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Effect of Seed Invigouration Treatments on Physiological Parameters and Nodulation of Grain Cowpea [*Vigna unguiculata* (L.) Walp]

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ABSTRACT

The experiment was conducted at Coconut Research Station, Balaramapuram with an objective to assess the effect of seed invigouration with $ZnSO_4$ and borax alone and along with *Trichoderma viride* on physiological parameters and nodulation of grain cowpea. Seed invigouration treatments had significant effect on physiological parameters as well as nodulation parameters in grain cowpea. Leaf area index and total chlorophyll content were found to be higher in seeds primed in $ZnSO_4$ 0.025 and 0.05 per cent for 4h at both 30 and 60 days after sowing (DAS). From 30 to 60 DAS, crop growth rate (CGR) and relative growth rate (RGR) were the highest in seeds pelleted with borax 50 mg kg^{-1} seed and from 60 DAS to harvest, seeds primed in $ZnSO_4$ 0.05 per cent for 4h registered the highest CGR and RGR. Total number of nodules and effective nodules per plant were found to be higher in seeds pelleted with borax 50 and 100 mg kg^{-1} seed. However, the nodule fresh and dry weight were the highest in seeds primed in $ZnSO_4$ 0.05 per cent for 4h. Hence it can be concluded that compared to seed pelleting with borax, seed priming with $ZnSO_4$ was found better for nodulation as well as better expression of physiological parameters in grain cowpea.

Key words: Borax, Chlorophyll content, Crop growth rate, Leaf area index, Nodulation, Relative growth rate, Zinc sulphate.

INTRODUCTION

Cowpea [*Vigna unguiculata* (L.) Walp] is the most widely cultivated pulse crop of Kerala. Although cowpea is grown throughout the year, over a variety of farming situations, a large gap exists between the supply and demand for grain cowpea within the state. Thus, there is an urgent need to boost the production and productivity of grain cowpea. There are certain challenges which reduce the yield and productivity of grain cowpea. The major challenges noticed are flower shedding, poor pod and seed setting and shrivelling of grains. These problems may mainly be ascribed due to the deficiencies of micro nutrients. Deficiencies of micronutrients especially zinc (Zn) and boron (B) are widely noticed in pulses. Zinc plays a vital role in plant metabolism and is known to be involved in nodule formation and improved N fixation (Shukla and Yadav, 1982). Boron is imperative for cell division, elongation and cell wall development in plants. It is also needed for the maintenance of nodule cell wall, membrane structure and development of symbiosome (Bolanos *et al*., 2001).

Seed priming and pelleting with micronutrients are the two simple inexpensive strategies to overcome the micronutrient deficiency. Advantage of seed pelleting and priming over soil and foliar application is that both the methods required very less quantity of micronutrients, it ensures uniformity in application and the uneven application can be avoided. Moreover, it is more cost effective than soil and foliar application. With, this background the present study is formulated with an objective to study the effect of seed invigouration with zinc sulphate and borax and its effect along with *Trichoderma viride* on physiological parameters and nodulation in grain cowpea.

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MATERIALS AND METHODS

The experiment was conducted at Coconut Research Station, Balaramapuram, Kerala, India located at 8° 22′ 52″ North latitude and 77° 1′ 47″ East longitude and at an altitude of 9 m above mean sea level. The experiment was conducted in randomized block design with eight seed invigouration treatments and a control (no seed invigouration) in three replications during November 2018 to February 2019. The variety used for the study was Bhagyalakshmy. The treatments comprised seed pelleting with borax 50 and 100 mg kg^{-1} seed; seed priming with $ZnSO_4$ 0.025 and 0.05 per cent for 4h; seed pelleting with borax 50 and 100 mg kg^{-1} seed + *Trichoderma viride* seed treatment 10 g kg^{-1} seed and seed priming with $ZnSO_4$ 0.025 and 0.05 per cent for 4h + *Trichoderma viride* seed treatment 10 g kg^{-1} seed and a control. For pelleting, borax was mixed thoroughly with 10 per cent as maida as adhesive and seeds were individually coated with this mixture and shade dried to eight per cent moisture. For priming, seeds were soaked in respective concentrations of $ZnSO_4$ solution and shade dried to eight per cent moisture. *Trichoderma viride* seed treatment was done by mixing the primed and pelleted seeds with *Trichoderma viride* 10 g kg^{-1} seed and little amount of water and kept for twenty minutes before sowing. Talc based formulation of *Trichoderma viride* having 2×10^6 CFU g^{-1} procured from Department of Agricultural Microbiology, College of Agriculture, Vellayani was used as the source. The crop was uniformly fertilized with farm yard manure (FYM) 20 t ha^{-1}, lime 250 kg ha^{-1} and N:P:K @ 20:30:10 kg ha^{-1}. Lime and FYM applied at the time of first ploughing. Half N, full P and K applied at the time of final ploughing and remaining half N at 15-20 DAS. The plant observation *viz*., number of green leaves per plant, length and breadth of leaves were recorded at 30 DAS and 60 DAS. The dry matter production of plant was recorded at 30 DAS, 60 DAS and at harvest. For dry matter production, five plants outside the net plot area were uprooted and initially shade dried for two days and then oven dried at 60 ± 5°C till a constant weight was obtained. The leaf area index (30 and 60 DAS) was worked out by the formula suggested by Watson (1972). The crop growth rate and relative growth rate were calculated by the formula developed by Watson (1958) and Evans (1972) respectively. The chlorophyll a, chlorophyll b and total chlorophyll content were determined at 30 and 60 DAS by DMSO method proposed by Yoshida *et al*. (1976). Total no. of nodules and effective nodules per plant, nodule dry weight and fresh weight, g per plant were recorded at 50 per cent flowering. The data were statistically analysed and the treatment means were compared at 5 per cent probability level.

RESULTS AND DISCUSSION

Effect on leaf area index

Leaf area index, an important indicator of plant growth was significantly influenced by seed invigouration treatments (Table 1). Seed invigouration treatments registered significantly higher LAI at 30 DAS and 60 DAS. The reason might be due to the fact that seed invigouration enhanced the vigour of seedlings which accelerates the leaf development resulting in higher number of leaves with larger leaf area. Chomontoswki *et al*. (2019) reported that seed priming accelerates the leaf development and significantly enhanced the LAI in sugar beet. Seed invigouration treatments also recorded higher number of effective nodules and nodule mass compared to control (Table 4), which will increase the N fixation and N supply to the plants. Shanti *et al*. (1997) observed that increased N supply enhanced the vegetative growth with more photosynthetic surface and higher LAI. At 30 DAS, the treatment T_4 (seed priming with $ZnSO_4$ 0.05 per cent for 4h) registered higher LAI and it was statistically on par with T_1 (seeds pelleted with borax 50 mg kg^{-1} seed), T_2 (seeds pelleted with borax 100 mg kg^{-1} seed), T_3 (seed priming with $ZnSO_4$ 0.025 per cent for 4 h)

Table 1: Effect of seed invigouration treatments on LAI at 30 DAS and 60 DAS.

Treatments	LAI 30 DAS	LAI 60 DAS
T_1	4.30	5.37
T_2	4.20	5.30
T_3	3.93	5.99
T_4	4.36	5.50
T_5	4.03	5.73
T_6	3.79	5.38
T_7	3.71	5.30
T_8	3.28	5.33
T_9	2.37	4.81
SEm (±)	0.17	0.18
CD (0.05)	0.514	0.547

T_1 and T_2- seed pelleting with borax 50 and 100 mg kg^{-1} seed, T_3 and T_4- seed priming with $ZnSO_4$ 0.025 and 0.05 per cent for 4h, T_5 and T_6- seed pelleting with borax 50 and 100 mg kg^{-1} seed + *Trichoderma viride* seed treatment 10 g kg^{-1} seed, T_7 and T_8- seed priming with $ZnSO_4$ 0.025 and 0.05 per cent for 4h + *Trichoderma viride* seed treatment 10 g kg^{-1} seed and T_9- control.

Table 2: Effect of seed invigouration treatments on chlorophyll content of leaves at 30 DAS and 60 DAS, mg g^{-1}.

Treatments	30 DAS			60 DAS		
	Chlorophyll a	Chlorophyll b	Total chlorophyll	Chlorophyll a	Chlorophyll b	Total chlorophyll
T_1	2.19	2.67	4.86	1.98	2.04	4.03
T_2	1.80	2.74	4.53	1.81	2.41	4.22
T_3	2.13	2.86	4.99	2.39	2.57	4.96
T_4	2.23	2.88	5.11	2.34	2.42	4.76
T_5	1.51	1.96	3.48	1.74	1.44	3.19
T_6	1.91	2.29	4.19	1.74	2.13	3.88
T_7	1.74	1.89	3.63	1.70	1.99	3.69
T_8	1.70	1.91	3.61	1.52	1.57	3.09
T_9	1.06	1.09	2.15	1.33	1.45	2.77
SE m (±)	0.07	0.16	0.18	0.10	0.16	0.16
CD (0.05)	0.209	0.490	0.528	0.314	0.493	0.481

and T_5 (T_1 + *Trichoderma viride* seed treatment 10 g kg^{-1} seed). However, at 60 DAS, T_3 registered the highest LAI and it was statistically comparable with T_4 and T_5. Higher LAI recorded in these treatments might be due to the better development of roots and increased availability of nutrients, which might have enabled the crop to absorb and translocate sufficient quantity of nutrients to establish good canopy with higher number of leaves and branches. The present result is in agreement with the findings of Shinde *et al*. (2017) who observed that seed polymer coating with micronutrients enhanced the seedling vigour resulting in good crop establishment leading to better crop growth with large leaf area index.

Effect on chlorophyll content

Seed invigouration treatments significantly influenced the chlorophyll content of leaf (Table 2). Similar to that of LAI, total chlorophyll content, chlorophyll a and b were higher in seed invigouration treatments. This was owing to the fact that fast and uniform germination and vigorous crop growth achieved due to seed invigouraion might have helped the plant to absorb required quantity of available plant nutrients from the soil which will favour the plant metabolism and chlorophyll synthesis and enhanced the photosynthetic activity. Sathiyanarayanan *et al*. (2015) opined that seed hardening enhanced the absorption of nutrients from the soil. The increased absorption and translocation of nutrients enhanced the chlorophyll synthesis and photosynthetic activity. Among the treatments, seed priming with $ZnSO_4$ 0.05 percent for 4 h recorded higher total chlorophyll (5.11 mg g^{-1}), chlorophyll a (2.23 mg g^{-1}) and chlorophyll b content (2.88 mg g^{-1}) and at 60 DAS, seed priming with $ZnSO_4$ 0.025 percent for 4 h recorded higher total chlorophyll (4.76 mg g^{-1}), chlorophyll a (2.39 mg g^{-1}) and chlorophyll b content (2.57 mg g^{-1}). The result was in accordance with the findings of Afzal *et al.* (2015) who pointed out that seed priming with Zn increased the chlorophyll content in spring maize. Seed priming with $ZnSO_4$ might have enhanced the availability of Zn right from seedling to subsequent growth stages of crop growth and increased the nodulation, N fixation, N availability and chlorophyll formation. Sharma *et al*. (2010) reported that Zn plays a major role in chlorophyll formation and enhanced the chlorophyll content of leaf.

Effect on crop growth rate and relative growth rate

Crop growth rate and RGR were also significantly influenced by seed invigouration treatments (Table 3). The increase in CGR and RGR values observed in seed invigouration treatments might be due to higher biomass production resulting from higher LAI and total chlorophyll content. At 30 DAS, the treatment T_1 (seeds pelleted with borax 50 mg kg^{-1} seed) recorded higher CGR (11.46 g m^{-2} day^{-1}) which was statistically at par with T_2 (seeds pelleted with borax 100 mg kg^{-1} seed) and T_6 (T_2 + *Trichoderma viride* seed treatment 10 g kg^{-1} seed). However, at 60 DAS, T_4 (seed priming with $ZnSO_4$ 0.05 per cent for 4 h) registered higher CGR (17.37 g m^{-2} day^{-1}) which was statistically comparable with T_3 (seeds primed in $ZnSO_4$ 0.025 percent for 4 h) and T_1. This might be due to increased DMP and dry matter partitioning observed in these treatments. Higher LAI (Table 1) and chlorophyll content (Table 2) observed in these treatments enhanced the photosynthesis which ultimately increased the dry matter production. The result is in line with the observation made by Amanullah *et al*. (2008) who reported that increase in LAI increases the light interception and total DMP at various growth stages. Afzal *et al*. (2013) reported that seed priming with $ZnSO_4$ 0.5 percent significantly enhanced the CGR in maize. Relative growth rate was found to decrease from 60 DAS to harvest stage in all treatments compared to 30 DAS to 60 DAS stage. Similar observation was also made by Arun *et al*. (2017) in cowpea. Similar to that of CGR, significantly higher RGR was observed in T_1 (seeds pelleted with borax 50 mg kg^{-1} seed) from 30 DAS to 60 DAS and T_4 (seeds primed in $ZnSO_4$ 0.05 percent) from 60 DAS to harvest stage. This might be due to increased DMP resulting from the enhanced photosynthesis as evident from the data on LAI (Table 1) and total chlorophyll content (Table 2).

Effect on nodulation

Total number of nodules, effective nodules and nodule fresh and dry weight per plant were significantly influenced by

Table 3: Effect of seed invigouration treatments on crop growth rate and relative growth rate of grain cowpea.

Treatments	CGR (g m^{-2} day^{-1})		RGR (mg g^{-1} day^{-1})	
	30 DAS to 60 DAS	60 DAS to harvest	30 DAS to 60 DAS	60 DAS to harvest
T_1	11.46	16.81	45.22	24.92
T_2	9.83	14.47	35.95	22.41
T_3	8.97	15.51	34.01	25.21
T_4	9.28	17.37	33.67	25.82
T_5	6.60	9.98	24.16	19.81
T_6	10.53	15.15	41.23	24.43
T_7	6.34	8.30	24.53	17.24
T_8	5.69	9.28	28.56	20.52
T_9	6.07	10.18	31.37	24.11
SEm (±)	0.56	0.62	0.97	0.80
CD (0.05)	1.698	1.888	2.935	2.681

Table 4: Effect of seed invigouration treatments on nodule parameters of grain cowpea at 50 per cent flowering.

Treatments	Total no. of nodules per plant	Total no. of effective nodules per plant	Nodule fresh weight (g per plant)	Nodule dry weight (g per plant)
T_1	32.7	27.3	0.33	0.06
T_2	32.7	29.0	0.43	0.08
T_3	20.3	17.0	0.45	0.08
T_4	25.3	22.3	0.60	0.09
T_5	18.0	14.7	0.22	0.03
T_6	29.7	26.0	0.25	0.06
T_7	17.3	15.7	0.29	0.04
T_8	22.7	20.0	0.33	0.05
T_9	12.0	8.0	0.15	0.03
SEm (±)	1.9	1.5	0.03	0.01
CD (0.05)	5.74	4.41	0.074	0.014

seed invigouration treatments (Table 4). Compared to control, seed invigouration treatments recorded higher number of nodules per plant, effective nodules per plant and nodule fresh and dry weight per plant. Early seedling emergence, better seedling establishment, better root development and root biomass production might have helped in the formation of more nodules in seed invigouration treatments. The present finding is in accordance with the observations of Lhungdim *et al*. (2014) who observed that seed invigouration enhanced the rhizobial population in lentil. Among the treatments, seeds pelleted with borax registered a greater number of total nodules and effective nodules per plant compared to seeds primed in $ZnSO_4$. This was due to the role of B in cell division in the formation of nodule (Brady and Weil, 2002). Zehirov and Georgiev (2003) also observed that B deficiency will inhibit the cell wall development and cell wall permeability which cause reduction in nodule number. Though the seeds primed in $ZnSO_4$ recorded lesser nodule number compared to seeds pelleted with borax, nodules were bigger in size and hence the fresh weight as well as dry weight of nodules were found to be more. Upadhyay and Singh (2016) reported that Zn has a significant role in N fixation through nodule formation. The growth of symbiotic bacteria inside the nodule depend on the sucrose transport from leaves to root nodule. The transport of sucrose from leaves to root nodule is mediated by Zn (Udvardi and Poole, 2013), also Zn plays a key role in the biosynthesis of leghaemoglobin (Das *et al*., 2012). Marsh and Waters (1985) reported that Zn deficiency in legumes reduces the size of nodules. Increase in nodule weight due to Zn fertilization was also reported by Desta *et al*. (2015) and Debnath *et al*. (2018).

CONCLUSION

It can be concluded from the results that seed priming with $ZnSO_4$ 0.05 per cent was found better for the expression of physiological parameters *viz*., LAI, chlorophyll content, CGR and RGR compared to seeds pelleted with borax. Nodule number was found to be higher in seed pelleted with borax 50 and 100 mg kg^{-1} seed, however the nodule fresh and dry weight were the highest in seed priming with $ZnSO_4$ 0.05 per cent for 4 h. Seed invigoration with borax and $ZnSO_4$ followed by seed treatment with *Trichoderma viride* recorded lesser values for physiological parameters and nodule parameters compared to seed pelleted with borax and seed priming with $ZnSO_4$ alone.

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