AN ABSTRACT OF THE THESIS OF

Andre Cunha Rosa for the degree of Master of Science in Crop Science presented on January 4, 1999.

Title: Pre-harvest Sprouting Tolerance of a Synthetic Hexaploid Wheat (*Triticum turgidum* L. × *Aegilops tauschii* Coss.)

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Abstract approved: _____

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Pre-harvest sprouting in wheat costs farmers millions of dollars every year. Pre-harvest sprouting tolerance (PST) has minimized this problem, but improvement of PST is still necessary. Synthetic hexaploid wheats (synthetics) have been used as sources of genes coding for many useful traits. Two studies evaluated the PST of a synthetic (Altar 84/*Aegilops tauschii*) and investigated its potential as a source of PST in crosses with wheat cultivars.

The first study compared the synthetic with selected wheat checks for PST and with its parent Altar 84 for the germination response of these genotypes to controlled wetting treatments applied to field-grown intact spikes and threshed seed. Spikes were rolled in wet germination paper and the percentages of germinated seed were determined after seven days. Threshed seeds in Petri dishes were wetted with water and vegetative floral tissues (chaff) extracts. Germinating seeds were counted daily for 14

days. The synthetic was more tolerant than Altar 84 and was classified as moderately sensitive. The improved PST of the synthetic over Altar 84 was attributed to *Aegilops tauschii*. Seed dormancy and water-soluble substances in the chaff of the synthetic and other genotypes appeared to contribute to their PST.

The second study used random inbred F_5 lines obtained from single and backcrosses between the synthetic (red-seeded) and the sensitive wheat cultivars Opata 85 (red-seeded) and Bacanora 88 (white-seeded). Seed coat color and germination responses of the F_5 lines subjected to a five-day spike wetting treatment were evaluated. Pre-harvest sprouting tolerance was moderately to highly inheritable and largely controlled by additive gene effects in the studied populations. An association between red seed coat color and PST was observed but white recombinant lines more tolerant than their sensitive parent were obtained. The synthetic can be used to improve wheats with red and white seed coats. The potential use of the synthetic as a PST source was discussed and a breeding strategy suggested.

PRE-HARVEST SPROUTING TOLERANCE OF A SYNTHETIC HEXAPLOID WHEAT (*Triticum turgidum* L. × *Aegilops tauschii* Coss.)

by

Andre Cunha Rosa

A THESIS

submitted to

Oregon State University

in partial fulfillment of
the requirements for the
degree of

Master of Science

Completed January 4, 1999

Commencement June, 1999

Master of Science thesis of Andre Cunha Rosa presented on January 4, 1999

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Andre Cunha Rosa, Author

ACKNOWLEDGEMENTS

I would like to express my sincere gratitude and appreciation to Dr. Warren E. Kronstad for his guidance, encouragement, support, patience, and friendship throughout my graduate studies at Oregon State University. Unfortunately, words can not express how much I am thankful to him.

Special thanks are extended to Drs. David Thomas, Patrick Hayes, and Gregory Perry, members of my committee for their time and assistance during the course of this research.

A very special thanks to Dr. Bob Metzger for his friendship, for his wish to share experience, and for his valuable suggestions. I wish to thank Mr. Dale Brown from the Oregon State Seed Laboratory for his friendship and help in obtaining laboratory supplies.

Special thanks to Mr. Mark Larson and Dr. Karim Ammar for their friendship, advice, and innumerous suggestions to this thesis. I would like to offer a special thanks to my friend and colleague Ms. Alicia Del Blanco for kindly providing the four wheat populations used in this study.

Sincere thanks and gratitude are extended to all staff and members of the Cereal Project, as well as my fellow graduate students. Your daily help and friendship were essential for the completion of this experience.

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IN DEDICATION

To

Gilda and Ottoni, my parents

Maria, my grandmother

and

to my wife, Erenice, whose help, patience,

perseverance, and love enabled me to carry out this study

PRE-HARVEST SPROUTING TOLERANCE OF A SYNTHETIC HEXAPLOID WHEAT (*Triticum turgidum* L. × *Aegilops tauschii* Coss.)

1. INTRODUCTION

The occurrence of rain before cereal crops are harvested may induce the kernels to sprout on the spike or panicle. This phenomenon is named pre-harvest sprouting and affects various cereal crops. On wheat (*Triticum aestivum* L.), pre-harvest sprouting reduces yield and test weight, and damages the end-use quality of the grain. Losses to sprouting cost farmers millions of dollars every year (Derera, 1989a). Countries most affected by pre-harvest sprouting include Argentina, Australia, Brazil, Canada, Kenya, Norway, Sweden, and the United States.

Much effort has been dedicated to develop cultivars with pre-harvest sprouting tolerance (PST), which can be defined as "the failure of viable kernels to germinate in intact spikes when subjected to favorable conditions of temperature, moisture and oxygen" (DePauw and McCaig, 1991). Pre-harvest sprouting tolerance is usually attributed to seed dormancy but, chemical inhibitors in the bracts, physical characteristics of plants and spikes, and other characteristics have also been shown to contribute to PST (Derera et al., 1976; Mares, 1987; Paterson et al., 1989). The development of cultivars with PST has alleviated the sprouting problem in many areas. However, most tolerant cultivars have red grains because of an association between red seed coat color and seed dormancy (DePauw and McCaig, 1983; Gfeller and Svejda, 1960). Therefore, white wheat growing areas tend to be more prone to sprouting

damage. Further enhancement of PST is still necessary in red and white wheats. This can be achieved by pyramiding genes already available or by introducing new sources of genetic tolerance.

Many synthetic hexaploid wheats (2n = 42, AABBDD) obtained by crossing various accessions of *Triticum turgidum* L. var. durum (2n = 28, AABB) and *Aegilops tauschii* Coss. (2n = 14, DD) have been produced and utilized as sources of many useful traits during the past decade (May and Lagudah, 1992; Mujeeb-Kazi et al., 1996; Villareal et al., 1996). Many wild species, such as *Aegilops tauschii*, have strong seed dormancy or other mechanisms to delay germination and ensure their survival (Mac Key, 1989; Xin-Jin et al., 1997; Gatford, 1998). Thus, it is reasonable to expect that the synthetic hexaploid wheats (hereafter called synthetics) may have contributed to the wheat gene pool some mechanisms to delay germination and improve PST. In fact, Xiu-Jin et al. (1997) has provided the prima facie evidence that PST can be found in *Aegilops tauschii* and can be transferred into wheat cultivars. The possibility of PST coming from durum wheat (*Triticum durum* L. var. durum) also deserve consideration due to previous reports showing that some of these wheats have some PST. Preliminary studies in the laboratories at Oregon State University suggested that, a synthetic (Altar 84/*Aegilops tauschii* - unknown accession) from the International Wheat and Maize Improvement Center (CIMMYT) has PST levels approaching those observed for Frontana, which is known for its excellent PST (data not shown).

The main purpose of this study was to evaluate the PST of the synthetic Altar 84/*Aegilops tauschii* (Altar/At) and determine the potential of this genotype as a source of PST in crosses with wheat cultivars. To accomplish this, two studies were conducted. In "Study I" the following objectives were considered: i) compare the PST of the synthetic Altar/At (synthetic) with the PST of check cultivars, ii) determine which parent(s) contributed to the PST in the synthetic, and iii) investigate the mechanism(s) that confer PST to the synthetic. "Study II" aimed to: i) determine the nature of the inheritance of PST and the heritability of this character in four F_5 random inbred populations from synthetic/wheat crosses, ii) investigate a possible association between seed coat color and PST in the selected populations, and iii) evaluate the synthetic as a source of PST for wheats with red and white seed coats.

2. LITERATURE REVIEW

2.1 THE SPROUTING PROBLEM

Pre-harvest sprouting can seriously affect the quantity and quality of the wheat crop. Grain yield losses occur during threshing because plumules, radicles, and light kernels are winnowed out. Test weight and seed viability may be also compromised. Qualitywise, the activation and *de novo* synthesis of hydrolytic enzymes, particularly α-amylase, during germination makes the wheat end use quality inappropriate for most end products. Sprouting damage is usually evaluated with the Falling Number apparatus (Hagberg, 1960) or with the more recently developed Rapid Visco-Analyser (RVA) (Ross et al., 1987). If sprouting damage (above a specific threshold) is detected, the wheat lot is downgraded or even considered as feed grain. In Australia, Derera (1980) reported that downgrading represents a loss of 15-40 dollars per ton, which translates to an estimated annual loss of 18 million dollars.

Bread made from sprout-damaged wheat exhibits a sticky crumb, pale crust, coarse texture, and sometimes the loaf volume collapses (Derera, 1989b; Sorrels et al., 1989). Other authors referred to a too-dark crust rather than a pale one (Orth and Moss, 1987; Mansour, 1992). The sticky crumb is a result of the excessive α-amylase activity, which causes an unbalanced ratio of α and β-amylases. This unbalance impairs the hydrolysis of dextrins by β-amylase, while the α-amylase continues degrading starch and producing low-molecular-weight dextrins. The accumulation of these dextrins is responsible for the crumb stickiness and the crust browning (Dapron and Godon, 1987).

There is considerable variation in sensitivity of various end products to sprout-damaged wheat. Orth and Moss (1987) found that Cantonese-style noodles were more sensitive than pan bread, while flat bread and chapattis were quite tolerant. Sorrels et al. (1989) demonstrated that cookies can tolerate high levels of sprouting with little effect on quality. According to Nagao (1996), the sensitivity of a specific product to sprout-damaged flour depends on the moisture content and temperature used to process the dough. The time during which the enzymes are allowed to degrade the flour before their inactivation by high temperatures of baking, cooking, or other similar process is critical. Finally, even when it is possible to produce some end products with acceptable quality using sprout-damaged wheat, mills have always preferred sound wheat because of its better milling and processing qualities (Orth and Moss, 1987; Sorrels et al., 1989; Nagao, 1996).

2.2 Mechanisms of Pre-Harvest Sprouting Tolerance

Pre-harvest sprouting tolerance (PST) is a complex trait. It is the result of a combination of mechanisms that can be divide into three classes: i) seed dormancy, ii) chemical inhibitors in vegetative floral tissues, and iii) spike and seed morphological characteristics.

2.2.1 Seed Dormancy

Seed dormancy can be defined as "any condition inherent in the seeds which prevents the germination of viable seeds for a definite period after harvest" (Gfeller and Svejda, 1960). Chemical or physical effects that originate outside the seed do not characterize seed dormancy.

Seed dormancy is the major mechanism responsible for PST in most of the existing wheat cultivars (Mares and Ellison, 1990; Mares, 1993). It is an efficient system to avoid pre-harvest sprouting damage, but presents limitations. The major limitation is the association between seed dormancy and red seed coat color, which impairs the production of tolerant wheats with white grain. Despite the difficulties, white wheats with reasonable levels of dormancy and PST have been obtained in the last decades. Some of the most promising include: Clarks Cream (Upadhyay et al., 1988), SC8021V2 (DePauw et al., 1992), and AUS1408 (Mares, 1987). Another limitation is that a strong dormancy may cause germination problems when the period between harvesting and sowing is short. This is particularly true in winter wheat production areas (Reddy, 1978), and for breeding programs that grow more than one generation per year (Rajaram et al., 1990). In such breeding programs, germination problems due to dormancy result in germplasm with higher levels of dormancy being discarded. Some breeding programs overcome this problem by breaking dormancy with a high temperature incubation (37°C) for seven days. Due to the limitations associated with dormancy, breeding efforts are in progress to develop alternative mechanisms, such as the presence of water-soluble chemical inhibitors in the glumes which,

hopefully, will not relate to seed color and will be easily removed by threshing (Gatford, 1998, personal communication).

Seed dormancy and therefore PST are affected by the interaction of a number of mechanisms. The mechanisms that control seed dormancy can be classified into two classes: seed-coat-related and embryo-related mechanisms.

2.2.1.1 Seed-Coat-Related Mechanisms

Physico-chemical barrier - the physical barrier to water uptake imposed by the seed coat has been the subject of few studies and does not appear to be an important mechanism in the control of seed dormancy in wheat (Ching and Foote, 1961; Belderok, 1976). More recently, this system was found to be working in *Aegilops tauschii* (Gatford, 1998, personal communication). According to Côme et al. (1984), dormancy in cereals is caused by a limitation in oxygen exchange imposed by the seed coat and pericarp. They postulated that the enzymatic oxidation of phenolic compounds on these integuments consumes most of the available oxygen, thus limiting embryo growth. The weakening of dormancy observed during after-ripening would be a result of a decrease in the efficiency of the oxygen diffusion barrier. Their conclusions were based on studies with barley. In wheat, this theory has been subject of discussion and remains to be proven (Belderok, 1976; Gordon, 1980; King, 1989).

Chemical inhibitors in the seed coat - the wheat seed coat has been shown to harbor water-soluble inhibitors, which interact with the embryo affecting seed dormancy. Miyamoto et al. (1961) identified catechin, catechin-tannins (CT), and alkaloids as water-soluble inhibitors from the wheat seed coat. Only traces of such compounds were detected in the pericarp. They found that wheats with dark-red grain had more CT than light-red wheats and twice as much as white wheats. Furthermore, as dormancy diminished with after-ripening, so did the amount of CT. Miyamoto and Everson (1958) suggested that the strong dormancy observed in red wheats is not caused directly by the seed coat pigment, namely phlobaphene, but by its precursors, the uncolored catechins and catechin tannins. In addition, the weakening of dormancy observed during after-ripening was considered to be caused by the inactivation of CT. Later studies demonstrated that genotypes clearly differed in the sensitivity of their embryos to CT (Stoy and Sundin, 1976) and this difference was controlled by a single gene (Stoy and Olsen, 1980).

Woodbury and Wiebe (1983) demonstrated that coumarins and other phenolic substances inhibit wheat germination under high moisture condition. At low moisture levels, the same inhibitors either had no effect or slightly increased germination. They suggested that as the water enters into the seed through cracks in the pericarp within the brush region, the inhibitors are then carried to the embryo. Effects of temperature and rate of drying on the final structure of the phenolic polymers were thought to be the basis of the response of dormancy to these two phenomena. The authors, however,

failed to indicate evidence of how coumarins and the other phenolics could be found in the seed integuments.

2.2.1.2. Embryo-Related Mechanisms

True embryo dormancy - true embryo dormancy is characterized by an inherent condition of the embryo to remain dormant and by the reversibility of this condition when cytokinins are applied (Belderok, 1976). This type of dormancy is known to occur in various tree species and Belderok (1976) has suggested its presence in wild oats, barley and wheat. While, experiments based on germination of excised embryos have indicated that wheat embryos germinate promptly when excised from the seed and placed on germination media (Miyamoto and Everson, 1958; Stoy and Sundin, 1976; McCrate et al., 1982). Recently, Gatford (1998, personal communication) encountered true embryo dormancy in *Aegilops tauschii* and transferred it to synthetic wheats. The embryos of the most dormant synthetic (870192/AUS18975) took 14 days to initiate germination after being placed on moist paper. However, the whole seeds of the same synthetic initiated germination in three days indicating a possible germination-promoting effect by the seed coat. Since a three-day period is thought to be adequate for Australian conditions, and true embryo dormancy is not expected to depend on seed coat color, there is hope that such trait can be introduced into white wheat cultivars.

Embryo inhibition/promotion - even though the excised embryos of wheat are ready to germinate when placed in moist media, a variety of substances external to the

embryo can inhibit this process. The effect of these "external" inhibitors is usually reversed by gibberelic acid (GA) rather than by cytokinins (Belderok, 1976). The effect of CT, coumarins, and other phenolics on wheat embryos was previously mentioned.

Others substances that have been reported to interact with wheat embryos are the plant growth regulators GA and abscisic acid (ABA). While GA promotes germination and production of α-amylase, ABA suppresses both phenomena and stimulates the synthesis of amylase inhibitors (Stoy and Sundin, 1976; King, 1989). Stoy and Sundin (1976) showed that the inhibition produced by ABA was stronger than that produced by CT, and that GA counteracted ABA more efficiently than it counteracted CT. Embryo sensitivity to ABA has been found to be proportional to seed dormancy. As seed dormancy was reduced by after-ripening, sensitivity of embryos to ABA was also reduced (Walker-Simmons et al., 1990). These results indicate that not only the amount and kind of inhibitors/promoters is relevant for seed dormancy control, but also that the sensitivity of the embryo to each of these substances is important.

Genotypic differences in embryo sensitivity to ABA and GA have been reported and used in breeding programs (Stoy and Sundin, 1976; McMaster, 1976; Flintham, 1990). The importance of GA insensitivity is not as related to dormancy enhancement as it is to the inhibition of α-amylase synthesis. Gibberelic acid insensitive genotypes can still produce enough α-amylase to sustain germination, but the overall α-amylase activity is reduced in both sound and sprouted samples (Flintham and Gale 1980; McMaster, 1976). Such reduction in a genotype may constitute the difference between

failure and success in the commercialization. Insensitivity to GA correlates with plant height (Derera et al., 1976). The genetic basis of this correlation will be reviewed in section 2.3.1.

2.2.2 Chemical Inhibitors in Vegetative Floral Tissues

The presence of water-soluble germination inhibitors in vegetative floral tissues of wheat and triticale has been demonstrated (Derera et al., 1976; Salmon et al., 1986; Trethowan et al., 1993). Vegetative floral tissues, commonly designated as chaff, are all the components of a wheat spike (glumes, lemmas, rachises, peleae, and awns) with the exception of the kernels. Derera et al. (1976) studied the germination response of after-ripened (80 days) seed of a group of cultivars to their own chaff inhibitors. They observed differential germination inhibition response among cultivars, varying from no inhibition to strong inhibition (in the case of the cultivar Kleiber). Using another set of cultivars, McCrate et al. (1982) demonstrated that during after-ripening the inhibition caused by chaff extracts decreases as seed dormancy is reduced until there is practically no inhibition. Thus, non-dormant seeds of the cultivar Kleiber should not have been inhibited. Possible explanations to conciliate results of both studies are that i) some dormancy was still present even after the 80 days, ii) Kleiber and the other responsive cultivars are different in this regard from the cultivars studied by McCrate et al. (1982), and iii) differences on concentration of inhibitors in the chaff caused the different responses. In any case, it seems clear that dormancy and chaff inhibitors combined

should provide a better protection to pre-harvest sprouting than any of these two mechanisms alone.

Some other findings have also contributed to a better understanding of chaff inhibitors and their effects. McCrate et al. (1982) found evidence that the decrease in germination inhibition with after-ripening was due to a loss of the embryo sensitivity and not to a decrease in the amount or activity of the inhibitor in the chaff. Such loss of embryo sensitivity is similar to that previously related for ABA. Recent studies concluded that the inhibitory substances on the glumes of a certain accession of *Aegilops tauschii* are a phenolic acid or a glycoside of either vanillic or *p*-coumaric acid (Gatford, 1998, personal communication).

2.2.3 Spike and Seed Morphological Characteristics

Spike morphological characteristics have been shown to affect rates of water absorption and consequently PST (King, 1987; King and Licis, 1990). In this regard, one of the most important spike morphological characteristics appears to be related to the awned or awnless phenotypes. King and Richards (1984) found that under simulated rain, awnless genotypes consistently absorbed less water and had reduced sprouting when compared with awned genotypes. Results were similar whether the comparison was made between groups of cultivars (awned vs. awnless) or between near-isogenic lines. Surprisingly however, mechanical removal of the awns did not reduce water uptake, indicating that the observed differences may not reside on the awns themselves.

Spike nodding angle has also been demonstrated to affect pre-harvest sprouting tolerance (PST). King and Licis (1990) concluded that wheat lines in which the spikes droop at maturity are less predisposed to pre-harvest sprouting. The advantage of the droopy lines seems to be mostly related to the projected surface area (or area directly exposed to the rain). Thus, spikes that are upright at maturity are still less predisposed to pre-harvest sprouting than spikes that acquire any near-horizontal position. In addition, the same authors did not find evidence that waxy spikes would be better than non-waxy in terms of PST and water absorption. Other spike traits that have been studied are glabrousness (King, 1987), floret openness, and glume tenacity (Hong, 1979 - cited in Paterson et al., 1989). Differences of up to two-fold in rate of water uptake by threshed seeds have been reported, however, this variation could not be attributed to differences in seed characteristics such as, seed coat color or hardiness (King, 1987).

One important limitation of the studies discussed in this section is that their measurements were made in a maximum period of 50 hours from the beginning of the wetting treatments. Therefore, the observed effects of spike and seed morphology may or may not be relevant if spikes are submitted to longer periods of rainy weather.

2.3 GENETICS

2.3.1 Association between Seed Coat Color and Seed Dormancy

Seed coat color is controlled by three independent *R* loci located on chromosome arms 3AL, 3BL and 3DL. The alleles that determine red color are dominant and symbolized as *R-A1b*, *R-B1b*, and *R-D1b* (Flintham and Gale, 1996). Various reports have indicated the existence of additional seed coat color alleles (Baker, 1981; Flintham and Humphray, 1993; Flintham and Gale, 1996).

Initially, it was assumed that there was a perfect association between dormancy and seed coat color, which could be due to tight linkages or pleiotropism. Selection for genotypes carrying the three alleles for redness would result in strong dormancy. It was suggested that no white dormant genotypes could be recovered from crosses with dormant red wheats (Gfeller and Svejda, 1960).

There is no doubt that an association between seed dormancy and red seed coat color exists. However, Reitan (1980) suggested that selection for red seed coat should only be used as a guide and not as a systematic method to select for PST. Although no white wheat as dormant as the most dormant red has been identified, the least dormant reds are not different from the least dormant whites (Mares and Ellison, 1990). Flintham and Gale (1996) compared the dormancy of five near-isogenic lines, each one carrying a different allele for red seed coat color, in a non-dormant white background. The lines were all more dormant than the white parent and did not differ among themselves.

Based on the these results, the authors concluded that the alleles for red coat color from anyone of the five donors had the same effect, and that the pigmentation produced by the alleles is apparently a dormancy factor itself. However, various dormant white lines have been recovered from different crosses between red dormant and white seed coat non-dormant parents (DePauw and McCaig, 1987; DePauw et al., 1992; Lawson et al., 1997).

2.3.2 Inheritance of Pre-Harvest Sprouting Tolerance and its Mechanisms

Genetic studies regarding PST have evaluated either PST per se or its individual mechanisms. Results from the study of PST have more practical application in breeding programs but usually lack the precision to identify the responsible mechanisms. In most cases, however, dormancy is the major underlying mechanism. Moderate to high broad sense heritability estimates of visual sprouting scores were observed (Upadhyay et al., 1988; Upadhyay and Paulsen, 1988). For seed dormancy in white wheats, moderate to low narrow sense heritability estimates were reported (Allan, 1992; Paterson and Sorrels, 1990). In every instance these authors suggested that progress could be achieved through selection.

A number of studies have addressed the inheritance of seed dormancy. The dormant red coat colored cultivar RL 4137 has been reported to carry at least two independent mechanisms for seed dormancy (DePauw and McCaig, 1987). The number of genes involved was not reported, but at least two genes must control the dormancy

character. Bath et al. (1983) reported two major recessive genes controlling dormancy of the white wheats Kenya 321 sib and Ford. Similar results were obtained by Mares (1992) studying AUS 1408, one of the best sources of dormancy in white wheats. In other cases, dormancy in white wheats was considered as a multigenic character (Upadhyay and Paulsen, 1988; Allan, 1992). Dormancy of the red wheat RL 4137 has been shown to be partially dominant, which would facilitate its transfer to other genotypes (Noll et al., 1982). The breeding of sprout tolerant cultivars, such as Columbus, has demonstrated the soundness of this approach (Campbell and Czarnecki, 1981). The fact that dormancy of various white dormant wheats is recessive and digenic or multigenic will make transferring dormancy from these sources to adapted cultivars more laborious and therefore, less attractive.

Derera and Bhatt (1980) found that 13% of the F3 and 21% of the F4 lines from a cross between Kleiber and Gamut had chaff with high level of inhibitory effect, being similar to that from Kleiber. The authors attributed this phenotype to the presence of germination inhibition. However, since the chaff of the F3 and F4 lines was tested on seed from the corresponding line and not on a unique seed source (Kleiber for example), the observed response can not be conclusively attributed to differences in chaff inhibitors. Differences in embryo response can not be ruled out. Stoy and Olsen (1980) reported embryo sensitivity to CT being controlled by a single semi-dominant gene while studying reciprocal crosses between Snabbe (insensitive) and U 67653 (sensitive).

Three dwarfing (*Rht*) alleles are known to affect GA sensitivity in wheat. These alleles may be linked or pleiotropic to the *Gai* alleles (Hu and Konzak, 1974; Flintham and Gale, 1980). The allele *Rht3* is stronger than *Rht1* or *Rht2*, but they all reduce GA-induced production of α-amylase (GA insensitivity) in the seed as well as plant height. Some agronomic problems have been associated to *Rht3.* Thus, *Rht1* and *Rht2* are widely used (Derera et al., 1976; McMaster, 1976; Flintham and Gale, 1980; Flintham, 1990). The existence of genes that cause premature production of α-amylase (even in absence of rain) has been reported in Australia and United Kingdom. Results are consistent with a single recessive gene hypothesis. Evidence indicates that the *Rht1* allele can largely overcome the effects of the pre-maturity α-amylase gene and provide commercially accepted levels of α-amylase in the grain (Gale et al., 1987; Mares and Gale, 1990).

2.3.3 Molecular Markers and Mapping

Molecular markers linked to important genes or quantitative trait loci (QTL) for PST have great potential as selection tools for breeders. Nevertheless, progress in developing pre-harvest sprouting tolerant wheats with assistance of molecular markers is still limited. Anderson et al. (1993) studied two populations of F_5 recombinant white winter wheat inbred lines for PST and for the segregation of RFLP (restriction fragment length polymorphism) makers. For each population, four different QTL's were positively associated with PST. Multiple regression models using such markers accounted for 44% and 51% of the genotypic variation in PST in each of the two

populations. These QTL were mapped to chromosomes 1AS, 2S, 2L, 5DL, 6BL, 3BL and 4AL.

In another study, 110 F_1-derived double haploid lines from a cross between the Japanese cultivars "Fukuho-komugi" (tolerant) and "Oligo culm" (sensitive) were tested for PST. Data analysis after 65 RAPD (randomly amplified polymorphic DNA) markers were scored identified ten markers positively associated with PST and five markers negatively associated (Ban et al., 1996).

More recently, Flintham et al. (1997, 1998) denominated a major gene controlling seed dormancy as *Phs*. The gene appears to be located on chromosome 1A and to have no effect on seed coat color. Finally, the mapping of three *R* loci for seed coat color using RFLP markers (Flintham and Gale, 1996) is likely to facilitate and increase precision of new PST mapping efforts. The precise definition of how many alleles each mapping line carries will allow scientists to account for the effect of *R* alleles on the PST data. It may also allow a better estimation of the effect of each *R* allele and their interactions.

2.4 Assessment of Pre-Harvest Sprouting Tolerance

2.4.1 Influence of Environment and Developmental Stage

Temperature needs to be taken under consideration to obtain an accurate assessment of PST. Temperature regimes prior and after maturity strongly affect seed

dormancy and, consequently, PST (Reddy, 1978; Mares, 1984). Low temperatures during seed development enhance seed dormancy while high temperatures reduce it (Reddy, 1978). In addition, fast drying caused by a low humidity environment has been demonstrated to reduce dormancy similarly to high temperatures (Gale et al., 1983). During after-ripening, the relationship with temperature is maintained and seeds stored at low temperatures lose dormancy at a slower rate. A practical example is the method of storage at -15°C proposed by Noll and Czarnecki (1980) to preserve seed dormancy for up to ten months. Their results greatly facilitated the study of PST. Samples can be frozen soon after harvest and wait to be analyzed at a more appropriate time.

The response to temperature, however, is reversed when seeds absorb water from rainfall in the field or from a wetting treatment in the laboratory. Under these conditions, low temperatures will break dormancy down, rather than preserve it. In contrast, high temperatures will allow full expression of dormancy (George, 1967; Mares, 1984). For example, George (1967) found that none of the genotypes studied showed dormancy at 10°C, but at 20°C differences among cultivars were clear. After a few weeks, with dormancy weakened by after-ripening, differentiation was possible only at 30°C. Consequently, there is no unique ideal temperature to detect differences in dormancy among genotypes. The germination temperature that will work best depends on the environmental conditions observed during development and on the potential of the genotypes to develop dormancy.

Other environmental factors that affect PST are rainfall, photoperiod, light quality, water stress, and nutritional factors (King, 1989; Mares, 1993). In addition, the stage of development in which spikes are harvested is critical. Mares (1984) measured dormancy parameters starting 20 days before harvest maturity. Dormancy was greatest at that particular time and decreased constantly through seed maturation and after-ripening.

2.4.2 Field Methods and Designs

To obtain a reliable assessment of PST, Mares (1989) recommended the use of an identifiable stage of the plant cycle as a reference point for the comparison of different genotypes. For that purpose, some authors prefer to tag the spikes at anthesis, others directly harvest them at physiological maturity or at a fixed moisture content (Plett and Larter, 1986; DePauw and McCaig, 1987; Lawson et al., 1997). To account for rainfall and temperature effects in the evaluation of breeding lines of different maturities, Mares (1989) used a set of check cultivars with similar PST, but different maturity dates. On the other hand, DePauw and McCaig (1987) found no significant correlation between days to heading and nine variables used to estimate PST.

Various kinds of field designs have been used for PST evaluations. Mares (1989) recommended the use of a randomized complete block design, which gave consistent results to his program. Other authors, however, have not found enough reduction in error variance due to this design and opted for complete randomized

designs or simply side-by-side rows (McCrate et al., 1981; Upadhyay et al., 1988; Paterson et al., 1989).

3. MATERIALS AND METHODS

3.1 STUDY I

3.1.1 Genotypes and Field Evaluations

A set of seven genotypes represented the experimental material used in Study I. Genotypes included the synthetic Altar/At, the durum wheat parent of the synthetic (Altar 84), and five wheat cultivars used as checks for PST. Seed coat color, pre-harvest sprouting tolerance (PST) and origin of the experimental material are provided in Table 1.

These genotypes were cultivated at Oregon State University Crop Science Field Laboratory at Hyslop Farm (Corvallis, OR) during the spring-summer of 1998. Random side-by-side plots (one per genotype) were hand-planted on April 15 at a rate of 75 seeds m^{-1}. A second planting date was sown on April 25 to allow synchronization of flowering of late and early genotypes. Plots consisted of three 16.5m- long rows spaced 0.25m apart for both dates of seeding. Each plot was divided into three 4.5m-long sub-plots by 1.5m-wide alleys to allow the mechanized application of agrochemicals. Plots were fertilized with 56 Kg of N ha^{-1} prior to planting and top-dressed with 15 Kg N ha^{-1} during tillering (Haun stage 3.6 to 3.9). In order to avoid leaf rust (*Puccinia recondita*) and stripe rust (*Puccinia striiformis*), three fungicide applications employed. Propiconazole (0.144 Kg A.i ha^{-1}) was used twice and Bayleton® (0.56 Kg ha^{-1}) once. Weed control was maintained by the application of MCPA (0.336 Kg A.i. ha^{-1}) plus

Table 1. List of genotypes in Study I with their seed coat color, pre-harvest sprouting tolerance (PST) and origin.

Genotypes	Seed Coat Color	PST	Origin
Altar 84	white	unknown	Mexico
Synthetic	red	unknown	Mexico
RL 4137	red	tolerant[1]	Canada
Frontana	red	tolerant[1]	Brazil
BR-35	red	moderately tolerant[2]	Brazil
BR-23	red	moderately sensitive[2]	Brazil
Bacanora 88	white	sensitive[3]	Mexico

1. Gale, (1989)
2. Comissão Centro-Sul Brasileira de Pesquisa de Trigo, (1996).
3. Based on results from preliminary experiments.

Bromoxynil (0.336 Kg A.i. ha^{-1}) and complemented by hoeing. Irrigation was used to avoid water stress.

All seven genotypes were headed on June 26, either in the first or in the second planting date. For each genotype, plots headed on June 26 were selected for tagging regardless of planting date. The remaining plots were discarded. For each sub-plot, 30 spikes that were half-emerged from the boot (Haun stage 10.5) were tagged with laboratory tape (Fisherbrand® Colored Label Tape - Fisher Scientific, Pittsburgh, PA). Tagged spikes from each sub-plot were hand-harvested when more than 50% had achieved physiological maturity (PM). Physiological maturity was determined by the complete lost of green color from the glumes (Hanft and Wych, 1982). After harvesting, spikes were bundled to dry at room temperature (24°C ± 6°C). After six days they were frozen and kept at - 8°C until analyzed. Spikes were thawed for 24 hrs at room temperature prior to analysis.

3.1.2 Laboratory Evaluations

A complete randomized design (CRD) with three replications was employed for all laboratory experiments (Study I and Study II). The 30-spike sample obtained from each sub-plot in the field constituted a replication in the laboratory experiments. It was assumed that the geographic position of a sub-plot in the field had little impact on the parameters evaluated in the laboratory.

3.1.2.1 Evaluation of Pre-Harvest Sprouting Tolerance

To assess PST a sub-sample of ten spikes from the 30-spike sample made up a experimental unit. Following immersion in distilled water for eight hours, each spike was sprayed with approximately one milliliter of Thiram Granuflo® 0.2% (1.5 g A.i. L^{-1}) and rolled between two sheets of germination paper (Anchor Paper Co., St. Paul, MN) previously imbibed in distilled water. To avoid drying, the ten rolls were placed into a reclosable plastic bag (33 x 47.5 cm). After incubation for seven days at 20°C (±1°C) in an upright position, the ten spikes were placed together into a dry paper bag and dried at 40°C for 48 hours. Following threshing, two variables were evaluated:

- Percentage of Germinated Seeds (PGS) - seeds with the pericarp ruptured by the radicle were counted as germinated.
- Wet Stirring Number (WSN) - seeds from the PGS determination were ground to pass a 0.5mm sieve. The whole-meal flour was then used to estimate starch damage as result of α-amylase activity in the Rapid Visco-Analyzer (RVA - Newport Scientific Instruments & Engineering, Narrabeen, Australia) using the Stirring Number method (American Association of Cereal Chemists, Method 22-08).

A second set of 10 spikes, which received no wetting treatment, was also threshed, ground, and used for determination of the "Dry Stirring Number" (DSN) in the RVA. This determination was conducted to verify if the synthetic had pre-mature production of α-amylase and also to serve as a basis for the interpretation of the WSN.

3.1.2.2. Evaluation of Dormancy and Chaff Inhibitors

A third sub-sample of ten spikes from each sub-plot was hand-threshed with the seed and chaff retained. The chaff was ground to pass a 0.5mm sieve and a sample of 4.2 g was stirred into 60 ml of distilled water and agitated for 24 hours to extract water-soluble chemical inhibitors. The extract was then centrifuged (Sorvall® RC-5B, Du Pont Instruments, Hoffman Estates, IL) at 3000 rpm to separate the solid matter. Seeds were surface-sterilized for 10 minutes in a 0.5% sodium hypochlorite solution and rinsed three times with distilled water. Ten Petri dishes (100 x 15mm) were each filled with 25 seeds. Seeds, with the crease down, were placed on top of two sheets of blue blotter paper. Five dishes received nine milliliters of distilled water and five received the same amount of chaff extract. Stacks of five dishes were placed into a closed plastic container and incubated at 20°C (±1°C) for 14 days.

Each stack of five dishes constituted an experimental unit. The experimental design was a Split Plot where genotypes constituted the main plot and media (water and chaff extract) constituted the sub-plots within each genotype. Germinated seeds (same criteria used for PGS) from each experimental unit were counted and discarded daily for 14 days. Non-germinated seeds remained for an additional 45 days (15 days at 5°C and 30 days at 20°C) in the germinator before being considered dead. Percentage of Germination (PG) was calculated cumulatively for each day taking into account the total number of viable seeds. Based on these percentages, the variables Germination Index (GI) (Hagemann and Ciha, 1984) and Area Under the Germination Progress Curve (AUGPC) (Mundt and Leonard, 1986) were calculated by the equations

$GI = (PG_1 \times 7) + (PG_2 \times 6) + \ldots + (PG_7 \times 1)$ and

$AUGPC = (\sum_{i=1}^{14} PG_i) - [(PG_1 + PG_{14})/2]$,

where PG_i = PG on the i^{th} day; and PG_1, $PG_{2,\ldots}$, PG_{14} = PG on day 1, day 2, ..., and day 14, respectively. Germination Index only used information of the first seven days and gave more weight for the first and less for the last days. Area Under the Germination Progress Curve used the 14 days of data equally.

3.1.3 Statistical Analysis

Statistical analysis utilized the SAS (SAS Institute Inc., 1995) statistical software. For the comparison of the synthetic and other cultivars the variables PGS, GI, WSN, and DSN were used. Original data of the variables PGS, GI and WSN violated the homogeneity of variance assumption of the analysis of variance (ANOVA). These variables were then rank transformed. Observations were ranked from the smallest to the largest value and ranks were assigned consecutively (1,2,..., n). Ties were assigned with average ranks (1, 2.5, 2.5, 4,..., n). Analyses of variance and Fisher's Protected Least Significant Difference (FPLSD) multiple comparison procedures were conducted on the rank transformed data as if they were common parametric data as indicated by Conover and Iman (1981). The variable DSN required no transformation.

The variable AUGPC was used to compare the effects of water and chaff extracts on dormancy of each genotype. In order to meet the ANOVA's assumptions, data analysis was performed separately in two groups, one with five genotypes and the

other with two. The variances within each group were homogeneous. Within each genotype, t-tests were performed to compare the AUGPC of water and chaff extract treatments. One-sided probability values were reported since chaff extracts are only expected to reduce AUGPC (Derera et al., 1976; McCrate et al., 1982; Salmon et al., 1986; Trethowan et al., 1993).

3.2 STUDY II

3.2.1 Genotypes and Field Evaluations

Random inbred $F_{2:5}$ lines from single and backcrosses between the synthetic Altar/At and the Mexican cultivars Opata 85 (Opata) and Bacanora 88 (BCN) were used in this study. Both Opata and BCN have little or no PST. Seed of Opata are red while BCN seed are white. F_5 lines heterogeneous for seed coat color were discarded. The total number of lines studied per cross included: i) 24 lines each from Altar/At//Opata and Altar/Ae//2*Opata populations, ii) 19 lines of Altar/At//BCN, and iii) 22 lines of the Altar/At//2*BCN population. These populations were respectively designated as Population 1, 2, 3 and 4.

The parent Altar/At was sent by the International Maize and Wheat Improvement Center (CIMMYT) to Oregon State University in 1994. Following single and backcrosses with Opata and BCN, the subsequent populations were advanced from F_2 to F_4 by single-seed-descend (SSD). Prior to every sowing, seed dormancy was removed by a 37°C-treatment for seven days. In 1997, F_4 lines were provided for the

present study and advanced to the F_5 in the same year at Crop Science Field Laboratory. From F_2 to F_5 no selection was applied other than discarding sterile or very late plants.

Each F_5 population accompanied by their respective parents was hand-planted at Hyslop Farm in side-by-side plots on April 16, 1998. Parents were replicated three times while the F_5 lines were not replicated. Each plot consisted of two 1.5m-long rows with 0.25m space between rows. A second planting date was sown ten days later in the same manner. Lines were sowed at a rate of 27 seeds m^{-2} or less, depending on seed availability. Cultural practices were identical to those described for Study I.

According to the heading dates, each genotype (lines and parents) was assigned to be harvested from only one of the two planting dates. Late heading genotypes were assigned to the first planting date while early genotypes were assigned to the second. This was done to better synchronize the stages of development of all genotypes and minimize error due to environmental influence on the parameters to be estimated. When at least 50% of the spikes in a plot had achieved PM, twelve spikes were hand-harvested. Spikes were handled until analysis as described previously. Additional four random spikes were collected to classify genotypes for seed coat color.

3.2.2 Laboratory Evaluations

Nine spikes from each plot were taken for the assessment of PST. A sub-sample of three spikes was considered as a replication. F_5 lines were replicated three times with nine replicates for the parents. Percentage of Germinated Seeds (PGS) was accessed

using intact spikes rolled in germination paper as described for Study I, except the period of incubation that was reduced from seven to five days.

Seed coat color was visually assessed with the help of a 1-M sodium hydroxide solution (Chemelar and Mostovoj, 1938 – cited by DePauw and McCaig, 1983) to emphasize contrast. Lines were classified as red or white.

3.2.3 Statistical Analysis

Statistical analysis was performed independently for each population using the software mentioned in Study I. Graphical assessment (residual plots) of data from P1 (Altar/At//Opata) and P2 (Altar/Ae//2*Opata) indicated that an arcsine square root transformation might be appropriate to minimize the slight heterogeneity of variance present in these two populations. Since the only significant change obtained with the transformation was a reduction in the coefficients of variation, statistical analysis of the four populations was performed using the non-transformed data. In addition, the analysis of a model were heading date was included as a covariate provided evidence that it was not necessary to account for this variable when estimating PGS means.

Narrow-sense heritability (h^2) estimates for each population were obtained according to Singh and Chaudhary (1977) by the formula

$$h^2 = \sigma^2 g / \sigma^2 p$$

where $\sigma^2 g$ = genetic variance or $(\sigma^2_L - \sigma^2_E)/r$, and $\sigma^2 p$ = phenotypic variance or $(\sigma^2 g + \sigma^2_E)$. From the ANOVA table σ^2_L = variance of the F_5 lines or expected mean square of lines; σ^2_E = variance of error or expected mean square of error; and r = number of replications. The estimates of h^2 were considered as narrow-sense because at the F_5 level of inbreeding, dominance variance is minimal and additive by additive epistatic variance can be included in the estimates.

The association between red seed coat color and PST was addressed through the use of single contrasts comparing the means of red and white lines in each population. Previous reports about this association and the hypothesis used to explain it indicate that the association between red seed coat color and PST is expected to be positive (Miyamoto and Everson, 1958; Gfeller and Svejda, 1960; DePauw and McMaig, 1983; Côme et al., 1984; Flintham and Gale, 1996; Lawson et al., 1997). Therefore, one-sided probability values were reported. A multiple comparison procedure (FPLSD) was used to compare lines and parents.

4. RESULTS AND DISCUSSION

4.1 STUDY I

4.1.1 Comparisons among the Synthetic and Check Cultivars

To evaluate the potential of the synthetic Altar/At as a source of pre-harvest sprouting tolerance (PST) it was necessary to compare it with wheat cultivars of known PST. Comparisons in this section were based on the variables PGS (Percentage of Germinated Seeds), GI (Germination Index) in water and GI in chaff extract. As PGS is measured from intact spikes subjected to a wetting treatment, it is influenced by various mechanisms that affect PST under natural conditions such as, dormancy, chaff inhibitors, and spike and seed morphological characteristics. For this reason, PGS is viewed as a better indicator of PST under natural conditions than germination tests in Petri dishes. However, a limitation of PGS is that it reflects the germination response at a specific moment (the seventh day in this study). Germination Index in water and chaff extract do not account for spike morphological differences but integrate data from several days giving more weight to the response on the first days. Therefore, the chosen variables complement each other to allow a more thorough evaluation of the PST of each genotype.

Temperatures during grain filling period were 1.9°C above average. Maximum daily temperatures of up to 39.4°C were observed near physiological maturity (for more detail refer to Appendix). High temperatures during seed development have been shown

to decrease seed dormancy and, consequently PST (Reddy, 1978). Comparisons made among genotypes were assumed to be valid since they were at similar developmental stages (all headed at the same day) when subjected to a high temperature period

Results for three variables involving five check genotypes are presented in Table 2. The rankings were consistent with those proposed based on previous reports (Gale, 1989; Comissão Centro-Sul Brasileira de Pesquisa de Trigo, 1996) and presented in Table 1. These results support the methodology utilized to assess PST and allowed the ranking of Altar 84 and the synthetic for this trait in comparison with known checks. Multiple comparison tests based on rank transformed data indicated that the synthetic was less tolerant than RL 4137 and Frontana (tolerant checks) and more tolerant than Bacanora 88 (sensitive check) for PGS, GI in water and chaff extract (Table 2). The cultivar BR-35 (moderately tolerant) was more tolerant than the synthetic according to PGS. In addition, BR 35 mean values for GI in water and chaff extract also indicated BR-35 as being more tolerant that the synthetic. Germination progress curves in water and chaff extract illustrate the responses of all genotypes (Figure 1).

The response of the synthetic and BR-23 (moderately sensitive) were similar and their relative ranking varied depending on which variable was considered. BR-23 was more tolerant when PGS was considered. However, for GI in water and chaff extract the synthetic ranked as more tolerant. The examination of the germination

Table 2. Non-transformed means for Germination Index in water and chaff extract, Percentage of Germinated Seeds (PGS), Wet Stirring Number (WSN), and Dry Stirring Number (DSN). Except for DSN, all variables result from wetting treatments of seven days at 20°C.

	Threshed seed in Petri dishes		Spikes in germination paper		
	Germination Index[1]		PGS	RVA Stirring Numbers	
Genotypes	Water	Extract	(%)	WSN	DSN
RL 4137	0.5[a 2]	0.2[e]	2.2[j]	106	113
Frontana	1.8[a]	2.3[e]	3.6[k]	71	115
BR-35	225[b]	82[f]	14.7[l]	11.3	118
Synthetic	444[bc]	184[fg]	40.3[m]	4.7	103
BR-23	590[c]	293[gh]	22.4[n]	6.0	126
Altar 84	538[c]	346[h]	65.2[o]	7.0	126
Bacanora 88	1057[d]	647[i]	89.3[p]	2.7	107
CV (%)[3]	20.9	17.7	9.1	18.1	7.5

1. Germination Index = $(PG_1 \times 7) + (PG_2 \times 6) + ... + (PG_7 \times 1)$ where PG_1, $PG_{2,...}$, PG_7 = percentages of germination on day 1, day 2,..., and day 7, respectively.
2. Means within a column followed by the same letter are not significantly different ($P>0.05$) according to a Fischer's Protected Least Significant Difference Test run with rank transformed data.
3. Except for variable DSN, Coefficients of Variation (CV) are based on analysis of rank transformed data.

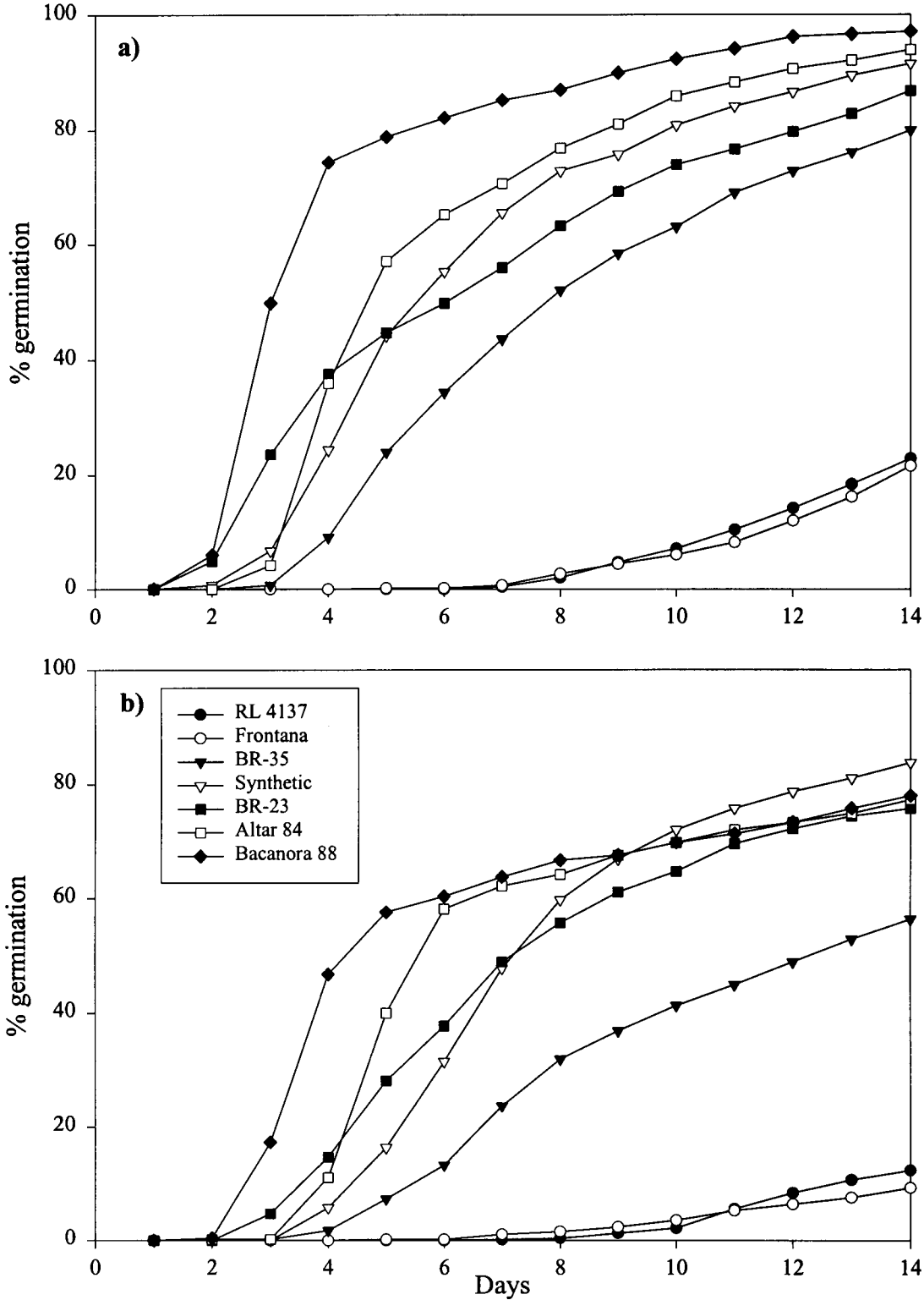

Figure 1. Germination progress curves of genotypes in Study I germinated in water (a) or chaff extract (b) for 14 days.

progress curves of these two genotypes helps to understand such contrasting responses (Figure 1). In both instances, percentage of germination at the seventh day for BR-23 was equal or lower than the synthetic. However, the examination of the curves from the second to the sixth day indicates that the synthetic was slower to germinate (more tolerant) during this period. This difference in initial response explains why the synthetic had a better PST if GI rather than PGS is considered. There is no assurance that the synthetic also had a slower rate of germination during the initial days within the intact spikes, but it seems to be a valid indication that the PGS of the synthetic would be relatively better if the test had been run for a shorter time. In any case, a clear conclusion can not be drawn from this data regarding the true relative ranking of these two genotypes. Both can be classified for PST as moderately sensitive.

Because the specific *Aegilops tauschii* genotype used to develop the synthetic is not known, it was necessary to compare the synthetic with its female parent Altar 84 for PST. Altar 84 was statistically more tolerant than Bacanora 88 for all three variables indicating that Altar 84 has some PST. The synthetic was ranked above Altar 84 for the three variables and was more tolerant for PGS and GI in chaff extract. The fact that the synthetic was found to be more tolerant than its parent Altar 84 provides evidence that *Aegilops tauschii* (D genome) contributed to the PST of the synthetic. Although it could not be proven, Altar 84 (A and B genomes) has probably contributed to the synthetic's PST as well.

The variable Wet Stirring Number (WSN) was used to indirectly assess the PST of the genotypes through the evaluation of their starch pasting properties as affected by the α-amylase activity developed during wetting treatments. Although the seven-day wetting treatment was effective in differentiating genotypes based on PGS, this treatment was too long to be able to differentiate the synthetic from wheat genotypes with similar levels of PST based on WSN. According to a reference table provided by the RVA manufacturer (Newport Scientific Instruments & Engineering, Narrabeen, Australia) a stirring number of 82 is equivalent to a Falling Number of 251. A Falling Number (FN) of 250 is commonly used as a threshold to decide whether a wheat lot is sprout-damaged or not. A lot with a FN at or above 250 is considered sound and appropriate for production of amylase sensitive end products such as pan bread. Data presented in Table 2 indicate that the only genotype that maintained a commercially acceptable stirring number (above 82) was RL 4137 (WSN = 106). Frontana remained at a level (WSN = 70, FN ≅ 227) that would still be acceptable for less amylase-sensitive wheat products or for mixing with "sound" grain. All the other genotypes had very low stirring numbers indicating a high α-amylase activity, which compromised flour quality.

Dry stirring number of all genotypes was higher than 100 indicating that low levels of α-amylase were present in the grains of non-wetted spikes. This indicates that, at least in the conditions in which this experiment was conduced, neither the synthetic nor the other genotypes had a pre-mature production of α-amylase.

Xiu-Jin et al. (1997) reported PST in a different synthetic. Their synthetic appeared to be more tolerant then all the checks employed. It had a mean percentage of germination at the seventh day of 6.06 % while the means of the checks ranged from 27.63 to 80.34%. No reference was made regarding the level of PST of their checks.

The PST level found for the synthetic Altar/At was less than that of RL 4137 or Frontana, but it may still be valuable. The cultivar BR-23, which had the PST level most similar to the synthetic, was cultivated in southern Brazil for many years. Even though this area is particularly prone to sprouting problems, BR-23 was a successful cultivar. Its area of cultivation decreased due to increased bread making quality requirements and not due to its level of PST. Therefore, the PST of the synthetic might be satisfactory in some regions. However, this tolerance would be more useful if added to the PST of other genotypes. This possibility is discussed in section 4.3.

4.1.2 The Effect of Chaff Extracts on Dormancy

Results of the ANOVAs for Area Under the Germination Progress Curve (AUGPC) are presented in Table 3. For each genotype, t-tests comparing AUGPC's means of water and chaff extract treatments provided evidence that chaff extracts reduced AUGPCs (Table 4). The germination progress curves of each genotype germinated in water and chaff extract are presented in Figure 2.

Table 3. Observed means squares for Area under the Germination Progress Curve (AUGPG) from ANOVAs in groups of two genotypes (RL 4137 and Frontana) and five genotypes (BR-35, Synthetic, BR-23, Altar 84, and Bacanora 88).

	Group of two			Group of five		
Sources of variation	df	AUGPC	P-value	df	AUGPC	P-value
Genotypes	1	99.8	0.78	4	131117	0.0014
Error (a)	4	1143.5		10	12605	
Media (Genotypes)[1]	2	2024.1	0.10	5	56463	0.0048
Error (b)	4	467.1		10	8133	
C.V. (%)		37.3			13.7	

1. Media refers to the two germination media employed: water and chaff extract.

Table 4. Means of AUGPC of genotypes in Study I germinated in water and in their own chaff extracts. One-sided probability values (P-value) refer to t-tests for the difference between water and chaff extract AUGPC means within each genotype.

Media	RL 4137	Frontana	BR-35	Synthetic	BR-23	Altar 84	Bacanora 88
Water	80	72	544	733	706	796	982
Extract	41	38	332	578	570	632	710
P-value	0.046	0.062	0.008	0.031	0.048	0.026	0.002

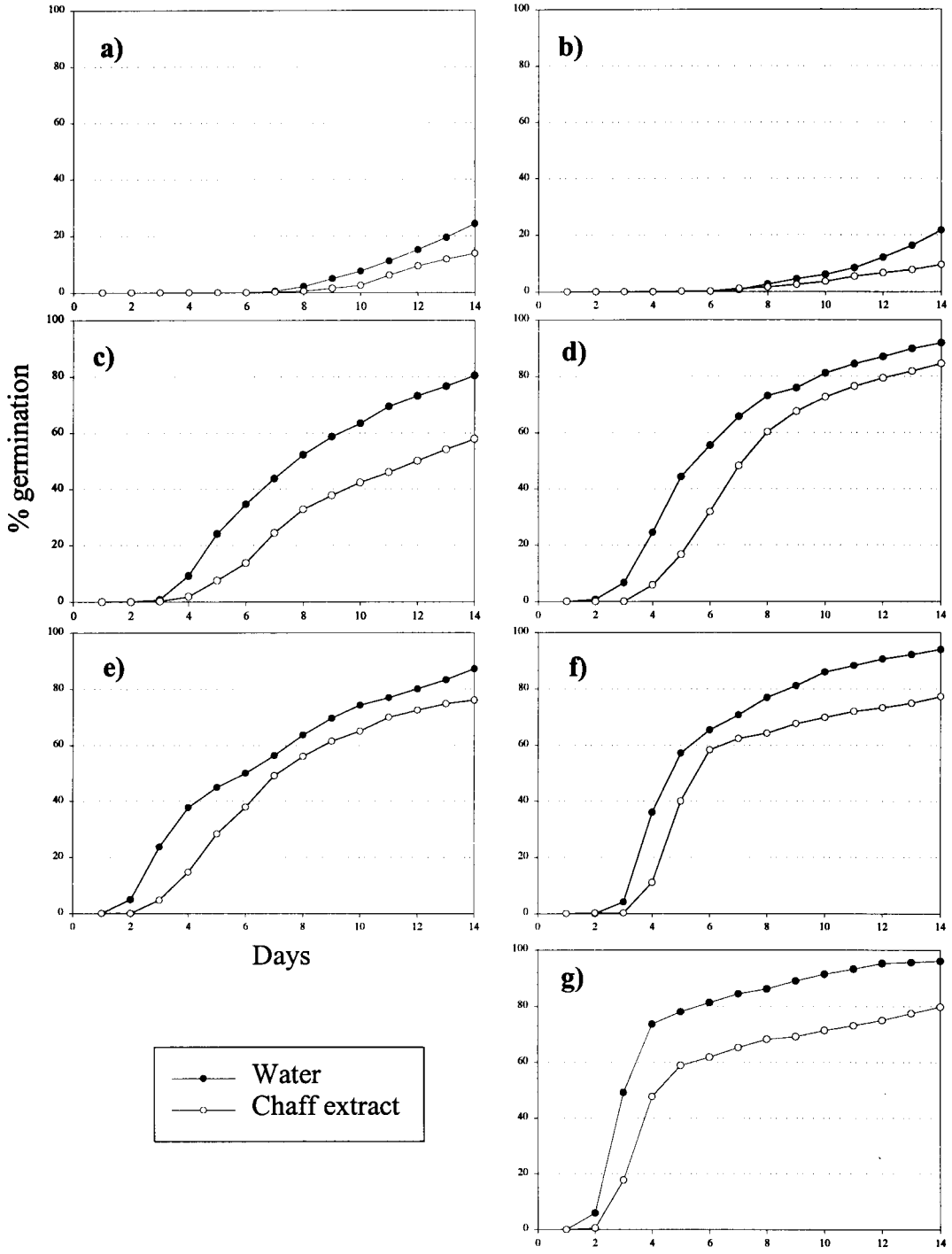

Figure 2. Germination progress curves of genotypes RL 4137 (a), Frontana (b), BR-35 (c), synthetic Altar/At (d), BR-23 (e), Altar 84 (f), and Bacanora 88 (g), germinated in water and chaff extract for 14 days.

The observed results indicating the existence of an inhibitory effect of chaff extracts on germination of red genotypes and white (Bacanora 88) are in agreement with results obtained by Derera et al. (1976) and McCrate et al. (1982). The inhibitory effect of chaff extracts on germination has also been observed in triticale (Salmon et al., 1986). From this study, there is evidence that this effect can be observed in durum wheat (Altar 84) and in synthetic hexaploid wheat as well. Seed dormancy and inhibitory substances in the chaff appear to contribute to the PST of all studied genotypes.

The inhibitory effects observed in this study depended not only on the inhibitors in the chaff, but also on the response of the embryos of each genotype to these inhibitors. It was expected that differences in these two components (inhibitors and embryos) would generate large differences among genotypes in terms of how they would respond to the chaff extracts (stronger or weaker inhibition). Instead, a visual assessment of the germination progress curves of each cultivar in water and chaff extract did not show major differences (Figure 2).

To demonstrate that chaff extracts can play a role in inhibiting germination in each genotype, it was necessary to test the chaff extract of these genotypes on their own seed. This would answer the question regardless the responsiveness of their embryos. However, to make comparisons among chaff extracts one should apply all chaff extracts to the seed of one or a few genotypes, as suggested by Salmon et al. (1986).

4.2 STUDY II

4.2.1 Analysis of Variance and Heritability Estimates

In this study, lines and parents did not head simultaneously, but the ranges of flowering and harvesting dates for harvested plots was reduced by using two planting dates. The influence of heading date on Percentage of Germinated Seeds (PGS) was tested for each population by the inclusion of heading date as a covariate in the ANOVA. Probability values for the significance of this effect in the four populations ranged from 0.46 to 0.70 indicating that heading date did not significantly influenced PGS. Visual assessment of scatterplots (PGS vs. heading date) also did not detect any influence. These results validate the comparison of PGS mean values made in sections 4.2.2 and 4.2.3 among genotypes that headed on different days.

F_5 lines showed a wide range of PGS mean values in all populations. The range in mean PGS (%) in each population varied between 76% (Population 1) to 87% (Population 2). Results of the analysis of variance of the F_5 lines (parents excluded) from populations 1, 2, 3, and 4 for the variable PGS, measured after five days of wetting treatment, are presented on Table 5. Significant differences were observed among F_5 lines in all populations. Coefficients of variation (CVs) were high (from 15.1 to 34.9). No systematic source of error variance could be identified. Despite the CVs, narrow sense heritability estimates for the four populations were moderate (63.9%) to high (82.3%) indicating that a large portion of the observed phenotypic variance was due to additive genetic variance. In a similar study, where three random inbred populations

Table 5. Observed means squares for Percentage of Germinated Seeds (PGS) of F_5 lines of Populations 1-4 accompanied by narrow sense heritability (h^2) estimates for the same trait.

Sources of	*Population 1*		*Population 2*		*Population 3*		*Population 4*	
Variation	df	PGS	df	PGS	df	PGS	df	PGS
F_5 lines	23	1462.7***	23	1695.6***	18	1516.5***	21	1791.3***
Error	48	195.3	48	113.8	38	240.3	44	151.2
C.V. (%)		34.9		15.1		29.8		20.5
h^2 (%)		68.4		82.3		63.9		78.3

*** Significant at the 0.001 probability level.

were evaluated under artificial rain, Lawson et al. (1997) observed similar heritabilities (46% to 92%) for visual sprouting scores. According to the heritability estimates observed in the present study, mean PGS values of F_5 lines are expected to be a good predictor of the future performance of their progenies. In other words, selection based on PGS can be efficient when applied to late generations. The efficiency of the selection based on PGS when applied to earlier generations is discussed in section 4.2.3.

4.2.2 Association between Seed Coat Color and Pre-Harvest Sprouting Tolerance

F_5 lines with red and white seed coats were evaluated in all four populations (Table 6). Since Populations 1 and 2 originated from crosses between two red parents (Altar/At and Opata), white lines in these populations indicate that alleles determining seed coat color are different in the parents. The segregation for seed coat color and PST observed in the four populations represents an opportunity to study the association between these two traits. If these traits segregated independently, the mean values of PGS (the estimator of PST in this study) of the red lines should not differ from the mean values of PGS of the white lines. Similar studies have found a positive association between the red seed coat color and PST. Despite this association, in some studies the PST of the tolerant red parent was partially recovered in white recombinant lines (DePauw and McMaig, 1983; Lawson et al., 1997). This apparent association was of interest in the present study for two reasons. First, previous reports on PST of synthetic wheats have not addressed the possible association between seed coat color and PST. Thus, mechanisms present on *Aegilops tauschii* may or may not be associated with its

Table 6. Summary of Percentage of Germinated Seeds (PGS) for F_5 lines with red and white seed coats from Populations 1-4 accompanied by the estimated differences in PGS mean values between red and white lines and the one-sided probability values (P-values) for the test of each difference.

Populations[1]	Red			White			Difference (%)	P- values
	Number of lines	Mean (%)	Range (%)	Number of lines	Mean (%)	Range (%)		
1	21	36	6 - 82	3	66	59 - 70	29	< 0.001
2	22	69	11 - 98	2	86	74 - 97	17	0.055
3	15	46	8 - 89	4	75	60 - 89	29	< 0.001
4	7	38	16 - 95	15	70	33 - 95	32	< 0.001

1- Population 1= Altar/At//Opata; Population 2 = Altar/ At//2*Opata; Population 3 = Altar/At//BCN; Population 4 = Altar/At//2*BCN.

red seed coat color. Second, since Altar 84 has no alleles for red color and some level of PST (identified in Study I), such tolerance may be passed to progeny independently of seed coat color.

Within each of the four populations, the mean values for PGS of the red lines was lower (low PGS indicates tolerance) than the mean PGS values of the white lines, indicating a positive association between red seed coat color and PST in these populations (Table 6). The tendency of the white lines to exhibit high PGS mean values can be observed graphically in Figures 3 and 4.

Seed coat color can be a useful tool to select for PST in crosses with the synthetic since red seed coat was found to be positively associated to PST in the studied populations. However, selection should not be based only on seed coat color because red seed coat was not sufficient to ensure tolerance. This can be demonstrated by the fact that, in all four populations, there were red lines less tolerant than the tolerant parent (synthetic) and not different from the sensitive parent (see Figures 3 and 4). This feature is especially noticeable in Population 2 (Figure 3c), where a group of lines is clustered near the sensitive parent BCN.

From the 24 white lines obtained in all four populations, one of them (in Population 4) was not significantly different from the synthetic. Caution must be taken in considering this line as a white recombinant with PST similar to the synthetic since it had a mean PGS value of 33% while the synthetic had a mean PGS value of 23%.

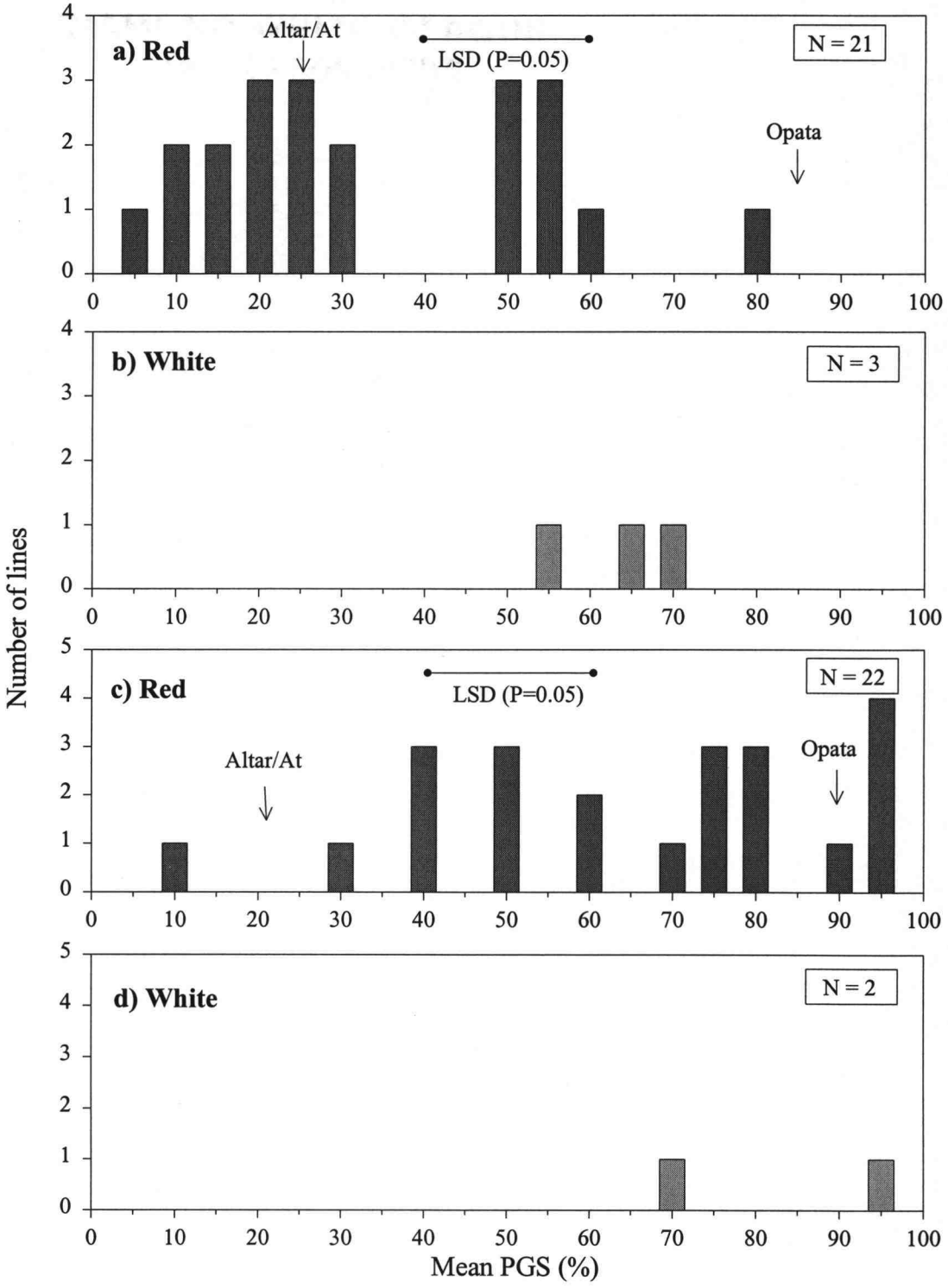

Figure 3. Frequency distribution of red and white-seeded F5 lines in (a,b) Population 1 (Altar/At//Opata) and (c,d) Population 2 (Altar/At//2*Opata), according to their mean Percentage of Germinated Seeds (PGS). Horizontal bars represent LSD at P = 0.05 for each population.

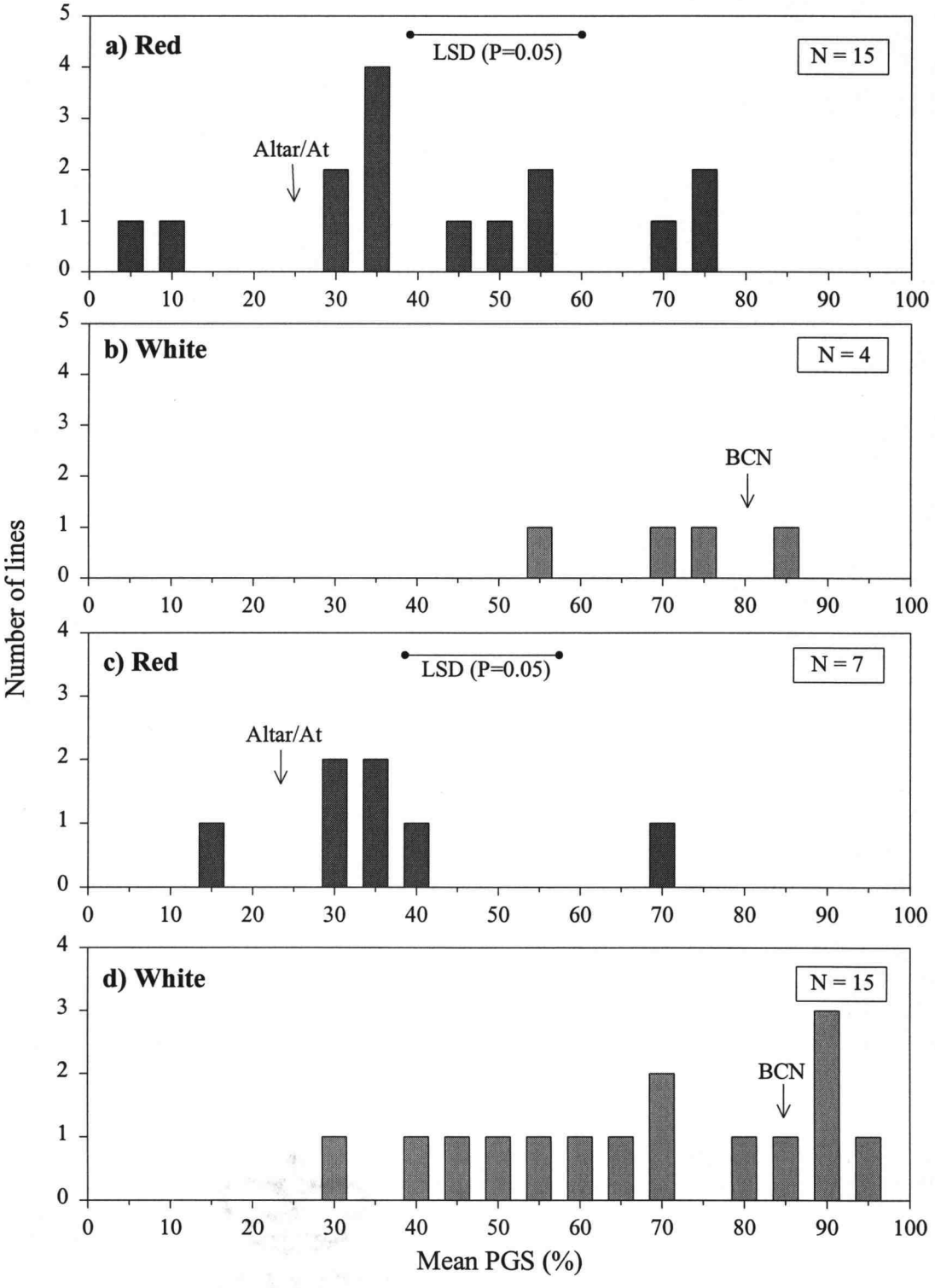

Figure 4. Frequency distribution of red and white-seeded F5 lines in (a,b) Population 3 (Altar/At//BCN) and (c,d) Population 4 (Altar/At//2*BCN), according to their mean Percentage of Germianted Seeds (PGS). Horizontal bars represent LSD at P = 0.05 for each population.

Whereas it is questionable that a white line as tolerant as the synthetic could be obtained in the present study, there is stronger evidence that some of the white lines were more tolerant than their sensitive parents. In Populations 1, 3, and 4, one, one, and six white lines, respectively, were significantly more tolerant than their sensitive parents. This indicates that at least part of the PST of the synthetic has been recombined with white seed coat color. Therefore, the synthetic can be used as a source of PST for white wheats. Pre-harvest sprouting tolerance in these recombinants may have originated from Altar 84. However, such hypothesis was not tested in the present study.

Findings in this section can be summarized as follows: i) progenies from crosses between the synthetic Altar/At and the sensitive parents (Opata and BCN) show a positive association between red seed coat color and PST, ii) this association is not strong enough to assure tolerance in all F_5 red lines and, iii) in spite of the association, white recombinant lines more tolerant than the sensitive parents can be obtained from single and backcrosses with the synthetic Altar/At. These findings are in agreement with those of DePauw and McMaig (1983) and Lawson et al. (1997) using different germplasm.

4.2.3 Pre-harvest sprouting tolerance in parents and F_5 lines

Mean values and ranges of PGS observed for the parents in the four populations are presented in Table 7. In all populations the synthetic had significantly lower PGS mean values than the respective sensitive parent, as illustrated in Figures 3 and 4. The

Table 7. Percentages of Germinated Seeds (PGS) means and ranges from parents of populations in Study II.

Population	Synthetic		Opata		Bacanora 88	
	Mean (%)	Range (%)	Mean (%)	Range (%)	Mean (%)	Range (%)
1	26	12 - 40	86	69 - 96	-	-
2	22	12 - 37	91	79 - 98	-	-
3	25	14 - 42	-	-	82	70-90
4	23	11 - 41	-	-	86	61-95

presence of lines significantly more tolerant than their respective sensitive parents in all populations demonstrates that the synthetic can be used to improve PST in crosses with wheat cultivars.

F_5 lines with absolute mean PGS values outside the range of the parents were identified in all populations, suggesting the occurrence of transgressive segregation for PGS. A substantial part of the phenotypic variance observed for PST in the populations studied was attributed to additive effects (section 4.2.1), therefore transgressive segregants would be expected. However, differences in PGS between these F_5 lines and the correspondent parents at either side of the parental range were not statistically significant. In addition, the existence of transgressive segregants would require the sensitive parents to have alleles conferring PST. Considering their consistently high PGS mean values (refer to Table 7), it is possible that the sensitive parents do not have alleles conferring PST. Therefore, until stronger evidence is available no conclusion should be drawn regarding transgressive segregation in the studied populations.

The numbers and percentages of lines that were not statistically different than the synthetic are presented in Table 8. The relatively high proportion of lines assumed to be as tolerant as the synthetic in each population is an indication that few genes control PST in the populations studied. Due to the limited number of lines available for this study it was not possible to estimate the number of genes involved. Xiu-Jin et al. (1997) reported one recessive gene controlling PST in a different synthetic wheat.

Table 8. Number and percentage of tolerant F_5 lines in each population. Tolerant lines had PGS mean values not significantly different (P>0.05) from the synthetic.

Population	Cross	Tolerant lines	
		Number	(%) of the total number of lines
1	Altar/At//Opata	13	54
2	Altar/At//2*Opata	3	13
3	Altar/At/BCN	8	42
4	Altar/At//2*BCN	7	32

The moderate to high narrow sense heritability estimates obtained (Section 4.2.1) and the small number of genes that seem to be involved in the genetic control of PST in the populations studied, indicate that selection would be effective in earlier generations than F_5. However, since in earlier generations smaller samples per plant are generally used selection efficiency is expected to be less efficient than in later generations.

4.3 Pre-harvest Sprouting Tolerance of the Synthetic - Potential and Breeding Strategy

Based on the information presented in the previous sections, it is now possible to make a comprehensive evaluation of the potential of the synthetic Altar/At as a source of PST. The level of PST observed in the synthetic (moderately sensitive) is not strong enough to make it a very attractive source of PST. However, it can be sufficient in areas less prone to pre-harvest sprouting. This limited level of PST may be compensated by the fact that the alleles conferring PST for the synthetic may be different from the alleles present in available sources of PST in wheat. Durum wheat and especially, *Aegilops tauschii*, have not been extensively used in crosses with wheat until recent years (Cox et al., 1990; Lange and Jochemsen, 1992; Mujeeb-Kazi et al., 1996; Xiu-Jin et al., 1997). The relative genetic isolation of the three gene pools has presumably contributed to the recent identification and transference of unique and useful alleles from *Aegilops tauschii* and durum wheat to the wheat gene pool (Lagudah and Halloran, 1988; Cox et al., 1990; Market et al., 1995; and Villareal et al., 1996). Therefore, the

alleles conferring PST in the synthetic may also be unique to the wheat gene pool. Different alleles represent a possibility of increasing the genetic diversity for the trait. Furthermore, as these alleles appear to have additive effects, it seems possible to enhance the tolerance of current sources of PST through crossing and selection. The improvement of sources of PST is especially important in the case of white wheats were the number of sources and the level of PST available is more limited. Results of this study indicate that the synthetic can also contribute to the PST of white wheats.

The potential use of the synthetic as a source of PST is also affected by how tolerance is inherited. Dominance, recessiveness, and potential interaction between alleles could not be assessed using F_5 lines (nearly homozygous). Nevertheless, the indication that few genes control PST in the synthetic can certainly facilitate the use of this tolerance.

Some other considerations about the use of the synthetic's PST for breeding purposes are noteworthy. First, the synthetic Altar/At has a poor agronomic plant type. The main agronomic problems in this study were related to threshability, lodging, and disease resistance. The backcrossed populations were, in general, agronomically more acceptable than those resulting from single crosses populations. If only few genes are responsible for the PST in the synthetic, the use of backcrosses seems to be an appropriate strategy to transfer the synthetic's PST to agronomically acceptable genotypes. The number of tolerant lines obtained in Population 2 and Population 4 (Table 8), which are derived from backcrosses to sensitive parents, reinforces the

appropriateness of this methodology. Second, tolerant lines obtained in this study may be re-tested and used in crosses with the genotypes of interest instead of using the synthetic. This strategy would certainly improve the agronomic plant type of the progenies facilitating the transference of PST.

Results in the present study support those of Xiu-Jin et al. (1997) and Gatford (1998, personal communication) to demonstrate that the synthetic hexaploid wheats represent a potentially important source of PST. A fruitful field for research relies on the identification of PST in *Aegilops tauschii* as well as in hundreds of synthetic wheats already available and on the transference of this PST to agronomically superior germplasm.

5. SUMMARY AND CONCLUSIONS

Two studies were conducted to evaluate the pre-harvest sprouting tolerance (PST) of the synthetic Altar/At and determine the potential of this genotype as a source of PST in crosses with hexaploid wheat cultivars. The first study compared the synthetic with selected PST checks and with the synthetic's durum wheat parent, Altar 84 (donor of genomes A and B). Comparisons were based on the germination response of the genotypes to wetting treatments applied under temperature controlled conditions to field-grown intact spikes and threshed seeds. The second study employed random inbred F_5 lines obtained from single and backcrosses between the synthetic and two sensitive wheat cultivars. Seed coat color and germination responses of the F_5 lines subjected to a spike wetting were evaluated. The following conclusions could be drawn:

1. The synthetic has a PST similar to that of the moderately sensitive check BR-23, and is more tolerant than its parent Altar 84.

2. The *Aegilops tauschii* parent (donor of D genome) contributed to the PST of the synthetic. The improvement of the synthetic over Altar 84 in terms of PST can be attributed to the *Aegilops tauschii* parent. Altar 84 has some PST and has probably contributed to the synthetic's PST as well.

3. Water-soluble substances present in the vegetative floral tissues (chaff) of the synthetic, Altar 84, and wheat checks, can inhibit germination of threshed seed of

these genotypes enhancing their dormancy. Seed dormancy and inhibitory substances in the chaff appear to contribute to the PST of these genotypes.

4. Pre-harvest sprouting tolerance as measured by Percentage of Germinated Seeds (PGS) in the selected populations had moderate heritable values suggesting this trait was controlled largely by additive gene effects. Few genes appeared to control PST, as indicated by the relatively high proportion of lines as tolerant as the synthetic. Selection for PST based on PGS would be expected to be effective in late generations and, to a lesser extent, in early generations as well.

5. Red seed coat was positively associated with PST in the populations studied. Seed coat color could be used as a selection tool for PST, but breeders can not rely only on seed color data since red coat color was found not only among the most tolerant F_5 lines, but also among the most sensitive.

6. The synthetic Altar/At can be used to improve PST of wheats with red and white seed coats. White recombinant lines more tolerant than their respective parent represented one third of all white lines obtained. One of these lines was not statistically different from the synthetic parent.

7. In order to utilize the tolerance from the synthetic to breed for improved PST, the use of the more tolerant lines obtained in this study as parents in backcrosses to agronomically superior germplasm appears to be an appropriate strategy.

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APPENDIX

Appendix 1. Summary of meteorological data for the month of July at Hyslop Farm during the wheat growing season of 1998.

Day	Temperature (°C)			Precipitation
	Maximum	Minimum	Mean	(mm)
1	21.1	12.8	19.6	0.0
2	21.1	13.3	17.2	0.0
3	24.4	12.2	18.3	0.0
4	19.4	13.9	16.7	0.0
5	21.7	12.8	17.2	0.0
6	25.6	13.9	19.7	0.0
7	29.4	12.8	21.1	0.0
8	27.2	10.6	18.9	0.0
9	28.3	12.8	20.6	0.0
10	29.4	11.7	20.6	1.3
11	24.4	9.4	16.9	0.3
12	27.2	11.1	19.2	0.0
13	25.6	9.4	17.5	0.0
14	27.8	11.7	19.7	0.0
15	28.3	11.1	19.7	0.0
16	31.1	11.7	21.4	0.0
17	35.0	10.6	22.8	0.0
18	32.2	11.7	21.9	0.0
19	28.9	11.1	20.0	0.0
20	26.7	12.2	19.4	0.0
21	30.0	11.7	20.8	0.0
22	33.3	12.2	22.8	0.0
23	33.9	11.7	22.8	0.0
24	30.6	11.7	21.1	0.0
25	28.9	10.6	19.7	0.0
26	30.6	16.1	23.3	0.0
27	**37.8**	**16.7**	**27.2**	0.0
28	**39.4**	**15.0**	**27.2**	0.0
29	**37.2**	**14.4**	**25.8**	0.0
30	27.8	10.6	19.2	1.0
31	26.7	15.0	20.8	0.0
Mean 1998	28.8	12.3	20.5	
Mean 1961-1990	26.8	10.5	18.6	
Departure	2.0	1.8	***1.9***	
Total 1998				2.6
Average 1961-1990				1.3