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Brief Report

Estimating Nitrogen Uptake Efficiency of Mango Varieties from Foliar KNO_3 Application Using a ^{15}N Tracer Technique

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Abstract: Commercial mango growers commonly spray potassium nitrate (KNO_3) solution to enhance flowering and fruit quality, yet there is limited information on the uptake efficiency of nitrogen (N) by mango cultivars through leaf cuticles. The study aimed to assess N uptake efficiency (NUpE) from foliar application of KNO_3 solution and compare NUpE among mango varieties. Mango cultivars were 'Kensington Pride' ('KP'), 'B74' ('Calypso®'), and 'NMBP 1201' ('AhHa!®'), 'NMBP 1243' ('Yess!®'), and 'NMBP 4069' ('Now®') grafted onto 'KP' seedlings. Leaves of six-month-old seedlings were dipped in ^{15}N-enriched KNO_3 solution and analyzed for total N and ^{15}N contents. A significant correlation was observed between the leaf area and the amount of solution retained after dipping the leaves in the KNO_3 solution. Moreover, leaves treated with the KNO_3 solution had higher ^{15}N levels than the natural ^{15}N abundance, indicating successful N uptake from the KNO_3 solution. The NUpE ranged from 27% to 44% and varied with variety. Cultivar 'NMBP 4069' had the highest NUE (44%) which was comparable with that of 'B74' (40%). 'NMBP 1201' showed the lowest (27%) NUpE which was comparable with that of 'NMBP 1243' (30%) and 'KP' (33%). These data on ^{15}N uptake through the mango leaf cuticle demonstrates the effectiveness of foliar application as a method of supplying N to mango trees, highlighting important varietal differences in foliar ^{15}N uptake efficiency. Considering these differences in NUpE among mango varieties will help in making informed decisions about cultivar selection and N management strategies for sustainable mango production.

Keywords: flowering induction; nitrogen use efficiency; mango cultivars; ^{15}N uptake; potassium nitrate

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1. Introduction

Sustainable Development Goal 2 of the United Nations aims to "end hunger, achieve food security and improved nutrition and promote sustainable agriculture" [1]. One of the targets in this goal focuses on "ensuring sustainable food production systems and implement resilient agricultural practices that increase productivity and production, that help maintain ecosystems, that strengthen capacity for adaptation to climate change, extreme weather, drought, flooding and other disasters and that progressively improve land and soil quality" [1]. Sustainability in agricultural systems involves safeguarding both resilience, which allows systems to withstand shocks and stresses, and persistence, which ensures their longevity. It also addresses broader economic, social, and environmental impacts [2,3].

Mango (*Mangifera indica* L.) orchards offer unique ecosystem functionalities for crop production and environmental protection. As an agroecosystem, mango trees produce fruits that are important sources of income, providing economic and food security. As a

forestry ecosystem, trees absorb CO_2 from the atmosphere and generate both above- and belowground tree biomass, contributing to carbon sequestration [4,5].

Mango is the second largest tropical fruit crop in the world and ranked fourth in total fruit consumed after bananas, citrus, and apples [6]. It is an important horticultural crop in Australia, and is grown in northern New South Wales, South Australia, Western Australia, Queensland, and the Northern Territory (NT) [7]. Mango growers in the NT bring the earliest fruits to the local market, contribute approximately AUD 102 million to the NT economy, and provide employment to over 3500 people [7,8]. However, mango production faces challenges, including the irregular bearing nature of the trees due to erratic and unpredictable flowering patterns [9,10].

Consistent flowering is crucial for mango production, as it initiates the series of events that set the stage for mango yield each year [11]. It is also important for mango growers in the NT, especially in the Darwin region, as it influences harvest timing, which directly impacts profitability. The early harvest in Darwin allows growers to access premium prices, but overlapping harvests with the Katherine region can cause logistical challenges due to high demand for labor and resources. Thus, by controlling flowering and crop timing, growers can improve harvest efficiency, reduce competition for resources, and enhance profitability, while also supporting the growth of an export market [12].

The flowering of fruit trees is a complex process influenced by both exogenous and endogenous factors [13]. Environmental factors, primarily temperature changes, as well as management practices such as the use of growth regulators, pruning, and irrigation, can induce the initiation of flowering in mango trees [11,14]. In Australia, several chemicals have been tested for their ability to support mango flowering, and foliar application of KNO_3 is an effective product registered for mangoes. Growers usually apply a diluted solution of KNO_3 before the flowering period to support floral induction and maximize fruit set and retention to maturity [12]. Under NT conditions, foliar application of KNO_3 is effective when temperatures drop to around 18 °C. However, when weather conditions are unfavorable, the effect of KNO_3 application is diminished. At this point, bud development can result in either leaves or flowers, depending on the temperature. If temperatures are not cool enough (above 18 °C), the buds will develop into vegetative growth instead of flowers [12,15]. This is because KNO_3 does not initiate flowering; rather, it promotes the development of buds [11,15].

Foliar uptake of agrochemicals occurs through diffusion based on Fick's Law, where solutes move down a concentration gradient into leaves, depending on the cuticle's permeability [16]. All vascular plant leaves feature a hydrophobic cuticle layer that acts as a barrier, managing water loss from the plant [17]. The cuticle on leaves, fruits, flowers, and stems shields plants from biotic and abiotic stresses such as insects and ultraviolet radiation [18,19]. This leaf cuticle acts as a barrier to any solution applied to leaves, with solution movement across the cuticle being passive [20]. Additionally, the cuticle contains stomatal openings, whose density and diameter vary significantly on both the upper and lower leaf surfaces, leading to highly variable and unpredictable solute penetration via stomata [21,22]. When N crosses the barrier of the cuticle and cell membranes into the cytoplasm, it is typically stored as NO_3^- in the vacuole [23]. The plant utilizes this stored N for essential morphological and physiological functions. Nitrogen is crucial for mangoes, as it enhances plant vigor, promotes vegetative growth, and supports physiological development. It is also a key regulator in many biological processes, including carbon metabolism, amino acid metabolism, and protein synthesis [10,24].

Applying KNO_3 to mangoes also benefits them due to the addition of potassium (K), which enhances the fruit set and allows for the maintenance of more fruit on the flowering panicles [25,26]. As an essential macronutrient, K is indispensable to plants and plays a crucial role in photosynthesis and carbohydrate translocation and metabolism [27,28]. It also regulates transpiration, respiration, and activates nitrate reductase and starch synthetase—enzymes that are essential for plant growth, development, and survival [29,30]. Moreover, K influences the mechanism of stomatal opening and closing by affecting cell

water potential and turgor, and photosynthesis, as well as the assimilation and transport of photosynthetic products, which directly impact crop productivity and fruit quality [31,32].

Farmers in the NT primarily grow mango varieties such as 'KP', 'B74', Honey Gold, and 'R2E2' [8]. Recently, the Australian National Mango Breeding Program (NMBP) has developed and released three commercial varieties that are now registered as 'Yess!' ('NMBP 1203'), 'AhHa!' ('NMBP 1201'), and 'Now!' ('NMBO 4069'). With significant commercial interest, there are new plantings going on across northern Australia to meet their demands [33]. However, there is limited information about how these varieties respond to foliar application of KNO_3. Therefore, understanding the response of these new mango varieties to foliar application of KNO_3 is important. This can be achieved using the ^{15}N tracer technique, which measures the rate of N uptake, partitioning, and cycling in the plant. It can also distinguish between N derived from applied sources and pre-existing N in the plant [10,34]. Thus, this study aimed to determine NUpE from the foliar application of ^{15}N-labeled KNO_3 and compare the NUpE among mango cultivars in the NT.

2. Materials and Methods

2.1. Study Site and Seedling Preparation

The study was conducted at the Berrimah Farm Science Precinct, NT, Australia (12°26′38.79″ S, 130°55′46.61″ E) from November 2018 to May 2019. The mango varieties used in the study were commercial varieties 'KP' and 'B74', as well as three new NMBP varieties: 'NMBP 1243', 'NMBP 1201', and 'NMBP 4069'. The origin, parentage, fruit characteristics, and other features of these varieties are shown in Table 1.

Table 1. Mango cultivars used in the study and their parentage, origin, and fruit characteristics.

Variety	Parentage	Origin	Fruit Characteristics	Other Features	References
'KP'	Unknown	Queensland, Australia	Ripens to a rich yellow with bright pink shoulders; sweet, low-fiber, medium-sized fruit; and preferred for its distinctive taste.	Polyembryonic, irregular bearing, high vigor tree	[35]
'B74'	'Sensation' x 'KP'	Queensland, Australia	Ripens to a yellow skin color with bright red shoulder; sweet with mild 'KP' flavor; firm flesh and free of fiber; medium-sized fruit.	Monoembryonic, consistent bearing, medium vigor tree	[35]
'NMBP 1201'	'Irwin' x 'KP'	Queensland, Australia	Ripens to a yellow background skin with a soft pink blush; rich in 'KP' flavor; yellow orange; soft texture, firm, very-low-fiber flesh	Monoembryonic, with tendency of biennial bearing, medium vigor, compact tree canopy	[36]
'NMBP 1243'	'Irwin' x 'KP'	Queensland, Australia	Ripens to a yellow background skin with a strong red/pink blush; a classical 'KP' flavor; light orange; soft texture, with firm, very slight fiber flesh	Monoembryonic, medium vigor, open tree canopy	[36]
'NMBP 4069'	'Van Dyke' x 'KP'	Queensland, Australia	Ripens to a yellow background skin with a soft pink blush; sweet and rich in 'KP' flavor; yellow/orange; soft texture, with firm, low-fiber flesh	Monoembryonic, with tendency of biennial bearing, medium vigor tree	[36]

Seeds of the 'KP' mango were extracted from fruits harvested at Coastal Plains Research Farm (12°35′36.77″ S, 131°18′14.3″ E) and sown individually in 2 L pots filled with potting mix. After four months of growth, the seedlings were grafted with scions of the five varieties that were collected from Katherine Research Station's NMBP mango orchard (14°27′58.48″ S, 132°18′58.48″ E). To prepare bud wood for grafting, leaves were removed from the tips of the stems to stimulate the formation of apical buds. Once these buds began to form and swell, stems measuring 10–20 cm in length were cut, wrapped in moist paper

towels, and placed in a cooler [37]. At the laboratory, the bud wood was wedge-grafted onto 'KP' rootstock, placed in a mist propagation house for one month, and then acclimatized for three months in both a shade house and under full sun conditions before the experiment commenced.

2.2. Treatments and Experimental Design

The treatments were with (+KNO_3) and without (−KNO_3) foliar application of 2% KNO_3 on five mango cultivars, with 10 replicates arranged in a completely randomized design. Control plants provided baseline information on the natural ^{15}N abundance levels in the experimental plants. Plants were watered to field capacity every morning during the experiment to maintain consistent water potential conditions for each tree.

During foliar application, 8–10 fully expanded, attached leaves at the top of each plant were loosely bunched together using a soft rubber band. The plant was then inverted to submerge the leaves completely into either the control solution of 0.4% LI 700® or the treatment solution of 2% KNO_3, which contained 2 atom % ^{15}N and 0.4% LI 700®. LI 700® is commonly used as an adjuvant in KNO_3 foliar applications, acting as a surfactant, penetrant, and acidifier of solutions. It temporarily loosens waxy components of leaf cuticles to facilitate the movement of various ions into the leaves [38,39].

Both solutions were prepared at room temperature (25 °C) using Millipore-filtered water with a pH of 3.18 for the control solution and 3.20 for the treatment solution. After leaf removal, excess solution was allowed to drain back into the container until dripping ceased. Leaves were gently released to prevent splashing, and the seedlings were returned to benches to air-dry before being returned to their original positions (Figure 1).

(**a**) (**b**)

Figure 1. Dipping leaves into ^{15}N-labeled KNO_3 solutions (**a**) to assess leaf N uptake, with mango varieties 'KP', 'B74', 'NMBP 1201', 'NMBP 1243' and 'NMBP 4069' that were grafted onto 'KP' rootstocks (**b**). Photo credit: D. Anson.

The amount of solution transferring to the leaves was measured by weighing the solution before and after each dipping event. Dipping was repeated 24 h later, and the dipped leaves were collected 46 h after the initial dipping. To remove dipping solution residues from leaf cuticles, leaf samples were washed individually and thoroughly by rubbing both sides of the leaf blade and along all venation under running tap water, then rinsing three times in Millipore filtered water.

The reproducibility and consistency of the dipping method was assessed by plotting the leaf area and the measured quantity of solution left on the leaves after two dipping events. Moreover, the efficiency of the washing method in removing the labeled dipping solution from leaves was determined by conducting an experiment with five plants of the 'B74' variety using the same labeled KNO_3 + LI 700 solution. Similarly, 8–10 leaves from each plant were dipped, removed from the plants, and carefully washed as described above.

An alternative method of remnant solution removal from leaves using cellulose acetate in acetone was trialed but discarded as unsuitable for the scale of this experiment [40]. This method is time-consuming and requires skilled personnel, which can be challenging with limited resources. It requires accuracy to prevent physical damage to leaf surfaces and the variability in the film's adherence and removal can lead to inconsistent results, complicating the assessment of solution retention.

2.3. Sample Preparation and Calculation

Leaf samples were blot-dried, passed through a digital planimeter (Paton Electronic Planimeter) to measure leaf area, and oven-dried at 60 °C until constant weight was attained (48 h), and weighed. Samples were ground in a ring mill (ROCKLABS, Dunedin, New Zealand) [41] and submitted to the Central Analytical Research Facility at QUT, Brisbane, QLD, Australia. Total N and ^{15}N contents were determined using the Elemental Analyzer–Isotope Ratio Mass Spectrometer (EA-IRMS, Sercon Limited, Crewe, UK). The samples are burned in the elemental analyzer, and the resulting gasses are transported to the IRMS using a helium carrier. Standards of reference materials range from 0.3% to 3.5% N. The amount of N in leaves derived from the labeled KNO_3 in the dipping solutions (Ndff, %) and NUpE (%) were calculated using the following formula [42]:

$$Ndff\ (\%) = \left(\frac{^{15}N(atom\ \%)\ in\ dipped\ leaves\ -\ ^{15}N(atom\ \%)\ in\ control\ leaves}{^{15}N(atom\ \%)\ in\ solution\ -\ ^{15}N(atom\ \%)\ in\ control\ leaves} \right) * 100 \quad (1)$$

$$NUpE\ (\%) = (N\ taken\ up\ into\ leaf\ (g)/quantity\ of\ N\ in\ solution\ (g)) * 100 \quad (2)$$

2.4. Statistical Analysis

Leaf area and leaf weight data were subjected to a two-way analysis of variance (ANOVA) using STAR statistical software version 2.0.1 [43] with KNO_3 treatment as the first factor and variety as the second factor. Moreover, a one-way ANOVA was carried out for Ndff and NUpE to determine if there were significant variations among cultivars.

Normality and homogeneity of variances were checked based on Bartlett's test and Shapiro–Wilk's test, respectively, and mean comparison was carried out based on Fisher's least significant difference test, with a 5% level of significance ($LSD_{0.05}$). Data visualization was carried out using GraphPad Prism version 10.0.0 software [44].

3. Results

3.1. Leaf Weight, Leaf Area, and Volume of Solution Taken by the Leaves

Figure 2 illustrates the leaf weight across various mango cultivars with and without the application of KNO_3 as well as the relationship between leaf area and the volume of solution retained on the leaves post-dipping. The results showed that leaf weight did not significantly vary with and without foliar N dipping, but there was significant variability in leaf weight among the different cultivars ($p < 0.001$). Specifically, the cultivar 'B74' exhibited the highest average leaf weight, followed by 'NMBP 1201', 'NMBP 1243', and 'KP'. The cultivar 'NMBP 4069' demonstrated the lowest average leaf weight, although this was statistically comparable to that of 'KP' (Figure 2a,b).

Linear regression analysis was carried out to evaluate the relationship between the volume of dipping solution and leaf area (Figure 2c). The analysis involved fitting lines of best fit to datasets from both the control and treatment groups, each consisting of 49 samples, where leaves were immersed in a solution with or without KNO_3. The comparison of the regression lines showed no significant difference in the slopes between the control and treatment groups ($p = 0.12$). The coefficient of determination (r^2) was 0.82 for the control group and 0.85 for the treatment group, indicating a strong correlation and a robust relationship between leaf area and solution retention.

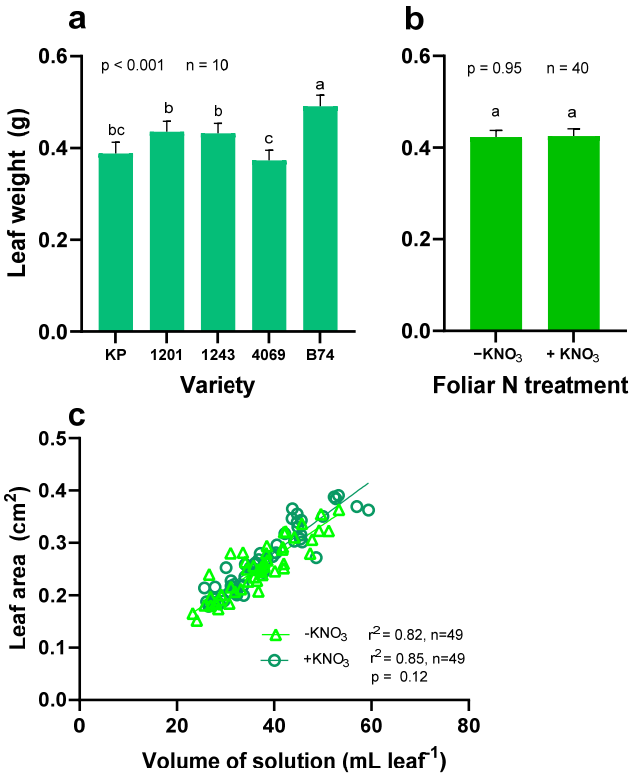

Figure 2. Leaf weight of mango cultivars (**a**), with and without KNO_3 application (**b**), and the relationship between leaf area and the volume of solution held on the leaves after dipping into the KNO_3 solution with ^{15}N-labeled fertilizer (**c**). In (**a**) and (**b**), vertical bars represent the standard error of 10 replicates. Bars with similar letters are not significantly different based on $LSD_{0.05}$.

3.2. Leaf Atom % ^{15}N Content

As shown in Figure 3, the atom % ^{15}N of leaf samples differs significantly among varieties and between KNO_3 treatments, but there were no significant interactions between varieties and KNO_3 treatments. Results showed that for leaves sampled from each variety, the mean leaf N content ranged between 0.98% and 1.25% in control plants and 0.98% to 1.30% in the treated plants. Also, the leaf ^{15}N content of the control plants was comparable with that of the natural ^{15}N abundance, indicating that plants did not receive any external N during treatment application.

In contrast, all varieties dipped in the ^{15}N-labeled KNO_3 solution showed a significant increase ($p < 0.001$) in ^{15}N in their leaves compared to the natural abundance of ^{15}N in leaves dipped in the control solution. This significant increase demonstrates the effectiveness of foliar application in delivering N to the leaves through the cuticles. The study highlighted the varying NUEs among the different mango cultivars, with 'NMBP 4069' exhibiting the highest NUE.

3.3. Ndff and NUpE

The leaf analysis showed that 4–6% of the total N in leaf content was derived from the dipping solution (Figure 4a). There were notable varietal differences, with leaves from 'NMBP 1201' and 'NMBP 1243' having 3.8–3.9% of their leaf N derived from the solution, which was significantly less than the 5.6–6.1% leaf N content in 'KP', 'B74', and 'NMBP 4069' ($p < 0.001$). Additionally, the study calculated leaf NUpE to determine the portion of N taken up from the KNO_3 dipping solution that dried on the leaves. Significant differences were again observed between the varieties ($p = 0.033$). 'NMBP 1201' showed the lowest mean uptake with 27%, while the highest N uptake across the cuticle occurred in leaves of 'NMBP 4069', with a mean uptake of 44% (Figure 4b).

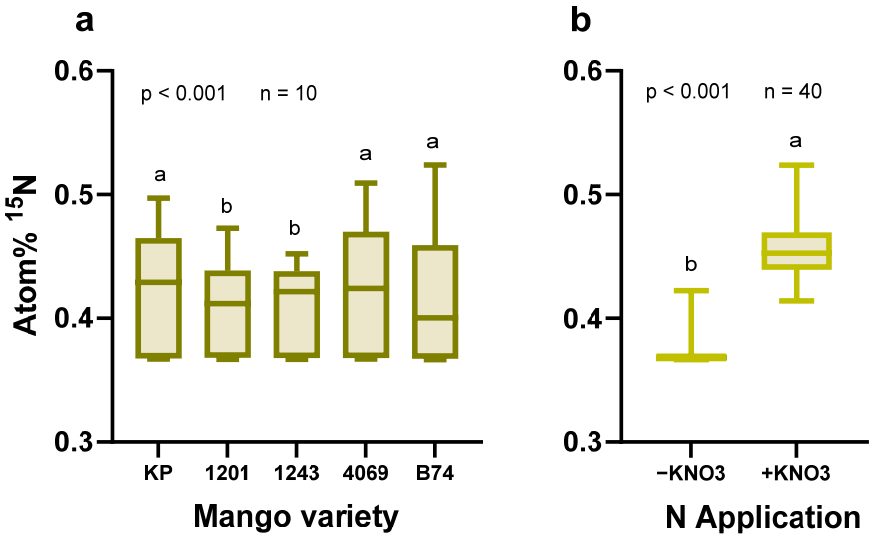

Figure 3. Atom % ^{15}N of leaf samples of mango varieties (**a**) dipped in solution with and without KNO_3 (**b**). Means with different letters are significantly different based on $LSD_{0.05}$. Vertical bars represent the standard error of the means.

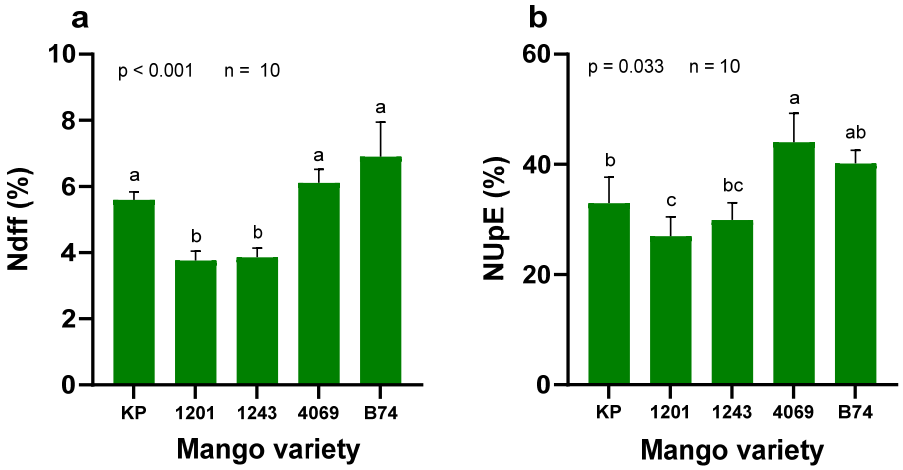

Figure 4. Varietal difference in the amount of N (**a**) and NUpE (**b**) from the KNO_3 dipping solution. Means with different letters are significantly different based on $LSD_{0.05}$. Vertical bars represent the standard error of the means.

4. Discussion

Aided by the ^{15}N tracer technique, this study provides direct and quantitative evidence of N uptake into mango leaves from the foliar application of a dilute KNO_3 plus adjuvant solution (Figures 3 and 4). The significant enrichment of ^{15}N in the leaf tissue demonstrates the effectiveness of foliar application in delivering N to the leaves through the cuticles. The uptake of ^{15}N is also rapid, which corroborates the reported translocation of ^{15}N foliar-applied urea within two days of application to young avocado 'Hass' trees [45]. An earlier study [39] on 'KP' mango cultivars using unlabeled KNO_3 also showed that foliar application of 3% KNO_3 increased the N content of leaf N from 24 to 48 h after spraying. The study also indicated that addition of at least 1 mL L^{-1} adjuvant in the spray solution further increases leaf N content from foliar application of KNO_3 [39].

Our study also showed that there were significant differences in NUpE with foliar N applications among the tested varieties, which ranged from 25% to 43% efficiency. These findings indicate that while all mango varieties absorbed N from the foliar KNO_3 application, the efficiency varied significantly among varieties. with 'NMBP 4069' demonstrating

superior N uptake efficiency, making it potentially more responsive to foliar N applications compared to the other tested varieties. This information is crucial for optimizing N management strategies tailored to specific mango cultivars, ultimately enhancing growth, yield, and fruit quality.

Foliar spray uptake is influenced by various environmental and plant-specific factors, such as leaf morphology, structure, positioning, sun exposure, and the plant's physiological processes. These factors collectively determine how effectively the spray is absorbed and utilized by the plant [20]. Since the study was conducted under similar environmental conditions, the differences in the NUpE among varieties could be attributed to variations in plant-specific phenotypes and physiological process. For example, 'KP' has an unknown parentage; 'B74' is derived from 'Sensation' and 'KP'; NMBP 1201 and 1243 are derived from 'Irwin' and 'KP'; and NMBP 4069 is derived from 'Van Dyke' and 'KP'. Thus, the hybrid vigor or specific adaptations of 'Van Dyke' influenced the efficiency of N assimilation and transport processes in NMB 4069 variety (Table 1).

Physiologically, Lu et al. [46] also reported significant variations in net photosynthesis and stomatal conductance for mango varieties grown in the Darwin region. These varietal differences became particularly pronounced during the dry season. Notably, the 'KP' variety struggled to maintain photosynthesis under the hot and dry conditions typical of this period [46]. This inability to sustain photosynthetic activity in adverse weather highlights the importance of selecting mango varieties that can thrive in specific climatic conditions. Future research will focus on understanding the physiological characteristics of these cultivars. The information on physiological responses will be critical to explain the varietal differences in NUpE, providing valuable insights for optimizing mango cultivation practices in regions like Darwin.

It is acknowledged that these results reflect the fact that both surfaces of the leaf were covered with the KNO_3 solution, whereas in-orchard spraying is likely to result in partial coverage. Besides potential localized leaf toxicity, the impact of repeated or frequent spray applications over time remains unknown. Thus, it is also important to account for cumulative amounts, as excessive inputs will be recycled in orchards through the litter and soil. However, some reports showed that along with other commercial fruit crops, foliar N application in mango orchards contributes to being efficient, targeted, and more environmentally friendly when compared to the uncontrolled losses of N from runoff and leaching associated with soil application [20,47]. They suggest that foliar application is comparatively efficient for applications of small amounts of N, likely to be rapidly available for use in trees. This is highly useful knowledge for mango producers and forms part of the crop N budget. It should be incorporated into annual fertilizer assessments and planning. Experimental work conducted in the 'KP' orchard at Katherine Research Station from 1995 to 1999 showed that although there was no significant annual differences in yield, the aggregated data over the five-year period indicated that foliar application of 140 g of N $tree^{-1}$ as KNO_3, either when fruits were golf ball-sized or during the post-harvest leaf flush, significantly increased marketable yields and fruit numbers compared to untreated trees [48]. This supports the use of foliar KNO_3 as an efficient method to address N deficiencies in an orchard and maximize yields.

To our knowledge, this is the first study attempting to estimate the uptake of N through foliar application using ^{15}N-labeled KNO_3 solution. Spraying mangoes with a low concentration of KNO_3 solution during inflorescence development is a conventional commercial practice to boost flowering and yields, although the frequency and concentration of solutions applied can vary [24,49,50]. However, N management in mangoes is crucial as excessive N in mango trees can lead to excessive foliage growth at the expense of fruit [10] and reduced post-harvest fruit quality, with skin remaining green when the fruit is ripe [51,52].

5. Conclusions

The study sought to quantitatively determine the NUpE from foliar applications of KNO_3 solution across different mango cultivars using the ^{15}N tracer technique. The results confirmed that foliar application of KNO_3 can effectively supply N to mango leaves, as evidenced by the consistent leaf area across N treatments and cultivars, with variability observed in leaf weight among cultivars. Among the cultivars tested, 'NMBP 4069' demonstrated the highest NUpE, underscoring its superior efficiency in utilizing foliar-applied N compared to other cultivars. In contrast, 'NMBP 1201' exhibited the lowest NUpE, highlighting significant varietal differences in response to foliar N applications. The results show that while foliar application of KNO_3 is generally effective for N delivery across mango cultivars, the efficiency of N uptake varies significantly between varieties. This suggests that certain cultivars are better suited for foliar N strategies, offering practical insights for mango production. The study provides empirical evidence of N uptake through mango leaf cuticles and highlights the efficacy of foliar KNO_3 applications in enhancing NUpE. These findings are valuable for informing future cultivar selection and optimizing agronomic practices aimed at improving NUpE in mango production. By identifying cultivars with superior NUpE, such as 'NMBP 4069', and 'B74', growers can tailor their fertilization strategies to achieve better yields and resource use efficiency.

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Data Availability Statement: The datasets generated and analyzed in the current study are available from the corresponding author upon request.

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