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Morphological and Chemical Characteristics of Black Gram (*Vigna mungo* L.) Sprouts Produced in a Modified Atmosphere Chamber at Four Seeding Densities

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ABSTRACT

A modified atmosphere with low oxygen and high carbon dioxide sprouting environments can be used to regulate sprout growth. By monitoring seeding density used for sprouting, stress ethylene is produced due to the compactness within sprouting pot. A study on the morphological and chemical characteristics and the total phenolic compounds of black gram sprouts (*Vigna mungo*), grown in a hermetically sealed chamber, was conducted using four seeding densities (50, 75, 100, and 125 g seeds L^{-1}). For this purpose, the selected pre-soaked seeds were put into a pot and placed in the chamber. The seeds were allowed to sprout for four days and watering was done every three hours for 20 minutes. Sprouts produced with lower seeding density (50 and 75 g seeds L^{-1}) were long and etiolated with long roots and higher sprout weight. In higher seeding density (100 and 125 g seeds L^{-1}), on the contrary, sprout length was shorter with short roots and lower sprout weight, and the ratio between hypocotyl and root length was 1:1. Meanwhile, there were no significant differences in hypocotyl diameter of sprouts produced. Sprouts produced at a lower seeding density had 2.7% lower soluble solids concentration, but they had 18% higher contents of ascorbic acid as compared to the ones produced in higher seeding density. Similarly, there were significant differences in the total phenolic compounds of sprouts and the contents decreased by 43% as the seeding densities increased. Over-crowding and lack of watering produced poor quality sprouts. Thus, seeding density and watering duration during sprouting in a hermetically sealed chamber need to be determined for sustainable sprout production, as well as to produce safe sprouts as demanded by consumers.

Keywords: Ethylene, phenolic compounds, soluble solids concentration, ascorbic acid, cotyledon, sprout length

INTRODUCTION

Bean sprouts or *tauge* are three- to five-day old germinated seedlings of green gram (*Vigna radiata*) or black gram (*Vigna mungo*) which are grown as soilless culture in darkness. The total amount of bean sprouts produced in Malaysia is estimated to be about 12 million metric tons a year, with an annual value of RM144 million. Bean sprouts can be cooked in many ways or eaten raw. They are rich in vitamins, minerals, and have been reported to contain important phytochemicals for disease prevention and health promoting benefits (Fernandez-Orozco *et al*., 2008). Bean sprouts are particularly popular in Asian cuisine and the consumption is increasing in Western countries.

Sprouting activates enzyme processes to sustain the later growth stages (Fernandez-Orozco *et al*., 2006). These chemical changes

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increase nutritive values and health qualities of sprouting seeds (Plaza *et al*., 2003). Storage nutrients such as concentrated starch and protein are mobilized into simpler carbohydrates and free amino acids (Ghazali and Cheng, 1991), which can be readily used by the human body (Sattar *et al*., 1988). Several studies have reported higher levels of amino acids, digestive protein, and available carbohydrates, as well as lower levels of non-nutritive factors such as trypsin inhibitors, phytic acid, and α-galactosides in legume sprouts as compared to their non-germinated seeds (Fernandez-Orozco *et al*., 2008; Vidal-Velverde *et al*., 2002).

Bean sprouts are conventionally grown in drums (blue-plastic or stainless steel), tanks (fibre-glass) or bins (garbage) and watered manually using rubber hoses or buckets. The sprouts produced are normally of poor quality with long (10-12 cm) and thin (1-1.5 mm) hypocotyls and long (7-8 cm) roots. A non-registered chemical known as 'Santoso' is widely used by local growers to produce bean sprouts with short (5-6 cm), thick (2-3 mm) and crispy hypocotyls, and short (1-2 cm) roots. Such short-rooted sprouts are highly demanded by Malaysians since they can just be cooked directly without having to remove their undesirable looking roots.

A preliminary work to study the use of 2,4-Dichlorophenoxyacetic acid (2,4-D) and Benzylaminopurine (BAP) as soaking solution on the growth characteristics of sprouts resulted in rootless sprouts with abnormal radial expansion at the hypocotyl which was similar to commercial sprouts. Bean sprouts that are too stunted and with over swollen hypocotyls are usually found among a batch of sprouts sold in the market, indicating indiscriminate and excessive use of this particular non-registered chemical. As indicated earlier, the sprouting process takes only three to five days. Thus, sprouts produced contain chemical residues that could be hazardous to human health in the long term.

Seeding density, which is the amount of seeds per litre of container, is important in sprout production as it determines sprout quality (Chang and Yeh, 1984). Low seeding density causes poor growth and produces thin and long sprouts. Meanwhile, high density causes overcrowding which results in short, curved sprouts and non-germinated seeds (Lee *et al*., 2007). Studies showed that ethylene is produced during seed germination and it acts as a growth inhibitor with the triple-response effects of dark-grown seedlings of *Pisum sativum* and *Arabidopsis* (Guo and Ecker, 2004). These responses consist of thickened hypocotyl, inhibition of both hypocotyl and root elongation, as well as exaggerated apical hook formation. The concentration of endogenous ethylene produced affects the quality of sprouts.

A hermetically sealed chamber was designed and constructed (Ahmad and Mohamed, 1988), and it could produce a modified environment for sprout growth. However, the chamber has not been fully tested and utilized commercially. Sprouting in the modified chamber environment has also been shown to produce high phenolic compounds as reported for tomatoes (Kubota *et al*., 2006). In plants, phenolic compounds are secondary metabolites which are synthesized through the glycolytic pathway, pentose phosphate pathway, shikimate and phenylpropanoid pathways (Randhir *et al*., 2004). They function as antimicrobial, antioxidant or chemical toxins in plant and repel would-be predators (Mccue and Shetty, 2001). Phenolics have nutraceutical importance to human who consumed plants containing them (Parr and Bolwell, 2000; Hasler, 1998). They are able to scavenge free radicals through electron-donating properties, generating a relatively stable phenoxyl radical or non-radical species (Lu and Foo, 2001).

The objective of this study was to determine the effects of seeding density, during sprouting in a chamber, on the morphological and chemical characteristics and the total phenolic compounds of sprouts. This is because the seeding density that produces sprouts with the optimum

morphological and chemical characteristics and total phenolic compounds could be used for commercial sprout production, and more importantly, without the use of any unregistered chemical to produce safe and desirable bean sprouts for consumption in Malaysia.

MATERIALS AND METHODS

Seeds and Sprouting Process

The black gram seeds used in the present study were those imported from Myanmar and purchased from a seed supplier in Malacca. These seeds were selected to remove imperfect seeds (e.g. broken or empty seeds) and inert matters (small stones, soil particles, and other debris), washed and sterilized in 10% sodium hypochlorite solution (NaCIO) (Clorox®) for 10 min. Then, the selected seeds were soaked in 150 mgL^{-1} calcium ($CaNO_3$) solution at room temperature for 12 h. Wholesome and fully imbibed seeds were put into a plastic pot (19.5 cm height x 16.5 cm diameter) which was perforated with holes (2 cm distance between the holes of 0.5 cm in diameter) at the bottom for drainage purposes.

Four seeding densities (50, 75, 100, and 125 g seeds L^{-1}) of soaked bean seeds, were put into each pot. The pots were arranged randomly in a stainless steel chamber equipped with an automatic watering system (Ahmad and Mohamed, 1988). Then, the chamber was hermetically sealed. The seeds were let to sprout in the chamber for four days, at 22-25°C and with 95-99% relative humidity. The sprouting seeds were watered for 20 minutes with a total volume of 9.6 L water at every three-hour interval. The sprouts were harvested after four days of sprouting for both the morphological and chemical characteristics measurement. Prior to sampling, the sprouts were separated into three equal layers, namely, top, middle, and bottom parts of each pot. Representative samples for the physical and chemical analysis were taken from the middle and bottom layers only, while the sprouts from the top layer were discarded because they were thin, long, and yellow.

MORPHOLOGICAL CHARACTERISTICS MEASUREMENT

Fifty sprouts were randomly selected in this study. The length of these sprouts was measured from the root to the base of the cotyledon, while the hypocotyl length was measured from just above the root to the base of the cotyledon. The length of root was measured from the end of the hypocotyl to the end of the root, while the hypocotyl diameter (thickness of sprout hypocotyls) was measured at the centre of each hypocotyl using an electronic calliper. The sprout fresh weight (i.e. the average fresh weight of 50 sprouts) and the cotyledon fresh weight (i.e. the average fresh weight of 50 cotyledons) were also determined.

CHEMICAL CHARACTERISTICS MEASUREMENT

Soluble Solids Concentration (SSC)

The soluble solids concentration (SSC) was determined according to Dadzie and Orchard (1997). Twenty gram of samples and 80 mL distilled water were homogenized with a blender (MX-798S, National, Malaysia) and filtered. Soluble solid concentration (SSC) was measured by dropping 1-2 drops of filtrate on the glass prism of a hand refractometer (Model N-1E, Atago, Japan). The reading was recorded and % SSC was calculated using the following formula:

[(refrectometer reading x dilution factor) + 0.28]

Meanwhile, the dilution factor was used as the sample was diluted in distilled water and the value was calculated using the following formula:

$$\frac{1 + \text{water volume (ml)}}{\text{Sample weight (g)}}$$

Titratable Acidity (TA)

For the titratable acidity measurement (Ranganna, 1977), 2 drops of 1% phenolphthalein was added into 5 mL of filtrate remaining from the SSC determination. The filtrate was titrated with

0.1 M NaOH until the colour turned pink. The results were converted to % citric acid using the following formula:

$$\frac{\text{(ml NaOH x 0.1 M NaOH x titrate vol (100 mL) x 64 g (equivalent weight of citric acid) x 100)}}{\text{sample weight (20 g) x sample vol for titration (5 mL) x 1000}}$$

pH

The balance of the filtrate from the determination of titratable acid was also used for the pH measurement using a pH meter (GLP 21, Crison, Barcelona). The glass electrode of pH meter was calibrated with buffer at pH 4.0, and this was followed by pH 7.0 before it was used. The glass electrode was washed with distilled water after calibration and then wiped with a soft tissue paper. It was then placed in the filtrate and a stabilized pH reading was recorded.

Ascorbic Acid (AA)

For the ascorbic acid determination (Ranganna, 1977), 20 g of sprout sample were homogenized with 80 mL of 3% cool metaphosphoric acid in a blender (MX-798S, National, Malaysia). Similarly, five ml from the filtrate was titrated with 2,6-diclorophenol-indophenol dye solution until the colour turned pink. The concentration of ascorbic acid was recorded as ascorbic acid (mg/100 g) using the following formula:

$$\frac{\text{ml-dye used x dye factor x titrate vol (100 mL) x 100}}{\text{sprout weight (20 g) x sample vol for titration (5 mL)}}$$

TOTAL PHENOLIC COMPOUNDS (TPC)

The total phenolic compounds were determined using Folin-Ciocalteau reagent according to the procedure proposed by Lin and Lai (2006). The harvested sprouts were freeze-dried, blended with a blender (MX-798S, National, Malaysia) and the fine powder obtained was stored in darkness in a freezer at -10°C until further use. The sprout powder (5 g) was suspended in 80% methanol solution (100 mL) and extracted at 60°C in a water bath with continuous shaking for 2 hours. After that, the extracted solvent was filtered through a filter paper. The filtrate (100 µL) was diluted with 100 µL of MeOH/0.3% HCl (6:4, v/v), mixed with 2% Na_2CO_3 solution (2 mL) and kept for 2 min. After the addition of 100 µL of 50% Folin-Ciocalteu's reagent to the mixture, the filtrate was incubated for 30 min at room temperature in the dark. The filtrate was vortexed and the absorbance at 750 nm was measured using a spectrophotometer (S1200 spectrawave, Cambridge, England). The determination of the TPC amount was repeated in triplicates and calculated from the standard curve of gallic acid (1-20 mgL^{-1}) (R^2=0.98) to give an absorbance range of 0.326-1.055. The results were expressed as gallic acid equivalents (mg gallic acid per g of dry meal).

EXPERIMENTAL DESIGN AND STATISTICAL ANALYSIS

The experiment was laid out in a completely randomized block design in order to determine the effects of seeding density on the morphological and chemical characteristics, as well as the total phenolic compounds of sprouts produced. Three out of the ten pots produced in the chamber of each seeding density were used for the analysis. The data were analyzed using the analysis of variance and the means were separated using the Duncan's multiple range tests at $P \leq 0.05$. Meanwhile, the regression analysis was carried out to describe the relationship between the morphological and chemical characteristics at different seeding densities. The correlation analysis was performed to indicate the strength of relationship within the morphological and chemical characteristics.

RESULTS AND DISCUSSION

Based on the data gathered in the experiment, there was a significant ($P \leq 0.01$) quadratic decrease in the length of sprouts as seeding density was increased (*Fig. 1A*). Initially, the sprout length showed a linear decrease when

50 to 75 g seeds L^{-1} were used for sprouting. However, as the seeding densities increased, the sprout length gradually decreased. There were also significant ($P \leq 0.01$) quadratic decreases of hypocotyl and root length (*Figs. 1B* and *1C*). Similarly, the lengths of hypocotyl and root showed linear decreases as seeding density was increased from 50 to 75 g seeds L^{-1} seeds and the length gradually grew shorter as the seeding densities increased.

The significant positive correlation (r^2=0.98) between the length of hypocotyls and root length indicated that a decrease in the hypocotyl length was followed by a decrease in the root length (Table 1). From the data obtained, the ratio between hypocotyl and root length was 1:1. These findings showed that during sprouting, reserves from cotyledon were used for both the growth of hypocotyl and root. In the correlation analysis, the length of sprouts had significant and positive correlations with the length of root

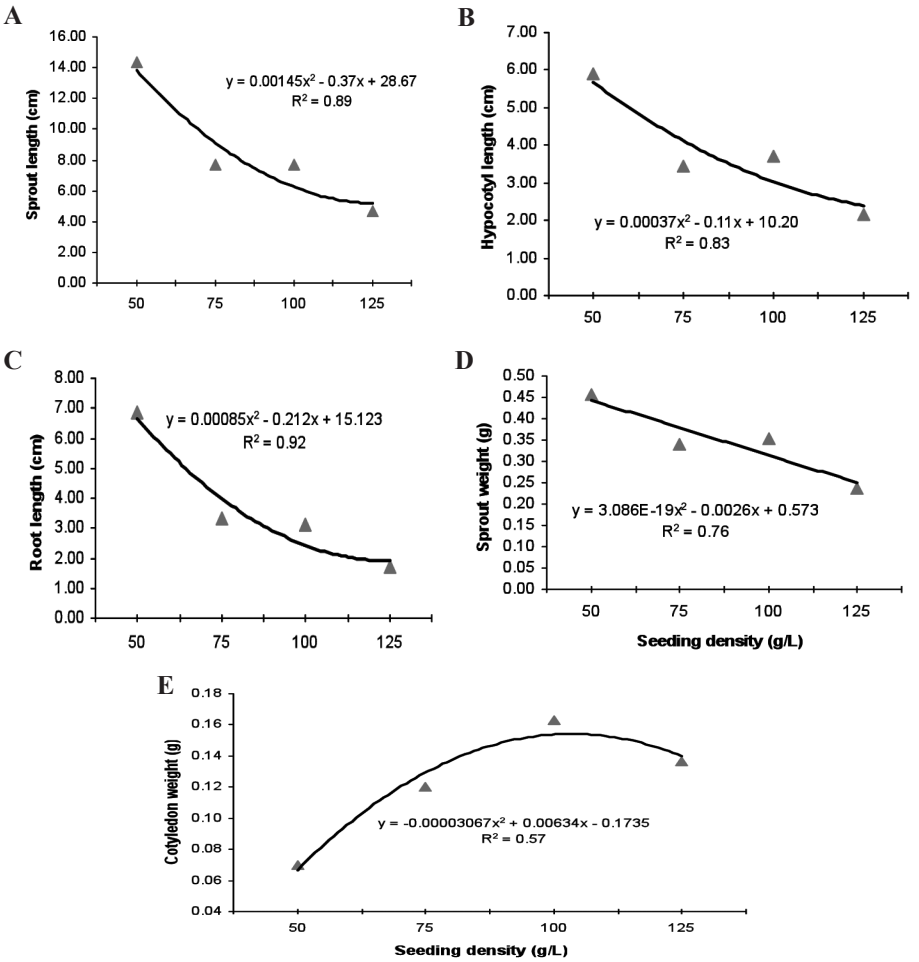

Fig. 1: The physical characteristics of sprouts produced; (A) sprout length, (B) hypocotyl length, (C) root length, (D) sprout weight, and (E) cotyledon weight. Data shown are the mean of 3 replicates of 100 sprouts (per replicate) collected from the two layers of sprouts in the sprouting pot

(r^2=0.99) and hypocotyl (r^2=0.98). Therefore, in this study, the increase in seeding densities resulted in shorter hypocotyl and root lengths of sprouts which affect the length of sprouts produced.

The decrease in the length of sprouts could be related to the ethylene effect during sprouting. When seeding density was increased, the sprouts grew closer to one another, and this led to compaction within the sprouting pot, and induced production of stress ethylene (Ahmad, 1985). The higher the seeding density used, the higher the production of stress ethylene would be. Several studies showed that restricting the elongation of soybean seedlings with a strain gauge resulted in a 3-7 fold increase in the ethylene production rate from hypocotyl which retarded elongation and increased hypocotyl expansion (Abeles *et al*., 1992). Meanwhile, pea epicotyls that were enclosed in the chambers, where the elongation was restricted by glass beads, were found to increase ethylene evolution that in turn induced radial expansion and decreased internode length (Abeles *et al*., 1992).

THE EFFECT OF SEEDING DENSITY AND WATERING DURATION ON HYPOCOTYL DIAMETER

Nonetheless, hypocotyl diameter was not affected by seeding densities as there was no significant linear or quadratic relationship found between hypocotyl diameter of sprout and seeding density used. The correlation analysis revealed that the relationships between the hypocotyl diameter with sprout length (r^2=0.42), hypocotyl length (r^2=0.44), root length (r^2=0.43), sprout weight (r^2=0.56), and cotyledon weight (r^2=0.15) were low (Table 1).

THE EFFECT OF SEEDING DENSITY AND WATERING DURATION ON SPROUT AND COTYLEDON FRESH WEIGHT

The results obtained indicated that there was a significant ($P \leq 0.01$) quadratic decrease of sprout weight as seeding density increased (*Fig. 1D*). When the seeding densities increased, the sprout weight reduced. The decrease of sprout weight was found to be related to the lengths of

TABLE 1
Correlation coefficients between sprout length (SL), hypocotyl length (HL), root length (RL), hypocotyl diameter (HD), sprout weight (SW) and cotyledon weight (CW) of the sprouts produced in four seeding densities

	SL	HL	RL	HD	SW	CW
SL	-					
HL	0.99*	-				
RL	0.99*	0.98*	-			
HD	0.42^{ns}	0.44^{ns}	0.43^{ns}	-		
SW	0.92*	0.95*	0.91*	0.56^{ns}	-	
CW	-0.60*	-0.55^{ns}	-0.61*	0.15^{ns}	-0.36^{ns}	-

For correlation coefficient, n=12.

^{ns} and * non-significant, significant at $P \leq 0.05$, respectively

sprouts, hypocotyl, and root. The correlation analysis also demonstrated that there were significant positive correlations between the weight of sprouts and sprout length (r^2=0.92), hypocotyl length (r^2=0.95), and root length (r^2=0.91). However, no significant correlation (r^2=0.56) was shown between sprout weight and hypocotyl diameter. This finding indicated that sprout weight was not affected by the diameter of hypocotyl.

Meanwhile, the decrease in sprout weight as a result of the increase in seeding densities indicated that when more seeds were used in the study, shorter sprouts were produced. Shorter sprout length in higher seeding densities occurred when there were more seeds in the containers during sprouting and led to over-crowding. In more specific, seeds from the bottom layers were unable to get sufficient water for growth and cooling purposes. In addition, the metabolic heat created during sprouting and the endogenous ethylene produced could also be the reasons that inhibited the sprout growth.

Apparently, there were significant negative correlations between the cotyledon weight with sprout length (r^2=-0.60) and root length (r^2=-0.61) (Table 1). On the other hand, the weight of cotyledon had no significant relationships with the weight of sprouts (r^2=0.36), hypocotyl length (r^2=0.55), and hypocotyl diameter (r^2=0.15). It is important to note that cotyledons are the food storage tissues of the embryo in dicots. The function of cotyledon is to supply the growing seedlings with nutrients until they are capable of producing their own food. In most species, the storage tissues shrivel and drop off as their reserve nutrients deplete (Elias, 2006).

The results from the study showed that the weight of cotyledon was heavier in sprouts from higher seeding densities (*Fig. 1E*). Meanwhile, the correlation analysis revealed that there were significant negative correlations (r^2=-0.60) between the weight of cotyledon and sprout length. In addition, there was also a significant negative correlation (r^2=-0.61) between the weight of cotyledon and length of roots (Table 1).

The decrease in the weight of cotyledon indicated that there was a translocation of soluble products to the axis for respiration (Karunagaran and Ramakrishna Rao, 1991). In the present study, however, no decrease was observed in the weight of cotyledon in higher seeding densities. These could have resulted from reduced mobilization of reserves from cotyledon or from their reduced utilization by the embryo axis (Promila and Kumar, 2000).

Sprouts which are produced under modified environment have shorter and thicker hypocotyls and shorter roots as compared to conventionally grown sprouts (Ahmad, 1985). It was reported that sprouting in an enclosed system restricted oxygen supply by the surrounding structures causing anaerobic respiration in sprouts, and resulting in reduced respiration rate. Peppelenbos and van't Leven (1996) found that bean sprouts displayed a high respiration rate during sprouting. However, high carbon dioxide concentrations, at ambient oxygen concentrations, were found to decrease respiration rates in mungbean sprouts. This was because the internal oxygen was consumed by the respiration of both cells and tissues with carbon dioxide production in the chamber (Varoquaux *et al*., 1996). When the respiration rate of sprouts slowed down, the reserves in the cotyledon were not fully utilized, and thus inhibited sprout growth (Bewley and Black, 1978).

THE EFFECT OF SEEDING DENSITY AND WATERING DURATION ON SOLUBLE SOLID CONCENTRATION, TITRATABLE ACID AND PH

There were significant ($P \leq 0.05$) quadratic increases of soluble solid concentration and pH in the sprouts produced (*Figs. 2A* and *2C*), while titratable acidity showed a significant ($P \leq 0.05$) quadratic decrease with increasing seeding density (*Fig. 2B*). The higher percentage of soluble solids concentration of sprouts, due to the increased seeding densities, also indicated an increased sweetness in sprouts. However, as the seeding densities increased, sprouts

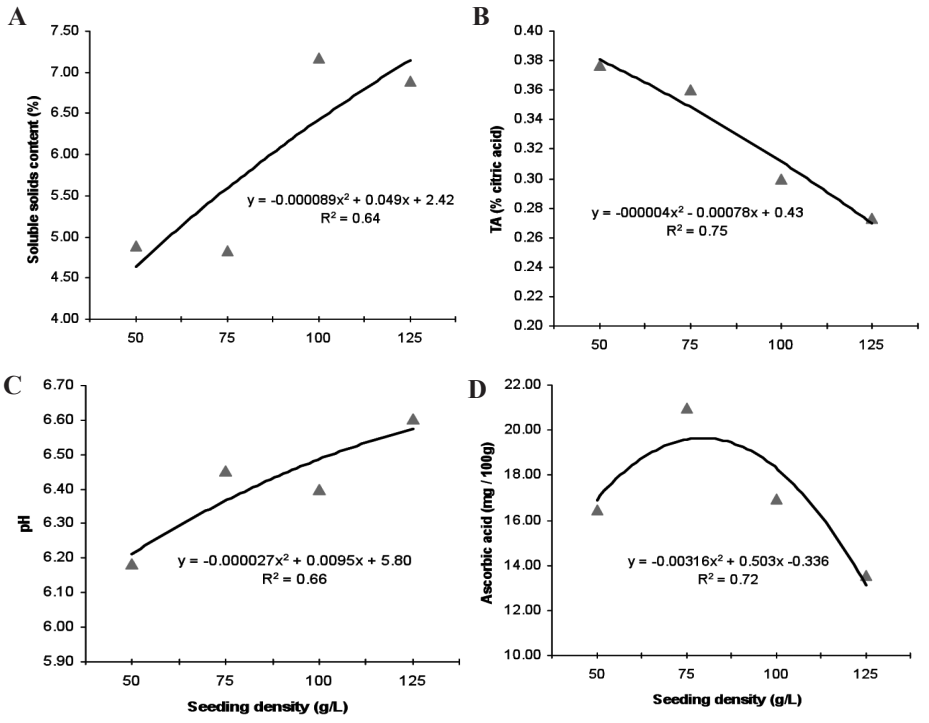

Fig. 2: The chemical characteristics of sprouts; (A) soluble solids concentration, (B) titratable acid, (C) pH, and (D) ascorbic acid. Data shown are the mean of 3 replicates of each treatment

produced showed a decrease of titratable acidity and an increase of the pH value. There was a significant negative correlation (r^2=-0.72) between the soluble solids concentration and titratable acidity (Table 2). The percentage of titratable acidity usually decreases as soluble solid concentration increases because they are respired and converted to starch and sugars (Knee and Smith, 1989).

During germination, the principal reserve materials (starch, proteins, and lipids) are degraded in the cotyledon and the products released are translocated to the developing axis (Karunagaran and Ramakrishna Rao, 1991). From the results, the sweetness of the sprouts was contributed by the contents of soluble sugar in the cotyledon. In more specific, higher cotyledon weight shows that more reserves are contained in it since cotyledon is the part where all the reserves are stored. Meanwhile, sprouting preceded by soaking the seeds in water activates the hydrolytic enzymes (Elias, 2006) and causes the reserve materials of the seeds to be degraded.

α-amylase is the major enzyme involved in the initial degradation of starch into the more soluble forms, while ß-amylase and phosphorylase assist in converting into free sugars in the cotyledon of germinated black gram seeds (Koshiba and Minamikawa, 1981) and mungbean seeds (Toyooka *et al*., 2001). During germination, amylase activity contributes significantly to soluble sugar of the cotyledon.

In horse gram, sugar presents in the cotyledon during the early period of growth is primarily responsible for growth of the embryo axis (Promila and Kumar, 2000) that controls the reserve mobilization in dicotyledonous seeds by affecting α-amylase activities of the cotyledon (De Klark, 1986). A similar situation was also found in mungbean sprouts (Morohashi,

TABLE 2
Correlation coefficients between soluble solids concentration (SSC), titratable acidity (TA), ascorbic acid content (AA) and pH of sprouts produced in four seeding densities

	SSC	TA	AA	pH
SSC	-			
TA	-0.72*	-		
AA	-0.49^{ns}	0.57^{ns}	-	
pH	0.55^{ns}	-0.64*	-0.23^{ns}	-

For correlation coefficient, n=12.
^{ns} * non-significant and significant at P≤0.05, respectively

1982). The degraded reserves are partly used for the respiration and synthesis of the new cell constituents of the developing embryonic axis during sprouting (Vidal-Valverde *et al*., 2002). It is important to note that this process causes important changes in biochemical, nutritional, and sensory characteristics in the sprouts.

In black gram seeds, starch constitutes 47.9% of the seed composition (Srinivasa, 1976). Ghazali and Cheng (1991) reported that the germination of black gram seeds led to a progressive increase in the total sugar content (glucose, sucrose, and fructose) in sprouts. The increase in the total sugar contents has been suggested to be the result of mobilization of oligosaccharides and starch into simple sugar by enzymes (Jaya and Venkataraman, 1980).

In buckwheat sprouts, Kim *et al*. (2004) reported that as seeding days progress, the contents of monosaccharides (fructose and glucose) increased while disaccharides (sucrose and maltose), trisaccharides and tetrasaccharide were decreased. These results illustrated that the di-, tri-, and tetrasaccharide were degraded to monosaccharides during sprouting in the cotyledon to provide the energy for seedling development. Based on the results gathered in the present study, higher cotyledon weight indicated that the soluble sugar in the cotyledon had not been fully used for sprout growth.

THE EFFECT OF SEEDING DENSITY AND WATERING DURATION ON ASCORBIC ACID

There was a significant (P≤0.05) quadratic decrease in the content of ascorbic acid as seeding density increased (*Fig. 2D*). Ascorbic acid is a water-soluble vitamin that acts as an antioxidant protecting against oxidative damage to DNA, membrane lipids, and proteins (Dekkers *et al*., 1996) by reducing small molecule antioxidants such as glutathione, tocopherols, and carotenes (Fernandez-Orozco *et al*., 2006). Germination has been found to increase ascorbic acid level (Kim *et al*., 2004). The increase in ascorbic acid level is due to the consequence of the reactivation of ascorbic acid biosynthesis in the seeds during germination (Mao *et al*., 2005; Xu *et al*., 2005).

In this study, the percentage of ascorbic acid showed the highest level at 75g seeds L^{-1}, while the sprouts from the other three seeding densities yielded lower levels. This might be due to the metabolic heat produced by the sprouting seeds that affected the ascorbic acid content of the sprouts. As the seeding density increased, less water could reach the bottom part of the sprout pot. At the same time, the metabolic heat produced inhibited the biosynthesis of the ascorbic acid, causing low ascorbic acid content of the sprouts produced in higher

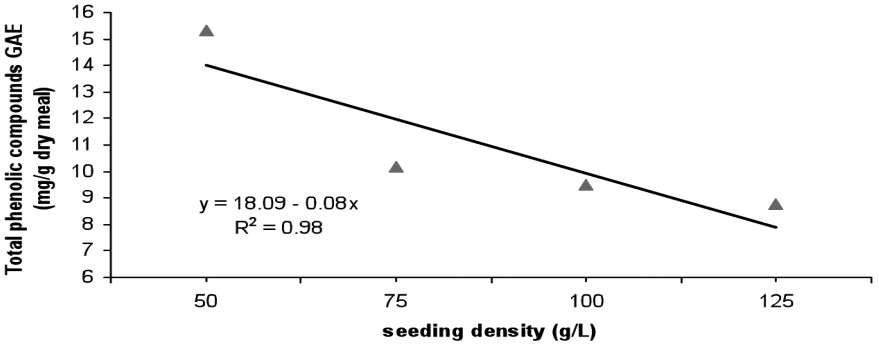

Fig. 3: The total phenolic compounds as GAE (mg/g dry meal) of sprouts. The sprout samples of each treatment from 3 replicates were analyzed in triplicates

seeding densities. Ghazali and Cheng (1991) reported that dry black gram seeds contained only trace amounts of ascorbic acid. However, the content of ascorbic acid increased as the germination proceeded. In lupin sprouts, Frias *et al*. (2005) found that the content of ascorbic acid was increased by 322% after 2 days and this continued to gradually rise up to 9 days where an increment of 866% was observed. In other legumes, such as soybean, Xu *et al*. (2005) found that the germination of soybean seeds for 4 days increased the content of ascorbic acid, but a subsequent germination up to 9 days caused it to decrease. Sattar *et al*. (1988) observed that sprouting temperature affected nutrients (protein, ascorbic acid, riboflavin, and thiamine) and anti-nutrients (phytate and trypsin inhibitor) of mungbean sprouts. Ascorbic acid of mungbean sprouts reached the maximum value after 48 hours of germination, i.e. at 20-35°C.

THE EFFECT OF SEEDING DENSITY AND WATERING DURATION ON THE TOTAL PHENOLIC COMPOUNDS

The TPC estimation is important to determine the antioxidant activity of the sprouts produced. TPC of sprouts produced at 50, 75, 100, and 125 g seeds L^{-1} were 15.3, 10.2, 9.5, and 8.7 of GAE (mg/g dry meal), respectively (*Fig. 3*). There were significant differences ($P \leq 0.05$) in terms of TPC in the sprouts produced using the different seeding densities. The variations of TPC in sprouts were apparently affected by cultivars, test method and environment stresses, such as sprouting temperature and drought (Kim *et al*., 2006). Fernandez-Orozco *et al*. (2008) found that mungbean seeds germinated for 7 days had increased the content of TPC in sprouts.

Randhir *et al*. (2004) stated that germination caused a decrease in TPC of mungbean seeds after 1 day of germination. The higher phenolics produced on Day 1 was utilized for guaiacol peroxidase-mediated polymerization to form polymeric phenolics and lignin during germination. The reduced TPC in the mungbean sprouts in the later stage of germination was due to the partitioning of lignifications and other developmental needs. In this study, sprouts in the higher densities produced lower TPC as compared to sprouts in the lower seeding densities, although they were subjected to a greater stress due to overcrowding. The reduced TPC could be attributed to the high demand for oxygen and phenolic compounds used to protect the cells from oxidation-induced deterioration (Randhir *et al*., 2004).

Xu and Chang (2008) reported that in common beans, the loss of TPC increased with the increasing hydration rate. Meanwhile, longer soaking time caused some polyphenols in the seed coat to be hydrolyzed and diffused into

the water. Thus, further studies on the effect of soaking duration on the sprouts of black bean should be carried out in order to maintain TPC content in sprouts produced.

CONCLUSIONS

In this study, the seeding densities used did not produce the desired sprouts having the characteristics of fat and thick hypocotyl diameter. At the same time, lower seeding densities (50 and 75 g seeds L^{-1}) used were not able to create the compactness in the pot, and thus less ethylene stress was produced. Instead, thin and etiolated sprouts were produced. At high seeding density (i.e. 125 g seeds L^{-1}) the sprouts growth were inhibited. The poor growth and quality of sprouts were due to the over-crowding of the sprouts in the pot, in addition to the condition whereby irrigation water could possibly not reach the bottom part of the sprouting pot and led to partially germinated or non-germinated seeds. Therefore, more studies need to be carried out on seeding density and watering duration during sprouting in a hermetically sealed chamber for sustainable sprout production, as well as produce safe sprouts for consumers.

REFERENCES

Abeles, F.B., Morgan, P.W. and Saltveit Jr, M.E. (1992). *Ethylene in Plant Biology* (2nd Edn.). California: Academic Press.

Ahmad, S.H. (1985). Effects of calcium nutrition and atmospheric carbon dioxide, oxygen and ethylene on the growth and quality of mungbean sprouts. Ph. D. Dissertation. Iowa State University, Ames Iowa, USA.

Ahmad, S.H. and Mohamed, M.T.M. (1988). Effect of atmospheric carbon dioxide, oxygen and ethylene on the growth and quality of mungbean sprouts. In *Proceedings of the Second International Symposium on Mungbean* (pp. 526-545).

Bewley, J.D. and Black, M. (1978). Physiology and biochemistry of seeds. In Relation to germination: Volume 1: *Development, germination and growth* (pp. 177-241). New York: Springer-Verlag.

Chang, D.C.N. and Yeh, W.J. (1984). Investigation and improvement of the sanitary conditions for mungbean sprout production. *Journal of The Chinese Society for Horticultural Science*, *30*(2), 120-125.

Dadzie, B.K. and Orchad, J.E (1997). Routine post-harvest screening of banana/plantation hybrids: criteria and method. In *Inibab Technical Guidelines*. International Plant Genetic Resources Institute.

De Klark, G.J. (1986). Axis control of amylase synthesis and secretion in cotyledons of *Agrostemma githago*. *Acta Botanica Neerlandica*, *35*, 143-152.

Dekkers, J.C., Van Doornen, L.J. and Kemper, H. C. (1996). The role of antioxidant vitamins and enzymes in the prevention of exercise-induced muscle damage. *Sport Medicine*, *21*, 213-238.

Elias, S. (2006). Seed quality testing. In A.S. Basra (Ed.), *Handbook of seed science and technology* (pp. 561-598). New York: Food Products Press.

Fernandez-Orozco, R., Piskula, M.K., Zielinski, H., Kozlowska, H., Frian, J. and Vidal-Valverde, C. (2006). Germination as a process to improve the antioxidant capacity of *Lupinus angustifolius* L. var. Zapaton. *European Food Resources Technology*, *223*, 495-502.

Fernandez-Orozco, R., Frias, J., Zielinski, H., Piskula, M.K., Kozlowska, H. and Vidal-Valverde, C. (2008). Kinetic study of the antioxidant compounds and antioxidant capacity during germination of *Vigna radiate* cv. *Emerald*, *Glycine max* cv. *Jutro* and *Glycine max* cv. *Merit*. *Food Chemistry*, *111*, 622-630.

Frias, J., Miranda, M.L., Doblado, R. and Vidal-Vaiverde, C. (2005). Effect of germination and fermentation on the antioxidant vitamin content and antioxidant capacity of *Lupinus albus* L. var. Multolupa. *Food Chemistry*, *92*, 211-220.

Ghazali, H.M. and Cheng, S.C. (1991). The effect of germination on the physico-chemical properties of black gram (*Vigna mungo* L.). *Food Chemistry*, *41*, 99-106.

Guo, H.W. and Ecker, J.R. (2004). The ethylene signaling and pathway: New insights. *Plant Biology*, *7*, 40-49.

Hasler, C.M. (1998). Functional foods: Their role in disease prevention and health promotion. *Food Technology*, *52*(11), 63-70.

Jaya, T.V. and Venkataraman, L.V. (1980). Effect of germination on the nitrogenous constituents, essential amino acids, carbohydrates, enzymes and antinutritional factors in chickpea and green gram. *Indian Food Packer*, *34*, 3-11.

Karunagaran, D. and Ramakrishna Rao, P. (1991). Model and control of starch mobilization during germination of seeds of horse gram. *Plant Science*, *73*, 155-159.

Kim, S.L., Kim, S.K. and Park, C.H. (2004). Introduction and nutritional evaluation of buckwheat sprouts as a new vegetable. *Food Research International*, *37*, 319-327.

Kim, E.H., Kim, S. H., Chung, J.I., Chi, H.Y., Kim, J.A. and Chung, I.M. (2006). Analysis of phenolic compounds and isoflavones in soybean seeds (*Glycine max* (L.) Merill) and sprouts grown under different conditions. *European Food Research and Technology*, *222*, 201-208.

Knee, M. and Smith, S.M. (1989). Variation in quality of apple fruits stored after harvest on different dates. *The Journal of Horticultural Science*, *64*, 413-419.

Koshiba, T. and Minamikawa, T. (1981). Purification by affinity chromatography of α-amylase a main amylase in cotyledons of *Vigna mungo* seeds. *Plant Cell Physiology*, *22*, 979-987.

Kubota, C., Thomson, C.A., Wu, M. and Javanmardi, J. (2006). Controlled environments for production of value-added food crops with high phytochemical concentrations: Laycopene in tomato as an example. *HortScience*, *41*, 522-525.

Lee, J.D., Shannon, J.G., Jeong, Y.S., Lee, J.M. and Hwang, Y.H. (2007). A simple method for evaluation of sprout characters in soybean. *Euphytica*, *153*, 171-180.

Lin, P.Y. and Lai, H.M. (2006). Bioactive compounds in legumes and their germinated products. *Journal of Agricultural and Food Chemistry*, *54*, 3807-3814.

Lu, Y. and Foo, L.Y. (2001). Antioxidant activities of polyphenols from sage (*Salvia officinalis*). *Food Chemistry*, *75*, 197-202.

Mao, J. X., Dong, J.F. and Zhu, M.Y. (2005). Effects of germination conditions on ascorbic acid level and yield of soybean sprouts. *Journal of the Science of Food and Agriculture*, *85*, 943-947.

Mccue, P. and Shetty, K. (2001). Clonal herbal extracts as elicitors of phenolic synthesis in dark-germinated mungbeans for improving nutritional value with implications for food safety. *Journal of Food Biochemistry*, *26*, 209-232.

Morohashi, Y. (1982). Control of development of amylolytic and proteolytic activities of germinating black gram seeds. *Physiology Plant*, *56*, 189-193.

Parr, A.J. and Bolwell, G.P. (2000). Phenols in the plant and in man. The potential for possible nutritional enhancement of the diet by modifying the phenols content or profile. *Journal of the Science of Food and Agriculture*, *80*, 985-1012.

Peppelenbos, H.W. and van't Leven, J. (1996). Evaluation of four types of inhibition for modeling the influence of carbon dioxide on oxygen consumption of fruits and vegetables. *Postharvest Biology and Technology*, *7*, 27-40.

Plaza, L., de Ancos, B. and Cano, M.P. (2003). Nutritional and health-related compounds in sprouts and seeds of soybean (*Glycine max*), wheat (*Triticum aestivum* L.) and alfalfa (*Medicago sativa*) treated by a new drying method. *European Food Research and Technology*, *216*, 138-144.

Promila, K. and Kumar, S. (2000). *Vigna radiate* seed germination under salinity. *Biologia Plantarum*, *43*(3), 423-426.

Randhir, R., Lin, Y. and Shetty, K. (2004). Stimulation of phenolics, antioxidant and antimicrobial activities in dark germinated mungbean sprouts in response to peptide and phytochemical elicitors. *Process Biochemistry*, *39*, 637-646.

Ranganna, S. (1977). *Manual of Analysis of Fruits and Vegetables Products*. New Delhi: McGraw Hill Pub. Co.

Sattar, A., Durrani, S.K., Mahmood, F., Ahmad, A. and Khan, I. (1988). Effect of soaking and germination temperatures on selected nutrients and antinutrients of mung bean. *Food Chemistry*, *34*, 111-120.

Srinivasa, R.P. (1976). Nature of carbohydrate in pulses. *Journal of Agricultural and Food Chemistry*, *24*, 958-961.

Toyooka, K., Okamoto, T. and Minamikawa, T. (2001). Cotyledon cells of *Vigna mungo* seedlings use at least two distinct autophagic machineries for degradation of starch granules and cellular components. *Journal of Cell Biology*, *154*(5), 973-982.

Vidal-Valverde, Frias, C.J., Sierra, I., Blázquez, I., Lambein, F. and Kuo, Y. (2002). New functional legume foods by germination: Effect on the nutritive value of beans, lentils and peas. *European Food Research and Technology*, *215*, 472-477.

Varoquaux, P., Albagnac, G., The, C. N. and Varoquaux, F. (1996). Modified atmosphere packaging of fresh beansprouts. *Journal of the Science of Food and Agriculture*, *70*, 224-230.

Xu, M., Dong, J. and Zhu, M. (2005). Effects of germination conditions on ascorbic acid level and yield of soybean sprouts. *Journal of the Science of Food and Agriculture*, *85*, 943-947.

Xu, B.J. and Chang, S.K.C. (2008). Total phenolic content and antioxidant properties of Eclipse Black beans (*Phaseolus vulgaris* L.) as affected by processing methods. *Journal of Food Science*, *73*, H19-H27.