Journal of Agriculture and Ecology Research International
11(1): 1-10, 2017; Article no.JAERI.30907
ISSN: 2394-1073

SCIENCEDOMAIN ***international***
www.sciencedomain.org

Comparison of Cationic and Anionic Forms of Nitrogen Fertilization on Growth and Calcium, Zinc and Iron Content in Shoots of Two Varieties of Vegetable Amaranth

Munene Rozy[1*], Changamu Evans[2], Korir Nicholas[1] and P. Gweyi-Onyango Joseph[1]

[1]Department of Agricultural Science and Technology, Kenyatta University, Nairobi, Kenya.
[2]Department of Chemistry, Kenyatta University, Nairobi, Kenya.

Authors' contributions

This work was carried out in collaboration between all authors. Authors MR and PGOJ designed the study and protocol. Author MR wrote the first draft of the manuscript. Author PGOJ read all the drafts. Author KN performed the statistical analysis. Author CE read the first draft. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/JAERI/2017/30907
Editor(s):
(1) Alejandro E. Ferrari, Department of Science and Technology, UNQ, Universidad Nacional de Quilmes, Argentina.
Reviewers:
(1) Iwona Konopka, University of Warmia and Masuria in Olsztyn, Poland.
(2) Congyan Wang, Institute of Environment and Ecology, Academy of Environmental Health and Ecological Security & School of the Environment and Safety Engineering, Jiangsu University, Jiangsu, China.
(3) Olaotan A. Adediran, Federal University of Technology, Minna, Nigeria.
Complete Peer review History: http://www.sciencedomain.org/review-history/17987

Original Research Article

Received 7th December 2016
Accepted 21st February 2017
Published 28th February 2017

ABSTRACT

Nitrogen (N) is an essential macro nutrient for plant growth and development. Plants absorb nitrogen in either ammonium (NH_4^+) or nitrate (NO_3^-) forms, which influence plant growth and absorption of nutrients in higher plants. The aim of the study was to determine the influence of different N forms on growth and tissue content accumulation of mineral elements; calcium, zinc and iron in vegetable amaranth. The study was conducted in Agriculture Science and Technology Departmental farm of Kenyatta University between March and May, 2015. Three N forms used were; sole ammonium (NH_4^+) stabilized with Piadin® as nitrification inhibitor, sole nitrate (NO_3^-),

**Corresponding author: E-mail: rozymunene@gmail.com, munenerozy@gmail.com;*

ammonium nitrate (NH_4NO_3) (also stabilized with Piadin®) and control as subplots on two amaranth varieties were AB6 and AB7 as main plots in randomized complete block design (RCBD) replicated three times in a split plot arrangement. Results indicated that ammonium treatment led to acidification of the rhizosphere compared to nitrate treatment, while nitrate nutrition led to a rise in rhizosphere pH. Nitrogen forms significantly (P=0.05) affected shoot and root dry weights and leaf area. Nitrate-treated plants accumulated shoot dry weight 3.7 fold in case of AB6 variety and 2.5 fold for AB7 variety as compared to control, while that of sole ammonium treatment was twofold for AB6 and 1.3 fold for AB7. Root dry weight reduced by 52% in AB6 and 46.7% in AB7 variety under ammonium treatment compared to nitrate treatment. Compared to the control, plants treated with nitrate-N form had leaf area four times higher while those treated with ammonium form were twice superior in both AB6 and AB7 varieties. Amaranths treated with sole nitrate markedly increased plant tissue (calcium, zinc and iron) concentrations compared to ammonium treated plants. Ammonium/nitrate mixture enhanced plant growth and mineral element concentration compared to sole ammonium treatment. It was therefore concluded that ammonium-induced pH acidification restricted plant biomass accumulation as well as calcium and zinc concentration compared with nitrate treatment which exhibited better growth in vegetable amaranth irrespective of variety.

Keywords: Nitrogen forms; ammonium; nitrate; rhizosphere; amaranth.

1. INTRODUCTION

Nitrogen (N) is one of the most important nutrients affecting the growth and development of plants [1]. Unlike other essential nutrient elements; plants take it in either as cation form; ammonium (NH_4^+), or anion form; nitrate (NO_3^-) [2]. However plant's response to a specific form of nitrogen varies from species to species [3]. In addition to the amount supplied, the form of the available nitrogen has a significant effect on the growth [4], yield and quality [5,6] of plants. Although ammonium should be the preferred N source, since its assimilation consumes less energy than that of nitrate [7], only a few species actually perform well when ammonium is provided as the only N source [3]. When ammonium is used as the only nitrogen source, most plant species show reduced growth, smaller leaves and a stunted root system compared to nitrate source [8,9], as reported in the case of tomatoes and spinach leaves. Restricted growth under sole NH_4^+ nutrition has been partly associated with acidification of the growth medium, resulting in NH_4^+-toxicity, antagonism in cation uptake, and/or alterations in osmotic balance [10,11].

The pH of the rooting medium is of paramount importance for plant growth as a large number of processes such as nutrient availability and availability of toxic ion species is closely related to this parameter [12]. Ammonium supply to plants leads to rhizosphere acidification while nitrate nutrition alkalinizes the rhizosphere [6,13], and risk of ammonium toxicity at low pH is attributed to NH_4^+ accumulation in plants photosynthetic tissues [14]. At the same time, ammonium N forms result in cations non-specific competition [7], that the uptake of NH_4^+ itself is higher than other cations and anions [14]. For instance, Borgognone et al. [8] observed that Ca^{2+} concentration significantly reduced under ammonium treatment compared to nitrate form. Chemical changes in the plant induced by NH_4^+N nutrition are; total tissue inhibition of essential cations such as potassium, calcium and magnesium and this decline in cations, other than NH_4^+, is accompanied by suppressed plant growth [15]. Ammonium nitrate mixture has been observed to not only stimulate plant growth but also up- regulates the uptake and concentration of essential mineral cations compared to sole ammonium in tomatoes and chive seedlings [3,5]. Rahayu et al. [16] and Walch-Liu et al. [17] reported that the possible reason for this observation was that very low concentrations of nitrate in nitrate/ammonium mixture to some extent alleviates toxicity associated with sole ammonium nutrition.

Vegetable amaranth (*Amaranthus species*) is one of the most preferred African leafy vegetables in the tropics [18,19]. It is an excellent source of numerous vitamins, proteins and mineral elements such as; iron, calcium and zinc [20,21]. Their nutritional properties have been reported to be of health protecting benefits [22] and nutritive value [23]. Nonetheless, their nutritive value may be compromised to some extent by a number of agronomical management practices. For instance the use of different nitrogen forms which greatly influences plant quality in terms of mineral element acquisition and accumulation [5] associated with rhizosphere pH shifts [24]. Ammonium induced disorders in pH regulation [17] coupled with deficiency of mineral cations (e.g. calcium) could be one the causes of hidden hunger within the society especially in women and children who are most vulnerable [25] which contributes to the increasing rates of illness and death from infectious diseases and disability [26]. Therefore the main objective of the study was to investigate the influence of nitrogen forms on growth, mineral elements accumulation in leafy amaranth for optimal derivation of both morphological and biochemical benefits from these vegetables.

2. MATERIALS AND METHODS

2.1 Experimental Design and Treatments

The experiments were laid out in a split plot arrangement on a Randomized Complete Block Design (RBCD) replicated three times. The main plot comprised of two vegetable amaranth varieties i.e. Abukutsa 6 (AB6) and Abukutsa 7 (AB7) while three N-forms (NO_3^-, $NH_4^+$and NH_4NO_3) and control (no N added) constituted the sub-plots. Sole NH_4^+ and NH_4NO_3 were stabilized with Piadin® as the nitrification inhibitor composed of a mixture of dicyandiamide and 3, 4 methylpyrazole phosphate.

2.2 Agronomical Practices

Amaranth seeds were obtained from Jomo Kenyatta University of Agriculture and Technology (JKUAT). The seeds were directly sowed at a spacing of 10 cm by 30 cm separated by 1 m path in Agriculture Science and Technology (AST) farm Kenyatta University. About 3 g of Triple superphosphate (TSP) was used as basal fertilizer.

2.3 Harvesting, Data Collection, and Preparation of Plant Samples

Harvesting of the plant samples was done by uprooting the whole plant where stratified sampling was used four weeks after treatment. The shoots and roots were separated and oven dried at 60-65℃ for 72 hours until the weight was constant and dry weight recorded. To obtain the leaf area transparent graph paper was used by summing up of the number of squares of all the leaves above 1 cm long. The dried plant samples were ground using a grinder-MIKA® to fine powder (0.2 mm) which was kept in zip lock polythene bags, appropriately labeled and stored awaiting mineral elements analysis.

2.4 Mineral Elements Analysis

Determination of mineral elements was by wet digestion and atomic absorption spectrophotometer (AAS), according to AOAC [27]. The minerals that were determined were calcium, zinc and iron. About 1 g of sample was placed in a 50 ml beaker; 10 ml of nitric acid was added. The beakers were placed on a hot plate in a fume hood for about 10-20 minutes. Distilled water was added, and then filtered using whatman filter paper No. 1. The filtrate was then transferred to a 250 ml volumetric flask and filled to the mark. The filtrate was kept in plastic bottles and appropriately labeled. The absorbance of the extracts was read by Atomic Absorption Spectrophotometer (AAS); Model A A-6200, Shimadzu, Corp., Kyoto, Japan. The various mineral standards were prepared to make the calibration curve.

2.5 Data Analysis

Data was subjected to analysis of variance (ANOVA) at 95% confidence level using SAS-computer software (SAS 2015; Version 9.0). Where there were significant differences, further mean separation was obtained by LSD.

3. RESULTS AND DISCUSSION

3.1 Effects of Nitrogen Forms on Rhizosphere pH

There was a significant (P=0.05) influence of nitrogen forms on the rhizosphere pH. Results indicated that sole ammonium treatment acidified the rhizosphere soil compared to nitrate treatment, which led to substantial alkanization of rhizosphere. Ammonium treatment led to reduction in rhizosphere pH value compared to nitrate treatment (Fig. 1). Such findings have been reported by other workers in maize [6], tomatoes [12] and strawberry [28] who observed that maximum soil pH was recorded in the pots where NO_3^- was supplied as only N source while drop in soil pH was evident under NH_4^+ treatment. Najafi and Parsazadeh, [13] and Ogut, [29] reported that rhizosphere acidification under ammonium source is due to excess uptake of cations over anions, while alkalization occurs when anion uptake exceeds cation uptake. The finding also revealed that pH values under the mixed form (ammonium nitrate) was between sole nitrate and ammonium which is in agreement with the findings of [30] who observed pH decrease at first was due to preferential uptake and depletion of NH_4^+, followed by pH increase. This maybe raises the question of stability of Piadin® under tropical conditions. The forms of nitrogen and its assimilation inside the plant cells are of great significance in the buildup of intracellular H^+ and $OH^−$, thus determining the pH shift of the external media [31]. Assimilation of NH_4^+ leads to decrease in cytoplasm pH which has to be stabilized by increased by H^+ efflux or decarboxylation of organic acid [7] - hence pH decrease. Maintenance of pH under high ammonium source is cost-incurring in terms of photosynthates such as sugars and water consumption [32]) hence poor root and shoot growth at low external pH.

3.2 Effects of Nitrogen Forms on Biomass Accumulation -Leaf Area, Shoot and Root Dry Weight

Nitrogen forms notably affected shoot and root dry mass accumulation. Ammonium as sole nitrogen source restricted shoots and root dry weights in both varieties compared to nitrate (Table 1). Compared to the control, provision of nitrate as sole source of nitrogen led to 3.7 fold increase in shoot dry weight in AB6 and 2.5 fold in AB7 while in the form of ammonium led to 2.1 fold increase in AB6 and 1.3 fold in AB7 as compared to control. Ammonium/nitrate mixture elicited the tendency to the highest shoot dry biomass accumulation (though not statically confirmed) (Table 1). Under sole nitrate treatments variety AB7 had enhanced shoot dry weight (20.5 g/m^2) than AB6 (18.0g/m^2), however greatest effect of this nitrogen form was observed in AB6 variety. Ammonium treatment led to 52% in AB6 and 46.7% in AB7 reduction in root dry weight in relation to nitrate treatment. This finding is similar to the previous results obtained by Borgognone et al. [8] and Zhang et al*.* [33] who observed that plants fed with NH_4^+ as sole N source resulted in decreased shoot and root dry weight as compared to those under NO_3^- N nutrition. Plants supplied with sole ammonium had significantly (p=0.05) smaller leaves unlike the plants supplied with nitrate-N form. Nitrate-treated plants showed enhanced leaf area, which was 1.6 fold higher than that of ammonium treated plants (Table 1). It was not surprising that amaranth plants provided with ammonium nitrate mixture led to leaf area statically similar to that of sole nitrate, even higher at some point, especially in AB7 variety. This is in concurrence with results of Zhang et al*.* [33] in maize and Gweyi- Onyango et al*.* [22] in tomatoes. Possible reasons for suppression of growth by ammonium nutrition could be due to a number of reasons: First, NH_4^+ N toxicity which develops when endogenous accumulation of free NH_4^+in the plant vacuoles exceeds the NH_4^+ -N assimilating capacity [14]. Second; sole NH_4^+ nutrition has been implicated with restricted cytokinins production [17], a hormone involved in the regulation of cell division and extensivity [34]. Rahayu et al. [16] observed that suppression in leaf growth rate was preceded by decline in cytokinins (Zeatin and Zeatin Riboside) concentrations in the xylem exudate and in young growing leaves. Third; availability of toxic ion species such as monomeric aluminium (Al^{3+}) due to increased Al_3^+solubilization in response to NH_4^+-induced rhizosphere acidification [12]. Fourth; nutritional disorders as a result of antagonism in cation uptake of NH_4^+ and H^+ at the cation uptake sites and/ or alterations and impairment in osmotic balance under ammonium application [11].

Conflicting results, however, were observed by Ruan et al*.* [35] and Gao et al*.* [36], who observed enhanced plant growth with NH_4^+ -N compared to other forms in *Camellia sinensis* and *Oryza species;* after which they concluded better adaptation of these plants to NH_4^+ - N regardless of the root-zone pH, while the latter is tolerant to NH_4^+-N source [3].

3.3 Effects of Nitrogen Forms on Plant Tissue Concentrations

3.3.1 Calcium concentration

Nitrogen forms highly significantly (p=0.01) affected plant tissue Ca concentrations in the shoots of the amaranth plants (Fig. 2). The results of the study revealed that ammonium treatment restricted Ca content compared to sole nitrate which on the contrary led to mineral element build up. Plants treated with nitrate as sole nitrogen source were above 2.5 fold in AB6 and 1.6 fold in AB7 superior in Ca concentration compared to ammonium treated plants. Variety AB7 had higher (15.2 mg/g) Ca concentration than AB6 (13.1 mg/g) in the shoots. Nonetheless; profound effects of N forms were evident in AB6 whereby nitrate application led to 60% increase in Ca accumulation compared to ammonium, whereas AB7 had 38% enhancement. Provision of ammonium/nitrate mixture enhanced Ca content in the amaranth shoots by 51% in AB6 and 21% in AB7 in relation to ammonium treatment. These findings concur with those of Roosta et al. [37] and Borgognone et al*.* [8] who worked on egg plants and tomatoes, respectively. These authors reported that Ca uptake and accumulation in plant tissues decreased with increasing external NH_4^+- N ratio in the nutrient solution. Ammonium treatment in contrast to nitrate treatment, restrains uptake of cations [38]. In this study the effects of N forms on Ca accumulation in the plant shoots may have been due to either: antagonism effect under NH_4^+- N source which reduces Ca uptake at the cation uptake sites explained by the fact that $NO3^-$ is quantitatively by far the most dominant anion in the xylem sap and is balanced by an equal concentration of cations hence increased uptake and translocation of Ca^{2+} to the shoots [39].

3.3.2 Zinc accumulation

Nitrogen forms markedly influenced zinc concentration in amaranth plants (Fig. 3). Nitrate nutrition elevated Zn concentration unlike the ammonium which resulted in inhibition of this element accumulation. When treated with sole nitrate; AB6 had higher shoot concentration of zinc (9.5 mg/Kg) than AB7 (8.4 mg/Kg). Generally used nitrate as sole treatment doubled Zn accumulation in both varieties. These results are in agreement with the previous results of Xie et al. [40] who found zinc concentration to be higher in the NO_3^- nutrition. Monsant et al*.* [41] observed that the highest shoot Zn accumulation occurred in the NO_3^- treatment, which was associated with the high rhizosphere pH in mildly acidic soils. Nitrogen form supplied to the plants may have influence on accumulation of Zn in plant shoots possibly by reducing the cation exchange capacity which leads to reversible adsorption of Zn^{2+}-ions on negatively charged sites on organic colloid surfaces, creating non-specific cation competition by NH_4^+source [42]. Another explanation is that increased translocation of the absorbed Zn from the roots to the shoot by is stimulated by NO_3^- availability and not necessarily rise in rhizosphere pH alone [43]). Serna et al. [44] reported that as the proportion of NH_4^+ in solution increased, Zn accumulation decreased in the leaves. However, in contrast to these results, Sabir et al*.* [6] recorded higher Zn concentration in maize shoots under treatment with sole NH_4^+compared with those of NO_3^--N fed plants. This could be probably because of increased soil pH of mostly above 6.5 under sole NO_3^- N source which results in reduced extractability and plant availability of soil Zn [45,29].

3.3.3 Iron accumulation

The results revealed no significant (P=0.05) differences between the nitrogen forms in iron content of the amaranth shoots (Table 2). Nitrate treatment revealed greater effect on Fe accumulation, which was however not statistically significant. Nitrate-treated variety AB7 exhibited higher (139 mg/kg) Fe content, than AB6 (133 mg/kg), however the differences were not statistically significant. These results concur with results of Rosen et al*.* [46], who found that levels of Fe in the shoot and root of *Vaccinium clones* were not affected by either N form nor pH. Roosta et al*.* [15] observed that

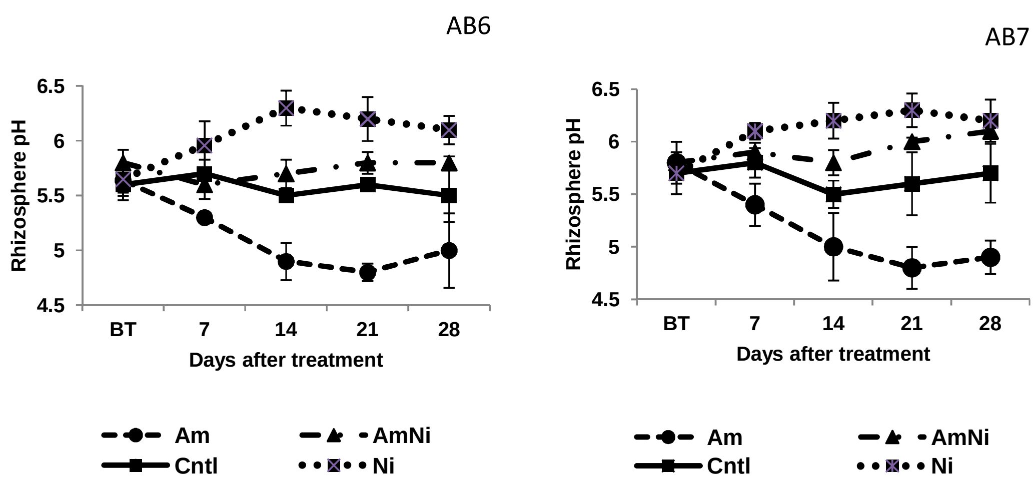

Fig. 1. Effects of different nitrogen forms on rhizosphere pH changes in field experiment
Cntl- control, Am -ammonium, AmNi -ammonium nitrate, Ni -nitrate

Table 1. Effects of nitrogen forms on plant biomass accumulation

Treatments		Shoot dry weight (g/m^2)	Root dry weight (g/m^2)	Leaf area (cm^2/m^2)
AB6	Cntl	4.9^{d}	0.8^{d}	9.7^{d}
	Am	10.4^{c}	1.2^{cd}	54.3^{cd}
	AmNi	19.4^{ab}	1.8^{b}	75.3^{bc}
	Ni	18.0^{b}	2.5^{a}	80.7^{abc}
AB7	Cntl	8.3^{cd}	1.0^{d}	32.0^{c}
	Am	11.1^{c}	1.6^{bc}	79.5^{abc}
	AmNi	22.1^{a}	2.7^{a}	132.0^{a}
	Ni	20.5^{ab}	3.0^{a}	125.0^{ab}
P value		0.003	0.001	0.001
LSD		3.5	0.5	56.1

Means followed by the same letter within the same column are not significantly different (p=0.05)
AB6-Abuktsa 6, AB7-Abuktsa 7, Cntl- control, Am – Ammonium, AmNi - Ammonium nitrate, Ni- nitrate

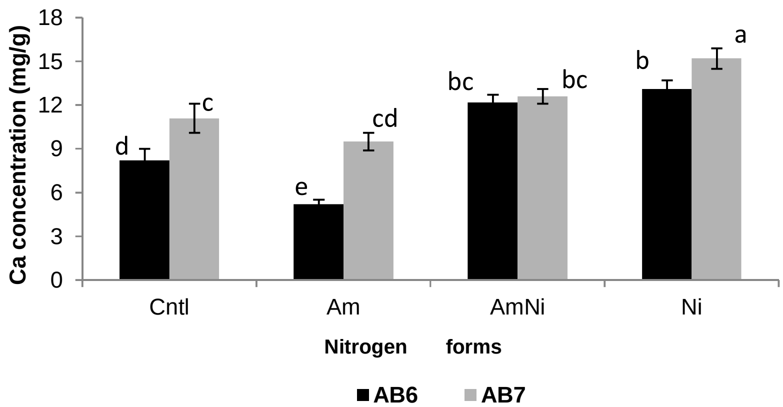

Fig. 2. Effects of different nitrogen forms on shoot calcium concentration in two amaranth varieties
AB6-Abuktsa 6, AB7-Abuktsa 7, Cntl- control, Am – Ammonium, AmNi - Ammonium nitrate, Ni- nitrate

nitrogen forms had less effect on iron accumulation in cucumber plants. Application of NO_3^--N led to increased levels of Fe in *Amaranthus tricolor* [47] which contradicts the findings of Carlisle et al. [48] who reported that plants supplied with NH_4^+-N had higher concentrations of total iron compared to plants supplied with NO_3^--N. This could be the result of limited uptake and shoot translocation of iron at very high pH in the nutrient solution [49] independent of N form, phenomena associated mostly with calcareous soils. Ammonium nutrition considerably enhanced the iron concentration in the aerial parts in comparison to the nitrate treatment in hydroponic medium enriched with bicarbonate to simulating calcareous soils [50 and 51]. Highly weathered, tropical soils are often characterized by a dominance of iron (Fe)-rich minerals which have potential for rapid rates of Fe reduction and re-oxidation [52]. In these conditions the availability of Fe to plants possible irrespective of the N- form under slightly acidic conditions.

Table 2. Effects of different nitrogen forms on shoot iron concentration in two amaranth varieties

Treatments		Iron concentration (mg/kg)
AB6	Cntl	132^{ab}
	Am	125^{c}
	AmNi	130^{bc}
	Ni	133^{abc}
AB7	Cntl	130^{bc}
	Am	127^{bc}
	AmNi	135^{ab}
	Ni	139^{a}
P value		0.19
LSD		8.6

Means followed by the same letter within the same column are not significantly different (P≤0.05)
AB6-Abuktsa 6, AB7-Abuktsa 7, Cntl- control, Am – Ammonium, AmNi - Ammonium nitrate, Ni- nitrate

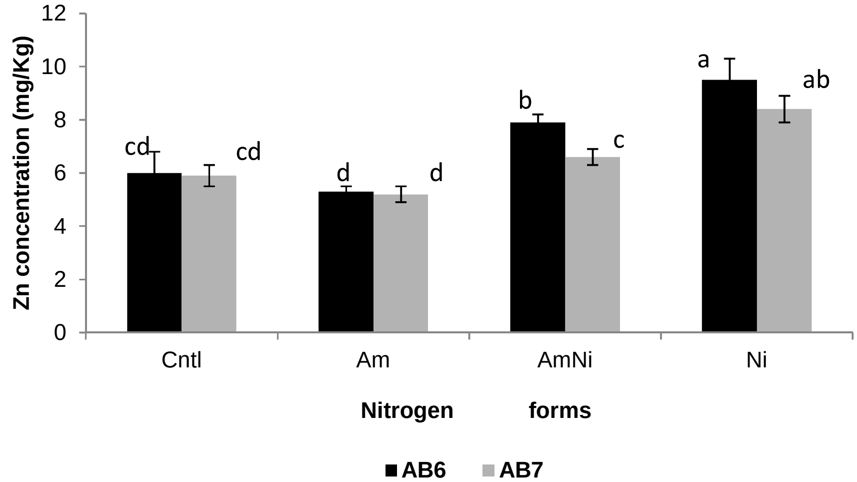

Fig. 3. Effects of different nitrogen forms on shoot zinc concentration in two amaranth varieties

AB6-Abuktsa 6, AB7-Abuktsa 7, Cntl- control, Am – Ammonium, AmNi - Ammonium nitrate, Ni- nitrate

Table 3. Pearson correlations coefficient between biomass accumulation, rhizosphere pH and mineral elements (Ca and Zn) concentrations in amaranth shoots

	Shoot dry weight	Root dry weight	Leaf area	Calcium (Ca)	Zinc (Zn)	pH
Shoot dry weight	1					
Root dry weight	0.92**	1				
Leaf Area	0.88*	0.68*	1			
Calcium (Ca)	0.76**	0.49*	0.59*	1		
Zinc (Zn)	0.65*	0.78*	0.64*	0.57	1	
pH	0.62*	0.64**	0.74*	0.59*	0.66 *	1

**and ** significant at P ≤ 0.05 or P ≤ 0.01, respectively*

3.4 Relationship between Plant Growth and Mineral Elements

Pearson correlation indicated a strong positive relationship between rhizosphere pH and plant dry weight (Table 3 above). This is in agreement with Gweyi- Onyango et al. [22] who observed that rhizosphere acidification by resulted to poor growth, while rise in rhizosphere pH was associated with superior growth in tomatoes. It is not surprising that plant biomass accumulation revealed a positive correlation with mineral elements concentrations in the amaranth plants (Table 2). This is in line with the findings of Roosta et al*.* [37] who demonstrated that enhanced plant growth was accompanied by increased concentrations of Ca in egg plants. Similarly Monsant et al*.* [43], reported that increase in external pH resulted to elevated buildup of zinc concentration in *Thlaspi caerulescens. This is an indication that* pH shift of the external medium influences biomass accumulation and mineral element composition in higher plants [39].

4. CONCLUSION

Amaranth plants responded differently to different nitrogen forms. Sole ammonium induced rhizosphere acidification while nitrate treatment resulted to rise in rhizosphere pH. This had different impact on availability and concentration of mineral elements (calcium, zinc and iron) in amaranth plants. Rhizosphere pH shift is of paramount importance in nutrient content accumulation not only for plant growth but also for optimal nutritional quality in vegetable amaranth. Use of nitrate N, both sole and mixed form -(ammonium nitrate), had better impact on plant growth and mineral element accumulation in amaranth biomass compared to ammonium treatment. Nitrate fertilized amaranth plants may be recommended -for production of leafy biomass for plant growth and nutritional benefits.

ACKNOWLEDGEMENTS

We sincerely thank Kenyatta University- Vice Chancellor's research Grant Programme. We also wish to greatly thank Prof Christ of Engels of Humboldt University, Berlin for providing us with Padin® and Prof Abukutsa-Onyango of JKUAT for the provision of amaranth seeds.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Qiang ZH, Fei XU, Rong-fu WA, Liang-zuo SH, Rui LI, De-yu ZH. Effects of nitrogen forms on the growth, yield and fruit quality of tomato under controlled alternate partial root zone irrigation. Yingyong Shengtai Xuebao. 2014;25:12.
2. Olfati, JA, Khasmakhi-Sabet, SA, Shabani, H. Nutrient Solutions on Yield and Quality of Basil and Cress. Interl J of Veg Sci, 2012;18:298-304.
3. Zhou YH, Zhang YL, Wang XM, Cui JX, Xia XJ, Shi K, Yu JQ. Effects of nitrogen form on growth, CO2 assimilation, chlorophyll fluorescence, and photosynthetic electron allocation in cucumber and rice plants. J of Zhej Univer Sci B. 2011;2:126-34.
4. Ali S, Farooq MA, Jahangir MM, Abbas F, Bharwana SA, Zhang GP. Effect of chromium and nitrogen form on photosynthesis and anti-oxidative system in barley. Biologia Plantarum. 2013;57: 758–63.
5. Sun YD, Lou WR, Liu HC. Effects of different nitrogen forms on the nutritional quality and physiological characteristics of Chinese chive seedlings. Plat Soil Environ. 2014;60:216–220.
6. Sabir M, Hanafi MM, Malik MT, Aziz T, Zia-ur-Rehman M, Ahmad HR, Hakeem KR, Shahid M. Differential effect of nitrogen forms on physiological parameters and micronutrient concentration in maize (*Zea mays* L.). Aust J of Crop Sci. 2013;7(12): 1836-42.
7. Marschner H. Mineral nutrition of higher plants. Academic Press London. 1995;889.
8. Borgognone D, Colla G, Rouphael Y, Cardarelli M, Rea E, Schwarz D. Effect of nitrogen form and nutrient solution pH on growth and mineral composition of self-grafted and grafted tomatoes. Scientia Horticulturae. 2013;149:61-9.
DOI: 10.1016/j.scienta.2012.02.012
9. Wang J, Zhou Y, Zhou C, Shen Q, Putheti R. Effects of NH4+-N/NO3--N ratios on growth, nitrate uptake and organic acid levels of spinach (*Spinacia oleracea* L.). Afr J of Biotech. 2009;8:15.
10. Britto DT, Kronzucker HJ, NH_4^+ toxicity in higher plants. J. Plant Physiol. 2002; 159(6):567-84.
DOI: 10.1078/ 0176-1617-0774
11. Horchani F, Hajri R, Aschi-Smiti S. Effect of ammonium or nitrate nutrition on photosynthesis, growth and nitrogen assimilation in tomato plants. J. Plant Nutr. Soil Sci. 2010;173:610–617.
12. Gweyi-Onyango JP, Neumann G, Romheld V. The role of nitrogen forms on solubilisation and utilisation of rock phosphate by tomato plants. In African Crop Science Conference Proceedings. 2006;7:1029-1032.
13. Najafi N, Parsazadeh M. Effect of nitrogen form and pH of nutrient solution on the changes in pH and EC of spinach rhizosphere in hydroponic culture. J of Sci and Tech of Greenhouse Culture. 2011; 2(5):29-44.
14. Roosta HR, Schjoerring JK. Effects of ammonium toxicity on nitrogen metabolism and elemental profile of cucumber plants. Journal of Plant Nutrition. 2007;30(11): 1933-51.
15. Roosta HR, Sajjadinia A, Rahimi A, Schjoerring JK. Responses of cucumber plant to NH 4+ and NO 3− nutrition: The relative addition rate technique vs. cultivation at constant nitrogen concentration. Scientia Horticulturae. 2009; 121(4):397-403.
16. Rahayu YS, Walch-Liu P, Neumann G, Römheld V, von Wirén N, Bangerth F. Root-derived cytokinins as long-distance signals for NO3−-induced stimulation of leaf growth. J of Exp Bot. 2005;56(414): 1143-52.
17. Walch-Liu P, Neumann G, Bangerth F, Engels C. Rapid effects of nitrogen form on leaf morphogenesis in tobacco. J of Exp Bot. 2000;51(343):227-37.
18. Munene R, Changamu E, Korir N, Gweyi-Onyango JP. Characterization and documentation of factors contributing to production and consumption of African

Leafy Vegetables (ALVs) in Kiambu and Kirinyaga Counties in Kenya. Asian Res J of Agri. 2016;1(3):1-9.
DOI: 10.9734/ARJA/2016/28734

19. Opiyo AM, Mungai NW, Nakhone LW and Lagat K. Production, status and impact of traditional leafy vegetables in household food security: A case study of Bondo district-Siaya county-Kenya. ARPN J of Agr and Biol. Sci. 2015;10(9)330-38.
20. Muriuki E, Sila D, Onyango A. Nutritional diversity of leafy amaranth species grown in Kenya J. Appl. Biosci. 2015;79:6818–25.
21. Habwe F. Walingo MK, Abukutsa-Onyango MO and Oluoch MO. Iron content of the formulated East African indigenous vegetable recipes 2009. Afri J Food Science. 2009;3(12):393-97.
22. Achigan – Dako E, Olga S, Maundu P. Current knowledge on *Amaranthus* spp. research avenues for improved nutritional value and yield in leafy amaranths in Sub Saharan Africa. Euphytica; 2014.
DOI: 10.1007/s10681-014-1081-9
23. Kwenin WK, Wolli M, Dzomeku BM. Assessing the nutritional value of some African indigenous green leafy vegetables in Ghana. J of Animal and Plant Sciences. 2011;10(2):1300-1305.
24. Gweyi-Onyango JP, Neumann G, Roemheld V. Effects of different forms of nitrogen on relative growth rate and growth components of tomato (*Lycopersicon esculentum* Mill.). African J. Hort. Sci. 2009;2:43-55.
25. Allen LH. Ending hidden hunger: The history of micronutrient deficiency control. Background Analysis for the World Bank-UNICEF Nutrition Assessment Project World Bank; 2000.
26. Black R. Micronutrient deficiency-an underlying cause of morbidity and mortality. Bulletin of World Health Organization. 2003;81:79-83.
27. Association of Official Analytical Chemists (AOAC). Official Methods of Analysis 16th Edition. Arlington; 1995.
28. Sas L, Marschner H, Römheld V, Mercik S. Effect of nitrogen forms on growth and chemical changes in the rhizosphere of strawberry plants. Acta Physiologiae Plantarum. 2003;25(3):241-47.
29. Ogut M, Er F, Neumann G. Increased proton extrusion of wheat roots by inoculation with phosphorus solubilising microorganims. Plant and Soil. 2011; 339(1-2):285-297.
30. Thomson CJ, Marschner H, Römheld V. Effect of nitrogen fertilizer form on pH of the bulk soil and rhizosphere, and on the growth, phosphorus, and micronutrient uptake of bean. J of Plant Nutr. 1993; 16(3):493-506.
DOI: 10.1080/01904169309364548
31. Turan M, Sevimli F. Influence of different nitrogen sources and levels on ion content of cabbage (*Brassica oleracea* var. capitate). New Z J of Crop and Hort Sc. 2005;33(3):241-49.
32. Marschner P. Marschner's mineral nutrition of higher plants. Academic Press; 2011.
33. Zhang LX, Zhai YO, Li Y, Zhao YO, Lv LI, Gao M, Liu J, Hu J. Effects of nitrogen forms and drought stress on growth, photosynthesis and some physico-chemical properties of stem juice of two maize cultivars (*Zea mays* L.) at elongation stage. Pak. J. Bot. 2012;44(4):1405-12.
34. Francis D, Sorrell DA. The interface between the cell cycle and plant growth regulators: A mini review. Plant Growth Regulation. 2001;33:1–12.
35. Ruan J, Gerenda J, Hardter R, Sattelmacher B. Effect of nitrogen form and root-zone pH on growth and nitrogen uptake of tea (*Camellia sinensis*) plants. Ann of Bot. 2007;99:301–10.
DOI: 10.1093/aob/mcl258
36. Gao Y, Li Y, Yang X, Li H, Shen Q, Guo S. Ammonium nutrition increases water absorption in rice seedlings (*Oryza sativa* L.) under water stress. Plant and Soil. 2010;331(1-2):193-201.
37. Roosta HR, Bagheri MH, Hamidpour M, Roozban MR. Interactive effects of nitrogen form and oxygen concentration on growth and nutritional status of eggplant in hydroponics. J of Agri Sci and Tech. 2016; 18(3):731-39.
38. Zhu YG, Smith SE, Smith FA. Plant growth and cation composition of two cultivars of spring wheat (*Triticum aestivum* L.) differing in P uptake efficiency. J of Exp Bot. 2001;52(359):1277-82.
39. Reid RJ, Smith FA. The cytoplasmic pH stat. In handbook of plant growth, ed. Z. Rengel. New York: Marcel Dekker, Inc. 2001;49–71.
40. Xie HL, Jiang RF, Zhang FS, McGrath SP, Zhao FJ. Effect of nitrogen form on the rhizosphere dynamics and uptake of cadmium and zinc by the hyper accumulator (*Thlaspi caerulescens)*. Plant and Soil. 2009;318(1-2):205-15.

41. Monsant AC, Tang C, Baker AJ. The effect of nitrogen form on rhizosphere soil pH and zinc phytoextraction by *Thlaspi caerulescens*. Chemosphere. 2008;73(5): 635-42.
42. Alloway BJ. Zinc in soils and crop nutrition. International Zinc Association (IZA). International Fertilizer Industry Association (IFA), Brussels; 2008.
43. Monsant AC, Wang Y, Tang C. Nitrate nutrition enhances zinc hyper accumulation in *Noccaea caerulescens* (Prayon). Plant and Soil. 2010;336(1-2): 391-404.
44. Serna MD, Borras R, Legaz F, Primo-Millo E. The influence of nitrogen concentration and ammonium/nitrate ratio on N-uptake, mineral composition and yield of citrus. Plant and Soil. 1992;147(1):13-23.
45. Rengel Z. Availability of Mn, Zn and Fe in the rhizosphere. Journal of soil science and plant nutrition. 2015;15(2): 397-409.
46. Rosen CJ, Allan DL, Luby JJ. Nitrogen form and solution pH influence growth and nutrition of two *Vaccinium* clones. J. Am. Soc. Hortic. Sci. 1990;115:83–89.
47. Makus DJ, Hettiarachy NS. Effects of nitrogen source and rate on vegetable amaranth leaf blade, mineral nutrients, pigments and oxalates. Subtropical Plant Science. 1999;51:10-15.
48. Carlisle E. Myers S. Raboy V. Bloom A. The effects of inorganic nitrogen form and CO2 concentration on wheat yield and nutrient accumulation and distribution. Frontiers in Plant Nutrition Science. 2012; 3(195):1-13.
49. Nikolic M, Ro¨mheld V. Nitrate Does Not result in iron inactivation in the apoplast of sunflower leaves. Plant Physiology. 2003; 132(3):1303-1314.
50. Assimakopoulou A. Effect of iron supply and nitrogen form on growth, nutritional status and ferric reducing activity of spinach in nutrient solution culture. Scientia Horticulturae. 2006;110(1):21–9. DOI: 10.1016/J.SCIENTA.2006.06.010
51. Barhoumi Z, Rabhi M, Gharsalli M. Effect of two nitrogen forms on the growth and iron nutrition of pea cultivated in presence of bicarbonate. J of Plant Nut. 2007;30(12): 1953-65.
DOI: 10.1080/01904160701700368
52. Chacon N, Silver WL, Dubinsky EA, Cusack DF. Iron reduction and soil phosphorus solubilization in humid tropical forests soils: the roles of labile carbon pools and an electron shuttle compound. Biogeochemistry. Springer. 2006;78:67–84.
DOI: 10.1007/s10533-005-2343-3

Peer-review history:
The peer review history for this paper can be accessed here:
http://sciencedomain.org/review-history/17987