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Mechanism of salt tolerance in wild rice (*Oryza coarctata Roxb*)

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Summary *Oryza coarctata*, a highly salt-resistant wild rice species, is commonly found on the banks of coastal rivers in India. This species can also withstand saline water (20 to 40 dSm^{-1} E.C) submergence for quite a long period. It was revealed that *O. coarctata* has some special unicellular salt hairs (trichomes) on the adaxial surface of the leaves, by which they efficiently maintain a low concentration of toxic salts in the plant tissue. Sodium and chloride were the dominant ions in the excreted material but they also excrete potassium, magnesium and calcium. With the increase in soil salinity sodium, magnesium and chloride excretion increased. *O. coarctata* maintained the optimum mineral concentration in its tissues. Maximum accumulation of potassium was observed in the leaves. With the increase in salt stress total biomass production and osmotic potential increased over control but there was no change in the moisture percentage of leaves.

Introduction

Oryza coarctata is an Asian wild rice species, occuring mostly in the coastal areas of India, on the sea shores and marshy bank of the tidal rivers. This species is highly salt resistant and can survive safely even up to 30 to 40 dSm^{-1} (ECe). It is also widely prevalent in almost all the islands of mangrove forests in Sunderban (West Bengal, India) where the soil is inundated twice a day with saline river or sea water of 20 to 40 dSm^{-1} (E.C). This species can withstand submergence with saline water for quite a long period. In the deep forests of Sunderban it is generally completely submerged by the tidal water for as long as 10–11 hours every day. The Mean Tidal Level (MTL) of this area is 1.10 m from the base of the plant. The plant grows up to a height of 1 m with very hard stem and thick leathery leaves. The leaves are without any midrib which is not generally observed in other *Oryza* species. The leaf blade of *O. coarctata* has a number of prominent ridges and furrows running longitudinally on the adaxial surface. Silica bodies on the abaxial epidermis of the blade were found to be oblong[8]. It is a tetraploid species with 2n = 48 chromosomes. Tateoka[10] found that each ridge contains one small vascular bundle nearer the adaxial surface and below it a larger one. Hedayetullah *et al.*[4] and Chalam[3] studied the mechanical system of *Oryza* spp. and confirmed that the highest development of mechanical tissues is in *O. coarctata* than in other *Oryza* spp. Richharia *et al.*[8] found that the root of this species show the usual structures of epidermis, cortex and stele as found in other species of *Oryza*. The mature roots, however, have a strongly developed sclerotic pith. Bal[2] showed that this species has an amino acid content which is much higher than in the cultivated rice varieties.

Many scientists have worked from time to time on the cytology, anatomy and stomatal behaviour of *O. coarctata* but there is no report on the salt tolerance mechanism and mineral uptake. Therefore the present study was undertaken to know the salt-tolerance mechanism of *O. coarctata*.

Material and methods

The experiment was carried out at Central Soil Salinity Research Institute, Regional Research Station, in the year 1982–83. Plants of *Oryza coarctata* collected from marshy river banks of Sunderban (India), were grown in porcelain pots with four replications under four levels of saline soil *i.e.* 2.7, 8.1, 16.5 and 31.7 dsm^{-1} ECe. The desired level of soil salinity was created artificially by adding saline river water from the river which is adjacent to the Institute farm.

At the time of peak vegetative growth, leaves were collected for anatomical studies and dehydrated in tertiary butyl alcohol following the method of Johansen[5]. The leaf samples were embedded in paraffin blocks and 12 μm thick transverse sections were cut with the help of a microtome. The sections were subsequently stained with safranin and hematoxylin and mounted in euparol. Microphotographs of the sections were taken by phase contrast.

The leaves to be sampled for collection of excreted materials were first washed in sterilised distilled water to wash away the already excreted material and the pots were kept in the green house. The leaves were cut off after 24 hrs and washed thoroughly with glass distilled water to collect the water soluble excreted salts; care was taken so that the cut point of the petiole did not come in contact with the washing to prevent any contamination by the xylem sap. The leaf washings were then estimated flame photometrically for the presence of sodium and potassium. Calcium and magnesium determined by EDTA complexometry and chloride was estimated with silver ion, using chromate ion as an indicator.

The leaf and stem tissue were dried and digested in diacidic mixture for determination of sodium, potassium and calcium. Chloride was estimated from the ground dry tissue.

Results and discussion

Anatomical studies of the leaf of *O. coarctata* (Plate 1) revealed that the adaxial surface possesses some special unicellular salt hairs (trichomes). These special unicellular structures are found in the furrows and are connected with the epidermal cells. The first important study on the physiology of salt excretion was carried out by Arisz *et al.*[1] using *Limonium latifolium*. They showed that sodium chloride could be secreted against a concentration gradient and that the process was temperature sensitive and required oxygen.

It was clear from the transverse section that *O. coarctata* leaves have many ridges and furrows and each ridge contains one small vascular

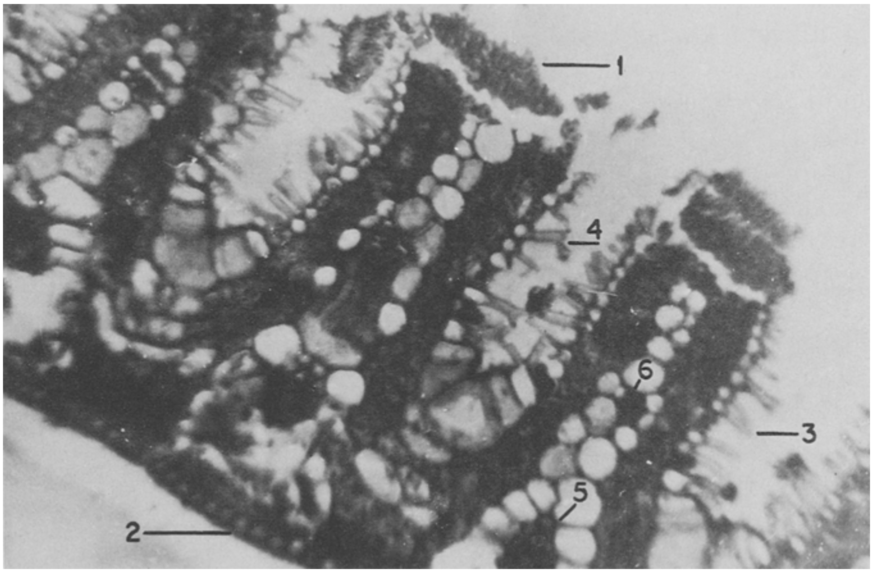

Plate 1. T.S. of the leaf of *O. coarctata* (Roxb) showing adaxial surface (1), abaxial surface (2), salt hairs in the furrows (3), bursted salt hairs (4) and vascular bundle (5, 6). (× 120).

towards the adaxial suraface and below it a larger one, wich confirms the report by Tateoka[10]. Both the adaxial and abaxial leaf epidermis are composed of short and long cells in rows. It was observed that the salt hairs are thin and small with blunt tips, not balloon shaped as found in *Atriplex* spp. The unicellular cells function only for short periods, when the optimum concentration of salts is reached within these cells (salt hairs) they burst and eliminate the salts. New cells are formed continuously in place of the bursted cells and through this process they efficiently maintain a low concentration of toxic salts in the tissue as reported in *Atriplex* spp. by Luttge[7]. Many halophytes and mangrove species have salt glands[6,7]; but these glands do not burst like the salt hairs of wild rice. The histology of salt glands has been described for *Statice gmelini*[9], *Avicennia* sp.[11].

The excreted materials on the adaxial leaf surface were collected in glass distilled water and analysed. It was found (Table 1) that sodium and chloride were the dominant ions followed by potassium, magnesium and calcium; carbonates and bicarbonates were found to be absent. The data indicated (Table 1) that with the increase in soil salinity sodium, magnesium and chloride excretion increased. Maximum excretion of sodium and chloride was observed at 31.7 dSm^{-1}, which was 59.5% of sodium and 54.5% of chloride above that of control.

However, there were no changes in concentration of excreted potassium and calcium ion with the increase in soil salinity. It might be that potassium is most essential for their better survival and growth under salinity stress. But potassium excretion was more than calcium. The reason may be due to the less uptake of calcium from the soil system.

There were no differences in sodium, potassium, calcium and chloride content in tissue of *O. coarctata* under different salinity stress (Table 2), its probably due to their salt excretion mechanism. They possibly maintain the mineral concentration in their tissues according to their requirement. It was observed (Table 2) that there was always a higher accumulation of potassium than sodium. Potassium was higher in the leaves compared to that in the stem but in case of sodium the reverse was true, there was no change with the increase in soil salinity. It was found that *O. coarctata* very efficiently maintained a lower chloride concentration in the leaf than in the stem. Calcium content was also lower in the leaf than stem. The accumulation of calcium was much lower than other elements.

Table 1. Composition of salts excreted through salt hairs in *O. coarctata* (mmol/Kg fresh leaves/24 hrs)

Salinity levels (ECe dSm^{-1})	Na ± SE	K ± SE	Ca ± SE	Mg ± SE	Cl ± SE
2.7	16.0 ± 0.16	6.6 ± 0.03	3.0 ± 0.100	1.9 ± 0.09	16.4 ± 0.23
8.1	21.7 ± 0.53	6.3 ± 0.07	2.0 ± 0.002	10.5 ± 0.06	17.9 ± 0.16
16.5	23.6 ± 0.28	6.3 ± 0.03	2.0 ± 0.100	14.5 ± 0.34	19.9 ± 0.16
31.7	25.6 ± 0.43	6.3 ± 0.05	2.0 ± 0.002	18.2 ± 0.27	25.5 ± 0.12

Table 2. Tissue mineral concentration of *O. coarctata* under different Salinity stress (Expressed in % dry matter)

Salinity levels	Na ± S.E.		K ± S.E.		Ca ± S.E.		Cl ± S.E.	
ECe dSm^{-1}	Stem	Leaf	Stem	Leaf	Stem	Leaf	Stem	Leaf
2.7	1.1 ± 0.01	0.87 ± 0.01	1.4 ± 0.01	4.3 ± 0.08	0.35 ± 0.02	0.04 ± 0.01	0.4 ± 0.04	0.2 ± 0.04
8.1	1.2 ± 0.02	1.00 ± 0.02	1.1 ± 0.05	4.2 ± 0.09	0.35 ± 0.01	0.04 ± 0.02	0.4 ± 0.03	0.2 ± 0.02
16.5	1.2 ± 0.04	1.00 ± 0.02	1.1 ± 0.10	4.2 ± 0.09	0.30 ± 0.02	0.04 ± 0.02	0.4 ± 0.08	0.2 ± 0.04
31.7	1.2 ± 0.09	1.00 ± 0.01	1.1 ± 0.06	4.2 ± 0.06	0.25 ± 0.02	0.06 ± 0.01	0.4 ± 0.06	0.2 ± 0.07

It was clear from Table 3 that moisture status of the leaf tissue did not change with the increase in soil salinity. Similarly, there was also not much change in osmotic potential of leaf sap except at 31.7 dSm^{-1} ECe), where it was slightly increased, this increment may have been due to the higher concentration of organic solutes in the tissues. It was interestingly noted that with the increase in soil salinity total biomass production increased markedly over control but this increment was maximum at (ECe) 8.1 dSm^{-1}, which was 65.0% over control. This study indicated that probably *O. coarctata* does not feel any stress even under higher salinity levels due to their unique salt hairs by which they efficiently excrete the excess amount of toxic elements. They also show the highest development of mechanical tissue which is much stronger than found in other *Oryza* spp.[3,5]. It is quite possible that this *O. coarctata* may be used as a parent for evolving better and truly salt resistant rice varieties. Due to its tetraploid nature, spikelet character and reportedly complete incompatibility with cultivated *Oryza* spp. this has not been possible till now by conventional breeding methods. However, it is felt that there is scope for evolving better resistant rice varieties through somatic hybridization.

Table 3. Total biomass production, moisture percentage and osmotic potential of *O. coarctata*

Salinity levels ECe dSm^{-1}	Biomass ± SE (g/pot)	Leaf moisture (%)	O.P. of Leaf sap (MPa)
2.7	63 ± 0.64	48	− 1.8
8.1	104 ± 0.92	48	− 1.8
16.5	70 ± 0.55	48	− 1.9
31.7	72 ± 0.63	48	− 2.0

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