

RESEARCH THE INFLUENCE OF SALINITY OVER THE GERMINATION OF THE SEEDS AND GROWTH OF PLANTS OF *OCIMUM BASILICUM* L.

AURA - MANUELA DAVID*,
NICOLETA BĂDĂLUȚĂ**

ABSTRACT

„The King of Spices” – basil is a herbal plant from Asia, tasting as the anise and having a strong sweet – acrid scent. As a consequence of the change of global climate, also in Romania have been detected a lot of natural limitative factors such as: the increasing of the salinity of the soil over the admissible limits, the exceeding dryness or increased acidity. We have studied the action of different concentrations of Sodium Chloride (NaCl) over the physiological processes, in the same time, at two forms of *Ocimum basilicum* L.: *bullatum* (the green basil) and *crispum* (the red basil). The research were performed over the seeds and little plants and it has been followed up the degree of germination of the seeds and the development of the little plants, using the biometrical measures of some morphological composition (root, hypocotile, cotyledon, weight) in conditions of salinity. By the results obtained it has been noticed that the green basil (the *bullatum* form) is more resistant at salinity than the red one (the *crispum* form).

Key words: seeds, plants, biometric measurements, salinity

Introduction

Taking into consideration that at the global level it is expected an annual increase of population with 1.5%, reaching approximate 11 billion inhabitants in the year 2050, it is very important to remove the causes which determine the decrease of crops and technical plants. The factors of abiotic stress represent one of the most important reasons for which the crops are decreasing. These factors can cause the decreasing of production to 70%. The development of crops which are resistant to the unfavorable environmental factors becomes a certain choice in order to attenuate the decreases of these losses.

The salinity of soil represents one of the most important factors of stress, which diminishes the productivity of the agricultural plants in many areas of the Earth. As an effect of the change of global climate, also in Romania there are found many restrictive natural factors. These are reflected in : excessive drought on approximate 7.000.000 ha, frequent excess of humidity in the condition of year 2005 on 3.781.000 ha, salted soils on 700.776.000 ha, little reserve of organic substance on 4.800.000 ha , increased acidity on 2.000.000 ha with low assurance of nutrient elements.

In this study I have the target to emphasize the effect of different concentrations of Sodium

Chloride (NaCl) over the germination of the seeds and the development of the little plants of *Ocimum basilicum* L.(*bullatum* and *crispum* forms), the comparison of the tolerance at salinity of these forms of *Ocimum basilicum* L. and their framing in classes of resistance at salinity.

Ocimum basilicum L.(2, 6, 7) is a part of Plantae rain, Magnoliophyta phylum, Magnoliopsida class, Asteridae subclass, Lamiales order, Lamiaceae family, *Ocimum* genus and *basilicum* species. It is a herbaceous annual plant, with the origins from tropical Asia, it measures 20-60 cm, it has got light green and silky leaves with the length of 1.5-5 cm and the width of 1-3 cm. The flowers are white and arranged in raceme having four stamens and one pistil and after the pollination with the help of the insects, the corollary develops four alkenes inside the bilabial calyx. The plant has got a taste similar to the anise and also it has got a strong sweet-acrid smell. It is very sensitive at cold and it grows in conditions of heat and moist on fertile soils with PH 4.5-7%. From this plant are extracted volatile oils (eugenol, citral, camfen, anetol). It is very well spread in Asia and North America in the spontaneous flora, and in our country it grows on small areas.

It is known on different popular names: basil, “plescaita” and it is used as spice, flavor, in different popular and Christian rituals. The name of this plant in Greek means king/royal and it is said that it would have grown in the place where the Emperors

* National Pedagogical College „Ștefan cel Mare” Bacău, Spiru Haret nr. 6, auralorelei13@yahoo.com

** „Vasile Alecsandri” University of Bacău, Calea Mărășești, 158, 600115, nicoletadogaru@yahoo.com

Constantin and Elena have discovered The Holy Cross (1,4).

The both studied forms are different by the shape of the leaves and the color of the flowers such as: the *bullatum* form has got bladder leaves and white flowers and it is known as “green basil” and the *crispum* form has got curly leaves and pinky flowers and it is known as “red basil” (it mustn’t be confused with *O. b. purpurascens* whose flowers have got reddish purple flowers).

A great amount of mineral salts in the upper horizons of the saturate soils are very harmful for almost all the crop plants or from the spontaneous flora in two ways: on one hand they are increasing the concentration of the soil solution, which leads to a heavy absorption of the water in roots, and on the other hand the excess of the salts from the soil has a toxic action over the plants. The increased concentration of the soil solution inhibits on the osmotic way the saturation and the seed germination, also the growth of the root system of little plants.

Under the dangerous influence of salts, the plants are suffering a lot of anatomical and morphological changes, visible at leaves, stalks and other organs of the plant, such as:



Fig.1 - Little plants of green basil

The seeds of green and red basil have been bought from a store which sells seed material (agricultural crop for obtaining the seeds) and the authenticity of seeds is guaranteed by the producer.

In order to determine the capacity of germination, initially the seeds have been washed with a solution of H_2SO_4 0.05% and energetic rinsed for several times with distilled water. The sulfuric acid was used in order to destroy the fungal spores which are found on the surface of the seeds and to prevent their stain during germination.

- the disturbance of the microscopic structure of the chloroplasts and the appearance of the phenomenon of the etiolation of leaves;
- the shrinking of leaves surface, especially at older leaves;
- the thickening of the cuticle and the palisade texture in leaves;
- the change of the sizes of somatic cells;
- the increase of the succulence of tissues;
- the decrease of the diameter of root and of the high of stalk, also the premature lignifications of the tissues.

Material and method

The researches were conducted on seeds and little plants of *Ocimum basilicum* L., *bulattum* and *crispum* forms in the laboratory of biochemistry and genetics of the Faculty of Biology from the University “Vasile Alecsandri” from Bacau city. In order to avoid confusions, I considered *Ocimum basilicum* L. *bullatum* form – the green basil (fig.1) and *Ocimum basilicum* L. *crispum* form – the red basil (fig.2), especially that the consecrated literature admits these terms.



Fig. 2 - Little plants of red basil

Later, the seeds have been put in disposables cups full of distilled water for 24 hours, in the dark, in order to increase the volume and foster the germination. The next day, the seeds were put in Petri vessel, on filter paper, around 60 seeds/ vessel.

The seeds were moistened daily with different concentrations of NaCl (0.2%; 0.35%; 0.65%; 1%; 1.25%; 1.5%; 2%; 2.5%; 3%), around 3 ml/vessel, excepting the seeds from the witness sample which were moistened with distilled water (fig.3).

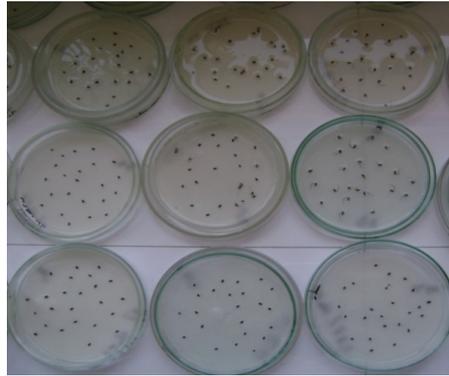


Fig. 3 - Seeds of *Ocimum basilicum* L. put on germination.

Later, the Petri vessels were introduced in the growth chamber in order that the physiological process develops in controlled conditions. In the growth chamber, the following factors were maintained constantly: the temperature of 20 degrees C, photoperiod 16 hours light - 8 hours dark, light intensity of 22 luxes. For each working version were made three repeats.

Daily, the number of germinated seeds / variant was followed up, in order to determine the efficiency of the germination in the conditions of salt stress.

I considered that the germinated seed is the seed whose roots have got the dimension equal to the length of the seed. The seeds of basil were photographed, in a preliminary stage, with a Panasonic digital photo camera (fig.4).



Fig. 4 - Germinated seeds *Ocimum basilicum* L.

The biometrical study was done on little red basil plants and also on green basil plants. After the germination of seeds, the Petri vessels were maintained in the growth chamber till the development of little plants from their seeds. The little plants were moistened daily with 3 ml solution of NaCl.

After 15 days from the beginning of the experiment, there were made some biometrical measurements, watching the length of the root and stalk, the width of seed lobe, the green weight of the little plants. The length of the root and stalk, the width of the seed lobe were measured in cm with a

ruler, the green weight of the little plants in grams using an analytical balance (ACCULAB).

The common dates were processed using Microsoft Excel program and were statistically interpreted. It was calculated the arithmetical average of the obtained variants. The arithmetical average represents the average obtained for the analyzed parameter, which results from the sum of the individual values divided at the total number of individuals.

Results and discussions

The percent of germination varied in wide limits, taking into consideration the first seeds

germinated after 2 – 4 days from the inoculation in Petri vessels (tab.1).

Table 1 - The percent of germination of the seeds of *Ocimum basilicum* L.

Nr. crt.	<i>Ocimum basilicum</i> L. sample to be analyzed	Percent of germination after 3 days (%)	Total percent of germination (%) after 10 days
<i>Green basil – bullatum form</i>			
1.	Witness sample	59	100
2.	NaCl solution 0,25%	51	87
3.	NaCl solution 0,35%	55	81
4.	NaCl solution 0,65%	55	75
5.	NaCl solution 1%	22	63
6.	NaCl solution 1,25%	-	-
7.	NaCl solution 1,50%	-	-
8.	NaCl solution 2%	-	-
9.	NaCl solution 2,50%	-	-
10.	NaCl solution 3%	-	-
<i>Red basil – crispum form</i>			
11.	Witness sample	11	68
12.	NaCl solution 0,25%	8	38
13.	NaCl solution 0,35%	10	35
14.	NaCl solution 0,65%	7	28
15.	NaCl solution 1%	2	13
16.	NaCl solution 1,25%	-	-
17.	NaCl solution 1,50%	-	-
18.	NaCl solution 2%	-	-
19.	NaCl solution 2,50%	-	-
20.	NaCl solution 3%	-	-

The moistened variants with saline solutions of NaCl in concentrations over 1% didn't germinate and, as the time has passed, they have degenerated. The phenomenon of inhibition of the germination of seeds was noticed at both forms of basil, green and red. The solutions of NaCl of 0.25%, 0.35%, 0.65% and 1% allowed the germination of both forms of basil after 3 days and also after 10 days, more pronounced in the case of the green basil.

From all the samples analyzed, it is noticed a high efficiency of the germinations of seeds of green basil compared with red basil, no matter what the conditions of germination were.

The witness sample and the germinated samples of the red basil, in conditions of salinity have emphasized half (50%) of the total percent of germination of the seeds of green basil, after 3 days (fig. 5) and also after 10 days (fig. 6). The efficiency of germination decreases in the same time with the increasing of the concentration of NaCl for both forms of basil.

The data obtained are according with the specific literature (3,5) which confirm the inhibitory effect of high concentrations of NaCl over the process of the germination of seeds and a reduced negative influence on low concentrations.

In what regards the biometric measurements, at samples of green basil, the witness sample has recorded at the length of the root some values between 1.1 and 3.8 cm, an average growth of hypocotyls of 0.7 cm, the seed lobe has biometrical values of 0.2-0.4 cm, and the average weight of the green little plants is 0.395 g.

At different concentrations of NaCl, the salinity has influenced in a positive way the growth of roots, it has reduced a little the length of hypocotyls at high value of 0.35% , it has reduced the dimensions of the seed lobe at concentrations over 0.25% comparing with the witness sample and the green biomass has decreased in the same time with the increasing of the concentration of the soil in salts (tab.2).

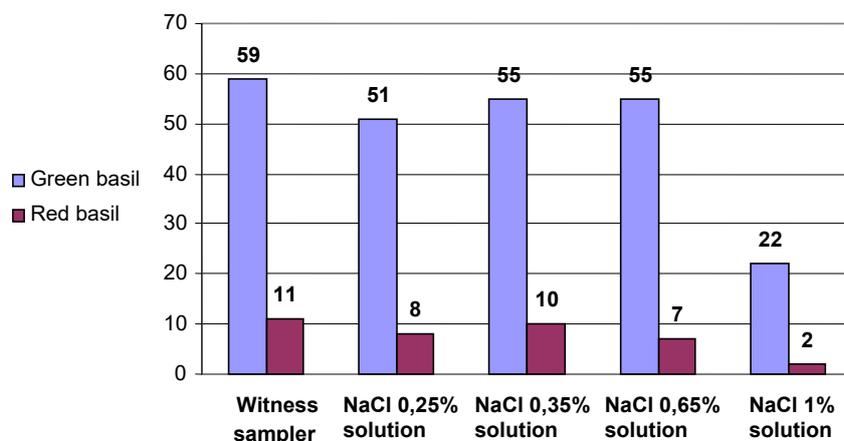


Fig. 5 - The percent of germination of seeds of green and red basil after 3 days.

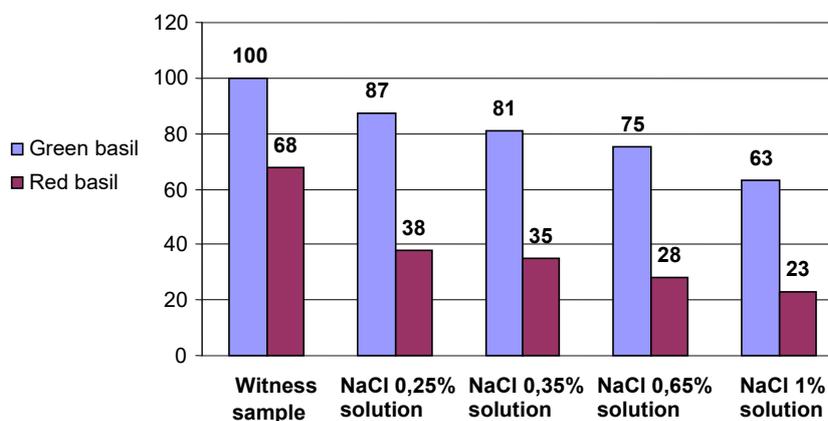


Fig. 6 - The percent of germination of seeds of green and red basil after 10 days.

Table 2 - The variations of some morphological features at green basil in conditions of salinity

Sample	Limits of the length of the little root (cm)	Limits of the length of hypocotyls (cm)	Limits of the width of the seed lobe (cm)	Limits of the average weight (g)
Witness sample	1,1 – 3,8	0,3 – 1,1	0,2 – 0,4	0,395
NaCl solution 0,25%	1,1 – 4,1	0,3 – 1,3	0,2 – 0,4	0,360
NaCl solution 0,35%	0,8 – 4,3	0,2 – 0,7	0,2 – 0,3	0,323
NaCl solution 0,65%	1,0 – 4,5	0,3 – 0,7	0,2 – 0,3	0,309
NaCl solution 1%	1,0 – 4,6	0,2 – 0,8	0,2 – 0,3	0,287

At the samples of red basil, the witness sample has registered in the length of the root 0.7-0.9 cm, the length of hypocotyls was between 0.3-0.8 cm, the seed lobe had 0.2-0.4 cm and the green biomass weighed 0.384 g.

At different concentrations of NaCl, the roots of the little plants of red basil have increased related

to the witness sample, it isn't noticed a significant increase of hypocotyls (for concentrations of 0.25 – 0.65% it is noticed an increase of length related to the witness sample), the increase of the aerial part of the little plant of red basil and the green biomass decreases in the same time with the increase of the concentration of salts in soil (tab. 3).

Table 3 - The variations of some morphological features at red basil in conditions of salinity

Sample	Limits of the length of the little root (cm)	Limits of the length of hypocotyls (cm)	Limits of the width of the seed lobe (cm)	Limits of the average weight (g)
Witness sample	0,7 – 3,9	0,3 – 0,8	0,2 – 0,4	0,384
NaCl solution 0,25%	0,9 – 4,0	0,3 – 1,0	0,2 – 0,4	0,339
NaCl solution 0,35%	1 – 4,1	0,2 – 1	0,2 – 0,3	0,294
NaCl solution 0,65%	1,1 – 4,3	0,3 – 1,1	0,2 – 0,3	0,215
NaCl solution 1%	1,1 – 4,6	0,2 – 0,8	0,2 – 0,3	0,198

Conclusions

The physiological investigations on *Ocimum basilicum* L., in conditions of salinity stress has led to the following conditions:

- The germination of seeds of red and green basil is spread on a short period of time (10 days) and the percent of germination was different according to the conditions of germination: 100% for the witness sample of green basil and 68% for the witness sample of red basil, over 60% for the samples germinated in conditions of salinity at green basil and around 20% for those of red basil.

- The salinity of soil has positively influenced the growth of the roots for both forms of basil: green and red, so at concentrations of 0.65% of NaCl, the root had the length of 4.5 – 4.6 cm for the green basil and 0.7 – 3.9 cm for the red basil.

- For the aerial biomass of the analyzed forms of basil, the salinity of the soil had a negative influence, the increase of concentration of NaCl has led to the decrease of the size of hypocotyls (0.2 – 0.8 cm) and of the seed lobe (0.2 – 0.3 cm).

- Red basil is less resistant at salinity which influences in a negative way some of the physiological processes (the germination, the growth of little plants)

- Green basil has a medium resistance to salinity, the influence of salts being more endurable comparing with the red basil, but it is obvious towards some physiological processes described above.

The necessity of doing such research can offer some precious information regarding the degree of accommodation and supportability of the plants which were grown on different types of soils, in order to find out the most optimally solutions of intervention and amelioration.

Rezumat

„Regele mirodeniilor” – busuiocul este o plantă ierboasă originară din Asia, cu gust asemănător anasonului și miros puternic dulceag-înțepător. Ca efect al schimbării climei globale și în România s-au semnalat o serie de factori naturali

restrictivi cum ar fi creșterea salinității solului peste limite acceptabile, secetă excesivă sau aciditate ridicată. Am studiat efectul diferitelor concentrații de clorură de sodiu (NaCl) asupra unor procese fiziologice, în paralel, la două forme de *Ocimum basilicum* L.: *bullatum* (busuiocul verde) și *crispum* (busuiocul roșu). Cercetările s-au realizat pe semințe și plantule și s-au urmărit rata germinării semințelor și dezvoltarea plantulelor prin măsurări biometrice ale unor caractere morfologice (rădăcină, hypocotil, cotiledon, greutate) în condiții de salinitate. Din rezultatele obținute s-a constatat că busuiocul verde (forma *bullatum*) este mai rezistent la salinitate în comparație cu cel roșu (forma *crispum*).

References

1. CRĂCIUN, F., BUJOR, O., ALEXAN, M., 1997 - *Farmacia naturii*, vol.II. Ed. Ceres, București, pag.125-128.
2. FISCHER, E., 1999 - *Dicționarul plantelor medicinale*. Ed. Gemma Peres, București.
3. GHIORGHITĂ, G., NICUȚĂ-PETRESCU, D., 2005 - *Biotehnologiile azi*. Ed. Junimea Iași.
4. GRIGORESCU, E., SILVA, F., 1997 - *De la etnomedicină la fitoterapie – Tezaurul verde al medicinei*. Ed. Spiru – Haret, București.
5. RAȚI, I., 2011 - *Fiziologia plantelor*, pag. 254-257.
6. STĂNESCU, U., MIRON, A., HĂNCIANU, M., APROTOSOAIE, C., 2004 - *Plante medicinale de la A la Z; Monografii ale produselor de interes terapeutic*, vol. I, Ed. „Gr.T. Popa”, U.M.F. Iași.
7. STĂNESCU, U., HĂNCIANU, M., MIRON, A., APROTOSOAIE, C., 2004 - *Plante medicinale de la A la Z; Monografii ale produselor de interes terapeutic*, vol. II, Ed. „Gr.T. Popa”, U.M.F. Iași.