Review

Unlocking plant defense: Exploring the nexus of biochar and Ca^{2+} signaling

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ABSTRACT

The interaction between biochar application and calcium ions (Ca^{2+}) in plants, in terms of activating plant defense mechanism would be useful to improve plant resilience and sustainable agriculture. This review aims to highlight the possible connection between biochar-induced changes in soil physicochemical properties, microbial interactions, and Ca^{2+} dynamics, ultimately leading to promote the plant defense mechanisms. We are also interested to discuss the role of Ca^{2+} signaling in coordinating plant responses to various biotic and abiotic stresses such as pathogen and insects attacks, cold or heat stress and drought stress as well as how Ca^{2+} fluxes, calcium-binding proteins, and ion channels are influenced by biochar application in the soil environment. Furthermore, we examine the impact of biochar on plant Ca^{2+} signaling pathways and how it can prime defense genes and strengthen call wall barriers to improve plant immunity. Despite significant progress, there is a need for interdisciplinary collaboration to fully sort out the mechanism of Ca^{2+} signaling in plants and induction of Ca^{2+} ions by biochar induction in soil environment. Advanced imaging techniques, proteomics and omics approaches could be helpful to unlock the complex interaction between biochar application and Ca^{2+} signaling. Overall, this review contributes substantially to the literature by describing the relationship between biochar and Ca^{2+} signaling and providing insights into novel approaches for enhancing plant defense mechanisms and development of sustainable agricultural solutions.

Abbreviation

ABA	Abscisic acid
cADPR	Cyclic ADP-ribose
CaM	Calmodulin
CBL	Calcineurin-B-like
CDPKs	Ca^{2+} dependent protein kinases
CEC	Cation exchange capacity
CML	CaM-like
CNGCs	Cyclic nucleotide-gated channels
cNMPs	Cyclic nucleotide monophosphates
ETI	Effector-triggered immunity
GLRs	Glutamate receptors
H$_2$O$_2$	Hydrogen peroxide
HACCs	Hyperpolarization-activated calcium channels
IP3	Inositol 1,4,5-trisphosphate
MAPKs	Mitogen-activated protein kinase
NOXs	NADPH oxidases
NO	Nitric oxide
OsCPK12	Rice Ca^{2+} dependent protein kinases
PAMP	Pathogen-associated molecular pattern
PTI	PAMP- triggered immunity
PP2C	Calcium-dependent protein phosphatase
ROS	Reactive oxygen species
SA	Salicylic acid
SOS	Salt overly sensitive
TPC1	Two-pore channels 1

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1. Introduction

In plants, calcium ion (Ca^{2+}) signaling plays a fundamental role in coordinating various physiological responses to a multitude of environmental stimuli, ranging from abiotic stresses to biotic stresses. Serving as a second messenger, Ca^{2+} directs complex signaling pathways in plant cells, converting external stimuli into appropriate cellular responses (Parmagnani and Maffei, 2022). Plants quickly mobilize Ca^{2+} ions from intracellular reserves or through influx from the extracellular space upon responses from environmental signals such as light, temperature, water availability, and pathogen attacks (Ghosh et al., 2023). This transient increase of cytosolic Ca^{2+} levels initiates a series of signaling events, such as activation of protein kinases, phosphatases, and transcription factors. These processes ultimately influence gene expression, metabolic pathways, and physiological processes (Naeem et al., 2020). In case of pathogen attacks, Ca^{2+} signaling serves as a front-line defense mechanism, regulating various defense responses such as the production of reactive oxygen species, activation of defense-related genes, synthesis of antimicrobial compounds, and reinforcement of cell walls (Tong et al., 2021). Therefore, it is important to understand the complexities of Ca^{2+} signaling in plant responses to environmental stimuli, particularly in case of pathogen interactions. Thus, it will further help us to better understand the plant defense mechanisms and crop resistance to biotic stresses. As global challenges such as climate change and emerging plant pathogens continue to threaten food security, there is a pressing need to explore innovative strategies to enhance plant defense mechanisms and resilience.

In last few decades, biochar has gained a lot of attention due to its heterogeneous nature and recalcitrant properties. Biochar is a solid carbon-rich product obtained after thermochemical conversion of organic biomass under complete or partial absence of oxygen (Sarfraz et al., 2023). Biochar has the ability to ameliorate soil nutrients status, cation exchange capacity (CEC), nutrient use efficiency (NUE), nutrient holding capacity and soil acidity. Due to large surface area and high porosity, biochar has the ability to hold a wide range of nutrients (e.g., K, Ca, Mg, Na, N, and P) (Sarfraz et al., 2019a). The influences of biochar on microbial activity are diverse, as it provides shelter for soil microbes with pore structures and surfaces. Biochar interrupts microbial intra and inter-specific communication between microbial cells via a combination of sorption and the hydrolysis of signaling molecules (Xu et al., 2016). Biochar application to soil can alter the Ca^{2+} signaling pathways in plants, which is essential for the priming of defense genes (Najafi-Ghiri et al., 2023). When biochar is introduced into the soil, it contributes to modify the soil physicochemical properties, thereby improving the availability of Ca^{2+} ions. In plants, these ions act as secondary messengers, initiating a signaling pathway that leads to the activation of defense genes (Moccia at al., 2023; Ahmad et al., 2023). As reported previously, kinases and transcription factors are activated when Ca^{2+} binds to sensor proteins like calmodulin (Iqbal et al., 2020). This process primes the plant's immune system, making it more responsive to future stressors. The presence of biochar thus indirectly influences the plant's ability to withstand biotic and abiotic challenges by affecting the Ca^{2+} signaling pathways that regulate stress-induced gene expression.

To our knowledge, however, the interplay between biochar application and Ca^{2+} signaling in modulating plant defense mechanisms remains largely unexplored. By revealing the complex mechanisms between biochar-induced changes in soil physicochemical properties, microbial interactions, and Ca^{2+} dynamics, this review aims to provide insights into novel approaches for enhancing plant defense. By exploring the synergistic effects of biochar and Ca^{2+} signaling, innovative strategies for sustainable agriculture can be developed. Biochar-based amendments or management practices that enhance crop resilience to both biotic stresses and abiotic challenges like salinity and drought can be introduced. Therefore, this review fills a critical gap in the literature by highlighting the connection between biochar and plant Ca^{2+} signaling, making a significant contribution to research on plant defense

and the advancement of sustainable agricultural practices.

2. Ca^{2+} signaling in plants

Ca^{2+} acts as a crucial intermediate in signaling pathways in plants. The "calcium signature" is the cytosolic or organelle Ca^{2+} concentration that can activate specific signaling sets, responsible for intermediates to carry out functions, and the fate of signal transduction determined by Ca^{2+} production (Bhar et al., 2023). As a mediator of the complex signaling network, Ca^{2+} concentration increases in the cell immediately or after a certain time as a response to environmental stimuli (Ranty et al., 2016). This Ca^{2+} signature is linked with different parameters including stimuli, and provides first-layer specificity (Allen et al., 2001). Ca^{2+} signal is decoded by Ca^{2+}-modulated proteins, and three main protein families are involved in this process: (i) Calmodulin (CaM) and CaM-like (CML); (ii) Calcineurin-B-Like (CBL); and (iii) Ca^{2+}-Dependent Protein Kinases (CDPKs) (La Verde et al., 2018). The binding of Ca^{2+} ions to the Ca^{2+} sensors trigger conformational change and affects downstream target proteins or directly stimulates kinase activity creating the second layer of specificity (Harmon et al., 2000).

Ca^{2+} signaling plays a complex role in plant defense against abiotic and biotic stresses, reconciling various physiological and molecular responses to combat invading microorganisms, pathogens, pests, and extreme environmental conditions to enhance plant survival (Fig. 1a). Among them, the primary function of Ca^{2+} is to stabilize plant cell walls and membranes. Similarly, Ca^{2+} can regulate the activity of various ion channels on the membrane, helping to maintain cellular balance. It can also activate or inhibit specific enzymes that are crucial for maintaining biochemical reactions under extreme environmental conditions. Further, Ca^{2+} can increase the expression rates of the anti-stress genes with changes in Ca^{2+} signaling (Li et al., 2022).

2.1. Ca^{2+} ion channels and transporters

Several channel proteins facilitate the exchange of Ca^{2+} ions in plants, including cyclic nucleotide-gated channels (CNGCs), two-pore channels 1 (TPC1), glutamate receptors (GLRs), and annexins. In Arabidopsis, around 150 cation transporters exist, with 20 being CNGC-class Ca^{2+} transporters that sense intracellular cyclic nucleotide monophosphates (cNMPs) such as cAMP and cGMP to regulate Ca^{2+} levels and transduce various signaling events (Xu et al., 2022; Yu et al., 2022). CNGCs play significant roles in plant immune responses; CNGC2 and CNGC4 trigger reactive oxygen species (ROS) generation and effector-triggered immunity (ETI) response upon recognition of the bacterial PAMP flg22 (Eichstadt et al., 2021). CNGC2, which is also known as DND1, regulates nitric oxide levels and defense responses, controlling hypersensitive response (HR)-mediated cell death (Zhao et al., 2021). Additionally, CNGC11 and CNGC12 participate in caspase-dependent programmed cell death (PCD) in response to pathogens, and CNGC20 interacts with BOTRYTIS INDUCED KINASE 1 (BIK1) to enhance immune responses (Ma et al., 2020).

The *cpr22* mutant, which contains a chimeric CNGC channel resulting from a deletion between *AtCNGC11* and *AtCNGC12*, exhibits constitutive expression of pathogenesis-related (PR) genes, providing resistance to the virulent pathogen *Hyaloperonospora parasitica*, although homozygosity for this mutant allele is lethal (Clough et al., 2000; Moeder et al., 2011). This mutant also suggests that Ca^{2+} ions are involved in Ca^{2+}-dependent cell death processes. Furthermore, plant GLR-type Ca^{2+} receptors, structurally similar to ionotropic glutamate receptors found in animals, contribute to ion homeostasis, immune signaling, and stress adaptations. Arabidopsis contains 20 GLR-type Ca^{2+} transporters, with the most performing developmental functions (Ghosh et al., 2022). Recent research highlights their involvement in immune signaling and stress responses, exhibiting a broader role in plant adaptation and defense mechanisms.
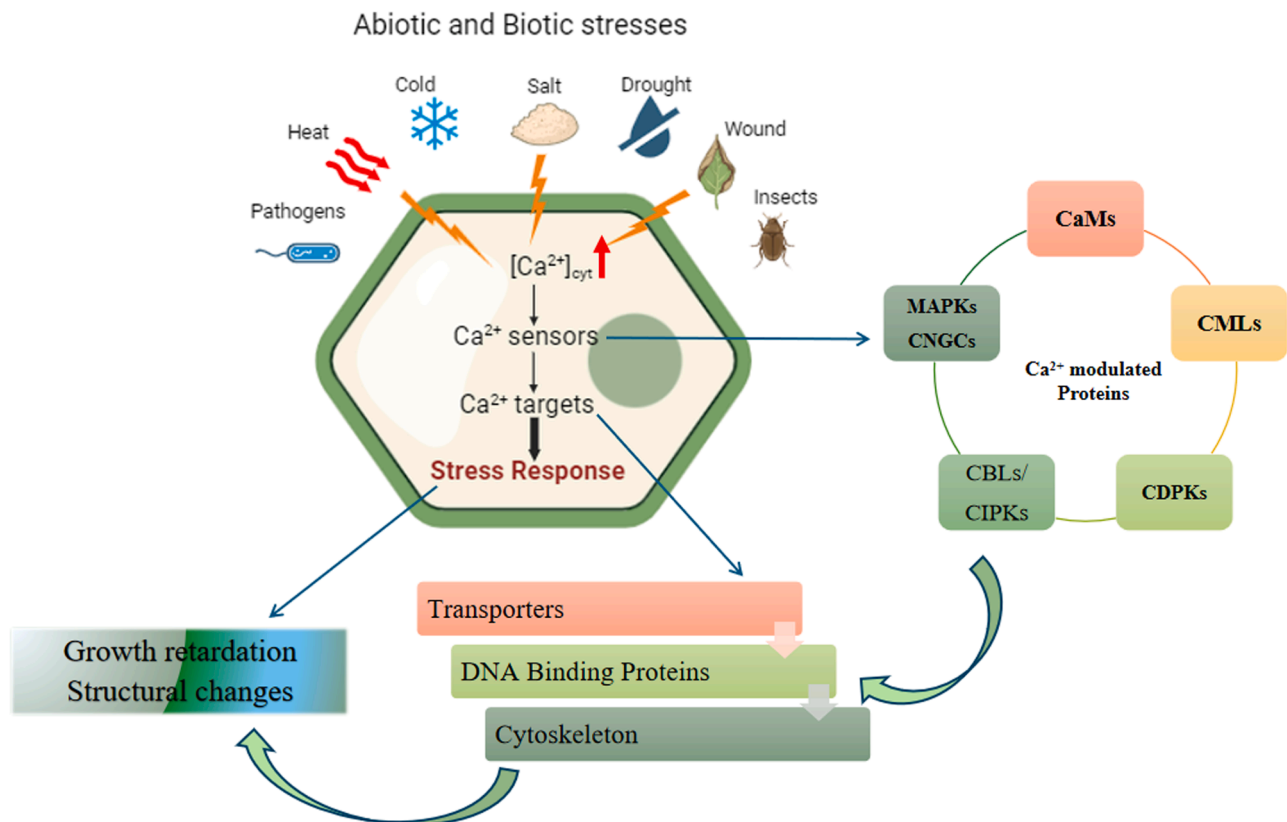
Abiotic and Biotic stresses

Fig. 1a. Effect of biotic and abiotic stress on Ca^{2+} signaling.

2.2. Ca^{2+} sensors and signal transduction

Different types of Calmodulins (CaMs) and CaM-like proteins (CMLs) are found in plants that contain Ca^{2+} sensor proteins having a common domain called EF-hand motifs. Approximately, 30 amino acids forming helix-loop-helix structure will make up these pairs of motifs which allow CMLs to sense Ca^{2+} in plant cells (Mohanta et al., 2015; Kaur and Upadhyay, 2022). When these proteins are disrupted, plants struggle more with both disease resistance and coping with environmental stress (Raina et al., 2021). Research has shown how important these proteins are for plant immunity. For example, when soybean CMLs are over-produced in tobacco plants, these plants become more resistant to bacteria, viruses, and fungi. Interestingly, these genetically modified plants also develop spots that resemble lesions and show signs of an immune response, even without the usual trigger, a chemical called salicylic acid (SA) (Heo et al., 1999). In some recent studies, *Arabidopsis thaliana,* alone is reported to contain 50 CML family members (Negi et al., 2023).

Recent research has demonstrated that the expression of individual CaM/CML genes is essential for defense responses. Overexpression of some CMLs, such soybean CMLs, enhances plant resistance to a wide variety of diseases. Likewise, research on loss-of-function has emphasized the significance of different CMLs in plant immunity. In particular, in Arabidopsis, CML8, CML9, CML24, and CML41 control reactions to various Pseudomonas syringae strains and aid in resistance to herbivores like Spodoptera littoralis (Leba et al., 2012; Zhu et al., 2017). Potential methods for improving crop protection against diseases and pests are provided by these Ca^{2+}-binding proteins, which allow plants to sense changes in Ca^{2+} levels and convert them into signaling pathways that trigger defense responses (Park et al., 2004).

Calcium-dependent protein kinases (CDPKs), with around 30 members in angiosperms, are diverse serine/threonine kinases. They consist of an N-terminal domain, a central kinase domain, and an activation domain containing EF-hands, which bind Ca^{2+} to activate kinase

activity. CDPKs regulate various cellular responses, including gene expression and protein degradation (Hu et al., 2016; Kaur and Upadhyay, 2022). They play significant roles in immune responses, such as PTI-induced resistance and stomatal closure, and can phosphorylate key enzymes like AtRBOHD and phenylalanine ammonia-lyase. Comparable studies in potatoes and tomatoes have shown CDPK involvement in defense against pathogens. CDPKs also regulate plant cell death, with some basic active CDPKs inducing programmed cell death, although activation alone is not always sufficient to trigger it (Dubiella et al., 2013).

2.3. Role of Ca^{2+} signaling in plant defense

2.3.1. Biotic stress

In unstimulated cells, cytosolic Ca^{2+} concentrations are typically around 100 nM. When plant cells encounter pathogens, an early response is an increase in cytosolic Ca^{2+} due to Ca^{2+} influx (Ma and Berkowitz, 2007). This elevation has been observed in both compatible and incompatible plant-pathogen interactions. For instance, in *Nicotiana plumbaginifolia* cells treated with cryptogein from *Phytophthora cryptogea*, Ca^{2+} transients occur (Lecourieux et al., 2002). Similar responses are induced by other elicitors like the Pep-13 peptide from *P. megasperma* and an oligopeptide from *P. sojae* in parsley cells. Additionally, during effector-triggered immunity (ETI), changes in Ca^{2+} levels are detected in interactions between *Pseudomonas syringae* pv. tomato and Arabidopsis RPM1 (Blume et al., 2000). Notably, the dynamics of Ca^{2+} changes differ between compatible and incompatible interactions, which might relate to the distinct defense mechanisms in pathogen-associated molecular pattern (PAMP)-triggered immunity (PTI) and ETI. These Ca^{2+} changes correlate with defense responses such as reactive oxygen species (ROS) and nitric oxide (NO) production, and PR gene expression. Recently, the tomato calcineurin B-like protein 10 (Cbl10)/ calcineurin B-like interacting protein kinase 6 (Cipk6)

signaling module is involved in ROS signaling during plant-pathogen interactions. Besides, 2 CIPKs, *OsCIPK14* and *OsCIPK15* have been shown to participate in PTI (Torre et al, 2013; Xiaolin et al., 2022).

AtSR1/CAMTA3 negatively regulates salicylic acid (SA)-mediated defense by inhibiting EDS1 expression, crucial for balancing defense responses. The early ethylene-responsive gene, *NtER1*, an ortholog of *AtSR1*, was first identified in tobacco plants. Later, 6 homologs of *NtER1*, *AtSR1–6/CAMTA1–6*, were identified in Arabidopsis and found to be induced rapidly and differently by various external stimuli (Bouche et al., 2002; Yang et al., 2002). Further functional analysis of one of these genes, *AtSR1*, connected Ca^{2+} signals directly to plant pathogen defense response through negative regulation of the activation of the pathway of the well-known plant defense hormone, salicylic acid (SA) (Du et al. 2009). Conversely, CBP60g, another CaMBP, positively regulates SA biosynthesis by activating ICS1. *AtNOS1* links Ca^{2+}/CaM signaling to nitric oxide (NO)-mediated defense, with mutants showing increased susceptibility. NAD kinase, activated by Ca^{2+}/CaM, enhances ROS production during pathogen response (Guo et al., 2003). CMLs, akin to CaM but with varied roles, include CML9, a positive regulator of defense against different strains of bacterial pathogen *P. syringae* (Leba et al., 2012).

Besides, a few CDPKs are negative regulators in plant defense responses. Overexpression of *OsCPK12* in rice led to hypersensitivity to both virulent and avirulent blast fungi, probably due to compromised ROS production (Asano et al., 2012). In summary, CDPKs serve as important Ca^{2+} sensors to translate the information to downstream processes during plant responses to pathogen challenges. CDPKs participate in plant defense by interacting and phosphorylating diverse substrates to regulate different aspects of immune responses.

2.3.2. Abiotic stress

The abscisic acid (ABA)-dependent Ca^{2+} signaling pathway is the primary mechanism through which plants respond to drought stress. A variety of secondary messengers, such as ROS, nitric oxide (NO), inositol 1,4,5-trisphosphate (IP3), and cyclic ADP-ribose (cADPR), assist in the release of Ca^{2+} from intracellular reserves when ABA activates plasma membrane Ca^{2+} channels. For instance, water deficiency leads to accumulation of ABA in leaves (Li et al., 2022). Conversely, it causes phospholipase C to break down IP3, which in turn activates intracellular Ca^{2+} pools in guard cells, causing the stomatal closure. Furthermore, hydrogen peroxide (H_2O_2) activated by ABA causes an increase in intracellular Ca^{2+} promoting hyperpolarization of the plasma membrane, in turn activating the vacuolar membrane Ca^{2+} channels and hyperpolarization-activated Ca^{2+} channels (HACCs) (Sun et al., 2017).

During drought conditions, there is a rise in the concentration of reactive oxygen species (ROS) within cells, causing membrane peroxidation and consequent damage to membrane structures (Li et al., 2022). However, plant cells can overcome such an increase in ROS by improving ROS scavenging mechanisms and regulating the activity of CDPKs and NADPH oxidases (NOXs) with the help of Ca^{2+} signaling (de Carvalho, 2008; Cousson, 2009; Wang et al. 2024).

During salt stress condition, Ca^{2+} inhibits Na^+ influx and the outflow of K^+ and initiates a salt overly sensitive (SOS) signal transduction pathway (Mahajan et al., 2008; Zhao et al., 2013). As per a previous study conducted on rice, at high salinity, Ca^{2+} did not affect Na^+ and K^+ accumulation, and K^+/Na^+ ratios in plants. This finding demonstrates that Ca^{2+} application significantly reduced both Na^+ efflux and influx in rice plants under low and high salinity conditions. Interestingly, the ratio of Na^+ efflux to influx was only diminished under low salinity stress. This suggests that Ca^{2+} might primarily regulate K^+/Na^+ homeostasis in rice at low salinity by enhancing the selectivity for K^+ over Na^+. It will reduce the Na^+ influx and efflux, minimizing unnecessary Na^+ cycling (Khan et al. 1992; Wu and Wang, 2012). Moreover, CDPKs can sense Ca^{2+} and directly activate target proteins, which affects various physiological processes in plants. For instance, OsCPK12 has been identified as a positive regulator of salt stress tolerance in rice (Pandit

et al., 2018)

Under low-temperature conditions, plants have the ability to open Ca^{2+} channels increasing cytosolic Ca^{2+} concentration and triggering Ca^{2+} signaling. Previously it is reported that tobacco plants treated with external Ca^{2+} under low-temperature stress could increase intracellular bound Ca^{2+} and improve the activities of catalase, superoxidase dismutase, peroxidase, and other antioxidant enzymes (Gao and Zhang, 2019). Application of calcium chloride ($CaCl_2$) could enhance enzyme and soluble protein activity while reducing malonic acid content in leaves (Li et al., 2009). Additionally, Ca^{2+} treatment would be helpful to improve net photosynthetic rate, transpiration rate, and stomatal conductance under high-temperature conditions, highlighting the importance of Ca^{2+} in abiotic stress responses (Kurusu et al., 2010; He et al., 2023).

High Ca^{2+} in the soil can improve the tolerance of plants to heavy metals (Li et al., 2022). At the same time, heavy metals create Ca^{2+} loss in plants and inhibit Ca^{2+} absorption and root growth. This suggests that soils contaminated with heavy metals needed remediation or Ca^{2+} external supply for better plant growth and development. A study conducted with soybeans showed endogenous salicylic acid (SA) and Ca^{2+} were required for the transmission of stress signals and suggested the importance of Ca^{2+} in stress tolerance (Plasencia et al., 2021). All this evidence suggests that Ca^{2+} signaling plays a vital role in the abiotic stress response in plants for survival. Not only abiotic stresses, but plants also must undergo several stress conditions triggered by biotic factors such as pathogens and insects (Fig. 1b). According to previous studies, Ca^{2+} has developed a response against pathogenic fungi, bacteria, etc. through several mechanisms such as CDPKs, MAPKs, and CNGCs pathways (Bhar et al., 2023). Further Ca^{2+} signaling is induced by herbivore-associated molecular patterns and activates Ca^{2+} permeable channels in the membrane. Insect attacks also enhance plant resistance to insects by inducing Ca^{2+} and ROS signaling pathways. Calcium-binding proteins also affect the biotic stress response mechanisms as they function during the abiotic stress resistance response.

3. Impact of biochar on soil environment

Biochar exhibits various effects on soil environments, both advantageous and detrimental depending on feedstock properties, pyrolysis conditions, application rate and soil indegenous properties. Through its inherent high cation exchange capacity (CEC), biochar helps to improve soil fertility by increasing the availability of nutrients such as nitrogen (N), phosphorus (P), potassium (K) and calcium (Ca) for plant uptake (He et al., 2018). Moreover, biochar has the ability to buffer soil pH by providing an optimum pH range crucial for nutrients availability and plant growth (Sarfraz et al., 2020). Biochar contributes to the amelioration of soil structure by improving soil aggregation and reducing soil compaction, consequently enhancing water infiltration rate, retention capacity, and promoting root penetration and aeration (Fig. 2).

Being a stable carbon reservoir, biochar has the capacity for long-term carbon sequestration, thereby serving as an eco-friendly approach to mitigate climate change (Tisserant et al., 2019). Bochar applications have been observed to mitigate greenhouse gasses emissions such as nitrous oxide and methane (Huang et al., 2019). Biochar has significant influence on soil microbial communities and enzyme activities, providing conducive habitats and nutrients for microorganisms, thereby enhancing soil biological activity and promoting plant growth (Sarfraz et al., 2019b). Biochar has the ability to immobilize certain contaminants in soil which make it a potential asset to remediate the contaminated soil (Khan et al., 2024). Hence, keeping in view the versatility and effectiveness of biochar, it could be a very useful strategy to improve soil health and plant development in future scenario.

3.1. Biochar as a source of Ca^{2+}

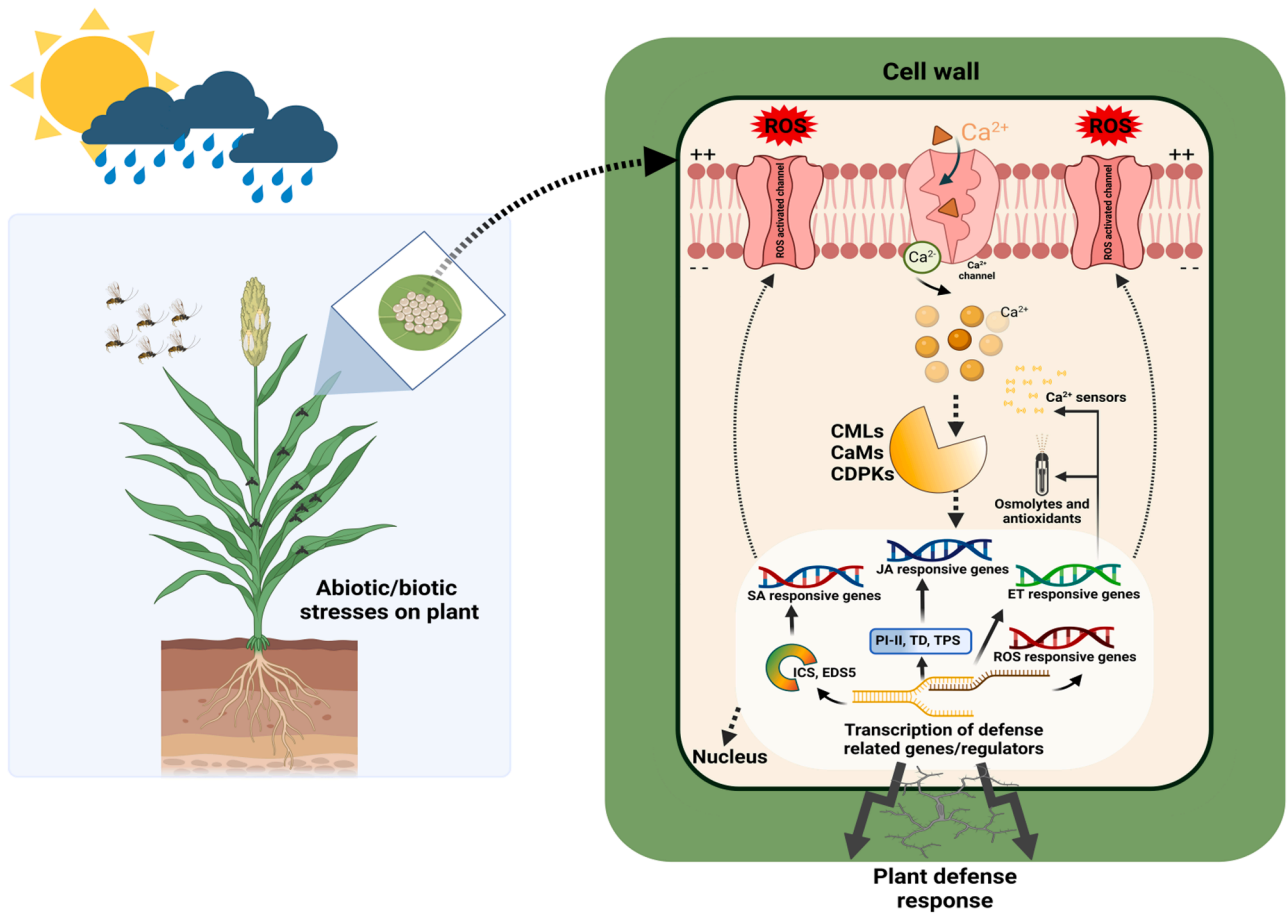Biochar obtained through the pyrolysis of organic feedstock, can

Fig. 1b. The cellular response of plant to biotic and abiotic stress: An insight on key components and signaling pathways.
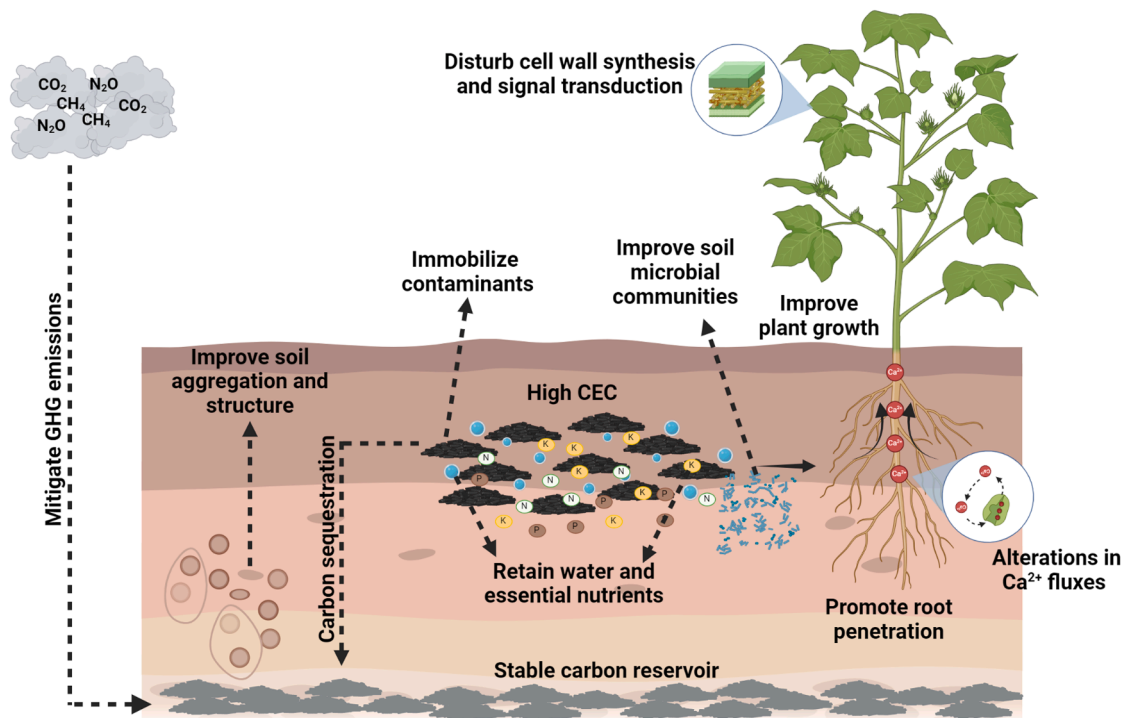

Fig. 2. The intricate mechanism of biochar application on greenhouse gasses, soil properties, carbon reservoirs, root penetration ultimately leading to improved Ca^{2+} fluxes and strengthening call wall barriers.

serve as a significant source of calcium (Ca), depending on the type of feedstock and specific pyrolysis conditions. The feedstock composition plays a crucial role such as sewage sludge, animal manures, spent mushroom substrates, paper and food wastes are often richer in Ca, making them preferable for generating Ca-enriched biochars (Table 1). Pyrolysis temperature and duration also markedly influence the Ca^{2+} content and its bioavailability in biochar. Higher pyrolysis temperatures (above 500°C) typically enhance the concentration of Ca^{2+} and other minerals due to the reduction of organic matter and the concentration of ash content. Ferreira et al. (2018) reported significant variability in the influence of biochar on Ca^{2+} content at different pyrolysis temperatures, and application rates. The study reported that biochar derived from elephant grass demonstrated a marked increase in soil Ca^{2+} levels at higher pyrolysis temperatures (600°C) (Ferreira et al., 2018). However, barley straw biochar, applied at 400°C and 10 ton ha^{-1}, produced a slight increase in silt loam soil Ca^{2+} content from 6.47 to 6.49 cmolc kg^{-1} (Kang et al., 2018). Biochar derived from brewery spent grain at 650°C, applied to maize, resulted in an increase in Ca^{2+} content in belowground tissues at lower dose (10.37 g kg^{-1} at 2 %) than higher dose (3.88 g kg^{-1}) (Manolikaki et al., 2020) (Table 2). Studies investigating the effects of biochar application on Ca^{2+} fluxes, calcium-binding proteins, and ion channels have reported various observations, albeit with some inconsistencies and context-specific outcomes. Some studies have noted alterations in Ca^{2+} fluxes following biochar application, suggesting potential impacts on Ca^{2+} dynamics in the soil environment. These changes in Ca^{2+} fluxes can influence various physiological processes in plants, including signaling pathways, enzyme activation, and cellular integrity.

A recent study investigated the effects of biochar on Ca^{2+} dynamics and CaM activity in *Arabidopsis thaliana*. They found that biochar application in soil increased the concentration of available Ca^{2+} ions in rhizosphere. The increase in Ca^{2+} availability also led to enhanced Ca^{2+} signaling within plant cells (Liang et al., 2023). The study demonstrated an upregulation of CaM gene expression and increased activity of CaM proteins in Arabidopsis plants grown in biochar-amended soil as compared to the control plants. The upregulation of CaM activity was associated with improved plant growth, stress tolerance, and defense responses. In another study the effects of biochar application on Ca^{2+} dynamics and CDPK activity in maize (Zea mays) was reported. The study reported that incorporation of biochar into soil resulted in increased levels of available Ca^{2+} ions and altered Ca^{2+} fluxes in maize roots (Ruan et al., 2023). Biochar treatment also induced changes in the expression and activity of CDPKs. The study also reported an upregulation of certain CDPK genes and increased phosphorylation activity of CDPK proteins in maize plants after biochar application (Wilkins et al., 2016). These changes in Ca^{2+} signaling and CDPK activity were associated with enhanced plant growth, nutrient uptake, and stress tolerance in maize subjected to biochar amendment. However, further research is needed to understand the underlying mechanisms and determine the efficacy of biochar amendment to improve Ca^{2+} signaling in plants across different soil types, plant species, and environmental conditions.

3.2. Influence of biochar on Plant Ca^{2+} signaling

Influence of biochar on plant Ca^{2+} signaling is a promising area of research with significant implications for agricultural sustainability and crop resilience. The application of biochar to soil has been shown to affect the Ca^{2+} signaling pathways, which are vital for plant stress responses (Ruan et al., 2023). Biochar can alter the soil physicochemical properties, thereby increasing the availability of Ca^{2+} ions that serve as secondary messengers in plant signaling networks. These ions play a crucial role in decoding environmental stimuli into physiological responses, including those to abiotic stresses such as drought, salinity, and extreme temperatures (Naeem et al., 2019). The presence of biochar can enhance the capacity of plants to maintain Ca^{2+} homeostasis, thereby improving their ability to resist and survive in adverse conditions

Table 1

Calcium (Ca^{2+}) contents in biochar obtained from various feedstocks at different pyrolysis temperatures.

Biochar Source	Pyrolysis Temperature (°C)	Ca^{2+} Contents in biochar (mg kg^{-1})	Notes	Reference
Chicken manure	250	19840	Moderate	Xiao et al. (2018)
	350	21710	Moderate	
	550	30250	Moderate	
Chicken manure *Ca	250	40500	Moderate	
	350	49100	High	
	550	59100	High	
Chicken manure *Mg	250	22370	Moderate	
	350	28090	Moderate	
	550	30940	Moderate	
Chicken manure *Fe	250	20320	Moderate	
	350	25300	Moderate	
	550	31800	Moderate	
Poultry litter	500-520	45400	High	Brantley et al. (2016)
Poultry litter	400	25000	Moderate	Macdonald et al. (2014)
Turkey litter	350	40400	High	Cantrell et al. (2012)
	700	56100	High	
Dairy manure	350	26700	Moderate	
	700	44800	High	
Paved-feedlot	350	22700	Moderate	
	700	35000	Moderate	
Swine Solid	350	39100	Moderate	
	700	61500	High	
Poultry litter	350	26600	Moderate	
	700	40200	High	
Swine manure	400	20300	Moderate	Subedi et al. (2016)
	600	28900	Moderate	
Poultry litter	400	28300	Moderate	
	600	35900	Moderate	
Pig manure	500	34700	Moderate	Zhao et al. (2018)
Sewage sludge	500	65700	High	
Waste mushroom substrate biochar (*Pleurotus eryngii*)	400	98650	High	Sarfraz et al. (2019a)
	500	105670	High	
	600	115470	High	
	700	117470	High	
Spent mushroom substrate (*Ganoderma lucidum*)	350	42600	High	Zhang et al. (2022)
	550	63800	High	
	750	89400	High	
Spent mushroom substrate (*Lentinus edodes*)	350	74800	High	
	550	96600	High	
	750	113500	High	
Bamboo	600	9000	Low	Hilioti et al. (2017)
Bull Manure	300	9412	Low	Enders et al. (2012)
	350	10518	Low	
	400	10088	Low	
	450	8450	Low	
	500	9432	Low	
	550	11109	Low	
	600	9386	Low	
Corn	300	6480	Low	
	350	6136	Low	
	400	7254	Low	
	450	7317	Low	
	500	11699	Low	
	550	9804	Low	
	600	9383	Low	
Dairy Manure	300	11094	Low	
	350	10859	Low	
	400	12808	Moderate	
	450	13473	Moderate	
	500	12569	Moderate	
	550	25702	Moderate	
	600	13997	Moderate	
Hazelnut	300	3726	Low	
	350	2580	Low	

Table 1 (*continued*)

Biochar Source	Pyrolysis Temperature (°C)	Ca^{2+} Contents in biochar (mg kg^{-1})	Notes	Reference
	400	2821	Low	
	450	2598	Low	
	500	2693	Low	
	550	2823	Low	
	600	3262	Low	
Oak	300	752	Low	
	350	1097	Low	
	400	1061	Low	
	450	1023	Low	
	500	1538	Low	
	550	1609	Low	
	600	1210	Low	
Pine	300	2927	Low	Enders et al. (2012)
	350	1940	Low	
	400	2247	Low	
	450	2194	Low	
	500	2741	Low	
	550	2255	Low	
	600	2167	Low	
Poultry Manure	300	157531	High	
	350	215648	High	
	400	265729	High	
	450	267804	High	
	500	204205	High	
	550	252608	High	
	600	242788	High	
Digested Dairy Manure	300	20185	Moderate	
	400	22552	Moderate	
	500	18505	Moderate	
	600	26518	Moderate	
Food	300	28177	Moderate	
	400	51745	High	
	500	53779	High	
	600	73534	High	
Paper	300	258128	High	
	400	266234	High	
	500	289226	High	
	600	311232	High	
Grass Clippings	500	20622	Moderate	
Sun flower husk pellet	400	9800	Low	Colantoni et al. (2016)
	500	12500	Low	
Grape vine pellet	400	15200	Moderate	
	500	17900	Moderate	
Elephant grass	400	10900	Low	Ferreira et al. (2018)
	500	12800	Moderate	
	600	14500	Moderate	

* $CaCl_2$, $MgCl_2 \cdot 6H_2O$, $FeCl_3 \cdot 6H_2O$

(DeFalco et al., 2023). Furthermore, biochar may influence the expression of Ca^{2+} transporters and sensors, potentially offering a novel approach to improve plant defense mechanisms through the optimization of Ca^{2+} signaling pathways. This section highlights how the effect of biochar application on Ca^{2+} signaling can be helpful for enhancing plant adaptation to environmental challenges and warrants further investigation to fully understand the possible mechanisms and practical applications in agriculture (Köster et al., 2022). At present, several studies discuss the role of environmental factors, biotic and abiotic stresses on Ca^{2+} signaling in plants however, application of biochar as soil amendment has always been neglected.

3.2.1. Biochar-mediated enhancement of plant defense

Biochar has been shown to stimulate the plants innate immune system, leading to the activation of systemic defense responses. This includes the up regulation of defense-related genes, such as pathogenesis-related (PR) genes, which encode proteins involved in plant defense mechanisms against pathogens (Cao et al., 2018). It is worth mentioning that biochar aren't always related to its ability to activate plant defenses, but rather to its ability to promote plant development. One notable

example is the use of biochar generated from poplar woodchips to the cultivation of lettuce and the model plant Arabidopsis thaliana. However, global gene expression arrays and metabolomics indicated that numerous classes of plant defense genes, including those involved in jasmonic acid production and those encoding the defensive response, were suppressed. These genes are all essential for a plant's resilience to pests and diseases (Yang et al., 2012).

Biochar has a significant influence on soil microbial communities which could promote beneficial interactions between plants and microbes. Some microorganisms produce secondary metabolites or volatile organic compounds after biochar application in soil directly or indirectly inducing plant defense responses or antagonizing pathogens and pests (Poveda et al., 2021). Improved nutrient availability and uptake in biochar-amended soils can enhance plant vigor and health, making them more resilient to pest and pathogen attacks (Abideen et al., 2020). Additionally, biochar has the ability to retain and slowly release nutrients providing a sustained source of nutrition for plants, supporting their defense mechanisms (Fig. 3).

The physical structure of biochar, particularly its porous nature, can create a physical barrier that impedes the movement of pathogens and pests in the soil, thus reducing their ability to infect plant roots or foliage. It is already discussed that biochar contributes substantially as a source of Ca^{2+} in soil which could help to improve plant signaling pathways (Tisserant et al., 201; Rajput et al., 2024). Biochar-induced changes in Ca^{2+} signaling can contribute to enhanced plant immunity through various mechanisms, including the priming of defense genes and reinforcement of cell wall barriers (Ruan et al., 2023). Her, we will discuss the possible pathways which may occur after biochar application in soil:

3.2.1.1. Priming of defense genes. Priming is the process by which defence mechanisms are pre-activated in response to a stress stimuli, allowing plants to generate a rapid defence response in case of a subsequent pathogen attack. As previously described, Ca^{2+} ions are essential signaling molecules involved in the activation of plant defense responses. Biochar application can improve Ca^{2+} signaling pathways in plants, leading to the priming of defense genes. Moreover, these changes in Ca^{2+} dynamics can trigger the expression of defense-related genes such as CDPK genes, CaM genes and PP2C genes, thereby enhancing the plant's ability to combat pathogens and enhance defense against various pathogens (Goher et al., 2024). It is reported that elevated Ca^{2+} levels activate CDPKs, which phosphorylate and activate transcription factors upregulating pathogenesis-related (PR) genes and enzymes involved in lignin biosynthesis, thereby priming the plant for a faster and stronger defense response. Upon CaM binding to Ca^{2+}, it undergoes a structural change that enables it to interact with CaM-binding proteins, including kinases and phosphatases. This interaction will boost up ROS production and activate additional defense genes (Putney et al., 2012; Singh et al., 2024), thereby preparing the plant for potential stress. PP2Cs help to maintain this priming process by dephosphorylating key signaling proteins, ensuring that defense responses are appropriately regulated and not prematurely activated, thus conserving resources. The coordinated actions of CDPKs, CaMs, and PP2Cs in response to biochar-induced Ca^{2+} signaling changes lead to the priming of defense genes, resulting in an abrupt and rapid activation of defense mechanisms when pathogens are detected, including the reinforcement of cell wall barriers, production of defense-related enzymes, and synthesis of structural proteins that hinders pathogen entry and spread.

3.2.1.2. Reinforcement of cell wall barriers. Lignin and callose are important components of plant cell wall which contribute in maintaining the plant structure and preventing against pathogens entrance. Biochar when applied in soil can lead to the deposition of callose and lignin enforcing the Ca^{2+} signaling channels. Callose deposition at plasmodesmata, microscopic channels connecting plant cells, can

Table 2
Effect of biochar application on soil and crop Ca^{2+} contents.

Biochar Source	Pyrolysis Temperature (°C)	Biochar Dose	Pre application Ca^{2+} contents in Soil/Crop	Post application exchangeable Ca^{2+}	Reference
Elephant grass	400	5 g L	Soil	0.72	Ferreira et al. (2018)
		15	0.4 ($cmol_c$ L^{-1})	0.80	
	500	5		0.82	
		15		1.16	
	600	5		1.54	
		15		1.44	
Barley straw	400	10 ton ha	Silt loam soil 6.47 ($cmol_c$ kg)	6.49	(Kang et al. (2018)
Eupatorium	450	5		3.54	Pandit et al. (2018)
		10	Silty loam soil	3.73	
		15	3.2 ($cmol_c$ kg)	4.47	
		25		4.61	
		40		5.74	
Wood	450	5 ton ha		3.97	Lusiba et al., (2017)
		10	Clay soil	4.06	
		15	5.84 (cmolc kg)	3.77	
		5 ton ha		0.77	
		10	Loamy Sand	0.66	
		15	0.91 (cmolc kg)	0.71	
Poultry litter	500	5 Mg ha^{-1}		2.5	Brantley et al. (2016)
		10	Loam soil 1.59 (mg g^{-1})	2.3	
Wheat straw and peanut shell	500	8 t ha-1		32.09	El-Naggar et al. (2018)
			Soil coarse sand 33.65 ($cmol_C$ kg^{-1})		
			Fine sand soil 29.90	29.24	
			Silt/clay soil 38.23	38.64	
Shrimp waste	300	5 g kg		2.3	Abo-Elyousr et al. (2022)
		10	Pearl millet shoots (%)	2.5	
		15		2.7	
		20		2.8	
		25		2.8	
Brewery spent grain	650	2 %	Maize (belowground tissues)	10.37	Manolikaki et al. (2020)
		5	(g kg)	3.88	
Surplus yeast		2		4.01	
		5		2.67	
Brewery spent grain	650	2		3.34	
		5	Maize (aboveground tissues)	3.29	
Surplus yeast		2		2.96	
		5		3.07	
peanut shells	350- 550	33.75 ton ha^{-1}		1848.09	Li et al. (2022)
		67.50	Soil 1815.89 (mg kg^{-1})	2056.38	
		101.25		2373.06	

restrict the spread of pathogens between cells (Blum, 2017). Moreover, lignification of cell walls can strengthen their resistance to any mechanical damage and enzymatic degradation by various pathogens (Fig. 3). Application of biochar can promote the acitivity of various key genes and enzymes involved in cell wall fortification. Pathognese resistance genes such as PR1, PR2 (β-1,3-glucanase), and PR3 (chitinase), degrade fungal cell walls and produce antimicrobial compounds (Santos and Franco, 2023) whereas enzymes such as expansins, peroxidases, and polygalacturonase-inhibiting proteins (PGIPs) modify and strengthen the cell wall. Biochar incoorporation into soil can improve the role of genes involved in lignin and callose biosynthesis, including PAL, C4H, 4CL, and GSL genes. These genes play vital roles in creating physical barriers against pathogen entry. Structural proteins like extensions and xyloglucan endotransglucosylase/hydrolases (XTHs) further enhance cell wall integrity (Moccia et al., 2023; Naeem et al., 2023a). For instance, fungal pathogens like Botrytis cinerea and Magnaporthe oryzae are countered by increased chitinase and β-1,3-glucanase activity, while bacterial pathogens such as Pseudomonas syringae and Xanthomonas oryzae are impeded by callose deposition and peroxidase-mediated lignification (Liu et al., 2021).

Briefly, biochar can enhance soil quality and nutrient uptake, including Ca^{2+}, Na+ and K+ thereby improving Ca^{2+}-dependent signaling pathways. The activation of cCDPKs, calmodulin CaM, and PP2Cs, will regulate the expression of defense-related genes, optimize resource allocation, and ensure effective and balanced plant immune responses (Gohar et al., 2024). Overall, the modulation of Ca^{2+} signaling pathways by biochar resulting in priming of defense genes and the reinforcement of cell wall barriers, will lead to improved plant immunity against pathogens. These mechanisms highlight the potential of biochar as a sustainable strategy for enhancing plant resistance to biotic stresses in agriculture (Naeem et al., 2023b). Further research is needed to clarify the specific molecular mechanisms underlying biochar-induced changes in Ca^{2+} signaling and their impact on plant immune responses.

4. Challenges and future directions

The interaction between biochar and Ca^{2+} signaling in plant defense presents several knowledge gaps and challenges that must be addressed in future studies:

1. The heterogeneous nature of biochar with diverse physicochemical properties can vary depending on feedstock typs, pyrolysis conditions, and pyrolysis times. However, the specific biochar properties influencing Ca^{2+} signaling and plant defense responses remain
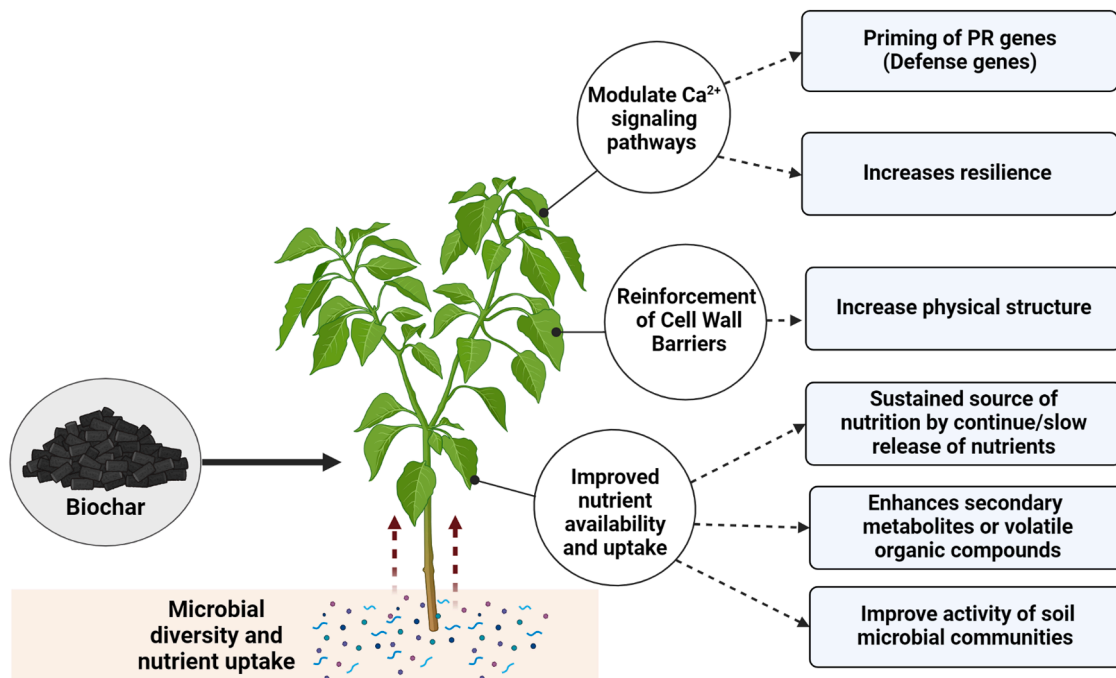
Fig. 3. Biochar-mediated enhancement of plant defense influencing gene priming and reinforcing cell wall barriers.

poorly understood. Therefore, systematic investigations are needed to establish the mechanism between biochar properties and their effects on Ca^{2+} dynamics and plant immunity.

2. There is a lack of standardized protocols to study the biochar-soil-plant interactions and Ca^{2+} signaling in plant defense. Variability in experimental conditions, such as biochar application rates, soil types, and plant species, hinders comparability between studies and the generalizability of findings. Establishing standardized experimental protocols and benchmarks will facilitate cross-study comparisons and meta-analyses to advance our understanding of biochar-mediated effects on Ca^{2+} signaling in plant defense.

3. Omics approaches hold immense potential in unlocking plant defense mechanisms, particularly when studying the complex interplay between biochar application, calcium (Ca^{2+}) signaling, and plant immunity. This has not yet been used in the biochar-related studies relating to the Ca^{2+} signaling. Genomics and transcriptomics can be used to identify genes regulated by biochar that influence Ca^{2+} signaling pathways and dynamic gene expression changes in response to biochar application and Ca^{2+} fluctuations. RNA sequencing is a main technique that can help to elucidate gene regulation under different biochar treatments under stress conditions. Therefore, omics will help discover and understand the role of genes involved in plant defense responses triggered by biochar and Ca^{2+} signaling. Furthermore, proteomics will be important in identifying and characterizing proteins involved in biochar-mediated defense, focusing on Ca^{2+}-binding proteins, enzymes, and transporters. Ca^{2+} imaging will further help to improve the knowledge by visualizing real-time Ca^{2+} dynamics within plant cells following biochar application. This can help understand the spatial and temporal patterns of Ca^{2+} signaling during defense activation.

Future research could explore several avenues to deepen our understanding of the molecular mechanisms underlying the interaction between biochar and Ca^{2+} signaling in plant defense. Advanced imaging techniques, including confocal microscopy and genetically encoded Ca^{2+} indicators, offer high-resolution visualization of Ca^{2+} dynamics in response to biochar amendments, providing insights into spatial and temporal signaling events. Genetic approaches, such as mutant analysis

and RNA interference, can reveal the roles of specific Ca^{2+}-related genes in mediating biochar-induced changes in plant immunity. Integrating omics technologies and bioinformatics tools can interpret global changes in gene expression, protein abundance, and metabolite profiles induced by biochar treatments, uncovering candidate genes and signaling pathways involved in Ca^{2+} signaling and defense responses. Field studies and long-term monitoring experiments are essential to assess the practical implications and sustainability of biochar-based interventions in agricultural ecosystems, validating laboratory findings and informing the development of innovative strategies for sustainable crop protection. By embracing multidisciplinary approaches and cutting-edge technologies, future research endeavors hold the promise of unraveling the complicated molecular mechanisms underlying the biochar-plant interaction and providing the way for sustainable agriculture practices.

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

No data was used for the research described in the article.

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