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Characterisation of two varieties of tomato (*Lycopersicon esculentum*)with saline resistant

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Abstract

During this study we have determine the effect of salt stress on the growth and biochemical parameters of plants. To do so, two tomato varieties were studied: *Rio Grande* and *Heinz* which differ in their origins and their adapting behaviour to abiotic stress. The two ecotypes originated from America. In order to select tomato genotypes that are more tolerant to salinity, an experiment was carried out and repeated three times. The salinity stress factor comprised two genotypes and three levels of salinity stress. The percentage of the dry and fresh matter and the stem length, the length of both young and old leaves, decreases with the rise in salinity in the two varieties with a slighter decrease in the *Rio Grande* variety compared to that of the Heinz. The foliar surface was more affected by salinity in the *Rio Grande* compared to the *Heinz*. Likewise, the contents in proline increased significantly more in the *Heinz* than in the *Rio Grande*. The results also show that the content of proteins is smaller in *Rio Grande* than in *Heinz*. These results seem to show that the *Rio Grande* variety studied is more sensitive to salinity within the tested limits than the *Heinz* variety. Static analyses show there is difference significant between salinity and the two varieties and between the vegetative and biochemical parameters, for the test of Fisher.

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Introduction

The functional biology of salt stress adaptation in plants is matter of current debate, in the 20th century, tomato (*Lycopersicon esculentum*) is eaten all over the world. It has become one of the most produced vegetables in the whole world (122 million tons in 2005): FAO Statistical database, 2008. Destined to be consumed fresh or after being modified industrially, tomatoes are an important source of minerals, vitamins, antioxidants and fibre in human diet.

The tomato has established itself as a model for several species with agronomical interest such as grapes, peaches, melon, apples or strawberries. Moreover, this species presents numerous biological advantages (short biological cycle, easy crossbreeding, genetic modification with *Agrobacterium,* small sizegenome, very large genetic diversity).

It is sensitive to moderate salt concentrations in the soil. However, many authors reveal a great variability amongst tomato genomes in their response to salinity according to Cuartero and Ferandez-Munez, 1999; Manaa *et al.*, 2011.

Salts present in the soil and in irrigation waters perturb the germination of grains, affect the growth of the seedlings and, consequently, the culture production. Na+ Cl- ions that are accumulated in plant tissues of above ground organs, can equally provoke toxicity in the plant, Munns and Teter, 2008. Moreover, their effects on the plant osmotic potential, high concentrations of NaCl cause a toxicity linked to excessive presence of Na+ ions which interfere with K+ ions. The salinity affects all physiological processes of the plant as well as its development and its growth.

The summary of organic compounds or osmoprotectants, is one of the strategies deployed by the plant in order to attenuate the effect of salinity. Proteins, soluble sugars, amino-acids and especially proline, count amongst these organic compounds which play a major role in the osmotic adjustment of the plant already perturbed by the excessive presence of ions. The tolerance of the plants to salt depends on their upholding of a sufficient absorption of essential nutrients such as K+, notably in presence of an excess in Na+. Shabala and al., 2005.

Genetic variability between species facilitates the screening and/or the selection of more tolerant plants at more or less high concentrations of salt. This variability is therefore necessary for any program aiming at selecting genotypes that are resistant to salt. Thus, it was indicated in several plant species: amongst 14 accessions of soft wheat Goudarzi and Pakniyat, 2008 ; 60 melon cultivars Kusvuran and al., 2007 ; 7 barley cultivars Chen *et al.*, 2005 and amongst 18 tomato cultivars Turhan and al., 2009. Thus, it has been reported in several plant species: between 14 accessions of soft wheat Goudarzi and Pakniyat, 2008 ; 60 melon cultivars Kusvuran *et al.*, 2007 ; 7 barley cultivars Chen and al., 2005 and between 18 tomato cultivars Turhan and al., 2009.

The objective of this work is the assessment of the degree of sensitivity ortolerance at the germination level first, and then at the vegetative stage of seedlings, of two tomato varieties of which one (*Heinz*) and the other (*Rio Grande*) to determine their behavior in the face of the increase in salt stress want to improving their productivity in the areas where they are grown.

Material and methods

Culture and treatment of tomato grains:

To study the effect of varying degrees of salinity on germination capacity, tomato seeds from 2 genotypes *Rio Grande* and *Heinz* were put to germinate during 48h at 22°C, in darkness. This was realised after disinfecting the seeds with sodium hypochlorite at 2% during 10 minutes, then rinsing them carefully with distilled water in petri boxes (30 grains) lined with 3 layers of filter paper soaked with distilled water for the « control » plants. By contrast, the « salinity » treatment consisted of bringing 0, 25, 50 or 150 mm of NaCl to the distilled water. Under controlled conditions of temperature, humidity and lighting, the number of germinated grains in each of the concentrations is collected every 24 hours for the evaluation of the effect of growing concentrations of NaCl on the percentage of germination.

In addition, the plants that were issued from germination in an environment free from NaCl, are transplanted in trays filled with inert coarse sand and are watered with Hoagland nutritive solution modified by Jemal and al. 2005, whose composition is as follows: 1,5 mM of Ca (NO_3) 4 H_2O, 0,5 mM of $MgSO_4$.7 H_2O, 1 mM of KNO_3, 1 mM of KH_2PO_4, 1 µM of KH_2PO_4. 7 H_2O, 30 µM of H_3BO_3, 50 µM of Fe-EDTA, 10 µM of M_nSO_4, 1µM of Z_nSO_4. $7H_2O$. At the 3-4 leaves stage, the seedlings are transplanted into plastic pots filled with nutritive solution in presence or absence of NaCl at the following concentrations (0, 17, 50, 85 et 130 mM). Culture media are aerated then regularly renewed.

After a period of 14 days in the hydroponic environment, the seedlings are removed, the number of leaves is counted and the foliar surface is measured. The length of the stem as well as the root, the length of young leaves and that of old ones are also measured. Then, the above-ground part is separated from the root part and the dry matter of the part above-ground is determined after drying during 48 hours at 80°C.

Next, the above ground part is separated from the root part and the dried matter of the aerial portion is fixed after drying during 48 hours at 80 °C. The weighing is carried out using electronic precision scales of the type Model- Citizen XK3190-A7M. Total protein synthesis is achieved according to the method of Bradford. 1976. Finally, the foliar proline content is determined according to the method of Bates *et al.*, 1973, Marin and al., 2009. The results have been subjected to analysis of Anova and the averages were compared with the help of the Fisher test. 1953. Baed on the LSDT method (Least Significant Difference test) using a software called XLSTAT 2012-2013 for Windows). Each average is assigned a letter. Averages followed by the same letter are not significantly different at the 5% probability level.

Results and discussion

We have noticed that salinity in the limit value of the examined concentrations significantly reduced the germinating capacity of grains from both varieties from the first concentration of 25 mM. These results are in agreement with some of the works already published, Lachhab and al., 2013, Ould Mohamdi and al., 2011.

Table 1. Represents the number of germinated grains at all concentrations with both varieties and their germination percentage.

Variety / Duration	Rio Grande				Heinz			
	0mM	25mM	50mM	150Mm	0mM	25mM	50mM	150mM
50h	23	8	2	0	29	15	5	0
70h	26	23	9	2	30	28	11	6
%Germination	86,6	76,6	30	6,6	100	93,3	36,6	20

Averages followed by different in the same column for each treatment differ significantly (probability < 0.05).

On the tomato in their work on cucumber Cuartero. 1999, Amini and Ehsanpour, 2005, reported that increasing saline concentrations in the medium, not only induce considerably reductions in the percentage of germination but they also slow down the speed of germination of a few tomato cultivars exposed to salt stress.

Effect of NaCl on the morphological parameters

The salinity effect is not homogenous for all organs and the morphological response of the latter are different, Hilal and al., 1998. And sometimes they are even opposite between the Juvenal and adult stages, Munns and al., 1986, Zid and al., 1989.

Table2. Represents the effect of NaCl on the morphological parameters.

Variety	NaCl (mM)	SL (cm)	RL (cm)	FM (mg)	DM (mg)	LA (cm²)	NL	OLL	YLL
Rio Grande	0	14,20±0,72	9,6±0,6	0,46±0,01	0,06±0,01	4,2±0 ,1	8±1	8,2±1	8,9±0,7
Rio Grande	25	12,4±2,33	10,4±0,5	0,37±0,02	0,03±0 ,01	2,7±0 ,2	7±0,6	7,3±0,6	7,1±0,25
Rio Grande	50	12±1	11,8±1,3	0,22±0,17	0,02±0 ,01	1,2±0 ,51	5,6±1	6,1±0,6	5,7±0,21
Rio Grande	150	9,3±1,41	13,6±0,5	0,11±0,01	0,02±0 ,00	0,9±0 ,05	2,4±1	4,9±0,6	3,1±0,25
Heinz	0	16,4±0,7	10,5±0,5	0,89±0,11	0,08±0 ,01	7,7±0 ,51	11±1	10,3±0,6	10±0,15
Heinz	25	15,2±0,7	11,6±0,3	0,57±0,08	0,07±0 ,02	6,5±0 ,29	9,3±1,15	8,9±1	8,5±1
Heinz	50	12,3±2,4	13,9±1,1	0,38±0,05	0,06±0 ,02	4,2±0 ,31	6±1	6,6±0,6	6,5±0,58
Heinz	150	11,3±0,9	15,8±0,9	0,27±0,03	0,04±0 ,01	2,5±0 ,45	4±0,6	5,5±0,6	4,7±0,58

SL=long stem, RL=long root, FM=fresh matter, DM=dry matter LA= leave area NL=number of leaf, OLL=ould leaves length, YLL=yield leaves longht.

On stem and root:

According to our results (Table 2), after 90h explain that the seedlings of the *Heinz* variety show a size of stem and roots larger than that of *Rio Grande*. At 0 mM of NaCl, the two varieties germinate but the *Heinz* presents a higher growth (of stem and root), indicating specific characteristics for each variety. On the other hand, at 25 and 50 mM of NaCl: The growth (of stem and root) has decreased with both varieties while remaining always higher with the Heinz variety compared with the *Rio Grande*. At 150 mM one notices a sharp decrease with *Rio Grande* compared to *Heinz* (Fig.1 and 2). Thus, the *Heinz* variety seems to have certain properties enabling it to have a better resistance to salt stress.

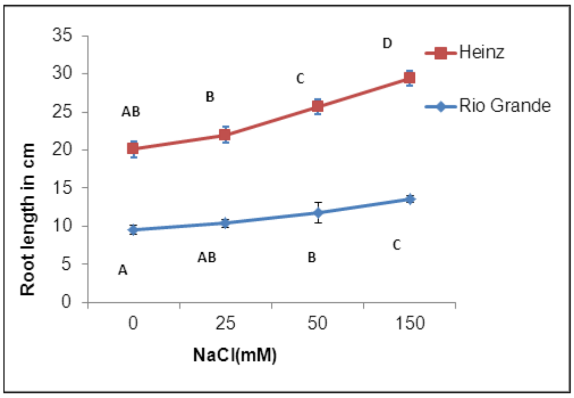

Fig. 1. Effect of the concentration of NaCl on the root length with both varieties of tomatoes. Each value represents an average of 3 out of 3 repetitions ± gap.

Salinity reduced the growth of the over-ground parts of the tomato more than it did its roots. This resistance of the tomato roots system to salt stress may be due to a reduction of carbon allocation for foliar growth in favour of roots growth, Brungnoli and al., 1992. The decrease in the growth may also be linked to some perturbations of growth regulating rates (Abscisic acid and cytokinins) induced by salt, Kuiper and al., 1990.

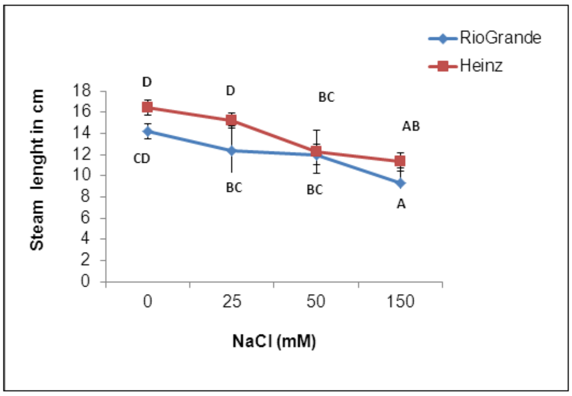

Fig. 2. Effect of the concentration of NaCl on the stem with both varieties of tomatoes. Each value represents an average of 3 out of 3 repetitions ± gap.

On dry and fresh matter

After a culture period of 80 days, fresh and dried matter of both varieties is determined (Table 2). Whether salt is present or not in the nutritive solution, the *Heinz* variety produces more fresh matter than the *Rio Grande* variety (Fig. 3 and 4). Similar results were reported by Ben Khaled and al., 2003. Indeed in the control solution, fresh matter with the *Heinz* variety equals (0.89 mg) and only (0.457 mg) with the *Rio Grande*, under saline constraint, NaCl concentration at 150 mM reduces significantly the production of fresh biomass with the

Heinz variety (22.97%) compared to the fresh biomass of the *Rio Grande* (30%) (Fig. 6).

According to Ben Ahmed and al., 2008, the depressive action of salt manifests itself equally by the reduction of the production of dry matter of the different organs of the plant. Also, it manifests itself equally by a reduction in the length of the plants according to Singh and Passad. 2009.

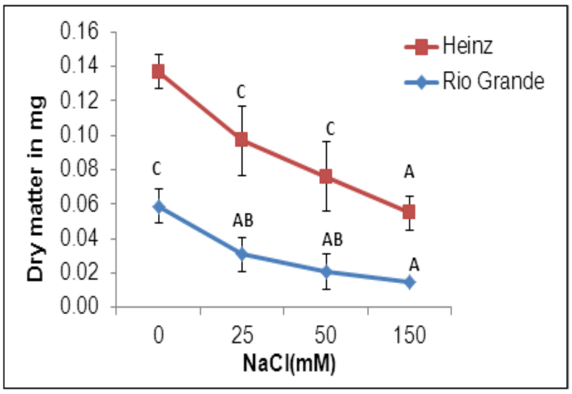

Fig. 3. Effect of the concentration of NaCl in dry matter with both tomato varieties. Each value represents an average of 3 repetitions ± gap.

The depressive action of salt manifests itself by a reduction in the production of dry matter by different organs of the plant and sometimes by a reduction in the photosynthesis capacity due to a decrease in CO_2 stomatal conduction under salt stress according to Santiago and al., 2000. It is attributed to a combination of the osmotic effect and the specific effects of Na+ and Cl- ions, Turan and al., 2007; Taffouo and al., 2010.

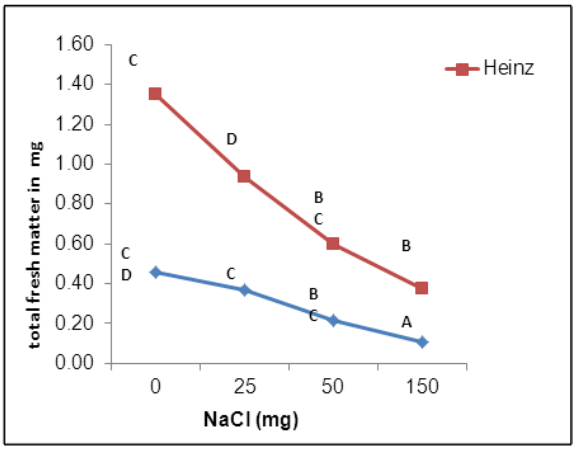

Fig. 4. Effect of the concentration of NaCl in fresch matter with both tomato varieties. Each value represents an average of 3 repetitions ± gap.

It is also established that salinity decreases the production of biomass through a limitation of the absorption of potassium and of calcium, Lachââl M and., 1995. And of their transport of ions Soltani and al., 1990. A supplement of Ca2+ in the culture medium corrects these effects, Rengel and al., 1992.

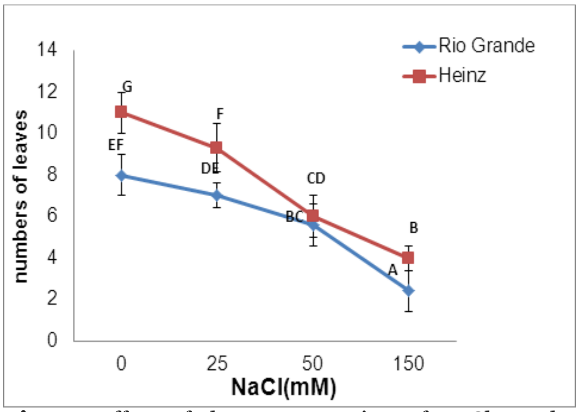

Fig. 5. Effect of the concentration of NaCl on the number of leaves with both tomato varieties. Each value represents an average of 3 repetitions ± gap.

On number of leaves and the leaf area:

In addition, salinity causes a similar and gradual decrease of foliar surface in both tomato varieties until it reaches 12%, compared to the controls at the highest do se of NaCl (150 mM) for the *Heinz* as well as for the *Rio Grande* (Fig. 6).

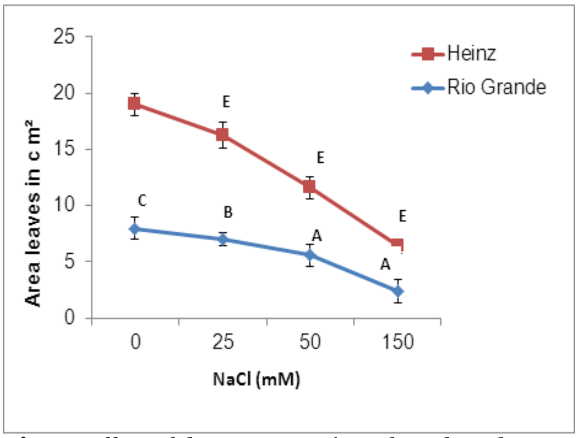

Fig. 6. Effect of the concentration of NaCl on the area leaves with both tomato varieties. Each value represents an average of 3 repetitions ± gap.

A rising colour gradient going from yellow-brown for old leaves to dark green for the young ones has been equally registered for both tomato varieties under a treatment of 150 mM of NaCl (Fig. 7 and 8). The number of leaves is higher with *Heinz* than with *Rio*

Grande for the three stress levels (Fig. 5).Similar results were reported by Ben Khaled and al., 2003. This may be attributed to high accumulation of toxic Na+ ions; according to Lachââl *et al.*, 1996.

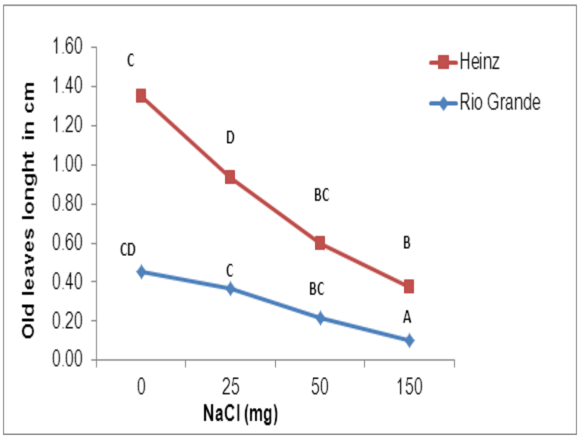

Fig. 7. Effect of the concentration of NaCl on old leaves with the two varieties of tomato. Each value represents an average of 3 repetitions ± gap.

Effect of NaCl on the proline conten

The results have shown a high proline content with plants coming from an environment where the ground is considered to be saline, the accumulation of proline being an adaptation mechanism to salinity with this species according to Morsy. 2008. (Fig.9) show that in the absence of salt stress, *Heinz* accumulates more proline in its leaves (0.273 μg/g), its content is significantly different from that of the *Rio Grande* (0.25 μg/g). On the other hand, in the presence of NaCl the *Rio Grande* accumulates more proline with a significant difference (37%) at the third NaCl level (150 nM).

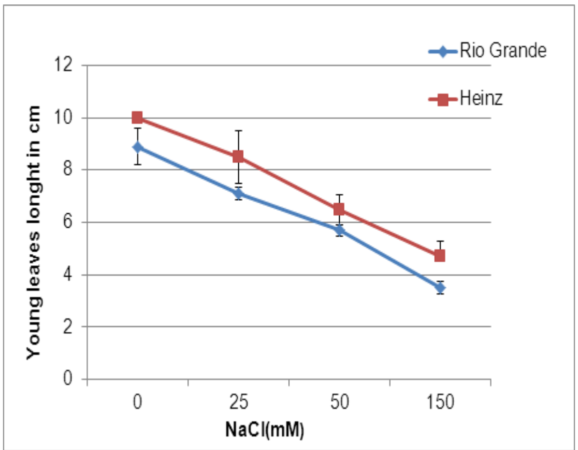

Fig. 8. Effect of the concentration of NaCl on young with the two varieties of tomato. Each value represents an average of 3 repetitions ± gap.

This figure shows also that it is severe salt stress that engendered the high proline accumulations, and it is *Rio Grande* that was most affected (Fig.9). It should be noted, that the moderate constraint did not lead to significant modification of this substance. The interaction of proline treatment being significant, it stipulates that each variety has behaved differently when faced with salt stress.

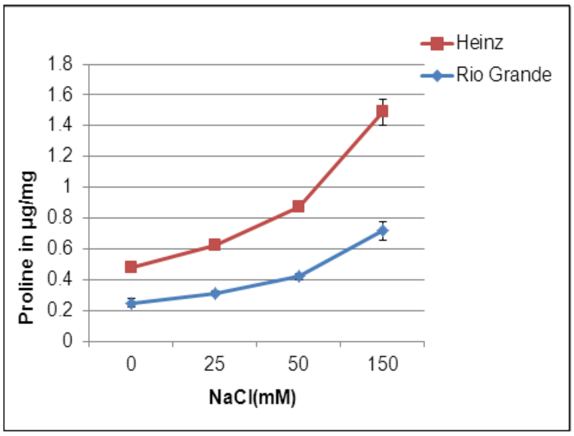

Fig. 9. The content of proline in the two species of tomato (*Rio Grande*, *Heinz*). Each value represents an average of 3 repetitions ± the gap.

The study of the nitrogen metabolism of a plant undergoing an abiotic constraint shows a varying global accumulation of amino acids, according to the nature of the applied stress and of the studied taxon, Ranieri and al., 1989; Belanger and al., 1990. The proline accumulation was independent of NaCl presence in the nutritive solution but it varied according to the varieties. Several authors, including Hernandez and *al.*, 2000; Khedr and *al.*, 2003; Claussen. 2005; Debnath and *al.*, 2008, had mentioned that this amino acid was part of the osmoticumsplants synthesise once they are exposed to water or salt stress. Its role is necessary for the osmotic adjustment in order to balance the osmotic potential of the ground in accordance with what has been demonstrated by other works including those of Gadallah. 1999. And Demir. 2000. Proline is considered to be an indicator of metabolic constraint. It is not specified (salinity constraint or thermal, water). Its accumulation is a common characteristic to numerous monocotyledons subjected to salt stress according to Moulineau. 1993; Zoumarou-Wallis. 1996; Nayar and Walia. 2003; Ashraf and Harris.

2004. The application of a severe salinity constraint has multiplied the proline content of both tomato varieties. It could be used as criteria for early selection of salinity-tolerant legumes, Messai and al. 2006.

Effect of NaCl on total proteins

By means of an electrophoresis of total proteins (Fig.10), on notices the presence of 5 major bands (A, B, C, D and E) which are present in the two varieties and only one band (F) exists with Heinz at the 100mM level of NaCl. These bands are light in the absence of NaCl (0 mM) and in the higher concentration of (150 mM of NaCl). By contrast, at average concentrations 25 and 50 mM in NaCl, the bands are clearly defined. Also, one observes a band (F) only with Heinz at the 100 mM level of NaCl, which means a specific protein for this variety. When there is germination, one notices a perceptible decrease of protein bands (A to F) in relation with the expression of proteases.

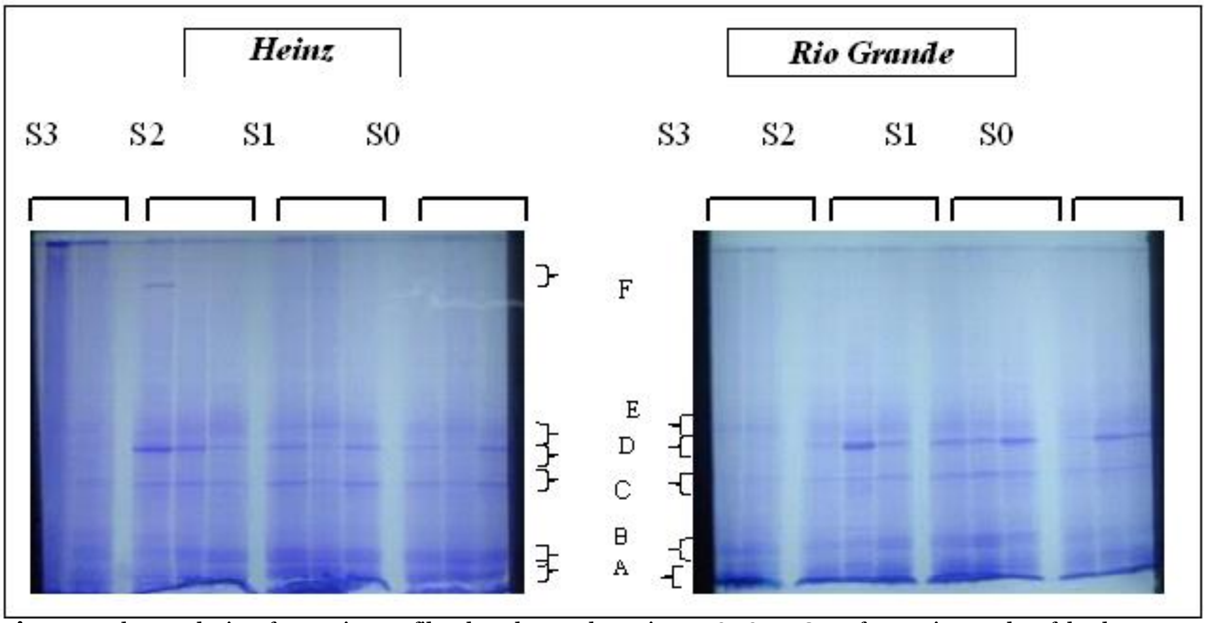

Fig. 10. The analysis of protein profiles by electrophoresis on SDS-PAGE of protein totals of both tomato varieties.

Evolution of the protein content in both varieties has also known a varying response amid the seedlings of both verities. The reduction in the content of the soluble protein totals under salt stress has been reported by several authors including Khosravinejad and *al.*, 2009, in their work on two varieties of barley Amini and Ehsanpour. 2007.

Mohameden and *al.,* 2011; in their work on the tomato variety (Campbell 33 and Mongal), the salinity induces the reduction of certain soluble proteins and that this variation in the proteins content does not necessarily give to the plant a tolerance to salt stress. These differences may correspond to the characteristics of each variety. One notices that there are different proteins which are squeezed out in the germination of both varieties. This indicates the presence of enzymatic activity protease which degrades these proteins. This is not the case for the profile corresponding to the concentration of 50 and 150 mM. In other words, with salt concentration of 150 mM where there is no germination, proteins remain intact and do not degrade. There is no protease expression at this level of salt concentration.

Conclusion

When faced with salinity, plants develop a series of mechanisms which can of morphological, biochemical, physiological or molecular order. The processes which favour tolerance to salt tress and which help to support an excess of ions can act in a supplementary or synergic manner. We propose to follow the physiological behaviour and the agronomic performance of the cultivated plants in nutritive solutions, and in particular, their growth, their development in saline conditions, under controlled laboratory conditions. The comparison between the varieties of plants cultivated in the nutritive solution or not, will enable us to evaluate the importance and the efficiency of the growth with the tolerance to salinity. In our study, we concerned ourselves with the accumulation of proline with the proteinic synthesis and a few vegetative parameters. Indeed, in the case of a saline constraint, the tomato adjusts its osmotic potential through accumulation of proline which increases proportionately to the applied salt stress. However, we should not neglect the significant role played by proline in the tolerance to stress where its potential role as an indicator of tolerance in the genetic enhancement programs for certain species. Furthermore, we have established that the reduction of certain proteins is a genetic characteristic.

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