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Proceeding Paper

Salinity Stress in Red Radish Crops †

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Abstract: Red radish (*Raphanus sativus* L.) has particular interest, not only for its particular taste, but also for its health-promoting properties. Most of them come from the presence of anthocyanins, glucosinolates and flavonols, whose benefits have been widely reported. However, little is known about how abiotic stress could affect the presence of these biomolecules in an adult plant of red radish. In the Region of Murcia, Spain, one of the mayor issues concerning due to edaphoclimatic and economic factors is salinity stress. For all that, this work aims to analyse the effect of salinity in red radish physiology and metabolism. To this point, a study based on hydroponic culture was designed to evaluate the effects of salinity (0, 40, 80 and 120 mM) in plant size, discerning between the bulb and the aerial part. Furthermore, RP-HPLC-DAD analysis was performed in order to determine secondary metabolites of red radish. Preliminary results suggest that salinity stress at high concentrations compromises the plant vegetative development. However, stresses are widely reported to stimulate the secondary metabolism, hindering the processes of finding a balance between nutritional value and production.

Keywords: glucosinolates; abiotic stress; red radish

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1. Introduction

Red radish (*Raphanus sativus* L.) is a root vegetable crop, member of the Brassicaceae family. Its root is consumed raw, in salad or pickled, and appreciated by its pungent flavor and crisp texture. In addition, it has been reported that this vegetable has plenty of phytomolecules that contribute to enhance human health, such as anthocyanins, vitamin C, and glucosinolates, as well as diverse micronutrients [1]. Specifically, glucosinolates not only regulate its final flavor (increasing its pungency from mild to strong), but also have been widely studied for its anti-tumorigenic, antioxidant and microbiome-regulating properties [2,3].

One of the main problems of soil degradation in culture areas under a semiarid Mediterraean climate is salinization. This process has been progressively increasing in the South-East of Spain during the last decades, drifting in a decrease in soil quality, yield and production, even leading to land abandonement [4]. Plant physiology and metabolites production could be affected by this changes in soil conditions, even the final flavor could be influenced if the glucosinolate production is altered [5]. Research performed in *Brassica* species have demonstrated that salinity mainly increased glucosinolates production, with few impact in its yield [6]. However, little to none information is available about the effect of soil salinity in red radish. Studies performed under drought stress reported a drecrease in total glucosinolate concentration [5].

In brief, the main aim of these work is to elucidate the effect of salinity stress in red radish physiological development and secondary metabolism.

2. Experiments

2.1. Growing Conditions

50 red radish seeds (*Raphanus sativus* L.) from SAKATA Seed Iberica S.L.U., were prehydrated and aireated in de-ionized water during 24 h. Then, seeds were transferred to vermiculite and kept in an incubator at 37 °C and darkness during 2 days. Seedlings were transferred to hydroponic culture with complete Hoagland Solution. When plants reached a 5–leaves stage (aprox. 3 weeks) they were harvested and leaves and the edible part (root) were processed separately. Samples were kept at −80 °C for further freeze-drying.

2.2. Extraction and Determination of Glucosinolates

100 mg of each freeze-dried powders were extracted with 1 mL methanol 70% (*v*/*v*) and heated in a bath at 70 °C for 30 min. Samples were centrifuged at 17,500× *g* for 15 min. Supernatants were collected, filtered thorugh a 0.22 µm Millex-millipore filter (Millipore, Billerica, MA, USA) and kept in vials. The ESI-HPLC-DAD-MS^2 fragmentation was analyzed in order to identify the different glucosinolates present in the red radish. The equipment employed was a Luna C18 100A column (150 × 1.0 mm, 3 µm particle size; Phenomenex Macclesfield, UK). Experimental conditions are described in Garcia-Ibañez et al. (2020). For further quantification intact glucosinolates were identified according their spectra and elution order, sinigrin and glucobrassicin were employed as external standards (Phytochem, Neu-Ulm, Germany).

2.3. Statistical Analysis

Statistical analysis was performed using a one-way ANOVA and a HSD Tukey as a *posthoc* test. All the analyses were performed in RStudio (version 4.0.2).

3. Results

3.1. Fresh Weigth Determination

Figure 1 shows the influence of threee different NaCl concentrations (40, 80, 120 mM) in the red radish fresh weight. According to the aereal part, a decrease by 2 times was observed when the treatments were compared with the control samples ($p < 0.05$). Nevertheless, no statistically significant differences were observed between the different saline treatments for the aereal part biomass ($p > 0.05$). Regarding to the roots, no statistically significant differences were observed betweeen control, 40 and 80 mM NaCl ($p > 0.05$). Howerver, when comparing the red radish roots from 120 mM NaCl treatment with the control, fresh weight decreased by a 38% ($p < 0.05$). Finally, whole plant (including the complete root, not only the edible part) fresh weight was meassured. An statistically significant decrease was observed between control plants and each treatment ($p < 0.05$). Specifically, treatments with 40 and 80 mM NaCl showed a similar decrease in a 35% when compared with control samples ($p < 0.05$). A decrease by a 63% was observed when compared the whole plant fresh weight from 120 mM NaCl with the control ($p < 0.05$).

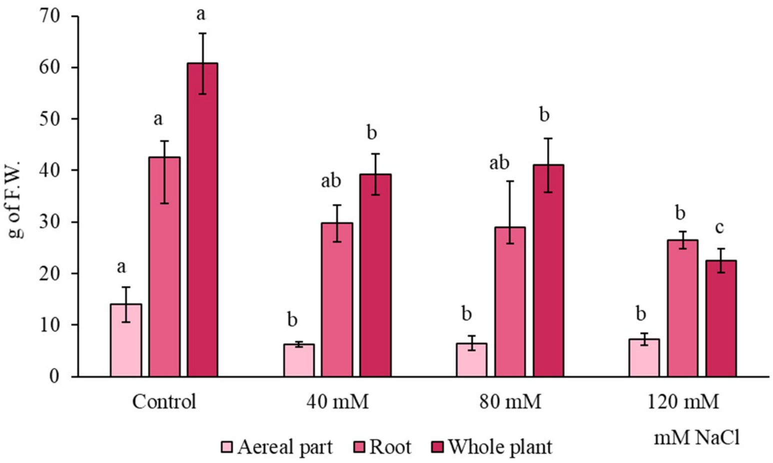

Figure 1. Influence of salinity (40, 80 and 120 mM) on fresh weight (g) from areal part, roots and whole plant of red radish (n = 5 ± SD). Different letters indicate statistically significant differences in the HSD Tukey test ($p < 0.05$).

3.2. Effect of Salinity in Glucosinolates Accumulation

Salinity effects in glucosinolate accumulation is showed in Figure 2. According to aliphatic glucosinolates accumulation (Figure 2A), concentrations were higer by 1.5 times in red radish roots under control conditions, when compared to the aereal part ($p < 0.05$). A 30% decrease was observed in the aereal part total aliphatic concentration when a treatment of 40 mM NaCl was applied ($p < 0.05$). Furthermore, under the same saline concentrations, a decrease of 55% was detected ($p < 0.05$). However, no remarkable statistically significant differences were present in the treatments with 80 and 120 mM NaCl when compaed to 40 mM samples ($p > 0.05$). Also, no differences were observed between the aereal part and the roots when the three salinity treatments were applied ($p > 0.05$).

Total indolic glucosinolate concentration is repreresented in Figure 2B. No statistically signigicant differences were found between the aereal part and roots in control plants ($p > 0.05$). Surprisingly, no indolic glucosinolates were detected in a quantifyable amount in roots from samples under salinity stress (40, 80 and 120 mM, $p < 0.05$). For the aereal part, an increase by 1.6 times was appreciated in red radish plans growed un 80 and 120 mM conditions ($p < 0.05$). However, no statistically significant differences were observed between 40 mM treatment and the control aereal parts ($p > 0.05$).

Finally, total glucosinolate concentration was analyzed (Figure 2C). Highest total concentrations were found in the control aereal part and roots ($p < 0.05$). A decrease by 26% was perciebed in the aereal part when a treatment of 40 mM NaCl was emplyed ($p < 0.05$). However, no differences were found for the aereal part under the three saline concentrations (40, 80 and 120 mM). A decrease by 2 times was observed when comparing 40 mM NaCl roots with control ones ($p < 0.05$). In adidtion, a decrease in a 15% was produce in total glucosinolate accumulation in roots when NaCl concentrations were increased up to 80 and 120 mM ($p < 0.05$). Nevertheless, no statistically significant differences were observed between those concentrations neither in roots nor the aereal part ($p > 0.05$).

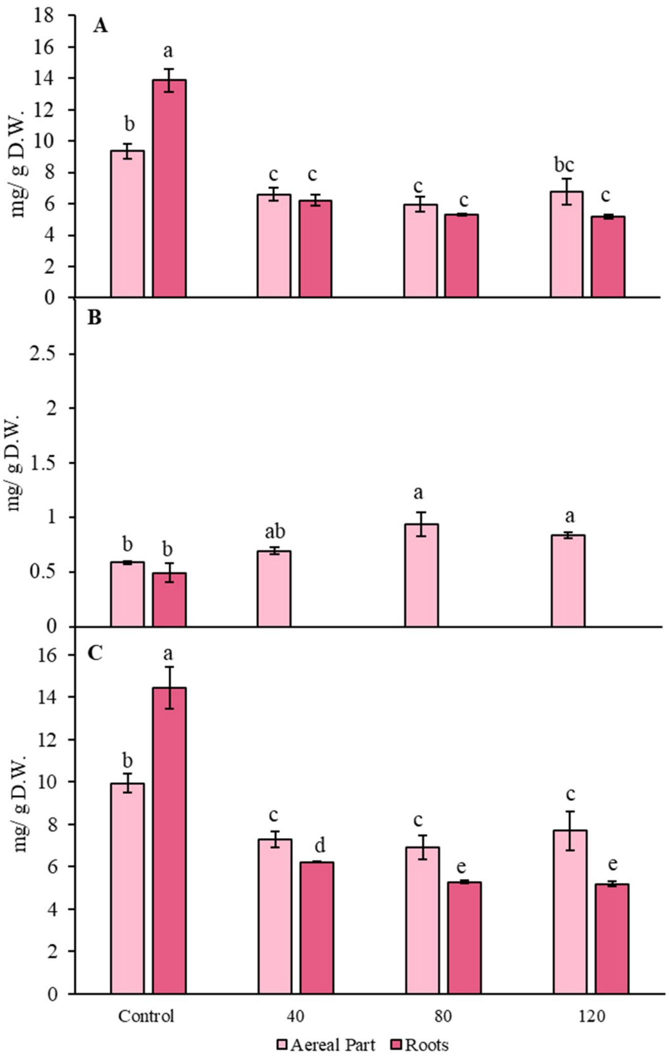

Figure 2. Presence of glucosinolates (mg/g D.W.) in the aereal part and roots under salinity conditions (0, 40, 80 and 120 mM). Quantifycation is from (**A**) Aliphatic; (**B**) Indolic and (**C**) total amount of glucosinolates (n = 5 ± SD). Different letters indicate statistically significant differences in the HSD Tukey test ($p < 0.05$).

4. Discussion

Salinity stress is one of the main concerns in the Region of Murcia agronomic practices, not only due to its increase by edaphoclimatic conditions, but also for its economical impact. Nevertheless, little information is available about the effect of salinity in red radish (*Rapahanus sativus* L.) yield and production.

Previous works performed in broccoli plants, showed a decrease by 60% in the fresh weight of the aereal part when 40 mM NaCl treamtents were applied [8]. In contrast, the results obtained in this work showed no statistically significant differences in the aereal part until 120 mM NaCl was applied. This could be due to the different physiognomy of both plants, even belonging to the same family (*Brassicaceae*).

About the presence of glucosinolates, broccoli experiments performed under field conditions in a semiarid Mediterranean climate showed no statistically significant differences between control and salinity treatments in both leaves and roots [9]. However, in our experiments, main decrease in total aliphatic glucosinolates concentration was observed between control and 40 mM NaCl plants (Figure 2A).

However, salinity in broccoli favoured the accumulation of indolic glucosinolates in the aereal part [9], which is similar to the pattern found in red raddish leaves (Figure 2B). Nevertheless, broccoli plants also showed an increase in roots measurements, which contrast the dramatically decrease observed in red radish roots. This could be due to the thickened root (bulb) morphology that shows the red radish. Similar results were observed in total glucosinolates content in broccoli demonstrating that this vegetable is able to accumulate this biomolecules under high salinity conditions [9]. Differences showed in red raddish could suggest a remobilization of these glucosinolates, maybe that are secreted to the media or soil under this stress condition Since glucosinolates exudation has been described under biostimulation conditions, it might be possible that saline stress triggers a similar response [10]. In addition, it must be taken into account that the experiments carried out in broccoli where perfomed in field where other factors might interfere, such as soil characteristics and climatic conditions. Since our experiment has been performed under controlled conditions, the results reflect only the effect of salinity in red radish metabolism.

5. Conclusions

In brief, our study has shown the negative the influence of high salinity concentrations in red radish biomass and yield. Furthermore, the type of glucosinolate which is sintethised under each salinity level and and in each organ could be an important factor of the metabolic response that should be studied deeply.

Author Contributions: the experiments were designed by D.A.M. and M.C., P.G.-I. carried out the experimental part. The formal data analysis was performed by P.G.-I. and D.A.M. P.G.-I. writted the first draft of the manuscript and D.A.M. and M.C. revised it. Funding adquisition by M.C. All authors have read and agreed to the published version of the manuscript.

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Conflicts of Interest: The authors declare no conflict of interest.

Abbreviations

The following abbreviations are used in this manuscript:

DW	dry weight
SD	standard deviation

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