

# FOLIAR METABOLISM AND OXIDATIVE STRESS INDICATORS OF RADISH (*RAPHANUS SATIVUS* L.) GROWN IN SALT CONTAMINATED SOIL

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**Abstract:** In order to elucidate the impact of soil salinity on foliar metabolism indicators of radish (*Raphanus sativus* L.), two genotypes of Radish "National" and "Cerise" were subjected to increasing salt treatments add ECE of soil (control (0.6 dS m<sup>-1</sup>), 5.63 and 12.18 dS m<sup>-1</sup>). The laboratory test showed that soil salinity had adverse effects on most of the physiological and biochemical parameters in the leaves of both radish varieties. Chlorophyll pigments decreased in the two radish varieties and the rate of chlorophyll degradation was higher in "Cerise" vr. under severe salinity stress. On the other hand, leaf carotenoids substantially decreased in "National" vr. The total soluble sugars and total soluble proteins decreased in both radish varieties under salinity stress. However, total soluble proteins were higher in the salt stressed leaves of "Cerise" vr. These results showed that greater salt tolerance in "National" vr. was associated with phenolics and antioxidant activity which indicated stimulation of foliar metabolism associated with antioxidant potential.

**Keywords:** radish (*Raphanus sativus* L.), salinity tolerance indicators, antioxidants, phenolics, soluble sugars.

## INTRODUCTION

Land salinization is one of the global problems to agriculture in many countries, including Algeria. It is estimated that one million hectares land are affected by salt stress, of which 49 thousand hectares are in the south of the country (Szabolcs, 1989). In these Saharan zones, as well known, the soil has a low level fertility. Although the water reserves are important, they are non-renewable and variously mineralized. Also, the water needs of crops are high because of the strong climatic demand (Daoud and Halitim, 1994). Such fragile ecosystems are characterized by a high irregularity of precipitation associated with important evaporation. Those two factors favorite the accumulation of soluble salts on the surface, and affect crop yields (Munns et al., 2006). High salinity reduces plant growth mainly by limiting water and nutrient absorption, and uptake of toxic ions which affect certain vital metabolic processes (Shabala and Munns, 2012).

Under salt stress conditions, plants develop various adaptive mechanisms which include accumulation of osmoprotectants protecting membrane integrity, stabilizing enzymes and the detoxification of reactive oxygen species (ROS) (Ashraf and Foolad, 2007; Ashraf et al., 2008). Some solutes perform an additional function to protect the cellular components from dehydration, such as soluble sugars, amino acids, quaternary ammonium compounds and polyamines (Shabala and Munns, 2012; Rasool et al., 2013). The antioxidant defense system in plants consists of enzymatic and non-enzymatic molecules (Karuppanapandian et al., 2011). The response of plant species to salt depends on their genotypes, salt

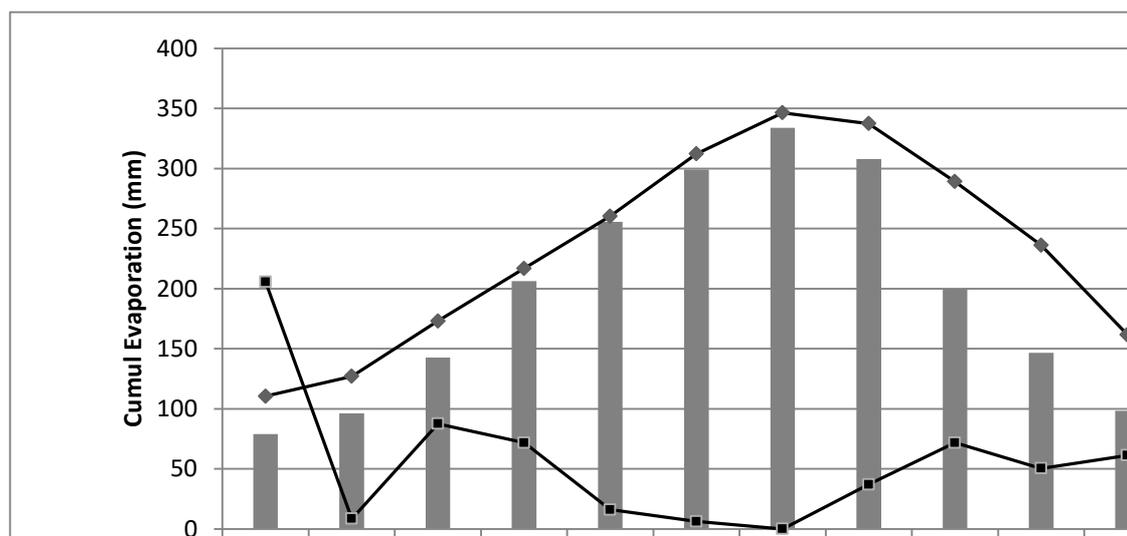
concentration, culture conditions, and stage of plant development (Rasool et al., 2013).

On the other hand, the monoculture of salt sensitive crops such as potato production led to the depletion of soil resources (ONFAA, 2014; DSA, 2016). In this context, it is important to encourage alternative salt tolerant crops such as root vegetable species, which have nutritional values and biological benefits such as radish, beetroot, carrot, turnip, etc. (Lintas, 1992). Radish (*Raphanus sativus* L.) belongs to Brassicaceae family (Jeong et al., 2014), characterized by its high nutritional value, as well as its therapeutic importance against many diseases (Singh and Singh, 2013). In view of the importance of radish, two varieties differing in their salt tolerance were subjected to salt stress to assess physiological indicators for salt tolerance in radish at the vegetative growth stage. Such studies will help in improving stress tolerance and productivity in Oued-Souf region (south-east of Algeria).

## MATERIALS AND METHODS

### Plant Material and Growth Conditions

Seeds of two French radish genotypes (*Raphanus sativus* L.) namely: "National" and "Cerise" were obtained from AGROSEED Company (France). The experiment was conducted in the Experimental Park of Life and Natural Sciences Faculty of El-Oued University, in the south-east of Algeria (33° 23' 51.1" N; 6° 51' 42.1" E), 80 m above sea level (ANAT, 2012), during 25 September to 31 October 2016. The climate of El-Oued region is hot and dry in summer (Fig. 1), and moderate in winter with low and irregular rain fall throughout the year.



**Fig. 1.** Monthly cumulative evaporation, precipitation and average air temperature during 2016 in El-Oued region, south-east of Algeria (ONM, 2017).

The trial site is characterized by sandy soils, low initial salinity and a weakly alkaline pH (Table 1). It is also very poor in organic matter.

**Table 1.**

**Soil chemical properties of the experimental site**

Parameter	Result	
pH	7.56	
Electrical Conductivity	0.6	mS Cm <sup>-1</sup>
Ammonium (NH <sub>4</sub> )	7.64	mg kg <sup>-1</sup>
Nitrate (NO <sub>3</sub> )	206.84	mg kg <sup>-1</sup>
Potassium (K <sub>2</sub> O)	94.89	mg kg <sup>-1</sup>
Phosphate (P <sub>2</sub> O <sub>5</sub> )	252.91	mg kg <sup>-1</sup>
Total carbonates	11.47	%
Organic matter	0.12	%

Squares cultivation plots (50 Cm × 50 Cm) were used. Planting plots have been supplying by organic matter (dried pigeon droppings), where it distributed 2 kg/plot unit. As has been mixing and stirring the soil parcels with organic matter added depth of 10 - 20 Cm. During the salt treatment application, the plants received a supply of mineral nitrogen fertilizer (Urea 46 %). The sowing is done at the rate of 20 seeds/plot unit, then at emergence, the number is reduced to ten plants/plot unit. The experiment was conducted in complete randomized design with two factors; three salinity levels, two radish genotypes and three replicates. In the irrigation process, the tap water was used. Its electrical conductivity (EC) was estimated at 3.55 mS Cm<sup>-1</sup> and its acidity was alkaline pH (8.18). Irrigation was done by submersion method, according to the plant growth stages:

- 8 L / plot unit: from the first day to day 25 of the plant growth.
- 10 L / plot unit: from day 26 to day 31 of the plant growth.
- 5 L / plot unit: from day 32 to day 40 of the plant growth.

Twenty-five days after the sowing, varying amounts of sodium chloride salt as powder were added homogeneously to the soil (0, 96 and 192 g NaCl per meter square), where soil salinity levels were estimated: control (0.6 dS m<sup>-1</sup>), 5.63 and 12.18 dS m<sup>-1</sup>, respectively.

After application of the saline (NaCl) soil treatment (period of 15 days), laboratory tests were performed, three mature leaf samples were collected at the same level for plants of each variety in each treatment, for each performed test.

#### Analysis of Photosynthetic Pigments

Chlorophyll A, chlorophyll B, and total carotenoids were determined according to the method of Shabala et al. (2005). Samples of 50 mg of fresh leaves were taken from the youngest but fully developed leaf. Extraction of the pigments was done with 80% acetone and absorbances at 663, 646 and 470 nm were read with a UV / visible spectrophotometer.

Content of chlorophylls and carotenoids were calculated according to Lichtenthaler and Wellburn (1983) using the following formulas:

$$- \text{Chlorophyll A} = 12.21 \text{ Abs}_{663} - 2.81 \text{ Abs}_{646};$$

- Chlorophyll B =  $20.13 \text{ Abs}_{646} - 5.03 \text{ Abs}_{663}$  ;

- Total Chlorophyll (A+B) = Chlorophyll A +

Chlorophyll B ;

- Carotenoids =  $(1000 \text{ Abs}_{470} - 3.27 \text{ Chlorophyll A} - 104 \text{ Chlorophyll B}) / 229$

The results were expressed in  $\mu\text{g g}^{-1}$  Leaf Fresh Weight (in  $\mu\text{g g}^{-1}$  FW).

Pigment degradation percentage was calculated using the following equation (Cha-Um and Kirdmanee, 2009):

$$Pd (\%) = (1 - \text{Salt treatment/Control}) \times 100$$

### Sugar Content

According to Dubois et al. (1956), 30 mg of dry extract of leaf samples was weighed and 1 mL of methanol and 4 mL of distilled water were added. Briefly, 50  $\mu\text{L}$  of an aqueous-alcoholic extract of the samples was taken, and 3 mL of sulfuric acid and 1 mL of phenol solution (5%) were added. The samples were placed in a water bath at a temperature of 30°C for 15 min, and then the samples were cooled and read in a spectrophotometer device at a wavelength of 490 nm.

In order to draw the calibration curve, the glucose solution ( $4 \text{ mg mL}^{-1}$ ) was prepared, and the following concentrations: 1.78, 1.19, 0.79, 0.53, 0.35, 0.23 and  $0.16 \text{ mg mL}^{-1}$  of the glucose solution were obtained.

The results were expressed as  $\mu\text{g}$  equivalent of glucose per mg of dry matter ( $\mu\text{g GE mg}^{-1} \text{ DM}$ ) relative to a calibration curve ( $y = 0.922x - 0.077$ ,  $R^2 = 0.995$ ).

### Protein Content

Briefly, the proteins were quantified according to the method of Bradford (1976), which uses the brilliant blue of Coomassie G250 and bovine serum albumin (BSA) as standard. The assay is performed on a spectrophotometer at the wavelength of 590 nm. The protein content is expressed in  $\mu\text{g g}^{-1}$  of fresh matter (FM) relative to a calibration curve made with known concentrations (1, 0.5, 0.25, 0.125,  $0.0625 \text{ mg mL}^{-1}$ ) of albumin solution.

### Leaf Phytochemical Analysis

The fresh mature leaves from each radish variety were dried in shade and were grinded by an electric mill. The methanol extract for the analysis was prepared using the method of Thaipong et al. (2006). Briefly, one gram (1 g) of grounded dry leaves were mixed with 20 mL methanol and homogenized. The homogenates were kept at 4°C for 48 h and then centrifuged. The supernatants were recovered and stored in the refrigerator until analysis.

### Total Phenolic Content

The total phenolic content (TPC) was determined using the Folin-Ciocalteu method (Scalbert et al., 1989). The absorbance was measured at 760 nm using a Shimadzu UV-VIS spectrophotometer (Shimadzu UV-1800). A calibration curve was performed using different concentrations of standard gallic acid solutions ( $15\text{-}200 \mu\text{g mL}^{-1}$ ,  $R^2 = 0.997$ ) and the concentration of TPC was expressed as  $\mu\text{g GAE mg}^{-1} \text{ DW}$  (Dry matter).

### Antioxidant Capacity Scavenging Effect Assay

The antioxidant capacity was assessed through the evaluation of the free radical scavenging effect on 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical (Blois, 1958). Briefly, aliquots of methanolic extracts were mixed with a methanolic DPPH solution (0.4%). After 30 minutes incubation at room temperature in dark, the absorbance of each sample was measured at 517nm against a blank of methanol.

The DPPH free radical scavenging activity was calculated using the following equation:

$$\% \text{ scavenging effect} = [(A_{\text{DPPH}} - A_{\text{Sample}}) / A_{\text{DPPH}}] \times 100$$

Where  $A_{\text{DPPH}}$  is the absorbance of the control reaction and  $A_{\text{Sample}}$  is the absorbance in the presence of plant extract.

All reagents and chemicals used were of the highest grade of purity commercially available.

### Statistical Analysis

All data were subjected to a two-way analysis of variance (ANOVA), significant different means were separated using the Fisher's L.S.D. test at the 5% level (Minitab 16).

## RESULTS

### Leaf Physiological Responses

#### Photosynthetic Pigments Contents

Soil contamination with NaCl salinity caused a significant decrease in photosynthetic pigments of plants in both varieties of radish (Table 2). Maximal reduction in chlorophyll A was found in "Cerise" vr. at the highest salinity level ( $12.18 \text{ dS m}^{-1}$ ), whereas the same result was recorded for chlorophyll B in the leaves of "National" vr. In addition total chlorophyll reduced maximally in "Cerise" vr. under the same salinity level. Similarly, carotenoids were decreased in both radish varieties due to increasing soil salinity. However, this reduction was more in the leaves of "National" vr. at  $12.18 \text{ dS m}^{-1}$  EC of soil salinity.

**Table 2.** Chlorophylls (Chl A, Chl B and Chl (A+B)), carotenoids (Car (x+c)) contents and pigments degradation (Pd) in leaves of radish (*Raphanus sativus* L.) varieties, "National" and "Cerise", under soil salt (NaCl) contamination levels

Varieties	Soil salinity levels (EC dS m <sup>-1</sup> )	Chl A (μg/g FM)	Chl B (μg/g FM)	Chl (A+B) (μg/g FM)	Pd <sub>Chl (A+B)</sub> (%)	Car (x+c) (μg/g FM)	Pd <sub>Car(x+c)</sub> (%)

	1)						
"National"	Control (0.6)	7.043 <sup>a</sup> ± 0.75	2.057 <sup>a</sup> ± 0.31	9.1 <sup>a</sup> ± 1.06	0	3.11 <sup>a</sup> ± 0.33	0
	5.63	4.113 <sup>c</sup> ± 0.32	1.671 <sup>b</sup> ± 0.38	5.459 <sup>c</sup> ± 0.44	40.01	1.836 <sup>bc</sup> ± 0.11	40.96
	12.18	2.886 <sup>d</sup> ± 0.37	1.032 <sup>d</sup> ± 0.19	4.095 <sup>d</sup> ± 0.76	55	1.585 <sup>c</sup> ± 0.27	49.04
"Cerise"	Control (0.6)	5.718 <sup>b</sup> ± 0.76	1.443 <sup>bc</sup> ± 0.15	7.161 <sup>b</sup> ± 0.9	0	2.16 <sup>b</sup> ± 0.22	0
	5.63	3.203 <sup>d</sup> ± 0.34	1.091 <sup>cd</sup> ± 0.17	4.294 <sup>d</sup> ± 0.5	40.04	1.578 <sup>c</sup> ± 0.88	26.99
	12.18	2.031 <sup>e</sup> ± 0.42	0.801 <sup>d</sup> ± 0.1	2.684 <sup>e</sup> ± 0.42	62.52	1.517 <sup>c</sup> ± 0.25	29.77
<b>Sources of variation</b>		<b>P value and significance level</b>					
<b>variety</b>		0.000***	0.000***	0.000***	/	0.000***	/
<b>salinity</b>		0.000***	0.000***	0.000***	/	0.000***	/
<b>variety × Salinity</b>		0.63 <sup>NS</sup>	0.231 <sup>NS</sup>	0.564 <sup>NS</sup>	/	0.004**	/

Values are expressed as mean ± standard deviation of three replicates for each variety × salt contamination level. Different letters in the same column indicate statistically significant differences (Fisher's test at  $P \leq 0.05$ ). NS, \*\*, \*\*\* non-significant or significant at  $P \leq 0.01$ , or  $P \leq 0.001$ .

### Leaf Biochemical Responses Sugar Content

The total soluble sugar content in leaves decreased in both radish varieties due to increasing soil salinity,

particularly in variety "National" (Table 3). However, total soluble sugars in the leaves of "Cerise" vr. decreased only at the highest level of soil salinity (EC 12.18 dS m<sup>-1</sup>).

**Table 3.**

**Sugar, proteins (Prot.), total phenolic contents (TPC) and antioxidant activity (AOA) in leaves of radish (*Raphanus sativus* L.) varieties, "National" and "Cerise", under soil salt (NaCl) contamination levels**

Varieties	Soil alinity levels (EC dS m <sup>-1</sup> ) 1)	Extract yield (%)	Sugar (µg GE/mg DM)	Prot. (µg BSA.E/mg DM)	TPC (µg GAE/mg DM)	AOA (%)
"National"	Control (0.6)	28.09	164.74 <sup>c</sup> ± 10.77	67 <sup>a</sup> ± 2.36	134 <sup>c</sup> ± 11.19	76.32 <sup>b</sup> ± 0.07
	5.63	31.45	79.54 <sup>e</sup> ± 5.7	64.79 <sup>ab</sup> ± 6.35	166.14 <sup>b</sup> ± 13.52	82.31 <sup>ab</sup> ± 8.65
	12.18	34.95	96.33 <sup>d</sup> ± 4.59	61.84 <sup>abc</sup> ± 3.69	191.82 <sup>a</sup> ± 6.97	86.27 <sup>a</sup> ± 1.22
"Cerise"	Control (0.6)	29.17	426.56 <sup>a</sup> ± 2.14	55.51 <sup>c</sup> ± 1.62	89.85 <sup>d</sup> ± 3.63	81.6 <sup>ab</sup> ± 7.01
	5.63	32.04	421 <sup>a</sup> ± 1.37	57.86 <sup>bc</sup> ± 4.73	94.13 <sup>d</sup> ± 3.12	83.06 <sup>ab</sup> ± 0.61
	12.18	35.36	190.67 <sup>b</sup> ± 7.99	57.7 <sup>bc</sup> ± 3.66	137.35 <sup>c</sup> ± 12.25	88.17 <sup>a</sup> ± 0.43
<b>Source of variation</b>		<b>P value and significance level</b>				
<b>salinity</b>			0.000***	0.758 <sup>NS</sup>	0.000***	0.028*
<b>variety</b>			0.000***	0.002**	0.000***	0.244 <sup>NS</sup>
<b>Salinity × variety</b>			0.000***	0.318 <sup>NS</sup>	0.010**	0.681 <sup>NS</sup>

Values are expressed as mean ± standard deviation of three replicates for each variety × salt contamination level. Different letters in the same column indicate statistically significant differences (Fisher's test at  $P \leq 0.05$ ). NS, \*, \*\*, \*\*\* non-significant or significant at  $P \leq 0.05$ ,  $P \leq 0.01$ , or  $P \leq 0.001$ .

### Protein Content

Salt stress reduced the total soluble proteins in the both radish cultivars (Table 3). However, greater

reduction in total soluble proteins was found in “National” vr. (8%) at the highest level of salt stress (12.18 dS m<sup>-1</sup>). On the other hand non-significant increase in protein in “Cerise” vr. was recorded under saline conditions.

## Leaf Phytochemical Responses

### Extraction Yield

Our results (Table 3) confirmed the increase in the yield of the methanolic extract of radish leaves while increasing the saline contamination level of the soil, in both studied genotypes.

### Total Phenolic Content (TPC)

The salt (NaCl) treatment of the soil has a very highly significant effect on the total phenolic contents in foliar extracts of both radish varieties (Table 3). Our results indicate that the total phenolic contents (TPC) of leaves increased with increasing the level of soil salinity. In addition, “Cerise” vr. had the highest foliar phenolic contents (an increase of 53%), in response to severe salt treatment (12.18 dS m<sup>-1</sup>). Although similar increase in phenolic contents had found in “National” vr., but this increase was lower (43%) under the same level of salt stress.

### Antioxidant Activity (AOA)

Imposition of salt stress had a significant increasing effect on antioxidant activity of leaves of both radish cultivars (Table 3), particularly at the highest level of soil salinity (EC 12.18 dS m<sup>-1</sup>).

## DISCUSSION

### Photosynthetic Pigments Stability

Photosynthetic pigments played a vital role in conversion of solar energy into biochemical energy and thus favoring CO<sub>2</sub> fixation rate. In the present study, photosynthetic pigments were reduced due to salinity stress in both radish cultivars. These findings are in agreement with some of previous studies (Jamil et al., 2007; Gao et al., 2015). Lewtt (1980) argued that salt stress cause excessive accumulation of Na<sup>+</sup> and Cl<sup>-</sup> ions in chloroplast which resulted in degradation of chlorophylls by an increase in chlorophyllase activity. In addition, such adverse effect of salt stress was more on chlorophyll A than on chlorophyll B in the leaves of both radish cultivars. This can be explained as greater sensitivity of chlorophyll A than chlorophyll B (Vodnik et al., 1999). In addition, genetic expression of the chlorophyllide a oxygenase, responsible for biosynthesis of the Chl B, is negatively regulated by salt stress thus increasing Chl A / Chl B ratio (Pattanayak et al., 2005; Biswal et al., 2012).

The decrease in photosynthetic rate under salt stress is normally attributed to the suppression of mesophyll conductance and stomatal closure (Gao et al., 2015). It could also be that the reduction of pigment formation is due either to the lack of differentiation of etioplasts to chloroplasts, or to lamellar and granular structures that do not differentiate or to enzymes related to the

biogenesis of pigments under stress (Ort and Baker, 2002).

Chlorophyll content is suggested as one of the parameters of salt stress tolerance in crop plants (Hernandez et al., 1995; Cuin et al., 2010). In addition, chlorophyll and its intermediate products are photodynamic toxic molecules that generate ROS (Krieger-Liszky et al., 2008; Turan and Tripathy, 2015). Hernandez et al. (1995) and Mitsuya et al. (2000) have observed at peas and potato a degradation of chloroplast thylakoid membranes in the mesophyll following an oxidative stress induced by salinity.

In the chloroplasts, O<sub>2</sub><sup>-</sup> and O<sub>2</sub> derivatives of H<sub>2</sub>O<sub>2</sub> are mainly produced by the electron acceptor of photosystem I (Salin, 1991; Asada, 1994). The chloroplastic CO<sub>2</sub> concentration decreases due to stomatal closure, which reflects a lesser availability of the NADP<sup>+</sup> to accept photosystem I electrons, thus initiating the O<sub>2</sub> reduction with a simultaneous generation of ROS (Halliwell, 1982).

The first line of defense against oxidative stress damage aims to limit the formation of ROS by regulating, in particular, the electron transport chains (Gratão et al., 2005). Thus, an increase in photorespiration, by consuming the excess of electrons not used by photosynthesis, partially avoids the formation of ROS (Wingler et al., 1999; Bai et al., 2008).

According to Uzilday et al. (2015), carotenoids are present in all photosynthetic organisms and are an integral part of the chloroplast thylakoid membrane. They play an important role in protecting against oxidative stress, the light energy absorbed by carotenoids is transferred to chlorophyll for photosynthesis, because of this role they are called accessory pigments. Carotenoids also have a protective role for plants against damage caused by light. They are particularly able to react with singlet oxygen product at the level of the chloroplast thus protecting the photosynthetic electron transport chain (Havaux et al., 2007).

In addition, carotenoids can inhibit the generation of free radicals (Peterman et al., 1995), where they associate with photosystem II (PSII) or the collector antenna contribute to the protection of the photosynthetic apparatus against ROS (Asada, 1994; Miller et al., 1996). Carotenoid cleavage products can also act in the defense processes of the plant (Bouvier et al., 2005). Abscisic acid (ABA) is derived from the cleavage of carotenoids (Nambara and Marion-Poll, 2005). The decrease of carotenoids under salt stress leads to the degradation of β-carotene and the formation of zeaxanthin which are apparently involved in the defense against photoinhibitions (Sharma and Hall, 1991).

### Sugar and Protein Contents in Leaves

The results obtained confirm that there is a correlation between the decrease in soluble sugars content and the rate of photosynthetic pigment degradation in the leaves of radish plants, which is evident in the “Cerise” variety under the effect of

severe salt treatment (EC 12.18 dS m<sup>-1</sup>). This is confirmed by the results of Fryer et al. (1998) and Gao et al. (2015), where the decrease of CO<sub>2</sub> assimilation rate in leaves is associated with inhibition of photosynthesis. We also mention that genes involved in photosynthesis and mobilization of stored reserves are repressed during an increase in sugar concentration, while the genes required for carbon catabolism metabolite are induced (Pego et al., 2000).

The evolution of the leaf protein content also showed a variable response in the plants of the two radish varieties. If "National" shows no significant reduction depending on soil salt stress intensity, there is a slight increase in proteins at "Cerise". The reduction of soluble protein content under the effect of salt stress is reported by several authors, including Khosravinejad et al. (2009), in their work on barley and Amini et al. (2007), in their work on tomato. These authors report that salinity induces the decrease of certain soluble proteins and that this variation of the protein content does not necessarily give the plant a salt stress tolerance.

This result can be explained by the fact that the plant seeks to protect its morphophysiological integrity, in response to the damage and the adverse effects of applied stress by developing enzymes and proteins whose role is to neutralize the molecules generated by this stress (Gardès-Albert et al., 2003).

Protein stimulation is due to an activation of a set of genes allowing the synthesis of specific proteins associated with stress "stress proteins" (Hamilton and Heckathorn, 2001) such as proteins "LEA" "AQP" and "HSP", which provide protection of the vital set of cellular proteins to maintain the protein and membrane structures of plant cells. These proteins are associated with plant stress tolerance in many crops (Kosová et al., 2013; Ahmad et al., 2016).

As noted, the accumulation of proteins involved in photosystems protection, such as DSP22 and DSP21, which are located in thylakoids and DSP34 located in stroma (Maury et al., 2011), as well as the accumulation of other proteins involved in carbon assimilation process, by means of its hydration to facilitate its diffusion and increase its concentration inside chloroplasts (Dubos, 2001).

### Total Phenolic Content and Antioxidant Activity

Our results have led to an examination of the response of radish plants to salinity through antioxidant activity to evaluate markers of oxidative stress.

The DPPH test, is specific for antioxidants capable of releasing a hydrogen atom, to stabilize the DPPH radical, and is therefore a good indicator of free radical-scavenging ability in plant cells (Ouhibi et al., 2014).

In addition, several studies show that salt stress induces an excessive production of reactive oxygen species (ROS) (Ksouri et al., 2007; Karray-Bourauoui et al., 2011), hence a ROS affecting different metabolic process which will induce an oxidative stress (Reginato et al., 2014).

Abiotic constraints are able to stimulate the biosynthesis of polyphenols (Akula and Ravishankar, 2011). In addition, it is possible that oxidative stress and secondary metabolism may be related (Hong et al., 2013).

The antiradical properties of phenolic compounds are comparable due to the presence of hydroxyl groups on the aromatic cycle able to give electrons or hydrogen atoms (Saqib et al., 2015).

Our results show that an accumulation of phenolic compounds evolves in leaves of both radish varieties in applying salt treatment (NaCl). At "National" variety, the synthesis of these compounds is significantly stimulated from the first level of saline (NaCl) soil treatment (EC 5.63 dS m<sup>-1</sup>); the second level of salt treatment (EC: 12.18 dS m<sup>-1</sup>) effect causes overproduction in polyphenols, particularly at "National" vr. compared to the "Cerise" vr.. This increase is strongly correlated with their ability to synthesize the antioxidants molecules needed to cope with ROS and maintain their low-level concentration in cells, which reflect the salt stress tolerance of plants (Reddy et al., 2004).

An increase in polyphenols, due to increased salinity, is reported in barley (Ali and Abbas, 2003) and maize (Hajlaoui et al., 2009). The work of De Abreu and Mazzafera (2005) conclude that responses of species vary considerably in their content of phenolic compounds related to their genetic variability and to their environment.

### CONCLUSION

In this experiment, we conclude that the stress due to soil contamination by sodium chloride (NaCl) salts is reflected by important changes in physiological, biochemical and antioxidant activity due to the imbalance of different metabolic processes in plants of the two studied radish (*Raphanus sativus* L.) genotypes. On the other hand, photosynthetic pigments stability, sugars accumulation, increase of protein content and antioxidants substances, in particular phenols in the leaves are good indicators of the plant's ability to resist stress and reduce metabolic disorders. "National" and "Cerise" varieties showed variable responses under conditions of the soil saline (NaCl) contamination.

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### AUTHORS CONTRIBUTIONS

Conceptualization: Acila S., Allioiu N.; Metodology: Acila S., Allioiu N.; Data validation and processing: Acila S.; Writing – original draft preparation: Acila S.; Writing – review and editing: Acila S., Allioiu N.

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## CONFLICT OF INTEREST

The authors declare no conflict of interest.

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