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Original Articles

Detection of nickel in maize roots: A novel nondestructive approach by reflectance spectroscopy and colorimetric models

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ABSTRACT

In this paper, a novel method capable of assessing nickel (Ni) uptake in intact plant roots is proposed and validated. Dimethylglyoxime (DMG) was used to stain roots of seven-day-old maize (*Zea mays* L.) seedlings grown in solutions containing 0, 0.025, 0.1, 0.2, and 0.5 mM Ni^{2+}. A nondestructive approach by using reflectance spectroscopy and *CIE XYZ* and *CIE L*a*b** color systems was used to investigate their optical properties. The maximum intensity in reflectance spectra at 545 nm (A_{545}) was used to monitor the development of DMG–Ni complexes. The values of A_{545} were polynomially related to the concentration of Ni^{2+} in the growing solution (R^2 = 0.98), and significant correlations were also observed between A_{545} and the two indicators of phytotoxicity, namely, root growth inhibition (*I*) and variations in the shoot-to-root ratio (*S/R*). The variation of dominant wavelength (λ_d) and excitation purity (P_e) graphically derived from the *x*, *y* color gamut in the *CIE XYZ* system paralleled the two highest Ni concentrations. Most of the color parameters in the *CIE L*a*b** system were closely related to both Ni^{2+} concentrations in plant growing medium and the values of (*I*) and (*S/R*) used here as markers of Ni toxicity. The revealed dependences confirm that the prelaminar method proposed here is capable of nondestructive estimating of Ni concentrations in intact root tissue. The values of A_{545} in the reflectance spectra of stained roots and color parameters, namely, opponent redness–greenness (a^*), integral color difference $(\Delta E)^*$, lightness color difference $(\Delta L)^*$, and hue angle difference $(\Delta H)^*$, showed the highest potential for diagnostic purposes.

1. Introduction

The importance of nickel (Ni) as a trace element for higher plants was established only in 1975, when it was discovered that Ni is a part of the active site of urease (Dixon et al., 1975; Boer et al., 2014), which is supposed to play a pivotal role in plant stress reactions (Fabiano et al., 2014). The concentrations of Ni range from ng to μg per kg of dry matter depending on plant species (reviewed by Yusuf et al., 2011). On the other hand, the excess of Ni availability in the soil (primarily due to anthropic sources) can induce a toxic effect on plants as well as introduce this harmful metal into the ecosystem and food chains (Reck et al., 2008).

A range of environmental factors affects the phytotoxicity caused by Ni-excess (Rooney et al., 2007). Soil properties (Li et al., 2011; Semenzin et al., 2007) and in particular the rhizosphere features (McGrath et al., 2001; Sessitsch et al., 2013), among others, strongly modulate Ni bioavailability. The rhizosphere microenvironment affects Ni behavior at the soil–root interface (Wenzel et al., 2003; Puschenreiter et al., 2005), which is crucial to understand both the initial Ni interaction with the roots (Hinsinger and Courchesne 2007) and further movements of Ni through apoplast and/or symplast in the root apparatus (Rejala et al., 2010). Moreover, rhizosphere may also influence the Ni phytoextraction ability by hyperaccumulator species (Wenzel, 2009; Alford et al., 2010), which has practical implications for the development of phytoremediation technologies (Ferret et al., 2014; Algreen et al., 2014). Therefore, Ni behavior at the soil–root interface and in outer root tissues plays an important role in the mechanism(s) of Ni uptake by plants (Hinsinger and Courchesne 2007; Luo et al., 2014).

To date, various approaches have been used to assess the Ni content in plant tissues. The information on the averaged Ni concentrations in the entire root system can be obtained by the conventional destructive methods, such as atomic absorption spectroscopy (AAS; Fargašová 2012) and inductively coupled plasma optical emission spectroscopy (ICP-OES; Dalir et al., 2017). However, it was observed that the Ni uptake and distribution might differ longitudinally in root zones, as well as in the different tissues of the root (L'Huillier et al., 1996). The simultaneous assessment of Ni in different plant tissues (Moradi et al., 2010b; Debeljak et al., 2013) can be carried out by laser ablation ICP-

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MS, but it does not allow *in vivo* investigations. The nondestructive methods such as NMR imaging (Moradi et al., 2010a) and synchrotron-based X-ray fluorescence microscopy are capable of studying Ni uptake by intact roots (Kopittke et al., 2011; Donner et al., 2012). These approaches are complicated and require expensive equipment though they have many advantages, so in a wider perspective, their application in the investigation of plant–Ni interactions is actually limited.

Considering that DMG is a selective metallochromic indicator for Ni (Gramlich et al., 2011), the staining of plant tissues with dimethylglyoxime (DMG) is widely used for histochemical mapping of Ni. Light microscopy is used to study DMG-stained sections of plant tissues/organs and the intensity of the staining reaction is used to assess Ni visually (L'Huillier et al., 1996; Seregin et al., 2003; Bhatia et al., 2004). However, conventional DMG staining is only qualitative and does not enable Ni assessment in the intact plants. Visible reflectance spectroscopy and color measurements, following similar methods to those already patented by the authors to assess metal accumulation in plant tissues by other metallochrome indicators (Fedenko et al., 2008; Fedenko and Shemet, 2014), could be used to assess the quantitative nondestructive indication of Ni uptake in intact plants.

The objective of this study was to develop a nondestructive method for Ni assessment by using reflectance spectroscopy and colorimetry parameters derived from DMG-stained roots. These methods have already been efficiently used as analytical techniques for quantifying DMG–Ni complexes in a solid phase (Tubino et al., 1997; Tubino et al., 1997; Ershova et al., 1999). Especially, reflectance spectroscopy, primarily used for the assessment of the surface of the tested specimen, is also efficient in quantifying chromophores in deep cells of intact roots (Shemet and Fedenko, 2005). Therefore, we can assume that this approach will enable the *in vivo* investigation of DMG–Ni complexes in not only root epidermis but also the deeper tissues of the root apparatus.

We established spectral criteria for the identification of DMG–Ni complexes in fresh intact roots of plants grown hydroponically with different Ni concentrations during the preliminary validation of the method. Maize (*Zea mays* L.) was chosen as the model excluder plant because it retains most of the Ni in the roots (Fargašová, 2012; L'Huiller et al., 1996). Moreover, the Ni distribution in maize has already been investigated with other (destructive) approaches, and the concentration of Ni applied in solution has been found to be linearly related to the concentration available in the root environment (L'Huillier et al., 1996; Yang et al., 1997).

The quantitative dependences between spectral parameters of DMG–Ni complexes and Ni^{2+} concentrations in growth solution were derived, allowing adequate estimation of the Ni concentration in the root environment. In addition, the relations between phytotoxicity indicators and optical parameters of the roots were also analyzed. The last step is of particular importance, which demonstrates that the method proposed in this paper allows adequate estimation of physiologically active Ni in roots.

2. Materials and methods

2.1. Plant growth and phytotoxicity parameters

The seeds of maize (*Zea mays* L., hybrid Kadr 267 MV) were provided by the Institute of Grain Crops of National Academy of Agrarian Sciences, Dnipro, Ukraine. The seeds were treated with $KMnO_4$ for 5 min and then placed between a double layer of a filter paper soaked in distilled water to allow germination and root emergency. The seedlings with at least 5 mm of the main root length were then grown hydroponically (using a 1.25-L container for each plant) for 7 days with distilled water (control) or aqueous solutions enriched with $Ni(NO_3)_2$ (0.025, 0.1, 0.2, and 0.5 mM) at 22–24°C, ~500 ± 50 μmol PAR $m^{-2}\,s^{-1}$ and 16:8 h (photoperiod, *L:D*). The different amounts of ammonium nitrate $(NH_4)(NO_3)$ (0.5 mM for controls, 0.475, 0.4, 0.2, and 0 mM, respectively) were used to equally maintain the supply of N among different treatments. The pH of the solutions was adjusted to 6.0 ± 0.1 with concentrated H_2SO_4 and monitored on a daily basis.

The growth inhibition of the main root (L_R, mm) due to its high sensitivity (Li et al., 2011) was used to assess the toxic effect of Ni and the "tolerance index" *I* (*I* shows the percentage variation between control and Ni-exposed root length), shoot length (L_S, mm), and the ratio of the shoot-to-root length (*S/R*; Agren and Franklin, 2003) were used as additional indicators of phytotoxicity.

2.2. DMG staining

Intact roots were stained with a DMG-containing solution after seven days of the experiment. In order to preserve the *in vivo* pattern of the Ni distribution in root tissues, the roots were then detached from the seedlings and immediately used for the reflectance and colorimetry measurements. In more detail, the intact roots of eight seedlings for both control and Ni-exposed treatments were rinsed for 5 min in the staining reagent: 1% (w/v) DMG in 1.5% (w/v) NaOH, prepared with 0.05 M borax ($Na_2B_4O_7{\cdot}10H_2O$), and with a pH value of 13 (Gramlich et al., 2011).

2.3. Reflectance spectroscopy

The diffuse reflectance spectra of DMG-stained roots were obtained in the range of 350–800 nm using a Specord M40 spectrophotometer (Carl Zeiss, Jena, Germany), equipped with an integrating sphere for diffuse reflectance measurements. The spectrophotometer was programmed to use its "Data Handling I" algorithm for the smoothing and differentiation of the spectra using the polynomial approximation with 9 spectral points (Savitzky and Golay, 1964). A baseline with 100% approximation was adjusted with an MgO standard and the optical zero baseline was adjusted with a black cavity standard. A standard spectrophotometer holder for solid samples was uniformly covered with roots, ensuring complete coverage of the holder surface, in order to provide identical conditions for optical measurements. The roots from eight seedlings from controls or each Ni-exposed variant were sampled for every measurement. According to the approach that was previously used for the investigation of natural pigments in plant roots (Shemet and Fedenko, 2005), reflectance spectra were registered in absorbance units (*A*). For each wavelength within the spectral range, difference spectra were calculated by subtracting absorbance in the control variant from the absorbance in Ni-exposed seedlings. For controls and every Ni-exposed variant, spectra of five independent samples were measured.

2.4. Color measurements

A Specord M40 spectrophotometer equipped with the same accessories as used for reflectance measurements and its "Color Measurement" algorithm were used for color measurements, where color parameters of the specimen were derived by the mathematical processing of spectral reflectance data. The color parameters, such as *X*, *Y*, *Z* color coordinates and chromaticity coordinates *x*, *y*, were measured in the *CIE XYZ* system. Moreover, the dominant wavelength λ_d (nm) and excitation purity P_e (%) were derived graphically from the *x, y* color gamut (Ohta and Robertson, 2006).

The same "Color Measurement" algorithm was used to express color data as the coordinates of the *CIE L*a*b** uniform color space. For difference colorimetry due to its uniformity in opponent color spaces, the *CIE L*a*b** color space was used, where *L** represents the lightness of colors, *a** value represents redness and greenness (*a** and $-a^*$, respectively), and *b** value represents yellowness and blueness (*b** and $-b^*$, respectively). These values were used to calculate the radial component, chroma [$C^* = (a^{*2} + b^{*2})^{1/2}$], and the angular component of the polar representation, hue angle [$H = \tan^{-1}(b^*/a^*)$] for the calculation of difference colorimetric parameters. The value of the