

In Vitro Culture as a Stressful Factor Triggers Changes in Polyphenols, Flavonoids and Antioxidant Activity in Somatic Hybrids between *Solanum tuberosum* and *S. bulbocastanum* and their Respective Parents

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Abstract

Phenols, important secondary metabolites in plants, are responsible for specific defence mechanisms against abiotic stress, due to their strong antioxidant activity. Flavonoids, as part of phenolic group, are also involved in plant stress responses, being primarily responsible for photo-protection against UV solar radiation. Based on these premises, the plant response to optimized *in vitro* culture was evaluated, by quantifying the total polyphenolic content, the total flavonoid content and the antioxidant activity, both under *in vitro* and *ex vitro* conditions. Four closely related potato genotypes were analysed: the wild species *Solanum bulbocatanum*, *S. tuberosum* cv. 'Rasant' and two somatic hybrids between them. For all genotypes, both total polyphenolic content and antioxidant activity were increasing under optimized *in vitro* culture. The shoot responses were genotype dependent and the two somatic hybrids were intermediate between the parents both as morphology and reaction to *in vitro* stress. The somatic hybrid 1508/5, having morphology similar to potato, was reacting as the wild species, while the somatic hybrid 1508/2, with a similar morphology to the wild species was reacting as potato to *in vitro* stress. The somatic hybrid 1508/5 being also resistant to late blight is of interest for further use in pre-breeding. Total flavonoid content is decreasing under *in vitro* as compared to *ex vitro* conditions, UV-B radiation, the major trigger of flavonoid biosynthesis being absent in the fluorescent light. This study reveals the effect of *in vitro* culture on flavonoid content and details aspects of the biochemical parameters involved in plant *in vitro* stress.

Keywords: biochemical parameters, electrofusion, optimized *in vitro* conditions, protoplasts, fusion hybrids, stress factors

Abbreviations: AA – Antioxidant activity; SH – Somatic hybrid; TFC – Total flavonoid content; TPC – Total polyphenolic content

Introduction

Stress, according to the state-change concept (Strasser, 1988), is represented by any modification of an environmental factor, biotic or abiotic, which forces a biological system away from its optimal state. The presence of a stressful factor generates genetic and epigenetic changes, especially modification at the gene expression level and as a result morphological, anatomical and physiological responses usually occur (Cramer *et al.*, 2011). One of the first such events happening at the cellular level is generation of reactive oxygen species (ROS), which alter gene expression and enzyme activity. ROS also have a role as signalling molecules for initiating

defence responses (Mittler *et al.*, 2011). Even though accumulation of ROS is an essential part of the signalling pathways used by the cell to make suitable adjustments in response to environmental changes, they are also harmful for the cell, producing oxidative stress and damaging important cellular components like lipids, proteins or nucleic acids (Foyer and Noctor, 2005). In this context, the quantity of intracellular ROS has to be controlled and that is achieved through the antioxidant system (with its enzymatic and non-enzymatic components), which protects the cell by scavenging ROS (Shao *et al.*, 2008).

One of the major classes of compounds, part of the non-enzymatic antioxidant system, playing an important role in

maintaining the redox state of the cell are the phenolic compounds, due to their strong antioxidant capacity. Phenols may be found constitutively in plant cells, but it was demonstrated that their quantity is rapidly increasing, by *de novo* synthesis, in the presence of a stressful factor (Jacobovelázquez and Cisneros-Zevallos, 2012; Mierziak *et al.*, 2014). In this way, phenolic compounds are directly involved in the plant defence mechanisms and in overcoming abiotic stress conditions (Ramakrishna and Ravishankar, 2011).

Among polyphenols, flavonoids are well known to have important roles in exceeding a wide range of stressful factors, but these compounds are primarily involved in photo-protection against short-wavelength UV-B solar radiation (over 280-315 nm waveband) (Agati and Tattini, 2010). The UV-B radiation, like all the environmental stressors, enhances the production of ROS at the cellular level, thus damaging cellular components. In response, plants synthesize higher levels of flavonoids (Sisa *et al.*, 2010), light being a major trigger of flavonoid biosynthesis. Moreover, a positive correlation between the total flavonoid content and the UV radiation was previously demonstrated (Caldwell, 1971). Flavonoids in plants act as sunscreens by absorbing the UV-B radiation and thus reducing the risk of ROS generation, but also as antioxidants by scavenging ROS once they are formed (Agati and Tattini, 2010). All these reports support the assumption that the total flavonoid content in plants is highly related to the UV-B radiation.

In vitro culture implies cultivation of shoots under special conditions regarding humidity, temperature, light exposure, aseptic environment and sucrose/nutrient containing medium. It is well known that, depending on these environmental influences, under *in vitro* conditions, plantlets are exposed to stress and might present abnormal morphology, anatomy or physiology (Hazarika, 2006). In extreme condition aberrant phenotype such as habituated and hyperhydric ('vitrified') explants might result (Balen *et al.*, 2009). One of the major causes for such phenomena is thought to be the oxidative stress damage occurring in plant tissues during explant preparation and cultivation (Cassells and Curry, 2001). In this context and taking into consideration the state-change concept, one can state that *in vitro* culture might be considered as a stressful factor for the shoots. However, all these external factors are usually well controlled and optimized for rapid growth and proper development of the explants. To achieve these goals, for each species subject to *in vitro* culture, specific optimal conditions have been identified, under which plantlets appear healthy and normal (Thorpe, 2007). The questions that remain are if under optimized *in vitro* culture conditions the explants are still subjected to stress and if so, how their cellular physiology is affected by it.

Pointing out how the *in vitro* culture influences the plantlets development and physiology is extremely relevant in many experimental designs. First of all, for all the studies being performed on *in vitro* cultivated shoots, with the main purpose to quantify different primary or secondary metabolites specific for a particular species/genotype, scientists must take into consideration the percentage of such compounds synthesized due to the environmental conditions. Secondly, for the studies that aim to characterize one or more species at the transcriptomic level, major differences between *in vitro* – *ex vitro* conditions may occur (Joyce *et al.*, 2003). Last but not

least, in the experiments focused on identifying the differences between genetically modified plants and their original counterparts, as part of the risk assessment strategy, an important percentage of the dissimilarities observed are caused by the *in vitro* procedure (Montero *et al.*, 2011; Fonseca *et al.*, 2015).

Plant responses to environmental stress, including accumulation of phenolic compounds, depend on the duration, severity and number of exposures to the stress factors, but also on plant characteristics, such as the tissue/organ in question, the stage of development or the genotype (Gaspar *et al.*, 2002). The genotype is a major factor regarding plant stress responses, thus even closely related genotypes might present significant differences in their reactions to stress. Asymmetric somatic hybrids obtained through protoplast electrofusion, are hybrids in which the nuclear genome consists of the full somatic complement of recipient fusion parent and a variable number of chromosomes from the donor one. These hybrids prove to be relevant resources for plant breeders due to their genetic make-up, several desirable genes being transferred from one fusion partner to another. Because of their atypical genetic constitution, their phenotypic traits are unpredictable, several outcomes being possible: the somatic hybrids might be inferior, intermediate or superior to their genitors from a specific phenotypic point of view (Shankar *et al.*, 2013). Therefore, we investigated the total phenolic and flavonoid content as well as the antioxidant activity under *in vitro* and *ex vitro* conditions for four different genotypes of *Solanum*: two asymmetric somatic hybrids (SH) and their parents. The aim of this study was to evaluate the specific response of those related genotypes to *in vitro* conditions in comparison to plants grown *ex vitro* at the level of polyphenol and flavonoid biosynthesis as well as antioxidant activity. Those data were correlated to previous results on cytogenetics, morphology and presence of two resistance genes to late blight/ resistant phenotype for better characterization of the somatic hybrids that are recommended for a pre-breeding program.

Materials and Methods

Plant material

Four different genotypes of *Solanum* were tested in this study: a wild diploid Mexican species *S. bulbocastanum* GLKS-31741 [blb41, Gross Lüsewitz Potato Collections (GLKS) of the IPK Gene Bank, Leibniz - Institute of Plant Genetics and Crop Plant Research, Germany]; a commercial potato *S. tuberosum* cv. 'Rasant' (Nordring-Kartoffelzucht-und Vermehrungs-GmbH Gross Lüsewitz, Germany); two asymmetric somatic hybrids – 1508/5 and 1508/2, produced by mesophyll protoplast electrofusion. Prior to this study, the hybridity of the plants was demonstrated through flow-cytometry and SSR markers, while the asymmetric status of the two somatic hybrids was demonstrated using AFLP markers (Rakosy-Tican *et al.*, 2015). Furthermore, the somatic hybrid 1508/5 proved to be resistant to late blight when tested in detached leaf assay (in two consecutive years) and a field, possessing two late blight resistance genes, *Rpi-blb1* and *Rpi-blb3*, known to induce durable resistance to this extremely adaptable and devastating pathogen (Rakosy-Tican, under publication).

Morphologically, the two somatic hybrids have intermediate phenotype when compared to the parents regarding their height and leaf morphology, as shown in Fig. 1. The 1508/5 somatic hybrid is more similar to the cultivated potato, while the 1508/2 hybrid is similar to *S. bulbocastanum*.

Six individuals for all four genotypes were cultivated *in vitro*, under controlled optimal environmental conditions for *Solanum* species [temperature: 21 °C; photoperiod: 16 h/8 h (light/dark); PPF - photosynthetic photon flux levels (light intensity): 135 $\mu\text{mol m}^{-2} \text{s}^{-1}$; light spectrum: 380-680 nm], on RMB5 medium [Duchefa Netherland - standard MS medium (Murashige and Skoog, 1962) enriched with Gamborg B5 vitamins (Gamborg *et al.*, 1968), 0.7% agar, pH = 5.8], for four weeks. The vessels used for the *in vitro* culture were clear glass jars, equipped with caps with sponges that allow the gas exchange. Each jar contained 100 ml RMB5 medium and 3 explants/jar were cultured. Three individuals for each genotype were transferred in a single jar and cultivated for an additional 4 weeks under the same *in vitro* conditions as mentioned before. Simultaneously, three individuals for each genotype were cultivated *ex vitro*, under uncontrolled environmental conditions (room temperature, 24 °C \pm 3 °C, photoperiod: approximately 12 h, in direct sunlight during May), in regular soil, for four weeks, including acclimatization. Therefore, the control group consisted in three individuals for each analysed genotype cultivated under *ex vitro* conditions, while the group of interest was formed from three individuals for each analysed genotype cultivated under *in vitro* conditions. Among the three individuals belonging to the same genotype, cultivated either under *in vitro* or *ex vitro* conditions, the growth was uniform. For each genotype, the *ex vitro* cultivated plants had a two to three times fold increase in biomass when compared to the *in vitro* plants.

Sample extraction

Each sample was obtained from three individuals belonging to one genotype, under one set of conditions, either *in vitro* or *ex vitro*. Therefore, eight samples were analysed in this study. For each sample extraction, the second, third and fourth leaf down from apex was collected from all three individuals



Fig. 1. Comparative leaf morphology of the parents (*Solanum bulbocastanum* and *S. tuberosum* cv. 'Rasant') and two asymmetric somatic hybrids (1508/2 and 1508/5), after eight weeks *ex vitro* growth. The third leaf down from apex was used for comparison

cultivated under *in vitro* or *ex vitro* conditions, belonging to each genotype. All the collected leaves belonging to one sample were milled and 1 g of the obtained powder was transferred in 10 ml extraction buffer [acidified methanol – 85: 15 v/v, MeOH: HCl] (El-Sayed and Hucl, 2003). The extraction was performed according to the procedure used by Bunea *et al.* (2011), through repeated extraction buffer addition, centrifugation and collection, the total extracts for all samples being dried afterwards in a vacuum rotary evaporator (Heidolph Laborota 4000 Efficient), at 40 °C and re-dissolved in 2 ml extraction buffer, prior to further analysis.

Total polyphenolic and flavonoid content determination

Total phenolic content (TPC), was determined using the colorimetric Folin-Cicalteu method (Singleton *et al.*, 1999). 25 μL of sample was used for the oxidation step with Folin-Cicalteu Reagent and a subsequent neutralization step with Na_2CO_3 . Sample absorbance was measured at 750 nm through spectrophotometry (BioTek Instruments, Winooski, VT) and the results were expressed as mg of gallic acid 100 g^{-1} fresh weight (FW). Each sample was measured three times.

The total flavonoid content (TFC) was evaluated for each sample based on the colorimetric method elaborated by Kim *et al.* (2003), by consecutive addition of 5% NaNO_2 , 10% AlCl_3 and 1N NaOH to each alcoholic extract. The absorbance was measured at 510 nm through spectrophotometry (JASCO V-630 series, International Co.). Each sample was measured three times and the results were expressed as mg quercetin equivalents 100 g^{-1} FW. Even though this method does not detect all flavonoid subclasses, it is one of the most widely used procedures to assess the flavonoid content in food and medicinal plant samples. It detects the flavonols and flavones in an extract, two major classes of flavonoids found in plants, but also some phenolic acids, such as the caffeic acid (Pekal and Pyrzynska, 2014). This protocol was chosen in these experiments also because it proved to be more reliable in determining the flavonoid content than other procedures (Pekal and Pyrzynska, 2014).

Antioxidant activity determination

The antioxidant capacity (AA) was determined through the ABTS assay (ABTS radical cation distaining assay), based on the capacity of the samples to scavenge ABTS radicals, in comparison with Trolox, a standard antioxidant (Arnao *et al.*, 2001). 20 μL of each sample extract and Trolox were added in 170 μL ABTS⁺ solution, which was prepared 16 h before the experiment. The absorbance was measured at 734 nm on a 96 well microplate, through spectrophotometric means (BioTek Instruments, Winooski, VT). The results were expressed as μmol Trolox equivalents 100 g^{-1} FW and for each genotype 3 measurements were performed.

Statistical analysis

Unpaired tow-tailed t-tests were used in order to determine if the differences observed in TPC, TFC and AA under *in vitro* and *ex vitro* conditions for each genotype are statistically significant. Furthermore, the same statistical analysis was performed to assess the differences between genotypes under *ex vitro* conditions (control), for each physiological trait. Significance of difference was defined at the 5% level ($p < 0.05$). Previously, our data were tested for their normal distribution

using the D'Agostino-Pearson omnibus normality test and for their variances using the F test. All statistical analyses were performed using the GraphPad Prism software (Graph Pad Software Inc; San Diego, CA, USA). The increased or decreased rate of TPC, TFC and AA (parameters) specific for each genotype between the *in vitro* and *ex vitro* (control) conditions was calculated as follows:

$$\text{Increased / decreased rate (\%)} = \left[\left(\frac{\text{parameter in } in\ vitro}{\text{parameter in } ex\ vitro} - 1 \right) \times 100 \right]$$

Results

Total polyphenolic content

The highest polyphenolic content, both *in vitro* and *ex vitro*, was found in the wild species *S. bulbocastanum*, while low TPC was revealed in the commercial *S. tuberosum* cv. 'Rasant' and in the somatic hybrid 1508/2 (Fig. 2). The 1508/5 genotype presents intermediate phenotype between the two parental species in *ex vitro* conditions regarding the TPC pattern, but because of its low increasing rate, in *in vitro* culture this hybrid is similar to its cultivated genitor, potato.

By comparing the total polyphenolic content under *ex vitro* conditions with TPC in *in vitro* culture, the accumulation of phenolic compounds becomes conspicuous for all four genotypes, even though the increasing rates are various. For all four genotypes, significant differences were found between the two sets of conditions. The highest increasing rates were found in *S. tuberosum* cv. 'Rasant' and in the 1508/2 hybrid,

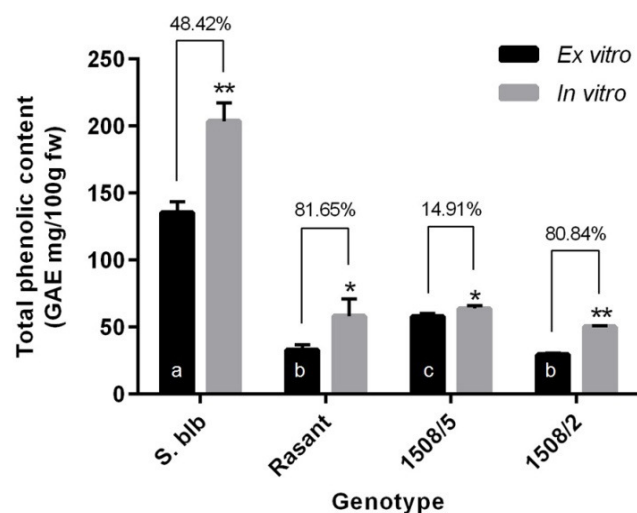


Fig. 2. Total phenolic content under *ex vitro* (black) and *in vitro* (gray) conditions in: *S. bulbocastanum* (*S. blb*), *S. tuberosum* cv. 'Rasant' and two somatic hybrids between them (1508/5 and 1508/2). Data are means \pm SD (n=3). For each measurement under *in vitro* conditions, * shows a statistically significant difference ($p < 0.05$) and ** shows a highly significant difference ($p < 0.01$) in TPC, when compared to *ex vitro* conditions. The increased rates (%) in TPC under *ex vitro* conditions for each genotype are shown above each set of data. Significant differences ($p < 0.05$) between genotypes under *ex vitro* (control) conditions are represented by different letters

genotypes that express the smallest quantities of constitutive polyphenols under *ex vitro* conditions. On the other hand, the somatic hybrid 1508/5, which synthesizes almost double quantities of constitutive phenolic compounds as compare to *S. tuberosum* cv. 'Rasant', it's characterized by the lowest growth rate, which translates into the smallest accumulation of TPC.

Total flavonoid content

The highest flavonoid content was determined in *S. bulbocastanum*, both *ex vitro* and *in vitro*, while the lowest amounts of flavonoids under *ex vitro* conditions were found in *S. tuberosum* cv. 'Rasant' (Fig. 3). The 1508/5 somatic hybrid presents intermediate flavonoid quantities when compared with the parents *in vitro*, as well as under *ex vitro* conditions.

When comparing the TFC for each genotype under *ex vitro* (control) and *in vitro* conditions, statistically significant differences were observed for *S. bulbocastanum* and for both somatic hybrids. However, in contrast with the TPC accumulation and the AA increase *in vitro*, the total flavonoid content is much higher under *ex vitro* conditions, not in the *in vitro* culture, as it might be expected.

Antioxidant activity

The highest AA, both under *ex vitro* and *in vitro* conditions, was revealed in *S. bulbocastanum*. Opposed to the wild species, in *ex vitro* culture the lowest antioxidant capacity was found in the somatic hybrid 1508/2. On the other hand, in *in vitro* culture, the cultivated species *S. tuberosum* cv. 'Rasant'

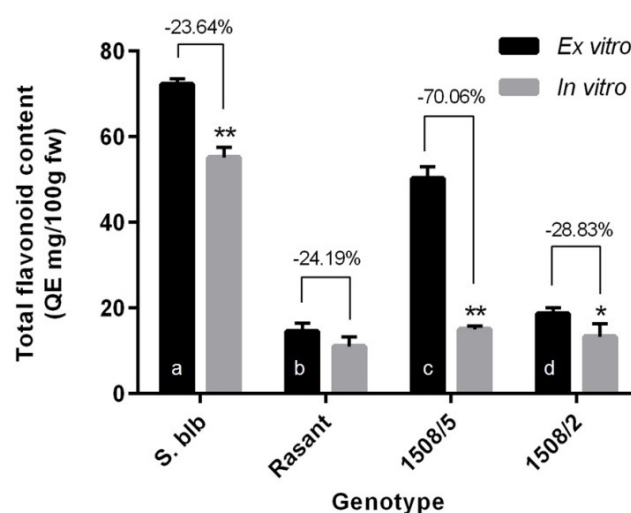


Fig. 3. Total flavonoid content under *ex vitro* (black) and *in vitro* (gray) conditions in: *S. bulbocastanum* (*S. blb*), *S. tuberosum* cv. 'Rasant' and two somatic hybrids between them (1508/5 and 1508/2). Data are means \pm SD (n=3). For each measurement under *in vitro* conditions, * shows a statistically significant difference ($p < 0.05$) and ** shows a highly significant difference ($p < 0.01$) in TFC, when compared to *ex vitro* conditions. The increased rates (%) in TFC under *ex vitro* conditions for each genotype are shown above each set of data. Significant differences ($p < 0.05$) between genotypes under *ex vitro* (control) conditions are represented by different letters

becomes the genotype with the poorest antioxidant activity. Compared to its genitors, the somatic hybrid 1508/5 holds an intermediate antioxidant capacity, both in *ex vitro* and in *in vitro* culture.

Except *S. bulbocastanum*, all the other genotypes present an increased antioxidant potential under *in vitro* conditions, with statistically significant differences ($p < 0.01$) when compared with the AA under *ex vitro* conditions. Between these 3 genotypes, the lowest increasing rate occurs in *S. tuberosum* cv. 'Rasant', while the highest is characteristic for the 1508/2 hybrid, which is doubling its antioxidant capacity under *in vitro* conditions.

Discussion

Based on our results, it can be noted that both under *in vitro* or *ex vitro* conditions, the highest polyphenol content, flavonoid content and the greatest antioxidant activity occur in *S. bulbocastanum*. This wild species has effective resistance mechanisms to different abiotic stress factors, but also to some biotic agents, like aphids (Alvarez *et al.*, 2006), nematodes (Austin *et al.*, 1993) or fungi (Lokossou *et al.*, 2010). In this context, higher TPC, TFC and AA were actually expected for the wild species, all three physiological traits taken into consideration being actively involved in the resistance mechanisms (Lattanzio *et al.*, 2006; Mierziak *et al.*, 2014).

The SH 1508/5 had intermediate TPC, TFC or AA when compared to its parents, while SH 1508/2 was similar in biochemical patterns with *S. tuberosum* cv. 'Rasant'. The two

somatic hybrids are asymmetric hybrids and thus their genetic makeup consists of two different parental genomes which have suffered uni- or bidirectional chromosome losses (Rakosy-Tican *et al.*, 2015). Therefore their phenotype with regard to TPC, TFC and AA was unpredictable. A relevant aspect that has to be highlighted is that the SH 1508/5 acquired new traits (higher TPC, TFC and AA) when compared to the cultivated potato. Corroborating these findings with previous data according to which this hybrid has gained resistance to *Phytophthora infestans* due to the presence of *Rpi-blb1* and *Rpi-blb3* genes in its genome (Rakosy-Tican, under publication), we can state that this hybrid has great potential for inclusion in future breeding programs.

The phenotypic appearance of the somatic hybrids were both intermediate between the two parental species, but SH 1508/2 was much similar to *S. bulbocastanum*, while SH 1508/5 had leaf morphology similar to the cultivated potato cv. 'Rasant'. The morphological features did not correspond in their similarity to biochemical characteristics, as shown before. However, the fact that the 1508/5 hybrid is more similar with *S. bulbocastanum* in regard to the resistance mechanisms and more alike *S. tuberosum* from a morphological perspective, strengthens this hybrid's potential for future potato breeding.

According to our results, optimized *in vitro* conditions represent a stressful factor for the plantlets. Plants respond to environmental stress by activating a number of defence mechanisms, including accumulation of phenolic compounds and increased antioxidant activity (Jacobo-Velázquez and Cisneros-Zevallos, 2012; Ramakrishna and Ravishankar, 2011). For all four analysed genotypes, both the polyphenol quantities and the antioxidant activity increase under *in vitro* conditions. These results demonstrate that *in vitro* conditions activate the plants defence mechanisms, by *de novo* synthesis of polyphenols, due to which an increased antioxidant capacity is observed. These results complement previous studies, which were based on gene expression using microarray analysis and metabolomics, showing that the *in vitro* tissue culture is perceived as a stressful factor by the explants (Desjardins *et al.*, 2009).

Several studies reported in the literature are focused on creating a stressful environment under *in vitro* explant culture, in order to improve the synthesis of important secondary metabolites (Patel and Krishnamurthy, 2013) or antioxidants (Matkowski, 2008), to assay the effects of abiotic stresses on different species (Terletskaia and Khailenko, 2014), to improve the efficiency of organogenesis in plant *in vitro* culture (Adamczuk *et al.*, 2012) or to develop biotechnological tools for crop breeding and *in vitro* stress selection (Pérez-Clemente and Gómez-Cadenas, 2012). None of these studies is taking into account the stress factor represented by the optimized *in vitro* culture itself.

The total flavonoid content was the only physiological trait examined in this study that decreased under *in vitro* conditions. Light is one of the major environmental factors that influences the flavonoid *de novo* biosynthesis, primarily short-wavelength UV solar radiation (Tegelberg *et al.*, 2001; Ryan *et al.*, 2002). The *in vitro* explants were grown in the absence of UV-B radiation because the light used had wavelengths in the range of 380-680 nm (Philips lamps – see: <http://www.lighting.philips.com>). On the other hand, the *ex vitro* plants were cultivated in direct sunlight, during May, where UV-B

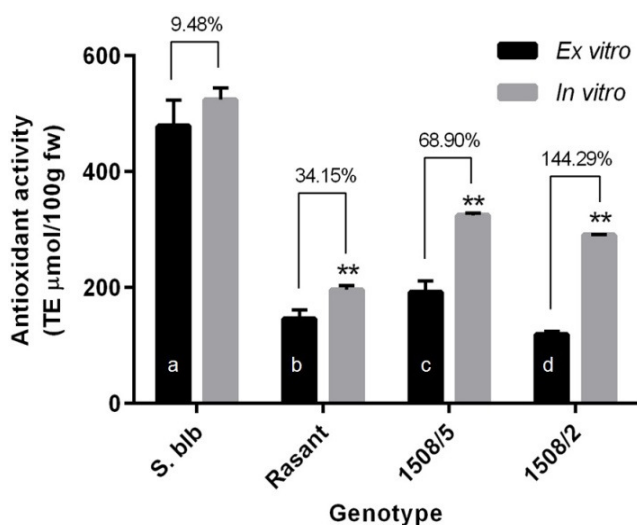


Fig. 4. Antioxidant activity under *ex vitro* (black) and *in vitro* (gray) conditions: *S. bulbocastanum* (*S. blb*), *S. tuberosum* cv. 'Rasant' and two somatic hybrids between them (1508/5 and 1508/2). Data are means \pm SD ($n=3$). For each measurement under *in vitro* conditions, * shows a statistically significant difference ($p < 0.05$) and ** shows a highly significant difference ($p < 0.01$) in AA, when compared to *ex vitro* conditions. The increased rates (%) in AA under *ex vitro* conditions for each genotype are shown above each set of data. Significant differences ($p < 0.05$) between genotypes under *ex vitro* (control) conditions are represented by different letters

radiation was present. Previous studies (Sisa *et al.*, 2010; Agati and Tattini, 2010), proving that flavonoid biosynthesis is strongly related with the presence and amount of UV-B radiation, support our results. The UV-B radiation present in the direct sunlight is activating the flavonoid biosynthesis, while the optimized *in vitro* culture produces the accumulation of polyphenols, others than flavonoids and increases the antioxidant activity. These results highlight the active involvement of other polyphenols than flavonoids in plant responses to *in vitro* stress.

Plant responses to environmental stress depend, among others, on the genotype of the organism (Gaspar *et al.*, 2002). Thus, the accumulation of phenolic compounds or flavonoids is also thought to be genotype dependent (Al-Saikhan *et al.*, 1995). The present study shows that, even in the case of four closely related organisms (2 species belonging to the same genus and their somatic hybrids), differences in phenolic compounds accumulation are present under *in vitro* conditions, both quantities and increasing rates being specific for each genotype. It was noticed that genotypes with the lowest levels of constitutive phenolic compounds under *ex vitro* conditions, namely *S. tuberosum* cv. 'Rasant' and the SH 1508/2, had much higher increasing rates. These results are consistent with previous reported data. Studies carried out on lettuce demonstrate that tissue with small quantities of polyphenols had a higher phenolic accumulation in response to heat shock stress (Salteit, 2000). The results presented here on potato somatic hybrids with the sexually incompatible species *Solanum bulbocastanum* might be applied to other wild species for better understanding of *in vitro* stress on plants with unique genetic constitutions and further application in breeding.

Conclusions

In this study the constitutive phenolic content, flavonoid content and the antioxidant activity of the potato cultivar *S. tuberosum* cv. 'Rasant', the wild species *S. bulbocastanum*, and two of their somatic hybrids, 1508/5 and 1508/2 were quantified in order to ask two questions:

- 1) Are there differences between related genotypes in their response to *in vitro* stress?
- 2) Which is the somatic hybrid that is most suited to be further used in a pre-breeding program?

The answers that can be drawn from the results are:

- 1) There are differences between the four genotypes analysed, in relation to polyphenol accumulation and antioxidant activity triggered by *in vitro* culture, but surprisingly the flavonoids analysed are reduced *in vitro* due to low level UV-B present in the fluorescent light;
- 2) The somatic hybrid 1508/5 proved to be superior when compared with the cultivated potato from the points of view of reaction to *in vitro* stress, antioxidant activity and previous results on late blight resistance and thus is under consideration for inclusion in a pre-breeding program.

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