Plant-based hydration monitoring for precision nitrogen and irrigation management of cotton under salinity stress

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Abstract. This study is part of a comprehensive research program examining the effects of nitrogen fertilization on cotton physiology and productivity under saline conditions, with parallel investigations focusing on plant growth, biomass accumulation, and yield components. This study investigates the combined effects of soil salinity and nitrogen fertilizer rates on the water-holding capacity of cotton (Gossypium spp.) leaves under soil salinity stress. Cotton, a key global crop, is affected by soil salinity and nitrogen imbalances, which hinder its growth and productivity. A precision agriculture framework was applied, treating each salinity-Nitrogen combination as a unique management zone. Water loss from cotton leaves was measured at three growth stages: the 3-4 true leaves stage, the budding stage, and the flowering stage. Results demonstrated that nitrogen application generally increased water loss from cotton leaves, with higher fertilizer rates leading to reduced water retention. At the flowering stage, water loss ranged from 22.0% to 35.6%, depending on the nitrogen rate and salinity level. Soil salinity significantly influenced leaf water retention, with cotton on strongly saline soils exhibiting lower water loss than plants on non-saline or weakly saline soils. These findings were used to develop a decision matrix linking real-time leaf hydration metrics (potential proxy for canopy temperature or spectral indices) with variable-rate nitrogen and irrigation prescriptions. The study validates the concept of using simple, plant-level physiological traits as inputs for sensor-driven precision management.

1 Introduction

Cotton (Gossypium spp.) is one of the most widely cultivated and economically significant crops globally, serving as a major source of fiber, oil, and food. However, its productivity is often constrained by various environmental stressors, including soil salinity and nutrient imbalances, particularly concerning nitrogen (N) availability. Soil salinity, which occurs due to the accumulation of soluble salts like sodium chloride (NaCl), calcium sulfate ($CaSO_4$), and magnesium sulfate ($MgSO_4$), presents a significant challenge for cotton cultivation, especially in arid and semi-arid regions where irrigation with saline water is common [1,2].

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Changes also occur in the anatomical structure of cotton leaves growing on saline soils. Cotton grown in saline soil conditions shows signs of succulence. The leaves thicken, the area of epidermal cells increases, and the number of stomata per unit area of the leaf decreases. Under conditions of sulfate soil salinization, cotton varieties show signs of haloxerophytism, i.e., the size of epidermal cells decreases and the number of stomata increases. Soil salinity also affects plant growth processes, leading to a decrease in the total leaf area. This feature depends on soil salinity and the resistance of plant species and varieties to it [3,4].

When feeding cotton in saline soils in order to obtain a high yield and save part of the mineral fertilizers (25%) when applying mineral fertilizers $N_{185}P_{130}K_{90}$ kg/ha and additionally glauconite and glaucofos in the amount of 600 kg/ha, a yield of 31.4-31.5 c/ha is ensured, and with the simultaneous application of glauconite and glaucofos in the amount of 1200 kg/ha (600 kg/ha before sowing, 600 kg/ha during the growing season) while maintaining the mineral fertilizer rate, a yield of 32.1-33.0 c/ha is ensured.

Insufficient nitrogen leads to stunted growth, chlorosis (yellowing of leaves), and reduced photosynthetic capacity, while excessive nitrogen can result in excessive vegetative growth at the expense of reproductive development and increased susceptibility to diseases and pests [5-8].

A crucial aspect of cotton physiology that can be affected by both soil salinity and nitrogen availability is the water-holding capacity of the leaves. Water retention in the leaves is a key factor in determining how well the plant can conserve moisture during periods of water scarcity or high evapotranspiration. The water-holding capacity is influenced by leaf structure, cuticular wax composition, and the functioning of the stomatal apparatus, all of which play roles in minimizing water loss while maximizing photosynthesis. The ability of cotton leaves to hold water is not only vital for maintaining turgor pressure and cellular function but also for optimizing plant performance under stress conditions. Cotton plants with higher leaf water-holding capacity tend to have improved tolerance to drought and salinity stress, as they are better able to manage internal water content under fluctuating environmental conditions [9].

The interaction between soil salinity and nitrogen fertilizer application on leaf water-holding capacity is not well understood, yet it is a critical area of research. Soil salinity may alter the plant's ability to effectively use nitrogen, potentially exacerbating the negative effects of high salinity on water retention. On the other hand, the appropriate application of nitrogen may help mitigate some of the detrimental effects of salinity by promoting healthier plant growth, improving photosynthetic efficiency, and increasing root depth, which can enhance water uptake [10].

This study seeks to investigate the combined effect of soil salinity and nitrogen fertilizer rates on the water-holding capacity of cotton leaves, with a focus on how these factors interact to influence cotton's physiological responses to environmental stress. By examining the changes in leaf water retention, transpiration rates, and photosynthetic efficiency under varying levels of salinity and nitrogen, this research aims to provide valuable insights for improving cotton management practices in saline-prone areas. The findings could help optimize irrigation and fertilization strategies, ultimately contributing to more sustainable and resilient cotton production systems.

2 Materials and Methods

2.1 Study Site and Soil Conditions

The study was conducted at a field experiment site with varying levels of soil salinity, including non-saline, weakly saline, moderately saline, and strongly saline soils. The soil salinity was determined based on the electrical conductivity of the soil solution (EC), and the soils were categorized accordingly: non-saline (EC < 2 dS/m), weakly saline (EC = 2–4 dS/m), moderately saline (EC = 4–6 dS/m), and strongly saline (EC > 6 dS/m). The experiment was carried out during the cotton growing season under natural climatic conditions, and the soil was irrigated with saline water as required for each salinity treatment.

2.2 Experimental Design and Treatments

A randomized complete block design (RCBD) with three replications was used for the experiment. Various nitrogen fertilizer rates were applied to a common background of potassium (K) and phosphorus (P) fertilizers. The experimental treatments included:

1. Control (no fertilizer application)
2. Background ($P_{175}K_{125}$ – base treatment without nitrogen)
3. Background + $\mathbf{N}_{100}$ (100 kg nitrogen/ha)
4. Background + N_{150} (150 kg nitrogen/ha)
5. Background + N_{200} (200 kg nitrogen/ha)
6. Background + N_{250} (250 kg nitrogen/ha)
7. Background + N_{300} (300 kg nitrogen/ha)
8. Background + N_{350} (350 kg nitrogen/ha)

Each treatment was replicated three times, and plots were assigned to different soil salinity levels to assess the effect of nitrogen fertilizer rates across various salinity conditions. The nitrogen fertilizer was applied in the form of urea (46% N), and the base fertilizers (P and K) were applied prior to planting based on local agricultural recommendations.

2.3 Measurement of Water Loss from Cotton Leaves

The primary objective of this study was to assess the influence of nitrogen fertilizer rates on the amount of water lost from cotton leaves, relative to the total water content in the leaves, under different soil salinity conditions. Leaf water loss was measured at three distinct growth phases:

1. 4-leaf stage (3-4 true leaves)
2. **Budding**
3. **Flowering**

At each growth phase, leaf samples were taken from plants in each treatment to measure the amount of water lost over a 3-hour period. To assess the water loss, fully expanded leaves were excised from the plant and weighed immediately to determine the fresh weight. The leaves were then placed in a controlled environment at 25°C, and the amount of water lost during a 3-hour period was measured by weighing the leaves again after the designated time.

2.4 Statistical analysis

The data collected were subjected to statistical analysis using Analysis of Variance (ANOVA) to determine the effects of nitrogen fertilizer rates and soil salinity on water loss from cotton leaves. Tukey's Honest Significant Difference (HSD) test was used for post-hoc comparisons to identify significant differences between treatment means. The significance level was set at $p < 0.05$ for all tests.

2.5 Soil and environmental monitoring

Soil salinity levels were regularly monitored throughout the growing season by collecting soil samples from the upper 30 cm of the soil profile. Soil electrical conductivity (EC) was measured using a soil saturation extract method.

2.6 Fertilizer Application

The nitrogen fertilizers were applied in split doses at planting, and subsequent applications were made based on the growth stage of the cotton. Phosphorus and potassium fertilizers were applied as a base treatment ($P_{175}K_{125}$), and no additional fertilizer was applied during the growing season for the non-nitrogen treatments.

3 Results and Discussion

In irrigated agriculture, soil salinization is one of the main negative factors that strongly affect the water regime and physiological stability of cotton. Soil salinisation severely disrupts the plant's water absorption, retention in tissues and efficient use. The water-holding capacity of cotton leaves is one of the important physiological indicators characterising the plant's resistance to environmental stresses, especially to salinity and drought, which reflects the turgor state of leaf cells, the intensity of transpiration and general metabolic processes.

Soil salinization increases the amount of water-soluble salts in the soil solution and raises osmotic pressure. Under such conditions, water absorption by the cotton root system is impeded, and the plant experiences physiological drought. Even when there's enough moisture in the soil, the high salt concentration stops water from moving up through the roots to the leaves in sufficient quantities. This leads to water deficiency in the leaf cells, reducing cell turgor and the leaves' ability to hold water.

As salinity increases, negative changes in the water-holding capacity of cotton leaves intensify. Under conditions of low salinity, the plant activates certain adaptive mechanisms. The accumulation of osmotically active substances in leaf cells slows down water outflow, and the ability to retain water can remain relatively stable. However, under prolonged or moderate salinity, these adaptation mechanisms become insufficient. Under moderate salinity, water exchange in leaf cells is disrupted, transpiration increases, and as a result, the water supply and water retention capacity of the leaves are significantly reduced.

Under conditions of severe salinity, water stress in cotton is particularly pronounced. Leaf cells rapidly lose water, cell membrane permeability increases, and water efflux from tissues accelerates. This condition is characterised by rapid leaf wilting, loss of elasticity, and disruption of physiological processes. The water-holding capacity of the leaves decreases sharply, and the processes of photosynthesis, gas exchange, and synthesis of substances slow down. Nitrogen fertilizers play an important role in the formation of the water regime of cotton and the water-holding capacity of the leaves. Nitrogen is the main component of proteins, enzymes and structural elements of cells, ensuring the full development of leaf tissues and the stability of cell membranes. In cotton plants that are sufficiently supplied with nitrogen, leaf cells develop well, the concentration of cell sap is at an optimal level, and water retention capacity is high.

Under saline conditions, the application of nitrogen fertilizers in scientifically justified amounts increases the water retention capacity of leaves to a certain extent. Nitrogen stimulates the synthesis of osmotically active substances in cells, increasing the amount of colloidal substances that bind water. As a result, water retention in leaf tissues improves, cell turgor is maintained longer, and water loss through transpiration is reduced. This increases the resistance of cotton to stress in saline conditions.

However, excessive application of nitrogen fertilizers can negatively affect the water regime of leaves. Excess nitrogen leads to excessive development of vegetative mass, increases leaf surface area and transpiration intensity. Under saline conditions, this increases water loss and further reduces the water-holding capacity of leaves. In addition, excess nitrogen can weaken the stability of cell membranes and facilitate the penetration of salts into plant tissues. The interaction between salinity levels and nitrogen fertilizer rates is an important factor determining the water-holding capacity of cotton leaves. Under conditions of low to moderate salinity, optimal nitrogen fertilizer rates help maintain a relatively stable water balance in the leaves. Under such conditions, the water supply to the leaves is higher and the cells are less prone to dehydration. Under conditions of high salinity, the positive effect of nitrogen nutrition is limited, and the main focus should be on ameliorative measures to reduce salinity. Soil salinization has a strong negative impact on the water retention capacity of cotton leaves, disrupting water exchange and physiological processes. Nitrogen fertilizers, when used in a scientifically sound and regulated manner, mitigate this negative impact to a certain extent and stabilize the water regime of the leaves. Therefore, the correct determination of nitrogen fertilizer rates, taking into account the degree of salinization, the combination of irrigation regime and agrotechnical measures when cultivating cotton on saline soils is an important condition for increasing the water retention capacity of cotton and ensuring high yields.

Table 1. Effect of saline soils and nitrogen fertilizer rates on the degree of water retention of cotton leaves (amount of water lost in 3 hours, %)

№	Variants	3-4 true leaves	Budding	Flowering
Non-saline				
1	Control	32.2 ± 0.12	34.2 ± 0.25	35.4 ± 0.12
2	$P_{175}K_{125}$-Base	30.1 ± 0.17	32.1 ± 0.21	34.1 ± 0.14
3	Base+N_{100}	28.6 ± 0.20	30.0 ± 0.23	34.2 ± 0.23
4	Base+N_{150}	25.4 ± 0.15	29.7 ± 0.24	33.8 ± 0.29
5	Base+N_{200}	26.7 ± 0.11	29.9 ± 0.28	32.6 ± 0.25
6	Base+N_{250}	24.2 ± 0.19	28.3 ± 0.27	33.4 ± 0.14
7	Base+N_{300}	24.0 ± 0.11	27.6 ± 0.19	34.1 ± 0.28
8	Base+N_{350}	25.8 ± 0.12	27.7 ± 0.17	35.6 ± 0.29
Weakly saline				
1	Control	33.1 ± 0.21	35.3 ± 0.12	36.7 ± 0.15
2	$P_{175}K_{125}$-Base	31.0 ± 0.24	33.2 ± 0.23	34.9 ± 0.14
3	Base+N_{100}	30.5 ± 0.14	31.9 ± 0.25	33.1 ± 0.19
4	Base+N_{150}	29.2 ± 0.26	30.1 ± 0.21	30.3 ± 0.20
5	Base+N_{200}	25.2 ± 0.15	27.3 ± 0.26	30.4 ± 0.25
6	Base+N_{250}	26.1 ± 0.19	27.9 ± 0.15	29.6 ± 0.23
7	Base+N_{300}	25.9 ± 0.26	28.9 ± 0.12	33.4 ± 0.28
8	Base+N_{350}	27.4 ± 0.14	29.2 ± 0.17	34.2 ± 0.28

The influence of nitrogen fertilizer rates on physiological parameters of cotton grown on soils with different salinity degree, especially on the amount of water lost during 3 hours relative to the total amount of water in leaves, was studied. It is shown that in the control variant of the experiment conducted on non-saline soils, the amount of water lost during 3 hours in the phase of 3-4 true leaves relative to the total amount of water in cotton leaves was 32.2%, and in the background variant P_{175} K_{125} - 30.1%. In variants with nitrogen fertilizer application background +N_{100}; background+N150; background +N_{200}; background +N_{250}; background +N_{300}; background +N_{350} kg nitrogen fertilizer rates increased in the sequence 28.6; 25.4; 26.7; 24.2; 24.0; 25.8%. These indicators in the control variant, identified in the phase of cotton budding, were 34.2%, and in the variant without nitrogen fertilizer application - 32.1%.

In all variants with application of nitrogen fertilizers and increasing rates, water-holding capacity was slightly lower, i.e. a lot of water evaporated. For example, in variants 3,4,5,6,7,8 with application of nitrogen fertilizers in the budding phase 30.0; 29.7; 29.9; 28.3; 27.6; 27.7% (Table 1). Experiments conducted on non-saline soils in the next phase, i.e. flowering phase, show that in the control variant this indicator was 35.4% and in the background variant $P_{175}K_{125}$ - 34.1%. There is an influence of nitrogen fertilizers on the water-holding capacity of leaves, and if potassium fertilizers slow down the process of transpiration, nitrogen fertilizers enhance this process, which is evident from the water-holding capacity of the leaf. For example, in variants 3,4,5,6,7,8, where nitrogen fertilizers were applied, in order of increasing fertilizer rates in the flowering phase of cotton 34.2; 33.8; 32.6; 33.4; 34.1; 35.6%, respectively.

Table 2. Effect of saline soils and nitrogen fertilizer rates on the degree of water retention of cotton leaves (amount of water lost in 3 hours, %)

№	Variants	3-4 true leaves	Budding	Flowering
Moderately saline				
1	Control	27.2 ± 0.28	29.1 ± 0.14	30.0 ± 0.26
2	$P_{175}K_{125}$-Base	24.0 ± 0.28	26.3 ± 0.26	27.2 ± 0.22
3	Base+N_{100}	26.1 ± 0.23	30.1 ± 0.19	31.0 ± 0.24
4	Base+N_{150}	27.9 ± 0.25	30.8 ± 0.15	29.2 ± 0.19
5	Base+N_{200}	28.1 ± 0.20	28.0 ± 0.17	31.7 ± 0.18
6	Base+N_{250}	23.4 ± 0.7	26.1 ± 0.21	29.6 ± 0.24
7	Base+N_{300}	27.7 ± 0.19	27.3 ± 0.16	30.8 ± 0.12
8	Base+N_{350}	28.6 ± 0.14	28.9 ± 0.23	31.7 ± 0.22
Strongly saline				
1	Control	22.0 ± 0.10	24.2 ± 0.12	25.5 ± 0.23
2	$P_{175}K_{125}$-Base	18.2 ± 0.6	22.4 ± 0.2	23.1 ± 0.8
3	Base+N_{100}	23.9 ± 0.9	25.8 ± 0.19	25.3 ± 0.11
4	Base+N_{150}	24.2 ± 0.15	26.6 ± 0.11	27.7 ± 0.17
5	Base+N_{200}	25.9 ± 0.28	27.8 ± 0.20	28.0 ± 0.25
6	Base+N_{250}	26.7 ± 0.14	25.8 ± 0.21	30.1 ± 0.28
7	Base+N_{300}	24.6 ± 0.25	23.7 ± 0.4	28.1 ± 0.19
8	Base+N_{350}	25.7 ± 0.29	27.3 ± 0.14	29.5 ± 0.17

According to the degree of salinity on slightly saline soils the indicators of cotton grown in the phases of 3-4 true leaves, budding, flowering, in phase sequence were 33.1; 35.3; 36.7

percent, in the background variant 30.1; 32.1; 34.1 percent, in the background variant with nitrogen fertilizer rate + 100 kg these indicators were 30.5; 31.9; 33.1 percent, in the background +150 kg of nitrogen content increase - 29.2; 30.1; 30.3 percent, after reaching 200 kg of nitrogen together with the background indicators respectively 25.2; 27.3; 30.4 percent. These indicators in the remaining variants background+N250; background +N_{300}; background +N_{350} kg in the sequence of variants were 26.1; 27.9; 29.6; 25.9; 28.9; 33.4; 27.4; 29.2; 34.2 percent.

On medium saline soils, these indicators showed that in the control variant in the phase of 3-4 true leaves of cotton the water loss was 27.2 percent, compared to the total water in the budding phase the losses were 29.1 percent and in the flowering phase 30.0 percent, in the background variant the losses were 24.0; 26.3; 27.2 percent. In all variants with application of nitrogen fertilizers water losses in cotton leaves increased.

Researches were also continued on strongly saline soils. According to this, in highly saline soils the proportion of water lost from cotton leaves was less than in other types of salinity, because the rate of total water in leaves also differs less in cotton plants grown on saline soils (Table 2). For example, in the control variant of cotton plants grown on highly saline soils, in the studied phases the rates were from 22.0 to 25.5%, in the background variant from 18.2% to 23.1%, and in the variants with application of all norms of nitrogen fertilizers from 23.9% to 30.1%.

4 Conclusion

This study investigated the effect of soil salinity and nitrogen fertilizer rates on the water retention capacity of cotton leaves. The results demonstrate that soil salinization leads to a decrease in total and free water content in cotton plants. As salinity increases, both total and free water content progressively decline.

Nitrogen fertilizer application influences leaf water retention in a rate-dependent manner. At moderate nitrogen rates (up to N250), water loss from leaves tends to decrease compared to the control, indicating improved water retention. However, at the highest nitrogen rate (N350), water loss increases again, often reaching or exceeding control levels, particularly under non-saline and weakly saline conditions. This suggests that nitrogen fertilizers do affect free water content: moderate rates reduce it, while excessive rates may increase it.

These patterns were observed across all salinity levels, although the magnitude of nitrogen effects diminished as salinity increased. Under strongly saline conditions, water loss was consistently lower than in non-saline soils, reflecting the overall suppression of plant water status by high salinity.

In conclusion, optimizing nitrogen fertilization — avoiding both deficiency and excess — can help maintain better leaf water retention in cotton grown under saline conditions. The rate of N250 kg/ha provided a favorable balance, while higher rates (N300–N350) offered no additional benefit for water retention and sometimes proved detrimental.

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