

Review

Drought-Induced Changes in Leaf Morphology and Anatomy: Overview, Implications and Perspectives

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Received: 4 September 2023

Accepted: 24 October 2023

Abstract

The global climate change scenario intensified various environmental factors, especially in arid and semi-arid regions. Drought is one of the most severe environmental stresses affecting plant productivity. Plants in the Mediterranean climate zone are exposed to heat and drought in summer, and these conditions have a significant effect on plant growth and development. However, in this case, the entry of CO₂ into mesophyll cells is prevented and therefore the rate of photosynthesis decreases which ultimately causes a reduction in plant growth. In order to acclimate to stressful environmental conditions, plants exhibit several structural modifications to cope with these harmful conditions. This review highlights some aspects of anatomical adaptive changes in plants under drought stress such as a reduction in leaf size and angle, stomatal position, epidermal thickness and deposition of the cuticle to prevent the loss of water from the leaf surface. Furthermore, it elaborates the role of bulliform cells in leaf rolling, structural adaptation in the mesophyll cells, and the presence of trichomes. Mesophyll cells and bulliform cells provide easier rolling of leaves in case of intense drought. In arid conditions, the economical use of water by plants is possible by closing the stomata and reducing transpiration.

Keywords: bulliform cell, drought, leaf rolling, mesophyll cells, stomata

Introduction

The beginning of 21st century intensified the various environmental factors, especially in arid and semi-arid regions, due to extreme and rapid changes in climatic conditions [1], viz, rising air temperatures, atmospheric CO₂ levels, and high wind, which is projected to accelerate global climate change in future [2, 3]. All these events produce changes in rainfall patterns and distribution. Along with extreme rainfall events, the destruction of the ecological balance and freshwater scarcity has become serious environmental problem worldwide [4]. Under natural and agricultural conditions, plant growth and development require an optimal level of soil moisture, fluctuation in soil moisture beyond the optimal level can affect plant productivity. However, low water availability in the rhizosphere slows plant growth and limits the plant's ability to absorb nutrients [5, 6] Water constitutes a significant proportion of total fresh biomass in plants, ranging from 80% to 95%, which plays an essential role in several morpho-physiological and biochemical processes, including plant growth, development, and metabolism [7, 8].

Drought is the condition of prolonged dryness, which adversely affects the anatomical, morpho-physiological and other fundamental processes in the plant leading to limited plant growth and productivity [9, 10]. It also affects the location and density of the stomata on the leaf surface. The deposition of a waxy layer (cuticle) and positive thickness in the epidermal cells are the important features, that plant leaves adapt under drought stress conditions [11, 12]. Under drought stress, plants undergo several anatomical and morphological changes at cell to organ levels to cope with stress conditions. In the early phases of drought stress, plants normally maintain water balance by boosting water intake via the root system and decreasing water loss via stomata closure to prevent transpiration [13]. In the lateral stage, when plants are subjected to severe drought stress, a variety of structural changes are triggered in plant leaves. These changes include an accumulation of solutes in the cells to maintain turgor pressure [14], a change in leaf size, angle, and thickness [15, 16], an increase in the thickness of the cuticle and the number of stomata to minimize transpiration and water loss [17, 18].

Plant leaves are the most important organs which are responsible life maintaining process like photosynthesis and plays a vital role in the transport of water and nutrients. Drought is one of the most severe abiotic stress and has detrimental affects on the morphological and anatomical characteristics of leaves which ultimately cause a reduction in crop growth and productivity. This review attempted to provide an overview of leaf morphological and anatomical responses under drought stress to maintain plant water status and crop productivity, mainly focusing on the role of leaf size and angle, the genesis of trichomes, the formation of bulliform cells, and their role in leaf rolling.

On the cellular level, functional traits associated with drought stress tolerance are also discussed.

Drought-Induced Changes in Plant Leaves

The changes observed in the plants during their vegetative periods of life by the harmful effects of drought stress are a gradual decline in the leaves' numbers and area, leaf wilting, cell elongation in the leaves, and enhanced senescence of leaves which ultimately cause a reduction in the total plant height. This reduction in plant height affects the morphology of the different plant essential organs. Plant leaves are the main organ for transpiration and act as an indicator for the water deficient condition. They have the capability to adapt their structural features such as increased thickness, tissue density, and reduce the area under such environments [19]. The first response of plants to drought is the change in the osmotic pressure of the cell. As the turgor pressure decreases, the cell water potential decreases, and cell expansion is limited, this is reflected in the morphological characteristics of the plant, and the first affected organ is the leaf, which is responsible for photosynthesis [20].

Specific leaf area is one of the morphological responses of the plant to drought stress. Specific leaf area components play an important role in photosynthesis, as they determine the flow and distribution of photons within leaves, limiting mesophyll to CO₂ diffusion to carboxylation sites in chloroplasts, especially under stress conditions [21]. Several previous studies reported that the reduction in the leaf area, change in the turgor, and canopy temperature, under drought, affect plant growth through a decline in the rate of photosynthesis [22]. Moreover, plants can roll their leaves which is the most important morphological feature of the leaves to cope with drought via loss the water in the upper epidermal cells which reduces press potential as a result of leaf roll as observed in the flag leaf of the wheat [23]. In severe harsh dry environments, some plants have thick film on their leaves and the rolling motion help to improve the loss of water under direct sunlight [24].

Since leaf morphology is important in plant growth and development, it significantly affects plant yield. The symptoms observed in the leaves in response to drought are important in the anatomical adaptation of the cultivars and are used in cultivar selection [10]. The responses of leaves to drought stress can be evaluated by looking at the leaf's anatomical features such as bulliform cells on the leaf surface, epidermal cells, and stomatal size. Drought affects the dehydration of leaf mesophyll cells by causing the closure of stomata and causes damage to photosynthetic organs [25].

Water-related morphological changes are the formation of fine roots, leaf rolling, and changes in growth patterns to increase water uptake from the soil [26]. Previous studies showed that drought stress significantly decreased the leaf numbers in maize [27],

and declined the total biomass and leaf area, and caused detrimental effects on photosynthesis [28, 29]. These complex modifications at the leaf level are an aspect to withstand drought, plants modify their growth patterns, distribute nutrients, and turn on stress-response genes. In addition to coping with water shortages, plants are able to secure their long-term resilience and capacity to recover when conditions improve due to their diverse adaptations.

Leaf Size and Angle under Drought Stress

The incline produced between the leaf blade and stem is referred to as the leaf angle [30, 31]. The cell wall composition, expansion, and division at the lamina junction that connects the leaf blade determine how the leaf angle forms which is regulated by the hormones [32]. There are two types of physiological mechanisms responsible for the leaf angle, first one is the change in the growth of the cells on the upper and lower surface of the petiole and the second mechanism is the change in turgor potential at the part present at the base of leaves called the pulvinus. Plants alter their leaf angle in response to environmental factors such as light, water, gravity, and carbon dioxide. A leaf curling under water stress is an extreme example of how various portions of a leaf may alter its angles at different speeds [33].

The relationship between leaf angle and leaf rolling is inverse [34]. Resistance to water stress is correlated with a change in leaf angle. A shift in leaf angle can lower leaf temperature, conductivity, and transpiration by reducing photosystem inhibition and affecting the efficient utilization of water. In response to drought stress, several grass species roll their leaf blades, minimizing their exposure to stress [35].

Leaf size is the morphological feature of the plants responsible for the photosynthesis efficiency connected to variation in the leaf size [36]. It has been observed in the wheat leaves that the photosynthetic rate is high due to the narrow, smaller, erect, and larger deposition of cuticular wax on the epidermis of the leaf. These abovementioned overcome the water loss in the plants facing water deficiency [37]. The narrow-sized leaves have more resistance to drought stress as compared to the large-sized leaves [38]. The plants have flag leaves smaller in size and erect leaf angles are more adaptive to improve photosynthesis and decline in water loss through the evaporation process [39]. Therefore, reducing leaf angle could serve as one of the breeding objectives for wheat growth to increase plant density, enhance light absorption, and boost chlorophyll levels [40].

The grass plants can overcome the adverse effects of drought stress at a moderate level, because they have narrow, small-sized, and erect-angle leaves which lead to the utilization of the radiation that perfectly comes from the sun and contribute to improving the process of photosynthesis [41]. Plants alter their leaf

size and angle in order to achieve an ideal equilibrium between absorbing sunlight for photosynthesis and preserving valuable water under drought stress. These modifications serve as a dynamic survival strategy that highlights the incredible ability of plants to harsh environmental conditions. Such flexibility increases the chance that plants will survive, enabling them to tolerate water under drought stress.

Drought and Leaf Thickness

The leaf thickness (LT), which measures the space between its top and bottom surfaces, reveals the optical route that light takes through it and determines whether it will be repelled or absorbed. LT is closely related to productivity, responses to drought stress, and biomass partitioning [42, 43].

Under water deficiency the leaves have two-way approaches a) increase the thickness, and b) decrease the thickness of the leaves. Palisade and spongy tissue growth, as well as a reduction in leaf and stomata size, are ways to increase the capacity of plants to store water and minimize water loss in the former [17, 44]. To boost the capacity of CO₂ and inorganic nutrients to penetrate the leaves as well as enhance the exchange of gases to repair and maintain respiration under stress, certain plants thin their leaves or develop unique leaves [45, 46]. In order to adapt the stomata and optimize transpiration under water stress, the leaves' internal framework is altered. However, the cause for this is unclear, necessitating additional research.

Leaf area, leaf thickness, leaf density, and stomatal structure in the plant vary depending on the plant's water content [47]. The increase in leaf thickness in plants in drought conditions is due to the increase in upper epidermis thickness. The thick epidermis layer in grass plants prevents high water loss from leaves in dry conditions. The leaf thickness of the varieties with moderate leaf rolling in arid conditions in rice decreased more than the ones with more leaf rolling [48]. Drought stress reduces leaf thickness in relation to the net carbon absorption and photosynthetic performance of plants [20]. If a plant has a high drought tolerance, it shows that biomass losses will be less [10].

In arid conditions, lamina thickness increased in the moderately drought-tolerant and highly tolerant sugarcane genotypes. The cell walls and cuticle thickness of the lamina epidermal cells of the genotype, which is moderately drought resistant, also increased. In moderately drought-tolerant sugarcane, with drought, stomata size increased in leaves, while a decrease was observed in the highly drought-tolerant genotype [18]. The complex interactions between drought and leaf thickness demonstrate the mechanisms that plants use to survive in harsh environments. By enabling plants to tolerate water stress, these adaptations not only benefit in immediate survival but also contribute to long-term resilience in the ecosystem.

Leaf Rolling under Drought Stress

Leaves are the most important organ in the plant body which are responsible for photosynthesis. Reduction in the leaf expansion, stomatal conductance and the assimilate produced through the photosynthesis in the leaves via the adverse effect of drought stress cause to reduce the yield of the crops. Morphological adaptation in the leaves such as leaf rolling, loss of turgor, and osmotic adjustment to mitigate the above-mentioned issues by the drought [49].

Leaf rolling is the most observed phenomenon caused by the change in the water potential in the bulliform cells present in the epidermis of the leaves. This phenomenon plays a vital role to slow down the process of transpiration and enhance the yield-contributing indices in plants that face water deficiency [50]. The top layer of the epidermis of the leaf loses water, which decreases the pressure potential, which makes the leaf roll. This process is beneficial by lowering leaf temperature, higher light absorption, and enhancing the rate of transpiration. Leaf area and leaf rolling were significantly increased in *Zea mays* L. (maize) leaves grown under drought stress regimes [51].

The primary indicator of plants is leaf rolling in response to drought stress involving the amount of thickness in the lamina and swelling of the epidermal cells [52]. By adjusting the leaf rolling, it is possible to control the efficiency of photosynthetic activity per unit leaf area. The amount of dry matter accumulation and transpiration may both be increased and decreased by minimizing the effect of sunlight on the leaves and achieving optimal leaf roll [10]. The rolling that occurs in the leaves also reduces the damage caused by light by reducing the areas exposed to the sun in the plant [53]. The water loss in rice is 36% in the case of partial curling of the leaf and 52% in the case of full rolling [54]. Therefore, a positive correlation was observed between the degree of leaf rolling and water loss.

The patterns of leaf rolling, which include rolling inward or outward, facilitate effective photosynthetic processes in leaves [55]. As compared to entirely rolled leaf behavior, the optimal expression of leaf rolling is helpful for increasing water usage efficiency [56]. A similar pattern of leaf rolling was observed in the wheat plant which contribute to reducing the loss of water from the leaf surface via lowering interaction between the leaf surface and direct sunlight, while simultaneously enabling light to penetrate farther into the canopy [57, 58]. Under drought stress, rice (*Oryza sativa* L.) leaves frequently show two leaf rolling (LR) patterns known as adaxial and abaxial rolling which is the inward and outward motion of the leaves [59, 60]. It is always believed that optimal/partial LR is a better option for mitigating the effects of dryness than fully rolling or fully flattened leaf appearances [53]. The optimal LR is the most effective strategy which takes part to improve the photosynthetic rate under drought stress and overcome water loss. This

phenomenon can be observed in the afternoon when the direct sunlight interaction with the surface of the leaves attains a high level [55, 61, 62].

In the rice plant exposed to drought, rolling occurs in the leaves due to bulliform cell shrinkage [10]. Similarly, a decrease in bulliform cell size is observed in rice varieties in arid conditions [20]. The decrease in cell size caused a decrease in transpiration rate in the plant and prevented high water losses. As a result of all these, rounding of the leaves of the plant has occurred. Vascular bundle size and amount were found to be associated with leaf rolling in rice under drought conditions [52].

Environment-related factors such as a shortage of water, a warm climate, and exposure to sunlight are the main causes of leaf rolling in plants. Changes in photosynthetic rates, ion levels, fluctuations in the systems that produce antioxidants, and cell forms are additional factors that contribute to leaf rolling. In plants that become more drought-resistant, a lack of water, an increase in temperature, and the rays of the sun accelerate leaf rolling, this process plays an important role in reducing the water loss from stomata [63]. Leaf rolling is a drought-induced response that helps plants maintain vital physiological functions and conserve moisture, allowing them to rely on it until favorable conditions return, thus increasing their chances of survival during drought periods.

Bulliform Cells and Their Positive Role in Leaf Rolling

Bulliform cells have been linked to the leaf-rolling response observed commonly in the leaves of grasses under drought stress. These cells develop in longitudinal strips on the adaxial leaf surface. During the water deficiency in the leaf, bulliform cells showed significant shrinkage in comparison to other epidermal cell types, offering a potential mechanism for facilitating leaf rolling [64]. Plants need an adequate amount of water for proper development during their vegetative stage of growth, its consumption varies in every stage of their life cycle, that's why LR acts as a vital adaptation under drought stress [13]. The change in the turgor pressure in the bulliform cell present on the upper layer of epidermal cells in the leaves cause leaf rolling to be a quick response against water-deficient conditions and prevents the leaves from large exposure to sunlight [65, 52, 64].

Bulliform, collenchymal, mesophyll, and vascular bundle cells are among the cells involved in LR. However, one of the main causes of LR in rice is the shrinking due to decreased turgor pressure of bulliform cells on both sides of the leaf [66, 67]. Bulliform cells expand in arid conditions in sugar beet [68]. The response to drought in sugarcane is the thickness of the cuticle layer, the enlargement of the vesicles in the bulliform cells, and the increase in the number of veins in the leaf. In addition, the lower and upper cuticle

thicknesses increased under drought stress. The number of green leaves, chloroplast content, chloroplast length, width, and width/length ratio are very important in selecting drought-resistant varieties in sugarcane [15]. Twelve days after drought in rice, a decrease in bulliform cell size, which is easily affected by turgor pressure, is observed. Thus, a high rate of evapotranspiration is prevented [25]. In short, bulliform cells are crucial in the physiological adaptation of leaf rolling, which improves water-saving behaviors and boosts the plant's tolerance to drought stress, making it an advantageous modification for plants.

Is Leaf Rolling Necessary in Plants?

However, since the leaf blade becomes flattened if rolling does not occur, greater amounts of sunlight will strike the leaf surface, enhancing transpiration through the stomata in order to prevent plant heating up [69]. As a consequence of this, plants will rapidly wilt. If stress takes a long time, it could also cause problems with photosynthetic activity and change in the metabolic function of the plant causing plant death. However, leaf rolling occurs in the leaves which contributes to improving the metabolic function in the leaves and induces resistance against water deficiency is a positive step toward the survival of the plants [6]. It has been reported in a number of studies that moderate LR enhances the photosynthetic efficiency, while high-level or complete rolling decrease this efficiency and causes adverse effects on the plant [70, 71].

Photosynthetic activity ceased by the extreme rolling of the leaves which is considered to not play a positive role in the other beneficial process in the plant. Therefore, the rolling approach of the leaves is the most critical process for the perfect functioning of the plants [72]. Bulliform cells are an example of a vital strategy to water conservation and photosynthesis enhancement. The crucial role of leaf rolling shows how plants can adapt to drought stress. Understanding the complex workings of bulliform cells not only increases our understanding of how resilient plants are but also stimulates new ideas for improving water use efficiency in agriculture and reducing the effects of water shortages in the face of global warming.

Drought Stress and Trichomes

Trichomes are the hair-like fine outgrowth on the surface of the leaves which have variability in their density, size, locality, and functioning according to plant species. There are two main types of trichomes first one is the glandular trichomes which secrete the chemicals to prevent the plants from external factors such as herbivorous and pathogens attacks and the second type is the non-glandular trichomes which function to act as barriers against harsh conditions by reflecting the rays from the sun, reduce the process of transpiration

and also absorbed water [73-75]. The two types have the capacity to secrete or store vast amounts of specialized metabolites, which increase a plant's resistance to harsh climatic circumstances [76].

In terms of morphology, leaves with more trichomes in terms of leaf area are more resistant to abiotic degradation because transpiration prevents excessive water loss and regulation of temperature [77]. According to a study, trichome numbers significantly increased in the dry season compared to the wet season, which affected the physiological processes of plants. A number of physiological functions including stomatal conductance, transpiration rate, and water consumption efficiency, can be significantly affected by the presence of leaf trichomes [78].

Because bulliform cells, which absorb and store water, are abundant in the basal section of the leaf trichomes in grasses provide water to leaves. Trichomes influence how water interacts with leaf surfaces by directing water droplets away from the stem and soil and towards them, which helps plants absorb water. By decreasing transpiration, modifying the energy balance, and lowering light absorption, it also improves water storage [79, 78, 80]. The connection between trichome density and physiological features under water-deficit stress in many plant species is still not evident even though this information is well-documented in many herbaceous crops. It will be easier to explain the growing susceptibility of plant species to drought if we have a better knowledge of this link.

Drought-Induced Changes in Stomata

Drought stress not only affects the morphological features of the plants but also has adverse effects on physiological attributes such as relative water content, relative water humidity, and stomatal conductance of the plants and also photosynthetic pigments which directly attach with the anatomical features of the leaves. Leaf anatomical features to be observed due to water deficiency can be used as important visual indicators to determine the sensitivity and tolerance of the plant to arid conditions [20].

Plants that have experienced a long period of drought stress have smaller stomata which are fewer in number to reduce water loss and maximize plant water consumption which leads to early and quick response against drought. Usually, controlling leaf vein density is used to attain this equilibrium [81]. The plants under stress conditions close their stomata to overcome the loss of water by the phenomena of transpiration, as a result, decrease in the amount of carbon dioxide (CO₂) in the internal part of leaves. During the light reaction of photosynthesis, the disturbance in the electron transport chain and the activation of the glycolate oxidase pathway produce a high amount of reactive oxygen species (ROS) which cause oxidative stress in the plants [20].

The opening and number of stomata decreased with the increase in the drought. These characteristics of the stomata such as size, density, and conductivity are very important to reduce the loss of water and the flow of CO₂ in the cells of plant leaves that take part in photosynthesis [15, 68]. Changes in the structure of stomata are closely related to abscisic acid (ABA), a hormone produced during times of stress. During dehydration in the root zone, ABA is rapidly synthesized and transported. This causes interactions with jasmonic acid and nitric oxide to stimulate stomatal closure. This occurs through changes in signal transduction, including changes in guard cell turgor and ion pumping through ion channels [20].

The plants of the family Poaceae have special anatomical features and are known as C₄ plants which regulate their stomata to prevent the loss of water and enhance carbon assimilation leading to positive help in the physiological process under water stress conditions [82]. So, the C₄ plants have higher water use efficiency and are considered to become drought tolerant than the plants belonging to the C₃ species. The number of stomata in grass plants demonstrated a substantial negative connection with the drought period [26].

Stomatal size plays an important role in the rice cultivars under water deficiency, the cultivars with large stomatal size have high transpiration which contributes to wilting more frequently. The reduction in the turgor pressure changes the size of the stomata. Therefore, the cultivars that have larger-sized stomata in drought stress are more sensitive [25]. In plants, stomatal density increases significantly under moderate stress [83] (Kofidis et al., 2004) and decreases under severe stress conditions [84]. The increase in stomatal density in dry conditions also helps to control sweating better [85].

Under conditions of water stress, the density of the stomata, which reduce moisture in the plant through the leaves, is crucial for plants. Different cotton (*Gossypium hirsutum* L.) genotypes have different stomatal densities in dry circumstances. Under drought stress, stomatal density in cotton genotypes decreased in the range of 10-23 mm² compared to control conditions. The maximum reduction in the number of stomata resulted in greater water retention in the plant due to less transpiration [86]. Plants have extensive mechanisms for optimizing gas exchange and preservation of water, which is highlighted by the extraordinary plasticity of stomata in response to drought. This illustrates their capacity to grow even in difficult circumstances.

Cuticular Changes under Drought

The outermost layer of the leaves is synthesized from the epidermal cells and is called the cuticle, which constitutes cutin and cuticular waxes. It protects the inner tissues from other environmental factors such as biotic and abiotic stress such as harmful radiation, fungus and pathogens effects, and water loss under

dry conditions [87, 88]. Cuticular waxes, on the other hand, play a major role in controlling non-stomatal water loss, making them an important adaptation in the development of terrestrial plants [89, 90]. It plays a vital role in the physiology of plants by delaying cellular water loss from the leaves and acts as a key adaptation [91].

Glaucousness, a bluish-white coloring caused by densely dispersed epicuticular wax crystalloids, is a common outcome of epicuticular wax accumulation on plant surfaces. These phenomena decrease the leaf temperature by the reflectance enhancement of radiations which is helpful for the survival of leaves in water-deficient environments [88, 92]. Xerophytic plants have more thickness in their cuticles to the enhancement in the production of cuticular waxes from the epidermal cells of the leaves [93]. It has been observed in the Arabidopsis plant that the increase in thickness of the cuticle per unit area of the leaf with the deposition of cuticular wax on the leaf surface [94]. This change in the cuticle surface decreases the permeability of the cuticle which is a positive step toward the stress via decline in the water loss.

The results of the previous studies witnessed that an increase in the deposition of the cuticular waxes on the surface of the leaves is the key adaptation to increase the resistance under water-deficient environments [95, 96]. Enhancement in the wax accumulation on the upper epidermal surface in the Arabidopsis (*Arabidopsis thaliana* L.) [97], alfalfa (*Medicago sativa* L.) [98], and Camelina (*Camelina sativa* L.) [99] through transcriptional factor overexpression under drought stress. The connection between the cuticle and drought stress was also observed in the barley plants which are bred to high tolerance and yield under this stress [100]. Cuticle and epidermal structures are effective features in the adaptation of plants to drought [25]. The continuation of functions of some species in arid soils may be due to various physiological changes such as an increase in cuticle thickness and a decrease in stomatal size and density [101, 26]. Drought-induced stress in plants leads to significant alterations in their tissues, such as increased wax accumulation, which is crucial for reducing water loss and enhancing their climatic resistance.

Cuticle Thickness and Stomatal Density Responses in Monocots vs Dicots

The formation of a hydrophobic cuticle on the outer surface of the leaves which plays a crucial role against external environmental changes, was one of the most crucial adaptive features for survival [102]. Deposition in the cuticle on the epidermal cells is one of the modifications that plants adopt to enhance tolerance in water-deficient conditions [95]. A positive increase in the thickness was observed in both monocot and dicot plant leaves such as *Hordeum vulgare* L. [103], *Gossypium*

hirsutum L. [104], *Nicotiana Tabacum* L. [105], *Glycine max* L. [106], *Triticum aestivum* L. [88], *Arabidopsis thaliana* L. [107] and *Sorghum bicolor* L. [108] under water stress. However, the thickness of the leaf cuticle is varying among the plant species or even in the same plants under different environmental conditions. In some cases, the monocot has thick cuticles as a key adaptation in response to drought stress, while in the same circumstance, the dicot develops thicker cuticle on the leaf surface. So, on a broad spectrum stating that the monocot has more thick cuticles than the dicot according to the external environment is not accurate and vice versa.

Stomata defines the ability of plants for gas exchange, including atmospheric carbon dioxide uptake for photosynthesis and water vapor release during transpiration. Stomata density may vary among plant species under different environmental conditions like drought stress [109]. A number of monocot species such as *Hordeum vulgare* L. [110, 111], *Oryza sativa* L. [112] and *Triticum aestivum* L. [113] may show a drop in stomatal density under drought stress. In dry situations, this can reduce transpiration to lower water loss and improve water conservation.

The dicot plants may also respond differently to drought stress in terms of their stomatal density, depending upon the species and its mechanisms of adaptation. Some dicot reduces their stomatal density like monocots to decline in water loss such as *Amaranthus viridis* L. [114] and *Vitis vinifera* L. [115]. Some dicot species that can withstand drought may continue to have larger stomatal densities to continue photosynthesis as well as development when water is limited [116, 117]. The comparative study of monocot and dicot in responses to cuticle thickness and stomatal density showed in Table.1.

Mesophyll Cells under Drought Stress

Mesophyll cells, the predominant cell type in leaves and the fundamental site of photosynthesis are organized in cylinders called palisade mesophyll cells (PMCs) on the upper side and spherical, spongy mesophyll cells (SMCs) on the lower side of the leaf. Modifications in leaf phenotypes result from PMCs and SMCs losing their dorsoventral differentiation [121].

In arid conditions, leaf thickness is associated with photosynthesis rate and plant growth. In drought-resistant plants, leaf thickness increased under drought-stress conditions, resulting in an increase in mesophyll density [18]. Changes in mesophyll thickness, vascular sheath, and sclerenchyma layer were observed in plants with drought stress [52, 20]. Compared to drought-susceptible plants, drought-adapted plants' leaves usually feature more densely packed, elongated cells, but thinner spongy mesophyll cells.

The influence of drought stress on the growth of plant leaves has been extensively investigated [122]. The growth of leaf epidermal cells and physical leaf shape are closely related phenomena [123]. The mesophyll, a kind of cell that experiences significant fluctuations in turgor status, can be found near the guard cells. This means it is the optimal tissue for converting rapid fluctuations in water stress into the quick ABA biosynthesis necessary to regulate stomatal responses [124, 125]. Additionally, many seed plant species depend on the numerous chloroplasts in mesophyll cells as a virtually endless source of carotenoid precursors to fuel the continuous production of ABA, which is necessary for keeping stomatal closure throughout protracted periods of soil water deficiency [126].

Mesophyll Palisade Cells

With drought stress, dense and smaller mesophyll palisade cells are observed in the plant [101, 25], and the space between mesophyll cells decreases

Table 1. Responses of cuticle thickness and stomatal density in monocot and dicot plants.

Group of plants	Cuticle thickness	Stomatal density	Plant species	Responses	References
Monocot	Increased	Decreased	Barley (<i>Hordeum vulgare</i> L.)	Reduced evaporation of water on the surface of leaves, high water use efficiency	[103, 110]
			Rice (<i>Oryza sativa</i> L.)	Enhanced tolerance against drought stress and improved yield	[118, 112]
			Wheat (<i>Triticum aestivum</i> L.)	Improved water use efficiency and yield	[88, 113]
Dicot	Increased	Increased	Arabidopsis (<i>Arabidopsis thaliana</i> L.)	-	[107, 119]
			Cotton (<i>Gossypium hirsutum</i> L.)	Improved physiological mechanisms to reduce drought stress effects	[120, 117]
	-	Decreased	Green amaranth (<i>Amaranthus viridis</i>)	Positive enhancement in the physiological indices	[114]

and increases the number of epidermal cells and mesophyll chlorenchyma cells [83]. It also significantly reduced leaf and epidermis thickness, number of palisade cells, length of palisade mesophyll, number of sponge cells, and length of sponge mesophyll in peanuts [127].

Leaf dry matter per area limits the photosynthetic efficiency by affecting the conductivity of mesophyll to CO₂ [128-130]. The conductivity of the mesophyll to CO₂ is largely determined by the anatomical features of the mesophyll in adapting to long-term stresses such as drought [131, 132]. Drought cell wall thickness increased the thickness between the palisade and spongy mesophyll. In drought conditions, olive also increases the number of mesophyll cells and sclereids in order to maintain photosynthesis as well as to reduce transpiration, the spaces between mesophyll cells are reduced and the amount of non-glandular surface in the leaf increases significantly [85].

According to a previous investigation, the plants that face the issue of drought stress have thicker and large numbers of palisade cells as compared to the well-watered plants [133]. Starch stores were depleted as a result of structural damage imposed by drought stress in palisade cells. The efficiency in exchanges of gases within the leaves and ultimately the creation of starch reserves is both impacted by damage to the vein network of the leaf [134, 135]. Lack of water can cause the leaves to shrink, which can seriously harm the vein networks, mesophyll tissues, and plastids, where starch is produced, structurally [136]. Previous research has shown that starch stores are crucial for preserving plant energy as well as growth and during the course of the photoperiod, plant starch stores can be increased and transformed into soluble sugars as osmolytes to sustain plant development during stress caused by drought [137]. Many plants have been shown to deplete starch stores in response to drought stress, but further research is needed to determine whether structural destruction of palisade cells occurs when the leaves shrink. Plants possess a remarkable capacity to overcome the adverse effects of harsh environmental conditions by modifying the structure of their cells and maintaining vital processes while decreasing the water loss. Mesophyll cells complex interaction reflects the complexity of its resistance and provides facts about plant survival mechanisms that might motivate environmentally friendly farming methods.

Epidermal Cells under Drought Stress

When plants are exposed to drought conditions, transpiration decreases and leaf epidermis thickness increases, cells, and intercellular spaces decrease, vascular tissue, root/shoot ratio increases [85, 138]. In dry conditions, the upper and lower epidermis and cuticle thickness decrease in the leaf. Drought reduces many anatomical features in the leaf, and differences are observed in drought-tolerant and sensitive species [139].

The smooth walls and small size of epidermal cells create resistance to dry conditions, while small epidermal cells show 20 times more resistance to dry conditions than large ones [85]. After drought stress, the thickness of the epidermis, which is the protector of photosynthetic organs, increases in rice cells [25]. The width/length of the upper epidermal cells ($\mu\text{m}/\mu\text{m}$) varied between 0.67 ± 0.13 under control conditions and 0.75 ± 0.11 under drought stress, while the width/length ($\mu\text{m}/\mu\text{m}$) of lower epidermal cells was 0.67 under control conditions. ± 0.11 while it was 0.7 ± 0.07 under stress conditions [140]. Plants in extreme drought conditions can enhance their survival by thickening epidermal cells, which store water and respond to stress through stomatal control and trichome growth, thereby enhancing their water conservation and drought resistance.

Conclusions

In arid and semi-arid regions, drought stress poses a significant global challenge, severely limiting both the quantity and quality of crop production. The morphological and structural strategies during drought stress hold significant importance concerning the enhancement of water use efficiency, drought tolerance, and crop plant productivity. Mesophyll and bulliform cells play a crucial role in the tolerance against drought stress. Mesophyll cells photosynthesize the leaf. During drought stress, mesophyll cells store osmolytes to maintain turgor pressure and boost antioxidant enzyme activity to decrease dehydration damage. Bulliform cells are thin-walled monocot epidermal cells that cover the leaf. Bulliform cells shriveled more than other epidermal cells and reduced water loss during leaf dryness. The combination of bulliform and mesophyll cells increases a plant's tolerance to drought, providing a potential mechanism in leaf rolling and storing osmolytes that helps plants to conserve water and protect their photosynthetic cells. In addition, drought-tolerant plants have evolved a range of other structural changes such as a change in leaf angle, size, and area, an increase in the thickness of the cuticle, and the number of stomata to minimize transpiration and water loss has been observed in response to drought stress. Moreover, this review illustrates how bulliform cells take part in leaf rolling which is the key trait to overcome drought stress.

In crux, this review will help researchers understand the morphological and anatomical traits in plants, but further studies are needed to fully comprehend its genetics and molecular mechanisms and its potential application in plant breeding. Further studies are also needed to understand the molecular and genetic mechanisms behind the leaf's anatomical changes of stressed plants, as they play a vital role in developing drought early warning systems, identifying crop risks, and minimizing crop damage. Genetic control of these features can enhance leaf modification for improved

agricultural productivity and water use efficiency, in conjunction with phonemics strategies.

Authors' Contributions

IY conceptualized the manuscript. IY, MAJ and KU prepared the initial manuscript. S.H. and MF reviewed the manuscript. All authors read and approved the final manuscript.

Conflict of Interests

The authors declare that they have no conflict of interest.

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