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Effect of bio and nutripriming on seed quality and yield in chia (*Salvia hispanica* L.)

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Abstract
The present work was conducted to standardize the seed priming duration in chia at Laboratory, Department of Seed Science and Technology, UAS, Dharwad during 2020. 20 minutes of seed priming duration recorded higher germination (91.75%), seedling length (13.53 cm) and seedling dry weight (1.03 g/ 10 seedlings),) compared to control. Another experiment was carried out to know the effect of biopriming and micronutrient priming on quality and yield parameters in chia at ARS, Bagalkot. Seed priming with Zinc (1.5%) recorded higher seed quality and growth parameters *viz*., germination (95.25%), seedling length (16.33 cm), seedling dry weight (1.260 g/10 seedlings), plant height (91.73) followed by *Azospirillum brasiliense* (20%). Seeds primed with Zinc (1.5%) showed higher yield attributing traits *viz.*, number of spikes per plant (18.53), spike length (16.30 cm), yield per plant (15.68 g), yield per hectare (752.38 kg ha^{-1}) and 1000 seed weight (1.332 g) followed by PSB (20%) over control. Hence, it can be concluded that seed priming with Zinc (1.5%), *Azospirillum brasiliense* (20%) and PSB (20%) enhances the quality, growth and seed yield in Chia.

Keywords: Chia, priming, duration, biopriming, micronutrient, quality, yield

Introduction

Chia (*Salvia hispanica* L.) is an herbaceous plant that belongs to the *Lamiaceae* family, which also includes mint (*Mentha spicata*), rosemary (*Rosmarinus officinalis*) and thyme (*Thymus vulgaris*). Chia grows up to one meter height and has oppositely arranged leaves. Chia produces white or purple small flowers (3 to 4 mm) with smaller corolla. Fused flower parts will contribute to higher self-pollination in Chia. The seed colour of chia varies from the grey, black and a black pointed to white and the shape is oval with the sizes ranging from one-two mm (Bresson *et al.*, 2009) [5]. It is mainly cultivated in the countries like Australia, Argentina, Bolivia, Colombia, Guatemala, Mexico and Peru. CHIA has repeatedly been cultivated in South-east Asian and Caribbean regions (Jansen *et al*., 1991) [13]. A chia seed contains protein (15 – 25%), fats (30 – 33%), high dietary fibre (18 – 30%), carbohydrates (26 – 41%), ash (4 – 5 per cent), vitamins, minerals, and dry matter (90 – 93%). It is also containing higher number of antioxidants (Ixtaina *et al*., 2008) [12]. Chia seeds are rich in Omega (ω) -3 and ω - 6 fatty acids which are the essential poly unsaturated fatty acids containing Alpha linolenic acid and linoleic acid. Seed priming has been reported to be one of the most significant advances in facilitating rapid and uniform seed germination and emergence and increasing the seed tolerance to adverse environmental conditions (Heydecker *et al.*, 1973; Harris *et al.*, 1999) [11, 9]. In seed priming, duration of priming is highly variable and critical, so standardization of priming duration is very important to exploit higher yield and quality parameters by imposing the treatments. In seed bio-priming, effect of priming agents is highly variable. Hence preliminary work on interaction of chia with bio priming agents is very important. Micronutrients are important for plant growth and human health. The potential of micronutrient seed priming for improving crop growth and grain nutrient enrichment can be utilised in increasing the productivity of chia.

Material and Methods

The laboratory experiment was conducted with an objective to standardize the seed priming duration in Chia. The experiment was carried out in the Seed science and technology department laboratory, University of Agricultural Sciences, Dharwad. The laboratory experiment was conducted by adapting completely randomized design with four replications.

Experiment consists of eight different treatments with different priming durations *viz*, T_1-Control (without priming), T_2-Hydropriming for 10 minutes, T_3-Hydropriming for 20 minutes, T_4-Hydropriming for 30 minutes, T_5-Hydropriming for 1 hour, T_6-Hydropriming for 2 hours, T_7-Hydropriming for 3 hours, T_8-Hydropriming for 4 hours. Seeds were analysed for their quality parameters.

Standard germination test was conducted as per the procedure given by International Seed Testing Association (Annon., 2017) [4]. Further observation on seed quality parameters such as seedling shoot length (cm), root length (cm), seedling dry weight (mg/seedling), seedling vigour index values were measured and analysed statistically. The seedling vigour index was calculated as the formula suggested by Abdul-Baki and Anderson (1973) [2] by multiplying the germination per cent with seedling shoot length and root length (cm). Electrical conductivity of seed leachate was determined by using five grams of seeds and 25 ml of distilled water was added to seeds and kept in the incubator maintained at 25°C ± 1°C temperature for twelve hours. The electrical conductivity of the leachate was measured in dSm^{-1} (Anon., 1995) [1].

Another experiment was carried out to know the effect of biopriming and micronutrient priming on seed quality and yield parameters in chia at ARS, Bagalkot. Experiment consisted of 10 priming treatments *viz.*, T_1-Control, T_2-*Azospirillum brasiliense* (20%), T_3-*Bacillus subtilis* at (0.6%), T_4-*Pseudomonas fluorescens* (20%), T_5-*Trichoderma harzianum* at (0.6%), T_6-Phosphorus solubilizing bacteria (20%), T_7-Zinc (1.5%), T_8-Boron (1%), T_9-Iron (1%), T_{10}-Manganese (1.5%) for 20 minutes duration. These primed seeds were used to check the quality parameters in laboratory and yield parameters. The field experiment was laid out in Randomized Complete Block Design having three replications.

The analysis and interpretation of the experimental data was done as suggested by Panse and Sukhatme (1985). The critical differences were calculated at one per cent and five per cent level of probability in laboratory and field respectively wherever ‘F’ test was found significant for various seed quality parameters under study.

Results and Discussion

Significant difference was observed due to different seed priming durations on seed quality parameters. Significantly higher seed quality parameters *viz.*, seed germination (91.75%), root length (3.76 cm), shoot length (9.77 cm), seedling length (13.53 cm), seedling dry weight (1.03 g/10 seedlings), seedling vigour index-I (1244.76) and seedling vigour index-II (94.31) was recorded in 20 minutes (S_3) seed priming duration compared to control (S_1-85.75%, 2.47 cm, 9.59 cm, 9.59 cm, 0.79 g/10 seedlings, 824.74 and 67.74 respectively) (Table 1).

The probable reason for early and higher germination of seeds soaked for 20 minutes (S_3) may be the completion of pre-germinative metabolic activities making the seed ready for radical protrusion consequently the seed germinated soon after incubating for germination test compared to the other priming durations and to the unprimed control. Emergence enhancement may be attributed to metabolic repair processes, a build-up of germination metabolites during 20 minutes priming duration.

The seed priming treatments improves the quality of seeds. Primed seeds tested for different seed quality parameters performed better than the unprimed control. Significantly higher germination percentage (95.25%), root length (4.76), shoot length (10.50), seedling length (14.77), seedling dry weight (1.203 g/10 seedlings), seedling vigour index-I (1555), seedling vigour index-II (1198) and lowest EC were observed in zinc at 1.5% (T_7) primed seeds followed by *Azospirillum brasiliense* at 20% (T_3-93.75%, 4.53 cm, 11.25 cm, 15.78 cm, 1.213, 1480, 112.0 respectively) and PSB primed seeds (T_6-92.75%, 4.27 cm, 10.50 cm, 14.77 cm, 1.158g/10 seedlings, 1370, 107.6, 0.20 dSm^{-1} respectively).

The probable reason for significant improvement of seed quality parameters primed with zinc could be justified by the role of zinc in enzymatic activities associated with auxin metabolism. Zinc plays a crucial role in the efficiency of growth regulation as a structural component and cofactor (Rudani *et al*., 2018) [28]. Mallikarjuna *et al*., 2020 [18] have given molecular evidences about direct correlation of zinc availability of plants with regulation of ethylene, auxin, gibberellins, and cytokinin like growth regulators hence implying the role in better growth and development of seedlings as depicted by current findings.

The improvement in seed germination and seedling vigour parameters under laboratory conditions due to seed biopriming with *Azospirillum brasiliense* 20% could be possible because of the production of germination accelerating and growth promoting substances by the Azospirillum. Similar observations were reported by Morgenstern and Okon (1987) [22], they reported that, auxin, gibberellins and cytokinin are synthesised and produced when the seeds were inoculated with Azospirillum. Tien *et al*. (1979), Cacciari *et al*. (1989) [6] and Tiwary *et al*. (1998) [32] also clearly established the production of gibberellins and cytokinins. Because of this Azospirillum inoculation, Okon and Kapulnik (1986) [24] noticed larger proportion of younger roots and seminal root elongation, which resulted in increased size and number of root hairs (Kapulnik *et al*., 1985). In addition, the Azospirillum colonies efficiently in the root hairs which improved the water uptake (Sarig *et al*., 1988) [29] in the early stages of growth. Ramamoorthy *et al*. (2000) [27] reported that, seed biofortication with Azospirillum enhanced seedling vigour encompassing speed of germination, seedling length and dry weight of high vigour in low vigour seed lots in rice.

The relative enhancement of germination and seedling vigour might be attributed to the role of phosphorus solubilising bacteria in enhancing the solubilisation of insoluble phosphorus and making it available to the germinating seed with consequent enhancement in the metabolic activity which resulted in higher germination (Cooper, 1979) [7]. According to Kavitha (2011) [16] seed biopriming with liquid phosphobacteria at 15% was found to be the best seed biopriming treatment for rice seed to enhance the germination rate, total germination percentage, seedling growth and vigour in ADT 43. Okra seeds bioprimed with liquid phosphobacteria -20% also resulted in higher germination percentage and seedling vigour (Mariselvam, 2012) [19]. Phosphobacteria at 20% concentration biopriming was found to improve the speed of germination, germination, root length, shoot length, dry matter production, total dry matter production and vigour index. (Karthika and Vanangamudi, 2013) [15].

Significantly higher growth parameters such as Initial plant stand-49.84, plant height-(10.5 cm, 22.2 cm, 65.4 cm, 91.73 cm at 15, 30, 60 DAS and at harvest stage respectively), number of branches per plant (5.33, 11.93, 14.06 at 30, 60 DAS, harvest stage respectively) and final plant stand-48.0 were recorded with zinc at 1.5%(T_7) priming followed by *Azospirillum brasiliense* at 20% (T_3) priming; Initial plant stand-49.28, plant height-(10.1 cm, 20.8 cm, 62.4 cm, 89.4 cm at 15, 30, 60 DAS and at harvest stage respectively), number of branches per plant (4.53, 10.33, 12.86 at 30, 60 DAS, harvest stage respectively) and final plant stand-46.3 and PSB(T_6) priming; Initial plant stand-48.72, plant height-(9.6 cm, 19.8 cm, 59.03 cm, 85.06 cm at 15, 30, 60 DAS and at harvest stage respectively), number of branches per plant (4.66, 10.86, 13.13 at 30, 60 DAS, harvest stage respectively) and final plant stand-47.3 compared to control(T_1) Initial plant stand (44.8), plant height (6.3 cm, 12.9cm, 39.6 cm, 72.6cm at 15, 30, 60 DAS and at harvest respectively), number of branches per plant (2.80, 7.06, 9.60 at 30, 60 DAS, harvest stage respectively) and final plant stand-42.7.

Zn encourages, cell multiplication, cell division and cell differentiation resulting in increased photosynthesis and translocation of food material which enhanced the plant height and is also improved root system of plants resulting in absorption of more water and nutrients and its utilization. Moreover, micronutrients activate several enzymes (catalase, carbonic dehydrogenize, tryptophan synthates etc.) and involved various physiological activities. $ZnSO_4$ is essential component of several dehydrogenase, proteinase, peptidase and promotes growth of hormones and closely associated with growth, all these factors contributed to cell multiplication, cell division and cell differentiation resulting in increased photosynthesis and translocation of food material which enhanced the number of branches.

Azospirillum regulates the concentration of IAA in the rhizosphere and a regulation of the concentration of ethylene within the roots (Sivakalai *et al.*, 2017) [31]. The beneficial effect of exogenous application of *Azospirillum brasilense* to seeds might be due to the translocation of GA_3 to the aerial part of plants and this perhaps occurs to an extent that is enough to increase epicotyl size and the consequent increase in first node height which is sufficient to positively affect plant height. The enhanced plant height may also be due to the improved and faster plant emergence in Azospirillum primed seeds which might have created nitrogen fixation by the plant, phosphorous solubilization and also cooperative competition among chia plants for light and resulted in taller plants. PSB improved the growth of chia compared to control. This could be due to high P-solubilising and mineralising ability from P-sources, production of growth promoting substances such as IAA and biocontrol compounds like HCN (Adithya *et al*., 2009) [3]. Significant increase in number of branches could be due to release of growth substances as well as mineralisation and solubilization of P-sources (Kumari *et al*., 2009) [17].

Significantly higher yield parameters such as spike length (16.3), number of spikes per plant (18.53), 1000 seed weight (1.332 g), seed yield per plant (15.68 g), seed yield per hectare (752.38 kg) were recorded with zinc at 1.5%(T_7) primed seeds followed by Phosphate solubilizing bacteria at 20% (T_6) primed seeds (15.8 cm, 17.53, 1.307g, 15.14g and 716.72 kg respectively) and *Azospirillum brasiliense* at 20% (T_3) primed seeds (15.23cm, 16.33, 1.275g, 14.75g and 683.95 kg respectively) compared to control (12.033, 9.93, 1.14g, 12.36g and 527.12 kg respectively).

The increase in yield parameters with Zinc priming may due to the reason that Zinc is structural component of many of the proteins (Marschner, 2012). The usage of Zn rich seeds positively affects the yield, promotes seedling growth and corrects to some extent soil induced Zn deficiency symptoms in wheat (Farooq *et al*., 2012; Prom-u-thai *et al*., 2012) [8, 26] and other field crops. It was reported that priming was superior over soil fertilizer treatments in improving the yield (Harris *et al*., 2004; Seddigh *et al*., 2016) [10, 30].

The positive influence of *Azospirillum brasiliense* priming on the grain yield might be through their effect on actively growing regions in such a way that they encourage nitrogen fixation, phosphorous solubilization and mobilize the nutrients absorbed elsewhere towards the shoot resulting in better vegetative growth and subsequent yield (Mohammad *et al.*, 2009) and (Nezarat and Gholami, 2009) [23]; the results of which are in agreement with the findings of the present investigation.

The possible reason for increase in yield and its contributing character of PSB primed seeds might be due to cumulative effect of phosphate solubilization and plant growth promotion activities. The coordinated expression of above activities might have been responsible for enhancing plant growth and yield in chia.

Table 1: Effect of seed priming durations on seed quality parameters *viz.*, seed germination, root length, shoot length, seedling length, seedling dry weight, seedling vigour indices and electrical conductivity in chia

Treatments	Germination (%)	Root length (cm)	Shoot length (cm)	Seedling length (cm)	Seedling dry weight (g/10 seedling)	Seedling vigour index−I	Seedling vigour index -II	EC (dSm^{-1})
S_1: Control	85.75(67.89)	2.47	7.12	9.59	0.79	824.7	67.7	0.212
S_2: Hydropriming 10 min.	89.25(71.05)	3.54	8.48	12.02	0.91	1069.7	81.2	0.229
S_3: Hydropriming 20 min	91.75(73.58)	3.76	9.77	13.53	1.03	1244.7	94.3	0.249
S_4: Hydropriming 30 min	87.75(69.63)	3.05	8.15	11.2	0.86	985.6	75.2	0.261
S_5: Hydropriming 1 h	84.25(66.65)	2.77	6.20	8.97	0.77	753.5	65.1	0.281
S_6: Hydropriming 2 h	81.25(64.38)	2.56	4.97	7.53	0.70	617.5	57.3	0.312
S_7: Hydropriming 3 h	77.50(61.72)	2.10	4.73	6.83	0.65	532.7	50.7	0.320
S_8: Hydropriming 4 h	73.75(59.19)	1.76	4.55	6.31	0.62	466.9	46.0	0.338
Mean	83.90 (66.76)	2.75	6.74	9.49	0.79	8111.93	67.2	0.275
S.Em±	1.44	0.09	0.14	0.19	0.017	16.86	5.03	0.006
C.D at 1%	5.70	0.39	0.55	0.77	0.069	66.79	19.89	0.024

* Figures in the parentheses indicates arcsine-transformed values

Table 2: Effect of bio priming and micronutrient priming on seed quality parameters *viz.*, seed germination, root length, shoot length, seedling length, seedling dry weight, seedling vigour indices and electrical conductivity in chia

Treatments	Germination (%)	Root length (cm)	Shoot length (cm)	Seedling length (cm)	Seedling dry weight (g/10 seedlings)	SVI-I	SVI-II	EC (dSm^{-1})
T_1: Control	86.25(68.21)	2.53	7.09	9.62	0.820	830	71.4	0.261
T_2: *Azospirillum brasilense* at 20%	93.75(75.52)	4.53	11.25	15.78	1.213	1480	112.0	0.189
T_3: *Bacillus subtilis* at 0.6%	86.75(68.63)	2.75	7.49	10.24	0.855	889	74.6	0.247
T_4: *Pseudomonas fluorescens* at 20%	91.25(72.81)	3.71	9.48	13.19	1.063	1203	95.6	0.213
T_5: *Trichoderma harzianum* at 0.6%	88.25(69.93)	3.06	8.09	11.15	0.918	984	80.5	0.242
T_6: Phosphorus solubilizing bacteria at 20%	92.75(74.37)	4.27	10.50	14.77	1.158	1370	107.6	0.198
T_7: Zinc at 1.5%	95.25(77.42)	4.76	11.56	16.33	1.260	1555	119.8	0.181
T_8: Boron at 1%	89.75(71.31)	3.53	9.06	12.59	1.020	1130	91.8	0.218
T_9: Iron at 1%	92.25(73.82)	4.00	10.06	14.06	1.125	1297	103.1	0.203
T_{10}: Manganese at 1.5%	89.5(71.08)	3.25	8.50	11.75	0.965	1052	85.9	0.226
Mean	90.57 (72.3)	3.64	9.31	12.94	1.030	1179	94.2	0.218
S.Em±	0.54	0.03	0.05	0.65	0.012	8.68	1.52	0.002
C.D at 1%	2.11	0.12	0.21	0.25	0.049	33.76	5.34	0.008

Table 3: Effect of biopriming and micronutrient priming on initial plant stand and plant height at different intervals in chia

Treatments	Initial plant stand (15 DAS)	Plant height (cm)			
		15 DAS	30 DAS	60 DAS	90 DAS
T_1: Control (No priming)	44.80	6.30	12.93	39.60	72.60
T_2: *Azospirillum brasilense* at 20%	49.28	10.10	20.80	62.40	89.40
T_3: *Bacillus subtilis* at 0.6%	45.36	6.60	13.60	42.40	74.40
T_4: *Pseudomonas fluorescens* at 20%	47.60	8.90	18.13	54.80	82.80
T_5: *Trichoderma harzianum* at 0.6%	45.92	7.10	15.13	45.40	76.40
T_6: Phosphorus solubilizing bacteria at 20%	48.72	9.60	19.80	59.06	85.06
T_7: Zinc at 1.5%	49.84	10.50	22.20	65.40	91.73
T_8: Boron at 1%	47.04	8.00	16.93	51.53	80.53
T_9: Iron at 1%	48.16	9.10	19.13	57.80	84.80
T_{10}: Manganese at 1.5%	46.48	7.50	15.60	48.26	78.26
Mean	47.32	14.83	17.42	52.66	81.6
S.Em±	0.93	0.22	0.34	1.37	1.29
C.D at 5%	2.74	0.64	1.01	4.05	3.80

Table 4: Effect of biopriming and micronutrient priming on number of branches per plant in chia at different intervals

Treatments	Number of branches per plant			Plant stand at harvest
	30 DAS	60 DAS	Harvest	
T_1: Control (No priming)	2.80	7.06	9.60	42.7
T_2: *Azospirillum brasilense* at 20%	4.53	10.33	12.86	46.3
T_3: *Bacillus subtilis* at 0.6%	3.00	7.40	9.80	43.0
T_4: *Pseudomonas fluorescens* at 20%	4.20	9.46	12.13	45.7
T_5: *Trichoderma harzianum* at 0.6%	3.20	7.86	10.13	44.0
T_6: Phosphorus solubilizing bacteria at 20%	4.66	10.86	13.13	47.3
T_7: Zinc at 1.5%	5.33	11.93	14.06	48.0
T_8: Boron at 1%	3.80	8.93	11.40	44.7
T_9: Iron at 1%	4.33	9.93	12.26	46.0
T_{10}: Manganese at 1.5%	3.60	8.46	11.00	44.3
Mean	3.94	9.22	11.64	45.2
S.Em±	0.12	0.35	0.52	0.96
C.D at 5%	0.35	1.05	1.55	2.84

DAS: Days after sowing

Table 5: Effect of biopriming and micronutrient priming on spike number per plant and spike length in chia

Treatments	Spike number/ plant	Spike length (cm)
T_1: Control (No priming)	9.93	12.03
T_2: *Azospirillum brasilense* at 20%	16.33	15.23
T_3: *Bacillus subtilis* at 0.6%	10.93	12.60
T_4: *Pseudomonas fluorescens* at 20%	15.20	14.56
T_5: *Trichoderma harzianum* at 0.6%	12.20	12.96
T_6: Phosphorus solubilizing bacteria at 20%	17.53	15.80
T_7: Zinc at 1.5%	18.53	16.30
T_8: Boron at 1%	13.86	13.93
T_9: Iron at 1%	15.73	14.93

T_{10}: Manganese at 1.5%	12.86	13.46
Mean	14.31	14.18
S.Em±	0.62	0.39
CD @ 5%	1.83	1.18

Table 6: Effect of biopriming and micronutrient priming on test weight, yield/plant and yield/hectare in chia

Treatments	Test weight (g)	Yield/plant (g)	Yield/ha (kg/ha)
T_1: Control (No priming)	1.140	12.36	527.12
T_2: *Azospirillum brasilense* at 20%	1.275	14.75	683.95
T_3: *Bacillus subtilis* at 0.6%	1.162	12.69	545.72
T_4: *Pseudomonas fluorescens* at 20%	1.242	13.96	638.12
T_5: *Trichoderma harzianum* at 0.6%	1.182	13.10	577.53
T_6: Phosphate solubilizing bacteria at 20%	1.307	15.14	716.72
T_7: Zinc at 1.5%	1.332	15.68	752.38
T_8: Boron at 1%	1.225	13.54	604.93
T_9: Iron at 1%	1.262	14.33	658.97
T_{10}: Manganese at 1.5%	1.202	13.32	590.86
Mean	1.233	13.89	629.63
S.Em±	0.031	0.288	19.65
CD @ 5%	0.091	0.857	58.41

Conclusion
From the findings of the research conducted the following conclusions can be made. 20 minutes primed chia seeds perform better than the control seeds for seed quality parameters. Micronutrients and bio-agents may be used to prime chia seeds especially Zn, *Azospirillum* and PSB for faster emergence and establishment, growth of plant, uniformity and synchrony in maturity and hence better seed quality and yield could be obtained.

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