

Understanding Heat and Drought Stress Adaptation Mechanisms in Wheat: A Combined Approach

Anjali Jha, Rinki Khobra*, Harohalli Masthigowda Mamrutha, Preeti Dohrey, Zeenat Wadhwa, Kapil Deswal, Gyanendra Singh and Gyanendra Pratap Singh

ICAR-Indian institute of Wheat and Barley Research, Karnal-132001

Article history:

Received: 17 Apr., 2022

Revised: 27 July, 2022

Accepted: 10 Aug., 2022

Citation:

Jha A, R Khobra, HM Mamrutha, P Dohrey, Z Wadhwa, K Deswal, G Singh, GP Singh. 2022. Understanding Heat and Drought Stress Adaptation Mechanisms in Wheat: A Combined Approach. *Journal of Cereal Research 14(Spl-1): 42-52*. <http://doi.org/10.25174/2582-2675/2022/125723>

*Corresponding author:

E-mail: Rinki@icar.gov.in

© Society for Advancement of Wheat and Barley Research

Abstract

Drought and heat are the key environmental stressors significantly decreasing wheat productivity (86 and 69%, respectively) and weakening food security in the major wheat growing regions worldwide. Wheat crops have regularly experienced combined (Heat+Drought) stress in the field and the joint effects are more detrimental to wheat growth than the effects of each stress separately. Drought and heat stress have shown synergistic, antagonistic, or hypo-additive impacts on growth, grain filling, and yield parameters when combined. In order to escape and/or tolerate these unfavorable environmental conditions, wheat has developed advanced responses at various levels. This review explores how physiological, morphological, biochemical and molecular traits work together to provide tolerance to coupled stresses. Importance of specific traits such as, canopy temperature, assimilate partitioning and water use efficiency along with reproductive traits which provide tolerance against these combined stressors has also been explained. This review also highlighted the potential of altered agronomic practices, application of micronutrients, biopriming of seeds (endophytes) against abiotic stresses. Further emphasize has been given to promising novel technologies like, genome editing (CRISPR), identification of novel QTL's and alleles to improve both heat and drought tolerance for sustainable wheat production.

Keywords: Wheat, drought, heat, stress tolerance

1. Introduction

Wheat being the most commonly cultivated cereal crops comes under the Poaceae family. It is having the world grain production of 30% and the world grain trade of 50% (Akter & Rafiqul Islam, 2017) resulting in catastrophic loss of wheat productivity. For each degree rise in temperature, wheat production is estimated to reduce by 6%. A detailed overview of morpho-physiological responses of wheat to heat stress may help formulating appropriate strategies for heat-stressed wheat yield improvement. Additionally, searching for possible management strategies may increase productivity and sustainability of growing wheat. The major findings from this review are as follows: (1. Presently

in India, the production of wheat reaches to 109.52 million tonnes and an area upto 30.55 million hectare including an average productivity of 3464 kg/ha (Devi *et al.*, 2022). In terms of area of cultivation, it is ranked first in rain-fed areas and second after rice in irrigated areas (Tricker *et al.*, 2018). In most of the regions of the world, wheat is said to be a major source of food and animal feed (Sallam *et al.*, 2019). It can be cultivated in a variety of agro-climatic conditions and wheat yields are highly susceptible to environmental and climatic changes (Mahrookashani *et al.*, 2017). Wheat had a origin from in South-Western Asia, but it has widely adaptability which leads to its growth



under different agro-climatic zone such as tropical zone, subtropical and temperate zone (Tyagi & Pandey, 2022).

It is evident from the studies that change in climate significantly affecting the yield defining traits and finally increasing the yield barriers hence, climate change is the key factor behind the reshaping of world agriculture (Dubey *et al.*, 2020). Among the multiple drivers of climate change, drought and heat, are the two most serious factors for sustainable agriculture across the world, leading to a yield losses of up to 86% and 69%, respectively (Tricker *et al.*, 2018). Semi-arid and hot growing regions in South Africa, Mexico, Argentina, Australia, Africa and Mediterranean countries as well as in high latitude, semi-arid growing areas of central and eastern Asia, Kazakhstan, USA and Canada are more prone to these stressors (Tricker *et al.*, 2018). Severe drought and heat waves has been spreads worldwide, which includes India, United States, Russia, Western Europe and Australia which are the major wheat producing areas. In Mediterranean climate zones such as Australia, the northwest of the US and southern Europe, wheat production is highly based on dry lands, which is characterized by combination of both high temperature with drought conditions. (Schmidt, Tricker, *et al.*, 2020). Wheat yields fall by 4.1% - 6.4% with rise in temperature for every 1°C due to climate change (B. Liu *et al.*, 2016).

Wheat consumption, on the other hand, has increased by over 30% in the next 40 years (Albergel *et al.*, 2019). As the world's population continues to rise year after year, and arable land continues to shrink, there is a higher requirement for wheat production (Li *et al.*, 2016). This happens because of the increased severity and soil destruction by abiotic environmental conditions. According to FAO, world would need additional wheat of 198 million tonnes by 2050, in order to fulfill the future demands. In order to achieve this, the production of wheat needs to be increased by 77% in the developing countries in upcoming years (Akter & Rafiqul Islam, 2017). Substantial research has been made to produce varieties with high yields but the impact of heat and drought is diminishing the overall crop yield. So it is a pre-requisite to understand the factors triggering plant processes due to elevated temperature and reduced water availability and the plant defense mechanisms to mitigate them simultaneously. The current time is indispensable to understand the wheat

phenology specifically during reproductive stage for mitigating the combined impacts of these stresses. Further to accelerate the research on abiotic stress tolerance basic understanding of the background traits/ mechanisms existing within the genotypes is very much required. All the crop improvement programs are highly dependent on sensible screening of already available stress tolerant high yield wheat genotypes. Hence, identification and utilization of abiotic stress (heat+drought) tolerant wheat varieties, genetic stocks and other utilizable breeding along with the trait associated for tolerance can expedite the wheat improvement programmes. This review highlights the impact of combined heat and drought stress on biochemical, morpho-physiological and molecular aspects of wheat varieties in an integrative manner. The critical plant traits and futuristic approaches are also elaborated so that the researchers focus either to develop new wheat varieties with innate tolerance or induce the tolerance with specific genome editing tools for these two crucial abiotic stresses.

2. Impact of Heat and Drought on Wheat growth and development

High temperatures and lack of water availability shows a significant impact on growth and yield output of crop (Beccari *et al.*, 2020). Based on IPCC analysis, high temperature and decreased water availability can worsen the conditions of crops (Sattar *et al.*, 2020). Cumulative effect of both heat and drought on the growth of wheat is predominant and more severe as compared to the effect of each stress individually. Both of them have a significant impact during the phase of grain filling of wheat development (Tahmasebi *et al.*, 2016). If both of these stressors occur simultaneously, they can have an irreversible effect on plant growth and development (Table 1) (González-Mendoza *et al.*, 2021). Over the period of time, plants have developed certain comprehensive and complex mechanisms in order to resist these adverse stress conditions which includes physiological, biochemical and molecular level of regulatory network (G. Zhang *et al.*, 2017).

Major mechanisms of regulation includes adaptation to water stress which induces reduction in water loss, enhancement of water uptake and the osmolyte production for sustaining cellular activities in drought conditions (G. Zhang *et al.*, 2017). When there is low



soil and atmosphere humidity and high ambient air temperature, it causes drought stress to the crops. This disturbs the balance between the water intake and evapotranspiration flux within the soil (Lamaoui *et al.*, 2018). High temperature beyond to optimum temperature, results in heat stress. However, it is difficult to observe most of the visible symptoms easily observed in the early phases of the plant growth under heat and drought conditions (González-Mendoza *et al.*, 2021). For monitoring and promoting crop health, it will be highly useful to detect the cumulative impacts of combined heat and drought stress on the transitional phases in growth of the plant (Wang *et al.*, 2021). These effects could be studied on the basis of the aspects described below.

2.1 Physiological changes under combined stress of heat and drought

It was suggested that the cumulative effects of both (heat and drought) stresses has either synergistic, antagonistic or hypo-additive effects on growth, filling of grain and yield factors (Mahrookashani *et al.*, 2017). Reduction in the yield has been observed under combined stress due to the direct effects of interaction of both of these stresses on stomatal movements. Plants respond with reduced photosynthetic rates, reduced stomatal aperture movements and increased the cellular content of damaging reactive oxygen species (ROS) specifically under these combined stresses (Sattar *et al.*, 2020). Plant closes its stomata under combined treatment of heat and drought, which causes osmotic imbalance (Qaseem *et al.*, 2018). Decreased content of leaf chlorophyll was observed by 49% under both drought and heat stress together, while it gets reduced by 27% and 9% on heat and drought stress respectively (Tricker *et al.*, 2018). There is a reduction by 56.47%, 53.05% and 44.66% in the grain yield under combined heat and drought stress with individual stress respectively. Combined treatment shows great impact on yield by reducing the synthesis and mobilization of reserves in developing leaves and grains (Qaseem *et al.*, 2019). However, disintegration of membrane structure with deformation of chlorophyll and protein molecules has been observed under heat stress than drought stress, whereas sink strength and water status of genotypes were more affected under drought than heat stress (Qaseem *et al.*, 2019). This shows reduced activity of water use efficiency and various photosynthetic enzymes, which directly stop the photosynthesis process

in wheat under combined effects of these stresses. It changes the primary metabolism of the crops (Li *et al.*, 2016) and therefore disturbs the balance of carbon and nitrogen in plants. There will be a penalty in the yield of the wheat productivity, when there is a prolonged drought conditions coinciding heat waves above 32°C (Ogbonnaya *et al.*, 2017).

High temperature supports plant growth and retards developmental stages, while drought stress slows down the growth of the plant by reducing the tillering and leaf expansion. However, under combined (drought and heat) stress, plant flower earlier and produces less biomass as compared to single stress. Each and every aspect of the wheat growth is affected by the high temperature and water deficient condition ranges from germination to maturity. Its yield highly depends on the stages of the plant development when there is a stress and the duration and the severity of the stress (Tricker *et al.*, 2018). Both stresses highly affect the transport of solutes and water together. There is a huge variation seen in the relative water contents (RWC) in different treatments. Both the stresses together decreased RWC by 42% as compared to control. But, individual (drought or heat) stress showed reduction of 23 and 25% in RWC than control.

The mechanism of osmotic regulation of plants under abiotic stress is associated with the soluble sugar accumulation. Increased soluble sugar content under stress provides resistance of plants to stress (Wang *et al.*, 2021). When compared to control, combined stressors result in a 67 percent increase in water potential. In terms of turgor potential (TP), the highest value (0.52 MPa) was found in control, while the lowest (0.23 MPa) in the heat stress treatment. Drought stress reduces turgor pressure by 34%, and both stressors together reduce TP by 36.5% as compared to control. In comparison to control, straw yields were reduced by 15-25% under heat stress, 21-56% under drought stress, and 26-60% in these combined treatments. Furthermore, maximum reduction in grain number per stem was reported under combined stress (91-98%) as compared to heat (14-28%) and drought (39-61%) separately (Mahrookashani *et al.*, 2017). Harvest index of any crop defines the ratio of grain to total shoot dry matter. A little difference has been noted among control and heat treatment, but were reduced by 2-30% for drought and 73-95% for the combined heat and drought (Mahrookashani



et al., 2017). These stresses can drive the chlorophyll (Chl) degradation and decrease its content (Wang *et al.*, 2021). There is a reduction seen in Chl a of 55.5, 51.6, and 55% under heat, drought and combined stresses, respectively than control. Amount of cChl b showed highest reduction of 67% under combined stresses however, Chl a/b ratio was increased with 27% in combined stresses than in control (Sattar *et al.*, 2020).

Compared to the vegetative parts reproductive tissues are more perceptive to combined heat and drought stress. Ovule function, grain weight and pistillate flower development gets inhibited by drought stress whereas grain number and pollen fertility of spring wheat gets affected by heat stress. Majorly, the combined effects of (heat and drought) stresses are more deleterious than the effects of single stress on reproductive tissues (Zandalinas *et al.*, 2017). Increased abortion of ovules results in low number of grains per spike in stress. Moreover, reduced size and weight of individual grains has been seen during filling of grain stage under combined stress. This happens by reducing the rate of division of endosperm cells and shortening the grain filling duration. Synergistic effects has been observed for the combination of both the stresses with respect to grain number per culm, grain weight, fertile spikelet number, yield and harvest index, whereas antagonistic or hypo-additive effects for straw yield (Mahrookashani *et al.*, 2017). Both stresses affect the growth of the wheat from their germination to maturity phase. Numerous traits are identified and measured in genetic mapping populations, which provides benefit for both the stresses (Tricker *et al.*, 2018).

2.2 Biochemical changes under combined stress of heat and drought

Various defensive molecules have been noted down, when the plant undergoes both heat or drought stress. Plants accumulate osmolytes, pigments & antioxidant enzymes in response to heat and drought stress. These protective molecules include MDA, soluble sugars, catalase (CAT), superoxide dismutase (SOD) and peroxidase (POX) (Wang *et al.*, 2021). These antioxidant enzymes accelerate internal defense system and plays an important role for ROS scavenging in plants during heat and drought stress conditions (Sattar *et al.*, 2020). Both of these stresses induces high amount of catalase activity in wheat as compared to control plants. The highest catalase activity

of 61% was recorded under combined stresses. Similarly, for SOD activity was observed in wheat under stress. Its activity increased significantly when exposed with stresses and the highest SOD activity of 54% has been observed under combined stress. Maximum increase of 68% of ascorbate peroxidase (APX) was seen under drought, while heat and combined stresses results in the increment of 39 and 55% in APX activity in comparison with control.

There has been a significant correlation of chlorophyll (Chl) content before and after anthesis, proline content (PC), water-soluble carbohydrates (WSC) and other parameters, which strengthens the grain yield, whereas days to anthesis (DTA) and days to maturity (DTM) showed negative impact (Qaseem *et al.*, 2019) on the grain yield under both stress. Current study reveals that combined treatment added hypo-additive effect over yield, which means that the effect of combined treatment was higher than its individual effects (Qaseem *et al.*, 2018). Both heat and drought stress elevated the ABA and jasmonic acid (JA) concentration significantly in wheat, when compared to the control.

Several modifications were done by the plants in response to combined (heat and drought) stress. Assimilation of carbohydrates and antioxidant defense mechanism is seen in flag leaf of the wheat against these stresses. Flag leaf majorly contributes to the grain yield. Thereby, in wheat it is highly focused to study the role and response of flag leaf in various biochemical processes i.e., photosynthetic pigments and osmolytes accumulation, water relations, antioxidants defense mechanism etc (Sattar *et al.*, 2020). Drought reduces photosynthetic rate through the regulation of stomatal (Choudhury *et al.*, 2017) and mesophyll conductance without affecting potential photosynthetic capacity, resulting in down regulation of photochemical processes and reduced carboxylation efficiency of ribulose-1,5-biphosphate carboxylase/oxygenase (Perdomo *et al.*, 2017).

Heat and drought stress collectively accumulates amino acids in the wheat. Heat stress leads to high glutamate, proline, aspartic acid, tryptophan, asparagine with low glycine amount in wheat (Xie *et al.*, 2021). However, drought stress causes high phenylalanine, proline, tyrosine, aspartic acid, glutamine, tryptophan while comparing with the well-watered conditions. The effects of individual stress on wheat were studied independently, but the combination



of two (heat and drought) stresses showed unique response and that cannot be generalized (Mahrookashani *et al.*, 2017). Few studies showed that both heat and drought stress shows significant positive interaction on valine, phenylalanine, aspartic acid and tyrosine, and negative interaction on glycine, alanine, arginine and threonine (Xie *et al.*, 2021).

The soluble sugar content of plants gets significantly affected by heat and drought stress. Various soluble proteins are also affected by these stresses. Usually minimum value of 1.46 mg g⁻¹ amount of soluble sugar found in the plant treated as control (Sattar *et al.*, 2020). But an increase amount of 4.3, 3.6, and 4.5 mg g⁻¹ was estimated under heat, drought, and combined stresses, respectively. Combined (heat and drought) stress also leads to reduction in thousand kernel weight (TKW) and glucan amount as compared to control. Under combined stress of heat and drought, there is a high contents of total arabinoxylan (TOT-AX) and proteins, whereas higher amount of water extractable arabinoxylan were observed in heat stress alone as compared to heat or drought and drought stress together (Rakszegi *et al.*, 2014).

Accumulated sugars, amino acids, and other substances coordinate with the activated molecular and physiological responses in plants, when grown under stress. Accumulation cause speedy recovery of plants after stress which mitigates the damage caused by the stress (Xie *et al.*, 2021). High concentration of proline are reported to be transported in cell organelles (chloroplasts, cytosol, and mitochondria) to hasten other metabolic plant reactions to enhance the abiotic stress tolerance. These accumulated amino acids also drive the metabolic process toward secondary metabolite productions and JA-dependent defenses in wheat. Proline contents get elevated with a highest increase of 69.8% under combined stresses as compared with heat (58.9%) and drought (53%) stress individually (Sattar *et al.*, 2020).

2.3 Molecular changes under combined stress of heat and drought

All of these above mentioned physiological and biochemical indices were used to assess the drought or

high temperature tolerance of wheat. Simultaneously, these alterations also modulate the expression of various genes involved in stress tolerance (Table 2). It can yield insight into the molecular mechanism associated with stress responses (Wang *et al.*, 2021). Several transcription factors and heat and drought-inducible genes perform function at the molecular level in the establishment of the stress tolerance network in wheat (G. Zhang *et al.*, 2017). Identification of key regulatory genes which involved in heat and drought tolerance network, not only assist in improved understanding of mechanism underlying the heat and drought adaptation, but also set forth a direction for improvement of abiotic stress tolerance of crops by changing genetic makeup (Li *et al.*, 2016).

Phosphoinositide-specific phospholipase C proteins (PI-PLCs) showed involvement in different stages of plant growth and stress responses. These proteins hydrolyzed phosphatidylinositol 4,5-disphosphate (PIP2) to synthesize diacylglycerol (DAG) and inositol triphosphate (IP3) (González-Mendoza *et al.*, 2021), which induce plant growth and stress signals by targeting specific molecules (Takáč *et al.*, 2019) which is required to generate rapid responses of plants to environmental cues. Moreover, they produce second messenger molecules, such as phosphatidic acid (PA). Moreover, IP3 releases stored Ca²⁺ by binding to the receptors on the ER membrane. Calcium regulated the abiotic stress responses including drought, salt or heat stress responses (Wang *et al.*, 2021).

The transcription factors (TFs) by interacting with cis-elements in the promoter region of downstream stress-related genes behaves as a regulators of stress responses. These TFs such as AtWRKY30, TaHSFA6e, AtHDG11, DREB1A and NAC, were found to play a crucial role in the regulation of stress-related genes expression (Haider *et al.*, 2021) the frequent occurrence of heat waves is drastically reducing the global crop yield. Molecular plant scientists can help crop breeders by providing genetic markers associated with stress resistance. Plant heat stress response (HSR). These genes have been usually overexpressed in heterologous and homologous systems in order to establish stress tolerance capacity to the plants (Li *et al.*, 2016).



Table 1: Summary of Wheat traits affected under combined heat and drought stress

Plant Traits	Wheat Species	Reference
Morphological Traits		
Plant height	<i>Triticumaestivum</i>	(Labuschagne <i>et al.</i> , 2021)
Shoot length	<i>Triticumaestivum</i>	(Labuschagne <i>et al.</i> , 2021)
Peduncle length	<i>Triticumaestivum</i>	(Labuschagne <i>et al.</i> , 2021)
Flag leaf width	<i>Triticumaestivum</i>	(Sattar <i>et al.</i> , 2020)
Phenological Traits		
Days to heading	<i>Triticumaestivum</i>	(Tahmasebi <i>et al.</i> , 2016)
Days to maturity	<i>Triticumaestivum</i>	(Qaseem <i>et al.</i> , 2019)
Days to anthesis	<i>Triticumaestivum</i>	(Qaseem <i>et al.</i> , 2019)
Days from heading to maturity	<i>Triticumaestivum</i>	(Tahmasebi <i>et al.</i> , 2016)
Early ground cover	<i>Triticumaestivum</i>	(Schmidt, Claussen, <i>et al.</i> , 2020)
Reproductive Traits		
Stigma Functionality	<i>Triticumaestivum</i>	(Fábián <i>et al.</i> , 2019)
Stigma Fertility	<i>Triticumaestivum</i>	(Fábián <i>et al.</i> , 2019)
Pollen viability	<i>Triticumaestivum</i>	(Zafra <i>et al.</i> , 2016)
Pistillate flower development	<i>Triticumaestivum</i>	(Zandalinas <i>et al.</i> , 2017)
Pollen fertility	<i>Triticumaestivum</i>	(Zandalinas <i>et al.</i> , 2017)
Stomatal traits		
Stomatal Closure	<i>Triticumaestivum</i>	(Xie <i>et al.</i> , 2021)
Guard cell area	<i>Triticumaestivum</i>	(Shahinnia <i>et al.</i> , 2016)
Stomatal density	<i>Triticumaestivum</i>	(Shahinnia <i>et al.</i> , 2016)
Stomatal index	<i>Triticumaestivum</i>	(Shahinnia <i>et al.</i> , 2016)
Guard cell length	<i>Triticumaestivum</i>	(Grieco <i>et al.</i> , 2020)
Stomatal aperture area	<i>Triticumaestivum</i>	(Shahinnia <i>et al.</i> , 2016)
Physiological traits		
Relative water content	<i>Triticum durum</i>	(Giusti <i>et al.</i> , 2017)
Photosynthesis Rates	<i>Triticumaestivum</i>	(Xie <i>et al.</i> , 2021)
Leaf temperature	<i>Triticum durum</i>	(Sattar <i>et al.</i> , 2020)
Photosynthetically active radiation	<i>Triticumaestivum</i>	(Grieco <i>et al.</i> , 2020)
Transpiration efficiency	<i>Triticumaestivum</i>	(G. Zhang <i>et al.</i> , 2017)
Flag leaf rolling	<i>Triticumaestivum</i>	(Sattar <i>et al.</i> , 2020)
Early vigour	<i>Triticumaestivum</i>	(Silva-Perez <i>et al.</i> , 2018)
Leaf osmotic potential	<i>Triticumaestivum</i>	(Sattar <i>et al.</i> , 2020)
Biochemical Traits		
Starch Synthesis	<i>Triticumaestivum</i>	(El Habi <i>et al.</i> , 2020)
B-glucan	<i>Triticumaestivum</i>	(Rakszegi <i>et al.</i> , 2014)
Proline Biosynthesis	<i>Triticumaestivum</i>	(Lamaoui <i>et al.</i> , 2018)
Glycine betane biosynthesis	<i>Triticumaestivum</i>	(Lamaoui <i>et al.</i> , 2018)
Reactive oxygen species generation	<i>Triticumaestivum</i>	(Fábián <i>et al.</i> , 2019)
Soluble protein content	<i>Triticumaestivum</i>	(H. Liu <i>et al.</i> , 2021)
Arabinoxylan	<i>Triticumaestivum</i>	(Rakszegi <i>et al.</i> , 2014)
Cysteine and methionine metabolism	<i>Triticumaestivum</i>	(H. Liu <i>et al.</i> , 2021)
Solvent Retention Capacity	<i>Triticum durum</i>	(Labuschagne <i>et al.</i> , 2021)



Gluten Protein	<i>Triticum durum</i>	(Labuschagne <i>et al.</i> , 2021)
Water soluble carbohydrates in grains	<i>Triticumaestivum</i>	(Qaseem <i>et al.</i> , 2019)
Fructan synthesis	<i>Triticumaestivum</i>	(Giusti <i>et al.</i> , 2017)
Yield Related Traits		
Grain yield	<i>Triticumaestivum</i>	(Qin <i>et al.</i> , 2016)
Grain number per plant	<i>Triticumaestivum</i>	(Silva-Perez <i>et al.</i> , 2018)
Grain number in the main spike	<i>Triticumaestivum</i>	(Silva-Perez <i>et al.</i> , 2018)
Number of Spikes	<i>Triticumaestivum</i>	(Schmidt, Tricker, <i>et al.</i> , 2020)
Spike weight	<i>Triticumaestivum</i>	(Schmidt, Tricker, <i>et al.</i> , 2020)
Grain yield	<i>Triticumaestivum</i>	(Qin <i>et al.</i> , 2016)
Spike harvest index	<i>Triticumaestivum</i>	(Shahinnia <i>et al.</i> , 2016)

Table 2: Effect of combined heat and drought stress at molecular level

Molecular Traits	Function	Reference
Phosphoenolpyruvate carboxylase (PEPC)	Provide tolerance to drought & high temperature stress in the transgenic wheat by increasing PEPC-responsive proteins namely asphosphatedikinese, chlorophyll AB binding protein, PAP fibrillin which involved in photosynthetic and cytoskeletal activity.	(Qin <i>et al.</i> , 2016)
TaHSFA6e (Heat shock factor) Transcriptional factors	Increase in guaiacol peroxidase (GPX), CAT, total antioxidant capacity and a decrease in peroxidation of lipid activity against both heat and drought stress together.	(Haider <i>et al.</i> , 2021)
AtWRKY30 Transcriptional factors	plant vigor, biomass, chl content, gas-exchange attributes, RWC, proline content and antioxidant enzymes' activity observed in overexpressed lines under combined stress.	(Haider <i>et al.</i> , 2021)
EF-Tu Elongation factor	Provide improved tolerance by Maize Ubiquitin 1 promoter using overexpressing these factors.	(Lamaoui <i>et al.</i> , 2018)
HVA1 (Late embryogenesis abundant protein)	Improved tolerance and field evaluation for combined stress.	(Lamaoui <i>et al.</i> , 2018)
AtHDG11 (Transcriptional factors)	Lower transpiration, lower stomatal density, more proline accumulation and increased activities of CAT and SOD observed in transgenic lines under stress.	(Li <i>et al.</i> , 2016)
DREB1A (Transcriptional factors)	Upregulated stress-inducible gene provides improved tolerance by using rd29A gene promoter.	(Haider <i>et al.</i> , 2021)
Stress-responsive NAC gene (Transcriptional factors)	Overexpressed lines showed enhanced stress tolerance.	(Li <i>et al.</i> , 2016)

3. Specific traits to be emphasized for tolerance against combined stressors

Abiotic stresses affect various physiological processes which causes oxidative damage due to the over-production of toxic reactive oxygen species (ROS). In order to overcome these harmful effects, plants adapt several physiological, morphological and genetic responses (El-Esawi *et al.*, 2019). Some specific traits crucial for combined stress tolerance are explained in Figure

1. Various transgenic approaches has been tried by over expressing certain genes and TFs, which showed the enhanced environmental stresses tolerance of crops by the induction of several downstream stress-associated genes (El-Esawi *et al.*, 2019). Following, promising traits can be considered in future, under combined stress tolerance to develop climate resilient crops.

Canopy temperature: Under heat stress, lowering of internal temperatures is the adaptive strategy of plant



and under drought plants closes their stomata partially/fully to prevent unnecessary water loss to maintain the thermostatis. By these mechanisms, the tolerant genotypes sustain, resulting in lower leaf and canopy temperatures in drought-stressed plants than in well-watered plants (Reynolds *et al.*, 2009). Cool canopies have traditionally been linked to higher yields (Pinto and Reynolds, 2015). Hence, canopy temperature should be counted as a reliable trait for screening in the wheat improvement programme under combined stress effect.

Assimilate partitioning: Photosynthesis is majorly affected in wheat under the combined heat and drought stress. It negatively affects the photosynthetic rates as compared to each of the two different stresses separately. Under continuous stress it becomes critical to allow nonstop assimilation and its partitioning to sink properly as these are dependent on the fine spatiotemporal regulation of gaseous exchange (Tricker *et al.*, 2018). Hence assimilate partitioning should be considered as a critical trait under stress.

Water use efficiency: Water use efficiency (WUE) is a measure of amount of carbon assimilated (biomass) per unit of water used by the crop. Water deficit cause different WUE responses in leaves. The physiological mechanisms governing CO₂ and H₂O gradients between the leaf and the air around the leaf, such as leaf:air vapour pressure deficits, are directly connected to WUE (leaf level). Improving WUE can help the plant system cope better with both stressors by improving the homeostatic process (Hatfield & Dold, 2019).

Comparative Yields: Grain yield is the highly valuable trait under any stress for breeders and farmers as well. Hence, at the initial levels, for screening a large population comparative yield losses under stress should be taken care crucially. The genotypes having a standard value of drought susceptible index (DSI) <0.5 and heat susceptible index HSI <1.0 should be given advantage in the breeding programmes as a crossing parent.

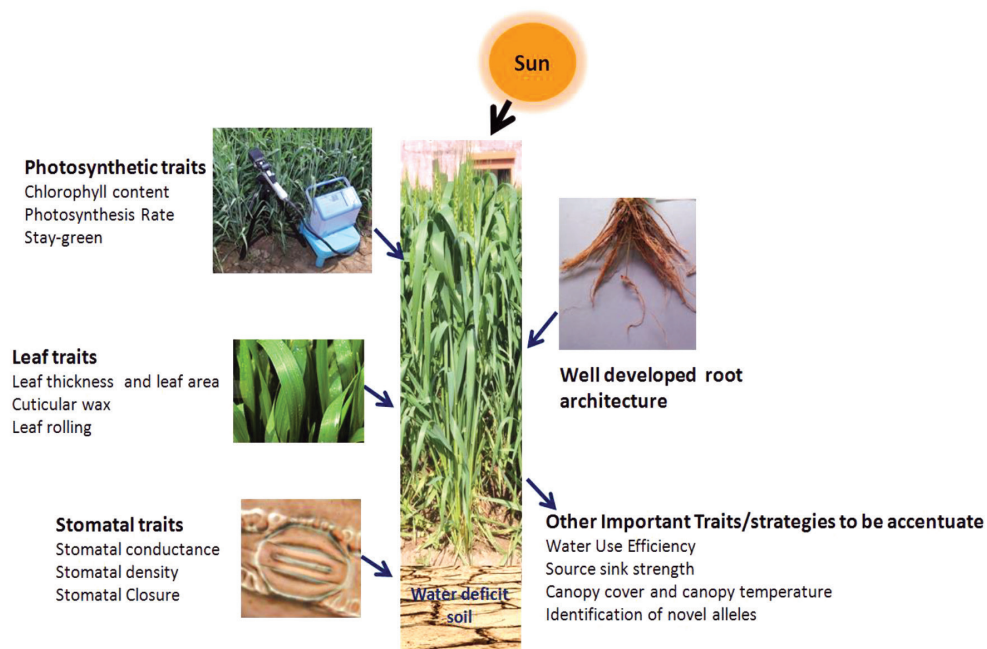


Figure 1: Traits associated with combined stress (heat and drought) tolerance

4. Futuristic approaches

Combined stress shows an additive negative impact and modify plant metabolism in novel ways as compared with individual stresses (Correia *et al.*, 2018). Agronomic methods are also important in the management of these abiotic variables. The use of certain micronutrients (Mn, Se, B, K, Ca), compost, and biochar to combat

the combined impacts of heat and drought has been recommended in the literature. Mulching and subsurface watering are also mentioned as viable strategies for combined stress mitigation (Lamaoui *et al.*, 2018). Endophytic fungi (e.g., SMCD 2206) have also been shown to be an effective technique for reducing these pressures (Zahra *et al.*, 2021).



In order to develop abiotic stress tolerant plants, numerous molecular studies have been carried out using several genes, regulatory and transcriptional factors which are associated with the heat and drought stress together. These genes are highly used as a target gene to design a transgenic crop. Through genome editing; specific genes can be targeted and get modified based on the tolerance capacity. It is very important to develop tolerance potential to improve high temperature and drought stress in the staple crops like wheat and corn. It is highly achieved by using genome editing (GE) technologies. Through these GE technologies which includes Zinc Finger (ZF) nucleases, Clustered regularly interspaced short palindromic repeats (CRISPR) and Transcription activator-like effector nucleases (TALENs) minor genome changes can be possible. These are having a potential to manipulate and change plant improvement strategies in a revolutionary manner (Kim *et al.*, 2018) particularly for plants possess complex genome. The recently discovered Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR). Several gene insertion and gene replacement mutants, knockout mutants were produced through these GE applications in maximum variety of plants. These mutants have been significantly found to be useful for the crop improvement (Zhang *et al.*, 2019). Identification of novel QTLs and alleles coupled with combined heat and drought tolerance is very much required in the coming future. Despite searching novel sources, exploitation of existing germplasm and use of identified parents for heat+drought could speed up the breeding programmes.

Acknowledgements: Authors thank Director ICAR-Indian Institute of Wheat and Barley Research, Karnal for constant encouragements and support.

Compliance with ethical standards: NA

Conflict of interest: No

Author's contribution: Conceptualization (RK, MHM); Preparation of the manuscript (AJ, RK, PD, ZW, KD); Review and editing (MHM, RK, GP); Editing and final approval (GPS). All authors read and approved the manuscript.

References:

1. Akter and M Islam Rafiqul. 2017. Heat stress effects and management in wheat. A review. *Agronomy for Sustainable Development* 37.
2. Albergel C, E Dutra, B Bonan, Y Zheng, S Munier, G Balsamo, de Rosnay P, J Muñoz-Sabater and JC Calvet. 2019. Monitoring and forecasting the impact of the 2018 summer heatwave on vegetation. *Remote Sensing* 11.
3. Beccari G, A Prodi, MT Senatore, V Balmas, F Tini, A Onofri, L Pedini, M Sulyok, L Brocca and L Covarelli. 2020. Cultivation area affects the presence of fungal communities and secondary metabolites in Italian durum wheat grains. *Toxins* 12.
4. Choudhury F K, Rivero R M, Blumwald E and Mittler R. 2017. Reactive oxygen species, abiotic stress and stress combination. *Plant Journal* 90: 856–867.
5. Correia B, RD Hancock, J Amaral, A Gomez-Cadenas, L Valledor and G Pinto. 2018. Combined drought and heat activates protective responses in eucalyptus globulus that are not activated when subjected to drought or heat stress alone. *Frontiers in Plant Science*, 9: 1–14.
6. Devi S, V Singh and N Naresh. 2022. Evaluation of wheat genotypes for yield potential under combined drought and heat stress conditions. *Journal of Cereal Research* 14: 37–43.
7. El-Esawi MA, AA Al-Ghamdi, HM Ali and M Ahmad. 2019. Overexpression of atWRKY30 transcription factor enhances heat and drought stress tolerance in wheat (*Triticum aestivum* L.). *Genes* 10:1–13.
8. El Habti A, D Fleury, N Jewell, T Garnett and PJ Tricker. 2020. Tolerance of Combined Drought and Heat Stress Is Associated With Transpiration Maintenance and Water Soluble Carbohydrates in Wheat Grains. *Frontiers in Plant Science* 11: 1–13.
9. Fábíán A, E Sáfrán, Szabó-Eitel G, B Barnabás and K Jäger. 2019. Stigma functionality and fertility are reduced by heat and drought co-stress in wheat. *Frontiers in Plant Science* 10:1–18.
10. Giusti L, E Mica, De E Bertolini, AM Leonardis, P Faccioli, L Cattivelli and C Crosatti. 2017. microRNAs differentially modulated in response to heat and drought stress in durum wheat cultivars with contrasting water use efficiency. *Functional and Integrative Genomics* 17:293–309.



11. González-Mendoza VM, ME Sánchez-Sandoval, LA Castro-Concha and SM Teresa Hernández-Sotomayor. 2021. Phospholipases c and d and their role in biotic and abiotic stresses. *Plants* **10**.
12. Grieco M, V Roustan, G Dermendjiev, S Rantala, A Jain, M Leonardelli, K Neumann, V Berger, D Engelmeier, G Bachmann, I Ebersberger, EM Aro, W Weckwerth and M Teige . 2020. Adjustment of photosynthetic activity to drought and fluctuating light in wheat. *Plant Cell and Environment* **43**:1484–1500.
13. Haider S, J Iqbal, S Naseer, M Shaukat, BA Abbasi, T Yaseen, SA Zahra and T Mahmood. 2021. Unfolding molecular switches in plant heat stress resistance: A comprehensive review. *Plant Cell Reports* **0123456789**.
14. Hatfield JL and C Dold. 2019. Water-use efficiency: Advances and challenges in a changing climate. *Frontiers in Plant Science* **10**: 1–14.
15. Kim D, B Alptekin and H Budak. 2018. CRISPR/Cas9 genome editing in wheat. *Functional and Integrative Genomics* **18**: 31–41.
16. Labuschagne M, C Guzmán, K Phakela, B Wentzel and A Van Biljon. 2021. Solvent retention capacity and gluten protein composition of durum wheat flour as influenced by drought and heat stress. *Plants* **10**:1–14.
17. Lamaoui M, M Jemo, R Datla and F Bekkaoui. 2018. Heat and drought stresses in crops and approaches for their mitigation. *Frontiers in Chemistry* **6**: 1–14.
18. Li L, M Zheng, G Deng, J Liang, H Zhang, Z Pan, H Long and M Yu. 2016. Overexpression of AtHDG11 enhanced drought tolerance in wheat (*Triticum aestivum* L.). *Molecular Breeding* **36**: 1–10.
19. Liu B, S Asseng, C Müller, F Ewert, J Elliott, DB Lobell, P Martre, AC Ruane, D Wallach, JW Jones, C Rosenzweig, PK Aggarwal, PD Alderman, J Anothai, B Basso, C Biernath, D Cammarano, A Challinor, D Deryng and Y Zhu. 2016. Similar estimates of temperature impacts on global wheat yield by three independent methods. *Nature Climate Change* **6**:1130–1136.
20. Liu H, AJ Able J and JA Able. 2021. Small rna, transcriptome and degradome analysis of the transgenerational heat stress response network in durum wheat. *International Journal of Molecular Sciences*, **22**(11). Mahrookashani, A., Siebert, S., Hüging, H., & Ewert, F. (2017). Independent and combined effects of high temperature and drought stress around anthesis on wheat. *Journal of Agronomy and Crop Science* **203**:453–463.
21. Ogonnaya FC, A Rasheed, EC Okechukwu, A Jighly, F Makdis, T Wuletaw, A Hagra, MI Uguru and CU Agbo. 2017. Genome-wide association study for agronomic and physiological traits in spring wheat evaluated in a range of heat prone environments. *Theoretical and Applied Genetics* **130**: 1819–1835.
22. Perdomo JA, S Capó-Bauçà, E Carmo-Silva and J Galmés. 2017. Rubisco and rubisco activase play an important role in the biochemical limitations of photosynthesis in rice, wheat, and maize under high temperature and water deficit. *Frontiers in Plant Science* **8**:1–15.
23. Qaseem MF, R Qureshi, QH Muqaddasi, H Shaheen, R Kousar and MS Röder. 2018. Genome-wide association mapping in bread wheat subjected to independent and combined high temperature and drought stress. *PLoS ONE* **13**:1–22.
24. Qaseem MF, R Qureshi and H Shaheen. 2019. Effects of Pre-Anthesis Drought, Heat and Their Combination on the Growth, Yield and Physiology of diverse Wheat (*Triticum aestivum* L.) Genotypes Varying in Sensitivity to Heat and drought stress. *Scientific Reports* **9**: 1–12.
25. Qin N, W Xu, L Hu, Y Li, H Wang, X Qi, Y Fang and X Hua. 2016. Drought tolerance and proteomics studies of transgenic wheat containing the maize C4 phosphoenolpyruvate carboxylase (PEPC) gene. *Protoplasma* **253**:1503–1512.
26. Rakszegi M, A Lovegrove, K Balla, L Láng, Z Bedo, O Veisz and PR Shewry. 2014. Effect of heat and drought stress on the structure and composition of arabinoxylan and -glucan in wheat grain. *Carbohydrate Polymers* **102**(1): 557–565.
27. Sallam A, AM Alqudah, MFA Dawood, PS Baenziger and A Börner. 2019. Drought stress tolerance in wheat and barley: Advances in



- physiology, breeding and genetics research. *International Journal of Molecular Sciences* **20**.
28. Sattar A, A Sher, M Ijaz, S Ul-Allah, MS Rizwan, M Hussain, K Jabran and MA Cheema. 2020. Terminal drought and heat stress alter physiological and biochemical attributes in flag leaf of bread wheat. *PLoS ONE* **15**: 1–14.
29. Schmidt J, J Claussen, N Wörlein, A Eggert, D Fleury, T Garnett and S Gerth. 2020. Drought and heat stress tolerance screening in wheat using computed tomography. *Plant Methods* **16**:1–12.
30. Schmidt J, PJ Tricker, P Eckermann, P Kalambettu, M Garcia and D Fleury. 2020. Novel Alleles for Combined Drought and Heat Stress Tolerance in Wheat. *Frontiers in Plant Science* **10**:1–14.
31. Shahinnia F, Le J Roy, B Laborde, B Sznajder, P Kalambettu, S Mahjourimajd, J Tilbrook and D Fleury. 2016. Genetic association of stomatal traits and yield in wheat grown in low rainfall environments. *BMC Plant Biology* **16**.
32. Silva-Perez V, G Molero, SP Serbin, AG Condon, MP Reynolds, RT Furban and JR Evans. 2018. Hyperspectral reflectance as a tool to measure biochemical and physiological traits in wheat. *Journal of Experimental Botany* **69**: 483–496.
33. Tahmasebi S, B Heidari, H Pakniyat, CL McIntyre and L Lukens. 2016. Mapping QTLs associated with agronomic and physiological traits under terminal drought and heat stress conditions in wheat (*Triticum aestivum* L.). *Genome* **60**:26–45.
34. Taká T, D Novák and J Šama. 2019. Recent advances in the cellular and developmental biology of phospholipases in plants. *Frontiers in Plant Science* **10**.
35. Tricker PJ, A Elhabti, J Schmidt and D Fleury. 2018. The physiological and genetic basis of combined drought and heat tolerance in wheat. *Journal of Experimental Botany* **69**: 3195–3210.
36. Tyagi M and GC Pandey. 2022. Physiology of heat and drought tolerance in wheat: An overview. *Journal of Cereal Research* **14**:13–25.
37. Wang X, X Yao, A Zhao, M Yang, W Zhao, MK LeTourneau, J Dong and X Gao. 2021. Phosphoinositide-specific phospholipase C gene involved in heat and drought tolerance in wheat (*Triticum aestivum* L.). *Genes and Genomics* **43**:1167–1177.
38. Xie H, J Shi, F Shi, H Xu, K He and Z Wang. 2021. Aphid fecundity and defenses in wheat exposed to a combination of heat and drought stress. *Journal of Experimental Botany* **71**:2713–2722.
39. Zafra A, JD Rejón, SJ HiscocJ and Alché J de D. 2016. Patterns of ROS accumulation in the stigmas of angiosperms and visions into their multifunctionality in plant reproduction. *Frontiers in Plant Science* **7**: 1–7.
40. Zahra N, A Wahid, MB Hafeez, A Ullah, KHM Siddique and M Farooq. 2021. Grain development in wheat under combined heat and drought stress: Plant responses and management. *Environmental and Experimental Botany* **188**, 104517.
41. Zandalinas SI, R Mittler, D Balfagón, V Arbona & A Gómez-Cadenas. 2017. Plant adaptations to the combination of drought and high temperatures - Zandalinas - 2017 - *Physiologia Plantarum* - Wiley Online Library. *Physiologia Plantarum* **15**: 2–15.
42. Zhang G, M Zhang, Z Zhao, Y Ren, Q Li and W Wang. 2017. Wheat TaPUB1 modulates plant drought stress resistance by improving antioxidant capability. *Scientific Reports* **7**:1–13.
43. Zhang Y, K Masse, ID Godwin and C Gao. 2019. Correction to: Applications and potential of genome editing in crop improvement. *Genome Biology* **20**: 1–11.

