Int.J.Curr.Microbiol.App.Sci (2017) *6*(11): 5471-5475

International Journal of Current Microbiology and Applied Sciences
ISSN: 2319-7706 **Volume 6 Number 11 (2017) pp. 5471-5475**
Journal homepage: http://www.ijcmas.com

Original Research Article https://doi.org/10.20546/ijcmas.2017.611.525

Proteomic analysis of growth promotional effects of biopriming in rice

M. Bhaskaran[1]*, M. Kokila[2], N. Jagadeeshselvam[3], Hifzur Rahman[3] and N. Senthil[4]

[1]Tamil Nadu Open University, Chennai; [2]Department of Seed Science and Technology, TNAU, Coimbatore, India; [3]Department of Plant Biotechnology, TNAU, Coimbatore, India; [4] Project Director, Centre of Innovation, AC&RI, Madurai, India
**Corresponding author*

Keywords
Proteomic analysis, biopriming in rice, *P. fluorescence*

Article Info
Accepted:
30 September 2017
Available Online:
10 November 2017

A B S T R A C T

To understand and identify the specific proteins which were responsible for improved speed of emergence and seedling vigour of primed seed, two-dimensional polyacrylamide gel electrophoresis (2D PAGE) study was conducted in 48 h germinated non-primed, hydro primed (12h) and bioprimed (4 % *P. fluorescence* for 12 h) seed of CORH4 rice hybrid. In the result, 29 proteins were differentially expressed in bio-primed seeds when compared to hydroprimed seeds. Among 29 proteins, 19 proteins were up-regulated and 4 proteins were down-regulated in bioprimed seeds and two proteins (27 and 28) were newly expressed only in hydroprimed seeds.

Introduction

In rice seeds, the food reserves are mainly stored in the form of lipids, proteins and starch in the endosperm. The protein and starch stored during seed maturation and desiccation, will be broken into small fragments and degraded gradually during germination. Protein degradation process contributes their amino acids to the biosynthesis of new proteins while starch degrading provides energy for cell multiplication during germination (Yang *et al*., 2007).

In primed seed germination, seedling vigour and speed of emergence were enhanced by advancement of endosperm weakening by hydrolase activities and storage proteins mobilization. Enzyme involved in mobilization of storage protein were either synthesized or activated during seed priming and degradation product of the β subunit of 11-s Globulin were accumulated during seed priming by an endo-proteolytic attack on the A-subunit in sugar beet seeds (Job *et al.*, 2000). Seed priming allow early protein synthesis which repair the damaged parts of the seeds (Entesari, *et al*., 2013) and induces the *de novo* synthesis of peptides (Wahid *et al*.,2008). Bio-priming is controlled hydration of seed with bio-liquid which initiate all germination-related activities, but prevent the actual emergence of radicle (McDonald, 2000). Hence, the study was initiated with aim of analysis of proteins which were

responsible for seedling vigour of bio-primed rice seed.

Materials and Methods

Nonprimed, hydroprimed and bioprimed seeds germinated for 48h used for the protein profile analysis on 2-DE.

Priming

For bio-priming seed treatment, the CORH4 rice seeds were imbibed in 4% *Pseudomas fluorescence* solution for the duration of 12 h and for hydro-priming seed treatment, the seeds were imbibed in water instead of *Pseudomas fluorescence* solution.

Preparation of total protein extracts for 2-DE

Nonprimed, hydroprimed and bioprimed seeds germinated for 48h of CORH 4 rice hybrid were homogenized separately in a pestle and mortar using liquid nitrogen. The ground sample was further extracted using 500 µl of cell lysis buffer (7 M urea, 2 M thiourea, and 4 % (w/v) CHAPS, 0.5 % (v/v) IPG buffer and 1 % dithiothreitol (DTT)). The extract was centrifuged at 14000 rpm for 15 min at 4 ^{0}C and supernatant was collected. To precipitate the proteins in the supernatant, 20 % TCA was added to the supernatant (1:1 ratio) and samples were incubated at 4 ^{0}C for 30 minutes. Protein concentrations in various extracts were quantified by the Non-Interfering™ protein assay kit (G-Biosciences, St. Louis, MO, USA), in accordance to the manufacturers protocol.

Two-dimensional electrophoresis (2-DE)

For the first dimension, 100 µg of proteins were rehydrated using 18 cm immobilized linear pH gradient (IPG) strips, pH 4–7, in a rehydration buffer (7 M urea, 2 M thiourea, 4 % (w/v) CHAPS and 0.002% Bromophenol blue). Isoelectric focusing was performed in the Ettan IPGphor 3 system (GE Healthcare) with following subsequent steps: 50 V for 1 h, 200 V for 1h, 500 V for 30 min, 4000 V for 30 min, 4000 V for 1 h 10,000 V for 1 h, 10,000 V for 13 h and 50 V for 3 h. Prior to the second dimension, the IPG strips were equilibrated twice for 30min each in 5 ml/strip of equilibration solution containing 6 M urea, 30 % glycerol (v/v), 2.5 % SDS (w/v), 0.15 M Bis-Tris and 0.1 M HCl, DTT (50 mM) for the first equilibration solution and 4 % iodoacetamide (w/v) was added to the second. Equilibrated gel strips were placed on top of 12 % vertical sodium dodecyl sulphate-polyacrylamide gels (10 % acrylamide, 0.33 % bisacrylamide, 15 ml of 4x resolving buffer, 10 % Sodium dodecyl sulphate, 10% APS and 60 µl TEMED). A denaturing solution (agarose sealing solution (0.075 g of low-melting agarose [Gibco BRL], 15 ml of SDS) was loaded onto gel strips. The electrophoresis was performed at 20 ^{0}C in a 1x electrophoresis SDS buffer at 30 mA/gel constant current. For each condition analyzed, 2D gels were made at least in duplicate and from two independent protein extractions. 2D gels were stained with silver nitrate according to Blum *et al*. (1987) for densitometric analyses. Image analysis was carried out with Image Master 2D Platinum Version 6.0 (GE Healthcare, Wisconsin, USA).

Results and Discussion

During seed germination, mobilization of the storage proteins is important event. In the present 2-DE analysis, 29 proteins differentially expressed in bioprimed seeds when compared to hydroprimed seeds. Out of 29 proteins, 19 proteins were up-regulated and 4 proteins were down-regulated in bioprimed seeds and two proteins (27 and 28) were newly expressed only in hydroprimed seeds (Fig. 1).

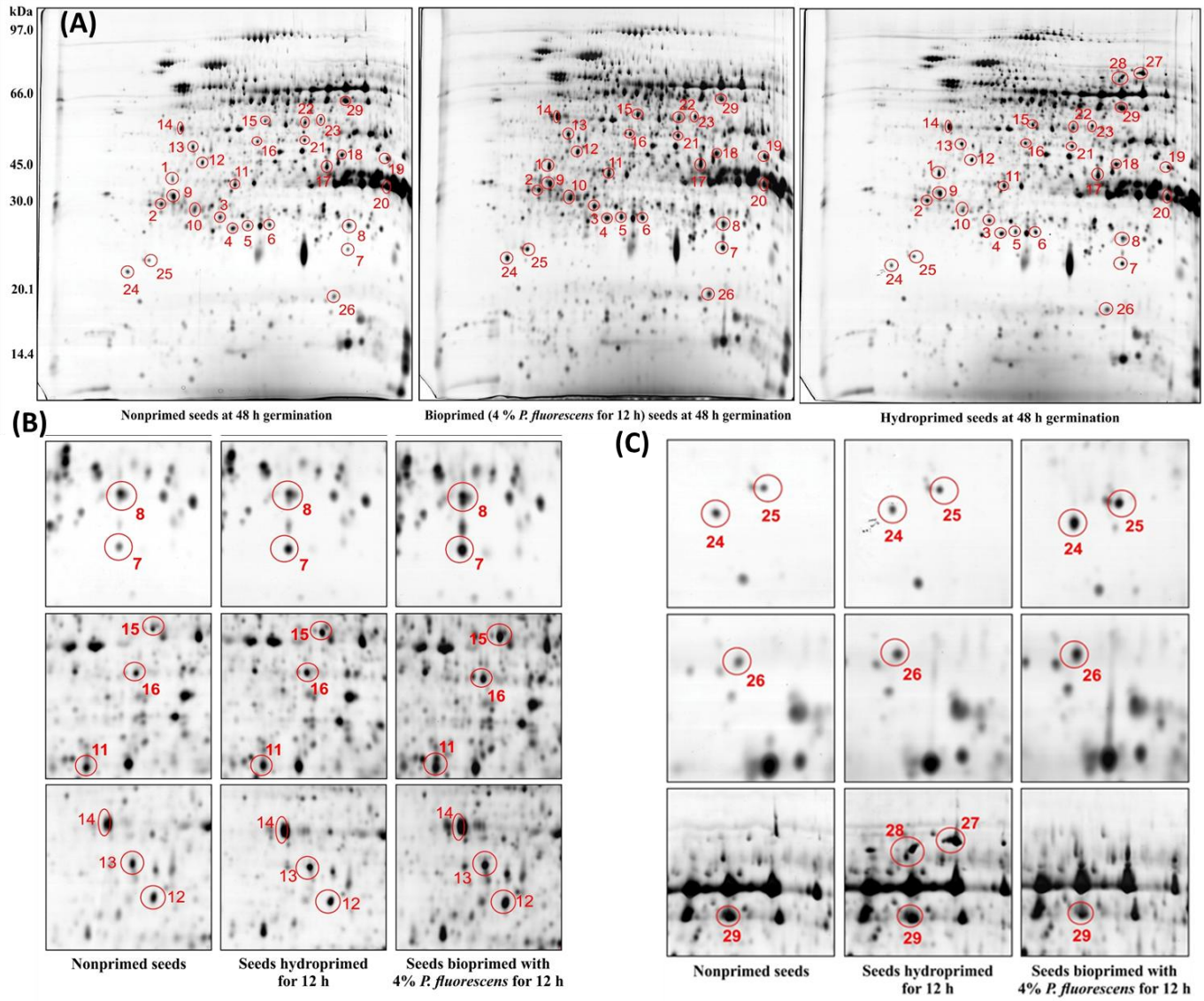

Fig.1 (A) Comparison between silver stained 2DE gel of non-primed seeds bioprimed and hydroprimed seeds. (B) and (C) Relative abundance of protein from 48 h germinated CORH 4 rice hybrid seeds in response to biopriming and hydropriming.

During rice seed germination, Yang *et al.* (2007) documented all the changed protein spots which were analyzed through MALDI-TOF MS and NCBI database searching. They identified 43 down-regulated, 58 up-regulated (including 14 induced proteins) and 8 proteins with complicated changes. According to the identification results, the changed proteins includes storage proteins such as globulin, glutelin, and seed allergen RA17, seed development and maturation associated proteins such as a seed maturation protein, an early embryogenesis protein, and a late embryogenesis abundant protein, desiccation-related proteins such as an abscisic acid-induced protein and a cold-regulated protein, carbon metabolism-associated proteins and some other functional proteins. Many down-regulated protein spots were identified as globulin or globulin like proteins. With the degradation of storage proteins, cysteine endopeptidase increased abundantly in the

process of rice seed germination. Seed maturation and desiccation associated proteins were also down-regulated upon imbibitions. In contrast to storage proteins, these proteins were drastically degraded at the early stage of phase II. The up-regulated proteins were mainly carbon metabolism associated proteins such as α-amylase and some enzymes involved in the glycolysis pathway.

In the present analysis, newly expressed two proteins (27 and 28) were observed in hydroprimed seeds (Fig. 1c). Similarly, hydropriming-specific protein was identified in Arabidopsis primed seed germination as a catalase isoform. Its abundance increased during hydropriming and continued to increase till the radicle emergence stage (Gallardo *et al*., 2001). Priming associated proteins were also identified in sugar beet seeds (Job *et al*., 1997).

Protein synthesis is an essential requisite for germination, starts several minutes after hydration of seed (Cheung *et al*.,1979).The storage proteins were synthesized during the process of seed maturation and broken into small fragments to release the energy and nitrogen resources for seed germination and subsequent seedling growth (Shewry *et al*.,1995).The storage proteins of globulin and glutelin were degraded during germination of rice seeds (Yang *et al*., 2007).

The advancement in cell division was occurred in primed seed due to an accumulation of β-tubulins during germination. Tubulin subunits were accumulated during priming in relation with reactivation of cell cycle activity (De Castro *et al*., 2000). The tubulin α and β subunits which were involved in cellular cytoskeleton and constituents of microtubules involved in cell division were abundant in primed Arabidopsis seed germination (Gallardo *et al*., 2001). Due to priming seed treatment, age induced damage to cellular protein was repaired (Kester, 1997). The level of free radical scavenging enzymes like superoxide dismutase increased during priming (Bailly *et al.*, 2000).

This study concluded that, the proteins which were differentially expressed in bioprimed seeds may be responsible for superior performance of bioprimed seeds over hydroprimed seeds.

References

Bailly, C., A. Benamar, F. Corbineau, and D. Come. 2000. Antioxidant systems in sunflower *(Helianthus annuus L*.) seeds as affected by priming. Seed Sci. Res., 10: 35–42.

Cheung, C.P., J. Wu and R. J. Suhadolnik. 1979. Dependence of protein synthesis on RNA synthesis during the early hours of germination of wheat embryos. Nature, 277: 66-67.

De Castro, R. D., V.A. A. M. Lammeren, S. P. C. Groot, R. J. Bino and H. W. M. Hilhorst. 2000. Cell division and subsequent radicle protrusion in tomato seeds are inhibited by osmotic stress but DNA synthesis and formation of microtubular cytoskeleton are not. Plant Physiol. 122: 327-336.

Entesari, M., F. Sharifzadeh, M. Ahmadzadeh and M. Farhangfar. 2013. Seed biopriming with *Trichoderma* species and *Pseudomonas fluorescens* on growth parameters, enzymes activity and nutritional status of soybean. I. J. Agronomy and Plant Prod., 4(4): 610-619.

Gallardo, K., C. Job, S. P. C. Groot, M. Puype, H. Demol, J. Vandekerckhove and D. Job. 2001. Proteomic analysis of Arabidopsis seed germination and priming. Plant Physiol., 126: 835-848.

Job, C., A. Kersulec, L. Ravasio, S. Chareyre and R. Pepin. 1997. The solubilization of the basic subunit of sugarbeet seed 11-S globulin during priming and early germination. Seed Sci. Res., 3: 225–243.

Job, D., I. Capron, C. Job, F. Dacher, F. Corbineau and D. Come. 2000. Identification of germination-specific protein markers and their use in seed priming technology. In: M. Black, K.J. Bradford and J. Vasquez-Ramos (eds.) Seed biology advances and application. CABI Publishing, Cambridge, UK, pp 449-459.

Kester, S.T., R.J. Geneve and R.L. Houtz. 1997. Priming and accelerated aging effect L- isoaspartylmethyl transferase activity in tomato *(Lycoperisicon esculentum* Mill) seed. J. Exp. Bot., 48: 943-949.

McDonald, M. B. 2000. Seed priming. In: Black, M. and J. D. Bewly (eds.) Seed Technology and its Biological Basis.

Shewry, P. R., J. A. Napier and A. S. Tatham. 1995. Plant Cell, 7: 945-956.

Wahid, A., A. Noreen, S. M. A. Basra, S. Gelani, M. Farooq. 2008. Priming induced metabolic changes in sunflower (*Helianthus annuus*) achenes improve germination and seedling growth. Bot. Stud., 49: 343-350.

Yang, P., X. Li, X. Wang , H. Chen, F. Chen and S. Shen. 2007. Proteomic analysis of rice (*Oryza sativa*) seeds during germination. Proteomics., 7 : 3358-3368.

Yang, P., X. Li, X. Wang , H. Chen, F. Chen and S. Shen. 2007. Proteomic analysis of rice (*Oryza sativa*) seeds during germination. Proteomics., 7 : 3358-3368.

How to cite this article:

Bhaskaran, M., M. Kokila, N. Jagadeeshselvam, Hifzur Rahman and Senthil, N. 2017. Proteomic analysis of growth promotional effects of biopriming in rice. *Int.J.Curr.Microbiol.App.Sci.* 6(11): 5471-5475. doi: https://doi.org/10.20546/ijcmas.2017.611.525