CHLOROPHYLL CONTENT INDEX OF SOME NE-ROMANIA *PHASEOLUS VULGARIS* L LOCAL CULTIVARS, UNDER SALT STRESS

INDICELE CONȚINUTULUI DE CLOROFILĂ A UNOR POPULAȚII LOCALE DE FASOLE DIN NE-ROMÂNIEI, EXPUSE STRESULUI SALIN

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Abstract. *Soil salinity is a significant limiting factor affecting crop productivity in many areas of the world. The development of salt-resistant crops has made very limited progress despite tremendous efforts. From this reason, the capitalization of salt tolerance variability in local landraces should be a first step in plant breeding for the improvement of salt tolerance of cultivated species. Phaseolus vulgaris L is a species particularly sensitive to salt stress A decrease in chlorophyll content under salt stress is reported as a commonly phenomenon in various studies. For this reason, the aim of this work was to determinate the effect of NaCl excess on chlorophyll content index at 19 common bean local landraces, at different growth stages, as an indicator of salt stress tolerance. After NaCl treatments, the most salt sensitive local landraces were Tudora 1, Balş 1, Copalău 2 and 3, Iezer 2, Moşna 6, and the most tolerant Coropceni 1 and 2, Iezer 4 and 5, Moşna 3 and 5, Săveni 1 and 7 and Truşeşti 5.*
Key words: *salt stress, Phaseolus vulgaris, chlorophyll content index*

Rezumat. *Salinitatea solului reprezintă un factor limitativ important pentru productivitatea culturilor agricole, în multe regiuni ale globului. Cu toate eforturile depuse, crearea de plante agricole rezistente la salinitate a înregistrat mici progrese. Din acest motiv, capitalizarea variabilității genetice, referitor la rezistența la salinitate a populațiilor locale, poate constitui un prim pas în lucrările de ameliorare, în vederea îmbunătățirii toleranței la salinitate a speciilor cultivate. Phaseolus vulgaris L este o specie deosebit de sensibilă la stres salin. Reducerea conținutului de clorofilă, sub efectul stresului salin, este descrisă ca un fenomen comun, în numeroase studii. Din acest considerent, scopul lucrării de fața a fost determinarea efectului excesului de NaCl asupra dinamicii indicelui conținutului de clorofilă în cazul a 19 populații locale de fasole, ca un indicator al toleranței la stres salin. După tratamentul cu NaCl, cele mai sensibile cultivare s-au dovedit a fi: Tudora 1, Balş1, Copalău2 şi 3, Iezer 2, Moşna 6, iar cele mai tolerante: Coropceni 1 şi 2, Iezer 4 şi 5, Moşna 3 şi 5, Săveni 1 şi 7 şi Truşeşti 5.*
Cuvinte cheie: *stres salin, Phaseolus vulgaris, indicele conținutului de clorofilă*

INTRODUCTION

Soil salinity is a significant factor affecting crop productivity in many areas of the world. The decreases of plant growth and yield, depend on the plant

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species, salinity levels, and ionic composition of the salts (Husain et al., 2010, Slabu et al., 2009).

Phaseolus vulgaris L. is one of the most important food plants, cultivated in several cultivars (Burescu and Toma, 2005), for its seeds rich in protein (15%), fat (2%) and starch (80%). Is a species particularly sensitive to the presence of NaC1 in the root zone (Greenway and Munns, 1980). During the last decades, the development of salt-resistant crops has made very limited progress despite tremendous efforts (Schubert et al., 2009). The investigation of stress exposed plants and subsequent identification of certain stress-specific biomarkers revealed important information about the limits of stress tolerance. (Gostin, 2007). The genetic variation in cultivated beans germplasm for salinity tolerance is limited (Gama et al., 2007). Although, it is important to test several cultivar regarding this quality. From this reason, the capitalization of salt tolerance variability in local landraces should by a first step in plant breeding for the improvement of salt tolerance of cultivated species.

Under salt stress, the decrease in chlorophyll content is a commonly reported phenomenon in various studies (Taffouo et al., 2009, Nazarbeygi et al., 2011, Mehr , 2013). Salinity and salt stress duration significantly affected photosynthesis (Bayuelo-Jimenez, 2012). For pinto bean found Ghassemi-Golezani et al. (2012) that under salt stress, the chlorophyll content index (CCI) of leaves diminished with progressing plant development at reproductive stages and during reproductive stages CCI of all cultivars decreased with increasing plant senescence. For faba bean found Slabu et al. (2009) that the reduction of chlorophyll in leaves after NaCl exposure may be explained as a result of high Cl^- concentrations in the chloroplasts, which is amplified by a simultaneously high Na^+ concentration. On the other hand, it is known that under salt stress, the plants have a reduced growth and an intense green color (Mengel, 1991), respectively a higher chlorophyll concentration in the leaves. These aspects can be explained by the biphasic response of plants to salinity, proposed by Munns (2002): a rapid, osmotic phase that inhibits growth of young leaves, and a slower, ionic phase that accelerates senescence of mature leaves. In the second phase of salt stress, NaCl decreased total chlorophyll concentration in the leaves by destruction of chloroplast structure and the instability of pigment protein complexes (Koyro, 2002). Plant adaptations to salinity are of three distinct types: osmotic stress tolerance, Na^+ or Cl^- exclusion, and the tolerance of tissue to accumulated Na+ or Cl^- (Munns and Tester, 2008). Therefore maintaining high levels of chlorophyll content in leaves can be an indication of greater salinity tolerance of plants.

The aim of this work was to determinate the effect of salt stress on chlorophyll content index at 19 common bean local landraces, at different growth stages, as an indicator for salt stress tolerance.

MATERIAL AND METHOD

A pot experiment with factorial arrangements on the bases of randomized complete block with three replications was conducted in 2014 to investigate the

salinity tolerance at 19 common bean local landraces. The cultivars were collected from NE Romania Iasi and Botosani districts from areas with saline excess. Six seeds were sown 3 cm deep in each pot, filled with 10 kg garden soil. The pots were placed in the greenhouse. The temperature variation in the greenhouse was 17- 30ºC. After the emergence were kept only three plants per pot. After the occurrence of the second true leaves, the plants were exposed to salt stress experimental variants: V1 – control, watered with water only; V2 - 4 watering with 1 liter 100 mM NaCl solution for each watering; V3 - 4 watering with 1 liter 200 mM NaCl solution for each watering. Plants were grown to full maturity.

Chlorophyll content index was measured in each plot by using a portable chlorophyll content meter (CCM-200, Opti-Sciences Inc., NH, USA). Ten measurements were taken for each pot. 4 determinations were made: d1 - after a week of exposure of plants to salt stress; d2 - alt flowering stage; d3 - beginning of pot development; d4 - ripening of fruit and seed.

RESULTS AND DISCUSSIONS

At the first determination (d1) control plants showed variations of CCI values between 11.7 (Truşeşti 5) and 21.0 (Copalău1). The treatment with 100 mM NaCl (V1) for one week led to an increase of the CCI values, 14.5 (Truşeşti) and 24.7 (Coropceni 1). The same behavior has been observed after the treatment with 200 mM NaCl, CCI values ranging from 12.0 (Coropceni 2) and 23.0 in Copalău 1 (table 1).

Table 1

Chlorophyll content index at vegtative growth stages (d1) and by flowering (d2)

	d1			d2		
Cultivars	**V1**	**V2**	**V3**	**V1**	**V2**	**V3**
Balş 1	17,9	19,2	19,5	21,1	24,1	24,0
Copalău 1	21,0	23,3	23,0	27,9	21,2	22,4
Copalău 2	15,2	17,1	17,6	15,3	17,2	16,1
Copalău 3	13,8	17,1	17,6	15,7	17,7	18,7
Codreni 1	16,3	17,3	13,0	14,4	14,1	13,1
Coropceni 1	17,5	24,7	17,2	24,3	19,2	16,7
Coropceni 2	17,7	17,7	12,0	21,0	16,6	17,7
Iezer 2	14,7	14,7	15,1	19,6	22,5	23,0
Iezer 4	14,3	18,7	18,1	20,0	24,3	17,4
Iezer 5	12,2	16,7	16,2	19,8	28,8	25,9
Moşna 1	18,6	23,9	17,8	17,9	25,6	17,0
Moşna 3	12,3	16,3	17,8	14,9	16,2	17,6
Moşna 4	13,4	17,7	14,7	14,7	23,2	20,7
Moşna 5	21,0	21,7	20,4	21,5	22,8	23,8
Moşna 6	12,3	17,0	13,7	16,7	19,4	18,0
Săveni 1	14,8	18,6	17,9	16,0	19,9	25,2

Săveni 7	12,2	18,4	19,5	16,3	16,4	25,5
Truşeşti 5	11,7	14,5	13,8	18,2	16,8	21,2
Tudora 1	16,6	17,8	18,8	19,6	22,6	17,8
MIN	**11,7**	**14,5**	**12,0**	**14,4**	**14,1**	**13,1**
MAX	**21,0**	**24,7**	**23,0**	**27,9**	**28,8**	**25,9**

The studies done by Beinsan C. et al, 2009 showed for the common beans an increase in chlorophyll content at lower level of stress and a decrease in more stressed variant. Dadkhah and Moghtader (2008) found for the sugar beet that under salt stress leaves appeared healthy and leaf chlorophyll content increased with increasing salinity. The increasing in chlorophyll can be explained due to salinity effect of leaf area. Leaves of stressed plants became thicker than unstressed plants. Thicker leaves contain more cells in a certain leaf area (Dadkhah and Moghtader, 2008). Also in the case of common bean salinity concentration caused considerable reduction of leaf area with differences between bean local landraces on different variant of osmotic stress (Beinsan et al., 2009).

The increase of CCI at vegetative growth phenophase could by explain by a leaf reduction under salt stress without a destruction of chloroplast.

At the flowering stage (d1) a growth of CCI was recorded in 11 of the analysed cultivars, in the rest of the cultivars was observed a reduction of CCI, after the treatment with 100 mM NaCl or with 200 mM NaCl (table 1).

At the beginning of pot development (d3), CCI values remain elevated in most cultivars, ranging between 14.3 and 28.4 (table 2) except cultivars Balş 1 and Tudora 1, wherein the plants are completely chlorotic.

Table 2

Chlorophyll content index at beginning of pot development (d3) and by ripening of fruit and seed (d4)

	d3			d4		
cultivars	**V1**	**V2**	**V3**	**V1**	**V2**	**V3**
Balş 1	22,0	3,3	2,5	12,4	0,0	0,0
Copalău 1	26,9	22,8	21,9	17,4	6,7	0,0
Copalău 2	21,6	17,8	16,7	6,3	0,0	0,0
Copalău 3	16,5	20,2	19,8	7,2	0,0	0,0
Codreni 1	14,3	12,2	11,1	6,5	8,9	0,0
Coropceni 1	28,4	25,5	24,5	14,4	10,0	4,6
Coropceni 2	23,8	18,1	21,1	9,3	7,4	6,0
Iezer 2	24,8	26,5	21,5	10,9	0,0	0,0
Iezer 4	16,3	20,1	15,1	12,2	6,1	6,5
Iezer 5	21,5	19,8	20,5	13,3	10,5	9,5
Moşna 1	22,9	18,1	17,8	0,0	0,0	0,0
Moşna 3	14,7	16,6	17,1	9,8	12,1	8,1

Moşna 4	17,0	20,1	14,8	9,9	3,9	0,0
Moşna 5	24,1	24,6	18,3	9,3	4,0	3,9
Moşna 6	17,3	20,3	14,1	9,2	0,0	0,0
Săveni 1	17,2	26,3	19,6	12,3	11,0	8,3
Săveni 7	16,6	22,1	23,4	10,3	5,0	5,9
Truşeşti 5	18,6	18,5	23,7	11,6	4,7	7,8
Tudora 1	20,4	0,0	0,0	17,6	0,0	0,0
MIN	**14,3**	**0,0**	**2,5**	**6,3**	**0,0**	**3,9**
MAX	**28,4**	**26,5**	**24,5**	**17,6**	**12,1**	**9,5**

At ripening of fruit and seed (table 2), normally occurs a decrease of CCI values by control variants, which are between 6.3 (Copalău 2) and 17.6 (Tudora 1). The treatments with 100 mM NaCl caused the drying of cultivars: Balş 1, Copalău 2 and 3, Iezer 2, Moşna 6. The treatments with 200 mM caused plants death from cultivars: Copalău 1, Codreni 1, Moşna 4. The decrease of CCI values after saline treatment may be explained by the toxic effect of Na^+ and Cl^- ions that disorganize the cells, especially chloroplasts, resulting a reduction of chlorophyll content.

After this salt treatments, the most tolerant cultivars, by which the chlorosis occurred less, were Coropceni 1 and 2, Iezer 4 and 5, Moşna 3 and 5, Săveni 1 and 7, and Truşeşti 5.

CONCLUSIONS

1. At the analysed cultivars, the control variants, watered with water only, the chlorophyll content index shows high variability.

2. NaCl treatment causes an increase in CCI, excepting of three local populations at 200 mM NaCl: Codreni 1, Coropceni 1 and Coropceni 2.

3. By the sensitive variants, elevated CCI is maintained until flowering and by the salinity tolerant variants until the pods formation.

4. After NaCl treatments, the salt sensitive local landraces were Tudora 1, Balş 1, Copalău 2 şi 3, Iezer 2, Moşna 6, and the most tolerant Coropceni 1 and 2, Iezer 4 and 5, Moşna 3 and 5, Săveni 1 and 7 and Truşeşti 5.

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