P 149

The activity of a new glutamic acid-based biostimulant on the mitigation of the drought stress damages in lettuce (*Lactuca sativa* L.).

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INTRODUCTION

Due to global climate change, drought periods are getting more frequent than in the past and occur in countries rarely affected by them until the last decades. Nowadays, most of the resources involved in plant physiology research are focused on understanding the behavior of crops exposed to a diverse range of abiotic stresses. The role of agrochemical companies is to channel the knowledge coming from research in order to get effective and sustainable solutions for farmers. The anti-stress activity of GHI_16_VHL (GREIT VG), a biostimulant based on plant-derived amino acids containing 15% of glutamic acid, has been recently investigated. Glutamic acid is an amino acid also known to be the precursor of a diverse range of other amino acids, among which proline, an amino acid playing a beneficial role in plants exposed to various stress conditions. In fact, under water deficit conditions, proline accumulation occurs at major part from glutamate (Delauney and Verma, 1993). The aim of this study was to evaluate the activity of GREIT VG in the mitigation of drought stress effects in lettuce (*Lactuca sativa* L.) under both agronomic and biochemical point of view. Biochemical parameters were investigated since the stress resilience in plants is often related to changes of endogenous ROS-scavenging activity. Furthermore, due to the above mentioned proline properties, the opportunity to boost the anti-stress activity of GREIT VG by adding L-proline was also taken into account.

Fig.1. Growth chamber of Green Has Italia

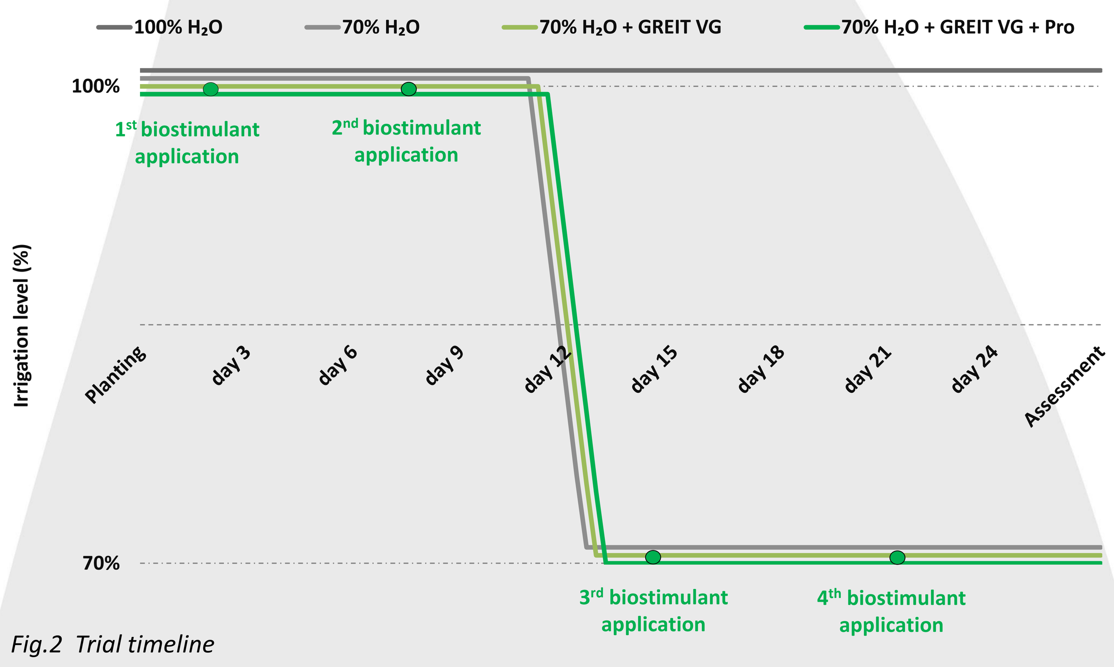

Fig.2 Trial timeline

MATERIALS AND METHODS

Eighty lettuce plug plants (cv. Canasta) were transplanted at 3-true-leaf stage in 1.5 l plastic pots filled with expanded clay (3-8 mm) and grown in a walk-in growth chamber under 15/9 h light/dark cycle, with 200 µmol photons/m²/s, at 25/20°C and 60% relative humidity. Half strength Hoagland solution was applied once a week (30 ml/pot). Plants were divided into four treatments: 1) 100% H_2O, 2) 70% H_2O, 3) 70% H_2O + GREIT VG , 4) 70% H_2O + GREIT VG + L-proline (Pro). A randomized block design with five replicates was adopted and each plot consisted in 4 lettuce plants (20 plants per treatment). For two weeks all plants were watered every second day with 50 ml of deionized water. Afterwards, control plants were grown with the same irrigation volume whereas the other plants were subjected to a 30% irrigation volume reduction in order to induce a water stress. The biostimulants (GREIT VG and GREIT VG + L-proline) were root applied four times with a 7-day interval, starting from the day after the transplanting, in mixture with Hoagland solution (30 ml/pot). GREIT VG was applied at 2.5 ml/l (0.25%). The optimal dosage of L-proline (0.1 mM) resulted from several tests where increasing dosages of L-proline (0.1, 0.5, 1, 5, 10 mM) were tested. Two biostimulant applications were carried out before the irrigation volume reduction in order to get a priming effect on plants whereas the other two applications were carried out once the drought stress was induced. The final assessment was carried out 7 days after the fourth application. At that moment, several biometric parameters were recorded (leaf and root biomass, number of leaves, rosette diameter, leaf dry matter content). Afterwards, fresh plant material was collected in order to determine the photosynthetic pigment content as well as the amount of the most important stress-related compounds (phenolic compounds, proteins, non-protein thiols, H_2O_2) and the ROS-scavenging enzyme activity in leaves (see methods listed in Fig.3)

RESULTS AND DISCUSSION

As a consequence of the drought stress, treatment 2 (70% H_2O) showed statistically lower rosette fresh weight and diameter when compared to the unstressed treatment (100% H_2O) whereas these parameters were not affected by stress when the biostimulants were applied. Four applications of GREIT VG or GREIT VG + L-proline mitigated the negative effects due to a 30% reduction in irrigation volume, leading the rosette diameter and fresh weight to a level statistically comparable to the unstressed treatment (100% H_2O). This anti-stress activity was also demonstrated by the increase of the estimated water use efficiency (mg leaf biomass / ml irrigation water). This parameter was statistically higher, if compared to the relative control (70% H_2O), in those plants where the biostimulants were applied. The determination of the leaf dry matter content showed statistically higher values in plants belonging to treatment 2 (70% H_2O) as a consequence of a slight plant wilting. However, the plants treated with GREIT VG showed a leaf dry matter content statistically higher than the unstressed plants (100% H_2O) without showing any stress symptoms. Both total chlorophyll and carotenoids did not show statistical differences between treatments even if a slight increase was observed in plants treated with the biostimulants if compared to the relative control (70% H_2O). Phenolic compounds resulted statistically higher in all the stressed plants as a result of an oxidative damage even if in treated plants these values were numerically lower if compared with the relative control (70% H_2O). Also the total protein content resulted statistically higher in treated plants. From the biochemical point of view, the plants treated with GREIT VG showed an increase in the activity of POX and a decrease in the activity of CAT and SOD if compared with the unstressed control (100% H_2O). The synergic ROS-scavenging activity of POX and CAT resulted in a statistically lower level of H_2O_2 detected in plants treated with GREIT VG, with or without L-proline, if compared to both unstressed and stressed control.

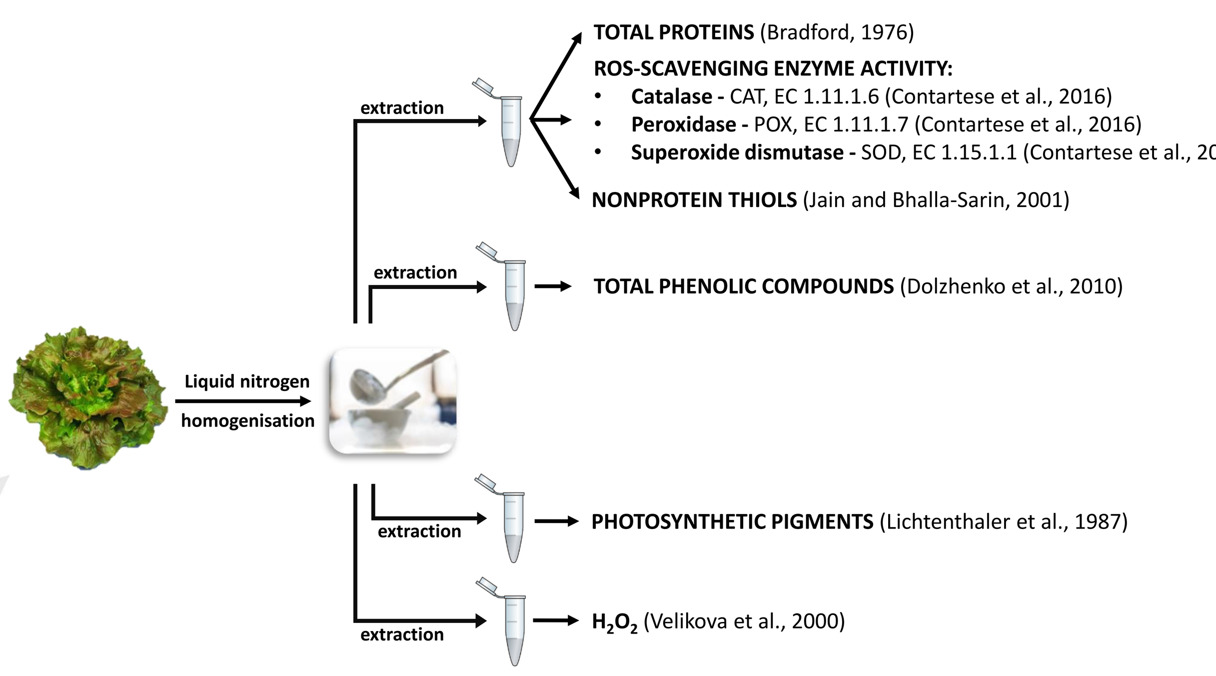

Fig.3 Analytical methods employed for the extraction and determination of stress-related compounds and ROS-scavenging enzyme activities

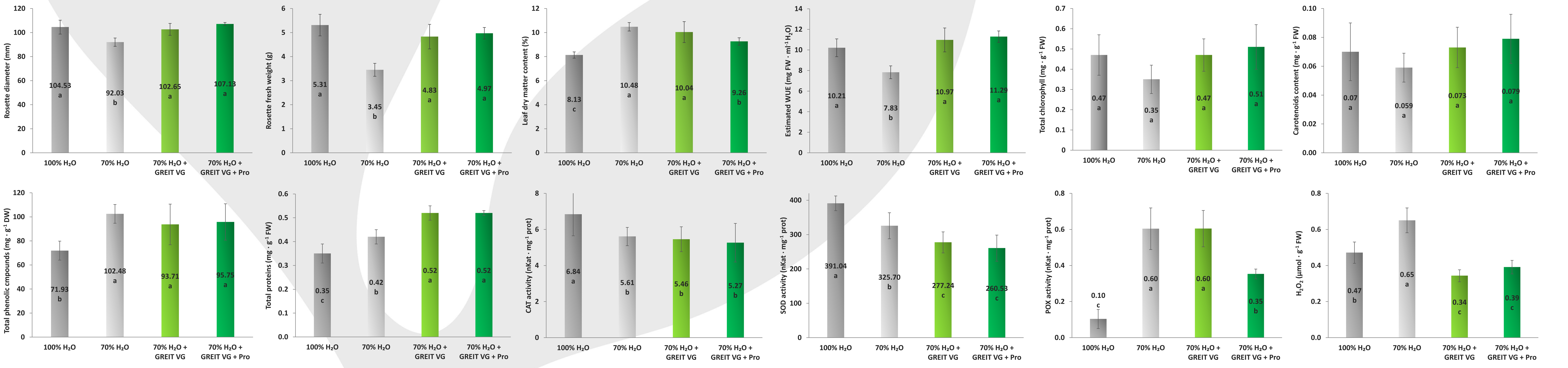

Fig.4 Effects of drought stress, Greit VG, L-proline (Pro) and their combination in biometric and biochemical parameters in lettuce. The bars represent the ± means standard deviations. Different letters indicate differences between treatments (Student-Newman-Keuls's Test, p=0.05)

CONCLUSIONS

The results of the current study demonstrate that four applications of GREIT VG on lettuce (two of which carried out to induce the priming effect) alleviate, and sometimes eliminate, the adverse effects of drought stress due to a 30% reduction in the irrigation volume. The anti-stress activity of GREIT VG is demonstrated by the biometric and biochemical parameters and confirmed by the enhanced plant water use efficiency (WUE). With regard to ROS-scavenging enzymes, the plants treated with GREIT VG showed an increase in the activity of POX and a decrease in the activity of CAT. Moreover, a decrease in the H_2O_2 content was observed probably as a consequence of the synergic activity of these two enzymes in the hydrogen peroxide detoxification. The activity of scavenger enzymes and the decrease in reactive oxygen species, including H_2O_2, might be related to a decrease in the level of stress promoted by the biostimulant application and confirmed by the good health of the treated plants. According to the results of these studies, as the addition of L-proline to GREIT VG did not generally show consistent booster effects on the anti-stress activity, we can state that GREIT VG can be applied on plants as stress reducer by itself, without the need for further upgrade.

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