Effects of Water Stress on Yield and Some Quality Parameters of Broccoli

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Abstract: Under increasing impacts of global warming, effective water use and using minimum amounts of water for irrigation have become the most critical issues to be considered in irrigated agriculture. In this research, effects of water stress on yield and some quality parameters of broccoli were investigated. Three different growing periods (early vegetative, late vegetative and flowering) and four different water deficit levels (80%, 60%, 40% and 20%) were applied to Maraton F1 broccoli cultivar. Yield per plant, total leaf area, total chlorophyll, total sugar content, and antioxidant activity have been determined. While a yield of 667,84 g/plant was obtained from control treatment with 100% irrigation without any water deficit, a yield of only 101,59 g/plant was obtained from 20% irrigation treatment. However, a yield of 591,01 g/plant was obtained from 80% irrigation treatment applied at early vegetative period.

Keywords: Broccoli, drought stress, yield

Introduction

Among the winter vegetables of Turkey, production and consumption of broccoli from magnoliopsida class and Bracicacea family have been increasing during the recent years (Eşiyok, 1996).

Broccoli is generally produced for sprouts but leaves of plant can also be consumed. There is an increasing interest in broccoli production in the world beside the cauliflower known all around the world. Average dry matter content of the plant is 10,3%. It has 24 cal nutritional value per 100 gram and contains 89,7% water, 3,3% protein, 0,2% fat ad 4,4-5,2% carbohydrate. Vitamin content is composed of 1542–2500 IU vitamin A, 0.07–0,1 mg/100 g B_1, 0.12–0.23 mg/100g B_2, 0.64–0,9 mg/100g Niacin ve 93,4–114 mg/100g vitamin C. Mineral content is composed of 48–105 mg/100g Ca, 0,9–1,3 mg/g Fe, 24 mg/100g mg, 66–82 mg/100g P, 325–464 mg/100g K ve 27 mg/100g Na (Vural et al., 2000).

Plants of cabbage-group have significant benefits for human health with their rich vitamin C, vitamin A and follic acid contents. Since they are also classified in fibrous foods, they can regulate the intestinal processes. Among these groups of plants, especially broccoli, cauliflower, cabbage, lady's smock and Brussels have been proven to be affective in prevention of several cancer diseases (Young and Wolf 1988, Farey et al. 2001, Zhao et al. 2001). Their preventive impacts against cancer diseases is due to their glicozinolate contents (Seow et al. 2002, Fowke et al. 2003, Sarıkamış et al., 2006). Glicozinolates are secondary metabolism products containing sugar and sulphur. Glucosinolates also serve as a defense mechanism against various ecological and biotic stress factors in plants (Ratzka, 2002).

Environmental conditions to which plants are exposed have significant effects on both yield and quality. These factors can be classified as climate factors, soil factors, artificial polluters, competition with animals and other plants. Optimum environmental conditions should be provided for a proper production with high yields. A deviation from these optimum requirements may cause a stress over the plants. Biologists adopted the word 'stress' for an unpleasant environmental condition for living organisms and called 'stress resistance' for ability to survive of plant against these unpleasant environmental conditions (Levitt, 1980).

Abiotic stress conditions like high temperature, drought, salinity and chemical toxicity and oxidative stress threats agricultural activities all around the world. Abiotic stress is the most significant cause for yield loses and may cause more than 50% loss in yield. It can cause morphological, physiological, biochemical and molecular changes and has negative impacts on plant growth and yield (Wang, 2003).

Drought stress is also among the most significant stress component effecting the plant growth. Synonymous to drought stress, water stress arises when the transpiration of plants are not met from the environment. Water makes up almost 85-90% of several plants. Water taken by roots of the plants is delivered through upper sections of plants based on osmotic rules. A negative pressure (tension) develops within xylems in case of lower root water uptakes than transpirated water and a competition starts among various parts of the plant (Kaçar et al. 2006).

Irrigation is an input in agriculture providing sustainability and stability, improving the efficiencies of other agricultural inputs and providing higher yields per unit area. The basic principle for receiving the desired benefit from irrigation is to provide the necessary amount of water to the root zones of the plants at proper times. Otherwise, plants get into stress due to water deficiency. Main reason for water stress is to have less water than the amount required for transpiration. Generally, the lower the water stress, the higher the yield (Reginato, 1983).

Bandurska, (2004) investigated the variation of proline amounts in leaves of plants grown under water stress and found out that broccoli leaves had the highest rational water loss and barley leaves had the lowest.

Meyer and Adam (2008) investigated the variations of glicosinote in red-cabbage and broccoli grown under organic and conventional conditions and concluded that glucoraphanine, glucobrassicine and neo-glucobrassicinine were dominant glucosinolates in broccoli. They also found lower amounts of glucobrassicinine in organic plants than conventional ones.

Since drought will increase parallel to global warming, negative impacts of drought on qualitative and quantitative parameters of plants should be prevented. In this study, changes in plants under stress conditions will be investigated, the best proper and economical method of growing will be determined; and some physiological and biochemical changes and changes in yield and quality of broccoli will be determined.

Material and Method

This study was carried out at experimental research fields of Agricultural Faculty of Çanakkale Onsekiz Mart University during the fall season of the year 2008. Maraton F1 broccoli cultivar, able to reach harvesting maturity in 90 days from the seedling plantation, was used as the plant material of the study. Seedlings were planted into each 10 liter-pots with sieved soil inside. Each treatment was repeated in 5 pots. Four different water deficits were applied based on growing periods. Experiments were carried out in randomized block design with 5 replications. Statistical method used in this study was summarized below:

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

μ = General population average

α = Water deficit levels (i:1,2,3,4)

β = Effect of growing periods (j:1,2,3,4)

$\alpha\beta$ = Effect of water deficit x Growing period interaction

ε = Error term

To determine the amounts of water deficits, initially the pots with sieved soil were saturated with irrigation water. Then the pots were left for seepage with gravity for 24 hours and weighed. This created 100% (control) treatment. Following the start of experiments, the amount lost by evaporation and plant utilization were determined by weighing the pots every 3 days and this amount was applied to pots as irrigation water. Other water deficit levels were determined based on the weight of control treatment and irrigation water applied accordingly. The first irrigation was performed right after the seedling plantation. Irrigations were performed at the same fashion for all treatments until the seedlings adapted to soil. Following the adaptation period, water deficit levels were applied and continued until the last economically harvestable broccoli is harvested.

Treatments and pot numbering were as follows:

	Early Vegetative	Late Vegetative	Flowering
1-	I_{100}	I_{100}	I_{100}
2-	I_{80}	I_{80}	I_{80}
3-	I_{60}	I_{60}	I_{60}
4-	I_{40}	I_{40}	I_{40}
5-	I_{20}	I_{20}	I_{20}
6-	I_{80}	I_{100}	I_{100}
7-	I_{60}	I_{100}	I_{100}
8-	I_{40}	I_{100}	I_{100}
9-	I_{20}	I_{100}	I_{100}
10-	I_{100}	I_{80}	I_{100}
11-	I_{100}	I_{60}	I_{100}
12-	I_{100}	I_{40}	I_{100}
13-	I_{100}	I_{20}	I_{100}
14-	I_{100}	I_{100}	I_{80}
15-	I_{100}	I_{100}	I_{60}
16-	I_{100}	I_{100}	I_{40}
17-	I_{100}	I_{100}	I_{20}

17*5=85 pots were used.

Total chlorophyll was determined by sampling 4 leaves from each plant. Samples were prepared by smashing the leaves in 90% 35 ml acetone solution. Solutions were filtered through Wattman No.2 filter paper. The filtered extract was completed to 50 ml with 90% acetone solution. Then, extracts were put into spectrophotometer tubes and readings were performed at 645, 652 and 663 nanometer wavelengths. In this way, amount of chlorophyll was determined in mg/100g (Holden 1976).

Total sugar for samples was determined as g/100 g in accordance with dinitrophenol method specified by Ross (1959). A 5g sample was taken from each sample representing each treatment and 5 ml 15% potassium ferrosynide and 5 ml 30% zinc sulphate were added. Then samples were completed to 250 ml with distilled water. Solutions were filtered through Wattman No2 filter paper. Extract of 0,5 ml was taken into test tubes, 1,5 ml distilled water and 6 ml dinitrophenol were added to test tubes and they were kept in 100^0C hot water bath for 6 minutes. Samples were then cooled under tap water for 3 minutes and absorbance readings were performed in T70 model PG Instruments brand spectrophotometers at 600 n wavelength. A 6ml dinitrophenol + 2 ml distilled water solution was used as the control of the method.

Antioxidant activities and radical cleaning power were determined by DPPH method. This method is based on spectrometric transition of characteristic color purple into yellow under the presence of antioxidant chemicals yielding electron or hydrogen atoms by cleaning free radical 2,2- Diphenyl-1-picryl hydrazyl (DPPH) with these chemicals. The more the antioxidant power, the brighter the color of methanolic DPPH solution. In this method, solutions of test extracts prepared in various concentrations (2,5 – 160 mg) of methanol are mixed with 3 mL 6.10^{-5} M DPPH solution. Following 15 minutes dark incubation period, sample absorbances were measured at 515 nm wavelength. Absorbance values were then evaluated against control and curve (methanol). Extract % inhibition values were calculated by using absorbance values of extract and empty control tests as follows:

$$\% \text{ Inh} = \frac{A_0 - (A - A_k)}{A_0} \times 100$$

A_0= DPPH absorbance at 515 nm.
A= Extract absorbance at 515 nm.
A_k= Metanol absorbance at 515 nm.

Calculated % inhibition values were plotted against extract concentrations prepared in mg/mL and IC_{50} of extracts were calculated. BHT and Ascorbic acid were used as positive control.

Total amounts of phenolic compounds were also determined in this study in accordance with Folin&Ciocalteu method. Gallic acid solutions at increasing concentrations were mixed with folin reactive and Na_2CO_3 solutions to draw the calibration curve. Solutions were then kept at 20 ^{0}C for 30 minutes, absorbance readings were performed at 765 nm and calibration curve was drawn. Extract was prepared as defined above by using the same reactives, kept under the same conditions for 1 hour and absorbance reading was made. Amount of total phenolic compound of plant methanol extract was calculated as Gallic acid equivalent (GAE) as follows:

$$C = c \times V / m$$

C= Total amount of phenolic compounds, mg/g plant extract (GAE)
c= Gallic acid concentration calculated from calibration curve, mg/mL
V= Volume of plant extract
m= Weight of plant extract

Calculated phenolic compound amounts (as Gallic acid equivalent) were compared with standard antioxidants BHT, α-tocopherol and ascorbic acid.

Results and Discussion

It was observed that broccoli was sensitive against water stress. It was also observed that water deficit at some growth periods of broccoli didn't statistically effect the yield values. Yield (g/plant), amount of applied irrigation water (L), leaf areas (cm^2), number of days passed until the harvest was given in Table 1. It can be seen from the table that all water deficits applied at early vegetative and late vegetative periods decreased the yield of broccoli, however these decreased were placed statistically in the same group. Leaf areas decreased with the water stress. Schreiner et al. (2009) indicated the impacts of water stress over the leaves of mustard at every growth period.

Treatments	**Yield (g/plant)**	**Irrigation Water (lt)**	**Leaf Area (cm^2)**	**Ripening Time (day)**
100% Irrigation	667,84 ± 29,72 a	44,14	6424.94 ± 32,78 a	110
80% Irrigation	473,12 ± 25,81bc	35,28	6058.97 ± 30,29 b	112
60% Irrigation	394,68 ± 33,43cd	26,46	5421.89 ± 42,46 c	114
40% Irrigation	195,68 ± 16,11ef	17,64	4825.54 ± 27,18 e	119
20% Irrigation	101,59 ± 10,51 f	8,81	3429.35 ± 60,53 j	127
E.V. 80% Irrigation	591,01± 26,19 ab	41,68	5055.91 ± 38,21 d	107
E.V. 60% Irrigation	583,89 ±22,10 bc	39,50	4784.82 ± 45,46 e	109
E.V. 40% Irrigation	566,86 ± 19,16ab	36,77	4337.51 ± 23,49 g	110
E.V. 20% Irrigation	573,61 ± 34,83 ab	34,28	4147.75 ± 25,36 h	110
L.V. 80% Irrigation	528,52 ± 21,26 abc	41,16	4662.83 ± 34,96 f	113
L.V. 60% Irrigation	521,83 ± 18,38ab	38,21	4622.16 ± 32,27 f	113
L.V. 40% Irrigation	497,47 ± 21,74abc	35,26	4608.61 ± 25,20 f	114
L.V. 20% Irrigation	359,83 ± 33,02ab	32,31	4296.85 ± 29,83 g	122
Flowering 80% Irrigation	453,13 ± 13,26 bcd	40,95	5327.01 ± 45,00 c	115
Flowering 60% Irrigation	475,92 ±40,40 bc	37,75	5083.02 ± 53,46 d	117
Flowering 40% Irrigation	359,37 ± 26,75cd	34,57	4676.38 ± 33,78 f	115
Flowering 20% Irrigation	301,18 ± 13,71de	31,36	3998.64 ± 43,77 ı	117

Table 1. Yield (g/plant), Amount of irrigation water (L), Leaf areas (cm^2), Ripening time (days)

Results of physical measurements made over broccoli plants were given in Table 2. Significant variations were not observed in diameter, height and perimeters of plants under water stress except the flowering period. The lowest values were obtained from 40 and 20% irrigation water applications at all growing periods. It was concluded that heavy water stress conditions exposed in flowering period could cause significant decreases in yield and quality.

Treatments	**Head Diameter X (cm)**	**Head Diameter Y (cm)**	**Head Height (cm)**	**Head Perimeter (cm)**
100% Irrigation	13.87 ± 0,475a	13.30 ± 0,644 a	10.89 ± 0,357 a	46.28 ± 2,066 a
80% Irrigation	12.55 ± 0,704 abc	12.65 ± 0,531 ab	9.06 ± 0,375 ab	41.68 ± 1,568 abc

60% Irrigation	12.23 ± 1,06 abc	12.00 ± 0,958 abc	9.46 ± 0,744 ab	39.82 ± 3,134 abc
40% Irrigation	8.87 ± 0,622d	8.91 ± 0,754 d	6.89 ± 0,681 cd	29.98 ± 2,371d
20% Irrigation	6.65 ± 1,06 e	6.31 ± 0,685 e	5.64 ±0,466 d	20.70 ± 3,158 e
E.V. 80% Irrigation	11.55 ±0 ,708 abc	9.47 ± 2,325 cd	8.59 ± 0,690 bc	40.36 ± 3,204abc
E.V. 60% Irrigation	12.13 ± 0,334 abc	12.60 ± 0,847 ab	9.59 ± 0,813 ab	42.30 ± 2,452 ab
E.V. 40% Irrigation	13.26 ± 0,985 ab	13.29 ± 0,429 a	9.20 ± 0,510 ab	44.94 ± 1,289 ab
E.V. 20% Irrigation	13.22 ± 0,442 ab	12.95 ± 0,738 ab	10.13 ± 0,723 ab	44.66 ± 2,309 ab
L.V. 80% Irrigation	12.12 ± 0,409 abc	12.64 ± 0,654 ab	9.57 ± 0,239 ab	41.44 ± 2,256 abc
L.V. 60% Irrigation	13.09 ± 0,438 ab	13.29 ± 0,402 a	10.17 ± 0,565 ab	45.04 ± 1,390 ab
L.V. 40% Irrigation	12.76 ± 1,005 ab	12.72 ± 0,310 ab	9.96 ± 0,916 ab	42.14 ± 0,573 ab
L.V. 20% Irrigation	11.66 ± 0,561 abc	11.77 ± 1,024 abcd	8.58 ± 0,677 bc	38.16 ± 2,858 abc
Flowering 80% Irrigation	13.05 ± 0,978 ab	13.08 ± 0,679 ab	10.17 ± 0,455 ab	42.86 ± 1,733 ab
Flowering 60% Irrigation	12.39 ± abc	11.74 ± 1,133 abcd	9.63 ± 0,881 ab	40.20 ± 3,454 abc
Flowering 40% Irrigation	10.46 ± 0,977 bcd	10.12 ± 0,943 abcd	8.49 ± 0,341 bc	35.28 ± 3,128 bcd
Flowering 20% Irrigation	10.15 ± 0,735 cd	10.08 ± 0,487 bcd	8.28 ± 0,345 bc	34.14 ± 1,984 cd

Table 2. Canopy diameter (cm), canopy height (cm) and canopy perimeter (cm)

Reduced and total sugar, total chlorophyll values of samples were given in Table 3 and Antioxidant activity (%), phenological compounds (mg/mL) and Flavonoids (mg/L) were given in Table 4. An increase was observed in amounts of reduced and total sugar with increased water stress. However, a decrease was observed in amounts of chlorophyll with increased water stress. In a fertilization study for broccoli, Sanwal et al. (2006) determined reduced sugar values between 17,42 – 20,41 and total sugar between 34,07 – 43,63. Murcia et al. (2000) determined the amount of chlorophyll for broccoli as 0,3 g kg^{-1} . Generally, amount of chlorophyll for broccoli varied between 0,32 – 0,75 g kg^{-1} in other studies (Hidaka et al. 1992, Hidaka Fukuda and Taniguchi, 1992).

Treatments	**Reduced Sugar (g/100g)**	**Total Sugar (g/100g)**	**Total Chlorophyll (mg/100g)**
100% Irrigation	1,263 ± 0,318 j	31,56 ± 0,260 ı	7.90 ± 0,055 a
80% Irrigation	1,886 ± 0,491 c	41,30 ± 0,346 f	7.77± 0,070 ab
60% Irrigation	1,920 ± 0,461 bc	41,66 ± 0,375 f	7.53 ± 0,125 bc
40% Irrigation	2,130 ± 0,288 a	42,16 ± 0,375 f	7.37 ± 0,140 c
20% Irrigation	1,436 ± 0,202 hı	44,20 ± 0,378 e	7.63 ± 0,034 abc
E.V. 80% Irrigation	1,530 ± 0,346 gh	32,80 ± 0,404 ı	7.06 ± 0,083 d
E.V. 60% Irrigation	1,773 ± 0,318 d	36,13 ± 0,433 h	6.96 ± 0,488 e
E.V. 40% Irrigation	1,833 ± 0,260 cd	37,53 ± 0,375 g	6.11 ± 0,085 fg
E.V. 20% Irrigation	1,603 ± 0,375 fg	37,73 ± 0,466 g	5.97 ± 0,141 g
L.V. 80% Irrigation	1,640 ± 0,346 f	29,76 ± 0,636 j	6.74 ± 0,087 de
L.V. 60% Irrigation	1,663 ± 0,318 ef	21,83 ± 0,433 k	6.14 ± 0,070 fg

L.V. 40% Irrigation	1,746 ± 0,260 de	29,53 ± 0,638 j	5.95 ± 0,080 fg
L.V. 20% Irrigation	1,640 ± 0,288 f	32,10 ± 0,435 ı	5.47 ± 0,120 h
Flowering 80% Irrigation	1,273 ± 0,260 j	49,40 ± 0,378 d	6.26 ± 0,100 f
Flowering 60% Irrigation	1,420 ± 0,404 ı	52,30 ± 0,635 c	6.03 ± 0,192 f
Flowering 40% Irrigation	1,986 ± 0,260 b	59,66 ± 0,753 b	5.68 ± 0,336 h
Flowering 20% Irrigation	2,123 ± 0,260 a	65,06 ± 0,523 a	5.17 ± 0,120 h

Table 3. Amounts of reduced, total sugar and chlorophyll

Antioxidant activity was investigated by DPPH method with radical cleaning effect. Based on DPPH method, radical cleaning powers between 8,1 – 17,3% were observed. It was found as significantly low. Varying percentages may be due to varying amounts of flavonoid and phenolic compounds and it was thought that these parameters could be analyzed by using other methods in future studies.

Treatments	**Antioxidant Activity (%)**	**Phenolic Compounds (mg/mL)**	**Flavonoids (mg/L)**
100% Irrigation	8,1	0,0020	9,14
80% Irrigation	10,3	0,0043	10,28
60% Irrigation	11,8	0,0098	12,98
40% Irrigation	15,9	0,0099	13,03
20% Irrigation	17,3	0,0102	13,35
E.V. 80% Irrigation	9,3	0,0025	9,58
E.V. 60% Irrigation	10,2	0,0032	9,77
E.V. 40% Irrigation	10,8	0,0030	9,82
E.V. 20% Irrigation	11,5	0,0034	9,95
L.V. 80% Irrigation	10,8	0,0045	10,95
L.V. 60% Irrigation	11,2	0,0051	11,29
L.V. 40% Irrigation	12,0	0,0054	11,74
L.V. 20% Irrigation	12,6	0,0059	12,08
Flowering 80% Irrigation	13,6	0,0065	11,88
Flowering 60% Irrigation	14,8	0,0072	12,32
Flowering 40% Irrigation	15,1	0,0081	12,56
Flowering 20% Irrigation	15,6	0,0093	12,97

Table 4. Antioxidant activity (%), phenolic compounds (mg/mL) and Flavonoid (mg/L) amounts

As a conclusion, water deficits applied at early and late vegetative periods will not cause significant decreases in yield and quality of broccoli plants grown under Çanakkale conditions. However, water deficit at flowering period will cause decreases in yield. Broccoli exhibits a tolerance against water deficit at early or late vegetative period. Deficit irrigation can be applied during these growing periods and production can be carried out over larger areas with the same amount o water and without causing significant losses in yield and quality.

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