EVALUATION OF ALPHA-AMYLASE ACTIVITY AND FALLING NUMBER FOR SOFT WHITE AND SOFT RED WHEAT VARIETIES ADAPTED TO MICHIGAN

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ABSTRACT

EVALUATION OF ALPHA-AMYLASE ACTIVITY AND FALLING NUMBER FOR SOFT WHITE AND SOFT RED WHEAT VARIETIES ADAPTED TO MICHIGAN

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Pre-Harvest Sprouting (PHS) is a major threat to Michigan soft white wheat, and recent epidemics were experienced in 2008 and 2009. Alpha-amylase is an important component of PHS and the falling number (FN) test is used by industry to identify sprouted wheat that is unacceptable for various food products. The objectives of this study were to evaluate wheat cultivars adapted to Michigan for the activity of α-amylase and FN values around physiological maturity (PM) in natural conditions and multiple artificial PHS induction treatments. Twenty-four soft winter wheat genotypes with varying levels of susceptibility to PHS were planted in α-lattice designs in two locations in three years. Spikes were collected three days before PM, at PM, and three days post PM. Treated samples were freeze-dried, processed and evaluated for α-amylase activity and FN values. Genetic differences existed for both responses at all maturity categories in the absence of PHS induction. A clear trend was observed in the reduction of α-amylase and the increase in FN during the maturation in non-PHS conditions. For the study of PHS induction methods, genetic differences among 24 cultivars existed for both responses at PM in all misting treatments. After-ripening period had a significant effect on breaking dormancy for some lines, and an effective protocol for PHS screening at MSU was identified. ‘Jupiter’, ‘Lowell’ and ‘Caledonia’ were highly susceptible, while ‘MSU E5024’ and AgriPro ‘W1062’ were two white wheat cultivars that showed consistent tolerance to PHS in this study.

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CHAPTER I

GENERAL INTRODUCTION

INTRODUCTION

The Importance of Wheat: from World to Michigan

Wheat is the one of the world's most important food crops and is produced widely around the globe. In 2011, world production of wheat was 690 million tons, making it the second most produced cereal after corn (861 million tons) (IGC, 2012). Wheat is grown more widely than any other commercial crop and holds the greatest world trade (FAO, 2002). Globally, it is the second major human food crop after rice. Wheat can be ground into flour, semolina or used as malts and groats, all of which are used in a wide variety of human foods, such as bread, pasta, cakes, cookies and so on. Wheat, which provides the majority of carbohydrates, is the main source of daily food in a lot of countries. Wheat is also the best grain to deliver gluten protein to the human diet. Starch and gluten from wheat also can be processed into animal feed, paper, adhesives and biofuels (Graybosch et al., 2009).

Wheat production is a major contributor to Michigan's economy and it is also a significant component of farming and the Agri-food industry. According to the National Agriculture Statistics Service, the production value of 226,720 ha Michigan wheat reached $164 million in 2009. Soft red and soft white winter wheat produced in Michigan are used in various products such as ready-to-eat cereals, pastries and baked goods, and soup thickeners. An economic review showed the total value of soft wheat food products manufacturing in Michigan was greater than $3.9 billion in 2002 (Peterson et al., 2006). Soft white wheat is primarily grown around the Great Lakes region of the U.S. and Canada (Michigan, New York, and Ontario) area and the Pacific Northwest. However, production of white wheat has dramatically declined in

New York and Ontario in the past 15 years, leaving Michigan as the only major producer of white wheat in the Eastern U.S. Soft white wheat is especially critical for cereal producers due to its less bitter taste and better color preference in the food processing compared to the red type. It is a primary raw material source of many whole grain wheat products, which become more popular to public with their growing fiber nutrition demand. Because of the quality difference and considerable shipping cost of the Pacific Northwest soft white wheat, a local material source is important for Eastern U.S. cereal industries. As a result, the continued presence of soft white winter wheat in Michigan is not only a critical and profitable role for the state economy, but is essential to the sustaining of the wheat industry. Increased protection of the white wheat crop and promotion of improvements in production will result in major economic benefits for Michigan.

Losses from Pre-harvest Sprouting

Pre-harvest sprouting (PHS) in wheat *(Triticum aestivum* L.*)* is a phenomenon whereby seeds germinate while still in the ear in the field. It usually happens when there is high relative humidity and cool temperatures prior to harvest. Pre-harvest Sprouting results in functional changes to the wheat flour and severely limits the end-use applications for wheat flour and results in substantial economic loss to farmers and food processors. In 2008 and 2009, Michigan wheat growing region experienced higher than average rainfall prior to harvest, resulting in high levels of PHS. The occurrence of PHS dramatically reduced the seed quality by the degradation of starch and protein, which leads to an economic loss for both farmers and end-users. Millers discount prices on PHS damaged grains when they bought wheat from farmers. In Canada, wheat with over 5% sprouted frequency can only be used as animal feed (Official Grain Grading Guide, 2006). The sprouted grains produce lower flour yield and decreased flour quality. Flour obtained from PHS grains loses tolerance for intense mixing and the baked products have poor volume and sticky crumb structure. While PHS affects both red and white wheat, PHS is a particularly serious threat to the soft white wheat.

Evaluation of Pre-Harvest Sprouting

Pre-harvest sprouting can be evaluated by several different methods. “Sprout Count” is a visual assessment of sprouted seeds, with sprouting occurring while seeds are in the head. “Germination Index” is an assessment of germination of seeds following harvest, threshing, and treatment with water. “Falling Number” (FN) is an industry-standard method used to quantify PHS by measuring the viscosity of the flour slurry. “Alpha-amylase activity test” directly measure the activity of the enzyme, which plays a major role in degrading endosperm starches and provides the source for seed germination. Unfortunately, although sprout count is the most easily assessed, it often does not effectively reflect the extent of internal damage to the seed (damage to the starches which affect grain/flour functionality), since many physiological processes occur prior to the external evidence of visual symptoms.

Objectives of the Study

As PHS susceptibility is a major threat to the wheat industry in Michigan and little information exists regarding PHS resistance for adapted cultivars, further evaluation research is needed to determine the resistance of adapted cultivars. In addition, a reliable and effective method for PHS evaluation is needed in Michigan to screen breeding germplasm for PHS resistance. Considering the limitations of visible evaluation methods, α-amylase and FN test that directly reflect changes in the seed irrespective of external symptoms are chosen to identify germplasm adapted to Michigan that have good resistance to PHS. Cultivars adapted to Michigan were assessed under natural condition (in the absence of PHS induction) to identify the base enzyme level during maturation with genetic variation. Multiple artificial weathering treatments were designed to evaluate the efficiency of the misting treatments and the variety performance in the different methods.

REVIEW OF LITERATURE

Wheat Domestication and Breeding

Recent evidence reveals that wheat domestication began over 10,000 – 12,000 years ago in the Fertile Crescent of the Near East (Gustafson et al., 2009). Modern wheat cultivars include two polyploid species: allohexaploid wheat (2n = 6× =42 chromosomes) (*Triticum aestivum*) and tetraploid wheat (2n = 4× = 28 chromosomes) (*Triticum turgidum*). The genome designations for hexaploid and tetraploid wheat are *AABBDD* and *AABB*, respectively. Wheat is a self pollinated crop and the chromosomes in *A, B, D* genomes are considered to be homoelogous, which are from a common ancestor (Gustafson et al., 2009). The *A* genome originates from the diploid species *T. urartu* (Huang et al., 2002). The *B* genome is hypothesized to originate from *Aegilops speltoides* (*Triticum speltoides*) (Johnson, 1972; Friebe et al., 1996; Sasanuma et al., 1996). Tetraploid durum wheat (*AABB*) (*T. turgidum*) evolved from the natural hybridization of the wild donors of genome *A* and *B*. Hexaploid wheat (*AABBDD*) derived from the crossing between the tetraploid wheat *T. turgidum* (*AABB*) and the wild diploid goat grass *Aegilops tauschii* (*Aegilops squarrosa*) (DD) (Kihara, 1954; Lubbers et al., 1991). A single locus *Ph1* on chromosome 5B, enables homologous chromosomes to pair and prevent homoelogous chromosomes from pairing during meiosis, to produce functional progeny (Poehlman and Sleper, 1995).

As early as the 19th century in France and Germany, the practice of hybridization followed by selection initiated the wheat breeding, which is currently improved to contain multiple elements world widely (Baenziger and DePauw, 2009). The genetic resources for breeding future cultivars could come from classical and biotechnological techniques, such as hybridization, mutations and tissue culture. In addition, transgenic technologies are now being used to develop the germplasm with traits including herbicide tolerance, pathogen resistance, abiotic stress tolerance and improved nutritional quality (Vasil, 2007). Synthetic wheat created by integrating source plants of genome A, B and D, followed by chromosome doubling, has been proven to be a successful method to improve genetic variantion (Villareal et al., 1994). Doubled haploid technology is used to reduce the time needed to obtain homozygous lines for breeding and genetic studies (Guzy-Wrobelska et al., 2007). Mapping the location of a desirable gene in wheat allows breeders to use marker-assisted selection (MAS) in early breeding generations to identify progeny with the gene of interest (Knox and Clarke, 2007). With the advent of next generation sequencing, SNP marker discovery has the potential to be a significant impact the wheat crop improvement in future (Berkman et al., 2012). A team of UK scientists has produced the first draft of wheat genome sequence (covered 95% of the all wheat genes), which is expected to have more annotated data and SNP identification in near future (BBSRC, 2010).

Wheat Classification

Hexaploid wheat is classified by kernel color, kernel texture and vernalization (cold temperature) requirements. Color is divided primarily into red and white, where "red" refers to the reddish brown color of the seed coat, while "white" wheat lacks this brown color and is a yellowish and tan color (McFall and Fowler, 2009). Wheat texture is classified as soft or hard. Winter wheat is planted in the fall; stays dormant through the vernalization period and is harvested the next summer. Spring wheat is planted in the spring and harvested in the early fall, without a vernalization period.

Seed Morphology

The major components of the wheat seed are the embryo, endosperm, and the seed coat. The embryo consists of the embryo axis, scutellum and epiblast. The scutellum is connected to the endosperm, which provides nutrition during germination. The fully developed endosperm contains starch and the aleurone layer. Starch dominates up to 75% of the endosperm and proteins usually occupy 6% to 16%. In addition, bioactive vitamins and nucleic acids in the seeds assist the enzyme synthesis during early maturity (Xiao et al, 1995). The testa (seed coat) is combined with the pericarp (fruit coat) in cereal grains, all of which are derived from ovular integuments. The testa is not only critical to provide protection for the embryo, but also assists in transportation of water, nutrition sources and other bioactive compounds from the maternal vascular system to storage places such as vacuoles and starch (Bewley and Black, 1994). The color and texture of the seed coat can be considered distinguishable characteristics of the seed.

Plant Development and Physiological Maturity

There are four stages of wheat growth after germination: tillering, stem extension, heading, and ripening. Tillering is the period when the plant starts to protrude shoots and tillers. In the second stage, stem elongation results in visible nodes and the wheat head begins to enlarge. Reproductive organs become mature and flowering is initiated after the heading phase. The seeds are filling and become firmer and drier during the ripening stage. The crop is ready to harvest after ripening. The time between germination to harvest varies by wheat species and environmental conditions, but it generally takes around nine months for winter wheat (Atwell, 2001).

The wheat kernel undergoes three phases spanning approximately four weeks to reach maturity. Endosperm cells accumulate in the milk stage and the dry weight also increases dramatically thereafter. During the third stage, the kernel approaches the end of grain filling and become solid. The kernel development can be affected by adverse environment conditions in these periods (Robson et al., 1995).

Physiological maturity (PM) is a time when crop seeds have reached maximum dry weight, after which the kernel merely loses water (Hanft and Wych, 1982). Zadoks et al. (1974) pointed out that the caryopsis development, included PM stage, is particular important for yield potential evaluation, quality and other environment affect risks, which suggested that the visual methods to determine PM in the field is valuable. Although this time point is defined by maximum dry weight, indirect measurements have been used to identify the time of PM. Grain moisture has been suggested as an indirect indicator, but measuring moisture to predict the time of PM is not appropriate because it varies in the range of 20% to 40% due to varieties and growing conditions (Wong and Baker, 1986). Theoretically, the most precise method to determine the time of PM is to track the dry weight, but field operation challenge drives people to use visual inspections to predict PM. Physiological maturity occurs very close to the time at which there is a complete loss of chlorophyll color from the glumes (Hanft and Wych, 1982; Falcinelli and Giannoni, 1985). The color judgment method is widely used in PHS research on wheat (Paterson and Sorrells, 1990; Humphreys and Noll, 2002; Hughes et al., 2010).

Seed Dormancy

A healthy seed is considered dormant if it does not germinate when it is in optimized conditions (such as presence of water and oxygen, suitable temperature and light) and is absent of inhibitory chemicals (Bewley and Black, 1994). Dormancy at harvest is a critical trait for most cereal crops to reduce undesired germination under cool moist conditions.

There are two types of dormancy in plants. In “coat-imposed” dormancy, the seed coat constrains the protrusion of the radicle, gas exchange and light filtration, and thereby prevents germination. In “embryo” dormancy, the dormancy is directly related to embryo itself (Bewley and Black, 1994). Environment factors, such as temperature, could affect the embryonic dormancy during wheat development (Walker-Simmons and Sesing, 1990). Phenolic compounds, especially proanthocyanidins (PAs) were found in *Arabidopsis* that could increase the seed coat thickness to improve coat-imposed seed dormancy (Bradford and Nonogaki, 2007). Kruger (1989) indicated that seed coat of red-grained wheat restricts embryo expanding and prevents degradation of the germination inhibitor. The effect of seed coat color on dormancy varies by the environmental conditions during kernel development and dormant white wheat was obtained by crossing red and white wheat (Torada and Amano, 2002).

There are multiple treatments that can release a seed from dormancy. After ripening period (ARP) refers to a period of time that the seeds broke dormancy in dry conditions after reaching PM and this effect depends on time length, moisture, temperature and oxygen level (Bewley and Black, 1994). Tavakkol-Afshari and Hucl (2002) observed that the durum wheat lost dormancy within 2 weeks of ARP in room temperature. Low temperature, usually below 10 °C, can break down wheat dormancy after 12 h (Bewley and Black, 1994). In sprouting tests, most soft whet cultivars showed inherent dormancy and needed to have ARP to initiate germination (Thomason et al., 2009).

Enzymes in Seed Development

Starch consists of linear amylose with mainly α-1, 4 bonds and branched amylopectin linked with α-1, 4 and α-1, 6 bonds. Starch hydrolytic enzymes such as α-amylase become increasingly active in germination and further catalyze endohydrolysis of α-1, 4 bonds in the starch (Van der Maarel et al., 2002). Beta-amylase cleaves the second α-1, 4 bonds after α-amylase action and de-branching enzyme is necessary to break down α-1, 6 bonds (Bewley and Black, 1994). Because β-amylase is not active prior to germination and is active under limited conditions, α-amylase is widely researched in cereal studies. Two α-amylase isozymes are present in developing wheat grains: *α-AMY-1* (also termed "germination amylase") and *α-AMY-2* (also termed "pericarp" or "green" amylases) (Olered, 1976). Flintham and Gale (1988) summarized three phases of α-amylase formation during seed development: (1) pre-mature state with relative high moisture in the absence of germination; (2) excess deposition of α-amylase in the endosperm cavity after maturity; and (3) germination after breaking down the dormancy. Another source of α-amylase presence is that the residual pericarp α-amylase does not degrade with ripening (Olered, 1976; Lunn et al., 2001). Upon germination, *α-AMY-1* was the first synthesized product and *α-AMY-2* formed at later stage (Lunn et al., 2001). However, without germination, the formation of *α-AMY-1* isozymes, also called late maturity amylase (LMA), is activated by a cool temperature shock (e.g. 18 °C day and 12 °C night) during the middle to later stages of grain development and ripening (25-30 days after anthesis) (Mares and Mrva, 2008).

Hormones in Seed Development

The plant hormones abscisic acid (ABA) and gibberellic acid (GA) control seed dormancy antagonistically. Abscisic acid plays an important role in seed dormancy development and maintenance (Walker-Simmons, 1987; Hilhorst and Karssen, 1992). Abscisic acid accumulates in the embryo during embryo development and decreases after maturity (Walker-Simmons, 1987). A series of publications reported the effect of ABA on embryos separated from the remainder of the seed and found that genetic and environmental conditions affected embryo sensitivity to ABA (Walker-Simmons, 1988; Walker-Simmons and Sesing, 1990). The effect of GA is focused on the production of α-amylase in aleurone tissues in cereal grains and α-amylase transcription activation is associated with the GA regulation (Gubler et al., 1995). The ABA and GA content ratio is essential in seed dormancy and the germination process (Bewley and Black, 1994).

Process of PHS

High relative humidity during the time between PM and harvest lead to precocious germination of the grain prior to harvest, with the consequence of yield loss and decreased grain quality. Absence of germination inhibitors, water and gas infiltration to the seeds, and activation of germination enzyme are major features involved in the PHS process (Xiao et al., 2005). Moisture differences between mature seeds and available water in the environment provide the potential of the water movement (Xiao et al., 2005).

Environment Effects on PHS

Susceptibility to PHS is dependent on both genotype and environmental conditions such as temperature and moisture (Nielsen et al., 1984; Barnard and Smith, 2009). In a study by Nielsen et al. (1984) , high temperature before maturity reduced sprouting resistance. Conversely, it is observed that cool temperature during the maturation period lead to higher levels of dormancy (Reddy et al., 1985). Similarly, greater dormancy was observed for cultivars maintained at 9 °C or lower during grain ripening (Mares, 1993). It was reported that 15 to 20 °C is the desired temperature range to study the dormancy issue for spring wheat cultivars (Nyachiro et al., 2002). However, Nakatsu et al. (2007) found that some genotypes are more likely to activate α-amylase and to have PHS under lower (15-17 °C) temperature in ripening period.

In addition to the effect of temperatures during maturation, the moisture greatly impacts the occurrence of PHS. In a study by Mares (1993), rainfall during the 20-day period before harvesting caused major variation on seed dormancy level. Conversely, drought and high temperature during grain filling has been shown to increase seed dormancy and non-dormant plants can be induced to show a dormant phenotype (Biddulph et al., 2005). In general, dry conditions combined with cooler temperatures just prior to maturity may also result in seed with higher dormancy, and cool and moist conditions after maturity are inclined to break dormancy and lead to PHS (Thomason et al., 2009).

Artificial misting is widely used to simulate rainfall in the field in PHS research (Mares, 1993; Humphreys and Noll, 2002; Hughes et al., 2010). FN results declined rapidly when wheat plants received 22mm of rainfall after harvest (Mares, 1993). From FN data, the use of artificial rain gives comparable results to natural rain conditions in the field for screening genotypes for resistance to PHS (Humphreys and Noll, 2002). Multiple wetting and drying cycles help water to penetrate the seed coat quickly (Thomason et al., 2009). However, there is no standard protocol to induce PHS post harvest in artificial environments.

PHS on Wheat and Product Quality

PHS can induce a series of physiological and biochemical changes, including the activation of plant hormones, α-amylase and proteases. The activities of α-amylase and protease degrade the seed starch and protein, respectively. If such seed is used for milling and baking, the seed is deemed as having poor grain quality and large economic losses are incurred.

In addition to end users, farmers are dramatically impacted by PHS damage. Sprouting not only results in loss of yield and lower test weights, but also farmers are paid less for sprouted grain due to the damage to the grain functional milling and baking characteristics (Xiao et al., 2005). If the damage is severe enough, the grain may be degraded to animal feed. Sprouted seed may also be unsustainable for planting, due to lower germination probabilities (Kruger, 1989).

Many grain quality characteristics are damaged in sprouted wheat. When milled, sprouted wheat has lower flour yields and higher ash content (Kruger, 1989). Reduced extensibility and maximum resistance showed in the properties of the dough from sprouted grains proved its weakness and would be detrimental to the end baking products (Buchanan and

Nicholas, 1980). The increased α-amylase activity deteriorates many baked products. Sticky crumb, collapsed loaves and compact interiors were observed in bread products made from sprouting damaged flour (Derera and Bhatt, 1980). Darken crusts were also observed, caused by caramelization of sugars (Bewley and Black, 1994). In addition, degraded starch has a strong ability to absorb water, but once released, the water would be too much to damage the intact flour and dextrin (Xiao et al., 2005). The hydrolysis of the protein and starch in the damaged flour resulted in noodle products with less elasticity, dark color and weak strength (Xiao et al., 2005). Generally, pan bread is more sensitive to high α-amylase activity compared to flat bread and bun, but sound flour definitely produces better food (Xiao et al., 2005).

PHS Assessment

Because of its high impact on wheat quality and the value of wheat grain, grain elevator and mill owners commonly evaluate PHS of the grains, when they purchase wheat from farmers. There are various approaches widely used around the world to assess for PHS and grain dormancy. Three main categories of these methods are visual evaluation, flour quality test and biochemical test.

A germination test of the seeds is widely used to detect seed dormancy directly (Reddy et al., 1985; Kruger, 1989). Seeds are placed in a petri dish and incubated under moisture condition for a controlled time. The numbers of germinated seeds are visually observed and can be directly calculated as germination percentage or the length of time to obtain the specific level of germination (Kruger, 1989).

The weighted germination index (WGI) gives higher weight to the seeds that germinate easily under standard condition and is calculated from the following formula: WGI = $(7\times n_1 +$

$6 \times n_2 + \ldots 1 \times n_7) / \text{total days of test} \times \text{total grains}$, where n_1, n_2, … n_7 are the number of grains that had germinated on day1, day 2, …day7, respectively (Reddy et al., 1985).

An intact-head sprouting test is preformed by treating heads with an artificial misting system or water saturation and, after a period of days, counting the sprouted grains. This method has been historically used in Sweden and China (Xiao et al., 2005). The spikes are selected at a fixed maturity time, usually at PM to reduce the effect of maturity on dormancy (Paterson and Sorrells, 1990). A sprouting score is used to rate the spike PHS severity after misting treatment (Paterson and Sorrells, 1990). However, a count of sprouted grains often does not effectively reflect the extent of starch damage inside the seed, since enzymatic processes occur prior to the external evidence of sprout extrusion. Sprouting tests may be affected with other mechanisms associated with the spike itself, such as husk germination inhibitors and spike morphology (King and Richards, 1984; Kato et al., 2002).

Falling Number test originated in Europe in 1960s and it has been approved as a standard evaluation method for sprouting damaged cereals (Hagberg, 1960). This test measures the weakness of the starch structure by recording the time required for the plunger to fall through a heated flour-water slurry. Typically, undamaged starches will cross-link to form a thick structure when mixed and heated with water, causing the plunger to fall slowly through the slurry. If the starches from the flour are degraded by α-amylase generated from PHS, cross-linking will be reduced according to the degree of degradation, and the plunger will fall more quickly through the slurry. Generally, 250 seconds is a widely accepted standard of acceptable grain quality for soft wheat flour using a FN test (Xiao et al., 2005). The FN value has been found to correlate well with the α-amylase activity (Perten, 1964). However, the correlation between sprouting scores and FN is not good and FN can provide more reliable estimation of PHS damage (Humphreys and Noll, 2002).

Rapid Visco-Analyzer (RVA) measures the starch viscosity by monitoring the stirring counter-force in real-time. The RVA test is widely used in Europe and Australia in wheat research as it needs only a small amount (3-4 g) of sample and short determination time (13-15 min) (Xiao et al., 2005).

McCleary and Shehan (1987) developed the α-amylase activity assay and it showed great correlation with FN and other α-amylase assays. The level of α-amylase activity has been widely used as an evaluation method of PHS in wheat, barley and rye (Singh et al., 2008; Masojc and Milczarski, 2009).

Physical Control of PHS

Spike morphology influences water absorption and drying in the spike, which affects PHS tolerance. The process of wetting is slower in awnless wheat compared to awned wheat, resulting in higher PHS resistance in awnless wheat (King and Richards, 1984). The existence and thickness of epicuticular waxes also impacts the water uptake rate (King and Von Wettstein-Knowles, 2000). King and Von Wettstein-Knowles (2000) also reported that wheat heads that remain upright were more likely to resist sprouting by shedding more water than lodged ones. Wheat with long and weak heads is more tolerant to PHS compared with the individual with short and strong heads (Zanetti et al., 2000). The position of the kernels also shows a relationship with PHS resistance. The seeds in the top of the spike germinated firstly, then the bottom ones, and finally germination proceeded to the central part of the spike (Hardesty and Elliott, 1956).

Genetic Control of PHS

Seed dormancy is a heritable trait and is controlled by dominant or recessive nuclear or cytoplasmic genes (Foley and Fennimore, 1998). The gene related to dormancy was found on chromosome 3D and it is also expected to associated with ABA sensitivity (Mares et al., 2002).

Seed coat color was proposed to have a relationship with seed dormancy and usually red kernel is associated more with dormancy (Xiao et al., 2005). Three *R1* genes (*R-A1*, *R-B1*, *R-D1*) control the level of seed coat red color, and only if all the *R1* genes have recessive alleles, the seed coat color is white (Metzger and Silbaugh, 1970; Flintham, 2000). Himi et al. (2002) reported that the *R1* genes increase the seed dormancy by increasing the sensitivity to ABA. The association between genes controlling seed coat color and dormancy may result from a pleiotropic effect or tight linkage with genes directly controlling seed dormancy (Groos et al., 2002). However, Himi et al. (2002) reported that the *R1* genes effect on dormancy was relatively small since they found that white wheat with *R1* gene derived by mutation showed a low level of dormancy. Gene dosage effects can explain the variation of seed coat color in the wheat kernel (Wang et al., 1999), but the differences in dormancy individuals that carry all three R1 dominant alleles can not be explained (Noll et al., 1982). The *R1* genes effect on PHS resistance varied by the evaluation measurement, such as sprout count, FN test and germination index (Groos et al., 2002). *R1* genes have been mapped on the long arm of group 3 chromosomes (3A, 3B and 3D) (McIntosh et al., 1998). Genetic analysis has identified a potential marker for the dormancy genes on chromosome 3D in white wheat populations (Mares et al., 2002). PCR markers of *Tamyb10* genes, the homologous to *TT2* gene that control the proanthocyanidin (related to seed coat pigment synthesis) production in *Arabidopsis*, have also been mapped at the same locus as *R1* genes in wheat (Himi et al., 2011).

There are three major genes controlling the α-amylase expression in wheat: *α-AMY-1*, *α-AMY-2* and *α-AMY-3*. Alpha-AMY-1 has high isoelectric point (pI) and is induced by GA during germination. However, during the later stage of maturity, this kind of enzyme can also be detected and usually it is called LMA (Mares and Mrva, 2008). The *α-AMY-1* gene is located on chromosome 6A, 6B and 6D. The recent association mapping study on LMA has confirmed that the existence of LMA gene on long arm of chromosome 6 (Emebiri et al., 2010). Alpha-AMY-2 has low pI and the gene is located on chromosome 7A, 7B and 7D. Alpha-AMY-3 only exists on the out layer of pericarp with high pI and the few gene copies were observed on chromosome 5 (Xiao et al., 2005). In addition, the barley α-amylase inhibitor gene was effectively expressed in wheat and could be used in developing transgenic PHS resistant wheat (Xiao et al, 1995).

It is important to identify linked molecular markers to assist selection of PHS resistance plants in breeding. PHS resistant QTLs have been mapped to wheat chromosome 1, 2, 3, 4, 5, 6, 7 and all the QTLs are on the short arm except the ones on chromosome 4A long arm (Flintham et al., 2002). These results suggest that the PHS resistance comes from multiple loci and the hypothetical gene could be found at similar QTL in wheat (Flintham et al., 2002).

In addition, the comparison genetics study found the homologous gene of *Vp1*, which encodes a transcriptional factor affecting maize (*Zea Mays*) dormancy, in wheat and this *TaVp1* gene has been mapped close to *R* loci (Bailey, McKibbin et al. 1999). The *TaVp1* gene is also validated to be associated with wheat seed dormancy and sprouting (Chang et al., 2010a; Chang et al., 2010b; Chang et al., 2011).

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CHAPTER II

EVALUATION OF ALPHA-AMYLASE ACTIVITY AND FALLING NUMBER FOR SOFT WHITE AND SOFT RED WHEAT VARIETIES ADAPTED TO MICHIGAN IN NATURAL CONDITONS

ABSTRACT

Michigan growers have experienced severe pre-harvest sprouting (PHS) problems on wheat in 2008 and 2009. Alpha-amylase is an important component of PHS and the falling number (FN) test is used by industry to identify sprouted wheat that is unacceptable for use in various food products. The objective of this study was to evaluate wheat cultivars adapted to Michigan for the activity of α-amylase and the corresponding FN values around physiological maturity (PM) time under natural conditions (in the absence of PHS induction). Twenty-four soft winter wheat genotypes (12 red and 12 white) with varying levels of susceptibility to PHS were planted in an α-lattice design in two locations from 2008 to 2010. Spikes were collected three days before PM, at PM, and three days post PM. Samples were freeze-dried, threshed, milled and evaluated for α-amylase activity and FN values. Genetic differences existed for both responses at all time points and treatments. A clear trend was observed in the reduction of α-amylase and the increase in FN during the maturation in non-PHS conditions. The genetic variability identified in natural α-amylase accumulation could be a standard for PHS research. It is conceivable that PM and 3 days post PM are reasonable maturity time-points to evaluate the effects of inducing PHS through artificial misting of heads, due to the converging base level for α-amylase activity and FN values.

INTRODUCTION

Soft white and soft red winter wheat production is economically important to the farming and food industries in Michigan. Due to its light color, less bitter taste and specific flour quality, soft white wheat is the basis of a wide variety of food products, including pastries, crackers, biscuits, cakes, cookies and breakfast cereals. An economic study by Peterson et al. (2006) showed the total value of these soft wheat products manufacturing in Michigan was greater than $3.9 billion in 2002. Soft white wheat is primarily grown in Great Lakes area and Pacific Northwest. However, declining soft wheat production in Ontario and New York has increased the importance of Michigan soft wheat in Great Lakes region.

Pre-harvest sprouting is the premature germination of wheat seeds while still in the wheat head in the field. Germination causes a sequence of physiological processes, which include changes in plant hormones and hydrolytic enzymes. Gibberellic acid (GA), in the soaked grain, will induce and increase synthesis and secretion of α-amylase and proteases (Gale and Lenton, 1987; Cornford et al., 1987). Starches and protein are hydrolyzed by increased α-amylase activity and subsequently flour quality is lower, which results in sticky crumb, compact interiors and off colors in baking products, which have harmful consequences for millers and end-users (Edwards et al., 1989). In addition, farmers are dramatically impacted by reduced yield and large discount for damaged grains (Xiao et al., 2005).

Physiological maturity (PM) is defined as the time when the seed reaches its maximum dry weight for wheat or grain crops (Hanft and Wych, 1982). Over cultivars and years, kernel moisture ranges from 32.4 to 43.6% at PM, which suggests the involvement of genetic and environment factors (Clarke, 1983). Zadoks et al. (1974) pointed out that the caryopsis development, included PM stage, is particular important for yield potential evaluation, quality and other environment affect risks, which suggested that the visual methods to determine PM in the field is valuable. PM occurs very close to the time at which there is a complete loss of chlorophyll color from the glumes (Hanft and Wych, 1982; Falcinelli and Giannoni, 1985). The color judgment method is widely used in PHS research (Paterson and Sorrells, 1990; Humphreys and Noll, 2002; Hughes et al., 2010)

Two α-amylase isozymes are present in developing wheat grains: *α-AMY-1* (also termed "germination amylase") and *α-AMY-2* (also termed "pericarp" or "green" amylases) (Olered, 1976). Both isozymes act by cleaving the α-1, 4-bond in starch, and their activity varies during different stages of formation and maturation, dormancy, and germination of the grain. Kruger (1989) reported that in red hard spring wheat kernels sampled in various stages ranged from early development stage to full maturity, the level of these α-amylases increases after pollination and then decreases to a low level when reaching full maturation. In addition, when immature seeds were harvested and bench-dried, the level of α-amylase would further decrease, while seeds that were frozen following harvest maintained their original α-amylase level (Kruger, 1989). In the dormant stage after maturation, the levels of α-amylase level are very low and varied in different cultivars (Kruger, 1989). Upon germination, *α-AMY-1* was the first synthesized product and *α-AMY-2* formed at a later stage (Lunn et al., 2001). However, in the absence of germination, the formation of *α-AMY-1* isozymes, also called late maturity amylase (LMA), is activated by a cool temperature shock (e.g. 18 °C day and 12 °C night) during the middle to later stages of grain development and ripening (25-30 days after anthesis) (Mares and Mrva, 2008).

A healthy seed is considered dormant if it does not germinate when it is in optimized conditions (Bewley and Black, 1994). It is critical for plant species to determine the best germination time and space. Pre-harvest Sprouting in wheat is associated with inadequate seed dormancy, which is affected by the seed coat and embryo (Kruger, 1989). Seed coat color and grain dormancy are pleiotropic effects of three *R* genes (*R-A1, R-B1, R-D1*) located at homoeologous loci on chromosomes 3A, 3B and 3D of hexaploid wheat (Flintham, 2000). Dominance at any one of these loci will result in red seed coat color (Metzger and Silbaugh, 1970). Wheat grain dormancy is a multigenic trait controlled by *R* genes as well other genes, at least one of which has a major effect (Flintham, 2000). Red wheat is generally more resistant to PHS than white wheat (Groos et al., 2002), though variation for PHS resistance exists within both red and white wheat (Flintham, 2000). Embryonic resistance is determined by the degree of sensitivity of the embryo to factors in the grain that inhibit germination, such as abscisic acid (ABA) (Gimbi and Kitabatake, 2002). Several researchers have reported the effect of ABA on embryos separated from the remainder of the seed and found that genetic and environmental conditions affected embryo sensitivity to ABA (Walker-Simmons 1987; Walker-Simmons 1988; Walker-Simmons and Sesing, 1990). ABA has antagonistic effects to Gibberellic acid (GA) and the ratio of the ABA and GA content is essential in seed dormancy and germination processes (Bewley and Black, 1994). Endogenous α-amylase inhibitor is active against native α-amylase. During germination, GA-induced *de novo* synthesis of α-amylase could be inhibited by the α-amylase inhibitor, which is induced by ABA (Mundy, 1984).

Various methods are widely used to evaluate PHS and grain dormancy. Visual methods include "sprout count", a visual assessment of sprouted seeds in the head (this measure has been used historically in the MSU Wheat Breeding Program), and "germination index", which is a calculation of the percent of seeds germinated after treatment with water. The Hagberg falling number (FN) test (Hagberg, 1960), which is the AACCI standard evaluation method for sprouting-damaged cereals (AACC International, 2000), measures the functional integrity of the starch structure. Since germination results in the starch being degraded by α-amylase, starch properties are reduced in PHS damaged wheat. Furthermore, α-amylase can be quantified directly using a chromogenic method (McCleary and Sheehan, 1987). The visual sprout count method is an unreliable indicators of PHS damage as assessed by baking tests, while FN and α-amylase activity tests are reliable (Moot and Every, 1990).

The objective of this work was to determine FN values and α-amylase activity at different levels of maturity under natural conditions (in the absence of PHS). Michigan wheat breeding germplasm has been evaluated, historically, for sprout count, but not for FN values or α-amylase activity, which are considered to be of greater importance for end-users. This study for the wheat varieties in the absence of PHS is also critical to understand the base value around PM and facilitate the prediction and selection of elite red and white genotypes adapted to Michigan with enchanced levels of dormancy performance.

MATERIALS AND METHODS

Plant Materials

In 2008, 10 soft white and 10 soft red winter wheat cultivars (Tables 1.1) adapted to growing in Michigan were selected for a study of α-amylase activity and falling number (FN). The selection was based on the goal of evaluating varying levels of PHS resistance in both white and red genotypes, according to previous sprouting performance studies (unpublished data). In 2009 and 2010, two additional soft white and two soft red winter wheat cultivars were added to the trials (Table 1.1).

Field Design

Field trials were planted in a three-replication α-lattice design at each location. An α-lattice design is a replicated design that uses incomplete blocks within each of the replicates to further strengthen the analysis by reducing the effect of field variability (Yau, 1997). Trials were planted in two locations each of the fall of year from 2008 to 2010, and the field information was provided in Table 1.2. In the fall of 2008, cultivars were planted with 1.27 cm in seed spacing, using a Hege® 95 planter at Michigan State University Agronomy Farm. The rest of the plantings were done by ALMANCO® Heavy Duty Drill planter at a rate of 15 million seeds per ha.

Physiological Maturity (PM) Determination and Harvesting

Flowering notes were taken when anthers were extruded for 50% of the plants for each plot. Three maturity time-points were identified for each plot: three days prior to PM (PM-3), PM and three days after PM (PM+3). The maturity time-points were determined by visualization of the chlorophyll loss for the plot. The following definitions were used: at PM-3 60% of heads in the plot had lost their chlorophyll; at PM 80% of the heads had lost their chlorophyll; PM+3 was three physical days passed the occurrence of PM. At each of the three time points, 100 spikes were sampled at from each plot. The samples were transported on ice to the greenhouse. They were stored in the freezer to maintain the metabolism level until they were freeze-dried.

Post-Harvest Processing

All spike samples were freeze-dried in a Genesis® 12EL (The Virtis Company, Gardiner, NY) in 2009, and a Tri-Philizer TM MP, (FTS Systems, Warminster, PA) in 2010 and 2011. Samples were then threshed using the gas-motored thresher (ALMACO LPR91001, Allen Machine Company, Nevada, IA). Threshed samples were subsequently cleaned using an air column cleaner (CB-2A, AGRICULEX Inc., Guelph, Ontario, Canada) with the ventilation scale set at 5.1. After cleaning, whole grain flour was obtained from approximately 40 g sample of grain using a UDY® Mill (UDY Cyclone Sample Mill, UDY Corporation, Fort Collins, CO) with a 0.5 mm sieve.

Determination of α-Amylase Activity

Alpha-amylase activity was measured according to AACCI Approved Method 22-02.01 (Ceralpha method) (AACC International, 2000). The enzyme extraction and assay was performed using the Megazyme® kit (K-CERA 08/05, Megazyme International Ltd. Ireland) with a modified protocol developed in USDA Soft White Wheat Quality Lab (Wooster, Ohio). Specifically, 3.0 g of flour and 20 mL of extraction buffer (50m*M* malic acid, 87.5 m*M* sodium hydroxide, 50m*M* sodium chloride, 2m*M* calcium chloride, 3m*M* sodium azide, pH 5.2) were added into a 50 mL centrifuge tube followed by vigorous stirring and incubation at 42 °C (Water Bath 20L, Fisher Scientific, Pittsburgh, PA) for 20 min with 5 min interval mixing. The mixture was then centrifuged at 1,500 g (SORVALL® RT7, Kendroll Laboratory Produces, Newton, CT) for 15 min at 35°C. Twenty µL aliquots of Ceralpha substrate (non-reducing-end blocked p-nitrophenyl maltoheptaoside, BPNPG7) solution were dispensed into each well of a 96 well plate and pre-incubated at 42 °C. Twnty µL of α-amylase extract was directly added to the bottom of the well. For each whole-grain sample, three replicates were conducted adjacent to each other on the 96 well plate. Since the enzyme reaction needed to be performed at 42 °C for exactly 20 min, 30 seconds intervals were kept between each group of three aliquots pipetted. At the end of the 20 min period for each well, 300 µL of the stopping reagent was added. Non-enzymatic control was obtained by following the order of adding the substrate after the stopping reagent. The absorbance of each well was read using a spectrophotometer (Synergy® HT Multi-Mode

Microplate Reader, BioTek, Winooski, VT) at 400nm, using a 340 uL distilled water as a control. The enzyme extract was diluted if the absorbance values were greater than 1.2, due to the possible saturation for the limited amount of substrate. The spectrophotometer was standardized and the extinction coefficient (E_{mM}) was determined with a dilution series of p-nitrophenol standard solution (Cat. 104-1, Sigma) in 1% tri-sodium phosphate. The absorbance of the solution was measured and then the concentration (mM/L) of the solution was divided by the absorbance to obtain the E_{mM}.

Determination of FN

In 2009, flour moisture content was determined following AACCI Approved Method 44-15.02 (AACC International, 2000), by calculating the loss of water for each sample after heating at 130 °C for 1 hour in the oven (ISOTEMP OVEN, Fisher Scientific, Pittsburgh, PA). In 2010 and 2011, flour moisture content was determined using Near-Infrared Spectroscopy (NIR) (Multi Purpose Analyzer, Bruker Optics, Billerica, MA). The appropriate amount of flour weight necessary for each FN test was calculated based on 7.0 g of flour at 14% moisture. According to AACCI Approved Method 56-81.03 (AACC International, 2000), a weighted flour sample was placed in the viscosity test tube with 25 ml of distilled water. Manual hand shaking (10 shakes) and automated shaking (SHAKEMATIC®, SM-1095, Perten Instruments, Kungens Kurva, Sweden) were used in 2009 and 2010-2011 respectively, to form a flour-water slurry without visualized dry flour in the bottom of the test tube. Immediately, the test tubes were placed into the FN apparatus to measure the seconds needed for the plunger to drop from the top of the tube to bottom by gravity force. The FN-1400 (Perten Instruments, Kungens Kurva, Sweden) was used in 2009 and the FN-1700 (Perten Instruments, Kungens Kurva, Sweden) was used is 2010 and 2011. Two replicates for each sample were performed in this test.

Statistical Analyses

Data were analyzed using SAS statistical software 9.2 (SAS Institute, Cary, NC). Analysis of variance (ANOVA) was carried out to check the effect of fixed factors and least square (LS) mean of α-amylase activity and FN values for each variety at each time points was generated using the mixed models. Data were logarithm transformed to reduce the heterogeneity of variance. Fisher's least significant difference (LSD) was calculated for each maturity category. Pairwise comparisons were performed using the Tukey's Honestly Significant Difference (HSD) test at a significance level of 0.05. All data points for α-amylase activity and FN values were fitting into a linear regression model to check the correlation coefficient for the two methods.

RESULTS

Planting Time, Flowering Time and PM Time

Some of the varieties ('MSU E2043', 'Jupiter', 'Coral') consistently showed relatively later maturity than others (data not showed). The summary of the time interval between planting date to PM and flowering date to PM for each location at 2010 and 2011 (Table 2.1), indicated that it took around 268 days (range of all genotypes) for these adapted soft red and soft white winter cultivars to reach PM, and 32 days after anthesis (DAA) average is required for both red and white wheat to reach PM.

Alpha-amylase Activity Test and FN test

Before the implementation of the chromogenic method to determine α-amylase activity, the linear absorbance changes for diluted standard solution were checked to define the E_{mM} of the following test. The absorbance at 400 nm of a series of diluted standardize solution and corresponding E_{mM} is shown in Table 2.2. The average E_{mM} 14.23 was used for further data conversion and analysis.

The effects of location, year, replication and blocks were not significant and the effects of genotype, maturity and genotype × maturity (PM-3, PM, PM+3) were significant ($p < 0.05$) for both α-amylase activity and FN test. Both the genotypes and PM maturity category contributed the variance of the α-amylase activity and FN values. The significant effect of genotype × maturity interaction indicated that the experimental varieties performed differently at all three PM times.

The means of each genotype for α-amylase activity and FN values at the three maturity categories around PM are presented in Figures 2.1 & 2.2. It is clear that all 24 cultivars showed a decreasing trend of α-amylase activity from PM-3 to PM+3. The date range of PM-3 to PM+3 was corresponding to approximately the period from 27 DAA to 37 DAA. The decrease between PM-3 to the time of PM was far greater than from PM to PM+3. There were significant differences among the 24 cultivars in terms of the α-amylase activity at all three time points, even though α-amylase activity levels converged towards similar values at PM+3 for all 24 genotypes. When examining the red and white cultivars, categorically, similar trends were observed and there were no specific differences in the pattern of red vs. white wheat (Fig 2.3).

For FN test, all 24 cultivars showed an increasing trend in FN from PM-3 to PM+3. In contrast to the converging trend observed over time for α-amylase activity, no strong convergence of values was observed for FN. The analysis of red vs. white wheat, categorically, also showed similar trends to each other (Fig 2.4).

Genotype Comparisons and Relative Grouping at PM-3

Pair-wise comparisons were made at PM-3 to determine significant differences between genotypes for both α-amylase activity and FN values. The least significant differences (LSDs) at three time points around PM for α-amylase activity were 0.122, 0.056, and 0.016, respectively

($p<0.05$) (Figure 2.1 & 2.2). The LSDs at these time points for FN values were 22.46, 22.12, and 13.60, respectively ($p<0.05$) (Figure 2.1 & 2.2).

In addition, α-amylase activity and FN values for 24 genotypes at PM-3 were selected to assign relative groups (low, middle and high) by percentile, due to only PM-3 had greatest differentiate strength of significant differences between genotypes (Table 2.3).

Correlation between α-Amylase Activity and FN Values

The linear regression for α-amylase activity and FN values is shown in Figure 2.5. The correlation coefficient (r^2) was 0.5743. The r^2 for red and white wheats were 0.5216 and 0.6881, respectively (Figure 2.6 & 2.7).

DISCUSSION

In this study, there was no heavy rainfall or visual PHS during the field sampling period, and consistent low α-amylase activity and high FN values at PM and PM+3 supported that all the wheat lines were absent of PHS. These results are in agreement with a study by Nishikawa and Watanabe (1988), which also showed progressively decreasing α-amylase activity in five out of six varieties at 14 to 35 DAA, due to imminent germination.

From the results it was clear that, irrespective of genotype, α-amylase accumulated in the seeds during the maturation process and decreased steadily as PM was neared and surpassed. This changing pattern is in agreement with the previous study on α-amylase activity in wheat kernels approaching maturity after pollination (Marchylo et al., 1980; Gale and Ainsworth, 1984; Reddy et al., 1985; Nishikawa and Watanabe, 1988). The increasing trend across the maturity categories (PM-3 to PM+3) for FN values, which indirectly reflected the α-amylase activity, was also consistent with another study on two wheat lines around PM, which is determined by the water content instead of visual judgment (Clarke, 1983).

Kruger (1989) indicated that the high level of α-amylase was found mainly in the pericarp during early stage of the kernel development. In our study, the highest α-amylase activity was observed at three days before PM, which is consistent with the physiological change that energy consumption is reduced when seed reaching PM. Physiological maturity was defined as the time when the kernel had the maximum weight. The decreasing enzyme activity contributed to the net starch deposition (Reddy et al., 1985). The converging trend of low α-amylase activity at PM and thereafter suggested seeds reducing starch degradation to provide energy, since the seeds had already reached maturity.

The decreasing enzyme activity during maturation was possibly due to multiple reasons: enzymatic protein degradation, insolublization and inhibition of the enzyme. Two immunochemical studies supported the theory that protein degradation caused the low activity of α-amylase (Daussant and Renard, 1976, 1987). The barley α-amylase inhibitor (BASI) (Afshari et al., 2011) were clearly observed 14 DAA and increased until 28 DAA without any further changes (Hill et al., 1995). In addition, the reduction of BASI results in the release the α-amylase protein in the germination study (Hill et al., 1995). The evidence of present endogenous α-amylase inhibitor in wheat seeds may explain the reason for decreasing α-amylase activity when seeds approach PM (30 to 34 DAA in our study) (Mundy, 1984; Weselake et al., 1985). The effect of α-amylase inhibitors isolated from sprouting resistant wheat lines suggested the role of α-amylase inhibitor in PHS (Abdulhussain and Paulsen, 1989). The variability of α-amylase activity of 24 genotypes at PM-3 might be associated with the function level of α-amylase inhibitor.

Significant differences in α-amylase activity were observed among the 24 cultivars, indicating a variation in genotypes for this enzyme. The genotypic distinction, especially prior to

PM, gives us the expectation that genetic differences would also be observed following the induction of sprouting, although the relative rank might be very different. Further studies need to be conducted to determine if the genetic variation observed in the absence of sprouting is associated with the variation in conditions that induce PHS.

In the absence of sprouting conditions, the wheat genotypes have relatively low levels of α-amylase at PM and thereafter in our study. The levels of α-amylase activity fall dramatically when wheat reaches full maturity (ready to harvest) and some lines showed no detectable α-amylase activity (Kruger, 1972). This result indirectly suggested that the low level of α-amylase activity was maintained from PM to the farmer's harvest time (7-10 days after PM). Because the α-amylase activity is highly converged at PM and PM+3, they are expected to be good starting time points to evaluate the effects of induced heads sprouting via misting in PHS research.

The correlation coefficient of the linear relationship between α-amylase activity and FN in our study is low compared the value of 0.998 in one study (Mathewson and Pomeranz 1978). The figure showed several outliers when FN value was low (<200) or α-amylase activity was high (>0.4). The plot also indicated that the correlation between α-amylase activity and FN values was poor when α-amylase activity was low (<0.2). In addition, recent research indicated that the correlation between alpha-amylase activity and FN is best for samples with FN values between 200 and 300 seconds (Souza et al., 2011). These evidence support the statement that starch quality became the determine factor for FN with low α-amylase activity (Ringlund, 1983). Hucl (1994) reported low repeatability of FN due to cultivar by year interaction. It was also suggested that FN should not be used as the only method to detect the PHS damage because it did not provide quantified and accurate protein composition and quality changes due to weathering (Barbeau et al., 2006).

Table 1.1. Soft white and soft red winter wheat cultivars and related references for PHS evaluation trial

Cultivar	Cultivar References
Ambassador	Lewis et al., 2010b
Aubrey	Private company†
Caledonia	Sorrells et al., 2004
Coral	Lewis et al., 2010a
Crystal	PVP‡ 200800367
Envoy	NR§, MSU
Jupiter	NR, MSU
Lowell	PVP 009500040
MSU D8006	PVP 200500308
MSU E2043	NR, MSU
MSU E5024	NR, MSU
W1062	PVP 200900411
Arena	N/A
Hopewell	Campbell et al., 2001
Hyland Emmit	NR, Hyland Seeds
MCIA Oasis	NR, Ohio State University
OH04-264-58	NR, Ohio State University
Pioneer Brand 25R47	PVP 200200232
Pioneer Brand 25R62	PVP 200700369
R045	Private company
R055	Private company
Red Ruby	PVP 200700409
Roane	Griffey et al., 2001
Tribute	Griffey et al., 2005

† Cultivars listed as "private company" were provided by private companies without information of their origin.
‡ Plant Variety Protection (PVP) number given
§ NR = Not Registered. Cultivars listed as NR have known origin, and these origins are indicated.

Table 1.2. Field location information by year, soil type, fertilizer & herbicide application and plot size for the trials

Trial Year	Field Name	Location Information	Soil Type	Fertilizer and Herbicide	Plot size
2009	Agronomy Farm	Ingham County East Lansing, MI	Capac Loam, 0-3 percent slopes	Pre-plant Fertilizer: None Spring Fertilizer: 89kg 46-0-0; 41kg N Herbicide: 15 ml Harmony Extra + NIS	4 rows, 1.5 m length, 2.1 m wide
2009	Clarksville	Ionia County Clarksville, MI	Lapeer Sandy Loam, 2-6 percent slopes	Pre-plant Fertilizer: None Spring Fertilizer: 89kg 46-0-0; 41kg N Herbicide: 15 ml Harmony Extra + NIS	6 rows, 3.6 m length, 1.1 m wide
2010	Agronomy Farm	Ingham County East Lansing, MI	Capac Loam, 0-3 percent slopes	Pre-plant Fertilizer: 45kg 6-24-24 Spring Fertilizer: 89kg 46-0-0; 41kg N Herbicide: 15 ml Harmony Extra + NIS	6 rows, 3.6 m length, 1.1 m wide
2010	Saginaw	Saginaw County Richville, MI	Tappan-Londo Loam, 0-2 percent slopes	Pre-plant Fertilizer: 90kg 7-12-28 Spring Fertilizer: 89kg 46-0-0; 41kg N Herbicide: 15 ml Harmony Extra + NIS	6 rows, 3.6 m length, 1.1 m wide
2011	Lenawee	Lenawee County Britton, MI	Silty Clay Loam, 0-3 percent slopes	Pre-plant Fertilizer: 135kg 9-23-30 Spring Fertilizer: 89kg 46-0-0; 41kg N Herbicide: 15 ml Harmony Extra + NIS	6 rows, 3.6 m length, 1.1 m wide
2011	Saginaw	Saginaw County Richivlle, MI	Tappan-Londo Loam, 0-2 percent slopes	Pre-plant Fertilizer: 101kg 7-12-27 Spring Fertilizer: 89kg 46-0-0; 41kg N Herbicide: 15 ml Harmony Extra + NIS	6 rows, 3.6 m length, 1.1 m wide

Table 2.1. Number of days between planting date to PM, flowering date and PM date for different locations in 2010 and 2011

Year	Location	Red		White	
		Planting to PM	Flowering to PM	Planting to PM	Flowering to PM
2010	Agronomy	251.3	34.6	252.3	34.6
	Saginaw	270.0	32.4	270.8	32.3
2011	Lenawee	271.3	30.4	271.9	30.4
	Saginaw	278.1	30.6	279.1	31.2

Table 2.2. Absorbance of a series of diluted standard solutions (p-nitrophenol in 1% tri-sodium phosphate) at 400 nm and corresponding E_{mM} value

Dilution fold	ΔE_{400} †	E_{mM}
50	2.937	14.69
100	1.460	14.60
150	0.964	14.18
300	0.709	13.83
600	0.231	13.86
Average -	-	14.23

† ΔE_{400} = Absorbance (reaction) – Absorbance (blank)

Table 2.3. The α-amylase activities for 24 wheat varieties at PM-3 in the absence of PHS were ranked from smallest to the largest; the FN values were ranked from largest to the smallest

Wheat Type	White		Red	
Test / Rank	α-amylase activity test	FN test	α-amylase activity test	FN test
1	MSU E5024	MSU E5024	Pioneer 25R62	Pioneer 25R62
2	Crystal	MSU E2043	Red Ruby	R055
3	MSU E2043	Jupiter	OH04-264-58	Red Ruby
4	Envoy	Envoy	R055	Hyland Emmit
5	Ambassador	Coral	Arena	Tribute
6	AgriProW1062	Ambassador	MCIA Oasis	OH04-264-58
7	Coral	Crystal	Roane	Roane
8	Jupiter	Lowell	R045	Pioneer 25R47
9	MSU D8006	MSU D8006	Pioneer 25R47	R045
10	Lowell	Aubrey	Tribute	Arena
11	Aubrey	AgriProW1062	Hopewell	Hopewell
12	Caledonia	Caledonia	Hyland Emmit	MCIA Oasis

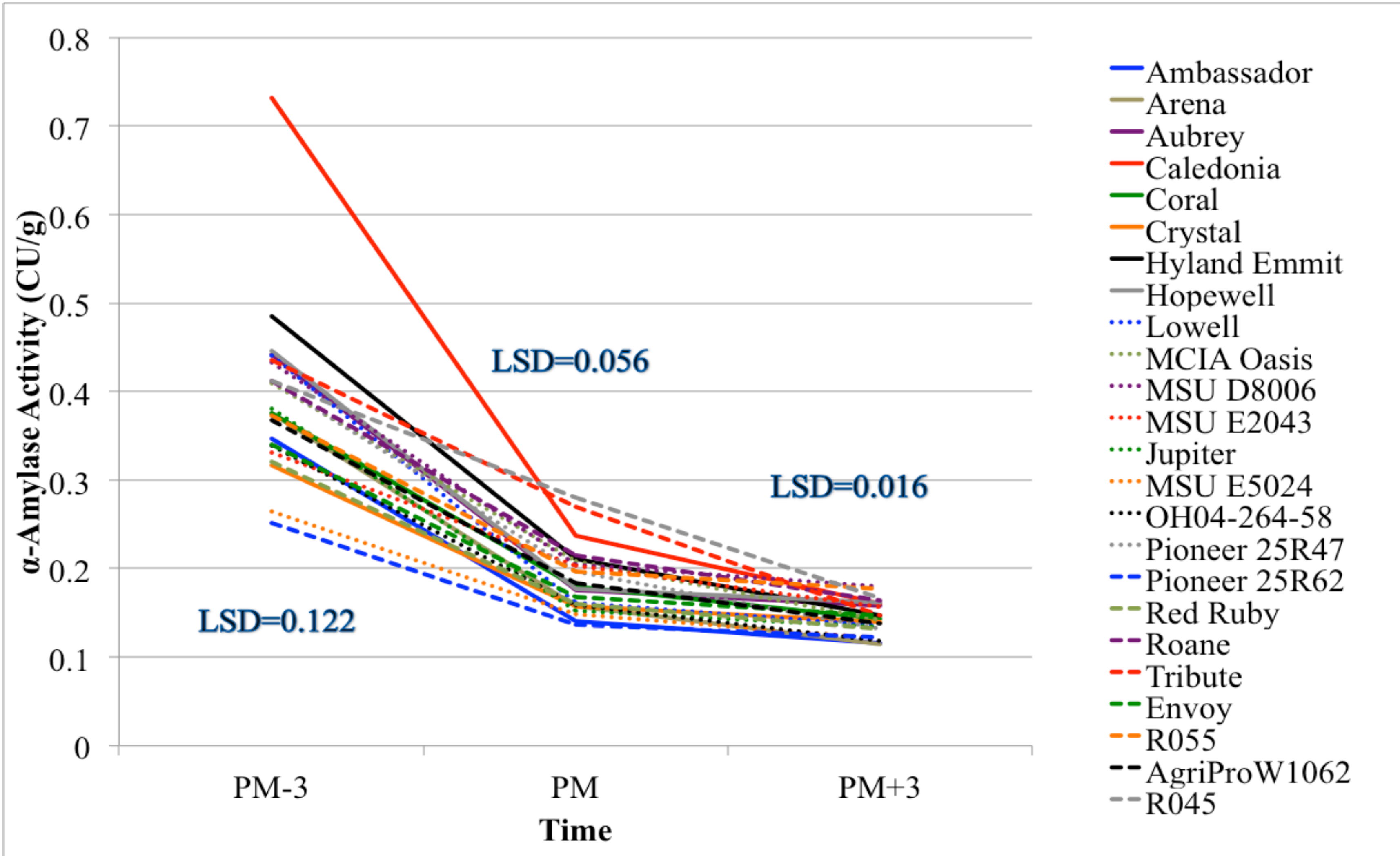

Figure 2.1. The α-amylase activity (CU/g) at three days before PM (PM-3), PM and three days after PM (PM+3) for 24 Michigan varieties, using grain frozen immediately after harvesting. LSD values are included at each time point ($p<0.05$).
(For interpretation of the references to color in this and all other figures, the reader is referred to the electronic version of this thesis.)

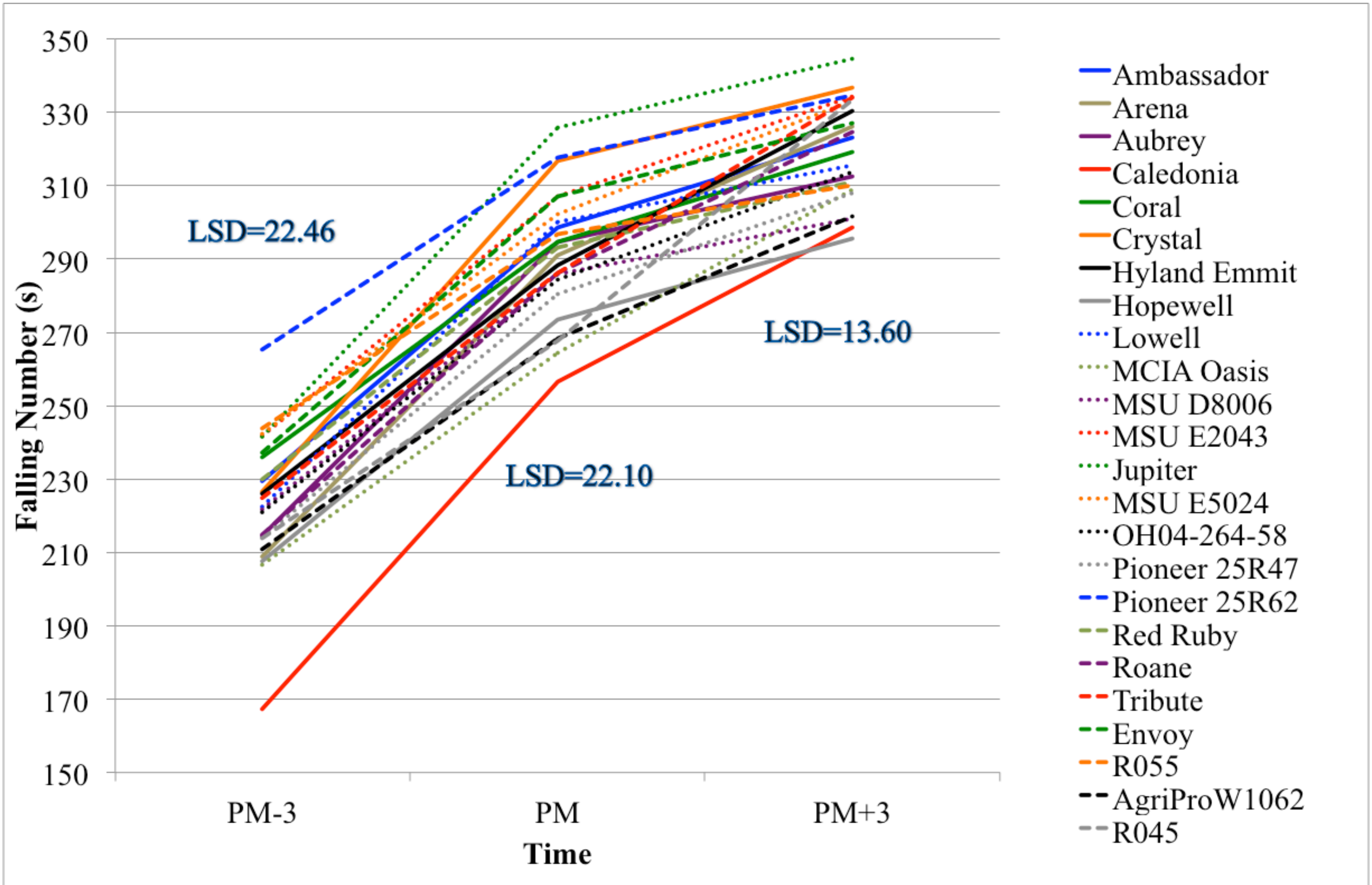

Figure 2.2. Falling Number values at three days before PM (PM-3), PM and three days after PM (PM+3) for 24 Michigan varieties, using grain frozen immediately after harvesting. LSD values are included at each time point (p<0.05).

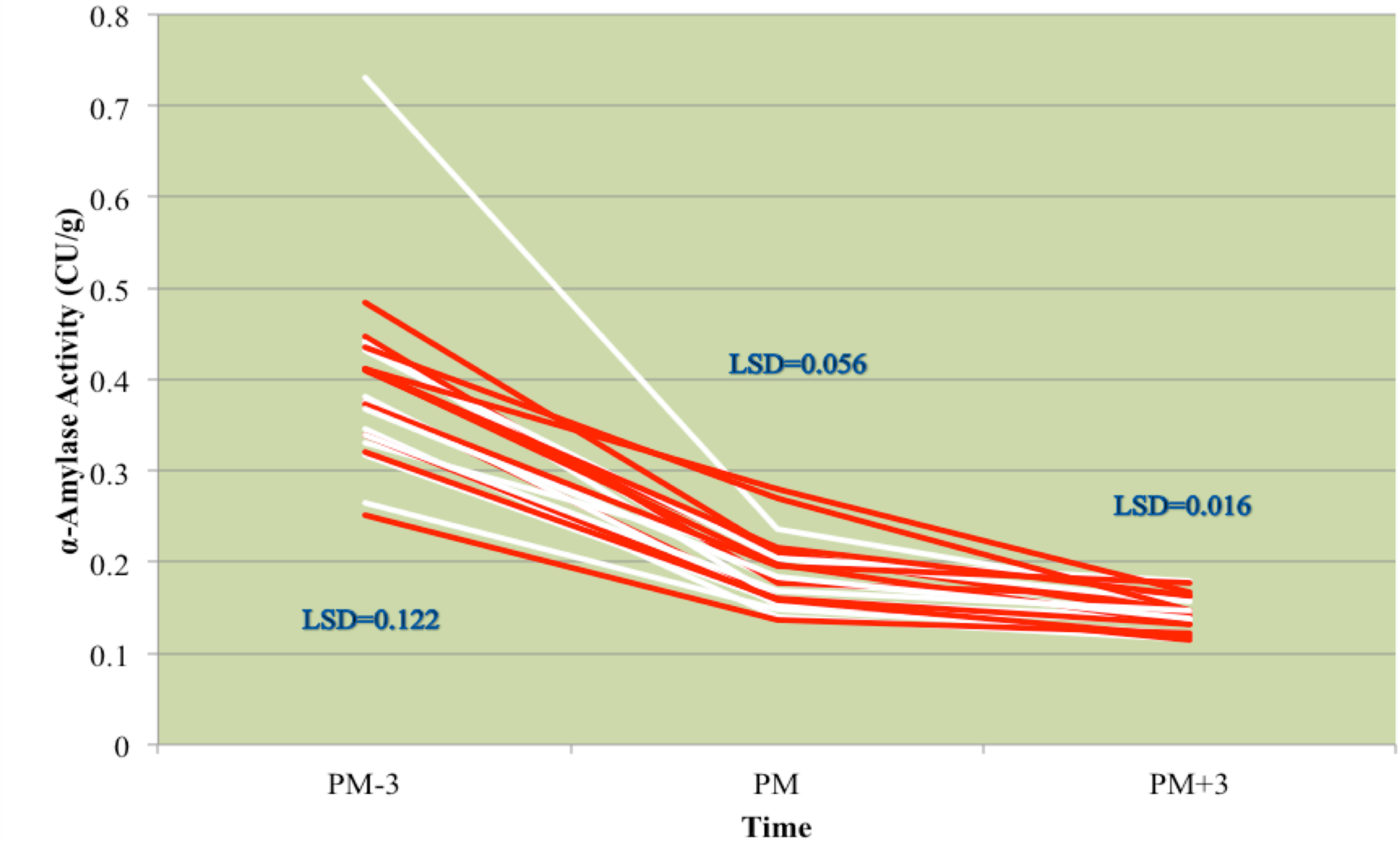

Figure 2.3. The α-amylase activity (CU/g) at three days before PM (PM-3), PM and three days after PM (PM+3) for 24 Michigan varieties, using grain frozen immediately after harvesting. LSD values are included at each time point ($p<0.05$). Red color represents red wheat lines, and white color represents white wheat lines.

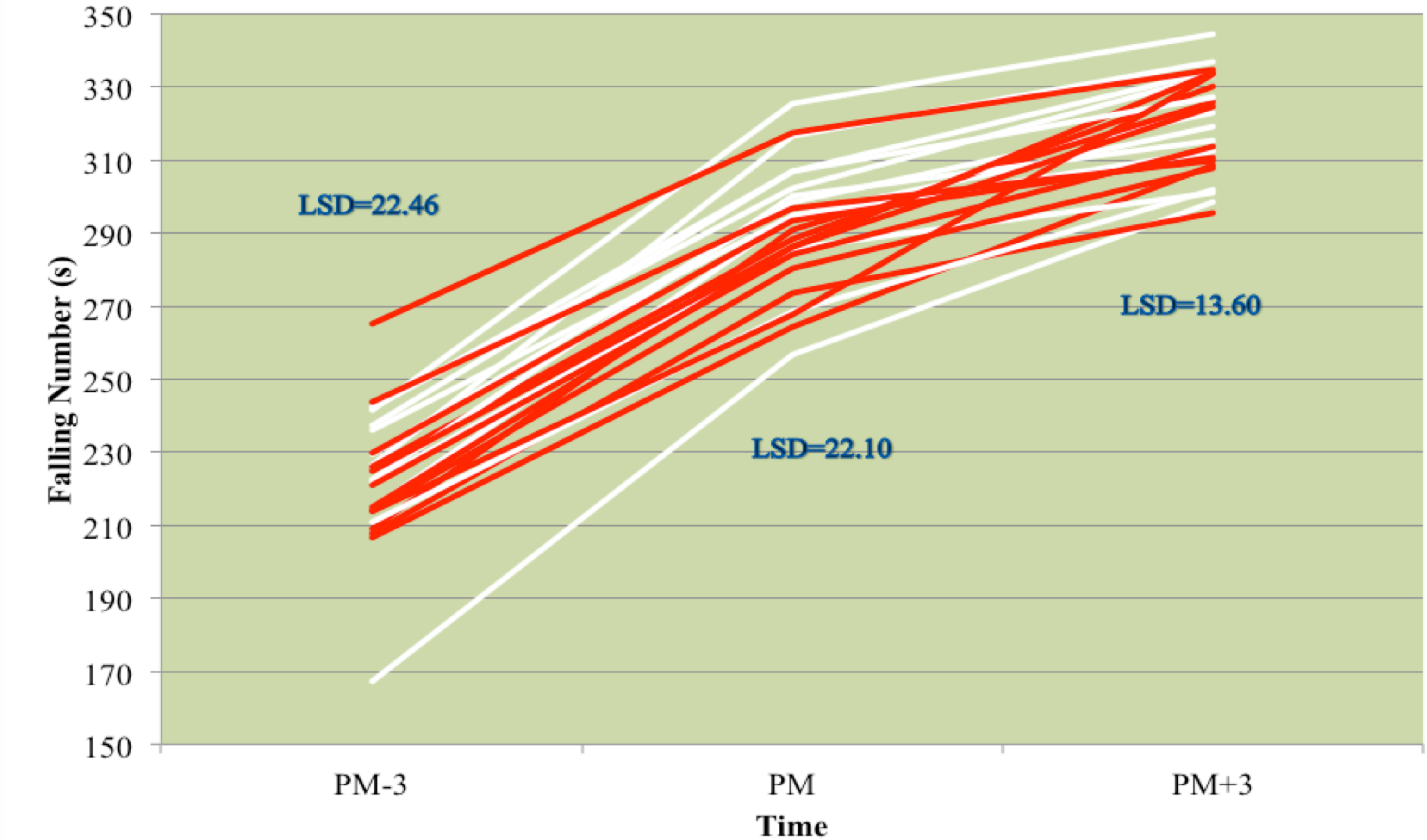

Figure 2.4. Falling Number values (s) at three days before PM (PM-3), PM and three days after PM (PM+3) for 24 Michigan varieties, using grain frozen immediately after harvesting. LSD values are included at each time point ($p<0.05$). Red color represents red wheat lines, and white color represents white wheat lines.

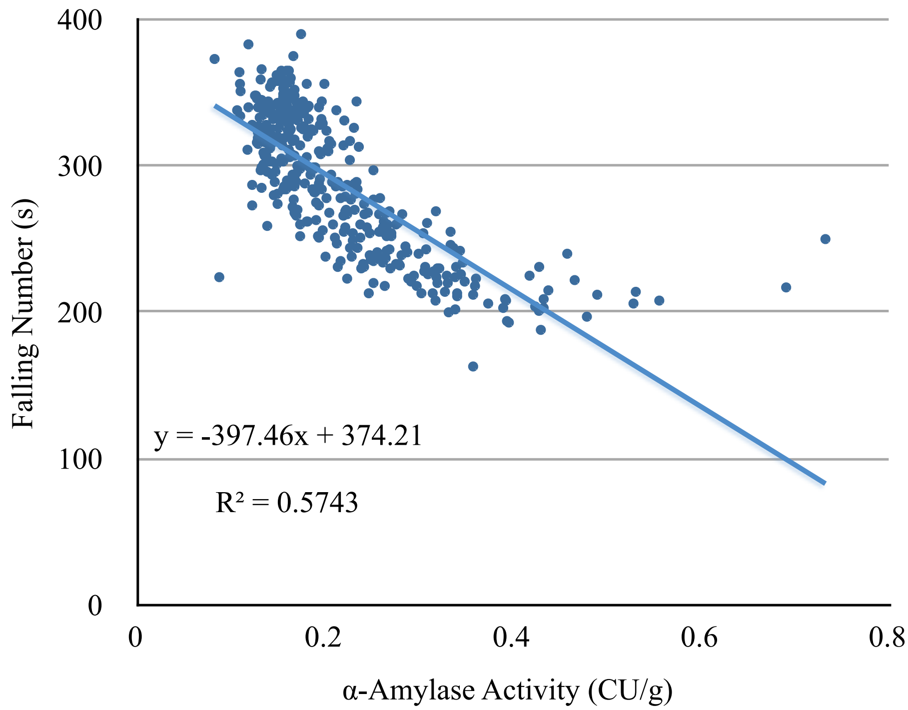

Figure 2.5. Correlation of α-amylase activity to FN for 24 wheat varieties.

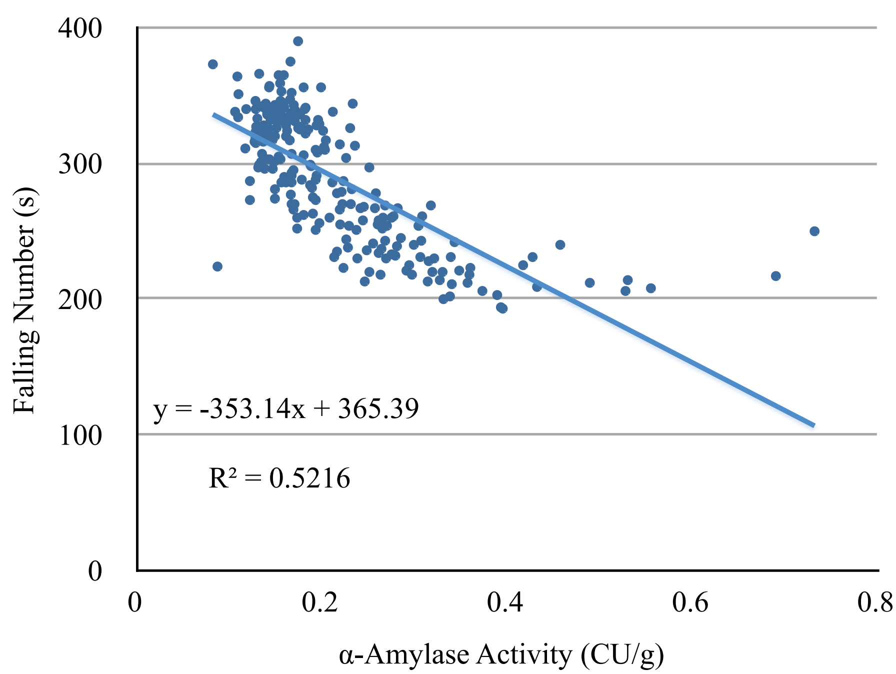

Figure 2.6. Correlation of α-amylase activity to FN for 12 red wheat varieties.

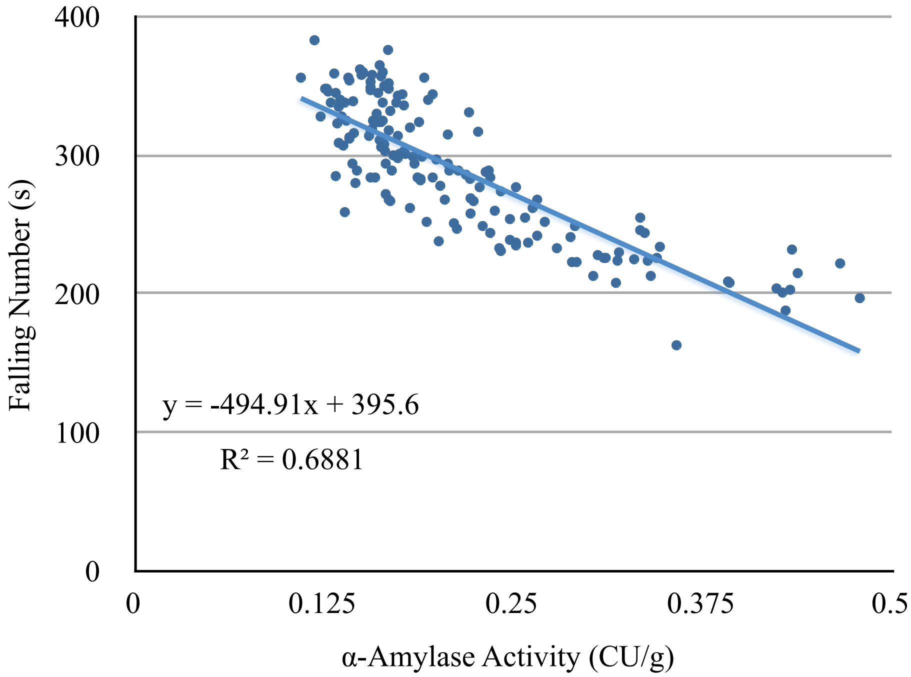

Figure 2.7. Correlation of α-amylase activity to FN for 12 white wheat varieties.

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CHAPTER III

EVALUATION OF ALPHA-AMYLASE ACTIVITY AND FALLING NUMBER FOR SOFT WHITE AND SOFT RED WHEAT VARIETIES ADAPTED TO MICHIGAN FOLLOWING MULTIPLE PHS INDUCTION TREATMENTS

ABSTRACT

Pre-Harvest Sprouting (PHS) on wheat is a major threat to Michigan, which is the primary soft wheat production area in Great Lakes. The objectives of this study were to evaluate adapted wheat cultivars for α-amylase activity and the corresponding FN values around physiological maturity (PM) time following artificial PHS induction treatments, determine the effect of after ripening period on PHS severity, and compare the impact of two misting procedures (severe and moderate) on tested wheat varieties. Twenty-four soft winter wheat genotypes (12 red and 12 white) with varying levels of susceptibility to PHS were planted in α-lattice design in two locations from 2009 to 2010. Samples were freeze-dried and evaluated for α-amylase activity and FN values. 'Lowell' and 'Jupiter' showed significant differences at PM for either α-amylase activity or FN values, compared between moderate misting and non-misting treatment. In 2010 trial, genetic differences among 24 cultivars existed for both responses at PM in all treatments. Five days of after-ripening period (ARP) has significant effect on dormancy breaking for some genotypes. Generally, red wheat is more resistant to PHS than white wheat. 'Jupiter', 'Lowell' and 'Caledonia' were three main white wheat varieties susceptible to PHS. White wheat varieties 'MSU E5024' and AgriPro 'W1062' showed PHS tolerance in this study. Red wheat 'Hopewell' and 'Red Ruby' exhibited PHS susceptibility after severe misting.

INTRODUCTION

Wheat is grown more widely than any other commercial crop and holds the greatest world trade (FAO, 2002). Globally, it is the second major human food crop after rice. One key motivation of the Michigan's wheat business is the dominant position for Michigan in soft wheat production in Great Lakes area. The other is that food industries that rely on soft wheat, including the headquarters of such major international cereal companies as Kellogg's®, Post® and Jiffy®, located in Michigan.

Pre-harvest sprouting (PHS) is the precocious germination of the grain prior to harvest in the field. Higher than average rainfall and cool temperature after grain maturation (i.e., after having reached physiological maturity) is the major cause of PHS. Pre-harvest sprouting in wheat results in the activation of enzymes, including α-amylase, which degrades starch, the major component of the endosperm (flour) of the grain. Such degradation results in poor grain quality, including reduced test weight, alkaline water retention capacity, as well as altered grain functionality (Sorrells et al., 1989; Barnard and Purchase 1998). Products made from PHS degraded flour will often be porous, sticky, misshapen, have poor loaf volume and be off-color (Ranhotra et al., 1977). Generally, the losses due to PHS are multifold, including loss of the grain itself due to rejection by millers and food companies, economic losses to the wheat business, and loss of wheat availability in future years because of the farmer avoidance of risk of more losses due to PHS.

Susceptibility to PHS is dependent on both genotype and environmental conditions such as temperature and moisture (Nielsen et al., 1984; Barnard and Smith, 2009). High temperatures before physiological maturity (PM) reduced sprouting tolerance (Nielsen et al., 1984). In general, cool temperature and moist conditions after PM are inclined to break dormancy and lead to PHS.

Monitored natural rainfall and artificial misting treatment have been widely used in PHS research (Mares 1993; Humphreys and Noll, 2002; Hughes et al., 2010). Multiple wetting and drying cycles help water to penetrate seeds quickly (Thomason et al., 2009). However, there is no standard protocol to induce PHS after harvest in artificial environment.

Seed dormancy is considered the main factor to generate resistance to wheat PHS. Two types of dormancy, seed coat-imposed dormancy and embryo dormancy, are present in wheat (Paterson and Sorrells, 1990; Flintham, 2000). Physical restriction, gas exchange interference, and the presence of inhibitors are major mechanisms to explain the seed coat effect contributing dormancy (Bewley and Black, 1994). The embryo dormancy is not affected by surrounding tissues. In cereals and other seeds, it is well established through physiological and genetic studies that abscisic acid (ABA) plays an important role in the induction and maintenance of dormancy (Gubler et al., 2005). During seed development, ABA content is low during the grain filling stages, peaks around mid-maturation, and then declines gradually during seed drying stage (after maturity) (Bewley and Black, 1994). Gibberellic acid (GA), which activates the production of α-amylase in aleurone tissues in cereal grains, has antagonistic effect to ABA on dormancy (Gubler et al., 2005).

Some morphology traits were also found to protect the grain from water. The presence of awns keeps more water on the heads and increased the sprouting probabilities, compared to the awnless lines (King and Richards, 1984). The existence and thickness of epicuticular waxes also impact the water uptake rate (King and von Wettstein-Knowles, 2000).

After ripening period (ARP) is an essential process for seed to start germination. After ripening period refers to a period of time that the seeds broke dormancy in dry conditions after reaching PM and this effect depends on time length, moisture, temperature and oxygen level

(Bewley and Black, 1994). The effect of breaking dormancy by six weeks ARP storage could be equivalent to the effect of 100 mol of GA, but ARP and GA break seed dormancy in different ways (Tavakkol-Afshari and Hucl, 2002). Abscisic acid decreased rapidly by hydration but the GA biosynthetic pathway was activated and GA accumulated during ARP storage, both of which are also effected by environment (Jacobsen et al., 2002). After ripening period has no effect on expression of ABA biosynthesis genes, but promotes expression of an ABA catabolism gene, a GA biosynthetic gene, and a GA catabolic gene following imbibition. In ARP, positive correlation of the seed dormancy and phenolic contents suggests its dormancy control effect in cereals (Weider et al., 1996). Cultivar and the ARP environment both affect the speed of losing seed dormancy during ARP, and high temperature during ARP was found to be a positive factor to accelerate the loss process (Hagemann and Ciha, 1987; Romagosa et al., 1999). Due to rapid loss of seed dormancy in tested wheat varieties, the aim of breeding for gradual loss of dormancy during after-ripening varieties to reduce PHS was proposed (Tavakkol-Afshari and Hucl, 2002).

The objective of this study was to evaluate the PHS resistance and susceptibility of the 24 wheat varieties adapted to Michigan, using α-amylase activity and Falling Number (FN) tests, following multiple PHS inductions such as artificial misting and added ARP treatment. The first misting experiment was conducted at three days before PM, at PM and three days after PM, to identify the relationship between maturity and PHS severity. Subsequently, ARP treatment and two different misting treatments were used to detect the effect of ARP on the 24 genotypes and the genetic variation on different misting categories. These results could provide some insight on line selection in breeding for PHS resistance and optimization of artificial PHS induction procedures for soft winter wheat breeding in Michigan.

MATERIAL AND METHODS

Plant Materials, Filed Design and Plot Sampling

In the fall of 2009 and 2010, 12 soft white and 12 soft red wheat varieties were grown in a three-replication α-lattice design in two locations in Michigan each year. The details of field design and planting has been described in material and methods part in chapter 2 of this thesis.

Physiological maturity (PM) was visually assessed within each season according to chlorophyll loss. In 2010, approximately 200 spikes with stem (30-50 cm) were sampled at three days prior to PM (PM-3), at PM and three days after PM (PM+3) from each plot. In 2011, approximately 600 spikes with stem were randomly sampled at PM from each plot.

Post-harvest Treatment

In 2010, samples were divided into two subsamples, one of which was treated with misting in the greenhouse to induce sprouting, while the other group was kept in the greenhouse at ambient temperature without misting. The plants in each group were arranged in plastic tubes (5.3 cm diameter and 25 cm deep), which were placed in racks so that stems were upright in tubes (to help mimic the natural architecture of the plant in the field). The subsample to be misted was placed on the greenhouse bench under a misting system made up of nozzles attached to the pipelines (1.4 m interval) hanging above the bench and the time controller (Plug-In Digital Timer, Model 15079, General Electric). The misting system was set to operate for 45 min every 6 h and the whole process lasts for 48 h, which is comparable to another recent study on PHS (Humphreys and Noll, 2002).

In 2011, five days after-ripening storage and additional misting treatments were added to the 2010 treatments. The details of the treatment arrangement are described in Table 3.1. Misting treatment I was performed with 1.4 m interval nozzles, controlled by 6-zone misting controller

(1626D, Phytochronics Inc., St. Louis, Mo), with the cycle of 45 min per 6 h, lasts for 48h. Misting treatment II was performed with 35-inch interval nozzles, controlled by another controller (Sterling, Superior Controls Co. Inc., Valencia, CA) in a separate room, with the cycle of 20 s per 2 min, lasts for 72 h. Relative humidity and temperature were recorded (Watchdog A150 Temp/RH Logger, Spectrum Technologies Inc., Plainfield, IL).

For both years, after misting treatment, all samples were trimmed to remove the stems and the remaining spikes were maintained the metabolism level at -20 °C until the samples were freeze-dried.

Sample processing, α-amylase and Falling Number measurements

After freeze-drying, cleaning and threshing, whole flour was obtained from 40 g of each sample using a UDY Laboratory Mill with a 0.5 mm sieve. Alpha-amylase activity and FN tests (AACCI Method 56-81.03) were conducted using the appropriate weight of each flour sample, calculated based on its moisture level according to a Near Infrared Reflectance Spectroscopy (NIRS) assessment. Alpha-amylase activity was assessed using the Ceralpha Method (AACCI Method 22-02.01).

Statistical Analysis

Analyses of variance (ANOVA) and least square (LS) means of α-amylase activity and FN values were performed using the PROC MIXED procedure of SAS for Windows, version 9.2 (Cary, NC). Bonferroni adjustment was used when the effect was not significant in ANOVA table. Fisher's least significant difference (LSD) was calculated for each specific treatment category in red and white wheat. Pairwise comparisons were performed by Tukey's Honestly Significant Difference (HSD) test at a significance level of 0.05.

RESULTS

PHS Severity vs. Maturity Categories: PM-3, PM, PM+3

The analysis of the misted and non-misted (ambient) treatment for 2010 data showed that the α-amylase activity and FN values of the 24 genotypes fluctuated across the three maturity time points (PM-3, PM, PM+3) and there no clear trends were observed (Figure 3.1, 3.2, 3.3 & 3.4). Fisher's Least Significant Differences (LSD) were calculated at each time point, at $p<0.05$ level. Although significant differences existed among cultivars at each of the three time points, the range of α-amylase activity and FN values were quite narrow in comparison with immediately frozen samples (Figure 2.1 & 2.2 in Chapter 2).

The ANOVA analysis indicated that genotype has significant effect on both α-amylase activity and FN test, which suggested that one part of the variance came from the genotypic effects ($p<0.05$). In contrast, the effect of genotype × maturity × misting treatment interaction is not significant, suggesting that this three way interactive effect may not contribute the variation of observations ($p<0.05$). There were no significant effects for year, location, replication and block in the ANOVA analysis ($p<0.05$). Due to our preplanned experimental hypothesis, it is necessary to use Bonferroni adjustment, in the following pairwise comparisons between misted and non-misted treatment for 24 genotypes at each maturity time (PM-3, PM and PM+3).

The 2010 results showed that only white wheat variety 'Lowell' at PM showed a significant difference on α-amylase activity between misting and non-misting treatments (Figure 3.5), and no other significances were found in FN test results or other maturity time points. However, the α-amylase activity of 'Lowell' at PM after misting treatment was still above the comparable commercially acceptable FN values (250) for sound soft wheat (Figure 2.1 & 2.2 in Chapter 2).

Moderate Misting Treatments at PM in 2010 and 2011

In 2011, only PM was selected as the time for sampling, and five days ARP combined with moderate misting or severe misting treatments were followed after sampling. The moderate misting treatment without ARP was equivalent to the misting treatment that used in 2010 study. The data at PM in 2010, and the data of treatment at moderate misting without ARP in 2011 were combined together for analysis. For both α-amylase activity and FN test, the effect of entry × treatment interaction is significant, suggesting that genotypes do not respond uniformly to the different misting treatments.

To conservatively estimate the differences between the misted and non-misted treatments, Tukey's Honestly Significant Difference (HSD) was used in the pairwise comparisons. The results showed that there is no significant difference found in α-amylase activity results. However, two white genotypes, 'Lowell' and 'Jupiter' were found to have significant differences in FN between misting and non-misting treatments (Figure 3.6). Even so, the FN results following misting were all above 250, which is the commercial acceptable line for sound soft wheat.

PHS Severity, ARP and Misting Treatments: Within Genotype Comparisons

From the ANOVA test, the effects of genotype, treatment (misting and ARP), and genotype × treatment interaction were significant ($p < 0.05$) for both α-amylase activity and FN test. The effects of location, year, replication and blocks were not significant ($p <0.05$). Significant differences were found within each genotype, between the following categories: severe misting effect, moderate misting effect and ARP effect, using Tukey's HSD method (Table 3.2, 3.3 & 3.4).

In table 3.2, when inspecting the α-amylase activity results of the severe misting treatment, it was clear that 10 out of 12 white wheat genotypes were sensitive to the severe misting with ARP, and seven out of 12 were sensitive to the severe misting without ARP. No red wheat genotypes showed significant differences in the severe misting and non-misting comparison for α-amylase activity test. For FN test, all 12 white wheat genotypes have significant differences under both severe misting with ARP and without ARP categories. The red wheat cultivars, 'Red Ruby', 'Tribute' and 'R055' showed significant differences between severe misting and non-misting, under both with ARP and without ARP categories. Pioneer '25R47', Pioneer '25R62', 'Roane' and 'R045' were only susceptible to the severe misting with ARP.

In table 3.3, in the moderate misting effect comparison for α-amylase activity test, 'Lowell' and 'Jupiter' showed significant differences between misting and non-misting, only when they were treated with ARP storage. For FN test, these two white wheat genotypes were significantly different compared to non-misting, under both severe misting with ARP and without ARP treatments. 'Aubrey' was susceptible to moderate misting when it was treated with ARP storage.

In table 3.4, the ARP effect comparison for α-amylase activity test, 'Lowell' and 'Jupiter' showed significant differences between with ARP and without ARP categories, for both misting treatments. The sensitivity to ARP for 'Ambassador', 'Aubrey', 'Caledonia', 'Coral' and 'MSU D8006' were only observed after severe misting. For FN test, only 'Lowell' was sensitive to ARP in both misting categories. Four white genotypes ('Aubrey', 'Caledonia', 'MSU E5024', AgriPro 'W1062') and two red genotypes (Pioneer '25R62' and 'R045') showed significant differences between with ARP and without ARP categories, following sever misting treatment.

However, there was no significant difference between non-misting with ARP and non-misting without ARP, for any of the 24 wheat genotypes.

PHS Severity, ARP and Misting Treatments: Within Treatment Comparisons

The comparisons were also made within each treatment, in order to identify the relative position for 24 genotypes for both α-amylase activity and FN values (Figure 3.7 – 3.18). Least significant differences (LSDs) were provided at each treatment for α-amylase activity and FN tests, and significant differences were found between genotypes for both white and red lines, at all six treatment categories: severe misting with ARP, severe misting without ARP, moderate misting with ARP, moderate misting without ARP, non-misting with ARP, non-misting without ARP.

For α-amylase activity test, following severe misting with ARP and severe misting without ARP, results varied below 0.50 CU/g for all the red genotypes, but the results of white genotypes ranged widely from 0.58 to 17.61 CU/g and 0.28 to 6.40 CU/g. The results for moderate misting (with ARP and without ARP), showed less variation for both red and white genotypes, and the majority are below 0.50 CU/g. The α-amylase activities of 24 wheat lines in the two control groups were consistently low below 0.25 CU/g.

For FN test under severe misting, the red genotypes ranged from 164 to 325, some of which were below the commercial acceptable value (250). The FN values of majority of white lines were around 62, which is the lowest theoretical FN value. A few white genotypes showed PHS tolerance with higher FN values, around 150. For moderate misting, the FN values of all red genotypes were above 250 and the white genotypes were varied from 200 to 300. The average FN values for both white and red varieties were higher without ARP treatment than with ARP treatment. Similar to the results in α-amylase activity test, two control groups showed high FN values (>300), for both red and white genotypes.

Genotype Ranking

Alpha-amylase activity and FN values of 12 white and 12 red wheat genotypes were ranked by percentile (percentage of scores in the frequency distribution) in each treatment category separately (Table 3.5 & 3.6). The top three resistant genotypes, which showed lowest α-amylase activity and highest FN, were in the top three rows of the table, and three most susceptible genotypes was in the three bottom rows. For each ARP class (with ARP or without ARP) and wheat color class, the times of genotype in the resistant group and susceptible group were counted to give a new rank and remarks were given if there was only one hit in these two groups (Table 3.7 – 3.10).

For the white wheat, the results showed that 'MSU E5024' and AgriPro 'W1062' were consistently the two most PHS tolerant lines in both misting categories, irrespective of ARP. 'Jupiter' was one of the most PHS susceptible white wheat for both treatments with or without ARP storage. 'Lowell' was another most susceptible genotype in the misting experiment with ARP storage. Several other genotypes performed differently depending on the ARP / misting treatments employed. 'Envoy' and 'Crystal' showed good resistance following ARP treatment. However, 'Envoy' did not perform well in the without ARP group. 'Caledonia' and 'Aubrey' showed good resistance to the misting at PM, but the former did badly in the misting experiment after ARP storage and the latter did badly in the misting without ARP.

Hyland 'Emmit' showed consistent resistance to PHS for both ARP treatments. 'OH04-264-58' and 'Arena' were also resistant red wheat lines in this study. MCIA 'Oasis', 'Roane', 'Tribute' and two Pioneer varieties varied in the resistant and susceptible group. Although the red wheat were more resistant to PHS than white wheat overall, 'Hopewell' and 'Red Ruby' consistently grouped within the PHS susceptible red genotypes.

Scatter Plot of the Experimental Data between α-Amylase Activity and FN Values

Using three years data points from all experiments, α-amylase activity and FN values were plotted to check their regression (Figure 3.19). It is clear that the correlation between α-amylase activity and FN values across the whole data range was not linear. Falling Number value did not correlate with α-amylase activity well ($r^2 = 0.5816$) when FN in the range of 200-300. The change of FN values was quite small and reached the minimum value (62), when α-amylase activity was over 3.0 CU/g. When the variation of α-amylase activity was relative small in the low range (0 to 0.4 CU/g), the FN values varied largely, which could be 150 to over 400.

DISCUSSION

In the summer of 2010 and 2011, there was no severe moisture condition in the field during sampling to induce PHS. No visual evidence of sprouting and the general low α-amylase activity and high FN values in the non-misting control groups validated the sound grain quality of our samples.

In 2010, the fluctuation of α-amylase activity and FN results between PM-3 and PM+3 for the misting and non-misting suggested that the maturity stage had little effect on the misting consequences. In the absence of PHS study, the converging trend of the α-amylase activity and FN results after PM was observed (Chapter 2). It is possible that the effect of non-misting control for two days was equivalent to the three days after PM in the field, on α-amylase activity level.

There was no significant difference found between misting and non-misting in FN test and one white wheat genotype, 'Lowell', had significantly higher α-amylase activity after misting treatment at PM, in 2010 study. For 2010 – 2011 experiment, which focused on PM, only white wheat cultivars, ‘Lowell’ and ‘Jupiter’, exhibited lower FN values after misting. Although Tukey’s HSD test detected significant difference for these comparisons, the mean values after misting for both α-amylase activity and FN value were still tend to be acceptable (>250). All red and white genotypes were selected based on the variation on PHS response from historical data, variation of response to the misting was expected. The results that only two lines had general high FN after misting, even significantly lower than non-misting, suggested that the artificial misting induction used in 2010 (moderate misting) might not be effective to the majority of genotypes in our specific environment. The results also suggested that genotype, environment and genotype × environment affected the PHS response. Since MSU previously conducted visual sprout count evaluations in the same greenhouse using the same misting system and during the same time of year, we knew that sprouting could occur using these facilities during the summer. In addition, large variation in FN was observed in the comparable artificial misting study on hard wheat (Humphreys and Noll, 2002). Industry reported that PHS can occur within hours in the field, but perhaps the same responses were not as rapid in the greenhouse and either a longer duration of wetting was needed, or more time was needed for the plant to respond to the wetting prior to freezing. The general lack of significant differences between the misted and ambient treatments within genotypes could have several different causes, such as specific temperature conditions in the field prior to harvest or in the greenhouse post-harvest that promoted higher levels of dormancy, drought condition before seed maturity, and insufficient relative humidity levels in the misting system. Previous studies suggested that the cool temperature during maturation led to higher levels of grain dormancy (Reddy et al., 1985). Drought during grain filling stage has been shown to increase seed dormancy and non-dormant plants can be induced to show a dormant phenotype (Biddulph et al., 2005). Thomason et al.

(2009) pointed out that cool temperatures prior to PM, combined with dry conditions, might result in higher dormancy of grain, and they also indicated that multiple wetting cycle help water penetrating out layers of grain, which provided better misting effect. While other related research (Paterson and Sorrells, 1990; Humphreys and Noll, 2002; Hughes et al., 2010) used air drying following misting, our use of freezing samples to stop metabolic changes soon after misting may have cut short the time for the grain to respond to the misting treatment. After ripening period was indicated to break seed dormancy, by ABA degradation and activation GA synthesis (Tavakkol-Afshari and Hucl, 2002). Short-term drying storage prior misting treatment was reported in several PHS researches (Paterson and Sorrells, 1990; Singh et al., 2008), which could be considered as equivalent to an ARP factor.

Significant difference among 24 genotypes to four different misting treatments for both α-amylase activity and FN values indicated genotypic variation in the response to the PHS induction. In two control treatments, significant differences were also observed in 24 genotypes, which were in agreement with the previous results of α-amylase activity and FN values at PM+3 time points, in the absence of PHS (Bradford and Nonogaki, 2007).

Both severe misting and moderate misting successfully induced significant changes for α-amylase activity and FN values in the 24 varieties. More varieties, especially the red wheat genotypes, showing significant difference after severe misting in the FN test, than α-amylase activity test, indicated that FN might be more easily to differentiate the changes in the PHS experiment for the red wheat. ‘Lowell’ and ‘Jupiter’ showed consistently significant difference even after moderate misting suggested that these two lines were susceptible to PHS.

Several genotypes receiving ARP treatment before misting, showed significantly higher α-amylase activity and lower FN values, compared to the group without ARP storage. It suggested that the dormancy of these wheat varieties were more easily broken, compared to other varieties, which showed no significant differences after ARP treatment. The dormancy reduction after ARP might be caused by the ABA degradation and GA precursor synthesis (Tavakkol-Afshari and Hucl, 2002). However, the ARP effect on dormancy was different between severe and moderate misting, even for the same genotype, which indicated that breaking dormancy was affected by both environment and ARP storage. ‘Lowell’ and ‘Jupiter’ showed consistently significant ARP effect for both severe and moderate misting, which implied that these two white wheat varieties were susceptible to the dormancy release factors, such as ARP and weathering.

The summary of the percentile rank for white and red genotypes, in terms of the misting responses, indicated two white wheat varieties, ‘MSU E5024’ and AgriPro ‘W1062’, are tolerant to PHS. ‘Jupiter’, ‘Lowell’, ‘MSU E2043’ were the lines which are very susceptible to PHS. ‘Envoy’ and ‘Crystal’ are moderately tolerant to PHS if they received ARP treatment, and ‘Caledonia’ and ‘Aubrey’ were moderately tolerant to PHS if they were directly weathered at PM. However, ‘Caledonia’ was susceptible to PHS after ARP storage, which validated the ARP effect on the dormancy reduction. The wheat varieties, such as AgriPro ‘W1062’ and ‘Aubrey’, showed susceptibility at PM and tolerance after ARP, suggested the dormancy might be obtained in the beginning term of ARP storage. For the red wheat, ‘Hopewell’, ‘Red Ruby’, ‘R055’ showed significantly reduced FN after misting, suggested these red varieties are not tolerant to the long term misting. However, the overall of red varieties showed significantly higher α-amylase activity and lower FN values than white wheat, in both severe misting and moderate misting group, which proved that red wheat were generally more resistant to PHS than white wheat. In this study, Hyland ‘Emmit’, ‘Arena’ and ‘OH04-264-58’ showed PHS tolerance consistently, suggested that those lines could be good source of PHS resistant for white wheat.

There were no obvious effects of the awn on the PHS tolerance in our study. We were not able to prove the previous conclusion that the awnless wheat was more resistant to sprouting than awned wheat (King and Richards, 1984). From the pedigree analysis, two PHS tolerant white wheat varieties, 'MSU E5024' and AgriPro 'W1062' shared the same pedigree, Pioneer '25W33'. The PHS susceptible wheat lines, 'Lowell', 'Caledonia' and 'Jupiter' all have genetic similarity from each other or 'Genesee'. The PHS resistant or susceptible genes might be brought to the progenies from the original pedigrees by genetic introgression.

The *viviparous-1 (VP1)* gene mutants in maize (Zea mays) have a defect in ABA biosynthesis or signaling, indicating a role for *vp1* in preventing precocious germination (McCarty 1995). Wheat homologs (*TaVP1*) of the maize *VP1* gene are also associated with dormancy and PHS (Chang et al., 2010a; Chang et al., 2010b; Chang et al., 2011). *VP1* gene was genotyped in 'MSU E5024', which suggested that the PHS tolerance might result from the *VP1* gene action (Lewis et al., 2011).

The scatter plots showed the similar results that significant changes in FN test could occur without increase in α-amylase activity when the latter in low range (< 0.25 CU/g) (Souza et al., 2011). The decline of FN may be due to swelling of the starch granules through hydration or it may be result from the effect of other enzymes, such as proteases or lipases, which degraded other large molecules in the grain (proteins and fats) to reduce the resistance of the plunger movement. However, our result was contradictory to the conclusion that FN correlated well with α-amylase activity in the range of FN 200 - 300 (Souza et al, 2011).

SUMMARY

Over years, locations and genotypes, significantly genetic differences were observed around physiological maturity (PM) time, especially three days prior to PM (PM+3), for α-amylase activity and FN values in 24 Michigan adapted soft white and soft red winter wheat. Pre-harvest sprouting (PHS) resistant, tolerant and susceptible soft white and soft red wheat genotypes have been identified through this study. However, repeated experiments in multiple years and locations are necessary to validate the evaluation results. The rank of base α-amylase activity identified in either red or white wheat, in the absence of PHS, did not match the rank of α-amylase activity under the artificial misting PHS induction treatments, except for the top and bottom ones, 'MSU E5024' and 'Caledonia'. This suggested that the enzyme accumulation level was affected by both genotypic and environment factors. The α-amylase activity test and FN test showed different power in analyzing PHS samples. For breeding purposes, using α-amylase activity test is better than FN test, especially for white wheat, since it could not only provide the direct enzyme activity information, but also having the unlimited range when the sample FN results in low range (<150). However, FN test is more convenient and faster than α-amylase activity test, and provided good differentiation in mid-high range (200-400). The average temperature record for the severe misting, moderate misting and non-misting was 31.65°C, 32.25°C, 37.75°C, which indicated that the two misting environment provided lower temperature conditions than non-misting control. The average relative humidity (RH) record for those three environments were 80.4%, 76.9%, 45.4%, and the close RH between severe misting and moderate misting implied that the frequency of watering might play an important role in breaking seed dormancy. The results suggested that severe misting plus ARP treatment is the effective approach to induce PHS for Michigan white and red wheat in greenhouse condition. The genotyping of 'MSU E5024' has proved the existence of vp1 gene and it would be interested genotyping its genetic similar line AgriPro 'W1062' and Pioneer '25W33'. Designing double haploid population of these genotypes is expected to map the PHS related gene.

Table 3.1. Post-harvesting treatment design of trial 2011

Treatment Code	After Ripening Period (ARP)	Misting Cycle
A	5 days	I: 20 seconds / 2 minutes, 72 hours total
B	None	I: 20 seconds / 2 minutes, 72 hours total
C	5 days	II: 45 minutes / 6 hours, 48 hours total
D	None	II: 45 minutes / 6 hours, 48 hours total
E	5 days	None
F	None	None

Table 3.2 Genotypes were shown in each treatment category when the pair wise comparisons between severe misting (20 s per 2 min, last for 72 h) and non-misting for α-amylase activity and FN tests were significant ($p<0.05$)

Test	α-Amylase activity test		FN test	
Treatment Category	Severe misting / Non-misting			
	With ARP	Without ARP	With ARP	Without ARP
White Wheat Genotypes	Ambassador		Ambassador	Ambassador
	Aubrey		Aubrey	Aubrey
	Caledonia		Caledonia	Caledonia
	Coral	Coral	Coral	Coral
	Crystal	Crystal	Crystal	Crystal
	Lowell	Lowell	Lowell	Lowell
	MSU D8006	MSU D8006	MSU D8006	MSU D8006
	MSU E2043	MSU E2043	MSU E2043	MSU E2043
	Jupiter	Jupiter	Jupiter	Jupiter
	Envoy	Envoy	Envoy	Envoy
			MSU E5024	MSU E5024
			AgriPro W1062	AgriPro W1062
Red Wheat Genotypes			Pioneer 25R47	
			Pioneer 25R62	Pioneer 25R62
			Red Ruby	Red Ruby
			Roane	
			Tribute	Tribute
			R055	R055
			R045	

Table 3.3 Genotypes were shown in each treatment category when the pair wise comparisons between moderate misting (45 min per 6 h, last for 48 h) and non-misting for α-amylase activity and FN tests were significant (p<0.05)

Test	α-Amylase activity test		FN test	
Treatment Category	Moderate misting / Non-misting			
	With ARP	Without ARP	With ARP	Without ARP
White Wheat Genotypes	Lowell Jupiter		Aubrey Lowell Jupiter	Lowell Jupiter

Table 3.4 Genotypes were shown in each treatment category when the pair wise comparisons between the group with ARP treatment and without ARP treatment for α-amylase activity and FN tests were significant (p<0.05)

Test	α-Amylase activity test		FN test	
Treatment Category	ARP effect for severe misting	ARP effect for moderate misting	ARP effect for severe misting	ARP effect for moderate misting
White Wheat Genotypes	Ambassador Aubrey Caledonia Coral Lowell Envoy MSU D8006 Jupiter	Lowell Jupiter	Aubrey Caledonia Lowell MSU E5024 AgriPro W1062	Lowell
Red Wheat Genotypes			Pioneer 25R62 R045	

Table 3.5. The α-amylase activities and FN values for 12 white wheat varieties at PM for misting and ARP treatment, were ranked from smallest to the largest; the FN values were ranked from largest to the smallest

Test	α-Amylase activity test		FN test		α-Amylase activity test		FN test	
Treatment / Rank	Severe misting w/ ARP	Moderate misting w/ ARP	Severe misting w/ ARP	Moderate misting w/ ARP	Severe misting w/o ARP	Moderate misting w/o ARP	Severe misting w/o ARP	Moderate misting w/o ARP
1	MSU E5024	MSU E5024	MSU E5024	MSU E2043	Aubrey	Ambassador	Aubrey	MSU D8006
2	Envoy	Crystal	Envoy	Crystal	AgriPro W1062	MSU E5024	AgriPro W1062	MSU E5024
3	AgriPro W1062	AgriPro W1062	AgriPro W1062	MSU E5024	Caledonia	AgriPro W1062	Caledonia	Envoy
4	Crystal	Envoy	MSU D8006	MSU D8006	Ambassador	MSU D8006	Ambassador	Crystal
5	Ambassador	MSU E2043	Crystal	Envoy	MSU E5024	Envoy	MSU E5024	Coral
6	MSU E2043	Aubrey	Aubrey	AgriPro W1062	Lowell	Lowell	Crystal	Ambassador
7	MSU D8006	MSU D8006	Ambassador	Coral	Coral	Caledonia	Lowell	Caledonia
8	Aubrey	Ambassador	Caledonia	Ambassador	Crystal	Coral	MSU D8006	Lowell
9	Coral	Coral	MSU E2043	Aubrey	MSU D8006	Crystal	MSU E2043	Aubrey
10	Jupiter	Caledonia	Coral	Caledonia	Envoy	Aubrey	Envoy	MSU E2043
11	Lowell	Lowell	Jupiter	Lowell	Jupiter	MSU E2043	Coral	AgriPro W1062
12	Caledonia	Jupiter	Lowell	Jupiter	MSU E2043	Jupiter	Jupiter	Jupiter

Table 3.6. The α-amylase activities and FN values for 12 red wheat varieties at PM for misting and ARP treatment, were ranked from smallest to the largest; the FN values were ranked from largest to the smallest

Test	α-Amylase activity test		FN test		α-Amylase activity test		FN test	
Treatment / Rank	Severe misting w/ ARP	Moderate misting w/ ARP	Severe misting w/ ARP	Moderate misting w/ ARP	Severe misting w/o ARP	Moderate misting w/o ARP	Severe misting w/o ARP	Moderate misting w/o ARP
1	MCIA Oasis	OH 04-264-58	Hyland Emmit	Hyland Emmit	Arena	OH 04-264-58	R045	Tribute
2	OH 04-264-58	Pioneer 25R62	Roane	Roane	Hyland Emmit	Pioneer 25R62	Hyland Emmit	Hyland Emmit
3	Hyland Emmit	Pioneer 25R47	MCIA Oasis	Tribute	Pioneer 25R62	Arena	Roane	Pioneer 25R62
4	R045	Arena	R045	R045	Pioneer 25R47	Pioneer 25R47	MCIA Oasis	Arena
5	Arena	MCIA Oasis	OH 04-264-58	Pioneer 25R62	OH 04-264-58	Hyland Emmit	Pioneer 25R47	Roane
6	Roane	Red Ruby	Arena	Red Ruby	R045	MCIA Oasis	OH 04-264-58	OH 04-264-58
7	R055	Hyland Emmit	Hopewell	OH 04-264-58	MCIA Oasis	R045	Pioneer 25R62	R045
8	Pioneer 25R47	Tribute	R055	Arena	Hopewell	Red Ruby	Hopewell	MCIA Oasis
9	Hopewell	R045	Pioneer 25R47	Pioneer 25R47	Roane	Tribute	Arena	R055
10	Tribute	R055	Tribute	R055	R055	Hopewell	R055	Hopewell
11	Red Ruby	Roane	Pioneer 25R62	MCIA Oasis	Tribute	R055	Tribute	Red Ruby
12	Pioneer 25R62	Hopewell	Red Ruby	Hopewell	Red Ruby	Roane	Red Ruby	Pioneer 25R47

Table 3.7. The summary of hit times and remarks for the genotypes listed in the top-three group of the percentile rank for white wheat in table 3.5

ARP	Rank	Genotype	Hit Times	Remark
W/	1	MSU E5024	4	
	2	AgriPro W1062	3	
	3	Envoy	2	
	3	Crystal	2	
	4	MSU E2043	1	Moderate Misting
	6			
W/O	1	AgriPro W1062	3	
	2	MSU E5024	2	
	2	Caledonia	2	
	2	Aubrey	2	
	5	Ambassador	1	Moderate Misting
	5	Envoy	1	Moderate Misting
	5	MSU D8006	1	Moderate Misting

Table 3.8. The summary of hit times and remarks for the genotypes listed in the top-three group of the percentile rank for red wheat in table 3.6

ARP	Rank	Genotype	Hit Times	Remark
W/	1	Hyland Emmit	3	
	2	OH04-264-58	2	
	2	MCIA Oasis	2	
	2	Roane	2	
	5	Pioneer 25R62	1	Moderate Misting
	5	Tribute	1	Moderate Misting
	5	Pioneer 25R47	1	Moderate Misting
W/O	1	Hyland Emmit	3	
	2	Pioneer 25R62	3	
	2	Arena	2	
	2	R045	1	Severe Misting
	5	OH04-264-58	1	Moderate Misting
	5	Roane	1	Severe Misting
	5	Tribute	1	Moderate Misting

Table 3.9. The summary of hit times and remarks for the genotypes listed in the bottom-three group of the percentile rank for white wheat in table 3.5

ARP	Rank	Genotype	Hit Times	Remark
W/	1	Jupiter	4	
	1	Lowell	4	
	3	Caledonia	3	
	4	Coral	1	Severe Misting
W/O	1	Jupiter	4	
	2	MSU E2043	3	
	3	Envoy	2	
	4	Coral	1	Severe Misting
	4	AgriPro W1062	1	Moderate Misting
	4	Aubery	1	Moderate Misting

Table 3.10. The summary of hit times and remarks for the genotypes listed in the bottom-three group of the percentile rank for red wheat in table 3.6

ARP	Rank	Genotype	Hit Times	Remark
W/	1	Hopewell	2	
	1	Pioneer 25R62	2	
	1	Red Ruby	2	
	1	Roane	2	
	1	R055	2	
	6	Tribute	1	Severe Misting
	6	MCIA Oasis	1	Moderate Misting
W/O	1	Red Ruby	3	
	2	R055	3	
	2	Hopewell	2	
	2	Tribute	2	
	5	Pioneer 25R47	1	Moderate Misting
	5	Roane	1	Moderate Misting

Table 3.11. Pedigree and awn information for white wheat

Color	Variety	Awn	Pedigree	Shared Genetic Source
White	MSU E5024	Awned	D6234 / Pioneer Brand 25W33	Pioneer Brand 25W33
	Agri Pro W1062	Awnless	Pioneer Brand 25W33/Caledonia	
	Ambassador	Awnless	Pioneer Brand 2737W/MSU Line D1148	Pioneer Brand 2737W, MSU Line D1148
	Crystal	Awned	Pioneer Brand 2737W/MSU Line D1148	
	MSU E2043	Awned	MSU Line DC076/ Pioneer Brand 2552	Pioneer Brand 2552, MSU Line DC076
	Envoy	Awned	MSU Line DC076/ Pioneer Brand 2552	
	Caledonia	Awnless	Ross Selection /3/(NY5207aB-2B-34) Burt // Genesee / CI 12658 /4/ Genesee	Genesee, Caledonia, Lowell
	Lowell	Awnless	Genesee / Winoka /3/ Suweon92 / Brevor // 5* Genesee /4/ Talbot / CI8487 /3/ Genesee *4 // Norin 10 / Brevor	
	Jupiter	Awned	Caledonia / NY88024-117	
	MSU D8006	Awned	Pioneer Brand 2555/Lowell	
	Coral	Awnless	MSU Line D3913/MSU Line D0331	
	Aubrey	Awnless	N/A (Private Company Line)	

Table 3.12. Pedigree and awn information for red wheat

Color	Variety	Awn	Pedigree	Shared Genetic Source
Red	Hyland Emmit	Awnless	Pioneer Brand 2510/Marilee	
	Arena	Awned	NASW84-345/Coker9835//OH419/OH389	
	OH04-264-58	Awned	OH645/HOPEWELL	Hopewell
	Hopewell	Awnless	Logan / Hart // 32270A / Rousalka /3/ TN1685 / IA22 // 6767 / 216-6-3	
	Roane	Awned	VA-71-54-147/Coker-68-15//IN-635309-C-1-18-2-3-2	
	Pioneer Brand 25R47	Awned	WBE2190B1/WBA416H2(Houser/MO9545//W4034D/Augusta)// Pioneer Brand 2552	Pioneer Brand 2552
	Red Ruby	Awned	Pioneer 2552/Pioneer 2737W	
	MCIA Oasis	Awnless	IL85-3132-1/Irena//OH449/VA86-54-290	
	Tribute	Awned	VA92-51-39/AL870365	
	Pioneer Brand 25R62	Awned	WBN0686C/WBJ0249B1	
	R045	Awned	N/A (Private Company Line)	
	R055	Awnless	N/A (Private Company Line)	

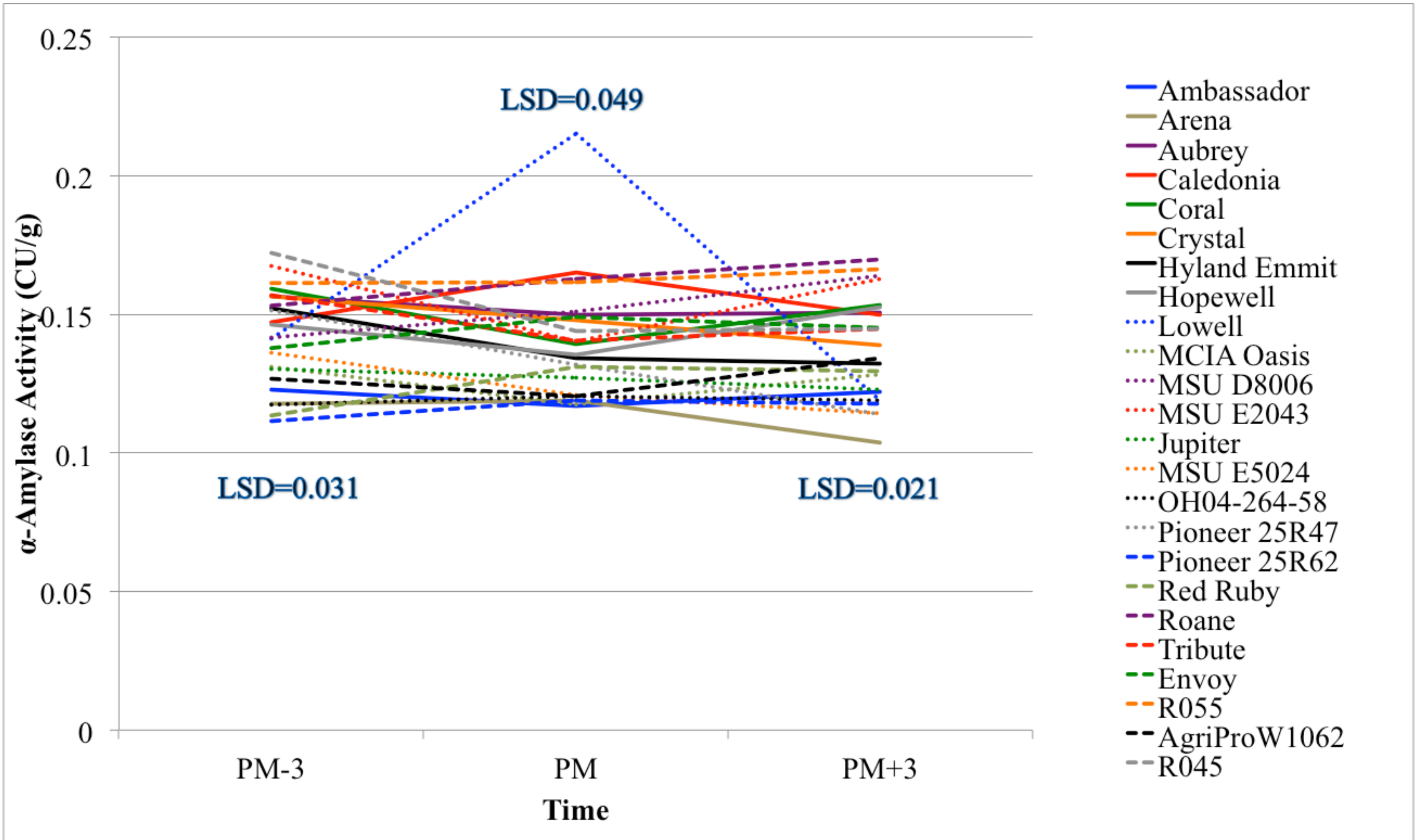

Figure 3.1. The α-amylase activity (CU/g) at three days before PM (PM-3), PM and three days after PM (PM+3) for 24 MI cultivars with artificial misting treatment immediately after harvesting in 2010.

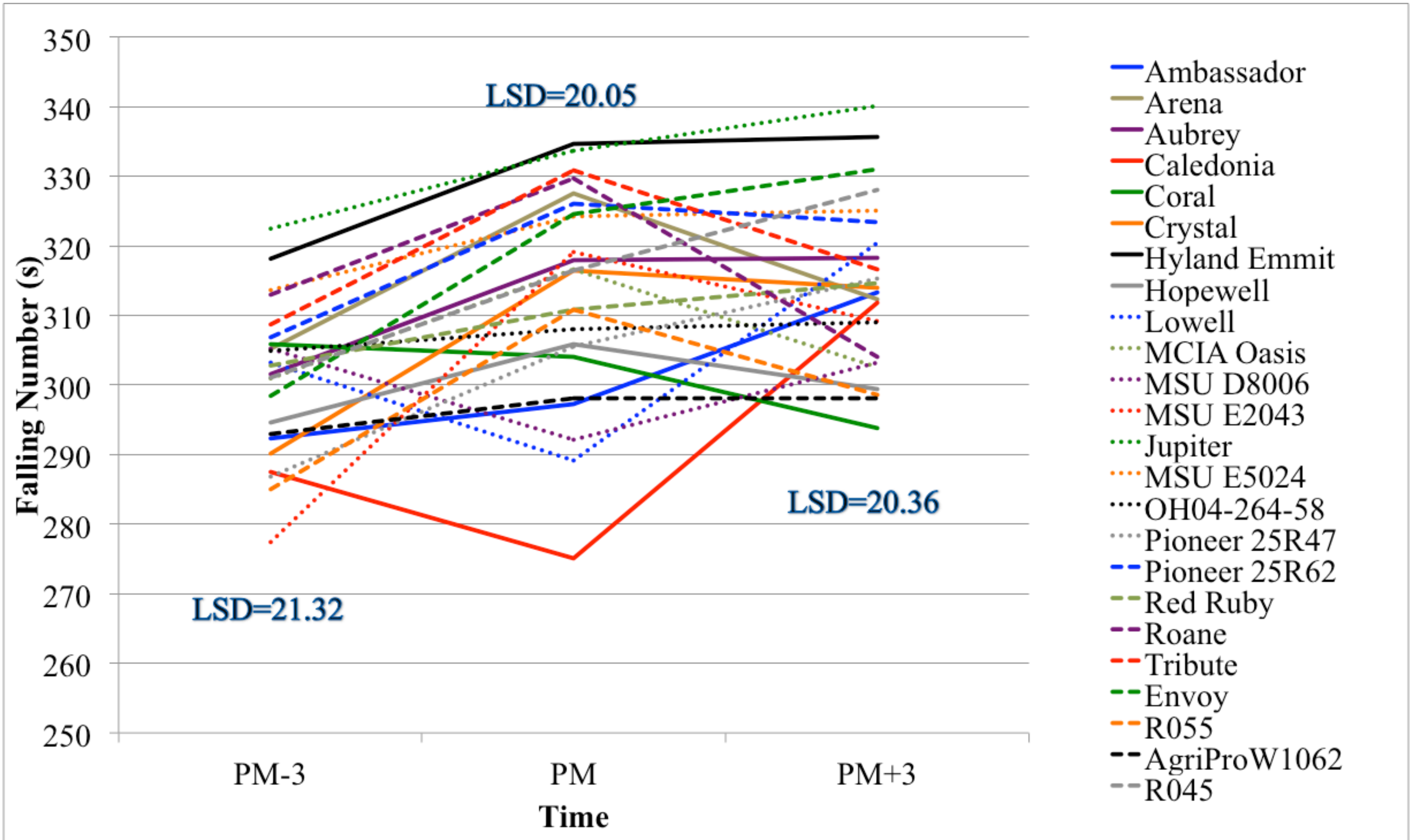

Figure 3.2. The Falling Number values (s) at three days before PM (PM-3), PM and three days after PM (PM+3) for 24 MI cultivars with artificial misting treatment immediately after harvesting in 2010.

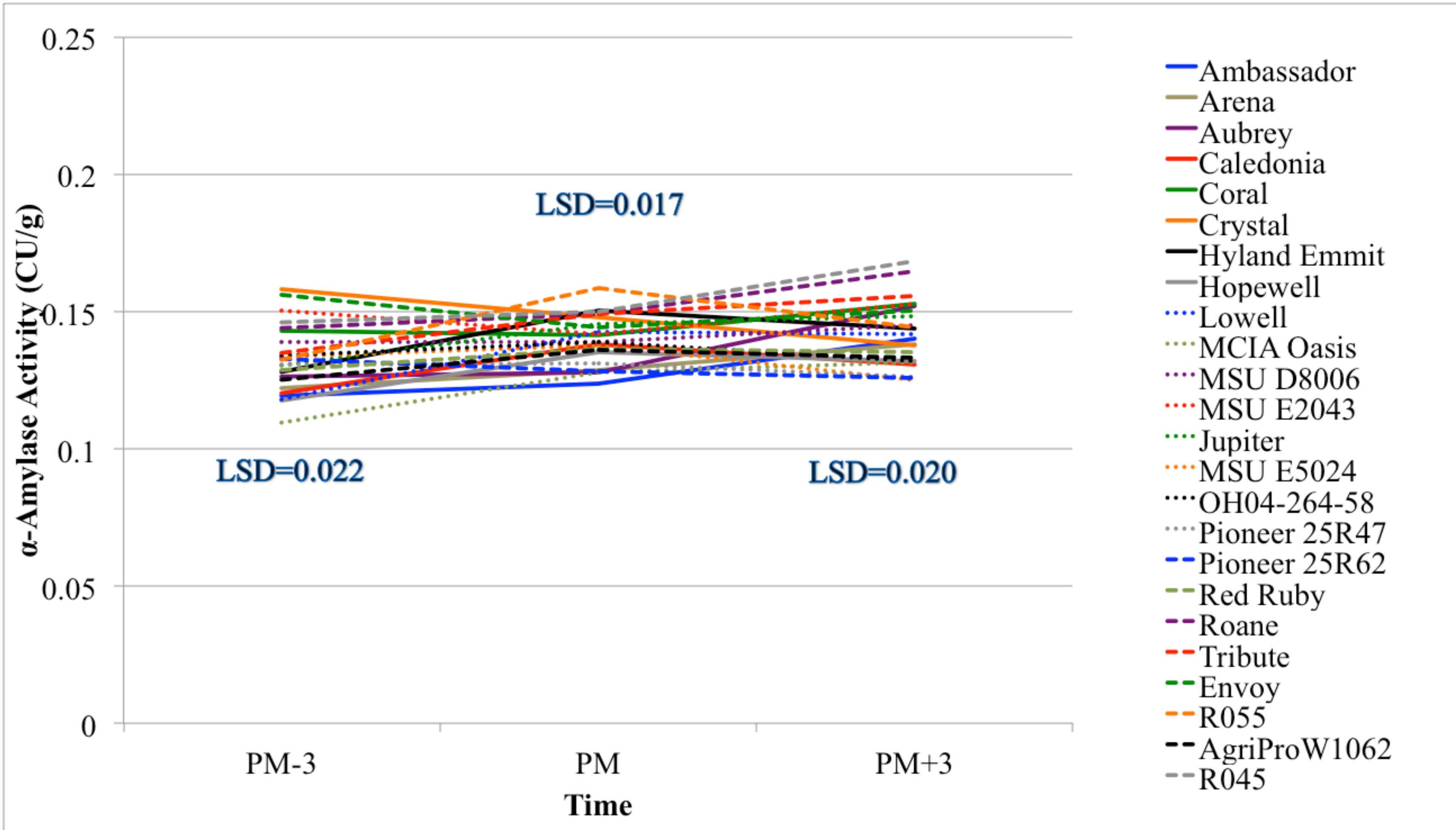

Figure 3.3. The α-amylase activity (CU/g) at three days before PM (PM-3), PM and three days after PM (PM+3) for 24 MI cultivars with non-misting treatment (ambient control) immediately after harvesting in 2010.

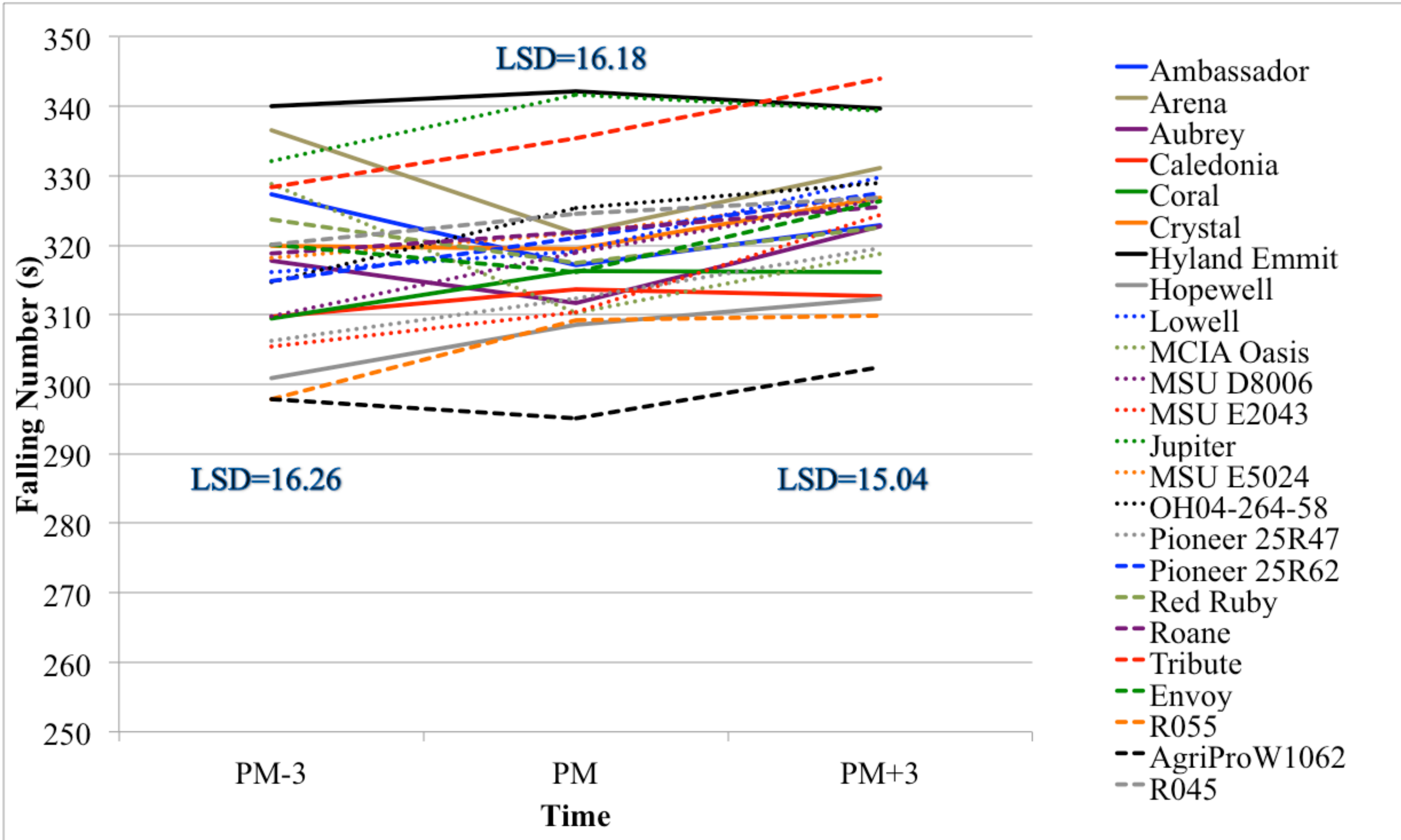

Figure 3.4. The Falling Number values (s) at three days before PM (PM-3), PM and three days after PM (PM+3) for 24 MI cultivars with non-misting treatment (ambient control) immediately after harvesting in 2010.

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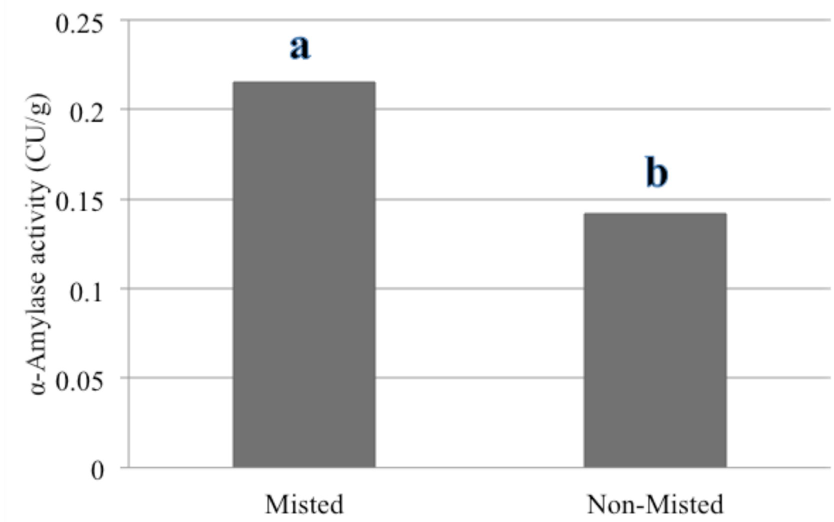

Figure 3.5. The α-amylase activity (CU/g) of genotype 'Lowell' at PM for misting and non-misting test in 2010. The letter indicated the significantly different group, at $p<0.05$ criteria.

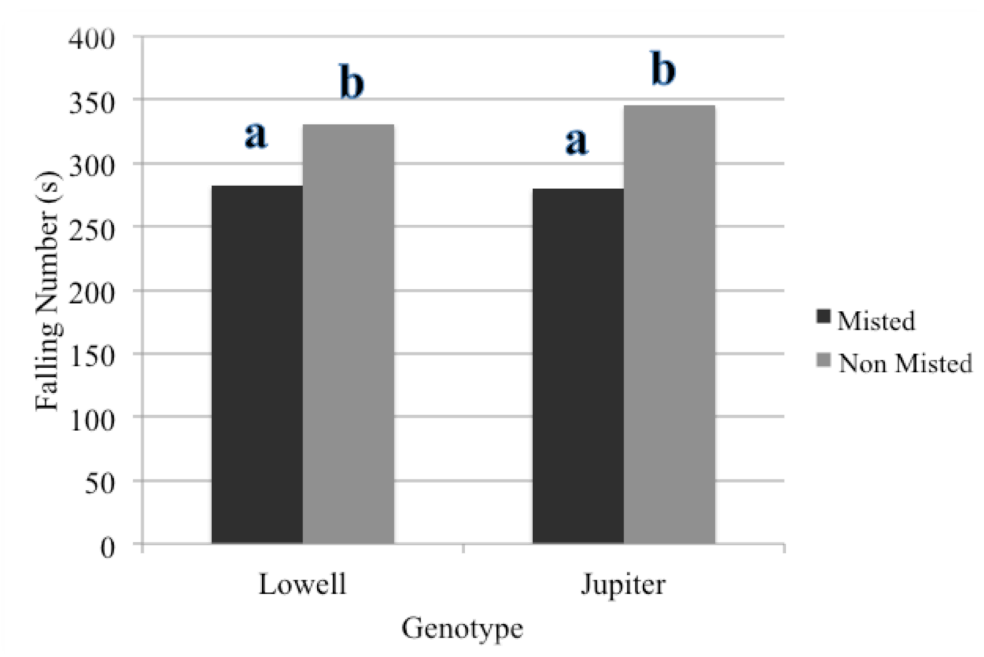

Figure 3.6. The Falling Number values (s) of genotype 'Lowell' and 'Jupiter' at PM for misting and non-misting test in 2010 and 2011. The letter indicated the significantly different group, at $p<0.05$ criteria.

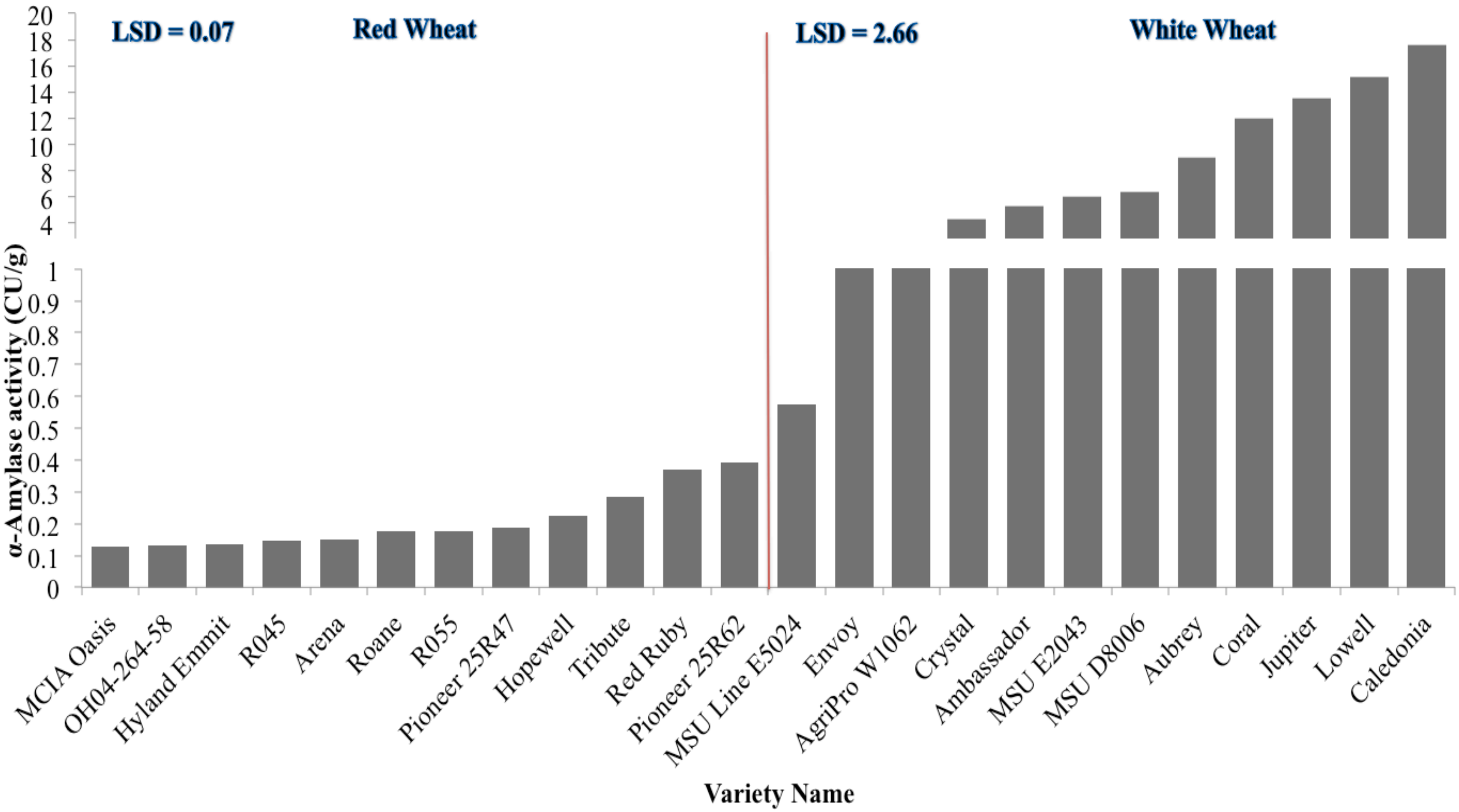

Figure 3.7. The α-amylase activity (CU/g) for 24 MI cultivars with severe misting treatment (20 s per 2 min for 72 h), after five days ARP storage in 2011. Left side showed red cultivars and right side showed white cultivars. In each part, the cultivars are ordered and LSDs are provided ($p<0.05$).

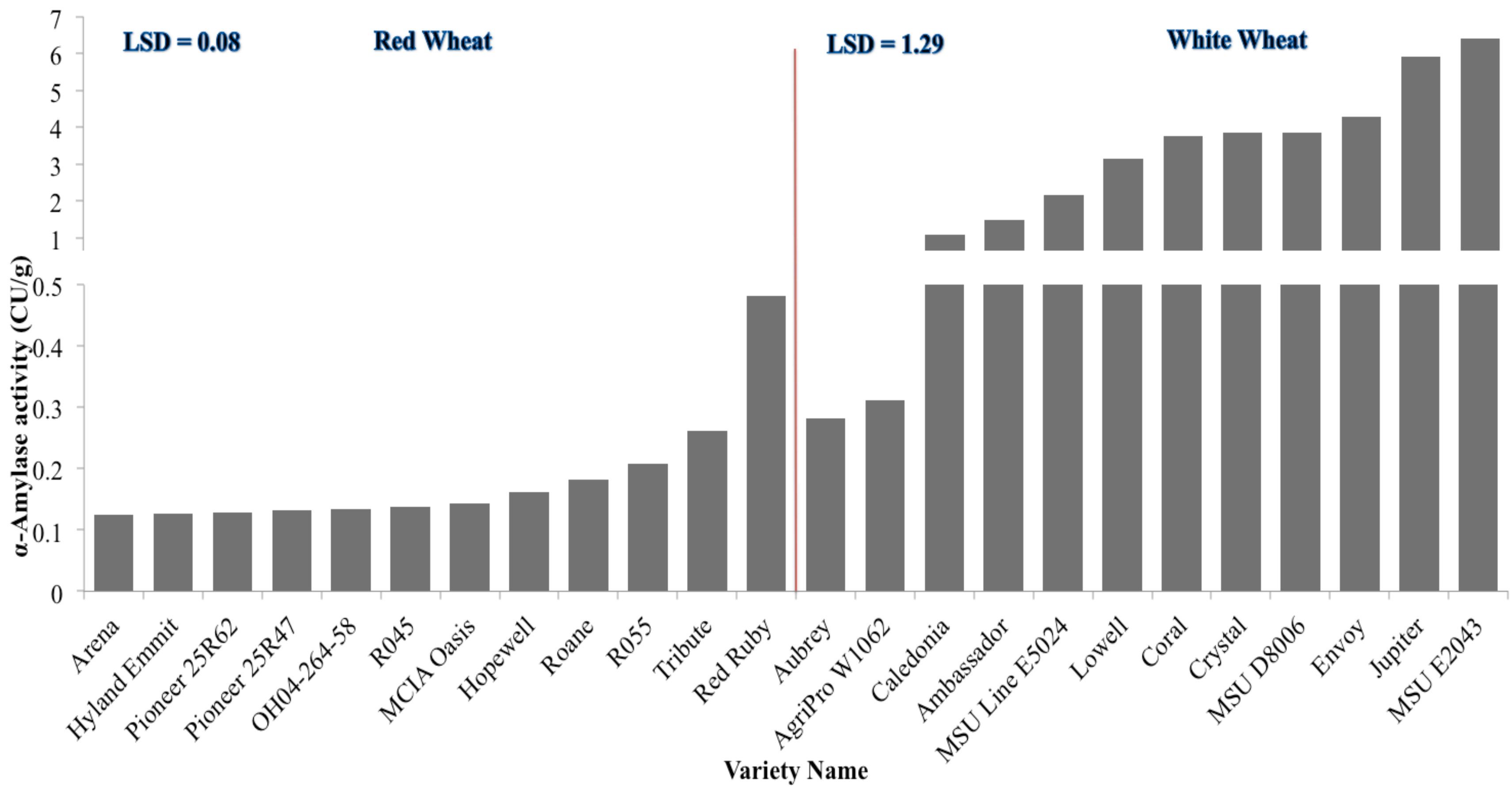

Figure 3.8. The α-amylase activity (CU/g) for 24 MI cultivars with severe misting treatment (20 s per 2 min for 72 h), immediately after harvesting in 2011. Left side showed red cultivars and right side showed white cultivars. In each part, the cultivars are ordered and LSDs are provided ($p<0.05$).

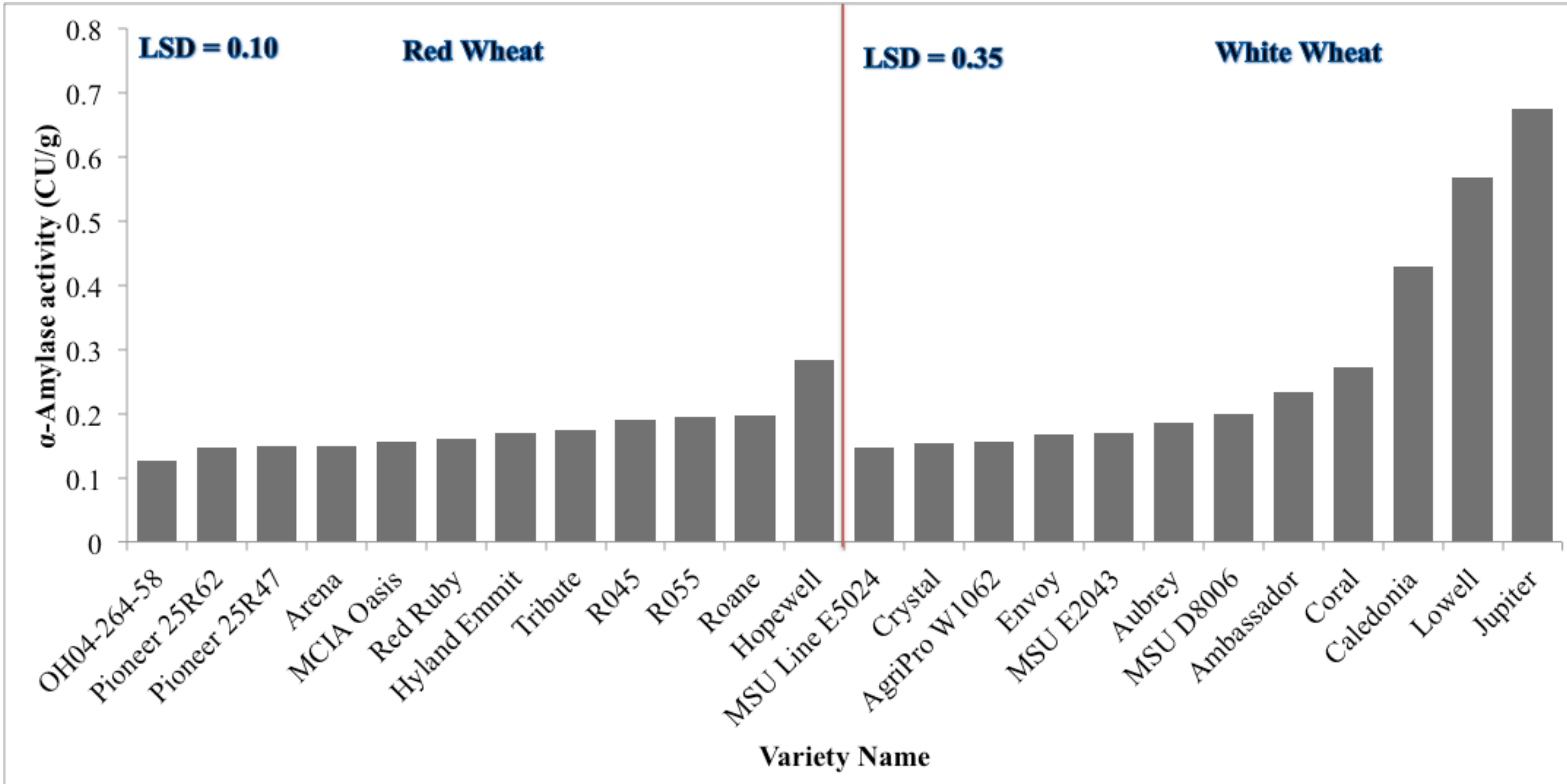

Figure 3.9. The α-amylase activity (CU/g) for 24 MI cultivars with moderate misting treatment (45 min per 6 h for 48 h), after five days ARP storage in 2011. Left side showed red cultivars and right side showed white cultivars. In each part, the cultivars are ordered and LSDs are provided ($p<0.05$).

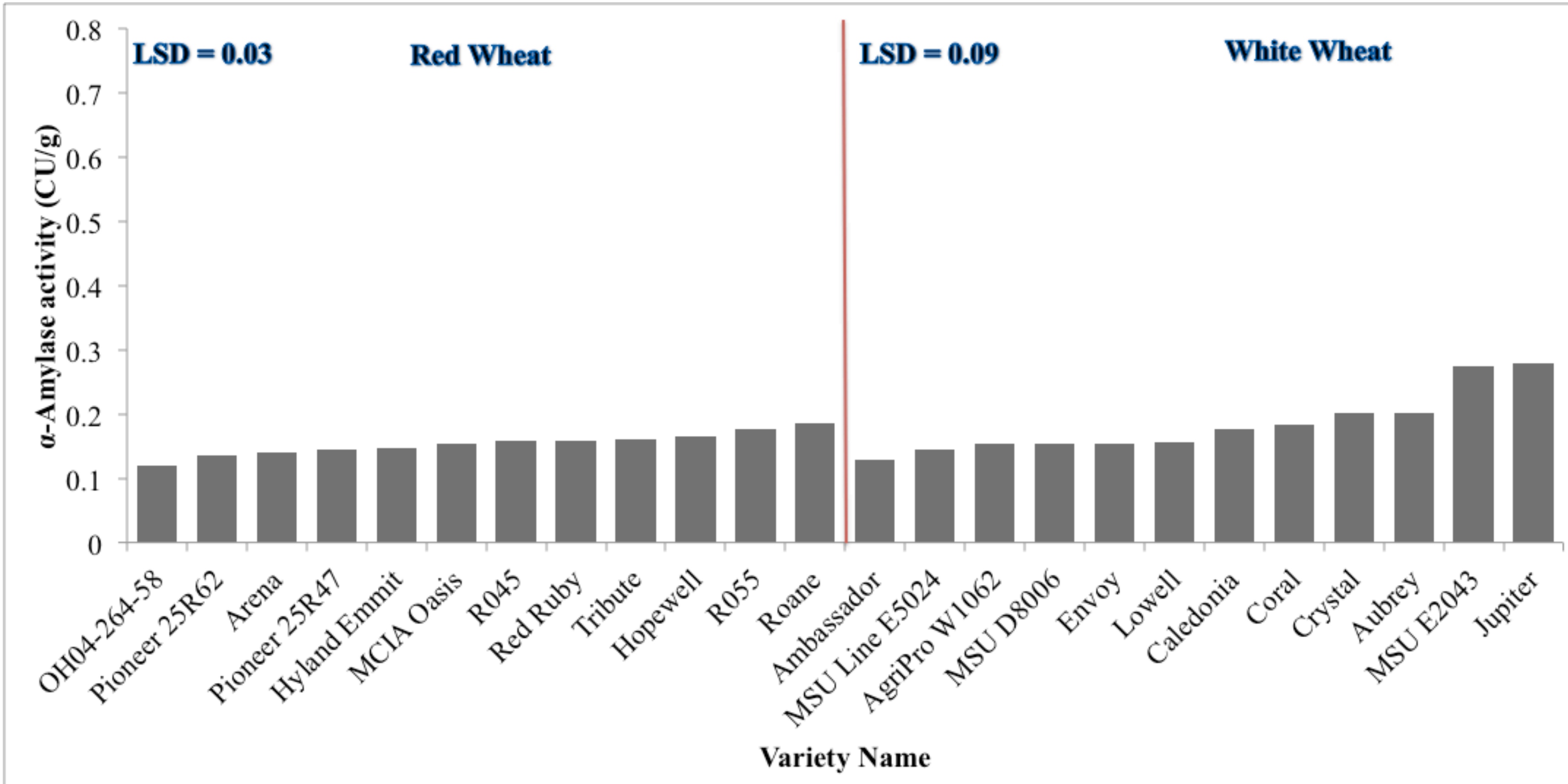

Figure 3.10. The α-amylase activity (CU/g) for 24 MI cultivars with moderate misting treatment (45 min per 6 h for 48 h), immediately after harvesting in 2011. Left side showed red cultivars and right side showed white cultivars. In each part, the cultivars are ordered and LSDs are provided ($p<0.05$).

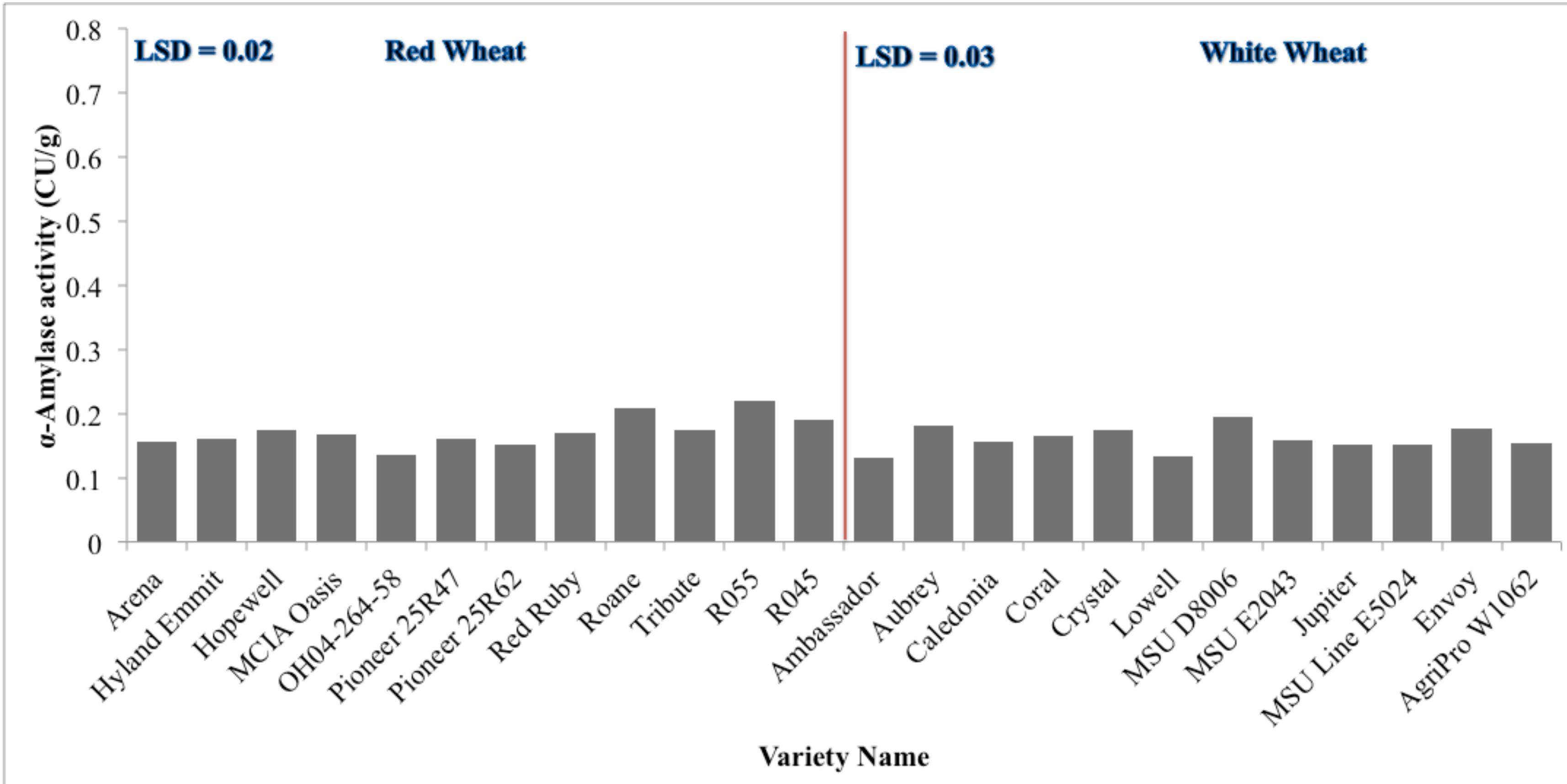

Figure 3.11. The α-amylase activity (CU/g) for 24 MI cultivars with non-misting treatment (ambient control), after five days ARP storage in 2011. Left side showed red cultivars and right side showed white cultivars. In each part, the cultivars are ordered and LSDs are provided ($p<0.05$).

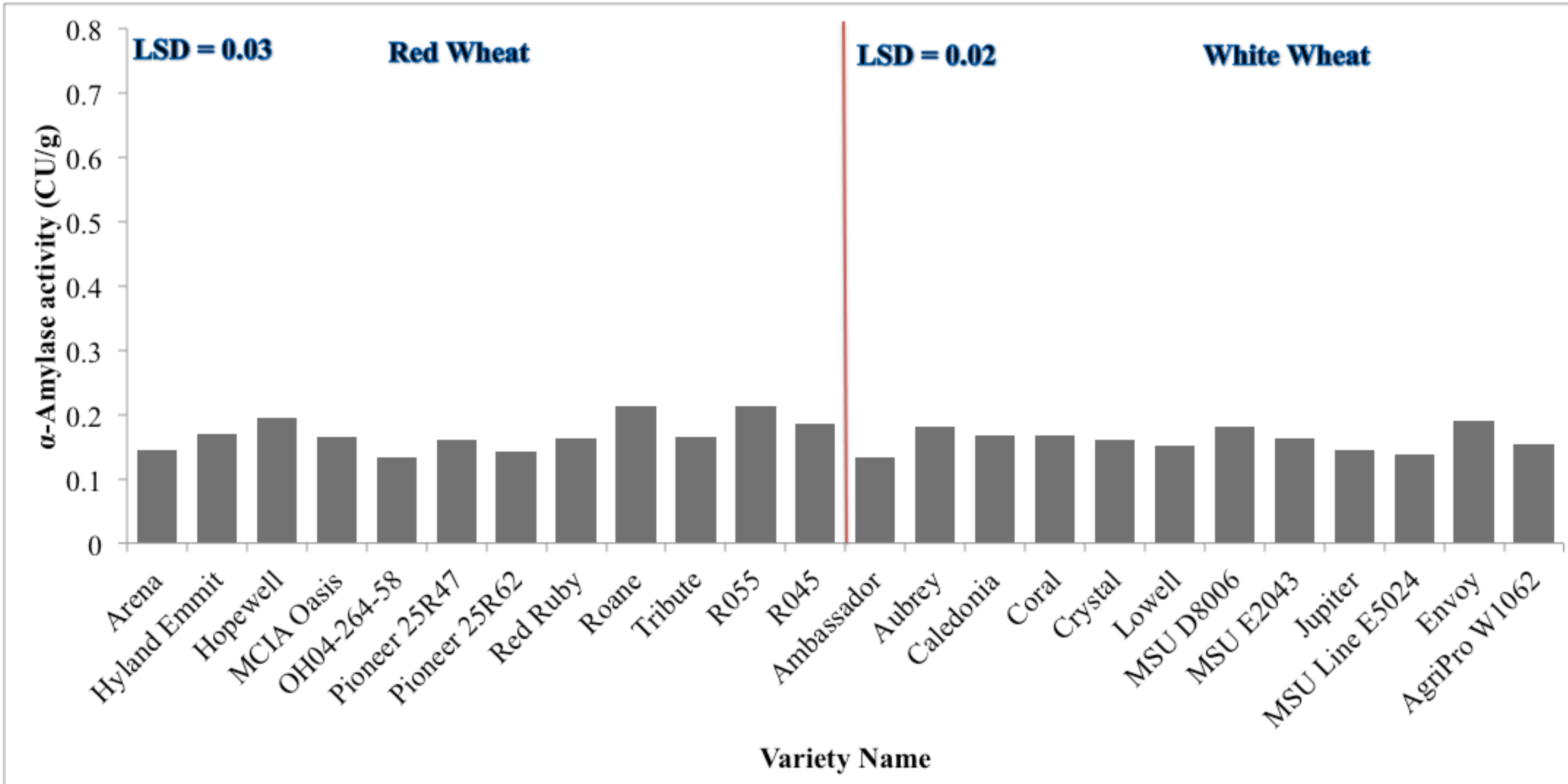

Figure 3.12. The α-amylase activity (CU/g) for 24 MI cultivars with non-misting treatment (ambient control), immediately after harvesting in 2011. Left side showed red cultivars and right side showed white cultivars. In each part, the cultivars are ordered and LSDs are provided ($p<0.05$).

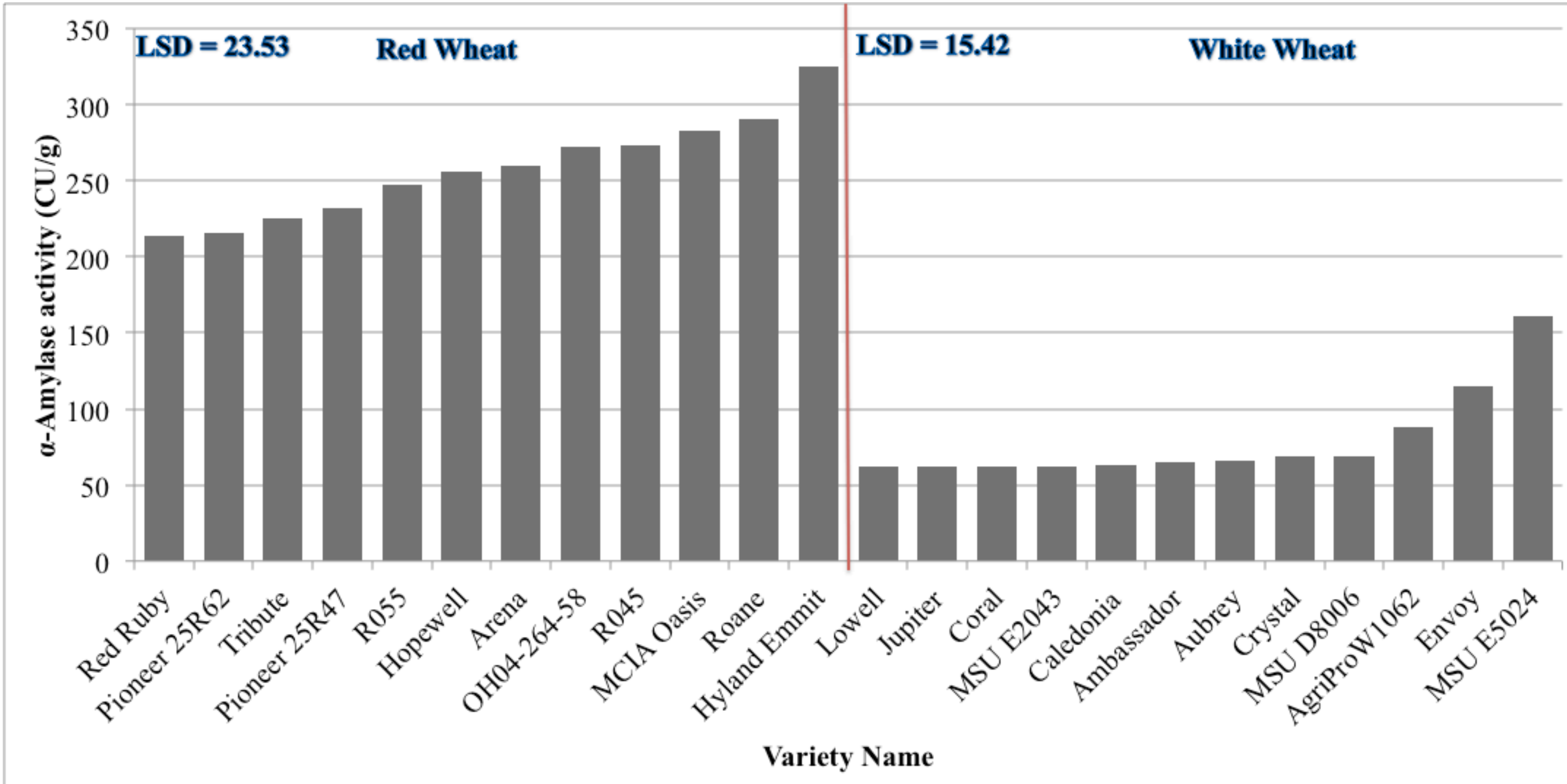

Figure 3.13. The Falling Number (FN) values (s) for 24 MI cultivars with severe misting treatment (20 s per 2 min for 72 h), after five days ARP storage in 2011. Left side showed red cultivars and right side showed white cultivars. In each part, the cultivars are ordered and LSDs are provided ($p<0.05$). The horizontal line at 250 indicated the commercial acceptance FN for soft wheat.

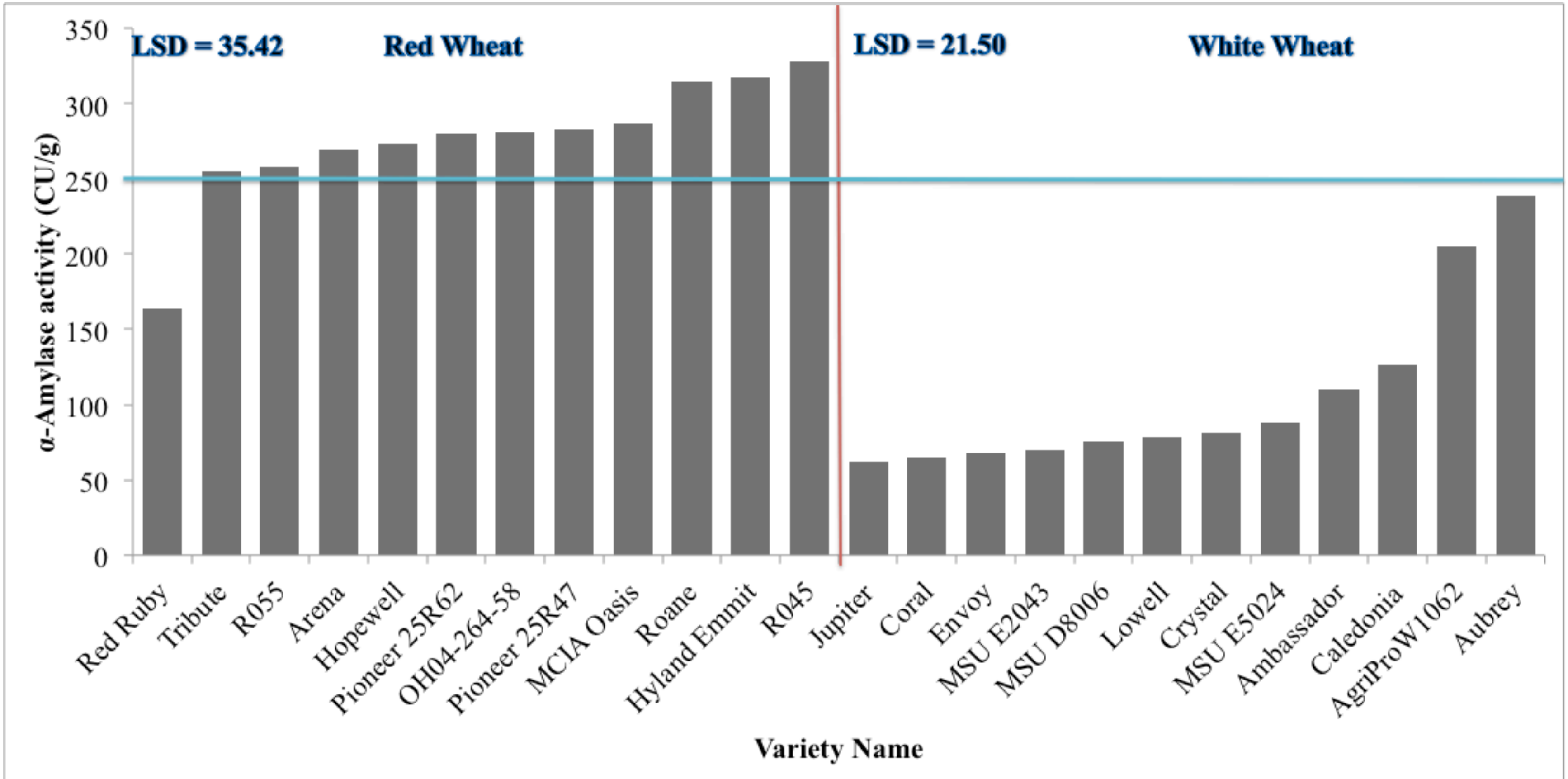

Figure 3.14. The Falling Number (FN) values (s) for 24 MI cultivars with severe misting treatment (20 s per 2 min for 72 h), immediately after harvesting in 2011. Left side showed red cultivars and right side showed white cultivars. In each part, the cultivars are ordered and LSDs are provided ($p<0.05$). The horizontal line at 250 indicated the commercial acceptance FN for soft wheat.

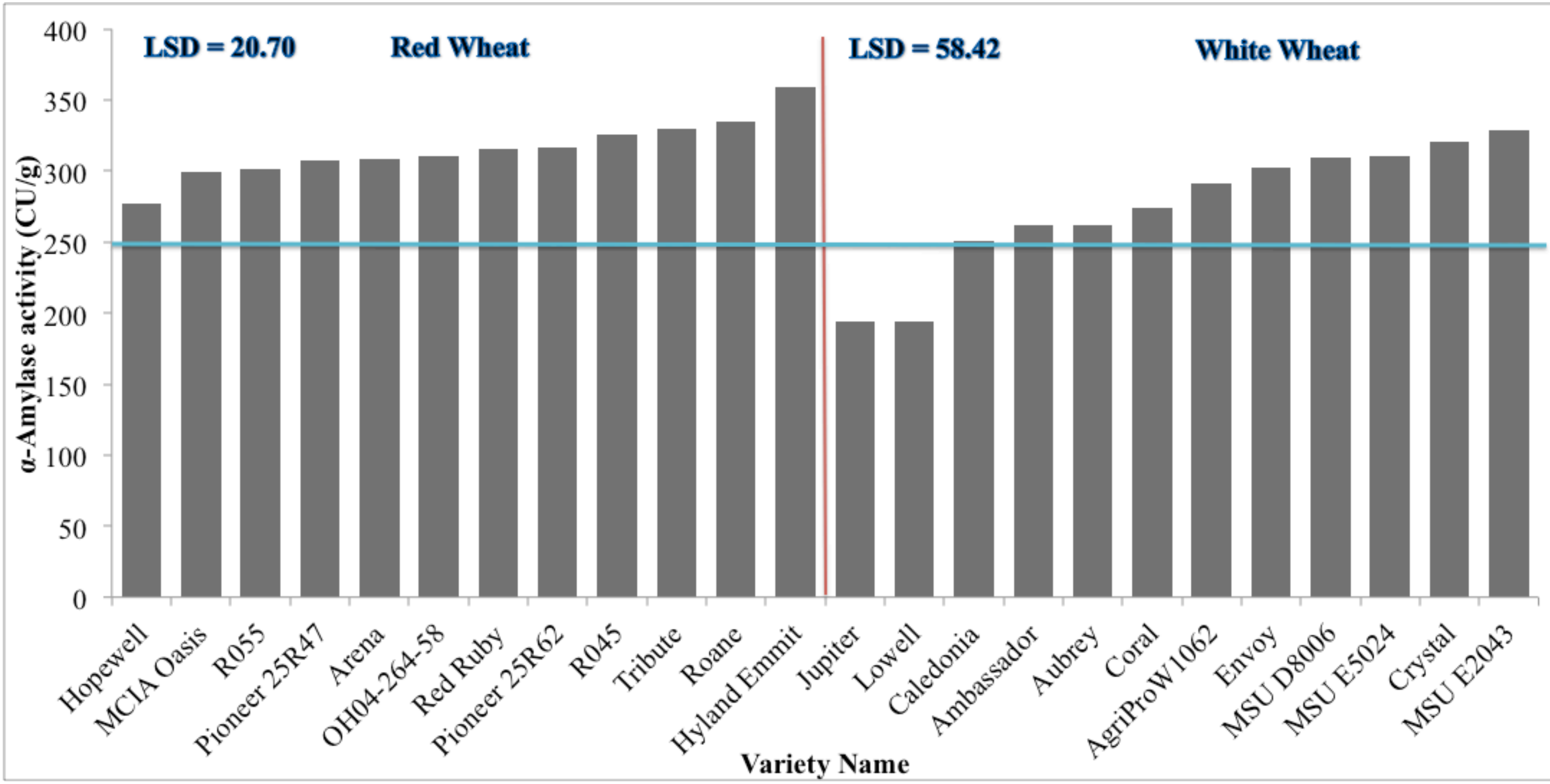

Figure 3.15. The Falling Number (FN) values (s) for 24 MI cultivars with moderate misting treatment (45 min per 6 h for 48 h), after five days ARP storage in 2011. Left side showed red cultivars and right side showed white cultivars. In each part, the cultivars are ordered and LSDs are provided ($p<0.05$). The horizontal line at 250 indicated the commercial acceptance FN for soft wheat.

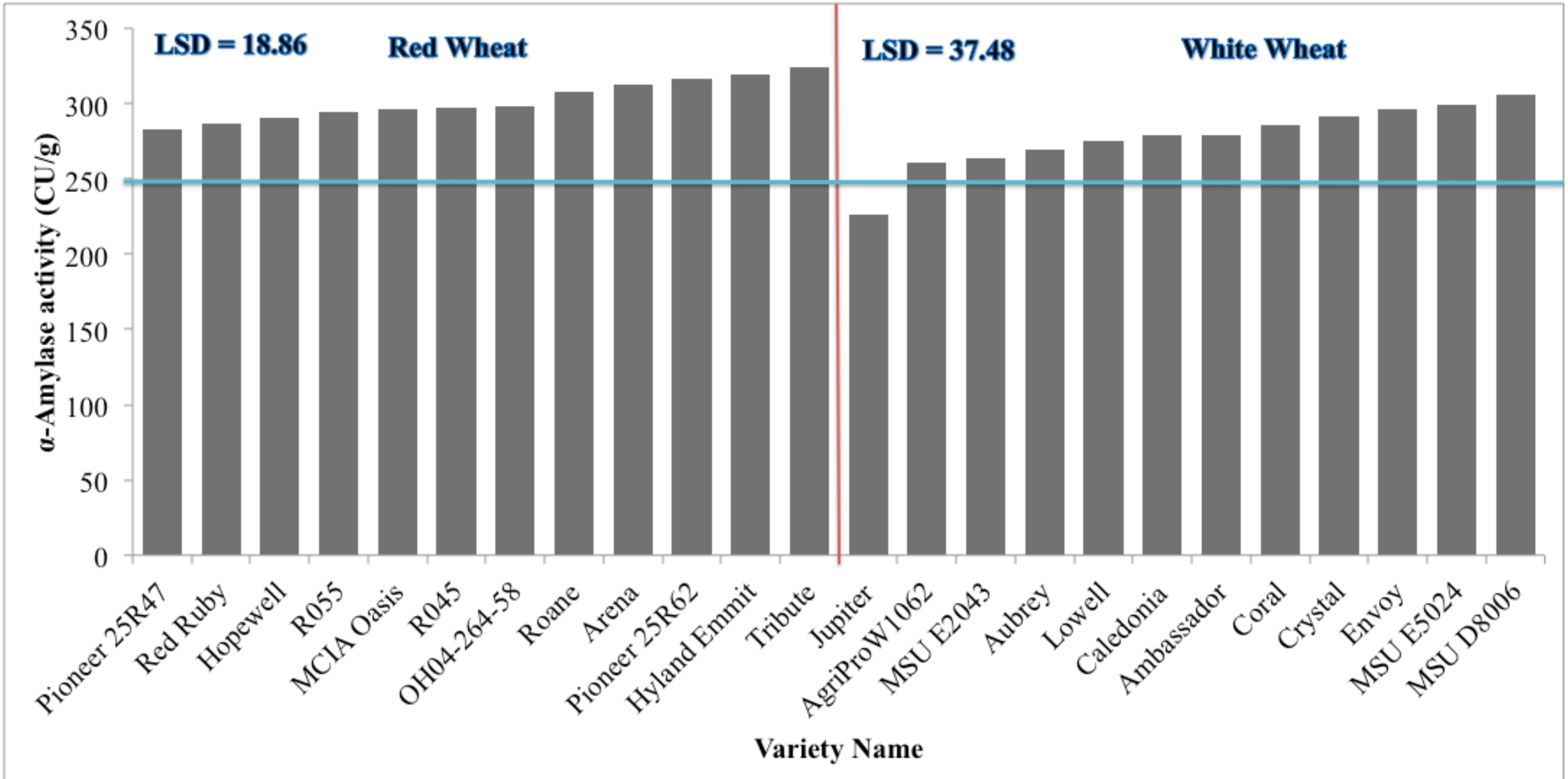

Figure 3.16. The Falling Number (FN) values (s) for 24 MI cultivars with moderate misting treatment (45 min per 6 h for 48 h), immediately after harvesting in 2011. Left side showed red cultivars and right side showed white cultivars. In each part, the cultivars are ordered and LSDs are provided (p<0.05). The horizontal line at 250 indicated the commercial acceptance FN for soft wheat.

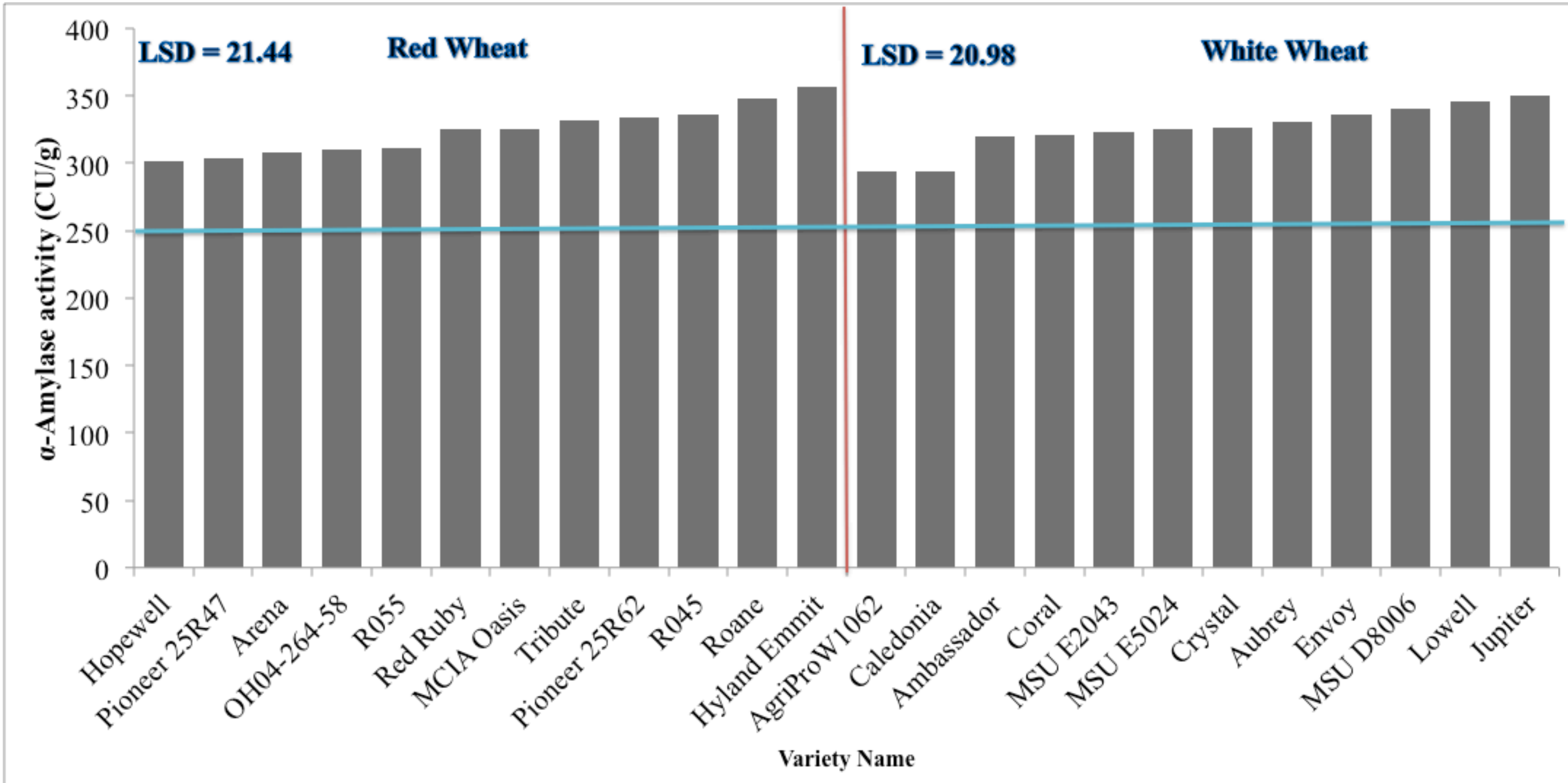

Figure 3.17. The Falling Number (FN) values (s) for 24 MI cultivars with non-misting treatment (ambient control), after five days ARP storage in 2011. Left side showed red cultivars and right side showed white cultivars. In each part, the cultivars are ordered and LSDs are provided ($p<0.05$). The horizontal line at 250 indicated the commercial acceptance FN for soft wheat.

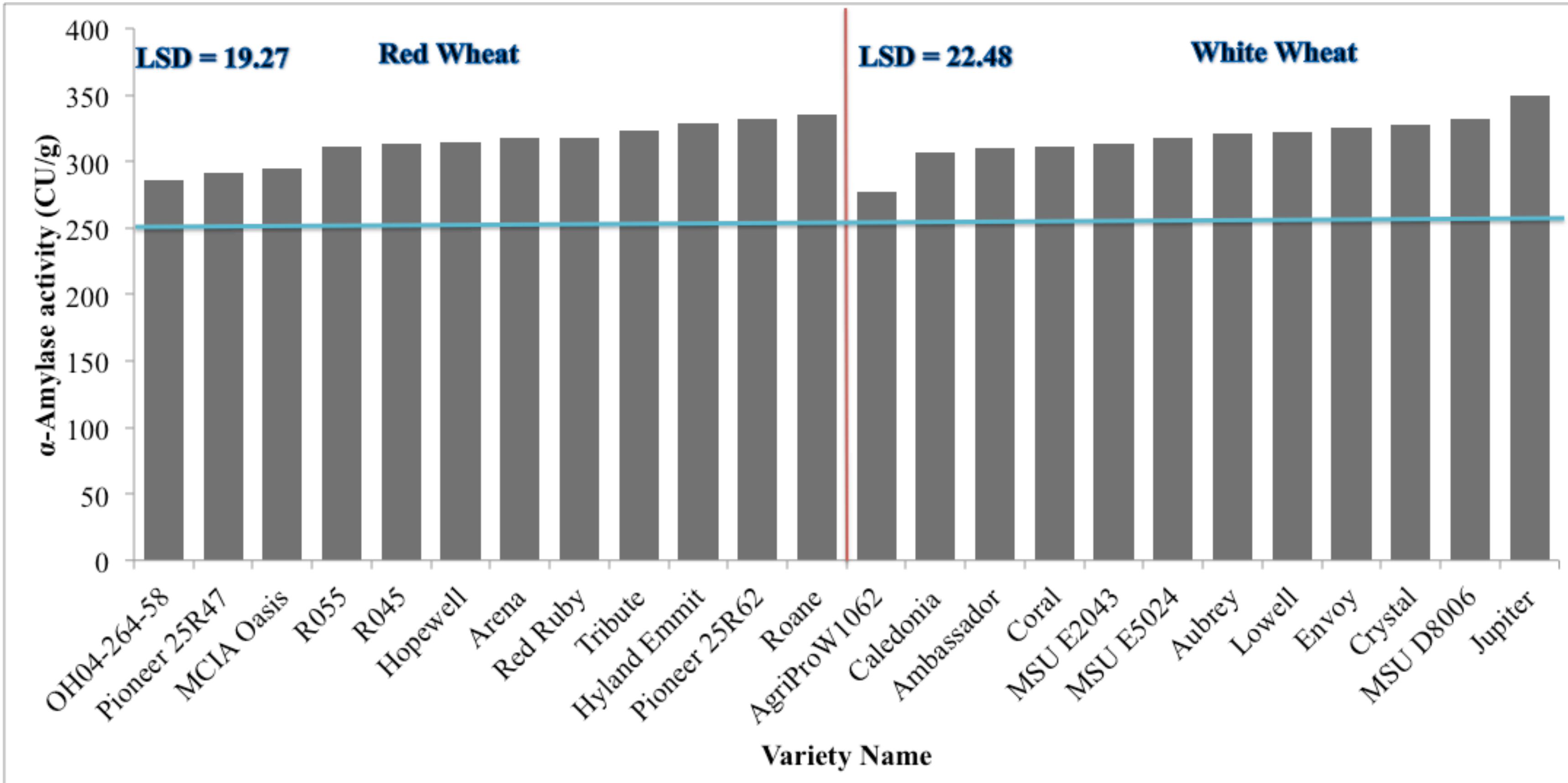

Figure 3.18. The Falling Number (FN) values (s) for 24 MI cultivars with non-misting treatment (ambient control), immediately after harvesting in 2011. Left side showed red cultivars and right side showed white cultivars. In each part, the cultivars are ordered and LSDs are provided ($p<0.05$). The horizontal line at 250 indicated the commercial acceptance FN for soft wheat.

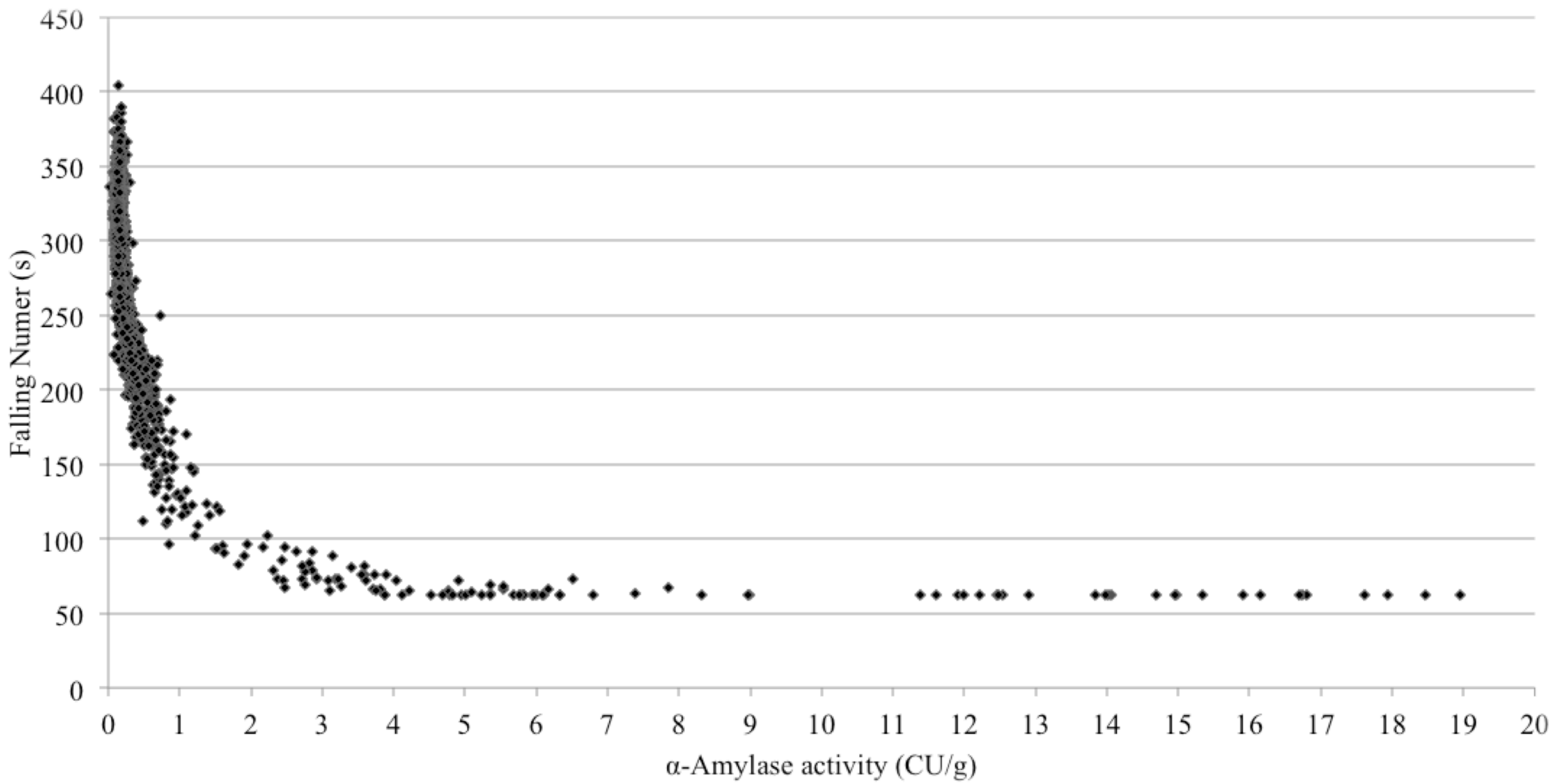

Figure 3.19. Scatter plot of α-amylase activity (CU/g) and Falling Number values (s) for all the data points collected in 2009-2011 studies.

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