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**Assessing Anther Extrusion and its Effect on US Hard Winter Wheat (*Triticum
aestivum* L.) Hybrid Seed Production**

By

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Assessing Anther Extrusion and its Effect on US Hard Winter Wheat (*Triticum aestivum* L.) Hybrid Seed Production

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University of Nebraska, 2017

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The promise of higher grain yields as a result of the development and production of hybrid wheat (*Triticum aestivum* L.) has not been fully realized primarily due to the high cost of seed production. Anther extrusion is a key trait that improves pollen availability, and thus, is expected to enhance hybrid wheat seed production yields. Hard winter wheat germplasm adapted to the US Great Plains was visually assessed for anther extrusion in the field and greenhouse environments. Significant genotypic differences were detected and high broad-sense heritability was calculated (ranging from 0.62 to 0.85) for anther extrusion in the field. Over 50% of the genotypes were visually assessed as 5 or higher (1 lowest to 9 highest extrusion) in both 2014 and 2015. Visual ratings made in the greenhouse were not highly correlated ($r=0.40^*$) with those made in the field, indicating that selection for anther extrusion should be conducted in the field. A chemical hybridizing agent, CROSOIR 100®, was used to induce male sterility and produce hybrid seed to determine the significance of anther extrusion on hybrid seed production. Hybrid seed yield as determined by weight was weakly correlated in 2015 ($r=0.60^*$) but not significantly correlated in 2016 with anther extrusion, indicating that anther extrusion likely improves hybrid seed set. However, hybrid seed set results must also be interpreted while considering the phytotoxic effects of the CHA, and its possible impact based on genotype.

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Introduction:

Wheat (*Triticum aestivum* L.) ranks third in global production behind maize (*Zea mays* L.) and rice (*Oryza sativa* L.) for food crops (FAOSTAT, 2017). It is important to improve wheat yields to meet the increasing demand associated with a rising global population which is estimated to reach 9 billion by the year 2050 and increased affluence (FAOSTAT, 2017). According to the Consultative Group on International Agricultural Research (CGIAR, 2016), food production will need to increase by at least 50 percent (1.4% per year) between 2010 and 2050. This increase may be especially difficult to achieve when considering the negative impact of climate change. To meet this demand, improved genetics coupled with the best agronomic practices must be developed. Improvements in genetics (exploiting heterosis in non-hybrid crops) will be critical for future grain yield improvements, but current plant breeding methodology has not provided the annual increases needed to meet projected future demand (CGIAR, 2016; Flavell, 2016). A study done to estimate grain yield increases from 1984 to 2008 for Great Plains wheats found that the increase in genetic gain was only about 1% per year (Graybosch and Peterson, 2010). Because the authors focused on genotypes adapted to the US Great Plains, results of this study demonstrates that a different breeding approach is needed to reach the goal of 1.4% per year as suggested by CGIAR (2016).

Hybrid wheat presents a new option to increase genetic gain over traditional cultivars, in that hybrid wheat should result in higher grain yields, improved resistance to pests and pathogens, and grain yield stability, particularly in marginal production environments (Boeven et al., 2016a; Cisar and Cooper, 2002). Longin et al. (2012) estimated heterosis in wheat ranged from 3.5% to 15%, while Zhao et al. (2015) reported

a hybrid which yielded 1 Mg ha⁻¹ more than a released cultivar ‘Tobak’ which is approximately 9% better. For hybrid wheat to be a commercial success, it must be economically feasible to produce enough hybrid seed at a price farmers are willing to pay based on increased yield or value over inbred cultivars. According to Longin et al. (2012), hybrid breeding and production of automatable cereal crops has not achieved the levels of maize because of the high seeding rates required, low heterosis, and a lack of economically viable hybrid seed production techniques in wheat. Although these problems have limited the success of hybrid wheat, both public and private sector initiatives have been started in recent years to improve hybrid performance and reduce production costs.

To improve the economic viability of hybrid wheat seed production, there must be a clear understanding of what morphological traits will help optimize seed set (or seed production) on the female parent while minimizing the need for large numbers of male parent plants (pollinators). The female parent should be male sterile, accomplished with a chemical hybridizing agent (CHA) or cytoplasmic male sterility (CMS), with the glumes open (gaping) that expose the receptive stigma to the pollen shed from the male parent for a long time period (Langer *et al.*, 2014). De Vries (1971) estimated that stigmas stay receptive between 2 and 6 days optimally but reported receptivity up to 13 days so extending the pollination window would increase the diversity of crosses that can be made due to differences in parental genotype anthesis date. The ideal male parent is a taller, exhibits differential flowering between tillers and has large anthers that exhibit excellent extrusion from the floret (measure of anthers outside the floret). It would also produce abundant pollen that stays viable for longer than half an hour (D’Souza, 1970;

Whitford et al., 2013). It is estimated that a wheat plant produces about 2.5% the total pollen that a maize tassel produces so increasing the amount of pollen and its availability (anther extrusion) is an important goal (De Vries, 1971). Male characteristics (specifically anther extrusion) have been the focus of recent research and have been reported as the limiting factor for hybrid wheat seed production (Boeven et al., 2016b).

Identifying ideal male parents has been a challenge since interest in hybrid wheat started in the 1960's. Researchers looked to change wheat floral characteristics to make it more compatible for cross-pollination. Research done by De Vries (1973), D'Souza (1970), Lucken (1986), and others focused on improving anther extrusion, anther length, and amount of pollen dispersed to produce hybrid wheat seed. Breeding for floral traits in wheat that foster outcrossing is difficult because the traits are difficult to characterize by phenotyping and the characterization is labor-intensive. Consequently, breeding for floral traits often requires additional labor costs (Langer *et al.*, 2014). A common trait which garnered the most interest is anther extrusion because exposing anthers outside the floret should increase the amount of pollen available to the female parent. Anther extrusion is thought to be ideal for initial selection of male parents as many genotypes can be visually rated relatively quickly in the field (Langer et al., 2014). Research from Europe suggests that anther extrusion is a quantitative trait under the control of genes with small effects but seems to be highly heritable with reported heritability ranging from 0.71 to 0.91 (Boeven et al., 2016b; Langer et al., 2014; Muqaddasi et al., 2016; Skinnies et al., 2010). With such high heritability, anther extrusion might be a candidate for selection in the greenhouse but with the genotype by environmental interactions reported this may not be possible (Boeven et al., 2016b; Langer et al., 2014). Although anther

extrusion is believed to be an important floral trait known to impact the success of outcrossing, there are few recently published results that characterize its impact on producing hybrid wheat seed using North American germplasm

The goals of this research were to: 1. utilize a diverse, adapted germplasm pool to identify variation for anther extrusion on the basis of a visual assessment 2. determine if anther extrusion in a greenhouse environment was predictive of anther extrusion under field conditions, and 3. assess the importance of anther extrusion to hybrid seed production.

Materials and Methods

Visual Assessment of Anther Extrusion

Visual assessment for anther extrusion was conducted on a total of 288 hard winter wheat genotypes (Table 1/Appendix Table 1). Both released and experimental genotypes were rated from breeding programs across the US Great Plains region with the majority of genotypes coming from the University of Nebraska Lincoln. Ratings were taken on the Triplicate (TRP), Nebraska Interstate Nursery (NIN), Irrigated/Dry (IRDR) yield trial, and Regional Performance Nursery (RPN, a nursery made by combining the Southern and Northern Regional Performance Nurseries and adding enough additional experimental genotypes to create a 90 entry trial) during 2014 and 2015 at two locations, Lincoln and Mead, Nebraska, USA (Table 2/ Appendix Table 2). These locations were chosen because of the relative ease of accessing them to repeatedly assess entries for anther extrusion. The trials were designed as alpha lattices with 3 to 4 replications per location depending on the trial and were created via Agrobase Gen II® Software (Agronomix, Inc. Winnipeg, Canada). Genotypes were planted in 3.0 m long four row

plots with 30 cm between rows in 2014 and in 3.0 m long five row plots with 22.8 cm between rows in 2015 at a seeding rate of 66 kg ha⁻¹. Twenty-nine entries from the NIN14 were also grown in the greenhouse and planted in 10 cm square pots. Greenhouse grown plants were planted in early November, vernalized in place between 6°C to 20°C with no supplemental light until January where the day length was gradually lengthened with artificial light to 16 h light, 8 h dark by the end of February and thereafter. These greenhouse grown genotypes were assessed for anther extrusion in March.

Anther Extrusion is a trait which can be assessed by visually rating to what extent anthers are presented outside of the glumes of the florets. Genotypes were visually rated for anther extrusion using a scale from one to nine with one indicating that little or only the tip of the anther is visible and nine indicating high number of anther fully presented outside of the floret (Figure 1). Visual assessment was chosen over more intensive metrics to maximize the number of genotypes which could be assessed in the limited time available. Anther extrusion was taken when 50 percent of the spikes had anthers showing and were shedding pollen, which is anthesis date. This protocol was done to standardize the timing of the assessment to decrease bias in the field. Factors which affected the genotypes' assessment included distribution of anthers along the wheat spike, the number of anthers seen per spikelet (maximum of nine is normally possible based on the assumption the primary, secondary, and tertiary florets have similar anthesis dates), and the variability of anthers extruded between flowering spikes within each plot (Figure 1).

Statistical analyses was completed using the ASREML 3.0 R package (Gilmour et al., 2009). Variance components were calculated using the restricted maximum

likelihood (REML) method, with all terms treated as random effects except replicates at single locations (three levels of measurement) and the location term (two levels of measurement) for multiple location analyses were treated as fixed effects. Significance testing was done with 95% confidence intervals for variance components using the *nadiv* R package (variance components were significant if the interval did not contain zero). Genotypes were treated as a random effect because no selection had been carried out for anther extrusion in any of the trials so it was reasonable to assume there was a representative sample of genotypes. Best linear unbiased predictions (BLUPs) along with mean adjusted BLUPs were calculated for genotypes at both single locations and across locations when possible. Broad sense heritability (H^2) was calculated for single locations and multi-location trials (Figure 2/Appendix Figure 2). Correlations between greenhouse assessments and mean adjusted BLUPs (combined analysis) from the NIN14 were calculated using SAS software 9.3 Proc Corr, copyright © 2002-2010 by SAS Institute Inc., Cary, NE, USA.

Hybrid Seed Production

In 2015 and 2016, to determine the relative impact of anther extrusion on hybrid seed production, a complete diallel crossing scheme which included 25 parental genotypes from the University of Nebraska and Texas A&M University wheat breeding programs was planted (Table 3). Genotypes with anther extrusion ratings of 5 or higher in previous breeding trials were included in the diallel scheme. To produce the necessary hybrid seed, 25 crossing blocks were created. Each crossing block was surrounded by a single male planted in a solid four row strip with 30 cm between rows at a seeding rate of 47 kg ha⁻¹ with 26 females planted in paired plots (13 x 2) 3.0 m long four row plots with

30 cm between rows at a seeding rate of 66 kg ha⁻¹ (Figure 3). Male parents were planted at a reduced seeding rate (25 g per plot) to encourage tillering which could potentially extend flowering. An extra genotype (NE10478-1) was added as a female parent to even out the number of female plots as there was space for 26 female plots in each crossing block. All 25 crossing blocks were originally planted at Lincoln, but a storm destroyed five of blocks just after planting. Those blocks were replanted at Mead due to lack of space at Lincoln.

In 2016, the seed production trial was repeated with 18 of the crossing blocks planted in Mead, NE and seven planted in Texas (only the 18 crossing blocks at Mead will be discussed for the 2016 analysis). As with the trial in 2015, each crossing block had one solid male strip surrounding 26 female plots. Plots and strips were planted with five rows with 22.8cm between rows instead of four rows with 30 cm between rows using the same seeding rates from 2015. Decreasing row width in the female plots was thought help increase tiller synchrony (reduce late tillering) for CHA optimization.

Male sterility was induced by the use of the CHA CROSOIR 100® (common name sintofen, 1-(4-chlorophenyl)-1,4-dihydro-5-(2-methoxyethoxy)-4-oxo-3-cinnolinecarboxylic acid, Saaten-Union Recherche, St. Denis, France). The timing and rate of application were as described on the product label. Two to three wax paper spike bags per plot were placed over single spikes to prevent cross pollination as a way to confirm male sterility and the efficacy of the CHA. Male parents were visually assessed for anther extrusion and anthesis date. Plants treated with CHA were confirmed as male sterile, and visually assessed for any phytotoxic effects of the CHA. The date at which

75% of the spikes in a plot had florets completely opened and receptive to pollen (personal communication A. Easterly) was also recorded.

An anther stigma interval (ASI) was determined for hybrid combinations, which was defined as the difference between the date of female gaping and the anthesis date of the male. The ASI was used to determine the optimal anthesis date for parental combinations and also to assess how anther extrusion affects pollination over time. However gape dates were difficult to record in 2015 because weather limited the number of days that data could be taken. May 2015 (when the gape dates needed to be taken) had rainfall in excess of 27 cm compared to the normal average around 10 cm (UNL School of Natural Resources, 2015). Early observations indicated that the difference between gape date and anthesis date was about two days due to the activity of the CHA and lack of self-pollination. Using this average, estimated gape dates were calculated for females, which were determined to have suspicious gape date data (caused by our not being able to measure gaping daily) by using the anthesis date of the male counterpart and adding two days to that value. The cutoff for successful cross-pollination was set at an ASI of seven days because it was assumed that later tillers would shed pollen no longer than seven days in Nebraska. De Vries (1971) reported depending on weather conditions, wheat spikes flowered over a period of four to five days. In Germany, with a longer grain filling period and generally lower temperatures during flowering, Langer et al. (2014) reported minimum flowering duration of 8 d and mean of 12 d. Hence, it was reasonable to assume pollination was possible seven days after anthesis date. The 2016 hybrid production trial did not have the weather issues experienced in 2015. Gape dates

from 2016 were determined to be accurate and the delay between gape date and anthesis date was determined to be between two and four days.

Female plots in the crossing blocks at Lincoln and Mead were harvested with a Wintersteiger USA Classic (Salt Lake City, Utah) plot combine to determine grain weight. The mean female grain weights (only weights from crosses which had compatible ASI) from each crossing block were correlated using SAS Software 9.3 Proc Corr to anther extrusion ratings from the corresponding male parents to determine if there was a relationship between anther extrusion and hybrid seed production at both locations separately and combined in 2015. The data from 2016 was correlated using only data from the Mead, NE location. Cross-pollination success rates were determined by comparing the average grain weight of the male parent plots and the grain weight of the hybrid counterpart, for example the average weight of the ‘Freeman’ male plots compared to the weight of Freeman x Freeman hybrid plot. Finally, reduced seed production capacity due to phytotoxicity was assessed by examining differences for ASI between 2015 and 2016 along with cross-pollination success rates from 2016.

Results and Discussion

Visual Assessment of Anther Extrusion

Evaluation for anther extrusion began in 2014 on the University of Nebraska’s elite yield trials at Lincoln and Mead, NE. The distribution of visual ratings for anther extrusion was checked for normality due to visual anther extrusion assessments being categorical and found to be approximately normal (Appendices) hence ANOVA could be used for analyses. In total, 288 genotypes were assessed between eight trials over two years with the trials having similar distributions (Figure 4). Significant differences for

anther extrusion were observed among genotypes but the genotype by location interaction was not significant as the 95 percent confidence interval contained the null hypothesis of zero (Table 4). In 2015, the Regional Performance Nursery was assessed along with elite yield trials at Lincoln and Mead, NE. Again all trials had significant differences between genotypes but the genotype by location interaction was not significant based on the confidence intervals (Table 4). The lack of genotype by location interaction for anther extrusion is understandable given that anther extrusion is a highly heritable trait (Langer et al., 2014) and Peterson (1992) found that Lincoln and Mead cluster together agronomically, hence are relatively similar testing locations.

High variability for anther extrusion was found among genotypes from the Nebraska breeding program and breeding programs across the Great Plains with over 75% of the trials having a mean rating of five or higher and genotype ratings ranging from one to nine (Figure 4). Greater than 50% of the genotypes scored above five for anther extrusion, which indicated that Nebraska and Great Plains germplasm have many candidates for male parental genotypes. Along with the large numbers of good genotypes, broad sense heritability for anther extrusion ranged from 0.62 to 0.85 (Table 4). High heritability values are consistent with previous findings from Boeven et al. (2016b), Langer et al. (2014), Muqaddasi et al. (2016), and Skinnes et al. (2010) in Europe and indicated that breeding for better anther extrusion should be possible and that individual genotype performance should be repeatable for anther extrusion.

Genotype performance was determined using predicted values from mean adjusted BLUPs (Table 2). Some genotypes were consistently high performers for anther

extrusion across years and locations. For example, in 2014 and 2015, Nebraska release Freeman was among the top five genotypes and had mean adjusted BLUPs ranging from 6.3 to 7.1 in the TRP14L, TRP15L, TRP15M, NIN14M, and NIN15M (Table 1, Table 2). Conversely, there were genotypes which were consistently among the poorest performers for anther extrusion. In 2014 and 2015, ‘Scout 66’ had mean adjusted BLUPs ranging from 3.6 to 4.7 in the NIN14L, NIN14M, and NIN15M. ‘Camelot’, only used as a check in 2014, was the most consistent low performing genotype with mean adjusted BLUPs ranging from 2.1 to 2.5 in the TRP14L, NIN14L, and NIN14M. Interestingly, other genotypes did not perform as consistently. For example, NHH11569 was the top ranked genotype in the NIN at Lincoln in 2014 (7.1) but ranked 23rd at Mead (6.5). NE13672 and NE13629 were ranked 14th and 15th in the TRP14L (6.2 and 6.1) but ranked 42 and 43 (4.7 and 4.6) in the NIN15L. The genotypes which did not perform as consistently indicated that environment has an effect on some genotypes, despite the locations and genotype x location being non-significant. Evaluating genotypes in more diverse environments may cause the environment to have a significant effect on anther extrusion performance.

Twenty nine entries of the NIN were assessed for anther extrusion in both the greenhouse and the field (NIN14 combined analysis). A correlation of $r=0.40^*$ between greenhouse and field anther extrusion assessments suggests that it may be difficult to utilize greenhouse anther extrusion evaluations of genotypes to predict performance for anther extrusion in the field. Upon examining individual genotype performance, a number of genotypes had large differences between the two assessments. For example,

NHH11569 had a field mean adjusted BLUP of 7.0 and a greenhouse assessment of 2.0, and NE12488 had a mean adjusted BLUP of 5.6 but a 2.0 in the greenhouse.

‘Goodstreak’ had a large difference between field (4.9) and greenhouse (7.0) indicating that the greenhouse did not have a consistent negative effect on anther extrusion.

Freeman and Camelot had close similarity between field and greenhouse assessments.

Freeman had a mean adjusted BLUP of 7.2 and a greenhouse assessment of 7.0. Camelot had a mean adjusted BLUP of 2.5 and a greenhouse assessment of 1.0. The relatively low correlation between field and greenhouse anther extrusion assessments suggests that breeders would not want to assess germplasm for anther extrusion in a greenhouse environment in the absence of also doing so in a field environment. To make selections based only on greenhouse anther extrusion data risks overlooking germplasm with better than average anther extrusion in the field, and it is the field environment where the commercial production of hybrid seed is expected to take place.

Anther Extrusion is a difficult trait to quantify. Although, with the help of a standardized visual rating scale, anther extrusion can be assessed quickly given that there is a small window of opportunity in which to make visual assessments. Also high wind and rain can dislodge anther from spikes before an adequate visual assessment can be made. As Langer et al. (2014) and Boeven et al. (2016b) reported, visual ratings data for anther extrusion are best used for initial selection since counting anthers outside of the floret is more labor intensive, and doing so would significantly reduce throughput. In the present study, anther extrusion assessments made in the field based on a standard visual scale were informative, and enabled excellent anther extruding genotypes to easily be

distinguished and separated from poor performing genotypes. Additionally, there were genotypes that could be consistently assessed as high, or low anther extrusion performers across environments, suggesting breeding progress can be made when selecting for improved anther extrusion in the field. As of yet, no major QTL or molecular markers have been identified as linked to anther extrusion, and in fact, reports are that anther extrusion is controlled by a large number of genes (Langer et al. 2014; Boeven et al. 2016b). This also emphasizes the importance of assessing and selecting for improved anther extrusion on a visual basis.

Hybrid Seed Production

Anther extrusion was examined in the hybrid production trials to determine the effect that it had on hybrid seed yields (cross pollination success). Genotypes which scored five or above for anther extrusion in 2014 were selected to be used in the hybrid production trial with the expectation that the score would be sufficient to produce large quantities of hybrid seed. To ensure the grain weights were an accurate measure of cross pollination, male sterility in the CHA treated plots was verified by bagging individual spikes to prevent cross pollination. In 2015 and 2016, male sterility achieved with the CHA was 80% to 100% (personal communication A. Easterly). The correlation between anther extrusion and female plot seed weight ($r = 0.60^*$, $p = 0.002$, $n = 25$) for the 2015 production trial at both Lincoln and Mead combined was significant. Since there were two locations, separate correlations were done for the trial. Both the Lincoln and Mead correlations were not significant ($r = 0.40$, $p = 0.08$, $n = 20$ and $r = 0.79$, $p = 0.11$, $n = 5$ respectively). The lack of significance at the individual locations (p not greatly higher than $\alpha = 0.05$) may be due to smaller sample size. The lack of significance may also be

explained by parental selection for generally good anther extrusion hence having good pollination capabilities (above a threshold for adequate pollination) and a lack of variability for anther extrusion (too small a range of anther extrusion). Interestingly, the highest mean crossing block female seed weight (768 g) had the highest performing male anther extrusion parent (Freeman which was rated an 8.0). In 2016, the correlation ($r = 0.32$, $p = 0.24$, $n = 15$) was not significant on the crossing blocks planted at Mead and the highest performing crossing block for mean female seed weight (555 g) had NE10683 as the male which had an anther extrusion score of 6.0 where Freeman (score 8.0) had a mean female seed weight of 473 g. The results from 2015 were interpreted to mean that an anther extrusion score of five or higher probably exposed enough anthers that cross pollination was possible. Evidence of phytotoxicity due to the CHA application was evident (data not shown), and this should be considered when interpreting the impact of anther extrusion on hybrid seed production. For example, when there is more phytotoxicity, or damage to female plants, a higher level of anther extrusion might be required to produce the same amount of hybrid seed compared with when there is little damage to the female plants as a result of a CHA treatment.

To assess the impact of the CHA on female seed set, a cross pollination success rate was calculated based on sib-crosses. In 2016 for example, the weight of the Freeman x Freeman hybrid plot was divided by the average weight of selfed Freeman male plots in that crossing block. Male plot weights were not recorded in 2015. The average cross pollination success rate among the 15 blocks that did not have male CHA damage (due to overspray) was 20% with a range from 12.5% to 40.0%. It should also be noted that the

average seed weights for each crossing block only ranged from 291 g to 555 g compared to 2015 which ranged from 212 g to 768 g further indicating that cross pollination potential was reduced most likely due to phytotoxicity or environmental factors. These findings are consistent with Pickett (1993) who reported cross pollination success ranging 6% to 20% in CHA treated experiments compared to approximately 50% in CMS trials. To get a better understanding of the effect that anther extrusion has on cross pollination it would be important to study anther extrusion and ASI with CMS females to compare to CHA treated females. Cisar and Cooper (2002) reported that seed set of CHA females was less than that of CMS females due to phytotoxicity from the CHA.

The low correlation between anther extrusion and female seed weight in 2015 indicated that potentially a high anther extrusion score (five to eight) was not enough to explain the differences for female seed weight. To investigate this further, the average female weights were compared to the ASI taking into account anther extrusion score from both 2015 and 2016. Since the gape dates were not accurate from 2015, only ASI comparisons (- 4 to 4 days) will be described for anther extrusion scores (4 to 7) as they were determined to be the most accurate (negative number indicating the female gaped earlier than male parent flowered). In the eight day interval, the average seed weight increased as anther extrusion increased (AE 4 [256 g], AE 5 [422 g], AE 6 [447 g], and AE 7 [456 g]). The difference between anther extrusion scores five and seven was only 34 g indicating that a score of five and higher did not greatly increase seed weight. In 2016, similar results were found (Table 5), ASI (-2 to 7 days) for anther extrusion (5 and 6) showed a 43 g difference for average seed weight (AE score 5 [442 g] and AE score 6

[485 g]). In both years there was an expected trend of decreasing seed weight as ASI got larger. Optimal ASI was determined to be between negative four and zero days. These results are consistent with Cisar and Cooper (2002) who reported that females being a few days earlier than males was ideal for seed set. Hybrid breeders of the past also reported only selecting males with anther extrusion of 6.0 or higher confirming that an anther extrusion score of six is required for seed production (personal communication G. Cisar).

Concluding Remarks

Visually assessing for anther extrusion in field environments can be a successfully employed to identify high performing genotypes that originate from breeding programs within the US Great Plains. Additionally, because the trait is highly heritable, progress can be made in selecting and breeding for genotypes with improved anther extrusion. Conversely, visually assessing for anther extrusion in a greenhouse environment will not accurately estimate performance in the field, which is the environment where hybrid seed production must take place. Although several factors impact hybrid seed production, anther extrusion is perhaps the most impactful. Hybrid seed production was likely influenced by the phytotoxic side-effects of a CHA application, results suggest that optimal production depends in part on utilizing a male with the best possible anther extrusion. Consequently, any breeding program with the objective of feasibly and economically producing hybrid wheat seed should concentrate on selecting parents that are measurably better than most wheat genotypes for anther extrusion.

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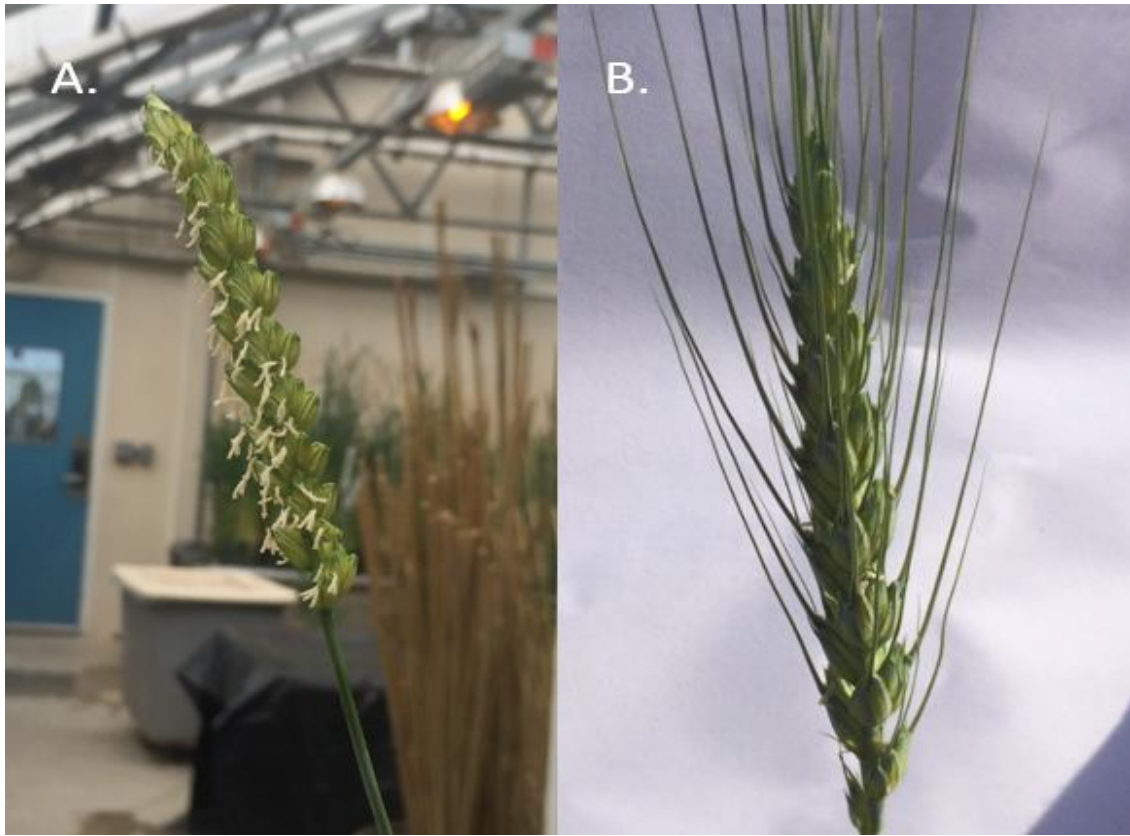


Figure 1 A. Genotype exhibiting excellent anther extrusion (rated 9) B. Genotype exhibiting poor anther extrusion (rated 1)

$$\text{A. } H^2 = \frac{\sigma_G^2}{\sigma_G^2 + \frac{\sigma_e^2}{R}} \quad \text{B. } H^2 = \frac{\sigma_G^2}{\sigma_G^2 + \frac{\sigma_{GE}^2}{L} + \frac{\sigma_e^2}{R \times L}}$$

Figure 2 A. Broad sense heritability equation for one location. B. Broad sense heritability equation for multi locations (He et al., 2016; IRRI, 2006)

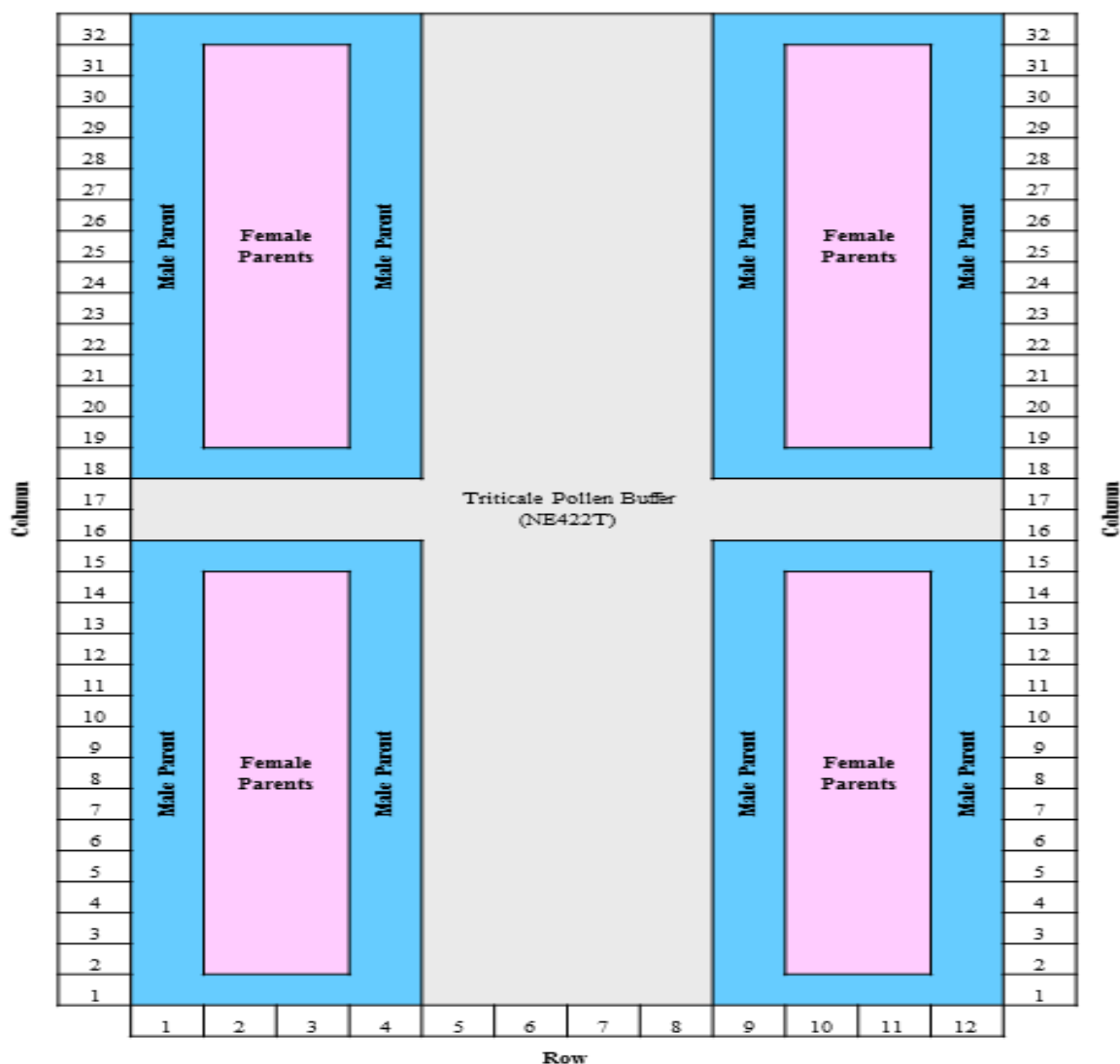


Figure 3 An example crossing block planting map, each crossing block is four plots wide with female plots planted side by side (pink area) and male strips surrounding (blue area). Each crossing block had two plots of isolation lengthwise and four plots of isolation lengthwise and four plots of isolation widthwise of triticales.

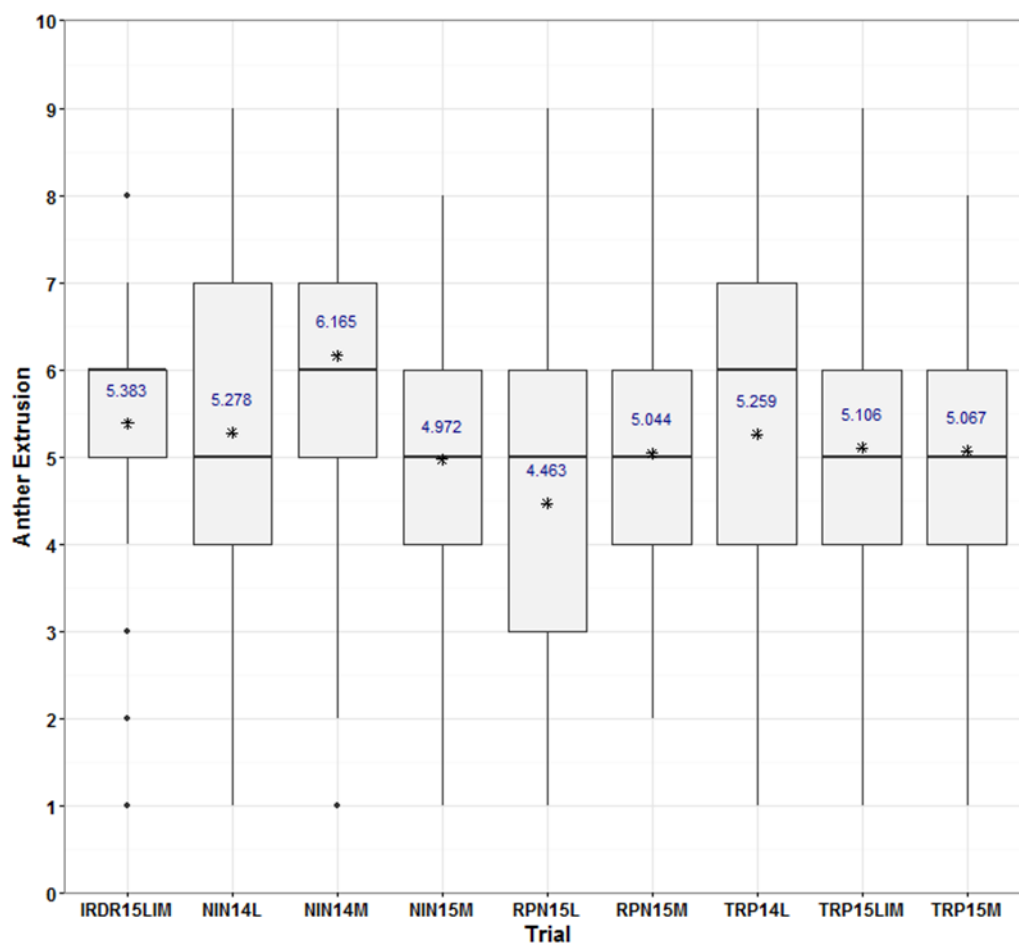


Figure 4 Anther Extrusion distribution boxplots by trial with means in blue marked with an asterisk. Trial descriptions can be found in Table 1.

Table 1 List of eight trials† with their descriptions that contained 288 winter wheat genotypes that were rated for anther extrusion in 2014 and 2015.

Trial Name	Trial	Trial Description	Generation	Year	Location	Number of Replications per Location	Incomplete Block Size	Number of Incomplete Blocks per Replication	Entries
Triplicate††	TRP14L‡	Advanced Yield Trial	F6	2014	Lincoln, NE	3	5	12	60
Triplicate	TRP15LIM	Advanced Yield Trial	F6	2015	Lincoln, NE	3	5	12	60
Triplicate	TRP15M	Advanced Yield Trial	F6	2015	Mead, NE	3	5	12	60
Nebraska Interstate Nursery	NIN14L	Elite Yield Trial	F7-8	2014	Lincoln, NE	4	5	12	60
Nebraska Interstate Nursery	NIN14M	Elite Yield Trial	F7-8	2014	Mead, NE	3	5	12	60
Nebraska Interstate Nursery	NIN15L	Elite Yield Trial	F7-8	2015	Mead, NE	3	5	12	60
Irrigated Dry	IRD15LIM	Irrigated/Dry yield trial (dryland conditions)	F6	2015	Lincoln, NE	3	5	8	40
Regional Performance Nursery	RPN15L	Combination of the Northern and Southern Regional Performance Nursery along with other experimental lines	F8-10	2015	Lincoln, NE	3	5	18	90

† Entries did not remain the same from 2014 to 2015 in either the TRP or the NIN.

†† All trials were alpha lattice designs.

‡ The yield trial code in the trial column represents the abbreviation of the trial, the year, and location.

Table 2. List of 288 genotypes rated for anther extrusion from 8 trials in 2014 and 2015 (Table 1) including the mean adjusted best linear unbiased predictions and standard errors

Name	Pedigree	Origin	TRP14L	SE	TRP15LIM	SE	TRP15M	SE	TRP15	SE	NIN14L	SE	NIN14M	SE	NIN14	SE	NIN15M	SE	IRDR15LIM	SE	RPN15L	SE
AAC Gateway	CDC Osprey/N95L1226																				6.30	0.46
Antelope	Pronghorn/Arlin	UNL																			5.47	0.52
BCS-11L00002-001		Bayer Crop Science																			5.24	0.52
BCS-12L00001		Bayer Crop Science																			5.74	0.52
BCS-12L00003		Bayer Crop Science																			4.63	0.52
BCS-12L00004		Bayer Crop Science																			5.19	0.52
BCS-12L00006		Bayer Crop Science																				
	KS91H184/ARLIN																					
	SIB/KS91HW293/NE82761/REDLAND																					
Camelot	(NE91631)/V/BFO168	UNL	2.30	0.77							2.72	0.63	2.11	0.66	2.49	0.68						
CHEYENNE	C18885	UNL									5.19	0.85	6.01	1.36	5.51	0.94	3.80	0.45				
CO11D1316W	Cowboy/Antero	CSU																			1.53	0.52
CO11D1353	CO050337-2/Byrd	CSU																			3.85	0.52
CO11D1397	CO050337-2/Byrd	CSU																			4.32	0.52
CO11D1539	Byrd/Antero	CSU																			5.50	0.52
CO11D1767	CO07MAS114/Cowboy	CSU																			3.85	0.52
CO11D446	CO050270/Byrd	CSU																			5.73	0.52
	KS92-946-B-15																					
Freeman	1=(AB186*3414/JAG/K92)/ALLIANCE	UNL	7.15	0.77	6.60	0.59	6.80	0.51	6.86	0.47	6.79	0.63	7.35	0.66	7.19	0.68	6.36	0.45				
	Len/Butte/ND526 (ND604)/6/(SD2971																					
)Agent/3/ND441/Waldron/Bluebird/4/Butte/5/																					
	Len																					
	(SD3055)/7/KS88H164/8/(NE89646)/COLT*2/																					
GOODSTREAK	PATRIZANKA	UNL	4.37	0.77	5.78	0.59	5.02	0.51	5.39	0.47	5.23	0.63	4.31	0.66	4.93	0.68	4.98	0.45				
HV9W10-0091	TX01V5314/P25R47/HV9W02-942R	Westbred																			5.48	0.52
	PFAU/WEAVER/3/MASON/2/GR/PECOS/4/																					
HV9W10-1002	FARMEC	Westbred																			7.17	0.52
Jagalone	JAGGER/ABILENE																				5.47	0.52
Jerry	Roughrider/W inoka/NB66425/3/Arapahoe																				4.34	0.52
Kharkof	Kharkof																				3.53	0.52
	KS00F5-20-3-2/KS980512-11-14/KS990160-4-3	KSU																			5.20	0.52
KS060084-M-4		KSU																			5.20	0.52
KS060106-M-11	KS940786-6-9A/CW04-417/Evenest																					
	KS940786-6-9EU/KS990011-1-21/KS990159-3-7	KSU																			4.96	0.52
KS060143-K-2	KS990031-3																					
KS060371-M-3	~1//Overley*3/Amadina/3/KS980512-11-19	KSU																			5.44	0.52
KS060476-M-6	KS990159-3-11/KS990031-3-11/KS970274-14-99	KSU																			4.35	0.52
	KS04HW101-																					
	3(98HW423)(JGR/93HW242)/98HW170(ARL/																					
KS11HW15-4-1	WGRC15)/RonL	KSU																			4.66	0.52
	KS04HW101-																					
	3(98HW423)(JGR/93HW242)/98HW170(ARL/																					
KS11HW18-1-6	WGRC15)/RonL	KSU																			4.63	0.52
	KS04HW101-																					
	3(98HW423/98HW170)/KS04HW119-																					
KS11HW39-5-4	3(TREGO*2/CO960293)	KSU																			6.60	0.52
	KS04HW119-																					
	3(TREGO*2/CO960293)/KS05HW122-																					
KS11HW53-1-6	3(KS99-5-16/STANTON/KS98HW423)	KSU																			4.32	0.52
LCH11-1117																					5.20	0.52
LCH12-012																					4.30	0.52
LCH13-054																					5.21	0.52
LCH13-092																					4.68	0.52
LCH13DH-20-87																					5.49	0.52
LCH13DH-3-31	NE04424 / Freeman																				3.54	0.52
LCH13DH-5-59	NE01481 / NE05496																				2.65	0.52
LCH13DH-7-3	NE01481 / Smokyhill																				2.13	0.52
LCH13NEDH-11-24	NE06469/Pronghorn																					
LCH13NEDH-3-31	NE04424 / NE06545																				5.69	0.45
LCH13DH-14-53W	NW03681 / SD07W084																				3.65	0.45
LCH13DH-4-16W	Mace / NE06545																				1.85	0.52
LCH13NEDH-14-53	NW03681 / SD07W084																				5.11	0.52
																					5.32	0.45

Table 2 continued. List of 288 genotypes rated for anther extrusion from 8 trials in 2014 and 2015 (Table 1) including the mean adjusted best linear unbiased predictions and standard errors

Name	Pedigree	Origin	TRP14L	SE	TRP15LIM	SE	TRP15M	SE	TRP15	SE	NIN14L	SE	NIN14M	SE	NIN14	SE	NIN15M	SE	IRDR15LIM	SE	RPN15L	SE
MT1117	Yellowstone*3/KS96W/GRC40																				1.62	0.52
MT1138	W99-194/2*Yellowstone																				2.40	0.52
N11MD2157W	Mace/SD98W175-1																				5.21	0.52
N11MD2166W	Mace/SD98W175-1																				6.62	0.52
NE01481	NE92458 (=OK83201/REDLAND)/Ike	UNL									5.40	0.63	7.41	0.66	6.22	0.68						
NE05548	NE97426 (=BRIGANTINA.2*ARAPAHOE)/NE98574 WESLEY (N95L158)=(KS831936- 3/COLT/CODY)/3/KS9U241/IKE/TXGH125	UNL									5.43	0.63	6.29	0.76	5.80	0.70	4.82	0.45				
NE06430	88-120*4/FS2	UNL									5.17	0.63	5.84	0.66	5.58	0.68						
NE07486	Lakin/Ok102 F3 5y3/(NE98574)	UNL									5.49	0.63	7.42	0.66	6.36	0.68						
NE07531	CO850267/RAWHIDE/4/HALLAM CDL 91185-1/NE99469	UNL									5.63	0.63	6.06	0.66	5.91	0.68			5.48	0.46		
NE08499	(=NE97V106/NE88588/KARL 92)	UNL									5.72	0.63	6.26	0.66	5.91	0.68						
NE09517	W96x1080- 21=(Jagger/Thunderbolt)/JAGALENE	UNL									5.20	0.63	4.74	0.66	5.13	0.68	4.03	0.45				
NE09517-1	W96x1080- 21=(Jagger/Thunderbolt)/JAGALENE	UNL															3.56	0.45				
NE09521	OK96717-99- 6756=(Abilene/2180/Chisholm)/NI01824=(IN TENSIVNAJA/NE92458 (=OK83201/REDLAND)/N BF0168)/NE0055 64 = (T81/NE91635 (=NE82761/NE82599))	UNL									5.36	0.63	6.31	0.66	5.89	0.68	5.78	0.45				
NE10478	NI03418/Camelot	UNL									4.17	0.68	6.81	0.66	5.28	0.70	4.52	0.45				
NE10478-1	NI03418/Camelot	UNL															4.85	0.45				
NE10507	NE01481=(OK83201/REDLAND)/Ike/Harry =(NE90614 (=BRL/4/PKR*4/AGT/BEL.198/LCR/3/NWT /BRL)/NE87612 (=NWT/WRR*5/AGT/3/NE69441))	UNL									5.07	0.63	6.28	0.66	5.68	0.68	5.65	0.45			5.20	0.52
NE10589	OK98697/Jagalene/Camelot	UNL									5.01	0.63	6.92	0.66	5.74	0.68	5.97	0.45				
NE10683	NE01481=(OK83201/REDLAND)/Ike/Harry =(NE90614 (=BRL/4/PKR*4/AGT/BEL.198/LCR/3/NWT /BRL)/NE87612 (=NWT/WRR*5/AGT/3/NE69441))	UNL									6.29	0.63	5.95	0.66	6.28	0.68	5.29	0.45			4.41	0.52
NE12408	KS03A1001/Harry/NI03418	UNL									5.61	0.63	7.70	0.66	6.64	0.68						
NE12409	NE02495/SHOCKER	UNL									6.03	0.63	6.16	0.66	6.10	0.68						
NE12429	NI04421/NE03458	UNL									4.67	0.68	6.36	0.66	5.35	0.70	4.25	0.45			3.18	0.52
NE12430	NI04421/NE03458	UNL									4.74	0.63	5.49	0.66	4.94	0.68						
NE12438	NI03427/HV9W00-1784R	UNL									5.11	0.63	6.47	0.66	5.58	0.68						
NE12438_HRD		UNL																			4.35	0.52
NE12439	NI03427/HV9W00-1784R	UNL									5.13	0.68	5.76	0.66	5.35	0.70						
NE12439_HRD		UNL																			6.05	0.52
NE12443	KS970197-8-9/NE01643	UNL									5.46	0.63	4.93	0.66	5.34	0.68	5.57	0.45			4.07	0.52
NE12444	KS980512-11-2/SHOCKER	UNL									5.97	0.63	6.56	0.66	6.41	0.68	5.85	0.45			5.48	0.52
NE12461	HV9W00-1784R/NE02495	UNL									5.06	0.63	4.93	0.66	5.01	0.68						
NE12464	DANBY/NE01643	UNL									4.87	0.63	6.66	0.66	5.71	0.68						
NE12483V	CO9600293/NW99L7068	UNL									5.74	0.63	6.13	0.66	5.98	0.68						

Table 2 continued. List of 288 genotypes rated for anther extrusion from 8 trials in 2014 and 2015 (Table 1) including the mean adjusted best linear unbiased predictions and standard errors

Name	Pedigree	Origin	TRP14L	SE	TRP15LIM	SE	TRP15M	SE	TRP15	SE	NIN14L	SE	NIN14M	SE	NIN14	SE	NIN15M	SE	IRDR15LIM	SE	RPN15L	SE
NE12488	Jagaleno/NE02495	UNL									5.48	0.63	5.61	0.66	5.57	0.68	5.59	0.45			4.67	0.52
NE12510	NW03681/BC97-ROM50W (NuDakota)	UNL									6.69	0.63	6.80	0.76	6.78	0.70						
NE12518	NE02549/NE01643	UNL									6.15	0.63	6.04	0.66	6.26	0.68						
NE12524	NI04421/NE03458	UNL									5.51	0.68	4.98	0.66	5.41	0.70						
NE12561	NI04420/NE00403	UNL									5.40	0.85	4.67	0.76	4.77	0.79	3.04	0.45			3.23	0.52
NE12571	OK00611W/NE01643/NE03458	UNL									4.67	0.63	4.59	0.66	4.91	0.68	4.84	0.45			5.10	0.52
NE12580	HV9W00-B361R/NE02495	UNL									4.45	0.68	3.35	0.76	4.04	0.73						
NE12589	CO97547-7/OVERLAND/CAMELOT	UNL									5.24	0.63	4.81	0.66	5.19	0.68	5.07	0.45			4.36	0.52
NE12630	SHARK/F4105W2.1/NE99656-1/ANTELOPE	UNL									5.44	0.63	6.94	0.66	6.04	0.68						
NE12637	CO00554/NE01643/NE02584	UNL									5.22	0.63	5.98	0.94	5.59	0.73						
NE12662	NE02558/NE02584	UNL									5.96	0.63	7.75	0.66	6.77	0.68						
NE12686	HV9W02-942R/CAMELOT	UNL									5.73	0.63	7.41	0.66	6.46	0.68						
NE13402	HV9W99-558/KS970197-8-9/NI04427	UNL	5.30	0.77																		
NE13405	TX00D1390/NE02495/NE05403	UNL	6.70	0.77													5.92	0.45				
NE13412	CO01385-A.1/NO2Y5117/Harry	UNL	5.52	0.77																		
NE13420	NI04420/TX01A.7326/NE03458	UNL	5.05	0.77																		
NE13425	3. INW0302/NE01643/NE02584	UNL	7.21	0.77													5.65	0.45				
NE13430	KS990159-3--7/NE01481	UNL	6.29	0.77																		
NE13434	TX00D1390/NE02495/McGill	UNL	4.45	0.77													5.26	0.45				
NE13438	NI04430/TX03M1179/NI04420	UNL	5.00	0.77																		
NE13443	NE02533/Glenn/NE04449	UNL	6.72	0.77																		
NE13445	NE04653/CO02322/McGill	UNL	5.53	0.77													5.35	0.45				
NE13470	T140/NE02495/NE03458	UNL																			4.62	0.52
NE13471	CO00016/CAMELOT/Wesley	UNL	3.92	0.77																		
NE13482	NE04424/Wesley	UNL	2.69	0.77																		
NE13483V	NE04550/NE05496	UNL	5.52	0.77													4.96	0.45				
NE13484V	NE05426/Harry	UNL	4.81	0.77																		
NE13510	T140/NE02495/NE03458	UNL	3.38	0.77																		
NE13511	CO00016/OVERLAND/NE03458	UNL	5.13	0.77													5.40	0.45				
NE13515	HV9W00-B267/NI04421/NI04427	UNL	5.29	0.77													4.49	0.45				
NE13544	NE03458/Glenn/NE02513	UNL	3.48	0.88																		
NE13545	27. 992151A1-2-2-5/NE01643/NE01643	UNL	5.62	0.88																		
NE13546	27. 992151A1-2-2-5/NE01643/NE01643	UNL	7.18	0.77																		
NE13550	CO00016/OVERLAND/NE03458	UNL	5.75	0.77																		
NE13554	OK01307/2*Overland	UNL	4.49	0.88													4.28	0.45				
NE13564	NE05425/NI04421	UNL	6.74	0.77																		
NE13583	MO980829/NE01604/NE03490	UNL	3.89	0.77																		
NE13585	VA04-474/NE01604/NE02584	UNL	4.41	0.77																		
NE13589	CLTR 8299=(MO12-2-3)/WAHOO/WAHOO	UNL	6.71	0.77																		
NE13593	CO00016/OVERLAND/Goodstreak	UNL	4.57	0.77													4.87	0.45				
NE13597	NE04424/Wesley	UNL	4.58	0.77													4.52	0.45				
NE13604	SD00258-1/McGill	UNL	4.54	0.77													5.29	0.45				
NE13624	OK01307/OVERLAND/OVERLAND	UNL	5.54	0.77																		
NE13625	W04-417/CAMELOT/Goodstreak	UNL	5.78	0.77													5.15	0.45			4.90	0.52
NE13629	SD01W064/NE02558/OVERLAND	UNL	6.08	0.88													4.58	0.45				
NE13660	WEATHERFORD/NUPLAINS/OVERLAND	UNL	5.54	0.77													3.92	0.45				
NE13672	MO980829/NE01604/NE03490	UNL	6.20	0.77													4.65	0.45				
NE13681	OK01307/OVERLAND/OVERLAND	UNL	4.58	0.77																		
NE13683	CO01212/NE03458/McGill	UNL	4.84	0.77													5.58	0.45				
NE13687	CLTR 8299=(MO12-2-3)/2*WAHOO	UNL	4.72	1.05													3.86	0.45				
NE14401	SD05179/NE04490	UNL			6.00	0.59	5.27	0.51	5.70	0.47												
	CO940610 (Yuma 05-05-06																					
	source)/NI04420/NE02584	UNL			3.72	0.59	2.47	0.51	2.99	0.47												
NE14412	NI04424/Overland	UNL			5.02	0.59	4.25	0.51	4.59	0.47												
NE14416	Cassiopea/NW03681/NE03490	UNL			3.22	0.59	3.49	0.51	3.23	0.47												
NE14419																						
NE14421	NE05426/Overland	UNL			5.32	0.59	4.76	0.51	5.00	0.47												
NE14427	SD98W175-1/NW03666/Freeman	UNL			5.86	0.59	6.03	0.51	5.96	0.47												

Table 2 continued. List of 288 genotypes rated for anther extrusion from 8 trials in 2014 and 2015 (Table 1) including the mean adjusted best linear unbiased predictions and standard errors

Name	Pedigree	Origin	TRP14L	SE	TRP15LIM	SE	TRP15M	SE	TRP15	SE	NIN14L	SE	NIN14M	SE	NIN14	SE	NIN15M	SE	IRDR15LIM	SE	RPN15L	SE
NE14428	Howard/OK04819/1(ND2928/Wesley/Wesley)																					
NE14431	JF3/Wesley F3	UNL			5.58	0.59	5.78	0.51	5.70	0.47												
NE14434	SD05W030/NW06655	UNL			5.81	0.59	5.52	0.51	5.68	0.47												
NE14436	SD98W175-1/NW03666/Freeman	UNL			6.49	0.59	6.29	0.51	6.53	0.47												
NE14436	SD05W030/NE02584	UNL			5.48	0.59	2.98	0.51	4.20	0.47												
NE14442	Glosa/NE01604/Santa Fe	UNL			5.58	0.59	5.52	0.51	5.63	0.47												
NE14448	NI06737/1(ND2928/Wesley/Wesley)F3/Wesley F3	UNL			5.02	0.59	5.78	0.51	5.42	0.47												
NE14449	NW05518/Wahoo/NW03654	UNL			4.58	0.59	5.27	0.51	4.92	0.47												
NE14457	CO03W239/ANTELOPE//Antelope	UNL			2.15	0.59	3.74	0.51	2.81	0.47												
NE14480	NI04424/Overland	UNL			5.50	0.59	5.27	0.51	5.39	0.47												
NE14484	NE04424/NH03614	UNL			5.02	0.59	4.76	0.51	4.90	0.47												
NE14492	SD05W018/NE99495	UNL			6.00	0.59	5.52	0.51	5.82	0.47												
NE14494	OK06822W/HV9W96-1383W//NW03681	UNL			4.51	0.59	5.27	0.51	4.85	0.47												
NE14495	Winterhawk/Bill Brown	UNL			5.50	0.59	5.27	0.51	5.36	0.47												
NE14496	KS04HW101-3/NW03670/NW06655	UNL			4.20	0.59	5.52	0.51	4.89	0.47												
NE14498	Millenium * Madsen/NI05714	UNL			3.75	0.59	4.25	0.51	3.89	0.47												
NE14500	CO940610 (Yuma 05-05-06 source)/NI04420/NE02584	UNL			2.52	0.59	2.73	0.51	2.48	0.47												
NE14502	CO03W239/ANTELOPE//Antelope	UNL			2.47	0.59	4.51	0.51	3.32	0.47												
NE14511	SNOWMASS/TAM 112/Hatcher	UNL			5.28	0.59	5.78	0.51	5.55	0.47												
NE14523	CO940610 (Yuma 05-05-06 source)/NI04420/NE02584	UNL			6.33	0.59	5.02	0.51	5.74	0.47												
NE14531	NI06737/1(ND2928/Wesley/Wesley)F3/Wesley F3	UNL			6.07	0.59	6.03	0.51	6.09	0.47												
NE14534	CO940610 (Yuma 05-05-06 source)/NI04420/NE02584	UNL			6.04	0.59	4.76	0.51	5.44	0.47												
NE14538	SD98W175-1/NW03666/Freeman	UNL			5.58	0.59	6.29	0.51	5.96	0.47												
NE14545	CO940610 (Yuma 05-05-06 source)/NI04420/NE02584	UNL			2.82	0.59	2.73	0.51	2.52	0.47												
NE14546	KS05HW15-2/NW03666	UNL			5.32	0.59	5.27	0.51	5.35	0.47												
NE14557	SD03164-2/Overland	UNL			4.51	0.59	4.25	0.51	4.37	0.47												
NE14561	KS05HW15-2/NW03666	UNL			4.23	0.59	4.76	0.51	4.44	0.47												
NE14563V	NE05699/NE01604	UNL			5.76	0.59	4.76	0.51	5.28	0.47												
NE14569	P98039G5-106/NI04421/NW06452	UNL			5.24	0.59	5.52	0.51	5.39	0.47												
NE14575	SD05191/PRONGHORN	UNL			4.72	0.59	4.76	0.51	4.71	0.47												
NE14594	SD05W030/NW06655	UNL			6.80	0.59	6.29	0.51	6.61	0.47												
NE14604	SD05W030/NW06655	UNL			5.83	0.59	6.03	0.51	5.98	0.47												
NE14605	OK03825-5403-6/NI04420	UNL			5.29	0.59	5.27	0.51	5.31	0.47												
NE14606	KS04HW101-3/NW03670/NW06655	UNL			6.01	0.59	6.03	0.51	6.13	0.47												
NE14607	SNOWMASS/TAM 112/Hatcher	UNL			4.27	0.59	3.49	0.51	3.79	0.47												
NE14617	MV-3/NE01604/Pronghorn	UNL			5.30	0.59	4.51	0.51	4.93	0.47												
NE14629	MV-3/NE01604/NE03490	UNL			5.00	0.59	4.51	0.51	4.71	0.47												
NE14632	Frument/NI04420/Overland	UNL			5.00	0.59	5.27	0.51	5.18	0.47												
NE14651	Kabul 2000/Pronghorn/Panhandle	UNL			5.78	0.59	5.27	0.51	5.59	0.47												
NE14654	SD05191/PRONGHORN	UNL			3.76	0.59	5.02	0.51	4.29	0.47												
NE14656	CO940610 (Yuma 05-05-06 source)/NI04420/NE04490	UNL			5.82	0.59	5.52	0.51	5.65	0.47												
NE14658	SD98W175-1/NW03666/NI04427	UNL			5.24	0.59	5.02	0.51	5.13	0.47												
NE14663	NI06737/1(ND2928/Wesley/Wesley)F3/Wesley F3	UNL			5.52	0.59	5.78	0.51	5.72	0.47												
NE14666	SD98W175-1/NW03666/Freeman	UNL			5.09	0.59	5.52	0.51	5.30	0.47												
NE14672	SD05191/PRONGHORN	UNL			4.75	0.59	5.78	0.51	5.31	0.47												
NE14674	KL Capricornio/NE04653/NE01604	UNL			5.56	0.59	5.78	0.51	5.74	0.47												
NE14686	KS04HW101-3/NW03670/NW06655	UNL			6.54	0.59	5.27	0.51	5.99	0.47												

Table 2 continued. List of 288 genotypes rated for anther extrusion from 8 trials in 2014 and 2015 (Table 1) including the mean adjusted best linear unbiased predictions and standard errors

Name	Pedigree	Origin	TRP14L	SE	TRP15LIM	SE	TRP15M	SE	TRP15	SE	NIN14L	SE	NIN14M	SE	NIN14	SE	NIN15M	SE	IRDR15LIM	SE	RPN15L	SE
	VA02W-																					
NE14688	713/Goodstreak//33(ND2710/Trego/Trego)F3/ Trego F3	UNL			5.51	0.59	4.76	0.51	5.13	0.47												
NE14695	NI06737/1(ND2928/Wesley/Wesley)F3/Wesley F3	UNL			5.49	0.59	5.52	0.51	5.53	0.47												
NE14696	NE05537/Overland	UNL			3.22	0.59	4.76	0.51	3.88	0.47												
NE14700	OK04505/NI04421//NE01481	UNL			5.85	0.59	5.78	0.51	5.90	0.47												
NE15420	HV9W02-942R/NW03666	UNL																	5.44	0.46		
NE15434	NW03681/NE97465 = Goodstreak/NW07534	UNL																	3.28	0.46		
NE15484	OR 2060097H/NW06452//NE07569	UNL																	5.81	0.46		
NE15558	OK03305/NE02584//NE07479	UNL																	4.74	0.46		
NH11489	NH01023/WESLEY//NE00403	UNL									5.64	0.63	7.12	0.66	6.27	0.68						
NH11490	NH01023/WESLEY//NE00403	UNL									5.48	0.63	6.87	0.66	6.10	0.68						
NHH11569	NH01048/TEAL//NH01036	UNL									7.13	0.63	6.48	0.66	6.95	0.68						
NHH14550	NH01045 x Teal/NE05537 NE96644(=ODESSKAYA P/CODY)/PAVON/*3SCOUT66/3Wahoo	UNL			6.04	0.59	5.52	0.51	5.85	0.47												
NI04420	(sib)	UNL									4.29	0.63	6.06	0.66	4.89	0.68						
NI04421	NE96644/Wahoo-sib	UNL			5.08	0.59	5.27	0.51	5.20	0.47	6.22	0.63	4.53	0.66	5.49	0.68	4.31	0.45	4.25	0.46		
NI08707	CO980829=(YumaT- 57//CO850034/3/4*Yuma/4/NEWS12)/wesley	UNL																	5.59	0.46		
NI09710H	NH01023/W97-234	UNL									4.63	0.68	3.47	0.94	4.31	0.76						
NI10718W	SD97W609=(Abilene/Karl)/NW98S097=(WA 691213-27//N86L177/AP-W189-163)	UNL																	6.23	0.46		
NI12702W	N03Y2014/NW03681//NuHills 10005 NH01023=(NE94489/NE94481//TXGH12588 8-120*4/PS2)/WESLEY=(KS831936-3 / NE86501 = Sumner sib (Plainsman V / Odesskaya 51)/ C	UNL									6.25	0.63	7.40	0.66	6.76	0.68	5.43	0.45			4.95	0.52
NI12709	OK02522W/NW03681	UNL									5.67	0.63	6.72	0.66	6.08	0.68						
NI12713W	TX01V5314/KS970197-8-9//NI03427	UNL									6.60	0.69	7.29	0.66	7.09	0.70			5.33	0.46		
NI13703	TX01V5314/KS970197-8-9//NI03427	UNL																	5.92	0.46		
NI13704	TX01V5314/KS970197-8-9//NI03427	UNL																	5.99	0.46		
NI13706	NI02425/HV9W99-558//NI04421	UNL			4.65	0.63	5.54	0.66	5.08	0.68	5.07	0.45									5.80	0.52
NI13708	HV9W94-CB94005R/NE00403//NE02513	UNL			4.60	0.68	5.16	0.66	4.87	0.70												
NI13717	MV-Gorsium/NI04421	UNL			4.75	0.63	5.28	0.66	4.93	0.68	4.55	0.45	4.86	0.46	4.07	0.52						
NI13720	NuHorizon/NW03666	UNL			5.18	0.63	6.20	0.66	5.67	0.68												
NI14719	KS00F5-20-3-2/TX01V5314//KS980512-11	UNL																	5.25	0.46		
NI14721	TX01A5936/BC97-ROM50W (NuDakota)/ANTELOPE	UNL															5.17	0.45	5.89	0.46		
NI14722	CO02W274/NE00403//NI03427	UNL																	5.73	0.46		
NI14727	NE05403/NE02584	UNL																	5.47	0.46		
NI14729	NE05426/Harry	UNL															6.33	0.45	5.94	0.46		
NI14732	CO01385-A1/NE05403	UNL																	5.43	0.46		
NI14733	MV-Regiment/NE04550	UNL															4.36	0.45	3.92	0.46		
NI14735	MV-Kolo/NUDAKOTA	UNL																	5.06	0.46		
NI15701	Alliance * KS96H10W10-3/NI04420	UNL																	5.46	0.46		
NI15702	SD05W030/NW03666/TAM111	UNL																	5.65	0.46		
NI15703	SD05W030/NW03666/TAM111	UNL																	2.68	0.46		
NI15704	HV9W02-942R/NW03666	UNL																	5.74	0.46		
NI15705	HV9W02-942R/NW03666	UNL																	5.03	0.46		
NI15706	HV9W02-942R/NW03666	UNL																	5.25	0.46		
NI15707	TA103/NE05403	UNL																	6.14	0.46		
NI15708	NE03458/NI07701	UNL																	5.92	0.46		
NI15709	VA02W-555/NW06649/NE01643	UNL																	5.50	0.46		
NI15710	KS05HW15-2/NW03681	UNL																	4.71	0.46		
NI15711	NI06737/HV9W03-539R	UNL																	5.69	0.46		

Table 2 continued. List of 288 genotypes rated for anther extrusion from 8 trials in 2014 and 2015 (Table 1) including the mean adjusted best linear unbiased predictions and standard errors

Name	Pedigree	Origin	TRP14L	SE	TRP15LIM	SE	TRP15M	SE	TRP15	SE	NIN14L	SE	NIN14M	SE	NIN14	SE	NIN15M	SE	IRDR15LIM	SE	RPN15L	SE
N115712	N106737/HV9W03-539R	UNL																	5.81	0.46		
N115713	CO03W139/NW06655/NW07534	UNL																	5.20	0.46		
Nord1301	Arapahoe/Danby	UNL																			2.40	0.52
NW03666	N94S097KS/NE93459	UNL									4.54	0.63	5.04	0.94	4.77	0.73						
NW07505	Trego/Thunderbolt F3	UNL									4.89	0.63	6.84	0.66	5.67	0.68	5.14	0.45				
NW07534	KS920709-B-5-2/NW98S061	UNL																	5.33	0.46		
NW09627	KS96HW115/NW99L068=(KS84HW1968/3/ RIOBLANCO/HBY762A/HALT)	UNL									4.66	0.75	7.38	0.66	5.87	0.72						
NW11511	NW97S139-1/KS02HW31/HV9W00-993W	UNL									4.52	0.68	6.26	0.66	5.16	0.70	4.22	0.45			4.08	0.52
NW13455	SD98W175-1/NW03666	UNL	7.38	0.77													5.24	0.45				
NW13457	KS04HW47-3/NW05518	UNL	6.23	0.77																		
NW13458	SD01W064/N104420	UNL	5.77	0.77																		
NW13480	N104436?	UNL	3.44	0.77																		
NW13491	SD98W175-1/NW03666	UNL	5.71	0.77																		
NW13493	SD98W175-1/NW03666	UNL	6.69	0.77													5.31	0.45				
NW13494	SD98W175-1/NW03666	UNL	7.08	0.77																		
NW13499	SD01W064/NW03670	UNL	4.13	0.77													5.32	0.45				
NW13502	HV9W02-323W/NW03670	UNL	5.55	0.77																		
NW13516	N104436?	UNL	3.87	0.77																		
NW13518	N104436?	UNL	5.11	0.77																		
NW13535	SD01W064/ANTELOPE	UNL	4.67	0.77																		
NW13536	NuHorizon/NW03666	UNL	5.10	0.77																		
NW13542	SD98W175-1-14/NW03670	UNL	4.06	0.88																		
NW13560	N104436?	UNL	3.90	0.77																		
NW13570	SD98W175-1/NW03666	UNL	5.99	0.77													6.22	0.45				
NW13574	KS04HW101-3/ANTELOPE	UNL	4.69	1.06													4.82	0.45				
NW13596	HV9W96-1270W-1/BC97-ROM50W (NuDakota)/NW03666	UNL	4.49	0.88																		
NW13647	KS02HW34/ANTELOPE	UNL	6.46	0.77																		
NW13669	SD98W175-1-14/NW03666	UNL	5.52	0.77													4.79	0.45				
NW15677	KS05HW15-2/NW03681	UNL																				
OK09125	Overley/TX98D1170	UNL																	5.91	0.46		
OK10126	OK00514/OK98680reseln	UNL																			5.43	0.52
OK1059060	(KAUZ/STAR)/U1254-1-5-1-1/TX89V4213	UNL																			5.18	0.52
OVERLAND	Millennium sib/(ND8974) Seward/Archer	UNL									4.70	0.63	6.72	0.66	5.67	0.68	4.97	0.45			4.38	0.52
OVERLAND_FHB1_1	overland*3[(7840/Jagger/Chokwang/JaggerF2) /Jagger]F4	UNL																			3.72	0.52
OVERLAND_FHB1_1C	overland*3[(7840/Jagger/Chokwang/JaggerF2) /Jagger]F4	UNL																			3.78	0.52
OVERLAND_FHB1_4	overland*3[(7840/Jagger/Chokwang/JaggerF2) /Jagger]F4	UNL																			2.88	0.52
OVERLAND_FHB1_5	overland*3[(7840/Jagger/Chokwang/JaggerF2) /Jagger]F4	UNL																			3.84	0.52
OVERLAND_FHB1_9	overland*3[(7840/Jagger/Chokwang/JaggerF2) /Jagger]F4	UNL																			3.28	0.52
PSB13NEDH-14-71W	NW03681 / SD07W084	UNL															5.72	0.45			4.96	0.52
PSB13NEDH-14-83W	NW03681 / SD07W084	UNL															4.71	0.45				
PSB13NEDH-15-58W	NW03681 / SD07W084	UNL																				
PSB13NEDH-7-140	SMOKEY HILL/NE01481	UNL																			3.45	0.52
SCOUT66	CT 3996										3.89	0.63	4.72	0.66	4.33	0.68	3.60	0.45			4.09	0.52
SD08200	Wesley/KS91048-L-2-1/NE93613/Wendy	SDSU																			5.80	0.52
SD09113	Jerry/Ning7840/KS920946-B-15-2/Wendy	SDSU																			3.47	0.52
SD09118	Wesley/Ning7840/SD97380-2/Wendy	SDSU																			4.55	0.52
SD09192	Harding/Trego/Trego/Wendy	SDSU																			3.52	0.52
SD09227	SD97088/KS920709-B-5-2/Jagakne/Wendy	SDSU																			5.98	0.52
SD10257-2	Ransom/SD96240-3-1	SDSU																			3.81	0.52
SD10W153	Wesley/OK00611W	SDSU																			7.19	0.52

Table 2 continued. List of 288 genotypes rated for anther extrusion from 8 trials in 2014 and 2015 (Table 1) including the mean adjusted best linear unbiased predictions and standard errors

Name	Pedigree	Origin	TRP14L	SE	TRP15LIM	SE	TRP15M	SE	TRP15	SE	NIN14L	SE	NIN14M	SE	NIN14	SE	NIN15M	SE	IRDR15LIM	SE	RPN15L	SE
SD110060-7	98X0435-15/Overland	SDSU																			4.05	0.52
SD110085-1	Arapahoe/W03-20/Danby	SDSU																			2.71	0.52
SD11023-8	Intrada/Alice/NW97S218-LT	SDSU																			5.69	0.52
Settler CL	Wesley sib/Millennium sib/Above sib	UNL									5.04	0.63	6.41	0.66	5.66	0.68	5.27	0.45	5.49	0.46		
SY Wolf		Syngenta																		6.14	0.46	
TAM107	TAM107	Texas A&M																			5.80	0.52
TX09V7315	TAM 304/TX02U2508	Texas A&M																			3.75	0.52
TX09V7352	TAM 112/TX02U2508	Texas A&M																			2.96	0.52
TX09V7446	TAM 112/KS03HW156-3	Texas A&M																			3.53	0.52
TX10A001099	TAM 303/TAM 112/TX99A0155	Texas A&M																			5.18	0.52
TX11A001295	TAM 112/TX02U2508	Texas A&M																			1.83	0.52
TX12M4004	TX12M4004	Texas A&M																			5.25	0.52
TX12M4063	TX12M4063	Texas A&M																			4.35	0.52
TX12M4065	TX12M4065	Texas A&M																			4.93	0.52
WB CEDAR		Westbred																		6.40	0.46	
WESLEY	KS831936-3/COLT/CODY	USDA									4.89	0.63	7.18	0.66	5.71	0.68	5.55	0.45	5.39	0.46	4.91	0.52

Table 2 List of genotypes used in the diallel mating design production trial in 2015 and 2016

Name	Pedigree	Origin
Freeman	KS92-946-B-15- 1=(ABI86*3414/JAG//K92)/ALLIANCE Len//Butte/ND526 (ND604)/6/(SD2971)Agent/3/ND441//Waldron/Bluebird/4/Butte/5/Len (SD3055)/7/KS88H164/8/(NE89646)COLT*2/PATRIZA	UNL
Goodstreak LCH13NED	NKA	UNL
H-11-24	NE06469/Pronghorn	UNL
NE07531	HBA142A/HBZ623A//ALE (HBK0630-4- 5)/3/(NE98574) CO850267/RAWHIDE/4/HALLAM	UNL
NE09517-1	W96x1080-21=(Jagger/Thunderbolt)/JAGALENE	UNL
NE10478-1	NI03418/Camelot	UNL
NE10589	OK98697/Jagalene//Camelot NE01481=(OK83201/REDLAND//IKE)/Harry=(NE9061 4(=BRL/4/PKR*4/AGT//BEL.198/LCR/3/NWT/BRL)/N E87612(=NWT//WRR*5/AGT/3/NE69441))	UNL
Overland	Millennium sib/(ND8974) Seward/Archer	UNL
Panhandle PSB13NEDH	NE97426 (=BRIGANTINA.2*ARAPAHOE)/NE98574	UNL
-15-58W	NW03681 / SD07W084	UNL
Robidoux (NI04421)	NE96644(=ODESSKAYA P./CODY)//PAVON/*3SCOUT66/3/Wahoo (sib)	UNL
Settler CL	Wesley sib//Millenium sib/Above sib	UNL
TX09D1172	TAM303/TAM112	Texas A&M
TX10D2063	OK99610/TX00V1131//TX02D5868	Texas A&M
TX10D2230	NW01L2019/TX96D1073//TX01D3215	Texas A&M
TX10D2363	OK99610/TAM 109//TAM 304	Texas A&M
TX11D3008	TX03M1004/TX02V7930	Texas A&M
TX11D3026	TX01V5425/KS03HW155-2//TX03M1004	Texas A&M
TX11D3049	TX96D1073/KSS9011-1-45 IP76//KS00F5-14-7	Texas A&M
TX11D3112	TX98V9628/TX02U2508	Texas A&M
TX11D3129	WBLL 1*2/TUKURU//OK BULLET	Texas A&M
TX12M4004	KS980478-3--5/FULLER	Texas A&M
TX12M4063	AP04TW9819/O3A-B3//KS980512-11-22	Texas A&M
TX12M4065	AP04TW1318/KS980512-11-9//KS06O3A~49	Texas A&M
Wesley	KS831936-3//COLT/CODY	UNL

Table 3 Restricted maximum likelihood variance component estimates, standard error (SE), and broad-sense heritability (H²) of genotypes rated for anther extrusion in 2014 and 2015.

Trial	GxL	SE	Loc	SE	Loc:Rep	SE	G	SE	Rep:Iblock	SE	Error	SE	H ²
NIN14L							0.96*	0.31	0.43	0.23	2.31	0.3	0.62
NIN14M							1.28*	0.38	0.35	0.20	1.52	0.25	0.72
NIN14 Combined	0.24	0.20	0.26	0.42	0.07	0.10	0.98*	0.29	0.12	0.10	2.27	0.21	0.69
NIN15M							0.73*	0.19	0.17	0.09	0.74	0.11	0.75
TRP14L							1.95*	0.54	0.09	0.21	2.40	0.37	0.71
TR15LIM							1.46*	0.36	0.06	0.11	1.32	0.20	0.77
TRP15M							1.10*	0.27	1.18e⁻⁸*	1.54e ⁻⁸	1.02	1.33	0.76
TRP15 Combined	0.14	0.10	1.23e⁻⁸*	1.23e ⁻⁸	0.05	0.05	1.17*	0.27	5.05e⁻²	4.50e ⁻²	1.15	0.11	0.82
IRDR15LIM							0.79*	0.24	0.18	0.12	0.72	0.13	0.77
RPN15L							1.72*	0.31	0.05	0.07	0.92	0.11	0.85

* significant based on 95% confidence interval

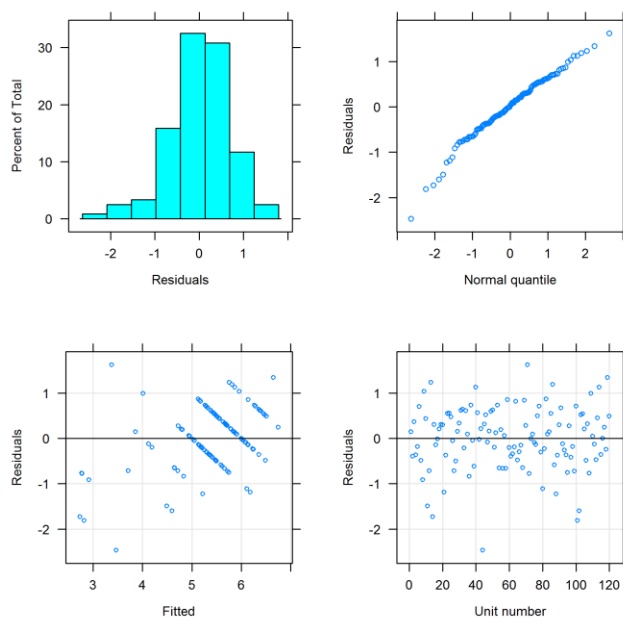
Table 4 The effect of anther stigma interval (ASI) on hybrid grain weight (grams) seperated by anther extrusion (rated 1 to 9) in 2016

ASI (days)	Anther Extrusion					Mean
	4	5	6	7	8	
	g					
-4		1101.0 (1)	936.0 (2)			991.0
-3		1034.3 (3)			856 (2)	963.0
-2		640.5 (2)	501.8 (4)		841 (1)	589.9
-1		775.8 (13)	697.0 (5)		612.5 (2)	739.8
0		575.3 (18)	610.6 (9)		418.2 (5)	560.7
1	641.3 (3)†	427.5 (19)	439.0 (5)		402.4 (5)	445.4
2	445.7 (3)	385.0 (29)	457.7 (6)			401.2
3	414.5 (4)	429.7 (17)	351.2 (10)			402.4
4	339.0 (2)	309.2 (10)	413.7 (11)		586 (2)	379.7
5		297.5 (15)	472.7 (20)		415 (3)	399.0
6		343.0 (21)	483.0 (11)		380.7 (3)	390.3
7	334.3 (3)	235.4 (17)	428.5 (12)	527.2 (2)	285.3 (3)	325.8
8	288.4 (5)	281.7 (10)	286.7 (13)	410.8 (12)		322.9
9	248.3 (4)	335.9 (7)	267.0 (9)	517.5 (8)		353.1
10	232.5 (2)		261.4 (11)	341.1 (4)		276.7

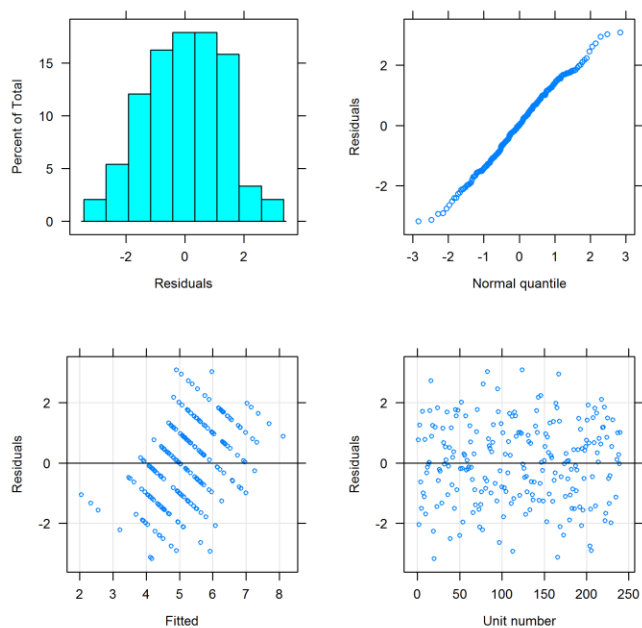
† The number in parenthesis indicated the number of hybrids in that category.

Appendices

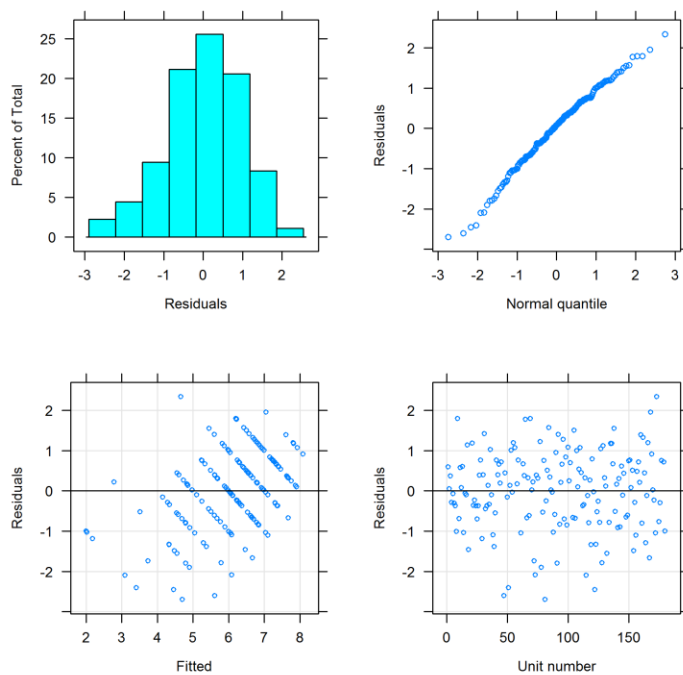
Appendix A IRDR15LIM Mixed Model Residual Plot



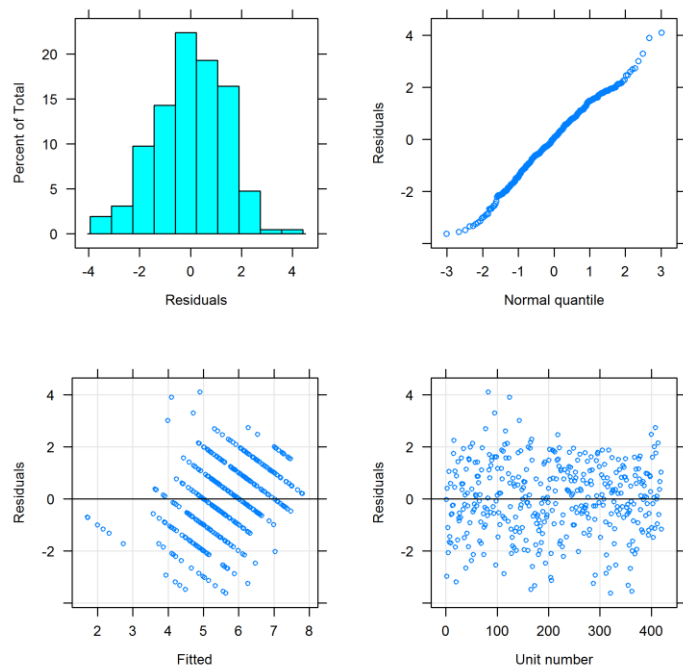
Appendix B NIN14L Mixed Model Residual Plots



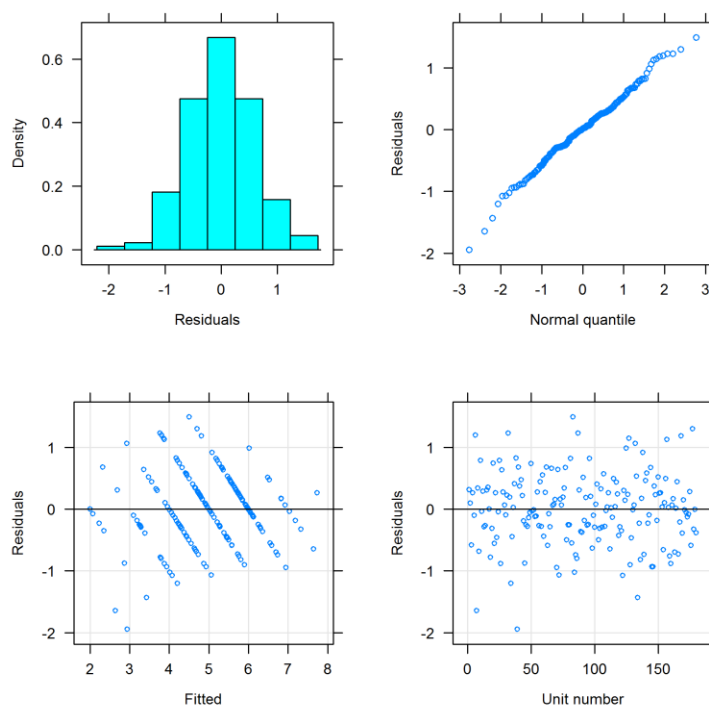
Appendix C NIN14M Mixed Model Residual Plots



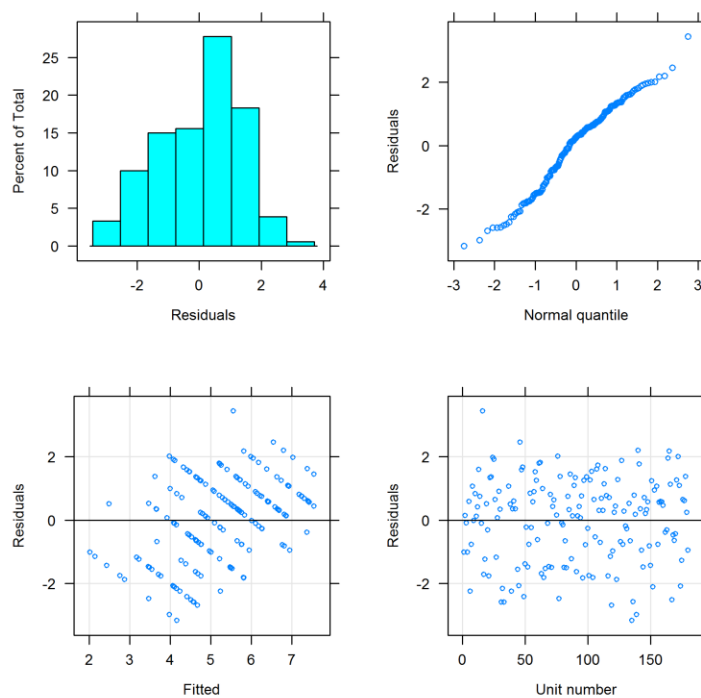
Appendix D NIN14 Lincoln and Mead Mixed Model Residual Plots



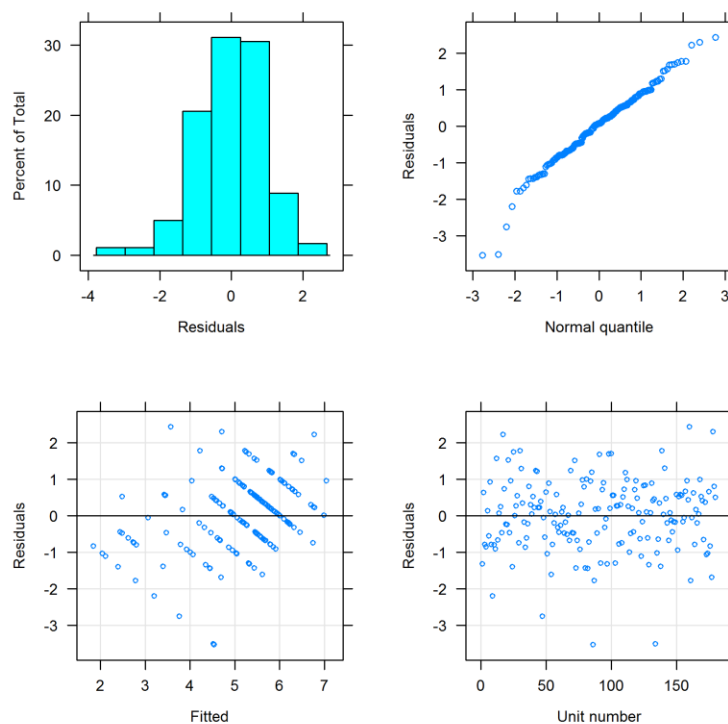
Appendix E NIN15M Mixed Model Residual Plots



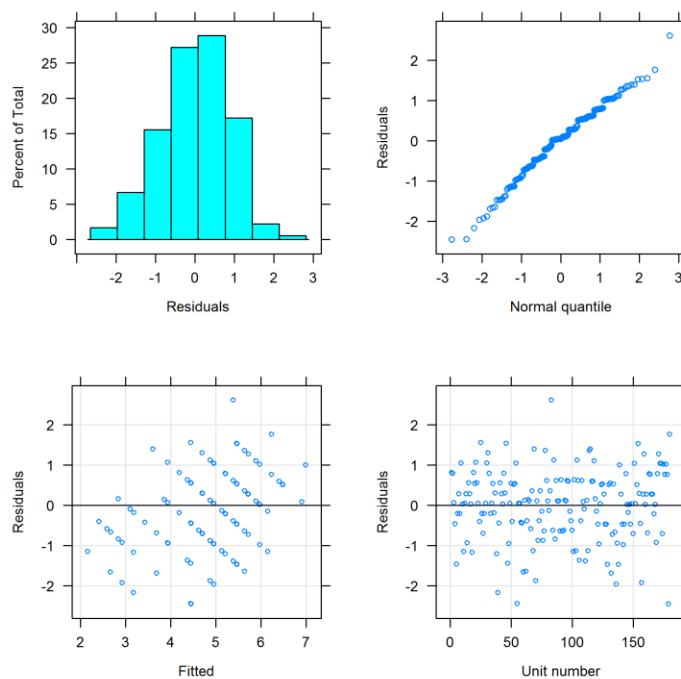
Appendix F TRP14L Mixed Model Residual Plots



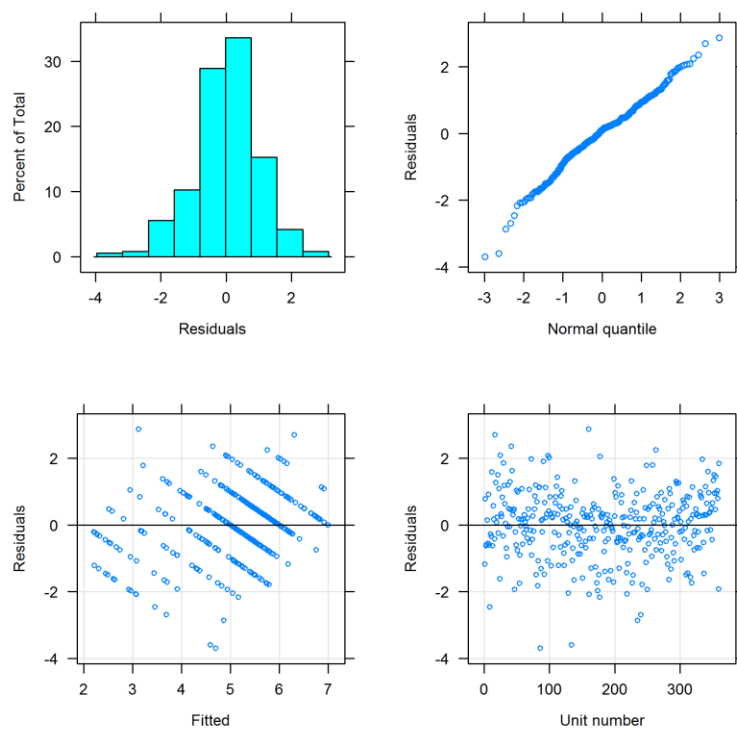
Appendix G TRP15LIM Mixed Model Residual Plots



Appendix H TRP15M Mixed Model Residual Plots



Appendix I TRP15 Lincoln and Mead Residual Plots



Appendix J RPN15L Mixed Model Residual Plots

