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Screening of canola (*Brassica napus* L.) cultivars for nickel stress tolerance

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Abstract
The present study was conducted to evaluate variation in nickel stress tolerance in a set of 10 canola cultivars (Leand, AC Excel, Faisal Canola, Shiralee, Oscar, Punjab Canola, CON-II, Rainbow, Dunkeld, and Cyclone) at the early growth stages, i.e., germination and seedling stage. The seeds of all canola cultivar were obtained from the Ayub Agricultural Research Institute, Faisalabad, Pakistan. Ten seeds of each cultivar were sown in each of Petri plates moistened with varying levels (15, 30 and 45 mg/L) of nickel in Hoagland nutrient solution. Data for different growth indices were recorded such as germination stress index (GSI), shoot length stress index (SLSI), root length stress index (RLSI), and seedling fresh (SFWI) and seedling dry weight indices (SDWI). Based on different growth indices, three canola cultivars, i.e., CON-II, Faisal Canola and Leand proved to be potential source of variation for Ni tolerance provided if they maintain their degree of Ni tolerance at the later growth stages. However, Oscar was the most sensitive to Ni stress of all 10 cultivars evaluated in the current study.

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Introduction

Among the heavy metals, nickel holds a special place, because other heavy metals are not the component of plant enzymes but nickel is a vital constituent of urease (Marschner, 1995). Small quantity of nickel (0.01 to 5 µg/g dry weight) is essential for some plant species. Nickel is an essential nutrient for plants, because plants cannot complete their life cycle in its absence, since it is actively involved in many biological functions and cannot be substituted by any other element (Gąszczak et al., 2021). Natural sources of nickel include weathering of rocks, metal mining, smelting, fossil fuel, vehicle emissions, burning, electrical batteries, municipal and industrial wastes, and metallurgical and anthropogenic activities (Meshram and Pandey, 2019). Like in case of other micro-nutrients, the high concentration of nickel has an inhibitory effect on plant growth and development due to the reason that it inhibits the activities of many enzymes and is involved in environmental pollution and human health risk (Maheshwari and Dubey, 2007). The toxic effects of nickel were investigated at many different levels and at different growth stages (Einhardt et al., 2021). Elevated concentrations of nickel adversely affect photosynthesis, plant water relations (Chen et al., 2009), activities of enzymes, mineral nutrition and respiration (Gajewska et al., 2006). Toxicity of nickel causes necrosis, wilting and stunted plant growth and also affects the quality and yield of fruit (Bhalerao et al., 2015). Nickel has been reported to both promote and inhibit the activities of antioxidant enzymes (Baccouch et al., 2001). As compared to other heavy metals, nickel receives a less attention from plant scientists due to its dual character and complex electronic chemistry, which is a major hurdle in discovering its toxicity mechanism in plants (Yusuf et al, 2011).

It was observed that seed germination and seedling growth of *Brassica juncea* was significantly

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reduced by nickel treatment (Sharma et al., 2008). Excess amount of nickel concentration also accelerates the reactive oxygen species and damages many cellular organelles, DNA, protein, lipids and chlorophyll pigments (Banjac et al., 2021).

The *Brassica* species is known as phylogenetically metal hyper-accumulator. Recently, it has been reported that *Brassica napus* L. has gained a lot of attention among *Brassica* species because it is one of the most common oil-producing and fast-growing crops. The oil produced from this plant has commercial applications due to its long shelf-life and cholesterol reducing properties (Ghobadi et al., 2019). Like several other crops, *B. napus* has the ability to accumulate high concentrations of metals (Angelova et al., 2017). Although *B. napus* is a metal accumulator, its growth and yield are adversely affected at high levels of metals including those of nickel. Moreover, canola germplasm has been screened for tolerance to a multitude of stresses, but it has not been screened for nickel tolerance. Thus, the main objective of the present study was to identify nickel tolerant cultivars from the existing crop germplasm of *Brassica napus*, which may grow well in soils containing high amount of nickel.

Materials and Methods

Screening experiment

The experiment was conducted to determine the tolerance limit of 10 cultivars of canola (Leand, AC Excel, Faisal Canola, Shiralee, Oscar, Punjab Canola, CON-II, Rainbow, Dunkeld, and Cyclone) under different nickel concentrations (0, 15, 30, and 45 mg/L). The seeds of all cultivars of canola were obtained from the Ayub Agricultural Research Institute, Faisalabad, Pakistan. Before sowing, the seeds were surface sterilized with 10% sodium hypochlorite solution for five minutes and then washed three times with distilled water. Ten seeds of each cultivar were used for sowing in each of Petri plates lined with moist filter paper. Different levels of nickel were prepared in Hoagland nutrient solution using $NiSO_4.6H_2O$ as a source of Ni metal. An aliquot (10 mL) of nickel solution from each treatment was applied to each Petri dish at the time of sowing. The solutions of the experimental Petri dishes were changed every day to ensure the constant level of nickel. The experiment was conducted in a growth chamber under continuous white fluorescent light (*PAR* 300 µmol m^{-2} s^{-1}) at 25 $^{\circ}$C. The experiment was set-up in a completely randomized manner with three replicates. Data for different parameters such as seed germination, shoot and root lengths, and fresh and dry weights were collected. The plants were dried at 65 ^{o}C for seventy two hours in an oven and their dry weights recorded. Root and shoot length stress tolerance index (RLSI and SLSI) and fresh and dry matter stress tolerance (FMSI and DMSI), respectively, were calculated according to the following equations/formulae:

PHSI = (Plant height of stressed plants / Plant height of control plants) x 100
RLSI = (Root length of stressed plants / Root length of control plants) x 100
SFSI = (Shoot fresh weight of stressed plants / Shoot fresh weight of control plants) x 100
RFSI = (Root fresh weight of stressed plants / Root fresh weight of control plants) x 100
SDSI = (Shoot dry weight of stressed plants / Shoot dry weight of control plants) x 100
RDSI = (Root dry weight of stressed plants / Root dry weight of control plants) x 100

Statistical analysis

The layout of the experiment was a completely randomized design (CRD) with three replications. The data obtained from the experiment was statistically analyzed (Steel et al., 1997). The Duncan's New Multiple Range test (DMRT) at the 5% level of probability was used to test the difference among the mean values (Steel and Torrie, 1986). Correlation among different variables was calculated following Snedecor and Cochran (1980).

Results

Germination stress tolerance index (GSI) gradually decreased with increase in external nickel concentration and it was 95.58%, 94.52% and 92.41% under 15, 30 and 45 mg/L nickel levels, respectively (**Table 1**). Overall, while categorizing the canola cultivars based on GSI, CON-II was found to be superior followed by Shiralee and Faisal Canola. However, Oscar was the poorest of all cultivars in terms of GSI under varying Ni levels.

Nickel stress inhibited shoot length of all canola cultivars, thereby influencing shoot length stress tolerance index (SLSI) of all canola cultivars (**Table 2**). The SLSI decreased significantly in all cultivars with increase in Ni concentration in the growth medium. Based on average performance of the cultivars in terms of SLSI, CON-II and Faisal Canola were the highest and AC Excel the lowest in SLSI of all cultivars.

Table 1. Germination stress tolerance index (GSI; %) of different canola cultivars

Cultivars	Ni treatment (mg/L)				
	15	30	45	Mean	Ranking
Leand	97.3	96.4	90.0	94.57 bcd	5
AC Excel	95.6	94.3	90.2	93.37 cd	6
Faisal Canola	96.8	94.5	94.0	95.10 bc	3
Shiralee	98.2	96.3	96.0	96.83 ab	2
Oscar	90.2	92.6	85.6	89.47 e	8
Punjab Canola	92.2	92.0	91.3	91.83 de	7
CON-II	98.6	98.0	98.0	98.20 a	1
Rainbow	97.5	92.3	92.0	93.93 cd	6
Dunkeld	94.3	94.2	93.0	93.83 cd	6
Cyclone	95.1	94.6	94.0	94.57 bcd	4

Root length stress tolerance index (RLSI) of all canola cultivars was significantly affected by nickel stress (**Table 3**). There was a consistent decrease in RLSI of all cultivars with increase in external Ni level. The RLSI was the highest in CON-II, Leand and Faisal Canola, whereas it was the lowest in Oscar of all cultivars.

Seedling fresh biomass stress tolerance index (SFSI) was significantly influenced by nickel stress (**Table 4**). Fresh weight gradually decreased by increasing nickel stress in all canola cultivars and the response of the cultivars to Ni stress varied at different Ni levels of the growth medium. Based on average performance of the cultivars in terms of SFSI, CON-II had the maximum SFSI, whereas the minimum was observed in Cyclone and Oscar.

Nickel stress significantly affected seedling dry biomass tolerance indices (SDSI) (**Table 5**). Significant differences among the cultivars were observed in SDSI at different external Ni levels (**Table 5**). Overall, ranking of the cultivars based on SDSI shows that AC Excel, Leand and CON-II were the highest, whereas Oscar being the lowest of all cultivars.

The correlation analysis indicated significant and positive correlations between GSI and SLSI, RLSI, FWSI and DWSI (**Table 6**). The same was the case with SLSI and RLSI, and FWSI and DWSI. Significant and positive correlations were also recorded between RLSI and FWSI and DWSI, and the relationship between FWSI and DWSI was also positive (**Table 6**). The data indicated that the cultivars with high correlation between GSI, SLSI, RLSI, FWSI and DWSI were tolerant to salt stress.

Table 2. Shoot length stress tolerance index (SLSI) of different canola cultivars

Cultivars	Ni treatment (mg/L)				
	15	30	45	Mean	Ranking
Leand	88.56	71.83	58.89	73.09 abc	3
AC Excel	73.04	60.23	48.50	60.59 d	7
Faisal Canola	103.51	70.24	66.42	80.06 a	1
Shiralee	78.09	59.39	59.77	65.75 bcd	5
Oscar	76.03	51.50	43.25	56.92 d	7
Punjab Canola	82.89	65.13	52.72	66.91 bcd	4
CON-II	92.57	81.65	74.70	82.97 a	1
Rainbow	84.03	59.44	54.18	65.88 bcd	5
Dunkeld	51.23	77.56	56.00	61.60 cd	6
Cyclone	83.19	75.49	67.71	75.46 ab	2

Table 3. Root length stress tolerance index (RLSI) of different canola cultivars

Cultivars	Ni treatment (mg/L)				
	15	30	45	Mean	Ranking
Leand	89.90	72.05	79.28	80.41 a	1
AC Excel	69.44	61.34	41.44	57.41 c	4
Faisal Canola	91.86	75.43	61.95	76.41 ab	2
Shiralee	73.00	73.00	55.92	67.31 bc	3
Oscar	41.06	34.02	51.42	42.17 d	5
Punjab Canola	110.71	78.09	54.89	81.23 a	1
CON-II	97.14	81.17	66.80	81.70 a	1
Rainbow	76.63	71.23	45.47	64.44 c	4
Dunkeld	68.04	67.08	40.59	58.57 c	4
Cyclone	66.48	57.02	49.46	57.65 c	4

Table 4. Fresh weight stress tolerance index (FWSI) of different canola cultivars

Cultivars	Ni treatment (mg/L)				
	15	30	45	Mean	Ranking
Leand	55.82	77.44	63.68	65.64 bcd	4
AC Excel	71.51	61.28	35.67	56.15 de	6
Faisal Canola	82.16	70.64	57.54	70.11 ab	2
Shiralee	67.50	68.20	58.11	64.60 bcd	4
Oscar	63.87	48.97	44.24	52.36 e	7
Punjab Canola	69.48	58.12	51.53	59.71 cde	5
CON-II	84.11	77.48	52.92	71.51 a	1
Rainbow	59.86	63.98	47.79	57.21 cde	5
Dunkeld	75.66	64.11	58.62	66.13 bc	3
Cyclone	67.30	53.15	42.64	54.36 e	7

Table 5. Dry weight stress tolerance index (DWSI) of different canola cultivars

Cultivars	Ni treatment (mg/L)				
	15	30	45	Mean	Ranking
Leand	60.58	71.73	62.26	64.85 a	1
AC Excel	70.63	72.53	60.95	68.04 a	1
Faisal Canola	84.34	66.46	37.00	62.60 ab	2
Shiralee	60.78	52.39	37.52	50.23 c	4
Oscar	45.98	35.28	22.01	34.42 d	5
Punjab Canola	58.84	58.04	34.53	50.47 c	4
CON-II	84.11	77.48	52.92	71.51 a	1
Rainbow	53.59	47.46	39.31	46.78 c	4
Dunkeld	57.68	47.51	36.11	47.10 c	4
Cyclone	59.77	51.17	45.15	52.03 bc	3

Table 6. Correlation among different attributes

Variables	GSI	SLSI	RLSI	FWSI
SLSI	0.7096^{*}			
RLSI	0.5748^{NS}	0.7072^{*}		
FWSI	0.7251^{*}	0.6395^{*}	0.7245^{*}	
DWSI	0.6546^{*}	0.6492^{*}	0.6434^{*}	0.5773^{NS}

** = Significant ($P < 0.01$); GSI = Germination stress tolerance index; SLSI = Shoot length stress tolerance index; RLSI = Root length stress tolerance index; DWSI = Dry weight stress tolerance index.

The cluster analysis categorized the 10 canola cultivars into three clusters (**Figure 1**). Clustering of nickel-tolerant (Cluster-1) contained Leand, Faisal Canola and CON-II; moderately nickel-tolerant (Cluster 2) comprised AC Excel, Rainbow, Cyclone, Shiralee, Dunkeld, and Punjab Canola, whereas the susceptible Cluster-3 contained only cv. Oscar. The results suggested that the exploitation of genetic variability for various morphological markers that contribute to nickel tolerance is important for varietal development with considerable nickel tolerance in early and terminal growth stages.

Discussion

High levels of metals and metalloids are known to alter the physiological, biochemical and cellular activities, thereby leading to severe damage to plants resulting in poor growth and productivity (Singh et al., 2015; Noor et al., 2022). Many scientists have reported that tolerance at the adult stage is reflected by the tolerance at the seedling stage of various crops (Zhang et al., 2020). Researchers mostly focus on uncovering the mechanism of metal tolerance in crops, but on the other hand, there is an increasing interest to breed crops to enhance their capacity to absorb and accumulate large quantity of metals in their tissues, so such plants are generally referred to as hyperaccumulators (Souza et al. 2013). However, although nickel at a low concentration is considered as an essential element for higher plants and is used as a functional component of eight different enzymes as well as being very important in nitrogen metabolism in plants (Ragsdale, 2009).

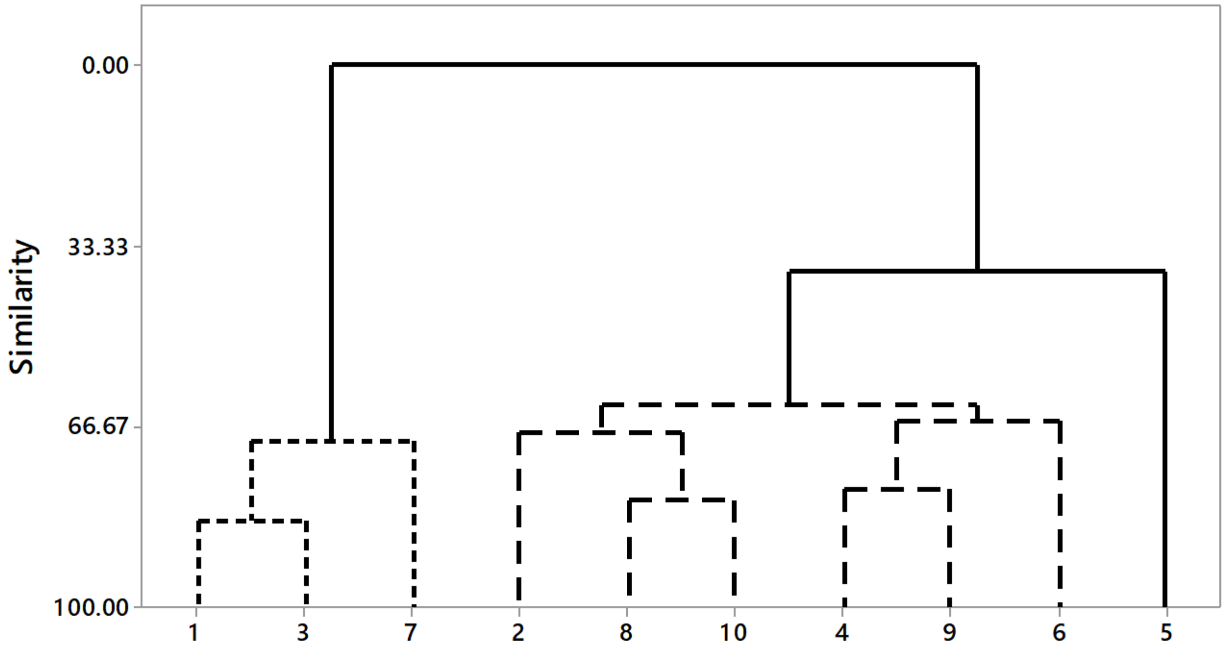

Figure 1. A dendrogram from cluster analysis for nickel tolerance in different canola cultivars based on biomass production. Clusters detail; Cluster 1: Leand, Faisal Canola, and CON-II. Cluster 2: AC Excel, Rainbow, Cyclone, Shiralee, Dunkeld, and Punjab Canola, Cluster 3: Oscar

Overall, Ni stress impeded the initial stage growth of all cultivars appraised in terms of seed germination and seedling growth. However, the response of each cultivar was specific and varied from that of other cultivars examined in terms of different growth indices such as shoot length, root length, and shoot and root fresh and dry weights. While categorizing the cultivars into different categories of Ni tolerance based on different indicators used in the current study, it was evident that cultivars differed significantly in their response to Ni stress, and cultivars such CON-II, Faisal Canola and Leand were superior to all cultivars in terms of Ni tolerance at the early growth stages. However, a question arises as to whether the tolerance observed at the early growth stage would be reflected at the adult growth stages. In this regard, Peralta-Videa et al. (2004) while assessing the effect of different metals on alfalfa at different growth stages, advocated to draw relationships among the effects of different metals on plants at different development stages. Likewise, Krupa and Moniak (1998) recorded significant and positive correlations among the efficiency of the leaf photosynthetic machinery, leaf maturity stage and the Cd sensitivity in rye seedlings. In other experiments with runner bean plants (*Phaseolus coccineus* L.) a positive correlation was recorded between Cu or Cd sensitivity and the plant growth stage (Maksymiec and Baszynski, 1996; Skorzynska-Polit and Baszynski, 1997; Tukendorf et al., 1997).

Overall, the three canola cultivars, i.e., CON-II, Faisal Canola and Leand could be a good source of variation for Ni tolerance provided if they maintain their degree of Ni tolerance at the adult stage.

Conclusion

The findings of this study indicated that physiological indices can be utilized to screen the canola variety for nickel tolerance. Positive and significant correlation among different indices and cluster analysis also proved that canola genotypes can be screened on the basis of physiological indices are nickel tolerant. Tolerant genotypes can directly be recommended for cultivation on nickel-polluted soil.

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This study does not involve Human/animal subjects and no ethical approval is needed.

Handling of bio-hazardous materials
The author(s) certify that all experimental materials were handled with care during collection and experimental procedures. After completion of experiment, all materials were properly discorded to minimize any types of bio-contamination(s).

Availability of primary data and martials
As per editorial policy, experimental materials, primary data or software codes are not submitted to the publisher. These are available with corresponding author and/or with other author(s) as declared by the corresponding author of this manuscript.

Author's consent
All authors contributed in designing and execution of the experiment. All contributors have critically read this manuscript and agree for publishing in IJAEB.

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