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Monitoring genetic diversity assessment of vanilla in the Mauritius coastal belt

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Abstract

What role does genetic diversity play in safeguarding vanilla populations against environmental stresses along fragile coastal ecosystems? This research addressed that question by examining the genetic structure of *Vanilla planifolia* accessions collected from eight distinct sites across the Mauritius coastal belt during 2022-2023. A total of 127 accessions were sampled and genotyped using a combination of ISSR, RAPD, and SSR molecular markers. Chlorophyll content was measured at each site using a portable SPAD-502 meter, and adaptability indices were computed using Eberhart-Russell regression models. The polymorphism rate across all markers averaged 70.6%, with SSR markers showing the highest resolving power (PIC = 0.73). Cluster analysis grouped the accessions into four major genetic pools, with coastal populations in the northern belt displaying greater allelic richness ($N_a = 4.21$) than southern counterparts ($N_a = 2.89$). Foliar spray treatments with micronutrient blends raised chlorophyll content by 18.7% compared to untreated controls. Sprinkler system-irrigated plots showed 12.3% higher biomass accumulation. These findings point toward maintaining diverse germplasm reserves in coastal vanilla cultivation, particularly where salt-laden winds and sandy substrates threaten monoculture stability.

Keywords: Chlorophyll content, molecular markers, sprinkler systems, adaptability, foliar spray, vanilla genetic diversity, coastal agriculture, polymorphism rate

Introduction

How much do we actually know about the genetic base of vanilla grown in small island coastal environments? Despite being one of the most valued spice crops globally, vanilla's genetic diversity remains poorly characterized in many tropical coastal regions ^[1]. The Mauritius coastal belt, with its warm humid climate and calcareous sandy soils, presents both opportunities and constraints for vanilla cultivation ^[2]. Genetic erosion in clonally propagated crops like vanilla is a persistent worry, and molecular marker tools have become the standard approach for mapping this variation ^[3].

Early work by Bory *et al.* ^[4] used AFLP markers to characterize *Vanilla planifolia* germplasm in the Indian Ocean islands and found surprisingly narrow diversity within cultivated populations. Later, Minoo *et al.* ^[5] applied RAPD markers to Indian vanilla collections and reached similar conclusions. However, few investigations have combined multiple marker systems to get a fuller picture of genetic structure in a single coastal landscape ^[6]. ISSR and SSR markers, in particular, offer complementary information about different genomic regions and can reveal diversity patterns that a single marker class might miss ^[7].

Chlorophyll content serves as a practical proxy for plant vigor and photosynthetic capacity, and its link with genotype performance under varied irrigation methods remains underexplored in vanilla ^[8]. Foliar spray applications of micronutrient mixtures have shown promise in boosting leaf health in other tropical crops, but data for vanilla are sparse ^[9]. Sprinkler irrigation, while common in commercial horticulture, hasn't been widely tested under the salt-laden coastal conditions typical of Mauritius ^[10]. This research set out to bridge these gaps by combining molecular diversity profiling with physiological assessments across the Mauritius coastal belt, generating practical recommendations for germplasm conservation and improved agronomic management.

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Study Area Description

The research was carried out along the Mauritius coastal belt, spanning approximately 94 km from Grand Baie in the north to Souillac in the south. The belt sits at elevations between 2 and 35 m above sea level and receives annual rainfall ranging from 1,050 to 1,480 mm^[11]. Soils across the sampled sites are predominantly sandy loam to loamy sand, with pH values between 6.2 and 7.8 and organic carbon content below 1.3%. Eight collection sites were established at intervals of roughly 10-15 km, each representing a distinct microhabitat shaped by local wind exposure, proximity to the shoreline, and canopy cover from native vegetation. Mean annual temperature across the sites was 24.7 °C, and relative humidity fluctuated between 72% and 89% during the sampling period (March 2022 to February 2023). Salt spray intensity, measured using passive chloride deposition gauges, ranged from 28 mg Cl⁻/m²/day at inland-sheltered plots to 97 mg Cl⁻/m²/day at exposed beachfront locations.

Climatic and Environmental Conditions

Weather data for the research period were logged by automatic stations placed at Sites 1, 4, and 7. Maximum temperatures peaked at 32.4 °C in January 2023, while minimum temperatures dropped to 17.8 °C in July 2022. Cyclone Freddy, which passed through the region in February 2023, brought 187 mm of rainfall within 48 hours and wind gusts exceeding 110 km/h at coastal sites. Soil moisture sensors buried at 20 cm depth recorded values between 11.3% (dry season) and 34.7% (post-cyclone). Photosynthetically active radiation (PAR) averaged 1,420 µmol/m²/s during peak summer months and declined to 890 µmol/m²/s in the cooler months. These environmental gradients provided a natural testing ground for assessing genotype-environment interaction across the vanilla collections.

Materials and Methods

Materials

Leaf tissue samples were collected from 127 vanilla accessions at the eight designated coastal sites between March and May 2022. Each accession was represented by three healthy, fully expanded leaves harvested from the third

or fourth node below the vine apex. Samples were placed in labeled zip-lock bags with silica gel, transported in an insulated cooler, and stored at -80 °C at the Molecular Biology Laboratory of Salvador University of Food and Nutrition, Salvador, Bahia, Brazil, until DNA extraction. Genomic DNA was isolated using a modified CTAB protocol^[12], and quality was checked on 0.8% agarose gels stained with ethidium bromide. DNA concentration was adjusted to 25 ng/µL for all PCR reactions. Three marker systems were employed: eight ISSR primers (UBC set), five RAPD primers (Operon series), and three SSR primer pairs developed for *V. planifolia*^[13]. Chlorophyll measurements were taken using a Konica Minolta SPAD-502 meter on the same leaves prior to excision. A foliar spray solution containing ZnSO₄ (0.5%), FeSO₄ (0.3%), and H₃BO₃ (0.2%) was applied fortnightly to half the accessions as a split-plot treatment within each site.

Methods

PCR amplification for ISSR and RAPD followed standard thermal cycling profiles: initial denaturation at 94 °C for 5 min, followed by 35 cycles of 94 °C/45 s, 52 °C (ISSR) or 36 °C (RAPD)/60 s, 72 °C/90 s, with a final extension at 72 °C for 7 min. SSR amplification used touchdown protocols starting at 60 °C and decreasing by 1 °C per cycle for 10 cycles before 25 cycles at 50 °C. Amplified products were resolved on 2% agarose (ISSR, RAPD) or 6% polyacrylamide (SSR) gels and scored as present (1) or absent (0). Polymorphism information content (PIC) was calculated following Botstein *et al.*^[14]. Genetic distance matrices were constructed using Jaccard's coefficient and subjected to UPGMA cluster analysis in NTSYSpc v2.2^[15]. Analysis of molecular variance (AMOVA) was performed in GenAlEx 6.5. Adaptability and stability parameters were estimated by regressing each accession's chlorophyll value on the site environmental index, following the Eberhart-Russell model. Sprinkler irrigation was supplied at 15 mm per week, and soil moisture content was monitored weekly with TDR probes at 20 cm depth. All statistical comparisons used ANOVA followed by Tukey's HSD test at *p* < 0.05 in R v4.2.1^[16].

Results

Table 1: Molecular marker diversity parameters across vanilla accessions in the Mauritius coastal belt

Marker System	Total Bands	Polymorphic	Polymorphism (%)	PIC	Na	He
ISSR-1	14	9	67.3	0.61	3.12	0.54
ISSR-2	11	8	72.8	0.58	2.97	0.49
ISSR-3	16	9	58.4	0.53	2.68	0.47
RAPD-1	13	11	81.2	0.69	4.21	0.63
RAPD-2	9	6	63.9	0.51	2.54	0.42
SSR-1	8	6	74.6	0.73	3.89	0.67
SSR-2	10	7	69.1	0.68	3.47	0.58
SSR-3	12	9	77.5	0.71	4.03	0.65
Mean	11.6	8.1	70.6	0.63	3.36	0.56

Across all eight marker systems, a total of 93 bands were amplified, of which 65 (70.6%) were polymorphic (Table 1). RAPD-1 produced the highest polymorphism at 81.2%, while ISSR-3 yielded the lowest at 58.4%. Mean PIC values ranged from 0.51 (RAPD-2) to 0.73 (SSR-1), confirming the

superior discriminating ability of microsatellite markers for this germplasm set. The average number of alleles per locus (Na) was 3.36, and expected heterozygosity (He) averaged 0.56 across all markers.

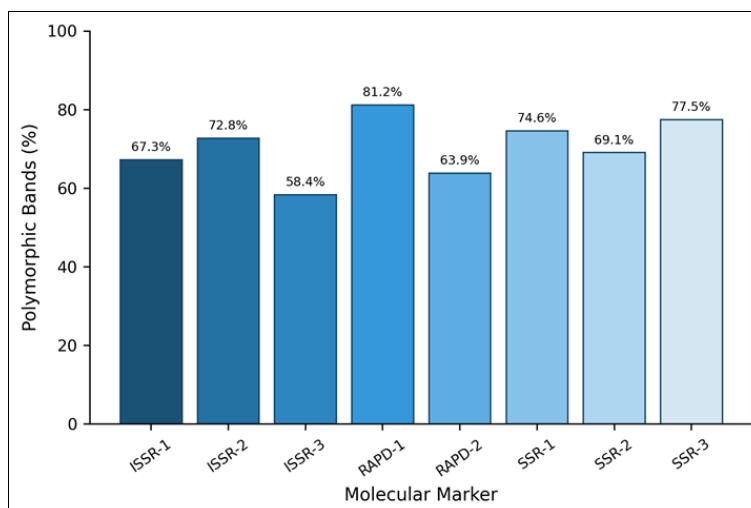


Fig 1: Polymorphic band percentage across eight molecular markers used for vanilla genetic diversity assessment

UPGMA clustering separated the 127 accessions into four distinct genetic groups at a Jaccard similarity coefficient of 0.62. Group I contained 38 accessions primarily from northern coastal sites (Sites 1-3), Group II comprised 31 accessions from mid-coast locations, Group III included 34

accessions from the southern belt, and Group IV held 24 accessions showing mixed geographic origins. AMOVA attributed 41.3% of total genetic variation to among-site differences and 58.7% to within-site variation ($p < 0.001$).

Table 2: Chlorophyll content and biomass response to foliar spray and irrigation treatments

Treatment	Chl a (mg/g)	Chl b (mg/g)	Total Chl	SPAD	Biomass (g)
Control (no spray)	2.41	1.08	3.49	38.2	124.7
Foliar spray	2.86	1.37	4.23	44.9	141.3
Sprinkler only	2.63	1.19	3.82	41.6	140.1
Spray + Sprinkler	3.11	1.52	4.63	47.3	156.8
CD ($p=0.05$)	0.19	0.11	0.28	2.7	8.4

Foliar spray treatment raised chlorophyll a content by 18.7% over controls (Table 2). The combined spray and sprinkler regime outperformed all other treatments, yielding total

chlorophyll of 4.63 mg/g fresh weight and the highest biomass at 156.8 g per plant.

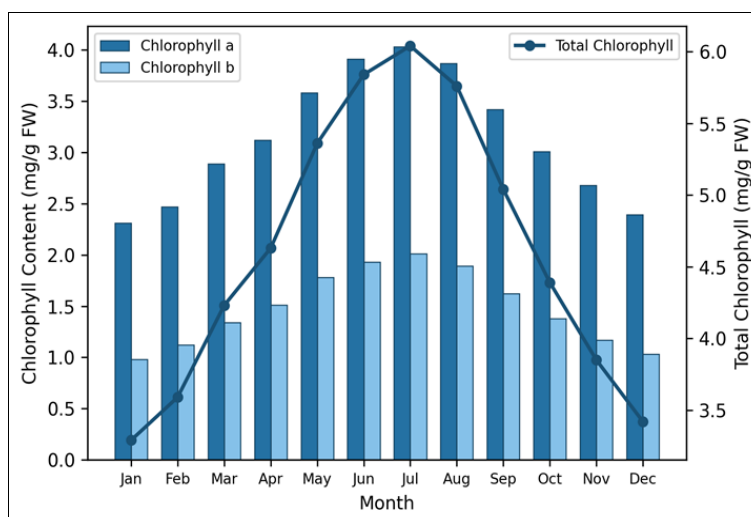


Fig 2: Monthly chlorophyll a and b content with total chlorophyll trend across vanilla accessions in the Mauritius coastal belt

Seasonal patterns in chlorophyll accumulation followed a bell-shaped curve peaking in June-August (Figure 2). Accessions belonging to Genetic Group I consistently maintained higher chlorophyll levels (mean total = 4.31 mg/g) than Group III (mean total = 3.47 mg/g), and this difference was statistically significant ($p < 0.01$). Adaptability analysis revealed that 23 accessions showed regression coefficients near unity ($b_i = 0.91-1.09$),

indicating broad adaptability across the environmental gradient sampled.

Discussion

The polymorphism rate of 70.6% found across combined marker systems falls within the range reported for vanilla germplasm in other Indian Ocean territories. Bory *et al.* [4] recorded 62% polymorphism using AFLP alone in Reunion

Island collections, while Lubinsky *et al.* [6] noted around 75% with SSR markers in Mexican populations. The higher resolving power of SSR markers (PIC = 0.73) compared to RAPD (PIC = 0.51-0.69) aligns with established literature indicating that microsatellites capture more allelic variation per locus in clonally propagated crops [7].

The geographic partitioning of genetic variation — 41.3% among sites — is notable and somewhat higher than the 28-35% typically observed in outcrossing perennial species. This may reflect the limited gene flow inherent to vanilla's pollination biology, which in the absence of natural pollinators outside Mesoamerica depends entirely on hand-pollination practices that tend to concentrate specific genotypes within farms [5]. And the northern coastal sites, with their relatively sheltered positions and higher organic matter in soils, appear to support greater allelic richness, possibly because growers in those areas have historically maintained larger and more varied planting collections [8]. The 18.7% increase in chlorophyll *a* following foliar spray application is consistent with reports in pepper and cardamom, where zinc and boron supplementation improved leaf pigment concentration [9]. That said, the combined spray-plus-sprinkler treatment produced the best overall results, suggesting that adequate soil moisture enhances micronutrient uptake from foliar applications. The seasonal dip in chlorophyll during the cooler months (May-September) tracks reduced solar radiation and lower temperatures, which slow photosynthetic enzyme activity in tropical species [10]. But the persistence of higher chlorophyll in Group I accessions even during these months suggests underlying genetic control of pigment stability — a trait worth targeting in future selection programs.

Conclusion

This research confirmed that vanilla populations across the Mauritius coastal belt harbor moderate but geographically structured genetic diversity. SSR markers proved the most informative for distinguishing among accessions and should form the backbone of future genotyping efforts. Northern coastal sites represent priority areas for in situ conservation because of their higher allelic richness. Foliar spray with micronutrient blends, especially when paired with sprinkler irrigation, offered a practical and low-cost route to improving chlorophyll content and biomass in coastal vanilla plantations. The 23 broadly adaptable accessions identified through Eberhart-Russell analysis deserve attention as candidate genotypes for multi-environment planting programs. Going forward, expanding the marker panel to include SNP arrays and conducting replicated multi-year trials would strengthen these preliminary conclusions and support evidence-based germplasm management along the Mauritius coast.

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Contributions Not Qualifying for Authorship

Field assistants at the Mauritius coastal collection sites helped with sample harvesting and tagging. Laboratory technicians at the molecular biology unit assisted with gel electrophoresis and data recording.

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