Current Journal of Applied Science and Technology

38(6): 1-7, 2019; Article no.CJAST.53307
ISSN: 2457-1024
(Past name: British Journal of Applied Science & Technology, Past ISSN: 2231-0843, NLM ID: 101664541)

Evaluating the Emergence and Biochemical Changes of Primed Seeds in Proso Millet (*Panicum miliaceum* L.)

R. Sridevi[1*] and V. Manonmani[1]

[1]Department of Seed Science and Technology, Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu, India.

Authors' contributions

This work was carried out in collaboration between both authors. Authors RS and VM designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors VM and RS managed the analyses of the study. Author RS managed the literature searches. Both authors read and approved the final manuscript.

Article Information

DOI: 10.9734/CJAST/2019/v38i630432
Editor(s):
(1) Dr. Bishun Deo Prasad, Assistant Professor, Department of Molecular Biology and Genetic Engineering, Bihar Agricultural College, Bihar Agricultural University, India.
Reviewers:
(1) Grace O. Tona, Ladoke Akintola University of Technology, Nigeria.
(2) Vipul Kumar, Lovely Professional University, India.
Complete Peer review History: http://www.sdiarticle4.com/review-history/53307

Original Research Article

Received 15 October 2019
Accepted 17 December 2019
Published 26 December 2019

ABSTRACT

The present study aimed in exploring the performance of primed seeds in enhancing the quality of proso millet. The primed seeds along with nonprimed seeds were evaluated for emergence, cell proliferation in radicle cells using scanning electron microscope and biochemical parameters. Seeds primed with *Pseudomonas fluorescens* 20% possessing higher germination and anatomical changes observed through scanning electron microscope revealed more cell proliferation which was found to show rapid cell elongation and cell division of the radicle when compared to nonprimed seeds. The biochemical causes responsible for higher invigorative effect were identified as enhanced enzyme activity recorded through α-amylase content, dehydrogenase activity, protein content, lipase activity, antioxidative enzymes like catalase activity, peroxidase activity and superoxide dismutase with lower electrical conductivity, free amino acid and free sugars of the seed leachate. It is concluded in this study that primed seeds of *Pseudomonas fluorescens* 20% performed better than other treatments through their exhibition of higher emergence, more cell proliferation and enhanced biochemical parameters.

**Corresponding author: E-mail: agrirose.28@gmail.com;*

Keywords: Primed seeds; emergence; cell proliferation; biochemical parameters; enzyme activity.

1. INTRODUCTION

Seed priming is the hydration of seeds at controlled level which facilitates the metabolic changes to take place as required for germination process but prevents actual emergence of radicle [1]. The useful effects of priming have been related to different cellular, biochemical, and molecular processes including synthesis of RNA and proteins. During seed priming several changes takes place in the seed such as imbibitions, removal o inhibitors which causes dormancy, activation of enzymes and metabolic events which are required for germination process to take place. This enhances the speed, uniformity of germination and stand establishment [2]. The process takes place during seed priming also persists even after desiccation of the seeds. The primed seeds when sown in the field could rapidly imbibe water and restore the seed metabolism resulting in higher germination rate and reduction in the heterogeneity in germination [3].

Biopriming is defined as the process of hydrating seeds in biological agents which includes the combination of seed priming and treating of seeds with useful microorganism to protect the seeds and improve their quality [4]. Biopriming provided an economically competitive delivery system, because only a relatively small amount of inoculum was needed for plant protection and crop improvement and also more stable field efficacy. Biopriming with biological agents promotes germination and growth of plants as these organisms produce plant growth promoters such as auxin, cytokinin and gibberellic acid. It not only promotes growth parameters but also colonizes the root zone and prevents them from being attacked by pathogens. Hence the current study aimed in finding the priming effect on seed quality parameters in proso millet.

2. MATERIALS AND METHODS

The genetically pure seeds of proso millet cv. CO (PV) 5 were used for this study. The proso millet seeds were treated with priming agents for the specified period of 8 hours and seed to solution ratio of 1:1 (w/v). The priming treatments *viz.,* T_1 - Nonprimed seed, T_2 – Hydropriming, T_3 - KH_2PO_4 2%, T_4 - *Pseudomonas fluorescens* 20% (Liquid formulation) reported by Iswariya et al. [5]. The biocontrol agent *Pseudomonas fluorescens Pf1* (Liquid formulation) was obtained from the Department of Plant Pathology, Tamil Nadu Agricultural University. The primed seeds were evaluated for the emergence of seedlings and biochemical parameters.

2.1 Emergence of Seedlings

The germination percentage of proso millet seeds were evaluated by the procedure given by [6]. Hundred (100) seeds in four replicates were used using top of paper method and allowed for seven days. After the germination period the germination percentage was calculated based on counting the seeds germinated out of total number of seeds sown.

2.2 Scanning Electron Microscopic (SEM) Observation

The anatomical changes of primed seeds were studied using SEM FEI QUANTA 250. The seeds primed with *Pseudomonas fluorescens* 20% of proso millet along with nonprimed seeds were scanned using Scanning Electron Microscopic (SEM) with attention to identify the cell proliferation in radicle cells.

2.3 Biochemical Parameters

The biochemical parameters were analysed using standard procedures. α-amylase content was analysed by the method given by Paul et al. [7], dehydrogenase activity [8], protein content [9], lipase activity [10], catalase activity [11], peroxidase activity [12], superoxide dismutase [13], electrical conductivity [14], free amino acid [15] and free sugars [16].

2.4 Statistical Analysis

The data obtained were subjected to statistical analysis using AGRES software by analysis of variance (ANOVA). The standard error and critical difference (CD) was calculated at 0.05 probability level.

3. RESULTS AND DISCUSSION

3.1 Effect of Seed Priming on Emergence and Anatomical Changes

Among the priming treatments, seeds primed with *Pseudomonas fluorescens* 20% recorded highest germination (90%) followed by seeds primed with

KH_2PO_4 2% (88 per cent). The poorest performance was registered by nonprimed seeds (85%) (Fig. 1). The highest germination in primed seeds might be due to the α-amylase enzyme activity. Amylase is one of the growth promoting enzymes which is involved in hydrolyzing the starch reserve in the seed and transport of sugars to the developing embryo. Quick resumption of α-amylase production immediately after imbibitions in primed seeds might be the reason for rapid increase in α-amylase activity during germination. This is in agreement with the findings of Iswariya et al. [5] who also made similar observation in barnyard millet variety.

The results on anatomical changes revealed that the proso millet seeds primed with *Pseudomonas fluorescens* 20% had more cell elongation and cell division of the radicle (Fig. 2). The radicle elongation in the primed seeds has been found to register twice larger than in the nonprimed seeds. Further, number of cells registered was 2-3 times higher in primed seeds in comparision to nonprimed seeds. This could be due to faster initiation of metabolic activity which activated the radicles cell expansion leading to early protrusion of radicles. The observation of this study is in agreement with McDonald [17] who stated that early and rapid imbibition would stimulate early metabolic activities and facilitate rapid multiplication and elongation of radicle in the primed seed. The *Pseudomonas fluorescens* would have increased the production of growth hormone such as gibberellins which have further triggered the amylase enzyme. These enzymes played a vital role in promoting early germination by increased starch assimilation [18]. This might also be due to the metabolic events like endosperm weakening by hydrolase activities, cell cycle related events and mobilization of storage reserves [19] which were also activated by the faster imbibition rate. These observations were also agreed by [20] in the onion. These researchers proposed that priming resulted in more rapid imbibition and higher radicle cell differentiation and enlargement.

3.2 Inluence of Seed Priming on Biochemical Changes

In the present study the highest α-amylase content (5.12 mg maltose min^{-1}), dehydrogenase activity of 0.348 OD value, protein content (11.76%), catalase activity of 1.90 m mol of H_2O_2 reduced min^{-1} g^{-1} $seed^{-1}$, peroxidase of 0.92 m mol of tetraguaicol formed min^{-1} g^{-1} $seed^{-1}$, superoxide dismutase activity of 0.99 Unit of enzyme, lipase activity of 0.018 meq min^{-1} g^{-1} of sample and lowest electrical conductivity of seed leachate (26.3 µS cm^{-1}), free amino acid of 32.36 µg 50 $seeds^{-1}$ 50 ml^{-1} leachate, free sugars (27.69 µg 50 $seeds^{-1}$ 50 ml^{-1} leachate) was quantified in seeds primed with *Pseudomonas fluorescens* 20%.

The increase in amylase activity was observed in primed seeds was due to rapid disintegration of starch which provides energy to the growing radicles. Thus the primed seeds were found to possess early germination than nonprimed seeds. The augmentation of α-amylase activity in primed seeds might be due to complete

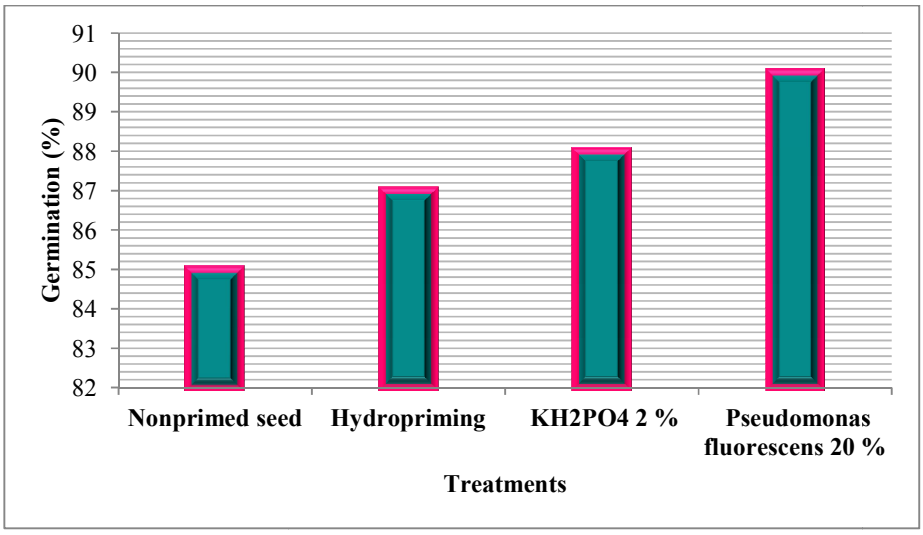

Fig. 1. Seed priming influence on germination in proso millet

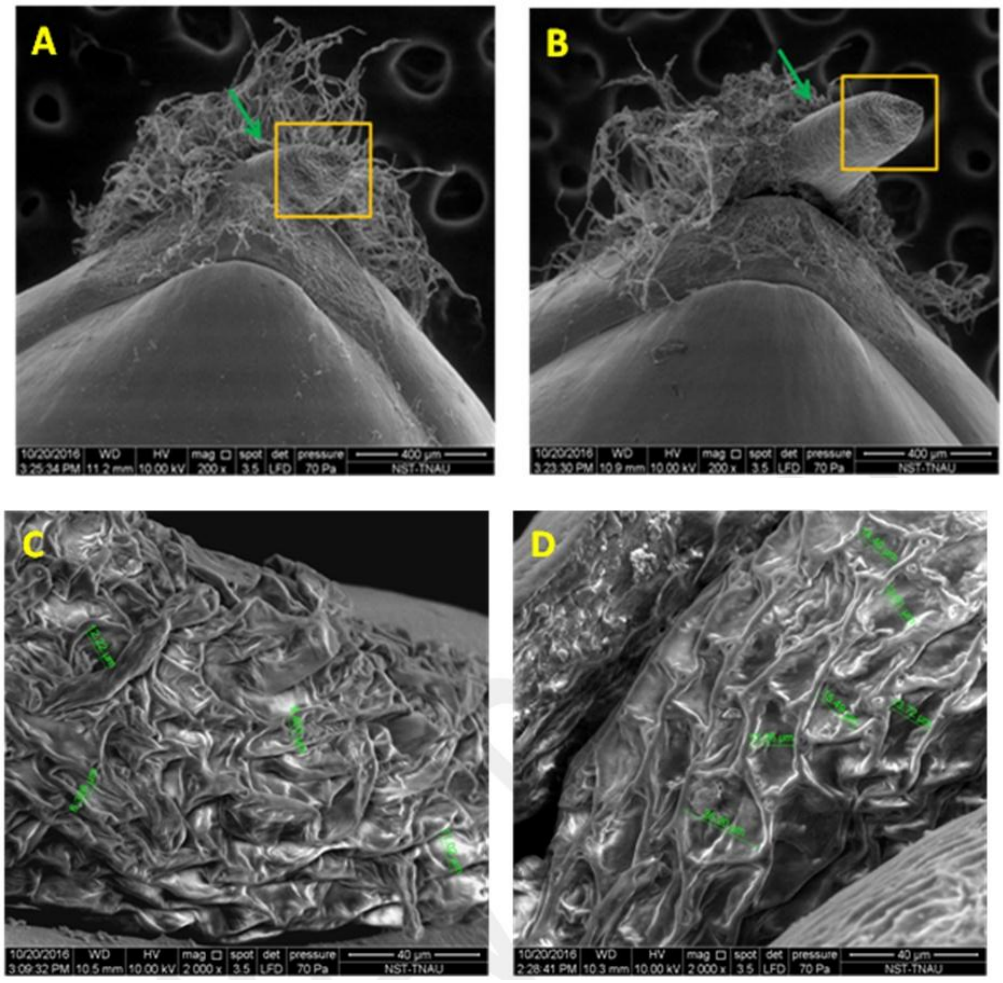

Fig. 2. Emergence of radicle and cell proliferation in radicle cells of proso millet viewed under Scanning Electron Microscope (SEM) (A) Radicle protrusion of nonprimed seeds (Arrow indicates initiation of radicle protrusion) (B) Radicle protrusion of seeds primed with *Pseudomonas fluorescens* 20% (Arrow indicates advancement of radicle protrusion than nonprimed seeds) (Square box indicate the scanned portion of radicle) (C) Cell proliferation of radicle cells of nonprimed seeds (D) Cell proliferation of radicle cells of seeds primed with *Pseudomonas fluorescens* 20%

hydration process at the time of imbibitions that increased hydrolysis of starch. The starch was then converted into reducing sugars which results in advancement of germination [21].

On valuation of biochemical changes, the electrical conductivity, free amino acids and free sugars of seed leachate was lower in seeds primed with *P. fluorescens* 20% than nonprimed seeds (Table 1) which may be due to the better integration of plasma membrane structure by slow hydration. Variation in the electrical conductivity indicated reduced compactness of seed coat, increased membrane integrity and deterioration of cell membranes. Similar findings were also reported in pearl millet [22]. The differential electrical conductivity values observed among the priming treatments indicates that the membrane integrity provided may not be uniform and results in varying electrical conductivity values.

In the present study, the electrical conductivity, free amino acids and free sugars of the seed leachate had negative association with seed quality which was supported by the enhanced α-amylase, dehydrogenase activity and anti-oxidative enzymes like catalase, peroxidise and

Table 1. Influence of seed priming on electrical conductivity, α-amylase content, dehydrogenase activity, protein content and free amino acid in proso millet

Seed priming treatments	Electrical conductivity ($\mu S\ cm^{-1}$)	α-amylase content (mg maltose min^{-1})	Dehydrogenase activity (OD value)	Protein content (%)	Free amino acid (µg 50 $seeds^{-1}$ 50 ml^{-1} leachate)
T_1 - Nonprimed seed	31.7	4.32	0.319	10.83	35.47
T_2 - Hydropriming	29.6	4.63	0.328	11.40	34.95
T_3 - KH_2PO_4 2%,	27.2	4.96	0.335	11.68	33.92
T_4 - *Pseudomonas fluorescens* 20%	26.3	5.12	0.348	11.76	32.36
Mean	**28.7**	**4.75**	**0.332**	**11.42**	**34.17**
SEd	0.191	0.034	0.0023	0.080	0.237
CD (P= 0.05)	0.406	0.072	0.0050	0.170	0.503

Table 2. Influence of seed priming on free sugars, catalase activity, peroxidase activity, superoxide dismutase and lipase activity in proso millet

Seed priming treatments	Free sugars (µg 50 $seeds^{-1}$ 50 ml^{-1} leachate)	Catalase activity (m mol of H_2O_2 reduced min^{-1} g^{-1} seed)	Peroxidase activity (m mol of tetraguaicol formed min^{-1} g^{-1} seed)	Superoxide dismutase (Unit of enzyme)	Lipase activity (meq min^{-1} g^{-1} of sample)
T_1 - Nonprimed seed	31.32	1.79	0.83	0.91	0.014
T_2 - Hydropriming	29.77	1.85	0.85	0.96	0.016
T_3 - KH_2PO_4 2%,	29.25	1.87	0.89	0.97	0.017
T_4 - *Pseudomonas fluorescens* 20%	27.69	1.90	0.92	0.99	0.018
Mean	**29.50**	**1.85**	**0.87**	**0.95**	**0.016**
SEd	0.205	0.012	0.006	0.006	0.0001
CD (P= 0.05)	0.435	0.026	0.014	0.014	0.0002

superoxide dismutase and protein content of seeds primed with *P. fluorescens* 20% (Table 2). The activity of all enzymes was at higher order highlighting the promotive action of priming technique as the primary cause for seed invigouration.

After initiation of imbibition of seeds the activity of hydrolytic and anti oxidative enzymes increased [23]. The increase in peroxidise activity might be due to the reason that degration of storage reserves and translocation to the growing embryo. Singh et al. [24] and Mathivanan et al. [25] also expressed that enzyme changes such as amylase content, catalase and peroxidise activity increased with primed seeds. Evangelina et al. [26] also reported that primed seeds showed higher superoxide dismutase and catalase activities than non-primed seeds. Peroxidases are the enzymes which plays a vital role in maintenance of seed quality by protecting the seeds against accumulation of peroxides that may be formed due to decomposition of hydrogen peroxide into water and oxygen. The increase in superoxide dismutase and catalase was also reported by Afzal [27] in primed seeds of tomato.

4. CONCLUSION

The proso millet seeds primed with *Pseudomonas fluorescens* 20% possessed higher germination percentage than the nonprimed seeds. Anatomical changes observed through scanning electron microscope revealed that *Pseudomonas fluorescens* 20% primed seeds produced more cell proliferation, rapid cell elongation of the radicle compared to nonprimed seeds. *Pseudomonas fluorescens* 20% primed seeds possessed enhanced enzyme activity of α-amylase content, dehydrogenase activity, protein content, lipase activity and antioxidative enzymes like catalase activity, peroxidase activity, superoxide dismutase with lower electrical conductivity, free amino acid and free sugars of the seed leachate.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Rinku V. Patel, Krishna Y. Pandya, Jasrai RT, Brahmbhatt N. Effect of hydropriming and biopriming on seed germination of brinjal and tomato seed. Research Journal of Agriculture and Forestry Sciences. 2017;5(6):1-14.
2. Khalil SK, Mexal JG, Rehman A, Khan AZ, Wahab S, Zubair M, Khalil IH, Mohammad F. Soybean mother plant exposure to temperature stress and its effect on germination under osmotic stress. Pak J Bot. 2010;42(1):213-225.
3. Rowse HR. Drum priming - A non-osmotic method of priming seeds. Seed Sci & Technol. 1995;24:281-294.
4. Bennett MAA. The use of biological methods to enhance vegetable seed quality. Seed Technol. 1998;20:198-208.
5. Iswariya S, Sujatha K, Subhashini R. Enhancement of Seedling Vigour through Bio-priming for Barnyard Millet Var. MDU 1. Int J Curr Microbiol App Sci. 2019; 8(4):2254-2259.
6. ISTA. International rules for seed testing. International Seed Testing Association, Bassersdorf, Switzerland; 2011.
7. Paul AK, Erji SM, Sircar SM. Metabolic changes in rice seeds during storage. Ind J Agric Sci. 1970;40(12):1031-1036.
8. Kittock PA, Law AG. Relationship of seedling vigour to respiration and tetrazolium chloride reduction of germinating wheat seeds. Agron J. 1968;60:286-288.
9. Ali-khan ST, Youngs CG. Variation in protein content of field peas. Can J Plant Sci. 1973;53:37-41.
10. Sadasivam S, Manickam A. Estimation of lipase content. In: Biochemical methods. New age international Ltd., New Delhi; 2008.
11. Aebi M. Catalase *in vitro*. In: Methods in Enzymology; 1984.
12. Castillo FI, Penel I, Greppin H. Peroxidase release induced by ozone in *Sedum album* leaves. Plant Physiology. 1984;74:846-851.
13. Dhindsa RH, Dhindsa RP. Thorpe: Leaf senescence correlated with increased level of membrane permeability, lipid peroxidation and decreased level of SOD and CAT. J Exp Bot. 1981;32:93-101.
14. Presley JT. Relationship of protoplast permeability of cotton seed viability and predisposition of seedling disease. Pl Dis Reptr. 1958;42:582.
15. Misra PS, Mertz ET, Glover DV. Biochemical analysis of total free amino acids. Cereal Chem. 1975;52:844.
16. Somogyi, M. Note on sugar determination. J Biol Chem. 1952;195:19-23.

17. McDonald MB. Seed deterioration: Physiology, repair and assessment. Seed Sci & Technol. 1999;27:177-237.
18. Bharathi R, Vivekananthan R, Harish S, Ramanathan A, Samiyappan R. Rhizobacteria based bioformulations for the management of fruit rot infection in chillies. Crop Protection. 2004;23(6):835-843.
19. Job D, Capron I, Job C, Corbineau F, Come D. Identification of germination-specific protein markers and their use in seed priming technology. In: Seed biology: Advances and applications. CAB International, Wallingford, UK. 2000;449-459.
20. Saranya N, Renugadevi J, Raja K, Rajashree V, Hemalatha G. Seed priming studies for vigour enhancement in onion CO onion (5). Journal of Pharmacognosy and Phytochemistry. 2017;6(3):77-82.
21. Afzal I, Basra SMA, Shahid M, Farooq M, Saleem M. Priming enhances germination of spring maize (*Zea mays* L.) under cool conditions. Seed Sci & Technol. 2008; 36:97-503.
22. Patil SK, Janpure SV, Shelar VR, Dumbre AD. Efficacy of insecticides and fungicides on seed germination, insect infestation and seed mycoflora on pearl millet during storage. Seed Res. 2004;32(2):189-192.
23. Umair A, Ali S, Muhammad MJ, Ali I, Tareen MN. Effect of seed priming on the antioxidant enzymes activity of mungbean (*Vigna radiata*) seedlings. Pakistan Journal of nutrition. 2012;11(2): 140-144.
24. Singh DP, Maurya S, Prakash OM, Singh UP. Phenolic composition and antifungal activity of culture filtrate of *Leptoxyphium axillatum*. Ind Phytopath. 2005;58:143–148.
25. Mathivanan S, Chidambaram ALA, Sundaramoorthy P, Baskaran L, Kalaikandhan R. The effect of plant growth promoting rhizobacteria on groundnut (*Arachis hypogaea* L.) seed germination and biochemical constituents. Int. J. Curr. Res. Acad. Rev. 2014;2(9):187-194.
26. Evangelina SE, Dionisio-Sese ML, Ismail AM. Seed pre-treatment in rice reduces damage, enhances carbohydrate mobilization and improves emergence and seedling establishment under flooded conditions. Oxford J. Life Sci; 2011. DOI: 10.1093/aobpla/plr007
27. Afzal IF, Munir CM, Basra SMA, Hameed A, Nawaz A. Changes in antioxidant enzymes, germination capacity and vigour of tomato seeds in response of priming with polyamines. Seed Sci & Technol. 2009;37:765-770.

Peer-review history:
The peer review history for this paper can be accessed here:
http://www.sdiarticle4.com/review-history/53307