REGISTRATION

Cultivar

Registration of 'Piranha CL+' soft white winter wheat

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Abstract

Soft white winter (SWW) wheat (*Triticum aestivum* L.) is commonly grown in Washington. In many production areas of the state, winter annual weeds have become prevalent in the fields. With only a few herbicide choices available, growers have used the Clearfield Production System technology as a tool to control grassy weeds. The use of the group 2 (amino acid/acetolactate synthase inhibitors) Beyond herbicide (imazamox) tolerant wheat has allowed growers effective weed control options but has also led to herbicide residual building up in the soil profile. The objective of this research was to develop a two-gene, Beyond-tolerant SWW cultivar with improved resistance to the stripe rust pathogen (caused by *Puccinia striiformis* Westend. f. sp. *tritici* Erikss.), tolerance to snow mold, improved end-use quality, and can maintain high grain yield across production regions and years. 'Piranha CL+' (Reg. no. CV-1214, PI 699241) is a SWW wheat cultivar developed and released in March 2020 by the Agricultural Research Center of Washington State University. Piranha CL+ was tested under the experimental designations MAS11295-1-1-0-2 and WA8305 CL+, assigned through progressive generations of advancement. Piranha CL+ is a two-gene Beyond tolerant semi-dwarf cultivar broadly adapted across the wheat production regions of Washington. Piranha CL+ has improved high-temperature adult-plant resistance to the stripe rust pathogen over the most commonly grown Clearfield cultivars, is tolerant to snow mold, has intermediate plant height with mid-season maturity, and has high grain volume weight. This cultivar has maintained high grain yield across multiple years of testing in Washington. The end-use quality properties of Piranha CL+ meet expectations of both domestic and export market standards.

Abbreviations: BASF, Baden Aniline and Soda Factory; IT, infection type; PNW, Pacific Northwest; SRC, solvent retention capacity; SWW, soft white winter.

Plain Language Summary

Soft white winter (SWW) wheat is commonly grown in Washington and surrounding states. In many production areas, winter annual weeds have become prevalent. The objective of this research was to develop a two-gene Beyond tolerant SWW cultivar, allowing herbicide application to kill weeds but allow the wheat to survive, and combine this trait with other agronomic and disease resistance traits needed for production in Washington. 'Piranha CL+' was developed and released by the Agricultural Research Center of Washington State University. Piranha CL+ has improved high-temperature adult-plant resistance to the stripe rust pathogen over the most commonly grown Clearfield cultivars, is tolerant to snow mold, has intermediate plant height with mid-season maturity, has high grain volume weight, and maintains high grain yield across multiple years of testing in Washington. The end-use quality properties of Piranha CL+ meet expectations of both domestic and export markets.

1 | INTRODUCTION

The winter wheat (*Triticum aestivum* L.) production areas of the state of Washington are typically characterized by the amount of annual precipitation occurring in each region (<400 mm rainfall, >400 mm rainfall, or irrigated). In the non-irrigated wheat producing regions, the amount of annual rainfall typically dictates the cropping systems which are employed. In the <400 mm rainfall areas, summer fallow and no-till fallow rotations are often used, with a winter wheat crop in the first year followed by a fallow year to conserve moisture for the subsequent crop. In the >400 mm rainfall areas, annual cropping with 2- to 4-year rotations are employed, often using minimum tillage and no-tillage practices to reduce soil erosion and improve soil health. These cropping systems, even with fallow or other crops rotated between winter wheat crops, has still led to an increase of winter annual weeds like downy brome (*Bromus tectorum* L.) and jointed goat grass (*Aegilops cylindrica* Host.), which have been difficult to control. The development and deployment of soft white winter (SWW) wheat cultivars with the Clearfield Production System technology has been an important tool for wheat producers to manage these weeds. This constant use of technology allows growers to spray the group 2 herbicide Beyond (imazamox; amino acid/acetolactate synthase inhibitors) on tolerant wheat but can led to a build-up of herbicide chemical residual in the soil (BASF, 2021).

The Pacific Northwest (PNW) of the United States is prone to many disease pressures during wheat production. Stripe rust (caused by *Puccinia striiformis* Westend. f. sp. *tritici* Erikss.) is present in wheat fields almost every year, and while fungicides can be sprayed to control the disease at an extra cost, yield losses are still often seen. The most economic method to control the disease is to plant resistant cultivars due to reduced fungicide applications. Other diseases, such as snow mold (caused by *Microdochium nivale*, *Typhula idahoensis*, *T. ishikariensis*, and *T. incarnata*), have no economical fungicides to control them, and thus genetic resistance/tolerance to the disease is the only option for their control. Depending on location within the state, these diseases can become very severe. Developing cultivars with resistance/tolerance to these pests is essential to ensure a broad adaptation of production.

'Piranha CL+' (Reg. no. CV-1214, PI 699241), a SWW wheat, was developed and released in March 2020 by the Agricultural Research Center of Washington State University. Piranha CL+ was released as a replacement for 'Curiosity CL+' (PI 675007; Gill et al., 2020b) and 'Mela CL+' (PI 675008; Gill et al., 2020a) in rainfed wheat production systems in Washington State which often see shorter crop production cycles and areas where grassy weeds are a major problem. This cultivar has broad adaptation across the state of Washington and has maintained high grain yield across drastically differing climactic events. The release of Piranha CL+ is based on (i) the presence of two mutations for enhanced tolerance to Beyond, (ii) high-temperature adult-plant resistance to the stripe rust pathogen, (iii) tolerance to snow mold, (iv) improved end-use quality, and (v) high grain yield across target production regions.

2 | METHODS

2.1 | Breeding design

Piranha CL+, tested under the experimental designations MAS11295-1-1-0-2 and WA8305 CL+, was derived from an $F_{3:4}$ head row selection from the cross OR2070011/Curiosity CL+. OR2070011 is an experimental soft white winter wheat line from Oregon State University that was not

commercialized. Curiosity CL+ is a line released from Washington State University in 2013 with the pedigree 'Eltan'*3/CL0618. Eltan (PI 536994; Peterson et al., 1991) is a SWW wheat cultivar grown in the state of Washington for multiple years and known for its tolerance to low rainfall conditions and snow mold. 'CL0618' is an Australian hard red spring two-gene mutant line that is a Baden Aniline and Soda Factory (BASF)-approved two-gene donor parent. This line carries a mutant allele at the *Als1* locus (D genome) and the *Als2* locus (B genome), which confers tolerance to imazamox, and is approved for use in Clearfield Production Systems (BASF, 2021). OR2070011 is an experimental breeding line from Oregon State University with the pedigree 'Tubbs' (PI 629114)/'Bitterroot' (PI 655042). Tubbs is a line developed and released by Oregon State University in 2002 and is known for its high agronomic characteristics in Oregon. Bitterroot (PVP 200800411) is a line developed and released by the University of Idaho in 2008 and is known for its high grain yield under Northern Idaho production, along with very good end-use quality attributes. The cross for this line was completed in the Plant Growth Facility (greenhouse) in Pullman, WA, in 2011.

Upon completion of the cross, the F_1 seed was harvested, and replanted into four 15-cm pots to establish an F_2 population (designated MAS11295) in 2012. Two weeks after planting, F_1 seedlings were placed in a growth chamber (Conviron), set at 4°C and an 8-h daylength photoperiod, for 8 weeks to satisfy vernalization requirements. After vernalization, F_1 plants were placed in a greenhouse bay set at 22–24°C and a 16-h daylength photoperiod. At maturity, F_1 plants were harvested, and F_2 seed was bulked (52 grams). From this F_2 bulked seed, 40 g of seed was planted in a 1.5- by 4.5-m plot in the fall of 2012. In the spring of 2013, when the plants were at the early tillering stage (Feekes 3; Large, 1954) seedlings were sprayed with Beyond herbicide (imazamox [2-(4,5-dihydro-4-methyl-4-(1-methylethyl)-5-oxo-1*H*-imidazol-2-yl)-5-(methoxymethyl)-3-pyridinecarboxylic acid]) at the rate of 105 g ai/ha^{-1}, with 2.0% (v/v) methylated seed oil and 5.0% (v/v) urea ammonium nitrate. Herbicide was applied using a CO_2 backpack sprayer with TeeJet 8002E flat-fan nozzles (TeeJet Spraying Systems) and set to deliver 112l ha^{-1} at 290 kPa at a walking speed of 4.5 kph. This method of application was used for all field trials and is a higher rate than the label recommendation, allowing confirmation of tolerance to the herbicide (BASF, 2021). Four weeks after spraying, plants were evaluated for visual injury, and those which appeared not to be injured were tagged for further evaluation. After further selection for heading date, plant height, and disease resistance, 20 plants remained and were left to grow until maturity, and one head per plant was harvested to generate F_3 seed.

These 20 F_3 selections, along with selections from other populations, were planted in non-replicated 1-m-long rows in the fall of 2013 at the Cook Agronomy Farm, Pullman, WA. The resistant (Curiosity CL+) and susceptible ('Brundage 96'; Zemetra et al., 2003) checks were planted every 40 rows. In the spring of 2014, at the Feekes 3 growth stage, plants were sprayed with Beyond at the rate of 105 g ai ha^{-1}, with 2.0% (v/v) methylated seed oil and 5.0% (v/v) urea ammonium nitrate. The lines were evaluated for tolerance to Beyond herbicide, disease resistance, heading date, and plant height. From this population, two F_3 rows were identified as meeting agronomic standards, and four heads were snapped from each row and eight rows were planted in the fall of 2014, and advanced to a non-replicated $F_{3:4}$ head row field trial in Pullman in 2015. The lines were once again sprayed and evaluated for tolerance to Beyond herbicide (as described previously), disease resistance, heading date, and plant height.

A total of four lines were selected from this population during harvest in the summer of 2015. A subsample of 20 g of these lines was sent to the USDA-ARS Western Wheat Quality Laboratory (WWQL), Pullman, and were analyzed for Single Kernel Characterization System (SKCS) hardness, milling characteristics, and flour properties (Solvent Retention Capacity [SRC] Water). Of these, all four lines were identified having acceptable end-use quality properties, replanted fall 2015, and were advanced to a $F_{3:5}$ non-replicated field trial at Pullman in 2016. This trial and subsequent herbicide tolerance qualification trials were sprayed with Beyond as previously described and per BASF protocols. All trial locations were evaluated for plant height, grain yield (Zürn 150 Combine, Zürn Harvesting GmbH & Co.), grain volume weight, disease resistance, and end-use quality. Using seed generated in the non-replicated field trial, MAS11295-1-1-0-2 was evaluated in both BASF approved replicated spray and replicated field breeding trials for 41 location-years between 2017 and 2022 in all precipitation zones in Washington state. All years of field testing utilized the same data collection strategy with a general α-lattice design (three replications; breeding trials; Mason et al., 2003) or a strip-plot (split-block) design (three replications; spray qualification trials; Steele & Torrie, 1980). Piranha CL+ was evaluated for Beyond herbicide tolerance from 2017 to 2020 in BASF approved field trials using the SWW varieties Curiosity CL+ as the two-gene resistant check and Brundage 96 as the susceptible check. Based on the breeding and spray qualification trial data from 2017 and 2018, MAS11295-1-1-0-2 was selected for testing on a regional basis and assigned the new identification number of WA8305 CL+. In 2019, the line was entered into the Washington State University Extension Uniform Cereal Variety Testing Program. In 2020, it was also entered into the Oregon State University Extension Uniform Cereal Variety Testing Program. Piranha CL+ was tested in these programs in 2021 and 2022.

2.2 | Disease resistance evaluation

In 2019 and 2020, WA8305 CL+ was evaluated for stripe rust resistance in naturally and artificially inoculated field trials by the USDA-ARS Wheat Health, Genetics, and Quality Research Unit. Field screening locations included the Plant Pathology, Palouse Conservation, and Spillman Farms near Pullman, WA, the Lind Dryland Research Station near Lind, WA, the Mount Vernon Research and Extension Center in Mt. Vernon, WA, and a grower's field in Walla Walla, WA, in 2019 and 2020. Greenhouse seedling tests for stripe rust resistance were conducted in 2019 and 2020 under low temperature cycles (diurnal temperature cycle gradually changing from 4°C to 20°C; Chen & Line, 1992) with races PSTv-4, 14, 37, 40, 51, and 198 of the wheat stripe rust pathogen. The selection of these races was determined by virulence and the commonly found races of the previous years. Adult-plant tests were conducted at high temperatures (diurnal temperature cycle gradually changing from 10°C to 30°C; Chen & Line, 1995) with races PSTv-14, 37, and 40. Data were collected for infection type (IT) on a scale of 0 to 9 (Line & Qayoum, 1992; McNeal et al., 1971), and disease severity on a scale of 0% to 100% (Peterson et al., 1948).

Snow mold (caused by *Microdochium nivale*, *Typhula idahoensis*, *T. ishikariensis*, and *T. incarnate*) evaluations were conducted in Waterville and Mansfield, WA, in 2019 to 2022. Single rows, replicated two times, were planted under field conditions with natural inoculation, with a score of 0 indicating highly susceptible plants, and a score of 9 for highly tolerant plants. Tolerance to low pH soils in fields near Rockford, WA, in 2019 and 2020 and was conducted on single rows, replicated three times, in a randomized complete block design. The field location where the testing took place had a soil pH of 5.2 in the top 15 cm, 4.8 in the next 15 cm, and was >6.1 below 30 cm. Observations resulted in ratings which were taken on a 1 to 5 scale, with 1 indicating severely stunted and discolored plants with no tillers, and 5 indicated green, healthy plants of normal growth and multiple tillers, similar to the protocols of Froese and Carter (2016). For both traits, the first rating took place as soon as the field was accessible in the spring, usually at Feekes 2, and a second rating about 1 month afterward, at Feekes 4 (Large, 1954).

WA8305 CL+ was tested for strawbreaker foot rot (caused by *Oculimacula acuformis* and *O. yallundae*) resistance during the 2020 field season in three-replicate inoculated field plots near Pullman as described by Murray and Sheng (2017) using 'Madsen' (PI 511673; Allan et al., 1989) as the resistant control and Eltan as the susceptible control. Disease incidence (0% to 100%) and severity (scale of 0 to 4, where 0 = no lesion and 4 = lesion girdling the stem) were determined by visual observation of stem bases. A disease index (0% to 100%) was calculated by multiplying the percentage of infected stems (disease incidence) by the disease severity of infected stems and dividing by four. WA8305 CL+ was also tested for Cephalosporium stripe tolerance during the 2020 field season in three-replicate inoculated field plots near Pullman as described in Murray and Sheng (2020). In these trials, Eltan was the tolerant check and 'Stephens' (CItr 17596; Kronstad et al., 1978) was the susceptible check. Lines in the trial were given a disease index (0% to 100%) score by multiplying the percentage of infected stems (disease incidence) by the disease severity (scale of 0 to 4, where 4 = symptoms detected in the flag leaf, 3, 2, or 1 = symptoms detected on the respective leaves fellow the flag leaf, and 0 = no visual symptoms) and divided by five.

2.3 | End-use quality testing

Starting in 2016, Piranha CL+ was evaluated for end-use quality, using seed from field trials, by the USDA-ARS Western Wheat Quality Laboratory (WWQL), Pullman, according to the Cereals and Grains Association Approved Methods of Analysis (Cereals & Grains Association, 2021). End-use quality trait targets are recommended by the Pacific Northwest Quality Council, and represent the general values needed to meet domestic and export quality standards. Before milling, samples were tempered to 14% moisture concentration, then milled on a modified Quadramat system (Jeffers & Rubenthaler, 1979). End-use quality traits included grain volume weight (Approved method 55-10), total flour yield (g kg^{-1} by weight of the total products recovered as straight-grade white flour), break flour yield (g kg^{-1} by weight of the total products recovered as flour off the break rolls of the mill), flour ash (Approved Method 08-01), grain protein concentration (Approved method 39-10 adjusted with Dumas combustion method), flour protein concentration (Approved Method 39-11), cookie diameter (Approved Method 10-50), and sponge cake volume (Approved Method 10-91). A derived trait, milling score (Morris et al., 2011), was calculated following the equation below.

$$\left(\left\{ 100 - \left[0.5 \times (16 - \text{temperlevel}) \right] + (80 - \text{flour yield}) + \left[50 \times (\text{flour ash} - 0.30) \right] \right\} \times 1.274 \right) - 21.602$$

Piranha CL+ was evaluated from 2019 to 2022 for low falling number (FN) using an international standard method (ICC 107/1. ISO 3093-2004, Approved Method56-81B) for detecting pre-harvest sprouting and late-maturity α-amylase damage in wheat (Sjoberg et al., 2020). Also known as the Hagberg-Perten Falling Number method, this test determines the enzyme (α-amylase, β amylase, and protease) activity, which confers the breakdown of starch and protein levels

within the wheat kernel. For the PNW area of the United States, a specification of 300 s is currently required by most domestic and export market customers.

2.4 | Genotypic information

Before release, Piranha CL+ was evaluated for the presence of the *Pch1* gene conferring resistance to strawbreaker foot rot. Marker analysis for the *Pch1* gene was conducted using a Kompetitive Allele Specific polymerase chain reaction (KASP) assay with marker, wMAS0000023 developed at the Johns Innes Centre (Wilkinson et al., 2012). The alleles for dwarfing genes *Rht-B1* and *Rht-D1* were evaluated with KASP markers based on the protocols found in Grogan et al. (2016), and for the glutenin subunits at the *Glu-D1* gene (Rasheed et al., 2016). The presence of stripe rust resistance gene *Yr17* was evaluated with the KASP marker referenced in Milus et al. (2015). All KASP assays were performed using PACE Genotyping Master Mix (3CR Bioscience) following the manufacturer's instructions (https://3crbio.com/wp-content/uploads/2020/01/PACE-User-Guide-v1.6.pdf) and endpoint genotyping was conducted from fluorescence using a Lightcycler 480 Instrument II (Roche). Piranha CL+ was confirmed to be homozygous for the mutant alleles at *Als1* and *Als2* conferring tolerance to imazamox using the DNA markers provided by BASF during the F_3 generation after field screening.

2.5 | Seed purification

Breeder seed of Piranha CL+ was produced by head row purification, based on phenotypic uniformity, of 1500 $F_{7:8}$ head rows grown under irrigation in Othello, WA, in 2019. Selected head rows (5% were discarded based on heading date, head type, and plant height) were bulked at harvest, resulting in the production of 3.6 metric tonnes of breeder seed. A 2.8-ha foundation seed increase was planted under irrigation in Moses Lake, WA, in the fall of 2019, with a subsequent 7-ha foundation seed increase planted in 2020.

2.6 | Statistical analyses

Yield trial data generated from 2017 to 2022 were analyzed with either the balanced strip-plot (strip-block) procedure or the general lattice procedure in Genovix version 1.5.2332.1 (Agronomix Software, Inc). Location means from 2017 to 2022 were generated via the general lattice adjusted mean, using genotypes as a fixed effect, and block and incomplete block as random effects. Adjusted means were then subjected to analysis of variance. Breeding lines were advanced

based on high grain yield within each location, across locations within a region, and across regions within a year. Once Piranha CL+ was selected for release, final data analysis used entries common to the trials across all years. For all data except end-use quality, significant differences were determined at $\alpha = 0.05$, whereas for end-use quality significance was determined at $\alpha = 0.01$. End-use quality data were analyzed using analysis of variance with PROC GLM (SAS v9.3; SAS Institute). Data were only analyzed from site-year locations where both lines and the respective check cultivars were in the same trial.

3 | CHARACTERISTICS

3.1 | General description

Piranha CL+ is an intermediate height, semi-dwarf (*Rht-B1b*, *Rht-D1a*) SWW wheat cultivar with semi-erect juvenile plant growth and anthocyanin absent on the coleoptile. The plant is green in color at the boot stage (Feekes 10.0), with an erect, twisted flag leaf absent of wax. The stem of Piranha CL+ is absent anthocyanin coloration, a waxy bloom is present, the last internode of the rachis is hollow, the auricle lacks anthocyanin pigmentation hairiness, and the peduncle is semi-erect. Piranha CL+ has a lax, tapering, and erect to inclined inflorescence with white awns and glumes, which are short and medium in width. The glume shoulder is oblique and narrow, and medium length acuminate beaks that are long in length and narrow to medium in width, with an absent pubescence. Piranha CL+ has ovate to elliptical kernels that are white and soft. The seed of Piranha CL+ has a medium to large germ with a crease width of 80% or less of the kernel and a depth of 25% or less of the kernel. The seed has rounded cheeks and a medium and collared brush.

3.2 | Agronomic performance

In the Washington State University Extension Uniform Cereal Variety Performance Trials conducted from 2019 through 2022, mean heading date, plant height, grain volume weight, and grain yield of Piranha CL+ were compared to similar non-Clearfield SWW cultivars 'Norwest Tandem' (PI 681649) and 'Norwest Duet' (PI 681650), and Clearfield cultivars Curiosity CL+ (<400 mm trials only), 'Stingray CL+' (PI 691557; Carter et al., 2021a), and 'UI Magic CL+' (PI 679629; registered as 'UI Magic' but marketed as UI Magic CL+ to indicate Clearfield herbicide tolerance), with data from the <400-mm annual precipitation trials in Table 1, and data from the >400-mm annual precipitation trials in Table 2. In both rainfall regions, Piranha CL+ was later in heading date than Norwest Tandem and UI Magic CL+, but earlier than Norwest

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TABLE 1 Head-to-head comparisons of Piranha CL+ to five widely grown cultivars from Washington State University Extension Uniform Cereal Variety Performance Trials grown from 2019 through 2022 in eastern Washington in the low-rainfall zone <400 mm annual precipitation.

Line	Heading date				Plant height				Grain yield				Grain volume weight			
	n	Line	Piranha CL+		n	Line	Piranha CL+		n	Line	Piranha CL+		n	Line	Piranha CL+	
			—Julian days—				—cm—				—kg ha^{-1}—				—kg hl^{-1}—	
Norwest Duet	38	155	152	***	38	91	87	***	38	4729	4840	ns[†]	38	73.9	75.1	***
Norwest Tandem	38	150	152	***	38	73	87	***	38	4671	4840	*	38	74.3	75.1	**
Curiosity CL+	42	156	152	***	42	87	87	ns	42	4233	4663	***	42	77.3	77.4	ns
Stingray CL+	30	152	152	ns	30	78	87	***	30	4202	4539	***	30	74.1	74.9	***
UI Magic CL+	34	150	152	***	34	76	88	***	34	4194	4932	***	34	74.5	74.7	ns

Note: Data on heading date, plant height, grain yield and grain weight volume were from rainfed trials (Almira, Anatone, Bickleton, Connell, Creston, Harrington, Horse Heaven Hills, Lamont, Lind, Reardan, and Ritzville, WA).

***Significantly different at the 0.01 probability level.
**Significantly different at the 0.05 probability level.
*Significantly different at the 0.1 probability level.
[†]ns, not significantly different at the $P = 0.05$ probability level.

TABLE 2 Head-to-head comparisons of Piranha CL+ to four widely grown cultivars from Washington State University Extension Uniform Cereal Variety Performance Trials grown from 2019 through 2022 in eastern Washington in the high-rainfall zone >400 mm annual precipitation.

Line	Heading date				Plant height				Grain yield				Grain volume weight			
	n	Line	Piranha CL+		n	Line	Piranha CL+		n	Line	Piranha CL+		n	Line	Piranha CL+	
			—Julian days—				—cm—				—kg ha^{-1}—				—kg hl^{-1}—	
Norwest Duet	26	163	161	***	26	97	94	***	26	7223	7599	***	26	74.7	74.7	ns[†]
Norwest Tandem	27	159	162	***	27	77	94	***	27	7093	7620	***	27	74.7	75.3	***
Stingray CL+	30	159	159	ns	30	86	95	***	31	7101	7532	***	30	74.5	75.3	***
UI Magic CL+	30	158	159	***	30	83	95	***	31	6501	7532	***	30	75.7	75.3	**

Note: Data on heading date, plant height, grain yield and grain weight volume were from rainfed trials (Colton, Dayton, Fairfield, Farmington, Mayview, Pullman, St. John, Walla Walla, WA).

***Significantly different at the 0.01 probability level.
**Significantly different at the 0.05 probability level.
[†]ns, not significantly different at the $P = 0.05$ probability level.

Duet and Curiosity CL+, with no difference when compared to Stingray CL+. The plant height of Piranha CL+ was taller than all comparison cultivars, except to Curiosity CL+ where they were equal height, and Norwest Duet, which was taller than Piranha CL+. The average grain volume weight of Piranha CL+ was significantly higher ($P < 0.05$) than all cultivars except for Curiosity CL+ and Norwest Duet, where no significant difference was found. Grain yield of Piranha CL+ in the low rainfall regions was greater ($P < 0.05$) than that of all check cultivars except for Norwest Duet, where Piranha CL+ had a higher grain yield, but not significantly different. In the high rainfall locations, the grain yield of Piranha CL+ was significantly higher than all four check cultivars.

Piranha CL+ was also tested in Oregon between 2020 and 2022 under the Oregon State University Extension Uniform Cereal Variety Performance Trials. The Oregon data can be found summarized online at https://cropandsoil.oregonstate.edu/wheat/variety-trials (accessed December 14, 2024). In general, when summarized across the low and high rainfall trials in Oregon, Piranha CL+ has grain yield similar to that of other commercially released Clearfield cultivars. In the intermediate rainfall trials, Piranha CL+ has a higher grain yield than the most commonly grown cultivars of UI Magic CL+ and Stingray CL+.

In the Washington State University Winter Wheat Program breeding trials conducted between 2018 and 2022, mean heading date, plant height, grain volume weight, and grain yield of Piranha CL+ were compared to SWW cultivars 'Devote' (PI 693628; Carter et al., 2021b), 'Jasper' (PI 678442; Carter et al., 2017), and 'Otto' (PI 667557; Carter et al., 2013), with data presented in Table 3 comprising the low precipitation locations. Piranha CL+ has a significantly earlier heading date than all check cultivars, ranging from 1 to 5 days earlier. The plant height of Piranha CL+ was significantly taller than Jasper and Devote, but equal to Otto. For the high precipitation locations, Piranha CL+ was compared to SWW cultivars Jasper, Norwest Tandem, and Stingray CL+, with data presented in Table 4. Piranha CL+ was 1 day earlier heading than Jasper and Stingray CL+, and 2 days later heading than Norwest Tandem. Plant height data indicated that Piranha CL+ is significantly taller than all three check cultivars. The grain yield of Piranha CL+ was not significantly higher than the three check cultivars, although it was numerically higher compared to all three. Piranha CL+ only had significantly higher grain volume weight than the cultivar Jasper.

Piranha CL+ was evaluated under BASF approved spray trials from 2019 through 2021 (7 site-years) using Brundage 96 as the susceptible check 'ORCF-102' (PI 641787) as the single-gene (*Als1* mutation) check, and Stingray CL+ and UI Magic CL+ as the two-gene (*Als1* and *Als2* mutation) checks. All data are presented in Table 5. Average grain yield data of Piranha CL+ was 7590 kg ha^{-1} untreated and 7570 kg ha^{-1} when treated with 105 g ai ha^{-1} (2X field rate) of Beyond

TABLE 3 Head-to-head comparisons of Piranha CL+ to three widely grown Washington cultivars from breeding trials in eastern Washington grown in the low-rainfall zone (<400 mm annual precipitation) between 2018–2022.

Line	Heading date				Plant height				Grain yield				Grain volume weight			
	n	Line	Piranha CL+		n	Line	Piranha CL+		n	Line	Piranha CL+		n	Line	Piranha CL+	
			Julian days				cm				kg ha^{-1}				kg hl^{-1}	
Jasper	30	152	151	*	30	82.3	85.1	***	32	4525	4707	*	27	78.1	79.5	***
Devote	29	152	149	***	29	80.0	84.6	***	31	4322	4660	***	25	80.8	79.4	***
Otto	32	152	147	***	32	83.3	84.1	ns†	34	4116	4539	***	28	79.4	79.4	ns

Note: Data on heading date, plant height, grain yield and grain weight volume were from rainfed trials (Davenport, Harrington, Kahlotus, Lind, Ritzville, and Waterville, WA).

***Significantly different at the 0.01 probability level.

*Significantly different at the 0.1 probability level.

†ns, not significantly different at the $P = 0.05$ probability level.

TABLE 4 Head-to-head comparisons of Piranha CL+ to three widely grown cultivars from breeding trials in eastern Washington grown in the high-rainfall zone >400 mm annual precipitation (2019–2022).

Line	Heading date				Plant height				Grain yield				Grain volume weight			
	n	Line	Piranha CL+		n	Line	Piranha CL+		n	Line	Piranha CL+		n	Line	Piranha CL+	
		Julian days				cm				kg ha^{-1}				kg hl^{-1}		
Jasper	21	155	154	***	21	98	102	***	24	7,428	7,445	ns†	21	78.4	79.3	**
Norwest Tandem	20	152	154	***	20	84	101	***	23	7,053	7,371	ns	20	79.1	79.3	ns
Stingray CL+	20	152	151	***	20	92	100	***	23	6,933	7,146	ns	18	78.5	79.0	ns

Note. Data on heading date, plant height, grain yield and grain weight volume were from rainfed trials (Dayton, Farmington, Pomeroy, Prescott, Pullman, and Walla Walla, WA).

***Significantly different at the 0.01 probability level.

**Significantly different at the 0.05 probability level.

†ns, not significantly different at the $P = 0.05$ probability level.

herbicide. The plant height of Piranha CL+ when treated was 95 cm, showing no difference from the untreated average. Heading date also showed no difference between treated and untreated plots, averaging 153 days after January 1. None of these values were significantly different ($P > 0.05$) between the treated and untreated plots. The other two-gene checks Stingray CL+ and UI Magic CL+ also showed no significant difference between treated and untreated plots. ORCF-102, had a significant decrease in plant height and grain yield when treated, and showed a 1-day delay in heading (Table 5). Grain yield of the susceptible check Brundage 96 was 6187 kg ha^{-1} untreated and no yield in the Beyond treated plots.

To evaluate herbicide injury, the two-gene cultivars UI Magic CL+ and Stingray CL+ were compared to Piranha CL+ from these same BASF approved trials and all data are presented in Table 5. These two check cultivars adapted to the growing area where Piranha CL+ is targeted. Three injury readings are reported, 14 days after treatment (DAT), 21 DAT, and 28 DAT. From 2019–2021 (7 site-years) the 14 DAT, 21 DAT, and 28 DAT injury of the three cultivars carrying two-gene mutations herbicide tolerance were not significantly different and did not average higher than 5%. DNA marker analysis indicates that Piranha CL+ is homozygous for the two mutations at *Als1* and *Als2* that confer tolerance to imazamox.

3.3 | Disease resistance

Piranha CL+ was evaluated for stripe rust resistance in various field locations in Washington state under natural infection and under controlled greenhouse conditions with selected races from 2019 to 2020. In 2019, stripe rust developed to adequate levels in the field for evaluation at Spillman Farm and Palouse Conservation Farm (both located near Pullman), where the check 'PS 279' (Chen, 2014) was susceptible (infection type [IT] 8; severity 10%–95%) and Piranha CL+ was rated as resistant (IT 2–4; severity 2%–20%) at the flowering (Feekes 10.51) and soft dough (Feekes 11.2) stage. In comparison, the two-gene cultivars Curiosity CL+ was rated as moderately susceptible (IT 2–8; severity 5%–40%) and UI Magic CL+ was also rated as moderately susceptible (IT 2–7; severity 5%–30%). In 2020, stripe rust developed to adequate levels for field evaluation at Spillman Farm, Plant Pathology Farm, Palouse Conservation Farm, Mount Vernon, Walla Walla, and Lind, where PS 279 displayed susceptible reactions (IT 9; severity 90%–100%). In this trial, Piranha CL+ displayed moderately resistant reactions (IT 2–5; severity 2%–30%), Curiosity CL+ displayed moderately susceptible reactions (IT 3–8; severity 15%–40%), and UI Magic CL+ displayed susceptible reactions (IT 8; severity 10%–80%) at the flowering and soft dough stage. The variation in IT values under field conditions is dependent on temperatures and the pathogen races present. Piranha CL+ was included in green-

TABLE 5 Mean percentage crop injury, plant height, and grain yield after application of 105 g ai ha^{-1} (2X rate) Beyond (imazamox) herbicide of soft white winter wheat cultivar Piranha CL+ and check cultivars in eastern Washington, 2019–2021 in the high-rainfall zone >400 mm annual precipitation.

| Cultivar | % Crop injury | | | | Plant height | | Heading date | | Grain yield | |
| | Untreated | 14 DAT | 21 DAT | 28 DAT | Untreated | Treated | Untreated | Treated | Untreated | Treated |
		%			cm		Julian days		kg ha^{-1}	
Piranha CL+	0	4.1	2.5	1.3	95	95	153	153	7,590	7,570
Stingray CL+	0	4.3	3.0	0.8	89	89	153	153	7,090	7,090
UI Magic CL+	0	4.3	4.25	2.25	85	84	151	151	5,649	5,678
ORCF-102	0	13	25.2	30.4	97	89	154	155	6,975	6,302
Brundage 96	0	51.4	70.0	91.8	88	–	153	–	6,187	–
LSD		6.1	8.3	15.3	7.3	4.6	2.4	1.5	504.4	638.9

Note: Data on mean percent crop injury, heading date, plant height, and grain yield were from seven rainfed trials (Cook Agronomy Farm, Pullman, WA, Palouse Conservation Field Station, Pullman, WA and a commercial field in Walla Walla, WA).

Abbreviation: DAT, days after treatment.

house seedling tests conducted with races PSTv-4, PSTv-14, PSTv-37, PSTv-40, and PSTv-51 in both 2019 and 2020, plus PSTv-198 in 2019. Piranha CL+, when tested at the low temperature cycles, had seedlings that were susceptible (IT 8) to races PSTv-4, PSTv-14, PSTv-37, and PSTv-51, but were resistant (IT 3) to PSTv-40 and moderately resistant (IT 5) to PSTv-198. UI Magic CL+ and PS 279 were both susceptible to all tested races at the seedling stage. In contrast, when tested at the adult-plant stage at high temperatures with races PSTv-14, PSTv-37, and PSTv-40, Piranha CL+ was rated moderately resistant (IT 2–3), whereas UI Magic CL+ was moderately susceptible (IT 2–5), and the check PS 279 was scored as susceptible (IT 8). These data indicate that Piranha CL+ has a moderate to high level of high-temperature adult-plant resistance to stripe rust. DNA marker analysis confirms that Piranha CL+ carries the gene *Yr17* for stripe rust resistance and is presumed to carry the high-temperature adult-plant resistance gene tightly linked to *Yr17* (Li et al., 2023) and other uncharacterized resistance genes based on field and greenhouse ratings.

Piranha CL+ was tested in inoculated field trials for strawbreaker foot rot in Washington in 2020. In these trials, Piranha CL+ showed a disease index of 56.4, which was significantly higher ($P > 0.05$) than the resistant control Madsen (26.8), but significantly lower than the susceptible control Eltan (70.7). DNA marker analysis indicates Piranha CL+ is heterogeneous for the *Pch1* gene, revealing 50% of the plants in the population carry the gene and 50% do not. This heterogeneity is the potential reason for the intermediate scores in field trials. In inoculated field trials for Cephalosporium stripe (caused by *Cephalosporium gramineum*), Piranha CL+ showed a disease index of 26.3, which was not significantly different than the tolerant control Eltan (39.9) but significantly lower than the susceptible control Stephens (49.0). This indicates that Piranha CL+ has a moderately high level of tolerance to Cephalosporium stripe.

Piranha CL+ has been tested for tolerance to snow mold under field conditions near Waterville and Mansfield, WA, between 2018 and 2022. Piranha CL+ is moderately tolerant (rating of 7) to this complex of snow molds found in Washington, whereas the most tolerant known cultivar Mela CL+ had a rating of 8. Due to the low pH soils found in Eastern Washington, Piranha CL+ was also evaluated for tolerance to low pH soils and was found to be moderately susceptible (score of 2.5). The tolerant cultivars used as checks had scores of 4.5.

3.4 | End-use quality

End-use quality of Piranha CL+ was assessed at the USDA-ARS Western Wheat Quality Laboratory in Pullman using grain produced in 46 breeding and commercial variety testing trials in Washington from 2017 through 2022, and all data are presented in Table 6. Stephens and Curiosity CL+ were used as checks in these evaluations. Stephens has a desired range of end-use quality values and is used as a check to generate placement in the Preferred Wheat Varieties brochure (https://wagrains.org/wp-content/uploads/2024/03/PreferredWheatVarieties2024_web.pdf, assessed December 14, 2024). Curiosity CL+ was compared as this is one of the targeted cultivars for replacement with Piranha CL+.

Grain and flour protein concentration of Piranha CL+ were significantly lower ($P > 0.01$) than those of Stephens and Curiosity CL+, which is an advantage for soft white winter wheat. Break flour yield of Piranha CL+ was significantly greater than Stephens and Curiosity CL+, whereas Piranha CL+ only had a significantly higher total flour yield compared to Curiosity CL+. Flour ash of Piranha CL+ was significantly lower than both checks, and combined with flour yield, lead to Piranha CL+ having a significantly higher milling score compared to both checks. Solvent retention capacity tests indicate a good profile for SWW products, with Piranha CL+

TABLE 6 End-use quality analysis of Piranha CL+ and check cultivars from winter wheat trials in eastern Washington between 2017 and 2022.

Cultivar	Grain protein concentration	Flour protein concentration	Total flour yield	Break flour yield	Flour ash concentration	Milling score	Cookie diameter	SRC water	Cake volume
			g kg^{-1}			unitless	cm	g kg^{-1}	L
Stephens	110	93	675	440	3.8	83.0	9.08	547	1.181
Piranha CL+	104	87	666	459	3.4	84.1	9.23	531	1.233
LSD ($\alpha = 0.01$)	2.1	1.6	3.9	3.6	0.1	0.8	0.04	4.6	0.015
$n = 103$									
Curiosity CL+	108	94	654	439	3.5	81.9	9.05	561	1.208
Piranha CL+	106	90	667	463	3.4	84.3	9.19	538	1.251
LSD ($\alpha = 0.01$)	3.8	3.1	3.8	3.4	0.1	0.7	0.06	7.8	0.015
$n = 46$									

being significantly lower in SRC Water. Regarding baking performance, the cookie diameter and cake volume of Piranha CL+ was greater ($P < 0.01$) than both Stephens and Curiosity CL+. Most of the SWW produced in the Pacific Northwest is exported to Pacific Rim countries. A diversity of products is made with soft white wheat flour, including cookies, crackers, sponge cake, and other confectionaries. The end-use quality of Piranha CL+ meets the export specifications and expectations for the soft winter wheat market class.

Falling number data has been collected since 2019 on Piranha CL+ using seed from the Washington State University Extension Uniform Cereal Variety Performance Trials. Data can be found summarized at steberlab.org/project7599. php (accessed December 14, 2024). In locations where low falling numbers were observed on susceptible checks, Piranha CL+ averaged a falling number 20-s higher than Curiosity CL+. Comparisons to other commercial cultivars can be found at the referenced website.

4 | AVAILABILITY

Foundation seed of Piranha CL+ will be maintained by the Washington State Crop Improvement Association under supervision of the Washington State University Department of Crop and Soil Sciences and the Washington State Agricultural Research Center. Small quantities of seed may be obtained for research purposes from the corresponding author for at least 5 years from the date of publication. A seed sample has been deposited with the National Plant Germplasm System, where it will thereafter be available for distribution. U.S. Plant Variety Protection status for this cultivar has been approved (PVP#202100485).

AUTHOR CONTRIBUTIONS
A. H. Carter: Conceptualization; data curation; formal analysis; funding acquisition; investigation; methodology; supervision; writing—original draft; writing—review and editing. **K. A. Balow**: Data curation; methodology; resources; writing—review and editing. **G. B. Shelton**: Data curation; investigation; methodology; resources; writing—review and editing. **A. B. Burke**: Data curation; investigation; methodology; resources; writing—review and editing. **K. E. Hagemeyer**: Data curation; investigation; methodology; resources. **A. Stowe**: Data curation; investigation; methodology; resources. **H. Wetzel**: Formal analysis; resources; writing—review and editing. **C. Neely**: Data curation; resources. **C. M. Steber**: Data curation; formal analysis; methodology; resources; writing—review and editing. **X. M. Chen**: Data curation; methodology; resources; writing—review and editing. **A. Kiszonas**: Data curation; investigation; methodology; resources; writing—review and editing.

CONFLICT OF INTEREST STATEMENT
The authors declare no conflict of interest.

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