Environmental Science and Pollution Research (2020) 27:3043–3064
https://doi.org/10.1007/s11356-019-07300-6

RESEARCH ARTICLE

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

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Received: 26 August 2019 / Accepted: 4 December 2019 / Published online: 14 December 2019

Abstract

Nickel (Ni) is an essential micronutrient but considered toxic for plant growth when present in excess in the soil. Polyamines (PAs) and arbuscular mycorrhiza (AM) play key roles in alleviating metal toxicity in plants. Present study compared the roles of AM and PAs in improving rhizobial symbiosis, ureide, and trehalose (Tre) metabolism under Ni stress in *Cajanus cajan* (pigeon pea) genotypes (Pusa 2001, AL 201). The results documented significant negative impacts of Ni on plant biomass, especially roots, more in AL 201 than Pusa 2001. Symbiotic efficiency with *Rhizobium* and AM declined under Ni stress, resulting in reduced AM colonization, N_2 fixation, and ureide biosynthesis. This decline was proportionate to increased Ni uptake in roots and nodules. Put-reduced Ni uptake improved plant growth and functional efficiency of nodules and ureides synthesis, with higher positive effects than other PAs. However, AM inoculations were most effective in enhancing nodulation, nitrogen fixing potential, and Tre synthesis under Ni toxicity. Combined applications of AM with respective PAs, especially +Put+AM, were highly beneficial in alleviating Ni-induced nodule senescence by arresting leghemoglobin degradation and improving functional efficiency of nodules by boosting Tre metabolism, especially in Pusa 2001. The study suggested use of Put along with AM as a promising approach in imparting Ni tolerance to pigeon pea plants.

Keywords Arbuscular mycorrhiza · Nickel · Pigeon pea · Polyamines · Trehalose · Ureides

Highlights
- Ni stress negatively affects growth and mycorrhizal and rhizobial symbioses in pigeon pea
- Among the three PAs (Put, Spd, Spm), Put is most effective in alleviating Ni stress
- AM is more effective than PAs in improving biomass, ureide, and trehalose biosynthesis
- PAs complemented AM by enhancing symbiotic efficiency and nutrient acquisition
- +Put+AM is identified as a promising approach in imparting Ni tolerance to pigeon pea

Responsible editor: Gangrong Shi

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Introduction

Nickel (Ni) is the 24th most available element in earth and belongs to transition series (group VIII B) in periodic table (Sachan and Lal 2017). The oxidation state, Ni (II), is considered the only accessible form for plants (Seregin and Kozhevnikova 2006; Bhalerao et al. 2015). It plays diverse roles in the metabolism of plants, e.g., ureolysis, methane biogenesis, hydrogen metabolism, and acetogenesis (Collard et al. 1994; Mulrooney and Hausinger 2003; Vatansever et al. 2017). Ni is required more for legumes than other crops because it plays a vital role in nodulation, nitrogen (N) fixation, as well as ureides (allantoin, ALN; allantoic acid, ALA) transport to other plant organs (Zrenner et al. 2006; Sprent and James 2007; González-Guerrero et al. 2014; Freitas et al. 2018). Ni acts as a cofactor for urease and catalyzes the conversion of urea into ammonium ions (NH_4^+), which is further used by plants as a source of N (Sakamoto and Bryant 2001; Barcelos et al. 2018).

Although Ni is required in numerous metabolic phenomena, its elevated levels in the soil, however, are highly toxic to plants and threaten agricultural productivity (Gajewska and Skłodowska 2008; Shafeeq et al. 2012; Jamil et al. 2014). Ni has been placed at 57th position in the list of hazardous substances by ATSDR (American Agency for Toxic Substances and Disease Registry 2017). Ni-affected areas in the world include Australia, Canada, Cuba, Indonesia, South Africa, the USA, etc. (National Academy of Sciences (NAS) 1975; Duke 1980; Kasprzak 1987; Eisler 1998; Sreekanth et al. 2013). India is largely affected by Ni toxicity because groundwater Ni levels have been reported in the range of 10 to 129 mg kg^{-1} (Panwar et al. 2010; Sharma et al. 2013). Main sources of Ni pollution include mining, electrical batteries, metallurgical and electroplating industries, and chemical and food industries (Rathor et al. 2014; Bhalerao et al. 2015). In the last decade, Ni toxicity has become a serious issue, since it had reached up to 26,000 ppm in contaminated soils (Yusuf et al. 2011; Alloway 2012; Bhalerao et al. 2015). Excess of Ni in soil reduces seed germination, plant biomass, nutrient absorption by roots, N_2 fixation, photosynthesis, respiration, yield, etc. (Pandey and Sharma 2002; Rahman et al. 2005; Gajewska et al. 2006; Vatansever et al. 2017; Shahzad et al. 2018). Moreover, it induces negative impact on legume-rhizobia symbiosis by disturbing nodulation and N_2 fixation process (Seregin and Kozhevnikova 2006; Polacco et al. 2013). Soluble Ni (II) is substantially absorbed by cation channels passively and competes with other essential ions of metals (Zn, Cu, and Fe) when absorbed by roots (Gray and Mclaren 2006; Page et al. 2006).

In recent years, trehalose (Tre) (an organic compatible solute) has been reported to act as a carbon (C) source and safeguards the free-living state of rhizobia under HMs and salt stress conditions (Garg and Pandey 2016; Garg and Singla 2016; Garg and Singh 2018). It is synthesized by bacteria such as *Rhizobium*, yeast, certain species of fungi such as arbuscular mycorrhiza (AM), and plants (Crowe et al. 1992; Zahran 2010) and has been detected in bacteroids as well as root nodules of legumes (Müller et al. 2001). Moreover, synthesis of Tre has been correlated to the efficient N_2 fixation and plant tolerance during drought, salinity, and metal stresses in legume species (Farías-Rodríguez et al. 1998; López et al. 2006; Garg and Singh 2018). It is synthesized by trehalose-6-phosphate synthase (T6PS) and trehalose-6-phosphatase (T6PP) enzyme in the nodules. Tre is then converted into two molecules of glucose by trehalase (TRE) (Jorge et al. 1997; Jules et al. 2008). However, reports on the impact of Ni on Tre biosynthesis in legumes are lacking.

Recent studies have indicated that exogenous application of polyamines (PAs), namely, putrescine (Put) [$NH_2(CH_2)_4NH_2$], spermidine (Spd) [$NH_2(CH_2)_3NH(CH_2)_4NH_2$], and spermine (Spm) [$NH_2(CH_2)_3NH(CH_2)_4NH(CH_2)_3NH_2$] has been reported to confer enhanced tolerance to heavy metals (HMs) stress in different crop plants (Bagni and Tassoni 2001; Duan et al. 2008; Gupta et al. 2013; Tiburcio et al. 2014). Nodules of legume crops accumulate 5–10 times higher PAs concentration than other plant organs (Fujihara et al. 1994; Efrose et al. 2008). Alterations in PA concentrations have been reported to modulate the control nodule number as well as biomass (Vassileva and Ignatov 1999; Terakado et al. 2006; Jiménez Bremont et al. 2014). Several researchers have indicated differential role of PAs in different crops under metal stress. Exogenous Spd and Spm have been found to reduce Cu stress in the leaves of *Nymphoides peltatum* (Wang et al. 2007), while in mung bean, Spm reduced the Cd stress (Nahar et al. 2016). In case of Ni stress, exogenous treatment of Put has been reported to stimulate accumulation of Spd and Spm, especially Put in *Brassica napus*, thus imparting stress tolerance (Shevyakova et al. 2011). However, reports on differential role of these three PAs (Put, Spd, and Spm) in alleviating Ni stress in case of legumes including pigeon pea are scanty.

In addition, arbuscular mycorrhiza (AM) are an integral part of the rhizosphere and can enhance the phytoremediation of HM-polluted soils through enhanced plant growth, water, nutrient uptake, as well as nitrogen fixation (Gonzalez-Chavez et al. 2002; Augé 2004; Javaid 2010; Shaker-Koohi 2014; Yang et al. 2015). The positive effects of AM inoculation on Ni tolerance in plants have been documented, with an AM *Funneliformis mosseae*-induced decline in Ni uptake in *Festuca arundinacea* (Shabani and Sabzalian 2016); *Sorghum vulgare* with two *Glomus etunicatum* isolates (Amir et al. 2013); *Costularia comosa* with *Glomus etunicatum* (Lagrange et al. 2011). Moreover, AM has also been reported to accumulate Tre in high concentration in the spores and sclerotia which serve as energy and carbohydrate reserves. Synthesis of Tre could help in phosphate release/transport to the plant, thereby improving nodulation potential (Ocón et al. 2007). Moreover, PAs have been reported to improve mycorrhizal colonization and infection in its initial stages of establishment (El Ghachtouli et al. 1995; Wu et al. 2012), since, various PAs especially, Put has been found in the spores of *Funneliformis mosseae* while Spd and Spm in *Gigaspora rosea* (El Ghachtouli et al. 1995; Sannazzaro et al. 2004). Additionally, exogenous Put, Spd, and Spm have been found to increase mycorrhizal colonization in *Citrus limonia* seedlings (Yao et al. 2010). However, relative and interactive effect of AM and PAs in enhancing N_2 fixation, ureide synthesis, and Tre metabolism in legume crops under Ni toxicity has not been explored and needs detailed investigations.

Cajanus cajan (L.) Millsp (pigeon pea) is a major legume crop of the tropical and subtropical regions, covering massive areas of developing countries from Africa to Asia to Latin America. It is the third important grain legume worldwide, currently cultivated on 7.02 million hectares with 6.81 million metric tons (MMT) annual production. India is the largest

producer with 5.38 million hectares under cultivation and 4.87 MMT annual production (FAOSTAT 2017). It is highly nutritional due to high quantity of proteins and amino acids such as methionine, lysine, and tryptophan. It has been extensively used in sustainable agricultural practices to enhance abiotic stress tolerance.

Therefore, the study was aimed to investigate the relative and interactive impacts of mycorrhization and/or three main PAs (Put, Spd, and Spm) in improving plant biomass, microbial symbiosis, and Tre metabolism, thereby imparting Ni tolerance in pigeon pea genotypes. The objectives of the study were to investigate (i) the impact of Ni toxicity on plant growth, mycorrhizal and rhizobial symbioses, ureide biosynthesis, and Tre metabolism in two differentially tolerant pigeon pea genotypes; (ii) the comparative impact of AM inoculation and PAs (Put, Spd, and Spm) priming in improving biomass accumulation and symbiotic efficiency under Ni stress; and (iii) functional complementarity between PAs and AM improving nitrogen-fixing potential under Ni stress in pigeon pea genotypes.

Materials and methods

Biological materials and experimental set-up

Experimental material taken for study included two differentially Ni-tolerant genotypes of *Cajanus cajan* L. (pigeon pea); relative tolerant, Pusa 2001; relative sensitive, AL 201, obtained from Indian Agricultural Research Institute (IARI), New Delhi, and Punjab Agricultural University (PAU), Ludhiana, India, respectively. Selection of these genotypes was based on screening of eight genotypes subjected to a range of $NiSO_4$ (50–300 mg/kg) in order to determine upper limit of metal tolerance (TI) for growth and survivability among them.

$$\left[\mathrm{TI} = 100 \text{ x (dry weight of stressed plant} - \text{dry weight of controlled plant)} / \text{dry weight of controlled plant}\right]$$

Pigeon pea-specific rhizobial inoculum (*Sinorhizobium fredii* AR-4) was procured from Department of Microbiology (IARI), New Delhi. Pure spores of mycorrhizal fungus *Rhizoglomus intraradices* were sourced from The Energy and Resource Institute (TERI), New Delhi, and the inoculum was prepared through trap cultures using three consecutive host plants species (*Sorghum bicolor* L., *Zea mays* L., *Coriandrum sativum* L). The inoculum consisted of colonized root segments, filamentous hypha, and spores. Experiments were conducted in the Department of Botany, Panjab University, Chandigarh, India, located at 30°45'N, 76°45′E and elevation 305–348 m above sea level, with a relative humidity ranging from 43 to 55% (morning) and 35–48% (afternoon), minimum temperature 21–28 °C, and maximum from 35 to 43 °C. Experimental soil consisted of sand and loam (1:1 *v*/v) was obtained from agricultural fields, pH 7.4, ECe 0.83.6 dS/m^{-1}, 0.41% total N (Nelson and Sommers 1973), 0.674% organic C (Estefan et al. 2013), 10.2 mg kg^{-1} P (Olsen and Sommers 1982), 0.79 meq/100 g Ca, 0.16 meq/100 g available K (Mehlich 1953), and 6.02 μg g^{-1} Ni concentration (Marguí et al. 2007).

Experiment design and nickel treatment

Circular earthenware pots (30 × 25 × 25 cm), sterilized with 70% ethanol, were lined with plastic bags and filled with 8 kg of autoclaved soil. Seeds were surface sterilized with 10% hydrogen peroxide (H_2O_2 *v*/v) solution for 10 min and then washed four to five times with distilled water. Seeds were priming with 0.5 mM Put, Spd, and Spm (concentration was selected on the basis of preliminary trails) at room temperature for 12 h and coated with the rhizobial inoculum of *Sinorhizobium fredii* AR-4. Soil-based fungal inoculum (50 g per pot) of *R. intraradices* was placed underneath (1.5 cm) the seeds before sowing. All non-AM treatments were supplemented with an equal quantity of sterilized inoculum to maintain uniformity. Fifteen-day-old seedlings were treated with 200 mg/kg $NiSO_4$ with/without Put, Spd, and Spm treatments and AM inoculations. Experimental units were organized in a completely randomized block design constituting factorial combination of 2 × 4 × 2 × 2 with two Ni concentrations [0 and 200 mg/kg]; two Put, Spd, and Spm treatments [0 mM (−) and 0.5 mM (+)]; two AM treatments [AM (+) and AM (−)]; and two pigeon pea genotypes (Pusa 2001 and AL 201). The plants were sampled at vegetative stage of 80 DAS (days after sowing), separated into shoots, roots, as well as nodules for analysis and oven dried at 70 °C for 72 h for dry weight measurements.

Mycorrhizal colonization and responsiveness

Mycorrhizal colonization (MC) was determined according to the method of McGonigle et al. (1990) following grid line intersect method. Root samples were autoclaved for 10 min with 10% (*w*/*v*) KOH solution, neutralized for 15 min in 20% HCl (*v*/v) and then stained with 0.05% trypan blue dye. The stained roots (approximately 1-cm length) were examined for root colonization using microscope, and per cent mycorrhizal colonization was recorded. Mycorrhizal responsiveness (MR) was calculated by using the method given by Hetrick et al. (1992).

$$\mathrm{MC}\ (\%) = 100 \left(\text{total number of colonized intersections} / \text{total number of intersections observed}\right)$$

$$\mathrm{MR}\ (\%) = 100\ (\text{dry weight of AM plants} - \text{dry weight of non-AM plants}) / \text{dry weight of non-AM plants}$$

Leghemoglobin (LHb) concentration and nitrogenase activity (acetylene reduction assay (ARA))

LHb concentration in nodules was analyzed by the method of Hartree (1957), based on the conversion of hematin to pyridine hemochromogen. Nitrogenase (N_2ase) activity was measured as acetylene reduction assay according to Herdina and Silsbury (1990). The rate of N_2ase activity was calculated as number of ethylene (C_2H_4) molecules produced per mg dry weight of nodules per hour (nmol C_2H_4 mg^{-1} nodule dry wt. h^{-1}).

Nutrients status, metal analysis, and membrane stability index (MSI) in nodules

Dry nodules were digested with sulfuric acid and perchloric acid for the estimation of nitrogen (N) concentration by using colorimetric method of Lindner (1944). Phosphorus (P) was estimated by vanadomolybdophosphoric colorimetric method of Chapman and Pratt (1961). The concentration of Ni (in roots and nodules), Cu, Fe, and Zn (in nodules) was analyzed according to Marguí et al. (2007) through WDXRF spectrometer (TIGER Bruker). MSI was calculated by the method of Sairam et al. (1997) in which the electrical conductivity of nodules (500 mg) in double distilled water was measured in two sets. Set I at 40 °C for 30 min and set II at 100 °C in boiling water bath for 15 min and their respective electrical conductivities (C1 and C2) were measured by using digital conductivity meter. MSI was calculated as follows: MSI = 100 [1–(C1/C2)] × 100.

Ureide metabolism

Total ureide concentrations and allantoinase (ALNase) activity

Total ureide concentrations (TUs) – ALN and ALA – were determined according to Vogels and van der Drift (1970). Nodules were ground in 50-mM potassium phosphate buffer (pH 7), homogenates were centrifuged at 18,000×g for 20 min at 4 °C, and supernatant was collected. About 0.5 mL of 0.5 N NaOH was added to the supernatant, and the mixture was heated for 30 min at 90 °C in water bath. After cooling, 0.5 mL of 0.65 M HCl was added and again incubated for 15 min. Thereafter, 3.5 mL of water, 0.4-M phosphate buffer (pH 7), and 1 mL of phenylhydrazine solution were added. Tubes were then placed in an ice-water bath, and 5 mL of cold concentrated HCl, freshly prepared 1 mL of a potassium ferricyanide [$K_3Fe(CN)_6$] solution was added. Absorbance was read at 535 nm. and ALN and ALA were determined.

Fresh nodules were extracted in 50-mM tricine buffer with 2 mM $MnSO_4$ and 35 mM β-mercaptoethanol and then incubated for 30 min at 30 °C. The incubated samples 250 μl were mixed with equal amount of 0.15 N HCl as well as 0.33% phenylhydrazine. Samples were kept in boiling water bath for 2 min and cooled before adding 1 ml cold concentrated HCl and 250 μl of 1.67% $K_3Fe(CN)_6$. The absorbance values were read at 520 nm to calculate the ALNase activity.

Urea concentration and urease activity

Modified procedure of Kyllingsbæk (1975) was used to estimate total urea concentration in the leaves. About 0.5 g of fresh material was extracted in 1.0 mL of 10-mM formic acid which was then centrifuged at 13,200×g for 5 min, at 4 °C. In 150 μL of aliquot, 3 mL of color developing reagent was added 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 for 15 min at 99 °C, then kept in ice-cooled system under dark for 5 min to determine urea concentrations and absorbance was read at 540 nm.

Leaf urease activity was determined by the modified method of Hogan et al. (1983). Homogenate was prepared with phosphate buffer (pH 7.4) and centrifuged at 18,000×g for 20 min at 4 °C and incubated for 1 h at 30 °C. 0.5 mL of aliquot added to 2.5 mL of reagent 1 (0.1 M phenol; 170 μM of sodium nitroprusside) and 2.5 mL of reagent 2 (0.125 M sodium hydroxide; 0.15 M dibasic sodium phosphate; sodium hypochlorite, 3% of Cl_2). Samples were then incubated at 37 °C for 35 min and read at 625 nm for determination of urease activity.

Trehalose metabolism

Tre concentration was determined through gas chromatography (Shimadzu GC-14B), according to the method of Streeter and Strimbu (1998). Fresh nodules were extracted in 80% methanol (*v*/v), incubated at 60 °C for 10 min, and extracts centrifuged at 13,000×g for 10 min. The pellet was re-extracted (thrice), and supernatant was vacuum dried. Solids were dissolved in equal amount of pyridine and STOX reagent. Derivatization of the samples was done by adding hexamethyldisilazane and trifluoroacetic acid (60 min). Trimethylsilyl (TXM)-oxime derivatives were separated using a gas chromatograph (packed column of 3% OV-17 on Chromosorb WHP). T6PS activity was measured by method of Salminen and Streeter (1986) and was read as release of UDP from UDP glucose consuming glucose-6-phosphate. T6PP activity was determined according to Padilla et al. (2004) by observing the liberation of phosphate (Pi) from trehalose-6-phosphate. TRE activity was estimated calorimetrically by quantifying the release of glucose according to method of Müller et al. (1994). Dinitrosulfosalicylic acid

method was used to measure the glucose released as described by (Miller 1959).

Statistical analysis

Data was subjected to statistically be analyzed using statistical software SPSS 25.0 for Windows (SPSS, Inc. Chicago, IL, USA) The data presented in figures and tables is expressed as mean values based on six replicates ± standard error (SE) per treatment. Data were analyzed by ANOVA for the main effects (Ni, Spm, Spd, Put, AM, G) and their interactions. Duncan multiple range test (DMRT, $p < 0.05$) was used to evaluate the differences among the treatments, after performing one-way ANOVA. Regression analysis was applied to compare the individual impacts of six independent variables or factors (Ni, Spm, Spd, Put, AM, G) on a particular parameter and expressed as standardized coefficient (β). Correlational analysis (Pearson's correlation coefficient-r) was carried out to investigate the correlations between relevant dependent variables for different parameters.

Results

Plant biomass and root/shoot ratio

Addition of Ni in the rooting medium had a strong negative effect on both root as well as shoot dry weights (RDW, SDW, Fig. 1), the effects being stronger in roots than shoots as evident by comparing standardized β coefficients through regression analysis (Table 1) [RDW β(Ni) = − 0.624, SDW β(Ni) = − 0.566]. Higher negative effects on roots resulted in disturbed root to shoot ratios (RSR) Table 2. Genotype AL 201 was highly sensitive and displayed greater reductions in shoot and root biomass by 41.30% and 57.04%, respectively. On the other hand, genotype Pusa 2001 had better ability to tolerate Ni stress with reductions of 22.12% and 29.72% observed in SDW and RDW, respectively (Fig. 1), and significant Ni x G interaction (Table 3). Exogenous applications of all three PAs were significantly effective in improving root as well as shoot biomass in the order Put > Spd > Spm [RDW − β(Put) = 0.283, β(Spd) = 0.180, β(Spm) = 0.99; SDW − β(Put) = 0.329, β(Spd) = 0.180, β(Spm) = 0.110]. A complete comparison of data revealed that AM treatment was more effective in improving RDW and SDW as well as able to alleviate maximum Ni stress [RDW − β(AM) = 0.434, SDW − β(AM) = 0.420 (Table 1)] than all PAs treatments with much higher positive effects on roots than shoots which led to stronger RSR in Pusa 2001 even when compared with unstressed controls. All the PAs when combined with AM inoculations prove to be highly beneficial in imparting tolerance to Ni toxicity with treatment +Put+AM providing greatest positive impact in terms of both root and shoot growth in Pusa 2001 with almost complete mitigation of negative effects of Ni toxicity and partial positive effects in AL 201 (significant Ni x Put and Ni x AM interaction (Table 3).

Mycorrhizal colonization (MC) and mycorrhizal responsiveness (MR)

Microscopic analysis of the root segments revealed a strong percent MC in both genotypes (Pusa 2001–79.21% and AL 201–75.67%) under unstressed control conditions when inoculated with *R. intraradices*, with no colonization observed in uninoculated pigeon pea plants (Fig. 2). However, with introduction of Ni in the rooting medium, a reduction in MC to 62.34% in Pusa 2001 and 48.68% in AL 201 was recorded and indicated negative impact of Ni on colonization. A negative correlation was observed between MC and Ni toxicity in both the genotypes (Pusa 2001 r(Ni − MC) = − 0.860, p = 0.01; AL 201 r(Ni − MC) = − 0.945, $p = 0.01$). A combined +Put+AM treatment was able to improve the colonizing ability of both genotypes (90.39% in Pusa 2001 and 84.83% in AL 201, respectively) under unstressed conditions. A complete mitigation of detrimental effects of Ni could be observed with Put treatment in Pusa 2001 where the data was at par with the control series of plants. Spd was relatively more effective and significant in improving MC than Spm under Ni stress in a genotype-dependent manner. On the other hand, MR increased under Ni stress with Pusa 2001 exhibiting greater responsiveness toward *R. intraradices* than AL 201. PAs (mainly Put) reduced the responsiveness of both the genotypes significantly under stress conditions with almost insignificant effects under unstressed controls.

Rhizobial symbiosis

The symbiotic potential of both genotypes [nodule number, NN; nodule dry weights, NDW (Fig. 3)] declined with addition of Ni in the rooting medium with higher negative impact on AL 201 than Pusa 2001 [NN − β(Ni) = − 0.371, NDW − β(Ni) = − 0.407]. The decline in nodule biomass under Ni had a direct correlation with the functional efficiency of nodules, with a significant decline observed in LHb concentration along with a decline in rate of nitrogenase activity – N_2ase (acetylene reduction activity, ARA) [Pusa 2001 r(NDW − LHb) = 0.968, $p = 0.01$; r(NDW − N_2ase) = 0.908, $p = 0.01$; AL 201 r(NDW − LHb) = 0.982, $p = 0.01$; r(NDW − N_2ase) = 0.929, $p = 0.01$]. The reduction observed in nodulation potential under stressed conditions could be directly correlated with reduced root biomass of both the genotypes [Pusa 2001 r(RDW − NDW) = 0.946, $p = 0.01$; AL 201 r(RDW − NDW) = 0.961, $p = 0.01$]. Exogenous Put application was more effective in improving NN under Ni stress, while AM-inoculated plants led higher nodule biomass as confirmed by comparing their standardized β coefficients [NN − β(Put) =

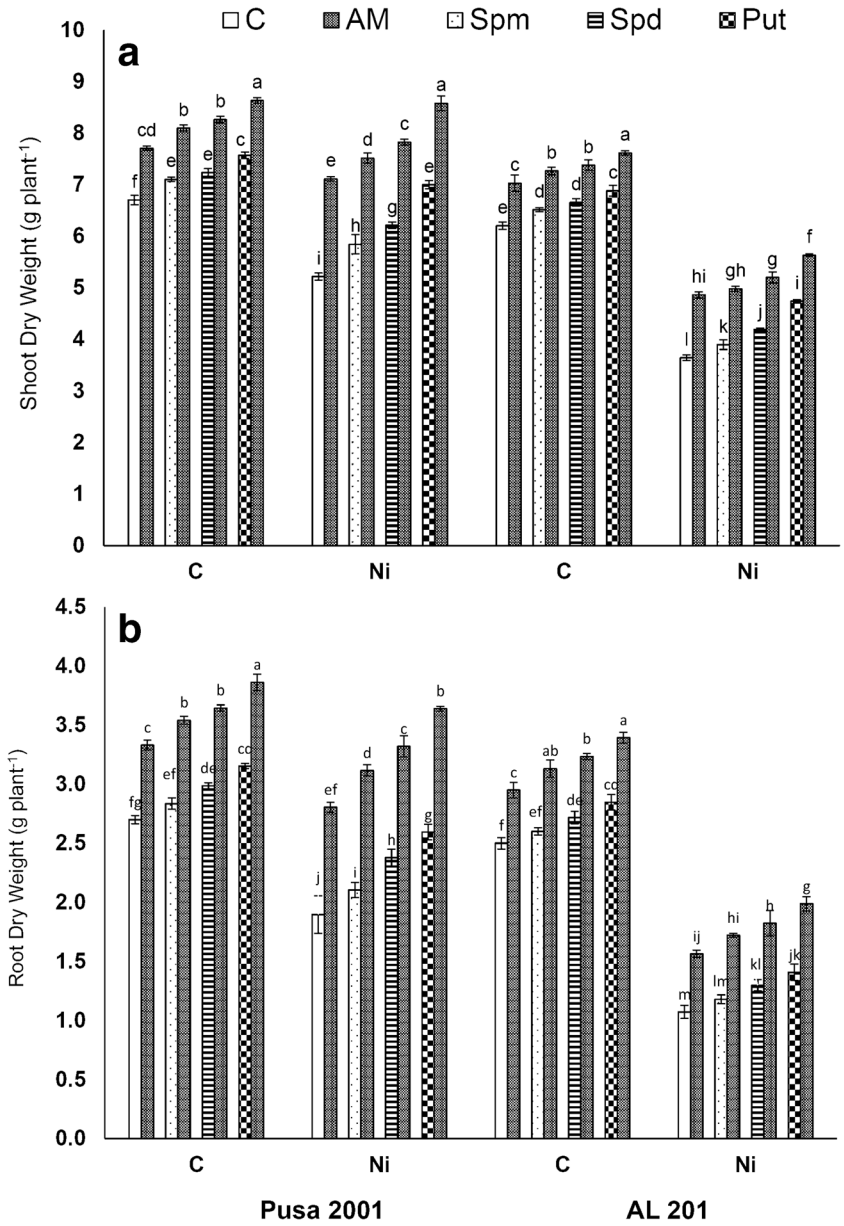

Fig. 1 Effect of polyamines (Put, Spd, Spm) and arbuscular mycorrhiza (AM, *Rhizoglomus intraradices*) on (**a**) shoot dry weight (SDW, g plant^{-1}) and (**b**) root dry weight (RDW, g plant^{-1}) in Pusa 2001 and AL 201 pigeon pea genotypes under Ni stress. Values are the mean of six replicates ± standard error (SE). Different letters above each bar indicate significant differences among the treatments, assessed by Duncan multiple range test, at $p \leq 0.05$. C=PAs and AM absent; Spm = 0.5 mM Spm added; Spd = 0.5 mM Spd added; Put = 0.5 mM Put added; AM = AM added; Ni = 200 mg/kg Ni added; Spm + AM = Spm and AM added; Spd + AM = Spd and AM added; Put+AM = Put and AM added

0.437, β(AM) = 0.402; NDW − β(Put) = 0.411, β(AM) = 0.561]. AM inoculated plants were able to restore LHb degeneration as well as ARA activity much more effectively than all the PAs under Ni stress [LHb − β(AM) = 0.456, β(Put) = 0.346, β(Spd) = 0.231, β(Spm) = 0.131; N_2ase − β(AM) = 0.418, β(Put) = 0.321, β(Spd) = 0.199, β(Spm) = 0.103]. Combined exogenous application of both +Put+AM proved to be the best in improving NN, NDW, as well as the N-fixing potential under Ni stress with more positive effects in Pusa 2001 than AL 201.

Nutrient status, Ni concentration, and MSI

The negative effects of Ni on both mycorrhizal and rhizobial symbiotic efficiencies had a direct impact on the nutrient status of nodules, analyzed in terms of N, P, Cu, Fe, and Zn (Table 4). The decline in N and P concentration in nodules was directly related with N_2ase and MC, respectively [Pusa 2001 r(N − N_2ase) = 0.891, $p = 0.01$; r(P − MC) = 0.932, $p = 0.01$; AL 201 r(N − N_2ase) = 0.946, $p = 0.01$; r(P − MC) = 0.983, $p = 0.01$]. The decline in Cu, Fe, and Zn concentration was proportionate to the increase in Ni concentrations in the nodules, and negative correlation was observed between them [Pusa 2001 r(Ni − Zn) = − 0.532, r(Ni − Cu) = − 0.709, r(Ni − Fe) = − 0.727; AL 201 r(Ni − Zn) = − 0.603, r(Ni − Cu) = − 0.835, r(Ni − Fe) = − 0.914]. Individually, exogenous Put and AM supplementation improved the Ni status of nodules and roots within the nutritional range under unstressed conditions (Table 2). However, there was a significant increase in Ni uptake in both nodule and roots (more in roots, Table 2) when

Table 1 Regression analysis to evaluate the relationship of dependent variable with all independent variables [Nickel (Ni), Spm, Spd, Put, arbuscular mycorrhiza (AM), and genotype (G)]

Parameters	Regression equation	Standardized coefficients (β)						R^2
		Ni	Spm	Spd	Put	AM	G	
SDW	$Y = 8.5 - 1.526(X1) + 0.343(X2) + 0.561(X3) + 1.024(X4) + 1.132(X5) - 1.497(X6)$	−0.566**	0.110**	0.180**	0.329**	0.420**	−0.556**	0.939
RDW	$Y = 3.671 - 0.970(X1) + 0.177(X2) + 0.323(X3) + 0.508(X4) + 0.675(X5) - 0.780(X6)$	−0.624**	0.099*	0.180**	0.283**	0.434**	−0.501**	0.941
RSR	$Y = 0.449 - 0.065(X1) + 0.007(X2) + 0.017(X3) + 0.017(X4) + 0.036(X5) - 0.037(X6)$	−0.696**	0.068	0.158**	0.157**	0.391**	−0.396**	0.904
NN	$Y = 68.889 - 19.505(X1) + 3.956(X2) + 8.166(X3) + 14.057(X4) + 11.206(X5) - 10.360(X6)$	−0.699**	0.123**	0.254**	0.437**	0.402**	−0.371**	0.963
NDW	$Y = 0.486–0.099(X1) + 0.022(X2) + 0.053(X3) + 0.089(X4) + 0.105(X5) - 0.076(X6)$	−0.530**	0.102*	0.243**	0.411**	0.561**	−0.407**	0.943
LHb	$Y = 192.473 - 46.736(X1) + 11.308(X2) + 20.039(X3) + 29.955(X4) + 34.199(X5) - 33.099(X6)$	−0.623**	0.131**	0.231**	0.346**	0.456**	−0.441**	0.937
N_2ase	$Y = 0.284 - 0.079(X1) + 0.014(X2) + 0.027(X3) + 0.044(X4) + 0.050(X5) - 0.054(X6)$	0.662**	0.103*	0.199**	0.321**	0.418**	−0.455**	0.946
MC	$Y = 8.350 - 8.128(X1) + 1.745(X2) + 4.073(X3) + 8.047(X4) + 73.407(X5) - 5.168(X6)$	−0.108**	0.020*	0.047*	0.092**	0.974**	−0.069**	0.986
MR	$Y = -0.125 + 7.998(X1) - 1.349(X2) - 2.518(X3) - 3.340(X4) + 23.354(X5) - 1.381(X6)$	0.302**	−0.044*	−0.082*	−0.109**	0.882**	−0.052*	0.939
Ni Concentration (Roots)	$Y = 5.507 + 37.227(X1) - 0.255(X2) - 4.764(X3) - 7.105(X4) - 7.269(X5) + 9.648(X6)$	0.873**	−0.052	−0.097*	−0.144**	−0.170**	0.226**	0.926
Ni Concentration (Nodules)	$Y = -0.833 + 24.258(X1) - 1.190(X2) - 2.686(X3) - 4.264(X4) - 4.050(X5) + 9.666(X6)$	0.798**	−0.034*	−0.077*	−0.122*	−0.133**	0.318**	0.876
MSI	$Y = 92.373 - 25.534\ (X1) + 3.859(X2) + 7.836(X3) + 12.191(X4) + 13.363(X5) - 15.056(X6)$	−0.717**	0.094*	0.191**	0.296**	0.375**	−0.423**	0.948
N Concentration	$Y = 25.762 - 5.480(X1) + 1.589(X2) + 3.126(X3) + 4.972(X4) + 6.359(X5) - 5.735(X6)$	−0.480**	0.121**	0.237**	0.377**	0.557**	−0.502**	0.947
P Concentration	$Y = 5.516 - 1.234(X1) + 0.326(X2) + 0.698(X3) + 1.124(X4) + 1.317(X5) - 0.937(X6)$	−0.533**	0.122**	0.261**	0.420**	0.569**	−0.405**	0.950
Zn Concentration	$Y = 4.098 - 0.575(X1) + 0.294(X2) + 0.705(X3) + 1.196(X4) + 1.416(X5) - 0.224(X6)$	−0.303**	0.134**	0.321**	0.545**	0.745**	−0.118**	0.941
Cu Concentration	$Y = 4.195 - 0.773(X1) + 0.232(X2) + 0.463(X3) + 0.710(X4) + 0.791(X5) - 0.536(X6)$	−0.542**	0.141**	0.281**	0.431**	0.555**	−0.376**	0.938
Fe Concentration	$Y = 5.097–1.078(X1) + 0.233(X2) + 0.501(X3) + 0.786(X4) + 0.840(X5)\text{-}1.163(X6)$	−0.532**	0.099**	0.214**	0.336**	0.414**	−0.573**	0.931
Total Ureides	$Y = 65.419 - 11.869(X1) + 2.903(X2) + 5.478(X3) + 8.655(X4) + 10.764(X5) - 9.815(X6)$	−0.560**	0.119**	0.224**	0.353**	0.508**	−0.463**	0.936
Allantoin	$Y = 11.152 + 4.381(X1) + 0.843(X2) + 1.809(X3) + 2.730(X4) + 3.027(X5) - 2.441$	0.674**	0.112**	0.241**	0.364**	0.466**	−0.375**	0.955
Allantoinase	$Y = 1.811 + 0.502(X1) + 0.080(X2) + 0.163(X3) + 0.271(X4) + 0.369(X5) - 0.301(X6)$	0.664**	0.091*	0.186**	0.310**	0.488**	−0.397**	0.953
Allantoic acid	$Y = 14.331 + 3.882(X1) + 0.915(X2) + 1.999(X3) + 2.975(X4) + 3.570(X5) - 1.644(X6)$	0.629**	0.128**	0.281**	0.418**	0.579**	−267**	0.967
Urea	$Y = 7.836 - 1.273(X1) + 0.479(X2) + 0.976(X3) + 1.407(X4) + 1.673(X5) - 0.791(X6)$	−0.479**	0.156**	0.318**	0.458**	0.629**	−0.297**	0.933
Urease	$Y = 5.645 - 0.900(X1) + 0.273(X2) + 0.519(X3) + 0.756(X4) + 0.944(X5) - 0.783(X6)$	−0.512**	0.134**	0.256**	0.372**	0.537**	−0.445**	0.922
Trehalose	$Y = 32.318 + 10.017(X1) + 1.353(X2) + 2.583(X3) + 4.372(X4) + 5.427(X5) - 7.575(X6)$	0.695**	0.081**	0.155**	0.263**	0.376**	−0.525**	0.974
Trehalase	$Y = 57.272–29.470(X1) - 3.218(X2) - 7.965(X3) - 12.040(X4) - 15.236(X5) + 13.426(X6)$	−0.792**	−0.075**	−0.185**	−0.280**	−0.409**	0.361**	0.992
T6PS	$Y = 20.885 + 7.962(X1) + 0.896(X2) + 1.924(X3) + 3.107(X4) + 3.849(X5) - 5.134(X6)$	0.741**	0.072**	0.155**	0.250**	0.358**	−0.478**	0.975
T6PP	$Y = 23.815 + 9.811(X1) + 1.174(X2) + 2.531(X3) + 4.152(X4) + 5.286(X5) - 4.804(X6)$	0.759**	0.079**	0.169**	0.278**	0.409**	−0.371**	0.968

Equations were derived from unstandardized coefficients of various independent variables, where *Y* is dependent variable and X1, X2, X3, X4, X5, and X6 denote independent variable – Ni, Spm, Spd, Put, AM, and G respectively. **coefficient is significant at $p < 0.01$; *coefficient is significant at $p < 0.05$ (2-tailed)

Table 2 Effect of polyamines (PAs) and arbuscular mycorrhiza (AM) on root to shoot ratio (R/S) and nickel (Ni) concentration in roots and nodules, membrane stability index (MSI) in Pusa 2001 and AL 201 genotypes under Ni stress

Parameters	Root to shoot ratio		Ni concentration (roots – mg g^{-1} DW)		Ni concentration (nodules – mg g^{-1} DW)		Membrane stability index (%)	
Treatments	Pusa 2001	AL 201	Pusa 2001	AL 201	Pusa 2001	AL 201	Pusa 2001	AL 201
Control	0.403 ± 0.004 f	0.403 ± 0.004 h	9.89 ± 0.137 o	10.53 ± 0.131 p	5.98 ± 0.234 l	6.12 ± 0.065 k	75.40 ± 0.207 i	71.62 ± 0.151 h
C + Spm	0.399 ± 0.006 f	0.399 ± 0.006 g	10.35 ± 0.110 no	10.97 ± 0.042 o	6.40 ± 0.076 kl	6.70 ± 0.120 jk	78.42 ± 0.245 h	74.02 ± 0.488 g
C + Spd	0.413 ± 0.002 e	0.408 ± 0.004 f	10.99 ± 0.139 mn	11.50 ± 0.047 n	6.90 ± 0.038 jkl	7.50 ± 0.132 jk	80.75 ± 0.361 g	76.20 ± 0.281 f
C + Put	0.417 ± 0.003 de	0.414 ± 0.006 ef	11.74 ± 0.075 m	12.19 ± 0.046 m	7.84 ± 0.056 jk	8.43 ± 0.072 ij	83.84 ± 0.077 e	78.93 ± 0.105 e
C + AM	0.432 ± 0.004 d	0.420 ± 0.005 e	12.67 ± 0.061 l	13.01 ± 0.159 l	9.33 ± 0.053 i	9.78 ± 0.149 i	85.65 ± 0.154 d	80.29 ± 0.294 d
C + Spm + AM	0.437 ± 0.006 b	0.431 ± 0.004 c	13.70 ± 0.102 j	13.96 ± 0.042 j	11.09 ± 0.053 g	11.75 ± 0.257 h	88.50 ± 0.292 b	83.17 ± 0.393 b
C + Spd + AM	0.441 ± 0.001 c	0.438 ± 0.006 d	14.84 ± 0.074 k	15.08 ± 0.047 k	12.93 ± 0.078 h	13.20 ± 0.414 h	91.69 ± 0.053 c	85.89 ± 0.311 c
C + Put+AM	0.447 ± 0.004 a	0.446 ± 0.008 b	16.09 ± 0.055 i	16.32 ± 0.036 i	14.55 ± 0.293 f	15.20 ± 0.225 g	95.68 ± 0.304 a	89.02 ± 0.433 a
N1	0.364 ± 0.012 m	0.295 ± 0.007 k	57.25 ± 0.393 a	74.51 ± 0.191 a	37.50 ± 1.026 a	52.65 ± 0.626 a	51.35 ± 0.052 n	32.59 ± 0.182 p
N1 + Spm	0.360 ± 0.002 l	0.303 ± 0.004 j	52.20 ± 0.557 b	68.78 ± 0.120 b	34.20 ± 0.777 b	50.78 ± 0.496 a	57.00 ± 0.112 m	35.82 ± 0.303 o
N1 + Spd	0.383 ± 0.016 k	0.310 ± 0.005 i	47.21 ± 0.111 c	64.93 ± 0.055 c	29.21 ± 0.711 c	47.93 ± 1.034 b	62.75 ± 0.237 l	39.56 ± 0.266 n
N1 + Put	0.371 ± 0.004 j	0.297 ± 0.004 h	44.14 ± 0.557 d	62.62 ± 0.057 d	26.40 ± 0.567 d	45.62 ± 0.167 c	67.36 ± 0.102 k	42.30 ± 0.267 m
N1 + AM	0.394 ± 0.008 j	0.322 ± 0.006 gh	42.06 ± 0.358 e	59.74 ± 0.121 e	25.06 ± 0.656 d	43.74 ± 0.820 c	68.45 ± 0.315 j	44.29 ± 0.367 l
N1 + Spm + AM	0.415 ± 0.010 i	0.346 ± 0.004 ef	34.38 ± 0.204 f	54.92 ± 0.059 f	19.80 ± 0.635 e	39.92 ± 0.811 d	75.12 ± 0.430 i	48.46 ± 0.306 k
N1 + Spd + AM	0.424 ± 0.009 h	0.351 ± 0.006 c	28.05 ± 0.275 g	48.95 ± 0.031 g	15.05 ± 0.410 f	35.95 ± 1.576 e	82.36 ± 0.265 f	53.12 ± 0.471 j
N1 + Put+AM	0.424 ± 0.010 g	0.353 ± 0.003 a	18.90 ± 0.104 h	40.82 ± 0.223 h	8.19 ± 0.344 ij	29.82 ± 1.091 f	90.94 ± 0.471 b	59.09 ± 0.471 i

Values are the mean of six replicates ± standard error (SE). Different letters in each column indicate significant differences among the treatments, assessed by Duncan multiple range test, at $p \leq 0.05$

Table 3 Results of the ANOVA test for independent variables [nickel (Ni), Spm, Spd, Put, arbuscular mycorrhiza (AM), and genotype (G)] and interactions among them in pigeon pea genotypes under Ni stress

Parameters	SDW	RDW	NN	NDW	LHb	N2ase	MC	MR	Ni (nodules)	Ni (Roots)	MSI	N	P	Fe	Zn	Cu	TUs	ALN	ALNase	ALA	Urea	Urease	Tre	TRE	T6PS	T6PP
Ni	*	*	*	*	*	*	–	–	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*
Spm	*	*	*	*	*	*	–	–	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*
Spd	*	*	*	*	*	*	–	–	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*
Put	*	*	*	*	*	*	–	–	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*
AM	*	*	*	*	*	*	–	–	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*
G	*	*	*	*	*	*	–	–	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*
Ni x Spm	ns	ns	*	ns	*	ns	–	–	*	*	*	ns	ns	*	ns	ns	ns	*	ns	ns	ns	*	ns	*	*	*
Ni x Spd	*	ns	*	ns	*	*	–	–	*	*	*	*	ns	*	*	*	*	*	*	*	ns	*	*	*	*	*
Ni x Put	*	*	*	*	*	*	–	–	*	*	*	*	*	*	*	*	*	*	*	*	ns	*	*	*	*	*
Ni x AM	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*
Ni x G	*	*	*	*	*	*	–	–	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	ns	*	*
Spm x AM	ns	ns	*	ns	ns	ns	*	*	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	*	*	*
Spm x G	*	ns	ns	ns	*	ns	–	–	ns	*	*	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	*	ns	*	ns
Spd x AM	ns	ns	*	ns	ns	ns	*	*	ns	*	*	*	*	*	*	ns	*	*	*	*	ns	*	*	*	*	*
Spd x G	*	*	*	ns	*	ns	–	–	*	*	*	*	*	*	ns	ns	*	*	ns	ns	ns	ns	*	ns	*	*
Put x AM	*	ns	*	*	ns	ns	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	ns	*	*
Put x G	*	*	*	ns	*	ns	–	–	*	*	*	*	*	*	ns	ns	*	*	ns	*	ns	*	*	*	*	*
AM x G	*	*	*	*	*	ns	*	*	*	*	*	*	*	*	*	ns	*	*	*	*	ns	*	*	*	ns	*	*
Ni x Spm x AM	ns	ns	ns	ns	ns	ns	ns	*	*	*	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	*	*	*	ns
Ni x Spm x G	ns	ns	ns	ns	*	ns	–	–	ns	*	*	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
Ni x Spd x AM	ns	ns	*	ns	ns	ns	*	*	*	*	ns	ns	ns	*	ns	*	ns	ns	ns	ns	ns	ns	*	*	*	*
Ni x Spd x G	ns	ns	ns	ns	*	ns	–	–	*	*	*	*	ns	*	ns	ns	ns	ns	ns	ns	ns	ns	*	ns	*	*
Ni x Put x AM	ns	ns	*	ns	ns	ns	*	*	*	*	*	ns	ns	*	ns	*	ns	ns	ns	ns	ns	ns	*	ns	*	*
Ni x Put x G	*	*	*	ns	*	ns	–	–	*	*	*	*	ns	*	ns	*	ns	*	ns	*	ns	ns	*	ns	*	*
Ni x AM x G	*	*	ns	ns	*	ns	*	ns	*	*	*	*	ns	*	*	*	*	*	ns	*	ns	*	*	ns	*	*
Spm x AM x G	ns	ns	ns	ns	ns	ns	ns	ns	ns	*	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	*	ns	ns	ns
Spd x AM x G	ns	ns	ns	ns	ns	ns	ns	ns	ns	*	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	*	ns	ns	ns
Put x AM x G	ns	ns	ns	ns	ns	ns	*	ns	ns	*	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	*	ns	*	ns

ns nonsignificant, – no interaction, * significance at $p \leq 0.05$, *SDW* shoot dry weight, *RDW* root dry weight, *NN* nodule number, *NDW* nodule dry weight, *LHb* Leghemoglobin, *N_2ase* nitrogenase activity, *MC* mycorrhizal colonization, *MR* mycorrhizal responsiveness, *Ni* nickel, *MSI* membrane stability index, *N* nitrogen, *P* phosphorus, *Fe* iron, *Zn* zinc, *Cu* copper, *TUs* total ureides, *ALN* allantoin, *ALNase* allantoinase, *ALA* allantoic acid, *Tre* trehalose, *TRE* trehalase activity, *T6PS* trehalose-6-phosphate synthase activity, *T6PP* trehalose-6-phosphatase activity

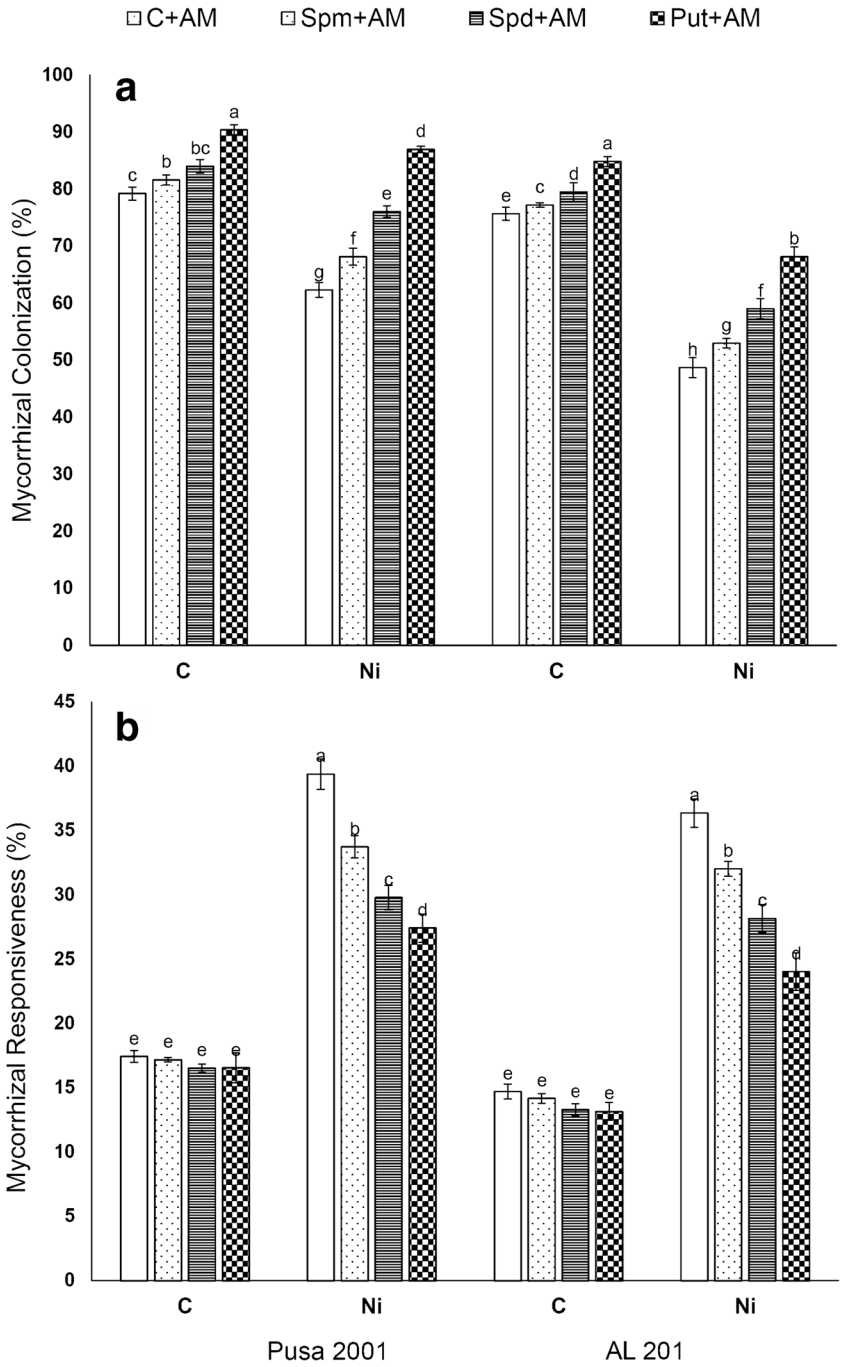

Fig. 2 Effect of PAs (Put, Spd, Spm) and arbuscular mycorrhiza (AM, *Rhizoglomus intraradices*) inoculation on (**a**) mycorrhizal colonization (MC, %) and (**b**) mycorrhizal responsiveness (MR, %) in Pusa 2001 and AL 201 pigeon pea genotypes under Ni stress. Values are the mean of six replicates ± standard error (SE). Different letters above each bar indicate significant differences among the treatments, assessed by Duncan multiple range test, at $p \leq 0.05$. C = PAs and AM absent; Spm = 0.5 mM Spm added; Spd = 0.5 mM Spd added; Put = 0.5 mM Put added; AM = AM added; Ni = 200 mg/kg Ni added; Spm + AM = Spm and AM added; Spd + AM = Spd and AM added; Put+AM = Put and AM added

the plants were supplemented with Ni beyond the permissible limits more in AL 201 than Pusa 2001 [nodules β(Ni) = 0.798; roots β(Ni) = 0.873;Table 1]. Put and AM were able to significantly reduce Ni concentration in the nodules as well as roots with a concomitant increase in nutrient concentrations in Ni-stressed plants (significant Put x AM, Put x G, and AM x G interactions, Table 3). AM was comparatively more effective in enhancing nutrient acquisition as compared to PAs application [N concentration − β(AM) = 0.557, β(Put) = 0.377, β(Spd) = 0.237, β(Spm) = 0.121; P concentration − β(AM) = 0.569, β(Put) = 0.420, β(Spd) = 0.261, β(Spm) = 0.122]. The plants gained maximum assistance, when both Put and AM were given in combination leading to significantly lower toxic ion concentrations. In addition, Ni decreases the MSI in nodules of both genotype of pigeon pea plants (Table 2). Thus, in nodules MSI declined from 75.4 to 51.35% in Pusa 2001 and from 71.62 to 32.59 in AL 201 genotype, respectively, under Ni. However, exogenous supplementation of PAs and mycorrhization significantly increased MSI when compared with stressed counterparts. AM

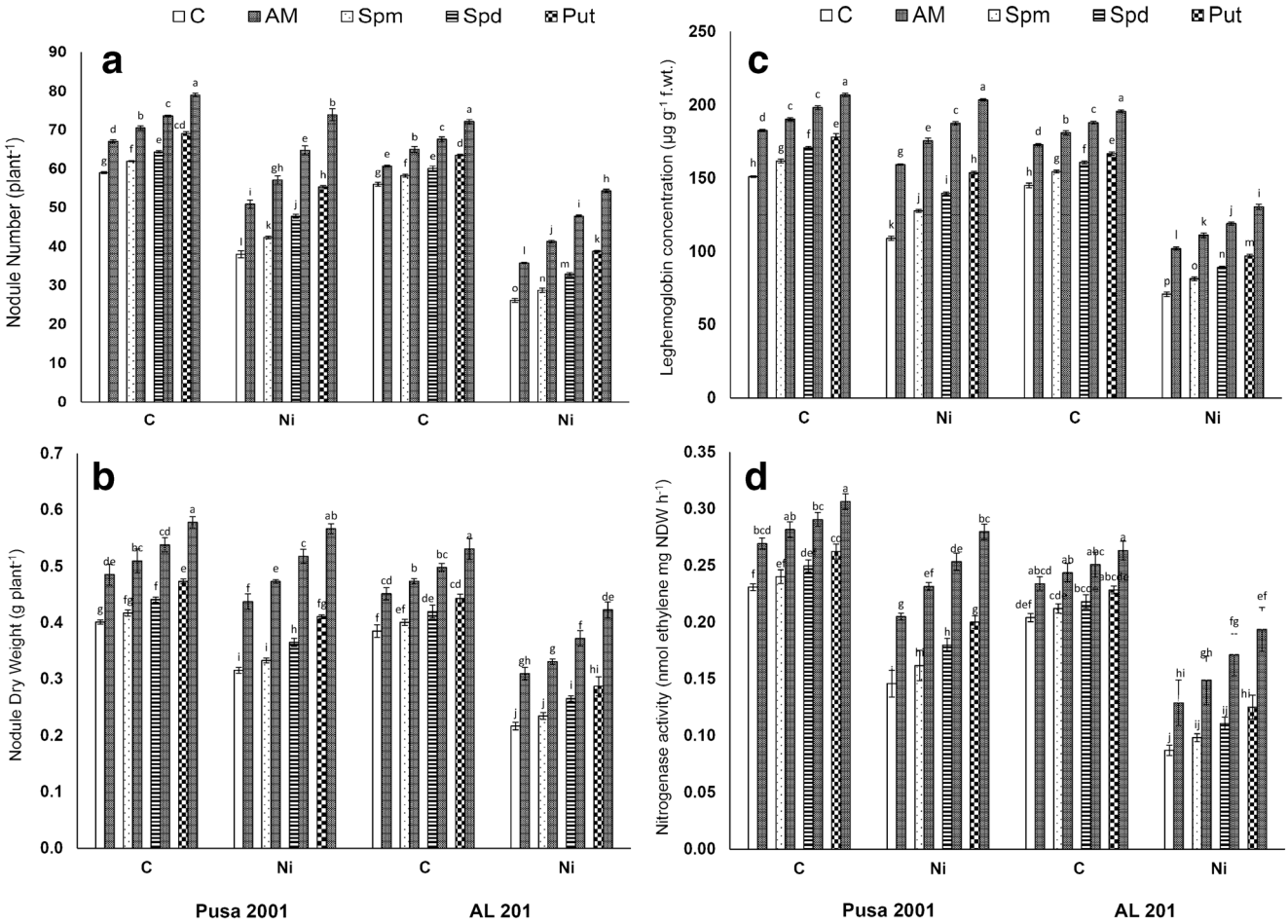

Fig. 3 Effect of PAs (Put, Spd, Spm) and arbuscular mycorrhiza (AM, *Rhizoglomus intraradices*) inoculation on (**a**) nodule number (NN) plant^{-1}, (**b**) dry weights of nodules (NDW, g plant^{-1}), (**c**) leghemoglobin concentration (LHb, µg g^{-1} nodule f. wt.) and (**d**) nitrogenase activity (ARA, nmol ethylene mg NDW h^{-1}) in Pusa 2001 and AL 201 pigeon pea genotypes under Ni stress. Values are the mean of six replicates ± standard error (SE). Different letters above each bar indicate significant differences among the treatments, assessed by Duncan multiple range test, at $p \leq 0.05$. C = PAs and AM absent; Spm = 0.5 mM Spm added; Spd = 0.5 mM Spd added; Put = 0.5 mM Put added; AM = AM added; Ni = 200 mg/kg Ni added; Spm + AM = Spm and AM added; Spd + AM = Spd and AM added; Put+AM = Put and AM added

inoculation was more effective than PAs in improving the stability of membranes as authenticated through regression analysis (Table 1). The decline in MSI was proportionate to increase Ni concentration in the nodules, and negative correlation was observed between them (Pusa 2001 r(Ni − MSI) = − 0.827; AL 201 r(Ni − MSI) = − 0.944). Thus, +AM plants improved MSI of nodules 68.45% and 44.24% in Pusa 2001 and AL 201, respectively, under Ni exposure. Maximum stability was recorded when +Put+AM were applied simultaneously in a genotype-dependent manner.

Ureides metabolism

Ni stress significantly decreased the total ureides (TUs) concentration in nodules, more in AL201 (37.92%) than Pusa 2001 (19.4%) as compared to unstressed controls (Fig. 4). The reduction observed in TUs under stressed conditions could be directly correlated with N concentration in nodules [Pusa 2001 r(TUs − N) = 0.984, $p = 0.01$; AL 201 r(TUs − N) = 0.992, $p = 0.01$]. Both PAs and AM were able to increase ureide concentration under unstressed as well as stressed conditions. AM proved to be much more beneficial than PAs and could nullify the negative effects of Ni stress completely [TUs − β(AM) = 0.508, β(Put) = 0.353, β(Spd) = 0.224, β(Spm) = 0.119]. The combined treatments of AM and PAs were able to increase the ureide concentration in the nodules, even more than unstressed controls in Pusa 2001 (31.04%) (significant Put x AM interaction; Table 3). On the other hand, AL 201 was less responsive to both treatments under Ni stress and could display only a partial improvement.

The major ureides (ALN and ALA) were analyzed in the nodules under Ni stress with and without PAs and AM (data is presented in Fig. 4). A moderately positive correlation was observed between the Ni concentration in the nodules and

Table 4 Effect of polyamines (PAs) and arbuscular mycorrhiza (AM) on N, P, Cu, Fe, and Zn concentration in nodules in Pusa 2001 and AL 201 genotypes under Ni stress

Parameters	N concentration (mg g^{-1} DW)		P concentration (mg g^{-1} DW)		Cu concentration (mg g^{-1} DW)		Fe concentration (mg g^{-1} DW)		Zn concentration (mg g^{-1} DW)	
Treatments	Pusa 2001	AL 201	Pusa 2001	AL 201	Pusa 2001	AL 201	Pusa 2001	AL 201	Pusa 2001	AL 201
Control	19.75 ± 0.180 g	17.57 ± 0.265 gh	4.45 ± 0.266 gh	4.28 ± 0.066 g	3.70 ± 0.093 h	3.45 ± 0.036 i	3.89 ± 0.087 h	3.32 ± 0.057 f	4.11 ± 0.121 ij	4.27 ± 0.080 f
C + Spm	20.74 ± 0.493 fg	18.27 ± 0.636 fg	4.75 ± 0.183 fg	4.54 ± 0.054 f	3.92 ± 0.032 fg	3.66 ± 0.055 gh	4.06 ± 0.033 g	3.47 ± 0.064 f	4.25 ± 0.060 hij	4.34 ± 0.044 ef
C + Spd	21.78 ± 0.573 f	19.15 ± 0.439 ef	5.01 ± 0.023 ef	4.71 ± 0.038 ef	4.07 ± 0.023 ef	3.87 ± 0.045 ef	4.26 ± 0.040 f	3.68 ± 0.040 e	4.50 ± 0.057 gh	4.63 ± 0.056 e
C + Put	23.13 ± 0.699 e	20.21 ± 0.665 de	5.30 ± 0.081 de	4.92 ± 0.038 e	4.17 ± 0.019 e	4.03 ± 0.015 de	4.41 ± 0.052 ef	3.82 ± 0.044 de	4.79 ± 0.036 fg	4.93 ± 0.053 d
C + AM	24.46 ± 0.429 d	21.38 ± 0.622 d	5.55 ± 0.096 cd	5.15 ± 0.061 d	4.22 ± 0.064 e	4.08 ± 0.049 cd	4.44 ± 0.052 e	3.89 ± 0.072 d	5.01 ± 0.294 ef	5.07 ± 0.132 d
C + Spm + AM	26.18 ± 0.261 b	22.84 ± 0.422 f	5.87 ± 0.079 b	5.43 ± 0.041 b	4.38 ± 0.055 c	4.23 ± 0.050 b	4.61 ± 0.039 c	4.07 ± 0.038 b	5.35 ± 0.091 cd	5.39 ± 0.050 b
C + Spd + AM	27.94 ± 0.520 c	24.42 ± 0.658 c	6.30 ± 0.055 c	5.74 ± 0.035 c	4.55 ± 0.054 d	4.43 ± 0.078 c	4.77 ± 0.023 d	4.26 ± 0.043 c	5.68 ± 0.168 de	5.77 ± 0.060 c
C + Put+AM	30.05 ± 0.551 a	26.28 ± 0.583 a	6.76 ± 0.115 a	6.14 ± 0.096 a	4.77 ± 0.050 b	4.66 ± 0.067 a	5.04 ± 0.033 b	4.45 ± 0.050 a	6.09 ± 0.040 b	6.12 ± 0.061 a
N1	13.83 ± 0.300 j	8.61 ± 0.296 m	3.34 ± 0.179 j	2.27 ± 0.156 m	2.87 ± 0.039 k	2.13 ± 0.038 n	2.75 ± 0.038 k	1.48 ± 0.081 m	3.22 ± 0.055 k	2.78 ± 0.085 i
N1 + Spm	16.18 ± 0.167 i	9.73 ± 0.135 lm	3.71 ± 0.040 i	2.52 ± 0.115 l	3.10 ± 0.038 j	2.33 ± 0.059 m	3.05 ± 0.042 j	1.67 ± 0.047 l	3.47 ± 0.068 k	3.10 ± 0.075 h
N1 + Spd	17.83 ± 0.247 h	10.78 ± 0.133 l	4.17 ± 0.101 h	2.78 ± 0.048 k	3.37 ± 0.078 i	2.53 ± 0.085 l	3.42 ± 0.036 i	1.88 ± 0.046 k	3.91 ± 0.133 j	3.58 ± 0.075 g
N1 + Put	19.89 ± 0.338 g	12.00 ± 0.058 k	4.66 ± 0.046 fg	3.08 ± 0.064 j	3.68 ± 0.035 h	2.80 ± 0.036 k	3.81 ± 0.041 h	2.11 ± 0.038 j	4.40 ± 0.125 hi	4.09 ± 0.050 f
N1 + AM	21.84 ± 0.329 f	13.34 ± 0.225 j	4.82 ± 0.088 fg	3.35 ± 0.084 i	3.83 ± 0.064 gh	2.92 ± 0.035 k	3.94 ± 0.087 gh	2.17 ± 0.033 j	4.79 ± 0.110 fg	4.21 ± 0.090 f
N1 + Spm + AM	24.68 ± 0.152 d	14.89 ± 0.105 i	5.28 ± 0.058 de	3.73 ± 0.102 h	4.16 ± 0.056 e	3.26 ± 0.059 j	4.38 ± 0.021 ef	2.41 ± 0.035 i	5.32 ± 0.130 de	4.59 ± 0.050 e
N1 + Spd + AM	27.33 ± 0.168 bc	16.55 ± 0.247 h	5.91 ± 0.035 c	4.17 ± 0.045 g	4.56 ± 0.064 c	3.52 ± 0.090 hi	4.88 ± 0.051 c	2.73 ± 0.073 h	5.90 ± 0.062 bc	5.13 ± 0.266 cd
N1 + Put+AM	30.59 ± 0.463 a	18.41 ± 0.189 fg	6.63 ± 0.215 ab	4.71 ± 0.053 ef	4.96 ± 0.034 a	3.80 ± 0.054 fg	5.41 ± 0.075 a	3.09 ± 0.044 g	6.70 ± 0.081 a	5.91 ± 0.060 ab

Values are the mean of six replicates ± standard error (SE). Different letters in each column indicate significant differences among the treatments, assessed by Duncan multiple range test, at $p \leq 0.05$

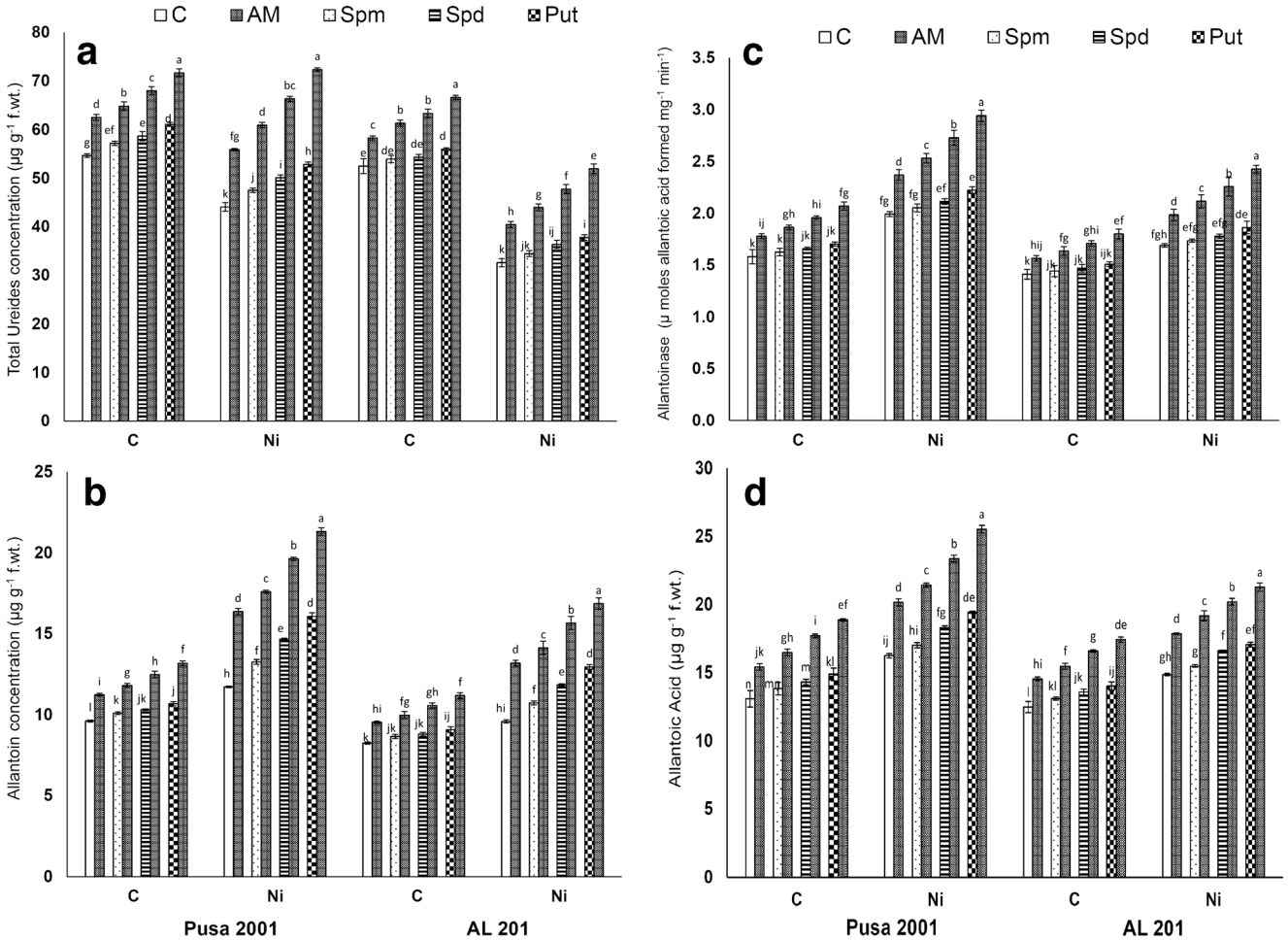

Fig. 4 Effect of PAs (Put, Spd, Spm) and arbuscular mycorrhiza (AM, *Rhizoglomus intraradices*) inoculation on (**a**) total ureides concentration (µg g^{-1} f.wt.), (**b**) allantoin concentration (µg g^{-1} f.wt.), (**c**) allantoinase (µ moles allantoic acid formed mg^{-1} min^{-1}), and (**d**) allantoic acid (µg g^{-1} f.wt.) in nodules of Pusa 2001 and AL 201 pigeon pea genotypes under Ni stress. Values are the mean of six replicates ± standard error (SE). Different letters above each bar indicate significant differences among the treatments, assessed by Duncan multiple range test, at $p \leq 0.05$. C = PAs and AM absent; Spm = 0.5 mM Spm added; Spd = 0.5 mM Spd added; Put = 0.5 mM Put added; AM = AM added; Ni = 200 mg/kg Ni added; Spm + AM = Spm and AM added; Spd + AM = Spd and AM added; Put+AM = Put and AM added

ALN, ALA under Ni stress [Pusa 2001 r(Ni − ALN) = 0.591, $p = 0.01$; r(Ni − ALA) = 0.529, p = 0.01]. On the other hand, a strong positive correlation was observed between ALN and ALA in the nodules under Ni stress in both the genotypes [Pusa 2001 r(ALN − ALA) = 0.978, $p = 0.01$; r(ALN − ALA) = 0.968, $p = 0.01$]. An increase in synthesis of ALA could be directly correlated to an increase in the activity of enzyme ALNase (EC 3.5.2.5) [Pusa 2001 r(ALA − ALNase) = 0.982, $p = 0.01$; AL 201 r(ALA − ALAase) = 0.973, $p = 0.01$]. A further increase in the concentrations of ALN and ALA along with a significant increase in ALNase was observed when pigeon pea plants were treated with the three PAs, Put being more effective than Spd and Spm [ALN − β(Put) = 0.364, β(Spd) = 0.241; β(Spm) = 0.112; ALA − β(Put) = 0.418; β(Spd) = 0.281, β(Spm) = 0.128; ALNase − β(Put) = 0.310, β(Spd) = 0.186; β(Spm) = 0.091]. Introduction of *R. intraradices* was the most efficient in increasing the concentration of ureides through increased enzyme activity [TUs − β(AM) = 0.508; ALN − β(AM) = 0.466; ALNase − β(AM) = 0.488; ALA − β(AM) = 0.579]. The combined treatments of +Put+AM further boosted the synthesis of ALA with an increase of 57.2% in Pusa 2001 and 43.02% in AL 201 over their stressed counterparts in the nodules.

Upon transport of ALA to the leaves, its conversion to urea was analyzed, and the data is presented in Fig. 5. The presence of Ni in the rooting medium decreased the urea concentration in the leaves in a genotype-dependent manner along with a greater decline in the activity of enzyme urease [urea − β(Ni) = − 0.479; urease−β(Ni) = − 0.512]. Exogenous application of PAs mainly Put and AM increased the urea synthesis more under stressed than control conditions, the effects being more prominent in Pusa 2001 than AL 201. However, AM inoculations were more effective in increasing urea synthesis (Pusa 2001–32%, AL 201–24.5%) as well as urease activity (Pusa 2001–33.04%, AL 201–20.4%) over their

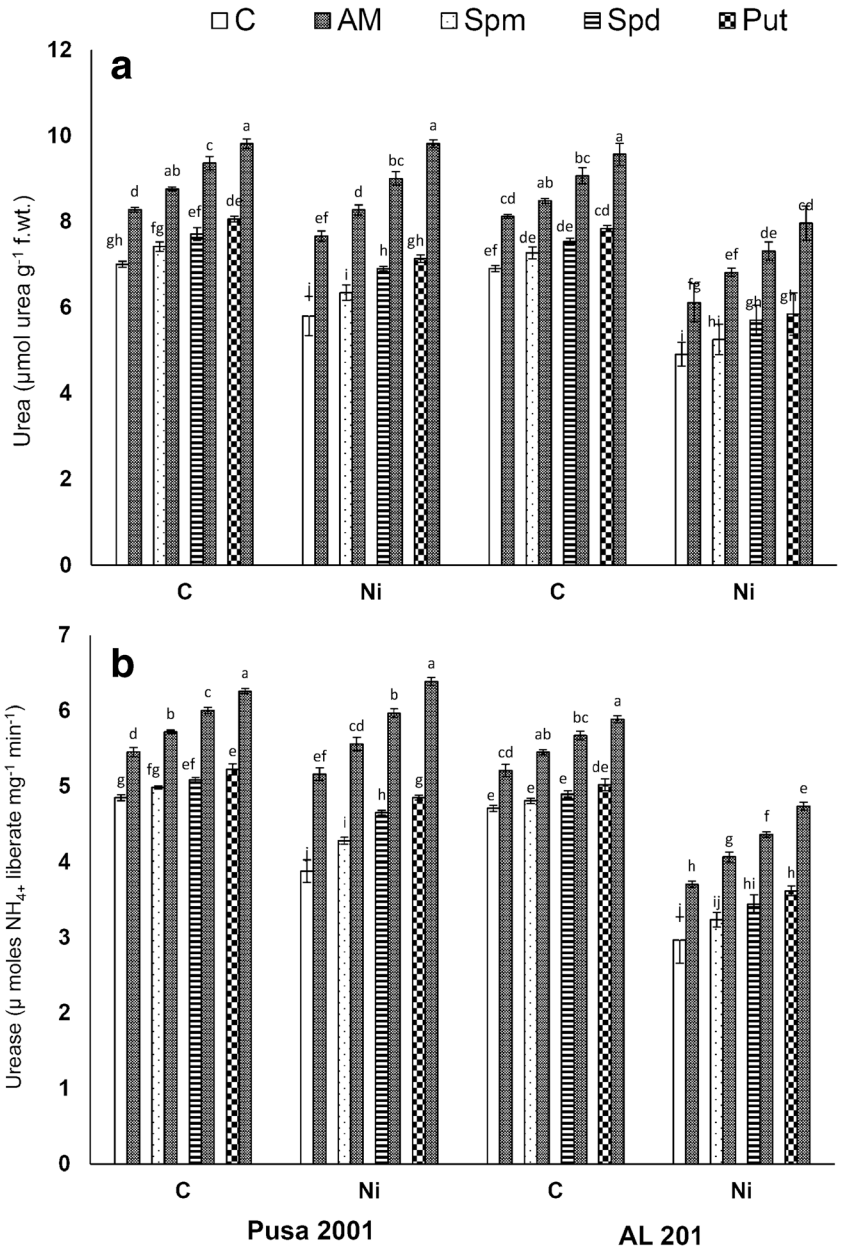

Fig. 5 Effect of PAs (Put, Spd, Spm) and arbuscular mycorrhiza (AM, *Rhizoglomus intraradices*) inoculation on (**a**) urea (μ moles urea g^{-1} f.wt.) and (**b**) urease (μ moles NH_4^+ liberate mg^{-1} min^{-1}) in Pusa 2001 and AL 201 pigeon pea genotypes under Ni stress. Values are the mean of six replicates ± standard error (SE). Different letters above each bar indicate significant differences among the treatments, assessed by Duncan multiple range test, at $p \leq 0.05$. C = PAs and AM absent; Spm = 0.5 mM Spm added; Spd = 0.5 mM Spd added; Put = 0.5 mM Put added; AM = AM added; Ni = 200 mg/kg Ni added; Spm + AM = Spm and AM added; Spd + AM = Spd and AM added; Put+AM = Put and AM added

respective stressed counterparts when compared with all the three PAs under Ni stress [urea − β(AM) = 0.629, β(Put) = 0.458, β(Spd) = 0.318, β(Spm) = 0.156; urease − β(AM) = 0.537, β(Put) = 0.372, β(Spd) = 0.256, β(Spm) = 0.134]. The combined treatment of Put and AM inoculation was most efficient in increasing urea as well as urease activity.

Trehalose metabolism

An increase in Tre concentration in the nodules was recorded when Ni was applied in the rooting medium (Fig. 6). This increase could be related to an upsurge in the activity of both biosynthetic enzymes, i.e.,T6PS and T6PP [Pusa 2001 r(Tre − T6PS) = 0.998, $p = 0.01$; r(Tre − T6PP) = 0.996, $p = 0.01$; AL 201 r(Tre − T6PS) = 0.989, $p = 0.01$; r(Tre − T6PP) = 0.985, $p = 0.01$]. On the other side, a negative correlation was observed between Tre synthesis and TRE activity [Pusa 2001 r(Tre − TRE) = − 0.992, $p = 0.01$; AL 201 r(Tre − TRE) = − 0.996, $p = 0.01$]. Nodules of Pusa 2001 showed better ability for osmotic adjustment by synthesizing higher amounts of Tre with greater reduction in TRE activity when compared to AL 201. AM inoculation further enhanced the activity of anabolic enzymes as well as Tre concentration in a genotype-dependent manner. However, PAs were relatively less effective in modulating Tre metabolism when compared with AM as validated through regression analysis [Tre, β(AM) = 0.376, β(Put) = 0.263, β(Spd) = 0.155, β(Spm) = 0.081; T6PP, β(AM) = 0.409, β(Put) = 0.278, β(Spd) = 0.169, β(Spm) = 0.079;

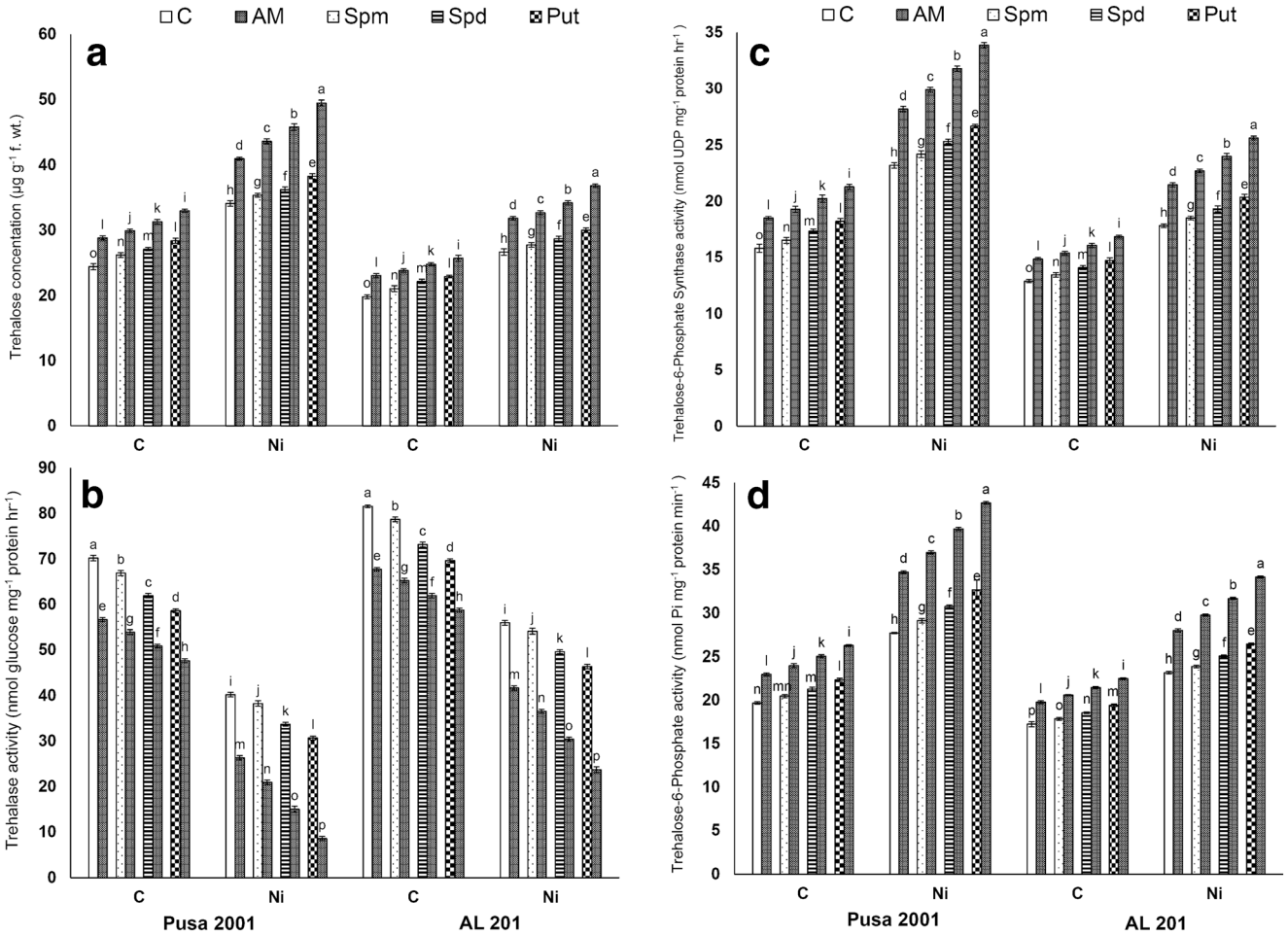

Fig. 6 Effect of PAs (Put, Spd, Spm) and arbuscular mycorrhiza (AM, *Rhizoglomus intraradices*) inoculation on (**a**) trehalose concentration (Tre, µg g^{-1} f.wt.), (**b**) trehalase activity (TRE, nmol glucose mg^{-1} protein hr^{-1}), (**c**) trehalose-6-phosphate synthase activity (T6PS, nmol UDP mg^{-1} protein hr^{-1}), and (**d**) trehalose-6-phosphatase activity (T6PP, nmol Pi mg^{-1} protein min^{-1}) in Pusa 2001 and AL 201 pigeon pea genotypes under Ni stress. Values are the mean of six replicates ± standard error (SE). Different letters above each bar indicate significant differences among the treatments, assessed by Duncan multiple range test, at $p \leq 0.05$. C = PAs and AM absent; Spm = 0.5 mM Spm added; Spd = 0.5 mM Spd added; Put = 0.5 mM Put added; AM = AM added; Ni = 200 mg/kg Ni added; Spm + AM = Spm and AM added; Spd + AM = Spd and AM added; Put+AM = Put and AM added

T6PS, β(AM) = 0.358, β(Put) = 0.250, β(Spd) = 0.155, β(Spm) = 0.072]. Moreover, a negative correlation observed between Tre and N_2ase activity indicated that nodules with reduced N_2ase activity tended to synthesize more Tre [Pusa 2001 $r(\text{Tre} - N_2\text{ase}) = -0.109$, $p = 0.01$; $r(\text{Tre} - N_2\text{ase}) = -0.345$, $p = 0.01$]. Maximum quantities of Tre could be attained when both +Put+AM were given together, especially in Pusa 2001 (significant interaction Put x AM x G; Table 3).

Discussion

The negative impact of Ni toxicity was elicited in the form of reduced plant biomass, the effects being more severe on RDW when compared with the SDW, thus resulting in reduced root to shoot ratios. The data also clearly demonstrated the differential response of two relatively tolerant pigeon pea genotypes under Ni stressed conditions, with higher reductions recorded in AL 201 than Pusa 2001. Roots are the first organ to face Ni stress, therefore experiencing higher harmful effects than shoots as reported in pigeon pea (Rao and Sresty 2000) and chickpea (Khan and Khan 2010). Reduction in plant biomass was proportionated to increased Ni concentrations which were significantly higher in roots than shoots in a genotype-dependent manner. The negative effects of Ni could also be attributed to modification of morphological and physiological characteristics (total biomass, leaves number, plant height, and root length) as observed in *Amaranthus viridis* (Joseph et al. 2018). The negative effects of Ni on root growth could also be due to retardation of cell division and alteration of mitosis in root apex zone (Kozhevnikova et al. 2007) as investigated in soybean, lentil (Jamal et al. 2002), tomato (Madhaiyan et al. 2007), as well as maize (Gopal et al. 2014). Moreover, approximately 50% of the absorbed Ni is stored in the roots because of its sequestration

inside walls of xylem parenchymatic cells via cation exchange and immobilization in vacuoles (Seregin and Kozhevnikova 2006; Sachan and Lal 2017).

The colonizing ability of pigeon pea roots with AM fungus *Rhizoglomus intraradices* had a negative correlation with the presence of Ni in the soil. Genotype Pusa 2001 had better ability to colonize and establish effective mycorrhizal symbiosis than AL 201. The symbiosis formed between plant and soil fungi varies in effectiveness based on the plant species and even the genotype of the same species (Singh et al. 2012; Bazghaleh et al. 2018). However, Ni-induced toxicity was more severe on plant growth when compared with mycorrhizal symbiosis. On the other hand, MR increased under Ni stress with Pusa 2001 exhibiting greater responsiveness toward AM than AL 201. The decline in MC could either be due to the direct effects of Ni on germination of the spore, hyphal branching, and development in soil or because of the adverse effects of Ni on root (Ker and Charest 2010; Twanabasu et al. 2013) as investigated in our study. However, ability of AM to effectively colonize even the sensitive genotype could be due to fact that AM fungal propagules never disappear completely even in highly HM-polluted soils (Vallino et al. 2006).

In addition to mycorrhizal symbiosis, the roots of both pigeon pea genotypes formed an efficient rhizobial symbiosis under unstressed control series. However, with the introduction of Ni to the rooting medium, the nodulation potential (NN, NDW) was reduced in line with the reduction in root biomass in a genotype-dependent manner. Although under control conditions, rhizobia were capable of actively infecting pigeon pea roots; the presence of HMs (such as Ni) might have prevented the root hair formation and affected the infection process, thereby limiting the number of active nodules in legume plants (Gage 2004; Ishtiaq and Mahmood 2011; Dudeja et al. 2012; de Macedo et al. 2016). In addition, the functional efficiency of nodules (ARA) also declined under Ni toxicity, with a direct correlation between LHb concentration and ARA, suggesting the determination of LHb as a good indicator of N_2-fixing efficiency. Poor root nodulation in soybean plants grown in Ni-stressed soils interfered in the N_2-fixing system causing direct toxic effects on rhizobia and inhibition of LHb synthesis (de Macedo et al. 2016). Inhibition of nodulation and nitrogenase activity under Ni toxicity has also been reported in *Frankia-Alnus* (Wheeler et al. 2001) and *Lens culinaris-Rhizobium leguminosarum* symbiosis (Saad et al. 2016). Haddad et al. (2015) reported significant decline in nodulation and N uptake with increasing HM concentrations (Ni, Co, Cr, Cd, Cu, and Pb) on three leguminous crops (*Vicia faba*, *Trifolium alexandrinum*, *Glycine max*). The observed decrease in N_2 fixation could be due to the reduction in O_2 flux into the nodule under environmental stress (Hunt and Layzell 1993) and also due to impaired carbon (C) metabolism which could limit for bacteroid respiration (González et al. 2001). This fact justifies the decreased atmospheric N_2 fixation due to low symbiotic activity of microbes at high Ni concentrations in soil.

The present study indicated that reduced mycorrhizal and rhizobial symbioses under Ni stress had a direct effect on nutrient (P and N) concentrations in the nodules. The decline in P and N was accompanied by reduction in Zn, Fe, and Cu concentrations as well. This nutrient imbalance was proportionate to the increase in Ni uptake due to loss of nodular membrane stability more in AL 201 than Pusa 2001. Garg and Bharti (2018) reported that salinity caused membrane damage in chickpea plants that could be a major reason for reduced growth and nutrient uptake, with greater membrane damage in the sensitive genotype than tolerant one. High concentration of Ni in nodules reduces influx of nutrient (N, P, K, Cu, Fe, and Zn) due to more accumulation of Ni inside the nodules which results in nutrient imbalance as observed in different crops such as barley, soybean, *Lens culinaris* (Rahman et al. 2005; de Macedo et al. 2016; Saad et al. 2016). In addition, Ni strongly competes with several cations (in particular Cu, Fe, and Zn) due to the same transporters (ABC, Nramp, CTR, and ZIP families of metal transporters) preventing them from being absorbed by plants, which ultimately leads to their deficiency (Pandey and Sharma 2002; Tamayo et al. 2014; Sachan and Lal 2017) as authenticated in our study as well.

Ureides (ALN and ALA) have been known as important N-rich compounds, playing a crucial role in the assimilation, metabolism, transport, and storage of N in several plant species such as *Vigna unguiculata* and *V. radiata* (Castro et al. 2001; Todd et al. 2005; Freitas et al. 2018). In this study, the effect of Ni toxicity on ureide metabolism in nodules of pigeon pea demonstrated that TUs concentration decreased under Ni stress which was in line with a significant decline in N_2-fixing efficiency and ultimate increased Ni concentration in nodules. The decreased TUs were however accompanied by an increase in ALN, ALA, and ALNase, the effects being stronger in Pusa 2001 as compared to AL 201. ALNase, a hydrolyzing enzyme, plays an important role in ureide metabolism, because this enzyme catalyzes the last step of ureide biosynthesis (i.e., conversion of ALN to ALA) in nodules (Pélissier and Tegeder 2007; Werner et al. 2010). The increased activity of ALNase under stress has been linked with a direct increase in ALA content in the nodules. Alamillo et al. (2010) has reported that induction of ureides (ALN and ALA) in nodules mitigated the negative impacts of drought stress and conferred stress resistance to *Phaseolus vulgaris* plants. To elucidate this, researchers proposed that ureides accumulation might be having a regulatory role and result in N-feedback inhibition of the N_2ase complex (Vadez et al. 2000; King and Purcell 2005). However, in our study, the increased ALN and ALA due to increased ALNase activity did not seem to be effective in ameliorating Ni-induced toxic symptoms in

the nodules of even the tolerant genotype. Ultimately, ureides get translocated to the leaves as urea (Zrenner et al. 2006; Ladrera et al. 2007). Urea is an important source of ammonia (NH_3), and the efficiency of urea is decreased after its hydrolysis with the enzyme urease, therefore reducing NH_3 emissions under stress leading to large N losses (Sanz-Cobena et al. 2008). Legumes require N in large quantities, and its limited availability negatively effects the plant growth (Masclaux-Daubresse et al. 2010; Takagi et al. 2018). In our study, reduction observed in urea accumulation in the leaves might have led to reduced urease activity as well as ultimate production of NH_4^+ ions consequently resulting in a decline in the available N source under Ni toxicity. Similar results (reduced leaf urea accumulation and urease activity) were also obtained in cucumber and soybean under Ni toxicity (Khoshgoftarmanesh et al. 2014; de Queiroz Barcelos et al. 2017).

Current study revealed that exogenous applications of both PAs (Put, Spd, and Spm) as well as AM (*Rhizoglomus intraradices*) led to a remarkable improvement in plant biomass, nutrient status, and nodulation potential of pigeon pea genotypes even under stressed conditions. Regression analysis displayed that comparatively AM inoculation was more effective in lowering the toxic effects of Ni when compared with PAs priming. Such beneficial effects could be direct – by adsorption or chelation of HMs – or indirect through improved nutrient status (Zhang et al. 2015). Higher improvement in plant biomass under Ni stress by AM inoculation may be attributed to its ability to decrease concentration of endogenous Ni in the nodules more efficiently than PAs. The beneficial effects were more discernible in Pusa 2001 due to its better potential to establish AM colonization than AL 201. AM associated extensive hyphal network in root rhizosphere of tolerant genotype was able to explore more soil area, thus leading to significant improvement in nutrient accumulation in the nodules, especially P and N, and the resultant higher beneficial effects on the functional efficiency of nodules. Increased P assimilation influences the N_2ase activity positively, which in turn enhances root as well as mycorrhizal growth (Stancheva et al. 2006). AM fungi increase the absorptive root surface by producing profuse hyphae and by taking up nutrients from relatively remote regions (Abbott and Robson 1991; Bolan 1991; Bitterlich et al. 2018). Our results indicated that exogenous application of AM enhanced root growth and improved root rhizosphere which resulted in the uptake of N, P, Zn, Fe, and Cu in nodules under Ni stress, by reducing the Ni uptake from the soil. PAs, especially Put and Spd, were also significantly effective in enhancing biomass, N-fixing potential, and reducing Ni uptake more in Pusa 2001 than AL 201. This enhanced plant biomass could be due to the involvement of PAs in cell division, replication, and transcription (Chen et al. 2018). PAs have been found to suppress the accumulation of HMs (Ni, Cd, Zn, Cu, and Pb) which resulted in an increase in the tolerance of wheat plants against these HMs (Aldesuquy et al. 2014). Among the three PAs, Put priming was relatively more effective than AM in increasing NN which might due to greater ability of rhizobia to infect pigeon pea roots. Similar results were reported by Vassileva and Ignatov (1999) where Put application increased NN and nodular biomass as well as induced N_2 fixation by enhancing the potential of *Rhizobium galegae* strain HAMBI540 to infect *Galega* roots. However, the nodules observed were small in size, suggesting that PAs were not very effective in increasing nodule growth and N-fixing potential. On the other hand, AM application was highly capable of increasing nodule development and improving nodule biomass as well as N_2-fixing efficacy. In one of our previous lab studies, AM was more effective in increasing NDW, LHb concentration, and N_2ase activity in which the Put treatment increases NN under salt stress in pigeon pea (Garg and Sharma 2019). In the current study, enhanced P status in AM-treated plant might have improved the energy status required for effective rhizobial symbiosis, thereby resulting in improved N_2 fixation more efficiently than all three PAs.

Existing study also revealed that AM inoculated plants could increase the accumulation of ureides, namely, ALN and ALA, in the nodules significantly and were able to mitigate the negative impact of Ni more than PAs. Put was more effective as compared to Spd and Spm but less effective than AM inoculation. Moreover, the exogenous application of AM and PAs treatments improved the urea accumulation in leaves and ultimately increased the activity of urease enzyme, making more N available to the plants and protecting them against Ni stress. In our study, the increased ALN and ALA synthesis and their degradation into urea with the help of increased urease activity more in AM inoculated plants than PAs, since AM could possibly be related to elevation in N_2 fixation which then resulted in high NH_3 concentration in the form of increased N status in pigeon pea plants.

In the present investigation, very low levels of Tre were observed in the nodules of the two genotypes under unstressed controls due to high activity of TRE. However, presence of Ni induced decline in TRE activity and increased Tre concentration to a certain extent only. Interestingly, negative correlation between Tre and N_2ase activity indicated that nodules with reduced N_2ase activity under Ni supply tended to synthesize more Tre, suggesting that more Tre is synthesized because of low demand for reduced C in the bacteroids (Streeter and Salminen 1988). Tre might also protect nitrogenase from inactivation since it has antioxidant effect (González-Párraga et al. 2003). Moreover, a significant decline in TRE activity was recorded with AM inoculations which resulted in significant improved Tre concentrations in the nodules. This increment in Tre was accompanied by increased activity of both enzyme T6PS and T6PP indicating direct role of AM in upregulating Tre biosynthesis. The genotype Pusa 2001 which

could establish a stronger association with AM and *Rhizobium* also displayed higher Tre concentration when compared with AL 201. Tre serves as energy source for the growth and survival of rhizobia in the deteriorating nodule (Zacarías et al. 2004; Iturriaga et al. 2009), and its enhanced synthesis through AM might have helped pigeon pea plants to overcome Ni stress as investigated in our study. Moreover, AM has also been reported to accumulate high concentrations of Tre in spores, extra radical mycelium, sclerotia where it functions as energy reserves, and intermediate C storage (Bécard et al. 1991; Ocón et al. 2007; Ballesteros-Almanza et al. 2010). In addition, high amount of Tre in the nodules of mycorrhizal plants was proportionate to higher P concentration, where it is present in the intra-radical hyphae of mycorrhiza which could favor phosphate production and translocation (Ocón et al. 2007). Although reports on the impact of Ni stress in Tre metabolism are lacking, exogenous supplementation of Tre has been observed to increase the endogenous Tre subjected to Cu stress in rice signifying its additional protective role in antioxidant induction (Mostofa et al. 2015). The overexpression of Tre synthesizing genes leading to greater T6PP activity as well as Tre accumulation has also been reported under Cd, Cu, and salt stress (Martins et al. 2014; Krasensky et al. 2014) stating its importance in imparting HMs stress tolerance. Increased Tre biosynthesis through the three PAs treatments (mainly Put), observed in the current study, revealed its indirect role in enhancing growth and mycorrhizal symbiosis in pigeon pea plants.

The combined application of PAs and AM inoculation was extremely beneficial in increasing root and shoot biomass, nodulation potential, and mycorrhizal effectiveness as well as boosting Tre synthesis when compared with their individual treatments. Among the various combinations of different PAs with their respective AM treatments, Put-primed AM-inoculated plants (*R. intraradices)* had higher MC which indicated that the combination of these two treatments was highly competent in enhancing the nodulation and N_2-fixing efficiency of pigeon pea plants. Exogenous PAs might have stimulated AM spore germination, hyphal growth, and branching which led to an efficient establishment of mycorrhizal symbiosis with *R. intraradices*, thereby increasing Tre biosynthesis and alleviating Ni toxicity through enhanced nutrient uptake. The improved nutrient status (especially P) resulted in improved ureides synthesis (ALN and ALA) in the nodules as well as their subsequent translocation and conversion into urea in the leaves for utilization as N source to growing pigeon pea plants.

Conclusion

The present study demonstrated that Ni had deleterious effects on plant growth and rhizobial and mycorrhizal symbioses. The resultant declined nutrient status of the nodules was proportionate to increased Ni uptake in the roots as well as its translocation to nodules. These negative effects had a direct bearing on ureide and urea synthesizing capacity of plants. Put priming facilitated mycorrhizal symbiosis which, in turn, improved the colonization ability of pigeon pea plants with rhizobia. Thus, present study highlighted the importance of PAs in complementing AM symbiosis, thereby modulating Tre and ureide metabolism, which could be a determining factor for efficient nodule functioning, especially in the tolerant genotype of pigeon pea subjected to Ni stress.

Acknowledgments We gratefully acknowledge the University Grants Commission (UGC) and the Department of Biotechnology, Government of India, for providing financial support in undertaking this research work. We are also thankful to PAU, Panjab; IARI, New Delhi, India; and The Energy and Resource Institute (TERI), New Delhi, for providing the biological research materials. The authors are also thankful to Sophisticated Analytical Instrumentation Facility (SAIF), Panjab University, Chandigarh, India, for WD-XRF analysis.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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