THE EFFECT OF IRRIGATION ON THE BIOCHEMICAL CONTENT OF LEAFY VEGETABLES

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Abstract. *Leafy vegetables contain a lot of vitamins and minerals Consumers more and more are thinking about a healthy and balanced diet. The aim of the research was to evaluate the effect of irrigation on the content of ascorbic acid and antioxidant activity in lettuce cv.'Grand Rapids' and leafy mustard cv 'Scala'. Experiments were arranged in high plastic tunnels in Pūre Horticultural Research Centre during spring and autumn of 2013. As control was used variant with optimal soil moisture, as experimental plot- with moisture deficit. Available for plants moisture in the soil was detected by tensiometers. The content of ascorbic acid was determined by using titration with 2, 6- dichlorophenolindophenol in acidic solution. Antioxidant activity was determined by using the DPPH assay. The higher ascorbic acid content and antioxidant activity was detected in the control variant with optimal soil moisture .Higher conent ofvitamin C was observed in leaf mustard – three to seven times more than in lettuce. Antioxidant activity did no show quite clear differences regarding vegetable species and growing periods.*

Key words*: leaf mustard, lettuce,vitamin C, antioxidant activity.*

INTRODUCTION

Leafy vegetables are good source of healthy nutrients in human diet. There is high percentage of carotene, ascorbic acid, riboflavin, folic acid, minerals and vitamins [1]. As human body is unable to synthesis ascorbic acid (vitamin C), it should be provided by diet. Vitamin C is a universal component of all green plants. Minimal suggested daily intake for adults a 60 mg vitamin C. Cabbages and leafy vegetables are the richest source of vitamin C among vegetables vitamin C [3]. Ascorbic acid and carotenoids are involved in ensuring of plants antioxidant properties [4]. Antioxidants protect plants from negative influence of free radicals. Also many human diseases are caused by free radical damages in the body. Vegetables usually contain natural antioxidants which can neutralize free radicals in the human body [5].

Leaf mustard (*Brassica juncea* var. *crispifolia* L.) is widely used for its special flavour and nutritional value. Major flavour components are mainly derived from gluosinoanalate hydrolysis [6]. Those are good source of ascorbic acid, β-carotene, chlorophylls, flavonoids and dietary fiber. 140 g leaf mustard provide with 60% vitamin A and 100% vitamin C of advisable daily dosage [7]. Mustard greens have high antioxidant activity; they can inhibit oxidative damage diseases, for example heart diseases, cancers [6]. Also lettuce (*Lactuca sativa* L.) is good source of carotene, vitamin C, vitamin E and is characteristic with good antioxidant activity [8]. Therefore it is significant source of dietary antioxidants as well. Antiradical activity of lettuce also derives from some phenolic compounds. Also C and E vitamin promote antioxidant activity in lettuce [9, 10].

Relationship between plants, soil and its water resources is tight and complicate. It is important to understand these relationships and use as much efficiently as possible to obtain not only biochemically rich, but also high yielding fresh commodities. One of the most important factors ensuring high quality yield is soil moisture. Irrigation is a basic requirement to ensure optimal soil moisture during the plants growing period. The highest yield and quality can be obtained in optimal moisture conditions during all vegetation season. It means – to have accurate, balanced and agronomical justified irrigation [10, 11]. It should be stressed that not only moisture and crop management influences yield amount and quality. Biological active compounds in plants are depend also on the climate conditions, seasonal changes, cultivar properties, maturity and post harvest treatments or storage conditions [12]. There were found results on investigations performed in other countries indicating that water stress reduce leafy vegetables yield and quality [10, 11]. Irrigation of vegetables is not often investigated in Latvia.

The aim of the study was evaluate effect of irrigation and sowing date on the content of ascorbic acid and antioxidant activity of leaf mustard and lettuce leaves.

MATERIALS AND METHODS

The investigations were carried out in Pūre Horticultural Research Centre in vegetation season of 2013. Experiments were arranged in high tunnels in two growing periods – spring and autumn. First period trials were sown on 8 May, but second – on 11 September. Two leafy vegetable species: lettuce cv.'Grand Rapids' and leaf mustard cv 'Scala' were grown in 3 replications. Two variants of irrigation were compared – experimental plot with moisture deficiency was compared to control variant – optimal soil moisture provided. Total area of each experimental plot was 1 m^2.

Soil moisture and temperature was fixed by soil tensionmeters and thermometers. After sowing all experiment plots were irrigated according to tensionmeters measurements, to provide optimal soil moisture for uniform seed germination. After germination, irrigation was reduced in control variant to keep moisture deficit in soil, but not achieve critical level for plant. In the first growing period from sowing till end of vegetation period optimal soil moisture was provided with irrigation performed 10 times. In the first decade of June weather was very hot and sunny. Average air temperature was +18.6 °C (max +28.1 °C) and therefore irrigation was done so often. In variant with moisture deficit irrigation was performed 8 times during growing period. Vegetables were harvested at 11 June. In the second growing period average air temperature was +10 °C and sunshine days were less. Irrigation was done 8 times in optimal variant, but 5 times in variant with moisture deficit. Yield was harvested at 28 October.

Biochemical analyses were done in Latvia University of Agriculture, Institute of Soil and Plant Sciences. Ascorbic acid and antiradical activity in lettuce and leaf mustard leaves were analysed. The content of ascorbic acid was determined by using titrimetric method with 2, 6 – dichlorophenolindophenol in acidic solution. Bulk sample of all replications was weighted and grinded by pestle and mortar. Two gram samples were placed in a graduated tube, added 50 mL of 1% HCl and 5% H3PO4 solution (1:1) and mixed. After 30 minutes solution was strained by paper filter. Then 10 mL filtrate titrated with 0, 0005 molar 2,6 – dichlorophenolindophenol until faintly rosy colour. Content of ascorbic acid (mg 100 g^{-1}) was calculated by equation (1):

$$m = \frac{V_{titr} \times 0.044 \times V_{sum} \times 100}{V_{anal.} \times m_{weighed}}, \quad (1)$$

where:

$V_{titr.}$ – for titrated used 2.6 – dichlorophenolindophenol volume, mL

0,044 – amount of ascorbic acid, which reduce 1 mL 0.0005 M 2,.6 dichlorophenolindophenol solution, mg

V_{sum} – amount of filtrate volume, mL

$V_{anal.}$ – amount of analysed solution volume, mL

$M_{weighed}$ – weighed amount of plant material [13].

Antiradical activity was determined using 1.1 – dyphenyl -2 picrylhydrazyl radical (DPPH*) method. An aliquot of the methanolic lettuce extract and leaf mustard extract was added to a DPHH methanolic solution and spectrophotometrically determined at 515nm wavelength. Antiradical activity (%) was calculated by equation (2):

$$ARA = 100 \times (1 - \frac{A_{ss}}{A_0} ARA = 100 \times (1 - \frac{A_{ss}}{A_0}), \quad (2)$$

where:

A_{ss} – absorbance of the solution at constant state, min

A_0 – absorbance of DPPH solution before antioxidant addition, min [12].

The results were analyzed using ANOVA at significance level of p = 0.05.

RESULTS AND DISCUSSION

Ascorbic acid content in mustard leaf shows significant differences between irrigation variants ($p = 0.02 \times 10^{-2}$) and growing periods ($p = 4.19 \times 10^{-5}$) (Fig.1).

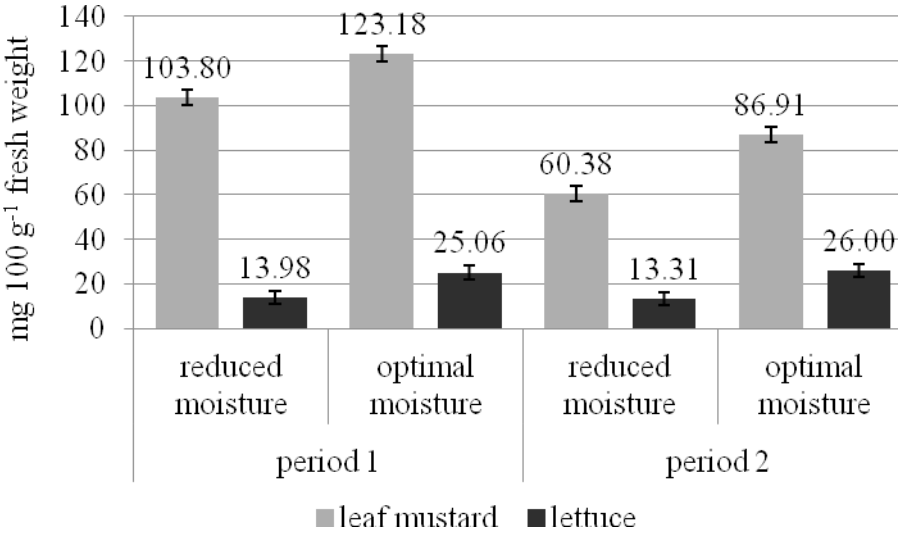

Figure 1. **Ascorbic acid content in lettuce and leaf mustard**

Higher content of vitamin C was observed in leaf mustard. Ascorbic acid content in the first growing period ranged between 103.8 and 123.18 mg 100 g^{-1} fresh weight. In the second growing period it was significant less than in the first growing period, it ranged between 60.38 and 86.91 mg 100 g^{-1} fresh weight. Vitamin C lettuce leaves contained three to seven times less ascorbic acid than leaf mustard leaves. Ascorbic acid content in lettuce leafs shows significant differences between irrigation variants ($p = 0.003$), but not significant differences between growing periods ($p = 0.93$). Ascorbic acid content in reduced moisture variant was two times less than in optimal soil moisture variant. In both periods vitamin C content in variant with reduced moisture and optimal moisture was practically identical.

Significant differences are not observed for antiradical activity in the lettuce and leaf mustard between both irrigation variants and growing periods (Fig.2).

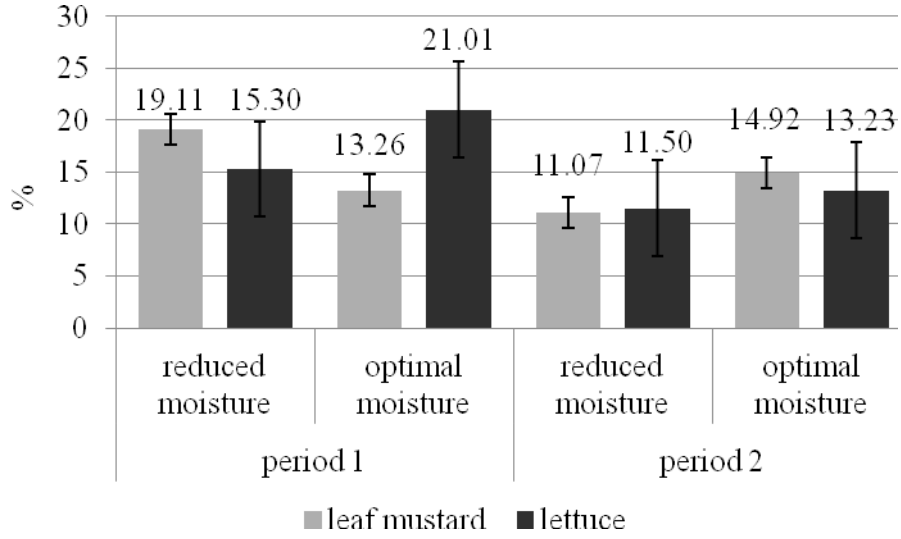

Figure 2. **Antiradical activity in lettuce and leaf mustard leaf**

Results of investigation did not show quite clear tendencies of antiradical activity changes in the lettuce and leaf mustard in two different growing periods and irrigations variants. In average for all samples antiradical activity was stated between 11.06 and 21.01%. If compare growing periods, in the first growing period for antiradical activity was observed tendency to be higher than in the second growing period.

Leafy vegetable production and consumption more and more interest growers because crop can be harvested several times during one vegetation period. Also our trial proved that it is possible to harvest leafy vegetables in Latvia even in September. Results obtained for vitamin C content are corresponding to those referred by

Cauni with colleagues, where content of ascorbic acid in lettuce in average was 24 mg 100 g^{-1} (detected by using titremetrical method) [1]. According to Liu and et al., in China vitamin C in lettuce leaves was in average 2.90 mg 100 g^{-1} fresh weight [14]. In our research vitamin C content in leaf mustard was higher than in lettuce leaves and ranged between 60.38 and 123.18 mg 100 g^{-1} fresh weight. Farnham with colleagues observed that vitamin C varied significantly among genotypes. In one variety content of vitamin C was 114 mg 100 g^{-1}, but in another – 162 mg 100 g^{-1} [15]. Other leafy vegetables also have high content of vitamin C. For example, vitamin C content in leafy parsley is 133 mg 100 g^{-1} and in celery leaves 85 mg 100 g^{-1} fresh weight [1].

C vitamin content depends on many factors, such as a cultivar, plant age, available photosynthetic radiation, soil type, indoor or outdoor culture [2]. Also in our research it was clearly observed that soil moisture and temperature influences content of ascorbic acid in leafy vegetables.

Obtained results are similar to findings of others in relation to the differences between growing periods. In USA were compared antiradical potential in lettuce when it was harvested in two different periods (July and September). In July it had higher antioxidant activity than in September [16]. Influence of growing conditions on the antiradical activity of leafy vegetables is reported also by Oh, et al. (2011), where higher antioxidant activity was found for vegetables grown under the open field conditions, compare to those grown in the high tunnels [9]. Our results indicates similar tendency that higher antioxidant activity was detected in better growing conditions – in the first growing period. In China it was stated that antioxidant activity was in average 39.57% in lettuce leaves and 21.80% in lettuce stems (by using DPPH assay) [14], which is higher than found in our investigations. In general, higher content of physiologically active compounds was observed in the first growing period with optimal soil moisture. Also irrigation influence on the leafy vegetables quality find in our trials is similar to findings of others. It is referred that irrigation increases leafy vegetables yield and quality. In Iran it was found that irrigation irrespective to water amounts used significantly increased mustard yield and quality, but the best results showed variants with higher water supply [11]. The same tendency was observed also for lettuce. The results investigation performed by Karam and colleague indicated that water stress caused by insufficient irrigation reduce leaf yield and quality [10].

In summary it can be concluded, that growing and management conditions (water supply, temperature, light conditions) directly influence biosynthesis of phytochemicals in plants. Growing conditions have effect on the quality of leaf vegetables, those influence leaf phenolic concentration and antioxidant activity. Our research does not confirm findings of Oh and colleagues, that leafy vegetable accumulate more antioxidants, if they are subjected to stress conditions [9]. Probably some particular stress conditions have positive influence on the changes of content of biologically active compounds, but they should be investigated more precisely in the agro–climatic conditions of Latvia.

CONCLUSIONS

Obtained results allow us to assume that water supply has influence on ascorbic acid content and antiradical activity of the leaf mustard and lettuce, but also other environment factors dependent on sowing period, such as temperature and light conditions can influence the amount of biochemical compounds in leafy vegetables. It is necessary to continue research about plant response on antioxidant and vitamin C accumulations in environmental stresses.

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REFERENCES

1. Caunii A., Cuciureanu R., Zakar A.M., Tonea E., Giuchici C. (2010) Chemical composition of common leafy vegetables. *Studia Universitatis*, 20 (2), pp. 45-48.
2. Davey M.W., Van Montagu M., Inze D., Sanmartin M., Kanellis A., Smirnoff N., Benzie I.J.J., Strain J.J., Fletcher J. (2000) Plant L-ascorbic acid: chemistry, function, metabolism, bioavailability and effects of processing. *Journal of the Science of Food and Agriculture.* 80 (7), pp. 825-860.
3. Zlotek U., Świeca M., Jakubczyk A. (2014) Effect of abiotic elicitation on main healt-promoting coumpounds, antioxidant activity and commercial quality of butter lettuce (Lactuca sativa L.). *Food Chemistry,* 148, pp. 253-260.

4. Kim M.H., Kim J.M.,Yoon K.Y. (2013) Effects of blanching on antioxidant activity and total phenolic content according to type of medicinal plants. *Food Science Biotehnology*, 22 (3), pp. 817-823.
5. Zhongxiang F., Yuxia H., Donghong L., Jianchu C., Xingqian Y. (2008) Changes of phenolic acids and antioxidant activities during potherb mustard (Brassica juncea, Coss.) pickling. *Food Chemistry*,108 (3), pp. 811-817.
6. Lian Y.J., Lin G.Z, Zhao H.M., Lim H.T. (2011) Production and genetic characterization of somatic hubrids between leaf mustard (Brassica juncea) and broccoli (Brassica oleracea). *In Vitro Cellular and Developmental Biology*, 47 (2), pp. 289-296.
7. Garg M., Garg C., Mukherjee P.K., Suresh B. (2004) Antioxidant potential of Lactuca sativa. *Ancient Science of Life*, 24 (1), pp. 6-10.
8. Martinez-Sanchez A., Gil-Izquierdo A., Gil M.I., Ferreres F.(2008) Coparative study of flavonoid copounds, vitamin C, and antioxidant properties of baby leaf Brassicaceae Species. *Journal of Agricultural and Food Chemistry*, 56(7), pp. 2330-2340.
9. Oh M.M., Carey E.E., Rajashekar C.B (2011) Antioxidant phytochemicals inlettuce grown in high tunnels and open field. *Horticultural, Environment,and Biotechnology*, 55 (2), pp. 133-139.
10. Karam F., Mounzer O., Sarkis F., Lahoud R. (2002) Yield and nitrogen recovery of lettuce under different irrigation regimes. *Journal of applied horticulture*, 4 (2), pp. 70-76.
11. Piri I., Nik M.M., Tavassoli A., Rastegaripour F. (2011). Effect of irrigation intervals and sulphur fertilizer on growth analyses and yield of *Brassica juncea*. *African Journal of Microbiology Research*, 5 (22), pp. 3640-3646.
12. Coria – Cayupans Y.S., Sanchez De Pinto M.I., Nazareno M.A. (2009) Variations in bioactive substance contents and crop yield of lettuce (Lactuca Sativa L.) cultivated in soils with different fertilization treatments. *Journal of Agricultural and Food Chemistry*, 57 (21), pp. 10122-10129.
13. Dūma M., Alsiņa I., Dubova L., Zeipiņa S. (2014) The comparison of titrimetrical methods for determination of ascorbic acid in vegetables. In: *Proceedings of the Scientific and Practical Conference „Balanced Agriculture*", Jelgava, Latvia, pp. 131-135.
14. Zhao Y., Li X., Jia J., Chen Y, Hua Z. (2014). Antioxidant capacities and main reducing substance contents in 100 fruits and vegetables eaten in China. *Food and Nutrition Sciences*, 5, pp. 293-307.
15. Farnhamn M.W., Lester G.E., Hassell R. (2007) Collard, mustard and turnip greens: effects of genotypes and leaf position on concentrations of ascorbic acid, folate, β-carotene, lutein and phylloquinone. *Journal of Food Composition and Analysis*, 27, pp. 1-7.
16. Liu X., Ardo S., Bunning M., Parry J., Zhou K., Stuhnoff C., Stinoker F, Yu L., Kendall P. (2007) Total phenolic content and DPPH radical savenging ativity of lettuce (Lactuca sativa L.) grown in Colorado. *LWT – Food Science and Tehnology*, 40 (3), pp. 552-557.