

¹Humboldt-Universität zu Berlin, Faculty of Life Sciences, Division Urban Plant Ecophysiology, Berlin, Germany

²Humboldt-Universität zu Berlin, Faculty of Life Sciences, Division Biosystems Engineering, Berlin, Germany

³Egerton University, Department of Crops, Horticulture and Soils, Egerton, Kenya

Impact of direct-electric-current on growth and bioactive compounds of African nightshade (*Solanum scabrum* Mill.) plants

Elisha Otieno Gogo^{1,3}, Susanne Huyskens-Keil^{1*}, Anja Krimlowski¹, Christian Ulrichs¹, Uwe Schmidt², Arnold Opiyo³, Dennis Dannehl²

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Summary

Production of indigenous African leafy vegetables such as African nightshade (*Solanum scabrum* Mill.), whose high nutritional and medicinal value is well documented is still limited due to insufficient preharvest techniques. Electric current is known to improve quality in food crops. Therefore, in the present study, the effects of direct-electric-current (DC) on growth and characteristic bioactive and health promoting compounds were evaluated in different morphological sections, i.e., leaves and stems of African nightshade cv. Olevolosi. Six weeks old plants were exposed to different DC applied with a voltage of 8 and 16 V, 10 h/day for 12 days. Non-treated plants served as control. Plant growth, primary and secondary plant compounds were evaluated. Applying DC increased leaf fresh (11.5-14.4%) and dry (12.1-24.2%) weight as well as marketable leaves (29.1-55.3%). Biosynthesis of chlorophylls and carotenoids was enhanced by increased DC. Furthermore, dietary fibre fractions such as hemicellulose was promoted (23.3-45.3%) by DC applications, while cellulose and lignin remained unaffected. Minerals accumulated with increasing DC. Alteration of cell membrane permeability due to DC may have enhanced physiological processes leading to the improved growth and acceleration of bioactive compounds in African nightshade leaves.

Introduction

African leafy vegetables such as African nightshade (*Solanum scabrum* Mill.) have the potential to contribute to poverty alleviation and nutritional security because of their ease to grow, minimum production input requirement, and being highly rich in minerals, vitamins, fiber and bioactive compounds (KAMGA et al., 2013). Recently, attention is being directed to these vegetables because of their high contents in bioactive phytochemicals (MIBEI et al., 2012). These chemicals possess strong antioxidant properties and have been implicated in prevention of diseases such as cancer, arteriosclerosis, diabetes and aging (VOUTILAINEN et al., 2006). Furthermore, diets rich in micronutrients and antioxidants are strongly recommended as supplements in medicinal therapy for fighting HIV/AIDS (FRIIS et al., 2002). As a result of changing lifestyle and eating habits, the protection against diseases such as cancer and diabetes are on the rise. Consequently, these circumstances are leading to increasing demands for high quality and healthy food products (ONIANG'O et al., 2008).

During postharvest processes, high electric fields were used to destroy plant cells in order to increase the bioavailability of consumed secondary plant compounds in processed food products (GONZALEZ and BARRETT, 2010). Moreover, the application of electricity is also known to stimulate plant growth (BLACK et al., 1971; COSTANZO,

2008), and has been used to protect plants from insect pests and diseases and in weed management (DIPROSE et al., 1984). However, information in terms of the effect of weak direct-electric-current (DC) treatments on growth, yield and quality of leafy vegetables are scarce or just currently being investigated. Internal electric fields of plants are affected by the externally applied electric fields, already early being reported (SCOTT, 1967), influencing crop physiology. It is assumed that electric fields accelerate mass transfer by affecting cell membrane permeability properties (BLACK et al., 1971; ADE-OMOWAYE et al., 2002).

Recently, different intensities of DC in the range of 200 to 1800 mA were applied to roots of radish plants (*Raphanus sativus* L.) and garden cress (*Lepidium sativum* L.) during growth, whereby primary and secondary bioactive compounds (phenols, carotenoids), without visible damages to the plants were increased (DANNEHL et al., 2009; DANNEHL et al., 2012). Previous studies during postharvest processes also noted that DC intensities between 100 and 500 mA with varied application times between 15 and 60 min resulted in the accumulation of carotenoids, phenolic compounds and an increase in antioxidant activity in tomatoes (*Solanum lycopersicum* L.) (DANNEHL et al., 2011). However, plant tissue is characterized by a heterogeneous structure and the uptake, as well as the availability of nutrients into plant cells are determined by the direction of the current flow (BEN-AMMAR et al., 2011). Cell size, cell size distribution, and cell orientation may have an impact on the effectiveness of the applied electric current (BEN-AMMAR et al., 2011). Therefore, the present study focused on the investigation of effects of DC on growth parameters, as well as primary (structural carbohydrates, mineral composition) and secondary bioactive (chlorophyll, carotenoid pattern) compounds in different morphological sections, i.e. leaves and stems of African nightshade (*Solanum scabrum* Mill.) cv. Olevolosi during plant growth. Additionally, the content of heavy metals in African nightshade was determined in order to examine a possible accumulation of toxic elements by DC, which might be caused by electrolysis of the metal electrodes used.

Materials and methods

Experimental site and plant material

Seeds of African nightshade (*Solanum scabrum* Mill.) cv. Olevolosi were procured from AVRDC (The World Vegetable Center, Eastern and Southern Africa Arusha, Tanzania). The African nightshade plants were grown in the experimental station under greenhouse conditions at Humboldt-Universität zu Berlin (Berlin-Dahlem, Germany). The experiment was conducted from 30.06.2014 until 19.08.2014. The weekly mean values of the microclimatic conditions are presented in Fig. 1. Average air temperature ranged between 15 °C to 27 °C (night and day), while the average relative humidity was recorded ranging between 60% and 85%. Daily light quantity ranged from 150 Wm⁻² to 300 Wm⁻².

* Corresponding author

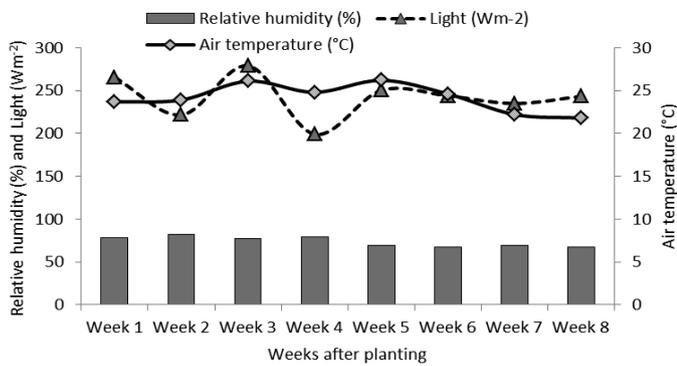


Fig. 1: Microclimate condition during greenhouse production of African nightshade plants.

Experimental set up and DC treatments

All plant boxes were equipped with stainless steel plates (EN-Standard: X5CrNi18-10; ThyssenKrupp Nirosta GmbH; Krefeld, Germany) with the dimensions of 90 cm × 8 cm × 0.1 cm (length × width × thickness), which were used as electrodes. To minimize the surface area of the box acting as conducting surfaces and thus altering the electric field pattern and distribution of current, the electrodes were centrally placed within each box. A part of the horticultural substrate (Profi-Substrate + Ton + Fe, Gramoflor GmbH & Co., Vechta, Germany) was first added to about 2 cm before the plate was placed into each box and more substrate was added to about 10 cm mark. Five seedlings were transplanted (3 weeks after sowing) in each box at a spacing of 20 cm apart. A power supply (Voltcraft, VLP 1303 pro, Hirschau, Germany) with an output of 0-30 V was integrated in this system. To close the electric circuit, wires were fixed at the stainless steel plate and at the top of each plant. As such, the DC flow was directed from the horticultural substrate to the top of the plant. The experiment was carried out in a completely randomized design with 3 replications. Excluding non-treated plants, the established plants were exposed to an electrical voltage of 8 and 16 V applied ten hours a day for 12 days. Output DC was monitored and recorded three times a day (8 am, noon and 6 pm) on a one-day interval from the start of the experiment until harvesting. Irrigation was done manually early in the morning on a daily basis ensuring an equal amount of water was applied to each treatment.

Determination of growth parameters

Plant height, total leaf number, stem diameter and number of primary shoots were determined on three central plants from each box at a 3-days interval from the beginning of the treatments until harvesting (12 days). After harvest, the leaves and stems of each plant per treatment were separated. Leaves per plant were used to determine leaf area (Leaf area meter-3100, Bachofer, Germany) of all plants, fresh weight, and amount of marketable and non-marketable leaves. Non-marketable leaves in this context were leaves which were considered not sellable due to discoloration. The plant compartments (stems and leaves) were used for further chemical analysis as described below.

Bioactive primary and secondary plant compound analysis

Three replicates of leaves and stems from freshly harvested plants of the control and each DC treatment were used for analysis of each of the phytochemical compound. One part of the harvested plants was immediately used for the determination of the dry matter. The other part of the harvested plant material was shock-frozen with liquid nitrogen and kept at -20 °C for subsequent analysis of chlorophylls and carotenoids. The remaining samples were freeze-dried for 48 h (Christ Alpha 1-4, Christ; Osterode, Germany), ground and mixed

to a fine homogenized powder and stored in a desiccator for further analysis such as structural carbohydrates, minerals elements and heavy metals.

Determination of dry matter, chlorophylls and carotenoids

To determine the dry matter, approximately 30 g fresh material per sample was placed in a drying oven at 105 °C for 24 h until constant weight was achieved. Subsequently, the dry weight of each sample was measured in order to calculate the dry matter by the ratio of the dry weight to the fresh weight of the samples. The results were expressed as % dry matter. The dry matter of each sample was further used to calculate all phytochemicals on a dry weight basis.

The extraction and determination of the chlorophylls and carotenoids content in African nightshade was conducted according to the method described by GOODWIN and BRITTON (1988). An aliquot of 0.5 g fresh material was homogenized with acetone/hexane (4:5, v:v) (Ultra-Turrax T 25, Jahnke & Kunkel, IKA-Labortechnik, Staufen, Germany) for 1 min at 18,000 rpm, and afterwards centrifuged for 10 min (4000 rpm). This procedure was carried out twice. The resulting supernatants were collected in a 25 ml volumetric flask and filled up with acetone/hexane (4:5, v:v). Three replications of the representative sample were measured twice with a spectrophotometer (UV-Vis spectrophotometer, UV-Mini-1240, Shimadzu, Japan) at wavelengths of 450 nm (total carotenoids), 453 nm (β -carotene), 505 nm (lycopene), 445 nm (lutein), as well as 663 nm and 645 nm for chlorophyll a and b, respectively. Carotenoids and chlorophylls were calculated as described by NAGATA and YAMASHITA (1992) and expressed as mg g⁻¹ DM.

Inductively coupled plasma-optical emission spectrometry (ICP-OES) analysis

For the determination of mineral elements (N, P, C, K, Ca, Mg, Na, Zn, Fe) and heavy metals (Cr, Cd, Pb and Ni), the ICP-OES analysis was applied for leaves and stems from each treatment in duplicates of three replications. For the microwave digestion, 0.2 g of each freeze dried sample was weighed into deionized containers. An aliquot of 5 ml HNO₃ (65%) and 3 ml H₂O₂ (30%) were added. The containers were then packed and placed into a microwave (MARS Xpress, CEM; North Carolina, USA) and digested according to the following program: step 1, 20 min to reach 200 °C; step 2, 5 min at 200 °C; step 3, 1 min to reach 210 °C; step 4, 5 min at 210 °C; step 5, 1 min to reach 220 °C; step 6, 5 min at 220 °C; and step 7, 30 min to cool down to room temperature. The resultant solution was transferred to 50 ml volumetric flasks using distilled water and finally filtrated into plastic flasks. Thereafter, the analysis of the elements in the digestion solution was conducted via ICP-OES with an ICP Emission Spectrometer (iCAP 6300 Duo MFC, Thermo; Waltham, USA). The operating conditions employed for ICP-OES were 1150W RF power, 0.55 l min⁻¹ nebulizer gas flow with argon employed as plasmogen as well as carrier gas and performed with a cross-flow nebulizer (MIRA MIST, Thermo Scientific; Cambridge England), in addition to radial (Ca, Mg) and axial (Fe, Zn, Cu, Al, Cd, Cr, Ni, Pb) view. For each element, a single-element standard solution (Roth, Karlsruhe, Germany) of 1000 mg l⁻¹ was used to prepare the reference analytic solutions in 1.4 mol l⁻¹ HNO₃. The calibration curves were established with the following reference solutions: blank 1.4 mol l⁻¹ HNO₃; 1-200 mg l⁻¹ of Ca; 0.5-50 mg l⁻¹ of Mg; 0.5-10 mg l⁻¹ of Al; 0.5-5 mg l⁻¹ of Cu, Zn, and Fe; 0.01-1 mg l⁻¹ of Cd, Cr, Ni, and Pb. The elements in the solutions were analyzed in duplicate at wavelength as follows: Ca at 317.9 nm, Mg at 279.0 nm, Fe at 259.9 nm, Zn at 213.8 nm, Cu at 324.7 nm, Al at 369.1 nm, Cd at 214.4 nm, Cr at 267.7 nm, Ni at 231.6 nm and Pb at 220.3 nm. The contents of macro- and micronutrients, as well as heavy metals in leaves and stems of African nightshade were expressed as mg g⁻¹ DM.

Determination of carbon and nitrogen content

Carbon and nitrogen analysis were determined using three replicates of freeze-dried leaves and stems per treatment using an elemental analyzer (Vario MAX, Elementar Analysensysteme GmbH; Hanau, Germany) according to DIN-ISO-10694 (1995) and DIN-ISO-13878 (1998). As such, an aliquot of 0.3 g of sample material was weighed into individual crucibles and catalytically combusted at 900 °C with pure oxygen. The combustion products and helium (as the carrier gas) passed through specific adsorption columns at a temperature of 830 °C to separate carbon and nitrogen. Based on the differences in thermal conductivity of these elements, carbon and nitrogen were determined successively with a thermal conductivity detector. Each analysis was performed twice and the results were calculated using glutamic acid as the standard reference. The results obtained were expressed as mg g⁻¹ DM.

Determination of structural carbohydrates content

Cellulose, hemicellulose and lignin were analyzed according to GOERING and VAN SOEST (1972), VAN SOEST et al. (1991) and AOAC (1999). One gram freeze-dried sample was extracted with 100 ml acid detergent fiber (ADF) reagent (*N*-cetyl-*N,N,N*-trimethyl-ammoniumbromid dissolved with 96% H₂SO₄) using a Fibertec System (M 1020, Tecator, Sweden). Thereafter, the solution was vacuum-filtered, washed with boiled, double-distilled water until removal of the acidity and again washed with 90% acetone. The residue was dried at 105 °C for 24 h, weighed, ash-dried at 500 °C for 24 h and weighed again to calculate ADF (acid detergent fiber). The dried ADF residue was used for acid detergent lignin (ADL) determination. Cellulose content was calculated as the difference between ADF and ADL. The contents of lignin and cellulose were expressed as mg g⁻¹ DM.

Using the neutral detergent fiber (NDF) approach (VAN SOEST and GOERING 1963), one gram of freeze-dried material was cooked in 100 ml of NDF mixture (Titriplex III, di-sodium borate, dodecyl-hydrogensulfate-Na, ethylene-glycol-monoethylester) to determine the hemicellulosic cell wall fraction. The solution was subsequently vacuum-filtered, and washed with demineralized water and with 90% acetone. The insoluble residue was dried at 105 °C for 24 h, weighed, ash-dried at 500 °C for 24 h and weighed again to calculate NDF. The hemicellulose content was obtained by subtracting ADF from NDF and expressed as mg g⁻¹ DM.

Statistical analysis

The impact of different DC treatments on plant growth and bioactive compounds, minerals and heavy metals in African nightshade was evaluated using analysis of variance (ANOVA) with the statistical program SAS (version 10). Proc-Univariate procedure was used to check for normality of data and all comparisons of the mentioned parameters were calculated using Tukey-tests at a significance level of $p < 0.05$. The same significance level was used for the calculation of differences between the electric current flows when a voltage of 8 and 16 V was applied using Fisher's t-test. Mean values labeled with different small letters indicate significant differences. The mean variability is shown as standard deviation.

Results

Changes in electrical current flow during African nightshade production

Results obtained from the voltmeter readings indicated that different voltage applications led to significant differences in DC flows during the experiment (Fig. 2). Doubling the voltage resulted in a maximum DC flow of 172 µA through plants, whereas a maximum flow of 73 µA was found at 8 V. There was a periodic fluctuation of the cur-

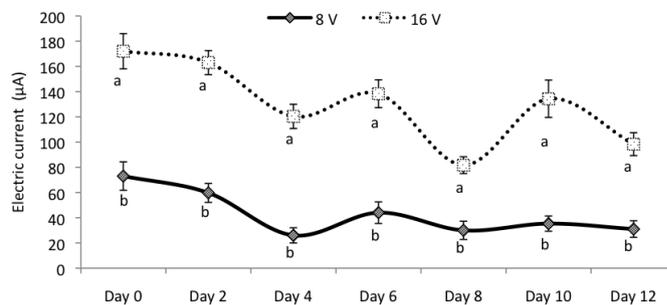


Fig. 2: Changes in current flow on African nightshade plants during production as influenced by DC intensity. Means (\pm standard deviation) followed by the same letter are not significantly different according to t-test ($p < 0.05$).

rent flow up to 56 µA from one day to another at a higher electrical voltage (16 V), while the current flow at a lower voltage intensity (8 V) remained relatively constant after 6 days of electrical treatments.

Effect of different electrical voltages on growth of African nightshade plants

As expected, growth of African nightshade plants increased with time. However, different electrical voltages did not influence plant height (Tab. 1A), leaf number (Tab. 1B), stem diameter (Tab. 1C), and primary shoots (Tab. 1D). Furthermore in terms of leaf area (LA), both DC treatments did not differ significantly from non-treated plants (Fig. 3). However, lower voltage (8 V) reduced LA by 12.4% compared to the higher voltage (16 V).

Effect of different electrical voltages on fresh and dry weight of African nightshade plants

Different electrical voltages had a significant influence on fresh weight of leaves, but not on stems (Fig. 4A). In terms of leaf fresh weight, the values indicated a significant increase by 11.5% and 14.4% at 8 V and 16 V treatments, respectively, when compared to the control plants. However, the reverse effect was found in the case of stem fresh weight, where this parameter decreased with increasing voltages. The control stems had the highest fresh weight with 58.2 g followed by those treated with a voltage of 8 V (53.9 g) and 16 V (49 g).

In terms of leaves, similar results were found on a dry weight basis (Fig. 4B). Thus, the same increase pattern was observed, where only the voltage treatment with 16 V caused a significant increase in leaf dry weight by 24.2% compared to the control leaves. On the other hand, a lower electrical voltage (8 V) tendentially increased stem dry weight by 5.8% while doubling voltage tended to reduce stem dry weight by 2.9% compared to the control.

Different voltage applications significantly influenced both marketable and non-marketable leaves harvested (Fig. 4C). A lower voltage application (8 V) increased marketable leaves by 55.3%, while a higher voltage (16 V) increased marketable leaves by 29.1% compared to plants grown under non-treated conditions. Regarding non-marketable leaves, the control had the highest amount with 5.7 leaves/plant, whereas number of leaves of DC at 8 V and 16 V was significantly reduced by 77.2% and 64.9%, respectively. No significant differences were observed between 8 V and 16 V.

Effect of different electrical voltages on chlorophyll and carotenoid contents of African nightshade plants

Varying responses of leaves and stems of African nightshade plants were noted due to different voltage treatments. Generally, a voltage

Tab. 1: Effect of different electrical voltages on growth parameters of African nightshade plants.

Days after treatment application				
DC	0	4	8	12
A. Plant height (cm)				
Control	41.4 ± 3.4 g	49.3 ± 3.6 f	58.4 ± 2.3 bcd	65.2 ± 3.0 ab
8 V	41.0 ± 3.5 g	52.1 ± 5.1 def	60.9 ± 5.6 bc	68.8 ± 5.5 a
16 V	40.6 ± 1.2 g	50.7 ± 1.8 ef	56.9 ± 3.2 cde	64.2 ± 4.2 ab
B. Leaf number (no./plant)				
Control	10.3 ± 0.3 e	12.3 ± 0.9 d	13.4 ± 0.7 bc	14.9 ± 0.7 a
8 V	10.2 ± 0.2 e	12.4 ± 0.5 cd	13.6 ± 0.4 b	14.8 ± 0.8 a
16 V	10.4 ± 0.8 e	12.8 ± 0.5 bcd	13.7 ± 0.7 b	14.8 ± 0.8 a
C. Stem diameter (mm)				
Control	9.6 ± 0.7 cd	10.0 ± 0.4 abcd	10.6 ± 0.7 abc	10.9 ± 1.0 a
8 V	9.2 ± 0.3 d	9.8 ± 0.4 bcd	10.4 ± 0.8 abcd	10.5 ± 0.7 abc
16 V	9.3 ± 0.6 d	9.8 ± 0.4 bcd	10.1 ± 0.5 abcd	10.1 ± 0.4 abc
D. Primary shoots (no./plant)				
Control	4.9 ± 0.8 de	5.4 ± 0.8 bcde	6.1 ± 0.4 abcd	6.9 ± 0.4 ab
8 V	4.1 ± 1.0 e	5.1 ± 1.3 de	6.7 ± 0.9 abc	7.6 ± 0.9 a
16 V	4.2 ± 1.0 e	5.3 ± 1.0 cde	6.8 ± 0.8 abc	7.6 ± 0.8 a

Mean values (\pm standard deviation) within a growth variable followed by the same letter are not significantly different according to Tukey-test ($p < 0.05$).

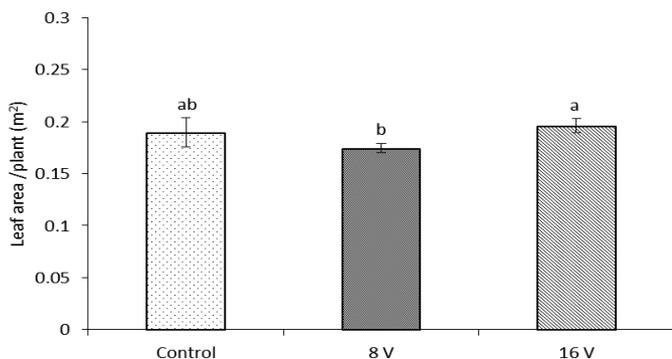


Fig. 3: Effect of different voltage applications on leaf area of African nightshade plants. Mean values (\pm standard deviation) followed by the same letter are not significantly different according to Tukey-test ($p < 0.05$).

of 16 V resulted in higher chlorophyll and carotenoid contents in African nightshade leaves, while a lower voltage treatment reduced both chlorophyll and carotenoids content. Leaves had higher chlorophyll and carotenoid contents compared with stems. Compared to the control, a voltage of 8 V significantly reduced the contents of lutein (15.6%), β -carotene (16.2%), total carotenoids (16.0%) and chlorophyll a (15.5%) in African nightshade leaves, whereas lycopene and chlorophyll b were not statistically different from control and a higher voltage treatment (Tab. 2A). On the other hand, compared to the control, a higher voltage of 16 V increased lutein (4.6%), β -carotene (4.4%), total carotenoids (4.5%) and chlorophyll a (4.0%) contents of African nightshade leaves, however, only tentatively.

Regarding African nightshade stems, an increase in voltage treatments resulted in a significant increase in almost all carotenoids and chlorophylls (Tab. 2B). When compared with the control plants, mainly the highest voltage treatment (16 V) increased lutein,

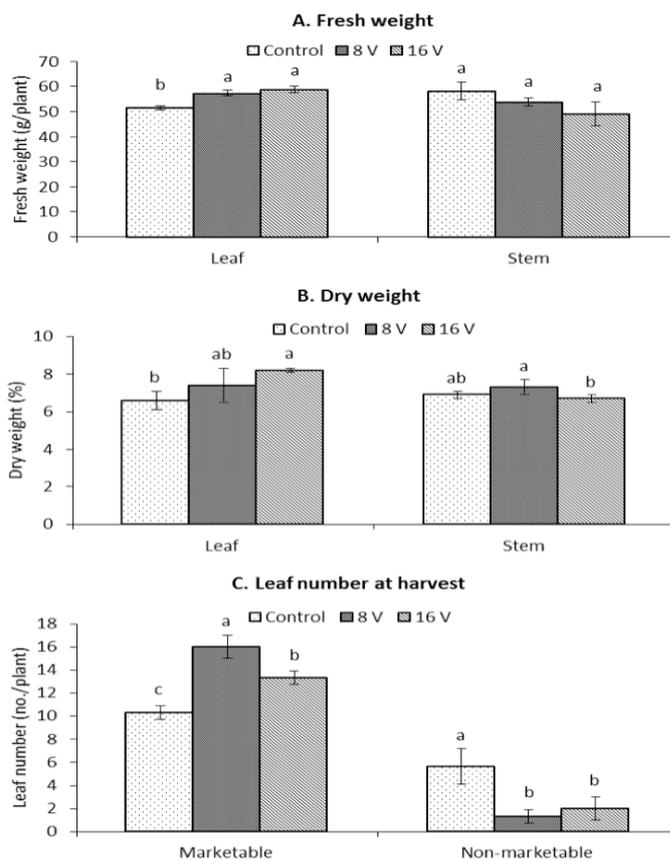


Fig. 4: Effect of different voltage applications on fresh (A) and dry (B) weight and marketable and non-marketable leaves (C) of African nightshade plants. Mean values (\pm standard deviation) within a specific variable followed by the same letter are not significantly different according to Tukey-test ($p < 0.05$).

Tab. 2: Effect of voltage applications on chlorophyll and carotenoid contents (mg/g DM) of African nightshade plants.

DC	Lutein	β -carotene	Lycopene	Total carotenoids	Chlorophyll a	Chlorophyll b
A. Leaves						
Control	2.0 \pm 0.15 a	2.1 \pm 0.16 a	1.5 $\times 10^{-1}$ \pm 0.01 a	8.2 \pm 0.62 a	16.9 \pm 1.52 a	7.5 \pm 0.74 a
8 V	1.7 \pm 0.24 b	1.7 \pm 0.24 b	1.5 $\times 10^{-1}$ \pm 0.02 a	6.9 \pm 0.97 b	14.3 \pm 1.58 b	6.9 \pm 0.54 a
16 V	2.1 \pm 0.10 a	2.2 \pm 0.10 a	1.6 $\times 10^{-1}$ \pm 0.01 a	8.6 \pm 0.40 a	17.6 \pm 0.70 a	7.6 \pm 1.01 a
B. Stems						
Control	1.0 $\times 10^{-1}$ \pm 0.01 b	1.0 $\times 10^{-1}$ \pm 0.01 b	7.9 $\times 10^{-3}$ \pm 0.00 a	4.1 $\times 10^{-1}$ \pm 0.03 b	7.8 $\times 10^{-1}$ \pm 0.07 b	4.1 $\times 10^{-1}$ \pm 0.02 b
8 V	1.1 $\times 10^{-1}$ \pm 0.01 ab	1.1 $\times 10^{-1}$ \pm 0.01 b	8.4 $\times 10^{-3}$ \pm 0.00 a	4.4 $\times 10^{-1}$ \pm 0.04 b	8.6 $\times 10^{-1}$ \pm 0.09 ab	4.8 $\times 10^{-1}$ \pm 0.01 ab
16 V	1.2 $\times 10^{-1}$ \pm 0.01 a	1.3 $\times 10^{-1}$ \pm 0.00 a	8.7 $\times 10^{-3}$ \pm 0.00 a	5.0 $\times 10^{-1}$ \pm 0.02 a	9.5 $\times 10^{-1}$ \pm 0.05 a	5.0 $\times 10^{-1}$ \pm 0.02 a

Mean values (\pm standard deviation) within a specific variable and plant part followed by the same letter are not significantly different according to Tukey-test ($p < 0.05$).

β -carotene, total carotenoids, chlorophyll a and b contents in stems by 20.0%, 30.0%, 22.0%, 21.8%, 22.0%, respectively, whereas lycopene was affected to a smaller extent (10.0%). Plants treated with a voltage of 8 and 16 V did not differ significantly from each other when bioactive compounds such as lutein, lycopene and chlorophylls were considered. However, doubling the voltage led to an increase in β -carotene by 18.2% and total carotenoids by 13.6% in stems compared with the lower voltage (8 V) applied on African nightshade plants.

Effect of different electrical voltages on structural carbohydrates of African nightshade plants

There was a similar response to different voltage in terms of structural carbohydrates in leaves and stems of African nightshade plants. A higher content of structural carbohydrates was obtained in stems compared to leaves. In leaves, only the treatment of 16 V resulted in a significant increase in hemicellulose (40.0%) content compared to the non-treated plants. In consideration of both DC treatments, no significant differences were found (Fig. 5A). However, neither 8 V nor 16 V influenced cellulose and lignin content in leaves significantly. Similar results were obtained in stems. The hemicellulose content was significantly increased by 52% and 38.7% when 8 V and 16 V were applied, respectively, compared to the control (Fig. 5B). Cellulose and lignin contents in stems were not significantly affected by different voltages.

Effect of different electrical voltages on mineral compounds and heavy metals in African nightshade plants

Voltage treatments differently affected minerals and heavy metals of African nightshade plants, in both leaves and stems. A general increase in voltage resulted in an increase in most of the mineral elements and heavy metals analyzed. Generally, a higher content of N, C, Ca, Mg, Fe, Zn, Ni, and Cd was observed in leaves while stems had higher K, Na, Pb and Cr contents. In leaves, a voltage of 8 V significantly influenced the contents of Ca, Pb and Cr which were increased by 20.2%, 42.1%, and 52.7%, respectively, compared to the contents observed in control leaves (Tab. 3A). A voltage of 16 V evoked nearly the same accumulations, except for the content of Ca which was increased by 27.5% when compared to the non-treated plants. A significant difference between lower and higher voltages occurred only when the content of Ca was considered. This was significantly increased by 6.1% when leaves were exposed to 16 V.

In stems, however, a voltage of 16 V significantly increased the contents of Mg (15.6%), Zn (244.4%), Ni (113.2%), Cd (36.8%) and Cr (288.5%) compared to the control. No significant difference between

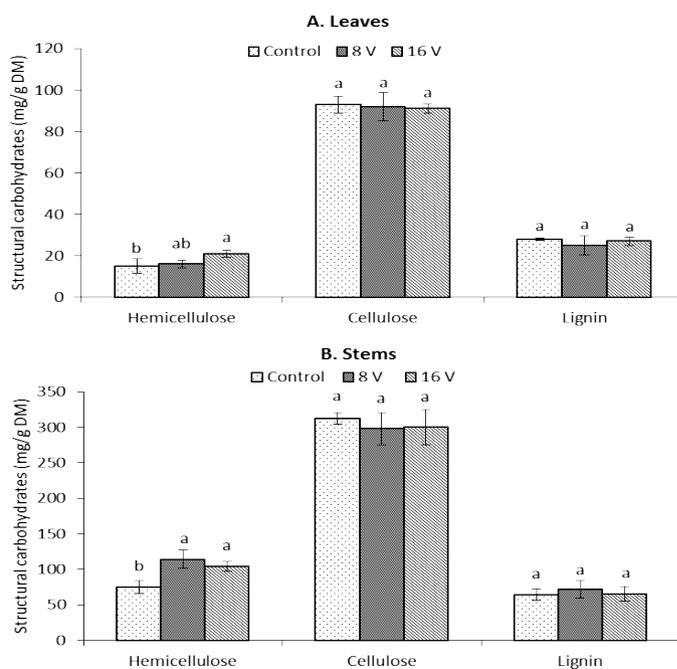


Fig. 5: Effect of different voltage applications on different structural carbohydrates on leaves (A) and Stems (B) of African nightshade plants. Mean values (\pm standard deviation) within a specific variable followed by the same letter are not significantly different according to Tukey-test ($p < 0.05$).

elements was achieved when control plants and plants exposed to 8 V were considered (Tab. 3B). Significant differences between both voltage treatments were only demonstrated in terms of Na, Zn and Ni. The content of these elements in stems grown under 16 V conditions was increased by 31.4%, 167.0% and 105.5%, respectively. However, different voltages were not able to influence the contents of N, C, P, K, Ca, Mg, Fe and Pb in stems of African nightshade plants significantly.

Discussion

Results from the present study have demonstrated that weak voltages may be used to improve growth and bioactive compounds in African nightshade plants. The different voltages had a significant influence on current flow through African nightshade plants. This was increased with increasing voltage. This circumstance could be attri-

Tab. 3: Effect of voltage applications on mineral element and heavy metal content (g/kg DM) of African nightshade plants.

Element	A. Leaves			B. Stems		
	Control	8 V	16 V	Control	8 V	16 V
N	29.60 ± 3.20 a	27.10 ± 3.40 a	28.60 ± 4.30 a	7.40 ± 0.80 a	6.79 ± 0.60 a	8.34 ± 1.30 a
C	425.50 ± 2.30 a	421.00 ± 3.80 a	424.40 ± 3.10 a	410.70 ± 5.80 a	413.10 ± 5.30 a	409.00 ± 4.80 a
P	6.13 ± 0.39 a	5.73 ± 0.54 a	6.02 ± 0.37 a	3.46 ± 0.18 a	3.6 ± 0.22 a	3.86 ± 0.22 a
K	33.35 ± 0.82 a	32.20 ± 3.17 a	32.56 ± 4.68 a	40.72 ± 2.68 a	39.37 ± 3.26 a	41.42 ± 3.64 a
Ca	21.56 ± 0.16 c	25.91 ± 0.77 b	27.49 ± 3.28 a	12.38 ± 1.02 a	12.90 ± 0.57 a	14.10 ± 1.18 a
Mg	3.12 ± 0.39 a	3.25 ± 0.39 a	3.07 ± 0.41 a	1.60 ± 0.05 b	1.76 ± 0.26 ab	1.85 ± 0.11 a
Na	1.10 × 10 ⁻¹ ± 0.00 a	0.90 × 10 ⁻¹ ± 0.00 a	0.97 × 10 ⁻¹ ± 0.00 a	2.38 × 10 ⁻¹ ± 0.03 ab	2.10 × 10 ⁻¹ ± 0.02 b	2.76 × 10 ⁻¹ ± 0.03 a
Fe	9.04 × 10 ⁻² ± 0.00 a	8.83 × 10 ⁻² ± 0.00 a	9.05 × 10 ⁻² ± 0.00 a	3.75 × 10 ⁻² ± 0.00 a	4.14 × 10 ⁻² ± 0.00 a	4.26 × 10 ⁻² ± 0.00 a
Zn	3.53 × 10 ⁻² ± 0.00 a	3.73 × 10 ⁻² ± 0.00 a	3.98 × 10 ⁻² ± 0.00 a	2.35 × 10 ⁻² ± 0.00 b	3.03 × 10 ⁻² ± 0.00 b	8.09 × 10 ⁻² ± 0.00 a
Ni	0.00 ± 0.00 a	1.19 × 10 ⁻⁶ ± 0.00 a	1.15 × 10 ⁻⁶ ± 0.00 a	0.53 × 10 ⁻⁶ ± 0.00 b	0.55 × 10 ⁻⁶ ± 0.00 b	1.13 × 10 ⁻⁶ ± 0.00 a
Pb	3.40 × 10 ⁻⁴ ± 0.00 b	4.83 × 10 ⁻⁴ ± 0.00 a	4.57 × 10 ⁻⁴ ± 0.00 ab	3.63 × 10 ⁻⁴ ± 0.00 a	4.10 × 10 ⁻⁴ ± 0.00 a	4.79 × 10 ⁻⁴ ± 0.00 a
Cd	4.87 × 10 ⁻⁴ ± 0.00 a	5.47 × 10 ⁻⁴ ± 0.00 a	5.43 × 10 ⁻⁴ ± 0.00 a	2.34 × 10 ⁻⁴ ± 0.00 b	2.58 × 10 ⁻⁴ ± 0.00 ab	3.20 × 10 ⁻⁴ ± 0.00 a
Cr	3.47 × 10 ⁻⁴ ± 0.00 b	5.30 × 10 ⁻⁴ ± 0.00 a	5.10 × 10 ⁻⁴ ± 0.00 a	5.2 × 10 ⁻⁴ ± 0.00 b	9.90 × 10 ⁻⁴ ± 0.00 ab	20.2 × 10 ⁻⁴ ± 0.00 a

Mean values (± standard deviation) within a row and plant part followed by the same letter are not significantly different according to Tukey-test ($p < 0.05$).

buted to varying resistance caused by African nightshade plants as a result of changes in growth and development with time. On the other hand, changes in water supply could result in variation in electrical conductivity followed by changes in electrical current flows.

Even though different voltages did not affect the plant growth (leaf number, plant height, stem diameter and number of primary shoots) of nightshade plants, LA was tendentially enhanced when a voltage of 16 V was applied compared to the control. However, KAREEM (1999) successfully demonstrated that a higher electric current (110 to 220 V) at varying time (10 to 30 s) was able to improve leaf length, leaf width and stem length of cowpea (*Vigna unguiculata* (L.) Walp.), guinea corn (*Sorghum bicolor* (L.) Moench) and groundnut (*Arachis hypogaea* L.), when seeds were treated using wet (seeds soaked in water inside aluminum cup connected to live wire) and dry (seeds with empty aluminum cup connected to live wire) application methods. KOTAKA and KRUEGER (1968) found that varying ion densities improved growth of barley (*Hordeum vulgare* L.), oats (*Avena sativa* L.) and lettuce (*Lactuca sativa* L.). These studies suggest that increases in ion density as a result of voltage effects may enhance the availability of nutrients in plant cells as observed in the current study. This phenomenon may result in improved physiological processes such as photosynthesis, whereby more photosynthates can be accumulated in leaves leading to a higher LA as tendentially observed in the present study at the 16 V treatments. OKAMURA et al. (2011) showed that growth of radish and thale cress (*Arabidopsis thaliana* (L.) Heynh.) was more promoted when seeds were treated with 10.0 kV/m DC than with 2.5 kV/m DC. It is also important to note that plant tissue is characterized by a heterogeneous structure with a high degree of anisotropy (BEN-AMMAR et al., 2011). Cell size, cell size distribution, and cell orientation have an impact on the effectiveness of electric currents on crops (BEN-AMMAR et al., 2011). These influencing factors could be taken into consideration to explain the reason why some growth parameters were not affected by different electric voltages as observed in the present study.

In the current study, the use of DC increased leaf fresh and dry weight of African nightshade plants. DC was considered to affect visual damage (yellowing) of plants, therefore, marketable and non-marketable leaves were recorded. It was found that DC reduced the amount of non-marketable leaves. MURR (1963) observed tip burn to orchard grass seedlings (*Dactylis glomerata* L.) due to electric

fields (40 kV/m) effect manifested by dark brown spots. Further, microscopic examination revealed chloroplast damage. However, the strength of electric field has an influence on the extent of plant damage. The results in terms of dry weight were corroborated by WARD (1996) who found that weak direct electric current (6 V) using wires as electrodes increased this parameter in greenhouse produced tomatoes. Electrical fields are reported to play a vital part in crop physiology (SCOTT, 1967). For example, an electrogenic proton pump helps in the regulation of cytoplasmic pH, active transport of mineral ions, hormones or organic metabolites (SPANSWICK, 1981). In this context, the externally applied voltages in the present study resulted in an increase in contents of mineral elements (e.g. Mg, Ca and Zn) and structural carbohydrates (hemicellulose) in a bid to cope with electric stress (GALL et al., 2015). This circumstance can promote physiological processes of African nightshade plants. For instance, Ca plays a major role in root system development associated with a better water and nutrient uptake (PICCHIONI et al., 2001) while Mg and Zn is involved in chlorophyll synthesis, which is important for photosynthesis (HU and SPARKS, 1991). The latter evidence could be the reason for a higher accumulation of carbohydrates as caused by increasing voltages in the present study, especially observed for hemicellulose content in leaves and stems. Hemicellulose facilitates cell wall thickening by reinforcement of secondary wall and hence reduction in water loss through transpiration (GALL et al., 2015). These plant responses were jointly responsible and sufficient, in order to increase fresh and dry weight in leaves as well as marketable leaves as observed on DC treated plants. Furthermore, the increase in mineral elements (e.g. Mg and Zn) in DC treated plants enhanced chlorophyll formation (green pigment), thus preventing yellowing (chlorosis) leading to reduced non-marketable leaves as reported in the present study.

As indicated earlier, a shift in consumer eating habits has led to increasing demand for high quality food products. In the present study, application of a voltage of 16 V through the whole plant significantly increased contents of chlorophyll a and b, as well as lutein, β -carotene and total carotenoids in stems and tendentially in leaves of African nightshade plants. These plant bioactive compounds have antioxidant potential and are therefore very important in maintaining human health. For instance, carotenoids have been reported to possess provitamin A activity (TANG, 2010); a precursor of vitamin

A, essential for the promotion of general growth, maintenance of visual function, regulation of differentiation of epithelial tissues and embryonic development (UNDERWOOD and ARTHUR, 1996). In addition, carotenoids have been reported to be necessary for the prevention of cardiovascular diseases and also have anticarcinogenic effects (VOUTILAINEN et al., 2006). Just like carotenoids, chlorophylls are important for health promotion such as enhancing oxygen uptake in the blood, which can increase energy, relieve fatigue and improve many blood disorders (FERRUZZI et al., 2001). Regarding electrophysiology, studies involving the use of intermittent-direct-current (IDC) between 200 and 1800 mA applied to plant roots increased phytochemical compounds in one week old garden cress sprouts compared to the control (DANNEHL et al., 2012). Previously, the same group of researchers demonstrated that different intensities of IDC (100 to 500 mA) with varied application times (15 to 60 min) during postharvest resulted in accumulation of carotenoids, phenolic compounds and an increase in antioxidant activity in tomatoes (DANNEHL et al., 2011). Similarly, they also reported that application of IDC (200 to 1000 mA) to roots of radish plants during growth resulted in an increase in phenolic compounds in radish tubers as well as in roots without visible damages to the plants (DANNEHL et al., 2009). Another report indicates that the application of DC (10V/12.5 μ A) resulted in an increase in peroxidase activity; an indicator of stress in spinach leaves (*Spinacia oleracea* L.) (MONTAVON et al., 1988). This stress response could be initiated when the plant recognizes a stimulus at the cellular level, as a result of the activity of specific ion channels such as voltage-gated ion channels embedded in a cell plasma membrane (GASPAR et al., 2002). Thus, perturbation of internal electric field as a result of applied DC could induce stress mediated changes in the electrochemical proton gradient or the cytoplasmic and vacuolar pH, among others. This in turn could result in an increase in biosynthesis of primary and secondary metabolites in order to rectify this plant state. In the present study, the higher contents of chlorophylls and carotenoids as a result of applied DC confirm this assumption.

In addition, DC improved content of hemicellulose, an important structural carbohydrate necessary in food digestion. However, DC did not significantly influence cellulose and lignin content. Stress induction by DC may result in various enzymatic activities, e.g. cell-wall bound peroxidases (DYMEK et al., 2014). This could lead to biosynthesis of structural carbohydrates such as hemicellulose in African nightshade plants as observed in the present study, in order to enhance plant resistance to stress. However, the enzymatic signaling pathways of various structural carbohydrates are compartment-specific or even cell-specific (DYMEK et al., 2014). This would explain why some structural carbohydrates were affected (hemicellulose) while others were not (cellulose and lignin).

Majority of the world population currently suffer from 'hidden hunger' (e.g. lacking various mineral elements, vitamin A and fiber content) in the diet (BIESALSKI, 2013). This is exacerbated by frequent consumption of food either lacking or having low content of such key mineral elements or phytochemicals. The present study revealed that DC treatments through the whole plant result in an increase in certain mineral elements such as Mg, Ca and Zn in African nightshade plants. However, other elements, for example, N, C, P, K and Fe were not significantly affected. An increased nutrient uptake of Mg and Ca was also found in greenhouse produced tomato treated with 6 V DC (WARD, 1996) while BLACK et al. (1971) reported a significant increase in K, Ca and P content in tomato when a much lower DC of 15 to 30 μ A was applied. As such, additional potential induced from external electric field in plants is reported to raise pressure on the membrane due to attraction between opposite charges on both sides of the membrane, whereby a faster transport of ions is induced simultaneously (ZIMMERMAN et al., 1974). This could cause an accumulation of elements, as this hypothesis is supported by in-

creased contents of minerals as observed in the present study. This rapid influx of mineral ions through cation channels imbedded in the plant membrane can induce stress in plants, which can promote the synthesis of secondary metabolites as well (DANNEHL et al., 2012). In order to ensure reversible membrane permeability and associated plant vitality, critical electric field strength must be taken into consideration, which is dependent on plant species, as well as cell geometry and size (HEINZ et al., 2002).

DC also increased the content of heavy metals, i.e. Cr, Ni, Cd and Pb of African nightshade plants, assumingly due to an electron transport between both electrodes used in the present study. Similar results were reported by DANNEHL et al. (2012) when DC (200 to 1800 mA) was applied to garden cress. However, the observed results are still within acceptable thresholds regulated by law for human consumption according to European Food Safety Authority (EFSA, 2008).

Conclusion and recommendation

In general, the findings of this study have demonstrated that the application of weak dosages of DC (8 and 16 V) can improve growth, and quality in terms of health protecting plant compounds of African nightshade plants. Plant specific carotenoids (β -carotene and lutein), chlorophyll a and b, as well as characteristic mineral elements such as Mg, Ca and Zn and structural carbohydrates (hemicellulose) in African nightshade were promoted by DC. The results showed that DC induced a defense response of plant cells and may have altered the cell membrane properties and hence physiological processes. Farmers of African leafy vegetables in Kenya commonly practice small-scale farming or kitchen gardening, where they cultivate vegetables in small plots or in pots, especially for home consumption. Therefore, the findings of the study may be beneficial to such farmers. This technology is also applicable in areas where electricity is absent or unstable which in most cases is the situation in rural areas as farmers can use alternative power sources, e.g. batteries, solar cells, or dynamos. Furthermore, emerging technologies such as DC could be used either as stand-alone or in synergistic combination with other technologies, in order to satisfy the increased consumer demand for high quality and healthy food products. As such, DC applications provide an alternative to enhance food security. However, it remains a need to adjust the intensity of DC and duration of exposure to various crop species and varieties for optimization of yield and quality. Furthermore, it has to be investigated in detail if such technologies are worthwhile in terms of economic aspects.

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Adresses of the authors:

Dr. Susanne Huyskens-Keil, Prof. Dr. Dr. Christian Ulrichs, MSc Anja Krimlowski, Humboldt-Universität zu Berlin, Faculty of Life Sciences, Division Urban Plant Ecophysiology, Lentzeallee 55-57, 14195 Berlin, Germany
E-mail: susanne.huyskens@agr.ar.hu-berlin.de

Prof. Dr. Uwe Schmidt, Dr. Dennis Dannehl, Humboldt-Universität zu Berlin, Faculty of Life Sciences, Division Biosystems Engineering Albrecht-Thaer Weg 3, 14195 Berlin, Germany

Dr. Arnold Opiyo, MSc Elisha Otieno Gogo, Egerton University, Department of Crops, Horticulture and Soils, P.O. Box 536, 20115 Egerton, Kenya

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