DOI: 10.31276/VJSTE.63(1).36-41

Influences of seed priming with *Spirulina platensis* extract on seed quality properties in black gram (*Vigna mungo* L.)

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Received 18 February 2019; accepted 24 June 2019

Abstract:

Black gram (*Vigna mungo* L.) is a high nutrient pulse crop. Its seed quickly and easily deteriorates after harvest, resulting in poor planting material production that must be improved accordingly by seed enhancement methods. The blue-green microalgae *Spirulina platensis* contains variously beneficial substances, including macro and micro nutrients, vitamins, amino acids and antioxidants. The aim of this study is to improve the vigour and germination of black gram by priming its seeds with *Spirulina platensis* extract to prevent seed deterioration. Medium vigour black gram seeds were subjected to hydropriming for three hours (T_2); other seeds were primed with *Spirulina platensis* extract at 1.5% for three hours (T_3) while unprimed seeds served as control (T_1). Then, the seeds were artificially aged under 40±1°C and 95±5% relative humidity for five days. The results revealed that the hydropriming and control of black gram seeds were significantly lower in all physiological and biochemical seed quality parameters than seeds primed with *Spirulina platensis* extract at 1.5% for three hours. Consequently, *Spirulina platensis* extract can effectively enhance black gram seeds and use agents in.

Keywords: **black gram, seed priming, seed quality, *Spirulina platensis* extract.**

Classification number: **3.1**

Introduction

Black gram (*Vigna mungo* L.) is an important food crop cultivated in most countries throughout Asia. India is the major producer and consumer of black gram, producing approximately 2.199 million tonnes of black gram in an area of 4.02 million hectares during 2015-2016. The crop is of great importance as about 70% of the world's black gram production comes from India [1] and comprises10-12% of India's total pulse crop production [2]. Black gram is highly nutritious containing 24% protein content, 983 mg/100 g of potassium, 138 mg/100 g of calcium, 7.57 mg/100 g of iron, 1.447 mg/100 g niacin, 0.273 mg/100 g of thiamine, and 0.254 mg/100 g of riboflavin. Black gram is an important part of diets in India and other Asian countries are because it complements essential amino acids, high protein, and carbohydrates. Additionally, black gram has been found to be useful in mitigating elevated cholesterol levels. It also improves soil fertility through biological nitrogen fixation and thus plays an important role in sustainable agriculture.

Cyanobacteria (blue-green microalgae) are found in alkaline volcanic lakes and warm water areas. *Spirulina platensis,* a biomass of blue-green algae, produces a protein called phycocyanin belonging to the photosynthetic apparatus; it contains soft cell walls made of complex sugars and proteins and includes antioxidant and free radical scavenging properties [3]. The component analysis revealed that the *Spirulina* extract contains 85.1 g/kg of flavonoids, 77.8 g/kg of b-carotene, 113.2 g/kg of vitamin A and 3.4 g/kg of α-tocopherol, which contribute significantly to their high antioxidant activity. The main fatty acids in the extract are palmitic acid (35.32%), linolenic acid (21.66%), and linoleic acid (20.58%). As a natural biofertilizer, cyanobacteria's ability to fix N_2 improves the growth and yield of crops through the production of growth promoters such as gibberellins, cytokinins, auxins, vitamins, antibiotics and amino acids [4].

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Seed priming is a hydration and dehydration process during pre-germination in which seeds absorb water and prevent radicle protrusion; the seeds are dried to their original moisture level [5]. Seed priming has been successfully demonstrated to improve germination and emergence in seeds of many crops. Primed seeds are able to complete the process of germination in a short time and can cope with environmental stresses [6]. Therefore, application of *Spirulina platensis* extract for seed priming to improve seed vigour and viability is a useful technology as it replaces the chemical utilities with organic nutrient supplements for seed quality improvement. Therefore, the present study is proposed to harness the potential use of *Spirulina platensis* extract to improve black gram seed quality.

Materials and methods

Seed material

Genetically pure, medium vigour seeds of black gram (*Vigna mungo* (L) Hepper) cv. CO 6 obtained from the Department of Pulses at Tamil Nadu Agricultural University, Coimbatore were used for this study.

Preparation of materials

Commercial *Spirulina platensis* powder taken from iGreen Firm, Coimbatore, Tamil Nadu was initially dried under sunlight followed by oven drying for 24 hours at 60°C and used for preparation of extract. Two hundred grams of *Spirulina platensis* powder was taken and added with 400 ml of acetone: methanol solvent [ratios of 1:1 (v/v)] and kept overnight after vigourous shaking. The solution was decanted and filtered through Whatman filter paper and stored in refrigerated conditions at 4°C until usage [7]. This *Spirulina platensis* extract was used as 100% concentrated stock for further studies.

Treatments

Black gram seeds were subjected to hydro-priming with distilled water for three hours and dried to their original moisture content of approximately 9% (T_2). Other black gram seeds were primed with *Spirulina platensis* extract at 1.5% concentration by soaking for three hours, then were dried to their original moisture content (T_3). The non-primed seeds were used as control (T_1). Then the seeds were artificially aged under 40±1°C and 95±5% RH (accelerated ageing) for five days.

Observations

Physiological and biochemical seed quality parameters were recorded in aged and non-aged seeds involving germination percentage, root length, shoot length, dry matter production [8], vigour index [9], speed of germination [10], dehydrogenase activity [11], free sugars in seed leachate [12], protein content [13], α-amylase activity [14], gibberellic acids content [15], and lipid peroxidation [16].

Statistical analysis

The data obtained from different treatments were analyzed for the 'F' test of significance following the methods described by Panse, et al. (1985) [17]. Wherever necessary, the percent values were transformed to angular (arcsine) values before analysis. The critical differences (CD) were calculated at 5% probability level. The data were tested for statistical significance.

Results and discussion

Physiological changes in black gram seeds due to priming with Spirulina platensis extract

The results revealed that the black gram seeds primed with *Spirulina platensis* extract at 1.5% concentration (T_3) recorded a maximum germination of 98%, higher than hydroprimed (T_2) and control (T_1) seeds, which recorded only 89 and 82% germination, respectively. The percentage of germination increased due to priming with *Spirulina platensis* extract was 9% and 16% over the hydropriming and control, respectively. Additionally, after accelerated ageing, the seeds primed with *Spirulina platensis* extract at 1.5% (T_3) recorded significantly higher germination (62%) which was 11% and 18% higher than the hydroprimed and control seeds, respectively.

Similarly, the root length of the seeds primed with *Spirulina platensis* extract at 1.5% (T_3) were significantly longer both before ageing (16.23 cm) and after ageing (11.97 cm) than the hydroprimed and non-primed seeds (control). The shoot lengths of the seedling were 14.67 cm before ageing and 10.17 cm after ageing in seeds primed with *Spirulina platensis* extract at 1.5%, which were significantly longer than those of the hydropriming and control seeds (Table 1). Soaking the seeds in *Spirulina platensis* extract might have improved not only the availability of seed food reverses but also maintained the cell structure, leading to increased seed vigour and germination in addition to preventing seed deterioration during accelerated ageing. Similar results were also reported by Ratan, et al. (1993) [18] in annona; Vijaya (1996) [19] in black gram and cowpea; Asgedom, et al. (2001) [20] in cereal; and Benaseer (2016) [21] in black gram hey concluded that sowing might have enhanced absorption of imbibition water and suitable nutrient concentration, resulting in quick initiation of the germination process and improving physiological parameters.

Table 1. Effects of seed priming with *Spirulina platensis* extract on seed germination, root length and shoot length in black gram CO 6.

Treatment	Germination (%)			Root length (cm)			Shoot length (cm)		
	Primed (P)	*Primed + Aged (A)*	*Mean*	*Primed (P)*	*Primed + Aged (A)*	*Mean*	*Primed (P)*	*Primed + Aged (A)*	*Mean*
T_1	82 (64.90)	44 (41.56)	63 (52.54)	14.23	11.00	12.62	13.03	8.93	10.89
T_2	89 (70.63)	51 (45.57)	70 (56.79)	15.20	11.10	13.15	13.57	9.93	11.75
T_3	98 (81.87)	62 (51.94)	80 (64.16)	16.23	11.97	14.10	14.67	10.17	12.42
Mean	90 (71.57)	52 (46.15)		15.22	11.36		13.76	9.68	
	P	A	PxA	P	A	PxA	P	A	PxA
SEd	0.236	0.192	0.333	0.036	0.029	0.051	0.038	0.031	0.054
CD (p=0.05)	0.514	0.419	0.726	0.078	0.064	0.111	0.068	0.166	0.119

Note: T_1: control, T_2: hydropriming, T_3: priming with *Spirulina platensis* extract at 1.5%. Figures in parenthesis indicate arcsine values.

Table 2. Effects of seed priming with *Spirulina platensis* extract on speed of germination, dry matter production and vigour index in black gram CO 6.

Treatment	Speed of germination			Dry matter production (mg/10 seedlings)			Vigour index		
	Primed (P)	*Primed + Aged (A)*	*Mean*	*Primed (P)*	*Primed + Aged (A)*	*Mean*	*Primed (P)*	*Primed + Aged (A)*	*Mean*
T_1	16.87	10.53	13.70	225.3	147.7	186.5	2168	855	1511
T_2	17.97	12.63	15.30	230.7	161.7	196.2	2501	1095	1798
T_3	22.13	15.87	19.35	250.0	173.0	211.5	2925	1393	2159
Mean	18.99	13.01		235.3	160.8		2531	1114	
	P	A	PxA	P	A	PxA	P	A	PxA
SEd	0.014	0.011	0.019	0.76	0.62	1.07	15.24	12.45	21.57
CD (P=0.05)	0.030	0.024	0.042	1.65	1.35	2.33	33.23	27.13	46.99

Note: T_1: control, T_2: hydropriming, T_3: priming with *Spirulina platensis* extract at 1.5%.

Speed of germination was one of the best methods of analyzing the vigour potential of seeds. The speed of germination of seeds primed with *Spirulina platensis* extract at 1.5% for three hours (T_3) was 22.13 in primed seeds before ageing and 15.87 after accelerated ageing, which was significantly higher than hydropriming (T_2) and control (T_1). *Spirulina platensis* extract consisting of biological chemicals and nutrients involved in the priming process might have triggered the germination process early in primed seeds (Table 2). Khalil, et al. (2010) [22] also reported that seed priming enhanced the speed and uniformity of germination.

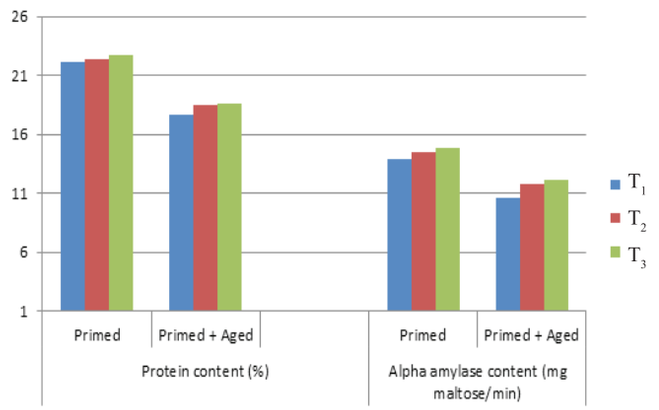
Seeds primed with *Spirulina platensis* extract at 1.5% recorded significantly higher dry matter production (250.0 mg 10 seedling^{-1} before ageing and 173.0 mg 10 seedling^{-1} after accelerated ageing) than hydroprimed and non-primed seeds. The computed vigour index value also recorded a similar trend as that of germination, root and shoot length, speed of germination, and dry matter production. The vigour index of seeds primed with *Spirulina platensis* extract at 1.5% recorded 2925 before ageing of primed seeds and 1393 after accelerated ageing, which was significantly higher than for hydroprimed and control seeds (Table 2). The results are in accordance with Benaseer (2016) [21], who reported that dry matter production and vigour index of seed priming with chitosan 400 ppm + PPFM 2% for three hours was higher than other treatments for black gram. These results demonstrated that physiological parameters of seeds primed with *Spirulina platensis* extract at 1.5% were superior to those in hydroprimed and control seeds.

Biochemical changes in black gram seeds due to priming with Spirulina platensis extract

The biochemical analysis of the black gram seeds revealed that the protein content in primed seeds was higher than that in non-primed seeds (control). However, seeds primed with *Spirulina platensis* extract at 1.5% for three hours (T_3) recorded the highest value of 22.78% while

control (T_1) seeds recorded 22.25% of protein content before ageing. After accelerated ageing, seeds primed with *Spirulina platensis* extract at 1.5% for three hours recorded 18.65% of protein content compared to 17.71% measured in the control (T_1) (Fig. 1). Koehler, et al. (1997) [23] reported that priming of seed promotes germination by repairing the damaged proteins, RNA, and DNA. In the present study as well, the vitamins, nutrients, and amino acids in the *Spirulina platensis* extract might have influenced the protein content of black gram seeds.

Amylase is an important enzyme that breaks down starch into the most commonly transportable form of sugar. Among the treatments, the α-amylase activity increased significantly in primed treatments compared to non-primed seeds. However, seeds primed with *Spirulina platensis* extract at 1.5% (T_3) registered the maximum α-amylase activity of 14.67 mg maltose/minutes while control seeds (T_1) recorded only 14.36 mg maltose/minutes. Additionally, after artificially ageing, the α-amylase activity was significantly higher in seeds primed with *Spirulina platensis* extract, which recorded 12.12 mg maltose/minute than the control and hydroprimed seeds. Seed priming with *Spirulina platensis* extract improved the seed structure leading it to maintain the seed metabolism to synthesis α-amylase during germination (Fig. 1). Zhan Li, et al. (2017) [24] also reported that seed priming enhanced α-amylase content under chilling stress in maize.



T_1: control, T_2: hydropriming, T_3: priming with *Spirulina platensis* extract 1.5%.

Fig. 1. Effects of seed priming with *Spirulina platensis* extract on protein content and alpha-amylase activity in black gram CO 6.

Gibberillic acid, which is considered an important growth hormone, is quite useful for promotion of seed germination. The seeds primed with *Spirulina platensis* extract at 1.5% (T_3) recorded the highest gibberellic acid content of 80.33 µg/g of fresh weight of seeds while control seeds (T1) recorded the lowest gibberellic acid content of 64.7 µg/g of fresh weight of seeds (Fig. 2). Similar results were also reported by Nakaune, et al. (2012) [25], which indicated that gibberellic acid is the major bioactive molecule involved in seed germination. The gibberellic acid content after accelerated ageing was also significantly higher in seeds primed with *Spirulina platensis* extract at 1.5% than in the hydroprimed seeds and non-primed control seeds. The *Spirulina platensis* contains more growth regulators, including gibberellic acids, which might be supplied to the seeds during the soaking and drying process.

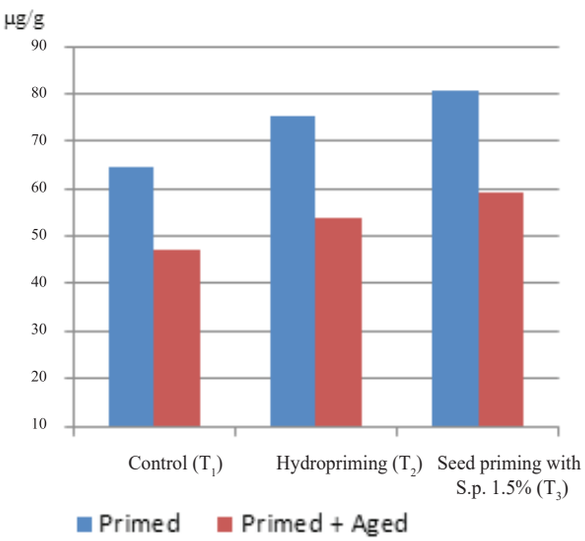


Fig. 2. Effects of seed-priming with *Spirulina platensis* extract on gibberellic acid content in black gram CO 6.

Dehydrogenase is an enzyme that seed germination [26]. All of the living seeds those respiration produce enzymes called dehydrogenase. The results of the study demonstrated that the dehydrogenase enzyme activity in the seeds primed with *Spirulina platensis* extract (T_3) was significantly higher (2.053) compared to hydroprimed (1.561) and control (1.353) seeds. Additionally, after accelerated ageing, seeds primed with *Spirulina platensis* extract recorded higher enzyme activity than hydroprimed and unprimed seeds. Since, *Spirulina platensis* extract includes antioxidants, growth regulators, vitamins, and macro and micro nutrients involved in repair maintenance of the cells, they contributed to promote the dehydrogenase activity in primed seeds and maintained it even under accelerated ageing (Fig. 3).

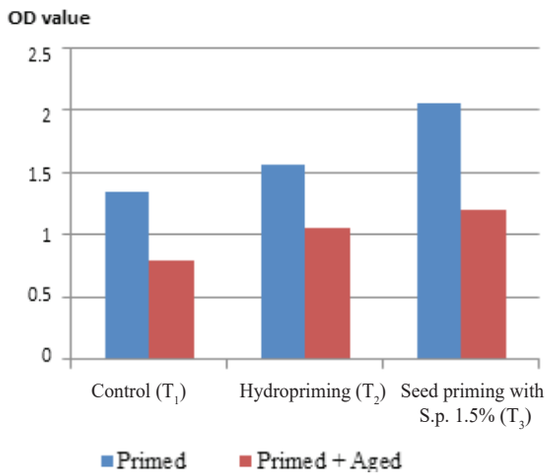
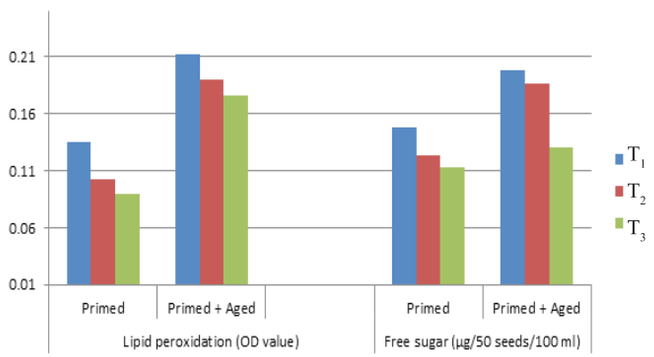


Fig. 3. Effects of seed-priming with *Spirulina platensis* extract on dehydrogenase activity in black gram CO 6.

Lipid peroxidation is the oxidative degradation of lipids. It is the process in which free radicals steal electrons from the lipids in cell membranes, resulting in cell damage. Therefore, lipid peroxidation is a factor indicated for degree of seed deterioration. In this study, seeds primed with *Spirulina platensis* extract (T_3) recorded a lower lipid peroxidation value (0.089) than the control (T_1) seeds, which recorded the highest value (0.135). Artificially aged seeds demonstrated an increase in lipid peroxidation, however, seeds primed with 1.5% *Spirulina platensis* extract recorded a minimum value (0.176), significantly lower than hydroprimed (0.190) and control (0.212) seeds. These results indicated that seeds primed with the *Spirulina platensis* extract demonstrated improved seed vigour by mitigating lipid peroxidation (significantly lower than in non-primed seeds) he seed vigour welleven under accelerated ageing. It might be due to the antioxidants in the *Spirulina platensis* extract react with lipids and the free radicals, leading to seed quality and reduced deterioration (Fig. 4).



T_1: control, T_2: hydropriming, T_3: priming with *Spirulina platensis* extract 1.5%.

Fig. 4. Effects of seed priming with *Spirulina platensis* extract on lipid peroxidation and free sugar in black gram CO 6.

Free sugar content in seed leachate is an index of cell membrane integrity and seed vigour. The leachate of seeds primed with *Spirulina platensis* extract for three hours (T_3) recorded the minimum free sugar content (0.113 µg/50 seeds/100 ml) while the control (T1) recorded 0.148 µg/50 seeds/100 ml. This trend was noticed even after accelerated ageing of seeds. Conversely, non-primed and hydroprimed seeds recorded higher free sugar content both before and after accelerated ageing (Fig. 4). It could be the seeds primed with *Spirulina platensis* extract maintained cell membrane integrity and tolerance to accelerated ageing. Chiu, et al. (2002) [27] reported that improvement in germination by priming might be due to enhanced repair of membranes that are disrupted during maturation drying. This is indirectly supported by the reduced leakage of electrolytes from primed seeds, since electrolyte leakage is in part a result of damaged cell membranes. However, electrolytes may be leaked out during priming, resulting in lower levels of electrolytes in primed seeds than in control seeds.

The results of these studies indicated that the seeds primed with *Spirulina platensis* extract at 1.5% for three hours might have trigger seed germination, vigour, and control over seed deterioration and the mechanism of seed ageing. Moreover, it is reasonable to assume that the priming also affected the rearrangement of cell membrane structure lost during seed ageing and increased the membrane integrity as suggested by Simon, et al. (1972) [28], Knyple, et al. (1980) [29], and Rudrapal, et al. (1988) [30].

Conclusions

From this study, it could be concluded that the seeds primed with *Spirulina platensis* extract at 1.5% for three hours significantly improved physiological parameters such as seed germination, speed of germination, dry matter production, seedling length, and vigour index. Additionally, the seeds primed with *Spirulina platensis* extract also recorded vigour associated with biochemical parameters such as protein content, gibberellic acids content, α-amylase activity, and dehydrogenase activity accompanied with lower lipid peroxidation and free sugar in seed leachate of black gram seeds.

COMPETING INTERESTS

The author declares that there is no conflict of interest regarding the publication of this article.

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