International Journal of Chemical Studies

P-ISSN: 2349–8528
E-ISSN: 2321–4902
IJCS 2019; 7(6): 46-49

Received: 25-09-2019
Accepted: 28-10-2019

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Effect of seed priming with chemicals, micronutrients and bio-inoculants on physiological attributes of resultant seed in foxtail millet (*Setaria italica* L.)

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Abstract
A laboratory experiment was conducted at department of seed science and technology, college of agriculture, Raichur during December-2018 to know the effect of seed priming with chemicals, micronutrients and bio-inoculants on seed quality parameters of resultant seed in foxtail millet. The experiment consisted of twelve treatment combinations. A laboratory experiment was conducted at department of seed science and technology, college of agriculture, Raichur during 2018-19 to know the influence of different seed priming methods on seed quality parameters in foxtail millet of resultant seeds. The seeds produced from the field experiment are evaluated in the laboratory for quality assessment. The seed quality parameters differed significantly between the treatments. Seed priming with *Azospirillum* (20 %) + *Pseudomonas fluorescens* (20 %) + *Phosphobacter* (20 %) + Zn SO_4 (0.1 %) + Boron (0.1 %) (T_{12}) recorded significantly higher seed germination percentage (90.67 %), seedling length (18.43 cm), seedling dry weight (24.33 mg), seedling vigour index I and II (1671 and 2206 respectively) and lower electrical conductivity (0.013 dSm^{-1}) compared to control (81.33 %., 16.12 cm, 17.03 mg, 1311, 1386 and 0.022d Sm^{-1} respectively).

Keywords: Priming, quality, *Setaria italica* L.

Introduction
Foxtail millet has been identified as major millet in terms of worldwide production. It was named foxtail Millet due to bushy, tail-like appearance of its immature panicles. Other alternative names for foxtail millet include Italian millet, German millet, Siberian millet and foxtail bristle grass. It is an important millet which sustains well in adverse conditions. Foxtail millet as compared to other cereals has several desirable nutritional attributes. Nutritionally it is superior to rice and wheat and therefore provides proteins, minerals and vitamins to the poor (Tylor and Emmanbux, 2008) [25]. It is consumed as stiff bread known as roti, after the dehulled grain has been milled into flour.

Seed is a basic input in agriculture in which 25% yield increase can be achieved by quality seeds. Quality seed is the key for successful agriculture, which demands each and every seed should be readily germinable and produce a vigorous seedling ensuring higher yield. To provide higher quality seeds, many researchers have developed new technologies called "Seed Enhancement Techniques".

Low quality seed lots frequently require more time for germination and seedling emergence. This makes the seedlings more sensitive to adverse climate conditions, reducing the final percentage of emergence and normally resulting in irregular seedling emergence. One of the most promising treatments is the osmo conditioning, also called seed priming, which consists of controlled hydration of seeds to a certain level to facilitate the initial germination process without the occurrence of root protrusion. Seed priming is a controlled hydration process that involves exposing seeds to low water potentials that restrict germination (radicle protrusion) but permits pre germinative physiological and biochemical changes to occur (Heydecker and Coolbear, 1977; Bradford, 1986; Khan, 1992) [8, 4, 11]. Upon rehydration, primed seeds may exhibit faster rate of germination, more uniform emergence, greater tolerance to environmental stresses and reduced dormancy in many species (Khan, 1992) [11]. Research on priming has proved that crop seeds primed with water germinated early, root and shoot development started rapidly, grew more vigorously and seedling length was also significantly greater than non-primed seeds.

This technique helps seeds to evenly germinate even under adverse soil conditions. Bio-priming could also reduce the amount of bio-control agents that must be applied to the seed (Singh *et al.* 2003) [20]. Bio-priming is a seed treatment system that involves coating the seed with fungal or bacterial bio-control agents. Bio-agents can be applied as seed treatment, seed coating, seed priming and soil drenching, of which, most effective technique is seed priming because it may be used for reducing diseases, improvement of germination, vigour, seedling establishment and yield in crops (Talebian *et al.* 2008) [24].

Materials and Methods

Treatment Details

T_1: Control
T_2: Hydro priming for 8 hours
T_3: Seed priming with KH_2PO_4 2% for 8 hours
T_4: Halo priming with NaCl 2% for 8 hours
T_5: Seed priming with *Azospirillum* sp. @ 20% for 8 hours
T_6: Seed priming with *Pseudomonas fluorescens* @ 20% for 8 hours
T_7: Seed priming with *Phosphobacter* @ 20% for 8 hours
T_8: Seed priming with *Azospirillum* sp. @ 20% + *Pseudomonas fluorescens* @ 20% + *Phosphobacter* @ 20% for 8 hours
T_9: Seed priming with $ZnSO_4$ (0.1%) for 8 hours
T_{10}: Seed priming with Boron (0.1%) for 8 hours
T_{11}: Seed priming with $ZnSO_4$ (0.1%) + Boron (0.1%) for 8 hours
T_{12}: $T_8 + T_{11}$

The resultant seeds produced in the field from these twelve treatments were collected from the experimental field and were evaluated in the laboratory for quality assessment.

Germination

The germination test was carried out in roll towel medium using 4 x 100 seeds (ISTA, 2007) [9] in a germination room maintained at 25 ± 2°C temperature and 95 ± 3 % RH. After the germination period of 7 days the seedlings were evaluated as normal seedling, abnormal seedling, hard seed and dead seed. Based on normal seedlings, the germination was calculated adopting the following formula and the mean expressed as percentage.

$$\text{Normal seedlings} = \frac{\text{Number of normal seedlings}}{\text{Total number of seedlings}} \times 100$$

Root Length

At the time of germination count, ten normal seedlings were selected at random from each of the crops and used for measuring the root length of seedlings. Root length was measured from the collar region to the tip of primary root. The mean values were calculated and expressed in centimetre.

Shoot Length

The seedlings used for measuring root length were also used for measuring shoot length. The shoot length was measured from the collar region to the tip of the primary leaves and the mean values were expressed in centimetre.

Seedling dry weight

Ten normal seedlings were selected randomly from each of the crops and dried in shade for 24 h and were kept in an oven maintained at 70^0C for 24 h. After the drying period, the seedlings were cooled in closed desiccator for 30 minutes and were weighed in a top pan balance and the mean expressed as g $seedlings^{-10}$ (Gupta, 1993) [7].

Seedling Vigour Index I and II

Vigour index (VI) was calculated using the method suggested by Abdul–Baki and Anderson (1973) and expressed in whole number.

SVI I =Germination (%) x [Root length (cm) + Shoot length (cm)].

SVI II = Germination (%) x Seedling dry weight.

Electrical conductivity

Five grams of seeds in four replications were soaked in acetone for half a minute and thoroughly washed in distilled water three times. Then, the seeds were soaked in 25 ml distilled water and kept in an incubator maintained at 25°C ± 1°C for 12 hours. The seed leachate was collected and the volume was made up to 25 ml by adding distilled water. The electrical conductivity of the seed leachate was measured in the digital conductivity bridge (ELICO) with a cell constant 1.0 and the mean values were expressed in decisions per meter (dSm^{-1}) (Milosevic *et al.*, 2010) [15].

Results and Discussion

All the seed quality parameters differed significantly due to seed priming treatments. Foxtail millet seeds primed with T12 (Seed priming with Azospirillum sp. @ 20% + *Pseudomonas fluorescens* @ 20% + Phosphobacter @ 20%+ ZnSO4 0.1% + Boron 0.1%) recorded significantly higher seed germination percentage (90.67 %), shoot length (9.27 cm), root length (9.16 cm) and seedling length (18.43 cm). While significantly minimum was recorded in control (T1) (81.33 %, 7.85 cm, 8.27 cm, and 16.12 cm respectively table 1.

Pseudomonas fluorescens helps to enhance the seed metabolic efficiency of primed seeds. The higher metabolic efficiency leading to mobilization of reserve food to the embryo for early initiation of germination was reported by many researchers (Wellman, 1961; Atia *et al.*, 2006; Job *et al.*, 2000) [27, 2, 10]. Biswas (1994) [3] in paddy stated that the mobilization rates known to affect the germination and seedling growth.

Table 1: Effect of seed priming with chemicals, micronutrients and bio-inoculants on germination (%), root length (cm) shoots length (cm) and seedling length (cm) of resultant seed in foxtail millet

Treatments	Germination (%)	Root length (cm)	Shoot length (cm)	Seedling length (cm)
T_1: Control	81.33	7.85	8.27	16.12
T_2: Hydro priming	82.33	8.04	8.31	16.35
T_3: Seed priming with KH_2PO_4 (2%)	85.00	8.15	8.57	16.72
T_4: Halo priming with NaCl (2%)	83.33	8.12	8.44	16.56
T_5: Seed priming with *Azospirillum* sp. @ 20%	85.67	8.45	8.63	17.08

T_6: Seed priming with *Pseudomonas fluorescens* @ 20%	85.33	8.39	8.63	17.02
T_7: Seed priming with *Phosphobacter* @ 20%	85.00	8.22	8.59	16.81
T_8: Seed priming with *Azospirillum* sp. @ 20 % +*Pseudomonas fluorescens* @ 20 % + *Phosphobacter* @ 20%	88.33	9.01	9.14	18.15
T_9: Seed priming with $ZnSO_4$ (0.1%)	86.67	8.45	8.82	17.26
T_{10}: Seed priming with Boron (0.1%)	86.67	8.45	8.70	17.15
T_{11}: Seed priming with $ZnSO_4$ (0.1%) + Boron (0.1%)	88.00	8.46	8.85	17.31
T_{12}: T_8 + T_{11}	90.67	9.16	9.27	18.43
Mean	85.67	8.40	8.68	17.08
S. Em.±	1.633	0.162	0.09	0.211
C.D. at 1 %	4.795	0.641	0.385	0.833

Pseudomonas fluorescens might have stimulated the hypocotyl and epicotyl growth and cell elongation by inducing secretion of GA_3 resulting in increased root length and shoot length. The results of present study is in agreement with the findings reported by Sakthivel *et al.,* 2009 [18] in tomato; Sivasankaridevi *et al.,* 2013 [22] in cucumber; Sivakalai and Krishnaveni, 2017 [21] in pumpkin; Sridevi and Manonmani, 2016 [23] in kodo millet and barnyard millet and Madhukeshwara and Sajjan, 2017 [13] in maize. The enhancement in the seedling growth recorded may be attributed to the suppression of deleterious microorganisms and pathogens; production of plant growth regulators such as gibberellins, cytokinins, and indole acetic acid; increased availability of minerals and other ions; and more water uptake as revealed by Van Loon *et al.*, (1998) [26] and Ramamoorthy *et al.*, (2001) [17].

Resultant seeds from T_{12} (Seed priming with *Azospirillum* sp. @ 20% + *Pseudomonas fluorescens* @ 20% + *Phosphobacter* @ 20%+ $ZnSO_4$ 0.1% + Boron 0.1%) recorded significantly higher seedling dry weight (24.33 mg), seedling vigour index I and II (1671 and 2206 respectively) and lower electrical conductivity (0.013 dSm^{-1}) While significantly minimum was recorded in control (T_1) (17.03 mg, 1311, 1386 and 0.022d Sm^{-1} respectively) (Table 2).

Pseudomonas fluorescens showed its beneficial effect on germination and seedling vigour expressed in this study might be due to the result of a synergism of priming effect with bacterial effect, since priming confers benefits such as completion of early germination phases, increasing the population of bio-protectants, rapid and uniform seedling emergence, facilitation of uptake of water and nutrients, protection against pathogens, potential defense responses such as early oxidation burst, incorporation of various phenolic compounds and polymers to the cell wall and secretion of phytoalexins as observed by Musa *et al.,* (2001) [16] in chick pea; Mathre *et al.,* 1999 [14] and Conrath *et al.,* (2002) [6] in arabidopsis. The similar results were noticed by Sridevi and Manonmani (2016) [23] in foxtail millet, proso millet and little millet.

Phosphorus Solubilizing Bacteria (PSB) played vital role in enhancing the seedling vigour which might be attributed to the role of Phosphobacteria in enhancing the solubilization of insoluble phosphorous and making it available to the germinating seed with consequent enhancement in the metabolic activity resulting in higher germination.

Significantly lowest electrical conductivity by priming might be due to enhanced repair of membrane, which is disrupted during maturation and drying. Since electrolyte leakage is a result of damage of Cell membranes. However, electrolytes may leak out more in non-primed seeds resulting in higher levels of electrolytes in non-primed seeds (Chiu *et al.* 1995) [5]. In the present, study the differential electrical conductivity values which were recorded among the seed priming treatments indicate the nature and extent of membrane protection offered, which may not be the same for all seed priming treatments, thus resulting in difference in electrical conductivity values as stated by Kurdikeri (1993) [12] in hybrid maize and Sandyarani (2002) [19] in cotton.

Table 2: Effect of seed priming with chemicals, micronutrients and bio-inoculants on seedling dry weight (mg), Seedling vigour index I, seedling vigour index II and electrical conductivity(dSm^{-1}) of resultant seed in foxtail millet

Treatments	**Seedling dry weight (mg)**	**Seedling vigour index-I**	**Seedling vigour index -II**	**Electrical conductivity (d Sm^{-1})**
T_1: Control	17.03	1311	1386	0.022
T_2: Hydro priming	17.18	1347	1414	0.020
T_3: Seed priming with KH_2PO_4 (2%)	18.57	1422	1577	0.019
T_4: Halo priming with NaCl (2%)	17.86	1380	1486	0.019
T_5: Seed priming with *Azospirillum* sp. @ 20%	19.58	1463	1673	0.017
T_6: Seed priming with *Pseudomonas fluorescens* @ 20%	19.57	1451	1673	0.017
T_7: Seed priming with *Phosphobacter* @ 20%	18.90	1428	1604	0.018
T_8: Seed priming with *Azospirillum* sp. @ 20% + *Pseudomonas fluorescens* @ 20 % + *Phosphobacter* @ 20 %	23.33	1603	2063	0.014
T_9: Seed priming with $ZnSO_4$ (0.1%)	19.93	1496	1728	0.016
T_{10}: Seed priming with Boron (0.1%)	19.93	1487	1727	0.017
T_{11}: Seed priming with $ZnSO_4$ (0.1%) + Boron (0.1%)	20.83	1523	1832	0.015
T_{12}: T_8 + T_{11}	24.33	1671	2206	0.013
Mean	19.76	1465	1697	0.017
S. Em.±	1.105	33.715	94.290	0.001
C.D. at 1%	4.37	133.36	372.96	0.002

Conclusion

Foxtail millet seeds primed with Seed priming with *Azospirillum* sp. @ 20% + *Pseudomonas fluorescens* @ 20% + *Phosphobacter* @ 20%+$ZnSO_4$ @0.1% + Boron @0.1% recorded higher seed quality attributes *viz.*, germination, shoot and root length, seedling length, seedling dry weight, vigour index and lower electrical conductivity.

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