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Plant Developmental Responses to Plasma-Synthesized Nitrate: Concentration- and pH-Dependent Effects on Root Architecture, Reproduction and Drought Tolerance

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Abstract:

Sustainable nitrogen fertilization requires alternatives that can reduce dependence on centralized, fossil-intensive ammonia production while providing nitrogen in a biologically effective form with reduced environmental impact. Although research on nitrogen fertilizer synthesis through thermal and non-thermal plasma routes is advancing rapidly, a comprehensive understanding of plant responses to plasma-derived fertilizers remains somewhat elusive, given the limited evidence available to date. Here, we evaluated the plant response to nitrate synthesized by an atmospheric-pressure catalytic thermal-plasma system using *Cicer arietinum* (chickpea) as a model crop. Soil and hydroponic experiments were combined to investigate nitrate- and pH-dependent responses from root development to reproduction and drought tolerance. Nitrate availability produced pronounced, reproducible, non-monotonic changes in root architecture and root–shoot coordination. Nitrogen limitation promoted a remarkably extensive exploratory root phenotype, whereas intermediate nitrate availability supported comparatively coordinated root and shoot development. Increasing nitrate beyond this range progressively altered root organization and restricted root-system expansion. Importantly, vegetative vigour and reproductive progression were not equivalent: under the experimental conditions, plants supplied with 35 ppm nitrate exhibited the most advanced transition toward fruit initiation, whereas higher nitrate concentrations exhibited delayed reproductive development. Root anatomical observations further revealed nitrate-associated structural differentiation. pH additionally modulated nitrate-associated root development and drought response substantially. Collectively, these findings identify a concentration- and pH-dependent developmental window for plasma-synthesized nitrate and provide a multi-scale framework linking fertilizer chemistry with root architecture, biochemical state, reproductive development and stress response.

1. Introduction

Nitrogen demand of modern agriculture is far beyond natural inputs [1-3], making industrial fixation absolutely essential for sustained crop production [1,4]. The Haber–Bosch (H–B) process revolutionized agriculture by enabling large-scale ammonia production, ~70% of which is used for fertilizers. While maturity and scalability of H-B is now an industrial benchmark, the process itself, relying on fossil derived feedstock is not sustainable as the feed stock reserve on earth is depleting fast and becoming increasingly costlier due to fast changing geopolitical scenario. Also, its substantial energy demand, and enormous CO_2 emissions impose significant environmental threats [5,6]. Subsequent losses of reactive nitrogen through volatilization, leaching and denitrification further contribute to greenhouse-gas emissions, eutrophication and disruption of the global nitrogen cycle [7,8]. The challenges have motivated the development of complementary CO_2 free nitrogen-fixation technologies in which atmospheric-pressure plasma route is particularly promising. It activates naturally abundant atmospheric N_2 and O_2 to generate reactive nitrogen species that can be converted into aqueous nitrate and nitrite without demanding fossil-derived feedstocks and a centralized infrastructure [9].

The research on plasma-derived nitrogen fertilizer has predominantly focused on non-thermal atmospheric-pressure plasmas and plasma-activated water (PAW). While effectiveness of PAW on seed germination, plant growth and physiological responses is reported in literature [10,11], actual agricultural application is severely hindered by its poor synthesis rate and issues with scaling up. Moving beyond device-level demonstrations toward integrated optimization of plasma reactors is the primary challenge. Also, systematic biological evaluation of the resulting nitrate fertilizer is an extreme necessity. A significant technological gap exists between efficient nitrogen fixation and practical agronomic functionality of the resulting fertilizer [9]. Most of the reported studies have concentrated primarily on plasma chemistry, seed germination or early plant growth, rather than following a defined plasma-derived nitrate fertilizer through extended vegetative and reproductive development. Little attention is paid on establishing a concentration-dependent response window for plasma-derived nitrate in which root, shoot and reproductive developments are considered together. Nitrate concentration and solution pH are tightly interlinked in any plasma synthesized fertilizer. Higher the nitrate concentration, lower is the pH value and both concentration and pH value has strong potential to influence the nutrient availability and root-zone physiology. As far as plasma synthesized fertilizer is concerned, this is a serious concern. However, this important fact has rarely taken the central stage in the reported studies. Root responses are mostly assessed from gross growth parameters. The relationship between nitrate-dependent root architecture, anatomical organization and biochemical state remains insufficiently explored. Also, the possible consequences of nitrate-dependent root architectural plasticity for plant response to subsequent water limitation remain poorly understood for plasma synthesized fertilizer.

Recently, Ghorui *et al.* followed an atmospheric pressure thermal plasma route and developed a portable catalytic thermal-plasma system for rapid synthesis of aqueous nitrate and nitrite from air and water [12,13]. Distinct differences with non-thermal plasma processes include several orders of magnitude higher nitrate production rate and reactive species generation, insignificant/zero H_2O_2 production, post synthesis auto conversion of nitrite into nitrate and operation in the thermal regime where electron and gas temperature both are in the eV range. The synthesis rate is high enough to make it directly adoptable for mass agricultural application. Fast synthesis, operability with renewable energy, zero CO_2 emission, feasible small-scale distributed production and less capital investment are the key attractive features of the developed system. With respect to H-B process, the system avoids safety hazards and costs related to the requirements of continuous energy resources, pressurized environment for synthesis, regulated storage, refrigeration need, transportation of raw materials and distribution of fertilizer. This development now shifts the central research question from whether atmospheric plasma can fix nitrogen to the more consequential question of whether the resulting plasma-synthesized nitrate can provide biologically effective nitrogen across the developmental life cycle of an agricultural crop.

With the mentioned gap areas in mind, the present study is designed to examine the response to thermal plasma synthesized nitrate [12,13] across soil and controlled hydroponic environments using *Cicer arietinum* (chickpea) as a model system. Chickpea is among the world's major food legumes and is cultivated extensively across South Asia, the Middle East, North Africa, Australia and other semi-arid and temperate regions [14]. Its agronomic importance, sensitivity to water availability and substantial dependence on coordinated root development make it an appropriate model crop to examine the biological functionality of alternative nitrogen-fertilization strategies. The experimental framework connects the fertilizer concentration and physicochemical conditions with root development, shoot growth, tissue organization, reproductive progression, drought response and biochemical signatures. Such an integrated design permits the biological response to be considered as a developmental continuum rather than as a collection of independent phenotypic observations.

Chemical production of nitrate does not necessarily translate directly into agronomic performance. Once supplied to plants, nitrate functions simultaneously as a nutrient and as a developmental signal. Plants sense nitrate through transporter–signalling systems including NRT1.1/NPF6.3 and NRT2-family transporters, and nitrate-responsive signalling interacts with calcium, auxin and transcriptional networks to regulate root-system architecture [15-17]. Consequently, plant responses to nitrate are not necessarily monotonic. Depending on concentration, spatial distribution and whole-plant nitrogen status, nitrate can stimulate lateral-root development and nutrient-foraging responses at one level while suppressing aspects of root growth at another [15-17]. Root-system architecture is therefore not simply an output of nitrogen nutrition; it represents a dynamic developmental response through which plants adjust their capacity to acquire water and nutrients from the surrounding environment. Nitrogen status influences shoot growth, branching, biomass allocation, developmental timing and reproductive transition through interactions involving carbon–nitrogen metabolism and multiple phytohormonal pathways. In the existing studies the effects of nitrogen on reproductive development are comparatively less integrated with studies of root responses [18]. Importantly, greater nitrogen availability does not necessarily result in proportionally greater whole-plant performance. Excessive or imbalanced nitrogen can alter developmental allocation and delay or modify vegetative vigor, root nutrient-foraging capacity and reproductive performance [18].

The study integrates soil and hydroponic experiments to evaluate the biological effectiveness of thermal-plasma-synthesized nitrate under agronomically relevant and controlled conditions, enabling systematic assessment of nitrate concentration and pH effects on plant development. Temporal analysis revealed pronounced nitrate-dependent plasticity in root architecture and root–shoot coordination, demonstrating that increased nitrate supply does not necessarily result in progressively greater growth and that extensive root development may reflect nutrient-foraging responses rather than superior whole-plant performance. Root anatomical analysis further linked morphological differences with changes in tissue organization and biochemical signatures. The study additionally examined reproductive progression and drought response, recognizing that optimal vegetative growth does not necessarily coincide with advanced reproductive development or improved drought performance. The principal novelty lies in integrating atmospheric-pressure thermal-plasma nitrate production with a multiscale assessment extending from nitrate concentration and pH through root biochemical state, root architecture, root–shoot development, flowering and fruit initiation, and drought response. Collectively, the findings support a concentration- and pH-dependent developmental window for the biological effectiveness of plasma-derived nitrate rather than a simple monotonic growth response, and establish an integrated framework connecting atmospheric thermal-plasma based N_2 activation, aqueous nitrate production, fertilizer chemistry and whole-plant function.

In the subsequent sections seven interconnected aspects of plasma-derived nitrate utilization in C. arietinum are presented: (i) nitrate-dependent vegetative performance under soil cultivation; (ii) temporal evolution of coordinated root–shoot development under controlled hydroponic conditions; (iii) nitrate-dependent plasticity of root architecture and root–shoot development; (iv) pH-dependent modulation of nitrate-associated root architecture and root–shoot coordination; v) nitrate-dependent alterations in root tissue organization and anatomical differentiation; (vi) nitrate-dependent progression of flowering and fruit initiation; and (vii) nitrate- and pH-dependent drought response and root–shoot coordination.

By integrating these measurements, the study seeks to establish a biologically grounded framework for evaluating thermal plasma synthesized nitrate as an alternative nitrogen source. More generally, the work demonstrates the importance of evaluating emerging fertilizer technologies across chemical, structural, biochemical, developmental and stress-response scales, rather than relying on a single growth endpoint. Such an integrated framework can inform future optimization of plasma-derived nitrate production, fertilizer formulation, concentration management and application strategies, while providing a basis for subsequent studies addressing nitrogen-use efficiency, field performance and crop yield.

2. Materials & Methods:

A combination of spectroscopic, chromatographic, microscopic, and physicochemical characterization techniques was employed to systematically investigate the plasma-generated nitrate fertilizer and its effects on Cicer arietinum. Optical emission spectroscopy was used to understand the spectral differences in natural sunlight and the light employed for the indoor study, whereas ion chromatography was employed for quantitative determination of nitrate and nitrite concentrations in the synthesized fertilizer. Optical microscopy was further employed to examine treatment-dependent changes in root microstructure and anatomical characteristics. In addition, pH levels were monitored to characterize the physicochemical environment of the fertilizer solutions. All measurements were performed using calibrated instruments

Optical emission spectroscopy (OES) was performed using an Ocean SR spectrometer (Ocean Optics, USA). The optical signal was collected through an optical fiber and coupled to the spectrometer, where the incident radiation was dispersed by a diffraction grating and detected using a linear detector array.

Microstructural features of the root sections were captured using an advanced trinocular optical microscope (Model DX-2153-APLi, Delphi-X Observer, Euromex Microscope BV, Netherlands) coupled with a Euromex Image Focus Plus digital camera tracking system. The instrument featured a high-intensity 100 W Halogen illumination system coupled with an infinity-corrected EIS optical layout. Image collection was executed utilizing high-numerical-aperture Plan Semi-Apochromatic (APLi) objectives (10x/0.30 oil immersion) to guarantee high resolving power and exact color rendering across a 25 mm field of view.

The pH of the solutions was determined using a portable, waterproof digital pen-type pH meter (Model AM-P-PH, Aquasol Digital, India). The instrument featured an operational measurement range of 0.00 to 14.00 pH, a resolution of 0.1 pH, and an accuracy profile within ±2% of full scale. To eliminate errors introduced by thermal variations, the unit utilized a built-in Automatic Temperature Compensation (ATC) system operating across a 0 to 80°C window. Prior to sample measurements, the meter was calibrated via an automatic 3-point calibration protocol using standard buffer solutions at pH 4.0, 7.0, and 10.0.

Root and shoot biomass were measured using a Sartorius CPA225D analytical balance (Sartorius AG, Göttingen, Germany) with a readability of 0.01 mg. Root and shoot samples were weighed separately after removing excess surface moisture, and the stable readings were recorded as the respective root and shoot masses.

Ion chromatography technique [19,20] was used to determine the concentrations of NO_2^- and NO_3^- ions in the plasma fertilizer used. A commercially available ion chromatography system (Metrohm 940 Professional IC Vario, Herisau, Switzerland) was used for this. The reported concentrations have a maximum analytical error of ±5%. Prior to instrumental analysis, the plasma-fertilizer samples were passed through 0.22 µm nylon membrane filters to remove any suspended particulate matter and were subsequently diluted appropriately with deionized water. The operating conditions used for the ion chromatographic analysis are summarized in Table 1. Identification and quantification of NO_2^- and NO_3^- were carried out by comparing the retention times and peak areas of the analytes with those obtained from the corresponding standard solutions. A commercially available multi-element ion chromatography anion standard solution (1.0 mg mL^{-1}) was used to prepare a series of mixed working standards in the concentration range of 0.05–10.0 mg L^{-1} for calibration and sample analysis. Ultrapure water with a resistivity of 18.2 MΩ·cm at 25 °C, obtained from a Millipore Synergy UV® ultrapure water purification system (USA), was used throughout the analysis.

Table 1. Ion chromatographic conditions used for the determination of NO_2^- and NO_3^- ions

Instrument	Metrohm-940 Professional IC Vario
Column	Metrosep A Supp-5- 250/4.0
Guard column	Metrosep A Supp-5- Guard/4.0
Analytical column temperature	27º C
Eluent composition	3.2 mM Na_2CO_3 + 1.0 mM $NaHCO_3$
Eluent Flow rate	0.7 mL min^{-1}
Sample volume	20 µL
Injection technique	Manual
Detection	Suppressed conductivity detection
Data analysis software	MagICNet (Version 4.0)

Initially, nitrogen fertilizer of desired concentration (mg/L of Nitrate +Nitrite) was prepared via thermal plasma synthesis route [12]. As reported in our previous study [12], the nitrite automatically gets converted into nitrate fast and nearly a complete conversion to nitrate is obtained within five days after synthesis. The final concentration of nitrate remains stable for months. The pH of the liquid nitrate fertilizer was adjusted using potassium hydroxide (KOH) of analytical reagent (AR) grade (>99% pure, Annexe Chem Pvt Ltd, Mumbai). A freshly prepared aqueous KOH solution was added gradually to the nitrate-containing solution under continuous stirring until the desired pH was attained. The use of AR-grade KOH minimized the introduction of extraneous ionic impurities that could potentially influence the chemical composition of the fertilizer solution or interfere with subsequent analytical measurements.

It should be noted that KOH addition primarily serves to adjust the pH of the fertilizer solution and simultaneously introduces K^+ ions. Therefore, the amount of KOH added was controlled carefully to achieve the target pH while avoiding unnecessary changes in the ionic composition of the fertilizer. The pH was measured using a calibrated pH meter after adequate mixing and equilibration of the solution.

For indoor cultivation of Cicer arietinum, white fluorescent tube lights (20 W, 2000 lm) were employed as artificial illumination sources in place of natural sunlight. The illumination was provided for 9.5 hours every day using programmed device. The spectral characteristics of the fluorescent lamp, shown in Fig. 1(b), exhibit a strong, narrow emission peak in the blue region (~430–450 nm) and a broad emission band extending through the green–yellow–red region (~500–650 nm). Together, these emissions provide substantial spectral coverage within the photosynthetically active radiation range (400–700 nm), which is

essential for plant photosynthesis and growth. Chlorophyll-a absorbs strongly in the blue (~430 nm) and red (~660–680 nm) regions, while chlorophyll-b extends light absorption particularly around the blue (~450–470 nm) and red-orange (~640–650 nm) regions, broadening the effective light-harvesting range [21].

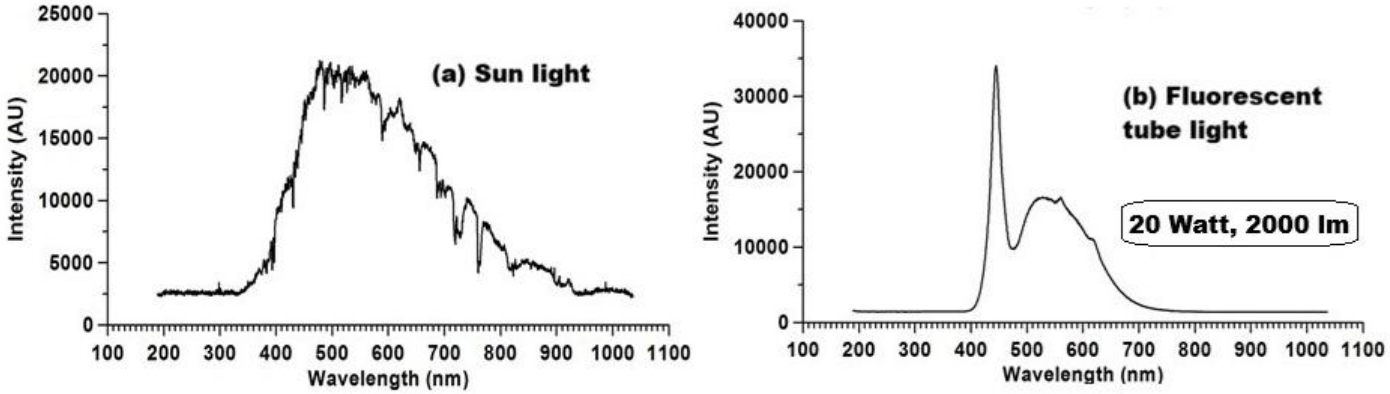


Figure 1. Spectral feature of the light used in indoor hydroponics

In comparison with the relatively continuous and broad spectral distribution of sunlight [Fig. 1(a)], the fluorescent lamp exhibits a more structured spectral profile, characterized by distinct emission features [Fig.1(b)]. Nevertheless, its output encompasses the major wavelength regions required for photosynthetic activity. The pronounced blue emission is particularly relevant to chlorophyll formation, photosynthetic development, stomatal regulation, and photomorphogenic responses, while the broad emission extending from the green to red region contributes to photosynthetic carbon assimilation and overall plant development.

The relatively limited emission beyond 700 nm compared with sunlight may also be advantageous for controlled indoor experiments, as it reduces the influence of far-red-mediated photomorphogenic responses, including excessive stem elongation. Furthermore, the use of a fixed artificial light source provides a stable and reproducible illumination environment, allowing the light exposure period and experimental conditions to be controlled independently of variations in natural sunlight intensity and spectral composition. The exposure period was precisely controlled by a microcontroller. Based on the spectral coverage within the photosynthetically active radiation region and its stable operating characteristics, the selected 20 W, 2000 lm fluorescent tube light provides a practical artificial illumination source for controlled indoor cultivation of C. arietinum. Its use facilitates systematic investigation of plant responses to experimental variables such as nitrate concentration and pH, while minimizing the day-to-day variability associated with natural solar illumination.

3. Results and Discussion

3.1 Nitrate-Driven Growth Optimum and Vegetative Performance of Cicer arietinum under Soil Conditions

Nitrogen availability is a major determinant of plant vegetative performance, influencing leaf formation, shoot elongation, biomass accumulation, and the allocation of resources between root and shoot systems. However, plant responses to nitrogen supply are not necessarily linear, as both insufficient and excessive nitrate availability can constrain growth through distinct nutritional and physiological mechanisms. To examine this response under soil-grown conditions, *Cicer arietinum* was cultivated across a broad range of externally supplied nitrate concentrations, and plant development was evaluated through complementary morphological and quantitative growth measurements. Identically germinated seeds were planted in six trays. Each tray contained 12 seeds under identical soil environment and the seedlings were allowed to grow with regular watering but no external fertilizer. After the seedlings grew above the soil level a natural statistical variation in heights of the seedlings was noticed as seen in Fig 2(a). Purposefully, the tray with highest average seedling height was chosen as control (labelled-A) and allowed to grow with regular watering alone without external fertilizer. Remaining trays with reducing seedling heights were labelled as ‘B’ to ‘F’ and subjected to application of plasma fertilizer (pH-7) of concentration 288, 143, 108, 68 and 35 ppm respectively. The day of application of plasma fertilizer was marked as ‘Day-1’. Corresponding seeding conditions before application of plasma fertilizer is seen in Fig.2(a). No external fertilizer was applied after one time application of this fertilizer till ‘Day-8’. Only regular watering was done. Particular emphasis was placed on identifying whether increasing nitrate progressively enhances growth or instead produces a distinct concentration-dependent optimum, thereby establishing the nitrate range associated with favorable vegetative development under the experimental conditions.

The combined morphological and quantitative analyses demonstrate a pronounced non-linear response of *Cicer arietinum* to externally supplied nitrate. Differences among treatments became progressively more evident during plant development, with the Day-8 photographs in Figure 2(c) showing substantial divergence in shoot height, leaf development and canopy architecture. These visual observations were quantitatively supported by the measured growth rates [Figure 3] averaged over 12 seedlings, which increased from approximately 13 mm day^{-1} in the nitrate-free treatment to a maximum of 27 ± ~1 mm day^{-1} at 35 ppm nitrate, corresponding to an approximately two-fold enhancement in growth rate. Importantly, increasing nitrate concentration beyond 35

ppm resulted in a progressive reduction in growth rate, reaching approximately 23, 20 and 17 mm day^{-1} at 68, 102 and 143 ppm, respectively. The 288-ppm treatment exhibited a modest recovery to approximately 18 mm day^{-1} but remained substantially below the 35-ppm value. Thus, within the investigated concentration range, the growth response was clearly non-monotonic, with 35 ppm representing an apparent optimum for shoot growth under the present experimental conditions.

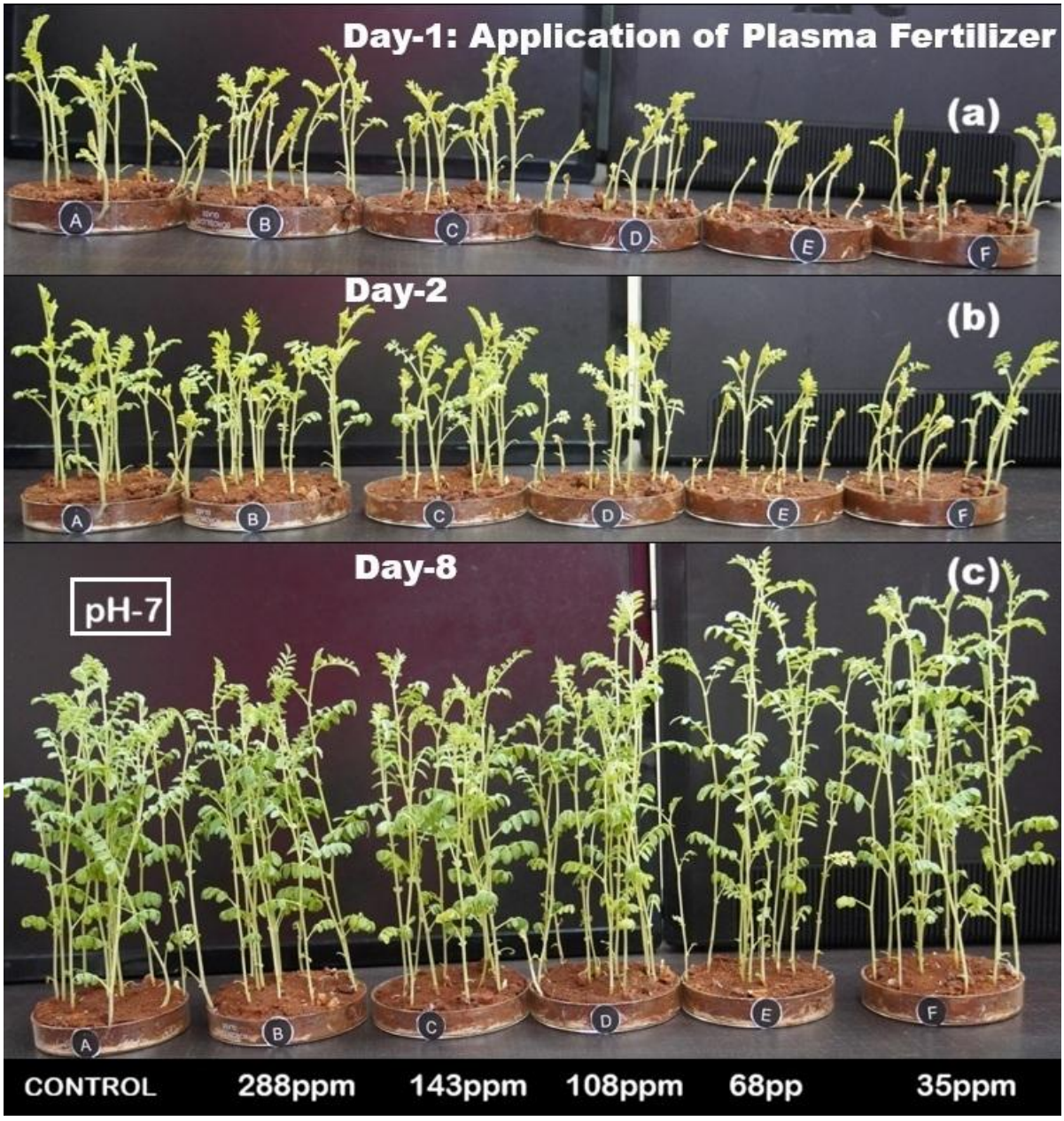


Figure 2. Growth in Soil System under application of plasma fertilizer of different concentration (pH-7)

The comparatively strong growth of the nitrate-free seedlings during the early developmental period is consistent with the utilization of endogenous seed reserves, whereas the enhanced growth at 35 ppm suggests that an appropriate level of external nitrate becomes increasingly important as seedling development progresses. Conversely, the reduction in growth at nitrate concentrations substantially above 35 ppm indicates that increased external nitrate availability does not translate into proportionally enhanced plant growth. At elevated nitrate concentrations, disturbances in ionic and cellular homeostasis, increased demands on nitrate assimilation, and imbalances in carbon–nitrogen metabolism may constrain the benefits of additional nitrogen [22-24]. These mechanisms remain to be experimentally resolved. Notably, the observed shoot-growth optimum coincides with the morphological phenotype of substantially greater canopy development at 35 ppm, while the higher-nitrate treatments exhibit visibly reduced vigour. When considered together with the observed suppression of root development at elevated nitrate concentrations, the results suggest that excessive nitrate may influence not only shoot growth but also the partitioning of plant resources between root and shoot systems.

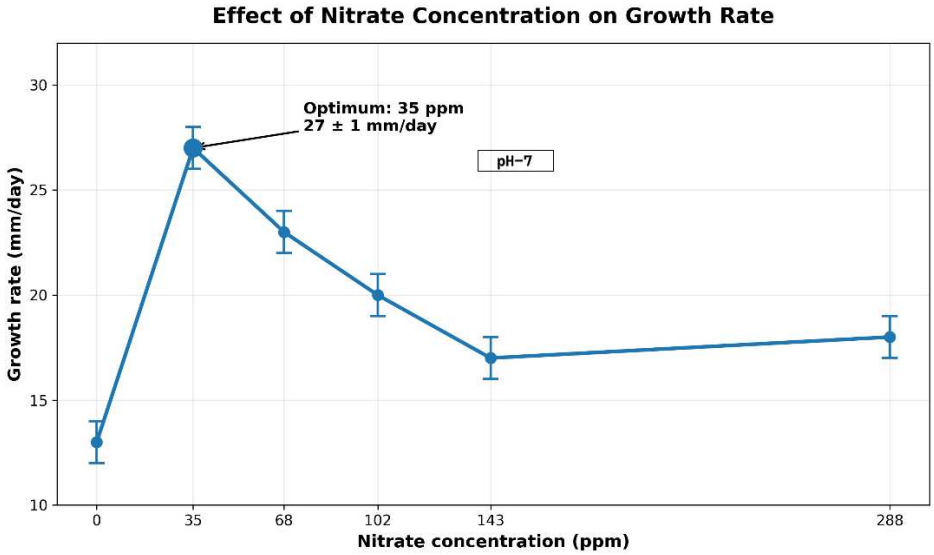


Figure 3. Plot of growth rate as a function of nitrate concentration

Overall, the data support a concentration-dependent optimum rather than a simple positive relationship between nitrate supply and plant growth. The pronounced enhancement at 35 ppm and subsequent decline at higher concentrations indicate that both nitrate deficiency and nitrate excess can constrain plant development, producing a distinct optimum within the experimental range. This finding is particularly relevant for plasma-derived nitrate fertilizer systems, because it demonstrates that increasing the nitrate concentration of the generated fertilizer solution should not automatically be equated with improved plant performance. Instead, fertilizer efficacy appears to depend on maintaining nitrate availability within a physiologically favourable concentration window. Further integration of growth rate with root/shoot biomass, root architecture presented in the subsequent sections supports the physiological basis of this optimum response more rigorously.

3.2 Temporal Evolution of Coordinated Root–Shoot Development of *Cicer arietinum* under Optimal Hydroponic Conditions

Plant development is a dynamic process involving the progressive establishment and coordination of below-ground and above-ground organs. In hydroponic systems, controlled nutrient availability provides an opportunity to examine how root-system architecture and shoot growth evolve over time under defined nutritional conditions. To characterize this developmental trajectory, the sequential growth of *Cicer arietinum* was monitored in Figure 4 from early seedling establishment through advanced vegetative development under pH 7 and 35 ppm nitrate. Particular emphasis was placed on the temporal progression of root elongation, lateral branching, spatial root occupation, and corresponding shoot expansion, providing a visual assessment of the coordination between root and shoot development throughout the growth period.

The sequential development of *Cicer arietinum* under hydroponic conditions demonstrates a progressive and coordinated establishment of the root and shoot systems over the 26-day growth period. At Day 8, the seedlings exhibited relatively limited shoot development accompanied by an emerging, sparsely branched root system. By Day 11, pronounced root elongation and lateral-root formation were evident, indicating rapid development of the absorptive root network. This architectural development became substantially more prominent by Day 17, when numerous elongated and branched roots occupied a significant fraction of the available nutrient-solution volume, coincident with marked shoot elongation and increased leaf development. By Day 26, the plant possessed a highly developed and extensively branched root system together with a substantially expanded shoot, demonstrating sustained coordination between below-ground and above-ground growth. The progressive increase in root branching and spatial occupation of the nutrient solution is particularly notable because it indicates that the developmental response involved not only root elongation but also increasing architectural complexity and potential absorptive capacity.

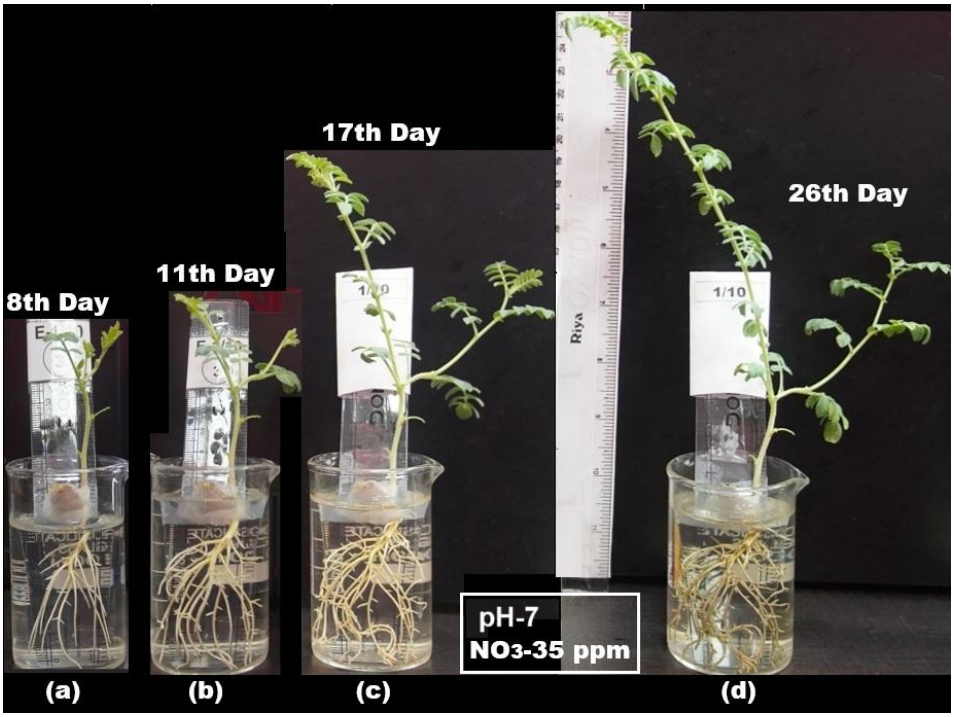


Figure 4. Temporal evolution of root and shoot development of Cicer arietinum under hydroponic conditions at pH 7 and 35 ppm nitrate. [Representative plant photographed on Days 8, 11, 17 and 26 demonstrates progressive shoot elongation, leaf development, primary-root extension and lateral-root proliferation. The increasing complexity and spatial extent of the root system are accompanied by coordinated expansion of the shoot, indicating progressive establishment of the root–shoot system during hydroponic growth.]

The temporal synchrony between root and shoot development suggests a coordinated root–shoot response to the hydroponic nutrient environment. Expansion of the shoot increases requirements for water and mineral nutrients, while development of the root network enhances the plant's capacity to acquire these resources. The extensive root and shoot development observed under 35 ppm nitrate is consistent with the independently measured maximum shoot growth rate of approximately 27 ± 1 mm day^{-1} at this concentration in soil plantation, thereby providing morphological support for the identification of 35 ppm as an apparent favourable nitrate concentration under the present experimental conditions.

Collectively, the observations indicate that nitrate availability under the investigated hydroponic conditions influences plant development at the level of the integrated root–shoot system rather than shoot growth alone. The progressive transition from root establishment at Day 8 to a highly branched root network and expanded shoot at Day 26 provides visual evidence of sustained vegetative development and offers a morphological framework for interpreting the concentration-dependent growth responses observed in the broader experiment.

3.3 Nitrate-Dependent Plasticity of Root Architecture and Root–Shoot Development

Root-system architecture is a highly plastic trait through which plants adjust nutrient acquisition, spatial exploration, and resource allocation in response to nutrient availability. In particular, nitrate availability can influence not only the magnitude of root growth but also root branching, spatial distribution, fineness, and the balance between below-ground and above-ground development. To examine this architectural response in Cicer arietinum, hydroponically grown plants were compared across a range of nitrate concentrations from complete nitrate deprivation to elevated nitrate supply. The analysis focused on the visible organization and spatial extent of the root system in relation to corresponding shoot development, providing a macroscopic perspective on how nitrate availability regulates root exploratory behavior and root–shoot coordination. This approach is particularly useful for distinguishing between increased root proliferation as a potential nutrient-foraging response under nitrate limitation and restricted root development associated with supra-optimal nitrate availability. When a root finds a "jackpot" of nitrogen, the plant stops growing the root longer and instead grows large numbers of tiny, hairy side branches right in that spot to suck up as much food as possible. When the soil is starving for nitrogen, the plant stops wasting energy on leaves and shoots above ground. Instead, it sends all its energy down into the roots, making them grow long and deep to search for food elsewhere.

The results of Day-26 hydroponic experiment in Figure 5 revealed pronounced nitrate-dependent plasticity in both root-system architecture and shoot development of Cicer arietinum. Striking differences were observed among plants supplied with 0, 35, 68, 102, 143 and 288 ppm nitrate, demonstrating that nitrate availability influenced not simply overall plant size but also the spatial organization and relative development of below- and above-ground organs. The root response was particularly prominent. The nitrate-free plants developed an extensive network of fine roots despite comparatively restricted shoot development, whereas plants supplied with intermediate nitrate concentrations, particularly 35–68 ppm, exhibited markedly developed and highly branched root systems together with substantially expanded shoots. At higher nitrate concentrations, especially 143 and 288 ppm, the visible

root systems became comparatively less extensive and the shoots also displayed reduced developmental vigour. The resulting phenotype therefore indicates a distinctly non-linear relationship between external nitrate concentration and plant development.

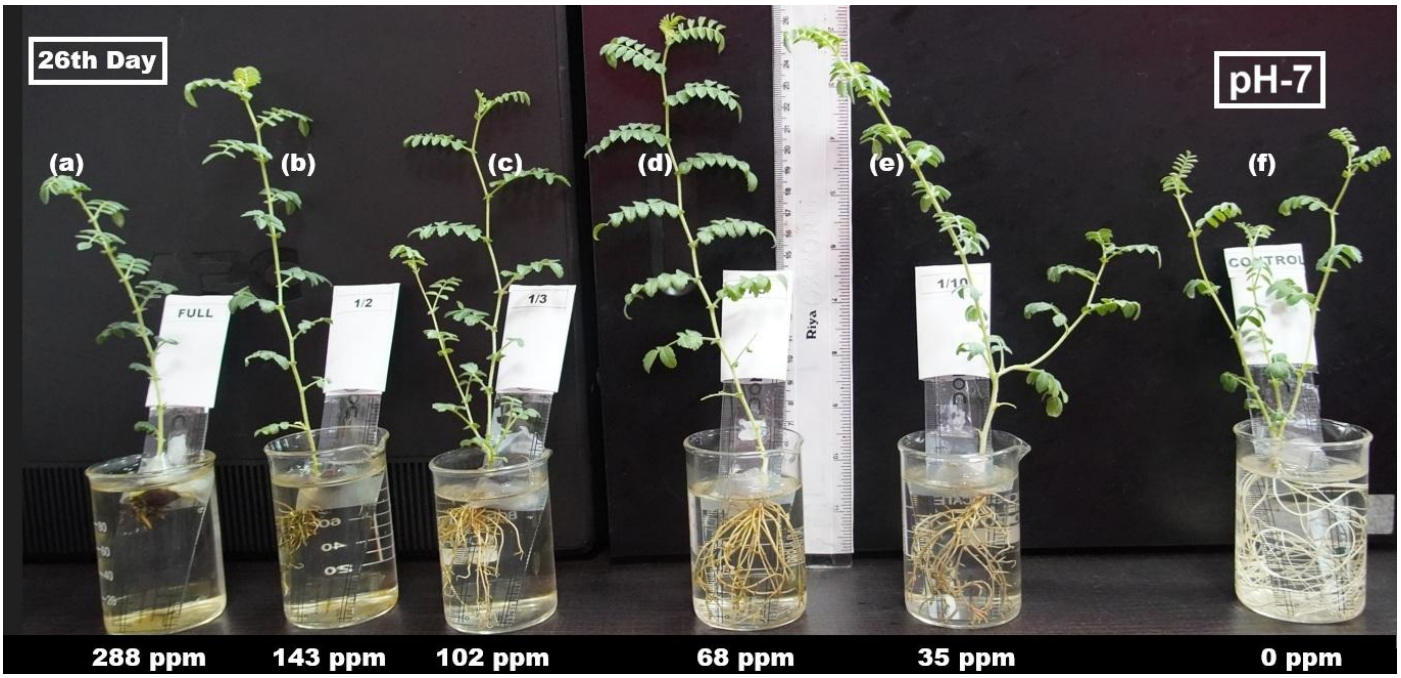


Figure 5. Strong variation in features of root and shoot development under different nitrate concentrations [pH-7]

Table 2. Variation of root and shoot phenotypes under different plasma nitrate concentration in hydroponics

Nitrate	Root phenotype visible in image	Shoot phenotype	Possible interpretation
0 ppm	Very extensive fine-root network	Relatively restricted	Strong root exploration under external N limitation
35 ppm	Extensive, branched roots	Strong shoot development	Apparent coordinated root–shoot development
68 ppm	Very extensive/dense root system	Strong shoot development	Strong root architectural response
102 ppm	Moderately developed root network	Reduced relative development	Beginning of reduced developmental response
143 ppm	More restricted root architecture	Reduced shoot development	Possible suppression at elevated nitrate
288 ppm	Highly restricted/concentrated root system	Altered/reduced development	Excess nitrate does not promote proportional growth

The pronounced root development under nitrate deprivation is noteworthy because it occurs in conjunction with comparatively limited shoot expansion. Such a phenotype may reflect enhanced allocation toward nutrient-acquisition capacity under nitrogen limitation. Conversely, the extensive root systems observed at intermediate nitrate concentrations suggest that an adequate nitrate supply can support both root proliferation and shoot development. The apparent reduction in root-system complexity at the highest nitrate concentrations is consistent with established evidence that nitrate functions not only as a nitrogen source but also as a signalling molecule regulating root-system architecture. Moderate nitrate availability can promote root growth, whereas excess nitrate can suppress lateral-root development through nitrate-sensing and downstream hormonal pathways involving auxin, cytokinin, ABA and related signalling networks. [16,25,26]

An important feature of the present observations is the partial decoupling of root and shoot responses. The concentration producing a visually extensive root system does not necessarily correspond to the phenotype with the greatest shoot development. This indicates that nitrate concentration may regulate developmental allocation between nutrient acquisition and above-ground biomass production rather than acting as a simple determinant of total plant size. The 0-ppm phenotype, for example, is characterized by extensive root exploration but comparatively limited shoot development, whereas intermediate nitrate availability supports simultaneous expansion of both organs. At the upper end of the nitrate range, additional nitrate does not result in proportionally greater root or shoot development. Such behaviour is consistent with nitrate-dependent root–shoot signalling, whereby information about external nitrate availability and internal nitrogen status is integrated to regulate root architecture and whole-plant growth. [15,27]

Collectively, these observations indicate that nitrate concentration acts as a developmental regulator of the integrated root–shoot system rather than simply as a fertilizer input. The hydroponic system provides particularly clear evidence of this plasticity because the roots are directly visible and their architectural response can be followed without soil interference. The apparent transition from an exploratory root phenotype under nitrate deprivation, through a highly developed root–shoot phenotype at intermediate nitrate availability, toward a more constrained root and shoot phenotype at elevated nitrate concentrations suggests the

existence of a concentration-dependent developmental window. The quantitative relationship through total root length, root biomass, shoot biomass, root-to-shoot ratio presented in the subsequent sections allow the visual phenotypes observed here converted into a mechanistically informative nitrate–root architecture–shoot growth relationship.

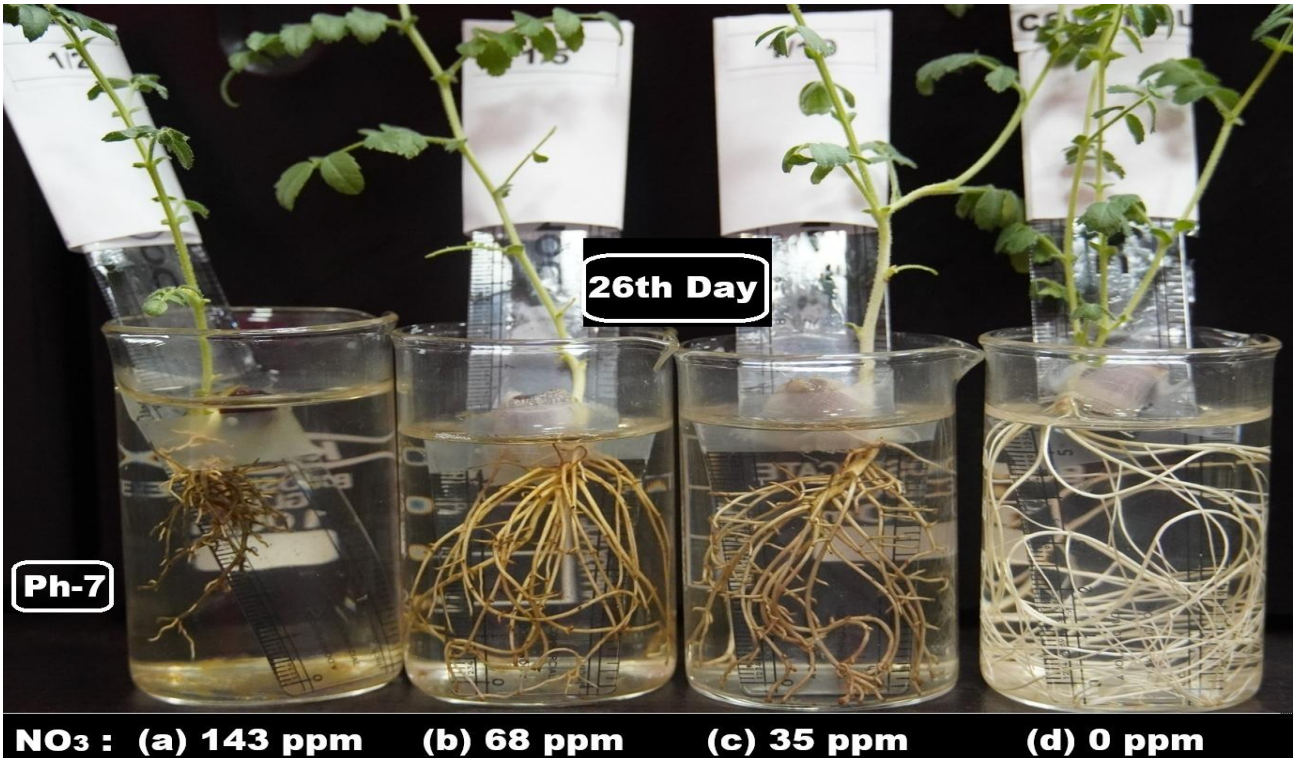


Figure 6. A closer view root expression under different nitrate concentration

A Closer examination of the hydroponically developed root systems in Figure 6 revealed that nitrate availability affected not only the extent of root growth but also root-system architecture, branching, spatial occupation and pigmentation. The nitrate-free treatment (0 ppm) produced a strikingly extensive network of predominantly fine, white roots, with roots extending deeply and laterally through much of the available aqueous volume. This phenotype, despite the absence of externally supplied nitrate, suggests pronounced root architectural plasticity and may reflect a nutrient-foraging strategy in which plants increase exploratory root extension under nitrogen limitation. However, the extensive below-ground development was not accompanied by proportionate shoot development, indicating that root proliferation under nitrate deprivation should not be equated with optimal whole-plant performance. At 35 ppm, the root system displayed a more complex architecture, combining substantial axial extension with pronounced lateral development and relatively extensive occupation of the growth medium. The 68-ppm treatment similarly exhibited extensive root development, although the network appeared more densely aggregated and irregularly looped. Together with the vigorous shoot development observed at these intermediate concentrations, these phenotypes suggest a range of nitrate availability within which root development and shoot growth are more effectively coordinated. In contrast, at 143 ppm, root-system expansion was markedly restricted, with roots becoming concentrated around the basal region rather than extensively exploring the available medium. The simultaneous increase in brownish root pigmentation with increasing nitrate concentration further indicates that nitrate availability may be associated with changes in root tissue composition or physiological status. Importantly, the contrasting architectures observed at 0 and 143 ppm indicate that nitrate limitation and supra-optimal nitrate availability induce fundamentally different root responses: the former is characterized predominantly by enhanced exploratory extension, whereas the latter is associated with contraction of the root system. Thus, the observed response cannot be adequately described by a simple nitrate-dependent increase or decrease in root biomass. Rather, the results indicate nitrate-dependent reprogramming of root-system architecture, with intermediate nitrate availability favouring coordinated root–shoot development and both nitrate deprivation and excessive nitrate producing distinct compensatory or restrictive phenotypes. Nitrate concentration appears to act as an architectural regulator of the chickpea root system, shifting the plant between an exploratory phenotype under nitrate deprivation, a coordinated developmental phenotype at intermediate nitrate availability, and a spatially restricted phenotype under supra-optimal nitrate.

The reproducibility study (Figure 7) conducted on day 26 independently confirms the concentration-dependent modification of root-system architecture observed in the initial hydroponic experiment. In the nitrate-free treatment (0 ppm), roots again developed as an extensive network of predominantly fine, white structures occupying a large fraction of the available aqueous volume. This reproducible phenotype indicates that the absence of externally supplied nitrate does not abolish root development; rather, it appears to promote an exploratory root architecture, potentially reflecting compensatory nutrient-foraging under nitrogen limitation. At 35 ppm nitrate, the root system exhibited pronounced axial elongation accompanied by lateral development and comparatively robust root structures, suggesting a shift from predominantly exploratory proliferation toward a more organized and functionally integrated root architecture. The 68-ppm treatment also maintained substantial root development, although the roots appeared more densely aggregated and architecturally complex. In contrast, 143 ppm produced a markedly restricted root system,

characterized by reduced spatial extension, concentration of roots near the basal region and pronounced brownish pigmentation. Importantly, this contrasting phenotype was reproduced in the independent experiment, strengthening the evidence that the observed architectural differences are associated with nitrate availability rather than random developmental variation. The opposing root phenotypes observed at 0 and 143 ppm are particularly noteworthy: nitrate deprivation was associated with extensive exploratory root proliferation, whereas elevated nitrate was associated with contraction of root-system expansion. Thus, the response cannot be adequately represented by a simple increase or decrease in root biomass with nitrate concentration. Rather, the results indicate nitrate-dependent root architectural plasticity involving changes in root extension, branching, spatial distribution and apparent tissue pigmentation. The intermediate nitrate treatments, particularly 35–68 ppm, supported substantial root development concurrently with vigorous shoot growth, suggesting a developmental window in which below-ground and above-ground resource allocation is more effectively coordinated. The reproducibility of these morphological responses provides a strong basis for subsequent correlation with quantitative root traits, root anatomical characteristics and biochemical signatures.

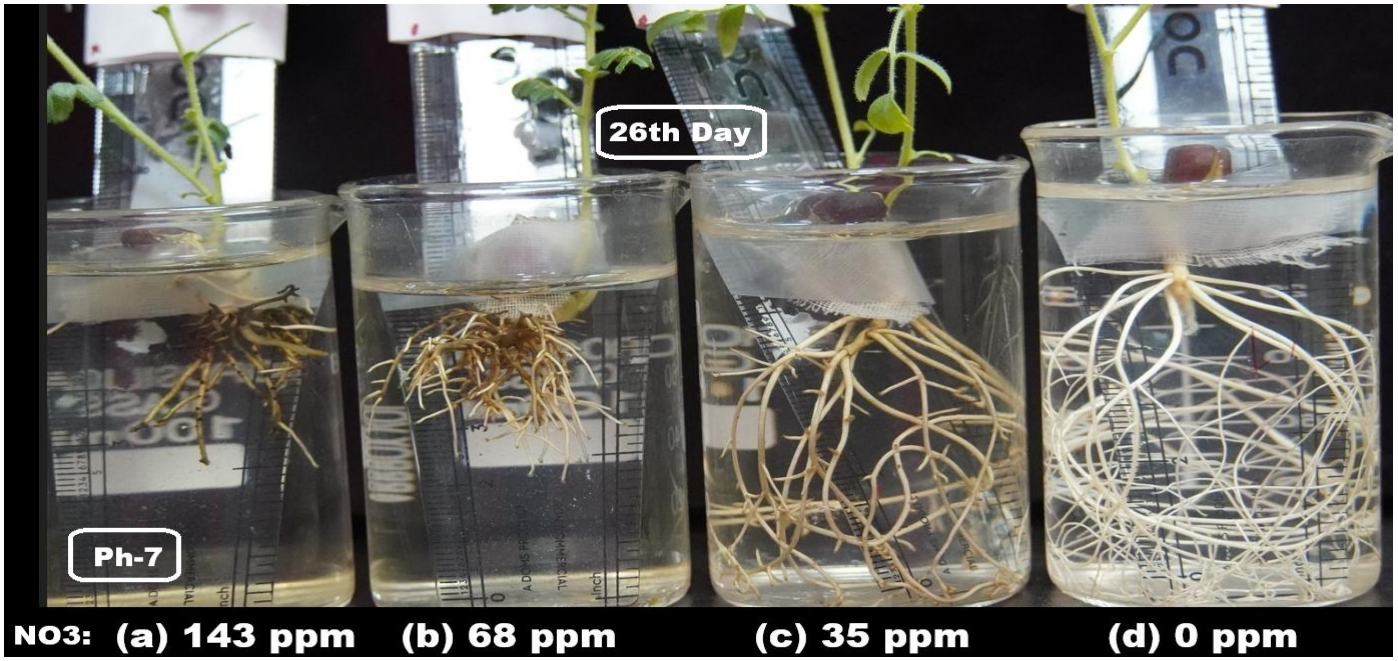


Figure 7. Root expression in a repeat study

The repeat experiment demonstrates that nitrate concentration functions not merely as a quantitative determinant of root growth, but as a regulator of root-system architecture. Nitrate deprivation induces an extensive exploratory phenotype, intermediate nitrate availability supports coordinated root development, whereas elevated nitrate progressively constrains root-system expansion and is accompanied by pronounced pigmentation.

3.4 Nitrate-Dependent Modulation of Root–Shoot Growth and Biomass Allocation in Hydroponics

The day-47 phenotype (Figure 8) revealed a pronounced, non-monotonic dependence of root and shoot development on nitrate availability as presented in Figure 9. Nitrate deprivation induced an exceptionally extensive root system (510 mm) despite relatively restricted shoot development (195 mm), accompanied by a high root-to-shoot biomass ratio (0.69), indicative of enhanced below-ground exploration under nitrogen limitation. Addition of only 35 ppm nitrate profoundly altered this allocation pattern: root length decreased to 80 mm, yet root biomass increased to 0.890 g, while shoot length and shoot biomass increased to 270 mm and 1.975 g, respectively, resulting in the highest total biomass among the treatments. Thus, nitrate supplementation appeared to shift root development from extensive exploratory elongation toward a more compact, biomass-rich architecture while simultaneously enhancing above-ground productivity. At 68 ppm, shoot elongation reached a maximum of 285 mm, but shoot and root biomass declined relative to 35 ppm, indicating that shoot elongation was not synonymous with maximal biomass accumulation. Further increases in nitrate to 143 and 288 ppm progressively restricted root development, with root length declining to 51 and 10 mm, respectively, and markedly reduced root-to-shoot biomass ratios. Collectively, these results demonstrate that nitrate availability regulates not only the magnitude of root and shoot growth but also the developmental allocation strategy between root exploration, root biomass investment and shoot production. The pronounced optimum around 35 ppm suggests that low-to-moderate nitrate availability can promote coordinated root–shoot development, whereas both nitrate deprivation and excessive nitrate availability drive the plant toward distinctly different, developmentally constrained phenotypes.

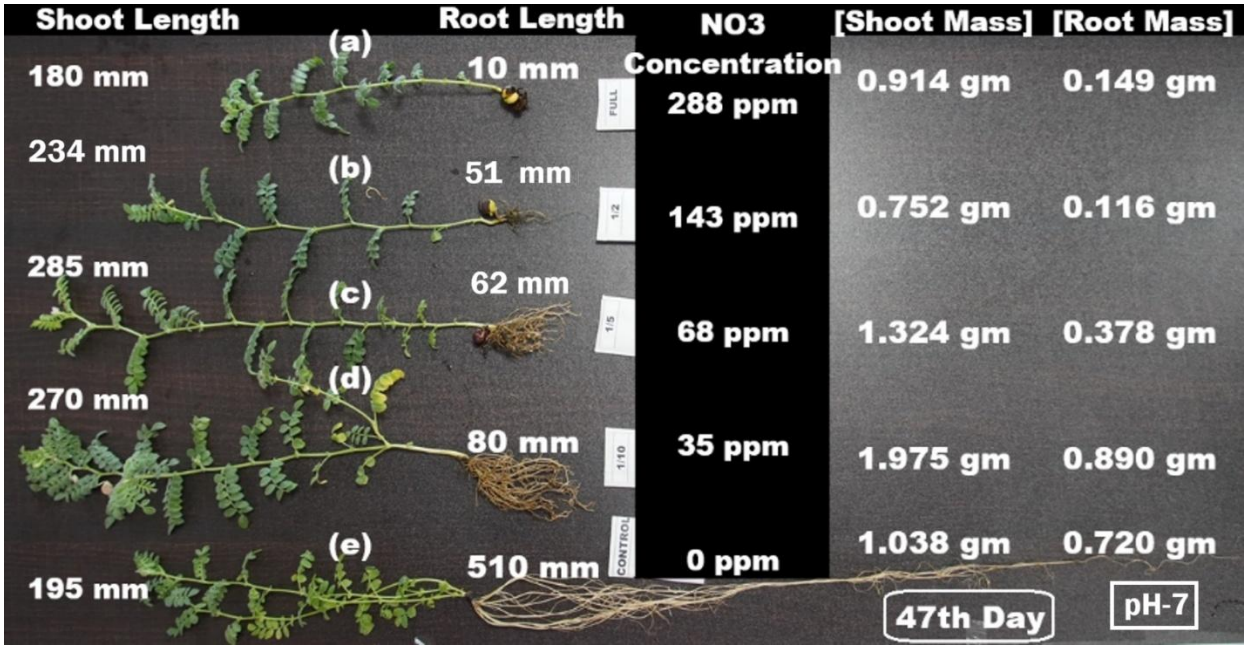


Figure 8. Physical measures of root and shoot under different nitrate concentrations [pH-7]

Table. 3 Quantitative physical response to nitrate concentration

NO_3^-	Shoot length (mm)	Root length (mm)	Shoot mass (g)	Root mass (g)	Root: shoot mass ratio	Total biomass (g)
0 ppm	195	510	1.038	0.720	0.69	1.758
35 ppm	270	80	1.975	0.890	0.45	2.865
68 ppm	285	62	1.324	0.378	0.29	1.702
143 ppm	234	51	0.752	0.116	0.15	0.868
288 ppm	180	10	0.914	0.149	0.16	1.063

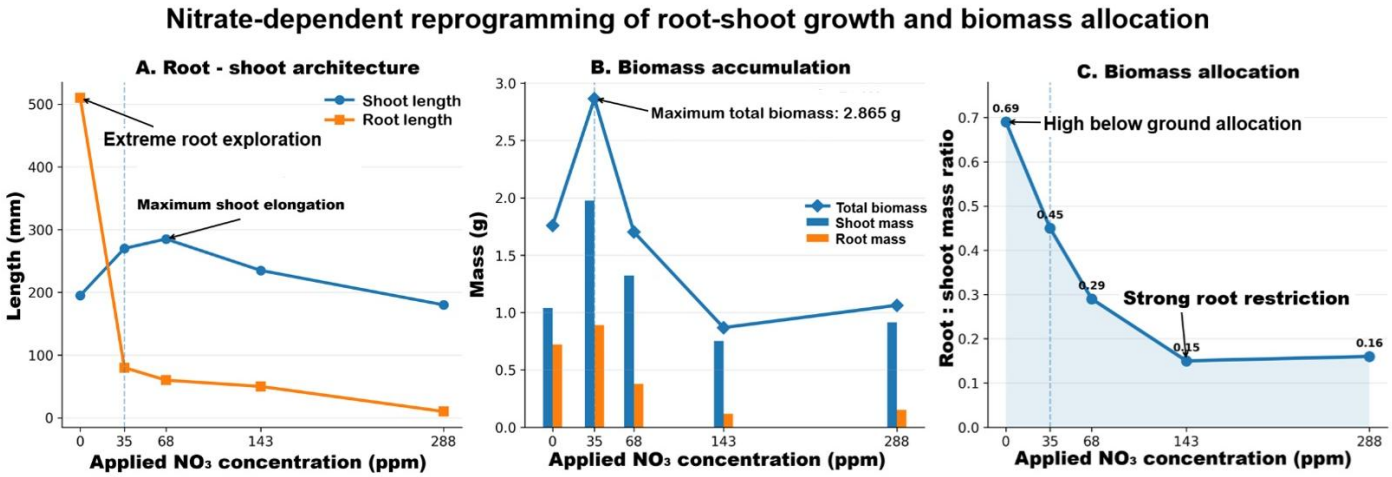


Fig. 9. Nitrate-Dependent Modulation of Root–Shoot Growth and Biomass Allocation in Hydroponics. Typical errors in length and mass measurement are ±2 mm and ±0.01mg respectively. A. Maximum root length, B. Total biomass, C. Root to shoot mass ratio.

The results are summarized in Table 3. It appears that 35 ppm is not simply the concentration producing "more growth"; it appears to represent a transition point at which the plant moves from an N-starvation-driven exploratory root strategy to coordinated, biomass-producing root–shoot development. This interpretation also fits very well with earlier root anatomical and flowering observations presented in the later sections.

3.5 pH-Dependent Modulation of Nitrate-Associated Root Architecture and Root–Shoot Coordination:

An important aspect of plasma synthesized nitrate is that higher the concentration of nitrate, more acidic is the chemical nature of the solution. In this study, for a given concentration of nitrate in the plasma synthesized fertilizer, pH level was adjusted to the desired value by adding KOH. Strong pH dependence of coordinated plant development in two independent studies is consistently demonstrated in the results presented in Figure 10 and Figure 11, where the nitrate concentrations were the same (35 ppm). At pH 5, plants exhibited markedly restricted root systems with reduced branching and limited spatial extension, accompanied by poor shoot development, indicating that acidic conditions strongly constrained overall growth. In contrast, pH 7 consistently supported vigorous shoot development together with an extensive, highly branched network of fine roots distributed throughout the available nutrient solution, suggesting comparatively coordinated root–shoot development and effective utilization of the supplied nitrate. At pH 9, both experiments showed pronounced proliferation of fine roots, with roots occupying a large fraction of the available aqueous volume; however, this extensive below-ground development was not accompanied by a proportionate enhancement of shoot growth, which was less vigorous and less coordinated than at pH 7. Importantly, the reproducibility of this contrasting pH-dependent phenotype in the repeat experiment (Figure 11) strengthens the interpretation that the observed differences are not attributable to random developmental variation. The results therefore indicate that pH regulates not only the magnitude of root growth but also the allocation and coordination of biomass between below- and above-ground organs. The extensive root proliferation at pH 9 may represent altered developmental allocation or a compensatory root response under alkaline conditions rather than inherently superior plant performance. Conversely, the strongly suppressed root and shoot development at pH 5 indicates that acidic conditions substantially limit the effective utilization of the supplied nitrate. Collectively, these observations demonstrate that the biological response to plasma-derived nitrate is governed by an interaction between nitrate concentration and the physicochemical environment in which it is supplied, with near-neutral pH producing the most balanced root–shoot phenotype, whereas strongly acidic or alkaline conditions induce distinct forms of developmental constraint or reallocation. The reproducibility across the two experiments (Figure 10 and Figure 11) further supports the conclusion that 35ppm nitrate at approximately neutral pH represents a condition favouring coordinated whole-plant development, whereas root-system size alone is insufficient to define optimal nitrate utilization.

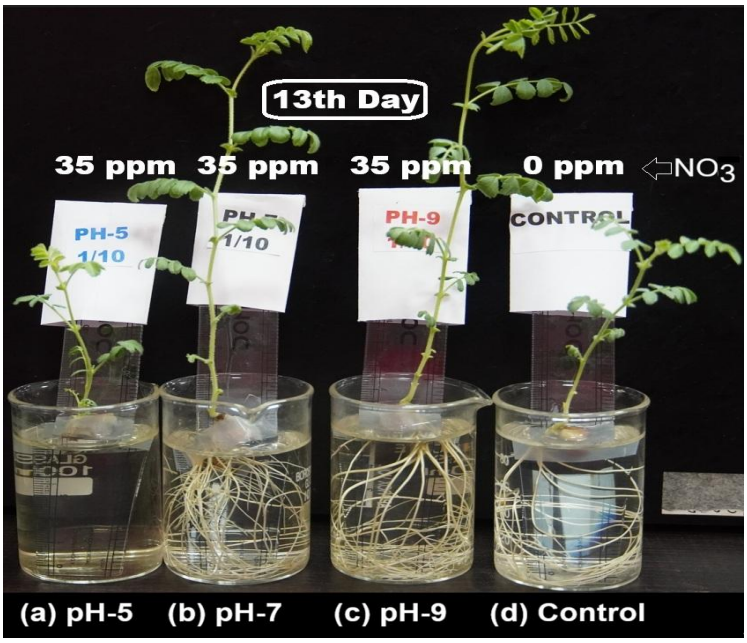


Figure 10. Root expression under different pH for a given nitrate concentration (35 ppm)

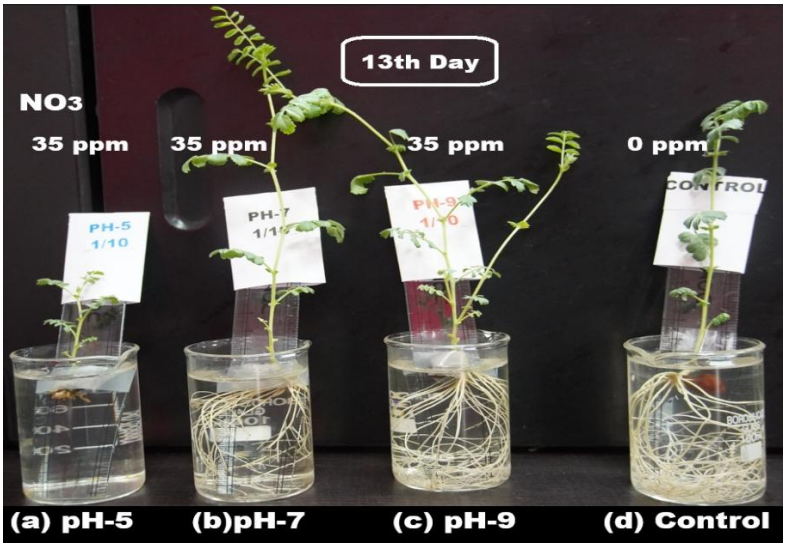


Figure 11. Root expression under different pH for a given nitrate concentration (35 ppm) in a repeat study

3.6 Nitrate-Dependent Alterations in Root Tissue Organization and Anatomical Differentiation

Root architecture and tissue organization are fundamental determinants of a plant's capacity for nutrient acquisition, water transport, mechanical support, and adaptation to changing environmental conditions. Because nitrate availability can influence both root growth and developmental differentiation, changes in external nitrate concentration may be reflected not only in root length and biomass but also in the organization of cortical and vascular tissues. To investigate this structural dimension of the nitrate response, transverse root sections of *Cicer arietinum* were examined on the 23rd day after planting under different nitrate concentrations. Comparative microscopic analysis was used to determine whether the pronounced differences in root growth were accompanied by corresponding changes in cortex–stele organization, cellular arrangement, and vascular differentiation. This anatomical assessment therefore provides a structural perspective on the observed nitrate-dependent modulation of root development and helps identify whether an optimum nitrate supply is associated with more coordinated root tissue development.

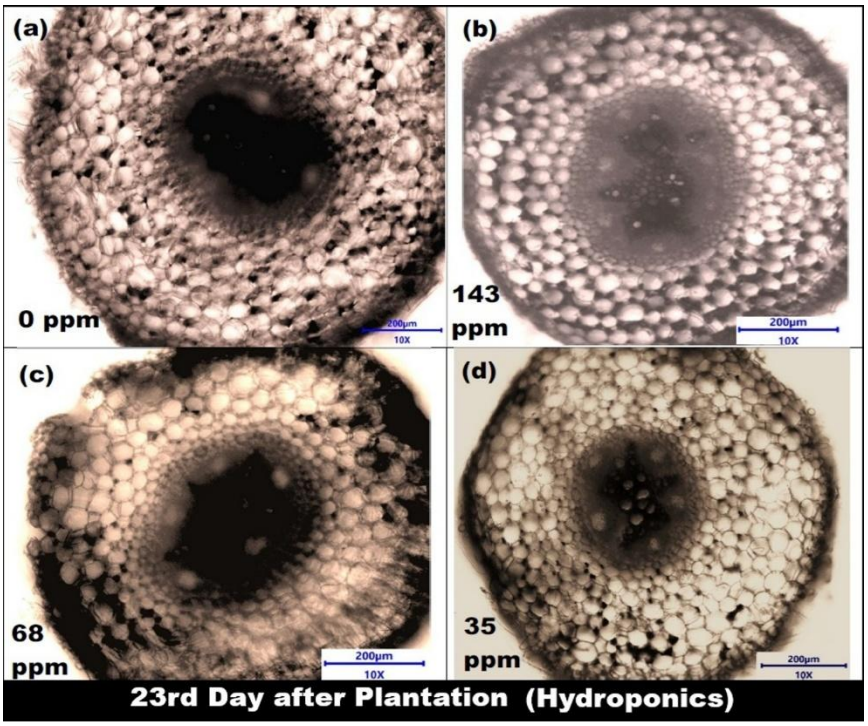


Figure 12. Anatomy of roots grown under different nitrate concentration

The transverse microscopic sections [Figure 12] provide clear evidence of nitrate-concentration-dependent differences in root anatomical development on the 23rd day after planting. All images were obtained at the same magnification and scale (200 µm). For 35 ppm, the root shows a well-developed and relatively well-organized anatomical structure. A broad cortex composed of large, fairly regular parenchymatous cells surrounds a clearly differentiated central stele. The vascular region appears compact and distinct, indicating comparatively mature and organized root development. This is consistent with the observation that 35 ppm produced the best overall root growth [Figure 12(d)]. For 68 ppm the section appears less regular and more heterogeneous. The

cortical cells show greater variation in size and arrangement, while the central stele appears relatively enlarged and irregular [Figure 12(c)]. The tissue organization is less distinct than at 35 ppm, suggesting that increasing nitrate concentration beyond the optimum may be associated with altered root tissue differentiation and development. For 143 ppm the section exhibits a relatively large and conspicuous cortical region with numerous enlarged cells, but the central vascular/stele region appears comparatively compact and less prominently differentiated [Figure 12(b)]. For 0 ppm the root displays a recognizable cortex and central stele, but the tissue arrangement appears comparatively heterogeneous with significant void regions in-between, with a strongly contrasted/dark central region [Figure 12(a)]. The absence of nitrate does not appear to support the same degree of organized development observed at 35 ppm.

Thus 35 ppm nitrate represents an anatomically favourable concentration, producing a relatively well-organized cortex–stele architecture and clear vascular differentiation. Both nitrate deficiency (0 ppm) and elevated nitrate concentrations (68–143 ppm) are associated with deviations from this organized anatomical pattern. The progressively altered cellular organization at higher nitrate concentrations may reflect nitrate-stress-associated changes in root development, rather than simply an increase in root tissue formation [17,25].

A particularly important point is that the anatomical observations complement, rather than replace, the root-length/root-growth measurements. The finding that higher nitrate concentrations resulted in significantly reduced root growth is therefore consistent with the microscopic evidence of altered tissue organization at elevated nitrate levels.

In summary, the root anatomical examination on the 23rd day after planting revealed pronounced differences in transverse tissue organization as a function of nitrate concentration. The 35ppm treatment exhibited comparatively well-organized root architecture, characterized by a broad, regularly arranged cortical region and a distinct, compact central stele with recognizable vascular differentiation. In contrast, roots exposed to 68 and 143 ppm nitrate showed greater heterogeneity in cortical cell size and arrangement together with altered organization of the central vascular region. The nitrate-deficient (0 ppm) root also exhibited comparatively heterogeneous tissue architecture. These observations indicate that nitrate availability influences not only root elongation but also the structural organization and differentiation of root tissues. The comparatively organized anatomy observed at 35 ppm agrees with the measured enhancement of root growth at this concentration, whereas the anatomical alterations at higher nitrate concentrations are consistent with the observed reduction in root development under elevated nitrate availability.

3.7 Dependency of flowering and fruiting stage on nitrate concentration

Reproductive transition represents a critical developmental checkpoint at which plants shift from vegetative biomass accumulation toward flowering, fruit initiation, and reproductive investment. Because this transition is tightly coupled to plant nutritional status, nitrate availability can influence not only vegetative growth and root development but also the timing and progression of reproductive development. In *Cicer arietinum*, an appropriate balance between nitrogen supply and developmental demand is particularly important because excessive or insufficient nitrogen may disrupt the coordination between vegetative growth and reproductive commitment. Therefore, the observed reproductive status at day 36 was examined across the nitrate treatments to determine whether nitrate availability produces a concentration-dependent or non-linear shift in the vegetative-to-reproductive transition and subsequent flower-to-fruit progression. The observations presented in Figure 13 and Table 4 revealed pronounced nitrate-dependent differences in reproductive stage at day 36, with plants spanning a continuum from complete absence of visible flowering to active flower initiation and, under the 35-ppm treatment, progression into early fruit formation.

Marked nitrate-dependent differences in reproductive developmental stage were evident at day 36, extending the observed effects of nitrate availability beyond root and shoot development to the transition from vegetative growth to reproduction. The 35-ppm treatment exhibited the most advanced reproductive phenotype, with plants progressing beyond flowering into the flower-to-fruit transition and showing visible fruit initiation. In comparison, plants receiving 68 ppm nitrate were at the earlier stage of floral initiation, indicating that reproductive development had commenced but had not progressed to fruit formation by the same observation time. Further elevation of nitrate to 143 ppm was associated with a predominantly pre-flowering phenotype, whereas no visible flowering was observed at 288 ppm. The nitrate-free treatment similarly showed no apparent flowering at day 36, despite the extensive exploratory root development observed under nitrate deprivation. Collectively, these observations reveal a distinctly non-linear relationship between nitrate availability and reproductive progression. Importantly, increasing nitrate from 35 to 68 ppm did not accelerate reproductive development; rather, the 35-ppm plants had progressed further, from flowering toward fruit formation, whereas the 68-ppm plants remained at floral initiation. This suggests that reproductive progression may depend on a favourable range of nitrogen availability rather than increasing monotonically with nitrate concentration. The delayed reproductive transition at 143 and 288 ppm further raises the possibility that supra-optimal nitrate availability may alter the balance between vegetative maintenance and reproductive commitment. Conversely, the absence of flowering at 0 ppm may reflect insufficient nitrogen availability to sustain the metabolic and structural requirements associated with reproductive transition. The contrasting phenotypes at the two extremes are particularly noteworthy because they were accompanied by distinct root responses: nitrate deprivation promoted extensive exploratory root development, whereas high nitrate concentrations were associated with restricted root-system expansion. Thus, both nitrate limitation and excessive nitrate appear capable of delaying reproductive development, but potentially through different physiological responses. The advanced flower-to-fruit transition at 35 ppm, together with the coordinated root and shoot development observed at this concentration, suggests that an intermediate nitrate supply may provide a favourable nutritional state for synchronizing vegetative growth with reproductive development under the present experimental conditions.

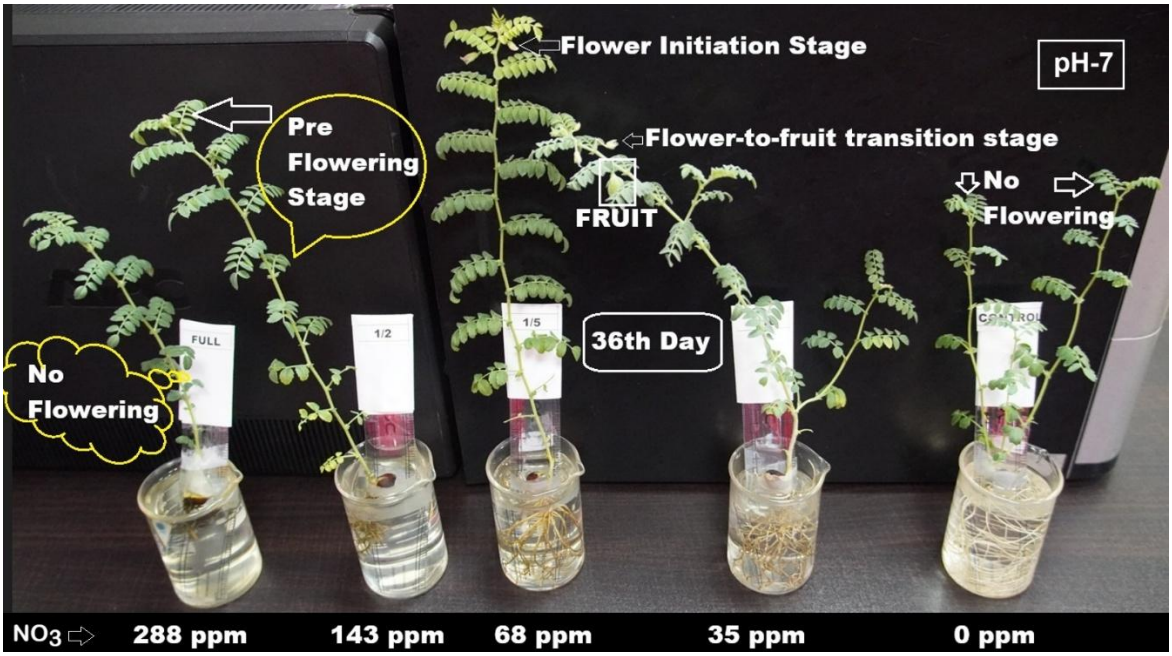


Figure 13. Variation of flowering and fruiting stage with variation in nitrate concentration

Table-4. Nitrate dependent reproductive status

NO_3^-	Reproductive status at day 36	Interpretation
0 ppm	No visible flowering	Reproductive transition substantially delayed
35 ppm	Flower → fruit transition; fruit visible	Most advanced reproductive development
68 ppm	Flower initiation	Reproductive transition underway, but earlier than 35 ppm
143 ppm	Pre-flowering	Reproductive transition delayed
288 ppm	No visible flowering	Strong reproductive delay

Nitrate availability regulates the timing and coordination of reproductive development in *Cicer arietinum*, with 35 ppm supporting the most advanced reproductive progression—from flowering to early fruit formation—by day 36, whereas increasing nitrate beyond this level progressively delayed reproductive progression.

3.10 Nitrate- and pH-Dependent Drought Response and Root–Shoot Coordination in Cicer arietinum

Draught resistance exhibited by plants grown under different nitrate concentrations and different pH is presented in Figure 14. Drought stress was imposed by withholding irrigation until soil moisture reached 20% of field capacity, corresponding to severe water-deficit conditions. The photograph was taken after 24 hours past the field capacity of 20% reached.

The pronounced differences in shoot architecture following exposure to severe water deficit reveal a strong dependence of drought-response phenotype on both nitrate availability and pH. Plants supplied with 35 and 68 ppm nitrate generally retained a more coherent and vertically organized canopy than plants receiving 143–288 ppm nitrate, particularly at pH 7 and pH 5. In contrast, the high-nitrate treatments frequently exhibited greater shoot biomass but substantially greater bending and loss of canopy integrity, indicating that biomass accumulation does not necessarily confer superior water-stress tolerance. The response was strongly modified by pH: the comparatively favorable phenotype at 35–68 ppm was most evident under pH 7, whereas alkaline pH 9 produced pronounced shoot bending in several higher-nitrate treatments. Acidic pH 5 was associated with additional visual symptoms, including reduced pigmentation and altered shoot organization in some high-nitrate treatments. Collectively, these observations indicate a complex nitrate × pH interaction in determining plant performance under water limitation and suggest the existence of a nutritionally favorable nitrate window rather than a monotonic relationship between nitrogen supply and drought tolerance. The comparatively effective maintenance of shoot architecture at 35–68 ppm may reflect a more balanced relationship between nitrogen-supported growth, ionic/osmotic status and plant water demand.

The nitrate-dependent variation in root cross-sectional architecture provides a potential anatomical basis for the differential drought-response phenotypes observed subsequently in soil-grown plants. Root sections obtained 23 days after hydroponic establishment revealed substantial differences in the organization of the cortical region and central vascular cylinder among nitrate treatments, indicating that nitrogen availability influenced root developmental patterning rather than merely total plant biomass. Notably, the 35-ppm treatment exhibited a comparatively well-organized radial architecture with a distinct central vascular region and substantial cortical tissue. This anatomical phenotype coincided with comparatively effective maintenance of shoot architecture under subsequent severe water deficit, particularly relative to several higher-nitrate treatments. Conversely, the higher nitrate treatments could produce substantial shoot development without correspondingly improved maintenance of canopy posture during drought, suggesting that enhanced biomass accumulation does not necessarily translate into greater whole-plant hydraulic resilience.

One possible explanation is that moderate nitrate availability establishes a more balanced root–shoot relationship, in which root structural development and water-acquisition capacity remain better matched to shoot transpirational demand. Such an interpretation remains mechanistic rather than demonstrative at this stage. Nevertheless, the convergence of root anatomical and drought-response phenotypes strongly supports further investigation of nitrate-dependent root–shoot coordination as a determinant of drought adaptation.

A moderate nitrate supply, particularly 35–68 ppm, appears to establish a favorable nutritional window for maintaining shoot turgor and canopy architecture during severe water deficit, whereas excessive nitrate supply produces greater vegetative biomass without proportionally improving drought-response capacity. The magnitude and phenotype of this response are strongly dependent on pH.

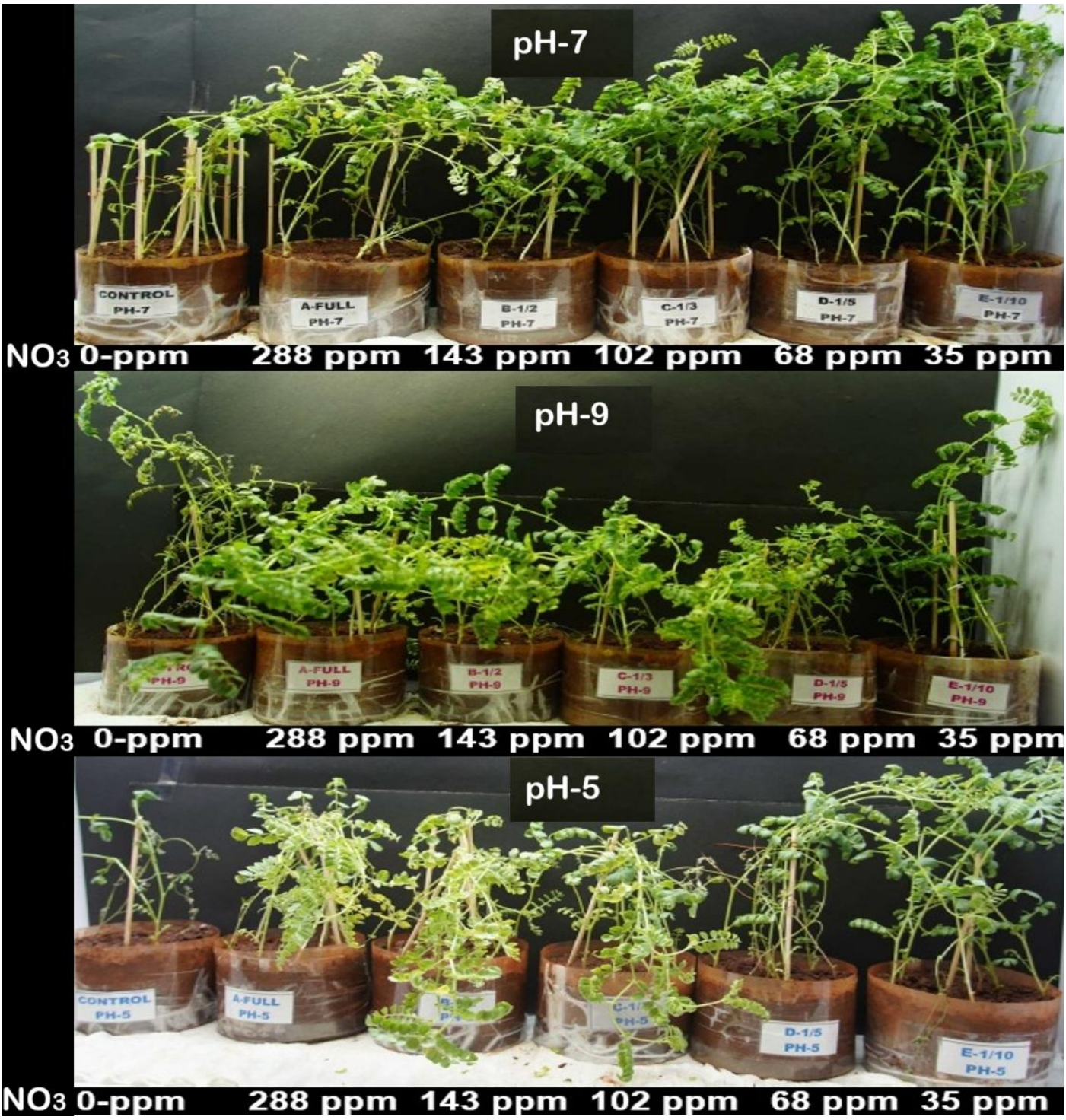


Figure 14. Draught resistance of plants grown under different nitrate concentrations and different pH.

4. Conclusions:

This study demonstrates that thermal-plasma-synthesized nitrate fertilizer markedly modulates *Cicer arietinum* development in a concentration- and pH-dependent manner. In hydroponics, nitrate deficiency promoted extensive root exploration, whereas 35 ppm nitrate produced the most coordinated root–shoot development, highest shoot and total biomass, and advanced reproductive transition. Increasing nitrate beyond this range progressively restricted root development and reduced overall biomass, highlighting a distinct optimum rather than a simple dose-dependent growth response. Solution pH strongly modified these responses, with pH 7 generally supporting vigorous root and shoot development, while acidic conditions restricted growth and alkaline conditions promoted disproportionate root proliferation. In soil, the nitrate treatments also influenced plant performance under severe water deficit, with lower-to-moderate nitrate availability retaining greater shoot integrity. Collectively, the findings establish that plasma-derived nitrate influences plant development from root architecture and biomass allocation to reproduction and drought response, supporting its potential as an alternative nitrogen-fertilizer platform while highlighting the importance of optimizing nitrate concentration and pH for agronomic application.

Credit authorship contribution statement:

S. Ghorui: Conceptualization, methodology, process, device design, device fabrication, installation, commissioning, experimental investigation, optimization, logistics, data curation, formal analysis, interpretation, project administration, writing—original draft, and writing—review and editing. **N. Tiwari:** Spectroscopic data acquisition, and review. **H. Parab:** Nitrate (NO_3^-) and nitrite (NO_2^-) concentration measurements by ion chromatography.

Declaration of competing interest:

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data Availability

The data that support the findings of this study are contained in the paper and available from the corresponding author upon reasonable request.

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