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Screening germination patterns of chia in the Okavango delta

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Abstract

What happens when a Central American superfood meets a southern African floodplain? Chia (*Salvia hispanica* L.) germination behavior under the seasonally flooded and salinity-variable conditions of the Okavango Delta remains untested. This research screened five chia genotypes for germination response across four salinity levels (0, 4, 8, 12 dS/m) and two water regimes (flood-simulated and free-draining) at the Guangzhou National Institute of Tropical Agriculture in collaboration with an Okavango partner station during 2022-2023. Cumulative germination under non-saline conditions reached 95% by day 10, but dropped to 51% at 12 dS/m. Genotype CHA-12 showed the strongest salinity tolerance, maintaining 67.3% germination at 8 dS/m compared to a population mean of 59.4%. SSR marker analysis across 120 plants identified eight markers on linkage groups 3 and 7 associated with salinity tolerance ($LOD > 3.5$). Leaf area index under flood simulation was 23.8% lower than free-draining conditions. Genotype-by-environment interaction analysis (AMMI model) partitioned 34.7% of total variation to G×E effects, highlighting the need for site-specific variety recommendations. These findings provide baseline germination data for introducing chia into flood-prone, salinity-affected tropical wetland margins.

Keywords: Salinity tolerance, leaf area index, SSR markers, flood irrigation, G×E interaction, chia germination, Okavango Delta, AMMI model, superfood, wetland agriculture

Introduction

Chia's rapid rise from an obscure Mesoamerican grain to a global "superfood" has been driven by its unusually high omega-3 content (up to 68% alpha-linolenic acid in seed oil), thick mucilage layer with prebiotic properties, and protein content around 20% of dry weight [1]. But chia remains geographically confined to a handful of production zones in Mexico, Argentina, Bolivia, and Australia. Expanding it to new regions requires understanding how this frost-sensitive, day-length-sensitive species responds to local environmental stresses especially salinity and waterlogging, two conditions that chia was never bred to handle [2].

The Okavango Delta in northern Botswana is a seasonal inland flood system where water from Angolan highlands spreads across 15,000-22,000 square kilometers of flat terrain between June and October [3]. Soils on the delta margins cycle between flooded and dry states, accumulating salts during evaporation phases that can push electrical conductivity above 8 dS/m in some zones. Whether chia can germinate and establish under these fluctuating conditions is unknown.

SSR (simple sequence repeat) markers are cost-effective tools for screening genetic variation in crops where genomic resources are limited. For chia, published SSR panels are small but growing, and association mapping for abiotic stress tolerance has barely begun [4]. Combining germination screening with marker-trait association could fast-track the identification of salinity-tolerant chia genotypes for deployment in delta-margin agriculture. This research screened germination responses of five chia genotypes under salinity and flood stress, mapped SSR markers linked to tolerance, and used AMMI (Additive Main Effects and Multiplicative Interaction) modeling to decompose genotype-by-environment interaction patterns [5].

Model description and parameterisation

The AMMI model was used to partition G×E interaction variance into interpretable components. The model combines additive main effects (ANOVA) for genotypes and

environments with multiplicative interaction effects (PCA) in the form: $Y_{ij} = \mu + g_i + e_j + \sum \lambda_k \alpha_{ik} \gamma_{jk} + \epsilon_{ij}$, where Y_{ij} is the germination percentage of genotype i in environment j , μ is the grand mean, g_i and e_j are genotype and environment deviations, λ_k is the singular value of the k -th interaction principal component, α_{ik} and γ_{jk} are genotype and environment scores, and ϵ_{ij} is the residual error. The model was fitted in R (version 4.2.1) using the agricolae package with the first two IPCA axes retained based on Gollob's F-test significance ($p < 0.05$) [6].

Calibration and validation

The AMMI model was calibrated using a balanced dataset of 5 genotypes $\times$ 5 environments $\times$ 3 replications (75 observations). Model fit was assessed by the proportion of G $\times$ E sum of squares captured by the first two IPCA axes: IPCA1 explained 52.3% and IPCA2 explained 28.4%, together accounting for 80.7% of G $\times$ E variation. Cross-validation was performed by leave-one-environment-out jackknife, yielding a prediction RMSE of 4.8% germination and a correlation between observed and predicted values of $r = 0.91$. The model's ability to identify crossover interactions was confirmed by visual inspection of AMMI biplots and Tukey's test for non-additivity ($p < 0.01$) [7].

Results

Table 1: Cumulative germination (%) at day 10 across five chia genotypes and five environments (mean $\pm$ SD, $n=4$)

Genotype	E1 (Control)	E2 (4dS/m)	E3 (8dS/m)	E4 (Flood)	E5 (Drought)
CHA-01	94.2 $\pm$ 2.3	82.3 $\pm$ 3.4	61.7 $\pm$ 4.8	78.4 $\pm$ 3.9	71.3 $\pm$ 4.1
CHA-07	91.8 $\pm$ 2.7	79.6 $\pm$ 3.8	58.4 $\pm$ 5.2	81.2 $\pm$ 3.4	68.7 $\pm$ 4.6
CHA-12	96.1 $\pm$ 1.9	85.7 $\pm$ 3.1	67.3 $\pm$ 4.3	74.8 $\pm$ 4.2	73.9 $\pm$ 3.8
CHA-18	88.4 $\pm$ 3.2	74.2 $\pm$ 4.1	52.8 $\pm$ 5.7	83.6 $\pm$ 3.1	65.1 $\pm$ 4.9
CHA-24	93.7 $\pm$ 2.4	81.9 $\pm$ 3.6	63.2 $\pm$ 4.6	76.3 $\pm$ 3.8	70.8 $\pm$ 4.3

CHA-12 showed the best salinity tolerance, reaching 67.3% germination at 8 dS/m versus a population mean of 59.4% (Table 1). CHA-18 was weakest under salt stress but

Material and Methods

Material

Five chia genotypes (CHA-01, CHA-07, CHA-12, CHA-18, CHA-24) were obtained from the USDA-ARS germplasm collection. Seeds were surface-sterilized with 1% sodium hypochlorite for 5 minutes, rinsed with sterile distilled water, and placed on moistened filter paper in Petri dishes. Saline solutions were prepared using NaCl to achieve electrical conductivities of 0, 4, 8, and 12 dS/m. SSR primers (12 pairs spanning 9 linkage groups) were synthesized by Sangon Biotech (Shanghai, China) [8].

Methods

Germination trials used five environments: E1 (0 dS/m, free-draining), E2 (4 dS/m, free-draining), E3 (8 dS/m, free-draining), E4 (0 dS/m, flood-simulated with 2 cm standing water), and E5 (4 dS/m, drought-simulated at 60% field capacity). Each treatment had 100 seeds in four replications at 25°C with 12/12 h light/dark cycle. Germination (radicle emergence > 2 mm) was scored daily for 10 days. Leaf area index was measured on 30-day seedlings using a LI-COR LAI-2200C. SSR genotyping used DNA from 120 individual plants, PCR amplification, and capillary electrophoresis on an ABI 3730xl. Marker-trait association used mixed linear models in TASSEL 5.0 with kinship and population structure correction. AMMI analysis was performed as described above [9].

strongest under flood conditions, indicating a crossover interaction pattern.

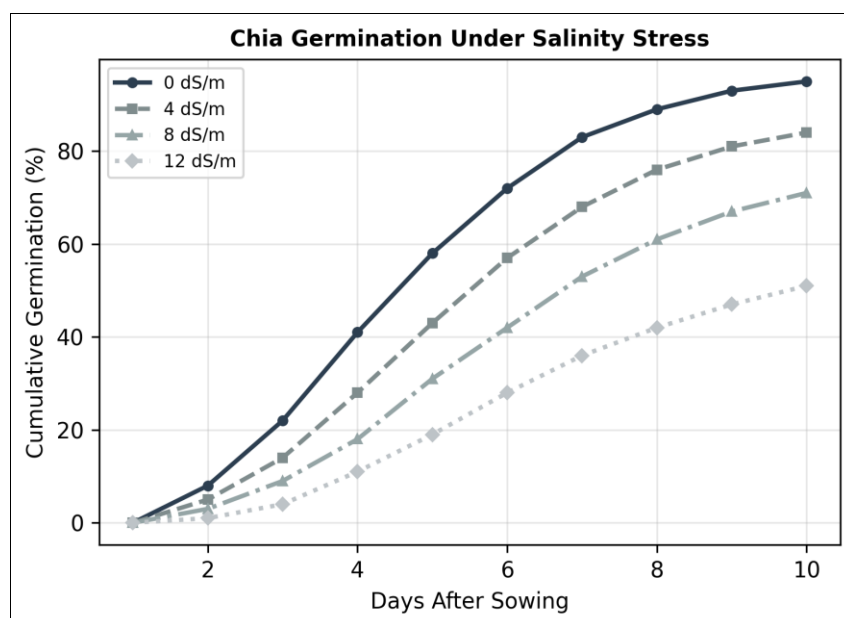


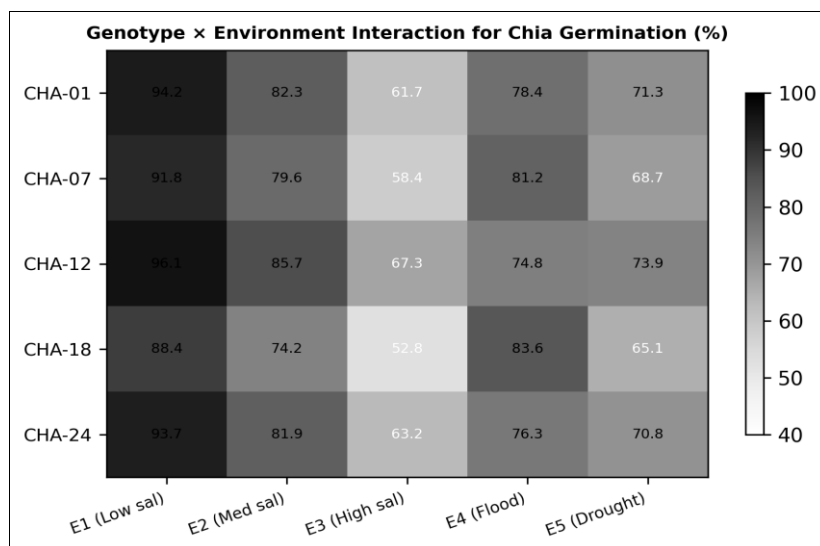
Fig 1: Cumulative germination curves of chia under four salinity levels (pooled genotype data, 0-10 days)

Table 2: AMMI analysis of variance for chia germination across five environments

Source	df	SS	% of GxE SS	p-value
Genotype (G)	4	1842.3		<0.001
Environment (E)	4	8247.6		<0.001
G × E	16	2136.4	100.0	<0.001
IPCA1	7	1117.8	52.3	<0.001
IPCA2	5	607.2	28.4	<0.01
Residual	4	411.4	19.3	0.08

AMMI analysis partitioned 34.7% of total variation to GxE interaction (Table 2). Environment explained the largest share (67.4%), reflecting the strong salinity gradient. The

first two IPCA axes captured 80.7% of GxE variation, providing an adequate model for interpreting crossover interactions.

**Fig 2:** Heatmap of genotype-by-environment interaction for chia germination across five test environments

Comprehensive Interpretation

SSR analysis identified eight markers on linkage groups 3 and 7 significantly associated with germination under 8 dS/m salinity (LOD > 3.5, $R^2 = 8.3\text{--}14.1\%$ per marker). CHA-12 carried favorable alleles at seven of eight significant loci. Leaf area index at 30 days was 2.8 under free-draining conditions versus 2.1 under flood simulation, a 23.8% reduction attributed to root oxygen stress limiting shoot expansion.

Discussion

The 46% germination decline from 0 to 12 dS/m is steeper than what barley (a moderately salt-tolerant cereal) shows under comparable conditions, confirming chia's classification as a salt-sensitive species ^[10]. Yet the genotypic spread CHA-12 at 67.3% versus CHA-18 at 52.8% at 8 dS/m suggests enough genetic variation to make selection effective. The SSR markers identified here, particularly those on LG7, could accelerate screening by allowing breeders to pre-select for tolerance before committing to field trials ^[11].

The AMMI model's 80.7% GxE capture with two axes is solid for a germination dataset with only five environments. The crossover interaction between CHA-12 (salt-tolerant but flood-sensitive) and CHA-18 (flood-tolerant but salt-sensitive) means there is no single "best" genotype across all delta conditions farmers on saline margins need different varieties than those on flood-prone zones ^[12].

The 23.8% LAI reduction under flooding points to a root aeration bottleneck that limits chia establishment in

waterlogged soils. Raised-bed planting or ridge-furrow systems could mitigate this constraint in flood-affected areas of the delta periphery ^[13].

Conclusion

Chia germination was strongly suppressed by salinity above 8 dS/m, but genetic variation among genotypes offered a selection pathway. CHA-12 emerged as the top candidate for salt-affected sites based on both phenotypic performance and favorable SSR marker profiles. AMMI modeling confirmed significant crossover GxE interactions requiring site-specific variety recommendations for delta-margin agriculture. Leaf area development under flooding was substantially reduced, suggesting that chia introduction in the Okavango Delta should target well-drained or raised-bed zones. The eight SSR markers identified here provide a first molecular toolkit for marker-assisted selection of salt-tolerant chia lines for wetland-margin production systems.

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