Journal of Plant Nutrition
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597277

Effect of Excess Nickel on Starch Mobilization in Germinating Rice Grains

Yu Ching Lin [a]; Ching Huei Kao [a]
[a] Department of Agronomy, National Taiwan University, Taiwan, Republic of China

To cite this Article Lin, Yu Ching and Kao, Ching Huei'Effect of Excess Nickel on Starch Mobilization in Germinating Rice Grains', Journal of Plant Nutrition, 29: 8, 1405 — 1412
To link to this Article: DOI: 10.1080/01904160600830225
URL: http://dx.doi.org/10.1080/01904160600830225

Journal of Plant Nutrition, 29: 1405–1412, 2006

ISSN: 0190-4167 print / 1532-4087 online
DOI: 10.1080/01904160600830225

Effect of Excess Nickel on Starch Mobilization in Germinating Rice Grains

Yu Ching Lin and Ching Huei Kao

Department of Agronomy, National Taiwan University, Taiwan, Republic of China

ABSTRACT

The effect of excess nickel (as $NiSO_4$) on starch mobilization of rice (*Oryza sativa* L.) grains or dehulled rice grains during germination was investigated. Excess $NiSO_4$ had no effect on starch content and α-amylase activity in endosperm of germinating rice grains or germinating dehulled rice grains. Evidence is provided to show that the hull is a barrier against influx of Ni^{2+} to endosperm; endosperm per se is less effective in Ni^{2+} uptake; and α-amylase extracted from the endosperm of germinating rice grains is highly resistant to excess Ni^{2+}.

Keywords: α-amylase, nickel, rice, starch mobilization

INTRODUCTION

Nickel (Ni) has long been considered a non-essential element, but recent evidence has shown that it is generally required in small amounts for normal plant growth and thus it has been included among the micronutrients (Brown et al., 1987; Dalton et al., 1988; Eskew et al., 1983; 1984). The phytotoxic effects induced by high concentrations of Ni^{2+} are known. The most common symptoms observed in plants exposed to phytotoxic levels of Ni^{2+} are chlorosis and necrosis of leaves, reduced root growth, inhibited germination, and retarded plant growth (Gabbrielli et al., 1999; Mishra and Kar, 1974).

Mobilization of seed reserves, which occurs during early seed germination, is crucial because it supplies substrates for the proper functioning of different metabolic processes that are essential for growth of the embryonic axis (Bewley, 1997; Mayer and Poljakoff-Mayber, 1975). Excess Ni^{2+} is known to inhibit

Received 12 January 2005; accepted 12 September 2005.
Address correspondence to Ching Huei Kao, Department of Agronomy, National Taiwan University, Taipei 106, Taiwan, Republic of China. E-mail: kaoch@ntu.edu.tw

seedling growth of rice (Wang et al., 2001). It appears that Ni^{2+} may inhibit starch mobilization in germinating rice grains. The present study examined the effect of Ni^{2+} (in the form of $NiSO_4$) on the changes in the content of starch and the activity of α-amylase of germinating rice grains and dehulled rice grains.

MATERIALS AND METHODS

Rice (*Oryza sativa* L., cv. 'Taichung Native 1') grains and dehulled rice grains were surface-sterilized in 2.5% sodium hypochlorite for 15 min, washed in distilled water, sown on moistened filter paper in a Petri dish (20 cm), and incubated at 37°C in the dark. After 24 h incubation, seedlings with 2 mm shoots were selected and transferred to Petri dishes (9 cm) containing two sheets of filter paper moistened with 10 mL of distilled water, $NiSO_4$, or 30 mM sucrose and allowed to grow at 27°C in the dark. Lin and Kao (2005) demonstrated that increasing concentrations of $NiSO_4$ (from 20 to 40 μM) progressively decreased root growth of rice seedlings and that no further decrease was observed at 60 μM $NiSO_4$. Thus, 40 μM $NiSO_4$ was used in the present study. Each Petri dish contained 10 germinated rice grains or dehulled rice grains. Each treatment was replicated four times.

Starch in endosperms was extracted twice with hot ethanol (80%, v/v). The tissue residues were suspended in 2 mM sodium phosphate buffer (pH 6.9) containing 6 mM NaCl and then boiled for 15 min to gelatinize the starch. Crude boiled homogenates were then used to determine starch content according to the method described previously (Hurng and Kao, 1993). Briefly, crude boiled homogenates containing starch were digested for 16 h at 37°C with 25 units of *Bacillus* α-amylase (Sigma) in sodium phosphate buffer. Blanks comprised sample aliquots and heat inactivated α-amylase. Maltose produced from starch was determined using dinitrosalicylic acid (Hurng and Kao, 1993). Starch content was expressed as milligrams maltose equivalent per one endosperm.

For α-amylase extraction, endosperm was homogenized in a pre-chilled pestle and mortar containing 3 mM $CaCl_2$ at 4°C. The homogenate was centrifuged at 12,000 *g* for 10 min at 4°C. Crude extract was used to determine α-amylase activity based on the method developed by Rinderknecht et al. (1967) using starch azure as substrate. The α-Amylase activity was calculated based on the change in A_{595}. One unit of enzyme activity was defined as an increase of 1 A_{595} min^{-1}.

For the determination of Ni, endosperms or hulls were dried at 65°C for 48 h. Dried materials were ashed at 550°C for 4 d. Ash residues were incubated with 17.5% (v/v) HNO_3 and 17.5% (v/v) H_2O_2 at 72°C for more than 10 h, then dissolved in double-distilled water for 3 h. Nickel was then quantified using an atomic absorption spectrophotometer (Model AA-680, Shimadzu, Kyoto, Japan).

Table 1
Changes in the concentration of starch and the activity of α-amylase in endosperm of germinating rice grains treated with $NiSO_4$

	Starch (mg endosperm^{-1})		α-Amylase (unit endosperm^{-1})	
Time (d)	Control	$NiSO_4$	Control	$NiSO_4$
0	9.8 ± 0.2		0.03 ± 0.01	
2	8.0 ± 0.6	8.2 ± 0.3	0.29 ± 0.01	0.26 ± 0.01
4	5.8 ± 0.7	6.5 ± 0.3	0.48 ± 0.01	0.45 ± 0.10
6	4.7 ± 0.7	5.5 ± 0.6	0.52 ± 0.01	0.51 ± 0.01

Rice grains were incubated in distilled water or $NiSO_4$ (40 μM). Means ± S.E. (n = 4).

Statistical differences between measurements of different treatments or at different times were analyzed by Duncan's multiple range test or Student's *t*-test.

RESULTS AND DISCUSSION

Starch is an important reserve in endosperm of rice grains. During germination, starch content in endosperm of rice grains decreased (Table 1). However, endosperm of rice grains treated with $NiSO_4$ had starch content similar to that treated with distilled water during germination (Table 1). Mobilization of starch in endosperm revealed a continuous increase in the activity of α-amylase during germination under both the control and $NiSO_4$ treatments (Table 1). However, endosperm of rice grains treated with $NiSO_4$ had α-amylase activity similar to that treated with distilled water during germination (Table 1). These results suggest that $NiSO_4$ had no effect on endosperm mobilization during germination of rice grains. This result is in contrast to our previous work, in which we demonstrated that NaCl and methyl jasmonate markedly inhibited the mobilization of starch in endosperm of germinating rice grains (Lin and Kao, 1995; Tsai and Kao, 1996).

Rice grain is the ripened ovary, with the lemma, palea, rachilla, sterile lemmas, and awn, if present, firmly attached to it. The lemma and palea themselves, and their associated structures including sterile lemmas, rachilla, and awn (if present), constitute the hull or husk. Recent observations (Wang and Kao, 2005) indicate that the hull is a barrier against influx of aluminum (Al) to the endosperm. It is not known whether the hull also acts as a barrier against the influx of Ni. To test this possibility, the Ni concentration was determined in the hull and endosperm, respectively, of germinating rice grains in the presence or absence of $NiSO_4$. It was observed that Ni concentration in the hull of $NiSO_4$ treated rice grains was much higher than that of distilled-water-treated ones

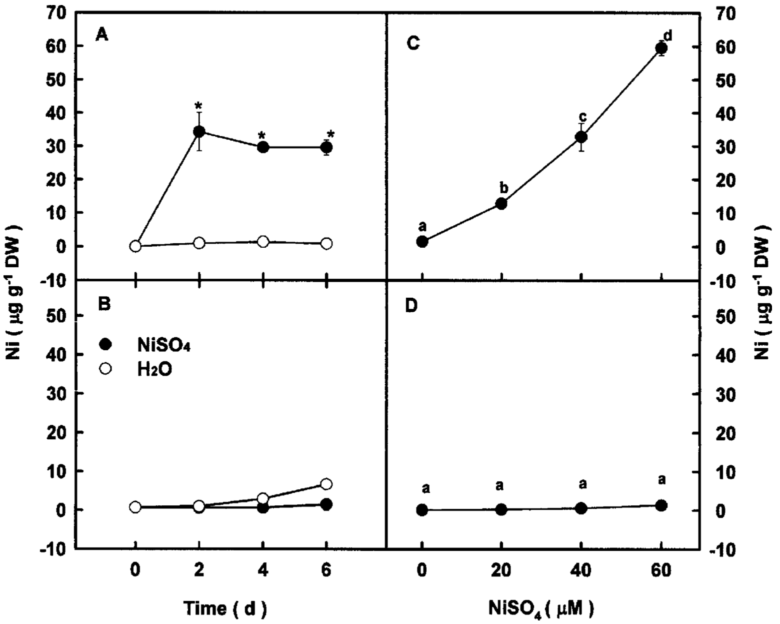

Figure 1. Changes in Ni content in the hull (A) and endosperm (B) of germinating rice grains treated with $NiSO_4$ and the effect of $NiSO_4$ concentrations on Ni content in the hull (C) and endosperm (D) of germinating rice grains. Bars indicate S.E. (n = 4). Asterisks indicate values that are significantly different from the controls at $P < 0.05$. Values with the same letter are not significantly different at $P < 0.05$.

(Figure 1A). However, much less Ni was observed in the endosperm of rice grains treated with $NiSO_4$ during germination (Figure 1B). Figure 1C shows that Ni content in the hull increased significantly with the increase in $NiSO_4$ concentration. However, Ni concentration in endosperm was not affected by $NiSO_4$ concentrations (Figure 1D). It appears that the hull of rice grains may act as a barrier against influx of Ni^{2+} to endosperm. Phytic acid (myoinositol hexaphosphte) is an important plant constituent. It has been shown that the immobilized phytic acid can adsorb heavy metal ions, such as cadmium (Cd), copper (Cu), lead (Pb), Ni, and zinc (Zn), from aqueous solution (Tsao et al., 1977). Wang et al. (1959) studied the distribution of phytic acid in sorghum (*Corghum vulgare* Per.) grains and found that the hull is rich in phytic acid. It is not known whether the hull of rice grains is also rich in phytic acid. If that is indeed the case, then it can explain why the hull contained higher amounts of Ni^{2+} in $NiSO_4$-treated rice grains. Further work is required to clarify this possibility.

The fact that $NiSO_4$ had no effect on starch mobilization in endosperm of germinating rice grains is possibly due to smaller amounts of Ni in the endosperm. If this suggestion is correct, then $NiSO_4$ may be expected to inhibit

Table 2
Changes in the content of starch and the activity of α-amylase in endosperm of germinating dehulled rice grains treated with $NiSO_4$

	Starch (mg endosperm^{-1})		α-Amylase (unit endosperm^{-1})	
Time (d)	Control	$NiSO_4$	Control	$NiSO_4$
0	10.5 ± 0.2		0.03 ± 0.003	
2	8.5 ± 0.3	8.9 ± 0.2	0.53 ± 0.02	0.57 ± 0.01
4	7.5 ± 0.2	8.0 ± 0.4	0.66 ± 0.01	0.65 ± 0.01
6	6.5 ± 0.2	6.9 ± 0.3	0.63 ± 0.01	0.64 ± 0.03

Dehulled rice grains were incubated in distilled water or $NiSO_4$ (40 μM) in the dark. Means ± S.E. (n = 4).

starch mobilization in germinating dehulled rice grains. However, when dehulled rice grains were treated with $NiSO_4$, it was found that $NiSO_4$ also failed to inhibit the decrease in starch content and the increase in α-amylase activity in endosperm (Table 2).

The failure of inhibition of starch mobilization by $NiSO_4$ in germinating dehulled rice grains suggests that endosperm per se is ineffective or less effective in Ni^{2+} uptake. When dehulled rice grains were treated with $NiSO_4$, only a small amount of Ni was observed in the endosperm (Figure 2), suggesting that Ni^{2+} content in endosperm is not high enough to inhibit starch mobilization. Alternatively, α-amylase in endosperm during germination of rice grains is highly resistant to Ni^{2+}. This possibility was tested by mixing enzyme extract of germinating endosperm with various concentrations of $NiSO_4$. The α-Amylase

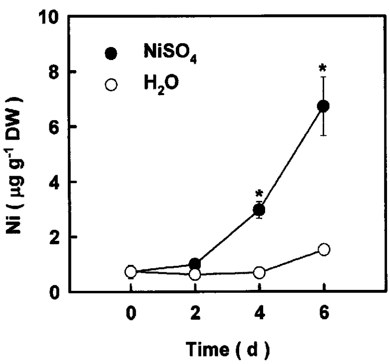

Figure 2. Changes in Ni content in the endosperm of germinating dehulled rice grains treated with $NiSO_4$. Bars indicate S.E. (n = 4). Asterisks indicate values that are significantly different from the controls at $P < 0.05$.

Table 3
Effect of $NiSO_4$ on the activity of α-amylase extracted from the endosperm of germinating rice grains

$NiSO_4$ (μM)	α-Amylase (A_{595})
0	1.22 ± 0.02
20	1.19 ± 0.04
40	1.18 ± 0.02
60	1.18 ± 0.02

Enzyme extracts were prepared from the endosperm of 4-d-old rice seedlings. Assays were performed 30 min after the addition of various concentrations of $NiSO_4$. Means $\pm$ S.E. (n = 4).

was then assayed 30 min after mixing. It was found that α-amylase was not inhibited by $NiSO_4$ (Table 3). These results indicate that α-amylase is highly resistant to Ni^{2+}.

Nickel sulfate ($NiSO_4$) is known to inhibit growth of rice roots (Wang et al., 2001). Here, we also found that $NiSO_4$ inhibited root growth of rice seedlings (Figure 3). Starch mobilization, which occurs during early seed germination, is important because it supplies sucrose for root and shoot growth (Bewley, 1997; Mayer and Poljakoff-Mayber, 1975). In the present study, it was found that $NiSO_4$ had no effect on starch mobilization in germinating rice grains; thus, the inhibition of root growth by $NiSO_4$ is probably not attributable to lack of sucrose, a product of starch mobilization. Because the addition of sucrose did not improve root growth of rice seedlings in $NiSO_4$ medium (Figure 4), it is

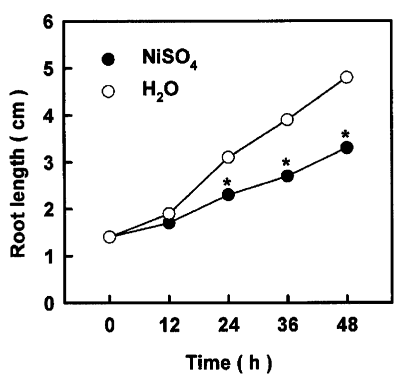

Figure 3. Changes in root length of germinating rice grains treated with $NiSO_4$. Bars indicate S.E. (n = 4). Asterisks indicate values that are significantly different from the controls at $P < 0.05$.

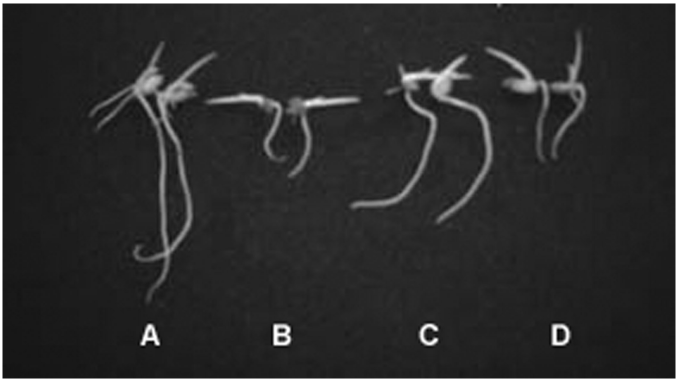

Figure 4. Effect of sucrose on $NiSO_4$-inhibited root growth of rice seedlings. All treatments contained 0.25 mg l^{-1} chloramphenical to inhibit bacterial growth. Photos were taken 2 d after treatment.

unlikely that the translocation of sucrose from scutellum into roots is inhibited by $NiSO_4$.

CONCLUSIONS

In conclusion, evidence is provided that Ni^{2+} has no effect on starch mobilization in germinating rice grains. The most novel aspects of this report are the findings that (1) the hull acts as a barrier against influx of Ni^{2+} to endosperm; (2) endosperm per se is less effective in Ni^{2+} uptake; and (3) α-amylase extracted from the endosperm of germinating rice grains is highly resistant to Ni^{2+}.

REFERENCES

Bewley, J. D. 1997. Seed germination and dormancy. *The Plant Cell* 9: 1055–1066.

Brown, P. H., R. M. Welch, and E. E. Cary. 1987. Nickel: A micronutrient essential for high plants. *Plant Physiology* 58: 801–803.

Dalton, D. A., S. A. Russel, and H. J. Evans. 1988. Nickel as a micronutrient element for plants. *BioFactors* 1: 11–16.

Eskew, D. L., B. M. Welch, and W. A. Norvell. 1984. Nickel in higher plants. Further evidence for an essential role. *Plant Physiology* 76: 691–693.

Eskew, D. L., R. M. Welch, and E. E. Cary. 1983. Nickel, as essential micronutrient for legumes and possibly all higher plants. *Science* 222: 691–693.

Gabbrielli, R., T. Pandolfini, L. Espen, and M. R. Palanddri. 1999. Growth, peroxidase activity and cytological modifications in *Pisum sativum* seedlings exposed to Ni^{2+} toxicity. *Journal of Plant Physiology* 155: 639–645.

Hurng, W. P., and C. H. Kao. 1993. Loss of starch and increase of α-amylase activity in leaves of flooded tobacco plants. *Plant and Cell Physiology* 34: 531–534.

Lin, C. C., and C. H. Kao. 1995. NaCl stress in rice seedlings: Starch mobilization and the influence of gibberellic acid on seedling growth. *Botanica Bulletin Academia Sinica* 36: 169–173.

Lin, Y.-C., and C. H. Kao. 2005. Nickel toxicity of rice seedlings: Cell wall peroxidase, lignin, and $NiSO_4$-inhibited root growth. *Crop, Environment and Bioinformatics* 2: 131–136.

Mayer, A. M., and A. Poljakoff-Mayber. 1975. *The germination of seeds*. New York: Pergamon Press.

Mishra, D., and M. Kar. 1974. Nickel in plant growth and metabolism. *Botanical Review* 40: 395–452.

Rinderknecht, H., P. Wilding, and B. J. Haverback. 1967. A new method for the determination of α-amylase. *Experientia* 23: 805.

Tsai, F.-Y., and C. H. Kao. 1996. Methyl jasmonate inhibits endosperm reserve mobilization in germinating rice seeds. *Physiology and Molecular Biology of Plants* 2: 67–70.

Tsao, G. T., Y. Zheng, J. Lu, and C. S. Gong. 1977. Adsorption of heavy metal ion immobilized phytic acid. *Applied Biochemistry and. Biotechnology* 63/65: 731–741.

Wang, C., H. C. Mitchell, and H. N. Barham. 1959. The phytin content of sorghum grain. *Trends of Kansas Academy of Science* 62: 208–211.

Wang, H.-H., J. Kang, F.-H. Zeng, and M.-Y. Jiang. 2001. Effect of nickel at high concentrations on growth and activities of enzymes of rice seedlings. *Acta Agronomica Sinica* 27: 953–957.

Wang, J.-W., and C. H. Kao. 2005. Effect of aluminium on endosperm reserve mobilization in germinating rice grains. *Biologia Plantarum* 49: 405–409.