

Evaluation of salt and UVB stress on tomato landraces from saline zones of Bihor County

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Abstract Over the past years awareness towards extreme weather conditions had increased and are predicted to happen in near future and together with growing world population and unequal food distribution indeed will bring a strong impact on overall crop production. For these reasons the signalling pathways leading to an appropriate and coordinated response to abiotic stress have been the target of intensive research in the past decades. Global warming and extensive farming make it necessary to understand the underlying mechanisms of these abiotic stresses and to increase plant stress tolerance through either selective breeding or by genetic modifications. This study has proposed to identify some tolerant local landraces of tomatoes to salt and UVB stress for utilization in breeding programs and as seeds for farmers. The seed material was collected from saline zones of Bihor County, in North-Western of Romania, and the experiments were employed on 3 out of 23 local landraces of tomatoes. Three types of abiotic stresses (salt stress, UVB radiation stress and a combination of both) were applied on early stage of plant development onto tomatoes plants grown from the seeds inside the greenhouse. A PAM fluorometer was used to measure chlorophyll fluorescence and from calculated chlorophyll fluorescence parameters (Fv/Fm, Y(II), ETR) the photosynthetic efficiency was determined. We found out that 2 of the studied local landraces were tolerant to high salinity and we propose that this cultivars to be used for breeding programs.

Key words

abiotic stress, tomatoes, local landraces, PAM fluorometry

In the last decade the awareness towards climate change was raised and upcoming generations can be influenced by the environmental factors [4]. On the other hand, growing world population, unequal food distribution, competing claims for land use are some of the reasons that challenges agriculture for crop production in the coming decades.

Recent predictions, made by the United Nations Food and Agriculture Organization, shows that cereal production must be doubled before 2050 to satisfy the demand for food by the growing world population, as well as the increasing competition for crops as sources of bio-energy, fiber and other industrial purposes (www.fao.org).

Under conditions of rapid climate change the plants that display more phenotypic plasticity rather than genetic diversity will be more successful in adaptation to their environments [14], [7]. The genotypes grown under various environmental conditions will exhibit a phenotypic plasticity, quite big under extreme conditions such as frost, drought and salinity [13]. Therefore is a demand for quantitative analyses of plant traits to accelerate the selection of crops that are better adapted to resource limited environments and soil

conditions, which is also a major constraint to global food production [5].

For these reasons the signalling pathways leading to an appropriate and coordinated response to abiotic stress have been the target of intensive research in the past decades.

Of the important abiotic stresses that affect plant growth are salinity, drought, excessive light, UVB radiations. Under upcoming climate conditions is of crucial importance to understand the underlying mechanisms of these abiotic stresses and to increase plant stress tolerance through either selective breeding or by genetic modifications. In plants, abiotic stresses often cause morphological, physiological, biochemical and molecular changes that unfavorably affect plant growth and development [9].

Salinity, one of the most important factor of stress, acts similarly to drought on plants by preventing roots of performing their osmotic activity [8]. Therefore, due to increased levels of salinity in the soil water, nutrients cannot move into the plant roots. In some cases, salinity also has a toxic effect on plants because of the high concentration of certain salts in the soil [7].

The degradation of the ozone layer, mostly due to the human activity, determines a specific increase of the ultraviolet-B (UVB) radiation reaching the Earth. There is a direct relation between UVB radiation (280-320 nm), terrestrial life and diminishing of the ozone layer [1]. In plants, UVB radiations represents another major factor of stress that causes damage of photosystems (PS I and PS II), deficiency in carbon fixing, decreasing of chlorophyll level, decreasing of sugar level [10].

There is a need, at global level, to know about the phenotype response to breed for increased yield and yield stability facing the climate change and environment [15]. For this reason is crucial to understand better plant reaction to different stress environments, to assess the performance of plant species [12].

Development of chlorophyll fluorescence imaging, in the last years, permits the study of the spatial-temporal heterogeneities in the fluorescence emission pattern within cells, leaves or whole plants [6]. This technique non-destructive, rapid and relative inexpensive, has an important advantage that makes it a valuable tool in plant research. One of these advantages is the potential to detect stresses before visual symptoms appear.

By screening of genotypes with certain tolerance to biotic or abiotic stresses it is possible to

identify those that have native resistance to different stress factors. An important advantage of this technique is that it can be used either to screen a large number of small plants simultaneously or a single plant [2], [3].

In this study, Pulse-amplitude-modulated (PAM) fluorometer, a fast and non-destructive tool, was used for screening the tolerance of studied plants to salinity and UVB radiation. We used chlorophyll *a* fluorescence measurements that provided data for evaluating the physiological status of plants. We evaluated the resistance of the studied landraces to salinity and UVB stress and purposed 2 of the tomatoes cultivars for future breeding programs.

Materials and Methods

Collection of material

The seed material was collected in 2013 from some localities in the western part Bihor County that have soils with a moderate salinity. Each landrace name comes from the name of the village and the house number where the seed were harvested from (Table 1). The seeds were collected from 16 locations. 14 different landraces have been found, that were cultivated by the peasants from the fruits selected from their gardens.

Table 1

Tomato landraces collected from Bihor Count

Sample no.	Adress	Fruit weight (g)	Tomato shape and color
1	Ateas 37	176	Red Ox heart
2	Ateas 37	325	Pink Ox heart
3	Ateas 38	160	Red ox heart
4	Martinaz 7	7	Red cherry
5	Martinaz 7	40	Long red
6	Cefa 450	180	Big red
7	Martinaz 51	35	Long red
8	Martinaz 51	240	Big red
9	Cefa 7	115	Red ox heart
10	Cefa 7	410	Big red round
11	Cefa 7	3	Red cherry
12	Cefa 7	8	Yellow cherry
7	Ateas 136	220	Big red ox heart
14	Ateas 136	5	Yellow cherry
15	Ateas 136	97	Yellow round
16	Ateas 136	270	Big red

Growing of tomatoes plants

The plants were obtained from collected seeds in the greenhouse from our institute. For the experiments were used 3 landraces - Ateas No 37, Ateas No 136, Cefa No 7- and the standard cultivar Marmande as control. From each cultivar were used 5 plants grown at 18-20°C and 60% relative humidity.

The growing substrate was peat with pH 6-6,5 that allowed a better control of salinity content during the treatments.

Design of the experiments

Two different abiotic stresses were applied: UVB radiation, salt and a combination of the two. For

the salt stress, two different concentrations of salt solution, 150 mM (S1) and 300 mM (S2), were employed. The salt solution was made of NaCl with a purity of 90%. The UVB radiation intensity of 0,400mW/cm²(UVB) was studied on the tomatoes plantlets. For the forth experiment, a combined stress of 0,400mW/cm²UVB radiation and 300 mM (S2) salt solution (UVB+S2) was used.

All treatments were applied on tomatoes plantles in early stage plant development, when the first leaves were well developed.

All experiments were done in triplicate with three independent replicas.

The UVB radiation was applied for three consecutive days at the same time of the day for 2 hours. The Fv/Fm measurements, with Open Fluor Cam, were made after every day of treatment.

The salt treatment consisted of weekly watering the plants with the above mentioned salt solution for three weeks.

The combined treatment was done as follows: the plants were watered with salt solution and after two salt treatments the UVB treatment was applied for three days, two hours daily.

Chlorophyll fluorescence measurements

Chlorophyll fluorescence imaging is a rapid and non-destructive technique, successfully used today in the evaluation of plant photosynthetic activity. It is very often applied in the diagnosis of biotic or abiotic stresses because it has a useful potential to detect stresses before visual symptoms appear; this is ideal in screening of genotypes for an early identification of

those with high tolerance to biotic and abiotic stresses [6].

For chlorophyll fluorescence measurements from our experiments we used the imaging fluorometer Open Fluor Cam. The photosynthetic efficiency was determined using Fv/Fm parameter, which represents the maximum quantum yield of PSII, after dark adaptation. All the chlorophyll fluorescence measurements were done after 15min dark adaptation of the tomatoes plants.

Results and Disscutions

The photosynthetic efficiency of tomatoes landraces treated with UVB radiation

We treated the tomatoes plantles with UVB radiation for 2 hours daily in three consecutive days as described in Material and methods. The control and the three UVB treatments correspond to the four groups of the collumns from figure 1. Each group of columns consists of measurements of Fv/Fm of the three landraces and one standard cultivar-Ateas_37, Ateas_136, Cefa_7 and Marmande. After the first day of treatment no significant changes have been observed. On the second day of treatment the studied cultivars reacted different: comparing with the standard cultivar, the ratio Fv/Fm has decreased on the three landraces. But the protective mechanisms from plants reacted and on the third day, Fv/Fm encreased significant comparing with the values from the first two days.

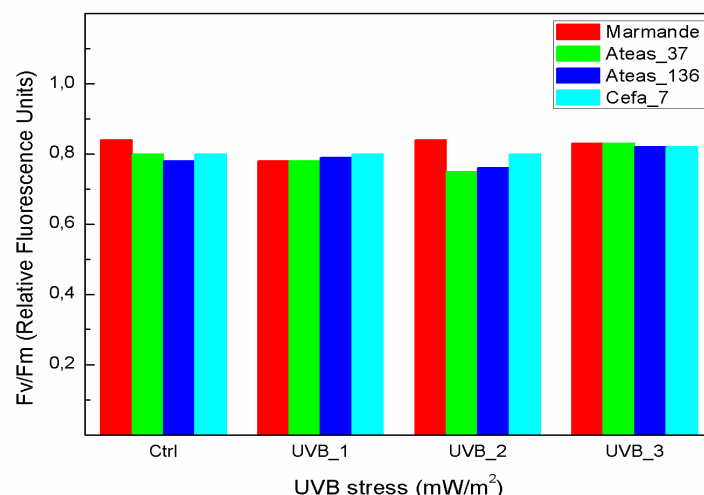


Fig. 1. Effect of UVB radiation on the Fv/Fm parameter at four tomato cultivars

The presence of 150mM salt in the soil did not induce significant difference in the growth of plants and also the photosynthetic efficiency was not affected under well watered conditions (60% soil water

content). When the amount of salt in the soil was increased to 300 mM, a significant decrease of green shoot/leaf area was observed, even under well watered conditions, but the photosynthetic efficiency remain

unchanged. Best tolerance against the effect of salt was observed in case of the Ateas 136 and Marmande varieties. Even the combined stress factors (UVB+S2)

have not decreased significantly the efficiency of photosynthesis (figure 2).

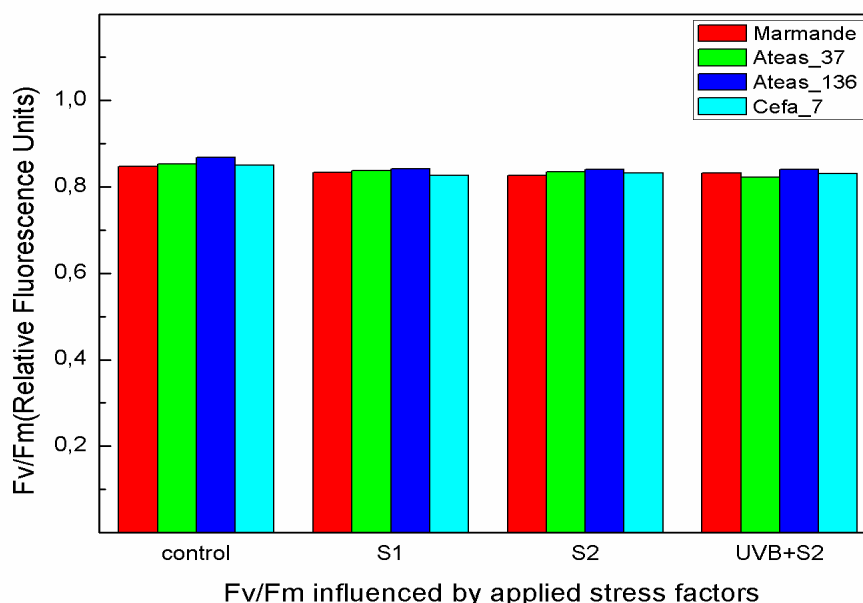


Fig. 2. Effect of different concentrations of salt and salt + UVB radiations on the Fv/Fm parameter at four tomato cultivars

Conclusions

After this experiment we can conclude that the amount of salt used for stressing plants doesn't produce significant damages at PS II level; the effect of used UVB intensities is counteracted by the protective mechanisms in tomatoes plants. Molecular analyses are needed for a better understanding of correlation between chlorophyll fluorescence measurements and the effect of stress factors on the studied plants.

We aimed to develop a phenotype screening of the best landraces, in relation to resistance traits, to compare their growth habit, morphology, salt and UVB tolerance and photosynthetic efficiency.

The three studied landraces are suitable for breeding programs, because of their native resistance to salt and UVB stress.

References

1. Allen, D. J., Nogués, S., & Baker, N. R. (1998). Ozone depletion and increased UV-B radiation: is there a real threat to photosynthesis?. *Journal of experimental Botany*, 49(328), 1775-1788.
2. Baker, N. R., & Rosenqvist, E. (2004). Applications of chlorophyll fluorescence can improve crop production strategies: an examination of future

possibilities. *Journal of experimental botany*, 55(403), 1607-1621.,

3. Chaerle, L., Hagenbeek, D., De Bruyne, E., & Van Der Straeten, D. (2007). Chlorophyll fluorescence imaging for disease-resistance screening of sugar beet. *Plant Cell, Tissue and Organ Culture*, 91(2), 97-106.

4. Dawson, T. P., Jackson, S. T., House, J. I., Prentice, I. C., & Mace, G. M. (2011). Beyond predictions: biodiversity conservation in a changing climate. *science*, 332(6025), 53-58.

5. Fiorani, F., & Schurr, U. (2013). Future scenarios for plant phenotyping. *Annual review of plant biology*, 64, 267-291.

6. Gorbe, E., & Calatayud, A. (2012). Applications of chlorophyll fluorescence imaging technique in horticultural research: a review. *Scientia Horticulturae*, 138, 24-35.,

7. Gratani, L. (2014). Plant phenotypic plasticity in response to environmental factors. *Advances in botany*, 2014. Plaut Z., 1995, Photosynthesis in plant/crops under water and salt stress, in Pessarakli M, ed. Handbook of Plant and Crop Physiology, New York, Marcel Dekker, p:587-603.

8. Komori, T., Yamada, S., Myers, P. N., & Imaseki, H. (2003). Biphasic response to elevated levels of NaCl in *Nicotiana occidentalis* subspecies *obliqua* Burbidge. *Plant Science*, 165(1), 159-165.

9. Maheswari, M., Yadav, S. K., Shanker, A. K., Kumar, M. A., & Venkateswarlu, B. (2012). Overview of plant stresses: Mechanisms, adaptations and research pursuit. In *Crop Stress and its Management: Perspectives and Strategies* (pp. 1-18). Springer, Dordrecht.
10. Rama Shanker Dubey, 2005, Photosynthesis in Plants under Stressful Conditions, Handbook of Photosynthesis, Second edition, p: 717-719.
11. Reynolds, M. P., **Pask, A. J. D.**, & Mullan, D. M. (2012). *Physiological breeding I: interdisciplinary approaches to improve crop adaptation*. CIMMYT.
12. Suter, L., & Widmer, A. (2013). Environmental heat and salt stress induce transgenerational phenotypic changes in *Arabidopsis thaliana*. *PloS one*, 8(4), e60364.
13. Tester, M., & Langridge, P. (2010). Breeding technologies to increase crop production in a changing world. *Science*, 327(5967), 818-822.
14. Vitasse, Y., Bresson, C. C., Kremer, A., Michalet, R., & Delzon, S. (2010). Quantifying phenological plasticity to temperature in two temperate tree species. *Functional Ecology*, 24(6), 1211-1218.
15. Wishart, J., George, T. S., Brown, L. K., White, P. J., Ramsay, G., Jones, H., & Gregory, P. J. (2014). Field phenotyping of potato to assess root and shoot characteristics associated with drought tolerance. *Plant and soil*, 378(1-2), 351-363.
16. www.fao.org,
FrontiersinPlantScience|www.frontiersin.org 1
August2015|Volume6|Article619, Rahaman et al.,
High-throughput plant phenotyping and data analysis