STUDIES ON THE PHYSIOLOGICAL CHARACTERISTICS OF THE HALOPHYTIC SPECIES *CRAMBE MARITIMA L.* AND THE POSSIBILITY OF ITS INTRODUCTION INTO CULTURE IN THE SOUTHERN AREA OF ROMANIA'S OLTENIA REGION

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Abstract
Crambe maritima L. is a perennial plant from the Brasicaceae family that grows spontaneously in Romania on the beaches of the Black Sea. Its tolerance to salt also makes it tolerant to soil drought. Therefore, it was considered that the sandy and dry soil in the southern part of the Oltenia region could be favorable for the cultivation of this plant, known in the world for its food value.
Studies show that the plant develops high values of osmotic pressure and suction force on dry and salty soil and thus manages to absorb optimal amounts of water.
Wax impregnation of the leaves reduces water losses and is thus another adaptive advantage. In conditions of moderate salinity or drought, the photosynthesis values are maintained at fairly high values and the net assimilation intensity also has values that ensure a moderate productivity.

Key words*: salt tolerance, suction force, photosynthesis*

INTRODUCTION

Sea kale (*Crambe maritima L.)* is a wild edible plant with forgotten and undiscovered potential as a field vegetable. Its natural habitat is gravel beaches in northern Europe and the Black Sea (Christensen,J et al, 2015).
C. maritima L. is of interest not only as a wild relative of other *Brassicaceous* crops and a crop in its own right, but also because it has potential as a new halotolerant crop. Studies of genetic variability have been conducted with the aim of diversifying vegetable crops and bringing *C. maritima* closer to commercial production, and also for conservation purposes in France, UK and Ireland. A breeding programme initiated in 1992 resulted from a systematic search for wild populations in France, along the Channel and Atlantic coasts, especially from Quiberon (south Brittany) to Dunkerque (north France near Belgium) that aimed to capture its genetic variability (Sanyal,A., Decocq G., 2015).
Sea-kale is rich in vitamin C, mineral salts, sulphur and iodine. It contains sulphur heteroside, recognized as having anti-cancer properties. Because of its high vitamin C content, it is used to prevent scurvy as well as viral infections. It has also been used as a purifier, diuretic, antiseptic and for its antifungal effects . The leaves have been used for healing wounds, the fruits for removing worms (Boullard 2001) and the raw juice of seeds to fight gastritis and gastric ulcers. The seeds are very rich in oil (41.7%) of potential economic value (Dolya et al. 1973) and are also used for cosmetic purposes. It is used as an ornamental plant and as a model for epigenetic studies (Sanyal,A., Decocq G., 2015).

Crambe maritima is found in cool, temperate oceanic climates and open sunny positions, often sheltered from strong winds (Huxley 1992). However, as its habitat is on sea shores, it is very tolerant of strong maritime winds, but less so of cold northerlies. Hill, Preston & Roy (2004) characterized it as a European temperate species. An Ellenberg indicator value of 9 has been ascribed to C. maritima, suggesting that it is found mostly in full sun (Hill, Preston & Roy 2004).

Sea-kale occurs primarily near sea level, occasionally on cliffs and has been recorded at a maximum altitude of 25 m (Scott & Randall 1976).

The coarse substrates supporting Sea-kale usually comprise pebbles and gravel derived from rock fragments, with colloidal elements, and traces of humus derived from the decomposition of algae (Géhu & Géhu 1969),

Crambe maritima grows singly or in small groups, usually occurring at the drift line (de Vos et al. 2010) on beaches, which have been stable over 5–20 year periods (Doody & Randall 2003).

Sea-kale can withstand temperatures as low as −20 °C (Phillips & Rix 1991). Plants may be partially killed by frost at −15 °C to −18 °C. The roots are damaged by temperatures below −15 °C (Péron 1990). Established plants are very drought tolerant (Chatto 1982). Excessive moisture and water logging result in diseases, such as the black rot of stems and black spot diseases of stems, leaves and fruits ((Scott & Randall, 1976)

Based on its growth response, *Crambe maritima* can be classified as a salt spray tolerant plant that is sensitive to root zone salinities exceeding 100 mM NaCl (de Vos et al, 2010).

MATERIALS AND METHODS

Crambe maritima is a plant with leaves arranged in a rosette and a fleshy taproot, which is the main perennial organ capable of vegetative propagation. Flowering can last 5-8 years when grown from seed. Reproduction is predominantly by seeds. Root and stem cuttings can be used for vegetative propagation. In winter, each branch terminates at ground level in a purple leafless bud, and in spring, it produces a succession of cabbage-like leaves just above ground level, the first being purple and the successive leaves becoming greener. *Crambe maritima* produces weakly protogynous hermaphrodite flowers. Self-pollination and cross-pollination occur. Pollination is done by insects, flies and bees. Plants typically produce 1000–10,000 seeds per year (Sanyal, A., Decocq G., 2015).

The study method consisted in determining some biophysical and physiological indices in plants grown on media with different concentrations of sodium chloride.

Salt tolerances are usually given as a function of plant growth stage within a range of electrical conductivity (EC) levels. Electrical conductivity is the ability of a solution to transmit an electrical current. To determine soil salinity according to EC, an electric current is introduced into a glass cell using two electrodes in a soil extract solution taken from the soil to be measured (soil salinity).

Units are usually given in decimens per meter (dS/m).

https://www1.agric.gov.ab.ca/$department/deptdocs.nsf/all/agdex3303

A high level of salt interferes with the germination of new seeds. Salinity acts like drought in plants, preventing roots from carrying out their osmotic activity where water and nutrients move from an area of low concentration to an area of high concentration. Therefore, due to the salt levels in the soil, water and nutrients cannot move into the plant roots.

As soil salinity levels increase, so does the stress on seedlings and sprouts. Perennials seem to tolerate salinity better than annuals. In some cases, salinity also has a toxic effect on plants due to the high concentration of certain salts in the soil. Salinity prevents plants from taking up the proper balance of nutrients needed for healthy growth.

https://www1.agric.gov.ab.ca/$department/deptdocs.nsf/all/agdex3303

The dominant salts in saline soils are calcium (Ca), magnesium (Mg), sodium cations (Na) and sulfate anions (SO4). If Na levels are high or not balanced with Ca and Mg, soil salinization may also be possible. The positively charged Na cations attach the negatively charged clay particles in the soil, causing the soil to be sticky when wet and hard and impermeable when dry.

Salinity thresholds are generally defined as the maximum amount of salt a plant can tolerate in the root zone without affecting growth. Other important thresholds indicate the highest level of plant salt tolerance associated with a decrease in yield or biomass (typically between 10-50%); zero yield thresholds specify the levels at which a plant can no longer survive. There is a continuum between extremes in salt tolerance, as demonstrated by the diverse spectrum of plants—those that thrive in seawater and higher salinities to those that cannot tolerate even minimal concentrations without significant decline.

http://www.biosalinity.org/salt-tolerant_plants.html

Generally, cultivated plants classified as salt sensitive have salinity thresholds of 1-3 dS/m and zero yields at 8-16 dS/m (or less), while "moderate" salt tolerance has thresholds of 5 -10 dS / m and zero returns at 16-24 dS / m. Not included in this list are a number of commercial crops that produce higher quality fruit and lower yields on saline soils or when irrigated with saline water between 3-5 dS/m, as well as others considered "moderately tolerant" to salt which, for practical purposes, can be seen as the line through which plants enter the halophytic zone. http://www.biosalinity.org/salt-tolerant_plants.html

he experiments were aimed at knowing the influence of different salt concentrations on seed germination, plant viability, growth intensity, respiration intensity, photosynthesis, transpiration and on suction force and osmotic pressure values.

The saline soil in which the plants grew had its conductivity measured at intervals using a Hanna conductivity meter. The values at which physiological determinations were made were: 4 dS/m, 8 dS/m, 16 dS/m, respectively 24 dS/m.

To determine the energy and germination capacity, whole seeds were chosen and germinated in Linhard type germinators.

Each variation had three repetitions.

The germination energy represents the speed with which the germination process takes place and was determined by counting the germinated seeds at the time interval corresponding to each species. The germination capacity expresses the total number of seeds capable of germination and was determined by counting the germinated seeds after 5 days from the determination of the germination energy.

In order to establish the plants' resistance to NaCl, the plants were cultivated on artificially saline soil with different concentrations of salts. The soil was sprinkled with water to maintain the moisture at approximately 60% of the minimum water holding capacity.

In the plants grown under these conditions, the following were determined:

- the intensity of photosynthesis
- the intensity of respiration
- the intensity of transpiration
- the suction force of the leaves
- the content of chlorophyll pigments in the leaves

To determine the suction force of the leaves, 10 ml of sucrose solution of different concentrations are introduced into 5 perfectly dry test tubes: 1M, 0.8M, 0.6M, 0.4M, 0.2M. Leave the leaves in the test tubes for about 2 hours, after which the final concentration of the sucrose solutions is determined. The initial and final values of the sucrose concentrations are entered in a table and finally analyzed, looking for the solution whose concentration remained unchanged, or changed the least. This solution is considered isotonic (with a concentration equal to that of the cell juice from the cells of the leaves used in the experiment). Knowing the isotonic solution and applying Avogadro's law (1 mol of a non-electrolyte solution develops an osmotic pressure equal to 22.41 atm), the osmotic pressure of the cell juice solution is calculated.

Transpiration, photosynthesis and respiration was determined with the portable Lci apparatus.

The quantity of chlorophyll pigments from the leaves has been determined with the Minolta chlorophyll meter.

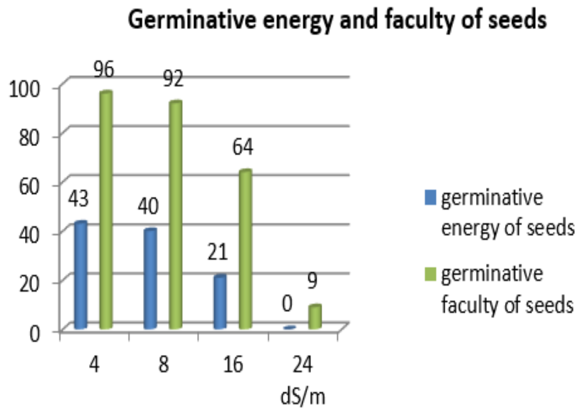
RESULTS AND DISCUSSIONS

The energy and germinative faculty of seeds

Analyzing the data from graph 1, it can be seen that the energy and germination capacity of *Crambe maritima* seeds had maximum values in the version with the lowest conductivity (4 dS/m), it decreases as the salt concentration of the solution increases. The lowest percentage of germinated seeds was determined for the variant with the conductivity of 24 dS/m.

In relation to the germination process, it can be said that in all the species studied, although they are adapted to conditions of high salinity, germination is optimal at low salt concentrations. This means that they are advantageous if germination occurs in spring periods after heavy rains, when the soil solution is considerably diluted.

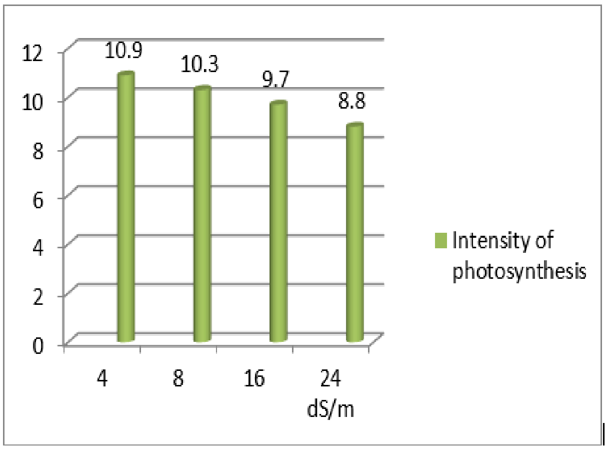
An adaptive eco-physiological role is attributed to such a germination phenomenon, which involves the unfolding of the young embryo from the testa immediately after contact with water, with an unusually high rate of cell elongation shortly after imbibition. Such rapid seed germination indicates an adaptive strategy taken by plants, as water with low NaCl content in the soil during the rainy season is only available for a short period of time. An increase in salinity leads to seed dormancy in halophytes and non-halophytes. Moreover, investigations with halophytes (Ungar, 1991) demonstrated that the seeds of several species remained dormant at maximum salinity, and they germinated when they were returned to distilled water.



Graph 1. Energy and germinative capacity of *Crambe maritima* seeds at different salt concentrations

The intensity of photosynthesis

The intensity of photosynthesis remained relatively constant in *Crambe maritima* plants, not being influenced by salt concentration in the soil (graph 2)

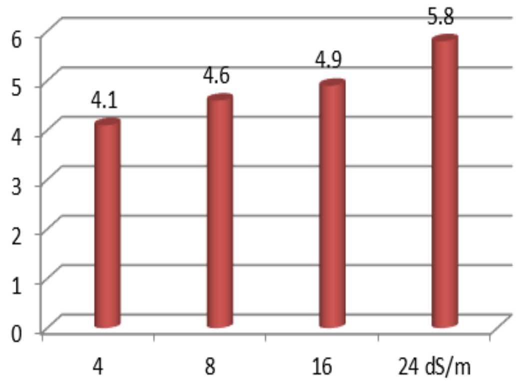


Graph 2. The value of leaf photosynthesis intensity (µmol/m2/s) in *Crambe maritima* at different soil salt concentrations

The intensity of respiration

The respiratory process is necessary to provide the energy that ensures the active absorption of water and mineral elements. In the case of halophilic plants, part of the energy is also used for the excretion of

excess salt, the excretion process being an active process.
Respiration intensifies as the concentration of salt in the soil increases(graph 3).



Graph 3. The value of the respiration intensity (µmol/m ^{2}/s) of *Crambe maritima* to different salt concentrations

The intensity of transpiration

In halophilic plants, transpiration is important because it ensures the creation of a sufficiently large suction force to ensure optimal absorption of water from the soil level. According to data from the specialized literature, as the transpiration process intensifies, so does the higher driving force, called the suction force, which ensures the ascent of the raw sap in the plant body (Atanasiu L., 1984). Therefore, in plants that grow on salty soils, high values of transpiration intensity can be considered an advantage.

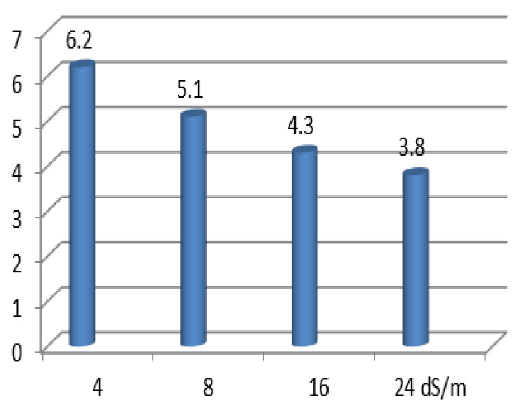
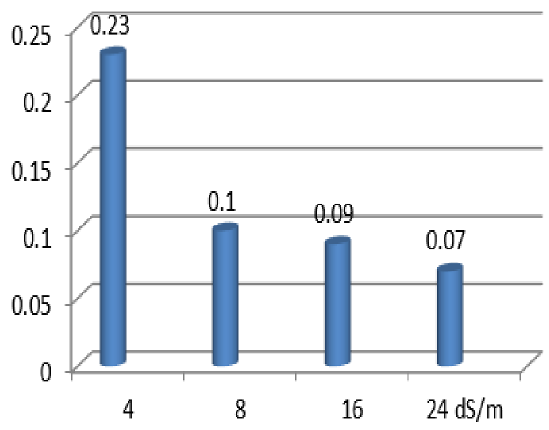


Chart 4. The value of transpiration intensity (mmol/m2/s) in *Crambe maritima* at different soil salt concentrations

The epidermis of *Crambe maritima* leaves is impregnated with wax, which greatly reduces the loss of water vapor in hot periods.
In relation to transpiration through the stomata, it is found that *Crambe maritima* Shows an reduction of transpiration directly proportional to concentration of salt soil solution. (graph 4).

The stomatal conductance

Stomatal conductance directly modifies plant water relations and photosynthesis.
Many studies have explored the relationship between drought stress and stomatal conductance. Through these experiments, researchers have found that a drought resistant plant regulates its transpiration rate via stomatal conductance. This minimizes water loss and allows the plant to survive under low water conditions (Li, Yuping, 2017).
While variations in light are the most diurnally fluctuating factor modulating gs in many environments, changes in other variables also influence gs; for example, stomata can exert a major control of assimilation under low humidity or high (Ball et al. 1987, Damour et al. 2010 quoted by Grace et al, 2016).
Graph 5 indicates a reduction in stomatal conductance values as the salt concentration increases. This can also explain the small reduction in the intensity of photosynthesis

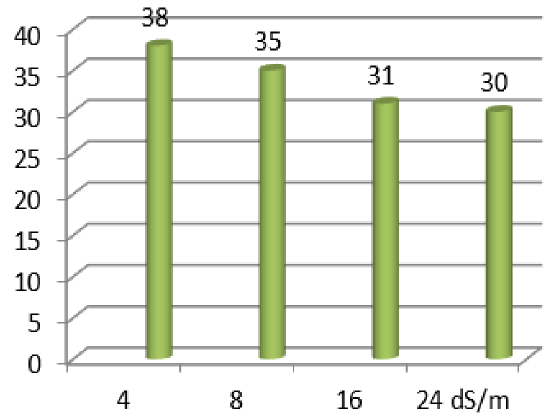


Graph 5. The stomatal conductance (mol /m^2 /s)

The chlorophyll content of the leaves

The change in the content of chlorophyll pigments in the case of plants growing on saline soils is known from the specialized literature (Weber et al., 2005, Yang et al., 2017). Even the appearance of plants changes on soils with higher salt concentrations, they have a lighter color, or, in some cases, a reddish appearance (Gaspari et al., 2016).

The determinations made indicated a progressive decrease in the chlorophyll content as the salt concentration increased (graph 6). This decrease can also explain the lower values of photosynthesis in these plants.

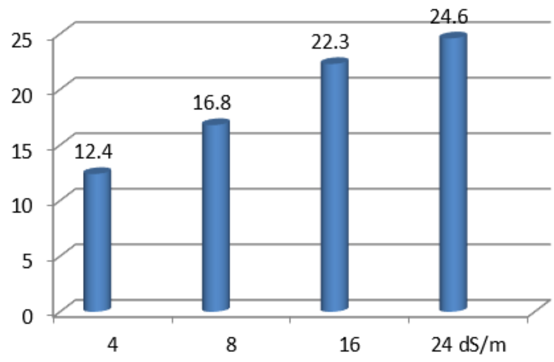


Gr. 6. The content of chlorophyll pigments under the influence of sodium chloride in the leaves of *Crambe maritima* (SPAD units)

The suction force of the vegetal parenchyma

The suction force of the plant parenchyma, resulting from the summation of the osmotic pressure with the cohesion force of the water molecules, from which the turgor pressure (membrane pressure) is subtracted, is very important in halophilic plants, ensuring the absorption of the concentrated soil solution in the plant body.

In plants grown under salinity-controlled conditions, the suction force of the plant parenchyma had different values. The highest values were recorded in plants grown in high salt concentrations (graph 7).

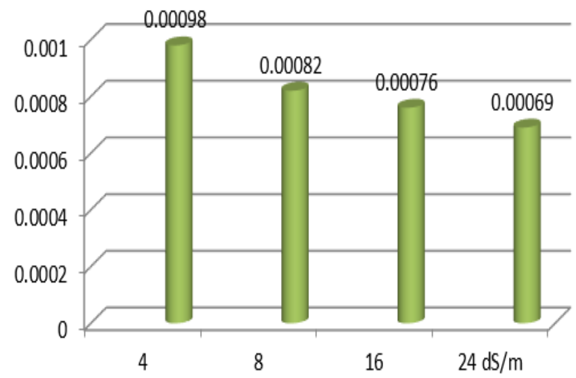


Graph7. The value of suction force (atm) in *Crambe maritima*

The net assimilation rate

NAR (net assimilation rate) is a measure of the average efficiency of leaf photosynthesis in a cultivated plant community, also known as the rate of accumulation of dry weight per unit leaf area per unit time

The determination of net assimilation rate indicates small differences between the variants (graph 8). This proves that *Crambe maritima* plants tolerate drought and salt stress. Even if the values are slightly lower at high salt concentrations, we cannot speak of a serious impact on productivity.



Graph 8. NAR (g/cm^2/day) under the influence of sodium chloride

CONCLUSIONS

High concentrations of NaCl inhibit the energy and germinative faculty, an effect manifested all the more significantly, the

higher the concentration of the salt solution
Photosynthesis decreases in intensity as the salt concentration of the soil increases, due to the increase in the viscosity of the protoplasm and the change in enzyme activity
The suction force of the plant parenchyma increases in direct proportion to the degree of salinity of the soil
The determination of net assimilation rate indicates small differences between the variants. This proves that *Crambe maritima* plants tolerate drought and salt stress. Even if the values are slightly lower at high salt concentrations, we cannot speak of a serious impact on productivity.

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