

PHYSIOLOGICAL DIVERSITY OF LETTUCE CULTIVARS EXPOSED TO SALINITY STRESS

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Abstract: Increased soil salinity is a major environmental stress factor for many plants, and limits crop yields. Lettuce is a frequently grown, moderately salt-sensitive crop plant, with many different cultivars. Their physiological diversity when exposed to salt stress enables breeders to efficiently select those cultivars more suitable to grow under conditions of salt stress associated with drought and global warming. The aim of this work is to study the physiological diversity of frequently cultivated lettuce varieties subjected to salinity stress, enabling an efficient selection of more tolerant cultivars. Different functional parameters of induced chlorophyll fluorescence, molar ratios between the main photosynthetic light-harvesting pigments, dry biomass accumulation and seed germination dynamics are evaluated in the context of physiological diversity of lettuce cultivars. Potential and effective light use efficiency is generally decreased only by severe salt stress (exposure to 150 mM NaCl for several days), the light reactions of photosynthesis being relatively resistant to dehydration and ion toxicity exerted by elevated salinity. The relative fluorescence decrease, also known as the vitality index of photosystem II, is one of the most suitable physiological markers of salt stress tolerance, making possible a good distinction between resistant, tolerant and sensitive lettuce cultivars. Germination energy is also a good functional indicator of differential salt sensitivity of lettuce cultivars. As a general compensatory mechanism of growth regulation and as a consequence of water deficit, dry biomass production is moderately increased by short-term mild salinity (50 mM NaCl). While the total chlorophyll content of lettuce leaves is decreased by salt stress, the molar ratio between chlorophylls a and b exhibits a moderate but statistically significant increase, because of a more pronounced disturbance of chlorophyll-b homeostasis. Determination of the evaluated physiological parameters is simple and cost-effective, being a good tool for screening lettuce varieties for efficient cultivation on soils with increasing salt content. Possible cross-tolerance for cold stress and salt stress is also discussed.

Keywords: germination energy, induced chlorophyll fluorescence, lettuce cultivars, photosynthetic pigments, salt tolerance

Introduction

Salt stress is a serious limiting factor for the development of terrestrial plants, as more than 800 million ha of land throughout the world are affected by salt. A significant proportion of recently cultivated agricultural land has become saline owing to land clearance or irrigation, both of which cause water tables to rise and concentrate salts in the root zone. Of the current 230 million ha of irrigated land, 45 million ha (20%) are salt-affected. Irrigated land accounts for only 15% of the total cultivated area, but because it has at least twice the productivity of rain-fed land, it produces approximately one third of the world's food supply [3, 15, 40].

Lettuce, an increasingly important component of a healthy diet, is a good source of vitamins, other antioxidants, essential mineral ions and several secondary metabolites such as flavonoids and other phenolic compounds. At present there are several promising attempts for using lettuce cultivars as eco-toxicological test organisms, as biomarkers of pollution, as well as an important source of health-promoting phytochemicals [2, 21, 23, 24, 25, 27, 35]. Lettuce is considered to be a moderately salt-sensitive crop, with a threshold electrical conductivity of 1.3 dS m⁻¹, but a wide range of salt tolerance can be identified among its different cultivars: e.g. the 'Romaine' or 'Cos' lettuce cultivars (*Lactuca sativa* var. *longifolia*) are considered far more tolerant of salinity than the 'Iceberg' cultivars (*Lactuca sativa* var. *capitata*) [10, 18, 28, 30].

The response of a plant to salinity stress occurs in two phases: a rapid response to the increase in external osmotic pressure, and a slower response due to the accumulation of Na^+ in leaves. Shoot growth is more sensitive than root growth, a phenomenon that also occurs in drying soils and for which there is as yet no mechanistic explanation [26]. Within many plant species, documented genetic variation exists in the rate of accumulation of Na^+ and Cl^- in leaves, as well as in the degree to which these ions can be tolerated [4, 8, 17]. The effect of osmotic stress is seen as a rapid inhibition of the rate of expansion of young leaves and reduced stomatal conductance of mature leaves. Ion-specific toxicity is seen as an increase in the rate of senescence of older leaves, due to either high leaf Na^+ concentrations or to low tolerance of the accumulated Na^+ [9, 16, 22, 32].

The effects of salinity on photosynthesis range from the restriction on CO_2 diffusion into the chloroplast (e.g. via limitations on stomatal opening) to alterations in leaf photochemistry and carbon metabolism. The main site of sodium toxicity for plants is the leaf-blade. Sodium has a strong inhibitory effect on K^+ uptake by cells, presumably by interfering with transporters in the root plasma membrane such as K^+ -selective ion channels and the transporters that mediate high-affinity and low-affinity K^+ transport at the plasma membrane and tonoplast. Most of the Na^+ that enters plant cells is pumped back out again via plasma membrane Na^+/H^+ antiporters or sequestered in the vacuoles by the proton pump-driven Na^+/H^+ antiporter of the tonoplast. High salinity also modulates the function of aquaporins, of certain heat shock proteins, anti-oxidative enzymes and other stress proteins [4, 5, 11, 26, 31, 36, 42]. At the whole plant level, acclimation to salt stress is mediated by abscisic acid, polyamines, salicylic acid, nitric oxide, brassinosteroids and other bioregulators [33, 37]. Response to salinity is a highly coordinated process, involving the induction of transcription of many genes. While constitutive over-expression of these genes often results in increased tolerance to abiotic stresses, it also frequently comes with growth inhibition under unstressed conditions [8, 17].

Plants that are hardened against some adverse conditions usually exhibit a certain degree of tolerance towards other environmental stress factors. This phenomenon is called cross-tolerance. Because there may be important differences in the salt tolerance of different intra-specific variants, it is important to identify the manifestations of physiological diversity of these variants when exposed to salt stress, in order to offer breeders a reliable tool for screening of the most suitable cultivars for the agricultural areas characterized by different degrees of soil salinity. Furthermore, physiological diversity under adverse environmental conditions offers an insight into the complex network of metabolic regulation and developmental integration of the whole plant and of plant communities [12, 14, 34, 40].

Because of its nutritional importance, there is an increasing interest for the cultivation of lettuce in new areas, many of these being affected by salinity. Many lettuce cultivars are characterized concerning their cold tolerance, but very little information exists on salt tolerance of these cultivars. Considering the possibility of cross-tolerance, common features of acclimation processes may be revealed even in a shorter timescale during the early developmental stage, providing breeders with useful tools for selection of the most tolerant cultivars under the prevailing conditions. Because germination and plantlet growth are crucial processes for survival of plants under high salinity, the study of short-term salt stress on young plants should be the first stage in the investigation of physiological mechanisms of different halo-tolerance of cultivars [4, 13, 26, 39]. The data obtained from short-term experiments will be complemented with the investigation of adaptive processes that occur during a longer period of time, in later developmental stages.

The aim of the present work is to study manifestations of physiological diversity of lettuce cultivars exposed to salt stress conditions, in order to identify parameters for a reliable selection of cultivars with different degrees of salinity tolerance in specific developmental stages.

Material and Methods

Investigation of physiological diversity in response to salt stress was performed with 11 cultivars of lettuce (*Lactuca sativa* L.). Five of these are considered cold-tolerant, early cultivars: Arctic King, May Queen, Parella Green, Valdor and Winter Butterkopf; another five are commercialized as cold-sensitive, late cultivars: Attractie, Bowl Red, Great Lake 118, Oak Leaf and Paris Island, while the 11th cultivar (Krolowa) is described as an amphi-tolerant cultivar with regard to temperature [13, 19, 38]. All of these lettuce variants are widely cultivated in European countries. Seeds were provided from a germ bank by B & T World Seeds (France).

Seeds were germinated in Linhard vessels (100 seeds per vessel, originated from a homogenous seed bank) for 9 days in a vegetation chamber with constant illumination ($90 \mu\text{M photons m}^{-2} \text{s}^{-1}$) at 20 °C. Seedlings were grown in pots filled with vermiculate, in an environmental test chamber, under controlled condition: a daily photoperiod with 12 hours of light and 12 hours of darkness, with a relative air humidity of 75%, a photon flux density of $135 \mu\text{M m}^{-2}\text{s}^{-1}$, and a temperature of 18°C during the light period and 16°C during the dark period. Control plants were supplied with ¼ strength Hoagland nutrient solution, while the experimental variants were watered with identical amounts of ¼ Hoagland solution supplemented with 50 mM, 100 mM and 150 mM NaCl (p. a.). For germination tests lower salt concentrations were required (40 mM and 80 mM NaCl). Three-week old plants were used for investigation of physiological reactions to salt stress, except for dry biomass, which was measured in 70 days old plants. *In vivo* chlorophyll fluorescence measurements and pigment extractions were performed on the same leaves (the third leaf from the base of the shoot).

Parameters of induced chlorophyll *a* fluorescence were measured with a pulse amplitude modulated chlorophyll fluorometer (PAM-FMS2, Hansatech). Leaves were dark adapted for 5 min. The modulated light was sufficiently weak ($0.04 \mu\text{M m}^{-2}\text{s}^{-1}$) so as not to produce any significant variable fluorescence. A single saturating flash ($2000 \mu\text{M m}^{-2}\text{s}^{-1}$ for 0.5 s) was applied to reach the maximal fluorescence F_m . After the decline of the signal, the actinic light was turned on ($100 \mu\text{M m}^{-2}\text{s}^{-1}$) to start the induction kinetics [6, 20, 41]. The determined parameters were initial fluorescence F_0 , maximal fluorescence F_m , the F_v/F_m ratio (F_v or variable fluorescence being the difference between the maximal and the initial fluorescence), the F_0/F_v ratio, modulated maximal fluorescence F_m' , steady state fluorescence F_s , the effective quantum use efficiency (Φ) representing the ratio $(F_m' - F_s)/F_m'$, as well as the vitality index (Rfd) expressed as the ratio $(F_m - F_s)/F_s$.

Extraction of photosynthetic pigments was performed in darkness with N,N-dimethylformamide. 0.1 g leaf was immersed in 4 ml of N,N-dimethylformamide and kept for 48 hours for complete extraction of pigments. The extracts were centrifuged for 5 min. at 7000 g, and the supernatant was used for measurement of optical density at 646.8 nm and 663.8 nm. Chlorophyll *a* and chlorophyll *b* content was determined spectro-photometrically [7, 29, 39].

Appearance of the root tip from the seed integument was considered the starting point of germination. Fresh and dry biomass of the plants was determined gravimetrically [1, 29], for dry weight measurement the sliced plants were kept in an electric dryer for 3 days at 80 °C (until the mass remained constant with an analytical precision).

Each determination was made with 4 replicates, when the means and standard errors were calculated. In data sets with normal distribution, significant differences between treatment means were determined using the post-ANOVA Tukey test, while in data sets with non-normal distribution, significant differences between means were established with the Kruskal-Wallis test followed by the Mann-Whitney U-test.

Results and Discussion

Germination of seeds is the first critical step in the ontogeny of spermatophytes, one that is very sensitive to adverse environmental conditions. Vegetative growth and differentiation of

the meristematic tissue of the embryo are highly regulated processes, on which the establishment of a new plant generation relies. This is why knowledge of the influence of salinity stress on the dynamics of germination is important for the selection of halo-tolerant intra-specific cultivars. Even without any salt stress, the timescale and frequency of germination vary among lettuce cultivars: in some cultivars germination of most seeds begins during the first day after hydration, in others there is a gradual germination preceded by a lag phase of several days. In most of the 11 lettuce cultivars examined, a mild salt stress (40 mM) only delays germination (decreases the germination energy), while a more pronounced salt stress (80 mM) diminishes the frequency of germinated seeds (Fig. 1).

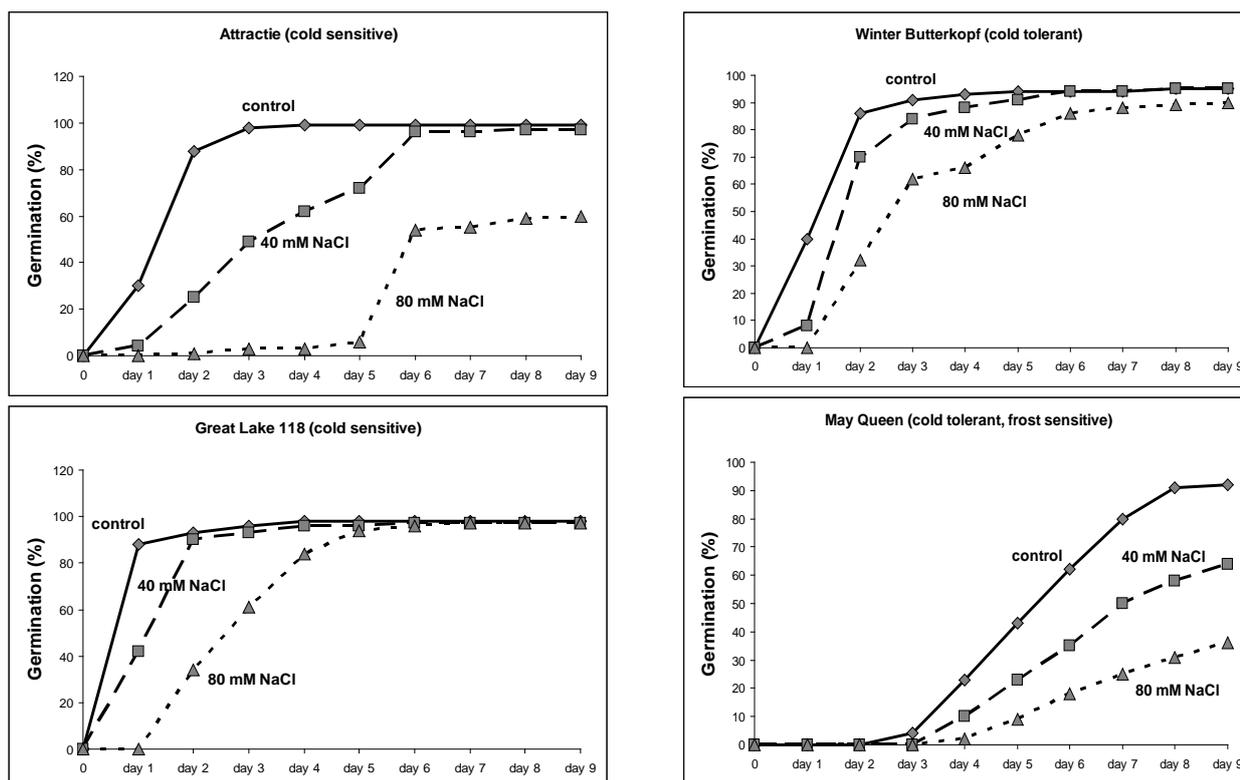


Fig. 1: Dynamics of seed germination for four lettuce cultivars exposed to mild and severe salt stress

The cold-sensitive *Attractie* cultivar was found to be less tolerant to salt stress, while the highest germination halo-tolerance was registered in the case of the cold-tolerant *Winter Butterkopf* cultivar.

Because salt stress exerts a simultaneous disturbance effect on growth, water relations and photosynthetic carbon assimilation, the dry biomass content and water content of plant organs varies in accordance with the degree of tolerance, e.g. in relation to osmoregulatory capacity, down-regulation of photosynthetic processes and a reprogrammed growth and development. Because salt stress induces a certain water deficit, the dry biomass content of the stressed plants is higher than in control plants. Accumulation of sodium and chloride ions in the plant tissues also has a small contribution to the increased percentage of dry (organic and inorganic) matter from the net fresh weight. In the sensitive lettuce cultivars (e.g. *Parella Green*) the dry biomass content of leaves increases even under the influence of lower salt concentrations (50 mM), while in more tolerant cultivars (e.g. *Oak Leaf*) this increment becomes significant only at higher salt concentrations (150 mM, Fig. 2). The same pattern was also observed in castor bean and in different maize cultivars exposed to salt stress [14, 29].

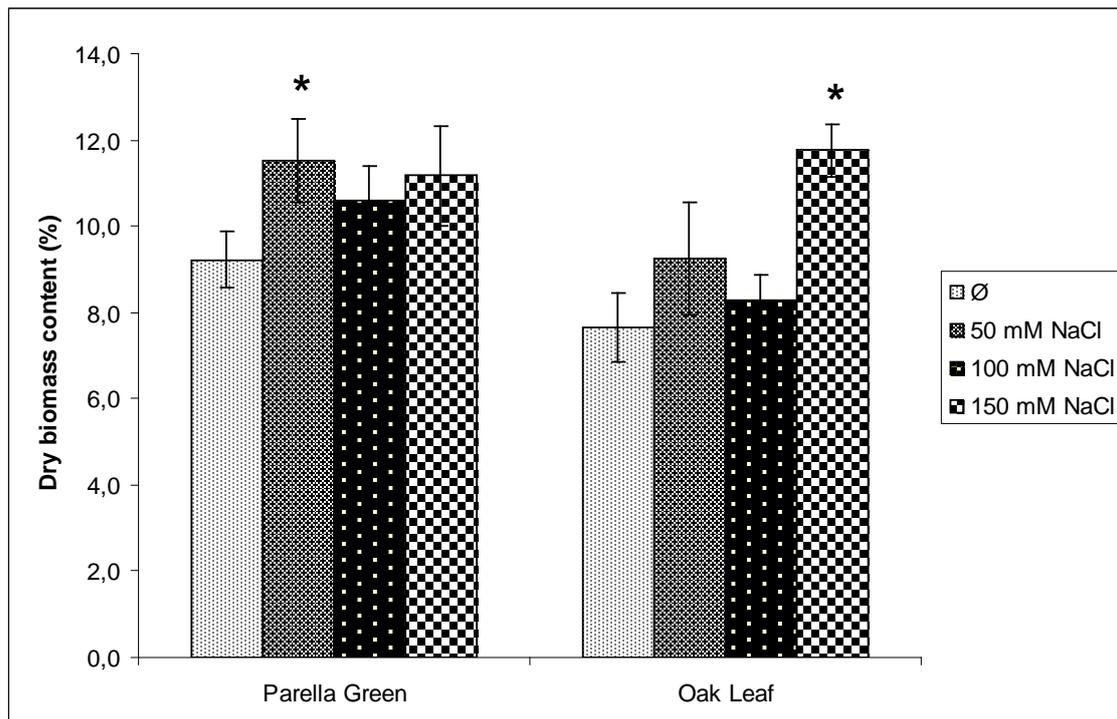


Fig. 2: Dry biomass content of 3-week old plantlets as a percentage of the total fresh weight, in two lettuce cultivars with different degrees of sensitivity to salt stress (n = 4, bars represent \pm SE from means, asterisk indicates significant difference from the control at $P < 0.01$)

As induced chlorophyll fluorescence is a very sensitive, non-destructive method for assessment of environmental stress effects on plants, the different parameters of the conventional and pulse amplification modulated chlorophyll fluorescence are reliable physiological indicators of the site and mode of action of stress factors that impair the primary energetic processes of photosynthesis.

A selection of the most relevant chlorophyll fluorescence parameters, for seven of the 11 lettuce cultivars that were assayed, is presented in Table 1.

The ground fluorescence (F_0) measured in dark-adapted leaves varies among cultivars but is not significantly influenced by different salt concentrations. This indicates that the amount of salt reaching the chloroplasts of leaves does not interfere significantly with the primary light-harvesting and energy transfer processes in the pigment-protein antenna of photosystem II (from where this fluorescence signal derives). This is also the case of the steady state fluorescence level (F_s) in leaves exposed to regular flashes of saturating light on the background of an actinic illumination. The transient maximal fluorescence level (F_m) in the dark-adapted leaves becomes obviously diminished upon severe salt stress, and the degree of this decrease indicates the degree of salt sensitivity of the electron transport processes on the acceptor side of photosystem II in the chloroplasts of the different lettuce cultivars. The highest disturbance was registered in the Paris Island and Oak Leaf cultivars at 150 mM NaCl, but the leaves of the Bowl Red, Krolowa and Valdor cultivars did not even survive under these conditions. A similar tendency can be also observed for the modulated maximal fluorescence (F_m'). The two most important energetic indicators of the light phase of photosynthesis – the potential (F_v/F_m) and the effective quantum yield efficiency (Φ_{PSII}) – are impaired only by strong salt stress (150 mM) and only in the less halo-tolerant lettuce cultivars. For example, the photochemical reactions of the cold-tolerant Parella Green cultivar are much less sensitive than in the case of the cold-sensitive Paris Island cultivar (Tab. 1).

Table 1: Parameters of induced chlorophyll fluorescence in intact leaves of different lettuce cultivars exposed to different salt concentrations (for abbreviations see Materials and Methods)

Cultivars	F _o	F _m	F _v /F _m	F _s	F _m '	Φ PSII	F _m /F _o	(F _m -F _s)/F _s
<i>Parella Green</i>								
∅ (control)	28.75	283.50	0.89	37.50	277.25	0.83	10.91	6.67
50mM NaCl	31.50	318.25	0.90	39.25	307.75	0.84	10.16	7.15
100mM NaCl	31.25	257.25	0.87	44	247.75	0.81	8.08	4.83
150mM NaCl	26	213.25	0.87	33.25	206	0.83	8.28	5.40
<i>Paris Island</i>								
∅	19	252.75	0.92	27	236.75	0.88	13.30	8.36
50mM NaCl	15.25	194.75	0.92	21.50	192.25	0.87	12.77	8.05
100mM NaCl	25	186.50	0.84	33	182.75	0.81	7.46	4.65
150mM NaCl	13	32	0.48	13.25	32.25	0.41	2.46	1.41
<i>Arctic King</i>								
∅	19.5	225	0.91	26.25	218	0.87	11.61	7.61
50mM NaCl	19.5	228.5	0.91	25	222.5	0.88	11.89	8.29
100mM NaCl	23.5	245	0.90	33.75	235.5	0.85	10.52	6.73
150mM NaCl	16	78.25	0.66	21	75.75	0.61	4.96	2.6
<i>Oak Leaf</i>								
∅	16.25	183.75	0.91	20.75	177.25	0.88	11.50	7.85
50mM NaCl	16.50	159.25	0.89	17.50	152.50	0.87	9.73	8.10
100mM NaCl	16.25	141.25	0.87	20.75	138.50	0.84	8.57	5.80
150mM NaCl	14	51.50	0.72	24.25	50.25	0.69	3.67	1.12
<i>Valdor</i>								
∅	25	274.75	0.90	35.5	274	0.87	10.99	6.73
50mM NaCl	17.75	219.75	0.91	27.5	218.75	0.87	12.38	6.99
100mM NaCl	22.25	270.75	0.91	33.5	263.5	0.85	12.16	7.08
<i>Bowl Red</i>								
∅	27.75	281.75	0.90	40.25	277.50	0.85	10.15	6
50mM NaCl	20	234	0.91	31	228.75	0.86	11.70	6.54
100mM NaCl	23.75	276.75	0.91	40	263	0.84	11.65	5.91
<i>Krolowa</i>								
∅	29.25	327.50	0.91	37	325.25	0.88	11.19	7.85
50mM NaCl	17.75	235.75	0.92	25.25	229	0.88	13.28	8.33
100mM NaCl	22.75	282	0.91	35.50	274.75	0.87	12.39	6.94

A smaller value of the F_m/F_o ratio usually indicates osmotic stress at the level of chloroplasts. In the most sensitive lettuce cultivars (e.g. Paris Island, Oak Leaf) this ratio decreases significantly, indicating a damaging effect of the osmotic component of salt stress and a weaker capacity of osmoregulation in the leaves exposed to partial dehydration [20, 39, 41]. The most useful parameter of chlorophyll fluorescence for distinguishing between salt tolerance capacities of lettuce cultivars was found to be the vitality index of photosystem II, i.e. the relative fluorescence decrease (F_m-F_s)/F_s. Its value decreases proportionally with the degree of salt stress over a wider range of salt concentrations, and this decrease is more pronounced in the more sensitive cultivars.

A molecular indicator related to the photosynthetic light-harvesting function is the ratio between the two types of chlorophyll that occur in the thylakoid membranes of eukaryotes. Because the different metabolic processes implied in the synthesis and in the degradation of these pigment molecules may be specifically influenced by external stress factors and by the

acclimation processes in photosynthesis, modifications of the molar ratio between chlorophyll *a* and *b* may indicate the degree of sensitivity of the light-harvesting complexes to adverse environmental conditions. This was observed in the case of several plant species exposed to long-term salt stress [20, 29, 36, 42]. The absolute amount of both chlorophyll *a* and chlorophyll *b* decreases upon salt stress (data not shown), but because chlorophyll *b* content diminishes more than chlorophyll *a* content (probably because of a smaller peripheral light-harvesting antenna), the chlorophyll *a* to chlorophyll *b* ratio increases moderately. In the more sensitive lettuce cultivars this increment is proportional to the salt concentration (e.g. Bowl Red), while in others there is no proportionality between the change in the chlorophyll *a/b* ratio and the degree of salt stress (Fig. 3).

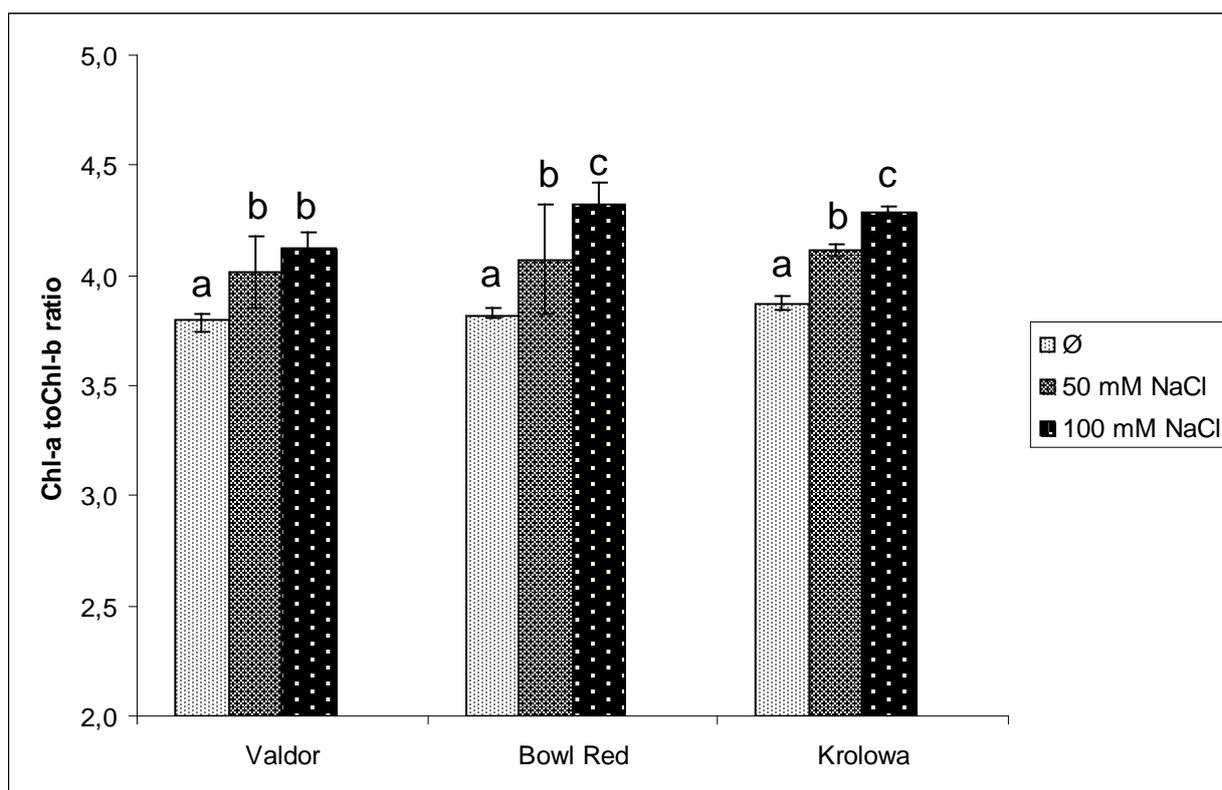


Fig. 3: Chlorophyll *a* (Chl-*a*) to chlorophyll *b* (Chl-*b*) ratio in leaves of three lettuce cultivars exposed to mild and severe salt stress (n = 4, bars represent \pm SE from means, different letters indicate significant differences at $P < 0.05$)

Conclusions

Seed germination of different lettuce variants exhibits a differential sensitivity to salinity stress, enabling a selection of more tolerant cultivars at this critical early developmental stage. It seems that germination of cold-sensitive cultivars (e.g. *Attractie*) is much more delayed by high salt concentration than in the case of cold-tolerant cultivars (e.g. *Winter Butterkopf*). This has a direct consequence on the availability of stored nutrients when the new plantlet starts its active life. In many cases a mild salt stress causes only a diminished germination energy, while higher amounts of sodium chloride significantly decrease the germination percentage of the seed bank. Of the 11 cultivars examined, *Winter Butterkopf* showed the least sensitivity of germination to increased salinity.

The highest dry matter content of the total biomass was registered for the *Oak Leaf* cultivar treated with 150 mM NaCl and for *Parella Green* grown in the presence of 50 mM NaCl.

In some lettuce cultivars the molar ratio between *a* and *b* type chlorophylls increases gradually with salt concentration, while in other cultivars there is no significant difference between the ratio of the two main light-harvesting photosynthetic pigment in the leaves exposed to 50 mM and 100 mM NaCl.

From among the many parameters of induced chlorophyll fluorescence recorded in the intact leaves, the relative fluorescence decrease ((Fm–Fs)/Fs) and the non-modulated maximal fluorescence yield (Fm) exhibit the highest variability among cultivars, while the values of the potential and effective quantum use efficiency (Fv/Fm and Φ) decrease with different degrees only in the presence of a severe salt stress exerted by 150 mM NaCl, a concentration at which the leaves of the most sensitive cultivars senesc prematurely and die.

The physiological parameters investigated provide a good tool for the screening of lettuce cultivars with different capacities for salt stress tolerance.

Of the 11 lettuce cultivars examined, we recommend that the cultivars Winter Butterkopf and Great Lake 118 be sown in soils where salinity does not exceed 40 mM. On more saline soils, Parella Green, Arctic King and Valdor cultivars proved to be sufficiently tolerant. Bowl Red, Oak Leaf and Paris Island cultivars are not recommended for cultivation on saline soils.

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**DIVERSITATEA FIZIOLOGICĂ A UNOR VARIETĂȚI DE *LACTUCA SATIVA* L.
EXPUSE STRESULUI DE SALINITATE****(Rezumat)**

Creșterea salinității solului afectează arii tot mai extinse în coralație cu accentuarea aridizării și cu schimbările climatice globale, provocând scăderi semnificative ale productivității plantelor cultivate, îndeosebi pe terenurile agricole irigate necorespunzător sau aflate în zona litorală. Stresul de salinitate prezintă numeroase manifestări caracteristice la nivelul proceselor fiziologice ale plantelor, declanșând reacții metabolice de contracarare în cursul dezvoltării halotoleranței. Salata cultivată (*Lactuca sativa* L.) este o specie sensibilă la stresul de salinitate, reprezentând o plantă model adecvată pentru studierea mecanismelor de acomodare fiziologică la salinitatea crescută. În prezent asistăm la o extindere a cultivării diferitelor soiuri de salată, datorită importanței sale crescânde în alimentația sănătoasă, datorită utilizării sale ca sursă naturală de substanțe profilactice (flavonoizi, polifenoli, vitamine și substanțe minerale cu efect antioxidant), precum și datorită utilizării sale în ecotoxicologie, ca organism indicator al poluării solului. Din acest motiv apare necesitatea cultivării eficiente a acestei plante pe arii tot mai extinse, care deseori sunt afectate de concentrația ridicată a clorurii de sodiu în mediul apos al solului. Odată cu aceasta, apare și necesitatea selectării unor varietăți halotolerante, pe cât posibil fără manipularea genetică a proprietăților metabolice. Numeroasele varietăți de salată cultivată prezintă o diversitate considerabilă în privința sensibilității față de salinitatea crescută a solului, cunoașterea reacțiilor diferențiate de apărare și de adaptare fiziologică permițând o selecție sigură și relativ rapidă a acelor varietăți care prezintă o halotoleranță crescută și au o dezvoltare corespunzătoare pentru a asigura rentabilitatea producției pe terenuri afectate de salinitate.

Scopul lucrării de față este studiul diversității fiziologice a 11 varietăți de salată dintre cele mai răspândite în Europa, în condiții de stres de salinitate, pentru o selectare mai ușoară a soiurilor halotolerante. Principalii parametri fiziologici urmăriți sunt energia germinativă, dinamica cantitativă a principalilor pigmenți fotosintetici, parametrii fluorescenței clorofilene induse, ca indicatori ai eficienței utilizării fotosintetice a energiei fotonice, precum și producția netă de substanță uscată din biomasa totală a cormului. Dintre cele 11 varietăți supuse studiului, 5 sunt soiuri timpurii tolerante la frig, alte 5 sunt soiuri târzii sensibile la frig, iar un soi este amfitolerant în privința temperaturii. Plantele au fost cultivate în cameră de vegetație, în condiții controlate de iluminare, umiditate atmosferică și temperatură. Ca substrat artificial s-a utilizat perlitul, acesta fiind udat cu soluție nutritivă Hoagland. Energia germinativă a semințelor diferitelor soiuri variază în mod diferit în prezența stresului de salinitate moderat și pronunțat, făcând posibilă o diferențiere a soiurilor mai tolerante și a celor mai sensibile. Germinația este în general întârziată proporțional cu concentrația clorurii de sodiu, iar procentul final de germinație scade semnificativ doar la o salinitate pronunțată. În privința germinației, cea mai halotolerantă a fost varietatea Winter Buttercopf. Soiurile mai puțin rezistente la frig s-au dovedit a fi mai sensibile și la stresul de salinitate, ceea ce sugerează o toleranță încrucișată față de cei doi factori de stres ambiental: temperatura scăzută și salinitatea crescută.

Deoarece homeostazia clorofilelor *b* este mai puternic afectată de salinitate decât cea a clorofilelor *a*, raportul molar dintre clorofilele *a* și *b* din frunze crește în condițiile stresului salin. La unele soiuri această creștere este proporțională cu concentrația NaCl, la altele această proporționalitate nu se observă. Dintre parametrii fluorescenței clorofilene induse *in situ* în frunzele intacte, cea mai mare variabilitate între diferitele cultivare de salată o prezintă indicele de vitalitate a sistemului fotochimic II și fluorescența maximă nemodulată. Randamentul cuantic potențial și cel efectiv al reacțiilor fotochimice scade semnificativ doar în prezența unui stres salin accentuat (100 mM și 150 mM NaCl). Parametrii fiziologici studiați permit o selecție corespunzătoare a soiurilor de salată mai halotolerante și oferă informații despre varietatea manifestării reacțiilor metabolice la stresul de salinitate.