

Inhibition of ribonuclease and protease activities in germinating rice seeds exposed to nickel

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Abstract When the seeds of two rice *cvs. Malviya-36 and Pant-12* were germinated up to 120 h in the presence of 200 and 400 μM NiSO_4 , a significant reduction in the germination of seeds occurred. Seeds germinating in the presence of 400 μM NiSO_4 showed about 12–20% decline in germination percent, about 20–53% decline in lengths and about 8–34% decline in fresh weights of roots and shoots at 120 h of germination. Ni^{2+} exposure of germinating seeds resulted in apparent increased levels of RNA, soluble proteins, and free amino acids in endosperms as well as embryo axes. A 400 μM Ni^{2+} treatment led to about 58–101% increase in the level of soluble proteins and about 39–107% increase in the level of free amino acids in embryo axes at 96 h of germination. Activities of ribonuclease and protease declined significantly with increasing levels of Ni^{2+} treatment. Isoenzyme profile of RNase as revealed by activity staining indicated decline in the intensities of 3–4 preexisting enzyme isoforms in embryo axes of both the rice cultivars and disappearance of one of the two isoforms in endosperms of *cv. Pant-12* due to 400 μM Ni^{2+} treatment. Results suggest that the presence of high level of Ni^{2+} in the medium of germinating rice seeds serves as a stress factor resulting in decreased hydrolysis as well as delayed mobilization of endospermic RNA and protein reserves and causing imbalance in the level of biomolecules like RNA, proteins, and amino acids in growing embryo axes. These events would ultimately

contribute to decreased germination of rice seeds in high Ni^{2+} containing environment.

Keywords Germination · Nickel · Protease · Ribonuclease · Rice · *Oryza sativa* L.

Introduction

Excessive levels of heavy metals in the soil environment adversely affect the germination of seeds, plant growth, alter the levels of biomolecules in the cells and interfere with the activities of many key enzymes related to normal metabolic and developmental processes (Jha and Dubey 2005; Li et al. 2005; Leon et al. 2005; Maheshwari and Dubey 2007; Ahsan et al. 2007; Kuriakose and Prasad 2008). Some heavy metals are essential micronutrients required for basic cellular processes, but their higher concentration causes potential toxic effects similar to those induced by nonessential metal ions (Krupa et al. 1993; Welch 1995).

Seed represents the most protective stage in the life cycle of plants and is well-protected against various environmental stresses. However, soon after imbibition and protrusion of embryonic axis, seeds become in general stress sensitive (Li et al. 2005). Germination of seed is a complex process involving series of events such as activation of respiration (Bewley and Black 1994), repair of macromolecules (Osborne 1993), reserve mobilization (Gallardo et al. 2001), etc. Breakdown of storage reserves and its mobilization are among the crucial events that govern seed germination following imbibition. Germination stage is thus highly sensitive to stressful conditions like salinity, desiccation, submergence, and toxicity due to heavy metals (Dubey and Rani 1989; Shah and Dubey

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1998; Mihoub et al. 2005; Ahsan et al. 2007; Kuriakose and Prasad 2008).

Ribonucleic acid (RNA) and proteins (either enzymatic or storage reserve) are synthesized during the process of seed development and maturation and are stored within specific storage tissues (Herman and Larkins 1999; Grilli et al. 2002). In rice two major classes of proteins, prolamine and globulin like gluteline are stored within the endosperm (Choi et al. 2000). These storage reserves serve as building blocks for the growing embryonic axis (Herman and Larkins 1999). At the onset of germination aleurone layers of cereal grains secrete several hydrolytic enzymes for the mobilization of storage reserves to fuel early seedling growth (Fincher 1989).

Ribonucleolytic and proteolytic events are important for seed germination and seedling establishment (Bewley and Black 1994). Several RNA hydrolyzing enzymes, RNases are involved in both RNA turnover and mobilization from storage tissues of seeds (Gomes-Filho et al. 1999) and exert major influence on gene expression during developmental and certain abiotic and biotic stressful conditions (Booker 2004). Protein breakdown and its recycling are essential for several developmental processes such as germination, morphogenesis, senescence, etc. (Palma et al. 2002). Proteases are expressed in germinating seeds and are required for the degradation of storage proteins to mobilize amino acids for the growth of embryonic axis (Yamauchi 2003).

Ni²⁺ is an essential micronutrient for plants and its beneficial role in seed germination has been reported in several nickel resistant and nickel hyperaccumulator plant species (Welch 1995; Rout et al. 2000). High concentration of nickel exerts toxic effects on various growth and metabolic processes in plants (Gabbrielli et al. 1999; Parida et al. 2003; Maheshwari and Dubey 2007). However, limited information is available related to the effect of nickel during the process of seed germination, especially in rice, which is a staple food crop of world population. Therefore the present study was undertaken with the objectives to determine the effect of increasing concentration of nickel on germination percent, growth, uptake of nickel, metabolism of RNA, and protein in germinating rice seeds with particular emphasis on the level of RNA, proteins, amino acids as well as behavior of the hydrolytic enzymes ribonuclease and protease in different parts of germinating rice seeds.

Materials and methods

Plant material

Seeds of two rice (*Oryza sativa* L.) cvs. Malviya-36 and Pant-12, commonly grown in India and sensitive to Ni²⁺

(Maheshwari and Dubey 2007) were used. Seeds were surface sterilized with 0.1% sodium hypochlorite solution for 10 min and then rinsed with double-distilled water. After soaking for 24 h in double-distilled water, seeds were spread in petriplates for 5 days at 28 ± 1°C with 80% relative humidity in BOD cum humidity incubator (Narang Scientific Works, New Delhi). Two different concentrations of NiSO₄·7H₂O solution, i.e., moderately toxic level of 200 μM (11.74 ppm) and highly toxic level of 400 μM (23.48 ppm) served as treatment solutions. Starting with 24 h soaked seeds (0 h of germination), germinated seeds were taken out at 24 h intervals up to 5 days. The endosperms and embryo axes were separated after dehusking the seeds and all the analyses were done in triplicate.

Determination of germination percent, growth, and Ni uptake in germinating rice seeds

Germination percent was determined using 50 healthy seeds from each cultivar in triplicate. For this, completely germinated seeds were counted at 24 h intervals up to 120 h, i.e., at 0, 24, 48, 72, 96, and 120th hour of germination. Fresh weight and length of emerging roots and shoots were recorded in germinating rice seeds at 24 h interval up to 120 h using ten random samples in triplicate. To determine Ni concentration in different parts of rice seeds, endosperms and embryo axes were separated and were oven dried (70°C for 4 days), ground to a fine powder, and digested in an acid mixture (HNO₃:H₂SO₄:HClO₄, 10:1:4) (Rao and Sresty 2000). Digested samples were dissolved in deionized double-distilled water and Ni concentration was determined using an atomic absorption spectrometer (Perkin–Elmer-2380, USA).

Extraction and estimation of RNA

RNA was extracted using 200 mg fresh endosperms and embryo axes samples at different hours of germination of seeds according to the method of Volkin and Cohn (1954) as modified by Mittal and Dubey (1991). Estimation of RNA was done using orcinol reagent (Schneider 1957). Dialyzed yeast RNA (Sigma) was used as standard and intensity of color was read at 660 nm in a spectrophotometer (ELICO, India Model SL-177).

Extraction and estimation of soluble proteins

About 200 mg fresh endosperms and embryo axes samples were extracted in 5 ml of 0.2 M sodium phosphate buffer pH 6.5 containing 0.5 M NaCl and centrifuged at 22,000×g for 10 min. Soluble proteins were precipitated from the supernatant with 1 ml of 60% (v/v) cold perchloric acid. Protein pellet was collected by centrifugation

at $22,000\times g$ for 5 min and was dissolved in 1 ml of 0.1 N NaOH. Estimation of proteins was done by the method of Lowry et al. (1951) using bovine serum albumin (BSA, Sigma) as standard.

Extraction and estimation of free amino acids

About 200 mg endosperms and embryo axes were oven dried at 70°C for 4 days and extracted in 5 ml of 80% ethanol by grinding thoroughly followed by heating at 70°C for 1 min and centrifugation at $22,000\times g$ for 10 min. Extraction was repeated thrice and ethanolic extracts were mixed together and dried at 80°C . Contents were dissolved in water and volume was made up to 10 ml. Amino acids were estimated according to the method of Rosen (1959) using leucine as standard. Values were expressed in terms of mg amino acids g^{-1} fresh wt. of the sample.

Assay of ribonuclease activity

The enzyme RNase was extracted and assayed following the method of Wilson (1967). About 200 mg fresh endosperms/embryo axes samples were homogenized in 5 ml of 0.1 mM sodium acetate buffer, pH 5.4 containing 0.1% polyvinyl pyrrolidone (PVP) using a chilled mortar and pestle. After centrifugation and dialysis, in the supernatants RNase activity was assayed using dialyzed yeast RNA (Sigma) as substrate. Released nucleotides were estimated by recording absorbance at 260 nm using Perkin–Elmer LAMBDA EZ-201 UV–VIS spectrophotometer. Enzyme specific activity is expressed in terms of nkat mg^{-1} protein. Adenine was used as standard for calculation of released nucleotides.

Assay of protease activity

Protease was extracted and the activity was assayed using 200 mg fresh endosperms/embryo axes samples according to Dubey (1982) using purified casein as substrate. Peptide fragments released due to protease activity were estimated by the method of Lowry et al. (1951) using BSA (Sigma) as standard. Enzyme specific activity is expressed as μg peptide fragments released min^{-1} mg^{-1} protein.

Isoenzyme profile of ribonuclease

Electrophoresis of anionic RNase isoforms was performed using dialyzed enzyme extract prepared from endosperms and embryo axes of 72 h germinated seeds of both the rice cvs. Pant-12 and Malviya-36. Native polyacrylamide gel electrophoresis (PAGE) was performed in vertical slab gels at 4°C following the method of Davis (1964) using 0.01 M Tris–glycine (pH 8.3) as electrode buffer and 7.5%

resolving and 3.5% stacking gels. Enzyme was extracted in 5 ml of 100 mM sodium acetate buffer (pH 5.4) containing 0.1% polyvinyl pyrrolidone (PVP). Enzyme extracts containing about 100 μg protein were mixed with glycerol and loaded on top of the gel. Electrophoretic run was completed using a current of 25 mA per slab. The RNase bands were located by incubating the gels initially in RNA solution (0.25% RNA in extraction buffer) followed by staining with 1% (w/v) toluidine blue (Sigma) in 30% methanol. To attain visibility of isoforms bands, gels were destained in 5% (v/v) acetic acid solution. RNase isoforms appeared as colorless bands on a blue background.

Protein concentration in all the enzyme extracts was determined according to the method of Bradford (1976) using bovine serum albumin (BSA, Sigma) as standard.

Statistical analysis

All the experiments were performed in triplicate. Values in the figures indicate mean \pm SD based on three independent determinations. Differences among control and treatments were analyzed by one factorial ANOVA followed by Tukey's test using software SPSS.10. Asterisks (* and **) were used to identify the level of significance of the difference between control and nickel treatments on the corresponding days as $P \leq 0.05$ (*) and $P \leq 0.01$ (**), respectively.

Results

Effect of Ni^{2+} on germination of rice seeds

Seeds of both the rice cultivars showed 100% germination by 96 h when germinated under distilled water (Fig. 1), whereas Ni^{2+} concentrations of 200 and 400 μM in the medium caused reduced germination of seeds. A significant inhibition in germination percent was noted after 48 h of germination. Under a higher Ni^{2+} treatment level of 400 μM about 12–20% inhibition in germination was noticed at 120 h of germination. During 0–120 h of germination of seeds, with increasing hours of germination, a continuous increase in length and fresh weight of roots and shoots was observed in germinating seeds of both rice cultivars in controls as well as nickel treatments (Fig. 2). A high Ni^{2+} treatment level of 400 μM led to about 48–53% decline in root length and about 20–29% decline in shoot length. Similarly, a marked decline in the fresh weights of roots and shoots was observed with Ni^{2+} treatment. In seeds germinating in the presence of 400 μM Ni^{2+} , at 120 h of germination, about 16–34% reduction in fresh weight of roots and about 8–15% reduction in fresh weight of shoots was observed compared to controls.

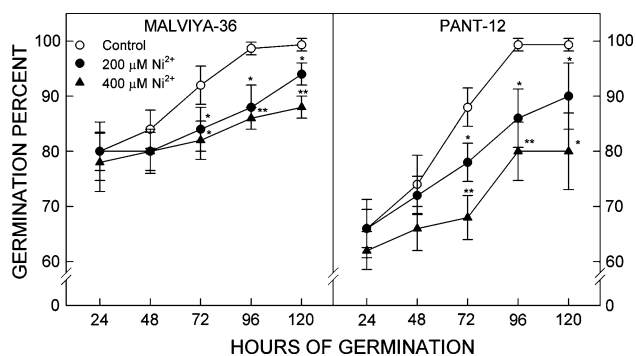


Fig. 1 Germination percent of seeds of rice cvs. Malviya-36 and Pant-12 at different hours of germination under increasing concentrations of NiSO₄. Values are mean ± SD based on three independent determinations and bars indicate standard deviations. Asterisks * and ** indicate significant differences compared to controls at $P \leq 0.05$ and $P \leq 0.01$, respectively, according to Tukey’s test

Figure 3 shows the level of absorbed Ni²⁺ in endosperms and embryo axes of rice seeds at different hours of germination under increasing concentration of NiSO₄ in the medium. As it is evident, in germinating seeds of both the rice cultivars, with increasing hours of germination a gradual increase in uptake of Ni²⁺ was noticed in endosperms as well as embryo axes with increasing concentration of Ni²⁺ in the medium. Seeds germinating without Ni²⁺ in the medium also possessed a very small amount of Ni²⁺ ranging from 0.01 to 0.05 μmol g⁻¹ dry wt. (equivalent to 0.6–3 ppm) in endosperms as well as embryo axes. In both the rice cultivars, during germination of seeds in the presence of Ni²⁺, higher content of Ni²⁺ was noted in embryo axes than in endosperms. Under 400 μM Ni²⁺ treatment, seeds germinating for 120 h, contained about 0.85–0.99 μmol Ni²⁺ g⁻¹ dry wt. equivalent to 49.89–58.1 ppm Ni²⁺ in

Fig. 2 Length and fresh weight of roots and shoots of rice cvs. Malviya-36 and Pant-12 at different hours of germination under increasing levels of NiSO₄. Values are mean ± SD based on three independent determinations and bars indicate standard deviations. Asterisks * and ** indicate significant differences compared to controls at $P \leq 0.05$ and $P \leq 0.01$, respectively, according to Tukey’s test

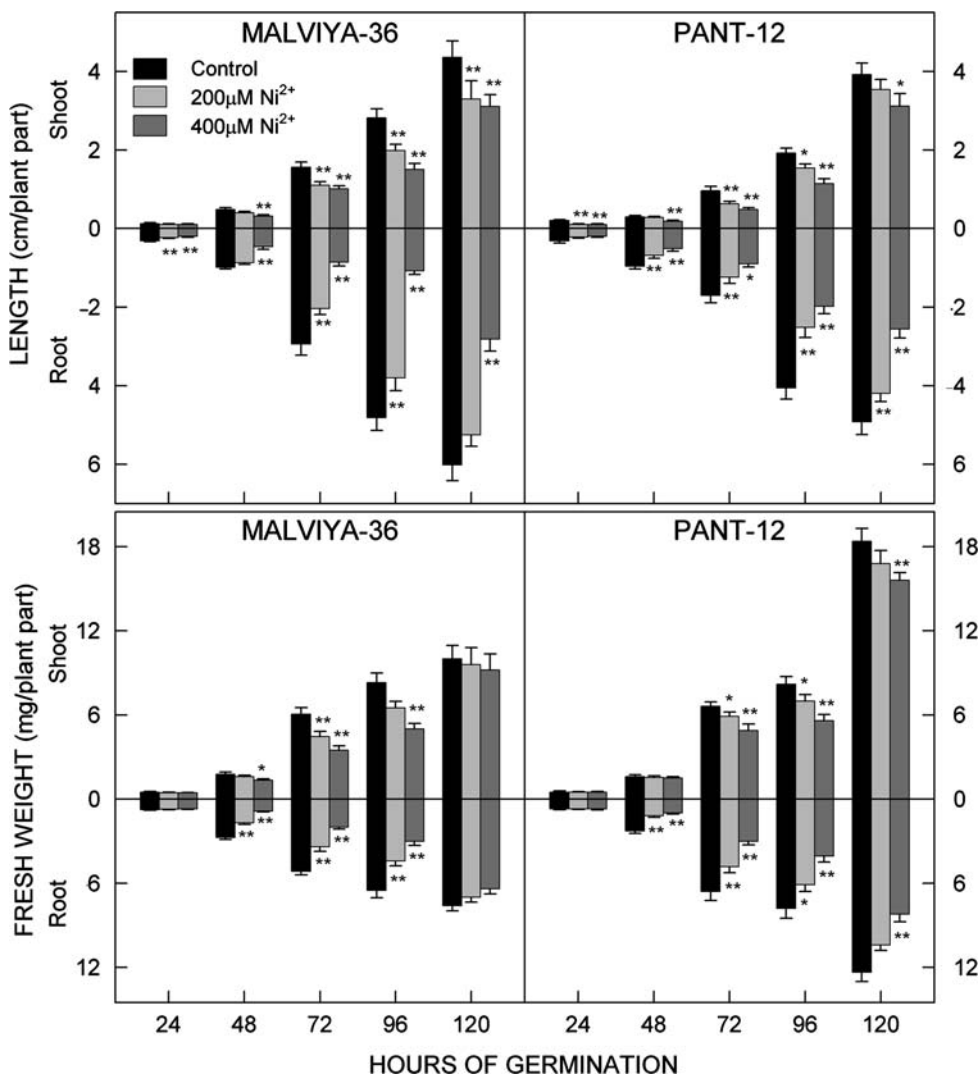
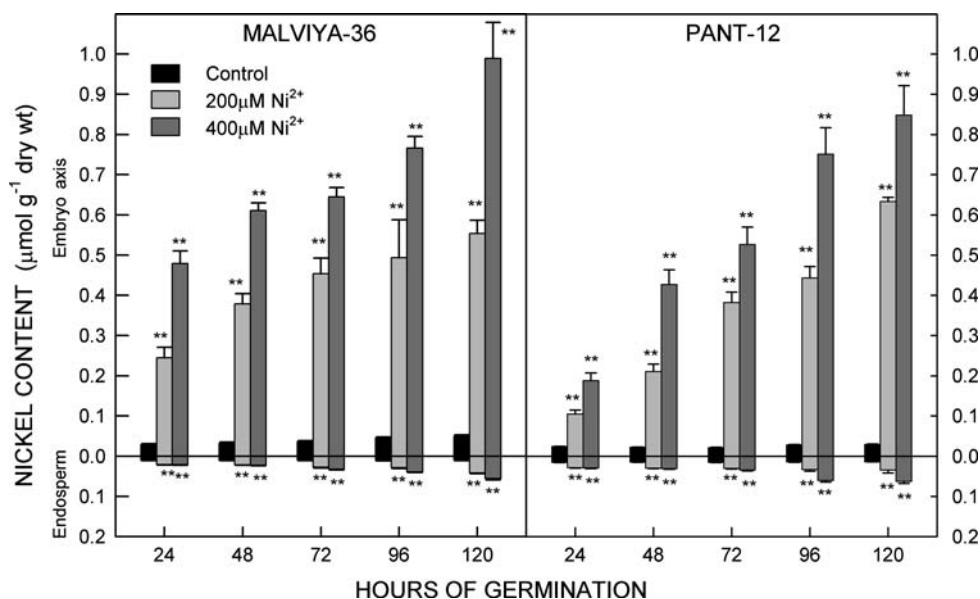


Fig. 3 Nickel content of endosperms and embryo axes of rice cvs. Malviya-36 and Pant-12 at different hours of germination under increasing levels of NiSO_4 . Values are mean \pm SD based on three independent determinations and bars indicate standard deviations. Asterisks * and ** indicate significant differences compared to controls at $P < 0.05$ and $P < 0.01$, respectively, according to Tukey's test



embryo axes and about 0.056–0.063 $\mu\text{mol Ni}^{2+} \text{ g}^{-1}$ dry wt. equivalent to 3.29–3.7 ppm Ni^{2+} in endosperms.

Effect of Ni^{2+} on level of RNA

In seeds germinating without Ni^{2+} in the medium, during a 0–96 h germination period, genotype specific alteration in the level of RNA was observed in endosperms as well as embryo axes (Fig. 4). In *cv.* Malviya-36 in both endosperms as well as embryo axes, there appeared a gradual increase in RNA level during 0–96 h of germination and it declined by 120 h, whereas in *cv.* Pant-12 no such regular trend could be seen. With increasing level of Ni^{2+} in the germination medium an increase in level of RNA was observed in both endosperms and embryo axes. Seeds germinating under Ni^{2+} treatment level of 400 μM showed about 42–64% increase in RNA level in endosperms and about 20–100% increased level in embryo axes at 96 h of germination.

Effect of Ni^{2+} on level of soluble proteins

Level of soluble proteins declined in endosperms with the onset of germination (during 0–48 h), increased thereafter at 72 h and declined further during 96–120 h of germination, while in embryo axes of both the rice cultivars level of soluble proteins increased concomitantly with increasing hours of germination from 0–120 h (Fig. 4). A marked increase in the level of soluble proteins was observed in both endosperms and embryo axes of rice seeds germinating under increasing level of nickel. A higher Ni^{2+} treatment level of 400 μM resulted in about 67–81% increase in the level of soluble proteins in endosperms and about 58–101% increase in the level in embryo axes during 96–120 h of germination.

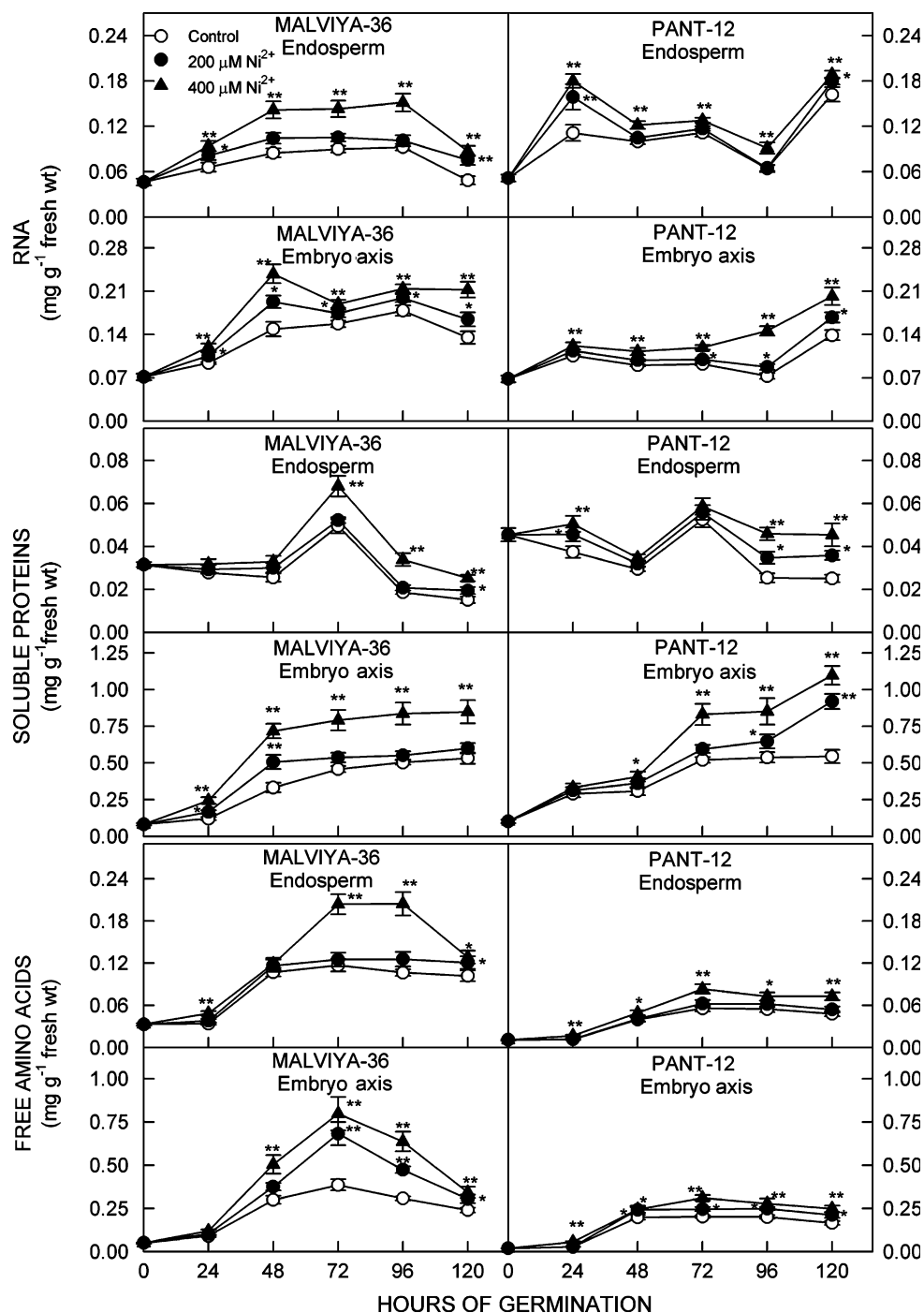
Effect of Ni^{2+} on level of free amino acids

Figure 4 shows the level of free amino acids in endosperms and embryo axes of seeds of both the rice cvs. Malviya-36 and Pant-12 as a function of time and increasing concentration of Ni^{2+} in the medium. As it is evident, the level of free amino acids increased in both endosperms and embryo axes with the start of germination during 0–72 h and it showed little decline thereafter. Presence of increasing concentration of Ni^{2+} in the germination medium resulted in a sharp increase in the level of free amino acids in both endosperms as well as embryo axes. The increase was more evident during 72–96 h period. A moderate Ni^{2+} treatment level of 200 μM resulted in about 13–18% increase in free amino acids level in endosperms and about 24–54% increase in embryo axes at 96 h of germination. While a higher Ni^{2+} treatment level of 400 μM resulted in about 33–92% increase in the level of free amino acids in endosperms and about 39–107% increase in embryo axes at 96 h of germination.

Effect of Ni^{2+} on ribonuclease activity

In endosperms of rice seeds, germinating without Ni^{2+} in the medium, RNase activity increased during early hours of germination and it was higher during 96–120 h, whereas in embryo axes it increased up to 72 h in *cv.* Malviya-36 and up to 96 h in Pant-12 and declined thereafter (Fig. 5). A significant drop in RNase activity was observed in both endosperms and embryo axes of the two rice cultivars with increasing level of Ni treatment. With 400 μM Ni^{2+} treatment about 21–32% inhibition in the activity of RNase was observed in endosperms and about 26–45% inhibition in embryo axes at 120 h of germination.

Fig. 4 Level of RNA, soluble proteins, and free amino acids in endosperms and embryo axes of germinating seeds of rice cvs. Malviya-36 and Pant-12 at increasing hours of germination under 0 (control), 200, and 400 μM NiSO_4 . Values are mean \pm SD based on three independent determinations and bars indicate standard deviations. Asterisks * and ** indicate significant differences compared to controls at $P \leq 0.05$ and $P \leq 0.01$, respectively, according to Tukey's test



Effect of Ni^{2+} on protease activity

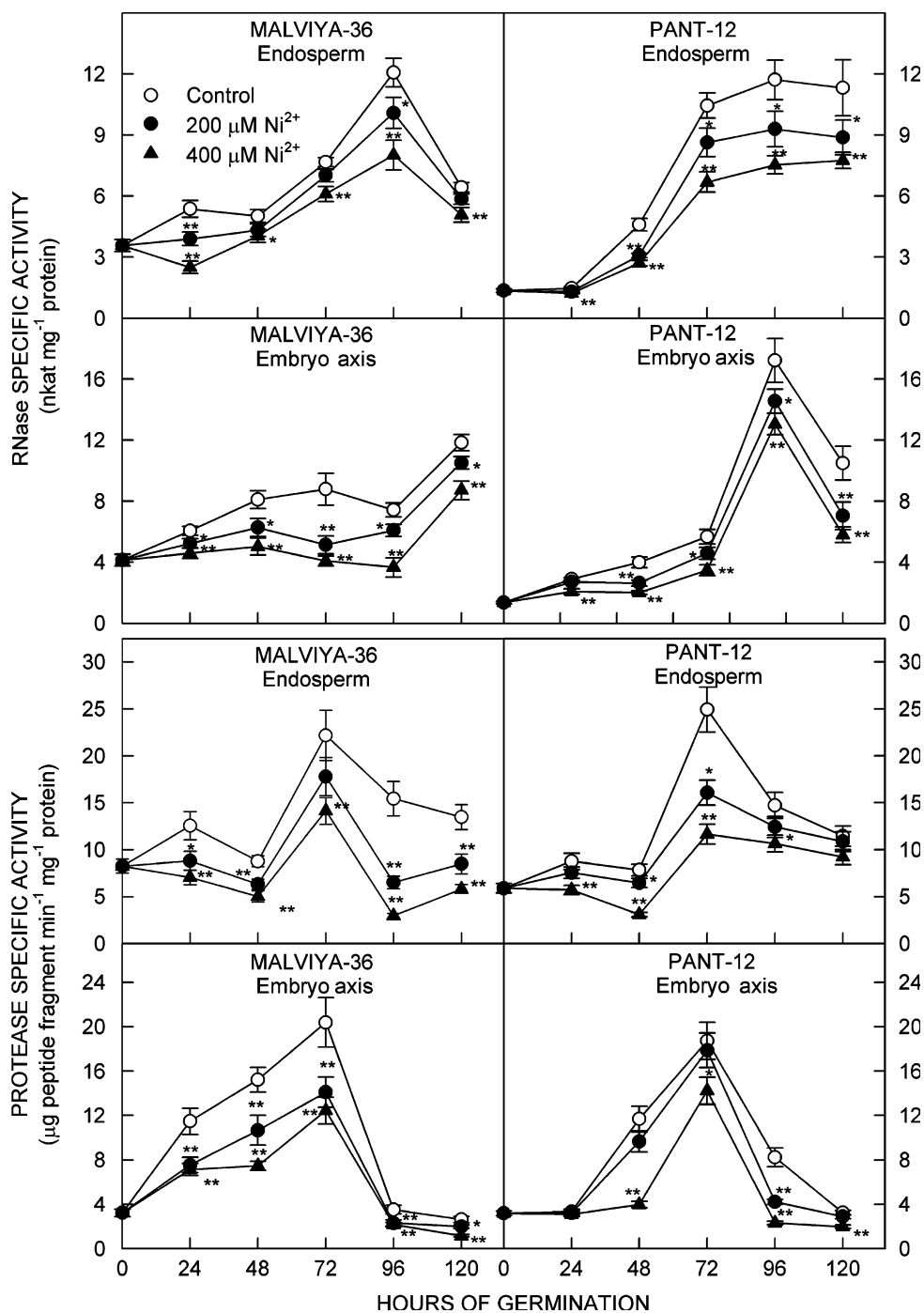
In germinating seeds of both the rice cultivars in endosperms as well as embryo axes activity of protease increased with the start of germination showing maximum activity at 72 h of germination and it declined thereafter (Fig. 5). A higher Ni^{2+} treatment level of 400 μM resulted in a marked decline in protease activity. At 72 h of germination under 400 μM Ni^{2+} treatment about 36–53%

decline in protease activity was observed in endosperms and about 24–39% decline in embryo axes compared to the activity in controls.

Effect of Ni^{2+} on isoenzyme profile of RNase

Figure 6 shows in-gel activity staining of RNase isoforms using dialyzed enzyme preparations prepared from

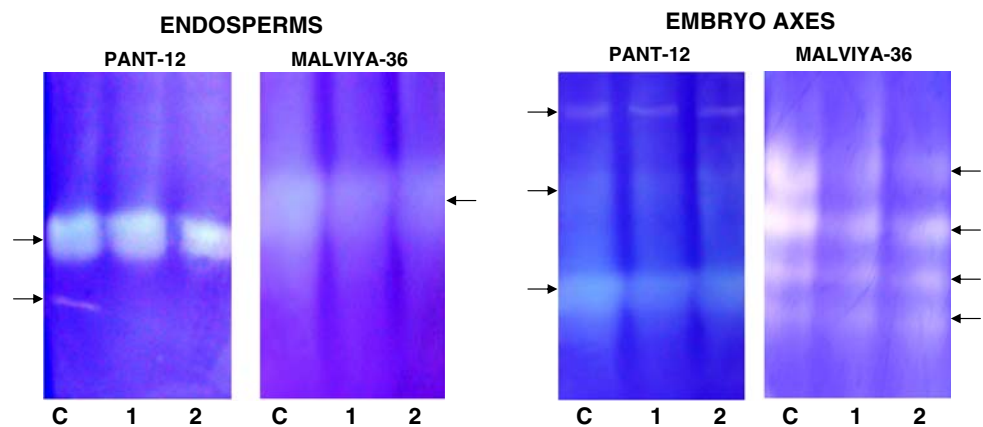
Fig. 5 Specific activities of ribonuclease and protease in endosperms and embryo axes of seeds of rice *cv.* Malviya-36 and Pant-12 at increasing hours of germination under 0 (control), 200, and 400 μM NiSO_4 . Values are mean \pm SD based on three independent determinations and *bars* indicate standard deviations. *Asterisks* * and ** indicate significant differences compared to controls at $P \leq 0.05$ and $P \leq 0.01$, respectively, according to Tukey's test



endosperms and embryo axes of 72 h germinated seeds of rice *cv.* Pant-12 and Malviya-36. One major and one minor RNase isoform bands were observed in enzyme preparations from endosperms of rice *cv.* Pant-12 and only one major band was detected from *cv.* Malviya-36, whereas three bands were observed from embryo axes of *cv.* Pant-12 and 4 from embryo axes of *cv.* Malviya-36

germinating without Ni^{2+} in the medium. At high level of Ni^{2+} (400 μM) treatment, the intensities of all the existing isoforms of RNase declined in both endosperms as well as embryo axes compared to the intensity in untreated controls. In endosperms the minor isoform band in *cv.* Pant-12 disappeared completely on nickel treatment.

Fig. 6 Isoenzyme profile of ribonuclease extracted from endosperms and embryo axes of 72 h germinated seeds of rice cvs. Pant-12 and Malviya-36. Seeds were germinated under increasing concentration of NiSO_4 (C, control; 1, 200 μM NiSO_4 ; 2, 400 μM NiSO_4). The positions of RNase bands are marked with *arrows*. 1–2 RNase bands were observed in enzyme preparations from endosperms whereas in embryo axes 3 (*cv.* Pant-12) or 4 (*cv.* Malviya-36) enzyme isoforms were visible



Discussion

Seed germination is sensitive to the nickel status of the environment because this mineral enters in the seeds during imbibition (Larcher 1995) and causes adverse effects on germination and subsequent seedling growth (Espen et al. 1997; Gupta et al. 2001; Parida et al. 2003; Leon et al. 2005). The present studies indicate decrease in germination percent as well as loss of seedling vigor when rice seeds are germinated in the presence of 200 or 400 μM Ni^{2+} in the medium. Further, Ni^{2+} is taken up by germinating rice seeds and it gets distributed differentially in different parts with its more distribution in embryo axes than in endosperms. Ribonucleolytic and proteolytic events are important for the process of seed germination and provide nutritional support to the germinating seed parts. Decreased hydrolysis of endospermic reserves due to a decline in the activities of hydrolytic enzymes followed by delayed transportation of mobilized reserves from endosperms to embryo axes are the key factors responsible for decreased germination of seeds and decreased seedling growth under various stressful conditions including metal toxicity (Dubey and Rani 1989; Mihoub et al. 2005; Ahsan et al. 2007; Kuriakose and Prasad 2008). The present results showed increased level of soluble protein and RNA and decline in the activities of protease and RNase in endosperms of nickel treated germinating rice seeds. This suggests that Ni^{2+} treatment possibly causes delayed mobilization of protein reserves from endosperms due to inhibition of protease activity, thereby showing an apparent higher level of protein in endosperms under Ni^{2+} treatment conditions, whereas increased level of endospermic RNA due to Ni^{2+} treatment could be possibly due to increased need for protein biosynthesis. The increased level of RNA and protein in Ni^{2+} treated germinating rice seeds, as observed in the present study, could also possibly be due to water loss upon Ni^{2+} treatment as a result of Ni^{2+} inhibition of aquaporin channels, as further evidenced by decreased

fresh weight of Ni^{2+} treated germinating rice seeds. Unlike our studies Lin and Kao (2006) showed that Ni^{2+} treatment could not alter endospermic starch content or α -amylase activity in germinating rice grains.

Similar to the present studies, increase in the levels of RNA and protein in both endosperms as well as embryo axes was also observed earlier in rice seeds germinated under high level of cadmium (Shah and Dubey 1995, 1998). Proteomics studies of germinating *Lepidium sativum* seeds showed inhibition of both storage protein catabolism and plant protein anabolism in response to cadmium exposure (Gianazza et al. 2007). In the present study, increased levels of RNA and soluble proteins in embryo axes of nickel treated germinating rice seeds could be either due to increased synthesis of stress-specific proteins and their corresponding RNAs or due to inhibition in their degradation due to decline in the activities of RNases and proteases under such conditions.

It was interesting to note in our studies that the activity of protease declined and protein level was higher in Ni^{2+} treated germinating seed parts, but at the same time the level of free amino acids increased. Such observations showing increased accumulation of free amino acids and decreased hydrolysis of protein reserves in Ni^{2+} treated rice seeds compared to controls indicate that free amino acids might be produced in stressed germinating seed parts at the cost of materials needed for the developmental processes (Alia and Saradhi 1991). A considerable increase in the level of amino acids has been reported in rice plants exposed to variety of stressful conditions such as salinity, toxicity due to heavy metals, etc., (Dubey and Rani 1989; Shah and Dubey 1998). It has been suggested that protein degradation might contribute to amino acid accumulation in metal stressed plants (Chen et al. 2001).

RNases and proteases play important role during germination of seeds and seedling establishment. RNases are secreted from the aleurone layer of cereal grains and have key role in the hydrolysis and mobilization of RNA in the

seed tissues (Gomes-Filho et al. 1999). Activity of RNases gets modulated under variety of stressful conditions like heat, chilling, salinity, and heavy metals (Shah and Dubey 1995; Chang and Gallie 1997; Gomes-Filho et al. 1999; Spanò et al. 2002; Mishra and Dubey 2006; Maheshwari and Dubey 2007). Proteases are expressed during seed development as well as germination (Zhang and Jones 1999). Protease activity is responsible for the hydrolysis of storage proteins and hydrolyzed products are mobilized to embryonic axis to support the growth of developing embryo axes during germination (Yamauchi 2003).

Activity of proteases gets altered in the presence of metals like Cu^{2+} , Cd^{2+} , As^{3+} , and Ni^{2+} in various plants (Nagoor and Vyass 1999; Balestrasse et al. 2003; Mishra and Dubey 2006; Maheshwari and Dubey 2007; Kuriakose and Prasad 2008). In the present study, the decline in the activities of total RNase and protease as observed in endosperms and embryo axes of Ni^{2+} treated germinating rice seeds, might be possibly due to binding of nickel with the functional groups of enzyme protein as a general heavy metal specific response (Van Assche and Clijsters 1990). Decline in the intensity of RNase isoform bands under Ni^{2+} treatment as observed in in-gel activity staining, with a parallel decline in the activity of total RNase in germinating seed parts suggests a clear down regulation of RNase activity in the presence of high concentration of Ni in the germination medium. Presence of two RNase isoforms in endosperms and three in embryo axes of *cv.* Pant-12 whereas one RNase isoform in endosperms and four in embryo axes in *cv.* Malviya-36 shows varietal difference in the two cultivars for RNase profile.

Present results suggest that excess Ni^{2+} in the germination medium of rice seeds operates as stress factor resulting in decline of RNase and protease activities leading to decreased mobilization of storage reserves from endosperms and imbalance in the levels of RNA, protein, and amino acids in germinating seed parts. This would ultimately contribute to decreased germination of seeds and inhibited growth of seedlings in nickel containing medium.

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