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Essential role of microelements in pollen germination and pollen tube growth of *Meistera muricarpa* (Elmer) Škorničk. & MF Newman

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Abstract

Determination of microelements essential for pollen viability is important for it serves as a key for successful germination and propagation of species, particularly for threatened species. Thus, this study was conducted to determine the microelements that are crucial to *Meistera muricarpa*'s pollen germination and tube growth. The pollen grains were subjected into five different treatments, namely: T_1 (No KCl); T_2 (No $MgSO_4$); T_3 (No H_3BO_3); T_4 (No $Ca(No_3)_2$), and T_5 (control). Afterward, a hanging-drop method was carried out; samples were then observed for pollen germination and tube growth for ten hours with one-hour interval. Results revealed that the highest average percentage of pollen germination (PPG) and the longest pollen tube growth (PTG) were observed on T_4 (17%; 72µm), attained at the 8th hour of the observation; while the lowest average percentage of germination and PTG were observed on T_3 (4%; 33µm) on the 9th hour. As for the statistical analysis, the PPG in T_1 to T_3 was noted to be significantly different from the control medium while the result in T_4 shows the opposite. While for the PTG, the results on T_2 to T_4 were revealed to have a significant difference against the control medium. Results imply that the microelements Boron and Magnesium in a form of Boric acid and Magnesium Sulfate are the most important microelements for *M. muricarpa*'s pollen germination and pollen tube growth.

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Introduction

Pollen grains are simple structured plant cells (Olgun and Dalgic, 2004) that house the male gametophyte generation of angiosperms, and play a vital role in plant breeding program (Patel and Mankad, 2012). In recent years, this simple structured cell has been used as a subject for an array of experiments through pollen tube formation for the advancement of plant developmental biology. This is for the reason that pollen tube formation and germination is a good and simple model of growth and development (Taylor and Hepler, 1997) and considered important research materials for morphological, physiological, biotechnological, ecological, evolutional, biochemical, and molecular biological studies (Ottayio *et al.*, 1992). Also, nowadays, it is used as materials for determining the importance of the cytoskeleton in cell growth and differentiation (Ma *et al.*, 2000). Studies on *in vitro* pollen germination and pollen tube growth are considered important also for understanding the fertilization and seed formation in flowering plants and deemed very useful for explaining any lack of plant fertility (Buyukkartal, 2003; Mendez and Acma, 2018).

The plant family Zingiberaceae (Gingers) is one of the diverse taxa in the angiosperm. Most species in this group are primarily used as medicine and spices for food especially in the Southeast Asian region (Waki *et al.*, 2013). In the Philippines, the group is represented in 14 genera with 107 species (Pelser *et al.*, 2011; Naive *et al.*, 2017). These plants are usually utilized for various applications such as food, spices, medicines, and ornaments. Among these species is *Meistera muricarpa* (Elmer) Škorničk. & M.F.Newman - a Philippine endemic ginger which is locally known as "Biraw" (Boer *et al.*, 2018; Dalisay *et al.*, 2018). This species has been reported to be an economically important species with various usage that cut-across the domain of cultural practices (Acma, 2010; Dalisay *et al.*, 2018) and reported to have a potential for pharmaceutics (Barbosa *et al.*, 2016). However, due to its demand in the different perspective in the community, the existence of this species could be compromised due to overharvesting and various environmental threats that are exemplified by habitat loss that is evident in the country (Sinha, 2013).

Its importance on the economic and medical aspect as well on its ecological facet serves as the reason for pursuing this study. Aside from the fact that *in vitro* germination and tube growth of pollen on gingers is not well established especially in the Philippines. Thus, this study was conducted with the aim to determine the significant factors – the microelements in particular that are essential to the pollen germination and tube growth of *M. muricarpa*; and provide further understanding on the developmental perspective of the species to be utilized for the advancement of the propagation and conservation of the species.

Materials and methods

The experimental procedures that include the preparation of stock solutions and sample processing were based on the modified procedures by Moctezuma (2008).

Preparation of Stock Solutions and Sample Collection

A 20ml stock solutions of 0.5M Potassium Chloride (KCl), 0.1M Magnesium Sulfate ($MgSO_4$), 1% Boric Acid (H_3BO_3), 0.5M Calcium Nitrate ($Ca(NO_3)_2$), 0.5M Potassium Hydroxide (KOH), and 10% Sucrose solution were prepared. From these solutions, five treatment media with a volume of 40ml per treatment were made. The treatments were labeled as T_1 (No KCl), T_2 (w/o $MgSO_4$), T_3 (w/o H_3BO_3), T4 (w/o $Ca(No_3)_2$), and lastly T_5 (complete: contains all the nutrients and served as a Control Media). Since the study was delimited on the botanical expedition, sample flowers of *M. muricarpa* were taken from the conservation garden (*ex-situ* conservation) of Acma's residence located at Musuan, Maramag, Bukidnon positioned at 7°51'31.79" N, 125°03'1.80" E. The samples were placed in a sealed moistened plastic container to avoid desiccation. Afterward, it was brought to the processing area of the Center for Biodiversity Research and Extension in Mindanao (CEBREM), Central Mindanao University for experimental procedures.

Experimental Procedures: Sample Processing and Modified Hanging Drop Method

The freshly collected flowers of *M. muricarpa* were examined under the dissecting microscope to carefully

locate the stamen. Then, the pollen grains were removed from the anther of the flower using forceps and plucked it towards the surface of the glass slide (1 sample/ slide). Afterward, it was added with a drop of treatment media and mixed (1 medium/ sample slide). The glass slides were then slowly flipped (treatment media with pollen) and placed on the top of the concave lid (with drops of the same treatment medium) sealing and letting the pollen to simply hang. After the abovementioned procedure, the pollen germination and pollen tube growth were observed for 10 hours with 1-hour interval (per slide) at the maintained temperature of 22^{0}C. A total of 50 pollen grains were examined and observed per slide per treatment under the light compound microscope. For this study, a total of 5 replicates per treatment were considered.

Data Analysis and Statistical Treatments

Pollen Germination Rate (PGR) was determined by counting the actual number of pollen grains that germinated after the contact with the media. The total was divided with the total number of pollens observed and multiplied by 100. For the calculation of the Average Pollen Germination Rate (APGR), the PGR of pollen grains under the same treatment of individual replicates was added and it was divided by the total number of sample replicates. For the Pollen Tube Growth (PTG) it was measured under the microscope with a calibrated micrometer eyepiece. After determining the PTG, the Average Pollen Tube Growth (APTG) was calculated by adding the PTG of the pollens under the same treatment and it was divided by the total number of replicates. Analysis of Variance Single Factor (One-way ANOVA) and Tukey's Honest Significance Test (HSD Test) were considered for the results gathered on the final observation (10th hour) to determine if there were a significant difference between the treatments result mean and point out which among the treatments are significantly different.

Results and discussion

Pollen germination and pollen tube growth were determined and found out that pollen grains of *M. muricarpum* subjected in all treatment media had a general slow response. The germination rate ranges only from 0 to 17% (Fig. 1A) with its pollen tube growth ranges from 0 to 72µm (Fig. 1B).

This entail that the physicochemical requirements of the pollen of *M. muricarpa* such as the concentration of nutrients in the media, level of pH, temperature, and other parameters were not totally met. This is in relation to the slow enzymatic activities observed all throughout the observations for all the replicates in relation to the response of pollen to the media. This is in consonance with Vasil (1960); Baker and Baker (1979); and Dane *et al.* (2004) that concentration of media used for pollen germination varies according to the plant species, thus, need more complicated media (Cetin *et al.*, 2000). This is further supported by Khan and Peerven (2006) that pollen grains of different plants require a varying range of growth media like water, sugar solution, inorganic salts, and vitamins for successful germination. In addition, the pH and temperature of the growth medium are two important factors that significantly affect germination and growth (Boavida and McCormick, 2007; Chebli and Geitmann, 2007; Ali, 2012). With this result, it could be derived also that even the medium that has all the nutrients necessary for pollen germination, in general, does not guarantee for this species to germinate successfully. Thus, modifications on the medium must be considered to test further the pollen germination activity of the species. Meanwhile, the highest average germination rate was observed in T_4(w/o $Ca(No_3)_2$) at the 9th and 10th hours of observation with 17% of the total pollen grains examined. The T_4 has also the longest average pollen tube growth with 72µm noted at the same time of observation. This is then followed in decreasing order with T_5 (complete) 16% and 63µm, T_1 (w/o KCl) 11% and 63µm, T_2 (w/o $MgSO_4$) 6% and 35µm, and T_3 (w/o H_3BO_3) 4% and 33µm during the observation. This implies that among the nutrients, Boron and Magnesium in the form of Boric acid and Magnesium Sulfate are the most valuable nutrients for *M. muricarpa*. It is in the sense that when these nutrients are absent, the tendency of *M. muricarpa*'s pollen to germinate were observed to have a significant decrease as compared with the other media and in reference to the control medium (Fig. 1: A and B).

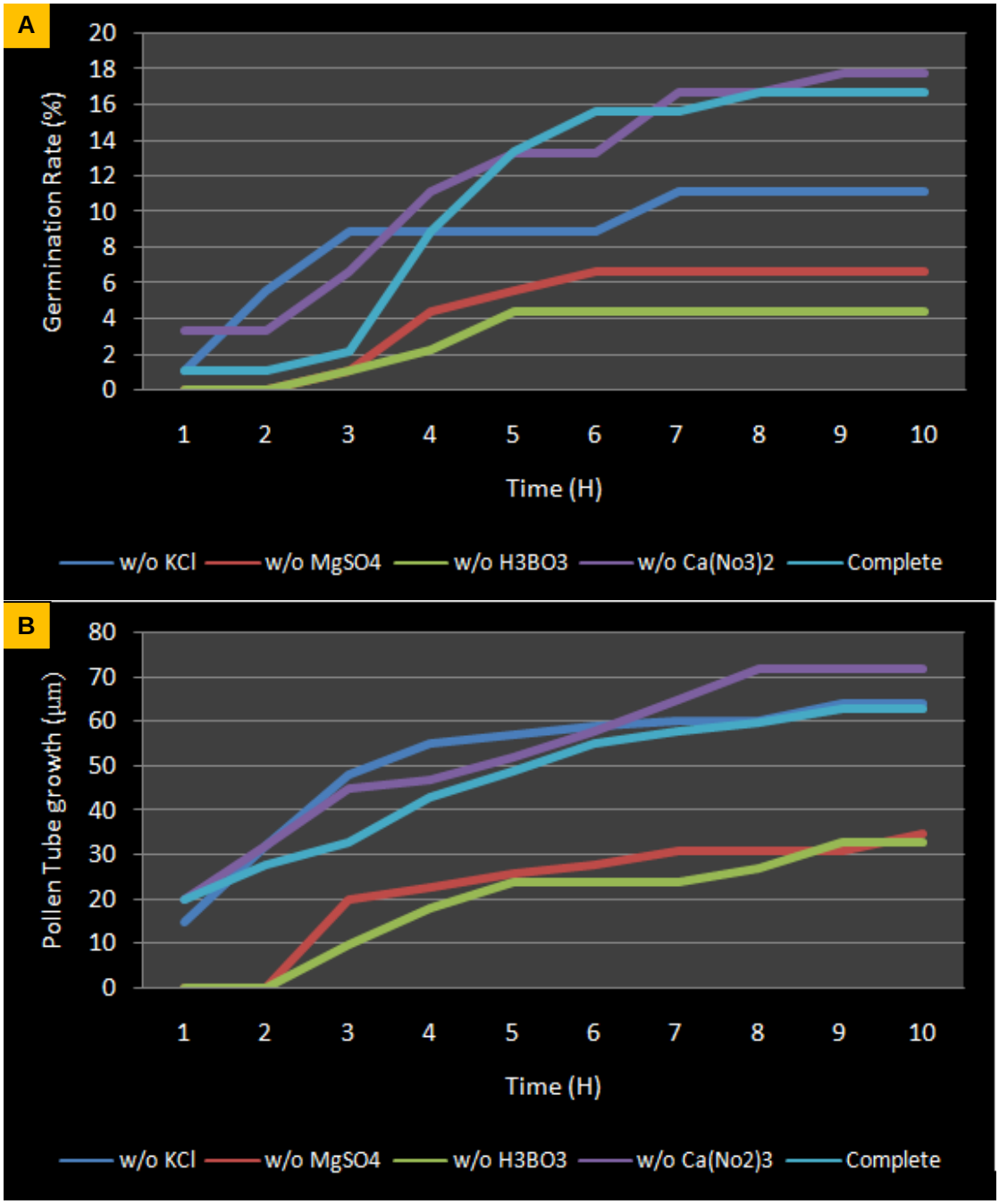

Fig. 1. Performance of *Meistera muricarpa* Elmer in five media (treatments) in relation to Pollen Germination (A) and Pollen Tube Growth formation (B).

To test if the means (results) are significantly different from each media and support the claim as mentioned above, One-way ANOVA was performed and revealed that the sample mean of the five treatments in pollen germination were significantly different with an F statistic of 203.55 against the F critical value of 2.86 at 5% (α=0.05) level of significance (Table 1). The Tukey's Honest Significance Test (HSD Test) were carried out as well to point-out which among the treatments showed significant difference with each other particularly against the control medium (Table 2). Results revealed that among the treatments 1 to 4, only treatment 4 showed no significant difference to the control medium on PPG with an absolute difference of 1.2 against the critical range of 1.75. This suggests that even though the nutrient Calcium is absent, pollen germination of *M. muricarpa* is not totally affected.

Table 1. One-way Analysis of Variance of Percentage of Pollen germination (PPG) of Treatment Media.

ANOVA						
Source of Variation	SS	df	MS	F	P-value	F crit.
Between Groups	700.24	4	175.06	203.55	6.75×10^{-16}	2.86
Within Groups	17.2	20	0.86			
Total	717.44	24				

Table 2. Tukey's Honest Significance Test (HSD Test) on Percentage of Pollen Germination (PPG).

Comparison	Absolute difference	Critical Range	Remarks
T^1-T^2	4.6	1.75	Mean is significantly different
T^1-T^3	6.8	1.75	Mean is significantly different
T^1-T^4	6.6	1.75	Mean is significantly different
T^1-T^5	5.4	1.75	Mean is significantly different
T^2-T^3	2.2	1.75	Mean is significantly different
T^2-T^4	11.2	1.75	Mean is significantly different
T^2-T^5	10.0	1.75	Mean is significantly different
T^3-T^4	13.4	1.75	Mean is significantly different
T^3-T^5	12.2	1.75	Mean is significantly different
T^4-T^5	1.2	1.75	Not significantly different

Meanwhile, as to the case of Treatments 1, 2, and 3, it showed significant difference against the control medium indicating the importance of Potassium, Boron, and Magnesium to the enzymatic activity of *M. muricarpa*'s pollen for successful germination. However, it can be observed that as to the absolute difference of these 3 treatments to the control medium, it can be noted that treatments 2 (10.0) and 3 (12.2) has a comparatively higher value than treatment 1 (5.4) which is double of treatment one's result. Thus, suggesting that even though the 3 nutrients are important for successful pollen germination of *M. muricarpa*, the nutrients Magnesium and Boron are far more crucial for the enzymatic activity of the aforementioned species with regards to PPG. As for the difference between treatments with the exclusion of control medium, the test shows significant difference across treatments, thus, entailing that the absence and presence of either one of the macronutrients has a different effect on the pollen germination of the sampled species.

Table 3. One-way Analysis of Variance of Pollen Tube Growth (PTG) of Treatment Media.

ANOVA						
Source of Variation	SS	df	MS	F	P-value	F crit.
Between Groups	6330	4	1582.5	236.19	1.58×10^{-16}	2.86
Within Groups	134	20	6.7			
Total	6464	24				

Table 4. Tukey's Honest Significance Test (HSD Test) on Pollen Tube Growth (PTG).

Comparison	Absolute difference	Critical Range	Remarks
T^1-T^2	27	4.89	Mean is significantly different
T^1-T^3	29	4.89	Mean is significantly different
T^1-T^4	10	4.89	Mean is significantly different
T^1-T^5	1	4.89	Not significantly different
T^2-T^3	2	4.89	Not significantly different
T^2-T^4	37	4.89	Mean is significantly different
T^2-T^5	28	4.89	Mean is significantly different
T^3-T^4	39	4.89	Mean is significantly different
T^3-T^5	30	4.89	Mean is significantly different
T^4-T^5	9	4.89	Mean is significantly different

As for the analysis of the results on Pollen Tube Growth (PTG), One-way ANOVA was also performed. Results show in table 3 that the acquired F statistic in the analysis is 236.19 which is greater than the F statistic critical value of 2.86 at 5% (α=0.05) level of significance. This implies that the means of the treatment media are significantly different. The HSD Test was also carried out and turned out that treatments 2, 3, and 4 are significantly different from the control medium with an absolute difference of 28, 30, and 9, respectively, which is greater than the critical range of 4.89 (Table 4). However, for the case of Treatment 4, it was noted based on its average mean that it has a significantly higher value (72µm)

as compared with the control medium (63µm), while the Treatments 2 and 3 showed the opposite result with 35µm and 33µm, accordingly.

With this, it could be derived that Treatment 4 is significantly different in a positive perspective because even for this treatment where Calcium is not present, the PTG is not compromised and tend to have a higher result than the control medium. Whereas Treatments 2 and 3 are considered significantly different in a negative perspective because it affects the pollen tube formation which is evidently shown in the result (Fig. 1: A and B). Thus, indicating that the absence of the nutrients Magnesium and Boron leads to a slow formation of *M. muricarpa*'s pollen tube. This is also strengthened by the fact that among the treatments with the exclusion of the control medium, only treatments 2 and 3 are reported to be not significantly different with an absolute difference of 2 against the critical range of 4.89. This result implies that it has a synonymous effect to the PTG of the sampled species if absent. Meanwhile, among the treatments, Treatment 1 is the only one that is not significantly different from the control medium with an absolute difference of 1 against the critical range of 4.89. The result simply entails that Potassium is not an important nutrient when it comes to the formation of the pollen tube of *M. muricarpa* because even if this macronutrient is absent, the formation of pollen tube is not totally affected with result that is almost equal to the control (T1(w/o potassium=62µm; T5(control)=63µm).

With all the analyses, it could be derived that Boron and Magnesium truly serve as an important factor that contributes to the success of pollen germination and tube formation. According to Obermeyer and Blatt (1995) and Patel and Mankad (2012) Boron is an essential microelement required for growth and development of vascular plants. This for the reason that this element facilitates sucrose uptake and it plays an important role in the production of protein at pollen tube (Kayand *et al.*, 2014). While excess and deficiency of Boron could result in the breaking of pollen grains (Rerkasem and Jamjod, 2004). Further, Wang *et al.* (2006) stated that Boron is believed to promote pollen germination by affecting H+-ATPase activity, which initiates pollen germination and tube growth.

On the other hand, the importance of Magnesium is attributed to its function as germination-stimulating substance (Khan and Perveen, 2006; Imani *et al.*, 2011; Mendez and Acma, 2017). Wherein the magnesium ions help promote the growth of the pollen tube (Moore and Jung, 1974; Biswas and Mondal, 2014). Bose *et al.* (2011) also reported that Magnesium is a pivotal nutrient for activating a large number of enzymes; hence, magnesium plays an important role in numerous physiological and biochemical processes affecting plant growth and development. Further, Bergman (1992) and Kasinath *et al.* (2014) stated that it acts as a cofactor and activator of many enzymes and substrate transfer reactions making it a vital component for plant growth and development.

Conclusion

Based on the result, it is concluded that Boron and Magnesium are the key elements for successful pollen germination and tube formation of *M. muricarpa*. It is for the reason that when these nutrients are absent, the enzymatic activity of the abovementioned species was at the very least as compared with the other treatments. On the other hand, Calcium is considered as not significant when it comes to pollen germination. While Potassium and Calcium are the nutrients that are not significant for the formation of pollen tube growth.

Recommendation

Since in general the enzymatic activities of the pollen test were slow for this study due to various factor that includes media complexity and time exposure to the culture medium. It is recommended to consider a more complex media with varying concentrations, especially for the concluded significant nutrients. Also, long exposure to the culture medium is recommended to further establish the ideal time for this species to successfully germinate.

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