Effect of Root Zone Temperature on Accumulation of Molybdenum and Nitrogen Metabolism in Potato Plants

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Abstract

Changes in root temperature caused by the application of plastic covers were studied in relation to the uptake and content of molybdenum (Mo) in the different organs of potato (*Solanum tuberosum* L. var. Spunta) plants (roots, tubers, stems, and leaves) and in relation to nitrogen (N) metabolism. For the semi-forcing technique of mulching, four different covers were used: T_1 (transparent polyethylene), T_2 (white polyethylene), T_3 (coextruded black and white polyethylene), and T_4 (black polythylene). The control treatment had no mulch. The results revealed a positive and significant effect of plastic covers on root temperatures: T_0 = 16°C, T_1 = 20°C, T_2 = 24°C, T_3 = 27°C, T_4 = 30°C. These thermal differences significantly influenced the Mo concentration, particularly in the T_2 and T_3 treatments in the leaves, roots, and tubers. The same temperatures significantly altered N metabolism in both the aerial and underground parts of the plants, and a strong interrelationship was found between Mo and nitrate reductase (NR) activity. The mulching of this crop proved to be a promising technique in phytoremediation.

Key Words: Solanum tuberosum; Mulch; N metabolism; Molybdenum; Phytoremediation; Root-zone temperature.

INTRODUCTION

Among the plant micronutrients, the amount of molybdenum (Mo) required by plants is very low,[1,2] and the best known Mo containing enzyme in plants is nitrate reductase (NR, E.C. 1.6.6.1), catalyzing the initial step of assimilatory nitrate pathway.[3,4]

Root-zone temperatures (RZT) strongly influences plants growth and nutrient uptake.[5–7] Economically important crops such as the pepper, potato, tomato, and melon require optimal temperatures in the root zone for maximum growth and yield.[8] Thus, temperature can influence both the Mo concentration and NR activity.[9]

One of the techniques used to increase and control the RZT is the application of polyethylene covers (soil mulching) of different colors and characteristics, which can generate a warmer microenvironment in the root zone for plants that benefit from such changes.[10]

In addition, a major current problem is agricultural soil and water pollution, which threaten human health and which can be partially solved by phytoremediation techniques.[11,12]

The aim of this work was to determine how different RZT generated under the different mulches affected the Mo concentration and the nitrogen (N) metabolism in potato plants (Solanum tuberosum L. var. Spunta) and thus the possibilities of phytoextraction using this crop.

MATERIAL AND METHODS

Crop Design

The experiment was conducted for 3 consecutive years (1993, 1994, and 1995) in the field (Granada, Spain), using *S. tuberosum* L. var. Spunta, planted at the beginning of March. The crop cycle was about 4 months. The climate was semiarid and the area was intensively used for agriculture. The soil used showed the following characteristics: 45.3% silt, 43.2% loam, and 11.2% clay, pH (H_2O 1 : 2.5) 8.6; electrical conductivity (E.C.) 1.10 dS m^{-1}; $CaCO_3$ 11.2%; total N (0.1%); total P_2O_5 (58 μg g^{-1}); total K_2O (115 μg g^{-1}); DTPA + TEA + $CaCl_2$ (pH 7.3) extractable Mo 0.62 μg g^{-1}. The characteristics of the irrigation water were: pH 7.6; E.C. 1.05 dS m^{-1}; Cl^- 58 mg L^{-1}; Na^+ 25 mg L^{-1}; K^+ 4 mg L^{-1}; H_2CO_3 369 mg L^{-1}, and 3 μg L^{-1} of Mo.

The experimental design was a factorial arrangement in a randomized complete block with five treatments replicated 4× (20 plots). Each plot occupied an area of 78.4 m^2, with a planting density of 4.2 plants m^{-2}. Plants were spaced 30 cm apart, with 80 cm between rows. The soil temperature was measured at the 15 cm in depth, using 107 temperature probes (Campbell Scientific Co., Logan, UT). Root zone temperature was measured (six measurements at 4-h intervals) every 3 days of the crop cycle.

The different treatments consisted of covering the soil surface of each plot with plastic mulches (polyethylene sheets), making a tight seal with the soil: the polyethylene sheets were transparent polyethylene (25 μm in thickness, T_1), white polyethylene (25 μm in thickness, T_2), coextruded black and white polyethylene (50 μm in thickness, T_3), and black polyethylene (25 μm in thickness, T_4). No plastic was applied for the control treatment (T_0).
The fertilization used was the same as is habitually applied by farmers in the zone. In the month of February in all 3 years, N (NH_4NO_3) and phosphorus (P) and potasium (K) (K_2HPO_4) were applied (27 g m^{-2}). At the end of April, 25 g m^{-2} of NH_4NO_3 were applied. Fertigation was complemented with the following micronutrients: Fe: 0.5 mg L^{-1}; B: 0.1 mg L^{-1}; Mn: 0.1 mg L^{-1}; Zn: 0.075 mg L^{-1}; Cu: 0.075 mg L^{-1}, and Mo: 0.05 mg L^{-1}. Iron was applied as FeEDDHA, B as H_3BO_3, and the remaining micronutrients as sulphates.

Plant Sampling

The plant material (stems, leaves, roots, and tubers) was sampled 6 times every 2 weeks, throughout the plant development for the 3 years of experiments. For each sampling, 10 plants were collected from each replicate per treatment. Leaf samples were taken only from plants with fully expanded leaves of the same size. Leaves were picked at about one third of the plant height from the plant apex. Roots, leaves, stems, and tubers were rinsed three times in distilled water after decontamination with non-ionic detergent at 1%,[13] and then blotted on filter paper.

At each sampling, fresh matter was used for assay of NR, amino acids and proteins, and then the sub sample was dried in a forced air oven at 70°C for 24 h, ground in a Wiley mill to pass through a 40-mesh screen and then placed in plastic bags for the further analyses.

Plant Analysis

NO_3^--N Determination

NO_3^--N was analyzed from an aqueous extraction of 0.2 g of dried and ground material in 10 mL of MILLIPORE-filtered water. A 100 µL aliquot was taken for NO_3^--N determination and added to 10% (w/v) salicylic acid in sulfuric acid at 96%. The NO_3^--N concentration was measured by spectrophotometry as performed by Cataldo et al.[14] The results were expressed as mg g^{-1} dry weight (dw).

Molybdenum Determination

For the assay of total Mo concentration, oven-dried and pulverized plant material was digested with concentrated nitric acid. Measurements were made using an atomic absorption spectrophotometer equipped with a graphite furnace.[15] Reagent blanks for analysis were also prepared performing the entire extraction procedure but in the absence of the samples. For the soluble Mo determination, dry matter (0.15 g) was extracted with 10 mL 1 M HCl for 30 min and then filtered, and determined using the method indicated above.

Detection of In Vivo Nitrate Reductase Activity

I n vivo NR activity (E.C. 1.6.6.1) was determined by the Bar-Akiva and Sternbaum[16] assay. Leaves were cut into 5 mm sections (100 mg) and placed in 10 mL of incubation buffer [100 mM K-phosphate buffer pH 7.5 and 1% (v/v) propanol]. The samples were infiltrated and the intracellular spaces of the tissues were flushed with buffer, using a vacuum (0.8 bar). After 5 min, the vacuum was released and the samples were re-evacuated, incubated at 30°C in darkness for 1 h, and finally placed in a boiling water bath to stop the NR activity. The resulting nitrite concentration was determined spectrophotometrically at 540 nm in a reaction mixture containing 2 mL of extract, 2 mL of 1% (w/v) sulfanylamide in 1.5 N ClH.[17] The activity induced by NO_3 (NR + NO_3) was determined following the same method as NR, with a modified incubation buffer, containing 50 mM KNO_3. The NR induced by Mo (NR + Mo) and NR induced by NO_3 and Mo (NR + NO_3 + Mo) were also determined using a modification of the incubation

buffer containing 2% Na_2MoO_4 (w/v), respectively. The resulting nitrite concentration was also determined by spectrophotometry.

Soluble Amino Acid and Soluble Proteins Determination

Fresh leaf and root samples (0.5 g) were crushed with cold phosphate buffer (50 mM KH_2PO_4, pH 7) and centrifuged at 12,000 g for 15 min. The resulting supernatant was used for the determination of total amino acids by the ninhydrin method as described by Yemm and Cocking.[18] Total free amino acids were expressed as mg glycine g^{-1} fw. Soluble proteins were measured by Bradford G-250 reagent[19] and expressed as mg bovine serum albumin g^{-1} fw.

Organic Nitrogen Determination

A 0.1 g dry weight sub-sample was digested with sulfuric acid and H_2O_2.[13],[22] After dilution with deionized water, a 1 mL aliquot of the digest was added to the reaction medium containing buffer [5% potassium sodium tartrate, 100 µM sodium phosphate and 5.4% (w/v) sodium hydroxide], 15%/0.03% (w/v) sodium salicylate/sodium nitroprusside, and 5.35% (v/v) sodium hypochlorite. Samples were incubated at 37°C for 15 min and organic ammonium (N-NH_4^+), was measured by spectrophotometry according to Baethgen and Alley.[20] For the determination of soluble N-NH_4^+, 0.15 g of dry matter was extracted with 10 mL of 1 M HCl for 30 min and then filtered, the concentration was measured by spectrophotometry.[20] The organic-N was calculated by subtracting the organic N-NH_4^+ from soluble N-NH_4^+. The results were expressed as mg g^{-1} dw.

Statistical Analyses

Analysis of variance was used to assess the significance of treatment means. Significant differences according the Duncan's Multiple Range Test (DMRT) are indicated with different letters in the tables. Levels of significance are represented at $p < 0.05$, $p < 0.01$, and $p < 0.001$.

RESULTS

Table 1 shows the mean values for RZT generated by the different cover treatments applied as well as for the open-air plots. The treatments significantly affected the mean RZT, giving the highest value at T_4 (30°C), and the lowest in T_0 (16°C).

Table 1 presents the results of biomass expressed as dry weight of the different organs of the potato plants, giving the mean value of 3 experimental years. The accumulation of dry material was significantly affected by some of the different root temperatures and in some of the organs. Thus, in the roots and tubers, T_3 registered value for dry weight, surpassing T_0 by 34% and 35%, respectively, while the lowest value was recorded in the T_1 treatment. In the stems, T_1 presented the highest dry weight values, exceeding T_0 by 20%, while T_3 values proved 7% lower than those of T_0. Finally, in the leaflets, T_3 reached the highest dry–weight values, exceeding T_0 by 15%, while the lowest values corresponded to T_1.

The results on the total Mo concentrations in the different organs analyzed (Table 2) reveal a positive effect of the treatments T_2 and T_3 on the total Mo

concentration in roots, tubers, stems, and leaves. The T_1 treatments negatively affected Mo, with the lowest concentrations in the roots, tubers, and leaves.

Soluble Mo (Table 2) in the roots reached the highest concentration in T_2 at 31% higher than T_0 values, while T_1 remained 5% lower than control. In tubers and stems, T_1 reached the highest concentration, at 10% and 27% higher than T_0 values. In the leaves, the concentration was higher in T_2, T_3, and T_4, while T_1 proved 11% lower than T_0.

Table 3 presents the relationship between RZT and Mo phytoaccumulation. Notable among the results, T_3 positively influenced phytoaccumulation of total Mo in the roots, tubers, and leaves, surpassing T_0 by 94%, 81%, and 20%, respectively, while in T_1 the phytoaccumulation in these organs proved negligible. In the stems, the highest phytoaccumulation was found in T_2, some 76% higher than in T_0.

In relation to the relative distribution of total Mo in the different organs, the leaves were the organs with the highest accumulation, registering between 35% and 48% of the plant total, while the lowest values were recorded for the stems, between 11% and 18%. Thus, the phytoaccumulation of total Mo followed the order: leaves > tubers > roots > stems. In the roots, leaves, and tubers, the T_3 treatment gave rise to the highest accumulation of soluble Mo (Table 3), this being 62%, 37%, and 23%, respectively, higher than T_0. In the stems, the effect of T_1 was notable for bringing about the accumulation of soluble Mo, surpassing T_0 by 53%. The phytoaccumulation of soluble Mo was greatest in the tubers, presenting 33% in T_1 and 41% in T_0.

Table 4 gives the NO_3^--N concentrations in the different organs of the potato plants. In the roots, the highest concentrations were registered by T_1 and T_3, surpassing T_0 by 11% and 9%, respectively. In the tubers, T_1 gave rise to the highest NO_3^- concentration. In the stems, no statistically significant differences appeared between treatments. Finally, in the leaves, the highest concentration were found in T_2, T_3, and T_4, surpassing T_0 values by 7%, 12%, and 15%, respectively, while the lowest value was found in T_1 (12% lower than in T_0).

Nitrate reductase catalyses the reduction of NO_3^- to NO_2^-,[21] a fundamental process for the conversion of mineral to organic N, which is considered limiting for plant growth and development.[22] The behavior of initial NR, which reduces the anion in reserve within the vacuole,[23] showed the highest activity in the roots in the T_3 treatment (25% higher than in T_0), while T_1 values were markedly lower (some 40%) than in T_0. In the leaves (Table 5), the T_2 treatment gave rise to the highest initial NR (some 18% higher than in control), while T_1 values also exceeded control (by 30%).

The NR activity infiltrated with NO_3^- (NR + NO_3^-) indicates the nutritional needs in NO_3^-, both in the leaves and roots, and thus a lower activity with respect to the initial value indicates excess endogenous NO_3^-.[24],[25] The NR + NO_3^- activity (Table 5) in the roots was the highest in T_0, while T_1 values

fell 29% below T_0 values. In the leaves this NR + NO_3^- was highest in T_1 and T_2 showed a 6% fall with regard to T_0.

In relation to NR infiltrated with Mo (NR + Mo), the roots (Table 5) showed the highest activity in T_1, while the other treatments did not statistically differ. In the leaves (Table 5), T_2 and T_3 registered sharply higher NR activity than T_0, while T_1 proved 27% lower.

The activity of NR infiltrated with NO_3^- and Mo (NR + NO_3^- + Mo) showed the nutritional needs with respect to both NO_3^- and Mo, and thus an activity of NR + NO_3^- + Mo higher than initial NR would reflect the physiological need of the two. As shown in Table 5; the highest NR + NO_3^- + Mo activity was recorded in T_2 and T_3, while the T_1 value was 19% lower than control. In the leaves, T_2 gave the highest activity value, with respect to control, and T_1 the lowest.

Notable among the products of the reduction of NR are amino acids and proteins.[26] Soluble amino acids in the roots (Table 6) showed the highest concentration in T_3 (37% higher than in T_0), while the lowest value was given in T_1 (39% lower than in T_0). In the leaves (Table 6) values did not statistically differ between treatments.

The levels of soluble proteins in the roots (Table 7) were highest in T_2 and T_3, exceeding T_0 by 13%. In the leaves, the highest protein concentration was found in T_1, some 6% higher than in T_0, while the lowest was in T_2, at 11% lower than control.

Organic N in the roots and tubers (Table 8) gave the highest concentrations in T_2 and T_3 but the lowest in T_1 (17% and 19% lower than T_0) values for the two organs, respectively). In the stems, T_1 proved 9% lower than T_0, while the rest of the treatments did not statistically differ. Finally, in the leaves, T_2 and T_3 surpassed T_0 by 9% and 11%, respectively while the lowest value was given by T_1.

DISCUSSION

Root-zone temperatures generated by different mulches were similar to those of Ham et al.[27] who reported that black polyethylene (similar to our T_4), absorb roughly 96% of the short-wave radiation while reflecting very little, and thus absorbed radiation warms the soil.[28] The white polyethylene (T_2) induced a cooler soil temperature than the black mulch, because the former reflected most wavelengths of solar radiation.[29] Schmidt and Worthington,[30] demonstrated that transparent mulches as T_1 do not cause soil warming, presenting mean temperatures of 18–20°C during the crop cycle whereas the white–black coextruded covers, generate higher RZT (27°C).

Similarly, Klock et al.[31] studying tomato plants, reported an increase in total biomass in plants within the RZT range of 24–27°C, while outside this range the dry weight fell. Engels and Marschner[5] found that corn plants grown at low root temperatures not only showed slower root growth, but also had less shoot

growth, as occurred in our experiment. However, the lower dry weight in T_4 was due to the high root temperatures.[32],[33]

The RZT strongly influenced the uptake and transport of total and soluble Mo (Table 2), with T_2 (23°C) and T_3 (27°C) being the most favorable in this respect for the organs studied. Hood and Mills[34] reported contradictory results, suggesting that Mo uptake in *Antirrhinum majus* L. was not influenced by RZT. However, our results agree with those of Follet and Barber,[35] who contended that higher root temperatures boosted Mo solubility in the soil, especially at temperature above 25°C, which favored uptake in the plant. Also, we found that Mo concentration was higher in the leaves, in agreement with the findings of Welch,[36] who suggested that this micronutrient is transported through the xylem, giving rise to high Mo concentrations in the aerial part of the plant.

Salt and Baker[37] proposed that ideal plants for phytoextraction should have rapid growth, efficient transport of elements from the roots to the sink, plus strong biomass production. In our experiment, the different organs of the potato plant in treatments T_2 and T_3 showed greatest efficiency in Mo accumulation (Table 3), suggesting that phytoaccumulation depends on the biomass of each organ. This supports the idea that the hyperaccumulation of elements requires large sinks for the storage of such quantities of pollutants.[12]

With regard to NO_3^- (Table 4), root concentrations proved especially high in T_1, while higher temperatures at T_2, T_3, and T_4 decreased NO_3^- concentration. By the way, the low NO_3^- concentrations found in the tubers, resulted of low transport and high accumulation in leaves, especially in T_2 and T_3. In the stems, the treatments had no significant effect. Finally, the high mobility in the xylem of NO_3^-[38] lead to those higher levels in stems and leaves.

As a biochemical indicator of the nutritional state of Mo, we studied NR activity,[39],[22] in its initial form NR, and infiltrated with NO_3^- (NR + NO_3^-), with Mo (NR + Mo) and with NO_3^- and Mo (NR + NO_3^- + Mo; Table 5). Wang et al.[9] suggested that NR activity diminishes in wheat plants with a low Mo supply and that this effect intensifies at low root temperatures. This idea could be applicable to the T_0 (16°C) and to the T_1 (20°C) treatment, which registered low NR activity in roots and leaves, while T_2 and T_3, with more adequate temperatures, showed higher NR values in roots. On the other hand, the highest temperatures at T_4 affected negatively the NR activity.

Du et al.[40] suggested that the N supply with low temperatures intensified the impact of Mo on plant growth by encouraging the inhibition of Mo uptake.[9] Thus, NR activity depends on the Mo concentrations and, according to Lavon and Goldschmidt,[39] the NR + Mo activity is stronger under conditions of Mo deficiency. Hence, the results above (Table 5) show that the highest NR activity corresponded to NR + NO_3^- + Mo, while NR + NO_3^- was lower than initial NR; this results suggests that our potato plants presented high endogenous NO_3^- and they did not show any deficiency symptom, while Mo was at deficient concentration, supported by the highest NR + Mo activity and NO_3^- concentration in leaves for these treatments, as well as interacting the Mo with NO_3^-.[38]

The lowest levels of amino acids and proteins in T_0 (16°C) and T_1 (20°C), apparently due to lower RZT (Table 6) that decreased the NO_3^- assimilation. The T_3 improved the NO_3^- assimilation, given that NO_3^- reduction require the NR activity, giving rise to amino acids and proteins.[41] The lowest level of amino acids and proteins in the roots of T_4 resulted of the too high temperatures which decrease the absorption and assimilation of NO_3^-.

Therefore, the highest organic N concentration at T_2 and T_3 in the studied organs (Table 8), due to the greater NR activity and higher reduction and assimilation of NO_3^-, raised the organic N. However, T_1 reflected how the decrease in the N assimilation takes place. Finally, highest temperatures in T_4 scarcely affected all these processes respect to the control plants. It was noteworthy that T_2 and T_3 positively affected the uptake and transport of Mo, boosting NR activity and thereby causing a strong reduction and assimilation of NO_3^-, which in turn led to high concentrations of amino acids, proteins, and organic N. The phytoaccumulation of Mo was favored in T_2 and T_3, which proved more efficient in dry weight production.

According to Pais and Jones,[42] the Mo concentrations in the plants can fluctuate from 0.1 to 3.0 mg kg^{-1} dw, with considerable variation between species. In tomato plants, Asher[43] and Jones[15] found the optimum range of Mo to be between 0.68 and 1 mg kg^{-1} dw, while in *Brassica rapa* L. var pekinensis, Mills and Jones[44] established the sufficient range for Mo at between 2.6 and 5.6 mg kg^{-1} dw, implying that the concentrations that we found here could be considered adequate for our potato crop. On the other hand, the toxic dosage for human consumption is not known.[45] Marschner[38] suggested that Mo does not usually present phytotoxicity problems, as the foliar concentration of Mo can reach 200 mg kg^{-1} dw without the leaves showing toxicity symptoms. In our experiment, the highest concentrations were recorded in the leaves (between 2 and 3 mg kg^{-1} dw).

According to Salt and Krämer,[46] a plant is a hyperaccumulator if the relationship (concentration of metal in the aerial part/concentration of metal in the root part) exceeds 1. In our study, the Mo proved greater than 1 in all the treatments, implying a potential of hyperaccumulation of Mo.[47] In potato, although the level is low, even 0.1% dw of the aerial part would represent an advantage in phytoremediation as opposed to techniques based on engineering that are costly and pollutant.[12] Therefore, it is worthwhile studying the advantages mulching to regulate RZT and thereby maximize phytoaccumulation in the removal of toxic elements from the soil during the cultivation of this crop as well as others.

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Table 1. Effect of mulch treatments on root-zone temperatures (RZT) and dry weight in potato organs

Treatments	RZT (°C)	Roots (g plant^{-1})	Tubers (g plant^{-1})	Stems (g plant^{-1})	Leaves (g plant^{-1})
T_0	16 e[a]	1.75 bc	19.94 c	1.82 b	2.48 b
T_1	20 d	1.04 c	10.89 d	2.19 a	1.93 c
T_2	23 c	1.97 b	22.42 b	1.85 b	2.59 b
T_3	27 b	2.34 a	26.93 a	1.70 b	2.84 a
T_4	30 a	1.63 bc	20.70 c	2.09 b	2.51 b

[a]Values followed by the same letter within a column were not significantly different at $p < 0.05$ according to Duncan's Multiple Range Test.

Table 2. Effect of root-zone temperatures on Mo concentration

	Total Mo (ng g^{-1} dw)				Soluble Mo (ng g^{-1} dw)			
Treatments	Roots	Tubers	Stems	Leaves	Roots	Tubers	Stems	Leaves
T_0	1,534 b[a]	151 b	853 b	2,338 ab	597 b	94 ab	402 b	350 b
T_1	1,447 b	147 b	771 b	2,253 b	566 c	103 a	511 a	312 b
T_2	2,318 a	183 a	1,476 a	2,446 a	785 a	85 b	354 c	410 a
T_3	2,220 a	202 a	1,338 a	2,442 a	725 ab	85 b	385 c	418 a
T_4	1,656 b	157 b	712 b	2,277 b	616 b	84 b	396 b	407 a

[a]Values followed by the same letter within a column were not significantly different at $p < 0.05$ according to Duncan's Multiple Range test.

Table 3. Effect of root-zone temperatures on Mo phytoaccumulation

	Total Mo (ng g^{-1} dw)				Soluble Mo (ng g^{-1} dw)			
Treatments	Roots	Tubers	Stems	Leaves	Roots	Tubers	Stems	Leaves
T_0	2,684 c[a]	3,011 c	1,552 c	5,798 c	1,045 b	1,864 b	731 bc	868 b
T_1	1,505 d	1,601 d	1,668 c	4,348 d	589 c	1,122 c	1,119 a	602 c
T_2	4,566 b	4,103 b	2,731 a	6,335 b	1,546 ab	1,906 b	655 bc	1,062 ab
T_3	5,195 a	5,440 a	2,275 b	6,935 a	1,696 a	2,289 a	609 c	1,187 a
T_4	2,699 c	3,251 c	1,488 c	5,715 cd	1,005 b	1,740 bc	828 b	1,022 ab

[a]Values followed by the same letter within a column were not significantly different at $p < 0.05$ according to Duncan's Multiple Range test

Table 4. Effect of root-zone temperatures on NO_3^- concentrations

Treatments	Roots (mg g^{-1} dw)	Tubers (mg g^{-1} dw)	Stems (mg g^{-1} dw)	Leaves (mg g^{-1} dw)
T_0	3.62 b[a]	0.24 c	9.19 a	2.50 ab
T_1	4.02 a	0.53 a	8.65 a	2.21 b
T_2	3.69 ab	0.25 c	8.98 a	2.68 a
T_3	3.95 a	0.33 b	9.21 a	2.79 a
T_4	3.68 ab	0.39 b	9.37 a	2.87 a

[a]Values followed by the same letter within a column were not significantly different at $p < 0.05$ according to Duncan's Multiple Range test.

Table 5. Effect of root-zone temperatures on nitrate reductase (NR) activity (μmol NO_2^- produced g^{-1} fw h^{-1})

	Roots				Leaves			
Treatments	Initial NR	NR + NO_3^-	NR + NO_3^- + Mo	NR + Mo	Initial NR	NR + NO_3^-	NR + NO_3^- + Mo	NR + Mo
T_0	0.67 bc[a]	0.35 a	0.73 ab	0.17 b	0.45 b	0.27 ab	0.63 bc	0.22 b
T_1	0.40 d	0.25 c	0.59 c	0.38 a	0.32 c	0.26 b	0.44 c	0.16 b
T_2	0.76 ab	0.3 b	0.81 a	0.20 b	0.53 a	0.26 b	1.03 a	0.56 a
T_3	0.83 a	0.33 ab	0.8 a	0.20 b	0.43 ab	0.3 a	0.93 ab	0.54 a
T_4	0.57 cd	0.28 bc	0.65 bc	0.16 b	0.46 ab	0.29 a	0.6 bc	0.20 b

[a]Values followed by the same letter within a column were not significantly different at $p < 0.05$ according to Duncan's Multiple Range test.

Table 6. Effect of root-zone temperatures on soluble amino acids

Treatments	Roots (mg g^{-1} fw)	Leaves (mg g^{-1} fw)
T_0	1.39 c[a]	3.74 a
T_1	0.85 d	3.46 a
T_2	1.68 b	3.65 a
T_3	1.91 a	3.88 a
T_4	1.46 bc	3.64 a

[a]Values followed by the same letter within a column were not significantly different at $p < 0.05$ according to Duncan's Multiple Range test.

Table 7. Effect of root-zone temperatures on soluble proteins

Treatments	Roots (mg g^{-1} fw)	Leaves (mg g^{-1} fw)
T_0	9.66 ab[a]	18.64 ab
T_1	7.94 b	19.74 a
T_2	10.88 a	16.61 c
T_3	10.89 a	17.34 bc
T_4	9.89 ab	18.57 ab

[a]Values followed by the same letter within a column were not significantly different at $p < 0.05$ according to Duncan's Multiple Range test.

Table 8. Effect of root-zone temperatures on organic N

Treatments	Roots (mg g^{-1} dw)	Tubers (mg g^{-1} dw)	Stems (mg g^{-1} dw)	Leaves (mg g^{-1} dw)
T_0	15.6 b[a]	14.64 b	13.52 a	12.2 b
T_1	13 c	11.93 c	12.39 b	9.8 c
T_2	16.9 a	15.90 a	14.07 a	13.24 a
T_3	17 a	15.72 a	13.47 a	13.5 a
T_4	15.3 b	14.52 b	13.48 a	12.08 b

[a]Values followed by the same letter within a column were not significantly different at $p < 0.05$ according to Duncan's Multiple Range test.