

## Chilling and drought stresses in maize: Mitigation strategies and potential management opportunities

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### Abstract

Maize (*Zea mays* L.) is a significant global staple crop, which is important for its nutritional and economic value. However, the negative effects of drought and chilling stress, induced by climate change, are rapidly increasing threats to compromise its healthy yield. The processes that are affected by chilling stress are reduced growth, impaired photosynthesis, increased susceptibility to disease and pests, late flowering and reduced leaf size. Growth components of plants such as gibberellic acid, calcium, abscisic acid and salicylic acid can control the maize response to chilling stress. Conversely, drought stress, which is brought on by insufficient water availability, interferes with essential functions like the roots' ability to absorb water and nutrients, increases transpiration losses, causes the leaves to roll, and delays senescence. To improve drought tolerance ability in maize, glycine betaine, potassium and foliar calcium spray are worthwhile. Events that combine freezing and drought present a difficult challenge and reduce maize productivity. Reduced yields of maize not only place food security at risk, but also have severe economic implications on farmers and countries that depend mainly on maize production. Implementing proactive approaches, including as breeding initiatives, precision agriculture, water management, farming systems can present workable ways to minimize the impacts these stresses and defend maize production under prevailing fluctuating environment. This review highlights the most recent findings on the impact of drought and cold weather on corn plants. Potential management strategies for regulating plant tolerance mechanisms to drought and cold stress are also outlined.

**Keywords:** chilling; drought; maize; photosynthesis; ROS; yield

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## Introduction

The main factors limiting the global output of maize (*Zea mays* L.) include abiotic stress factors like salinity, temperature, and drought. Abiotic stress is the negative impact of non-living components on living organisms within a certain environment. In regions, where predisposed strategy to avoid various stresses, is adopted, the development of stress-tolerant agricultural types will be advantageous (Rane *et al.*, 2021). Drought stress damages photosynthesis pigments, which reduces the effectiveness of light absorption. Under drought stress, the administration of 2(3, 4-Dichlorophenoxy) Triethylamine (DCPTA) accelerates plant photosynthetic activity and growth. Drought stress had a greater detrimental effect on maize plants than heat stress did (Killi *et al.*, 2017). The main reasons why plant growth and productivity are reduced in saline soil are osmotic stress and ion poisoning. The crop growing season has been shortened due to the rise in global temperatures, although this can be mitigated by planting cultivars that require longer days to mature (Yang *et al.*, 2017).

Maize is a major grain crop that is widely used for food, feed, and the synthesis of biofuels. After wheat and rice, it is the third-most important crop (Madhukar *et al.*, 2020). It is grown twice a year, in the seasons of spring and fall. It's a crop of miracles and is used to bake cakes, for making porridge and bread across the world, especially, in America, Africa and Asia (Dabija *et al.*, 2021). Vitamin B complex, vitamin C, the antioxidant vitamin E, and vitamin A are just a few of the critical vitamins and minerals found in maize grains (Garg *et al.*, 2021). Pakistan's land and climate are favorable for maize cultivation, but compared to other nations that produce maize worldwide, its per hectare yield as a fodder crop is extremely poor, which get higher yields with proper fertilizer application, which may increase fodder content on per unit area basis. However, prolonged occurrence and severity of drought and cold conditions brought about by climate change is a big challenge to healthy maize production (Duchenne-Moutien *et al.*, 2021). High air temperatures and an increase in moderate to severe droughts jeopardize maize output. Recovering of resistant traits against abiotic stress is the main focus of current studies on maize (Gilliam *et al.*, 2017).

Drought is one of the most active and harmful abiotic stresses on the growth and development of plants (Ahmad *et al.*, 2022). In actual, drought is a state in which plants are deficient in water. Drought inhibits crop growth and development by affecting a series of intricate physio-biochemical and metabolic processes at the genetic and molecular levels. These processes include respiration, photosynthesis, uptake of water and nutrients, enzyme functioning, metabolism of organic materials, and over- or suppressed expression of genes encoding transcription factors and stress proteins (Muhammad *et al.*, 2021). Drought stress is negatively affecting maize production, as it is other crops. Although there is no discernible delay in anthesis, drought in maize seems to cause a delay in silking throughout the reproductive phase of the plant. This leads to an increase in the anthesis to silking period, which in turn causes a yield loss under drought stress (Naseer *et al.*, 2023). In response to extended moderate drought stress, plants shut down their metabolism, which is followed by their mortality from stomatal closure and impeded gaseous exchange. When drought conditions are extreme, maize reproductive growth stages are somewhat more vulnerable to the effects of drought, potentially leading to barren ear formation (Ortez *et al.*, 2022). Foliar calcium spray, potassium, and glycine betaine are used to reduce the effect of drought stress in maize (Shemi *et al.*, 2021).

The growth, development, nutritional, and water relations of maize plants are impacted by chilling stress. Chilling stress is defined as temperature in a range low enough to restrict development deprived of terminating cellular processes. Water, salinity, temperature, and light are just a few of the abiotic challenges that plants must cope with. Chilling stress is known to cause a number of problems at different organizational levels of cells (Kaushal *et al.*, 2016). For various species, chilling temperatures may vary. For example, temperate plants might be able to withstand colder temperatures than tropical plants. Low temperatures generally are seen as having a continual chilling impact for a specific amount of time on sensitive plants (Zheng *et al.*, 2016).

Effects of chilling stress include enhanced expression of antioxidants, damaged membranes, increased reactive oxygen species, reduced cellular respiration, elevated abscisic acid levels and cryoprotectants. Chilling stress on maize can be lessened by using salicylic acid and calcium (Li *et al.*, 2017). The rate of plant development, leaf elongation, photosynthesis, reactive oxygen species formation, mineral and water intake, stomatal conductance, antioxidant activity, and changes in membrane characteristics are all signs of chilling stress (Chaudhry *et al.*, 2022).

This article goal is to determine the impacts of drought and chilling stress on maize plants, as well as mitigating strategies and potential management opportunities that can be used to ensure sustainable production of maize. To date, various novel approaches have been tested in minimizing the negative effects of these stresses. Thus, future researches particularly using biotechnological and molecular approaches should be carried out to develop genetically engineered plants with enhanced tolerance against these stress factors.

### Chilling stress effects on the growth and development of maize

Chilling stress stunts maize development and growth, resulting in smaller organs, reduced flower, and grain output. The reduced buildup of the enzymes results in the synthesis of sucrose and starch, which causes a decrease in grain filling (Ullah *et al.*, 2022). It is a given that a plant's response to cold stress is contingent upon several factors, including growth rate, duration, severity, and time of day. Cold stress eventually causes stomata to close, which decreases net photosynthesis at the same time (Li *et al.*, 2018). Produced by organelles like peroxisomes, mitochondria, and chloroplasts, ROS (Reactive oxygen species) is one of the primary causes of the decreased growth and efficiency of plants under cold stress. ROS cause the lipids in cellular membranes to peroxide and protein enzymes and nucleic acids to degrade (Gaschler *et al.*, 2017).

Thermal thresholds for ideal physiological activity, morphological growth, and biochemical processes regulate maize development and metabolism. Cell and tissue harm results from this, which lowers the heritably anticipated production potential. Temperature range between 25 and 28 °C is considered optimal for maize development (Waqas *et al.*, 2021). Low temperatures during spring sowing are typically dangerous for long-cycle and productive maize varieties in chilly climates because they increase the average time to seed germination and seedling emergence (Hunt *et al.*, 2021). The creation of vegetative and reproductive shoots as well as the growth of leaves are significantly influenced by temperature. Low temperatures slow down both cell division and elongation. Plants are less capable of absorbing minerals and water under mild cold stress, which has the following nutritional implications on plant growth (Van Oosten *et al.*, 2017). A young root system may prevent certain nutrients from being absorbed. Roots that have grown swelled past the tip have denser axes, fewer lateral roots, and more seminal roots (Postma *et al.*, 2014) (Table 1).

**Table 1.** Chilling stress influences on growth parameters of young maize plants (mark in text)

Mechanisms	25 °C (control)	10 °C	References
Growth	Optimal plant growth	Stunted plant growth	(Quan <i>et al.</i> , 2023)
Cell division	Normal cell division	Inhibition of cell division	(Kaur <i>et al.</i> , 2023)
Embryo	Optimal development of embryo	Stunted development of embryo	(Deng <i>et al.</i> , 2022)
Fruit	Optimal development of fruit	Stunted development of fruit	(Wu <i>et al.</i> , 2022)
Water content	More water content	Reduce water content	(Vita <i>et al.</i> , 2021)
ABA	Normal or slightly decreased	Increased ABA	(Xia <i>et al.</i> , 2021)
Antioxidants	Baseline antioxidant enzyme activity	Increased antioxidant	(Odukoya <i>et al.</i> , 2021)
Photosynthesis	High photosynthetic activity	Poor photosynthetic activity	(Stefanov <i>et al.</i> , 2021)
Respiration	Enhanced respiration rate	Reduced respiration rate	(Lo <i>et al.</i> , 2022)
Nutrient relations	Increased nutrient uptake and transport	Decreased nutrient uptake and transport	(Wijewardana <i>et al.</i> , 2016)

ABA, abscisic acid

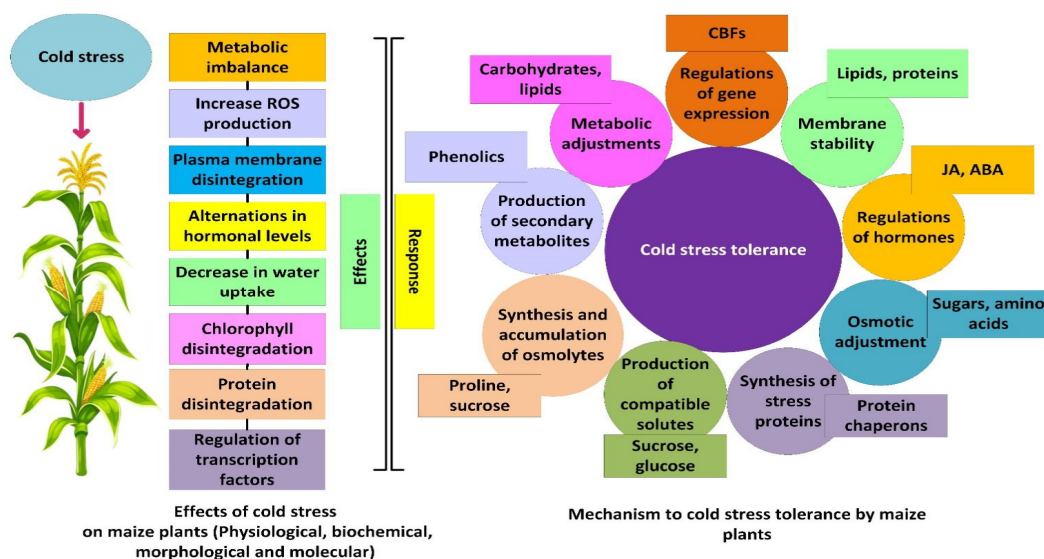
Chilling stress influences various plant variants including growth, cell division, embryo, fruit, water content, ABA, photosynthesis, respiration, nutrient relations. All these variants are affected by chilling temperature as seen in Table 1. Maize growth and development decreased at chilling temperature. Lobell *et al.* (2013) thought that growth of maize is influenced by temperature. The threshold temperature for maize root extension was 178 °C, slight variations in heat in the apical region had a significant impact on overall performance. As temperature dropped below the thermal threshold, the level of metabolic disruption, growth inhibition, and tissue damage increased (Sehgal *et al.*, 2017).

### **Effect of chilling on nutrient and water relations**

Mild cold stress causes roots to lose some of their capacity to absorb water and nutrients, which affects how plants develop nutritionally. The intake of vital nutrients could be restricted by less developed and ineffective root systems. The rate at which maize roots absorb phosphate (P) or potassium (K) is significantly influenced by temperature (Li *et al.*, 2020). Similarly, maize seedlings were shown to experience limited shoot growth due to decreased supply of nutrients at insufficient root-zone temperatures, which has direct effect on shoot meristems (Calleja-Cabrera *et al.*, 2020). Low temperatures in the root zone lowered growth rates and nutrient intake, which in turn reduced nutritional achievement. Frequently, prolonged contact of plant roots to low temperatures increases the ability of all predicted ions to be taken up by the roots, probably due to an increase in the number of ion transporter rather than their activity. Important factors that affect plant water relations include relative canopy temperature, amount of water absorbed, rate of evaporation, leaf potential for water, leaf temperature, and stomatal resistance (Sexton *et al.*, 2021). Plant water interactions are harmed by cooling in the root region due to lower root hydrological loss of stomatal regulation and conductivity. Chilling stress impairs root cell membrane function and structure at the cellular level. As a result of its thermophilic nature, maize face water deficits as the temperature drops (Rasheed *et al.*, 2021). A greater reduction in water uptake through roots as compared to its loss through leaf rate of transpiration causes this water deficit. Chilling-sensitive plants, like maize, show symptoms of water stress initially due to reduced root water flow and later significant decreases in water turgor potential at the cellular level (Lu *et al.*, 2021).

### **Mechanism for Chilling Tolerance**

The complicated mechanism of chilling tolerance involves several biochemical and physiological processes that take place at the level of individual cells, tissues, organs, and entire plants. This mechanism is active at different phases of plant growth (Muchate *et al.*, 2016). These strategies can reduce water loss through raising stomatal resistance, enhance water absorption by growing big, deep-rooted systems, and accumulate osmolytes and osmoprotectants. Plant growth hormones SA (salicylic acid) and ABA (abscisic acid) are particularly crucial for cold tolerance as shown in Figure 1. Important mechanisms of maize tolerance include the production of aquaporins and stress proteins, cell membrane integrity, and ROS scavenging by enzymatic and non-enzymatic systems Kaur *et al.* (2017). Effects of chilling stress can be controlled, among other things, by growing the best plant genotypes, priming seeds, using regulators of plant growth, and using osmoprotectants. External glycine betaine, proline, which is and other compatible solvents can be used effectively and beneficially as inducers of cold tolerance, but their ideal doses, modes of action and suitable plant growing phases to aim must all be sensibly recognized (Kaya *et al.*, 2023).



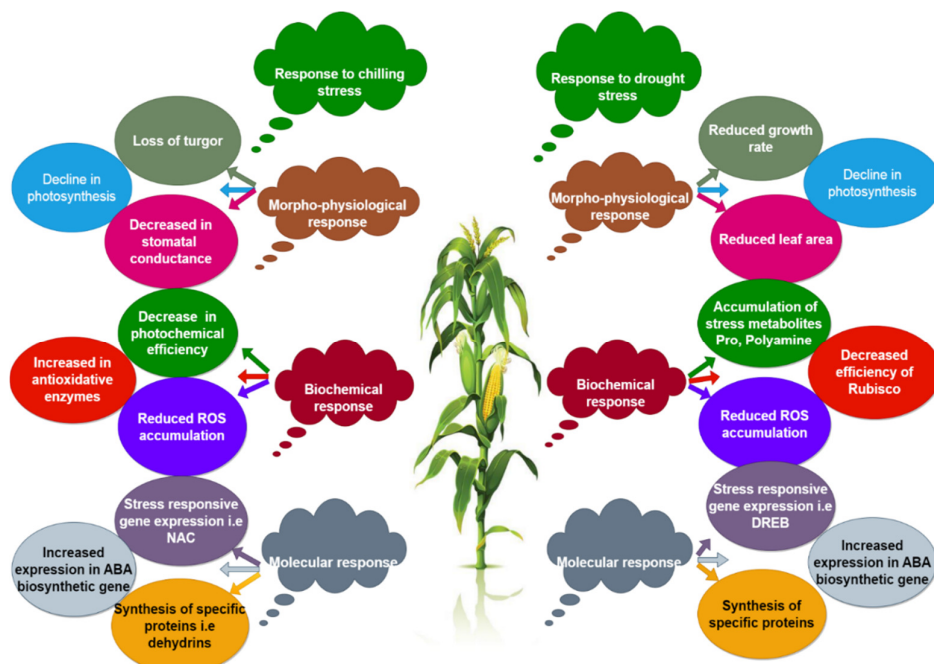
**Figure 1.** The impact of chilling stress on the physiological, biochemical, morphological, and molecular characteristics of maize plants, illustrates the complex defense mechanisms and adaptive responses used by maize plants to reduce chilling stress, demonstrating their endurance and survival mechanisms.

### Management for Chilling Stress

Maize is a crop that is vulnerable to chilling, environmental stresses like low temperatures at critical developmental stages may be harmful to later crop establishment and yield. Therefore, developing molecular, biochemical, and physical defenses against such environmental threats is crucial (Jiao *et al.*, 2022). It is essential to develop unique strategies or modify plant architecture in accordance with the environmental conditions that are beneficial to the plant to reduce losses brought about by chilling (López *et al.*, 2017).

### The physiological and biochemical responses of maize to cold stress

In maize plants, cold stress causes substantial alterations in gene expression (Figure 2). While some genes linked to typical development and growth may be down regulated, others linked with stress response and endurance may be increased (Shafi, 2016).



**Figure 2.** Morphophysiological, biochemical, and molecular responses of maize to drought and cold stresses. In order to overcome the effects of chilling and drought, plants induce biochemical, physiological and molecular modifications that improve tolerance against these stresses. As a result, these methods can enhance the complex and effective network that plants use to respond to challenges like drought and cold, which will increase plant productivity and tolerance

Low temperature can negatively impact the fluidity of cellular membranes, affecting their integrity and function. To counter this, maize plants adjust the composition of membrane lipids to maintain appropriate fluidity, ensuring proper cellular processes (Rawat *et al.*, 2021). Stress from the cold might cause a water imbalance within cells, which could lead to dehydration. Sugars and proline are collected by maize plants as a reaction to water stress in order to reserve cellular water potential while avoiding cell damage (Karalija *et al.*, 2018). Chilling stress can alter cell wall properties, affecting the overall mechanical strength and flexibility of plant tissues. Maize plants may undergo changes in the composition of cell wall components to maintain structural integrity as shown in Figure 2 (Ezquer *et al.*, 2020). Chilling stress can inhibit photosynthesis due to reduced enzyme activity and disrupted electron transport. Maize plant respond by adjusting the expression and activity of photosynthetic proteins to optimize photosynthesis below low temperature conditions (Nouri *et al.*, 2015). Maize plants may regulate respiratory pathways to minimize energy wastage (Aliyari Rad *et al.*, 2021). Below chilling stress, a number of transcription factors are indispensable for controlling gene expression. They act as genetic switches that control the production of genes sensitive to stress and that synchronize the resilience of the plant (Manna *et al.*, 2021). Denaturation and misfolding can result from cooling stress. Heat shock proteins are turned on in maize plants to help with protein folding and avoid aggregation, safeguarding vital cellular processes (Priya *et al.*, 2019). Chilling stress can affect the balance of gibberellins and abscisic acid, two plant hormones. Their leaves change to adapt to the stress conditions, and these hormones have functions in signaling stress and growth regulation, respectively (Podlešáková *et al.*, 2019).

### **Chilling stress alleviation**

As with many other abiotic stimuli, chilling modifies the morphology and biochemistry of developing plants. Many methods have been employed to increase plants' resistance to cold, some of them are described below (Madani *et al.*, 2019).

### **Agronomic management**

The use of genomic platforms and molecular markers provides exceptional prospects for the discovery, choosing, and replication of maize gene to enhance tolerance to chilling in maize, a mainly quantitative trait. Additionally, the creation of transgenics is yet another practical strategy for enhancing maize's chilling tolerance (Moon *et al.*, 2022). According to Melicher *et al.* (2022), methyl viologen and cold tolerance in maize were enhanced by transfer and overproduction of the iron super oxide dismutase (FeSOD) from the plant *Arabidopsis thaliana*, as measured by oxidative stress and development rates. *NP1K1* (*Nicotiana protein kinase*) transgenic maize that expressed CBF3 (Centromere Binding Factor 3) and CBF1 (Centromere Binding Factor 1) from *A. thaliana* was able to survive temperatures that were 28 °C lower than the controls. According to Bezerra-Neto *et al.* (2019) using *Agrobacterium*-mediated transfer of the bet, four of the five maize lines a gene in *Escherichia coli* had superior survival rates below freezing (108 °C) than its wildtype counterparts due to lower damage to cell membranes serine. Post-harvest techniques that enhance the development of seedling germination, or both can make it easier to supply seeds and supplementary supplies at the time of sowing. Despite more recent references to post-harvest seed treatments in the scientific literature, this phrase is still used in the business world (Shukla *et al.*, 2019). The purpose of these treatments is to compress the seedling population in order to protect seed both abiotic and biotic threats by the development of seedlings and to synchronize emergence (Marthandan *et al.*, 2020).

### **Calcium role for improving chilling tolerance in maize**

Abiotic stresses like cold impede plant development and growth primarily by causing oxidative damage (Xie *et al.*, 2019). Priming the seeds with CaCl<sub>2</sub> was used to lessen the harm hybrid maize suffered from chilling stress. Before being dried, Hyson 8288 hybrid maize seeds were soaked in solutions of CaCl<sub>2</sub> comprising 50, 100, and 150 mg/l for a full day. Seeds were sown at the optimal temperature of 27 °C and a cold stress temperature of 15 °C under carefully monitored conditions. Seed priming using CaCl<sub>2</sub> increased the growth rate and also greatly reduced the chilling damage. CaCl<sub>2</sub> seems to promote cold tolerance by maintaining tissue glucose metabolism, water content and decreasing membrane leakage. CaCl<sub>2</sub> seed priming at a concentration of 100 mg/l improved hybrid maize performance both under ideal and stressful conditions (Tamindžić *et al.*, 2023). Germination of Hybrid maize and early developments of seedlings were both severely reduced by chilling stress, but the chilling resistance was successfully produced by seed priming through CaCl<sub>2</sub>. These included larger seedlings fresh and dry weights, longer shoots and roots, and seed priming with CaCl<sub>2</sub> (Demidchik *et al.*, 2014). On the other hand, seed priming significantly decreased membrane leakage, possibly as a result of higher antioxidant activity. It is generally recognized that calcium helps to preserve membrane function and structure, possibly through preserving intra- and intermolecular connections (Wang *et al.*, 2022).

### **Mechanism of drought stress**

Drought is defined as "a temporary drop in humidity accessibility, where the amount of water that is available is significantly less than usual for a predetermined amount of time." Drought specifically impacts the physiological functioning of plants directly (Ansari *et al.*, 2019). Desiccation results in a great deal of water, which interferes with cell structure and metabolism before halting enzyme-catalyzed actions. Plants that can tolerate or delay the beginning of severe shortages of water are considered drought-resistant (Chourasia, 2017). One of the main problems restricting crop production worldwide, particularly in warm arid regions, is drought. Water stress lowers average crop yields of significant crops by more than 50%. As a result, tolerance to drought is a crucial yield trait. Agro-ecosystems and food crops are both significantly impacted by drought stress. In plant breeding processes, increasing yield during drought stress conditions becomes a major challenge. Stem elongation, development of leaves, and stomatal movement all slow down under drought stress (Yang *et al.*, 2021). Furthermore, it limits chemical change and, as a result, plant production by interfering with physiological and chemical processes that regulate plant growth and productivity. When water loss from transpiration outweighs the water taken in from the soil, drought stress results (Sinacore *et al.*, 2019). A plant understands drought stress while the water supply to the roots develops overly great. Every year, drought disturbs many different regions of the world, typically having a disastrous effect on crop productivity (Lobell *et al.*, 2015).

### **Effect of drought on seedling establishment and vegetative growth of maize**

To adapt to low water potential, maize seedlings have been shown to increase the walls in the apical part of their roots. This is brought on by an increase in growth activity, among other complex processes. If it happens at the seedling stage, drought stress-induced root adaptability and growth of hybrid maize are improved (Liu *et al.*, 2021). Drought has an important influence on the development of establishment and seedling in its early stages. Thus, the growth of seedlings is stopped once cell expansion and elongation stop (Anjum *et al.*, 2017). Figure 3 illustrates how drought stress affects both vegetative and reproductive growth. Cell growth is tremendously sensitive to conditions of water deprivation. Under drought stress, maize stem lengthened less during the vegetative stage.

According to research, drought-tolerant cultivars produce more dry and fresh stem weights than sensitive ones. When under drought stress, drought-resistant maize hybrids developed larger leaf area as opposed to drought sensitive varieties (Adee *et al.*, 2016).

### **Impact of drought on maize root growth**

The best signs and traits for identifying drought resistance of agricultural plants, such as maize, were determined to be highest root fresh weight in the most resistant to drought kinds (Kour *et al.*, 2020). Additionally, it was found that, in comparison to less drought-resistant kinds, the M351 and De Kalb C42y cultivars had longer roots, larger root weight, and volume. Nada *et al.* (2018) observed that when water supply was limited, plants' roots to shoots ratio improved. The ratio increased because of roots being less sensitive to water shortage conditions than shoot growth. It was ruled out that the xyloglucan endotransglucosylases, peroxidase, and other wall enzymes in plant roots were responsible for this adaptation to low water potential (Wilmowicz *et al.*, 2022). The question of whether more brace roots and extendable fine root development permit the creation of shoot biomass has received increased attention in the research (Liu, 2021).



### Drought effect on reactive oxygen species (ROS)

Drought influences maize, which in turn, results in yield reduction at various growth stages as shown in Table 2. Due to the low light energy consumption in this situation, excitation energy also transfers to O<sub>2</sub>, increasing the amount of ROS that is added in the chloroplast producing highly hazardous ROS (Foyer *et al.*, 2022).

**Table 2.** Reduced yield due to drought at various phases of crop growth

Crop	Stage of growth	Reduction in yield	References
Maize	Reproductive	47-70%	(Seleiman <i>et al.</i> , 2023)
Maize	Reproductive	63-87%	(Osae <i>et al.</i> , 2022)
Maize	Reproductive	32-92%	(Diédhiou <i>et al.</i> , 2022)
Maize	Grain filling (Mild stress)	79-81%	(Mehmood, 2021)
Maize	Vegetative	25-60%	(Gao <i>et al.</i> , 2021)
Rice	Reproductive (Lightly stressed)	53-92%	(Kommana <i>et al.</i> , 2023)
Rice	Grain filling and flowering (Long-term, extreme stress)	84%	(Sujariya <i>et al.</i> , 2023)
Rice	Filling with grain (High stress)	60%	(Zheng <i>et al.</i> , 2022)
Rice	Reproductive under extreme stress	48-94%	(Ashraf, 2021)
Rice	Grain filling and flowering (Long-term, mild stress)	52%	(Ali <i>et al.</i> , 2021)
Rice	Reproductive	24-84%	(Badawy <i>et al.</i> , 2021)
Rice	Flowering (Brief, intense stress)	54%	(Wu <i>et al.</i> , 2020)
Rice	Filling the grain (Little stress)	30-55%	(Prathap <i>et al.</i> , 2020)
Wheat	Pre-anthesis (Long-term, moderate stress)	18-53%	(Thapa <i>et al.</i> , 2022)
Wheat	Reproductive (Long-term, moderate stress)	50-66%	(Qayyum <i>et al.</i> , 2021)
Wheat	Vegetative growth stage	40%	(Feng <i>et al.</i> , 2021)
Wheat	Reproductive growth stage	4%	(Yang <i>et al.</i> , 2020)
Wheat	Post-anthesis (Long-term, moderate stress)	13-38%	(Alhabbar <i>et al.</i> , 2018)
Barley	Seed filling	49-57%	(Mwendwa <i>et al.</i> , 2022)
Chickpea	Ripening stage	49-54%	(Sagar <i>et al.</i> , 2023)
Chickpea	Reproductive	45-69%	(Khatun <i>et al.</i> , 2021)
Chickpea	Anthesis stage	27-40%	(Dar <i>et al.</i> , 2021)
Pigeon pea	Reproductive	40-55%	(Varshney, 2016)
Sunflower	Reproductive	60%	(Stefanic <i>et al.</i> , 2023)
Potato	Flowering	13%	(Jama-Rodzenska <i>et al.</i> , 2021)
Soybean	Reproductive	46-71%	(Ciampitti <i>et al.</i> , 2018)

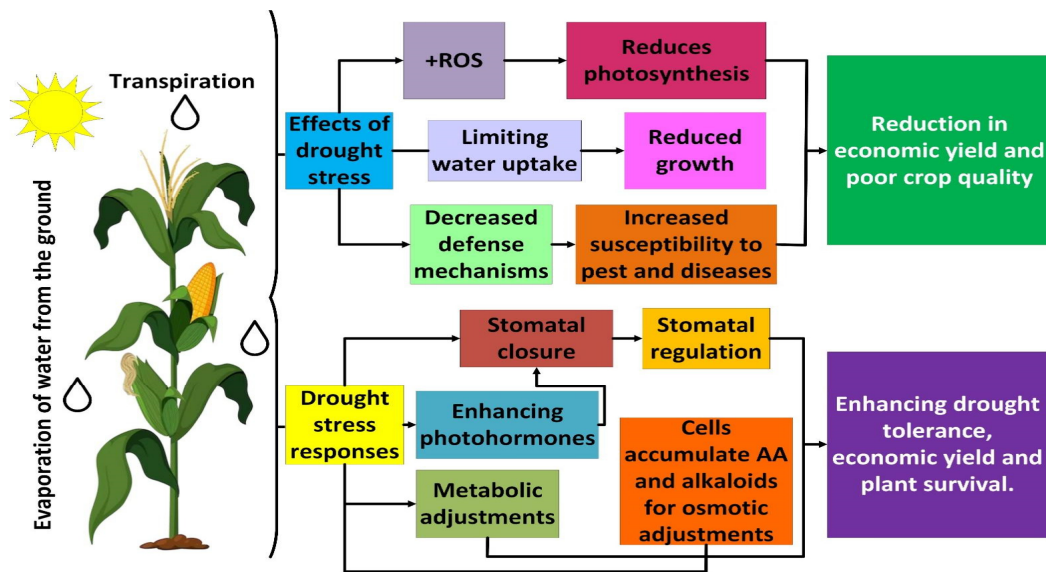
### Drought effect on reproductive growth

It is well known that a plant's need for carbon decreases when it undergoes drought stress throughout its reproductive phase because the size of its sink expands less. Degradation of tillers, rotting of pollens and breaking off flowers are some of the disorders, which may result in the abortion of the ovules (Feng *et al.*, 2018). The natural qualities of maize could indicate greater possibilities of kernels, ovules, and ear as related to the product at the peak of adulthood. The ovule may die if silks are not formed because of slow development. Abortion of kernels casts a shade over the pollination process. According to Pacini *et al.* (2019), pollination is not prevented by low potential of water at the moment of pollen shedding, but embryo growth is constrained

since photosynthesis is not occurring. When the amount of assimilates reaching the ear is less than what is necessary for ovule development, the whole kernels cease to grow, leaving the cob barren. In order to investigate the variables impacting grain production, the size and number of the kernels have to be established (Rafique, 2020).

### Drought impacts on grain yield

Drought stress negatively impacts maize yield characteristics (Figure 4). Both the length and intensity of water restrictions along with the stage of plant growth have an impact on maize yield during drought conditions. Drought stress limits the growth and germination of early seedlings (Queiroz *et al.*, 2019). The anthesis process is more impacted by the prolonged and severe drought throughout the reproductive phase than it is at the vegetative stage, lowering the grain output of the maize crop. A lack of water during the crucial growth stages, from tasseling to grain, will decrease the amount of grain that the maize crop produces. Wijewardana *et al.* (2018) described that minor water stress affected yield as well as the development and growth of all hybrids at various phases of the maize crop. Ultimately, drought lowers output, however under drought conditions, some genotypes of hybrid and open-pollinated cultivars fared slightly better than landraces. Higher leaf ear, total biomass, and leaf stem are found in improved genotypes compared to landscape (Varshney *et al.*, 2021).



**Figure 4.** Effects of drought stress on the physiology, yield characteristics, and drought-resistant responses in maize plants, demonstrating the complex defensive mechanisms and adaptive responses used by maize plants to fend off drought stress and indicating their flexibility and survival mechanisms

### Inducing drought tolerance in maize

Recognizing how plants may withstand abiotic pressures requires an understanding of the complex molecular, physiological, and biochemical events that interfere with agricultural plants' ability to develop and flourish. A crop by the highest yield potential could nonetheless function effectively below mild drought stress (Boorboori *et al.*, 2022). In developing nations, it has been regarded as a severe yield-limiting factor. There are numerous biological and physiological processes involved in the mechanism for drought resistance (Usmani *et*

*al.*, 2020), which include increasing root depth and water intake, decreasing water loss by improving stomatal resistance, depositing osmolytes, and producing osmoprotectants. The unique processes of drought tolerance include the detoxification of ROS using non-enzymatic and enzymatic methods, maintaining stability of plasma membrane and producing stress proteins and aquaporins (Zia *et al.*, 2021).

### **Responses of maize to drought stress on morphology, biochemistry, and physiology**

In order to help themselves adapt to their surroundings, plants undergo biochemical, morphological and physiological alterations in response to drought stress. While a plant's drought tolerance refers to its stability in maintaining normal activities even when tissue levels are low, stress avoidance signifies that a plant has the capacity to sustain high water potential tissue during dry conditions. Usually, plants must undergo morphological changes in order to avoid drought, such as decreased stomatal conductance, smaller leaves, the development of higher root-shooting ratios and large root systems (Parray *et al.*, 2019). Through biochemical, molecular, and physiological pathways of specific tissues and cells, including targeted protein accumulation and gene expression, drought tolerance is achieved. Plants modify their interactions with water, physiological and biochemical functions, the structure of membranes, and subcellular organelles ultrastructure in response to drought. Further research is necessary to fully understand the strategies drought-tolerant plants use to defend themselves against water shortages (Mukarram *et al.*, 2021).

### **Utilizing potassium to withstand drought**

If we compare the losses affected by stresses, both abiotic and biotic, the losses affected by abiotic stresses are particularly large. Added drought stress, nutrient shortage, and toxicity are the main causes of severe agricultural output losses worldwide (Mansoor *et al.*, 2021). Among various minerals, K is crucial for the body of the plant to acquire tolerance. When K is in scarce, leaf chlorosis and necrosis occur, and plants subjected to high light intensities experience growth disruption. The low K content of the plant body reduces carbon metabolism, photosynthetic activity, and the uptake of fixed carbon sources because of the significant amount of carbohydrates that are stored in the source leaves (MacNeill *et al.*, 2017). It is crucial to keep in mind that while a potassium deficit may be seen on a plant's leaves, it cannot be corrected as the plant grows. Application of potassium may increase shelling percentage, yield, and 1000 grain weight as compared to controls (Gomaa *et al.*, 2021). For a plant to develop and grow, potassium, a crucial element, is intricate in the activation of over sixty enzymes. Potassium helps plants develop resistance to diseases, drought, and hot temperatures (Zhang *et al.*, 2019). According to crop plant studies, potassium increases the length of roots and the stem as well as the shelf life of fruit. Lacking K, plants damaged by drought are especially vulnerable to strong light and rapidly become necrotic and chlorotic (Souri *et al.*, 2021). In plants that are K deficient and subjected to high light intensities, chlorosis and necrosis occur quickly (Hasanuzzaman *et al.*, 2018). The preservation of photosynthesis along with other associated mechanisms depends heavily on potassium. The reduction in photosynthesis caused by lower K status of plants is more pronounced when plants are exposed to high amounts of O<sub>3</sub> and CO<sub>2</sub>. This makes it clear that plants require more K when their surroundings are CO<sub>2</sub> rich (Soares *et al.*, 2019).

### **Drought tolerance in maize improves by glycine betaine**

Although the capacity of different maize cultivars to accumulate glycine betaine varies, glycine betaine is essential to maize, in conditions of abiotic stress. The *Escherichia coli* beta gene was given to the maize inbred line DH4866, coding for enzymes for production of glycine betaine from choline. The transgenic maize plants collected more glycine betaine than wild type plant (Maazou *et al.*, 2016). Most remarkably, after being subjected to drought, transgenic plants generated grain at a considerably higher level of complexity than wild-type plants. When associated to wild-type plants, transgenic maize's amplified glycine betaine accumulation offers stronger protection for the structural reliability of the cell membrane and higher enzyme activity (Hussain Wani *et al.*, 2013).

### **Foliarly applied calcium increases maize's tolerance to drought stress**

Limitations of nutrients and water prevent maize from growing in semi-arid and arid climates. Below drought stress (DS), calcium ( $\text{Ca}^{2+}$ ) uptake is significantly impacted. Two maize cultivars, the Yousafwala-hybrid (drought-sensitive) and the 'Dekalb-6525' (drought-tolerant) were studied. The applied DS significantly decreased grain nutrient content, hydration status, photosynthesis, and maize growth (Naeem *et al.*, 2018). Foliar  $\text{Ca}^{2+}$  significantly improved plant development, transpiration rate, turgor potential, water potential, photosynthesis, stomatal conductivity, and accumulation of total soluble sugars in all cultivars under DS while lowering  $\text{H}_2\text{O}_2$  content (23%). Furthermore, in water-scarce conditions, adjusted  $\text{Ca}^{2+}$  rate increased maize grain yield and quality. Over 'Yousafwala'-hybrid, cultivar 'Dekalb-6525' showed a considerable responsiveness to  $\text{Ca}^{2+}$ . According to the study's findings, feeding plants with  $\text{Ca}^{2+}$  through their leaves is a good way to provide them the strength they need to survive in water shortages (Nawaz *et al.*, 2021).

### **Combined chilling and drought stress**

When applied separately, drought stress has more detrimental effects than chilling stress. Chilling stress had a considerably more detrimental impact on the chlorophyll concentration than drought stress did. The amount of carbon dioxide inside cells rose significantly when there was less heat stress and dryness (Maghsoudi *et al.*, 2016). The rate of transpiration varied depending on the stressor. It expanded in response to heat stress, contracted in response to drought stress, and shrank even more in response to a combination of heat and drought stress. Although the concentration of soluble protein dropped during all combined and individual drought circumstances, that of free protein, heat shock protein, and soluble sugar improved during drought as well as combined chilling stress and drought (Zahra *et al.*, 2021). In contrast to the control, chilling stress had no effect on the nitrogen content in the stem, root, or leaves, whereas drought stress drastically decreased it. Individual temperature and drought stress had a negligibly lower content of potassium and phosphorus than usual. However, in conditions of combined drought and heat stress, concentrations of leaf potassium, stem phosphorus, root, stem, and root nitrogen were significantly lower (Correia *et al.*, 2018). A decrease in photosynthetic pigments and structural growth was caused by the combined and individual effects of drought and chilling stressors, which encouraged the generation of  $\text{O}_2$ ,  $\text{H}_2\text{O}_2$ ,  $\text{OH}$ , and increased malondialdehyde (MDA) levels. To balance the oxidative damage, suitable antioxidants, osmolytes and ROS detoxifying proteins were induced by the stress conditions of chilling, drought, and drought + chilling. It was discovered that the combination of chilling and dryness stress was more deadly for corn seedling growth than either stress alone (Tayyab *et al.*, 2020). However, the XD889 and XD319 hybrid maize cultivars outperformed the Yu13 and Yu37 inbred cultivars in terms of performance. By interfering with normal plant metabolism, drought and cold

stress significantly reduce plant growth and yield qualities. For instance, the insufficient temperature results in water shortage in maize seedlings by significantly reducing root water intake in conjunction with leaves transpiration (Waqas *et al.*, 2021). On the other side, drought-pretreatment causes maize's stomata to close and decrease transpiration. Both stresses generally have similar effects on the morphological characteristics of plants, but the ways in which they alter the physio-biochemical functions of plants may differ. Under stressful circumstances, osmo-regulation via accumulation of various osmolytes is crucial for maintaining turgor and safeguarding macromolecules in dehydration cells (Nawaz *et al.*, 2022). According to Hussain *et al.* (2020), plants' ability to withstand drought and frost is strongly associated with their increased antioxidant levels under stress. The ideal growing temperature for maize, a thermophilic crop with a preference for tropical climates, is around 28 °C. However, the maize seedling is very vulnerable to dehydration and low temperatures during the emergence and initial growth stages (Khaeim *et al.*, 2022).

### Photosynthetic characteristics and oxidative stress defenses

In the current investigation, two genotypes of maize (*Zea mays* L.) with varying levels of tolerance to chilling (sensitive, Penjalinan, and tolerant, Z7) were cooled to 5 °C for five days, regardless of whether they had received a pretreatment with drought. The Penjalinan plants' extreme quantum output of photosystem II and necrotic leaf area were measured as signs of chilling injury, and these symptoms were reduced by the drought pretreatment (Maiti *et al.*, 2017). In comparison to undrought-pretreated plants, drought-pretreated Penjalinan and Z7 plants revealed greater net photosynthetic rates. After being saved from chilling for one day, penjalinan plants are less likely to produce reactive oxygen species. There was a correlation between increased net photosynthesis and higher NADP-malate dehydrogenase activity (Kandoi *et al.*, 2018). Antioxidant enzyme activity and the de-epoxidation state of the xanthophyll cycle were the same in all cold plant groups. However, chilled Penjalinan plants that had not been subjected to a pre-drought condition showed a sharp decline in ascorbate content. Given that we observed an increase in H<sub>2</sub>O<sub>2</sub> concentration following drought pretreatment, we propose that it serves as a signal for the drought's ability to raise maize's freezing tolerance (Nawaz *et al.*, 2020).

The *NAC* transcription factor family plays a significant role in environmental stressors such as drought stress (Table 3).

**Table 3.** Different genes related to drought and cold tolerance in maize

Gene category	Gene	Phenotype	Reference
<i>Beta</i>	<i>betA</i> ( <i>E. coli</i> )	Chilling and drought tolerance	(Yan <i>et al.</i> , 2023)
<i>NF-YB</i>	<i>NF-YB2</i>	Drought tolerance	(Izadi-Darbandi <i>et al.</i> , 2023)
<i>PI-PLC1</i>	<i>ZmPLC1</i>	Drought tolerance	(Sheoran <i>et al.</i> , 2022)
<i>CSP</i>	<i>CspB</i>	Tolerance for cold, heat, and drought	(Wang <i>et al.</i> , 2021)
<i>PARP</i>	<i>hypAtPARP1</i>	Drought, heat and high light tolerance	(Waititu <i>et al.</i> , 2021)
<i>MAPK</i>	<i>NPK1</i>	Drought tolerance	(Zhao <i>et al.</i> , 2021)
<i>NAC</i>	<i>ZmNAC49</i>	Tolerance to drought	(Xiang <i>et al.</i> , 2021)

One important *NAC* transcription factor involved in drought tolerance is *ZmNAC49* (Wang *et al.*, 2020). *Beta* gene present in *E. coli* that is use for drought tolerance. *NF-YB* proteins has important role in various stress responses in maize including heat, drought and cold stress. *NF-YB2* is a member of the *NF-YB* family and an essential component in drought stress. Phospholipase C1 in maize plants is called *ZmPLC1*. *ZmPLC1* plays a significant part in drought resistance (Zhu *et al.*, 2021). The cold shock protein, or *CSP*, is essential for resilience to heat and drought. One member of the *CSP* family that is found in bacteria, *CspB*, is

crucial to maize's ability to withstand drought. Poly ADP ribose polymerase, or PARP, is an enzyme that aids in DNA repair. A key component of maize drought resistance is *HypAtPARP1*, a member of the *PARP1* family. Plant resistance to drought is mostly dependent on mitogen-activated protein kinase, or *MAPK*. *NPK1* plays a role in initiating *MAPK* signaling pathways, which are essential for drought resistance in maize (Jagodzik *et al.*, 2018).

## Conclusions

Two abiotic stresses that negatively affect crop plants include drought and low temperatures. Drought and chilling conditions coexisting, rather than any one stress, is the main factor limiting crop output in field settings. Another characteristic shared by both stresses is a noticeable change in plant development and growth, from seed germination to yield. Plants' ability to withstand particular stresses varies depending on the species. A plant's response to multiple stresses differs from its response to a single stressor, as evidenced by the data presented above. More detrimental changes to root morphology can result from chilling stress than from drought stress. Conversely, coupled stressors lead to smaller seeds, reduced growth of the flower and pod, increased abortion of the flower and pod, and lower crop yields because of insufficient dry matter accumulation. Some noteworthy outcomes include the way that nutrients diffusely absorb them, the way that CO<sub>2</sub> diffuses into chloroplasts, and how plants use their photosynthetic system. The combination of cold and drought has been found to have cumulative effects on almost all physiological and developmental traits of plants, and when combined with additional stresses, can cause much more damage. In order to strengthen their resistance against the damaging effects of cold and drought, plants go through physiological, molecular, and biochemical alterations. The co-occurrence of drought and cold results in both oxidative and osmotic stress. Plants offset these detrimental effects by increasing good solutes, detoxifying ROS-absorbing proteins, and regulating the activity of antioxidant enzymes. Some genes are solely regulated by drought, whereas others are only activated by cold temperatures. Both drought and chilling stress change the expression of these genes. So far, several novel approaches have been tried to lessen the negative effects of combination stressors. Notwithstanding the significant progress made, there is still much room for improvement in the combination of cold and drought tolerance. A thorough understanding of the ways in which plants react to cold and drought stress requires that the studies be designed under field conditions. Future studies should also focus on using molecular and biotechnological methods to create genetically engineered plants that can respond specifically to drought and cold stress. Bioinformatics, genome sequencing, transcriptomics and proteomics analysis, and other methods can be used to identify the common and unique genes that are changed under stress. These tactics help improve the intricate and functional network that plants employ to adapt to environmental challenges like cold and drought, which in turn increases plant tolerance and productivity.

## Authors' Contributions

Conceptualization: AM and JMA, Writing original draft: AF and AM. Reviewing and editing: MMJ, AM, HFNA, HBA, MIA, OA, BA, WFS, Funding acquisition; JMA. All authors read and approved the final manuscript.

## Ethical approval (for researches involving animals or humans)

Not applicable.

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## Conflict of Interests

The authors declare that there are no conflicts of interest related to this article.

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