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Progress in Developing Perennial Wheats for Grain and Grazing

for ***Crop and Pasture Science*** special issue

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Abstract

Dual-purpose cereals have been important for increasing the flexibility and profitability of mixed farming enterprises in southern Australia, providing winter feed when pasture dry matter production is low and recovering to produce grain. A perennial dual-purpose cereal could confer additional economic and environmental benefits. For the first time we establish that selected perennial cereals were able to achieve up to ten-fold greater below ground biomass at the end of a second growth season compared to a resown annual wheat. We review and expand the data on available diverse perennial wheat-derived germplasm, confirming that perenniality is achievable but that further improvements are essential through targeted breeding. Although not yet commercially deployable, the grain yields and dry matter production of the best performing lines approach the benchmarks predicted by Bell *et al.* (2008) to achieve profitability. After reviewing the genomic composition of the most promising wheat-derived perennials, we conclude that the best near term prospect of a productive breeding program for a perennial wheat-derived cereal will utilise a diploid perennial donor species, and the most promising one thus far is *Thinopyrum elongatum*. Furthermore the breeding should be aimed at complete wheat-*Th.elongatum* amphiploids, a hybrid synthetic crop analogous to triticale. We advocate the generation of many primary amphiploids involving a diversity of *Th.elongatum* accessions and a diversity of adapted annual wheat cultivars. Primary perennial amphiploids would be intercrossed and advanced with heavy early generation selection for traits such as semi-dwarf plant height, non-shattering heads, large seed size and good self-fertility, followed by later generation selection for robust perenniality, days to flowering, grain yield, forage yield, stability of grain yield across seasons, and disease resistance.

1. Introduction.

~~Global population and demand for food are increasing, while arable land is limited and faces increasing risk of degradation. To ensure food and ecosystem security, would be served by the~~ development of perennial grain crops ~~could provide~~ more options under diverse and generally more marginal conditions (Glover et al 2010 a,b). Perennial grains should offer more stable surface cover against soil erosion, and improved nutrient balance against soil acidification, rising water tables and salinity, thereby improving ecosystem services. Systems which include perennial grains should also offer farmers greater flexibility and diversity of enterprise, and greater stability of income.

In Southern Australia, deep-rooted native perennial vegetation was removed, and replaced with shallow-rooted annual cropping systems based on wheat and self-regenerating annual legume pastures for sheep. System stability was threatened by water and nitrates moving below the root zone of annual crops, resulting in water table rise, acidification, and salinity, on soils which were often already fragile and low in fertility. Development of perennial wheat should offer prospects to address these concerns. Bell et al (2008) used MIDAS, a bioeconomic model of a mixed crop/livestock farming system, to explore what role perennial wheat may play in the system. Perennial wheat used solely for grain production was not selected as part of an optimal farm plan under the standard assumptions. In contrast, dual-purpose perennial wheat that produces grain and additional forage during summer and autumn could increase farm profitability substantially (A\$20/ha over the whole farm) and 20% of farm area was selected for perennial wheat production on the optimal farm plan under standard assumptions. Forage from perennial wheat replaced stubble over summer and grain supplement at break of season and increased farm stock numbers. The additional value added by grazing reduced the relative yield required for perennial wheat to be profitable. This analysis suggested dual-purpose perennial wheat would be a profitable option for mixed crop/livestock farmers.

The challenges in developing a perennial wheat for Australia were reviewed by Bell et al (2010), following ground-breaking work in the United States to generate amphiploids between annual *Triticum* and perennial *Thinopyrum* species (Murphy et al 2009; Cox et al 2010). Experimental efforts in Australia commenced by evaluating a diverse array of putative perennial wheat derivatives including germplasm imported from the US perennial wheat programs, Russian and Chinese wide cross germplasm, and assorted lines from the Australian Winter Cereals Collection. This initial evaluation of over 150 wheat x wheatgrass derivatives assessed capacity to regrow post-harvest, and yield grain over successive years, thereby identifying characteristics common to surviving breeding lines (Hayes et al 2012). Several entries persisted to produce grain over three successive years. Regrowth was associated with the presence of at least one whole-genome equivalent (14 chromosomes) from the perennial donor species. This research established that developing a perennial wheat may be feasible, though existing germplasm was not intended for Australian conditions, nor was it yet sufficiently developed to be deployed commercially.

The objective of this paper is to build upon the foundations laid by Hayes et al (2012), by reporting further on field evaluations of perennial wheat derivatives, including previously untested germplasm, and an additional fourth year of those entries surviving from the original report. In addition, three more-detailed experiments are examined, which used a common set of six genotypes to evaluate forage-biomass production under serial defoliation, changes in root-shoot partitioning of dry matter over regrowth cycles, and dehydration tolerance and survival under severe water deficit and rewatering. Based on this evidence, the paper then explores breeding approaches suitable for generating a perennial wheat for Australian farmers. The paper concludes by identifying priorities for perennial wheat research to achieve the release of a perennial wheat to Australian farmers by 2030.

2. Current Germplasm – Perenniality and Grain (Experiment 1)

Previously we reported our assessment in the field of a large collection of germplasm bred internationally by crosses between annual wheat and perennial Triticeae species, many already selected for some capacity to regrow after grain harvest. We report here an extension of that study including an expansion of germplasm entries. Hayes *et al.* (2012) reported 4 hybrid derived entries to be still surviving in June 2011 after sowing in June 2008 (Fig. 1a in Hayes *et al.* 2012). We have since been able to monitor the longevity of these entries beyond the measurement period reported in the previous paper, though by years 3 and 4 entries were often represented by only single individual plants in the original short rows. Entries CPI 147251b, a *Th. elongatum* derivative, and CPI 147257b, a *Th. intermedium* derivative, persisted and yielded grain over 4 consecutive years (Table 1). Only the *Th. ponticum* control, Dundas, persisted beyond the fourth summer. Grain yields tended to decline with time for all experimental lines, a reflection of increased mortality with time. The fact grain yields were recorded for 4 consecutive years is a significant biological finding and highlights the likely potential to select promising lines with enhanced longevity by expanding the genetic diversity within one or more of the hybrid breeding strategies represented in the experiment.

The performance of 58 entries not reported in Hayes *et al.* (2012) were monitored in a field experiment from May 2011 – June 2013, referred to as Experiment 1 hereafter, with the results reported below. The experiment had two objectives; first, to test the longevity and grain attributes of novel lines not previously tested, including 3 previously unseen lines from the Centre for Molecular Biotechnology, Moscow, Russia. This is consistent with a key objective of the Hayes *et al.* (2012) study which aimed to benchmark the performance of existing perennial wheat germplasm in order to determine promising donor perennial species, desirable genomic compositions, and the level of improvement still required to achieve a commercially deployable perennial crop. The second objective of Experiment 1 is hereafter referred to as the ‘heterogeneity test’. A limitation of the Hayes *et al.* (2012) study was that most lines included in that evaluation needed to be imported into Australia and therefore grown in quarantine glasshouses prior to field evaluation. Only seed harvested from the quarantine glasshouse could be included in field experiments. Due to the large number of lines being imported, only 2 seeds per line (marked *a* and *b*) were grown in the glasshouse and the authors were forced to assume that the performance of the progeny of those 2 seeds was an accurate assessment of the potential of that entire line. However, some early data showed significant differences between the *a* and *b* progeny of particular lines suggesting that there was genetic heterogeneity in the imported seed which would not necessarily have been assessed in the initial experiments. This would particularly have been the case for the *Th. intermedium* accessions because this is an outcrossing species coming from mixed stands. The amphiploids and partial amphiploids generally appeared to be self-pollinating like wheat itself. The heterogeneity test in Experiment 1 described below was aimed to quantify the likely variability within 3 promising lines to determine whether a more comprehensive screening of previously developed germplasm was warranted.

The field experiment was sown at the Agricultural Research and Advisory Station, Cowra, NSW (S 33°48.211' E 148°42.236'; 385 m altitude) on 3 May 2011. The experiment was a randomised complete block design with 3 replicates. Twenty five seeds of each ‘entry’ were sown by hand in a single 1 m row (plot) and were managed similarly to the previous ‘row nurseries’ described in Hayes *et al.* (2012). A total of 84 entries were sown in Experiment 1; 58 previously untested entries as well as 26 controls or ‘promising’ entries from the initial evaluation study. The entries are detailed in Table 2. No irrigation was applied to the site during the experimental period.

One key difference between this and the previous row nurseries was the method used to quantify post-harvest regrowth. Previously, regrowth was assessed using an uncalibrated visual scoring system which was adequate to determine relative differences between treatments at a given

sampling but of course has limitations when comparing results between experiments, particularly where different operators were involved. The current study assessed regrowth more objectively with an adaptation of the plant basal frequency measure used commonly by scientists assessing perennial pastures (e.g. Li *et al.* 2008). The method involved laying a 1 m quadrat, split into forty 25 x 25 mm squares, immediately adjacent to the sown row. The operator counted the number of squares which were adjacent to a perennial plant base, either a crown or tiller emerging from the soil surface, expressed as a percentage. This approach differs from standard measures of basal frequency as the quadrat lies beside the plot rather than on top of it. However, this adaptation was required because it becomes impossible to ascertain the location of the base of perennial plants by looking down through the canopy of a tall cereal crop.

Plant density of each plot was assessed 6 weeks after sowing. The date of anthesis of each plot was recorded and time between sowing and anthesis in year 1 calculated to determine maturity of individual entries. No defoliation of single rows occurred until grain harvest in the summer of year 1. At harvest, plant biomass was cut at 100 mm from the soil surface. The date of harvest varied according to the maturity of the individual entry and was undertaken when the stem immediately below the majority of spikes had senesced and turned a brown colour. The harvested biomass was dried at 60 °C and weighed to determine dry matter production. Stem mass of 20 representative tillers was recorded for each plot and length of 5 representative tillers recorded. Spikes were then removed and the grain threshed in a Hanford stationary thresher (Beverley, South Australia) to determine total grain weight, 1000 kernel weight and harvest index (HI). Post-harvest regrowth was monitored using the technique described above in the months following grain harvest. The first regrowth score reported below (Table 3) is the average of assessments taken on 3 dates during the first summer and following autumn (i.e. the period prior to the second winter growing season), then averaged across the three replicate rows. The second regrowth score (Table 4) is an assessment of survival over the second summer and is derived from only one assessment date undertaken in June 2013 and averaged across three row replicates. First year grain yield is expressed as g/plant to account for different plant densities between plots. However, due to the inability to monitor plant number as they mature, grain yields beyond the first summer are expressed on a g/m (or g/plot) basis.

First year grain attributes and post-harvest regrowth during and after the first summer are reported in Table 3. The highest yielding entries in the first summer were the two conventional wheat cultivars, Wedgetail and Naparoo. Entries such as 6754, 6755 and OK7254112 had a similar cumulative grain yield as the wheat controls after only two years in addition to their exhibiting relatively high post-harvest regrowth scores (Fig 1). Some new wheat × wheatgrass derivatives were able to achieve good levels of post-harvest regrowth, such as entries 915, 916 and CPI-147244c, but grain yields were only 19- 32% of that achieved by the control, Wedgetail. Similarly, some new lines such as CPI-147235c, CPI-149796 and CPI-149800 were able to achieve approximately 75% of the grain yielded by Wedgetail in year 1, but these lines had nil or negligible regrowth beyond the first harvest (Table 3). The performance of the following lines are notable as these were able to achieve a grain yield > 40% that of Wedgetail as well as a regrowth score in excess of 25% following the first summer: OK7211542, 11955, CPI-147236a, 6755, CPI-147247a and 6754. Each of these entries was previously identified as being of interest in the initial evaluation (Hayes *et al.* 2012). CPI-147244d and CPI-147244e were the only 'new' lines to achieve this combination of first year grain yield and regrowth over the first summer.

As in the Hayes *et al.* (2012) evaluation, variability was a common theme in the assessment of these new lines. For any generalisations made about the agronomic characteristics of the novel germplasm, there were invariably exceptions to the rule. For example, there was no correlation between first year grain yield and post-harvest regrowth over the first summer. Maturity of the novel germplasm tended to be longer than for the conventional wheats, except for 6754 and CPI-149799 which had similar maturity to the earliest maturing control, Wedgetail. Harvest index was

usually lower for the novel germplasm than for the annual wheat controls, except for CPI-149796 which had a HI similar to both controls. The novel germplasm tended to have taller tillers than the wheat controls, but there were many examples of entries where this generalisation did not apply (Table 3). Similarly, there were a number of examples of novel entries that had similar grain size as the conventional wheat controls. Some of the novel entries produced a grain yield in the autumn when plants were approximately 12 months old, only 3-4 months following the main summer grain harvest in year 1 (Table 3).

By the end of the second summer, the only entries surviving were the pure *Th. intermedium* selections (entries CPI-1480: 37-62), as well as a small number of surviving plants from the CPI-147235a and 6754 lines. This was a higher level of mortality than was recorded in the previous evaluation and we have shown some of the same lines which failed to survive into year 3 in Experiment 1 were capable of surviving for 4 years when sown at the same site several years previously. The likely reason for this apparent inconsistency is the different seasonal conditions experienced during the period 2008-13. The second summer (2012/13) experienced during Experiment 1 happened to be one of the hottest summers on record for that location which was in contrast to some of the wettest summers on record experienced during 2010/11 and 2011/12.

The *Th. intermedium* selections are worthy of special consideration given their superior longevity relative to the wheat × wheatgrass derivatives (Table 4). Some entries, such as CPI-148037 had significantly lower regrowth scores than other *Th. intermedium* entries indicating that even within the pure perennial grass cohort there is substantial opportunity to select for enhanced longevity. However, all of the *Th. intermedium* entries had a 1000 kernel weight of 5.9-7.8 g compared to 35.2-40.3 g for the wheat controls (Table 3); a 6-fold difference. This highlights the substantial challenge remaining in developing pure *Th. intermedium* lines as viable perennial cereal crops.

The heterogeneity test revealed that the relative performance of the 'a' and 'b' entries of the CPI-147235, CPI-147236 and CPI-147244 lines measured in Experiment 1 were generally consistent with that reported in the initial evaluation (Hayes *et al.* 2012). For line CPI-147235, entry a in the initial evaluation showed considerable capacity for regrowth in contrast to entry b which did not regrow at all; consistent with Experiment 1. Entries c – i were not superior to entry a in terms of regrowth (Table 3), though entry c had significantly higher 1000 kernel weights than either entries a or b. Some entries within this line had lower grain yields or taller tillers or later maturity than either entries a or b, indicating the a and b lines did not encompass the full range of variability within this line; however, these characteristics are unlikely to be desirable in the development of a perennial cereal crop. **Tall plants are more prone to lodging and therefore detrimental to grain harvesting.** A similar story exists for line CPI-147236 where in both Experiment 1 and the initial evaluation, both a and b entries showed reasonable capacity for post-harvest regrowth. Table 3 suggests that the a or b entries were as good as any entry within that line for agronomic parameters measured. For the final grouping, line CPI-147244, entry e had significantly greater regrowth over the first summer whilst achieving a grain yield in year 1 that was 60% of the grain yield of Wedgetail. In this example entry e was able to combine the superior grain yield of the a entry whilst surpassing the impressive regrowth capacity of the b entry. In this regard, the initial evaluation underestimated the potential of this particular line as a dual purpose perennial crop. However, given no entries from this line persisted beyond the second summer (in contrast to CPI-147235a for example; Table 4), there is still no compelling case to suggest that significantly superior germplasm exists in the cohort of material still with Australian Quarantine compared to the broad range of material ~~evaluated~~ considered within the initial evaluation (Hayes *et al.* 2012).

3. Current Germplasm – Forage Biomass (Experiment 2)

The use of dual-purpose cereals has been an important strategy for increasing the flexibility and profitability of mixed farming enterprises in southern Australia. The ability of these crops to increase

feed availability in winter, when dry matter production from pastures is low and then recover to produce grain yield has been well documented (McMullen and Virgona 2009). For perennial cereals, their dual-purpose ability will also be important for profitable adoption in a farming system (Bell *et al.* 2008; Reeling *et al.* 2012). Flexibility in being able to change between forage production and grain recovery may also be an advantage of perennial crops in adapting to variable weather patterns from predicted climate change (Soussana *et al.* 2010).

In annual crops a large proportion of photosynthetic products and nutrient accumulation are diverted into grain production. In perennial species there is a significant allocation of these resources into perenniating structures as well as grain development. A desktop study of whole farm budgets has suggested that for perennial cereals to be economically viable, they would need to achieve grain yields at a minimum of 40% of annual wheat and offset the lower grain recovery by providing an extra 800 kg/ha of dry matter above the forage production of an annual crop (Bell *et al.* 2008)

Consequently, an experiment was sown in April 2011 at Cowra, to compare grain yield and dry matter production of perennial wheat hybrids (Newell *et al.* 2013c). The experiment included *Triticum* spp. x *Thinopyrum* spp lines: 11955; CPI-147235a; CPI-1472280b; and OK7211542, the dual-purpose annual winter wheat cv. EGA Wedgetail (*Triticum aestivum*: Wedgetail); and the perennial grass *Th. intermedium* (line CPI-148055) which are all detailed in Table 2. Note that the same six entries were also used in Experiments 3 and 4 which follow. CPI-148055 was included in Experiment 1 above and although was not the line with the highest regrowth score, was the *Th. intermedium* selection with the highest 1000 kernel mass (7.8 g; Table 3). Each entry was defoliated by cutting to simulate four grazing intensities of nil (D₀; i.e., grain-only), one defoliation (D₁), two defoliations (D₂), and two defoliations followed by a simulated hay cut (D₃) This study demonstrated the lower grain yield potential of the perennial hybrid entries compared to annual wheat. Under nil defoliation the yield of the majority of hybrid entries was less than 40% of the annual wheat control Wedgetail (Figure 2). However, as defoliation intensity increased, the yields of most perennial entries exceeded 40% of the annual wheat, particularly in the D₃ grazing treatment. This demonstrates that perennial wheat lines were less sensitive to defoliation than annual wheat in terms of grain yield.

The grazing annual wheat cultivar, Wedgetail, produced greater levels of biomass in the winter-spring growing season compared to the other entries under all defoliation intensities (Table 5). However, post-harvest regrowth by the perennial entries allowed substantial biomass production in late summer and autumn. This cumulative biomass exceeded the production from the annual wheat cultivar from the previous spring, particularly from entries 11955 and OK 72. There was also better dry matter production by the perennial grass entry during the autumn period, compared to the previous spring, presumably due to its already established roots and crown. Cumulative biomass production across both spring and summer, in most defoliation treatments and perennial entries, exceeded the dry matter produced by Wedgetail (Table 5).

Defoliation intensity had no significant effect on plant survival post grain harvest. However, some entries suffered greater levels of plant mortality post harvest and into the second growing season ($P < 0.001$, Table 6). The perennial grass entry maintained its plant numbers throughout the assessment period. All remaining plants from the April assessment date survived through to maturity in the second year post sowing. Table 6 reflects plant survival in the field of all 6 entries towards the end of the first 12 months post sowing.

This study has shown that among the early generation perennial wheat derivatives available, there is promise to develop dual purpose crops. Although not yet commercially deployable, their grain yields and dry matter production approach the benchmarks for perennial cereals as described by Bell *et al.* (2008). Vigorous autumn regrowth among wheat-*Th. elongatum* derived perennials, as well as a perennial rye, has also been observed in one year of an informative study by Jaikumar *et al.* (2012). It is possible that with further selection and breeding, higher dry matter production and grain yields could be achieved. However, selection for improved grain yield will need to be tempered by the

need to maximise plant longevity to ensure post-year 1 production is maintained or improved. All wheat × wheatgrass derivatives showed an incremental decrease in plant density within 12 months of sowing. This supports the postulation in Hayes *et al.* (2012) and in Experiment 1 above that declining grain yields of perennial wheat lines with time is more a reflection of plant mortality than of intrinsic yield decline of the germplasm. Therefore, in future breeding initiatives, a key aspect of improving grain yields of elite germplasm will be to improve plant longevity. The effect of defoliation on grain yield and plant survival in subsequent years is still under investigation

4. Current Germplasm – Root/Shoot DM Partitioning (Experiment 3)

Under Australian conditions, to fulfil the ‘perennial wheat concept’ will require plants to be tolerant to water deficit and adaptable to poor soil conditions, in order to survive and regrow in the next cycle, while presumably providing an environmental advantage by capturing resources normally lost below the root zone of the annual wheat. The desirable attributes of perennial wheats with these levels of adaptability may include deeper roots, increased root length density and root longevity for water and nitrate acquisition, facilitated perhaps by an altered priority in dry matter allocation as a result of the perennial growth habit. Dry matter partitioning will likely be important in perennial wheat, since at different stages of the life cycle, priorities may need to alternate between forage and grain production on one hand, and investment in roots and perenniating structures on the other. Currently, there is scant evidence whether any of the currently available perennial wheat amphiploids have any of these traits (Table 2).

We report here on the first study of the relative investment in above-ground and below-ground biomass and its partitioning across two growing seasons using four selected wheat-derived perennial amphiploids (OK7211542, CPI-147280b, CPI-147235a, 11955), compared with an accession of *Th. intermedium* (CPI-148055), and a long-season adapted annual wheat cv. Wedgetail (*T. aestivum*), which as a resown annual, was present for comparison during wet seasons only. Note these are the same six entries used in Experiments 2 and 4, which were also present in Experiment 1.

Single plants were grown in 150 cm PVC tubes with a 10 cm diameter in an open-top and side-netted enclosure at ambient conditions. The experimental design was composed of 6 genotypes, 4 harvests and arranged in 3 replicates for a total of 72 pots. All 6 genotypes were sown on May 2011 and they were watered every 2 weeks and fertilized monthly through the duration of their life. At harvest time the shoot was cut at the soil surface and separated into crowns, leaves, stems and spikes. The soils cores were separated in 10 cm intervals and their roots washed separately. All plant samples were dried in a dehydrator at 70 °C. For the purposes of this report we present the combined above and below ground dry matter.

During the first year the 1st harvest was done at anthesis (GS 65) (between October and November 2011) but was dependent upon the growth stage of the individual genotype. The 2nd harvest was done at the ripening stage (GS 89) (between November and December) and again it was dependent upon the growth stage of the individual genotype. These 2 harvests represented the plant growth during the ‘wet season’ of the first year (WS1), the typical wheat growing season in South Eastern Australia. At the 2nd harvest all remaining plants were also defoliated at 8 to 10 cm from the soil surface. This would allow quantification of the re-growth potential of genotypes during the summer and their recovery during year 2.

After the summer season (dry season: DS1) the 3rd harvest took place in April 2012 when most of genotypes were at anthesis (GS 65) except for the annual wheat which was not grown during the dry season. Again, the remaining pots were also defoliated as described above. Also, the annual wheat cv. Wedgetail was re-sown in May 2012. The 4th and final harvest took place at maturity (GS 89) and again it was dependent upon the growth stage of the individual genotype and it ranged between

November and December 2012. This harvest represented plant growth during the 'wet season' of the second year (WS2).

The above- and below-ground dry matter at all four growth stages for annual wheat cv. Wedgetail, perennial grass (CPI-148055) and the four wheat-derived perennial amphiploids are shown in Figure 3. On average, above-ground dry matter increased from about 25 to 40g/plant from flowering to maturity in Wet Season 1 (WS1), but dry season (DS1) regrowth was only about 15g/plant. In Wet Season 2 (WS2), average dry matter increased to 50g/plant. Root dry weight was similar among entries until the second wet season (WS2).

The annual wheat, Wedgetail, had less above - ground dry matter than other entries at flowering and maturity in Wet Season 1 (WS1), though at maturity, its dry matter was reduced by bird damage. In the second wet season (WS2), Wedgetail had similar above-ground dry matter to its production in the first wet season (WS1). The perennial grass CPI-148055 lagged in dry matter behind the perennial wheat derivatives in Wet Season 1(WS1), was similar to them in dry-season (DS) regrowth, but by the second wet season (WS2), substantially increased its dry matter above-ground, and in particular, below ground.

The perennial wheat derivatives produced more dry matter than Wedgetail and CPI-148055 at flowering in Wet Season 1 (WS1), but by maturity in that season, CPI-148055 was recovering after a slow start, and by the end of dry- season (DS) regrowth, CPI-148055 was similar in above-ground regrowth to the four amphiploids.

For root dry weight, there was little difference among entries during wet season 1 (WS1) and dry season (DS) regrowth, although, by the end of dry season regrowth, CPI-148055 had a tendency to a greater root dry weight. By maturity in the second wet season (WS2), however, CPI-148055 showed substantially greater root dry weight than the four amphiploids, which in turn had more root dry weight than Wedgetail. Consequently, CPI-148055 at maturity in wet season 2 (WS2) was similar in above- ground dry matter to CPI-147235a, the heaviest of the perennial wheat derivatives, but greatly exceeded it in root dry weight.

This study showed that wheat-derived perennial amphiploids over time invested in greater root biomass compared with annual wheat, and their root systems were able to regenerate over growing seasons which was a similar response to the perennial grass, though to a lesser extent. This ability to persist and increase root biomass may potentially provide increased soil water extraction during the dry season, providing an environmental advantage as a result of its perennial characteristics. Research is underway to quantify these differential responses.

5. Current Germplasm – Dehydration Tolerance

In most of southern Australia the level of stress due to water deficit over the summer is usually so severe that it precludes growing crops over this season unless they receive the majority of their moisture requirements from irrigation. Temperate perennial herbaceous plants able to survive these summers generally have to express one or a combination of the traits of summer dormancy (Volaire and Norton 2006), dehydration avoidance (Norton *et al.* 2012) or dehydration tolerance (Volaire *et al.* 2001) to ensure survival. The perennial wheat germplasm in our studies showed no evidence of expressing summer dormancy. Consequently a trial was established to evaluate and compare the perennial wheat derivative lines CPI-147280b, CPI-147235a, OK7211542, '11955', *Th. intermedium* '055' and *T. aestivum* cv. Wedgetail for dehydration tolerance, the same six entries as in Experiments 2 and 3 above.

Following the screening protocols of Volaire *et al.* (2001), and to avoid the possibility of confounding dehydration tolerance with summer dormancy, the trial took place in a heated glasshouse in winter (early July to early September) at CSIRO, Canberra, Australia. Twenty two plants of each line were

grown from seed in 0.6 m deep tubes (0.1 m diameter; two plants per tube) with each containing approximately 4.5 kg (oven dried) of substrate consisting primarily of river sand. The drying cycle to which the plants were subjected did not commence until plant roots were observed at the bottom of the tubes. The soils were then brought to field capacity by watering generously and allowing tubes to drain for 24 hours. The weight of all tubes was taken and assumed to represent field capacity. The bottoms of the tubes were then sealed and the drying cycle was commenced. Four tubes of each line were weighed every second or third day to estimate plant water use. Eight plants (four tubes) of each of the lines were maintained at or near field capacity as control plants in an adjacent block throughout the duration of the trial.

Tubes were subjected to a drying cycle of between 50 to 58 days depending upon the genotype. The aim with tube dehydration was to achieve approximately 50% mortality in each line although this can always only be estimated because the last surviving organ of these grasses, the apical meristem, is not visible being enclosed in senescent leaf sheaths. The drying cycle was terminated earlier for genotypes that appeared to be dying quicker than others. When it was considered that each genotype had reached 50% mortality, tubes were weighed and then rehydrated. Observations of the presence or absence of regrowth were then made over the next 10-14 days.

The perennial *Th. intermedium* '055' had the highest level of survival (88%), followed by the perennial derivative CPI 147235a (77%). The derivative '11955' was next with 41% survival followed by OK7211542 (36%) which was no different from CPI 147280b or the annual wheat cv. Wedgetail with both having only 27% of their initial plants surviving (Table 7). These results indicate wheat x wheatgrass derivatives have a lower level of dehydration tolerance than the intermediate wheatgrass, '055'. However, the derivative CPI 147235a expressed higher dehydration tolerance than any other of the derivatives tested. This is the same derivative line which achieved the highest level of post-harvest regrowth in Experiment 1 (Table 4) and was amongst the longest lived of derivatives in the initial evaluation (Table 1). *Th. intermedium* '055' used the greatest amount of water over the duration of the dehydration tolerance trial, followed by Wedgetail. Wedgetail had contrasting survival to '055' but also high water use. The reason for this apparent anomaly is unclear, but may be due to greater leaf area of Wedgetail and a higher initial rate of water use leading to a longer period of low soil moisture that the plant had to survive, in comparison to '055'. This hypothesis needs to be tested, and is scheduled for a following experiment. There was no difference in cumulative water use between the 4 derivative lines (Table 7). The fact that *Th. intermedium* '055' had the highest survival, the greatest cumulative water use and was subjected to the longest drying cycle indicates that this genotype survives low soil moisture content well and therefore expresses a higher level of dehydration tolerance. This work will continue aiming to better characterise lethal soil moisture levels of each line while also extending to identify other sources of dehydration tolerance, by exploring mechanisms and screening germplasm for alternative survival strategies.

6. Future Germplasm – Traits and Breeding

The studies reported above, and in recent literature, with the available perennial wheat genotypes may be considered to have established the feasibility, perhaps even the proof-of-principle for the concept, of perennial wheat. Capacity to survive multiple seasons, to provide valuable forage as well as grain, and the environmental benefits that should accrue after the first season, through greater investment in root biomass have been demonstrated in these studies. Nevertheless the genotypes used in these initial studies were those that were available, and were not developed with parental germplasm adapted to the Australian environments in which they were tested. And it is evident that the available germplasm falls short of the ideal phenotype; the perennial habit was not expressed as reliably as [in the](#) perennial grasses, grain yields were lower than annual wheat, and declined rapidly in subsequent seasons, and root systems were not as extensive as good perennial grasses. While

there is potential for a perennial wheat in Australia and globally (Bell *et al.* 2010), the early available lines may not be suited to grain growing areas outside of the high rainfall zone (> 650mm average annual rainfall; Hayes *et al.* 2012). Murphy *et al.* (2010) and Cox *et al.* (2010) came to similar conclusions in a North American environment, namely that the available germplasm has demonstrated the feasibility of the concept but that more genetic diversity and strong selection is required to deliver on the promise potential. Developing perennial cereals for a wider range of Australian environments would require a higher level of drought tolerance for success. Wide hybridisation and allopolyploid formation has occurred many times naturally in the Triticeae and has the potential to combine traits associated with domestication, such as in annual wheat, with those associated with the perennial habit, such as tall wheatgrass. Here we explore the question of the best approach to achieve these breeding objectives which may be viewed as a method of directing and shortening the domestication process (DeHaan *et al.* 2005).

There was some encouragement from earlier studies that it might be possible to introgress perenniality from Triticeae relatives of wheat such as *Th. elongatum* into a true wheat. Lammer *et al.* (2004) examined single chromosome addition and substitution lines from *Th. elongatum* to Chinese Spring wheat and found chromosome 4E conferred some capacity for post-harvest regrowth on wheat. It seems that at least one influential gene resides on 4ES, however, the ability to regrow and reflower (they termed *polycarpy*) was not as strong as the perennial amphiploid progenitor carrying all the *Th. elongatum* chromosomes. It is likely that many genes are required to confer fully the perennial habit (Zhao *et al.* 2012). It has become evident from breeding attempts in the Russian Academy of Science, Washington State University and The Land Institute, and from our own assessment of a wide range of germplasm derived from wheat x perennial Triticeae crosses, that reasonable rates of post-harvest regrowth are only observed when many chromosomes are added to wheat from the perennial donor species (Hayes *et al.* 2012 and here, Fig. 4). Some lines were able to perennialise in one or both field environments where they were grown, and a few were able to regrow through four seasons. The only wheat-derived lines that could perennialise contained seven or more pairs of chromosomes from the perennial parent. Individual Triticeae genomes have seven pairs of chromosomes and therefore the chromosome constitutions of stable allopolyploids, both naturally occurring and synthetic amphiploids, stabilise at multiples of 14. Many fertile and stable wheat derived amphiploids have been formed at the octoploid level ($2n=56$; Mujeeb-Kazi 1995) and this seems to be a benchmark of stability.

Successful donor perennial parents in the production of perennial amphiploids have included *Th. ponticum* ($2n=70$, decaploid) (Berezhnoi 1987; Zhang *et al.* 1996; Chen *et al.* 1998), *Th. intermedium* ($2n=42$, hexaploid) (Tsitsin and Lubimova 1959; Caudeyron 1966; Sun 1981; Jones *et al.* 1999; Cox *et al.* 2002) and *Th. elongatum* ($2n=14$, diploid) (Jenkins and Mochizuki 1957; Rommel and Jenkins 1959; Cai *et al.* 2001; Murphy *et al.* 2007; Mujeeb-Kazi *et al.* 2008). We conclude that the best near-term prospect for a perennial wheat-like grain crop is a full or partial amphiploid, containing the full set of tetraploid (AABB) or hexaploid (AABBDD) wheat chromosomes plus one genome equivalent (XX) from the donor. If the perennial donor is a polyploid, the extra genome is usually a synthetic genome, consisting of a mixture of chromosomes from the polyploid parent genomes, but where each of the seven homoeologous groups are represented in the synthetic genome. This creates a significant difficulty in that each time a primary partial amphiploid is produced, the synthetic genome may consist of a different mix of donor chromosomes (Jones *et al.* 1999; Han *et al.* 2004; Liu *et al.* 2005), and therefore interbreeding of these primary partial amphiploids may result in significant infertility and loss of the donor chromosomes. This problem was demonstrated for wheat-*Th. intermedium* partial amphiploids by Banks *et al.* (1993). The mismatch of chromosomes in independently derived partial amphiploids becomes problematic in establishing a breeding program. That is not to argue that robust perennial habit cannot be achieved in partial amphiploids from a polyploid perennial donor. The Land Institute, for example, has made excellent progress using *Th. intermedium* (6x), many of which performed favourably in the Australian evaluation (Fig 4B). However, ongoing breeding will need the ability to intercross compatible primary amphiploids in

order to generate genetic segregation on which to impose heavy selection. This is not insurmountable, but an extra challenge in a breeding context that is already very challenging.

There is a small group of perennial *Triticeae* grasses, endemic or naturalised in Australia. These vary widely in their distribution and adaptation to the varying environments found in this country. A number of these species are native grasses which may show adaptation to drought stress and low fertility soils. The native temperate grass, *Elymus scaber* ($2n = 6x = 42$, StYW), has the widest distribution across all regions of the southern Australian wheat belt (see Table 8). Accessions of this species have been characterised as highly persistent with consistent herbage production and suitability as an alternative pasture species (Mitchell *et al.* 2001; Garden *et al.* 2005). Its range of distribution makes it a potential candidate to be used in the development of perennial cereals adapted to Australian conditions.

E. scaber populations have been described as highly summer dormant (Mitchell *et al.* 2001). This is a rare trait among native grasses (Culvenor 2009). The majority of native grass vegetation in Australia is comprised of C_4 species, which have active growth over summer. However, summer dormancy has proved to be a powerful mechanism, aiding survival and persistence of C_3 temperate grasses, particularly in southern environments which experience unreliable summer rainfall (e.g. Hayes *et al.* 2010; Norton *et al.* 2012). More recent investigations into survival mechanisms in *E. scaber* have found that the species displays only partial summer dormancy (Newell *et al.* 2013b). This may limit the adaptability of hybrids based on this species to the wetter zones of the cropping belt. Attempts to hybridise wheat with *E. scaber* have shown that pollen from this perennial grass can pollinate wheat florets and readily trigger seed development (Newell *et al.* 2013a). However post-zygotic barriers are strong in this cross resulting in embryo abortion. Very early embryo rescue techniques are required to salvage hybrid embryos produced when hybridising these species. Successful attempts to cross wheat and *E. scaber* haven't been reported by Ahmad and Comeau (1991) and Torabinejad and Muller (1993). The yield of plants per cross attempted was less than one percent, and all hybrid progeny were sterile. Nevertheless Torabinejad and Muller (1993) report that their hybrids with Australian *Elymus* species were perennial. Even if successful perennial partial amphiploids could be produced between wheat and the hexaploid *E. scaber*, there would be difficulties in establishing a long term breeding program as outlined in the next paragraph.

Despite the existence of perennial allopolyploid *Triticeae* with excellent adaptation to target environments, we believe the best prospects for a productive breeding program in the medium term would be to base it on full amphiploids between wheat (either tetraploid or hexaploid wheat) and a diploid perennial donor, such as *Th. elongatum* (EE). This is analogous to triticales breeding. The amphiploids produced would have genome constitutions AABBEE (analogous to hexaploid triticales, AABBRR) or AABBDEE (analogous to octoploid triticales, AABBDDRR). It is noteworthy that triticales took some decades of selection before it delivered on its promise as a high yielding crop. Extensive genome modifications were required to achieve genomic accommodation in newly formed allopolyploids (Ma and Gustafson 2008; Tang *et al.* 2008; Ozkan and Feldman 2009; Feldman and Levy 2012). The observed changes in triticales were mainly losses of AFLP and RFLP bands, predominantly from the rye genome and largely involving repetitive sequences. A positive relationship was found between increased bivalent pairing, improved fertility, and elimination of low-copy non-coding DNA sequences (Ma and Feldman 2009). Some chromosomal rearrangements may have been required to permit the wheat and rye genomes to time their division together. Qi *et al.* (2010) studied the extensive genomic rearrangements associated with allopolyploidization in wheat, observing both genetic and epigenetic changes in newly synthesized allotetraploid wheat lines with diverse genome compositions. Some of the changes seemed to be required (which they called *directed*) while others were highly variable (which they called *stochastic*). Feldman and Levy (2012) refer to rapid essential changes required in new allopolyploids as *revolutionary*. Required changes included the non-random loss of non-coding, low- and high-copy sequence to the extent of 2-10% of the sum of the parents. In the case of triticales, the loss was about 9% in the octoploid and

28-30% in the hexaploid triticale. Intergenomic invasion by sequences such as transposons, and elimination of rRNA and 5S RNA genes are also noted as revolutionary genetic changes occurring rapidly after allopolyploidisation. Epigenetic changes were also observed. More recently Hu et al (2012) looked specifically at newly formed wheat x *Th. elongatum* amphiploids using genome specific molecular markers and found chromosome rearrangements and duplications occurring.

Most of the wheat x *Th. elongatum* amphiploids we have examined were octoploid (2n=56, presumably AABBDEE) provided by Washington State University (WSU) and apparently derived from the one primary amphiploid. However one of the WSU lines, CPI147232 in Fig 4B, is hexaploid (2n=42) and requires further examination. Accession 20238 (Table 2) is cv. Stewart (*T. turgidum*) x *Th. elongatum* amphiploid (AABBEE)(Jenkins and Mochizuki, 1957); this line is very tall but should be crossable to CPI147232 to generate useful variation from which to select improvements at the hexaploid level. Almouslem and Amleh (1999) also report a durum wheat x *Th. elongatum* hybrid.

Two recently acquired accessions of bread wheat x *Th. elongatum* octoploid amphiploids from CIMMYT used wheat cv. Goshawk (Mujeeb-Kazi et al 2008) and had good semi-dwarf stature, strong straw and reasonable post-harvest regrowth in the glasshouse. They are not in Table 2 and have not been assessed in the field. However in the glasshouse their fertility was reduced and the heads shattered badly (Larkin, unpublished). Shattering was not a problem with the perennial wheat-*Th. elongatum* amphiploids from WSU. Therefore we have initiated successful crosses between two wheat x *Thinopyrum elongatum* types, the Goshawk derivative and CPI147242b, with the prospect of being able to select over the next few generations types with beneficial combinations of traits. *Th. elongatum* accessions have been obtained from dry locations such as in Israel and Mediterranean France, which are likely to experience and be adapted to harsh dry summers as in the target Australian environments. We therefore believe this diploid species will exhibit the type of summer survival and perenniality which is required.

Of course *Th. elongatum* is not the only perennial diploid that might serve the purpose described above. It is just the one that has been most extensively used to date. It is noteworthy that most species of the Triticeae are perennial (Barkworth et al. 2009) with about 10 basic genome types represented, including *Australopyrum retrofractum* (W), *Agropyrum cristatum* (P), *Thinopyrum bessarabicum* (E^b), *Dasyphyrum villosum* (V), *Psathyrostachys hushanica* (Ns), *Pseudoroegneria spicata* (St); and wheat can be hybridised to these species. So there should be many other possibilities for generating perennial amphiploids. The diploid *Australopyrum* species (2n = 2x = 14, WW) might appear to be attractive perennial donors in an Australian context, however there are no reports of hybrids with wheat and these species lack the broader adaptation of *E. scaber* to various climatic regions of southern Australia. Other Australian native grasses have previously proven difficult to domesticate either as forages (Cole and Johnston 2006) or as perennial crops (Davies et al., 2005) and it is difficult to predict whether the diploid *Australopyrum* species will be any different.

Concern has been expressed that a perennial cereal crop would represent an unacceptable disease threat to the annual cereal crops. In particular a “green bridge” is envisaged carrying disease between growing seasons. However the types of perennial grain crops exemplified in our studies are not evergreen and do not form green bridges; all above ground tissues senesce between growing seasons. Furthermore the wheatgrass donors of perenniality are also excellent sources of disease resistance as we and others have demonstrated (Cox et al. 2002; 2010; Hayes et al. 2012). Nevertheless vigilance will be required in the selection for disease resistance, especially crown and root diseases that are more likely to carry over.

Some attention has been given to perennial triticale produced using perennial rye, *Secale montanum*. Schlegel (1980) produced F1 hybrids and amphiploids from wheat x *S. montanum* crosses; the amphiploids at meiosis had an average of 26.55 bivalents compared to 27.30 in an established octoploid triticale. Schlegel observed a correlation between the amount of telomeric

heterochromatin on the rye chromosomes and the frequency of univalents in amphiploid meiosis, suggesting the heterochromatin difference between the wheat and rye chromosomes was an impediment to full fertility. Delayed DNA replication at the heterochromatic telomeres may be a problem for coordinating wheat and rye chromosome behaviour in meiosis. The reduced amount of telomeric heterochromatin in *S. montanum* relative to *S. cereale* is postulated to explain why wheat-*S. montanum* amphiploids were more regular in pairing than primary wheat-*S. cereale* amphiploids (Thomas and Kaltsikes 1974; Schlegel 1980). Established fully fertile triticales have lost much of the telomeric heterochromatin on the rye chromosomes. This is another example of a genetic change required to enable newly formed allopolyploids to be fully fertile.

There has been some success in breeding perennial rye as a grain crop through intercrossing *S. cereale* and *S. montanum* and the release of tetraploid cvs. Permontra and Sopertra, and diploid cv. Benmonta in Germany (Reimann-Philipp, 1995) and diploid cvs. Perenne and Kriszta in Hungary (Kotvics *et al.* 2001; Füle *et al.* 2005; Sipos and Halasz 2007). Cv. ACE-1 was selected in Canada from German germplasm (Acharya *et al.*, 2004). Initial attempts had difficulties with fertility and retention of perenniality which seemed to be inversely related. In Australia a perennial rye cv. Black Mountain was bred from the *S. cereale* x *S. montanum* cross with two backcrosses to the perennial parent; selection for this release was based mainly on perenniality and forage yield, rather than grain yield. Following cell culture and six generations of selection for fertility, non-shattering types and grain yield, a higher grain yield perennial selection was recovered called *Family 10* (Oram 1996, and pers.comm. 2010). Small quantities of seed of this accession are still available; it was used as a perennial grass control in the initial evaluation of perennial wheat derivatives in Australia (Hayes *et al.* 2012), but it has never been commercialised and there are no immediate plans to do so (R. A. Culvenor, Pers. Comms).

The rapid development of genomic analyses may lead to greater genetic insight to which types of genes are responsible for the perennial habit. For example transcriptomic comparisons between annual and perennial *Brachypodium* species, might reveal a tractable number of genes whose manipulation could confer perenniality in wheat or some other cereal. Other potential comparisons that might deepen the genetic insights are annual and perennial rice (*Oryza rufipogon*) (Zhao *et al.* 2012), *Sorghum bicolor* and *S. halepense*, *Hordeum vulgare* and *H. bulbosum*, *Panicum miliaceum* and *P. turgidum*. Eventually this could lead to informed and targeted attempts to engineer perenniality into a cereal crop. In the meantime the best near term prospect of a productive breeding program for a perennial wheat-derived cereal will involve the following steps:

1. The generation of many primary amphiploids between wheat and a perennial diploid such as *Th. elongatum*. Importantly this should involve a diversity of *Th. elongatum* accessions and a diversity of adapted annual wheat cultivars.
2. Intercrossing primary amphiploids and advancing segregating populations with selection.
3. Early generation selection at F2 to F4 would emphasise traits such as semi-dwarf plant height, non-shattering heads, large seed size, good self-fertility and regrowth in pots.
4. Later generation selections would emphasise traits such as days to flowering, capability of post-harvest longevity, grain yield, forage yield, stability of grain yield across seasons, disease resistance.

A number of rounds of such breeding cycles may be required with vigilance to look for and select for the *revolutionary* genetic changes so frequently observed in newly forming allopolyploids (Feldman and Levy 2012) that signal the accommodation between genomes and the rise in fertility and stability. From the experience with other allopolyploids, it might even be possible to devise molecular assays to detect these events and impose early marker-assisted selection for the domestication events. Triticale took 40 to 50 years to begin to deliver on its potential, but that timeframe could be greatly compressed with the tools available now that could be applied to the development of a perennial cereal.

CONCLUSIONS

This manuscript has established that development of perennial wheat should be feasible, as the best currently-available germplasm survived for up to four years in the field, and contributed up to 40 % of grain yield and cumulatively, comparable forage dry matter to Wedgetail, the annual wheat control over the first year. Under controlled conditions, perennial wheat derivatives allocated more dry matter to roots in the second season, indicating a potential for enhanced water extraction in subsequent cycles. There were also indications of enhanced dehydration tolerance and survival in controlled conditions. These results are encouraging, as they are derived from germplasm developed overseas, which were not intended for adaptation under Australian conditions. Consequently, we have proposed a breeding program be commenced, based on intercrossing of primary amphiploids derived from crosses between a diversity of adapted wheat and a diverse collection of a perennial diploid such as *Thinopyrum elongatum*. Segregating populations would be selected for traits resulting in desired agronomic types, combining survival and regrowth attributes with forage and grain contributions. Selection progress should be feasible, to generate perennial wheat derivatives with survival, root and forage characteristics of the perennial grass and with the crop, stubble and grain characteristics of the wheat. A systems approach with multidisciplinary collaboration will be essential to secure the release of an adapted perennial wheat for Australia by 2030. The availability of an adapted perennial wheat would greatly increase the flexibility of farmer enterprises, allowing a greater diversity and stability in farming systems for the future.

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Table 1. Grain yields (g/m) of the longest surviving entries from the initial field evaluation, sown in single rows in 2008 and sampled in December of 4 consecutive years

| Entry | 2008 | 2009 | 2010 | 2011 |
|--------------|---------------|-------------|--------------|-------------|
| CPI 147235a | 73.5 | 25.9 | 0.0 | 0.0 |
| CPI 147236a | 81.7 | 45.9 | 11.2 | 0.0 |
| CPI 147236b | 65.9 | 8.9 | 0.0 | 0.0 |
| CPI 147251b | 70.9 | 9.4 | 0.5 | 0.4 |
| CPI 147257b | 7.8 | 12.6 | 4.7 | 0.7 |
| CPI 147258a | 4.7 | 0.3 | 0.3 | 0.0 |
| CPI 147286a | 36.3 | 0.4 | 0.0 | 0.0 |
| Dundas | 0.0 | 9.4 | 207.7 | 268.8 |
| | <i>l.s.d.</i> | | <i>45.74</i> | |

Table 2. Description of germplasm assessed in Experiment 1.

| No. | Entry | Reported pedigree ¹ | Source ² | Source reference | Previously assessed ³ |
|-----|--------------------|------------------------------------|---------------------|---------------------------|----------------------------------|
| 1 | CPI-148037 | <i>Th. intermedium</i> | TLI | Cycle 2 selection bulk | No |
| 2 | CPI-148041 | <i>Th. intermedium</i> | TLI | IWG 892 | No |
| 3 | CPI-148042 | <i>Th. intermedium</i> | TLI | IWG 1706 | Yes |
| 4 | CPI-148043 | <i>Th. intermedium</i> | TLI | IWG 1793 | No |
| 5 | CPI-148044 | <i>Th. intermedium</i> | TLI | IWG 1843 | No |
| 6 | CPI-148045 | <i>Th. intermedium</i> | TLI | IWG 1871 | No |
| 7 | CPI-148048 | <i>Th. intermedium</i> | TLI | IWG 2402 | Yes |
| 8 | CPI-148049 | <i>Th. intermedium</i> | TLI | IWG 2692 | No |
| 9 | CPI-148051 | <i>Th. intermedium</i> | TLI | IWG 2858 | No |
| 10 | CPI-148052 | <i>Th. intermedium</i> | TLI | IWG 3026 | Yes |
| 11 | CPI-148055 | <i>Th. intermedium</i> | TLI | IWG 3182 | Yes |
| 12 | CPI-148056 | <i>Th. intermedium</i> | TLI | IWG 3221 | Yes |
| 13 | CPI-148057 | <i>Th. intermedium</i> | TLI | IWG 3228 | No |
| 14 | CPI-148058 | <i>Th. intermedium</i> | TLI | IWG 3515 | No |
| 15 | CPI-148062 | <i>Th. intermedium</i> | TLI | IWG 4226 | No |
| 16 | Ostankinskaya | <i>T. aestivum/Th. intermedium</i> | CMB | (Anon. 2002) ⁴ | No |
| 17 | Zernokormovaya-169 | <i>T. aestivum/Th. intermedium</i> | CMB | (Anon. 2002) | No |
| 18 | Istra-1 | <i>T. aestivum/Th. intermedium</i> | CMB | (Anon. 2002) | No |

¹ Abbreviations: Ti, *Th. intermedium*; Ta, *T. aestivum*; TC, *Triticum carthlicum*; CS, wheat variety Chinese Spring; M, wheat variety Madsen; S, durum wheat cv. Stewart; Agroyron, undefined wheatgrass species; *Ag. junceum*, as in received pedigree description, likely to be *Th. junceum*; *Ag. elongatum*, as in received pedigree, could be *Th. elongatum* or *Th. ponticum*.

² TLI – The Land Institute, Kansas, USA; CMB – Centre for Molecular Biotechnology, Moscow, Russia (originally from N.V. Tsitsin Main Botanical Garden, Russian Academy of Science); WSU – Washington State University, Washington State, USA, AWCC – Australian Winter Cereals Collection, New South Wales, Australia

³ In Hayes *et al.* (2012)

⁴ Anon. (2002) Vera Fedorovna Lyubimova (1906–2002). Russian Journal of Genetics **38**: 1217-1218

| | | | | | |
|----|-------------|---|-----|-------------------|-----|
| 19 | CPI-149776 | <i>T. aestivum</i> 2*/ <i>Th. intermedium</i> | TLI | B307-19-49-3 | No |
| 20 | CPI-149777 | <i>T. aestivum</i> 2*/ <i>Th. intermedium</i> | TLI | B307-67-7-1 | No |
| 21 | CPI-149778 | <i>T. aestivum</i> 2*/ <i>Th. intermedium</i> | TLI | B373-4-30-3-6-1-1 | No |
| 22 | CPI-149779 | <i>Triticum carthlicum/Th. junceum</i> | TLI | B875-12-12-12 | No |
| 23 | CPI-149780 | <i>Triticum carthlicum/Th. junceum</i> | TLI | B875-12-1-34 | No |
| 24 | CPI-149781 | <i>T. durum/Th. junceum</i> | TLI | B913(3)-12-27 | No |
| 25 | CPI-149784 | <i>T. durum/Th. junceum</i> | TLI | B913(3)-1-3 | No |
| 26 | CPI-149785 | <i>T. durum/Th. junceum</i> | TLI | B913(3)-14-29 | No |
| 27 | CPI-149786 | <i>T. durum/Th. junceum</i> | TLI | B913(3)-14-5 | No |
| 28 | CPI-149787 | <i>T. durum/Th. junceum</i> | TLI | B913(3)-1-63 | No |
| 29 | CPI-149789 | <i>T. durum/Th. junceum</i> | TLI | B913(3)-2-8 | No |
| 30 | CPI-149793 | <i>T. durum/Th. junceum</i> | TLI | B913(3)-6-1 | No |
| 31 | CPI-149796 | Ta 3*/ <i>Agropyron</i> hybrid//Ti | TLI | B938(13)-18 | No |
| 32 | CPI-149797 | Ta 3*/ <i>Agropyron</i> hybrid//Ti | TLI | B938(16)-8 | No |
| 33 | CPI-149798 | Ta 3*/ <i>Agropyron</i> hybrid//Ti | TLI | B938(23)-8 | No |
| 34 | CPI-149799 | Ta 3*/ <i>Agropyron</i> hybrid//Ti | TLI | B938(24)-8 | No |
| 35 | CPI-149800 | Ta 3*/ <i>Agropyron</i> hybrid//Ti | TLI | B938(24)-9 | No |
| 36 | CPI-149801 | Ta 3*/ <i>Agropyron</i> hybrid//Ti | TLI | B938(27)-8 | No |
| 37 | CPI-149804 | Tc/Ti//Ta/3/Ti | TLI | B1085(3)-14 | No |
| 38 | CPI-149808 | <i>T. durum/Ag. junceum</i> //unknown | TLI | B1107(2)-14 | No |
| 39 | CPI-149809 | <i>T. durum/Ag. junceum</i> //unknown | TLI | B1107(4)13 | No |
| 40 | CPI-149810 | <i>T. durum/Ag. junceum</i> //unknown | TLI | B1107(4)-8 | No |
| 41 | CPI-149813 | (Ta 2*/Ti) //Ta /3/ Ti /4/ (Ta 2*/Ti) | TLI | B1126(11) | No |
| 42 | CPI-149815 | Tc / 3* Ti // (Ta 2* /Ti) | TLI | B1152(1) | No |
| 43 | CPI-147231a | <i>T. aestivum</i> (CS)/ <i>Th. elongatum</i> // <i>T. aestivum</i> (M) | WSU | PF5RI-0005 | Yes |
| 44 | CPI-147235a | <i>T. aestivum</i> (CS)/ <i>Th. elongatum</i> // <i>T. aestivum</i> (M) | WSU | PF5RI-0009 | Yes |
| 45 | CPI-147235b | <i>T. aestivum</i> (CS)/ <i>Th. elongatum</i> // <i>T. aestivum</i> (M) | WSU | PF5RI-0009 | Yes |
| 46 | CPI-147235c | <i>T. aestivum</i> (CS)/ <i>Th. elongatum</i> // <i>T. aestivum</i> (M) | WSU | PF5RI-0009 | No |
| 47 | CPI-147235d | <i>T. aestivum</i> (CS)/ <i>Th. elongatum</i> // <i>T. aestivum</i> (M) | WSU | PF5RI-0009 | No |
| 48 | CPI-147235e | <i>T. aestivum</i> (CS)/ <i>Th. elongatum</i> // <i>T. aestivum</i> (M) | WSU | PF5RI-0009 | No |
| 49 | CPI-147235f | <i>T. aestivum</i> (CS)/ <i>Th. elongatum</i> // <i>T. aestivum</i> (M) | WSU | PF5RI-0009 | No |
| 50 | CPI-147235g | <i>T. aestivum</i> (CS)/ <i>Th. elongatum</i> // <i>T. aestivum</i> (M) | WSU | PF5RI-0009 | No |
| 51 | CPI-147235h | <i>T. aestivum</i> (CS)/ <i>Th. elongatum</i> // <i>T. aestivum</i> (M) | WSU | PF5RI-0009 | No |

| | | | | | |
|----|-------------|---|-------------|-----------------|-----|
| 52 | CPI-147235i | <i>T. aestivum</i> (CS)/ <i>Th. elongatum</i> // <i>T. aestivum</i> (M) | WSU | PF5RI-0009 | No |
| 53 | CPI-147236a | <i>T. aestivum</i> (CS)/ <i>Th. elongatum</i> // <i>T. aestivum</i> (M) | WSU | PF5RI-0010 | Yes |
| 54 | CPI-147236b | <i>T. aestivum</i> (CS)/ <i>Th. elongatum</i> // <i>T. aestivum</i> (M) | WSU | PF5RI-0010 | Yes |
| 55 | CPI-147236c | <i>T. aestivum</i> (CS)/ <i>Th. elongatum</i> // <i>T. aestivum</i> (M) | WSU | PF5RI-0010 | No |
| 56 | CPI-147236d | <i>T. aestivum</i> (CS)/ <i>Th. elongatum</i> // <i>T. aestivum</i> (M) | WSU | PF5RI-0010 | No |
| 57 | CPI-147236e | <i>T. aestivum</i> (CS)/ <i>Th. elongatum</i> // <i>T. aestivum</i> (M) | WSU | PF5RI-0010 | No |
| 58 | CPI-147236f | <i>T. aestivum</i> (CS)/ <i>Th. elongatum</i> // <i>T. aestivum</i> (M) | WSU | PF5RI-0010 | No |
| 59 | CPI-147241a | <i>T. aestivum</i> (CS)/ <i>Th. elongatum</i> // <i>T. aestivum</i> (M) | WSU | PF5RI-0015 | No |
| 60 | CPI-147242b | <i>T. aestivum</i> (CS)/ <i>Th. elongatum</i> // <i>T. aestivum</i> (M) | WSU | PF5RI-0016 | Yes |
| 61 | CPI-147244a | <i>T. aestivum</i> (CS)/ <i>Th. elongatum</i> // <i>T. aestivum</i> (M) | WSU | PF5RI-0018 | Yes |
| 62 | CPI-147244b | <i>T. aestivum</i> (CS)/ <i>Th. elongatum</i> // <i>T. aestivum</i> (M) | WSU | PF5RI-0018 | Yes |
| 63 | CPI-147244c | <i>T. aestivum</i> (CS)/ <i>Th. elongatum</i> // <i>T. aestivum</i> (M) | WSU | PF5RI-0018 | No |
| 64 | CPI-147244d | <i>T. aestivum</i> (CS)/ <i>Th. elongatum</i> // <i>T. aestivum</i> (M) | WSU | PF5RI-0018 | No |
| 65 | CPI-147244e | <i>T. aestivum</i> (CS)/ <i>Th. elongatum</i> // <i>T. aestivum</i> (M) | WSU | PF5RI-0018 | No |
| 66 | CPI-147244f | <i>T. aestivum</i> (CS)/ <i>Th. elongatum</i> // <i>T. aestivum</i> (M) | WSU | PF5RI-0018 | No |
| 67 | CPI-147247a | <i>T. aestivum</i> (CS)/ <i>Th. elongatum</i> // <i>T. aestivum</i> (M) | WSU | PF5RI-0021 | Yes |
| 68 | CPI-147251b | <i>T. aestivum</i> (CS)/ <i>Th. elongatum</i> // <i>T. aestivum</i> (M) | WSU | PF5RI-0025 | Yes |
| 69 | CPI-147257b | <i>T. carthlicum</i> / <i>Th. intermedium</i> // <i>T. aestivum</i> | TLI | B137-1-10 | Yes |
| 70 | CPI-147271b | <i>T. aestivum</i> / <i>Th. intermedium</i> // <i>T. aestivum</i> | TLI | B307-25-X | Yes |
| 71 | CPI-147278a | <i>T.carthlicum</i> / <i>Th. intermedium</i> // <i>T. aestivum</i> | TLI | B330-3-12 | No |
| 72 | CPI-147278b | <i>T.carthlicum</i> / <i>Th. intermedium</i> // <i>T. aestivum</i> | TLI | B330-3-12 | No |
| 73 | CPI-147282a | <i>T. carthlicum</i> / <i>Th. intermedium</i> | TLI | B378-10-14 | No |
| 74 | CPI-147289a | <i>T. carthlicum</i> / <i>Th. intermedium</i> // 3* <i>T.aestivum</i> | TLI | B556-4-5 | No |
| 75 | CPI-147289b | <i>T. carthlicum</i> / <i>Th. intermedium</i> // 3* <i>T.aestivum</i> | TLI | B556-4-5 | No |
| 76 | 6754 | <i>T.aestivum</i> (CS) 2*/ <i>Ag. elongatum</i> | AWCC | AUS6754 | Yes |
| 77 | 6755 | <i>T.aestivum</i> (CS) 2*/ <i>Ag. elongatum</i> | AWCC | AUS6755 | Yes |
| 78 | 11955 | <i>Triticum</i> spp/ <i>Agropyron</i> spp. hybrid | USDA/AWCC | AUS11955 | Yes |
| 79 | 20238 | <i>T. durum</i> (S)/ <i>Ag. elongatum</i> | Mexico/AWCC | AUS20238 | Yes |
| 80 | OK7211542 | Partial amphiploid from <i>T. aestivum</i> / <i>Th. ponticum</i> | (USA) | (Sando 1935) | Yes |
| 81 | TAF 46 | Partial amphiploid from <i>T. aestivum</i> / <i>Th. intermedium</i> | (France) | (Cauderon 1966) | Yes |
| 82 | Dundas | <i>Th. ponticum</i> | (Australia) | (Cultivar) | Yes |
| 83 | Naparoo | <i>T. aestivum</i> | (Australia) | (Cultivar) | Yes |
| 84 | Wedgetail | <i>T. aestivum</i> | (Australia) | (Cultivar) | Yes |

Table 3. Grain yield (g/plant), grain size (g/1000 kernal), harvest index, maturity (days post sowing to anthesis), tiller height (cm), mass of 20 tillers (g), summer/autumn regrowth (approximate basal frequency [%]), autumn grain yield (g/m) and autumn harvest index of 84 entries during the first 12 months of growth in Experiment 1. All measures are the means across three replicate rows. (Data sorted on grain yield in descending order except for entries within heterogeneity test groupings; note the different units in the two 'grain yield' columns).

| Entry | Grain yield (g/plant) | Grain size (1000 kernel; g) | Harvest index (HI) | Average tiller height (cm) | Maturity (Days to flowering) | Mass of 20 tillers (g) | Regrowth score (%) | Autumn grain yield (g/m) | Autumn HI |
|-------------|-----------------------|-----------------------------|--------------------|----------------------------|------------------------------|------------------------|--------------------|--------------------------|-----------|
| Naparoo | 40.3 | 33.5 | 0.49 | 64.6 | 156 | 88.3 | 0 | - | - |
| Wedgetail | 35.2 | 39.8 | 0.52 | 65.3 | 149 | 88.9 | 0 | - | - |
| CPI-149796 | 26.4 | 31.3 | 0.48 | 80.6 | 159 | 64.9 | 2 | 0.0 | - |
| CPI-149800 | 25.7 | 28.1 | 0.38 | 64.8 | 162 | 74.8 | 0 | - | - |
| 11955 | 19.4 | 31.7 | 0.25 | 91.2 | 174 | 88.4 | 51 | 41.2 | 0.27 |
| 6755 | 17.1 | 23.0 | 0.34 | 137.2 | 165 | 86.1 | 44 | 42.6 | 0.33 |
| CPI-149778 | 16.8 | 33.5 | 0.30 | 65.7 | 171 | 64.2 | 13 | 1.0 | 0.12 |
| 6754 | 16.5 | 28.1 | 0.34 | 105.5 | 149 | 91.0 | 26 | 19.5 | 0.33 |
| CPI-147247a | 15.0 | 27.4 | 0.25 | 76.7 | 183 | 73.6 | 34 | 2.2 | 0.09 |
| OK7211542 | 14.8 | 26.7 | 0.29 | 88.1 | 173 | 72.6 | 64 | 50.2 | 0.26 |
| CPI-149801 | 14.5 | 21.3 | 0.35 | 67.8 | 163 | 60.5 | 0 | - | - |
| CPI-149779 | 14.0 | 28.5 | 0.32 | 91.2 | 164 | 57.5 | 1 | 0.0 | - |
| CPI-149781 | 13.5 | 31.5 | 0.19 | 74.3 | 168 | 85.5 | 0 | - | - |
| CPI-149804 | 13.3 | 31.6 | 0.33 | 95.1 | 169 | 68.8 | 0 | - | - |
| CPI-147289a | 12.8 | 32.1 | 0.28 | 70.4 | 169 | 74.3 | 0 | - | - |
| CPI-149799 | 12.2 | 26.1 | 0.35 | 55.9 | 157 | 51.1 | 0 | - | - |
| 20238 | 12.1 | 28.2 | 0.18 | 122.3 | 175 | 135.6 | 36 | 9.6 | 0.16 |
| CPI-147241a | 12.0 | 24.0 | 0.19 | 91.9 | 185 | 79.6 | 9 | 0.0 | - |
| CPI-149810 | 11.5 | 30.5 | 0.22 | 56.8 | 164 | 76.1 | 0 | - | - |
| CPI-149808 | 11.3 | 23.2 | 0.26 | 67.1 | 164 | 74.0 | 0 | - | - |
| CPI-149789 | 11.2 | 24.8 | 0.19 | 85.6 | 171 | 84.2 | 0 | - | - |
| CPI-147251b | 10.9 | 24.6 | 0.24 | 100.7 | 187 | 82.5 | 38 | 1.8 | 0.03 |
| CPI-149776 | 10.1 | 36.8 | 0.27 | 88.2 | 165 | 86.9 | 0 | - | - |
| CPI-149777 | 10.1 | 29.7 | 0.25 | 82.4 | 164 | 54.3 | 5 | 0.0 | - |
| CPI-149809 | 9.3 | 25.7 | 0.22 | 73.3 | 165 | 67.8 | 1 | 0.0 | - |

| | | | | | | | | | |
|-------------|-----|------|------|-------|-----|------|----|------|------|
| CPI-147271b | 9.1 | 46.6 | 0.22 | 83.9 | 163 | 71.1 | 8 | 0.1 | 0.03 |
| CPI-147231a | 9.0 | 25.9 | 0.19 | 76.9 | 190 | 68.4 | 29 | 2.5 | 0.08 |
| CPI-149787 | 8.8 | 21.2 | 0.18 | 68.1 | 173 | 63.5 | 3 | 0.0 | - |
| CPI-147242b | 8.4 | 27.5 | 0.17 | 87.8 | 181 | 82.0 | 34 | 0.4 | 0.02 |
| 917 | 8.0 | 28.6 | 0.22 | 91.7 | 187 | 67.8 | 34 | 8.3 | 0.11 |
| CPI-147289b | 8.0 | 32.9 | 0.23 | 69.9 | 172 | 86.5 | 4 | 0.0 | - |
| 916 | 7.4 | 22.4 | 0.17 | 94.8 | 195 | 70.9 | 67 | 5.7 | 0.07 |
| 915 | 6.8 | 26.5 | 0.17 | 110.3 | 190 | 73.4 | 55 | 13.1 | 0.11 |
| CPI-149793 | 6.1 | 26.1 | 0.17 | 68.0 | 181 | 52.7 | 22 | 0.6 | 0.04 |
| CPI-149786 | 5.9 | 24.4 | 0.16 | 79.0 | 176 | 61.8 | 4 | 0.0 | - |
| CPI-149784 | 5.1 | 23.8 | 0.14 | 86.3 | 175 | 71.7 | 8 | 0.0 | - |
| CPI-149798 | 4.9 | 27.2 | 0.18 | 61.8 | 166 | 60.4 | 1 | 0.0 | - |
| CPI-149785 | 4.7 | 18.9 | 0.14 | 72.9 | 170 | 65.1 | 8 | 0.3 | 0.07 |
| CPI-148041 | 4.6 | 7.5 | 0.06 | 100.9 | 205 | 47.4 | 84 | 0.0 | - |
| CPI-148062 | 4.5 | 7.1 | 0.07 | 100.4 | 196 | 75.0 | 80 | 0.0 | - |
| CPI-149815 | 4.4 | 29.1 | 0.13 | 62.2 | 175 | 46.5 | 14 | 0.5 | 0.04 |
| CPI-147282a | 4.0 | 24.1 | 0.15 | 60.5 | 173 | 34.9 | 16 | 0.7 | 0.06 |
| CPI-149813 | 3.8 | 28.8 | 0.16 | 59.6 | 163 | 60.0 | 21 | 1.2 | 0.05 |
| CPI-148056 | 3.5 | 6.1 | 0.07 | 103.6 | 185 | 46.6 | 76 | 0.0 | - |
| CPI-149780 | 3.1 | 21.6 | 0.16 | 82.3 | 170 | 50.4 | 0 | 0.0 | - |
| CPI-148042 | 2.9 | 6.8 | 0.10 | 97.0 | 202 | 55.1 | 77 | 0.0 | - |
| CPI-148051 | 2.8 | 7.5 | 0.06 | 95.1 | 186 | 61.5 | 89 | 0.0 | - |
| CPI-148043 | 2.6 | 7.0 | 0.06 | 85.1 | 203 | 51.2 | 86 | 0.0 | - |
| CPI-148049 | 2.6 | 7.5 | 0.07 | 98.3 | 199 | 58.8 | 80 | 0.0 | - |
| CPI-148045 | 2.5 | 7.2 | 0.07 | 93.1 | 199 | 47.3 | 93 | 0.0 | - |
| TAF 46 | 2.4 | 26.5 | 0.08 | 73.7 | 183 | 60.2 | 57 | 4.9 | 0.13 |
| CPI-148055 | 2.3 | 7.8 | 0.04 | 103.5 | 199 | 62.5 | 72 | 0.0 | - |
| CPI-147278b | 2.0 | 18.3 | 0.13 | 44.6 | 180 | 24.8 | 2 | 0.0 | - |
| CPI-149797 | 2.0 | 30.0 | 0.07 | 91.8 | 164 | 43.5 | 1 | 0.0 | - |
| CPI-148057 | 1.9 | 6.9 | 0.07 | 92.4 | 196 | 56.3 | 81 | 0.0 | - |
| CPI-147278a | 1.9 | 18.4 | 0.10 | 47.8 | 173 | 26.0 | 6 | 0.1 | 0.01 |
| CPI-148037 | 1.9 | 7.6 | 0.02 | 85.0 | 210 | 50.9 | 36 | 0.0 | - |
| Dundas | 1.9 | 7.2 | 0.04 | 110.1 | 235 | 71.8 | 72 | 0.0 | - |

| | | | | | | | | | |
|-------------------------------------|------|------|------|-------|-----|-------|------|-------|-------|
| CPI-148044 | 1.8 | 5.9 | 0.06 | 84.6 | 197 | 44.5 | 79 | 0.0 | - |
| CPI-148048 | 1.6 | 7.4 | 0.05 | 86.9 | 196 | 46.3 | 91 | 0.0 | - |
| CPI-148058 | 1.2 | 7.1 | 0.05 | 81.1 | 203 | 50.4 | 78 | 0.0 | - |
| CPI-147257b | 1.1 | 16.1 | 0.06 | 71.9 | 178 | 47.1 | 49 | 0.7 | 0.01 |
| CPI-148052 | 0.8 | 6.6 | 0.07 | 85.7 | 195 | 43.3 | 91 | 0.0 | - |
| <i>Heterogeneity test groupings</i> | | | | | | | | | |
| CPI-147235a | 11.7 | 24.8 | 0.21 | 89.9 | 183 | 94.2 | 53 | 22.7 | 0.14 |
| CPI-147235b | 25.8 | 36.1 | 0.34 | 80.6 | 175 | 76.3 | 0 | - | - |
| CPI-147235c | 25.5 | 43.0 | 0.32 | 86.9 | 173 | 79.8 | 0 | - | - |
| CPI-147235d | 5.9 | 29.8 | 0.15 | 83.3 | 182 | 80.4 | 48 | 15.5 | 0.12 |
| CPI-147235e | 3.7 | 20.5 | 0.17 | 77.7 | 192 | 41.1 | 23 | 2.4 | 0.05 |
| CPI-147235f | 6.2 | 18.1 | 0.16 | 84.2 | 183 | 83.4 | 12 | 0.2 | 0.01 |
| CPI-147235g | 8.9 | 22.2 | 0.11 | 83.6 | 191 | 88.7 | 39 | 2.7 | 0.06 |
| CPI-147235h | 10.1 | 22.9 | 0.18 | 92.4 | 186 | 75.5 | 41 | 5.9 | 0.11 |
| CPI-147235i | 9.6 | 19.9 | 0.15 | 81.9 | 189 | 81.8 | 50 | 4.0 | 0.31 |
| CPI-147236a | 15.0 | 26.9 | 0.22 | 84.7 | 183 | 96.9 | 48 | 7.8 | 0.12 |
| CPI-147236b | 11.4 | 29.8 | 0.22 | 79.1 | 181 | 70.8 | 27 | 0.9 | 0.02 |
| CPI-147236c | 11.1 | 23.1 | 0.21 | 97.3 | 180 | 76.9 | 36 | 10.3 | 0.15 |
| CPI-147236d | 11.5 | 26.0 | 0.22 | 82.0 | 178 | 72.4 | 31 | 3.7 | 0.08 |
| CPI-147236e | 5.7 | 18.8 | 0.18 | 72.7 | 186 | 34.0 | 6 | 0.0 | - |
| CPI-147236f | 5.7 | 24.2 | 0.15 | 67.7 | 181 | 53.0 | 15 | 0.0 | - |
| CPI-147244a | 23.3 | 36.9 | 0.40 | 77.5 | 172 | 88.6 | 0 | - | - |
| CPI-147244b | 2.0 | 29.7 | 0.13 | 85.4 | 181 | 45.3 | 44 | 11.5 | 0.08 |
| CPI-147244c | 11.2 | 30.9 | 0.19 | 95.5 | 186 | 94.8 | 49 | 28.6 | 0.21 |
| CPI-147244d | 14.8 | 34.1 | 0.21 | 87.2 | 181 | 91.4 | 49 | 5.2 | 0.08 |
| CPI-147244e | 21.2 | 31.8 | 0.21 | 98.3 | 183 | 112.9 | 59 | 7.9 | 0.26 |
| CPI-147244f | 4.4 | 37.5 | 0.12 | 79.5 | 187 | 70.1 | 28 | 6.2 | 0.04 |
| l.s.d. | 7.42 | 2.46 | 0.04 | 20.87 | 8.6 | 18.22 | 17.3 | 10.85 | 0.142 |

Table 4. Second year summer grain yield (g/m) and regrowth score (%) following the second summer of entries in Experiment 1. Entries that did not persist beyond year 1 are excluded from this table, other than those included in the heterogeneity test groupings.

| Heterogeneity test groupings | | | Other entries | | |
|-------------------------------------|------------------------------|-------------------------------|----------------------|------------------------------|-------------------------------|
| <i>Entry</i> | <i>Grain yield (g/m)</i> | <i>Regrowth score (%)</i> | <i>Entry</i> | <i>Grain yield (g/m)</i> | <i>Regrowth score (%)</i> |
| CPI-147235a | 58.2 | 5.8 | CPI-148041 | 49.7 | 99.2 |
| CPI-147235b | 0.0 | 0.0 | CPI-148052 | 43.6 | 98.3 |
| CPI-147235c | 0.0 | 0.0 | CPI-148048 | 51.1 | 96.7 |
| CPI-147235d | 52.5 | 0.0 | CPI-148043 | 56.4 | 95.8 |
| CPI-147235e | 0.0 | 0.0 | Dundas | 71.9 | 94.2 |
| CPI-147235f | 0.0 | 0.0 | CPI-148056 | 58.9 | 93.3 |
| CPI-147235g | 66.7 | 0.0 | CPI-148058 | 69.6 | 93.3 |
| CPI-147235h | 17.6 | 0.0 | CPI-148062 | 84.5 | 93.3 |
| CPI-147235i | 24.1 | 0.0 | CPI-148045 | 52.4 | 92.5 |
| | | | CPI-148057 | 64.9 | 92.5 |
| CPI-147236a | 20.0 | 0.0 | CPI-148049 | 52.3 | 90.8 |
| CPI-147236b | 1.1 | 0.0 | CPI-148055 | 43.4 | 85.8 |
| CPI-147236c | 38.4 | 0.0 | CPI-148042 | 41.1 | 84.2 |
| CPI-147236d | 1.1 | 0.0 | CPI-148044 | 64.7 | 76.7 |
| CPI-147236e | 0.0 | 0.0 | CPI-148051 | 72.0 | 63.3 |
| CPI-147236f | 0.0 | 0.0 | CPI-148037 | 32.7 | 61.7 |
| | | | 6754 | 9.8 | 5.0 |
| | | | 915 | 54.7 | 0.0 |
| CPI-147244a | 0.0 | 0.0 | 916 | 51.7 | 0.0 |
| CPI-147244b | 6.3 | 0.0 | 917 | 4.4 | 0.0 |
| CPI-147244c | 62.6 | 0.0 | 6755 | 53.2 | 0.0 |
| CPI-147244d | 27.0 | 0.0 | 11955 | 65.9 | 0.0 |
| CPI-147244e | 88.7 | 0.0 | 20238 | 16.1 | 0.0 |
| CPI-147244f | 4.1 | 0.0 | CPI-147242b | 13.0 | 0.0 |
| | | | CPI-147251b | 16.4 | 0.0 |
| | | | CPI-147257b | 4.0 | 0.0 |
| | | | CPI-149777 | 8.6 | 0.0 |
| | | | CPI-149787 | 7.8 | 0.0 |
| | | | CPI-149796 | 6.9 | 0.0 |
| | | | OK7211542 | 78.8 | 0.0 |
| | | | TAF 46 | 57.9 | 0.0 |
| <i>l.s.d.</i> | <i>36.39</i> | <i>13.62</i> | | <i>36.39</i> | <i>13.62</i> |

Table 5. Spring and post grain harvest (summer/autumn) dry matter (DM) production (g) for 6 entries under 4 defoliation regimes, D₀- D₃ (reproduced from Newell *et al.* 2013c).

| Entries | Spring 2011 DM | | | | Post-harvest DM 2011/2012 | | | | Cumulative DM | | | | |
|--|----------------|----------------|----------------|----------------|---------------------------|----------------|----------------|----------------|----------------|----------------|----------------|----------------|--|
| | D ₀ | D ₁ | D ₂ | D ₃ | D ₀ | D ₁ | D ₂ | D ₃ | D ₀ | D ₁ | D ₂ | D ₃ | |
| CPI-148055 | - | 3 | 2 | 39 | 239 | 213 | 299 | 230 | 239 | 215 | 301 | 269 | |
| 11955 | - | 41 | 27 | 226 | 315 | 318 | 337 | 211 | 315 | 359 | 364 | 436 | |
| CPI-147235a | - | 17 | 14 | 150 | 145 | 50 | 111 | 52 | 145 | 67 | 125 | 201 | |
| CPI-147280b | - | 19 | 13 | 111 | 92 | 55 | 118 | 17 | 92 | 74 | 131 | 128 | |
| OK7211542 | - | 37 | 27 | 252 | 149 | 216 | 205 | 67 | 149 | 254 | 231 | 319 | |
| Wedgetail | - | 100 | 70 | 316 | 0 | 0 | 0 | 0 | 0 | 100 | 70 | 316 | |
| <i>lsd</i> (<i>P</i> = 0.05) ^a | | | | | | 100.2 | | | | | | | |
| <i>lsd</i> (<i>P</i> = 0.05) ^b | | | | | | 89.2 | | | | | | | |

^a when comparing means within a defoliation treatment

^b when comparing means with the same level of entry

Table 6. Average number of plants remaining (from the original 10 planted) of 6 entries under 4 defoliation treatments post grain harvest from December 2011 to April 2012 (reproduced from Newell *et al.* 2013c)

| Entries | Assessment date | | | |
|--|-----------------|----------|----------|------------|
| | Dec 2011 | Jan 2012 | Feb 2012 | April 2012 |
| CPI-148055 | 9 | 9 | 9 | 9 |
| 11955 | 10 | 10 | 10 | 8 |
| CPI-147235a | 10 | 10 | 9 | 5 |
| CPI-147280b | 10 | 10 | 7 | 4 |
| OK 72 | 10 | 10 | 10 | 6 |
| Wedgetail | 0 | 0 | 0 | 0 |
| <i>lsd</i> ($P = 0.05$) ^a | 1.3 | | | |
| <i>lsd</i> ($P = 0.05$) ^b | 1.0 | | | |

^a when comparing means within assessment date

^b when comparing means with the same level of entry

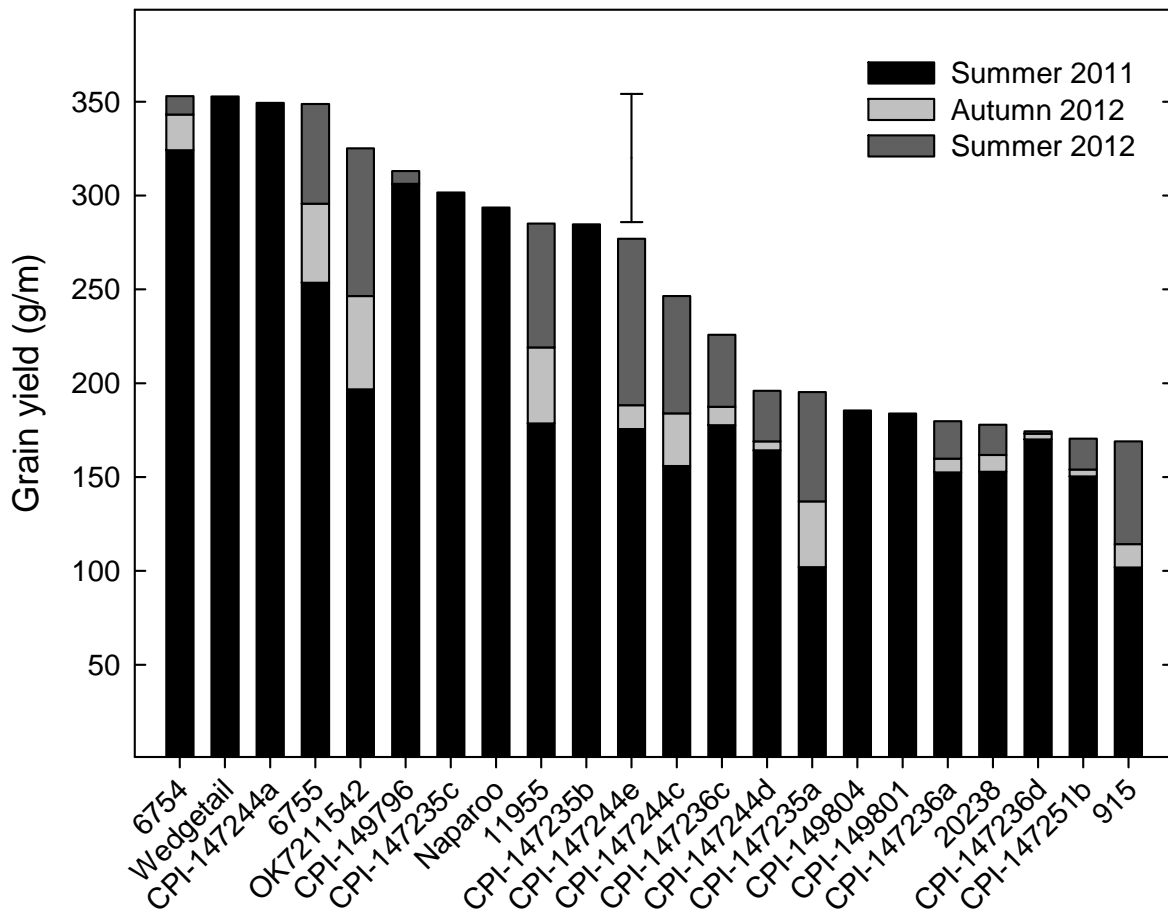
Table 7. Proportion of live plants (survival), the associated water consumption and the length of the drying cycle to which perennial wheat derivative lines CPI 147280b, CPI 147235a, OK72, '11955', *Thinopyrum intermedium* '055' and *Triticum aestivum* cv. EGA Wedgetail were subjected. Means followed by the same superscripts are not significantly different ($P=0.05$).

| Genotype | Survival (proportion) | Total water consumption (g) | Days of drying cycle |
|----------------------------|--------------------------|--------------------------------|-------------------------|
| CPI 147280b | 0.27 ^a | 681.0 ^a | 51.9 ^b |
| CPI147235a | 0.77 ^c | 696.1 ^a | 51.9 ^b |
| OK72 | 0.36 ^{ab} | 679.9 ^a | 51.9 ^b |
| 11955 | 0.41 ^b | 685.9 ^a | 51.9 ^b |
| <i>Th. intermedium</i> 055 | 0.88 ^d | 742.3 ^c | 56.6 ^a |
| Wedgetail | 0.27 ^a | 715.2 ^b | 51.9 ^b |

Table 8. Examples of perennial grasses from the *Triticeae* tribe found in Australia. (Information summarised from PlantNET; Cunningham *et al.* 1981; Wheeler *et al.* 1990).

| Species | Origin | Distribution |
|---|------------|--|
| <i>Australopyrum pectinatum</i> | Native | Generally found on black soil plains at higher altitudes of the northern and southern tablelands NSW. Also Victoria and Tasmania |
| <i>Australopyrum retrofractem</i> | Native | Found in alpine regions in southern Australia. |
| <i>Australopyrum velutinum</i> : | Native | Grows in high altitude forests and grasslands of NSW, Victoria and Tasmania |
| <i>Elymus scaber</i> var. <i>scaber</i> . | Native | Wide distribution in NSW from coastal to inland environments. Very common in all southern regions of Australia. |
| <i>Elymus scaber</i> var. <i>plurinevus</i> | Native | Restricted to self mulching soils of northwest slopes NSW and in Queensland |
| <i>Elymus multiflorus</i> | Native | Distribution limited to coastal and riparian areas of NSW, Queensland and Western Australia. |
| <i>Elymus rectisetus</i> | Native | Found in most divisions of NSW except the south coast. Also in areas of Victoria, South Australia and Queensland |
| <i>Elymus fertilus</i> | Native | Found on basaltic and alluvial soils of the northwest plains of NSW and in Queensland |
| <i>Elytrigia repens</i> | Introduced | Common in cooler tableland and slopes areas of NSW. Also Vic, Tasmania, South Australia & Western Australia. Has been used in wheatgrass improvement programs in the past. |
| <i>Leymus arenarius</i> | Introduced | Small number of plants in Tasmania and Victoria only |
| <i>Thinopyrum ponticum</i> | Introduced | Scattered on saline areas where used as a pasture species in southern states. |
| <i>Thinopyrum junceiforme</i> | Introduced | Grows between the strandline and the fore-dune of beaches or along the sandy banks of estuaries Victoria and Tasmania. |
| <i>Thinopyrum distichum</i> | Introduced | Coastal areas of Western Australia. |

Fig 1. Cumulative yield (g/plot) of grain harvested in the first summer (2011), the first autumn and the second summer after planting of the twenty highest yielding experimental entries in Experiment 1. Error bar indicates significant differences between total cumulative yields at $P = 0.05$.



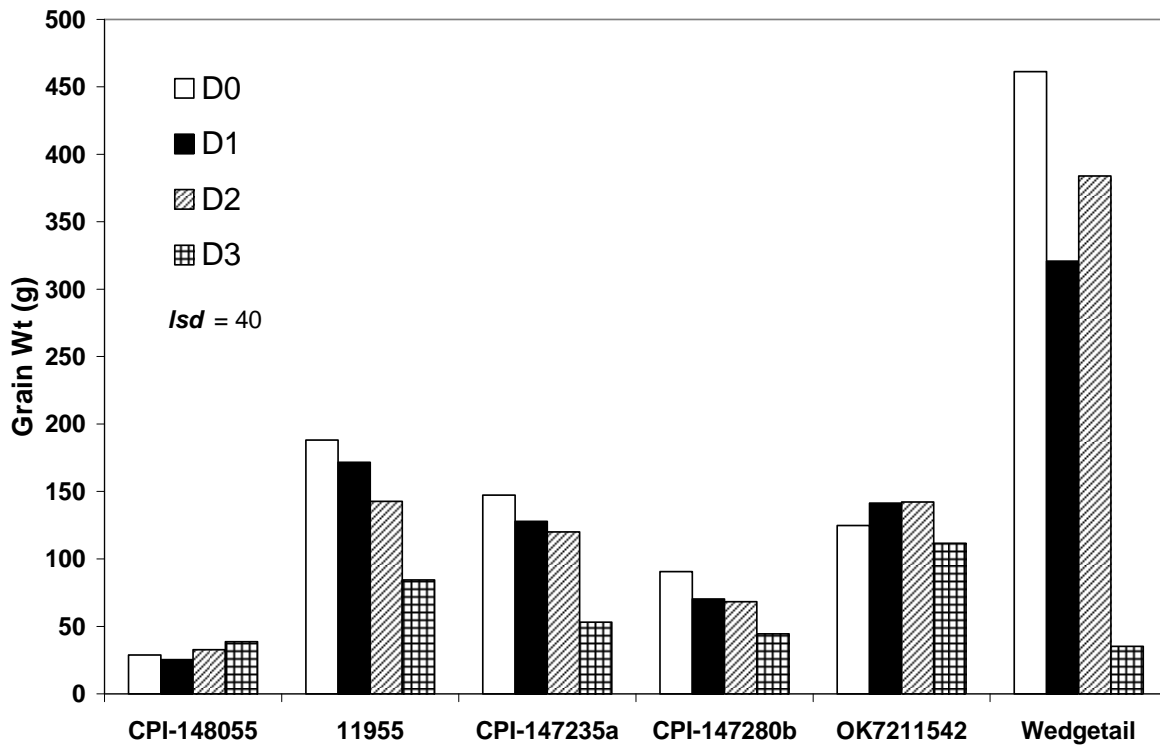


Figure 2 First year grain yield (g/plot = g/10 plants) of one *Th. intermedium* line, 4 wheat x wheatgrass hybrids and an annual wheat under 4 defoliation treatments (**D₀**: no defoliation, **D₁**: defoliated once in the season, **D₂**: defoliated twice, **D₃**: defoliated twice followed by a third defoliation to simulate a hay cut).

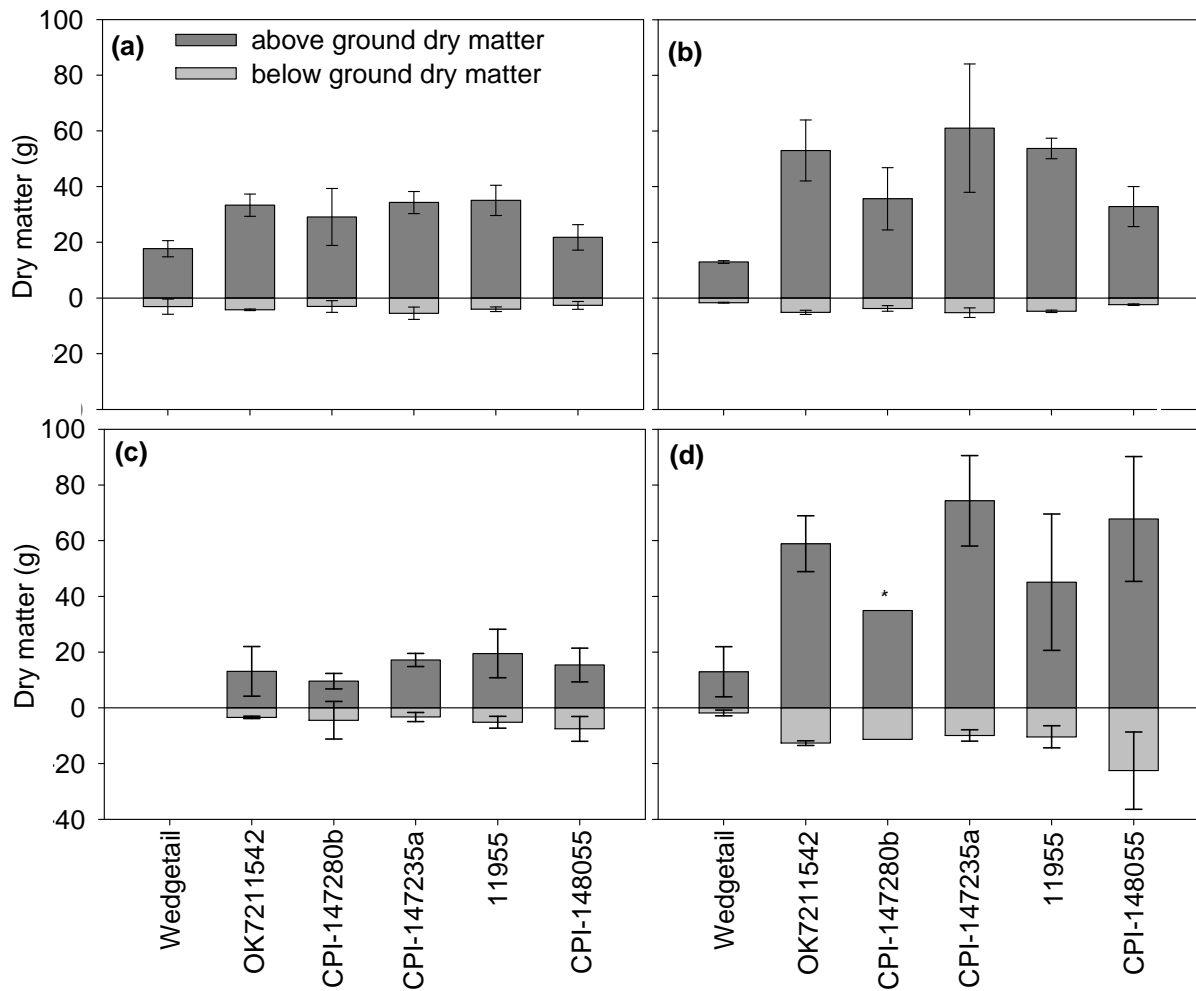


Figure 3. Above-ground (leaf, straw, stem, spike) and below-ground (total roots) dry matter (g/plant) of one annual wheat (cv. Wedgetail), one perennial grass (CPI-148055) and four perennial wheat derivatives (OK7211542, CPI-147280b, CPI-147235a, and 11955) grown in large soil columns at Wagga Wagga under well watered conditions at four growth stages: a) flowering of first wet season (WS1) crop; b) maturity of first wet season (WS1) crop; c) end of dry season (DS) regrowth; and d) maturity of second wet season (WS2) crop. Bars are \pm SEM. CPI-147280b was represented by 1 replicate only in the final harvest (d).

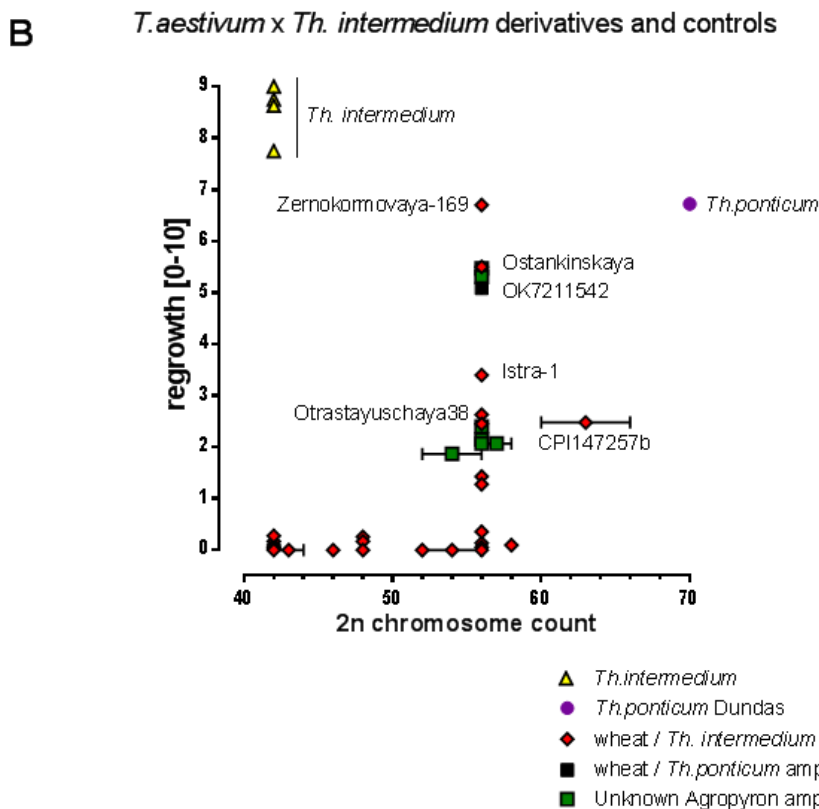
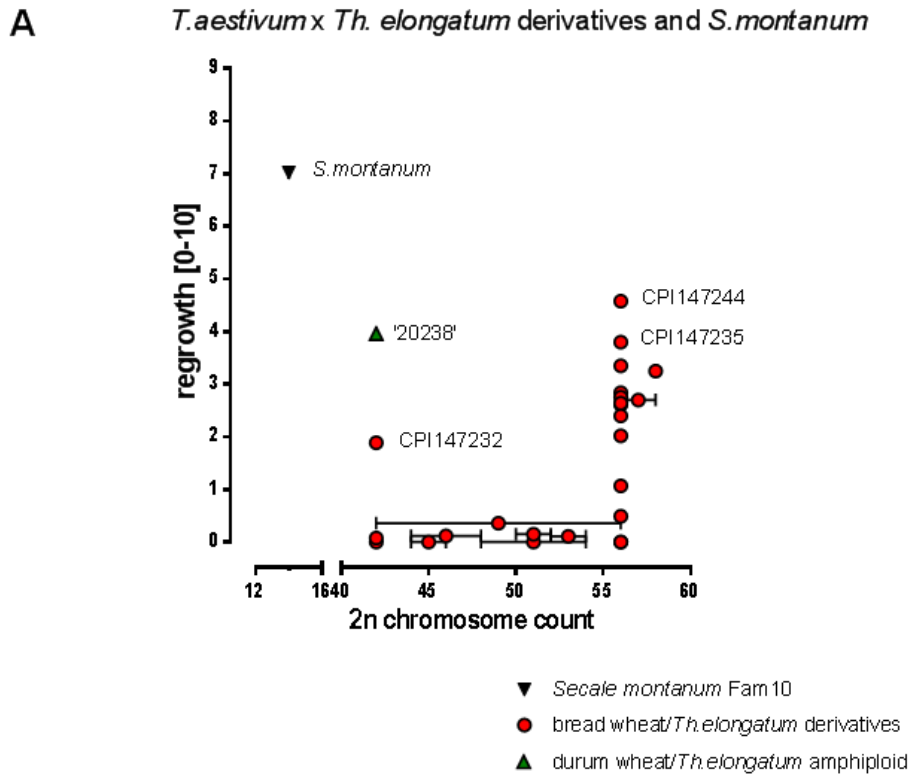


Figure 4. 2n chromosome counts and post-harvest regrowth score in the field for a range of control perennial species and wheat hybrid derivatives. The bars show the range where variable chromosome counts were observed. Some of the data plotted is from Hayes et al (2012). A. show mainly wheat x *Th.elongatum* hybrid derivatives. B. shows mainly wheat x *Th intermedium* derivatives.