Journal of Applied Horticulture, 18(3): 221-227, 2016

Improving pollen viability of ginger (*Zingiber officinale* Rosc.) by application of boron and zinc and its impact on rhizome yield

Melati[1], E.R. Palupi[2]*, S. Ilyas[2] and A.D. Susila[2]

*[1]Indonesian Spices and Medicinal Crops Research Institute, Bogor, Indonesia 16111. [2]Department of Agronomy and Horticulture, Bogor Agricultural University, Bogor, Indonesia 16680. *E-mail: erpalupi@yahoo.co.id*

Abstract

Ginger production is hindered by lack of high quality seeds due to seed-borne pests and rhizome disease. Therefore, ginger true seed (GTS) is considered as one of the potential alternatives to solve the problem. However, GTS production is constrained because lack of flowering and low pollen viability. This research aimed to improve pollen viability by applying boron and zinc and study its impact on yield. This study was arranged in randomized complete block design with two factors and four replications. The first factor was dosage of boron at 0, 2, 4 kg ha^{-1} and zinc at 0, 1, 2 kg ha^{-1}. Boron and zinc were applied once a week for three weeks started at one week after generative bud appearance. Parameters observed were plant growth (plant height, numbers of tillers, stem diameter and number of leaves) and pollen viability. The dosages of boron and zinc had no significant effect on plant growth parameters. Boron at 2 kg ha^{-1} improved pollen viability up to 275% compared to control. Cross pollination using pollen from boron treated plants resulted in delaying flower fall. Existence of flowers had no effect on rhizome formation.

Key words: Generative bud, ginger true seed, pollen viability, pollination

Introduction

The main problem of ginger production is lack of high quality seeds. Ginger varieties that have been released are prone to bacterial wilt disease caused by *Ralstonia solanacearum.* The disease is mainly seed-borne causing rhizome rot and difficult to control, hence reducing yield. The infected rhizomes are difficult to sterilize. Thus, the use of ginger true seed (GTS) has potential for solving the problem. The advantages of using GTS are: less voluminous hence easy to handle, have longer storability, and mostly free of seed-borne pests and diseases. However, ginger plants produce less flowers under normal cultivation conditions, thus requires flower induction. Melati *et al* (2011) reported that the application of paclobutrazol 100 ppm induced flowering in 50% of ginger population. However, pollen viability was still low (about 31%), hence the seed set may not occur.

Boron (B) is a non-metal micronutrient required for growth and development of plants. It is required for cell wall formation, cell membrane integrity, cell division, Ca uptake and sugar translocation, flowering, pollen germination and pollen tube growth, and fruit development (Munawar, 2011). Boron is relatively immobile in plant, hence its availability is essential at all stages of growth, especially during fruit/seed development. Boron deficiency caused abnormality in pollen (empty, deformed). Lordkaew *et al.* (2011) found that boron deficiency reduced corn seed set. Pollen tubes may burst possibly due to boron role in the cell wall structure of the pollen tube (Dell and Huang 1977; Brown *et al.*, 2002).

Zinc has important function in protein synthesis, carbohydrate metabolism and stem growth. Plant with Zn deficiency indicated delayed flowering, premature bud abscission, reduced seed set and seed yield. Zinc is required by a number of enzymes including alcohol dehydrogenase functioning in meristematic areas such as shoots and roots (Marschner, 1995). Pandey *et al.* (2013) reported that spraying the leaves and the base of plants of *Vigna mungo* with 1.0 µM zinc increased viability of pollen up to 95%, increased seed set (145 grains plant^{-1}), higher than those sprayed with 0.01 µM zinc (32% and 44 grains plant^{-1}), respectively. Morshedi and Farahbakhsh (2010) reported the application of zinc 40 kg ha^{-1} in wheat increased number of seed per spike, weight of 1000 seed, and yield. Kumawat *et al.* (2015) stated that zinc also functioned in the initiation of reproductive primordia and flowering, hence increasing the number of flower. It also played important role in photosynthate partitioning. Similar result was found in fenugreek (Jakhar *et al.*, 2014).

Crossing of *Alpinia purpurata* and *Etlingera elatior* (ornamental ginger) resulted in low seed set with only 25% viable seeds (Luc-Cayol and Fereol, 1997). Yunira (2009) stated that self-pollination of *A. purpurata* var 'Jungle King' (JK) or *A. purpurata* 'Jungle Queen' (JQ) produced no seed but successfully set fruit (55 %) when crossed between the two varieties (*A. purpurata*: JK x JQ and JQ x JK). Currently, information on breeding system of edible ginger (*Zingiber officinale)* is not available because open and controlled cross pollination of edible ginger generated no seed and flowers aborted about 12 h after anthesis (Melati *et al.*, 2015).

The research aimed to improve pollen viability of ginger by boron and zinc application and its impact on plant growth and rhizome yield. It was expected that boron and zinc would improve pollen viability and eventually would be beneficial for GTS production.

Materials and methods

The experiment was conducted in the green house and Seed Technology Laboratory of Indonesia Spice and Medicinal Crops Research Institute (ISMCRI) in Bogor, West Java (200 m asl) from November 2013 to August 2014.

Seed nursery: Seed rhizomes were selected for firmness, weight (50-60 g), and number of shoot bud (2-3 buds) and free from seed borne pests and diseases. The selected seed rhizomes were soaked for 4 hours in fungicide (Dithane) 2 g L^{-1} and bactericide (Agrept) 1 g L^{-1} prior to seeding. Cocopeat as planting medium, was filled into plastic boxes. The germinated seed rhizomes were transplanted into polybags one month after seeding using planting media: a mixture of soil, sand, and manure (2:1:1) (± 30 kg per polybag). The planting media was treated previously with fungicide (Dithane) at concentration of 4 g L^{-1} 500 mL per polybag. Granule NPK (16:16:16) was applied on 30, 60, 90 days after transplanting with a dosage of 3.5 g per polybag.

Boron and zinc application: The treatment was arranged in Randomized Complete Block Design (RCBD) with two factors and four replications. The first factor was boron at 0, 2, 4 kg ha^{-1} equivalent to dissolved B_2O_3 at 1 g L^{-1} and 2 g L^{-1} and untreated seed rhizomes as control. The second factor was zinc at 0, 1, 2 kg ha^{-1} equivalent to dissolved $ZnSO_47H_2O$ at 1.1 g L^{-1} and 2.2 g L^{-1} and untreated seed rhizomes as a control. The 100 mL of boron and zinc solution were applied to every generative buds as soil drench one week after generative buds emergence.. The application of boron and zinc was conducted once a week for three weeks. Generative buds can be determined by its rounded shape (Melati *et al.,* 2015). Control plants were drenched with 100 mL of water. Each treatment was repeated four times, hence there were 36 plots with six plants/plot.

Flowering and pollen viability: Data were collected every two weeks, during 18-22 weeks after planting (WAP). Flowering parameters observed were time to first and last spike emergence, percentage of flowering plant, number spike/plant and number of blooming flowers per spike. Pollen viability was observed twice at anthesis and 20-30 minutes after anthesis. Time of anthesis was determined according to Melati *et al.* (2015). Pollen was then cultured in medium containing 10% sucrose, 100 ppm H_3BO_4, 300 ppm $Ca(NO_3)_2.H_2O$, 200 ppm $MgSO_4.7H_2O$, 100 ppm KNO_3 desolved in 1000 mL water (Brewbaker and Kwack, 1964). Pollen was categorized as viable when 75% of the pollen was visually dark after 24 hours incubation. Olympus light microscope observation at 40× magnification was used to observe the dark color of the pollen.

Plant growth and seed rhizomes yield: Plant growth parameters observed were plant height, number of tillers, number of leaves, and stem diameter. Rhizome production per plant was observed at 9 months after planting (MAP).

Crossing: Cross pollination was carried out to examine the possibility of obtaining GTS using pollen with the highest viability by cross-pollinating it into flowers of other plants.

Data analysis: The data were analyzed according to F test (ANOVA) and the significant differences among treatments were observed using Duncan's multiple range test at $P < 0.05$.

Results and discussion

Plant growth: The application of boron (B) and zinc (Zn) at various dosages showed no significant effect on vegetative growth of ginger as indicated by growth parameters and also there were no interctions between B and Zn (Table 1 and 2). Single treatment of B or Zn were not significantly different among dosages in all growth parameters at 18, 20 and 22 WAP except for the number of leaves as a response to 2 kg Zn ha^{-1} at 22 WAP. Treatment of 2 kg ha^{-1} Zn showed slower growth than other treatments,.

Table 1. Effect of B and Zn on plant height and stem diameter at 18, 20, and 22 weeks after planting (WAP)

Treatment	Plant height (cm)			Stem diameter (mm)		
	18 WAP	20 WAP	22 WAP	18 WAP	20 WAP	22 WAP
B dosage (kg B_2O_3 ha^{-1})						
0	84.2	82.3	91.5	8.2	8.1	7.5
2	95.6	93.8	96.0	9.4	9.0	8.9
4	92.3	88.8	91.0	9.3	8.8	8.9
Zn dosage (kg $ZnSO_47H_2O$ ha^{-1})						
0	90.7	89.6	90.7	9.0	8.7	8.6
1	94.6	89.7	93.1	9.1	8.9	8.7
2	86.8	85.6	84.7	8.7	8.3	7.8
Boron x zinc	ns	ns	ns	ns	ns	ns

The application of B in this study primarily aimed to improve the viability of pollen, while the application of Zn was to enhance flowering. The application of B and Zn was not to improve vegetative growth. Thus, B application showed no effect on plant growth parameters. However, Asad *et al.* (2003) revealed the foliar application of B (28-1200 mM) at the late vegetative stage increased sunflower vegetative biomass. Halder *et al.* (2007) also reported that application of B (3 kg ha^{-1} and Zn (4.5 kg ha^{-1}) in final land preparation of local ginger variety in Bangladesh could enhance plant height and leaves number. The mix finding occured presumably because of different soil chemical properties. Allegedly, planting media used in this study already had micro-compound (B and Zn) needed to support vegetative growth, hence the addition of micro compound did not affect plant growth. Results of soil analysis showed that the content of Zn and B was high 155.57 ppm and 292.94 ppm, respectively. The high content of B in the planting media was because the media constisted of mixture of soil: sand: manure and sandy soil has high B content.

Table 2. The effect of B and Zn on number of shoots and number of leaves on 18, 20, and 22 weeks after planting (WAP)

Treatment	Number of tillers			Number of leaves		
	WAP			WAP		
	18	20	22	18	20	22
B dosage						
0	5.3	6.4	6.5	21.3	21.9	20.8
2	4.8	5.6	5.2	23.1	23.5	23.7
4	5.2	6.6	6.8	23.2	22.9	22.8
Zn dosage						
0	4.6	5.7	6.1	23.1	22.9	22.7ab
1	5.0	7.1	7.2	23.4	23.8	24.2a
2	5.7	5.8	5.3	20.9	21.6	20.4b
Boron x zinc	ns	ns	ns	ns	ns	ns

Numbers followed by the same letter in the same column were not significantly different at 5%. ns : non significant.

Flowering period, percentage of flowering plants and number of spike: The environmental condition (Appendix 1) in the trial location was suitable to induce flowering. The temperature at 2 MAP during daylight was relatively high of 36°C. Temperatures in the third month (3 MAP) on 8 AM, 12 AM and 4 PM were 25, 27 and 26 °C, respectively, with fairly high humidity 89%. The suitable environmental conditions were capable to induce

Table 3. The effect of B and Zn at various dosages on number of spikes and number of blooming flowers

Treatment	Number of spikes					Number of blooming flowers*
	Flowering periode (WAP)					
	14	16	18	20	22	
B dosage						
0	0	1.1	1.6 ab	2.0	0.8	2.2 a
2	0	1.6	2.7 a	2.7	1.2	2.2 a
4	0	1.0	1.9 ab	2.1	1.1	2.1 a
Zn dosage						
0	0	1.1	1.9	1.9	0.5	1.8 b
1	0	1.4	2.5	2.7	1.7	2.4 a
2	0	1.3	1.9	2.2	0.9	2.2 ab
Boron x Zinc	ns	ns	ns	ns	ns	ns

Numbers followed by the same letter in the same column were not significantly different at 5%. ns: non significant, *Transformation $(\sqrt{x}+ 5)$

flowering without a particular treatment. Flower initiation occurred at 16 WAP. The application of B and Zn on generative shoots was carried out three consecutive weeks starting at 17 WAP.

The addition of B and Zn did not affect flower induction, as indicated by number of spike parameter which was not significantly different among treatments until the end of observation (Table 3). Single treatment of B and Zn also had no effect on flower formation. Moreover, there was no interaction between B and Zn on the parameters. One of the Zn role is in IAA synthesis, primordia initiation of reproductive organ and photosynthate partition. The addition of Zn was expected to change photosynthate partition, to support generative growth (flowering), althouh this did not occur in this study. Photosynthate partition on ginger was also used for rhizome development, in addition to vegetative growth and flowering. Zn addition had no effect on flowering induction.Tsconev and Lindon (2012) reported that the availability of Zn in soil could increase the soil pH.

The application of B and Zn at apperance of generative shoots were not able to induce the formation of new generative shoots. The period of flowering (first spike and last spike), as well as flowering percentage, were not significantly different among treatments (Table 4). This indicated that flower induction was more affected by enviromental conditions than by B and Zn application. Ginger flowering was strongly triggered by temperature and humidity. In suitable environment, ginger will generate flower without any additional treatment. Melati *et al.* (2011) stated that natural flower induction on ginger occurred at the temperature (21-36 °C) and humidity (51-89%). The peak spike formation occured at 20 WAP and flowering decreased at the end of the observation (22 WAP). This happened because some spikes withered. Differences in the number of flowers was thought to be caused by environmental conditions, as shown by the number of spikes that were not significantly different from the early to the end of the observation.

Table 4. Effect of boron and zinc on first and last spike emergence and the percentage of flowering

Treatment	First spike emergence (WAP)	Last spike emergence (WAP)	Percentage of flowering plants (%)
B dosage (kg B_2O_3 ha^{-1})			
0	16.0	21.0	89.5 a
2	15.8	21.2	74.8 b
4	15.6	20.2	84.0 ab
Zn dosage (kg $ZnSO_47H_2O$ ha^{-1})			
0	15.0	20.2	78.4 a
1	16.0	20.2	74.8 a
2	16.0	19.7	77.8 ab
Boron x Zinc	ns	ns	ns

Numbers followed by the same letter in the same column were not significantly different at 5%. ns: non significant

Viability and number of pollen: Pollen viability altered by the time of pollen collection. Pollen was taken shortly after anthesis showed no germination. Pollen collected at the beginning of anthesis was difficult to separate and formed blob due to the pollen stickiness and its high water content. In general, omitting the treatment effect, pollen viability improved up to 22.06% when collected 20-30 minutes after anthesis (Table 5). This implied that anther dehisced ± 15 minutes after anthesis. Pollen water content reduces when exposed to air and makes it separate easily from one another. Previously, Melati *et al.* (2015) reported pollen was started viable 15 minutes after anthesis and increased gradually until 45 minutes after anthesis. The duration of pollen viability was brief (30 min) . Pollen viability of large white ginger is lower than the other ginger. Hamidou *et al.* (2011) studied some ornamental gingers (*Hedycium* spp) and recorded viability of the pollen upto 88.5%.

There was interaction beween B and Zn on pollen viability and number of pollen per anther collected at 20-30 minute after anthesis (Table 5). The highest viability (40.93%) was obtained at 2 kg ha^{-1} B. Pollen viability improved 275% compared to without B treatment. The application of 1-2 kg ha^{-1} B and Zn increased number of pollen ± 8.3-22.9% (Table 5). The higher addition of B (4 kg ha^{-1}), either singly or with Zn might reduce pollen viability. B served to improve the quality of vegetative and generative organs (Cakmak and Romheld, 1997; Blevins and Lukaszewski, 1998; Uraguchi *et al.*, 2014). In this study, the addition of B is able to improved pollen viability. Commonly, pollen contains a low quantity of B. The addition of B through spray at generative buds was expected to increase the content of B in pollen. High B content in the pollen can lengthen pollen tube to support pollination. B was essential in plant reproductive cycle, including pollen production and germination (Keefe, 1998). However, excessive B concentration will cause pollen unviability and decreasing pollen number due to physiological depression and protoplasm damage. Garg *et al.* (1979) found that rice that have low pollen viability can be enhanced by the addition of B. The adequate B availability will enhance sugar, enzymatic activity, and respiration that needed to repair the growth of pollen. Rosliani *et al.* (2012) found that pollen of *Allium cepa* var *ascalonicum* increased with application of B (4 g ha^{-1}). Several researches show that application of B affects the reproductive organs (Rerkasem *et al.*, 1993; Obermeyer *et al.*, 1996; Rowe and Eckhert 1999; Wang *et al.*, 2003). B affects the physiological condition of the plant to interact with other compounds in complex manner and can be antagonistic (Tariq and Mott, 2007).

The highest number of pollen per anther was obtained from application of Zn (1 kg ha^{-1}) without B addition (Table 5). Zn

Table 5. Interaction of boron and zinc dosages on pollen viability and number of pollen per anther at 20-30 minutes after anthesis

Treatment		Pollen viability (%)*	Number of pollens/anther
B dosages (kg B_2O_3 ha^{-1})	Zn dosages (kg $ZnSO_47H_2O$ ha^{-1})		
0	0	14.89 e	598.5 bcd
	1	15.05 e	877.5 a
	2	14.02 e	724.5 bc
2	0	40.93 a	291.0 e
	1	29.24 b	648.0 bcd
	2	24.25 c	735.0 b
4	0	22.73 c	589.5 cd
	1	16.85 de	546.7 d
	2	21.54 cd	526.5 d

*Observation was carried out 20-30 minute after anthesis. Pollen viability at anthesis was 0 %.

Numbers followed by the same letter in the same column were not significantly different at α=5%

acts as catalyst for several enzyme. Zn deficiency will interfere the metabolism of N path, which in turn will affect flowering namely abnormal pollen development. Zinc is involved in the development and function of the flower tissue, for example pollen, tapetum, and secretory tissue in the ovary, thus play an important role in normal flower set and fruit formation. The addition of Zn in appropriate dose may increase the amount of pollen on ginger, and expected to generate flower with normal reproductive development. Zinc fuctions in activating plant enzymes for metabolism of carbohydrates, maintaining the membrane integrity, protein synthesis and the formation of pollen tubes (Hafeez *et al.*, 2013).

There was no correlation between pollen viability and the number of pollen per anther. The correlation coefficient was negative and statistically not significant. That means the amount of pollen per anther cannot be predicted by considering the viability of pollen (Fig.1). Pollen viability was affected by the dose of B ($R^2 = 0.626$) with quadratic pattern effect. This suggested the effect of B dose on the viability of pollen was 62.6%, while the residual influence by other factors was not included in this model.

Pollen viability was indicated by its ability to absorb germination media. The observation under microscope showed that pollen was viable and not empty, black indicating the presence of dense cytoplasm. Pollen tube formation was not found in all treatments. This is presumably because the germination media was not suitable for ginger pollen to germinate (Fig. 2) or due to the thickness of pollen exine and the absence of pollen pores hence it was difficult to be penetrated by the pollen tube. Melati *et al.* (2015) stated that the size of pollen of large white ginger was 59.1 ± 8:52 µm x 58.25 ± 5.74 µm, rounded on one side and concave on the other side, have no ornaments and unporate.

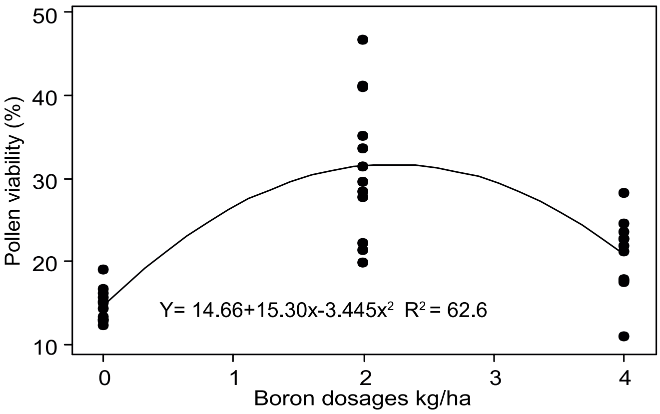

Fig. 1. Response curve of pollen viability for B dosages

Cross Pollination: Pollen from plants treated with B 2 kg ha^{-1} was used for cross pollination into other plants. The result showed that the pollinated flowers remained intact on the following day (Fig 3b). Conversely, flower developed from natural pollination or hand pollination using pollen from untreated were dropped on the next day (12-18 hours after anthesis) (Fig. 3a).

The delay of flower abscission could to be due to higher pollen viability which generated tube formation in style. The pollen tube growth in the style would eventually induced IAA accumulation in the pistil. Auxin inhibited the formation of abscission layer (Kovaleva and Zakhora, 2003). Aloni *et al.* (2006) stated that the growth of pollen tubes increased in the presence of IAA in pistil stalk. IAA affected flower (Zhao, 2011) and delayed abscission of flowers and fruit. Perica *et al.* (2001) suggested that B application might increase fruit set in some species and boron could delay abscission of style. The addition of Zn also delayed the flowering. Pathak *et al.* (2012) reported that Zn application might delay the death of chickpea flower for 4-5 days hence increasing fruit formation.

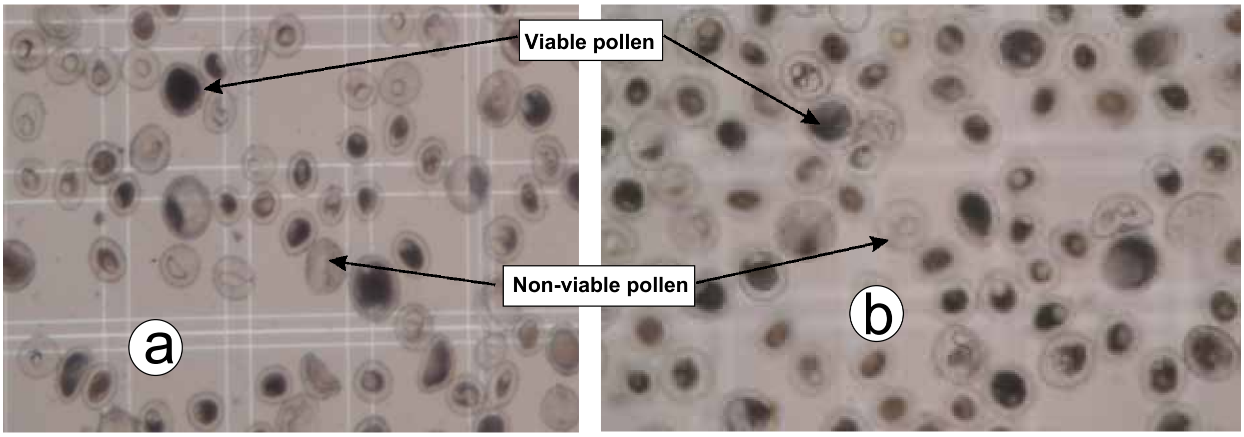

Fig. 2. Viable pollen and non-viable pollen, a) control, b) boron 2 kg/ha.

Fig. 3. Ginger flower after cross pollination on 12-18 h using a) pollen from untreated plants, b) pollen from plants at 2 kg ha $^{-1}$ B application.

Yield and correlation analysis between flowering and growth paramater: There was no interaction between B and Zn to rhizomes yield parameter. The application of Zn, with or without B application, gave better result (Table 6). The lowest rhizome production (242.3 g plant^{-1}) was generated from plants without Zn application. The addition of B aimed to improve pollen viability, not to increase of rhizome yield, hence B application had no significant effect on rhizome yield. However, Zn gave significant effect to rhizome yield (Table 6).

Table 6. Effect of boron and zinc on rhizome yield at 9 months after planting

Treatment	Rhizome yield (g plant $^{-1}$)
B dosages (kg B_2O_3 ha^{-1})	
B 0	273.8 a
B 2	379.6 a
B 4	312.5 a
Zn dosages (kg $ZnSO_47H_2O$ ha^{-1})	
Zn 0	242.3 b
Zn 1	386.1 a
Zn 2	337.5 a
Boron x Zinc	ns

Numbers followed by the same letter in the same column were not significantly different at 5%. ns : non significant.

One of Zn functions is to set the partition of photosynthate. Addition of Zn was able to divert photosynthate accumulation from vegetative growth to rhizome formation, thereby increasing rhizomess yield. However, Halder *et al.* (2007) showed that the addition of B and Zn in local ginger in Bangladesh could enhance the growth of plant height and number of leaves. This result might occur due to differences in soils chemical properties. Soil media used in this study already had sufficient micro-nutrients (B and Zn), hence the addition of micro nutrient might not have affected plant growth. However, Zn application gave better effect to increase rhizomes yield. Zn in media was only able to support vegetative growth, hence required addition of Zn played role in photosynthate partitioning. This is presumably due to the availability of shoots as organs that support photosynthesis, sufficient to the needs for rhizome development.

There was no significant correlation between growth parameters, and numbers of flowers with rhizomes yield (Table 7). This suggests that existence of flowers had no effect on rhizome formation in ginger.

Table 7. Correlation coefficient among vegetative and generative parameters

	BF	FR	NT	NS	NL	SH	PH
BF	1.000	0.376	0.253	0.681**	0.183	0.012	-0.059
FR		1.000	0.444	0.266	-0.199	-0.183	-0.173
NT			1.000	0.097	0.095	-0.229	-0.268
NS				1.000	0.190	0.076	0.074
NL					1.000	0.691**	0.665**
SH						1.000	0.988**
PH							1.000

Blooming flowers (BF), Fresh rhizomes (FR), Number of shoot (NT),Number of spike(NS), Numbers of leaves (NL), Stems height (SH), Plants height(PH)

Ginger plant growth (plant height, number of leaves, number of tillers, stem diameter) was not affected by doses of B and Zn. Spike formation was strongly influenced by environmental factors such as temperature and humidity, not by application of B and Zn. The addition of B @ 2 kg ha^{-1} was able to improve pollen viability up to 275% compared to controls. The amount of pollen per anther increased with Zn addition @ 1 kg ha^{-1}.

The treatment of B and Zn have not been able to enhance pollen tube formation when germinated in media. Pollen from plants treated with B (2 kg ha^{-1}) were able to delay flowers abscission but the seed set took place. Application of boron (2 kg ha^{-1}) has good prospect to support ginger breeding system development. There was no correlation between flower number and rhizome yield, hence the existence of flowers had no effect on the formation of ginger rhizomes. Further research is necessary to determine the cause of seeds formation failure in large white ginger.

References

Adaniya, S. and M. Shoda, 1998. Variation in pollen fertility and germinability in ginger. *J. Japan. Soc. Hort. Sci*., 67(6): 872-874.

Aloni, R., E. Aloni, M. Langhans and C.I. Ullrich, 2006. Role of auxin in regulating Arabidopsis flower development. *Planta,* 223: 315-328.

Anon. 2012. Agronomy note: The role of boron in flowering and fruit, nut and seed formation. (www.riotintominerals.com).

Asad, A., F.P.C. Blamey and D.G. Edwards, 2003. Effects of boron foliar applications on vegetative and reproductive growth of sunflower. *Ann Bot*., 92: 565-570.

Barclay, G.F. and C.R. McDavid, 1998. Effect of benzylaminopurine on fruit set and seed development in pigeonpea (*Cajanus cajan*). *Sci. Hort.,* 72 (2): 81-86.

Blevins, D.G. and K.M. Lukaszewski, 1998. Boron in plant structure and function. *Annu. Rev. Plant Physiol. Plant Mol. Biol*., 49: 481-500.

Brewbaker, J.L. and B.H. Kwack, 1964. The calcium ion and subtances influecing pollen growth. In: *Pollen Physiology and Fertilization.* H.F. Linskens (Ed). North-Holland Publishing Company. Amsterdam

Brown, P.H., N. Bellaloui, M.A .Wimmer, E.S. Bassil, J. Ruiz. H. Hu, H. Peffer, F. Dannel and V. Romheld, 2002. Boron in plant biology. *Plant Biology,* 4: 205-223.

Cakmak, I. and V. Romheld, 1997. Boron deficiency-induced impairments of cellular functions in plants. *Plant Soil,* 193: 71-83.

Dale, G., D.G. Blevins and K.M. Lukaszewski, 1998. Boron in plant structure and function. *Annu. Rev. Plant Physiol. Plant Mol. Biol*., 49: 481-500.

Dell, B. and L. Huang, 1997. Physiologycal response of plants to low boron. *Plant and Soil,* 193: 103-120.

Dordas, C. 2005. Foliar boron application improves seed set, seed yield and seed quality of alfalfa. *J. Agron.,* 98(4): 907-913.

Garg, O.K., A.N. Sharma and G.R.S.S. Kona, 1979. Effect of boron on the pollen vitality and yield of rice plants (*Oryza sativa* L. var. Jaya). *Plant Soil*, (52)4: 591-94.

Hafeez, B., Y.M. Khanif and M. Saleem, 2013. Role of zinc in plant nutrition- A Review. *Amer. J. of Expt. Agr.,* 3(2): 374-39.

Halder, N.K., N.C. Shill, N.A., Siddiky, R. Gomes and J. Sarkar, 2007. Response of ginger to zinc and boron fertilization. *Asi. J. Plant Sci*., 6(2): 394-398.

Hamidou, F., H.F. Sakhanokho and K. Rajasekaran, 2011. Pollen biology of ornamental ginger (*Hedychium* spp. J. Koenig). *Sci. Hort*., 125: 129-135.

Jakhar, R.K., B.L.Yadav and A. Kumawat, 2014. Response of fenugreek (*Trigonella foenum-graecum*) to different waters and zinc fertilization. *Agric. Sci. Digest.,* 34(1): 67-69.

Keefe, S. 1998. In the green update on boron's role as an essential plant micronutrient, Borax Pioneer.

Kovaleva, L. and E. Zakharova, 2003. Hormonal status of the pollen pistil system at the progamic phase of fertilization after compatible and incompatible pollination in *Petunia hybrida* L. *Sex. Plant Repro.*,16: 191-196.

Krudnak, A., S. Wonprasaid and T. Machikowa, 2013. Boron affects pollen viability and seed set in sunflowers. *Afr. J. Agri. Res.*, 8(2): 162-166.

Kumawat, S.K., B.L. Yadav and S.R. Kumawat, 2015. Response of fennel (*Foeniculum vulgare* Mill.) to phosphorus and zinc fertilization in a loamy sand soil. *J. Spices. and Aromatic Crops*. 24(1): 23-27.

Kun Hua, W., M.J. Hua, H.H. Ping and G.S. Li, 2011. Generation of autotetraploid plant of ginger (*Zingiber officinale* Rosc.) and its quality evaluation. *Pharmacogn Mag*., 7(27): 200-206.

Lordkaew, S., B. Dell, S. Jamjod and B. Rerkasem, 2011. Boron deficiency in maize. *Plant Soil*, 342(1-2): 207-220.

Luc-Cayol, F. and L. Fereol, 1997. *Alpingera martinica* (Zingiberaceae) an intergeneric hybrid between *Alpinia purpurata* and *Etlingera elatior*. *Hort Sci.,* 32(5): 915-915.

Marchner, H. 1995. *Mineral Nutrition of Higher Plant*. Academic Press. London. 680p.

Meena, 2010. Effect of boron on growth yield and quality tomato (*Lycopersium esculentum*) on growth under semi arid condition. *Int. J. Chem. Eng. Res*, 2(2): 162-172.

Melati, 2011. Induksi pembungaan dan biologi bunga pada tanaman jahe putih besar (*Zingiber officinale* Rosc.). Thesis. Bogor Agricultural Univ (in Indonesian).

Melati, E.R. Palupi and N. Bermawie, 2011. Induction of flowering in large white ginger (*Zingiber officinale* Rosc.) using paclobutrazol. Proceedings of the 2nd International Symposium on Temulawak. pp52.

Melati, E.R. Palupi and N. Bermawie, 2015. Floral biology of ginger (*Zingiber officinale* Rosc). *Int. J. Curr. Res. Biosci. Plant Biol.*, 2(4): 1-10.

Morshedi, A. and H. Farahbakhsh, 2010. Effects of potassium and zinc on grain protein contents and yield of two wheat genotypes under soil and water salinity and alkalinity stresses. *Plant Ecop*., 2: 67-72.

Munawar, A. 2011. Kesuburan tanah dan nutrisi tanaman. IPB Press. Bogor. p 237 (in Indonesian).

Obermeyer, G., R. Kriechbaumer, D. Strasser, A. Maschessning and F.W. Bentrup, 1996. Boric acid stimulates the plasma membrane H+-ATPase of ungerminated lily pollen grains. *Physiol. Plant*., 98: 281-290.

Pandey, N., B. Gupta and G.C. Pathak, 2013. Foliar application of Zn at flowering stage improves plant s performance, yield and yield attributes of black gram. *Indian J. Expt. Biol*., 5: 548-555.

Pathak, G.C., B. Gupta and N. Pandey, 2012. Improving reproductive efficiency of chickpea by foliar application of zinc. *Braz. J. Plant Physiol.,* 24(3): 173-180

Perica, Slavko, Patrick H. Brown, Joseph H. Connell, Agnes M.S. Nyomora, Christos Dordas, and Hening Hu, 2001. Foliar boron application improves flower fertility and fruit set of olive. *HortScience,* 36(4): 714-716.

Ramachandran, K. 1982. Polyploidy induced in ginger by colchisine treatment. *Curr. Sci*., 51: 288-289.

Ravindran, P.N., K.N. Babu and K.N. Shiva, 2005. Botany and crop Improvement of ginger. In: *The Genus Zingiber*. P.N. Ravindran and K.N. Babu (ed.). Washington DC. CRC Press. p.552.

Rerkasem, B., R. Netsangtip, S. Lordkaew and C. Cheng, 1993. Grain set failure in boron deficient wheat. *Plant Soil*., 156: 309-312.

Rosliani, R., E.R. Palupi and Y. Hilman, 2012. Penggunaan benzil amino purin dan boron untuk meningkatkan produksi dan mutu benih true shallots seed bawang merah (*Allium cepa* var *ascalonicum* di dataran tinggi. *J. Hort.,* 22(3): 242-250 (in Indonesian).

Rowe, R.I. and C.D. Eckhert, 1999. Boron is required for zebrafish embryogenesis. *J. Exp. Biol.,* 202: 1649-1654.

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

Supriadi, J.G. Elphinstone, S.J. Edengreen and S.Y. Hartati, 1995. Physiological, serological and pathological variation amongst isolates of *Pseudomonas solanacearum* from ginger and other host in Indonesia. *J. Penel. Tanaman Industri,* 1(2): 88-89 (in Indonesian)

Tariq, M. and C.J.B. Mott, 2007. The significance of boron in plant nutrition and environment-a review. *J. Agron.,* 6: 1-10.

Tsonev, T. and F.J.C. Lidon, 2012. Zinc in plants - An overview. *Emir. J. Food Agric.,* 24(4): 322-333

Uraguchi, S., Y. Kato, H. Hanaoka, K. Miwa and T. Fujiwara, 2014. Generation of boron deficiency tolerant tomato by overexpressing an *Arabidopsis thaliana* borate transporter *AtBOR 1. Plant Sci*., 5 (125): 1-7.

Wang, Q., L. Lu, X. Wu, Y. Lin and J. Lin, 2003. Boron influences pollen germination and pollen tube growth in *Picea meyeri*. *Tree Physiol.,* 23: 345-351.

Wu, J.Z., Y. Lin, X.L. Zhang, P.D. Pang and J. Zhao, 2008. IAA stimulates pollen tube growth and mediates the modification of its wall composition and structure in *Torenia fournieri. J. Expt. Bot.*, 59(9): 2529-2543

Yunira, I. 2009. Keberhasilan reproduksi *Alpinia* sp dan *Zingiber* sp: penyerbukan alami dan penyerbukan buatan. Skripsi. Bogor Agricultural Univ. (in Indonesian)

Zhao, Y. 2010. Auxin biosynthesis and its role in plant development. *Annu Rev Plant Biol.,* 2(61): 49-64.

Received: June, 2016; Revised: June, 2016; Accepted: June, 2016

Appendix 1. The average daily temperature and humidity during the induction period and flowering

Date	8.00 am		12.00 am		4.00 pm	
	RH(%)	TEMP (^{0}C)	RH	TEMP (^{0}C)	RH(%)	TEMP (^{0}C)
January (2 MAP)	85.53	25.65	55.00	36.32	76.84	27.79
Februar (3 MAP)	89.95	25.26	58.47	27.21	85.21	26.32
March (4 MAP)	80.28	25.60	63.20	29.95	76.57	28.09
April (5 MAP)	71.95	26.25	56.95	31.00	82.26	25.88
May (6 MAP)	64.73	27.84	51.86	31.69	74.40	26.75

MAP : Month After Planting

TEMP: Temperature