

## Effects of Different Nitrogen Application Rates on Apparent Soil Nutrient Balance and Tuber Yield in Tiger Nut Cropland (Postprint)

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### Abstract

This study investigated the apparent nutrient balance and tuber yield of tiger nuts (*Cyperus esculentus*) in sandy soil under different nitrogen application levels, providing a theoretical basis for scientific nitrogen fertilization of tiger nuts in sandy soil. Using the tiger nut variety “Zhongyousha No. 1” as the experimental material, four nitrogen application levels were established:  $0 \text{ kg} \cdot \text{hm}^{-2}$  (N0),  $75 \text{ kg} \cdot \text{hm}^{-2}$  (N1),  $150 \text{ kg} \cdot \text{hm}^{-2}$  (N2), and  $225 \text{ kg} \cdot \text{hm}^{-2}$  (N3). The effects of the four nitrogen application levels on tiger nut agronomic traits, soil apparent nutrient balance, and tuber yield were analyzed. The results showed that with increasing nitrogen application, tiller number, plant height, and single leaf area of tiger nuts in both locations increased; however, excessive nitrogen application caused excessive vegetative growth of above-ground parts, leading to yield reduction. At the nitrogen application rate of  $150 \text{ kg} \cdot \text{hm}^{-2}$  (N2), agronomic traits such as maximum root length and root volume were optimal, whole-plant dry weight and tuber yield were highest, with fresh tuber yield reaching  $9298.87\text{--}10336.06 \text{ kg} \cdot \text{hm}^{-2}$ . The apparent nitrogen surplus rates in both locations were negative at the  $0 \text{ kg} \cdot \text{hm}^{-2}$  (N0) and  $75 \text{ kg} \cdot \text{hm}^{-2}$  (N1) levels, and positive at the  $150 \text{ kg} \cdot \text{hm}^{-2}$  (N2) and  $225 \text{ kg} \cdot \text{hm}^{-2}$  (N3) levels, indicating that nitrogen balance was achieved at the N2 level ( $150 \text{ kg} \cdot \text{hm}^{-2}$  nitrogen application) in both locations. Redundancy analysis also indicated that tiger nut tiller number, maximum root length, root volume, and nitrogen removal were the main factors driving dry matter and tuber yield formation. Therefore, under northern sandy soil conditions, a nitrogen application rate of  $150 \text{ kg} \cdot \text{hm}^{-2}$  can promote nutrient uptake by tiger nuts, maintain soil apparent nutrient balance, and is conducive to healthy growth and development and yield improvement.

## Full Text

### Abstract

This study investigated the apparent soil nutrient balance and tuber yield of *Cyperus esculentus* in sandy farmland under different nitrogen application levels to provide a theoretical basis for scientific nitrogen fertilization of *C. esculentus* in sandy soils. The experiment was conducted at two sites in Inner Mongolia: Dengkou County, Bayannur City, and Toketo County, Hohhot City. Four nitrogen fertilizer treatments were applied:  $0 \text{ kg} \cdot \text{hm}^{-2}$  (N0),  $75 \text{ kg} \cdot \text{hm}^{-2}$  (N1),  $150 \text{ kg} \cdot \text{hm}^{-2}$  (N2), and  $225 \text{ kg} \cdot \text{hm}^{-2}$  (N3). The effects of these nitrogen levels on agronomic traits, tuber yield, and soil nutrient balance were analyzed. The results showed that as nitrogen application increased, the number of tillers, plant height, and single leaf area of *C. esculentus* at both sites increased, but excessive nitrogen caused excessive above-ground growth and reduced yield. At the application rate of  $150 \text{ kg} \cdot \text{hm}^{-2}$  (N2), *C. esculentus* exhibited optimal agronomic traits including maximum root length and root volume, the highest whole-plant dry weight and tuber yield, with fresh tuber yields reaching  $9298.87\text{-}10336.06 \text{ kg} \cdot \text{hm}^{-2}$ . The apparent nitrogen surplus rates were negative at the N0 and N1 levels but positive at the N2 and N3 levels at both locations, indicating that nitrogen reached a balanced state at the N2 level. Redundancy analysis also revealed that tiller number, maximum root length, root volume, and nitrogen uptake were the main factors driving dry matter and tuber yield formation in *C. esculentus*. Therefore, under northern sandy soil conditions, nitrogen application at  $150 \text{ kg} \cdot \text{hm}^{-2}$  can promote nutrient absorption by *C. esculentus*, maintain apparent soil nutrient balance, and support favorable growth, development, and yield improvement.

**Keywords:** *Cyperus esculentus*; nitrogen application levels; agronomic traits; tuber yield; soil apparent nutrient balance

### Introduction

*Cyperus esculentus*, also known as tiger nut or chufa, is a novel economic crop that integrates oil, grain, food, medicine, feed, and landscaping applications, and serves as an ideal green plant for windbreak and sand fixation, soil fertility improvement, and marginal land utilization [1-3]. Its underground tubers are rich in oil, carbohydrates, proteins, minerals, and vitamins [4], and the crop is cultivated on nearly all continents (Africa, Europe, Asia, America, and Oceania) [5]. In China, it is planted in severely desertified regions of the northwest, northeast, and Huang-Huai areas, with the planting area maintained at approximately  $1.7 \times 10^4 \text{ hm}^2$  in recent years [6]. The crop can be grown in sandy, saline-alkali, and black soils, with sandy soil being the most suitable. Research indicates that after *C. esculentus* matures, its leaves, roots, and tubers can be buried underground as a nutrient source for soil improvement, enabling degraded plant communities to recover to optimal conditions within 3-5 years [7]. Additionally, foreign reports have documented that a single *C. esculentus* seed

can produce new plants and nearly 2000 new tubers within one year on 2 m<sup>2</sup> of land, significantly improving the ecological environment [8].

Fertilization is the most rapid and primary measure for increasing crop yield, with nitrogen fertilizer playing a crucial role in high-yield cultivation [9]. Nitrogen application promotes crop growth, increases yield, and affects nutritional quality [10]. However, even with the best cultivation techniques, only 30-50% of nitrogen applied as urea is absorbed by crops [11], while excessive nitrogen not only fails to increase economic benefits but also causes waste and pollution [12]. Therefore, rational nitrogen application is a key measure for improving fertilizer utilization efficiency and increasing crop economic benefits. Previous studies on *C. esculentus* fertilization have shown that increasing nitrogen application can improve the number of tubers per plant and total tuber yield [13]. Research on potato nutrient requirements has demonstrated that increased nitrogen application enhances nutrient absorption, with medium nitrogen levels producing the highest nutrient content in various organs [14]. Studies on soil nutrient balance have indicated that nitrogen application rates affect both crop yield and soil nutrient balance [15], though research on nutrient apparent balance in sandy soils remains incomplete.

In recent years, research on *C. esculentus* has focused primarily on cultivation patterns and variety screening, with few studies addressing nitrogen application rates and soil nutrient apparent balance in northern sandy soils. Previous investigations into the nitrogen requirements of *C. esculentus* itself have been insufficient, and studies on soil nutrient apparent balance need improvement. To address the unclear optimal nitrogen application rate for *C. esculentus* in sandy soils, this study established two experimental sites in Dengkou County, Bayannur City, and Toketo County, Hohhot City, Inner Mongolia. Four nitrogen application levels (0, 75, 150, and 225 kg · hm<sup>-2</sup>) were established to analyze the effects of different nitrogen levels on agronomic traits, whole-plant dry weight, tuber yield, and soil nutrient apparent balance. The objective was to determine the optimal nitrogen application rate for *C. esculentus* in sandy soils, improve tuber yield, and maintain soil nutrient apparent balance, providing a scientific basis for cultivation.

## 1. Materials and Methods

### 1.1 Experimental Site Overview

The experiment was conducted in 2021 at two locations: Xingtai Family Farm in Dengkou County, Bayannur City (106°56 E, 40°32 N) and the Toketo County Experimental Station of the Inner Mongolia Academy of Agricultural and Animal Husbandry Sciences in Hohhot City (111°25 E, 40°30 N). Both regions have a temperate continental monsoon climate with similar ecological types, abundant sunlight, and rich heat resources. The average annual temperature is 6-8°C, with effective accumulated temperatures of 3015°C and 2795°C, respectively. Annual evaporation rates are 1784 mm and 1650 mm, and average wind

speeds are  $2.6 \text{ m} \cdot \text{s}^{-1}$  and  $1.6 \text{ m} \cdot \text{s}^{-1}$ , respectively. The topsoil fertility is generally poor at both sites, with sandy soil as the soil type and corn as the previous crop. Specific physical and chemical properties are shown in .

## 1.2 Experimental Design

A randomized block design was employed with three replications per treatment. Each plot measured  $4 \text{ m} \times 6 \text{ m}$  ( $24 \text{ m}^2$ ). The planting method was hole-sowing with row spacing of 50 cm and plant spacing of 15 cm, resulting in a planting density of  $13.33 \times 10^4$  plants  $\cdot \text{hm}^{-2}$ , with 2-3 seeds per hole. The tested variety was “Zhongyousha No. 1”. Sowing occurred on May 20, 2021, and harvest on October 5, 2021. Drip irrigation was used with a total water application of  $1200 \text{ m}^3 \cdot \text{hm}^{-2}$ . Field management including tillage, irrigation, and weed control was consistent throughout the growth period.

Four nitrogen treatments were established:  $0 \text{ kg} \cdot \text{hm}^{-2}$  (N0),  $75 \text{ kg} \cdot \text{hm}^{-2}$  (N1),  $150 \text{ kg} \cdot \text{hm}^{-2}$  (N2), and  $225 \text{ kg} \cdot \text{hm}^{-2}$  (N3). The N1 and N2 levels were determined based on local *C. esculentus* nitrogen application rates and soil baseline fertility, with N0 and N3 representing  $75 \text{ kg} \cdot \text{hm}^{-2}$  reductions and increases from the N2 baseline, respectively. Phosphorus ( $\text{P}_2\text{O}_5$ ) and potassium ( $\text{K}_2\text{O}$ ) applications were uniform across all treatments at  $240 \text{ kg} \cdot \text{hm}^{-2}$  and  $120 \text{ kg} \cdot \text{hm}^{-2}$ , respectively. Nitrogen was applied as urea (pure N  $\geq 46\%$ ), phosphorus as superphosphate ( $\text{P}_2\text{O}_5 \geq 16\%$ ), and potassium as potassium sulfate ( $\text{K}_2\text{O} \geq 50\%$ ). All fertilizers were applied as base fertilizer at sowing.

## 1.3 Measurements

**1.3.1 Agronomic Trait Measurement** Before harvest (late September 2021), five representative plants were selected from each plot to measure plant height, tiller number, single leaf area, maximum root length, and root volume. Plant height and tiller number were measured directly. Since tiller number increases rapidly after the fast tillering stage and cannot be completely collected from a single plant, tiller number was defined as all tillers from a single plant within a  $0.3 \text{ m} \times 0.3 \text{ m}$  area. The complete root system with soil was excavated using the full-dig method, and soil was shaken off. Maximum root length was measured with a ruler. Roots were washed clean, all tubers were removed, and root volume was determined using the water displacement method [16]. Single leaf area was calculated using the length-width coefficient method [17]. After harvest, plants were dried at  $105^\circ\text{C}$  for 0.5 hours, then at  $80^\circ\text{C}$  to constant weight to determine whole-plant dry weight.

**1.3.2 Plant Nutrient Determination** Dried *C. esculentus* plants were separated into stems/leaves, roots, and tubers, then crushed and passed through a 0.5 mm sieve for nutrient analysis. Plant samples were digested with  $\text{H}_2\text{SO}_4$ - $\text{H}_2\text{O}_2$ . Total nitrogen was determined by the Kjeldahl method, total phosphorus by the vanadium-molybdenum yellow colorimetric method, and total potassium by flame photometry.

**1.3.3 Tuber Yield Determination** Due to the strong tillering ability of *C. esculentus* and the spatial expansion and crossing capability of tiller plants, a small-area multi-point measurement method was used to ensure representative sampling. Tuber yield was determined from the middle zone (0.5 m × 0.5 m) of each plot.

#### 1.4 Data Analysis and Processing

Nutrient balance was calculated using the apparent balance method [18]: - Nutrient balance value ( $\text{kg} \cdot \text{hm}^{-2}$ ) = Nutrient input ( $\text{kg} \cdot \text{hm}^{-2}$ ) - Crop uptake ( $\text{kg} \cdot \text{hm}^{-2}$ ) - Nutrient surplus rate (%) = [(Nutrient balance value) / (Nutrient input)] × 100

Nutrient input included only chemical fertilizer application, while uptake included only nutrients removed by crop harvest. Data were organized and analyzed using Excel 2010. Significance testing was performed using SPSS Statistics 22 software with one-way ANOVA and Duncan's multiple range test for comparison among treatment means. Origin 2021 was used for graphing and redundancy analysis (RDA).

## 2. Results

### 2.1 Effects of Different Nitrogen Levels on Agronomic Traits of *Cyperus esculentus*

As shown in , nitrogen application improved not only above-ground agronomic traits such as plant height, tiller number, and single leaf area but also below-ground traits including root length and root volume. With increasing nitrogen application, tiller number, plant height, and single leaf area showed upward trends, peaking at the N2 level and differing significantly from the N0 level ( $P < 0.05$ ). Compared with N0, tiller numbers at N2 increased by 38.89% and 25.00% at Toketo and Dengkou, respectively; plant height increased by 26.09% and 20.83%; and single leaf area increased by 12.79% and 18.50%. At the N3 level, these traits were lower than at N2 but still higher than at N0.

For below-ground traits, maximum root length and root volume increased with nitrogen application up to 150  $\text{kg} \cdot \text{hm}^{-2}$ , reaching maximum values at N2. Maximum root length at N2 increased by 13.95% and 14.54% compared with N0 at Toketo and Dengkou, respectively, while root volume increased by 11.16% and 22.18%. However, differences in maximum root length among levels were not significant.

### 2.2 Effects of Different Nitrogen Levels on Whole-Plant Dry Weight and Tuber Yield of *Cyperus esculentus*

As shown in , whole-plant dry weight was lowest at N0 at both sites and showed a trend of increasing then decreasing with nitrogen application, peaking at N2. At Toketo and Dengkou, whole-plant dry weights at N2 were 132.40  $\text{g} \cdot \text{plant}^{-1}$

and  $138.81 \text{ g} \cdot \text{plant}^{-1}$ , respectively, significantly higher than at N0 but not significantly different from N3. Tuber fresh yield also increased then decreased with nitrogen application, reaching maximum values at N2 that were significantly higher than other levels. Fresh tuber yields at N2 were  $9298.87 \text{ kg} \cdot \text{hm}^{-2}$  at Toketo and  $10336.06 \text{ kg} \cdot \text{hm}^{-2}$  at Dengkou, representing increases of 26.57% and 16.47% compared with N0, and 5.82% and 7.01% compared with N1.

### 2.3 Soil Nutrient Balance Under Different Nitrogen Levels

As nitrogen application increased, nitrogen uptake by *C. esculentus* at both sites showed a trend of increasing then decreasing. Nitrogen uptake ranged from  $108.56\text{--}132.94 \text{ kg} \cdot \text{hm}^{-2}$  at Toketo and  $128.07\text{--}139.54 \text{ kg} \cdot \text{hm}^{-2}$  at Dengkou, with maximum values at N2. Compared with N0, nitrogen uptake at N2 increased by 22.46% and 8.96% at the two sites, respectively. The apparent nitrogen balance value was negative at N0 and N1 but positive at N2 and N3 at both locations, with  $\text{N3} > \text{N2} > \text{N1} > \text{N0}$ . The apparent nitrogen surplus rate was negative at N0 and N1 but positive at N2 and N3, indicating that nitrogen reached a balanced state at the N2 level ( $150 \text{ kg} \cdot \text{hm}^{-2}$ ) at both sites.

Nitrogen application level also affected phosphorus and potassium balance. Phosphorus and potassium uptake at both sites showed similar increasing then decreasing trends. Phosphorus uptake ranged from  $22.75\text{--}31.68 \text{ kg} \cdot \text{hm}^{-2}$ , while potassium uptake ranged from  $87.70\text{--}167.59 \text{ kg} \cdot \text{hm}^{-2}$ . The apparent phosphorus surplus rate was positive at all nitrogen levels at both sites, indicating phosphorus surplus. However, the apparent potassium surplus rate was negative at all levels at Toketo but positive at all levels at Dengkou, likely due to differences in baseline soil conditions. This demonstrates that rational nitrogen application promotes crop nutrient absorption, providing a foundation for high yield and quality.

### 2.4 Redundancy Analysis of Agronomic Traits, Nutrient Uptake, Dry Weight, and Yield

RDA was performed to elucidate relationships among agronomic traits, nutrient uptake, dry weight, and yield. At Toketo, RDA axes 1 and 2 explained 85.92% and 14.08% of the variation, respectively; at Dengkou, they explained 85.00% and 15.00%, respectively. The analysis revealed that at Toketo, nitrogen uptake, phosphorus uptake, maximum root length, plant height, root volume, and phosphorus uptake were the main drivers of dry matter and tuber yield formation, followed by single leaf area and potassium uptake. At Dengkou, tiller number, root volume, single leaf area, maximum root length, and nitrogen uptake were the primary drivers, followed by plant height, phosphorus uptake, and potassium uptake. Both sites showed that tiller number, maximum root length, root volume, and nitrogen uptake were significantly positively correlated with dry matter and tuber yield. This indicates that favorable agronomic traits and nitrogen absorption contribute to yield increases, and rational nitrogen application promotes nutrient uptake, providing the material basis for dry matter and

yield accumulation.

### 3. Discussion

#### 3.1 Effects of Different Nitrogen Levels on Agronomic Traits of *Cyperus esculentus*

Fertilizers affect crop growth and development differently, thereby influencing agronomic traits and yield [19]. Nitrogen is an essential element for all organisms and plays a necessary role in crop growth and development [20]. Nitrogen deficiency inhibits new cell synthesis, slowing or stopping growth [21]. Nitrogen generally has the greatest impact on leaf development; deficiency inhibits both above-ground and root growth [22], while adequate nitrogen promotes robust development and accelerates leaf growth [23].

Our results showed that compared with the no-nitrogen treatment (N0), nitrogen application improved agronomic traits of *C. esculentus* at both sites. Above-ground traits including plant height, tiller number, and single leaf area increased with nitrogen rates from 0–225 kg · hm<sup>-2</sup>, with significant differences from N0. Previous studies on crops such as corn and rice under different nitrogen environments have shown that plants can alter root architecture to respond to rhizosphere nitrogen conditions [24]. In this study, below-ground traits including maximum root length and root volume increased with nitrogen application from 0–150 kg · hm<sup>-2</sup>, peaking at N2 with significant differences from N0. This indicates that favorable root architecture is an important pathway for efficient nutrient absorption. *Cyperus esculentus* can increase maximum root length and root volume to enhance nitrogen absorption under low nitrogen conditions, while reducing these traits under high nitrogen to regulate nitrogen uptake and balance plant nutrition. Therefore, rational nitrogen application improves agronomic traits and increases yield, resulting in better economic benefits.

#### 3.2 Effects of Different Nitrogen Levels on *Cyperus esculentus* Yield

Among the many factors affecting crop yield and its components, fertilizer is the most effective and fastest-acting variable [25] and can influence and constrain yield through effects on crop population structure and production performance [26]. *Cyperus esculentus* yield depends on the expansion degree and number of underground tubers, which are directly affected by plant growth status. Shen [27] found that as nitrogen application increased, above-ground dry matter mass of stems and leaves increased, but yield did not increase correspondingly, with maximum yield occurring at 67.5 kg · hm<sup>-2</sup>. Sun et al. [28] reported that when nitrogen application reached 120 kg · hm<sup>-2</sup>, leaf physiological characteristics, grain quality, and yield traits of *C. esculentus* reached maximum values. Our study found that whole-plant dry weight and tuber yield increased then decreased with nitrogen application, peaking at N2. At this level, dry weights were 132.40 g · plant<sup>-1</sup> and 138.81 g · plant<sup>-1</sup>, and tuber yields were 9298.87 kg · hm<sup>-2</sup> and 10336.06 kg · hm<sup>-2</sup> at Toketo and Dengkou, respectively.

Excessive nitrogen application ( $225 \text{ kg} \cdot \text{hm}^{-2}$ ) caused vigorous above-ground vegetative growth, increased ineffective tillers, and reduced transport of photosynthates to underground stems and roots, leading to insufficient tuber filling, low seed setting rates, and decreased yield [29]. Additionally, at the same nitrogen level, yield at Toketo was lower than at Dengkou, possibly due to soil salinization issues at Toketo [30]. Studies have shown that under saline-alkali stress, plants increase overall height to resist stress, and malondialdehyde content increases significantly [31].

### 3.3 Effects of Different Nitrogen Levels on Nutrient Balance

Soil nutrient balance reflects farmland nutrient demand and status, providing critical guidance for nutrient resource management and scientific fertilization [32]. Fertilizer rates for major crops in China are generally calculated through soil testing and target yield nutrient consumption, but current nitrogen rates for *C. esculentus* rely largely on experience or reference to similar crops, easily leading to excessive nitrogen residue and potassium deficiency. Therefore, determining rational nitrogen application rates is essential for improving fertilizer utilization while reducing losses and environmental pollution [33].

Our data from both experimental sites showed that nitrogen uptake increased then decreased with nitrogen application, peaking at N2. The apparent nitrogen balance value was negative at N0 and N1 but positive at N2 and N3, with nitrogen reaching a balanced state at N2 ( $150 \text{ kg} \cdot \text{hm}^{-2}$ ). A negative balance at N0 and N1 indicates soil nitrogen consumption, while a positive balance at N2 and N3 indicates nitrogen surplus. Research suggests that nitrogen surplus rates greater than 40% may pose environmental risks [34], making rational nitrogen application important for ecological protection. Potassium surplus rates in this study fell within the reasonable range of allowable potassium surplus [35]. However, the apparent phosphorus surplus was substantial, possibly related to phosphorus loss and fixation [36], which warrants further investigation.

## 4. Conclusion

Nitrogen application improved agronomic traits of *Cyperus esculentus* including tiller number, plant height, and single leaf area. At the N2 level ( $150 \text{ kg} \cdot \text{hm}^{-2}$ ), maximum root length, root volume, and other agronomic traits were optimal at both sites, with significant differences from the no-fertilizer treatment. Nitrogen application increased whole-plant dry weight and tuber yield, with maximum values at N2 producing tuber yields of  $9298.87\text{--}10336.06 \text{ kg} \cdot \text{hm}^{-2}$ . The apparent nitrogen surplus rate was negative at N0 and N1 but positive at N2 and N3, indicating that nitrogen reached a balanced state at the N2 level ( $150 \text{ kg} \cdot \text{hm}^{-2}$ ). Based on this experimental design, nitrogen application at approximately  $150 \text{ kg} \cdot \text{hm}^{-2}$  in northern sandy soils is beneficial for *C. esculentus* growth and development, promotes nutrient absorption, maintains soil nutrient balance, and supports yield formation.

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*Note: Figure translations are in progress. See original paper for figures.*

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