Kali komarovii (Amaranthaceae) is a xero-halophyte with facultative NADP-ME subtype of C₄ photosynthesis

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Kali komarovii is a representative of C₄ NADP-ME annual species of sect. Kali (subfam. Salsoiloideae of fam. Amaranthaceae). This species is genetically close (Ney's distance is 0.16–0.17) to K. paulsenii and K. tragus, which are similar species of this section of the Central Asian desert flora. The difference is that K. komarovii inhabits Japanese Sea coasts and occurs at 9000–10,000 km away from Central Asia. Comparative analysis of K. komarovii and arid NADP-ME xero-halophytes (K. paulsenii, K. tragus) and NAD-ME halophytes (Caroxylon incanecens, Climacoptera lamata) was carried out using anatomical, physiological and population genetic methods aimed to reveal structural and functional rearrangements, which provide the adaptation of NADP-ME species to saline, wet and cold conditions of sea coasts. The analysis of changes in Na⁺ and K⁺ accumulations, the Na⁺/K⁺ ratio, water content and quantitative parameters of photosynthetic apparatus in K. komarovii showed less expressed NADP-ME, but more expressed NAD-ME features. A unique characteristic of K. komarovii is the formation of specific structural-functional subtype C₄ photosynthesis related to adaptation to low temperatures, which differs from desert ancient NAD-ME subtype. Thus K. komarovii is identified as a species with a facultative NADP-ME or intermediate NADP-ME – NAD-ME subtype C₄ photosynthesis based on anatomical, biochemical and genetic characteristics.

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1. Introduction

The appearance of the C₄ syndrome was an important pre-adaptation for expansion in a wide range of environmental conditions and formation of taxonomic diversity in many groups of herbaceous plants (Pyankov and Mokronosov, 1993; Liu and Osborne, 2015). The C₄ photosynthesis pathway is estimated to have evolved independently in more than 66 lineages of flowering plants (Sage et al., 2012). Under changed environments more efficient photosynthesis has required more profound ecological specialization of species with different types of structural and biochemical CO₂ fixation. The Salsoiloideae subfamily (Amaranthaceae) comprises the largest number of C₄ species among eudicots, and 10 C₄ lineages have been recognized there (Akhan et al., 1997; Pyankov et al., 2001a; Kadereit et al., 2003; Akhan et al., 2007). Species of the tribe Salsoiloideae are characterized by anatomical and biochemical diversity (Pyankov et al., 2001b; Wen and Zhang, 2011). Intermediate C₃-C₄ species were found in various C₄ lineages, which suggests the existence of different evolutionary scenarios, including C₃-C₄ reversion (Pyankov et al., 2001a,b; Voznesenskaya et al., 2013; Wen and Zhang, 2015).

According to the theory of C₄ photosynthesis evolution in the tribe Salsoiloideae, proposed by V.I. Pyankov (Pyankov and Mokronosov, 1993; Pyankov et al., 2001a,b), the most likely geographical center of origin of “Kranz syndrome” is Central Asia. The greatest ecological, morphological and biochemical diversity, and the presence of species with C₃ and C₃-C₄ intermediate type of photosynthesis were discovered there. The NAD-ME (aspartate) subtype is considered an evolutionarily most ancient subtype of C₄ photosynthesis, which is adapted to saline and dry environments. Particularly, it is widespread on the salt marshes and in rocky and gravelly soils of Africa, Asia and Europe. The NADP-ME (malate) subtype of photosynthesis represents the evolutionary lineages facilitated by the occupation of new ecological niches. Therefore, the NADP-ME...
subtype of photosynthesis was found in almost all psammophytic C₄ species of the Central Asian deserts. These species are characterized by salsoloid type “Kranz” leaf anatomy with granal system, which developed in chloroplasts of mesophyll and agranal chloroplasts in the bundle sheath cells. Moreover, there is a predominance of malate in primary products of CO₂ fixation and a higher photosynthetic activity over that in NAD-ME species (Carolin et al., 1975; Voznesenskaya and Gamalev, 1986; Pyankov et al., 2001b).

It is known that within C₄ species of Salsoloideae there are various “intermediate” modes of malate and aspartate subtypes of photosynthesis associated with different ratios of aspartate aminotransferase (AAT) and NADP-ME activity (Pyankov et al., 1992a). Analysis of ecological distribution of C₄ species has shown that these parameters are important for determining their ecological niche. The enzyme status of C₄ species in Salsoloideae is characterized as higher AAT activity when compared with other C₄ plants (Pyankov et al., 1992a; Pyankov and Mokronosov, 1993). Many C₄ chenopods with the NADP-ME subtype have some features of NAD-ME subtype. The presence of mixed mechanisms of decarboxylation confirms the flexibility of C₄ photosynthesis (Wang et al., 2014). It was suggested that the colonization of saline both sandy and clay desert soils by facultative NADP-ME species might have happened due to the gradual reduction of CO₂ malate transport pathways and the expression of NAD-ME in them (Pyankov and Vakhrusheva, 1989; Pyankov and Mokronosov, 1993). Under worsening conditions the occurrence of biochemical subtypes of plants changes in the direction NADP-ME → NADP/ME → NAD-ME. High AAT activity in C₄ Salsoloideae provided greater lability of transition of NADP-ME to NAD-ME-pathway in extreme conditions (for example, salinity) (Pyankov and Mokronosov, 1993). It is known that the distribution of C₄ species is limited to warm habitats. However, some C₄ chenopods overcome this limitation and their photosynthetic apparatus has acclimated to low temperatures (Pyankov et al., 1992b; Long and Spence, 2013). This was the consequence of osmoregulation activation (Liu and Osborne, 2015) and significant biochemical adaptation: increase in the content of Rubisco (ribulose 1,5-bisphosphate carboxylase/oxygenase), its cold-stable isoenzymes, pyruvate phosphate dikinase (Yamori et al., 2014) and C₄ cycle enzymes (Sage and Kubien, 2007; Long and Spence, 2013). The temperature reduction to 6–8°C has not resulted in a significant decrease in the intensity of photosynthesis in C₄ species of Salsoloideae. An increase in aspartate and malate contents has been observed in some species (Galdwell et al., 1977), while in other C₄ species (Kali australis (Brown) Akhani and Roalson) the decrease in malate and the increase in aspartate contents have been detected (Pyankov et al., 1992b; as Salsola australis).

It is well known that leaf anatomical structures play an important role in plant ecology. The mesostructure of the photosynthetic apparatus characterizes the potential photosynthetic capacity of leaves and can be used as a marker of photosynthesis type and environmental strategy of species (Pyankov et al., 1998). Comparative studies of the leaf mesostructure of arid NAD-ME halophytes and NADP-ME xero-halophytes have found significant differences in the number of phototrophic cells, the ratio of mesophyll and bundle sheath cells and the internal assimilation surface area (Pyankov et al., 1993, 1997). The aspartate channel transport of CO₂ has been enhanced during the expansion of NADP-ME species of the genus Kali to the cold high mountains of the Pamirs. Moreover, activity reduction of NADP-ME and substantial adaptive adjustment of the photosynthetic apparatus mesostructure (7–9 fold increase in cell size and reduced cell number per leaf area unit) have been observed (Pyankov et al., 1993, 1997). Thus, the study of the leaf mesostructure of Kali komarovi (Ilijin) Akhani and Roalson while considering the adaptation to wet and cool coastal climate is of great interest.

Genetic polymorphism plays an important role in successful survival of populations under changeable environmental conditions. Various isozymes are widely used as molecular genetic markers in the genetic studies of populations, since they may be affected by environmental conditions (Spooner et al., 2005; Marden, 2013) and characterize the degree of ecological plasticity of the species.

The tribe Salsoleae is a well-known “model system” for the evolutionary study of C₄ photosynthesis and adaptation mechanisms of C₄ species under extreme conditions of deserts (Pyankov et al., 2001a; Akhani et al., 2007; Voznesenskaya et al., 2013). In this term the species of the youngest genus Kali are the most perspective. This genus consists of annual species with symmetric salsoloid type anatomy of leaves and cotyledons. Currently, they are widespread in Central Asia, and can be found in Europe, Siberia, in the cold deserts of Mongolia, in the highlands of the Pamirs and in North America (Pyankov et al., 1997, 2000; Ryan and Ayres, 2000; Pyankov et al., 2010). In the Russian Far East three species of the genus Kali are known: K. australis, K. collina and K. komarovi. The first two are rare, invasive, but K. komarovi is considered a typical sea coastal natural species. This species represents a unique case of extremely distant distribution of representatives of the tribe Salsoleae from the primary origin area to more wet and cool climate. Kali komarovi has a NADP-ME type of photosynthesis and salsoloid type leaf anatomy (Muhaitd et al., 2007; as Salsola komarovi).

The current study is aimed to investigate the structural and functional changes which ensure the adaptation of the xero-halophytic NADP-ME species K. komarovi to saline, wet and cold conditions of the seacoast (Japanese Sea). Another goal is to carry out a comparative analysis of the leaf mesostructure, water–salt balance and the genetic polymorphism of K. komarovi with other species of the tribe Salsoleae with NADP-ME and NAD-ME subtypes of photosynthesis.

2. Materials and methods

2.1. Study species and sites

Sea coastal species K. komarovi and four arid annual Salsoleae species (Fig. 1, Table 1) – representatives of two independent lines of the phylogenetic evolution of NADP-ME (K. tragus and K. paulseni) and NAD-ME (Carosylon incanescens and Climacoptera lanata) – were studied. Plant shoots and seeds were collected in 19 populations: two populations of K. komarovi, three of K. tragus, 5 of K. paulseni, 5 of C. incanescens and 4 of C. lanata (Fig. 1). Taxonomic and nomenclature for genera and species of plants: Akhani et al. (2007).

2.2. Isozyme analysis

On the basis of starch gel electrophoresis of isozymes from randomly chosen embryos variability of the following enzymatic systems was studied: glutamate oxaloacetate transaminase (GOT, E.C. 2.6.1.1), diaphorase (DIA, E.C. 1.6.99), glutamate dehydrogenase (GDH, E.C. 1.4.1.2), superoxide dismutase (SOD, E.C. 1.15.1.1), glucose–6-phosphate dehydrogenase (G6PD, E.C. 1.1.1.49), 6-phosphogluconate dehydrogenase (6PGD, E.C. 1.1.1.44), malate dehydrogenase (MDH, E.C. 1.1.1.37), and malic enzyme (ME, E.C. 1.1.1.40). Seeds (100 per population) were collected from 10 to 15 mother plants of each population and mixed together to make the seed pool. The seeds were cleaned of their wings and soaked in water for 12 h, and homogenized in 80 µL of Tris–HCl buffer (pH 7.5) with KCl, MgCl₂, EDTA, Triton X-100, and PVP. Enzymes were separated in 10% starch gel using two buffer systems (Muona and Szmidt, 1985; Wojnicka-Półtorak et al., 2002). Staining of particular enzymes as well as genetic interpretation of the results followed standard techniques (Muona and Szmidt, 1985; Solits and Solits, 1990).
<table>
<thead>
<tr>
<th>Species</th>
<th>Distribution</th>
<th>Habitat</th>
<th>Ecotype</th>
<th>Subtype of C₄</th>
<th>Study sites</th>
<th>Na⁺ and K⁺ contents in soil (mmol g⁻¹)</th>
<th>Tₑaverage</th>
<th>Tₚ₀ (℃)</th>
<th>Pₑaverage (mm)</th>
<th>Soil Moisture (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Kali komarovii</em> (Iljin) Akhani and Roalson (=<em>Salsola komarovii</em> Iljin)</td>
<td>Japan, China, Korea, Russia (Sakhalin, Kuril Islands, Primorski Krai) C and SW, and Middle Asia, SW Europe (C and S parts of Russia), Siberia, Caucasus, Asia Minor, Mongolia, N China; naturalized in S Africa, Australia and N and S America</td>
<td>sandy soils on riversides, saline lake shores, seashores</td>
<td>xero-halophyte</td>
<td>NADP</td>
<td>Primorski Krai</td>
<td>0.003 ± 0.001</td>
<td>5.5</td>
<td>830</td>
<td>618</td>
<td>6.43</td>
</tr>
<tr>
<td><em>Kali tragus</em> (Linn. Scopoli (=<em>Salsola pestifer</em> A. Nelson)</td>
<td>Afghanistan, Aral-Caspian region, SE Europe, W Mongolia, C, Middle, and SW Asia; naturalized in SW North America</td>
<td>on clay slopes, around villages, fields, along roads</td>
<td>xero-halophyte</td>
<td>NADP</td>
<td>Zarafshan valley (foothill semi desert conditions, Uzbekistan)</td>
<td>0.002 ± 0.001</td>
<td>14</td>
<td>300</td>
<td>70</td>
<td>5.08</td>
</tr>
<tr>
<td><em>Kali paulsenii</em> (Litvinov) Akhani and Roalson (=<em>Salsola paulsenii</em> Litv.)</td>
<td>Afghanistan, Central part of Kyzylkum desert (Uzbekistan)</td>
<td>sandy soils, low saline sandy places</td>
<td>xero-halophyte</td>
<td>NADP</td>
<td>Central part of Kyzylkum desert (Uzbekistan)</td>
<td>&lt;0.001</td>
<td>17–18</td>
<td>160–180</td>
<td>0.74</td>
<td></td>
</tr>
<tr>
<td><em>Caroxylon incanescens</em> (C.A. Mey.) Akhani and Roalson (=<em>Salsola incanescens</em> Cam.)</td>
<td>Middle Asia, Iran, Caucasus</td>
<td>salt marshes, saline clay, and clay-sandy soils</td>
<td>halophyte</td>
<td>NAD</td>
<td>Central part of Kyzylkum desert (Uzbekistan)</td>
<td>0.04 ± 0.002</td>
<td>17–18</td>
<td>160–180</td>
<td>3.03</td>
<td></td>
</tr>
<tr>
<td><em>Climacoptera lanata</em> (Pall.) Botsch.</td>
<td>Afghanistan, Middle Asia, Pakistan, Mongolia, Russia, Iran, China (N Xinjiang)</td>
<td>salt marshes, takyrs, saline sand and clay soils</td>
<td>halophyte</td>
<td>NAD</td>
<td>Central part of Kyzylkum desert (Uzbekistan)</td>
<td>0.07 ± 0.03</td>
<td>17–18</td>
<td>160–180</td>
<td>5.67</td>
<td></td>
</tr>
</tbody>
</table>

* Tₑaverage - average annual temperature, Tₚ₀ - average temperatures during the growing season (May–September), Pₑaverage - annual precipitation, Pₚ₀ - precipitation during the growing season (May–September).
2.3. Water content

The soil samples from three horizons (0–20 cm, 20–40 cm, 40–60 cm) were dried at a temperature of 100 °C until reaching a constant mass to determine the soil moisture. Plant samples were dried at 80 °C for two days until reaching a constant mass in order to measure quantitatively the dry shoot matter. The water content in soil samples and the shoots for each species was calculated as WC = (FM – DM)/DM, with FM representing fresh mass and DM dry mass of the samples.

2.4. Ion contents

Contents of Na⁺ and K⁺ in the shoots and soil were determined in water extracts from 100 mg dry samples (in a ratio of sample:water = 1:20) by atomic absorption spectrometry (Hitachi 207, Japan). Determination of Na⁺ content in the shoots of halophytes (Caroxylon incanescens and Climacoptera lanata) required additional dilution of the extracts (2–5-fold).

2.5. Quantitative leaf anatomy

The quantitative characteristics of photosynthesizing tissues (mesostructure) were examined according to Mokronosov (1981) and Burundukova et al. (2009) with some modifications. Counting of the cell number per leaf area and chloroplast number per cell was carried out on the material fixed with 3.5% glutaraldehyde in 0.05 M phosphate buffer (pH 7.2). Pieces of leaves with known area, carved out of the middle part of 5 leaves, were macerated by heating briefly in 50% KOH at 80–90 °C. Cell number was counted in a Goryaev hemocytometric chamber under light microscope, separately for mesophyll cells (M) and bundle sheath (BS) cells clearly

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Fig. 1. The location of 19 sampled populations of Kali komarovii (K.k), K. paulsenii (K.p), K. tragus (K.tr), Caroxylon incanescens (C.in) and Climacoptera lanata (C.lan).
distinguishable by shape and size (20 replicates). The ratio of mesophyll and bundle sheath cells number (M/BS) was calculated.

The number of chloroplasts, in 30 M and 30 BS cells, width and length of 30 cells of each type were determined in a cell suspension made from leaf pieces carved out of the middle part of 5 leaves macerated in 5% CrO₃ in 1 N HCl. Preparations of cell suspension were examined with a Zeiss Axiostar-40 light microscope and photographed with a Zeiss AxioCam (HR) digital camera using AxioVision ver. 4.8.3. Some measurements (leaf thickness, cell length and width) were taken from microscope images. Cell volume and cell surface area were calculated using the geometric equations describing a cylinder for M cells and rotation ellipsoid for BS cells.

The integral characteristics were determined as follows: the number of chloroplasts per leaf area unit was calculated by multiplying the number of chloroplasts per cell by the number of cells per leaf area unit; the cell membrane surface was calculated by multiplying surface area of M and BS cells and respectively in their quantity per leaf area unit (cells per leaf area unit). Multivariate principal component (PCA) method was used in comparative studies of our experimental data and literature data.

2.6. Statistical analysis

All of the physiological measurements were performed four times, and the means and standard errors (SE) were calculated using Sigma Plot 12.0 statistical program. Comparisons of parameters were made between treatments using analysis of variance (ANOVA) with a post-hoc Tukey test. Differences were considered significant at P < 0.05. The level of genetic variation was estimated by calculating the following parameters: the proportion of polymorphic markers, the average observed (Hₒ) and expected (Hₑ) heterozygosities. Nei’s (1987) genetic distances (D) were estimated between all pairs of populations to generate average clusterings using the UPGMA methods (modified from NEIGHBOR procedure of PHYLIP Version 3.5) (Yeh et al., 1999). Allele frequencies and standard genetic diversity parameters were estimated following Nei and Roychoudhury (1974) and Hedrick (1985) using the software POPGENE 1.32 (Yeh et al., 1999). One-dimensional analysis was used to test the significance of the results. Data reduction by principal components analysis (PCA) was carried out using Statistica 10.

3. Results

3.1. Genetic distance and polymorphism

Analysis of eight enzyme systems in the sea coastal species K. komarovii and four arid species (K. tragus, K. paulsenii, C. incanescens and C. lanata) showed that in K. komarovii and K. tragus they were coded by similar 14 loci. In K. paulsenii an additional locus on Gdh was found. In C. incanescens these enzymes were coded by 13 loci, in C. lanata by 16 loci. Eight loci (Gdh-2, Sod-1, Dia-2, Gpd-2, Mdh-1, Mdh-3, Me-1, Me-3) were similar (by electrophoretic mobility) in all studied species. Genetic distances (Nei, 1987) between K. komarovii and the arid NADP-ME xero-halophytes K. tragus and K. paulsenii were 0.16 and 0.17, respectively (Fig. 2). Genetic distances between K. komarovii and the arid NAD-ME species (C. incanescens and C. lanata) were significantly higher (D = 0.75 and 1.9, respectively) (Fig. 2). Estimation of genetic polymorphism in 19 populations of the studied five species showed that only two of them, K. komarovii island population and one population of K. paulsenii, were monomorphic across all studied loci. The other 17 populations were polymorphic. In a second population of K. komarovii 29% of loci were polymorphic (P), mean expected heterozygosity (Hₑ) was 0.021 and mean observed heterozygosity (Hₒ) was 0.017 (Fig. 3). Similar estimates of genetic diversity were observed in populations of the NAD-ME species C. incanescens (Fig. 3). Populations of the NADP-ME species were characterized by higher levels of heterozygosity (Hₑ = 0.076 ± 0.009, Hₒ = 0.083 ± 0.012 in K. tragus and Hₑ = 0.036 ± 0.024, Hₒ = 0.048 ± 0.030 in K. paulsenii; mean ± SE, n = 5–9 populations), but lower percentage of polymorphic loci (Fig. 3). There were no heterozygotes detected in populations of the NAD-ME halophyte C. lanata, although expected heterozygosity ranged from 0.032 to 0.22.

3.2. Moisture and Na⁺ and K⁺ contents of soil

Chemical analysis of the soils showed that the sea coastal species K. komarovii grows at very low sodium content in the soil, as well as the arid NADP-ME xero-halophytes K. tragus and K. paulsenii (Table 1). The arid NAD-ME halophytes C. incanescens and C. lanata grow at a significantly higher salinity (approximately 10- and 20-fold, respectively). Soil moisture was 6.4% in habitats of K. komarovii, which is significantly higher than in K. paulsenii and C. incanescens habitats, but comparable with K. tragus and C. lanata habitats. K⁺ content was very low in the soils of all studied habitats (Table 1).

3.3. Water and ion contents in shoots

Na⁺ content in shoots of K. komarovii was approximately 2–3-fold higher than in arid NADP-ME xero-halophytes, but approximately 6–8-fold lower than in studied NAD-ME species (Fig. 4a,b). Kali komarovii accumulated approximately the same amount of K⁺ as NAD-ME species, but lower (3-fold) as compared with NADP-ME (Fig. 4a,b). K. komarovii had an intermediate value of Na⁺/K⁺ ratio (2.1) between NADP-ME (Na⁺/K⁺ = 0.1–0.4) and NAD-ME (Na⁺/K⁺ = 10.4–12.5) species. Water content in shoots of K. komarovii was approximately 1.6-fold and 2–3-fold higher than that in NAD-ME and NADP-ME species respectively (Fig. 5).

3.4. Quantitative anatomical characteristics

The comparative anatomical study of photosynthetic apparatus of leaves showed that the sea coastal species K. komarovii had greater cell size of chlorenchyma, especially bundle sheath cell (Fig. 6a,b) as compared with arid species. Mesophyll and bundle sheath cell volumes in K. komarovii were 3–5-fold higher than those in C. incanescens and C. lanata (NAD-ME). Mesophyll cell volumes were similar in K. komarovii and K. tragus and K. paulsenii (NADP-ME), but bundle sheath cell volumes were 3–4-fold higher in K. komarovii as compared with K. tragus and K. paulsenii (NADP-ME). Arid NADP-ME species had 4–7-fold higher sum of mesophyll and bundle sheath cells per unit of leaf area and integral characteristics.
Genetic polymorphism (percent of polymorphic loci and observed heterozygosity) in populations of five studied species (Kalli komarovii, K. paulsenii, K. tragus, Caroxylon incanescens and Climacoptera lanata).

The number of chloroplasts per unit of leaf area and cell membrane surfaces as compared with desert NAD-ME species (Fig. 6c,e,f). K. komarovii had 2–3-fold higher values of these parameters than desert NAD-ME species. The M/BS ratio was significantly lower in K. komarovii than in NADP-ME species, but it was higher than in NAD-ME species (Fig. 6d). Thus, K. komarovii took an intermediate place between desert NADP-ME and NAD-ME species based on the quantitative parameters of the photosynthetic apparatus.

4. Discussion

It is known that the geographical distribution of C₄ species is related to the regional climatic conditions, such as the average daily temperature, precipitation and aridity index (Vogel et al., 1986; Pyankov and Mokronosov, 1993), as well as to edaphic factors (soil moisture, salinity and content of nitrogen in soil) (Pyankov and Mokronosov, 1993; Feldman et al., 2008; Osborne, 2008). Additionally, it has been found that diversity of C₄ species of

Water content in shoots of five studied species. The values are means (±SE) of four replicates. Letters above the bars represent significant differences at P<0.05 (Tukey’s pairwise comparison).
Fig. 6. The quantitative characteristics of photosynthetic apparatus (mesostructure) of *Kali komarovii* (1) (grey column), NADP-ME xero-halophytes *K. tragus* (2) and *K. paulsenii* (3) (white columns), NAD-ME halophytes *Coroxylon incanescens* (4) and *Climacoptera lanata* (5) (black columns). The values are means (±SE) of 30 cells. Letters above the bars represent significant differences at *P*<0.05 (Tukey’s pairwise comparison).

Chenopodioideae s. str. is highly correlated with the precipitation and aridity index rather than temperature (Pyankov et al., 2000; Pyankov et al., 2010). Perhaps, the weakening of the temperature dependence has enabled *K. komarovii* to adapt to the difficult conditions of the sea coasts of East Asia. The areal of *K. komarovii* includes the peninsula of Korea (from Hamgyong-pukto to Jeollanam-do and Gyeonggi on the Yellow Sea), the Jeju island, Japan (Honshu and Hokkaido), the Russian Far East (southern and northern Sakhalin, the South Kuriles, Primorski Kraj) and Northeast China (Liaoning, Shandong, Hebei, Jiangsu, Zhejiang) (Ohwi, 1965; Ignatov, 1988; Chung, 2007).
Isozyme analysis revealed significant genetic identity at the subspecies level; (Nei, 1987) of sea coastal species K. komarovii and arid xero-halophytes K. tragus and K. paulsenii (Fig. 2). Mean genetic estimates in studied K. komarovii populations were lower (P=0 and 29%, Hs = 0 and 0.20), than those in the Korean populations (P=29.76%, Hs = 0.113, Hs = 0.116) (Kim and Chung, 1995), as well as in other annuals with a predominantly wind-outcrossing mode of reproduction, widespread geographic range, and sexual mode of reproduction (P of 40%, Hs of 0.132) (Hamrick and Godt, 1989). Kim and Chung (1995) consider the strongly directional natural selection toward genetic monomorphism in homogeneous beach and sand dune habitats (“niches” hypothesis) as one of the possible reasons of loss of genetic polymorphism in K. komarovii. The reasons for low genetic polymorphism in the Russian coastal populations of K. komarovii may be the “bottleneck” effect and further inbreeding, as well as new environmental conditions. In Primorski Krai (Vladivostok), the average annual and growing season temperature (Tavg = 5°C and Tg = 17°C) is significantly lower than in South Korea (Tavg = 13°C and Tg = 22°C) (Chung et al., 2004) and in semidesert and desert conditions (Tavg = 14–18°C and Tg = 23–27°C, Uzbekistan) (Table 1). In invasive K. tragus populations (in USA) the genetic polymorphism was lower (P = 31%, Hs = 0.038; Ryan and Ayres, 2000; as S. tragus) as compared with Asian populations K. tragus (Fig. 3). Moreover the estimates of genetic variation in K. komarovii populations were closer to C4 NAD-ME halophytes than to NADP-ME xero-halophytes (Fig. 3). Low genetic polymorphism in halophytes may be related with their restricted ecological distribution (growing only in open habitats in sea coastal and inland salt marshes) (Wolff and Jeffers, 1987). It is known that geographic range is associated with the level of genetic variation maintained within populations (Hamrick et al., 1992).

Close relationship of K. komarovii with widespread Central Asian and Eurasian species (Wen et al., 2010) indicates a relatively recent isolation from ancestral forms. The similarity of population-genetic estimates of K. komarovii and arid NAD-ME halophytes suggests the possibility of partial reversion to the ancient NAD-ME structural and functional subtype of photosynthesis in evolution of NADP-ME xero-halophytes. The results of our comparative studies of K. komarovii and arid NADP-ME and NAD-ME species with the use of structural, physiological and biochemical features (Na+ and K+ accumulation, water content, leaf mesostructure) confirm this (Table 2, Figs. 4–6).

<table>
<thead>
<tr>
<th>Parameters</th>
<th>K. paulsenii, K. tragus</th>
<th>K. komarovii</th>
<th>C. lanata, C. incarnescens</th>
</tr>
</thead>
<tbody>
<tr>
<td>NADP-ME</td>
<td>836 ± 80a</td>
<td>811 ± 55a</td>
<td>163 ± 14b</td>
</tr>
<tr>
<td>NADP-ME to NAD-ME</td>
<td>4.5 ± 0.4a</td>
<td>3.1 ± 0.3b</td>
<td>2.2 ± 0.1c</td>
</tr>
<tr>
<td>Ratio number of mesophyll cells/number of bundle sheath cells (M/BS)</td>
<td>20.4 ± 4a</td>
<td>13.2 ± 2b</td>
<td>6.1 ± 1a</td>
</tr>
<tr>
<td>Number of chloroplasts per mm² of leaf surface (× 10⁴)</td>
<td>31 ± 8b</td>
<td>24 ± 5b</td>
<td>5 ± 3a</td>
</tr>
<tr>
<td>Na+ content in the shoots (mmol/g dry mass)</td>
<td>0.4 ± 0.06b</td>
<td>1.1 ± 0.07b</td>
<td>7.2 ± 0.1b</td>
</tr>
<tr>
<td>Na+/K+ ratio</td>
<td>0.3 ± 0.1a</td>
<td>2.5 ± 0.5b</td>
<td>11.5 ± 0.9c</td>
</tr>
<tr>
<td>Adaptive strategy</td>
<td>ruderall plant (R)</td>
<td>ruderall – stress tolerant (RS)</td>
<td>stress tolerant (S)</td>
</tr>
</tbody>
</table>

Similarly, K. komarovii occupies an intermediate position between NAD-ME halophytes and NADP-ME xero-halophytes on the basis of leaf mesostructure: the number of cells and chloroplasts per unit of leaf area, and cell membrane surfaces. However, regarding the values of mesophyll and bundle sheath cell volume K. komarovii differs significantly from the desert NAD-ME and NADP-ME species (Fig. 6, Table 2). These quantitative anatomical data indicate not just a reversion, but the development of specific adaptive traits in K. komarovii and a different evolutionary way.

A comparative multivariate analysis (PCA) of our experimental and literature data on leaf mesostructure of many annual Salsoleae species from Central Asia, Pamir and the Urals was carried out to identify patterns of evolution of the quantitative anatomy of photosynthetic apparatus and its role in a wide geographic distribution of species of Kali (Fig. 7). The PCA score plot in Fig. 7 allowed to form two groups: NAD-ME and NADP-ME species. NAD-ME species were compactly grouped, which indicates a low interspecific variation in quantitative anatomical parameters of chlorenchyma and the similarity of their structural adaptations. On the contrary, for the NADP-ME species high intraspecific and interspecific variations in the leaf mesostructure parameters were found (Fig. 7). Wide geographic distribution and ecological plasticity of NADP-ME species were manifested at the tissue and cellular level, first of all, at the variation in cell size and number (Fig. 6). The arrows in Fig. 7 indicate the direction of structural rearrangements, providing adaptation to the cold climate of the Pamir and the Urals, and saline soils on the coast of the Japan Sea (Primorski Krai). The quantitative characteristics of leaf mesostructure in K. collina and K. australis of midlands western Pamirs (Pyankov et al., 1993; Pyankov et al., 1997; as Salsola collina, S. australis) are close to those in K. komarovii; K. collina and K. australis occupy an intermediate position between arid NAD-ME and NADP-ME Salsoleae species regarding the number of cells and chloroplasts per unit of leaf and cell membrane surfaces. Moreover K. komarovii differs from desert species by larger size of cells, particularly by larger bundle sheath cells (Fig. 6 b). The largest cell size has been observed in NADP-ME Salsoleae species in the Pamir Mountain and in desert species expanded to the Urals – at the cold temperature limit of C4 species distribution (Pyankov et al., 1993; Pyankov et al., 1997; Pyankov et al., 1999).

Perhaps the increase in cell size of K. komarovii as compared with desert species is associated with adaptation to the conditions of cool and monsoon climate (Table 1). However in K. australis found in Pamirs at 3600 m above sea level such an increase in cell size is expressed even more (mesophyll and bundle sheath cells volume reached 26 and 56 thousand mm³, respectively) (Pyankov et al., 1993). Also, another reason of the cell volume increase may be polyploidy, i.e. an increase in the number of chromosomes or DNA endoreplication (Lundgren et al., 2014). In Kali diploid, tetraploid and hexaploid cytotypes have been reported (Chaffari et al., 2015). Among the desert xerophytes the diploid number of chromosomes (2n = 18) are more common, although the tetraploids are known, for example in K. paulsenii (Zakharyeva, 1985). Tetraploid chromosome number is known (2n = 36) in ruderal species, which can

Table 2 Parameters of leaf mesostructure and ion contents (mean ± SE, n = 6–10) in the shoots of NADP-ME xero-halophytes Kali komarovii, K. paulsenii, K. tragus and NAD-ME halophytes Caroxylon incanescens, Climacoptera lanata. Letters represent significant differences at P < 0.05 (Tukey’s pairwise comparison).
occupy the seashore, such as K. tragus (Michalkova and Roman, 2014) and coastal halophytes K. soda and K. komarovi (Lomonosova et al., 2005; Michalkova and Roman, 2014; Probatova et al., 2014). Probably polyploidy has played an important role in adaptation to saline coastal habitats. In the genus Salicornia L., it was found that polyploidy contributed to adapting species to coastal salt marshes (Rozema and Schat, 2013).

The evolution of photosynthetic systems under conditions of high salinity and low temperatures is accompanied by a reduction of malate pathways of CO2 and increased aspartate pathways. It is also connected with the restructuring of the leaf mesostructure (decrease in the number of cells and chloroplasts per leaf area, cell membrane surfaces) and with the change in the ratio of mesophyll and bundle sheath cells (4–8 mesophyll cells per bundle sheath cell in NADP-ME species and 1–3 in NAD-ME species) (Pyankov et al., 1993). The ratio of mesophyll and bundle sheath cells (M/BS) in the succulent desert NADP-ME species Hammada leptoclada (M. Pop. ex Iljin) Iljin was lower (2.7) in cold conditions of midland Pamir as compared with desert habitats (6) (Pyankov et al., 1993). Desert shrubs with NADP-ME type of photosynthesis (Xylosalsola richteri (Mocz.) Akhani and Roalson and species of Anabasis L., Haloxylon Bunge et al.) are more resistant to salinity, have lower NADP-ME and higher AAT activities as compared with annual NADP-ME xero-halophytes. This has allowed to assume the existence of an intermediate NADP-ME – NAD-ME subtype of photosynthesis in these species (Pyankov et al., 1992a). These species have lower values of M/BS ratio (2.2–2.7) as compared with annual NADP-ME xero-halophytes. Kali komarovi has 3.1 of M/BS (Table 2), which gives reason to suggest a low activity of NADP-ME in it. Based on published data on the leaf mesostructure and activity of AAT and NADP-ME enzymes (Pyankov et al., 1992a; Pyankov et al., 1993), we calculated the index M/BS and AAT/NADP-ME for 10 annual and perennial Salsoleae species and found a significant negative correlation between them \( r = -0.63, P < 0.05 \) (Fig. 8). Regression analysis allowed hypothetical evaluation of the value of AAT/NADP-ME for K. komarovi – it could be approximately 50–150 (Fig. 8). This value is significantly higher than that in NADP-ME annual xerohalophytes, but it is lower than that in NAD-ME halophytes (Fig. 8), which points to the intermediate NADP-ME – NAD-ME subtype of photosynthesis in K. komarovi.
Quantitative anatomical characteristics of photosynthesizing tissues allow to identify the type of ecological strategy and to estimate the degree of the expression of stress-tolerance and ruderal properties (Pyankov et al., 1998). In general, high values of cell membrane surfaces and the number of cells and chloroplasts per leaf area in the annual desert NADP-ME xero- and mesophytes K. tragus and K. pautsenii indicate considerable expressing ruderal properties. In contrast, low values of these parameters in the halophyte C. inamenceas and, in particular, C. lanata indicate the prevalence of stress-tolerant-properties and stress-tolerant-type of environmental strategy (Fig. 6). Decrease in the values of these traits in K. komarovi indicates weakening ruderal and enhancing stress tolerance characteristics, i.e. a mixed type (RS) of environmental strategy (Table 2).

Thus, the results of our study show that K. komarovi occupies an intermediate position between the NADP-ME xero- and mesophytes and NAD-ME halophytes based on a number of structural, physiological and genetic parameters (Table 2).

5. Conclusion

Results of our research confirmed that under saline conditions or low temperatures an important element in the adaptation of NADP-ME subtype in Salsoleae is a variability of the primary CO2 fixation pathways, which is realized in a partial inhibition of malate and increase of aspartate pathways, i.e. a partial reversion to an ancient NAD-ME subtype. Adaptation of K. komarovi to the sea coasts was accomplished by strengthening the stress-tolerant properties (cool and salt tolerance) and the restructuring of the structural and functional characteristics of the photosynthetic apparatus in the direction to NAD-ME subtype photosynthesis. This was accompanied by an increase in hydration of shoot and in chlorenchyma cell volume, and by maintenance of relatively high number of chloroplasts per leaf area unit, which allowed to keep a high level of CO2 assimilation. Moreover, there was a change in ionic balance (higher Na+/K+ ratio) to increase salt tolerance (Table 2). The NADP-ME subtype of photosynthesis has more capability to adapt to new conditions and unfavorable factors than the ancient NAD-ME subtype. It allows species of the younger genus Kali to adapt to changing habitat conditions and expand their range.

Our results provide evidence that the development of intermediate NADP-ME – NAD-ME or facultative NADP-ME subtype in K. komarovi is caused by the influence of a combination of various specific factors, such as high humidity, low temperature and salinity.

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References


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