Available in: http://www.redalyc.org/articulo.oa?id=303026202002

NaCl and *Phaeomoniella chlamydospora* affect differently starch and sucrose metabolism in grapevines

Helena Oliveira, Armando Costa and Conceição Santos[*]

*Departamento de Biologia, Centro de Estudos do Ambiente e do Mar, Universidade de Aveiro, Campus Universitário de Santiago, 3810-193, Aveiro, Portugal. *Author for correspondence. E-mail: csantos@ua.pt*

ABSTRACT. Vineyards production is often affected by diseases as esca and Petri disease and by excess of salt. *Phaeomoniella chlamydospora* is one of the pathogenic fungi associated to esca disease, but its interaction with the plant under excess of salt remains unknown. Under controlled *in vitro* conditions, *Vitis vinifera* L. plants were exposed to 0, 20 and 100 mM NaCl and inoculated with *P. chlamydospora.* Both inoculation and salt stress decreased the levels of chlorophylls, which was aggravated when both factors were combined. NaCl 100 mM and, mostly, inoculation, decreased maximum fluorescence (F_m), variable fluorescence (F_v) and F_v/F_m. The activity of α-amylase decreased in plants exposed to 100 mM or inoculated but no synergic effect of these factors was observed; the activity of sucrose synthase was inhibited only by inoculations, whereas invertase was stimulated at 20 mM, but decreased with inoculation. Data support that both excess of salt and fungi inoculation negatively affect photosynthesis and sucrose metabolism, and that they also decrease amylase activity, which may play an important role in the increased levels of starch found in inoculated plants.

Keywords: grapevine, *Phaeomoniella chlamydospora*, salt stress, starch metabolism, amylase, sucrose metabolism.

NaCl e *Phaeomoniella chlamydospora* afetam de forma diferente o metabolismo do amido e da sacarose em videiras

RESUMO. A produtividade das vinhas é frequentemente comprometida por doenças como a esca e a doença de Petri, e por injúria salina. *Phaeomoniella chlamydospora* é um dos fungos patogénicos associados à esca, mas a sua interação com a planta em condições salinas é praticamente desconhecida. Em condições *in vitro* controladas, plantas de *Vitis vinifera* L foram expostas a 0, 20 e 100 mM NaCl e inoculadas com *P. chlamydospora.* A inoculação e o stress salino mais severo decresceram os conteúdos de clorofilas a e b, sendo o efeito agravado pela combinação dos dois fatores. A salinidade (100 mM) e, sobretudo, a inoculação, diminuíram a fluorescência máxima (F_m), fluorescência variável (F_v) e F_v/F_m. A atividade da α-amilase decresceu em plantas expostas a 100 mM ou inoculadas, não se registando efeitos sinérgicos dos dois fatores. A atividade da sintase da sacarose foi inibida apenas pela inoculação; a atividade da invertase sofreu um estímulo em plantas expostas a 20 mM, salinidade moderada; mas decresceu significativamente com a inoculação. Demonstramos aqui que os dois danos, embora afetarem negativamente a fotossíntese e o metabolismo da sacarose, também inibem a amilase o que explicará o aumento de níveis de amido nestas plantas.

Palavras chave: videira, *Phaeomoniella chlamydospora*, salinidade, metabolismo do amido, amilase, metabolismo da sacarose.

Introduction

Vitis vinifera is the source of wine industry having an enormous economic importance in the Mediterranean (OLIVEIRA et al., 2009), South Africa (GOSZCZYNSKI, 2010), Australia, and North and South America (GADOURY et al., 2012) regions. Unfortunately, most of these regions present a combination of diseases and salinization, with high economical costs (OLIVEIRA et al., 2009).

The effects of salinity and osmotic stress in plants are well studied with the description of several physiological and biochemical disturbances (COELHO et al., 2010; FERNANDES et al., 2011; FLOWERS, 2004), such as increase of membrane permeability and lipid peroxidation (BRITO et al., 2003; MITTOVA et al., 2004, OLIVEIRA et al., 2009), decrease of both chlorophylls content and fluorescence (BARKA et al., 2004; SANTOS et al., 2005) or nutritional imbalances (SANTOS et al., 2002). However, there are still few reports on the effects of salt stress in grapevines (ALIZADEH et al., 2010; OLIVEIRA; SANTOS, 2011; OLIVEIRA et al., 2009; SINGH et al., 2000).

In some regions, as in many maritime coasts, vineyards grow on salt rich soils and simultaneously may also be exposed to esca/Petri disease related fungi.

Phaeomoniella chlamydospora and several species of *Phaeoacremonium* are associated with Petri disease, which affects the young grapevines. Esca is a more complex disease usually associated to older grapevines and besides *P. chlamydospora* and *Phaeoacremonium* sp. other fungi species are involved as *Fomitiporia punctata*, *F. mediterranea* and *Stereum hirsutum* (SANTOS et al., 2005). Recently, Gramaje et al. (2010) compared several species of fungi involved in esca and Petri disease and found that *P. chlamydospora* showed the highest ability to induce severe symptoms of those diseases.

We have described elsewhere some senescence effects observed in *in vitro* grapevines after inoculation with *Phaeoacremonium* species and *P. chlamydospora* (SANTOS et al., 2005; 2006a and b). We have also demonstrated by FT-IR that *in vitro* grapevines exposed to salt and *P. chlamydospora* showed the main changes in structural and non structural carbohydrates in leaves, with decreases of uronic acids contents and increases of glucose insoluble fraction levels, together with an increase of starch content (OLIVEIRA et al., 2009). We then hypothesized that the observed changes in FT-IR measured sugar levels could be due to stress-induced changes in starch metabolism (OLIVEIRA et al., 2009). Starch synthesis depends on photosynthesis, and it may be formed from sucrose. This sugar is a primary transport carbohydrate in higher plants, and subsequently converted into starch, by a series of enzymatic steps where sucrose synthase and invertases play primary roles (COUNCE; GRAVOIS, 2006; SCHAFFER; PETREIKOV, 1997).

In vitro cultures are not only excellent tools to micropropagate economically important species (BRONDANI et al., 2011; COUTINHO et al., 2011) but also provide optimization of extremely controlled conditions (ASSIS et al., 2012), minimizing somaclonal variation (MIGUEL; MARUM, 2011). These optimized conditions for plant growth are ideal to study the response of plants to environment, in particular plant response to salt stress (DOGAN et al., 2010) or to pathogens (SMALLEY; GURIES, 1993) because they minimize the effects of the complex environment present in the 'real scenario' (ZANZOTTO et al., 2008), and were recommended, preceding field assays (SANTOS et al., 2005, 2006b). This method also allows screening a large number of genotypes in short period and small areas. The more resistant lines could then be used for assays in the field.

The aim of this work was to test the hypothesis above reported, i.e., whether the increase of starch content induced by these two types of injuries previously described by Oliveira et al. (2009) were due to changes either in starch degradation, or in starch synthesis. For that, we used highly controlled conditions (*in vitro* culture) to ensure that any detected change was exclusively due to the biotic/abiotic factor in study.

Material and methods

Fungal material

Phaeomoniella chlamydospora (strain 1AS) was isolated from grapevines from the Bairrada Appellation of Portugal showing esca symptoms and was grown routinely on Malt Extract Agar (MEA) at 25°C in the dark as described by Santos et al. (2005, 2006a and b).

Plant material and growth conditions

Grapevine plants (*Vitis vinifera* L. cv. Baga) were supplied by Estação Vitivinícola da Bairrada (Portugal). After plant material disinfection in ethanol 70% (30 sec) followed by NaOCl 20% (20 min.), shoots were grown for 20 days on modified Murashige and Skoog medium (Duchefa) supplemented with 30 g L^{-1} sucrose, 8 g L^{-1} agar, 1.0 mg L^{-1} 6-benzilaminopurine (BAP) and the pH adjusted to 5.7 and then transferred to MS modified medium supplied with 0.02 mg L^{-1} 3-indolebutyric acid (IBA). Two-month-old plants were exposed to 20 and 100 mM NaCl for one month under controlled conditions. Groups of these plants growing in excess of salt were inoculated at the base of the stem with 4 µL of a suspension of conidia (10^7 conidia mL^{-1}) of *P. chlamydospora*. The control plants were inoculated with the same volume of sterilized distilled water. All plant cultures were kept under controlled conditions of temperature 24 ± 1°C, 75% relative humidity (%RH), a photoperiod of 16 h and a total light intensity of 488 μmol m^{-2} s^{-1} emitted by Osram 18W Lamps (OLIVEIRA et al., 2009). Samples from all conditions were harvested 0, 15 and 30 days after the beginning of exposure.

Chlorophyll content and fluorescence

For fluorescence analysis, plants were dark adapted for 30 min. in a growth chamber and fluorescence was monitored in expanded leaves using a Plant Efficiency Analyser (Hansatech Instruments Ltd., UK) by illuminating leaves with saturating light intensity. The basal non-variable chlorophyll fluorescence (F_0) and the maximal fluorescence induction (F_m) and the variable fluorescence ($F_v = F_m$-F_0) were determined under the conditions described by Azevedo et al. (2005) and then the maximum quantum yield of PSII (F_v/F_m) was estimated. From leaves used for fluorescence analyses, chlorophylls were extracted in 80% acetone and determined as described by Sims and Gamon (2002).

Enzyme activities assays

For enzyme activities determination, fresh samples were harvested and immediately treated: invertase (E.C.3.2.1.26) was determined spectrophotometrically according to Pfeiffer and Kutschera (1995). For the α-amylase (E.C.3.2.1.1) assay, samples were ground in acetate buffer 0.2 M (pH 4.8) at 4ºC for protein extraction and activity was determined spectrophotometrically according to Rich and Stegbauer (1984). For the sucrose synthase (EC 2.4.1.13) activity, samples were homogenized and enzyme activity determined spectrophometrically as described by Counce and Gravois (2006). Proteins were determined in protein extracts using a comassie blue kit (Sigma, USA) and using bovine serum albumin as standard.

Calculations and Statistical analysis

For each parameter, values are given as Mean ± Standard Deviation (SD) as calculated from data from two independent experiences (with at least six replicates in each experiment). The comparison between the treatments was made using a one-way Anova followed by Tuckey test. The correlations between some parameters analyzed were performed by the Pearson Correlation test. The level of statistical significance was set at p ≤ 0.05. All statistical procedure was performed using the software package SigmaStat (SigmaStat for Windows, v. 2.03).

Results

Plant morphology

Shoots from plants grown at 20 mM NaCl did not differ from those of controls, showing green leaves, whereas roots showed lower growth. Plants grown at 100 mM NaCl had reduced growth, leaf chlorosis with reddish colour appearing one week after the beginning of the experiment, and severe root growth inhibition. All these symptoms aggravated with time, and at the end of the experience, small necrotic spots were also visible. Inoculation alone induced intense reddish colour and necrotic spots in leaves (which appeared one week after the beginning of the experiment), and a decrease in the root system development. Excess of salt combined with inoculation worsened these symptoms, with inoculation and 100 mM NaCl leading to necrotic shoots and roots (Figure 1).

Chlorophyll content and fluorescence

Excess of salt decreased chlorophylls a and b contents in leaves through time, with minimum values at the end of the experiment (Figure 2).

Figure 1. *In vitro* grapevine plants 30 days after starting experiment: A) 0 mM (control); B) 20 mM and C) 100 mM NaCl treated plants; D) *P. chlamydospora* + 0 mM exposed plants; E) *P. chlamydospora* +20 mM exposed plants; F) *P. chlamydospora* + 100 mM exposed plants.
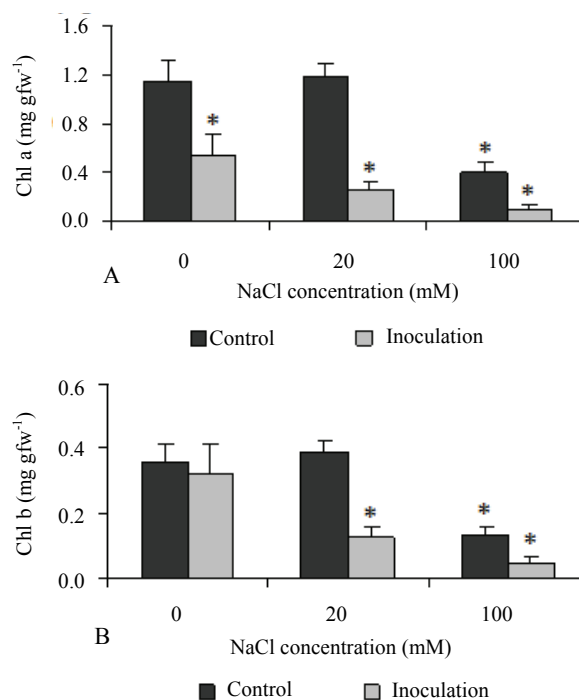

Figure 2. Effect of salt treatment and *P. chlamydospora* inoculation on *in vitro* grapevine leaf chlorophyll content: A) chlorophyll *a* (chl*a*); B) chlorophyll *b* (chl*b*);. Symbols ★ and ★★ indicate significantly different means between control and salt stressed and/or fungi inoculated individuals (p < 0.05 or p<0.01). Vertical bars represent the SD of mean (with at least six replicates each).

Inoculation also significantly reduced chlorophyll a and chlorophyll b contents and the ratio chlorophyll a/b ($p < 0.05$) during and after the treatment (Figure 2). The combination of salt stress and inoculation aggravated the decrease of chlorophyll content ($p < 0.05$) respecting to controls.

Inoculation and/or NaCl did not affect chlorophyll basal fluorescence ($p > 0.05$; Figure 3a). Contrarily, the F_m steadily decreased mostly in 100 mM NaCl treated plants and, mostly, in inoculated ones (Figure 3b). These negative changes were worsened in plants exposed to both conditions ($p < 0.001$). Therefore, decreases of F_v (i.e., F_m-F_0) and of the ratio F_v/F_m were observed in plants exposed to both salt and inoculation (Figure 3c and d).

Starch and sucrose metabolism

The highest dose of NaCl significantly decreased α-amylase activity ($p < 0.05$). Similarly, all inoculation conditions (with or without NaCl exposure) decreased α-amylase activity (Figure 4a). Sucrose synthase activity was in general not affected ($p > 0.05$) by moderate salt stress alone (20 mM), and only slightly by 100 mM. However, this enzyme activity was severely inhibited by inoculation ($p < 0.05$) (Figure 4b). Concerning invertase, an increase of its activity was observed for the mild salt stress 20 mM ($p < 0.05$), decreasing thereafter (Figure 4b). Similarly to sucrose synthase, the most severe effects were observed for inoculated plants ($p < 0.05$). At 100 mM the combined inoculation and salt excess led to the highest decrease of invertase activity ($p < 0.001$).

Discussion

The general analysis of plant growth in response to both stress conditions is in accordance with the quantitative and qualitative descriptions by Santos et al. (2005, 2006a and b) and Oliveira et al. (2009). The chlorosis found in leaves of stressed plants is related with the reduction in chlorophyll contents as a result of a decrease in chlorophyll synthesis or an increase in chlorophyll degradation or even due to a reduction in the chloroplast number. Salt stress and infection in combination aggravated the decrease of chlorophyll content respecting to controls.

The reduction in chlorophyll content by NaCl stress was also observed in some *Vitis vinifera* cultivars such as Cardinal, Delight, Beauty Seedless and Baga (OLIVEIRA et al., 2009) and in grape rootstocks Dogridge, SO4, H-144 and 3309C (ALIZADEH et al., 2010). Also the decrease of chlorophyll contents during an inoculation process with *Phaeoacremonium* sp. was previously recorded for grapevine (OLIVEIRA et al., 2009; SANTOS et al., 2005), and supports that

photosynthetic machinery suffers an impairment with inoculation and severe salt stress.
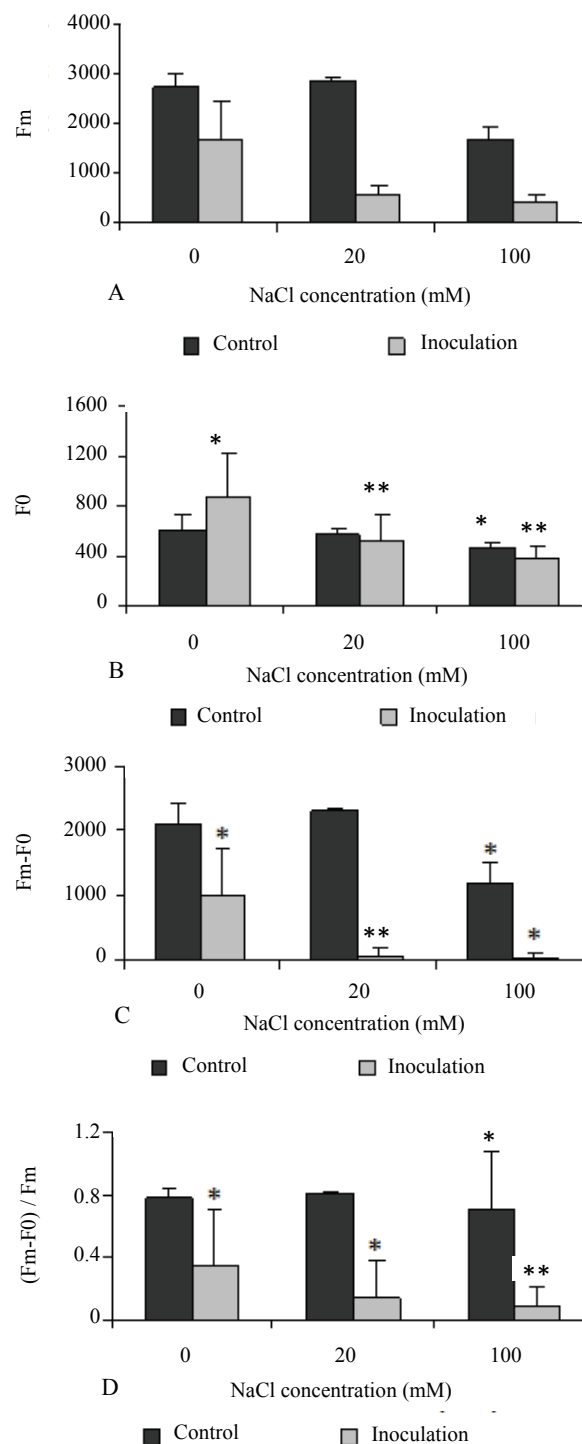

Figure 3. Effect of salt treatment and *P. chlamydospora* inoculation on *in vitro* grapevine leaf chlorophyll fluorescence: A) basal fluorescence (F_0); B): maximum fluorescence (F_m); C) variable fluorescence ($F_v = F_m$-F_0); D) ratio F_m-F_0/F_m. Symbols ★ and ★★ indicate significantly different means between control and salt stressed and/or fungi inoculated individuals for $p < 0.05$ and $p < 0.001$, respectively. Vertical bars represent the SD of mean (with at least six replicates each).
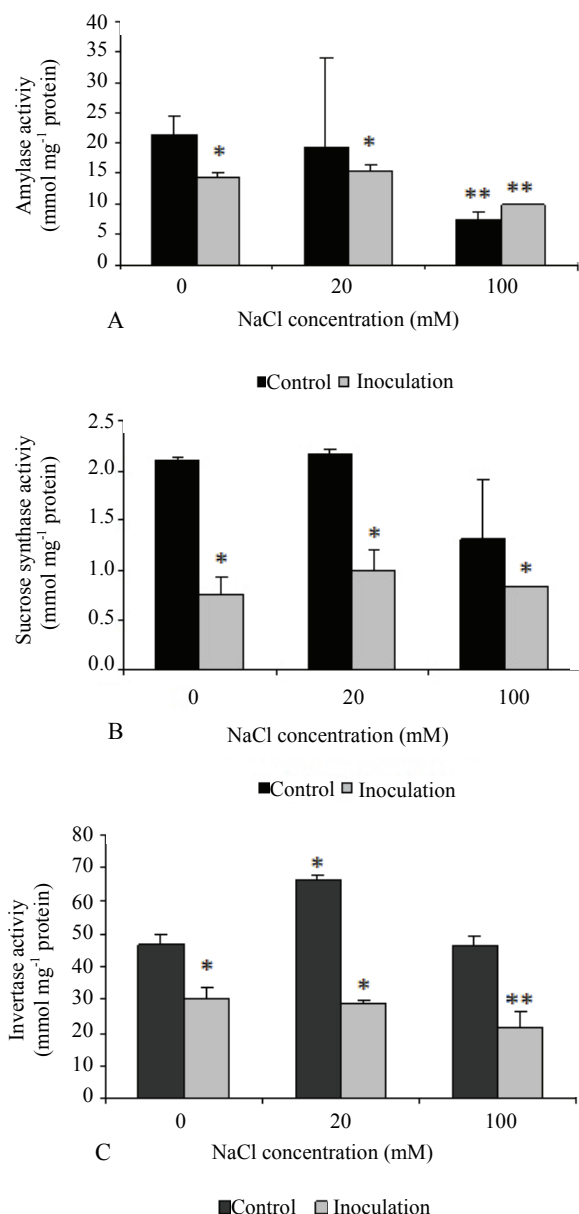
Figure 4. Effect of salt treatment and P. chlamydospora inoculation on *in vitro* grapevine α-amylase (A), sucrose synthase (B) and invertase activity (C). Symbols ★ and ★★ indicate significantly different m   eans between control and salt stressed and/or fungi inoculated individuals for p < 0.05 and p < 0.001, respectively. Vertical bars mean the SD of mean (with at least three replicates each).

No significant effect was found in F_0 in this assay, while a slight decrease was described by Santos et al. (2005). This apparent discrepancy may be related with factors as the cultivar genotypes or fungus strain, as these factors are important in host sensitivity to the fungus (SANTOS et al. 2005, 2006a and b). The maximum fluorescence (F_m) was a more sensitive parameter to both salt and inoculation stresses either alone or combined. Data presented here are in accordance with those obtained by Santos et al. (2005).

The decrease in the ratio F_v/F_m indicates variations on the photosynthetic efficiency of the Photosystem II and is also related with the decrease in F_m, reflecting an increase of energy dissipation by the destruction of the photosynthetic apparatus (MAXWELL; JOHNSON, 2000). These results demonstrate that inoculation with *P. chlamydospora* significantly decreases chlorophyll content and fluorescence, so affecting photosynthesis.

Moreover, the decrease in total chlorophyll content could correlate with a decrease in the F_v values (Pearson correlation, r = 0.929; p = 0.0073) and in those of F_m (Pearson correlation, r = 0.905; p = 0.0131). Most of these correlations were due to the changes in chlorophyll a (Pearson correlation, r = 0.939; p = 0.00551).

We have demonstrated elsewhere that malondialdehyde content and membrane integrity (solute and electrolyte leakages) were affected by salt stress and inoculation (OLIVEIRA et al., 2009). By analyzing the data reported by Oliveira et al. (2009) and the results presented here, we found that the loss of membrane integrity correlated negatively with chlorophyll maximum fluorescence (Pearson correlation, r = -0.882; p = 0.0199) and chlorophyll content (r = -0.959; p = 0.00250) suggesting that the membrane/thylakoid degradation occurred in the chloroplasts together with the loss of chlorophyll content and fluorescence.

Our previous studies also demonstrated that starch contents in grapevine leaves increased by *P. chlamydospora* inoculation, when compared with control plants (OLIVEIRA et al., 2009). Starch is the most common storage carbohydrate in plants and therefore, changes in its levels under stress indicate unbalances in the enzymes regulating starch biosynthesis and/or degradation. Sucrose is *in vivo* the main molecule of carbon transport, and is highly abundant in *in vitro* growing media. A main step of starch synthesis implies utilizing the hexoses of sucrose hydrolysis and ultimately the conversion of that carbon into starch (DALE; HOUSLEY, 1986). In our previous work, we found that some light dependent reactions of the photosynthetic process were affected in grapevines inoculated with this fungus (OLIVEIRA et al., 2009; SANTOS et al., 2005). We then hypothesised that once *P. chlamydospora* decreases the light dependent reactions of photosynthesis, affecting photosynthesis, then the increase of starch levels observed in leaves was mostly due to severe interference in starch metabolism, by reducing amylase activity and/or increasing starch synthesis. Starch may be produced from sucrose (an abundant component of any *in vitro* plant culture media),

where sucrose synthase and invertase may play important roles (SCHAFFER; PETREIKOV, 1997).

Our data support that the increase of starch is mostly due to the decrease of α-amylase as demonstrated here. Moreover, both sucrose metabolizing enzymes are more affected by fungus inoculation than by the excess of salt, suggesting therefore, that they also are more sensitive to infection than to salt stress. These enzymes are recognized to be important in tissues undergoing active growth, once they require hexoses as substrates for metabolic processes (e.g., glycolysis, biosynthesis of starch, triacylglycerides, or cell expansion) (HO, 1988; WINTER; HUBER, 2000). In particular, changes in sucrose synthase activity have been correlated with changes of cellulose content and of cell wall structure of wheat roots (ALBRECHT; MUSTROPH, 2003), and it should be interesting to research the activities of this enzyme in the roots of inoculated plants, which showed such reduced root system. The high susceptibility to inoculation of these enzymes represents constraints to these processes, namely starch synthesis from sucrose in infected plants. The putative correlation between these changes with the previously described changes in structural and non structural carbohydrates in leaves of inoculated grapevine plants also still needs further studies.

Conclusion

As conclusion our data support that inoculation with *P. chlamydospora* and salt stress function in synergy on some parameters of the photosynthetic apparatus, so reducing photosynthesis. Also, inoculation alone or combined with salt stress negatively affects two enzymes of the sucrose metabolism, sucrose synthase and invertase, jeopardizing its further use in starch synthesis. So, our data support that the previously reported increase of starch in inoculated plants is mostly due to a reduction of amylase activity.

Acknowledgements

References

ALBRECHT, G.; MUSTROPH, A. Sucrose utilization via invertase and sucrose synthase with respect to accumulation of cellulose and callose in wheat roots under oxygen deficiency. **Russian Journal of Plant Physiology**, v. 50, n. 6, p. 813-820, 2003.

ALIZADEH, M.; SINGH, S. K.; PATEL, V. B.; BHATTACHARYA, R. C.; YADAV, B. P. *In vitro* responses of grape rootstocks to NaCl. **Biologia Plantarum**, v. 54, n. 2, p. 381-385, 2010.

ASSIS, K. C.; PEREIRA, F. D.; CABRAL, J. S. R.; SILVA, F. G.; SILVA, J. W.; CORREA DOS SANTOS, S. *In vitro* cultivation of *Anacardium othonianum* Rizz.: effects of salt concentration and culture medium volume. **Acta Scientiarum. Agronomy**. v. 34, n. 1, p. 77-81, 2012.

AZEVEDO, H.; GOMES, C.; PINTO, G.; FERNANDES, C.; FERNANDES, J.; LOUREIRO, S.; SANTOS, C. Cadmium effects on sunflower growth and photosynthesis. **Journal of Plant Nutrition**, v. 28, n. 12, p. 2211-2220, 2005.

BARKA, E.; EULLAFFROY, P.; CLEMENT, C.; VERNET, G. Chitosan improves development and protects *Vitis vinifera* against *Botrytis cinerea*. **Plant Cell Reports**, v. 22, n. 8, p. 608-614, 2004.

BRITO, G.; COSTA, A.; FONSECA, H.; SANTOS, C. Response of *Olea europaea* ssp. *maderensis in vitro* shoots exposed to osmotic stress. **Scientia Horticulturae**, v. 97, n. 3-4, p. 411-417, 2003.

BRONDANI, G. E.; DUTRA, L. F.; WENDLING, I.; GROSSI, F.; HANSEL, F. A.; ARAUJO, M. A. Micropropagation of an *Eucalyptus* hybrid (*Eucalyptus benthamii* x *Eucalyptus dunnii*). **Acta Scientiarum. Agronomy**, v. 33, n. 4, p. 655-663, 2011.

COELHO, D. L. M.; TORQUATO DE AGOSTINI, E. A.; GUABERTO, L. M.; NETO, N. B. M.; CUSTÓDIO, C. C. Estresse hídrico com diferentes osmóticos em sementes de feijão e expressão diferencial de proteínas durante a germinação. **Acta Scientiarum. Agronomy**, v. 32, n. 3, p. 491-499, 2010.

COUNCE, P.; GRAVOIS, K. Sucrose synthase activity as a potential indicator of high rice grain yield. **Crop Science**, v. 46, n. 4, p. 1501-1507, 2006.

COUTINHO, O. L.; REGO, M. M.; RAMALHO DO REGO, E.; KITAMURA, M. C.; MARQUES L. F.; FILHO, L. P. F. Desenvolvimento de protocolo para microenxertia do tomateiro *Lycopersicon esculentum* Mill. **Acta Scientiarum. Agronomy**, v. 32, n. 1, p. 87-92, 2011.

DALE, E.; HOUSLEY, T. Sucrose synthase activity in developing wheat endosperms differing in maximum weight. **Plant Physiology**, v. 82, n. 1, p. 7-10, 1986.

DOGAN, M.; TIPIRDAMAZ, R.; DEMIR, Y. Effective salt criteria in callus-cultured tomato genotypes. **Zeitschrift für Naturforschung C, Journal of Biosciences**, v. 65, n. 9-10, p. 613-618, 2010.

FERNANDES, P. D.; BRITO, M. E. B.; GHEYI, H. R.; FILHO, W. S. S.; MELO, A. S.; CARNEIRO, P. T. Crescimento de híbridos e variedades porta-enxerto de citros sob salinidade. **Acta Scientiarum. Agronomy**, v. 33, n. 2, p. 259-267, 2011.

FLOWERS, T. Improving crop salt tolerance. **Journal of Experimental Botany**, v. 55, n. 396, p. 396-409, 2004.

GADOURY, D.; CADLE-DAVIDSON, L.; WILCOX, W.; DRY, I.; SEEM, R.; MILGROOM, M. Grapevine powdery mildew (*Erysiphe necator*): a fascinating system for the study of the biology, ecology and epidemiology of an obligate biotroph. **Molecular Plant Pathology**, v. 13, n. 1, p. 1-16, 2012.

GOSZCZYNSKI, D. Rugose wood-associated viruses do not appear to be involved in Shiraz (Syrah) decline in South Africa. **Archives of Virology**, v. 155, n. 9, p. 1463-1469, 2010.

GRAMAJE, D.; GARCÍA-JIMÉNEZ, J.; ARMENGOL, J. Field Evaluation of Grapevine Rootstocks Inoculated with Fungi Associated with Petri Disease and Esca. **American Journal of Enology and Viticulture**, v. 61, n. 4, p. 512-520, 2010.

HO, L. Metabolism and compartmentation of imported sugar in sink organs in regulation to sink strength. **Annual Review of Plant Physiology and Plant Molecular Biology**, v. 39, p. 355-378, 1988.

MAXWELL, K.; JOHNSON, N. Chlorophyll fluorescence: a practical guide. **Journal of Experimental Botany**, v. 51, n. 345, p. 659-668, 2000.

MIGUEL, C.; MARUM, L. An epigenetic view of plant cells cultured in vitro: somaclonal variation and beyond. **Journal of Experimental Botany**, v. 62, n. 11, p. 3713-3725, 2011.

MITTOVA, V.; GUY, M.; TAL, M.; VOLOKITA, M. Salinity up regulates the antioxidative system in root mitochondria and peroxisomes of the wild salt tolerant tomato species *Lycopersicon poennellii*. **Journal of Experimental Botany**, v. 55, n. 399, p. 1105-1113, 2004.

OLIVEIRA, H.; SANTOS, C. An integrative view of sodium chloride stress and *Phaeomoniella* sp. Inoculation on growth and nutrient accumulation and patterning in *in vitro* grapevine plants. **Journal of Plant Nutrition** v. 34, n. 4, p. 557-572, 2011.

OLIVEIRA, H.; BARROS, A.; DELGADILLO, I.; COIMBRA, M.; SANTOS, C. Fourier transformation infrared spectroscopy analysis of salt stressed and fungi infected *in vitro* grapevine plants. **Environmental and Experimental Botany** v. 65, p. 1-10, 2009.

PFEIFFER, I.; KUTSCHERA, U. Sucrose metabolism and cell elongation in developing sunflower hypocotyls. **Journal of Experimental Botany**, v. 46, n. 6, p. 631-636, 1995.

RICH, W.; STEGBAUER, H. P. a-Amylase measurement of reducing groups. In: BERGMEYER, H. V. (Ed.). **Methods of enzymatic analysis**. New York: Verlag Chimie Academic Press Inc., 1984. v. 2, p. 285-288.

SANTOS, C.; FRAGOEIRO, S.; PHILLIPS, A. Physiological response of grapevine cultivars and a rootstock to infection with *Phaeoacremonium* and *Phaeomoniella* isolates: an *in vitro* approach using plants and calluses. **Scientia Horticulturae**, v. 103, p. 187-198, 2005.

SANTOS, C.; SILVA, S.; PHILLIPS, A. Phenotypic characterisation of *Phaeoacremonium* and *Phaeomoniella* strains isolated from grapevines: enzyme production and virulence of extra-cellular filtrate on grapevine calluses. **Scientia Horticulturae**, v. 107, n. 2, p. 123-130, 2006a.

SANTOS, C. V.; FALCÃO, I.; PINTO, G.; OLIVEIRA, H.; LOUREIRO, J. Nutrient responses and glutamate and proline metabolism in sunflower plants and calli under Na_2SO_4 stress. **Journal of Plant Nutrition and Soil Science**, v. 165, n. 3, p. 366-372, 2002.

SANTOS, C.; FRAGOEIRO, S.; SILVA, S.; OLIVEIRA, H.; PHILLIPS, A. Response of *Vitis vinifera* L. plants inoculated with *Phaeoacremonium angustius* and *Phaeomoniella chlamydospora* to thiabendazole, resveratrol and sodium arsenite. **Scientia Horticulturae**, v. 107, n. 2, p. 131-136, 2006b.

SCHAFFER, A.; PETREIKOV, M. Sucrose-to-Starch metabolism in tomato fruit undergoing transient starch accumulation. **Plant Physiology**, v. 11, n. 3, p. 739-746, 1997.

SIMS, D.; GAMON, J. Relationships between leaf pigment content and spectral reflectance across a wide range of species, leaf structures and developmental stages. **Remote Sensing of Environment**, v. 81, n. 2-3, p. 337-354, 2002.

SINGH, S.; SHARMA, H.; GOSWAMI, A.; DATTA, S.; SINGH, S. *In vitro* growth and leaf composition of grapevine cultivars as affected by sodium chloride. **Biologia Plantarum,** v. 43, n. 2, p. 283-286, 2000.

SMALLEY, E. B.; GURIES, R. Breeding elms for resistance to Dutch Elm Disease. **Annual Review of Phytopathology**, v. 31, p. 325-352, 1993.

WINTER, H.; HUBER, S. C. Regulation of sucrose metabolism in higher plants: Localization and regulation of activity of key enzymes. **Critical Reviews in Plant Sciences**, v. 35, n. 4, p. 253-289, 2000.

ZANZOTTO, A.; GARDIMAN, M.; LOBAT, L. Effect of *Phaeomoniella chlamydospora* and *Phaeoacremonium* sp. on *in vitro* grapevine plants. **Scientia Horticulturae**, v. 116, n. 4, p. 404-408, 2008.