THE SIMULTANEOUS EFFECT OF HEAT STRESS AND WATER SUPPLY ON TOTAL POLYPHENOL CONTENT OF EGGPLANT

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Abstract. Eggplant is considered as one of the healthiest vegetables due to its nutritional values. A 2-year open field experiment was set up in 2011 and 2012 to observe the effects of heat stress and different irrigation volumes on the amount of phenolic compounds, fibre and dry matter in eggplant fruits. The optimal irrigation volume (IO) was calculated from the daily potential evapotranspiration and was compared to a treatment utilising 50% of the optimal water volume (I50). We concluded that there was no significant difference between the irrigation levels in the aspect of total polyphenol and dry matter, but there was a decrease in fibre content. Yield was increased by 19.6% with irrigation in 2011 and by 4.44% in 2012. The effect of heat stress is closely related to harvest time. In our study we proved that more heat stress resulted in more total polyphenol content in eggplant, the equation describing this relation is $y=39.322+0.501x$. Heat stress also stimulated dry matter and fibre content, and this influence was also significant.

Keywords: *eggplant, abiotic stress, fibre content*

Introduction

The harvested area of eggplant was estimated at 1.72 million hectare worldwide, in 2010, and 1.82 million in 2011. The vast majority of production is in China and India (Faostat, 2012). In Bangladesh economically eggplant is the most important vegetable, because it provides direct income throughout the year for family farmers, even in extreme hot and humid seasons (Rashid et al., 2003) and counts as one of the main vegetables in Iran (Khanamani et al., 2014). It is cultivated in greenhouses, even in soilless culture (Hamdy et al., 2004) on the field alike (Acciarri et al., 2002; Muñoz-Falcón et al., 2008), with conventional and organic agricultural practices (Luthria et al., 2010). Even though there are plenty of studies concerning eggplant few of them explain how growing conditions can enhance the amount of polyphenol substances, fibre and dry matter in the fruits as they have beneficial properties for human health (Ma et al.,

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2011; Kritchevsky et al., 1975). In this study we conducted an experiment to investigate the relationship between an abiotic stress (heat) and total polyphenol, fibre, dry matter content of eggplant fruits at two different irrigation levels.

Review of literature

Nutritional value of eggplant

It is well documented that the quantity and quality of phenolic substances present in the fruits of eggplant are not homogeneous (Whitaker and Stommel, 2003). Noda et al. (2000) found that the peel had the highest value of nasunin that belongs to the flavonoids and induces its dark purple colour. According to Matsuzoe et al. (1999) nasunin is responsible for 70%-90% of total anthocyanin in the peel. As well as the peel, the pulp is also rich in polyphenols, particularly in chlorogenic acid, which belongs to the hidroxybenzoic acids and gives the 70-95% of the total phenolic content in the flesh (Stommel and Whitaker, 2003).

Cai et al. (2004) measured 166.9 μmol/100 g antioxidant capacity (Trolox Equivalent Antioxidant Capacity, TEAC) in dry weight and 1.08 g GAE/100g in dry weight total phenolic content of the fruit. They also revealed a positive and highly significant linear correlation between the amount of total polyphenol content and antioxidant capacity by testing different herbs, including eggplant as well. Stefanovits-Bányai et al. (2005) reported 1.37-2.27 mmol/l ascorbic acid equivalent antioxidant capacity (Ferric Reducing Ability of Plasma, FRAP) from the fresh juice of the fruit. The calyx, pulp and peel has different antioxidant activity, according to Boubekri et al. (2012) the dark purple cultivar’s peel contained 66.78 mg/g followed by the pulp 16.54 mg/g and then the calyx 14.82 mg/g, expressed in ascorbic acid antioxidant capacities.

Eggplant also counts as a potassium, calcium and phosphate rich vegetable (Raigón et al., 2008; Flick et al., 1978). The measurements of Inthichack et al. (2013) range the value of potassium of the fruits among 3.29% and 4.69% per dry weight. Adamczewska-Sowińska and Krygier (2010) compared the dry matter content of optimal mature and overripe fruits in five commercial cultivars; they concluded the optimal mature crop contained significantly higher dry matter, while the difference of the cultivars were not significant. Eggplant is also known as a fibre source vegetable its fibre content was reported to be 6,6 g/100g (Dhingra et al., 2012; Hanson et al., 2006), but different coloured varieties show different contents of crude fibre (Flick et al., 1978).

Storage and processing occured changes

After slicing up eggplant fruits the cutting surfaces suffer natural browning which is caused by the activation of polyphenol oxidase enzyme (Ramírez et al., 2002). A principal breeding aim is to moderate the degree of browning beside maintaining the high amount of polyphenol content, since a positive correlation exists among the concentration of phenol substances and the degree of browning (Prohens et al., 2007). The experiment of Boubekri et al. (2013) investigated the effect of freezing and drying on the total polyphenol content separately on the peel, and on the whole fruit. They concluded the fresh peel of purple cultivar contained the most (548.77 mg GA/g), after the frozen (106.11 mg GA/g) and the least was measured in the dried sample (93.48 mg GA/g). Scalzo et al. (2010) found that an exact cooking process resulted in richer

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chlorogenic and caffeic acid content fruit comparing to the raw eggplants. A study on storing eggplant found the fruits kept at 10 °C were intact, while the others kept at 5 °C and 0 °C suffered chilling injury (Concellón et al., 2004).

Health protective effect of eggplant

Crude extract of eggplant against several pathogenic microorganisms show potential inhibitory effect, especially the extract from the fruit and root of the plant (Al-Janabi and Al-Rubeey, 2010). Hepatoprotective effect was described by Akanitapichat et al. (2010). They also found a significant correlation between hepatoprotective activities and total phenolic content. An in vitro experiment by Kahlon et al. (2007) concluded that eggplant has positive bile acid binding ability. Anti-cancer effect was described by Azevedo et al. (2007) they found that the anthocyanin content of the peel has a reducing effect on cell mutagenity, also Node et al. (2000) found oxigen scavenger function of nasunin which is the major component of anthocyanin in the peel. Phenolic compounds extracted from eggplant significantly lowered cholesterol-level in rats (Sudheesh et al., 1997). Kritchevsky et al. (1975) reported a hypocholesteremic effect in rabbits with the absorption of dietary cholesterol. The findings of Kwon et al. (2008) and Hanhineva et al. (2010) revealed eggplant has an impact on Type 2 diabetes by controlling glucose absorption.

Polyphenol in stress context

The ability of polyphenol synthesis is genetically determined, so a particular eggplant variety shows consistent phenolic content among years (Prohens et al., 2007). However, the circumstances of the cultivation including temperature, water supply and soil conditions influence the development of eggplant fruits and consequently effects polyphenol content as well (Ajay et al., 2009). Previous observations showed that plants are able to adapt to stress by changing certain biochemical pathways and evolve resistance by synthesizing polyphenol compounds (Wang et al., 2014). Several studies have shown that abiotic stress stimulates the accumulation of polyphenols in various plants (Abdallah et al., 2013; Khavari-Nejad et al., 2006). After pesticide utilization a high activity of polyphenol oxidase was measured to reduce toxic substances in tomato (Nasrabadi et al., 2011). An experiment on apples after fungal infection indicates elated chlorogenic acid and phloridzin concentration as a defensing mechanism in apple peel (Schovankova and Opatova, 2011).

Rivero et al. (2001) found that growing tomato at optimal temperature resulted in lower amount of polyphenols than at cooler and warmer temperature treatment levels. According to Lee et al. (2013) on chicory and garland chrysanthemum the optimum had a greater influence than the unfavourable temperature on flavanoid and total polyphenol content. In lettuce Boo et al. (2011) found that the lowest 13/10°C day/night temperature resulted in the highest total anthocyanin content, however in strawberry the highest 30/22 °C day/night temperature set caused the most phenolic content as well (Wang and Zheng, 2001).

Optimum temperature of eggplant

The optimum temperature of eggplant is 22±7 ºC, this is the temperature range under which the plant is not suffering any damage caused by temperature as Markov and Haev had described (Somos, 1983). However, according to Boyer (1982) the

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optimum temperature of eggplant ranges between 22-30 ºC. Moreover, different eggplant varieties are likely to have different optimum temperature ranges as suggested by others (Minghua et al., 2001; Li and Yu, 2004). An experiment with temperature alternations in different developmental stages showed that optimal daytime temperature is between 20-31.3 ºC, whereas optimal night temperature varies between 15-20 ºC for proper vegetative growth. They also revealed that the combination of 25/15 ºC day/night temperatures resulted in the lowest amount of dry matter in eggplant (Inthichack et al., 2013).

Materials and methods

Experimental field conditions

The study was conducted at the experimental field of Institute of Horticulture, Szent István University, located in Gödöllő, Hungary (lat. 47°61’ N, long. 19°32’ E). The soil of the experimental field is sandy loam classified as Cambisol with 1.8-2% humus content and pH value around 7. Electric conductivity was measured as 0.26 dS/m in 2011, and 0.18 dS/m in 2012. The inorganic content of the soil are represented in *Table 1*.

Table 1. *The average (n=3) of inorganic content of the experimental field in 2011 and 2012. All values are given in mg/kg.*

	NO_3-N [mg/kg]	P_2O_5 [mg/kg]	K_2O [mg/kg]	Ca [mg/kg]	Mg [mg/kg]	Fe [mg/kg]	Cu [mg/kg]	B [mg/kg]	SO_4-S [mg/kg]	Cl [mg/kg]	HCO_3 [mg/kg]	Na [mg/kg]
2011	15	(868) 19	48	150.3	30.4	227.8	4.28	0.35	6	0	533.7	70
2012	7.1	(736) 34	54	140.3	30.4	132.2	11	0.90	43	0	534	10

The climate of this area is dry continental; climatic parameters (daily temperature and precipitation) were logged by a Campbell CR21X meteorological instrument (Campbell Scientific Inc., Loughbourgh, U.K.) throughout the study. *Figures 1-2* show the minimum and maximum temperature (ºC) and precipitation (mm) for the whole vegetation in 2011 and 2012.

In both years we used Barcelona F1 hybrid, which fits to the Hungarian market demand, because it has medium sized, drop-shaped, dark purple coloured fruits, and prickeless calyx. The colour of the skin remains vivid purple even in hot temperature. It is also characterized with strong vegetative growth, well-yielding and early ripening. It gives quite long storable fruits after harvest (Semillasfito, 2012). Seeds were sown at the end of April, while transplantation of the seedlings was made at the beginning of May each year in simple row with a plant density of 4.2 plants/m^2. Irrigation was applied using drip irrigation tubes, one lateral for each row, with discharge rates of 4 L/h. Half of the culture received optimal water supply (irrigation optimal: ‘IO’), whereas the other half obtained 50% water supply (irrigation 50%: ‘I50’) after transplantation. IO plants were irrigated to daily potential evapotranspiration of eggplant. The algorithm of the irrigation is the following one:

$$\frac{Tmin+Tmax}{2} \times 0.2 = Et_{pot} \qquad \text{(Eq.1)}$$

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The amount of potential daily evapotranspiration (Et_{pot}) was estimated from the sum of the expected daily maximum (T_{min}) and maximum (T_{max}) temperature (in °C) divided by two and multiplied with 0.2 and after expressed in millimetre according to a previous study of Helyes and Varga (1994). Plants were irrigated with the calculated amount of water in the morning of every Monday, Wednesday and Friday until harvesting. Total amounts of available water were 489 and 331 mm (including 131 mm precipitation) in 2011, and 596 and 408 mm (including 240 mm precipitation) in 2012 for plants in the IO and I50 treatments, respectively.

Technical ripened eggplant fruits were harvested every week from July 26th to October 6th in 2011, and from July 28th to October 10th in 2012. Samples for the analytical measurement were collected on July 26th and October 6th in 2011 and Aug 30th and October 10th in 2012. In both irrigation treatment groups four replicates were formed, each consisted of the fruits of 10 plants, which were transported for analytical preparation immediately after harvesting. Marketable yield was calculated from the weight of marketable (i.e. no sign of physical injury or any diseases) eggplant fruits in these replicates and summarized for each harvest time and extrapolated to one hectare.

Chemical analysis

For all analytical measurements we used all harvested fruits in each replicate. After arrival the laboratory the samples were immediately cleaned, chopped into small pieces and freeze-dried. After lyophilisation, samples were allowed to equilibrate in open air and ground to pass a 0.5-mm sieve. The samples were stored at -25 ^{o}C for less than 2 months until analysed. Analytics included the measurement of total polyphenol, dry matter and fibre content. Total dry matter content was measured by A.O.A.C. (1984) method. 5 g of each sample was dried at 105 °C until constant weight to calculate dried fruit weight, which then was used to estimate dry matter content as the proportion of fresh and dried fruit weight.

The analyses of total polyphenols were completed according to Folin-Denis method by spectrophotometry at 760 nm using catechin as standard and Folin-Ciocalteu’s phenol reagent (A.O.A.C., 1990). 20 ml 60% ethanol was added to 1 g lyophilised eggplant powder, mixed well and then filtered. Folin-Denis reagent (0.5 ml) was added to 1 ml filtered sample and the content of the tube was mixed thoroughly. After 3 min, 1 ml of saturated Na_2CO_3 was added. The mixture was completed to 10 ml with distilled water and it was allowed to stand for 30 min at room temperature. The absorbance was determined at 760 nm using catechin as standard. Total polyphenol contents are given as mg/100 g fresh weight. Fibre content was measured by a digestion treatment following the protocol of A.O.A.C. 985.29 Megazyme International Ireland Limited, Total dietary fibre assay procedure (2000). For the measurement a Megazyme TDF K-TDFR 01/05 Total Dietary Fiber Assay Kit was used consisting heat stable alpha-amylase (gelatinize), protease (remove protein) and amyloglucosidase (remove starch). Fiber content data are corrected with protein and ash content of the sample. All values are given in 100 g fresh weight (*Table 2*).

Calculation method

Besides the irrigation treatment, we also investigated how weather conditions contributed to the potential differences in total polyphenol content of eggplant in this experiment. We adopted Markov-Haev temperature range (Somos, 1983) where 22±7

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ºC was considered as optimum to calculate heat stress values for each harvest time in both years. According to the applied method, we used the minimum and maximum daily temperatures of each day ($T_{\min}$ and $T_{\max}$, respectively) in an 8 day-long period before each harvest time, and calculated the stress values as follows:

$$Heat\ stress\ \ value = (T_{max} - 29°C) + (15°C - T_{min}) \quad \text{(Eq.2)}$$
$$only\ if\ T_{max} > 29°C\ and\ T_{min} < 15°C$$

On those days when $T_{\max}$ was lower than 29 ºC and/or $T_{\min}$ was higher than 15 ºC, the corresponding term in the equation was set to zero.

In all tests α was set to 0.05. We fitted linear models (LM) for total polyphenol, fibre and dry matter as dependent variables and included 'irrigation level' (as fixed factor), 'heat stress' (as covariate), and their interaction as potential predictors. We applied a stepwise backward elimination procedure to choose the best model. Prior to model fitting residual homogeneity and the normally distribution was checked by plot diagnostic. One data point was removed, and detected as an outlier (*Table 2*. October 10th 2012, IO irrigation treatment). Statistical analyses were performed in IBM SPSS 22 software (IBM Co., New York) and Microsoft ® Excel 2007 Analysis Toolpack (Microsoft Corporation., Redmond, Washington).

Results

Table 2*. Mean and standard deviation of the measured total polyphenol, fibre and dry matter contents (n=4). All value is given in 100 g fresh weight. IO is the optimal irrigation set, I50 indicates half of optimal irrigation level.*

Harvest	Irrigation treatment	Fibre [g/100g]	Dry matter [g/100g]	Total polyphenol [mg/100g]
July 26th 2011	I50	3.42 ±0.42	6.98 ±0.26	45.4 ±2.92
	IO	2.60 ±0.21	6.86 ±0.17	46.5 ±1.69
October 6th 2011	I50	4.29 ±0.30	8.64 ±0.61	66.6 ±9.73
	IO	4.67±0.67	8.40 ±0.74	72.7 ±6.69
August 30th 2012	I50	4.06±0.19	9.36±0.40	52.8±9.72
	IO	3.73±0.21	8.12±0.43	48.6±1.93
October 10th 2012	I50	4.19±0.42	8.14±0.58	61.9±3.33
	IO	5.01 ± 0.56*	8.49 ± 1.13*	63.73 ± 12.35*

*(n=3)

We found a significant effect of the heat stress value on total polyphenol content in eggplant fruits ($F_{1,31}$=61.375, p<0.001): the higher the stress values calculated for each harvest, the more polyphenol the fruits contained. The highest polyphenol amount was observed in 2011 October 6th in the optimal irrigation with 72.7 ± 6.69 where the corresponding heat stress value was 59. In 2012 October 10th the total polyphenol

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content decreased to 63.73 ± 12.35 with a 46 heat stress value in the optimal irrigation set. The difference between the weather conditions are indicated in *Figures 1-2*.

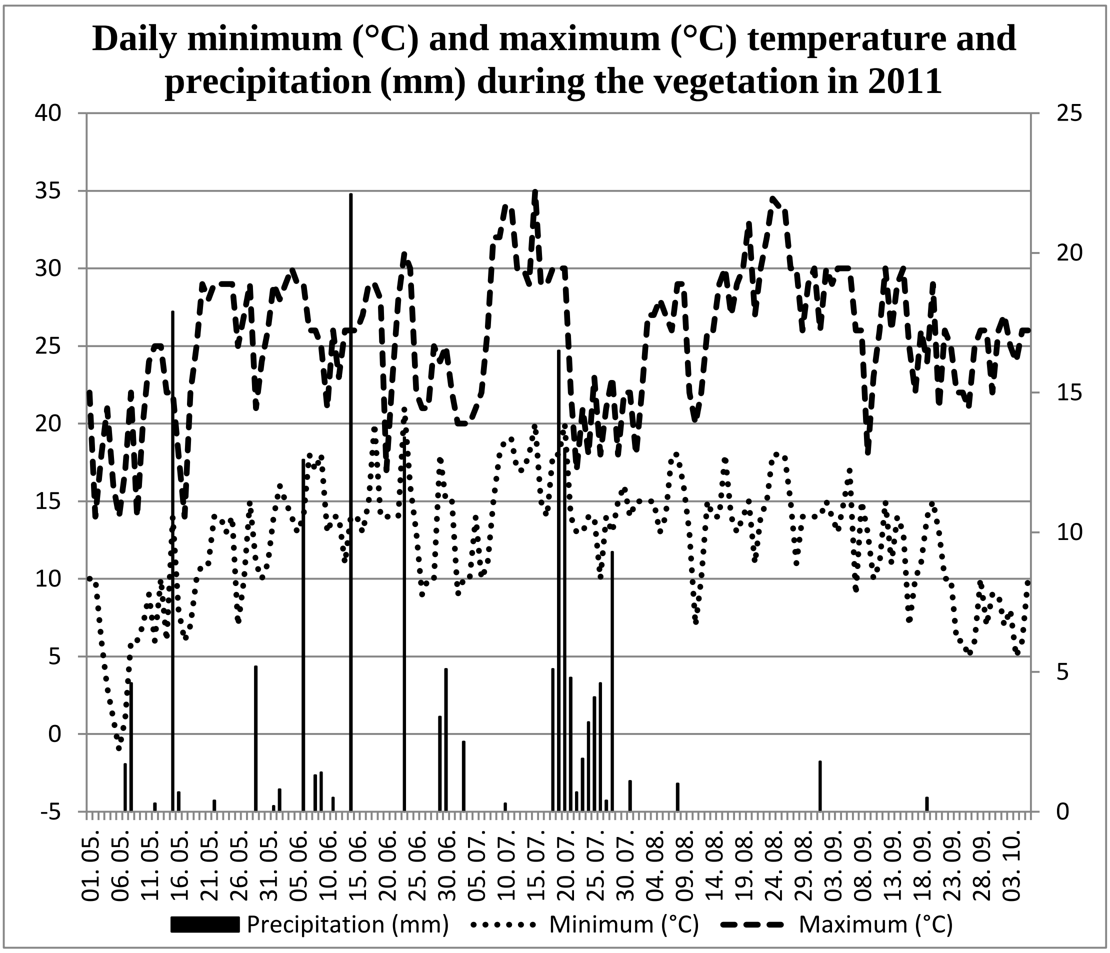

Figure 1. *Daily minimum and maximum temperature (ºC) and precipitation (mm) during the vegetation period of eggplant in 2011. The y-axis on the left side shows the temperature scale, the y-axis on the right the amount of precipitation.*

The fluctuation before the autumn harvest in 2011 was among 5-10°C in the previous nights of the harvest, in 2012 the minimum reached -1°C for one night and even increase to 14°C. To understand the relationship of the measured total polyphenol and heat stress value a linear regression was made (*Figure 3*).

The equation describing the relation is y=39.322+0.501x (r^2=0.679). The calculated heat stress value and its influence on total polyphenol content showed a positive linear relation. Irrigation, on the other hand, had no significant effect either alone ($F_{1,31}$=0.219, p=0.644) or in interaction with heat stress values ($F_{1,31}$=0.878, p=0.357).

Irrigation negatively affected fibre content as it was expected. The amount of fibre is significantly influenced by the interaction of heat stress and water supply ($F_{1,31}$=10.614, p=0.003). *Figure 4.* shows the relation of heat stress and fibre content separately by irrigation treatments. When IO was set, the heat stress determined 73.9% of fibre content, in the case I50 this value was 48.7% which difference came from the interaction, so the irrigation affected in different ways because of the heat stress.

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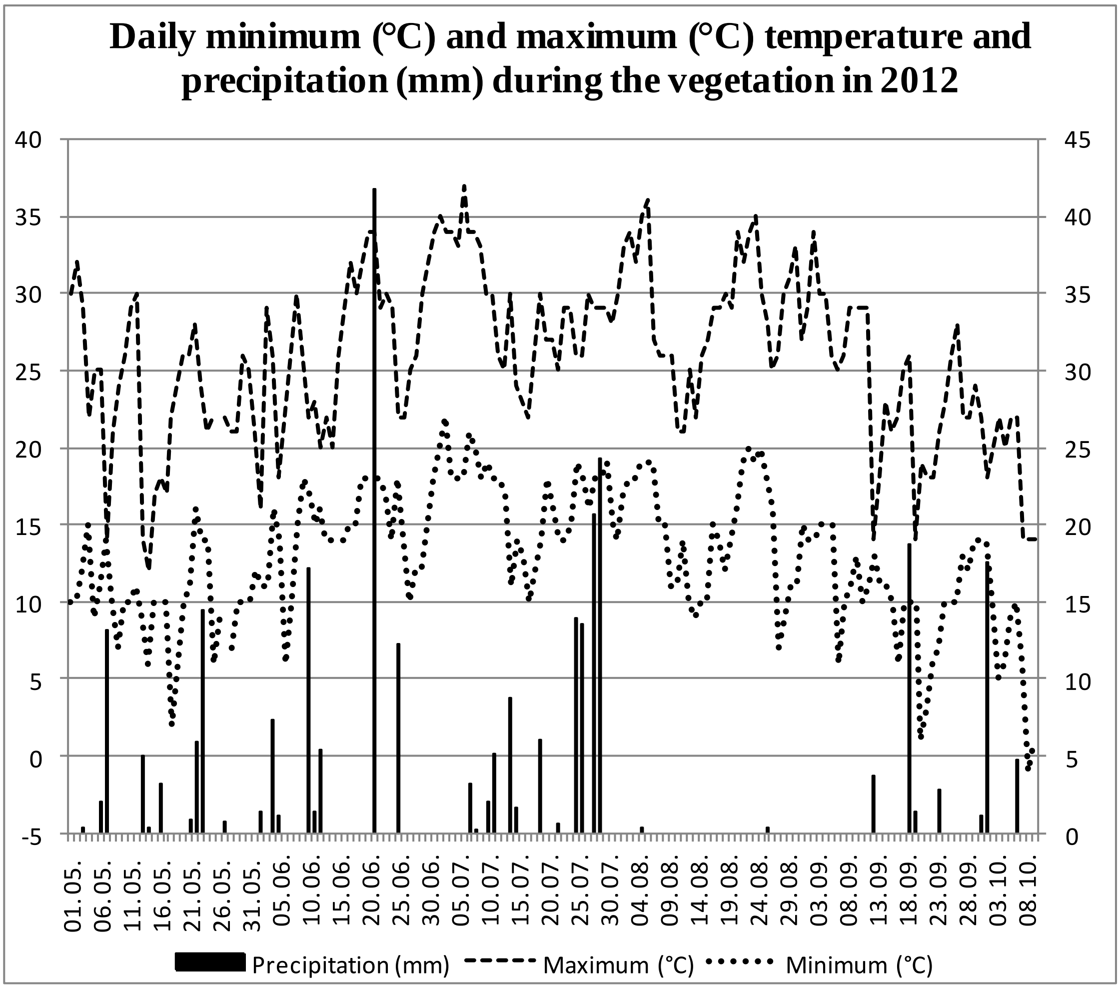

***Figure 2**. Daily minimum and maximum temperature (ºC) and precipitation (mm) during the vegetation period of eggplant in 2012.The y-axis on the left side shows the temperature scale, the y-axis on the right the amount of precipitation.*

In the case of dry matter heat stress had significant effect ($F_{1,31}$=12.201, p=0.002). There was a bigger difference (*Table 2*) between the summer and autumn harvested fruits in 2011 than in 2012. The highest amount was measured in 2012 October 10th 9.36±0.4 at the I50 treatment, the lowest value was 6.86±0.17 in 2011 at IO treatment. Irrigation, on the other hand, had no significant effect either in itself ($F_{1,31}$=1.282, p=0.267) or in interaction with heat stress values ($F_{1,31}$=0.228, p=0.637).

Comparing yield parameters of IO and I50 in 2011; 83 t/ha, 69.4 t/ha and in 2012; 47 t/ha, 45 t/ha was calculated. IO gave 19.6% more yield in 2011; while in 2012 it gave 4.44% more yield than the less irrigated treatment.

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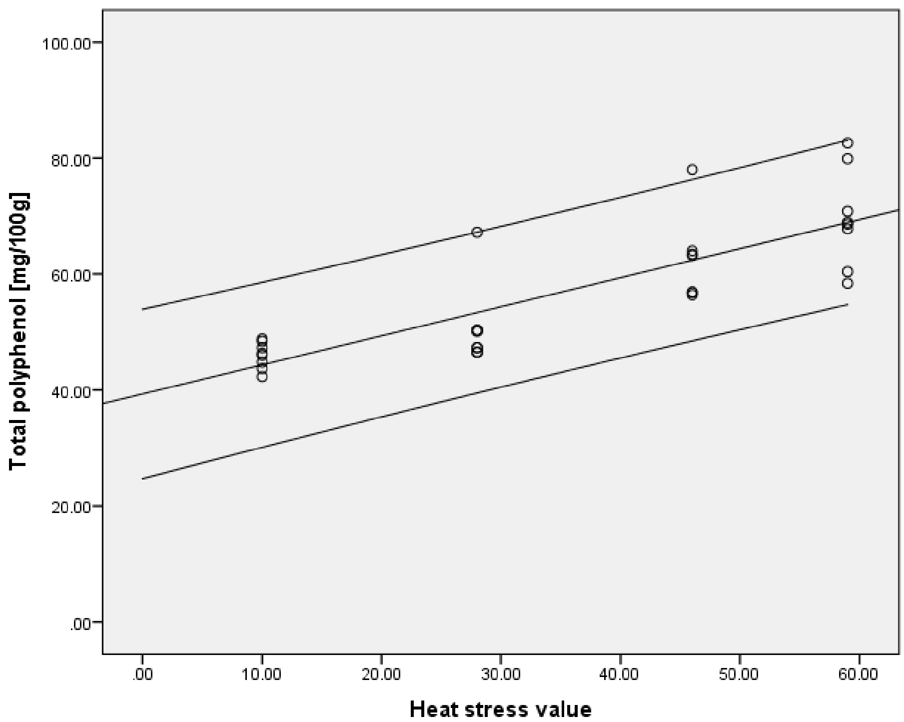

Figure 3. *Heat stress value determined 67.9% (r^2=0.679) of total polyphenol content of the eggplant fruits. Confident interval of 95% is indicated.*

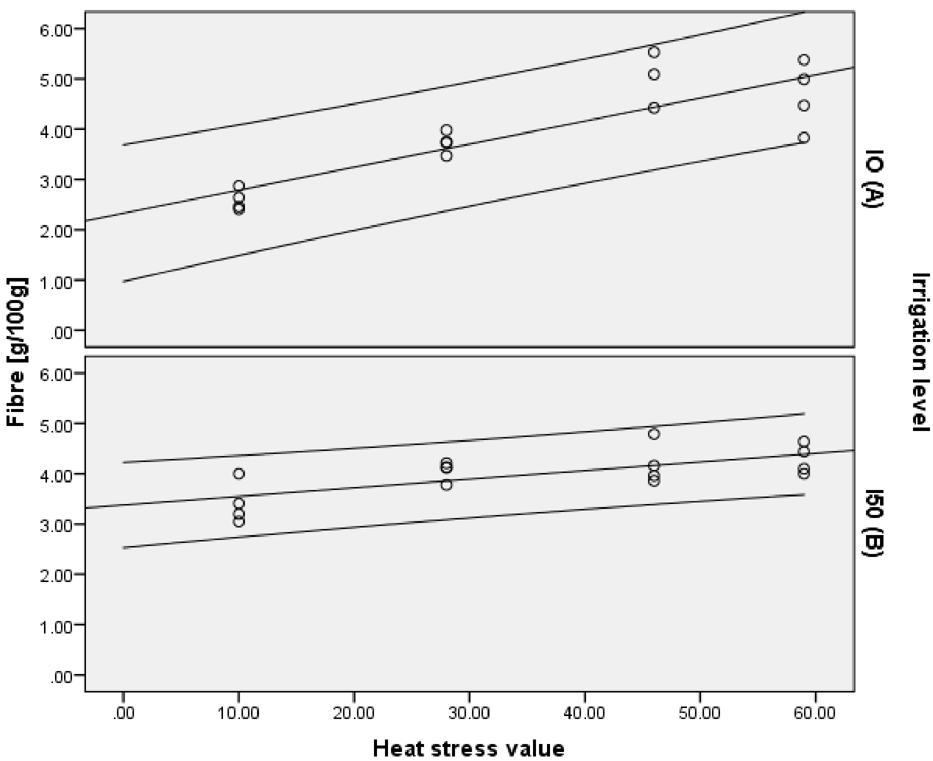

Figure 4. *When optimal irrigation was set (A), the heat stress determined 73.9% of fibre content (r^2=0.739), but in the case of 50% of optimal irrigation volume (B) this value decreased to 48.7% (r^2=0.487). Confident interval of 95% is indicated.*

Discussion

According to our results the of the measured total polyphenol content in 2011 and 2012 a better quality eggplant could be harvested in autumn after some relatively cold days, than in the summer season under our climate. Our results regarding the relation of total polyphenol and heat stress is consistent with the findings of Rivero et al. (2001). Each harvest has a different calculated heat stress value, and in both years the value was higher in autumn than in the summer harvest period. From another point of view the total polyphenol content showed a wide range of standard deviation in our measurements, which is undesirable for statistical analysis. The finding of Whitaker and Stommel (2003) draws attention on the uneven distribution of polyphenol contents in the different parts of the pulp and in the whole fruit which possibly cause difficulties that we experienced at the analytical measurements. The content of fibre was influenced by the interaction of irrigation and heat stress, so neither the irrigation levels nor the heat stress can be accountable in an unequivocal way to explain the fibre content.
To maintain profitable yield in eggplant irrigation is essential (Aujla et al., 2007). The optimal water supply gave 19.6% and 4.44% more yield (in 2011 and 2012 respectively) than the less irrigated plots.

As a conclusion considering the effect of the IO and I50 on total polyphenol and dry matter we could not detect significant differences, so the irrigation levels in our utilisation was not a matter of the quality of eggplant. Therefore half volume irrigation was enough to provide the same quality as the optimal for eggplant crop.

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APPENDIX

Electronic Appendix 1: Basic Data

Electronic Appendix 2: Experimental Plot – Barcelona

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