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Cultivar

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## Registration of 'Clara CL' Wheat

Terry J. Martin, Guorong Zhang,\* Allan K. Fritz, Rebecca Miller, and Ming-Shun Chen

### ABSTRACT

'Clara CL' (Reg. No. CV-XX; PI 665948) hard white winter wheat (*Triticum aestivum* L.) cultivar was developed at the Agricultural Research Center–Hays, Kansas State University and released by the Kansas Agricultural Experiment Station in 2011. Clara CL carries one Clearfield gene and has tolerance to imazamox herbicide. Clara CL was selected from a single cross of KS03HW154/KS03HW1 using a modified bulk breeding method. Both parental lines are unreleased hard white winter experimental lines from Kansas State University. The objective of the cross was to develop a hard white winter wheat variety with herbicide resistance and adaptation to the semiarid area in western Kansas. Clara CL was released because of its tolerance to imazamox herbicide, high grain yield potential under nonirrigated conditions in western Kansas, preharvest sprouting tolerance, and good disease and insect resistance.

T.J. Martin and G. Zhang, Agricultural Research Center–Hays, Kansas State Univ., Hays, KS 67601; A.K. Fritz, Dep. of Agronomy, Kansas State Univ., Manhattan, KS 66506; R. Miller, Dep. of Grain Science and Industry, Kansas State Univ., Manhattan, KS 66506; M.-S. Chen, USDA–ARS, Hard Winter Wheat Genetics Research Unit, Kansas State Univ., Manhattan, KS 66506. Registration by CSSA. Received 23 July 2013. \*Corresponding author (gzhang@ksu.edu).

Abbreviations: ARCH, Agricultural Research Center–Hays; AYN, advanced yield nursery; HWWW, hard white winter wheat; KIN, Kansas Intra-State Nursery; KSU, Kansas State University; PPO, polyphenol oxidase; PYN, preliminary yield nursery; SBMV, *Soil-borne mosaic virus*; SRPN, Southern Regional Performance Nursery; WQC, Wheat Quality Council; WSMV, *Wheat streak mosaic virus*.

'Clara CL' (Reg. No. CV-XX; PI 665948) is a hard white winter wheat (*Triticum aestivum* L.) (HWWW) variety developed at the Agricultural Research Center–Hays (ARCH), Kansas State University (KSU), and released by the Kansas Agricultural Experiment Station in 2011. Clara CL was selected from the single cross KS03HW154/KS03HW1. Both parental lines, KS03HW154 and KS03HW1, are unreleased hard white winter experimental lines developed at ARCH. KS03HW154 is a sister line to 'RonL' (PI 648020) with the pedigree 'Trego' (PI 612576; Martin et al., 2001)/CO960293. KS03HW1 was derived from the four-way cross FS2/KS97HW150//KS97HW349/3/Trego. FS2 is a proprietary wheat germplasm developed by BASF Corporation (formerly American Cyanamid) through induced mutagenesis, and it carries *Als1* locus (D genome) conferring tolerance to the imidazolinone class of herbicides (also known as Beyond herbicide) (Newhouse et al., 1992). Clara CL is an F<sub>6</sub>–derived line, which was tested as experimental line number KS08HW35-1. Clara CL was released because of its imazamox herbicide tolerance, high grain yield potential in the dryland production system in western Kansas, preharvest sprouting tolerance, and good resistance to the major diseases in western Kansas. CL in the name stands for Clearfield, the trademark for Beyond herbicide tolerance.

### METHODS

Clara CL was developed using a modified bulk breeding method. All the early generation populations and line development was conducted at Hays, KS. The cross

(KS03HW154/KS03HW1) was made in the greenhouse in fall 2002. The F<sub>1</sub> seed was planted in the greenhouse in spring 2003 and bulk harvested. The F<sub>2</sub> seed was planted in a field plot under nonirrigated conditions in fall 2003. At the four-leaf stage, the F<sub>2</sub> field plot was sprayed with imazamox herbicide [2-(4,5-dihydro-4-methyl-4-(1-methylethyl)-5-oxo-1h-imidazol-2-yl)-5-(methoxymethyl)-3-pyridinecarboxylic acid] at a rate of 105 g a.i. ha<sup>-1</sup>. The herbicide was applied with 0.25% (v:v) nonionic surfactant and 1.5% (v:v) urea-ammonium nitrate fertilizer (UAN 28). Plants showing tolerance or resistance to the herbicide, stripe rust (caused by *Puccinia striiformis* Westend.), and leaf rust (caused by *Puccinia triticina* Ericks.) were selected and bulk harvested. A subsample of the F<sub>3</sub> seed was planted in the field under nonirrigated conditions in fall 2004. In 2005, approximately 150 spikes were selected from the F<sub>3</sub> population based on plant height, lodging resistance, and head size. These spikes were threshed individually and planted in an F<sub>4</sub> head-row nursery under nonirrigated conditions in fall 2005. Head rows were subjected to selection based on winter injury, plant height, maturity, lodging, grain shattering, stripe and leaf rust resistance, test weight, and grain yield. One head row (row 100 in pass 27), which later became Clara CL, was selected for a yield test in the first preliminary yield nursery (PYN1). Six head-selections were made from that row and the remainder of the row was bulk harvested. The head-selections (reselections) were threshed individually and planted as head rows in the reselection nursery under irrigated conditions. The bulk seed (F<sub>3:5</sub>) from that row was planted in the PYN1 as entry number 5067 in fall 2006.

Entry 5067 performed well in the PYN1 and one reselection row (5067-5) was selected for further testing in 2008 in the second preliminary yield nursery (PYN2). Six head-selections were made from 5067-5, and the remainder of the row was bulk harvested. The head-selections were individually threshed and planted as head rows in the reselection nursery. The bulk seed (F<sub>4:6</sub>) was used for the PYN2 test and one seed-increase plot. The reselection was tested in the 2008 PYN2 as entry number 6035. Entry 6035 performed well and was selected for further testing in the advanced yield nursery (AYN) in 2009. Two reselections (-1, -2) of entry 6035 were selected for advancement. Six head-selections were made from each reselection row, and the remainder of each row was bulk harvested for seed increase (two plots for each reselection). The head-selections were planted in the reselection nursery. The bulk seed (F<sub>4:7</sub>) from the seed increase plot of entry 6035 was grown in 2009 AYN under the experimental line number KS08HW35.

In 2010, both reselections of KS08HW35 were advanced into Kansas Intra-State Nursery (KIN) for further testing under the experimental line names KS08HW35-1 and KS08HW35-2. Three reselection rows from each were selected for advancement. Again, six head-selections were made from each reselection row and the remainder of each row was bulk harvested. The head-selections were planted in the reselection nursery, and bulk seed (F<sub>6:8</sub>) from each reselection row was planted in two seed increase plots in 2010. On the basis of the 2010 testing results, KS08HW35-1 was selected for further testing in the 2011 KIN and also in the Southern Regional Performance Nursery (SRPN). KS08HW35-1 was grown on 0.04 ha together with check cultivars Danby (PI 648010) and Tiger (PI 661995; Martin et al., 2013) to obtain grain samples for quality evaluation by the Wheat Quality Council (WQC). In addition, 200 head rows of KS08HW35-1 were grown for breeder seed and 1.6 ha were planted for foundation seed. In July 2011, KS08HW35-1 was released under the name of Clara CL.

Both PYN1 and PYN2 were unreplicated yield trials with an augmented design. PYN1 was grown at one location (Hays), whereas PYN2 was grown at three locations (Hays, Colby, and

Garden City) in western Kansas. The AYN, KIN, and SRPN were replicated yield trials with a randomized complete block design (RCBD). The AYN and KIN are statewide yield trials, and the SRPN is a regional yield trial. All yield trials except two of the 2011 KIN trials were conducted under nonirrigated conditions. Entries in yield trials and their reselections were screened for yield, test weight, winter injury, maturity, grain shattering, lodging, polyphenol oxidase (PPO) activity, herbicide tolerance, and pest resistance, which includes stripe rust, leaf rust, *Wheat streak mosaic virus* (WSMV), *Soil-borne mosaic virus* (SBMV), and Hessian fly [*Mayetiola destructor* (Say)]. Entries to be advanced were further tested for kernel characteristics (size, weight, color, and protein content), milling quality, alkaline noodle color stability, and bread baking quality in the KSU Wheat Quality Laboratory in Manhattan, KS. In 2011, the seed and end-use quality of Clara CL was also tested by the WQC. Seed purification of Clara CL started in 2007 by visual identification and manual removal of off-types from seed increase plots. Additionally, the field growing for breeder seed and foundation seed was treated with imazamox herbicide for purification.

To meet the qualifications to be a Clearfield wheat variety, Clara CL was tested in six Clearfield Wheat Qualification trials in Kansas and Colorado in 2010 and 2011. Trials were conducted using a factorial arrangement with variety and herbicide rate (no herbicide, 1× label rate, and 2× label rate) as two factors. Each trial was laid out in a RCBD with three replicates.

Data were analyzed using SAS 9.1 (SAS Institute, Cary, NC). Grain yield and agronomic traits from AYN, KIN, and SRPN trials were subjected to analysis of variance across locations within years, and combined analysis across year-locations. For KIN trials, data from western Kansas locations and eastern Kansas locations were analyzed separately; only results from western Kansas are reported in this paper. The combined analysis only included the common entries across year-locations. A mixed model was used in the analysis with genotypes as fixed effects, and years, or year-locations, and replications as random effects. An F-protected LSD test ( $\alpha = 0.05$ ) was used to compare the least square means for genotype effects. Clearfield Wheat Qualification trials were analyzed separately for each trial. In the KIN trial, grain from the four replications was bulked for seed and end-use quality evaluation. Therefore, analysis for the KIN quality data was conducted using location × genotype as experimental error. The WQC quality data was analyzed with the Student's paired *t* test because the grain sample was tested by multiple cooperators.

## CHARACTERISTICS

### Agronomic and Botanical Description

Clara CL is an awned, white chaffed, and white-seeded wheat variety. It has a medium late maturity (13.8 d to heading from 1 May; Table 1), which is similar to that of Danby and Tiger, and 1 to 3 d later ( $p < 0.05$ ) than that of 'Hatcher' (PI 638512; Haley et al., 2005) and 'Armour'. Clara CL is medium tall (87.8 cm), which is similar to that of Danby and Hatcher (87.4 cm), and 9 to 10 cm taller ( $p < 0.05$ ) than that of Tiger (78.7 cm) and Armour (77.7 cm). Clara CL has good preharvest sprouting tolerance (17.8% sprouted on the eighth day of the germination test), which is not significantly different from that of Hatcher (22.6%), Armour (16.2%), and Danby (12.3%). No objective data are available for the straw strength and winter hardiness of Clara CL,

but field observations in Kansas show that it has good winter hardiness and moderate straw strength.

Clara CL has a prostrate juvenile growth habit. Its coleoptile, stem, and auricle lack anthocyanin. The plants are green at the boot stage and have a waxy bloom. The flag leaves are erect, not twisted, and waxy while stems are hollow and hairless. Clara CL has erect peduncles with a length of about 25 cm. The heads are tapered, mid-dense, and inclined at maturity. Its glumes lack pubescence and are medium sized with square shoulders and medium-width acuminate beaks. The kernels are ovate and have medium sized germs, angular shaped cheeks, medium wide and medium deep creases, and medium long and noncollared brushes.

Clara CL has been observed for over six generations (F<sub>4</sub> to F<sub>9</sub>) from 2006 to 2011. It has been uniform and stable during the last two generations. Clara CL remains essentially unchanged in its primary and distinctive characteristics following sexual reproduction. Observed variants include slightly taller plants (<0.1%), plants with brown glumes (<0.1%), and plants with red kernels (<0.5%). However, these variants are commercially acceptable.

### Field Performance

In the 2010 KIN, Clara CL was tested in seven dryland trials across western Kansas and six of them were harvested. In the 2011 KIN, Clara CL was tested in five trials (four dryland and one irrigated) in western Kansas and all of them were harvested. In both years, Clara CL was the highest yielding line among the lines tested (data not shown). In the combined analysis across the ten dryland trials in western Kansas in 2 yr, the average yield of Clara CL (4839 kg ha<sup>-1</sup>) was significantly ( $p < 0.05$ ) higher than those of all the four check cultivars Hatcher (4367 kg ha<sup>-1</sup>), Armour (4320 kg ha<sup>-1</sup>), Danby (4171 kg ha<sup>-1</sup>), and Tiger (4085 kg ha<sup>-1</sup>) (Table 1). Additionally, Clara CL had a high test weight (817 kg m<sup>-3</sup>), which was similar to that of Danby (818 kg m<sup>-3</sup>), but significantly higher than those of other three check cultivars Hatcher (800 kg m<sup>-3</sup>), Tiger (797 kg m<sup>-3</sup>), and Armour (786 kg m<sup>-3</sup>). Clara CL was only tested in one irrigated trial in western Kansas within the 2 yr. However, Clara CL was the highest yielding line in that trial (data not shown).

In the 2011 SRPN, Clara CL was tested in the 26 trials across seven states of New Mexico, Texas, Oklahoma, Kansas, Colorado, Nebraska, South Dakota, Missouri, and Montana. The average yield of Clara CL was 3266 kg ha<sup>-1</sup>, which was slightly lower ( $p > 0.05$ ) than the trial mean (3382 kg ha<sup>-1</sup>) (SRPN data is available at <http://www.ars.usda.gov/Research/docs.htm?docid=11932>). However, Clara CL was the fourth highest-yielding line across the three Kansas locations with an average yield of 3682 kg ha<sup>-1</sup>.

In two of the six Clearfield Wheat Qualification trials, Clara CL showed some crop injury after the herbicide application. However, the degree of injury on Clara CL was comparable to that of the Clearfield check cultivar Above (PI 631449; Haley et al., 2003) (data not shown). In addition, herbicide application did not cause adverse effects on flowering date, test weight, and grain yield in all the six trials (data not shown). Therefore, Clara CL meets the qualification standards as a Clearfield wheat variety.

### Seed and End-Use Quality

Kernel characteristics, milling, bread baking, and noodle quality of Clara CL and check cultivars Danby, Tiger, Hatcher, and Armour were evaluated using American Association of Cereal Chemists International approved methods (AACCI, 2000) by the KSU Wheat Quality Laboratory and the WQC. In 2010, grain samples from two locations, each a composite of four replications, were evaluated by the KSU Wheat Quality Laboratory. The thousand kernel weight of Clara CL (29.4 g) was comparable to those of all the checks except Danby (24.4 g) (Table 2). The kernel hardness index of Clara CL (82.5) was similar to that of Danby (81.3), and higher ( $p < 0.05$ ) than those of the other three checks. The grain protein of Clara CL (13.8%) was similar to that of Armour (13.4%), and higher ( $p < 0.05$ ) than those of the other checks. Clara CL has overall good milling and baking quality. The flour yield (71.0%) and flour ash content (0.39%) of Clara CL was not significantly ( $p = 0.05$ ) different from those of all the check cultivars except Armour. In the baking test, Clara CL was not significantly ( $p = 0.05$ ) different from all check cultivars in mixing time, water absorption, and loaf volume. Noodle color stability was tested for the white varieties (Clara CL, Danby, and Tiger). The brightness (L value) change over 24 h for the noodle made with Clara CL was similar to that of Danby and greater ( $p < 0.05$ ) than that of Tiger, which may be explained by the medium high PPO level in both Clara and Danby (data not shown). In 2011, Clara CL and check cultivars Danby and Tiger were further tested for milling, baking, and noodle qualities by the WQC. The results showed that the overall baking quality of Clara CL was similar to that of Danby but inferior to that of Tiger (WQC data available at <http://www.wheatqualitycouncil.org/>). The PPO concentration in Clara CL was 0.671 (spectrophotometric absorbance at 475 nm), similar to that of Danby (0.644) and higher than that of Tiger (0.224). The noodle color stability of Clara CL was less than Tiger, which makes it less desirable for alkaline noodle production. However, the WQC test showed that Clara CL was good for making white salted noodles because of its bright color, good chewiness, and high water uptake.

### Disease and Insect Resistance

Clara CL was characterized for disease and insect resistance in the Kansas trials and through cooperative evaluations in the SRPN (SRPN data available at <http://www.ars.usda.gov/Research/docs.htm?docid=11932>). Clara CL was developed for western Kansas, where the major disease and insects are WSMV, leaf rust, stripe rust, SBMV, and Hessian fly. Clara CL showed good WSMV resistance in the greenhouse tests in Hays, KS, which is similar to that of RonL. Clara CL also showed SBMV resistance in naturally infested field trials in Manhattan, KS. Clara CL showed moderate resistance to the Great Plain biotype of Hessian fly in seedling evaluations under greenhouse conditions in Manhattan, KS. In the 2010 KIN at Hays, KS, Clara CL showed resistant reactions to leaf rust while check cultivars Danby and Hatcher were found to be susceptible with severity ranging from 10 to 30%. In the same nursery, Clara CL showed moderate resistance to stripe rust while check cultivar Danby was rated as susceptible. In the 2011 SRPN, Clara CL was further tested against various pathogens through cooperative evaluations. Leaf rust, stripe rust, and stem rust (caused by *Puccinia graminis* Pers.:Pers f. sp. *tritici* Eriks. & E. Henn.) were evaluated under both greenhouse and field conditions. Clara CL showed resistance to leaf rust in the field trial in St. Paul, MN. Seedling reaction to leaf rust was tested in the greenhouse at the USDA Cereal Disease Laboratory in St. Paul, MN, and Clara CL showed resistance to the eight isolates tested,

including 10US1–1MLDS, MFPS, TDBG, TMGJ, MHDS, TNRJ, 10US3–1TFBJ, and KFBJ. Clara CL was evaluated at both seedling and adult plant stages with various stripe rust isolates in the greenhouse in Pullman, WA. Clara CL was susceptible to all the isolates (PST-37, PST-45, PST-100, PST-114, PST-127) tested in the seedling stage, but it showed resistance (infection type 2 to 3, on a scale of 0–9 where 0 = most resistant, and 9 = most susceptible) to isolates PST-100, PST-114, and PST-127 tested during the adult plant tests, indicating it has high-temperature, adult-plant resistance. In eight field tests conducted in the states of Kansas, North Carolina, Washington, and Montana and in Njoro, Kenya, Clara CL showed various reactions with infection types ranging from 3 to 8. In the greenhouse seedling tests for stem rust resistance, Clara CL showed resistance to stem rust races QFCSC, MCCFC, RCRSC, TPMKC, SCCSC, and TRTTF. In a field trial in St. Paul, MN, Clara CL showed moderate resistance to stem rust with severity of 30%. However, Clara CL was susceptible to the stem rust in a Kenya field trial, indicating its susceptibility to the Ug99 race. Cooperative evaluations in the 2011 SRPN also showed that Clara CL was moderately resistant to SBMV and Hessian fly, but susceptible to the Russian aphid (*Diuraphis noxia*) biotype 1 and greenbug (*Schizaphis graminum*).

## SEED AVAILABILITY

Clara CL contains a patented herbicide tolerance trait owned by BASF Corporation that confers tolerance to imidazolinone herbicides, such as imazamox. Any use of Clara CL requires a Material Transfer Agreement (for research use only) or a Commercial License to the trait, as well as permission from the originator (KSU). The KSU ARCH will maintain the breeder seed of Clara CL. Multiplication and distribution rights of other classes of certified seed have been transferred from KSU to the Kansas Wheat Alliance (1990 Kimball Ave., Manhattan, KS 66502). Clara CL has been submitted for US Plant Variety Protection (PVP). Foundation, registered, and certified seed of Clara CL are protected under the PVP Act. A seed sample has been deposited with the National Plant Germplasm System (NPGS), where it will be available for distribution by the NPGS 20 years after the date of publication. For seed requests of Clara CL, contact the corresponding author, who will forward the requests to BASF Corporation. No seed will be distributed without written permission from both BASF Corporation and the originator (KSU).

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**Table 1. Summary of yield and agronomic traits for wheat cultivar Clara CL and check cultivars Hatcher, Armour, Danby, and Tiger grown in Kansas Intra-State Nursery across western Kansas locations† in 2010 and 2011.**

Cultivar	Class‡	Grain yield	Test weight	Plant height	Heading date	Preharvest sprouting
		kg ha <sup>-1</sup>	kg m <sup>-3</sup>	cm	d from 1 May	%
Clara CL	HWWW	4839	817	87.8	13.3	17.8
Hatcher	HRWW	4367	800	87.4	11.9	22.6
Armour	HRWW	4320	786	77.7	10.1	16.2
Danby	HWWW	4171	818	87.4	13.7	12.3
Tiger	HWWW	4085	797	78.7	13.3	100
CV (%)		8.2	0.9	3.0	5.1	28.1
LSD (0.05)		158.5	3.3	2.1	0.5	9.8

† Grain yield and test weight are the average of six dryland locations in 2010 and four dryland locations in 2011. Plant height and heading date are the average of two locations in 2010 and one location in 2011. Preharvest sprouting data is the average of Hays location in 2010 and 2011.

‡ HWWW, hard white winter wheat; HRWW, hard red winter wheat.

**Table 2. Quality parameters† for wheat cultivar Clara CL and check cultivars Danby, Tiger, Hatcher, and Armour grown in the Kansas Intra-State Nursery at Hays, KS, and Garden City, KS in 2010.**

Cultivar	TKW	KHI	KP	FA	FY	MT	WA	LV
	g			%		min	%	cc
Clara CL	29.4	82.5	13.8	0.39	71.0	4.8	66.0	941.5
Danby	24.4	81.3	13.0	0.35	71.0	4.0	64.5	1028.0
Tiger	30.7	70.1	13.1	0.34	70.7	6.3	66.0	956.5
Hatcher	28.8	72.3	13.1	0.36	71.5	5.8	65.0	951.5
Armour	28.5	69.3	13.4	0.32	72.6	4.6	66.5	1032.5
CV (%)	3.8	2.5	1.3	0.7	6.0	21.5	2.1	3.4
LSD (0.05)	3.0	5.1	0.5	0.06	1.4	–‡	–	–

† TKW, thousand kernel weight; KHI, kernel hardness index; KP, kernel protein; FA, flour ash; FY, flour yield; MT, WA, and LV, mixing time, water absorption, and loaf volume in the bread baking test.

‡ –, no significant difference at  $p = 0.05$ .