PRE-HARVEST SPROUTING TOLERANCE IN HARD WHITE WINTER WHEAT

by

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ABSTRACT

In many countries producers have been growing varieties of hard white winter (HWW) wheat since decades. The cause of concern is most varieties of HWW wheat are susceptible to pre-harvest sprouting (PHS) which affects grain quality. Environmental conditions like high humidity, precipitation, heavy dew and hormonal activity at physiological maturity stimulate PHS in HWW. To alleviate these conditions research was carried out at KSU.

KS01HW163-4, a sprouting tolerance line was crossed with Heyne, a sprout susceptible cultivar. A total of 224 doubled haploid (DH) lines thus produced were phenotyped in the present study through experiments conducted in controlled environments. The objectives of this research were to (i) characterize and phenotype the doubled haploid lines for PHS in controlled environments; (ii) understand the impact of growth environment (high temperature and/or drought) and; (iii) impact of exogenous application of growth hormones on tolerance to pre-harvest sprouting in the parental lines of the doubled haploid population. The phenotypic data collected from this research will be ultimately combined with the genotypic data to identify DNA markers related to PHS tolerance and provide DNA markers for marker assisted selection.

Based on my results of the germination percentages from the 224 DH lines, the population was distributed as susceptible, and tolerant to PHS showing a bimodal distribution and χ^2 analysis indicating a complimentary gene action. From the study of the influence of environmental factors on PHS, my results confirmed a definite influence of stress on sprouting. Under optimum temperature (OT), KS01HS163-4 was tolerant to PHS, but at HT and/or drought it became susceptible to PHS. Growth under stressed

conditions changed the tolerance levels to PHS. Seed dry-weight, and harvest index were also influenced negatively due to stress. Therefore multi-location tests must be conducted with variable environments to test the stability of a variety to PHS. From the study of the influence of phytohormones on PHS, the results suggest that tolerance to sprouting was seen in seeds from plants sprayed with abscisic acid (ABA) and paclobutrazol (GA-inhibitor) treatments where as those from gibberellic acid (GA) treatment showed susceptibility to sprouting.

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CHAPTER 1 - Pre-harvest Sprouting in Hard White Winter Wheat

Review of Literature

One fifth of the total wheat produced in the United States (US) is from Kansas, making it the largest producer of wheat in the country. An important wheat variety introduced in 1874 that quickly became predominant for its "excellent adaptation" to the climate and soils of Kansas was 'Turkey' hard red wheat (Paulsen, 1998). The genetic heritage of 'Turkey' is still retained by the modern varieties of hard red winter wheat, which are more productive and have increased resistance to biotic and abiotic stresses compared to 'Turkey.' The greatest percentage of wheat grown in Kansas is hard red winter wheat. However, in the 1990's, a change "from red grain to white grain" was predicted for the state for the coming decades, and we see that change happening now (Paulsen, 1998). Hard white winter (HWW) wheat differs from red winter wheat only in the color of the seed coat or bran, but it is similar to the later in its other agronomic aspects and economic utility. In fact, HWW is preferred over red winter wheat (Paulsen, 1998).

Although Kansas almost always tops in the US in producing and exporting red grain, the demand for hard white wheat from millers, bakers, and consumers necessitates increased production of hard white wheat from Kansas farms. It is believed that most red wheat in Kansas will soon be replaced by white wheat due to its advantages over red wheat for better milling and baking characteristics (Kansas Farmer, 2006). Hard white wheat has a great potential in Kansas, because of its high end-use qualities. Its production will make the Kansas farmer more competitive in the

world market. Also, the production practices are similar to those of hard red wheat, which makes the transition inexpensive. Research is being carried out to improve white grain, because the importance of producing hard white wheat has been recognized by the Kansas Agricultural Experiment Station as well as by Department of Agronomy at Kansas State University (Paulsen, 1998).

For many years, research has been carried out in Kansas as well as in other states with the goal of developing improved varieties of HWW wheat. Kansas State University has one of the best breeding programs for hard white wheat in the US. The yields of hard white wheat varieties are similar to those of hard red wheat grown in the state, which shows its adaptability to Kansas. 'Trego,' released in year 2000, and the recently released 'Danby' (in 2006) are two hard white wheat varieties developed by Dr. Joe Martin, a Kansas State University wheat breeder. However, yields were affected in the past due to the agronomic problem of pre-harvest sprouting, which was especially prevalent in 2004 (Kansas Farmer, 2006). It persists as the major problem affecting hard white wheat.

The condition where grain germinates on the spike before it is harvested is called pre-harvest sprouting (PHS) (Paulsen, 1998). Environmental conditions, like high humidity, precipitation, heavy dew, as well as hormonal activity at physiological maturity are the major factors that stimulate PHS in hard white wheat. These conditions occur more in eastern and central Kansas than in western Kansas. The cause of concern is the fact that most of the available varieties of hard white wheat are susceptible to sprouting, and this affects the grain quality. Consequently many sectors of the wheat industry are affected, such as operators of grain elevators, millers, and farmers.

Sprouted grains lose their starch content, which makes the flour unacceptable for baking purposes (Roozeboom, 1999).

The problem of PHS in HWW wheat is complex. It exists because of poor dormancy observed in the white grain. Failure of the seeds to germinate due to non-optimal conditions, such as too low or too high temperatures, lack of oxygen, or inadequate or excessive amounts of water, is called dormany. Non-dormant seeds germinate easily under such conditions (Gosling et al., 1983; King, 1993). When the seed coat or bran surrounding the embryo ruptures breaking the dormancy of the seed in the kernel, sprouting is initiated (Wu et al., 1999). The physiology of pre-harvest sprouting is discussed below.

1.1 The Developing Wheat Grain

1.1.1 Grain Structure

A mature, intact cereal grain of wheat, which shows no sign of germination, consists of the following structure: a major portion of nonliving starchy endosperm filled with protein and carbohydrate as reserves, and the living tissues of embryo, aleurone, and scutellum that play a role in the mobilization of these reserves (Ashford and Gubler, 1984; Percival, 1921). The caryopsis, which is what the small, dry, one seeded fruit of wheat is called, is enveloped by two maternal tissues or integuments, namely the outer pericarp, and the inner testa that are non-living at maturity. Prior to maturity, 50% of the grain protein is in the integuments. However, at maturity 90% of the protein is in the endosperm (Rijven and Cohen, 1961). The pericarp is colorless, but the testa contains the phenolic compounds that determine color of the seed coat (Koornneef et al., 2000). The cuticular layer present below the testa consists of lipid-like compounds that control

the mobility of solutes in and out of the caryopsis. Activity of enzymes, like α-amylase, is seen in the pericarp until ten days after anthesis. At maturity, in the absence of germination, little enzyme activity is detected.

1.1.2 Grain Development

The concepts of harvest maturity, and physiological maturity and/or ripeness are not clearly defined. However, the maturity of the HWW wheat caryopsis is recognized by the drying of the peduncle, as it turns green to yellow just below the head. The instance of grain maturity and the extent of its drying are markedly significant in relation to the event of pre-harvest sprouting. A half-mature embryo of wheat grain that is approximately 25-days old may show tendencies of germination when exposed to favorable conditions. However, the maturity of the aleurone layer happens at 65-70% of grain maturity, and this layer's ability to synthesize α-amylase is suppressed until total grain development and drying (King, 1976, 1983). This shift from an inactive aleurone to an active aleurone is clearly apparent under conditions of pre-harvest sprouting, when the grain germinates on the spike.

Gale et al. (1983) studied many varieties of triticale. In situations of slow grain drying and favorable conditions for germination (such as occurrence of rainfall, optimal humidity, and temperature), the grain germinates on the spike, initiating the enzymatic activity that accompanies degradation of protein and starch in the endosperm. The rate of grain desiccation not only determines propensity for grain germination on the spike but also, in cases of escape from PHS, the future ability of the grain to germinate, the extent of enzyme activity thereafter, and the responses to the levels of growth regulators, and inhibitors such as gibberellins, and abscisic acid.

From the studies of Karssen (2002), it is evident that immature seeds are rich in abscisic acid (ABA) and that dormancy is heavily reduced in seeds from "ABA-insensitive mutants". Abscisic acid plays an important role in dormancy induction during grain development. Nevertheless, the event of PHS results from factors such as the temperature, humidity, and rainfall in addition to the rate of grain desiccation.

1.2 Event of Sprouting

1.2.1 Rainfall and Ear Wetting

Rainfall results in wetting of the spike and, consequently, the grain within. The incidence of wetting during an event of rainfall is complicated, when considered either naturally or during its simulation. The amount of wetting is, to a certain extent, controlled by grain morphology. Studies of King and Richard (1984) on ear characteristics and water uptake suggested that, when natural rainfall is considered, limitation of wetting is related to the position of the spike and its morphology. The amount of wetting and water uptake is determined by ear characters such as presence or absence of awns, husk structure, seed coat thickness, ear waxiness, hairiness, and the "ear-nodding angle," as described in studies on barley (*Hordeum vulgare*) by Brinkman and Luk (1979). King and Richard (1984) showed that awned grain imbibed more water than awnless grain, which resulted in a sprouting delay of 12 hours in awnless grain. Furthermore, the difference in the sprouting time could be attributed to the husk structure. Depending on its structure, there was evidence of that grain wetting was delayed as long as 8 hours after a rain simulation.

However, the situation of a rainfall simulation includes, in addition to the constraints laid by the ear morphology, the lack of kinetic energy, detachment power,

and terminal velocity of natural rain and the automatic decreasing and increasing randomness in rain drop sizes, which occurs with the intensity variations associated with natural rain (Kinnell, 1973; Hardy and Dingle, 1960). In the current study (to be reported in this thesis), rainfall simulation was not used but germination percentages were calculated by germinating the seed *in vitro* and by normalizing the data for quantification of dormancy, and sprouting rate.

1.2.2 Water Uptake and Germination in the Wheat Grain

Owing to the low water potential in cereal grains and the presence of a nonliving tissue inside the kernel, the initial water intake by grains is through physical imbibition and the process is rather quick (Shaykewich, 1973). This early water uptake is similar in living caryopses as well, confirming that primary imbibition is a physical process (Clarke, 1980; Owen, 1952a). The early increase in the volume of the grain after imbibition is low in wheat (40%) as compared to other seeds, and it is later increased after the embryo starts absorbing the water (Leopold, 1983; Wellington, 1956). The potential physical water uptake and the consequent rates of germination are influenced by factors such as availability of water, temperature (5% increase in volume per every 10°C), and humidity (Murphy and Noland, 1982). Free availability of water facilitates obtaining the critical water content in 3 hours. For wheat, 50% germination is possible at water potential greater than -0.8 to -1.0 MPa (Lush et al., 1981; Kaufmann and Ross, 1970; Owen, 1952b). When water uptake, and germination are estimated in terms of humidity, maximum germination results at relative humidities above 97% and at 20°C (Owen, 1952b).

From a study of 49 cultivars of wheat, King (1984) observed that the cultivars differed in amount of water imbibed after the first two hours, and in the overall germination rates after 30 hours, and the two were correlated. However, the factors controlling this correlation were not known. Although dormancy is a multigenic trait, it is controlled partially by a set of three *R* genes related to the red testa pigmentation, indicating that the seed coat plays an important role in grain dormancy and germination (Bassoi and Flitham, 2005). The traits of red coat color and enhanced grain dormancy are inherited together on the alleles of an *R* gene with evidence of linkage between the two traits. From the studies of Wellington (1956), the relationship between seed coat color and water uptake was suggested. The embryos of red grain exhibited resistance to further expansion and uptake of water after 24 hours. Further germination in these experiments was brought about by tearing open the pericarp.

The role of embryo resistance or seed coat resistance to water uptake is not clearly demarcated and understood. In addition to the above studies, there are divergent arguments and conclusions drawn from studies conducted on grain hardiness to pre-harvest sprouting and the influence of seed coat on water imbibition by King (1984), Butcher and Stenver (1973), and Moss (1973). The latter three authors propose a possible relationship, but the studies of King (1984) show no such relation. Further studies showed differences in water uptake and germination due to variation in size of the grain; smaller grains absorb water faster (Marshall et al., 1984; King, 1984). Another factor that constrains water imbibition is the movement of water in the grain through the endosperm. Lateral movement is forced, due to the seed coat restricting the transverse

flow of water (Hinton, 1955). However the factors that affect grain water imbibition, discussed above, need further experimentation to answer the existing controversies.

Further, the optimal conditions for embryo germination in different lines and cultivars need to be established to understand their dormancy mechanisms. High or low and optimal temperatures, different levels of humidity, and the amounts of available water influence germination. These factors interact with the control of germination and dormancy (Briggs, 1978). Germination reaches a significant point only 24 hours after the cereal grain imbibes water. Cell division is started leading to the initiation of the radical and the plumule (Akalehiywot and Bewley, 1980).

Mobilization of reserves such as proteins, carbohydrates, and nutrients from the endosperm starts later, after 24 to 48 hours. In the event of grain germination, the physiological mechanisms of respiration and reserve mobilization, and the biochemical mechanisms of protein and RNA synthesis, followed by starch degradation, occur in the grain (Marcus et al., 1966; Chen and Varner, 1969). This is when the hydrolyzing enzymes such as α-amylase and proteases are synthesized and secreted in the endosperm (Bewley and Black, 1978). The phytohormonal regulation of reserve mobilization under the influence of gibberellins initiates the production of α-amylase. Gibberellins play a major role during grain germination. Abscisic acid plays a major role during grain development, and it induces dormancy in the maturing kernel.

1.2.3 Role of Phytohomones in Grain Development and Sprouting

All phytohomones, i.e., abscisic acid (ABA), gibberellins (GA), auxins, cytokinins, and ethylene, play an important role in activities that facilitate plant growth and development, such as solute mobilization, dormancy induction, germination, and many

others. Of these, ABA and GA are associated with the process of grain development, dormancy, and germination. Studies made by Wareing and Saunders (1971) showed that ABA and GA function simultaneously but antagonistically in dormancy induction, maintenance, and termination. However, later studies emphasized the role of ABA during seed development in dormancy induction, while GA is important in promotion of germination in non-dormant seeds. Also, the levels of GA required for germination at maturity is directly proportional to the levels of ABA in the seed during development, which implies that greater amounts of ABA lead to reduced non-dormancy (Hilhorst and Karssen, 1992; Karssen, 1995). Biosynthesis of ABA is greatly increased by the over-expression of the corresponding genes leading to increased dormancy (Nambara and Marion-Poll, 2003). In a study by Gonzalez-Guzman et al. (2004), reduced seed ABA biosynthesis showed reduced dormancy. Although ABA induces dormancy, the amounts of ABA found in the dormant and non-dormant seeds seemed to be the same. So its role of maintaining dormancy in seeds is not completely understood (Bewley, 1997).

In the developing grain, ABA is in higher concentrations in the embryo than in the endosperm. The pericarp also contains large amounts of ABA as compared to endosperm, and this difference in the content, percentage between pericarp and endosperm occurs even at maturity (Radley, 1976). Immature seeds are rich in ABA (Karssen, 2002). The amount of ABA present in the grain diminishes when it reaches desiccation, in the same pattern as seen with the accumulation of dry matter (Schopfer and Plachy, 1985). Unlike GA, ABA inhibits germination and enzymatic activity in the mature grain. It was found that, after the dormancy-inductive influence of ABA is

completed, GA's act as promoters of germination and growth. A period of warm storage at room temperature (~ 25°C) after harvest aids in dormancy release. The kernel gradually becomes insensitive to ABA, which is the positive regulator of dormancy induction and possibly maintenance, and it becomes sensitive to GA. Consequently, there is a transition from dormant to non-dormant condition. When maturity is reached, the developing kernels lose the initial amount of GA and ABA during the process of desiccation. The existing ABA content in the dry grain matches with levels of dormancy exhibited by the grain. The activity levels of GA are resumed when the grain comes into contact with water. The sites, and times at which ABA, and GA work are different (Karssen and Lacka, 1986).

Gibberellins play an important role in dormancy release. However, biosynthesis of GA during seed growth aids mostly in seed development, including fertilization, embryo growth, reserve mobilization, and fruit growth, but not in establishing primary dormancy in seeds, which occurs with ABA. Evidence of reserve mobilization from the endosperm to the embryo has been known for a long time. Also the involvement of GA in fostering this process is not a recent discovery (Brown and Morris, 1980).

The presence of GA is observed in developing wheat kernels. However, the species of GA's in the developing kernel are different from those in a germinating grain. Although many species of GA's are seen in a developing grain, their activity is low as compared to that in a sprouted kernel (Pharis and King, 1985; Lenton and Gale, 1987). The developing grain consists of less polar bioactive GA's, such as $GA_{9,15,20,24}$, and a slightly more polar $GA_{19,44}$ and five C-1 β-OH GA's, such as $GA_{54,55,60,61,62}$ of which GA_{54} is present in excess and occurs at a concentration of about 10 ng per seed (Gaskin et

al., 1980; Lenton and Gale, 1987). The active GA species such as GA_1 and GA_3, seen in large quantity in seedlings, are present in extremely small quantities in the developing grain, and, in contrast, GA_{54}, present in the developing grain, is hardly detectable in germinating seeds (Lenton and Gale, 1987; Metzger, 1983). It is not evident if GA_{54} or a similar species is stored in the developed seed until it transforms into some form of active GA species in the seedling (Gaskin et al., 1983). However, studies show that there seems to be GA carryover from the developmental stage to the seedling stage (Black and Naylor, 1959). The functions of GA species found in a germinating seedling are different from those seen in a developing kernel.

Studies propose two functions of GA during germination: 1) GA facilitates an increase in embryo growth potential, and 2) GA weakens the tissues around the radicle and reduces the mechanical restraint from the seed coat (Hilhorst, 1995; Yamaguchi et al., 2001; Lubner-Metzger, 2003). This is accompanied by the GA_3-induced α-amylase production from the aleurone, and the scutellum. It is, however, not known if the scutellum or the aleurone produces the greater amount of amylase, although studies by Palmer (1982) showed that only limited amounts of amylase are produced by aleurone in the presence or absence of GA_3 .

When exposed to heavy rainfall between the post-physiological maturity stage and the pre-harvest stage, grains germinate in the spike, which is, as discussed earlier, influenced by several factors including the morphological characteristics of the spike. This is followed by a chain of physiological processes leading to the rapid release of the enzyme α-amylase by the aleurone layer and the embryo, which results in the digestion of starch (Mares, 1989). Gibberellins produced in the embryo trigger the production of

the hydrolytic enzymes. The role of the embryo in GA secretion is emphasized by studies revealing that an isolated endosperm synthesizes α-amylase only when treated with GA (Derera, 1989). A complex interaction with ABA and the environmental factors bring about the control of GA in dormancy release, and germination initiation.

1.2.4 Dormancy and Sprouting

Dormancy is a condition where the seed cannot germinate in a specified time, given any right combination of physical environmental factors such as temperature, moisture, light, and humidity, which would have otherwise brought about its germination at the end of its dormancy (Baskin and Baskin, 2004). A non-dormant seed, on the contrary, germinates with the availability of any of the wide range of favorable environmental factor combinations for it (Karssen, 1995). Primary dormancy, which is developed during seed maturation, exists when the seed is still on the plant. After harvest, the seed eventually moves to secondary dormancy (Hilhorst, 1995).

Seed scientists world-wide have classified the diversity that exists in seed dormancy due to different seed structural system (Harper, 1957, 1977; Nikolaeva, 1969, 1977, 2001; Lang et al., 1985, 1987). One of the classifications that includes most of the kinds of dormancy seen in different seeds is the 'Nikolaeva's scheme,' as modified by Baskin and Baskin (2004). The scheme classifies dormancy as follows: physiological (PD), morphological (MD), morphophysiological (MPD), physical (PY), and combinational (PY+PD) dormancy. Physiological dormancy is further classified into deep, intermediate, and non-deep dormancy based on the emerging seedling (normal/abnormal), the physiological responses of seeds to varying temperatures required for germination, and the activity of the growth promoter GA. In deep PD, the

seedling develops abnormally, GA does not promote growth, and the seed requires a greater period of cold stratification to germinate. Seeds with intermediate PD produce normal seedlings, require less time for cold stratification to emerge, and GA promotes growth.

Non-deep dormancy is seen in most seeds with PD. They produce normal seedlings, GA promotes germination, and seeds vary in their need for cold or warm stratification depending on the species. Breakage of dormancy and the rate of germination in this type of seed is accelerated or retarded as a consequence of increase or decrease in temperature. Various factors, under the influence of changing temperatures, bring about a number of physiological responses in seeds with non-deep PD. This can be indicated as follows, as given by Baskin and Baskin (1985): seed development → induction of primary dormancy (Sp) → mature seed (Sp) → Sc1 (conditional or relative dormancy) → Sc2 → Sc3 → Sc4 → Sc5 → non-dormancy (Sn) Sc5 → Sc4 → Sc3 → Sc2 → Sc1 → secondary dormancy (Ss) → Sc1 and so on, where Sc1 through Sc5 are the transitional physiological states in the seed during the 'dormancy continuum,' which is the period of its development from primary dormancy to the non-dormant state and from the non-dormant state to secondary dormancy. The range of environmental conditions favorable for dormancy break widen as the seed moves from Sp to Sn; they narrow down as the seed moves from Sn to Ss, and a seed under Sc is relatively less likely to germinate as compared to a seed under Sn (Baskin and Baskin, 1985).

Seeds with MD have under-developed embryos (Baskin and Baskin, 1998). They do not require environmental conditions that promote dormancy breakage but need only

the time for complete growth, which is followed by emergence. Seeds with MPD have under-developed embryos with a need for temperature stratification and involvement of a GA promoter for dormancy breakage and germination. One other kind of dormancy, called PY, is characterized by the presence of a water impermeable seed coat, which resists water absorption and thus fosters dormancy. A condition that combines both PY and PD is termed combinational dormancy, wherein seed dormancy is due to the seed coat as well as the physiological characteristics of the seed (Baskin and Baskin, 2004).

In pre-harvest sprouting the seed lacks primary dormancy. It is in a quiescent state for the physical environmental conditions to be appropriate for germination, and no internal barriers for germination exist. The variation in the duration of dormancy observed in HWW wheat is related to many other traits in addition to the lack of primary dormancy. As mentioned earlier, PHS is also associated with many traits such as ear and grain characters, genetic traits of the seed coat color, interaction with environmental factors, and activity of growth regulators during grain development and maturation.

1.2.5 Sprouting and Environment

The different environmental variables that affect germination, either individually or in combination are light, temperature, rainfall, humidity, and nutrition. Some scientists have shown that dormancy is light dependent, and others have shown that light is one of the many environmental factors needed to promote germination (Bewley and Black, 1994; Vleeshouwers et al., 1995). Effect of light on germination involves the presence of the pigment phytochrome in the seed coat. If phytochrome is in its far-red form (P_{fr}), then germination becomes light dependent. Although there is no requirement of light for cereal seed germination, pigmentation due to chlorophyll and/or other

pigments in the seed coat affects the incident light and there arises a need for light (Cresswell and Grime, 1981). Baskin and Baskin (2004) concluded from their studies that only 'non-dormant light-requiring seeds' need light for germination initiation. Because white wheat has no pigmentation in its seed coat, light exerts no restraint in germination.

Temperature is one of the key factors that plays an important role in the induction of seed dormancy during grain growth and aids in expression of dormancy after maturity (Weisner and Grabe, 1972; Olsson and Mattson, 1976). Although high temperatures increase rate of germination, studies also have shown that low temperatures break seed dormancy (Harrington, 1923). Low temperatures prevailing during grain development induce dormancy in grain, while low temperatures prevailing during germination break dormancy. Conversely, high temperatures during grain development hasten grain growth, decrease the chance of dormancy induction, and thus shorten the period of dormancy after physiological maturation (MacKey, 1976). When high temperatures are followed by low temperatures, germination is fostered (Robertson and Curtis, 1967). High temperatures induce germination in less-dormant or non-dormant seeds (Nyachiro et al., 2002). Low temperatures and rainfall accompanied by high humidity at the grain maturation stage bring about pre-harvest sprouting.

Grain nutrition plays a role in grain sprouting. Tanner (1978) demonstrated pre-harvest sprouting in corn (*Zea mays* L.) grown in acidic soils. Lower level of molybdenum in the kernels resulted in formation of nitrate, a germination promoter (Tanner, 1978). Presence of high grain protein also favors germination, which makes it

difficult for the breeder to select the desirable traits for breeding a crop (Morris and Paulsen, 1985).

1.3 Sprouting and Economic Value

Pre-harvest emergence is a detrimental trait, which leads to non-dormant seeds that have reduced economic value and low quality. It starts during the period of seed development, and it can occur any time from seed formation to seed maturity. It takes place when the seed imbibes water after being exposed to rainfall at maturity, but before harvest (Nyachiro, 2002). Pre-harvest sprouting of hard white wheat decreases grain quantity, and quality. Consequently, the seed can be graded low because of lack of functional attributes needed for good milling, and baking (Paulsen, 1998; Kansas Farmer, 2006). It also affects agronomic attributes, such as test weight, and grain yield (Paulsen, 1998; Roozeboom, 1999). The farmer has to choose between grain mixing or, in extreme cases of damage, using the grain to feed livestock. Grain mixing brings poor returns to the producer.

1.4 Objectives of Current Work

To alleviate the detrimental attributes of HWW wheat, it is important to identify tolerance to PHS and to develop resistant genotypes through breeding techniques. The breeding program at Kansas State University has been working constantly with this objective for decades and has released resistant varieties in the past. The program is upgraded continually with identification of new experimental lines and cultivars. In the present study, 224 doubled haploid (DH) lines produced from a cross between ‘KS01HW163-4,’ a line reported to be highly sprouting-resistant, and ‘Heyne,’ a

sprouting susceptible cultivar, were screened for tolerance to PHS. Doubled haploid technique is one of the recent and simple technologies for crop improvement today. The technique uses pollen/anther culture to produce doubled haploid plants that are homozygous, a condition which is difficult to be obtained by other breeding methods in such shorter time. It is a cost effective technique enabling prospects in breeding for pre-harvest sprouting resistance but, is influenced by the methods of tissue culture used and the material being cultured. The DH seed used in this experiment was developed by Agri Pro (Berthoud, CO, USA).

Comparative studies of grain quality obtained from these controlled environments with that obtained from field experiments will be carried out later to observe the correlation between the two methods of screening. Phenotypic data combined with genotypic data can be used to identify the genes related to PHS tolerance and provide DNA markers for marker assisted selection.

Three experiments were conducted to estimate sprouting, and sprout damage. In all three experiments, germination percentages and falling number were calculated. Falling number is a special number calculated to evaluate starch decay levels. They are quantified using a “viscometric enzyme activity as falling number” (FN) value, which is deteremined from the Hagberg Falling Number (HFN) test (Sodkiewicz, 1999). This number quantifies the level of the alpha-amylase-enzyme activity in the gelatinized wheat flour/water suspension. A high FN value indicates good quality, while a low FN value is a sign of poor quality in the grain because the starch that gelatinizes the suspension has been degraded due to germination or sprouting (Humphreys, 2002). In the current work, the FN values and germination rates of the seed from the

experimental lines were evaluated. Simultaneous study was also done to observe the effect of environmental factors such as temperature and drought, and exogenous application of various growth hormones on grain development in the parental lines of the DH population. The specific objectives of this research were:

(1) To determine sprouting and the falling numbers in the 224 lines of the doubled haploid population

(2) To determine the influence of high temperature and drought stress during seed development on PHS

(3) To evaluate the influence of exogenous application of growth hormones (germination stimulants and germination retardants) on PHS

(4) To phenotype the 224 lines of the doubled haploid population

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CHAPTER 2 - Screening Doubled Haploid Lines for Tolerance to Pre-harvest Sprouting

ABSTRACT

Research has been started in Kansas as well as in other states, regarding development of improved varieties of hard white winter (HWW) wheat. However, yields were being affected due to pre harvest sprouting (PHS) damage (in 2004) and the condition persists (Kansas Farmer, 2006). KS01HW163-4, an experimental line reported to have a high degree of sprouting tolerance was crossed with Heyne, a sprout susceptible cultivar from KSU and 224 doubled haploid (DH) lines were produced in the Spring of 2006. The main objective of this experiment is to phenotype the DH population under controlled environment conditions. This is for a better understanding of phenotypic traits and distribution of sprouting index and falling numbers in the selected population and also eventually to combine this data with the genotypic data for determination of molecular markers. Based on the germination percentages the population was distributed as susceptible, and tolerant to PHS. χ^2 analysis of the DH population resulted in a 1:3 ratio indicating a complimentary gene action.

INTRODUCTION

Kansas produces one fifth of the total wheat in the United States, making the state the largest producer of wheat in the country. Although hard red winter wheat is mostly produced, there has been a shift from the traditional red grain to white grain in the recent years. Hard white winter (HWW) wheat differs from hard red winter wheat only in color of seed coat or bran. Except for pre-harvest sprouting (to be described in the next paragraphs), HWW is similar to hard red winter wheat in its agronomic aspects and economic utility. This has made the shift from hard red winter wheat to HWW wheat possible (Paulsen, 1998). Yields of HWW wheat varieties are similar to those of hard red winter wheat grown in the state, showing its adaptability to Kansas.

It is believed that most of hard red winter wheat in Kansas will soon be replaced by white wheat, because of its better milling and baking characteristics (Kansas Farmer, 2006). In many countries of Asia, Europe, and Australia, where the climate is favorable, producers have been growing varieties of HWW wheat for decades. With the growing demand in the international and the domestic markets for white wheat rather than red wheat, the United States wheat industry is currently producing and exporting HWW wheat to foreign markets every year (KACC, 2005). However, the production does not equal the current demand of the national and international markets. At a recent International Grain Program Conference, emphasis was made to increase HWW wheat production in the United States (KSRE News, 2005).

To meet these demands and to develop improved varieties of HWW wheat, research has been carried out in Kansas and in other states for many years. Kansas State University has one of the best breeding programs for hard white wheat in the

United States. ‘Trego,” released in 2001, a widely grown variety in Kansas, and the recently released cultivar ‘Danby’ (in 2006) are two hard white winter wheat varieties developed by Joe Martin, a Kansas State University wheat breeder. Yields and quality of HWW wheat were affected in the past due to the agronomic problem of pre-harvest sprouting, which was especially prevalent in 2004 (Kansas Farmer, 2006). It persists as the major problem affecting hard white winter wheat.

The condition where grain germinates on the spike before it is harvested is called pre-harvest sprouting (PHS) (Paulsen, 1998). It is a cause for concern, because most of the available varieties of HWW wheat are susceptible to PHS. It affects grain quality because sprouted grains lose their starch content, which makes the flour unacceptable for baking purposes (Roozeboom, 1999). Grains with PHS have low quality due to poor functional attributes needed for good milling and baking, and they have low agronomic attributes such as test weight and grain yield.

Pre-harvest sprouting in HWW wheat is stimulated by environmental conditions like high humidity, precipitation, heavy dew, and hormonal activity at physiological maturity. High precipitation after maturity hinders or delays harvest and thus, exposes the seed to wetting. These conditions occur more in eastern Kansas than in central and western Kansas.

During precipitation, grains absorb water retained in the spike and swell. Swelling breaks the seed coat and facilitates the inflow of necessary elements for germination, water and oxygen, which initiate enzyme activity (Roozeboom, 1999). This is followed by a chain of physiological activities leading to the release of gibberellins by the embryo

and the enzyme alpha-amylase by the aleurone layer, which results in the digestion of starch (Mares, 1989).

Establishment of dormancy during grain development also plays an important role in influencing PHS. Higher temperatures during grain development lead to formation of grain with lower dormancy levels. Grains from a sprouting-susceptible genotype readily germinate under optimal conditions of germination, while those from a sprouting-resistant genotype do not. In pre-harvest sprouting, the seed lacks primary dormancy. It is in a quiescent state and ready for the physical environmental conditions to be appropriate for germination, and no internal barriers for germination exist.

To alleviate the detrimental conditions prevalent in HWW wheat, it is important to identify tolerance to PHS and to develop resistant genotypes through breeding techniques. The literature indicates that work is being done to develop improved, sprouting-tolerant varieties through techniques of genetics and breeding in those parts of the world where hard white wheat is produced. The breeding program at Kansas State University has been working constantly with this objective and has released resistant varieties in the past. The program tries to upgrade continually its experimental lines and cultivars.

In the present study, 224 doubled haploid (DH) lines produced from a cross between ‘KS01HW163-4,’ a line reportedly having a high level of sprouting-resistance, and ‘Heyne,’ a sprouting-susceptible cultivar, were screened for tolerance to PHS. The objective of the experiment was to screen the DH lines for the trait of pre-harvest sprouting tolerance by determining the sprouting percentages, and falling number values of these lines as well as to multiply the seed for field studies.

The phenotypic data collected from this experiment will be combined with the genotypic data to help identify the genes related to PHS tolerance and to provide DNA markers for marker-assisted selection.

MATERIALS AND METHODS

The study was conducted in a greenhouse of the Department of Agronomy at Kansas State University during the spring of 2007, using 224 doubled haploid (DH) experimental lines. The 224 DH lines are categorized as CAP - 7 and CAP - 8 with 136 and 88 lines in each respectively. CAP - 7 and CAP- 8 involved different individual plants of KS01HW163-4 and Heyne in their respective crosses. Coordinated Agricultural Projects (CAP) is a program funded by USDA-CSREES. The DH lines in the current study are within this project. The goal of this project is to increase the competitiveness of public wheat breeding programs through use of modern selection technologies, like Marker Assisted Selection (MAS).

2.2.1 Plant Husbandry and Growth Conditions

Seeds were thoroughly cleaned before sowing and were sown by hand in perforated plastic trays filled with soil (Metro Mix 200, Hummert International, Topeka, Kansas). The plastic trays were then placed in metal trays filled with water. The metal trays were watered frequently enough to keep the soil in the plastic trays wet and to give a continued supply of water to the seeds and the seedlings that eventually emerged. A week after emergence the plastic trays with plants were moved to a vernalization chamber run continuously at 4°C. The plants were kept at this temperature for vernalization for 6 weeks. Immediately after being taken out of the vernalization

chamber, the trays were placed in the corridors of the greenhouse, where the temperature was about 12 – 15°C to let the plants get acclimatized to the temperature change.

The plants were then transplanted into plastic pots at a soil depth of 5 cm. The diameters of each pot at the top and bottom were 21 cm and 16 cm, respectively, and the height of each pot was 20 cm. Four plants were transplanted into each pot. Soil used as rooting medium consisted of a potting mix (Metro Mix 200, Hummert International, Topeka, Kansas). Osmocote Plus (15-9-12, $N\text{-}P_2O_5\text{-}K_2O$) controlled release fertilizer (Scotts-Sierra Horticultural Products Company, Maryville, Ohio) was mixed into the soil at a rate of 15 grams per pot before transplanting.

Pots had perforations at the bottom and were placed in plastic trays, eight pots per tray. The trays were moved to the greenhouse and placed on benches. All plants were placed in the same greenhouse to provide similar environmental conditions of temperature, and humidity. Trays were watered regularly. Each pot was considered a replication and there were four replications for most of the experimental lines. However, some lines had only few seeds, and they had only one or two replications. The four replicated pots of each DH line were labeled with the line number to avoid mixing of replications between lines.

All plants were grown at an optimum temperature of 20/15°C day/night, with 16/8 hours day/night period, and at 85% humidity until physiological maturity. The trays were randomized periodically to ensure similar treatment was given to all plants.

2.2.2 Data Collection

Growth and yield traits were measured on all four replications of the 224 lines. Data on phenology (time to booting, flowering, seed-set, physiological, and harvest maturity) were recorded during the period of plant growth. After harvest, data on plant biomass, and grain yield were also recorded. Germination tests, and falling number test also were conducted on the grain obtained from the experimental lines.

2.2.3 Growth and Yield Traits

Physiological maturity was recognized by clearing of green and appearance of yellow just below the spike. At harvest, plant height (base of the plant to the tip), tiller number, and ear number were recorded for all the plants. Harvested tillers were dried at 65°C for 10 d and harvested spikes were dried at 28°C, for 7 d. Dry weights of the tillers and spikes were collected, and the total dry weights (tiller + spikes) were calculated. Spikes were threshed, and grain number and grain dry weight were recorded. Seed size was then calculated as the ratio of grain dry weight over gain number.

2.2.4 Germination Percentages

At harvest, grains from the four replications were bagged together in a single bag, dried, and stored at -24°C. This was done to randomize the replications. All samples were harvested, dried, and threshed, and the seeds were bagged and stored in the freezer. Germination tests were carried out five times, taking 20 seeds each time from each DH line, making five replicated tests per line. Each replicated test started at the end of the previous test, so all replicated tests were not carried out simultaneously, however, the environmental conditions for the germination tests were similar each time.

To carry out the germination test, bags were thawed and 20 seeds from each line were taken at a time. This was considered one replication of the germination test. Germination tests were set up in petri dishes that were cleaned with doubled distilled water and 70% ethanol to create aseptic conditions and to prevent fungal or bacterial growth. Seeds were placed on a germination paper in the clean petri dishes. Water was sprayed on the seeds until the paper was completely wet, but no excess water was left in the petri dish. Petri dishes were placed in an incubation chamber maintained at 20°C. Germinated seeds were counted and removed each day from the third day to the twelfth day and cumulative day-wise germination percentages were calculated.

2.2.5 Falling Number Test

Falling number was obtained by using a machine that determines falling number (Falling Number Model 1800, Perten Instruments, Sweden). The Hagberg Falling Number (HFN) test was carried out by using the flour obtained from the grain (Sodkiewicz, 1999). Two samples could be run at a time. 7 g of flour was put in the tubes; 25 ml of distilled water was dispensed into each tube and each tube was shaken vigorously 20 times to allow complete blending of flour, and water. The tubes were placed in a hot water bath immediately. When the machine was switched on, stirrers rigorously stirred the flour, and water for 60 seconds for thorough blending and formation of slurry. The stirrers were then left at the top of the slurry to fall freely to the bottom of the tube. Falling number directly corresponds to the number of seconds taken for the stirrer to reach the bottom of the tube. A lower falling number value indicates less viscosity, and greater sprout damage, and a larger falling number value indicates healthy grain with less damage.

2.2.6 Data Analysis

Data from the experiment were analyzed by analysis of variance using the 2003 PROC ANOVA procedure in Statistical Analysis System software (SAS Institute, Cary, NC). Randomized complete block designs were used for the experimental design in all parts of the study. There were four replicated pots representing each line. The abbreviation ln (experimental line number) was used as class variable, and the influence of ln was considered during analysis of germination rates, and yield traits.

A margin criterion for germination rate was set at 90% and the lines were evaluated for the day on which the margin criterion was reached starting from the third day (D3) after the germination experiment was set through day twelve (D12). A dependent variable 'germ,' used during analysis is the day when the 90% margin criterion for germination was met. If the line germinated within the observation period of 3 to 12 days, the variable 'germ' took a value from 3 to 12 corresponding to the day at which germination level reached the target margin. If the target germination margin was not reached, the variable 'germ' took a value 13. In this analysis, the mean percentage on all replications per line was used as the data for analysis. The lines were grouped by the day the mean germination rates reached the 90% germination and graphs were plotted, starting day three (D3) through day thirteen (D13). $\chi 2$ analysis test was conducted on the populations of CAP-7 and CAP-8 to understand the segregation ratio of the trait of PHS within the populations. Yield traits were measured on the four plants from the four pots of each line, that is, sixteen plants per experimental line. There were a total of five replicated germination tests for each experimental line. Standard errors of the means were calculated for all variables to represent the variability.

RESULTS & DISCUSSION

2.3.1 Phenology

The duration to various phenological stages during plant development, from the time the plants were taken out from vernalization chamber and moved to the environment of the green house, were similar in both CAP - 7 and CAP - 8. The duration for emergence of boot leaf, emergence of the spike, flowering, seed-set, and physiological maturity over the 224 DH lines ranged between 45 – 55 d, 51 – 61 d, 58 – 67 d, 66 – 75 d, and 102 – 119 d respectively.

2.3.2 Germination/Sprouting Percentages

There were significant variances among the DH lines in total germination, sprouting %, and rate of germination. The DH lines in CAP - 8 showed greater germination rate as compared to those in CAP - 7. Population from CAP - 7 and CAP - 8 were combined together to plot graph for germination percentages. Lines from the entire population were grouped by the day they reached the target 90% germination. Graph of germination ratio was plotted indicating the day (from D3 to D12) when the experimental lines reached the target 90% germination. The lines shown on D13 are those that did not reach 90% germination within the observation time.

Since dormancy levels are greater in plants from controlled environment studies as compared to those from field studies, seeds that germinated until D10 were considered susceptible and those that germinated further than that were considered tolerant. Accordingly the entire DH population of both CAP - 7and CAP - 8 was be divided into two classes – susceptible (those germinated by D10), and tolerant (those

germinated from D11 and further) to PHS, based on the germination rates. This classification was based on final germination percentages. The graph indicated a bimodal distribution.

Maximum number of lines reached the target 90% germination on D6 and/or D7 (Fig. 2.1). Number of lines reaching the target germination in CAP - 8 was distributed over D6 and D7 (Fig. 2.2), where as in CAP - 7 greater number of lines reached target germination rate on D7 (Fig. 2.3).

X^2 analysis of the DH population of CAP - 7 and CAP - 8 resulted in 1:3 ratio suggesting that the trait is controlled by two genes indicating a complimentary gene action (Table 2.1). Studies of Andreoli et al. (2006) on gene control and PHS in Brazilian wheat cultivars. Detailed germination ratio data of CAP - 7 and CAP - 8 is provided in Appendix – I.

2.3.5 Falling Number values

The Falling number values ranged from 248 – 551 in CAP-7, and from 243 – 668 in CAP- 8, indicative of lines being susceptible, moderately tolerant, or most tolerant. Detailed germination ratio data of CAP - 7 and CAP - 8 is provided in Appendix – I. Falling number values did not correlate well with the germination ratios of the population (Fig. 2.4).

2.3.3 Correlations

Seed dry weight, seed number and seed size did not correlate with germination ratios and falling number values, indicating no relationship between these traits and rate of germination, in the entire DH population (Figures 2.5, 2.6, 2.7, 2.8, 2.9, and 2.10).

This suggests that seed dry weight, seed number, and seed size do not influence the extent of PHS tolerance/susceptibility of a genotype and may not be used to determine PHS.

2.3.4 Seed Weights and Seed Numbers

In the DH population, seed dry weight increased with increase in seed number (Figure 2.11) suggesting a positive correlation between the two traits. Earlier studies on peanut indicated that pod dry weight, seed dry weight, pod number per plant and seed number per plant were positively correlated with each other suggesting similar results as obtained in this study (Phudenpa, 2004).

The data obtained from this study will be used to characterize and phenotype the doubled haploid lines in controlled environments and later, compare these results to field studies. The phenotypic data collected from this experiment also will eventually be combined with the genotypic data to help identify the genes related to PHS tolerance and provide DNA markers for marker assisted selection.

Table 2.1. χ2 analysis table indicating the day of germination (Day), observed number of lines (O) reaching the target germination ratio (0.9), the expected number of lines (E) that should reach the target germination to fit a 1:3 ratio and the χ2 values for CAP-7 and CAP-8 population.

CAP-7					
Day	O	E	$(O-E)^2$	$(O-E)^2/E$	
3-10	98	100.5	6.25	0.062	
11-13	36	33.5	6.25	0.187	
				$\chi2 = 0.249$	0.95-0.50 > 0.05
					Fits 1:3 ratio
CAP-8					
Day	O	E	$(O-E)^2$	$(O-E)^2/E$	
3-10	57	63	36	0.571	
11-13	27	21	36	1.714	
				$\chi2 =2.285$	0.20-0.10 > 0.05
					Fits 1:3 ratio

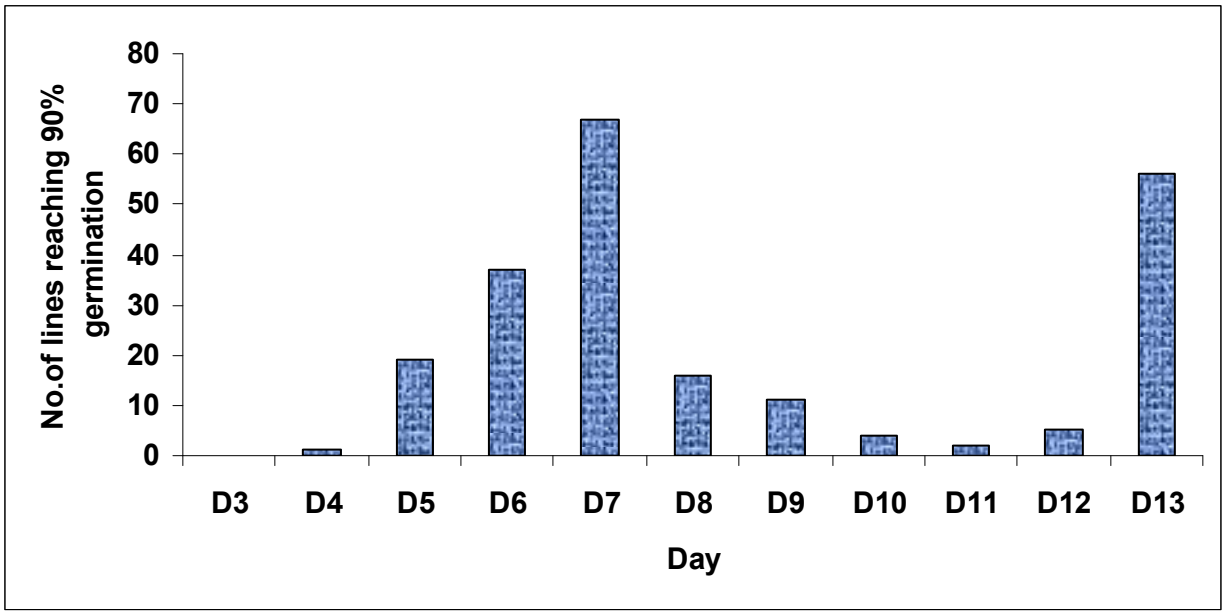

Figure 2.1. Distribution of germination ratio in the entire double haploid population of Cooperated Agricultural Projects (CAP) – 7 (136 lines) and CAP – 8 (88 lines) combined. D13 represents the number of lines which did not reach 90% germination until day 13.

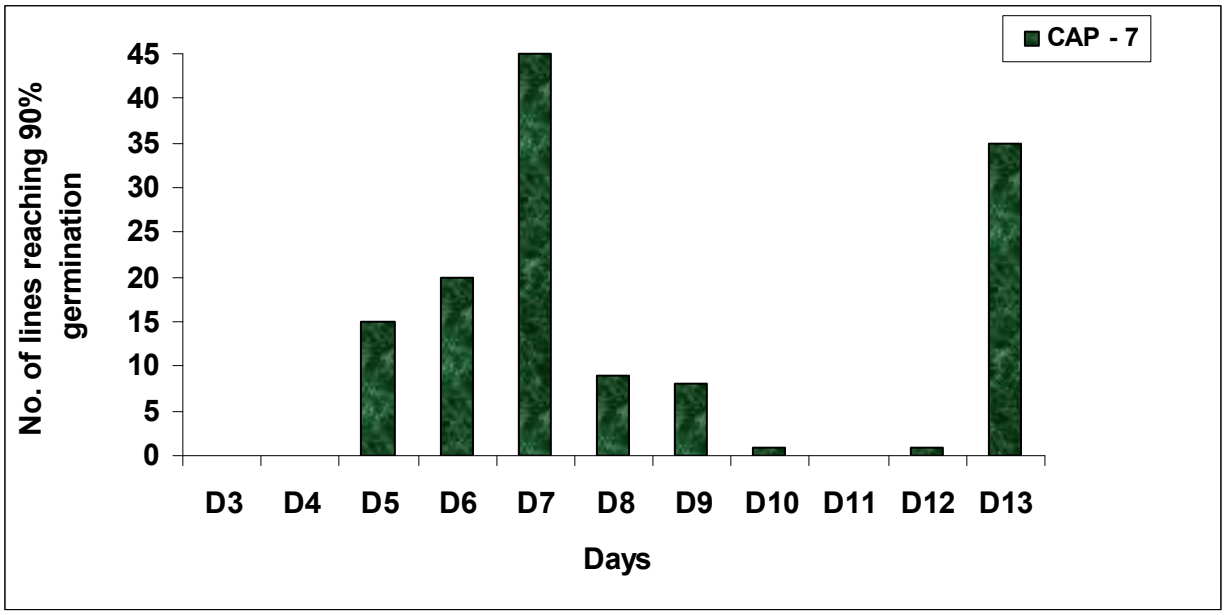

Figure 2.2. Distribution of germination ratio of double haploid population in CAP – 7 (136 lines). D13 represents the number of lines which did not reach 90% germination until day 13.

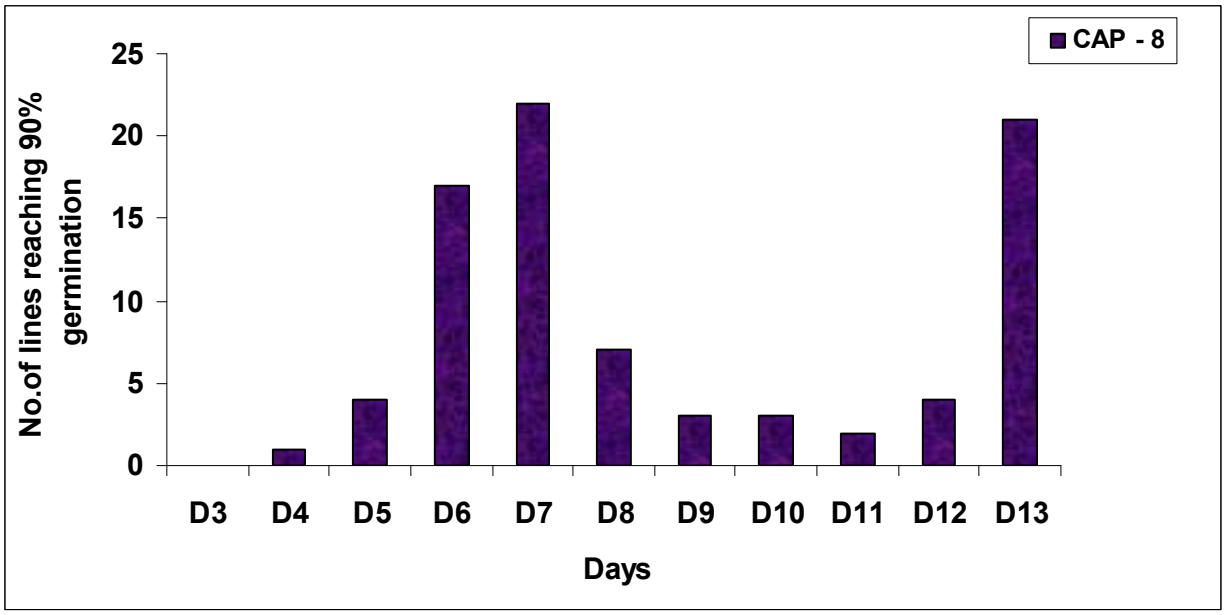

Figure 2.3. Distribution of germination ratio of double haploid population in CAP – 8 (88 lines). D13 represents the number of lines which did not reach 90% germination until day 13.

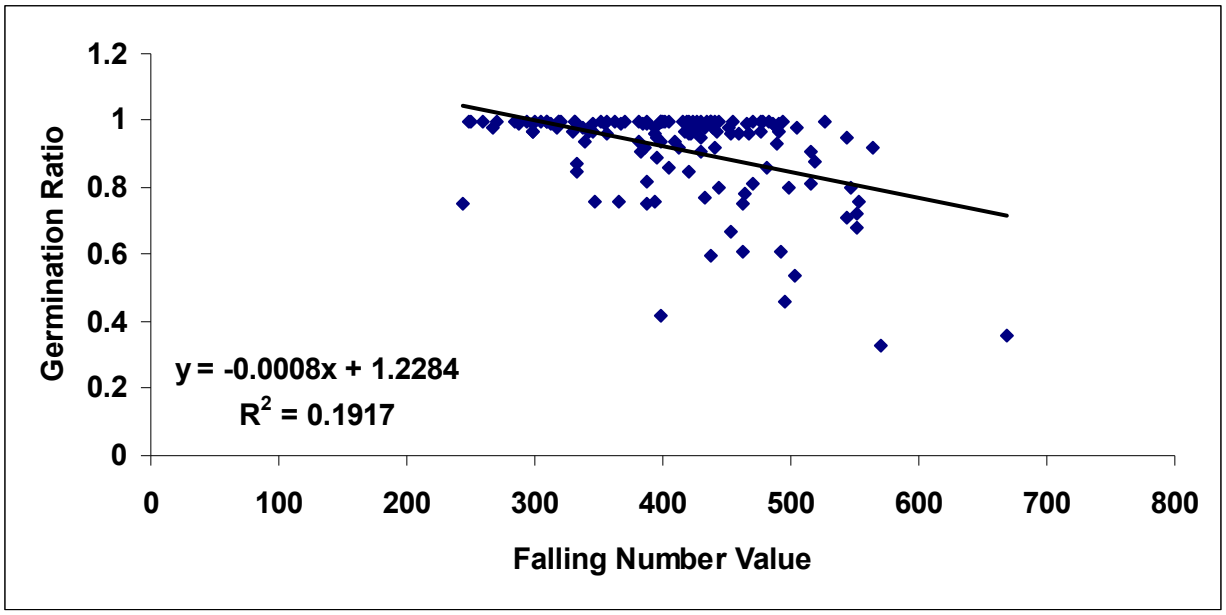

Figure 2.4. Correlation between germination ratio and falling number values of the entire double haploid population of CAP – 7 (136 lines) and CAP – 8 (88 lines).

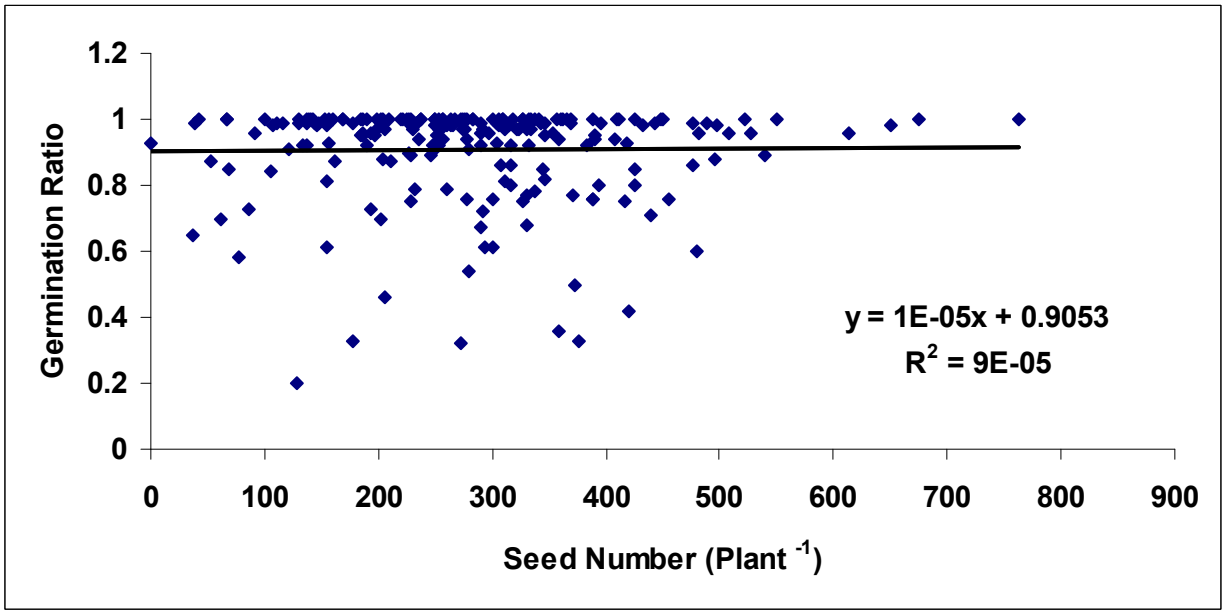

Figure 2.5. Correlation between seed number and germination ratio of the entire double haploid population of CAP – 7 (136 lines) and CAP – 8 (88 lines).

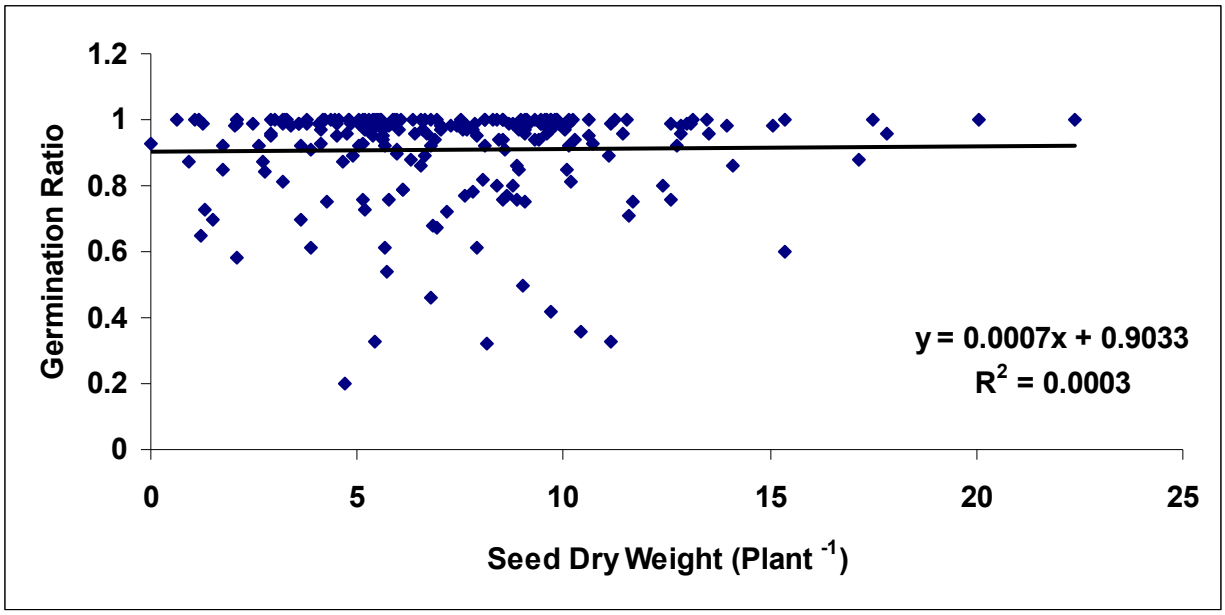

Figure 2.6. Correlation between seed dry weight and germination ratio of the entire double haploid population of CAP – 7 (136 lines) and CAP – 8 (88 lines)

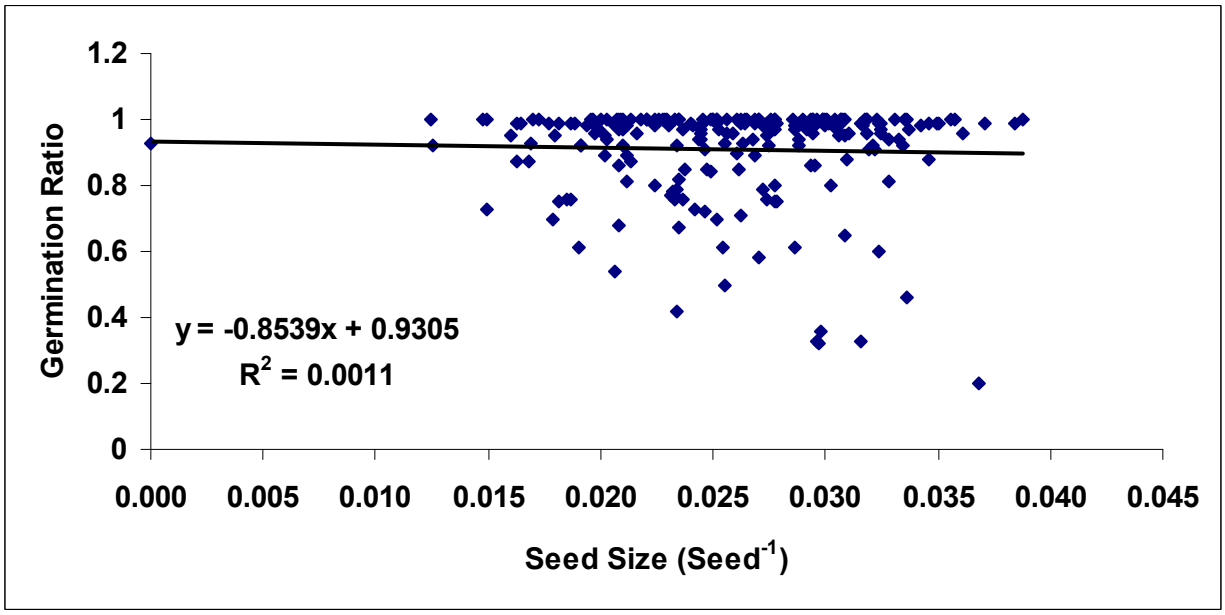

Figure 2.7. Correlation between seed size and germination ratio of the entire double haploid population of CAP – 7 (136 lines) and CAP – 8 (88 lines)

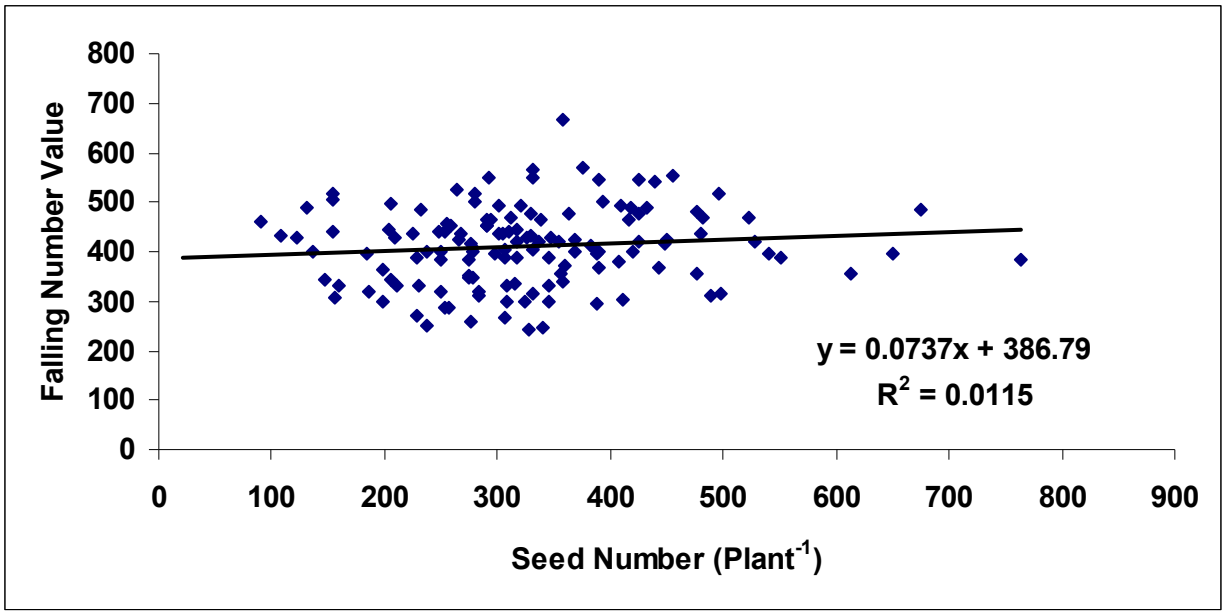

Figure 2.8. Correlation between seed number and falling number values of the entire double haploid population of CAP – 7 (136 lines) and CAP – 8 (88 lines)

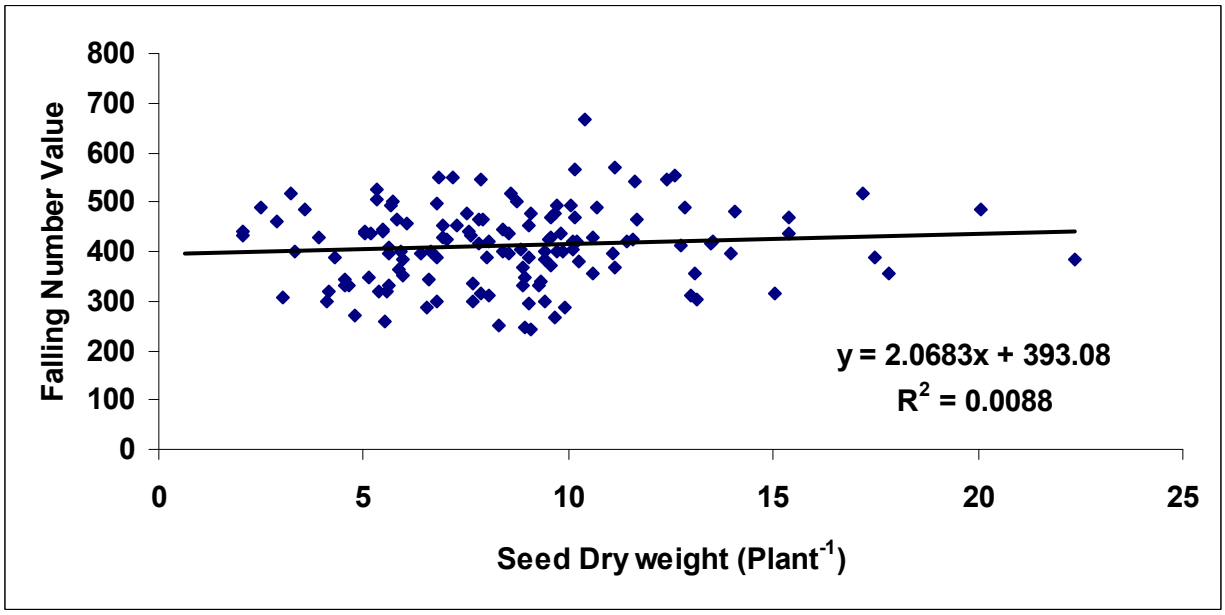

Figure 2.9. Correlation between seed dry weight and falling number values of the entire double haploid population of CAP – 7 (136 lines) and CAP – 8 (88 lines)

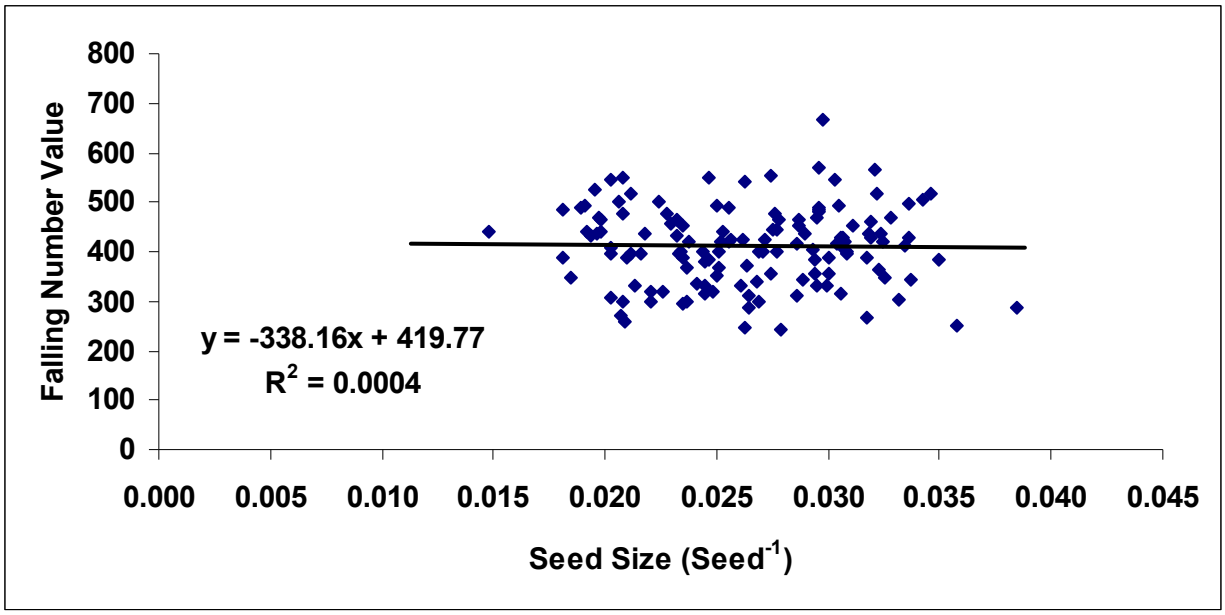

Figure 2.10. Correlation between seed size and falling number values of the entire double haploid population of CAP – 7 (136 lines) and CAP – 8 (88 lines)

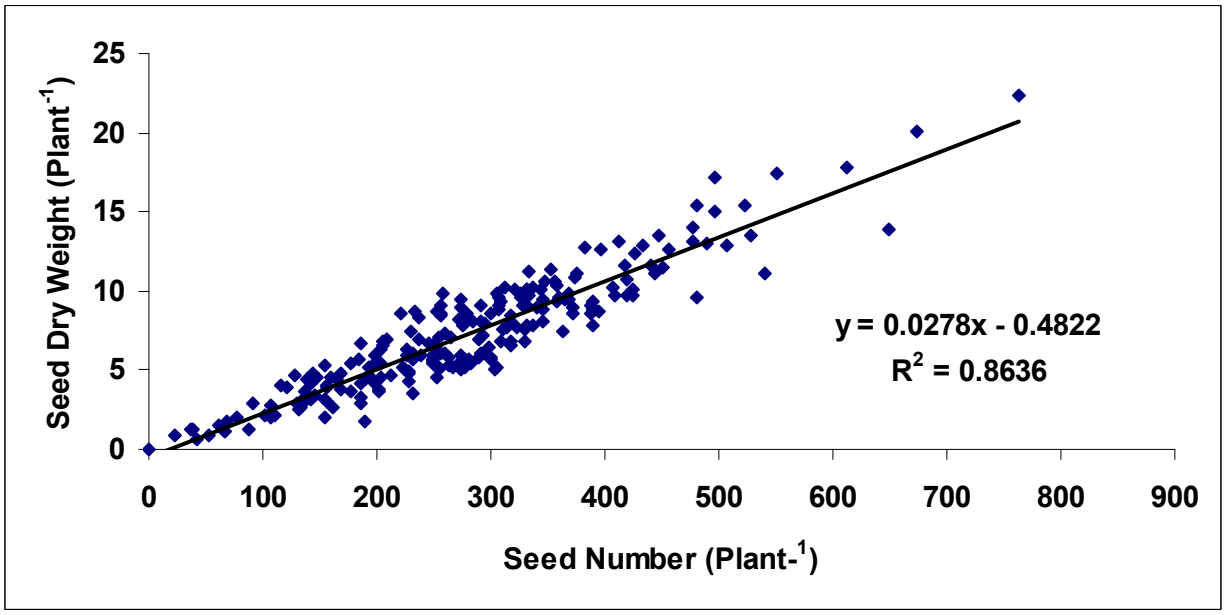

Figure 2.11. Correlation between seed number and seed dry weight of the entire double haploid population of CAP – 7 (136 lines) and CAP – 8 (88 lines)

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CHAPTER 3 - Understanding the Impact of Environmental Factors during Seed Development on Pre-harvest Sprouting

ABSTRACT

Pre-harvest sprouting (PHS) is the major problem affecting hard white winter (HWW) wheat which could decrease yields and grain quality. The condition where grain germinates on the spike before it is harvested is called PHS. Environmental factors play a great role in inducing PHS. The current study was conducted on the parental lines, KS01HW163-4, (PHS tolerant) and Henye (PHS susceptible)- two HWW wheat cultivars, of a set of DH lines being screened for PHS. The objective was to look into the influence of varying environmental factors of temperature, drought and temperature by drought interaction during grain development, on physiological, growth, and yield traits, and also on the process of germination and PHS tolerance. After seed set, plants are exposed to two temperatures, optimum temperature (OT), 20/15°C; and high temperatures (HT), 25/20°C initially and 30/20°C later, and to two water regimes, fully irrigated (I, irrigated 2 d intervals) and drought stressed (D, irrigated at 7 d intervals) until physiological maturity. Data on phenology, physiological, growth and yield traits were determined. Germination percentages and falling number test were conducted on the harvested seed. Phenology was significantly influenced by environmental factors. Days from seed-set to maturity were reduced by 8 and 20 d due to high temperature alone and combination of high temperature and drought, respectively. Seed dry-weight, and harvest index were also influenced negatively due to stress. Under OT, KS01HS163-4, the tolerant cultivar, was tolerant to PHS, but at HT and/or drought it became susceptible to PHS and Heyne, the susceptible cultivar showed greater

tolerance to PHS. Growth under stressed conditions changed the tolerance levels to PHS. Therefore multi-location tests must be conducted with variable environments to test the stability of a variety to PHS.

INTRODUCTION

Considerable amounts of hard white winter (HWW) wheat are grown in North America, South America, China, Russia, and Australia. However, one problem faced by the industry is that most HWW wheat cultivars are susceptible to pre-harvest sprouting (PHS) (Paulsen, 1998). Conditions favoring PHS often occur in these areas leading to yield losses and poor grain quality. Pre-harvest sprouting occurs with some frequency in some parts of Kansas in HWW wheat.

Kansas is the largest producer of wheat in the United States. In recent years, due to the increasing demand for white grain as compared to red grain, increased acreage is being planted to HWW wheat. White wheat is preferred over red because of the color, and quality of bread, and noodles made from it (Paulsen, 1998; Roozeboom, 1999). Environmental conditions encountered during grain development, and those during physiological maturity cause the grain to become susceptible to PHS (Nyachiro et al., 2002; Biddulph et al., 2005; Biddulph et al., 2008). The traits of color and quality of bread, flat bread, and noodles are greatly affected when sprouted grain is processed (Derera, 1989). Consequently, farmers incur great losses in the years that result in PHS.

Unfavorable environmental conditions, such as high temperatures, and drought during grain development and low temperatures and precipitation at physiological maturity, coupled with insufficient grain dormancy, can lead to PHS in white wheat cultivars. White wheat genotypes vary in dormancy levels and resistance to PHS. Hence, some genotypes do not have the capability to stop the hydrated grain from germinating (Nyachiro et al., 2002). Grains from a sprouting-susceptible genotype

readily germinate under optimal conditions of germination, while those from a sprouting-resistant genotype do not. Germination of the seed in the spike of a genotype exhibiting a short dormancy period also can be attributed to enzymatic and hormonal activity influenced by environmental factors (Pagano et al., 1997).

Tolerance to PHS is induced by appropriate environmental conditions that occur during grain development, and this tolerance is expressed at physiological maturity and after harvest. In genotypes susceptible to PHS, high amounts of precipitation during post maturity hinder or delay harvest, thus exposing the seed to wetting. Grains absorb the water retained in the spike and swell. Swelling breaks the seed coat and facilitates the inflow of necessary elements for germination (water and oxygen) which initiate the enzymatic activity (Roozeboom, 1999). Under these conditions favorable for PHS, gibberellins are produced which trigger alpha-amylase activity that degrades the starch in the grain resulting in grain with poor end-use quality.

Most crop species rapidly germinate with an increase in temperature. However, in some crop species, like wheat, low temperatures break seed dormancy (Harrington, 1923). Reddy et al. (1985) also showed that temperature variations at germination can determine the dormancy potential of genotypes. Induction of dormancy during seed development and its expression during seed germination are influenced by temperature along with other environmental factors (Olsson and Mattson, 1976; Nyachiro et al., 2002; Biddulph et al., 2005). Although it has been found that low temperatures break dormancy in wheat, Belderok's work (1961) suggested that dry and high temperature conditions during grain development lead to short dormancy periods. His later work (1968), in which the plants were moved between different glasshouses and exposed to

different temperature sums during grain development, showed that a short dormancy period is related to “large accumulated temperature sums during development.”

Although wheat is a versatile crop grown world-wide and is adapted to tropical, temperate, and subtropical temperatures, it is known that the high temperatures and drought experienced during post-anthesis result in detrimental effects on yield (Tewolde et al., 2006). In a study by Al-Khatib and Paulsen (1990), it was found that cultivars susceptible to temperature stress showed a yield loss of >40%, whereas the yield levels of tolerant varieties were not much affected. Drought due to limited rainfall, accompanied by high temperatures during the grain-filling stage of wheat, is common in most areas where wheat is grown around the world.

Breeding winter wheat cultivars with thermotolerance may lead to short term survival during stress (Machado and Paulsen, 2001). However, influence of temperature during grain filling and its effects on PHS in recently bred HWW wheat varieties is yet to be explored. In Kansas, low temperatures during physiological maturity break dormancy in HWW wheat. Conditions of high humidity also facilitate the process. In addition, grain maturity and harvest dates influence PHS (Paulsen, 1998).

Developing varieties resistant to sprouting minimizes the problem of PHS. The literature indicates that work is being done to develop improved, sprouting-tolerant varieties through techniques of genetics and breeding in those parts of the world where hard white wheat is produced. Interesting and result-oriented work has been done by wheat breeders at Kansas State University. They have developed sprouting-resistant varieties suitable for Kansas. High yielding cultivars with sprout-resistant traits have been developed in the past. Work is focused on the continuous improvement of cultivars

for their agronomic qualities, such as increased disease resistance and sprouting tolerance.

Two HWW wheat cultivars, 'KS01HW163-4,' a cultivar with reportedly high degree of sprouting tolerance, was crossed with 'Heyne,' a sprout-susceptible cultivar from Kansas to produce 224 doubled haploid lines in the spring 2006. The current project, carried out under controlled environmental conditions in growth chambers, studied these haploid lines. The research was conducted to understand the influence of varying environmental conditions of temperature and drought during grain development and on the subsequent process of germination. The specific objectives of this research were:

1) To determine the influence of high temperature and/or drought stress during seed development on physiological, growth, and yield traits in the parental cultivars of the DH HWW wheat lines
2) To determine the influence of high temperature and drought stress during seed development on PHS
3) To better understand the influence of high temperature and/or drought stress to model possible impact of growth temperature on PHS

MATERIALS AND METHODS

The study was conducted in controlled environmental growth chambers in the Department of Agronomy at Kansas State University in the Summer of 2007. Two HWW wheat cultivars, one sprouting-resistant, 'KS01HW163-4', and the other sprouting- susceptible, 'Heyne,' were used in the experiment. The study was done to

determine the influence of the varying environmental conditions of temperature, and/or drought during grain development and on the subsequent process of germination.

3.2.1 Plant Husbandry and Growth Conditions

Seeds from the two HWW wheat cultivars, 'KS01HW163-4' and 'Heyne,' were thoroughly cleaned before sowing. Seeds were sown by hand in perforated a plastic tray filled with soil (Metro Mix 200, Hummert International, Topeka, Kansas). The plastic tray was then placed in a metal tray filled with water. The metal tray was watered frequently enough to keep the soil in the plastic tray wet and to give a continued supply of water to the seeds, and the seedlings, which eventually developed. A week after emergence the plastic tray with plants was moved to a vernalization chamber run at 4°C. The plants were kept at the vernalization temperature for 6 weeks. Immediately after being taken out of the vernalization chamber, the tray was placed in a corridor of the greenhouse, where the temperature was about 12 – 15°C, to let the plants get acclimatized to the temperature change.

The plants were then transplanted into plastic pots at a soil depth of 5 cm. The diameters of each pot at the top and bottom were 21 cm and 16 cm, respectively, and the height of each pot was 20 cm. Three plants were put in each pot. Soil used as rooting medium consisted of a potting mix (Metro Mix 200, Hummert International, Topeka, Kansas). Osmocote Plus (15-9-12, N-P_2O_5-K_2O) controlled release fertilizer (Scotts-Sierra Horticultural Products Company, Maryville, Ohio) was mixed into the soil at the rate of 15 gm per pot before transplanting.

Four growth chambers (Conviron Model E15, Winnipeg, Manitoba, Canada) were used in the experiment. Pots were moved into the four growth chambers that were set

at the same temperature and humidity. Pots were watered regularly. Each pot was considered a replication, and there were 20 pots of each cultivar in each growth chamber making a total of 40 pots per chamber. The plants in all growth chambers were grown at an optimum temperature of 20/15°C day/night, with 16/8 hours day/night until seed-set [i.e., 50% of plants of each cultivar in each chamber had reached seed-set (Feekes stage 10.5.4, Large, 1954)]. The chambers were maintained at 85% humidity. From seed-set to physiological maturity, the plants were exposed to different temperatures and drought treatments.

At seed-set (Feekes stage 10.5.4) (Large, 1954), two chambers were set at the optimum temperature of 20/15°C and two were set at a high temperature, 30/20°C. Within each temperature treatment, there were two watering regimes: fully irrigated (irrigated every 2 days) and drought stressed (irrigated at 7 d intervals). The pots in all the chambers were randomized every 10 days.

3.2.2 Data Collection

Five plants of each genotype, under the four treatments (two temperatures and two watering regimes), were randomly tagged at the beginning of the treatments. Physiological traits, growth, and yield were measured on these tagged plants. Data on phenology (time to booting, flowering, seed-set, physiological, and harvest maturity) were recorded during plant growth. After harvest, data on plant biomass and grain yield were recorded. Germination tests and falling number tests also were conducted on the grain obtained from the different treatments.

3.2.3 Physiological Traits

Physiological measurements were taken on the fully expanded flag leaf of the tagged plants at ambient CO_2 and growth temperatures during noon or early afternoon. Measurements were taken 0, 2, 4, 6, 8, 10, and 12 days after the beginning of the treatments. Physiological measurements included chlorophyll fluorescence, leaf chlorophyll content, leaf temperature, and relative water content. Leaf chlorophyll content was measured using a self-calibrating chlorophyll meter (SPAD, Model 502, Spectrum Technologies, Plainfield, IL, USA). Leaf temperature was measured using a infrared thermometer (OS534 handheld infrared thermometer, Omega Engineering, Inc., Stamford, Connecticut, USA) by shooting a beam of infrared light on the topmost fully expanded leaf exposed to light. Chlorophyll fluorescence, a measure of the damage caused to the thylakoid membranes in the leaf tissue, was measured using a fluorometer (OS 30, OptiScience, Hudson, NH, USA). Data loggers (HOBO U12 Temp/RH/Light/External Data Logger, Onset, Bourne, MA, USA) were set in the growth chambers to record the humidity, temperature, and light intensity at 20 min intervals.

3.2.4 Growth and Yield Traits

Physiological maturity was recognized by clearing of green and appearance of yellow just below the spike. At harvest, plant height (base of the plant to the tip), tiller number, and ear number were recorded on the five tagged plants of each genotype within each treatment. Harvested tillers were dried at 65°C for 7 days, and spikes were dried at 35°C for 10 days. Dry weights of the tillers, and spikes were collected, and the total dry weight (tiller + spikes) was calculated. Spikes were hand-threshed, and seed

number, and seed dry weight were recorded. Harvest index was calculated as the ratio of seed dry weight, over total dry weight (above ground biomass).

3.2.5 Germination Percentages

At harvest, grains from each replicated pot were bagged separately, dried, and stored at -24°C. All samples were harvested, dried, and threshed, and seeds were bagged and stored in the freezer. To carry out the germination test, bags were thawed, and 20 seeds from each replication were taken. Germination tests were done in petri dishes that were cleaned with doubled distilled water, and 70% ethanol to create aseptic conditions and to prevent fungal, and bacterial growth. Seeds were placed on a germination paper in the clean petri dishes. Water was sprayed on the seeds until the paper was completely wet, but no excess water was left in the petri dish. Petri dishes were placed in a chamber running at 20°C. Germinated seeds were counted and removed, starting on the third day and continuing until the twelfth day after the beginning of germination. Cumulative day-wise germination percentages were calculated.

3.2.6 Falling Number Test

The Hagberg Falling Number (HFN) test was carried out by using the flour obtained from the grain (Sodkiewicz, 1999). Falling number values are obtained by using the falling number machine (Falling Number Model No.1800; Perten Instruments, Sweden). Two samples could be run at a time. 7 g of flour was put in the tubes, and 25 ml of distilled water was added. The tubes were shaken vigorously 20 times to allow for complete blending of the water and the flour. The tubes were placed in a hot water bath

immediately. When the machine was switched on, stirrers rigorously stirred the flour and water for 60 seconds for thorough blending and formation of slurry. The stirrers were then left at the top of the slurry to fall freely to the bottom of the tube. Falling number directly corresponded to the number of seconds taken for the stirrer to reach the bottom of the tube (http://www.northern-crops.com/technical/fallingnumber.htm). A low number indicated less viscosity and greater sprout damage, and a larger number indicated healthy grain with less/no damage.

3.2.7 Data Analysis

Data from the experiment were subjected to analysis of variance using the 2003 PROC ANOVA procedure in Statistical Analysis System software (SAS Institute, Cary, NC). Randomized split-plot design, with environment as main plot and cultivar as sub-plot within the main plot, was used for the experimental design in the study. There were a total of fifteen plants representing each of the two genotypes from the four treatment environments. A combined analysis was done over all the treatment environments. The abbreviations cul (cultivar), and trt (treatment) were used as class variables and the influence of cul, trt, and the cul*trt interaction were considered during analysis of various physiological, growth, and yield traits. The statistical model used was

$$Yijk = \mu + \alpha_i + \beta_j + Y_{ij} + e_{ijk}$$

Six replicated measurements were taken for the physiological traits from the five tagged pots of each genotype. Growth and yield traits were measured on three plants from each of the five tagged pots, that is, fifteen plants per genotype per treatment. Standard errors of the means were calculated for all variables to represent the variability.

RESULTS

The mean daily temperatures during the experiment for the environments of HT/D, HT/I were 27.0, and 26.3°C and those for the treatments of OT/D, and OT/I were 13.9, and 12.3°C respectively. Relative humidity in the growth chambers was 65±5% for all environments and it was the same during the day time and the night time. The mean of soil moisture measurements taken during the experiment at the different environments of HT/D, HT/I, OT/D, AND OT/I were 26.8, 44.2, 28.2, and 45.1 respectively. The mean leaf relative water content was 0.5060, 0.8020, 0.7986, and 0.8418 at the above mentioned respective environments.

3.3.1 Phenology

In both cultivars, the duration to various phenological stages during the plant development, from the time the plants were taken out of vernalization and moved into the environments, were similar. It was 35 and 37 d, 40 and 42 d, 47 and 49 d, and 53 and 55 d for emergence of boot leaf, emergence of the spike, flowering, and seed-set for Heyne and KS01HW163-4, respectively.

However, at seed-set after the temperature and drought treatments were started there was significant difference in the duration of time to reach physiological maturity in all the environments. The interaction between environment and cultivar was not significant. Plants reached physiological maturity at 70 d, 72 d, 78 d and 90 d under the environments of HT/D, HT/I, OT/D, and OT/I respectively. Exposure to drought alone decreased the duration of seed-set by 12 d, exposure to high temperature alone by 18 d and exposure to high temperature accompanied with drought decreased seed filling duration by 20 d as compared to the optimal conditions.

3.3.2 Analysis of Variance (ANOVA) Table

Data from the analysis of variance table indicates that the responses of both cultivars, for most physiological, growth, and yield traits were very similar with no significant differences. Plant height, total dry weight, seed dry weight, and harvest index were the traits that differed between the cultivars (Table.3.1). However, the influences of environment, and interaction between cultivar and environment (CXE) were significant on most traits. Chlorophyll fluorescence, plant height, total dry weight, seed dry weight, and harvest index were significantly different among cultivars (Table.3.2).

3.3.3 Physiological Traits

Both the cultivars responded similarly to all physiological traits (Table.3.2). However, there were significant effects of temperature and/or drought on most of the physiological traits (Fig.3.1). Leaf temperature was 25±3°C in the HT environments as compared to the 13±2°C in OT environments (Fig. 3.1A). Influence of combination of HT/D stresses on leaf temperature was similarly to that of HT alone however was greater than D alone. Leaf chlorophyll content was 10 and 12 % lower under HT and combination of HT and D respectively (Fig. 3.1C). There was no effect of drought on chlorophyll content. Maximum chlorophyll florescence was observed at combination of HT/D, followed by HT alone and drought alone. Plants from HT/D environment indicated greatest damage to the thylakoid tissue (40% greater as compared to the OT/I) followed by those at HT alone, and D alone (Fig. 3.1D). Interaction of HT/D had significantly harmful influence on chlorophyll content, relative water content (RWC), and chlorophyll florescence (Fig. 3.1C, D, E). It was also understood that HT alone had greater influence than drought alone on all physiological traits.

Cultivar by environment responses indicated that the cultivars responded similarly for all treatments environments except at OT/D (Figure. 3.2A-E). Cultivar KS01HW163-4 showed greater RWC and lower chlorophyll fluorescence, and chlorophyll content compared to cultivar Heyne.

3.3.4 Growth and Yield Traits

The cultivars did not differ in the number of tillers produced, but plant height was significantly higher in Heyne as compared to KS01HW163-4. Though environment did not have a significant influence on plant height, tiller number was reduced by 35% in plants under combination of stresses HT/D environment as compared to those at OT/I (Figure.3.3A, B).

When yield traits are considered, Heyne had a higher seed dry weight, seed number, and harvest index as compared to KS01HW163-4 (Table.3.2). Different treatment environment did not influence total dry weight. However, seed dry weight, seed number, and harvest index were significantly influenced by the different environments (Fig. 3.3D, E, F). Decrease in seed number, seed dry weight and harvest index due to HT alone was 2%, 41%, and 42% respectively, where as by D alone was 36%, 13%, and 12% respectively as compared to the optimal conditions. However, combination of HT/D stresses together decreased seed number, seed dry weight, and harvest index by 39%, 46%, and 44% respectively.

When cultivar by environment responses are considered for growth traits, cultivars responded differently for some traits (Figure. 3.4). Differences in tiller number were seen only at OT/D environment with Heyne showing (2-3) more number of tillers as compared to KS01HW163-4. When yield traits are considered, cultivars responded

differently at all environments for total dry weight and harvest index (Fig. 3.4 C, F). Heyne showed (approx. 10-15%) greater total dry weight under HT/D, and KS01HW163-4 showed (12%) greater total dry weight at OT/I treatment. Higher (25%) seed dry weight was observed in KS01HW163-4 at OT/D (Fig. 3.4 D). For both cultivars, harvest index was increased as the stress decreased, and Heyne showed a greater harvest index under all environments. The percentage difference was greater (30-35%, HT/I-OT/I) under irrigated treatments as compared to that (10-15%, HT/D-OT/D) under drought. There was no variation in seed number between the cultivars.

Grain filling rate, and grain filling duration were also influence in the plants under stress. The plants under the three treatments of either high temperature, or drought, or both were accelerated towards physiological maturity as compared to the plant under optimal conditions. Grain filling duration was decreased by 20, 18, and 12 d in HT/D, HT/I, and OT/D treatments respectively as compared to the grain filling duration in plants at OT/I.

3.3.5 Germination/Sprouting Percentages

Cultivars did not differ significantly in their germination ratios. When measurements were averaged by treatment, germination percent was not significantly different between the two temperatures treatments; however there was greater germination under irrigated treatments (5%) as compared to those at drought. Interaction of HT and D also showed decreased germination (5%) as compared to OT/D (Fig.3.5). Day-wise germination ratios, when considered under cultivar by environment interaction, indicated higher rate of germination and lower levels of PHS tolerance in cultivar KS01HW163-4, the tolerant cultivar, under drought treatments. Where as

cultivar Heyne, the susceptible cultivar, showed higher germination rate under irrigated treatments (Fig.3.6). High temperature alone did not influence PHS of cultivars.

3.3.6 Falling Number Value

Responses from interaction between treatment and cultivar indicated grain from OT/I treatment showing higher falling number (FN) value over grain from OT/D (Table.3.3). However, grain from cultivar KS01HW163-4 showed a lower FN value over grain from cultivar Heyne under the stressed condition as opposed to optimum condition where grain from KS01HW163-4 showed a greater FN value. Due to inadequacy in grain available from the high temperature treatment regime, the FN values could not be determined.

DISCUSSION

Nyachiro et al. (2002) reported dormancy differences in genotypes due to varying germination temperatures and concluded that lower temperatures favor quick germination and lower dormancy. However in the current study, keeping the temperature during germination constant, temperature variation was applied during grain development to study the influence of stress on germination percent. Results of the study demonstrated that high temperature and/or drought during the final stage of grain development (grain filling) not only alter the levels of sprouting tolerance in different genotypes but also lead to yield losses.

The observations from the current study showed higher chlorophyll fluorescence measurements at high temperature regime which is indicative of damage to the chlorophyll tissue and thylokoid membrane. The influence of stress is reflected in the

photosynthetic organelles and in the thylokoid membrane activity. High temperature influences the membrane photochemical stability, damages photosynthetic tissue, and thus decreased photosynthesis (Santarius and Muller, 1979; Berry and Bjorkman, 1980; Al-Khatib and Paulsen, 1999). Similarly, lower chlorophyll content at the high temperature treatments in the current study suggests loss of chlorophyll tissue. High temperature and/or drought stress led to loss of existing chlorophyll tissue and decreased its synthesis (Tewari and Tripathy, 1998). This could be due to membrane damage (Ristic et al., 2007) or leaf senescence (Prasad et al., 2008).

Many photosynthetic processes are adversely affected by supra-optimal temperatures, due to the reduction of activity of ribulose bisphosphate carboxylase and many other enzymes in the chloroplast and damage to the photosynthetic membranes and organelles in the chloroplast. With drought, photosynthesis declines (Al-Khatib and Paulsen, 1984; Paulsen, 1994) and photorespiration and dark respiration increase (Lowlor, 1979). Such conditions in the leaf tissue during grain filling result in reduction of photosynthesis and leaf area. This accelerates senescence and leaf deterioration and leads to disruption of the transport capacity of the PSII system (Harding et al, 1990).

Temperature/drought stress often decreases seed number per spike (Prasad et al., 2008). However in the current study decrease in seed number was more obvious in treatments of drought stress under the two temperature regimes as compared to the irrigated treatments. Since the stress treatment was started after seed-set in the current study, the influence of this was expressed by the decrease in the subsequent grain dry weight and harvest index. Grain dry weight and harvest index decreased as the intensity of stress increased. Grain filling in wheat is dependent on the following three factors: 1)

production of photosynthates by the leaves and stem, 2) mobilization of these carbohydrates and nitrogen compounds within these organs and their transportation to the spike and the growing caryopsis, and 3) production of photosynthates by the ear (Plaut et al, 2004). All or one of these processes might have been decreased in this study.

Negative effects of high temperature during plant growth and grain development were studied in crops like wheat (Saini et al., 1983), rice (Prasad et al., 2006a), sorghum (Prasad et al., 2006b), and peanut (Prasad et al., 2003). Stress after anthesis accelerates growth and reduces the duration of grain filling, which reduces kernel dry weight at maturity (Aggarwal and Sinha, 1984; Khanna-Chopra et al., 1994; Wardlaw and Willenbrink, 2000). In the current study the duration of grain filling was decreased significantly and almost similarly by HT stress as well as by the combination of HT and D stress. Grain filling duration was greater under stress due to drought alone, as compared to that under HT alone (6 d) or that under interaction of HT and D (8 d).

Reduction in the production and utilization of plant solutes like glucose, coupled with increase in rate of evapotransportation (Gates, 1968), affects plant osmotic potential. The expression of these stresses is seen in the developing kernel by their impact on yield, grain size, weight, and number, and on flour quality (Altenbach et al, 2003). In the current study there was significant influence of HT and D on seed number, seed dry weight and harvest index. Seed number was decreased by 2, 36, and 38 % by high temperature, drought and combination of HT/D respectively as compared to optimal conditions, where as the corresponding decrease in seed dry weight was 41, 13, and 46 % and in harvest index was 42, 12, and 44 %. Yield data from the current

study suggests that influence due to exposure to combination of stresses is more negative than that to either HT or D stress alone. Also HT stress influence seed dry weight and harvest index greatly but not seed number where as D stress influenced seed number significantly.

Based on the results of this study, the optimum temperature regime favored relatively higher germination as compared to the higher temperature regime. Although HT induced tolerance to PHS, under drought stress environment, tolerance to PHS decreased in the resistant cultivar (KS10HW163-4) as compared to the susceptible cultivar (Heyne). This is indicative of the fact that the cultivars tested showed greater tolerance under HT which could be due to the excessive secretion of ABA during stressed grain development. However, the lack of tolerance to PHS under drought when temperature was optimum could not be explained by this experiment and needs further study.

The rate of photosynthesis and the chlorophyll content in the leaf are negatively correlated with ABA. Further, greater amounts of ABA in the stems and leaves reduce transport of sucrose into the grain and lower the grain's ability to synthesize starch. Decrease in duration of seed filling due to accelerated growth towards maturity in the stress treatments, decrease in production and transfer of photosynthates from the senescing leaves to the sinks, and shrinkage of the sink cells due to drying, all attributed to the decrease in the starch and protein accumulation in the grain. With lower amounts of starch in the wheat grain, lower α-amylase activity is observed and lower is the germination and higher is the falling number.

However, it was also observed that under the drought treatments, in both the temperature regimes, the dormancy levels of the two cultivars were altered. The cultivar susceptible to PHS showed greater levels of grain dormancy as compared to the resistant cultivar. The higher FN value of Heyne over that of KS01HW163-4 at the OT/D treatment also emphasizes and reiterates this finding.

In conclusion temperature/drought stress in the current study decreased yield. Response of tolerance to sprouting was altered in the cultivars tested, but over all germination was decreased in the stress treatments.

Table 3.1. Significance of various treatments and their interaction on physiological, growth, yield and germination traits.

TRAIT	Cultivar (C)	Environment(E)	C X E
Leaf temperature (°C)	NS	***	**
Chlorophyll Content (SPAD units)	NS	***	*
Soil Moisture	**	***	*
Relative Water Content	*	*	NS
Chlorophyll Fluorescence (Fo/Fm)	NS	***	**
Plant Height (cm)	***	NS	NS
Tiller Number (plant^{-1})	NS	*	*
Total Dry Weight (g plant^{-1})	*	*	NS
Seed Dry Weight (g plant^{-1})	***	***	**
Harvest Index (%)	**	***	*
Seed Number (plant^{-1})	NS	*	**
Germination Rate	NS	NS	*

***, **, *, corresponding to P< 0.001, 0.01 and .05 significance levels.

Table 3.2. Influence of cultivar on physiological, growth, and yield traits. Data are means of temperature and drought treatments.

TRAIT	KS01HW163-4	Heyne	LSD
Leaf temperature (°C)	19.83^{A}	19.37^{A}	NS
Chlorophyll Content (SPAD units)	48.18^{A}	47.52^{A}	NS
Soil Moisture (%)	38.01^{A}	36.16^{B}	1.2024
Relative Water Content (ratio)	0.3846^{A}	0.3931^{A}	NS
Chlorophyll Fluorescence (Fo/Fm)	0.78^{B}	0.74^{A}	0.0356
Plant Height (cm)	44.03^{B}	52.78^{A}	1.8686
Tiller Number (plant^{-1})	15.15^{A}	15.9^{A}	NS
Total Dry Weight (g plant^{-1})	11.98^{B}	12.98^{A}	0.908
Seed Dry Weight (g plant^{-1})	4.14^{B}	5.87^{A}	0.524
Harvest Index (%)	0.35^{B}	0.46^{A}	0.0422
Seed Number (plant^{-1})	124.85^{A}	133.65^{A}	NS
Germination Ratio	0.98^{A}	0.96^{A}	NS

Table 3.3. Falling number (FN) values of the two cultivars at various temperature and drought treatments. High Temperature/Drought (HT/D), High Temp/Irrigated (HT/I), Optimum Temp/Drought (OT/D), and Optimum Temp/Irrigated (OT/I) represent the different treatment environments.

Cultivar	Environment	Falling number (FN)
Heyne	HT/D	Not Analyzed (NA)
Heyne	HT/I	NA
Heyne	OT/D	349
Heyne	OT/I	431
KS01HW163-4	HT/D	NA
KS01HW163-4	HT/I	NA
KS01HW163-4	OT/D	277
KS01HW163-4	OT/I	440

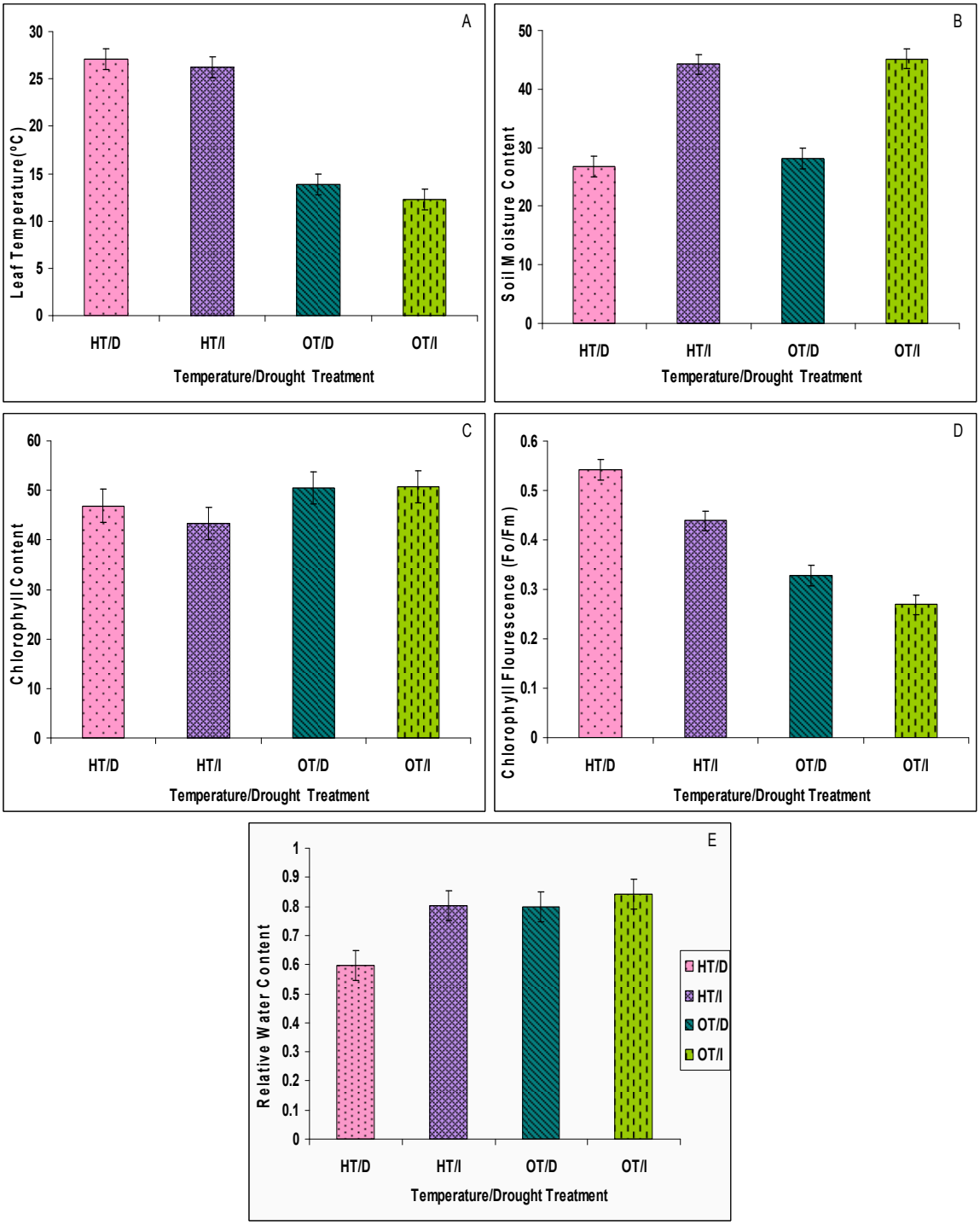

Figure 3.1. Influence of environment on physiological traits. Data are means of cultivars. Error bars provide LSD for comparison of treatment means.

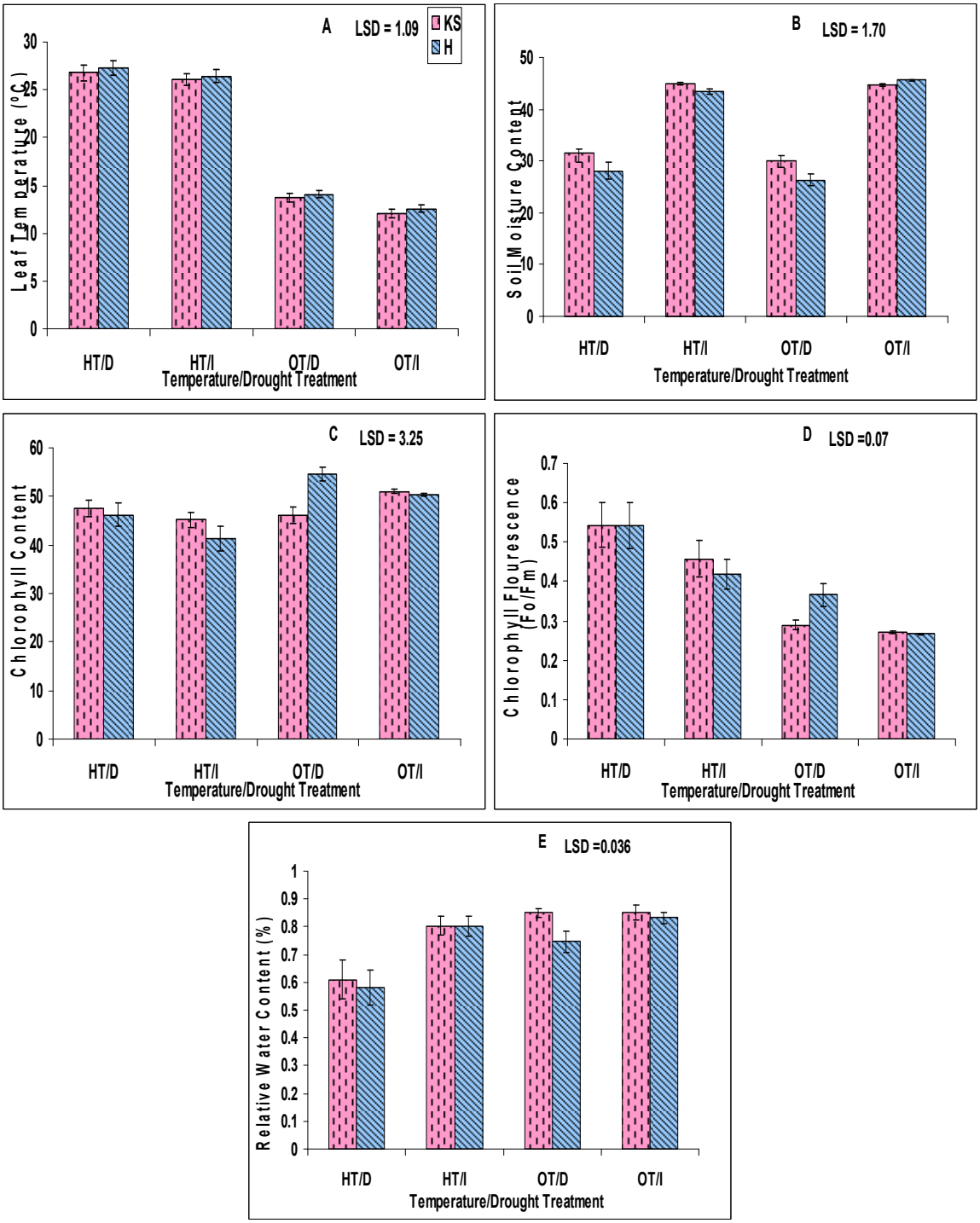

Figure 3.2. Influence of cultivar [KS01HW163-4 (KS) and Heyne (H)] by environment interaction on physiological traits. Data are means of five replicated measurements. Error bars provide SE and the LSD value is given in the box for comparison of treatment means.

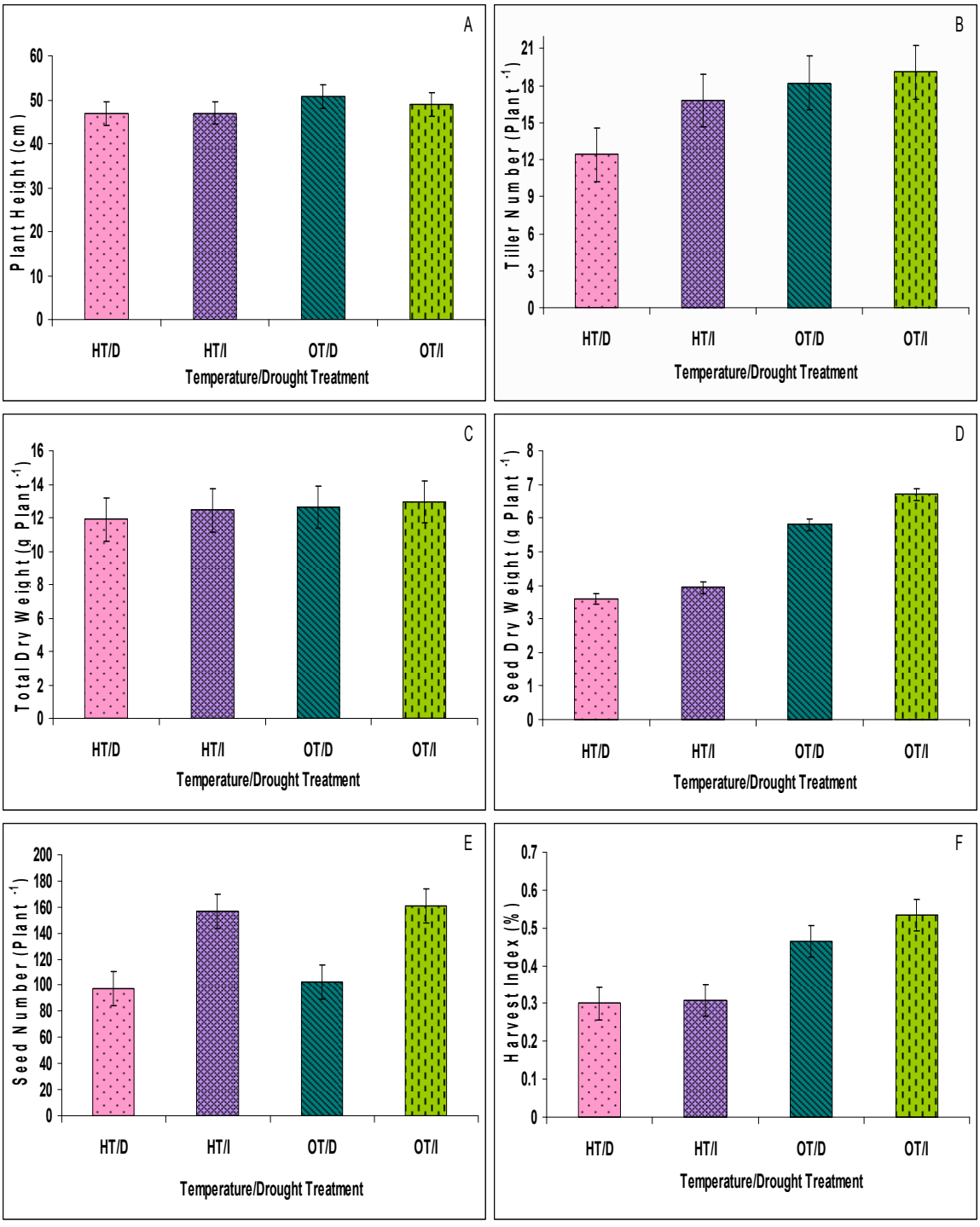

Figure 3.3. Influence of environment on growth and yield traits. Data are means of cultivars. Error bars provide LSD for comparison of treatment means.

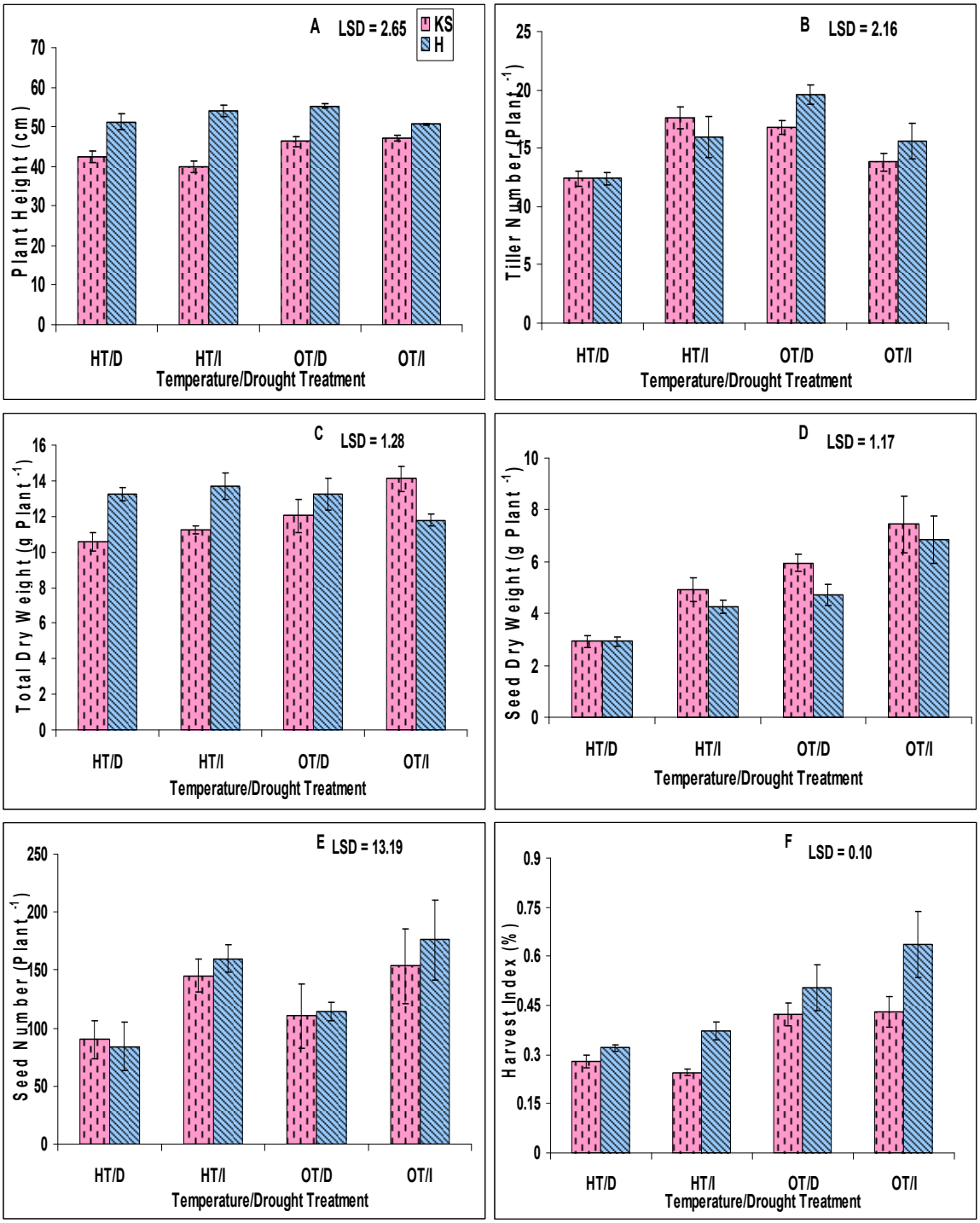

Figure 3.4. Influence of cultivar [KS01HW163-4 (KS) and Heyne (H)] by environment interaction on yield traits. Data are means of five replicated measurements. Error bars provide SE and the LSD value is given in the box for comparison of treatment means.

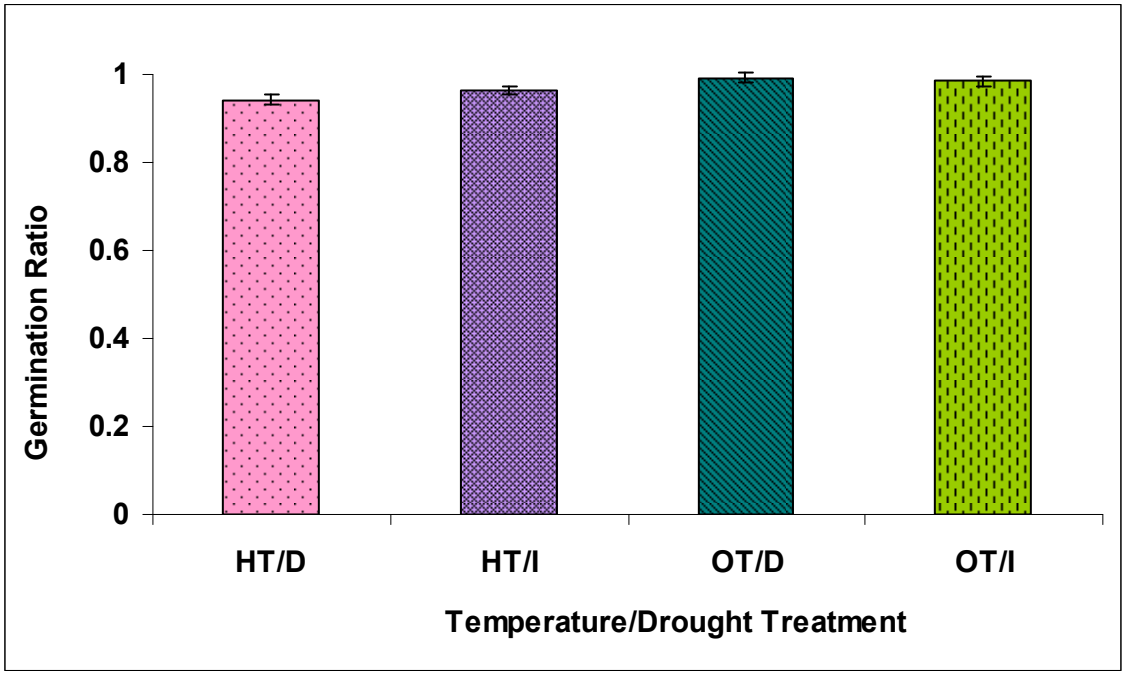

Figure 3.5. Influence of environment on germination rate. Data are means of cultivars. Error bars provide LSD for comparison of treatment means.

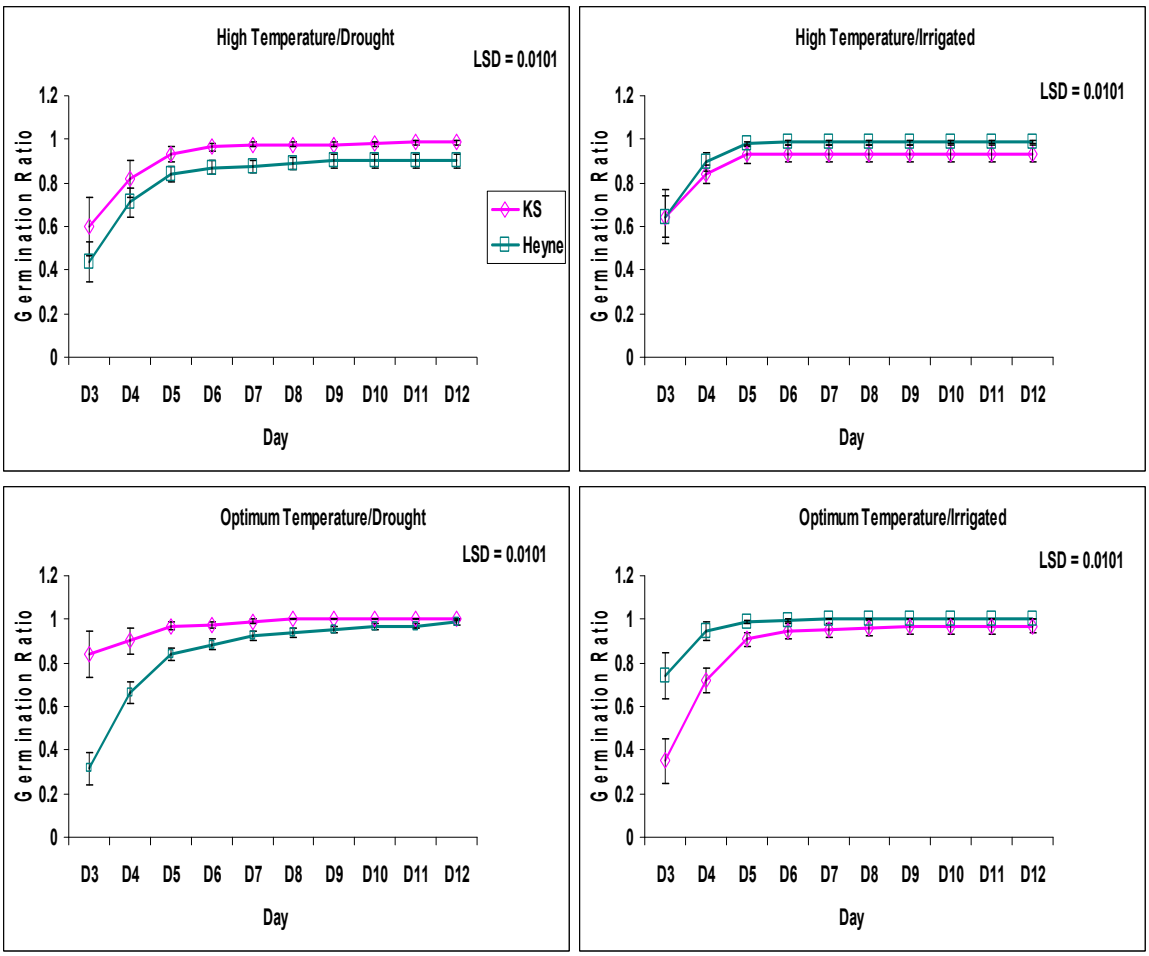

Figure 3.6. Influence of cultivar [KS01HW163-4 (KS) and Heyne (H)] by environment interaction on germination rate. Data are means of five replicated measurements. Error bars provide SE and the LSD value is given in the box for comparison of treatment means.

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CHAPTER 4 - Influence of Exogenous Application of Growth Hormones on Pre-harvest Sprouting

ABSTRACT

Pre-harvest sprouting (PHS) is a problem affecting hard white winter (HWW) wheat causing yield loses. The condition where grain germinates on the spike before it is harvested is called PHS. A complex phytohormonal interaction, in addition to environmental factors, brings about PHS. The current study is conducted on KS01HW163-4 (PHS tolerant) and Henye (PHS susceptible) - two HWW wheat cultivars. Objectives were to study the influence of (a) exogenous application of growth hormones, and (b) interaction of hormones and stress environments, on PHS. Plants were treated with either Gibberellins (GA) or abscisic acid (ABA) or paclobutrazol or no hormone twice - at flowering, and at a week after seed set. Data on phenology (time to panicle emergence, flowering and maturity), physiological, growth, and yield traits were determined. Germination percentages and falling number tests were conducted on the harvested seed. Influence of growth hormones was not significant on physiological and growth traits of both cultivars. However, rate of germination in both cultivars was influenced by application of growth hormones. Application of GA increased germination percentage germination across both cultivars and all environments. Paclobutrazol showed lowest germination percentages across all stressed treatments. Tolerance to sprouting was seen in seeds of plants from ABA and paclobutrazol treatments where as those from GA treatment showed susceptibility to sprouting. Temperature/drought stress in the current study also decreased yield similar to that observed in the previous study.

INTRODUCTION

Hard white winter wheat is grown in many countries of the world like Australia, China, Russia, and countries of South America, where the climate is favorable. With the growing demand for white wheat compared to red wheat, the U.S. wheat industry is interested in augmenting the production of HWW wheat (KACC, 2005). Greater emphasis is being made on increasing production of hard white wheat in the U.S., so it will not lose its markets to other countries (KSRE News, 2005). But, poor productivity and reduced yields have been recorded with certain frequency in these regions of the world which can be frequently attributed to pre-harvest sprouting (PHS). Although PHS has been an intermittent problem in Kansas, it has been occurring with some rate of recurrence.

In a study made on pre-harvest sprouting in sorghum, it was found that the susceptible varieties showed lower dormancy levels from an early stage of development compared to the resistant varieties (Steibach et al., 1995). The embryo and the seed coat are the two components that control PHS tolerance. Abscisic acid (ABA) in the embryo inhibits germination, and the seed coat prevents flow of water and oxygen into the seed (Biddulph, 2005). Abscisic acid is a growth inhibitory hormone (Tanner, 1980; Walton 1980), and GA are growth stimulatory hormones, and they are associated with the process of grain development, dormancy, and germination.

In addition, stresses like heat and water stress increase the production of ABA in leaves (Ober and Setter, 1990; Westgate, 1996), floral organs (Saini and Aspinall, 1982; Westgate, 1996), and developing grains (Goldbach and Goldbach, 1977; Haeder and Beringer, 1981; Ober et al., 1991). This may lead to reduced grain filling and loss of

viability of the grain (Trewavas and Jones, 1991). Earlier studies emphasized the role of ABA in development of dormancy induction in seeds and the role of GA in germination promotion in non-dormant seeds. Levels of GA required for germination at maturity are directly proportional to the levels of ABA in the seed during development, which implies that greater amounts of ABA lead to non-dormancy (Hilhorst and Karssen, 1992; Karssen, 1995).

The amount of ABA present in the grain diminishes when it reaches desiccation. It decreases while dry matter accumulates (Schopfer and Plachy, 1985). The kernel gradually becomes insensitive to ABA, which is the positive regulator of dormancy induction and possibly maintenance. The kernel becomes sensitive to GA showing a transition from a dormant to a non-dormant condition. Unlike GA, ABA inhibits germination and enzymatic activity in the mature grain. It was found that, after the dormancy-inductive influence of ABA is completed, GAs act as promoters of germination and growth. The activity levels of GA are resumed when the grain comes into contact with water.

The sites and times at which ABA and GA work are different (Karssen and Lacka, 1986). Gibberellins play an important role in dormancy release. However, biosynthesis of GA during seed growth aids in seed development, including fertilization, embryo growth, reserve mobilization, and fruit growth. But it does not establish primary dormancy in seeds, like ABA does. Evidence of reserve mobilization from the endosperm to the embryo has been long been confirmed. However, the involvement of GA in fostering this process is a recent discovery (Brown and Morris, 1980). Paclobutrazol is a plant growth retardant that blocks GA biosynthesis and has been

used in studies on influence of GA in α-amylase production in wheat (Garcia-Maya et al., 1990). In the current study paclobutrazol is applied to plants at anthesis and also at seed set to understand the influence of GA retardation on PHS.

When exposed to high rainfall at physiological maturity and pre-harvest stage, grains germinate in the spike leading to rapid release of enzyme α-amylase by the aleurone layer and the embryo, which results in the digestion of starch resulting in pre-harvest sprouting (Mares, 1989). The current project, which was carried out in growth chambers to understand the influence of exogenous application of growth hormones on PHS. Two HWW wheat cultivars, 'KS01HW163-4,' a sprouting tolerant and 'Heyne,' a sprout susceptible cultivar were used in the study. The specific objectives of this research were:

1) Understanding the influence of exogenous application of growth hormones accompanied by HT and/or drought environments on regulating PHS in HWW wheat
2) Studying the possibilities of using exogenous application of germination retardant to increase dormancy duration in HWW wheat.

MATERIALS AND METHODS

The study was conducted in controlled environmental growth chambers of the Department of Agronomy at Kansas State University in the spring of 2007. Two HWW wheat cultivars, one sprout-resistant, 'KS01HW163-4,' and the other sprout-susceptible, 'Heyne, were used in the experiment. The study was done to determine the influence of exogenous application of growth hormones [growth stimulants (gibberillic acid; GA); GA-inhibitor (paclobutrazol) and germination retardant (abscisic acid ABA)] on dormancy induction, and germination. The experiment was conducted in conjunction with

application of varying environmental conditions of temperature, drought, and combination of temperature and drought during grain development. The study involved hormone by environment interaction to understand the combined influence of hormone and different environmental conditions on grain development, and on the subsequent process of germination.

4.2.1 Plant Husbandry and Growth Conditions

Seeds from control treatments of Experiment 2, described in the previous chapter, were used in this experiment. Seeds of the two HWW wheat cultivars, 'KS01HW163-4' and 'Heyne,' were thoroughly cleaned before sowing. Seeds were sown by hand in perforated plastic trays filled with soil (Metro Mix 200, Hummert International, Topeka, Kansas). The plastic trays were then placed in a metal tray filled with water. The metal tray was watered frequently enough to keep the soil in the plastic tray wet and to give a continued supply of water to the seeds and the seedlings that eventually developed. A week after emergence, the plastic tray with plants was moved to a vernalization chamber run at 4°C. The plants were kept at the vernalization temperature for six weeks. Immediately after being taken out of the vernalization chamber, the tray was placed in a corridor of the greenhouse, where the temperature was about 12 – 15°C, to let the plants get acclimatized to the temperature change.

The plants were then transplanted into plastic pots at a soil depth of 5 cm in the pot. Each pot had a diameter of 21 cm, and 16 cm at the top, and bottom respectively. The height of each pot was 20 cm. Three plants were put in each pot. Soil used as rooting medium consisted of a potting mix (Metro Mix 200, Hummert International, Topeka, Kansas). Osmocote Plus (15-9-12, N-P_2O_5-K_2O) controlled release fertilizer

(Scotts-Sierra Horticultural Products Company, Maryville, Ohio) was mixed to the soil at the rate of 15 g per pot before transplanting.

Four growth chambers (Conviron Model E15, Winnipeg, Manitoba, Canada) were used in the experiment. Pots were moved into the four growth chambers that were set at same temperature, and humidity. Pots were watered regularly. Each pot was considered a replication, and there were 16 pots of each cultivar in each growth chamber making a total of 32 pots per chamber. The chambers were run at an optimum temperature of 20/15°C day/night with 16/8 hours day/night. They were maintained at 85% humidity.

At flowering (Feekes stage 10), the two genotypes in all chambers were exposed to four different hormonal treatments: (1) plants with GA (growth stimulant) applied, (2) plants with paclobutrazol (GA inhibitor) applied, (3) plants with ABA (germination retardant) applied, 4) control – plants with no hormones applied. The hormones were sprayed at a concentration of 50 ppm, and these concentrations were based on concentrations found in literature. There were four replications of each genotype for all treatments in each chamber.

At seed set (i.e., 50% of plants of each cultivar in each chamber had reached seed-set), plants were exposed to two temperature regimes from seed set (Feekes stage 10.1) to physiological maturity: an optimum temperature of 20/15°C and a high temperature of 30/20°C. There were two watering regimes from seed set to physiological maturity: fully irrigated (irrigated every 2 days) and drought stressed (irrigated at 7 d intervals). A second application of the growth hormones was made at seed set. The pots in all the chambers were randomized every 10 days.

4.2.2 Data Collection

Four replications of each genotype under the four hormonal treatments within the four temperature/drought environments were randomly tagged at the beginning of the hormonal treatments. Physiological traits, growth, and yield were measured on these tagged plants. Data on phenology (time to booting, flowering, seed-set, physiological, and harvest maturity) was recorded during the period of plant growth. After harvest, data on plant biomass, and grain yield were recorded. Germination rates, and falling number values were recorded.

4.2.3 Physiological Traits

Physiological measurements were taken on the fully expanded flag leaf of the tagged plants at ambient CO_2 and growth temperatures at noon or during the early afternoon. Measurements were taken 0, 2, 4, 6, 8, 10, and 12 days after the beginning of the treatments. Physiological measurements included leaf chlorophyll content, leaf temperature, and chlorophyll fluorescence. Leaf chlorophyll content was measured using a self-calibrating chlorophyll meter (SPAD, Model 502, Spectrum Technologies, Plainfield, IL, USA). Leaf temperature was measured using an infrared thermometer (OS534 handheld infrared thermometer, Omega Engineering, Inc., Stamford, Connecticut, USA) by shooting a beam of infrared light on the topmost fully expanded leaf exposed to light. Chlorophyll fluorescence, which is a measure of the damage caused to the thylakoid membranes in the leaf tissue, was measured using a fluorometer (OS 30, OptiScience, Hudson, NH, USA). Data loggers (HOBO U12 Temp/RH/Light/External Data Logger, Onset, Bourne, MA, USA) were set in the growth chambers to record the humidity, temperature, and light intensity.

4.2.4 Growth and Yield Traits

Physiological maturity was recognized by clearing of green and appearance of yellow just below the spike. At harvest, plant height (base of the plant to the tip), tiller number, and ear number were recorded from all the three plants in the four pots of each genotype within all treatment. The harvested tillers were dried at 65°C for 10 days, and the spikes were dried at 28°C for 7 days. Dry weights of the tillers and spikes were collected, and the total dry weight (tiller + spikes) was calculated. Spikes were hand-threshed, and grain number and grain dry weight were recorded. Harvest index was calculated as the ratio of grain dry weight over total dry weight (above ground biomass).

4.2.5 Germination Percentages

At harvest, grains from each replicated pot were bagged separately, dried, and stored at -24°C. All samples were harvested, dried, and threshed, and seeds were bagged and stored in the freezer. To carry out the germination test, bags were thawed and 20 seeds from each replication were taken. Germination tests were done in petri dishes that were cleaned with doubled distilled water and 70% ethanol to create aseptic conditions and to prevent fungal and bacterial growth. Seeds were placed on a germination paper in the clean petri dishes. Water was sprayed on the seeds until the paper was completely wet, but no excess water was left in the petri dish. Petri dishes were placed in a chamber running at 20°C. Germinated seeds were counted and removed starting on the third day after the beginning of germination until the twelfth day. Cumulative day-wise germination percentages were calculated.

4.2.6 Falling Number Test

The Hagberg Falling Number (HFN) test was carried out by using 7 grams of the flour obtained from the grain (Sodkiewicz, 1999). Falling number is obtained using a macine (Falling Number 1800; Perten Instruments, Sweden). Flour was put in the tubes, and 25 ml of distilled water was dispensed into each tube. They were shaken vigorously for 20 times to allow for complete blending of water, and flour. The tubes were placed in a hot water bath immediately. When the machine was switched on, stirrers rigorously stirred the flour and water for 60 seconds for thorough blending and formation of slurry. The stirrers were then left at the top of the slurry to fall freely to the bottom of the tube. Falling number directly corresponds to the number of seconds taken for the stirrer to reach the bottom of the tube. A low number indicates less viscosity and greater sprout damage, and a large number indicates healthy grain with less damage.

4.2.7 Phytohormone Analysis

At 7 days after spraying, leaf samples were collected from both cultivars under the four hormonal treatments, but only from three treatment environments. The plants in the chamber with high temperature and drought dried out and reached maturity faster, leaving no time to collect the leaf samples. The collected leaves from the other environments were immediately stored in liquid nitrogen and kept at -80°C.

4.2.8 Data Analysis

Data from the experiment were analyzed by analysis of variance using the 2003 PROC ANOVA procedure in Statistical Analysis System software (SAS Institute, Cary, NC). Randomized split-split plot design, with environment as main plot, cultivar as sub-

plot and hormone treatments as sub-sub-plot, was used for the experimental design in the study. There were a total of four replicated pots representing each of the two genotypes for the each of the four hormonal treatments within the four temperature/drought treatments. Plants from these pots were used for measuring physiological traits. A combined analysis was done over all the treatment environments. The abbreviations cul (cultivar), and trt1 (temperature/drought treatment) and trt2 (phytohormone treatment) were used as class variables and the influence of cul, trt1, trt2 and the influence of interactions - trt1*cul, trt2*cul, and trt1*trt2 were considered during analysis of various physiological, growth, and yield traits. The statistical model used was

$$Yijk = \mu + \alpha_i + \beta_j + Y_{ij} + e_{ijk}$$

There were four replicated measurements of each trait. Growth, and yield traits were measured on the four pots (three plants in each pot, that is, twelve plants per genotype per treatment) under each treatment. Standard errors of the means were calculated for all variables to represent the variability.

RESULTS

The treatments of HT/D, HT/I had mean daily temperatures of 20.6 and 19.3°C where as the treatments of OT/D and OT/I had 16.8 and 15.5°C respectively. Relative humidity on all chambers was set at 85±5%.

4.3.1 Phenology

The duration to various phenological stages from when the plants were taken out of vernalization chamber until seed-set was similar in both cultivars and in all

treatments. It took 41 and 39 d, 47 and 45 d, 52 and 50 d, 60 and 58 d for boot leaf appearance, spike emergence, flowering, and seed-set in the cultivars of Heyne and KS01HW163-4 respectively.

After the temperature and hormonal treatments were started from seed-set, the time to reach physiological maturity was significantly different in treatments at high temperature as compared to optimum temperature. The interaction between cultivar and treatment were not significant. Plant reached physiological maturity at 87, 92, 98, and 110d in HT/D, HT/I, OT/D, and OT/I treatments respectively. Exposure to HT/D, HT/I, and OT/D reduced grain filling duration by 23, 18, and 12 d respectively.

4.3.2 Analysis of Variance (ANOVA) Table

The responses of cultivar to most physiological, growth, and yield traits were significantly different. For leaf temperature, tiller number, seed number, and germination rate however the traits responded similarly (Table.4.1). Responses of environment averaged over both cultivars, and phytohomones, for all traits, were significant except for tiller number. All measurements averaged by hormone across all environments and cultivars showed similar responses for most traits except for leaf chlorophyll content, plant height, seed number and germination rate. Interaction between environment and cultivar, and interaction between environment and phytohormone showed significant differences for all traits. However, the responses from interaction between phytohormone and cultivar were similar showing no significance for any traits except for seed number (Table. 4.1). The three way interaction between cultivar, environment, and phytohormone was not significant.

4.3.3 Cultivar

When physiological traits were considered with responses averaged across both treatments (environment and phytohormone) by cultivar, plants of cultivar Heyne had lower chlorophyll content, and chlorophyll fluorescence as compared to plants of cultivar KS01HW163-4 (Table 4.2).

Analysis from growth and yield traits showed plant height, seed dry weight, and total dry weight being lower, but harvest index being higher in cultivar Heyne as compared to plants of cultivar KS01HW163-4 (Table 4.2).

4.3.4 Environment

When responses were considered by environment alone, leaf temperature, and chlorophyll fluorescence responded similarly, there was a consistent decrease in the intensity of the traits from HT/D-HT/I-OT/D-OT/I (Fig. 4.1A-C). There was a 5°C increase in temperature and 30% increase in chlorophyll fluorescence in HT/D as compared to the OT/I. Chlorophyll content was (8%) lower at high temperature environments as compared to that at OT/I.

When growth, traits were considered, exposure to HT increased tiller number by 25% when compared to the OT, but plant height was not effected (Fig. 4.2A,B). Similarly, among the yield traits, decrease in seed number (50%), seed dry weight (40%) and harvest index (48%) was observed under HT/D as compared to OT/I (Fig. 4.2D, E, F). Total dry weight was lower (15%) in drought treatments as compared to irrigated treatments.

4.3.5 Phytohormone

When similar analysis was done by phytohormone treatment, plants treated with ABA showed slightly lower (8%) chlorophyll content and plants treated with GA had lower (4%) chlorophyll fluorescence when compared to the other three treatments. No significant differences were observed in leaf temperature among treatments (Fig. 4.3A-C).

Within growth and yield traits, there were no significant differences among the four phytohormone treatments for total dry weight, seed dry weight, and harvest index (Fig. 4.4A-F). However, seed number was higher (10%) in plants treated with GA as compared to the other treatments.

4.3.6 Cultivar by Environment Interaction

The interaction of cultivar by environment showed no significant cultivar differences in the optimum temperature regime for any of the physiological traits (Fig. 4.5A-C). However, within the high temperature regime, KS01HW163-4 showed lower chlorophyll content (12%) and chlorophyll fluorescence (25%) as compared to Heyne. Leaf temperature was not affected by cultivar by environment interaction.

Significant differences were seen between cultivars in response to stress for plant height. Heyne had higher (15-25%) plant height as compared to KS01HW163-4 under all environments (Fig. 4.6A,B). Tiller number was different between cultivars only at OT/D with Heyne showing a lesser (19%) tillers. For yield traits, cultivar Heyne had significantly higher total dry weight in the irrigated treatments (30%) as compared to the drought (15%) (Fig. 4.6C-F). Under all environments, seed dry weight was higher (14-20%) in Heyne. However, KS01HW163-4 showed a greater (22-26%) harvest index

under all environments except OT/D where the cultivars responded similarly (Fig. 4.6F). The cultivars did not differ in seed numbers.

4.3.7 Cultivar by Phytohormone Interaction

The interaction of cultivar by phytohormone indicated lower chlorophyll content (SPAD), and higher chlorophyll fluorescence (Fo/Fm) in KS01HW163-4 under all hormone treatments (Fig. 4.7A-C). The difference between cultivars was higher (26% for Fo/Fm, 12% for SPAD) in paclobutrazol and ABA treatments and lowest (14% for Fo/Fm, 4% for SPAD) in GA treatment. Cultivars responded similarly to leaf temperature.

Higher plant height (10-20%) was observed in Heyne, whereas, cultivars responded similarly to tiller number under all treatments (Fig. 4.8A, B). Plant height difference was least (10%) in paclobutrazol treatment. Among yield treats, total dry weight was higher in Heyne than in KS01HW163-4 (Fig. 4.8C-F). The difference in responses to hormone between cultivars was more significant under ABA, paclobutrazol, and control treatments (28%) as compared to that under GA (13%). Seed dry weight was significantly different between cultivars in ABA (25%), and control (22%) treatments and was higher in Heyne (Fig. 4.8D). Difference in seed number was significant only under influence of ABA (Fig. 4.8E). Greater seed number was observed in Heyne. Cultivar responses were significantly different for harvest index under paclobutrazol (23%) and GA (20%) treatments. Harvest Index was higher in KS01HW163-4 (Fig. 4.6F).

4.3.8 Environment by Phytohormone Interaction

The interaction of environment by phytohormone indicated significant differences for most traits more with respect to environment than with respect to phytohormone treatments within each environment. Also, the trait responses among the four phytohomones within a particular environment could not be very well related to those within the other. Phytohormone treatments within HT/I alone responded differently for leaf temperature (Fig.4.9A). Plants treated with ABA had greater (20°C) leaf temperature as compared to others and those treated with GA had the lowest (18°C). Chlorophyll content was higher and chlorophyll fluorescence lower, in GA treatments as compared to those in ABA treatments of all environments except HT/D (Fig. 4.9B, C).

Responses for growth traits indicated higher plant height among GA, and control treatment applications when compared to those of ABA or paclobutrazol, under all stressed environments (Fig. 4.10A, B). Application of GA increased plant height (7%) when compared to all other treatments at HT/D (Fig. 4.10C). Under OT/I all phytohomone treatments responded similarly for plant height except paclobutrazol where the plant height was lower. Responses for tiller number indicated maximum tillers under paclobutrazol application in HT/D, under control in HT/I, under GA in OT/D, and under ABA in OT/I (Fig. 4.10B).

Plants in control (no hormone) application and plants treated with GA responded similarly to total dry weight under all treatment environments (Fig. 4.10C). Hormone treatments with in all environments responded similarly for seed dry weight except in OT/I where GA and control treatments showed maximum values (Fig. 4.10D). Plants treated with GA had highest seed dry weight and seed number under irrigated

environments (Fig. 4.10D, E). Harvest index was highest among plants treated with GA under all environments (Fig.4.10F).

4.3.9 Germination/Sprouting Percentages

Analysis of germination measurements, when averaged by cultivar across all treatment types indicated similar response in both cultivars (Table 4.2). However, environment had significant influence on germination with plants under OT/I showing maximum germination and those under HT/D showing minimum sprouting (Fig. 4.11). The influence of hormone was also significant. Plants treated with GA showed maximum germination and those treated with paclobutrazol showed minimum germination (Fig. 4.12).

When interaction between cultivar by environment was considered for germination percentages, the cultivars responded similarly, showing greater amount of germination at OT as compared to that at HT. At high temperature regime, Heyne, the susceptible cultivar, showed lower germination ratio than KS01HW163-4. However, at optimum temperature regime, Heyne had a greater germination ratio as compared to KS01HW163-4 (Fig. 4.13). When day-wise germination ratios were looked at, in Heyne, the graph indicated a flip in germination ratio between HT/I and OT/D, with OT/D treated plants showing greater germination until day eight (D8) (Fig. 4.14). However, from D8 through D12 plants from HT/I showed greater germination ratio.

When interaction between cultivar by phytohormone was observed, with in the four phytohormone treatments for Heyne, the plants treated with ABA had maximum germination rates, those treated with control and GA had similar germination and those treated with paclobutrazol had minimum germination. However, for KS01HW163-04, the

plants treated with GA showed maximum germination followed by ABA, control, and then paclobutrazol (Fig. 4.15). In both the cultivars the response to paclobutrazol was similar and the lowest of all treatments. Graph of day-wise germination ratio indicated a greater spread in the ratio at the end of the germination period than at the beginning among all the phytohormone treatments (Fig. 4.16).

When interaction between environment by phytohormone was considered, in the irrigated treatments of both the temperature regimes, plants treated with GA showed maximum germination followed by ABA, and control responding similarly and paclobutrazol showing minimum germination (Fig. 4.17). In the drought treatments however, GA showed maximum germination followed by ABA, control, and paclobutrazol sequentially. Grain from plants treated with paclobutrazol showed minimum germination and those treated with GA showed maximum germination ratios under all treatment conditions.

4.3.10 Falling Number (FN) Value

Due to unavailability of sufficient grain from specific treatment within treatments, conclusions about FN value could not be arrived at (Table 4.3).

DISCUSSION

Results obtained from this study suggest significant differences in the responses among the environments and phytohormone treatments applied. The responses were more significant by environment than by phytohormone treatment. Within each environment, the influence of phytohomones was significant in physiological and yield traits. The influence of high temperature environments as compared to the optimum,

showed intense negative effects on physiological, and yield traits of both cultivars similarly. The influence of environment was similar to that discussed in the previous chapter.

Damage to the chlorophyll tissue, and thylakoid membrane is evident from the increased chlorophyll fluorescence measurements observed at the stress treatments. Within all environments, the ABA treatment showed lower chlorophyll content indicating a possible decrease in the chlorophyll tissue due to increased concentration of ABA in the leaf tissue (Upreti et al., 1997).

Total dry weight was lower in the drought environments as compared to those at irrigated, under all phytohormone treatments indicative of greater remobilization of assimilates from the straw to the grains (Plaut et al., 2004). Under high temperature regime, plants treated with ABA showed lower total dry weight as compared to those at other phytohormone treatments.

Grain filling is closely related to whole plant senescence during environmental stress when the availability of current assimilates from photosynthesis is heavily lowered due to disturbed physiological processes (Yang and Zhang, 2006). In wheat, mobilization of carbohydrates from the stem, leaves, and spike to the grain is enhanced at high temperature, however the limited availability of assimilates to be remobilized causes decrease in grain dry weight.

Seed germination responses to exogenous application of phytohormones differed among environments and genotypes evidencing the role of hormones in controlling germination. Results suggest that irrespective of the genotypes tested, grain from plants treated with paclobutrazol, inhibitor of GA-biosynthesis and ABA showed minimum

germination rates in most stress treatments. Contrastingly, it was also observed that sprouting susceptibility of the grain obtained from plants treated with GA was maximum under all stress treatments. The influence of GA is antagonistic to ABA during seed development and germination (Jacobsen et al., 1995). Role played by GA in induction of germination and seedling development is significant (Thomas and Roddriguez, 1994). It has been recognized to induce genes coding for α-amylase in many species.

Paclobutrazol was used in the experiment to observe the influence of GA-inhibitor, to subsequently understand the involvement of GA in initiating the process of germination. Capacity of the grain to retain sufficient levels of dormancy determines its resistance to PHS (Steinbach et al., 1995). In the absence of dormancy moisture induces degradation of starch by enzyme α-amylase produced due to the hormone GA. The secretion of α-amylase is dependent on the levels of GA released under favorable conditions of moisture, temperature, and humidity. Reduced germination in paclobutrazol treatment in the current study complies with studies of Garcia-Martinez et al., (1987) who observed decrease in GA with paclobutrazol treatment in peas. The influence of paclobutrazol is evident when applied after pollination, as done in our study, facilitating its accumulation in the grain and also causing an early inhibition of GA biosynthesis (Pagano et al., 1997). Pagano et al., (1997) reported lack of inhibitor effect of paclobutrazol when applied during seed incubation. Influence of ABA caused similar results as paclobutrazol in that the latter inhibits GA biosynthesis, but the former limits α-amylase activity. Seeds with lower embryonic sensitivity to ABA show higher α-amylase activity (Steinbach et al., 1995). Chandler et al., (1991) observed that ABA decreased production of α-amylase in barley. Results from the study of Pagano et al.,

(1997) on sorghum also suggest the involvement of ABA in controlling α-amylase activity. However, in stress treatments of the current study, in addition to the exogenously applied ABA, increased endogenous levels of ABA in the grain caused a twofold effect in the grain ABA accumulation. This caused lowering of germination rates in ABA treatment as much as those seen in paclobutrazol in this study.

In conclusion, phytohormone treatments under all treatment environments significantly influenced germination ratios. ABA and paclobutrazol decreased germination percentages where as GA increased sprouting. Plants treated with paclobutrazol showed lowest germination ratio and GA treated plants showed maximum under all environments. Influence of environments was similar to that seen in the previous study.

Table 4.1. Significance of various treatments and their interaction on physiological, growth, yield and germination traits;

Traits	Cultivar (C)	Environment (E)	Hormone (H)	C X E	C X H	E X H
Leaf Temperature (° C)	NS	***	NS	***	NS	***
Chlorophyll Content (SPAD Units)	***	***	**	***	NS	**
Chlorophyll Fluorescence (Fo/Fm)	***	**	NS	***	NS	***
Plant Height (cm)	***	*	***	***	NS	*
Tiller Number (plant^{-1})	NS	NS	NS	***	NS	**
Total Dry Weight (g plant^{-1})	***	***	NS	***	NS	***
Seed Dry Weight (g plant^{-1})	**	***	NS	***	NS	***
Harvest Index (%)	**	***	NS	***	NS	***
Seed Number (plant^{-1})	NS	**	***	***	*	***
Germination Ratio	NS	***	*	*	NS	**

***, **, *, Corresponding to P< 0.001, 0.01 and .05 significance levels.

Table 4.2. Influence of cultivar on physiological, growth, and yield traits. Data are means of environment and phytohormone treatments.

Traits	KS01HW163-4	Heyne	LSD
Leaf Temperature (° C)	18.04^{A}	17.65^{A}	NS
Chlorophyll Content (SPAD Units)	51.54^{A}	48.32^{B}	1.2606
Chlorophyll Fluorescence (Fo/Fm)	0.33^{A}	0.26^{B}	0.0326
Plant Height (cm)	61.33^{A}	49.57^{B}	1.3261
Tiller Number (plant^{-1})	15.72^{A}	16.73^{A}	NS
Total Dry Weight (g plant^{-1})	17.41^{A}	13.34^{B}	1.4157
Seed Dry Weight (g plant^{-1})	3.58^{A}	3.21^{B}	0.2314
Harvest Index (%)	0.22^{B}	0.25^{A}	0.0233
Seed Number (plant^{-1})	140.86^{A}	135.27^{A}	21.041
Germination Ratio	0.62^{A}	0.58^{A}	NS

Table 4.3. Falling number (FN) values of the two cultivars at various environment and phytohormone treatments. Abscisic Acid (A), Paclobutrazol (P), Gibberellic Acid (G), and Control (C) represent the phytohormone treatments. High Temperature/Drought (HT/D), High Temp. /Irrigated (HT/I), Optimum Temp./Drought (OT/D), and Optimum Temp. /Irrigated (OT/I) represent the different environments.

Environment	Hormone	KS01HW163-4	Heyne
HT/D	A	Not Available (NA)	NA
HT/D	P	NA	194
HT/D	G	NA	158
HT/D	C	NA	NA
HT/I	A	NA	NA
HT/I	P	NA	NA
HT/I	G	NA	NA
HT/I	C	NA	NA
OT/D	A	NA	196
OT/D	P	NA	NA
OT/D	G	NA	NA
OT/D	C	NA	162
OT/I	A	238	186
OT/I	P	200	254
OT/I	G	119	85
OT/I	C	186	NA

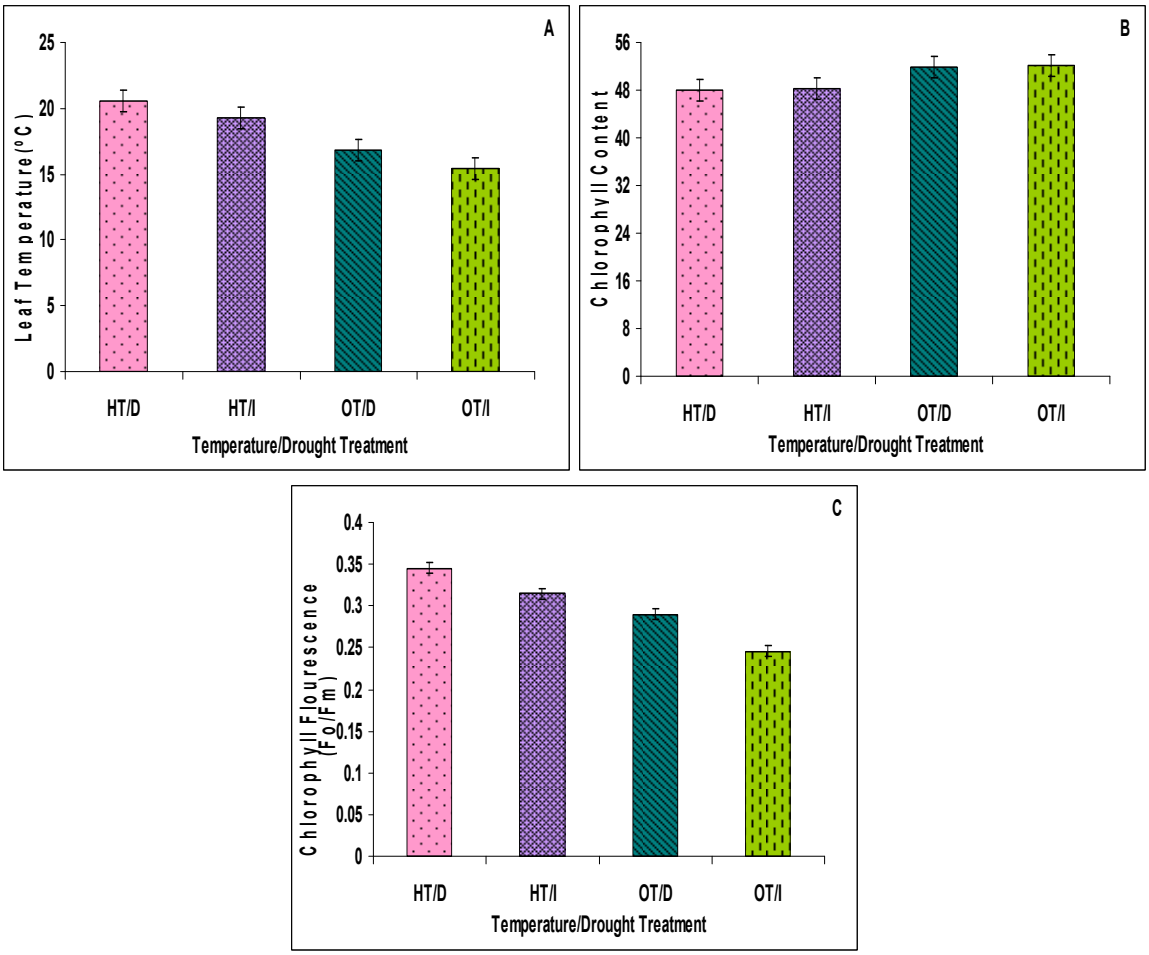

Figure 4.1. Influence of environment on physiological traits. Data are means of cultivar and phytohormone. High Temperature/Drought (HT/D), High Temp. /Irrigated (HT/I), Optimum Temp./Drought (OT/D), and Optimum Temp. /Irrigated (OT/I) represent the different environments. Error bars provide standard error (SE) and least significant difference (LSD) value is given for comparison of treatment means.

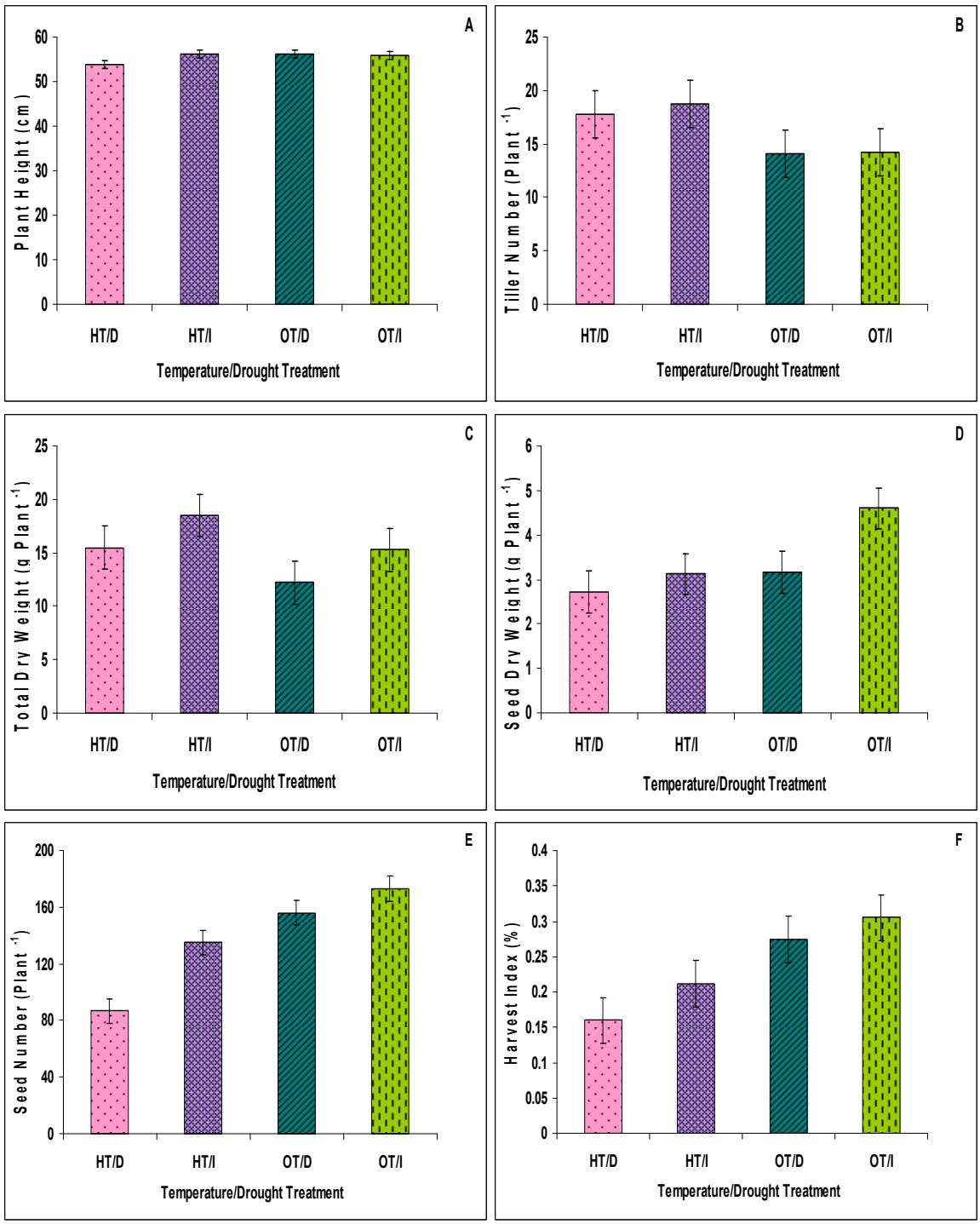

Figure 4.2. Influence of environment on growth and yield traits. Data are means of cultivar and phytohormone. Error bars provide LSD for comparison of treatment means. See Fig. 4.1 for abbreviations.

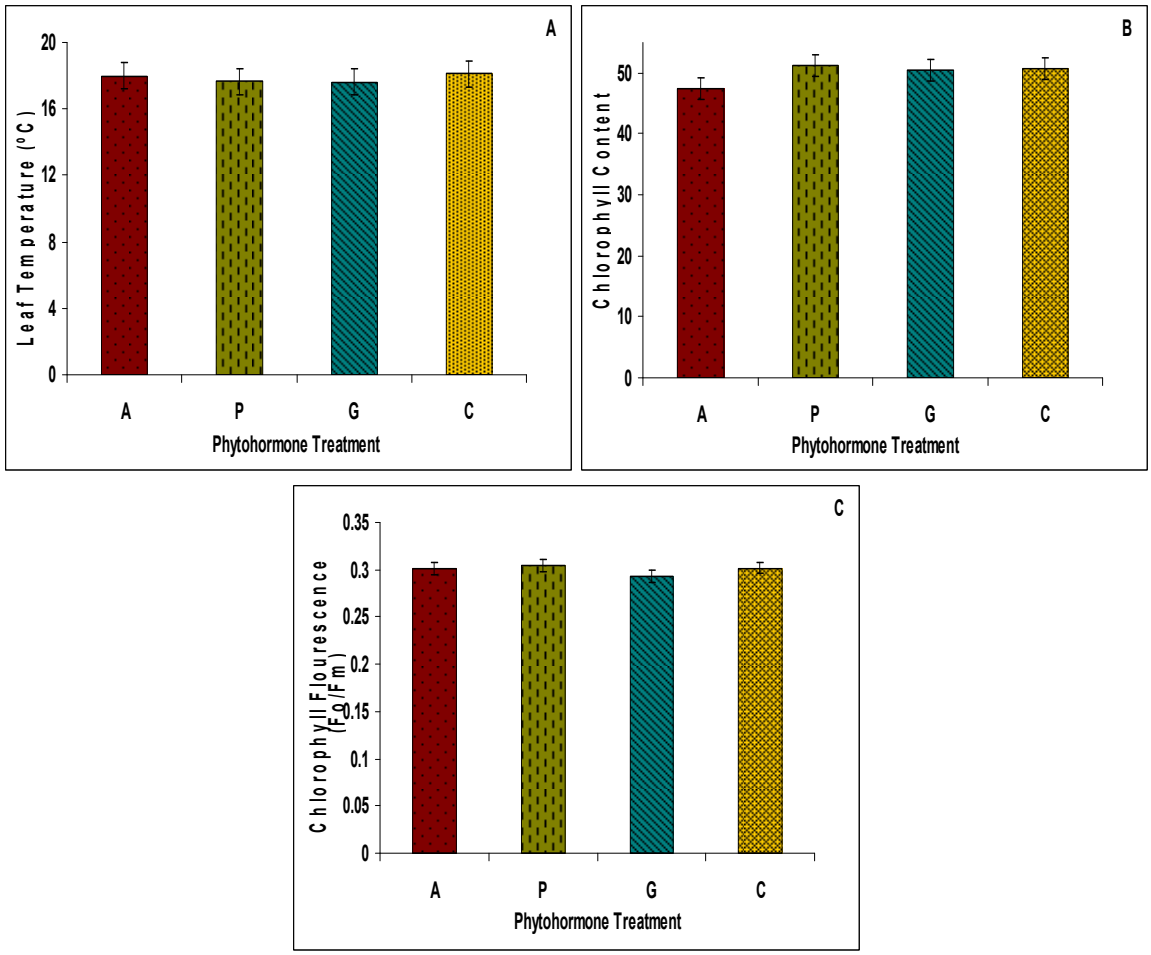

Figure 4.3. Influence of phytohormone on physiological traits. Data are means of cultivar and environment. Abscisic Acid (A), Paclobutrazol (P), Gibberellic Acid (G), and Control (C) represent the phytohormone treatments. Error bars provide least significant difference (LSD) for comparison of treatment means.

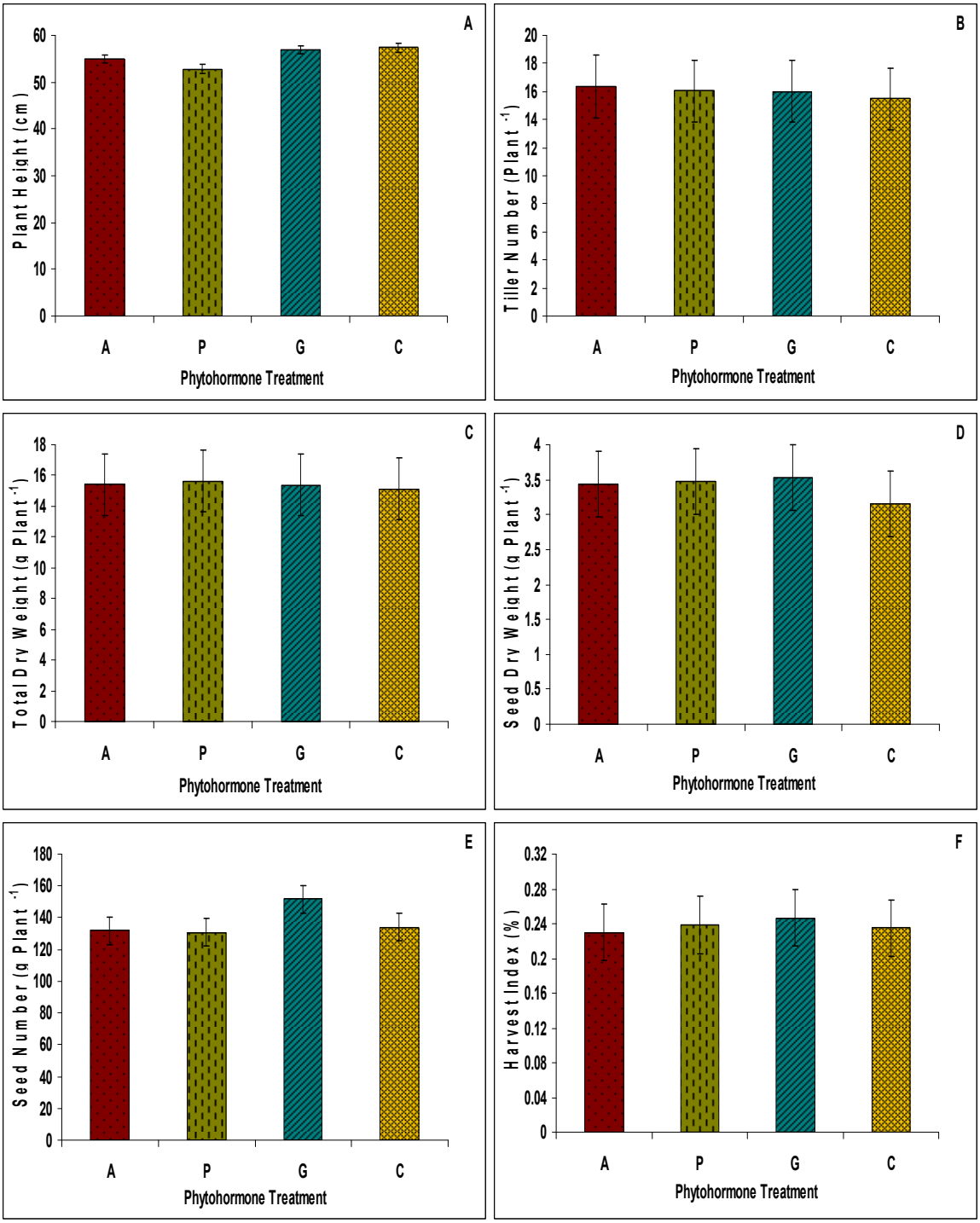

Figure 4.4. Influence of phytohormone on growth and yield traits. Data are means of cultivar and environment. Error bars provide LSD for comparison of treatment means. See Fig. 4.3 for abbreviations.

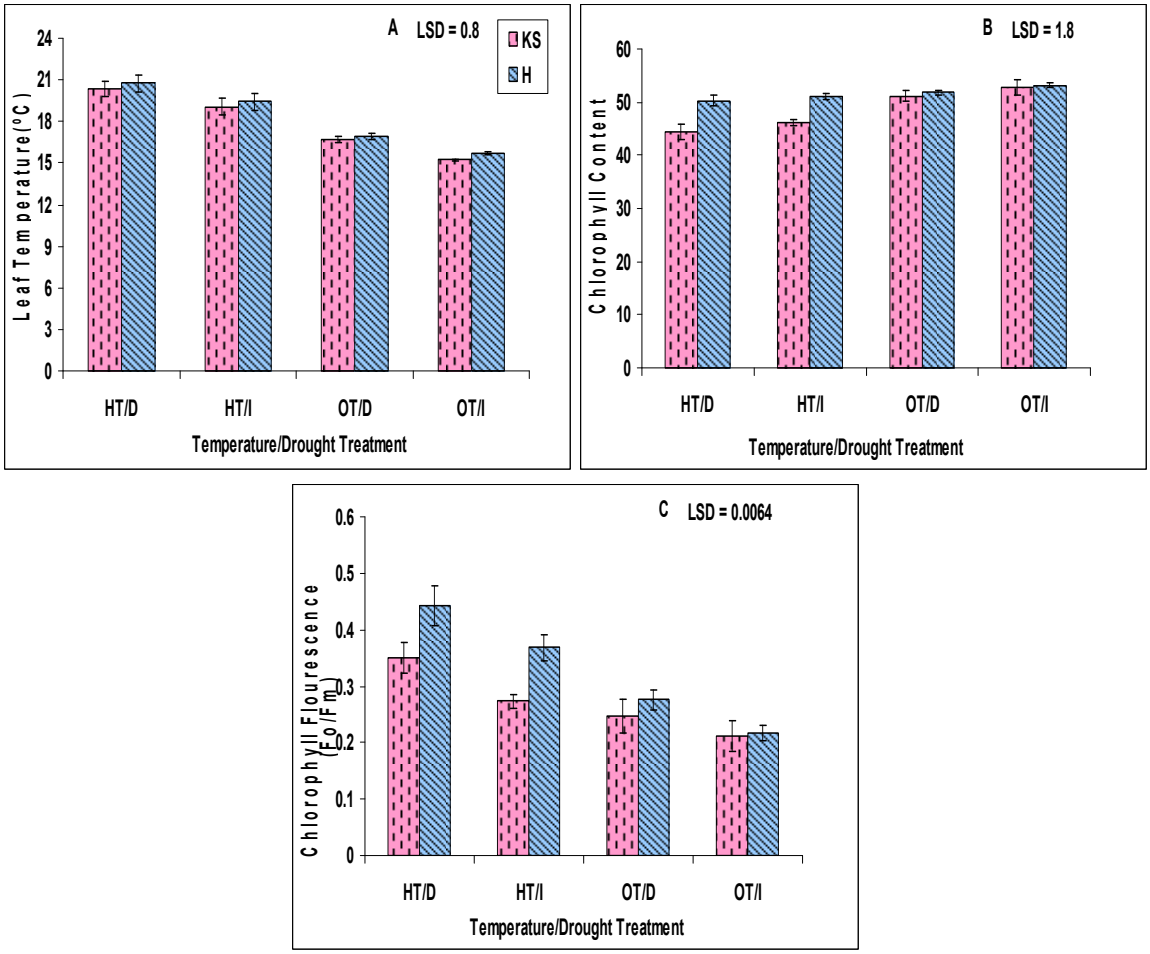

Figure 4.5. Influence of cultivar [KS01HW163-4 (KS) and Heyne (H)] by environment interaction on physiological traits. Data are means of five replicated measurements. Error bars provide SE and the LSD value is given for comparison of treatment means. See Fig. 4.1 for abbreviations.

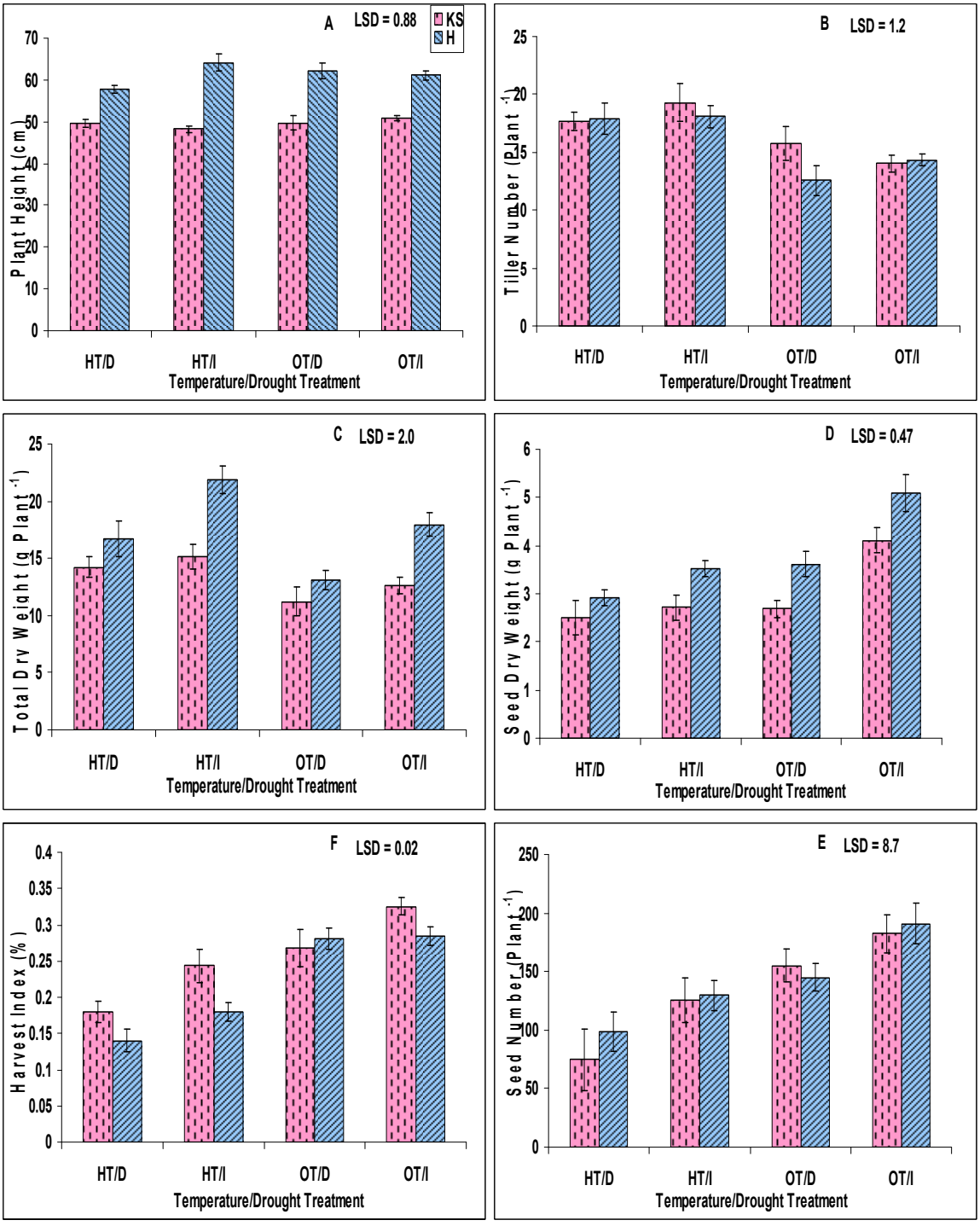

Figure 4.6. Influence of cultivar [KS01HW163-4 (KS) and Heyne (H)] by environment interaction on growth and yield traits. Data are means of five replicated measurements. Error bars provide SE and the LSD value is given for comparison of treatment means. See Fig. 4.1 for abbreviations.

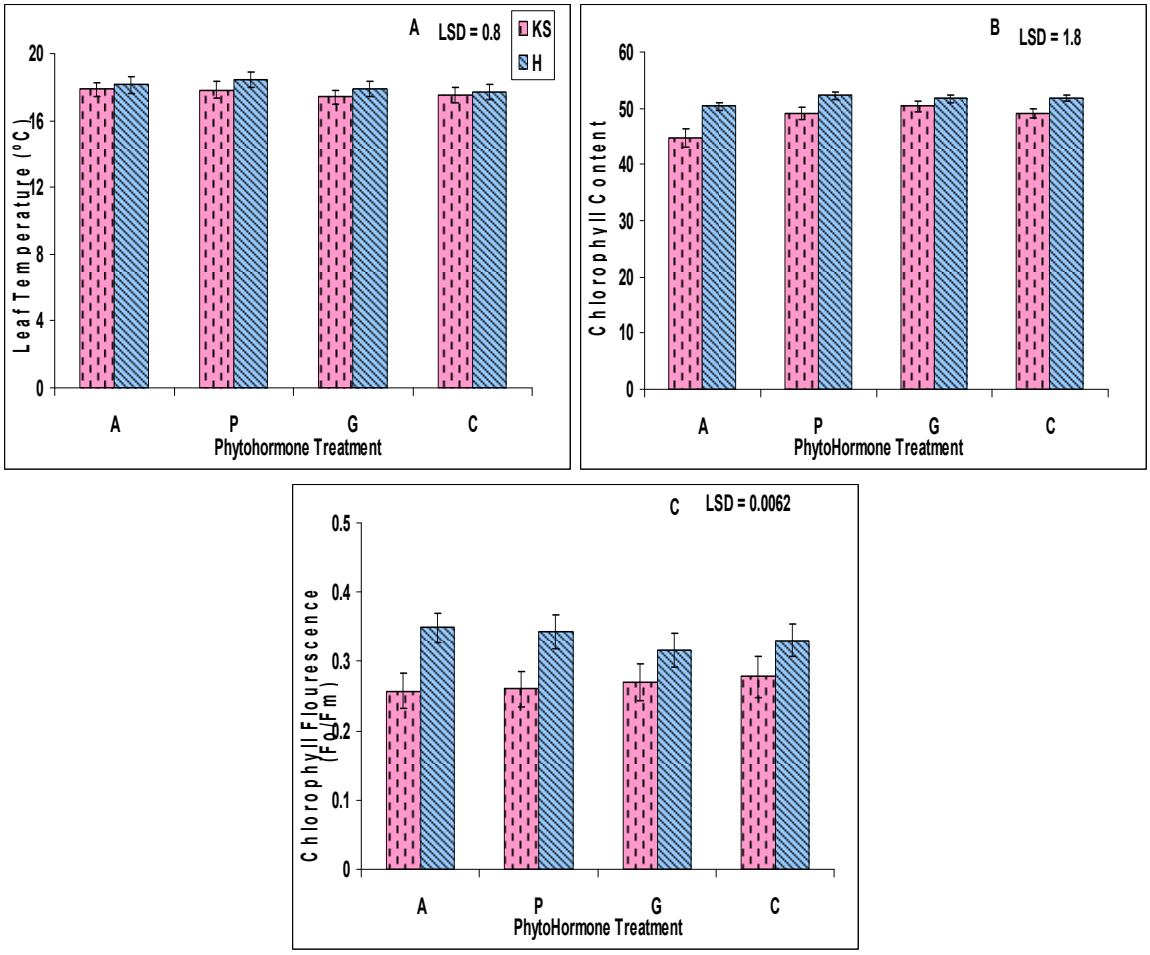

Figure 4.7. Influence of cultivar [KS01HW163-4 (KS) and Heyne (H)] by phytohormone interaction on physiological traits. Data are means of five replicated measurements. Error bars provide standard error (SE) and the LSD value is given for comparison of treatment means. See Fig. 4.3 for abbreviations.

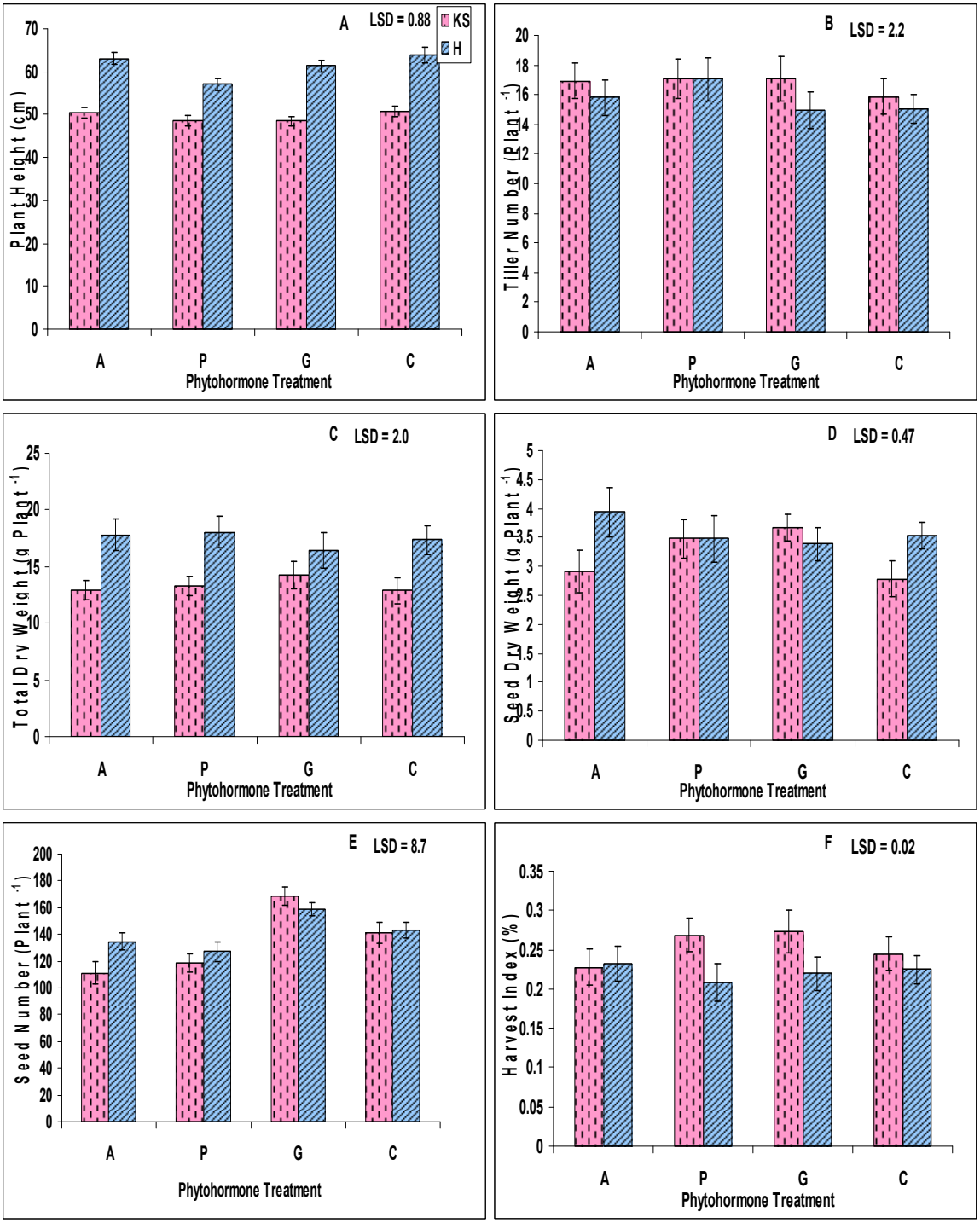

Figure 4.8. Influence cultivar [KS01HW163-4 (KS) and Heyne (H)] by phytohormone interaction on growth and yield traits. Data are means of five replicated measurements. Error bars provide SE and the LSD value is given for comparison of treatment means. See Fig. 4.7 for abbreviations.

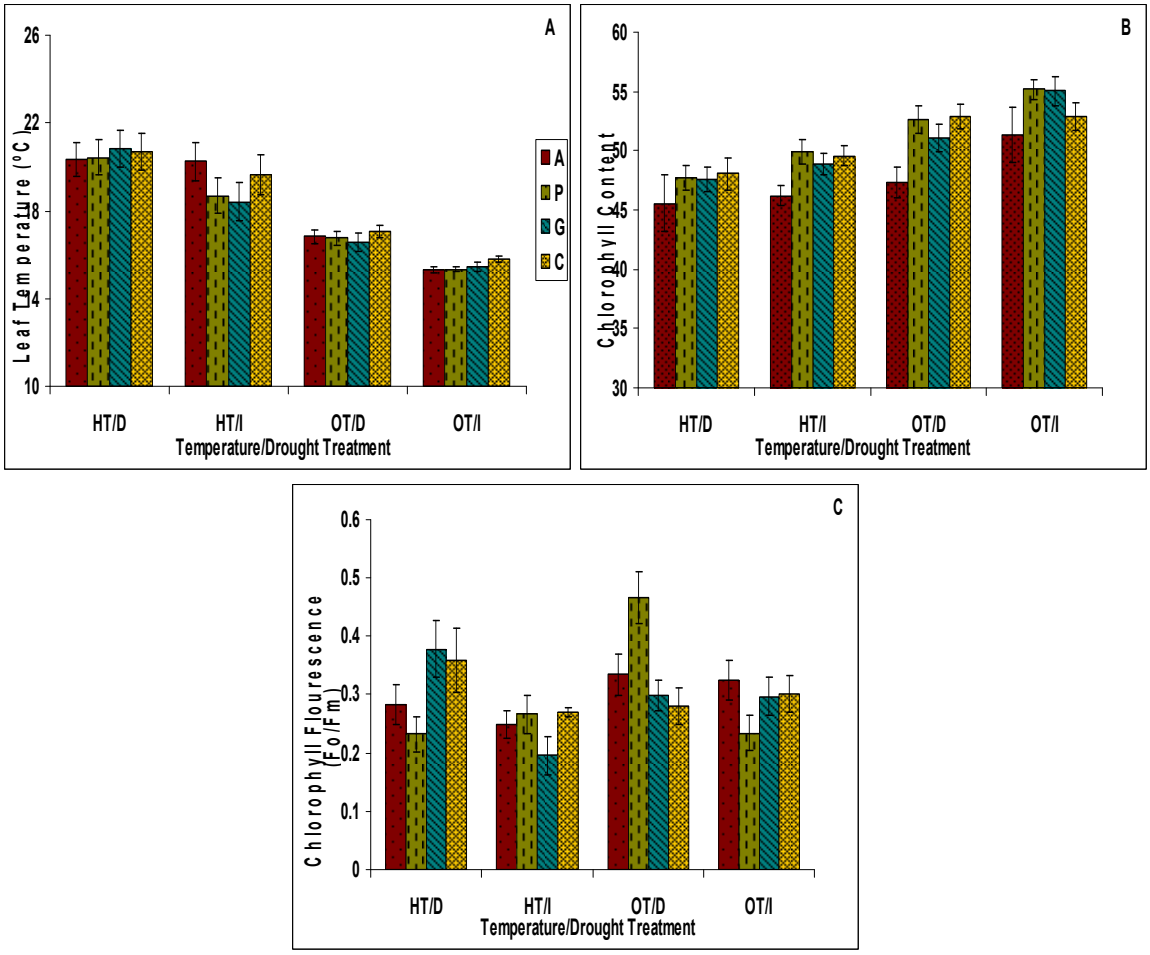

Figure 4.9. Influence of environment by phytohormone interaction on physiological traits. Data are means of replicated measurements. Error bars provide SE. See Fig. 4.1 and Fig. 4.3 for abbreviations.

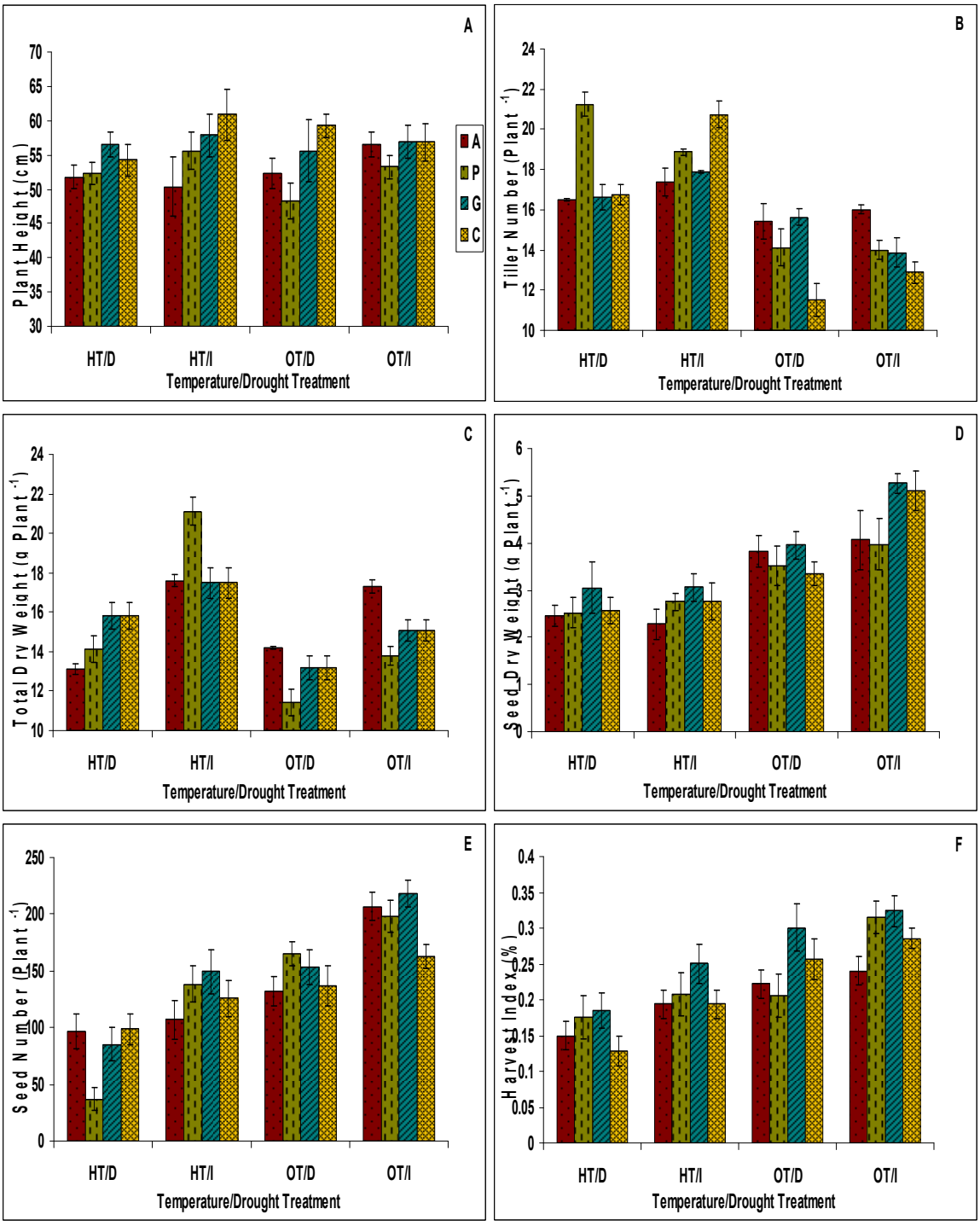

Figure 4.10. Influence environment by phytohormone interaction on yield traits. Data are means of replicated measurements. Error bars provide SE. See Fig. 4.1 and Fig. 4.3 for abbreviations.

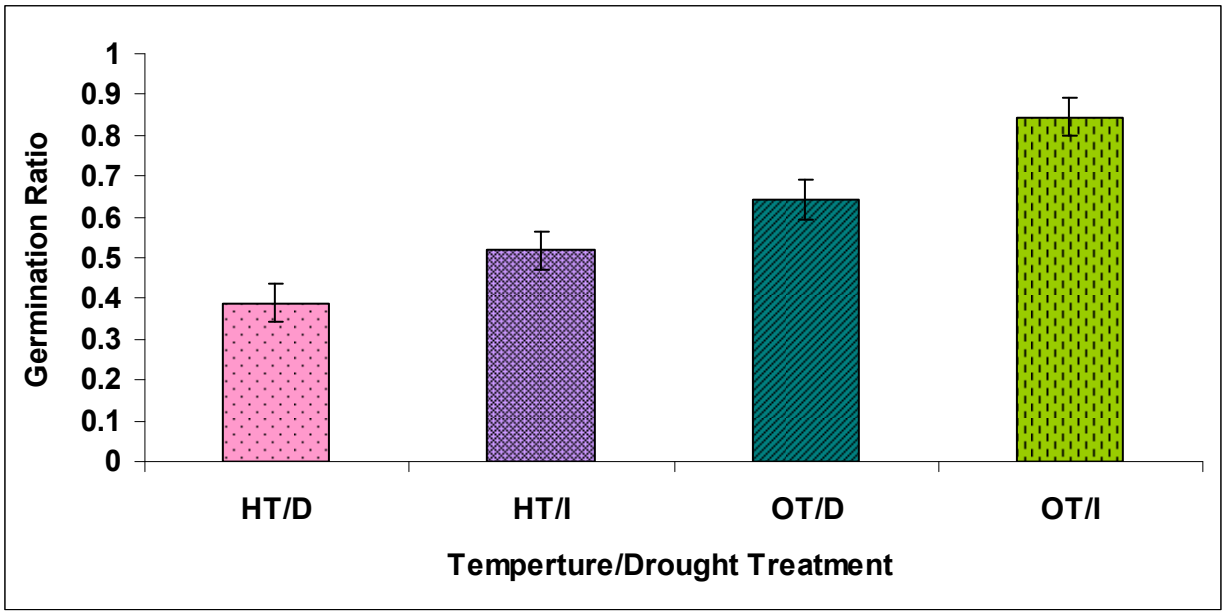

Figure 4.11. Influence of environment on germination ratio. Data are means across cultivar and Phytohormone. Error bars provide LSD for comparison of treatment means. See Fig. 4.1 for abbreviations.

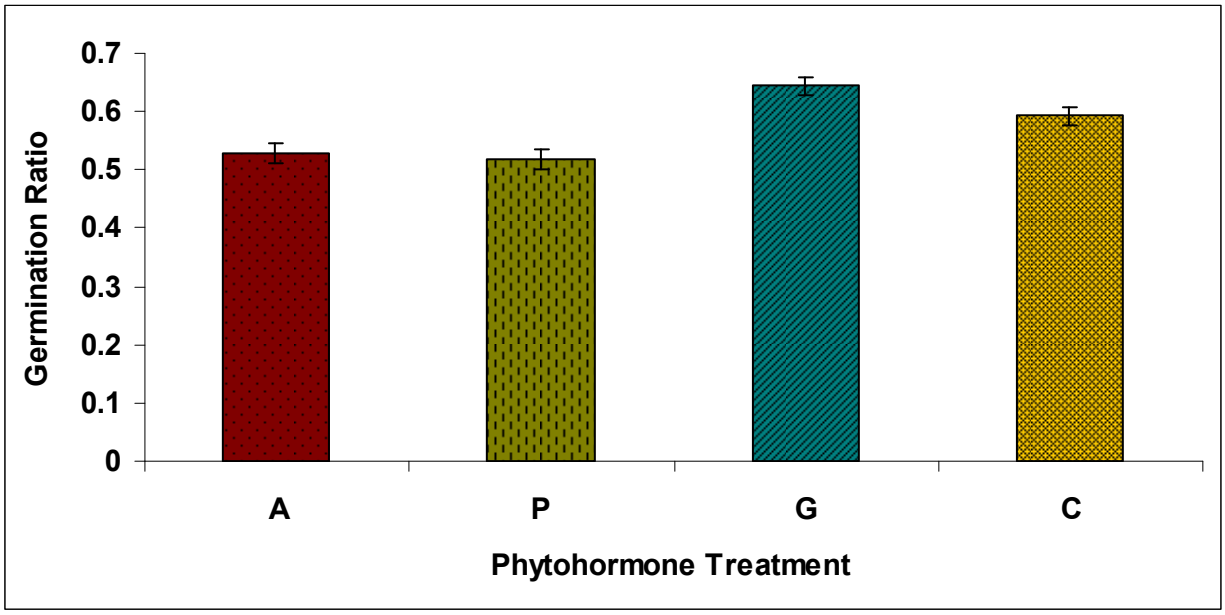

Figure 4.12. Influence of phytohormone on germination ratio. Data are means across cultivar and Environment. Error bars provide LSD for comparison of treatment means. See Fig. 4.3 for abbreviations.

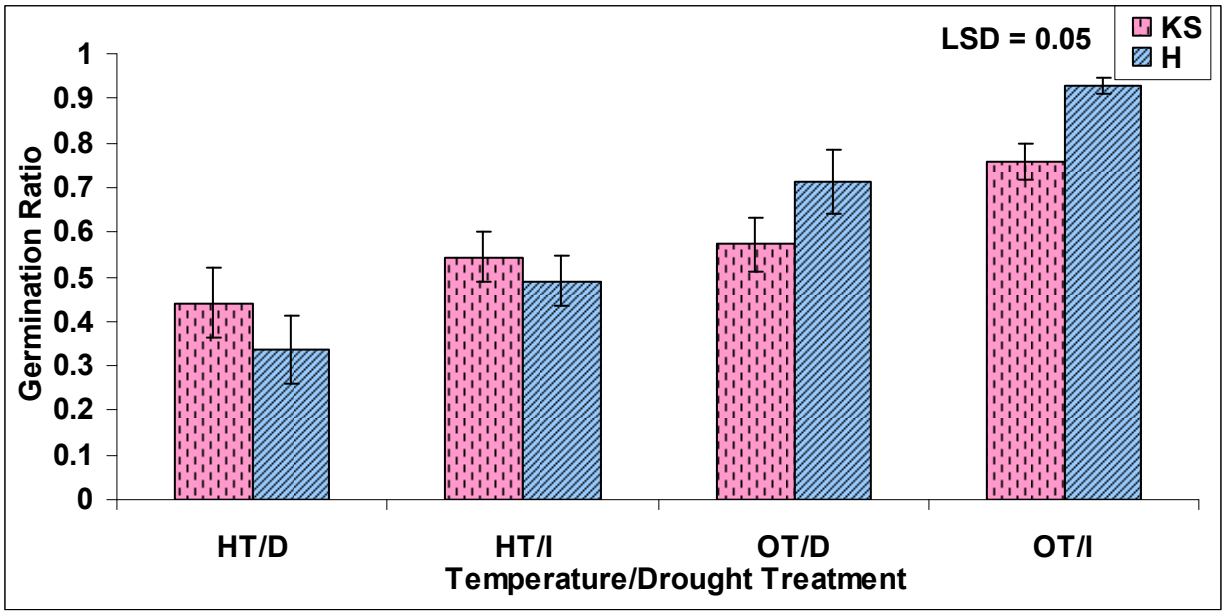

Figure 4.13. Influence of cultivar [KS01HW163-4 (KS) and Heyne (H)] by environment interaction on total germination ratio. Data are means of five replicated measurements. Error bars provide SE and the LSD value is given for comparison of treatment means. See Fig. 4.1 for abbreviations.

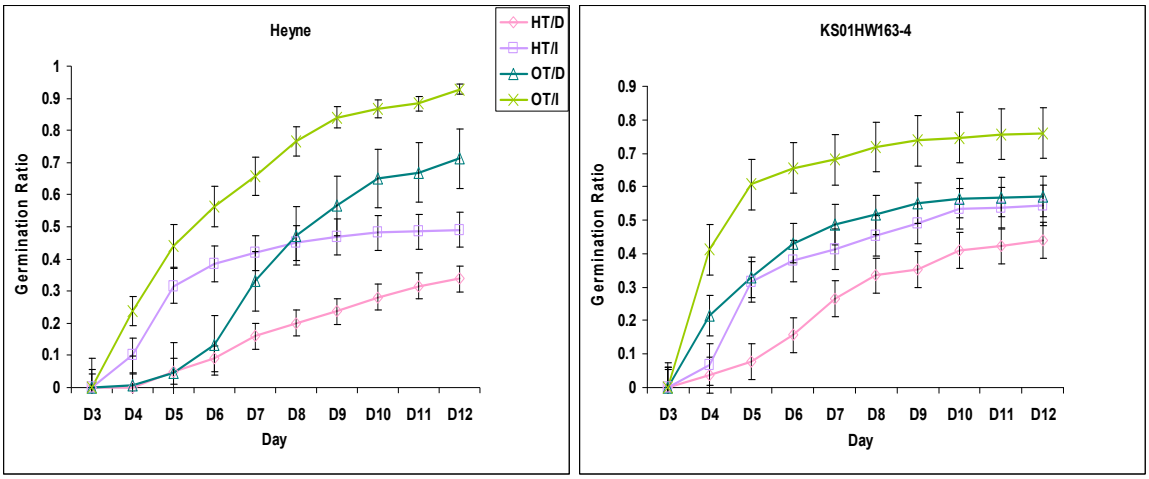

Figure 4.14. Influence of cultivar [KS01HW163-4 (KS) and Heyne (H)] by environment interaction on day-wise germination ratio. Data are means of five replicated measurements. Error bars provide SE. See Fig. 4.1 for abbreviations.

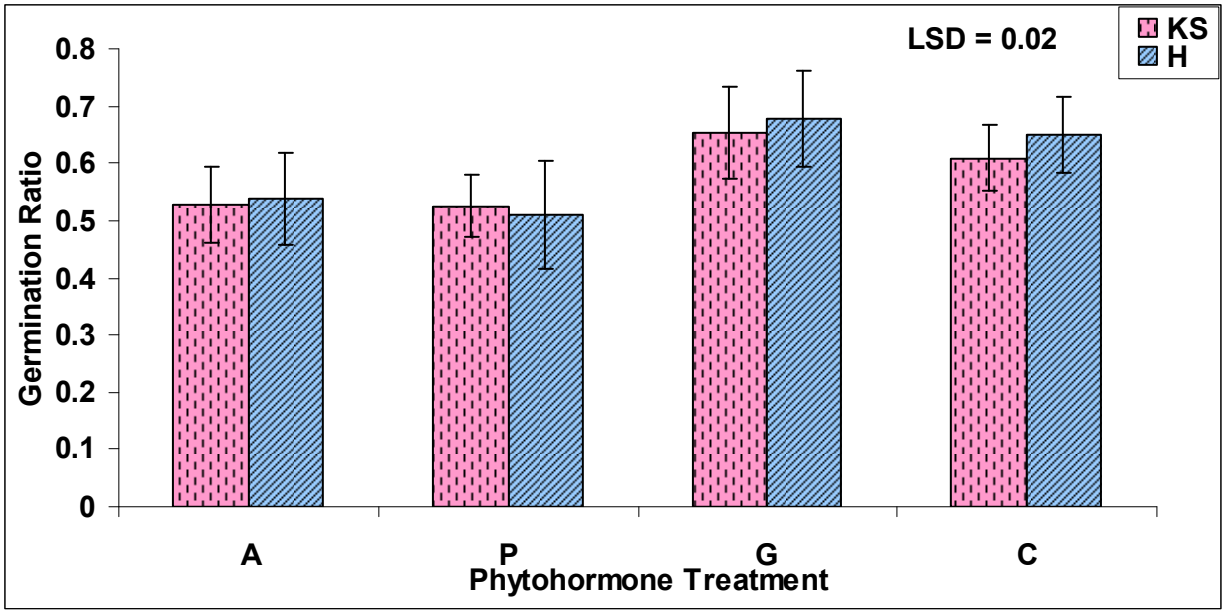

Figure 4.15. Influence of cultivar [KS01HW163-4 (KS) and Heyne (H)] by phytohormone interaction on total germination ratio. Data are means of five replicated measurements. Error bars provide SE and the LSD value is given for comparison of treatment means. See Fig. 4.3 for abbreviations.

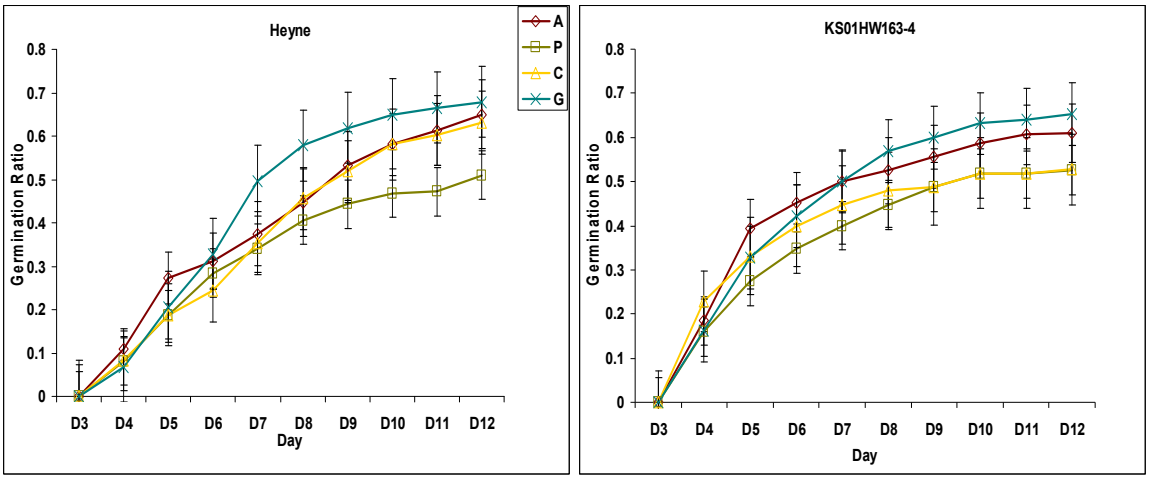

Figure 4.16. Influence of cultivar [KS01HW163-4 (KS) and Heyne (H)] by phytohormone interaction on day-wise germination ratio. Data are means of five replicated measurements. Error bars provide SE. See Fig. 4.3 for abbreviations.

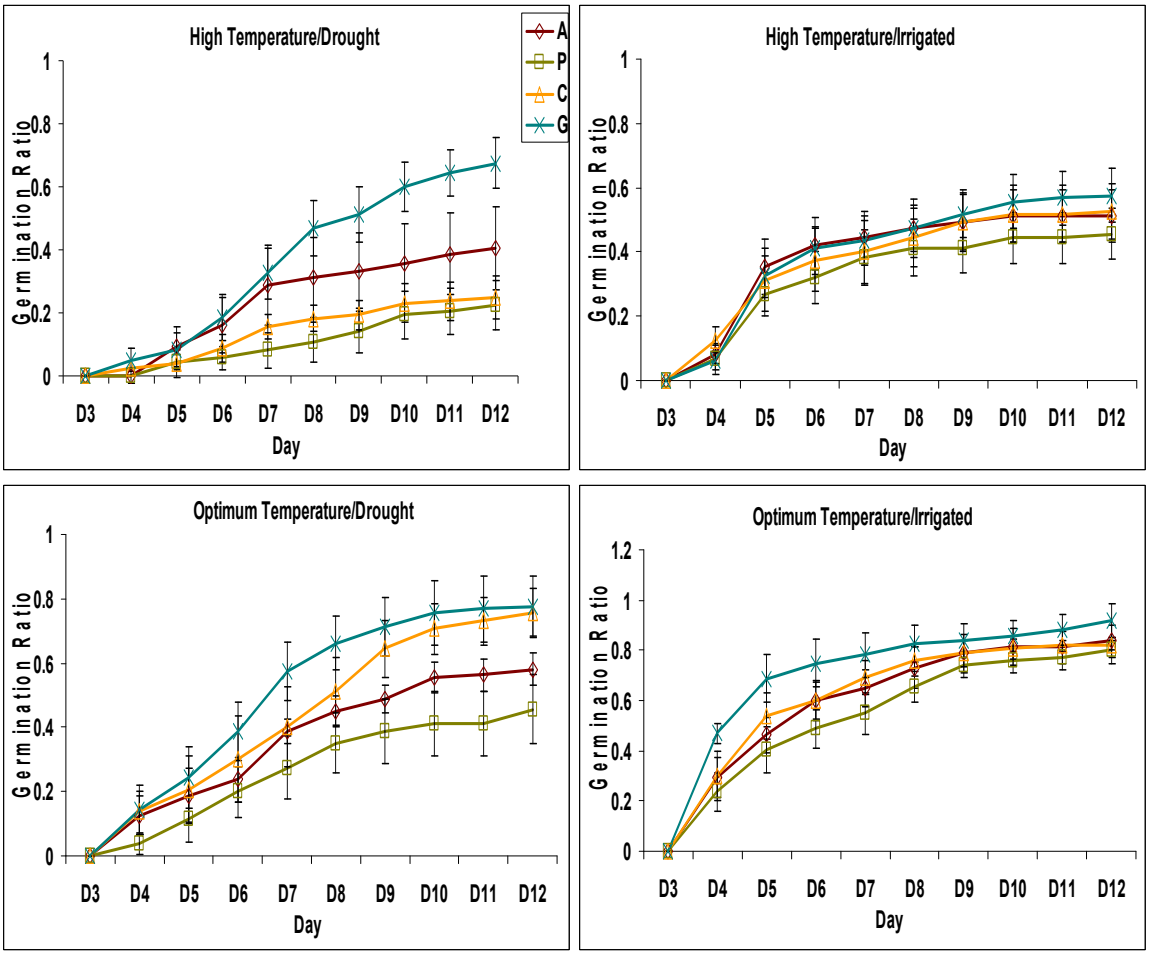

Figure 4.17. Influence of environment by phytohormone interaction on day-wise germination ratio. Data are means of five replicated measurements. Error bars provide SE. See Fig. 4.3 for abbreviations.

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Appendix A - Chapter 2 Tables

Table A.1 Seed dry weights (SDW, g Plant $^{-1}$) of population in CAP-7 (136 lines).

Data are means of four replications

Line Number	SDW
18547	17.2
19140	15.4
19282	15.4
18828	15.1
18591	14.1
19053	14.0
19196	13.5
18827	13.5
19880	13.1
19028	13.1
18426	13.0
20270	12.4
20313	11.6
18198	11.6
17069	11.3
19055	11.1
18224	10.6
19086	10.6
19202	10.3
20733	10.2
18249	10.1
17034	10.1
17466	9.9
19284	9.8
18422	9.7
19079	9.7
20268	9.7
19038	9.7
18592	9.6
19046	9.6
18527	9.6
18014	9.3
20266	9.3
20263	9.2
19883	9.1
17456	9.1
18425	9.1
18662	9.0
18057	8.9
20271	8.9
19027	8.8
18174	8.6
18593	8.6
20737	8.4
17068	8.4
19084	8.2

Line Number	SDW
18888	8.1
18169	8.1
18690	8.1
18693	8.0
18534	7.9
19200	7.7
18999	7.6
19878	7.4
19131	7.0
19281	7.0
19274	6.9
17671	6.9
18833	6.8
19201	6.8
18548	6.7
18229	6.6
19198	6.6
18173	6.5
18531	6.4
18245	6.3
18130	6.3
18121	6.0
18728	5.9
18319	5.9
18131	5.8
19130	5.8
17672	5.8
18664	5.7
17447	5.6
18312	5.6
19036	5.6
18123	5.6
17670	5.6
18013	5.5
18830	5.5
18227	5.5
19048	5.5
18315	5.4
19040	5.4
18659	5.3
18579	5.2
18590	5.2
17668	5.2
19051	5.2
18530	5.1
18552	5.1

Line Number	SDW
19044	5.0
18248	4.8
18429	4.8
19190	4.8
18507	4.7
18578	4.7
19029	4.6
18834	4.5
19273	4.5
18200	4.5
19204	4.4
18234	4.3
19074	4.2
18660	4.1
19080	4.1
18171	4.1
19075	3.9
18129	3.8
18133	3.8
18666	3.7
19043	3.6
19906	3.6
18656	3.4
18232	3.3
19194	3.2
17037	2.9
18256	2.8
19034	2.7
17452	2.6
17450	2.1
18258	2.1
19081	2.1
17469	1.8
18132	1.7
17489	1.5
18196	1.3
18255	1.3
19009	1.2
17030	1.2
17669	1.1
18651	0.9
19042	0.9
19195	0.6

Table A.2. Seed numbers (SNO, g Plant $^{-1}$) of population in CAP-7 (136 lines). Data are means of four replications

Line Number	SNO
19053	650.0
19055	539.8
19196	527.3
19140	522.3
18828	497.3
18547	496.3
18426	489.5
19046	481.5
19282	480.5
19028	477.0
18591	476.5
18198	450.5
18827	448.0
20313	439.8
20270	426.0
18249	425.0
18422	419.5
19880	411.5
20268	409.3
19202	407.5
18534	389.5
20271	389.0
18425	388.5
18593	388.0
18527	359.5
18014	357.8
18224	356.0
19086	347.0
18693	345.5
18057	345.0
20733	343.3
18662	340.5
19079	334.0
17069	333.5
20263	331.5
17671	330.5
18999	329.8
19883	329.8
18592	326.3
19200	323.5
17034	321.5
18690	317.5
20737	317.3
19198	317.0
19201	316.8
20266	308.5

Line Number	SNO
17456	307.0
19038	306.5
19284	305.3
19051	304.8
18530	304.3
19130	300.3
18531	297.8
18121	291.5
18888	290.5
18131	289.8
18169	283.5
19040	283.0
17068	278.3
17668	277.5
19036	277.5
19044	273.5
17670	273.0
19084	272.0
18579	267.3
19281	265.8
18659	263.3
17672	263.0
17466	257.8
19131	255.3
18552	254.0
18173	252.8
19027	252.5
18123	249.5
18229	249.5
18013	248.8
18664	248.5
18728	238.0
19274	236.0
17447	231.0
19878	229.0
18234	228.0
18429	228.0
18245	226.8
18174	220.5
18833	205.8
18130	203.5
18830	203.5
18133	201.5
19043	201.5
19048	201.5
18660	198.8

Line Number	SNO
18319	198.0
18315	197.8
18507	193.5
18590	193.0
19273	190.3
18132	189.0
18232	186.8
18548	186.5
18312	184.3
18227	177.5
19906	177.0
18129	168.8
19190	168.0
18200	164.5
19034	161.0
19029	160.3
19080	156.0
19075	155.5
18834	147.5
18656	145.8
18248	144.5
19074	141.3
19204	138.5
19194	137.8
18666	136.5
17452	133.8
18578	128.0
18171	115.5
18258	110.5
18256	106.3
17450	101.0
17037	91.0
18196	87.0
19081	77.3
17469	68.5
17669	67.5
17030	66.8
17489	61.5
18651	53.0
19195	42.8
18255	38.8
19009	36.8
19042	22.0

Table A.3 Seed size (SS, mg Kernal $^{-1}$) of population in CAP-7 (136 lines). Data are means of four replications

Line Number	SS
18174	0.0388
17466	0.0385
18578	0.0368
18548	0.0361
18171	0.0350
18547	0.0346
19027	0.0346
18833	0.0336
17069	0.0335
19880	0.0331
18248	0.0325
19282	0.0324
18319	0.0323
17037	0.0319
19284	0.0318
19204	0.0318
19038	0.0317
18255	0.0316
18227	0.0315
18130	0.0309
17068	0.0309
18312	0.0308
19009	0.0308
18592	0.0307
19086	0.0306
18828	0.0306
17034	0.0305
18827	0.0304
20270	0.0303
20733	0.0301
18224	0.0301
17456	0.0300
19878	0.0300
20266	0.0299
19074	0.0299
19084	0.0297
18591	0.0295
19140	0.0295
19029	0.0295
19079	0.0292
18666	0.0288
18834	0.0288
19274	0.0288
18169	0.0286
20263	0.0279
20737	0.0277

Line Number	SS
19190	0.0277
19131	0.0277
19883	0.0276
18830	0.0275
18888	0.0275
19028	0.0274
18315	0.0274
19281	0.0272
18200	0.0271
19048	0.0270
19081	0.0270
18229	0.0269
18014	0.0268
18173	0.0265
18426	0.0264
18527	0.0264
19080	0.0263
18662	0.0263
20313	0.0262
18057	0.0261
18198	0.0256
19196	0.0256
19075	0.0255
18690	0.0252
17489	0.0252
18728	0.0251
20268	0.0250
18256	0.0249
19040	0.0249
17469	0.0247
18245	0.0245
17447	0.0245
19202	0.0245
18507	0.0244
18590	0.0242
19194	0.0240
18249	0.0237
19200	0.0237
20271	0.0236
19273	0.0235
18425	0.0235
18693	0.0235
18422	0.0234
18664	0.0234
18593	0.0233
18999	0.0233

Line Number	SS
18656	0.0231
17672	0.0224
18123	0.0221
18531	0.0216
18129	0.0213
19053	0.0212
17450	0.0210
19201	0.0210
18121	0.0210
19198	0.0208
18660	0.0208
17671	0.0208
18429	0.0207
19906	0.0206
19036	0.0203
19055	0.0202
18534	0.0202
17670	0.0201
18131	0.0198
18013	0.0198
19046	0.0197
18579	0.0197
18659	0.0196
18552	0.0191
17452	0.0191
18258	0.0187
19130	0.0187
17668	0.0185
18234	0.0181
19043	0.0179
19044	0.0177
18232	0.0172
17030	0.0169
19051	0.0169
19034	0.0168
18133	0.0165
18530	0.0163
18651	0.0163
18196	0.0149
19195	0.0149
18132	0.0125
17669	0.0125
19042	0.0113

Table A.4 Seed dry weights (SDW, g Plant $^{-1}$) of population in CAP-8 (88 lines).

Data are means of four replications

Line Number	SDW
19950	22.4
18733	20.1
17413	17.8
17810	17.5
18523	12.8
18708	12.8
19953	12.8
17479	12.6
20517	12.6
18641	11.7
17817	11.4
17396	11.2
18621	11.1
18096	10.8
17412	10.7
18617	10.4
20495	10.2
19989	10.2
18599	10.2
17482	10.1
18638	9.9
18237	9.8
17411	9.7
18781	9.5
18524	9.4
18686	9.4
20492	9.4
20124	9.4
18522	9.1
19189	9.1
18335	9.1
18117	9.0
20121	8.9
18727	8.9
18620	8.8
17461	8.7
19308	8.6
18740	8.6
18730	8.5
20493	8.5
19988	8.4
18674	8.3
17715	7.9
18618	7.9
18525	7.8
19307	7.8

Line Number	SDW
18218	7.7
18557	7.6
18773	7.5
17414	7.3
17719	7.2
18732	7.0
20118	6.9
18685	6.8
18104	6.7
18344	6.6
18737	6.1
18862	6.1
17462	6.1
17397	6.0
18116	6.0
18329	6.0
18779	5.7
18712	5.7
18559	5.5
19304	5.3
18776	5.2
18673	5.1
22373	5.1
20527	4.9
20117	4.7
18520	4.5
18644	4.5
18098	4.2
19951	3.9
18221	3.6
18521	3.3
20529	3.2
17815	3.2
18675	3.0
17816	2.9
17991	2.9
18642	2.5
20190	2.1
20494	2.1

Table A.5 Seed numbers (SNO, number Plant $^{-1}$) of population in CAP-8 (88 lines).

Data are means of four replications

Line Number	SNO
19950	763.3
18733	674.3
17413	612.8
17810	550.5
18523	507.5
17479	456.0
18621	443.5
18708	433.0
17411	424.8
17412	418.8
18641	417.0
20517	396.3
18620	394.0
20124	389.8
19953	383.0
17396	375.8
18096	374.0
18117	372.5
19308	371.5
18638	368.8
18781	368.5
18686	365.5
18773	362.5
18617	358.5
17817	353.5
18524	346.0
18525	337.5
20495	337.0
17482	332.0
18599	332.0
18618	331.5
19189	327.5
18237	327.0
18218	314.8
19989	311.5
18557	310.8
18685	308.8
18727	307.3
18730	300.8
18712	300.5
17715	294.0
17719	292.5
18335	290.5
18732	290.0
18779	280.0
18740	279.8

Line Number	SNO
18559	276.8
19307	275.5
20121	274.5
20492	274.5
18329	274.3
18862	260.0
17414	259.5
19988	257.0
17462	256.0
18522	256.0
20493	255.8
18776	254.0
18644	252.0
17397	249.3
18104	246.0
18674	237.5
17461	233.5
18221	231.5
18737	231.5
20527	228.0
18116	227.0
22373	225.0
18673	222.0
20117	211.5
20118	209.3
18344	205.8
18520	203.5
17991	185.5
18098	185.3
18675	156.0
19304	155.0
20529	154.0
20190	153.8
17815	142.8
18521	136.5
18642	130.8
17816	129.5
19951	121.5
20494	107.8

Table A.6 Seed size (SS, mg Kernel $^{-1}$) of population in CAP-8 (88 lines). Data are means of four replications

Line Number	SS
17461	0.0371
18674	0.0358
18522	0.0355
20492	0.0349
19304	0.0343
18344	0.0337
20118	0.0336
19953	0.0335
20493	0.0333
19989	0.0328
19988	0.0328
20121	0.0325
17817	0.0325
20517	0.0324
18096	0.0323
18740	0.0322
18599	0.0321
19951	0.0320
17810	0.0317
18335	0.0311
20495	0.0307
17482	0.0307
18237	0.0299
18617	0.0298
17396	0.0296
18733	0.0296
18708	0.0296
17413	0.0294
19950	0.0294
18727	0.0293
18730	0.0290
17414	0.0287
17715	0.0286
19307	0.0286
19189	0.0279
18641	0.0278
18521	0.0277
17479	0.0274
18737	0.0272
18638	0.0270
18524	0.0268
18104	0.0268
18781	0.0262
18116	0.0261
18686	0.0260
18523	0.0258

Line Number	SS
17412	0.0256
18117	0.0255
18557	0.0253
18621	0.0251
18329	0.0250
17397	0.0247
17719	0.0246
18618	0.0245
20124	0.0244
18218	0.0241
18732	0.0235
18862	0.0234
18673	0.0233
18525	0.0232
19308	0.0231
17462	0.0229
17815	0.0229
17411	0.0227
18098	0.0226
17816	0.0224
18620	0.0224
18520	0.0223
18685	0.0220
22373	0.0218
20117	0.0214
20527	0.0212
20529	0.0212
18559	0.0209
18773	0.0208
18779	0.0206
18675	0.0203
18776	0.0199
20494	0.0194
18712	0.0191
18642	0.0189
18221	0.0181
18644	0.0180
17991	0.0160
20190	0.0148

Table A.7. Daily summation percentages and days to germination margin (0.9); data are means of five replications

Cap	line	d3	d4	d5	d6	d7	d8	d9	d10	d11	d12	germ	margin
7	18133	0.45	0.74	0.94	0.99	0.99	0.99	0.99	0.99	0.99	0.99	5	0.9
7	18169	0.26	0.56	0.91	0.95	1	1	1	1	1	1	5	0.9
7	18171	0.45	0.64	0.95	0.98	0.99	0.99	0.99	0.99	0.99	0.99	5	0.9
7	18173	0.49	0.76	0.99	1	1	1	1	1	1	1	5	0.9
7	18232	0.49	0.79	0.98	1	1	1	1	1	1	1	5	0.9
7	18245	0.3	0.48	0.95	1	1	1	1	1	1	1	5	0.9
7	18425	0.57	0.88	0.98	1	1	1	1	1	1	1	5	0.9
7	18426	0.25	0.67	0.9	0.97	0.97	0.97	0.99	0.99	0.99	0.99	5	0.9
7	18530	0.56	0.79	0.96	0.99	0.99	0.99	0.99	0.99	0.99	0.99	5	0.9
7	18659	0.54	0.89	0.97	0.98	0.99	1	1	1	1	1	5	0.9
7	18828	0.55	0.73	0.93	0.98	0.98	0.98	0.98	0.98	0.98	0.98	5	0.9
7	18830	0.66	0.8	0.97	1	1	1	1	1	1	1	5	0.9
7	19027	0.29	0.61	0.92	0.98	0.98	0.98	0.99	0.99	0.99	0.99	5	0.9
7	19190	0.66	0.79	0.93	0.94	0.99	1	1	1	1	1	5	0.9
7	20268	0.46	0.71	0.94	0.99	1	1	1	1	1	1	5	0.9
7	17669	0.4	0.66	0.88	1	1	1	1	1	1	1	6	0.9
7	17670	0.34	0.42	0.89	1	1	1	1	1	1	1	6	0.9
7	17672	0.14	0.43	0.81	0.92	0.94	0.95	0.96	0.97	0.98	0.98	6	0.9
7	18121	0.39	0.59	0.8	0.95	0.95	0.96	0.97	0.97	0.97	0.97	6	0.9
7	18224	0.27	0.56	0.89	0.95	0.97	0.98	1	1	1	1	6	0.9
7	18258	0.45	0.6	0.82	0.98	0.99	0.99	0.99	0.99	0.99	0.99	6	0.9
7	18319	0.38	0.61	0.88	0.97	0.98	1	1	1	1	1	6	0.9
7	18592	0.43	0.51	0.83	0.92	0.98	1	1	1	1	1	6	0.9
7	18662	0.32	0.56	0.65	0.94	1	1	1	1	1	1	6	0.9
7	19036	0.37	0.47	0.83	0.93	0.94	0.94	0.94	0.94	0.94	0.94	6	0.9
7	19038	0.35	0.6	0.83	0.94	0.98	0.98	0.98	0.98	0.98	0.98	6	0.9
7	19040	0.46	0.52	0.83	0.97	0.97	0.98	0.99	1	1	1	6	0.9
7	19044	0.41	0.43	0.74	0.9	0.95	0.96	0.96	0.97	0.99	0.99	6	0.9
7	19048	0.45	0.65	0.89	0.94	0.98	1	1	1	1	1	6	0.9
7	19053	0.08	0.33	0.68	0.91	0.95	0.96	0.97	0.97	0.98	0.98	6	0.9
7	19194	0.29	0.6	0.83	0.93	0.98	0.99	0.99	0.99	0.99	0.99	6	0.9
7	19195	0.2	0.62	0.87	0.94	0.97	0.99	1	1	1	1	6	0.9
7	19878	0.53	0.58	0.76	0.95	0.98	0.98	0.98	0.98	0.98	0.98	6	0.9
7	19880	0.17	0.47	0.81	0.9	1	1	1	1	1	1	6	0.9
7	19906	0.43	0.64	0.89	0.98	0.98	0.99	0.99	0.99	0.99	0.99	6	0.9
7	17030	0.21	0.36	0.5	0.66	0.93	0.94	1	1	1	1	7	0.9
7	17034	0.05	0.28	0.71	0.85	0.94	0.95	0.96	0.96	0.96	0.97	7	0.9
7	17037	0.11	0.44	0.58	0.7	0.93	0.96	0.96	0.96	0.96	0.96	7	0.9
7	17068	0.05	0.24	0.58	0.79	0.96	0.97	0.99	0.99	0.99	1	7	0.9
7	17069	0.31	0.59	0.63	0.79	0.95	1	1	1	1	1	7	0.9
7	17450	0.34	0.59	0.8	0.84	0.94	1	1	1	1	1	7	0.9
7	17466	0.18	0.25	0.42	0.62	0.95	0.95	0.97	0.98	0.99	0.99	7	0.9
7	18013	0.29	0.45	0.63	0.85	0.98	0.98	0.98	0.98	0.98	0.98	7	0.9
7	18014	0.14	0.43	0.63	0.83	0.9	0.91	0.91	0.91	0.92	0.94	7	0.9
7	18123	0.47	0.59	0.78	0.83	0.93	1	1	1	1	1	7	0.9
7	18129	0.03	0.33	0.7	0.78	0.91	0.99	1	1	1	1	7	0.9

Table A.7 Continued.

Cap	line	d3	d4	d5	d6	d7	d8	d9	d10	d11	d12	germ	margin
7	18131	0.21	0.41	0.74	0.78	0.94	0.98	0.98	0.99	0.99	0.99	7	0.9
7	18174	0.1	0.29	0.63	0.81	0.93	0.95	0.99	1	1	1	7	0.9
7	18198	0.26	0.6	0.76	0.84	0.98	0.99	1	1	1	1	7	0.9
7	18229	0.25	0.57	0.75	0.85	0.94	0.99	1	1	1	1	7	0.9
7	18248	0.29	0.54	0.71	0.79	0.99	0.99	0.99	0.99	0.99	0.99	7	0.9
7	18315	0.31	0.5	0.75	0.86	0.91	0.92	0.95	0.95	0.95	0.95	7	0.9
7	18429	0.48	0.71	0.79	0.87	0.99	0.99	1	1	1	1	7	0.9
7	18507	0.25	0.44	0.74	0.85	0.94	0.94	0.94	0.96	0.96	0.96	7	0.9
7	18534	0.24	0.46	0.78	0.86	0.91	0.95	0.95	0.95	0.95	0.95	7	0.9
7	18548	0.12	0.39	0.68	0.82	0.93	0.94	0.96	0.96	0.96	0.96	7	0.9
7	18552	0.28	0.42	0.73	0.88	0.91	0.91	0.92	0.92	0.92	0.92	7	0.9
7	18579	0.15	0.35	0.59	0.8	0.93	0.98	0.98	0.98	0.99	1	7	0.9
7	18656	0.04	0.33	0.66	0.85	0.94	0.95	0.97	0.97	0.97	0.98	7	0.9
7	18666	0.45	0.64	0.79	0.89	0.9	0.9	0.9	0.91	0.92	0.92	7	0.9
7	18690	0.33	0.44	0.76	0.8	1	1	1	1	1	1	7	0.9
7	18728	0.39	0.64	0.8	0.87	1	1	1	1	1	1	7	0.9
7	18827	0.25	0.4	0.76	0.85	0.99	0.99	1	1	1	1	7	0.9
7	18834	0.08	0.29	0.63	0.71	0.9	0.9	0.95	0.99	0.99	0.99	7	0.9
7	19028	0.07	0.29	0.51	0.73	0.93	0.99	0.99	0.99	0.99	0.99	7	0.9
7	19029	0.22	0.35	0.71	0.88	0.95	1	1	1	1	1	7	0.9
7	19046	0.21	0.44	0.7	0.89	0.93	0.93	0.95	0.95	0.96	0.96	7	0.9
7	19074	0.28	0.41	0.63	0.89	0.97	0.99	1	1	1	1	7	0.9
7	19086	0.14	0.39	0.71	0.86	0.91	0.94	0.95	0.95	0.95	0.95	7	0.9
7	19131	0.43	0.57	0.75	0.86	0.91	0.97	0.97	0.97	0.97	0.97	7	0.9
7	19200	0.03	0.09	0.42	0.64	0.93	0.96	0.97	0.97	0.97	0.97	7	0.9
7	19202	0.24	0.42	0.59	0.78	0.9	0.92	0.93	0.94	0.94	0.94	7	0.9
7	19204	0.18	0.44	0.63	0.74	0.9	0.97	0.97	0.99	1	1	7	0.9
7	19273	0.28	0.54	0.71	0.81	0.9	1	1	1	1	1	7	0.9
7	19274	0.06	0.31	0.69	0.84	0.9	0.91	0.92	0.92	0.94	0.94	7	0.9
7	19281	0.17	0.29	0.53	0.82	0.94	0.96	0.98	0.98	0.98	0.98	7	0.9
7	19284	0.08	0.27	0.5	0.76	0.97	0.97	0.99	0.99	1	1	7	0.9
7	20263	0.15	0.25	0.56	0.85	0.93	0.97	0.98	0.99	0.99	0.99	7	0.9
7	20266	0.2	0.46	0.62	0.83	0.97	0.99	0.99	1	1	1	7	0.9
7	20733	0.4	0.54	0.74	0.76	0.91	0.99	0.99	0.99	0.99	0.99	7	0.9
7	17447	0.29	0.51	0.71	0.73	0.86	0.94	0.97	0.97	0.97	0.97	8	0.9
7	18255	0.17	0.45	0.57	0.69	0.87	0.96	0.97	0.98	0.98	0.99	8	0.9
7	18312	0	0.15	0.61	0.74	0.86	0.91	0.94	0.95	0.95	0.95	8	0.9
7	18527	0.46	0.6	0.71	0.8	0.89	1	1	1	1	1	8	0.9
7	18531	0.16	0.46	0.71	0.78	0.83	0.94	0.95	0.96	0.96	0.96	8	0.9
7	18660	0.23	0.38	0.49	0.64	0.88	0.9	0.94	0.96	0.97	0.97	8	0.9
7	18688	0.22	0.45	0.54	0.75	0.89	0.91	0.91	0.92	0.92	0.93	8	0.9
7	19079	0.15	0.32	0.62	0.82	0.87	0.92	0.94	0.95	0.97	0.97	8	0.9
7	19140	0.21	0.33	0.52	0.65	0.84	1	1	1	1	1	8	0.9
7	17452	0.25	0.48	0.63	0.65	0.84	0.89	0.91	0.91	0.92	0.92	9	0.9
7	17456	0.28	0.41	0.67	0.74	0.77	0.8	0.99	0.99	0.99	0.99	9	0.9
7	18888	0.2	0.46	0.51	0.61	0.77	0.84	0.92	0.92	0.92	0.92	9	0.9
7	19051	0.19	0.4	0.6	0.76	0.88	0.89	0.9	0.91	0.92	0.93	9	0.9
7	19080	0.3	0.48	0.76	0.85	0.87	0.87	0.92	0.92	0.92	0.93	9	0.9
7	19196	0.23	0.4	0.57	0.78	0.85	0.89	0.91	0.94	0.95	0.96	9	0.9

Table A.7 Continued.

Cap	line	d3	d4	d5	d6	d7	d8	d9	d10	d11	d12	germ	margin
7	18131	0.21	0.41	0.74	0.78	0.94	0.98	0.98	0.99	0.99	0.99	7	0.9
7	18174	0.1	0.29	0.63	0.81	0.93	0.95	0.99	1	1	1	7	0.9
7	18198	0.26	0.6	0.76	0.84	0.98	0.99	1	1	1	1	7	0.9
7	18664	0.12	0.33	0.49	0.62	0.73	0.79	0.82	0.84	0.88	0.92	12	0.9
7	17469	0.18	0.25	0.45	0.66	0.79	0.81	0.84	0.84	0.84	0.85	13	0.9
7	17489	0.31	0.41	0.61	0.68	0.7	0.7	0.7	0.7	0.7	0.7	13	0.9
7	17668	0.08	0.21	0.37	0.47	0.58	0.68	0.72	0.72	0.75	0.76	13	0.9
7	17671	0.17	0.33	0.51	0.6	0.64	0.66	0.66	0.68	0.68	0.68	13	0.9
7	18057	0.08	0.25	0.52	0.61	0.76	0.82	0.83	0.85	0.85	0.85	13	0.9
7	18130	0.25	0.39	0.51	0.6	0.78	0.85	0.88	0.88	0.88	0.88	13	0.9
7	18196	0.23	0.4	0.54	0.63	0.66	0.66	0.7	0.7	0.7	0.73	13	0.9
7	18200												
7	18227	0.12	0.13	0.18	0.18	0.18	0.33	0.33	0.33	0.33	0.33	13	0.9
7	18234	0.1	0.34	0.58	0.63	0.7	0.72	0.72	0.73	0.74	0.75	13	0.9
7	18249	0.16	0.33	0.49	0.66	0.76	0.83	0.85	0.85	0.85	0.85	13	0.9
7	18256	0.11	0.24	0.42	0.6	0.81	0.83	0.84	0.84	0.84	0.84	13	0.9
7	18422	0.05	0.12	0.17	0.2	0.23	0.32	0.38	0.4	0.42	0.42	13	0.9
7	18547	0.01	0.1	0.31	0.59	0.77	0.82	0.86	0.88	0.88	0.88	13	0.9
7	18578	0	0	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	13	0.9
7	18590	0.01	0.05	0.19	0.33	0.54	0.59	0.65	0.68	0.71	0.73	13	0.9
7	18591	0.08	0.26	0.51	0.62	0.71	0.79	0.81	0.82	0.85	0.86	13	0.9
7	18593	0.17	0.29	0.46	0.57	0.7	0.72	0.74	0.76	0.76	0.76	13	0.9
7	18651	0.03	0.16	0.33	0.42	0.56	0.7	0.78	0.86	0.86	0.87	13	0.9
7	18693	0.32	0.49	0.56	0.76	0.81	0.81	0.82	0.82	0.82	0.82	13	0.9
7	18833	0.01	0.05	0.1	0.17	0.34	0.4	0.43	0.46	0.46	0.46	13	0.9
7	18999	0.08	0.24	0.39	0.5	0.61	0.7	0.72	0.76	0.76	0.77	13	0.9
7	19009	0	0.12	0.28	0.34	0.54	0.59	0.62	0.63	0.65	0.65	13	0.9
7	19034	0.28	0.45	0.63	0.69	0.79	0.8	0.81	0.84	0.87	0.87	13	0.9
7	19042												
7	19043	0.14	0.22	0.48	0.62	0.64	0.67	0.68	0.7	0.7	0.7	13	0.9
7	19055	0.11	0.3	0.65	0.74	0.81	0.83	0.84	0.86	0.88	0.89	13	0.9
7	19075	0.18	0.34	0.49	0.52	0.53	0.59	0.6	0.6	0.61	0.61	13	0.9
7	19081	0.11	0.25	0.48	0.48	0.54	0.55	0.57	0.58	0.58	0.58	13	0.9
7	19084	0	0.02	0.02	0.05	0.17	0.29	0.31	0.31	0.31	0.32	13	0.9
7	19130	0.07	0.19	0.37	0.51	0.65	0.72	0.75	0.75	0.76	0.76	13	0.9
7	19198	0.16	0.3	0.59	0.68	0.76	0.78	0.86	0.86	0.86	0.86	13	0.9
7	19282	0	0.06	0.14	0.32	0.53	0.54	0.55	0.56	0.58	0.6	13	0.9
7	20270	0.06	0.19	0.38	0.6	0.67	0.72	0.75	0.78	0.78	0.8	13	0.9
7	20271	0.11	0.26	0.47	0.66	0.71	0.72	0.75	0.76	0.76	0.76	13	0.9
7	20313	0.06	0.17	0.37	0.57	0.64	0.67	0.71	0.71	0.71	0.71	13	0.9
7	20737	0.09	0.24	0.37	0.47	0.67	0.75	0.78	0.78	0.79	0.8	13	0.9

Table A.8. CAP-8 Daily summation percentages and days to germination margin (0.9); data are means of five replications

Cap	line	d3	d4	d5	d6	d7	d8	d9	d10	d11	d12	germ	margin
8	19307	0.45	0.9	0.97	1	1	1	1	1	1	1	4	0.9
8	17411	0.37	0.59	0.92	0.99	0.99	0.99	0.99	1	1	1	5	0.9
8	18221	0.55	0.73	0.95	0.97	0.97	0.99	1	1	1	1	5	0.9
8	18520	0.63	0.73	0.92	0.99	1	1	1	1	1	1	5	0.9
8	19189	0.43	0.67	0.93	0.97	0.97	0.97	0.97	0.97	0.97	0.97	5	0.9
8	17461	0.14	0.32	0.69	0.91	0.94	0.95	0.95	0.97	0.98	0.99	6	0.9
8	17462	0.25	0.57	0.88	0.98	1	1	1	1	1	1	6	0.9
8	18096	0.4	0.65	0.8	1	1	1	1	1	1	1	6	0.9
8	18237	0.33	0.51	0.7	0.92	0.96	0.99	1	1	1	1	6	0.9
8	18525	0.08	0.37	0.63	0.93	0.97	0.97	1	1	1	1	6	0.9
8	18618	0.47	0.58	0.87	0.98	0.99	0.99	0.99	0.99	0.99	0.99	6	0.9
8	18620	0.43	0.57	0.84	0.96	0.96	0.98	0.99	1	1	1	6	0.9
8	18638	0.4	0.68	0.86	0.97	0.99	0.99	0.99	0.99	0.99	0.99	6	0.9
8	18642	0.22	0.5	0.75	0.95	0.98	0.98	1	1	1	1	6	0.9
8	18673	0.37	0.55	0.66	0.91	0.95	0.97	0.98	0.99	0.99	1	6	0.9
8	18712	0.42	0.54	0.77	0.9	0.99	0.99	0.99	1	1	1	6	0.9
8	18737	0.14	0.55	0.82	1	1	1	1	1	1	1	6	0.9
8	19989	0.1	0.27	0.74	0.96	1	1	1	1	1	1	6	0.9
8	20124	0.22	0.46	0.77	0.95	0.98	0.98	0.99	0.99	0.99	0.99	6	0.9
8	20493	0.26	0.58	0.82	0.93	0.99	0.99	1	1	1	1	6	0.9
8	20494	0.44	0.62	0.82	0.97	0.98	0.99	0.99	0.99	0.99	0.99	6	0.9
8	20529	0.42	0.65	0.88	0.96	0.96	0.96	0.99	1	1	1	6	0.9
8	17413	0.06	0.27	0.57	0.73	0.92	0.94	0.95	0.96	0.96	0.96	7	0.9
8	17414	0	0.07	0.39	0.64	0.9	0.93	0.94	0.95	0.96	0.98	7	0.9
8	17482	0.15	0.38	0.74	0.86	0.99	0.99	0.99	1	1	1	7	0.9
8	17810	0	0.14	0.51	0.71	0.96	0.96	0.98	0.98	0.98	1	7	0.9
8	17816	0	0.22	0.69	0.78	0.98	0.98	0.98	1	1	1	7	0.9
8	18117	0.3	0.53	0.61	0.75	0.98	0.98	0.98	0.98	0.98	0.98	7	0.9
8	18218	0.08	0.23	0.62	0.87	0.95	0.97	0.97	0.99	0.99	0.99	7	0.9
8	18329	0.06	0.22	0.56	0.8	0.96	0.96	0.96	0.96	0.96	0.96	7	0.9
8	18344	0.05	0.31	0.53	0.78	0.97	0.99	1	1	1	1	7	0.9
8	18521	0.17	0.42	0.59	0.77	0.93	0.93	0.96	0.96	0.96	0.96	7	0.9
8	18524	0.22	0.47	0.6	0.85	0.95	0.96	0.97	0.97	0.97	0.97	7	0.9
8	18599	0.06	0.22	0.61	0.79	0.94	0.96	0.99	0.99	0.99	0.99	7	0.9
8	18644	0.46	0.69	0.79	0.79	0.95	0.99	1	1	1	1	7	0.9
8	18674	0.18	0.37	0.63	0.84	0.92	0.94	1	1	1	1	7	0.9
8	18675	0.49	0.62	0.76	0.83	0.98	0.98	0.99	1	1	1	7	0.9
8	18685	0.15	0.38	0.64	0.83	0.92	0.95	0.97	0.97	0.97	0.98	7	0.9
8	18730	0.05	0.28	0.69	0.77	0.94	0.99	1	1	1	1	7	0.9
8	18776	0.25	0.57	0.73	0.83	0.96	0.97	0.99	0.99	0.99	0.99	7	0.9
8	18862	0.09	0.2	0.57	0.71	0.92	0.95	0.95	0.97	0.98	0.98	7	0.9
8	20117	0.17	0.32	0.74	0.84	0.94	0.94	0.96	0.97	0.97	0.97	7	0.9
8	20118	0.04	0.16	0.42	0.78	0.92	0.92	0.93	0.93	0.94	0.94	7	0.9
8	20121	0.12	0.31	0.66	0.76	0.96	0.98	1	1	1	1	7	0.9
8	17815	0.02	0.16	0.59	0.77	0.87	0.97	0.99	1	1	1	8	0.9
8	17817	0	0.09	0.42	0.68	0.87	0.95	0.96	0.96	0.96	0.96	8	0.9
8	18335	0.08	0.39	0.57	0.71	0.8	0.94	0.97	0.97	0.97	0.97	8	0.9
8	18522	0.16	0.35	0.57	0.77	0.86	0.95	0.99	0.99	0.99	0.99	8	0.9

Table A.8. Continued.

Cap	line	d3	d4	d5	d6	d7	d8	d9	d10	d11	d12	germ	margin
8	18740	0.11	0.2	0.6	0.72	0.87	0.96	0.96	0.96	0.97	0.97	8	0.9
8	19308	0.42	0.7	0.81	0.82	0.88	0.9	0.91	0.91	0.91	0.91	8	0.9
8	20492	0.14	0.34	0.59	0.75	0.81	0.97	0.98	0.98	0.98	0.98	8	0.9
8	17412	0.02	0.14	0.53	0.76	0.85	0.86	0.91	0.93	0.93	0.93	9	0.9
8	17991	0.22	0.35	0.63	0.83	0.87	0.89	0.9	0.92	0.95	0.95	9	0.9
8	22373	0	0.1	0.22	0.48	0.66	0.89	0.91	0.94	0.94	0.96	9	0.9
8	18557	0.06	0.22	0.44	0.77	0.85	0.86	0.89	0.91	0.91	0.92	10	0.9
8	19951	0	0.11	0.24	0.48	0.67	0.76	0.86	0.93	0.94	0.94	10	0.9
8	20190	0.34	0.58	0.71	0.78	0.81	0.84	0.89	0.92	0.92	0.94	10	0.9
8	18104	0.21	0.41	0.57	0.66	0.78	0.8	0.88	0.88	0.9	0.9	11	0.9
8	18641	0.07	0.29	0.52	0.65	0.75	0.81	0.82	0.87	0.95	0.95	11	0.9
8	17397	0	0.02	0.22	0.45	0.71	0.81	0.87	0.89	0.89	0.91	12	0.9
8	18733	0.02	0.14	0.34	0.58	0.78	0.84	0.89	0.89	0.89	0.91	12	0.9
8	18773	0.09	0.18	0.19	1.03	1.49	0.75	0.75	0.51	0.53	2.54	12	0.9
8	19950	0.06	0.24	0.45	0.56	0.83	0.87	0.89	0.89	0.89	0.92	12	0.9
8	17396	0	0	0.02	0.03	0.06	0.13	0.31	0.31	0.31	0.33	13	0.9
8	17479	0	0.04	0.09	0.2	0.51	0.53	0.63	0.69	0.72	0.76	13	0.9
8	17715	0.01	0.05	0.15	0.31	0.35	0.44	0.48	0.53	0.55	0.61	13	0.9
8	17719	0.12	0.31	0.43	0.61	0.66	0.69	0.71	0.72	0.72	0.72	13	0.9
8	18008												
8	18098	0.05	0.19	0.47	0.64	0.72	0.8	0.83	0.88	0.89	0.89	13	0.9
8	18116	0	0.06	0.27	0.36	0.38	0.4	0.41	0.46	0.49	0.5	13	0.9
8	18343												
8	18523	0.08	0.16	0.41	0.49	0.7	0.73	0.76	0.78	0.78	0.78	13	0.9
8	18559	0.01	0.03	0.06	0.12	0.21	0.23	0.27	0.32	0.34	0.36	13	0.9
8	18617	0.05	0.21	0.47	0.61	0.72	0.76	0.77	0.79	0.79	0.8	13	0.9
8	18621	0.05	0.17	0.3	0.45	0.59	0.63	0.7	0.71	0.73	0.75	13	0.9
8	18686	0.01	0.08	0.16	0.35	0.5	0.55	0.57	0.59	0.6	0.61	13	0.9
8	18708	0.05	0.14	0.35	0.52	0.73	0.84	0.85	0.86	0.86	0.86	13	0.9
8	18727	0.02	0.11	0.29	0.42	0.63	0.65	0.66	0.66	0.66	0.67	13	0.9
8	18732	0.05	0.15	0.46	0.62	0.71	0.72	0.78	0.79	0.79	0.79	13	0.9
8	18779	0.23	0.33	0.31	0.39	0.66	0.68	0.68	0.79	0.79	0.79	13	0.9
8	18781	0.25	0.36	0.44	0.5	0.75	0.75	0.75	0.75	0.75	0.75	13	0.9
8	19304	0.33	0.52	0.65	0.75	0.77	0.77	0.77	0.77	0.77	0.77	13	0.9
8	19953	0	0.06	0.25	0.41	0.66	0.68	0.78	0.81	0.81	0.81	13	0.9
8	19988	0.03	0.12	0.41	0.67	0.71	0.79	0.82	0.86	0.87	0.87	13	0.9
8	20495	0.09	0.21	0.41	0.61	0.76	0.79	0.82	0.88	0.89	0.89	13	0.9
8	20517	0.11	0.31	0.42	0.64	0.78	0.79	0.81	0.81	0.81	0.81	13	0.9

Table A.9. Falling number (FN) values of population in CAP – 7 (136 lines). Data are means of two replications.

Line Number	FN
18662	248
19038	267
18429	271
18173	285
17466	288
18425	294
18660	299
19200	299
19880	305
18169	310
18426	313
18828	317
19040	318
18123	319
17447	330
19029	332
20266	332
18057	333
18014	339
18834	345
17668	347
19028	355
18224	357
18319	363
20271	366
18527	371
19202	381
19201	386
18234	388
17456	388
18693	388
18531	394
19053	394
18593	394
18312	396
19055	396
18422	398
18728	398
17068	399
18229	402
19036	409
18827	415
18690	419
18249	421

Line Number	FN
19196	422
18198	423
19281	426
19086	430
18592	430
18999	433
18579	437
19284	437
19282	438
18013	439
18552	440
20737	443
18830	444
17037	460
18131	465
19046	467
19140	470
19883	477
18591	482
17034	491
20268	494
18833	495
18547	519
18659	526
20313	543
18534	544
20270	547
17671	551
18315	.
18248	.
18245	.
18227	.
19075	.
19084	.
19081	.
19080	.
19079	.
19074	.
18174	.
18171	.
18232	.
19195	.
19194	.
18200	.

Line Number	FN
18133	.
18132	.
19190	.
18664	.
17672	.
18666	.
19274	.
19204	.
19273	.
18651	.
18656	.
17452	.
18130	.
18121	.
17450	.
18129	.
17489	.
18196	.
18888	.
18530	.
18578	.
19034	.
19043	.
19048	.
19042	.
19130	.
19131	.
17670	.
17669	.
20733	.
19051	.
19044	.
19009	.
18590	.
19027	.
18548	.
17030	.
18507	.
18688	.
18258	.
18256	.
18255	.
19906	.
19878	.

Table A.10. Falling number (FN) values of population in CAP – 8 (88 lines). Data are means of two replications.

Line Number	FN
18781	243
18644	250
18525	259
18522	299
18674	300
18673	309
18599	317
18096	320
19988	333
18117	337
18335	342
20117	346
18237	352
17413	356
18618	367
19307	382
17397	383
20124	384
17810	387
18344	398
20118	398
18620	400
17482	404
18708	405
19950	412
19189	417
17817	421
20493	421
18776	425
19989	427
19308	429
20492	433
18712	435
20517	437
20121	441
18524	442
17414	451
18329	453
18727	453
17462	455
18621	463
17715	463
18523	464
19953	470

Line Number	FN
18737	477
17411	478
18730	483
18218	486
17412	489
18685	489
18638	490
18686	492
18617	499
18773	503
18862	504
18733	516
20495	516
17719	551
17479	553
18557	564
17396	570
18559	668
17815	.
17816	.
18732	.
22373	.
18740	.
19951	.
18521	.
18520	.
20527	.
18779	.
18641	.
18642	.
18098	.
18104	.
18116	.
17461	.
17991	.
18008	.
18221	.
20494	.
20190	.
19304	.
20494	.
20529	.
18343	.
18675	.