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Impacts of deficit irrigaton applied at different growth stages on vitamin contents of broccoli

Farklı Gelişme Dönemlerinde Uygulanan Su Kısıntılarının Brokkolinin Vitamin İçeriğine Etkileri

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Anahtar Sözcükler:

Brassica oleracea L. var. *Italica*, L-ascorbic acid, retinol, α-tocopherol

ABSTRACT

This research was conducted in order to determine the impacts of deficit irrigation applied at different growth stages on some vitamin contents of broccoli during 2010 growing season. Variations in L-ascorbic acid (vitamin C), retinol (vitamin A) and α-tocopherol (vitamin E) contents were determined by using high-pressure liquid chromatography (HPLC) method. Maraton Broccoli was grown as the plant material of the study and 5 different water deficit levels (80, 60, 40, 20%, and a control treatment with 100%) were applied during 3 different growth stages (early vegetative, late vegetative and flowering periods) of broccoli plants. Results revealed significant variations in L-ascorbic acid (vitamin C), retinol (vitamin A) and α-tocopherol (vitamin E) contents of broccoli heads with deficit irrigation applications at different growth stages. Results revealed significant variations in L-ascorbic acid (vitamin C), retinol (vitamin A) and α-tocopherol (vitamin E) contents of broccoli heads with deficit irrigation applications at different growth stages.

Key Words:

Brassica oleracea L. var. *Italica*, L-askorbik asit, retinol, α-tokoferol

ÖZET

Araştırma, brokoli taçlarında farklı su kısıntılarında yüksek basınçlı likit kromotografi (HPLC) metoduyla L-askorbik asit (vitamin C), retinol (vitamin A) ve α-tokoferol (vitamin E) miktarlarındaki değişimlerin belirlenmesi amacıyla 2010 yılında yürütülmüştür. Maraton brokkoli çeşidi kullanılarak kontrol (%100) ile birlikte 5 farklı su kısıntısı (%80, %60, %40 ve %20) bitkinin 3 değişik gelişme döneminde (erken vegetatif, geç vegetatif ve çiçeklenme dönemi) uygulanmıştır. Araştırma sonuçlarına göre su kısıntısı uygulamaları ile birlikte brokkoli taçlarında meydana gelen L-askorbik asit (vitamin C), Retinol (vitamin A) ve α-tokoferol (vitamin E) miktarlarında farklı gelişme dönemlerine göre değişimlerin olduğu tespit edilmiştir.

INTRODUCTION

For nearly a century, fruits and vegetables have been recognized as a good source of vitamins and minerals. They have been especially valuable for their ability to prevent vitamin C and vitamin A deficiencies (Groff et al., 1995; Ross, 1999).

In their peculiarity *Brassica* vegetables received widespread attention with their nutritive values and significant attributes. Broccoli (*Brassica oleracea* var. *italica*) is an excellent source of beta-carotene, vitamin C, vitamin E, calcium (Ca), magnesium (Mg), folate, flavonoids, and fiber (Kurilich et al., 1999; Rosa and Rodrigues, 2001; Souci et al., 1994).

Researches of the past 20 years have proven that fruits and vegetables not only prevent malnutrition but also support maintaining optimum health through a host of chemical components that are still being identified, tested, and measured (Anonymous, 2011).

Several carotenes, tocopherol, and ascorbate act both as traditional vitamins and as antioxidants (Kurilich et al., 1999).

On the other side Vitamin C is the most significant vitamin source of fruits and vegetable in human nutrition (Lee and Kader, 2000).

The potential antioxidant effect of vitamin C and carotenoids was the subject of number of studies. Byers and Perry (1992) indicated that vitamin C prevents cancer by inhibiting the formation of N-nitroso compounds in the stomach, and by stimulating the immune system. The same authors also stated that carotenoids are required for human epithelial cellular differentiation. In addition, some epidemiological studies have established an inverse relationship between the risk of laryngeal, lung, and colon cancers and consumption of foods containing carotenoids (Block et al. 1992; Steimetz and Potter, 1993).

Vitamin E is composed of 4 tocopherol and tocotienol and contains unsaturated fatty acids. Most of the plant-originated foods, especially vegetables and fruits, contain medium-low levels of vitamin E, therefore they are usually included into diets as source of vitamin E (Chun et al., 2006; Eitenmiller and Lee, 2004).

The increase in tocopherol content is reported to increases stress tolerance of the plants (Blokhina et al., 2003; Munne-Bosch and Alegre, 2000) and as result of the studies it is determined that drought-tolerant plants have higher tocopherol contents than the other plants (Price and Hendry, 1989). While carotenoids are accepted as the precursors of vitamin A (retinol), the term retinoid refers to any compound that is structurally similar to retinal (aldehyde), retinol (alcohol), or any other substance that exhibits vitamin A activity (Anoynomous, 2011).

It could be concluded that L-ascorbic acid (vitamin C), Retinol (vitamin A) and α-tocopherol (vitamin E) all are significant vitamins in human nutrition and health, and are generally supplied from plant-originated foods (Donaldson, 2004; Sahni et al., 2010).

The experimental crop (Broccoli) is considered as a good source of these vitamins. Although variations in some secondary metabolites were determined under abiotic stress conditions, variations in these metabolites under stress conditions at different growth stages have not been quantified yet. Only few studies relate these variations with plant metabolism together with environmental factors (Hamidou et al., 2007; Vinocur and Altman, 2005).

This study was carried out to determine the variations in Retinol (Vitamin A), α-tocopherol (vitamin E) and L-ascorbic acid (Vitamin C) contents of broccoli under deficit irrigations at different growth stages.

MATERIAL and METHOD

EXPERIMENT AREA AND GROWING MEDIA

Experiments were carried out in 10-liter pots placed and prevented from precipitations at research fields of the Agricultural Faculty of Çanakkale Onsekiz Mart University, during the fall season of 2010 experimental year. Research site is located between 40° 06′ north latitude and 26° 24′ east longitude.

Sandy-loam soil mixture with an electrical conductivity of 0.72 dS m^{-1}, and pH of 7.6 was placed into pots at a ratio of 2:1:1 (soil, sand and manure respectively). 5.28 g N, 4.8 g P_2O_5 and 4.8 g K_2O was added to each pot for plant nutrition based on soil analyses results.

Municipality tap water with an electrical conductivity of 0.33 dS m^{-1}, and pH of 7.6 was used as irrigation water source for all treatments.

Meteorological parameters for the research period are presented in Table 1 (Anonymous 2011). Maraton broccoli variety was used as plant material of the research. The length of the growing season (period from seedling to harvest) is 90 days.

Table 1. Meteorological parameters for research period

Months	Temperature (°C)	Average Relative Humidity (%)	Average Wind Speed*	Solar Radiation (h)	Amount of evaporation** mm/day	Precipitation (mm)
August	28.07	62.94	3.76	9.9	9.7	-
September	21.90	65.86	3.81	6.8	6.5	21.4
October	15.16	78.78	3.44	2.9	2.8	333.3
November	16.21	81.61	4.96	3.3	2.5	38.1
December	10.31	78.29	4.81	1.4	-	90,5

* : measured at 2 m elevation
** : measured from Class- A evaporation pan

EXPERIMENTAL DESIGN

A total of 5 different irrigation levels (100, 80, 60, 40, 20%) were applied to experimental plants during the growth season. Control treatment was 100% replenishment of deficit water calculated by weighing the pots. Water amounts applied to rest of the treatments were calculated on the base of the fully irrigated control treatment and were determined as 80, 60, 40 and 20 % of water applied for the control. Experiments were carried out in randomized plots factorial experimental design with 5 replications. The statistical experimental model was defined below:

$Y_{ijk} = \mu + \alpha_i + \beta_j + \alpha\beta_{ij} + \varepsilon_{ijk}$

μ = General population mean

α = Water deficit levels (i:1,2,3,4)

β = Effect of growth stages (j:1,2,3,4)

αβ = Effect of water deficit x growth stage interaction

ε = Error term

Treatments used in this research;

1) $\underline{\mathbf{E_{100}L_{100}F_{100}}}$: Control treatment grown under optimum moisture conditions without any stress 100% (**C**),

2) $\underline{\mathbf{E_{80}L_{100}F_{100}}}$: 20% water deficit from seedling to formation of rosette leaves, then full irrigation during consecutive periods, (**D1**)

3) $\underline{\mathbf{E_{60}L_{100}F_{100}}}$: 40% water deficit from seedling to formation of rosette leaves, then full irrigation during consecutive periods, (**D2**)

4) $\underline{\mathbf{E_{40}L_{100}F_{100}}}$: 60% water deficit from seedling to formation of rosette leaves, then full irrigation during consecutive periods (**D3**)

5) $\underline{\mathbf{E_{20}L_{100}F_{100}}}$: 80% water deficit from seedling to formation of rosette leaves, then full irrigation during consecutive periods, (**D4**)

6) $\underline{\mathbf{E_{100}L_{80}F_{100}}}$: 20% water deficit between formation of rosette leaves and formation of flower heads, full irrigation during previous and consecutive period, (**D5**)

7) $\underline{\mathbf{E_{100}L_{60}F_{100}}}$: 40% water deficit between formation of rosette leaves and formation of flower heads, full irrigation during previous and consecutive period, (**D6**)

8) $\underline{\mathbf{E_{100}L_{40}F_{100}}}$: 60% water deficit between formation of rosette leaves and formation of flower heads, full irrigation during previous and consecutive period, (**D7**)

9) $\underline{\mathbf{E_{100}L_{20}F_{100}}}$: 80% water deficit between formation of rosette leaves and formation of flower heads, full irrigation during previous and consecutive period, (**D8**)

10) $\underline{\mathbf{E_{100}L_{100}F_{80}}}$: 20% water deficit between formation of flower heads and harvest, full irrigation during previous periods, (**D9**)

11) $\underline{\mathbf{E_{100}L_{100}F_{60}}}$: 40% water deficit between formation of flower heads and harvest, full irrigation during previous periods, (**D10**)

12) $\underline{\mathbf{E_{100}L_{100}F_{40}}}$: 60% water deficit between formation of flower heads and harvest, full irrigation during previous periods, (**D11**)

13) $\underline{\mathbf{E_{100}L_{100}F_{20}}}$: 80% water deficit between formation of flower heads and harvest, full irrigation during previous periods, (**D12**)

PREPARATION of SAMPLES

Initially, water holding capacity of pot soils with pre-known weights was determined. The pots were saturated and left for seepage under gravity for 24 hours and pots were weighed. The weight of the pots filled with mixture was accepted as weight of the pot at filled full capacity of the mixture and was accepted as the weight of 100% (control) irrigation treatment. Following the initiation of experiments, pots were weighed every 3 days to measure both evaporation from the pots and water used by plants. Weight loss was taken as the amount of irrigation water to be applied.

For deficit irrigations, amount of irrigation water to be applied was determined based on control treatment with 100% irrigation. Irrigation water for any experimental treatment was determined multiplying the amount of water applied to control treatment by the rate of water supply.

In order to provide full seedling setting, the total of 10.50 lt irrigation water was equally applied to each pot of the experiment before the initiation of the experimental treatments. The application of deficit irrigation programs were started just after full setting and continued until the harvest of last plant.

ANALYSIS of SAMPLES

Chemicals used in analysis were all (Merck GmbH Germany) HPLC Grade chemicals.

1/1 broccoli juice / methaphosphoric acid mixture was prepared to determine vitamin C content. Solutions taken from centrifuged samples were passed through syringe filter and prepared for HPLC analysis (Nollet, 1992).

For vitamin A and E contents, chopped broccoli samples were extracted with hexane. Then, 20 g broccoli puree was subjected to extraction with 250 ml hexane in socsolete set. Resultant hexane-plant extract was volatilized in an evaporator. Following the separation of hexane and fruit extract, they were filtered through syringe filter and purified for HPLC analysis.

Retinol and α-tocopherol determination were performed by using HPLC-UV detector (Jasco 820-FP), Lutegratör (HP-3394 A), LC-20AD prominence model pump, SIL-20A prominence autosampler, 100 µl injection unit, Hypersil ODS (5µm-150x 4.6 mm, RCM 100 RP18 (Supelco. Inc. Supelco Park. Bellofanta, PA16823–0048) column. Driving phase methanol and hexane (72:28) solutions were used at 1ml/min. flow rate (Burns et al., 2003; Rodas et al., 2003; Karpinska et al. 2006).

Calibration curves were prepared by using vitamin A and E (Fluka) standards (Gimeno et al., 2000; Hiroshi 2000; Moreno and Salvado, 2000).

STATISTICAL ANALYSIS

Kruskal-Wallis test was used to compare water deficit treatments in regard to vitamins (A, E and C). Dunn-Z multiple comparison test was used to determine the different water deficits. MDS statistical analysis was employed to determine the differences among vitamin A, E and C contents based on differences in applied irrigation water. Beside the multiple comparison tests, Retinol (vitamin A), α-tocopherol (vitamin E), and L-ascorbic acid (vitamin C) contents of harvested broccoli were also evaluated by using Multi Dimensional Scaling (MDS) statistical analysis method.

RESULTS and DISCUSSION

Average retinol (vitamin A), α-tocopherol (vitamin E) and L-ascorbic acid (vitamin C) values and statistical analysis results are presented in Table 2. From results in table it could be seen that, while there were not significant differences among water deficits in terms of α-tocopherol (vitamin E) (p= 0.313), significant differences were observed in regard to effect of water stress on retinol (vitamin A) (p= 0.004) and L-ascorbic acid (vitamin C) (p= 0.013).

Dunn-Z multiple comparison test performed on data obtained from the study revealed that the lowest vitamin A value (0.1210 mg/100g) was observed in control treatment C, while the highest value (0.3697 mg/100g) was obtained from treatment D12, exposed to most several stress during the last growth stage of the plant. Differences among treatments with regard to variations in vitamin A contents due to applied water stress of different level during various growth stages were found to be significant (p= 0.004). In addition, although the treatment D12 seemed to have the highest retinol (vitamin A) value of 0.393 mg/100 g, the difference between the mentioned treatment and D1, D2, D3, D5, D11 was not proved statistically. Therefore, it can be concluded that the treatment D12 and the treatments D1, D2, D3, D5 and D11 had higher retinol (vitamin A) value than the other treatments.

Several researchers indicated different vitamin A contents for different growth stages of plants and also observed fluctuations especially in β-carotene values under stress conditions (Munne-Bosch and Alegre 2003; Olivan and Munne-Bosch 2010; Keles and Oncel 2002). Kurilich et al., (1999) reported that the vitamin A contents of broccoli varieties varies in the ranges of 0.393 and 1.502 mg/100g.

Similar results as given above were obtained in terms of L-ascorbic acid (vitamin C). Just as in the case of Vitamin A, the highest L-ascorbic acid (vitamin C) value of 102.21 mg/100 g was observed in plants under conditions of treatment D12, while the lowest value of 77.21 mg/100 g was recorded for the plants of treatment C.

Dunn-Z multiple comparison test results indicated significantly higher vitamin-C values for treatment D12 than the other deficit irrigation treatments. Similar differences were also observed among L-ascorbic acid values under deficit irrigation conditions at flowering period. It can be concluded that entire irrigation deficiencies applied at flowering period of broccoli caused an increase in L-ascorbic acid content. Also, it can be pointed out that different deficit irrigations at early and late vegetative periods caused similar changes and differed from flowering period.

Results revealed increasing vitamin C contents with increased water stress. Increases in vitamin C contents of different plants with increasing water stress were also pointed out by other researchers (Başaran, 1979). The highest Vitamin C values obtained in our study are comparable with those of 89.0 mg/100g published earlier by Vallejo et al., (2002).

Data included in table 2 demonstrates the similar effect of induced water stress on α-tocopherol (vitamin E) content of broccoli. As in the case of Vitamin A and Vitamin C, the lowest (1.833 mg/100g) α-tocopherol (vitamin E) content was observed in treatment C, while the highest value (2.597 mg/100g) was determined in the plants grown under treatment D12 conditions. According to Dunn-Z multiple comparison test results, α-tocopherol (vitamin E) contents exhibited significant differences with deficit irrigation applications at different growth stages (p= 0.313). Vanderslice et al., (1990); carried out a research on brassica varieties and observed the vitamin E content of broccoli varied in the ranges of 0.22–0.68 mg/100g. Moreover (Kurilich et al., 1999) determined the vitamin E content of broccoli to vary between 0.48 and 4.93 mg/100g. Several researchers reported that plants exposed to drought usually increase endogenous a-tocopherol and carotenoid levels to cope with oxidative stress (Fryer, 1992; Eskling et al., 1997; Depka et al., 1998).

Table 2. Average Retinol (vitamin A), α-tocopherol (vitamin E) and L-ascorbic acid (vitamin C) values

Treatment	Retinol (mg/100 g) $\bar{X} \pm S_{\bar{X}}$	Median	L-ascorbic acid (mg/100 g) $\bar{X} \pm S_{\bar{X}}$	Median	α-tocopherol (mg/100 g) $\bar{X} \pm S_{\bar{X}}$	Median
C	0.1210 ± 0.00751 E	0.1210	77.21 ± 2.68 D	75.86	1.833 ± 0.215	1.866
D1	0.2603 ± 0.0251 AB	0.2620	79.28 ± 2.90 CD	79.88	2.126 ± 0.310	1.888
D2	0.2207 ± 0.0153 ABC	0.2210	79.66 ± 2.35 CD	80.54	1.939 ± 0.175	1.792
D3	0.2157 ± 0.0314 ABCD	0.2230	77.55 ± 0.432 D	77.98	2.080 ± 0.181	2.225
D4	0.1720 ± 0.0234 BCDE	0.1690	81.64 ± 0.296 BCD	81.34	2.082 ± 0.204	2.213
D5	0.1817 ± 0.0200 ABCDE	0.1840	78.61 ± 2.39 D	77.39	2.058 ± 0.147	2.197
D6	0.1680 ± 0.0156 BCDE	0.1660	78.93 ± 2.33 CD	78.32	1.976 ± 0.094	2.050
D7	0.1437 ± 0.00441 DE	0.1420	80.61 ± 2.03 CD	80.95	2.343 ± 0.256	2.585
D8	0.1483 ± 0.00470 CDE	0.1520	80.91 ± 3.02 BCD	82.66	2.301 ± 0.241	2.313
D9	0.1810 ± 0.0182 BCDE	0.1780	85.47 ± 1.80 ABCD	84.52	2.360 ± 0.196	2.375
D10	0.1580 ± 0.0107 BCDE	0.1590	88.36 ± 1.76 ABC	87.75	2.333 ± 0.206	2.336
D11	0.2613 ± 0.0416 AB	0.2640	98.72 ± 2.97 AB	97.36	2.438 ± 0.206	2.451
D12	0.3697 ± 0.0534 A	0.3700	102.21 ± 2.05 A	102.85	2.597 ± 0.137	2.590

Not: Differences among vitamin means indicated with different capital letters are significant at (P ≤ 0,05) level

MDS evaluations over experimental results revealed compliance with linear form and a linear relationship was determined between observa-tional distances and differences.

Multi Dimensional Scaling (MDS) analysis, performed to evaluate the relationships among irrigation water levels and relevant vitamins, revealed a stress coefficient of 0.00021 and R^2 of 99.2%. These values indicate that MDS method can readily be used to evaluate the relationships among irrigation water levels with regard to relevant parameters. Results of MDS analysis carried out to determine the relationships among deficit irrigations at different growth stages with regard to retinol (vitamin A), α-tocopherol (vitamin E) and L-ascorbic acid (vitamin C) were presented in Figure 1.

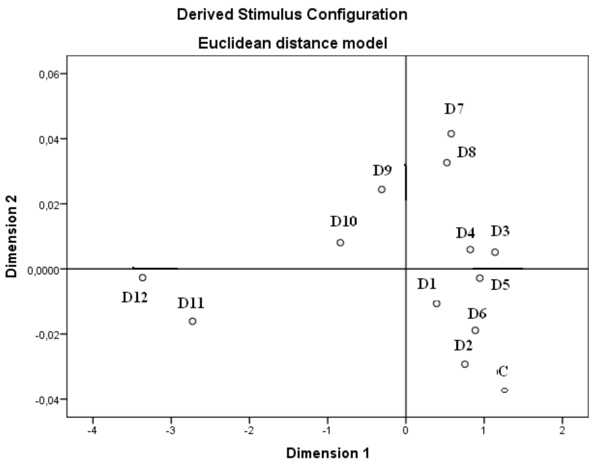

Figure 1. Relationships among irrigation water levels with regard to relevant vitamins

It is possible to mention about the variations in vitamin contents that they haven't presented differences when the water deficit was applied at early or late vegetative period when the deficit level was 20 and 40% (D1-D2-D5-D6). Results also revealed significant effects of 60 and 80% water deficit (D11-D12) at flowering period of broccoli with regard to vitamin contents. On the other hand, 60 and 80% deficit irrigations applied at each growth stage exhibited similarities with control treatment. Also, MDS analysis indicated that differences with regard to vitamins in 20 and 40% deficit irrigation application in all growth stages were considered to be similar (D1-D2-D5-D6-D9-D10).

In conclusion, water stress affecting entire physiological characteristics of broccoli also causes changes in vitamin content of the plant. Neverthless; researchers stated that not only harvest season can substantially affect antioxidant components e.g. ascorbic acid, carotenoids and chlorophylls, but also irrigation and cultivation period affect the phenolic content of broccoli florets, although the different components were inconsistent in their effects in different environmental conditions (Pek et al., 2013). Broccoli crop owing a significant place in human nutrition, recently has an increasing economical value for the regional growers. In addition the content of nitrogenous-sulfurous compounds increases the nutritive value of the crop which provides significant contribution to its economical value. Current research revealed that severe water deficit applied at flowering period could increase the content of vitamin A and vitamin C of broccoli.

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