

## Registration of 'Matterhorn' Hard White Waxy Winter Wheat

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### Abstract

'Matterhorn' (Reg. No. CV-1151, PI 687896) hard white winter waxy wheat (*Triticum aestivum* L.) was developed cooperatively by the USDA-ARS and the Nebraska Agricultural Experiment Station and released in 2018. Matterhorn, a sibling of the hard red waxy cultivar Mattern, has white grain color and waxy (amylose-free) endosperm starch. It was released primarily for its unique end-use quality attributes and for grain yield competitiveness with currently grown Nebraska-adapted cultivars. The waxy starch is conditioned by the presence of three naturally occurring mutations that eliminate production of the enzyme granule-bound starch synthase. Granule-bound starch synthase synthesizes amylose in typical wheats and other cereal crops. Matterhorn (tested as NX04Y2107W) was selected from the heterogeneous red/white-seeded experimental line NX04Y2107 derived from the cross NW98S061/99Y1442.

WAXY WHEATS (*Triticum aestivum* L.), carrying null alleles at the three *Wx* loci, produce endosperm starch free of amylose (Nakamura et al., 1993, 1995; Slade et al., 2005). The resultant waxy starch, consisting of nearly 100% amylopectin, imparts radically different functional properties compared with wild-type starches (Graybosch, 1998; Akashi et al., 2000; Hatta et al., 2000) and can be used to develop novel whole grain products (Wilson et al., 2011), as a more efficient substrate for ethanol production (Zhao et al., 2009), and can provide unique functional properties to modified food starches (Hansen et al., 2010; Graybosch and Hansen, 2016). To date, only two waxy wheat cultivars, 'Waxy-Pen' (Morris and King, 2007) adapted to the Pacific Northwest, and the hard red waxy 'Mattern' (Graybosch et al., 2014), adapted to the Great Plains, have been released in the United States.

Market classes of wheat are largely based on endosperm texture (grain hardness), grain and flour protein concentrations, and grain color (Carson and Edwards, 2009). The Great Plains of North America now produces two types of winter wheat, hard red and hard white. The red color in the grain is a result of condensed tannins or phlobaphenes, present in the seed coat. White wheats lack condensed tannins. White wheats have some potential advantages over red wheats, including wider application in non-pasta noodles and steamed wheat products, and the production of higher extraction flours (Taylor et al., 2005). The lack of condensed tannins imparts a slightly sweeter, less bitter taste to whole grain products. Whole grain products from white wheats can be significantly brighter in color, with greater appeal to consumers, than those from red wheats (Ambalamaatil et al., 2002). To fill potential demand from commercial entities for a hard white waxy wheat adapted to the Great Plains, the USDA-ARS, in cooperation with the University of Nebraska, developed and released the hard white waxy winter wheat 'Matterhorn' (Reg. No. CV-1151, PI 687896).

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**Abbreviations:** NRPN, Northern Regional Performance Nursery.

## Methods

### Selection, Seed Purification, and Increase

Matterhorn, tested experimentally as NX04Y2107W, was selected from the heterogeneous red/white-seeded experimental line NX04Y2107 derived from the cross NW98S061/99Y1442. NW98S061 is a hard white winter wheat breeding line developed by USDA-ARS at Lincoln, NE, with the pedigree 'Jagger' (PI 593688)/'Nekota' (PI 584997). 99Y1442 is a waxy experimental line, also developed by USDA-ARS, Lincoln, with the pedigree 'BaiHuo'/'Kanto107'(PI 631445)/'Ike'(PI 574488)/3/96MD7413-10. 96MD7413-10 is a partial waxy breeding line developed by USDA-ARS, with the pedigree NE90616/Ike. White and red seed of NX04Y2107 were separated by mechanical sorting using a Satake ScanMaster IE 200 Automated Seed Sorter housed at the Seed Science Center, Iowa State University. Red-seeded segregates of NX04Y2107 were used to develop the cultivar Mattern (Graybosch et al., 2014). White-seeded progeny were bulked to form NX04Y2107W. In 2012, spikes (heads) were obtained from a field planting of NX04Y2107W grown near Mead, NE. Heads were individually threshed with identity maintained and seed screened for grain color and the presence of waxy endosperm. One hundred white-seeded waxy-endosperm head selections were used to seed single-plant-progeny rows at Yuma, AZ. After harvest, seed of each row was retested for grain color and endosperm characteristics. All waxy/white-seeded samples were composited to form the breeding line NX04Y2107W, which was increased via a bulk planting at Yuma in 2014.

### Evaluation and Statistical Analysis

Grain yield and additional agronomic traits of Matterhorn (as NX04Y2107W) were evaluated in the 2014 USDA-ARS coordinated Northern Regional Performance Nursery (NRPN; USDA, 2014). Trials were planted in randomized complete block designs with three replications at 15 locations. States and provinces hosting study sites, along with number in each, were Kansas (1), Nebraska (4), South Dakota (3), Minnesota (2), Montana (2), North Dakota (1), Wyoming (1) and Alberta (1). The NRPN contains advanced experimental lines from Great Plains wheat breeding programs. Matterhorn performance is presented relative to the control cultivars 'Overland' (PI 647959), 'Wesley'(PI 605742), 'Jagalene' (PI 631376), and 'Jerry' (PI 632433), though all trial entries were used in the analysis of variance and calculation of least significant differences (LSD,  $\alpha = 0.05$ ) for grain yields. Grain volume weights, plant heights, and days to heading (from 1 Jan. 2014) are presented as means without statistical analysis, due to incomplete reporting from cooperators. Matterhorn (as NX04Y2107W) also was entered in the University of Nebraska Winter Wheat Variety Test (University of Nebraska, 2016) from 2014 to 2016. Matterhorn was tested at 40 location-year environments over the 3-yr period, in randomized complete block designs with six blocks per site. All entries were used in analyses of variance of grain yield, grain volume weight and plant heights, with results presented comparing Matterhorn to the control cultivars 'Freeman' (PI 667038), Overland, 'Ruth'(PI 675998), and Wesley, as well as to its sibling Mattern. Mean responses and LSD ( $\alpha = 0.05$ ) were computed separately for each year.

Regression analysis (Eberhart and Russell, 1966) was used to evaluate grain yield stability across environments in both the NRPN and across the 40 site-year environments of the University of Nebraska trials, years 2014 to 2016. Grain yield of each entry was used as the dependent variable, and the average grain yield of all entries at each location was used as the independent variable (environmental index). The Test statement in SAS Proc Reg was used to determine if the slope of the regression equation for each entry significantly deviated from 1.0. All trial entries were used for calculations, while only those of the control cultivars noted above are presented. Regression analysis also was used to evaluate performance of Matterhorn relative to that of its sibling Mattern across the Nebraska environments. SAS version 9.4 (SAS Institute, 2013) was used for all statistical computations.

Responses to wheat pathogens, molecular marker data, and evaluation of grain quality traits were conducted as part of the 2014 NRPN (USDA, 2014). Molecular markers and procedures are described in detail in USDA (2016). Seedling responses to stem rust (caused by *Puccinia graminis* Pers.:Pers. f. sp. *tritici* Eriks. & E. Henn.), races QFCSC, QTHJC, MCCFC, RCRSC, RKQQC, TPMK, CTTTTF, GFMNC, QCCSM, and TTKSK, and leaf rust (caused by *P. triticina* Eriks.), races TNBGJ, MCTNB, MFPSB, TBBGJ, KFBGJ, MBDS, TFBJQ, MHDSB, TCRKG, and PBLRG, were obtained using procedures established at the USDA-ARS Cereal Disease Laboratory, St. Paul, MN (USDA, 2018a). Field adult plant responses to pathogens wheat stripe rust (caused by *P. striiformis* Westend. f. sp. *tritici* Eriks.), leaf rust, stem rust, *Wheat soilborne mosaic virus*, *Wheat streak mosaic virus*, additional fungal and bacterial pathogens, and acid soils were provided by cooperators in the NRPN.

Tolerance to preharvest sprouting was evaluated using procedures described by Fakthongphan et al. (2016). Samples were collected from field plantings at the University of Nebraska Eastern Nebraska Research and Extension Center near Mead. Matterhorn, along with a collection of hard red and hard white winter wheat cultivars, was planted in a randomized complete block design with three replications over two harvest seasons, 2013 and 2014. Mean responses were compared via analysis of variance and computation of LSD (0.05) and reported separately for each season, due to large and significant effects of year and entry  $\times$  year interactions. Grain and flour quality traits were evaluated on Matterhorn samples from the 2014 NRPN using procedures established by the USDA-ARS Hard Winter Wheat Quality Laboratory, Manhattan, KS (USDA, 2018b). Composite samples were milled from locations within three Great Plains agroecological production zones (Peterson, 1992), treated as replications and analyzed as a completely random design with mean comparisons by LSD (0.05). High molecular weight glutenin subunit composition was determined as per Blechl and Anderson (1996).

## Characteristics

### Agronomic and Botanical Description

Matterhorn has a white coleoptile and erect juvenile growth pattern. The foliage is blue-green with a light waxy bloom. The leaves are glabrous. The spike is tapering, narrow, midlong, and dense. The glume is short and narrow, and the glume shoulder

is oblique. The beak is long with an acuminate tip. Kernels are white colored, hard textured, and mainly ovate in shape. Matterhorn is an awned, tan-glumed, semidwarf cultivar that contains the *Rht-B1b* allele. The kernel has no collar, a large brush, rounded cheeks, with a midsize germ. Endosperm starch lacks amylose and, like all waxy starches, stains reddish-brown with I<sub>2</sub>KI (Nakamura et al., 1995).

## Field Performance

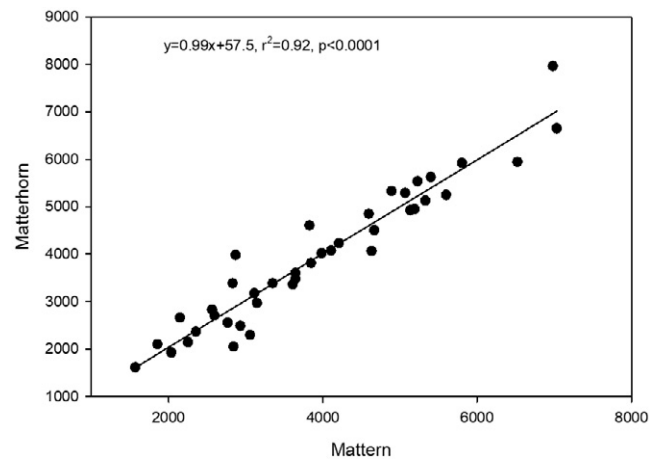
In 2014, Matterhorn was entered (as NX04Y2107W) in the USDA-ARS coordinated NRPN (USDA, 2014). Average grain yield (Table 1), across 15 northern Great Plains locations, was 4123 kg ha<sup>-1</sup>, not significantly different from grain yields (kg ha<sup>-1</sup>) of modern check cultivars Overland (4372), Wesley (4188); Jagalene (4235), and Jerry (4275).

In the University of Nebraska Winter Wheat Variety Tests (Table 1), Matterhorn's statewide grain yield was significantly lower than the control cultivar Ruth in 2014 and 2015, significantly lower than Overland in 2014, and significantly lower than Freeman in 2016. In all other years, grain yield of Matterhorn

**Table 1. Grain yield and other agronomic traits of wheat cultivar Matterhorn compared with control cultivars.**

Entry	Grain yield	Grain volume weight	Plant height	Days to heading
	kg ha <sup>-1</sup>	kg hL <sup>-1</sup>	cm	d from 1 Jan.
<b>2014, NRPN†</b>				
Overland	4372	76.0	79.1	164
Wesley	4188	74.2	70.6	162
Jagalene	4235	75.1	77.1	164
Jerry	4275	74.6	86.6	167
Matterhorn	4123	72.9	78.1	161
LSD (0.05)	383			
<b>2014, NE State‡</b>				
Freeman	3871	74.7	71.3	
Mattern	3752	73.4	78.5	
Matterhorn	3614	74.2	75.0	
Overland	3970	75.7	76.6	
Ruth	4115	75.6	76.2	
Wesley	3679	74.1	70.6	
LSD	278	0.9	3.2	
<b>2015, NE State</b>				
Freeman	3324	67.1	86.4	
Mattern	2880	65.5	81.0	
Matterhorn	3022	65.5	78.9	
Overland	3316	70.1	78.5	
Ruth	3549	69.5	78.6	
Wesley	2773	65.2	68.8	
LSD (0.05)	319	3.1	14.0	
<b>2016, NE State</b>				
Freeman	4595	73.6	91.8	
Mattern	4056	74.7	93.2	
Matterhorn	4016	74.3	92.2	
Overland	3530	75.2	93.7	
Ruth	4246	76.2	95.0	
Wesley	3598	73.2	87.0	
LSD (0.05)	294	1.6	2.3	

† NRPN = Northern Regional Performance Nursery; NE State = University of Nebraska Winter Wheat Variety Trial.



**Fig. 1. Correlation of grain yields (kg ha<sup>-1</sup>), Matterhorn vs. Mattern.**

did not differ from these cultivars. Matterhorn's grain yield was significantly higher than Wesley's in 2016. Across all Nebraska test environments, Matterhorn never differed significantly in grain yield from its sibling Mattern. Grain yields of these two cultivars were highly correlated (Fig. 1), indicating no grain yield penalty associated with the presence/absence of grain pigmentation.

In stability analysis, the average response of all entries is, by definition, 1.0. Any deviation in slope from this value indicates a deviation from the typical response to changing environmental conditions. The slope of Matterhorn's regression in both the NRPN and the Nebraska Winter Wheat Variety trial did not deviate from 1.0 (Table 2), nor did the slopes of any of the control cultivars, with the exception of Wesley in the Nebraska trials. This indicates Matterhorn responds to changes in environmental productivity in a similar fashion to the control cultivars.

## Disease Resistance

Observations in the 2014 NRPN (USDA, 2014) and in field trials in Nebraska indicate that Matterhorn's response to stripe rust ranges from moderately susceptible to resistant, dependent on prevalent races in the test locale. Molecular marker analysis revealed the presence of the *Lr37/Sr38/Yr17* complex on the 2NS:2AS chromosomal translocation from *Aegilops ventricosa*

**Table 2. Stability analysis of wheat cultivar Matterhorn relative to control cultivars.†**

Study‡	Entry	b value	r <sup>2</sup>	p
NRPN	Overland	0.99	0.92	0.95
	Wesley	0.88	0.82	0.30
	Jagalene	1.04	0.88	0.71
	Jerry	0.97	0.71	0.87
	Matterhorn	0.95	0.83	0.69
NE State	Freeman	0.95	0.92	0.34
	Mattern	1.01	0.94	0.97
	Matterhorn	1.02	0.91	0.78
	Overland	0.88	0.86	0.08
	Ruth	0.96	0.92	0.34
	Wesley	1.11	0.96	0.01

† Regression parameters presented: b value (slope), r<sup>2</sup> = coefficient of determination, p = probability slope (b value) equals 1.0.

‡ NRPN = Northern Regional Performance Nursery, NE State = University of Nebraska Winter Wheat Variety Trial.

(Zhuk.) Chennav. Matterhorn is susceptible to most prevalent races of leaf rust but is postulated to carry the seedling resistance genes *Lr1* and *Lr14a*. Seedling stem rust resistance is present to races QFCSC, QTHJC, MCCFC, RCRSC, TPMKC, and QCCSM, though Matterhorn is susceptible to stem rust race Ug99 and its derivatives. Matterhorn is tolerant of acid soil conditions and carries the Al4DL+ allele (Ma et al., 2005) for this trait, based on marker WMC00331. Matterhorn demonstrates field tolerance to the *Wheat soilborne mosaic virus/Wheat spindle streak mosaic virus* complex, bacterial streak [caused by *Xanthomonas translucens* pv. *undulosa*, (Xtu.)], and dwarf bunt (caused by *Tilletia controversa* Kuhn). It is susceptible to wheat curl mite (*Aceria tosichella* Keifer), Hessian fly [*Mayetiola destructor* (Say)] biotype GP, greenbug [*Schizaphis graminum* (Rondani)] biotype E, and Russian wheat aphid [*Diuraphis noxia* (Kurdjumov)] biotype 2.

## End-Use Quality

End-use quality attributes of Matterhorn, due to the presence of amylose-free (waxy) starch, differ markedly from those of wild-type wheats (Table 3). Flour yield was significantly lower than in the control cultivars, a characteristic typical of waxy wheats (Graybosch et al., 2003). Flour pasting properties, as measured by the Rapid Viscoanalyzer, also differed markedly from the controls and from typical wheats in general (Table 3).

The observed low stirring number and short pasting time are typical of waxy wheats, are independent of  $\alpha$ -amylase activity (Graybosch et al., 2000), and are not indicative of preharvest sprouting. Polyphenol oxidase activity is not low and is similar to the red wheat controls. Matterhorn's hardness score is typical of hard wheats and equal to that of Wesley and Jerry. Grain and flour protein concentrations, mixograph scores, bake mix time, and loaf volume of Matterhorn fall within the ranges observed for wild-type wheats (Table 3). Baked loaves of Matterhorn will

collapse on cooling, another trait typical of waxy wheats. Matterhorn displayed significantly higher mixograph water absorption than all of the controls and significantly higher bake water absorption than all controls except Wesley. Preharvest sprouting tolerance of Matterhorn, using a surrogate assay (Fakhongphan et al., 2016), is intermediate, significantly greater than susceptible white wheats such as 'Arrowsmith' and 'Anton' (Table 4), statistically equal to that of its red wheat sibling Mattern, equal to known tolerant white wheats such as 'Danby', but significantly lower than the tolerant red wheat 'Camelot' and the tolerant white wheat 'Clark's Cream'. Molecular marker analyses using PHS-4A-34586\_92 (USDA, 2014) indicate that Matterhorn carries a marker allele linked to preharvest sprouting tolerance on 4AL. Both Matterhorn and Mattern produce the high-molecular-weight glutenin protein subunits 2\*, 7+9, 5+10.

## Availability

The US Department of Agriculture will not have seed for commercial distribution but will accept requests for licensing. The seed classes will be breeder, foundation, registered, and certified. Matterhorn has been submitted (application #201800398) for US Plant Variety Protection under P. L. 10577 with the certification option. Seed of Matterhorn has been deposited into the USDA-ARS National Laboratory for Genetic Resources Preservation, where it will be available for research purposes immediately upon publication.

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**Table 3. Grain and flour quality attributes of Matterhorn compared to control cultivars, 2014 Northern Regional Performance Nursery (NRPN).**

Entry	Grain hardness	Grain protein concentration	Flour yield	Flour ash	Flour protein concentration	Flour polyphenol oxidase (OD†)	Flour absorption	Mixograph mix time	Mixograph tolerance‡
	hardness units	g kg <sup>-1</sup>			g kg <sup>-1</sup>			min	0–5
Jagalene	68	134.7	685	4.50	122.0	0.54	632	4.3	3.7
Jerry	54	134.7	696	3.97	123.3	0.61	632	3.6	3.0
Kharkof	39	148.7	653	4.27	135.0	0.59	630	3.5	3.3
Matterhorn	57	138.0	588	4.13	125.0	0.67	659	2.5	1.0
Overland	63	131.3	688	4.17	117.0	0.58	624	2.5	0.7
Wesley	56	140.7	700	4.00	130.7	0.69	643	4.0	2.7
LSD (0.05)	3	5.1	15	0.38	5.1	0.06	3	0.5	1.0
Entry	RVA§ pasting time	RVA stirring number	RVA peak viscosity	RVA final viscosity	Bake water absorption	Bake mix time	Loaf volume		
	min	units	units	units	g kg <sup>-1</sup>	min	mL		
Jagalene	6.2	119	235	283	632	5.2	943		
Jerry	6.2	135	214	277	628	4.8	885		
Kharkof	6.3	142	245	288	628	4.7	987		
Matterhorn	3.4	71	102	24	651	4.1	985		
Overland	6.2	134	233	284	610	3.0	845		
Wesley	6.2	126	227	280	643	6.3	990		
LSD (0.05)	0.09	11	20	21.7	13	0.7	44		

† OD = optical density.

‡ Mixograph tolerance scored on a scale of 0 to 5, with 0 = no tolerance, 5 = greatest tolerance.

§ RVA = Rapid Viscoanalyzer.



**Table 4. Sprouting tolerance (delta†) of Matterhorn compared to a collection of Great Plains hard red and hard white winter wheats.**

Entry	PI no.	Market class‡	2013	2014
Arrowsmith	PI 633911	HW	130.7	79.6
Anton	PI 651044	HW	107.8	85.2
Platte	PI 596297	HW	126.7	56.3
Antelope	PI 633910	HW	132.4	40.9
Alliance	PI 573096	HRW	106.7	55.3
Alice	PI 644223	HW	111.5	37.4
NuDakota	PI 643089	HW	99.8	45.9
McGill	PI 659689	HRW	111.3	33.5
Millennium	PI 612390	HRW	105.7	24.9
Overland	PI 647959	HRW	91.5	34.2
Snowmass	PI 658597	HW	97.0	28.1
Pronghorn	PI 593047	HRW	59.9	59.1
Nuplains	PI 605741	HW	66.2	49.9
TAM 111	PI 631352	HRW	83.3	25.5
Settler CL	PI 653833	HRW	79.4	26.3
Trego	PI 612576	HW	71.5	24.0
Danby	PI 648010	HW	78.6	24.1
Mattern	PI 665947	hard red waxy	66.1	30.0
Matterhorn	PI 687896	hard white waxy	60.2	33.5
Robidoux	PI 659690	HRW	53.1	32.7
TAM 107	PI 495594	HRW	64.6	19.6
Niobrara	PI 584996	HRW	55.3	23.2
Wesley	PI 605742	HRW	44.0	17.9
Jagalene	PI 631376	HRW	42.8	17.4
Mace	PI 651043	HRW	36.7	22.0
Camelot	PI 653832	HRW	37.0	16.3
Clark's Cream	PI 476305	HWW	10.1	12.6
Mean			78.9	35.4
LSD (0.05)			35.0	12.1

† Delta = change in shoot and root area of seed within heads after misting treatment. Higher values = greater susceptibility to preharvest sprouting.

‡ HW = hard white; HRW = hard red winter.

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