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Influence of nickel stress on growth and some important physiological/biochemical attributes in some diverse canola (*Brassica napus* L.) cultivars

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ABSTRACT

To assess the effect of nickel on six canola cultivars a series of experiments were conducted. On the basis of shoot dry weight cvs. Shiralee and Range found to be nickel tolerant, Dunkeld and Ester as nickel sensitive, while the remaining cultivars intermediate. Nickel accumulation in shoots was lower in the nickel sensitive cultivars followed by that in the tolerant ones. Leaf water and osmotic potentials decreased significantly due to high concentration of Ni^{2+}. Decrease in osmotic potential was positively associated with accumulation of total free amino acids. By comparing accumulation of individual amino acids, pattern of accumulation of the amino acids was different in different cultivars. However, only histidine, serine and cysteine increased in appreciable amount in the xylem sap of different canola cultivars. Overall, nickel tolerant cultivars Shiralee and Range showed higher levels of histidine, serine and cysteine under varying levels of nickel than the others. This higher accumulation of histidine, serine and cysteine was positively related to nickel tolerance in all canola cultivars. Thus, differential nickel tolerance in canola cultivars proposed to be associated with relative detoxification of Ni by developing complexes with histidine, serine and cysteine and can be used as potential indicators of nickel tolerance in canola.

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1. Introduction

Of many heavy metals known in nature, nickel (Ni) is essential as trace element for normal plant growth and development, because it is constituent of some important enzymes such as urease. However, high concentration of Ni in growth medium can lead to toxicity symptoms and reduced growth of plants [1]. Toxic effects of high concentrations of Ni in growth medium on plants include alteration in uptake of essential nutrients, chlorosis, reduced CO_2 uptake, disturbances in gas exchange, alterations in water uptake and generation of free radicals and reactive oxygen species that produce oxidative stress [1–4].

There are a variety of mechanisms by which plants can endure high concentration of heavy metals, including restricted uptake and/or translocation of metals, exclusion of toxic heavy metals from cells by ion-selective metal transporters, excretion or compartmentation of heavy metals, production of heavy metal binding factors such as proteins, peptides, amines, amino acids, and formation of complexes with these binding factors and metals to detoxify metals [5–6]. For example, metal tolerant plants accumulated higher proline in response to heavy metal stress as compared to metal sensitive plants, and this accumulation of proline in stressed plants was found to be associated with reduced damage to membranes and proteins [7–8]. In another study, nicotianamine has been shown to chelate Ni and enhanced nickel tolerance in *Thlaspi goesingense* [9]. From these reports it is evident that different amino acids can have an important role in regulating metal toxicity in plants and thus it needs an extensive study. Furthermore, genetic variation and some degree of heritability for Ni stress tolerance have also been reported in Ni hyperaccumulator *Thlaspi spp.* [9–11]. In view of all these reports, the present study was aimed to assess variability for Ni stress tolerance in some elite canola cultivars and to examine up to what extent accumulation of different amino acids has a role in Ni stress tolerance in canola cultivars. In general, the work reported in manuscript is to identify the potential indicators responsible for the tolerance of nickel stress, which could be used in future breeding programs to evolve high yielding canola varieties with improved characters.

2. Materials and methods

Four independent experiments were conducted to assess the response of six selected canola cultivars to varying nickel concentrations. In the first experiment, LD_{50} (Lethal dose at 50% growth)

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concentration of Ni was determined for canola cultivars. In the second experiment, screening of canola cultivars for Ni tolerance was carried out and cultivars were grouped as tolerant, moderately tolerant and sensitive on the basis of shoot dry weight at the adult stage. In the third experiment, physiological and biochemical responses of canola cultivars were assessed under varying concentrations of nickel. In the fourth experiment, accumulation of Ni and amino acids in xylem sap was determined and parallels were drawn between them to assess their role in Ni tolerance.

3. Experiment 1: Optimization of LD_{50} concentration of Ni for canola

In order to determine LD_{50} (nickel concentration where 50% growth was reduced), 20 seeds of each cultivar were allowed to germinate for 15 days under varying concentrations of Ni (0, 10, 20, 30, 40 ... 150 mg L^{-1}). After 15 days, germination percentage, percent viable population size, shoot fresh and dry weights were recorded. The Ni concentration 150 mg L^{-1} was the most toxic concentration, which severely inhibited the growth. At Ni concentration 100 mg L^{-1}, 50% reduction in percent viable population size, and shoot fresh and dry weights was observed and considered as LD_{50}. This Ni concentration was used for further experimentation to screen the canola cultivars.

4. Experiment 2: Assessment of variation in tolerance of canola cultivars to Ni

Seeds of 10 canola cultivars (Shiralee, Range, Torch, Rainbow, Dunkeld, Ester, Con-I, Con-II, Tobin, Frontier) were obtained from Ayub Agricultural Research Institute, Faisalabad, Pakistan. Twenty seeds were sown in earthen of 30 cm diameter containing 6.0 kg homogenously mixed sun-dried sandy clay loam soil with a completely randomized design (CRD) and four replications. After germination the plants were thinned to maintain five seedlings in each pot. After 3 weeks, all plants were subjected to 100 mg L^{-1} Ni as $NiCl_2$ for 4 weeks. On the basis of shoot dry weight in 100 mg L^{-1} Ni, Shiralee and Range, Torch and Rain, and Dunkeld and Ester were categorized as tolerant, moderately tolerant, and sensitive to Ni, respectively, while the other cultivars were found to be intermediate in Ni tolerance. The cultivars so selected were further used to evaluate Ni tolerance on the basis of physiological and biochemical attributes.

5. Experiment 3: Physiological and biochemical responses of selected canola cultivars to Ni

A pot experiment was conducted during the winter 2004–2005 in a net-house at the Botanic Garden of the University of Agriculture, Faisalabad, Pakistan (latitude 31°30 N, longitude 73°10 E and altitude 213 m), with 10/14 light/dark period at 800–1100 μmol m^{-2} s^{-1} PPFD, a day/night temperature cycle of 28/17 °C and 65 ± 5% relative humidity. About 50 seeds of each of six canola cultivar were sown in each earthen pot (30 cm diameter and 20 cm in depth) filled with 6 kg sandy clay loam soil (soil saturation percentage 33%; pH 7.8; EC_e 2.21 mS cm^{-1}). For determining available Ni, soil was extracted in 1 N ammonium acetate solution (1:5) ratio following Allen et al. [16] and for total Ni in the soil samples were digested in a mixture of sulphuric acid and hydrogen peroxide following Wolf [17]. The mean available and total Ni concentration levels in the soil were 0.14 and 29.5 mg kg^{-1}, respectively. After 1 week, the seedlings of comparable size growing equidistantly were thinned to maintain five seedlings per pot. The experiment was arranged in a completely randomized design with four replicates, four nickel treatments (0, 50, 100, 150 mg L^{-1}) and six cultivars. The plants were irrigated with distilled water for 3 weeks before the start of varying Ni treatments as nickel chloride ($NiCl_2 \cdot 6H_2O$) for further 58 d after which time three plants out of five were harvested. Uprooted plants were washed with distilled water and separated into shoots and roots, and then blotted dry before recording their fresh weights. All plants parts were dried at 65 °C until constant dry weight, and their dry weights recorded. Before harvesting the plants for determination of plant biomass, the following physiological parameters were measured:

5.1. Water relations

The 2nd leaf from each plant was excised at 7.00 a.m., and the leaf water potential measurements were made with a Scholander type pressure chamber (Arimad, UK). A proportion of the same leaf used for water potential measurements, was frozen into 2 cm^3 polypropylene tubes at −40 °C in an ultra-low freezer for 2 weeks, after which time plant material was thawed and the frozen sap was extracted by crushing the material with a glass rod. After centrifugation (8000 × g) for 4 min, the sap was directly used for osmotic potential determination using a vapor pressure osmometer (Wescor 5500). Leaf turgor pressure was calculated as the difference between leaf water potential and leaf osmotic potential values.

5.2. Total soluble proteins

Total soluble proteins were determined as described by Lowry et al. [12]. Fresh leaf material (0.2 g) was homogenized in 4 mL of sodium phosphate buffer solution (pH 7.0) and centrifuged. The extract was used for the estimation of soluble proteins and free amino acids. The sample extracts were reacted with a Folin phenol reagent and the optical densities read at 620 nm using a spectrophotometer (Hitachi U-2000).

5.3. Total free amino acids

Total free amino acids were determined following the procedure of Hamilton and Van Slyke [13]. For estimation of total free amino acids, 1 mL of each sample as extracted for soluble protein determinations was treated with 1 mL of 10% pyridine and 1 mL of 2% ninhydrin solution. The optical densities of the solutions were read at 570 nm using a spectrophotometer (Hitachi U-2000).

5.4. Total soluble sugars

Total soluble sugars were estimated following the procedures of Malik and Srivastava [14]. Well ground dry leaf material (0.1 g) of each sample was homogenized in 80% ethanol and centrifuged at 3000 × g. The residue was retained and repeatedly washed with 80% ethanol to remove all traces of soluble sugars. The resulted filtrate was diluted up to 100 mL with distilled deionized water and reacted with anthrone reagent. The aborbance of the colored solutions was read at 625 nm using a spectrophotometer (Hitachi U-2000).

5.5. Qualitative and quantitative estimation of individual amino acids

Amino acid profile was estimated according to the method of Braithwaite and Smith [15]. One gram fresh leaves were chopped in 2 mL of 6 N HCl in sealed test tubes and incubated at 125 °C in a heating chamber for 24 h. The resulting paste was centrifuged at 15,000 rpm at 15 °C for 10 min. The supernatant was used for separation of amino acid profile through paper chromatography. Individual amino acids were identified by comparing Rf values of

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