UNIVERSITI PUTRA MALAYSIA

EFFECTS OF SEEDING DENSITY AND WATERING DURATION ON SPROUTING ATMOSPHERE, QUALITY CHARACTERISTICS AND ANTIOXIDANTS OF BLACK GRAM (*VIGNA MUNGO* L.) SPROUTS

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CHOON SEA YEAT

Thesis submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfilment of the Requirements for the Degree of Master of Science

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EFFECTS OF SEEDING DENSITY AND WATERING DURATION ON SPROUTING ATMOSPHERE, QUALITY CHARACTERISTICS AND ANTIOXIDANTS OF BLACK GRAM (*VIGNA MUNGO* L.) SPROUTS

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A modified atmosphere containing low oxygen and high carbon dioxide in the sprouting environment could be used to regulate sprouts growth. By manipulating the seeding density and watering duration, the gases atmosphere can be changed. A study on sprouting atmosphere, morphological and chemical characteristics, and phytochemical contents of *Vigna mungo* sprouts grown in a hermatically sealed chamber was conducted using three seeding densities (75, 100 and 125 g seeds L^{-1}) and three watering durations (10, 15 and 20 min with 3 h interval). The experiment was conducted in a RCBD in split-plot arrangement with watering duration as main plot and seeding density as sub-plot in three replications.

Seeds were presoaked in 150 mg Ca L^{-1} for 12 h and put into a pot and the pot was placed into the hermatically sealed chamber to sprout for 96 h. Sprouting atmosphere in the chamber was measured every 12 h throughout the sprouting period to determine the production of carbon dioxide and ethylene gas. Hypocotyl

and root length, hypocotyl diameter, sprout and cotyledon fresh weights, soluble solids concentration, titratable acidity and pH were measured to determine morphological and chemical characteristics of sprouts. Ascorbic acid, total phenolic compounds and antioxidant activities were analyzed to determine phytochemical contents and antioxidant activities of sprouts produced.

There were significant ($P \leq 0.05$) interaction effects of seeding density and watering duration on sprouting atmosphere, morphological and chemical characteristics, and phytochemical contents of sprouts produced. For sprouts that were produced at 75, 100 and 125 g seeds L^{-1}, carbon dioxide production rate showed linear decreases as sprouting progressed. There was a gradual increase of ethylene production rate from 12 to 60 h after imbibition followed by a decrease until the day of harvest.

The sprouting atmosphere modified by different seeding density and watering duration created a modified atmosphere that can be used to regulate sprouts growth. At higher seeding densities, hypocotyl and root length decreased as watering duration increased. Hypocotyl diameter of sprouts produced at 100 g seeds L^{-1} increased as watering duration increased. However, for sprouts that were produced at 75 and 125 g seeds L^{-1}, maximum hypocotyl diameter was produced at 15 min/3 h watering duration and decreased at 20 min/3 h watering duration.

Increased in seeding density and watering duration increased the soluble solids concentration, ascorbic acid content and total phenolic compounds of sprouts produced. Optimum water volume that was suitable for best sprouts growth was at 15 min/3 h watering duration. Too much watering reduced the soluble solids

concentration, ascorbic acid content and total phenolic compounds at higher seeding density used. Sprouts produced at 100 g seeds L^{-1} and 15 min/3 h watering duration had the highest percentage of soluble solids concentration, ascorbic acid content and total phenolic compounds. Thus, by regulating seeding density and watering during sprouting, healthy sprouts with acceptable appearance could be produced without the use of chemicals.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Master Sains

KESAN KEPADATAN BIJI BENIH DAN KEKERAPAN PENYIRAMAN TERHADAP ATMOSFERA TERUBAH SUAI, CIRI KUALITI DAN ANTIPENGOKSIDA BAGI TAUGE KACANG HITAM (*Vigna mungo* L.)

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Atmosfera terubah suai yang mengandungi kandungan oksigen yang rendah dan karbon dioksida yang tinggi semasa percambahan boleh digunakan untuk mengawal kadar pertumbuhan tauge. Dengan menggunakan kepadatan biji benih dan kekerapan penyiraman yang sesuai, atmosfera gas dapat diubahkan. Kajian terhadap atmosphera percambahan, ciri-ciri morfologi dan kimia, dan kandungan fitokimia bagi tauge *Vigna mungo* yang dicambah dalam kebuk kedap udara telah dijalankan dengan menggunakan tiga kadar biji benih (75, 100 dan 125 g biji benih L^{-1}) dan tiga kekerapan penyiraman (10, 15 dan 20 min setiap 3 j). Eksperimen dijalankan dengan rekabentuk 'split-plot' di mana kekerapan penyiraman digunakan sebagai plot utama dan kepadatan biji benih sebagai sub-plot, dalam tiga replikasi.

Biji benih direndam terlebih dahulu dalam larutan 150 mg Ca L^{-1} selama 12 j dan dimasukkan ke dalam bekas percambahan. Bekas percambahan kemudian dimasukkan ke dalam kebuk kedap udara untuk percambahan selama 96 j. Atmosfera percambahan dalam kebuk kedap udara disukat setiap 12 j sepanjang

tempoh percambahan untuk menentukan kadar penghasilan gas karbon dioksida dan etilena. Kepanjangan hipokotil dan akar, diameter hipokotil, berat basah tauge dan kotiledon, kandungan pepejal terlarut, keasidan tertitrat dan pH telah diukur untuk menentukan ciri-ciri morfologi dan kimia tauge yang dihasilkan. Asid askorbik, kandungan fenolik dan aktiviti antipengoksida juga dianalisa untuk menentukan kandungan fitokimia dan aktiviti antipengoksida bagi tauge yang dihasilkan.

Bagi penentuan atmosfera percambahan, ciri-ciri morfologi dan kimia, serta kandungan fitokimia tauge yang dihasilkan, terdapat kesan interaksi yang bererti ($P \leq 0.05$) bagi kepadatan biji benih dan kekerapan penyiraman yang digunakan dalam kajian ini. Tauge yang dihasilkan dengan kepadatan 75, 100 dan 125 g biji benih L^{-1} menunjukkan kadar penghasilan karbon dioksida menurun secara linear sepanjang tempoh percambahan. Kadar penghasilan etilena hanya bertambah beransur-beransur daripada 12 hingga 60 j selepas penyerapan air dan kadar penghasilan mengurang selepas itu sehingga hari penuaian.

Atmosfera terubah suai yang dihasil daripada kepadatan biji benih dan kekerapan penyiraman yang digunakan dapat mengawal kadar pertumbuhan tauge. Pada kepadatan biji benih yang tinggi, kepanjangan hipokotil dan akar berkurangan apabila kekerapan penyiraman meningkat. Diameter hipokotil bagi tauge yang dihasilkan dengan 100 g biji benih L^{-1} meningkat apabila kekerapan penyiraman meningkat. Bagi tauge yang dihasilkan dengan kepadatan biji benih 75 dan 125 g biji benih L^{-1}, diameter hipokotil yang maksimum dapat dihasilkan apabila kekerapan penyiraman 15 min/3 j digunakan dan diameter hipokotil menurun apabila kekerapan penyiraman bertambahn kepada 20 min/3 j.

Peningkatan kepadatan biji benih dan kekerapan penyiraman meningkatkan kandungan pepejal terlarut, kandungan asid askorbik dan kandungan fenolik bagi tauge yang dihasilkan. Kekerapan penyiraman yang sesuai untuk pertumbuhan kualiti tauge yang terbaik adalah pada 15 min/3 j. Penyiraman yang terlalu kerap mengurangkan kandungan pepejal terlarut, kandungan asid askorbik dan kandungan fenolik pada kepadatan biji benih yang tinggi. Tauge yang dihasilkan dengan 100 g biji benih L^{-1} dan kekerapan penyiraman 15 min/3 j mengandungi kandungan pepejal terlarut, kandungan asid askorbik dan kandungan fenolik yang tinggi. Oleh itu, dengan mempelbagaikan kepadatan biji benih dan kekerapan penyiraman, tauge yang selamat dimakan dengan ciri-ciri morfologi yang diterima oleh pengguna dapat dihasilkan tanpa penggunaan bahan kimia.

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2,4-D	:	2,4-dichlorophenoxyacetic acid
AA	:	Ascorbic acid
ABTS	:	2,2'-azinobis-(3-ethyl-benzothiazoline-6-sulfonic acid)
ACC	:	1-aminocyclopropene-1-carboxylic acid
ACO	:	1-aminocyclopropene carboxylic acid oxidase
AOS	:	Active oxygen species
ANOVA	:	Analysis of variance
6-BAP	:	6-Benzylaminopurine
ACS	:	1-aminocyclopropene carboxylic acid synthase
C_2H_4	:	Ethylene
CO_2	:	Carbon dioxide
CRBD	:	Completely randomized block design
CZE	:	Capillary zone electrophoresis
DMRT	:	Duncan's multiple range tests
DPPH	:	2,2-diphenyl-1-picrylhydrazyl
Fe^{III}-TPTZ	:	Ferric-tripyridyltriazine
FID	:	Flame ionization detector
FRAP	:	Ferric reducing antioxidant power
fw	:	fresh weight
GAE	:	Gallic acid equivalents
GC	:	Gas chromatography
h	:	Hour
HPLC	:	High-performance liquid chromatography

HPO_3	:	Metaphosphoric acid
MACC	:	N-malonyl ACC
NaCIO	:	Sodium hypochlorite
NaOH	:	Sodium hydroxide
ns	:	Non significant
O_2	:	Oxygen
r^2	:	Correlation coefficient
ROS	:	Reactive oxygen species
SAM	:	S-adenosyl methionine
SAS	:	Statistical analysis system
SD	:	Seeding density
SSC	:	Soluble solids concentration
TCD	:	Thermal conductivity detector
TEAC	:	Trolox equivalent antioxidant capacity
TPC	:	Total phenolic compounds
TPTZ	:	2,4,6-tris (1-pyridyl)-5-triazine
Trolox	:	6-hydroxy-2,5,7,8-tetramethychroman-2-carboxylic acid
TSS	:	Total soluble solids
WD	:	Watering duration

CHAPTER 1

GENERAL INTRODUCTION

Bean sprouts are dark-germinated seedlings that are rich in dietary fibers, vitamin B and C, beta-carotene, and bioactive compounds. They are commonly served as one of the popular vegetables in Asian cuisine as soup, salad and side dishes (Lin and Lai 2006). The consumption of bean sprout is increasing in western countries (Fernandez-Orozco et al. 2008; Liu et al. 2008) due to their fresh, crunchy and sweet characteristics and health benefits (Fernandez-Orozco et al. 2008). They have been reported to contain important phytochemicals for disease prevention and health promoting benefits. In Malaysia, black gram (*Vigna mungo*) has primarily been used for bean sprouts production. Total amount of bean sprouts produced is estimated to be about 12 million metric tons with an annual value of RM 144 million.

Consumers prefer bean sprouts which are short (5-6 cm), thick (2-3 mm) and white with crispy hypocotyls and short roots (1-2 cm). Sprouts with its seed coat removed from the cotyledons are preferred as fresh vegetable in commercial markets (Kim et al. 2004). The local conventional method of sprout production, using blue plastic drum, fiber glass tank, garbage bin or stainless steel drum as containers, with manual watering compared to the more sophisticated use of pumps with automatic sprinklers, produced poor quality sprouts with long and thin hypocotyls and long roots (Lee and Lee 1992) which is the typical of sprouts that are grown in darkness. Light is not necessary in the germination process as it causes greening or the development of chlorophyll pigment in the primary leaf which is considered a defect in sprouts (Schrader 2002).

Hence, controlling the growth of sprouts to minimize the etiolation effect becomes an important factor in producing sprouts with short and thick hypocotyls, as required by the consumers. Thus, growers resort to the use of non-registered chemicals containing plant growth regulators such as 2,4-dichlorophenoxyacetic acid (2,4-D) and 6-Benzylaminopurine (6-BAP) to obtain consumers' preferred sprouts. Preliminary work to study the use of 2,4-D and 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 the sprouts sold in the market. In fact, 2,4-D is used as herbicide in agriculture to control broad-leaved weeds and woody plants. Studies showed that the presence of 2,4-D modulated the DNA and caused cellular damage in human lymphocytes (Soloneski et al. 2007). Considering the fact that the sprouting process takes only 3-5 days, the sprouts are expected to contain high chemical residues, thus, defeating the purpose of consuming sprouts for health benefits.

In Malaysia, bean sprouts are commercially produced in residential areas and growers have little knowledge of basic hygiene and food safety. There is potential for pathogen growth during the sprouting process. Sprouts grown in warm and humid environment for 3-5 days provide an ideal condition for the growth of pathogenic microorganisms such as *Salmonella* spp. and *Escherichia coli* O157. These food-borne pathogens may cause food-borne diseases and affect sprouts quality by inducing rapid deterioration of sprouts, resulting in short storage life. The symptoms of sprouts deterioration include darkening of root and cotyledon, dark streaks on hypocotyl, decay and musty odor (DeEll et al. 2000).

Foodborne pathogens could be introduced to sprouts by seeds, water and lack of sanitation during the sprouting practices. In commercial sprout production, well water is normally used without any test for microbes. The unhygenic facilities and broken equipments used during sprouting are potential ways in which food-borne pathogens such as *Salmonella* species can grow (Mccue and Shetty 2002; http://hort-devel-nwrec.hort.oregonstate.edu/beansprt.html). In order to eliminate bacterial contamination in germinating sprouts, seeds are first treated with bleach followed by a fresh water rinse to reduce the number of viable *Salmonella* population in order to reduce bacterial infection during germination (Mccue and Shetty 2002). It must be done carefully to ensure there is no remaining chemical residue on germinating seeds. As a result from the use of unsanitary sprouting practices in the commercial sprouts production, there is a need to improve the quality of sprouts by improving cleanliness during sprouting.

High oxygen (O_2) concentrations and temperatures are unfavourable for the production of desirable sprouts because they induce high respiration rate. Rapid respiration rate will produce etiolated sprouts with thin hypocotyl and long root (Peppelenbos and van't Leven 1996). Lacking the ability to harness photosynthesis to generate new substrates for biosynthesis (growth) reaction, dark-germinated seedlings would have to depend on stored starch and lipids to generate glucose-derived precursors required for the biosynthetic reactions of typical seedling germination (Mccue and Shetty 2002). By reducing the respiration rate, sprouts could be induced to grow slowly. Stored food in the cotyledon would not be consumed completely and would be translocated slowly to the elongating hypocotyl

and remain stored there as carbohydrate or consumed in the production of soft, succulent tissue (Ahmad and Mohamed 1988).

Seeds generate metabolic heat during sprouting process due to respiration. Sprouting temperatures from 26-30 ºC resulted in slightly quicker growth but produced elongated sprouts. For best sprouts quality, temperature should be maintained at 21-26 ºC during the sprouting period (Schrader 2002). Sprouting temperature can be manipulated using watering frequency. Watering is important for seed growth and development, and to flush accumulated carbon dioxide (CO_2) and metabolic wastes during sprouting to provide adequate aeration and O_2 flow (Schrader 2002).

Studies by Ahmad and Mohamed (1988) showed that hermetically sealed chamber with an automatic water supply system could be used to regulate sprout growth. Hermetically sealed chamber could cause changes in gas composition resulting from sprout metabolic processes that creates a modified atmosphere during sprouting. This modified atmosphere can provide a suitable combination of ethylene (C_2H_4), CO_2 and O_2 to control sprout growth during sprouting when manipulated accordingly. The desired effect is achieved through the lower level of O_2 (<10%) and a high level of CO_2 (>3%) in the modified atmosphere system which can regulate the respiration rate of sprouts and ultimately their growth to produce shorter, large-diameter hypocotyl and short roots (Ahmad and Mohamed 1988) .

The interactions of the seeding density and watering frequency used during sprouting could be used to modify sprouting atmosphere in the hermetically sealed

chamber. It is hypothesized that sprout compaction by high seeding density could cause changes in gas composition and produce stress C_2H_4. Stress C_2H_4 is responsible for alteration of growth, development and differentiation in plants (Pierik et al. 2006; Abeles et al. 1992). Ethylene produced during sprouting acts as a plant growth regulator in shortening the hypocotyl and root length, and stimulating radial hypocotyl swelling of sprouts. The concentration of endogenous C_2H_4 that was produced plays an important role in determining the sprouts quality (Ahmad 1985).

Phytochemical concentrations in plants are affected by environmental conditions during cultivation and postharvest handling (Kubota et al. 2006). Plant phenolic compounds are secondary metabolites that do not serve a primary metabolic role in a plant and are not specifically required for basic plant functions of energy metabolism. They are produced because they confer some sort of benefit to the plant, usually an antimicrobial or antioxidant function or as chemical toxins that deter predators (Mccue and Shetty 2002). Because unsanitary sprouting processes induce the growth of pathogenic microorganisms, and treating or germinating sprouts with pesticides may result in undesirable residues that remain on sprouts for human consumption (Mccue and Shetty 2002); therefore, stimulating the endogenous antioxidant content production in sprouts via the modified atmosphere resulted from oxidative stress could be one of the methods to produce clean sprouts.

There is little knowledge about phenolic compounds and their antioxidative activities during the growth of *Vigna mungo* sprouts. It is assumed that the production of bean sprouts under modified atmosphere would cause oxidative stress