COST ACTION FA1204

Vegetable Grafting to Improve Yield and Fruit Quality Under Biotic and Abiotic Stress Conditions

STSM Scientific Report

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STSM Topic: Interactions of shading and rootstock/scion combination on physiological and antioxidant parameters and fruit quality aspects of grafted pepper plants grown in greenhouse in a spring-summer crop

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1. Aim of the STSM

Bell pepper (*Capsicum annuum* L.) is a vegetable crop that when grown during summer in greenhouse conditions in the Mediterranean Basin responds positively to the application of shading technique. During summer season, shading allows to reduce the thermal stress and to prevent damage to the photosynthetic apparatus. However, shading causes a reduction of photo-assimilates which may reduce shoot and root growth and consequently the absorption of water and nutrients. It can be hypotized that grafting combinations can respond in a different way to shading depending on the shoot and root demand of photo-assimilates for growth and maintenance respiration. Moreover, shading can also affect fruit quality reducing photo-assimilate availability for fruit growth and accumulation of sugars. Therefore, the objective of this research is to study the responses of different pepper rootstock/scion combinations to different solar radiation intensities with respect to yield, fruit quality, and several physiological parameters related to the antioxidant defense mechanisms, in a spring-summer crop in greenhouse. The results of the STSM will be of great interest for all working groups in particular to WG3 (Rootstock-mediated resistance to biotic and abiotic stresses) and WG4 (Rootstock-mediated improvement of fruit quality) of the COST Action FA1204.

2. Research activity

The experiment was carried out at the experimental greenhouse of the Department of Crop Science, Laboratory of Vegetable Crops-University of Athens, located in Athens (Greece).'Sondela' and

'Orangery' F1 bell peppers self-grafted and grafted onto 'Rocal' rootstock were compared under non-shaded and shaded conditions. The experiment was conducted into two greenhouse compartments (non-shaded and shaded). In each compartment, 12 NFT (nutrient-film-technique) closed-loop hydroponic circuits (experimental plots) were used (Fig.1). Each hydroponic circuit was an independent NFT unit comprising one channel that was fed with nutrient solution (NS) from a dedicated 'supply-tank' via a pump. The level of NS in the supply-tank was fed by a 'replenishment tank' positioned above it to compensate for the NS consumed by the plants through the transpiration process. All the basic NS used in this experiment were prepared using water having an EC of 0.32 dS/m and a pH of 7.3. The pH, the electrical conductivity (EC) and the mineral composition were 5.6 and 2.6 dS/m, and 5.6 and 2.1 dS/m in the starter and replenishment NS, respectively. The pH and EC of the recirculating NS were measured every two days and if necessary, they were adjusted to the target values. Solar radiation intensity, temperature and relative humidity inside and outside the greenhouse were measured in real-time by sensor and recorded in a data-base. Treatments were defined by a two factorial experiment in three randomized replications. The first factor had two levels (shaded and non-shaded conditions), whereas the second factor had four levels (self-grafted 'Orangery', self-grafted 'Sondela', 'Orangery'/'Rocal', and 'Sondela'/'Rocal'). Each experimental unit consisted of nine plants. The experiment in the greenhouse started on 21/04/2016 and ended on 18/07/2016.

My activity involved the laboratory analysis, through use the atomic absorption spectrophotometer for measurements (Ca, Mg, Fe, Mn and Zn), flame photometer (K and Na) and spectrophotometer (B, P and NO_3) measurements. For P and K determination samples of plant tissue were dried in a forced-air oven at 70^{o}C to constant weight. Subsequently sub-samples of all dried plant tissue sample were used for chemical analysis to determine the P and K concentrations. The dried tissue samples were powdered and then the powdered material was placed in a muffle furnace at 550 ^{o}C for 5 hours and the ash was used to extract P and K by means of 1 N HCl. P was measured photometrically as phosphomolybdate blue complex at 880 nm using a 96-position microplate spectrophotometer (Anthos Zenyth 200; Biochrom, USA). The K concentrations in the aqueous extracts were determined by flame photometry using a Sherwood Model 420 (Sherwood Scientific, Cambridge UK). Ca, Mg, Fe, Mn and Zn content were determined by atomic absorption spectrophotometer (Perkin Elmer 110 B, Waltham, MA) and Na, K content by flame photometry (Sherwood Model 410, Cambridge, UK). The NO_3 and NH_4 concentrations in the sample extracts were measured by UV/VIS spectroscopy at 220 and 653 nm respectively, using a 96-position microplate spectrophotometer (Anthos Zenyth 200; Biochrom, USA). The nitrate NO_3 in the nutrient solution was determined by applying the copperized cadmium reduction method (Griess-llosvay procedure), as described by Page et al. (1982) at the different

devolopmental stages. At the same sampling dates, determination of ammonium NH_4 in nutrient solution samples was conducted using the indophenol blue method as described by Page et al. (1982). Furthermore my activity include the assessment of the total phenolic content and total antioxidant capacity of fruits. In order to assess the total phenolic content and antioxidant capacity of the fruits was a necessary preliminary extraction. It has been used a method that employs an ethanol/water 70:30 solution, which is reported to have good extraction capacity of the compounds (Rababah T.M. et al., 2010). The procedure used was the following: solubilization of 1g of extract in 8 ml of ethanol/water and agitation with vortex; incubation in the thermostatic bath at 40 C^o for 20 minutes and agitation by vortex after 10 minutes; centrifugation at 3000 g for 5 minutes; filtration and collection of the supernatant; union of the supernatant. Then it proceeded to the evaluation of the total phenolic content using the method Folin-Ciocalteau (Singleton V.L. and Rossi J. A., 1965), with some modifications that allow the use of 96 well plates as described by Dicko M.H. et al. (2002). The reagent of Folin-Ciocalteau consists of a mixture of phosphotungstic acid and phosphomolybdic acid. The hydroxyl groups of the phenolic compounds contained in the sample, in moderately alkaline medium, are oxidized by the reagent to quinone while the acids of the reagent are reduced giving a blue complex. The blue coloration produced has a maximum absorption around to 760 nm. Initially they were pipette into each well 45 µl of water, and then were added 5 µl of the extract and 25 µl of reagent Folin- Ciocalteau 50% in water (v/v). After 5 minutes of incubation, they were added 25 µl of Na_2CO_3 20% and 100 µl of water up to a final volume in each well of 200 µl. The white whose absorbance was subtracted from each reading, was prepared by replacing the water to the sample. The absorbance was measured after 60 minutes at 750 nm with a microplate reader Infinite M200 (Tecan Mannedorf , Switzerland). The gallic acid was used for calibaration. The final concentration of phenols was expressed in mg gallic acid equivalents, chosen as the reference phenol, for g of sample. Such equivalence is made possible by the conversion of the values made on the basis of a calibration curve constructed from a series of solutions of known concentration and gallic acid variable. Instead the evaluation of total antioxidant capacity was carried out using the method ABTS (Re R. et al., 1999), spared on the ability of antioxidant molecules present in the sample to perform an action of quenching against the radical cation 2,2'-azino-bis (3 etilbenzotiazolin-6-sulfonico), a blue-green chromophore that has a characteristic absorption at 734 nm. The radicalization of ABTS was performed by the addition of potassium persulphate to an aqueous solution of ABTS. The solution thus obtained was kept in the dark for at least 6 hours before use to allow complete radicalization. Is the reaction with the antioxidants of the sample to cause the ABTS reaction that deradicalizes and then decolorized , with consequent variation of absorption. The intensity of the coloration is proportional to the concentration of antioxidant present, in accordance with the law of Lambert-Beer. The anti-radical

action of the fruits was evaluated by comparing with a suitable standard such as trolox, a water-soluble analogue of Vitamin E, and was expressed in µmoli of trolox equivalents TE/g of fresh product. The calibration curve was constructed using trolox solution in ethanol at scalar concentrations (0,5 mM, 1 mM, and 1,5 mM).

Currently the results obtained from the analyzes are being statistical processing.

Figure 1. Experimental plots

3. Future collaboration with the host institution (if applicable):

The research work conducted within the frame work of this STSM is considered a good basis for future collaboration between the home and the host in situation on topics related to grafting of vegetables. Discussions about this possibility have resulted in some plans for joint research activity and joint submission of research projects in the near future.

4. Foreseen publications/articles resulting from the STSM (if applicable):

After completion of the experiment and the laboratory analyses, the data are expected to be used for writing a paper on the responses of grafted vs. non-grafted pepper plants to the thermal stress in greenhouses.

5. Confirmation by the host institution of the successful execution of the STSM:

This is to certify, that the STSM applicant, Dr. Guido Bernabei has worked successfully on the experimental plan he presented in his STSM application. Dr. Guido Bernabei effectively contributed to the establishment of the experiment and carried out a major part of the experimental work and the measurements there after. He participated in many discussions about the ongoing experimental work and attended some lectures related to vegetable production and grafting. Although the experiment is going on, regarding the statistical analysis, the first findings are promising and we expect that they can be a sound basis for a good publication in a reputable international scientific journal.

The host scientist

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