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Physiological and biochemical adaptations of *Cynodon dactylon* (L.) Pers. from the Salt Range (Pakistan) to salinity stress

Mansoor Hameed*, Muhammad Ashraf

Department of Botany, University of Agriculture, Faisalabad 38040, Pakistan

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Abstract

Naturally adapted salt tolerant populations provide a valuable material for exploring the adaptive components of salt tolerance. Under this aspect, two populations of Cynodon dactylon (L.) Pers. were subjected to salt stress in hydroponics. One was collected from a heavily salt-affected soil in the vicinity of a natural salt lake, Uchhali Lake, in the Salt Range of the Punjab province of Pakistan, and the other from a normal non-saline habitat from the Faisalabad region. The NaCl treatments in Hoagland's nutrient solution were: Control (no salt), 50, 100, 150 and 200 mM of NaCl. After 8 weeks of growth in hydroponics produced biomass, ion relations, and photosynthetic capacity were measured in the differently adapted ecotypes. In the ecotype of C. dactylon from the Salt Range, shoot dry weight was only slightly affected by varying levels of salt. However, in contrast, its root weight was markedly increased. On the other hand, the ecotype from Faisalabad (non-saline habitat) showed a marked decrease in shoot and root dry weights under saline regimes. The ecotype from the Salt Range accumulated relatively less amount of Na in the shoot than did that from Faisalabad, particularly at higher salt levels. Shoot or root K and Ca²⁺ contents varied inconsistently in both ecotypes under salt stress. All the photosynthetic parameters, leaf water potential and osmotic potential, and chlorophyll content in both ecotypes were adversely affected by salt stress, but all these physiological attributes except turgor potential and soluble sugars were less affected at high salinities in the salt tolerant ecotype from Salt Range. This ecotype accumulated significantly higher organic osmotica (total free amino acids, proline, total soluble proteins, and total soluble sugars) under saline conditions than its intolerant counterpart. Overall, the salt tolerant ecotype of *C. dactylon* from the Salt Range showed high salt tolerance due to its restricted uptake of Na⁺ accompanied by an increased uptake of K⁺ and Ca²⁺ in the roots as well as shoot due to its higher photosynthetic capacity and accumulation of organic osmotica such as free amino acids and proline under saline conditions.

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Introduction

Tolerance to salinity has been evolved naturally in numerous turfgrasses including members of *Agrostis*, *Festuca*, *Lolium* and *Poa* (Acharya et al., 1992; Humphreys et al., 1986). The evolution of salt tolerance

E-mail address: hameedmansoor@yahoo.com (M. Hameed).

^{*}Corresponding author.

in several genera of turfgrasses (Zovsia, Buchloe, Cynodon, etc.) has occurred in coastal marshes and rocky alpine habitats. Natural selection pressure in the form of salinity stress drives selection coefficients, due to which alleles vary in number in natural populations and the eventual extent to which salt tolerance evolves (Ahmad et al., 1981; Wu, 1981). Natural populations can vary dramatically in salt tolerance across distances as little as 10 m (Casler and Duncan, 2003). Salt tolerant genotypes of turfgrasses have an ability to evolve a form of salt dependence, as illustrated by greater increases in root and shoot growth under high- vs. low-saline growth conditions (Ashraf et al., 1986; Grieve et al., 2004). Salt tolerant plants have the ability to minimize these detrimental effects by producing a series of anatomical, morphological and physiological adaptations (Poljakoff-Mayber, 1988), such as an extensive root system and salt secreting glands on the leaf surface (Gorham et al., 1985; Marcum and Murdoch, 1990; Marcum et al., 1998; Sinha et al., 1986) and restricting the uptake of toxic ions (Ashraf, 2004).

Salt Range, located between longitude 71°30′-73°30′ east and between latitude 32°23′-33°00′ north, forms a very prominent geomorphologic and ecological feature as it is between the Thar desert in the west and the Potohar Plateau in the north east (McKerrow et al., 1992; Yeats et al., 1984). The climate of the Salt Range is characterized by relatively low precipitation, about 50 cm annually, and most of the rain is confined to the months of July, August and September (Ahmad et al., 2002). Vegetation of the Salt Range mainly comprises sub-tropical dry evergreen scrub forest. Chaudhry et al. (2001) have reported over 40 grass species in addition to a number of trees, shrubs and herbs from the Salt Range area; some of them are highly palatable and nutritious. Dominant grasses are Chrysopogon serrulatus, Heteropogon contortus, Dichanthium annulatum, D. foveolatum, Digitaria sanguinalis, Aristida mutabilis, A. adscendens and Panicum antidotale, while Cynodon dactylon, Sporobolus arabicus, Imperata cylindrica, Aeluropus lagopoides dominate the saline or saline-arid habitats. These grasses are expected to be well adapted against high salinities in view of the length of time they have been growing there. Thus, one of them, C. dactylon, was chosen for the present studies.

C. dactylon (L.) Pers. (Bermuda or Couch grass) is a perennial grass. Its distribution is very wide but it occurs predominantly in tropical and warm temperate regions throughout the world (Chaudhary, 1989). Although it is a favorite lawn grass, it makes an excellent hay and is considered as a potential fodder plant in Australia, Pakistan and Southeast Asia (Chaudhary, 1989; Jacobs, 2003). The grass is known for its extremely variable habit and adaptation to a variety of habitat types. For example, it is frequently found growing on sandy or saline soils of open sites, including road sides, on

agricultural fields, along irrigation canals, in orchards, waste places and metal-contaminated wastelands, the latter particularly with high loads of Pb, Zn and Cu (Gleason and Cronquist, 1991; Río-Celestino et al., 2006; Shu et al., 2002; Wu et al., 2006).

Natural populations of Bermuda grass possess a great magnitude of genetic variation for growth traits, including erect vs. prostrate stems, root penetration and tolerance of extreme soil temperatures, salinity and drought (Grattan et al., 2004; Wu et al., 2006). Some ecological and distribution patterns are correlated well with the ploidy levels of this grass (De Silva and Snaydon, 1995; Wu et al., 2006). Bermuda grass has a good tolerance to salinity, but it shows slow growth on salt affected soils. Moderate salinities may increase its yield, though it is capable of resisting relatively high salinities (Grattan et al., 2004; Grieve et al., 2004).

Naturally adapted salt tolerant plants provide an excellent material for investigating the adaptive mechanisms they use to encounter high concentrations of salt. By using these plants as a model, research can play an important role in improving the tolerance of non-halophytic plants. The present studies were focused to examine pattern and extent of physiological adaptations in a *C. dactylon* population adapted to high-saline conditions. In view of the considerable time period the population has been under the exposure of high selection pressure from salinity, it was expected that it must have evolved traits related to quite a good salt tolerance.

Materials and methods

C. dactylon (L.) Pers. was collected from the heavily salt affected soil in the vicinity of the natural salt lake, Uchhali Lake, in the Salt Range, Punjab (ECe 19.92 dS m⁻¹, pH 6.62, coordinates 32°36′34.59″N, 72°13′53.72″E). This population seemed to be highly salt tolerant as it grows in a habitat which is in direct contact with highly saline water of the lake. An ecotype of this grass was also collected from a normal, nonsaline habitat within the Faisalabad region (ECe 1.82 dS m⁻¹, pH 8.20, coordinates 31° 25′ 42.87″ N, 73° 04′ 11.46″ E). This population was considered as the non-salt-tolerant control.

Plants of both populations of *C. dactylon* were grown in normal non-saline soil for a period of 6 months. Earthen pots (22.5 cm high) filled with loam and sand in equal quantities were used for growing the plants of both populations. The plants were kept under full sunlight and irrigated daily with good-quality irrigation water till their establishment under the Faisalabad climatic conditions (coordinates 30°35′47″N 74°73′40″E/30.59′64″E, 75.22′78″N, altitude 300 m AMSL, max temp. 48 °C in June, min temp. is

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