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EXOGENOUS APPLICATION OF PROLINE AND GLYCINE BETAINE MITIGATES NICKEL TOXICITY IN MUNG BEAN PLANTS BY UP-REGULATING GROWTH, PHYSIOLOGICAL AND YIELD ATTRIBUTES

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Abstract

Nickel (Ni) toxicity is a serious threat to plant yield, especially when present in the soil, and its amount is increasing with each passing day. This study addresses the mitigation of this toxicity using proline and glycine betaine. The effect of exogenous glycine betaine (50, 100 mM) and proline (30, 60 mM) application on the morpho-physiological and yield attributes of Mung bean [*Vigna radiata* (L.) Wilczek] under varying levels of nickel stress (0, 100, 125, and 150 mg Ni/kg of soil) were investigated in this study. Data for growth and yield parameters were collected after 30, 45, and 65 days, respectively. The results showed that under nickel stress, root length, shoot length, fresh weight, chlorophyll contents, gas exchange characteristics, and yield attributes were reduced compared to the control. Foliar applications of proline (30, 60 mM) and glycine betaine (50 mM) improved all growth parameters, gas exchange characteristics, leaf carotenoid content, and yield attributes. The root length, shoot length, and gas exchange characteristics all correlated positively and significantly. On the other hand, root length, chlorophyll, and photosynthetic rate had a negative correlation. In Ni-stressed plants, the application of 100 mM glycine betaine caused additional damage. The exogenous application of 30 mM proline appeared to be the most effective treatment for nickel toxicity in mung bean plants. The results show that nickel toxicity has a negative impact on mung bean growth and yield, whereas optimal doses of both osmolytes have a high potential to alleviate nickel toxicity. The growth of mung bean was enhanced by foliar sprays of proline and glycine betaine, which improved gas exchange and increased chlorophyll and carotenoid levels. In the future, the identified treatments can optimally be used to obtain a high yield after the resilience development.

Key words: Nickel stress, Mung bean, Osmolytes, Photosynthesis, Transpiration rate.

Introduction

Heavy metals, including nickel (Ni), have contaminated Pakistan's agricultural land. Nickel concentrations exceed permissible limits in many parts of the country (Waseem *et al.*, 2014). Increased Ni toxicity has become a significant concern in recent decades, negatively impacting crop production, and appropriate remedial approaches are urgently needed (Kumar *et al.*, 2021). Ni stress is well known for impairing plant development, yield, photosynthetic pigments (Yusuf *et al.*, 2019), chlorosis and necrosis of leaves (Hassan *et al.*, 2019) and carotenoid content (Selvaraj, 2018). Excess Ni disrupts transpiration, net photosynthetic rate (Shafeeq *et al.*, 2012) and stomatal conductance (Ouzounidou *et al.*, 2006). Nickel toxicity is increasing in agricultural soils worldwide due to anthropogenic activities such as the combustion of fossil fuels, and the manufacture of nickel compounds, alloys, and industrial wastes. Some natural processes, such as rock weathering, also contribute to nickel toxicity in soil and water.

Exogenous application of various compounds can improve stress tolerance in plants. Glycinebetaine and proline are two well-known stress-mitigating compounds. Glycine betaine is a quaternary ammonium compound that is non-toxic, environmental friendly, and water-soluble, and it is found in many plant species at varying concentrations (Kumar *et al.*, 2017). Abiotic stress causes plants to synthesize proline de-novo (Kishor *et al.*, 2015). Both osmolytes are widely used for alleviating heavy metal toxicity in many crops, including Cu-stressed wheat plants (*Triticum aestivum*) (Noreen *et al.*, 2018) and Cd-stressed cotton plants (*Gossypium arboreum*) (Farooq *et al.*, 2016). Exogenous glycine betaine is said to benefit plants in combating abiotic stress by modulating the activity of many enzymatic and non-enzymatic antioxidants (Hasanuzzaman *et al.*, 2014), whereas proline functions as a free radical scavenger and stabilizes cellular integrity and structure (Hossain *et al.*, 2015). It reduces cytoplasmic acidosis and maintains an appropriate $NAPD^+$/NADPH ratio that are well-suited to metabolism (Zali & Ehsanzadeh, 2018).

Mung bean [*Vigna radiata* (L.) Wilczek] is an effective pulse produced and consumed in Pakistan. Mung bean is sensitive to Ni stress, as evidenced by reduced growth, necrosis, and chlorosis of leaves, as well as decreased photosynthetic pigments and productivity (Ali *et al.*, 2015). Only a few studies have been conducted to determine the effects of proline and glycine betaine on mung bean at the seedling stage (Hossain & Fujita, 2010). However, no comprehensive study of the effects of exogenous proline and glycine betaine at different stages of development has been conducted, and the mechanisms underlying their protective function have yet to be discovered. Therefore, this study aimed to examine the effect of proline and glycine betaine on mung bean growth, physicochemical properties, and yield at the seedling, vegetative, and reproductive stages when exposed to nickel stress.

Materials and Methods

Experimental details and treatments

Experimental Site: The experiment was conducted in a net house of the Department of Botany at the University of Sargodha in Sargodha, Pakistan, between July and September of the 2017-2018. Mung bean seeds (NM-2006) were obtained from the Ayub Agricultural Research Institute (AARI), Faisalabad. Nine surface sterilized seeds were directly sown in 30 cm diameter and 35 cm high glazed pots containing 10 kg of sandy loam soil. Following germination, three healthy and uniform seedlings were retained in each pot with three replicates in a completely randomized design. Plants were supplied a half–strength nutrient solution (Hoagland & Arnon, 1950) at all stages of growth. The Soil and Water Testing Laboratory for Research, Sargodha Department of Agriculture, Government of Punjab, Pakistan, conducted soil and water analysis and determined that it was suitable for agricultural use.

Treatments: To induce different levels of toxicity, three doses of Ni (0,100, 125, and 150 mg/kg of soil) in the form of $NiCl_2$ were applied to the soil before sowing. Tween-20 (0.1%) was used as a surfactant, and foliar sprays of glycine betaine (50 and 100 mM) and proline (30 and 60 mM) were applied after 20 and 30 days of germination, respectively. Irrigation was done with tap water as needed.

Plant harvesting: The plants were harvested after 45 days for physiochemical and morphological examination. The shoot length was measured with a meter rod from the base of the shoot to the tip of the root, while the root length was measured with a meter rod from the base of the shoot to the tip of the root. Plant samples were washed with water, blot dried, and their fresh weight was recorded. Both parts were oven-dried separately for seven days at 70°C until they reached a consistent weight, and the dry weights were recorded. A meter rod was used to measure the plant's height from where the root begins to grow to the leaf base of the fully extended highest leaf. Each plant's leaves were counted, and the leaf area meter was used to measure the leaf area (cm^2). An infrared Gas Analyzer (LI-6800) was used to determine the photosynthetic rate (Pn) and transpiration rate (E) of leaves. The Davis (1976) method was used to calculate carotenoids and Arnon (1949) method for total chlorophyll. At maturity, 15 plants were harvested to examine yield characteristics such as the number of pods per plant, seeds per pod, and total seed yield per plant (g). A total of 100 seeds were chosen at random for a total weight of 100 grains.

Statistical analysis: For statistical analysis, SPSS software version SPSS-21 was used to analyze the results. Using randomized complete block design (RCBD), two-factor analysis of variance was applied, and as post-hoc, the Tukey HSD (honestly significant difference) test was used. The standard deviation was calculated, and the validity of the data was recorded at a 5% probability level. Correlation analysis was done by using the MINITAB 17.

Results

Plants supplemented with different levels of Ni (100, 125, and 150 mg Ni/kg soil) showed concentration-dependent reduction in growth parameters (root and shoot length, leaf area, and number of leaves). Exogenous application of 30 mM proline completely neutralized the toxicity of plants treated with 100 mg Ni/kg of soil and partially neutralized the toxicity of plants treated with 125 and 150 mg Ni/kg of soil over control plants out of all doses of glycine betaine (50, 100 mM) and proline (30, 60 mM). This was followed by proline at 60 mM and glycine betaine at 50 mM. All growth parameters were reduced after foliar application of 100 mM glycine betaine. Plants treated with 125 mg Ni/kg soil had mild necrotic spots on the leaves, while plants treated with 150 mg Ni/kg soil had more necrotic spots (Table 1).

Carotenoid and total chlorophyll content decreased linearly as soil Ni levels increased. At 45 days of growth, plants with the highest Ni levels (150 mg Ni/kg soil) showed the greatest reduction, with carotenoids and chlorophyll contents reduced by 18.53% and 14.11% less than control plants, respectively. On the other hand, exogenous application of 30 mM proline resulted in the greatest restoration of their levels at the 45-day growth stage compared to their respective controls. This pattern was followed by 60 mM proline and 50 mM glycine betaine, resulting in partial restoration. However, 100 mM glycine betaine reduced their levels in all Ni-stressed plants (Table 2).

Exogenous application of 30 mM proline resulted in a complete restoration of the rate of transpiration (E) and net photosynthetic rate (Pn) in plants treated with 100 mg Ni/kg soil at the 45-day growth stage. This trend was followed by 60 mM Proline and 50 mM glycine betaine, except for 100 mM glycine betaine, which caused further reductions in gas exchange parameters (Table 3).

Exogenous application of both doses of proline and 50 mM glycine betaine significantly increased yield attributes (number of pods per plant, number of seeds per pod, seed yield per plant, 100 grains weight). The application of 30mM proline resulted in the restoration of all yield attributes in plants with lower Ni stress (100 mg/kg) and partially in plants with higher Ni stress (125 and 150 mg/kg). Plants treated with 150 mg Ni/kg soil and 100 mM glycine betaine, on the other hand, showed the maximum reduction in all yield attributes (Table 4).

The correlation data revealed that shoot length, root length, and gas exchange characteristics had significant and positive correlations with growth and morpho-physiological and yield attributes. A negative and non-significant correlation was found between seed yield per plant and the number of seeds per pod and carotenoids (Table 5).

Table 1. Effect of treatment of glycine betaine (GB) and proline on growth attributes of mung bean plants.

Treatment	Nickel				Mean
	0 mg/kg	100 mg/kg	125 mg/kg	150 mg/kg	
	Shoot length (cm)				
Control	41.67 ± 0.15b	39.67 ± 0.42cde	35.17 ± 0.63ij	31.33 ± 0.52m	36.96 ± 1.23c
30 mM P	43.00 ± 0.44a	41.67 ± 0.42b	38.50 ± 0.30ef	35.33 ± 0.65ij	39.63 ± 0.92a
60 mM P	42.00 ± 0.32ab	40.00 ± 0.28cd	36.83 ± 0.34gh	34.67 ± 0.29jk	38.38 ± 0.86b
50 mM GB	40.42 ± 0.13c	39.17 ± 0.56de	36.67 ± 0.20gh	33.67 ± 0.56kl	37.48 ± 0.80c
100 mM GB	41.67 ± 0.25b	37.50 ± 0.30fg	36.17 ± 0.48hi	32.50 ± 0.58lm	36.96 ± 1.00c
Mean	41.75 ± 0.25a	39.60 ± 0.39B	36.67 ± 0.33c	33.50 ± 0.44d	
	Root length (cm)				
Control	7.12 ± 0.03bc	5.57 ± 0.14ghi	5.38 ±0.13hi	4.12 ± 0.10lm	5.55 ± 0.32d
30 mM P	8.28 ± 0.09a	6.88 ± 0.10c	6.02 ± 0.12ef	4.72 ± 0.05j	6.48 ± 0.39a
60 mM P	7.22 ± 0.09b	6.30 ± 0.11de	5.77 ± 0.05fg	4.58 ± 0.04jk	5.97 ± 0.29b
50 mM GB	7.03 ± 0.12bc	6.13 ± 0.12e	5.67 ± 0.13gh	4.35 ± 0.10kl	5.80 ± 0.30c
100 mM GB	6.43 ± 0.17d	5.83 ± 0.08fg	5.33 ± 0.08i	4.03 ± 0.10m	5.41 ± 0.27d
Mean	7.22 ± 0.17a	6.14 ± 0.13b	5.63 ± 0.08c	4.36 ± 0.08d	
	Number of leaves per plant				
Control	21.17 ± 0.07b	18.50 ± 0.52de	15.17 ± 0.43i	13.17 ± 0.10j	17.00 ± 0.94c
30 mM P	23.17 ± 0.08a	20.17 ± 0.07c	18.17 ± 0.08ef	16.17 ± 0.09h	19.42 ± 0.78a
60 mM P	21.67 ± 0.40b	18.95 ± 0.29d	16.83 ± 0.50gh	15.17 ± 0.09i	18.16 ± 0.75b
50 mM GB	21.67 ± 0.32b	18.90 ± 0.34de	16.67 ± 0.13h	15.17 ± 0.19i	18.10 ± 0.75b
100 mM GB	20.00 ± 0.06c	17.50 ± 0.25fg	15.17 ± 0.11i	12.50 ± 0.29j	16.29 ± 0.84d
Mean	21.54 ± 0.29a	18.80 ± 0.26B	16.40 ± 0.32c	14.44 ± 0.37d	
	Leaf area per plant (cm^2)				
Control	6.52 ± 0.12	6.43 ± 0.11	6.12 ± 0.10	5.70 ± 0.06	6.19 ± 0.11bc
30 mM P	6.85 ± 0.10	6.55 ± 0.09	6.35 ± 0.06	5.98 ± 0.03	6.43 ± 0.10a
60 mM P	6.72 ± 0.09	6.38 ± 0.09	6.23 ± 0.16	5.92 ± 0.07	6.31 ± 0.10ab
50 mM GB	6.60 ± 0.02	6.38 ± 0.20	6.22 ± 0.14	5.88 ± 0.09	6.27 ± 0.10bc
100 mM GB	6.52 ± 0.10	6.32 ± 0.09	5.98 ± 0.09	5.77 ± 0.05	6.15 ± 0.09c
Mean	6.64 ± 0.05a	6.41 ± 0.05b	6.18 ± 0.05c	5.85 ± 0.04d	

Data is the mean of three replicates. The means sharing similar letters in a row or a column are statistically non-significant (p>0.05). Small letters represent comparison among interaction means, and capital letters are used for overall mean, P= Proline, GB= Glycine Betaine

Table 2. Effect of treatment of glycine betaine (GB) and proline on total chlorophyll and carotenoids.

Treatment	Nickel				Mean
	0 mg/kg	100 mg/kg	125 mg/kg	150 mg/kg	
	Total chlorophyll (mg/g of tissue)				
Control	0.163 ± 0.002c	0.160 ± 0.003cd	0.147 ± 0.002g	0.140 ± 0.002h	0.153 ± 0.003b
30 mM P	0.183 ± 0.003a	0.160 ± 0.002cd	0.150 ± 0.003fg	0.150 ± 0.002fg	0.161 ± 0.004a
60 mM P	0.180 ± 0.003a	0.157 ± 0.002de	0.150 ± 0.002fg	0.149 ± 0.002fg	0.159 ± 0.004a
50 mM GB	0.170 ± 0.003b	0.153 ± 0.002ef	0.150 ± 0.001fg	0.148 ± 0.002fg	0.155 ± 0.003b
100 mM GB	0.170 ± 0.002b	0.153 ± 0.002ef	0.147 ± 0.002g	0.147 ± 0.003g	0.154 ± 0.003b
Mean	0.173 ± 0.002a	0.157 ± 0.001b	0.149 ± 0.001c	0.147 ± 0.001c	
	Total carotenoids (mg/g of tissue)				
Control	0.340 ± 0.003	0.320 ± 0.006	0.290 ± 0.003	0.277 ± 0.002	0.307 ± 0.008b
30 mM P	0.357 ± 0.006	0.313 ± 0.004	0.297 ± 0.003	0.287 ± 0.006	0.313 ± 0.008a
60 mM P	0.347 ± 0.003	0.307 ± 0.003	0.290 ± 0.006	0.286 ± 0.003	0.307 ± 0.007ab
50 mM GB	0.343 ± 0.003	0.303 ± 0.006	0.287 ± 0.007	0.285 ± 0.003	0.305 ± 0.007b
100 mM GB	0.340 ± 0.003	0.300 ± 0.006	0.287 ± 0.003	0.282 ± 0.004	0.302 ± 0.007b
Mean	0.345 ± 0.002a	0.309 ± 0.003b	0.290 ± 0.002c	0.283 ± 0.002d	

Data is the mean of three replicates. Means sharing similar letters in a row or a column are statistically non-significant (p>0.05). Small letters represent comparison among interaction means, and capital letters are used for overall mean, P= Proline, GB= Glycine Betaine

Table 3. Effect of treatment of glycine betaine (GB) and proline on gas exchange parameters.

Treatment	Nickel				Mean
	0 mg/kg	100 mg/kg	125 mg/kg	150 mg/kg	
	Transpiration rate (mmol m^{-2} s^{-1})				
Control	11.66 ± 0.16abc	10.87 ± 0.11d	9.08 ± 0.08g	8.04 ± 0.16h	9.91 ± 0.43d
30 mM P	11.44 ± 0.16cd	12.21 ± 0.21a	10.88 ± 0.12d	9.19 ± 0.06fg	10.93 ± 0.34a
60 mM P	11.00 ± 0.44d	12.03 ± 0.05ab	10.26 ± 0.06e	8.97 ± 0.09g	10.57 ± 0.35b
50 mM GB	11.10 ± 0.32cd	11.45 ± 0.02bcd	9.74 ± 0.13ef	8.74 ± 0.10g	10.26 ± 0.34c
100 mM GB	11.05 ± 0.09d	11.00 ± 0.09d	8.96 ± 0.56g	8.69 ± 0.19g	9.93 ± 0.36d
Mean	11.25 ± 0.12a	11.51 ± 0.15a	9.78 ± 0.22b	8.73 ± 0.11c	
	Photosynthetic rate (µmol m^{-2} s^{-1})				
Control	13.09 ± 0.12bcd	12.82 ± 0.09cd	10.67 ± 0.17i	9.72 ± 0.21j	11.58 ± 0.43c
30 mM P	13.08 ± 0.30bcd	15.46 ± 0.09a	12.51 ± 0.16def	10.77 ± 0.17hi	12.96 ± 0.51a
60 mM P	12.94 ± 0.57bcd	13.50 ± 0.26b	11.39 ± 0.25gh	10.45 ± 0.21i	12.07 ± 0.40b
50 mM GB	12.72 ± 0.36cde	13.16 ± 0.07bcd	12.14 ± 0.21ef	9.76 ± 0.16j	11.94 ± 0.41b
100 mM GB	13.30 ± 0.26bc	12.00 ± 0.18fg	10.60 ± 0.24i	9.10 ± 0.13j	11.25 ± 0.48c
Mean	13.03 ± 0.14b	13.39±0.31a	11.46 ± 0.22c	9.96 ± 0.17d	

Data is mean of three replicates. Means sharing similar letter in a row or in a column are statistically non-significant (p>0.05). Small letters represent comparison among interaction means and capital letters are used for overall mean, P= Proline, GB= Glycine Betaine

Table 4. Effect of treatment of glycine betaine (GB) and proline on yield parameters of mung bean.

Treatment	Nickel				Mean
	0 mg/kg	100 mg/kg	125 mg/kg	150 mg/kg	
	No. of pods /plant				
Control	14.67 ± 0.18a	12.67 ± 0.16c	9.67 ± 0.10h	8.67 ± 0.22i	11.42 ± 0.72d
30 mM P	14.67 ± 0.16a	13.67 ± 0.19b	12.67 ± 0.16c	12.40 ± 0.17cd	13.35 ± 0.28a
60 mM P	14.70 ± 0.14a	12.90 ± 0.16c	12.00 ± 0.13de	12.00 ± 0.30de	12.90 ± 0.34b
50 mM GB	12.50 ± 0.27cd	12.67 ± 0.21c	11.67 ± 0.13e	11.67 ± 0.20e	12.13 ± 0.16c
100 mM GB	11.00 ± 0.17f	10.67 ± 0.07fg	10.40 ± 0.23g	8.72 ± 0.16i	10.20 ± 0.27e
Mean	13.51 ± 0.41a	12.52 ± 0.27b	11.28 ± 0.30c	10.69 ± 0.45d	
	No. of seeds /pod				
Control	6.67 ± 0.21a	6.00 ± 0.13de	5.00 ± 0.12h	4.00 ± 0.13j	5.42 ± 0.31c
30 mM P	6.70 ± 0.17a	6.67 ± 0.22a	6.45 ± 0.09abc	6.00 ± 0.14de	6.46 ± 0.11a
60 mM P	6.62 ± 0.15ab	6.20 ± 0.16bcd	5.67 ± 0.13ef	5.67 ± 0.17ef	6.04 ± 0.14b
50 mM GB	6.69 ± 0.06a	6.17 ± 0.13cd	5.45 ± 0.18fg	5.03 ± 0.12gh	5.83 ± 0.20b
100 mM GB	5.89 ± 0.08de	4.90 ± 0.06hi	4.67 ± 0.13hi	4.50 ± 0.24i	4.99 ± 0.17d
Mean	6.51 ± 0.10a	5.98 ± 0.14b	5.44 ± 0.13c	5.04 ± 0.21d	
	100 grains weight (g)				
Control	2.16 ± 0.05de	1.79 ± 0.02gh	1.29 ± 0.03j	1.08 ± 0.06kl	1.58 ± 0.13d
30 mM P	2.45 ± 0.03a	2.29 ± 0.02bc	2.00 ± 0.05f	1.83 ± 0.04g	2.14 ± 0.07a
60 mM P	2.38 ± 0.01ab	2.12 ± 0.05ef	1.83 ± 0.04g	1.43 ± 0.03i	1.94 ± 0.11b
50 mM GB	2.25 ± 0.03cd	2.06 ± 0.07ef	1.73 ± 0.03gh	1.30 ± 0.01j	1.84 ± 0.11c
100 mM GB	2.10 ± 0.07ef	1.68 ± 0.05h	1.19 ± 0.05jk	1.00 ± 0.03l	1.49 ± 0.13e
Mean	2.27 ± 0.04a	1.99 ± 0.06b	1.61 ± 0.09c	1.33 ± 0.08d	
	Seed yield /plant				
Control	2.24 ± 0.03bc	1.75 ± 0.04f	1.20 ± 0.02i	1.02 ± 0.03l	1.55 ± 0.15c
30 mM P	2.35 ± 0.06a	2.00 ± 0.05d	1.39 ± 0.05h	1.20 ± 0.02i	1.74 ± 0.14a
60 mM P	2.28 ± 0.05ab	1.90 ± 0.02e	1.30 ± 0.02h	1.14 ± 0.05ijk	1.66 ± 0.14b
50 mM GB	2.20 ± 0.03bc	1.84 ± 0.02ef	1.15 ± 0.02ij	1.08 ± 0.05jkl	1.57 ± 0.14c
100 mM GB	2.18± 0.01c	1.59 ± 0.01g	1.05 ± 0.03kl	0.99 ± 0.02l	1.45 ± 0.15d
Mean	2.25 ± 0.02a	1.82 ± 0.04b	1.22 ± 0.03c	1.09 ± 0.02d	

Data is mean of three replicates. Means sharing similar letter in a row or in a column are statistically non-significant (p>0.05). Small letters represent comparison among interaction means and capital letters are used for overall mean, P= Proline, GB= Glycine Betaine.

Table 5. Correlation among different morpho-physiological and yield attributes of mung bean.

	Shoot length	Root length	Root fresh weight	No. of leaves/Plant	Leaf area	Total chl.	Carotenoids	Transpiration rate	Photosynthetic rate	No. of pods /plant	No. of seeds /pod	100 grains weight	Seed yield /plant
Shoot length	1												
Root length	0.946*	1											
No. of leaves/plant	0.966*	0.960*	0.924*	1									
Leaf area	0.972**	0.968**	0.910	0.973**	1								
Total chlorophyll	-0.863	0.866**	0.753	-0.903	0.890**	1							
Carotenoids	0.892*	-0.883	0.782**	-0.928	0.901**	0.966	1						
Transpiration rate	0.918**	0.850*	0.903	-0.871	0.889	0.672**	0.720**	1					
Photosynthetic rate	-0.910	0.829**	-0.907	0.849	-0.869	0.645	0.683*	0.944*	1				
No. of pods /plant	0.807	-0.773	0.816*	0.845**	0.783**	-0.711	0.695*	0.739	0.721**	1			
No. of seeds /pod	0.708*	-0.755	0.729**	0.743*	-0.725	0.620*	0.610**	-0.592	0.632	0.764*	1		
100 grains wt.	0.940*	0.908**	-0.864	-0.956	0.930**	0.909**	0.954*	-0.865	0.825*	0.738	0.608*	1	
Seed yield /plant	0.930	0.903	0.909*	0.955	0.930*	0.818	-0.815	0.892*	0.883**	0.869	-0.792	0.893*	1

**= Significant ($p<0.01$); shoot length, root length, no. of leaves per plant, lead area, total chlorophyll, carotenoids, transpiration rate, photosynthetic rate, no. of pods per plant, no. of seeds per pod, 100 grain weight and seed yield per plant

Discussion

The results of this study clearly showed that morphological parameters, gas exchange characteristics, and physiological and yield attributes were reduced in mung bean crops at varying nickel concentrations (0, 50, 100, 150 mg/kg of soil) (Tables 1-5). Exogenous application of proline (30 and 60 mM) and glycine betaine (50 mM) improved these morpho-physiological and yield parameters of mung bean. Nickel stress reduced the root/shoot length and decreased the fresh weight of all mung bean plants (Table 1). These findings are consistent with the findings of Nawaz & Ashraf (2019) in abiotically stressed mung bean plants. However, this decrease in growth characteristics is linked to a higher Ni concentration in the soil. Excess Ni in the soil can disrupt plant morphological parameters (root and shoot biomass) (Ali *et al*., 2015) by lowering cell membrane permeability and cell division rates (Khan *et al*., 2019). The number of leaves and leaf area were also reduced in all mung bean plants. A decrease in chlorophyll and carotenoid content was also observed in the NM-2006 mung bean variety (Table 2).

Improved plant growth with exogenous glycine betaine under stress may be attributed to increased nutrient uptake in aerial parts (Shahbaz & Zia, 2012). Since it is primarily found in chloroplasts, it increases photosynthetic efficiency by causing less damage to the chloroplast ultrastructure and thylakoid membrane (Wang *et al*., 2010). The increased photosynthetic rate is due to its role in stabilizing the extrinsic PSII complex protein link (Sakamoto & Murata, 2002). It maintains CO_2 fixation by protecting CO_2-fixing enzymes like Rubisco, resulting in reduced ROS generation. However, overall accumulation of glycine betaine reduces photo-inhibition of PSII, which may have exacerbated plant stress conditions.

Nickel stress causes yield loss in mung bean plants due to decreased growth, reduced gas exchange attributes, and a low photosynthetic rate (Table 4). However, applying proline and glycine betaine favoured all of these characteristics, ultimately improving mung bean plant yield. The beneficial effect of glycine betaine on yield could also be attributed to its role in reducing Ni uptake to plant aerial parts (Xalxo *et al*., 2017) and protecting cell membranes (Giri, 2011). Exogenous glycine betaine application may have resulted in intracellular glycine betaine accumulation (Zhang *et al*., 2014), which can alleviate stress by degrading to the nitrogen pool in plants (Ashraf & Foolad, 2007). Exogenous proline also has a protective role in maintaining osmotic adjustment, preserving ionic homeostasis, effectively scavenging ROS, stabilizing antioxidant enzymes that minimize oxidative damage, increasing the photosynthetic rate of the plant, and improving growth and yield (Zouari *et al*., 2019).

Conclusion

Exogenous proline and glycine betaine spray can alleviate nickel toxicity in mung bean plants exposed to 100 mg Ni/kg of soil, but only in a dose-dependent manner. Under oxidative stress, 30 mM proline can be used to up-regulate mung bean plants' growth, physiological, and yield attributes. These findings can help farmers in planting in Ni toxic soils so that an ample yield can be obtained using our reported methods. As a result of this, we recommend that the application of glycine betaine spray be used to mitigate this abiotic stress. These methods can be validated, modified, and applied in the future to get more benefits.

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