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# Nickel: A Micronutrient Essential for Higher Plants[1]

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## ABSTRACT

**Nickel was established as an essential micronutrient for the growth of temperate cereal crops. Grain from barley (*Hordeum vulgare* L. cv 'Onda'; containing 40 to 80 nanograms of Ni per gram dry weight) grown in solution culture with negligible Ni concentrations (< 30 nanograms of Ni per liter) exhibited greatly reduced germination rates (*i.e.* 50% less than grain from Ni-adequate plants) and seedling vigor of the viable grain was greatly depressed. Grain containing less than 30 nanograms per gram dry weight was inviable. Under Ni-deficient conditions, barley plants fail to produce viable grain because of a disruption of the maternal plant's normal grain-filling and maturation processes that occur following formation of the grain embryo. The observations that (a) barley plants fail to complete their life cycle in the absence of Ni and (b) addition of Ni to the growth medium completely alleviates deficiency symptoms in the maternal plants satisfies the essentiality criteria; thus, Ni should be considered a micronutrient for cereals. Because Ni is required by legumes, and is now established as essential for cereals, we conclude that Ni should be added to the list of micronutrients essential for all higher plant growth.**

The discovery in 1975 that Ni is a component of the enzyme urease (6), which is present in a wide range of plant species (15), led to renewed scientific interest and research concerning the role of Ni in higher plants. Several researchers have since reported growth responses of plants to Ni fertilization under field conditions (for a review see Ref. 15) and in plants grown in nutrient solutions (13) or in tissue culture media furnished with urea as the sole N source (12). Eskew *et al.* (7) reported that Ni-deficient soybean (*Glycine max* L.) accumulate toxic levels of urea in their leaflet tips because of a depression in urease activity in their leaves. Walker *et al.* (14), working with cowpeas (*Vigna unguiculata* L. Walp), suggested that Ni (and urease) participates in N metabolism of legumes during the reproductive phase of growth. Checkai *et al.* (4) reported that Ni-deficient tomato plants (*Lycopersicon esculentum* L.) developed chlorosis in the newest leaves and, ultimately, necrosis of the meristem.

The earliest report of a growth response to Ni additions under controlled experimental conditions (2) indicated that Ni deficiency has a wide range of effects on plant growth and metabolism. These include effects on (a) plant growth, (b) plant senescence, (c) N metabolism, and (d) Fe uptake. Preliminary investigations also indicate that Ni may have a role in phytoalexin synthesis and plant disease resistance (9). Thus, low levels of Ni are known to be beneficial to plant growth. Previously, however, no study has satisfied all of the essentiality criteria for establishment of Ni as an essential element for all higher plants. For an element to be proven essential, one must demonstrate that a plant cannot complete its life cycle in the absence of the element, and that no other element can substitute for the test element (1). We report here that Ni satisfies these criteria and, therefore, should be classified as a micronutrient element essential for all higher plant growth.

## MATERIALS AND METHODS

Establishing a trace element as essential requires techniques capable of reducing its levels in the nutrient media and environment to below those levels required by the plant. For investigations involving Ni, this involves lowering Ni in the seed or grain to a sufficiently low level by growing plants for several generations under low-Ni conditions and upon maintaining controlled, low levels of Ni in the growth medium (*i.e.* <30 ng Ni $L^{-1}$).

**Plant Material and Growth Conditions.** Details of solution culture techniques and procedures used to minimize Ni contamination were described elsewhere (2). Briefly, to reduce Ni contamination, macronutrient salt stock solutions and deionized water supplied to growing plants were purified by column chromatography using an ion exchange column packed with 8-hydroxyquinoline controlled-pore glass beads (Pierce[2]) and by using only very high purity micronutrient chemicals and reagents (Ultrex, J. T. Baker; Puratronic and Specpure, Johnson Matthey; 7)

Barley plants (*Hordeum vulgare* L. cv 'Onda') were grown for three generations in purified nutrient solutions supplemented with 0, 0.6, and 1.0 $\mu$M $NiSO_4$ (40 plants per treatment). Grain from the third generation plants were used in the experiments reported here.

Yield responses to the addition of Ni to the growth medium and Ni deficiency symptoms in third generation plants used to produce the grain for studies reported here, were described elsewhere (2).

**Germination.** Germination tests were performed by imbibing grain in deionized water for 24 h and then placing the grain on wetted filter paper in sealed Petri dishes at 25°C for 36 h in the dark. In a second procedure, grain were first imbibed in a weak $NiSO_4$ solution (1.0 $\mu$M Ni; pH 4.9), which is an effective method of supplying micronutrients to germinating grain (10).

**Nickel Determination.** Concentrations of Ni in 5 to 10 grain (0.5–1.0 g total dry weight) were determined by an isotope dilution mass spectrometry technique as follows. Twenty ng of

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[2] Mention of a trademark, proprietary product, or vendor does not constitute a guarantee or warranty of the product by the United States Department of Agriculture and does not imply its approval to the exclusion of other products or vendors that may also be suitable.

Table I. *Effect of Ni Supply on Grain Yield of Third Generation Barley Plants Grown at Three Ni Levels*

| Ni Supplied in Nutrient Solution | Total Grain Wt[a] | Total Grains[a] per Plant |
|---|---|---|
| *μM* | *g dry wt* | |
| 0 | 7.3 ± 1.3 | 175 ± 26 |
| 0.6 | 7.5 ± 0.9 | 179 ± 35 |
| 1.0 | 8.4 ± 1.5 | 195 ± 41 |

[a] Mean ± SD: $n = 20$; treatment means do not differ significantly ($P > 0.05$).

Table II. *Effects of Ni Supply on the Germination of Grain Produced by Third Generation Barley Plants Grown at Three Ni Levels*

| Ni Supplied in Nutrient Solution | Germination* | Nickel Concentration* |
|---|---|---|
| *μM* | % | *ng/g dry wt* |
| 0 | 11.6 ± 11.6[a] | 7.0 ± 11.7[a] |
| 0.6 | 56.6 ± 16.3[b] | 63.8 ± 21.6[b] |
| 1.0 | 94.0 ± 8.9[c] | 129.2 ± 72.7[c] |

* Mean ± SD: $n = 12$; means with different superscripts differ significantly ($P < 0.005$).

$^{60}Ni$ were added to each sample; grain were dry ashed at 550°C; the ash was dissolved in 5 ml of 1 N $HNO_3$. Five ml of 10% sodium citrate and 3 ml of 1% DMG[3] were added to each sample and the pH was adjusted to 7.5 with $NH_4OH$. The Ni-DMG complex formed was then extracted into 3 ml of chloroform and the aqueous phase discarded. Five ml of 1 N $HNO_3$ was added to the chloroform phase and the chloroform phase discarded. The 1 N $HNO_3$ phase was dried at 55°C for 8 h (to evaporate dissolved chloroform) and then made to a volume of 3 ml with 1 N $HNO_3$. Ni concentrations in these samples were determined by inductively coupled, argon-plasma, mass spectrometry (Sciex, Canada). This procedure allowed quantitative determination of Ni in grain digests containing Ni concentrations as low as 0.05 ng $ml^{-1}$.

*Viability and Vigor Testing.* Grain viability and vigor was estimated qualitatively using a TTC stain test (11). Grain ($n = 100$) from plants supplied either with 1.0 μM Ni or without Ni were imbibed for 16 h in deionized water and then dissected longitudinally through the embryonic axis. Grains were then placed in Petri dishes and covered with 15 ml of 0.05% TTC and placed in an incubator, at 35°C, for 4.5 h. Grains were then scored as follows: not viable, embryo not stained and aleurone layer may be lightly stained; low vigor, embryo unevenly stained, shoot or more than two root-primordia stained unevenly or not stained at all, and aleurone layer brightly stained; high vigor, embryo brightly stained, shoot and root-primordia evenly stained, and aleurone layer brightly stained.

## RESULTS AND DISCUSSION

Third generation grain yield per plant (g dry weight), total grain number per plant, and average grain weight did not differ significantly between treatments (Table I).

Germination rates depended on the level of Ni supplied to the maternal plant in the three preceding generations (Table II). Plants grown without Ni additions to their growth media produced grain with very low percent germination whereas plants grown with 1.0 μM Ni in their nutrient solutions produced grain with germination percentages in excess of 95%. Grain of plants supplied 0.6 μM Ni had intermediate percent germination.

[3] Abbreviations: DMG, dimethylglyoxime; TTC, triphenyl-tetrazolium-chloride.

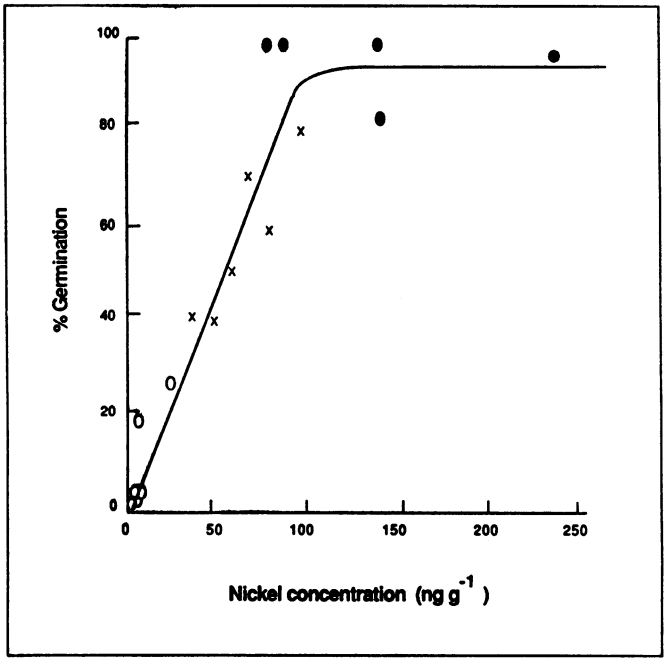


FIG. 1. Effect of barley grain-Ni concentration on germination. Curve joining data points was determined by using a cubic spline function generated by 'Cricket Graph' software. Each point represents percent germination and Ni concentration (dry weight bases) of from 10 to 15 grain from a single plant. Maternal plants were supplied either: (O), 0 μM Ni; (×), 0.6 μM Ni; or (●), 1.0 μM Ni.

Table III. *Effect of $NiSO_4$ Imbibition of Barley-Grain on Per cent Germination*

| Ni Supplied to Maternal Plant | Imbibing Solution | |
|---|---|---|
| | 0 μM $NiSO_4$* | 1 μM $NiSO_4$* |
| *μM* | % | |
| 0 | 9.1 ± 10[a] | 11.5 ± 10.3[a] |
| 0.6 | 55.2 ± 17[b] | 51.2 ± 20.3[b] |
| 1.0 | 98.2 ± 6.7[c] | 97.1 ± 9.1[c] |

* Mean ± SD; means with different superscripts differ significantly ($P < 0.001$).

Table IV. *Effect of Ni Supplied to the Maternal Plant on Vigor and Viability of Barley Grain as Estimated by TTC Staining Technique (% of all Grain)*

| Viability and Vigor Categories* | Ni Supplied in Nutrient Solution | |
|---|---|---|
| | 0 μM† | 1.0 μM† |
| | *% of seed in each category** | |
| Not viable | 23 ± 5[a] | 0[a] |
| Low vigor | 70 ± 12[b] | 40 ± 15[b] |
| High vigor | 7 ± 7[c] | 60 ± 22[c] |

* Categories were as follows: Not viable, embryo not stained and aleurone layer may be lightly stained; low vigor, embryo unevenly stained, shoot or more than two root-primordia stained unevenly or not stained at all, and aleurone layer brightly stained; high vigor, embryo brightly stained, shoot and root-primordia evenly stained, and aleurone layer brightly stained. † Mean ± SD; means with same superscripts were compared; all means compared differed significantly ($P < 0.05$).

Figure 1 shows the relationship between grain-Ni concentration and percent germination for 12 individual plants from each of three Ni treatments. The data fits a curve typical of nutrient-yield responses for an essential element (8). A critical value (defined as that plant tissue concentration of a mineral nutrient that results in a 15% reduction in the optimum yield of the plant; [8]) for Ni of 90 ± 10 ng $g^{-1}$ dry weight was determined for barley grains from the data presented (Fig. 1).

The observed effects of Ni depletion on grain viability could be the result of an effect of Ni deficiency on diminishing the ability of the maternal plant to produce viable grain or, alternatively, by an absolute requirement for Ni in embryo metabolism during germination. To determine if there was an absolute requirement for Ni during germination, grains from the 0, 0.6, and 1 μM Ni-treated plants were imbibed in a weak $NiSO_4$ solution (1.0 μM; pH 4.9). Imbibition with $NiSO_4$ did not improve the percent germination of grain from any of the Ni treatments (Table III), suggesting that the availability of Ni for essential processes in the grain is not limiting germination. However, there is the possibility that Ni in the imbibition treatment was not available for uptake by the embryo.

Possibly, Ni deficiency affected germination by disrupting grain development during maturation of the maternal plant. An effect of Ni deficiency on anthesis, fertilization, or early grain development was not indicated, however, as grain numbers and grain yield did not differ significantly between Ni treatments (Table I).

The viability and vigor of seeds and grain can be estimated qualitatively using a TTC stain (11). TTC stains the grain embryonic tissue bright red if active dehydrogenases (enzymes abundant in the cells of the embryo and aleurone layer of viable grain; [11]) are present.

TTC staining of grain from plants supplied 1.0 μM Ni and those grown without Ni supplementation demonstrated that dehydrogenase activity was decreased or absent in grain from Ni-deficient plants. Embryonic tissue did not stain in Ni-deficient grain. In addition, approximately 10% of the grain from Ni-deficient plants retained green palea at harvest. These green grains were from lateral tillers that developed late in the growth of the Ni-deficient plants.

Histological inspection of the split grain indicated that Ni deficiency inhibited the development of the embryo soon after the formation of the shoot primordia; the root primordia of Ni-deficient grain were poorly developed or absent at harvest. In addition, the endosperm tissue of Ni-deficient grain was generally transparent, flaccid, and dull white whereas in healthy, Ni-sufficient grain, the endosperm was turgid and bright and the root primordia where well formed.

The TTC staining method was used to classify grain with respect to vigor and viability based on a range of qualitative characters (Table IV). Ninety-three percent of the grain from plants not supplied Ni during growth were damaged during development as shown by the TTC test. Seventy percent showed some dehydrogenase activity, indicating that most of the Ni deficient grain had developed enough to contain some active dehydrogenases. All grain from plants supplied Ni exhibited some dehydrogenase activity. The high percentage of seeds from Ni-supplied plants that were classified as having low vigor (40%) did not correlate with the observed germination percentages. This may be a result of poor resolution of the TTC test.

The roles of Ni in plant metabolism remain mostly unknown. The broad range of effects attributable to a Ni deficiency suggest that it may be involved in several physiological processes. These may include the transport of nutrients to the seed or grain and movement of Fe into plant cells as well as the various metabolic effects described earlier (2, 7, 11, 14). Evidence presented here, and in the literature, indicate that Ni may also have essential functions in grain maturation and plant senescence (2).

Nickel is not likely to be deficient in experiments using conventional solution culture techniques because of contaminant-Ni in the nutrient salts used to prepare the nutrient solutions and/or in the water used for irrigation. High levels of chelating agents (*e.g.* EDTA), particularly when used together with low Fe (2) may, however, result in incipient Ni deficiency that might go unrecognized, especially when nutrient salts of very high purity are used. Under these conditions, we recommended that nutrient solutions be supplemented with at least 1.0 μM $NiSO_4$ to negate the possibility of Ni deficiency.

The evidence here demonstrates that barley plants cannot complete their life cycle without adequate Ni. Further, Eskew *et al.* (7) demonstrated that Ni could not be replaced by Al, Cd, Sn, V for the growth of soybeans. This evidence in conjunction with the findings that Ni is essential for cowpeas, produces beneficial growth responses in oats (*Avena sativa* L.; [2]), wheat (*Triticum aestivum* L.; [2]), tomato (4) and other plant species (13, 15) provides conclusive evidence that Ni must now be considered essential for all higher plants. This is the first micronutrient to be discovered to be essential since Cl was reported to be essential in 1954 (3).

The significance of Ni deficiency in agriculture has yet to be investigated intensively. The potential exists, however, for low Ni availability under some soil conditions (5) and therefore, Ni deficiency in food and feed crops is a possibility. Since Ni is required for normal grain development, agricultural scientists should be cognizant of the potential implications of Ni deficiency on grain quality.

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