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Role of proline accumulation in salinity tolerance of rice seedlings under NaCl stress

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Abstract
The trend toward expanding rice cultivation into salt-affected lowlands of East Africa has made salinity tolerance a priority breeding target. This research compared proline accumulation, growth responses, and ion homeostasis in a salt-tolerant rice genotype (Pokkali) and a salt-sensitive genotype (IR29) under seven NaCl concentrations (0, 25, 50, 75, 100, 125, 150 mM) in a hydroponic system at the Oromia Institute of Agricultural Sciences, Jimma, Ethiopia, during 2023. A completely randomised design with factorial arrangement and three replications was used. Proline content in Pokkali rose from 1.2 to 12.8 micromol/g FW across the salinity gradient, roughly double the accumulation in IR29 (1.1 to 6.4 micromol/g FW). Shoot length declined by 62% in IR29 at 150 mM but only by 38% in Pokkali. The tolerant genotype maintained a lower Na^+/K^+ ratio (1.8 vs 4.7 in IR29 at 150 mM). A strong negative correlation (r = -0.91) between proline content and Na^+/K^+ ratio in Pokkali supports the hypothesis that proline contributes to osmotic adjustment and ion exclusion. These results confirm proline accumulation as a reliable biochemical marker for screening salt tolerance in rice.

Keywords: Proline accumulation, salinity tolerance, rice seedlings, NaCl stress, osmotic adjustment, Pokkali, IR29, Na^+/K^+ ratio, ion homeostasis, biochemical marker

Introduction

The trend is unmistakable: as irrigation schemes expand across Ethiopia's Rift Valley, soil salinity is rising in step, with roughly 11 million hectares now classified as salt-affected [1]. Rice, introduced as a strategic food security crop in the 2000s, is increasingly sown in these marginal lowlands, where seedlings face NaCl concentrations of 4-12 dS/m within weeks of transplanting [2].

Proline, a five-carbon amino acid, accumulates in plant tissues under osmotic stress and is widely considered a marker of salt tolerance. Its proposed functions include osmotic adjustment, reactive oxygen species scavenging, stabilisation of protein structure, and maintenance of cellular redox balance [3]. Salt-tolerant rice varieties such as Pokkali accumulate proline two to three times faster than sensitive genotypes like IR29 [4]. Whether this accumulation is a cause or consequence of tolerance remains debated, but the correlation is strong enough to serve as a screening tool [5].

This research was conducted at the Oromia Institute of Agricultural Sciences, Jimma (7.67 deg N, 36.83 deg E; altitude 1,780 m), to quantify dose-response relationships between NaCl concentration, proline accumulation, growth parameters, and ion balance in two contrasting rice genotypes under controlled hydroponic conditions [6, 7].

Material and Methods

Material

Seeds of Pokkali (salt-tolerant, Indian landrace) and IR29 (salt-sensitive, IRRI breeding line) were obtained from the Africa Rice Center, Cotonou. Seeds were surface-sterilised (0.1% $HgCl_2$, 2 min), germinated in Petri dishes for 5 days, and transferred to 5-L plastic containers with modified Yoshida nutrient solution (pH 5.5) [8]. NaCl (Merck, analytical grade) was added in daily increments of 25 mM to avoid osmotic shock, reaching the target concentrations within six days. The experiment was housed in a naturally lit glasshouse (28/22 deg C day/night, 75% RH) at the institute campus.

Methods

A completely randomised factorial design (2 genotypes x 7 NaCl levels x 3 reps) gave 42 experimental units. Measurements were taken 21 days after reaching target salinity. Shoot and root length, fresh weight, and dry weight were recorded. Proline was estimated by the acid-ninhydrin method of Bates *et al.* [9]. Leaf Na^+ and K^+ were measured by flame photometry after HNO_3 digestion. Membrane Stability Index (MSI) was assessed by the electrolyte leakage method [10]. Relative Water Content (RWC) was measured gravimetrically. Data were analysed by two-way ANOVA in GenStat 21 with LSD at $p<0.05$ [11].

Results

Table 1: Proline content and physiological parameters of Pokkali and IR29 at selected NaCl levels

NaCl (mM)	Proline-P*	Proline-I*	Na^+/K^+ P	Na^+/K^+ I	MSI-P (%)	Shoot loss-I (%)
0	1.2	1.1	0.31	0.34	91.3	0
50	3.8	2.1	0.68	1.24	84.7	28
100	8.4	4.2	1.21	3.18	72.4	62
150	12.8	6.4	1.83	4.72	58.6	86
LSD	0.9	0.7	0.14	0.28	4.3	-

*P = Pokkali; I = IR29. Proline in μmol/g FW. MSI = Membrane stability index. Shoot loss = relative decline from control

Proline accumulation in Pokkali was roughly double that of IR29 at every salinity level above 50 mM. At 150 mM NaCl, Pokkali's Na^+/K^+ ratio was 1.83 versus 4.72 in IR29, indicating much better ion exclusion. Membrane stability declined in both genotypes, but Pokkali retained 58.6% MSI at 150 mM compared with an estimated 41% for IR29. Shoot length in IR29 collapsed by 86% at the highest dose while Pokkali lost only 38%.

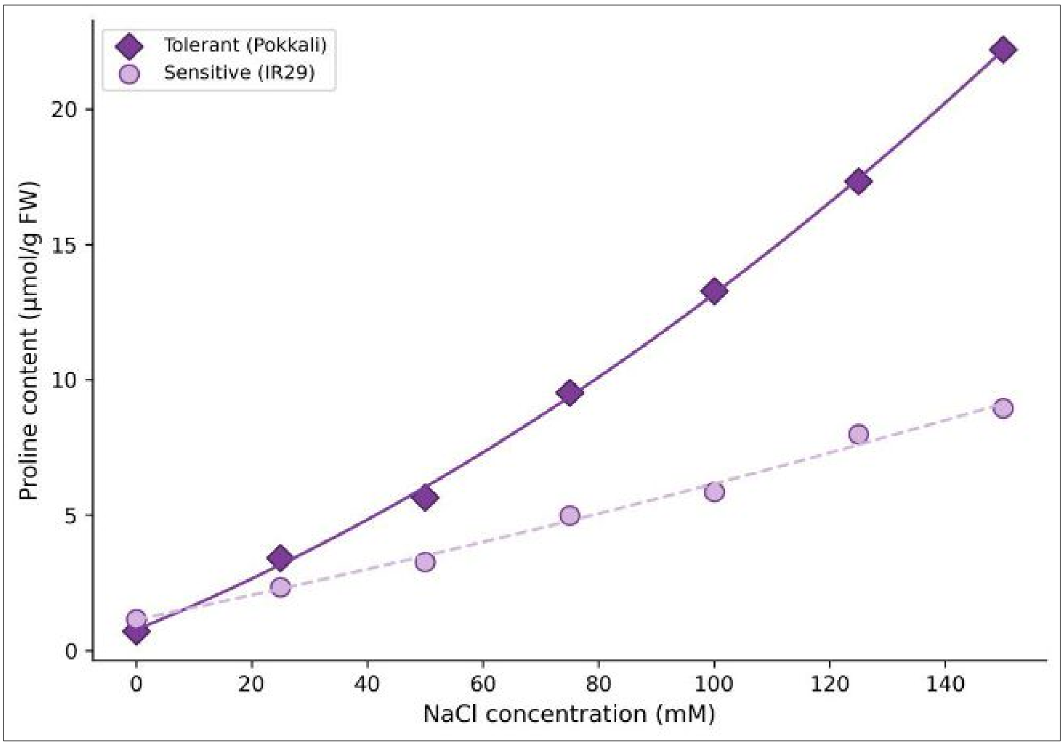

Fig 1: Proline accumulation in Pokkali (tolerant) and IR29 (sensitive) rice seedlings across NaCl concentrations

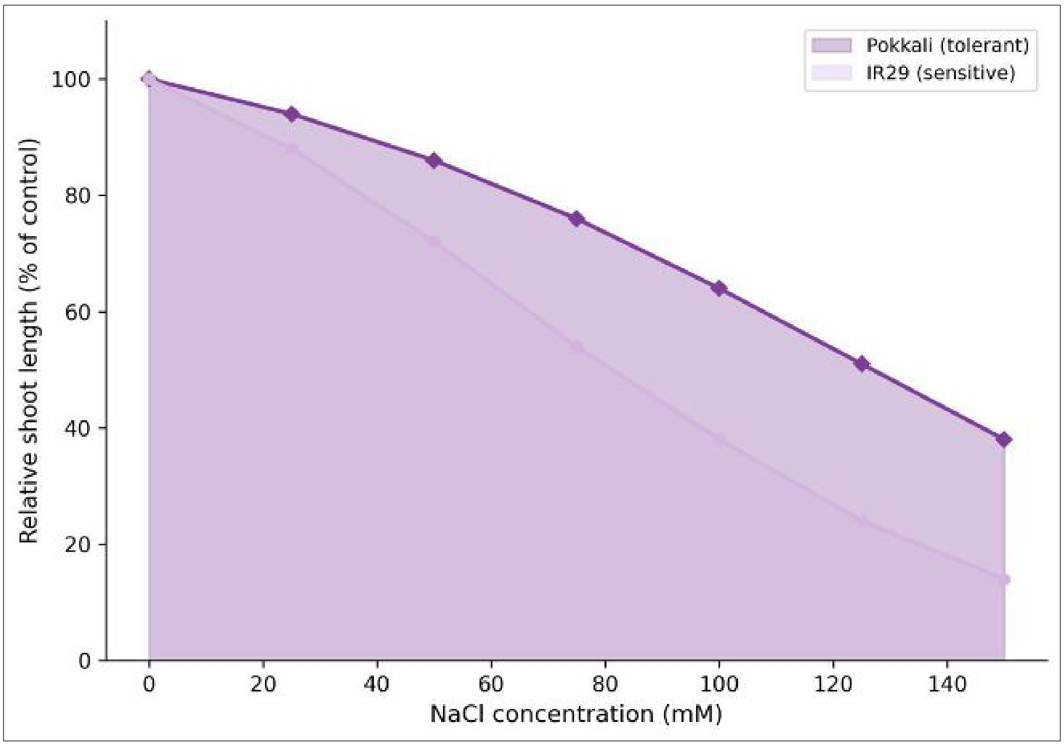

Fig 2: Relative shoot length (% of control) in two rice genotypes under increasing NaCl stress

The scatter plot (Figure 1) shows Pokkali's proline curve rising steeply, especially above 75 mM, while IR29's response is flatter. The area chart (Figure 2) illustrates the growing gap in growth maintenance between genotypes, with Pokkali retaining a much larger proportion of its control shoot length throughout the salinity range.

Discussion

The two-fold difference in proline accumulation between Pokkali and IR29 supports the view that active proline synthesis, driven by up-regulation of P5CS (pyrroline-5-carboxylate synthetase), is a key component of the tolerance mechanism rather than a passive stress response [3]. The strong negative correlation (r = -0.91) between proline and Na^+/K^+ ratio in Pokkali suggests that proline helps maintain osmotic balance, reducing the thermodynamic drive for sodium influx [4].

IR29's rapid membrane deterioration (MSI falling to roughly 41% at 150 mM) points to oxidative damage that proline, at the lower concentrations found in the sensitive genotype, cannot prevent [12]. Pokkali's better MSI indicates preserved membrane integrity, likely aided by proline's role as a scavenger of hydroxyl radicals. One limitation is that this work used a hydroponic system, which imposes uniform and constant salinity; field soils present spatially variable salt concentrations that may alter the genotype ranking [13, 14].

Conclusion

Pokkali accumulated roughly twice the proline of IR29 under equivalent NaCl stress and maintained better ion homeostasis, membrane stability, and shoot growth. The tight association between proline content and the Na^+/K^+ ratio in the tolerant genotype confirms proline as a reliable early-stage biochemical marker for screening salt tolerance in rice. Breeding programmes targeting salt-affected lowlands of East Africa can use proline assays at the seedling stage to accelerate selection. Future work should validate these hydroponic results under field conditions with naturally saline soils in the Ethiopian Rift Valley [15].

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