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A NEW SPECIES OF WHEAT THAT CONTINUES TO GROW AFTER HARVEST

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ABSTRACT

Crosses with hexaploid wheat and intermediate wheat grasses (*Thinopyrum intermedium*) were used to develop perennial wheat that exhibits post sexual cycle regrowth. These lines were bred to senesce fully after seed development and then regrown after a dormant cycle. Some plants however exhibited continuous growth in areas with mild winters and wet autumn months such as the Pacific Northwest areas of Washington State in the United States. Plants with continuous growth were at first discarded but are now being selected as a possible forage and grain multi-use crop for animal production. Forage quality is as high as wheat hay but the tonnage per acre is much greater. The chromosome constitution of the lines are stable at 56 chromosomes. Forty

two are wheat and the other 14 are at this point unidentified. There are awned and awnless types and seed colour is red, white or blue. Height can exceed 2 metres. These lines seem to have great promise in short term rotations (2 to 3 years) where large amounts of organic matter is needed and flexibility on end-uses ranging from straw to hay to grain is desired.

Keywords: wheat, *Thinopyrum intermedium*, awn, Salish Blue

Beginning in 1995, crosses with hexaploid wheat and intermediate wheat grasses (*Thinopyrum intermedium,* -Host- Barkworth & Dewey) were used to develop perennial wheat that exhibits post-sexual cycle regrowth. These lines were bred to senesce fully after seed development and then regrow after a dormant cycle. Some plants however exhibited continuous growth in areas with mild winters and wet autumn months such as the Pacific Northwest areas of Washington State in the United States. Plants with continuous growth were at first discarded but are now being selected as a possible forage and grain multi-use crop for animal production. We have named an exemplary breeding line from this population "Salish Blue." Salish Blue is an awnless, blue-seeded derivative of these breeding efforts. Forage quality is as high as wheat hay but the tonnage per acre is much greater. The chromosome constitutions of the lines are stable at 56 chromosomes. Forty two are wheat and the other 14 are at this point *Th. intermedium* of unidentified homoeology groups. There are awned and awnless types and seed color is red, white or blue. Height can exceed 2 metres. These lines seem to have great promise in short term rotations (2 to 3 years) where a large amount of organic matter is needed and flexibility on end-uses ranging from straw to hay to grain are desired.

In situ Hybridization

We performed fluorescent genomic *in situ* hybridization (FGISH) on root tip cells from Salish Blue using biotinylated genomic DNA from *Thinopyrum ponticum* Barkworth and Dewey as a probe. gDNA of *Th. ponticum* was used because our previous studies indicated that the 10n *Th. ponticum* genome is derived from each of the principal diploid ancestral genomes for all of the *Thinopyrum* species and thus is an ideal all-purpose probe for detecting *Thinopyrum* chromatin (Arterburn *et al.* 2011). Signal detection was accomplished using avidin-fluorescein and biotinylated anti-avidin. The FGISH probe bound strongly to the alien chromosomes, even compared to positive controls (metaphase cells of the *Thinopyrum* amphiploid AgCS). Fluorescent signals clearly indicate that 14 of the 56 chromosomes of Salish Blue are of alien origin, and the efficacy of probe binding indicates a member of the *Thinopyrum* species as the alien donor (Figure 1). Six replicates produced identical results, suggesting that Salish Blue is stable at 56 chromosomes. Because the wild parent of Salish Blue is the hexaploid *Th. intermedium*, FGISH is insufficient to determine which specific chromosomes of

the parent have been retained in this amphiploid and which were lost during backcross breeding efforts. Because our previous investigations have confirmed that *Th. intermedium* is a descendant of *Th. elongatum* (2n = 14, EE), *Th. bessarabicum* (2n =14, JJ) and *Pseudoroegneria spicata* (2n = 14, StSt), we sought a means to use DNA evidence to indicate which specific homoeologous pairs from these donor genomes are present in Salish Blue (Arterburn *et al.* 2011).

MARKER ANALYSIS

We sought to identify polymorphisms in Salish Blue that correspond to known polymorphic loci on specific chromosomes from the E, J or St genomes. To accomplish this, we analyzed 24 DNA markers that have been localized to specific chromosomes in those diploid *Thinopyrum* species that are related to likely alien chromosome donors of Salish Blue (e.g. *Thinopyrum intermedium*). The markers analyzed were a combination of SSR polymorphisms detected on chromosomes of the E genome of *Th. elongatum*, and cleaved amplified polymorphic sequence (CAPS) polymorphisms detected on chromosomes of the St genome of *Ps. spicata* (Hu *et al.* 2012; Mullan *et al.* 2005). There are a further 20 SSR and CAPS markers available that we intend to assay. The results of this marker analysis can be seen in Table 1.

During this analysis, we identified five polymorphisms specific to Salish Blue. Curiously, only two of these amplicon/fragment size polymorphisms matched with a putative alien donor. A 315 bp polymorphism, amplified by SSR primers associated with chromosome 1E is shared between Salish Blue and the hexaploid *Th. junceum*. A 435 bp polymorphism, amplified with SSR primers associated with chromosome 3E, is shared between Salish Blue and the diploids *Th. elongatum* and *Th. bessarabicum*. While not conclusive evidence on its own, this suggests that two alien pairs in Salish Blue belong to homoeology groups 1 and 3 respectively. Two other polymorphisms detected in Salish Blue were amplified by primers associated with chromosome 7E, although the fragment sizes were subtly different from those detected in *Th. elongatum*, possibly due to additional microsatellite expansion in Salish Blue. A restriction cut-site polymorphism was detected in Salish Blue for a CAPS marker associated with chromosome 1St, although the fragment generated was distinct from the polymorphism associated with *Ps. spicata*.

This preliminary evidence indicates that alien chromosomes of homoeology groups 1, 3 and 7 may be present in Salish Blue. Additional marker and sequence work will be able to confirm this as well as elucidate the origins of the remaining four chromosomes pairs present in this line.

NUCLEAR GENE SEQUENCING EFFORTS

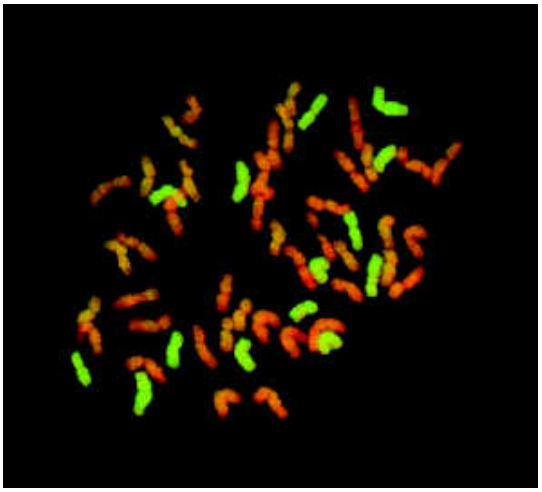
To provide further evidence of *Th. intermedium* chromosomes in Salish Blue, we are in the process of cloning and sequencing the various alleles of the beta-amylase I (bmyI) gene and the granule-bound starch-synthase (GBSSI) gene present in this amphiploid line. We have used this method successfully in the past to detect genome origins and have identified specific polymorphisms

associated with distinct *Th. intermedium* (Arterburn *et al.* 2011). Identification of bmyI and GBSSI alleles matching those found in *Th. intermedium* will also confirm the presence of alien homoeology groups 4 and 7, respectively, in Salish Blue. This method is work-intensive in amphiploid samples such as Salish Blue because it requires sequencing of many clones from multiple PCR products to ensure that all alleles are detectable and free of background heterogeneous signal.

CONCLUSION

The genomic origin of the additional 14 chromosomes will lead to the naming of a new species of wheat. This new species and improved varieties within this species will have value in perennial wheat breeding programmes. Identification of the chromosomes will also lead to more efficient mapping and tagging of genes that control traits of interest such as regrowth and stay-green.

FIGURE 1. FLUORESCENT GENOMIC *IN SITU* HYBRIDIZATION (FGISH) OF SALISH BLUE



Identification of the chromosomes will also lead to more efficient mapping and tagging of genes controlling traits of perenniality in crops

TABLE 1. SUMMARY OF SSR AND CAPS MARKER ANALYSIS. MARKERS WHICH DETECTED NO *THINOPYRUM* POLYMORPHISMS ARE EXCLUDED

MARKER NAME	CHROMOSOME LOCATION	MARKER TYPE	POLYMORPHISMS DETECTED IN OUR STUDY
MWG634	4ES	STS	*Th. elongatum* = 450 bp
			Salish Blue = No polymorphic band
Xedm17	1E	SSR	*Th. elongatum* = 250 bp
Xedm28	2ES	SSR	*Th. bessarabicum* = 200 bp
			Th. elongatum = 200 bp

MARKER NAME	CHROMOSOME LOCATION	MARKER TYPE	POLYMORPHISMS DETECTED IN OUR STUDY
Xedm54	5ES	SSR	*Th. elongatum* = 185 bp
			Th. elongatum = 185 bp
			Salish Blue = No polymorphic band
Xedm74	1EL	SSR	*Th. bessarabicum* = 325 bp and 285 bp
			***Th. junceum* = 315 bp and 285**
			Salish Blue = 315 bp
			Th. elongatum = 275 bp
Xedm105	7EL	SSR	*Th. elongatum* = No polymorphic band
			Th. bessarabicum = No polymorphic band
			Salish Blue = 340 bp
Xedm109	3E	SSR	***Th. elongatum* = 435 bp**
			***Th. bessarabicum* = 435 bp**
			Salish Blue = 435 bp
Xedm149	6EL	SSR	*Th. elongatum* = 175 bp
Xedm156	7ES	SSR	*Th. elongatum* = 260 bp
			Th. bessarabicum = 270 bp and 295 bp
			Salish Blue = 280 bp
TNAC1001	1St	CAPS	Salish Blue = 275 bp
TNAC1102	2St	CAPS	*Th. bessarabicum* = 975 bp
			Th. junceum = 975 bp
			Th. intermedium = 1 000 bp
			Salish Blue = No polymorphic band
TNAC1178	2St	CAPS	*Th. bessarabicum* = 900 bp
			Th. intermedium = 900 bp
			Salish Blue = No polymorphic band
TNAC1248	3St	CAPS	*Th. elongatum* = 800 bp
			Th. intermedium = 750 bp
			Salish Blue = No polymorphic band
TNAC1408	4St	CAPS	*Th. intermedium* = 700 bp
			Salish Blue = No polymorphic band
TNAC1485	5St	CAPS	*Th. elongatum* = 1 000 bp
			Th. bessarabicum = 640 bp
			Th. intermedium = 640 bp
			Salish Blue = No polymorphic band
TNAC1674	6St	CAPS	*Th. elongatum* = 550 bp
			Th. bessarabicum = 775 bp
			Th. intermedium = 525 bp
			Salish Blue = No polymorphic band

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