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IMPACT OF DIFFERENT WATER SUPPLY LEVELS ON YIELD AND BIOCHEMICAL INGREDIENTS IN BROCCOLI

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Abstract

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This research was conducted to determine the impacts of different water supply levels on the yield and biochemical ingredients of the broccoli plant during the 2008 and 2009 growing seasons under the environmental conditions of Canakkale province, Turkey. The Maraton broccoli (*Brassica oleracea* L. var. *Italica*) species was grown in 10 lt pots with five different irrigation levels (80%, 60%, 40%, 20%), including the control treatment (100%), where the total quantity of moisture deficiency was replenished with each irrigation application.

The results show there was a decline in yield parallel to the reduction in amount of irrigation water. During the first and second years of the research, the respective yields obtained were between 667.84 - 101.68 g plant^{-1} and 775.87 - 109.29 g plant^{-1}. Data on the total leaf surface area, electrolytic conductivity of the tissues, and amount of sulfur and proline were also determined and used to estimate the water-yield, irrigation water-proline and irrigation water-sulfur interactions.

Key words: Broccoli, water stress, sulfur, proline, glucosinolate

Introductıon

Biotic and abiotic stress conditions such as high temperature, drought, salinity and chemical toxicity have been a threat to agriculture and agricultural fields in many parts of the world (Wang, 2004; Vinocur and Altman, 2005; Berenguer et al., 2009).

There is a need to limit the use of water resources in arid and semi-arid climates, particularly due to the threat of climate change. Decreased water reserves due to global warming accompanied by irregular seasonal and annual precipitations have increased the importance of irrigation scheduling, including deficit irrigation programs (Anonymous, 2011a).

One of the most important stress factors that influences plant growth is stress occurring because of limited water application (Steele et al., 1997; Wanjura et al., 1990; Imtiyaz et al., 2000; Kaçar et al., 2006).

Broccoli is a valuable crop for human health owing to its nutritional values (Krauss et al., 1996). Furthermore, it is known that consumption of broccoli may reduce some types of cancer (Cohen et al., 2000; Spitz et al., 2000; Joseph et al., 2004; Sarıkamış et al., 2005; Moreno et al., 2006; Berenguer et al., 2009). Some studies in recent literature show that the anti-carcinogenic properties of broccoli result from glucosinolate production synthesized in broccoli florets (Sarıkamış et al., 2005).

The glucosinolates collected by plants under stress conditions are amino acid derived chemicals that can be found in all species of the Caparales order. Similar to other secondary compounds, glucosinolates show variation between the different tissues (roots, leaves, generative organs, seeds, etc.) of each plant, influenced by their development period and environmental factors, although the genetic structure of the product is the most determining factor (Rosa and Rodrigues, 2001; Martinez-Ballesta etal., 2004). It is further known that the eco-physiological elements of water and nutrition affect phytochemicals (Rosa and Rodrigues, 2001). Glucosinolate precursors, which are secondary metabolites appearing un-

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der low turgor pressure in tissues facing drought stress, are thought to be produced for future use (Larkin, 1976).

Studies carried out on the Brassica species show that the glucosinolate concentrations increase with drought. Moreover, glucosinolate concentrations that decrease or do not change under low drought conditions show the degree of drought is a determinative factor. These contradictory results support the theory that climatic adaptation of broccoli against water scarcity is also influenced by the genotype and its development periods. It has been further determined by the same researchers that water stress during the vegetative period also influences the glucosinolate concentrations in seeds well (Jensen et al., 1996).

This research was conducted in order to determine the changes that occur during the synthesis of some secondary metabolites in the broccoli plant under different water stress levels, and to investigate their relationship to yield.

Materials and Method

This research was carried out at the Experimental and Research Farm of Çanakkale Onsekiz Mart University during the fall of 2008 and 2009 in 10-liter pots under controlled precipitation. The research site is located between 40° 06′ latitude north and 26° 24′ longitude east.

Physical and chemical characteristics of the pot soil mixtures used during the experimental years are presented in Tables 1 and 2.

Although the table values reveal rather low organic material content, the soil appears to be suitable for growing broccoli. An appropriate fertilization program for broccoli was prepared based on chemical analysis results and fertilizers were applied to each pot separately.

Irrigation water with an electrical conductivity range of 0.17 - 0.33 dS m^{-1} and pH range of 7.0 - 7.6 was used during the experiments.

According to the long-term average of the nearest Çanakkale Meteorological Station, the annual temperature at the research site was 14.8°C. The lowest average monthly temperature (6.2°C) was observed in January while the highest average value (24.6°C) was determined in August. Average annual precipitation of the experimental site was reported as 608.9 mm (Karagöz, 2001).

Meteorological parameters for both research years during the growth season are presented in Table 3 (Anonymous, 2011).

Maraton broccoli cv. was used as the plant material of the research. The variety has a 90-day growth period from seedling to harvest. It has a dome-like shape with dark green color and is a high-yield edible variety especially in demand by exporters. The broccoli used in the pot experiments is a variety particularly recommended for field farming (Anonymous, 2010). 5 different irrigation levels (100%, 80%, 60%, 40%, 20%) were applied to the plants during the growth season. Control treatment was 100% replenishment of deficit water calculated by weighing the pots. The water amounts applied to the rest of the treatments were calculated based on the fully irrigated control treatment and were determined as 80%, 60%, 40% and 20% of the water applied to the control. Experiments were carried out in randomized plots following a factorial experimental design with 5 replications. The statistical experimental model is 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)
$\alpha\beta$ = Effect of water deficit x growth stage interaction
ε = Error term

Firstly, the water holding capacity of the pot soils with pre-known weights was determined. The pots were saturated and left for seepage under gravity for 24 hours then the pots were weighed. The weight of the pots filled with the mixture

Table 1
Physical characteristics of pot soils

Year	EC dS. m^{-1}	pH	Clay	Sand	Silt	Texture	Gravimetric moisture%	Lime %
2008	0.74	7.5	19.1	59.5	21.4	Sandy-Loam	6.53	8.6
2009	0.68	7.3	18.7	58.2	20.8	Sandy-Loam	6.25	8.2

Table 2
Chemical characteristics of pot soils

Year	Organic material %	P_2O_5 (ppm)	K_2O (ppm)	Mg (ppm)	Ca (ppm)	Na (ppm)	Fe (ppm)	Zn (ppm)	Cu (ppm)	Mn (ppm)	B (ppm)	Mo (ppm)
2008	1.77	268.02	654.87	758.95	5696.59	258.55	1.892	1.048	0.097	11.879	0.077	0.001
2009	1.75	254.63	671.83	787.41	5539.69	251.25	1.825	1.102	0.088	11.769	0.085	0.001

was accepted as the weight of the pot at full capacity. This was taken as the weight of the 100% (control) irrigation treatment. Following the start of experiments, pots were weighed every 3 days to measure both evaporation from the pots and the water used by the plants. Weight loss was taken as the amount of irrigation water to be applied.

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

In order to provide full seedling setting, a total of 7.20 and 8.80 lt irrigation water was applied equally to each pot in the first and second year respectively of the experiments before initiation of the experimental treatments. Application of the deficit irrigation programs started just after the full setting and continued until harvesting of the last plant.

Plants were harvested when the primary shoots reached their maximum weight (just before the blossoming of flower buds). Then, the yield (g plant^{-1}), leaf area (cm^2) (CL-202 Leaf Area Meter), tissue electrical conductivity (Fan and Sokorai, 2005), proline accumulation (Bates et al., 1973) and glucosinolate amounts (Krumbein et al., 2005) were determined. The wet-ashing method was used to determine sulfur content (%) of the broccoli in different treatments and the element amounts were read from an ICP device (Ryan et al., 2001).

ANOVA and Duncan tests were carried out using Minitab 13 statistical software for evaluation of yield, leaf area, and tissue electrical conductivity data. The relationships between irrigation water-yield, water-proline, water-glucosinolate and water-sulfur were tested by regression analysis and confidence tests.

Results and Discussion

During the period of the experiment, 51.34 lt and 60.60 lt of irrigation water per pot were applied to the 100% control treatment in 2008 and 2009, respectively. The amount of irrigation water applied to the experimental treatments with allowed water deficit is given in Table 4.

The yield values obtained from the primary and secondary shoots of all treatments during the research years and results of variance analysis on yield data are presented in Table 5. Significant decreases (95%) were observed in yield values with decreasing amounts of irrigation water in both research years. Moreover, each treatment with a different deficit level was statistically placed in a different Duncan group. Results revealed that decreasing irrigation levels caused significant yield losses in the broccoli.

Statistical evaluations in terms of the research years are presented in Table 4 with lower-case letters. A significant difference was determined for the treatment with 80% water supply or 20% irrigation deficit. While the total yield was 473.18

Table 4
Amount of irrigation water applied to pots (l)

Treatment	Years	
	2008	2009
100%	51.34	60.6
80%	41.07	48.48
60%	30.8	36.36
40%	20.54	24.24
20%	10.27	12.12

Table 5
Total head and side branch weights

Treatment	2008 yield per plant, g	2009 yield per plant, g
100%	667.84 ± 3.95 Ab	775.87 ± 4.28 Aa
80%	473.18 ± 11.6 Bb	549.54 ± 7.21 Ba
60%	394.69 ± 7.18 Cb	457.1 ± 16.90 Ca
40%	195.69 ± 5.49 Db	296.9 ± 10.90 Da
20%	101.68 ± 12.5 Ea	10929 ± 3.80 Ea

* Difference between means indicated by capital letters is significant ($P \leq 0,05$).
** Differences between year averages indicated by lower-case letters in same water deficit are significant ($P \leq 0.05$).

Table 3
Meteorological parameters during growth season 2008 and 2009

Month	Temperature, °C		Average relative humidity, %		Average wind speed*		Solar radiation, h		Amount of evaporation** mm/day		Precipitation, mm	
	2008	2009	2008	2009	2009	2008	2008	2009	2008	2009	2008	2009
August	26.2	25.3	60.6	57.3	57.3	4.6	10.4	11	10.6	10	34.1	-
September	20.6	20.4	68.3	67.9	67.9	3.7	7.3	7	6	5.5	32.2	39.8
October	16.5	17.7	74.8	75.8	75.8	3.7	6.5	5.7	3.9	3.5	55.5	63.6
November	13.1	12.5	78.4	79.4	79.4	3.9	3.8	5	2.2	1.9	43.2	58.8
December	8.7	11.2	77	80.3	80.3	3.7	2.1	1.4	-	-	58.2	176.7

g in 2008, the value was 549.54 g in 2009. Similar findings were also observed for the treatments consisting of 40% and 60% water application but no statistically proved difference was observed between the years for the most stressed treatment with a 20% deficit level.

Plant leaf area and tissue electrical conductivity were measured at the end of the growing period in both research years. The leaf area and tissue electrical conductivity with regard to year and water deficit level are presented in Table 6. A decrease was observed in plant leaf area with increasing water deficit in both research years. Statistical analysis revealed that each water deficit level formed a different Duncan group. The difference between years was found to be significant with regard to leaf area. The highest leaf area values were observed in treatments for 2009.

The amount of irrigation water applied to all treatments and the yield of each treatment were compared and the relationships between these two parameters are presented in Figure 1. The highest amount of irrigation water was applied to the full water supply (100%) treatment and the lowest was applied to the treatment with 20% water application, which underwent the most severe water stress during the experiment. Various research carried out in Turkey to determine seasonal plant water consumption and irrigation water requirements shows that a linear relationship exists between yield and amount of irrigation water (Yildirim et al., 1994; Gençoğlan and Yazar, 1999; Çakir, 1999; Kadayifçi and Yildirim, 2000; Ertek and Kanber, 2000; **Oktem et al., 2003;** Çakir, 2004; Şimşek et al., 2005; Şimşek and Gerçek, 2005; Şehirali et al., 2005; Çakir and Çebi, 2010a).

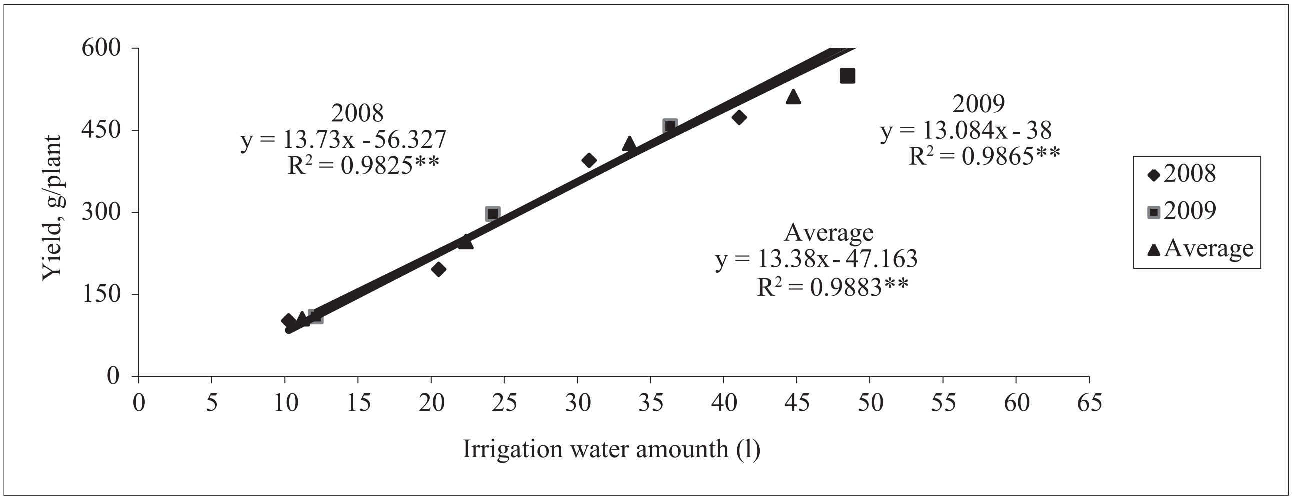
Water-yield relationships for broccoli under Çanakkale area conditions were investigated by using data from the obtained yield and the applied irrigation water values for all treatments in both research years. The relationships are presented in Figure 1 for each experimental year and averaged over two years.

Statistical significance tests carried out using R^2 and r-values for the studied years revealed p values to be between

Table 6
Leaf area (cm^2) and tissue electrical conductivities (%) by year

Treatment	Leaf area, cm^2		Tissue electrical conductivities	
	2008	2009	2008	2009
100%	6424.9 ± 7.59 Ab	7075.60 ± 16.80 Aa	2.23 ± 0.04 Eb	1.87 ± 0.04 Ca
80%	6059.0 ± 8.66 Bb	6750.30 ± 9.60 Ba	2.74 ± 0.05 Db	2.15 ± 0.01 Ca
60%	5421.90 ± 3.99 Cb	6113.20 ± 9.22 Ca	3.58 ± 0.08 Ca	2.07 ± 0.06 Cb
40%	4825.50 ± 3.99 Db	5255.90 ± 28.90 Da	6.70 ± 0.03 Ba	4.49 ± 0.14 Bb
20%	3429.40 ± 3.67 Eb	3903.80 ± 43.30 Ea	8.30 ± 0.04 Aa	5.37 ± 0.05 Ab

* Difference between means indicated by capital letters is significant ($P \leq 0,05$).
** Differences between year averages indicated by lower-case letters in same water deficit are significant ($P \leq 0.05$).



0.000984 and 0.00067, while p values were found to be 0.000541 when the two years were evaluated together. It was observed that the p values obtained for each year and two-year average could be used with 95% confidence. Proline accumulation and sulfur content of the broccoli leaves in each research year are given in Table 7.

The highest proline accumulation during the two study years was obtained from the treatment including application of 20% water applied to the control treatment. The highest value (53.38 µmol/g) was observed under the most severe stress conditions of the mentioned treatment during the second experimental year (2009), while the lowest value (11.44 µmol/g) was determined under the most favorable conditions of the control treatment during the same experimental year. Research has indicated a correlation between decreasing proteins and increasing amino acids with water stress (Gorbanli et al., 1998). The distinctive effects of deficit soil moisture on leaf proline accumulation have also been reported researchers (Hsiao et al., 1973; Yoshiba et al., 1997; Ain-Lhout et al., 2001). An increase in proline accumulation in broccoli leaves due to water deficit and consequent water stress is reported to play a significant role in adaptation to ambient conditions (Sivaramakrishnan et al., 1988; Yoshiba et al., 1997; Heuer and Nadler, 1998; Sarker et al., 2005, Handa et al., 1983).

Table 7
Proline (µmol/g) and sulphur contents (%) of broccoli leaves

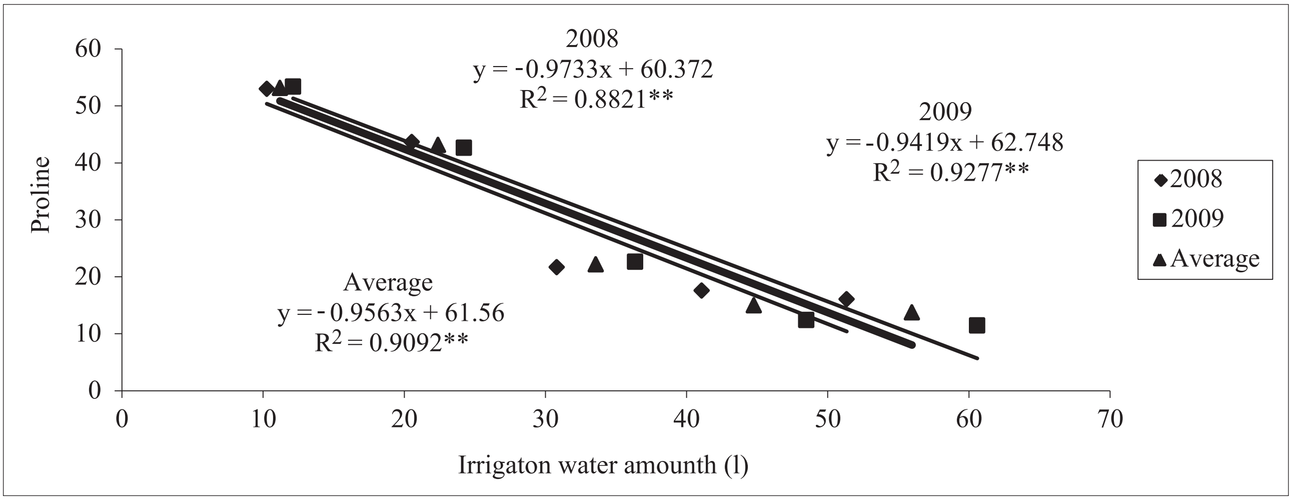
Treatment	Proline		Sulfur	
	2008	2009	2008	2009
100%	16.1	11.4	0.87	0.95
80%	17.6	12.4	0.9	0.93
60%	21.7	22.6	1.05	1.11
40%	43.7	42.7	1.15	1.34
20%	53	53.4	1.28	1.45

Ashraf (2004), reported that proline was commonly observed in taller plants and accumulation was higher than other amino acids in plants under salt stress (Wyn Jones, 1981; Ashraf, 1993; Ashraf, 1994a; Ali et al., 1999; Abraham et al., 2003). Proline accumulation increase due to water stress is as high as that which appears due to salt stress (Yamaya and Matsumoto, 1989). Therefore, proline accumulation is not considered a medium level specific response (Ashraf, 1994b). Proline is highly active in the osmotic regulation of available nitrogen accumulation (Ashraf, 1994a).

Results of the regression analysis between the amount of applied irrigation water and proline accumulation in broccoli leaves are plotted in Figure 2. Increasing proline accumulation was observed with increasing water deficit. This increase has mostly been reported as a defense mechanism of the plant against water stress (Sivaramakrishnan et al., 1988; Raggi, 1994; Ruilian and Gang, 1997; Upreti et al., 1998; Sarker et al., 2005; Handa et al., 1983; Yoshiba et al., 1997; Heuer and Nadler, 1998; Gorbanli et al., 1998; Bandurska, 2004; Ahire et al., 2005; Sankar et al., 2007; Hamidou et al., 2007).

Statistical significance tests carried out using R^2 and r values showed that close regression relationships exist between proline content and applied irrigation water. Values of p for 2008, 2009 and the two-year average were determined as 0.017831, 0. 00843 and 0.011945, respectively. The p values indicated that the relationships between proline and irrigation water were statistically valid for both years.

The relationships between the amount of applied irrigation water and the glucosinolate content of broccoli are presented

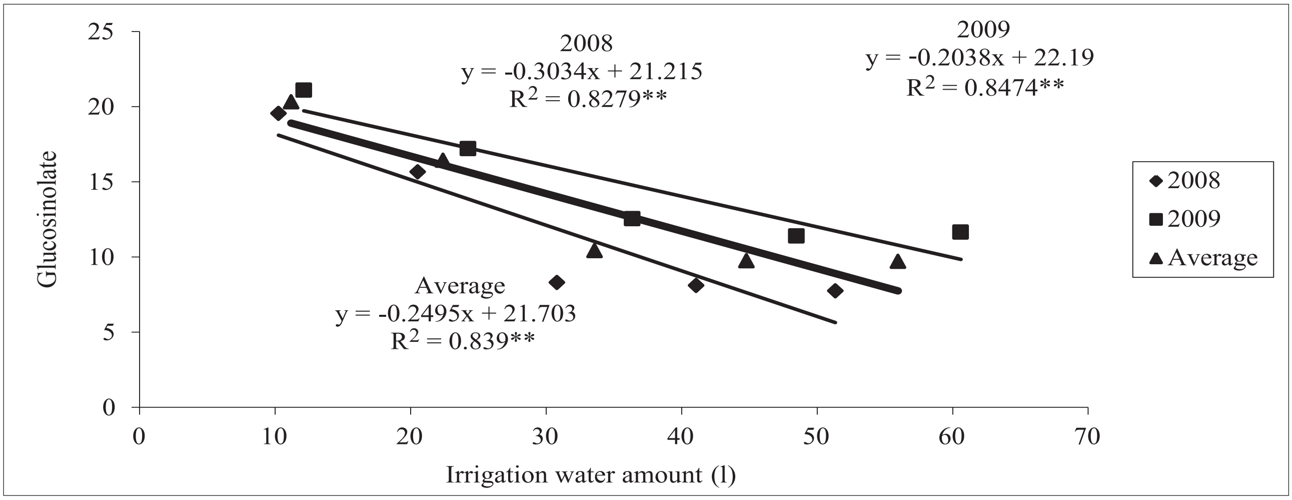


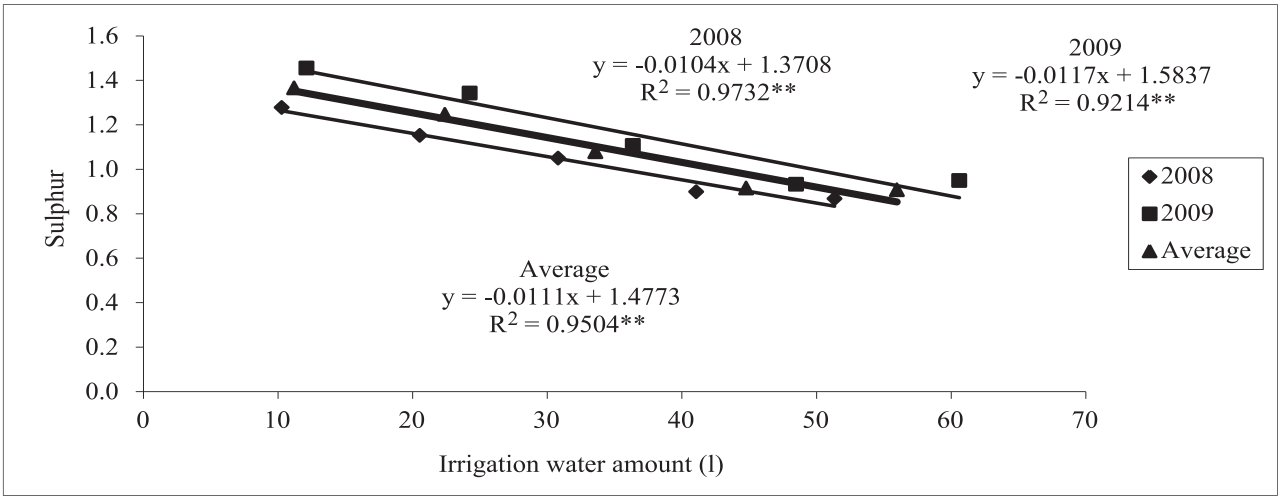
in Figure 2. The results obtained from the experimental treatment for glucosinolate content indicated that glucosinolate content accumulation increases with a decreasing amount of irrigation water. An inverse relationship was determined between the amount of applied irrigation water and glucosinolates. In other words, a decrease in irrigation water and consequent increase in water stress increased the glucosinolate synthesized as a secondary metabolite during the growth and development of the plant. During drought seasons, leaves lose their turgor pressure, primary metabolism products are limited under low water potential and secondary metabolism products and glucosinolates increase (Stroaher et al., 1995; Jensen et al., 1996).

Similar results were observed in the current study at different deficit irrigation levels. An increase in secondary metabolite glucosinolates was seen with decreasing irrigation water (Figure 3), that is, increased water stress increased glucosinolate accumulation in broccoli heads. An increase in synthesis of different metabolites with increasing water stress levels was also reported for other plants (Assimi et al., 2004; Biglouei et al., 2010; Çakır and Çebi, 2011b).

Tests of statistical significance using R^2 and r values determined by regression analysis comparing glucosinolate content and applied irrigation water revealed p values of 0.032029 and 0.026555 for the first and second experimental year, and 0,028861 for both study years evaluated together.

Sulfur is among the elements with multi-way impacts on living organisms (Hell, 1997). It plays a role in electron transfer in plants and exists in secondary metabolites. Glucosinolates, mostly dependant on sulfate assimilation, are known as





supportive compounds for human health and are the members of the Brassicaceae family (Zareba and Serradelf, 2004; Finley, 2005).

Relationships between the amount of applied irrigation water and sulfur accumulation are shown in Figure 4. As in the case of glucosinolate content of broccoli heads, the sulfur content also increased with decreasing amounts of irrigation water. Regression analysis revealed R^2 values of 0.9732 and 0.9214 for the years 2008 and 2009 and 0.9504 for the two-year average. All R^2 values were determined as valid for 95% significance level.

Tests of confidence carried out using R^2 and r-values determined by regression analysis between the sulfur content and applied irrigation water revealed p values of 0.00188 and 0.009578 for 2008 and 2009. The p value was found to be 0.004764 when the two years were evaluated together. The p values indicated that the relationships between sulfur accumulation and irrigation water were statistically valid at 95% confidence level for both years.

Conclusions and Recommendations

Data obtained from treatments with various water deficit levels during the growing season revealed that the water deficit caused yield losses in broccoli grown under the climatic conditions of Çanakkale province. The amount of irrigation water applied to the experimental treatments varied in the range of 51.34-10.27 lt during 2008 and 60.60–12.12 lt in 2009. The yield response of plants to variations in irrigation water was between 667.84–101.68 g/plant in 2008 and 775.87–109.29 g/plant in 2009. The findings of this study indicate that severe levels of water deficit should not be implemented for an economical broccoli culture in Çanakkale. An increase was observed in glucosinolate content under stress conditions. Glucosinolate content under different stress conditions varied between 7.75 and 19.55 for 2008 and between 11.65 and 21.10 in 2009. In addition, significant increases with increasing water stress levels were also observed in the proline synthesis of broccoli.

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