RESISTANCE TO COMMON BUNT IN THE USDA *AEGILOPS TAUSCHII* COLLECTION

By

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A thesis submitted in partial fulfillment of
the requirements for the degree of

MASTER OF SCIENCE IN CROP SCIENCE

Washington State University
Department of Crop and Soil Sciences

December 2009

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Acknowledgements

I thank my advisor and chair, Dr. Stephen Jones, for taking a chance on me and pushing me to accomplish what he knew I was capable of. I have learned a lot from you.

Many thanks to Dr. Clare Coyne for unflagging support and advice throughout my time here and the writing of this thesis. You kept me going and excited, for which I am very grateful.

To my on-campus advisor, Dr. Kimberly Campbell, I convey deep thanks. Your level head and sage advice kept me on track and out of (too much) trouble.

A great deal of thanks goes to Dr. Lori Carris, who introduced me to the world of fungi. I enjoyed our talks about orchids and benefited greatly from your input on my work.

I am incredibly grateful for the help of our three technicians, Kerry, Meg, and Steve. You all helped me through the peaks and valleys of graduate school with insights and assistance, even when things were going crazy for all of you. I also thank all of the great undergraduate students working with them.

To Dr. Deven See in the Western Regional Small Grains Genotyping Laboratory and Dr. Ted Kisha and Lisa Taylor in the Western Regional Plant Germplasm Introduction and Testing Center, I convey great thanks for all of the help and advice with the TRAPs and DNA work.

I owe a great debt of gratitude to Dr. Robert Metzger and Blair Goates of the USDA. Their advice and technical consultation on smuts helped form the basis of these experiments. The materials they provided were also invaluable in conducting them.

Many thanks to the postdocs in our lab: Dr. Jennifer Moran, Dr. Lori Hoagland, and Dr. Kevin Murphy. You helped me navigate through the degree and provided me with constant food for thought.

G. Janet Matanguihan, salamat po. You were a perpetual fount of information about bunts and smuts, as well as many other things, and I am grateful for how willing you have been to share.

To my co-worker, neighbor, and partner in crime, Lucas Patzek, I tip my glass. From Mexico to Portland to Pullman, you kept things interesting (in a good way). The door's always open, buddy. And, of course, I thank Sarah for not only tolerating, but participating in our escapades.

Mahalo nui loa to my friends from the Pacific Islanders Club. You've kept this haole young. One of these days maybe I'll even learn a dance.

To other friends here and abroad, I thank you for your support and companionship. A degree would be a terrible thing without good friends.

Finally, I thank my family, specifically my loving parents, and my tee-rak, Git. You have supported me no matter which time zone I've been in and still kept me in the loop. I promise that one of these days I'll be back for more than a week. Three generations of Keach Cougars, wow!

'There is something fascinating about science. One gets such wholesale returns of conjecture out of such a trifling investment of fact'.

Mark Twain

RESISTANCE TO COMMON BUNT IN THE USDA *AEGILOPS TAUSCHII* COLLECTION

Abstract

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December 2009

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Common bunt or 'stinking smut', caused by *Tilletia caries* or *T. laevis*, is re-emerging as a major pathogen to common wheat, *Triticum aestivum* when grown in low-input or organic systems. In addition to reducing yields by up to 30%, it imparts a smell like 'rotting fish' that makes contaminated grain unsuitable for export or consumption. The objective of this study was to screen *Aegilops tauschii* for resistance to 10 races of common bunt, representing virulence against the most common resistance genes, for use in future wheat breeding. *Aeg. tauschii* is the D-genome donor of wheat and a source of resistance to various other pathogens. Seeds of 117 accessions of *Aeg. tauschii* from the USDA-ARS National Plant Germplasm System National Small Grains Collection and four susceptible *T. aestivum* cultivars, as well as one putative resistant cultivar, were inoculated with a mixture of spores from the 10 races of common bunt and maintained under appropriate conditions for disease development. When the plants were between Zadoks stages 73-87, developing seeds were removed and examined for the presence of bunt spores. Accessions with any infected plants were marked as susceptible and excluded from future experiments. The putative resistant lines from each experiment, as well as the lines which did not germinate, were re-tested in the subsequent experiments. A cold stratification performed on all of the seeds for the third and fourth experiments improved germination, permitting screening of larger populations and more accessions. Genotyping of all of the accessions using the Target Region Amplified Polymorphism (TRAP) marker technique, as well as with several

standard primers, is also being conducted to elucidate relationships among the accessions and identify other traits. Eighteen resistant accessions were identified, with eight having high probability of not being escapes from infection. All resistant accessions were from Turkey or Iran. A discussion of methods to integrate this resistance, as well as what the resistance might mean, and why infection by this pathogen has not been observed *in vivo* is included. This is the first known screening of multiple accessions of *Aeg. tauschii* for resistance to known races of common bunt.

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This work is dedicated to the memory of

Dr. Robert J. Metzger
April 11, 1925 - June 24, 2008

whose advice and encouragement inspired
this work and myself.

Literature Review

Common wheat, *Triticum aestivum* L., is the source of most food energy consumed across the world (FAO, 2008). Over 226 million hectares are cropped in it each year (Vocke, 2009). Although originating in the Middle East, it has spread due to its versatility in climatic tolerance and is now cultivated on all of the continents except Antarctica. This flexibility is partially due to being an allohexaploid, comprising of three genomes which each contribute unique characteristics.

The origin of wheat's three genomes has been well researched, although some debate still exists as to the specifics. A wide body of evidence (i.e. Dvorak et al., 1993; Jiang & Gill, 1994) supports the theory that the A genome was contributed by *T. monococcum* L. or one of its close relatives. This is visible in its resemblance to common wheat, *T. aestivum* L., and its cross-compatibility with it. Like *T. aestivum*, *T. monococcum* is also grown as a food source, referred to as 'einkorn wheat'. The cultivation of this species may have paved the way for the cultivation of the morphologically similar polyploid wheats, with each increase in ploidy level also leading to increased seed size.

Of all of wheat's genomes, the B genome has been the source of the most debate. Morphological (Parkar & Stebbins, 1956), cytological (Maestra & Naranjo, 1998), and molecular data (Daud & Gustafson, 1996; Jiang & Gill, 1994) suggest that it comes from a common ancestor with *Aegilops speltoides* Tausch, and that structural rearrangements and/or mutations account for the current differences and lack of complete homology between *Aeg. speltoides*' S^B genome and wheat's B genome. The B genome is the source of the 'pairing homeologous' or *Ph1* gene, which allows cultivated wheat to function as a fertile allopolyploid by suppressing intergenomic recombination.

The most recent genomic addition is the D genome from *Aeg. tauschii* Coss. (Sears & McFadden, 1946; Kihara, 1944 in Japanese, summarized in English by Kihara, 1982), whose presence is the difference between *T. durum* Desf. and *T. aestivum*. The addition of this genome led to tolerance to wider range of environmental conditions and improved baking ability (Heiser, 1981), which expanded the range of cultivated wheat (Hancock, 2004) and increased its value to humans. This genome also shows the least genetic diversity within cultivated wheat, although greater diversity exists within the *Aeg. tauschii* species than in the D genome of *T. aestivum* (Lagudah, 1991).

The diversity in *Aeg. tauschii* has been integrated into many breeding programs. Sears & McFadden (1946) postulated that *T. spelta* L., the more primitive form of *T. aestivum,* was a result of hybridization between *T. durum* and *Aeg. tauschii* and backed up this claim by recreating the hybridization event and comparing the hybrids to *T. spelta*. These hybrids were dubbed 'synthetic hexaploids' and formed a new method for improving wheat. Analysis of these, and synthetic hexaploids created later by other groups, showed improved pathogen and abiotic stress resistance, as well as unique morphological traits and baking qualities. They have been utilized extensively in the breeding program at the International Maize and Wheat Improvement Center (CIMMYT) (Dreisigacker, 2008; Mujeeb-Kazi & Hettel, 1995), which has led, through germplasm exchange, to their integration into breeding programs worldwide, as well as production of new synthetic hexaploids at various research institutions. A recent modification to the production of synthetic hexaploids, as outlined by Mujeeb-Kazi & Hettel (1995), is the extraction of the AABB component of hexaploid wheat, using the method initially described by Kerber (1964) and developed further by Kaltsikes et al. (1969) and Yang et al. (1999). This provides an agronomically adapted AABB genome donor, minimizing the load of unwanted

genetic variability. Cox et al. (1995) used direct crosses between *T. aestivum* and *Aeg. tauschii*, claiming more rapid integration of desired traits and recombination between the D genomes, but cautioned that meiotic irregularities may occur and that the lines would not be immediately true breeding.

Aegilops tauschii and the synthetic hexaploids produced from it have contributed a variety of agronomic traits to cultivated wheat germplasm. Cox and his collaborators documented resistance to Hessian fly (*Mayetiola destructor* Say) and soilborne mosaic virus (Cox et al., 1990), as well as leaf rust (*Puccinia recondita* Rob. ex Desm.) resistance and higher grain protein content (Cox et al., 1995) in backcross progeny of hybrids between *Aeg. tauschii* and wheat. Gill et al. (1986) reported resistance to greenbug (*Schizaphis graminum* Rond.), powdery mildew (*Blumeria graminis* [DC.] Speer), as well as Hessian fly and leaf rust in the *Aeg. tauschii* accessions they screened. Limin and Fowler (1980) and Le et al. (1986) found several accessions of *Aeg. tauschii* to possess better freezing or cold tolerance than their cultivated checks. Yildrim et al. (1995) screened over 279 accessions for resistance to both stripe rust (*Puccinia striiformis* Westend.) and eyespot (*Pseudocercosporella herpotrichoides* Fron.), and found varying degrees of resistance to both pathogens. The contribution of this and other *Aegilops* species to wheat improvement is reviewed by Schneider et al. (2008).

Perhaps most important to developing nations is the resistance in *Aeg. tauschii* to Karnal bunt, *T. indica* Mitra, a disease which directly infects heads of wheat grown throughout the Indian subcontinent, and has spread to other parts of the tropical and subtropical world. It is rapidly disseminated, resulting in unpredictable infection patterns and relatively low loss in yield, but the disease can weaken plants and harm flour quality and palatability (Fuentes-Davila et al., 2002; Wilcoxson & Saari, 1996). Resistance to this pathogen has been identified in

cultivated wheat and synthetic hexaploid lines developed by CIMMYT. The synthetic hexaploid resistance has been transferred into a cultivated wheat background and released in cultivars such as 'CIGM 90.257-1', 'CIGM 91.61-1', 'CIGM 90.462', 'CIGM 90.248-1', 'CIGM 90.250-2', and 'CIGM 90.412' (Mujeeb-Kazi et al., 2001). Recently, Chhuneja et al. (2008) screened 183 accessions of *Aeg. tauschii* for alternative forms of resistance to Karnal bunt and were able to find usable resistance in 28 of them. While Karnal bunt is a major pathogen of warmer areas, it has not been an issue in the cooler climates of the northern United States, where much of the wheat production occurs. The disease has been controlled throughout the United States by strict quarantines. However, the co-generic pathogen common bunt, caused by *T. caries* [DC.] Tul. & C. Tul or *T. laevis* Kuhn, has historically been a devastating pathogen for wheat in the Pacific Northwest of the United States and is reemerging under modern methods of cropping.

The life cycle of common bunt has been well characterized and elucidates the detrimental effects of the fungus on wheat yield and end-use quality (Wilcoxson & Saari, 1996). Germination of teliospores occurs in the soil or on seed surfaces under moist conditions at temperatures optimally between 5-10°C, but as high as 22°C. Non-germinated teliospores can survive in wet soil for two years, and much longer in dry soil (Agrios, 2005). Basidiospores form at the end of a basidium emerging from the teliospore and fuse to form 'H-cells', from which infective dikaryotic mycelium develop. The hyphae enter the plant through the coleoptile, proceed through the bases of the leaves, and inhabit the apical meristem and the area directly below it. This must occur before internode elongation, or the fungus will not be able to continue growing. In winter wheat, the fungus is dormant within the plant during the winter and starts growing again when the plant resumes growth. Slight stunting sometimes results as a result of infection. The fungus modifies the seeds to form a sorus, retaining the pericarp and populating the inside

with teliospores. Disease expression may be variable within a plant, leading to partially infected kernels, heads, or sets of tillers, especially when some genetic resistance is present. Similarity in size and shape between the sorus and the wheat seeds facilitates distribution of the spores during threshing, allowing significant pathogen infestations from a relatively small amount of inoculum (Bruehl, 1989). Teliospore traits can be used to identify seed contamination. Both species have teliospores with thick, three-layered walls and which contain spherical, translucent lipid bodies when dormant. In *T. caries* the teliospores range in color from yellow to gray or a reddish brown, are generally globose with a diameter of 14-23.5µm and have a net-like, reticule exospore. Those of *T. laevis* are pale to dark olivaceous brown, globose or ovoid in shape, and have a diameter of 14-22µm and a smooth exospore (Wilcoxson & Saari, 1996).

Until 1956, common bunt was a major problem in the Pacific Northwest. A combination of the local climate and agricultural practices provided a near ideal environment for common bunt infection and growth (Bruehl, 1989). Work at land grant universities by both USDA and state researchers helped characterize the growth habits of the pathogen and identified several genes in wheat that provided resistance to specific races of common bunt. In 1954 it was discovered that treating seeds with hexachlorobenzine before planting would prevent infection, and most of the basic common bunt research was discontinued as this and other chemical treatments became more popular. However, starting in the late 1970s a shift among some farmers to low-input or organic production (Rawson, 2007) reduced the use of chemical seed treatments, and, coupled with re-adoption of practices such as saving of non-certified seed, the pathogen has begun to reemerge as a potential problem. In 2006, a major outbreak of common bunt occurred in Kansas, resulting in seed rejection by grain elevators (Jardine, Extension Factsheet 'Common Bunt of Wheat').

Common bunt incidence has also increased in Europe, and become a major concern. Liatukas & Ruzgas (2006) link the increase directly to organic agriculture, and farmers saving and replanting seed from their crops. This practice of seed saving is an attempt by farmers to fulfill requirements that certified organic seed must be used to plant organic crops of wheat in the European Union, although a shortage of that seed currently exists. One smutted head bears approximately 150 million spores (Kochanova et al., 2004), and with several countries in the EU requiring less than 10 spores per seed for quarantine (Waldow & Jahn, 2007), a small amount of contamination can be economically devastating. The problem of common bunt infection has also begun affecting yields across Europe. At the International Symposium on Wheat Yield Potential, Reynolds et al. (2008) reported that in several European and Middle Eastern countries over 30% of yield loss can be attributed to common bunt. This is corroborated by van Bueren et al. (2003), who cite common bunt as the primary reason for seed lots being disposed of in the Netherlands. A paper titled 'High Consequence Plant Pathogens' (Gamliel, 2008) in the North Atlantic Treaty Organization's (NATO) Crop Biosecurity even goes so far as to suggest that common bunt should be regarded as a potential biological weapon, due to the ease of obtaining and dispersing it in combination with its devastating effects on wheat cultivation. There is some evidence (Suffert et al., 2009), that Iraq may have investigated this possibility in their war against Iran. This has highlighted the need for new research into non-chemical methods for common bunt control and prevention, including new sources of genetic resistance.

Metzger reported novel resistance to common bunt in several wild relatives and progenitors of wheat as narratives on accessions in the NPGS-GRIN (http://www.ars-grin.gov/npgs/acc/acc_queries.html). This includes the accession CIae 23 of *Aeg. tauschii*, #2144 from the Kyoto University Scientific Expedition (KUSE), which he suggests carries at

least one common bunt resistance gene, conferring resistance to many races. This accession was utilized by him to create two synthetic hexaploids, M82-3668 (PI 542503) and M82-3676 (PI 542507) with *T. carthlicum* Nevski. While this suggests that *Aeg. tauschii* may be a source of resistance to common bunt, the races tested and genes conferring resistance are not listed, and the experiments demonstrating resistance are not referenced or found in other known publications.

Work conducted by Reichert (1931) also suggests that *Aeg. tauschii* may possess resistance to some races of common bunt. To expand upon work conducted by Vavilov (1918) on other species of *Aegilops*, Reichert obtained one unnamed accession of *Aeg. squarrosa* (= *Aeg. tauschii*), in addition to single accessions of 19 other species in the genus, from Eig. He inoculated these with a Palestinian landrace of common bunt and found resistance in all of the plants except *Aeg. ventricosa* Tausch. However, he cautioned that the results he obtained for resistance in *Aeg. cylindrica* Host conflicted with Vavilov's, and so differences in virulence may exist between their strains of the fungus. As his study predated the separation of common bunt into races, the actual virulence of the strain he used is unknown.

In 2000, Babayants et al. reported characterization of new sources of bunt resistance introgressed from *Aeg. cylindrica* (CCDD), whose existence they had suggested in a previous publication in Russian. Segregation in crosses with other *Bt* (bunt resistance) genes indicated that at least two new, independent genes were present in their introgression lines. They postulated that these were donated from the D genome of *Aeg. cylindrica,* as it is also believed to have originated from *Aeg. tauschii*, and so shares homology with the D genome of common wheat. However, in a 2006 study by Galaev et al. utilizing a portion of the same germplasm, one of the genes was localized to the telomeric region of chromosome 1BL. They suggest this

placement may be a result of structural or chromosomal rearrangements, but do not explain why it is not found on one of the D genome chromosomes, as would have been expected. It is possible that the gene may have originated on the D genome but was rearranged, due to the presence of gametocidal genes on the C genome (Endo, 1979).

Fifteen genes for resistance to common bunt have been identified and characterized (Wilcoxson & Saari, 1996). Different genes confer resistance to different combinations of races, and the distribution of races allows certain genes to continue to be used for long periods in one area, even if they may have been overcome elsewhere. Genes *Bt10* and *Bt8* are examples of this, as the former is still in use in Canada (Laroche et al., 2000) despite being overcome by over five races elsewhere (Wilcoxson & Saari, 1996), and the latter forms the basis for most resistance in the United States while it has been overcome in Iran (Mardoukhi & Torabi-Anghaji, 2003). *Bt10* and *Bt8* have also been overcome by the synthetic races R39 and R43, respectively, which were produced by hybridization (Metzger, personal communication, and descriptor data for 'Common Bunt' in 'Wheat' on the USDA National Plant Germplasm System Genetic Resources Information Network [NPGS-GRIN]). No races have yet been identified that are virulent against *Bt11* or *Bt12*. Modes of inheritance of *Bt* genes 1-10 are known, as summarized in Wilcoxson & Saari (1996), with *Bt3* being recessive, *Bt1, Bt5, Bt8, Bt9, & Bt10* being completely dominant, and *Bt2, Bt4, Bt6, & Bt7* expressing full resistance only when both copies of the genes are present. Additional genes conferring partial resistance exist, but are not actively used in breeding programs as they permit infection that is sufficient to contaminate seedlots (Wilcoxson & Saari, 1996; Metzger, personal communication). Some accessions contain multiple *Bt* genes, such as PI 178383, which is the source of *Bt8, Bt9,* and *Bt10*, as well as a gene for partial resistance. This type of gene pyramiding would be advantageous to integrate into commercial cultivars, but

is difficult to do solely through phenotypic selection, as the different genes can each produce the same phenotype.

Marker-assisted selection allows pyramiding of multiple genes that share a similar phenotype into a single cultivar. Work by Laroche et al. (2000) identified two polymorphic bands of wheat DNA, generated by primers derived from enzyme restrictions, which were present in lines carrying *Bt10* and could identify whether the gene was in a homozygous or heterozygous state by the intensity of the band. Previous work by Menzies et al. (2006) had placed *Bt10* on the short arm of chromosome 6 of the D genome. Wang et al. (2009) developed markers capable of distinguishing between lines carrying the unnamed source of common bunt resistance in 'Blizzard' with those that did not carry it, and were able to determine that the resistance factor is inherited as a single dominant trait on the short arm of chromosome 1 of the B genome. Cichy (unpublished, 2009), however, reported difficulties in achieving reliable screening using both Laroche et al. (2000) and Wang et al.'s (2009) markers, as well as with markers described in Romanian by Ciuca et al. in 2007. Fofana et al. (2008) identified three quantitative trait loci (QTLs) related to bunt resistance, contributed by 'AC Domain' and discovered in a segregating F_2 between it and 'RL4452', and suggested other populations should be investigated for bunt resistance QTLs. Further work on more reliable markers, as well as information on the chromosomal location and modes of action of more of these genes, would facilitate future screening of wheat and its relatives for known sources of resistance.

Hu & Vick (2003) described a new technique for creating markers, known as Target Region Amplified Polymorphism (TRAP). One primer, the 'fixed primer', is designed using a known sequence from the organism of interest, while the other 'random primers' use AT- or GC-nucleotide-rich sequences to anneal to exons or introns, respectively. The random primers are

also labeled using 700 or 800nm infrared dye to permit running on an automated polyacrylamide gel electrophoresis (PAGE) machine, such as a Li-Cor sequencer (Li-Cor Biosciences, Lincoln, NE). Due to the presence of multiple introns and exons throughout the genome of a plant, the random primers create bands of varying length when anchored by the fixed primer. Bands are also created from imperfect sequence matches due to low annealing temperatures, as well as from fixed/fixed and random/random primer combinations. The process is comparatively cheap and easy, compared to methods like amplified fragment length polymorphism (AFLP) markers, which require enzyme digestion of the DNA, and has better reproducibility than Random Amplified Polymorphic DNA (RAPD) markers. In 2006, Hu published improvements to his earlier protocol and expanded upon it by suggesting the use of fixed primers that match sequences from the telomeric regions of chromosomes, which are typically conserved across organisms. Hu also compared different random primer combinations, identifying which produced the most polymorphic bands in combination with one of the telomeric fixed primers. These polymorphic bands can be used for QTL discovery by association mapping or to establish relationships between accessions based on shared polymorphism. The TRAP protocol has now been run on a variety of species for both of these purposes, including *Pelargonium* (Palumbo et al., 2007), sunflower (Hu, 2006; Yue et al., 2008; Yue et al., 2009; Yue et al., in press), sugarcane (Alwala et al., 2006), lettuce (Hu et al., 2005), and wheat (Liu et al., 2005; Li et al., 2006; Chu et al., 2008).

In Liu et al.'s (2005) investigation of wheat with TRAP markers, they described adequate resolution of the A and B genomes, but had some difficulty getting coverage of the D genome. They discussed that this was likely due to homogeneity within cultivated wheat's D genome. Through their marker work, they were able to identify QTLs for days to heading and reduced

plant height on chromosomes 5A and 4B, respectively. Li et al. (2006) found that by screening the D genome chromosomes when substituted into a line of *T. durum* (AABB) 'Langdon', in an attempt to characterize the lines and develop TRAP markers, they were able to achieve improved resolution, although it was still not as good of that of the A or B genomes. Chu et al. (2008) were able to use TRAP marker screening of a doubled haploid population to find QTLs for days to heading (on chromosomes 5A and 5B), plant height (on chromosomes 4D and 5A), and spike characteristics (on chromosomes 3D, 4A, 4D, 5A, and 5B), indicating that sufficient resolution of the D genome to achieve some QTL mapping is possible. Faris and Friesner (2005) used TRAP marker mapping to identify two QTLs related to tan spot (*Pyrenophora tritici-repentis*) resistance in wheat, accounting for up to 70% of the phenotypic variation, and were able to associate them to 1BS and 3BL. Additional work in wheat and in its D genome donor, *Aeg. tauschii,* might elucidate D genome polymorphism and provide a simple method of screening for more agronomic traits, such as common bunt resistance.

In the present study, *Aeg. tauschii* was screened for resistance to common bunt. The resistance discovered is discussed, along with its potential implications for breeding. Ongoing work is outlined, including the possible integration of this resistance into breeding programs and genetic characterization of the accessions screened. Other potential projects, derived from conclusions in this study, are also detailed for future researchers.

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RESISTANCE TO COMMON BUNT IN THE USDA *AEGILOPS TAUSCHII* COLLECTION

Introduction

Common bunt, caused by *Tilletia caries* (DC.) Tul. & C. Tul. or *T. laevis* Kuhn, is a reemerging pathogen threat in wheat, *Triticum aestivum* L.. Reynolds et al. (2008) report yield losses of up to 30% in several European and Middle Eastern countries due to common bunt infection. Flour produced from common bunt-infested grain is rendered unmarketable due to a color change from the presence of spores and a fish-like odor from trimethylamine (Hanna et al., 1932). Common bunt teliospores can survive on either seed or soil, and germinate into infective dikaryotic mycelia during cool, moist weather. While common bunt is controlled in some areas through chemical seed treatments (Bruehl, 1989; Wilcoxson & Saari, 1996), this is not feasible or possible in some developing areas of the world or anywhere under organic or low-input cropping systems. Genetic resistance, which exists in some cultivars of wheat and its relatives, provides an alternative control strategy.

Fifteen genes for resistance to common bunt have been characterized in wheat (Wilcoxson & Saari, 1996). These offer resistance against all currently characterized naturally occurring races. However, genes 1-10, including the commercially popular *Bt8*, have been overcome by synthetic races produced by hybridization (Wilcoxson & Saari, 1996; Goates, personal communication; descriptor data for 'Common Bunt' in 'Wheat' on NPGS-GRIN http://www.ars-grin.gov/cgi-bin/npgs/html/desc.pl?65032), and a recent report has described races with new virulence combinations (Mardoukhi & Torabi-Anghaji, 2003) The possibility exists that more races may similarly develop virulence against currently used sources of genetic resistance.

Wild relatives and progenitors of wheat have served as new sources of resistance to various pathogens in many breeding programs. The International Maize and Wheat Improvement Center (CIMMYT) has been very active in integrating traits from these relatives into cultivars and breeding lines, especially by producing synthetic hexaploids from crosses of *T. durum* Desf., the donor of the A and B genomes in *T. aestivum*, with *Aegilops tauschii* Coss., the D genome donor. Dreisigacker et al. (2008) review the history of this work, as well as the direction it is headed, and describe some of its use in breeding and germplasm improvement. *Aeg. tauschii* has been screened and utilized as a source of resistance to a range of pathogens, as reviewed in Schneider et al. (2008) including Hessian fly (*Mayetiola destructor* [Say]) (Gill et al., 1986; Cox et al., 1990), greenbug (*Schizaphis graminum* [Rond.]) (Gill et al., 1986), soilborne mosaic virus (Cox et al., 1990), leaf rust (*Puccinia recondita* [Rob. ex Desm.]) (Cox et al., 1995; Gill et al., 1986), stripe rust (*Puccinia striiformis* [Westend.]) (Yildrim et al., 1995), powdery mildew (*Blumeria graminis* [DC.] Speer) (Gill et al., 1986), and eyespot (*Pseudocercosporella herpotrichoides* [Fron.]) (Yildrim et al., 1995). CIMMYT and other groups (Chhuneja et al., 2008; Multani et al., 1988; Villareal et al., 1994) have also observed resistance to Karnal bunt (*T. indica* [Mitra] Mundkur), a pathogen co-generic to common bunt but having a different mode of infection, in *Aeg. tauschii* and synthetic hexaploids derived from it.

Metzger reported novel resistance to common bunt in several wild relatives and progenitors of wheat as narratives on accessions in the USDA National Plant Germplasm System Genetic Resources Information Network (NPGS-GRIN, http://www.ars-grin.gov/npgs/acc/acc_queries.html). This includes the accession CIae 23 of *Aeg. tauschii*, #2144 from the Kyoto University Scientific Expedition (KUSE), which he suggests carries at

least one common bunt resistance gene, conferring resistance to many races. This accession was utilized by him to create two synthetic hexaploids, M82-3668 (PI 542503) and M82-3676 (PI 542507), with *T. carthlicum* Nevski. While this suggests that *Aeg. tauschii* may be a source of resistance to common bunt, the races tested and genes conferring resistance are not listed, and the experiments demonstrating resistance are not referenced or found in other known publications.

Work conducted by Reichert (1931) also suggests that *Aeg. tauschii* may possess resistance to some races of common bunt. To expand upon work conducted by Vavilov (1918) on other species of *Aegilops*, Reichert obtained one unnamed accession of *Aeg. squarrosa* (= *Aeg. tauschii*), in addition to single accessions of 19 other species in the genus, from Eig. He inoculated these with a Palestinian landrace of common bunt and found resistance in all of the plants except *Aeg. ventricosa* Tausch. However, he cautioned that the results he obtained for resistance in *Aeg. cylindrica* Host conflicted with Vavilov's, and so differences in virulence may exist between their strains of the fungus. As his study predated the separation of common bunt into races, the actual virulence of the strain he used is unknown.

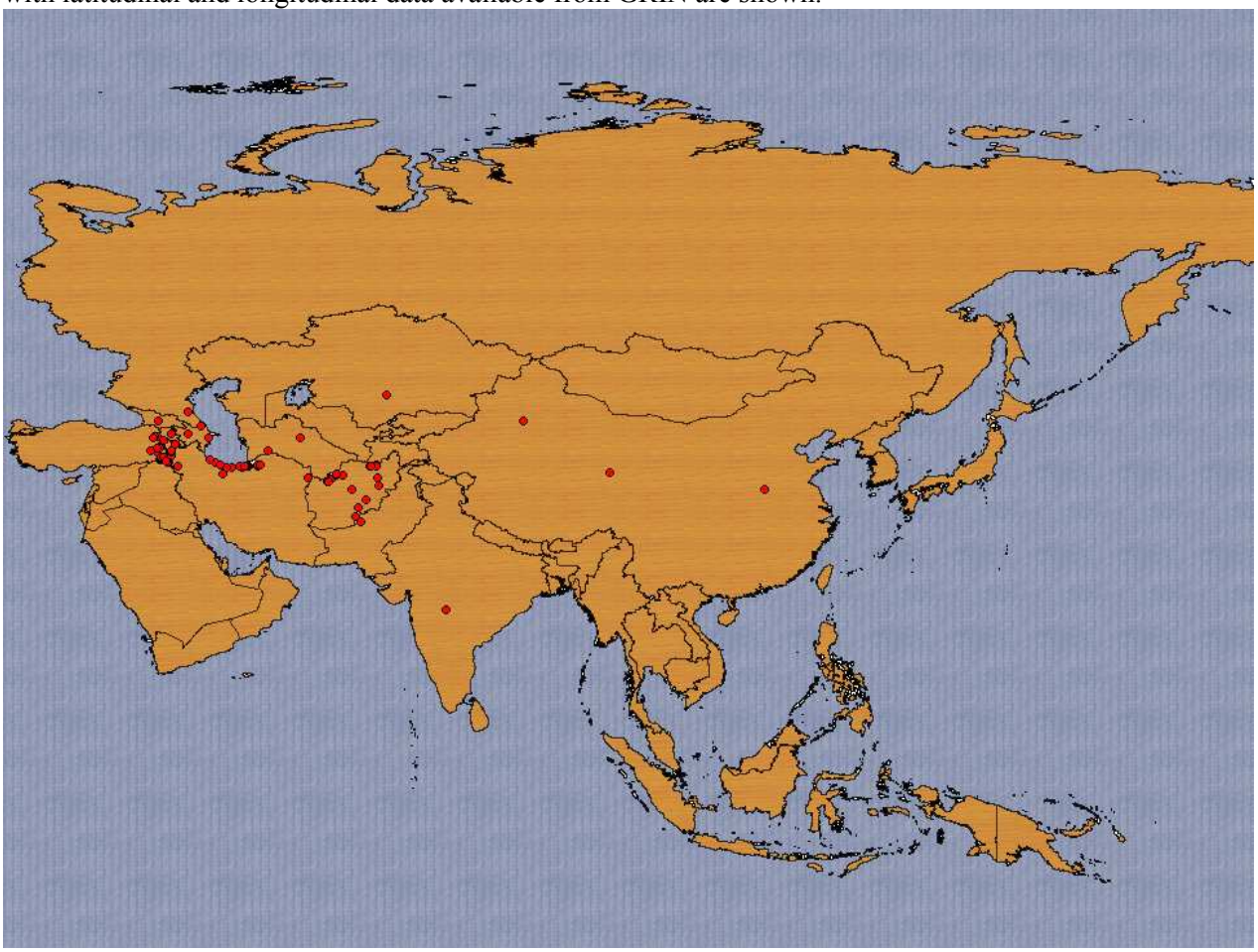
The objective of this study was to screen 117 accessions of *Aeg. tauschii* from the USDA NPGS using known races of common bunt, to identify resistance for utilization in breeding programs.

Materials and Methods

Germplasm Used

Seeds of 117 available accessions of *Aeg. tauschii* were obtained from the United States Department of Agriculture's National Small Grains Collection (USDA NSGC). These accessions represent collection events in over 10 countries (Appendix 1, Figure 1). Cultivars 'Eltan' (Peterson et al., 1991), 'Elgin', 'Red Bobs' (Clark et al., 1926), and 'TetraCanthatch' (Kerber, 1964) were used as susceptible controls. A hybrid between *T. aestivum* and *T. urartu* Thumanian ex Gandilyan, made by Dr. Robert Metzger and maintained by Blair Goates, was used as a resistant control (#122).

Figure 1. Map of accessions used in this study. Each red dot represents one accession. Only accessions with latitudinal and longitudinal data available from GRIN are shown.



Vernalization and Growth

Four experiments were conducted over the course of one year. For each experiment two seeds were planted into the bottom of each #727 Jiffy peat pellet (Jiffy-7; Shippagan, Canada), with sixteen seeds total of each accession being planted in Experiment 1 and twelve seeds total of each accession being planted in the subsequent experiments. All accessions were planted in Experiment 1, and only accessions that showed putative resistance or did not germinate were planted for Experiment 2; this process was repeated with each of the subsequent experiments, limiting planting to only putative resistant or non-germinating accessions. The seed was germinated at 5-10ºC with a 16/8 hour light/dark schedule for Experiment 1 and 23/1 hour light/dark for the subsequent experiments. After germination, the seedlings were thinned to a maximum of eight plants per accession in Experiment 1 and six plants per accession in the subsequent experiments, and grown under the same conditions as above for eight weeks to vernalize. Vernalized seedlings were transplanted into 1 gallon pots, with one to two pellets per pot, using LC1 potting mix (Sun Gro Horticulture; Bellevue, WA) supplemented with 15-20g of 90550 slow release fertilizer (14N-14P-14K; Osmocote; Marysville, OH) and grown in greenhouses maintained at 21-24ºC/15-18ºC day/night and with a 16/8 light/dark schedule. Throughout all stages of the study, watering was performed when dry media was observed.

Inoculation of Seeds

Prior to planting, the seed was hand-threshed to remove the glumes and then seeds of each accession were surface sterilized using a 15 minute exposure to 70% ethanol followed by a rinse with distilled, de-ionized water (ddH_20). The seed was then dried overnight using a chemical fume hood for Experiments 1 and 2. In Experiments 3 and 4, some moisture was retained and the seed was stored at 4ºC for three days to break dormancy. Ground teliospores from a mix of sori of 10 *Tilletia* races, obtained from Blair Goates (USDA NSGC) via Glafera Matanguihan (Washington State University) and representing virulence against ten of the more common bunt resistance genes (Table 1), were added in quantities sufficient to coat the seeds and vortexed briefly in a microcentrifuge tube. For Experiment 4, the spores were suspended in ddH20, due to concerns about unequal seed coating from residual moisture on the seeds.

Table 1. Races of *Tilletia* included in the study. Races starting with 'T' are *T. caries*, 'L' *T. laevis*, and 'R' the result of artificial hybridization between races. An 'x' marks the bunt resistance (Bt) genes they are virulent against. (Wilcoxson & Saari, 1996; descriptor data for 'Common Bunt' in 'Wheat' on NPGS-GRIN http://www.ars-grin.gov/cgi-bin/npgs/html/desc.pl?65032)

	Bt1	*Bt2*	*Bt3*	*Bt4*	*Bt5*	*Bt6*	*Bt7*	*Bt8*	*Bt9*	*Bt10*	*Bt11*	*Bt12*	*Bt13*	*Bt14*	*Bt15*
T1							x								
T16		x		x	x	x	x								
T19	x	x	x				x								
T23	x	x		x		x	x		x						
T27	x	x		x		x	x			x					
T29	x	x					x		x	x					
T30	x	x		x		x	x		x	x					
L8		x		x		x	x		x						
L16	x	x		x		x	x								
R43		x			x		x	x							

Screening of Inoculated Plants

Each plant was considered to be an experimental unit. The inflorescences were observed at Zadoks stages 59-69 (Zadoks et al, 1974) for any signs of sori formation and growth abnormalities. Seed was removed from the glumes of each plant at approximately Zadoks stages 73-87 and dissected. Dark teliospore masses in the seed were considered signs of *Tilletia* infection and plants containing these were scored as 'susceptible'. The number of susceptible plants for a given accession was recorded as a fraction of the total number of plants present for that accession in that experiment. Plants exhibiting late head formation were also noted, and maintained until data could be taken in Experiments 1 and 2 but discarded due to time and external disease constraints in Experiments 3 and 4. Accessions marked as susceptible during one experiment were excluded from growth or screening in subsequent experiments.

All of the data for the different susceptible checks was pooled from across the experiments and used to calculate the probability that susceptibility would not be observed in a susceptible accession (i.e. an ‘escape’). The number of susceptible plants from this data, 6, was taken out of the total number of plants screened, 18, and simplified to generate the probability of susceptibility being observed in a susceptible accession, 1/3. The probability that susceptibility would not be observed in a susceptible accession, 2/3, was raised to the number of plants screened for each putative resistant accession, producing each accession’s overall probability of escaping infection. This data suggested whether or not each accession had received sufficient screening to observe a susceptible plant.

Results

Out of the 117 accessions of *Aeg. tauschii*, 18 accessions were identified as showing resistance across all conducted experiments to all 10 races of common bunt used in this study (Table 2). Eight of these putative resistant accessions had a probability lower than 5% of being a susceptible 'escape'. Ninety-seven accessions were susceptible to at least one race of common bunt used in this study. Data could not be collected on three accessions due to a lack of germination. At least one susceptible check was observed as being infected in each experiment, except in Experiment 4, where none of the checks germinated. In Experiment 1 the resistant check was also observed to have a few infected kernels in an otherwise healthy head. Complete data for all accessions, by experiment, is listed in Appendix 1.

Several morphological differences were observed in infected plants, which may be useful in separating resistant and susceptible plants in breeding programs and future experiments. Most pronounced was the splaying of florets (Figure 2), which was present to some degree on all infected plants and was especially noticeable as the heads dried down. Reduction or absence of extruded anthers at anthesis also was frequently observed on infected heads, although some variability for this existed within each plant. Sori development was also modified, with sori on several accessions bursting upon maturity (Figure 3).

Table 2. Data on accession origin from NPGS-GRIN and performance in screening. A '-' indicates no germination. Data highlighted in green shows accessions where no susceptible plants were found. a = Total number of plants screened. b = Total number of susceptible plants. c = Probability susceptible exists but not observed in a putative resistant accession.

#	Variety	TAXON	PLANTID	COUNTRY	STATE	Total # Plants[a]	Total # Sus[b]	p(Sus not obs)[c]
1	CIae 1	*Aegilops tauschii*	2001	Pakistan	Baluchistan	2	1	
2	CIae 2	*Aegilops tauschii*	2016	Pakistan	Baluchistan	4	1	
3	CIae 3	*Aegilops tauschii*	2036	Afghanistan	Zabul	3	2	
4	CIae 4	*Aegilops tauschii*	2038	Afghanistan	Ghazni	5	2	
5	CIae 5	*Aegilops tauschii*	2051-2	Afghanistan	Baghlan	2	2	
6	CIae 6	*Aegilops tauschii*	2095	Afghanistan	Badghis	3	3	
7	CIae 8	*Aegilops tauschii*	2111	Iran	Mazandaran	3	3	
8	CIae 9	*Aegilops tauschii*	2112	Iran	Mazandaran	4	2	
9	CIae 10	*Aegilops tauschii*	2115	Iran	Mazandaran	9	4	
10	CIae 11	*Aegilops tauschii*	2118	Iran	Mazandaran	2	1	
11	CIae 12	*Aegilops tauschii*	2119	Iran	Mazandaran	4	2	
12	CIae 13	*Aegilops tauschii*	2123	Iran	Mazandaran	5	5	
13	CIae 14	*Aegilops tauschii*	2128	Iran	Mazandaran	3	2	
14	CIae 15	*Aegilops tauschii*	2131	Iran	Khorasan	3	3	
15	CIae 16	*Aegilops tauschii*	2133	Iran	Mazandaran	2	2	
16	CIae 17	*Aegilops tauschii*	2134	Iran	Mazandaran	3	1	
17	CIae 18	*Aegilops tauschii*	2137	Iran	Mazandaran	8	4	
18	CIae 19	*Aegilops tauschii*	2139	Iran	Mazandaran	2	0	0.4444

#	Variety	TAXON	PLANTID	COUNTRY	STATE	Total # Plants[a]	Total # Sus[b]	
19	CIae 20	*Aegilops tauschii*	2140	Iran	Mazandaran	-	-	
20	CIae 21	*Aegilops tauschii*	2141	Iran	Mazandaran	11	4	
21	CIae 22	*Aegilops tauschii*	2142	Iran	Gilan	7	7	
22	CIae 23	*Aegilops tauschii*	2144	Iran	Gilan	1	1	
23	CIae 24	*Aegilops tauschii*	2146	Iran	Gilan	5	3	
24	CIae 25	*Aegilops tauschii*	2147	Iran	Gilan	5	5	
25	CIae 26	*Aegilops tauschii*	2152	Iran	Gilan	5	5	
26	CIae 27	*Aegilops tauschii*	2168	Iran	West Azerbaijan	9	0	0.0260
27	CIae 28	*Aegilops tauschii*	2170	Iran	West Azerbaijan	1	0	0.6667
28	CIae 30	*Aegilops tauschii*	2402	Unknown		2	1	
29	CIae 50	*Aegilops tauschii*	Sando 206	Unknown		4	1	
30	CIae 51	*Aegilops tauschii*	Sando 208	Unknown		4	2	
31	CIae 68	*Aegilops tauschii*	7612a	Turkey	Kars	1	1	
32	CIae 71	*Aegilops tauschii*	M7-262	Unknown		7	6	
33	CIae 72	*Aegilops tauschii*	0-623	Unknown		5	4	
34	PI 210987	*Aegilops tauschii*	12862	Afghanistan	Kondoz	2	2	
35	PI 220326	*Aegilops tauschii*	416	Afghanistan	Kondoz	1	1	
36	PI 220331	*Aegilops tauschii*	552	Afghanistan	Faryab	6	3	
37	PI 220641	*Aegilops tauschii*	475	Afghanistan	Kondoz	4	4	
38	PI 220642	*Aegilops tauschii*	545	Afghanistan	Faryab	3	3	
39	PI 268210	*Aegilops tauschii*	134	Iran	Mazandaran	3	3	

#	Variety	TAXON	PLANTID	COUNTRY	STATE	Total # Plants[a]	Total # Sus[b]	
40	PI 276975	*Aegilops tauschii*	M7	Turkistan		5	3	
41	PI 276980	*Aegilops tauschii*	16	Former Soviet Union	Caucasus	1	1	
42	PI 276985	*Aegilops tauschii*	Meyeri	Iran	Mazandaran	3	3	
43	PI 317392	*Aegilops tauschii*	337	Afghanistan	Badghis	3	3	
44	PI 317394	*Aegilops tauschii*	378	Afghanistan	Badghis	2	1	
45	PI 330489	*Aegilops tauschii*		Unknown		5	5	
46	PI 349037	*Aegilops tauschii*	WIR 115	Azerbaijan		3	3	
47	PI 369627	*Aegilops tauschii*	D.I.V. 16185	Unknown		3	2	
48	PI 428563	*Aegilops tauschii*	WIR 1216	Georgia		3	1	
49	PI 428564	*Aegilops tauschii*	WIR 1405	Azerbaijan		3	2	
50	PI 431598	*Aegilops tauschii*	WIR 33	Turkmenistan		3	2	
51	PI 431599	*Aegilops tauschii*	WIR 109	Azerbaijan		1	1	
52	PI 431600	*Aegilops tauschii*	WIR 246	Russian Federation	Dagestan	5	5	
53	PI 431601	*Aegilops tauschii*	WIR 299	Azerbaijan		4	3	
54	PI 431602	*Aegilops tauschii*	WIR 433	Turkmenistan		6	4	
55	PI 431603	*Aegilops tauschii*	WIR 467	Azerbaijan	Naxcivan	5	5	
56	PI 452130	*Aegilops tauschii*		China	Henan	5	4	
57	PI 452131	*Aegilops tauschii*		China	Qinghai	5	4	
58	PI 476874	*Aegilops tauschii*	WIS 2086	Afghanistan		1	1	
59	PI 486265	*Aegilops tauschii*	79TK057-317	Turkey	Hakkari	1	1	
60	PI 486266	*Aegilops tauschii*	79TK057-318	Turkey	Hakkari	3	1	

#	Variety	TAXON	PLANTID	COUNTRY	STATE	Total # Plants[a]	Total # Sus[b]	
61	PI 486267	*Aegilops tauschii*	79TK057-322-1	Turkey	Hakkari	3	0	0.2963
62	PI 486271	*Aegilops tauschii*	79TK075-400	Turkey	Van	3	0	0.2963
63	PI 486272	*Aegilops tauschii*	79TK075-405	Turkey	Van	3	0	0.2963
64	PI 486274	*Aegilops tauschii*	79TK091-455-1	Turkey	Kars	4	0	0.1975
65	PI 486275	*Aegilops tauschii*	79TK091-455-2	Turkey	Kars	2	2	
66	PI 486276	*Aegilops tauschii*	79TK092-467-1	Turkey	Kars	6	1	
67	PI 486277	*Aegilops tauschii*	79TK093-471	Turkey	Kars	3	1	
68	PI 499262	*Aegilops tauschii*	82-Ae 3	China	Xinjiang	5	4	
69	PI 508263	*Aegilops tauschii*	Ae-41	China	Shaanxi	5	5	
70	PI 508264	*Aegilops tauschii*	Ae-46	China	Henan	6	5	
71	PI 511363	*Aegilops tauschii*	KU-2059	Afghanistan	Faryab	1	1	
72	PI 511365	*Aegilops tauschii*	KU-2001	Pakistan	Baluchistan	4	4	
73	PI 511366	*Aegilops tauschii*	KU-2013	Afghanistan	Zabul	6	3	
74	PI 511367	*Aegilops tauschii*	KU-2019	Afghanistan	Kabul	4	4	
75	PI 511368	*Aegilops tauschii*	KU-2069	Iran	Tehran	5	5	
76	PI 511369	*Aegilops tauschii*	KU-2082	Iran	Mazandaran	2	2	
77	PI 511370	*Aegilops tauschii*	KU-2083	Iran	Mazandaran	4	2	
78	PI 511375	*Aegilops tauschii*	KU-20-2	Unknown		4	3	
79	PI 511378	*Aegilops tauschii*	KU-2117	Iran	West Azerbaijan	4	3	
80	PI 511379	*Aegilops tauschii*	KU-2118	Iran	West Azerbaijan	9	6	
81	PI 511380	*Aegilops tauschii*	KU-2126	Iran	Mazandaran	7	0	0.0585

#	Variety	TAXON	PLANTID	COUNTRY	STATE	Total # Plants[a]	Total # Sus[b]	
82	PI 511381	*Aegilops tauschii*	KU-2073	Iran	Mazandaran	10	7	
83	PI 511382	*Aegilops tauschii*	KU-2074	Iran	Mazandaran	5	4	
84	PI 511383	*Aegilops tauschii*	KU-2075	Iran	Mazandaran	6	1	
85	PI 542277	*Aegilops tauschii*	84TK154-015	Turkey	Izmir	5	1	
86	PI 542278	*Aegilops tauschii*	84TK154-043	Turkey	Izmir	1	1	
87	PI 554310	*Aegilops tauschii*	84TK501-003	Turkey	Van	9	0	0.0260
88	PI 554313	*Aegilops tauschii*	84TK501-009	Turkey	Van	8	0	0.0390
89	PI 554315	*Aegilops tauschii*	84TK501-012	Turkey	Van	11	0	0.0116
90	PI 554316	*Aegilops tauschii*	84TK501-012	Turkey	Van	0	-	
91	PI 554318	*Aegilops tauschii*	84TK530-002	Turkey	Hakkari	5	1	
92	PI 554319	*Aegilops tauschii*	84TK532-001	Turkey	Hakkari	4	1	
93	PI 554320	*Aegilops tauschii*	84TK534-004	Turkey	Hakkari	13	0	0.0051
94	PI 554321	*Aegilops tauschii*	84TK562-005	Turkey	Hakkari	5	2	
95	PI 554322	*Aegilops tauschii*	84TK572-004.00	Turkey	Van	0	-	
96	PI 554323	*Aegilops tauschii*	84TK573.1-001	Turkey	Van	2	0	0.4444
97	PI 560534	*Aegilops tauschii*	TU85-007-01	Turkey	Hakkari	4	0	0.1975
98	PI 560535	*Aegilops tauschii*	TU85-018-02	Turkey	Hakkari	9	0	0.0260
99	PI 560536	*Aegilops tauschii*	TU85-034-03	Turkey	Van	11	0	0.0116
100	PI 560538	*Aegilops tauschii*	TU85-052-01	Turkey	Bitlis	7	0	0.0585
101	PI 560754	*Aegilops tauschii*	TU86-12-02	Turkey	Hakkari	4	1	
102	PI 560755	*Aegilops tauschii*	TU86-36-02	Turkey	Hakkari	4	1	

#	Variety	TAXON	PLANTID	COUNTRY	STATE	Total # Plants[a]	Total # Sus[b]	
103	PI 574465	*Aegilops tauschii*	WIR 78	Azerbaijan		5	5	
104	PI 574467	*Aegilops tauschii*	WIR 366	Russian Federation	Dagestan	6	5	
105	PI 574468	*Aegilops tauschii*	WIR 415	Armenia		3	3	
106	PI 574469	*Aegilops tauschii*	WIR 912	India		4	3	
107	PI 603220	*Aegilops tauschii*	TA 1578	Western Asia		3	1	
108	PI 603221	*Aegilops tauschii*	TA 1597	Western Asia		4	2	
109	PI 603223	*Aegilops tauschii*	TA 1600	Iran	Mazandaran	5	2	
110	PI 603224	*Aegilops tauschii*	TA 1616	Russian Federation	Dagestan	1	1	
111	PI 603225	*Aegilops tauschii*	TA 1617	Turkmenistan	Balkan	4	2	
112	PI 603233	*Aegilops tauschii*	TA 1669	Azerbaijan		6	4	
113	PI 603235	*Aegilops tauschii*	TA 1671	Azerbaijan		6	4	
114	PI 603246	*Aegilops tauschii*	TA 1712	Portugal		9	6	
115	PI 603249	*Aegilops tauschii*	TA 2375	Iran	Tehran	4	4	
116	PI 603252	*Aegilops tauschii*	TA 2486	Iran	West Azerbaijan	13	0	0.0051
117	PI 603255	*Aegilops tauschii*	TA 2570	Armenia	Erevan	6	2	
118	PI 536994	*Triticum aestivum*	Eltan	USA	Washington	8	2	
119	CItr 11755	*Triticum aestivum*	Elgin	USA	Oregon	3	0	
120	CItr 6255	*Triticum aestivum*	Red Bob	Canada	Saskatchewan	5	3	
121	PI 583718	*Triticum aestivum*	Tetra Canthatch	Canada		2	1	
122	Metzger urartu	*Triticum* hybrid		USA	Oregon	4	1	

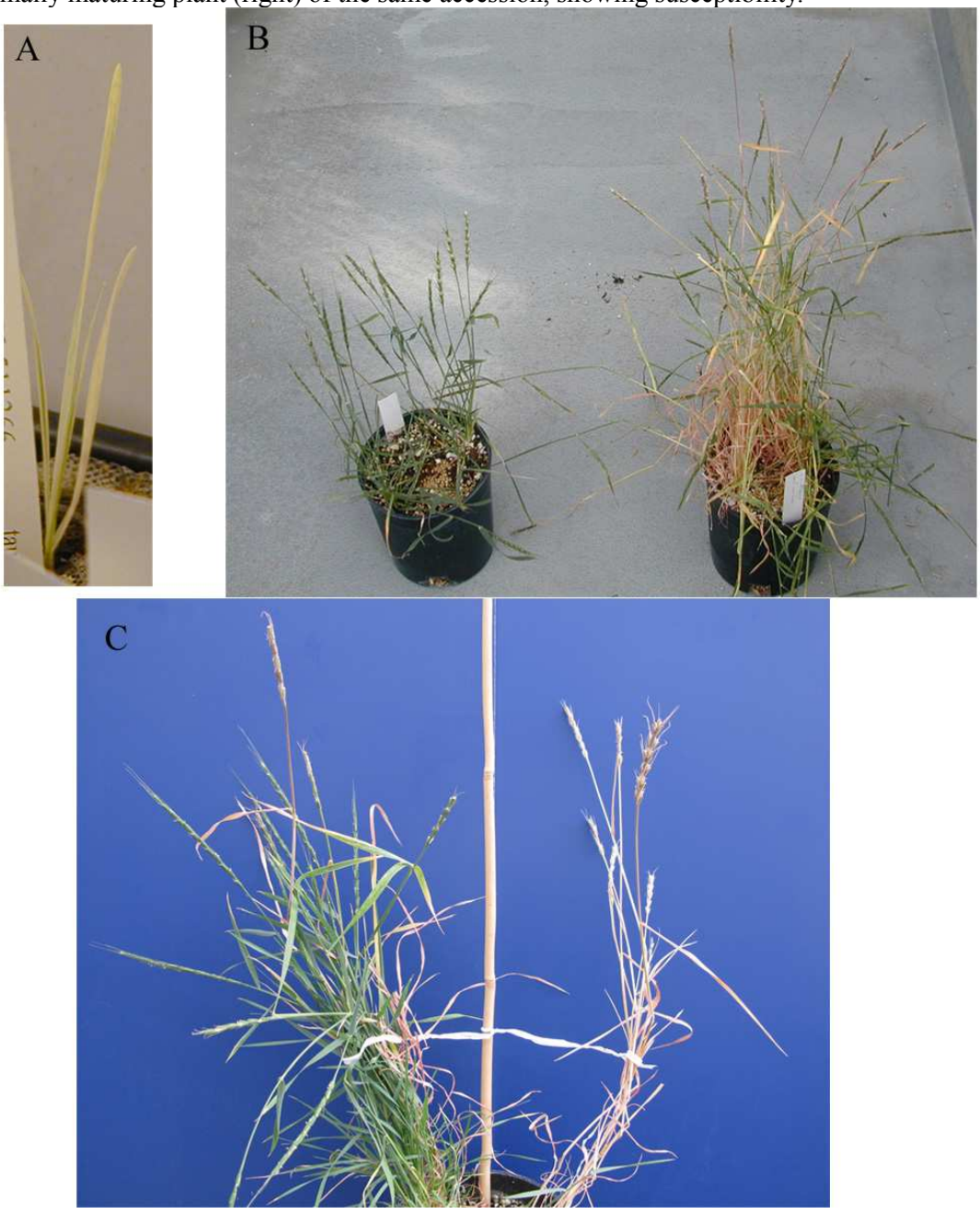
During plant development and vernalization, some accessions showed partial juvenile albinism or xanthism (Figure 4A), which in all cases disappeared when the seedlings were transplanted to the greenhouse. Dwarfing was observed in some infected plants when compared to uninfected plants of the same accession, which had been grown under the same conditions for a seed increase (Figure 4B). It is possible that aspects of the dwarfed plant development and albinism could also have resulted from sustained growth at low temperatures for periods of more than 12 weeks, compared to the 8 week vernalization normally employed for wheat.

Eighty-four accessions in Experiment 1, as well as one in Experiment 2, exhibited heavy tillering and late flowering, consistent with insufficient vernalization. These plants were maintained until sufficient heads were produced for observation. To ameliorate this, the hours of light while under vernalization conditions were increased in Experiments 2-4. Several accessions did not germinate in Experiments 1 and 2, resulting in incomplete retest data, so a stratification protocol was implemented to break dormancy in Experiments 3 and 4. Data on the number of late-maturing plants were recorded and are included in Appendix 1; late maturing data for Experiments 3 and 4 indicate plants which germinated but where no data was taken due to delayed development and infection by pathogens unrelated to this experiment. In some accessions, plants that appeared to be delayed in their development, but still appeared to have vernalized, expressed a resistant phenotype while other, normally maturing plants in the same accession were clearly susceptible (Figure 4C).

Figure 2. Splaying of florets. A – An infected head (left) of accession PI 574465, compared to an healthy head of the same accession (right). B – Floret from a healthy head of PI 486275.
C – Floret from an infected head of PI 486275.

Figure 3. Burst sori. Dark masses are released teliospores. A – Spike of PI 554321 showing burst sori in several florets. B – Detail of floret with burst sorus in PI 554321. C – Detail of floret with burst sorus in PI 511379. D – Top view of burst sori in PI 511382.

Figure 4. Effect of common bunt infection on plant morphology. A - Partial juvenile albinism on a seedling of PI 511366 growing in the vernalization chamber. B - Dwarfing of an infected plant of accession PI 574467 (left), compared to a uninoculated plant of the same accession (right). C - Developmentally delayed plant of PI 486275 (left), showing a lack of infection, compared to a normally maturing plant (right) of the same accession, showing susceptibility.

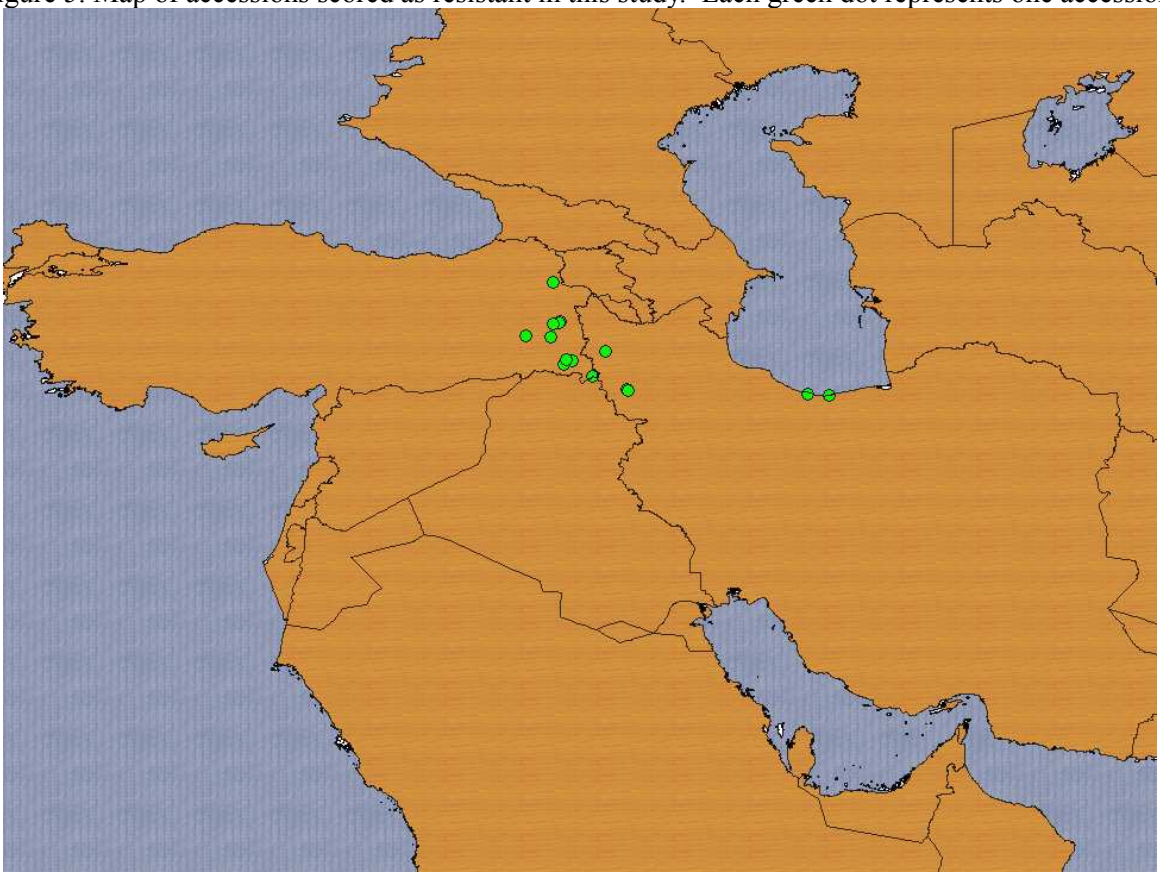


Discussion

The resistance observed in 18 accessions throughout all four experiments indicate that levels of resistance to common bunt usable for commercial purposes may exist in *Aeg. tauschii*. This is the first published account of screening multiple accessions of *Aeg. tauschii* with known races of common bunt. As the races used to screen these accessions are virulent against *Bt1-Bt10*, the resistance found here is mostly likely the result of either one of the other *Bt* genes, *Bt11-Bt14*, or a new source of resistance. Further studies could be conducted using races T13 (virulent against *Bt1*, *Bt2*, *Bt3*, and *Bt13*) and L7 (virulent against *Bt1*, *Bt2*, *Bt7*, and *Bt14*) to narrow down the source of resistance, although this would still leave the possibility of resistance from *Bt11* or *Bt12*, which have not yet been reported as being overcome by any races. While races virulent to *Bt15* were not used in this study, it is unlikely to be the source of resistance, as the cultivar it was described on, 'Carleton', is a durum (*T. durum* Desf., AABB) and so lacks the D genome of *Aeg. tauschii.* It may be possible to combine the resistance found in the accessions identified in this study with the *Bt15* from Carleton by crossing them to produce a synthetic hexaploid.

Some correlations between collection location and resistance can be surmised. All of the accessions found to be resistant in this study are from regions near the border between Turkey and Iran (Figure 5). These locations may suggest future areas of interest for collecting common bunt-resistant germplasm and could represent different lineages. It would also be interesting to get local accounts of how large of a role common bunt plays as a pathogen in these regions and see whether this could have led to natural selection for these genotypes.

Figure 5. Map of accessions scored as resistant in this study. Each green dot represents one accession.



The presence of some bunted florets on the otherwise uninfected resistant check raises questions as to the true level of its resistance in this source, as well as to which race was able to overcome it. Limited, partial infection of heads is common in resistant genotypes (Wilcoxson & Saari, 1996). Metzger (personal communication) indicated that quantitative resistance to common bunt can be found in some tetraploid wheat AABB genome species, reducing the extent of infection but not totally eliminating it. The resistant check is descended from a cross of *T. urartu* (AA) and *T. aestivum*, suggestive that the A genome might be contributing some of the quantitative resistance. The total resistance exhibited by this germplasm in most experiments would also suggest the presence of one of the *Bt* genes. Metzger cautioned that quantitative

resistance is not sufficient for breeding resistant cultivars, because partial infection can result in seed that is unmarketable. Unfortunately, the exact pedigree of the resistant check is unknown. Eight accessions of *T. aestivum* hybrids with *T. urartu,* created by Metzger and listed as having strong common bunt resistance, are maintained in the USDA NPGS. A comparison between the morphological or molecular traits of these eight accessions and the resistant check used in this study, as well as evaluation of their resistance to individual races of common bunt, may clarify their ancestry and relatedness.

Metzger's observation of common bunt resistance in KUSE 2144 (CIae 23), as reported in the narratives for PI 542503 and PI 542507, disagree with the data for this accession in the current study. Since the races used to make this determination are not mentioned in his observations, it is likely that KUSE 2144 contains one of the *Bt* genes against which the races in this study are virulent (e.g. *Bt1-10*). Similarly, other accessions marked as susceptible in this study may exhibit resistance to common bunt under field conditions, as *in situ* race combinations likely do not cover the range of virulence employed by the combination of races in this study. Tests for alleleism to known *Bt* genes in both resistant and susceptible accessions would provide further information for breeders.

Effects of development, unrelated to resistance genes, may also have played a role in the 'resistance' found in some of the accessions. As mentioned earlier, seed dormancy was an issue in the earlier experiments, with some accessions failing to germinate and others showing a delay of several weeks in germination. While efforts were made to only record data from plants that had been actively growing during the full vernalization period, and to discard plants that had germinated right before transplanting as they would not have had sufficient exposure to the pathogen, it is possible that late germination followed by rapid plant growth could fascimilate the

appearance of an established plant. This could account for the appearance of so many late-flowering accessions in Experiment 1. The increased hours of light while under vernalization for Experiments 2-4 contributed to improved vernalization and reduced the number of late heading plants. The presence of resistance in some later-developing plants, showing some delay but not of the type from insufficient vernalization, may suggest that late development could be advantageous in escaping common bunt infection. This might be useful in breeding new cultivars and when designing planting schemes.

The decision was made to use seeds from the USDA NPGS collection as they are made freely available to researchers worldwide, and so any accessions that carry resistance can be easily obtained by interested breeders. Several details can be inferred about the accessions' background. First, NPGS accessions, unless otherwise specified, represent collections of a species from one location, which may contain a range of genotypes. Accessions that were heterogeneous for resistance would have been scored as susceptible according to this study's screening criteria. Some of this was ameliorated for Experiments 2-4 which, due to insufficient seed stock, were preceded by a single-plant increase of each accession and so included a mix of seed from the NPGS and the increase.

The number of accessions scored as susceptible in this study show that common bunt is capable of infecting and growing on *Aeg. tauschii.* No accounts have been published of common bunt infection in wild stands of *Aeg. tauschii*, or jointed goatgrass, *Aeg. cylindrica* (CCDD). Susceptibility in jointed goatgrass would be of particular concern, as it is a noxious weed common in wheat fields. If it is capable of acting as a host for common bunt, it could be a source of inoculum to infect the wheat it infests, in addition to the other challenges it poses as a weed. In this study, seed of *Aeg. tauschii* was removed from the glumes prior to inoculation to

improve the chances of spores being able to contact the coleoptile prior to emergence from the soil. In the seed increase, however, it was observed that heads of *Aeg. tauschii* shatter and new plants germinate directly from the glumes, on the soil surface. In the field, *Aeg. cylindrica* and *Aeg. tauschii* would germinate from seeds with intact glumes which may act as a barrier to infection. Also, the presence of burst sori on some accessions, described previously, may be a clue into why infection of this species had not been documented. This phenomenon might aide in dissemination, but would appear to ultimately be non-adaptive for the fungus, as it eliminates the protection conferred through the outer wall of the sorus and exposes the spores to possibly adverse environmental conditions. Infection of *Aeg. tauschii* might naturally occur, but would be selected against, as the races infecting it would not be able to survive adverse conditions as long as those in intact sori on *T. aestivum*.

Summary

Common bunt, caused by *T. caries* and *T. laevis*, is a reemerging pathogen of wheat. Genetic resistance provides control where seeds treatments are not possible. Eighteen accessions of *Aeg. tauschii*, a progenitor of cultivated wheat, show resistance to a range of virulence. Eight of the resistant accessions have a less than 5% probability of being susceptible escapes. All of the resistant accessions were collected in either Iran or Turkey. This resistance may correspond to existing resistance genes *Bt11-14*, but further studies must be undertaken to test for alleleism. Infected accessions showed distinct changes in morphological characteristics that may be useful in future screening studies. This is the first documented screening of multiple *Aeg. tauschii* accessions using known races of common bunt.

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Conclusions & Future Research

Conclusions from Current Research

Eighteen accessions of *Aeg. tauschii* with putative resistance to 10 races of common bunt were identified in the present study. These accessions were all either collected in Iran or Turkey, indicating a possible center of origin for the resistance. Eight of the putative resistant accessions had a probability lower than 5% of being a susceptible 'escape'. Susceptibility in 94 accessions demonstrates that *Aeg. tauschii* is capable of acting as a host for the fungus, and causes changes in morphological characteristics. The low germination rates for many accessions, and failure of three accessions to germinate at all, suggests that dormancy and strong vernalization requirements exist in *Aeg. tauschii.* Overall, data on common bunt resistance was collected on 114 of the 117 accessions in this study.

Use of Aeg. tauschii in Wheat Improvement & Methods of Integration

The work presented in this thesis suggests that *Aeg. tauschii* is a possible source of resistance to common bunt. Work by other researchers (Chhuneja et al., 2008; Cox et al., 1990; Dreisigacker et al., 2008; Hall et al., 2009; Miranda et al., 2007; Multani et al., 1988; Villareal et al., 1994; Yildrim et al., 1995) demonstrates that lines developed from this species may also possess improved tolerance or resistance to a range of biotic and abiotic stresses. Breeding programs in areas affected by this disease may benefit from integrating *Aeg. tauschii* into their pool of germplasm, as an alternative to chemical seed treatments and for farmers interested in

engaging in organic or low input cropping systems.

To utilize the resistance in *Aeg. tauschii*, it must be moved into the agronomic species *T. aestivum* (AABBDD), for which *Aeg. tauschii* (DD) is the D genome donor. The *Bt15* resistance gene is already known to exist in 'Carleton' durum (AABB) and could be combined in a synthetic hexaploid with the resistance found in *Aeg. tauschii* (DD). The development of synthetic hexaploids has been the preferred method at CIMMYT to use the diversity present in the *Aeg. tauschii* (Mujeeb-Kazi & Hettel, 1995; Kishii, personal communication). As mentioned previously, this method was created by Sears & McFadden in 1946 as part of their attempt to recreate *T. spelta.* Other groups, including those at CIMMYT, have used the technique developed by Kerber (1964), and expanded upon by Kaltsikes et al. (1969) and Yang et al. (1999), to extract the AABB component of popular *T. aestivum* cultivars for use in synthetic hexaploid production. This provides agronomically adapted AABB genome donors, minimizing the load of unwanted genetic variability, but still requiring some backcrossing into cultivated wheat. The alternative, discussed at length by Cox et al. (1995), is crossing *Aeg. tauschii* directly with *T. aestivum* and then selfing to reconstitute a hexaploid state and allow recombination between the two D genomes. While Cox's method would appear to have some advantages, especially in that it has a comparatively small amount of genetic variation segregating, there are also potential downsides in forms such as possible meiotic irregularities, leading to aneuploidy, and the fact the hybrids are not immediately true breeding, although they can become true breeding through backcrossing. Also, the same genetic diversity that may make a synthetic hexaploid more difficult to develop into an agronomically fit wheat cultivar can provide some traits of possible economic importance, such as improved pathogen interactions, growth habits, or end use quality, that might not be found in direct cross progeny.

Existing Germplasm Resources & Genetic Characterization

Crossing within a wild progenitor species, such as *Aeg. tauschii*, provides a method to consolidate different agronomically valuable traits into a smaller number of lines, allowing easier integration into commercial cultivars and contributing less deleterious traits to be screened against. Existing literature, such as Chhuneja et al. (2008) and screening studies like this one, point to individual accessions that show promise for improving specific problems in wheat.

Use of known molecular markers allows detection of a wide range of important agronomic traits in many plants. This is more efficient economically, spatially, and temporally than engaging in multiple phenotypic screening protocols or monitoring and recording detailed observations about the plant. Germplasm holdings of *Aeg. tauschii* could be screened to identify accessions bearing good combinations of important genes, allowing breeders to make informed decisions when integrating the germplasm into their breeding programs. Relationships between the accessions may also be discovered, permitting collection curators to better group their stock, and giving researchers information on which accessions may be related to an accession they are already using or where sources of resistance appear to originate.

Genotype screening is currently being conducted on the accessions used in this study, to locate resistance to a range of other pathogens and identify important agronomic and quality traits, such as vernalization or baking protein genes. This work will use primers employed by the Western Regional Small Grains Genotyping Lab, such as those for leaf rust resistance or grain hardness, in combination with recently published primers for *Soilborne Wheat Mosaic Virus* (Hall et al., 2009) and powdery mildew (Miranda et al., 2007). The marker for *Bt10* (Laroche et al., 2000) will also be tested, as this gene is found on the short arm of chromosome 6 of the D

genome (Menzies et al., 2006). The results will be run out on a capillary system. All data derived from this screening will be shared with the curator for the *Aeg. tauschii* collection so that it may be posted as observation or narrative data on GRIN.

Large collections exist for many crop species and their relatives, which could be subjected to marker investigation. Examples of groups that hold large collections of *Aeg. tauschii* include the Wheat Genomic and Genetic Resources Center (WGGRC) with 555 accessions, the USDA NPGS with 143, the European Cooperative Programme for Plant Genetic Resources (ECPGR) with 1154 accessions, and the System-wide Information Network for Genetic Resources (SINGER) with 449 accessions. A listing of more groups can be found at http://www.k-state.edu/wgrc/Germplasm/links.html .

One factor that must be considered when working with germplasm collections is that the accessions often represent genetically heterogeneous populations collected in one location, and so may be segregating for important characteristics. This can be ameliorated by selecting single plants out of each accession and increasing them, producing lines that should be more homogenous. If these lines are found to be useful, especially for a range of attributes, they can then each be returned to the germplasm repository as individual accessions. Different accessions of the same species may also be widely diverse. This is evidenced in not only agronomic aspects, such as the disease resistance investigated in this study, but also in morphological and genetic characteristics. Naghavi et al. (2009) observed high levels of genetic diversity in Iranian *Aeg. tauschii* accessions using microsatellite markers, with large amounts of variation occurring between different collection locations, and also reported that this variation was much greater than that present in the D genome of *T. aestivum.*

To evaluate the variation of the accessions used in this study, and to establish

relationships between them, Target Region Amplified Polymorphism (TRAP) markers are being run (Appendix 2). This work is using the telomeric primers created and discussed in Hu (2006), and a modification of its methodology. TRAP markers were chosen due to the fact that they can be conducted rapidly, easily, and with very little expense. Also, previous work (Liu et al., 2005) has shown that TRAPs demonstrate polymorphism in wheat, but with less resolution of the D genome due to homogeneity within cultivated wheat's D genome; although some improvement in resolution was found when chromosomes in a *T. durum* (AABB) line were substituted with D genome chromosomes (Li et al., 2006). Furthermore, Faris and Friesen (2005) were able to use the polymorphism displayed by TRAP markers to map QTLs associated with resistance to tan spot to specific chromosome arms in wheat. This suggests that TRAP markers may have some utility for correlating phenotypic data to genetic information associated with resistance. By screening the more heterogeneous D genome of *Aeg. tauschii* alongside wheat lines nullisomic or ditelosomic for different D genome chromosomes, clarification of D genome polymorphism will be obtained that can be pinpointed to specific chromosomal regions. Polymorphism that is unique to those accessions which showed resistance in the common bunt screening experiment may also provide TRAP markers to rapidly assess future germplasm collections for resistance to common bunt. This could provide information on why some accessions from the same location show different reactions to the common bunt pathogen, and whether certain geographic areas should be subjected to further collection efforts.

Other Possible Sources of Genetic Resistance

Existing data, written as narratives for accessions in the NPGS-GRIN, suggests that

resistance to common bunt may exist in other wheat species. As with the narratives for *Aeg. tauschii*, little of this data contains references or specifics on the genes or races involved, but the results of this study indicate there may be some plausibility to these claims. One of the most promising may be *T. urartu* (AA), which was the parent of the resistant check used in this study. Hybrids between *T. urartu* and *T. aestivum* are already held by the NPGS and documented to possess excellent resistance. Similarly, *T. monococcum* L. (AA) is documented to express resistance. As both of these species are A genome donors, it may also make sense to screen *T. boeticum* Boiss. (AA), which also has one resistant hybrid documented in GRIN. Although no GRIN or published data on common bunt resistance exists for them, *T. turanicum* Jakubz. (AABB) and *T. polonicum* L. (AABB) may also be worth screening. They have not been utilized in CIMMYT's wild relative introgression program (M Kishii, personal communication), and only recently (Zhang et al., 2008; Kang et al., 2009) were used by other groups in the production of synthetic hexaploids. These may also provide other novel traits of interest to breeders, due to their phenotypic divergence from other AB genome wheats.

The identification of common bunt resistance in lines derived from *Aeg. cylindrica* (CCDD) (Babayants et al., 2000; Galaev et al., 2006) demonstrates that it may also be a source of common bunt resistance. However, while *Aeg. cylindrica* possesses a D genome homologous to that in cultivated wheat, it also contains a copy of the C genome, which is known to contain gametocidal genes (Endo, 1979). These may complicate introgression of desired genes in the breeding process.

Further Work on Common Bunt & Resistance

Further worth on elucidating the form of resistance could be conducted on the accessions used in this study. As discussed previously, none of the races used in the screening were virulent against *Bt13* or *Bt14*, and the re-screening of the resistant accessions with races T13 (virulent against *Bt1*, *Bt2*, *Bt3*, and *Bt13*) and L7 (virulent against *Bt1*, *Bt2*, *Bt7*, and *Bt14*) could help determine whether these accessions possess those genes, or are expressing a novel form of resistance. Additionally, crosses between the resistant accessions, followed by screening of the segregating F_2 generation, would show whether the same gene or gene combinations are responsible for resistance in all of the accessions. If multiple forms of resistance exist, work could be done to pyramid them into one accession for use in future breeding; this would work especially well if the TRAP markers described above or another type of marker could aid in detecting the presence of the resistance factor and permit for it to be screened molecularly.

Those accessions which were determined to be susceptible in this study could also be studied further, using one race of common bunt at a time to determine if *Bt1-Bt10*, overcome by the races used in the screening, are present. While resistance that has not been overcome is preferable, the other *Bt* genes are still useful in areas where the races virulent against them have not been introduced, as is the case with the continued use of *Bt10* in Canada (Laroche et al., 2000). Also, the introduction of these races into a host not normally infected in nature may have resulted in unusual events such as virulence shift or hybridization, so the races infecting the plants of *Aeg. tauschii* should be isolated and tested against a set of *Bt* gene differentials. Similarly, it would be good to clarify which race caused the partial infection of the 'resistant' *T. urartu*-derived line in Experiment 1, and determine whether it has undergone any changes. It is

possible that infection of this line may not occur again, as evidenced by the lack of infection in Experiments 2-4, and so the infected head has been kept for future analysis by interested parties.

The presence of resistance to common bunt in *Aeg. tauschii* presents possibilities for future research. Specifics about the resistance and information on the resistant accessions must be elaborated through further experimentation and molecular screening, to create an effective pre-breeding strategy. This will allow the germplasm to be better utilized in research, as well as in the creation of new commercial cultivars, possessing resistance to common bunt and a range of new traits introgressed from wild relatives.

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Appendix 1

Detailed data from the experiments

Complete data is reported for Experiments 1-4. 'Variety' and 'Country' information is from accession information in NPGS-GRIN (http://www.ars-grin.gov/npgs/acc/acc_queries.html); further data is available for each accession when accessed using the variety name. The '#' column shows the number assigned to that accession during the experiment, and referenced in the TRAP data (Appendix 2) and elsewhere.

Late maturing (LM) data for Experiments 1 and 2 indicates plants which headed late, but whose data is included in total number of plants. The late maturing data for Experiments 3 and 4 indicates plants which germinated but where no data was taken, and is not included in the total number of plants for these experiments.

Table 3. Complete data on accession origin from NPGS-GRIN and performance in screening. A '-' indicates no germination, while '--' indicates that it was not re-planted due to susceptibility. LM stands for 'Late Maturing'; these plants are counted in the totals for Experiments 1 and 2, but in Experiments 3 and 4 did not have data collected due to constraints from time and external pathogens.

#	Variety	TAXON	COUNTRY	Exp 1 - # Plants	Exp 1 - # Sus	Exp 1 - LM	Exp 2 - # Plants	Exp 2 - # Sus	Exp 2 - LM	Exp 3 - # Plants	Exp 3 - # Sus	Exp 3 - LM	Exp 4 - # Plants	Exp 4 - # Sus	Exp 4 - LM	Total # Plants	Total # Sus
1	CIae 1	Aegilops tauschii	Pakistan	2	1	-	--	--	-	--	--	-	--	--	-	2	1
2	CIae 2	Aegilops tauschii	Pakistan	4	1	-	--	--	-	--	--	-	--	--	-	4	1
3	CIae 3	Aegilops tauschii	Afghanistan	3	2	-	--	--	-	--	--	-	--	--	-	3	2
4	CIae 4	Aegilops tauschii	Afghanistan	5	2	-	--	--	-	--	--	-	--	--	-	5	2
5	CIae 5	Aegilops tauschii	Afghanistan	2	2	-	--	--	-	--	--	-	--	--	-	2	2
6	CIae 6	Aegilops tauschii	Afghanistan	3	3	-	--	--	-	--	--	-	--	--	-	3	3
7	CIae 8	Aegilops tauschii	Iran	3	3	-	--	--	-	--	--	-	--	--	-	3	3
8	CIae 9	Aegilops tauschii	Iran	4	2	-	--	--	-	--	--	-	--	--	-	4	2
9	CIae 10	Aegilops tauschii	Iran	5	0	-	4	4	-	--	--	-	--	--	-	9	4
10	CIae 11	Aegilops tauschii	Iran	1	0	-	1	1	-	--	--	-	--	--	-	2	1
11	CIae 12	Aegilops tauschii	Iran	4	2	-	--	--	-	--	--	-	--	--	-	4	2
12	CIae 13	Aegilops tauschii	Iran	5	5	1	--	--	-	--	--	-	--	--	-	5	5

#	Variety	TAXON	COUNTRY	Exp 1 - # Plants	Exp 1 - # Sus	Exp 1 - LM	Exp 2 - # Plants	Exp 2 - # Sus	Exp 2 - LM	Exp 3 - # Plants	Exp 3 - # Sus	Exp 3 - LM	Exp 4 - # Plants	Exp 4 - # Sus	Exp 4 - LM	Total # Plants	Total # Sus
13	CIae 14	Aegilops tauschii	Iran	-	-	-	3	2	-	--	--	-	--	--	-	3	2
14	CIae 15	Aegilops tauschii	Iran	3	3	1	--	--	-	--	--	-	--	--	-	3	3
15	CIae 16	Aegilops tauschii	Iran	2	2	-	--	--	-	--	--	-	--	--	-	2	2
16	CIae 17	Aegilops tauschii	Iran	3	1	-	--	--	-	--	--	-	--	--	-	3	1
17	CIae 18	Aegilops tauschii	Iran	1	0	-	-	-	-	4	3	-	3	1	-	8	4
18	CIae 19	Aegilops tauschii	Iran	2	0	-	-	-	-	-	-	-	-	-	-	2	0
19	CIae 20	Aegilops tauschii	Iran	-	-	-	-	-	-	-	-	-	-	-	-	-	-
20	CIae 21	Aegilops tauschii	Iran	5	0	-	-	-	-	3	2	-	3	2	-	11	4
21	CIae 22	Aegilops tauschii	Iran	-	-	-	-	-	-	3	3	1	4	4	-	7	7
22	CIae 23	Aegilops tauschii	Iran	1	1	-	--	--	-	--	--	-	--	--	-	1	1
23	CIae 24	Aegilops tauschii	Iran	5	3	-	--	--	-	--	--	-	--	--	-	5	3
24	CIae 25	Aegilops tauschii	Iran	5	5	1	--	--	-	--	--	-	--	--	-	5	5
25	CIae 26	Aegilops tauschii	Iran	5	5	-	--	--	-	--	--	-	--	--	-	5	5
26	CIae 27	Aegilops tauschii	Iran	1	0	-	-	-	-	4	0	-	4	0	-	9	0
27	CIae 28	Aegilops tauschii	Iran	-	-	-	1	0	-	-	-	-			3	1	0
28	CIae 30	Aegilops tauschii	Unknown	2	1	2	--	--	-	--	--	-	--	--	-	2	1
29	CIae 50	Aegilops tauschii	Unknown	-	-	-	-	-	-	1	0	-	3	1	-	4	1

#	Variety	TAXON	COUNTRY	Exp 1 - # Plants	Exp 1 - # Sus	Exp 1 - LM	Exp 2 - # Plants	Exp 2 - # Sus	Exp 2 - LM	Exp 3 - # Plants	Exp 3 - # Sus	Exp 3 - LM	Exp 4 - # Plants	Exp 4 - # Sus	Exp 4 - LM	Total # Plants	Total # Sus
30	CIae 51	Aegilops tauschii	Unknown	-	-	-	-	-	-	-	-	-	4	2	-	4	2
31	CIae 68	Aegilops tauschii	Turkey	-	-	-	-	-	-	-	-	-	1	1	-	1	1
32	CIae 71	Aegilops tauschii	Unknown	-	-	-	-	-	-	3	2	-	4	4	-	7	6
33	CIae 72	Aegilops tauschii	Unknown	5	4	-	--	--	-	--	--	-	--	--	-	5	4
34	PI 210987	Aegilops tauschii	Afghanistan	2	2	-	--	--	-	--	--	-	--	--	-	2	2
35	PI 220326	Aegilops tauschii	Afghanistan	1	1	-	--	--	-	--	--	-	--	--	-	1	1
36	PI 220331	Aegilops tauschii	Afghanistan	6	3	-	--	--	-	--	--	-	--	--	-	6	3
37	PI 220641	Aegilops tauschii	Afghanistan	4	4	-	--	--	-	--	--	-	--	--	-	4	4
38	PI 220642	Aegilops tauschii	Afghanistan	3	3	-	--	--	-	--	--	-	--	--	-	3	3
39	PI 268210	Aegilops tauschii	Iran	3	3	-	--	--	-	--	--	-	--	--	-	3	3
40	PI 276975	Aegilops tauschii	Turkistan	5	3	-	--	--	-	--	--	-	--	--	-	5	3
41	PI 276980	Aegilops tauschii	Former Soviet Union	1	1	1	--	--	-	--	--	-	--	--	-	1	1
42	PI 276985	Aegilops tauschii	Iran	3	3	-	--	--	-	--	--	-	--	--	-	3	3
43	PI 317392	Aegilops tauschii	Afghanistan	3	3	-	--	--	-	--	--	-	--	--	-	3	3
44	PI 317394	Aegilops tauschii	Afghanistan	2	1	-	--	--	-	--	--	-	--	--	-	2	1
45	PI 330489	Aegilops tauschii	Unknown	5	5	-	--	--	-	--	--	-	--	--	-	5	5
46	PI 349037	Aegilops tauschii	Azerbaijan	3	3	-	--	--	-	--	--	-	--	--	-	3	3

#	Variety	TAXON	COUNTRY	Exp 1 - # Plants	Exp 1 - # Sus	Exp 1 - LM	Exp 2 - # Plants	Exp 2 - # Sus	Exp 2 - LM	Exp 3 - # Plants	Exp 3 - # Sus	Exp 3 - LM	Exp 4 - # Plants	Exp 4 - # Sus	Exp 4 - LM	Total # Plants	Total # Sus
47	PI 369627	Aegilops tauschii	Unknown	3	2	-	--	--	-	--	--	-	--	--	-	3	2
48	PI 428563	Aegilops tauschii	Georgia	2	1	2	1	0	-	--	--	-	--	--	-	3	1
49	PI 428564	Aegilops tauschii	Azerbaijan	3	2	-	--	--	-	--	--	-	--	--	-	3	2
50	PI 431598	Aegilops tauschii	Turkmenistan	3	2	-	--	--	-	--	--	-	--	--	-	3	2
51	PI 431599	Aegilops tauschii	Azerbaijan	1	1	-	--	--	-	--	--	-	--	--	-	1	1
52	PI 431600	Aegilops tauschii	Russian Federation	5	5	-	--	--	-	--	--	-	--	--	-	5	5
53	PI 431601	Aegilops tauschii	Azerbaijan	4	3	-	--	--	-	--	--	-	--	--	-	4	3
54	PI 431602	Aegilops tauschii	Turkmenistan	6	4	-	--	--	-	--	--	-	--	--	-	6	4
55	PI 431603	Aegilops tauschii	Azerbaijan	5	5	-	--	--	-	--	--	-	--	--	-	5	5
56	PI 452130	Aegilops tauschii	China	5	4	-	--	--	-	--	--	-	--	--	-	5	4
57	PI 452131	Aegilops tauschii	China	5	4	-	--	--	-	--	--	-	--	--	-	5	4
58	PI 476874	Aegilops tauschii	Afghanistan	1	1	-	--	--	-	--	--	-	--	--	-	1	1
59	PI 486265	Aegilops tauschii	Turkey	1	1	-	--	--	-	--	--	-	--	--	-	1	1
60	PI 486266	Aegilops tauschii	Turkey	2	1	1	1	0	-	--	--	-	--	--	-	3	1
61	PI 486267	Aegilops tauschii	Turkey	1	0	1	2	0	-			5			4	3	0
62	PI 486271	Aegilops tauschii	Turkey	3	0	3	-	-	-	-	-	-			4	3	0
63	PI 486272	Aegilops tauschii	Turkey	1	0	1	-	-	-	2	0	-			1	3	0

#	Variety	TAXON	COUNTRY	Exp 1 - # Plants	Exp 1 - # Sus	Exp 1 - LM	Exp 2 - # Plants	Exp 2 - # Sus	Exp 2 - LM	Exp 3 - # Plants	Exp 3 - # Sus	Exp 3 - LM	Exp 4 - # Plants	Exp 4 - # Sus	Exp 4 - LM	Total # Plants	Total # Sus
64	PI 486274	Aegilops tauschii	Turkey	3	0	3	1	0	-	-	-	-	-	-	-	4	0
65	PI 486275	Aegilops tauschii	Turkey	2	2	2	--	--	-	--	--	-	--	--	-	2	2
66	PI 486276	Aegilops tauschii	Turkey	4	0	4	2	1	-	--	--	-	--	--	-	6	1
67	PI 486277	Aegilops tauschii	Turkey	-	-	-	2	0	-	1	1	-			2	3	1
68	PI 499262	Aegilops tauschii	China	4	3	4	1	1	-	--	--	-	--	--	-	5	4
69	PI 508263	Aegilops tauschii	China	5	5	-	--	--	-	--	--	-	--	--	-	5	5
70	PI 508264	Aegilops tauschii	China	6	5	-	--	--	-	--	--	-	--	--	-	6	5
71	PI 511363	Aegilops tauschii	Afghanistan	1	1	-	--	--	-	--	--	-	--	--	-	1	1
72	PI 511365	Aegilops tauschii	Pakistan	4	4	-	--	--	-	--	--	-	--	--	-	4	4
73	PI 511366	Aegilops tauschii	Afghanistan	6	3	-	--	--	-	--	--	-	--	--	-	6	3
74	PI 511367	Aegilops tauschii	Afghanistan	4	4	-	--	--	-	--	--	-	--	--	-	4	4
75	PI 511368	Aegilops tauschii	Iran	5	5	-	--	--	-	--	--	-	--	--	-	5	5
76	PI 511369	Aegilops tauschii	Iran	2	2	-	--	--	-	--	--	-	--	--	-	2	2
77	PI 511370	Aegilops tauschii	Iran	4	2	-	--	--	-	--	--	-	--	--	-	4	2
78	PI 511375	Aegilops tauschii	Unknown	4	3	-	--	--	-	--	--	-	--	--	-	4	3
79	PI 511378	Aegilops tauschii	Iran	4	3	-	--	--	-	--	--	-	--	--	-	4	3
80	PI 511379	Aegilops tauschii	Iran	3	0	-	6	6	-	--	--	-	--	--	-	9	6

#	Variety	TAXON	COUNTRY	Exp 1 - # Plants	Exp 1 - # Sus	Exp 1 - LM	Exp 2 - # Plants	Exp 2 - # Sus	Exp 2 - LM	Exp 3 - # Plants	Exp 3 - # Sus	Exp 3 - LM	Exp 4 - # Plants	Exp 4 - # Sus	Exp 4 - LM	Total # Plants	Total # Sus
81	PI 511380	Aegilops tauschii	Iran	3	0	-	-	-	-	-	-	-	4	0	-	7	0
82	PI 511381	Aegilops tauschii	Iran	2	0	-	-	-	-	4	4	-	4	3	-	10	7
83	PI 511382	Aegilops tauschii	Iran	5	4	-	--	--	-	--	--	-	--	--	-	5	4
84	PI 511383	Aegilops tauschii	Iran	5	0	-	1	1	-	--	--	-	--	--	-	6	1
85	PI 542277	Aegilops tauschii	Turkey	5	1	-	--	--	-	--	--	-	--	--	-	5	1
86	PI 542278	Aegilops tauschii	Turkey	-	-	-	1	1	-	--	--	-	--	--	-	1	1
87	PI 554310	Aegilops tauschii	Turkey	5	0	5	2	0	-	2	0	1			3	9	0
88	PI 554313	Aegilops tauschii	Turkey	3	0	1	2	0	-	3	0	-			4	8	0
89	PI 554315	Aegilops tauschii	Turkey	5	0	5	4	0	-	2	0	1			3	11	0
90	PI 554316	Aegilops tauschii	Turkey	-	-	-	-	-	-	-	-	-	-	-	-	0	-
91	PI 554318	Aegilops tauschii	Turkey	4	0	4	1	1	-	--	--	-	--	--	-	5	1
92	PI 554319	Aegilops tauschii	Turkey	3	1	3	1	0	-	-	-	-	-	-	-	4	1
93	PI 554320	Aegilops tauschii	Turkey	6	0	2	3	0	-	-	-	-	4	0	-	13	0
94	PI 554321	Aegilops tauschii	Turkey	3	0	-	2	2	-	--	--	-	--	--	-	5	2
95	PI 554322	Aegilops tauschii	Turkey	-	-	-	-	-	-	-	-	-			1	0	-
96	PI 554323	Aegilops tauschii	Turkey	2	0	2	-	-	-	-	-	-			1	2	0
97	PI 560534	Aegilops tauschii	Turkey	3	0	3	-	-	-	1	0	-			1	4	0

#	Variety	TAXON	COUNTRY	Exp 1 - # Plants	Exp 1 - # Sus	Exp 1 - LM	Exp 2 - # Plants	Exp 2 - # Sus	Exp 2 - LM	Exp 3 - # Plants	Exp 3 - # Sus	Exp 3 - LM	Exp 4 - # Plants	Exp 4 - # Sus	Exp 4 - LM	Total # Plants	Total # Sus
98	PI 560535	Aegilops tauschii	Turkey	4	0	2	2	0	-	-	-	-	3	0	1	9	0
99	PI 560536	Aegilops tauschii	Turkey	4	0	4	-	-	-	7	0	-			4	11	0
100	PI 560538	Aegilops tauschii	Turkey	5	0	1	-	-	-	2	0	-			4	7	0
101	PI 560754	Aegilops tauschii	Turkey	4	1	-	--	--	-	--	--	-	--	--	-	4	1
102	PI 560755	Aegilops tauschii	Turkey	4	1	-	--	--	-	--	--	-	--	--	-	4	1
103	PI 574465	Aegilops tauschii	Azerbaijan	5	5	-	--	--	-	--	--	-	--	--	-	5	5
104	PI 574467	Aegilops tauschii	Russian Federation	6	5	-	--	--	-	--	--	-	--	--	-	6	5
105	PI 574468	Aegilops tauschii	Armenia	3	3	-	--	--	-	--	--	-	--	--	-	3	3
106	PI 574469	Aegilops tauschii	India	4	3	-	--	--	-	--	--	-	--	--	-	4	3
107	PI 603220	Aegilops tauschii	Western Asia	3	1	-	--	--	-	--	--	-	--	--	-	3	1
108	PI 603221	Aegilops tauschii	Western Asia	4	2	-	--	--	-	--	--	-	--	--	-	4	2
109	PI 603223	Aegilops tauschii	Iran	5	2	-	--	--	-	--	--	-	--	--	-	5	2
110	PI 603224	Aegilops tauschii	Russian Federation	1	1	1	--	--	-	--	--	-	--	--	-	1	1
111	PI 603225	Aegilops tauschii	Turkmenistan	4	2	-	--	--	-	--	--	-	--	--	-	4	2
112	PI 603233	Aegilops tauschii	Azerbaijan	5	3	5	1	1	-	--	--	-	--	--	-	6	4
113	PI 603235	Aegilops tauschii	Azerbaijan	6	4	-	--	--	-	--	--	-	--	--	-	6	4
114	PI 603246	Aegilops tauschii	Portugal	5	3	6	4	3	-	--	--	-	--	--	-	9	6

#	Variety	TAXON	COUNTRY	Exp 1 - # Plants	Exp 1 - # Sus	Exp 1 - LM	Exp 2 - # Plants	Exp 2 - # Sus	Exp 2 - LM	Exp 3 - # Plants	Exp 3 - # Sus	Exp 3 - LM	Exp 4 - # Plants	Exp 4 - # Sus	Exp 4 - LM	Total # Plants	Total # Sus
115	PI 603249	Aegilops tauschii	Iran	4	4	1	--	--	-	--	--	-	--	--	-	4	4
116	PI 603252	Aegilops tauschii	Iran	4	0	-	4	0	-	4	0	-	1	0	-	13	0
117	PI 603255	Aegilops tauschii	Armenia	6	2	1	--	--	-	--	--	-	--	--	-	6	2
118	Eltan	Triticum aestivum	USA	6	2	6	2	0	-			4	-	-	-	8	2
119	Elgin	Triticum aestivum	USA	1	0	1	-	-	-	2	0	-	-	-	-	3	0
120	Red Bob	Triticum aestivum	Canada	4	2	2	-	-	-	1	1	-	-	-	-	5	3
121	Tetra Canthatch	Triticum aestivum	Canada	1	1	1	1	0	-	-	-	-	-	-	-	2	1
122	Metzger urartu	Triticum hybrid	USA	2	1	1	2	0	-	-	-	-	-	-	-	4	1

Appendix 2

Completed Gels for TRAP Markers

Telomeric fixed markers TeloTRG (Hu, 2006) and B14-61413 (Hu, personal communication) were used in combination with random primers Sa12 (700nm dye) and Ga5 (800nm dye). All gels have a 700bp ladder on each side. Accessions are in numerical order from left to right. In Figures 8-13, the 18 lanes on the right are DNA from #118, #122, the ditelosomic series for the D genome, 'Chinese Spring', and 'Wichita' (see order below). The DNA of the ditelosomics for 3DS and 5DS were unavailable, so nulli-telosomics, having the D genome chromosome replaced with telosomic extra copies of the corresponding B genome chromosomes, were used instead. These will allow inference about arms location when compared to the ditelosomics for the 3DL and 5DL chromosome arms.

Order of DNA checks

Left to Right in right-most rows for Figures 8-13: 118 (Eltan), 122 (Metzger *T. urartu* hybrid), 1DL ditelosomic, 1DS ditelosomic, 2DL ditelosomic, 2DS ditelosomic, 3DL ditelosomic, nullisomic 3D – telosomic 3B, 4DL ditelosomic, 4DS ditelosomic, 5DL ditelosomic, nullisomic 5D – telosomic 5B, 6DL ditelosomic, 6DS ditelosomic, 7DL ditelosomic, 7DS ditelosomic, Chinese Spring, Wichita

Figure 6. Accessions #1-64 using the TeloTRG fixed primer and Sa12 random primer with 700nm dye.

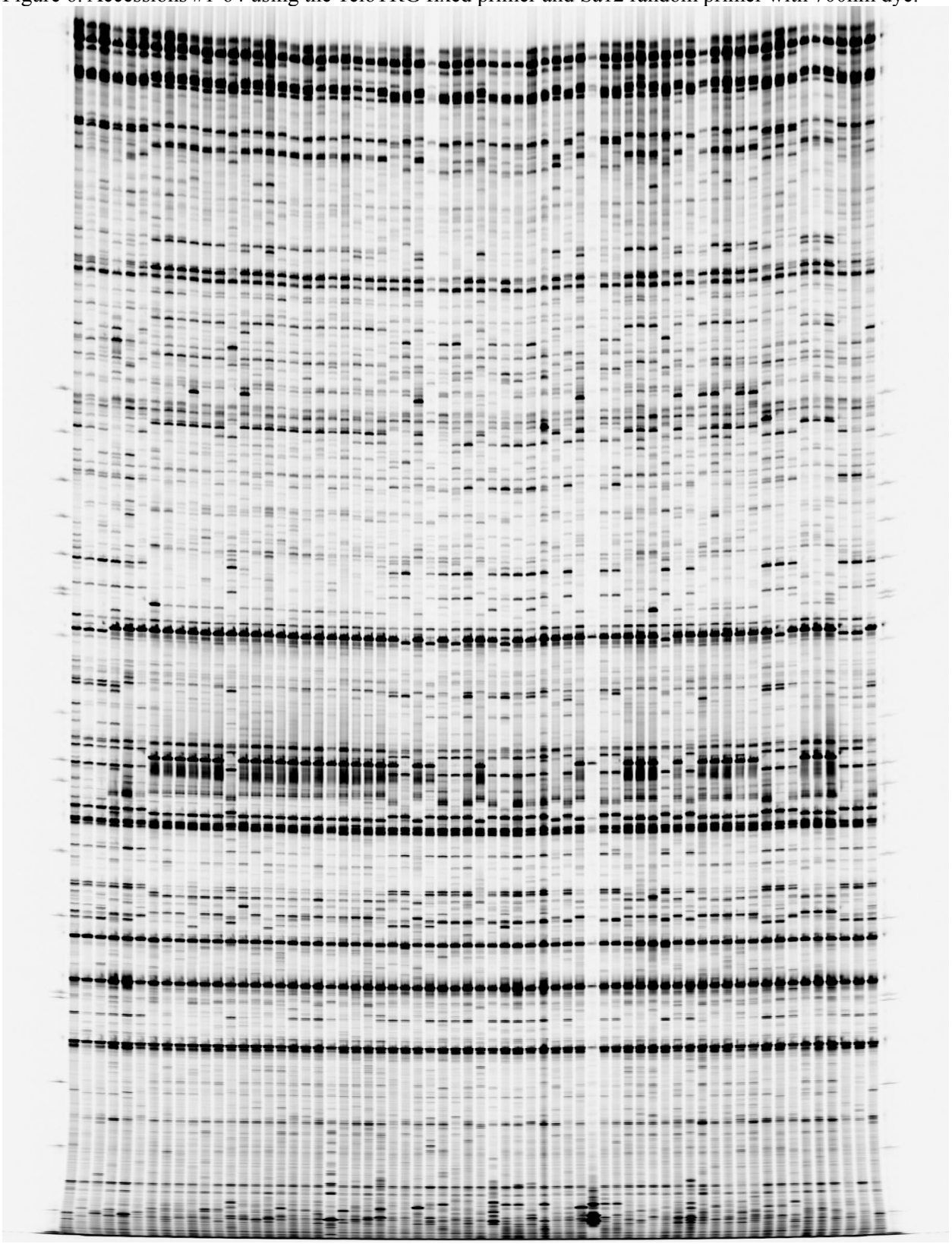

Figure 7. Accessions #65-122 using the TeloTRG fixed primer and Sa12 random primer with 700nm dye.

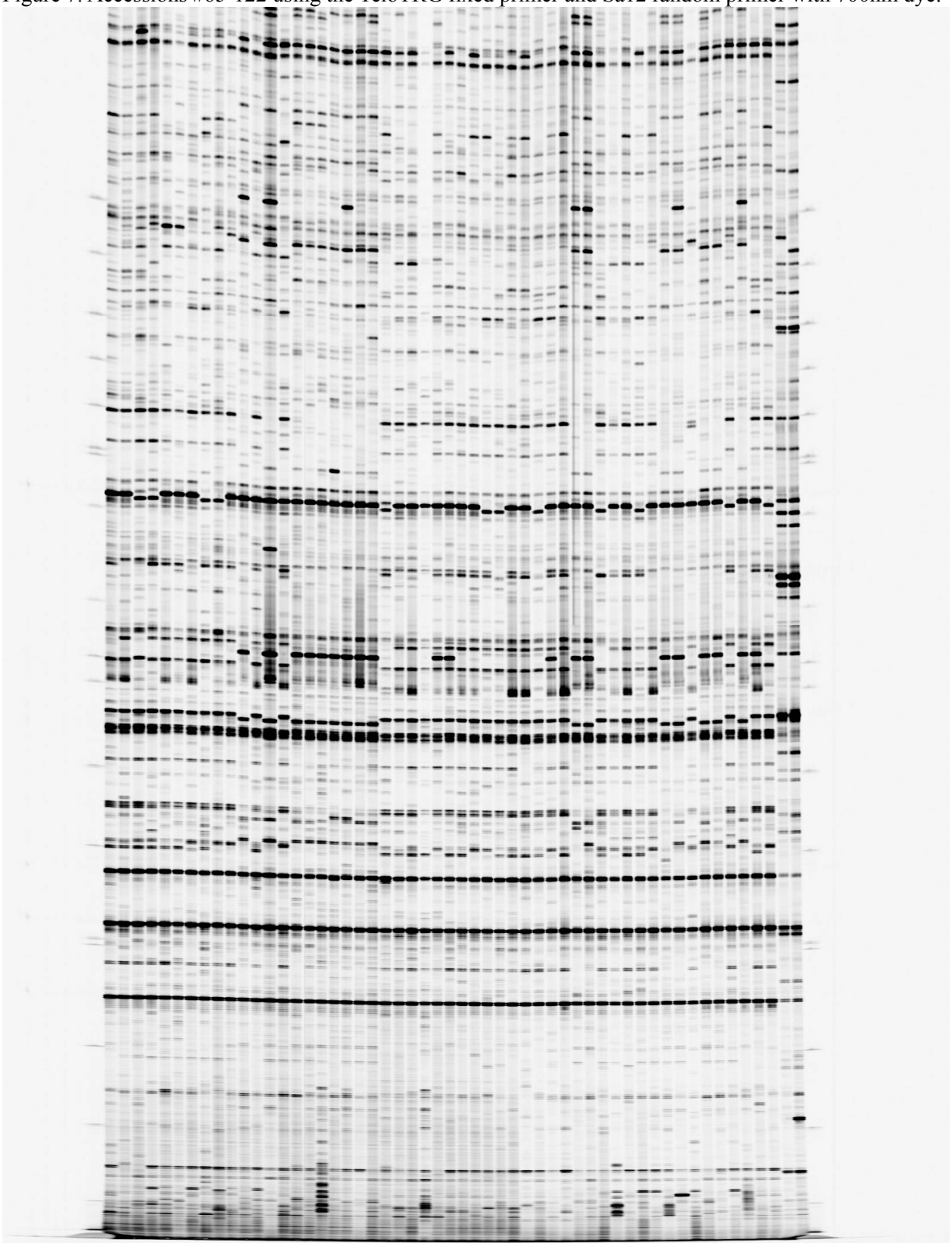

Figure 8. Accessions #1-64 using the TeloTRG fixed primer and Ga5 random primer with 800nm dye.

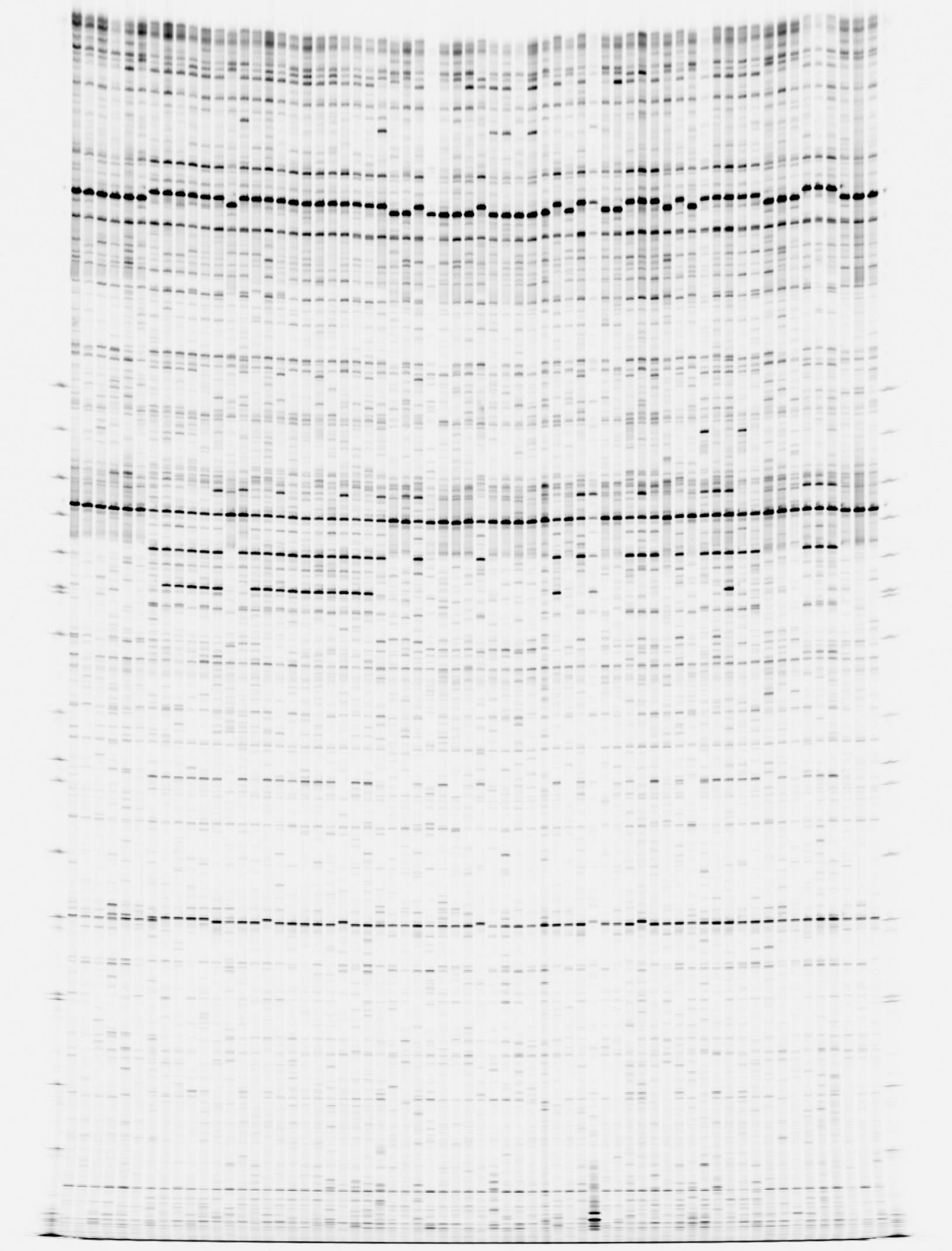

Figure 9. Accessions #65-122 using the TeloTRG fixed primer and Ga5 random primer with 800nm dye.

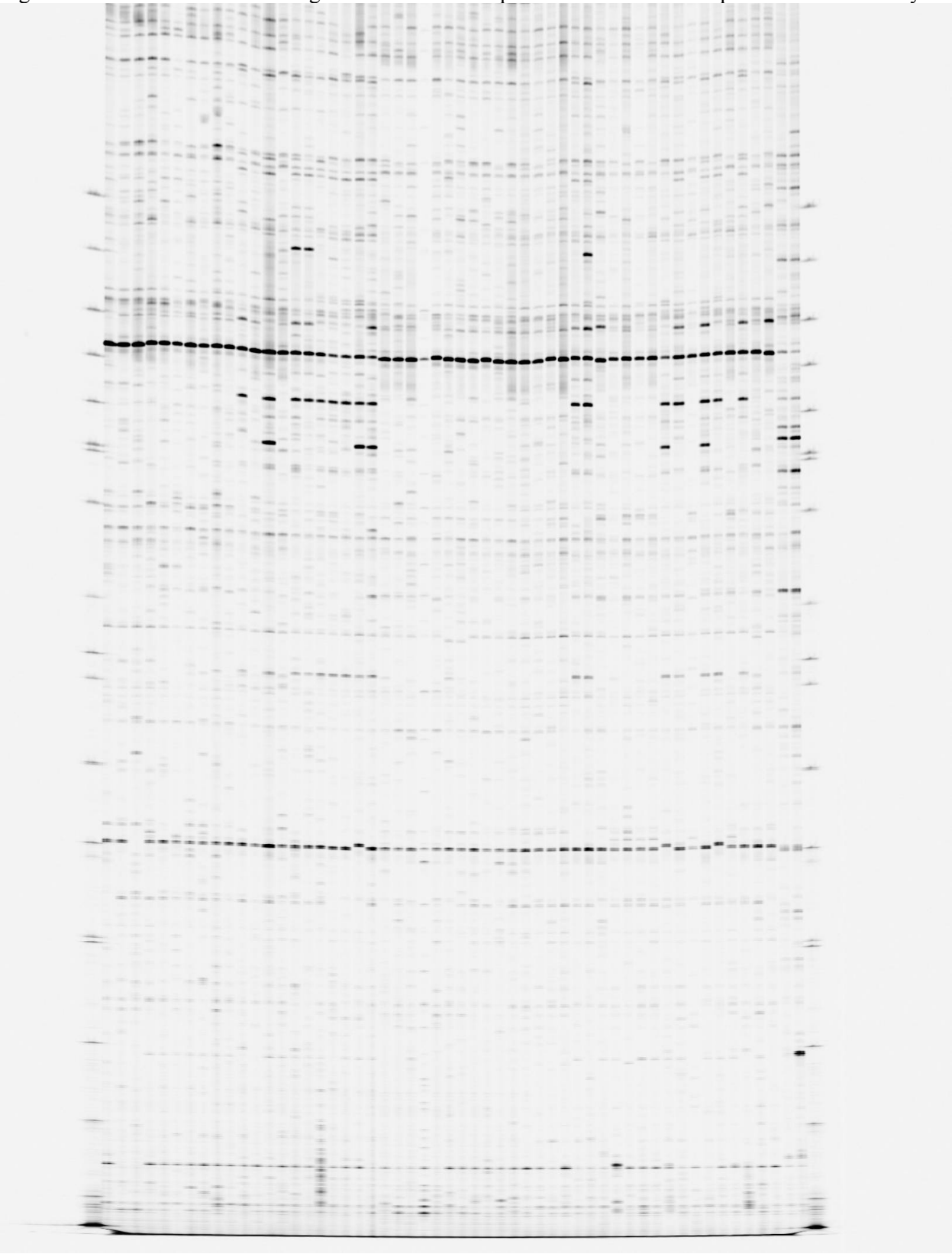

Figure 10. Accessions #1-46 using the B14-61413 fixed primer and Sa12 random primer with 700nm dye.

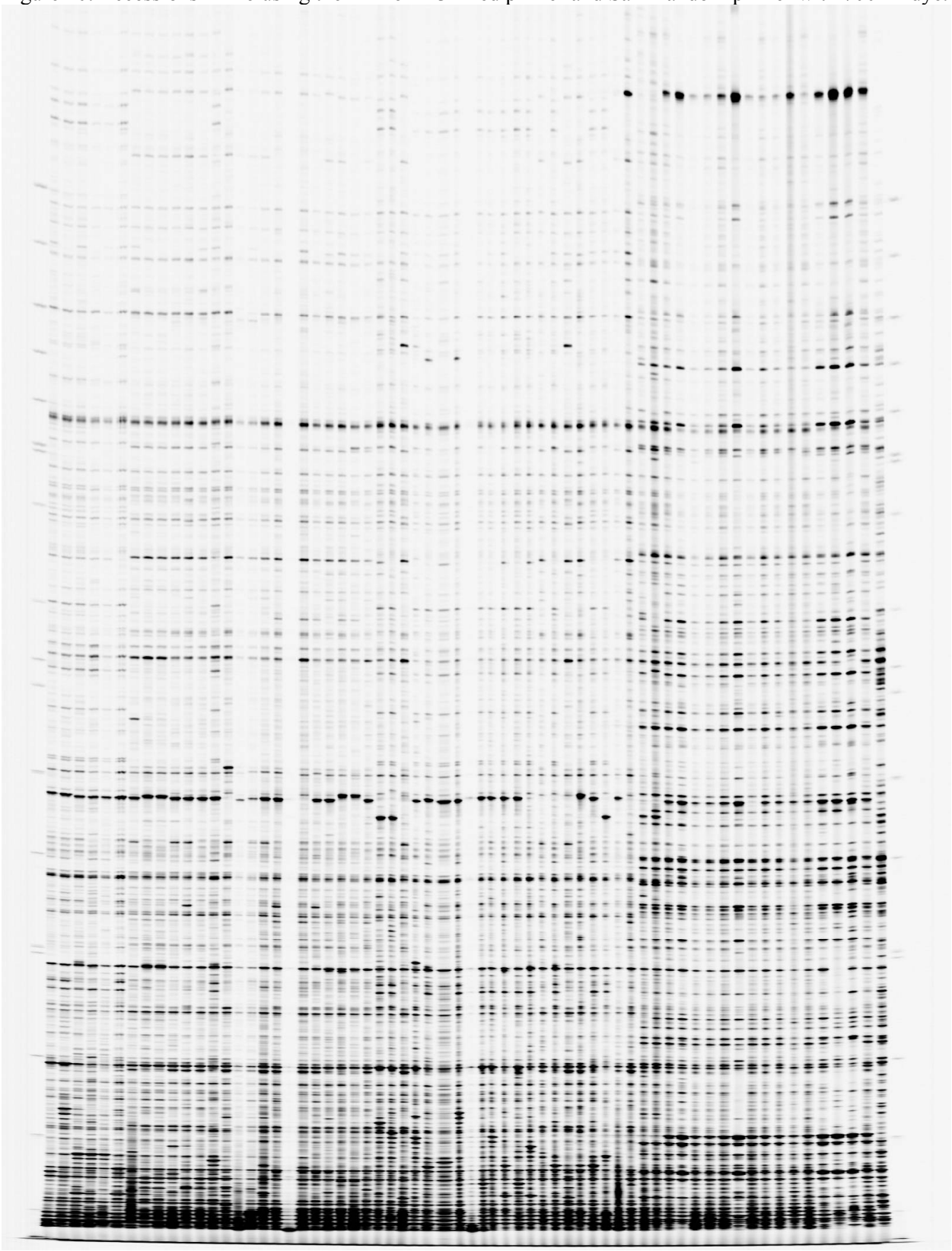

Figure 11. Accessions #47-92 using the B14-61413 fixed primer and Sa12 random primer with 700nm dye.

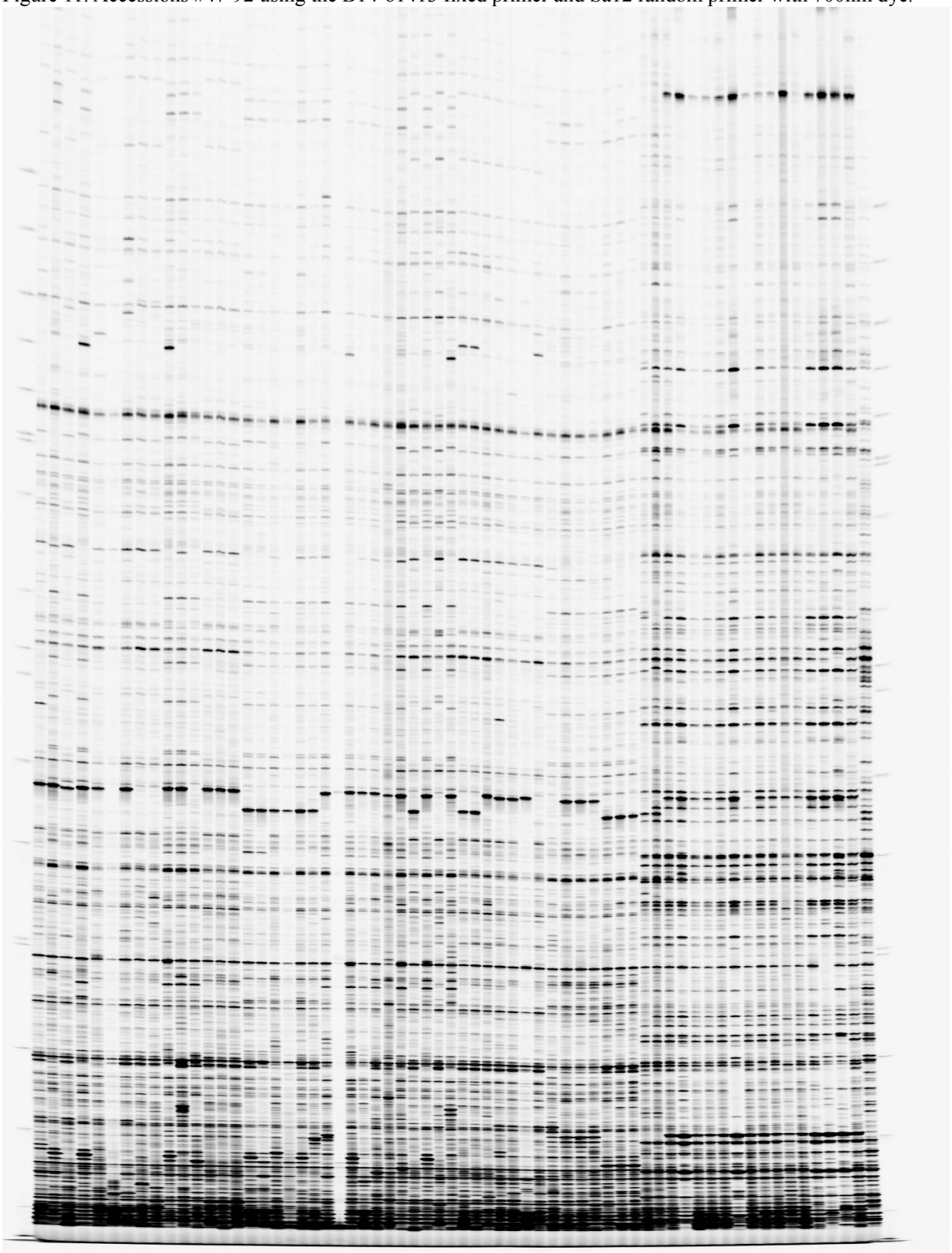

Figure 12. Accessions #93-117 using the B14-61413 fixed primer and Sa12 random primer with 700nm dye.

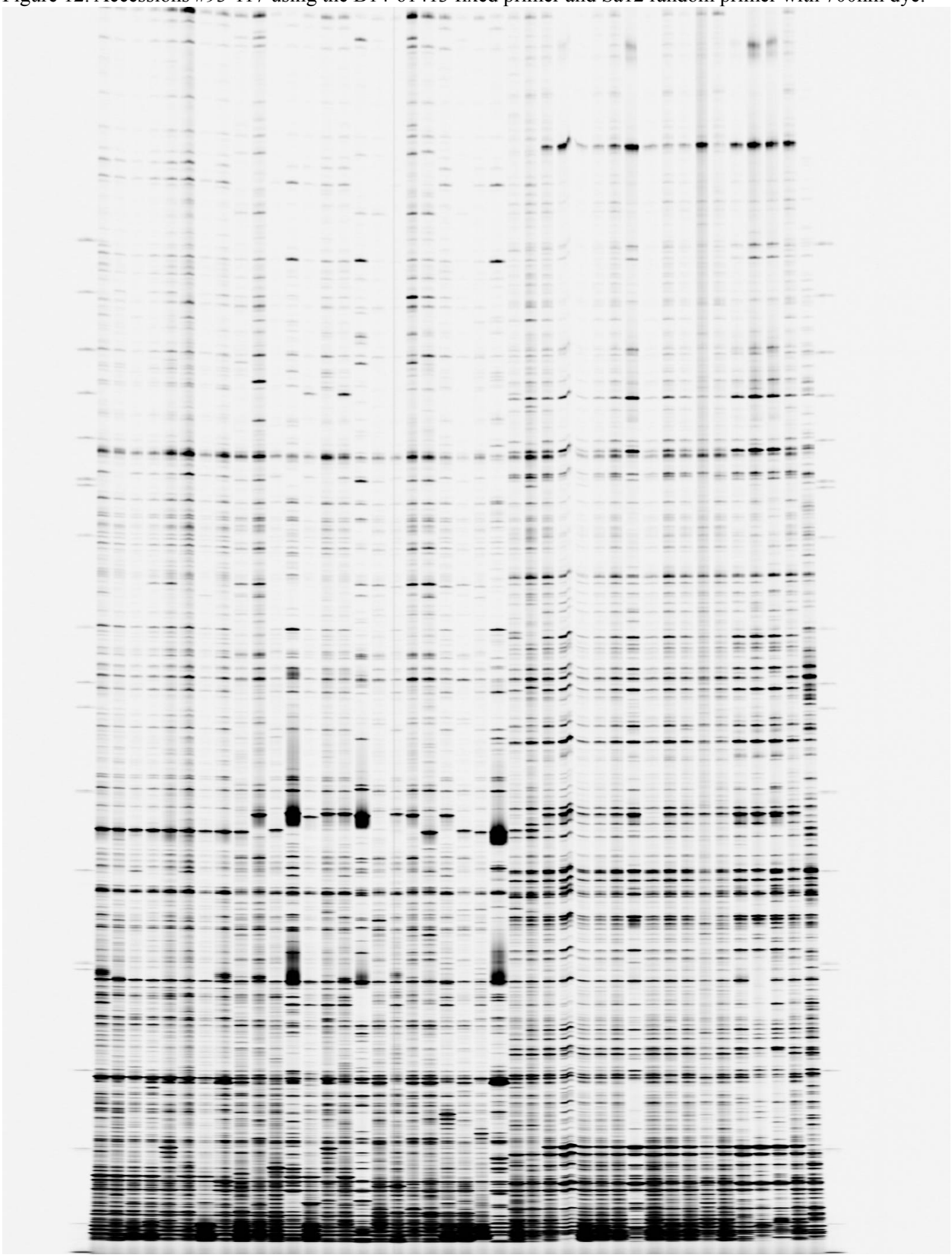

Figure 13. Accessions #1-46 using the B14-61413 fixed primer and Ga5 random primer with 800nm dye.

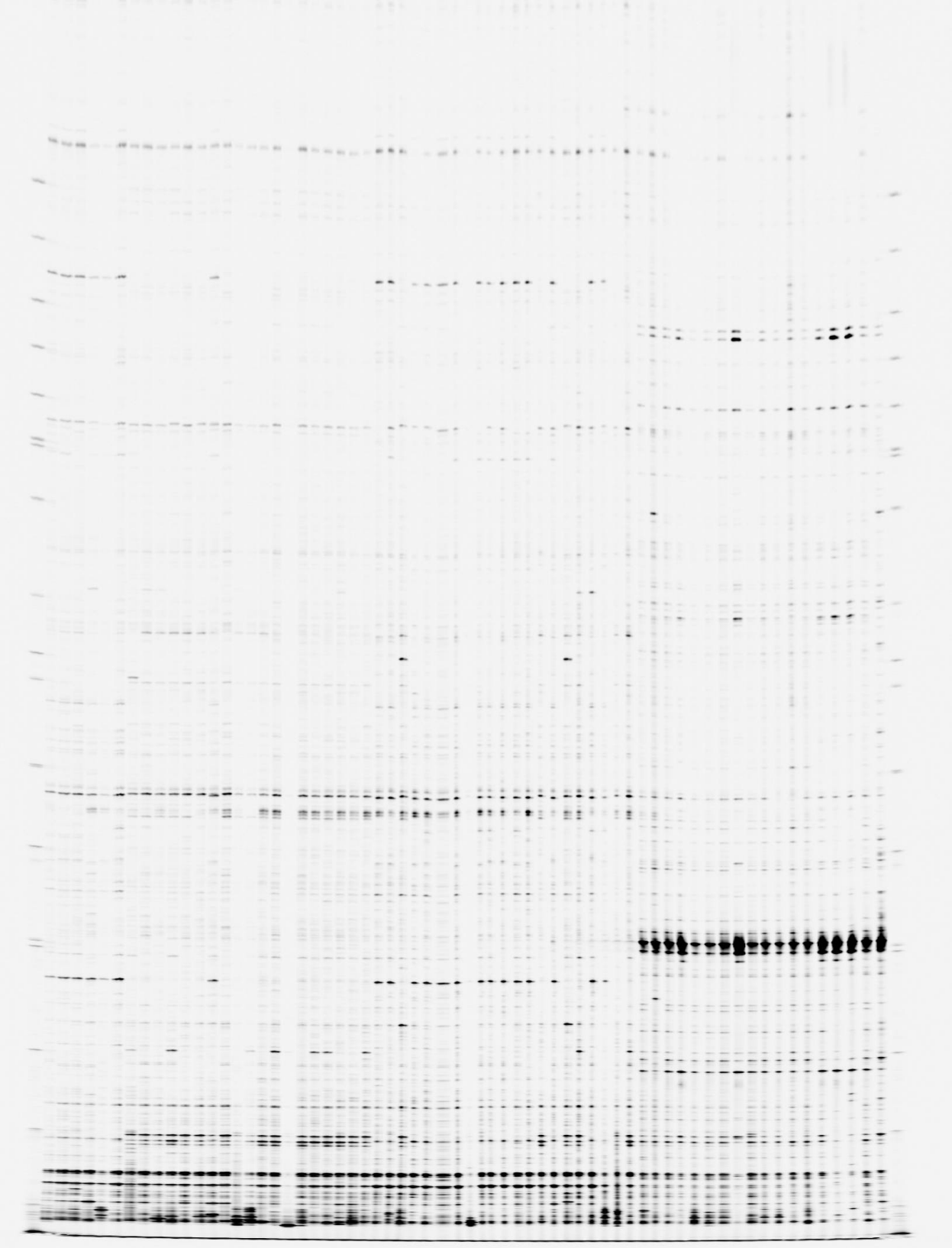

Figure 14. Accessions #47-92 using the B14-61413 fixed primer and Ga5 random primer with 800nm dye.

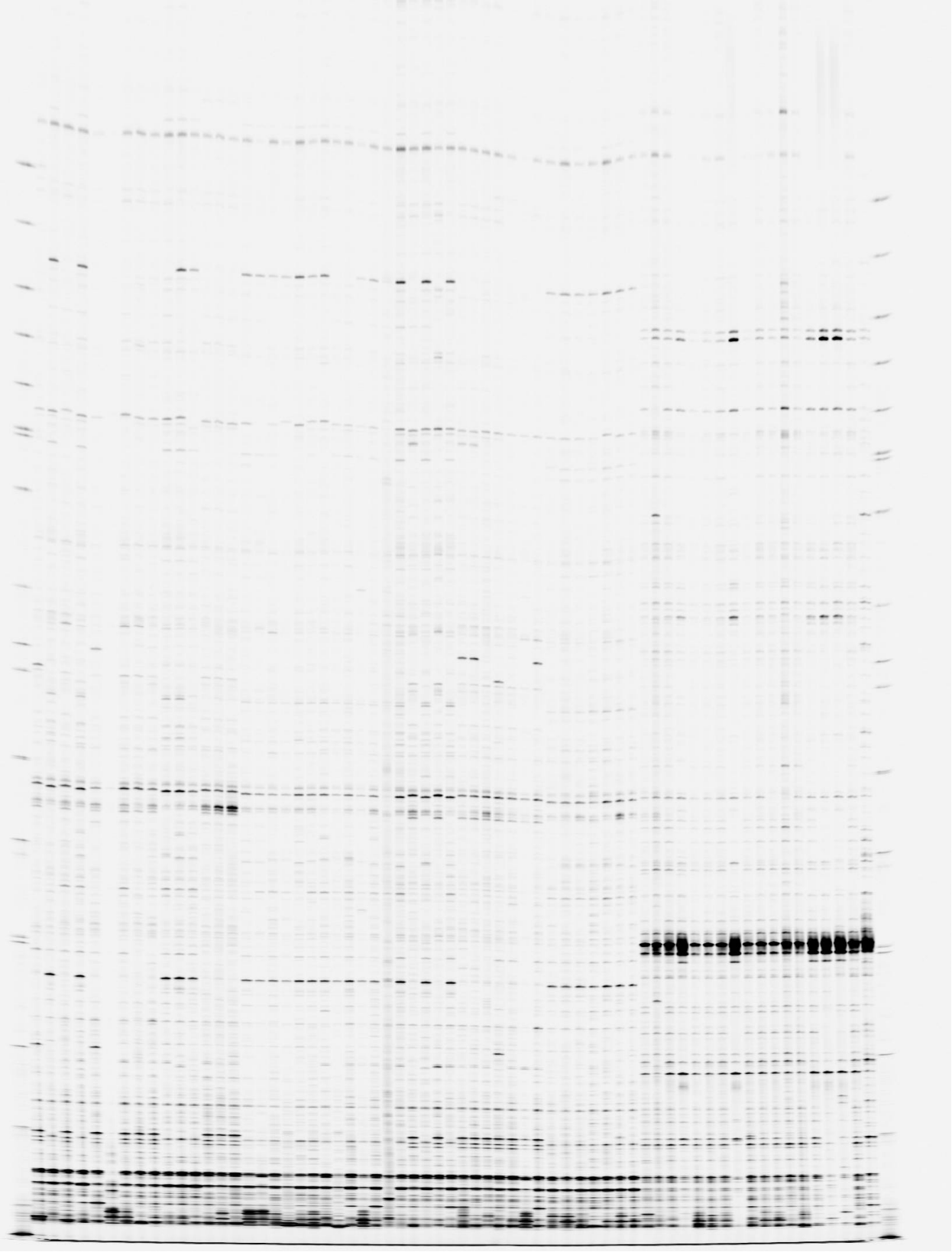

Figure 15. Accessions #93-117 using the B14-61413 fixed primer and Ga5 random primer with 800nm dye.