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RESEARCH

Differential Role of Silicon and *Rhizoglomus intraradices* in Modulating Amide and Ureide Metabolism of Seasonally Different Legume Species Subjected to Nickel Toxicity

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Abstract
Nickel (Ni) toxicity disrupts the cellular processes and is detrimental for plant growth and development. Silicon (Si) and AMF (arbuscular mycorrhizal fungi) alleviate heavy metal stress induced toxic responses in plants. Although Fabaceae members are considered low Si accumulators, however, variations in their ability for Si uptake have been reported. Present study compared the individual and cumulative roles of Si and AM (*R. irregularis*) in alleviating Ni (150 mg/kg) toxicity in three seasonally different legume species namely chickpea (HC3), mungbean (Pusa-9531) and pigeonpea (Pusa-2002). Presence of Ni in the root rhizosphere declined growth, nitrogen fixing ability, N-assimilation and yield attributes, with chickpea displaying highest sensitivity, mungbean showcasing moderate tolerance, while pigeonpea having maximum resistance against metal stress. AM and Si were highly beneficial in mitigating the toxic effects of Ni especially in pigeonpea followed by mungbean and chickpea. The higher beneficial effects of AM could be related to its ability in improving soil enzymatic activities, nutrient availability and reduced metal uptake. Moreover, AM symbiosis complemented rhizobial symbiosis by improving nodulation potential, trehalose turnover, thus leading to higher ammonia assimilation, ureide and amide synthesis as well as their transport. Interestingly, mycorrhization significantly induced Si uptake and therefore, their co-applications (+ Si + AM) proved to be most effective in alleviating Ni toxicity in all three legume species with maximum positive impacts displayed by pigeonpea. Hence, study suggested the need of exploring more legume species having an ability to uptake Si and establish efficient AM symbiosis in order to reduce Ni toxicity.

Keywords Nickel · Silicon · Arbuscular mycorrhizal fungi · Legumes · Amide · Ureide

1 Introduction

Heavy metals (HMs) are considered as one of the main threats due to their non-degradable nature and deposition into the agricultural soils [1]. Among the HMs, Nickel (Ni), a trace metal, is considered as 22nd extensively present element on earth's crust [2]. Ni-contaminated areas in the world include, Canada, Australia, Indonesia, South Africa, North America as well as India [3–6]. At low concentration i.e., 0.05–10 mg/kg dry weight, it acts as an essential element for plant growth as it is a fundamental part of enzyme namely urease that plays a key role in nitrogen (N) metabolism in higher plants [7]. However, excess of Ni acts as a potential phyto-toxic metal and can adversely affect normal functioning of plant cells. High levels of Ni hinder multiple processes inside the plants such as inhibition of photosynthetic activity, generation of reactive oxygen species (ROS), decline in dry mass production, leaf necrosis and chlorosis [8] as well asrespiration, mineral acquisition (Cu, Fe, Mn and Zn) [9, 10] and transport of assimilates [11]. In legumes Ni restricts the formation of nodules and decreases their number leading to decline in nitrogen fixation affecting the overall crop productivity [12, 13]. Nickel enters the environment through factory waste of ferrous and nonferrous metallurgy, cement clinker production, mine tailings and metallurgical factories, organic and mineral fertilizers and pesticides [14]. In polluted soils, the uptake of Ni is chiefly carried out via passive diffusion (through cation channels via Mg ion transport system) and active transport through the root system of plants [15]. The overall uptake of Ni by plants depends on the concentration of Ni^{2+}, the acidity of

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soil, occurrence of other metals and plant metabolism [16]. Furthermore, Ni^{2+} may also compete with other important metal ions (Fe^{3+}, Co^{2+}, Ca^{2+}, Mg^{2+}, NH_4^+, K^+, Na^+) when absorbed by roots [17]. Almost 50% of Ni that is absorbed by the plant is stored in the roots thus inhibiting their growth to a larger extent than shoots [18]. This retention occurs due to the sequestration of Ni at cation exchange sites of vessel walls and xylem parenchyma [15].

In response to these stresses, plants exhibit complex, quantitative traits that include accumulation of low-molecular weight organic compounds, known as compatible solutes or osmolytes. Trehalose (Tre) is one such compound, which bulks under several abiotic stresses in many organisms, including bacteria, yeast, plants, and invertebrates [19]. Trehalose biosynthesis involves two enzymes namely, trehalose-6-phosphate synthase (T6PS) and trehalose-6-phosphatase (T6PP). On the other hand, Trehalase (TRE) is the enzyme that hydrolyses trehalose into two glucose residues [20]. Trehalose safeguards the membrane and acts as a chemical chaperone, preventing acetylation and glycation of protein under stress [21]. Interestingly, symbiotic nitrogen-fixing bacteria such as rhizobium and related genera can synthesize trehalose under unfavourable conditions for protection against desiccation and osmotic shock [22]. Its accumulation has been found in *Medicago trunculata*[23]) and chickpea nodules [24]under salt stress. Inoculation of *Rhizobium etli* strains in *Phaseolus vulgaris*, led to the overexpression of T6PS and improved number of nodules and N fixing potential [25]. Moreover, various studies have also indicated that rhizobial trehalose plays an important role in improving growth and yield of legumes and helps in adaptation towards abiotic stress [26–28].

Legumes are an important source of food, nutrition across the world, as they provide essential nutrients including protein, minerals anddietary fibre because of their ability to fix atmospheric N_2 in their nodules [29]. The fixed nitrogen can be exported to the aerial parts in form of amides and ureides depending upon the plant species, with *Cicer arietinum* L. (chickpea) exporting amides (glutamine-Gln, asparagine -Asn) while others such as *Vigna radiata* L. (mungbean) and *Cajanus cajan* L. (pigeonpea) as ureides (allantoin and allantoic acid) [30]. Ureide synthesis takes place with the help of xanthine dehydrogenase (XDH) that converts xanthine into uric acid which is then oxidised by uricase into allantoin (ALN). ALN is the biologically active form in plants, that is converted into allantoic acid (ALA) by allantoinase (ALNase) enzyme. ALA is further transported to the leaves where it is converted into urea and ammonia through the enzyme urease. In case of amide synthesis two key enzymes, aspartate aminotransferase (AspAT) and asparagine synthetase (AS) are involved that lead to Asn-synthesis. The ureide and amide synthesis take place in conjunction with the enzymes namely,glutamine synthetase (GS) and glutamate synthase (GOGAT), collectively called as the GS-GOGAT pathway. The synthesis of ureides and amides have been reported to impart tolerance in legumes under abiotic stress [31, 32].

In the recent years, application of (Si) has come to light as one of the effective approaches to mitigate abiotic stresses [33, 34]. Si is the second most abundant element after oxygen in the earth's crust [35] and is regarded as a quasi-essential element as it is useful for overall development of higher plants. The range of Si content could vary from 1 to 45% in soil dry weight and it can further accumulate or redistribute in soil during soil development [36]. Siis absorbed indirectly and usually in the form of soluble silicic acid [$Si(OH)_4$] [37] that is normally present at a concentration of 0.1–0.6 mM in soil solution (pH 1–9) [38]. When $Si(OH)_4$ is transported to the shoot, it isfurther polymerized to form colloidal $Si(OH)_4$ through loss of water (transpiration) and finally into silica gel [39]. In plants, concentration of Si can greatly vary among different species and genotypes ranging from 0.1 to 10.0% of dry weight in above ground plant parts [40]. This wide variation is chiefly because of disparities in the processes that involve uptake and transportation of Si [41]. These processes are described as active, passive, or rejective andbased on them plants are known as high-, intermediate-, or non-silicon accumulators [42]. In general, Poaceae members absorb more Si than other species, while legumes are considered low silicon accumulators as most of the Si uptake occurs passively [43–45]. Tansportation of Si is facilitated by membrane intrinsic proteins, namely aquaporins (AQP), more specifically NIP2s (NIP-III) which are known to transport Si in high-Si accumulator species. The presence of NIP-IIIs is strongly correlated to enhanced uptake of Si in plants [39, 46, 47]. Ma et al. [39] found Si transporters in rice (Lsi1 (OsNIP2.1) and Lsi6 (OsNIP2.2)), and later in maize, barley, wheat, soybean, cucumber and pumpkin. Moreover, based upon the presence of CcNIP2-1 [48–50] pigeonpea has also been found to be a Si accumulator species. Till date, except for soyabean and pigeonpea, Si transporters have not been identified in any other legume species, indicating towards the variability among them for uptake of silicon and its positive impacts.Si acts as a physical barrier in plants due to its deposition in the form of silica gel leading to hinderance in metal uptake and [51], thereby improving their growth and yield due to enhanced photosynthetic rate, increased water status, higher osmotic adjustment and lowered transpiration [52]. While Si is recognized for its role in imparting tolerance towards stress, very less is known about its positive role on N_2-fixation of legumes in response to metal stress.

Legumes can form a unique symbiotic relationship with arbuscular mycorrhizal fungi (AMF) and that can enhance plant fitness under environmental stresses [53, 54] by improving the availability of inorganic nutrients specifically

phosphorus (P) and nitrogen (N) [55, 56]) by stimulating the activities of enzymes such as phosphatases (PHAs)and urease (URE) even in contaminated soil [57]. AMF inoculated pea plants subjected to arsenic As(V) displayed high chlorophyll content, Mg and relative water content (RWC), thus proving the role of AM symbiosis in sustaining Chl biosynthesis, lower leaf chlorosis and high turgor [58, 59]. Moreover, AMF can stimulate the uptake and accumulation of Si as reported in mycorrhiza-inoculated plants like *Glycine max* [60]), *Zea mays* [61], and *Cajanus cajan*[62, 63]. Hammer et al. [64] also observed increased content of Si in *Rhizophagus irregularis* (spores and hyphae) and its transfer to the roots of *Acacia cyanophylla*. In addition, Si nutrition has also been reported to enhance AMF symbiosis by reducing the main released phenolics as well as polymerization and lignin synthesis in strawberry [65]. Furthermore, mycorrhizal symbiosis can also improve trehalose production by stimulating enzymes involved in its metabolism leading to trehalose accumulation [66–68], thus imparting stress tolerance.

India is a major producer of important legumes accounting for 9.075 million tonnes of chickpea, 15.91 lakh tonnes of mungbean and contributing 28.66 lakh tonnes of world's pigeonpea production [69, 70]. As these legumes are seasonally different, their sensitivity towards nickel stress varies, along with the differences in their response towards Sinutrition and AMF colonization. Although, positive role of Si and AMF has been reported but a comparative insight among these legumes needs to be investigated in order to compare the benefits provided in a species-specific manner. Thus, this study aimed to investigate the functional complementarity between Si and AMF in imparting Ni tolerance among three legume species.

The objectives of study were to investigate the: (1) effect of Ni toxicity on growth, yield, rhizobial symbiosis, ammonia assimilation and trehalose metabolism in chickpea, mungbean and pigeonpea (2) relative and cumulative roles of Si and AMF in alleviating Ni stress by regulating amide and ureide metabolism (3) role of Si and AMF in regulating soil enzyme activities and nutrient bioavailability in the Ni stressed rhizosphere soils of legume species.

2 Material and Methods

2.1 Plant Materialand Biological Inoculants

Seeds of *C. arietinum* were obtained from Haryana Agriculture University (HAU), Hisar, India and *V. radiata*, *C. cajan* from Pulse Laboratory, Indian Agriculture Research Institute (IARI), New Dehli, India. Tolerant genotypes of *C. arietinum* (HC3), *V. radiata* (Pusa-9531), *C. cajan* (Pusa-2002) were selected based on screening experiments and were used for further research purpose. Specific rhizobial strains for chickpea- *Mesorhizobiumciceri* PF:75, mungbean-*Rhizobium radiobacter* VBCK1062, pigeonpea-*Sinorhizobium fredii*AR-4 were obtained from Department of Microbiology, IARI, New Delhi. Pure spores of AMF-*Rhizoglomus intraradices*(Ri) were obtained from The Energy and Resource Institute (TERI), New Delhi. The mycorrhizal inoculum of *R. intraradices* was bulked and maintained in pot cultures with *Zea mays* L., *Coriandrum sativum* L., and *Sorghum bicolor* L. as consecutive hosts. A mixture of soil, spores and roots was used as an inoculum in the pot experiments.

2.2 Experimental Setup and Ni Application

The earthenware pots (30 × 26 × 26 cm) were washed, decontaminated (70% ethanol) and then lined with polythene bags to avoid metal leaching. Each pot was filled with 10 kg of autoclaved soil containing sand and loam in the ratio 1:1. The properties of soil were as follows:pH 7.55, 0.68% organic carbon (Estefan et al. 2013), 0.43% total nitrogen, 9.8 mg kg^{-1} phosphorus, 160 mg kg^{-1} potassium, 15.67 mg kg^{-1} calcium, 6.03 µg g^{-1} Ni, 0.07 mg g^{-1} silicon, and ECe 0.87 dS m^{-1}). The seeds of each genotype were pre-treated with hydrogen peroxide (10%) for 10 min and then washed thoroughly with distilled water. The seeds of each species were coated with their specific rhizobia and left for drying for 1 h. The mycorrhizal inoculum (50 g/pot) was placed beneath the coated seeds (1.5 cm deep) before sowing. They were treated with nickel ($NiSO_4$) solution (150 ml/pot) at the rate of 150 mg/kg of dry soil with/without Si treatment and AM inoculation at 15 days after emergence (DAE). The experimental setup comprised of a complete randomized design with combination of three factors: Two Ni concentrations (0, 150 mg/kg), two Si concentrations (0, 300 mg/kg) and two AMF treatments (Control/Non-AM, *R.irregularis*) accounting to eight (2 × 2 × 2) treatments with six replicates each for each species. Siwas given in the form of potassium silicate (K_2SiO_3) solution (150 ml/pot) at a concentration of 300 mg/kg of dry soil one week after Ni treatment. Sampling of plants was done just before the flowering stage i.e., as for chickpea 90 DAS (days of sowing), mungbean 60 DAS and pigeonpea90 DAS. Out of the 18 pots (per treatment) 6 pots were maintained for harvest analysis, other six kept in deep freezer (-80 °C) and another six oven dried (72 h) at 70 °C for various fresh and dry estimations respectively.

2.3 Data Collection

2.3.1 Biomass and Yield

Biomass of the plants was calculated in terms of shoot dry weight (SDW) and root dry weight (RDW) by oven drying

the shoots and roots separately at 70 °C for 72 h. To determine the yield of the three species their flower number, seed and pod number and their fry weights were noted, and harvest index (HI) was also evaluated (Leport et al. [71]

$$HI = \frac{Dry\ weight\ of\ seeds\ perplant}{Above\ ground\ biomass\ at\ harvest}$$

2.3.2 Mycorrhizal Attributes

Roots of plants with mycorrhizal inoculum were selected randomly and autoclaved using 10% potassium hydroxide (KOH). Thereafter, they were dipped in HCL (20%) and later rinsed with water followed by staining with trypan blue. After 24 h 100 sections (~1 cm) were checked under the microscope for per-cent root colonization (MC) (Giovannetti and Mosse [72]) whereas mycorrhizal responsiveness (MR) was determined by the methodology of Hetrick et al. [73].

$$\mathrm{MC} = \frac{Total\ number\ of\ colonized\ intersections}{Total\ number\ of\ intersections\ observed} \times 100$$

$$MR = \frac{Dry\ weight\ of\ AM\ plants - Dry\ weight\ of\ non-AM\ plants}{Dry\ weight\ of\ non-AM\ plants} \times 100$$

2.3.3 Nutrient Status,Si and Ni content

Oven dried plant samples were grounded into fine powder for analysis of various nutrients, Si and Ni content in soil. Roots and nodules with the help of LA-ICP-MS (Laser Ablation Inductively Coupled Plasma Mass Spectrometry) located in at SAIF (Sophisticated Analytical Instrumentation Facility), Panjab University, Chandigarh.

2.3.4 ROS Generation, MDA Content and Electrolyte Leakage (EL)

Estimation of hydrogen peroxide (H_2O_2) was done according to the method suggested by Velikova et al. [74]. 0.1% TCA (w/v) was used to homogenize the nodules, followed by its centrifugation ((12,000 × *g* for 15 min). The supernatant was separated and phosphate buffer and 1 M potassium iodide were added to it. The content of H_2O_2 was measured by reading the absorbance at 390 nm using an extinction coefficient.

Superoxide radical ($O_2^{\bullet-}$) estimation was done according to Doke [75] and was determined based on its potential to reduce nitro blue tetrazolium (NBT). Nodules were homogenized in 10 Mm phosphate buffer (pH 7.8) containing NBT (0.05 per cent (w/v)) and sodium nitrate (10 Mm), followed by incubation at room temperature for 1 h. The obtained solution was heated (85 °C) and increase in absorbance was read at 580 nm.

Malondialdehyde (MDA) that is the product of lipid peroxidation was recorded by the method of Heath and Packer [76]. Nodules were grounded in a solution containing trichloroacetic acid (TCA, 0.1%)) and the mixture was centrifuged (15,000 × *g*, 15 min). The supernatant was separated followed by addition of thiobarbituric acid (TBA, 0.5%) and TCA (20%). The solution obtained was incubated in boiling water and later cooled and centrifuged (10,000 × *g*, 10 min). The absorbance was taken at 532and 600 nm and MDA was computed by deducting the absorption at 600 nm from the absorption at 532 nm with the help of an extinction coefficient.

The ion leakage from the leaves and roots was assessed through the method given by Zwiazek and Blake [77] using the equation:

$$EL = \frac{Electrical\ conductivity\ of\ solution\ before\ heating}{Electrical\ conductivity\ of\ solution\ after\ heating} \times 100$$

2.3.5 Nodular Parameters [Nodule Number, Nodule Dry Weight, Leghaemoglobin (LHb) Concentration and Nitrogenase Activity (Acetylene Reduction Assay (ARA)]

Nodule number (NN) per plant was counted for each treatment at the time of sampling and later they were oven dried (70 °C,72 h) for determining their dry weight. Leghaemoglobin (LHb) content was measured with the help of method given by Hartree [78], based on the formation of hemochromogen from hematin. The activity of nitrogenase (N_2ase) was evaluated as acetylene reduction assay (ARA) by the method of Herdina and Silsbury [79]. The rate of N_2ase activity was measured as number of ethylene (C_2H_4) molecules formed per mg dry weight of nodules per hour (nmol C_2H_4 mg^{-1} nodule dry wt.h^{-1}).

2.3.6 Trehalose Metabolism

Trehalose (Tre) content in nodules was measured by the methodology given by Streeter and Strimbu [80]. The extraction of fresh nodules was done in methanol (80%, v/v), were later incubated (60 °C, 10 min) and then centrifuged(10 min, 13,000 × g). Re-extraction of pellet was done three times and the supernatant obtained was vacuum dried. Equal amount of pyridine and STOX reagent was used to dissolve the solids. Hexamethyldisilazane and trifluoroacetic acid were added to the samples for their derivatization (60 min).Activity of Trehalose-6-Phosphate synthase (T6PS) was determined according to Salminen and Streeter [81] based on the liberation of UDP from UDP glucose in the presence of glucose-6-phosphate. The activity of Trehalose-6-phosphate phosphatase (T6PP) was based on the release of phosphate (Pi)

from trehalose-6-phosphate [82]. Activity of trehalase (TRE) was determined according to Müller et al. [83] that involved calorimetric estimation by quantifying the liberation of glucose. The released glucose was measured with the help of dinitrosulfosalicylic acid method as given by Miller [84].

2.3.7 Soil enzymes (Urease, phosphatases, Dehydrogenase)

Methodology of May and Douglas [85] was used to determine the activity of urease (URE) by using 10% urea solution as a substrate. Fresh soil samples were mixed with citrate solution (5 mL, pH-6.7) and were kept for 24 h at 37 ◦C. The mixture obtained was diluted with distilled water (50 mL) and filtered after incubation. To 1 mL of supernatant, sodium hypochlorite (0.9%, 3 mL) and sodium phenol solution (4 mL) was added. The activity was measured at 578 nm based on the ammonium ions released from hydrolysis of urea. Alkaline Phsopahatase (Alk. PHA) was evaluated according to Eivazi and Tabatabai [86] methods. (Alk.) PHA was determined by using Tris–HCl buffer (100 mM, pH 8.6) comprising of sodium acetate (100 mM), $MgCl_2$ (10 mM), para-nitrophenyl phosphate (pNPP) (0.03 M). This solution was kept for 20 min in ice cold water and later centrifuged followed by addition of $CaCl_2$ (5 mM) and NaOH (0.5 M). The absorbance was read at 405 nm spectrophotometrically. Dehydrogenase (DHase) activity was analysed according to Casida et al. [87]. Soil (2.5 g) was suspended in solution containing calcium carbonate (25 mg), 2,3,5-triphenyltetrazolium chloride (1%, 1.5 mL) and distilled water (1 mL). Incubation of the above mixture was done at 37 ◦C for 24 h followed by adding methanol (5 mL). The solution was then filtered and diluted upto 50 mL with methanol. The absorbance of red colour was measured at 485 nm.

2.3.8 Ammonia Assimilation

Glutamate synthetase (GS) activity was estimated by extracting fresh nodules in potassium phosphate buffer (0.1 M, pH 7.8) containing sucrose (0.4 M), dithiothreitol (10 mM), KCl (10 mM), $MgCl_2$ (1 mM) and EDTA (10 mM). The above mixture was centrifuged (10,000 × g, 20 min) and the supernatant obtained was used for the enzymatic assay, as suggested by Thimmaiah [88]. The reaction mixture contained Tris-maleate buffer (50 mM, pH 7.5), L-glutamine (80 mM), hydroxylamine (67 mM), ATP (8 mM) and EDTA (4 mM) to which enzyme extract was added. The activity was measured at 540 nm spectrophotometrically and was compared with a calibration curve plotted by using pure g-glutamyl hydroxamate. Determination of Glutamine oxoglutarate aminotransferase (GOGAT) and Glutamate dehydrogenase (GDH) activity was done by extracting fresh nodules in extraction buffer (3 ml) that contained Tris HCl (0.05 M, pH 7.5), b-mercaptethanol (0.01 M) and sucrose (0.4 M). The above solution was centrifuged (10,000 × g, 20 min) and enzyme assay was done as described by Thimmaiah [88]. 1 ml of supernatant was added to reaction mixture consisting of Tris–HCl buffer (0.1 M, pH 7.5), 2-oxoglutarate (0.33 M, pH 6.0), NADH (1 mM) and L-glutamine (0.3 M) or NH_4Cl (3 M) for GOGAT and GDH respectively. The absorbance was read at 340 nm that was based on the NADH oxidation in the reaction medium.

2.3.9 Ureide Metabolism

Xanthine dehydrogenase (XDH) and uricase were analysed by extracting fresh nodules in TES-KOH buffer (25 mM, pH 7.5) containing polyvinylpolypyrrolidone (10% w/w). The above mixture was centrifuged (1100 × g, 15 min). Activities of XDH and uricase activities were by Schubert [89] methodology. For XDH the reaction mixture consisted of NAD^+(3.5 mM) and hypoxanthine (0.5 mM) in TES-KOH buffer (50 mM, pH 8.4) and the crude extract. The absorbance was taken at 340 nm on a spectrophotometrically and was based on hypoxanthine dependent formation of NADH. For determining uricase activity reaction medium contained uric acid (50 µM) in glycine-KOH buffer (pH 9) and the crude extract. The absorbance was measured at 293 nm based on oxidation of uric acid.

Analysis of allantoinase (ALAase) was done according to Schubert [89] by extracting the fresh nodules in tricine buffer (50 mM) that contained $MnSO_4$ (2 mM), β-mercaptoethanol (35 mM) followed by incubation (30 min, 30 °C). These samples were later mixed with HCl (0.15 N) and phenylhydrazine (0.33%). The samples were kept in water bath (boiling) for 2 min and kept for cooling. 1 ml HCL (conc.) and 250 µl $K_3Fe(CN)_6$ (1.67%) were added to the samples. The absorbance was measured at 520 nm for activity of ALNase.

Allantoin (ALN) and allantoic acid (ALA) concentrations were analyzed according to the method given by Vogels and van der Drift [90]. Nodules were extracted in potassium phosphate buffer (50 mM, pH 7) and centrifuged (18,000 × g, 20 min). To the supernatant, NaOH (0.5 N) was added and the solution was heated (30 min, 90 min) in water bath. The mixture was cooled down and HCL (0.65 M) was added followed by incubation (15 min). Later on, water, phosphate buffer (0.4-M, pH 7) and phenylhydrazine solution was poured into the above mixture. Thereafter, the tubes were kept in ice water bath to which 5 ml of HCl (conc.) and and 1 ml of $K_3Fe(CN)_6$ was added. The absorbance was measured at 535 nm.

Urea concentration in leaves was estimated by modified method of Kyllingsbæk [91]. 1 ml of extraction medium containing 10 mM formic acid was used to homogenize 0.5 g of leaves that was later centrifuged (13,200 × g, 5 min, 4 °C).

Supernatant was separated and mixed with a colour developing reagent which was prepared by using a 1:1 proportion of the colorimetric reagent (7%, 0.2 M diacetyl monoxime; 7%,0.05 M thiosemicarbazide) and the acid reagent (20%, H_2SO_4; 0.06%, 74 mM $FeCl_3$ hexahydrate; 9%, orthophosphoric acid). The samples were incubated (15 min, 99 °C) and later kept in ice cooled system under dark for 10 min and urea concentration was spectrophotometrically analysed at 540 nm.

Modified method of Hogan et al. [92] was used to study the leaf urease activity. The material was homogenized in 7.4 pH phosphate buffer and then centrifuged (18,000 × g, 20 min, 4 °C). The homogenate was incubated for 1 h at 30 °C and later supernatant was separated to which 2.5 mL of reagent 1 containing 0.1 M phenol + 170 µM of sodium nitroprusside and 2.5 mL of reagent 2 containing 0.125 M sodium hydroxide + 0.15 M dibasic sodium phosphate + sodium hypochlorite, 3% of Cl_2 was added. Samples were incubated for 35 min at 37 °C and readings were taken at 625 nm for determining urease activity.

2.3.10 Amide Metabolism

Activity of Aspartate amino transferase (AspAT) activity was measured by homogenising the fresh nodules in Tris–HCl (50 mM, pH 7.8) consisting of $MgCl_2$(4 mM), aspartic acid (10 mM), NADH (0.2 mM)and 2-oxoglutarate (1 mM) [93]. For analysis of Asparagine synthetase (AS) fresh nodules were extracted in 5 ml of 100 mM imidazole–HCl of pH 7.5 and mercaptoethanol (10 mM) followed by centrifugation (5 min, 15,000 × g). The supernatant was used as enzymatic extract and the assay was done according to method given by Rognes [94]. The crude extract was added to a mixture containing L-aspartate (10 µm), ATP (15 µm), $MgCl_2$ (400 µm), NH_2OH-HCl (brought to pH 7.6 with Tris) and dithiothreitol (2 µm). The above mixture was incubated (60 min, 37 °C) and the absorbance was read at 540 nm after the addition of $FeCl_3$ reagent. Asparagine (Asn) content quantification was done by extracting the dry nodules in phosphate buffer (10 mM, pH 7.5) and incubating the mixture (5 min, 30 °C) followed by centrifugation (10 min, 12,000 g). The methodology used for the assay was according to the Vadez, Sinclair & Sarraj [95]. The reaction mixture comprised of phosphate buffer (14.5 mM, pH 7.5), α-ketoglutarate (600 Mm), NADH (10 mM), asparaginase (2.5 U), aspartate aminotransferase (5 U), malate dehydrogenase (5 U) and enzyme extract. The absorbance was recorded at 340 nm spectrophotometrically.

2.4 Statistical Analysis

The experimental setup consisted of eight treatments and six replications. Data presented as mean ± standard error (SE) and analysed through Statistical Package for Social Science (SPSS,version 25.0) software with Ni stress (Ni), Si levels (Si), AMF inoculations (AM) and legume species (G) as main factors. All data was analyzed by four-way analysis of variance (ANOVA) followed by Duncan multiple range test (DMRT, $p \leq 0.05$). Regression analysis was executed to find out the relationship between the independent variables (Ni, Si, AM and G) on dependent factors. To study the relationship between the dependent variables of two parameters of the three species, Pearson's correlation coefficients (r) were calculated.

3 Results

3.1 Mycorrhizal (MC, MR) and Growth Attributes

No root colonization was recorded in uninoculated roots of all the three legume species. Upon inoculation with AM species i.e., *R. irregularis* extensive colonization was observed with pigeonpea displaying highest degree of MC (74%), followed by mungbean (71%) and then chickpea (67%) under unstressed conditions (Fig. 1a). However, with the introduction of Ni in the rooting medium there was a significant decline in the colonizing ability and MC was in the following order chickpea (49%), mungbean (58%) and pigeonpea (65%). Amendment of soil with Si nutrition significantly increased the colonizing ability of the three species with maximum MC displayed by roots of pigeonpea and mungbean with least in chickpea under Ni stress (ESM Table 1). Further, maximum mycorrhizal responsiveness was also recorded in Si + AM inoculated pigeonpea plants.

Presence of Ni significantly hampered the growth potential of legume species with higher toxic effects on roots than shoots evident through regression analysis [RDW β(Ni) = -0.128, SDW β(Ni) = -0.090]. Chickpea was the most sensitive to Ni stress when compared to other two species, while pigeonpea was relatively more tolerant and was able to retain maximum plant biomass with mungbean displaying moderate tolerance (Table 1). Amendment of soil with Si enhanced SDW and RDW significantly in pigeonpea and mungbean with minimum positive impacts in chickpea (7.3%, 8.6%; 14.2%, 15.8%; 15.5%,17.8% respectively) over their stressed counterparts. On the other hand, AM inoculations were highly beneficial in reducing the Ni induced negative responses in all the three species, with higher positive effects on roots than shoots, thus improving their biomass and balancing R/S ratios [RDW β(AM) = 0.121, SDW β(AM) = 0.100]. These effects had a direct corelation with per cent MC in the three species [r(RDW – MC) chickpea 0.908; mungbean 0.990; pigeonpea 0.996, $p = 0.01$]. Interestingly, the collective application of Si + AM proved to be the most beneficial in increasing root and shoot biomass in

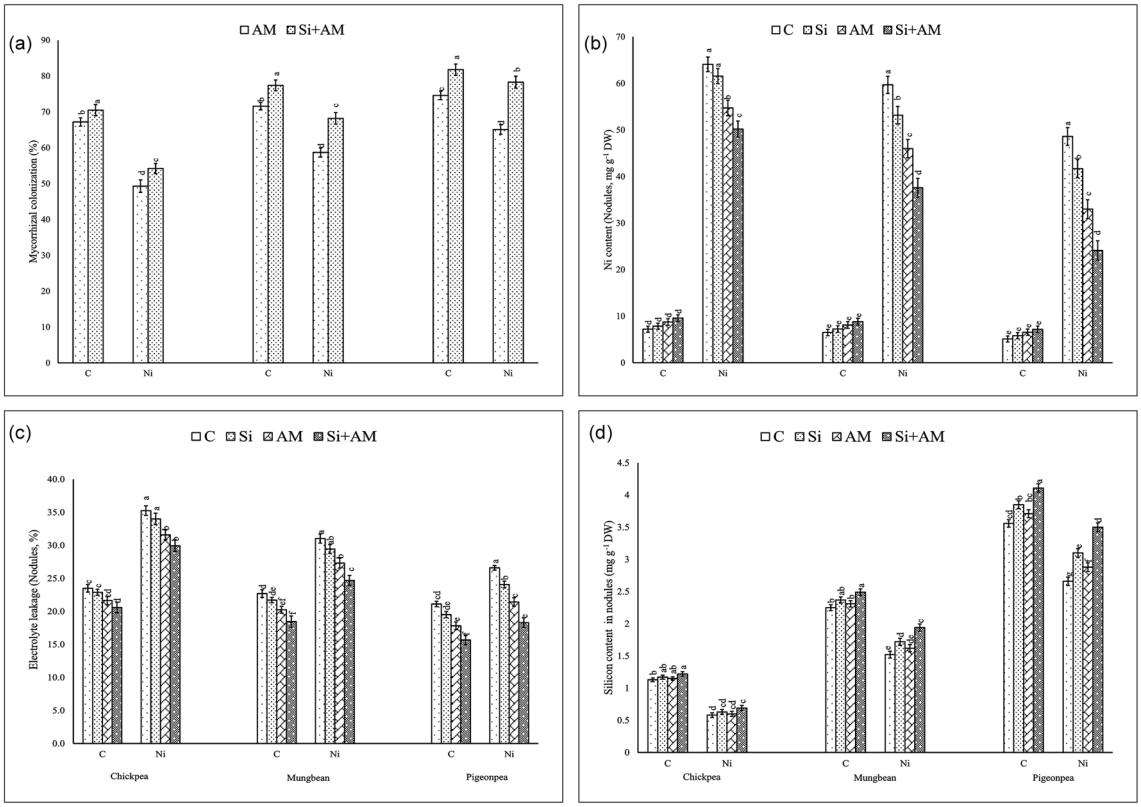

Fig. 1 Effect of silicon (Si) and arbuscular mycorrhiza fungi (AMF) on **(a)** mycorrhizal colonization (%) **(b)** nickel content (mg g^{-1} DW)**(c)**electrolyte leakage (%) **(d)** silicon content (mg g^{-1} DW) under nickel (Ni—150 mg/kg) concentration in chickpea, mungbean and pigeonpea nodules. Values are mean of 6 replicates ± standard error (SE). Different letters above the bar indicate significant differences among the treatments assessed by Duncan multiple range test at $p \leq 0.05$. C = Si and AM absent, + Si = Si present, + AM = arbuscular mycorrhiza present, + Si + AM = Si with AM present

pigeonpea and mungbean. However, cumulative effects were not significant in chickpea when compared with individual AM treatments (Table 1).

3.2 Ni Uptake, EL and Si content

Presence of high concentrations of Ni in the soil led to its enhanced uptake both in nodules and roots (Fig. 1a; ESM Fig. 1), with higher accumulation in roots (ESM Table 1) with chickpea displaying maximum Ni content followed by mungbean and then pigeonpea. Enhanced metal uptake could be directly corelated to reduced biomass under Ni stress, in legume species [chickpea r(Ni-RDW) = − 0.933; mungbean r(Ni-RDW) = − 0.818; pigeonpea r(Ni-RDW) = − 0.752, $p = 0.01$)]. Metal stress induced ROS generation and lipid peroxidation that was expressed in terms of H_2O_2, $O_2^{\bullet-}$ and MDA content respectively and was in accordance with the metal uptake in the nodules in the order: chickpea (MDA-43.9%) > mungbean (MDA-36.8%) > pigeonpea (MDA-30.7%) (Table 2). Ni toxicity further led to the degradation of root and nodule plasma membrane which accelerated electrolyte leakage that was in proportion to metal uptake [Roots β(Ni) = -0.682; nodules β(Ni) = -0.679]. Si nutrition was able to reduce Ni uptake significantly under stressed conditions, the impact being more prominent in pigeonpea than mungbean with least positive effects displayed by chickpea. The three legumes were more responsive to AM inoculations than Si in reducing Ni uptake. Both these amendments could proportionately strengthen the membranes, decrease the EL in nodules and roots (Fig. 1b; ESM Fig. 1), reduced the oxidative outburst, with AM being more effective than Si (ESM Table 1). Least metal uptake could be detected when Si + AM were given in combination especially in pigeonpea and mungbean. Maximum amount of Si content was detected in pigeonpea, closely followed by mungbean and least Si in chickpea nodules and roots (Fig. 1c; ESM Fig. 1). A significant decline in Si uptake was recorded with the introduction of Ni in the soil (ESM Table 1) with greater decline in chickpea. Addition of Si to the soil enhanced its content more in roots than nodules, maximum being detected in pigeonpea followed by mungbean and then chickpea. Interestingly, introduction of AM to soils significantly enhanced Si uptake in the order pigoenpea > mungbean > chickpea. However, addition of

Table 1 Effect of silicon (Si) and arbuscular mycorrhiza fungi (AMF) on root dry weight (RDW, g plant^{-1}), shoot dry weight (SDW, (g plant^{-1}) and root to shoot ratio (RSR) and mycorrhizal responsiveness (%) under nickel (Ni—150 mg/kg) concentration in chickpea, mungbean and pigeonpea

Parameters	RDW			SDW			RSR			Mycorrhizal Responsiveness		
Treatments	Chickpea	Mungbean	Pigeonpea	Chickpea	Mungbean	Pigeonpea	Chickpea	Mungbean	Pigeonpea	Chickpea	Mungbean	Pigeonpea
C	0.45 ± 0.005b	0.40 ± 0.009c	2.46 ± 0.092c	1.52 ± 0.017c	1.30 ± 0.016d	6.56 ± 0.115d	0.299 ± 0.002abc	0.315 ± 0.003bcd	0.376 ± 0.005bcd	-	-	-
C + Si	0.47 ± 0.006b	0.43 ± 0.010bc	2.67 ± 0.052bc	1.56 ± 0.019c	1.37 ± 0.017bc	7.02 ± 0.179 cd	0.303 ± 0.004abc	0.319 ± 0.002abc	0.380 ± 0.006abc	-	-	-
C + AM	0.50 ± 0.007a	0.46 ± 0.011ab	2.92 ± 0.046b	1.63 ± 0.022ab	1.42 ± 0.018b	7.40 ± 0.127bc	0.307 ± 0.010ab	0.330 ± 0.004ab	0.395 ± 0.004ab	7.8 ± 0.44c	10.5 ± 0.87c	14.4 ± 0.81c
C + Si + AM	0.52 ± 0.008a	0.50 ± 0.012a	3.15 ± 0.075a	1.68 ± 0.026a	1.49 ± 0.020a	7.89 ± 0.185a	0.311 ± 0.006a	0.336 ± 0.005a	0.399 ± 0.007a	8.4 ± 0.47c	10.2 ± 0.58c	13.9 ± 0.69c
C + Ni	0.24 ± 0.009e	0.26 ± 0.013f	1.80 ± 0.091e	0.90 ± 0.030f	0.93 ± 0.021 g	5.15 ± 0.144f	0.269 ± 0.005e	0.284 ± 0.008f	0.349 ± 0.009f	-	-	-
C + Ni + Si	0.26 ± 0.010e	0.30 ± 0.014e	2.12 ± 0.064d	0.96 ± 0.032f	1.05 ± 0.022f	5.95 ± 0.133e	0.273 ± 0.007de	0.289 ± 0.006ef	0.356 ± 0.008ef	-	-	-
C + Ni + AM	0.32 ± 0.012d	0.35 ± 0.015d	2.48 ± 0.040c	1.13 ± 0.035e	1.18 ± 0.023e	6.71 ± 0.156d	0.284 ± 0.008cde	0.299 ± 0.007def	0.370 ± 0.010def	27.0 ± 0.82b	30.4 ± 1.04a	32.2 ± 1.13a
C + Ni + Si + AM	0.35 ± 0.013c	0.41 ± 0.017c	2.90 ± 0.087b	1.22 ± 0.038d	1.35 ± 0.024d	7.68 ± 0.104ab	0.291 ± 0.009bcd	0.306 ± 0.009cde	0.378 ± 0.011cde	28.7 ± 1.05a	29.3 ± 1.63b	31.1 ± 1.44b

Values are mean of 6 replicates ± standard error (SE). Different letters in each column indicate significant differences among the treatments assessed by Duncan multiple range test at $p \leq 0.05$. C = Si and AM absent, + Si = Si present, + AM = arbuscular mycorrhiza present, + Si + AM = Si with AM present

Si to the soil resulted in higher Si uptake when compared to only AM inoculation (+ AM-Si) as evident through beta coefficient (ESM Table 1). The cumulative treatment of Si and AM proved to be the most efficient in restoring the Si content with pigeonpea and mungbean displaying higher responsiveness than chickpea. Variability in Si content in the plant roots could be directly corelated with differential ability for MC among the three species [chickpea r(Si – MC) = 0.812; mungbean r(Si – MC) = 0.984; pigeonpea r(Si – MC) = 0.997].

3.3 Nodular Parameters

Establishment of host-rhizobium symbiosis recorded in terms of NN and NDW per plant declined in the presence of Ni (Fig. 2a and b), which was proportionate to decline in RDW [chickpea r(NDW-RDW) = 0.996; mungbean r(NDW-RDW) = 0.990; pigeonpea r(NDW-RDW) = 0.989, $p = 0.01$)]. Chickpea displayed the highest sensitivity towards metal stress in terms of nodulation potential (NN -44.39%; NDW -42.23%) than the other two species. Nodule senescence was also a result of lipid peroxidation caused byNi induced ROS generation and MDA formation [Nodule MDA β(Ni) = 0.635]. Both Si and AM improved the NDW of the species, however the beneficial effects of Si were limited when compared with significant positive impacts of AM. Amongst the species pigeonpea was most responsive to Si as well as AM followed by mungbean with least impacts observed in chickpea which could be corelated to their respective root colonizing abilities as well as Si uptake [chickpea r(MC-NDW) = 0.945, r(Si-NDW) = 0.797; mungbean r(MC-NDW) = 0.988, r(Si-NDW) = 0.810; pigeonpea r(MC-NDW) = 0.992, r(Si-NDW) = 0.954 $p = 0.01$)]. However, the positive effects of Si were significantly enhanced in the presence of AM (Si + AM) in all three legumes species. Decline in nodular potential under Ni treatment had a negative impact on the functioning of nodules in terms of leghaemoglobin content (LHb) and rate of nitrogenase (N_2ase) activity (Fig. 2c and d) more in chickpea [r(NDW-LHb) = 0.996; r(NDW- N_2ase) = 0.998, $p = 0.01$] than mungbean [r(NDW-LHb) = 0.992; r(NDW- N_2ase) = 0.996, $p = 0.01$;] and pigeonpea [r(NDW-LHb) = 0.990; r(NDW-N_2ase) = 0.987, $p = 0.01$]. Si nutrition remarkably improved N_2ase and LHb in a species dependent manner with AM inoculations were relatively more effective. The combination of the two amendments (Si + AM) was the most effective in improving the nodular parameters, with best effects recorded in pigeonpea (N_2ase -71.9%) than the other two legumes (mungbean- N_2ase 58.9%; chickpea- N_2ase 45.6%) over their stressed counterparts. Clearly, the establishment of rhizobial symbiosis was proportional to the mycorrhizal colonization ability and Si uptake with more remarkable effects on pigeonpea.

Table 2 Effect of silicon (Si) and arbuscular mycorrhiza fungi (AMF) on hydrogen peroxide (H_2O_2, µmol g^{-1} FW), superoxide radical ($O_2^{\cdot-}$, $\Delta A_{580} \times g^{-1}$ FW) and malondialdehyde (MDA, µmol g^{-1} FW)) under nickel (Ni—150 mg/kg) concentration in chickpea, mungbean and pigeonpea nodules

Parameters	H_2O_2			$O_2^{\cdot-}$			MDA		
Treatments	Chickpea	Mungbean	Pigeonpea	Chickpea	Mungbean	Pigeonpea	Chickpea	Mungbean	Pigeonpea
C	7.54 ± 0.300c	8.01 ± 0.237d	7.88 ± 0.335c	0.40 ± 0.005c	0.35 ± 0.009c	0.34 ± 0.011bc	0.91 ± 0.018c	0.86 ± 0.020d	0.82 ± 0.018c
C + Si	7.21 ± 0.312c	7.52 ± 0.242de	7.17 ± 0.387 cd	0.39 ± 0.006c	0.33 ± 0.018 cd	0.31 ± 0.012 cd	0.89 ± 0.017c	0.81 ± 0.019de	0.75 ± 0.017 cd
C + AM	6.71 ± 0.323 cd	6.85 ± 0.254ef	6.42 ± 0.358de	0.37 ± 0.008d	0.30 ± 0.019de	0.27 ± 0.013de	0.86 ± 0.015d	0.75 ± 0.018e	0.67 ± 0.016de
C + Si + AM	6.19 ± 0.335d	6.170 ± 0.231f	5.60 ± 0.300e	0.35 ± 0.009d	0.28 ± 0.012e	0.24 ± 0.014e	0.83 ± 0.014d	0.68 ± 0.017f	0.60 ± 0.014e
C + Ni	11.06 ± 0.346a	10.91 ± 0.266a	10.21 ± 0.329a	0.58 ± 0.010a	0.47 ± 0.013a	0.43 ± 0.016a	1.32 ± 0.023a	1.01 ± 0.025a	1.08 ± 0.025a
C + Ni + Si	10.57 ± 0.358a	10.08 ± 0.294b	9.06 ± 0.318b	0.56 ± 0.011a	0.43 ± 0.014ab	0.38 ± 0.017b	1.25 ± 0.021a	1.08 ± 0.024b	0.96 ± 0.026b
C + Ni + AM	9.28 ± 0.370bc	9.13 ± 0.306c	7.87 ± 0.306c	0.52 ± 0.012b	0.39 ± 0.016b	0.33 ± 0.018c	1.10 ± 0.024b	0.96 ± 0.023c	0.81 ± 0.027c
C + Ni + Si + AM	8.65 ± 0.375c	7.98 ± 0.318d	6.61 ± 0.346de	0.49 ± 0.013b	0.34 ± 0.017 cd	0.28 ± 0.19de	1.03 ± 0.025b	0.83 ± 0.022d	0.67 ± 0.028de

Values are mean of 6 replicates ± standard error (SE). Different letters in each column indicate significant differences among the treatments assessed by Duncan multiple range test at $p \leq 0.05$. C = Si and AM absent, + Si = Si present, + AM = arbuscular mycorrhiza present, + Si + AM = Si with AM present

3.4 Soil Enzymes and Nutrient Availability

Presence of Ni had a negative corelation with soil enzymes activities with higher sensitivity in terms of URE followed by dehydrogenases (DEH) and alkaline phosphatase (AlkPHA) [Fig. 3a-c]. This decline in enzymatic activities led to reduced nutrient availability in the soil in terms of N and P ((Fig. 3d and e) ultimately leading to decline in

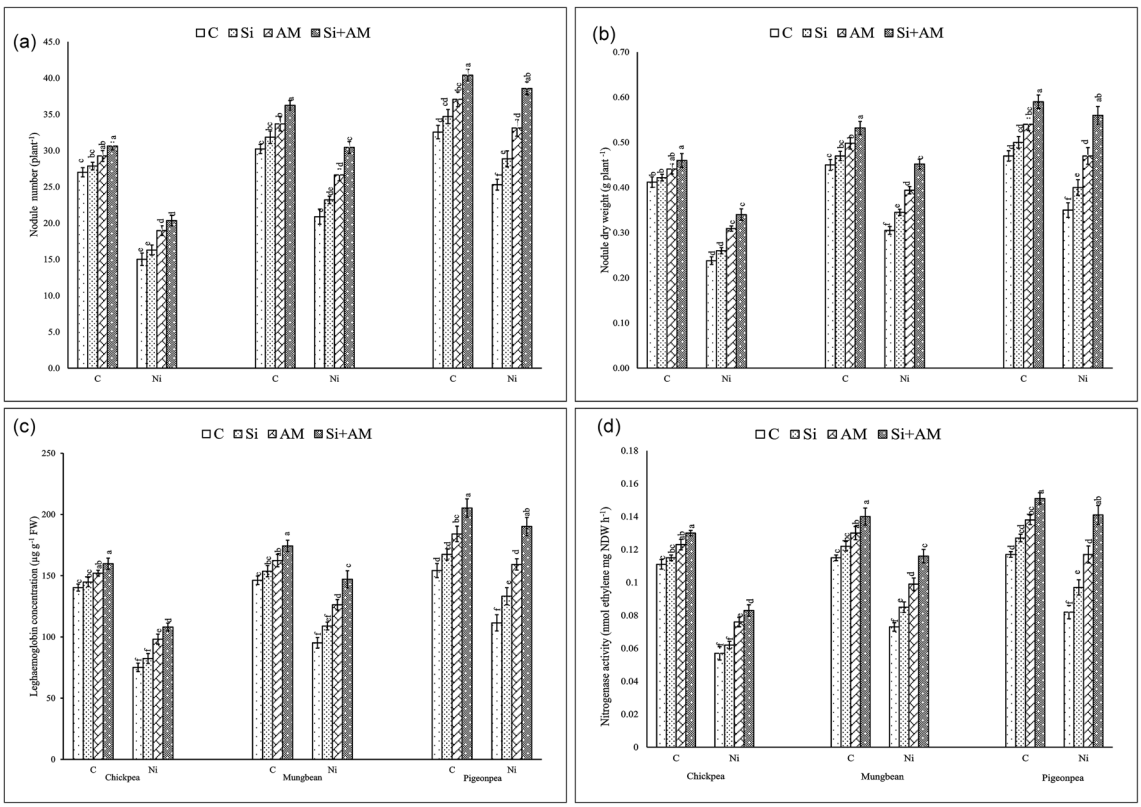

Fig. 2 Effect of silicon (Si) and arbuscular mycorrhiza fungi (AMF) on **(a)** nodule number (NN, $plant^{-1}$) **(b)** nodule dry weight (NDW, g $plant^{-1}$) **(c)** leghaemoglobin (LHb, µg g^{-1} FW) **(d)** nitrogenase activity (N_2ase, nmol ethylene mg NDW h^{-1}) under nickel (Ni—150 mg/kg) concentration in chickpea, mungbean and pigeonpea. Values are mean of 6 replicates ± standard error (SE). Different letters above the bar indicate significant differences among the treatments assessed by Duncan multiple range test at $p \leq 0.05$. C = Si and AM absent, + Si = Si present, + AM = arbuscular mycorrhiza present, + Si + AM = Si with AM present

their content in nodules and roots (Table 3; ESM Table 2) with maximum decrement in chickpea. Si and AM applications helped to improve enzyme activities in the rhizosphere of legume species with AM proving to be superior than Si in alleviating the Ni induced detrimental effects on soil enzymes as authenticated through regression analysis [URE $\beta(Si) = 0.211$; $\beta(AM) = 0.400$; DEH $\beta(Si) = 0.215$; $\beta(AM) = 0.431$; AlkPHA $\beta(Si) = 0.207$; $\beta(AM) = 0.437$]. The soil enzymatic activities were further improved when the species were treated with Si and AM in combination, the effects being more positive in soil environment of pigeonpea and mungbean.

An increase in soil enzymatic activities resulted in a proportionate increase in the nutrient uptake by roots and their translocation to nodules upon addition of Si and AMF, with AMF relatively more efficient. AMF seemed to play a direct role in improving the nutrient content of the soil and the resultant nutrient status of plants under Ni stress in a species dependent manner, as evidenced by correlation values (ESM Table3a-c). Pigeonpea and mungbean displayed higher responsiveness towards Si nutrition, with almost negligible positive effects displayed by chickpea. Maximum increment in nutrient content of roots and nodules was observed when the plants were supplemented with both Si and AM with significant enhancement in pigeonpea followed by mungbean and least in chickpea.

3.5 Trehalose Metabolism

Addition of Ni to the pot soils brought about a slight increase in the Tre content due to high concentrations of TRE as well as insignificant increase in the activities of its biosynthetic enzymes namely T6PS and T6PP in the nodules of all the three species (Fig. 4a-d). Individual applications of Si or AM had a significant impact in increasing the biosynthesis of trehalose under unstressed and stressed

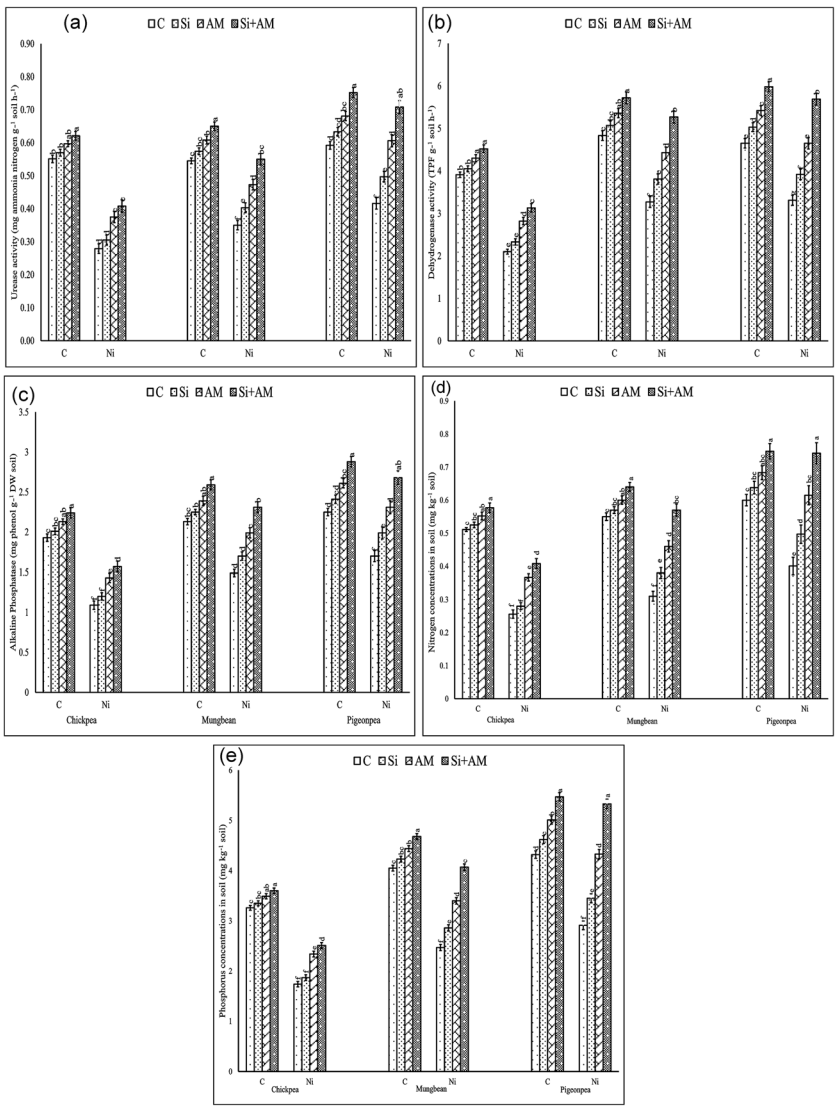

Fig. 3 Effect of silicon (Si) and arbuscular mycorrhiza fungi (AMF) on **(a)** urease (URE, mg ammonia nitrogen g^{-1} soil h^{-1}) **(b)** dehydrogenase (DHA, TPF g^{-1} soil h^{-1})**(c)** alkaline phosphatase (Alk. Phosphatase, mg phenol g^{-1} DW soil) **(d)** nitrogen (N, mg kg^{-1} soil) **(e)** phosphorus (P, mg kg^{-1} soil) in soil under nickel (Ni—150 mg/kg) concentration of chickpea, mungbean and pigeonpea. Values are mean of 6 replicates ± standard error (SE). Different letters above the bar indicate significant differences among the treatments assessed by Duncan multiple range test at $p \leq 0.05$. C = Si and AM absent, + Si = Si present, + AM = arbuscular mycorrhiza present, + Si + AM = Si with AM present

Table 3 Effect of silicon (Si) and arbuscular mycorrhiza fungi (AMF) on nitrogen (N, mg g^{-1} DW), phosphorus (P, mg g^{-1} DW), iron (Fe, mg g^{-1} DW) and copper (Cu, mg g^{-1} DW) in nodules under nickel (Ni—150 mg/kg) concentration of chickpea, mungbean and pigeonpea. Values are mean of 6 replicates ± standard error (SE). Different letters in each column indicate significant differences among the treatments assessed by Duncan multiple range test at $p \leq 0.05$. C = Si and AM absent, + Si = Si present, + AM = arbuscular mycorrhiza present, + Si + AM = Si with AM present

Parameters	N			P			Fe			Cu		
Treatments	Chickpea	Mungbean	Pigeonpea	Chickpea	Mungbean	Pigeonpea	Chickpea	Mungbean	Pigeonpea	Chickpea	Mungbean	Pigeonpea
C	13.45 ± 0.260c	15.02 ± 0.290c	17.55 ± 0.318c	2.93 ± 0.06b	3.21 ± 0.07c	3.91 ± 0.13 cd	2.23 ± 0.040ab	2.83 ± 0.035c	3.13 ± 0.047d	1.27 ± 0.042c	1.67 ± 0.052c	1.78 ± 0.064c
C + Si	13.80 ± 0.242c	15.61 ± 0.300bc	18.59 ± 0.346bc	3.02 ± 0.07b	3.37 ± 0.05bc	4.20 ± 0.14bc	2.30 ± 0.043ab	2.95 ± 0.036bc	3.30 ± 0.052 cd	1.31 ± 0.045c	1.74 ± 0.053bc	1.90 ± 0/069bc
C + AM	14.34 ± 0.277ab	16.29 ± 0.312ab	19.68 ± 0.358b	3.17 ± 0.08a	3.55 ± 0.06ab	4.54 ± 0.15abc	2.40 ± 0.046a	3.08 ± 0.037ab	3.50 ± 0.050bc	1.38 ± 0.047ab	1.83 ± 0.054ab	2.00 ± 0.075ab
C + Si + AM	14.72 ± 0.289a	17.08 ± 0.323a	21.02 ± 0.370a	3.25 ± 0.09a	3.76 ± 0.08a	4.95 ± 0.16a	2.49 ± 0.049a	3.23 ± 0.038a	3.77 ± 0.053a	1.43 ± 0.049a	1.94 ± 0.055a	2.15 ± 0.081a
C + Ni	7.30 ± 0.248e	9.58 ± 0.326f	12.79 ± 0.387e	1.72 ± 0.11d	2.15 ± 0.13f	2.91 ± 0.17e	1.25 ± 0.052d	1.86 ± 0.039f	2.20 ± 0.051f	0.63 ± 0.051e	1.11 ± 0.056f	1.31 ± 0.087e
C + Ni + Si	7.80 ± 0.266e	11.15 ± 0.341e	15.20 ± 0.398d	1.84 ± 0.10d	2.46 ± 0.10e	3.42 ± 0.20d	1.35 ± 0.053 cd	2.16 ± 0.040e	2.62 ± 0.054e	0.69 ± 0.052e	1.27 ± 0.057e	1.53 ± 0.092d
C + Ni + AM	9.18 ± 0.300d	12.97 ± 0.346d	17.80 ± 0.410c	2.19 ± 0.12c	2.79 ± 0.11d	4.00 ± 0.18c	1.67 ± 0.051bc	2.51 ± 0.041d	3.15 ± 0.055d	0.82 ± 0.053d	1.46 ± 0.058d	1.75 ± 0.098c
C + Ni + Si + AM	9.86 ± 0.312d	15.03 ± 0.358c	20.94 ± 0.421a	2.37 ± 0.13c	3.21 ± 0.12c	4.69 ± 0.21ab	1.81 ± 0.055b	2.91 ± 0.042bc	3.70 ± 0.056ab	0.92 ± 0.054d	1.70 ± 0.059c	2.06 ± 0.104ab

conditions, with AM more effective in modulating trehalose metabolism as validated through regression analysis (ESM Table1). However, species level differences were prominent where both the amendments were able to boost Tre synthesis to the maximum level in pigeonpea, moderate in mungbean and least positive effects in chickpea. Moreover, Si and AM stimulated Tre accumulation, was directly linked to decrease in H_2O_2, $O_2^{\bullet-}$ content ultimately leading to low MDA formation [pigeonpea r(Tre-MDA) = 0.970; mungbean r(Tre- MDA) = 0.959; chickpea r(Tre- MDA) = 0.924, $p = 0.01$]. Higher increase in Tre synthesis through mycorrhization could be directly related to improved N_2ase activity of the nodules under stress in a species dependent manner [pigeonpea r(Tre-N_2ase) = -0.627; mungbean r(Tre- N_2ase) = -0.666; chickpea r(Tre- N_2ase) = -0.697, $p = 0.01$. The combined effect of Si + AM under stress proved to be most beneficial in improving the synthesis of storage carbohydrate and maintaining the osmotic balance.

3.6 Ammonia Assimilation Enzymes

Treatment of legumes with Ni negatively affected the activities of enzymes involved in the ammonia assimilation pathway (Fig. 5a-c) by significantly damaging their activity, especially in chickpea (GS -42.29%; GOGAT -40.11%) with a concomitant increase in GDH activity (12.9%) in nodules over unstressed control. Treatment of Si elevated the GS-GOGAT pathway more than GDH under stress with a maximum increment in pigeonpea followed by mungbean and chickpea [pigeonpea r(Si-GS) = 0.956; r(Si-GOGAT) = 0.921; r(Si-GDH) = 0.881; mungbean r(Si-GS) = 0.809; r(Si-GOGAT) = 0.821; r(Si-GDH) = 0.779, $p = 0.01$; chickpea r(Si-GS) = 0.758,; r(Si-GOGAT) = 0.798; r(Si-GDH) = 0.732, $p = 0.01$]. AM proved to be more efficient in improving the enzymatic activities in a species dependent manner which could be corelated to their root colonizing abilities [pigeonpea (MC-GS) = 0.997; r(MC-GOGAT) = 0.996; r(MC-GDH) = 0.989, $p = 0.01$; mungbean r(MC-GS) = 0.985,; r(MC-GOGAT) = 0.988,; r(MC-GDH) = 0.979, $p = 0.01$;chickpea r(MC-GS) = 0.978; r(MC-GOGAT) = 0.983; (MC-GDH) = 0.964, $p = 0.01$]. The combined application of Si and AM proved to be the most beneficial in upregulating GS-GOGAT under Ni stress with pigeonpea being more receptive towards the individual and cumulative treatments of Si and AM than the other two legumes.

3.7 Ureide Metabolism

The nodules of both pigeonpea and mungbean displayed a slight increment in the ureides contents (ALN and

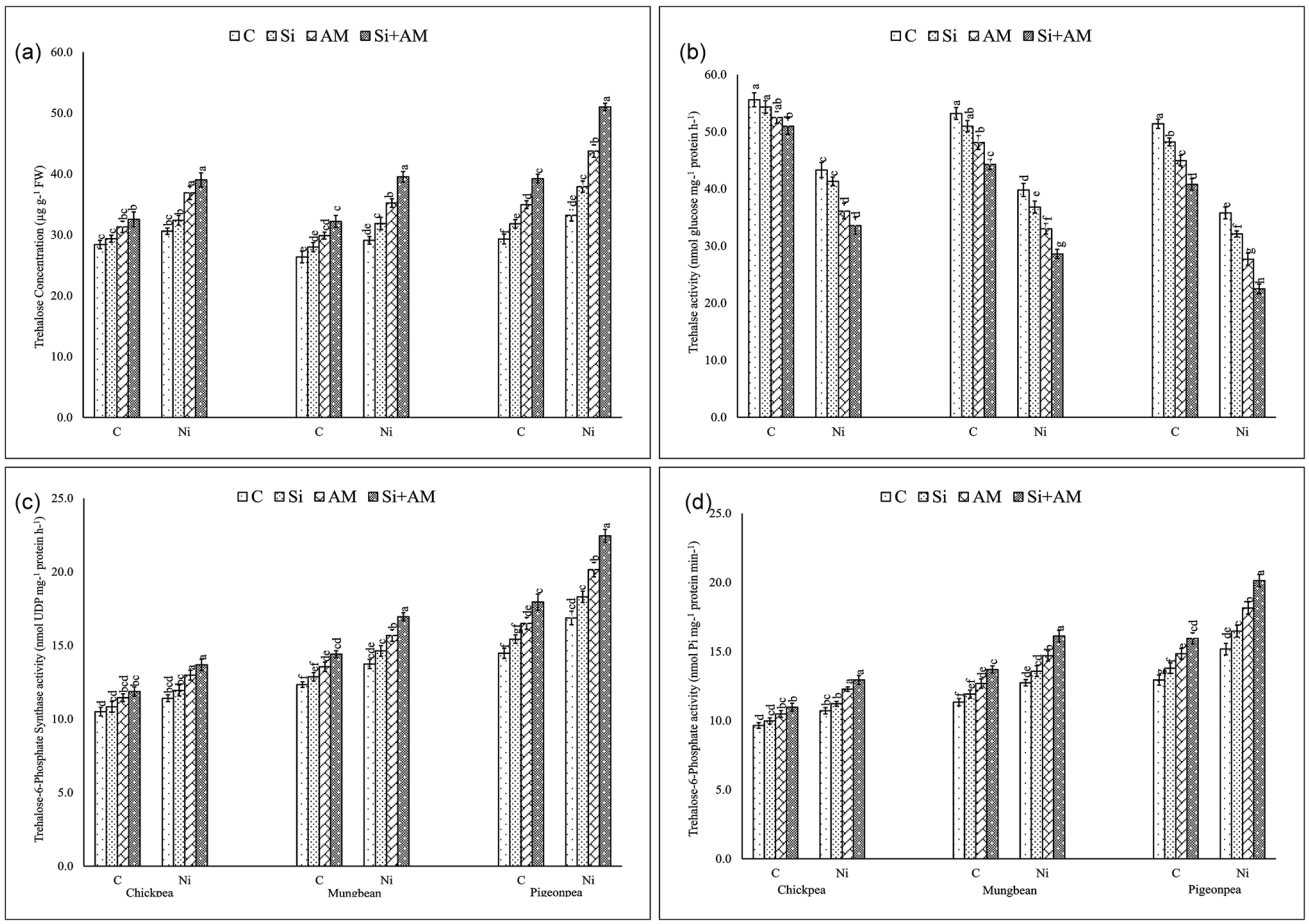

Fig. 4 Effect of silicon (Si) and arbuscular mycorrhiza fungi (AMF) on **(a)** trehalose concentration (Tre, µg g^{-1} FW) **(b)** Trehalse (TRE, nmol glucose mg^{-1} protein h^{-1}) **(c)** Trehalose-6- phosphate synthase (T6PS,nmol UDP mg^{-1} protein h^{-1}) **(d)** Trehalose-6- phosphatase (T6PP, nmol Pi mg^{-1} protein min^{-1}) under nickel (Ni—150 mg/kg) concentration of chickpea, mungbean and pigeonpea. Values are mean of 6 replicates ± standard error (SE). Different letters above the bar indicate significant differences among the treatments assessed by Duncan multiple range test at $p \leq 0.05$. C = Si and AM absent, + Si = Si present, + AM = arbuscular mycorrhiza present, + Si + AM = Si with AM present

ALA) as well as the enzymes involved in their synthesis (XDH, Uricase, ALNase)with a decline in urease under Ni stress with no data recorded in chickpea (Fig. 6a-d; Fig. 7a). Increase in ALA was directly corelated to increase in ALNase activity that was significantly more in pigeonpea [r(ALA-ALNase) = 0.983, $p = 0.01$] than mungbean [r(ALA-ALNase) = 0.935, $p = 0.01$] under Ni stress. Si and AM positively impacted the enzyme activities and the resultant ureide content with more beneficial impacts of AM especially in pigeonpea (ESM Table 1). Apparently, ALA concentration and N_2ase activity displayed a negative corelation in stressed nodules [pigeonpea r(ALA-N_2ase) = -0.818, $p = 0.01$; mungbean r(ALA-N_2ase) = -0.906. $p = 0.01$]. Combined treatment of Si + AM was the most effective as it significantly enhanced ureide synthesis under stress, with pigeonpea displaying better results than mungbean. Moreover, production of urea and its conversion into NH_3 and CO_2 declined due to reduced urease activity in the leaves under Ni stress (Fig. 7b and c) Si supplementation boosted the urease activity and improved the urea content more in pigeonpea than mungbean as evident through corelation coefficient [pigeonpea r(Si-Urease) = 0.989; r(Si-Urea) = 0.957 $p = 0.01$; mungbean r(Si-Urease) = 0.944; r(Si-Urea) = 0.933, $p = 0.01$]. However, AM colonization was more effective in increasing the synthesis of urea by significantly increasing the urease activity [pigeonpea r(MC-Urease) = 0.998, r(MC-Urea) = 0.990, $p = 0.01$; mungbean r(MC-Urease) = 0.968, r(MC-Urea) = 0.970, $p = 0.01$]. Si + AM treatment was the most efficient in enhancing the urease activity and helped to enrich the urea pool especially in pigeonpea.

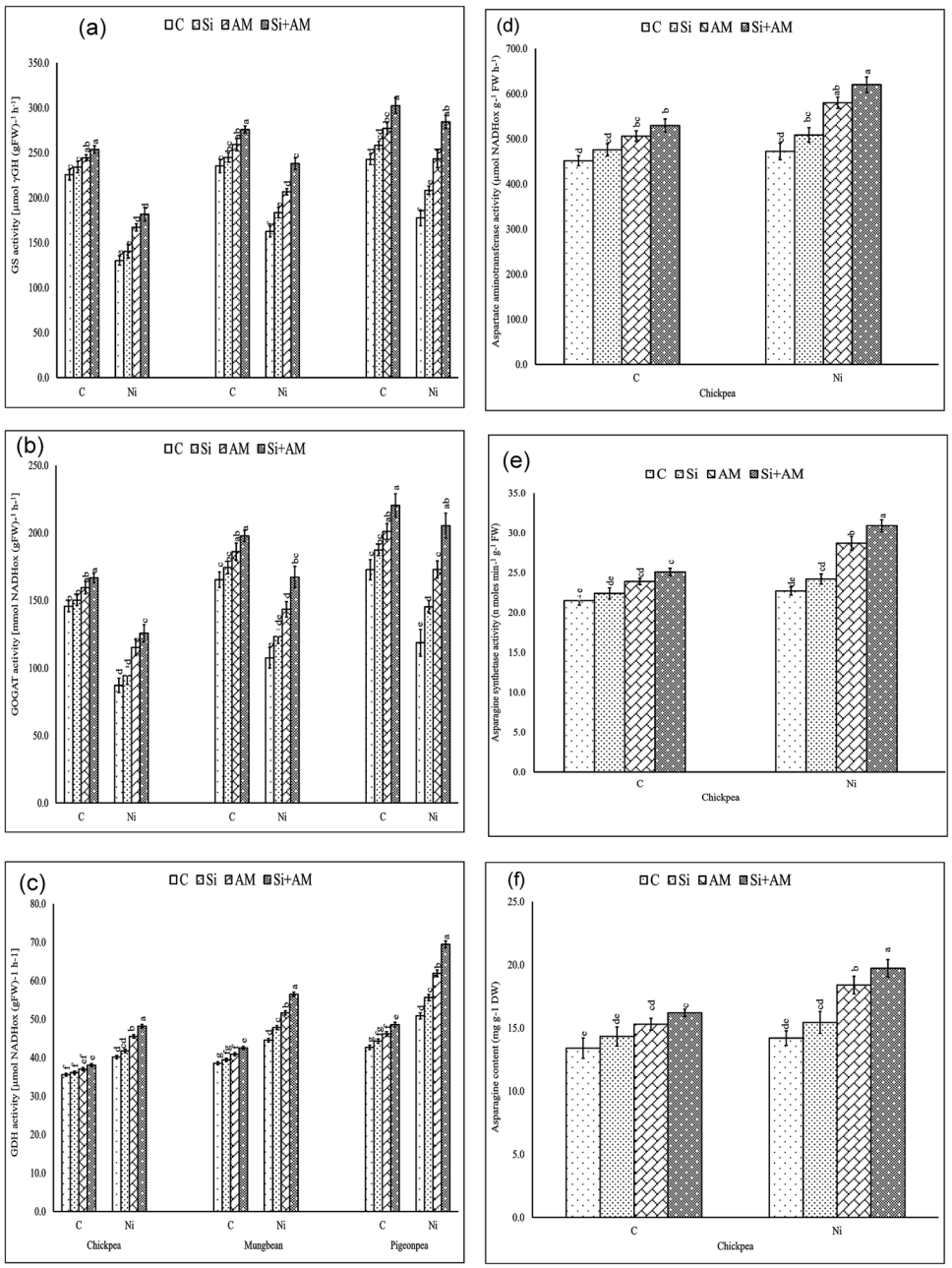

Fig. 5 Effect of silicon (Si) and arbuscular mycorrhiza fungi (AMF) on **(a)** glutamine synthetase (GS, µmol γGH $(gFW)^{-1}$ h^{-1}) **(b)** glutamate synthase (GOGAT, mmol $NADH_{ox}(g\ FW)^{-1}$ h^{-1}) **(c)** GDH (Glutamate dehydrogenase, µmol $NADH_{ox}(g\ FW)^{-1}$ h^{-1}) in chickpea, mungbean and pigeonpea. **(d)** Aspartate aminotransferase activity (AAT, µmol $NADH_{ox}g^{-1}$ FW h^{-1}) **(e)** asparagine synthetase activity (AS, n moles min^{-1} g^{-1} FW) **(f)** asparagine concentration (Asn, mg g^{-1} DW) under nickel (Ni—150 mg/kg) concentration in chickpea. Values are mean of 6 replicates ± standard error (SE). Different letters above the bar indicate significant differences among the treatments assessed by Duncan multiple range test at $p \leq 0.05$. C = Si and AM absent, + Si = Si present, + AM = arbuscular mycorrhiza present, + Si + AM = Si with AM present

3.8 Asparagine Metabolism

Significant amide (Asn) synthesis was observed in unstressed chickpea plants, which increased further with the introduction of Ni in the rooting medium (Fig. 5d-f). This increase was accompanied by an increment in the activities of its biosynthetic enzymes (AspAT and AS) which could be authenticated through corelation values [chickpea r(Asn-AspAT) = 0.988; r(Asn-AS) = 0.984, $p = 0.01$]. Addition of Si did not bring about any significant improvement in terms of Asn synthesis. On the other hand, AM inoculations were highly efficient in improving the Asn synthesis (ESM Table 1). Interestingly, Asn content had a negative corelation with N_2ase activity advocating higher Asn synthesis in metal stressed nodules with decreased N_2ase activity [Chickpea r (Asn- N_2ase) = -0.840, $p = 0.01$]. The combined (Si + AM) treatments were almost at par in enhancing Asn content with those of individual AM treatments under metal stress.

3.9 Yield Attributes

Reduction in root and shoot biomass led to decline in crop yield elicited in the form of decrease in flower, pod

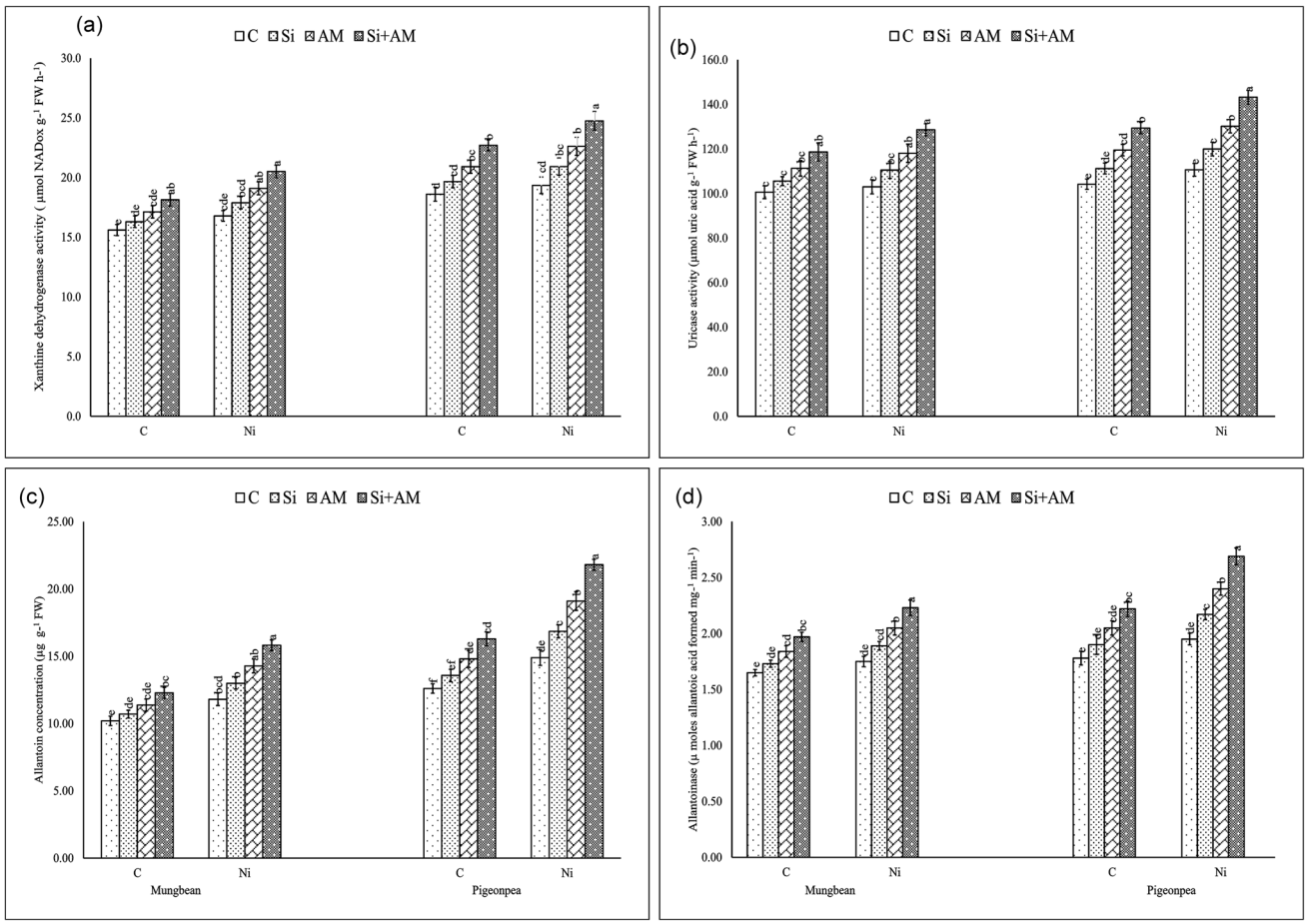

Fig. 6 Effect of silicon (Si) and arbuscular mycorrhiza fungi (AMF) on **(a)** xanthine dehydrogenase (XDH, µmol NAD_{ox} g^{-1} FW h^{-1}) **(b)** uricase (µmol uric acid g^{-1} FW h^{-1})**(c)** allantoin (ALN, (µg g^{-1} FW) **(d)** allantoinase (ALNase, µ moles allantoic acid formed mg^{-1} min^{-1}),under nickel (Ni—150 mg/kg) concentration in mungbean and pigeonpea. Values are mean of 6 replicates ± standard error (SE). Different letters above the bar indicate significant differences among the treatments assessed by Duncan multiple range test at $p \leq 0.05$. C = Si and AM absent, + Si = Si present, + AM = arbuscular mycorrhiza present, + Si + AM = Si with AM present

and seed no. as well as seed dry weight and above ground biomass, thus resulting in reduced HI (ESM Table4; Table 4). On addition of Si to soil, there was a significant improvement in the productive potential of plants, maximum in pigeonpea followed by mungbean and least in chickpea. However, AM inoculations were much more beneficial in arresting flower abortion and subsequently improving the seed dry weights as well as HI when compared with the added Si nutrition in all three species [HI β(AM) = 0.565; β(Si) = 0.235]. The increment in productive potential due to Si application and AM inoculations can be attributed to increase in nutrient uptake by the legumes. Based on the above data pigeonpea and mungbean were significantly more responsive towards Si and Si + AM in terms of improving yield under Ni stress when compared to chickpea.

4 Discussion

The present study compared the sensitivity of the three legume species chickpea, mungbean and pigeonpeain Ni contaminated soil. MC declined on introduction of Ni in the rooting medium with maximum decrease recorded in chickpea followed by mungbean whereas pigeonpea was relatively more tolerant and displayed least decline. However, even under metal contaminated soils AMF species i.e., *R. irregularis* was able to form and retain symbiosis due to the endurance of AMF spores towards metal

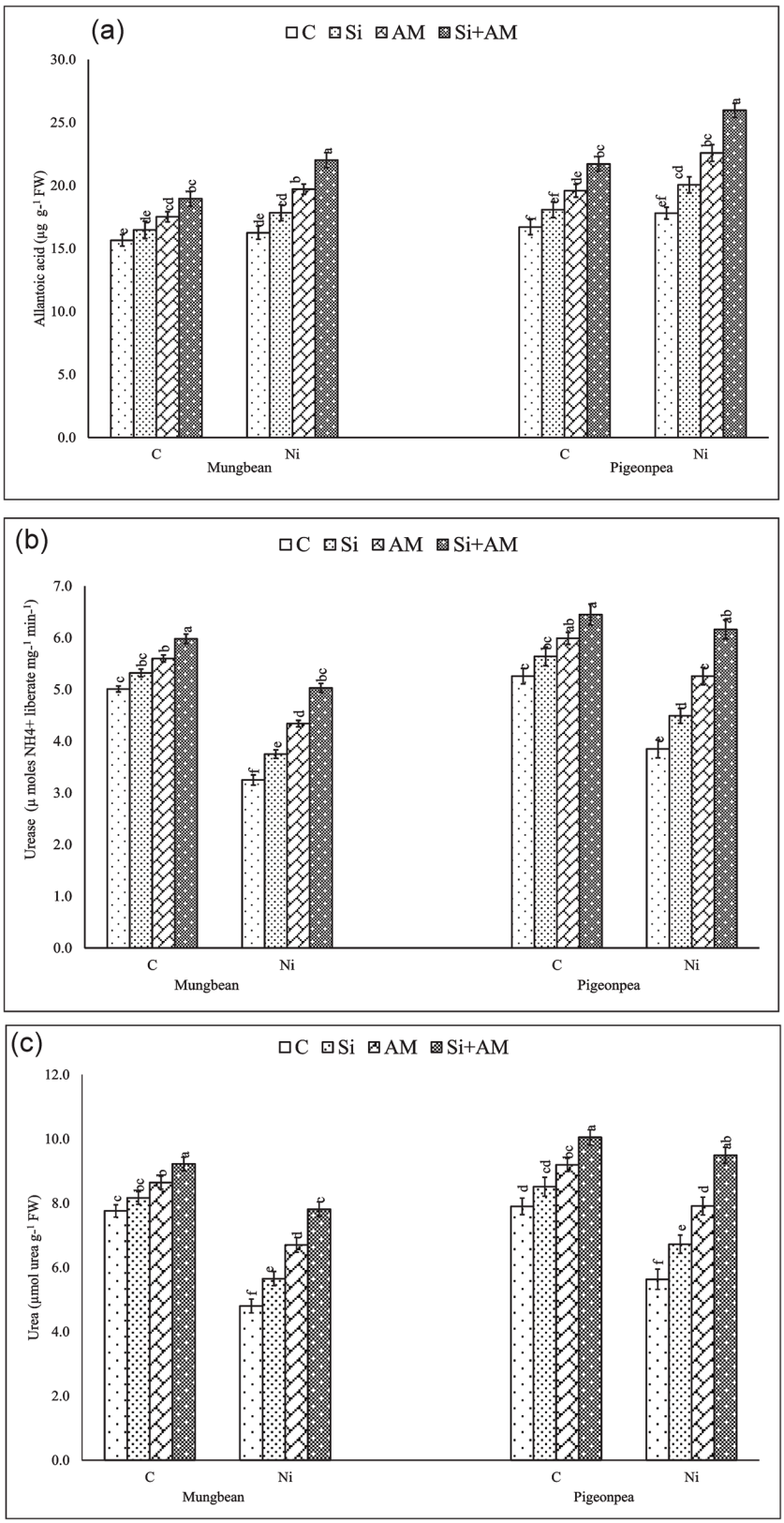

Fig. 7 Effect of silicon (Si) and arbuscular mycorrhiza fungi (AMF) on **(a)** allantoic acid (ALA, µg g-1 FW) **(b)** urease (µ moles NH_4^+ liberate mg^{-1} min^{-1}) **(c)** urea (µmol urea g^{-1} FW)under nickel (Ni—150 mg/kg) concentration in mungbean and pigeonpea. Values are mean of 6 replicates ± standard error (SE). Different letters above the bar indicate significant differences among the treatments assessed by Duncan multiple range test at $p \leq 0.05$. C = Si and AM absent, + Si = Si present, + AM = arbuscular mycorrhiza present, + Si + AM = Si with AM present

Table 4 Effect of silicon (Si) and arbuscular mycorrhiza fungi (AMF) on seed dry weight (g plant^{-1}), above ground biomass (Abv.GB, g plant^{-1}) and harvest index under nickel (Ni—150 mg/kg) concentration in chickpea, mungbean and pigeonpea

Parameters	Seed Dry Weight			Abv.GB			Harvest Index		
Treatments	Chickpea	Mungbean	Pigeonpea	Chickpea	Mungbean	Pigeonpea	Chickpea	Mungbean	Pigeonpea
C	1.16 ± 0.014b	1.07 ± 0.012c	3.32 ± 0.058d	3.86 ± 0.052c	3.48 ± 0.058c	8.10 ± 0.173d	0.301 ± 0.002b	0.308 ± 0.003bc	0.410 ± 0.004c
C + Si	1.20 ± 0.023b	1.13 ± 0.017bc	3.58 ± 0.069bc	3.97 ± 0.064bc	3.63 ± 0.052bc	8.63 ± 0.182 cd	0.302 ± 0.004b	0.311 ± 0.003b	0.415 ± 0.005bc
C + AM	1.27 ± 0.018ab	1.19 ± 0.021b	3.87 ± 0.081b	4.18 ± 0.075ab	3.80 ± 0.075ab	9.22 ± 0.208bc	0.304 ± 0.001ab	0.313 ± 0.002ab	0.420 ± 0.002b
C + Si + AM	1.33 ± -.030a	1.28 ± 0.024a	4.21 ± 0.092a	4.35 ± 0.078a	4.01 ± 0.087a	9.92 ± 0.219a	0.306 ± 0.003a	0.319 ± 0.004a	0.424 ± 0.003a
C + Ni	0.66 ± 0.020d	0.73 ± 0.014f	2.49 ± 0.064f	2.25 ± 0.058f	2.40 ± 0.069f	6.19 ± 0.191f	0.294 ± 0.007d	0.304 ± 0.005d	0.402 ± 0.009d
C + Ni + Si	0.71 ± 0.029d	0.84 ± 0.020e	2.90 ± 0.075e	2.40 ± 0.069f	2.72 ± 0.064e	7.18 ± 0.196e	0.297 ± 0.006c	0.307 ± 0.007c	0.404 ± 0.006 cd
C + Ni + AM	0.85 ± 0.024c	0.96 ± 0.023d	3.42 ± 0.087 cd	2.85 ± 0.081e	3.12 ± 0.081d	8.35 ± 0.214d	0.299 ± 0.008bc	0.308 ± 0.006bc	0.410 ± 0.010c
C + Ni + Si + AM	0.93 ± 0.031c	1.11 ± 0.026b	4.01 ± 0.100b	3.08 ± 0.084d	3.59 ± 0.080bc	9.63 ± 0.231ab	0.300 ± 0.005bc	0.309 ± 0.008bc	0.416 ± 0.008bc

Values are mean of 6 replicates ± standard error (SE). Different letters in each column indicate significant differences among the treatments assessed by Duncan multiple rangetest at $p \leq 0.05$. C = Si and AM absent, + Si = Si present, + AM = arbuscular mycorrhiza present, + Si + AM = Si with AM present

toxicity since the spores never completely vanish, thus leading to significant root colonization even under stressed conditions [96, 97]. Further, Ni toxicity led to reductions in root and shoot dry weights, the impacts being more severe on roots than shoots in a species dependent manner which ultimately declined the root to shoot ratio. Roots are severely affected as they store majority of Ni and do not allow much of it to be transported to the shoots [13]. Moreover, high Ni concentration in the roots interferes with various physiological activities including cell division, formation of root hairs etc. [14].

The three species were able to form rhizobial symbiosis and develop nodules for efficient nitrogen fixation under unstressed conditions. However, addition of Ni had a negative impact on the nodulation potential in terms of NN and NDW with maximum sensitivity shown by chickpea followed by mungbean and pigeonpea, which was proportionate to their respective declines in root weights. The decreased NN could be attributed to a number of factors such as reduced root biomass, less root hair formation, depletion of rhizobia in soil and altered *nod* gene expression [98] [99]. Decrement in NDW was followed by reduction in the synthesis of LHb which was accompanied by a decrease in the rate of nitrogenase activity (ARA) in a species dependent manner. The depletion in LHb, exposes N_2ase to excess O_2 that ultimately diminishes its activity [100]. A negative corelation between Ni stress and nitrogen fixation could also be related to the damaged C (carbon) metabolism that ultimately might have affected bacteroid respiration [101]. The decline in root and nodule biomass was proportionate to the uptake of Ni by these organs which was maximum in chickpea while pigeonpea had the ability to limit the same. Furthermore, accumulation of Ni escalated EL of root plasma membrane which led to significant reductions in root biomass. The NDW, LHb content as well as N_2ase activity decreased along with increased ROS generation and MDA content as a result of Ni uptake. In addition, a significant decline in Si content in the roots and nodules was also recorded under Ni stress which was species specific. Interestingly, no study has been carried out to compare the relative and selective uptake of Si in legume species under any abiotic stress.

Apart from reducing plant biomass accumulation, Ni also negatively affected the enzymatic activities of the rhizosphere soils of the three species in the following sequence: URE > DEH > AlkPHA. HMs restrict enzymatic reactions by complexing with substrate or obstructing the functional groups of enzymes or reacting with enzyme–substrate complex [102]. This decrease in enzyme activities resulted in reduced nutrient availability as well as uptake (N and P) especially P which might have decreased the nodular efficiency as P is required for mitochondrial and symbiosome membrane synthesis during nodule development and ATP synthesis [104]. The decline in these nutrients was accompanied by the decreased uptake of Cu and Fe because Ni competes with these ions due to similar transporters (Nramp, ABC, CTR, and ZIP families of metal transporters), thus, preventing their uptake by plant roots especially those of chickpea [105]. Consequently, the plant biomass production strained as a result of decreased nutrient acquisition and soil enzymatic activity.

Metal toxicity impaired the GS-GOGAT pathway of ammonia assimilation severely in chickpea with moderate effects observed in mungbean and pigeonpea. The decrease in the activity of GOGAT and GS enzymes indicated the disturbance in NH_4 + assimilation induced by metal toxicity, which was supported by decreasing N content. However, the activity of the GDH enzyme increased under metal stress as an alternate pathway to overcome stressful environments. under impaired GS/GOGAGT system. GDH can reduce the accumulation of toxic levels of NH_4^+ and provide the glutamate required for several defensive biomolecules [106].

The overall decline in GS and GOGAT activity was a consequence of oxidative damage that further degraded LHb [107]. Furthermore, reduced GOGAT activity is closely corelated to decrease in N_2ase activity as demonstrated by Khadri et al. [108] in *Phaseolus vulgaris* under salt stress. Amides and ureides play an important role in storage-transport of N and for combatting stresses [32, 109, 110]. Though the ureide and amide synthesis was increased to an extent under Ni toxicity more in pigeonpea than mungbean, but their transport to leaves was hindered that ultimately led to decline in N assimilation.Translocation of ALA (ureide) to the leaves was obstructed which eventually led to less urea formation.Finally, in leaves urea is converted into NH_3 and CO_2 with the help of urease enzyme. Ni is a constituent of the urease prosthetic group and its supply increases the enzyme activity but at high Ni concentrations, urease activity declines [111] as also recorded in the present study. The decrease in urease activity and urea level was more prominent in mungbean than pigeonpea. Therefore, low urea availability and decline in urease activity ultimately decreased NH_4^+ production consequently leading to large N losses.

The present study indicated that Ni induced high flower abortion leading to reduction in pod no., seed no., seed dry weight and HI in the three legumes with maximum negative impacts on chickpea followed by mungbean and pigeonpea. Abiotic stress constricts the pollen tube growth, causes abnormal functioning of gametes (male and female) and flower abortion [112]. Higher decline in plant yield in chickpea could be due to higher Ni uptake, reduced plant biomass and decrease in nutrient content of plant especially in terms on N and P as observed in the present study. Irfan et al. [113] reported that application of Cd to the soil significantly decreased yield parameters in *Brassica juncea* L. while that of *Hordeum vulgare* L. under Cr (VI) [114]. These studies suggest that HM stress restricts the physiological processes, growth and yield attributes, reducing overall crop production [115]. The differential sensitivity of the three legumes towards Ni toxicity could be due to their genetic makeup and their relative ability to uptake and transfer the metal in different plant parts.

Current study revealed that Si and AMF improved growth and biochemical parameters in all the three legumes, with AMF being more beneficial than Si in ameliorating the toxic effects due to its more effective role in reducing metal uptake than Si. Si nutrition displayed differential effectiveness in reducing the metal stress symptoms with highest positive impacts observed in pigeonpea followed by mungbean and least in chickpea.

The reduction in metal uptake could be corelated to the relative root colonizing ability of the host species with *R. irregularis*.Si aids in co-precipitation of metals, metal ions chelation, compartmentation and structural modifications of plant tissues that leads to metal detoxification thus aiding in enhanced RDW [116]. Higher beneficial effects of AMF on root growth and nutrient uptake owing to its direct role in inducing the development of healthy roots and enhanced number of root hair and surface area for the acquisition of nutrients under abiotic stresses [117, 118]). Si and AMF were highly beneficial in strengthening the plasma membranes and reducing the EL in roots and nodules thereby bringing down Ni uptake. Si-HMs co-precipitation aids in cell wall thickening through the formation of strong silica barriers that bind and obstruct the transport of toxic metals [116]. AM proved to be more beneficial in lowering metal content in all the three legumes, more in pigeonpea and mungbean than chickpea due to their differential colonizing rates. Si uptake enhanced with the supplementation of Si nutrition with maximum Si content observed in pigeonpea roots and nodules. This can be attributed to the presence of transporters CcNIP2-1 (an AQP) predicted to transport Si in pigeonpea [119] that led to increased uptake of Si from the soil whereas no Si transporters have been reported in the other two species till date. Limited Si uptake in chickpea and mungbean could be due to the passive or active uptake of Si rather than through transporters as most of the legumes are low Si accumulators [120].

Si uptake enhanced upon mycorrhization with pigeonpea most responsive to these treatments. AMF plays an important role in the uptake of Si, its transport from the external solution into the intraradical mycelium, and transfer from the fungal cells to the root cells. Although the mechanisms are still not clear, but active transport might be the involved via transporters present in the extraradical hyphae at the soil-fungus interface for Si uptake and at the plant–fungal interface (arbuscule) for its translocation across the peri–arbuscular interface in the plant cells [121]. Interestingly, supplementation with Si nutrition to the soil enhanced the MC of all the three species with least positive effects recorded in chickpea. This could be attributed to the disparity in terms of Si uptake by the three species.

There was a significant improvement in the functional efficiency of nodules (LHb, ARA) upon supplementation with Si and AM along with increase in NN and NDW, with AMF being more beneficial than Si in all the three legume species, while Si synergistically effective in pigeonpea and mungbean. Putra et al. [122] observed that Si increased the concentrations of specific flavonoids in *Medicago truncatula* that function as Nod-gene regulators along with higher synthesis of free amino acids, total soluble protein and total N thus resulting in enhanced nodulation. AMF inoculation improved the nodulation by increasing absorption and translocation of nutrients (especially P) as also observed by Gough et al. [123] in *Vigna radiata*. Si enhances N_2 fixation [124] as its deposition changes the permeability of nodule that may impact the solute transport and gaseous (e.g. oxygen and nitrogen) diffusion though the exact mechanism

is still not clear [125, 126]. Furthermore, enhancement of oxygen and nitrogen fluxes could have accelerated bacteroid metabolism (respiration and nitrogen fixation) inside the root nodules [125]. AMF proved to be more beneficial than Si in upregulating the LHb content and N_2ase activity in all three species especially pigeonpea due to maximum P uptake.

In the present study, Si and AMF inoculations increased the Tre synthesis by upregulating T6PS and T6PP activity and lowering the activity of TRE ultimately reducing the ROS and MDA content.Tre is a common reserve carbohydrate but also a molecule involved in defence actions as it detoxifies ROS [127]. Pigeonpea displayed higher synthesis of Tre which could be due to more per-cent MC than the other two legumes. Calonne et al. [128] reported that in response to chemical (disodium arsenate) or heat stress AMF activated transcriptionally and/or post-transcriptionally the tre metabolism enzymes, which led to tre accumulation. Si supplementation also led to increased tre synthesis with higher accumulation in pigeonpea, moderate in mungbean and lowest in chickpea. Tre and N_2ase activities were observed to have a negative corelation with one another suggesting that the nodules under stress i.e., with reduced N_2ase activity produced more tre to mitigate metal induced oxidative damage. Thus, tre might have played an important role in protecting N_2ase from the free radicals due to its antioxidant nature [129]. Luo et al. [130] observed a direct role of tre in reducing H_2O_2 and $O_2^{\bullet-}$ in heat stressed wheat plants. In the present study the increase in nodular parameters, including NDW and N_2ase activity could be directly related to Si and AMF stimulated tre synthesis that decreased the generation of ROS.

Increased soil enzymatic activities such as URE, AlkPHA and DEH enhanced nutrient availability (N and P) and their uptake by host plant in the presence of *R. irregularis.* AMF increases the soil enzyme activity by improving microbial activities in the plant rhizosphere [131]. These results were strongly supported by the findings of Qian et al. [132] who reported increment in the activities of various soil enzymes in AMF inoculated ryegrass grown in mine soils. Furthermore, fungal association also increased the uptake of nutrients namely Fe and Cu and increased their content in roots and nodules. Si exhibited lower effects than AMF on soil enzymes which could be due to its sparingly soluble nature [133]. However, Si supplementation increased the nutrient status of plant with higher positive impact on pigeonpea followed by mungbean and chickpea. There have been reports regarding its involvement in changing the microbial environment leading to improved soil properties (pH and enzymatic activities) and availability of mineral nutrients to the plants [38]. This might be due to Si stimulated upregulation of the genes that are related with NPK transport and utilization [134]. Fe and Cu uptake and concentrations also increased with Si nutrition as it supressed the Ni uptake from the soil thus increasing the mineral content of the legumes.

Application of Si and AMF positively revived the GS/GOGAT pathway with little increase in GDH, signifying the importance of GS-GOGAT in eliminating Ni induced toxic effects. Pigeonpea was highly responsive to both the treatments which could be related to its ability to absorb Si more efficiently as well as establish a more effective AMF symbiosis than mungbean with chickpea displaying positive impacts with AM only. Calcium silicate ($CaSiO_3$) proved to be beneficial in upregulating the GDH activity in Zn stressed barley plants [135]. In the present study, AMF was more beneficial in improving ammonia assimilation due to the functional complementarity between the two symbiosome. The direct role of AMF on these pathways is still unclear, however the increase in their activity can be attributed to the ability of AMF to impart overall stress tolerance. *R. irregularis* promoted GOGAT and GS activities in chickpea under beryllium stress as reported by Sheteiwy et al. [136].

The present study observed that introduction of Si and AMF modulated ureide metabolism and significantly increased the ureide content under Ni stress, more in pigeonpea than mungbean. The mechanism through which Si and AMF enhance the ureide synthesis is still unclear but it could be due to upregulation of their biosynthetic enzymes and inhibition of the catabolic enzymes. Similar findings were observed in *Arabidopsis* that reported low expression of the ALN degrading enzyme gene, *allantoinase* with a concurrent increase in the expression of ALN biosynthesis enzyme gene, *uricase* under abiotic stress [137–140, 142]. Several studies suggested that ALN may function as an antioxidant due to its role in eliminating ROS-induced oxidative damage in plants [141]. The translocation of ureides to the leaves was restored through the application of Si and AMF thus increasing urea synthesis, more so in AMF inoculated pigeonpea plants, along with enhanced urease activity that ultimately improved the urea catabolism and NH_4^+ production.Chickpea, being an amide transporter displayed a significant increase in Asn content in the nodules with AM supplemented stressed plants while, Si did not seem to play any significant role due to relatively low Si uptake. The enzymes AspAT and AS involved in the synthesis were upregulated that ultimately increased the Asn concentration. Andrade et al. [142] reported high levels of Asn in AMF inoculated jack bean plants subjected to Cu stress.Moreover, supplementation of Si and AMF enhanced the productivity potential of all three legumes, with AMF displaying higher efficiency than Si. AMF inoculations were more beneficial in improving the flower no., pod formation, seed biomass

and overall yield of all three species while Si was effective in pigeonpea and mungbean. This could be attributed to the AM aided increased root biomass, increased nutrient uptake especially P, N_2 fixation and efficient translocation of nitrogen metabolites (amides and ureides). He et al. [143] indicated that AMF (*Glomus mosseae*) decreased the yield losses in tomato under salt stress.

The combined applications of Si + AM completely nullified the negative effects of Ni stress, especially in pigeonpea and mungbean, due to enhanced Si uptake aided by fungal symbiosis. The cumulative treatments not only enhanced the root and shoot biomass, they also positively influenced the nodulation potential, trehalose metabolism, soil enzymes and nutrient acquisition, leading to increased productivity of all three legumes species. Moreover, ammonia assimilation and nitrogen transport were greatly enhanced by their dual application with pigeonpea benefitting to a maximum extent due to highest mycorrhization and silicon uptake, followed by moderate positive effects on mungbean. However, no significant functional complementarity between Si and AMF was observed in case of chickpea.

5 Conclusion

Application of Si helped in the re-establishment of the three legume species subjected to Ni stress with pigeonpea most responsive towards Si nutrition, closely followed by mungbean, with least positive effects displayed by chickpea. The differential impacts of Si nutrition could be related to the disparities in terms of relative Si uptake abilities among the species. AM, on the other hand, was efficient in all experimental species and improved the nitrogen fixing potential and seed yield, with pigeonpea able to establish a stronger AM symbiosis when compared with other legumes. Combined application of + Si + AM proved to be the most beneficial treatment whichhad a cumulative effect in nullifying the harmful effects of Ni stress in all three species, especially in pigeonpea, which could be attributed to maximum AM mediated Si uptake. The findings suggested the importance of identifying particular legume species, able to establish an effective AM symbiosis and benefit from Si nutrition in order to alleviate Ni toxicity in the contaminated soils.

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Data Availability The datasets used and/or analyzed during the currentstudy are available from the corresponding author on request.

Declarations

Ethics Approval Not applicable.

Consent to Participate Not applicable.

Consent for Publication Not applicable.

Competing Interests The authors declare no competing interests.

References

1. Nagajyoti PC, Lee KD, Sreekanth T (2010) Heavy metals, occurrence and toxicity for plants: a review. Environ Chem Lett 8:199–216. https://doi.org/10.1007/s10311-010-0297-8
2. Hussain MB, Ali S, Azam A, Hina S, Farooq MA, Ali B, Bharwana SA, Gill MB (2013) Morphological, physiological and biochemical responses of plants to nickel stress: a review. Afr J Agric Res 8:1596–1602. https://doi.org/10.5897/AJAR12.407
3. National Academy of Sciences (NAS) (1975) Nickel, medical and biological effects of environmental pollutants. National Research Council, National Academy of Sciences, Washington
4. Duke JM (1980) Production and uses of nickel. In: Nriagu JO (ed) Nickel in the environment. Wiley, New York
5. Kasprzak KS (1987) Nickel. Adv Mod Environ Toxicol 11:145–183
6. Eisler R (1998) Nickel hazards to fish, wildlife, and invertebrates: a synoptic review. Biological science report GS/BRD/BSR- 1998–0001, Patuxent Wildlife Research Center, U.S. Geological Survey, Laurel
7. Mustafiz A, Ghosh A, Tripathi AK, Kaur C, Ganguly AK, Bhavesh NS, Tripathi JK, Pareek A, Sopory SK, Singla-Pareek SL (2014) A unique Ni^{2+}-dependent and methylglyoxal- inducible rice glyoxalase I possesses a single active site and functions in abiotic stress response. Plant J 78:951–963. https://doi.org/10.1111/tpj.12521
8. Gajewska E, Słaba M, Andrzejewska R, Skłodowska M (2006) Nickel-induced inhibition of wheat root growth is related to H_2O_2 production, but not to lipid peroxidation. Plant Growth Regul 49:95–103. https://doi.org/10.1007/s10725-006-0018-2
9. Taylor GJ, Stadt KJ (1990) Interactive effects of cadmium, copper, manganese, nickel and zinc on root growth of wheat (*Triticum aestivum*) in solution culture. Plant Soil Sci 4:317–322. https://doi.org/10.1007/978-94-009-0585-6_54
10. Yang X, Baligar VC, Martens DC, Clark RB (1996) Plant tolerance to Ni toxicity. I. Influx, transport and accumulation of Ni in four species. J Plant Nutr 19:73–85. https://doi.org/10.1080/01904169609365108

11. Sreekanth TVM, Nagajyothi PC, Lee KD, Prasad TNVKV (2013) Occurrence, physiological responses and toxicity of nickel in plants. Int J Environ Sci Technol 10:1129–1140. https://doi.org/10.1007/s13762-013-0245-9
12. Athar R, Ahmad M (2002) Heavy metal toxicity in legume-microsymbiont system. J Plant Nutr 25:369–386. https://doi.org/10.1081/PLN-100108842
13. Garg N, Saroy K (2020) Interactive effects of polyamines and arbuscular mycorrhiza in modulating plant biomass, N_2 fixation, ureide, and trehalose metabolism in *Cajanus cajan* (L.) Millsp. genotypes under nickel stress. Environ Sci Pollut Res 27:3043–3064. https://doi.org/10.1007/s11356-019-07300-6
14. Bhalerao SA, Sharma AS, Poojari AC (2015) Toxicity of nickel in plants. Int J Pure ApplBiosci 3:345–355
15. Seregin IV, Kozhevnikova AD (2006) Physiological role of nickel and its toxic effects on higher plants. Russ J Plant Physiol 53:257–277. https://doi.org/10.1134/S1021443706020178
16. Chen C, Huang D, Liu J (2009) Functions and toxicity of nickel in plants: recent advances and future prospects. Clean 37:304–313. https://doi.org/10.1002/clen.200800199
17. Temp GA (1991) Nickel in plants and its toxicity: Ustoichivost' ktyazhelymmetallamdikorastushchikhvidov (Resistance of wild species to heavy metals). In: Alekseeva-Popova NV (ed). Lenuprizdat, Leningrad
18. Seregin IV, Kozhevnikova AD, Kazyumina EM, Ivanov VB (2003) Nickel toxicity and distribution in maize roots. Russ J Plant Physiol 50:1–7
19. El-Bashiti T, Hamamcı H, Öktem HA, Yücel M (2005) Biochemical analysis of trehalose and its metabolizing enzymes in wheat under abiotic stress conditions. Plant Sci 169:47–54. https://doi.org/10.1016/j.plantsci.2005.02.024
20. Jules M, Beltran G, Francois J, Parrou JL (2008) New insights into trehalose metabolism by Saccharomyces cerevisiae: NTH2 encodes a functional cytosolic trehalase, and deletion of TPS1 reveals ATH1p-dependent trehalose mobilization. Appl Environ Microbiol 74:605–614. https://doi.org/10.1128/AEM.00557-07
21. Sharma MP, Grover M, Chourasiya D, Bharti A, Agnihotri R, Maheshwari HS, Pareek A, Buyer JS, Sharma SK, Schütz L, Bagyaraj DJ (2020) Deciphering the role of trehalose in tripartite symbiosis among rhizobia, arbuscular mycorrhizal fungi, and legumes for enhancing abiotic stress tolerance in crop plants. Front Microbiol 11:509919. https://doi.org/10.3389/fmicb.2020.509919
22. Vriezen JAC, De Bruijn FJ, Nüsslein K (2007) Responses of rhizobia to desiccation in relation to osmotic stress, oxygen, and temperature. Appl Environ Microbiol 73:3451–3459. https://doi.org/10.1128/AEM.02991-06
23. Lopez M, Tejera NA, Iribarne C, Lluch C, Herrera-Cervera JA (2008) Trehalose and trehalase in root nodules of *Medicago truncatula* and *Phaseolus vulgaris* in response to salt stress. Physiol Plant 134:575–582. https://doi.org/10.1111/j.1399-3054.2008.01162.x
24. Garg N, Singla P (2016) Stimulation of nitrogen fixation and trehalose biosynthesis by naringenin (Nar) and arbuscular mycorrhiza (AM) in chickpea under salinity stress. Plant Growth Regul 80:5–22. https://doi.org/10.1007/s10725-016-0146-2
25. Suárez R, Wong A, Ramírez M, Barraza A, Orozco MDC, Cevallos MA, Lara M, Hernández G, Iturriaga G (2008) Improvement of drought tolerance and grain yield in common bean by overexpressing trehalose-6-phosphate synthase in rhizobia. Mol Plant Microbe Interact 21:958–966. https://doi.org/10.1094/MPMI-21-7-0958
26. Domínguez-Ferreras A, Soto MJ, Pérez-Arnedo R, Olivares J, Sanjuán J (2009) Importance of trehalose biosynthesis for *Sinorhizobiummelilotio*smotolerance and nodulation of alfalfa roots. J Bacteriol 191:7490–7499. https://doi.org/10.1128/JB.00725-09
27. Sugawara M, Cytryn EJ, Sadowsky MJ (2010) Functional role of *Bradyrhizobium japonicum* trehalose biosynthesis and metabolism genes during physiological stress and nodulation. Appl Environ Microbiol 76:1071–1081. https://doi.org/10.1128/AEM.02483-09
28. Fernandez-Aunián C, Hamouda TB, Iglesias-Guerra F, Argandõa M, Reina- Bueno M, Nieto JJ, Aouani ME, Vargas C (2010) Biosynthesis of compatible solutes in rhizobial strains isolated from *Phaseolus vulgaris* nodules in Tunisian fields. BMC Microbiol 10:192. https://doi.org/10.1186/1471-2180-10-192
29. Kouris-Blazos A, Belski R (2016) Health benefits of legumes and pulses with a focus on Australian sweet lupins. Asia Pac J Clin Nutr 25:1–17
30. Quiles FA, Galvez-Valdivieso G, Guerrero-Casado J, Pineda M, Piedras P (2019) Relationship between ureidic/amidic metabolism and antioxidant enzymatic activities in legume seedlings. Plant PhysiolBiochem 138:1–8. https://doi.org/10.1016/j.plaphy.2019.02.016
31. Sulieman S, Tran LSP (2013) Asparagine: an amide of particular distinction in the regulation of symbiotic nitrogen fixation of legumes. Crit Rev Biotechnol 33:309–327. https://doi.org/10.3109/07388551.2012.695770
32. Kaur H, Chowrasia S, Gaur VS, Mondal TK (2021) Allantoin: emerging role in plant abiotic stress tolerance. Plant Mol Biol Report 39:648–661. https://doi.org/10.1007/s11105-021-01280-z
33. Ashfaque F, Inam A, Inam A, Iqbal S, Sahay S (2017) Response of silicon on metal accumulation, photosynthetic inhibition and oxidative stress in chromium-induced mustard (*Brassica juncea L.*). South Afr J Bot 111:153–160. https://doi.org/10.1016/j.sajb.2017.03.002
34. Deshmukh RK, Ma JF, B´elanger RR, (2017) Role of silicon in plants. Front Plant Sci 8:1858. https://doi.org/10.3389/fpls.2017.01858
35. Epstein E (1999) Silicon. Annu Rev Plant Physiol 50:641–644. https://doi.org/10.1146/annurev.arplant.50.1.641
36. Sommer M, Kaczorek D, Kuzyakov Y, Breuer J (2006) Silicon pools and fluxes in soils and Landscapes: A review. J Plant Nutr Soil Sci 169:310–329. https://doi.org/10.1002/jpln.200521981
37. Mitani N, Ma JF, Iwashita T (2005) Identification of the silicon form in xylem sap of rice (*Oryza sativa* L.). Plant Cell Physiol 46:279–283. https://doi.org/10.1093/pcp/pci018
38. Ma JF, Tamai K, Yamaji N, Mitani N, Konishi S, Katsuhara M, Yano MA (2006) Silicon transporter in rice. Nature 440:688–691. https://doi.org/10.1038/nature04590
39. Ma JF, Yamaji N (2006) Silicon uptake and accumulation in higher plants. Trends Plant Sci 11:392–397. https://doi.org/10.1016/j.tplants.2006.06.007
40. Ma JF, Yamaji N (2008) Functions and transport of silicon in plants. Cell Mol Life Sci 65:3049–3057. https://doi.org/10.1007/s00018-008-7580-x
41. Epstein E (1994) The anomaly of Si in plant biology. Proc Natl Acad Sci 91:11–17. https://doi.org/10.1073/pnas.91.1.1
42. Henriet C, Draye X, Oppitz I, Swennen R, Delvaux B (2006) Effects, distribution and uptake of silicon in banana (Musa spp.) under controlled conditions. Plant Soil 287:359–374. https://doi.org/10.1007/s11104-006-9085-4
43. Liang YC, Zhang WH, Chen Q, Liu YL, Ding RX (2006) Effect of exogenous silicon (Si) on HÞ-ATPase activity, phospholipids and fluidity of plasma membrane in leaves of salt-stressed barley (*Hordeum vulgare* L.). Environ Exp Bot 57:212–219. https://doi.org/10.1016/j.envexpbot.2005.05.012

44. Ma JF, Miyake Y, Takahashi E (2001) Si as a beneficial element for crop plants. Stud Plant Sci 8:17–39. https://doi.org/10.1016/S0928-3420(01)80006-9
45. Liang YC, Si J, Ro€mheld V (2005) Si uptake and transport is an active process in Cucumis sativus L. New Phytol 67:797–804. https://doi.org/10.1111/j.1469-8137.2005.01463.x
46. Ouellette S, Goyette MH, Labb´e C, Laur, J, Gaudreau L, Gosselin A, Dorais M, Deshmukh RK, B´elanger RR (2017) Silicon transporters and effects of silicon amendments in strawberry under high tunnel and field conditions. Front Plant Sci 8:949. https://doi.org/10.3389/fpls.2017.00949
47. Zargar SM, Nagar P, Deshmukh R, Nazir M, Wani AA, Masoodi KZ, Agrawal GK, Rakwal R (2017) Aquaporins as potential drought tolerance inducing proteins: towards instigating stress tolerance. J Proteom 169:233–238. https://doi.org/10.1016/j.jprot.2017.04.010
48. Shivaraj SM, Mandlik R, Bhat JA, Raturi G, Elbaum R, Alexander L, Tripathi DK, Deshmukh R, Sonah H (2021) Outstanding questions on the beneficial role of silicon in crop plants. Plant Cell Physiol 63:4–18. https://doi.org/10.1093/pcp/pcab145
49. Mandlik R, Thakral V, Raturi G, Shinde S, Nikoli´c M, Tripathi DK, Sonah H, Deshmukh R, (2020) Significance of silicon uptake, transport, and deposition in plants. J Exp Bot 71:6703–6718. https://doi.org/10.1093/jxb/eraa301
50. Lee JK, Kozono D, Remis J, Kitagawa Y, Agre P, Stroud RM (2005) Structural Basis for Conductance by the Archaeal Aquaporin AqpM at 1.68 Å. Proc Natl Acad Sci 102:18932–18937. https://doi.org/10.1073/pnas.0509469102
51. Liang Y, Sun W, Zhu YG, Christie P (2007) Mechanisms of silicon-mediated alleviation of abiotic stresses in higher plants: a review. Environ Pollu 147:422–428. https://doi.org/10.1016/j.envpol.2006.06.008
52. Amin M, Ahmad R, Ali A, Aslam M, Lee DJ (2016) Silicon fertilization improves the maize (*Zea mays* L.) performance under limited moisture supply. Cereal Res Comm 44:172–185. https://doi.org/10.1556/0806.43.2015.035
53. Antunes PM, Goss MJ (2005) Communication in the tripartite symbiosis formed by arbuscular mycorrhizal fungi, rhizobia and legume plants: a review. Roots and Soil Management: Interactions between Roots and the Soil 48:199–222. https://doi.org/10.2134/agronmonogr48.c11
54. Antunes PM, De Varennes A, Zhang T, Goss MJ (2006) The tripartite symbiosis formed by indigenous arbuscular mycorrhizal fungi, *Bradyrhizobium japonicum* and soybean under field conditions. J Agron Crop Sci 192:373–378. https://doi.org/10.1111/j.1439-037X.2006.00223.x
55. Smith SE, Smith FA, Jakobsen I (2003) Mycorrhizal fungi can dominate phosphate supply to plants irrespective of growth responses. Plant Physiol 133:16–20. https://doi.org/10.1104/pp.103.024380
56. Nell M, Wawrosch C, Steinkellner S, Vierheilig H, Kopp B, Lössl A (2010) Root colonization by symbiotic arbuscular mycorrhizal fungi increases sesquiterpenic acid concentrations in *Valeriana officinalis* L. Planta Med 76:393–398. https://doi.org/10.1055/s-0029-1186180
57. Garg N, Cheema A (2021) Relative roles of Arbuscular Mycorrhizae in establishing a correlation between soil properties, carbohydrate utilization and yield in Cicer arietinum L under As stress. Ecotox Environ Safe 207:111196. https://doi.org/10.1016/j.ecoenv.2020.111196
58. Garg N, Singla P (2012) The role of *Glomus mosseae* on key physiological and biochemical parameters of pea plants grown in arsenic contaminated soil. Sci Hortic 143:92–101. https://doi.org/10.1016/j.scienta.2012.06.010
59. Latef AAHA, Hashem A, Rasool S, Abd-Allah EF, Alqarawi AA, Egamberdieva D, Jan S, Anjum NA, Ahmad P (2016) Plants mycorrhizal symbiosis and abiotic stress in plants: A Review. J Plant Biol 59:407–426. https://doi.org/10.1007/s12374-016-0237-7
60. Yost RS, Fox RL (1982) Influence of mycorrhizae on the mineral contents of cowpea and soybean grown in an oxisol. Agron J 74:475–481. https://doi.org/10.2134/agronj1982.00021962007400030018x
61. Clark RB, Zeto SK (2000) Mineral acquisition by arbuscular mycorrhizal plants. J Plant Nutr 23:867–902. https://doi.org/10.1080/01904160009382068
62. Garg N, Singh S (2017) Arbuscular mycorrhiza *Rhizophagusirregularis*, and silicon modulate growth, proline biosynthesis and yield in *Cajanus cajan,* L. Millsp. (pigeon pea) genotypes under cadmium and zinc stress. J Plant Growth Regul 37:1–18. https://doi.org/10.1007/s00344-017-9708-4
63. Garg N, Kashyap L (2019) Joint effects of Si and mycorrhiza on the antioxidant metabolism of two pigeonpea genotypes under As (III) and (V) stress. Environ Sci Pollut Res 26:7821–7839. https://doi.org/10.1007/s11356-019-04256-5
64. Hammer EC, Nasr H, Pallon J, Olsson PA, Wallander H (2011) Elemental composition of arbuscular mycorrhizal fungi at high salinity. Mycorrhiza 21:117–129. https://doi.org/10.1007/s00572-010-0316-4
65. Hajiboland R, Moradtalab N, Aliasgharzad N, Eshaghi Z, Feizy J (2018) Silicon influences growth and mycorrhizal responsiveness in strawberry plants. Physiol Mol Biol Plants 24:1103–1115. https://doi.org/10.1007/s12298-018-0533-4
66. Fillinger S, Chaveroche M, Van Dijck P, De Vries R, Ruijter G, Thevelein J, d'Enfert C (2001) Trehalose is required for the acquisition of tolerance to a variety of stresses in the filamentous fungus *Aspergillus nidulans*. Microbiol 147:1851–1862. https://doi.org/10.1099/00221287-147-7-1851
67. Gancedo C, Flores C (2004) The importance of a functional trehalose biosynthetic pathway for the life of yeasts and fungi. FEMS Yeast Res 4:351–359. https://doi.org/10.1016/S1567-1356(03)00222-8
68. Van Dijck P, De Rop L, Szlufcik K, Van Ael E, Thevelein JM (2002) Disruption of the Candida albicans TPS2 gene encoding trehalose-6-phosphate phosphatase decreases infectivity without affecting hypha formation. Infect Immun 147:1772–1782. https://doi.org/10.1128/IAI.70.4.1772-1782.2002
69. Merga B, Haji J (2019) Economic importance of chickpea: Production, value, and world trade. Cogent Food Agric 5:1615718. https://doi.org/10.1080/23311932.2019.1615718
70. Pandey AK, Burlakoti RR, Kenyon L, Nair RM (2018) Perspectives and challenges for sustainable management of fungal diseases of mungbean [*Vigna radiata* (L.) R. Wilczek var. radiata]: a review. Front Environ Sci 6:53. https://doi.org/10.3389/fenvs.2018.00053
71. Leport L, Turner NC, Davies SL, Siddique KHM (2006) Variation in pod production and abortion among chickpea cultivars under terminal drought. Eur J Agron 24:236–246. https://doi.org/10.1016/j.eja.2005.08.005
72. Giovannetti M, Mosse B (1980) An evaluation of techniques formeasuring vesicular arbuscular mycorrhizal infection in roots. New Phytol 84:489–500
73. Hetrick BAD, Wilson GWT, Todd TC (1992) Relationships of mycorrhizal symbiosis, rooting strategy, and phenology among tallgrass prairie forbs. Can J Bot 70:1521–1528. https://doi.org/10.1139/b92-191
74. Velikova V, Yordanov I, Edreva A (2000) Oxidative stress and some antioxidant systems in acid rain treated bean plants. Plant Sci 151:59–66. https://doi.org/10.1016/S0168-9452(99)00197-1
75. Doke N (1983) Involvement of superoxide anion generation in the hypersensitive response of potato tuber tissues to infection with

an incompatible race of *Phytophthora infestans* and to the hyphal wall components. Physiol Plant Pathol 23:345–357. https://doi.org/10.1016/0048-4059(83)90019-X
76. Heath RL, Packer I (1968) Photoperoxidation in isolated chloroplast I, kinetics and stochiometry of fatty acid peroxidation. Arch BiochemBiophys 125:189–198. https://doi.org/10.1016/0003-9861(68)90654-1
77. Zwiazek JJ, Blake TJ (1991) Early detection of membrane injury in black spruce (P*iceamariana*). Can J For Res 21:401–404. https://doi.org/10.1139/x91-050
78. Hartree EF (1957) Haematin compounds. In: Paech K, Tracey MV (eds) Modern methods of plant analysis. Springer-Verlag, Germany, Berlin
79. Herdina JA, Silsbury JH (1990) Estimating nitrogenase activity of faba bean (*Vicia faba*) by acetylene reduction (AR) assay. Aust J Plant Physiol 17:489–502
80. Streeter JG, Strimbu CE (1998) Simultaneous extraction and derivatization of carbohydrates from green plant tissues for analysis by gas–liquid chromatography. Anal Biochem 259:253–257. https://doi.org/10.1006/abio.1998.2675
81. Salminen SO, Streeter JG (1986) Enzymes of alpha, alpha-trehalose metabolism in soybean nodules. Plant Physiol 81:538–541
82. Padilla L, Krämer R, Stephanopoulos G, Agosin E (2004) Overproduction of trehalose: heterologous expression of Escherichia coli trehalose-6-phosphate synthase and trehalose-6- phosphate phosphatase in *Corynebacterium glutamicum*. Appl Environ Microbiol 70:370–376. https://doi.org/10.1128/AEM.70.1.370-376.2004
83. Müller J, Xie ZP, Staehelin C, Mellor RB, Boller T, Wiemken A (1994) Trehalose and trehalase in root nodules from various legumes. Physiol Plant 90:86–92. https://doi.org/10.1111/j.1399-3054.1994.tb02196.x
84. Miller GL (1959) Use of dinitrosulphosalicylic acid (DNSA) reagent for determination of reducing sugar. Anal Chem 31:426–428. https://doi.org/10.1021/ac60147a030
85. May PB, Douglas LA (1976) Assay for soil urease activity. Plant Soil 45:301–305. https://doi.org/10.1007/BF00011156
86. Eivazi F, Tabatabai MA (1977) Phosphatases in soils. Soil BiolBiochem 9:167–172. https://doi.org/10.1016/0038-0717(77)90070-0
87. Casida LE Jr, Klein DA, Santoro T (1964) Soil dehydrogenase activity. Soil Sci 98:371–376
88. Thimmaiah SR (1999) Standard methods of biochemical analysis. Kalyani Publisher, New Delhi
89. Schubert KR (1981) Enzymes of purine biosynthesis and catabolism in *Glycine max*: Comparison of activities with N_2 fixation and composition of xylem exudates during nodule development. Plant Physiol 68:1115–1122. https://doi.org/10.1104/pp.68.5.1115
90. Vogels GD, Van der Drift C (1970) Differential analyses of glyoxylate derivatives. Anal Biochem 33:143–157. https://doi.org/10.1016/0003-2697(70)90448-3
91. Kyllingsbæk A (1975) Extraction and colorimetric determination of urea in plants. Acta Agric Scand 25:109–112. https://doi.org/10.1080/00015127509436239
92. Hogan ME, Swift IE, Done J (1983) Urease assay and ammonia release from leaf tissues. Phytochemistry 22:663–667. https://doi.org/10.1016/S0031-9422(00)86958-7
93. Gonza´ lez EM, Gordon AJ, James CL, Arrese-Igor C, (1995) The role of sucrose synthase in the response of soybean nodules to drought. J Exp Bot 46:1515–1523. https://doi.org/10.1093/jxb/46.10.1515
94. Rognes SE (1975) Glutamine-dependent asparagine synthetase from *Lupinus luteus*. Phytochemistry 14:1–8. https://doi.org/10.1016/0031-9422(75)83108-6
95. Vadez V, Sinclair TR, Sarraj R (2000) Asparagine and ureide accumulation in nodules and shoots as feedback inhibitors of N_2 fixation in soybean. Physiol Plant 110:215–233. https://doi.org/10.1034/j.1399-3054.2000.110211.x
96. Yang Y, Song Y, Scheller HV, Ghosh A, Ban Y, Chen H, Tang M (2015) Community structure of arbuscular mycorrhizal fungi associated with *Robiniapseudoacacia* in uncontaminated and heavy metal contaminated soils. Soil BiolBiochem 86:146–158. https://doi.org/10.1016/j.soilbio.2015.03.018
97. Philippot L, Raaijmakers JM, Lemanceau P, van der Putten WH (2013) Going back to the roots: the microbial ecology of the rhizosphere. Nat Rev Microbiol 11:789–799. https://doi.org/10.1038/nrmicro3109
98. Abd-Alla MH, Bashandy SR, Bagy MK, El-enany AWE (2014) *Rhizobium tibeticum* activated with a mixture of flavonoids alleviates nickel toxicity in symbiosis with fenugreek (*Trigonella foenum graecum* L.). Ecotoxicology 23:946–959. https://doi.org/10.1007/s10646-014-1239-1
99. Stambulska UY, Bayliak MM, Lushchak VI (2018) Chromium (VI) toxicity in legume plants: modulation effects of rhizobial symbiosis. BioMed Res Int. https://doi.org/10.1155/2018/8031213
100. Ali B, Hayat S, Hayat Q, Ahmad A (2010) Cobalt stress affects nitrogen metabolism, photosynthesis and antioxidant system in chickpea (*Cicer arietinum* L.). J Plant Interact 5:223–231. https://doi.org/10.1080/17429140903370584
101. Gonzalez EM, Galvez RL, Aparicio-Tejo PM, Arrese-lgor C (2001) Insights into the regulation of nitrogen fixation in pea nodules: lessons from drought, abscissic acid and increased photo-assimilate availability. Agronomie 21:607–613. https://doi.org/10.1051/agro:2001151
102. Sethi S, Gupta S (2015) Responses of soil enzymes to different heavy metals. Biolife 3:147–153
103. Hussain RM (2017) The effect of phosphorus in nitrogen fixation in legumes. J Plant Nutr Soil Sci 167:125–137. https://doi.org/10.19080/ARTOAJ.2017.04.555654
104. Hassan MU, Chattha MU, Khan I, Chattha MB, Aamer M, Nawaz M, Ali A, Khan MA, Khan TA (2019) Nickel toxicity in plants: reasons, toxic effects, tolerance mechanisms, and remediation possibilities—a review. Environ Sci Pollut Res 26:12673–12688. https://doi.org/10.1007/s11356-019-04892-x
105. Haider FU, Wang X, Farooq M, Hussain S, Cheema SA, Ul Ain N, Virk AL, Ejaz M, Janyshova U, Liqun C (2022) Biochar application for the remediation of trace metals in contaminated soils: Implications for stress tolerance and crop production. Ecotox Environ Safe 230:113165. https://doi.org/10.1016/j.ecoenv.2022.113165
106. Rizwan M, Usman K, Alsafran M, Jabri HA, Samreen T, Saleem MH, Tu S (2022) Nickel Toxicity Interferes with NO3−/NH4+ Uptake and Nitrogen Metabolic Enzyme Activity in Rice (Oryza sativa L.). Plants 11:1401. https://doi.org/10.1016/j.ecoenv.2022.113165
107. Balestrasse KB, Benavides MP, Gallego SM, Tomaro ML (2003) Effect of cadmium stress on nitrogen metabolism in nodules and roots of soybean plants. Funct Plant Biol 30:57–64. https://doi.org/10.1071/FP02074
108. Khadri M, Pliego L, Soussi M, Lluch C, Ocaña A (2001) Ammonium assimilation and ureide metabolism in common bean (*Phaseolus vulgaris*) nodules under salt stress. Agronomie 21:635–643
109. Todd CD, Tipton PA, Blevins DG, Piedras P, Pineda M, Polacco JC (2006) Update on ureide degradation in legumes. J Expl Bot 57:5–12. https://doi.org/10.1093/jxb/erj013
110. Dresler S, Hawrylak-Nowak B, Kováčik J, Pochwatka M, Hanaka A, Strzemski M, Sowa I, Wójciak-Kosior M (2019) Allantoin attenuates cadmium-induced toxicity in cucumber

plants. Ecotox Environ Safe 170:120–126. https://doi.org/10.1016/j.ecoenv.2018.11.119
111. Barcelos JPQ, Reis HPG, Godoy CV, Gratão PL, Furlani Junior E, Putti FF, Campos M, Reis AR (2018) Impact of foliar nickel application on urease activity, antioxidant metabolism and control of powdery mildew (*Microsphaeradiffusa*) in soybean plants. Plant Pathol 67:1502–1513. https://doi.org/10.1111/ppa.12871
112. Ali S, Abbas Z, Seleiman MF, Rizwan M, YavaŞ İ, Alhammad BA, Shami A, Hasanuzzaman M, Kalderis D (2020) Glycine betaine accumulation, significance and interests for heavy metal tolerance in plants. Plants 9:896. https://doi.org/10.3390/plants9070896
113. Irfan M, Hasan SA, Hayat S, Ahmad A (2015) Photosynthetic variation and yield attributes of two mustard varieties against cadmium phytotoxicity. Cogent Food Agric 1:1106186. https://doi.org/10.1080/23311932.2015.1106186
114. Wyszkowski M, Radziemska M (2010) Effects of chromium (III and VI) on spring barley and maize biomass yield and content of nitrogenous compounds. J Toxicol Environ Health 73:1274–1282. https://doi.org/10.1080/15287394.2010.492016
115. Kumar S, Shah SH, Vimala Y, Jatav HS, Ahmad P, Chen Y, Siddique KH (2022) Abscisic acid: Metabolism, transport, crosstalk with other plant growth regulators, and its role in heavy metal stress mitigation. Front Plant Sci 13:972856. https://doi.org/10.3389/fpls.2022.972856
116. Bhat JA, Shivaraj SM, Singh P, Navadagi DB, Tripathi DK, Dash PK, Solanke AU, Sonah H, Deshmukh R (2019) Role of silicon in mitigation of heavy metal stresses in crop plants. Plants 8:71. https://doi.org/10.3390/plants8030071
117. Riaz M, Kamran M, Fang Y, Wang Q, Cao H, Yang G, Deng L, Wang Y, Zhou Y, Anastopoulos I, Wang X (2021) Arbuscular mycorrhizal fungi-induced mitigation of heavy metal phytotoxicity in metal contaminated soils: A critical review. J Hazard Mater 402:123919. https://doi.org/10.1016/j.jhazmat.2020.123919
118. Chandrasekaran M (2022) Arbuscular mycorrhizal fungi mediated enhanced biomass, root morphological traits and nutrient uptake under drought stress: A meta-analysis. J Fungi 8:660. https://doi.org/10.3390/jof8070660
119. Mandlik R, Singla P, Kumawat S, Khatri P, Ansari W, Singh A, Sharma Y, Singh A, Solanke A, Nadaf A, Sonah H, Deshmukh R (2022) Understanding aquaporin regulation defining silicon uptake and role in arsenic, antimony and germanium stress in pigeonpea (*Cajanus cajan*). Environ Pollut 294:118606. https://doi.org/10.1016/j.envpol.2021.118606
120. Meena VD, Dotaniya ML, Coumar V, Rajendiran S, Kundu S, RaoAS, (2014) A case for silicon fertilization to improve crop yieldsin tropical soils. Proc Natl Acad Sci, India Sect b: Biol Sci 84:505–518. https://doi.org/10.1007/s40011-013-0270-y
121. Etesami H, Jeong BR, Glick BR (2021) Contribution of arbuscular mycorrhizal fungi, phosphate–solubilizing bacteria, and silicon to P uptake by plant. Front Plant Sci 12:699618. https://doi.org/10.3389/fpls.2021.699618
122. Putra R, Waterman JM, Mathesius U, Wojtalewicz D, Powell JR, Hartley SE, Johnson SN (2022) Benefits of silicon-enhanced root nodulation in a model legume are contingent upon rhizobial efficacy. Plant Soil 77:201–217. https://doi.org/10.1007/s11104-022-05358-9
123. Gough EC, Owen KJ, Zwart RS, Thompson JP (2021) Arbuscular mycorrhizal fungi acted synergistically with *Bradyrhizobium* sp. to improve nodulation, nitrogen fixation, plant growth and seed yield of mung bean (*Vigna radiata*) but increased the population density of the root-lesion nematode *Pratylenchusthornei*. Plant Soil 465:431–452. https://doi.org/10.1007/s11104-021-05007-7
124. Putra R, Powell JR, Hartley SE, Johnson SN (2020) Is it time to include legumes in plant silicon research? FunctEcol 34:1142–1157. https://doi.org/10.1111/1365-2435.13565
125. Dakora F, Atkins C (1989) Diffusion of oxygen in relation to structure and function in legume root nodules. Aust J Plant Physiol 16:131–140. https://doi.org/10.1071/PP9890131
126. Sheehy JE, Minchin FR, Witty JF (1985) Control of nitrogen fixation in a legume nodule: An analysis of the role of oxygen diffusion in relation to nodule structure. Annal Bot 55:549–562. https://doi.org/10.1093/oxfordjournals.aob.a086930
127. Wang K, Li F, Gao M, Huang Y, Song Z (2020) Mechanisms of trehalose-mediated mitigation of Cd toxicity in rice seedlings. J Clean Prod 267:121982. https://doi.org/10.1016/j.jclepro.2020.121982
128. Calonne M, Fontaine J, Debiane D, Laruelle F, Grandmougin-Ferjani A, Sahraoui ALH (2014) The arbuscular mycorrhizal *Rhizophagusirregularis* activates storage lipid biosynthesis to cope with the benzo [a] pyrene oxidative stress. Phytochemistry 97:30–37. https://doi.org/10.1016/j.phytochem.2013.10.014
129. González-Párraga P, Hernández JA, Argüelles JC (2003) Role of antioxidant enzymatic defences against oxidative stress (H_2O_2) and the acquisition of oxidative tolerance in *Candida albicans*. Yeast 20:1161–1169. https://doi.org/10.1002/yea.1029
130. Luo Y, Li WM, Wang W (2008) Trehalose: protector of antioxidant enzymes or reactive oxygen species scavenger under heat stress? Environ Exp Bot 63:378–384. https://doi.org/10.1016/j.envexpbot.2007.11.016
131. Kaiser C, Kilburn MR, Clode PL, Fuchslueger L, Koranda M, Cliff JB, Solaiman ZM, Murphy DV (2015) Exploring the transfer of recent plant photosynthates to soil microbes: mycorrhizal pathway vs direct root exudation. New Phytolo 205:1537–1551. https://doi.org/10.1111/nph.13138
132. Qian K, Wang L, Yin N (2012) Effects of AMF on soil enzyme activity and carbon sequestration capacity in reclaimed mine soil. Int J Min Sci Technol 22(4):553–557. https://doi.org/10.1016/j.ijmst.2012.01.019
133. Bhalla S, Garg N (2021) Arbuscular mycorrhizae and silicon alleviate arsenic toxicity by enhancing soil nutrient availability, starch degradation and productivity in *Cajanus cajan* (L.) Millsp. Mycorrhiza 31:735–754. https://doi.org/10.1007/s00572-021-01056-z
134. Khan E, Gupta M (2018) Arsenic–silicon priming of rice (*Oryza sativa* L.) seeds influence mineral nutrient uptake and biochemical responses through modulation of Lsi-1, Lsi-2, Lsi-6 and nutrient transporter genes. Sci Rep 8:1–16. https://doi.org/10.1038/s41598-018-28712-3
135. Paradisone V, Navarro-León E, Ruiz JM, Esposito S, Blasco B (2021) Calcium silicate ameliorates zinc deficiency and toxicity symptoms in barley plants through improvements in nitrogen metabolism and photosynthesis. Acta Physiol Plant 43:1–11. https://doi.org/10.1007/s11738-021-
136. Sheteiwy MS, Ahmed M, Korany SM, Alsherif EA, Mowafy AM, Chen J, ośko I, Selim S AbdElgawad H, (2022) Arbuscular Mycorrhizal Fungus "*Rhizophagusirregularis*" impacts on physiological and biochemical responses of ryegrass and chickpea plants under beryllium stress. Environ Pollut 315:120356. https://doi.org/10.1016/j.micres.2022.127254
137. Irani S, Todd CD (2016) Ureide metabolism under abiotic stress in *Arabidopsis thaliana*. Plant Physiol 199:87–95. https://doi.org/10.1016/j.jplph.2016.05.011
138. Lescano CI, Martini C, González CA, Desimone M (2016) Allantoin accumulation mediated by allantoinase down regulation and transport by Ureide Permease 5 confers salt stress tolerance to *Arabidopsis* plants. Plant Mol Biol 91:581–595. https://doi.org/10.1007/s11103-016-0490-7

139. Nourimand M, Todd CD (2016) Allantoin increases cadmium tolerance in *Arabidopsis* via activation of antioxidant mechanisms. Plant Cell Physiol 57:2485–2496. https://doi.org/10.1093/pcp/pcw162
140. Irani S, Lobo JM, Gray GR, Todd CD (2018) Allantoin accumulation in response to increased growth irradiance in *Arabidopsis thaliana*. Biol Plant 62:181–187. https://doi.org/10.1007/s10535-017-0747-2
141. Brychkova G, Alikulov Z, Fluhr R, Sagi M (2008) A critical role for ureides in dark and senescence-induced purine remobilization is unmasked in the Atxdh1 *Arabidopsis* mutant. Plant J 54:496–509. https://doi.org/10.1111/j.1365-313X.2008.03440.x
142. Andrade SA, Gratao PL, Azevedo RA, Silveira AP, Schiavinato MA, Mazzafera P (2010) Biochemical and physiological changes in jack bean under mycorrhizal symbiosis growing in soil with increasing Cu concentrations. Environ Exp Bot 68:198–207. https://doi.org/10.1016/j.envexpbot.2009.11.009
143. He Z, He C, Zhang Z, Zou Z, Wang H (2007) Changes of antioxidative enzymes and cell membrane osmosis in tomato colonized by arbuscular mycorrhizae under NaCl stress. Colloids Surf B: Biointerfaces 59:128–133. https://doi.org/10.1016/j.colsurfb.2007.04.023