# Registration of Great Northern Common Bean Cultivar 'Coyne' with Enhanced Disease Resistance to Common Bacterial Blight and Bean Rust

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#### **ABSTRACT**

Great northern common bean (*Phaseolus vulgaris* L.) 'Coyne' (Reg. No. CV-287, PI 655574) was developed by the dry bean breeding program at the University of Nebraska Agricultural Research Division and released in 2008. It was bred specifically for adaptation to Nebraska growing conditions and for enhanced resistance to common bacterial blight (CBB), a major disease of common bean caused by the seed-borne bacterium *Xanthomonas campestris* pv. *phaseoli* (Smith) Dye, and bean common rust *Uromyces appendiculatus* (Pers.:Pers) Unger. Coyne is a great northern F<sub>7:8</sub> line derived from a three-way cross (G95023/Weihing//BelMiNeb-RMR-11). The first cross was made in winter 2003. The F<sub>7:8</sub> was tested in advanced yield trials at Scottsbluff and Mitchell, NE, and in growers' fields in Nebraska. Yield of Coyne was only 47 kg ha<sup>-1</sup> lower than 'Marquis' in Morrill and Scotts Bluff, NE, counties. Reaction of Coyne to CBB under field conditions was consistent across 3 yr at the West Central Research and Extension Center, North Platte, NE, where field disease ratings of 3.2, 3.5, and 4.4 were recorded in 2005, 2006, and 2007, respectively. Coyne has the *Ur-3* and *Ur-6* genes for resistance to common bean rust and carries the single dominant hypersensitive *I* gene that provides resistance to all non-necrotic strains of *Bean common mosaic virus*. Coyne has bright white seed, blooms 44 d after planting, and is a midseason bean, maturing 91 d after planting.

Great northern common bean (*Phaseolus vulgaris* L.) Coyne' (Reg. No. CV-287, PI 655574) was developed by the dry bean breeding program at the University of Nebraska Agricultural Research Division and released in 2008. It is named in honor of Dermot P. Coyne, who was the bean breeder at the University of Nebraska for about 30

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**Abbreviations:** BCMNV, Bean common mosaic necrosis virus; BCMV, Bean common mosaic virus; CBB, common bacterial blight; MRPN, Mid-West Regional Performance Nursery; PCR, polymerase chain reaction; QTL, quantitative trait locus; SCAR, sequence characterized amplified region; WCREC, West Central Research and Extension Center; WRBT, Western Regional Bean Trial.

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years before retiring in 2001. He died in 2002. During his career at the University of Nebraska, Coyne and his cooperators developed 22 dry bean releases (Steadman and Janick, 2003). Coyne was also noted for his work on the genetics of resistance to bacterial diseases of common bean (Steadman and Janick, 2003). Several of the lines he developed are included in the pedigree of Coyne. This cultivar was bred specifically for adaptation to the common bean growing conditions of Nebraska and for enhanced resistance to common bacterial blight (CBB), a major disease of common bean caused by the seed-borne bacterium Xanthomonas campestris pv. phaseoli (Smith) Dye [syn. X. axanopodis pv. phaseoli (Smith) Vauterin et al.] (Xap), and the brown pigmented variant X. axanopodis pv. phaseoli var. fuscans (Xapf), and for improved resistance to common bean rust caused by Uromyces appendiculatus (Pers.:Pers) Unger.

Coyne is a great northern F<sub>7:8</sub> line derived from a three-way cross (G95023/Weihing//BelMiNeb-RMR-11). G95023 is a great northern line developed by the Michigan Agricultural Experiment Station derived from G91213/7/G90123/P86295/3/WM1-85-45/Sierra//P86241/4/90MS-36/G2913. 'Weihing' (PI 614779) is a great northern cultivar derived from a cross between two great northern breeding lines (NE6-91-115 and NE6-91-73) from the University of Nebraska dry bean breeding program. Weihing has the *Ur-3* and *Ur-6* rust-resistance genes and resistance to the halo blight pathogen, caused by the bacterium *Pseudomonas syringae* pv. *phaseolicola* (Burkholder) Young et al. combined with partial

avoidance of white mold [caused by Sclerotinia sclerotorium (Lib.) de Bary] due to its upright and porous plant architecture (Coyne et al., 2000). Weihing also has excellent seed quality and possesses the I gene for resistance to Bean common mosaic virus (BCMV). BelMiNeb-RMR-11 (PI 642017) is a great northern line developed by the USDA-ARS Beltsville Agricultural Research Center, Beltsville, MD, in cooperation with the Michigan Agricultural Experiment Station and the University of Nebraska Agricultural Research Division (Pastor-Corrales et al., 2007). BelMiNeb-RMR-11 combines four genes for resistance to the bean common rust pathogen (Ur3, Ur4, Ur-6, and Ur-11), with two genes for resistance to BCMV and Bean common mosaic necrosis virus (BCMNV). BelMiNeb-RMR-11 was derived from bulked F<sub>s</sub> generation from Kodiak/9/P94232\*2/8/92 BR-3-10-1084B/7/BR3-1006B/6/88-011-03\*2/5/Aztec/4/87-039-34\*2/3/POX10// Fiesta/PI 190078. Coyne has high yield potential, broad adaptation to Nebraska, and good seed quality and resistance to the CBB, rust, and BCMV pathogens.

### Methods

Coyne is an F<sub>7.8</sub> line obtained from the cross (G95023/Weihing//BelMiNeb-RMR-11). The first cross was made in winter 2003 in the greenhouse. The  $F_1$  was advanced to  $F_2$  and  $F_3$  in 2004, and about 50 individual plant selections were made and sent to Isabela, Puerto Rico, for generation advancement  $(F_{3\cdot4})$ . Plants were selected for upright to semi-upright plant architecture, earliness, and freedom from disease. The F<sub>3.5</sub> F<sub>3.6</sub> bulks were evaluated in two replicated yield trials at Scottsbluff (41°53.6' N, 103°40.7' W, 1200 m elevation) and Mitchell (41°56.6′ N, 103°41.9′ W, 1240 m elevation), NE, in 2005 and 2006, respectively. In 2006, about 150 individual plant selections were sent to New Zealand and the seed was bulked based on uniformity to get F<sub>7-8</sub>. In 2007, the F<sub>7-8</sub> bulk was tested in five advanced yield trials at Scottsbluff and Mitchell with the common bean cultivars Orion, Beryl-R, 999-131, and 99-136 included as references. In addition, "Mother and Baby" trials (Bänziger and De Meyer, 2002) were conducted in four growers' fields in Morrill and Scotts Bluff, NE, counties using the common bean cultivar Marquis as a reference. For these trials, each entry was planted in four 45- to 121-m long rows spaced 56 or 76 cm apart. Mother and Baby trials are a form of Participatory Variety Selection developed by the International Center for Wheat and Maize Improvement (CIMMYT). New lines are evaluated using a combination of Mother trials, where all new lines are grown together in on-station research plots, and Baby trials, where one or two lines are compared with a local cultivar in grower fields. Additionally, in 2007 Coyne was tested in the Western Regional Bean Trial (WRBT) at Fort Collins, CO, Kimberly, ID, Mitchell, NE, and Roza, WA, and the Mid-West Regional Performance Nursery (MRPN) at Fort Collins, CO, Saginaw, MI, Mitchell, NE, and Johnstown, ND.

Common bacterial blight screening was conducted in the field at the West Central Research and Extension Center (WCREC), North Platte, NE, in 2005, 2006, and 2007. At flowering, plants were inoculated with a bacterial suspension containing  $3 \times 10^7$  colony forming units mL<sup>-1</sup> of Nebraska Xap strains SC-4A and LB-2 using a backpack sprayer (Schuster and Coyne, 1981). The plants were evaluated 14 d after inoculation using a 1 to 9 scale, where 1 = immune and 9 = very susceptible (van Schoonhoven and Pastor-Corrales, 1987). Reactions from 1 to 3 were considered resistant, 4 to 6 intermediate, and 7 to 9 susceptible.

Rust was evaluated in greenhouse facilities at the USDA-ARS Beltsville Agricultural Research Center, Beltsville, MD, from 2005 to 2007 using races 41, 44, 47, 49, 53, 67, 73, and 108 maintained at Beltsville, MD. These races are used because they reliably identify the presence of rust resistance genes *Ur-3*, *Ur-4*, *Ur-6*, and *Ur-11*. 'Pinto UI 114', 'Aurora' (*Ur-3*), 'Early Gallatin' (*Ur-4*), 'Golden Gate Wax' (*Ur-6*), PI 1819886 (*Ur-11*), Weihing (*Ur3*, *Ur6*), and other beans were used as checks (Table 1). A rust evaluation scale that considers pustule size and intensity of infection was used (Stavely et al., 1983). The reaction grades used were 1 to 6, where 1 = no visible symptoms, 2 = necrotic spots without sporulation, 3 = small sporulating pustules less than 0.3 mm in diameter, 4 = midsize sporulating pustules 0.31 to 0.5

Table 1. Reaction of the great northern common bean cultivar Coyne and other bean cultivars and lines with known rust resistance genes to eight races of the common bean rust pathogen (*Uromyces appendiculatus*) used to identify rust resistance genes.

Common bean cultivar or line	Resistance gene	Reactions to selected races of bean rust pathogen <sup>†</sup>							
		41	44	47	49	53	67	73	108
		1-6							
'Coyne'	(Ur-3 & Ur-6)	2,2+/5	2,2+/5	2+,2/5	4,5,6/5	2,2+/5	4,5/6	f2/4	2/3
'Aurora'	(Ur-3)	2/4	4,5/4	4,5/4	4,5/5	2,2+/5	4,5/5	5,4/4	2/2
'Golden Gate Wax'	(Ur-6)	2/2	2,2+/5	2+,2++/5	4,5/5	4,5/4	4,5/3	2/2	5/2
Other checks									
'Pinto 114'	(none)	4,5,6/5	4,5,6/4	4,5/5	4,5,6/4	4,5,6/5	4,5/4	4,5/4	4,5/5
'Early Gallatin'	(Ur-4)	4/5	2++/4	4/4	2/4	4/5	4/5	2+,2/4	2/3
PI 190078	(Ur-11)	f2/2	f2/2	f2/2	f2/2	f2,2/3	f2/3	f2/3	5,4/3
BelMiNeb-RMR-11	(Ur-3,-4,-6,-11)	2/4	f2/2	f2,2/3	f2,2/3	2/4	3,f2/4	2/2	2/2

<sup>†1 =</sup> immune, no visible symptoms; 2 = highly resistant, necrotic spots (NS) <0.3 mm in diameter; 2<sup>++</sup> = NS between 0.3 and 1 mm in diameter; 2<sup>++</sup> = NS between 1 and 3 mm in diameter; 2<sup>++</sup> = NS >0.3 mm in diameter; f2 = faint chlorotic spots without sporulation; 3 = resistant, uredinia (sporulating spores) present, <0.3 mm in diameter; 4 = moderately resistant, uredinia 0.3 – 0.5 mm in diameter; 5 = moderately susceptible, uredinia 0.5 – 0.8 mm in diameter; 6 = susceptible, uredinia >0.8 mm in diameter. Number(s) before the backslash corresponds to the type of reaction. Number after backslash corresponds to the intensity of the reaction. High numbers (e.g., 5) indicate many pustules or necrotic spots were present; lower numbers (e.g., 2) indicate few.

mm in diameter, 5 = large sporulating pustules 5.1 to 0.8 mm in diameter, and 6 = very large sporulating pustules larger than 0.81 mm in diameter. It is not unusual to have two or more pustule types on the same leaf. When several pustule grades were present, they were recorded in order of predominance, with the most prevalent type listed first.

In addition to phenotypic selection for CBB and rust resistance, Coyne was screened using sequence characterized amplified region (SCAR) DNA markers to identify genes and quantitative trait locus (QTL) markers linked to CBB and bean rust resistance. A single DNA sample was extracted from an individual leaf disk punched from a newly opened trifoliolate leaf using a modified (Afanador et al., 1993) mini-prep procedure, and assayed for the presence of CBB and rust resistance-linked markers. The polymerase chain reaction (PCR) protocol for amplification of the SCAR marker SAP 6 linked with a major CBB resistance QTL derived from Montana No. 5 was as described by Miklas et al. (2000): 34 cycles of 10 s at 94°C, 40 s at 55°C, and 120 s at 72°C; followed by one cycle of 5 min at 72°C. The PCR protocol for the SCAR marker SK 14 linked with a major bean common rust resistance QTL was described by Miklas et al. (2002): 34 cycles of 10 s at 94°C, 40 s at 63°C, and 120 s at 72°C; followed by one cycle of 5 min at 72°C. The band was visualized on 1.4% (w/v) agarose gels containing ethidium bromide (0.5 µg mL<sup>-1</sup>) for 5 h at 3 V cm<sup>-1</sup> constant voltage.

The NL3 strain of BCMNV was used to screen bean lines for resistance to both BCMV and BCMNV. At the primary leaf stage, plants were mechanically inoculated with infected source tissue macerated in 50 mM potassium phosphate (pH 7.4) containing 10 mM sodium sulfite. Disease reaction was evaluated 14 d after inoculation.

### **Characteristics**

The CBB reaction of Coyne was consistent across 3 yr at the WCREC in North Platte, NE, where field disease ratings of 3.6, 3.5, and 4.4 were recorded in 2005, 2006, and 2007, respectively (Table 1). This was similar to the reaction of Marquis (2.5 and 4.1 in 2006 and 2007, respectively) and Beryl-R (1.7, 4.5, and 5.4 in 2005, 2006, and 2007, respectively) (Table 1). Conversely, the susceptible great northern Orion scored 6.0, 8.5, and 9.0 in 2005, 2006, and 2007, respectively (Table 2). Across years, Neb#1-Sel 27 showed the lowest CBB reaction followed by USPT-CBB-1, ABCP-8 (Mutlu et al., 2005), and ABC-Weihing (Mutlu et al., 2008) (Table 2). The difference in CBB reaction among sources of CBB resistance and Coyne was not significant. Coyne carries the SAP6 SCAR marker (Miklas et al., 2000) linked with the major QTL for CBB resistance derived from Montana No. 5 (via Weihing).

Inoculation of Coyne with races 41, 44, 47, 49, 53, 67, 73, and 108 under greenhouse conditions at Beltsville, MD, from 2005 to 2007 provided evidence for the presence of *Ur-3* and *Ur-6* genes for resistance to common bean rust (Table 1). Coyne also carries the SK14 SCAR marker (Miklas et al., 2002) linked to the QTL for *Ur-3* common bean rust resistance.

Table 2. Reaction of current dry bean cultivars grown in western Nebraska and sources of common bacterial blight (CBB) resistance to Nebraskan Xanhomonas campestris pv. phaseoli strains SC-4A and LB-2 at the West Central Regional and Extension Center, North Platte, NE, from 2005 to 2007.

	CBB reaction						
Genotypes	2005	2006	2007	Means†			
	1_9 <sup>‡</sup>						
Great Northern cultivars							
Beryl-R	1.7	4.5	5.4	3.9			
Coyne	3.2	3.5	4.4	3.7			
Marquis	-	2.5	4.1	3.3			
Orion	6.0	8.5	9.0	7.8			
Sources of CBB resistance							
ABCP-8	1.5	2.0	3.5	2.3			
ABC-Weihing	3.5	2.0	2.5	2.7			
Neb#1-Sel.27	2.0	1.5	2.0	1.8			
USDK-CBB-15	1.5	3.0	6.0	3.5			
USPT-CBB-1	1.5	2.0	3.0	2.2			
XAN 159	3.9	2.0	5.0	3.6			
LSD <sub>0.05</sub>		<del></del> 4.0 <del></del>					

<sup>†</sup>Genotype means were calculated across years.

Based on top necrosis reaction to NL-3 strain of BCMNV, it was determined that Coyne carries the single dominant hypersensitive I gene that provides resistance to all nonnecrotic strains of BCMV but that it is hypersensitive to the temperature-dependent necrosis-inducing strains of BCMV and to the temperature-independent necrosis inducing strains of BCMNV. Coyne has the same partial avoidance of white mold as Weihing due to its semi-upright and porous plant architecture in field nurseries.

Coyne exhibits a semi-upright Type 2b indeterminate growth habit. Plants averaged 57 cm in height during 2007 with excellent lodging resistance. Coyne has white flowers and blooms 44 d after planting. Coyne is a midseason bean maturing 91 d after planting (range 90–92 d). The seed coat of Coyne is bright white.

Average seed size for Coyne (36.7 g 100 seeds<sup>-1</sup>) was slightly larger than Orion (34.3 g 100 seeds<sup>-1</sup>) and larger than Beryl-R (28.5 g 100 seeds<sup>-1</sup>) in the intermediate, advanced, and growers' field trials grown from 2005 to 2007. For the same trials, Coyne (3147 kg ha<sup>-1</sup>) had a slightly lower yield than Marquis (3204 kg ha<sup>-1</sup>) (Table 3). Although Coyne had lower yields than Orion and Matterhorn in the WRBT and MRPN, respectively, the difference in yields was not significant (Table 4).

# **Availability**

Husker Genetics Foundation Seed Program, University of Nebraska–Lincoln, will maintain a small quantity of Breeder seed of Coyne. An application will be filed for cultivar protection under Title V of the U.S. Plant Variety Protection Act. A small quantity of seed of Coyne is available

<sup>&</sup>lt;sup>‡</sup>1 = immune and 9 = very susceptible. Reactions from 1 to 4 were considered resistant and from 5 to 9 susceptible (van Schoonhoven and Pastor-Corrales, 1987).

Table 3. Overall yield of Coyne compared to the standard great northern cultivars grown from 2005 to 2007 in onstation and on-farm trials.

Cultivar		Yield			100-seed weight	
	On-station <sup>†</sup>	On-farm <sup>‡</sup>	Average	On-station	On-farm	Average
		kg ha <sup>-1</sup>			g	
Beryl-R	2966	-	2966	28.5	_	28.5
Coyne	3139	3155	3147	36.1	37.2	36.7
Gemini	2959	_	2959	26.1	_	26.1
Orion	3064	-	3064	34.3	_	34.3
Marquis	3166	3242	3204	26.5	31.3	28.9
LSD <sub>0.05</sub>	974	750		5.5	5.8	

<sup>&</sup>lt;sup>†</sup>Nine yield trials grown at Mitchell and Scottsbluff, NE, from 2005 to 2007.

Table 4. Overall yield of Coyne compared to the standard great northern cultivars in the Western Regional Bean Trial (WRBT) and Mid-West Regional Performance Nursery (MRPN) in 2007.

Cultium	Yie	eld	100-seed weight		
Cultivar	WRBT†	MRPN <sup>‡</sup>	WRBT	MRPN	
	—— kg l	na <sup>-1</sup> ——	g		
Coyne	2211	2268	35.6	35.3	
Orion	2938	-	35.5	-	
Matterhorn	_	2538	-	34.7	
LSD <sub>0.05</sub>	1079	1296	5.4	4.3	

<sup>†</sup>WRBT, four environments in 2007, in Colorado, Idaho, Nebraska, and Washington.

for research purposes from the corresponding author for the first five years. University of Nebraska–Lincoln approval will be required to market a new cultivar that is 25% or more Coyne. This will include a negotiated license agreement and fee structure. We ask that appropriate recognition of source be given when this cultivar contributes to the development of a new cultivar.

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<sup>\*</sup>Mother and Baby trials grown in four growers' fields located at Morrill and Scotts Bluff, NE, counties during 2007.

<sup>&</sup>lt;sup>‡</sup>MRPN, four environments in 2007, in Colorado, Michigan, Nebraska, and North Dakota.