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Effects of KNO_3 on Germination Rate and Seedling Vigor of Macadamia Seeds in Morogoro, Tanzania

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Abstract

This study evaluated the effects of potassium nitrate (KNO_3) priming on the germination and early seedling development of macadamia (Macadamia integrifolia) seeds. Four KNO_3 concentrations—0.19 M (C1), 0.25 M (C2), 0.40 M (C3), and a control (0 M, C4)—were tested in a completely randomized design with three replications. Data collected included germination percentage (GM), number of normal and abnormal seedlings (NS, ABS), seedling vigor index (SVI), and seedling growth parameters such as shoot and root height, growth rates, biomass, and stem/root girth. Statistical analysis was conducted using analysis of variance (ANOVA) in Origin Pro 2024 (Origin Lab Corporation) and R version 4.4.2 (R Core Team, R Foundation for Statistical Computing). Results showed that germination percentage was not significantly affected across treatments ($p = 0.4$); however, KNO_3 concentration significantly influenced the number of normal seedlings ($p = 0.01$), abnormal seedlings ($p = 0.03$), and seedling vigor index ($p = 0.00025$). The highest vigor and lowest abnormality rates were observed in C1 and C4, while the highest abnormality (10%) and lowest vigor occurred in C3, indicating that excessive nitrate concentrations may induce stress. No significant differences ($p > 0.05$) were found in shoot and root elongation, biomass, or tissue girth, suggesting that while KNO_3 influences early metabolic and physiological activation, it does not significantly alter short-term morphological growth parameters. Principal Component Analysis (PCA) revealed that SVI, shoot growth rate (SGR), and root growth rate (RGR) were key contributors to early seedling vigor, with C1 and C4 treatments aligning positively along the principal component associated with vigorous growth. These findings support the use of moderate KNO_3 concentrations to enhance seedling vigor and quality in macadamia, while highlighting the importance of avoiding excessive nitrate levels that may compromise seedling development.

Subject Areas

Plant Science

Keywords

Macadamia integrifolia, Potassium Nitrate, Seed Germination, Seedling Vigor

1. Introduction

Macadamia (Macadamia integrifolia) is a high value nut crop that is increasingly sought after due to its nutritional and economic importance. However, successful propagation of macadamia seeds remains challenging, as low germination rates and poor seedling vigor often constrain large scale cultivation. To enhance seed germination and seedling growth, pre-sowing treatments have been widely investigated. Among these, the application of potassium nitrate (KNO_3) as a seed priming agent has demonstrated potential to improve seed performance by alleviating dormancy and stimulating metabolic processes essential for germination [1] [2]. Potassium nitrate (KNO_3) enhances germination by facilitating water uptake and activating enzymes involved in the early stages of seed metabolism [3]. It has been extensively applied in a range of species to overcome dormancy and promote uniform germination [4]. The influence of KNO_3 on germination is concentration dependent: while suitable levels can accelerate germination and improve seedling vigor, excessive concentrations may induce inhibitory effects [5].

The present study aims to evaluate the effects of KNO_3 pre-sowing treatments on germination and seedling vigor of macadamia seeds. Three concentrations of KNO_3 (0.19 M, 0.25 M, and 40 M) were selected to provide a gradient of nitrate exposure and to assess their differential effects on seed performance. These concentrations were chosen based on the premise that macadamia seeds, characterized by a hard seed coat and inherent dormancy, may require stronger chemical stimuli to facilitate germination. The lower concentrations (0.19 M and 0.25 M) represent moderate treatments that may enhance germination without imposing stress, whereas the higher concentration (40 M) was included to evaluate whether more intensive exposure can effectively overcome both physical and physiological dormancy barriers. Determining the impacts of these KNO_3 treatments on germination dynamics and seedling vigor will provide essential knowledge for optimizing propagation protocols and supporting the development of consistent and sustainable macadamia cultivation systems.

2. Materials and Methods

2.1. Seed Collection and Preparation

Macadamia seeds (*Macadamia integrifolia*) were collected from mature trees in the Mbeya region, Tanzania. A total of 300 seeds were selected based on uniform size, shape, and appearance, with no visible signs of damage or disease. The seeds were extracted from fresh nuts, cleaned, and air dried at room temperature for 24 hours before being subjected to the pre-sowing treatments. Seeds showing signs of damage or defects were discarded to maintain consistency in the experiment.

2.2. Experimental Design and Replications

The experiment followed a completely randomized design (CRD) with a single factor: the concentration of potassium nitrate (KNO_3). Three different concentrations of KNO_3 were used: 0.19 M, 0.25 M, 40 M and control. The seeds were soaked in each KNO_3 solution for 24 hours.

Each treatment combination was replicated three times, with 25 seeds per replication. This resulted in a total of 300 seeds (4 KNO_3 concentrations × 3 replications × 25 seeds per replication). The experimental units (pots with seeds) were randomly assigned within the laboratory to minimize any environmental bias.

2.3. Pre-Sowing Treatment with Potassium Nitrate (KNO_3)

Seeds were subjected to potassium nitrate (KNO_3) pre-sowing treatments. Three different concentrations of KNO_3 were prepared by dissolving KNO_3 in distilled water to achieve final concentrations of 0.19 M, 0.25 M, and 40 M. For each treatment, seeds were soaked in the respective KNO_3 solution for 24 hours. After soaking, seeds were briefly rinsed in distilled water to remove any excess KNO_3 solution and placed on paper towels to dry for 10 minutes before sowing [5].

2.4. Germination Setup

The experiment was conducted in a laboratory located in Morogoro, Tanzania, in February, where controlled conditions of temperature (28˚C ± 2˚C) and humidity (75%) were maintained. Sterilized sand was used in each pot as the substrate for seed sowing. A total of 25 seeds were planted per pot, ensuring adequate spacing between seeds to prevent overcrowding. Pots were randomly assigned within the laboratory environment to avoid bias. Regular watering was done to maintain proper moisture levels, and no additional fertilizers were used during the experimental period. Germination was monitored daily, with the emergence of the radicle being considered as the criterion for germination.

2.5. Germination Rate and Seedling Vigor Assessment

Germination process was observed over a period of 30 days. Germination was recorded daily, and the germination rate (GR) was calculated as the percentage of seeds that successfully germinated relative to the total number of seeds sown for each treatment. The formula for calculating germination rate is as follows:

$$\begin{aligned}&\text{Germination Rate}\\&=\left(\text{Number of germinated seeds/Total number of seeds sown}\right)\times 100\end{aligned}$$

Seedling vigor was quantified as the mean of observations collected on days 3, 30, and 60 post-germination. Seedling height was measured from the base of the seed to the tip of the shoot using a ruler. Root length was defined as the length of the longest root from each seedling. Dry weight was determined by harvesting, cleaning, and drying the seedlings in an oven at 65˚C for 48 hours. The dried seedlings were then weighed to obtain their dry weight.

A vigor index was calculated to assess the overall seedling development, using the formula:

$$\text{Vigor Index} = \text{Seedling Height}(\text{cm}) \times \text{Root Length}(\text{cm})$$

This index provides an estimate of seedling vigor, with higher values indicating better growth and development of the seedlings [3] [5].

Seedlings were classified as abnormal (ABS) if KNO_3 treatment caused damage to the apical meristem, indicated by necrosis or browning of the shoot tip, leading to cessation of apical growth. Abnormal seedlings exhibited compensatory lateral branching from nodes below the damaged apex. Observations were conducted every days for 120 days, recording shoot elongation, number of lateral branches, and leaf morphology to ensure reproducibility [6] [7].

2.6. Data Analysis

The collected data was subjected to analysis of variance (ANOVA) using standard procedures in Origin Pro 2024 (Origin Lab Corporation) and R version 4.4.2 (developed by the R Core Team under the R Foundation for Statistical Computing) [3].

3. Results and Discussion

3.1. Analysis of Variance

The data presented on **Table 1**, demonstrates the influence of varying concentrations of potassium nitrate (KNO_3) on the germination and early seedling development of macadamia seeds. Germination percentage (GM) remained statistically unaffected across treatments, ranging from 85% to 96%. This suggests that while KNO_3 can support germination, macadamia seeds may exhibit a degree of resilience or insensitivity to nitrate induced germination triggers. This aligns with findings in Capsicum annuum and Brassica napus, where KNO_3 priming improved germination only under specific conditions, such as high dormancy levels or stress environments [8] [9]. Additionally, Kaya *et al.*, [10] found that nitrate responsiveness varies widely among species depending on their seed coat permeability and metabolic readiness. Abnormal seedlings (ABS) and the number of normal seedlings (NS) were significantly influenced by KNO_3 concentration. The highest abnormality rate occurred at C3 (10%), whereas C1 and C4 treatments showed minimal abnormalities, suggesting that either too high or unbalanced concentrations of nitrate may induce metabolic stress or disrupt hormonal regulation during seedling differentiation. These findings are supported by Demir and Mavi [11], who observed similar trends in melon and tomato seeds, where excess nitrates led to malformed seedlings due to potential ionic toxicity and osmotic stress. Hacisalihoglu *et al.*, [12] also reported that high nitrate exposure could impair cellular division and elongation during early morphogenesis.

Table 1. Effects of potassium nitrate (KNO_3) on the germinations and growth of macadamia seedlings.

FL	50% GM	GM (%)	ABS	NS	SVI	ASH	ARH	SGR	RGR	LGR	SBI	ARG	ASG
C1	39a	96a	0.33c	22a	255785b	16.2a	6.8a	513a	77a	14a	12a	0.6a	0.4a
C2	41a	85a	7b	14b	219375b	14.8a	7.9a	458a	145a	14a	12a	0.5a	0.3a
C3	39a	86a	10a	11b	139087c	12.4a	6.7a	460a	107a	16a	14a	0.5a	0.4a
C4	43a	88a	0c	22a	308923a	12.8a	8.4a	413a	78a	13a	12a	0.5a	0.4a
Mean	41	89	4.5	17.7	230792.8	14.1	7.5	462	102	14.2	12.4	0.54	0.4
P (value)	0.1ns	0.4ns	0.03*	0.01*	0.00025*	0.3	0.7	0.29	0.21	0.82	0.11	0.49	0.5
CV	0.06	0.08	0.26	0.15	0.11	0.18	0.1	0.1	0.4	0.2	0.1	0.4	0

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C2	41a	85a	7b	14b	219375b	14.8a	7.9a	458a	145a	14a	12a	0.5a	0.3a
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C4	43a	88a	0c	22a	308923a	12.8a	8.4a	413a	78a	13a	12a	0.5a	0.4a
Mean	41	89	4.5	17.7	230792.8	14.1	7.5	462	102	14.2	12.4	0.54	0.4
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CV	0.06	0.08	0.26	0.15	0.11	0.18	0.1	0.1	0.4	0.2	0.1	0.4	0

FL-Factor level, C-KNO_3 Concentration (C1-0.19 M KNO_3, C2-0.25 M KNO_3, C3-0.40 M KNO_3, and C4-0 M KNO_3 (control)),GM-germination percentage, ABS abnormal seedlings, NM-Normal seedlings, SVI-Seedling vigor index, ASH-Average shoot height, ARH-Average root height, SGR-Shoot growth rate, RGR-Root growth rate, LGR-Leaf growth rate, SBI-Seedling biomass, ARG-Average root girth, ASG-Average stem girth, CV-Coefficient of variations.

The seedling vigor index (SVI), which combines germination percentage and seedling length, was significantly affected, with C4 and C1 showing the highest vigor. This suggests that moderate nitrate concentrations enhanced seed metabolic activity, likely via the stimulation of nitrate reductase and related enzymatic systems critical for early energy metabolism [13]. Zheng *et al.*, [14] further noted that nitrate priming in wheat increased the antioxidative capacity and accelerated reserve mobilization, resulting in stronger seedling establishment. Similar benefits have been documented in sorghum and maize, where nitrate priming improved radicle protrusion and energy efficiency under both optimal and sub-optimal conditions [15] [16]. Interestingly, shoot height (ASH), root height (ARH), and growth rates (SGR, RGR, LGR) were not significantly different across treatments, although numerically some variation was present. For example, root growth rate

(RGR) varied considerably, particularly in C2, suggesting individual seed variability and possibly localized microenvironmental effects. This type of variability is common in woody perennials like macadamia, whose seeds are classified as recalcitrant and physiologically heterogeneous [17]. The lack of significant differences in shoot and root elongation may indicate that KNO_3 primarily exerts its influence during the early metabolic activation phase rather than in sustained structural growth during the first few weeks. Seedling biomass (SBI), root girth (ARG), and stem girth (ASG) also did not show significant variation among treatments.

Confirming the value of nitrate priming in promoting early-stage seedling health. This suggests that potassium nitrate treatments may not influence tissue thickening and biomass accumulation in the short term. Similar conclusions were drawn by Sadeghian and Yavari [18] in sugar beet, where nitrate priming improved germination but had minimal impact on early shoot and root mass under non-stress conditions. In the present case, the macadamia seedlings may require longer growth periods before nitrate-induced differences in biomass become evident. In conclusion, moderate concentrations of potassium nitrate (particularly in treatments C1 and C4) positively influenced normal seedling development and vigor while minimizing abnormality rates, confirming the value of nitrate priming in promoting early stage seedling health. However, care must be taken not to exceed optimal concentration thresholds, as this could result in negative outcomes such as increased abnormal seedlings or suppressed growth. These findings are consistent with previous reports on nitrate priming in a range of crops, including rice, wheat, and legumes, where low to moderate concentrations improved early seedling metrics, but excessive levels were either ineffective or detrimental [15] [19] [20]. Further research is needed to investigate the long term growth effects of KNO_3 treatments in macadamia and to optimize protocols for nursery level seedling production under varying environmental conditions.

3.2. Principal Component Analysis of Parameters for Macadamia Seedlings

The Principal Component Analysis (PCA) biplot illustrates the multivariate relationships among various seedling growth parameters and the responses of macadamia seeds to different potassium nitrate (KNO_3) concentrations. The first two principal components (PC1 and PC2) capture most of the variance in the data, offering insights into how the measured traits co-vary and respond to treatments. PC1 primarily reflects a “vigorous growth” dimension, as it shows strong positive loadings for parameters such as Seedling Vigor Index (SVI), Shoot Growth Rate (SGR), and Root Growth Rate (RGR). These traits are essential indicators of early seedling performance and biomass accumulation. Treatments such as C4, which lies closer to the direction of these vectors, demonstrate superior overall vigor and growth potential. This suggests that C4’s potassium nitrate concentration promotes physiological and metabolic activities favorable for early seedling establishment. Similar findings have been reported by Kaya *et al.* [10], where PCA of primed sunflower seeds under stress identified SVI and root growth as major contributors to early vigor, particularly under KNO_3 priming. Likewise, Farooq *et al.* [13] showed that nitrate-primed rice seeds clustered along a principal component linked to early metabolic activation and enhanced growth, further validating our observations with macadamia.

On the other hand, PC2 appears to be more influenced by parameters like Abnormal Seedlings (ABS) and Average Root Height (ARH), suggesting it captures variability related to morphological irregularities or stress responses. Treatment C3, which aligns more closely with this axis, exhibited a higher rate of abnormal seedlings and relatively poor seedling vigor, implying that its nitrate concentration may have exceeded the physiological threshold for optimal development. Such negative outcomes at higher nitrate levels have been documented in other crops as well; for instance, Sivritepe *et al.*, [20] reported that excessive nitrate concentrations in melon led to stress symptoms and inhibited growth. In maize, similar patterns were identified through PCA where higher abnormality rates were associated with stress-related components rather than growth performance, Adebisi *et al.* [21].

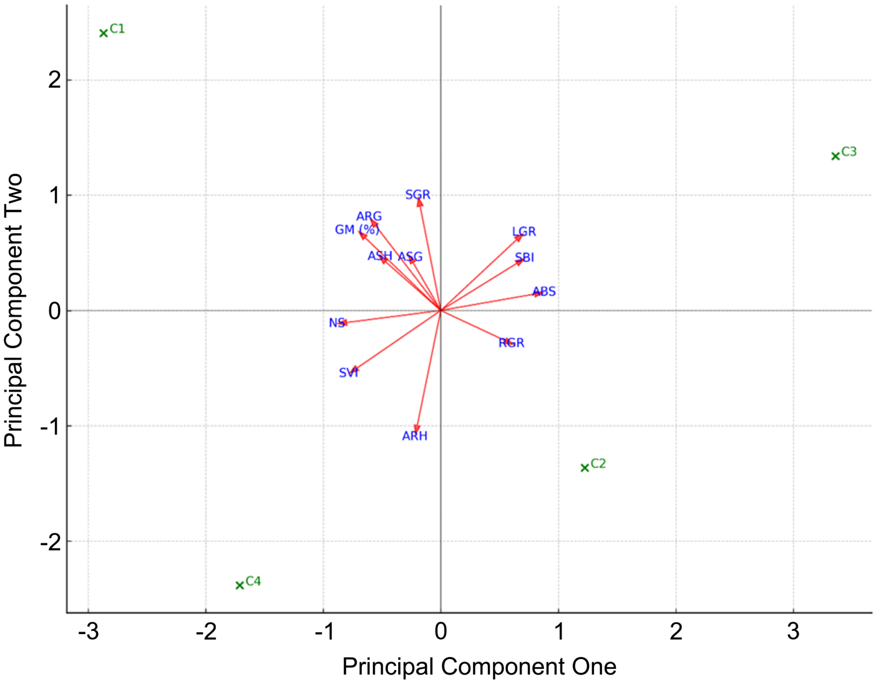

Figure 1. PCA Biplot of seedling growth parameters and treatments.

Treatment C1, closely aligned with shoot-related parameters such as SGR and Average Shoot Height (ASH), suggests a stronger effect on vertical elongation and early canopy development. This aligns with the work of Zheng *et al.* [14], who found that nitrate treatments significantly enhanced shoot elongation and antioxidant activity in wheat, favouring early biomass accumulation. C2, meanwhile, occupies a more central position in the biplot, indicating it has a more neutral or moderate influence across most traits. Its lack of strong association with any extreme trait implies it does not strongly promote or inhibit any specific aspect of seedling growth. Overall, the PCA biplot effectively separates treatments based on their influence on seedling performance traits, providing a nuanced understanding of the concentration-dependent effects of potassium nitrate. The alignment of traits such as SVI, RGR, and SGR on PC1 confirms that this axis is a robust indicator of beneficial responses to KNO_3, as corroborated in several other studies involving leguminous and cereal crops [10] [22]. Treatments like C4 and C1, which lie along this axis, are therefore ideal for promoting macadamia seedling vigor. The use of PCA in this context thus proves to be an effective tool for selecting optimal seed priming concentrations based on a suite of growth traits, ensuring better decision-making in macadamia nursery management (See **Figure 1**).

4. Conclusions

This study provides evidence that potassium nitrate (KNO_3) has significant effects on early seedling development of macadamia (Macadamia integrifolia), even though it does not significantly alter the overall germination percentage. Moderate KNO_3 concentrations, especially 0.19 M (C1), resulted in the highest seedling vigor index and lowest abnormal seedling rates. In contrast, the highest concentration (0.40 M, C3) was associated with increased abnormalities and reduced seedling vigor, suggesting stress-induced morphological disruption. These findings are in agreement with recent studies showing that excessive nitrate levels can impair seedling growth through metabolic and oxidative stress mechanisms [4] [12].

Principal Component Analysis (PCA) revealed that key parameters such as seedling vigor index, shoot growth rate, and root growth rate were positively associated with moderate KNO_3 concentrations. Treatments C1 and C4 were closely aligned with these traits, indicating improved physiological responses and more uniform seedling development. These results align with observations in tropical and temperate crops where moderate nitrate priming enhances enzymatic activation and antioxidant defenses [5] [13] [14].

5. Recommendations

Based on the results of this study, it is recommended that potassium nitrate be used at a concentration of 0.19 M for seed priming in macadamia to enhance seedling vigor and reduce abnormal seedling formation. This treatment offers an effective and practical solution for improving early seedling performance in nursery settings. The use of higher KNO_3 concentrations, particularly 0.40 M, should be avoided due to their adverse effects on seedling morphology and health. For practical nursery operations, adopting 0.19 M KNO_3 priming provides a cost-effective and scalable approach to improving macadamia seedling quality.

Further studies are recommended to evaluate the long-term field performance of macadamia seedlings raised from nitrate-primed seeds and to explore the biochemical mechanisms that regulate nitrate uptake and utilization during early seedling development.

Author Contributions

Conceptualization and investigation (Mariam Nyasasi), Supervision and guidance (Dr. Richard Madege and Dr. Beatrice Mwaipopo), Methodology and analysis (Mariam Nyasasi), Writing original draft (Mariam Nyasasi), Writing, review and editing (Dr. Richard Madege and Dr. Beatrice Mwaipopo). All authors have read and approved the final manuscript.

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Data Availability

Data supporting this study are available from the corresponding author upon request.

Conflicts of Interest

The authors declare no conflicts of interest.

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