

Review

Drought Stress in Quinoa: Effects, Responsive Mechanisms, and Management through Biochar Amended Soil: A Review

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Abstract: *Chenopodium quinoa* Willd. (quinoa), a highly nutritious pseudocereal, is a promising crop to address global food insecurity challenges intensified by population growth and climate change. However, drought stress remains a significant constraint for quinoa cultivation. The plant exhibits several morphophysiological adaptations to water stress conditions, including root system modifications, reduced growth rate, leaf abscission, and stomatal closure. While these adaptations enhance drought tolerance, they can also negatively impact plant growth, potentially through alterations in root architecture, physiological changes, e.g., stomatal regulations, and anatomical changes. Different studies have suggested that soil amendment with biochar, a pyrolyzed organic material, can improve quinoa growth and productivity under drought stress conditions. Biochar application to the soil significantly enhances soil physiochemical characteristics and maintains plant water status, thereby promoting plant growth and potentially mitigating the negative consequences of drought on quinoa production. This review focuses on the current understanding of quinoa behavior under drought stress and the potential of soil amendment with biochar as a management strategy. We summarize existing research on applying biochar-amended soil to alleviate quinoa drought stress.

Keywords: drought; *Chenopodium quinoa* Willd.; morphology; physiology; anatomy; biochar; soil properties; plant growth



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1. Introduction

Current agricultural practices include excessive use of chemical fertilizers, pesticides, herbicides, untreated sewage, and industrial wastes that cause pollution and severe soil degradation [1]. Thus, while thinking about food production, the attention must also be towards the reclamation of soil-degraded properties, the efficient usage of environmental resources, and sustainability to avoid further damage [2]. Under natural field conditions, plants can face adverse environmental conditions like excess salts, water deficiency, heavy metal accumulation, high temperature, or light that can alter average plant growth [3]. Global warming causes water deficiencies through changes in rainfall patterns that significantly impact sustainable crop production [4].

Quinoa (*Chenopodium quinoa* Willd.) is a pseudocereal, nutrient-diversified crop known as a super and functional food with promising potential to meet the global food demand and aid in reducing poverty due to population growth and global warming [5]. It originated from the Andean region, where it is consumed as a staple food, and is ranked as a climate-proof crop due to its ability to withstand adverse climate conditions [6]. Its remarkable agronomic adaptation to adverse climatic conditions makes it appropriate for cultivating in regions susceptible to climate change's effects [7]. Quinoa's superior adaptability enables

its cultivation in a diverse range of environments, including lowlands, marginal lands, deserts, and areas above 4000 m of sea level [8].

Recently, it has been gaining global attention because of its gluten-free and superior nutrition profile [9]. The year 2013 was declared by the United Nations the “International Year of Quinoa” due to the importance of this crop in ensuring food security and quality. A significant increase in quinoa production worldwide was evident after that [10]. In 2020, Peru was the leading quinoa producer, yielding 134,400 metric tons, followed by Bolivia with a production of 88,500 metric tons [11].

Quinoa’s remarkable adaptability to diverse environments is attributable to its extensive genetic variability [12]. The plant shows numerous adaptations to drought conditions through its morphology and physiology by exhibiting different drought responses, including escape, avoidance, and tolerance [12]. The different responses involve changes in root architecture and growth, leaf dropping, and stomatal closure and might include changes in gene expression [13]. Quinoa is important for current and future challenges and is a source of genes with major bio-technological applications [14].

Drought appears to be the most recurring natural disaster whose impacts on available water resources depend upon timing, duration, frequency and intensity [15]. The Mediterranean region has faced these trends since the 1960s and is also known as the region most vulnerable to climatic changes and global warming [16]. Recently, drought has spread globally in two-thirds of all cultivation area, which is foreseen to increase due to global warming [17]. Drought stress significantly affects plant growth [18], physiology (stomatal conductance, respiration) [19], morphology (leaf dropping, root architecture) [20] and biochemistry (increased production of reactive oxygen species) [21], which ultimately creates serious threats for future food security [22]. The current focus is on using potential organic amendments, specifically biochar, that can simultaneously enhance crop growth, productivity, and soil properties under drought conditions and contribute to future food security.

Biochar is a carbonaceous material obtained from pyrolysis conducted at high/moderately high temperatures (250 to 900 °C) [23]. Historical perspective gives the main reason for manufacturing activated carbon (biochar) as improving crop productivity through soil application [24]. Biochar is highly recalcitrant in soil, and its persistence in soil is approximately 10–1000 times longer than most soil organic amendments [25]. In recognition of its potential to improve soil’s physicochemical properties and crop production, biochar has become a significant focus for scientists and farmers in modern agriculture [2]. Plant growth stimulation through biochar addition is attributed to enhanced soil water retention capacity and mineral content [26], increased soil cation exchange capacity [27], neutralized phytotoxic compounds in soil [28], decreased soil bulk density [29], improved soil hydraulic conductivity [30], drainage and aeration [31], modified soil pH [32,33], flourishing soil microbiota, and induced systemic resistance in plants against diseases [34]. Various studies have shown biochar’s capacity to immobilize natural and inorganic contaminants in soil [35]. It has been reported that biochar significantly enhances the biomass, growth, physiology, water use efficiency, and drought tolerance in quinoa [36–38]. A study previously revealed that soil amendment with biochar increased quinoa biomass and yield-contributing traits up to 21% and 50%, respectively [38]. In this review, we will discuss: (1) quinoa botanical characteristics and responses to drought stress; (2) recent advancements (from the last decade) in using biochar to alleviate drought stress through soil property improvements and enhance plant growth under water shortage conditions; and (3) quinoa’s responses to biochar application under drought conditions.

2. Quinoa’s Botanical Characteristics and Germplasm Diversity

A quinoa plant ranges between 0.3 to 3 m in height, while its stems are thick, erect, and hollow. It exhibits various colors, i.e., white, yellow, purple, pink, and black. At the start, its leaves show a green color, turning yellow, red, or purple with time, depending on the genotype [39]. The leaves are alternate, generally pubescent, of different sizes on the same

plant, and have a goosefoot shape with a lanceolate to triangular outline [40]. The seeds are cylindrical-lenticular-shaped, with two flat surfaces, and are round on both sides, with a diameter of 0.28–2.1 cm [41]. Like the plants, the seeds are also diverse in color, varying from yellow to orange, pink, red, and black [42]. Its panicle is of three different shapes, i.e., Glomerulate, Intermediate, and Amarantiform [43]. The panicle density is classified into three types: lax, intermediate, and compact. From this, cultivars with compact panicle density usually yield high [44]. Pollen grains are monads, medium in size, from 42.99 to 51.30 μm , polypanoporate, scabrate (microechinate), and have an exine thickness of 2.08–2.40 μm [45].

Mature quinoa plants can have a main panicle length of 30 to 60 cm [43] and 100-seed weights ranging from 0.2 to 0.5 g based on the different genotypes [46]. Flowering typically occurs after 40–50 days, with some quicker varieties reaching this stage in just 25–30 days. Commonly known quinoa varieties, including the Italian variety Quipu [47], Chilean variety Regalona [48], and Pakistani variety UAFQ7 (V7) [49], take 150 to 180 days to mature, while some faster-growing varieties, like the Danish varieties Titicaca and Baer, can be ready in just 90–100 days [50]. It has a pivotal, vigorous, deep root system reaching up to 1.8 m deep in the soil. The roots are well-branched and play an essential role against drought and in the stability of the plants [12]. Akram et al. [49] tested 13 superior genotypes from 128 USDA lines under agroecological conditions in Faisalabad. They reported that UAFQ7 was recorded to have longer root growth and higher yield than the other genotypes studied. UAFQ7 was later approved as the first quinoa variety in Pakistan.

Undoubtedly, the center of origin for quinoa is the Andean Altiplano and its extended areas, i.e., Bolivia, Peru, Ecuador, Northern Chile, and Colombia [6]. Huge phenotypic diversity and inter-varietal variations were observed among quinoa germplasm [40,51–53]. Given the vast genetic variability of the different ecotypes, there is a need to screen the germplasm for specific objectives, like drought tolerance. The germplasm evaluation is the first step to assessing quinoa suitability and adaptability potential to new environmental conditions [51]. According to a study conducted by Gomez-Pando et al. [54], greater diversity was found between the Cuzco, Peru, and Bolivia ecotypes, with the largest number of landraces located in the Andean area and its extensions, including Lake Titicaca in Bolivia and Peru. Evaluating germplasm collected from various locations is essential for the effective agricultural management of this crop [55].

Based on ecotypes, quinoa is classified into five classes: (i) Valley, (ii) Altiplano, (iii) Salares, (iv) Subtropical/Yungas, and (v) Sea level. These ecotypes may contribute to unique and remarkable adaptations to drought [6]. Depending on the ecotype, quinoa exhibits different drought-responsive mechanisms, i.e., drought escape, avoidance, and tolerance. Most ecotypes from Valley and Altiplano are of short-duration varieties, like Titicaca [50]. They complete their growth cycle earlier to avoid the negative effects of drought at later growth stages, called terminal drought, exhibiting drought-escape mechanisms. On the contrary, ecotypes from Salares outlined deeper and more well-established root systems [12] and stomatal regulations than other ecotypes, exhibiting drought avoidance. Commonly used varieties from these ecotypes are UAFQ7 and Quipu. On the other hand, Subtropical and Sea level ecotypes exhibited drought-tolerance mechanisms through tissue elasticity and low osmotic potential. They accumulated higher proline levels along with the induction of ornithine and raffinose pathways that affect nitrogen-associated enzymes [56]. Notable varieties from these ecotypes are Regalona [57] and PI665283 accession [51].

Currently, quinoa evaluation for its adaptability under diverse environmental conditions is undergone globally, especially in the U.S., Australia, Canada, China, E.U., India, Israel, Middle East, Pakistan, and Turkey, by collection of its germplasm all over the world [58]. Expanding the cultivation of this crop by identifying high-yielding quinoa cultivars adaptable to different agro-ecological zones is a primary approach to meeting the increasing global demand for food [59].

3. Quinoa Responses to Drought

Insufficient soil moisture content in the soil, referred to as agricultural drought, significantly reduces plant production [60]. Drought frequency has increased for the past few years, with detrimental impacts on crop production and the agricultural system, which is the backbone of food security. Globally, quinoa is considered a drought-tolerant crop that can grow in arid and semi-arid regions receiving less than 200 mm of annual rainfall, including Chile, Northwest Argentina, Peru, and Bolivia [61]. Apart from these areas, studies conducted outside of the Andean region have shown that quinoa has the potential for adaptation and seed production in other arid and semi-arid environments, such as the Mediterranean area, Asia, and North Africa [62,63]. Its exceptional ability to grow under water limitations is due to its low water requirements and capacity to maintain gaseous exchanges during the occurrence of water stress conditions [64]. Quinoa confronts these conditions and produces substantial yields [65].

Drought response in quinoa is attributed to its deep extended root system, which can penetrate up to 1.5 m in sandy soils [12], and the presence of calcium oxalate vesicles on leaves, which help to reduce transpiration [66]. The plant can also avoid the negative consequences of drought through leaf-area reduction and small cells with thick walls that can preserve water and release it during the dry period to maintain its water status [67]. Additionally, due to its immense diversity in genotypes and ecotypes, quinoa's drought responses are divided into drought escape, avoidance, and tolerance [68]. Usually, short-duration cultivars develop drought escape mechanisms by shortening their life cycle through precocity [69]. This phenomenon is essential in those areas where the drought instance risk is higher at the end of the growing season, called terminal drought, such as the Altiplano and Valley regions [70].

Alternatively, drought avoidance creates an equilibrium between water uptake and loss [60]. Plants accumulate excess osmolytes to lower the tissue water potential and enhance water uptake [71]. Moreover, for water conservation, stomatal closure reduces transpiration [12]. Drought avoidance mechanisms become insufficient under severe stress conditions, and the stress tolerance mechanisms aim to protect against cell damage. They usually involve the detoxification of reactive oxygen species (ROS) and the accumulation of late embryogenesis abundant (LEA) proteins, as well as other solutes, especially proline, that act as osmolytes and osmoprotectants simultaneously [72]. Both mechanisms follow the ABA-dependent pathway that includes DREB proteins [73]. As drought conditions diminish, quinoa can resume its former photosynthetic level and leaf area [74].

3.1. Morphological Responses of Quinoa

Several morphological adaptations were reported for quinoa under drought stress, significantly reducing leaf area through leaf shedding [75] and extending the root system [76]. The inhibition of leaf growth and reduction of leaf area appeared to reduce water loss through transpiration to ensure plant survival under water limitations [12]. However, in severe drought, leaves wilt and are shed to reduce transpiration and avoid direct exposure to sunlight [46]. A rapid and sharp decrease in leaf elongation in quinoa has been reported due to drought conditions termed acute growth inhibition. In contrast, after drought termination, the recovery of new steady-state growth, referred to as acclimation, has been observed. Moreover, quinoa's leaf expansion rate is reduced by 30–50% under water-limited conditions, compared to well-watered plants [69].

Quinoa root architecture and morphology are important in determining its ability to face drought conditions. The root architecture is generally classified into two types: dichotomic, i.e., without any predominant root axis, and herringbone, with the main root axis supporting lateral roots [77,78]. Under drought conditions, quinoa reveals a herringbone root architecture pattern [79] that helps the plant to efficiently explore deep soil layers for water and nutrients [80]. Under limited water availability, a rapid primary root elongation was observed that slowed down afterwards, giving good support to the above-ground plant part [81]. However, quinoa root development is strongly influenced by

ecotypes [79]. Compared to Salares ecotypes, coastal ecotypes show slower root growth [69]. A study revealed that when both ecotypes were at 6 to 9 cm of shoot length, the Salares ecotypes reached 1 m deep in the soil, as compared to the coastal ecotype that attained the same length after 1 to 2 weeks [82]. When drought conditions arise at early plant growth stages (vegetative growing cycle), primary roots are significantly longer, which is helpful in the exploitation of deeper and more-reliable water resources through deeper penetration in the soil [83].

Water limitations undoubtedly reduce the total root length, but less reduction has been recorded in the Salares ecotype than in other ecotypes. As a response to drought, primary root elongation is stimulated in all ecotypes but more pronounced in Salares than others. The faster primary root elongation allows this ecotype to produce lateral roots evenly distributed in the deeper soil from 50 to 75 cm, like the root density of plants grown under humid conditions. Instead, coastal ecotypes reach only 5 to 50 cm, with lower root densities [84].

3.2. Physiological and Biochemical Responses of Quinoa

Studies have revealed that quinoa adapts different physiological and biochemical strategies to counter the negative consequences of drought [76,85]. From these strategies, osmotic adjustments are crucial in maintaining turgor pressure during water limitations [13,86]. The accumulation of osmolytes like glucose, trehalose, free amino acids, proline, and total soluble sugar significantly increases, directly stimulating antioxidant enzymes and decreasing lipid peroxidation and hydrogen peroxide content during drought stress [87]. Other mechanisms, such as the accumulation of calcium oxalate, enhanced protein stability, and thermostability of chlorophyll machinery [88], also enhance drought tolerance in quinoa. Drought conditions cause a significant reduction in gaseous exchange traits, including stomatal conductance, transpiration rate, and photosynthesis rate, along with leaf water potential [12]. A rapid closure of stomata is evident with a two-thirds reduction in transpiration and photosynthesis, and it becomes stable until water potential does not drop below -4 MPa and drought conditions remain. Importantly, unless severe drought conditions exist, quinoa does not respond to abscisic acid, and even if stomata close, they can photosynthesize at very low water levels for three days [74] through oxalic acid conversion to carbon dioxide for photosynthesis. Stomatal closure in quinoa has been reported to occur at leaf water potential below -1.2 MPa [89]. The leaf water relation is characterized by low osmotic pressure and a low turgid weight to dry weight ratio, which sustains the potential gradient for water movement from soil to leaves to maintain turgor [38,89].

Another possible approach in quinoa for stomatal closure is the production of anti-transpirant compounds other than abscisic acid, particularly cytokinin, that act antagonistically to abscisic acid [90]. When its transport in the xylem is reduced, stomatal sensitivity to xylem abscisic acid increases accordingly [74]. It is reported that quinoa plants close their stomata to maintain leaf water potential and photosynthetic activity [83]. Apart from this, the accumulation of soluble sugars, proline, and glycine betaine, also reported for quinoa under drought conditions, plays a role in osmotic adjustment [91]. Furthermore, proline accumulation has a dual purpose, i.e., osmotic adjustment and osmoprotection simultaneously [72]. It scavenges free radicals and prevents membrane protein denaturation because of osmotic stress caused by severe water limitations [92]. Moreover, proline and sugar accumulation act as compatible osmolytes and maintain the cell turgor pressure required for cell expansion. Additionally, quinoa tolerates drought through growth plasticity and tissue elasticity [93]. Recently, it has been reported that enhanced drought resistance in quinoa is also characterized by its ability to resume photosynthetic activity after eliminating drought conditions [94].

3.3. Quinoa Anatomical Responses

Quinoa exhibits massive leaf senescence and bladders or glands in the leaves and stems, the volume of which depends upon the severity of drought [93]. It can rapidly recover from the consequences of drought by forming new leaves for leaf area expansion.

Quinoa has smooth leaf surfaces without trichomes in mature leaves while containing a thicker cuticular epidermis [95]. Moreover, the young leaves and stems are covered with bladders containing calcium oxalate and silicic anhydride that are hygroscopic in nature and help reduce transpiration [96]. Additionally, epidermal bladder cells on the shoot, especially on younger parts of the plant [67], are used as additional reservoirs for moisture conservation that are released during water-limited conditions to maintain turgor pressure. Stomata are deeply sunken in the epidermis, aiding drought tolerance [95].

Al-Naggar et al. [97] reported a significant decrease in leaf thickness and upper and lower epidermis under water stress. Alternatively, under severe water stress, the leaves exhibit a substantial increase in palisade and spongy layer thickness, which helps in enhancing mesophyll conductance that ultimately aids in more CO₂ diffusion, whose role in increasing photosynthesis is crucial. Moreover, photosynthesis occurs in the palisade, so increasing its thickness increases the photosynthetic activity and the production of carbohydrates [98].

4. Biochar Production and Usage for Management of Drought Stress

Since 2009, the quantity and quality of research on biochar increased, just after the COP15 event organized by the European Commission in Copenhagen [99]. Afterwards, from 2014, a dramatic increase in articles on biochar was evident, about three times more than any other organic amendment [100]. These articles characterize biochar's role in circular economy and role under different conditions of abiotic stress, including drought, salinity, heavy metals, and biotic stress (Figure 1). Biochar is a porous, high carbon-content product obtained through pyrolysis of different biomasses, such as plants, animals, and municipal wastes (forestry, agricultural and agro-industrial residues, manure, and sewage sludge), at elevated temperatures (250 to 900 °C) and under low- or no-oxygen conditions [23]. It has the ability to sequester atmospheric CO₂, thus helping in climate change mitigation [101]. It significantly affects soil's physical [31], hydraulic [30], chemical [33], and biological properties [34,35]. Biochar's presence neutralizes phytotoxic compounds in soil [28]. Plant growth stimulation is attributed to biochar's unique physicochemical properties, such as its highly porous structure, large inner surface area, greater negative surface charge, and charge density, which result in enhanced soil water retention capacity and mineral contents [26], increased soil cation exchange capacity [27], and decreased soil bulk density [29].

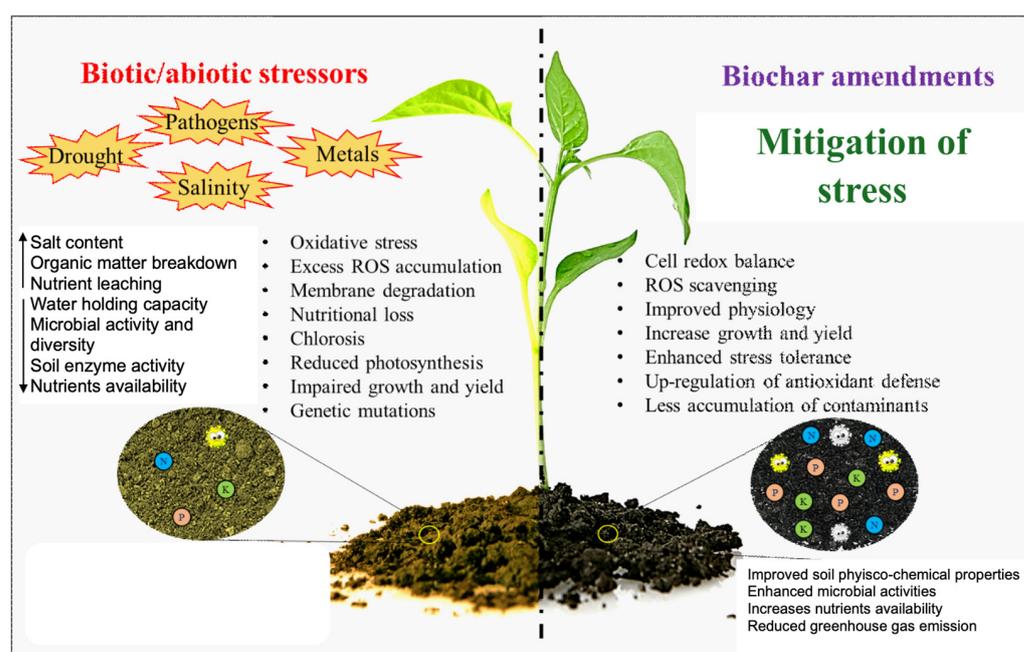


Figure 1. Role of biochar under biotic [102,103] and abiotic stress [104–106]. Upward arrows indicate increased levels of the indicated parameter, while downward arrows represent decreased levels.

Recently, biochar has gained considerable attention because of its role in water retention and soil remediation [107]. Biochar is an essential soil amendment, as it binds all toxic elements and pollutants in the soil and increases crop production rates [108]. It prevents water and nutrients from leaching, can retain moisture longer than non-amended soils [109], and helps in reducing the adverse effects of drought. The multidimensional applications of biochar make it suitable for agricultural improvements and solid waste management [108]. Presently, synthetic and other bio-based fertilizers dominate the agricultural sector. However, due to several initiatives, farmers are gradually spreading awareness to include biochar in farming activities [110], thus creating huge avenues for market growth in the coming years.

Biochar's effects depend on different factors, including original feedstock, pyrolysis temperature, soil type, application rate, and plant species [111]. Biochar addition may positively affect the soil C sequestration and, thus, act as a sink and long-term storage of C due to its long residence time in the soil, ranging from 100 to 1000 years [112]. In particular, woody biochar, which has high carbon, is being recognized by scientists for its potential role [113]. Although biochar contains higher C contents of up to 80%, it depends upon the pyrolysis conditions in which it was manufactured [114].

Significant variation exists among the characteristics of different biochars. For instance, biochar from pyrolysis conducted at a higher temperature ($>350\text{ }^{\circ}\text{C}$) is characterized by higher pH, cation exchange capacity (CEC), and extractable NO_3 . In contrast, biochar obtained at a lower temperature ($<350\text{ }^{\circ}\text{C}$) has a higher concentration of extractable phenols, NH_4 , and P [115]. Furthermore, low pyrolysis temperature degrades the cellulose of the feedstock materials and may cause a considerable reduction in mass by volatilization, producing a hard, shapeless C matrix. Pyrolytic temperature is directly related to the concentration of aromatic C in biochar; i.e., an increase in temperature increases the proportion of aromatic C due to reduced volatile matter [116]. Typically, biochar has two main morphological stages: crystalline graphene sheets in the form of layers and shapeless aromatic structures that are haphazardly arranged. Hydrogen (H), oxygen (O), nitrogen (N), phosphorus (P), and sulfur (S) are mainly present integrated within the aromatic rings as heteroatoms [117]. The presence of heteroatoms imparts great heterogeneous surface chemistry and reactivity to biochar.

Furthermore, the biochar application rate to the soil exerts a significant impact on the soil's characteristics and plant growth. For instance, higher biochar doses than optimum may lock up ions, making them unavailable for plant uptake [113,118]. A study revealed that larger biochar doses have a highly volatile matter that reduces plant nitrogen uptake and reduces plant growth [113]. Similarly, these doses can reduce the population of beneficial mycorrhizal fungi due to changes in soil pH and the toxic effects of salts and heavy metal contents present in biochar [119]. Conversely, high application rates of biochar can offer environmental advantages, such as carbon storage and reduced nitrate pollution, but the absence of economic incentives may hinder its widespread adoption in the short term [120]. However, these environmental benefits can contribute to long-term sustainability [121]. Thus, any counterproductive effects should be comprehensively studied before adding biochar on a large scale.

During pyrolysis, formulation of several functional groups [e.g., hydroxyl (-OH), amino (NH_2), ketone (-OR), ester ($-(\text{C}=\text{O})\text{OR}$), nitro ($-\text{NO}_2$), aldehyde ($-(\text{C}=\text{O})\text{H}$), carboxyl ($-(\text{C}=\text{O})\text{OH}$)] takes place mainly on the outside of the graphene layers and porous surfaces [122]. Some of these groups behave as electron donors. Contrastingly, some act as electron acceptors determining the biochar state, either acidic/basic or hydrophilic/hydrophobic [123]. Assimilation of H, N, O, P, and S in the aromatic rings is used to determine the electronegativity of the biochar, thereby improving CEC. The type of interaction between biochar and other soil components, such as soil particles, dissolved organic matter, gases, microorganisms, and water, is influenced by surface charge [26].

These biochar characteristics enhance the soil's physicochemical properties, increase soil fertility, and provide a large area for crop cultivation [124]. When the biochar particles

remain in the soil for a long time, they increase the oxidation process and negatively charge at the soil surface, enhancing soil CEC [26]. Biochar particles, after oxidation, may attach to soil minerals by interacting with clay and silt-sized minerals, which might increase the sorption capacity of biochar to adhere to organic contaminants in the soil [26]. The small pore size of biochar particles increases their surface area and adsorption ability. Mesopores play a significant role in liquid-solid adsorption, while larger pores are necessary for air passage [125]. At the same time, macropores are essential for aeration, hydrology, movement of roots, and bulk soil structure [126].

5. Biochar-Amended Soils Under Drought Stress

Biochar-amended soils have demonstrated potential for ameliorating the negative impacts of drought stress. By enhancing soil's physical properties, such as water retention and infiltration, biochar contributes to improved soil water availability for plants [127]. Additionally, biochar can indirectly enhance drought resilience by stimulating beneficial microbial communities [128], which can positively influence plant growth and development under water-limited conditions [105]. As discussed in detail below, incorporating biochar plays a crucial role in mitigating the drastic effect of water limitations.

5.1. Effect of Biochar on Soil Properties under Drought Stress

Many studies have reported that biochar addition improves the physicochemical properties of soil under limited water supply and drought conditions [129,130]. Biochar application to different soil types with different textures reduces the bulk density and improves the soil water contents [131]. Guo et al. [132] reported that a 5% biochar application rate substantially enhanced plant growth and soil hydraulic properties during drying. The decrease in soil bulk density due to adding biochar enhances the soil's water-holding capacity (WHC), significantly improving plant growth and yield under a limited water supply [133]. Its addition to the soil significantly increases the WHC of the soil because of the formation of macroaggregates with a specific higher surface area and porosity that provide more binding sites for water molecules [134]. Biochar's WHC is co-controlled by multiple properties of biochar working together, including its chemical makeup (elements and surface groups), carbon structure (graphitic structure and aliphatic structure), pore volume, and specific surface area [135]. However, the quantitative contributions of the various properties of biochar to the WHC remain unknown, which hinders the understanding of the dominant mechanism controlling the WHC of biochar and the regulation of biochar-producing conditions to improve its WHC.

Ruan et al. [136] reported that 100 t/ha application of maize straw biochar significantly decreased the root osmolality and malonaldehyde content. In contrast, root water potential, ascorbate peroxidase activity, and plant fresh weight were increased considerably under severe drought conditions. Similarly, Gullap et al. [137] revealed that applying hazelnut shell biochar to the sandy loam soil under drought conditions significantly enhances malonaldehyde, hydrogen peroxide, proline, sucrose, and antioxidant enzyme activities, including peroxidase, catalase, superoxide dismutase, and abscisic acid contents in soybean leaves. It has been demonstrated that the application of 15 t/ha from agricultural bio-waste (tomato plant residues) significantly improves barley and wheat growth by enhancing soil and plant nutrients N, P, and K, organic matter, and soil water retention capacity [138]. Furthermore, under water limitations, the application of 10% (*w/w*) wheat straw biochar significantly eliminates the harmful impacts of drought by enhancing wheat growth and yield, as well as physiological plant attributes, including stomatal conductance, transpiration rate, water use efficiency, and chlorophyll content [139]. The increase in the cumulative constancy of sandy-clay soil after biochar addition is influential in increasing soil water retention, specifically in drought stress conditions [140]. It is evident from these studies that soil biochar application effectively improves soil water-holding capacity. However, further details are needed to explain the role of biochar in enhancing soil water-holding capacity under different environmental conditions.

Biochar application and suitable microbial inoculants further improve plant growth and biomass under drought conditions [141,142]. An indirect interaction of biochar and microbes to reduce drought stress has been reported, e.g., by altering soil properties such as pH [143]. Biochar can shelter microbes, which attach themselves to porous biochar surfaces. It also serves as a nutrient source for microbial growth and can positively modify soil physicochemical properties such as pH, water content, organic matter, etc. [144]. Several reports indicate enhanced microbial communities with biochar addition under water-stressed conditions [145]. The uptake of N and P, growth, biomass, and nodulation in the lupin seedlings grown in a restricted water supply were enhanced after adding biochar combined with *Bradyrhizobium* sp., compared to the inoculation of microbes only [146]. The application of 2% woodchip biochar to sandy loam soil significantly reduced the bulk density and modified the soil pH and EC, which ultimately enhanced vegetative growth and quinoa biomass [32]. Therefore, it can be anticipated that applying biochar in combination with microbes may effectively decrease the water scarcity effect in plants and improve plant growth; however, it largely depends on microbial properties, soil conditions, and plant species.

5.2. Effect of Biochar-Amended Soils on Plant Growth under Drought Stress

Biochar application improves the growth and biomass of plants growing under a limited water supply. The most common plant traits that were enhanced with biochar additions are morphology, biomass, pigment content, nutrient contents, water use efficiency, stomatal pore aperture, stomatal density, photosynthetic rate, relative water content, and membrane stability index. It has been shown that a 3% application rate of biochar from sesame residue to sandy soil enhances the morphological traits, leaf gas exchange, plant water status, yield, and water use efficiency while reducing proline levels in tomato plants in semi-arid and arid areas [147]. Similarly, among two biochars tested (woodchip and vineyard pruning biochar), 2% woodchip biochar in sandy loam soil significantly enhanced the plant biomass by 23% while yielding contributing traits by 50% in quinoa under drought conditions [38]. Similarly, the 2% woodchip biochar application under water shortage conditions enhanced *Titicaca* quinoa's morphological and yield attributes [148].

Different studies revealed that biochar helps plants cope with drought stress by improving the plants' physiological status through chlorophyll content, photosynthetic rate, stomatal conductance, water use efficiency, and relative water contents. However, Afshar et al. [149] reported that the chlorophyll content, transpiration rate, plant biomass, stem height, and leaf area were not affected by increasing the biochar application rate from 1.0 to 2.0% in milk thistle seedlings grown in fine sandy loam soil under moderate (60% of control) and severe (40% of control) drought stress, compared to the control (50% of field capacity: FC). Similarly, a study reported that the production of the vine was improved after the application of biochar (22 t ha⁻¹) to the field, specifically during the years of reduced rainfall, while the characteristic parameters of grapes, such as total soluble solids (°Brix), total acidity, and anthocyanins, were not altered [150]. Recently, Zhang et al. [151] reported an increase in grain yield and water use efficiency and a reduced negative effect of drought stress on *Glycine max* productivity under biochar amendments (10 g/kg).

Abideen et al. [152] reported a one-fold increment of *Phragmites karka* biomass root/shoot ratio under the application of 0.75% of peanut husk biochar produced at high temperature (750 °C). Plant cells adjust osmotically under biochar application by accumulating organic solutes, which aids in water influx, reduces efflux, maintains plant water status, and inhibits the reactive oxygen species' (ROS) harmful effects, protecting biomembranes and photosystem II efficiency [153]. Applying biochar to the soil improves essential nutrient availability and enzyme activity and enhances root traits under drought stress, especially diameter, surface area, volume, and density [154]. Its addition is also helpful for the rhizosphere environment because it stimulates the proportion of different bacterial communities, including Proteobacteria, which is responsible for symbiotic nitrogen fixation, and Acidobacteria, which aids in nutrient cycling remineralization [155,156]. Importantly, biochar

could help delay root senescence, as reported by Han et al. [157], who found that under biochar application, root length was maintained during grain filling stages, whereas a 21–34% reduction was observed in non-amended soils. Different studies highlighted that biochar could enhance the water absorption capacity of crops because of the stimulation of finer root growth and length under water-limited conditions [158,159].

Biochar addition to the soil not only improves the nutrient contents and structure in the soil but also enhances the absorption and utilization of nitrogen fertilizers that are important for grain quality, such as its appearance and its starch and protein contents [160]. The higher nitrogen availability under biochar application promotes the total amino acid content in the grain [2]. As a soil conditioner, it significantly enhances fruit flowering, growth, quality, and yield. Sharma et al. [161] reported that biochar addition in soil is associated with potential benefits for the growth and yield of fruit plants such as grapes and apples. Moreover, a significant reduction in the total acidity of Red Globe grapes and increased protein and firmness was observed under biochar application [162], while glucose, fructose, ascorbic acid, total acidity, and lycopene in tomatoes increased significantly [163]. The enhanced water availability under drought conditions because of biochar application is its vital benefit in sustaining gas exchange and the antioxidant defense system. The strategic usage of biochar during drought to sustain plant functionality and, ultimately, crop productivity is important in arid areas. Applying biochar in addition to Arbuscular mycorrhizal fungi positively regulates the fluorescein diacetate dehydrogenase and alkaline phosphatase activity, soil nutrients, growth, and physiological properties in turmeric plants [164]. The studies reporting the biochar effect on growth and physiology of different plant species subjected to water deficit are summarized in Table 1.

Table 1. Application of biochar to alleviate drought stress on different crop species (selected studies were organized according to their bibliographic references, with the most recent ones listed first).

Feedstock	Pyrolysis T. (°C)	Plant Species	Application Rate	Water Stress Application Method	Effects on Plants	Ref.
Hazelnut shells (<i>Corylus avellana</i> L.)	500	<i>Glycine max</i> L.	3 and 6% w/w	75% and 50% of FC	Boosted growth and chlorophyll content	[137]
Maize (<i>Zea mays</i> L.)	400	<i>Zea mays</i> L.	100 t/ha	40% and 20% of FC	Increased K ⁺ concentration, less Ca ²⁺ efflux, and increased apoplastic pH in roots.	[136]
Acai seeds (<i>Euterpe oleracea</i> Mart.)	700	<i>Glycine max</i> L.	2.5 to 10% w/w	With-held watering (8 days)	Increased biomass, including leaf and root DM, PN, WUE, PN/Ci, and gs.	[165]
Rice husk (<i>Oryza sativa</i> L.)	500	<i>Lolium perenne</i> L.	5 and 10% w/w	25% of FC	Enhanced root and shoot growth, leaf RWC and nutrient status, chlorophyll contents, and photosynthetic efficiency while reducing proline, H ₂ O ₂ , and MDA.	[166]
Natural wood	-	<i>Solanum lycopersicum</i> L.	20 g/kg	75% and 45% of FC	Enhanced morphological parameters, such as PH, LA, NB, FW, DW, and productivity.	[167]
Apple wood (<i>Malus domestica</i> (Suckow) Borkh.)	400	<i>Onobrychis viciifolia</i> L.	0.8 to 4% w/w	80 to 40% of FC	Enhanced leaf RWC and reduced MDA and H ₂ O ₂ accumulation.	[168]
Timber waste	390	<i>Triticum aestivum</i> L.	2% w/w	75 and 35% of FC	Increased flavonoids, anthocyanin, phenolics, proteins, GB, APX, POD and SOD.	[169]
Date palm (<i>Phoenix dactylifera</i> L.) and pistachio (<i>Pistacia vera</i> L.)	560	<i>Solanum melongena</i> L.	500 g/m ²	100 to 50% of FC	Improved growth, yield, and WUE.	[170]
-	700	<i>Triticum aestivum</i> L.	28 and 38 g/kg	Skip irrigation at tillering and grain formation	Higher mineral nutrient, Bray P, exchangeable K, soil C, N mineralization and respiration in the soil along with enhanced microbial activity.	[171]
Oak wood (<i>Quercus robur</i> L.)	400	<i>Ehretia Asperula</i> L.	5 to 20 t/ha	With-held watering (10 days)	Increased PH, NL, FW and DW of roots, shoots, and leaves, chlorophyll content, and leaf RWC. Reduced relative ion leakage	[172]
Wheat straw (<i>Triticum aestivum</i> L.)	550	<i>Glycine max</i> L.	5 and 10 g/kg	80 to 25% of FC	Increased GY and improved WUE while reducing negative impact on productivity.	[151]

Table 1. Cont.

Feedstock	Pyrolysis T. (°C)	Plant Species	Application Rate	Water Stress Application Method	Effects on Plants	Ref.
Corn straw (<i>Zea mays</i> L.)	500	<i>Chenopodium quinoa</i> Willd.	5% w/w	DI and ARD by restoring water to 100% FC when consumed 90% of water	Improved water relations and growth and ameliorated plant water status. Creating a balance between chemical signal (leaf ABA) and hydraulic signal (Ψ).	[173]
Cattle manure	600	<i>Glycine max</i> L.	1.25 to 5% w/w	100 and 55% of FC	Improvement in plant growth and morphology increased g_s . Higher rates (2.5, 5%) adversely affected the plant growth and production due to excessive salinity.	[174]
Woody branches of button mangrove (<i>Conocarpus erectus</i> L.)	450	<i>Cicer arietinum</i> L.	3% w/w	60% throughout the experiment and half exposed to 50% of FC for 6 weeks	Increased plant growth, PH, LA, enhanced chlorophyll content and carotenoids, increased stomatal pore aperture, g_s , P_n , RWC, membrane stability index, and nutrient content.	[175]
Eucalyptus bark (<i>Eucalyptus</i> sp.)	350	<i>Zea mays</i> L.	5 to 60 g/kg	With-held watering (4 days)	Raised water retention, and micro/macropore ratio while reducing BD, additionally, increased PH, plant nutritional status, and growth. However, raised water retention due to biochar could not overcome the drought problems in soil	[176]
Rice straw (<i>Oryza sativa</i> L.)	450	<i>Oryza sativa</i> L.	3 and 5% w/w	50% and 35% of FC	Increased DM production, chlorophyll contents, P_n , g_s , WUE, E , reduction in H_2O_2 content, MDA and electrolyte leakage, enhanced enzymatic defense system.	[177]
Rice straw (<i>Oryza sativa</i> L.)	450–550	<i>Triticum aestivum</i> L.	3 and 5% w/w	70%, and 35% of FC	Increased plant growth and yield, chlorophyll contents, E , P_n , g_s , WUE, increased antioxidant activity, decreased MDA, H_2O_2 and electrolyte leakage.	[178]
Pinewood (<i>Pinus</i> L.), sewage sludge, paper sludge, and grapevine wood (<i>Vitis vinifera</i> L.)	550–620	<i>Helianthus annuus</i> L.	Two experiments I: 15 t/ha II: 1.5 t/ha	-	Increased LA, PH, wider inflorescences, enhanced vegetative growth, seed production, and reduced g_s with greater WUE.	[179]

Table 1. Cont.

Feedstock	Pyrolysis T. (°C)	Plant Species	Application Rate	Water Stress Application Method	Effects on Plants	Ref.
Woodchip	500–600	<i>Zea mays</i> L.	1.5 and 3% <i>w/w</i>	60% and 25% of FC	Increased biomass, WUE, leaf RWC, and $\Psi\pi$, N use efficiency, photosynthesis because of stimulated electron transport rate of PSII.	[180]
Lantana stems (<i>Lantana camara</i> L.)	450	<i>Abelmoschus esculentus</i> L.	1 and 3% <i>w/w</i>	100 and 60% of FC	Improved WUE, g_s , E , P_n , WUE, plant DM.	[181]
Cotton sticks (<i>Gossypium arboreum</i> L.)	385	<i>Vitis vinifera</i> L.	1 and 2% <i>w/w</i>	100% and 50% watering restoration	Increased PH, FW, DW, RL, chlorophyll, carotenoids and anthocyanin, and WHC.	[182]

T—temperature, FC—field capacity, DI—deficit irrigation, ARD—alternate root-zone drying, DM—dry matter, PH—plant height, LA—leaf area, NL—number of leaves, NB—number of branches, FW—fresh weight, DW—dry weight, GY—grain yield, RL—root length, WUE—water use efficiency, RWC—relative water content, Ψ —total water potential, $\Psi\pi$ —osmotic potential, P_n —net photosynthetic rate, g_s —stomatal conductance, P_n —photosynthesis rate, E —respiration, P_n/C_i —carboxylation efficiency, MDA—malondialdehyde, GB—glycine betaine, APX—ascorbate peroxidase, POD—peroxidase, SOD—superoxide dismutase, BD—bulk density, WHC—water holding capacity.

6. Role of Soils Amended with Biochar in Alleviating Drought Stress in Quinoa

According to the literature, few studies have described biochar application's role in enhancing quinoa's drought tolerance. However, most experiments are concerned with soil amended with biochar under drought imposed during the reproductive growing cycle, especially during flowering [36,173]. The results revealed that biochar application under drought significantly enhances quinoa biomass [36]. They also reported that a higher dose of biochar could negatively affect quinoa growth because of excess negative charge on the soil surface. Its addition plays a significant role in greenhouse gas mitigation and C sequestration. They demonstrated that biochar addition stimulates taproot growth, increasing water uptake and reducing water loss by transpiration; however, lower content of proline and higher values of leaf osmotic potential increase drought tolerance. On the other hand, biochar-grown plants exhibit higher leaf areas than non-amended plants.

Similarly, Ramzani et al. [37] introduced the deficit irrigation concept to cope with the negative consequences of less water application and sustain growth and productivity through biochar addition. They highlighted that the water deficit negatively affects the yield and overall plant growth. However, the application of 2% biochar effectively counters the adverse impact of drought. They also reported that a 2% biochar application could save up to 20% of the water without significantly impacting physiology, growth, and water use efficiency. Furthermore, a significant increment in yield-contributing traits and growth of quinoa was observed with woodchip biochar application under drought stress [38]. Unlike the woodchip biochar, vineyard pruning biochar application negatively impacts quinoa growth, which aligns with the findings that biochar effectiveness depends upon the feedstock used [183]. Biochar addition to soil further regulates the soil's physicochemical properties, including pH and electrical conductivity, and reduces soil bulk density [28,32], helping the extensive root system development for deep penetration in the soil for more water and nutrient uptake during severe water-limited conditions. The growth- and yield-contributing trait enhancement was evident in quinoa experiencing water shortage conditions, starting from the emergence of the 2% woodchip biochar application [148]. The study found that a 2% woodchip biochar application was most effective in mitigating the adverse effects of water shortage, compared to a 4% dose, which showed no benefit or a negative effect. Under moderate water-limited conditions (60% of FC), biochar application to the soil significantly enhanced the bioavailability and uptake of the nutrients by quinoa roots, and significant effects were evident on plant biomass and yield enhancement [184,185].

Until now, most studies have focused on the role of biochar application under moderate water stress during quinoa's reproductive growing cycle, mainly at the flowering and grain-filling stages. However, severe water stress application during the vegetative stage can have a significant impact on plant growth, particularly roots, depending on the quinoa variety [186]. According to Geerts et al. [187], the drought period during the vegetative growing cycle should be mitigated with a potential watering strategy, as water stress during this period can significantly reduce water use efficiency, plant growth, and grain yield. Applying biochar to the soil offers a promising strategy to combat quinoa growth problems caused by water stress and avoid drought's adverse effects. Therefore, future studies should investigate biochar's ability to enhance the vegetative growth of quinoa plants under severe drought conditions. A comprehensive overview of existing research investigating the effects of biochar application on quinoa cultivation is presented in Table 2.

Table 2. Effect of biochar under drought stress applied at different growth stages in quinoa (*Chenopodium quinoa* Willd.) (studies examining the effects of biochar-amended soils on quinoa compiled chronologically, from the earliest research to the most recent).

Feedstock	Pyrolysis T. (°C)	Application Rate	Water Stress Application Stage	Water Stress Application Method	Effects on Plants	Ref.
Peanut hull residue (<i>Arachis hypogaea</i> L.)	498	100 and 200 t/ha	After 27 days of sowing (flowering)	60 and 20% of FC	Increased growth, LA, DT, leaf N, and WUE.	[36]
Maize cob (<i>Zea mays</i> L.)	350	1% w/w	With-held watering every two weeks from the leaf development stage until maturity	15–20% of FC	Improved plant growth, yield, physiological, chemical, and biochemical processes. Reduced anti-nutrients (phytate and polyphenols) and BD in soil, enhance bioavailability, and translocate essential nutrients from soil to plant.	[37]
Corn straw (<i>Zea mays</i> L.)	500	5% w/w	Flowering	DI and ARD	ARD with biochar under salinity enhanced PH, SB, and grain. Balanced chemical signal (leaf ABA) and Ψ and increased iWUE.	[173]
Pistachio tree (<i>Pistacia vera</i> L.)	-	20 t/ha	From emergence	70, 100 and 130 mm of pan evaporation	Applying biochar + vermicompost enhanced chlorophyll, LAI, PH, PL, 1000 SW, and GY while decreasing proline content.	[188]
Rice straw (<i>Oryza sativa</i> L.)	300	0.4, 0.8% w/w	4 leaf stage and flowering	30% of FC	Increased concentration of macro and micronutrients in soil.	[189]
Woodchip (Bw) and vineyard (<i>Vitis vinifera</i> L.) pruning (Bv)	-	2% w/w	12-leaf stage	2 successive water stress cycles from FC to PWP	Bw increased plant growth, LA, FW and DW, main PL, NSP, and WUE. Reduced leaf Ψπ and TW:DW. Bv negatively affects the plant growth	[38]
Woodchip	-	2 and 4% w/w	From E to FI	Restoration 100% and 50% ET	Enhanced NL, LA, biomass, DM, PL, and NSP.	[148]
Woodchip (Bw) and vineyard (<i>Vitis vinifera</i> L.) pruning (Bv)	-	I: 2% w/w Bw and Bv II: 2 and 4% Bw	I: at 12-leaf stage II: from E to FI	I: two successive water stress cycles from FC to PWP. II: restoring 100% and 50% ET	I: Bw enhanced biomass, DM, PL, NSP. II: 2% Bw modified soil pH, and EC and reduced BD.	[32]
Northern forest tree	300	2, 4% w/w	From 8-leaf stage	100, 80, 60% of FC	Increased P, K, N pH and EC of soil, while reducing the actual specific gravity and the apparent specific gravity of soil.	[184]

Table 2. Cont.

Feedstock	Pyrolysis T. (°C)	Application Rate	Water Stress Application Stage	Water Stress Application Method	Effects on Plants	Ref.
Dried forest leaves	-	2, 4% w/w	After plant establishment	100, 80, 60% of FC	Increased growth, PW, 1000 SW, and LAI.	[185]
Woodchip	-	2% w/w	At 12 leaf stage	2 successive water stress cycles from WHC to PWP	Increased root growth and development, plant growth and yield contributing traits	[186]

ET—evapotranspiration, DI—deficit irrigation, ARD—alternate root-zone drying, FC—field capacity, WHC—water holding capacity, PWP—permanent wilting point, E—emergence, FI—flowering initiation, LA—leaf area, PH—plant height, NL—number of leaves, SW—seed weight, SB—shoot biomass, LAI—leaf area index, PL—panicle length, NSP—number of sub panicles, PW—panicle weight, SW—grain weight, GY—grain yield, DT—drought tolerance, WUE—water use efficiency, iWUE—intrinsic water use efficiency, $\Psi\pi$ —osmotic potential, TW:DW—turgid weight to dry weight ratio, EC—electrical conductivity, BD—bulk density, N—nitrogen, P—phosphorous, K—potassium, ABA—abscisic acid.

7. Conclusions and Future Perspectives

Although quinoa exhibits drought tolerance mechanisms, current climate change scenarios significantly impact both traditional and climate-proof crops, including quinoa. Different studies showed that quinoa positively responds to biochar addition in the soil regarding root development, gaseous exchange parameters, and alleviation of osmotic stress (production of reactive oxygen species) through enhanced antioxidant activity. It is evident from the above discussion that the characteristics of biochar can differ significantly because of feedstock, pyrolysis conditions, application rate, soil type, and plant species. Therefore, it is necessary to know biochar's properties and expected outcomes before its application to the soil. Additionally, most of the research work has been done under controlled conditions, with few studies conducted in field conditions. So, the focus must be diverted towards planning biochar usage under field conditions. Finally, future research should be focused on the following points:

- (i) Long-term field investigations are needed to elucidate the intricate interplay between biochar soil application and soil–plant systems under drought conditions.
- (ii) Exploring synergistic interactions between biochar application and established agricultural practices could unveil novel avenues for achieving sustainable agricultural production.
- (iii) Quinoa varietal response to biochar-amended soils under water stress conditions should be assessed through morphology, physiology and anatomical changes.

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