

# Multi-Omic Insights into Proline-Mediated Salt Stress Responses in Moth Bean (*Vigna aconitifolia*): Unraveling Metabolomic and Proteomic Dynamics

## Research Article

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### Abstract

The study examines the impact of salinity on two varieties of moth beans (*Vigna aconitifolia*). Jacq, V1 (Matki) and V2 (Gauranmatki) focus on the morphological parameters, proline, and protein content. Salinity stress is increasingly relevant in the context of climate change and its potential impact on agricultural productivity. We investigate how these two varieties respond to different concentrations of sodium chloride (NaCl) and their suitability for future sustainable agriculture. The accumulation of osmolyte compounds is often proposed as a solution to overcome the negative effects of water shortages in crop production, which is proposed as an adaptive mechanism for drought and salt tolerance. This finding is consistent with what metabolomics studies often reveal. The accumulation of proline is considered an osmoprotective strategy used by plants under osmotic stress conditions, including salinity. Changes in morphological parameters, including the length of the shoot and root, the number of leaves, and the fresh/dry weight, in response to varying salt concentrations. Proteomic analyses reveal how the expression of specific proteins changes in response to salt stress, providing insights into the molecular adaptations that enable plants to tolerate salt. Meanwhile, metabolomic data may highlight changes in metabolites linked to proline metabolism, providing insight into the intricate processes and interactions involving proline in salt-stressed plants.

**Keywords:** *Vigna Aconitifolia*; Climate Change; Salinity; Metabolomics; Proteomics

### Introduction

The 21<sup>st</sup> century is marked by challenges such as water scarcity, environmental pollution, and soil salinization, exacerbated by climate change. With a growing global population and the need for a 70% increase in food production by 2050 [1]. Soil salinity has become a major constraint to agricultural productivity. Poor-quality irrigation water is a leading cause of soil salinity, affecting over 20% of cultivated land. Thus, salinity is one of the most brutal environmental stresses that hamper crop productivity worldwide [2]. Soil salinity reduces plant growth and yield, making it crucial to identify salt-tolerant crop varieties for sustainable agriculture.

Soil salinity arises from various factors, with one of the primary culprits being the use of poor-quality irrigation water [3]. This

phenomenon is not limited to arid lands and semi-arid regions; it extends to various corners of the globe. Excessive soil salinity can severely limit the growth and spread of plants in their natural habitats, making it a global environmental concern [4,5].

In this context, the emerging field of omics, including genomics, proteomics, and metabolomics, has revolutionized our ability to dissect the molecular underpinnings of plant responses to salinity stress. Omics approaches allow us to comprehensively study the genes, proteins, and metabolites involved in salt stress responses, providing insights that were previously inaccessible. These omics technologies enable us to explore the intricate networks of molecular interactions that govern a plant's ability to thrive or survive in saline conditions [6].

Moth bean also known as Matki bean is a versatile crop used for food, fodder, and green manure, making it essential for addressing nutritional deficiencies.

This study delves into the salt stress responses of two Matki beans also known as moth bean (*Vigna aconitifolia*) varieties, V1 (Matki) and V2(GauranMatki), with a focus on morphological parameters, proline accumulation, and protein content. Moreover, we aim to relate these responses to broader metabolic and proteomic dynamics, shedding light on the intricate pathways and interactions that underlie salt stress tolerance in plants.

### Materials and Methods

- **Collection of Seed:** Seeds of two different varieties of moth bean i.e *Vigna aconitifolia* locally called as Matki and gauranmatki were obtained from a local shop and experimentally named V1(Matki) And V2(GauranMatki).
- **Morphological Study:** Seeds were subjected to salt stress by irrigating them with different NaCl concentrations (15-75 mM), with a control group receiving distilled water. Morphological parameters were analyzed on the 10<sup>th</sup> and 20th day. Five plantlets were collected from each treatment and measured for root length, shoot length, plant height, leaf length, and the number of leaves.
- **Fresh and dry weight (g):** Fresh weights of plantlets were measured using an electric balance. Dry weights were determined after drying samples at 80°C for 24 hours.

### Biochemical Analysis

- **Protein Estimation:** Protein content in plant material was estimated by the modified Lowry method [7].
- **Proline Estimation:** Proline measurements were performed using the modified protocol of Bates [8].

### Results

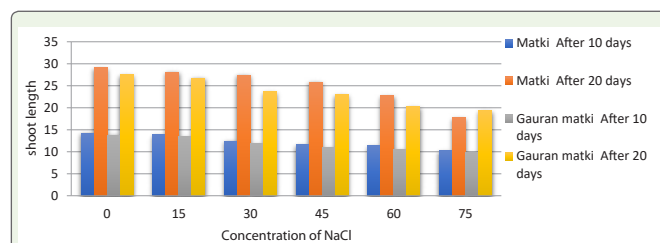
**Shoot and Root Lengths:** Increasing salinity had a significant negative impact on shoot and root lengths, with maximum reductions observed at 75 mM NaCl (Graphs 1,2). Comparing the two varieties, V2(GauranMatki) was more affected by salinity than V1(Matki), ultimately leading to the demise of V2(GauranMatki) plants after 20 days. The reduction in root and shoot development may be due to the toxic effects of higher levels of NaCl concentration and unbalanced nutrient uptake.

**Number of Leaves:** Higher levels of salinity decreased the number of leaves throughout the experiment. This decline may be attributed to sodium chloride accumulation in older leaves (Graph 3).

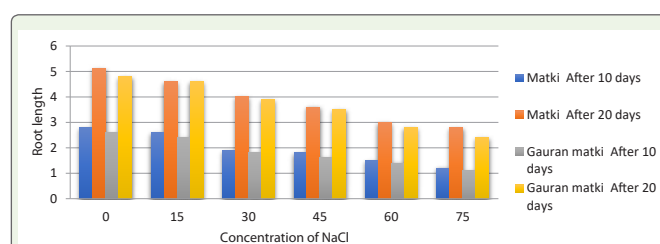
**Fresh and Dry Weight:** Salt stress negatively affected the fresh and dry weights of both *V. aconitifolia* varieties, with greater reductions at higher NaCl concentrations. These differences were more pronounced at higher salinity levels (Table 1).

**Proline Content:** Proline accumulation was more pronounced in V1(Matki) than V2(GauranMatki), with the highest increase at 75 mM NaCl. Proline acts as an osmoprotectant, contributing to osmotic adjustment and protecting cellular structures (Table 2).

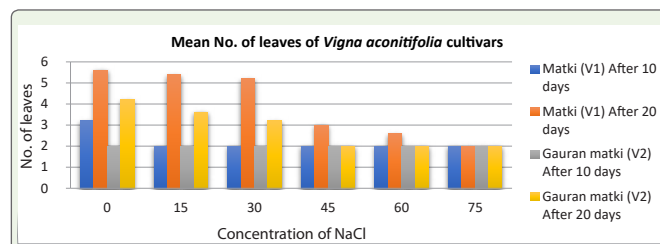
**Protein Content:** Protein content increased with salt concentration in both varieties. The variety V1(Matki) exhibited higher tolerance to salinity stress compared to V2(GauranMatki), as indicated by its greater increase in protein content. (Table 3)



Graph 1: Shoot Length (In Cm) Of *Vigna Aconitifolia* Cultivars.



Graph 2: Root Length (In Cm) Of *Vigna Aconitifolia* Cultivars.



Graph 3: Mean No. Of Leaves of *Vigna Aconitifolia* Cultivars

Table 1: Fresh and Dry Weight of *Vigna Aconitifolia* Cultivars Under Different Salt Concentrations

Salt concentration (mM)	V1(MATKI)		V2 (GAURAN MATKI)	
	Fresh weight (mg)	Dry weight (mg)	Fresh weight (mg)	Dry weight (mg)
00	249.2	170.8	136	77.4
15	208.2	148.8	126.0	67.0
30	128.0	62.8	89.0	22.8
45	104.4	39.2	71.0	21.6
60	88.0	28.8	68.0	20.4
75	82.0	26.0	48.0	19.2

Table 2: Proline Content (Mg/Gm Fresh Weight) Of *Vigna Aconitifolia* Cultivars Under Different Salt Concentrations

Salt concentration (mM)	V1(MATKI)		V2 (GAURAN MATKI)	
	After 10 days	After 20 days	After 10 days	After 20 days
00	0.08	0.42	0.04	0.48
15	0.17	0.69	0.08	0.58
30	0.41	0.72	0.10	0.68
45	0.21	0.79	0.08	0.71
60	0.26	0.82	0.13	0.74
75	0.27	0.90	0.16	0.81

**Table 3:** Protein Content (Mg/Gm Fresh Weight) Of *Vigna Aconitifolia* Cultivars Under Different Salt Concentrations

Salt concentration (mM)	V1(MATKI)		V2(GAURAN MATKI)	
	After 10 days	After 20 days	After 10 days	After 20 days
00	2.56	2.52	1.20	1.56
15	3.40	2.68	1.36	2.24
90	3.52	2.84	3.40	2.40
45	4.20	2.88	3.56	2.52
60	4.44	3.32	3.84	2.84
75	4.76	3.52	3.96	3.00



**Figure 1:** Seeds of Moth Beans (Matki V1, GauranMatki V2)



**Figure 2:** Seedling Growth in Matki (V1)



**Figure 3:** Seedling Growth in GauranMatki V2

### Discussions

In recent years, omics-based approaches have transformed our understanding of how plants respond to environmental stressors, including salinity. These approaches allow us to dissect the intricate molecular mechanisms that govern plant responses at the genomic, proteomic, and metabolomic levels.

### Proline and Metabolomics

Proline, a naturally occurring amino acid, plays a pivotal role in the response of plants to salinity stress. It is a key osmoprotectant that contributes significantly to cytoplasmic osmotic adjustment [9]. Proline also plays multiple roles beyond osmotic regulation, including stabilizing sub-cellular structures, scavenging free radicals, and buffering cellular redox potential under stress. Metabolomic studies can help unravel the intricate pathways and interactions involving proline in salt-stressed plants. Proline accumulation under stress is associated with various metabolic adjustments, including alterations in nitrogen metabolism and changes in the profiles of other osmolytes and antioxidants [10]. A metabolomics approach could elucidate how proline fits into these metabolic networks and its interactions with other metabolites involved in salt stress responses.

### Protein and Proteomics

The proteomic landscape of plants under salt stress is equally complex and informative. Proteomic analyses reveal how the expression of specific proteins changes in response to salt stress, providing insights into the molecular adaptations that enable plants to tolerate salinity.

Proteomics can help identify the key proteins involved in salt stress responses, such as ion transporters, chaperones, and enzymes involved in osmolyte biosynthesis. These proteins are part of a coordinated response that helps maintain cellular ion homeostasis, counteract protein denaturation, and regulate osmotic balance [11].

Integrating omics data can provide a holistic view of salt stress responses. For instance, proteomic analyses may reveal that certain proteins involved in proline biosynthesis are upregulated in response to salt stress, aligning with the observed increase in proline content. Meanwhile, metabolomic data may highlight changes in metabolites linked to proline metabolism, shedding light on the metabolic pathways involved.

### Conclusion

This study underscores the importance of selecting salt-tolerant crop varieties. Out of the differential responses of moth bean varieties, V1 and V2, to salt stress, V1 appears to be more salt-tolerant, making it a potential candidate for future sustainable agriculture under changing climate conditions. Proline and protein content analysis revealed their positive response to salinity, offering potential avenues for enhancing nutritional value in moth bean. Future research should focus on unravelling the molecular mechanisms of salt tolerance, exploring genetic engineering techniques, and developing holistic strategies for mitigating soil salinity's long-term impacts on crop yields and soil health.

we observed a significant increase in proline content in response to salt stress in both moth bean varieties (V1 and V2). This finding is consistent with what metabolomics studies often uncover. Proline accumulation is considered an osmoprotectant strategy employed by plants under osmotic stress conditions, including salinity. This suggests that proline is a key metabolite involved in the plant's response to salt stress.

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