Climate's Impact on Agriculture

Climate's Impact on Agriculture:

A Growing Challenge

Edited by

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Cambridge
Scholars
Publishing

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This book first published 2025

Cambridge Scholars Publishing

Lady Stephenson Library, Newcastle upon Tyne, NE6 2PA, UK

British Library Cataloguing in Publication Data
A catalogue record for this book is available from the British Library

ISBN: 978-1-0364-4995-7
ISBN (Ebook): 978-1-0364-4996-4

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CLIMATE CHANGE: A MENACE TO FARMING

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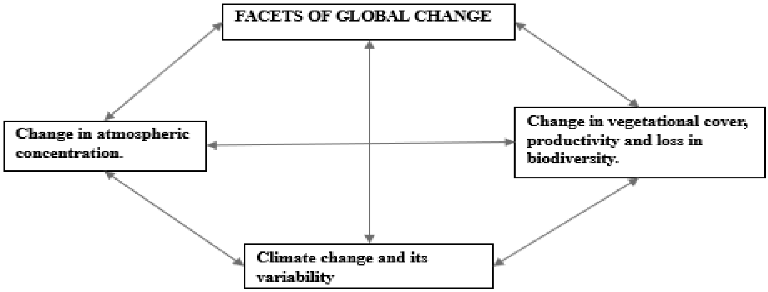
> "There's one issue that will define the contours of this century more dramatically than any other, and that is the urgent threat of a changing climate." - Barack Obama

Introduction

The last few decades have witnessed a sharp rise in global change awareness, which has led to widespread concern among various governments, non-government organizations, and scientists. A prolonged alteration in the statistical distribution of weather patterns is referred to as climate change. Therefore, variations like El Nino that last for a few decades or less do not indicate climate change. In the broadest sense, Earth's equilibrium temperature and climate are determined by the rate at which energy is acquired from the sun and the rate at which it departs into space. Earth's climate has an impact on human life. It controls the availability of food and water, as well as energy consumption, the spread of disease, and other facets of human health and well-being. Major characteristics of worldwide change are climatic variation, different concentrations of atmospheric elements (such as carbon dioxide (CO_2), methane (CH_4), etc.), land surface cover (e.g., desertification, deforestation), and biodiversity (National Research Council, 2010). The shift in these characteristics is of major concern to us because of its effect on human beings and the resources essential for their survival. However, shifts in these aspects have many manmade components, e.g., an increase in concentration of CO_2 due to fossil fuel burning, deforestation, and loss of biodiversity. It is crucial to remember that a lot of these modifications are interconnected (Fig. 1). Thus, emissions of greenhouse gases and global warming are thought to be the major causes of the unfavorable acceleration of climate change. Anthropogenic activities are increasing rapidly giving rise to a global temperature of 0.9^{o} C since the 19^{th} century, and it is predicted to rise by 1.5^{o} C by 2050 (Eftekhari, 2022). Since 1751, global CO_2 emissions have been approximately 1.5 trillion metric tonnes. Regional differences exist in the emission, though. With 514 billion metric tonnes of CO_2 emissions, Europe has become the biggest contributor to CO_2 emissions. Asia and the continent of North America occur second and third, respectively, with 457 billion metric tonnes of cumulative CO_2 emissions.

China has contributed 200 billion metric tonnes of CO_2 emissions, but the United States has contributed 399 billion metric tonnes, or 25 percent of all historical emissions since 1751. Twenty-eight nations that make up the European Union (EU-28), which sets cooperative goals, have contributed twenty-two percent of the total historical CO_2 emissions. Due to low CO_2 emissions per capita, Africa only contributes 3 percent of the world's total CO_2 emissions. But in the current scenario, nations with historically lower emissions, such as Brazil and India, notably increase their overall emissions (CDIAC). Numerous and ongoing increases in greenhouse gas emissions are having a significant and irreversible negative impact on freshwater, terrestrial, and marine ecosystems. These greenhouse gases (GHGs) trap heat by preventing the transmission of infrared radiation that tries to elude the atmosphere. The potential for global warming possessed by CH_4 is the highest, approximately 300 times greater than that of CO_2 and 20 times greater than that of N_2O. Fossil fuel combustion, application of nitrogen fertilizers, managing soil, flooded rice fields and manure management are the main initiators of greenhouse emissions. Crop fertilization increases as atmospheric CO_2 levels rise, and warming causes a reduction in crop energy requirements. Climate change emerges to be a serious threat in the 21^{st} century, with detrimental externalities affecting the developed and developing nations as well (Tol, 2013). The United Nations Framework Convention on Climate Change (UNFCCC) officially defines climate change as a change in climate that is attributed directly or indirectly to human activity altering the composition of the global atmosphere in addition to natural climatic variability observed over equivalent periods. However, the Intergovernmental Panel on Climate Change (IPCC) states climate change is a change in the state of the climate that can be identified by changes in the mean and/or the variability of its properties and that persists for an extended period, typically decades or longer.



Gadgil (1995)
Fig. 1: Major aspects of global change caused by and having an impact on human life

1. Climate and Weather

According to the World Meteorological Organization (2023), climate is the mean weather conditions for a given location over a long period, ranging from months to thousands or millions of years. WMO utilizes about 30 years to determine the average climate. In 2022, the global mean temperature was about 1.15 C above the 1850-1900 average and according to the data set used, this year was recorded as the 5^{th} and 6^{th} warmest year. Also, the global sea level reached a new high of 107 mm (4.2 in), and based on WMO datasets, 2016 is still the warmest year on record, having begun during a strong El Nino. The meteorological conditions, including wind, rain, snow, sunshine, temperature, and so forth, at a specific time and location are referred to as the weather. On the other hand, a place's climate refers to the general long-term features of its meteorological conditions. A region's ecosystem, agriculture, means of subsistence, and population density are strongly influenced by its climate. Thus, it can be viewed as a long-term synopsis of weather patterns that accounts for both the variability and average of these patterns. Climate variability includes both the year-to-year variations and the statistics of extreme events, like powerful storms or exceptionally hot seasons. The geological validation of ice ages and changes in sea level in addition to the records of human history spanning over hundreds of years, reveal how drastically the climate on earth has changed over time.

While the exact causes of historical changes are occasionally unknown, alterations in solar activity, ocean currents, volcanic eruptions, and other natural phenomena are generally accepted to be accountable. The current difference is that during the prior few decades, an unusually rapid rise in global temperatures has been observed. The IPCC's sixth assessment outlines that the upcoming decades will see a surge in climate change throughout the world. About 1° C or 1.5° C represents an average across the planet, but even at 1° C or 1.5° C of global warming, there will be more heat waves, longer warm seasons, and shorter but severe cold seasons, which will get stronger at 2° C of warming. Thus, without a doubt, global warming exists. The atmospheric and oceanic temperatures are higher now than they have been for the past five centuries, if not longer. It has long been understood by scientists that the greenhouse gases (GHGs) in the atmosphere function as a blanket, trapping solar radiation and maintaining Earth's surface temperature above its ambient temperature. They also know that an increase in GHGs in the atmosphere would result in further warming. Since global warming is one of the most critical indexes of global change, these terms are very often used reciprocally with climate change. The term "global warming" describes a surge in the mean global temperature that has been affiliated with utmost effects on ecosystems, wildlife, and humans worldwide. Since the 20^{th} century, noted warming drifts have been primarily caused by anthropogenic activities. As of early 2020, the amount of carbon dioxide in the atmosphere has risen from 280 parts per million (ppm) in pre-industrial times to 413 ppm approx. There has never been carbon dioxide at this concentration in history. Since 1990, carbon dioxide (CO_2) emissions have increased globally by nearly 50 percent. According to scientific reports, stabilizing global warming will require us to come back to a "safe" concentration of 350 parts per million by the year 2100.

At present time, every nation is being influenced by the change in climate and its variations. It is destroying lives, troubling the economy of nations, and causing significant financial losses for every individual, group, and nation both now and in the coming times. Serious effects of climate change are being perceived by humans, such as altered weather patterns, an increase in mean sea levels, and extreme weather events. Climate change is primarily the result of greenhouse gas emissions from anthropogenic activities increasing continuously. Thus, they are at their highest level compared to past decades. In the absence of intervention, the global temperature is predicted to increase by more than 3 degrees celsius during this century, with certain regions of the world experiencing even more warming. The most vulnerable and destitute individuals are highly impacted.

2. Impact of Climate Change on Agriculture

Climate change has a direct and indirect effect likewise on agricultural production across the globe. The climate and weather have a significant impact on agriculture. Despite their propensity for adapting to varying weather conditions and annual fluctuations, farmers have developed a high degree of local climate adaptation through established farming practices, specialized equipment, and personal experience. Therefore, it is rational to predict that climate change will affect agriculture, possibly endangering established components of farming systems while also offering chances for advancement. Many crops can have shorter growing seasons and lower final yields as a result of an increase in the mean seasonal temperature. By 2080, it is predicted that global agricultural productivity will decrease by 3 to 16%. Enzymes are known to require optimal temperatures to function, and the loss of even one essential enzyme system can prevent the growth of plants or other organisms. Crop growth and maturity are directly impacted by variations in temperature and precipitation, which expose the crops to a range of biotic and abiotic stress (Chaudhry *et al*., 2022). The recurrence and effects of global warming are predicted to increase with a surge in human population and industrialization; in the end, these effects won't be limited to any one area but will instead be distributed throughout the world's ecosystems. The hazardous effects of climate change on crop yields have the potential to jeopardize global food security. Therefore, it can be said that two of the 21st century's greatest challenges are food insecurity and climate change (Neupane *et al*., 2022).

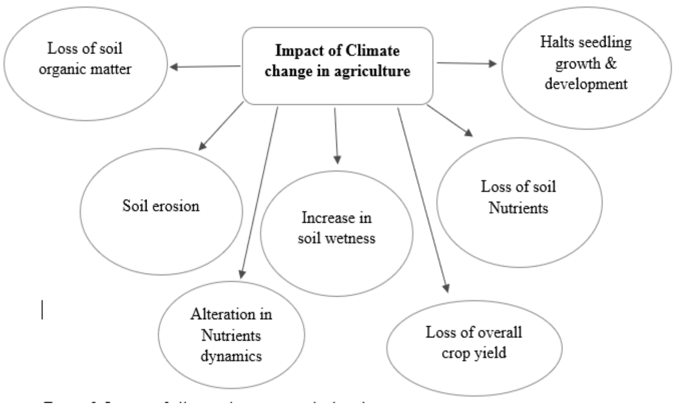


Fig. 2: Impact of climate change on crops

3. Possible negative effect on agriculture

- Changes in agricultural yield and geographic shifts
- Decrease in the amount of water accessible for irrigation
- land loss as a result of salinization and sea level rise

4. Impact on soil properties

Climate change is not only directly harming plants, but it is also negatively influencing soil systems. The direct effect of climate change on the soil is that it disturbs the structure of the soil by the destructive splashes of raindrops, filtering waters, and surface run-off; on the contrary, the biological characteristics of the soil, such as termites and earthworms' susceptibility to these climate changes, and the fluctuations in vegetation patterns led to the indirect effects. Climate conditions directly affect soil texture, bulk density, organic matter content, and salinization. Soil compaction is exacerbated by the loss of organic matter brought on by soil microbial activity and soil erosion, which raises the bulk density of the soil. Compaction and bulk density of the soil prevent plant roots from growing, which combined leads to low crop yield (Yu *et al.*, 2019). Extreme weather events also have an impact on soil chemical properties, including Ph, the amount and distribution of soluble salts, nutrients, and carbonates, as well as the cation exchange capacity and base saturation value. Changes in the physiology and growth of soil microorganisms can result from variations in temperature, moisture content, wet-drying, and freeze-thawing cycles, among other factors.

Table 1: Effect of stress due to high temperature in different crops Hasanuzzaman *et al*. (2013)

Crops	Heat treatment	Growth stage	Major effects
Chili pepper (*Capsicum annum*)	38/30 °C	Reproductive, maturity, and harvesting stage	Fruit weight and width are reduced, increasing the proportion of abnormal seeds per fruit.
Tobacco (*Nicotiana tabacum*)	43°C, 2 hrs	Early growth stage	A decrease in net photosynthesis rate, and stomatal conductance as well as the apparent quantum yield (AQY), Reduced the activity of antioxidant enzymes.
Rice (*Oryza sativa*)	25- 42.5°C	Vegetative growth stage	Decrease in the CO2 assimilation rate.
Wheat (*Triticum aestivum*)	38°C, 24 to 48 hrs	Seedling stage	Decreased relative water content (RWC), and diminished antioxidant capacity.
Mango (*Mangifera indica)*	Day temp- 18/10 °C Night temp- 30/25°C	Flowering stage	Late flowering.

5. Impact on crop growth and yield

Crop growth and yield are influenced by several critical variables, including soil toxin buildup, salinity, precipitation patterns, and amounts, and atmospheric temperature and CO_2 levels. With the rise in global temperature, several changes have been observed like runoff, evapotranspiration, groundwater, and soil moisture. High temperatures and heat stress have been linked to several crop physiochemical processes, including oxidative stress, membrane lipid peroxidation, and cellular damage. An estimate states that future crop yields could drop dramatically by 5–10% for a 1 °C surge in temperature. Plants are forced to finish their growth cycle faster in higher temperatures having less time to reproduce, which eventually causes a significant loss in yield. However, the optimum temperature differs for different crops.

6. Impact on fruit crops

Temperature has a known effect on respiration and photosynthesis, and a high ratio of these two processes is necessary to produce a high yield (Moretti *et al.*, 2010). As temperature rises to a certain point, the photosynthetic activity also increases, but above that certain point, it is affected by enzyme inactivation, which ultimately decreases its capacity to tolerate heat stress. Since ethylene production is suspended at temperatures above 35° C, climacteric fruit is thought to stop ripening above that temperature. In the case of peaches, it was found that, after treating the fruit with hot water, there was an elevated response to heat stress (43%) in terms of protein spots. An earlier harvest was also a result of higher temperatures during the growing season. Hormones necessary for the growth and development of a fruit tree are altered by temperature. Bud differentiation, flowering, and fruit sets are impacted by temperature, as in mango and litchi, where symptoms such as early blooming and advanced crop harvest have been noticed (Hribar and Vidrih, 2014). The most significant factors that could be linked to the impact of climate change on fruits are changes in harvesting dates, decline in irrigation water, enhanced irrigation costs, alteration in cultivar suitability and availability for current and future production, rise in physiological disorders, outbreaks of new pests and diseases, extreme event damage, and adverse effects on soil due to immoderate rainfall and temperature.

Alterations in fruit productivity, flowering, and quality from year to year are influenced by shifts in the distribution of rainfall. In bananas, a dry spell during the emergence of flower and fruit sets has also been linked to reduced crop duration and yield. An increase in temperature of 1-2 °C above 25-30 °C encourages vegetative flushes rather than flowering ones in citrus, while premature winter rains influence flower initiation and favor Psylla incidence. Mangoes may exhibit both early and delayed flowering in response to climatic changes. Thus, it has been noted that in January, low temperatures (4 to 11.5 °C), high humidity (> 80%), and cloudy weather delay the emergence of panicles, while low temperatures during inflorescence development decrease the number of perfect flowers. Tropical and subtropical climates are ideal for citrus plant growth; extremely high temperatures are not beneficial. Raised temperature may elevate water requirements in tropical conditions, and reduce flowering in subtropical conditions where plants are put to flower under cold stress. Citrus scale and mite pests may benefit from warming due to the conditions becoming drier and dustier. Temperature increases above 30° C encourage vegetative flushes rather than flowering ones (Nath *et al*., 2018).

7. Impact on insect/ pests

There is a greater chance of suffering losses as a result of weeds, insects, and diseases. As natural ecosystems adapt to changes in temperature and precipitation profiles, the range of many insects will shift or expand, and new combinations of diseases and pests may appear. The effect of other variables, such as the overuse of pesticides and the loss of biodiversity, which already contribute to plant pests and disease outbreaks, may be amplified by the effect of climate on pests.

8. Possible positive effects

- Agricultural productivity increases due to carbon dioxide fertilization.
- In some plants their growth and transpiration rate show positive response due to atmospheric CO_2.
- Higher CO_2 levels may also enable crop plants to use water more effectively.
- Rise in the level of CO_2 generally results in benefitting many C_3 plants.
- In comparison to C_3, C_4 plants are less likely to be benefitted, and CAM plants are the other form of C_4 plants but they are not at all affected by the elevation of CO_2.
- In some parts of the globe, a temperature rise brings a positive effect not only on high-altitude farming as well as on high-latitude regions which positively affects the yield and new varieties can be released.
- In certain places, more rainfall may also lead to increased productivity and more water available for irrigation.

Table 2: Climate change's effects on agriculture over the next 50 years [Source: Climate Change and Agriculture, MAFF (2000)]

Climatic factors	**Changes expected by 2050's**	**Confidence in Prediction**	**Impact on Agriculture**
Carbon dioxide (CO_2)	Rise from 360 ppm to 450-600 ppm (in 2023 CO_2 level was 417.06 ppm)	Very high	Photosynthesis increased and reduced water use. Hence, considered good for crops.
Rise in sea level	Rise by 10-15cm. Increased in the south and offset in the north by natural subsistence/rebound	Very high	Loss of land, coastal erosion, flooding, and salinization of groundwater
Temperature	Rise by 1-2°C. Winter warming more than summer. Increased frequency in heat waves	High	Faster, shorter, earlier growing seasons, range moving north and to higher altitudes, heat stress risk, increased evapotranspiration.
Precipitation	Seasonal changes by +- 10%	Low	Impacts on drought risk, soil workability, water logging, irrigation supply, transpiration
Storminess	Increased wind speeds, especially in the north, more intense rainfall events	Very low	Lodging, soil erosion, reduced infiltration of rainfall
Variability	Increases across most climatic variables. Predictions uncertain	Very low	Changing risk of damaging events (heat waves, frost, droughts floods) which affect crops and timing of farm operations.

9. Mitigation progress

Green agricultural techniques must address increased crop yields, better soil health, and environmental issues related to climate change all at once in order to become successful in contemporary agriculture. To maximize both adaptation and mitigation, combining the two approaches is the most effective way. The land use, forestry, and agriculture sectors have enough room to address mitigation and adaptation simultaneously because each creates chances for the other.

- **Biochar**: The primary constituents of biochar, a stable solid black carbon substance, are carbon, moisture, volatile matter, and minerals. It influences in support of earthworm activity that is beneficial to soil systems. The enormous capacity of biochar to sequester CO_2 and stop carbon from being released back into the atmosphere during its breakdown. It plays a vital role in improving soil health (by lowering methane emissions and nitrous oxides from soil) and crop productivity by minimizing $1/8^{th}$ of CO_2 emissions. Thus, Biochar has a great potential to lessen the effects of climate change on agriculture, soil, and eventually crop yields.
- **Biostimulants**: Emerging biological techniques known as biostimulants can reduce biotic and abiotic stress in plants without negatively affecting the environment, plant growth, or soil health. Microbes, organic substances, or a combination of the two are known as biostimulants, and they can aid in controlling the growth of plants. It is a safe method for increasing crop productivity and nutritional value.

Table 3: Effect of biostimulants in mitigating the impact of climate change and in enhancing crop yield

Biostimulants	Crop	Effect of treatment on crop	Reference
Silicate Compound and antagonistic bacteria *Bacillus* sp.	Banana	Bananas treated with this biostimulant showed increased physiological growth performance and significant resistance to Fusarium wilt. Fusarium wilt incidence lowered by 56.25%.	Zakaria *et al*. (2020)
Natural organic matter-based Biostimulant	Tomatoes Avocados	Plants became resistant to drought stress and increased the growth of plant shoots (27%) and roots (36%) Plants became resistant to salt and drought, which increased yield by 45%	Sleighter *et al*. (2023)
Ascophyllum nodosum	Watermelon	Plants treated with biostimulants exhibited a positive phenotypic response to salt stress.	Bantis *et al*. (2023)

- The creation and application of adaptable cultivars and cultural practices is the most straightforward adaptation strategy to preserve fruit crops.
- Enriching soil organic carbon is the most significant adaptation and mitigation strategy in tropical production systems because it offers resilience against climate change and has the largest potential for mitigation based on agriculture.
- One of the best adaptation strategies is crop and variety change in response to climate change. More biotic and abiotic stress-tolerant crop varieties contribute to increased climate resilience. It has been discovered that the (drought-tolerant) Pomegranate hybrid Ruby, the (drought-tolerant) Annona hybrid Arka Sahan, and the (*Vitis Champine*) grape rootstock Dog ridge exhibit potential.
- It is possible to abate the effects of severe weather events by altering the microclimate. The most common method for modifying the microclimate to protect against both heat and cold stress is water. Options for adapting to extreme weather include water channels, shade nets, mid-canopy sprinklers, overhead irrigation, and canopy management (Bakshi *et al*., 2020).
- Soil is particularly susceptible to erosion and degradation due to factors such as increased temperatures, low soil organic matter, inadequate vegetation cover, and increased precipitation in a changing climate. To protect against frequent droughts and food shortages, it is crucial to implement soil and water conservation techniques such as leveling, bunding, terracing, trenching, and incorporating in situ moisture conservation techniques like mulching.

Conclusion

Global food production is directly or indirectly affected by the changing climate in today's scenario. Climate change cannot be divided by borders in the world, which is why it is said that emissions anywhere have effects everywhere in the world. Increased photosynthesis, decreased photorespiration, and decline in stomatal conductance can all help to boost crop growth and yield when CO_2 concentrations are higher. C3 plants will likely compete with C_4 crops even more fiercely than they do now, and vice versa. Multidisciplinary strategies for various problems are required in all fields of agriculture to collaborate to identify long-term strategies for maintaining agricultural output. The tactical investigation is necessary to improve the resilience of Indian agriculture, which comprises crops, insects and pests, horticulture, and natural resource management. This will aid in the development and implementation of better production and risk management technologies. The use of various biostimulants, and biochar, release of new varieties tolerant to various biotic and abiotic stresses, proper use of land, and availability of water for irrigation are a few of the mitigation strategies that can help in controlling the detrimental impact of climatic variations on agriculture. The future of agroecosystems can be saved by futuristic planning and the implementation of these mitigation tactics in an interdisciplinary manner. They can also be utilized as biological tools to combat the unexpected effects of climate change on agriculture.

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TARGETED GENETIC MODIFICATION FOR CLIMATE SMART AGRICULTURE

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Abstract

The productivity of agricultural practices is negatively affected by climate change. Abiotic stresses, including heat, cold, drought, and salt, harm agroecological conditions. Reduced sustainable production is caused by global climate change. Worldwide, agricultural output takes a major hit when weather conditions aren't ideal. An enormous problem for the scientific community is to adapt to changing climate circumstances while meeting the needs of an ever-increasing global population. While conventional breeding techniques have helped crops become more resistant to abiotic stress, increasing agricultural yields is dependent on developing new cultivars with stress adaptation traits as soon as possible. The conventional approach to breeding stress-tolerant cultivars may take years, further slowing agricultural researchers' ability to meet the evolving demands of farmers, producers, and businesses. Breeding crops that can withstand abiotic stress is becoming more common via the use of genome editing tools and RNA interference technologies like CRISPR/Cas9. This approach is effective in climate-smart agriculture. Here we look at the inner workings of RNAi and CRISPR, how they control stress, the limitations they have, and the opportunities that follow.

Keywords: Abiotic stress, climate change, climate-smart agriculture, CRISPR, RNAi, stress tolerance

Introduction

Crop yields are reduced by several abiotic variables. Resistant crops will be in high demand since the globe is becoming hotter and drier by the day

(Battisti and Naylor 2009). Unpredictable rainfall and falling groundwater levels provide for an ecosystem that is often stressed by drought, which reduces plant grain yields generally. Takeda and Matsuoka (2008) identified a decrease in sustainable production as a result of a shrinking quantity of farmable land and water, an increasing world population, and the consequences of climate change. A large amount of agricultural output is lost due to adverse climatic conditions on a global scale. The continued struggle of agriculture to increase productivity in the face of rising demand and the threat of climate change and global warming is particularly pressing because food consumption is expected to almost quadruple by the 2050s (Tilman et al., 2011). Consequently, it is critical to understand the role of climate in affecting agricultural output and to use modern breeding methods to develop crops that can withstand the effects of climate change. The reason traditional breeding doesn't substantially make crops more resistant to climate change is due to complicated inheritance and a high level of genotype × environment interaction, even if it is still crucial for agricultural progress (Bhat et al. 2016). Since genomics allows for direct analysis of the genotype and its link with the phenotype, a method that integrates genetic engineering with omics technologies has been suggested as a practical approach (Tester and Langridge 2010). The marketing and progress of genetically modified (GM) crops have been impeded by public concerns about their safety and efficacy (Prado et al. 2014; Raman 2017). One promising strategy for reducing the impact of environmental stresses is the use of RNA interference (RNAi) and genome editing technologies, such as CRISPR/Cas9. New opportunities for the creation of climate-resilient crops have arisen as a result of advances in RNAi and the CRISPR/Cas system, as well as a deeper comprehension of the molecular pathways underlying the responses of crops to abiotic stress. The current chapter delves into the inner workings of RNAi and CRISPR, offering a synopsis of the vast majority of potential applications of this genome editing approach in agricultural plants to manage abiotic challenges including drought, salt, heat, and cold. It will also summarise the potential and limitations of using RNAi and CRISPR/Cas-based systems to create crop types that can withstand stress.

Discovery of RNAi

RNA interference was unintentionally found in *Petunia*. In 1990, Jorgensen et al. introduced an exogenous transgene into *Petunia* to enhance the activity of a chalcone synthase gene that was driven by 35S promoters. The goal was to increase the production of pigments in light pink or violet flowers.

The expectation was that both the transgenic and native gene plants would express the enzyme, leading to deeper-coloured flowers (Ali, et al. 2010). Rather than a rich purple, the flower coloration showed surprising variegations, with some areas losing all colour. This indicates that the inserted transgene changed the expression of endogenous loci and was also inactive. The word used to describe this occurrence was co-suppression. Further research by Guo and Kemphus (1995) examined the function of the *par1* gene in the worm *C. elegans* and found that its expression was suppressed upon introduction of either sense or antisense RNA. Additionally, it was shown by (Andrew Fire and Craig Mello 1998) that a combination of sense and antisense strands of double-stranded RNA is more efficient in silencing the target gene than either strand alone (Tabara et al. 1998). For RNA interference research, this was a watershed moment (Ali et al. 2010).

Components of RNAi

RNA interference system relies on Dicer, one of its key components. Dicer, a complex ribonuclease enzyme belonging to the RNase III family, was first discovered in *Drosophila* by (Bernstein et al. 2001). It has four unique domains, each with a highly specialized function: the N-terminal helicase, dual RNase III motifs, C-terminal double-stranded RNA binding domain, and PAZ (Piwi/Argonaute/Zwille) domain (Williams et al. 2004; Agrawal et al. 2003). According to Williams et al. (2004), the specific 5' phosphate and 3' hydroxyl residues of siRNA are generated when the double-stranded RNA is broken by the use of dual RNase III motifs. At a constant spacing of 21 to 25 base pairs, Dicer cleaves double-stranded RNA. During the first step of the RNAi pathway, the dicer plays a catalytic role by helping to initiate the synthesis of the RNA-induced silencing complex (RISC). According to Kumar et al. (2012), the catalytic component of Dicer, known as agronaute, may break down messenger RNA that is complementary to the small interfering RNA guide strand. A crucial part of the RNA interference system, RISC targets and degrades cellular messenger RNA (mRNA) using small interfering RNAs (siRNAs). According to Williams et al. (2004), RISC consists of both RNA and protein. The target RNA is cleaved when the RNAase enzyme is activated by RISC after it finds mRNA that is complementary to siRNA. With a binding affinity for RISC, small interfering RNAs (siRNAs) ranging in length from 20 to 23 base pairs may be guided to their target messenger RNAs (mRNAs), where they can be combined and degraded due to a decrease in protein translation and a subsequent reduction of gene activity (Kumar et al. 2012). According to

Schwartz et al. (2004), RISC acts as a catalyst that disrupts a single phosphodiester link in the target messenger RNA.

RNAi Mechanism

Small interfering RNA (siRNA) and microRNA (miRNA) are two types of small RNAs that are part of the RNA interference pathway. There are a lot of similarities between miRNA and siRNA. Both are 20-30 bp in size, originate from double-stranded structures, and undergo processing by an enzyme similar to DICER (DCL1, DCL2). Both serve as target sequences for RISC, which uses them to control gene silencing after transcription. They are distinct from one another according to their origins. miRNA originates from genomic DNA, while siRNA is generated by slicing dsRNA into smaller parts. The two stages of active miRNA are pre-miRNA and primary miRNA, or pri-RNA. A hairpin structure is shared by both. The cytoplasm and the nucleus are both involved in the processing of miRNA (Williams et al. 2004). A transcript with a miRNA gene will have two arms of about the same length and be 42 to 60 base pairs long, resembling a hairpin shape. To generate functional miRNA, one of these strands employs DICER.

The RNA interference process consists of four primary steps: step one: dsRNA cleaves by dicer; step two: siRNA enters the RISC complex; step three: activation of the silencing complex; and step four: degradation of messenger RNA (Ali et al. 2010). The first step of RNA interference is for the DICER enzyme to identify the target gene-homologous dsRNA that has been introduced into the cell (Fig 1). The dicer enzyme uses ATP to convert the dsRNA into the 21–25 nucleotide double-stranded siRNA. The RNA-induced silencing complex is formed when the dicer's siRNA is integrated with the multicomponent nucleus complex; however, this complex is currently unable to perform RNAi (Nykänen et al. 2001). The unwinding of the DNA strand was formerly attributed to the ATP-dependent helicase; however, this ATP-independent activity is carried out by the protein component of the RISC. The agronaute family protein and single-stranded siRNA are the two main components of the RISC ribonucleoprotein complex (Kumar et al. 2012). The next step is the destruction of messenger RNA. Argonaute protein, an endonuclease, is the key component of RISCs. As a result, RISCs gain "silencer" activity because they cleave the complementary mRNA strand to the associated siRNA. Once the dsRNA is diced, the agronaute protein attaches to the guide strand of the short siRNA and guides gene silencing. Fig. 1 shows that after mRNA identification and cleavage are over, the RISC departs, and the siRNA may be used again in another cycle. Interestingly, RNAi seems to have catalytic properties. The amount of amplification caused by dicer's cleavage of double-stranded RNA into tiny siRNA is not enough to induce continuous degradation of messenger RNA, even if this is the case (Lipardi et al., 2001; Sijen et al., 2001). In particular, it provides biochemical and genetic evidence that strongly suggests RNA-dependent RNA polymerase is critical for amplifying the RNAi impact. The RNA-dependent RNA polymerase enzyme RdRp uses siRNAs as primers to synthesize new double-stranded RNA (dsRNA), which may then be cleaved into more siRNAs (Ahlquist 2002). One promising new approach is RNA interference (RNAi) technology, which uses short double-stranded RNA to inhibit gene expression by inducing the homologous sequence of the target messenger RNA in the cell nucleus.

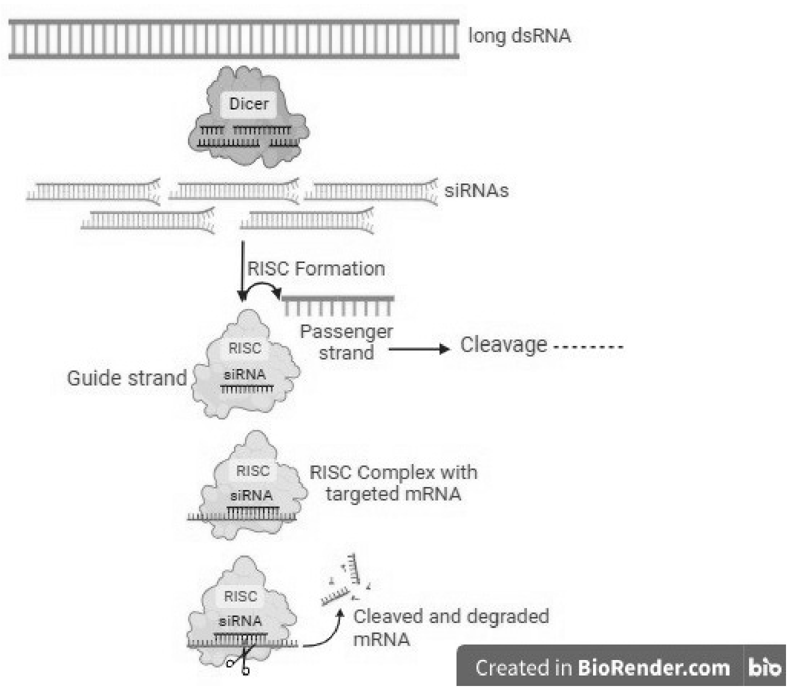

Fig 1: Diagrammatic representation of the RNAi pathway

Mechanism of CRISPR/Cas9-based genome editing

The CRISPR/Cas system is based on the adaptive immune system that is present in bacterial and archaeal genomes and is used to protect against the invasion of foreign DNA or plasmids (Marraffini and Sontheimer, 2010). According to Cong et al. (2013), the CRISPR/Cas9 system consists of two parts: CRISPR-associated protein 9 (Cas9) and a single guide RNA (sgRNA). The synthetic combination of the transactivating crRNA and the protospacer-matching crRNA, both of which are necessary for CRISPR action, results in the sgRNA. The twenty nucleotides that make up the 5' end of a small guide RNA (sgRNA) bind to the specific genomic region that has to be targeted by the Cas9 complex. For *Streptococcus pyogenes* SpCas9, this specific target site must be located just upstream of the protospacer adjacent motif (PAM; NGG). A small, conserved DNA region that lies downstream of the cleavage site; its size varies between bacterial species, but typically ranges from two to five base pairs. The SpCas9 multi-domain DNA endonuclease is a large (1368 amino acid) enzyme that cleaves DNA in the genome, leading to blunt-ended double-strand breaks (DSBs). According to Mei et al. (2016), the DSB is eventually repaired by the host cellular machinery. The Cas-9 protein causes double-strand breaks (DSBs), which may be repaired in two ways: homology-directed repair (HDR), which employs a homologous DNA template and is very exact, and nonhomologous end joining (NHEJ) processes (Liu et al. 2018). Since HDR is most active in the late S and G2 cell cycle stages, it necessitates a large number of donor DNA templates containing a target DNA sequence. To insert or replace the desired gene, a donor DNA template with a similar sequence is inserted into the expected DSB location (Liu et al. 2018; Yang et al. 2020). Speeding up DSB repairs, non-homologous end-joining is an enzyme mechanism that links DNA fragments without the requirement for exogenous homologous DNA. The mechanism of CRISPR/Cs9 is shown schematically in Fig. 2.

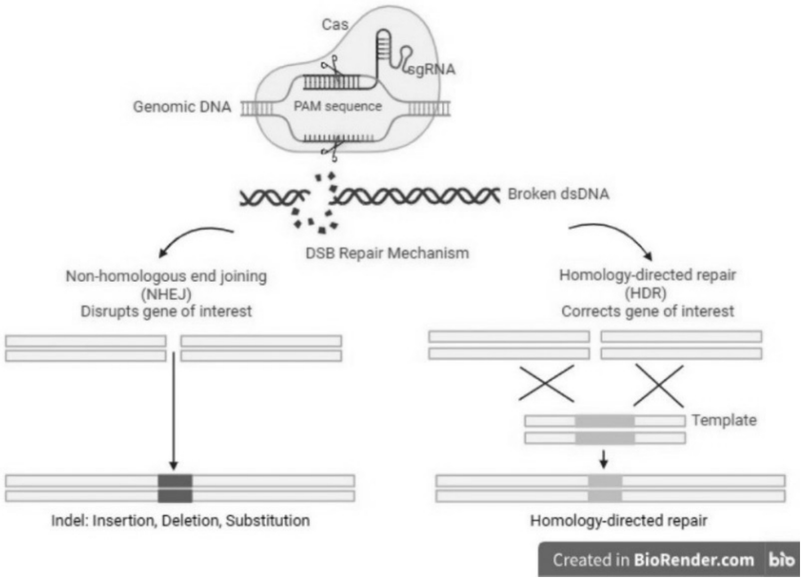

Fig 2: Diagrammatic representation of CRISPR/Cas9 mechanism

The most effective method of gene editing is usually homology-directed repair, which is carried out via homologous recombination (HR). For flawless editing, it may use a donor template-provided sequence. For biological reasons, such as knock-in or precise mutagenesis, it is one of the most used genome editing technologies. The low frequency of native HR, however, remains the main impediment to performing successful genome editing in plants. To enhance plant stress response, HDR-mediated Cas9 introduces resistance (R) genes. The more precise HDR repair process requires homologous donor DNA compared to the less precise NHEJ DNA repair method. Although homologous recombination (HDR) allows for the introduction of targeted point mutations or desirable sequences, NHEJ creates indels (Jain 2015).

RNAi strategy for abiotic stress tolerance

Abiotic stress tolerance is a defence mechanism that plants have evolved to deal with sudden changes in their environment, which might reduce seed output. Research on RNA interference (RNAi) has demonstrated promise as a tool for crop breeding in response to abiotic stress via post-transcriptional regulation in several different crops and abiotic stress conditions (Vanderauwera et al. 2007; Jagtap et al. 2011; Younis et al. 2014; Abhary and Rezk 2015; Khare et al. 2018; Dalakouras et al. 2020). The use of RNA interference has been successful in engineering crops to be more resistant to abiotic stress (Jagtap et al. 2011). The role of microRNAs in plants' ability to withstand abiotic stress is well-established. Some examples of microRNAs that crops use to tolerate abiotic stress are shown in Table 1.

Drought tolerance

One of the most detrimental abiotic stimuli that plants face is drought, which stunts their development and growth. Canola (*Brassica napus* L.) plants that have an RNA interference construct driven by the AtHPR1 promoter reduce levels of farnesyltransferase, which in turn protects yield against drought stress, as reported in a 2009 study by Wang et al. To increase the transgenic rice line's drought tolerance compared to a non-transgenic line, (Li et al., 2009) used RNA interference (RNAi) to suppress the receptor for the activated C kinase 1 (RACK1) gene. Drought stress-induced microRNA expression profiling in many plant species, including *Arabidopsis*, *Populus trichocarpa*, and *Oryza sativa*, has uncovered drought-responsive miRNAs such as miR169, miR396, miR171, miR319, miR393, miR156, and miR158 (Liu et al. 2008; Younis et al. 2014). According to Jian et al. (2010) and Zhou et al. (2010), the miR169g and miR393 genes were shown to be active in rice when subjected to drought stress. At various stages of development, from tiller formation to inflorescence, drought-studied rice underwent miRNA analysis and genome sequencing profiling using a microarray technology. Drought stress allegedly led to the downregulation of sixteen microRNAs (miR1126, miR1050, miR1035, miR1030, miR896, miR529, miR408, miR156, miR171, miR170, miR168, miR159, miR397, miR396, miR319, miR172, and miRNA1088) (Liu et al. 2008). Wheat has also shown drought-responsive microRNAs (Singroha et al., 2021). Because of the mutation, tae-miR9657b-5p is no longer able to attach or be targeted. This resulted in a rise in tae-miR9657b-5p expression levels during the drought, which supports the idea that miRNA responses to drought stress may be mediated by post-transcriptional RNA modification (Pan et al. 2022). The first publication on the durum wheat degradation genome also revealed the discovery of novel regulatory pairs between miRNAs and their targets. Durum wheat showed an upregulation of NDH and PGR5 expression in response to a decrease in the abundance of ata-miR396c-5p and osa-miR160f-5p. Activation of NDH and PGR5 was significantly upregulated in response to drought stress. These two miRNA-target modules improve drought tolerance by shielding the photosynthetic apparatus from ROS accumulation and lowering their levels. Redox homeostasis may also be supported by the ata-miR528-5p-target module (Liu et al. 2020). These modules could be the best bet for making durum wheat more drought-resistant. During drought stress and maize dwarf mosaic virus (MDMV) infection, another research examined the transcript activity of four ZmRDR genes, five ZmDCL genes, and seventeen ZmAGO genes in maize. Both biotic and abiotic stressors caused these genes to express themselves differently (Balassa et al. 2022).

Salt tolerance

Another abiotic factor that significantly affects plant health is salinity. A disturbance in ion homeostasis and an imbalance in water potential are the results of excessive salinity stress, which affects the plant as a whole. This molecular and cellular shift causes several morphological changes in plants, including leaf yellowing and withering, growth arrest, wilting, and plant death. As a result of the changes in physiology brought on by salt stress, ABA is synthesised and then transferred to the guard cells, where it closes the stomata. According to Mangrauthia et al. (2013), this leads to oxidative damage and a decrease in photosynthesis. According to Shriram et al. (2016), many plant species display different small RNA behaviours when exposed to salt stress. This leads to changes in the expression levels of miRNAs and related genes. Under salt stress, the pattern of miRNA expression changes with the length of time the stress is present. Several miRNA expression patterns were identified in a specific study of barley that was exposed to salt stress at different phases of its developmental maturation. According to Deng et al. (2015), miR444 showed a decrease in expression after 8 and 27 hours of stress, but an increase in expression towards the end of the stress period. The miR393 gene was up-regulated in *Arabidopsis* in response to abiotic stresses including increased salt, dehydration, cold, and abscisic acid (ABA). In addition, according to Jagtap et al. (2011), the expression of miR402, miR319c, miR397b, and miR389a was influenced by the degree of abiotic stress in *Arabidopsis*. A microarray study was carried out to comprehend the dissimilarities between the miRNA profiles of a salt-tolerant and a salt-sensitive maize line. Under salt stress, maize roots showed down-regulation of miR396, miR156, miR167, and miR164 family members and up-regulation of miR474, miR162, miR395, and miR168 family members (Ding et al. 2009). When exposed to 150 mM sodium chloride (NaCl) for 7 days, overexpression of miR397 from *Arabidopsis* (AtMIR397) or *Setaria viridis* (SvMIR397) suppressed the expression of the LACCASE (LAC) gene, namely LAC2, LAC4, and

LAC17. This resulted in *Arabidopsis* having a lower lignin concentration and a higher resistance to salt (Nguyen et al. 2020). In addition, miR393 regulates salt stress via the ABA signalling pathway, which is mediated by the scaffolding protein RACK1A, when *Arabidopsis* seedlings are exposed to 100 mM NaCl salt externally. It is believed that RACK1 acts as a negative regulator of ABA due to the fact that miR393 targets TIR1/AFB2. According to Denver and Ullah (2019), MiR393 may have a role in salt acclimation by regulating the antagonistic auxin response and the ABA-mediated salt stress. Some plants, including alfalfa, cotton, apples, switchgrass, Jerusalem artichoke, tamarisk trees, and cereal crops, defend themselves against salt damage by regulating miRNAs (Arshad et al. 2020; Wang et al. 2019; Ma et al. 2021; Liu et al. 2019; Wen et al. 2020; Ye et al. 2020; Cheng et al. 2021).

Heat tolerance

Heat stress is now a major component influencing plant development and growth as a result of climate change and global warming. The stability of agriculture is threatened by high temperatures. In order to mitigate the detrimental impacts of heat stress, which may impact processes such as photosynthesis, respiration, nitrogen, nucleic acid, and lipid metabolism, as well as protein production, researchers have been looking at heat-sensitive miRNAs (Grover et al., 2013; Zuo et al., 2021; Begum 2022). As a result of heat stress, 102 microRNAs were identified in maize (Zhang et al. 2019), with 41 of them being new miRNAs and 61 being established miRNAs. They also used cluster analysis to look at 30 miRNA-mRNA couples that showed notable inverted expression patterns. Under heat stress, these interaction pairs may serve as crucial modules for miRNA-mRNA regulation in maize. The SPL gene family is regulated by the miR156-SPL module in bananas, and another research discovered that miR535 and miR156 target this family. A pattern of downregulation in multiple miRNAs was seen in bananas that were subjected to heat stress. Ultimately, heat stress has the potential to influence the expression of microRNAs (Zhu et al. 2019). Wheat has been shown to have 32 distinct miRNA families. Thermosensitivity was found in nine different miRNA families. According to Xin et al. (2010), when heat stress is present, some microRNAs, such miR166 and miR393, are up-regulated, whereas miR172 is down-regulated. Li et al. (2015), Liu et al. (2017), Mangrauthia et al. (2017), and Hivrale et al. (2016) found heat-sensitive miRNAs in rice, among other crops. Kumar et al. (2015) discovered that six wheat miRNAs are heat sensitive.