

SESSION 5

CONSEQUENCES OF GENE FLOW BETWEEN SPECIES – 2

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Predicting the environmental impact of transgene outcrossing to wild and weedy rices in Asia

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ABSTRACT

Asia is the center of origin of Asian cultivated rice, *Oryza sativa*, and is also home to numerous wild species of *Oryza*. Two wild species, *O. rufipogon* and *O. nivara*, are abundant in many parts of Asia and are known to hybridize with *O. sativa* under natural conditions. Numerous types of weedy rice also occur in Asia, derived from *O. sativa*, wild species, and hybrids between wild rices and *O. sativa*. Genetic engineering of rice for resistance to insects, diseases, and abiotic stresses has been rapidly advancing, although no transgenic varieties have as yet been widely field tested. We propose an interdisciplinary set of research activities to help predict the consequences of transgene outcrossing to wild and weedy rices, before transgenic varieties with enhanced stress resistance are extensively released into the environment.

INTRODUCTION

Rice is the staple food of half the world's population (Lampe, 1995). It provides 20% of global human per capita energy and 15% of per capita protein. Of 25 major rice-producing nations, 17 are located within South, Southeast and East Asia. Agricultural population densities on Asia's rice-producing lands are among the highest in the world and continue to increase at a remarkable rate. Rapid population growth puts increasing pressure on the already strained food-producing resources. By the year 2025, it is estimated that the world will need an additional 13 million tons of rough rice each year. With accelerating loss of productive riceland due to rising sea levels, salinization, erosion, and human settlements, the problem becomes one of increasing yields under ever-more trying circumstances (Dowling *et al.*, 1998).

Genetic engineering offers new opportunities to substantially increase rice yields per hectare. Extensive research on genetic engineering of rice is underway in dozens of laboratories around the world, in both the private and public sectors. Rice has been transformed with gene constructs to confer improved resistance to insects, pathogens, herbicides, and submergence, and to alter plant morphology and grain quality (Khush, 1996). Genetic engineering for other traits, including nutritional quality, and resistance to drought, salinity, and aluminum toxicity can be expected in the near future. Asia is the center of origin of Asian cultivated rice, *Oryza sativa*. Asia is also home to numerous wild and weedy species of *Oryza*, some of which are known to hybridize with *O. sativa* under natural conditions (Oka, 1991). Given the occurrence and environmental persistence of hybrids between cultivated and wild and weedy

rices, the extensive amount of land that may eventually be planted to transgenic rice, and the large populations of wild and weedy rices in many rice growing areas, it must be assumed that transgenes will escape to wild and weedy relatives. In this paper, we analyze the scope of the potential problem and outline an integrated set of research activities that will help to predict the consequences of outcrossing of transgenes to wild and weedy rices in Asia.

TAXONOMY AND ECOLOGY OF CULTIVATED, WILD, AND WEEDY RICES

Rice and its wild relatives are classified in the genus *Oryza*. The 22 species of wild rice are distributed throughout the tropics of Asia, Africa, Australia, Oceania, and Latin America (Vaughan, 1994; Bellon *et al.*, 1998). Several species complexes, based primarily on genomic identities, are recognized within the genus. The *O. sativa* complex consists of eight species which share the AA genome, including two wild species widespread in Asia, *O. rufipogon* and *O. nivara*. These two taxa are considered by some to be perennial and annual forms of the same species, and indeed the relationships among *O. sativa*, *O. nivara*, and *O. rufipogon* are complex (Oka, 1991). *O. sativa* is able to hybridize under natural conditions with other *Oryza* species that have the AA genome (Oka, 1991). The incidence of pollination of *O. rufipogon* and *O. nivara* by cultivated rice is increased by the fact that these wild species have a much higher incidence of outcrossing than *O. sativa*, which is primarily self-pollinating. Oka & Chang (1961) described "hybrid swarms" of plants consisting of F₁ hybrids and backcross progeny between *O. sativa* and *O. rufipogon*, clearly indicating that such hybrids can arise and persist under natural conditions. The ability to cross wild AA genome species with *O. sativa* has also facilitated their use in rice breeding (Bellon *et al.*, 1998). *O. nivara* is the source of a widely used gene for resistance to rice grassy stunt virus, and *O. rufipogon* has been a source of cytoplasmic male sterility for use in hybrid rice seed production.

In addition to wild rices, a diversity of weedy rices occurs in Asia. Weedy rices are becoming an increasingly severe problem as farmers switch their method of crop establishment from transplanting to direct seeding. Weedy rices are superior competitors to modern cultivars due to early vigor, greater tillering and height of plants. Close mimics of rice especially in the early stages of the crop, they are most easily recognized in the field as "off-types" at harvest by their height and by examination of panicles (easy shattering) and grain (having awns and red pericarps). Three main mechanisms may be hypothesized for the genesis of weedy rice in farmers fields: invasion and persistence of annual wild rices (aided by contaminated farmer seed, machinery movement); segregation and selection of "weedy" characters in existing cultivars; and introgression of genes from wild, annual or perennial, rice into sown cultivars. Recent genetic work supports the last two mechanisms (Suh *et al.*, 1997).

In Asia, extensive populations of *O. nivara* and/or *O. rufipogon* occur in India, Sri Lanka, Laos, Indonesia, Thailand, Cambodia, and Vietnam (Vaughan, 1994). Smaller

populations can be found in China, Nepal, and Bangladesh, among other countries. *O. nivara* and *O. rufipogon* are still common vegetation in irrigation ditches, swamps, and riverbanks interspersed within rice-growing areas, although the size and number of these populations has been dramatically reduced by urbanization and agricultural intensification. In some areas, *in situ* conservation programs will be necessary to prevent local extinction of wild rices. Such programs have already begun in China and Thailand. Management of weedy rices and outcrossing of transgenes to wild and weedy rices are intertwined with conservation issues. For example, *in situ* conservation of *O. nivara* and *O. rufipogon* populations may increase local weedy rice problems, or be perceived to do so by farmers. Outcrossing of transgenes will change the genetic background of conserved wild rice populations and in some cases may change their ecology.

GENETIC ENGINEERING OF RICE FOR STRESS RESISTANCE

Much of the initial work in genetic engineering of rice, as with other crops, has concerned genes for resistance to *biotic stresses*, e.g. plant pathogens and insect pests. This is in part because, in many cases, high levels of resistance can be achieved with single genes that are already available and well studied. For example, rice has been transformed with *Bacillus thuringiensis* (Bt) toxin genes for resistance to stem borers (Lepidoptera: Pyralidae) (e.g., Cheng *et al.*, 1998) and modified plant chitinase genes for resistance to sheath blight, *Rhizoctonia solani* (Lin *et al.*, 1995). Genetic engineering for resistance to stem borers and sheath blight has been a priority not only because of their widespread importance in Asia, but also because high levels of resistance to these organisms has not been found in the gene pool of AA genome rice species. One further consequence of the lack of genes for high levels of resistance to stem borers and sheath blight in AA genome rice species is that transgenes conferring such resistance will be truly novel additions to the ecosystem. This is in contrast with genes for resistance to other insects and diseases, such as brown planthopper (*Nilaparvata lugens*) and rice blast (*Pyricularia oryzae*), for which resistance genes are common in traditional rice varieties and wild rices, and have been long deployed in improved rice varieties. Anecdotal observations of wild and weedy rice populations suggest that insects and diseases do not appreciably constrain the distribution or abundance of these plants. However, to the best of our knowledge, no systematic study of the population dynamics of insects or pathogens on wild or weedy rices has been published.

Genetic engineering for resistance to *abiotic stresses*, such as drought, submergence, and salinity has proceeded more slowly than that for biotic stresses because genes and promoters that can confer such resistances have not yet, or only recently, been identified and cloned. Progress in understanding proteins and pathways conferring resistance to submergence (Setter *et al.*, 1997) and salinity (Schachtman & Schroeder, 1994) indicates that plant transformation for resistance to these stresses is not far off. Resistance to acid sulfate/aluminum toxicity (de la Fuente *et al.*, 1997), and drought (Iuchi *et al.*, 1996) will take longer, but eventual success is highly probable. The adaptive range of rice species and cultivars is largely determined by water regime, and

thus ecosystems for rice are defined on the basis of agrohydrology, e.g. irrigated, rainfed lowland, floodprone/deepwater, and upland (Garrity *et al.*, 1986). In rainfed conditions, the greatest yield losses are due to drought and submergence. *O. rufipogon* and *O. nivara* occur widely in rainfed lowland rice growing areas and *O. rufipogon* is abundant in some areas with acid sulfate soils, although these two species do not generally occur in saline soils. Because of the importance of water availability and soil chemistry on the distribution and abundance of rices, it is probable that the outcrossing of transgenes for abiotic stresses will have significant effects on the ecology of some populations of *O. rufipogon* and *O. nivara*.

A PROPOSED RESEARCH AGENDA

To predict the consequences of transgene outcrossing to wild and weedy rices will require an interdisciplinary research program involving germplasm specialists, plant ecologists and physiologists, and pest management and social scientists. Some of this research will also contribute to assessing the origins of weedy rices, the current conservation status of wild rices, and the potential for success of *in situ* conservation programs. We propose the following five linked research activities.

1. Survey the ecogeographical distribution and diversity of target species

There is a need for an updated and more comprehensive inventory of the distribution and conservation status of *O. rufipogon* and *O. nivara* populations. This will require updating information on previously-identified populations and visits to areas that have been inadequately surveyed but are likely to harbor wild rice populations. Data collection at each site should include climate and micro-climate, topography, associated vegetation, soil type, estimated conservation status, use and potential use of the plants by local people, local land use and agricultural practices. The transgenic traits that are likely to be most in demand by local farmers and the transgenic varieties that are likely to be popular in the area should also be identified. Cuttings and seeds should be collected for herbarium specimens and for analysis with genetic markers.

2. Identify key factors that determine the distribution and abundance of target species

To predict the impact of the outcrossing of a particular transgenic trait into a wild or weedy rice population, it is necessary to understand the ecological factors that constrain the distribution and abundance of that population. This will require monitoring the habitat and demographic characteristics of selected populations on at least a monthly basis, quantifying biotic and abiotic factors, e.g. identity and density of associated plant species, and of insect herbivores and plant pathogens occurring on the target species; soil nutrient status, water depth, shading, and micro-climate. This information can be used to identify candidate factors that determine population levels. However, because this approach cannot explain the mechanisms by which species abundance is actually regulated, there is also a need to manipulate habitat characteristics experimentally to confirm their role as key factors influencing

population growth rate. This will involve deliberate additions and removals of plants from populations to examine density compensation mechanisms, and experimental alterations of habitat variables. These variables should be chosen on the basis of the initial one or two years of surveys, and are likely to include manipulation of insect herbivore density and abiotic variables such as water and nutrient levels.

3. Analyze gene flow among wild, weedy, and cultivated rices

Previous work with DNA and allozyme markers has indicated that patterns of gene flow among wild, weedy, and cultivated rices differs among locations (e.g. Suh *et al.*, 1997). There is a need to expand gene flow studies to additional sites. Studies using markers for the mitochondrial and chloroplast genomes, in addition to the nuclear genome, could further elucidate the genetic origins of weedy rices and how transgenes might spread among wild and weedy populations.

4. Determine fitness of hybrids between improved varieties and target species

To estimate fitness of the hybrids between improved varieties and target wild and weedy species, a comparison of selected vegetative and reproductive characters of the parents, F_1 hybrids, and F_2 and backcrossing progenies will be conducted under controlled greenhouse conditions, and under field conditions. These are particularly important to measure for hybrids between perennial wild rices and cultivated (annual) rices. If the hybrids are not perennial but have limited capability of sexual reproduction, then they will have little ability to spread or persist in wild populations.

5. Quantify tolerance of target species to selected abiotic stresses

An indirect approach can be taken to study the possible effects of transgene outcrossing on wild and weedy rices, by quantifying the existing tolerance of wild and weedy rices to selected abiotic stresses. The initial focus should be on drought and submergence because of their great agronomic importance and the potential for environmental impact of transgenes conferring resistance to these stresses. There are several kinds of drought and submergence stress. These result from the continuum of water regimes that occur in rice growing areas: from drought on continuously aerobic soils in the uplands, to the drought-prone rainfed lowlands where drought generally follows ponding of water on previously anaerobic soils; to favorable rainfed and irrigated water regimes, and then to short-term flooding in submergence-prone rainfed lowland, and finally to deepwater in flood-prone ecosystems. Greenhouse experiments can be used to test the tolerance of wild and weedy rices and F_1 hybrids to water stress regimes characteristic of the areas in which the plants are found. Dependent upon progress and results from the drought and submergence tests, treatments of salinity and/or acid sulfate/aluminum toxicity could be added to the water regimes in a factorial design.

PERSPECTIVES

Regulations for field testing of genetically modified organisms have been established in China, Thailand, and the Philippines, among other countries in Asia. These regulations generally require that proposals for field testing provide information about the potential for, and the possible harmful effects of, outcrossing. To the best of our knowledge, field tests of transgenic rice have so far taken place only in Japan and China, but it is likely that the number of field tests will soon begin to increase rapidly in several countries. While initial field testing of transgenic rice can be confined to areas where wild and weedy relatives do not occur or have been removed, or held in plots isolated by distance and bordering vegetation, it clearly will not be practical to enforce such restrictions once large scale field tests begin or transgenic varieties are released to farmers. Outcrossing was a focus of the first conference on rice biosafety, where it was concluded that "Further study of the ecology and distribution of wild and weedy rices is recommended, but this need not and should not delay field tests in areas where the ecology is well known" (Clegg *et al.*, 1993). Hopefully, when promising transgenic rice varieties are ready for extensive field testing and release to farmers, sufficient information on the consequences of transgene outcrossing will be available to permit regulatory bodies to make well-informed decisions about possible risks in all affected rice-growing areas.

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The production of herbicide-resistant jointed goatgrass (*Aegilops cylindrica*) x wheat (*Triticum aestivum*) hybrids in the field by natural hybridization and management strategies to reduce their occurrence

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ABSTRACT

Two imazamox-resistant hybrids from a cross between *A. cylindrica* and an imazamox-resistant wheat ('cv' FS-4 IR wheat) were discovered in a research plot where FS-4 IR wheat seed had been replanted from the harvest of an efficacy study conducted the year before. Seven seeds (BC_1) were produced by these plants. Six of the seven seedlings from these seeds survived an application of 72 g ai ha⁻¹ imazamox. Management strategies to reduce the occurrence of herbicide-resistant *A. cylindrica* x wheat hybrids can be incorporated before, during, and after the crop year when the herbicide-resistant wheat is planted. Strategies to control hybrids that do occur are discussed. These strategies take advantage of the biology of the *A. cylindrica* x wheat hybrid plant.

INTRODUCTION

A. cylindrica ($2N = 4x = 28$) is an important winter annual grass weed problem in winter wheat ($2N = 6x = 42$) in the western United States. Because *A. cylindrica* shares the D genome with winter wheat (Kimber & Sears, 1987) (Figure 1), no selective herbicides have been developed. Recently, a soft red winter wheat (cv. 'Fidel') was mutagenized and plants resistant to imazamox, an imidazolinone herbicide, were selected from the mutagenized population (cv FS-4 IR wheat) (White & Morrison, 1998). Field experiments in Washington, USA and Oregon, USA (Ball *et al.*, 1999) have documented high levels of resistance to imazamox by the FS-4 IR wheat.

A concern in the development of herbicide-resistant crops is the potential transfer of the resistance gene to other plant species (Harrison, 1992). Studies in Idaho, USA have demonstrated that glufosinate-resistant canola could out-cross to *Brassica rapa* to produce glufosinate-resistant hybrids in both greenhouse (Brown & Brown, 1996)

and field studies (Brown *et al.*, 1995). It has been known for 70 yr that *A. cylindrica* crosses with wheat to produce hybrids that are phenotypically intermediate and sterile (Johnston & Parker, 1929; Mayfield, 1927). Only recently has it been shown that the *A. cylindrica* x wheat hybrids will produce viable seed with two to three percent fertility (Zemetra *et al.*, 1998).

In a 1995-96 herbicide efficacy field study, *A. cylindrica* pollen fertilized some FS-4 IR wheat plants at the Palouse Conservation Field Station near Pullman, WA (Ball *et al.*, 1999). Seed harvested from this experiment was cleaned by hand of *A. cylindrica* spikelets and replanted for a second imazamox efficacy trial (1996-91) at a second location in the Palouse Conservation Field Station. Just before harvest, two hybrid plants (SSS201 and SSS405) from a winter wheat x *A. cylindrica* cross were discovered in plots where imazamox at the two highest rates (53 and 69 g ai ha⁻¹) was applied. All imazamox applications included 0.25 % R-II (v:v) a surfactant, and 380 ml ha⁻¹ 'Prefer'. Imazamox at the two high rates killed all *A. cylindrica*, but had no observable effect on the FS-4 IR wheat. The hybrid plants were growing in the wheat row, indicating that the hybrid seed was in the seed lot, had been planted, and resembled winter wheat in appearance. The hybrid plants were 15 cm taller and more robust than the surrounding FS-4 IR wheat and had 8 and 10 tillers. About 2 % of the spikelets on these hybrid plants had viable BC₁ seed (four from one plant and three from the other). The objective of this research was to document that the gene which imparts resistance to imazamox had been transferred to hybrids between *A. cylindrica* and wheat and to outline management strategies which would reduce this occurrence.

MATERIALS AND METHODS

The seven BC₁ seeds were germinated and mitotic chromosomes were analyzed in root tip cells as described in Seefeldt *et al.* (1998). After the root-tip squashes, the seven BC₁ seedlings were transplanted to small pots (0.5 L) and placed in a greenhouse (22 C and 16:8 h day:night). At the 3 to 4 leaf stage, the BC₁ plants, six FS-4 IR wheat plants, and six *A. cylindrica* plants were sprayed with 72 g ai ha⁻¹ imazamox with 0.25 % R-11¹ (v:v) and 380 ml ha⁻¹ 'Prefer' in a spray chamber. The plants were then vernalized for 8 wk at 4°C in a growth chamber.

DISCUSSION

Transfer of the resistance gene

Winter wheat/*A. cylindrica* hybrids will have 35 chromosomes, (21 from wheat and 14 from *A. cylindrica*) (Figure 1) and the BC₁ generation may result from backcrosses with wheat or *A. cylindrica* (Zemetra *et al.*, 1998). Fertility in the hybrid is reduced compared to wheat because of the lack of bivalent formation with the three unpaired genomes. When the hybrid backcrosses with wheat, subsequent selfing would tend to return the chromosome number to 42, whereas backcrosses with *A. cylindrica* would tend to return the chromosome number to 28. From the two sets of root squashes, the

chromosome counts were $2n = 39, 39, 42,$ and 45 for SSS201 and $39, 40$ and 54 for SSS405 (Seefeldt *et al.*, 1998).

All FS-4 IR wheat plants and six of the seven BC_1 plants survived imazamox at 72 g ai ha^{-1} . All of the *A. cylindrica* plants were killed. The BC_1 plant that did not survive (chromosome count $2N=45$) may have been a backcross to a susceptible *A. cylindrica* plant or the resistance gene may have been lost during meiosis (Figure 1). It is clear that the FS-4 IR wheat was originally pollinated by *A. cylindrica*, the gene for resistance to imazamox was in the hybrids, and the majority of the seed produced by the hybrids possessed the resistance gene.

There was a range of phenotypic variation in the six surviving BC_1 plants, with two similar to the FS-4 IR wheat, one similar to *A. cylindrica*, and three intermediate in appearance. Although F_1 hybrids are more vigorous than the parent wheat or *A. cylindrica*, the BC_1 plants, after being treated with imazamox, were less vigorous, particularly at the seedling stage, than the treated FS-4 IR wheat plants. The BC_1 plants were allowed to self-pollinate. However no seed was produced indicating that the BC_1 plants from this study were not self-fertile (Seefeldt *et al.*, 1998).

Management strategies

The gene for resistance to imazamox in FS-4 IR wheat is thought to be located in the D genome which occurs in both wheat and *A. cylindrica*. This location would increase the potential for transfer of the resistance gene from wheat to *A. cylindrica* (Figure 1). If the resistance gene was located in the wheat A or B genome there is reduced probability of establishment of the resistance gene in *A. cylindrica* backcrosses as the unpaired A and B chromosomes tend to get lost with each additional backcross (Zemetra *et al.*, 1998). There is however, a low probability that the gene could persist through translocation from the A or B genome to the C genome in *A. cylindrica*. Since the D genome has homologous pairs of chromosomes, it is unlikely that translocation would occur within this genome. It is possible that a pair of A or B chromosomes could undergo reduced meiosis which would result in a substitution line ($2N=28$) or an addition line ($2N=30$ or $2N=32$). *A. cylindrica* plants that result from either scenario would probably be sterile or non-competitive. However, under herbicide selection, stabilization of the A or B genome chromosome pair is possible. While the 30 and 32 chromosome plant may be self-fertile because of normal bivalent formation, resistance transfer by outcrossing to *A. cylindrica* would be reduced due to the differences in chromosome number in the two plants (30 or 32 and 28). Backcrosses to FS-4 IR would, of course, remain resistant.

The rapid transfer of a herbicide-resistance trait from wheat to *A. cylindrica* is a problem with serious implications for growers who would use herbicide-resistant wheat in areas infested with *A. cylindrica*. However, it might be possible to mitigate the problem with management strategies that take advantage of aspects of the hybridization process. These strategies are listed below with reference to the planted resistant wheat.

Preplant

1. If the weed infestation is heavy consider a one-time burn on non-highly erosive land to kill *A. cylindrica* seed on the surface followed by a spring crop or fallow to further reduce the *A. cylindrica* seed bank. If the land is highly erodible one could chisel plow at intervals to reduce erosion.

Crop year

2. Growers must use herbicide-resistant wheat that comes from 'certified' wheat fields where there is no *A. cylindrica* within half a mile of the field.
3. Ensure that the herbicide-resistant wheat is a variety that is competitive against *A. cylindrica*.
4. Use high seeding rates and narrow row spacing to increase crop competitiveness.
5. The herbicide should be applied for maximum efficacy to the field and to the field borders to reduce cross-pollination of off-site *A. cylindrica*.

Harvest

6. As much wheat and *A. cylindrica* seed as possible should be harvested to remove the resistance gene from the field.
7. Grain trucks hauling herbicide-resistant seed from the field should be covered to prevent herbicide-resistant seed from blowing off the truck and being introduced to other fields and roadsides.
8. All farm machinery should be thoroughly cleaned before moving off the field.

Following year

9. A non winter wheat crop should be planted that allows the use of alternative methods to control any *A. cylindrica*, herbicide-resistant wheat, and herbicide-resistant hybrids.
10. Because the increased size of the F₁ hybrids makes them easy to see in the field, hand-weeding should be used where practical to remove hybrids in order to prevent backcrosses with *A. cylindrica* and wheat. Removed plants should be destroyed.
11. Adding an additional year of spring crops before may drive the *A. cylindrica* seed bank to near extinction.
12. The next winter wheat crop should be one that is not herbicide resistant which will limit backcrosses to susceptible genes.
13. Any small infestations of *A. cylindrica* should be killed.

Growers, once made aware of the ease of transfer of the resistance gene to winter wheat/*A. cylindrica* hybrids and the progeny of those hybrids, will want to adopt strategies that reduce the movement of the resistance gene to *A. cylindrica*. These strategies will ensure that herbicide-resistant wheats will be useful tools for the control of problem weeds such as *A. cylindrica* for years to come.

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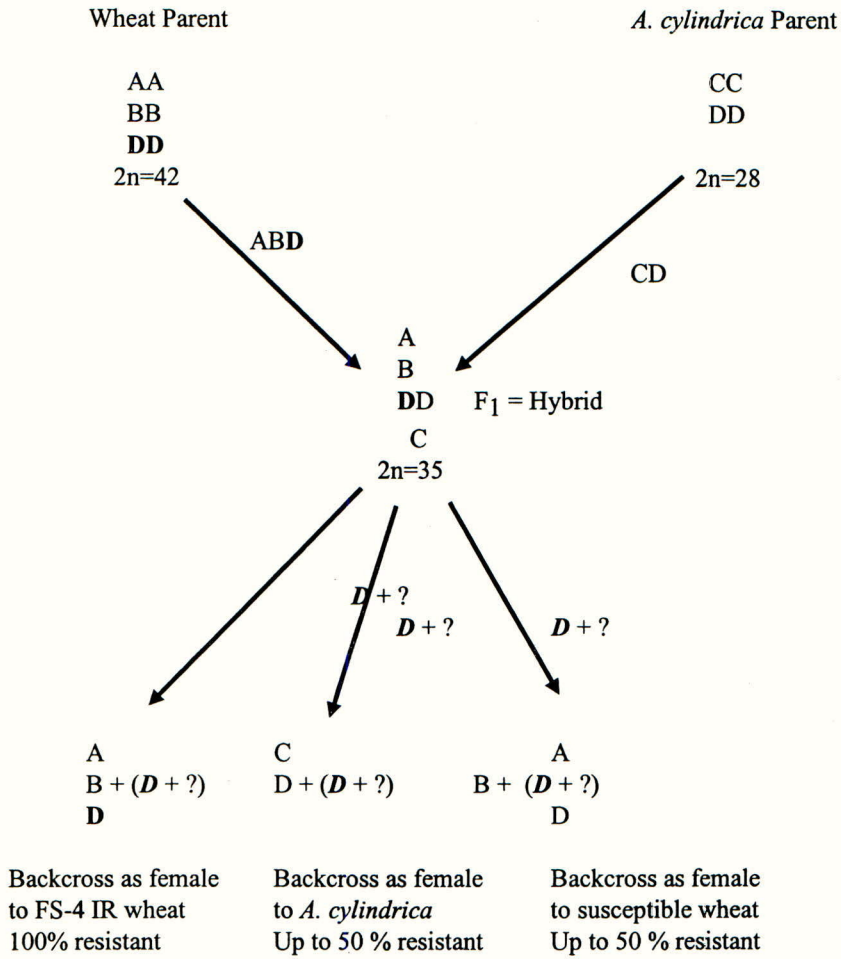


Figure 1. Movement of resistance gene from wheat to *A. cylindrica* where **D** = resistance gene in the D genome and ***D*** = 50 % probability of resistance gene.

Potential for gene flow between wheat (*Triticum aestivum*) and jointed goatgrass (*Aegilops cylindrica*) in the field

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ABSTRACT

Aegilops cylindrica is a major weed in the wheat producing areas of the western United States. One method proposed to control *Aegilops cylindrica* is the use of herbicide resistant wheat. Of concern is whether a herbicide resistance gene could be transferred from wheat to *Aegilops cylindrica*. *Aegilops cylindrica* shares the D genome with wheat and interspecific hybrids between the two species occur in the field. In previous greenhouse research, partial self-fertility was restored in the BC₂ generation after backcrossing to *Aegilops cylindrica*. The objective of this research was to determine if backcrossing of the wheat x *Aegilops cylindrica* hybrid could occur in the field. Hybrids were transplanted with various combinations of wheat and *Aegilops cylindrica* plants. Field derived BC₁ plants were transplanted into stands of *Aegilops cylindrica*. Both BC₁ and BC₂ seeds were produced in the field by natural backcrossing. Seed harvested off the hybrid plants had a high germination rate. Four BC₁ plants were found to be partially self-fertile. The production of BC₂ seed in the field and the low level of self-fertility of BC₁ plants indicates that hybrids could serve as a bridge in the transfer of genes between wheat and *Aegilops cylindrica* in the field. Any release of a herbicide resistant wheat should be accompanied by a management plan that would minimize the potential for gene movement between these species.

INTRODUCTION

Aegilops cylindrica is a major weed of winter wheat in the western United States. Its similarity to wheat makes control very difficult once *Aegilops cylindrica* is introduced into a wheat field (Donald & Ogg, 1991). Herbicide-resistant wheat may provide a means to selectively control *Aegilops cylindrica* in wheat fields. An imidazolinone herbicide-resistant wheat has been developed and is currently being field tested in the Pacific Northwest of the United States (Mallory-Smith *et al.*, 1996). While the use of herbicides in combination with herbicide resistant wheat may provide a control method for *Aegilops cylindrica*, the sexual transfer of the herbicide resistance gene from wheat to *Aegilops*

cylindrica would eliminate this benefit.

Hybrids can occur between *Aegilops cylindrica* and wheat in the field because they are related species that share a common genome (Kimber & Sears, 1987). The shared D genome between wheat and *Aegilops cylindrica* allows hybrids between the species to be produced in the field. These hybrids were believed to be sterile but viable seed was found on the hybrids (Mallory-Smith *et al.*, 1996). In the greenhouse, the interspecific hybrids showed a low level of female fertility (2%) that allowed for backcrossing to occur between the hybrid and either *Aegilops cylindrica* or wheat (Zemetra *et al.*, 1998). Partial female fertility increased with subsequent backcrosses with partial self-fertility restored after two backcrosses to *Aegilops cylindrica*. The restoration of self-fertility after two backcrosses raises the question whether the hybrid could serve as a bridge to transfer a herbicide resistance gene from wheat to *Aegilops cylindrica*. Because the current source of imidazolinone herbicide resistance in the wheat being field tested is controlled by a single, semidominant gene (Newhouse *et al.*, 1992), the risk of transfer is increased if backcrossing can occur. The objective of this research was to determine if BC₁ and BC₂ seed could be produced in the field and if the rate of seed production in the backcross generations was similar to that observed in the greenhouse.

MATERIALS AND METHODS

Hybrid and backcross seed was produced in the greenhouse by crossing a native collection of *Aegilops cylindrica* with the soft white winter wheat cultivar 'Madsen' (Allan *et al.*, 1989). The wheat was used as the female parent and the approach method was used for pollination. For the field BC₁ seed production trial, hybrid seed was germinated and transplanted into the field in Corvallis, Oregon in the fall of 1995 and 1996. There were four replications of one, five, or ten hybrids transplanted into plots seeded with one of five mixtures of *Aegilops cylindrica* and Madsen wheat (150/0, 100/50, 75/75, 50/100, and 0/150). Hybrid heads were collected in July, 1996 and July, 1997 and a maximum of 100 heads per plot were hand-threshed to determine percent seed set. Seed found in these trials was tested to determine the germination rate by germinating the seed in the dark at 24 C for one week. The BC₁ plants from the germination trial were planted in the greenhouse and heads were collected to determine level of self-fertility. For the field BC₂ production trial, a set of BC₁ seed from the 100% *Aegilops cylindrica* plots were germinated and transplanted into plots of 100% *Aegilops cylindrica* in Corvallis, Oregon in the fall 1996 and 1997. Heads were harvested off the BC₁ plants and hand-threshed to determine number of seed set. Standard ANOVA was run on BC₁ seed production trial for percent seed set and percent germination to determine if differences existed among the wheat - *Aegilops cylindrica* combinations.

RESULTS AND DISCUSSION

Seed set occurred on the hybrid plants in both 1996 and 1997 (Table 1). The mean percent seed set was 2.2% in 1996 and was 3.7% in 1997. This is equal or greater than that observed for seed set on hybrids in the greenhouse (Zemetra *et al.*, 1998). No

differences for percent seed set were observed among the different plots of wheat and *Aegilops cylindrica* in 1996 or 1997. This demonstrates BC₁ seed can be produced in the field and confirms our greenhouse results that percent seed set is related to the partial female fertility of the *Aegilops cylindrica* x wheat hybrid and not the species providing the pollen.

BC₁ seed produced in the 1995-96 and 1996-97 field trials were germinated and grown out in greenhouses in Oregon and Idaho. The average germination was 85% in 1995-96 and 89% in 1996-97. No differences were found among the wheat - *Aegilops cylindrica* combinations for percent germination. This indicates that the seed set on the hybrid plants in the field have a high likelihood of germinating in the field the next growing season.

Table 1. Percent seed set on *Aegilops cylindrica* x wheat hybrids grown among various mixtures of *Aegilops cylindrica* and wheat.

<i>Aegilops</i>		Number of <i>Aegilops cylindrica</i> x wheat hybrids					
		1		5		10	
<i>cylindrica</i>	Wheat	1996	1997	1996	1997	1996	1997
150	0	2.3	4.0	2.2	3.5	2.2	2.9
100	50	2.9	2.1	2.3	3.6	2.3	3.8
75	75	3.2	1.8	2.2	4.4	2.1	2.8
50	100	1.6	3.3	1.7	4.2	2.4	4.0
0	150	1.2	5.7	2.1	4.8	2.5	5.2

Heads of the greenhouse grown BC₁ plants were checked for seed to determine if any of the BC₁ plants were self-fertile. Four BC₁ plants were found to have a high level of self-fertility (Table 2). Two of the four plants appeared to be phenotypically like wheat, one like *Aegilops cylindrica* and the fourth plant was more similar to the hybrids in appearance. The presence of self-fertility in the BC₁ generation is one generation earlier than observed in the greenhouse (Zemetra et al. 1998) but this may be related to the greater number of plants evaluated in the field trial.

Table 2. The restoration of fertility in wheat x *Aegilops cylindrica* F₁ hybrids and their backcross progenies.

	Chromosome mean	Number range	Number of plants	Number of plants setting seed	Seed set (%)	
					Mean	Range
F ₁ and backcross progenies used as male parent						
F ₁	35	35	10	0	0	0
BC ₁	45	41-49	4	1	2.4	0-9.5
BC ₂	34	28-39	6	3	13.0	0-38.5
F ₁ and backcross progenies used as female parent						
F ₁	35	35	10	10	0.9	/*
BC ₁	45	41-49	4	4	4.4	0.8-13.2
BC ₂	34	28-39	5**	5	26.8	5.6-81.1

* F₁ plants were harvested in bulk.

** One of BC₂ plant grew poorly and was only used as male parent.

In the 1996-97 BC₂ seed production trial, the mean frequency of seed set on the BC₁ plants was 2.5% and ranged from 0.2% to 5.2%. In the 1997-98 BC₂ seed production trial, the mean frequency of seed set on the BC₁ plants was 7.5% and ranged from 1.6% to 20.3%. In 1996-97, the mean frequency of seed set on the field grown BC₁ plants was lower than that observed in the greenhouse but was higher in the 1997-98 field trial. The field harvested BC₂ seeds are currently being germinated and the BC₂ plants will be grown out in the greenhouse to determine the level of self-fertility for this backcross generation.

Based on the results of this study BC₂ plants, originating from a wheat x *Aegilops cylindrica* hybrid can be produced in the field. Based on our greenhouse study results where self-fertility was restored in the BC₂ generation of *Aegilops cylindrica* parents and the low number of partially self-fertile BC₁ plants found in this study, there is a good potential for gene movement from wheat to *Aegilops cylindrica* in the field. Management practices and genome placement of herbicide resistance genes will need to be taken into account to minimize the movement of herbicide resistance from wheat to *Aegilops cylindrica*.

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