

Matthew P. Reynolds  
Hans-Joachim Braun *Editors*

# Wheat Improvement

Food Security in a Changing Climate



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# Chapter 22

## Heat and Climate Change Mitigation



Dirk B. Hays, Ilse Barrios-Perez, and Fatima Camarillo-Castillo

**Abstract** High temperature stress is a primary constraint to maximal yield in wheat, as in nearly all cultivated crops. High temperature stress occurs in varied ecoregions where wheat is cultivated, as either a daily chronic metabolic stress or as an acute episodic high heat shock during critical periods of reproductive development. This chapter focuses on defining the key biochemical processes regulating a plant's response to heat stress while highlighting and defining strategies to mitigate stress and stabilize maximal yield during high temperature conditions. It will weigh the advantages and disadvantages of heat stress adaptive trait breeding strategies versus simpler integrated phenotypic selection strategies. Novel remote sensing and marker-assisted selection strategies that can be employed to combine multiple heat stress tolerant adaptive traits will be discussed in terms of their efficacy. In addition, this chapter will explore how wheat can be re-envisioned, not only as a staple food, but also as a critical opportunity to reverse climate change through unique subsurface roots and rhizomes that greatly increase wheat's carbon sequestration.

**Keywords** Climate change mitigation · Respiration · Heat shock · Ethylene · Leaf epicuticular wax · Source and sink relationships

### 22.1 Learning Objectives

- Identify factors responsible for yield loss during acute high temperature stress heat shock.
- Define alternate hormonal yield pathways that maximize and/or limit wheat yield during acute high temperature stress.

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D. B. Hays (✉) · I. Barrios-Perez  
Soil and Crop Sciences, Texas A&M University, College Station, TX, USA  
e-mail: [dbhays@tamu.edu](mailto:dbhays@tamu.edu); [ibarrios@tamu.edu](mailto:ibarrios@tamu.edu)

F. Camarillo-Castillo  
International Maize and Wheat Improvement Center (CIMMYT), Texcoco, Mexico  
e-mail: [f.camarillo@cgiar.org](mailto:f.camarillo@cgiar.org)

- Define unique adaptive traits that suppress the induction of yield-limiting signal transduction pathways during yield formation.
- Define the impact of nighttime high temperature stress on respiration-derived yield limitations in wheat, and explore current strategies to minimize it.
- Identify key traits that increase wheat's climate change mitigation capacity.
- Contrast the breeding efficiency of employing physiological and idiotypic based trait introgression versus integrated yield based selection strategies.

## 22.2 Introduction

*Global warming* – The steadily growing concentration of atmospheric carbon dioxide (CO<sub>2</sub>) has currently reached an excess of 409 ppm or 720 gigatons of carbon (C) (GtC), its highest point in more than 800,000 years (NOAA). As is widely known, increasing atmospheric CO<sub>2</sub>, along with methane (CH<sub>4</sub>), and nitrous oxide (N<sub>2</sub>O) are the primary factors raising global temperatures. Warming temperatures are already constraining local agricultural production, thus inflating food prices nationally and globally. If rapid mitigation strategies are not implemented on a global scale, we can expect constraints on our food systems to increase in frequency and severity, leading to regional conflicts and migration events. The gravity of these negative consequences partly depends on our ability to adapt current cultivars to increasing temperatures, while also modifying our agro-ecological practices to mitigate these challenges. In the past decade alone (2008–2017) anthropogenic activity has resulted in the release of 9.4 GtC y<sup>-1</sup> via fossil fuel combustion. Land use changes, including modern agricultural cultivation, emit an additional 1.3 GtC y<sup>-1</sup>. Oceans and terrestrial lands sequester more than half of these anthropogenic emissions (2.4 and 3.0 GtC y<sup>-1</sup>, respectively) as primary carbon sinks. The remaining net emissions are lingering in the atmosphere, further contributing to global warming at an annual increase of 4.7 GtC y<sup>-1</sup> [1]. Under current global practices, agriculture thus contributes a sizeable (12%) fraction to the net atmospheric CO<sub>2</sub> emissions [2]. Given high temperature stress damage to wheat is a common issue globally, agriculture scientists must adapt practices and varieties to mitigate its impact on yield, while also transforming agriculture from a source of C emissions to compensated sinks.

Heat stress can reduce wheat yields throughout the crop's life cycle, either as an acute, chronic and nighttime stress. During early seedling establishment, heat stress reduces coleoptile elongation, impairing emergence. During vegetative development it accelerates the transition to flowering, increasing frost damage risks. While during inflorescence heat stress can accelerate grain development and ablate tillering meristems reducing grain number and yield [3]. During microgametogenesis and embryo development, acute heat stress can sterilize pollen and result in embryo abortion resulting in reduced grain number [4]. While heat stress during grain maturation can hasten the transition to the dry seed stage negatively impacting end-use quality.

The aforementioned effects are regulated in many instances by a heat stress induced increase in the plant hormone ethylene [5]. As such, breeding for heat tolerance in wheat should focus on the introgression of traits that moderate heat stress in wheat's internal tissues, thus allowing the maximal expression of growth and yield conducive regulatory pathways, while minimizing the induction of yield limiting pathways regulated by ethylene. This approach is in line with Passiourra's focus on optimizing traits that confer growth conducive conditions versus yield limiting plant survival traits [6].

High night temperatures (HNT) are important concerns of global warming. Data suggest that nighttime temperatures are rising at 1.4 times the rate of daytime temperatures [7]. HNT are often longer in duration during crop development and occur over broader geographic regions compared to chronic and episodic daytime heat stress. The physiological basis for HNT yield decline and current strategies to select for increased yield in response to HNT will be explored. In addition, this chapter will also explore how the idiosyncrasy of wheat could be re-envisioned into an asset in the world's arsenal of climate change mitigation through wheat root-derived CO<sub>2</sub> sequestration.

### **22.3 Factors Responsible for Yield Loss During Acute High Temperature Stress**

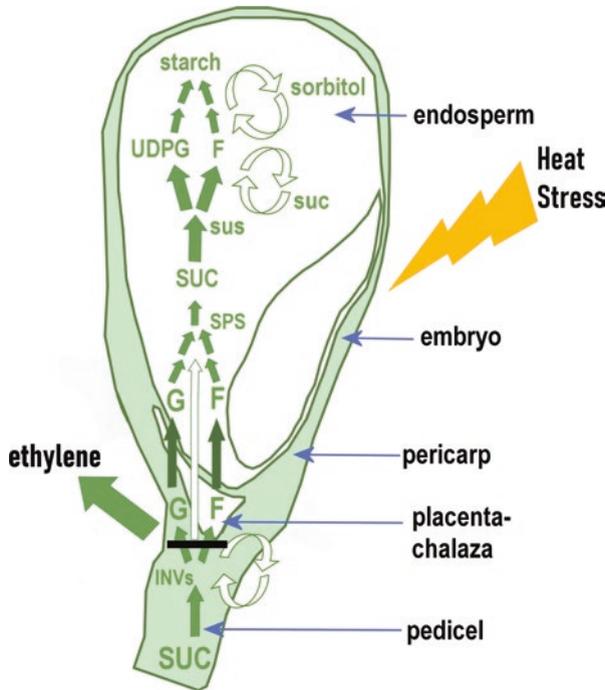
Optimal temperatures for wheat growth and development have been defined by numerous studies to range between 17 to 23 °C [8]. Above 37 °C, growth stops, while temperatures above 48 °C are lethal to most wheat genotypes. When occurring prior to or shortly after anthesis, temperatures above 30 °C cause pollen and floret sterility through early embryo abortion, both of which reduce grain number. Additionally, temperatures above 30 °C during maturation can reduce overall grain fill, and end-use quality by reducing starch deposition and altering high and low molecular weight glutenin and gliadin ratios [9]. The expression of genes involved in carbohydrate metabolism during grain maturation such as sucrose synthase, soluble starch synthase, phosphoglycerate kinase, starch branching enzyme have been shown to be highly sensitive to acute heat stress. Genes for several  $\alpha$ -amylase inhibitors also are down-regulated, as are a number of genes for gluten proteins, including  $\alpha$ -gliadins, LMW-GSs and a few HMW-GSs and  $\gamma$ -gliadins [9].

Also detrimental to wheat yield is chronic high temperature stress, which is confounded with high night temperatures. Chronic heat stress is differentiated from acute heat shock injury which occurs as a rapid increase in temperatures over a few days. The physiological basis for lower yields due to chronic heat stress is poorly understood, yet in other crops enhanced development is recognized as a function of growing degree days. In addition, beyond screening for increased yield, few trait that confer tolerance to chronic heat have been defined.

In temperate wheat growing climates such as North America, where fluctuations in warm versus cold fronts are common, chronic heat stress or early acute heat stress can hasten transitions to reproductive development leaving wheat vulnerable to frost damage and reproductive sterility. In these environments selection of earliness to avoid acute periods of heat stress may be worthwhile to combat continuing trends in global warming, however in the near-term earliness may not be a viable option.

## 22.4 The Role of Ethylene in Regulating High Temperature Stress Responses in Wheat

Ethylene is a gaseous plant hormone, that has been shown to exhibit time and dose-dependent effects on plants during heat stress. Under low concentrations, the developmental induction of ethylene positively regulates leaf and cotyledon expansion, lateral root growth and dormancy release in seeds and buds. However, high concentrations of ethylene can also impose an inhibitory, or senescence effect which is deleterious to growth and yield expression. Heat stress induced ethylene has been shown to regulate early leaf senescence, and result in reduced spikelet fertility through both pollen sterility and embryo abortion. Its production in embryos has been shown to accelerate senescence [5] and reduce total seed progeny, likely as an important conserved mechanism to optimize resource allocation during stress [10]. Similarly, heat stress ethylene has been reported in developing pollen and flowers where it induces sterility [11]. In other systems, ethylene has been shown to regulate leaf abscission in response to reduced auxin flux and to increase in response to reduced glucose levels, which promote the synthesis of abscisic acid (ABA) through glucose signalling [12]. In wheat responses to acute heat stress, it is unclear whether the yield limiting impacts of increased ethylene are induced pathways in embryos and grains themselves, an ethylene elevation resulting from the reduction in photosynthetic glucose, or a reduction in glucose and fructose from diminished sucrose hydrolysis via invertases or sucrose synthases in sink tissues (Fig. 22.1). In maize, this latter scenario is referred to as the Shannon hypothesis for sink strength. In this hypothesis, sucrose is hydrolysed by cell wall and vacuolar invertases in the phloem unloading zone at the pedicel and placenta-chalazal connection to the seed nucellus [13]. These enzymes have been shown to be sensitive to drought stress in early developing maize kernels, which results in plant cell death (PCD) at this connective abscission zone [14]. An analogous event regulates the tapetum connections to developing pollen microspores. In this case, drought and heat stress reduce cell wall invertase gene expression in rice and wheat tapetum resulting in premature PCD in the tapetum's connection to microspores and loss of sugar (glucose and fructose) translocation [15, 16]. Both early embryo, seed and microspore development are dependent on a sink-signalled supply of sucrose to facilitate early developmental growth, cell expansion, and starch deposition to maintain viability. Loss of invertase gene expression in pedicel and tapetum may be a critical feature of heat and drought



**Fig. 22.1** The Shannon hypothesis (in green) with the addition of heat stress induced ethylene (in black) proposes that sucrose (SUC) first cleaved by cell wall invertases (INV) in the pedicel, the placenta-chalazal, and the basal endosperm transfer layer. Hexoses glucose (G) and fructose (F) enter the endosperm across the endosperm transfer layer. In the endosperm and embryo, SUC is resynthesized by sucrose phosphate synthase (SPS), and transferred within the endosperm and embryo where it is re-hydrolyzed by sucrose synthase (SUS) for metabolism into starch. Reduction in INV or SUS by heat stress in either in the placenta-chalazal, endosperm, or embryo could reduce glucose derived suppression of ethylene synthesis regulated by ABA resulting in abscission at the placenta chalazal and an early developmental senescence of the endosperm and embryo. (Modified with permission from Ref. [13])

sensitive wheat genotypes [4]. The role of invertases in the basal pedicel regions of developing kernels is also consistent with phloem unloading being an important central feature in promoting turgor gradients and pressure driven sucrose movement and establishing sink strength in developing seed and microspore sinks [17]. At present, the sequence of regulatory responses to acute heat stress leading to increased ethylene, decreased invertase activity, reduced starch deposition and early microspore and kernel senescence requires clarification.

The negative role ethylene can play in response to acute heat stress in wheat and other cereals, can however be inhibited using competitive inhibitors of ethylene response such as 1-methyl cyclopropane (1-MCP) or ethylene biosynthesis inhibitor. Wheat genotypes have also been identified that either don't show increased ethylene in response to heat stress or are insensitive to ethylene during reproductive

development as it relates to yield [6]. In addition, recent studies have shown that negative regulation of the ethylene response via ARGOS8 using CRISPR-CAS9 or other genetic engineering technologies can be used to improve yield in maize under drought [18].

## 22.5 Traits that Suppress Stress Pathway Induction

Because responses to heat stress have been shown to be regulated by independent and convergent signal transduction pathways, a prudent approach should also seek to combine multiple heat adaptive traits that moderate the internalization of ethylene inducing high temperature. Traits that reduce both excess photosynthetic and high temperature conferring solar radiation should be prioritized. This approach has been used inadvertently during humanity's domestication of wheat, by regional and multi-locational breeding programs in heat stress prone environments, and more recently through targeted introgression of specific traits conferring adaptive advantages [4].

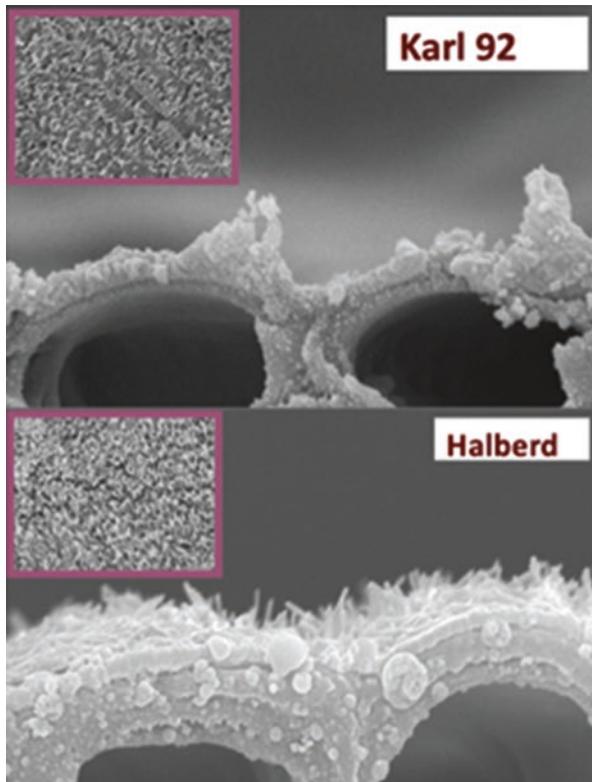
Targeted screening for lower canopy temperatures (CT) or high canopy temperature depression (CTD) during high temperature stress is one such approach that has been demonstrated to confer improved yields [19]. However, measuring CT is a proxy for both water use traits such as stomatal conductance and root depth and solar radiation avoidance traits such as incident leaf angle, leaf rolling, and leaf epicuticular wax. Each of the traits contributing to lower CT are developmentally regulated and highly responsive to time of day and microclimate fluctuations such as cloud cover changes and wind gusts, which can render measurements unstable [4].

An alternate approach is to focus on the selection and pyramiding of traits that reduce CT, by increasing the crops *albedo*, which is the ratio of incidence to reflected radiation, in a manner that reduces water use for transpirational cooling during heat stress. Specific traits with these qualities include increased epicuticular wax (EW), erect leaves and in some cases, enhanced leaf rolling. Improved root morphology such as deeper root angles can extend transpirational cooling and growth during heat and drought stress, yet should be selected in combination with traits that reduce solar radiation and preserve limited soil water for the duration of growth and grain development.

Selection for increased EW has been shown to double the proportion of solar radiation that is reflected, reducing photo-inhibition and leaf burning, while significantly reducing leaf and spike temperatures. Increased leaf and glume EW has also been associated with an increased harvest index (HI), residual leaf water content while lowering transpiration and stomatal conductance in a manner that improves water and radiation use efficiencies (WUE and RUE) [20].

Visual selection for glaucousness, which is the light blue grey bloom on leaves, stems and glumes, is often used as a proxy for high EW or as a heat tolerance trait itself. The genetic control of glaucousness, is however independent of total EW content or reflectance. EW is a complex mixture of long chain hydrocarbons of

varying length with unique functional groups consisting of alcohols, aldehydes, ketones, and methyl groups. Glauousness is a function of a higher ratios of alcohols, aldehydes and ketone functional groups arranged as erect glaucous rods and plates [21] versus reduced methyl functional groups arranged as non-glaucous flat plates. As such, glaucousness is not a suitable selection proxy for high EW content, and the associated physiological benefits. Wheat genotypes with higher EW, cooler CT and higher reflectance can be identified with high or low degrees of glaucousness (Fig. 22.2). Accurate selection of high EW wheat genotypes should utilize new hyperspectral indices for EW [22] or the chemical extraction of EW content assay developed by Ebercron [23]. Recent studies have used the latter method to identify novel genetic loci regulating increased EW in wheat. Increased EW QTL are also co-associated with measures of cooler leaf and spike temperatures (CT), and improved yield component stability during heat and drought stress (referred to as heat or drought susceptibility index (HSI or DSI) which is the ratio of the mean



**Fig. 22.2** Scanning electron microscopy cross section of leaves of two glaucous wheat lines with varying epicuticular content. The glaucous wheat cultivar ‘Karl 92’ is heat susceptible with a thin epicuticular wax layer, while the glaucous wheat cultivar ‘Halbred’ is heat tolerant with a thick epicuticular wax

yield under stress versus control conditions). The QTL identified are located on chromosomes 1A, 2B, 3B, 6B, 7A and 7B [24]. While a gene cluster regulating glaucousness has been identified on wheat chromosomes 2BS and 2DS where S refers to the short arm [25]. It should be noted that the inheritance of high EW and glaucousness on flag leaves and the glumes appears independent and care is needed to select for high EW on both leaves and glumes.

Genotypes with erect leaves that reduce high incidence solar radiation or that express leaf rolling in contrast to prostrate or droopy flag leaves have been suggested to be protected from heat stress injury in wheat, barley and other cereals [3]. However few studies have found strong associations between the trait and improved yield under low water or high temperature stress.

## **22.6 Nighttime High Temperature Stress Impacts on Wheat Yield**

The impact of high day temperature on wheat yield has been well documented in numerous studies. However, fewer studies beyond selection for yield, have identified critical selection targets for tolerance to HNT. The biochemical basis for HNT yield decline has been predominately attributed to increasing nighttime respiration which is presumed to decrease the amount of stored photoassimilates available for plant growth and grain filling. In this case, metabolite profiling showing increases in tricarboxylic acid (TCA) intermediates in leaves exposed to HNT is highlighted as evidence [7]. It should be noted that high night respiration may however represent an increase in nighttime ATP dependent photoassimilate deposition and an accelerated seed maturation rate.

Yield declines of 4% per increase in °C above 14 °C night temperatures have been defined. These studies attributed the decline in yield and yield components to alterations in the grain maturation process and overall shortening of the grain development period rather than photosynthetic or photoassimilate source limitations that might be attributed to elevated night respiration [26]. Discriminating this difference is an important consideration for developing the most effective HNT tolerance screening protocols. The current available literature suggests focusing on grain filling rate as a probable target for selection for HNT in addition to yield and kernel specific yield components.

## **22.7 Climate Change Mitigation via High Root Biomass**

Internationally, there is growing consensus among the scientific, governmental, and political establishment that managed agricultural soils represent a viable opportunity to reverse climate change by sequestering atmospheric CO<sub>2</sub> into soil C via

underground root biomass [2]. The known synergistic benefits of soil C include improved soil health, increased crop resilience to abiotic stressors (such as those caused by climate change), and reduced soil erosion and runoff. The C input from plants is cycled into the surrounding soils through two main mechanisms: the continual growth and dieback of small-diameter roots (root turnover), and the final deposition of the shoot and root systems following senescence. The root turnover mechanism is temperature dependent, with higher and lower rates of turnover being related to tropical and low-temperature environments, respectively. This turnover represents a continual deposition of C in soils. Modeling results, as well as the high presence of suberin (protective layer of plant roots) in the soil organic matter (SOM), indicate that a majority of long-lasting material is derived from roots rather than shoots [27]. Emission of CO<sub>2</sub> from these stocks into the atmosphere via soil organisms is accelerated by increasing temperatures [28], with the C sources that are inherently resistant to degradation generally being labeled as 'recalcitrant' [29]. The sequestered material requires maintenance, as decomposition will release the stored C back into the atmosphere. Given this understanding, a reasonable course of action for successful sequestration is to focus efforts on directing root growth into deeper subsoils and larger structures such as rhizomes, as the residence time for C below 30 cm has been shown to be on the order of millennia owing to the spatial separation between microorganisms and SOM [30].

In order to gain a true understanding of the efficacy of this strategy, a quick means of measuring belowground plant traits is needed. Most current methods are slow, cumbersome (e.g., full destructive excavation) or sacrifice accuracy of measurements for speed. As a compromise between speed and accuracy, researchers have pivoted towards ground penetrating radar (GPR) or new technologies such as magnetic resonance imaging (MRI) or X ray computed tomography (X ray CT) as a proxy measure for both high root, and rhizome biomass and high soil organic C.

## 22.8 Climate Change Mitigation and Potential of High-Root Biomass Grain Crops

The morphology of wheat is highly variable with heights ranging from 0.5 to 1.5 m. The species is generally noted for its extensive fibrous root system. Unfortunately, wheat along with other fibrous root annual grain crops (such as annual sorghum, annual maize, wheat, rice and millet) can sequester only  $\sim 0.48$  GtC y<sup>-1</sup> on a global basis, primarily through crop root biomass. This sequestration potential is not sufficient to mitigate the current increase in C emissions as it represents roughly 10% of the current 4.7 GtC y<sup>-1</sup> net emissions [2].

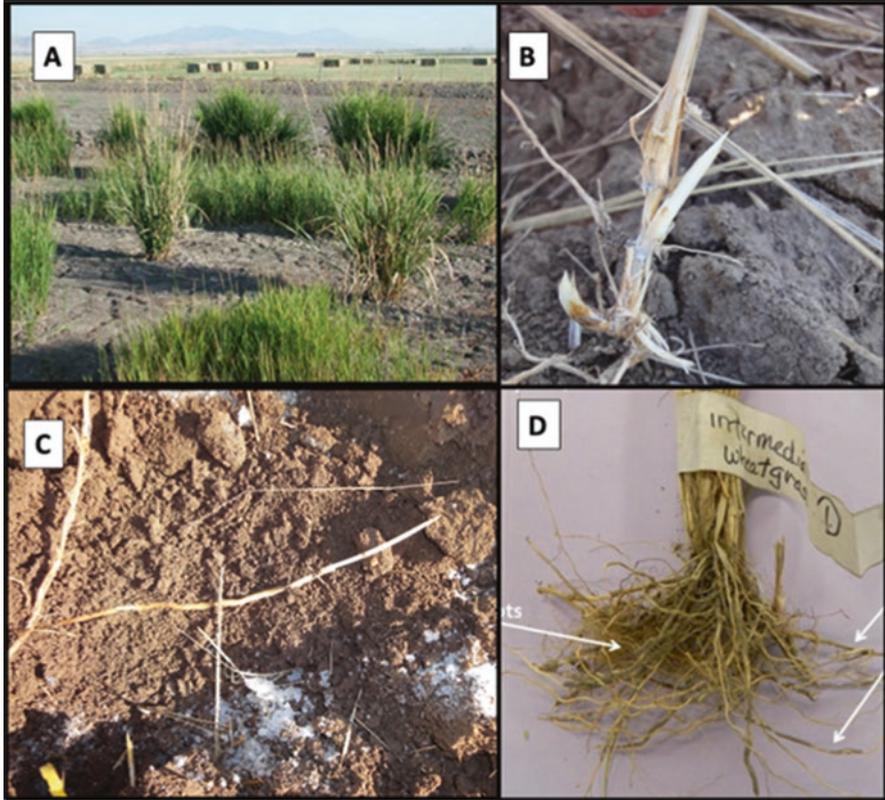
Rhizomes are an alternative subsurface anatomical feature common to wild ancestors of our staple crops such as rice, wheat, and sorghum that could provide the solution to increased agricultural C sequestration. Rhizomes are axillary shoots that develop from axillary buds. Axillary shoots that develop from buds in aerial nodes

are known as branches, while those that develop from buds close to the soil surface in the grasses are known as tillers or rhizomes. Transcriptome analysis have shown that expressed genes in the rhizome tip were more similar to above ground stems in *Sorghum propinquum* and *S. halepense* [31]. Thus, rhizomes appear to function as stems. This has important benefits for subsurface carbon storage. As subsurface stems, their apical meristem likely supply auxin and as such are not targeted for senescence following above ground biomass harvest. Rhizomes thus persist from season to season, resulting in maintenance of sequestered C both in the rhizomes and in the rhizome node derived roots. Similarly, because source-sink relationships are diverged within a rhizomatous grain system, and increase in biomass following grain harvest, they may have less photoassimilate competition to grain yield than vascular and source-sink-connected large root biomass.

While not known to occur in wheat per se, close relatives of wheat, which have been used as donors for traits like disease resistance or perenniality, are also known to possess significant rhizome biomass. These include perennial rye's *Leymus triticoides* and *Leymus cinereus* [32]. In addition, perennial wheat grass *Thinopyrum* has been used to produce rhizomatous hybrids of wheat [33] (Fig. 22.3).

As an example, hybrids of cultivated Sorghum (*Sorghum bicolor*) with *S. halepense* (PS) or *S. propinquum* (RAS) are being used to derive grain- and forage-type hybrids that produce rhizomes with a diameter of 1 cm. Root and rhizome biomass in these hybrids has been recorded to reach 24 MT ha<sup>-1</sup>, with 90% of it derived from the rhizome. Millet hybridized to Napier grass produces rhizome root biomass in excess of 70 MT ha<sup>-1</sup> [34]. The rhizomes increase subsurface C sequestration 10 to 30-fold compared to their non-rhizomatous annual crop counterparts. If grown globally as replacements for current annuals, high root: rhizome biomass grain crops have the potential to sequester more than 4.7 GtC y<sup>-1</sup> (unpublished extrapolations), and could negate and begin to reverse current net C emissions. Given the global urgency, the challenge is to devise the most efficient method to transfer the high soil C-sequestration potential in rhizomes from perennial wheat relatives into a yield-competitive perennial or annual grain-types.

Genetic analysis have identified two complementary dominant genes, Rhz2 and Rhz3, that control tillering and rhizome development – including rhizome number, branching, internode number and length in *O. longistaminata* [35]. Loss of function of either Rhz2 or Rhz3 inhibits rhizome development. However, to our knowledge, Rhz2 and Rhz3 are not yet cloned. QTLs that control rhizome development in *S. propinquum* correspond to most of the *O. longistaminata* QTLs. Modern remote sensing-based selection, gene editing and gene-based, marker-assisted selection tools and strategies can be employed to breed yield competitive rhizomatous wheat and other grain crops as high C sequestration replacements for current low root biomass grain crop cultivars.



**Fig. 22.3** (a) Plots of wildryes *Leymus triticoides* and *Leymus cinereus*; (b) Rhizomes in a hybrid of *L. triticoides* and *L. cinereus*; (c) Rhizome in *L. triticoides*; (d) Roots of *Thinopyrum intermedium* (intermediate wheatgrass) with thicker rhizomes. (Figure reprinted with permission from Ref. [36])

## 22.9 High Throughput Phenotyping Selection Strategies to Introgress Multiple Heat Stress Adaptive Traits

Independent conceptual models for grain yield (GY) under heat and drought have been proposed based on the following main drivers: light interception (LI), RUE, partitioning of total assimilates, WUE and harvest index [37]. Each of these main drivers contains genetically determined traits that can potentially lead to an additive genetic effect for resilience to heat when combined through strategic crossing [38]. Physiological traits such as canopy temperature (CT) are already utilized as a selection criteria. Other key traits such as EW remain underutilized except for selection of donors for favorable alleles because of the expensive and laborious methods for phenotyping.

Limitations on field phenotyping restrict our capacity to unravel complex morphological and physiological traits. One important consideration that requires understanding is that numerous physiological traits that have been defined as key targets for selection are developmentally programmed and exhibit temporal variation. As such, high throughput phenotyping selection trials should employ strategies that compensate for temporal and phenological variation (in this case defined as variations in flowering time). This requires partitioning trials into genotypes with narrow ranges of flowering times and conducting high throughput phenotyping scans in narrow temporal windows. This can help avoid confounding phenotypic measures associated with phenology or variations in time of day measured rather than the intended physiological selection.

In breeding programs, high-precision phenotyping can enable the screening of segregating material, advanced lines and germplasm [39]. Increasing the accuracy of phenotyping can provide more reliable estimates of heritability and variance components, facilitate gene discovery and enable prediction of complex traits using genomic selection. The strong association of spectral secondary traits with GY and in season biomass highlights the potential of hyperspectral canopy reflectance to increase productivity in wheat.

New UAV systems are available that combine both hyperspectral, thermal and LIDAR sensors, which should open the door to ideal integrative selection regimes. These are ideal systems for physiological breeding, however at present they remain cost prohibitive even for well-funded breeding programs (Fig. 22.4).

Spectral vegetation indices (SVI) are a quick, easy and inexpensive method of transforming light reflectance into simple indicators of photosynthetic and canopy variations. The simple ratio index (SR) and the normalized difference vegetation index (NDVI) are two of the first SVIs developed for detecting green vegetation. Both indices combine the percentage of reflectance at the wavelengths where plants absorb (~750 to 800 nm) and reflect (800 to 2500 nm) light. Several other SVIs have been derived for sensing the water content of plants, photosynthetic radiation, carotenoid pigments, plant height, leaf area, and diseases [22].

Recent studies have developed new spectral indices for selection of EW with accuracies reaching 65% [22]. EW indices combine narrow wavelength at 625 nm, with narrow wavelength at 736 nm and 832 nm reflectance for direct measures of leaves. Other indices were derived for canopy level reflectance indices derived from 2 narrow bands at 617 and 718 nm with EW prediction accuracies of 71%. It is worth emphasizing that selection for ideal leaf and glume EW should target genotypes which express high levels of EW in response to developmentally programmed cues that are irrespective and prior to heat or drought stress cues and not in response to stress cues. The rationale is that genotypes that express high EW in response to developmental cues will be more heat tolerant, while genotypes which increase EW content in response to stress have already incurred the yield penalty through induction of the heat and drought stress pathways regulated by ethylene. Given this understanding, selection for ideal EW requires critical attention to regularly monitor EW from flag leaf development to late maturation using multi-location selections.



**Fig. 22.4** VNIR/SWIR Hyperspectral spectrometer with integrated LIDAR for quantifying leaf wax and other unique heat stress adaptive traits

## 22.10 High Throughput Phenotyping Selection to Introgress Roots and Rhizomes

Unlike above ground foliar traits, roots are obscured by their growth medium. As such, measurement of root system traits has historically been an invasive process. Invasive techniques include the excavation of root systems, or ‘shovelomics’, soil coring, and rhizotrons. Noninvasive observations are attractive as it does not interfere with plant growth. Several techniques have been proposed, tested, and validated to different extents. These methods include MRI, X ray CT, and GPR. As high throughput selection tools the development of a field-based MRI or X ray CT are still in their infancy in terms of commercially available solutions. While coarser in terms of root imaging, both high throughput field compatible GPR instruments and the data processing methods needed to phenotype both total root biomass and root architecture traits are well developed and commercially available (see Crop Phenomics at [cropphenomics.com](http://cropphenomics.com)).

MRI is an imaging technique most widely known for its application in medicine and chemical analysis. Signals are largely derived from  $^1\text{H}$  protons which are abundant in water molecules and thus living tissues. Results of root trait (root length, root mass, root diameter, growth angles) quantification using MRI have been shown to be comparable to conventional methods.

X ray CT scans operate by projecting EM radiation in the X ray region of the spectrum through a sample from multiple angles. Elements within the sample attenuate the signal to different degrees depending on the density of their electrons. The resulting data is then used to construct a 3D image of belowground root architecture. Both X ray CT scans and MRI scans have mainly been used to evaluate root traits within pot-grown plants. A direct comparison of the two methods showed that CT scans tended to have a higher spatial resolution than MRI.

GPR also uses electromagnetic (EM) radiation. Returned information is similar in nature to seismic data, and data has traditionally been examined using seismic analysis methods. GPR has several characteristics that make it ideal for examining belowground biological materials: It is non-invasive, non-destructive, and data collection is rapid. The emitted waves reside in the radio/microwave portion of the EM spectrum and can record data at a range of depths depending on the frequency of the output signal, the soil matrix environment, and the antenna design. As a rule of thumb, higher frequencies (greater than 1.0 GHz) result in lower penetration depths. This is due to greater energy absorption of free water and scattering. Penetration depth and frequency are inversely related, and range from 100.0 m at 50 MHz to 0.1 m at 50 GHz. Penetration depths of agricultural soil subsurfaces are complicated by higher average water content signal attenuation. One benefit of higher soil water content is an increase in resolution in the time domain. As the signal velocity varies with dielectric permittivity, smaller distances are traversed with the same number of collected samples. High-intensity reflections are created when the EM energy encounters a media interface with a high difference in relative dielectric. Water, with a relative dielectric of 81, creates a high contrast with the surrounding soil which typically has a dielectric of ~4. This fact can be leveraged for small root detection.

## 22.11 Ground Penetrating Radar Application in Life Sciences

Due to their larger size, the coarse roots of trees are more easily detectable by GPR. As such, the majority of early root studies using GPR have been used to mensurate tree root biomass or the biomass of large tuber-like roots of cassava (*Manihot esculenta*) [40]. However, Liu et al. [41] recently performed a study which used GPR to detect the roots of wheat (*Triticum aestivum* L.) in field conditions, while Wolfe 2021 [34] developed both a novel GPR instrument, and new data processing methods to quantitatively discriminated fibrous root versus rhizome biomass.

## 22.12 Trait Introgression Versus Integrated Yield Selection Strategies for Heat Stress Tolerance

As discussed in detail in Chap. 23 on drought, a careful analysis of the efficacy of breeding specific adaptive traits versus direct selection for yield components is a worthwhile exercise. In traditional breeding programs, selection for disease resistance and flowering time attributes are prioritized while selection for heat stress adaptive physiological and morphological traits are rare. Direct selection for yield under multi-locational trials is considered the most efficient method for combining the best adaptive traits for heat stress and water-stress. However, identifying novel physiological and morphological traits in adapted genotypes for introgression into elite breeding lines is a valid method to improve overall heat stress tolerance in breeding programs. Following introgression, high throughput remote sensing based phenotypic selection of novel heat adaptive traits can be used as a compliment to direct yield selection to discriminate the potential yield benefit of the given traits. Markers for key adaptive traits such as high EW and glaucousness have been identified. Markers linked to developmentally regulated EW deposition should be prioritized. As well, introgression of high EW can utilize new hyperspectral EW spectral indices [22]. When selecting for yield, attention should be given to selecting for both high yield and ideal high single kernel weight and kernel dimension stability across multi-location high temperature trials. Lines which show yield instability and high variation in single kernel weight and dimensions should be discarded as heat susceptible and responsive to heat stress ethylene regulated early transitions to the dry seed stage. Novel high throughput digital kernel weight and dimension instruments can be used to improve the accuracy of direct yield selection for heat stress tolerance.

## 22.13 Key Concepts

- High confidence predictions state that increasing use of fossil fuels and inorganic fertilizers will continue to increase global temperatures, further challenging wheat productivity and its capacity to meet a growing population's food needs.
- Both acute and chronic high temperature stress in addition to high night temperatures are recognized as important limiting factors affecting wheat productivity.
- Heat stress impairs emergence by reducing coleoptile elongation, speeds the transition to flowering thus exposing wheat to spring frost injury. During reproductive development acute heat stress can suppress sucrose hydrolyzing invertases reducing assimilate translocation to developing pollen and embryos resulting in pollen sterility, kernel abortion, or a transition to early dry seed stage thus negatively effecting yield and end-use quality.
- Many of the negative impacts of heat stress are regulated by the plant hormone ethylene.

- Development or selection of heat stress ethylene insensitive genotypes for improving heat tolerance has demonstrated efficacy in improving heat tolerance in other cereals.
- Novel traits which reflect excess solar radiation, such as increased EW and glaucousness, or avoid direct solar radiation, such as erect leaves, have been shown to be useful in reducing wheat leaf and glume temperatures and are important breeding targets for improving heat tolerance.
- Both molecular markers for marker-assisted selection and hyperspectral crop indices which select for leaf EW have been developed. Both can be used to discriminate between heat tolerant, developmentally regulated high EW genotypes versus genotypes exhibiting stress induced EW.
- Novel ground penetrating radar tools and data processing software have been developed to aid in the selection of deep root wheat lines, with high root biomass for enhanced CO<sub>2</sub> sequestration into soils.
- Traits such as rhizomes should be transferred from wheat relatives to significantly increase wheat's soil carbon sequestration potential and contribution to climate change mitigation.

## 22.14 Summary

Novel strategies can be employed to define unique traits that confer improved adaptation to heat stress in combination with improved drought stress. Traits such as deeper root architectures, erect leaves and increase epicuticular leaf and glume wax help reflect or avoid heat stress by reflecting excess photosynthetic solar and thermal infrared radiation. These traits common to heat adapted species when optimized in wheat, help moderate internal plant temperatures, avoid induction of yield limiting hormone stress pathways regulated by ethylene while conserving excess water loss through transpirational cooling. In this manner, heat stress adaptive traits help conserve water for optimal growth and yield. When combined with traits that increase wheat's soil carbon sequestration potential, they may improve wheat's role as an essential food staple for the earth's growing population.

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