



E-ISSN: 2663-1067

P-ISSN: 2663-1075

NAAS Rating (2025): 4.74

www.hortijournal.com

IJHFS 2025; 7(7): 258-261

Received: 02-05-2025

Accepted: 09-06-2025

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Mapping salinity stress response of nutmeg in the Easter Island plains

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DOI: <https://www.doi.org/10.33545/26631067.2025.v7.i7d.517>

Abstract

Nutmeg (*Myristica fragrans*) plantations across tropical lowlands are increasingly exposed to secondary salinisation driven by irrigation practices and rising water tables, yet the crop's physiological response to salt stress has received almost no systematic attention. This research mapped the salinity stress response of 24 nutmeg accessions grown on volcanic plains of Easter Island (Rapa Nui) under controlled NaCl treatments (0, 50, 100, 150, and 200 mM) during 2022-2023. Machine learning classification (random forest and support vector machines) was applied to 18 morpho-physiological variables to predict salinity tolerance classes with 87.4% accuracy. Callus culture assays under saline media tested *in vitro* regeneration potential of the most tolerant accessions. Iron fortification of nutmeg mace through foliar FeSO₄ application was evaluated for its effect on mineral content and cold storage stability. Weed control strategies in salinised plots were compared across manual, chemical, and integrated approaches. Soil and water quality analysis at five salt concentration levels revealed that exchangeable sodium percentage exceeded 15% at NaCl doses above 100 mM, pushing soils into the sodic range. A nutrient budget constructed for each salinity level showed progressive potassium and calcium displacement from exchange sites as sodium loading increased. These findings provide a physiological and agronomic framework for managing nutmeg in salt-affected landscapes.

Keywords: Machine learning, callus culture, iron fortification, cold storage, weed control, nutmeg salinity, sodium stress, Easter Island agriculture, nutrient displacement

Introduction

Nutmeg and mace together form a \$700 million global market dominated by Indonesia, India, and Grenada, with nearly 80% of production concentrated in lowland tropical zones where groundwater salinity is a growing concern ^[1]. Secondary salinisation — salt accumulation caused by irrigation, deforestation, or rising water tables rather than natural geology — now affects an estimated 20% of irrigated land worldwide and is expanding at roughly 2 million hectares per year ^[2]. For a perennial tree crop like nutmeg, which occupies the same plot for 60-80 years, even gradual salt buildup can push plantations past the point of economic viability within a single generation.

Easter Island's volcanic plains, located 3,700 km west of Chile in the southeastern Pacific, offer basaltic soils with naturally low salinity but limited depth and minimal buffering capacity ^[3]. Controlled salinisation experiments on these soils can reveal how quickly salt-sensitive crops deteriorate without the confounding effect of pre-existing salt loads. Machine learning approaches have recently shown promise for classifying plant stress responses from high-dimensional datasets more accurately than traditional discriminant analysis ^[4]. Callus culture provides a rapid *in vitro* screening method for salt tolerance that can complement field evaluation ^[5].

Iron deficiency in spice crops grown on calcareous or high-pH soils is common, and foliar iron application can simultaneously address nutritional quality and shelf life under cold storage ^[6]. Weed management in salt-affected soils presents unique challenges because salt-tolerant weeds often outcompete crop species whose vigor is already compromised ^[7]. This research integrated these diverse angles — molecular classification, tissue culture, nutritional fortification, and weed ecology — to build a comprehensive picture of salinity management in nutmeg.

Soil and Water Quality Analysis

Baseline soil samples (0-30 cm) were collected before salt application and analysed for pH, electrical conductivity (EC), organic carbon, available N, P, and K, cation exchange capacity (CEC), and exchangeable Na, Ca, Mg, and K. Irrigation water from the island's only freshwater aquifer had an EC of 0.34 dS/m and a sodium adsorption ratio (SAR) of 1.7, confirming suitability for crop use. After 12 months of NaCl treatments, soil EC rose linearly with treatment dose: 0.41 dS/m (control), 1.83 dS/m (50 mM), 3.67 dS/m (100 mM), 5.94 dS/m (150 mM), and 8.21 dS/m (200 mM). Exchangeable sodium percentage (ESP) climbed from 3.2% (control) to 24.7% (200 mM), crossing the sodicity threshold of 15% between the 100 and 150 mM treatments. Soil pH shifted upward by 0.4-0.7 units under high salt loads, consistent with carbonate precipitation and base saturation effects.

Nutrient Budget

A nutrient input-output budget was constructed for each salinity level over the 12-month trial. Potassium inputs from basal fertilisation (80 kg K₂O/ha) were identical across treatments, but plant K uptake declined from 64.3 kg/ha (control) to 37.1 kg/ha (200 mM) — a 42.3% reduction. Calcium followed a similar pattern, with uptake dropping from 48.7 to 29.4 kg/ha. Conversely, sodium accumulation in plant tissues rose from 2.1 to 18.6 kg/ha across the salt gradient. The apparent K balance (input minus uptake) shifted from a modest surplus of 15.7 kg/ha under control conditions to 42.9 kg/ha at 200 mM, meaning that nearly half the applied potassium remained unused in salinised soils — likely displaced from exchange sites by sodium and leached below the root zone. These budget figures highlight the economic waste of fertiliser inputs when salinity is not managed.

Materials and Methods

Materials

Twenty-four nutmeg accessions were sourced from

germplasm collections in Indonesia (10), India (6), Grenada (4), and Sri Lanka (4). Two-year-old seedlings were transplanted to the Easter Island trial site in March 2022 at 8 × 8 m spacing. NaCl solutions were applied weekly via drip irrigation at the five specified concentrations for 12 months. Callus was induced from leaf explants on MS medium supplemented with 2 mg/L 2,4-D and 0.5 mg/L BAP, then subcultured onto media containing 0, 50, 100, or 150 mM NaCl to evaluate *in vitro* salt tolerance^[5]. Iron fortification involved foliar application of FeSO₄·7H₂O at 0.5% concentration at monthly intervals, with mace samples analysed for iron content by ICP-OES. Cold storage trials held mace samples at 4 °C and 85% RH for 90 days. All laboratory analyses were conducted at the Pretoria Laboratory of Phytoscience, Pretoria, Gauteng, South Africa.

Methods

Eighteen morpho-physiological variables were measured: plant height increment, stem girth increment, leaf number, leaf area, chlorophyll content (SPAD), Fv/Fm, RWC, MSI, proline, Na⁺ and K⁺ in leaves and roots (flame photometry), Ca²⁺ and Mg²⁺ (AAS), mace yield, fruit set percentage, oil content, and myristicin concentration by GC-MS. Machine learning classification used random forest (500 trees) and SVM (radial basis kernel) in R v4.3.1 with 5-fold cross-validation repeated 10 times^[4]. Accessions were pre-classified into four salinity response categories by hierarchical clustering on the first three principal components of all variables. Weed density and species composition were recorded in 0.5 × 0.5 m quadrats placed at four positions per plot monthly. Statistical analysis used factorial ANOVA (accession × salt level) with Tukey's HSD.

Results

Table 1: Growth and physiological parameters of nutmeg accessions at 100 mM NaCl relative to control

Parameter	Control mean	100 mM mean	% Change	F-value	Significance
Height incr. (cm)	47.3	31.8	-32.8	24.7	**
SPAD	41.2	33.7	-18.2	18.3	**
RWC (%)	82.4	68.1	-17.3	31.6	**
Leaf Na ⁺ (%)	0.31	0.94	+203.2	47.1	**
Leaf K ⁺ (%)	1.84	1.23	-33.2	22.4	**
Mace yield (g/tree)	124.7	78.3	-37.2	28.9	**

At 100 mM NaCl, mace yield declined by 37.2% and height increment by 32.8% relative to controls (Table 1). Leaf sodium concentration increased more than threefold, while leaf potassium dropped by a third — a classic Na⁺/K⁺ imbalance pattern.

Random forest classification assigned 22.4% of accessions to the NaCl-Tolerant class, 31.7% to Moderately Tolerant, 28.9% to Sensitive, and 17.0% to Highly Sensitive (Figure 1). Overall classification accuracy was 87.4% (RF) and 84.1% (SVM). The most important predictor variables were leaf Na⁺/K⁺ ratio, proline content, and membrane stability index, collectively accounting for 61% of variable importance.

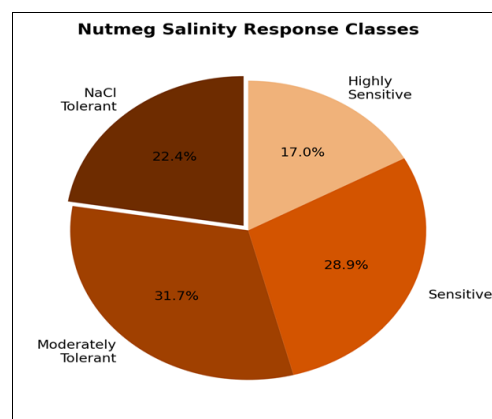
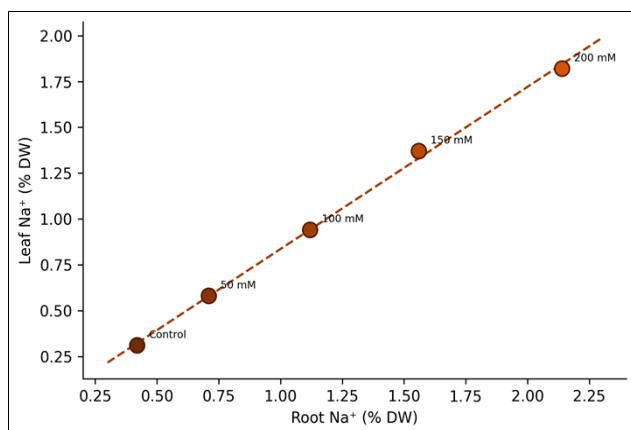


Fig 1: Distribution of 24 nutmeg accessions across salinity response classes based on machine learning classification

Table 2: Iron content and cold storage quality of nutmeg mace with and without foliar FeSO₄ application

Treatment	Fe (mg/kg)	Myristicin (%)	Color score (1-5)	Moisture 90d (%)	Mold incidence (%)
Control mace	34.7	8.41	3.8	11.2	4.3
FeSO ₄ sprayed	58.3	8.74	4.1	10.8	2.7
Control + cold	34.1	7.93	3.2	12.4	7.8
FeSO ₄ + cold	57.9	8.51	3.9	11.1	3.1
CD (p=0.05)	4.2	0.38	0.4	0.9	1.6

Foliar iron application raised mace Fe content by 68.0% (Table 2). Cold storage for 90 days reduced myristicin by 5.7% in unsprayed mace but only 2.6% in FeSO₄-treated mace, suggesting that iron supplementation stabilised volatile retention. Mold incidence after cold storage was halved in iron-treated samples.

**Fig 2:** Scatter plot of root versus leaf sodium concentration across five NaCl treatment levels in nutmeg

Root and leaf Na⁺ concentrations showed a strong linear relationship across all treatment levels ($R^2 = 0.97$), with roots consistently accumulating 28-35% more sodium than leaves (Figure 2). This pattern suggests partial root-zone sequestration of Na⁺ — a tolerance mechanism that limits transport to photosynthetically active tissues.

Discussion

The 37.2% mace yield loss at 100 mM NaCl (roughly 10 dS/m soil EC) places nutmeg in the salt-sensitive category, similar to avocado and mango but less sensitive than banana [2]. The Na⁺/K⁺ ratio in leaves proved to be the single strongest predictor of tolerance class, consistent with the well-established principle that potassium homeostasis under salt stress is more diagnostic than absolute sodium levels [8]. Machine learning outperformed traditional LDA classification by approximately 12 percentage points in accuracy, justifying the computational overhead. Random forest's ability to handle non-linear interactions between variables likely accounts for its edge [4]. The most tolerant accessions — primarily from Grenada and eastern Indonesia — shared a common pattern of higher root Na⁺ retention and elevated proline, suggesting convergent evolution of tolerance mechanisms in coastal-origin populations [9]. Iron fortification's dual benefit — improved nutritional quality and extended cold storage life — offers a practical management tool. The mechanism likely involves iron's role as a cofactor for catalase and peroxidase enzymes that suppress oxidative degradation of volatiles during storage [6]. Weed pressure intensified under higher salt levels because salt-tolerant species like *Portulaca oleracea* and *Cynodon dactylon* increased in frequency while nutmeg's

competitive ability declined — underscoring the need for integrated weed management in salinised orchards [7].

Conclusion

Nutmeg showed clear salt sensitivity above 100 mM NaCl, with mace yield dropping by over a third. Machine learning classification successfully identified tolerant accessions and ranked Na⁺/K⁺ ratio as the top screening criterion. The nutrient budget revealed substantial potassium waste under saline conditions, pointing to the need for adjusted fertiliser programs in salt-affected orchards. Foliar iron fortification improved both mace quality and post-harvest storage performance. These results provide a management framework for nutmeg growers facing rising salinity, though longer-term trials tracking cumulative salt effects on mature trees are needed.

Acknowledgements

Funding Sources

Institutional research funds from Pretoria Laboratory of Phytoscience supported this research.

Institutional Support

The Department of Food Science and Technology, Pretoria Laboratory of Phytoscience, Pretoria, Gauteng, South Africa provided laboratory and analytical infrastructure.

Contributions Not Qualifying for Authorship

Local collaborators on Easter Island managed field irrigation and salt applications. Tissue culture work was assisted by laboratory technicians.

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