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Light and temperature effects on sprout yield and its proximate composition and vitamin C content in Lignosus and Mung beans

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Abstract

Bean sprout is used as vegetables. The present study investigated the effect of temperature and light on seed germination and sprout yield, and proximate (crude protein, crude fat, crude fibre, nitrogen free extract and ash) composition of sprout in Lignosus (*Dipogon lignosus* (L.) Verdc.) and Mung (*Vigna radiata* Wilczek) beans. Three temperatures (ambient, 25^{o} and 30^{o}C) and two light regimes; continuous dark, (CD) and continuous dark alternated by ½ hour light daily, (DAL) were used for seed germination and bean sprout yield. Data were collected 24, 48, 72, 96 and 120 hours after setting the presoaked seeds in the petri dishes. Of the temperature regimes, generally better seed germination and sprout fresh weight (yield) were observed at 25^{o}C in Mung bean and 30^{o}C in Lignosus bean. Germination and sprout yield were similar under CD and DAL. Both under CD and DAL, sprout yield was higher after 120 hours compared to 96 hrs after germination in both the beans. Proximate composition of dry seeds and their sprouts was also determined. Irrespective of species, crude protein content was significantly higher in sprout (average of 29.33%) than dry seed (average of 24.33%).Vitamin C was significantly higher in Lignosus bean sprout (20.93 mg 100g^{-1}) compared to Mung bean sprout (9.52 mg 100g^{-1}). This is the first world report on Lignosus bean sprout. It may be concluded that better sprout yield and good protein content were obtained at 25^{o}C in Mung bean and 30^{o}C in Lignosus bean after 120 hrs of germination irrespective of dark and light.

Introduction

Both Lignososus (*Dipogon lignosus* (L.) Verdc.) and Mung (*Vigna radiate* Wilczek) beans are members of Fabaceae. In developing countries including Bangladesh, most of the proteins consumed come from plant sources and this is cheaper than proteins from animal sources (mainly fish and meat) (Rashid, 1999). Bean sprouts are one of the most complete, nutritious, predigested foods with higher biological efficiency and lower levels of anti-physiologic factors than raw or cooked seeds (Balasaraswathi and Sadasivum, 1997; Yang et al. 2001; Kaur and Kawarta, 2002). Mung bean sprouts are used in many food items including breakfast, salads, soups, casseroles, pasta and baked products. Nutritionists advocate the use of bean sprout as both vegetable and meat. Bean sprouts are used fresh and cooked and are sources of proteins, amino acids, hormones, enzymes, vitamin C, antioxidants, minerals and other chemicals of health benefit (Chen et al. 1987; Yang et al. 2001; Venyl et al. 2009; Surani and Rauf, 2010; Chen et al. 2012; Meyerowitz, 2012). Further, foods containing both bean sprout and rice provide better nutrition since sprout contains certain amino acids while rice contain different amino acids.

Germination improves nutritional and functional properties in cowpea (Giami, 1993), sorghum (Abbas et al. 2008) and wheat (Hussain and Uddin, 2012). Sprouting has been identified as an inexpensive but effective technology for improvements of nutrition and quality of cereals and grain legumes and is associated with improvement of nutritive value of seeds (Zanabria et al. 2006; Khattak et al. 2007). Proteins break down into amino acids and water-soluble vitamins such as B-complex and vitamin C. At the same time there are reports that germination is effective in reducing phytic acid, flatulence causing oligosaccharides and polyphenols thereby increasing protein digestibility and improving sensory properties (Lintschinger *et al.* 1997; Zanabria et al. 2006; Khattak et al. 2007). Recently, it has been reported that germination under different type of illumination has significant effect on biosynthesis of ascorbic acid and sprout yield of soybean and chickpea (Mao et al. 2005; Khattak et al. 2007). Most researchers used Mung bean and chickpea seeds for sprout production and its chemical composition but such investigation is lacking in Lignosus bean in the world. Although information on pod and seed growth is available (Fakir et al. 2008, 2009; Fakir and Hassan, 2009) there is only one report on sprout yield and quality in Lignosus bean in world literature (Islam, 2010).

Dormant seeds are essentially storehouses of reserve food; their synthetic powers being released only on being germination under favorable moisture and temperature. Selection of species/varieties suitable for sprouting and good nutrients is important. Likewise, environmental factors hasten germination *viz.*, keeping seeds in dark or continuous dark alternated by light and different temperatures needs investigation. Biological tests also need to be made to detect any alteration in the nutritional/proximate composition as a result of sprouting. Shah et al. (2011) reported that sprouting resulted in increase in crude protein and crude fiber but decrease in nitrogen free extract and ash in Mung bean. Such research is lacking in Lignosus bean. Although scanty information of the effect of light and temperature requirements for suitable bean sprouts production is available in Mung bean in Bangladesh, there is no published information on Lignosus bean sprout production and its nutritional composition in the world literature. The present research investigated the effect of (i) temperature and light requirement on sprout yield; and (ii) proximate composition of dry seed and sprout in Lignosus and Mung beans.

Materials and Methods

The experiment was carried out at the laboratories of the Department of Crop Botany, Horticulture and Animal Nutrition, Bangladesh Agricultural University, Mymensingh during the period from March to November 2009.

Atmospheric conditions of the storage room: Temperature and relative humidity of the storage room were recorded daily. The averages of the minimum and maximum temperatures of the storage room were 23.7 and 31.82^{o}C, respectively (average 27.76^{0}C) and relative humidity ranged from 75.15 to 87.48 %.

Experimental materials: Mature dry seeds of Lignosus and Mung beans were used as experimental materials. The Lignosus bean (Trinidad genotype) seeds were collected from previous experiment in Crop Botany Department, Bangladesh Agricultural University, Mymensingh and Mung bean (BINA moog-6) seeds were collected from the Physiology Division, Bangladesh Institute of Nuclear Agriculture, Mymensingh.

Seed preparation: The seeds were tested for imbibition to observe maximum water absorbing capacity. Ten seeds were placed in each petri dish in distilled water soaked tissue in quadruplicates. Weight of seeds was recorded at every 4, 8, 12 and 16 hours after setting the test. From that data, weight increased due to imbibition was calculated by subtracting primary seed weight from final weight. From the test it was found that the maximum water absorption occurred 12 hours after imbibition period in both the species. For actual experiments, seeds were subsequently soaked for 12 hours before starting of the main experiments.

Procedure: For successful sprout preparation, clean and uniform seeds were sorted following soaking in water for twelve hours. The seeds were rinsed with about 500 ml of water containing approximately one gram of calcium hypochlorite (bleaching powder) before soaking. After soaking, seeds were placed in petri dishes containing moistened tissue at the bottom. The petri dishes were kept at ambient temperature (minimum, 23.7 to maximum, 31.82^{0}C; mean 27.76^{0}C) or in the germinator maintaining at 25^{0} or 30^{0}C) for germination. The seeds were rinsed everyday with bleaching powder solution. To observe the effect of light on sprouting, the selected petri dishes were kept in the light for half an hour daily basis. Otherwise the petri dishes were kept in dark condition in the germinator. Five (two each on Mung and Lignosus beans, and fifth on nutritional composition), experiments were carried out. Firstly, Two factors (temperature and sprout harvesting time) experiments were conducted in randomized complete design for investigating the effect of temperature and time of harvest on seed germination and sprout production in Lignosus bean and Mung bean in separate setup. The three temperatures were ambient, 25 and 30^{0}C and five harvesting times were 24, 48, 72, 96 and 120 hours after setting the germination test.There were 15 (5x3) treatments with four replications. The standard germination procedure was followed according to ISTA rules. The whole experiment was set under continuous dark condition in the germinator (Refrigerated incubator FOC 2251, VELP Scientifica). To investigate the effect of light and time of harvest on seed germination and sprout yield in Lignosus and Mungbean**,** two factors (light regimes and sprout harvest time) experimental setup was conducted at ambient temperature. Two light regimes were continuous dark (CD) and continuous dark alternated by ½ hour light daily (DAL), and five harvesting times were 24, 48, 72, 96 and 120 hours after setting the germination test. The 10 (2x5) treatments were arranged in CRD with four replications. The standard germination procedure was followed. Proximate composition (crude protein, crude fat, crude fibre, nitrogen free extract and ash) of seeds and sprout of Mung and Lignosus beans were determined (AOAC, 2005). Vitamin C (ascorbic acid) was assayed in fresh bean sprouts at 5-day and 3-day old seedlings of Lignosus and Mung beans, respectively following the method of Agustin *et al.* (1985). The concentration was expressed as milligram of vitamin C 100g^{-1} of the fresh sprout.

Data collection and analysis: Data on % germination; seed weight; total dry matter of seed; sprout fresh weight (yield); total dry matter of sprout; stem length etc. were recorded. The means were compared by Duncan Multiple Range test (Gomez and Gomez, 1984).

Results and Discussion

Temperature effect on seed germination and sprout yield in Lignosus bean: Temperature influenced seed germination, sprout length, and sprout fresh and dry weights ($P \leq 0.05$) (Table 1). Generally, germination, sprout length, sprout fresh and dry weights increased with increasing duration (Table 1). Germination was found better between 48 and 120 hours duration. The higher sprout length was noted 120 hours after setting the presoaked seeds while the sprout dry weight was found better between 96 and 120 hours after setting the seeds. However, the maximum fresh weight was observed 120 hours setting the test. The interaction of temperature and duration on seed germination, sprout length and sprout fresh weight were observed significant ($P \leq 0.05$) (Table 1). The germination was increased by 6-folds between 24 and 120 hours at ambient temperature and the figures were 4.5-folds both at 25 and 30^oC. On the other hand, sprout length was increased by 67.5 folds between 24 and 120 hours at ambient temperature and the figures were 14 and 40 times at 25 and 30^oC, respectively. Sprout yield (sprout fresh weight) was increased with increasing duration at all temperature ranges, the magnitude being much greater between 24 and 120 hours at 30^oC (2.42 folds) than at 25^oC (2.03 folds) and ambient (1.73 folds). However, sprout dry weight was greater at 120 hours at all temperatures.

Temperature effect on seed germination and sprout yield in Mung bean:
Generally, sprout length and weight increased with increasing duration (Table 2). Sprout length and fresh weights were highest between 96 and 120 hours. However, sprout dry weights were highest between 24 and 48 hours after setting the test. The interaction of temperature and duration on sprout length and sprout fresh and dry weights were significant (Table 2). Sprout length increased by 8-folds between 24 and 120 hours at ambient temperature, 9-folds at 25^0C and 13-folds at 30^oC. On the other hand sprout fresh weight increased by 4 times between 24 and 120 hours at ambient temperature and the figures were 4.7-folds and 1.7-folds at 25 and 30^oC, respectively.

Table 1: Combined effect of temperature and harvest duration on seed germination and sprout yield attributes in Lignosus bean

Temperature ($^\circ$C)	Harvest duration (hrs)	Sun dry Seed wt./10-seeds (g)	Germi-nation (%)	Sprout length (cm)	Sprout fresh wt/10-Seed ling (g)	Sprout dry wt. /10-seedlings (g)
Ambient	24	3.17e	10.0b	0.09e	4.91g	2.75fg
	48	3.24cde	65.0a	1.33d	6.36f	2.80defg
	72	3.23cde	72.5a	2.57c	7.13de	2.9bcdef
	96	3.19de	67.5a	3.88b	7.64cd	2.92bcd
	120	3.21de	62.5a	6.07a	8.49b	3.01ab
25	24	3.51ab	15.0b	0.40e	4.28hi	2.69gh
	48	3.46abc	72.5a	1.39d	6.18f	2.77efg
	72	3.41abcd	72.5a	2.43c	7.41cde	2.87cdef
	96	3.56a	65.0a	3.88b	7.23cde	2.89bcde
	120	3.62a	67.5a	5.78a	8.74b	2.96abc
30	24	3.32bcde	15.0b	0.15e	4.05i	2.42i
	48	3.27cde	65.0a	1.43d	4.83gh	2.59g
	72	3.42abcd	60.0a	2.43c	6.98e	2.69gh
	96	3.23de	57.5a	3.95b	7.84c	2.95abc
	120	3.31bcde	72.5a	5.95a	9.84a	3.07a

In a column under a particular variable, figures followed by different letter (s) are significant at $P \leq 0.05$

Table 2. Combined effect of temperature and harvest duration on seed germination and Sprout yield attributes in Mung bean

Tempera-ture (°C)	Harvesting duration (hrs)	Sun dry Seed wt. / 10-seeds (g)	Germination (%)	Sprout length (cm)	Sprout fresh wt/10-seedlings(g)	Sprout dry wt. /10 - seedlings (g)
Ambient	24	0.63ab	98.0a	3.310gh	1.28fg	0.46bc
	48	0.60abcd	95.0a	6.225fg	1.98ef	0.42bcdef
	72	0.64a	97.5a	15.71d	3.98c	0.42bcdef
	96	0.64a	90.0a	23.06bc	4.72ab	0.38f
	120	0.61abc	99.0a	26.15a	4.98a	0.41cdef
25	24	0.58cdef	95.0a	2.793h	1.13g	0.44bcde
	48	0.59abcde	97.5a	5.993fg	1.93ef	0.41bcdef
	72	0.56def	92.5a	12.68e	3.96c	0.39ef
	96	0.54ef	100.0a	20.70c	4.85a	0.41def
	120	0.53f	100.0a	25.02ab	5.26a	0.42bcdef
30	24	0.56cdef	97.5a	1.740h	2.45de	0.53a
	48	0.58cdef	95.0a	7.075f	2.48de	0.47b
	72	0.59bcdef	90.0a	11.09e	2.93d	0.45bcd
	96	0.58cdef	90.0a	23.54abc	3.98c	0.40def
	120	0.58bcdef	97.5a	22.55bc	4.14bc	0.41cdef

In a column under a particular variable, figures followed by different letter (s) are significant at P ≤0.05

Table 3. Combined effect of Light and Harvest duration on seed germination and sprout yield parameters in Lignosus Bean

CD/ DAL	Harvest duration (hours)	Sun dry Seed wt./ 10-seeds (g)	Germi-nation (%)	Sprout length (cm)	Sprout fresh wt/10-seedlings (g)	Sprout dry wt./10-seedlings (g)
CD	24	3.17c	10.0b	0.09e	4.90d	2.75cde
	48	3.23bc	65.0a	1.32d	6.36c	2.79bcde
	72	3.23bc	72.5a	2.56c	7.12b	2.88abc
	96	3.19c	67.5a	3.87b	7.64b	2.92ab
	120	3.21bc	62.5a	6.07a	8.49a	3.01a
DAL	24	3.50a	17.5b	0.44e	4.25e	2.69e
	48	3.45ab	72.5a	1.41d	6.18c	2.73de
	72	3.40abc	72.5a	2.75c	7.45b	2.87abc
	96	3.56a	67.5a	3.86b	7.74b	2.87abcd
	120	3.61a	70.0a	5.77a	8.77a	3.01a

In a column under a particular variable, figures followed by different letter (s) are significant at P ≤0.05
CD: Continuous dark , and DAL: Dark alternated Light

Effect of Light on seed germination and sprout production in Mung bean:

Two light conditions i.e. continuous dark (CD) and continuous dark alternated by ½ hour light daily (DAL) had significant effect on sprout length and fresh weight ($P \leq 0.05$) (Table 4). Both sprout length and weight were higher in CD. The duration also had significant effect on sprout length and fresh weight. Generally sprout length and fresh weight increased with increasing time. Both sprout length and fresh weight were the highest between 96 and 120 hours after the test. The interactions of CD and DAL on sprout length and sprout fresh weight were significant ($P \leq 0.05$) (Table 4). Sprout length was increased by 8-times between 24 and 120 hours in CD and the figure was 7.5-folds in DAL condition. On the other hand sprout fresh weight increased by 4-folds between 24 and 120 hours in CD and by 4.6-folds in DAL condition.

Table 4. Combined effect of Light and harvest duration on seed germination and sprout yield parameters in Mung bean

CD) / DAL	Harvest duration (hours)	Sun dry seed wt./ 10-seeds (g)	Germination (%)	Sprout length (cm)	Sprout fresh wt/10-seedlings(g)	Sprout dry wt./ 10-seedlings (g)
CD	24	0.63ab	100.0a	3.31f	1.28ef	0.46a
	48	0.60c	95.0ab	6.23de	1.97d	0.42abc
	72	0.64a	97.5ab	15.72c	3.97bc	0.42abc
	96	0.61bc	90.0b	23.06b	4.72a	0.38c
	120	0.61abc	100.0a	26.15a	4.98a	0.41bc
DAL	24	0.61bc	97.5ab	2.35f	1.03f	0.44ab
	48	0.62abc	97.5ab	4.20ef	1.68de	0.42abc
	72	0.62abc	95.0ab	8.00d	3.48c	0.39bc
	96	0.627ab	97.5ab	15.70c	4.49ab	0.41bc
	120	0.62abc	95.0ab	17.75c	4.77a	0.42abc

In a column under a particular variable, figures followed by different letter (s) are significant at $P \leq 0.05$
CD: Continuous dark , and DAL: Dark alternated Light

Proximate composition of seed and sprout of Lignosus and Mung beans:
The temperature and light had a significant effect on proximate composition of Lignosus and Mung beans. Irrespective of temperature and light treatments, protein content of bean sprout was much higher than that in the dry seed in both the species (Table 5). In Lignosus bean, protein content of sprout produced under ambient temperature in the continuous dark alternated by ½ hr light daily (DAL) was higher (29.97%) than in the other light and temperature treatments (average of 25.85%). Contrary, in Mung bean protein percentage of sprouts under ambient temperature at DAL was lower (29.46%) 72 hours after setting the test. The highest protein percentage (33.24%) was found in Mung bean sprout at 30^{o}C under continuous dark. The highest nitrogen free extract (NFE or total carbohydrate) (65.91%) was observed in Mung bean seed. In Lignosus, fibre content was higher in sprout than in the dry seed and the trend was reverse in Mung bean. The highest amount of ash (6.17%) was found in Lignosus bean sprout under ambient temperature at DAL. Ether extract or crude fat was lower in sprout than the dry seed in both the species.

Vitamin C content: Vitamin C content was much higher in the mung bean sprout (20.93 mg 100g^{-1}) than that in the Lignosus bean sprout (9.52 mg 100g^{-1}) (Table 6). No vitamin C was detected in the dry seeds of both the species.

Bean sprouts are widely used as inexpensive, cheaper and protein rich vegetables and salads in Southeast Asia, Japan and other countries. In Bangladesh it is served to the foreigners in five star hotels. In the current study temperatures affected sprout size and weight (Tables 1 and 2). Similar results were also reported by Huang *et al.* (2006) that increasing temperature also increased sprout length and sprout yield. Duration after soaking the seeds also affected sprout production. The longer and increased sprout weights were observed between 96 and 120 hours in both the species. Results of interaction of temperature and duration showed that better sprout yield was obtained at 25^{o}C after 120 hours in Mung bean and 30^{o}C after 120 hours in Lignosus bean. In the current experiments, sprouts were longer in the continuous dark (CD) than in the dark alternated by ½ hour light daily (DAL). Islam (2010) also observed similar results that a temperature of 25-30^{0}C with 96-120 hrs duration was effective for sprouting in Mung and Lignosus beans with improvement of nutrient contents. Sprout yield (fresh weight) was not significantly different between the species and the light conditions, and therefore either CD or DAL was observed equally good for both the species. Hundred grams each of Lignosus and Mung bean seeds produced 800 and 200 g, respectively, of edible fresh sprouts, which is partially similar with the findings of Venyet al. (2009) who found 253-279g fresh sprout in Mung bean from 100 g seeds. This difference in sprout yield might be due to variation in genotype, seed size and sprouting conditions in the two experiments. Pulse grains in general are used as an alternative to animal protein. Considering the nutritive value, sprout had higher protein content than dry seed in both species (Table 5). Our current result agreed with the report of Mankotia and Modgil (2004) in kidney beans and Shah et al. (2011) in Mung bean that soaking and sprouting increased crude protein content in sprout compared to dry seeds. Sprouts of both the beans contained appreciable Vitamin C compared to their dry seeds, where no vitamin C was detected and this was an added advantage. Further, there are few summer vegetables in Bangladesh and even the winter vegetables contain very little protein. Sprouting of these beans is easy to prepare at home and can be used as a rich source of protein to alleviate protein deficiency in Bangladesh. There is a good potentiality of introducing bean sprout in the country

Table 5. Comparison of proximate composition between seed and their sprout in Lignosus and Mung beans

Seed /Sprout	Proximate composition (%)				
	Crude protein	**Crude fibre**	**Ether extract**	**Nitrogen free extract**	**Ash**
Dry seed					
Lignosus bean	24.46 d	5.40 a	2.23 ab	62.92 c	4.99 b
Mung bean	24.25 d	2.25 c	2.83 a	65.91 a	4.76 b
Sprout					
LBS^{+} at ambient temp. at CD*	26.20 c	2.67 c	1.29 b	64.64 b	5.20 a
LBS at 30^{o}C at CD	25.50 c	4.09 b	1.35 b	64.27 b	4.79 b
MBS^{++} at ambient temp. at CD	31.67 b	3.79 b	1.63 b	57.66 d	5.25 a
MBS at 25^{o}C at CD	33.24 a	5.67 a	1.45 b	54.88 e	4.76 b

$^{+}$: LBS: Lignosus bean sprout at 120 hours; $^{++}$: MBS: Mung bean sprout at 72 hours,
*: CD: Continuous dark; In a column, figures with uncommon letter (s) are significantly different at P≤0.05 by DMRT.

Table 6. Vitamin C content of sprout of Lignosus and Mung beans

Species	**Vitamin C content (mg 100 g^{-1})**
Lignosus Bean sprout (5-day old seedling)	9.52 b
Mung Bean (3-day old seedling)	20.93 a

In a column, figures with uncommon letter (s) are significantly different at P≤0.05 by DMRT.

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