EFFECT OF SOIL SALINITY ON THE LIPID COMPOSITION OF HALOPHYTE PLANTS FROM THE SAND BAR OF POMORIE

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Summary. Seven samples of halophyte plants (*Bassia hirsuta* Aschers, *Euphorbia peplis* L., *Salicornia europeae* L., *Calystegia soldanella*, *Calystegia sepium*, *Stachys maritima* Gonan and *Suaeda maritima* Dumort,) were collected from a sand bar near the town of Pomorie (Bulgaria). The soil salinity ranged between 650 and 850 mg salts in 100 g soil. For comparison, two other samples from the species *Calistegia sepium* were collected also from soils with lower salinities (330 and 90 mg salts in 100 g soil) in different regions of Bulgaria. The results showed almost a linear increase of linoleic/linolenic acid ratio with increasing soil salinity.

Key words: Fatty acids, halophyte plants, lipids, salt stress.

Abbreviations: TAG – triacylglycerols, GL – glycolipids, PL – phospholipids, FAME – fatty acids methyl esters, PTLC – preparative thin-layer chromatography, GC – gas chromatography, FA – fatty acids, 18:2 – linoleic acid, 18:3 – linolenic acid.

INTRODUCTION

Five percent of cultivated lands are affected by salinity, with salt stress being one of the most serious environmental factors limiting the productivity of crop plants. Saline conditions reduce the ability of plants to absorb water, causing rapid reduction in growth rate and induce changes in plant physiology.

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Halophytes are plants, which are able to live in elevated salinity of their growth media. These plants are important subjects for investigations since enlarging of desert and semi-desert territories is an importantl problem. A number of halophyte plants have been tested for their possible use as an alternative source of alternative energy (Villalobos and Correal, 1992), waxes (Hemmers and Gьlz, 1986), hydrocarbons and phytochemicals (Sekar and Francis, 1998). Halophytes from the cosmopolitan family *Convolvulaceae* have being used in folk medicine (Tori et al., 2000). Some species from this family (genus *Calystegia*) were found to contain alkaloids (Asano et al., 2001), caffeic and coumaric acid esters (Tori et al., 2000), and acylated tetrasaccharide macrolactone (Gaspar, 2001). Genus *Euphorbia* (family *Euphorbiaceae*) was found to contain terpenoids (Gotta et al., 1984, Connolly and Hill, 1996), flavonoids (Rizk et al., 1980), and a digalactosyl diacylglycerol, which possesses an anti-inflammatory activity (Cateni et al., 2000).

Although Bulgaria is situated in the moderate climatic zone, there are some areas along the Black Sea where the soil may contain up to 900 mg salts in 100 g soil.

Lake Pomorie is a hyper saline naturally formed lagoon situated along the town of Pomorie. It has up to 70 ‰ salinity and is separated from the Black Sea (salinity 16-18 ‰) by a manmade dike and a sand bar. The lake surface area is 850 ha, but its depth is less than 2m. For more than 1500 years the lake has been a well-known SPA place. The very high salinity and the content of sulfur and dihydrogen sulfide are useful in treating arthritis, radiculites, rheumatism, discal hernia, lumbago and sciatica.

As a result of increasing urbanization and industrialization, the gulf of Bourgas is now heavily polluted. Part of the pollution is due to petroleum, coming from partially treated industrial wastewater of a big factory and by discharging of petrol products from the ships.

Lake Pomorie and Black Sea are separated from a sand bar, in which the soil salinity of the sand bar varies from 650 to 850 mg salts in 100 g soil. More than 40 different halophyte plants grow in this area and some of them are endemites for the Black Sea shore.

MATERIALS AND METHODS

Plant material

Seven samples of halophyte plants were collected from a sand bar near the town of Pomorie (Bulgaria). The soil salinity ranged between 650 and 850 mg salts in 100 g soil. For comparison samples of one halophyte plant (*Calistegia sepium*) were collected also from soil with lower salinities in other regions of Bulgaria (Table 1).

Table 1 Investigated plants

№	Family	Name	Soil salinity [mg salts.100^{-1} g soil]
1	*Chenopodiaceae*	*Bassia hirsuta* Aschers	840
2	*Euphorbiaceae*	*Euphorbia peplis* L.	800
3	*Chenopodiaceae*	*Salicornia europeae* L.	690
4	*Convolvulaceae*	Calystegia soldanella	680
5	*Convolvulaceae*	Calystegia sepium	680
6	*Lamiaceae*	*Stachys maritima* Gonan	680
7	*Chenopodiaceae*	*Suaeda maritima* Dumort	660
8	*Convolvulaceae*	Calystegia sepium*	330
9	*Convolvulaceae*	*Calystegia sepium**	<90

* Samples from other regions

Extraction

All leaf samples (8.5-12.5 g) were cut to small pieces and extracted twice with 65 ml chloroform – methanol (1:1 v/v) according to Bligh and Dyer (1959). The extracts were combined and diluted with water until two layers appeared. The lower layer, containing the lipophilic compounds, was evaporated under vacuum and kept at –30 °C.

Analysis of the lipophilic extracts

Part of lipophilic extracts (50 mg from each sample) was transferred to small vials with teflon screw caps. Five ml of 15 % acetyl chloride in absolute methanol was added and the vials were heated for 4 h at 55 °C (Christie, 1989). After cooling, the samples were diluted with water and the obtained FAME were extracted twice with hexane (2 x 5 ml). The FAME in combined hexane extracts were purified by PTLC on 20 x 20 cm silica gel G (Merck) plates (layer thickness 0.5 mm) with hexane – acetone (95:5 v/v). The spots of the FAME were visualized under UV light, scrapped off with the silica-gel layer and eluted with hexane. The amount of each sample was determined gravimetrically.

The FAME were analyzed by GC using Hewlett Packard 5890 (Hewlett Packard, Palo Alto, California, USA) equipped with FID and capillary column SP WAX 52CB (30 m x 0.25 mm). The temperature was programmed from 165 to 230 °C at a rate of 4 °C min^{-1} and a 10 min hold at 230 °C. The temperature of injector was 260 °C, and of the detector was 280 °C. The carrier gas was nitrogen (1.45 x 10^{-3} Pa).

RESULTS AND DISCUSSION

There was no relation between the amounts of the main lipid classes and the soil salinity of the investigated plants (Table 2). Phospholipids represent the main lipid class in lipid membranes of plant leaves. Although triacylglycerols are not membrane constituents, their concentration is relatively high in some halophytes from the same region – Pomorie (Ivanova et al., 2000). It was also found that the content of triacylglycerols increased in some desiccation resistant plants during water stress (Stefanov et al., 1992). It is possible that halophyte plants could react to harmful environment by rearrangement of the lipid membranes as well as by changes in their fatty acid composition.

The vegetation formation processes of coast differ from those on lands. The main factors affecting coastal and sand dune vegetation are the inundation of periodic seawater and variation of soil sand contents with time and location (Min and Je, 2002). The membrane fluidity of the plants depends on the content of the unsaturated 18:2 and 18:3 acids. The 18:2/18:3 ratio is related to the salt resistance and it is reduced when the salt resistance of halophyte plants decreases (Zarrouk and Cherif, 1983). As a confirmation of this, the same trend was observed in the halophyte plants from the Black Sea shore near Pomorie. Fig. 2 represents the 18:2/18:3 ratio in different plants from this region. The PL is the main lipid class in lipid membranes of plant leaves. To improve the resistance to salt stress, the lipid membrane decreases its permeability, i.e. with increasing the amount of saturated FA. With the increase of salinity, the amount of 18:3 acid increases, but not as much as 18:2. As a result, the 18:2/18:3 ratio itself increases twice. An almost linear correlation was observed between linoleic/linolenic acid ratio and soil salinity in the investigated plants (Fig. 1). This fact could be explained only with the assumption that the halophyte plants could react to harmful environment by reducing membrane permeabil-

Table 2 Amounts of the main lipid classes in the investigated halophyte plants

№	Samples	Lipid content (wt % of total)*		
		TAG	GL	PL
1	*Bassia hirsuta* Aschers	12.1 ± 1.0	4.8 ± 0.4	83.1 ± 6.6
2	*Euphorbia peplis* L.	8.9 ± 0.7	66.0 ± 5.3	25.1 ± 2.0
3	*Salicornia europeae* L.	10.3 ± 0.8	6.8 ± 0.5	82.9 ± 6.6
4	Calystegia soldanella	32.5 ± 2.6	39.0 ± 3.1	28.5 ± 2.3
5	Calystegia sepium	17.2 ± 1.4	32.8 ± 2.6	50.0 ± 4.0
6	*Stachys maritima* Gonan	14.3 ± 1.1	52.4 ± 4.2	33.3 ± 2.7
7	*Suaeda maritima* Dumort	4.1 ± 0.3	77.3 ± 6.2	18.6 ± 1.5
8	*Calystegia sepium*	17.6 ± 1.4	34.2 ± 2.7	48.2 ± 3.9
9	*Calystegia sepium*	13.5 ± 1.1	19.5 ± 1.6	67.0 ± 5.4

* Values are mean ± SD from three samples

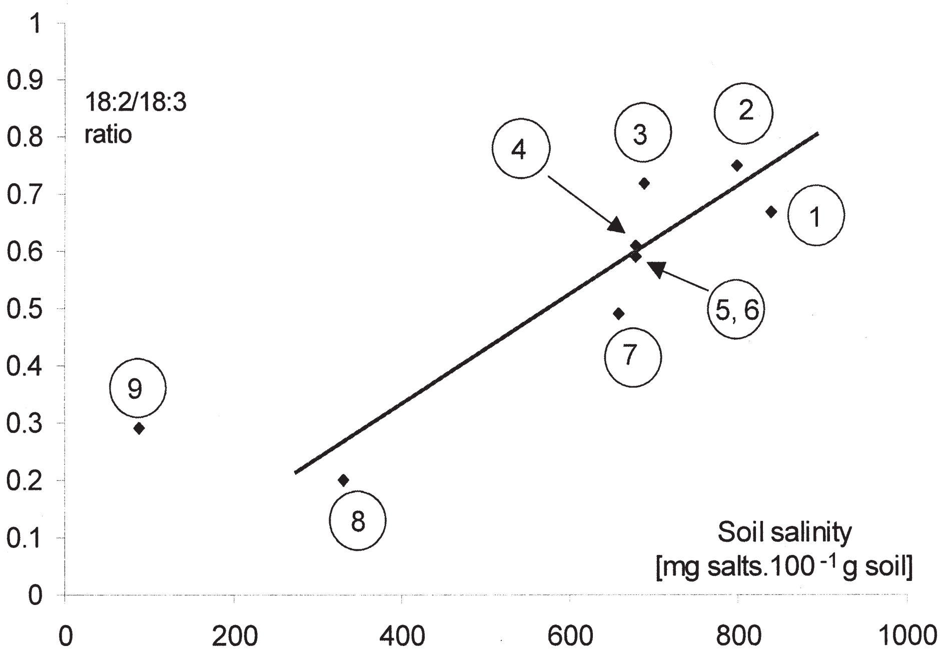

Fig. 1. Effect of soil salinity on the 18:2/18:3 fatty acids ratio in the investigated halophyte plants (see also Table 2)

ity, using 18:2/18:3 ratio as a regulating mechanism. The changes of membrane permeability seems not to be the only mechanism of plant resistance against salt stress. The investigations of sand dune vegetation could give also some valuable data for restoration of degraded coastal ecosystems. The efforts may be focused on the control of some factors due to human impacts, damaging the coastal habitats.

Research topics

1. Halophytes can be grown as forage for cattle in saline conditions. They can be used as sand-binding plants. In some cases, they could be employed to generate oil seeds.

2. For the future studies on the potential utilization of halophytes more precise data are required about the physiology of salinity tolerance and their possibilities to accommodate the lipid membranes to the harmful environment.

3. An important question that needs to be answered is whether the plants are capable of retaining halophytic trails without a loss of their productivity? Transgenic approaches for increasing plant salt tolerance are promising.

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