

## Registration of 'Egan' Wheat with Resistance to Orange Wheat Blossom Midge

N. K. Blake, R. N. Stougaard, B. Bohannon, D. K. Weaver, H.-Y. Heo, P. F. Lamb, D. Nash, D. M. Wichman, K. D. Kephart, J. H. Miller, G. V. P. Reddy, J. L. Eckhoff, W. E. Grey, S. P. Lanning, J. D. Sherman, and L. E. Talbert\*

### ABSTRACT

'Egan' hard red spring wheat (*Triticum aestivum* L.) (Reg. No. 1102, PI 671855) was developed by the Montana Agricultural Experiment Station and released in 2014. Egan is intended for production in areas of Montana infested with the orange wheat blossom midge (OWBM) (*Sitodiplosis mosellana* Géhin). Egan is resistant to OWBM due to antibiosis conferred by resistance gene *Sm1*. Egan also contains a chromosome segment originally introgressed into wheat from *T. turgidum* ssp. *dicoccoides* containing a gene for high protein (*Gpc-B1*) and a gene for stripe rust (caused by *Puccinia striiformis* Westend. f. sp. *tritici*) resistance (*Yr36*). Egan has shown high yield potential and high grain protein in nurseries grown under OWBM pressure in the Flathead Valley of Montana. Egan is the first hard red spring wheat cultivar with resistance to OWBM developed for Montana.

A NEWLY RECOGNIZED pest in the intermountain valleys of western Montana is the orange wheat blossom midge (OWBM) (*Sitodiplosis mosellana* Géhin). Sporadic outbreaks have occurred in North America, Europe, and Asia (Oakley et al., 1998), with severe damage to spring wheat (*Triticum aestivum* L.) occurring in Saskatchewan during 1983. The OWBM spread throughout western Canada and the northern Great Plains of the United States by the early 1990s. Significant damage to spring wheat crops has been reported in Alberta, Saskatchewan, Manitoba, Minnesota, Montana, and North Dakota. Patterns of damage to the local wheat crop in western Montana have been difficult to establish, as adjacent fields planted at similar times often differ markedly for levels of infestation and damage. This has caused difficulty in developing widely applicable control recommendations.

Adult OWBM emerge at the time of wheat heading and lay eggs on the spikelets. Larvae feed on young kernels, causing kernel damage as well as low yield and poor seed quality (Olfert et al., 1985; Lamb et al., 2000). Larvae leave the florets at the end of the season, drop from the plant, form a cocoon, and overwinter in the soil. Larvae migrate to the soil surface in the following spring, re-enter the soil to pupate, and then emerge from the soil as adults. Environmental factors play a key role in the development of OWBM and its potential impact on a wheat crop (Oakley et al., 1998). Requirements for maximum OWBM activity include temperatures below 10°C for 3 mo for diapause breaking, a wet soil surface for pupation, temperatures above 15°C in wet soils for emergence, and then warming temperatures (above 15°C) for flight at dusk and subsequent egg laying (Oakley et al., 1998).

Genetic resistance to OWBM is available. A single gene for resistance, termed *Sm1*, was identified in some North American winter wheat varieties (McKenzie et al., 2002). *Sm1* confers

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Journal of Plant Registrations 8:298–302 (2014).  
doi: 10.3198/jpr2014.04.0022crc  
Received 15 Apr. 2014. Registration by CSSA.  
5585 Guilford Rd., Madison, WI 53711 USA  
\*Corresponding author (usslt@montana.edu)

N.K. Blake, H.-Y. Heo, D. Nash, W.E. Grey, S.P. Lanning, J.D. Sherman, and L.E. Talbert, Plant Sciences and Plant Pathology Dep., Montana State Univ., Bozeman, MT 59717; R.N. Stougaard and B. Bohannon, Northwestern Agric. Res. Center, 4570 Montana 35, Kalispell, MT 59901; D.K. Weaver, Land Resources and Environmental Sciences Dep., Montana State Univ., Bozeman, MT 59717; P.F. Lamb, Northern Agric. Res. Center, 3710 Assiniboine Rd., Havre, MT 59501; D.M. Wichman, Central Agric. Res. Center, 52583 US Hwy. 87, Moccasin, MT 59462; K.D. Kephart, Southern Agric. Res. Center, 748 Railroad Hwy., Huntley, MT 50037; J.H. Miller and G.V.P. Reddy, Western Triangle Res. Center, P.O. Box 1474, Conrad, MT 59425. J.L. Eckhoff, Eastern Agric. Res. Center, Box 393, Sidney, MT 59270.

**Abbreviations:** AYT, advanced yield trial; OWBM, orange wheat blossom midge; PCR, polymerase chain reaction.

resistance due to antibiosis, resulting in death of the hatched larvae (McKenzie et al., 2002). Thomas et al. (2005) developed polymerase chain reaction (PCR)-based molecular markers closely linked to the *Sm1* gene. One of the markers (*WMI*) is a dominant marker less than 2 cM proximal to the gene. An additional marker, *Xgwm210*, is located on the distal side of *Sm1*, providing flanking markers for the gene. In addition to antibiotic resistance, spring wheat lines showing antixenosis for oviposition by the OWBM have been identified (Lamb et al., 2002). Blake et al. (2011) developed molecular markers for a major quantitative trait locus influencing the amount of oviposition.

Resistance to OWBM is a new objective for hard red spring wheat breeding specific for northwestern Montana. Cultivars for this area also need to have high yield potential under relatively high precipitation conditions (>30 cm yr<sup>-1</sup>), semidwarf growth habit, resistance to stripe rust (caused by *Puccinia striiformis* Westend. f. sp. *tritici*), and high grain protein. A chromosome segment introgressed into wheat from *T. turgidum* ssp. *dicoccoides* provides an opportunity to address the latter two objectives. This segment contains a gene for high grain protein, *Gpc-B1*, which has resulted in an average increase in grain protein of 14 g kg<sup>-1</sup> in several wheat backgrounds (Mesfin et al., 1999; Chee et al., 2001; Sherman et al., 2008; Brevis and Dubcovsky, 2010). *Gpc-B1* is also associated with accelerated leaf senescence in some genetic backgrounds (Uauy et al., 2006; Brevis and Dubcovsky, 2010). The *Gpc-B1* locus is tightly linked to the stripe rust resistance gene *Yr36* (Uauy et al., 2005). This gene confers high-temperature adult plant resistance to several stripe rust races.

## Methods Germplasm

The donor parent for *Gpc-B1* and *Yr36* was hard red spring wheat cultivar Glupro (PI 592759), which contains a translocation whereby a portion of the short arm of chromosome 6B of wheat is replaced by a segment from *T. turgidum* ssp. *dicoccoides* (Mesfin et al., 1999). A total of six backcrosses were made to introduce the *Gpc-B1* allele from Glupro into four hard wheat backgrounds (Sherman et al., 2008), one of which was the widely grown cultivar McNeal (Lanning et al., 1994). McNeal contains an allele for high gluten strength linked to the *Gli-B1* locus (Nash et al., 2006). A McNeal-derived line with *Gpc-B1* was backcrossed once to a line with pedigree CAP19/Choteau. Choteau is a semidwarf hard red spring wheat cultivar with solid stems (Lanning et al., 2004). CAP19 is a *Sm1*-containing line derived from the cross 'Reeder'/BW-277. Reeder (PI 613586) is a semidwarf hard red spring wheat cultivar from North Dakota State University, and BW277 is a hard red spring wheat experimental line from Agriculture and Agri-Food Canada. BW277 contains the *Sm1* allele for OWBM resistance.

## Breeding Procedure

A single-seed descent program beginning at the F<sub>2</sub> generation was conducted following the first backcross of CAP19/Choteau to McNeal\*7/Glupro. Marker-assisted selection for the *Sm1* gene was conducted during the backcross program using the dominant marker, *Xgwm210* (Thomas et al., 2005). We performed PCR on BC<sub>1</sub>F<sub>1</sub> plants using *Xgwm210*, and those with a band of approximately 175 bp were selected. F<sub>3</sub> lines

were grown under OWBM pressure in Kalispell, MT, in 2008, and 16 lines were selected for lack of OWBM damage. F<sub>4</sub> lines were increased in the greenhouse and F<sub>5</sub> lines evaluated for agronomic traits in an unreplicated trial at Bozeman, MT, in 2009. Due to limited seed, OWBM and stripe rust resistance were evaluated at Kalispell in 2009 using F<sub>4</sub> seed. In 2010 and 2011, *Sm1*-containing lines, including a (McNeal\*7/Glupro)\*2//CAP19/Choteau line, designated CAP400, were evaluated in three-replication trials at Bozeman and Kalispell for agronomic, quality, disease, and midge resistance traits.

## Cultivar Evaluation

CAP400, hereafter referred to as 'Egan' (Reg. No. 1102, PI 671855), was entered in to the Montana Spring Wheat Advanced Yield Trial (AYT) in 2012 and 2013. Three-replication lattice designs containing 64 entries were planted at the Northwestern Agricultural Research Center (48°10'N, 114°15'W) in Kalispell from 2012 and 2013. Site elevation is 881 m, and soil texture is a Kalispell silt loam (coarse-loamy, mixed, superactive, frigid Typic Haplustolls). Data were also obtained from rainfed sites at Havre, Sidney, Moccasin, Huntley, and Bozeman and irrigated sites at Sidney and Bozeman in 2012. Data were obtained in 2013 from rainfed sites in Havre, Moccasin, Huntley, Conrad, and Bozeman in 2013. Data collected in all years included date of heading (days from planting date), plant height, grain yield, test weight, and grain protein. Plant height, excluding awns, was measured as mean height of plants within a row. Heading date was measured as the day after 1 January when 50% of the heads within a row were completely emerged. Test weight was measured from a sample of cleaned grain on a Seedburo test weight scale. Grain protein concentration was obtained on whole grain samples using a Foss Infratec 1241 (Tecator).

Additional data on stripe rust infection and OWBM infestation were also obtained for the AYT planted at Kalispell. Stripe rust infection was measured as percentage leaf area infected in all three replications. We assessed OWBM numbers by dissecting three heads per plot and counting the number of larvae from plants collected approximately 3 wk after heading from the first replication in 2012. The AYT was sprayed with chlorpyrifos to control the OWBM in 2013, and thus OWBM numbers were not assessed. In addition, OWBM numbers were similarly assessed from three replications in a separate trial containing a smaller number of entries, including experimental lines with *Sm1* as well as susceptible cultivars. This trial was grown in 2011, 2012, and 2013.

Grain harvested from four sites was tested for bread-making quality in the Montana Cereal Quality Laboratory for both 2012 and 2013 (AACC, 2000). Whole grain protein was determined by near infrared transmittance using an Infratec 1225 Grain Analyzer (Foss North America) (AACC Method 39-21). Wheat grain was tempered to 15% moisture before milling (AACC Method 26-10A). A Brabender Quadromat Senior Mill (C.W. Brabender Instruments, Inc.) was used to obtain straight grade flour. Whole wheat meal was obtained by grinding wheat through a UDY Cyclone Mill (UDY Corporation) equipped with a 0.5-mm screen. Flour protein (14% moisture basis) was determined by near infrared reflectance using a Technicon InfraAnalyzer 400 (Technicon Industrial Systems) (AACC Method 39-11). Moisture was determined by the oven method (AACC Method 44-15A),

and results were adjusted to 14% moisture basis (AACC Method 44-01). Dough properties were measured using the mixograph (AACC Method 54-40). Mixing tolerance was scored on a 1-to-7 scale, with 1 being low tolerance and 7 being high tolerance. A standard bake test methodology was used to measure bread-making properties (AACC Method 10-10B). Statistical analysis was conducted based on genotype means for each location and included genotype and location as sources of variation, with location × genotype as error term (SAS Institute, 2004).

## Molecular Genotyping

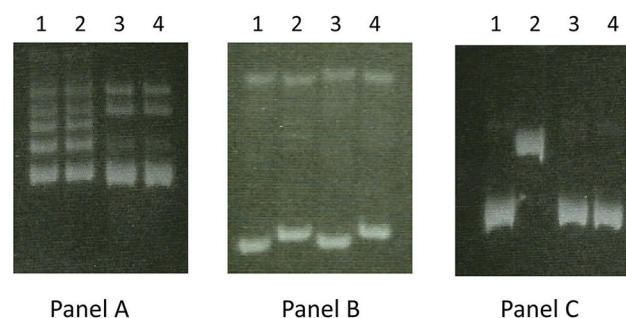
Egan was genotyped for *Gpc-B1*, *Yr36*, and *Gli-B1* genes. Codominant PCR marker *Xuhw89* was used to confirm the presence of the linked genes *Gpc-B1* and *Yr36* (Khan et al., 2000; Distelfeld et al., 2006). The wheat microsatellite marker *Xpsp2* was used to screen for the high gluten strength allele of *Gli-B1* (Manifesto et al., 1998).

## Characteristics

Polymerase chain reaction was used to assay Egan for the presence of major genes *Gpc-B1* for high grain protein content, *Yr36* for stripe rust resistance, *Gli-B1* for strong gluten, and *Sm1* for OWBM resistance. As indicated in Fig. 1, Egan contains *Sm1* from CAP19, *Gpc-B1* and *Yr36* from McNeal\*7/Glupro, and *Gli-B1* from McNeal.

Egan showed excellent resistance to the OWBM in several nurseries grown in Kalispell. First, F<sub>4</sub> and F<sub>5</sub> head rows were selected as being free from OWBM compared with check susceptible cultivars that had as many as 100 OWBM per head. A 2011 replicated nursery showed 0.3 OWBM larvae per spike for Egan, while susceptible cultivars Hank and Reeder had 151 and 62 larvae per spike, respectively. The LSD (0.05) for this nursery was 2.5. A 2012 nursery showed 0.0 OWBM larvae on Egan, while susceptible cultivars Hank and Reeder had 102 and 46 OWBM per spike, respectively. The LSD (0.05) for this nursery was 30. A 2013 nursery also showed 0.0 OWBM per spike for Egan, while Hank and Reeder had 27 and 7 OWBM per spike, respectively. The LSD (0.05) was 4.6.

Yield performance over locations for the 2012 AYT (Table 1) showed Egan to be at or below the mean nursery performance for most environments. However, Egan was the highest-yielding line grown at Northwestern Agricultural Research Center near Kalispell. The agronomic summary for 2012 at the Kalispell site is shown in Table 2. Levels of OWBM were modest at



**Fig. 1.** Marker genotypes for Egan and parents CAP19, McNeal\*7/Glupro, and McNeal for genes *Sm1*, *Gpc-B1*, *Yr36* and *Gli-B1*. Lane 1: Egan. Lane 2: CAP19. Lane 3: McNeal\*7/Glupro. Lane 4: McNeal. Panel A: Amplified DNA from polymerase chain reaction (PCR) using primers for *Xgwm210*; Egan and CAP19 have bands of approximately 175 bp, indicating presence of *Sm1*. Panel B: Amplified DNA from PCR using primers for *Xuhw89*; Egan and McNeal\*7/Glupro have a band of 126 bp, indicating presence of *Gpc-B1* and *Yr36*. Panel C: Amplified DNA from PCR using primers for *Xpsp2*; Egan, McNeal\*7/Glupro, and McNeal have a band of approximately 240 bp, indicating presence of the high-strength allele at *Gli-B1*.

this nursery, ranging from no OWBM per spike for Egan to 15 OWBM per spike for the most heavily infested line. The mean number of OWBM per spike was 2.0. The nursery was heavily infected by stripe rust, with an average percentage leaf coverage of 87.4%. Egan showed the lowest percentage infection in the nursery, 27.2%, significantly lower than all other entries ( $P < 0.05$ ). Test weight, heading date, and height of Egan were similar to its parent McNeal. Grain protein for Egan was among the highest in the nursery. Grain protein data were obtained on a single replication; thus statistical comparison is not possible.

Grain yield at most sites in 2013 (Table 3) was typically average or below average for Egan, except at Kalispell, where it was one of the highest-yielding lines. Data for the Kalispell nursery in 2013 are summarized in Table 4. Egan was among the top-performing lines in this nursery. Other lines performed similarly for grain yield to Egan in 2013, likely because the 2013 nursery was treated with chlorpyrifos to control the OWBM and with pyraclostrobin to control stripe rust. Test weight, heading date, and height of Egan were similar to its parent McNeal. Grain from four locations of the AYT was tested for milling and baking properties in both 2012 and 2013. Grain protein of Egan was 171 g kg<sup>-1</sup> in the 2012, significantly higher than all of the other cultivars ( $P > 0.05$ ). Other baking properties for Egan were most similar to McNeal, with relatively high mixing tolerance, bake mixing time, bake water absorption, and loaf volume

**Table 1.** Grain yield summary for the advanced yield trials grown across Montana in 2012.

Cultivar	Havre rainfed	Sidney rainfed	Sidney irrigated	Moccasin rainfed	Huntley rainfed	Conrad rainfed	Bozeman rainfed	Bozeman irrigated	Meant†	Kalispell rainfed
kg ha <sup>-1</sup>										
Egan	2117	2345	3239	1707	3582	4805	3488	6216	3441	5665
Fortuna	2197	2453	3380	1452	3521	4207	3098	4039	3044	3972
Reeder	2110	2775	4785	1640	4133	4986	3723	5564	3716	4281
McNeal	2231	2406	4556	1606	3709	5006	3656	6035	3649	3790
Choteau	2050	2507	3783	1270	3636	5040	3642	6532	3555	2896
Vida	2379	2688	4489	1626	4032	5369	4348	6176	3891	3588
Duclair	2278	2675	4079	1465	4146	4993	3723	6149	3689	3830
Mean (n = 64)	2191	2460	4092	1519	3844	5067	3743	6122	3629	3434
LSD (0.05)	283	355	1099	361	472	508	390	978	327	566

† Data from Kalispell were not included to calculate the mean due to the severe impact of orange wheat blossom midge infestation on many lines.



**Table 2. Agronomic summary for Egan relative to other lines grown in the advanced yield trial in Kalispell in 2012.**

Cultivar	Grain yield	Test weight	Heading date	Height	Protein	Stripe rust
	kg ha <sup>-1</sup>	kg m <sup>-3</sup>	d from 1 Jan.	cm	g kg <sup>-1</sup>	%
Egan	5664	781.7	184.5	95.5	162	26.2
Fortuna	3971	793.3	183.0	108.7	132	94.5
Reeder	4280	785.6	182.1	100.6	149	67.5
McNeal	3790	757.2	183.7	95.8	138	88.6
Choteau	2896	736.6	183.1	81.5	144	91.4
Vida	3588	750.8	183.1	89.1	149	65.9
Duclair	3830	734.0	180.4	87.6	152	87.2
Mean (n = 64)	3433	745.6	181.4	87.4	143	87.4
LSD (0.05)	846	27.1	1.6	6.1	NA†	14.0

† Not available. LSD was not calculated for grain protein as data were obtained on only one replication.

**Table 3. Grain yield summary for the advanced yield trial grown in rainfed nurseries across Montana in 2013.**

Cultivar	Havre	Moccasin	Huntley	Conrad	Bozeman	Kalispell	Mean
	kg ha <sup>-1</sup>						
Egan	3736	2594	2843	4859	3515	7500	4173
Fortuna	3313	2634	2890	5006	3266	5934	3837
Reeder	3562	2903	3353	5598	3111	7231	4294
McNeal	4213	2762	3024	5181	3326	6895	4234
Choteau	3622	2916	3273	5000	3078	7170	4180
Vida	4516	3521	3454	5349	3145	7365	4556
Duclair	4146	3286	3353	5000	3441	7580	4469
Mean (n = 64)	3985	3011	3172	4993	3320	7137	4274
LSD (0.05)	497	585	349	1102	780	598	450

**Table 4. Agronomic summary for Egan relative to other lines grown in Kalispell in 2013.**

Cultivar	Grain yield	Test weight	Heading date	Height	Protein	Stripe rust
	kg ha <sup>-1</sup>	kg m <sup>-3</sup>	d from 1 Jan.	cm	g kg <sup>-1</sup>	%
Egan	7500	797	178.7	96.8	167	1.0
Fortuna	5934	797	176.7	107.7	155	3.0
Reeder	6895	803	175.3	99.3	155	4.3
McNeal	7231	792	178.7	101.1	162	17.7
Choteau	7170	788	177.0	92.7	154	0.0
Vida	7365	772	176.3	97.0	153	11.7
Duclair	7580	788	173.0	90.7	146	8.3
Mean (n = 64)	7137	793	175.7	91.2	151	9.1
LSD (0.05)	598	NA†	1.6	10.2	NA	13.6

† Not available. LSD was not calculated for test weight or grain protein as data were from a single replication.

(Table 5). Similar results were obtained in 2013 (Table 6). Egan had grain protein level of 161 g kg<sup>-1</sup>, significantly higher than the other cultivars ( $P < 0.05$ ). Egan also showed strong dough characteristics and high loaf volume similar to McNeal.

Breeder seed of Egan was developed by selection for uniformity among approximately 400 head rows planted in 2012. Seed from selected head rows was sown in four-row plots in 2013, and selected rows were bulk harvested to form breeder seed of Egan.

## Discussion

Egan is the first hard red spring wheat cultivar with OWBM resistance due to *Sm1* developed for Montana. Additional desirable characteristics of Egan for the region include resistance to stripe rust, semidwarf stature, and high grain protein levels. Egan will also be beneficial in locations where the OWBM and the wheat stem sawfly (*Cephus cinctus* Norton) occur

together, as spraying to manage OWBM could adversely impact economically important endemic sympatric braconid parasitoids, which play an important role in suppressing wheat stem sawfly larval populations (Peterson et al., 2011).

## Availability

Breeder, foundation, registered, and certified classes of seed for Egan are all recognized. Breeder and foundation seed will be maintained by the Department of Plant Sciences and Plant Pathology, Montana Agricultural Experiment Station, Bozeman, MT 59717. Application will be made for U.S. Plant Variety Protection with the certification option. Contact the corresponding author for all seed requests. No seed will be distributed without written permission for 20 years from date of publication in *Journal of Plant Registrations* by the Montana Agricultural Experiment Station, at which time seed will also be available from the National Plant Germplasm System (NPGS).

**Table 5. Mill and bake data for Egan relative to other cultivars based on four locations in 2012.**

Cultivar	Wheat protein	Flour yield	Flour protein	Flour ash	Mixing tolerance	Bake mixing time	Bake water absorption	Loaf volume
	g kg <sup>-1</sup>			%	1–7†	min	g kg <sup>-1</sup>	cm <sup>3</sup>
Egan	171	622	149	0.39	6.5	15.9	798	1258
Fortuna	158	592	142	0.43	3.8	6.8	757	1195
McNeal	162	561	147	0.44	6.0	11.7	786	1310
Reeder	158	631	141	0.38	3.5	5.8	746	1215
Choteau	161	632	144	0.39	4.75	6.2	757	1216
Vida	156	661	138	0.39	3.25	5.7	748	1170
Duclair	159	620	140	0.40	4.75	8.4	751	1258
Mean (n = 64)	160	627	142	0.40	4.3	7.7	758	1189
LSD (0.05)	8.0	32	6.5	0.02	1.1	2.5	20.0	56

† Mixing tolerance was determined from mixograph analysis, where 1 indicates low tolerance and 7 indicates high tolerance.

**Table 6. Mill and bake data for Egan relative to other cultivars based on four locations in 2013.**

Cultivar	Wheat protein	Flour yield	Flour protein	Flour ash	Mixing tolerance†	Bake mixing time	Bake water absorption	Loaf volume
	g kg <sup>-1</sup>			%	1–7	min	g kg <sup>-1</sup>	cm <sup>3</sup>
Egan	161	629	139	0.39	6.0	13.6	788	1282
Fortuna	152	669	132	0.40	4.0	5.8	743	1193
McNeal	149	626	131	0.41	5.5	11.7	776	1232
Reeder	153	628	134	0.37	4.0	5.9	755	1200
Choteau	149	631	138	0.38	4.5	8.0	769	1218
Vida	141	677	125	0.38	3.3	7.8	757	1163
Duclair	149	625	130	0.38	4.5	9.6	758	1227
Mean (n = 64)	149	640	131	0.38	4.3	10.0	76.	1168
LSD (0.05)	6.3	24.1	6.1	0.02	1.1	3.1	21.0	59

† Mixing tolerance was determined from mixograph analysis, where 1 indicates low tolerance and 7 indicates high tolerance.

## Acknowledgments

This research was supported by grants from the Montana Wheat and Barley Committee and by USDA National Institute of Food and Agriculture award 2011-68002-30029.

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