

Some biochemical changes of early germinated Arabica coffee seeds (*Coffea arabica* L.) after treated by brassin-like substance

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ABSTRACT: Effect of Brassin-like substance on chemical compositions of Arabica coffee seeds was studied during early germination. The substance has played an important role in promoting seed germination and chemical composition during germination. In this study, coffee seeds were soaked in four Brassin-like substance (BS) concentrations 0, 0.5, 1.0 and 2.0 mg/L for 24 hours before germination test. Total sugars, protein and fat contents were determined at 0, 2, 4, 6 and 8 days after soaking. The study was conducted as 4 × 5 factorial in completely randomized design: CRD. The results revealed that BS application significantly effected on nutritional quality during early germination. Total sugars reached the highest amount at day 8 when soaked in 1.0 and 2.0 mg/L BS. As for protein content, coffee seeds in every treatment showed the greatest amount at day 4 of germination especially, of coffee seeds for ones soaked in 1.0 and 2.0 mg/L BS. Fat content gradually increased upon the first 4 days of germination. Coffee seeds in 1.0 and 2.0 mg/L BS showed higher fat content than the seeds soaked in 0 and 0.5 mg/L BS. Therefore, BS application could be a promising alternative regulator for enhancing coffee seed quality during early germination.

Keywords: Arabica coffee, nutritional quality, brassin-like substance, early germination

Introduction

Brassinosteroids (BRs) have been found to play an important role in plant physiological responses including seed germination, seedling growth, cell division and differentiation (Clouse and Sasse, 1998; Xi et al., 2013). Bewley and Black (1982) found that endogenous BRs naturally breakdown dormancy and trigger germination in seeds of several genera. The hormones also have inhibitory effects of abscisic acid (ABA) during seed germination, seedling growth and development (Zheng et al., 2009). Moreover, exogenous BRs analogues application promoted total yield and regulated biosynthesis of antioxidant compounds in endives (*Cichorium*

endivia L.) (Serna et al., 2012), nucleic acids and reducing sugars in radish (*Raphanus sativus* L.) (Vardhini et al., 2012), soluble proteins, fat contents and soluble sugars in pea (*Phaseolus vulgaris*) and lupin (*Lupinus luteus* L.) (Jolanta et al., 2014). Recently, the synthetic compound or brassin-like substance (BS) has been discovered and studied its positive effects, i.e. fruit size and quality of some fruit types such as longan (*Dimocarpus longan*), papaya (*Carica papaya*), mango (*Mangifera indica*) and Arabica coffee (*Coffea arabica* L.) by applying BS concentration between 0.01 to 1.50 mg/L (Pankasemsuk, 2007; Praphutphitthaya and Pankasemsuk, 2012). BS has similar chemical structure as BRs and like wisely functions. However, limited works have

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reported about the effect of BS applications on seed germination and quality especially for Arabica coffee.

Nowadays, Arabica coffee (*Coffea arabica* L.) has become a crucial beverage crop in Thailand as its export values run over 3.1 million USD (Office of Agricultural Economics, 2015). It has not only attractive aroma and flavor, but also enriches in nutritive values along with several health promoting compounds (Yeretziana et al., 2003; Farah, 2012). In germinated seed, significant alterations of nutrient compositions typically occur immediately after water imbibitions (Vidal-Valverde et al., 2002). During biological process, vastly release and generate important compounds essential to embryonic growth and development such as proteins, fatty acids and sugars (Marcone, 2004; Gulewicz et al., 2008). The quality of coffee used for beverage is related to the chemical composition of the roasted beans, which is affected by the composition of the green beans and roasting conditions (Banks *et al.*, 1999). As aforementioned, no previous research has been reported on nutritional quality changes during coffee seed germination. Therefore, this work was to determine the effect of BS applications and germination time on the total sugar, protein and fat contents of Arabica coffee seed during germination.

Materials and methods

Plant material

Arabica coffee fruit (*Coffea arabica* L. cv. Catimor) were obtained from Highland Research and Training Center (Khun Chang Kien Station), Faculty of Agriculture, Chiang Mai University,

Thailand. The seeds were removed from a fruit berry, left fermented for 48 hours at ambient temperature. Subsequently, a part called mucilage was removed by hand and rinsed off using tap water. The seeds were dried (until moisture content became as low as 12%), and the endocarp was removed thereafter.

Germination procedure

Firstly, in order to control contamination, coffee seeds were soaked in 1% sodium hypochlorite (NaOCl) solution for 2 minutes, and subsequently rinsed off by distilled water. Four BS soaking treatments were made on this experiment, which were (i) distilled water (control treatment), (ii) 0.5 mg/L BS (Chaiwatana, Thailand), (iii) 1.0 mg/L BS and (iv) 2.0 mg/L BS. Soaking time was 24 hours, under the room temperature. Each treatment consisted of 3 replications, and each replication was conducted by 100 coffee seeds. Germination procedures described by Valio (1976) were used. Coffee seeds were placed between 2 layered-paper in the aluminum tray (width × length = 18.0 × 29.0 cm). Each tray was moistened by spraying distilled water at an amount of 2.5 times of its weight (paper and seeds altogether). All trays were then incubated in a germination chamber Model 620 RHS, P6 Contherm, New Zealand (30.0 ± 1.0 °C, $85.0 \pm 5.0\%$ RH) under dark condition. At day 0 (before soaking BS), 2, 4, 6 and 8 days after germination, coffee seeds (100 seeds) from each treatment were randomly sampled for determining nutritional quality namely total sugars, protein and fat contents. The following determinations were expressed as percent of dried matter basis.

Determination of total sugars, protein and fat contents

Total sugars

Total sugars were extracted and quantified by the methods described by James (1995). One gram of dried ground coffee seed was added into 10 mL of 1.5 N H_2SO_4 solution (RCI Labscan, Thailand). The sample was boiled at 100 °C for 20 minutes in water bath (Waterbath WNB, Germany) and cooled rapidly thereafter. Twelve mL of 10% NaOH solution was added into the mixture and stirred thoroughly prior to filtrating through filter paper (Whatman no.4). The sample's volume was made up to 100 mL using distilled water. One mL of the aforementioned mixture was again adjusted to 250 mL by distilled water. Lastly, one mL of sample was used to determine total sugars by adding 1 mL of DNS solution and 2 mL of distilled water. The mixture was boiled at 100 °C for 5 minutes and cooled rapidly prior to adjusting final volume to 25 mL by distilled water. The solution was collected an absorbance at 540 nm by spectrophotometer (Genesys 20 Thermo Science, USA) and calculated against total sugars standard curve.

Protein content

Protein content in coffee seed extract was determined by Lowry method (Lowry et al., 1951). Protein extract was prepared by homogenizing 1 g of dry coffee seed with 10 mL of 20% trichloro acetic acid and centrifuged at 6000 rpm for 15 minutes. Supernatant was discarded prior to adding 5 mL of 0.1 N NaOH into the residue and letting to centrifuge at 6000 rpm for 15 minutes. Supernatant was thereafter collected and used for protein content analysis.

For analysis, dissolving 2 g of Na_2CO_3 in 100 mL of 0.1 N NaOH (reagent A), and mixing 1 mL of 0.5% $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ and 1 mL of 1% sodium potassium tartrate solution (reagent B). Reagent C was obtained by mixing reagent A and reagent B at a ratio of 1 : 1. An amount of 0.5 mL of supernatant collected from the previous paragraph was added into 5 mL of reagent C and left at room temperature for 10 minutes. Folin-Ciocalteu reagent (Fluka, Switzerland) (0.5 mL) was added into the solution and strongly stirred prior to keeping the sample under dark lighting condition for 20 minutes. Absorbance was recorded at 660 nm by spectrophotometer (Genesys 20 Thermo Science, USA) and calculated against protein standard curve.

Fat content

Fat content of coffee seed was extracted and quantified according to AOAC (2000) using a continuous solvent extraction of Soxhlet apparatus (Gerhardt, Germany). Dried ground coffee seed sample was weighed for 2 g and put into a thimble and placed in a Soxhlet extraction tools. Fat content was extracted by 200 mL of dichloromethane solvent (RCI Labscan, Thailand) for 8 hours. Sample was subsequently evaporated, dried and weighed. Percentage of fat was calculated using the following equation:

$$\text{Fat content (\%)} = 100W \times (W2 - W1)$$

Where $W2$ = Weight of extraction flask and oil extract

$W1$ = Weight of empty extraction flask

W = Weight of sample

Statistical Analysis

In order to obtain the effect of BS application on Arabica coffee seed germination, the experiment was designed as 4×5 factorial in completely randomized design. Duncan's multiple range test (DMRT) was used to interpret significant difference among the means ($P < 0.05$).

Results and discussion

Total sugars

Total sugars in coffee seed tended to increase during early germination especially for the seeds applied by 1.0 mg/L BS, and resulted in significantly higher amount of total sugars (210.90 mg/g DW) among all BS treatments. Total sugars content had the increased tendency during day 2 to day 8 after germination. Herein, the final amount of total sugars in germinated coffee seeds was significantly higher than the amount of non-germinated seeds, which were 239.79 and 116.37 mg/g DW, respectively. The results confirmed that total sugars in coffee seed endosperm sharply increased immediately after imbibitions had started. The endosperm is the principal reserve tissue of the seed where utmost chemical compounds are preserved for embryonic growth and development (Borem et al., 2008). The stored compounds are degraded, commonly used for respiration and synthesis of new cells prior to developing embryo during early germination (Bewley and Black, 1994; Vidal-Valverde et al., 2002). In germinated seeds, several nutrients markedly increase, such as soluble carbohydrates and proteins (Gulewicz et al., 2008). In the case

of carbohydrate, sucrose is a major sugar found in coffee seeds and considered as a crucial precursor for producing coffee aroma, flavor and color of roasted coffee (Flament, 1968; Kolling-Speer and Speer, 2005). Moreover, when roasted, sugars are essential to Maillard reaction (when reacted with amino acids in order to form aliphatic acids, hydroxy-methylfurfural, pyrazine and carbonyl compounds) which contribute uniqueness to coffee (Grosch, 2001). Interestingly, our obtained results found that total sugars of coffee seeds in all treatment were considerably doubled from their initial values (**Table 1**).

Significant interactions between BS application and germination time were observed. Coffee seeds treated by 1.0 and 2.0 mg/L BS showed the greatest total sugars at day 8 of germination (255.41 and 253.40 mg/g DW, respectively). The BS application at these aforementioned concentrations might enhance seed germination, thus resulting in marked increase in soluble sugars (Clouse and Sasse, 1998; Gulewicz et al., 2008). Similar results were reported by Satyanarayana et al. (2011) whom studied the changes of chemical compositions during gum karaya (*Sterculia urens* Roxb.) seed germination. The authors found that soluble sugars increased rapidly within 6 days of germination since growing plant tissues typically needed energy from sugar-based respiration. Generally, endogenous BRs appear to help break seed dormancy and enhance germination via inducing endosperm rupture and catabolism of preserved metabolites (Yokota et al., 1996; Leubner-Metzger, 2003).

Protein content

Application of BS greatly affected protein content in germinated coffee seeds. The seeds treated by 1.0 and 2.0 mg/L BS (101.71 and 100.23 mg/g DW, respectively) had significantly higher protein contents than ones treated by 0 and 0.5 mg/L BS (**Table 1**). The content significantly increased during the half period of early germination (within 4 days). The peaks of protein contents in all treated seed clearly appeared on the same day, however, at different amounts. Kalinich et al. (1986) suggested that BS probably enhanced notable enzymes activities, RNA and DNA polymerases that engaged in plant physiological responses to BRs hormone family. Those activated polymerases helped to increase level of protein content in certain tissue. The authors also reported that BRs application effectively triggered protein synthesis in pea (*Phaseolus vulgaris*) and lupin (*Lupinus angustifolius*) seeds. Nonetheless, our results showed that after protein contents reached their peaks, they began to decline continually. This might be due to embryonic development and/or new cells synthesis (Vidal-Valverde et al., 2002). In typical Arabica coffee seeds, protein content represents about 10% of dry weight with 45% of storage proteins (Rogers et al., 1999). Glutamic acid and asparagine are the major free amino acids found in coffee seed endosperm (Arnold and Ludwig, 1996). Sangronis et al. (2006) suggested that metabolic enzyme called proteinases are activated during germination, thus free amino acids are literally freed. The amino acids typically react with sugars through "Maillard" reactions, thus volatile compounds are subsequently formed when roasted (Maillard,

1912; Montavon et al., 2003).

The interactions among combinations were also obtained from 2 experimental factors. BS application at levels of 1.0 and 2.0 mg/L provided the greatest amount of protein contents at day 4 of germination (135.74 and 134.27 mg/g DW). Typically, protein breakdown occurs during germination as newly developed cells use storage proteins as nitrogen and carbon sources for metabolic synthesis (Wang et al., 2007). BS, as previous discussed, probably enhanced protein synthesis (enzymes) and protein breakdown of storage protein preserved in vacuole via an increase activation of proteinases (Sangronis et al., 2006; Wang et al., 2007). These phenomena coincided with several literatures who studied on germinated rice and brown rice (*Oryza sativa*) (Trachoo et al., 2006), sweet lupin (*Lupinus angustifolius* L.) (Rumiyati et al., 2012), soybean (*Glycine max*) (Mostafa et al., 1987) and mung bean (*Phaseolus vulgaris*) (Sangronis and Machado, 2007).

Fat content

It was found the tendency of fat content during early germination of Arabica coffee seeds. Lipids are typically stored in coffee seed endosperm (Wolfrom and Patin, 1964; Da Silva et al., 2004). Stephanucci (1979) reported that Arabica coffee seed has lipid content about 14 g/100 g dry matter. Lipid fraction of coffee is composed mainly of triacylglycerols (approximately 75%), free fatty acids, sterols, unesterified and esterified with fatty acids, and tocopherols (Kolling-Speer and Speer 2005; Folstar, 1985). The fatty compounds typically enhance coffee beverage palatability in terms of flavor and aroma

when roasted. (Vaast et al., 2006). Our results showed that fat content tended to decline especially in the later period of early germination. BS application at concentrations of 1.0 and 2.0 mg/L significantly delayed fat content diminutions. However, it indicated that no significant interaction between BS application and germination time. Nonetheless, the application of BS at 1.0 and 2.0 mg/L provided statistically higher seed fat contents (96.98 and 95.01%, respectively) than the uses of lower BS concentrations (88.37 and 89.02%, respectively). In the case of germination date, the fat content appeared its peak at 4 days after germination (105.33%) (Table 1).

Brassinosteroids family is known to promote endosperm rupture in several plant seeds, such as tobacco (*Nicotiana tabacum*) (Leubner-Metzger, 2001) and mung bean (*Phaseolus aureus*) (Srivastava et al., 2011). They also promote enzymes involving carbohydrate, protein and fat catabolism. In which, during germination, fatty acids are usually oxidized to carbon dioxide and water for developing embryonic cells (Hahm

et al. 2008). Our results of fat content corresponded to Satyanarayana et al. (2011) who found that lipid content increased during initial stage of germination (day 0 to 6) and gradually declined afterward. Likewise, Rumiyati et al., (2012) discovered that germination of Australian sweet lupin (*Lupinus angustifolius* L.) seed contributed to 70% fat content diminution while protein content increased by 38%. Several studies also reported similar trends of fat content in germinated seeds, for examples, mung bean (*Phaseolus vulgaris*) (El-Adawy et al., 2003), chick pea (*Cicer arietinum*) (Ghavidel and Prakash, 2007) and sesame (*Sesamum indicum*) (Hahm et al., 2008).

In conclusion, the application of BS seems to enhance the nutritional composition in early germination of Arabica coffee seed. This finding might be benefit for coffee beverage because the nutritional compounds directly help to promote coffee aroma and flavor of coffee beans when roasted.

Table 1 Effect of different brassin-like substance concentration and germination time on the total sugars, protein and fat contents of early germinated Arabica coffee seeds

BS (mg/L)	Total sugars (mg/g DW)					
	0 DAG	2 DAG	4 DAG	6 DAG	8 DAG	Mean (BS)
0	116.52 ± 0.90 ^J	163.04 ± 1.40 ^I	171.56 ± 0.99 ^H	207.19 ± 0.94 ^E	224.80 ± 3.37 ^C	176.62 ± 18.81 ^D
0.5	116.0 ± 0.79 ^J	174.59 ± 0.60 ^H	193.24 ± 1.16 ^F	210.00 ± 1.69 ^E	225.57 ± 0.79 ^C	183.94 ± 18.92 ^C
1.0	116.11 ± 0.91 ^J	227.19 ± 4.04 ^{BC}	224.41 ± 2.23 ^C	231.38 ± 2.04 ^B	255.41 ± 1.99 ^A	210.90 ± 24.32 ^A
2.0	116.55 ± 0.91 ^J	186.44 ± 0.79 ^G	197.49 ± 1.06 ^F	214.74 ± 0.60 ^D	253.40 ± 0.92 ^A	193.72 ± 22.39 ^B
Mean (DAG)	116.37 ± 0.10 ^e	187.81 ± 13.97 ^d	196.67 ± 10.85 ^c	215.83 ± 5.41 ^b	239.79 ± 8.45 ^a	
	Protein content (mg/g DW)					
	0 DAG	2 DAG	4 DAG	6 DAG	8 DAG	Mean (BS)
0	73.91 ± 3.03 ^H	81.20 ± 4.16 ^{HI}	102.96 ± 6.12 ^{CDE}	77.17 ± 1.86 ^I	75.19 ± 6.98 ^I	82.08 ± 4.80 ^C
0.5	74.14 ± 4.54 ^I	100.74 ± 3.76 ^{CDEF}	116.71 ± 2.24 ^B	95.25 ± 1.49 ^{EFG}	77.99 ± 4.87 ^{HI}	92.96 ± 6.95 ^B
1.0	74.55 ± 4.18 ^I	108.44 ± 1.65 ^{BC}	135.74 ± 4.94 ^A	97.53 ± 3.67 ^{DEFG}	92.34 ± 2.44 ^{FG}	101.71 ± 9.05 ^A
2.0	73.85 ± 2.93 ^I	107.39 ± 3.15 ^{BCD}	134.27 ± 2.89 ^A	97.24 ± 2.36 ^{DEFG}	88.43 ± 2.71 ^{GH}	100.23 ± 9.06 ^A
Mean (DAG)	74.10 ± 0.16 ^e	99.44 ± 6.32 ^b	122.42 ± 7.79 ^a	91.79 ± 4.90 ^c	83.48 ± 4.10 ^d	
	Fat content (mg/g DW)					
	0 DAG	2 DAG	4 DAG	6 DAG	8 DAG	Mean (BS)
0	97.71 ± 0.25	93.50 ± 6.26	104.26 ± 4.82	77.30 ± 2.91	69.07 ± 2.42	88.37 ± 6.56 ^B
0.5	98.02 ± 0.88	96.04 ± 0.37	104.72 ± 5.24	77.44 ± 0.59	68.86 ± 6.19	89.02 ± 6.77 ^B
1.0	97.52 ± 0.88	100.10 ± 2.76	104.90 ± 9.81	91.77 ± 8.45	90.62 ± 6.96	96.98 ± 2.65 ^A
2.0	97.84 ± 1.81	99.02 ± 2.71	107.45 ± 4.32	88.42 ± 2.97	82.29 ± 4.60	95.01 ± 4.38 ^A
Mean (DAG)	97.77 ± 0.11 ^b	97.17 ± 1.49 ^b	105.33 ± 0.72 ^a	83.73 ± 3.74 ^c	77.71 ± 5.33 ^c	
	Total sugar		Protein content		Fat content	
BS	*		*		*	
DAG	*		*		*	
BS × DAG	*		*		NS	
C.V. (%)	2.14		9.82		8.67	

Means within the column followed by different uppercase letters, means within the row followed by different lowercase letters, and interaction effects means followed by different italic uppercase letters showed significant difference by Duncan's multiple range test (DMRT) at $P < 0.05$.

"*" Significant difference between treatments (BS), days after germination (DAG) and interaction effects.

"NS" No significant difference.

Conclusion

BS soaking treatment effected on nutritional compounds of Arabica seed during early germination. Total sugars and protein content were promoted by using BS concentrations of 1.0

and 2.0 mg/L. Protein content showed the greatest peak within 4 days in seeds treated by BS 1.0 and 2.0 mg/L. Fat content slightly declined in BS-treated seeds. Soaking Arabica coffee seed with BS solution had potential to enhance seed quality at early germination period.

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