generally associated with a decrease in stomatal conductance (increase in mesophyll resistance to $CO_2$), decreased enzyme activities that are involved in $CO_2$ fixation and the increased accumulation of carbohydrates (Cakmak and Kirkby, 2008; Hariadi and Shabala, 2004; Laing *et al.*, 2000).

Boron (B) is a micronutrient element that is essential for the growth and development of plant (Shaaban, 2010; Shaaban *et al.*, 2004). Deficiency of B is followed by a decrease in leaf stomatal conductance, net photosynthetic rate and translocation of carbohydrates from the leaves to the fruit (Pinho *et al.*, 2010; Shaaban, 2010; Han *et al.*, 2008; Halder *et al.*, 2007; Shaaban *et al.*, 2006; Huang *et al.*, 2005; Oosterhuis and Zhao, 2001). The decrease in the stomatal conductance in plant with B deficiency is caused by the high oxidative damage in leaves (Huang *et al.*, 2005).

Meanwhile, the low net photosynthesis rate has a close relationship with the decrease in stomatal conductance. In addition, low stomatal conductance also decreases transpiration rate (Han *et al*., 2008).

Silicon (Si) is a beneficial element for monocots. Some experiments carried out on rice and wheat showed that the application of Si had positive effects on plant growth, as well as yield and plant resistance to pests and diseases (Gorecki and Danielski-Busch, 2009; Almeida *et al*., 2009; Ma, 2004; Basagli *et al*., 2003). However, the positive effect of the Si application in the plant is generally not too obvious under optimum conditions, but it is most evident when the plant is under stressed condition (Ahmed *et al*., 2008; Henriet *et al*., 2006; Epstein, 1994). Although, Si has several positive effects on plants, this element has been found to decrease stomatal conductance and transpiration rate. It was associated with the changes in stomatal morphology and density. In addition, Si application increased the concentration of this element in the leaf, especially in the walls of epidermal cells. Silicon accumulates in the cell wall in the form of $SiO_2$ polymers (Zuccarini, 2008; Gao *et al*., 2006; Agarie *et al*., 1998). The accumulation of $SiO_2$ in the epidermal cell wall is expected to increase the strength of cells. The application of Si has also been found to affect the cuticle on the leaf surface. Cuticle is significantly thickened by the addition of Si. Thickening occurs because of the formation of a double-layer cuticle-silica on the surface of epidermal cells. The thickening of the cuticle layer on the leaf surface affects stomatal regulation through restrictions of water conditions in the epidermal cells, such as regulation of water transport in the epidermal system and loss of water from the guard cell complex (Zuccarini, 2008; Al-Rawahy *et al*., 2007; Gao *et al*., 2006; Agarie *et al*., 1998).

The objectives of this study were: (1) to determine the stomatal morphology, conductance and transpiration rate of the *Musa* sp. cv. Rastali (later referred to as Rastali) accessions, (2) to determine stomatal responses of Rastali accessions to the applications of Mg, B and Si and (3) to elucidate the relationship between stomatal morphology, stomatal conductance and transpiration rate of the Rastali accessions in relation to the availability of Mg, B and Si.

## MATERIALS AND METHODS

The experiment was done at Universiti Putra Malaysia on May 2009-May 2010 under field conditions arranged in the split-split plot design with four blocks. The main factors were NPK fertilizer (as recommended by the Department of Agriculture Malaysia, DOA) [N, 660.00 g/plant/planting session, $P_2O_5$, 660.00 g/plant/planting session, $K_2O$, 810.00 g/plant/planting session] and United Plantation Berhad (UPB) [N, 432.00 g/plant/planting session, $P_2O_5$, 180 g/plant/planting session, $K_2O$, 360.00 g/plant/planting session]. The sub-factors were the application and non-application (as a control) of Mg, B and Si. The applications of Mg, B and Si were as follows: Mg, 43.70 g/plant/planting session, B, 0.97 g/plant/planting session and Si, 0.69 g/plant/planting session. N, P, K, Mg, B and Si were soil applied. The sub factors were Rastali accessions, consisting of four accessions, namely; Accessions R08, R12, R34 and R62. These four accessions were chosen because they have different morphological and molecular characters based on the previous studies. A different cultivar, namely *Musa* sp. cv. Berangan (later referred to as Berangan) accession, was included as a control treatment.

In this study, the observations were carried out on the stomatal morphology, stomatal conductance and transpiration rate. All the variables were observed from the mother plant at the middle part of the lamina on the third leaf from the top, at flowering (during the first finger initiation). The stomatal morphology includes the density, length, width and length to width ratio.

Scanning Electron Microscope (SEM) (JEOL model JSM-5610 LV, Datum Ltd, Tokyo, Japan) was used to observe the stomatal morphology at high vacuum acceleration voltage of 15 kV with a working distance of 23 mm. Stomatal morphology was observed from the abaxial and adaxial leaf surfaces. The leaf samples were cut into 1×1 $cm^2$. The samples were then soaked in 70% ethanol for a minimum of 24 h (fixation) and air-dried at room temperature (hydration process). Next, these fixed and hydrated samples were mounted on aluminium stubs, coated with gold twice for two minutes, and finally viewed under the SEM. Stomatal width, stomatal length, stomatal density and stomatal ratio were measured from the SEM micrographs. The stomatal ratio was derived from a comparison between stomatal density at abaxial and adaxial surfaces. Meanwhile, stomatal conductance and transpiration rate were determined using a Porometer (type AP-4, Delta-T Devices Ltd, Cambridge, United Kingdom). The observations were conducted at 10.00-12.00 noon, with light intensity, leaf temperature and relative humidity at 99.62-100.20 kilo lux, 39-40°C and 68-70%, respectively.

**Statistical analysis:** The data were analyzed using the analysis of variance (ANOVA) at 5% level and were continued with the Least Significant Differences (LSD) to determine the interactions between NPK fertilizer, Rastali accessions and the applications of Mg, B and Si on the stomatal morphology, stomatal conductance and transpiration rate of Rastali. Meanwhile, the relationship patterns between the parameters were determined using regression analysis. All the analyses were performed using the General Linear Model Procedure (PROC GLM) (SAS Institute Inc., 1990).

## RESULTS

The statistical analysis showed that there was no interaction among the main factors (NPK fertilizer), sub-factors (Mg, B and Si) and sub-sub factors (Rastali accessions) on the stomatal morphology and conductance and transpiration rate ($p \le 0.05$). Meanwhile, there was an interaction between the main and sub-factor for stomatal morphology (except for stomatal density and ratio), stomatal conductance and transpiration rate ($p \le 0.05$). In general, stomatal morphology was influenced by the interaction between the NPK fertilizer and Mg, B and Si, except for density (abaxial and adaxial surfaces) and stomata ratio ($p \le 0.05$).

Stomatal length and width (abaxial and adaxial surfaces) significantly increased in Rastali accessions and Berangan which had been applied with the NPK fertilizer following the doses suggested by UPB, with the applications of Mg, B and Si (Table 1). Meanwhile, stomatal length and width were not significantly different in Rastali accessions and Berangan which had been applied with the NPK fertilizer following the doses suggested by DOA Malaysia, with or without the applications of Mg, B and Si. This indicated that the applications of Mg, B and Si were not significant at high doses of NPK fertilizer. The dose of NPK in the fertilizer from DOA Malaysia was much higher than UPB.

In contrast, stomatal density and ratio were not influenced by the interactions between NPK fertilizer and Mg, B and Si ($p \le 0.05$). However, stomatal density and ratio were significantly influenced by the cultivars of *Musa* spp., where the numbers of stomata of Berangan on both the leaf surfaces were denser than of Rastali (Table 2). Moreover, there was a different trend in the stomatal ratio, whereby Rastali had a higher ratio compared to Berangan. The significant difference in the stomatal density and ratio only occurred among cultivars, but did not occur within cultivars. It is crucial to note that the Rastali accessions (R08, R12, R34 and R62) had similar stomatal density and ratio.

Table 1: Stomatal morphology, conductance and transpiration rate of Rastali accessions and Berangan in relation to the applications of NPK fertilizer and Mg, B and Si

| Stomatal morphology, conductance and transpiration rate | NPK fertilizer Recommended by DOA | | NPK fertilizer Recommended by UPB | |
|---|---|---|---|---|
| | With Mg, B, Si | Without Mg, B, Si | With Mg, B, Si | Without Mg, B, Si |
| Stomatal density at abaxial surface ($\mu m^{-2}$) | 395.74[a] | 397.16[a] | 396.89[a] | 392.05[a] |
| Stomatal density at adaxial surface ($\mu m^{-2}$) | 210.38[a] | 214.82[a] | 214.21[a] | 218.32[a] |
| Stomatal ratio (abaxial/adaxial surface) | 1.89[a] | 1.85[a] | 1.86[a] | 1.82[a] |
| Stomatal length at abaxial surface (μm) | 37.48[a] | 39.64[a] | 39.01[a] | 26.81[b] |
| Stomatal length at adaxial surface (μm) | 55.46[a] | 59.10[a] | 58.45[a] | 43.56[b] |
| Stomatal width at abaxial surface (μm) | 8.75[a] | 10.94[a] | 10.52[a] | 3.99[b] |
| Stomatal width at adaxial surface (μm) | 10.39[a] | 12.40[a] | 12.31[a] | 6.42[b] |
| Stomatal conductance (cm $sec^{-1}$) | 0.59[ab] | 0.97[a] | 0.89[a] | 0.25[b] |
| Transpiration rate ($\mu mol/m^2$ $sec^{-1}$) | 6145.00[ab] | 9624.00[a] | 8933.00[a] | 3182.00[b] |

Means in the same row, followed by different letters are significantly different, according to LSD test ($p \leq 0.05$)

Table 2: Stomatal morphology, conductance and transpiration rate of Rastali accessions and Berangan

| Stomatal morphology, conductance and transpiration rate | Rastali accessions | | | | |
|---|---|---|---|---|---|
| | R08 | R62 | R34 | R12 | Berangan (as a control) |
| Stomatal density at abaxial surface ($\mu m^{-2}$) | 388.97[b] | 389.96[b] | 389.98[b] | 390.37[b] | 418.02[a] |
| Stomatal density at adaxial surface ($\mu m^{-2}$) | 208.92[b] | 208.59[b] | 207.68[b] | 210.23[b] | 236.74[a] |
| Stomatal ratio (abaxial/adaxial surface) | 1.87[a] | 1.87[a] | 1.88[a] | 1.86[a] | 1.78[b] |
| Stomatal length at abaxial surface (μm) | 35.51[a] | 36.11[a] | 35.61[a] | 34.02[a] | 37.42[a] |
| Stomatal length at adaxial surface (μm) | 54.03[a] | 54.58[a] | 54.15[a] | 52.50[a] | 55.45[a] |
| Stomatal width at abaxial surface (μm) | 8.38[a] | 8.67[a] | 8.63[a] | 7.60[a] | 9.46[a] |
| Stomatal width at adaxial surface (μm) | 10.10[a] | 10.38[a] | 10.67[a] | 9.73[a] | 11.01[a] |
| Stomatal conductance (cm $sec^{-1}$) | 0.67[a] | 0.65[a] | 0.69[a] | 0.58[a] | 0.80[a] |
| Transpiration rate ($\mu mol/m^2$ $sec^{-1}$) | 7898.00[a] | 6959.00[a] | 6908.00[a] | 6330.00[a] | 7898.00[a] |

Means in the same row, followed by different letters are significantly different according to the LSD test ($p \leq 0.05$)

Stomatal conductance was significantly influenced by the interaction between NPK fertilizer with Mg, B and Si ($p \leq 0.05$). Stomatal conductance increased in Rastali accessions and Berangan with an increase in the dose of the NPK fertilizer, with or without Mg, B and Si (Table 1). This was shown by the higher stomatal conductance observed in plants which had been applied with the dosage of NPK fertilizer from DOA (higher dose) compared to the one from UPB (lower dose). The data also showed that the additions of Mg, B and Si did not significantly affect the stomatal conductance of plants which were given high doses of NPK fertilizer. Meanwhile, stomatal conductance was significantly improved at low doses of NPK fertilizer (UPB), with the applications Mg, B and Si. Low doses of NPK fertilizer, in combination with the applications of Mg, B and Si, were found to have improved stomatal conductance that was similar to the effect of high dose of NPK fertilizer. Without the combinations with Mg, B and Si, a low dose of NPK fertilizer decreased stomatal conductance. Meanwhile, stomatal conductance was not affected by the cultivars of *Musa* spp. ($p \leq 0.05$), especially between Rastali and Berangan cultivars. Berangan has a similar stomatal conductance with Rastali (Table 2). The similarity in terms of stomatal conductance did not only occur among the cultivars, but also within the Rastali accessions. The R08, R12, R34 and R62 have similar stomatal conductance.

There was interaction between the NPK fertilizer with Mg, B and Si in terms of transpiration rate ($p \le 0.05$). At higher doses of NPK fertilizer (DOA), however, the applications of Mg, B and Si did not have any significant effect on the transpiration rate (Table 1). Transpiration rate was found to be similar, with or without the applications of Mg, B and Si at high doses of NPK fertilizer (DOA). At low dose of NPK fertilizer (UPB), the combination with Mg, B and Si was shown to have accelerated the transpiration rate, which was similar to the effect of high doses of NPK fertilizer. Meanwhile, the low doses of NPK fertilizer, without any combination of Mg, B and Si, impeded the transpiration rate of Rastali accessions and Berangan.

The transpiration rate also showed the same trend with that of the stomatal conductance. An insignificant impact of the *Musa* spp. cultivars ($p \le 0.05$) was observed. Berangan exhibited similar transpiration rate to that of Rastali (Table 2). The transpiration rate was also similar within the Rastali accessions (namely R08, R12, R34 and R62).

The relationship between stomatal width (abaxial) and stomatal conductance was shown to be linear (Fig. 1). Meanwhile, the regression between stomatal width and stomatal conductance was given by the equation Y = 0.1951X-1.1639. The regression had a positive slope (0.1951), so a rise in the stomatal width will be followed by a rise in the stomatal conductance. The regression between the stomatal width and transpiration rate had the same pattern as that of the stomatal width and stomatal conductance, with the equation given by Y = 2290.1X-11168 (Fig. 2). That equation has a positive slope (2290.1) indicating that an increase in the stomatal width will be followed by an increase in the transpiration rate. Meanwhile, the same trends were found in the regression

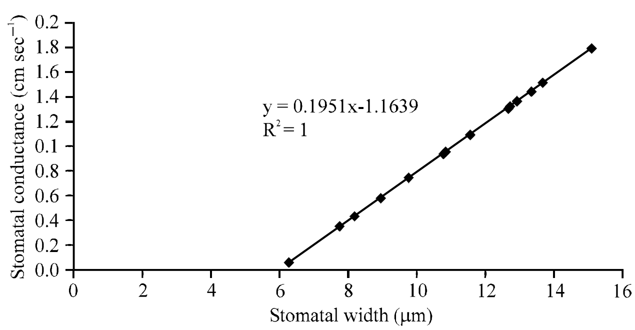


Fig. 1: The relationship between the abaxial stomatal width and stomatal conductance of Rastali accessions and Berangan

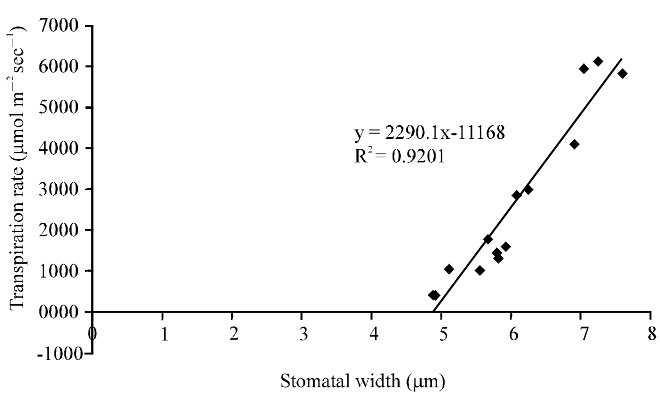


Fig. 2: The relationship between the abaxial stomatal width and transpiration rate of Rastali accessions and Berangan

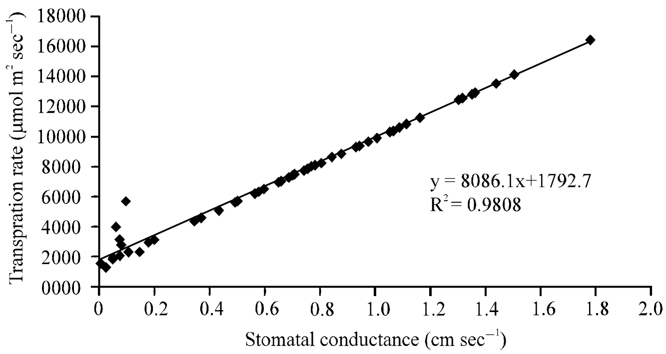


Fig. 3: The relationship between the stomatal conductance and transpiration rate of Rastali accessions and Berangan

between the stomatal conductance and transpiration rate, with the following equation, Y = 8926.4 X+938.15 (Fig. 3). The equation also has a high positive slope (8926.4), suggesting that an increase in the stomatal conductance will be followed by an increase in transpiration rate.

## DISCUSSION

Rastali accessions and Berangan that were applied with a high dose of NPK fertilizer (with or without Mg, B and Si) and low doses of NPK fertilizer (in combination with Mg, B and Si), had longer and wider stomata, higher stomatal conductance and faster transpiration rate compared to Rastali accessions and Berangan exposed to low doses of NPK fertilizer, without any applications of Mg, B and Si. This finding shows that Mg, B and Si have a significant effect at low level of NPK fertilizer. Meanwhile, the addition of Mg, B and Si have no significant effect on the stomatal length and width, stomatal conductance and transpiration rate in the presence of high levels of NPK fertilizer. On the other hand, stomatal density was different between the cultivars (Rastali accessions and Berangan) and not affected by the application of nutrient. This indicates that stomatal density is genetically controlled and morphologically unique as it is not influenced by environmental factors.

Length and width of the stomatal opening, stomatal conductance and transpiration rate were not increased when a high dose of NPK fertilizer (DOA) was applied in combination with Mg, B and Si. Length and width of the stomatal opening, stomatal conductance and transpiration rate were not influenced by the applications of Mg, B and Si at a high dose of NPK fertilizer. As a result of K fertilization, the K concentration in the plant tissues, especially in the guard cells, increased. Potassium is a nutrient that controls the stomatal opening (Kim and Lee, 2007; Cakmak, 2005). Increasing the concentration of K in guard cells decreased the water potential in the tissues, causing the water to enter the guard cells. Water entering the guard cells increases its turgidity and causes them to open. Increased stomatal opening was then followed by an increase in the stomatal conductance, which in turn accelerated the transpiration rate. Hence, it is clear that at high doses of NPK fertilizer, K has a direct role in controlling the activity of stomata compared to N and P. This is in line with the results of some previous studies reported by Kim and Lee (2007) and Cakmak (2005).

At low levels of NPK (UPB), an application of Mg directly enhanced the role of N, particularly in the synthesis of chlorophyll. Indirectly, the application of Mg improved plant efficiency with the use of N, due to increased plant N uptake (Mostafa *et al.*, 2007). Although, N was given at a low dose, the plant could still use it optimally because of the improved N uptake by roots. The plants which were applied with low N levels remained capable of synthesize chlorophyll at normal amounts. It was observed that the leaves with normal chlorophyll content absorbed solar radiation optimally and stimulated the stomata to open wider. Once the stomata have wider openings, both the conductance and transpiration rate will also be increased. In addition, the application of Mg at low level of N increased the carbohydrate translocation from leaves to other organs such as roots, fruits and seeds (Cakmak and Kirkby, 2008; Hermans *et al.*, 2006; Hermans and Verbruggen, 2005). Increased translocation prevented the accumulation of carbohydrate in the leaves, which could inhibit photosynthesis (feedback effect). Therefore, the application of Mg would ensure the process of photosynthesis (Upadhyay and Patra, 2011; Choi and Latigui, 2008) and thus keeping stomatal conductance and transpiration rate at a high level.

The application of Boron with low levels of N, P and K has the same response as Mg, i.e., an increase in the photosynthesis rate. Increasing photosynthesis rate has a close relationship with high stomatal conductance in the presence of an adequate supply of B (Han *et al.*, 2008; Oosterhuis and Zhao, 2001). Huang *et al.* (2005) stated that B deficiency induced stomatal disfunction and sensitivity to photo-oxidative damage on leaf cells. Meanwhile, high stomatal conductance was associated with longer and wider stomatal openings on plants with adequate level of B. High stomatal conductance was also followed by increased transpiration rate. This indicates that the application of B in the plant has affected or altered the morphology of the stomata, particularly its length and width. Stomata became longer and wider due to increased deposit of B in cell walls, especially the guard cells (Shaaban, 2010). This is in line with Fernandez *et al.* (2008), who declared that the deficiency of minerals, especially micro-elements would alter the morphology and anatomy of leaf surface in particular cuticle and stomata.

Besides high stomatal conductance, increased photosynthesis rate was also supported by increased leaf chlorophyll content (Pinho *et al.*, 2010). Previous research showed that the application of B on banana increased the chlorophyll content (Pinho *et al.*, 2010; Han *et al.*, 2008). The increase in the leaf chlorophyll content stimulated the stomata to open wider, so that gasses could be circulated well, specifically the flow of $CO_2$ from the environment into the leaf to be used in photosynthesis.

In relation to the applications of Mg, B and Si, specifically from the aspect of Si, the results obtained from this study seem to be different from the findings of previous studies (Zuccarini, 2008; Gao *et al.*, 2006; Agarie *et al.*, 1998). In fact, the applications of Si on Rastali accessions and Berangan were actually found to have increased the length and width of the stomatal opening, stomatal conductance and transpiration rate, particularly at low doses of NPK fertilizer. Meanwhile, the results of several previous studies showed that the application of Si on rice, wheat and barley decreased the length and width of stomatal opening, stomatal conductance and transpiration rate (Zuccarini, 2008; Gao *et al.*, 2006; Agarie *et al.*, 1998). Therefore, the application of Si is often used to increase plant resistance to drought because of the increase in water use efficiency. The application of Si was found to increase water use efficiency as a result of the following: (1) Si reduced the length and width of stomatal openings, (2) It decreased stomatal conductance and (3) It slowed down the transpiration rate. The length and width of the stomatal opening decreased through the application of Si because of its deposition in the leaves, especially the guard cells (Morikawa and Saigusa, 2004). The deposition of Si in the guard cells reinforced the guard cells, while the thickening of the guard cells automatically reduced the length and width of stomatal opening.

The applications of Si in Rastali gave a different response compared to its applications in rice, wheat and barley. As it is known that Rastali is a triploid plant with AAB genome, whereas, rice, wheat and barley are generally diploid. There are differences in the stomatal characteristics between diploid and triploid plants. The stomatal density decreased but the size increased with the increasing levels of ploidy (Khazaei *et al.*, 2010; Aryavand *et al.*, 2003; Rajendra *et al.*, 1978). Therefore, Rastali has lower stomatal density although it is bigger in size compared to rice, wheat and barley. These characteristics have caused different responses of Rastali towards the Si applications, compared to rice, wheat and barley.

There was a relationship between stomatal width and conductance, stomatal width and transpiration rate and stomatal conductance with transpiration rate. All these relationships have a positive slope. Wider stomatal openings are highly conductive for gas exchange and water vapour (transpiration). It is logical because with wider opening, the way which could be passed by both gas and water vapour would also be larger and facilitate a smoother exchange of gasses and water vapour. This is in line with the results of the previous studies by several researchers who have shown positive correlation between the width of stomatal opening, stomatal conductance and transpiration rate (McDowell *et al.*, 2008; Keel *et al.*, 2007; Tricker *et al.*, 2005; Jones, 1998). As for the transpiration rate, the data from previous studies have also revealed that the majority of transpiration is dependent on the condition of the stomata, i.e., disturbances on the stomata are directly followed by a decrease in the transpiration rate (Ilgin and Caglar, 2009). This interference causes the closure of stomata and decreases stomatal conductance. The findings of the previous studies have also indicated that in the long run, the increase or decrease in transpiration is largely determined by a wider opening and conductance of stomata, rather than its density (Tricker *et al.*, 2005). Transpiration also takes place through the cuticle, other than through the stomata, however, this only occurs in very small proportions. Therefore, transpiration rate remains high as long as stomata open wider to the optimal conductance, despite the resistance by the cuticle.

## CONCLUSION

The results showed that high-dose applications of NPK fertilizer enhanced stomatal length and width (abaxial and adaxial), stomatal conductance and transpiration rate, with or without Mg, B and Si. Similarly, in low doses, NPK fertilizers added with Mg, B and Si significantly enhanced stomatal length and width, stomatal conductance and transpiration rate. On the contrary, stomatal density (abaxial and adaxial) and ratio were not influenced by NPK fertilizer, Mg-B-Si or their combinations. Moreover, stomatal density and ratio were found to be different among the cultivars (Berangan and Rastali), but similar within the Rastali accessions. In particular, Berangan had a high stomatal density (abaxial and adaxial) compared Rastali, so stomatal ratio became smaller. There were linear relationships between stomatal width, stomatal conductance and transpiration rate, whereby wider stomata were shown to have resulted in higher conductance and transpiration rate.

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**REFERENCES**

Agarie, S., H. Uchida, W. Agata, F. Kubota and P.B. Kaufman, 1998. Effects of silicon on transpiration and leaf conductance in rice plants (*Oryza sativa* L.). Plant Prod. Sci., 1: 89-95.

Ahmed, A.H.H., E.M. Harb, M.A. Higazy and S.H. Morgan, 2008. Effect of silicon and boron foliar applications on wheat plants grown under saline soil conditions. Int. J. Agric. Res., 3: 1-26.

Ainsworth, E.A. and A. Rogers, 2007. The response of photosynthesis and stomatal conductance to rising $CO_2$: Mechanisms and environmental interactions. Plant Cell Environ., 30: 258-270.

Al-Rawahy, S.H., H. Al-Amri, A. Al-Hinai and N. Sherwani, 2007. Growth and stomatal conductance of *Prosopis cineraria* (Ghaff Tree) exposed to sulphur dioxide. Asian J. Plant Sci., 6: 673-677.

Allen, M.T. and R.W. Pearcy, 2000. Stomatal behavior and photosynthetic performance under dynamic light regimes in a seasonally dry tropical rain forest. Oecologia, 122: 470-478.

Almeida, G.D., D. Pratissoli, J.C. Zanuncio, V.B. Vicentini, A.M. Holtz and J.E. Serrao, 2009. Calcium silicate and organic mineral fertilizer increase the resistance of tomato plants to *Frankliniella schultzei*. Phytoparasition, 37: 225-230.

Aryavand, A., B. Ehdaie, B. Tran and J.G. Waines, 2003. Stomatal frequency and size differentiate ploidy levels in *Aegilops neglecta*. Genet. Resour. Crop Evol., 50: 175-182.

Basagli, M.A.B., J.C. Moraes, G.A. Carvalho, C.C. Ecole and R.C.R. Goncalves-Gervasio, 2003. Effect of sodium silicate application on the resistance of wheat plants to the green-aphids *Schizaphis graminum* (Rond.) (Hemiptera: Aphididae). Neotropical Entomol., 32: 659-663.

Bunce, J.A., 2000. Contrasting effects of carbon dioxide and irradiance on the acclimation of photosynthesis in developing soybean leaves. Photosynthetica, 38: 83-89.

Cakmak, I., 2005. The role of potassium in alleviating detrimental effects of abiotic stresses in plants. J. Plant Nutr. Soil Sci., 168: 521-530.

Cakmak, I. and E.A. Kirkby, 2008. Role of magnesium in carbon partitioning and alleviating photooxidative damage. Physiol. Plant., 133: 692-704.

Choi, J.M. and A. Latigui, 2008. Effect of various magnesium concentrations on the quantity of chlorophyll of 4 varieties of strawberry plants (*Fragaria ananassas* D.) cultivated in inert media. J. Agron., 7: 244-250.

Dhir, B., Mahmooduzzafar, T.O. Siddiqi and M. Iqbal, 2001. Stomatal and photosynthetic responses of *Cichorium intybus* leaves to sulfur dioxide treatment at different stages of plant development. J. Plant Biol., 44: 97-102.

Diaz-Espejo, A., E. Nicolas and J.E. Fernandez, 2007. Seasonal evolution of diffusional limitations and photosynthetic capacity in olive under drought. Plant Cell Environ., 30: 922-933.

Epstein, E., 1994. The anomaly of silicon in plant biology. Proc. Nat. Acad. Sci., 91: 11-17.

Epstein, E. and A.J. Bloom, 2004. Mineral Nutrition of Plants: Principles and Perspectives. 2nd Edn., Sinauer Associates, Sunderland, pp: 400.

Fernandez, V., T. Eichert, V. Del Rio, G. Lopez-Casado and J.A. Heredia-Guerrero *et al*., 2008. Leaf structural changes associated with iron deficiency chlorosis in field-grown pear and peach: Physiological implications. Plant Soil, 311: 161-172.

Flexas, J., M. Ribas-Carbo, D.T. Hanson, J. Bota, B. Otto and J. Cifre *et al*., 2006. Tobacco aquaporin NtAQP1 is involved in mesophyll conductance to $CO_2$ *in vivo*. The Plant J., 48: 427-439.

Gao, X., C. Zou, L. Wang and F. Zhang, 2006. Silicon decreases transpiration rate and conductance from stomata of maize plants. J. Plant Nutr., 29: 1637-2647.

Gorecki, R.S. and W. Danielski-Busch, 2009. Effect of silicate fertilizers on yielding of greenhouse cucumber (*Cucumis sativus* L.) in container cultivation. J. Elementol., 14: 71-78.

Grassi, G. and F. Magnani, 2005. Stomatal, mesophyll conductance and biochemical limitations to photosynthesis as affected by drought and leaf ontogeny in ash and oak trees. Plant Cell Environ., 28: 834-849.

Guo, P., X. Xia And W.L. Yin, 2011. A role for stomata in regulating water use efficiency in *Populus* x *euramericana* and characterization of a related gene, *PdERECTA*. Afr. J. Biotechnol., 10: 6904-6912.

Halder, N.K., M.A. Hossain, M.A. Siddiky, N. Nasreen and M.H. Ullah, 2007. Response of wheat varieties to boron application in calcareous brown floodplain soil at southern region of Bangladesh. J. Agron., 6: 21-24.

Han, S., L.S. Chen, H.X. Jiang, B.R. Smith, L.T. Yang and C.Y. Xie, 2008. Boron deficiency decreases growth and photosynthesis, and increases starch and hexoses in leaves of citrus seedlings. J. Plant Physiol., 165: 1331-1341.

Hardy, J.P., V.J. Anderson and J.S. Gardner, 1995. Stomatal characteristics, conductance ratios, and drought-induced leaf modifications of semiarid grassland species. Am. J. Bot., 82: 1-7.

Hariadi, Y. and S. Shabala, 2004. Screening broad beans (*Vicia faba*) for magnesium deficiency. II. Photosynthetic performance and leaf bioelectrical responses. Funct. Plant Biol., 31: 539-549.

Henriet, C., X. Draye, L. Oppitz, R. Swennen and B. Delvaux, 2006. Effects, distribution and uaptake of silicon in banana (*Musa* spp.) under controlled conditions. Plant Soil, 287: 359-374.

Hermans, C. and N. Verbruggen, 2005. Physiological characterization of Mg deficiency in *Arabidopsis thaliana*. J. Exp. Bot., 56: 2153-2161.

Hermans, C., J.P. Hammond, P.J. White and N. Verbruggen, 2006. How do plants respond to nutrient shortage by biomass allocation? Trends Plant Sci., 11: 610-617.

Huang, L., Y. Zhengqian, W.R. Bell and B. Dell, 2005. Boron nutrition and chilling tolerance of warm climate crop species. Ann. Bot., 96: 755-767.

Ichihashi, S., T. Higuchi, H. Shibayama, Y. Tesima, Y. Nishiwaki and K. Ota, 2008. Aspects of CO2 uptake in the crassulacean acid metabolism orchid *Phalaenopsis*. Acta Hortic., 766: 245-256.

Ilgin, M. and S. Caglar, 2009. Comparison of leaf stomatal features in some local and foreign apricot (*Prunus armeniaca* L.) genotypes. Afr. J. Biotechnol., 8: 1074-1077.

Ismail, M.R., M.K. Yusoff and M. Mahmood, 2004. Growth, water relations, stomatal conductance and proline concentration in water stressed banana (*Musa* sp.) plants. Asian J. Plant Sci., 3: 709-713.

Jones, H.G., 1998. Stomatal control of photosynthesis and transpiration. J. Exp. Bot., 49: 387-398.

Keel, S.G., S. Pepin, S. Leuzinger and C. Korner, 2007. Stomatal conductance in mature deciduous forest trees exposed to elevated CO2. Trees, 21: 151-159.

Khazaei, H., P. Monneveux, S. Hongbo and S. Mohammady, 2010. Variation for stomatal characteristics and water use efficiency among diploid, tetraploid and hexaploid Iranian wheat landraces. Genet. Resour. Crop Evol., 57: 307-314.

Kim, D.J. and J.S. Lee, 2007. Current theories for mechanism of stomatal opening: Influence of blue light: Mesophyll cells and sucrose. J. Plant Biol., 50: 523-556.

Kosobryukhov, A.A., 2009. Activity of the photosynthetic apparatus at periodic elevation of CO2 concentration. Russ. J. Plant Physiol., 56: 6-13.

Laing, W., D. Greer, O. Sun, P. Beets, A. Lowe and T. Payn, 2000. Physiological impacts of Mg deficiency in *Pinus radiata*: Growth and photosynthesis. New Phytol., 146: 47-57.

Leakey, A.D.B., M. Uribelarrea, E.A. Ainsworth, S.L. Naidu, A. Rogers, D.R. Ort and S.P. Long, 2006. Photosynthesis, productivity and yield of maize are not affected by open-air elevation of CO2 concentration in the absence of drought. Plant Physiol., 140: 779-790.

Long, S.P., E.A. Ainsworth, A. Rogers and D.R. Ort, 2004. Rising atmospheric carbon dioxide: Plants FACE the future. Ann. Rev. Plant Biol., 55: 591-628.

Ma, J.F., 2004. Role of silicon in enhancing the resistance of plants to biotic and abiotic stresses. Soil Sci. Plant Nutr., 50: 11-18.

McDowell, N., W.T. Pockman, C.D. Allen, D.D. Breshears and N. Cobb *et al*., 2008. Mechanisms of plant survival and mortality during drought: Why do some plants survive while others succumb to drought? New Phytol., 178: 719-739.

Melgar, J.C., J.P. Syvertsen and F. Garcia-Sanchez, 2008. Can elevated $CO_2$ improve salt tolerance in olive trees? J. Plant Physiol., 165: 631-640.

Morikawa, C.K. and M. Saigusa, 2004. Mineral composition and accumulation of silicon in tissues of brueberry (*Vaccinum corybosus* cv. Bluecrop) cuttings. Plant Soil, 258: 1-8.

Mostafa, E.A.M., M.M.S. Saleh and M.M.M. Abd-El-Migeed, 2007. Response of banana plants to soil and foliar applications of magnesium. Am. Eurasian J. Agric. Environ. Sci. 2: 141-146.

Oosterhuis, D.M. and D. Zhao, 2001. Effect of Boron Deficiency on the Growth and Carbohydrate Metabolism of Cotton. In: Plant Nutrition-Food Security and Sustainability of Agro-ecosystems, Horst, W.J., M.K. Schenk, A. Burkert, N. Claassen and H. Flessa *et al*., (Eds.). Kluwer Academic Publishers, London, pp:166-167.

Pinho, L.G.R, E. Campostrini, P.H. Monnerat, A.T. Netto, A.A. Pires, C.R. Marciano and Y.J.B Soares, 2010. Boron deficiency affects gas exchange and photochemical efficiency (JPI test parameters) in green dwarf coconut. J. Plant Nutr., 33: 439-451.

Rajendra, B.R., K.A. Mujeeb and L.S. Bates, 1978. Relationships between *2X hordeum* sp., *2X Secale* sp. and *2X, 4X, 6X triticum* spp. for stomatal frequency, size and distribution. Environ. Exp. Bot., 18: 33-37.

SAS Institute Inc., 1990. SAS/STAT Users Guide. SAS Publishing, North Caroline.

Shaaban, M.M., M.M. El-Fouly and A.W.A. Abdel-Maguid, 2004. Zinc-boron relationship in wheat plants grown under low or high levels of calcium carbonate in the soil. Pak. J. Biol. Sci., 7: 633-639.

Shaaban, M.M., F.E. Abdalla, E.A.A. Abou El-Nour and A.M. El-Saady, 2006. Boron/Nitrogen interaction effect on growth and yield of faba bean plants grown under sandy soil conditions. Int. J. Agric. Res., 1: 322-330.

Shaaban, M.M., 2010. Role of boron in plant nutrition and human health. Am. J. Plant Physiol., 5: 224-240.

Silim, S., R. Nash, D. Reynard, B. White and W. Schroeder, 2009. Leaf gas exchange and water potential responses to drought in nine poplar (*Populus* spp.) clones with contrasting drought tolerance. Trees Struct. Funct., 23: 959-969.

Singh, V.K., D.K. Singh and S.M. Pathak, 2006. Relationship of leaf and fruit transpiration rates to the incidence of softening of tissue in mango (*Mangifera indica* L.) cultivars. Am. J. Plant Physiol., 1: 28-33.

Tricker, P.J., H. Trewin, O. Kull, G.J. Clarkson and E. Eensalu *et al*., 2005. Stomatal conductance and not stomatal density determines the long-term reduction in leaf transpiration of poplar in elevated $CO_2$. Oecologia, 143: 652-660.

Upadhyay, R.K. and D.D. Patra, 2011. Influence of secondary plant nutrients (Ca and Mg) on growth and yield of chamomile (*Matricaria recutita* L.). Asian J. Crop Sci., 3: 151-157.

Xuan, X., Y. Wang, S. Ma and X. Ye, 2011. Comparisons of stomatal parameters between normal and abnormal leaf of *Bougainvillea spectabilis* Willd. Afr. J. Biotechnol., 10: 6973-6978.

Yu, Q., Y. Zhang, Y. Liu and P. Shi, 2004. Simulation of the stomatal conductance of winter wheat in response to light, temperature and CO2 changes. Ann. Bot., 93: 435-441.

Zhang, Z.B., H.B. Shao, P. Xu, L.Y. Chu, Z.H. Lu and J.Y. Tian, 2007. On evolution and perspectives of bio-watersaving. Biointerfaces, 55: 1-9.

Zuccarini, P., 2008. Effects of silicon on photosynthesis, water relations and nutrient uptake of *Phaseolus vulgaris* under NaCl stress. Biol. Plant., 52: 157-160.