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Optimization of priming time and concentration of bioprimers and their influence on bajra seed

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ABSTRACT

A series of experiments were carried out to optimize the suitable concentration and duration of seed bioprimers like *Azotobacter chroococcum* and *Azospirillum lipoferum* to improve seed germination and seedling vigour. The seeds bioprimed with *A chroococcum Azo* 311 cells (T_1-T_5) enhanced germination by 30-35 per cent over control. High concentrations of *A lipoferum Azs* 301 (T_8, T_9, T_{10}) significantly enhanced germination by 30 per cent over control. *Pennisetum* seeds primed for 30 min with biofertilizers ranging from 0.8-4.8 x 10^6 colony forming units (CFUs)/seed increased germination by 30-35 per cent over non-primed seeds and reduced radical emergence time RT50 by 24 h. Highest root length was registered in seeds primed with *A chroococcum Azo* 311 by 66 per cent followed by *A lipoferum Azs* 301 inoculation by 60 per cent and *Bradyrhizobium* sp *Rbg* 314 by 33 per cent over control. Twenty four hours of contact with *A lipoferum Azs* 301 significantly increased shoot length by 28.5 per cent while *A chroococcum Azo* 311 priming enhanced shoot length by 33.3 per cent over control. The results revealed that seed biopriming along with recommended dose of nitrogen fertilizer (24 kg) yielded the best vegetative growth in terms of root and shoot length and weight over non-primed seeds.

Keywords: *Pennisetum*; biopriming; *Azotobacter*; *Azospirillum*; seedling vigour

INTRODUCTION

Pennisetum glaucum (L) R Br, a minor millet and commonly known as Bajra is an underutilized and neglected crop despite its marvellous potential to be established as a viable and cheaper source of nutraceuticals in daily diet. Millets are highly nutritious, non-glutinous and non-acid forming grains. Millets are a rich source of dietary fibre and micronutrients containing essential amino acids; are rich in oil content and can provide an assay of health benefits making it a superior choice over regularly consumed cereals like rice and wheat (Gopalan et al 2004, Parameswaran and Sadasivan 1994, Leder 2004, Singh and Raghuvanshi 2012).

Though bajra is a good source of nutrients gap between the yield of bajra in Andhra Pradesh and highest yielding states in the country is very high indicating a possible potential for enhancement of productivity. The cultivation of bajra in most of the villages of Andhra Pradesh remains to be rainfed without additional inputs due to the poor economic conditions of the farmers. As *Pennisetum* responds favourably to addition of chemical fertilizers use of potential bio-fertilizers would narrow down the gap between production and productivity of the crop.

Bio-fertilizers like *Azotobacter, Azospirillum* and *Bradyrhizobium* have been extensively used as effective inoculums to improve plant growth and pest control on sustainable basis (Abhilash et al 2016). Though marked effects of bio-inoculation on plants have been reported the major hurdle for popularizing bio-fertilizers is their inconsistent performance. Optimum concentration of inoculum needs to be established for each study as high concentrations of inoculum are known to have inhibitory effect while too little inoculum

has no effect. The primary aim while working with bio-inoculants is to find out the best microbial consortium for the conscious effect on the target crop and to design a specific inoculant formulation and a method of practical application.

The present study was therefore aimed at evaluating the consistent performance of three bio-inculants viz *Bradyrhizobium, Azotobacter* and *Azospirillum* on the response of bajra under in vitro and in vivo conditions. Thus the study was conducted to optimize priming time and inoculum density for improving germination and to evaluate the effect of various bio-priming agents on root and shoot growth of *Pennisetum*.

MATERIAL and METHODS

The present study was carried out in the Department of Microbiology and Food Science and Technology, Gitam university, Visakhapatnam, Andhra Pradesh during 2013. The plant variety selected was pearl millet, *P glaucum* (L) R Br obtained from the local stores. Seeds were pre-treated with 5 per cent NaOCl (sodium hypochlorite) and distilled water to avoid fungal invasion. Subsequently seeds were hydroprimed in distilled water for 10 h. Pre-soaked seeds were then bio-primed. The bio-primers used were *Azotobacter chroococcum Azo* 311, *Azospirillum lipoferum Azs* 301, and *Bradyrhizobium* species *Rbg* 314 obtained from biological control unit, Acharya NG Ranga Agricultural Research Station, Amaravati, Andhra Pradesh. The slurry for seed treatment was prepared by suspending known amount of peat-based bio-primers (*A chroococcum Azo* 311, *A lipoferum Azs* 301 and *Bradyrhizobium* species *Rbg* 314) in equal volumes of water and mixed thoroughly. The inoculum size ranged from low (2-10 mg/ml) to high (50 mg/ml). Table 1 depicts the range of treatments given in the present study.

Effect of priming on seed germination of pearl millet under in vitro conditions

Germination tests were conducted by the paper towel method (Anon 1993). Five levels of each bio-primer (10, 20, 30, 40 and 50 mg/ml) and three levels of soaking time (30 min, 1 h and 24 h) were taken singly and in combination thus making total number of treatment combinations fifteen. Hydro-primed seeds soaked in equal volumes of sterilized bio-fertilizers served as control treatment. Fifty seeds for each treatment with two replicates were seeded equidistantly on to paper towels soaked in distilled water and maintained for germination under laboratory conditions at 28 ± 1ºC. In order to maintain moisture another pre-soaked paper towel was placed over the first, rolled up and wrapped with polythene. The time taken for emergence of the radical in 50 per cent of the seeds defined as radical emergence time (RT50) was observed from the second day of incubation to the seventh day.

Table 1. Bio-priming agents and their concentrations used for seed soaking for 30 min, 1 h and 24 h

Agent	Concentration (%)	Average CFU/ml
Non-primed seed (control)	Water containing sterilized peat	-
Azotobacter (*Azo* 311)	10 *Azo*	0.8×10^6
	20 *Azo*	1.5×10^6
	30 *Azo*	2.3×10^6
	40 *Azo*	4.5×10^6
	50 *Azo*	4.6×10^6
Azospirillum (*Azs* 301)	10 *Azs*	0.7×10^6
	20 *Azs*	1.69×10^6
	30 *Azs*	2.7×10^6
	40 *Azs*	3.4×10^6
	50 *Azs*	4.4×10^6
	C	0
Bradyrhizobium (*Rbg* 314)	2 *Rbg*	1.9×10^5
	5 *Rbg*	4.6×10^5
	10 *Rbg*	0.7×10^6
	15 *Rbg*	1.3×10^6
	20 *Rbg*	1.8×10^6

The numbers 2, 5, 10, 15, 20, 30, 40 and 50 for each treatment depict the milligrams of peat-based formulation per ml of water; CFU= Colony forming unit

Table 2. Effect of seed priming with *A chroococcum Azo* 311, *A lipoferum Azs* 301 and *Bradyrhizobium* sp *Rbg* 314 on germination of *Pennisetum* seeds (proportion test)

Treatment	Concentration of inoculum	Seed germination (%)	Z-value	p-value	Significance
T_0	C	60	-	-	-
T_1	10 Azo	85	-1.84	0.065	NS
T_2	20 Azo	90	-2.34	0.020	S
T_3	30 Azo	100	-3.65	0.000	S
T_4	40 Azo	90	-2.34	0.020	S
T_5	50 Azo	90	-2.34	0.020	S
T_6	10 Azs	70	-0.67	0.505	NS
T_7	20 Azs	85	-1.84	0.065	NS
T_8	30 Azs	90	-2.34	0.020	S
T_9	40 Azs	90	-2.34	0.020	S
T_{10}	50 Azs	90	-2.34	0.020	S
T_{11}	2 Rbg	50	0.64	0.523	NS
T_{12}	5 Rbg	70	-0.67	0.505	NS
T_{13}	10 Rbg	60	0.00	1.000	NS
T_{14}	15 Rbg	65	-0.33	0.744	NS
T_{15}	20 Rbg	60	0.00	1.000	S

Azo= Azotobacter, Azs= Azospirillum, Rbg= Bradyrhizobium
Numerals 2-50 represent concentration of inoculum in g/kg seed

The number of seeds germinated was calculated and represented in percentage (Abdul Baki and Anderson 1973) calculated as udner and the number of treatments and per cent germination are tabulated in Table 2.

$$\text{Germination percentage (\%)} = \frac{\text{Number of germinated seeds}}{\text{Total number of seeds}} \times 100$$

Effect of priming on vegetative growth of pearl millet under green house

For evaluation of efficacy of bio-primer in combination with N fertilizer on growth promotion under greenhouse conditions, seeds primed with *Azotobacter* were sown in each earthen pot filled with sandy loam soil where nitrogen was applied in different amounts and watered daily. Non-primed seeds served as control. There were three replicates for treatments arranged in a randomized complete block design.

At time period of 10, 20 and 40 days after seeding plants were randomly picked from each replication and the length of the roots and shoots was measured. Root length was taken from the point of attachment of seed to the tip of primary root. Shoot length was measured from the point of attachment of seed to the tip of the leaf. The mean shoot and root length values were expressed in centimetres. The data were tested for statistical significance, per cent germination by proportion test and other experiments were analysed by means of two-way ANOVA test.

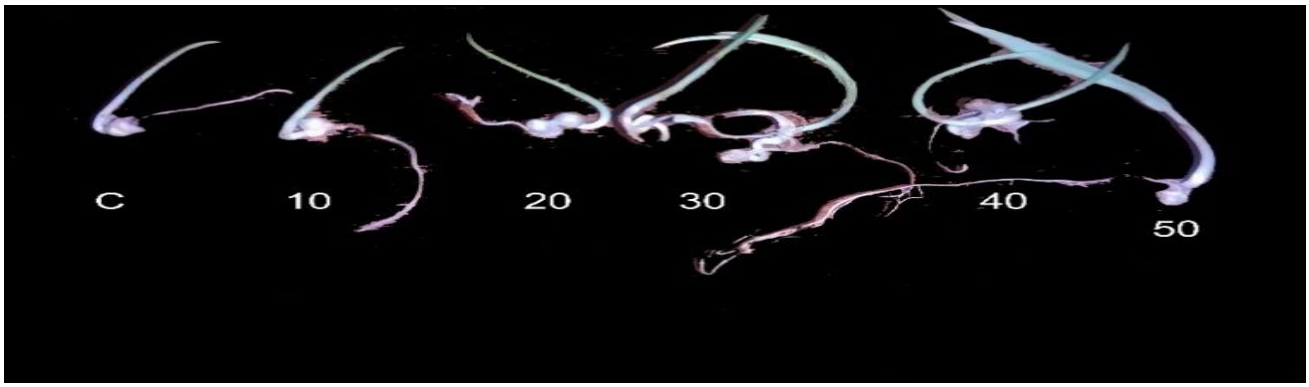
RESULTS and DISCUSSION

Effect of priming on in vitro seed germination of pearl millet

Priming had a significant effect on radical emergence time RT50 and germination percentage of *Pennisetum*. In general priming reduced the time for radical emergence by 24 h compared to non-prime controls (Fig 1a-d). Seeds were incubated for bacterization for a period of 30 min, 1 h or 24 h.

The observations suggested that increased incubation time does not necessarily bring an increase in germination. Significant differences were observed among the incubation time and among the treatments but the interaction was insignificant ($p < 0.02$). Fig 1d suggests that increased incubation time up to 24 h had reduced root length and thus 30 min incubation time was optimized for all further experiments.

(a)



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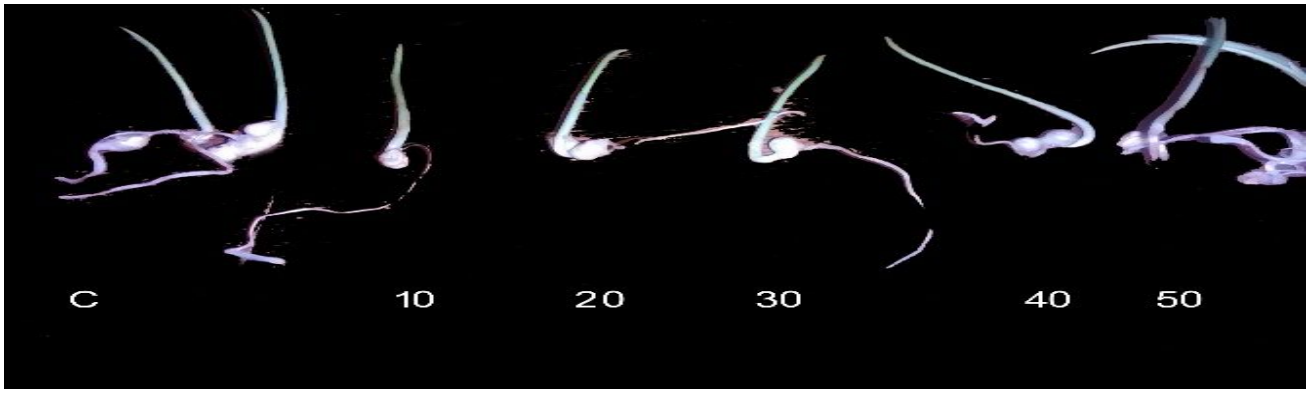

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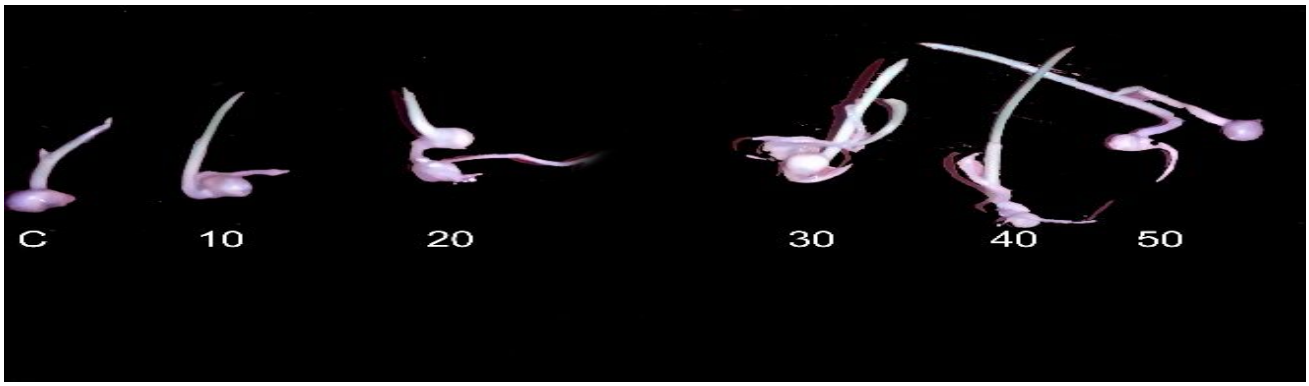

(d)

Fig 1. Effect of priming by different concentrations of ***A chroococcum Azo*** **311 on seed germination of pearl millet (a) Non-primed seeds, (b) Seeds bio-primed for 30 minutes, (c) Seeds bio-primed for 1 h, (d) Seeds bio-primed for 24 h**

Priming seeds with *A chroococcum Azo* 311 cells (T_1-T_5) enhanced seed germination significantly over control (Table 2). Priming enhanced per cent germination by 30-35 per cent over control seeds. However no significant variations in germination percentage among different treatments were visible suggesting that the range of inoculum size ie 10-50 g/ kg seed was appropriate for germination and did not produce lethal effects. Priming with *A lipoferum Azs* 301 at lower concentrations (T_6 and T_7) enhanced germination over control which was not statistically significant. However high concentrations of *A lipoferum Azs* 301 (T_8, T_9 and T_{10}) significantly enhanced germination by 30 per cent over control (Table 2).

Priming seeds with *Bradyrhizobial* cells at a concentration as low as 2 g/kg seed reduced germination suggesting that inoculum size of 10^{-8} had an inhibitory effect on seed vigour. *Pennisetum* seeds coated with bacterial inoculum ranging between 0.8-4.8 x 10^6 CFU/ seed increased germination by 30-35 per cent over non-primed seeds and reduced radical emergence time RT50 by 24 h. However no visible difference was seen between the treatments suggesting that range of inoculum size (0.8 to 4.5 x 10^6) was appropriate for germination and did not produce lethal effects. The findings are in conformity with the observations made by Hohmann et al (2011) who opined that an optimum inoculum range existed for plant growth activity. Paulitz (2000) reported that an inoculum size of 10^5 CFU/g root was optimum and further increase in size did not necessarily bring higher biological activity.

Effect of incubation time on root length of *Pennisetum*

Fig 2 suggests that priming enhanced root length of seedlings significantly. Significant differences in root length were observed among the treatments. Highest root length was observed in seeds primed with *A chroococcum Azo* 311 which enhanced root length by 66 per cent over control. *A lipoferum Azs* 301 inoculation enhanced root length by 60 per cent and *Bradyrhizobium* sp *Rbg* 314 by 33 per cent over control.

Effect of incubation time on shoot length of *Pennisetum*

Bradyrhizobium sp *Rbg* 314 inoculation did not improve shoot length over control. *A lipoferum Azs* 301 and *A chroococcum Azo* 311 enhanced shoot growth significantly over control. Incubation time had no significant effect on shoot length of *Pennisetum.* Twenty four hours of contact with *A lipoferum Azs* 301 significantly increased shoot length by 28.5 per cent while *A chroococcum Azo* 311 priming by 33.3 per cent over control (Fig 3).

Effect of priming on vegetative growth under greenhouse conditions

Effect of priming on various vegetative parameters was observed up to 45 days under greenhouse conditions. Preliminary studies in lab had suggested that a combination of inorganic and biological fertilizers had significantly enhanced fodder yield and growth of *Pennisetum* local variety (Sunitha et al 2015).

Based on this study nine treatments T_1-T_9 were given to evaluate the effect of combined use of bio-inoculation and chemical fertilizers on vegetative growth of *Pennisetum* (Table 3). Recommended dose of nitrogen fertilizer (24 kg) yielded the best vegetative growth in terms of root length, root weight, shoot length and shoot weight (treatment T_6). A comparison of treatments T_5 and T_6 suggests that inoculation with biofertilizer significantly enhanced shoot weight by 35-40 per cent. Inoculation thus provided an additional impetus to growth in terms of shoot weight. Similar growth increase was recorded in pearl millet with application of 100 per cent RDN through 75 per cent urea and 25 per cent vermicompost along with seed treatment with *Azotobacter* which was par with that of treatment 100 per cent RDN through urea with *Azotobacter* inoculation (Patel et al 2014).

As evident from Table 3 treatments T_7 and T_8 suggest that an increase in chemical fertilizer from 20 to 40 kg had reduced the vegetative growth by 50 per cent irrespective of the bio-fertilizer concentration used. At low level of nitrogen (7 kg/ha) treatments T_2 and T_3 high inoculation doses significantly enhanced root and shoot weight. Hence positive and significant response of *Azotobacter* is evident with lower doses of inorganic fertilizer than at higher doses. This is in agreement with the work of Singh et al (2010) who reported that combinations of low input system of integrated nutrient management could be most beneficial to obtain high yields of pearl millet hybrids.

In the present study *Pennisetum* seeds were pre-soaked in distilled water for 8-10 h and primed just before radical emergence. It is possible that short

Table 3. Effect of priming on vegetative growth under in vitro conditions (pot experiment)

S/N	Nitrogen concentration (kg/ha)	*A chroococcum Azo* 311 concentration	Root length (cm)	Shoot length (cm)	Root weight (g)	Shoot weight (g)
T_1	0	20	9.00[d]	100.00[c]	4.34[b]	11.31[b]
T_2	7.09	34.14	13.92[c]	90.15[c]	2.85[c]	9.64[c]
T_3	7.09	5.86	12.28[c]	155.24[a]	1.82[c]	4.56[d]
T_4	20	40	16.00[b]	93.25[c]	1.827[c]	14.56[a]
T_5	24	20	18.50[a]	140.00[b]	6.53[a]	15.36[a]
T_6	24	0	18.89[a]	165.00a	7.13[a]	11.87[b]
T_7	40.97	5.86	16.76[b]	94.93[c]	4.62[b]	12.32[b]
T_8	40.97	34.14	10.81[d]	62.30[d]	1.34[d]	3.67[d]
T_9	48	20	9.23[d]	50.50[d]	2.82[c]	7.32[c]

Means (n= 10) for each treatment followed by the same letter in the columns are not significantly different (P <0.05) according to DMR test, Numerals 0, 5, 7, 20, 30, 40 represent concentration of N kg/ha and g/kg seed of *A chroococcum*

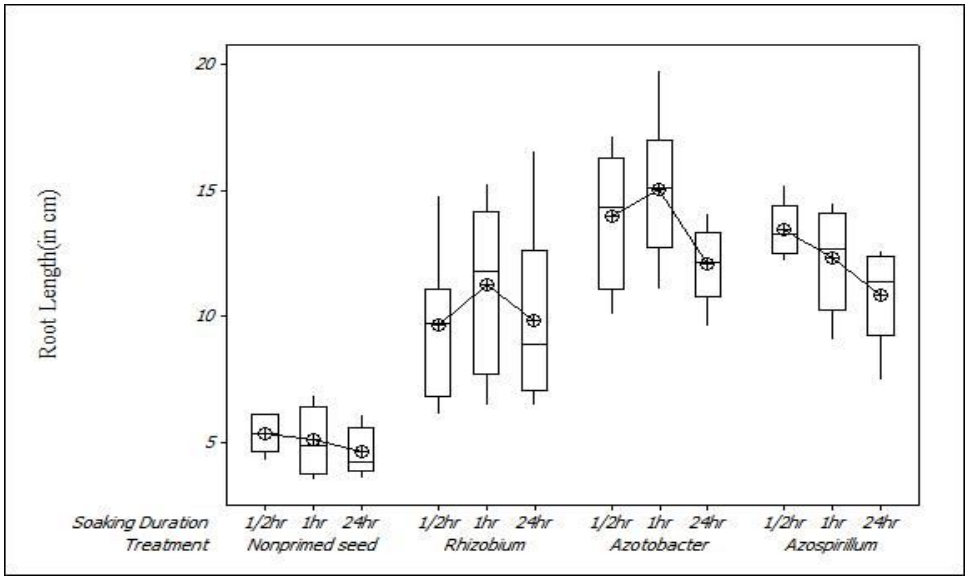

Fig 2. Effect of incubation time on root length of five-day old *Pennisetum* seedlings

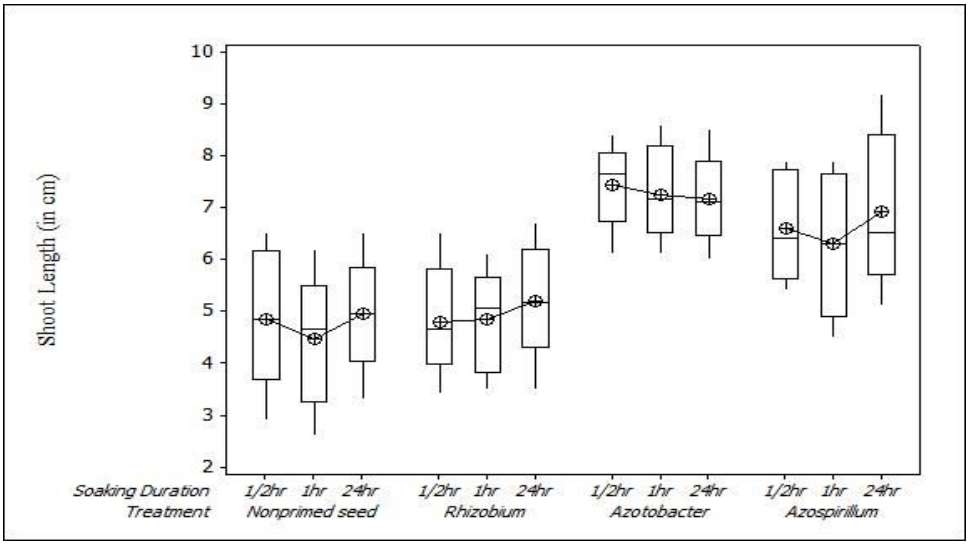

Fig 3. Effect of incubation time on shoot length of five-day old *Pennisetum* seedlings

priming time of 30 min and 1 h was sufficient for inducing growth and germination. In fact prolonged incubation for 24 h had an inhibitory effect on germination. Thus the study optimized priming time and inoculum density for improving seed germination and enhancing root and shoot growth of *Pennisetum.*

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