

EXPLORATION OF GENETIC VARIATION FOR HEAT TOLERANCE
IN WHEAT: A GLOBAL CORE COLLECTION AND
EMS MUTAGENESIS APPROACH

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DEDICATION

This thesis is dedicated to my grandfather, Dr. William Jellema. His love of education, particularly teaching, was evident throughout his lifetime. Thank you, Grandad, for loving your family so well and for inspiring me, and countless others, to pursue knowledge.

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ABSTRACT

Wheat, one of the world's most important crops, faces unprecedented challenges as climatic variability increases and finite resources such as soil and water degrade. Although wheat occupies the largest total harvested area among cereals, its total productivity remains the lowest due to abiotic stress-induced losses. The main challenge for breeders lies in developing wheat cultivars that can sustain or boost their productivity amidst stressful conditions. To address this challenge, screening methods at various developmental stages are needed to evaluate wheat lines under heat stress. Screening was conducted under controlled conditions in growth chambers at the seedling stage under a continuous heat stress of 40°C for four days and at all developmental stages under a continuous diurnal heat stress of 35/28°C (day/night) temperatures for the entire life cycle. To evaluate existing and novel genetic variation for heat tolerance, both screens utilized a global germplasm collection and an EMS (ethyl methanesulfonate) population using Montana cultivars MT Sidney and Dagmar. The goal was to identify and leverage genetic diversity in heat tolerance for eventual development of Montana-adapted, heat tolerant wheat varieties. The seedling stage screen of the germplasm collection revealed 51 heat tolerant wheat lines, three of which exhibited repetitive tolerance. Using the same germplasm collection, the full lifecycle screen revealed 27 heat tolerant wheat lines that maintained vegetative, reproductive, and post-reproductive growth and development under diurnal heat stress conditions. Four of these lines correlated with the seedling stage screen and thus may exhibit tolerance under heat stress conditions from seedling stage all the way until senescence. Mutagenesis revealed ten MT Sidney M₂ lines that outperformed all controls during the seedling stress screen. Additionally, five Dagmar M₂ lines maintained vegetative, reproductive, and post-reproductive tolerance under full lifecycle heat stress conditions. Because both MT Sidney and Dagmar M₂ lines exhibited better heat tolerance than their respective controls, some sort of heat response advantage was likely conferred via EMS mutagenesis. This study emphasizes the importance of germplasm material for identifying abiotic stress tolerance using various screening methods. Mutagenesis techniques are also pivotal for the development of abiotic stress tolerant crops.

CHAPTER ONE

LITERATURE REVIEW

Introduction

Plants are sessile organisms that are forced to acclimate and adapt to their changing environments (Luhua et al., 2013) physiologically and molecularly (Hikaru Sato, Mizoi, Shinozaki, & Yamaguchi-Shinozaki). Short-term acclimation responses can develop into an array of intricate and diverse long-term adaptation mechanisms at different levels (Tinashe Zenda, Nan Wang, Anyi Dong, Yuzhi Zhou, & Huijun Duan, 2022). To adapt, plants perceive environmental cues through primary sensory mechanisms which then trigger pathways to coordinate physiological and morphological adaptations for acclimation and survival (Lamers, van der Meer, & Testerink, 2020). For crop plants, the ability to adapt to changing environmental conditions is crucial for ensuring growth and productivity and, like other plants, crop plants undergo physiological and molecular changes to cope with challenging conditions. Understanding the primary sensory mechanisms and signaling pathways involved in stress perception is essential for developing tolerant crop varieties through breeding or genetic engineering (Lamers et al., 2020).

Impact of Abiotic Stresses on Crop Productivity

Variations in abiotic stresses, such as temperature and precipitation, severely limit crop plant development and productivity (Sewelam et al., 2020), more so than any biotic stresses the plants may encounter (Kumar Abhinandan et al., 2018). For context, yield losses due to abiotic stresses are reported around and above 50% while losses due to biotic stresses are reported

around 35% (Baillo, Kimotho, Zhang, & Xu, 2019). Recent climate models predict severe losses in overall global crop production due to increasing temperatures, however in wheat, this loss can be as large as 42 metric tons per one degree Celsius (Kumar Abhinandan et al., 2018). As global temperatures increase, the frequency of other disastrous climatic events such as droughts and heatwaves will also increase (Sewelam et al., 2020) especially in terrestrial regions (Tinashe Zenda et al., 2022). These abiotic stresses in conjunction with the continuing degradation of our soil and water supplies present a unique challenge to producers and breeders as they try to sustain crop productivity amidst a failing environment (Kumar Abhinandan et al., 2018). To successfully face this challenge, it is crucial to identify and manipulate the genetic mechanisms conferring stress tolerance in crops. Only then can we improve agricultural sustainability and food security in the face of climate change.

Globally, cereal grain crops such as wheat, maize, and rice are most important for providing daily calories and protein (Kumar Abhinandan et al., 2018). Among United States field crops, wheat ranks third in planted acreage, production, and gross farm receipts (Andrew Sowell, 2023). In the marketing years 2023 and 2024 alone, U.S. farmers produced 1.8 billion bushels of winter, durum, and spring wheat from a harvested area of 37.3 million acres (Andrew Sowell, 2023). Although occupying the largest total harvested area among cereal crops, wheat has the lowest overall productivity due to abiotic stresses such as drought, salinity, and high temperatures (Kumar Abhinandan et al., 2018). Given wheat's significant global presence in agriculture, the impact of abiotic stresses on its productivity warrants producers' attention. For instance, a simulation model based in Australia predicted reduced wheat yield up to 50% when growing season temperature was two degrees Celsius higher than the average (Fu, Bowden,

Jagadish, & Prasad, 2023). This highlights the need for understanding the underlying genetic mechanisms involved in abiotic stress response in wheat.

Plant Responses to Heat Stress

Heat stress occurs when temperatures exceed a threshold, usually by 10-15°C, long enough to cause irreversible damage to the growth and development of the plant (Fu et al., 2023) such as plasma membrane injury, reactive oxygen species overproduction, and protein denaturation (Tinashe Zenda et al., 2022). These periods of heat stress are transient, occurring in heat waves, (Tinashe Zenda et al., 2022) and often coincide with the sensitive stages of wheat development, such as flowering and reproduction, which decreases single seed weight (Fu et al., 2023) and ultimately leads to significant losses in grain yield (Ullah, Nadeem, Nawaz, Siddique, & Farooq, 2022).

Due to early domestication and plant breeding prior to the Green Revolution, the modern bread wheat (*T. aestivum*) has lost considerable natural genetic variation (Reif et al., 2005). This makes it challenging to breed or select for heat-tolerant varieties, necessitating targeted research and breeding efforts to enhance the crop's tolerance to high temperatures. Addressing these challenges becomes even more difficult when considering that heat stress is often compounded by concurrent stresses such as drought and salinity. These combined stresses have significant consequences for cereal production, as evidenced by a 10% U.S. cereal yield reduction between 1964 and 2007 due to drought and heat stress (Sewelam et al., 2020). While combined stressors like drought and salinity often converge in agricultural settings, compounding the challenges faced by crop yields, it's imperative to recognize the dominant role of heat stress in this scenario. Studies in *Arabidopsis* highlight heat's dominant impact on global gene expression and

metabolite synthesis, where the simultaneous induction of heat, drought, and salinity stress accounted for 46% of gene expression variation in response to heat stress, with it significantly inducing the expression of over 1,000 genes and reduced the expression of almost 700 (Sewelam et al., 2020).

Mechanisms of Heat Tolerance

Genetic elements and mechanisms responsible for conferring heat tolerance range from single, specific proteins to generalized modulations of gene expression. The plant cell uses many complex and not-well understood signaling processes to confer this tolerance. These specialized networks are able to coordinate sensing, response, and acclimation to fluctuations in water, soil and atmosphere temperature, nutrient toxicities, and more (Luhua et al., 2013). They also coordinate with plant growth and reproduction networks to optimize plant performance and survivability given their limited conditions (Luhua et al., 2013). Unraveling the processes that key genetic elements and tolerance mechanisms operate in is vital to breeding heat tolerant crops.

When screening for abiotic stress tolerance via phenotype, it is important to distinguish tolerance from avoidance stress responses. Common avoidance stress responses involve stomatal closure to minimize transpiration in the plant and conserve water (Hikaru Sato et al.), petiole elongation, accelerated flowering and hyponasty (Tinashe Zenda et al., 2022). These avoidance responses are temporary and short-lived and are characterized by alterations in morphology and development (Tinashe Zenda et al., 2022). Wheat can certainly exhibit these avoidance mechanisms, one of the most common being its tendency to mature early under heat stress conditions, and its ability to alter the time of day it flowers to avoid the hottest hours of the day

(Tinashe Zenda et al., 2022). Heat tolerance is defined as “a special trait ensuring the maintenance of essential plant functions at high ambient temperatures and contributes to the fitness and the productivity of the genotype” (Tinashe Zenda et al., 2022). Common tolerance stress responses include activation of reactive oxygen species (ROS) pathways, the accumulation of protective molecules such as HSPs, or elevated synthesis of compatible solutes (Hikaru Sato et al.). The mechanisms in which plants use these responses include the antioxidant defense system, transcriptional regulation, and the initiation of heat stress response genes (Tinashe Zenda et al., 2022). Over long periods of time, metabolic changes and gene expression modulation during many periods of stress acclimation can result in a more tolerant plant (Hikaru Sato et al.) through transgenerational memory mediated by transposons (Tinashe Zenda et al., 2022).

Throughout the entire plant, heat stress causes the dysfunction of membrane systems and biochemical pathways. When membrane structures break down, the proteins responsible for membrane management are denatured and metabolic imbalances occur when these toxic proteins accumulate in the cell (Hikaru Sato et al.). Plant photosynthetic pathways are severely damaged under heat stress conditions in this way, as indicated by the damaged structure and function of thermosensing proteins such as light receptors PHYTOCHROME B (PHYB), CRYPTOCHROMES (CRYs), AND UVB-resistance 8 (UVR8) which are inactivated under heat stress conditions (Hikaru Sato et al.). Another plant cell process impacted by heat stress is cell division. In eukaryotes, cyclin-dependent kinases and their associated activators, cyclins, and cell division cycle proteins work together to control the cell cycle. During heat stress in *Arabidopsis*, cell cycle genes as well as DNA synthesis and repair genes were all down-regulated (Sewelam et al., 2020). In addition, 234 genes coding for ribosomal proteins that localize to the cytosol after

synthesis were significantly repressed during heat treatment (Sewelam et al., 2020). Heat stress often reduces transcription of genes coding for photosynthetic proteins and proteins regulating the cell life cycle, while up-regulating genes coding for the degradation of proteins (Sewelam et al., 2020). This suggests that under heat stress, plants may arrest their cell cycles (Sewelam et al., 2020) and emphasizes the need for tolerant plants that are able to mitigate the impacts of heat stress on the cellular and molecular levels.

Antioxidant defense systems can be utilized by the cell to mitigate ROS accumulation under abiotic stress conditions. Under normal biological conditions, plant cells can naturally preserve equilibrium between ROS generation and equilibrium (Islam et al., 2024). Under heat stress however, plant cell membranes experience changes in membrane fluidity which lead to immediate and overgeneration of ROS and other solutes (Kumar Abhinandan et al., 2018). ROS proliferation is not limited to a specific location but instead occurs in many plant organelles including chloroplasts, peroxisomes, mitochondria, and the apoplast (Hikaru Sato et al.). This results in oxidative damage to cellular membranes, proteins, nucleic acid within the cell and can ultimately result in cell death (Hikaru Sato et al.). Heat stress tolerance mechanisms that respond to cellular ROS accumulation can employ enzymatic antioxidant systems (Tinashe Zenda et al., 2022). The enzymatic systems synthesize and distribute enzymes responsible for scavenging and detoxifying. Examples of these ROS-scavengers include superoxide dismutase (SOD), catalase, ascorbate peroxidase, and glutathione peroxidase (Hikaru Sato et al.; Tinashe Zenda et al., 2022). SODs often act as the first line of defense for ROS accumulation and respond by specifically converting superoxide radicals into less harmful hydrogen peroxide (H_2O_2) (Islam et al., 2024). This enhanced activity of antioxidant enzymes and reduced accumulation of ROS in maturing

anthers have been linked to an increased tolerance to heat stress, thereby preserving grain yield in wheat (Tinashe Zenda et al., 2022).

Plant cells also respond to heat stress via transcriptional regulation, post-transcriptional regulation, and protein production. To regulate transcriptionally, gene expression can be altered through alternative forms of histones such as H2A.Z directly overseeing the unwrapping and occupancy of heat regulated gene expression in *Arabidopsis* (Kumar Abhinandan et al., 2018). When this heat regulated gene expression is activated, genes such as *miR* genes may begin synthesis of micro RNAs that help contribute to thermotolerance within the plant by post-transcriptionally regulating heat stress responses products. Plant microRNAs are part of the heat response mechanism as they are employed to post-transcriptionally regulate genes through the cleavage of their respective mRNAs or through translation inhibition (Islam et al., 2024). Such miRNA families include the miR156 microRNA family which can promote and strengthen heat stress memory within plants (Islam et al., 2024). Additionally, chaperone proteins may activate under elevated temperatures and detach from heat-stress response transcription factors to allow the downstream heat stress response (Kumar Abhinandan et al., 2018). Similarly, ROS accumulation can also activate heat shock transcription factor HsfA1 which is often referred to as the master-regulator of plant heat response (Kumar Abhinandan et al., 2018).

The primary proteins that function in response to elevated temperatures are HSPs. HSPs represent the basic mechanism of thermotolerance in the plant due to their ability to prevent protein denaturation as well as refold damaged proteins (Hikaru Sato et al.). By protecting the protein structures, they act as master regulatory agents by limiting the potential for disrupted biochemical processes (Sewelam et al., 2020). The transcription factors responsible for HSP

production share a well-conserved structure comprising of an N-terminal binding domain that, when bound to heat shock elements, immediately induces HSP expression (Tinashe Zenda et al., 2022). As reported, HSFA1s function as the main transcriptional activators of heat stress response factors. They are upregulated when the plant encounters heat stress and subsequently modulated by the variation in high temperature (Tinashe Zenda et al., 2022). HSFA1 has been reported to interact with BRASSINOSTEROID INSENSITIVE 1 EMS-SUPPRESSOR 1 (BES1) in *Arabidopsis* which resulted in enhanced heat stress tolerance (Tinashe Zenda et al., 2022). Furthermore, overexpression of wheat HSF gene *TaHSFA6f* enhanced heat tolerance as well as other abiotic stresses (Tinashe Zenda et al., 2022).

In response to heat stress, the plant cell can also activate the adaptive unfolded protein response (UPR) mechanism which functions in the endoplasmic reticulum (ER) as well as the cytosol, depending on where the misfolded proteins aggregate (Tinashe Zenda et al., 2022). The ER UPR enhances the cell's ability to protect the folding proteins, preserves quality control of the synthesized proteins, and maintains degradation of the damaged proteins. These damaged proteins are then retrotranslocated to the cytosol to be degraded by the ubiquitin-proteasome machinery (Tinashe Zenda et al., 2022). Here in the cytosol, the cytosolic UPR mechanism takes place and functions differently than the ER UPR. When heat shock triggers HSFs, HSP gene expression is induced to preserve cytosolic protein homeostasis. This gene-dependent mechanism also can repair or remove damaged proteins to prevent irreversible damage to the cell (Tinashe Zenda et al., 2022). A small HSP gene involved in this mechanism is the wheat gene *TaHSP23.9*. When overexpressed transgenically in *Arabidopsis*, *TaHSP23.9* conferred enhanced tolerance to both heat and salt stress, suggesting a chaperone role in positively regulated plant heat stress

response (Tinashe Zenda et al., 2022). In another *Arabidopsis* study where heat stress was induced along with drought and salinity, it was found that most members of all HSP families were specifically induced by the induction of heat stress (Sewelam et al., 2020). Because of the extreme damage of cellular proteins and the subsequent expression of these HSPs due to heat stress, the authors concluded that elevated temperature is the most severe environmental stress (Sewelam et al., 2020).

Oftentimes, signal transduction pathways result in the synthesis of molecules such as proteins and solutes to help mediate the tolerance response. The influx of free cytosolic calcium levels is one of the most common responses to abiotic stress in plant cells (Kumar Abhinandan et al., 2018). In *Arabidopsis*, CYCLIC NUCLEOTIDE-GATED CALCIUM CHANNEL 2 (CNGC2), CNGC24, AND CNGC4 are all reported to help the influx of heat-shock-dependent Ca^{2+} into the cytosol (Finka, Cuendet, Maathuis, Saidi, & Goloubinoff, 2012; Gao et al., 2012; S. Lu et al., 2022). Calcium, however, is not the only molecule that helps mediate the heat response in plant cells. The accumulation of compatible solutes such as raffinose family oligosaccharides (RFOs), nontoxic low-molecular-weight solutes that lower the cellular water potential, can also confer tolerance under abiotic stress (Hikaru Sato et al.). These important metabolites are shown to operate under induction of heat stress and help confer thermotolerance (Serrano, Ling, Bahieldin, & Mahfouz, 2019; Song, Chung, & Lim, 2016). Specifically in cell membranes, the proportion of polyunsaturated fatty acids decreases under high temperatures to reduce membrane fluidity and maintain turgor pressure (Hikaru Sato et al.). In *Arabidopsis*, heat stress induction resulted in the accumulation of free amino acids, reflecting the plants effort to compensate for the down-regulated photosynthetic processes (Sewelam et al., 2020). By catabolizing proteins

and producing free amino acids, the plant confers an osmotic protective effect against heat stress (Wang, Vinocur, & Altman, 2003).

Hormone Signaling and Regulation

Hormone signaling processes also rapidly respond to heat stress. Hormones that are upregulated in response to heat stress include abscisic acid (ABA), ethylene, salicylic acid, and jasmonic acid (Kumar Abhinandan et al., 2018). ABA is suggested to play a role in plant acclimation to heat and salt stress. For example, *Arabidopsis* mutants deficient in ABA synthesis and signaling exhibit greater susceptibility to combined heat and salt stress (Tinashe Zenda et al., 2022). This enhanced susceptibility may be, in part, because ABA signaling can elicit activation of downstream effector HSFA6b in *Arabidopsis*, which is involved in the heat stress response (Tinashe Zenda et al., 2022). Ethylene-mediated signaling is also responsible for conferring heat stress tolerance. Specifically, the ethylene pathway transcriptionally regulates HSFs, such as *HSFA1a* and *HSFA2a*, as well as ethylene signaling related genes (Tinashe Zenda et al., 2022), acting primarily as a regulator of the heat stress response. Salicylic acid (SA) and jasmonic acid (JA) can collaborate to confer basal thermotolerance in wild type *Arabidopsis*. When methyl Jasmonate (MeJa) was exogenously applied to wild type plants under heat stress, the plants accumulated an array of jasmonates which activated the antioxidant defense system (Tinashe Zenda et al., 2022), enhancing the plants' inherent ability to survive above optimum temperatures. After accumulation of the jasmonates and activation of the antioxidant enzyme activities, salicylic acid begins a helpful regulatory role and assists JA in conferring heat tolerance. Additionally, in wheat, SA can help maintain photosynthetic processes under heat stress conditions by enhancing amino acid Pro biosynthesis via the enhanced λ -glutamyl kinase

and decreased proline oxidase activity (Tinashe Zenda et al., 2022). This is specifically accomplished through the promotion of osmotic potential and water potential and through the restricted formation of ethylene in heat stressed plants. Overall, SA interacts with proline metabolism and ethylene formation to alleviate heat stress effects in wheat (M. I. Khan, Iqbal, Masood, Per, & Khan, 2013).

While the upregulation of certain hormones can help initiate a protective response under heat stress, the downregulation of others suggests a strategic reallocation of resources within the plant, resulting in nuanced regulatory mechanisms at the molecular level. Phytohormones that are downregulated include auxins, cytokinins, gibberellic acid, strigolactones, and brassinosteroids (Kumar Abhinandan et al., 2018). Adding to this complexity, temperature-sensitive miRNAs play a significant role by fine-tuning the hormonal heat stress response through post-transcriptional regulation. By post-transcriptionally regulating these signaling pathways, there are major differential expression patterns across wheat cultivars with varying degrees of heat tolerance (Islam et al., 2024). These intricate and extremely variable heat stress responses observed in wheat underscore the critical need for a deeper understanding of the mechanisms underlying the heat stress response. Moreover, it emphasizes the necessity for standardized screening approaches to accurately identify and select tolerant wheat lines, thus aiding in the development of heat-tolerant cultivars.

High-throughput Screening and Characterization

High-throughput screening technologies play interconnected roles in studying and understanding the molecular mechanisms underlying abiotic heat stress responses in plants, including crops like wheat. Through the application of DNA microarray technology, it is possible

to identify genes/genomic regions with altered mRNA abundance after abiotic stress induction. Unfortunately, many of the identified genes are unknown or poorly characterized, hindering the understanding of abiotic stress signaling (Horan et al., 2008). Similarly, identification and characterization of miRNAs that function in response to heat stress is also limited (Islam et al., 2024). As of 2021, over 600 plant genomes have been sequenced but still little is known about the specific plant genes responsible for the complex processes the genomes code for (Depuydt & Vandepoele, 2021).

To understand the mechanistic complexity of wheat stress response, there is a need for thorough gene function characterization, a critical step in unraveling the complex signaling cascades that govern plant stress responses (Depuydt & Vandepoele, 2021). The two major approaches for identifying and characterizing gene function are the forward- and reverse-genetic approaches (Hikaru Sato et al.). Forward-genetic approaches such as the use of germplasm collections, sequencing pangenomes, and high-throughput phenotyping allow us to identify important genes and alleles while reverse-genetic approaches such as the use of the *Arabidopsis* model system, omics, and transgenics may offer more conclusive characterization (Hikaru Sato et al.). Ultimately, it falls upon the researcher or breeder to discern which functional study approaches are best aligned with the specific requirements and objectives of the project, ensuring effective elucidation of wheat stress response mechanisms, and facilitating the development of tolerant cultivars.

The large number of undefined or poorly characterized genes involved in wheat regulatory networks coupled with the expansive genome that wheat boasts, severely limits the current scope of understanding (Luhua et al., 2013). Despite recent advances in biotechnology,

there remains a significant gap in the functional characterization of genes involved in wheat stress responses. Omics-supported functional annotations in *Arabidopsis* may help with a general understanding of developmental and molecular processes, including plant responses to abiotic stress (Depuydt & Vandepoele, 2021). However, a recent study of various abiotic stress treatments on *Arabidopsis* resulted in almost 40% of the differentially expressed genes being of unknown function (Sewelam et al., 2020). While *Arabidopsis*, rice and Brachypodium can offer enlightening fundamental network knowledge, functional studies in wheat remain limited (Kumar Abhinandan et al., 2018) and are needed for more precise understanding.

Because of the limitations of functional characterization, much of conventional breeding still relies on the classical approach, where breeders advance populations and conduct selection on their trait of interest (Crossa et al., 2017). Often, this is a phenotype to genotype approach, where the identity and heritability of the trait's genetic component is methodically determined. With classical breeding, selection is often conducted phenotypically. Furthermore, classical breeding approaches utilize breeding material or cultivars to make breeding populations, limiting the availability of genetic variation for the trait of interest since these lines are mostly fixed for many traits. The classical approach is time-consuming and costly because it involves the maintenance and selection of extremely large populations over a long period of time, often lasting 10-15 years. Classical breeding has evolved over the years, now utilizing conventional methods such as mutagenesis, synthetics, and transgenics to develop abiotic stress tolerant lines. These methods remain the most successful way to mitigate heat stress crop damage in the US (Fu et al., 2023) by introducing additional genetic variation and helping transfer desirable traits into cultivars. Despite this success, conventional breeding is still challenged by the evaluation of

large plant populations in various environments as well as in varying stages of development (Fu et al., 2023). This process is still quite labor and time intensive due to the mechanistic complexity of multigene families (Kumar Abhinandan et al., 2018) and the phenotypic variability that is characteristic of such a complex mechanistic response. Technological advances such as genomic selection certainly can optimize this process; however, genotyping costs and the undefined guidelines as to where genomic selection can be applied in breeding programs remain two primary challenges (Crossa et al., 2017).

To develop a standardized protocol for abiotic stress screening of wheat lines, screening protocols need to be tested at various developmental stages (seedling vs. reproductive), in various environments (controlled vs. field), and under varying heat stress conditions (continuous vs. diurnal), to name a few. Additionally, the screening of abiotic stresses alone vs. combined stresses would provide valuable insight into plant response. Expansive testing would help functionally characterize wheat traits involved in the stress response and develop optimized screening approaches. Given the pressing demand for abiotic stress-tolerant crops, there are abundant practical examples demonstrating the application of these principles in the development of abiotic stress screening protocols.

In one study, to develop a phenotyping protocol for combined drought and salinity stress at the seedling stage in rice, phenotypic characterization was conducted at the seedling stage with an experimental tray system inside a growth chamber, as well as in a greenhouse growth room throughout the entire life cycle of the rice plants. The intention was to characterize stress responses of the rice cultivars and evaluate different measures of heat stress tolerance that could be used in future seedling stage stress screening (S. Kota et al., 2023). It was found that rice

cultivars had distinct stress responses under the combined stresses when compared to drought or salinity stress alone. Furthermore, under full lifecycle heat stress conditions, they identified chlorophyll fluorescence during the vegetative stage as a reliable indicator of later grain yield (S. Kota et al., 2023). This study illustrates the beneficial impact of incorporating various developmental stages into screening approaches, demonstrating how phenotyping protocols conducted at multiple stages can reveal potential indicators for crop performance under challenging conditions.

Germplasm Resources and Mutagenized Populations

Utilizing germplasm resources that offer plenty of genetic variation is a key step in identifying tolerant lines. A multiple abiotic stress tolerance screening study on rice seedlings utilized a germplasm collection composed of native landraces, breeding material, and cultivars to identify stress tolerant lines. The average genetic distance between the germplasm lines was 0.443, demonstrating the wide array of genetic diversity present in this study. Using defined indices and known QTLs, they successfully identified four potential germplasm lines for abiotic stress tolerance (Kumar et al., 2024). This study emphasizes the potential success for breeding programs to consider germplasm collections when developing abiotic stress tolerant lines.

An invaluable resource of existing genetic variation lies in the USDA-ARS National Plant Germplasm System collections. These collections of wheat accessions vary in their habit, origin, subspecies differentiation, level of improvement and more, making it possible to access a wider base of genetic diversity for abiotic stress studies. It is important to note that plant breeding does not necessarily lead to a reduction in genetic diversity if intensive selection is counteracted by introgression of novel germplasm (Reif et al., 2005). However, historically, the

decrease in genetic diversity via domestication experienced two main drivers: the initial samples chosen for domestication represented only a small subset of the wild gene pool and the subsequent selection pressure reduced population sizes through the effects of substitution load (Allaby, Ware, & Kistler, 2019). However, the immediate challenges of utilizing germplasm are the genomic redundancy of wheat and the limited biotechnological resources available for understanding gene function compared to the resources available in *Arabidopsis* and *Brachypodium* (Kumar Abhinandan et al., 2018). By increasing the amount and type of screening studies conducted on abiotic stress tolerance, these limitations can be targeted for easier selection of heat tolerance in crops.

Another source of genetic variation in crops lies in the creation of artificial mutant populations, where mutations are induced throughout the crop genome. A mutation is “a sudden, heritable alteration in genetic material of a living cell induced by mutagens” (Raina et al., 2021). When spontaneous mutations occur in the natural environment and confer an advantageous trait, they are naturally selected for and contribute to the genetic variation we see in the environments around us.

To create novel genetic variation in breeding populations, chemical mutagens can be utilized. As mentioned prior, mutagenesis is a popular, conventional technique that when mixed with classical plant breeding techniques, can offer monumental advantages to breeders. The primary advantage lies in the fact that genetic variation is created in and selected for in high-yielding, locally adapted cultivars. There is no need for intensive selection of the trait of interest amidst an undesirable genetic background, as the cultivars already contain genetic material adapted to the local region. Additionally, mutagenesis provides an alternative to transgenics, is

quick and cheap, and has proven successful already in many crops. Recent EMS induced line developments include Clearfield rice for herbicide resistance (Sudianto et al., 2013) and salinity tolerance (Arun, Choudhary, Dharminder, & Kuldeep, 2021), wheat for salinity tolerance (Lethin et al., 2022) and drought tolerance (Zahra et al., 2022), and silage maize for salinity tolerance (Cho et al., 2020).

Ethyl methanesulfonate (EMS) mutagenesis is a popular method of chemical mutagenesis and is characterized by the high multitude of random point mutations it randomly induces throughout the genome. EMS acts on deoxyribonucleic acid (DNA) by randomly alkylating guanine bases through the addition of an ethyl group and subsequent modification of its hydrogen bonds. This addition and modification mechanism results in the guanine base pairing with thymine instead of cytosine which subsequently results in an A-T base pair instead of a C-G (L. Chen et al., 2022; Raina et al., 2021). EMS is a frequently used chemical mutagen because of its efficacy and efficiency. Furthermore, it creates a broader spectrum of mutations than other chemical mutagens available (Raina et al., 2021).

Conclusion

This literature review elucidates the various mechanisms through which plants, especially crop species like wheat, rice, and maize, adapt to abiotic stresses—a significant concern due to climate change's impact on agriculture. Plants have developed complex systems to sense environmental stresses and initiate molecular and physiological responses. Acclimation strategies, which might initially be short-term, can lead to long-term adaptive changes in plants at multiple biological levels. With abiotic factors such as temperature and precipitation

drastically affecting crop productivity, understanding the molecular and genetic basis for stress responses is critical for developing tolerant crop varieties.

Abiotic stresses, particularly temperature extremes, are more detrimental to crop yield than biotic stresses, with predictions indicating substantial losses in global crop production due to rising temperatures. For instance, wheat, a major staple, is susceptible to reduced yield because of its sensitivity to heat, drought, and other stressors, highlighting the urgent need for genetic studies aimed at enhancing heat tolerance. Modern wheat varieties, having undergone extensive breeding, have lost much of their genetic diversity, making it challenging to select for traits such as heat tolerance. As such, research into genetic mechanisms conferring stress tolerance, including the use of *Arabidopsis* as a model organism, is paramount.

The review also discusses the role of various proteins and signaling pathways that mediate plant responses to heat stress. Heat stress proteins (HSPs), transcriptional and post-transcriptional regulatory mechanisms, and antioxidants are all part of the plant's arsenal against cellular damage caused by elevated temperatures. Advanced techniques such as high-throughput screening and the study of microRNAs are critical in unraveling these complex responses, despite the challenges presented by the vast number of uncharacterized genes. Ultimately, integrating forward- and reverse-genetic approaches, along with conventional breeding and modern biotechnology, is suggested to enhance our understanding and improve the heat stress tolerance of crop plants.

This review underscores the need for a multi-faceted approach to address the challenges of climate change on agriculture. By dissecting and manipulating the complex genetic and molecular networks that govern plant stress responses, the goal of developing crops capable of

thriving under adverse conditions may be realized, thus ensuring food security in an increasingly unpredictable global climate.

CHAPTER TWO

DEVELOPMENT AND UTILIZATION OF A SEEDLING
STAGE SCREENING HEAT STRESS PROTOCOL TO
IDENTIFY HEAT TOLERANT LINESAbstract

Identifying and characterizing crop abiotic stress tolerance is of vital importance as global climate variability intensifies and tolerant, versatile crops are needed to meet food demands. Despite advances in genomic technology, breeding efficiency is still delayed by the population advancement, precise selection, and successful introgression of the traits into popular cultivars which can take over a decade. The identification of heat-tolerant wheat genotypes through early-stage screening would optimize breeding programs by allowing early selection of the trait of interest. Early selection would result in smaller, yet representative populations to work with thus saving time and resources. This study presents the findings from a comprehensive seedling stage heat stress screen in wheat (*Triticum aestivum*). Utilizing a core collection of 1,095 wheat accessions encompassing a diverse range of origins, habits, and levels of improvement, we subjected these accessions to continuous heat stress of 40°C for four days beginning at the one/two leaf stage. The objective was to discern which germplasm lines exhibited seedling stage tolerance to heat stress. Germplasm lines that exhibit heat tolerance at the seedling stage under controlled conditions may also exhibit tolerance during crop establishment under field conditions. Through visual assessment of heat stress symptoms and the absence or presence of the second leaf, we identified lines that exhibited early tolerance to

extreme, continuous heat stress. Most identified tolerant lines were spring landraces originated from the Middle East. The results of this seedling stage screen were compared with data from a full lifecycle heat stress screen to evaluate consistency in stress tolerance across different developmental stages. This approach enables a nuanced understanding of heat stress tolerance mechanisms in wheat at early developmental stages, facilitating quicker identification of tolerant wheat lines suitable for breeding programs.

Background

Temperatures are becoming increasingly variable. In Montana from 1950 to 2007, March temperatures significantly rose by 0.08 degrees annually (Lanning et al., 2010). Rising early-season temperatures results in earlier planting dates for producers; since 1950, the spring wheat planting date in Montana has advanced almost two weeks, allowing producers to take advantage of the longer growing period (Lanning et al., 2010). While these conditions temporarily confer advantage by lengthening the growing season, this advantage certainly will not last. As temperatures slowly increase, climatic variability will also increase (Lanning et al., 2010). Historically, the strategy to mitigate heat stress in crops has involved optimizing planting schedules where feasible. By carefully selecting the spring sowing date, it is possible to align anthesis with cooler temperatures while also avoiding late-season frosts (Thistlethwaite, Tan, Bokshi, Ullah, & Trethowan, 2020). For winter forage wheat planted early in the fall, heat stress coincides with crop establishment reducing forage and grain yield (Maulana et al., 2018). In the context of a changing climate characterized by increasing variability, precisely timing sowing to evade heat stress is becoming more challenging, complicating the prediction and avoidance of

heat stress events. Herein lies the need for heat tolerant crops that exhibit tolerance not only at the reproductive stage, but also in early crop establishment and biomass production.

Early selection for abiotic stress tolerance in wheat is being explored for heat, cold, drought, salinity, and cadmium (Lone et al., 2022; Lu, Liu, Wu, & Yan, 2022; Shahzad, Gul, Ahsan, Wang, & Fahad, 2023; Shilin et al., 2021; Tao et al., 2021). Abiotic stresses limit crop production worldwide and when experienced simultaneously, can elicit greater damage on crop yields than when experienced alone (Suneetha Kota et al., 2023). Developing tolerant crop cultivars capable of maintaining early growth and development while also sustaining their yield is of the utmost importance considering the ever-present, increasing climatic variability. Historically, selection of tolerant varieties has been conducted at the reproductive stage which is considered the most sensitive stage of crop development to abiotic stresses (Fu et al., 2023). While this selection approach has merit, it requires much more time and space to conduct selection, and ultimately delays the breeding process. Additionally, mechanisms conferring heat tolerance may be present throughout the entire life cycle; thus, early selection would be advantageous. Overall, seedling stage screening for abiotic stress tolerance would quicken identification of tolerant lines and narrow down breeding populations faster through efficient phenotyping and high-throughput characterization (L. Lu et al., 2022). Breeders could then verify the abiotic stress tolerance during other developmental stages as well as in different environments, under varying intensities and/or durations of the stress. Currently, a variety of protocols are being explored for early screening of abiotic stresses, including drought and salinity tolerance in rice (Suneetha Kota et al., 2023; Kumar et al., 2024), drought and salinity

tolerance in maize (Akram, Mohsan, Ashraf, Ahmad, & Waraich, 2010; Avramova et al., 2016), and more.

The overall goal of breeders is to reduce cycle time for their programs (Merrick, Herr, Sandhu, Lozada, & Carter, 2022). Incorporating genomic selection into early-stage screening approaches is a dual approach that may meet this need. Without genomic selection, but with doubled haploids and offseason nurseries, maize still needs a minimum of two years for test cross performance selection (Heffner, Sorrells, & Jannink, 2009). In this situation, genomic selection could reduce unit time per selection cycle by reducing the need for progeny test data in every cycle (Heffner et al., 2009). An even more extreme case is the oil palm, a crop that needs at least 19 years for a selection cycle without genomic selection (Tester & Langridge, 2010). With genomic selection, this cycle could be decreased to six years (Heffner et al., 2009). Other than the extensive time needed for selection cycles, the conservation of yield stability is vital for the release of a variety. Before release, the crop needs to be fixed for yield stability which is harder to select for than improved yield. Ensuring that a variety has stable yields before releasing requires many years and many sites for evaluations (Tester & Langridge, 2010).

The strategic use of germplasm material is essential in the context of limited genetic diversity resulting from intensive domestication, that is so common in popular cultivars. Germplasm banks, like the USDA-ARS-NPGS, serve as crucial reserves of genetic variation, preserving ancestral traits of major crops. These traits, once identified and selected through targeted screening, can be incorporated into local cultivars through breeding programs, potentially enriching the genetic landscape of cultivars, and enhancing abiotic stress tolerance.

Materials and Methods

Plant Materials

The selected core collection of wheat accessions utilized in this study was obtained from the United States Department of Agriculture - Agricultural Research Service - National Plant Germplasm System (USDA-ARS-NPGS). This core collection encompasses a total of 1,095 wheat accessions, offering a broad spectrum of genetic diversity reflective of a wide range of geographical origins, levels of improvement, subspecies differentiation, and growth habits. The diversity encapsulated within this collection provides a unique and valuable resource for exploring genetic variability in response to abiotic stresses, including heat stress. The accessions within this collection were carefully selected to represent the global diversity of wheat, ensuring a comprehensive genetic base for the study.

Protocol Development

The development of our heat stress tolerance assay for *Triticum aestivum* began with a preliminary protocol that used three-centimeter diameter, cylindrical, 20-cell trays with no drainage. Cells were filled with pre-moistened medium. The medium used was a blend of Canadian Sphagnum peat moss and horticultural grade Perlite. This medium was buffered with Dolomitic lime and contains a starter fertilizer charge and wetting agent. The medium is prepared at the Plant Growth Center at Montana State University. The tested pH of the medium is around 5.8 and electrical conductivity is 1.5 dS/m. Seeds were sown one per cell and four seeds of each germplasm accession were planted. They were then lightly covered with medium. The cells were lightly sprayed with tap water daily throughout the entire protocol to maintain field capacity and avoid over/undersaturation. Stratification was initially conducted at 4°C for one day in a

Precision Scientific Low Temperature BOD Incubator. Immediately afterwards, the cells entered a germination period at 25°C for three days in a growth chamber (model CU-14L4). In this same chamber, heat stress was then induced at 42°C continuously for five days. It is important to note that each of the three periods were consecutively executed.

The final, optimized protocol for screening heat stress tolerance in *Triticum aestivum* seedlings used 72-cell trays set inside 1020 trays. Four seeds of each accession were screened. Distilled (DI) water was used to pre-moisten the medium and to subsequently top and bottom water the cells throughout the screen. Proper soil moisture levels were maintained throughout the entire protocol to prevent selection for drought stress. The seeds were stratified at 4°C for two days with DI water spraying at the 24-hour mark. Immediately after, trays were transferred to the Percival chamber for germination and growth at 25°C for three days. Consistent moisture levels were maintained through bottom watering via the 1020 trays. At this point, germination rates were recorded, and any additional germination afterwards was also noted. In the same chamber, seedlings were then exposed to 40°C continuously for four days of heat stress, with germination rates and any heat stress symptoms meticulously documented each day of stress. After the heat stress period, the chamber temperature was decreased back to 25°C for two days to facilitate seedling acclimation before transplanting into the greenhouse. Greenhouse conditions were 22/18°C day/night temperatures with an extended photoperiod of 16 hours.

Fully acclimated seedlings were transplanted into six-inch diameter pots with the same pre-moistened medium. Winter accessions had a one-to-two-week recovery in the greenhouse before a seven-week vernalization period at 4°C. During this period, winter lines were watered once a week. Spring habit seedlings grew in greenhouse conditions and were fertilized weekly

with 20-20-20 fertilizer until senescence. Winter habit seedlings followed the same protocol after their vernalization period. Upon reaching senescence, seedheads were harvested, dried, and threshed. This protocol was conducted for all 1,095 lines initially and then four replications were conducted of the selected top tolerant and top susceptible lines.

Visual Characterization of Lines During Heat Stress

The most common symptoms of heat stress for wheat seedlings were chlorosis and leaf curling and rolling. Often, all three symptoms were observed on susceptible seedlings. The presence and exacerbation of these symptoms was recorded each day during the heat stress period for all 1,095 germplasm accessions. The top tolerant lines were selected based off the qualitative, phenotypic observations noted for all lines. First, all lines that exhibited heat tolerance throughout the initial screen were selected. These lines had produced at least one heat tolerant transplant from the heat stress screen, but sometimes as much as all four seedlings survived. Secondly, accessions that exhibited heat tolerance throughout the heat stress period but did not necessarily survive past the acclimation period or transplantation into the greenhouse were selected. Thirdly, accessions that maintained greenness throughout the leaves for most of the heat stress period were selected. Ultimately, these three rounds of selection resulted in the 81 top tolerant lines. These heat tolerant lines consistently exhibited “recovery phenotypes” where, despite the presence of symptoms, the seedling was still capable of maintaining growth and development. The two main indicators of a “recovery phenotype” were the absence of major rolling throughout the first leaf and the maintained growth of the second leaf. Seedlings exhibiting a recovery phenotype after transplanting varied little in terms of recovery. The only

noticeable variation was a slight difference in length of time before new leaf growth in some seedlings. All lines that recovered eventually went to seed.

Controls

Four wheat cultivars were used as checks in replications three and four to help discern relative tolerance and survival of germplasm lines when compared to popular Montana cultivars. Two spring cultivars - MT Sidney and Vida (Lanning, Carlson, Nash, Wichman, & et al., 2006) - and two winter cultivars - Bobcat and Yellowstone (Bruckner et al.) - were planted in the same trays as germplasm lines and treated according to the same protocol. Four seeds of each cultivar check were planted in each screen.

Harvesting

At senescence, all seedheads were harvested, including those that had not fully emerged. Observations such as the presence or absence of awns, whether the spike fully emerged or not, and any spike color were noted. The spikes were dried at 45°C for 48 hours and then threshed. If seed count was less than 10 seeds, that germplasm line was also noted.

Results

Germination Rates

After the heat stress screening protocol was finalized, all 1,095 lines of the selected core collection were screened. During this first run through, the average germination rate of all lines was 75.75%. Using the qualitative, phenotypic data from each day of heat stress and subsequent acclimation and transplant survival or death of the lines, the top heat tolerant lines were selected. Eighty-one lines were selected and rescreened four times under the same protocol to confirm true

tolerance. Notably, the increased average germination rate of the selected tolerant lines may indicate that lines with higher germination rates are more likely to be selected for heat stress tolerance (Table 1). Average germination rates of the susceptible lines during all four replications were slightly below the average germination rate of all lines (Table 1).

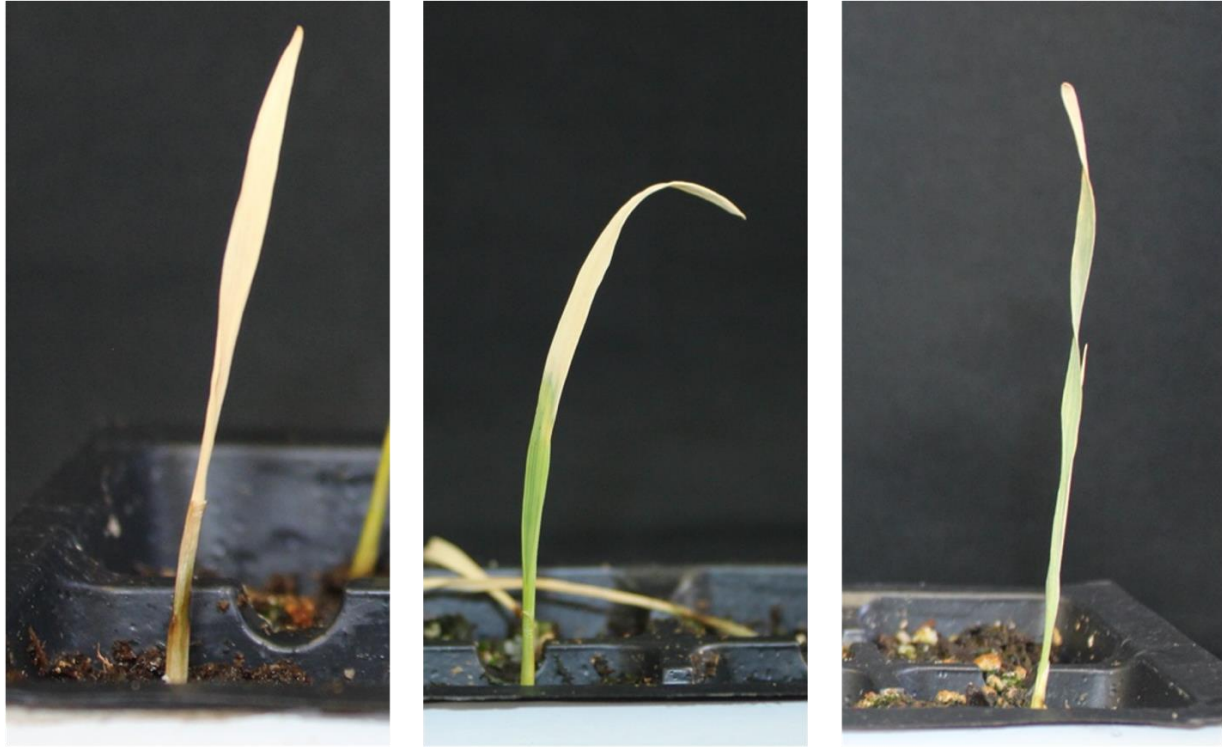
Table 1: Average germination rates of selected tolerant and susceptible lines throughout all four replications.

Average Germination Rates of Selected Lines		
Replication #	Tolerant Lines (81)	Susceptible Lines (84)
1	87%	69.3%
2	87%	74.5%
3	87.5%	73.5%
4	81.3%	75%

Phenotypic Characterization of Germplasm Lines

To characterize germplasm lines as either heat tolerant or susceptible during the seedling stage screen, observations of heat stress symptoms were noted for each day of the heat stress period. Chlorosis, curling, and rolling were the primary symptoms indicating that the seedling was heat susceptible (Figure 1). While rolling and curling can indicate avoidance mechanisms and be favorable under field conditions, it was discovered that seedlings exhibiting these phenotypes rarely survived the acclimation period and transplant to greenhouse, indicating lack of tolerance. Most often, susceptible seedlings exhibited a range of these symptoms before dying. Tolerant seedlings consistently exhibited recovery phenotypes, where heat stress symptoms were sometimes present, but did not ultimately prevent growth and development.

Figure 1: Common heat stress symptoms at the seedling stage. From left to right: chlorosis, curling, and rolling. All three symptoms were consistent indicators of seedling susceptibility.



Selected Tolerant Lines

The 81 selected heat tolerant lines had extremely diverse origins. Many of them originated around the Middle East (Figure 1), which is unsurprising considering *Triticum aestivum*'s ancient domestication in this area. Landraces consisted of 58.5% of the entire germplasm collection so naturally, most of the selected heat tolerant lines were landraces with a spring habit (Figure 2). These landraces exhibited a variety of wild, weakly domesticated phenotypes while being grown in the greenhouse. Examples of weakly domesticated phenotypes include extreme stem length variation in single plants, extreme lodging, and low number of spikelets.

Figure 2: Origins of the 81 selected tolerant lines. Colors indicate average surface temperatures.

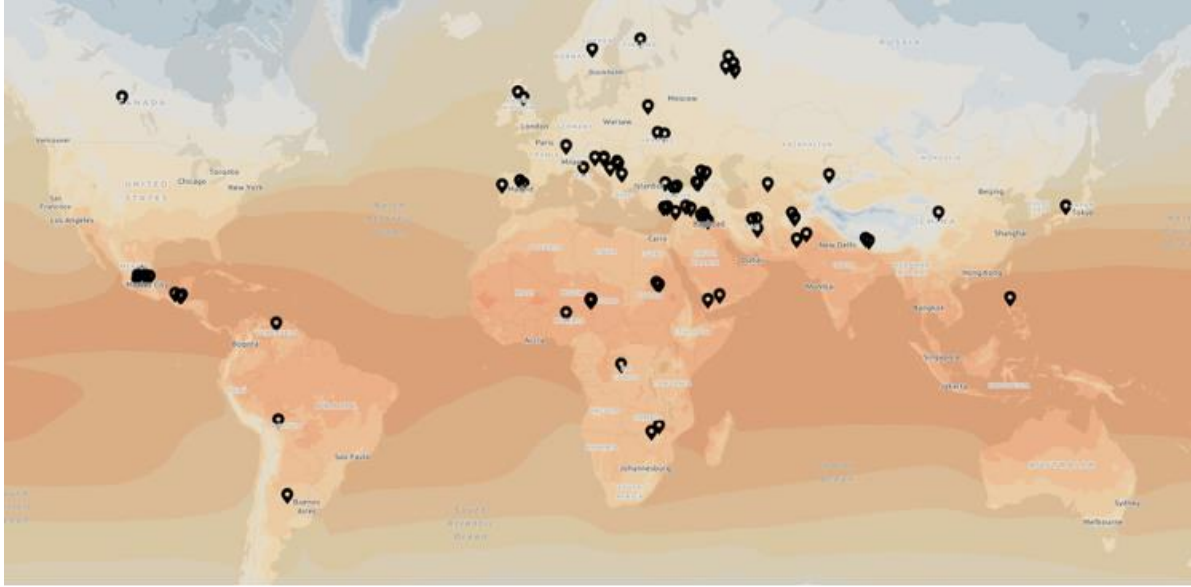
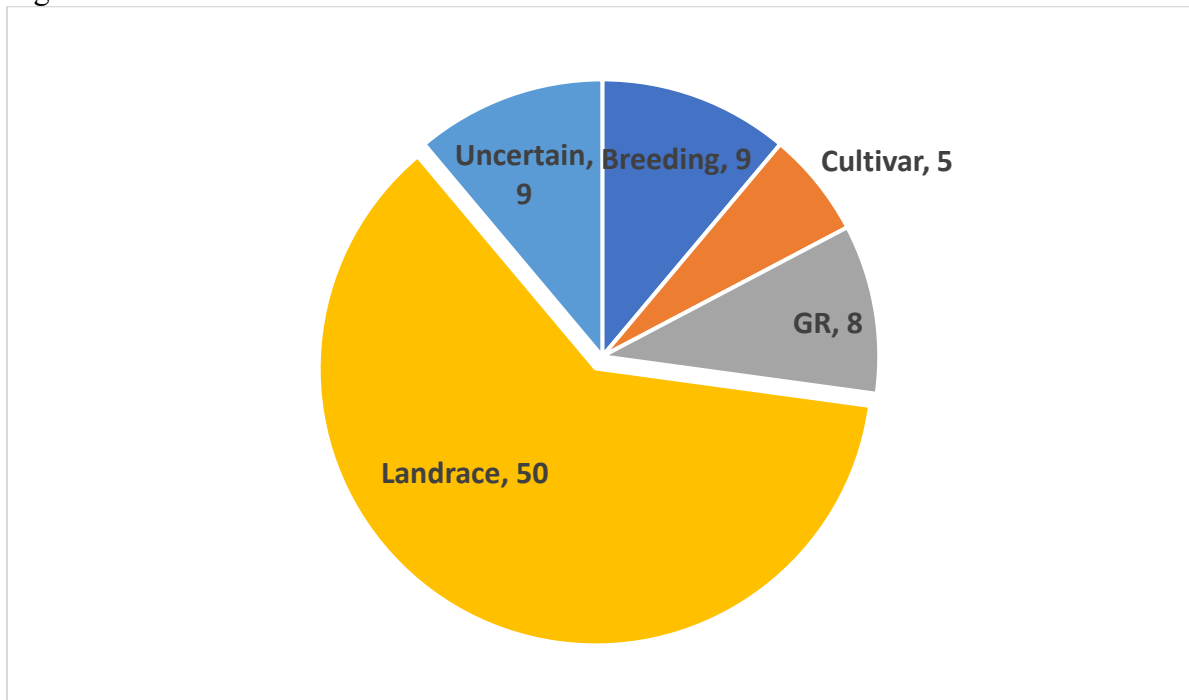


Figure 3: Habits of the 81 selected tolerant lines. GR indicates Post-Green Revolution lines.



The top ten heat tolerant lines were selected after the initial screen of the entire germplasm collection and four replications of the selected 81 lines (Table 2). Altogether, five

screens were conducted in the same fashion to determine which lines exhibited true heat tolerance. True heat tolerance, in this study, is defined as lines that consistently produced at least one out of four heat tolerant seedlings that survived the stress and acclimation periods, as well as transplant. Fifty-one lines were selected true heat tolerance, and out of those, the top ten are listed. The number of screens where heat tolerance was exhibited for each line is listed in the last column. Most of the true heat tolerant lines are spring landraces but as mentioned, of extremely variable origin. One line, 158, exhibited heat tolerance in four out of five heat stress screens. Any of the spring lines listed are potential candidates for introgression of early heat tolerance into Montana spring varieties. As such, any of the winter lines, namely lines 9 and 649 are potential candidates for introgression of early heat tolerance in Montana winter forage varieties (Table 2).

None of the spring (Vida and MT Sidney) or winter (Bobcat and Yellowstone) control lines survived through the heat stress period, acclimation period, or transplant to the greenhouse.

Table 2: Top 10 heat tolerant lines after one initial screen of entire germplasm collection and four replications of selected tolerant lines.

Line #	Accession #	Origin	Improvement	Habit	Screens
158	PI 62366	Venezuela	Landrace	S	4
431	PI 207104	Iran	Landrace	S	3
734	PI 374684	Bosnia & Herzegovina	Landrace	S	3
1017	PI 565386	Turkmenistan	Landrace	S	2
9	Cltr 6698	Canada	Breeding	W	2
596	PI 321889	Turkey	Landrace	S	2
593	PI 314924	Zimbabwe	Breeding	S	2
649	PI 349683	Denmark	Uncertain	W-S	2
723	PI 374247	Chad	Landrace	S	2
5	Cltr 5018	Former Soviet Union	Landrace	F	2

Discussion

Initial challenges encountered during protocol optimization included inconsistent water distribution and prevalent mold growth on the soil surface and around the seedling base, both leading to early seedling mortality. To address these issues, several optimizations were implemented. We transitioned to greenhouse 72-cell trays with bottom holes coupled with 1020 trays to facilitate drainage. We adopted both top and bottom watering to prevent oversaturation and substituted tap water with DI water to reduce potential contaminants. We also extended the stratification period to two days. These adjustments resulted in more uniform soil saturation and reduced mold incidence. The use of larger trays also allowed for a more consistent spatial arrangement within the growth chamber, which further optimized screening.

Despite these improvements, the first completed screening failed to identify heat tolerant lines, prompting a reduction in the heat stress temperature to 40°C. Lowering the heat stress temperature led to a decreased incidence of chlorosis and the identification of four potential heat tolerant lines, although these did not survive transplanting into the greenhouse. The heat stress period was decreased to four days and an acclimation period at 25°C for two days post-stress was added at the end of the protocol. The next completed screen resulted in successful growth and development in the greenhouse after screening and transplanting. No further optimizations were deemed necessary.

Increasing the stratification period from one to two days at 4°C did not greatly increase average germination rates of the germplasm collection. An average germination rate of 75.75% was representative of all stratification and germination periods, except for the selected top susceptible lines that were rescreened four times to confirm susceptibility. These lines may have

been predisposed for susceptibility to heat stress because of their decreased germination rate and sometimes delayed germination under the same protocol. After the three-day germination and growth period, all seedlings immediately underwent heat stress which resulted in them perceiving and responding to the heat stress while at slightly different seedling stages, ranging from the one to two leaf stage. While this is only a small developmental difference, wheat seedlings with less growth at the time of heat stress may not have the resources to respond in as optimized of a manner. In contrast, seedlings that germinated early and had more time for growth, may have more resources to mitigate heat stress effects with specific heat stress response mechanisms. Furthermore, more developed above ground biomass in wheat seedlings may signify increased root biomass, which can significantly impact the heat stress response (L. Lu et al., 2022). Heat tolerant wheat seedlings also experience a smaller decrease in root length when compared to heat susceptible wheat seedlings (L. Lu et al., 2022). Unfortunately, screening widely diverse germplasm means that treating all accessions the exact same in terms of heat stress induction is quite difficult. Modified screening protocols could address this issue by screening only at the two-leaf stage and letting delayed germinating seedlings have more time before heat stress induction. This would require more than one Percival chamber, however.

The way phenotypic characterization was conducted proved to be a reliable selection process for tolerant and susceptible lines. Surprisingly, chlorosis proved to be an unreliable indicator of susceptibility, as some heat tolerant lines exhibited complete chlorosis in the first leaf and no chlorosis in the second leaf. Initial assessment of lines with this phenotype resulted in classifying them as susceptible. However, the growth and development of the healthy second leaf indicated otherwise, thus changing subsequent phenotypic characterizations of the seedlings.

Curling and rolling of the seedling leaves is a common avoidance phenotype in many crops (Fierro et al., 2015; Kadioglu & Terzi, 2007; Kadioglu, Terzi, Saruhan, & Saglam, 2012). It was important to select for true heat tolerance, so selecting against lines that exhibited these temporary avoidance strategies was necessary. Furthermore, lines that exhibited potential avoidance phenotypes rarely survived acclimation after heat stress and transplant into the greenhouse, indicating that they are not capable of maintaining internal processes under continuous heat stress. A potential reliable indicator of heat tolerance at the seedling stage is the absence of rolling throughout the first leaf. Despite many germplasm lines staying green and remaining upright during the heat stress period, major rolling in these lines almost always resulted in later death. This was a common observance in germplasm lines that also exhibited the red stem phenotype at seedling stage.

The discovery that the tolerant lines were primarily spring landraces from the Middle East aligns with the evolutionary history of *Triticum aestivum*, suggesting that these ancient genotypes may carry beneficial alleles for heat tolerance. Wheat landraces often exhibit stress tolerance because of the thousands of years of cultivation in their respective location, usually characterized by extremely variable environmental conditions (Lopes et al., 2015). Furthermore, landraces are often cultivated under low input farming systems resulting in conservation of favorable traits amidst unfavorable environments (Lopes et al., 2015). This makes sense when considering that 50 out of the 81 selected heat tolerant lines are landraces. The landraces' broad geographic origins underscore the potential of these genotypes to contribute to the abiotic stress tolerance improvement, however later breeding challenges may relate to landraces' often lower yields when compared to cultivars (Denčić, Kastori, Kobiljski, & Duggan, 2000).

Seedling stage heat stress screens for wheat are currently being explored with the intention of being able to identify tolerance earlier than at the reproductive stages of wheat development. Early screening would save time, space, and money for breeders. Successful seedling stage screens would identify tolerant lines that could later be confirmed at the reproductive stage for true heat tolerance. One study heat stressed wheat seedlings hydroponically at 35°C and adult plants under greenhouse conditions at a 37/27°C day/night temperature. By calculating seedling root length and final yield, a general positive relationship of heat tolerance at seedling stage and at adult stage was established (L. Lu et al., 2022), indicating that early-stage screening can be a successful approach for identifying wheat lines that exhibit tolerance throughout the lifecycle. Optimized screening approaches could identify the presence of these mechanisms early and save time for the breeding program.

There are many potential heat response mechanisms that may confer tolerance at the seedling stage in wheat. High temperature stress at the seedling stage results in damage at the physiological, biochemical, and molecular levels. Physiological mechanisms include HSP activation, calcium influx, hormonal regulation, and compatible solute accumulation. When heat stress is induced, heat shock protein synthesis is activated and housekeeping proteins' synthesis decreases (Essemine, Ammar, & Bouzid, 2010). This molecular switch of protein synthesis is most pronounced at heat stress temperatures of 45°C (Essemine et al., 2010). While this seedling stage screen induced heat stress at a continuous temperature of 40°C, lines that exhibit heat tolerance may employ this mechanism of HSP activation to maintain growth and development despite ROS accumulation, protein denaturation, and membrane fluidity changes. Calcium can also play a pivotal role in the heat stress response at the seedling stage. In maize seedlings,

external calcium treatment can significantly enhance heat shock induced thermotolerance, indicating that the influx of extracellular calcium into cell cytosols helps mitigate heat stress damage (Gong, Chen, Song, & Li, 1997). Hormonal modulation such as the induction of abscisic acid (ABA) is an important component of plant cell thermotolerance, playing a variety of roles such as inducing HSP synthesis (HSP70) and cooperation with HSPs to recover denatured housekeeping proteins (Essemine et al., 2010). Many other hormones are involved in the heat stress response and are covered in the literature review. Lastly, compatible solute accumulation in the plant cytosol can help mitigate osmotic stress (Essemine et al., 2010).

Biochemical heat stress mechanisms include peroxidase activity which, like ABA, crosstalks with HSPs to confer thermotolerance in the plant cell (Essemine et al., 2010). Maintaining cellular homeostasis under heat stress is coordinated by intricate molecular processes and components. Multiple signal transduction pathways in various organelles of the cells can work together to confer thermotolerance, often involving many of the aforementioned molecules such as HSPs, calcium and its related transporters, antioxidants, and more.

When these tolerance mechanisms are present in wheat at the seedling stage, the seedlings exhibit a “recovery phenotype” and are distinguishable from susceptible lines. That is, despite the presence of some heat stress symptoms, their growth and development is maintained under heat stress. Common symptoms such as chlorosis, rolling, and curling are present but limited. Many of these mechanisms, such as HSP restoration of housekeeping proteins, may be activated inside the plant cells, and prevent further stress damage. In this screen, heat stress symptoms were often present at the tip of the first leaf but absent throughout the rest of the seedling. Additionally, many heat tolerant lines sustained healthy growth of the second leaf

despite heat stress symptoms affecting the first leaf. This could suggest allocation of heat stress response resources to the second leaf.

One of the additional advantages of wheat seedling stage heat stress screening is the identification of tolerant lines that would, theoretically, display the same thermotolerance during crop establishment under field conditions. By streamlining the selection process and focusing on genotypes that exhibit early-stage tolerance, breeders can more quickly develop varieties that can withstand the variable and warming climates. Such traits are not just beneficial for maintaining yield but also for ensuring the sustainability of wheat production systems.

Crop establishment, along with the subsequent biomass production, are important indicators of grain yield and quality. Developing wheat lines that exhibit heat tolerance during this early developmental stage would help maintain later yields under limiting conditions. Lines that exhibit this heat tolerance would be candidates for introgression of the trait into Montana cultivars. Through an initial cross of the germplasm line and the Montana cultivar, selection can be conducted on the hybrid F_1 population. Then, selfing and more selection can occur to isolate the heat tolerance trait. The selected F_2 plants can be backcrossed (trait introgression by recurring selection) into the Montana cultivar repetitively to select for heat tolerance and the high yielding and local adaptation traits of the cultivar. This can all be done without quantitative trait loci (QTL) mapping and/or marker assisted selection (MAS) because the heat tolerance trait can be phenotypically selected via heat stress screens. If introgressed into Montana varieties, the heat tolerance would help ensure healthy crop establishment and thus, robust grain yield and quality. The potential to introduce these traits into local cultivars offers a practical pathway to enhance

crop tolerance, a prospect of great importance to regions such as Montana, where climate trends are already influencing agricultural practices.

This study elucidates the crucial role of early-stage heat stress screening in identifying wheat genotypes with enhanced tolerance, which is increasingly important in the face of increasing climate variability. By examining a diverse array of wheat germplasm lines under controlled heat stress conditions, this protocol provides valuable insights into seedling stage tolerance that could be indicative of overall tolerance during crop establishment under field conditions.

CHAPTER THREE

DEVELOPMENT AND UTILIZATION OF A FULL LIFECYCLE

SCREENING HEAT STRESS PROTOCOL

Abstract

Worldwide, abiotic stresses negatively impact wheat development and subsequent grain yield and quality. Temperature stress at the grain filling stage is the leading cause for single seed weight decrease and thus, grain yield reduction, in wheat. The development of heat-tolerant wheat genotypes through screening is of utmost priority for meeting crop consumer demands. This study presents the findings from a comprehensive lifecycle screen for heat stress in *Triticum aestivum*. Utilizing a selected core collection of 1,095 wheat accessions encompassing a diverse range of origins, habits, and levels of improvement, we subjected these accessions to a diurnal heat stress environment of 35/28°C day/night temperature throughout their entire lifecycle. The objective was to discern which germplasm lines exhibited heat stress tolerance vegetatively, reproductively, and post-reproductively, increasingly pertinent traits given global climate trends. Through phenotypic assessment and seed count analyses, we identified lines that exhibited true tolerance to full lifecycle heat stress. The results of this screen were compared with data from the preceding seedling stage heat stress screen to evaluate consistency in stress tolerance across different developmental stages. Lines that exhibited stress in the seedling stage screen as well as in all three developmental stages of the full lifecycle screen are candidates for introgression into Montana cultivars. These candidates would exhibit heat tolerance from crop establishment all the way through senescence under field conditions. This approach enabled a nuanced understanding

of heat stress tolerance mechanisms in wheat, facilitating the identification of potentially tolerant wheat lines suitable for breeding programs aimed at enhancing crop sustainability in the face of climatic challenges.

Background

Reproductive stage heat stress presents a major challenge to global cereal crop production by damaging important processes such as anthesis, pollen development and grain fill, photosynthesis, and crop yields (T. Zenda, N. Wang, A. Dong, Y. Zhou, & H. Duan, 2022). Heat stress at these late stages of development is the leading cause for single seed weight decrease and subsequent grain yield reduction (Fu et al., 2023). The extent of heat stress damage on the crop depends on the species or genotype, stage of development, stress intensity and duration (T. Zenda et al., 2022). Under field conditions, heat stress most commonly occurs in transient waves, only impacting crops for short periods of time. As climatic variability intensifies, however, these waves will occur with greater frequency and duration over most terrestrial regions (Fu et al., 2023; T. Zenda et al., 2022). It is important to develop crops that exhibit heat tolerance from the crop establishment period all the way through senescence to ensure crop yields are maintained under stressful conditions throughout the crop lifecycle. To do so, there is a need for better understanding of the molecular and physiological mechanisms underlying reproductive stage heat stress response in cereals.

Optimum temperatures for normal growth and development in wheat ranges between 12 and 24°C (Bergkamp, Impa, Asebedo, Fritz, & Jagadish, 2018). Temperatures such as 10-15°C above the optimum mean may result in severe cellular structural damage, disruption of vital cellular processes, and loss of pollen viability at the reproductive stage (T. Zenda et al., 2022).

Factors that specifically lead to pollen death include loss of membrane integrity, ROS accumulation, and altered carbohydrate and lipid metabolism in mature pollen (T. Zenda et al., 2022). Furthermore, photosynthetic processes are disrupted which negatively impacts plant vegetative growth, thereby minimizing photosynthetic products being translocated to reproductive sinks. The decline in grain yield under heat stress conditions can sometimes also be attributed to accelerated senescence of the leaves, a common response of heat stress in wheat (T. Zenda et al., 2022).

Prior to anthesis, high temperatures can cause wheat pollen development to be disrupted (K. Abhinandan et al., 2018). Disruption at this stage can cause male sterility and increase the number of structurally abnormal and nonfunctional florets (Fu et al., 2023). Specifically, the degradation of the tapetum tissues during microspore meiosis is what causes pollen sterility (I. Khan, Wu, & Sajjad, 2022). Overall, both meiosis and gametogenesis are highly thermosensitive processes during reproduction (Thistlethwaite et al., 2020). Even heat stress temperatures of 30° or higher for three consecutive days during pollen development will significantly decrease seed setting and thus, grain yield of wheat (I. Khan et al., 2022).

The optimum temperature for wheat anthesis varies from 15 to 20°C (Djanaguiraman, Narayanan, Erdayani, & Prasad, 2020); for spring wheat, optimum temperature for anthesis and grain fill is from 12 to 22°C (I. Khan et al., 2022). Temperature exceeding the optimum threshold of each wheat variety will result in reduced grain yield and quality by minimizing the ability of the plant to grain fill. Specifically, the reduced assimilate availability, the reduced translocation of photosynthates to grain, and the reduced synthesis and deposition of starch in developing grains is what decreases grain fill under heat stress conditions (Thistlethwaite et al., 2020). This

was seen in wheat anthesis and grain fill under diurnal heat stress conditions (32/22 °C day/night) where photosynthetic rate decreased by 17 and 25%, respectively, and grain yield decreased by 29 and 44%, respectively, when compared to controlled conditions (T. Zenda et al., 2022). Additionally, post-anthesis heat stress grain yield may also decrease due to the shortened grain fill period. Under controlled greenhouse conditions, wheat plants subjected to a continuous high temperature regime ranging from 30-40°C exhibited premature senescence, thereby cutting short the grain filling period and consequently diminishing yield potential (K. Abhinandan et al., 2018).

Developing a variety of heat stress screening protocols using different environments and developmental stages is vital for successfully identifying germplasm lines with significant tolerance. Both growth chamber-based and field-based protocols have previously been reported for successful identification of tolerant lines (Asana & Williams, 1965; Bányai et al., 2014; Bergkamp et al., 2018). Chamber-based studies can be limited by the relatively small number of genotypes that can be screened at one time. Furthermore, selected tolerant lines may not actually exhibit true tolerance because they were selected from a relatively small pool of genotypes. Field-based screening is not without its issues either; the genotype and environment interaction results in a dynamic and diverse display of heat stress response phenotypes, causing inaccurate and imprecise selection. To make matters worse, it is much more difficult to distinguish stress effects from one another under field conditions. For example, ensuring that the field is sufficiently and evenly irrigated throughout the heat stress screen is vital to avoid selection for drought stress (Fu et al., 2023). The variety of genotypes tested under field conditions, the lack of controlled conditions to impose heat stress, and the introduction of unavoidable additional

stresses such as drought and salinity indicate that growth chamber-based screens may be of more advantage to breeders.

Reproductive stage screening successfully selects for germplasm lines that, under heat stress during anthesis and grain fill, can maintain seed production and produce viable seed. This is of great importance, as most heat waves occur during the grain filling period. For Kansas winter wheat production, heat wave temperatures of above 30°C throughout the grain filling months of May and June annually limit crop productivity (Bergkamp et al., 2018). Heat stress issues like this occur in crop production throughout the United States and emphasize the need for heat tolerant crops.

Reproductive stage heat stress critically undermines cereal production by impairing key developmental processes like anthesis and grain filling, leading to reduced crop yields. Optimal growth temperatures for wheat span 12 to 24°C; however, rising temperatures due to climatic variability are causing more frequent and prolonged heatwaves, potentially resulting in structural cellular damage, compromised photosynthesis, and reduced pollen viability. Disruption of wheat pollen development prior to anthesis contributes to male sterility. A temperature rise beyond optimal thresholds during wheat anthesis and grain fill can diminish yield and quality, primarily due to decreased assimilate availability and starch deposition. Field-based heat stress screening, while valuable, often suffers from imprecision due to the complex genotype-environment interaction and is less controlled compared to chamber-based studies. Hence, there's an increasing need for diverse and controlled screening protocols to identify wheat lines tolerant to heat throughout their lifecycle, an essential step towards securing grain yield stability amid escalating thermal stress.

Materials and Methods

Plant Materials

The selected core collection of wheat accessions utilized in this study was obtained from the United States Department of Agriculture - Agricultural Research Service - National Plant Germplasm System (USDA-ARS-NPGS). This core collection encompasses a total of 1,095 wheat accessions, offering a broad spectrum of genetic diversity reflective of a wide range of geographical origins, levels of improvement, subspecies differentiation, and growth habits. The diversity encapsulated within this collection provides a unique and valuable resource for exploring genetic variability in response to abiotic stresses, including heat stress. The accessions within this collection were carefully selected to represent the global diversity of wheat, ensuring a comprehensive genetic base for the study.

Plant Growth Conditions and Heat Stress Treatment

To identify heat stress tolerant lines throughout the full life cycle instead of just as the seedling stage, we screened wheat plants throughout their vegetative and reproductive developmental stages. Three seeds of each accession were planted in six-inch diameter pots filled with pre-moistened medium. The medium used was a blend of Canadian Sphagnum peat moss and horticultural grade Perlite. This medium was buffered with Dolomitic lime and contains a starter fertilizer charge and wetting agent. The tested pH of the medium is around 5.8 and electrical conductivity is 1.5 dS/m. Stratification was conducted at 4°C for two days with light watering at the 24-hour mark to ensure the medium did not dry out. After stratification, they immediately were transferred to a greenhouse growth room to germinate and grow for one week. Greenhouse conditions were 22/18°C day/night temperatures with an extended photoperiod of 16

hours. At this point, germination rates were recorded, and any additional germination afterwards was also noted. After germination and growth, the spring habit plants were transferred to a greenhouse heat stress chamber for the rest of their lifecycle. In this chamber, heat stress was induced diurnally at 35/28°C day/night temperatures. Plants were watered thoroughly six to seven days a week and fertilized bi-weekly at 200 parts per million with 20-20-20 fertilizer. The soil moisture levels were successfully maintained to ensure selection for drought stress did not occur. Additionally, two dehumidifiers were placed in the room to mitigate humidity, reaching 70% humidity. Winter habit plants were vernalized for a seven-week period at 4°C after the one-week growth period and before the heat stress period.

After 45 days of diurnal heat stress, selection was conducted. Plants whose flag leaves had fully emerged (Feeke's stage 9 of stem extension) were selected while plants that failed to fully emerge the flag leaf were removed. The selected plants continued developing under heat stress conditions until senescence. After complete senescence, seedheads were harvested, dried for two days at 45°C, and threshed.

Results

All lines exhibiting a spring, spring-facultative, or facultative habit were treated without a vernalization period while winter, winter-spring, or winter-facultative lines underwent a vernalization period for seven weeks at 4°C (Table 1). Notably, Post-Green Revolution lines exhibit a spring-facultative habit and were treated as such.

Table 1: Treatment of various habits for screening approach.

Habit	Treated
S, S-F, F	No vernalization
W, W-S, W-F	7-week vernalization at 4°C

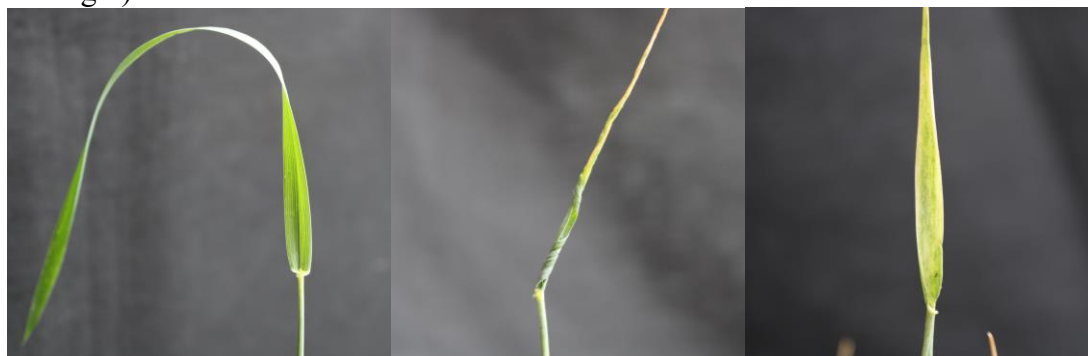
Table 2: Average germination rates of spring and winter screens during full lifecycle diurnal heat stress screen.

Average Germination Rates		
Screen #	Spring	Winter
1	83%	81%
2	90%	81%
3	82.7%	81.3%
4	86.7%	74%
5	77.3%	-
6	65.3%	-

After a stratification period of two days at 4°C, the average germination rates of all lines in the germplasm collection varied minimally. The most noticeable variation in germination rate was exhibited in spring screen six, which included most of the Post-Green Revolution Lines. Post-Green Revolution lines exhibit a spring habit; however, they have a facultative genetic background. The lower germination rate of 65.3% in this screen indicates that the Post-Green Revolution lines may have benefited from a winter vernalization.

Out of 1,095 wheat germplasm lines, 291 maintained growth and development under diurnal heat stress conditions of 35/28°C (Table 3) until at least Feeke's stage nine which is full emergence of the flagleaf (Figure 1). These lines can be classified as vegetatively tolerant, as they can sustain vegetative growth under at least 45 days of intense heat stress.

Figure 1: Examples of fully emerged flag leaves – one control (left) and two heat stressed (center and right).



Out of the 291 lines that exhibited vegetative tolerance, 31 lines maintained reproductive development (Table 3). These lines produced varying amounts of seed under heat stress conditions for the entire lifecycle. Out of these 31 lines, a germination test resulted in 27 of them producing viable seed (Table 3). These 27 lines are considered the most heat tolerant, as they exhibited tolerance vegetatively, reproductively, and post-reproductively. Maintaining normal growth, pollen viability, and viable seed production under such intense heat stress conditions is indicative of true heat tolerance. While pollen viability was not tested, the ability of the wheat plants to produce seed indirectly alludes to viable pollen and functional ovules.

Table 3: Amount of vegetative, reproductive, and post-reproductive heat tolerant lines selected from the full lifecycle screen.

Stage Tolerance Exhibited At	# of Lines	Percentage
Vegetative	291	26.58%
Reproductive	31	2.83%
Post-reproductive	27	2.47%

Out of the 27 lines that exhibited heat tolerance vegetatively, reproductively, and post-reproductively, Table 4 lists the top producing lines. Most lines in this category are landraces,

just like the selected lines in the seedling stage heat stress screen; however, this is unsurprising as 58.5% of the entire germplasm collection were landraces. There were also a few cultivars and Post-Green Revolution lines such as line 983 from India and line 1065 from Mexico City, Mexico, respectively. The habits and origins of these lines varied considerably.

Table 4: Top seed producing germplasm lines with viable seed from full lifecycle screen.

Line #	Accession #	Origin	Improvement	Habit	Seed #
602	PI 325845	Japan	Landrace	W	49
694	PI 361853	Denmark	Uncertain	W-S	41
588	PI 306676	Nigeria	Uncertain	S	15
983	PI 537060	India	Cultivar	S	11
603	PI 326308	China	Landrace	W	11
904	PI 490403	Mali	Uncertain	S	10
1065	PI 591780	Mexico City, MX	Post-Green Revolution	S-F	10
44	Cltr 14143	Georgia	Landrace	S	9
906	PI 491405	Mali	Uncertain	S	7

Discussion

Throughout the comprehensive full lifecycle screening, many accessions that exhibited vegetative and reproductive tolerance also advanced to seed set and senescence early, with some reaching senescence as early as five weeks post-germination. This phenomenon is consistent with previous findings, as elevated temperatures can induce early senescence by disrupting photosynthetic processes, thereby diminishing the assimilates necessary for proper grain filling (K. Abhinandan et al., 2018; Fu et al., 2023).

Additionally, many wheat accessions succumbed completely to necrosis prior to the 45-day selection mark, which was unsurprising given the deleterious impact of sustained high

temperatures observed in previous studies conducted under greenhouse conditions (K. Abhinandan et al., 2018). Such an extreme and consistent heat stress, as simulated in this study, does not typically occur in natural field environments, where heat stress generally presents as transient heat waves, rather than prolonged stress. This contrast between the natural environment and the controlled environment emphasizes the rigor of the screening protocol, designed to identify only those germplasm lines with exceptional, sustained heat tolerance. Consequently, the germplasm lines susceptible to heat succumbed rather rapidly under sustained stress conditions. Further screening may consider the potential trade-offs between selecting for extreme tolerance and the adaptability of such traits under realistic agronomic conditions.

It is important to note a few things when considering the seed production of the most tolerant lines from the full lifecycle screen (Table 4). Firstly, the protocol applied imposes conditions that are far more stringent than those typically encountered in field settings. For reference, one study induced a diurnal heat stress of 32/22°C at anthesis under controlled conditions for 14 days. Under only 14 days of less intense heat stress conditions, the wheat plants experienced a 28% reduction in seed set and a 29% reduction in grain yield when compared to wheat plants that experienced optimal temperatures of 22/14°C (Djanaguiraman et al., 2020). Consequently, the ability of the most heat tolerant lines in the full lifecycle screen to set seed under such rigorous testing conditions is remarkable.

Secondly, the average grain yield of landrace wheat is usually less than that of cultivars. One study focusing on grain yield of landraces and cultivars under normal and drought stress conditions found that under both conditions, cultivars produced significantly more seed than the landraces (Denčić et al., 2000). This is unsurprising because cultivars experienced intense

selection for high yields while landraces are usually not characterized for their high yields, but rather their environmental adaptations.

These results provide potential germplasm candidates for heat tolerance introgression into Montana cultivars. The first group of candidates were selected for their ability to yield large amounts of grain under the diurnal heat stress conditions throughout their full lifecycle. These lines, while not correlating with the seedling stage heat stress screen results, still exhibit impressive tolerance and should be considered for further screens as well as potential introgression candidates. These candidates are lines 602 and 694 (Table 4). They are both of winter habit and produced over 40 seed each by the end of their lifecycles.

The second group of candidates were selected in both the seedling stage heat stress screen and throughout the entire full lifecycle heat stress screen. These candidates are lines 44 and 1065. Line 44 is a spring landrace while line 1065 is a Post-Green Revolution line with a spring habit and facultative background. The difference in level of improvement and habit of these lines provides two alternatives for introgression. Line 1065 may be a better introgression candidate because of its already domesticated genetic background, in contrast to line 44 which would potentially be troublesome to introduce into a breeding program.

CHAPTER FOUR

UTILIZATION OF EMS MUTAGENESIS TO SCREEN FOR
NOVEL HEAT TOLERANCE TRAITS IN TWO MONTANA
CULTIVARSAbstract

Increasing climatic variability impairs crop growth and development and results in instable food supply because of decreased grain yields and quality. To address these limiting factors and meet consumer demands, crop tolerance to abiotic stresses is needed. Crop tolerance can quickly be achieved via transgenics; however, consumer acceptance of transgenic crops in the United States is low and cultivation of genetically modified crops is prohibited so breeders are forced to look at alternative options. One alternative, ethyl methanesulfonate (EMS) mutagenesis, induces genetic variation randomly throughout the genome and is used on locally adapted, high yielding cultivars so no later introgression is needed. This project explores the innovative application of EMS mutagenesis to induce novel genetic variations in two cultivated wheat varieties in Montana: MT Sidney and Dagmar. We systematically screened the M₂ generations of both cultivars for heat tolerance. MT Sidney M₂ plants underwent seedling stage screening and Dagmar M₂ plants underwent full lifecycle screening. The seedling stage heat stress screen resulted in 3.8% of the M₂ MT Sidney plants exhibiting seedling stage tolerance while the full lifecycle heat stress screen resulted in 2% of the M₂ Dagmar plants exhibiting vegetative, reproductive, and post-reproductive heat tolerance. These tolerant M₂ plants from both cultivars under both screening methods consistently outperformed control plants. These

findings affirm that EMS mutagenesis stands as a successful and versatile strategy for inducing genetic variation and bestowing abiotic stress tolerance, thereby offering a promising alternative to transgenic methods in the quest for cultivating resilient crops under variable climate conditions.

Background

EMS mutagenesis is a powerful, non-transgenic tool for generating novel genetic variation in a particular trait of interest. EMS mutates genetic material by alkylating and modifying nucleotides which ultimately results in a base change. Most commonly, EMS induces G/C to A/T nucleotide base transversions (Todd, Brouwer, & Glickman, 1981). In most cases, mutant phenotypes result from premature stop codons, splice site disruption, deleterious amino acid substitutions, and loss of gene function (Sabetta, Alba, Blanco, & Montemurro, 2011). These mutations occur randomly throughout the genome both in non-coding and coding regions (Lethin et al., 2020).

EMS mutagenesis can help identify unknown genes and characterize gene function to understand the molecular mechanisms of desirable agronomic traits (Liuzhu Chen et al., 2023). EMS is a commonly used chemical mutagen because of its high mutagen load and wide distribution of mutations throughout polyploidy crops' sub-genomes (Lethin et al., 2020). Another advantage that is characteristic of all mutation breeding is the potential to improve one or a few varietal traits without changing the genetic background, such as local adaptation or high yielding traits (Ke et al., 2019). Conducting EMS mutagenesis in popular, already high-yielding cultivars thus presents enormous potential for plant breeders. This form of mutagenesis has been successfully applied to many crops already such as oat (Chawade et al., 2010), barley (Caldwell

et al., 2004), maize (Till et al., 2004), and wheat (Slade, Fuerstenberg, Loeffler, Steine, & Facciotti, 2005). Thus, EMS mutagenesis is a critical approach to enhance crop genetic diversity, helping the discovery and development of novel traits vital for agriculture.

When conducting EMS mutagenesis in plants, two critical factors to consider are the genetic uniformity of the starting material and the optimal balance between induced mutation rate and seed viability. Utilizing isogenic seeds, which are genetically identical and true-breeding, is crucial for the success of the experiment (Liuzhu Chen et al., 2023). This uniformity is necessary to ensure that any observed phenotypic variations in subsequent generations are the result of induced mutations rather than inherent genetic diversity.

Moreover, calibrating the EMS dosage is a delicate task that requires careful attention. The aim is to induce a mutation rate that is high enough to produce discernible mutant phenotypes, without excessively reducing seed viability (Liuzhu Chen et al., 2023). Doses that lead to a 25-50% reduction in viability are typically deemed optimal, as they strike a balance between generating a sufficient number of mutations for analysis and maintaining enough viable seeds for experimental progress (Ke et al., 2019). A lethality rate beyond this range could significantly diminish the number of seeds available for planting, while a rate below this threshold may not induce an adequate number of mutations to facilitate the identification of desired phenotypic changes. Therefore, meticulous dosage determination is imperative to ensure a successful mutagenesis outcome and the generation of valuable mutant lines for further study.

Furthermore, the concept of 'dose' in the context of chemical mutagenesis, particularly with EMS, is subject to ongoing debate and is not as straightforward as one might assume. The potency of a mutagenic treatment arises not only from the concentration of the chemical mutagen

but also from the duration of exposure (Ke et al., 2019). Additionally, the absorption of the mutagen by the plant tissue, which is a pivotal step in the mutagenesis process, is influenced by a multitude of factors, including the water content of the seeds (Ke et al., 2019). Consequently, identical exposure conditions can lead to significant variation in the actual uptake of the mutagen by the seeds, and thus in the mutagenic effect. This complexity necessitates a nuanced approach to defining 'dose', considering both the conditions of the treatment and the physiological state of the plant material. Such considerations are essential to ensure consistency and repeatability in the outcomes of mutagenesis experiments.

EMS mutagenesis is a vital technique for generating novel genetic variation, focusing on specific traits within crops without altering their entire genetic makeup. This approach, which induces mutations through nucleotide alterations, is particularly effective due to its capacity to create widespread genetic changes that facilitate the analysis of gene functions related to desirable agronomic traits. The crux of successful EMS mutagenesis lies in using isogenic seeds to ensure true mutations are observed, coupled with a carefully modulated dosage that balances mutation induction with seed survival. EMS's strategic application has already led to significant advancements in various crops, emphasizing its importance in agricultural innovation and the pursuit of crop abiotic stress tolerance.

Materials and Methods

Plant Materials

The selected wheat cultivars used for mutagenesis are MT Sidney (Reg. no. CV-1199; PI 699957) (Crutcher et al., 2023) and Dagmar (Reg. no. CV-1158; PI 690450) (Heo et al., 2020).

The M₀ seeds were sourced from the Spring Wheat Breeding Lab at MSU.

EMS Preparation

EMS solutions were prepared at specific concentrations to induce mutations. Initially, 0.8% was used for both cultivars, however a lower dose was needed for MT Sidney. For MT Sidney, a concentration of 0.6% was deemed optimal; thus, 60 μ L of EMS was combined with 99.4mL of distilled (DI) water. For the Dagmar variety, a 0.8% EMS solution was prepared by pipetting 80 μ L of EMS into a glass media bottle containing 99.2mL of DI water, ensuring the mixture reached the desired concentration for effective mutagenesis. These varying EMS preparations are critical for the mutagenesis process, differing in concentration to accommodate the distinct genetic backgrounds and mutagenic tolerance of the two wheat varieties.

Mutagenesis and Planting of Cultivars

One hundred seeds from each cultivar were placed into a 250mL flask and submerged in DI water, ensuring all seeds were fully covered. This soaking process, lasting 30 minutes, also served as a viability test, with floating seeds deemed non-viable and discarded. Following the water soak, seeds were drained and then submerged in the EMS solution in shaker tubes. In addition to the decrease in EMS concentration, MT Sidney also had a decreased shaking time of six hours. Dagmar's shaking time was nine hours. After shaking in the EMS solution, the EMS was safely discarded into a chemical waste bin, secured with a strainer to prevent seed loss. The seeds then underwent four 30 second washes with DI water to remove any residual EMS from the seed coat, which involved gentle manual shaking and a 24-hour period of additional shaking with periodic water changes. After this 24-hour period, we watched the seeds for germination while they continued to soak in DI water in the shaker. Once germination of most of the seeds was observed, seeds were planted in greenhouse trays filled with Sunshine medium. This

generally occurred 2-3 days after the 24-hour washing period. To accommodate varying germination times, seeds were initially planted shallowly and, upon germination, transplanted into pots at a density of three seeds per pot. This protocol underscores the comprehensive approach taken to induce genetic variations for heat tolerance, leveraging EMS mutagenesis within a controlled experimental framework.

Advancement of Mutagenized Cultivars

M₁ plants grew and developed in greenhouse conditions until senescence. Greenhouse conditions were 22/18°C day/night temperatures with an extended photoperiod of 16 hours. Throughout the M₁ plants' development, dominant mutations were observed such as red stem and leaf curling mutant phenotypes, which confirmed that mutagenesis was successful. At maturity, all seedheads were harvested, dried for two days at 45°C, and then M₂ seeds were threshed. The M₂ plants were heat stressed and also retained for advancement.

Heat Stress Screening of M₂ Plants

For MT Sidney, four seeds of each M₁ plant were sowed into 72-cell trays placed inside of 1020 trays. 232 M₂ seeds in total were used for the seedling stage screen. They were stratified at 4°C for two days, and then transferred to the Percival chamber to grow and develop at 25°C for three days. After this period of development, the M₂ plants began heat stress in the same chamber at 40°C for four days. (See Chapter 2 Seedling Screen Materials and Methods).

For Dagmar, five seeds of each pot of M₁ plants were sowed into six-inch diameter pots. In total, 250 M₂ seed were used for the full lifecycle screen. They were stratified at 4°C for two days, and then transferred to the greenhouse to grow and develop under normal conditions for one week. After this period of development, the M₂ plants were transferred into a diurnal heat

stress chamber at 35/28°C day/night temperatures and left to develop for the rest of their lifecycle. (See Chapter 3 Full Lifecycle Screen Materials and Methods).

Results

Optimizing EMS dosage demonstrates the fine balance needed in selecting mutagen concentrations and exposure times to achieve favorable germination rates. The initial protocol utilized an EMS concentration of 0.8% with a shaking time of nine hours for both Dagmar and MT Sidney. While Dagmar M₁ average germination rates were favorable, MT Sidney seeds germinated at a much lower rate (Table 1). EMS concentration was decreased to 0.6% and shaking time was decreased to six hours for MT Sidney which resulted in an average germination rate of 51% (Table 1). The modification to a 0.6% EMS concentration and reduced exposure for MT Sidney highlights the specificity required for each cultivar's tolerance to mutagenic treatment.

Table 1: Average germination rates of Dagmar and MT Sidney M₁ seed. 500 seed were mutagenized for each cultivar.

Cultivar	EMS Concentration	Shaking Time	M₁ Germination Rate
Dagmar	0.8%	9 hours	83%
MT Sidney	0.8%	9 hours	17%
	0.6%	6 hours	51%

After M₁ plants were grown to maturity, all spikes were harvested. Dagmar spikes were harvested per pot which resulted in three M₁ plants being harvested together. The harvesting method then evolved and MT Sidney M₁ lines were harvested individually to better trace later mutant phenotypes to their parental source. These harvesting methods were followed by drying

and threshing. Afterwards, MT Sidney M_2 seed was planted four seeds per line for the seedling stage screen and Dagmar M_2 seed was planted five seeds per pot for the full lifecycle screen.

Oftentimes, confirmation that EMS did result in mutant phenotypes is exhibited in the M_1 plants. At this stage in advancement, only dominant mutant phenotypes can be observed. Figure 1 shows a Dagmar M_1 line which exhibited an early, red stem mutant phenotype. While phenotypes such as this can be shown early, all phenotypes (dominant and recessive) are present in the M_2 generations which is why screening is conducted on this generation. Figure 2 shows a mutant phenotype in a MT Sidney M_2 line that resulted in increased susceptibility to heat stress at the seedling stage.

Figure 1: Dagmar M_1 line exhibiting a red stem mutant phenotype.

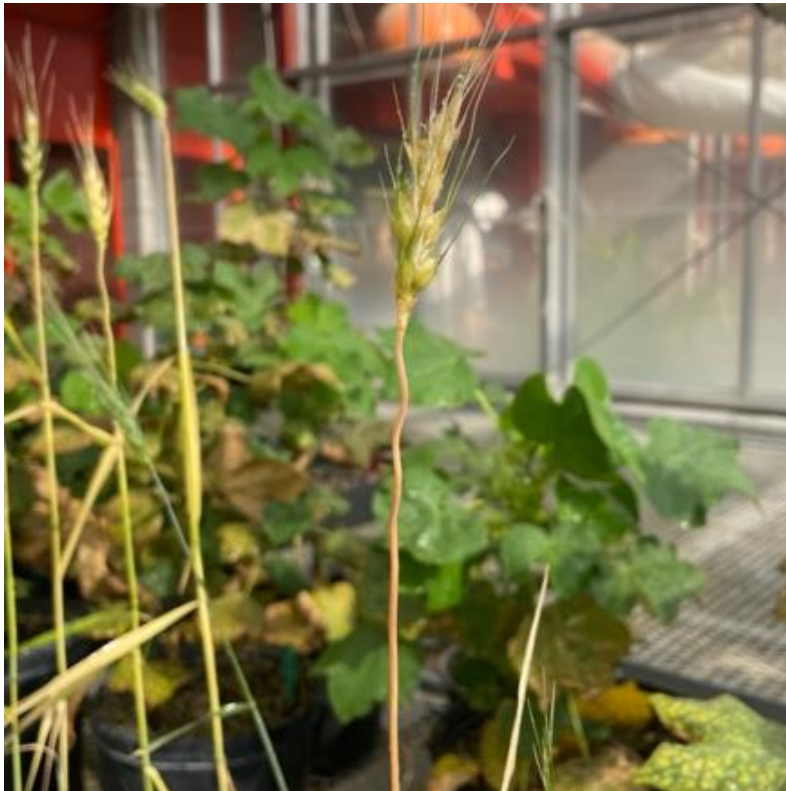
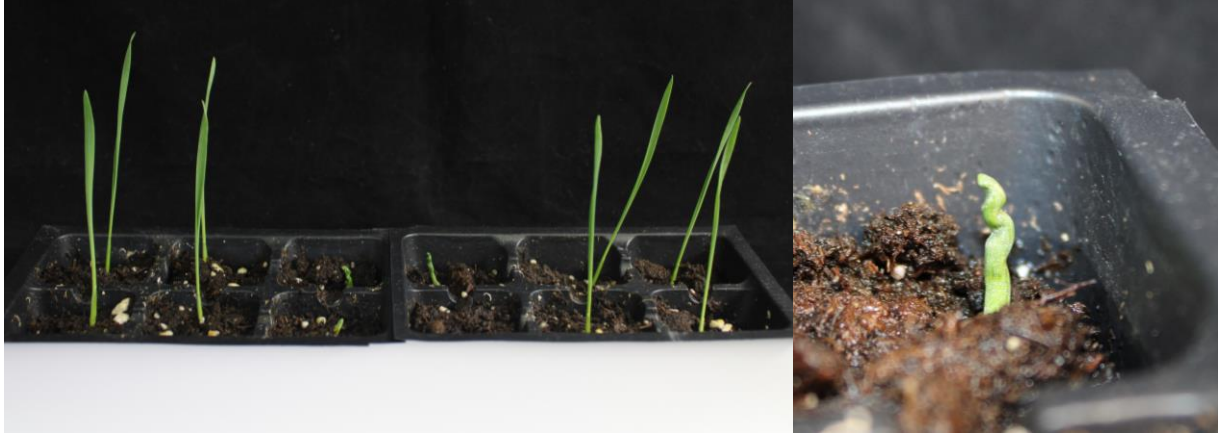


Figure 2: MT Sidney M₂ line exhibiting a mutant phenotype at the seedling stage.



After screening all 232 MT Sidney M₂ seedlings, 14 were identified as heat tolerant but only ten survived transplant to the greenhouse (Table 2). These mutagenized seedlings maintained normal growth and development under continuous heat stress conditions in contrast to the control lines, which all died.

Table 2: MT Sidney M₂ lines that exhibited seedling stage tolerance and survived transplant.

Line # (Screen #)	Seedling Stage Tolerance (Y/N)	Transplant
14 (1)	Y	Survived
26 (2)	Y	Survived
36b (2)	Y	Survived
36 (4)	Y	Survived
37 (4)	Y	Survived
13b (5)	Y	Survived
13c (5)	Y	Survived
16 (5)	Y	Survived
19 (5)	Y	Survived
32a (5)	Y	Survived

After the full lifecycle screen, 72.22% of Dagmar M₂ lines exhibited vegetative tolerance reaching at least Feeke's stage 9, full emergence of the flagleaf, under diurnal heat stress conditions of 35/28°C. Out of these lines, a smaller subset advanced to seed production. Specifically, eight lines produced seed and a viability test resulted in five lines producing viable seed. The decrease in tolerance percentages follows a similar pattern as the germplasm lines under full lifecycle heat stress conditions (See Chapter 3). These mutagenized Dagmar plants exhibited better heat stress tolerance than their controls.

Table 3: Exhibited vegetative, reproductive, and post-reproductive heat tolerance of Dagmar M₂ populations throughout lifecycle.

Stage Tolerance Exhibited At	# of Populations	Percentage of Populations
Vegetative	39	72.22%
Reproductive	8	14.81%
Post-reproductive	5	9.26%

Discussion

EMS mutagenesis has emerged as an indispensable technique in the realm of crop improvement, particularly for enhancing abiotic stress tolerance. By introducing random genetic mutations, EMS mutagenesis creates a diverse array of genotypes, which may include individuals with novel traits like enhanced heat tolerance. In this study, the two cultivars elected for EMS mutagenesis were MT Sidney and Dagmar. Both cultivars are hard red spring wheat (*Triticum aestivum*) but vary in their environmental tolerances. Dagmar is known for its excellent yield potential under semi-arid agriculture, its solid stems, and its high end-use quality. Dagmar also exhibits similar grain yield potential to 'Vida', one of the most popular cultivars in Montana. Its ability to maintain yields under semi-arid production systems emphasizes its inherent drought

tolerance (Heo et al., 2020). Objectives focused on in the development of this variety included long green leaf duration after heading as well as an overall early heading date compared to Vida. Additionally, Dagmar exhibits more solid, pith-filled stems which may increase wheat stem sawfly resistance (Heo et al., 2020). As drought and heat tolerance response mechanisms are intrinsically connected, Dagmar presents a good candidate for EMS mutagenesis as the more tolerant variety. MT Sidney was selected as a top yielding variety in advanced field trials that included Vida. Furthermore, it has a significantly higher test weight and an earlier heading date than Vida (Crutcher et al., 2023). Comprehensive Fusarium head blight (FHB) resistance screening indicated that MT Sidney was moderately resistant. It was then released on the basis of high yield potential, high test weight, early heading date, and moderate FHB resistance (Crutcher et al., 2023). MT Sidney presents a good candidate for EMS mutagenesis as the more susceptible variety.

Two key parameters to consider when mutagenizing with EMS are the homogeneity of the material and the optimization of EMS dose (Liuzhu Chen et al., 2023). Both Dagmar and MT Sidney M₀ seed were sourced from the Montana State University Spring Wheat Breeding Lab and are true breeding, representative of what Montana producers grow in field. Secondly, optimizing EMS dose must be troubleshooted, as EMS concentration varies across species and even variety (Ke et al., 2019).

In the case of the wheat cultivars MT Sidney and Dagmar, the observed variation in response to heat stress suggests that EMS mutagenesis can indeed induce genetic changes that enhance an organism's stress tolerance. It is noteworthy that while Dagmar inherently possesses a degree of (drought) stress tolerance, and both control cultivars fared poorly under extreme heat

stress conditions, a select few M₂ lines exhibited improved performance. This enhanced tolerance may be attributed to EMS-induced mutations in critical genes encoding proteins or transcription factors that regulate stress responses. Mutations in such loci, for example, the release of a repressor's control, could shift the plant's physiological balance toward improved survival and reproductive success under thermal stress.

The potential of EMS to induce beneficial mutations hinges on the concept of genetic redundancy and the multifaceted nature of plant stress responses. Within the complex network of plant stress physiology, numerous pathways and gene products interact to determine the organism's tolerance to heat. Wheat's hexaploidy nature emphasizes this complexity as there are multiple copies of genes in the three subgenomes (A, B, and D) (Zhou et al., 2020). Therefore, the subtle heat tolerance observed in some mutagenized M₂ lines may result from mutations that either bolster the plant's defense mechanisms or mitigate detrimental stress effects. For instance, changes in the expression or function of heat shock proteins, ion transporters, or osmoprotectant biosynthesis enzymes could all contribute to an enhanced heat stress response (H. Sato, Mizoi, Shinozaki, & Yamaguchi-Shinozaki, 2024; T. Zenda et al., 2022). The performance of these mutagenized lines, surpassing that of control plants, implies that EMS mutagenesis can serve as a valuable strategy for identifying and harnessing these intricate genetic variations. As such, it provides a non-transgenic approach to breed crops like wheat, which are not only vital for food security but also increasingly subjected to the whims of a changing climate.

CHAPTER FIVE

CONCLUSION

Developing stress tolerant crops capable of maintaining high grain yield and quality is of the utmost importance in the next few decades. Gradually rising March temperatures coupled with climatic variability throughout the entire growing season will result in increasingly unfavorable conditions for Montana wheat producers. To maintain production and meet demands, Montana producers must meet or increase current yields. However, with changing environmental conditions such as temperature, this becomes increasingly difficult. Montana's characteristic semi-arid environment results in more complicated challenges for producers. While heat stress has a more prominent effect on gene expression than drought, both abiotic stresses often work in conjunction with one another under field conditions. The development of heat tolerant wheat varieties would potentially enable the crop to better withstand drought stress as well.

Most of the identified seedling stage tolerant lines from the NPGS germplasm collection were spring landraces originating from the Middle Eastern region. Much of this region is classified as semi-arid, the same as Montana, so the morpho-physiological adaptations of these landraces are theoretically suitable for the Montana environment. As seen in the seedling stage heat stress screen, these Middle Eastern landraces exhibit heat tolerance early in the lifecycle, even under an intense, continuous heat stress of 40°C. Maintaining internal processes such as photosynthesis and the structural integrity of the membrane under heat stress conditions at the seedling stage is indicative of intrinsic, lifecycle mechanisms that may confer heat tolerance throughout the entire growth and development of the plant. Under field conditions, this early

seedling stage is vital because it determines the success of crop establishment, which is an indicator of later grain yield and quality. Additionally, with increasing March temperatures, producers need to ensure that spring wheat varieties are tolerant early on and not just during anthesis and grain filling later in the season where heat stress is more common. Additionally, winter lines could benefit from early season heat tolerance due to their sometimes-early fall planting dates. Incorporating these traits into locally adapted cultivars could prove essential for sustaining crop establishment and overall yield.

The results of this study advocate for a strategic shift in wheat breeding approaches. Early-stage screening, as demonstrated, can accelerate the breeding cycle by quickly identifying genotypes with inherent tolerance to heat stress. This has practical implications for Montana's semi-arid farming practices, allowing producers to develop robust planting strategies and potentially reducing the risk of crop failure due to increasingly unpredictable temperatures.

Among the heat tolerant lines selected from the full lifecycle screen, there was considerable variability for lifecycle length. Some tolerant lines finished their lifecycle quickly while maintaining pollen viability and adequate grain fill to produce viable seed. These germplasm lines reached senescence as early as two months post planting. The ability of these lines to reach early senescence without a decline in seed viability suggests a potential breeding strategy for developing early maturing varieties that complete their life cycle before the onset of peak summer heat, a strategy that could be increasingly beneficial as temperatures are unpredictable.

Additionally, some tolerant lines extended their lifecycle and took as long as four months to reach senescence. Fewer of these were able to maintain pollen viability and produce viable

grain. It may be advantageous to rescreen this germplasm material under similar, if not the same, conditions and focus on lifecycle length as well as heat tolerance and seed production. Breeders could then select germplasm lines that exhibited heat tolerance throughout the entire lifecycle and reached senescence early. If these traits were introgressed into Montana wheat cultivars, the cultivars would exhibit heat tolerance throughout the entire lifecycle and would reach harvest maturity before the onset of mid to late season heat stress.

This study's findings emphasize the importance of identifying wheat varieties that maintain tolerance to temperature stress across all developmental stages, from tillering to post-reproduction. In Montana, where early spring temperatures are on the rise, these findings are particularly relevant for ensuring that both early sown and late maturing wheat varieties can withstand the full range of temperature variations they are likely to encounter throughout the growing season. The full lifecycle screening process not only identified germplasm lines exhibiting heat tolerance in various developmental stages but also indicated which full lifecycle tolerant lines correlated with seedling stage screen lines.

Lines that were selected for heat tolerance during both screens are listed in Table 1. These lines exhibited tolerance during the seedling stage screen and vegetatively, reproductively, and post-reproductively throughout the full lifecycle screen. These are the best candidates for introgression of the heat tolerance trait into Montana cultivars as they would confer tolerance throughout each stage of wheat growth and development. Out of these five candidates, 44 and 1065 exhibited the best heat tolerance because, under full lifecycle heat stress conditions of 35/28°C, they produced more seed than their counterparts and exhibited 100% germination rates when three of each seed were planted. Lines 12 and 250 produced only one seed each during the

full lifecycle screen but could still be suitable lines for heat tolerance introgression. Line 1017 also produced only one seed which was nonviable when planted. However, this does not mean that 1017 is incapable of producing viable seed under full lifecycle stress conditions. Further replicative screening should be conducted with all selected heat tolerant lines.

Table 1: Germplasm lines that exhibited tolerance during both seedling stage and full lifecycle screens.

Line #	Accession #	Origin	Improvement	Habit	Seed Viability
12	Cltr 7611	Georgia	Landrace	S	Yes
44	Cltr 14143	Russian Federation	Landrace	S	Yes
250	PI 137750	Iran	Landrace	S	Yes
1017	PI 565386	Turkmenistan	Landrace	S	No
1065	PI 591780	Mexico City, MX	Post-Green Revolution	S-F	Yes

Both the seedling stage and full lifecycle heat stress screens proved successful for identification of heat tolerant and susceptible germplasm lines. Because of the seedling stage protocol, it was very easy and cost effective to screen over 1,000 lines (over 4,000 plants total) for heat tolerance under controlled conditions. In the future, large germplasm collections could be screened in this way and then selected lines could be confirmed for heat tolerance later using various reproductive stage heat stress screens.

The vast genetic diversity for abiotic stress tolerance found in germplasm material is of great advantage to breeders. By utilizing efficient screening methods such as this seedling stage screen protocol, it may be easier for breeders to utilize this germplasm material. Furthermore, implementing genome selection and characterizing tolerant lines via molecular markers would

optimize breeding time. Not only would the development of abiotic stress tolerant lines be quicker, but identification and understanding would be easier.

EMS mutagenesis stands out as a potent non-transgenic methodology, fostering genetic diversity within wheat cultivars through the random induction of mutations. Given the consumer resistance to genetically modified crops in the United States and the legal constraints, EMS is a strategic alternative, especially relevant for Montana, where climatic conditions are becoming increasingly variable. The mutations induced in the local cultivars Dagmar and MT Sidney aim to leverage their inherent traits—like stress tolerance and early maturity—which are particularly advantageous for semi-arid farming practices in Montana.

This approach, by promoting base changes such as G/C to A/T transversions, opens the door to a myriad of genetic variations that could translate into phenotypes with improved abiotic stress tolerance. Remarkably, the application of EMS mutagenesis to Montana's wheat varieties Dagmar and MT Sidney has underlined the method's potential; the identification of heat-tolerant lines within the M₂ generation—particularly those derived from Dagmar—signals progress toward breeding wheat capable of withstanding the harsh realities of heat stress.

The observed survival and seed production of Dagmar M₂ lines under rigorous heat conditions represent a significant success for EMS mutagenesis. These lines, enduring a diurnal heat stress environment which simulates extreme climatic conditions, provide a hopeful glimpse into the future of wheat breeding strategies that prioritize tolerance to temperature fluctuations. It is within this context that the forthcoming conclusions for MT Sidney's response to EMS mutagenesis will further clarify the role of genetic background and mutagenic specificity in developing heat-tolerant wheat varieties. The anticipation of these results fosters a bridge

between experimental mutagenesis and practical breeding programs, aimed at securing food supplies against the backdrop of climate change.

In conclusion, EMS mutagenesis, by generating a wide range of genetic diversity, allows for the precise enhancement, identification, and introgression of desirable agronomic traits, such as heat tolerance, without altering the cultivar's well-adapted genetic background. As the need for sustainable agricultural practices intensifies, the strategic use of EMS mutagenesis is well becoming a cornerstone of modern crop improvement programs.

Ultimately, the findings underscore the critical need for wheat varieties that consistently express tolerance to temperature stress throughout their development, ensuring tolerance from seedling stage to post-reproduction. As such, the germplasm lines displaying heat tolerance both in early seedling stages and throughout the lifecycle—namely lines 12, 44, 250, 1017, and 1065—emerge as primary candidates for introgression. Among these, lines 44 and 1065 stand out, demonstrating not only a high germination rate but also substantial seed production under the duress of full lifecycle heat stress.

This research advocates for comprehensive breeding strategies, integrating the vast genetic diversity of germplasm resources, efficient screening protocols, and mutagenesis techniques. Such an approach could revolutionize the development of abiotic stress-tolerant wheat lines, shortening breeding timelines while enhancing the understanding of complex traits.

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APPENDIX

NPGS GLOBAL CORE COLLECTION OF *TRITICUM AESTIVUM*

Table A1: List of wheat germplasm accessions and their respective name, origin, improvement level, and habit

Line #	Accession #	Plant Name	Origin	Improvement Level	Habit
1	CItr2804	CI 2804	Mexico	LANDRACE	S
2	CItr4175	Saracen (CANDEAL)	Philippines	LANDRACE	S
3	CItr4203	CI 4203	Honduras	UNCERTAIN	S
4	CItr4646	Schlandstedts Sommerweizen	Germany	CULTIVAR	F
5	CItr5018	Vierny	Former Soviet Union	LANDRACE	F
6	CItr5069	White Straw Tuscan	New Zealand	UNCERTAIN	F
7	CItr6316	Gold Drop	United Kingdom	CULTIVAR	W
8	CItr6678	Tanganika	Zaire	UNCERTAIN	S
9	CItr6698	CI 6698	Canada	BREEDING	W
10	CItr7590	CI 7590 (FHB 51886)	Russian Federation	LANDRACE	F
11	CItr7605	CI 7605 (FHB 51901)	Russian Federation	LANDRACE	S
12	CItr7611	CI 7611 (FHB 51907)	Russian Federation	LANDRACE	S
13	CItr7635	CI 7635 (FHB 51932)	Russian Federation	LANDRACE	S
14	CItr7648	CI 7648 (FHB 51945)	Russian Federation	LANDRACE	F
15	CItr7652	CI 7652 (FHB 51949)	Russian Federation	LANDRACE	S
16	CItr7664	CI 7664 (FHB 51961)	Russian Federation	LANDRACE	F
17	CItr7703	Chernishivka	Russian Federation	LANDRACE	S
18	CItr8160	56	Ethiopia	LANDRACE	S
19	CItr8442	Favorito	Argentina	CULTIVAR	S
20	CItr8625	Banatka	Ukraine	UNCERTAIN	S
21	CItr9340	910R22	Czechoslovakia	LANDRACE	W
22	CItr9348	Swan	Sweden	UNCERTAIN	S
23	CItr9358	914R1	Finland	LANDRACE	W

24	CItr9362	914R5	Finland	LANDRACE	W
25	CItr11223	Croatia 1	Croatia	LANDRACE	S
26	CItr11254	Greece 13	Greece	LANDRACE	S
27	CItr11262	Greece 21	Greece	LANDRACE	S
28	CItr 11477	Golden ball	South Africa	CULTIVAR	S
29	CItr11478	Ulka No. 1	Former Soviet Union	LANDRACE	S
30	CItr11614	Graecum 10245	Russian Federation	CULTIVAR	S
31	CItr11862	OAC 104	Canada	CULTIVAR	W
32	CItr12345	Red Egyptian	Egypt	UNCERTAIN	S
33	CItr12808	Australith	Israel	UNCERTAIN	W
34	CItr13270	Sel. 18	Canada	BREEDING	S
35	CItr13332	Pembina	Canada	CULTIVAR	S
36	CItr 13448	Gaines	United States, Washington	Post-Green Revolution	S-F
37	CItr 13924	Penjamo 62	Mexico, Ciudad de México	Post-Green Revolution	S-F
38	CItr 13926	Mayo 64	Mexico, Ciudad de México	Post-Green Revolution	S-F
39	CItr 13927	Pitic 62	Mexico, Ciudad de México	Post-Green Revolution	S-F
40	CItr 13928	Sonora 63	Mexico, Ciudad de México	Post-Green Revolution	S-F
41	CItr 13929	Lerma Rojo 64	Mexico, Ciudad de México	Post-Green Revolution	S-F
42	CItr 13930	Sonora 64	Mexico, Ciudad de México	Post-Green Revolution	S-F
43	CItr 13968	Nugaines	United States, Washington	Post-Green Revolution	S-F
44	CItr14143	17425	Georgia	LANDRACE	S
45	CItr14151	CI 14151	Former Soviet Union	UNCERTAIN	F
46	CItr14172	Isogenic: ISr11-Sa	United States	GENETIC	S
47	CItr 14293	Huamantla Rojo	Mexico, Ciudad de México	Post-Green Revolution	S-F
48	CItr14346	Betmark	South Africa	CULTIVAR	S
49	CItr14350	H 648	Argentina	BREEDING	S

50	CItr14351	Prima	Belgium	CULTIVAR	F
51	CItr14353	II-50-150	Paraguay	BREEDING	S
52	CItr14442	R24	Ethiopia	LANDRACE	S
53	CItr14451	P494	Ethiopia	LANDRACE	S
54	CItr 14483	Coulee	United States, Washington	Post-Green Revolution	S-F
55	CItr 14485	Paha	United States, Washington	Post-Green Revolution	S-F
56	CItr 14586	Luke	United States, Washington	Post-Green Revolution	S-F
57	CItr14607	ELS 6404-63-4	Ethiopia	LANDRACE	S
58	CItr14811	ELS 6404-125	Eritrea	LANDRACE	S
59	CItr15035	26.402	Afghanistan	LANDRACE	S
60	CItr15088	Sr 13	Canada	GENETIC	S
61	CItr15134	Local White	Pakistan	LANDRACE	S
62	CItr15135	Hudeiba 164	Sudan	UNCERTAIN	S
63	CItr15136	American 378	Sudan	BREEDING	S
64	CItr15142	Maiah	Saudi Arabia	LANDRACE	S
65	CItr15144	Hallany	Saudi Arabia	LANDRACE	S
66	CItr15172	Picota 11	Bolivia	BREEDING	S
67	CItr15200	Modesty	Kenya	UNCERTAIN	S
68	CItr15203	Ebro	Spain	CULTIVAR	W
69	CItr15204	Gredos	Spain	CULTIVAR	F
70	CItr15212	Deir Alla	Jordan	UNCERTAIN	S
71	CItr15226	Jo-03057	Finland	BREEDING	W
72	CItr15310	Darash Hosha	Afghanistan	LANDRACE	W
73	CItr15382	Syrimex	Syria	CULTIVAR	S
74	CItr 15388	Potam 70	Mexico, Ciudad de México	Post-Green Revolution	S-F
75	CItr 15390	Yecora 70	Mexico, Ciudad de México	Post-Green Revolution	S-F

76	CItr15412	3144-19	Tunisia	LANDRACE	S
77	CItr15416	Frigui	Tunisia	LANDRACE	S
78	CItr15434	Hmira	Tunisia	LANDRACE	S
79	CItr15456	3362-80	Tunisia	LANDRACE	S
80	CItr15467	Hmira	Tunisia	LANDRACE	S
81	CItr15468	Ajili	Tunisia	LANDRACE	S
82	CItr15478	Arbi	Tunisia	LANDRACE	S
83	CItr15517	Fritissa	Tunisia	LANDRACE	S
84	CItr 15851	Nadadores 63	Mexico, Ciudad de México	Post-Green Revolution	S-F
85	CItr 15883	Jaral 66	Mexico, Ciudad de México	Post-Green Revolution	S-F
86	CItr15942	CAR 16	Chile	BREEDING	W
87	CItr16226	CAR 299	Chile	BREEDING	W-S
88	CItr16742	CAR 815	Chile	BREEDING	F
89	PI278635	Macedonian 14	Macedonia	LANDRACE	W
90	CItr 17391	Noroeste 66	Mexico, Ciudad de México	Post-Green Revolution	S-F
91	CItr 17416	Tanori 71	Mexico, México	Post-Green Revolution	S-F
92	CItr 17417	Barbee	United States, Washington	Post-Green Revolution	S-F
93	CItr 17418	Raeder	United States, Washington	Post-Green Revolution	S-F
94	CItr 17419	Daws	United States, Washington	Post-Green Revolution	S-F
95	CItr17808	Isogenic: Rht1 Rht2 (WBXP A77154)	United States	GENETIC	W
96	CItr 17909	Lewjain	United States, Washington	Post-Green Revolution	S-F
97	PI7430	Gejar	Spain	LANDRACE	S
98	PI8813	Kurd	Iraq	LANDRACE	S
99	PI9131	Chul	Uzbekistan	CULTIVAR	S
100	PI9781	CItr 2401	Tajikistan	LANDRACE	W

101	PI9791	Yantagbay	Uzbekistan	LANDRACE	S
102	PI9871	Erivan	Armenia	CULTIVAR	S
103	PI10611	Talimka	Turkmenistan	CULTIVAR	S
104	PI24484	Turbat	Uzbekistan	LANDRACE	S
105	PI24485	Kara Bugdai	Uzbekistan	LANDRACE	S
106	PI24486	131	Turkmenistan	LANDRACE	S
107	PI25989	Medeah	Algeria	LANDRACE	S
108	PI34126	CItr 4075	Spain	UNCERTAIN	S
109	PI36390	396	Bolivia	LANDRACE	S
110	PI36500	357 (341)	Uzbekistan	LANDRACE	S
111	PI36501	370 (357)	Uzbekistan	LANDRACE	S
112	PI41020	Kizil Bogara	Uzbekistan	LANDRACE	F
113	PI41021	Poltavka	Ukraine	LANDRACE	F
114	PI41032	Allorca	Tunisia	CULTIVAR	S
115	PI42006	3561	India	LANDRACE	S
116	PI42016	Compactum	India	LANDRACE	S
117	PI 42424	Golden ball	South Africa	CULTIVAR	S
118	PI43250	CItr 5478	Greece	LANDRACE	S
119	PI43355	Pelon	Uruguay	LANDRACE	S
120	PI43364	Pelon	Uruguay	LANDRACE	F
121	PI44952	Red Bearded Autumn	France	UNCERTAIN	F
122	PI45369	Du Toits	South Africa	UNCERTAIN	F
123	PI45403	Red Egyptian	South Africa	UNCERTAIN	S
124	PI45435	Wol Koren	South Africa	UNCERTAIN	F
125	PI46038	Blanco	Venezuela	LANDRACE	S

126	PI46041	Macarron	Venezuela	LANDRACE	S
127	PI47544	CI 6500	Iran	LANDRACE	S
128	PI48097	Transvaal Wolkoren	South Africa	UNCERTAIN	S
129	PI48149	CItr 6530	Spain	LANDRACE	S
130	PI51688	Geldersche Ris	Netherlands	LANDRACE	W
131	PI52227	Mugami	Sudan	LANDRACE	S
132	PI52557	Hard Federation 24	Australia	BREEDING	S
133	PI54431	Triminia	Libya	LANDRACE	S
134	PI55884	Liguleless	Tajikistan	LANDRACE	S
135	PI56053	Tulun 418	Russian Federation	BREEDING	S
136	PI57145	CI 7092	Georgia	LANDRACE	W
137	PI57147	CI 7094	Georgia	LANDRACE	S
138	PI57157	CI 7104	Georgia	LANDRACE	W
139	PI57159	CI 7106	Georgia	LANDRACE	W-F
140	PI57162	CI 7109	Georgia	LANDRACE	F
141	PI57164	CI 7111	Georgia	LANDRACE	W
142	PI57165	CI 7112	Georgia	LANDRACE	F
143	PI57166	CI 7113	Georgia	LANDRACE	S
144	PI57171	CI 7118	Georgia	LANDRACE	F-S
145	PI57172	CItr 7119	Georgia	LANDRACE	F
146	PI57176	CI 7123	Georgia	LANDRACE	W-S
147	PI57181	CI 7128	Georgia	LANDRACE	W
148	PI57657	65	Egypt	LANDRACE	S
149	PI57660	95	Egypt	LANDRACE	S
150	PI57814	123	India	LANDRACE	S

151	PI57825	134	India	LANDRACE	S
152	PI57988	175	India	LANDRACE	W
153	PI58559	Burgoyne Fife	United Kingdom	UNCERTAIN	F
154	PI58560	Svalof	Sweden	BREEDING	S
155	PI62362	Grande del Norte	Venezuela	LANDRACE	S
156	PI62363	La Clase	Venezuela	LANDRACE	S
157	PI62364	Lebarata	Venezuela	LANDRACE	S
158	PI62366	CI 8361	Venezuela	LANDRACE	S
159	PI62368	CI 8363	Venezuela	LANDRACE	W
160	PI65564	Cologna Veneto	Italy	LANDRACE	W
161	PI68012	5496	China	LANDRACE	W-S
162	PI69749	8015	China	LANDRACE	S
163	PI70694	79	Iraq	LANDRACE	S
164	PI70697	84	Iraq	LANDRACE	S
165	PI70698	87	Iraq	LANDRACE	S
166	PI70700	92	Iraq	LANDRACE	S
167	PI70702	99	Iraq	LANDRACE	S
168	PI70704	156	Iraq	LANDRACE	S
169	PI70706	176	Iraq	LANDRACE	S
170	PI70713	568	Iraq	LANDRACE	S
171	PI70714	572	Iraq	LANDRACE	S
172	PI71114	Sary Maghis	Uzbekistan	LANDRACE	S
173	PI73311	CItr 9182	Azerbaijan	BREEDING	W
174	PI73322	Kanarskaia	Azerbaijan	LANDRACE	W
175	PI73324	2894	Azerbaijan	BREEDING	W

176	PI73329	Karmir	Armenia	LANDRACE	S
177	PI73335	Sari Boogda	Azerbaijan	LANDRACE	W-F
178	PI73338	2908	Azerbaijan	LANDRACE	W
179	PI73361	Kirda Boogda	Azerbaijan	LANDRACE	W
180	PI73362	1650 (Khurgeno Boogda)	Azerbaijan	LANDRACE	W
181	PI73364	Chernovka	Azerbaijan	LANDRACE	S
182	PI74298	492	China	LANDRACE	W
183	PI74305	533	China	LANDRACE	W
184	PI74318	647	China	LANDRACE	S
185	PI74345	981	China	LANDRACE	W-F
186	PI74752	1975	China	LANDRACE	W
187	PI74801	2641	China	LANDRACE	S
188	PI78814	CI 10112	Georgia	LANDRACE	S
189	PI82469	Poubiru	Korea, North	LANDRACE	S
190	PI82846	B37B	China	LANDRACE	W
191	PI82912	C32	China	LANDRACE	W
192	PI83251	F27	China	LANDRACE	W
193	PI83275	G6A	China	LANDRACE	F
194	PI83436	I45	China	LANDRACE	W
195	PI83481	J25	China	LANDRACE	S
196	PI83515	L1B	China	LANDRACE	W
197	PI83733	Tiszagyongye No. 164	Hungary	CULTIVAR	S
198	PI84527	176	Uzbekistan	LANDRACE	W
199	PI87115	Komugi Zairaishu	Korea, South	LANDRACE	W
200	PI87116	Nulsu Misu	Korea, South	LANDRACE	W

201	PI87987	Rainugi	Korea, North	LANDRACE	W
202	PI93767	Samanta 1252	Romania	CULTIVAR	W-F
203	PI94345	Reasante	Armenia	LANDRACE	W
204	PI94356	41	Armenia	LANDRACE	W
205	PI94364	59BSW	Armenia	LANDRACE	F
206	PI94367	61BP	Armenia	LANDRACE	S
207	PI94375	66BPR	Armenia	LANDRACE	W
208	PI94377	66BPW	Armenia	LANDRACE	S
209	PI94380	66BSR	Armenia	LANDRACE	W
210	PI94442	123	Ukraine	LANDRACE	W
211	PI94450	131a	Former Soviet Union	LANDRACE	W
212	PI94457	137	Ukraine	LANDRACE	W
213	PI94464	144	Ukraine	LANDRACE	W
214	PI94468	148	Ukraine	LANDRACE	W
215	PI94479	159	Kazakhstan	LANDRACE	W
216	PI94481	161	Russian Federation	LANDRACE	W
217	PI94484	164	Former Soviet Union	LANDRACE	W
218	PI94489	169	Uzbekistan	LANDRACE	W
219	PI94499	179	Ukraine	LANDRACE	W
220	PI94503	183	Ukraine	LANDRACE	W-F
221	PI94520	200	Azerbaijan	LANDRACE	S
222	PI94538	218	Turkmenistan	LANDRACE	S
223	PI94555	375	Mongolia	UNCERTAIN	S
224	PI94563	395 (17329-1929)	Israel	LANDRACE	S
225	PI94564	396	Ancient Palestine	LANDRACE	S

226	PI94565	397 (17329)	Israel	LANDRACE	S
227	PI94567	399	Israel	LANDRACE	S
228	PI94568	400 (17380)	Israel	LANDRACE	S
229	PI94570	403 (17723)	Greece	LANDRACE	S
230	PI94572	Leda Collection A47	Former Soviet Union	LANDRACE	S
231	PI94590	448	Uzbekistan	LANDRACE	S
232	PI94757	310	Armenia	LANDRACE	S
233	PI108981	Solid Straw Tuscan Selection 13/28	New Zealand	BREEDING	F
234	PI113916	Currawa	Australia	CULTIVAR	S
235	PI115838	4453/31	Germany	BREEDING	S
236	PI225158	G-54326	Greece	BREEDING	S
237	PI116232	Solid Straw Tuscan	New Zealand	BREEDING	W
238	PI116330	Varonne	Australia	CULTIVAR	W-S
239	PI118896	C9906 (C9965)	Kenya	BREEDING	W
240	PI124336	378	China	LANDRACE	F
241	PI124353	462	China	LANDRACE	W
242	PI125342	Maparcha	Afghanistan	LANDRACE	W-F
243	PI125364	3039	Afghanistan	LANDRACE	F
244	PI125387	Bahari	Afghanistan	LANDRACE	S
245	PI131267	231	Australia	BREEDING	W-S
246	PI132863	San Giorgio	Italy	CULTIVAR	F
247	PI134116	33	Afghanistan	LANDRACE	W
248	PI134871	Erythrospermum	Portugal	LANDRACE	S
249	PI137748	Gandum	Iran	LANDRACE	W-S
250	PI137750	Gandumi Rishi	Iran	LANDRACE	S

251	PI137753	5536	Iran	LANDRACE	S
252	PI142414	481-2-G-40	Australia	BREEDING	W
253	PI145718	6-month wheat	Saudi Arabia	LANDRACE	S
254	PI145719	24	Saudi Arabia	LANDRACE	S
255	PI145723	36	Saudi Arabia	LANDRACE	S
256	PI145724	37	Saudi Arabia	LANDRACE	S
257	PI153785	Goiana	Brazil	LANDRACE	S
258	PI154279	8191	Algeria	BREEDING	S
259	PI154282	9290	Algeria	BREEDING	S
260	PI157569	Eshima No. 55	Korea, South	UNCERTAIN	W-S
261	PI157580	Nang Rim No. 38	Korea, South	UNCERTAIN	W-F
262	PI157902	Florio	Italy	CULTIVAR	S
263	PI157906	GR 2	Italy	UNCERTAIN	F
264	PI157949	Romano	Italy	LANDRACE	F
265	PI162590	Drott Red	United Kingdom	UNCERTAIN	W
266	PI163052	Mandel Gehun	India	LANDRACE	S
267	PI164165	8877	India	LANDRACE	S
268	PI164518	Kanak	India	LANDRACE	S
269	PI165912	9453	India	LANDRACE	S
270	PI165930	9472 (Gehun)	India	LANDRACE	S
271	PI166013	9558	India	LANDRACE	S
272	PI166130	9676	India	LANDRACE	S
273	PI166180	9726	India	LANDRACE	S
274	PI166280	Terian	Turkey	LANDRACE	S
275	PI166333	Mahlut	Turkey	LANDRACE	S

276	PI166494	Ak	Turkey	LANDRACE	S
277	PI166583	Kizilca	Turkey	LANDRACE	S
278	PI166636	Sert	Turkey	LANDRACE	S
279	PI166667	Bolu	Turkey	LANDRACE	S
280	PI166727	Ak	Turkey	LANDRACE	S
281	PI166863	Kirmizi Yazlik	Turkey	LANDRACE	S
282	PI166946	Digrak	Turkey	LANDRACE	S
283	PI167441	1513	Turkey	LANDRACE	S
284	PI167636	Ak Sunter	Turkey	LANDRACE	S
285	PI167706	3905	Turkey	LANDRACE	S
286	PI168480	9361a	India	LANDRACE	S
287	PI168711	Kanhard Selection Buck	Argentina	CULTIVAR	W
288	PI168789	Rescote	Peru	UNCERTAIN	S
289	PI171063	4282	Turkey	LANDRACE	F
290	PI171113	O.S.B.	Peru	UNCERTAIN	W
291	PI172582	8728	Turkey	LANDRACE	W
292	PI173488	8328	Turkey	LANDRACE	S
293	PI173497	Asure	Turkey	LANDRACE	W
294	PI174594	Annie	France	CULTIVAR	W
295	PI174606	Bon Fermal	France	CULTIVAR	F
296	PI174639	Gentil Rosso	Italy	LANDRACE	F
297	PI44952	Red Bearded Autumn	France	UNCERTAIN	F
298	PI175514	Olympia	Finland	CULTIVAR	W
299	PI175517	Sampo	Finland	CULTIVAR	S
300	PI176225	9544a	India	LANDRACE	S

301	PI176234	Rajeh	Nepal	LANDRACE	W
302	PI176272	Safed Pissi	India	LANDRACE	S
303	PI176325	10056	India	LANDRACE	S
304	PI176334	10120	India	LANDRACE	W
305	PI176356	10282	India	LANDRACE	S
306	PI177943	Akova	Turkey	LANDRACE	S
307	PI178055	4494	Turkey	LANDRACE	S
308	PI178075	5348	Turkey	LANDRACE	S
309	PI178184	Tir	Turkey	LANDRACE	W
310	PI178188	7775	Turkey	LANDRACE	S
311	PI178705	175/2	Turkey	BREEDING	S
312	PI178752	Hamra	Iraq	LANDRACE	S
313	PI178755	Husainiya	Iraq	LANDRACE	S
314	PI178788	Hevidik	Turkey	LANDRACE	S
315	PI180581	Hauters II	Germany	CULTIVAR	W
316	PI180610	Strain No. 1827/47	Germany	BREEDING	W
317	PI181251	9	Afghanistan	LANDRACE	S
318	PI181356	123	Afghanistan	LANDRACE	F
319	PI181398	166	Afghanistan	LANDRACE	W
320	PI181433	204	Afghanistan	LANDRACE	S
321	PI 182098	S-24	Pakistan	-	S
322	PI182525	Skorospelka	Russian Federation	CULTIVAR	S
323	PI182564	Norin 9	Japan	CULTIVAR	W
324	PI182565	Haya Komugi	Japan	LANDRACE	S
325	PI182665	9915 (BEKAA)	Lebanon	LANDRACE	F

326	PI182670	9934 (BIKAI)	Lebanon	LANDRACE	F
327	PI182672	9945 (BIKAI)	Lebanon	LANDRACE	F
328	PI182673	Salamouni	Lebanon	LANDRACE	S
329	PI182703	B-5 (GHABAGHEB)	Syria	LANDRACE	S
330	PI182711	Kaundouhari	Syria	LANDRACE	S
331	PI182751	10446	Turkey	LANDRACE	S
332	PI182850	Diosecka 777	Slovakia	CULTIVAR	W
333	PI182851	Hodominska Holica	Czech Republic	CULTIVAR	W
334	PI183528	11193	India	LANDRACE	S
335	PI184150	Brkulja	Bosnia and Herzegovina	LANDRACE	W
336	PI184160	Domaca	Bosnia and Herzegovina	LANDRACE	S
337	PI184161	Brkulja	Bosnia and Herzegovina	LANDRACE	W
338	PI184162	Domaca	Bosnia and Herzegovina	LANDRACE	W
339	PI184168	Crvenica	Serbia	LANDRACE	F
340	PI184185	Ranka	Bosnia and Herzegovina	LANDRACE	W-S
341	PI184208	BR 18	Serbia	LANDRACE	W
342	PI184258	R 14	Croatia	UNCERTAIN	W
343	PI184260	Banatka	Serbia	LANDRACE	W
344	PI184598	Uruguay	Uruguay	UNCERTAIN	S
345	PI184602	3CX3C	Spain	UNCERTAIN	F
346	PI184611	3245	Portugal	BREEDING	S
347	PI184631	Webster	Russian Federation	UNCERTAIN	S
348	PI184634	Heines Kolben	Germany	CULTIVAR	S
349	PI184846	Criollo	Guatemala	UNCERTAIN	S
350	PI184857	Loosdorfer Winterweizen	Austria	CULTIVAR	W

351	PI184993	M-36	Norway	UNCERTAIN	S
352	PI185108	0617-26	Norway	BREEDING	S
353	PI185190	3194	Portugal	UNCERTAIN	W
354	PI185198	Beirao	Portugal	CULTIVAR	S
355	PI185356	Florence 193	Portugal	UNCERTAIN	S
356	PI185713	Ideal	Portugal	CULTIVAR	S
357	PI185715	Ruivo	Portugal	LANDRACE	S
358	PI189629	Colonias	Brazil	CULTIVAR	S
359	PI189632	Trintani	Brazil	CULTIVAR	W-S
360	PI189830	Sel. 49-2852 H1073	Argentina	BREEDING	F
361	PI190196	Ciento Cinquento	Ecuador	UNCERTAIN	S
362	PI190914	Pondus	Sweden	CULTIVAR	S
363	PI190985	Akagomugh Involcable Vilmorin	Spain	UNCERTAIN	W
364	PI191006	2029	Spain	BREEDING	W-S
365	PI191007	2030	Spain	BREEDING	W
366	PI191014	Ballobar	Spain	LANDRACE	W
367	PI191016	Barbilla	Spain	LANDRACE	S
368	PI191039	2083	Spain	BREEDING	S
369	PI191075	Chamorro de Hellin	Spain	LANDRACE	S
370	PI191180	Pelade de Artajona	Spain	LANDRACE	S
371	PI191202	Rojo de Pamplona	Spain	LANDRACE	W
372	PI191215	Sierra Nevada	Spain	LANDRACE	F
373	PI191226	2418	Spain	BREEDING	W-S
374	PI191240	Jeja de Conesa	Spain	LANDRACE	W
375	PI191272	Blanc Precoce	Belgium	UNCERTAIN	W

376	PI191275	Moskovskaja	Russian Federation	CULTIVAR	W
377	PI191406	Mocho	Morocco	LANDRACE	S
378	PI191453	Hundi	Spain	UNCERTAIN	S
379	PI191483	Trigo 229	Spain	CULTIVAR	S
380	PI191506	Rosafe Argentino	United Kingdom	UNCERTAIN	F
381	PI191530	HNAD 12231	Portugal	UNCERTAIN	F
382	PI191565	H 15 H 13344	Portugal	UNCERTAIN	F
383	PI191748	3119	Tunisia	BREEDING	S
384	PI191961	Magvarovax	Hungary	UNCERTAIN	S
385	PI191985	Roemer	Germany	UNCERTAIN	F
386	PI191987	Mocho de Espiga Ruiva	Portugal	LANDRACE	S
387	PI191996	Granadero FS	Mozambique	CULTIVAR	S
388	PI192001	Cana Roxa	Angola	LANDRACE	S
389	PI192027	3430	Mozambique	BREEDING	S
390	PI192030	Roemer	Germany	UNCERTAIN	W
391	PI192038	Roemer	Germany	UNCERTAIN	S
392	PI192048	3456	Mozambique	UNCERTAIN	S
393	PI192080	Regional de Angola	Mozambique	UNCERTAIN	S
394	PI192147	Matte Lungo	Ethiopia	LANDRACE	S
395	PI192169	Precoce	Portugal	LANDRACE	S
396	PI192198	Regional Mocho	Angola	UNCERTAIN	S
397	PI192208	Rumanien	Romania	UNCERTAIN	S
398	PI192225	Postolopytska Presivkakmen 58	Czechoslovakia	CULTIVAR	F
399	PI192269	3728	Sweden	BREEDING	S
400	PI192350	Mirandez	Portugal	LANDRACE	W

401	PI192383	Litaniskt	Lithuania	UNCERTAIN	S
402	PI192416	Ankar II	Sweden	CULTIVAR	W
403	PI192460	Tremesino	Spain	LANDRACE	S
404	PI192466	Pioneer	Mozambique	UNCERTAIN	S
405	PI192475	Veadeiro	Brazil	UNCERTAIN	S
406	PI192480	2021A	Morocco	BREEDING	S
407	PI192493	Praganoso de Muscapa	Mozambique	UNCERTAIN	S
408	PI192569	Forma Vinda de Varmland	Sweden	LANDRACE	S
409	PI192579	Stendes	Estonia	UNCERTAIN	F
410	PI192632	Jacto	Angola	UNCERTAIN	S
411	PI192646	Matopo	Mozambique	UNCERTAIN	S
412	PI192648	2288A	Mozambique	BREEDING	S
413	PI192716	T de Lituania	Lithuania	UNCERTAIN	W
414	PI192737	Richelle Rosso	Ethiopia	LANDRACE	S
415	PI192802	Ruivo	Portugal	LANDRACE	S
416	PI192808	Tremes Arroxeado	Portugal	LANDRACE	S
417	PI192864	R 023	Finland	UNCERTAIN	W-S
418	PI192981	San Pedro	Guatemala	LANDRACE	S
419	PI193937	Inter-Generic	Colombia	BREEDING	S
420	PI195413	2710	Guatemala	LANDRACE	F
421	PI195414	2726	Guatemala	LANDRACE	S
422	PI195415	2743	Guatemala	LANDRACE	F
423	PI196900	10021	Ethiopia	LANDRACE	S
424	PI200762	3418	Honduras	LANDRACE	S
425	PI202790	Frontana	Peru	CULTIVAR	S

426	PI203083	Wabian	Paraguay	UNCERTAIN	S
427	PI203084	Burpian	Paraguay	UNCERTAIN	S
428	PI203085	Kawain	Paraguay	UNCERTAIN	S
429	PI203086	Frombina	Paraguay	UNCERTAIN	S
430	PI205714	31	Peru	BREEDING	S
431	PI207104	65o	Iran	LANDRACE	S
432	PI207115	158e	Iran	LANDRACE	S
433	PI207123	169c	Iran	LANDRACE	W
434	PI210485	3883	Iran	LANDRACE	W
435	PI210945	Scalavatis	Cyprus	LANDRACE	S
436	PI210969	Croisement	Cyprus	UNCERTAIN	S
437	PI211684	Sertak 52	Turkey	CULTIVAR	S
438	PI213679	Buck 12/52	Argentina	BREEDING	W
439	PI219750	14144	Pakistan	LANDRACE	S
440	PI220127	110	Afghanistan	LANDRACE	S
441	PI220357	Gandum-i-Siahkltoshah	Afghanistan	LANDRACE	F
442	PI220431	Mokhtar	Egypt	LANDRACE	S
443	PI220843	Gandum	Afghanistan	LANDRACE	W-S
444	PI221354	Hodoniska Holice	Slovakia	CULTIVAR	W
445	PI221384	U-1	Croatia	CULTIVAR	W
446	PI221699	3	Indonesia	UNCERTAIN	W
447	PI222650	1620	Iran	LANDRACE	W
448	PI222671	1662	Iran	LANDRACE	F
449	PI223899	Gandom	Afghanistan	LANDRACE	W
450	PI225158	G-54326	Greece	BREEDING	S

451	PI225238	52	Iran	LANDRACE	W
452	PI225256	70	Iran	LANDRACE	W
453	PI225265	79	Iran	LANDRACE	W
454	PI225275	89	Iran	LANDRACE	W
455	PI225282	96	Iran	LANDRACE	S
456	PI225371	Petirrojo	Uruguay	CULTIVAR	S
457	PI225461	102-71	Uruguay	BREEDING	S
458	PI230652	Jhiaveva	Paraguay	UNCERTAIN	S
459	PI231117	II-2809-1c1x4x1x0	Guatemala	BREEDING	S
460	PI231366	Duecentodieci	Italy	CULTIVAR	W
461	PI234385	Salti Naimya	Jordan	LANDRACE	S
462	PI235221	Himekei No. 428-1	Japan	BREEDING	S
463	PI237650	Psathas	Cyprus	LANDRACE	S
464	PI237655	Kenya Governor	Kenya	CULTIVAR	S
465	PI243650	5357	Iran	LANDRACE	W
466	PI243679	5401	Iran	LANDRACE	S
467	PI243709	5446	Iraq	LANDRACE	S
468	PI243777	5520	Iran	LANDRACE	W
469	PI243792	5410	Iran	LANDRACE	S
470	PI245368	Criollo Santa Irene	Guatemala	LANDRACE	S
471	PI245397	3075	Afghanistan	LANDRACE	S
472	PI245514	3344	Afghanistan	LANDRACE	W
473	PI247907	Andes 55	Colombia	CULTIVAR	S
474	PI247914	Maipofen	Chile	CULTIVAR	S
475	PI247922	Vilufen	Chile	CULTIVAR	S

476	PI253799	K911	Iraq	LANDRACE	S
477	PI253803	K1761	Afghanistan	LANDRACE	S
478	PI254118	503.L.I.A.4	Kenya	BREEDING	S
479	PI258425	Arawa	New Zealand	CULTIVAR	S
480	PI259881	U-14	Croatia	CULTIVAR	S
481	PI260797	1385-3331	Egypt	BREEDING	S
482	PI260895	Atson	United Kingdom	CULTIVAR	S
483	PI261822	Gerbya	Saudi Arabia	UNCERTAIN	S
484	PI261824	Samma	Saudi Arabia	LANDRACE	S
485	PI262594	Vysokelitovskaya	Belarus	CULTIVAR	W
486	PI262596	Dankovi	Lithuania	CULTIVAR	W
487	PI262603	Hydri Bugda	Azerbaijan	LANDRACE	W
488	PI262605	Karagach	Turkmenistan	LANDRACE	W
489	PI262611	Kysyldzha	Turkmenistan	LANDRACE	S
490	PI262615	Todireshti	Moldova	LANDRACE	W
491	PI262619	Upkli	Georgia	LANDRACE	W-S
492	PI262620	Ak Bugdai	Kyrgyzstan	LANDRACE	S
493	PI262623	Bukovinka	Ukraine	LANDRACE	S
494	PI262624	Karmraat	Armenia	LANDRACE	S
495	PI262653	Beltskaya Mestnaya	Moldova	LANDRACE	W
496	PI262654	Batkan Krasnaya	Kyrgyzstan	CULTIVAR	W
497	PI262659	Safedak	Tajikistan	LANDRACE	S
498	PI262664	Kamchatka	Armenia	LANDRACE	S
499	PI264275	Norrone	Norway	CULTIVAR	S
500	PI264278	Fylgia II	Sweden	CULTIVAR	F

501	PI264927	220	Greece	LANDRACE	S
502	PI264937	81	Greece	LANDRACE	S
503	PI264998	628	Greece	LANDRACE	S
504	PI266862	Cross 7	New Zealand	CULTIVAR	F
505	PI266882	Tainui	New Zealand	CULTIVAR	S
506	PI266895	Galego Rapado	Portugal	LANDRACE	S
507	PI268009	Lir	Portugal	UNCERTAIN	S
508	PI269407	322	Afghanistan	LANDRACE	W
509	PI270041	C271	Pakistan	CULTIVAR	S
510	PI271130	Mult 764	Peru	BREEDING	S
511	PI272308	Cruz Alta	Brazil	BREEDING	S
512	PI272374	Barabas-Fele	Hungary	LANDRACE	W
513	PI272384	Arpadhalmi 267	Hungary	CULTIVAR	W
514	PI272468	Bankuti 23-46	Hungary	BREEDING	W
515	PI272490	Perbetei 35/419	Hungary	BREEDING	W
516	PI272494	Veneny 3621	Hungary	UNCERTAIN	S
517	PI273990	1864	Ethiopia	LANDRACE	S
518	PI274505	-	Thailand	UNCERTAIN	S
519	PI274656	Barbarossa Podolanka	Poland	CULTIVAR	W
520	PI278196	Varna 2	Bulgaria	LANDRACE	W
521	PI278199	Varna 7	Bulgaria	LANDRACE	W
522	PI278213	Sinai 1	Egypt	LANDRACE	S
523	PI278249	Ostmarker	Germany	LANDRACE	W
524	PI278279	Greece 22	Greece	LANDRACE	S
525	PI278289	Salonika 12	Greece	LANDRACE	W

526	PI278297	Crete 11	Greece	LANDRACE	S
527	PI278301	Crete 15	Greece	LANDRACE	S
528	PI278310	Lovink	Netherlands	CULTIVAR	W
529	PI278311	Mansholt White	Netherlands	CULTIVAR	S
530	PI278318	Hungarian 3	Hungary	LANDRACE	W
531	PI278392	Palestine 1	Ancient Palestine	LANDRACE	S
532	PI278405	Barbela Grosso	Portugal	LANDRACE	S
533	PI278421	Roumanian Autumn	Romania	UNCERTAIN	W
534	PI278424	Balan de Figanesti	Former Soviet Union	LANDRACE	W
535	PI278432	Lukasievici	Romania	UNCERTAIN	W
536	PI278458	Dymchatka	Former Soviet Union	UNCERTAIN	W
537	PI278468	460	Russian Federation	UNCERTAIN	F
538	PI278478	2338	Former Soviet Union	UNCERTAIN	S
539	PI278490	2441	Tajikistan	LANDRACE	S
540	PI278491	2447	Uzbekistan	LANDRACE	W
541	PI278531	Beyrouth 11	Lebanon	LANDRACE	S
542	PI278533	Beyrouth 3	Lebanon	LANDRACE	W
543	PI278537	Damaskus 12	Syria	LANDRACE	S
544	PI278539	Aleppo 10	Syria	LANDRACE	W
545	PI278540	Aleppo 21	Syria	LANDRACE	S
546	PI278542	Aleppo 25	Syria	LANDRACE	S
547	PI278545	Aleppo 28	Syria	LANDRACE	S
548	PI278546	Aleppo 30	Syria	LANDRACE	S
549	PI278550	Cyprus 2	Cyprus	LANDRACE	S
550	PI278559	Tunis 11	Tunisia	LANDRACE	S

551	PI278560	Tunis 23	Tunisia	LANDRACE	S
552	PI278589	White Victoria	United Kingdom	LANDRACE	W
553	PI278590	Winter Bearded	United Kingdom	UNCERTAIN	W
554	PI278612	Sarajevo 18	Bosnia and Herzegovina	LANDRACE	W
555	PI278614	Dalmatia 3	Croatia	LANDRACE	W
556	PI278619	Crotia 5	Croatia	LANDRACE	W-S
557	PI278623	Crotia 11	Croatia	LANDRACE	W
558	PI278627	Belgrade 1	Serbia	LANDRACE	W
559	PI278635	Macedonian 14	Macedonia	LANDRACE	W
560	PI278638	Fehorri 25031 Kazak	Kazakhstan	UNCERTAIN	S
561	PI280844	II-66-13E-3E-1E	Paraguay	BREEDING	S
562	PI283147	Dorziyeh Karak	Jordan	LANDRACE	S
563	PI283148	F 8	Jordan	CULTIVAR	S
564	PI283865	Solid-Straw Velvet	New Zealand	CULTIVAR	W
565	PI283866	Sensation	New Zealand	UNCERTAIN	W
566	PI283883	Echinoides	Afghanistan	LANDRACE	W
567	PI285757	Knusiku	Poland	UNCERTAIN	S
568	PI285819	Betana	Poland	UNCERTAIN	S
569	PI285823	Czerwona Z Zsrr	Poland	CULTIVAR	W-S
570	PI285827	Erythrospermum 0211	Former Soviet Union	CULTIVAR	W
571	PI285896	Akakkowa Aka	Japan	UNCERTAIN	W
572	PI285921	Stylowa	Poland	UNCERTAIN	W
573	PI286017	Wisclawicka Wczesna	Poland	UNCERTAIN	W
574	PI286051	Viestura	Poland	UNCERTAIN	W
575	PI286526	TB 106/40	United Kingdom	BREEDING	W

576	PI286544	Colorado	Ecuador	UNCERTAIN	S
577	PI292998	TP 114/78-79	United Kingdom	BREEDING	W
578	PI294566	Beacon	Kenya	CULTIVAR	F
579	PI294888	Pudmericka Cervenoclasa	Bulgaria	UNCERTAIN	W
580	PI294911	Karnobatska Ranasreika	Bulgaria	CULTIVAR	S
581	PI294915	Kroatien	Bulgaria	UNCERTAIN	W
582	PI294918	Leschnikowzi	Bulgaria	UNCERTAIN	F
583	PI294932	Ubileina III	Bulgaria	UNCERTAIN	S
584	PI294940	Dsali Sura 35-3	Bulgaria	UNCERTAIN	W
585	PI295963	W 4-242	Israel	BREEDING	S
586	PI304090	Baragan 12	Romania	CULTIVAR	W
587	PI304101	Nr. 301	Bulgaria	CULTIVAR	W
588	PI306676	Danbatta	Nigeria	UNCERTAIN	S
589	PI308133	Rode Ris	Netherlands	LANDRACE	W
590	PI313098	Quern	Ireland	CULTIVAR	S
591	PI313109	Tres Granos	Colombia	UNCERTAIN	S
592	PI314909	Kafue	Zimbabwe	CULTIVAR	S
593	PI314924	S948A1	Zimbabwe	BREEDING	S
594	PI316428	Bc3898/62	Serbia	BREEDING	W
595	PI 320109	Sonora 64A	Mexico, Ciudad de México	Post-Green Revolution	S-F
596	PI321889	B-858	Turkey	LANDRACE	S
597	PI321902	B-871	Turkey	LANDRACE	W
598	PI321968	B-942	Turkey	LANDRACE	S
599	PI 323093	Xelaju 66	Mexico, Ciudad de México	Post-Green Revolution	S-F
600	PI323413	Vigorka 37/16	Croatia	UNCERTAIN	W
601	PI323634	NS 732	Serbia	CULTIVAR	W

602	PI325845	Shiro-Daruma	Japan	LANDRACE	W
603	PI326308	Tczy-Gan-U-Man	China	LANDRACE	W
604	PI329235	Najah	Lebanon	CULTIVAR	S
605	PI330535	Hostianum	United Kingdom	UNCERTAIN	S
606	PI331245	Sinday	Eritrea	LANDRACE	S
607	PI337650	Surkhcha Paghmani	Afghanistan	LANDRACE	W
608	PI338530	Niki	Greece	CULTIVAR	S
609	PI338533	Pindos	Greece	CULTIVAR	W
610	PI 338915	Ciano F67	Mexico, Ciudad de México	Post-Green Revolution	S-F
611	PI 338916	Inia 66	Mexico, Ciudad de México	Post-Green Revolution	S-F
612	PI 338920	Norteno 67	Mexico, Ciudad de México	Post-Green Revolution	S-F
613	PI 338921	Siete Cerros 66	Mexico, Ciudad de México	Post-Green Revolution	S-F
614	PI340665	Albania V	Netherlands	UNCERTAIN	W
615	PI340762	Trifolium	Denmark	CULTIVAR	W
616	PI341364	Kislik	Turkey	LANDRACE	S
617	PI341431	Bugday	Turkey	LANDRACE	S
618	PI341442	Ziraat	Turkey	LANDRACE	W
619	PI341625	Sunter	Turkey	LANDRACE	S
620	PI343737	Atacazo	Ecuador	CULTIVAR	S
621	PI343738	Ruminahui	Ecuador	CULTIVAR	S
622	PI344018	Mistura Pinto	Angola	CULTIVAR	S
623	PI345159	158-V/5-A	Serbia	LANDRACE	W
624	PI345205	204-VI/9-A	Serbia	LANDRACE	W
625	PI345238	237-VII/2	Macedonia	LANDRACE	W
626	PI345244	243-VII/4	Macedonia	LANDRACE	W
627	PI345269	268-VII/16	Macedonia	LANDRACE	S

628	PI345319	319-VII/38	Macedonia	LANDRACE	W
629	PI345355	354A-I/7	Montenegro	LANDRACE	W
630	PI345356	355-I/8	Montenegro	LANDRACE	W
631	PI345408	407-IV/60	Bosnia and Herzegovina	LANDRACE	F
632	PI345409	408-IV/61	Bosnia and Herzegovina	LANDRACE	W
633	PI345468	466a	Serbia	LANDRACE	W
634	PI345474	471	Serbia	LANDRACE	S
635	PI345476	473	Serbia	LANDRACE	W
636	PI345693	Minskaja	Belarus	CULTIVAR	S
637	PI346424	29/60	Former Yugoslavia	UNCERTAIN	W
638	PI347110	Bahri Sirhosha	Afghanistan	LANDRACE	S
639	PI347169	Tirmai	Afghanistan	LANDRACE	S
640	PI347180	Sirhosha	Afghanistan	LANDRACE	W
641	PI347188	Garma	Afghanistan	LANDRACE	W
642	PI347196	Kalabudundak	Afghanistan	LANDRACE	S
643	PI349444	1876D	Switzerland	LANDRACE	S
644	PI349476	135D	Switzerland	LANDRACE	S
645	PI349512	2045A	Switzerland	LANDRACE	S
646	PI349521	1271B	Switzerland	LANDRACE	S
647	PI349531	1294B	Switzerland	LANDRACE	S
648	PI349539	381F	Switzerland	LANDRACE	S
649	PI349683	HA 9-39-1	Finland	LANDRACE	S
650	PI349959	547-III/3	Serbia	LANDRACE	W
651	PI349995	590-III/13	Serbia	LANDRACE	W-S
652	PI350150	772-VII/14	Macedonia	LANDRACE	W

653	PI350264	908-IX/4	Bosnia and Herzegovina	LANDRACE	W
654	PI350269	914-IX/6	Bosnia and Herzegovina	LANDRACE	W
655	PI350727	69Z108.1	Austria	LANDRACE	S
656	PI350744	69Z108.18	Austria	LANDRACE	S
657	PI350748	69Z108.22	Austria	LANDRACE	S
658	PI350822	69Z108.117	Austria	LANDRACE	S
659	PI350831	69Z108.126	Austria	LANDRACE	S
660	PI350832	69Z108.127	Austria	LANDRACE	S
661	PI350847	69Z108.142	Austria	LANDRACE	S
662	PI350850	69Z108.145	Austria	LANDRACE	S
663	PI350856	69Z108.151	Austria	LANDRACE	S
664	PI350974	69Z2.104/672A	Switzerland	LANDRACE	W
665	PI351046	69Z2.179/1380C	Austria	LANDRACE	W
666	PI351217	Nevodvorska Niva	Czechoslovakia	UNCERTAIN	W
667	PI351313	Abed 8-41	Denmark	BREEDING	W
668	PI351342	T-976	Austria	BREEDING	W
669	PI351362	Pailly	Switzerland	CULTIVAR	W
670	PI351461	T-1164	Italy	BREEDING	W
671	PI351463	Prince Leopold	Belgium	CULTIVAR	W
672	PI351474	Reval	Estonia	UNCERTAIN	W
673	PI351580	Moystad 0944	Norway	CULTIVAR	W
674	PI351595	Chitral	India	LANDRACE	W
675	PI351626	Udvaros 2	Hungary	CULTIVAR	W
676	PI351660	Ulka	Switzerland	UNCERTAIN	S
677	PI351669	St 8/12	Italy	BREEDING	W

678	PI351748	Jasi 10T	Romania	CULTIVAR	S
679	PI351872	JC 73	Burundi	UNCERTAIN	S
680	PI351878	Kiska 9	Burundi	UNCERTAIN	S
681	PI351994	Z.88.116	Switzerland	BREEDING	S
682	PI352021	A 394	Switzerland	BREEDING	W
683	PI352095	SV 59559	Sweden	BREEDING	W
684	PI352105	JC 73 Selection	Burundi	UNCERTAIN	S
685	PI352226	Niederndorferberg 1380C	Austria	LANDRACE	W
686	PI352286	Leningrad 0 479 (LUTESCENS 479)	Russian Federation	BREEDING	S
687	PI355088	No. 9	Iran	LANDRACE	S
688	PI358339	2020/70	Croatia	BREEDING	S
689	PI361713	XIII T.I	Denmark	UNCERTAIN	W
690	PI361776	Torado Fam. 29	Denmark	UNCERTAIN	W
691	PI361796	Saunders I-47-4	Denmark	UNCERTAIN	F
692	PI361810	Blak. Fra. Prof. Tschkem.	Denmark	UNCERTAIN	W
693	PI361837	Otofte L56	Denmark	CULTIVAR	W
694	PI361853	Puun	Denmark	UNCERTAIN	W-S
695	PI361868	63/1-7-15-12	Denmark	BREEDING	S
696	PI361953	A 15 Marcelesti Selection	Romania	BREEDING	W
697	PI362184	Seu Yuk 126	Korea, South	CULTIVAR	W
698	PI362185	Su Kae No. 158	Korea, South	CULTIVAR	S
699	PI362412	II/7-B	Serbia	LANDRACE	W
700	PI362456	III/22-B	Serbia	LANDRACE	W
701	PI362486	IV/9-E	Serbia	LANDRACE	W

702	PI362526	V/2-A	Serbia	LANDRACE	W
703	PI362546	VI/3-A	Serbia	LANDRACE	W
704	PI362612	VII/8-B	Guatemala	-	W
705	PI362693	VIII/21-A	Montenegro	LANDRACE	W
706	PI366063	Giza 156	Egypt	CULTIVAR	S
707	PI366072	V-3	Egypt	LANDRACE	S
708	PI366083	V-14	Egypt	LANDRACE	S
709	PI 366128	Nadadores 169	Mexico	Post-Green Revolution	S-F
710	PI366685	756	Afghanistan	LANDRACE	S
711	PI367244	472/71	Croatia	BREEDING	W
712	PI368010	187-199-VI/8 A	Former Yugoslavia	UNCERTAIN	W
713	PI 369726	Mexicano 1481	Mexico, Ciudad de México	Post-Green Revolution	S-F
714	PI372125	Alabasskaja	Kazakhstan	CULTIVAR	W
715	PI372137	Lutescens 491	Ukraine	CULTIVAR	S
716	PI372150	Ferrugineum 67	Tajikistan	CULTIVAR	S
717	PI372434	FAO 29.930	Cyprus	LANDRACE	S
718	PI372473	FAO 29.949	Greece	LANDRACE	W
719	PI374243	BOL-11	Chad	LANDRACE	S
720	PI374244	BOL-12	Chad	LANDRACE	S
721	PI374245	BOL-13	Chad	LANDRACE	S
722	PI374246	BOL-14	Chad	LANDRACE	S
723	PI374247	BOL-15	Chad	LANDRACE	S
724	PI374248	BOL-17	Chad	LANDRACE	S
725	PI374249	BOL-19	Chad	LANDRACE	S
726	PI374254	Jan-94 (34335)	Mali	UNCERTAIN	S
727	PI374257	Niana	Mali	CULTIVAR	S

728	PI374617	155/71	Macedonia	LANDRACE	W
729	PI374670	208/71	Bosnia and Herzegovina	LANDRACE	S
730	NO PACKET	N/A	N/A	N/A	N/A
731	PI374672	210/71	Bosnia and Herzegovina	LANDRACE	S
732	PI374678	216/71	Bosnia and Herzegovina	LANDRACE	W
733	PI374680	218/71	Bosnia and Herzegovina	LANDRACE	W
734	PI374684	222/71	Bosnia and Herzegovina	LANDRACE	S
735	PI374692	230/71	Bosnia and Herzegovina	LANDRACE	W
736	PI378276	1548	Serbia	LANDRACE	W
737	PI378323	1596	Montenegro	LANDRACE	W
738	PI378353	1627	Macedonia	LANDRACE	W
739	PI378355	1630	Macedonia	LANDRACE	W
740	PI378412	1687	Macedonia	LANDRACE	W
741	PI378430	1705	Macedonia	LANDRACE	F
742	PI378455	1730	Macedonia	LANDRACE	S
743	PI378500	1775	Macedonia	LANDRACE	W
744	PI378527	1802	Serbia	LANDRACE	W
745	PI378912	13440	Colombia	UNCERTAIN	S
746	PI378917	C38-1	Philippines	UNCERTAIN	S
747	PI378922	Florence Aurore	Philippines	CULTIVAR	S
748	PI381963	15	Iran	LANDRACE	S
749	PI382048	125	Iran	LANDRACE	S
750	PI382150	Inayama	Japan	LANDRACE	S
751	PI383991	Hitsumi Komugi	Japan	CULTIVAR	F
752	PI384033	Mivhor 1177	Israel	CULTIVAR	S

753	PI384101	GAW 8-5	Ethiopia	LANDRACE	S
754	PI384351	Dikwa 6	Nigeria	UNCERTAIN	S
755	PI384396	Hadejia 2	Nigeria	UNCERTAIN	S
756	PI384399	Nigeria-2	Nigeria	UNCERTAIN	S
757	PI384403	Wurno 4	Nigeria	UNCERTAIN	S
758	PI384421	Zaria 1	Nigeria	UNCERTAIN	S
759	PI387588	IAR/W/126-4	Ethiopia	LANDRACE	S
760	PI387594	IAR/W/128-5	Ethiopia	LANDRACE	S
761	PI388066	FAO 33.202	Pakistan	LANDRACE	S
762	PI404235	Bal'bugda	Azerbaijan	CULTIVAR	S
763	PI406486	18	Nepal	LANDRACE	S
764	PI406490	26	Nepal	LANDRACE	W
765	PI406517	48B	Nepal	LANDRACE	S
766	PI406523	55	Nepal	LANDRACE	S
767	PI406530	77	Nepal	LANDRACE	W
768	PI410566	W25	Pakistan	LANDRACE	F
769	PI410595	W47	Pakistan	LANDRACE	F
770	PI410596	W48A	Pakistan	LANDRACE	F
771	PI410630	W79	Pakistan	LANDRACE	S
772	PI410632	W81	Pakistan	LANDRACE	F
773	PI410899	Gloria	Morocco	CULTIVAR	S
774	PI410909	Boudahaika	Morocco	LANDRACE	S
775	PI 410915	Potam	Mexico	Post-Green Revolution	S-F
776	PI 412954	Bluebird 2	Mexico, Ciudad de México	Post-Green Revolution	S-F
777	PI 412955	Cajeme 71	Mexico, Ciudad de México	Post-Green Revolution	S-F

778	PI414087	Chinoli 70	Bolivia	CULTIVAR	S
779	PI414975	No. 6	Indonesia	UNCERTAIN	S
780	PI414993	Perico 'S'	Mexico	BREEDING	S
781	PI415421	I/79	Macedonia	LANDRACE	F
782	PI415430	I/88	Macedonia	LANDRACE	W
783	PI 415999	Tordo	Mexico, Ciudad de México	Post-Green Revolution	S-F
784	PI418571	Przevalskaja	Kyrgyzstan	CULTIVAR	W
785	PI420618	I/140	Macedonia	LANDRACE	F
786	PI420932	25	Jordan	LANDRACE	W
787	PI422301	Vers 8-1	Egypt	BREEDING	S
788	PI422438	Lushnja	Albania	UNCERTAIN	W
789	PI422440	Mysegeja	Albania	UNCERTAIN	S
790	PI422512	Cora	Belgium	CULTIVAR	W
791	PI 428414	Cocoraque F75	Mexico, Ciudad de México	Post-Green Revolution	S-F
792	PI 428429	Sapsucker	Mexico, Ciudad de México	Post-Green Revolution	S-F
792	PI 428428	Zaragoza 75	Mexico, Ciudad de México	Post-Green Revolution	S-F
793	PI428649	Prerovska Alba	Czech Republic	CULTIVAR	W-S
794	PI428666	Detenicka Vouska	Czech Republic	CULTIVAR	S
795	PI429315	Bir Bouni	Yemen	LANDRACE	S
796	PI429318	Mesri	Yemen	UNCERTAIN	S
797	PI429323	R.R. 21	Yemen	BREEDING	S
798	PI429396	86	-	-	W
799	PI429627	NW10A	Nepal	LANDRACE	S
800	PI429641	NW25A	Nepal	LANDRACE	S
801	PI429657	NW45A	Nepal	LANDRACE	S
802	PI429659	NW48A	Nepal	LANDRACE	S

803	PI429667	NW56A	Nepal	LANDRACE	S
804	PI429670	NW59A	Nepal	LANDRACE	S
805	PI429689	NW86A	Nepal	LANDRACE	W
806	PI429696	NW93A	Nepal	LANDRACE	S
807	PI429699	NW96A	Nepal	LANDRACE	S
808	PI429707	NW103A	Nepal	LANDRACE	W
809	PI430030	W142	India	LANDRACE	F
810	PI430067	W195	India	LANDRACE	S
811	PI430075	W204	India	LANDRACE	W
812	PI 433766	Jauhara 76	Mexico, Ciudad de México	Post-Green Revolution	S-F
813	PI 433767	Pima 77	Mexico, Ciudad de México	Post-Green Revolution	S-F
814	PI 433768	Tezapaco 76	Mexico, Ciudad de México	Post-Green Revolution	S-F
815	PI 433769	Torim 73	Mexico, Ciudad de México	Post-Green Revolution	S-F
816	PI434673	NS 50-25	Serbia	BREEDING	W
817	PI434915	Giza 23	Egypt	LANDRACE	F
818	PI436228	49	Chile	LANDRACE	W
819	PI436241	74	Chile	LANDRACE	F
820	PI436300	159	Chile	LANDRACE	W
821	PI436314	177	Chile	LANDRACE	W
822	PI436327	212	Chile	LANDRACE	W
823	PI436425	381	Chile	LANDRACE	S
824	PI436435	403	Chile	LANDRACE	W
825	PI436512	500	Chile	LANDRACE	W
826	PI438964	Karagandinskaja 2	Kazakhstan	CULTIVAR	F
827	PI442901	Egret	Australia	CULTIVAR	F
828	PI445714	56 (SANO CHILE)	Nepal	LANDRACE	S

829	PI445732	143 (HASHA)	Nepal	LANDRACE	S
830	PI445734	145	Nepal	LANDRACE	S
831	PI445736	148 (DUDHE MUDULO)	Nepal	LANDRACE	S
832	PI445746	167	Nepal	LANDRACE	S
833	PI445747	169	Nepal	LANDRACE	S
834	PI445748	172 (RAJI)	Nepal	LANDRACE	S
835	PI445754	180 (DOTIL)	Nepal	LANDRACE	S
836	PI445763	206	Nepal	LANDRACE	S
837	PI445764	207	Nepal	LANDRACE	W
838	PI445778	J-29	Nepal	LANDRACE	S
839	PI462106	Bohr Gamh	Yemen	UNCERTAIN	S
840	PI469005	MG 27061	Greece	LANDRACE	S
841	PI469020	MG 27174	Spain	LANDRACE	W
842	PI470595	79TK113-1076	Turkey	LANDRACE	F
843	PI470884	MG 18036	Algeria	LANDRACE	S
844	PI470906	MG 18061	Algeria	LANDRACE	F
845	PI 471919	Genaro F81	Mexico, Ciudad de México	Post-Green Revolution	S-F
846	PI 471920	Glennson M81	Mexico, Ciudad de México	Post-Green Revolution	S-F
847	PI 471921	Sonoita F81	Mexico, Ciudad de México	Post-Green Revolution	S-F
848	PI 471923	Ures T81	Mexico, Ciudad de México	Post-Green Revolution	S-F
849	PI477899	Trigo (PLT 76-2017)	Peru	LANDRACE	S
850	PI478031	PNE 196-2	Pakistan	LANDRACE	S
851	PI478037	PNE 198-6	Pakistan	LANDRACE	S
852	PI478054	PNE 212-2	Pakistan	LANDRACE	S
853	PI478071	PNE 225-1	Pakistan	LANDRACE	W
854	PI478083	PNE 229-4	Pakistan	LANDRACE	F

855	PI478197	PNE 374-4	Pakistan	LANDRACE	F
856	PI478199	PNE 377-1	Pakistan	LANDRACE	W-S
857	PI478203	PNE 381-1	Pakistan	LANDRACE	S
858	PI478204	PNE 382-1	Pakistan	LANDRACE	S
859	PI478286	PNE 244-2	Pakistan	LANDRACE	S
860	PI479870	MG 31441	Ethiopia	LANDRACE	S
861	PI479876	MG 31450	Ethiopia	LANDRACE	S
862	PI479890	Soprino	Ethiopia	LANDRACE	S
863	PI479901	MG 31613	Ethiopia	LANDRACE	S
864	PI480161	Sindi	Ethiopia	LANDRACE	S
865	PI480285	MG 31507	Ethiopia	LANDRACE	S
866	PI480364	Soprino	Ethiopia	LANDRACE	F
867	PI480480	R-124	Bolivia	LANDRACE	S
868	PI481562	IQ 14	Iraq	LANDRACE	S
869	PI481563	IQ 53	Iraq	LANDRACE	S
870	PI481564	IQ 54	Iraq	LANDRACE	S
871	PI481567	IQ 83	Iraq	LANDRACE	S
872	PI481708	Ka	Bhutan	LANDRACE	S
873	PI481710	Ka	Bhutan	LANDRACE	W-S
874	PI481711	Jho	Bhutan	LANDRACE	W-S
875	PI481712	Jho (Ka)	Bhutan	LANDRACE	S
876	PI481713	41	Bhutan	LANDRACE	W
877	PI481718	101	Bhutan	LANDRACE	S
878	PI481719	Ka	Bhutan	LANDRACE	S
879	PI481720	Ka	Bhutan	LANDRACE	F

880	PI481721	148	Bhutan	LANDRACE	F
881	PI481722	151	Bhutan	LANDRACE	W
882	PI481723	Go	Bhutan	LANDRACE	F
883	PI481725	Go	Bhutan	LANDRACE	W
884	PI481726	Go	Bhutan	LANDRACE	W-S
885	PI481727	Dosherbong	Bhutan	LANDRACE	S
886	PI481728	Bong	Bhutan	LANDRACE	S
887	PI481729	Bithpu Bong	Bhutan	LANDRACE	F
888	PI481916	109	Sudan	LANDRACE	S
889	PI481917	110	Sudan	LANDRACE	S
890	PI481918	111	Sudan	LANDRACE	S
891	PI481919	112	Sudan	LANDRACE	S
892	PI481920	114	Sudan	LANDRACE	S
893	PI481921	119	Sudan	LANDRACE	S
894	PI481922	122	Sudan	LANDRACE	S
895	PI481926	126	Sudan	LANDRACE	S
896	PI481927	127	Sudan	LANDRACE	S
897	PI481928	129	Sudan	LANDRACE	S
898	PI481929	130	Sudan	LANDRACE	S
899	PI481930	131	Sudan	LANDRACE	S
900	PI 486429	Dusty	United States, Washington	Post-Green Revolution	S-F
901	PI487288	SY 267	Jordan	LANDRACE	S
902	PI487292	SY 270	Jordan	LANDRACE	S
903	PI490402	Alkamu beri	Mali	UNCERTAIN	S
904	PI490403	Alkamu tire	Mali	UNCERTAIN	S

905	PI490404	79	Mali	UNCERTAIN	S
906	PI490405	Koira alkuna	Mali	UNCERTAIN	S
907	PI491378	PK 15896 (PNE 401-1)	Pakistan	LANDRACE	W-S
908	PI 494186	Broadbill	Mexico, Ciudad de México	Post-Green Revolution	S-F
909	PI494927	ZFA 3629	Zambia	UNCERTAIN	S
910	PI499964	KU 1523	Azerbaijan	LANDRACE	W
911	PI499967	KU 1630	Armenia	LANDRACE	S
912	PI499971	KU 1806	Georgia	LANDRACE	S
913	PI508387	V882-F22-F2-F3-F2-J2	Israel	BREEDING	S
914	PI518885	P 8045-78	Austria	BREEDING	W
915	PI519098	L 227-H 3	Romania	BREEDING	W
916	PI519185	Sort 11-32-1145	Bulgaria	BREEDING	W
917	PI 519315	Azteca F67	Mexico, Ciudad de México	Post-Green Revolution	S-F
918	PI 519316	Bajio F67	Mexico, Ciudad de México	Post-Green Revolution	S-F
919	PI519465	SA 79003-9-2	Zimbabwe	BREEDING	S
920	PI519487	TP 250-2	United Kingdom	BREEDING	S
921	PI519489	VI 121.2.13B.2T.2B	Colombia	BREEDING	S
922	PI519629	VI-91-2-2-25B-6T-1B	Ecuador	BREEDING	S
923	PI519653	R 100171-78	Brazil	BREEDING	S
924	PI519683	A4920-52P-2P-2P	Chile	BREEDING	S
925	PI519711	LM72-14-75	Iran	BREEDING	S
926	PI 519847	Pavon F76	Mexico, Ciudad de México	Post-Green Revolution	S-F
927	PI519912	W 5885-2-M-1-LM	South Africa	BREEDING	S
928	PI519950	M 1169-1-49-164	Iran	BREEDING	S
929	PI 519986	Bonza 65	Mexico, Ciudad de México	Post-Green Revolution	S-F
930	PI 520076	Rousalka	Mexico, Ciudad de México	Post-Green Revolution	S-F

931	PI520093	SE 424-5S-2S-0S	Turkey	BREEDING	S
932	PI520128	HD 2171	India	CULTIVAR	S
933	PI520156	SWN 2984-M-1Y-1M-2Y-0M-0MN	Mexico	BREEDING	S
934	PI 520167	Ceyhan 75	Mexico, Ciudad de México	Post-Green Revolution	S-F
935	PI 520171	Alondra	Mexico, Ciudad de México	Post-Green Revolution	S-F
936	PI520194	WA 6510	United States	BREEDING	S
937	PI 520248	Veery No. 2	Mexico, Ciudad de México	Post-Green Revolution	S-F
938	PI 520249	Veery No. 3	Mexico, Ciudad de México	Post-Green Revolution	S-F
939	PI 520259	Ciano T79	Mexico, Ciudad de México	Post-Green Revolution	S-F
940	PI520373	Code 70-133	Syria	BREEDING	S
941	PI 520553	Veery No. 3	Mexico, Ciudad de México	Post-Green Revolution	S-F
942	PI 525198	Dollarbird	Mexico, Ciudad de México	Post-Green Revolution	S-F
943	PI525211	1004b	Morocco	LANDRACE	S
944	PI525219	1018b	Morocco	LANDRACE	S
945	PI525221	1018d	Morocco	LANDRACE	S
946	PI525241	1049	Morocco	LANDRACE	S
947	PI525246	1054a	Morocco	LANDRACE	S
948	PI525253	1066	Morocco	LANDRACE	S
949	PI525280	1127	Morocco	LANDRACE	S
950	PI525282	1130	Morocco	LANDRACE	S
951	PI525283	1137	Morocco	LANDRACE	S
952	PI525284	1139	Morocco	LANDRACE	S
953	PI525295	1162	Morocco	LANDRACE	S
954	PI525318	1254	Morocco	LANDRACE	S
955	PI525326	1359	Morocco	LANDRACE	S

956	PI525346	1083c	Morocco	LANDRACE	S
957	PI525361	1134	Morocco	LANDRACE	S
958	PI525434	1372	Morocco	LANDRACE	S
959	PI527451	Kammah	Algeria	LANDRACE	S
960	PI532067	907	Egypt	LANDRACE	S
961	PI532107	2137	Egypt	LANDRACE	S
962	PI532116	2355	Egypt	LANDRACE	S
963	PI 532243	Hamira	Oman	LANDRACE	S
964	PI532244	Gredha	Oman	LANDRACE	S
965	PI532246	Saraya	Oman	LANDRACE	S
966	PI532248	Kule	Oman	LANDRACE	S
967	PI532255	Mufseggha	Oman	LANDRACE	S
968	PI532258	Hamira	Oman	LANDRACE	S
969	PI532263	Musane	Oman	LANDRACE	S
970	PI532277	Sariya	Oman	LANDRACE	S
971	PI532278	Kule	Oman	LANDRACE	S
972	PI532280	Musane	Oman	LANDRACE	S
973	PI532283	Sariya	Oman	LANDRACE	S
974	PI532284	7442	Oman	LANDRACE	S
975	PI532286	Saluta	Oman	LANDRACE	S
976	PI532293	Shallot	Oman	LANDRACE	S
977	PI532296	Musane	Oman	LANDRACE	S
978	PI534284	Tucursindi	Ethiopia	LANDRACE	S
979	PI534323	Sindi	Ethiopia	LANDRACE	S
980	PI534350	Chili	Tunisia	LANDRACE	S

981	PI534352	MG 18140	Tunisia	LANDRACE	S
982	PI534388	Florxaurore	Algeria	LANDRACE	S
983	PI537060	Ajantha	India	CULTIVAR	S
984	PI 542434	Pato	Mexico, Ciudad de México	Post-Green Revolution	S-F
985	PI542450	H82-710-1	Poland	BREEDING	W
986	PI542666	Ghati	Algeria	LANDRACE	S
987	PI542668	Hamra	Algeria	LANDRACE	S
988	PI548117	Ecsi	Hungary	CULTIVAR	W
989	PI548119	Erpataki	Hungary	LANDRACE	W
990	PI548134	Marcaltoi	Hungary	LANDRACE	W
991	PI559598	84TK541-001.0	Turkey	LANDRACE	S
992	PI559660	Erget	Serbia	CULTIVAR	S
993	PI559967	1116	Morocco	LANDRACE	S
994	PI560637	TU85-048-03-1	Turkey	LANDRACE	F
995	PI561910	OK91G158	United States	GENETIC	S
996	PI565204	Mura Huma	Bolivia	LANDRACE	S
997	PI565209	Mocho	Bolivia	LANDRACE	S
998	PI565211	Lerma	Bolivia	LANDRACE	S
999	PI565213	Florentino	Bolivia	LANDRACE	S
1000	PI565221	Trigo	Bolivia	LANDRACE	S
1001	PI565222	Gabo	Bolivia	LANDRACE	S
1002	PI565223	Australiano	Bolivia	LANDRACE	S
1003	PI565230	Americano	Bolivia	LANDRACE	S
1004	PI565235	Trigo Australiano	Bolivia	LANDRACE	S
1005	PI565236	Jaral	Bolivia	LANDRACE	S

1006	PI565238	Quimora	Bolivia	LANDRACE	S
1007	PI565241	Saguayo	Bolivia	LANDRACE	S
1008	PI565246	Criollo	Bolivia	LANDRACE	S
1009	PI565254	Khumpu	Bolivia	LANDRACE	S
1010	PI565336	Kzyl Bugdai	Uzbekistan	LANDRACE	S
1011	PI565353	Buhara Bugdai	Uzbekistan	LANDRACE	S
1012	PI565355	Buhara Bugdai	Uzbekistan	LANDRACE	W
1013	PI565356	Kusary	Azerbaijan	CULTIVAR	W
1014	PI565358	Teremai Bugdai	Uzbekistan	LANDRACE	W
1015	PI565381	WIR 26382	Uzbekistan	LANDRACE	W
1016	PI565385	Kuzgiu Bugdai	Kyrgyzstan	LANDRACE	W-S
1017	PI565386	Guzlyk	Turkmenistan	LANDRACE	S
1018	PI565429	Tedzenskaja 60	Turkmenistan	CULTIVAR	F
1019	PI572621	Al'borubrum B-21	Belarus	CULTIVAR	W
1020	PI572638	Saljut	Ukraine	CULTIVAR	W
1021	PI572642	Mil'turum 1	Moldova	CULTIVAR	W
1022	PI572650	Galliaaral'skaja 3	Uzbekistan	CULTIVAR	S
1023	PI572661	AW 6637A/87 (SN-Z32a)	Georgia	LANDRACE	S
1024	PI572677	HW 6558/85 (SN-Z20)	Georgia	LANDRACE	S
1025	PI572680	HW 7033/88 (SN-1905)	Georgia	LANDRACE	W
1026	PI572683	HW 7038/88 (SN-2023)	Georgia	LANDRACE	W
1027	PI572685	AW 7040/90 (SN-2073)	Georgia	LANDRACE	S
1028	PI572698	Shrike	Australia	CULTIVAR	W-S
1029	PI572720	I-906	Nepal	LANDRACE	S
1030	PI 572728	Tobari 66	Mexico, Ciudad de México	Post-Green Revolution	S-F
1031	PI 572729	Nacozari F76	Mexico, Ciudad de México	Post-Green Revolution	S-F

1032	PI 572730	Chapingo VF74	Mexico, Ciudad de México	Post-Green Revolution	S-F
1033	PI572764	86PK1281-001.02	Pakistan	LANDRACE	S
1034	PI572844	86PK1330-001.00	Pakistan	LANDRACE	S
1035	PI573754	NSGC 531	Honduras	LANDRACE	S
1036	PI574344	15023	Saudi Arabia	UNCERTAIN	S
1037	PI574379	1183	Yemen	UNCERTAIN	S
1038	PI576639	2262-12	Tunisia	LANDRACE	S
1039	PI576667	84TK046-118.1	Turkey	LANDRACE	S
1040	PI576700	84TK293-008.1	Turkey	LANDRACE	S
1041	PI577755	MG 4471	Italy	LANDRACE	S
1042	PI583694	Xelaju	Guatemala	CULTIVAR	S
1043	PI 583709	Saric 70	Mexico, Ciudad de México	Post-Green Revolution	S-F
1044	PI 583710	Gallo	Mexico, Ciudad de México	Post-Green Revolution	S-F
1045	PI 583715	Obregon	Mexico, Ciudad de México	Post-Green Revolution	S-F
1046	PI 583717	Merav	Mexico	Post-Green Revolution	S-F
1047	PI585211	1076	Yemen	UNCERTAIN	S
1048	PI 591762	Carrizo T89	Mexico, Ciudad de México	Post-Green Revolution	S-F
1049	PI 591764	Guasave F81	Mexico, Ciudad de México	Post-Green Revolution	S-F
1050	PI 591765	Delicias 81	Mexico, Ciudad de México	Post-Green Revolution	S-F
1051	PI 591766	Jupateco 73	Mexico, Ciudad de México	Post-Green Revolution	S-F
1052	PI 591767	Galvez 87	Mexico, Ciudad de México	Post-Green Revolution	S-F
1053	PI 591768	Mexico 82	Mexico, Ciudad de México	Post-Green Revolution	S-F
1054	PI 591769	Culiacan T89	Mexico, Ciudad de México	Post-Green Revolution	S-F
1055	PI 591770	Tesia 79	Mexico, Ciudad de México	Post-Green Revolution	S-F
1056	PI 591771	Imuris 79	Mexico, Ciudad de México	Post-Green Revolution	S-F
1057	PI 591772	Tonichi S81	Mexico, Ciudad de México	Post-Green Revolution	S-F

1058	PI 591773	Jahuara 77	Mexico, Ciudad de México	Post-Green Revolution	S-F
1059	PI 591774	Seri 82	Mexico, Ciudad de México	Post-Green Revolution	S-F
1060	PI 591775	Tepoca 89	Mexico, Ciudad de México	Post-Green Revolution	S-F
1061	PI 591776	Opata 85	Mexico, Ciudad de México	Post-Green Revolution	S-F
1062	PI 591777	Cleopatra 74	Mexico, Ciudad de México	Post-Green Revolution	S-F
1063	PI 591778	Nuri 70	Mexico, Ciudad de México	Post-Green Revolution	S-F
1064	PI 591779	Angostura F88	Mexico, Ciudad de México	Post-Green Revolution	S-F
1065	PI 591780	Temporalera 87	Mexico, Ciudad de México	Post-Green Revolution	S-F
1066	PI 591782	Papago 86	Mexico, Ciudad de México	Post-Green Revolution	S-F
1067	PI 591783	Bacanora 88	Mexico, Ciudad de México	Post-Green Revolution	S-F
1068	PI 591784	Rayon 89	Mexico, Ciudad de México	Post-Green Revolution	S-F
1069	PI 591785	Ocoroni 86	Mexico, Ciudad de México	Post-Green Revolution	S-F
1070	PI 591786	Cumpas 88	Mexico, Ciudad de México	Post-Green Revolution	S-F
1071	PI 591787	Anahuac 75	Mexico, Ciudad de México	Post-Green Revolution	S-F
1072	PI 591788	Cucurpe 86	Mexico, Ciudad de México	Post-Green Revolution	S-F
1073	PI 591789	Esmeralda 86	Mexico, Ciudad de México	Post-Green Revolution	S-F
1074	PI 591790	Huasteco M81	Mexico, Ciudad de México	Post-Green Revolution	S-F
1075	PI 591791	Mochis 88	Mexico, Ciudad de México	Post-Green Revolution	S-F
1076	PI 591792	Hermosillo 77	Mexico, Ciudad de México	Post-Green Revolution	S-F
1077	PI591867	AW 6637C/86 (SN-Z32c)	Georgia	LANDRACE	S
1078	PI591870	HW 6554/86 (SN-Z17a)	Georgia	LANDRACE	W
1079	PI591997	Hong Mang Mai	China	LANDRACE	W
1080	PI 603213	Batan F96	Mexico, México	Post-Green Revolution	S-F
1081	PI615369	RCAT000491	Hungary	LANDRACE	W
1082	PI615373	RCAT003313	Hungary	LANDRACE	W
1083	PI615375	RCAT003418	Hungary	LANDRACE	W
1084	PI615386	RCAT004555	Hungary	LANDRACE	W

1085	PI 619633	Nahuatl F2000	Mexico, México	Post-Green Revolution	S-F
1086	PI 619634	Tlaxcala F2000	Mexico, México	Post-Green Revolution	S-F
1087	PI 619635	Juchi F2000	Mexico, México	Post-Green Revolution	S-F
1088	PI620902	IWA8606156	Iran	LANDRACE	W
1089	PI623097	IWA8600064	Iran	LANDRACE	S
1090	PI630982	93-11-2-3-2	Canada	BREEDING	S
1091	PI639339	TJK03-91	Tajikistan	LANDRACE	S
1092	PI639349	TJK03-123	Tajikistan	LANDRACE	S
1093	PI639364	TJK03-151	Tajikistan	LANDRACE	S
1094	PI639458	Karabalykskaya 92	Kazakhstan	CULTIVAR	S
1095	PI 652453	S-24	Pakistan	-	S