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Influence of Age of the Plant and Season on Nitrate Reductase Activity in Leaves of Alphonso Mango (*Mangifera Indica* L.) Grafts

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Abstract

Nitrate reductase (NR) activity, a key indicator of nitrogen assimilation, was evaluated in the leaves of Alphonso mango grafts of different ages across rainy, winter, and summer seasons. The results revealed significant variation in NR activity with both graft age and season. Across all seasons, 6-year-old grafts consistently exhibited higher NR activity than 1-year-old grafts, indicating enhanced nitrogen assimilation capacity with advancing plant age. Seasonally, NR activity followed the trend winter > rainy > summer, irrespective of graft age. The highest NR activity was recorded during winter, reflecting favorable physiological conditions such as moderate temperatures and improved internal nitrogen balance. Rainy season values were intermediate, likely due to increased soil nitrate availability, while summer recorded the lowest activity, possibly due to heat stress and moisture deficits. The progressive increase in NR activity with graft age suggests improved root development and sink–source relationships in mature grafts. These findings emphasize the importance of age- and season-specific nitrogen management strategies to enhance nutrient use efficiency and productivity in Alphonso mango orchards.

Introduction

In the present study, nitrate reductase activity in the leaves of Alphonso mango grafts of different ages (1–6 years) was evaluated across three distinct seasons—rainy, winter, and summer and the results are illustrated in Figure. During the rainy season, nitrate reductase activity in the leaves ranged from 2.26 to 2.80 µg NO_2 g^{-1} h^{-1} across the different age groups. In the winter season, comparatively higher enzyme activity was recorded, with values ranging from 2.52 to 3.30 µg NO_2 g^{-1} h^{-1}. In contrast, during the summer season, nitrate reductase activity remained relatively lower, varying between 2.26 and 2.80 µg NO_2 g^{-1} h^{-1}.

In comparison to the summer and wet seasons, nitrate reductase activity was often higher in the winter. Improved internal nitrogen balance and better physiological circumstances for nitrogen assimilation during the winter may be responsible for this seasonal improvement. Additionally, the data show that nitrate reductase activity gradually rose as the grafts grew older, indicating improved

metabolic capability during growth and development.

In addition, nitrate reductase activity increased by 23.89% over the rainy season, from 2.26 µg NO_2 g^{-1} h^{-1} in 1-year-old grafts to 2.80 µg NO_2 g^{-1} h^{-1} in 6-year-old grafts. Similarly, between 1- and 6-year-old grafts, enzyme activity increased from 2.52 to 3.04 µg NO_2 g^{-1} h^{-1} throughout the winter, a 20.63% increase. whereas the rise in nitrate reductase activity with graft age was very slight during the summer, rising by only 11.11% from 2.52 to 2.80 µg NO_2 g^{-1} h^{-1}.

These results unequivocally show that nitrate reductase activity in Alphonso mango leaves rises with graft age, with the increase being particularly noticeable in the winter and rainy seasons. The relatively smaller enhancement seen in the summer indicates lower nitrogen absorption efficiency, which may be brought on by heat stress and decreased physiological activity, which would result in less nitrogenous compound production.

Materials and Methods:

The activity of enzyme nitrate reductase was assayed according to the *in vivo* method described by Evans (1982) from leaf samples. Phosphate buffer 0.1 M (pH 7.5), 0.02 M KNO_3, 5% Propanol, 1% Sulphanil amide, 0.02% NEEDA (N-1-Naphthyl ethylene diamine dihydrochloride) reagents were used.

0.5 g of leaf material was cut into small discs. In test tubes 5 ml of 0.1 M phosphate buffer (pH 7.5), 1 ml of 0.02 M KNO_3 and 1 ml of 5 per cent propanol were taken. To this test tubes the leaf material was added. After shaking vigorously the test tube were incubated for 60 minutes in dark at $30^{O}C$ temperature. After incubation the test tubes were kept in boiling water bath for 2 minutes. After cooling test tubes, 2 ml of test tube solutions were pipette out in another test tubes in which 1 ml of sulphanil amide reagent was added, which is followed by 1 ml of NEEDA. The test tubes were kept in dark for 30 minutes. The colours developed were measured at 540 nm with the help of spectrophotometer. For blank test tube was prepared as above except the leaf material.

Standard curve was prepared following different concentrations of $NaNO_2$ (10, 20, 30, 40 mg) by using same procedure of NO_2 estimation. With the help of graph, enzyme activity was calculated.

Results and Discussion

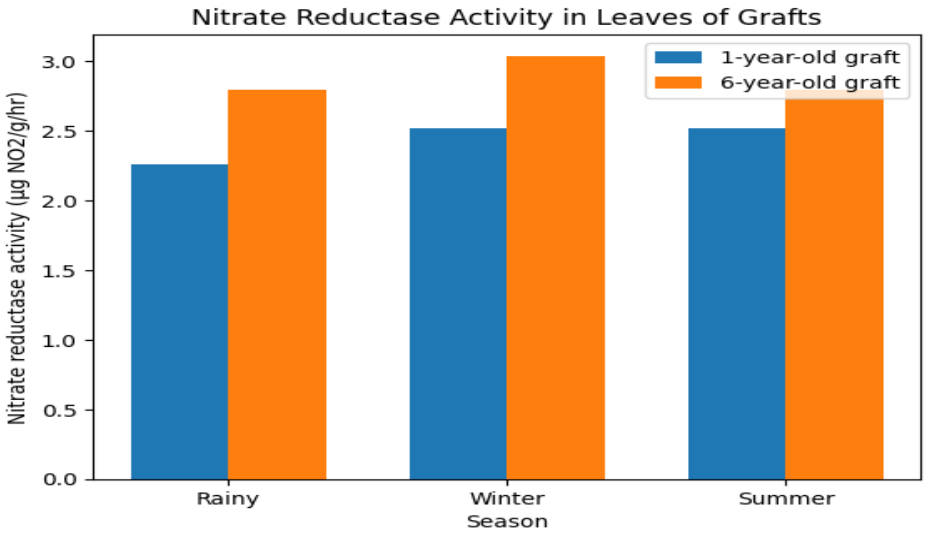

Figure 1: Nitrate reductase activity in leaves of grafts

Graft age and season had a significant effect on the nitrate reductase (NR) activity in Alphonso mango graft leaves (Figure1). 6-year-old grafts persistently showed higher NR activity than 1-year-old grafts during all seasons, indicating that with age of plants , their ability to assimilate nitrogen increases.

During the rainy season, NR activity was recorded at approximately 2.26 µg NO_2 g^{-1} h^{-1} in 1-year-old grafts, whereas it increased to 2.80 µg NO_2 g^{-1} h^{-1} in 6-year-old grafts. In the winter season, the highest NR activity was observed, with values of 2.52 µg NO_2 g^{-1} h^{-1} in 1-year-old grafts and 3.04 µg NO_2 g^{-1} h^{-1} in 6-year-old grafts. Conversely, during the summer season, NR activity remained comparatively lower, recording 2.52 µg NO_2 g^{-1} h^{-1} in 1-year-old grafts and 2.80 µg NO_2 g^{-1} h^{-1} in 6-year-old grafts.

The nitrate reductase (NR) activity in Alphonso mango graft leaves are fluctuated by graft age and season (Figure). In every season, 6-year-old grafts consistently displayed greater NR activity than 1-year-old grafts, suggesting that plants' capacity to absorb nitrogen grows with age.

Nitrate reductase is a pivotal enzyme regulating nitrate assimilation and nitrogen use efficiently in perennial fruit crops, including mango. In perennial fruit crops, such as mangos, nitrate reductase is an essential enzyme that controls nitrate uptake and nitrogen usage effectively. A steady improvement in nitrogen metabolism with increasing plant age is suggested by the continuously increased NR activity shown in older (6-year-old) grafts relative to younger grafts. Mature mango grafts may have better root

development, larger leaf areas, and improved sink-source connections, all of which contribute to increased nitrogen uptake and assimilation (Laxman et al., 2018; Singh et al., 2020).

For the mango, a perennial evergreen fruit crop, the maximal NR activity during the winter season found in this study is physiologically significant. Moderate temperatures, reduced respiration losses, and improved internal nitrogen balance are characteristics of tropical and subtropical winter regions which encourage nitrate assimilation. Mango and other fruit crops like citrus and guava have been shown to have similar seasonal elevation of nitrogen metabolic enzymes during the cooler months (Reddy et al., 2017; Kumar et al., 2019).

On the other hand, high temperature stress, increased photorespiration, and moisture shortages, which adversely affect nitrogen metabolism and enzyme stability, may be linked to the reduced NR activity during the summer. Reduced nitrogen efficiency in absorption in horticulture crops is known to result from heat stress's inhibition of nitrate reductase synthesis and activity (Xu et al., 2020). Another sign of physiological constraints on nitrogen metabolism under stress is the notably smaller increase in NR activity between young and old grafts in the summer.

The rainy season showed intermediate NR activity, which can be associated with enhanced soil nitrate availability due to increased mineralization and improved soil moisture status. However, excessive rainfall may also lead to nitrate leaching, partially offsetting the benefits of higher nitrogen availability. Similar seasonal responses of nitrogen metabolism have been reported in mango orchards under monsoonal climates (Patel et al., 2016; Singh & Singh, 2021).

Overall, the observed increase in nitrate reductase activity with graft age and its seasonal modulation highlight the importance of age-specific and season-specific nitrogen management strategies in mango cultivation. Enhanced NR activity during winter and rainy seasons suggests these periods are physiologically favorable for nitrogen uptake and assimilation, which can be strategically utilized to improve growth, productivity, and nutrient use efficiency in Alphonso mango orchards.

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