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Evaluation of crossability between triticale (X *Triticosecale* Wittmack) and common wheat, durum wheat and rye

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Development of transgenic triticale as a platform for novel bio-industrial products is predicated on an environmental biosafety assessment that quantifies the potential risks associated with its release. Pollen-mediated gene flow to related species and conventional triticale varieties is one pathway for transgene movement. A tier 1 quantification of triticale hybridization was conducted by emasculating and hand pollinating flowers under greenhouse conditions. Approximately 2000 manual pollinations were conducted for each cross and its reciprocal between two triticale genotypes: a modern triticale cultivar (AC Alta) and primary triticale (89TT108), and common wheat, durum wheat and rye. The frequency of outcrossing, hybrid seed appearance and weight, and F_1 emergence and fertility were recorded. Outcrossing, F_1 emergence and fertility rates were high from crosses between triticale genotypes. Outcrossing in inter-specific crosses was influenced by the species, and the genotype and gender of the triticale parent. In crosses to common and durum wheat where triticale was the male parent, outcrossing was ≥ 73.0% and ≥ 69.5%, respectively, but ≤ 23.9% and ≤ 3.0% when triticale was the female parent. Overall, outcrossing with rye was lower than with common and durum wheat. F_1 hybrid emergence was greater when triticale was the female parent. With the exception of a single seed, all wheat-triticale F_1 hybrid seeds were non-viable when triticale was the male parent in the cross. Only seven durum wheat-triticale F_1 hybrids emerged from 163 seeds sown, and all were produced with triticale 89TT108 as female parent. With rye, 8 F_1 hybrids emerged from 38 seeds sown, and all were produced from crosses to AC Alta; five with AC Alta as the female parent and three as the male. Interspecific F_1 hybrids were self-sterile, with the exception of those produced in crosses between common wheat and triticale where triticale was the female parent. Tier 2 hybridization quantification will be conducted under field conditions.

Keywords: triticale / outcrossing / risk assessment / biosafety / hybridization / rye / wheat / crossability

INTRODUCTION

Triticale (X *Triticosecale* Wittmack) is being evaluated as a candidate for the production of novel bioproducts in Canada. Improvement of triticale through genetic modification necessitates that potential risks associated with the release of transgenic cultivars be assessed. Triticale is an intergeneric hybrid between wheat (*Triticum* ssp.) as the female parent and rye (*Secale* ssp.) as the male parent. Reduced fertility and shriveled kernels were common in early triticale, but breeding programs were successful in improving these traits (Oettler et al., 2005). The first commercial triticale cultivars were released in the late 1960s. Triticale production increased almost 50% worldwide from 1991 to 2001, and in 2004 triticale was grown on over 3 million ha in 28 countries (http://faostat.fao.org/). In Canada, triticale acreage reached a maximum in 2002 with 87 000 ha. It is used primarily for animal feed, but small amounts enter the human food chain either intentionally or unintentionally through mixing with other grains. Triticale has many favorable agronomic qualities for production in Canada: it is more productive than other cereals under abiotic stress conditions, less susceptible to most diseases, and is competitive with other cereals in terms of grain yield (Alberta Agriculture, Food and Rural Development, 2005). Furthermore, triticale has excellent potential for improvements through breeding. Recent studies suggest that significant improvements in yield

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are possible through hybrid breeding (Oettler et al., 2003; 2005) and breeding programs are underway to improve additional traits including disease and lodging resistance and starch profile (Green, 2007). There is also the potential to enhance breeding programs using existing genetic markers (Gonzalez et al., 2005; Kuleung et al., 2006; Tams et al., 2004; 2005; Zhang et al., 2005). Successful transformation of triticale was achieved using microprojectile bombardment and *Agrobacterium*-mediated transformation (Nadolska-Orczyk et al., 2005; Zimny et al., 1995). Currently, the traits expressed by transgenic triticale in the published literature are limited to reporter genes, such as antibiotic resistance, used to identify successful transformation events. Development of transformation protocols for triticale allows the introduction of a number of novel traits.

Triticale may be a valuable platform for bioproducts. Bio-industrial farming opportunities encompass existing and emerging markets including bioenergy, biorefining and biomaterials. Conventional triticale has already been identified as a suitable resource for biofuel production (Plochl and Heiermann, 2006; Rosenberger, 2005; Rosenberger et al., 2002; Wang et al., 1997). Biotechnology may provide opportunities to improve triticale as a biofuel resource and to introduce new traits, expanding its applications with bio-industrial farming.

The use of triticale as a platform for bioproducts requires an environmental risk assessment. The current small acreage of triticale reduces the opportunities for transgenic, or novel, triticale to outcross with conventional triticale. In addition, triticale has limited use in products destined for human consumption, reducing the challenges associated with segregating bioproducts from conventional products. One concern regarding the introduction of a transgenic crop is gene flow to related species. If pollen from transgenic triticale can successfully produce hybrid seed with similar or related conventional crops (*i.e.* triticale, wheat, or rye) and transgene introgression occurs, the transgene could enter the conventional food chain. This could result in market harm to conventional crops. The potential for pollen-mediated gene flow from triticale to other crops, and the potential impact on their weediness or invasiveness needs to be assessed.

Triticale may be tetraploid, hexaploid or octoploid, but hexaploid varieties are the most commercially successful. Hexaploid hybrids of Triticale ($2n = 6x = 42$; AABBRR) may be developed by crossing durum wheat (*Triticum turgidum* ssp. *durum*; $2n = 4x = 28$; AABB) and rye (*Secale cereale*; $2n = 2x = 14$; RR). Additionally, octoploid triticales often revert to the hexaploid level (Oettler, 2005). Although triticale is primarily selfing, considerable outcrossing may occur, and fertilization was detected as far as 30 m from a pollen source (Yeung and Larter, 1972). The potential for pollen-mediated gene flow over long distances in triticale is unknown; however, a recent publication examining pollination from common wheat to neighboring fields within a 10 km radius of a central pollinator field detected gene flow at distances up to 2.75 km (Matus-Cadiz et al., 2007). Triticale was previously reported to outcross with common wheat (*Triticum aestivum*; $2n = 6x = 42$; AABBDD) and rye (Chaubey and Khanna 1986; Guedes-Pinto et al., 2001; Lelley, 1992), though crossability was higher with wheat than with rye (Lelley, 1992). Crosses between triticale and common wheat where triticale was the female parent (ABR × ABD) produced viable F_1 seed, but the reciprocal cross (ABD × ABR) produced non-viable seed (Bizimungu et al., 1998; Chaubey and Khanna, 1986; Khanna, 1990; Nkongolo et al., 1991). Although the seed is non-viable, common wheat × triticale (ABD × ABR) hybrids can be obtained using *in vitro* embryo rescue (Kapila and Sethi, 1993). Hybridization was influenced by the genotypes of the parents used in the interspecific cross (Bizimungu et al., 1998; Guedes-Pinto et al., 2001; Lelley, 1992; Lima-Brito and Guedes-Pinto, 1998; Nkongolo et al., 1991). In addition to genetic factors, observations of variation in hybridization success in different years and at different temperatures suggest an environmental influence on hybridization (Guedes-Pinto et al., 2001; Nkongolo et al., 1991).

In order to quantify the potential for pollen-mediated gene flow, we are conducting a tiered evaluation of outcrossing in triticale. This evaluation will include the potential for outcrossing between triticale varieties, as well as between triticale and related crops. Assessing the potential for hybridization in a tiered manner was described by Raybould and Cooper (2005). This approach involves several tiers of testing, the first tier involving a simple study in the laboratory or greenhouse under conservative "worst case" assumptions. The results of tier 1 testing determine if testing moves to a second tier under more complex and environmentally realistic conditions. In testing for potential hybridization, tier 1 testing is carried out under optimal controlled greenhouse conditions where self-fertilization is prevented by emasculation and pollen is transferred manually. The removal of pollen competition through emasculation enhances the potential for hybridization. As a result, hybridization is a very conservative measure of potential gene movement. In the case of successful hybridization at the tier 1 level of testing, tier 2 assessment is recommended. Tier 2 testing includes tests for "spontaneous" hybrid production in the lab or field (Raybould and Cooper, 2005).

This research documents the results of tier 1 testing on the intra- and inter-specific outcrossing frequencies between triticale cultivars and between triticale and its relatives: common wheat, durum wheat and rye.

The frequency of outcrossing, hybrid seed production and weight, and F_1 emergence and fertility were quantified.

RESULTS

Outcrossing frequency

Approximately 2000 flowers were emasculated and hand pollinated (data not shown) under greenhouse conditions for each intra- and inter-specific cross and its reciprocal cross. The study used two triticale genotypes (cv. AC Alta and 89TT108, ABR), common wheat (AC Barrie, ABD), durum wheat (Kyle, AB) and rye (Rogo, R). Crosses were carried out over a period of 10 months. The mean outcrossing for the results obtained at each time point was determined and standard error calculated. Seasonal environmental fluctuations were anticipated for conditions such as light quality, and all representative crosses were performed at regular intervals. In addition to the inter-specific crosses, crosses between triticale genotypes and crosses between plants of a single triticale genotype were carried out. Common wheat, durum wheat and rye were also crossed to themselves to evaluate the success of emasculation and pollination. Percentage outcrossing (*OC*) was determined for each intra- and inter-specific hybridization, correcting for unsuccessful hybridizations due to factors such as mechanical damage during emasculation and pollination. In the inter-specific crosses not involving triticale, *OC* was highest in crosses between durum and common wheat where durum wheat was the female parent (52.0% ± 2.6%) (Tab. 1). In crosses involving rye *OC* was highest in crosses to durum wheat (15.3% ± 2%) where rye was the female parent.

The *OC* for reciprocal crosses between the two triticale genotypes and between triticale and common wheat, durum wheat, and rye were analyzed (Fig. 1). Crosses between the two triticale genotypes AC Alta and 89TT108 demonstrated that *OC* was not reduced compared to crosses between plants of the same genotype ($p < 0.05$), and *OC* was not affected by which triticale was used as the female parent ($p < 0.05$). In crosses between triticale and both common and durum wheat, the direction of the crosses affected the number of F_1 hybrid seeds produced, with *OC* higher when triticale was the male parent for both triticale genotypes ($p < 0.05$). A significantly higher *OC* was obtained when triticale AC Alta was the male parent in crosses with common wheat (86.0% ± 4.0%) compared to when it was the female parent (20.9% ± 1.4%). Similar results were obtained with triticale 89TT108. *OC* was higher when triticale AC Alta was the male parent in crosses with durum wheat (89.4% ± 5.7%) than when it was the female parent (1.4% ± 0.5%). Again, similar results were obtained with triticale 89TT108. In crosses between triticale 89TT108 and rye, outcrossing was higher when triticale was the male parent (21.1% ± 1.7% *vs.* 4.7% ± 0.9%). However, no difference in *OC* for reciprocal crosses between triticale AC Alta and rye was observed ($p < 0.05$) with an *OC* of 15% for crosses in both directions.

In general, the *OC* for crosses between triticale and common wheat, durum wheat, and rye were significantly lower than the *OC* obtained when plants of a triticale genotype were crossed to each other ($p < 0.05$), with the exception that the *OC* of crosses between triticale AC Alta and common wheat and durum wheat, when AC Alta was the male parent, were not significantly lower.

Seed weight and appearance

F_1 hybrid seed was harvested from all species, photographed (Fig. 2), and weighed to determine thousand kernel weight (TKW) (Tab. 2). When plants of a cultivar were crossed to the same cultivar, the TKWs were as follows: triticale AC Alta 47.6 g, triticale 89TT108 46.3 g, common wheat AC Barrie 24.5 g, durum wheat Kyle 26.6 g, rye Rogo 39.4 g. Crosses between triticale genotypes resulted in plump seed with a TKW of ≥ 45.7 g. Crosses between common or durum wheat and triticale, when wheat was the female parent, produced F_1 hybrid seeds that appeared shriveled, reflected in the low TKW. F_1 seeds produced from triticale × common wheat had a TKW of ≥ 18.5 g and appeared healthy. Seeds from the reciprocal cross (ABD × ABR) had a TKW of ≤ 5.9 g and appeared shriveled. F_1 seeds produced from durum wheat (AB) and triticale (ABR) had a TKW of ≤ 2.8 g from crosses in both directions, and all seeds appeared shriveled. The TKW of F_1 seeds for crosses between rye and triticale were also low, with a maximal TKW of 7.3 g obtained with AC Alta × Rogo, and seeds appeared shriveled.

F_1 hybrid emergence

Sprouting resistance was overcome and F_1 seed was planted at uniform depth. Emergence was recorded as a percentage of the total seeds planted (Tabs. 1 and 3). The results for emergence when plants of a cultivar were crossed to each other were as follows: triticale AC Alta 99%, triticale 89TT108 100%, common wheat AC Barrie 97%, durum wheat Kyle 88%, rye Rogo 100%. F_1 hybrid emergence was variable for inter-specific crosses between common wheat, durum wheat and rye (Tab. 1), and emergence was highest for the F_1 hybrids produced by inter-specific crosses where rye was the female parent in crosses to both common and durum wheat. F_1 emergence from crosses between the two triticale cultivars was 100% (Tab. 3). F_1 emergence for triticale × common

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Table 1. Results of inter-specific crosses between common wheat, durum wheat and rye.

♀ Parent	♂ Parent	Outcrossing (%)	F_1 hybrid emergence (%)	F_1 hybrid fertility (%)
Common wheat (ABD)	Durum wheat (AB)	12.0 (± 1.6)	45	0
	Rye (R)	0.6 (± 0.3)	0	-
Durum wheat (AB)	Common wheat (ABD)	52.0 (± 2.6)	12	0
	Rye (R)	1.0 (± 0.4)	25	0
Rye (R)	Common wheat (ABD)	4.0 (± 0.9)	67	0
	Durum wheat (AB)	15.3 (± 2.0)	88	0

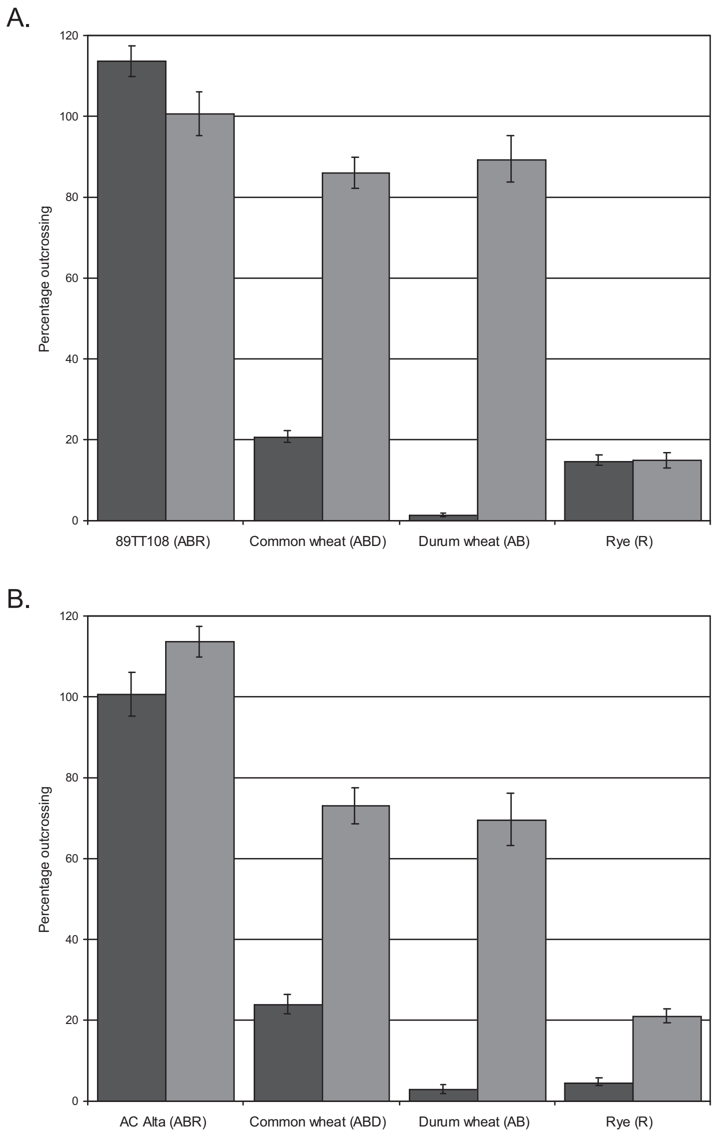

Figure 1. Percentage outcrossing (*OC*) for triticale inter- and intra-specific crosses. Outcrossing between two triticale varieties, AC Alta (A.) and 89TT108 (B.), common wheat, durum wheat and rye, was quantified for each cross and its reciprocal. Dark bars depict results obtained when triticale was the female parent in the cross, and light bars when triticale was the male parent. Mean *OC* and the corresponding standard error bars are shown.

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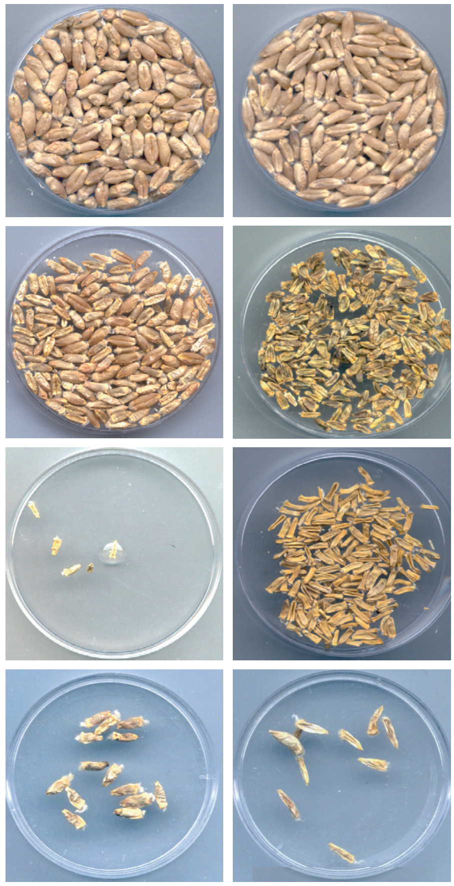

Figure 2. Appearance of F1 hybrid seeds at maturity.

wheat was > 90%, while emergence of the reciprocal cross was ≤ 1% with only one F_1 plant emerging. Seed weight and appearance were good predictors of emergence for crosses between common wheat and triticale (Fig. 2, Tab. 2). F_1 emergence for durum wheat × triticale was 0%. The reciprocal cross resulted in 0% F_1 seed emergence with AC Alta as the female parent, and 41% with 89TT108 as the female parent. F_1 emergence was 0% in crosses between rye and 89TT108 in both directions. In contrast, F_1 seed produced from crosses between rye and AC Alta where AC Alta was the female parent or male parent showed emergence of 50% and 38%, respectively. TKW was not a good indicator of emergence for any crosses between triticale and durum wheat or rye.

F_1 hybrid fertility

When the F_1 hybrid plants flowered, spike sterility was recorded. Only F_1 hybrids formed from the triticale × common wheat crosses were self-fertile, with 95% and 90% fertility recorded for the 89TT108 and AC Alta crosses, respectively. Spikes of all other hybrids did not produce seed (data not shown and Tab. 1).

DISCUSSION

This study initiates the quantification of triticale crossability through measurements of intra- and inter-specific outcrossing frequency, hybrid seed production, and F_1 emergence and fertility. Tier 1 tests of hybridization under conservative conditions, where self-fertilization is prevented by emasculation and pollen is transferred manually, provides information to make informed decisions regarding the need for further testing. Hybrid seed formation does not equate to transgene introgression, or potential gene flow, but does suggest a need for further testing. If introgression can occur and the F_1 hybrid is sterile, gene flow is prevented, but the transgenic seed

Table 2. Thousand kernel weight (TKW) of F_1 hybrid seed.

Parent 1	Parent 2	TKW (g)	
		♀ Triticale	♂ Triticale
Triticale AC Alta (ABR)	Common wheat (ABD)	20.6	5.5
	Durum wheat (AB)	2.7	2.8
	Rye (R)	7.3	3.8
	Triticale 89TT108 (ABR)	50.9	45.7
Triticale 89TT108 (ABR)	Common wheat (ABD)	18.5	5.9
	Durum wheat (AB)	2.6	0.3
	Rye (R)	3.5	4.0
	Triticale AC Alta (ABR)	45.7	50.9

Table 3. Percentage of F_1 hybrid seed emergence.

Parent 1	Parent 2	♀ Triticale	♂ Triticale
Triticale AC Alta (ABR)	Common wheat (ABD)	91	1
	Durum wheat (AB)	0	0
	Rye (R)	50	38
	Triticale 89TT108 (ABR)	100	100
Triticale 89TT108 (ABR)	Common wheat (ABD)	97	0
	Durum wheat (AB)	41	0
	Rye	0	0
	Triticale AC Alta (ABR)	100	100

could potentially be harvested with conventional food crops. However, hybrid seed carrying a transgene, even if non-viable, may be detectable and pose a regulatory concern despite its inability to contribute to transgene spread. Seed size becomes critical in this instance, because small, light seed may be left in the field as a result of the seed size and weight selectivity of harvesting machinery. If a fertile transgenic hybrid is formed, gene flow may occur *via* volunteers produced from seed lost during harvest and/or replanting of contaminated seed lots.

As expected, significant outcrossing was observed between the two triticale cultivars, indicating the potential for outcrossing between transgenic and conventional triticale crops. Observations of hybrid emergence and fertility further emphasize the potential for temporal and spatial transgene movement. The rate and distance of triticale outcrossing will be further investigated in tier 2 studies under field conditions.

Common wheat is the most widely grown and exported crop in Canada with approximately 25.0 million acres of common wheat grown annually in Canada. A high percentage of inter-specific outcrossing was observed between common wheat and triticale (Fig. 1). Outcrossing was significantly higher when triticale acted as the male parent ($\geq 73\%$) than observed when triticale was the female parent ($\leq 23\%$). Although crossing between wheat and triticale with triticale as the male parent produced seed, only a single F_1 plant emerged and was not fertile. Therefore, although outcrossing with common wheat where triticale was the male parent occurred at a higher frequency, these outcrosses rarely produced viable seed. With the exception of the single emergent F_1, these results support previous observations that hybrid seed produced from crosses between triticale and common wheat are only viable when triticale acts as the female parent in the cross (Bizimungu et al., 1998; Chaubey and Khanna, 1986; Khanna, 1990; Nkongolo et al., 1991). While crossability between triticale and common wheat is minimal, the scale of wheat acreage and the importance of this crop suggest testing should continue.

Approximately 5.6 million acres of durum wheat are grown in Canada annually and exported worldwide. Durum wheat is grown in similar areas to triticale, and flowering periods are likely to be synchronous. Outcrossing between durum wheat and triticale was primarily observed with triticale as the male parent, with a total of 509 seeds produced from 2471 pollinated and emasculated flowers; these seeds were not viable. F_1 emergence was only observed from crosses with triticale as the female parent. None of the F_1 hybrids that emerged were self-fertile. Tier 2 tests will continue to quantify seed production in the field.

Outcrossing between triticale and rye was low for crosses in both directions, indicating that the potential for outcrossing with rye is limited. Crossability was previously shown to be lower between triticale and rye than between triticale and common wheat (Lelley, 1992). The moderate emergence observed for the F_1 hybrids indicates the potential for successful hybrid production between triticale and rye, but the hybrids were infertile

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indicating that transgene spread as a result of hybridization with rye is unlikely. With the exception of the single emergent common wheat-triticale F_1 hybrid, rye was the only species to produce viable, though infertile, F_1 hybrids with triticale as the male parent. This observation illustrates the potential for pollen-mediated gene flow from triticale to rye. However, the low rate of outcrossing and the lack of fertile F_1 hybrids suggest there is minimal risk for triticale pollen-mediated gene flow to rye. Only 0.5 million acres of rye grown annually in Canada, and the majority is winter rye. Flowering synchrony between winter rye and spring triticale is unlikely. Spring rye, which may flower synchronously with spring triticale, is rarely grown, reducing the potential for gene flow under field conditions.

Triticale is a potential platform for bio-industrial products. In order to grow transgenic triticale in the field, the risk of pollen-mediated gene flow to conventional triticale and its related crops must be quantified. Because of the frequency of cereals in the western Canadian crop rotation, there is a high probability that transgenic triticale could be grown in proximity to a related crop or conventional triticale if it is released. The lack of self-fertile inter-specific F_1 hybrids produced with triticale as the male parent suggest that significant transgene spread through triticale pollen-mediated gene flow to related species is unlikely. However, we did not evaluate the mechanism of sterility and cannot reject the possibility that these self-sterile F_1 hybrids might act as female parents in outcrosses. Similarly, the hybrid pollen was not tested for its ability to successfully fertilize non-hybrid plants. Transgene movement between triticale cultivars is of a greater concern due to the production of fertile intra-specific hybrids.

AC Alta is an elite hexaploid cultivar and has been the subject of extensive breeding efforts, whereas 89TT108 is a primary triticale synthesized as an octoploid and then selfed for five generations. During this process, 89TT108 reverted to the hexaploid level. Differences were observed in the results obtained with the two triticale genotypes, AC Alta and 89TT108. For example, when AC Alta was the female parent in crosses to common and durum wheat, outcrossing was higher than that observed with 89TT108. Furthermore, AC Alta-Rye F_1 hybrid emergence was $\geq 38\%$, but no 89TT108-Rye F_1 hybrid plants emerged. In contrast, emergence of 89TT108-durum wheat F_1hybrids was 41% where triticale was the female parent, but no AC Alta-durum wheat F_1 hybrids emerged. A genetic component to crossability has been established in previous research (Bizimungu et al., 1998; Guedes-Pinto et al., 2001; Lelley, 1992; Lima-Brito and Guedes-Pinto, 1998; Nkongolo et al., 1991) and these results are not unexpected. Observations that outcrossing is influenced by differences in cultivars as well as environmental factors suggest that it may be advisable to conduct tier 2 testing with more cultivars in several environments. Furthermore, future decisions regarding the genetic background to use for transgenic applications should take into consideration the outcrossing potential of the genotype. Tier 2 testing in the field will accommodate the need to observe hybridization in a field environment with environmental fluctuations. Analysis of how the results of tier 1 risk assessment reflect on those at the tier 2 level will be valuable in making future risk assessment decisions, as well as providing valuable data on the potential for triticale pollen-mediated gene flow.

MATERIALS AND METHODS

Plant material

Plant materials used were as follows: triticale cultivar AC Alta (6x, ABR); triticale 89TT108 from CIMMYT which was synthesized from a wheat × rye cross and then selfed through five generations and confirmed to be $2n = 6x = 42$ (ABR) including 14 intact rye chromosomes (George Fedak, personal communication); common wheat cultivar AC Barrie (ABD); durum wheat cultivar Kyle (AB); and rye cultivar Rogo (R). Plants were grown in 1-gallon pots filled with soil-less Cornell mix (Boodley and Sheldrak, 1977) in a single greenhouse at AAFC Lethbridge, Alberta (49° 41' 51.91" N 112° 46' 24.82" W; elevation 909 m). Temperature was maintained at 21/18 °C and humidity was 40–50%. Photoperiod (18 h light/6 h dark) was provided by natural light supplemented with electronically determined amounts of artificial light to reduce crossing variability from September to June. Plants were staked at 3 weeks of age to prevent slumping and breaking of stems. Watering was carried out daily according to need and plants were fertilized every second week with liquid 20-20-20 fertilizer. Plants received a preventative propiconazole (Tilt®) and imidacloprid (Impower®) treatment at the 5–7 leaves stage to prevent leaf disease and insect damage.

Hybridization

Cultivar AC Alta and CIMMYT genotype 89TT108 (ABR) were crossed to Canadian cultivars of related species; AC Barrie (ABD), Kyle (AB), and Rogo (R). Similar numbers of reciprocal crosses were made with a target of 2000 emasculated and hand pollinated flowers (data not shown) for each intra- and inter-specific cross and its reciprocal cross. Inter-specific crosses among the wheat and rye species were made as well (Tab. 1). Emasculation was begun when plants were 8 weeks old

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(± 1 week) and/or just prior to anthesis: common wheat (Barrie) was emasculated when the spike was 4–7 cm out of boot; durum wheat (Kyle) at 6–9 cm; triticale (AC Alta) at 7–10 cm; triticale (89TT108) at 7–10 cm; spring rye (Rogo) at 3–5 cm. Following emasculation, 4–6 days were allowed for flowers to mature to the receptive stage where they begin to open. Scar tissue caused by the emasculation process was clipped to relieve tension on the flower and allow it to open fully. Flowers were pollinated with a spike of the desired male plant. Spikes for pollinating were collected at the following stages of development; common wheat when fresh yellow anthers were showing from centre flowers along 1/2 the length of the spike, durum wheat when pollen was showing along 3/4 of the length of spike and flowers had begun to swell slightly, triticale AC Alta when pollen was showing on no more than 1/3 the length of the spike; triticale 89TT108 when pollen was showing on only 6–8 flowers; rye when pollen was showing on 1/4 to 1/3 the length of the spike. Each week approximately 30 crosses were carried out between plants of the same cultivar to document manual pollination efficiency. Glassine bags (5 cm × 19 cm), surgical scissors, tweezers and # 1 paper clips were used for manual emasculation and pollination (Allen, 1980). In addition, emasculated spikes were bagged and not manually pollinated to document the frequency of selfing. Bags were removed 10 days after pollination. The spike was allowed to mature for 6 weeks and then dry off for 2 weeks, after which it was cut and allowed to dry for 1 week prior to threshing by hand. Seeds were collected at full maturity, counted, and weighed to determine the thousand kernel weight. Percentage outcrossing (*OC*) was determined using the following equation:

$$OC = \left[\frac{HS/F}{P}\right] \times 100$$

where HS = the number of F_1 hybrid seeds produced, F = the number of flowers emasculated and pollinated, and P is the percentage outcrossing of the female parent genotype when plants of this genotype were crossed to each other and was determined using the following equation, where S is the number of seeds produced:

$$P = \frac{S}{F}.$$

χ^2 analysis was used to determine if there was a significant effect of *OC* when triticale was the female parent compared to when triticale was the male parent. χ^2 analysis was also used to determine whether *OC* differed significantly in the intra- and inter-specific crosses when compared to the *OC* obtained when crosses were carried out between plants of a single triticale genotype. The number, quality, and thousand kernel weight of seeds were then recorded.

F_1 emergence and fertility

Sprouting resistance was overcome by placing F_1 hybrid seed in alternating refrigerator (4 °C) and room temperatures for 2 days at each temperature for 7 cycles. Seeds were placed in RootTrainers (Nursery Supplies Inc.) with one seed per cell planted at a uniform depth covered with 1 cm of cornell mix (Boodley and Sheldrak, 1977). Approximately 70 F_1 hybrid seeds were sown for each cross and its reciprocal where possible. Where crossability was low, fewer seeds were sown. Seeds were watered as required. The percentage emergence was determined as:

$$Emergence\ (\%) = \frac{F_1\ plants\ emerged}{seeds\ planted} \times 100.$$

At four weeks, plants were transferred into 1-gallon pots. When plants flowered, sterility was observed and recorded.

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