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RESEARCH NOTE

Effect of seed bio priming with endophytes on seed quality of soybean under induced drought stress

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Abstract: A lab experiment was conducted during 2021-22 at Raichur Karnataka to study the effect of seed bio priming with endophytes was examined on seed quality parameters of soybean (JS-335). The experiment was laid out in two factorial completely randomized design with four replications. Results showed that seed bio priming with the endophyte *Trichoderma hamatum* (1 x 10^3 conidia ml^{-1}) recorded significantly higher seed germination (81.2%), shoot length (15.9 cm), root length (9.5 cm), seedling vigour index-I (2073), growth rate index (24.08), peak value (893), mean daily germination (10.15) under drought stress as compared to control.

Key words: Endophytes, Seed biopriming, Seed quality, Soybean

Introduction

Soybean is known as a world's most important crop due to high protein as well as oil content (Bellaloui, 2012). It is the most important source of vegetable oil in India that occupies 35-65 percent of total oilseed crop in the country. According to the reports of Anon (2020) the cultivation of soybean is now practiced almost all over the world, including India. In India it occupies an area of 12.09 million hectare with a production of 11.22 million tonnes and 928 kg per hectare productivity.

Nutritionally, soybean is rich in proteins, carbohydrates, fatty acids, minerals and secondary metabolites such as is of lavones, saponins, anthocyanins and lignin (Garcia *et al*, 1997). The improvement of seed quality in soybean [*Glycine max* (L.) Merril] by seed priming treatments is attributed to primary reduction of lipid peroxidation and quantitative changes in biochemical activities inducing greater amylase activity and enhancing sugar percentage during seed germination.

Soybean is grown in different agro-ecological conditions, hence seed germination and vigour are also influenced by various unfavourable environmental factors such as extreme temperature, drought, untimely planting, low or high light intensity and deficiency or toxic levels of nutrients have huge impacts on crop productivity, seed germination and vigour (Lichtenthaler, 1996).

Now a day's drought stress has became a greater challenge to agricultural production worldwide (Rahdari and Hoseini, 2012). Around 50 per cent of the world's area is affected by drought stress as it intervene the normal biochemical, physiological, and morphological processes of plants by reducing leaf size, stem extension, root proliferation, photosynthesis, nutrient absorption and water relation. Further, decrease in the availability of the nutrients is affected by drought stress and ultimately leading to poor growth, development and productivity of crop plants.

Among the different seed priming methods, seed biopriming is found to be the most appropriate one as it ensures the entrance of endophytic bacteria into the seeds and also by avoiding the effect of high temperature (Moeinzadeh *et al.* 2010) and thereby ensuring good emergence and better establishment of crop (Reddy, 2013). Seed bio-priming promote quick and even germination as well as better plant growth (Moeinzadeh *et al.* 2010). As the endophytes might interact more closely with the host plant and therefore, could be used as an efficient biological control agent and offer a unique opportunity for crop protection and biological control (Melnick *et al.*, 2011).

Thus it is necessary to improve quality of other external inputs by utilizing useful endophytes through seed biopriming for enhancing the planting value, germination, uptake of inorganic phosphate, plant development, seed yield and its quality. Thus the objective of this study was to assess the effect of seed bio priming with endophytes on seed quality parameters under induced drought stress conditions.

Material and methods

The present laboratory investigation was carried out by using soybean *cv.* JS-335 variety seeds and the pure cultures of bio agents were obtained from National Bureau of Agriculturally Important Microorganisms (ICAR-NBAIM), Mau, Uttar Pradesh. The laboratory experiment was carried out in the Bio Control Unit and Seed Testing Laboratory, Seed Unit, University of Agricultural Sciences, Raichur, in the year of 2022. The study was aimed to know the influence of seed bio priming with endophytes on seed quality parameters of soybean under induced drought stress conditions.

The experiment consisted with 32 treatment combinations laid out in two Factorial Completely Randomized Block Design with first factor having four PEG concentration *viz.,* D_1-0 bars, D_2-2 bars, D_3-4 bars, D_4-6 bars and second factor consisting of six bio agents *viz.,* B_1: *Piriformospora indica* (fungal culture 1 x 10^3 conidia ml^{-1}), B_2:*Trichoderma hamatum* (fungal culture 1 x 10^3 conidia ml^{-1}), B_3: *Paenibacillus polymyxa* (bacterial culture 1 x 10^8 cfu ml^{-1}), B_4: *Bacillus amyloliquefaciens* (bacterial culture 1 x 10^8 cfu ml^{-1}), B_5: *Bacillus subtilis* (bacterial culture 1 x 10^8 cfu ml^{-1}), B_6; *Stenotrophomonas maltophilia* (bacterial culture 1 x 10^8 cfu ml^{-1}), including hydro priming and a check, in four replications.

Initially the micro-organisms cultures were prepared by inoculating the suspension from the mother cultures into the freshly prepared potato dextrose broth (PDB) and nutrient broth (NB) for fungal and bacterial cultures, respectively and observed for the growth of microorganisms. Afterwards the seeds were primed (seed to solution ratio of 1: 0.3, Miladinov *et al.* 2015) with different bio priming agents for a duration of 3 hours based on preliminary trial and then the primed seeds were subjected for drying (24 hrs) and brought back to original moisture content and then put for seed germination test using the germination papers immersed in different PEG solutions. The observations were recorded under laboratory condition on seven quality parameters *viz*., standard germination, shoot length, root length, seedling vigour index-I, growth rate index, peak value, mean daily germination.

The seeds of variety JS-335 as 4 ×100 were germinated in between paper (B.P.) method as per the recommendations of ISTA (2013). Then the samples were placed at 25C in the seed germinator. At the end of the germination period, the normal seedlings were counted on 8th day and the seed germination was reported in percentage by adopting the following formula as per the standard procedure (ISTA, 2013).

Germination per cent = (Normal seedlings / Total number of seed) X 100

Among the normal seedlings ten seedlings were randomly selected on 8th day of germination test from each replication from each treatment and measured with the help of a scale for root and shoot length and the mean was expressed in centimeter. The seedling vigour index I was calculated as per the following formula Seedling vigour index-I = Standard germination (%) ´ Shoot length (cm) + root length (cm)

Further to calculate the vigour indices, the seed was observed for its growth on daily basis up to the end of test period and the growth rate index, peak value, mean daily germination were calculated as per the below mentioned formulas.

$$\text{Growth rate index} = \frac{G1}{T1} + \frac{G2}{T2} + \frac{G3}{T3} + \ldots \frac{GN}{TN}$$

GN= Percent germination at last day

TN= Day after planting

$$\text{Peak value} = \frac{\text{Peak germination}}{D} \text{ x } 100$$

D = Day of peak germination

$$\text{Mean daily germination} = \frac{\text{Final germination}}{\text{Total number of the days of test}}$$

The critical difference at 1 per cent level of significance was calculated to compare the mean different treatment.

Results and discussion

Under laboratory conditions the results revealed that the seed germination, shoot and root length, seedling vigour and vigour indices were significantly influenced by different bio-agents under drought stress. Among all the bio-agents, *Trichoderma hamatum* showed positive influence for most of the quality parameter and showed significant differences due to drought stress levels, bioagents as well as drought levels (D) ´ bioagents (B).

Seed germination (%)

Among the drought stress levels (Table 1) the control D_1 (0 bars) recorded significantly higher seed germination (77.6%) followed by D_2 (75.8%) and D_3 (74.3%) and lowest was reported in control D_4 (72.3%). The seed germination is severly affected by the presence of various kinds of abiotic stresses, among them drought stress is one of the most important one that greatly hampers the seed germination and further seedling development. Seed germination is potentially the most critical stage for drought stress (Ahmad *et al*., 2009). Water availability and movement into the seeds are very important to promote germination. The high negative osmotic potential created by drought stress affect the seeds water uptake, making seed germination not possible (Meneses *et al*., 2011). The lesser seed germination under drought was in conformity with the reports of Khodarahmpour, (2011) in corn and , Shahi *et al.* (2015) in wheat.

Among the six different bio control agents, *T. hamatum* showed significantly higher seed germination (81.2%), followed by *B. amyloliquifaciens* (76.7%) and significantly lowest seed germination (70.3%) was recorded by the control. (Table 1). The interaction due to drought stress levels and endophytes showed a non-significant difference for seed germination. Further, among the different endophytes *T. hamatum* showed significantly higher seed germination. Similar findings were also reported by Shukla *et al.* (2015) in wheat and Guler *et al.* (2016) in maize. The possible reason may be due to production of certain hormones like GA_3 by the endophyte which help in uptake of adequate water required for the seeds to germinate and induce osmolyte synthesis to protect the seeds from osmotic stress (Farooq *et al.* 2009).

Shoot and Root length (cm)

The shoot and root length had shown a decreasing trend (Table 1) with an increase in drought levels. Significantly highest shoot (16.1 cm) and root (9.9 cm) length was recorded in control (D_1, 0 bars), while the least shoot (11.8 cm) and root (6.7 cm) length was recorded at highest drought level, D_4 (-6 bars).

The present study revealed that both shoot and root length showed a decreasing trend with an increase in drought stress levels. The possible reason for reduced shoot & root length under drought stress may be due limited supply of metabolites to the growing tissue as the metabolic activity was significantly reduced due to low water availability (Kafi, 2009) resulting in impediment of cell division and elongation leading to tuberization (Fraser *et al.* 1990). The reduced shoot and root length under drought stress has been earlier reported by Omar (2012) in wheat and Ibrahim *et al.* (2000) in sorghum & reduced root length by Yucel *et al.* (2010) in chickpea and Partheeban *et al.* (2017) in maize.

Among the six different bio control agents, *T. hamatum* showed significantly higher shoot (15.9 cm) and root (9.5 cm) length. Further, the lowest shoot (12.1 cm) and root (6.7 cm) length was recorded by control. The interaction effect due to bio control agents and drought levels were non-significant for both shoot and root length.

Table 1. Effect of drought stress and seed bio priming agents and interaction effect on seed quality parameters.

	Seed germination (%)	Shoot length (cm)	Root length (cm)	Seedling vigour index-I	Growth rate index	Peak value	Mean daily germination
D1	77.6	16.1	9.9	2033	21.97	846	9.70
D2	75.8	14.2	8.6	1745	20.79	790	9.48
D3	74	13.2	7.9	1577	19.94	746	9.25
D4	72.3	11.8	6.7	1355	19.38	696	9.03
Mean	74.92	13.82	8.27	1677.5	20.52	769.5	9.36
SEM	0.4	0.2	0.2	26	0.15	7	0.07
C D @ 1%	1.1	0.6	0.6	74	0.46	20	0.14
B1	75.2	13.8	8.5	1695	21.10	800	9.40
B2	81.2	15.9	9.5	2073	24.08	893	10.15
B3	73.6	13.0	7.9	1556	19.71	725	9.20
B4	76.7	15.7	9.1	1912	22.57	862	9.59
B5	75.8	14.2	8.6	1747	21.37	831	9.48
B6	73.7	13.6	8.2	1623	20.33	800	9.21
B7	72.8	12.4	7.4	1457	18.43	650	9.10
B8	70.3	12.1	7.1	1358	21.10	600	8.79
Mean	74.9	13.8	8.2	1677	21.08	770	9.44
SEM	0.5	0.3	0.3	37	0.22	10	0.10
C D @ 1%	1.6	0.8	0.8	104	0.65	29	0.20
D1B1	77.5	15.7	10.3	2023	22.79	875	9.68
D1B2	83.0	18.4	11.2	2459	25.21	950	10.37
D1B3	76.5	15.2	9.3	1883	21.26	775	9.56
D1B4	80.0	18.1	11.0	2333	24.12	975	10.00
D1B5	78.5	16.0	10.4	2080	22.91	925	9.81
D1B6	76.5	15.7	9.8	1961	21.71	875	9.56
D1B7	75.5	15.0	8.8	1805	19.37	725	9.43
D1B8	73.5	14.6	8.7	1719	18.46	675	9.18
D2B1	75.5	14.4	9.0	1766	20.77	825	9.43
D2B2	82.0	16.5	9.8	2166	24.47	925	10.25
D2B3	74.5	12.9	8.3	1585	19.86	725	9.31
D2B4	78.0	16.3	9.1	1989	22.63	875	9.75
D2B5	76.0	14.6	9.0	1799	21.84	850	9.50
D2B6	75.5	14.3	8.6	1740	20.81	825	9.43
D2B7	74.0	12.8	7.8	1531	19.30	675	9.25
D2B8	71.5	12.1	7.1	1385	16.53	625	8.93
D3B1	74.5	13.3	8.0	1598	20.65	775	9.31
D3B2	81.0	14.9	9.2	1958	23.96	875	10.12
D3B3	73.0	12.8	7.8	1510	18.81	725	9.12
D3B4	75.0	14.8	8.5	1758	21.93	825	9.37
D3B5	75.0	14.2	8.1	1682	20.44	800	9.37
D3B6	72.5	12.9	7.9	1513	19.46	775	9.06
D3B7	72.0	11.6	6.9	1341	17.93	625	9.00
D3B8	69.5	11.1	7.0	1257	16.53	575	8.68
D4B1	73.5	11.9	7.0	1392	20.18	725	9.18
D4B2	79.0	13.7	7.8	1710	22.69	825	9.87
D4B3	70.5	11.3	6.3	1244	18.91	675	8.81
D4B4	74.0	13.4	7.6	1567	21.60	775	9.25
D4B5	74.0	12.0	7.1	1426	20.28	750	9.25
D4B6	70.5	11.7	6.3	1278	19.34	725	8.81
D4B7	70.0	10.4	6.0	1151	17.11	575	8.75
D4B8	67.0	10.2	5.7	1072	14.76	525	8.37
Mean	74.9	13.8	8.2	1677	20.51	770	9.25
SEM	1.1	0.6	0.6	74	0.45	21	0.21
C D @ 1%	NS	NS	NS	NS	NS	NS	NS

Bio priming of seeds with *T. hamatum* showed significantly higher shoot & root length. Inoculation of *T. hamatum* endophyte triggers certain kind of detoxifying proteins which acts a scavenging enzymes and play central role in protecting the cell from oxidative damage under stress conditions (Shukla *et al.* 2012). Further, *T. hamatum* under drought stress might have helped in production of several hormones such GA_3, especially indole-3 acetic acid by the endophyte which is a precursor of auxin that stimulates certain metabolites (water and food) required for cell development (Gravel *et al.* 2007) and there by enhanced the shoot & root length. Similar findings were also observed by Okoth *et al.* (2011) in maize and Pandey *et al.* (2016) in wheat.

Seedling vigour index-I

The control (D_1, 0 bars) recorded significantly highest (Table1) seedling vigour index-I (2033) compared to all other drought levels (Table 1). The seedling vigour index-I had shown a decreasing trend with an increase in drought levels *viz*., D_2 (1745), D_3 (1577), D_4 (1355). Comparing different levels of drought stress, better shoot length (cm), root length (cm) and seed germination (%) was observed in D_1 (0 bars) as the effect of drought stress was not there which finally resulted in higher seedling vigour index-I and better performance of seedlings (Koskosidis *et al.* 2020). Similar findings were also observed by Muscolo *et al.* (2014) in lentil.

Among the six different bio control agents *T. hamatum* showed significantly higher (2073) seedling vigour index-I followed by *B. amyloliquifaciens* (1912) and the lowest seedling vigour index (1358) was recorded by control (Table 1). The interaction effect due to bio agents and drought stress levels showed a non-significant difference for seedling vigour index-I. Bio priming of seeds with *T. hamatum* showed significantly higher seedling vigour index-I as compared to control. *T. hamatum* inoculation to seed increases certain antioxidants levels which would degrade higher amounts of ROS (reactive oxygen species) and therefore protect photosynthesis which in turn maintains the vigour of the seedling (Ahmed *et al.* 2015). Similar results were also noticed by Zheng *et al.* (2016) in rice and Mustafa *et al.* (2017) in chickpea.

Vigour indices (GRI, PV, MDG)

The vigour indices have shown a decreasing trend with an increase in drought levels. Among the drought levels D_1 (control) has recorded the highest value for growth rate index (21.97), peak value (846) and mean daily germination (9.70) (Table 1). While, D_4 (-6bars) has recorded the least values (19.38, 696 & 9.03) for these parameters, respectively.

The present study also indicated that the growth rate index (GRI), peak value (PV), mean daily germination (MDG) had shown decreasing trend with an increase in drought level. This might be due to highly negative osmotic potential that affect the water uptake of the seeds, which is the first step to occur during the germination process (*i.e*., imbibition) (Marcos-Filho, 2005). In addition drought stress affects the starch synthesis reactions and energy production process (adenosine triphosphate-ATP) through respiration, resulting in reduced growth rate index and germination percentage (Oliveira and Gomes-Filho, 2009). Similar findings were in accordance with Queiroz *et al.* (2019) in sorghum and Hadas (1976) in leguminous seeds.

While comparing the different bio control agents, *T. hamatum* showed significantly higher (24.08) growth rate index, higher (893) peak value and significantly higher (10.15) mean daily germination compared to all other treatments and control (16.57, 600 & 8.79, respectively). The interaction effect due to bio agents and drought stress levels showed a non-significant difference for vigour indices.

Further the present investigation also reported that *T. hamatum* showed an increase in GRI, PV & MDG among different endophytes (Table 1). The possible reason is that as it secretes certain hormones such as cytokinin and auxin, which stimulates for better absorption of water which in turn helps in germination rate of seeds (Zahir *et al*., 2004). Similar results were also observed Piri *et al.* (2019) in cumin.

Conclusion

From the results it was observed that the seed quality parameters decreased with an increase in drought stress levels. Among the different seed biopriming treatments seed bio priming for 3 hours (seed to solution ratio of 1: 0.3) with *Trichoderma hamatum* (1 x 10^3 conidia ml^{-1}) followed by *Bacillus amyloliquifaciens* (1 x 10^8 cfu ml^{-1}) and *Bacillus subtilis* (1 x 10^8 cfu ml^{-1}) had improved the seed germination (%) and seedling vigour under drought stress conditions.

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