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Research Article

EFFECTS OF SEED PRIMING WITH *Spirulina platensis* EXTRACT ON PHYSIOLOGICAL AND BIOCHEMICAL SEED QUALITY PARAMETERS IN BLACKGRAM (*Vigna mungo* L.) CV. CO6

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Abstract: Seeds of blackgram (*Vigna mungo* L.) deteriorate at a rate sufficiently rapid to make them a poor planting material soon after the attainment of physiological maturity but can be minimisal by seed enhancement methods. The blue-green micro algae *Spirulina platensis* contains more beneficial nutrients including macro and micro nutrients, vitamins, amino acids and antioxidants. The aim is to exploit *Splirulina plantensis* for seed priming to prevent seed deterioration and to improve germination and vigour of blackgram seeds. Medium vigour black gram seeds were subjected to hydropriming for 3 hours (T2); priming with *Splirulina plantensis* extract at 1.5% for 3 hours (T3) while unprimed seeds served as control (T1). Then, the seeds were artificial aged under 40± 1°C and 95± 5 % for five days. The results revealed that the seed priming with *Spirulina platensis* extract at 1.5% for 3 hours had significantly improved all the physiological and biochemical seed quality parameters than the hydropriming and control. Thus, *Spirulina platensis* extract can be used for organic seed treatments and seed quality enhancement in blackgram.

Keywords: *Blackgram, Seed priming, Spirulina platensis extract, Seed quality*

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Introduction

Blackgram (*Vigna mungo* L.) is an important food crop cultivated in Indian subcontinent. India is the major producer and consumer of black gram and produced about 2.199 million tonnes of blackgram in an area of 4.02 million ha during 2015-2016.Important states producing black gram are Maharashtra, Uttar Pradesh, Andhra Pradesh, Orissa, Tamil Nadu, Rajasthan, Chhattisgarh and Madhya Pradesh. The crop is of great importance as about 70% of world's black gram production comes from India [1] and contributed 10 to 12% national share among the total pulse production [2]. Black gram is very nutritious as it contains high levels of protein (24g/100g), potassium (983 mg/100g), calcium (138 mg/100g), iron (7.57 mg/100g), niacin (1.447 mg/100g), thiamine (0.273 mg/100g) and riboflavin (0.254 mg/100g). Black gram complements the essential amino acids provided in most cereals and plays an important role in the diets of the people of India and Asian countries. Black gram has been found to be useful in mitigating elevated cholesterol levels. It maintains soil fertility through biological nitrogen fixation in soil and thus, plays a vital role in furthering sustainable agriculture. *Spirulina platensis* is a blue-green micro alga or cyanobacterium found in warm water and alkaline volcanic lakes. Spirulina has a soft cell wall made of complex sugars and proteins. Recent studies have demonstrated that in *Spirulina platensis*, a blue protein called phycocyanin, belonging to the photosynthetic apparatus has antioxidant and free radical scavenging properties [3]. The component analysis showed 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 greatly to their high antioxidant activity. The main fatty acids in the extract were palmitic acid (35.32%), linolenic acid (21.66%) and linoleic acid (20.58%). As a natural bio-fertilizer, cyanobacteria owed the positive effect of N_2 fixing and improved the growth and yield of crops by the production of growthpromoting substances, *i.e.*, gibberellins, cytokinins, auxins, vitamins, antibiotics and amino acids [4]. Seed priming is a pre-germination treatment in which seeds are held at a water potential that allows imbibition but prevents radicle extension and then seeds are dried back to the 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 cope with environmental stresses [6]. Therefore, application of Spirulina platensis extract for seed priming to improve of seed vigour and viability is a useful technology as it would replace the chemical utilities by organic nutrient supplements for seed quality improvement. Hence, the present study is proposed to harness the potential use of *Spirulina platensis* extract towards improvement of seed quality of blackgram.

Material and Methods

Seed material

Genetically pure, medium vigour seeds of Blackgram (*Vigna mungo*(L) cv. CO6 obtained from Department of Pulses, Tamil Nadu Agricultural University, Coimbatore were used for this study.

Preparation of materials

Commercial *Spirulina platensis* powder bought from iGreen Firm manufacturer, Coimbatore, Tamil Nadu was initially dried under sun followed by oven drying for 24 hours at 60°C and used for preparation of extract. Two hundred gram 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 what man filter paper and stored under refrigerated condition at 40 C till usage[7]. This *Spirulina platensis* extract was used as 100 % concentrated stock for further studies.

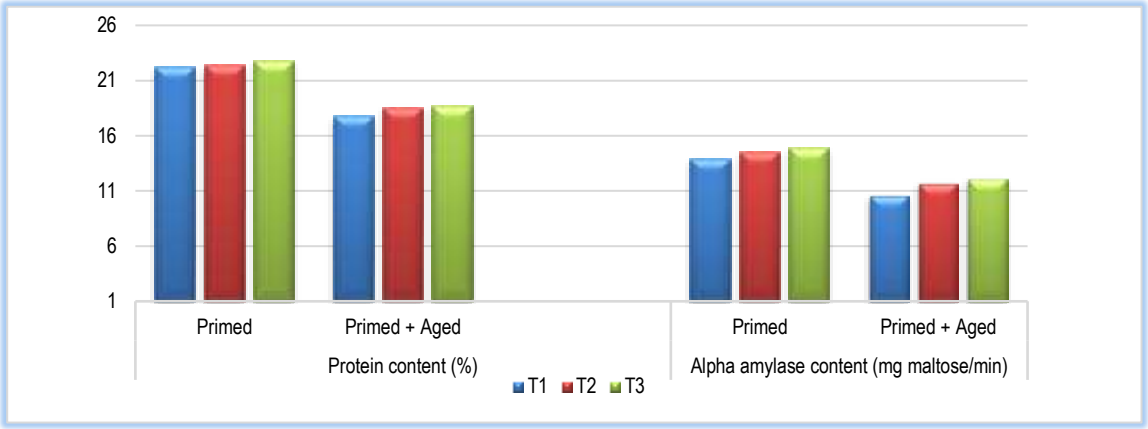
Treatments

Blackgram seeds were subjected to hydro-priming with distilled water for 3 hours and dried back to original moisture content of about 9.0% (T2); primed with *Spirulina platensis* extract at 1.5% concentration by soaking for 3 hours then dried

Table-1 *Effects of seed priming with Spirulina platensis extract on seed germination, root length, shoot length, speed of germination, dry matter production and vigour index in Blackgram CO6*

Treatment	Germination (%)			Root length (cm)			Shoot length (cm)			Speed of germination			DMP (mg/10 seedlings)			Vigour index		
	Primed (P)	Primed + Aged (A)	Mean	Primed (P)	Primed + Aged (A)	Mean	Primed (P)	Primed + Aged (A)	Mean	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	16.87	10.53	13.70	225.3	147.7	186.5	2168	855	1511
T_2	89 (70.63)	51 (45.57)	70 (56.79)	15.20	11.10	13.15	13.57	9.93	11.75	17.97	12.63	15.30	230.7	161.7	196.2	2501	1095	1798
T_3	98 (81.87)	62 (51.94)	80 (64.16)	16.23	11.97	14.10	14.67	10.17	12.42	22.13	15.87	19.35	250.0	173.0	211.5	2925	1393	2159
Mean	90 (71.57)	52 (46.15)		15.22	11.36		13.76	9.68		18.99	13.01		235.3	160.8		2531	1114	
	P	A	PxA	P	A	PxA	P	A	PxA	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	0.014	0.011	0.019	0.76	0.62	1.07	15.24	12.45	21.57
CD(P=0.05)	0.514*	0.419*	0.726*	0.078*	0.064*	0.111*	0.068*	0.166*	0.119*	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%, Figures in parenthesis indicate arcsine values, DMP- dry matter production



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 and alpha amylase activity in Blackgram CO6.

back to original moisture content (T3) and the non primed seeds were used as control (T1). Then, the seeds were artificially aged under 40±1oC and 95±5% RH (accelerated ageing) for five days.

Observations

Physiological and biochemical seed quality parameters were recorded in aged and non-aged seeds involved 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 analysed for the 'F' test of significance following the methods described [17]. Wherever necessary, the percent values were transformed to angular (Arc-sine) values before analysis. The critical differences (CD) were calculated at 5 percent probability level. The data were tested for statistical significance.

Results and Discussion

Physiological changes in blackgram seeds due to priming with *Spirulina platensis* extract

The results revealed that the blackgram seed primed with *Spirulina platensis* extract at 1.5% concentration (T3) recorded the maximum germination of 98% when compared to hydropriming (T2) and control (T1) which recorded only 89 % and 82 % germination, respectively. The percentage increase in germination due to priming with *Spirulina platensis* extract was 9.0 % and 16 % over the hydropriming and control, respectively. After accelerated ageing also, the seeds primed with *Spirulina platensis* extract at 1.5% (T3) recorded significantly higher germination (62%) which was 11 % and 18 % higher than the hydropriming and control, respectively. Similarly, the root length of the seeds primed with *Spirulina platensis* extract at 1.5% (T3) were significantly longer both before ageing (16.23 cm) and after ageing (11.97 cm) than the hydroprimed and nonprime control seeds. The shoot length of the seedling was 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 the hydropriming and control seeds [Table-1]. Due to soaking the seeds in *Spirulina platensis* extract might have improved the availability of seed food reverses and also maintained the cell structure leading to increased seed vigour and germination, besides preventing the seed deterioration during accelerated ageing. Similar results were also reported and concluded that pre-sowing soaking might have helped in imbibing enough quantity of water and suitable nutrient concentration resulting in quick initiation of germination process and improving physiological parameters in annona [18], in blackgram and cowpea [19], in cereal [20]and in blackgram [21]. Speed of germination was termed as one of the best test to analyse the vigour potential of seeds. The speed of germination of seeds primed with *Spirulina platensis* extract at 1.5% for 3 hours (T3) was 22.13 in primed seeds before ageing and 15.87 after accelerated ageing which were significantly higher than hydropriming (T2) and control (T1). *Spirulina platensis* extract having biological chemicals and nutrients involved in priming process might have triggered the germination process early in seed primed seeds [Table-1]. The result was accordance to a report that seed priming enhances speed and uniformity of germination [22]. Seed primed with *Spirulina platensis* extract at 1.5% recorded significant higher dry matter production of 250.0 mg 10 seedling-1 before ageing and 173.0 mg 10 seedling-1 after accelerated ageing than seed hydropriming and control. The computed vigour index value was also recorded similar trend as that of germination, root and shoot length, speed of germination and dry matter production. 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 were significantly higher than hydropriming and control [Table-1]. The results were similar to a reported that dry matter production and vigour index of seed priming with chitosan 400 ppm + PPFM 2% was super higher than hydropriming and control in blackgram [21]. These results showed that physiological parameters of seeds priming with *Spirulina platensis* extract at 1.5% were superior to than hydropriming and control.

Biochemical changes in blackgram seeds due to priming with *Spirulina platensis* extract

The biochemical analysis of the blackgram seeds showed that the protein content was increased due to priming when compare to control. However, seeds priming with *Spirulina platensis* extract at 1.5% for 3 hours (T3) recorded the highest value of 22.78 % while control (T1) was 22.25% of protein content before ageing.

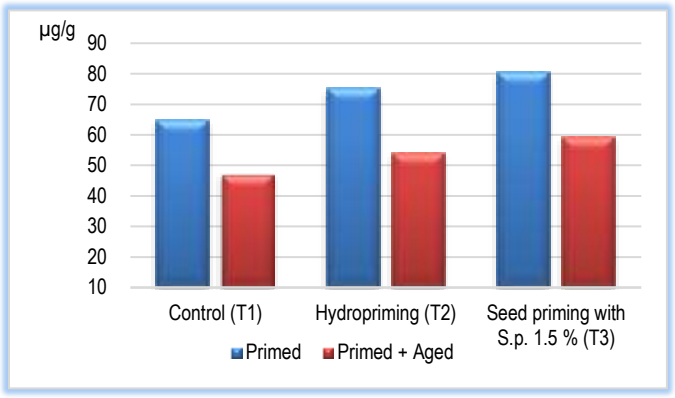


Fig-2 Effects of seed priming with Spirulina platensis extract on gibberellic acid content in Blackgram CO6

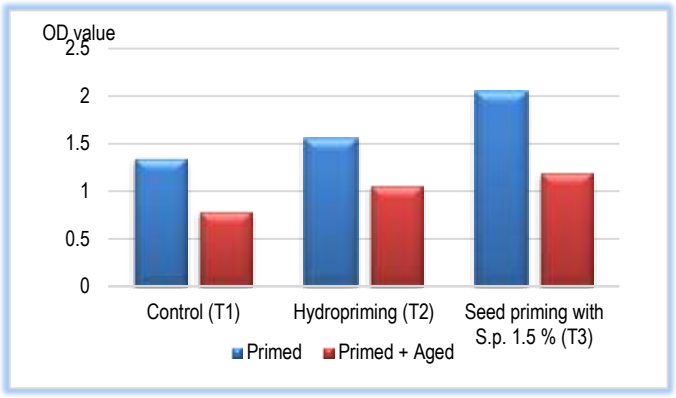
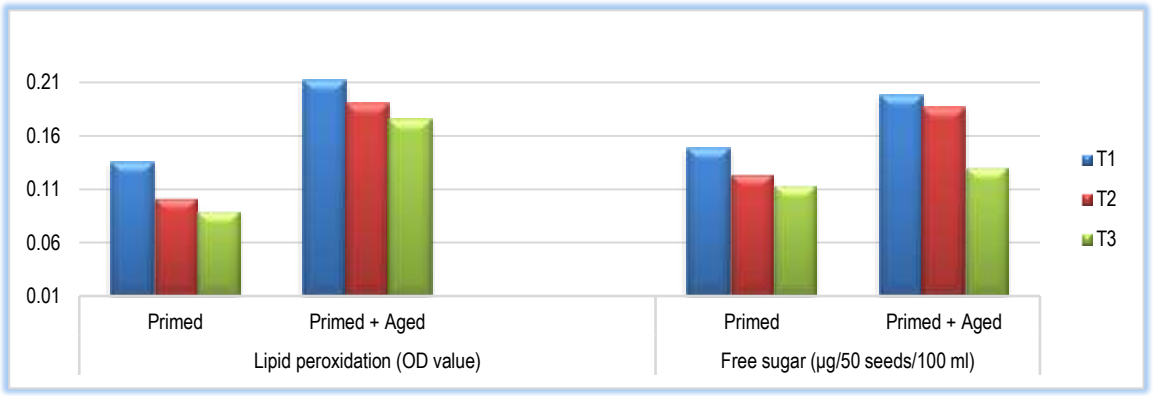


Fig-3 Effects of seed priming with Spirulina platensis extract on Dehydrogenase activity in Blackgram CO6



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 Blackgram CO6.

After accelerated ageing, seeds priming with *Spirulina platensis* extract at 1.5% for 3 hours recorded 18.65 % of protein content while control (T1) was measured 17.71 % of protein content [Fig-1]. The result was similar to are port that priming of seed promotes germination by repair of the damaged proteins, RNA and DNA [23]. In the present study, as well, *Spirulina platensis* extract having vitamins, nutrients and amino acids might have influenced in increasing the protein content of blackgram seeds. Amylase is an important enzyme which breakdown starch in to the most common transportable form of sugar. Among the treatments, the α-amylase activity increased significantly in primed treatments compared to non-primed seeds, but seeds priming with *Spirulina platensis* extract at 1.5% (T3) had registered the maximum α-amylase activity of 14.67 mg maltose/minutes while control (T1) recorded only 14.36 mg maltose/minutes. After artificially ageing also, 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 hydropriming. Seed priming with *Spirulina platensis* extract had improved seed structure leading to maintain the seed metabolism to synthesis α-amylase during germination [Fig-1]. The result was accordance to a report that seed priming enhanced α-amylase content under chilling stress in maize [24]. Gibberillic acid which is an important growth hormone is very much important for promotion of seed germination. The seeds primed with *Spirulina platensis* extract at 1.5% (T3) recorded the highest gibberellic acids content of 80.33 µg/g of seed while control (T1) recorded the lowest gibberellic acid content of 64.7 µg/g of fresh weight of seeds [Fig-2]. Similar result was also a report which indicated that the gibberellic acid is the major bioactive molecule involved in seed germination [25]. The gibberellic acids content after accelerated ageing was also significantly higher in seeds primed with *Spirulina platensis* extract at 1.5%than in the hydro-primed seeds and non-primed control seeds. Because, the *Spirulina platensis* contains more growth regulators including gibberellic acids which might be supplied to the seeds during soaking and drying process. Dehydrogenase is an enzyme which contributes towards improving seed germination and all the living seeds those respire produce enzymes called dehydrogenase [26]. The results of the study showed that the dehydrogenase enzyme activity in the seeds priming with *Spirulina platensis* extract(T3) was significantly higher (2.053)when compare to hydropriming (1.561) and control (1.3503). After accelerated ageing also, seeds primed with *Spirulina platensis* extract recorded higher enzyme activity when compare to hydropriming and control. *Spirulina platensis* extract having antioxidants, growth regulators, vitamins, macro and micro nutrients might have involved in repair mechanism and maintained the cells and thus increased the dehydrogenase activity in primed seeds and maintained it even under accelerated ageing [Fig-3]. Lipid peroxidation is the oxidative degradation of lipids. It is the process in which free radicals stealelectrons from the lipids in cell membranes, resulting in cell damage. Therefore, lipid peroxidation is a measure of degree of seed deterioration. In the present study, seeds primed with *Spirulina platensis* extract (T3) recorded the lowest lipid peroxidation value of 0.089than the control (T1) which showed the highest value of 0.135. Artificially aged seeds showed an increase in lipid peroxidation, however, seed priming with *Spirulina platensis* extractrecorded minimum value of 0.176 than hydropriming (0.190) and control (0.212) with significance. These results indicated that seed priming with *Spirulina platensis* extracthas improved the seed vigour by mitigating lipid peroxidation in primed seeds much lower than non-primed seeds and maintained the seed vigour better than non-primed seeds even under accelerated ageing. It might be due to the reason that the *Spirulina platensis* extract having antioxidants might have reacted with lipid and quenched the free radicals leading to improve the seed quality and reduce deterioration [Fig-4]. Free sugar content in seed leachate is an index of cell membrane integrity and seed vigour. The leachate of seeds primed with *Spirulina platensis* extractfor 3 hours (T3) 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. One the other hand, non primed and hydroprimed seeds recorded higher free sugar content in both before and after accelerated ageing [Fig-4]. It might be due to the seeds primed with *Spirulina platensis* extract maintained the cell membrane integrity in primed seeds and tolerance to accelerated ageing.

The result was similar to are port that improvement in germination by priming might be due to enhanced repair of membranes which are disrupted during maturation drying[27]. This is indirectly supported by the reduced leakage of electrolytes from primed seeds, since electrolyte leakage is in part a result of damage cell membranes. However, electrolytes may be leaked out during priming, resulting in lower levels of electrolytes in primed seeds than in control. The results of these studies indicated that the seed priming with Spirulina *platensis* extract at 1.5% for 3 hours might have resulted in the triggering of seed germination, vigour and control over the seed deterioration and mechanism of seed ageing. Moreover, it is reasonable to assume that the priming have also involved in the rearrangement of cell membrane structure which lost during seed ageing and increased the membrane integrity [28], vigour was increased chiefly by modifications in cell membrane structure and also by activation of protein synthesis during priming process[29] and the efficacy of priming treatment on vigour and viability maintenance [30].

Conclusion

From this study, it could be concluded that the seeds primed with *Spirulina platensis* extract at 1.5% for 3 hours significantly improved all the physiological parameters *viz*., seed germination, speed of germination, dry matter production, seedling length and vigour index and also vigour associated biochemical parameters like protein content, gibberellic acids content, α-amylase activity and dehydrogenase activity accompanied with lower lipid peroxidation, free sugar in seed leachate of blackgram seeds.

Application of research: The findings of the research will be useful for the seed's men and researchers who are involved in seed preservation and also to apply organic treatments.

Research Category: Seed Science and Technology

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Conflict of Interest: None declared

Ethical approval: This article does not contain any studies with human participants or animals performed by any of the authors.

Sample Collection: Seeds of Blackgram (*Vigna mungo* (L) cv. CO6 obtained from Department of Pulses, Tamil Nadu Agricultural University and *Spirulina platensis* powder bought from iGreen Firm manufacturer, Coimbatore for this study.

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