Flowering, Growth and Fruit Setting in Greenhouse Bell Pepper under Water Stress

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Abstract: In this study, the effects of water stress at different growth stages on dynamics of flowering and fruit production of greenhouse grown pepper plants were investigated. Irrigation treatments included complete restoration of water use during the whole growing season (control), water deficit during the vegetative phase (vegetative stressed stage) and water deficit during the reproductive phase (reproductive stressed stage). Soil water content was periodically measured; leaf water and osmotic potential, net assimilation rate, stomatal conductance, transpiration rate, intercellular CO₂ concentration were also measured on pepper leaves. Flowers were monitored till the end of the crop cycle to calculate fruit setting. Leaf water and osmotic potential changed with time according to the soil water content of stressed treatments. Photosynthesis was lower in vegetative stressed stage and reproductive stressed stage treatments during the water stress periods compared to the well watered control. During the vegetative phase, water stress significantly reduced the number of flowers per plant, decreasing from 30.9 to 10.7 in the control and vegetative stressed stage treatments, respectively. Unlike flowering, setting was not affected by water stress, when it was imposed during both the vegetative and reproductive phases. Total pepper yield gradually decreased going from the fully irrigated treatment to the treatment stressed during the vegetative phase, getting to the lowest value in the treatment stressed during the reproductive phase (5644, 2040 and 875 g m⁻², respectively). Since setting was not affected by water stress, the attention on irrigation scheduling should not be paid only on reproductive phase, but on the whole growing cycle.

Key words: Capsicum annuum L., leaf water potential, photosynthesis, pollination, stomatal conductance, water use efficiency

INTRODUCTION

Water stress is the main factor limiting crop production in rainfed farming systems in arid and semi-arid environments (Debaeke and Aboudrare, 2004). According to increasing water needs for agriculture, threats to natural water resources and climatic change scenarios, water scarcity and decreased allocation of water to irrigation will occur in the near future (Kirda and Kanber, 1999). To cope with limited, variable and often chronically deficient rainfall, the farmer must seek the best combination of various management and varietal options. Among them we find optimizing the water use pattern throughout the crop cycle.

The research on plant-water relations has always been object of large interest to better understand the role of water in crop growth and productivity. There is an enormous amount of studies in literature, especially about field crops as corn, wheat and sorghum. However relatively less attention has been paid to vegetable crops, where the production is often associated with an abundance of water use.

Water stress can be very critical for the yield response during particular phenological phases: This is very important for scheduling irrigation in those areas where a considerable water saving is necessary (Jones, 2004). When insufficient amount and/or poor rainfall distribution throughout the season are unable to fulfill crop water requirements, a continuously optimal water regime can be obtained by supplemental irrigation. Under limited available water conditions, the challenge is to satisfy crop water demand at the critical (and most responsive) stages (Debaeke and Aboudrare, 2004).

Pepper (Capsicum annuum) growing is very common in Mediterranean regions where is considered one of the most susceptible crops to water stress in horticulture (Gonzalez-Dugo et al., 2007; Showemimo and Olarewaju, 2007; Katerji et al., 1993). Many studies confirmed that reductions in water supply during pepper growth have an adverse effect on final yield. For high yields, an adequate water supply is required during the whole crop cycle (Delfine et al., 2001; Dorji et al., 2005; Gonzalez-Dugo et al., 2007; Sezen et al., 2006). Dalla Costa and Gianquinto (2002) reported that continuous water

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stress significantly reduced total fresh weight of fruits and Antony and Singandhupa (2004) concluded that total pepper yield was poor at lower levels of irrigation. Plant reproduction is an important and delicate phenological phase of plants, especially for grain and fruit crops. Agricultural yield largely depends on the success of plant reproduction, which depends on many environmental biotic and abiotic factors. Among them, water stress is probably the most important factor affecting crop productivity, reducing number of grains or fruits per plant at harvest (Broyer and Westgate, 2004; Saini and Lalonde, 1998). Westgate and Thomson Grant (1989) and Saini and Lalonde (1998) attribute the reduced number of fruits in water stressed crops to the failure of pollination. But Hsiao (1993) individuates the restricted plant size and reduced assimilate availability at the time of fruit development and maturation caused by water stress, as the major causes of the reduced number of fruits.

Many researches have been carried out on maize, rice and wheat while a minor number of studies is available for dicotyledoneous species, which are also sensitive to severe drought during their reproductive phases (Westgate and Peterson, 1993). For indeterminate crops, such as pepper, is more difficult to analyze their long period of reproduction. Few information are available on the susceptibility of pepper to drought during the different stages of growing, both in vegetative and reproductive phases. Jaimez et al. (2000) underlined that an important aspect to consider is how the length of the periods of water deficit affects flowering dynamics and subsequent fruit production in this crop. Dagdelen et al. (2004) reported high sensitivity during the early phase of reproduction at flowering. Ghanem and Shafeek (2005) reported growth data for pepper grown in protected environment.

The aim of this experiment is to study the effect of water stress applied during different growing stages on gas exchange, dynamics of flowering and fruit production of bell pepper, grown in a protected environment.

**MATERIALS AND METHODS**

The experiment was carried out from April 17th to August 1st in a heated/ventilated glasshouse (8×15 m) of the University of Basilicata, Potenza, Italy (40°63′ N, 15° 81′E). Glasshouse was maintained at 24°C during the day and 16°C at night. The soil used for the experiment is a clay-silty soil (35.4% clay, 36.5% silt, 27.8% sand), rich in organic matter (2.0%), 1.23 bulk density, with a good chemical fertility and 27.5% gravimetric moisture at field capacity and 15.7% at wilting point (determined in lab at -0.03 MPa and at -1.5 MPa, respectively). Bell pepper seedlings (cv Peppone) were transplanted in pots (1 m diameter and 0.4 m height); each pot was filled with 260 kg of dry soil, three small plants were placed in the middle, 40 cm spaced. Plants underwent fertiritration (18 g di N, 12 g di P2O5, e 18 g di K2O per each pot).

The following irrigation treatments were applied: (a) complete restoration of water use during the whole growing season (Control treatment, C); (b) water deficit during the vegetative phase (Vegetative Stressed Stage treatment, VSS) withholding water during vegetative phase (until 53 Days After Transplantation, DAT) and then watering with complete restoration of water use until the end of the crop cycle; c) water deficit during the reproductive phase (Reproductive Stressed Stage treatment, RSS) watering with complete restoration of water use during vegetative phase and withholding water from 53 DAT to the end of the crop cycle. Irrigation turn was weekly. As for the control treatment, soil water depletion never exceeded 40% available soil water as recommended by Doorenbos and Kassam (1986) for pepper; in the water stressed treatments, soil water depletion was kept between 15 and 35% available water. Each treatment included three repetitions (i.e., three pots, 27 plants) randomly distributed in the glasshouse.

During the whole experimental period, meteorological data were recorded by an automatic weather station placed in the greenhouse. Measured parameters were mean air temperature, air humidity and global radiation (Table 1); they were acquired every 10 sec, averaged and registered every 30 min by a Sky data logger. All data were downloaded from the data logger by a laptop and processed in order to obtain the daily averages.

**Soil water content**: Soil water content was measured gravimetrically by daily weighing the pots by a weighing transpallet, whose error forgiveness was 0.500 kg.

**Plant water status**: Total water potential ($\Psi_t$) and osmotic potential ($\Psi_s$) were measured at 42, 49 and 62 days after transplanting on the youngest uppermost fully-expanded leaf using a Peltier-cooled thermocouple psychrometer (Tre Pse SC10X; Decagon Devices, Pullman, WA, USA). Leaf discs were removed from one side of the margin of the lamina and sealed in the psychrometer chamber. Samples were allowed to equilibrate for 2 h before $\Psi$ readings were made. Leaf discs were then frozen in a cold room at -20°C for 24 h to rupture cell membranes and then returned to the chamber to measure $\Psi_s$.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>April</th>
<th>May</th>
<th>June</th>
<th>July</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean air temperature (°C)</td>
<td>22.3</td>
<td>25.4</td>
<td>26.5</td>
<td>26.9</td>
</tr>
<tr>
<td>Air humidity (%)</td>
<td>34.3</td>
<td>36.6</td>
<td>37.2</td>
<td>35.8</td>
</tr>
<tr>
<td>Global radiation (μmol/m²/sec)</td>
<td>480</td>
<td>528</td>
<td>725</td>
<td>780</td>
</tr>
</tbody>
</table>

Values are monthly means of measurements made during the experimental period.
Gas exchange analysis: Leaf gas exchange was weekly measured on the abaxial surface of a well developed and fully expanded leaf located to the upper part using a portable gas analyser system LI-COR 6400 (LI-COR, Lincoln, NE, USA), weekly from 11.00-12.00 a.m. Photosynthesis rate (A), stomatal conductance (g) transpiration and internal concentration of CO₂ of the leaves were measured.

Yield and reproductive structures: Pepper fruits were harvested at 78 and 92 Days After Transplantation (DAT) and both fresh and dry weight were measured. After 105 DAT, at the end of the experiment, all plants were taken to determine green leaf area (LA) by an electronic area meter (Model 3100, LI-COR, Lincoln, NE, USA). Shoot dry weight (DW) was determined by weighing samples that had been oven dried at 70°C. Water use efficiency at leaf level (WUE L) was calculated as the ratio between assimilated CO₂ and transpired H₂O (μmol CO₂ mmol⁻¹ H₂O). Whole plant water use efficiency (WUE P) was calculated as the ratio between dry weight (DW) at harvest and total water use (g L⁻¹). Yield Water Use Efficiency (YWUE) was calculated as the ratio between yielded peppers and total water use (g L⁻¹).

To evaluate the effects of water stress on the reproductive structures, all flowers and buds of each plant of the three different treatments were periodically tagged. Tagged flowers were then monitored till the end of the crop cycle to calculate the fruit set as the ratio between total yielded peppers and the total reproductive structures (peppers, scars, buds and flowers).

Statistical analysis has been performed according to Analysis of Variance (ANOVA) and mean discrimination was performed according to the Duncan’s multiple range test using the MSTAT 2.0.

**RESULTS**

Average soil moisture content in the three different treatments over the experimental period is shown in Fig. 1. Significant difference of soil water content between treatments was measured during stress periods. While the level of water content in the control treatment was maintained in the range of 40% of soil available water, in the vegetative stressed stage treatment it progressively reached the range of 30-15% of available water. Since 58 days after transplanting (at the beginning of flowering) the soil water content was maintained at the same level of the Control treatment. In the Reproductive Stressed Stage treatment, the soil water content in the vegetative part of the crop cycle was equal to the control, while since 50 DAT, in the reproductive phase, it was maintained in the range of 15-30% of available water.

The leaf water and osmotic potential changed with time according to the soil water content of stressed treatments (Table 2). In the VSS treatment the lowest value of leaf water potential was reached 49 DAT and successively it began to increase progressively till the end of the crop cycle. In the RSS treatment leaf water content was equal to the control treatment until 49 DAT, when it began to reduce. The lowest value of leaf water potential observed in VSS and RSS treatments during the whole experimental period was -1.32 and -1.29 MPa, respectively. Osmotic potential values are reported in Table 2. In control plants the average value of osmotic potential during all the experimental period was -1.33 MPa while in the stressed plants it came down to -1.81 and -1.65 MPa in the VSS and RSS treatments, respectively.

Photosynthesis was lower in VSS and RSS treatments during the water stress periods compared to the well-watered control (Fig 2a). The minimum level reached during the stress phase was 17.0 and 5.47 μmol CO₂ m⁻²sec in VSS and RSS, respectively. After re-watering, the level of CO₂ assimilation in the VSS treatment was not different from the control, until the end of experimental period.

Stomatal conductance (Fig. 2b), had the same trend as the leaf photosynthetic rate. Since the first days after transplantation stomatal conductance was 0.13 mmol H₂O/ m²sec in the VSS treatment, while it was significantly higher in C and RSS treatments. Transpiration (Fig 2c) and intercellular CO₂ concentration (Fig 2d) were lower in VSS and RSS treatments during the water stress periods than in the full watered control, as well as leaf photosynthesis. Leaf WUE was higher in VSS and RSS treatments during water stress periods than in the control plants (Fig 3).
Fig. 2: Leaf gas exchange; (a) assimilation rate, (b) stomatal conductance, (c) transpiration, (d) leaf internal CO₂ concentration measured in the three treatments (C, VSS and RSS) during experimental period. Vertical bars are standard errors of the means

Table 2: Leaf water potential (Ψw), osmotic potential (Ψs), turgor potential (Ψp) measured in Control (C), Vegetative Stressed Stage (VSS) and Reproductive Stressed Stage (RSS) treatments at 42, 49 and 62 days after transplanting.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Ψw (MPa)</th>
<th>Ψs (MPa)</th>
<th>Ψp (MPa)</th>
</tr>
</thead>
<tbody>
<tr>
<td>At 42 days</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>-0.83±0.01</td>
<td>-1.27±0.03</td>
<td>0.44±0.03</td>
</tr>
<tr>
<td>VSS</td>
<td>-0.97±0.08</td>
<td>-1.38±0.03</td>
<td>0.41±0.06</td>
</tr>
<tr>
<td>RSS</td>
<td>-0.85±0.09</td>
<td>-1.18±0.02</td>
<td>0.32±0.03</td>
</tr>
<tr>
<td>At 49 days</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>-0.88±0.05</td>
<td>-1.33±0.01</td>
<td>0.44±0.05</td>
</tr>
<tr>
<td>VSS</td>
<td>-1.32±0.07</td>
<td>-1.81±0.03</td>
<td>0.48±0.06</td>
</tr>
<tr>
<td>RSS</td>
<td>-0.86±0.02</td>
<td>-1.34±0.03</td>
<td>0.47±0.04</td>
</tr>
<tr>
<td>At 62 days</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>-0.96±0.02</td>
<td>-1.40±0.05</td>
<td>0.44±0.03</td>
</tr>
<tr>
<td>VSS</td>
<td>-1.08±0.05</td>
<td>-1.41±0.05</td>
<td>0.32±0.02</td>
</tr>
<tr>
<td>RSS</td>
<td>-1.79±0.14</td>
<td>-1.65±0.1</td>
<td>0.35±0.04</td>
</tr>
</tbody>
</table>

Values are expressed as Mean±SE

Maximum value of WUE was 5.5 and 4.8 µmmol CO₂/mmol H₂O in VSS and RSS, respectively, while in the Control plants it was 2.95 µmmol CO₂/mmol H₂O, on average over the whole experimental period.

Total evapotranspiration from the pots of the three treatments is shown in Table 3. The control used over the whole trial period 0.42 m³/pot of water, while the VSS and RSS treatments used 0.34 and 0.24 m³/pot of water, respectively. Leaf Area (LA) resulted significantly different only between RSS and C treatments. Drought reduced significantly (p<0.05) the dry weight of both stressed treatments (Table 3). In VSS treatment, drought reduced the dry weight by 22.5% compared to the C treatment, while in RSS treatment dry weight was reduced by 60.5%. WUEp and YWUE did not vary significantly between VSS and control treatments, while they reduce significantly in the RSS treatment.

In Table 4, the effect of the induced water stress during the vegetative (VSS) and reproductive (RSS)
Table 3: Evapotranspiration, Leaf Area, Total Dry Matter (TDM), Water Use Efficiency (WUE), Yield Water Use Efficiency (YWUE) measured in the three treatments

<table>
<thead>
<tr>
<th>Treatment</th>
<th>ET (mm)</th>
<th>LA (m²)</th>
<th>TDM (g)</th>
<th>WUE (kg m⁻²)</th>
<th>YWUE (kg m⁻²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>C</td>
<td>0.42</td>
<td>0.97a</td>
<td>285a</td>
<td>0.68a</td>
<td>0.36a</td>
</tr>
<tr>
<td>VSS</td>
<td>0.34</td>
<td>0.86a</td>
<td>221b</td>
<td>0.66a</td>
<td>0.33a</td>
</tr>
<tr>
<td>ARSS</td>
<td>0.24</td>
<td>0.52b</td>
<td>103c</td>
<td>0.43b</td>
<td>0.06b</td>
</tr>
</tbody>
</table>

Values are means of measurements made during the experimental period. Values within a column followed by different letters are significantly different at p<0.01 according to Duncan's multiple range test.

Table 4: Tagged flowers per plant, percentage of fruit set, number of pepper per plant, average weight of pepper, total yield and percentage of marketable pepper measured in the three treatments

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Tagged flowers plant⁻¹</th>
<th>No. of pepper plant⁻¹</th>
<th>Fruit set (%)</th>
<th>Average weight pepper (g) (g m⁻²)</th>
<th>Marketable pepper (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>C</td>
<td>30.5a</td>
<td>28.4a</td>
<td>8.9a</td>
<td>166a</td>
<td>584a</td>
</tr>
<tr>
<td>VSS</td>
<td>10.7b</td>
<td>27.3b</td>
<td>2.9b</td>
<td>184a</td>
<td>204b</td>
</tr>
<tr>
<td>ARSS</td>
<td>25.6a</td>
<td>23.4a</td>
<td>5.9a</td>
<td>39b</td>
<td>875c</td>
</tr>
</tbody>
</table>

Values are means of measurements made during the experimental period. Values within a column followed by different capital letters are significantly different at p<0.01 and by different lower case letters are significantly different at p<0.05 according to Duncan's multiple range test.

Discussion

The lack of irrigation during the vegetative and reproductive phases considerably reduced the soil water content and leaf water potential of pepper. Apparently, under drought conditions, an urgent need for plants would be to increase the uptake of water by lowering leaf water potential. Actually, osmotic adjustment allows plants to absorb water also under low soil water availability conditions. Photosynthesis and stomatal conductance were lower in water stressed treatments than in the full watered control. Under drought or any other abiotic stress, there is a significant decrease in photosynthesis and, consequently, a reduction in the amount of metabolites and energy (Kulkarni and Prakul, 2009). In this experiment, bell pepper under drought conditions had a small but significant decrease in leaf water potential and a higher decrease in gas exchange parameters. This is so because stomatal closure in some crops is a drought avoidance response that allows leaf water content maintenance (Ludlow, 1980) but the consequent decrease of internal CO₂ concentration (Ci) limits photosynthesis (Chaves, 1991; Cornic, 2000; Flexas and Medrano, 2002; Lawlor, 2002; Lawlor and Cornic, 2002). This decrease in Ci value may induce reversible inhibition of some enzymes such as Sucrose-Phosphate Synthase (SPS), a highly regulated enzyme that plays a key role in plant source-sink relationships. At the same time, starch content decreases and reducing sugars are maintained or even increased and this change in the carbohydrate status can lead to alterations of gene expression (Chaves and Oliveira, 2004). Photosynthetic limitation was sufficient to reduce plant growth and fruit yield as we observed in our experiment. In fact fruits are a stronger sink for assimilates than vegetative parts of plant.

The analysis of data shown in Table 4, concerning the effect of water stress during the vegetative (VSS treatment) and reproductive (VSS treatment) phases on flowering, setting and pepper yield, point out that, although for different reasons, both phases are equally sensitive to water stress in this crop.

The significant yield decrease in the treatment stressed during the vegetative phase compared to the C treatment has to be related to the lower number of flowers...
per plant measured in the first treatment. This is due to the fact that the water stress imposed in the latter treatment significantly reduced leaf number and area and also the branch number that dyecomically form during this phase as a function of water availability. Considering that flowers develop from leaf and branch axils, it is clear that the lower plant growth due to water stress in the vegetative phase inevitably damaged the potential productivity, despite optimal water availability was restored during the next reproductive phase.

On the contrary, the significant yield decrease measured in the stressed treatment during the reproductive stage was related to the dramatic drop of pepper average weight which, together with the great incidence of tip rot, have practically nullified the marketable yield. In fact, optimal water conditions maintained in this treatment during the vegetative phase allowed plants to produce a great number of flowers. From flowering on, this determined a great competition for produced assimilates which were significantly reduced by the concurrent water stress, as shown by the photosynthetic measurements. Under water stress conditions, the inability to adequately feed the fruits during the pepper growth phase was then the main reason for the observed significant yield decrease. In both treatments (VSS and RSS), the set flower percentage did not significantly differ from the control treatment. Such result is equal to that recorded in eggplant, in similar experimental conditions (Lovelli et al., 2007), this would point out a good tolerance of setting process to water stress. However, these results require further studies because they seem to be offbeat in comparison with what is generally reported in literature (Katerji et al., 1993; Dorji et al., 2005), about the great sensitiveness of that phase to water stress. According to Katerji et al. (1993) pepper plants are sensitive to water stress during flowering and fruit growth. Dorji et al. (2005) reported similar results studying partial root drying in hot pepper under water stress.

Actually, even if the periods previous to flowering and flowering itself, have been described as the most susceptible stages to water deficit in several crops resulting in lower yields, some authors (Jaimez et al., 2000) underlined that low water availability during the period between flowering and fruit development reduces final fruit yield. Even though water availability is determinant during both stages, a water deficit during fruit development affects final fruit yield to a greater extent (Jaimez et al., 2000). Maybe, competition for water from developing reproductive sinks during fruit growth may have caused the final fruit size reduction. On the contrary, Saini and Westgate (2000) underlined that even though all the reproductive sub-phases are sensitive to water deficit, a water stress occurring during the earliest reproductive stages causes seed and/or flower number reduction. However, water deficit conditions which take place later on, during the reproductive phase, determine a dimension rather than seed and/or flower number decrease. The values reported in Table 4 show that in bell pepper, water deficit effect on final production is significant.

Being flowering stopped in this species, the susceptibility period to water stress is extended, and the water shortage negatively and markedly affects all yield components, as we already measured on eggplant (Lovelli et al., 2007). On Bell pepper, water stress during the vegetative and fruiting stage decreases LA, dry matter and yield. Similar results were obtained by Kulkarni and Phalke (2009), Dorji et al. (2005), Kang et al. (2001) and Ismail et al. (2002) in pepper, sweet pepper and hot pepper, reporting a consequently yield decrease under water stress conditions. In our study, in the RSS treatment, dry matter was reduced more than in the VSS treatment probably because the length of water stress period was greater in the first treatment (32 and 43 days for VSS and RSS treatments, respectively). At leaf level, water use efficiency is higher when water supply is reduced. In study, water use efficiency and Yield Water Use Efficiency, are basically conservative parameters, according to Hanks (1983) and Steduto and Albrizio, (2005). Yield water use efficiency was smaller in the RSS treatment due to the unavoidable not marketable yield occurring at harvest.

CONCLUSION

Obtained results show a great sensitiveness of this horticultural crop to water stress. Water stress negatively affected most of the studied physiological parameters, both during vegetative and reproductive phases. Unlike what is generally reported in literature, in our study setting was scarcely affected by water stress. However, the result of our study requires a further in-depth study at field level. If this would be confirmed the attention on irrigation scheduling should not be mainly paid on this phase, as it is usually done, but on the whole growing crop cycle.

REFERENCES
