Registration of ‘StandClear CLP’ hard red winter wheat

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Abstract
‘StandClear CLP’ (Reg. no. CV-1162, PI 693236) hard red winter (HRW) wheat (Triticum aestivum L.) was developed and released by the Montana Agricultural Experiment Station and exclusively licensed to Loveland Products, Inc., in 2020. StandClear CLP is a two-gene Clearfield, semisolid-stem wheat intended for use with the selective imidazolinone (IMI) herbicide imazamox. StandClear CLP resulted from a cross of MTS0531 to an IMI herbicide tolerant F1 plant from a population segregating for two acetohydroxyacid synthase (AHAS) genes [TaAHAS1D and TaAHAS1B]. Original herbicide tolerance donors were IMI ‘Fidel’ (TX12588*4/FS2, BASF) for allele TaAHAS1D via descended experimental lines MTCL0309 and MTCL0510, and proprietary hard red spring wheat line CDC Teal 1IA (BASF Corporation) for allele TaAHAS1B. StandClear CLP was selected as a F6:7 headrow in 2014 following multiple cycles of phenotypic mass selection for IMI herbicide tolerance and stem solidness. StandClear CLP was tested under the experimental number MTCS1601 from 2016 to 2019 in Montana for field performance, herbicide tolerance, and end-use quality. StandClear CLP is a high-yielding, Clearfield HRW wheat cultivar with intermediate stem solidness, moderate host plant resistance to wheat stem sawfly, and acceptable milling and baking quality.

Abbreviations: AACC, American Association of Cereal Chemists; AHAS, acetohydroxyacid synthase; HRW, hard red winter; IMI, imidazolinone; IT, infection type; MSO, methylated seed oil; UAN, urea-ammonium nitrate.
1 | INTRODUCTION

The Montana winter wheat (Triticum aestivum L.) breeding program has worked in cooperation with BASF Corporation to develop adapted cultivars of herbicide-tolerant Clearfield winter wheat for Montana (Berg et al., 2014a; Carlson et al., 2007; Kephart et al., 2007; Stougaard et al., 2007). The Clearfield Wheat Production System includes imidazolinone (IMI) herbicide–tolerant wheat cultivars and imazamox (Beyond, BASF Corporation) herbicide. Imazamox herbicide provides selective weed control of several winter annual weeds in Montana, including jointed goatgrass (Aegilops cylindrica Host) (Ball, Young, & Ogg, 1999) and downy brome (Bromus tectorum L.) (Stougaard, Mallory-Smith, & Mickelson, 2004). This herbicide inhibits activity of acetohydroxyacid synthase (AHAS), the first enzyme in synthesis of the branched chain amino acids valine, leucine, and isoleucine. The IMI herbicides are an attractive weed control option because they have a broad spectrum of weed control activity, flexibility in timing of application, high biological potency that makes them effective at low application rates, and low mammalian toxicity (Newhouse, Smith, Starrett, Schaefer, & Singh, 1992). Three homoeologous AHAS genes conferring tolerance to IMI herbicides have been identified in wheat: TaAHASID, located on chromosome 6DL; TaA-HASIB, on chromosome 6BL; and TaAHASIA, on chromosome 6AL. (Tan, Evans, Dahmer, Singh, & Shaner, 2005). Pozniak and Huc (2004) demonstrated higher levels of imidazolinone resistance in wheat could be achieved by stacking two or more genes into a single genotype. The objective of this research was to transfer herbicide tolerance alleles TaAHASID and TaAHASIB into a solid-stem background to provide the Clearfield grass weed control option along with enhanced tolerance to weed stem sawfly (Cephus cinctus Nort.), the major biotic limitation to wheat production in Montana.

2 | METHODS

2.1 | Pedigree and breeding history

‘StandClear CLP’ (Reg. no. CV-1162, PI 693236) hard red winter (HRW) wheat (tested as MTCS1601) was derived from a 2007 cross of MTS0531 (‘L’Govskaya 167’/Rampart’ [PI 593889; Bruckner et al., 1997]/MT9409 ['Tiber’ (PI 517194; Kisha et al., 1992)/MT8030]) to an IMI herbicide tolerance plant from a population (MTS0532/6/96X17E69/3/MTCLO309/CDC Teal 11A/MTW01143/4/MTCLO510/5/MTS0531) that was sprayed in the 2007 greenhouse with a 157 g a.i. ha⁻¹ rate of imazamox herbicide with 0.25% (v/v) nonionic surfactant and 2.5% (v/v) urea-ammonium nitrate (UAN) fertilizer. The predominant genetic background is from solid-stem sister lines MTS0531 and MTS0532. The potential herbicide tolerance trait donors are MTCLO309 (TaAHASID; MT9409*2/IMI Fidel [FS2]) or MTCLO510 (TaAHASID; Rampart*3/IMI Fidel [FS2]/MTS9720/MT7863) and CDC Teal 11A (TaAHASID). 96X17E69, MTW01143, MT8030, MTS9720, and MT7863 are unreleased Montana experimental lines.

The F₁ population was grown in the field near Bozeman, MT, in 2008. All Clearfield breeding nurseries grown near Bozeman from 2009 to 2014 were sprayed with imazamox herbicide at a rate of 105 g a.i. ha⁻¹ plus 1% v/v methylated seed oil (MSO) and 1.5% v/v UAN. F₂, F₃, F₄, and F₅ bulk populations were grown at Bozeman in 2009 and 2010, Williston, ND, in 2011, and Bozeman in 2012, respectively, using a modified bulk breeding method, with multiple cycles of phenotypic mass selection primarily for herbicide tolerance and stem solidness, but also additional selection for winter survival, reduced plant height, stripe rust (caused by Puccinia striiformis Westend. f. sp. tritici Eriks.) resistance, and kernel plumpness when dictated by the environment. In each generation, individual plants were selected and threshed in bulk, and the seed was sieved using appropriately sized screens to retain the plumpest seed fraction for replanting. Heads (110) were selected from the F₅ population and subsequently grown as F₆ headrows at Fort Ellis, MT, in 2013. Headrow 07X349D10 was selected based on the evaluation of stem solidness, visual criteria for herbicide tolerance (absence of phytotoxicity, chlorosis, stunting), uniformity, productivity, and acceptable agronomic type. Four heads of 07X349D10 were retained for reselection. In 2014, one of these reselections, 07X349D10-1, judged to be a superior solid-stem, IMI herbicide–tolerant F₆ headrow was selected and harvested in bulk. In 2015, 07X349D10-1 and cohorts were tested at five Montana sites in single replication Sawfly and Clearfield field trials.

2.2 | Line selection and evaluation

Line 07X349D10-1 was selected and designated MTCS1601 for further testing in the Montana Sawfly Yield Trial (20 location-years) and the Montana Clearfield Qualification Trial (9 location-years) from 2016 to 2019, and in the Montana Advanced Trial planted in 2017 (6 location-years). MTCS1601 was evaluated in the Montana Intrastate Trial (18 location-years), and in the Montana Off-Station Nursery (27 location-years) planted from 2018 to 2019.

The Montana Intrastate Trial consisted of 49 entries arranged in a 7 × 7 partially balanced, incomplete block, triple lattice design (Cochran & Cox, 1957). Plot size, row number, and row spacing varied by location. Seeding rate
was approximately 2.15 million kernels ha\(^{-1}\) at all locations. The Montana Off-station Trial, planted at approximately 15 locations each year, consisted of 25 entries, arranged as a 5 × 5 partially balanced, incomplete block, triple lattice design and planted at 2.15 million kernels ha\(^{-1}\). The Montana Sawfly Trial, planted at three to six locations each year, consisted of 49 entries, arranged as a 7 × 7 partially balanced, incomplete block, simple lattice and planted at 2.15 million kernels ha\(^{-1}\). Grain yield, volume weight, plant height (distance from ground to top of spike excluding awns), and grain protein concentration were measured in all environments. Days to heading (50% of plot heads completely emerged from boot) were recorded in most on-station trials. Cutting by wheat stem sawfly (\% incidence) was recorded in those environments where significant infestation by the pest was documented.

Stem solidness was determined in selected environments using five stems per plot, sampled randomly near crop maturity using a method like that reported by McKenzie (1965). Five internodes per stem were cross-sectionally cut and visually rated on a semi-quantitative scale of 1–5, where 1 designates a hollow (normal) stem and 5 designates a solid stem. Internode scores were summed for each stem and averaged over five stems, resulting in composite stem solidness scores of 5 (hollow) to 25 (completely solid).

Tolerance to imazamox herbicide was tested in comparison to ‘SY Clearstone 2CL’ (PI 668090; Berg et al., 2014a) in nine trials at Bozeman, Fort Ellis, Kalispell, and Huntley, MT, from 2016 to 2019. Standard protocol involved an untreated control or a 2× imazamox herbicide application at a rate of 105 g a.i. ha\(^{-1}\) plus 1% v/v MSO and 1.5% v/v UAN. Trials were planted in a split plot arrangement with control and imazamox treatments as whole plots and cultivars as subplots, with three replications. Crop injury (\%) was visibly estimated at 14–21 and 28–35 d after treatment. Heading date, plant height, grain yield, grain volume weight, and grain protein concentration were recorded dependent variables.

Millling and baking quality were evaluated in multilocation Montana trials since 2016. Milling and baking characteristics were determined by the Montana State University Cereal Quality Laboratory using methods approved by the American Association for Cereal Chemists International (AACC, 2000). Grain protein concentration was determined using an Infratec 1241 Grain Analyzer (Foss Analytical). Kernel hardness was determined using a single-kernel characterization system (SKCS-4100, Perten Instruments). Composite grain samples harvested from four environments (2018) of the Montana Intrastate trial and four environments from the Montana Sawfly trial (2016–2018) were milled on a Brabender Quadramat Sr. mill (C.W. Brabender), and the flour was used to determine bake water absorption, mix time, and loaf volume using AACC Method 10-10B (AACC, 2000). MTCS1601 was also evaluated in the 2019 Hard Winter Wheat Quality Council evaluation (Wheat Quality Council, 2019).

Analysis of variance was conducted on data from individual environments and across environments using SAS version 9.2 (SAS Institute, 2009). Mean comparison of traits using a protected LSD (\(\alpha = 0.05\)) test was made to identify significant differences among genotypes. The genotype × environment mean square was used to estimate the standard error of differences when comparing genotype means across environments.

### 2.3 Seed purification and increase

Increase of MTCS1601 began in 2017 when a phenotypically uniform, herbicide-treated field plot (F\(_{10}\)) was hand harvested and threshed as a source of breeder seed. Breeder seed was increased at Fort Ellis in 2018 and by Northern Seed LLC under a material transfer agreement in 2019 using Clearfield seed increase protocols. StandClear CLP has been genetically uniform and stable over three generations of seed increase. StandClear CLP contains tall, hollow stem, dark chaff, and awnless variants at frequencies less than 5, 5, 5, and 3 per 10,000 spikes, respectively, and a white kernel variant at a frequency less than 30 per 10,000 kernels.

### 3 CHARACTERISTICS

#### 3.1 Agronomic characteristics

StandClear CLP is an awned, white-chaffed, solid-stem, semidwarf, imazamox-tolerant HRW wheat. Relative to other solid-stem cultivars, StandClear CLP has medium maturity, 164 d heading from 1 January, similar to ‘Judee’ (PI 665227; Carlson et al., 2013) and ‘Warhorse’ (PI 670157; Berg et al., 2014b) and earlier than ‘Loma’ (PI 680576; Bruckner et al., 2017; Table 1). StandClear CLP is medium in height (76 cm, \(n = 62\)), similar in plant height to Judee and significantly taller than Warhorse and Loma. StandClear CLP has intermediate stem solidness, averaging 19.5 on the 5 (hollow) to 25 (solid) stem-solidness scale, significantly less solid than Warhorse (22.0), Judee (20.7), and Loma (20.7) (Table 1). Cutting by wheat stem sawfly in 23 environments with sawfly infestations averaged 36% for StandClear CLP, similar to Loma (30%) and Judee (36%) and significantly higher than Warhorse (10%, Table 1).

Relative to hollow-stem Clearfield check cultivars, StandClear CLP is 7 d later in heading and 5 cm taller than ‘Brawl CL Plus’ (PI 664255; Haley et al., 2012) and 1 d earlier in heading and 7 cm shorter than SY Clearstone
TABLE 1  Mean performance of StandClear CLP and solid-stem hard red winter wheat check cultivars in 65 Montana environments, 2016–2019

<table>
<thead>
<tr>
<th>Cultivar</th>
<th>Grain yield</th>
<th>Volume weight</th>
<th>Heading date</th>
<th>Plant height</th>
<th>Grain protein</th>
<th>Stem solidnessa</th>
<th>Sawfly cutting</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>kg ha⁻¹</td>
<td>kg m⁻³</td>
<td>d from 1 Jan.</td>
<td>cm</td>
<td>g kg⁻¹</td>
<td>5–25 % incidence</td>
<td></td>
</tr>
<tr>
<td>Loma</td>
<td>4,818</td>
<td>777</td>
<td>166</td>
<td>73</td>
<td>127</td>
<td>20.7</td>
<td>30</td>
</tr>
<tr>
<td>StandClear CLP</td>
<td>4,804</td>
<td>793</td>
<td>164</td>
<td>76</td>
<td>127</td>
<td>19.5</td>
<td>36</td>
</tr>
<tr>
<td>Judee</td>
<td>4,600</td>
<td>794</td>
<td>163</td>
<td>77</td>
<td>131</td>
<td>20.7</td>
<td>36</td>
</tr>
<tr>
<td>Warhorse</td>
<td>4,549</td>
<td>782</td>
<td>165</td>
<td>75</td>
<td>133</td>
<td>22.0</td>
<td>10</td>
</tr>
<tr>
<td>LSD (0.05)</td>
<td>134</td>
<td>3</td>
<td>1</td>
<td>1</td>
<td>2</td>
<td>0.7</td>
<td>7</td>
</tr>
<tr>
<td>No. of environments</td>
<td>65</td>
<td>63</td>
<td>25</td>
<td>62</td>
<td>63</td>
<td>30</td>
<td>23</td>
</tr>
</tbody>
</table>

a Stem solidness score where 5 = hollow and 25 = completely solid.

TABLE 2  Mean performance of StandClear CLP and hollow stem hard red winter wheat check cultivars in 45 Montana environments, 2016–2019

<table>
<thead>
<tr>
<th>Cultivar</th>
<th>Grain yield</th>
<th>Volume weight</th>
<th>Heading date</th>
<th>Plant height</th>
<th>Grain protein</th>
<th>Sawfly cutting</th>
</tr>
</thead>
<tbody>
<tr>
<td>SY Clearstone 2CL</td>
<td>5,012</td>
<td>775</td>
<td>165</td>
<td>83</td>
<td>125</td>
<td>61</td>
</tr>
<tr>
<td>Yellowstone</td>
<td>4,952</td>
<td>777</td>
<td>166</td>
<td>81</td>
<td>125</td>
<td>58</td>
</tr>
<tr>
<td>StandClear CLP</td>
<td>4,858</td>
<td>797</td>
<td>164</td>
<td>76</td>
<td>129</td>
<td>35</td>
</tr>
<tr>
<td>Brawl CL Plus</td>
<td>4,522</td>
<td>804</td>
<td>157</td>
<td>71</td>
<td>135</td>
<td>33</td>
</tr>
<tr>
<td>LSD (0.05)</td>
<td>181</td>
<td>4</td>
<td>1</td>
<td>1</td>
<td>2</td>
<td>11</td>
</tr>
<tr>
<td>No. of environments</td>
<td>45</td>
<td>45</td>
<td>17</td>
<td>44</td>
<td>45</td>
<td>14</td>
</tr>
</tbody>
</table>

2CL (Table 2). Cutting by wheat stem sawfly was similar for Brawl CL Plus and StandClear CLP and significantly lower than cutting of SY Clearstone 2CL and ‘Yellowstone’ (PI 643428; Bruckner et al., 2007).

StandClear CLP displayed tolerance to herbicide injury after application of Beyond herbicide at the 2× rate of 105 g a.i. ha⁻¹ plus 1% v/v MSO and 1.5% v/v UAN based on crop injury visibly estimated 14–21 and 28–35 d postapplication in a total of nine trials from 2016 to 2019. There were no significant differences between herbicide-treated and untreated StandClear CLP plots for crop injury, grain yield, volume weight, and grain protein concentration across trials (P > .05, data not shown).

3.2  Field performance

In 65 location-years of testing in the Montana Winter Wheat Intrastate, Off-station, and Sawfly nurseries, the average yield of StandClear CLP (4,804 kg ha⁻¹) was high, similar to the yield of Loma and slightly higher than predominantly grown solid-stem cultivars Judee and Warhorse (Table 1). Volume weight of StandClear CLP (793 kg m⁻³) was medium to high, similar to Judee and significantly higher than Loma and Warhorse (Table 1). Grain protein concentration of StandClear CLP was lower than Warhorse and Judee and similar to that of Loma (Table 1). Relative to hollow-stem check cultivars, StandClear CLP was significantly higher in yield and lower in volume weight and grain protein concentration than Brawl CL Plus (Table 2). StandClear CLP was similar in yield and higher in volume weight and grain protein concentration than SY Clearstone 2CL and Yellowstone (Table 2).

3.3  Disease and insect resistance

StandClear CLP is moderately resistant to wheat stem sawfly due to intermediate stem solidness (Tables 1 and 2). StandClear CLP was tested in the Montana Winter Wheat Nursery for reactions to stripe rust in Pullman and Mount Vernon, WA, from 2016 to 2019 under natural infections of P. striiformis f. sp. tritici, except 2019 Pullman, which was artificially inoculated with urediniospores collected from the same location in 2018, mostly of race PSTv-37. Infection type (IT; based on a 0–9 scale where IT 0–3 is considered resistant, 4–6 intermediate, and 7–9 susceptible; Line & Qayoum, 1992) and severity (0–100%) were recorded for each entry. In all Washington field experiments, the susceptible check (PS279) was highly susceptible (IT 8) with 80–100% severity in the late growth seasons, demonstrating that stripe rust pressure was sufficient...
to differentiate resistant from susceptible reactions. The infection type of StandClear CLP was mostly IT 2–3 (highly resistant) with severity 5–20%. Intermediate reaction (IT 5) was observed in the Pullman field in 2016 (25% severity) and 2017 (30% severity). In the 2019 test at Mount Vernon, StandClear CLP had IT 3 in the early season and IT 2 in the late stage. The slight reduction of reaction may indicate the presence of high-temperature adult-plant resistance in StandClear CLP. Stripe rust resistance of StandClear CLP is similar to that of Yellowstone HRW wheat. In seedling evaluations at the USDA Cereal Disease Laboratory in 2016, StandClear CLP was resistant to several stem rust (caused by *Puccinia graminis* Pers.:Pers. f. sp. *tritici* Erikss. & E. Henn.) races from the United States, including QCCSM, QFCSC, and TPMKC, but susceptible to QTHJC, RKRQC, and TTTTF.

### 3.4 | End-use quality

Based on evaluation of kernel hardness and milling and baking qualities over 13 environments, StandClear CLP has acceptable quality for U.S. HRW wheat domestic and export markets (Table 3). StandClear CLP has relatively high flour yield and low flour ash compared to other Montana check varieties, with intermediate mixograph tolerance and mixing time. Bake water absorption and loaf volume of StandClear CLP are similar to Yellowstone (Table 3).

### 4 | Availability

StandClear CLP contains patented herbicide tolerance traits owned by BASF Corporation that confer tolerance to imidazolinone herbicides. Use of StandClear CLP requires a Material Transfer Agreement (research use only) or a commercial license to the traits, as well as permission from the originator (Montana Agricultural Experiment Station). Seed requests sent to the corresponding author will be forwarded to BASF Corporation. Seed of StandClear CLP has been deposited in the USDA-ARS National Plant Germplasm System, where it will be available for distribution after the expiration of applicable patents and Plant Variety Protection. StandClear CLP has been exclusively licensed to Love-land Products, Inc., who will maintain seedstocks and produce foundation seed. Application has been made for U.S. Plant Variety Protection with the certification option (PVP202000183).

### ACKNOWLEDGMENTS

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### CONFLICT OF INTEREST

The authors declare no conflict of interest.

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