Int. Agrophysics, 2009, 23, 61-65

INTERNATIONAL
Agrophysics
www.international-agrophysics.org

Effect of various NPK fertilizer doses on total antioxidant capacity of soil and amaranth leaves (*Amaranthus cruentus* L.)

B. Skwaryło-Bednarz and A. Krzepiłko*

Faculty of Agricultural Sciences in Zamość, University of Life Sciences in Lublin, Szczebrzeska 102, 22-400 Zamość, Poland

Received May 21, 2008; accepted August 14, 2008

A b s t r a c t. A field experiment was set up in 2007 in a split-plot design to investigate the effect of various NPK fertilizer doses on the total antioxidant capacity (TAC) of fresh leaf tissue of the Polish amaranth varieties Rawa and Aztek, cultivated for seed, and of the soil they were cultivated on. The experiment was conducted with three replicates. The following combinations of macroelement doses were applied: I: N – 50 , P – 40, K – 40 kg ha^{-1}; II: N – 90, P – 60, K – 60 kg ha^{-1}; and III: N – 130, P – 70, K – 70 kg ha^{-1}. The total antioxidant capacity (TAC) of the soil and amaranth leaves was determined using the method described by Rice-Evans and Miller, as modified by Bartosz. Organic carbon content was determined as well. In most of the cases the fertilization (NPK) caused an increase in total antioxidant capacity of the soil under amaranth cultivation. The highest total antioxidant capacity in the soil examined (compared with the control) was found with combination III of NPK fertilizers for soil under Rawa cultivation, and with combination I for the Aztek soil. The total antioxidant capacity of amaranth per unit fresh leaf mass was dependent on the variety and on the doses of the macroelements applied. The highest total antioxidant capacity in Rawa per unit fresh leaf mass was observed with combination III, while for the Aztek variety the highest TAC was found with combination I. Statistical analysis showed significant dependencies between TAC of the soils and leaves and the level of fertilization, as well as between the TAC of the soil and of amaranth leaves.

K e y w o r d s: total antioxidant capacity, amaranth, leaves, soil

INTRODUCTION

Soil fertilization is one of the main factors increasing the yield of plants (Kołodziej, 2006). It affects the accumulation, mineralization and humification of organic matter added to the soil (Łoginow *et al*., 1991), and determines plant production potential (Demmler, 1998). The amount of fertilizer introduced into the soil, including mineral fertilizers, affects the amount of mineral nitrogen available to plants and the organic carbon content of the soil (Bijlsma and Lambers, 2000). Compounds contained in soil can take part in redox reactions. In recent years, soil testing has been expanded to include measurement of the total antioxidant capacity of soils, which makes it possible to determine the intensity of redox processes taking place in the soil environment. Total antioxidant capacity depends on a variety of factors: type of soil, content of organic compounds, especially of humus, microbiological activity, and also the species of plant cultivated. Through different chemical composition of root exudates, plants can stimulate or inhibit the development and activity of soil microflora, and thus the total antioxidant capacity of soils. A special environment for interactions between microorganisms and plants is the rhizosphere (Wielgosz and Szember, 2006). The speed and type of chemical processes taking place in the rhizosphere, particularly transport of water and nutrients, oxidation and reduction reactions, exudation of organic acids, sugars, phenols and amino acids by plant roots, and the formation of chelates, are affected by antioxidant substances (Baran *et al*., 1999), especially humic acids contained in humus (Rimmer, 2006; Schepetkin *et al*., 2002). Unpublished preliminary research by the authors of this paper indicates that amaranth has a positive effect on the numbers and activity of soil microorganisms. This suggests that the soil environment under amaranth cultivation is characterized by high content of antioxidant substances. These compounds are taken up from the soil by plants, where they then become components of plant products and can be beneficial to human health (Olivier, 1997). Numerous epidemiological studies confirm that antioxidants present in

*Corresponding author's e-mail: bskwarylo@wnr.edu.pl

food have a beneficial effect on the course of many diseases associated with the occurrence of oxidative stress, such as neoplasms, cardiovascular diseases, osteoporosis, inflammations, Alzheimer's disease, Parkinson's disease, and others (Martinez-Cayuela, 1995).

The aim of this study was to assess the effect of various doses of NPK fertilizer on the total antioxidant capacity of soil and of leaves of two Polish varieties of amaranth, Rawa and Aztek, cultivated for seed.

MATERIAL AND METHODS

A field experiment was conducted in 2007, on a field belonging to a farmer, situated near Zamość. Rawa and Aztek varieties of Amaranth were grown on brown soil with very high N content and high P, K and Mg content. Soil pH was slightly acidic (pH_{KCl} 5.9). Sorption capacity, determined using the Kappen method, was 189.2 mmol(+) kg^{-1}. The control soil contained 29.0-29.4 g kg^{-1} of organic carbon (SOC).

The experiment was set up in a random split-plot design with three replicates. The following combinations of NPK doses were used: I) nitrogen – 50, phosphorus – 40, potassium – 40 kg ha^{-1}; II) nitrogen – 90, phosphorus – 60, potassium – 60 kg ha^{-1}; III) nitrogen – 130, phosphorus – 70, potassium – 70 kg ha^{-1}. The soil was fertilized with nitrogen in the form of ammonium nitrate twice, before sowing and during the intensive growth period. Phosphorus and potassium fertilizers were applied before sowing – P in the form of Polifoska and K in the form of potassium chloride. The results obtained were compared with the control (without NPK fertilization). Amaranth seeds were sown during the last decade of May, at wide row spacing (every 30 cm). The area of the experimental microplots from which the plants were to be harvested was 1 m^2. The plants were cultivated in accordance with proper horticulture procedures.

A soil extract for determining total antioxidant capacity was prepared according to the procedure described by Baran (2000). An extract from fresh amaranth leaves was prepared by the method described by Sas-Piotrowska *et al*. (1996).

Total antioxidant capacity was determined using the Rice-Evans and Miller method (1994) as modified by Bartosz (2003). This method uses $ABTS^+$ (2,2-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid radical) as an indicator substance. The $ABTS^+$ solution is green. Adding an antioxidant substance causes $ABTS^+$ to lose its colour. The decrease in absorbance after 30 min is a measure of the total content of all antioxidants in the sample. In this study, soil extract or amaranth leaf extract was added to $ABTS^+$ radical cation solution. After 30 min the decrease in absorbance was measured at a wavelength of 414 nm. A standard curve was used to read the Trolox concentration corresponding to the change in absorbance in the sample.

RESULTS

The organic carbon content in the soil increased successively with the amount of NPK. This tendency was observed with both varieties of amaranth (Table 1).

The total antioxidant capacity of the soil was dependent on the NPK fertilization applied and also on the variety of amaranth (Table 1). The soil under Rawa cultivation with fertilizer combination III had the highest total antioxidant capacity – 64.8% higher than the unfertilized soil (control). The soils fertilized with combination I showed a 21.4% increase in TAC, while a 40.5% increase was attained with combination II. The soil on which the Aztek variety was grown had the highest TAC with fertilizer combination I – 39.4% higher than the control. The TAC increase was 38.6% with combination II, and 38.7% with combination III, compared with the unfertilized soil (Table 1).

T a b l e 1. Effect of NPK fertilization on organic carbon content in soil and TAC of soil and amaranth leaves (mean values)

Macroelement doses	SOC (g kg^{-1})	TAC of soil (µM trolox cm^{-3} g^{-1}soil)	TAC of leaves (µM trolox cm^{-3} g^{-1} leaf extract)
Rawa variety			
Combination I	29.8	147.83	9.47
Combination II	31.3	171.09	9.59
Combination III	32.7	200.65	14.55
Control	29.2	121.74	7.35
Aztek variety			
Combination I	30.9	209.13	12.50
Combination II	31.2	207.83	9.83
Combination III	31.8	208.04	9.74
Control	29.3	150.00	8.23

The TAC of the amaranth leaves was also found to be dependent on the amaranth variety and on the dose of macroelements applied (Table 1). Leaves of the Rawa variety had the highest TAC for combination III of macroelements (TAC nearly 98% higher than in the leaves from plants growing on unfertilized soil). The TAC increase in the leaves was 28.8% for NPK combination I, and 30.5% for combination II, compared with the control (Table 1). All the fertilizer doses applied caused an increase in the TAC of Aztek leaves. The TAC increase was about 52% for combination I, about 19.5% for combination II and 18.3% for combination III.

Statistical analysis revealed significant dependencies between the antioxidant properties of the soils and the different doses of NPK fertilizers. For the Rawa variety, the TAC of the soil was significantly positively correlated with the doses of macroelements applied (correlation coefficient $r = +0.788$, significance level $p = 0.01$) and with its organic carbon content ($r = +0.986$, $p = 0.01$). Similar dependencies were found for the Aztek variety: the correlation coefficient between the TAC of the soil and the level of NPK fertilization applied was $r = +0.531$ with $p = 0.01$. A positive correlation was also found between the TAC of the soil and organic carbon content ($r = +0.531$, $p = 0.01$).

The TAC of Rawa amaranth leaves was significantly dependent on the amount of fertilizers applied to the soil ($r = +0.950$, $p = 0.05$). TAC of leaves of this variety positively correlated with organic carbon content ($r = +0.936$, $p = 0.01$). Positive values for the correlation coefficient, but lower than for the Rawa variety, were obtained for the Aztek variety ($r = +0.239$, $p = 0.05$). Furthermore, significant correlations were found between TAC of Aztek leaves and organic carbon content in the soil ($r = +0.707$, $p = 0.01$).

DISCUSSION

Measurement of total antioxidant capacity has been used to determine the antioxidant capacity of samples with diverse chemical composition, such as food and drinks (Gorinstein *et al*., 2003), or plant extracts used as parapharmaceuticals (Mantle *et al*., 2003). Many studies indicate the potential of this method for blood analysis in diagnosing diseases associated with generation of reactive forms of oxygen, such as Alzheimer's disease (Pulido *et al*., 2005) or type II diabetes (Opara *et al*., 1999). Methods for measuring TAC make use of various indicator substances, and the amount of antioxidants contained in the sample is compared to the activity of such antioxidants as trolox, ascorbic acid or ferulic acid. It was pointed out that it can be difficult to compare results obtained using different methods for measuring antioxidant capacity because different types of antioxidant molecules react with indicator substances at varying rates (Arts *et al*., 2004). However, if we consider results obtained using one method in strictly defined conditions, they are repeatable and comparable.

Soil type significantly affects the chemical composition of plants. Studies on the content of antioxidant compounds in kale have confirmed that plants grown on lessive soil under certain conditions have higher antioxidant content than when grown on alluvial soil. Cultivation on lessive soil favours increased synthesis of ascorbate and small-molecule thiol compounds in kale. In the leaves of the plants grown on lessive soil, the average concentrations of ascorbate and glutathione were 11 and 12% higher than in plants grown on alluvial soil. Soil type had the least effect on total phenol content and anthocyanin content (5 and 2% increase), while the greatest effect was on concentration of γ-glutamylcysteine, a precursor of glutathione (33% increase). The FRAP index was about 13% higher in the case of lessive soil (Łata and Wińska-Krysiak, 2006).

Soil composition not only affects plant yield, but also determines the nutritional value of plants for animals and people. Antioxidant substances present in soil, particularly humic compounds in humus, can affect the biological activity of the soil, redox reactions and plant-soil interactions taking place in the rhizosphere (Rimmer, 2006; Schepetkin *et al*., 2002).

A number of factors influence total antioxidant capacity. The type of soil and content of humic compounds in it have a decisive effect. The higher the content of these in the soil, the more potent its antioxidant properties (Rimmer, 2006). In this study an attempt was made to determine the total antioxidant capacity of soil extracts with varied doses of NPK fertilizers from soil on which two varieties of amaranth were cultivated. The TAC of the soil is positively correlated with its organic carbon content.

In a previous study by these authors, TAC of three types of soil was compared: brown rendzina, lessive soil and acid podzolic soil (Skwaryło-Bednarz and Krzepiłko, 2007). Brown rendzina was found to have the highest total antioxidant capacity. The TAC value was over 30% higher than for lessive soil and nearly 65% higher than for acid podzolic soil. Fresh soils had higher TAC than air-dry soils, probably due to activity of soil microorganisms. Statistical analysis revealed that the numbers of the microorganisms studied were significantly positively correlated with the total antioxidant capacity of the soils studied.

Soil substrata rich in humic compounds increase the antioxidant content of plants produced on them (Jarosz, 2006; Rimmer, 2006; Shiow *et al*., 2003). Research by Jarosz (2006) confirms that tomatoes of the Cunero F_1 variety grown on peat had considerably more vitamin C, total sugars, total nitrogen and potassium than tomatoes grown on mineral wool or sand. In addition, non-chloride medium increased vitamin C content in comparison with medium containing chlorine (Jarosz, 2006). Cultivation of two varieties of strawberry – Allstar and Honeoye – on soil supplemented with compost (at a 1:1 ratio), or on compost alone, substantially increased the concentration of ascorbic acid and glutathione, as well as total antioxidant capacity, in their fruits (Shiow *et al*., 2003). Content of antioxidant compounds in the leaves of this plant was also tested. Many chemically diverse compounds, such as polyphenols, flavonoids and anthocyanins, have antioxidant functions in plants. In amaranth plants, most of these compounds accumulate in the seeds and leaves. β-carotene content is particularly high. The vegetative parts and seeds of amaranth have also been found to contain large amounts of phenols (Acar *et al*., 1988). Of particular value is the oil obtained from the seeds, which contains potent antioxidants such as squalene, tocopherols or tocotrienols (Nalborczyk *et al*., 1994). It is recommended that the leaves of this plant, which are rich in antioxidant substances, should be consumed as a vegetable, like spinach or lettuce. Amaranth leaves are a source of many mineral nutrient, such as calcium, phosphorus, potassium, magnesium, iron, as well as vitamins A, B_1, B_2, C and E (Nalborczyk *et al*., 1994). Measuring TAC of amaranth leaf extract reveals that the leaves of this plant have high TAC, and that the value of this parameter is dependent on the fertilizer combinations applied and on the variety of amaranth. Studies by other authors confirm that mineral fertilization not only affects the content of mineral components in the plant, but also the content of antioxidant substances (Nurzyńska-Wierdak, 2006). A frequently used indicator of the antioxidant properties of fruits and vegetables is vitamin C content which increases when a mineral fertilizer is used, particularly nitrogen. Properly selected nitrogen fertilization applied to Erucca sativa grown in autumn (0.2-0.25 g N dm^{-3}) made it possible to obtain very high biomass yield with very high vitamin C content (Nurzyńska-Wierdak, 2006).

CONCLUSIONS

1. Fertilization with increasing doses of macroelements (NPK) caused an increase in total antioxidant capability of the soil in which the analysed varieties of amaranth were cultivated, as well as in fresh leaf mass.

2. The highest total antioxidant capability of soil (as referred to the control) in which amaranth (Rawa variety) was cultivated was observed at combination III of NPK fertilization, and in the case of Aztek variety at combination I of NPK fertilization.

3. The doses of macroelements that were used (as referred to the control) positively influenced the increase of total antioxidant capability of the leaves of amaranth, both Rawa and Aztek varieties. The highest total antioxidant capability for the plants of amaranth (Rawa variety) was observed at combination III of macroelements dose, and for Aztek variety at combination I.

4. The statistical analysis that was carried out revealed many significant correlations between total antioxidant capability of the soils in which two varieties of amaranth were cultivated and the level of fertilization that was used, and between the total antioxidant capability of the investigated soils and the leaves of amaranth and the content of organic carbon in the soil.

REFERENCES

Acar N., Vohra P., Becker R., Hanners G.D., and Sauders R.M., 1988. Nutritional evaluation of grain amaranth for growing chicken. Poultry Sci., 67(8), 1166-1173.

Arts M.J.T.J., Haenen G.R.M.M., Voss H.-P., and Bast A., 2004. Antioxidant capacity of reaction products limits the applicability of the trolox equivalent antioxidant capacity (TEAC) assay. Food Chem. Toxicol., 42(1), 45-49.

Baran S., 2000. Estimation of degree of soil degradation and reclamation (in Polish). Univ. Life Sci. Press, Lublin, Poland.

Baran S., Martyn W., Krzepiłko A., Skwaryło B., Święciło A., and Onuch-Amborska J., 1999. Adaptation of Catherine Rice-Evans and Nicholas Miller's method to marking antioxidant activity of water solution of wermicompost (in Polish). Fol. Univ Stetin. Agriculturae, 200(77), 11-14.

Bartosz G., 2003. Another 'Face' of Oxygen (in Polish). PWN Press, Warsaw, Poland.

Bijlsma R.J. and Lambers H., 2000. A dynamic whole-plant model of integrated metabolism of nitrogen and carbon. Plant and Soil, 220, 71-87.

Demmler D., 1998. Gründüngung zur Stabilisierung der Bodenfruchtbarkeit bzw. zur Verringerung von N-Verlusten. Kartoffelbau, 49(6), 232-235.

Gorinstein S., Martin-Belloso O., Katrich E., Lojek A., Ciz M., Gligelmo-Miguel N., Haruenkit R., Park Y.S., Jung S.T., and Trakhtenberg S., 2003. Comparison of the contents of the main biochemical compounds and the antioxidant activity of some Spanish olive oils as determined by four different radical scavenging tests. J. Nutr. Biochem., 14(3), 154-9.

Jarosz Z., 2006. Effect of differents types of potassium fertilization on the Fielding of greenhouse tomatoes grown in various substrates (in Polish). Acta Scientiarum Polonorum - Hortorum Cultus, Horticulture, 5(1), 11-18.

Kołodziej B., 2006. Effect of mineral fertilization on ribwort plantain (*Plantago lanceolata* L.) yielding (in Polish). Acta Agrophysica, 141, 637-647.

Łata B. and Wińska-Krysiak M., 2006. Chemical composition kale cultivated on two types of soils (in Polish). Acta Agrophysica, 134, 663-670.

Łoginow W., Andrzejewski J., and Janowiak J., 1991. Role of organic fertilization in maintenance of stock of organic stuff in soil (in Polish). Roczn. Glebozn., 42, 3/4, 19-25.

Mantle D., Wilkins R.M., and Gok M.A., 2003. Comparison of antioxidant activity in commercial *Ginkgo biloba* preparations. J. Altern. Compl. Med., 9(5), 625-629.

Martinez-Cayuela M., 1995. Oxygen free radicals and human disease. Biochimie, 77, 147-161.

Nalborczyk E., ``Wróblewska E., Marcinkowska E., and Roszewski R., 1994. Amaranthus – prospect cultivation and utilization (in Polish). SGGW Press, Warsaw, Poland.

Nurzyńska-Wierdak R., 2006. The effect of nitrogen fertilization on yield and chemicial composition of garden rocket (*Eruca sativa* Mill.) in autumn cultivation (in Polish). Acta Scientiarum Polonorum - Hortorum Cultus, 5(2),53-63.

Olivier M.A., 1997. Soil and human health a revive. Eur. J. Soil Sci., 48, 573-592.

Opara E.C., Abdel-Rahman E., Soliman S., Kamel W.A., Souka S., Lowe J.E., and Abdel-Aleem S., 1999. Depletion of total antioxidant capacity in type 2 diabetes. Metabolism, 48(11), 1414-1447.

Pulido R., Jimenez-Escrig A., Orensanz L., Saura-Calixto F., Jimenezescrig A., 2005. Study of plasma antioxidant status in Alzheimer's disease. Eur. J. Neurol., 12(7), 531-535.

Rice-Evans C. and Miller N.J., 1994. Total antioxidant status in plasma and body fluids. Methods in Enzymology, 234, 279-293.

Rimmer D.L., 2006. Free radicals, antioxidants, and soil organic matter recalcitrance. Eur. J. Soil Sci., 57, 91-94.

Sas-Piotrowska B., Piotrowski W., and Misiak M., 1996. The growth and development of potato pathogens on the media with extracts from Polygonaceae plants. Phytopathologia Polonica, 11, 103-109.

Schepetkin I., Khlebnikov A., and Kwon B.S., 2002. Medical drugs from humus matter: Focus on mumie. Drug Develop. Res., 40-159.

Shiow Y. Wang and Hsin-Shan Lin, 2003. Compost as a soil supplement increases the level of antioxidant compounds and oxygen radical absorbance, capacity in strawberries. J. Agric. Food Chem., 51, 60-78.

Skwaryło-Bednarz B. and Krzepiłko A., 2007. Biological and antioxidant properties of soils from the protected zone of Roztocze Nat. Park. Polish J. Environ. Stud., 16, 3A, 251-254.

Wielgosz E. and Szember A., 2006. The effect of selected plants on the number and activity of soil microorganisms (in Polish). Annales UMCS, E, 61, 107-119.