



Araştırma Makalesi/Research Article

Yield and Quality Compounds of Broccoli (*Brassica oleracea* L.cv. Beaumont) as Affected by Different Irrigation Levels

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Abstract

The field experiment was carried out at the Dardanos Agricultural Research Station of Canakkale Onsekiz Mart University in 2015, near Dardanelles straits in Canakkale province, Turkey. Irrigation interval (4-day) was fixed for all treatments and evaporation was determined by Class-A pan. In the full treatment ($I_{1.0}$), water in the root zone was refilled according to the amount of water evaporated from Class-A pan. In the deficit treatments, the water applied was 70% ($I_{0.7}$), 30% ($I_{0.3}$) and 0% ($I_{0.0}$) of full irrigation. It is concluded that broccoli (*Brassica oleracea* L.cv. Beaumont) gave the highest yield and quality in terms of that water need ($I_{1.0}$, 361 mm) was fully met. If there is water scarcity in the field where broccoli is cultivated, an economical income from broccoli production can be obtained by saving 30% water or applying water of 253 ($I_{0.7}$) mm. However, if water stress exceeds more than 30%, the yield and especially the quality of broccoli can be significantly lost.

Keywords: Broccoli, Water Stress, Drip Irrigation, Yield, Dardanelles, Evaporation pan,

Öz

Farklı Sulama Düzeylerinin Brokoli (*Brassica oleracea* L.cv. Beaumont) Bitkisinin Verim ve Kalite Özellikleri Üzerine Etkisi

Bu çalışma, 2015 yılında Çanakkale Onsekiz Mart Üniversitesi Ziraat Fakültesi Dardanos Araştırma ve Uygulama alanında yürütülmüştür. Çanakkale koşullarında yapılan bu çalışmada, tüm sulama konularına 4 günde bir A sınıfı buharlaşma kabından meydana gelen buharlaşma miktarına göre sulama suyu uygulanmıştır. Deneme de 4 farklı sulama düzeyi oluşturulmuştur. Bunlar sırasıyla; bitkiyi su stresine sokmayacak tam sulama ($I_{1.0}$), ve diğer stres konuları da tam sulama konusunda uygulanan suyun %70'i ($I_{0.7}$, orta stres) , %30'u ve %0'nın ($I_{0.3}$ ve $I_{0.0}$ ağır stres) uygulandığı sulama konularıdır. Deneme sonucunda, brokoli (*Brassica oleracea* L.cv. Beaumont) bitkisi su ihtiyacının tam karşılandığı sulama konusunda ($I_{1.0}$, 361mm) en yüksek verim ve kalite değerlerine ulaşıldığı görülmüştür. Brokoli bitkisinin yetiştirildiği alanda su kısıtının olması durumunda, %30'luk su tasarrufu yapılarak ($I_{0.7}$, 253 mm) ekonomik oranda verim elde edilebileceği, ancak su stresinin %30'un üzerine çıkması durumunda verim ve özellikle kalitede önemli kayıpların olabileceği belirlenmiştir.

Anahtar Kelimeler: Brokoli, Su Stresi, Damla Sulama, Verim, Dardanos, Buharlaşma Kabı.

Introduction

Irrigation is the most important factor in increasing crop yield and water has becoming an extremely important strategic resource because of climate change since it is already occurring and represents one of the greatest environmental threats facing our planet (Anonymous, 2010).

Irrigation and fertigation have an extremely important effect on yield and quality in crop production. Water resources are decreasing in quantity and quality day by day. Increasing water demands are increasing because of the population growth, which creates heavy burden on existing water resources. Hence, the objectives and processes of water management have been changing. Nowadays, research carried out on irrigation emphasizes the optimum water requirements during drought conditions rather than obtaining the highest yield for sustainable agriculture, and also identification of drought resistance varieties of all crops for each climatic condition has been becoming an important issue.

Many research studies have recently been carried out to determine bioactive components since it was reported that broccoli may prevent chronic disease, also some nutrients inside in broccoli play a dual role in human health and in plant metabolism (Jeffery et al. 2003). Plants are seriously affected by some environmental factors (climate, available organism, geologic materials, soil, atmospheric conditions and so on) during all phases of growth and development (Haferkamp, 1987). Sarah et al.



(2011) observed green color and bioactive compounds of broccoli (*Brassica Oleracea* L.) under low and optimal soil water content in greenhouse conditions. Pek et al. (2012) reported that the contents of glucosinolate and flavonoids changed under different water applications, except for sulphoropane content, but the harvesting time has an effect on the amount of sulphoropane, especially harvesting in fall period increases the amount of it. Broccoli has a wide variety of nutritional and medicinal benefits (Sinha et al., 2011).

The health benefits of broccoli are derived from the unique mixture of nutrients, minerals and vitamins and organic compounds that are found in broccoli. Organic acids are a group of organic compounds containing carboxylic groups. Malic and citric acids are predominant in plants. Organic acids have important functions as flavor enhancers and natural antimicrobial agents. Organic acids are used in food preservation because of their effects on bacteria, since they can penetrate the bacteria cell wall and disrupt the normal physiology of certain types of bacteria (Irkin et al., 2015).

The main objectives of this research were to determine the optimum water requirement of broccoli and changes in plant development and quality parameters under different irrigation levels.

Materials and Methods

Experimental site and soil description: This experiment was conducted at the Agricultural Research Station of Canakkale Onsekiz Mart University, Turkey. The soil was clay-loam with an available soil moisture holding capacity of 36.6% (P_w) or 167.7 mm in the depth between 0 and 90 cm below the soil surface. Broccoli seedlings (*Brassica oleracea* L.cv. Beaumont) were transplanted on 10th of July and harvested on the 12nd November in 2015. The experiment was laid out using a randomized block with 3 replications for each treatment. Broccoli plants were arranged with a distance of 0.6 m between the rows and 0.4 m between the plants in row and crop density in each plot was 40.

Climate: The climate parameters; solar radiation ($MJ\ m^{-2}\ day^{-1}$), temperature ($^{\circ}C$) and relative humidity (%) at the site were measured above the canopy of the plants. All data were measured by a mini-weather station (HOBO U12 including sensors and data logger-MicroDAQ com Inc.). Data were saved into the data logger at 1-hour intervals throughout the experiment. Climate parameters; temperatures (min., max., and mean), relative humidity and solar radiation are given in fig.1. Average values of temperature and relative humidity were almost 20-25 $^{\circ}C$ and 60-70 %, respectively. Solar radiation was regularly being decreased from transplanting almost near 350 $MJ\ m^{-2}\ day^{-1}$ to the harvest date as 50 $MJ\ m^{-2}\ day^{-1}$.

Irrigation management : Each plot in the experiment took the same amount of fertilizer; it included N% (20%), Amonium NH_4-N (3.4%), Nitric NH_3-N (5.3%), Ureic NH_2-N (11.3%), P_2O_5 (water soluble) (20%), K_2O (water soluble) (20%), B (0.01%), Cu(0.01%), Fe(0.05%), Mn(0.02%), Mo(0.001%), Zn(0.02%). The total amount of urea was applied at three times, first at planting then at 15 and 20-day intervals. Hence, the irrigation treatments included four gradient irrigation levels from full water to severe water stress. Only in the full irrigation ($I_{1.0}$) was water refilled in the root zone up to field capacity at 4-day intervals. In the deficit treatments, water was applied at 70% ($I_{0.7}$), at 30% ($I_{0.3}$), and at 0% (I_0) of full irrigation. All treatments were equally irrigated for 20 days after transplanting in order to root development of all plants, then water was applied according to the irrigation treatments. A Class-A Pan was installed next to the experimental plot. The amount of 4-day irrigation water was estimated based on the cumulative evaporation from class-A pan, given by (Ertek and Kanber, 2000). All amounts of evaporation from the class-A pan throughout the whole growing season were measured every 4 days for all growing period.

$$I = A \times E_{pan} \times Kpc \cdot P \quad (1)$$

where I is the amount of irrigation water applied (mm), A is the plot area, E_{pan} is the cumulative evaporation at irrigation interval (mm), Kpc is the pan coefficient (Kpc=1 in the $I_{1.0}$, Kpc=0.7 in the $I_{0.7}$, Kpc=0.3 in the $I_{0.3}$ and Kpc=0 in the I_0), P is the percentage of wetted area (%). Evapotranspiration (ET) was calculated by the water balance equation below:

$$ET = I + P \pm \Delta SW \quad (2)$$



where ET is evapotranspiration (mm), P is rainfall (mm) and ΔSW is the change in soil water content (mm) at a depth of 60 cm from the soil surface, and soil water content was determined by the gravimetric method at 30 cm intervals.

Water use efficiency (WUE) (kg/m^3) was estimated according to Howell (2001) as:

$$WUE = Y / ET \quad (3)$$

$$IWUE = Y / I \quad (4)$$

where Y is yield (t ha^{-1}), and ET is the same as described above and I is the applied irrigation water (mm).

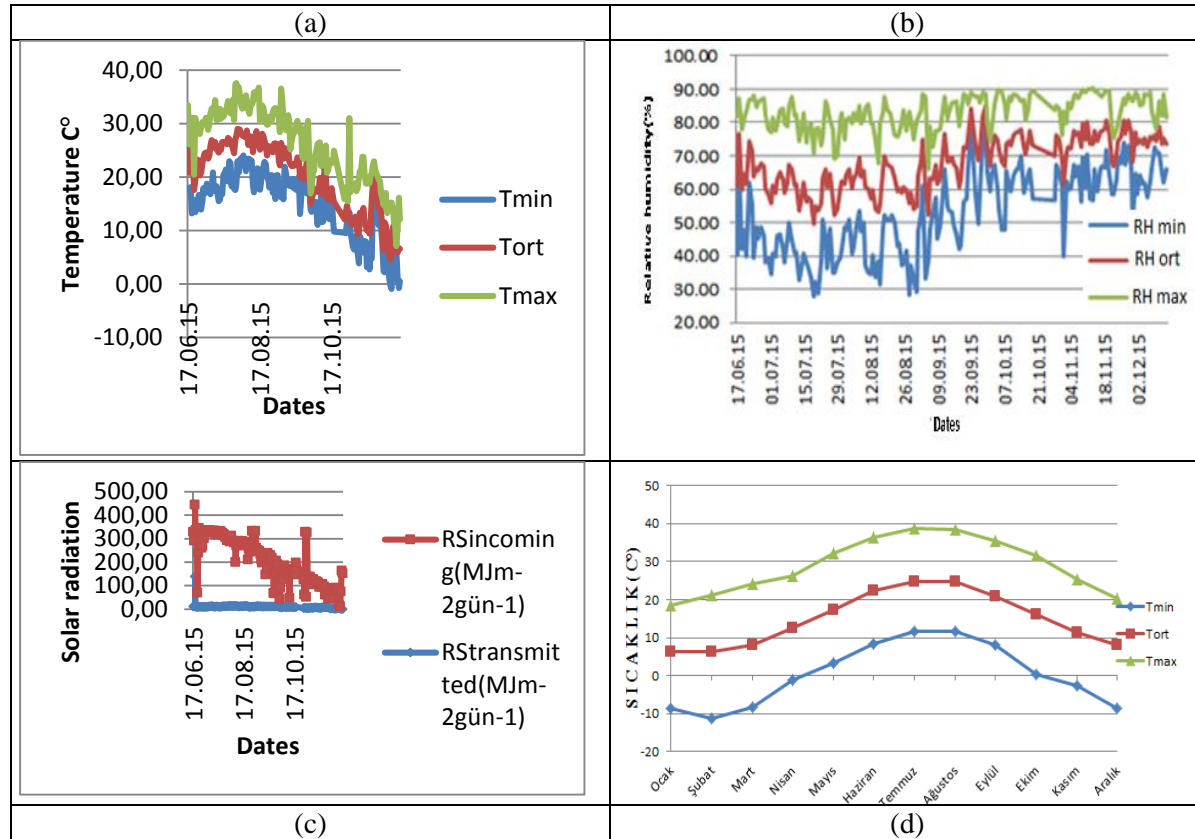


Figure 1. Meteorological data for period of experiment (a) Temperatures(°C), (b) Relative humidity (%), (c) Solar radiation(MJm^{-2}), (d) prolonged temperatures parameters (1975-2000).

Plant and fruit quality parameters : Total and reduced sugar concentration were determined by dinitrophenyl method given by (Ross, 1959). Leaf area was determined by a CI-202 Portable Laser area meter (CID, Inc., USA) as cm^2 , all leaves of each plant were collected in all treatments. All plant weights (stem, leaf and fruit) were determined using a digital balance (± 0.01 g) and diameters were measured with a digital clipper (± 0.01 mm). Fresh weights (stem, leaf and fruit) were determined separately by weighing. After that, they were all oven-dried to a constant weight at about 70°C for two days to determine the dry weight of whole plants in each treatment.

Proline : Chopped leaves were frozen in nitrogen and kept under -15°C until lyophilization, then 0.5 g of broccoli leaves was mixed with 2 ml of 3% sulfosalicylic acid. The culture medium was filtered with a double membrane filter. For proline colorimetric determinations, a 2:2:2 ml solution of culture medium, ninhydrin acid and glacial acetic acid was incubated at 100°C for 1 hour. The reaction was arrested in an iced bath and chromophore was extracted with 4 ml toluene and its absorbance at 520 nm was determined in a spectrophotometer. The method was calibrated for each determination with standard proline solutions within the detection range of the method (Bates et al., 1973).



Organic acid extraction and analysis : Broccoli pulp (3 g) was extracted by stirring with 30 mL of metaphosphoric acid for 15 minutes, then mixed with distilled water to 50 mL and subsequently filtered through Whatman no.4 paper (Vazquez et al.,1994). The volume was adjusted to 10 ml and passed through a 0.45µm filter before examination. The process was performed for each sample using a HPLC system with a UV/VIS detector. The reagents used included chromatography-grade standards for ascorbic, oxalic, tartaric, malic, lactic and citric acids. Each sample was measured in triplicate and the figures were then averaged.

All collected data were analyzed by SPSS statistical package software, and then were separated by Duncan's Multiple Range Test at the probability level of 0.1%, 1% and 5% ($p<0.01$, $p<0.05$ and $p<0.001$).

Results and Discussion

Irrigation water and soil moisture variations: Irrigation amounts, soil moisture fluctuations within the effective root depth of 60cm and rainfall amounts and dates throughout the calendar days were given in Fig 2. As seen in Fig. 2, total precipitation is lower than average evapotranspiration during the growing period, hence supplemental irrigation is unavoidable. Therefore, even broccoli is a winter vegetable crop, it needs supplemental irrigation uniformly to get an economical yield. One another important point in the experiment was that even though irrigation scheduling was performed to Class-A pan, the soil moisture level, especially after the 224th of the year, never reached up to field capacity, even in the full treatment ($I_{1.0}$). The irrigation events were performed between 209th and 293th days of the year. Broccoli (*Brassica Oleracea L. cv. Belstar*) were harvested at the 315th day of the year, hence broccoli development period lasted 106 days. In this experiment, it can be considered that full water application seems like deficit irrigation and more water application than 360.9 mm should increase the yield of broccoli. Therefore, if we add more water to the broccoli root area, they will have a higher yield to the unit increment of water.

Irrigation and evapotranspiration amounts and yield: The irrigation amounts (I), evapotranspiration (ET_c), yield, WUE and IWUE parameters for the field-grown broccoli (*Brassica oleracea L.cv. Beaumont*) are given in Table 1. The total amount of water applied varied from 46.8 to 360.9 mm between the treatments. The highest quantities of applied water and evapotranspiration were obtained from the treatment of $I_{1.0}$ and the lowest 46.8 and 151 mm, respectively, from the treatment of $I_{0.0}$. Evapotranspiration increased as the amount of applied water increased. These findings are well agree with the findings of Erken et al.(2013) and Erdem et al. (2010).

Water use efficiency increased as the applied water decreased according to the irrigation treatments. Therefore, the highest WUE was obtained from the treatment to which the least water was applied ($I_{0.3}$), but the yield in $I_{0.3}$ treatment placed almost in the lowest group. For this reason, the amount of irrigation should have not been less than 252.6 mm ($I_{0.7}$) to obtain an economical yield, which can be considered as the critical level for broccoli production. If we take the full irrigation treatment as a reference, yields were 34%, 46% and 66% less in the $I_{0.7}$, $I_{0.3}$, $I_{0.0}$ treatments, respectively (Table 1.).

Yield components were significantly reduced by deficit irrigation. In the treatment of $I_{0.7}$, crop evapotranspiration reduced fresh head weight and total yield decreased as the ratio of 34%. However, if water scarcity exists, almost 30% of irrigation water can be saved by the $I_{0.7}$ treatment. Hence, severe stress treatments ($I_{0.3}$ and $I_{0.0}$) causes significant decrement in the marketable and total yield also. The amount of rainfall had a significant positive effect on the yield, even it made the WUE ($I_{0.3}$) to be higher than other treatments. The changes in WUE were well agree with Sezen et al.(2006) obtained the highest WUE in the stress treatment.

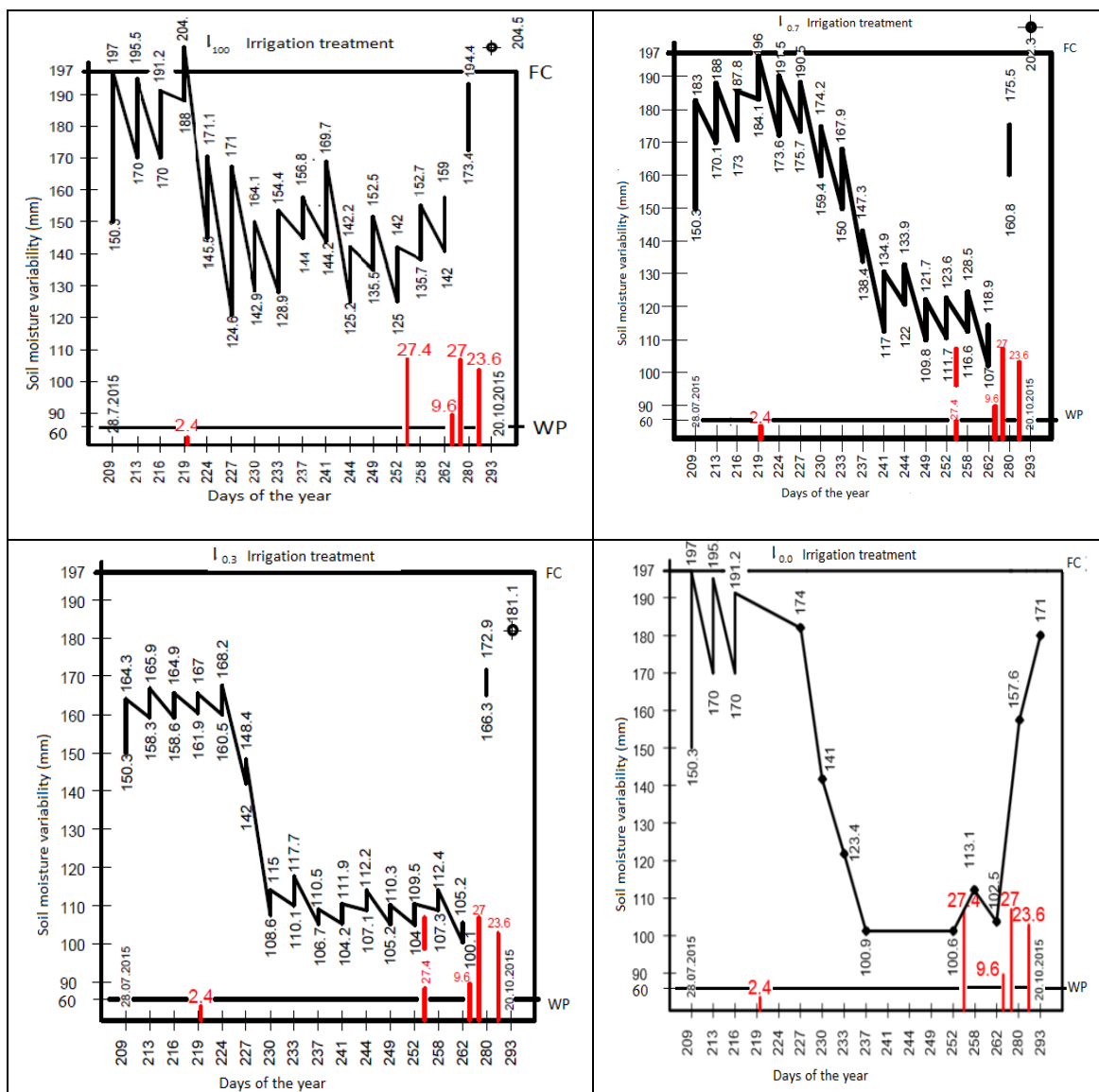


Figure 2. The soil moisture fluctuation throughout the effective root depth (0-60cm) and rain amounts dates given as red bars.

Yield and quality parameters: Measurements of plant development parameters for the whole growing season are given in Table 2. Parameters related to plant development; fresh and dry head weights, floret number per plant, leaf area were negatively affected as the amount of water decreased from 360.9 to 46.8 mm. Plant sizes in terms of stem and leaf and also floret numbers were reduced by 30% ET_c irrigation as compared to 100% ET_c irrigation (Table 2.). Mean fruit diameter and length were not affected even the applied water reduced up to 108.3 mm in the I_{0.30} treatment. However, yield and floret number were significantly reduced when the applied water dropped to 108.3 mm. Therefore, deficit irrigation at 70% ET_c can be considered as the threshold level in terms of both yield and quality to obtain an economical yield.

The various sugars perform different functions in the tubers, but they all can provide energy, glucose is the main source of energy because the most complex sugars and carbohydrates breakdown into glucose (Yildirim et al, 2009). The total and reduced sugar contents were the highest in the I_{0.0} as 7.42 and 1.85 g/100g, respectively. Therefore, deficit treatments, especially in the severe stress treatments, caused broccoli plants to store more energy. This finding is very similar with Ashraf and Haris (2004), reported that carbohydrates in plants increases especially under drought conditions.



Table 1. Measured irrigation depth (I), evapotranspiration (ET), yield, water use efficiency (WUE) and irrigation water use efficiency (IWUE).

Treatments	Irrigation depth (mm)	Evapo transpiration (mm)	Yield ^{***} (kg ha ⁻¹)	WUE (kg m ⁻³)	IWUE (kg m ⁻³)
I _{1.0}	360.9	429.4	10560 ^a	2.46	2.93
I _{0.7}	252.6	308.5	6960 ^b	2.26	2.76
I _{0.3}	108.3	180.3	5693 ^{bc}	3.16	5.26
I _{0.0}	46.8	151	3640 ^c	2.54	7.78

*P<0.05, **P<0.01, ***P<0.001

Table 2. Effect of different water levels on yield and fruit quality parameters.

Plant development parameters	I _{1.0}		I _{0.70}		I _{0.30}		I _{0.0}	
	Fresh weight	Dry weight	Fresh weight	Dry weight	Fresh weight	Dry weight	Fresh weight	Dry weight
Yield per plant (g plant ⁻¹)**	264 ^a	70.5	174 ^b	60.1	142 ^{bc}	63.1	91 ^c	52.7
Stem weight (g)	477	77.3	435.4	50.2	276.4	48.3	226.8	40.3
Leaves weight (g)	572.1	43.1	527.9	27.9	499.1	40.2	415.8	16.4
Mean fruit diameter (cm)**	12.12 ^a		11.66 ^a		11.73 ^a		8.73 ^b	
Mean fruit length (cm)**	13.13 ^a		13.13 ^a		12.8 ^a		9.48 ^b	
Floret number***	13.4 ^a		12.4 ^a		11.2 ^b		4.8 ^c	
Leaf area cm ² ***	3639.2 ^a		2891.3 ^b		2401.3 ^c		1902.1 ^d	
Leaf number **	161 ^a		116 ^b		119 ^b		69 ^c	
Total sugar (g/100g)	4.57		5.27		5.62		7.42	
Reduced sugar(g/100g)	1.23		1.36		1.56		1.85	

*P<0.05, **P<0.01, ***P<0.001

Proline : Proline is a proteinogenic amino acid with an exceptional conformational rigidity, and is essential for primary metabolism. Proline accumulates in many plant species because of environmental stresses. Proline accumulation has been reported during conditions of drought, high salinity, high light, heavy metals and in response to biotic stresses (Szabados and Savoure, 2009). Glutamate is converted to proline in two stages. It is thought that transformation have occurred under physiological conditions and nitrogen deficiency or osmotic stress (Sivaramakrishnan et al. 1988).

In the experiment, decreasing amount of water from 360.9 mm to 46.8 mm increased the proline content from 14.42 µmol/g in the I_{1.0} treatment to 32.54 µmol/g in the I_{0.0} treatment as seen in fig.3. Proline contents were very close to each other in the treatments of I_{1.0} and I_{0.70}, indicating the reason of why an economical yield can be obtained in the I_{0.70} treatment. Therefore, our finding is well agree with the literature, since proline content increased during drought conditions.

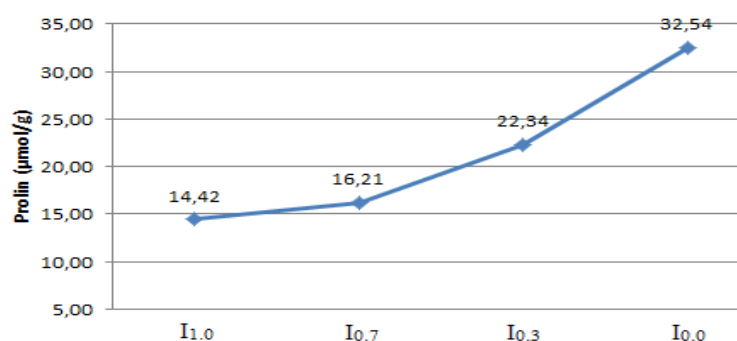


Figure 3. Changes in proline content under different water levels.

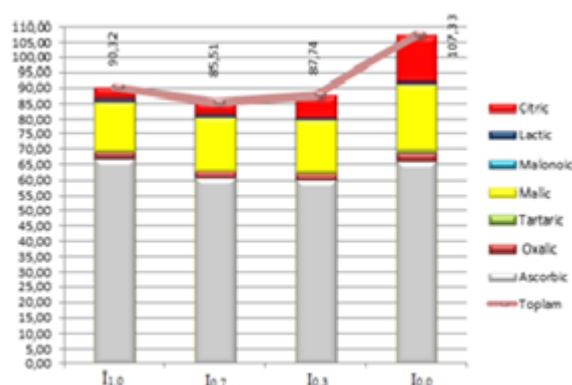


Organic acids : The health benefits of broccoli are derived from the unique mixture of nutrients, minerals, vitamins and organic compounds that are found in broccoli (Nelson and Mottern, 1931). Organic acids have important functions as flavor enhancers and natural antimicrobial agents. Organic acids influence the color of vegetables since many pigments are natural pH indicators (Sinha et al., 2011). The organic acids (ascorbic, oxalic, tartaric, malic, lactic, citric acid) in broccoli (*Brassica Oleracea* L. cv. Belstar) are given in Table 3. The organic acid contents in broccoli varied from 83.32 mg g⁻¹ in the treatment that full water demand of broccoli was met to 107.33 mg g⁻¹ in the severe stress treatment. As seen, the amount of total organic acid increased when the level of water stress increased.

Table 3. Organic acids in broccoli.

Organic Acids	I _{1.0}	I _{0.7}	I _{0.3}	I _{0.0}
Ascorbic *	60.24 ^b	60.88 ^b	60.28 ^b	66.17 ^a
Oxalic *	1.83 ^b	1.93 ^b	1.96 ^b	2.49 ^a
Tartaric *	0.29 ^b	0.31 ^b	0.36 ^b	0.95 ^a
Malic *	16.18 ^b	17.87 ^{ab}	17.35 ^{ab}	21.84 ^a
Malonoic **	0.01 ^b	0.03 ^{ab}	0.05 ^a	0.06 ^a
Lactic *	1.17 ^a	0.90 ^{ab}	0.85 ^b	0.76 ^b
Citric ***	3.43 ^b	3.68 ^b	6.96 ^{ab}	15.06 ^a
Total	83.32	85.51	87.74	107.33

*p<0.05, **p<0.01, ***p<0.001



The dominant organic acids in broccoli (*Brassica Oleracea* L. cv. Belstar) were ascorbic, malic and citric acid, respectively. The ascorbic acid content exhibited the highest content as compared with other organic acids given in Table 3. The ascorbic acid ranged from 60.24 mg g⁻¹ in the full water application to 66.17 mg g⁻¹ in the severe stress treatment (I_{0.0}), which was almost accounting of 68.5% of the total organic acid content, malic and citric acid were that of 20.1% and 7.6% of total acid. The minor organic acids in broccoli were tartaric (0.29 to 0.95 mg g⁻¹), lactic (0.76 to 1.17 mg g⁻¹) and oxalic acid (1.83 to 2.49 mg g⁻¹), and these accounted for 3.83% of total acids. The content of each individual organic acid increased with increasing water stress, even though the differences between the individual organic acids were not significant. Soyer et al. (2003) found that the dominant organic acid in the 11 different white grape cultivars was tartaric acid (4.07 to 4.92 g L⁻¹) and citric acid (31 to 181 mg L⁻¹), malic acid (1.36 to 3.47 g L⁻¹), respectively. Sha et al. (2011) determined 10 organic acids in the pear fruit (*P. ussuriensis*) and the total organic acid content ranged from 3.04 to 9.13 mg L⁻¹. Organic acid content in broccoli (*Brassica Oleracea* L. cv. Belstar) was higher than other fruits. Ascorbic acid known as vitamin C is organic acid with antioxidant properties. Vitamin C is involved in the absorption of iron and calcium. It assists in the healing of wounds and burns, in preventing blood clotting and in strengthening the walls of capillaries (Carr and Frei, 1999). Therefore, broccoli can be considered as a good source in terms of ascorbic acid.

Conclusion

In the experiment, broccoli (*Brassica Oleracea* L. cv. Beamount) exhibited to be a water-sensitive plant to water shortage, Yield of broccoli was affected according to the different water levels and produced the highest yields in I_{1.0} and I_{0.7} treatments, in which the amount of water applied were 360.9 mm and 252.6 mm, respectively.

*Applying irrigation water for broccoli between 252 and 360 mm seems to be more appropriate level for getting an economical yield.

*Supplemental irrigation for broccoli, even being a winter plant, is an obligatory to provide a uniform plant development and to get a reasonable economic yield.

*An increment in the organic acid content was observed, as water stress level increased, but were no statistical differences in each organic acid content under different irrigation levels.

*Within the organic acids, ascorbic acid (Vitamin C) was dominant in broccoli and also the content of it was very high as compared with many fruits. Therefore, broccoli can be considered as an important antioxidant in the foods in terms of ascorbic acid.



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