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Effects of Soil pH Stress on Plant Development: From Seed Germination to Early Seedling Growth

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Abstract:

Seed germination and early seedling growth are critical stages in a plant's life cycle, heavily influenced by internal physiological processes and external environmental factors. Internal factors such as phytohormones like gibberellins (GA) and abscisic acid (ABA) regulate dormancy and germination, while external factors including soil pH play pivotal roles in nutrient availability and root development. Soil pH affects the availability of essential nutrients and can introduce toxic elements like aluminium, impacting root growth and overall plant health. This review explores the effects of pH on seed germination and early seedling development, discussing mechanisms involving nutrient uptake, hormone balance, and microbial activity. Furthermore, strategies to ameliorate pH effects, such as biochar application, are investigated for their potential to enhance soil fertility, mitigate acidity, and promote sustainable crop growth. Specifically, the use of biochar types like Kolakhar demonstrates promising results in alleviating soil acidity and enhancing nutrient availability, thereby improving plant resilience to environmental stresses. Understanding these interactions is crucial for optimizing agricultural practices and ensuring food security amidst changing global climates and soil conditions.

Key Words: *Seed germination, early seedling growth, Soil pH, Kolakhar*

Introduction

Seed germination is an early and key stage in a plant's life cycle. It describes the physiological and biochemical processes that begin with the dry seeds' intake of water and conclude with the protrusion of the radicle. These processes include a sequence of signal transduction and gene expression regulation (Bewley, 1997). Germination increases the bioavailability of substances like proteins and carbohydrates. Antioxidants and gamma aminobutyric acid (GABA), two beneficial substances, have been observed to rise. Additionally, germination raises the amount of fibre, promotes the synthesis of beneficial chemicals, and decreases the amount of substances like phytates which are unnecessary or detrimental to human health (Xia et al, 2017). The minimum conditions necessary for seed germination are almost same, but each species has unique needs that change depending on temperature, time, intensity, and other elements like nutrition and light. As soon as the grain meets the necessary requirements—moisture content of 15 to 45%, temperature above 4 degrees Celsius, atmospheric air and lack of germination inhibitors—the germination process starts. Because light is essential to plants' ability to produce energy and to signal a variety of physiological reactions, plants also needs enough light (Dziki et al., 2015).

In the first step, seeds soak up water through tiny holes and a special opening. Proteins and fibres are primarily responsible for the water being stored inside the seed. The second stage occurs when the seed coat splits open close to the sprout and the rate of water absorption decreases. When the sprout's root finally begins to grow, the water consumption rises once more. The root's cells proliferate quickly as it expands (Nelson et al., 2013).

Factors influencing germination of seed

The emergence of seedling from a seed is a critical stage comprising of multiple developmental processes which are regulated by internal and external components. Internal factors include seed proteins, phytohormones (auxin, gibberellins /ABA balance, ethylene), chromatin modifications (methylation, acetylation, histone ubiquitination), genes related to maturation, hormone and epigenetics-regulating genes, non-enzymatic procedures, seed size, seed age, and structural elements (endosperm and seed coat). Beyond internal components, seed germination is significantly influenced by a number of environmental elements, including moisture, light, salinity, temperature, acidity, and nutrients (Li et al., 2011).

Internal factors:

Seed germination and seedling growth are controlled by two key phytohormones, Gibberellins and ABA. ABA acts as a germination inhibitor, promoting seed dormancy. Conversely, GA plays a key role in breaking dormancy, promoting seed germination and subsequent seedling growth. But hormone level is not the key to controlling the commencement of germination and the breaking of seed dormancy, but rather the ratio of the two hormones. Dormancy or seed germination is decided by the GA/ABA ratio. Gibberellins lead to synthesis and production of α-amylase, proteases, and β-glucanases, which causes seed germination. GA also activates the genes responsible for the embryo cell proliferation and weaken of the endosperm. To safeguard against germination occurring within the fruit (viviparous germination), ABA acts as an antagonist to GA. It achieves this by suppressing the expression of genes encoding essential hydrolytic enzymes, hindering water uptake, and consequently preventing cell wall loosening, a critical step for germination to initiate (White et al.,2000).

Gaseous hormone ethylene is engaged in a number of functions, one of which is the positive regulation of seed germination. Ethylene promotes seed germination by overcoming both primary and secondary dormancy through the reduction of ABA levels or sensitivity within the seed. Ethylene is secreted in response to auxin and brassinosteroids (BRs), and also in combination with GAs ethylene promote germination. Auxin promotes seed germination by increasing tolerance to ABA through two mechanisms: overexpression of microRNAs and interaction with gibberellins (GAs). While phytohormones are recognized for their role in seed dormancy and germination, additional signaling molecules, such as nitric oxide (NO) and reactive oxygen species (ROS), also contribute to this process. Seed germination is critically regulated by reactive oxygen species (ROS) due to their widespread interactions within the cell. ROS not only target key signalling molecules like ABA and GA, but also interact with crucial biomolecules such as lipids, DNA, and proteins. Seed water uptake triggers biochemical and cellular reactions, leading to the generation of reactive oxygen species (ROS). However, a balance in the amount of ROS is crucial as ROS can act as signalling molecules promoting germination, but excessive ROS cause oxidative damage delaying germination. This balance is based upon the cell's antioxidant machinery, specifically its ability to precisely regulate the accumulation of hydrogen peroxide (H_2O_2). Particularly, H_2O_2 acts as a central signalling hub, playing a critical role in managing seed dormancy and germination. According to Li et al. (2011), nitrogen compounds, such as NO, increase amylase activities, modify the K+/Na+ balance, and promotes seed germination by improving seed respiration and ATP synthesis.

External factors:

Soil moisture and texture: Moisture is necessary for the germination of seeds. The amount of organic matter and soil texture affect soil's water retention capacity, which affects seed germination. In sandy soils, poor seed-to-soil contact might result in inadequate moisture for germination of seeds. Seed germination is best achieved when the soil texture allows for firm contact between moist soil particles and the seed itself (Lamichhane et al., 2018).

Soil pH: Depending on the species, different soil pHs are needed for crop plants to flourish properly. It is almost neutral (7.0) for the majority of crops, ranging from slightly acidic (6.5) and slightly alkaline (7.5). Rice (*Oryza sativa*), cassava (*Manihot esculenta*), mango (*Mangifera indica*), cashew (*Anacardium occidentale*), citrus, pineapple (*Ananas comosus*), cowpeas (*Vigna unguiculata*), blueberries (*Vaccinium* spp.), and some grasses are among the crops that can be grown in acidic soils (pH less than 5.5).

Soil salinity: Saline soils are found worldwide in arid and semi-arid environments. High soil salinity inhibits seed germination because of the elevated salt concentrations in the surrounding water which prevents seeds from absorbing enough water to initiate the imbibing process, that is necessary for seed germination (Welbaum et al., 1990). The amount of soil salinity that different crop species and cultivars can withstand varies (Hosseini et al., 2003).

Oxygen: The process of respiration takes place inside the seed, which uses oxygen to stay alive and releases carbon dioxide to store food that is eventually turned into energy. To protect the seeds, minimal respiration is maintained during storage in a cool, dry environment. When a seed is exposed to moisture, warm air, and oxygen, it will begin to respire more quickly. Oxygen is restricted in the pores of soil that are very tiny or filled with water, facilitating gas exchange and oxygen intake by seeds and soil. For most crops, this results in a cessation of respiration and germination. Excessive compaction or wetness can lead to oxygen-limiting situations. High clay content, poor organic matter, and the disintegration of soil structure and particles due to excessive tillage are frequently linked to over compaction. Flooding, waterlogging, and inadequate drainage—which is associated with compaction and low areas in a field—are the causes of excessively wet conditions. When fully grown, semi-aquatic crops like rice and aquatic crops like water spinach (*Ipomoea aquatica*) can withstand floods, although they may still be sensitive when the seeds germinate (Lamichhane et al., 2018).

Light: While seeds with innate dormancy require an extra stimulation, seeds lacking innate dormancy germinate when exposed to appropriate temperatures, water, and oxygen (Frankland and Taylorson, 1983). One possible germination stimulant that certain crops need in order to start germination is light. Plants that are grown in very dark environments grow lanky (long with few leaves) as a result of their constant search for light. The crop is stressed as a result, and the stem structure becomes weaker.

Temperature: Temperatures ranging from 20°C and 25°C are generally ideal for seed germination (Gilroy, 1986). While a minimum temperature of 24°C is generally required for seed germination in most plants, certain delicate species may fail to germinate even with slight deviations from their preferred temperature range. The germination process should be conducted at constant temperatures, minimizing large fluctuations (Ashworth, 2002).

Seed microbiota: The term "seed microbiota" refers to the variety of bacteria that live inside seeds (Hardoim et al., 2012). The seed microbiome composition is ultimately shaped by a combination of factors: inheritance from the mother plant through internal or floral pathways, environmental influences like climate of the soil, and even human interventions. (Klaedtke et al., 2016). Variation in seed microbiota, both between and within species, arises from a complex interplay of factors. These include the mother plant's growth environment, inherent differences in seed characteristics like size, and colonization events occurring throughout seed development and maturation (Shade et al., 2017). The microbiota found in seeds consists of bacteria that are either beneficial (plant pathogens or biocontrol agents) or neutral (commensals) to the fitness of plants (Barret et al., 2016).Because they can produce a variety of plant hormones, enzymes, antimicrobial compounds, and some other secondary metabolites, as well as because they can transmit vertically and enhance seed germination under stressful conditions, beneficial microbes living inside seeds as seed endophytes are becoming more and more interesting.

Effect of pH on seed germination

Soil pH, a measure of hydrogen ion concentration (acidity or alkalinity), emerges as a critical environmental stress factor due to its significant impact on plant germination, development, and overall performance (Heidari, 2013; McCall and Watanabe, 1980). The Danish biochemist Soren Peter Lauritz Sorensen first proposed this definition of pH in 1909 (Kohlmann, 2003). Soil pH exerts a widespread influence on seed germination and plant growth. It can directly affect nutrient availability for plant uptake, hinder water absorption, elevate specific elements to toxic levels, and indirectly impact microbial activity and other soil characteristics.

In addition, the pH of the soil can influence how hormones pass through cellular membranes, as well as a root's capacity to alter its microenvironment and draw water and nutrients from the soil, all of which can interfere with a seedling's germination process and early seedling growth (Deska and Jankowshi, 2011). The carbon, nitrogen and sulphur cycle effects that come from inherently occurring biological activities in soils are primarily responsible for the pH variation in soils. However, through industrial and agricultural processes including fertilizer application, irrigation, and acid rain, human activity contributes significantly to quickening the pace of change (Adeoye et al., 2004).

The influence of soil pH on seed germination is a well-established area of research, with numerous studies investigating its effects on various plant species. Some of them are-

Irregular soil pH can limit plant nutrient availability, which can impact maize germination and development, according to a research from Illinois State University and Utah State University. The study clarified that the pH of soil is one of several other variables that limit nutrient availability and uptake. The study also demonstrates that soil pH values higher than 7.4 have lower nutrient availability, especially for P, Zn, Fe, and Mn. The researchers offer methods for using iron or aluminium sulphate to bring pH down to the proper values. Reducing the pH of the soil hasn't been demonstrated to be cost-effective for growing agronomic crops like maize, but (Cox and Koenig, 2010).

Performance of crop is indirectly impacted by low pH of soil due to manganese and aluminium toxicity brought on by the soil's excessive acidity. According to some research, too alkaline soil can lead to issues like iron and zinc deficiencies, which can impair seedling germination and early development. Conversely, acidic soil can cause poor bacterial growth and deficiencies in magnesium and calcium.

The effects of pH on the metabolic processes and germination rate of Acinos alpinos subsp. Meridionalis (Satureja) seeds were investigated (Cherrate et al., 2023). Tests for seed germination in an aqueous media were conducted in vitro. They were time-dependently observed at various pH values. At pH = 7, the highest germination rate of 85.3% was attained. However, at an acidic pH (pH <3.5), there was no germination. Analysis using Fourier Transform Infrared Spectroscopy (FTIR) reports remarkable variation in the chemical makeup of seeds that had germinated. In fact, there were notable variations in the amounts and structures of chemical components between the treatments' absorbance peaks and the control groups, which could account for why some extremely abiotic circumstances prevent seeds from germinating.

The effect of pH on seed germination from *Origanum compactum* (Benth), a Moroccan medicinal plant, was investigated by Laghmouchi et al., (2017). After the flowers were finished, mature seeds were separated from *O. compactum* that had been gathered from two locations within the province of Ouezzane. The in vitro seed germination experiments were conducted with an aqueous medium on Petri dishes under dark conditions. The study assessed the impact of pH by monitoring the germination process over an extended period of time. The findings indicated that at an ideal pH of 7, there was a maximum germination percentage of 81%. Null germination of the seeds occurred at an acidic pH (pH <3.5).

Early seedling stage

Essential phases in the creation of a new plant are successful germination and seedling growth.
The cell cycle must be activated during the germination stage in order for seedlings to form. Cell growth and division occur through a series of precisely regulated stages known as the cell cycle. The progression of the cell cycle is regulated by interactions between protein kinases, phosphatases, cyclin-dependent kinases (CDKs), and their regulatory subunits, cyclins (CYCs) that works together in an orderly manner through reversible phosphorylation, ensuring the replication and cell division at the appropriate time. Seedling growth initially relies on cell expansion in the embryonic axis, not cell division. Later growth comes from new cells produced by the shoot and root tips (meristems) of the mature embryo. Root development and seedling establishment depends on the activation of the embryo's root meristem. (Wolny E. et al., 2018).

A seedling is developed when the first true leaf appears. The coleoptile, a crucial component in grass seedlings, shields and propels the plumule through the earth. Mesocotyl elongation begins as soon as the coleoptile emerges from the seed, pushing the base of the coleoptile upward and toward the soil's surface. Among grasses, there are two distinct seedling growth processes. A mesocotyl is an elongated region located directly below the coleoptile in most grass species. Certain grasses, like crested wheatgrass, have a long coleoptile but no elongation in the mesocotyl, which is the region below the coleoptile. The mesocotyl stops elongating when the coleoptile breaks through the soil crust and is exposed to light, especially red and/or far-red light. The mesocotyl may continue elongating to the point where it pushes the base of the coleoptile above the soil surface if the coleoptile is protected from red light, as in the case of extreme shade. As a result, the seedling lodges and eventually dies from inadequate crown development. The mesocotyl begins at the base of the coleoptile and ends at the embryonic axis, also known as the cotyledonary or scutellar node. Mesocotyl elongation is dependent on the seed's energy stores. When seedling leaves are exposed to light, the process of photosynthesis starts, supplying energy. At this point, the seedling can obtain its food without the seed. The primary root and the closely related seminal roots make up a root system that can provide the seedling with short-term access to water and inorganic nutrients. These roots serve a purpose until adventitious roots—which grow from the tissue of the crown—form the permanent root structure. Compared to the primary, seminal root system, the more hairy adventitious roots are more effective and penetrate a greater amount of soil (Wolny E. et al., 2018).

Effect of pH on early seedling development

Awasthi et al., (2022) examined rice seedling's varying reaction to an acidic pH. In order to distinguish between the detrimental effects of acidity on plant growth and the combined effects of stress, researchers looked at the effects of acidity on plants. They demonstrated it in rice seedlings of Disang and Joymati using a mix of morphological and physiological assays, including growth, reactive oxygen species (ROS), and parameters associated to photosynthesis under acidic and non-acidic rhizospheric conditions. Root length reductions of up to

31% and 17%, respectively, were seen in Joymati and Disang varieties; conversely, cultivars of Joymati showed a 9% reduction in root-relative water content and a 3% reduction in Disang cultivars. Overall, they found that diversity had little impact on morphometric characteristics such root length, biomass, and chlorophyll content. However, it was found that ROS buildup had considerably risen, with Joymati (a sensitive variety) showing greater accumulation than Disang (a tolerant variety). Chlorophyll fluorescence measurements show that photosynthesis was severely impacted even though the amount of chlorophyll decreased relatively little.

Pradhan et al., (2023) noted that Phosphorus (P) deficiency and Aluminium (Al) toxicity are the significant effects to plants grown in acidic soils (pH ≤ 4.5). Root growth suppression was seen in susceptible cultivars at a rate of about 24.2–28.9%, while tolerant cultivars showed just 2.69–4.32% inhibition. In contrast, the percentage Al uptake in tolerant cultivars was 1.58–1.9 times greater than that of sensitive cultivars, ranging from 1.90–3.25% higher than that of the control. Sensitive cultivars showed more root deformation, whereas tolerant cultivars showed less.

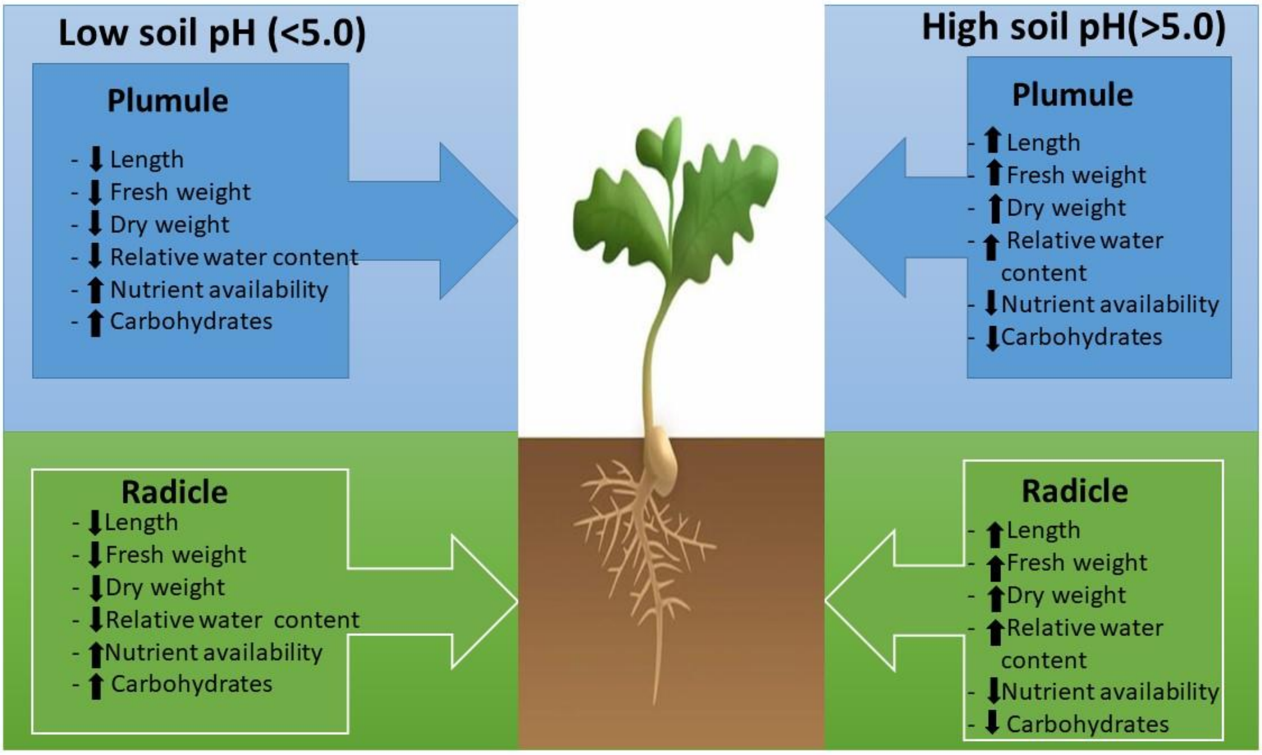

Fig 1. Overview of pH effects on seed germination and early seedling growth in plants

Amelioration of pH-induced damages in plant development

Since, acidic soil is a major problem in, and the low pH soil adversely affects the crop productivity including indigenous rice cultivars. It has become a crucial factor to find out ways for the amelioration of highly acidic soil for better production and proper growth of the crop plants. Biochar is a popular mitigating agent for acidic stress conditions in soil, that has received great attention in the last two decades by the scientists (Shi et al., 2019). Biochar is made by pyrolysis of various organic materials like crop and vegetable residual, animal and poultry waste by heating at varied temperatures. Adding biochar to acidic soil creates a more favourable environment for plants by reducing acidity, enhancing nutrient retention, and promoting beneficial microbial activity. (Rondon et al., 2007). Moreover, application of biochar also reduces the concentration of hydrogen ions, aluminium ions and some toxic metals present in the acidic soil and favours plant growth (Borchard et al., 2012). There are several alkaline biochars used by the researchers to ameliorate acidic soil (Huang et al., 2023).

Kinnunen et al., (2021) studied the capacity of biochar to mitigate acidity and adsorb metals for acid sulphate soil drainage water. To determine whether biocahar has any potential as a water protection strategy, a 96-hour laboratory experiment was conducted. Three types of biochar were mixed with water from the acid sulphate site: ash, lime, and biochar. From the start of the experiment to its conclusion, they periodically measured the concentrations of water Al, S, Fe, Cu, Co, Ni, and Zn as well as the overall pH. The outcome shown that biochar may both raise the pH of water and adsorb elements including Al, Fe, Co, Ni, and Zn.

Wang et al., (2014) studied the amelioration of soil acidity of strongly acidic tea garden soils by the application of biochar produced from crop residue. Strongly acidic soil (pH less than 5.0) is bad for the quality and productivity of tea. The use of biochar dramatically raised the pH and exchangeable cations of the soil after 65 days of incubation at 25 °C, while also lowering the Al saturation of tea soil. It was probably primarily the H+ ion's association with biochar and decarboxylation reactions that neutralized the acidity of the soil. Additionally, the use of biochar reduced the H+ ion produced from the nitrogen cycle.

Kolakhar, a traditional food preservative used in Northeast India, particularly by the Assamese people, emerges as a potential natural biochar for mitigating soil acidity and is prepared from banana stems, peels, and suckers (Kalita et al., 2015). Kolakhar is also used in rural Assam for treating stomach and respiratory ailments, and as an antibacterial agent. Studies by Hemanta et al., 2014 analysing its physico-chemical properties revealed high levels of alkali elements, vanadium, and zinc, which likely contribute to Kolakhar's high pH and bioactivity. This high alkalinity, attributed to elements like potassium, sodium, calcium, carbonate, and chloride. Application of Kolakhar to the soil can significantly ameliorate the acidic condition of the soil and therefore may lead to improvement in the growth and yield of the crop plants.

Shandilya (2022) investigated Kolakhar's role as a major factor in reducing soil acidity. This experiment evaluated the growth response of five traditional rice varieties with varying aluminum (Al) tolerance and phosphorus (P) deficiency levels. The treatments included application of traditionally made organic biochar from banana stem, peel and suckers (kolakhar), commercially available biochar (biokhar), and a combination of both. Rice seedlings treated with kolakhar exhibited significant improvements in biomass production, photosynthetic efficiency, and the activation of antioxidant defense mechanisms. Under kolakhar, seedlings showed increased synthesis of ascorbate peroxidase, guaiacol peroxidase, and other enzymes, suggesting that the plants may have established a stress-coping mechanism. The ultimate pH of any treated oils with ameliorants was found to change to pH 6 nearly similar to normal soil from pH 4.2 to 4.5. The soil treated with 250 mg kg^{-1} of kolakhar was found to have the highest amount of phosphorous. The Al availability also showed highest reduction from 2.107 to 0.018 mg kg^{-1} after 30 days of treatment with kolakhar.

Conclusion

In conclusion, soil pH significantly influences seed germination and early seedling development by affecting nutrient availability, hormone balance, and microbial activity. Acidic soils, with their higher levels of toxic elements like aluminum, prevents root growth and overall plant health. Strategies such as biochar application offer effective solutions by improving soil pH, enhancing nutrient uptake, and mitigating toxicity stress. Specifically, biochar types like Kolakhar have shown promise in alleviating acidity and promoting healthier crop growth. These highlight the importance of managing soil pH for sustainable agriculture and future food security amid changing environmental conditions.

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