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Changes in the Major Antioxidant Compounds of Red Cabbage Under Water Stress Applied at Different Vegetative Growth Periods

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ABSTRACT

Effective and efficient use of water resources has become an important issue in recent studies, where the impacts of climate change has become more apparent and alternative solutions are discussed. There, however, are limited studies that look at the impacts of water stress at different vegetative periods. For this reason, in this study, different levels of water treatment (0%, 30%, 70%) were applied to red cabbage at two stages of development (early and late vegetative) in a two-year field study. The effect of water stress on the major antioxidant compounds, as well as on yield and some morphological parameters were investigated.

According to the findings, the least yield loss (22%) occurred in the early vegetative period of the second-year trial where 70% irrigation water was applied, while the highest yield loss (56%) was obtained during the early vegetative period of the first-year trial where no irrigation was applied. Biochemical analyses revealed that the highest accumulation of flavonoids, 0.83 mg g^{-1}, and anthocyanins, 1.51 mg g^{-1}, occurred in the early vegetative period with the trial that received no irrigation treatment. The phenolic compound content was determined as 1.62 mg g^{-1}, and the antioxidant capacity was found to be 1.93 mg g^{-1} during the late vegetative period in the trials without irrigation treatment. These findings suggest that in regions with limited water resources, water conservation can be practiced during different vegetative periods in order to get higher biochemical benefits with a lower yield loss when cultivating red cabbage.

Keywords: Vegetative period, Antioxidant, Anthocyanin, Flavonoids, Phenolic compounds

1. Introduction

Food security is a major public concern, which has gotten worse with the increase of the population and pressure caused by environmental stress. Abiotic stress aggravated by the impacts of climate change has further influenced the yield and quality of crops (Haghighi et al. 2020) threatening food security. Approximately 40% of the world's available land is estimated to be affected by drought because of the climate change (Zhang et al. 2014). In summer months, particularly in arid and semi-arid regions, agricultural crops often face water shortages and erratic rainfall, leading to a significant loss of crops. The necessary amount of water required by these crops cannot be met, resulting in adverse effects on agricultural productivity (Kusvuran & Abak 2012). There is a need, therefore, for improvements in irrigation scheduling to maximize productivity under limited water conditions (Dhungel et al. 2023; Semiz et al. 2023).

Water shortage imposes several constraints on agricultural production unless precisely managed through conservation methods (Saha et al. 2021). In the future, there will be even greater pressure on freshwater resources with the additional need to provide food for future generations, in which the world population is predicted to increase by 65% (3.7 billion) by 2050 (Wallace 2000). In addition to the adverse effects of water shortages on crops, there is an increasing consumer demand for food that can be used as functional ingredients that promise to provide better well-being and health (Janabi et al. 2020). According to Siro et al. (2008), consumers are increasingly aware of the direct contribution of food on health.

Brassica species, which are among the fibrous and easily digestible vegetables, are widely consumed vegetables worldwide known for their nutritional benefits, of which vegetables such as cauliflower, broccoli, brussel sprouts and kale possess both antioxidant and anticarcinogenic properties (Cohen et al. 2000; Chu et al. 2002). Red cabbage, in particular, is a vegetable that offers a variety of beneficial effects on human health. It contains vitamins and minerals and is commonly consumed as a raw vegetable salad (Majkowska & Wierzbicka 2008). Red cabbage is also a valuable source of acylated anthocyanins that exhibit potent antioxidant activity. It can be utilized as a natural food colorant and may contribute to the prevention of diseases linked to oxidative damage (Wiczkowski et al. 2013).

Red cabbage also has been shown to prevent oxidative stress in the livers and brains of animals exposed to paraquat, as demonstrated in study by Igarashi et al. (2000). Additionally, red cabbage includes various bioactive substances like anthocyanins, flavanols and glucosinolates. These bioactive compounds are known to have a positive impact on human health (Podsedek 2007; Volden et al. 2008; Lobos et al. 2017).

Red cabbage is a cool-season vegetable that is grown in the summer season in semi-arid regions such as Turkey. The changes in climate patterns in the summer season require the more effective management of the water resources while maximizing the nutritional benefits of the crops. Looking at previous research, there are some studies that investigate the impact of water stress on the yield parameters of red cabbage (Hajiboland & Amirazad 2010; Beacham et al. 2017; Shinde et al. 2020; Kishor et al. 2023). Other studies have investigated the impacts of water stress on some of the biochemical properties of the plant (Şahin et al. 2018; Haghighi et al. 2020; Erken 2022).

There, however, is no known study that investigates the impact of water stress applied at different growth stages on the yield, quality and some biochemical properties of red cabbage. This study is a two-year field study that investigates the effect of water stress applied at different growth stages of red cabbage (*Brassica oleracea* L var. Rubra). The more specific objective of this paper is to observe the variation of yield, quality, and some biochemical compounds such as flavonoids, anthocyanins, and antioxidant activity, in red cabbage under water stress applied during different growth stages of the plant.

2. Material and Methods

2.1. Experiment site

In this study, field experiments were carried out both in the summer of 2021 and 2022, where crops were harvested at the end of each year. Experimental plots were set up at the Dardanelles Research Extension Station of Çanakkale Onsekiz Mart located in Dardanos, Çanakkale. The soil at the experiment site was clay-loam with a water holding capacity of 167.7 mm at 90 cm depth. The rainfall data (Table 1) throughout the growth period was taken from the meteorology station at approximately 10 km from the experiment site.

Table 1- Meteorological data for the experiment period of 2021, 2022 and long-term average (MGM 2023)

Months	***Average temperature (°C)***			***Rainfall (mm)***			***Evaporation (mm)***	
	2021	***2022***	***1940-2022***	***2021***	***2022***	***1940-2022***	***2021***	***2022***
August	28.3	26.2	25.1	0.0	111.6	11.3	479.2	491.9
September	23.1	22.0	21.1	8.9	1.2	24.1	359.7	400.5
October	18.1	17.6	16.2	75.9	0.6	55.2	206.0	301.3
November	15.8	14.8	12.2	26.7	33.2	83.2	156.2	276.8
December	12.2	11.5	8.4	121.0	43.2	103.2	211.1	209.2

The total amount of rainfall from August to December in 2021 was 232.5 mm, while in 2022, the total amount decreased to 189.8 mm (Table 1). During the second year, a significantly higher amount of rainfall occurred during the seedling period, while the total rainfall was lower in the other growing stages of red cabbage. This difference in rainfall amounts offered an important opportunity for comparison.

2.2. Experimental design

The plots were laid out in a randomized complete block design with three replications. Seven different levels of irrigation treatment were applied, of which the experiment layout is provided in Figure 1 for the entire trial. While one plot consisted of a control treatment, three plots were irrigated with 70%, 30% and 0% water in the early vegetative (EV) period and the remaining three plots received 70%, 30% and 0% water in the late vegetative (LV) period.

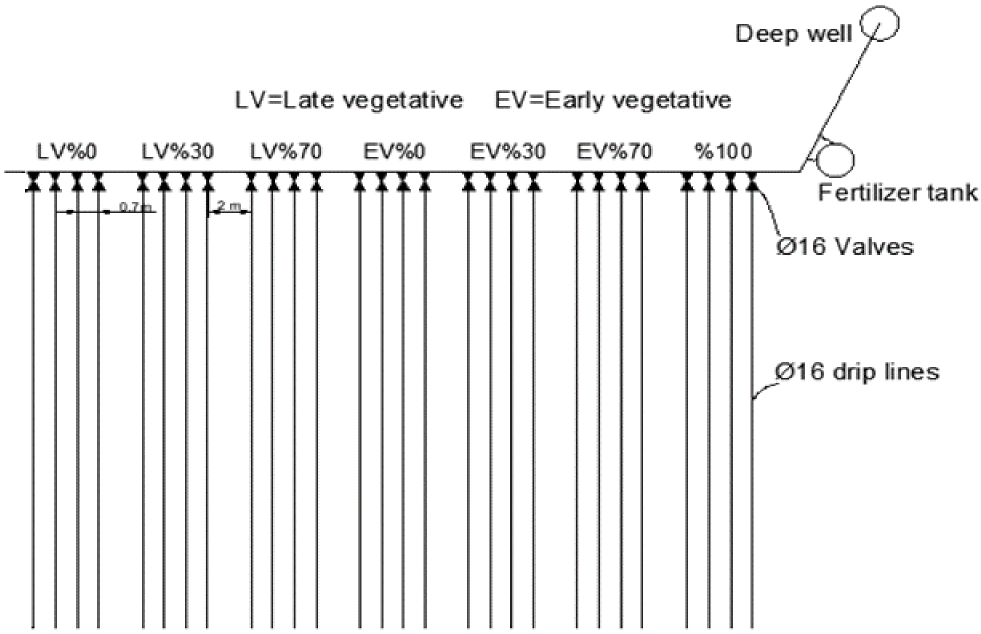

Figure 1- The experimental layout indicating the drip lines for all treatments

In the experiment, a total of 588 seedlings were planted in 7 plots, with 28 seedlings in each repeated application and 84 seedlings in each plot. Both the replications and the plots were kept 2 m apart and the seedlings were planted with 70 cm x 33 cm spacing. The red cabbage seedlings were first transplanted on August 7, 2021 and July 29, 2022. The EV period covered the dates between September 24, 2021 and December 22, 2021 in the first year and lasted from September 7, 2022 to December 13, 22 in the second year of the experiment. The LV period lasted from December 23, 2021 to November 23, 2021 in the first year and from December 14, 2022 to November 14, 2022 in the second year. All water treatments ended at the end date of the LV period. Crops were harvested on January 5 in 2022 and January 9 in 2023.

All experimental plots were applied with the same amount of fertilizer of 10 kg N, 5 kg P and 10 kg of K per decare (1 decare=1000 m^2). Fertilization was carried out three times; first at planting, next in 20 days and last in 15 days after the second application.

Each row was set up with a single drip line where the emitter had a normal discharge of 4L h^{-1} under the pressure of 1-1.5 atm. The irrigation water used in the experiment had an electrical conductivity (ECw) of 0.941 dS m^{-1}, which was measured with an EC59 meter (Martini Institute). This value was in the moderately tolerable range and had previously been used at the site for irrigation purposes.

All experimental plots were irrigated with 4-day intervals throughout the experiment. Following transplantation, each plot was irrigated equally for 20 days until the early vegetative period in order to allow for equal root development.

The irrigation amount was estimated using the following equation (Ertek & Kanber 2000):

$$I = A \times Epan \times Kcp \times P$$

Where: I, irrigation water amount (mm); A, the area of plot (m^2); Epan, the cumulative evaporation at irrigation intervals (mm); Kcp, the crop-pan coefficient; P, the percentage of wetted area (%)

2.3. Yield and quality parameters

Yield and quality parameters were measured from the plants harvested from the center of each treated plot. While a digital balance (±0.01 g) was used to weigh the plants, a digital clipper (±0.01 mm) was used to measure the quality parameters and a refractometer was used to determine the soluble solids. Following the measurement of the fresh plants, random samples from each plant were oven dried for 48 hours at 70 °C.

2.4. Determination of some biochemical properties

Total flavonoids, phenolic content, antioxidant capacity and the anthocyanin content of the red cabbage plants were estimated from fresh samples refrigerated at -18 °C after harvest. All analysis were carried out at the Laboratory of the Center for Plant and Herbal Products Research-Development located in Istanbul University using international standard (ISO, AOAC) methods.

2.5. Total flavonoids

The flavonoid concentrations were estimated using an aluminium based colorimetric assay as explained by Shraim et al. (2021). 100 µL of leaf extract was mixed with equal amounts of 1 M potassium acetate and 10% aluminium nitrate, as well as 4.4 mL of 96% ethanol, which were then incubated at dark room temperature for 40 minutes. The absorbance values were read at 415 nm using a UV-vis spectrophotometer (Thermo Aquamate). The total flavonoid content was expressed in "mg g^{-1} FW."

2.6. Total phenolic content

The high-throughput assay to assess the Folin-Ciocalteau reducing capacity as described by Magalhaes et al. (2010) was used to measure total phenolic content. 50 µL of gallic acid standard solution and 50 µL of Folin-Ciocalteau reagent diluted in water (1:5, v/v) were mixed. 100 µL of sodium hydroxide solution (0.35 M) was introduced into the mixture. Absorbance was measured at 760 nm at 1-minute intervals until it reached its maximum value (optimally achieved in 3 minutes). In order to assess the intrinsic absorption of the sample, the Folin-Ciocalteau reagent was replaced with 50 µL of 0.4 M acid solution and the reagent blank was prepared with 50 µL of water instead of the standard solution. The total phenolic content of the samples was quantified as GAE (gallic acid equivalents) in mg per g FW (fresh weight).

2.7. Antioxidant capacity

The antioxidant capacity of the plant samples was determined using two methods: the cubric reducing antioxidant capacity (CUPRAC) assay and the 1.1-diphenyl-2-picrylhydrazyl (DPPH) free radical scavenging activity assay.

The CUPRAC assay, developed by Apak et al. (2004) utilizes an electron-transfer method to assess the plant samples' ability to reduce cupric ions (Cu^{+2}). Using this assay, we incubated 40 µL of the plant extract with 0.01 M copper(II) chloride (1 mL), 7.5 × 10–3 neocuproine (1 mL), 1 M pH 7 ammonium acetate (1 mL), and distilled water (1060 µL). After incubating the mixture for 30 minutes at 20 °C, the final volume of the mixture was 4100 µL. The absorbance measured at 450 nm and the antioxidant activity of fresh weight of leaves was quantified using a standard calibration curve, which was expressed in mg trolox 100 g^{-1} FW.

The DPPH radical scavenging activity was evaluated following the method outlined by Brand-Williams et al. (1995) and Ak & Türker (2018). Methanol was used to dilute frozen plant samples until a concentration of 1 mg mL^{-1} were reached. Subsequently, the diluted sample (0.1 mL) was thoroughly blended with the DPPH solution (3.9 mL) at room temperature for 30 minutes to facilitate the reaction between the plant extracts and DPPH. Following the incubation, the absorbance of the samples was measured using a spectrophotometer at 515 nm. The absorbance measurement provided an indication of the level of DPPH radical scavenging activity exhibited by the plant extracts.

The equation used to estimate DPPH radical scavenging activity is provided below:

$$DPPH\ scavenging\ (\%) = \left[(Acontrol - Asample) / Acontrol \right] \times 100$$

In this equation, Asample stands for the absorbance of the sample once it reaches a plateau after leaving still for15 minutes. Acontrol stands for the absorbance of DPPH alone. The IC50 inhibition values, which showed the concentration of the compounds that inhibit 50% of the total DPPH radicals, were estimated. Lower IC50 inhibition values indicated higher antioxidant activity.

2.8. Anthocyanin content

The total anthocyanin content of the samples was estimated with the pH differential method (Giusti et al. 1998; Benvenuti et al. 2004). Following this method, two buffer systems were utilized for anthocyanin extraction (0.025 M potassium chloride (pH 1.0) and 0.4 M sodium acetate (pH 4.5)). Anthocyanin extraction was conducted for all four parallel samples obtained from each treatment plot. Each extraction was diffused with a pH 1.0 and a pH 4.5 buffer separately and incubated for 20 minutes in room temperature. This extraction process was replicated three times.

The absorbance measurements were taken at 520 nm and 700 nm using a UV-vis spectrophotometer. The absorbance values and the anthocyanin content were estimated using the following equations: [Please provide the relevant equations for absorbance and anthocyanin content as they were not provided in the initial statement.

$$A = (A520 - A700)\ pH1.0 - (A520 - A700)\ pH4.5$$

$$Monometric\ anthocyanin\ pigment \left(\frac{mg}{lt}\right) = (A \times MW \times DF \times 1000) / (\varepsilon \times 1)$$

In this equation, the symbol A represents the absorbance of the diluted sample, MW stands for the molecular weight, DF denotes the dilution factor, and ε is the molar absorptivity. The MW and ε values for the specific compound cyanidin-3-glucoside were 449.2 and 26,900, respectively.

2.9. Statistical analysis

Statistical analysis was made using the Duncan's Multiple Range Test. Differences were considered statistically significant at the probability level of 5% ($P<0.05$) which the SPSS statistical package software were used to analyse the data.

3. Results

3.1. Effects of water stress on yield

The amount of water used for irrigation and the corresponding fresh head weights for two consecutive years (2021 and 2022) based on the measurements of pan evaporation are provided in Table 2. The corresponding fresh weights of the water stressed red cabbage heads differed statistically significantly from control plants in both years.

Table 2- The amount of irrigation water applied and corresponding yield

Treatments	*Irrigation water (mm)*		*Fresh head weight (g/plant)*	
	2021	*2022*	*2021*	*2022*
$I_{1.0}$	433	495	$1514±118.7^{a}$	$1252.6±41.6^{a}$
$EV_{0.7}$	406	444	$818.8±41.6^{bc}$	$972.3±115.5^{ab}$
$EV_{0.3}$	370	376	$717.2±152.7^{bc}$	$708±184.6^{b}$
$EV_{0.0}$	343	325	$661.5±174.4^{c}$	$650.6±145.6^{b}$
$LV_{0.7}$	420	466	$975.9±57.4^{b}$	$923.8±226.9^{b}$
$LV_{0.3}$	413	439	$951.7±169.4^{b}$	$769.7±49.5^{b}$
$LV_{0.0}$	410	419	$825.1±181.7^{bc}$	$765.5±119.5^{b}$

*: Significant differences between treatments in both years are indicated by different letters ($P<0.05$).

According to the results, when water restriction was applied during different growth stages, the yield still decreased significantly in comparison to the fully irrigated ($I_{1.0}$-control treatment) plants. The highest yield, therefore, were obtained from the control plants with 1514 g plant^{-1} in 2021 and 1252.6 g plant^{-1} in 2022. The irrigation water applied during these periods were 433 mm in 2021 and 495 mm in 2022, respectively.

When the meteorological data for the first year of the experiment is examined (Table 1), it can be observed that the autumn season had above-average rainfall. The reason for achieving higher yields with less water usage in the first year could be attributed to the relatively abundant rainfall during the autumn season.

The plant quality indicators measured at the end of harvest are provided in Tables 3 and 4. According to the data, there was no significant difference in diameter development among different treatments in 2021 (Table 3), while this was not the situation in the following year (Table 4). The likely reason for this is that the rainfall was higher in 2021 when compared to 2022, resulting in a less pronounced impact of drought created by irrigation treatments on diameter development.

Table 3- Quality parameters of red cabbage in 2021

Treatments	***Diameter-x (cm)***	***Diameter-y (cm)***	***Height (cm)***	***Circumference (cm)***	***Leaf Number***	***Total Soluble Solid (%)***	***Dry weight Percent (%)***
$\mathbf{I_{1.0}}$	12.83^{ns}	12.59^{ns}	16.51^{a}	45.70^{a}	29.40^{ns}	8.77^{ns}	9.26^{bc}
$\mathbf{EV_{0.7}}$	10.52^{ns}	10.04^{ns}	13.85^{b}	36.68^{bc}	25.00^{ns}	9.25^{ns}	8.25^{bc}
$\mathbf{EV_{0.3}}$	11.58^{ns}	11.00^{ns}	14.50^{ab}	42.06^{ab}	26.56^{ns}	9.43^{ns}	9.79^{b}
$\mathbf{EV_{0.0}}$	9.25^{ns}	8.87^{ns}	12.28^{b}	34.84^{c}	25.67^{ns}	9.45^{ns}	7.93^{c}
$\mathbf{LV_{0.7}}$	10.86^{ns}	10.73^{ns}	14.30^{ab}	39.01^{bc}	27.53^{ns}	8.93^{ns}	11.41^{a}
$\mathbf{LV_{0.3}}$	10.78^{ns}	10.50^{ns}	14.54^{ab}	38.89^{bc}	27.13^{ns}	10.05^{ns}	9.86^{b}
$\mathbf{LV_{0.0}}$	10.40^{ns}	10.18^{ns}	13.79^{b}	37.19^{bc}	26.20^{ns}	9.05^{ns}	8.90^{bc}

*: Significant differences between treatments are indicated by different letters ($P<0.05$).

Table 4- Quality parameters of red cabbage in 2022

Treatments	*Diameter-x (cm)*	*Diameter-y (cm)*	*Height (cm)*	*Circumference (cm)*	*Leaf Number*	*Total Soluble solid (%)*	*Dry weight percent (%)*
I$_{1.0}$	14.02^{a}	13.96^{a}	15.33^{ns}	41.96^{a}	31.44^{ns}	8.77^{ns}	9.26^{bc}
EV$_{0.7}$	11.92^{ab}	11.31^{ab}	14.30^{ns}	38.67^{ab}	32.67^{ns}	8.17^{ns}	8.37^{c}
EV$_{0.3}$	10.67^{bc}	10.48^{bc}	13.33^{ns}	34.46^{bc}	33.56^{ns}	9.67^{ns}	9.40^{bc}
EV$_{0.0}$	8.10^{c}	8.05^{c}	12.19^{ns}	33.35^{c}	33.33^{ns}	9.63^{ns}	9.43^{bc}
LV$_{0.7}$	12.08^{ab}	11.85^{ab}	13.33^{ns}	37.91^{bc}	32.56^{ns}	9.90^{ns}	9.76^{bc}
LV$_{0.3}$	11.37^{ab}	11.39^{ab}	13.00^{ns}	35.95^{bc}	32.89^{ns}	9.85^{ns}	11.07^{b}
LV$_{0.0}$	11.61^{ab}	10.97^{bc}	12.91^{ns}	35.77^{bc}	31.22^{ns}	10.17^{ns}	13.82^{a}

*: Significant differences between treatments are indicated by different letters ($P<0.05$)

In 2022, however, lower amount of rainfall during the plant growth period indicated the effects of water stress on diameter development highlighting a significant difference in plant diameter growth. Water restriction applied during the EV period had a more pronounced negative impact on plant growth compared to the LV period. This indicates that implementing water restriction during the late vegetative period is more suitable for plant development, because in the EV period, compared to the LV period, the diameters were the smallest with 8.10 cm in x, and 8.05 cm in y directions. Compensating full water demand of the crops in the control treatment ensured that the highest values in diameter were obtained as 14.02 cm in x and 13.96 cm in y directions.

Significant differences were observed in height values in 2021, while no significant difference was observed in 2022. In both years, plant height was the highest in control treatment and the lowest values were obtained in the treatment that water restrictions applied in EV period. Limited irrigation also significantly affected the circumference of red cabbage in all phases in 2021. In 2022, plant circumference was higher in the control treatment than the EV$_{0.0}$ and all LV treatments. Differences in irrigation had no significant effect on the leaf number and total soluble solids in red cabbage in both years.

With respect to dry weight values, in 2021, the highest value was achieved with 11.42% in the LV0.7 stage and in 2022, the highest value was obtained with 13.82% in the LV$_{0.0}$ period. The situation can be explained as follows; in red cabbage, when the plant's water demand is fully met until the end of the EV period, there is a significant increase in fruit weight. In the LV period, however, there is no statistically significant difference in fruit weight increase in both years, as observed in Table 2.

3.2. Effects of water stress on major antioxidant compounds

The amount of flavonoids, phenolic compounds, antioxidant capacity using the CUPRAC and DPPH methods, and anthocyanin levels in red cabbages exposed to seasonal water stress during the two-year experiment are given in Table 5.

Table 5- Biochemical changes under different irrigation treatments

Treatments	*Flavonoid (mg g^{-1})*		*Phenolic (mg g^{-1})*		*CUPRAC (mg g^{-1})*		*DPPH (mg g^{-1})*		*Anthocyanin (mg g^{-1})*	
	2021	*2022*	*2021*	*2022*	*2021*	*2022*	*2021*	*2022*	*2021*	*2022*
I$_{1.0}$(Control)	0.25^{d}	0.47^{bc}	0.73^{c}	0.84^{c}	1.08^{c}	1.38^{b}	2.05^{a}	3.01^{a}	0.6^{d}	0.5^{d}
EV$_{0.7}$	0.34^{ab}	0.53^{bc}	1.14^{b}	1.03^{bc}	1.47^{b}	1.38^{b}	1.94^{a}	2.86^{ab}	0.96^{ab}	1.05^{c}
EV$_{0.3}$	0.35^{ab}	0.59^{b}	1.14^{b}	1.09^{bc}	1.49^{ab}	1.54^{b}	1.97^{a}	2.64^{b}	0.79^{c}	1.21^{bc}
EV$_{0.0}$	*0.36^{a}*	*0.83^{a}*	1.03^{b}	1.11^{b}	1.47^{b}	1.54^{b}	1.63^{b}	2.73^{ab}	*1.04^{a}*	*1.51^{a}*
LV$_{0.7}$	0.29^{c}	0.38^{c}	1.2^{b}	1.19^{bc}	1.4^{b}	1.35^{b}	1.69^{b}	2.62^{b}	0.88^{bc}	1.15^{bc}
LV$_{0.3}$	0.32^{bc}	0.49^{bc}	1.42^{a}	1.25^{b}	1.61^{ab}	1.55^{b}	1.52^{b}	2.56^{b}	0.88^{bc}	1.33^{ab}
LV$_{0.0}$	0.28^{cd}	0.52^{bc}	*1.54^{a}*	*1.62^{a}*	*1.7^{a}*	*1.93^{a}*	*1.45^{b}*	*2.57^{b}*	0.92^{bc}	1.45^{a}

*: Significant differences between treatments in both years are indicated by different letters ($P<0.05$); **: Numbers in bold represent the highest value within each column

When considering the flavonoid levels in red cabbage plants, those exposed to EV water stress showed significant differences compared to both the control group and the experimental groups subjected to LV water stress in both years. Overall, it was found that water stress experienced by red cabbage during early development leads to increased flavonoid levels.

In the 2022 early vegetative period, the highest flavonoid content of 0.83 mg g^{-1} Fresh Weight (FW) was recorded in the treatment (EV$_{0.0}$) where no irrigation was applied. It is worth noting, however, that this treatment also resulted in a considerable decrease in fruit weight of 650 grams, indicating an approximate 50% yield loss. Thus, while water stress enhanced flavonoid accumulation, it also caused economically significant yield reductions. Addressing this issue, implementing a 30% water saving irrigation practice during the EV period (EV$_{0.7}$) resulted in an average fruit weight of 972 grams corresponding to a 22% decrease in yield, enabling producers to achieve an economically viable, also producing a high flavonoid content of 0.53 mg/g, beneficial for human health. Moreover, applying a 70% water saving treatment ($EV_{0.3}$) during the EV period in red cabbage led to higher flavonoid content of 0.59 mg g^{-1} compared to other treatments. This treatment also showed an average yield reduction of 43%, resulting in a yield of 708 grams, which remains statistically and economically viable.

Looking at the phenolic compound levels, a variation between 0.73 and 1.54 mg g^{-1} in 2021 and 0.84 and 1.62 mg g^{-1} in 2022 was observed. The lowest value in terms of phenolic compounds was obtained in the control treatment where the plant water needs were fully met. It was determined that the highest accumulation of phenolic compounds occurred in red cabbage with the water restrictions applied during LV period. The highest values were obtained during the LV period, with 1.42 mg g^{-1} for the treatment with 30% fulfillment of the plant's water needs ($LV_{0.3}$) and 1.54 mg g^{-1} for the treatment where no water was provided ($LV_{0.0}$). In the subsequent year, these values were obtained as 1.25 for the $LV_{0.3}$ treatment and 1.62 mg g^{-1} for the $LV_{0.0}$ treatment.

The antioxidant levels in the experimented red cabbage plants (Table 5) were also determined using two methods, namely the CUPRAC and DPPH methods. The highest antioxidant level, as determined by the CUPRAC method, was found in the $LV_{0.0}$ experimental group in both years. Similarly, the DPPH method showed lower radical scavenging activity in the $LV_{0.0}$ treatment, indicating higher antioxidant content in the water stressed plants in both years.

The study results also showed variations in anthocyanin levels among experimental groups subjected to different levels of water stress during different growth stages. The highest anthocyanin synthesis of 1.04 mg g^{-1} and 1.51 mg g^{-1} were obtained from the $EV_{0.0}$ experimental group in the first year (2021) and second year (2022), respectively. The lowest anthocyanin amount was obtained from the control plants in both years.

4. Discussion

Results showed that there is slight difference between the yield obtained from plants applied with water stress in the EV and LV periods. Head weights were slightly higher in the plants applied with restricted water in the LV period compared to the EV period. Similarly, a review carried out by Bute et al. (2021) have stated that it is critical to keep red cabbage irrigated when the seedlings have 6-7 leaves and when head formation starts. This indicates that if water restriction is to be applied, it would be more suitable to impose it during the late vegetative period in terms of yield.

Furthermore, the parameters such as head diameter in the x and y directions (cm), circumference (cm), and dry weight (%) indicated that plant development was also negatively affected as the amount of water decreased at different growth phases. Both the yield and quality parameters in the water stressed red cabbage indicate that full irrigation should be applied until the end of the EV period if the objective is to get marketable yield. Alternatively, if marketable yield is not the concern and if water conservation is required, 30% of the plant's water demand could be restricted in the LV period.

Significant increments in flavonoid levels were observed in treatments with water stress during the early vegetative period during both years of the experiment. In comparison to previous studies, Lin et al. (2008) identified the amount of flavonoids in red cabbage between 0.6-2.1 g $100g^{-1}$. These results showed similar outcomes with the control plants of the present study. In another study carried out on tomatoes (Kumar et al. 2015), drought stress increased flavonoid levels statistically significantly although a reduction in yield occurred. This reduction in yield, however, was lowest when water stress was implemented in the vegetative period, rather than the flowering or fruiting periods.

In a study carried out to understand flavonoid response of Mediterranean species, Laoué et al. (2022) have stated that the accumulation of these defensive chemicals is highest in constrained environments due to drought, high temperatures and UV radiations. This mechanism would explain the higher flavonoid levels accumulated in the water stressed EV period of this study, which had the highest temperatures throughout the experiment.

The phenolic compounds also increased statistically significantly, particularly when water stress occurred during the LV period in this research. Comparing to previous studies, while Erken (2022) found an increase in the phenolic content of red cabbage subjected to long-term water-stress, both Shawon et al. (2020) and Šola et al. (2021) reported that the polyphenol content did not differ significantly between the control and drought stressed Chinese cabbage plants. While Shawon et al. (2020) applied short-term drought stress, Šola et al. (2021) implemented longer-term drought stress. The results altogether indicate that polyphenol induction in drought stressed cabbage may occur when stress is applied at the late vegetative period. This result, however, may also be inconclusive due to differences in the species in the abovementioned studies.

Further results of the study showed that the lowest antioxidant content obtained using both the CUPRAC and DPPH methods was in the control plants where the red cabbage plants were fully irrigated. According to Valifard et al. (2017), plants synthesize and accumulate natural antioxidants in response to abiotic stress. The greater antioxidant capacity in the water stressed red cabbage is an indication of its higher ability to stabilize free radicals (Reyes et al. 2017).

Similar to our results, in a two-year study carried out by Hegazi & El-Shraiy (2017), the antioxidant enzyme activity in red cabbage increased statistically significantly under salt stress. Previous studies (Jafari et al. 2019; Erken 2022) have also found that long term water stress influences the biochemical properties of red cabbage significantly.

The results of this study further suggest that, in order to get the highest antioxidant benefits from red cabbage, water restriction can be applied at the LV phase as the highest antioxidant amount was obtained from the $LV_{0.0}$ treatment. These results are similar to the accumulation of phenolic compounds, which increased in red cabbage as water restriction approached harvest time.

The anthocyanin values obtained from the cabbage in this experiment ranged from 0.5 to 1.51 mg g^{-1}. Mazza & Minati (1993) reported that the anthocyanin content for red cabbage lie within the wide range from 25 to 495 mg 100 g^{-1} FW (0.25 to 4.95 mg g^{-1} FW). Ahmadiani et al. (2014) found that the total anthocyanin content of seven red cabbage cultivars at different harvest times ranged from 109 to 170 mg Cy3G 100 g^{-1} FW when harvested in the 13th week, and from 104 to 188 mg Cy3G 100 g^{-1} 1FW when harvested at the 21st week. The amount of anthocyanin decreased in some red cabbage cultivars, while it decreased in others as the harvest time increased.

Similar to the flavonoids, the results of this study showed that the highest accumulation of anthocyanin was measured in red cabbages exposed to water stress in the EV stage. In a research carried out by Erken (2022) the anthocyanin levels increased statistically significantly (from 30.72 to 51.27 mg Cy3G 100g^{-1} FW) with water stress in red cabbage. Hegazi & El-Shraiy (2017) also found increased anthocyanin levels in salt stressed red cabbage.

While there are no known studies that specifically investigate the impacts of water stress at different vegetative phases, this study has shown that the increase in the anthocyanin content of the plants is higher when stress is applied during the late vegetative phase.

5. Conclusions

The findings of this study revealed that yield loss was least with the 30% water saving practice applied in the late vegetative phase (LV0.7). Results also showed that implementing a 30% water saving practice (EV0.7) during the early vegetative period lead to an elevation in flavonoid and phenolic compound levels. The antioxidant and anthocyanin amount in the water stressed red cabbage, on the other hand, were highest in the LV0.0 treatments. These findings could assist irrigation management strategies regarding red cabbage cultivation for different purposes.

Conflicts of interest

The authors declare that there is no conflict of interest to disclose.

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