Note

Breeding and brewing quality of the Canadian malting barley variety 'CDC Goldstar' lacking lipoxygenase-1

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Various types of malt quality profiles have been investigated to benefit the North American brewing industry. Herein, we report the development and brewing quality of the hulled, two-row malting barley (*Hordeum vulgare* L.) variety 'CDC Goldstar' lacking lipoxygenase-1 (LOX-1-less). This new variety offers a novel malt type for the improvement of beer flavor stability. The agronomic performance of 'CDC Goldstar' was tested in the Western Cooperative Two Row Barley Registration Trials during 2013–2014. In addition to high lodging tolerance, the new variety showed 6% higher yield than the current leading variety 'CDC Copeland'. The malt quality of 'CDC Goldstar' showed higher diastatic power and lower wort β -glucan content than 'CDC Copeland' and controllable proteolytic modification (soluble nitrogen and Kolbach Index). Pilot-(100 L) and commercial-scale (5,000 L) brewing trials were conducted using 'CDC PlatinumStar', another LOX-1-less variety with a low enzymatic profile, as the control variety. Absence of the LOX-1 trait from 'CDC Goldstar' maintained *trans*-2-nonenal levels in aged beers as low as those in other LOX-1-less varieties without affecting major beer parameters, such as ester and aldehyde content or foam stability. The newly developed 'CDC Goldstar' malting barley provides added value for the beer industry and consumers.

Key Words: malting barley, lipoxygenase-1, flavor stability, trans-2-nonenal, CDC Goldstar.

Introduction

Several enzymes related to lipid oxidation in beer production have been reported, among which, the concerted reaction between lipase (EC.3.1.1.3) and lipoxygenase (LOX; EC.1.13.11.12) was identified as a major reaction in the pathway (Vanderhaegen et al. 2006). To date, two lipoxygenase isozymes have been identified and characterized in barley (Hordeum vulgare L.), namely, LOX-1 and LOX-2 (Yang and Schwarz 1995). LOX-1 mainly catalyzes the formation of 9-hydroxyperoxide (9-HPOD), whereas LOX-2 mainly catalyzes the formation of 13-HPOD (Davies and Nilesen 1986, Drost et al. 1990). In turn, 9-HPOD can be transformed into beer-deteriorating substances, such as trans-2-nonenal (T2N) and trihydroxyoctadecenoic acid (THOD), which negatively affect the freshness and foam stability of beer, respectively (Kobayashi et al. 1993, Kuroda et al. 2002, 2003). Therefore, developing new barley varieties that lack LOX-1 is considered a priority for the improvement of brewing quality.

We discovered several barley landraces lacking LOX-1 in the barley germplasm collection at Okayama University (Hirota et al. 2005), and in 2008 released the first LOX-1less barley variety, 'CDC PolarStar', in Canada (Hoki et al. 2013). 'CDC PolarStar' was developed by backcross breeding with molecular marker-assisted selection techniques using 'CDC Kendall' as the recurrent parent. Pilot- and commercial-scale brewing trials revealed that T2N and THOD content in aged beers made from 'CDC PolarStar' malts were reduced by approximately 1/2 and 1/3, respectively, compared to beers made from the control malts without affecting any other beer characteristic (Hoki et al. 2013). Sapporo Breweries has also released LOX-1-less barley varieties in Australia ('SouthernStar') and Japan ('Satuiku 2 go') with backcross breeding (Hoki et al. 2018a, 2018b). Furthermore, another LOX-1-less barley variety 'New Sachiho Golden', originated from a different mutant, has been developed to improve beer qualities in Japan (Oozeki et al. 2017).

The craft beer market has been expanding in North America, where malt types used for brewing craft beers are

Communicated by Koji Murai

Received August 25, 2020. Accepted October 6, 2020.

First Published Online in J-STAGE on February 25, 2021.

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characterized mainly by high extract, low soluble nitrogen content, and low diastatic power. Additionally, craft breweries prefer different types of malts to those used for adjunct brewing (using unmalted grains). The barley variety 'CDC Copeland' is currently the leading variety in Canada as it is preferred by most craft breweries for its lower enzymatic profile compared to other varieties such as 'AC Metcalfe'. Furthermore, beer freshness has become increasingly important as craft breweries expand their business to cover wider areas. 'CDC PlatinumStar', a backcrossbased variety with 'CDC Copeland' as the recurrent parent, was developed and released in 2014. Although backcross breeding accelerates the introduction of a new trait, the method is limited in its ability to match the agronomic performance of other varieties developed by other breeding techniques that don't rely on a recurrent parent. Increasing the diversity or number of LOX-1-less varieties will contribute to a wider range of beer types. Thus, the aim of this study was to develop a new LOX-1-less barley variety that is competitive with the current leading varieties in Canada by using a bulk population method of breeding.

Here, we describe the breeding of the new malting barley variety 'CDC Goldstar' and report the malting and brewingquality characteristics of this new variety through pilot- and commercial-scale brewing trials.

Materials and Methods

Breeding process and field trials

The original cross between 'CDC Reserve' and F₁ seed from the cross, 'TR03375'/'CDC PlatinumStar' was carried out in a greenhouse at Sapporo Breweries, in Gunma, Japan (36°16'N, 139°18'E) during the winter in 2006. 'CDC PlatinumStar' is a Canadian malting-barley variety developed through backcross breeding using 'CDC Copeland' as the recurrent parent and donor of the LOX-1less trait for 'CDC Goldstar'. 'CDC Reserve' is a Canadian malting barley variety developed under the joint breeding program of University of Saskatchewan and Prairie Malt. It has good agronomic performance, moreover it is tolerant to pre-harvest sprouting. 'TR03375' is a high-yield variety, and it was bred by University of Saskatchewan for feed purpose. The F₁ generation was grown as a bulk population in a summer nursery in Hokkaido, Japan (43°55'N, 144°23'E), which is a spring sown area, in 2006. From 2006 to 2008, the F_2 to F_4 generations were bulked using contra-season growing methods between Canada and New Zealand. The F₅ generation was grown in Canada as a space-planted population in the summer of 2008. Selected spikes were checked for the LOX-1-less trait using molecular marker-assisted selection techniques according to Hirota et al. (2005), with selected lines grown as F_6 hill-plots in 2009. One of the selected lines from the F₆ hill-plots was named 'SM105054' and subsequently tested in yield trials by the University of Saskatchewan from 2010 to 2012. The line was subsequently renamed 'TR13812' and tested in the Western Cooperative Two Row Barley Registration Trials (2RCOOP) during 2013 and 2014. The trials were conducted at 16–17 sites in Western Canada, which included locations in the black, black/grey, and brown soil zones, with each trial site consisting of three replications in a completely randomized block design. The agronomic performance was evaluated in accordance with the protocols of the Prairie Recommending Committee for Oat and Barley in Canada.

Micro-malting system based evaluation of malt quality

Barley samples collected in the 2RCOOP were malted on a micro-scale (250 g barley grain samples) using an automated micro-malting machine (Phoenix Biosystems, Adelaide, Australia). Micro-malting and malt analysis were conducted according to Hoki *et al.* (2018a).

Pilot-scale brewing trials

Pilot-scale (100 L) brewing trials were conducted according to the standard methods of Sapporo Breweries. Barley varieties 'CDC Goldstar' and 'CDC PlatinumStar' (control) were harvested in 2015 from the test fields at the University of Saskatchewan, and malted using the automated micro-malting machine. Wort was prepared from malts (78%, w/w), starch, corn, rice, and hops using a 100-L mashing apparatus. Mashing was conducted for 20 min at 60°C, followed by 30 min at 67°C, and then 5 min at 75°C. The wort was boiled for 90 min and then diluted to a concentration of ca. 11.6% of extract with hot water. After cooling, 15.0×10^6 cells mL⁻¹ of lager yeast was added to the wort to start fermentation, which was carried out at 9.0–12.0°C for 6–7 days followed by maturation for 8 days at 12.0°C, and then 25 days at 0°C.

Commercial-scale brewing trials

Commercial-scale brewing trials (5,000 L) used the same method described for the "Pilot-scale brewing trials" with slight modification. Mashing was conducted for 5 min at 50°C, followed by 40 min at 65°C, and then 3 min at 75°C. Fermentation was carried out at 8.5-10.5°C for 6-7 days followed by maturation for 6 days at 10.5°C, cooling for 5 days (-2°C/day), and then for 17 days at -0.5°C. Grain of 'CDC Goldstar' for this trial was grown at a commercial farm in Saskatchewan, Canada, in 2018, and the harvested grain was malted in commercial malting facilities at Prairie Malt, Saskatchewan, Canada. Commercial malts produced from 'CDC PolarStar' and 'CDC PlatinumStar' blended at Prairie Malt, were used as control malts. Analytical methods for parameters related to wort and beer quality in brewing trials were based on EBC methods (European Brewery Convention 2010).

Measurement of T2N and THOD

T2N and THOD contents were determined by the methods described by Hirota *et al.* (2006a, 2006b) and Kobayashi *et al.* (2000), respectively.

 Table 1.
 Agronomic performance of 'CDC Goldstar' and check varieties in the Western Cooperative Two Row Barley Registration Trials during 2013–2014. Values are means of 16 (2013) and 17 (2014) sites in each year

Variety	Grain yield		Days to	Days to	Height	Lodging	Test weight	TGW	Plumpness
	(kg/ha)	(%; Metcalfe)	heading (days)	maturity (days)	(cm)	score (1–9) ^a	(kg/hL)	(g)	(% > 6/64 inch)
(2013 growing sease	on)								
AC Metcalfe	6001	100	51.7	95.0	85.2	4.7	67.3	46.7	91.6
CDC Copeland	6504	108	53.4	96.1	88.5	3.4	65.7	48.4	92.2
Xena	6884	115	51.0	94.9	83.3	3.2	68.0	51.0	92.9
CDC Goldstar	6630	110	52.9	95.6	84.8	2.6	67.1	48.1	93.4
(2014 growing sease	on)								
AC Metcalfe	5692	100	56.0	89.6	82.8	4.3	65.5	45.4	86.7
CDC Copeland	5842	103	56.8	89.4	84.4	4.8	63.8	45.7	87.8
Xena	6471	114	55.0	89.3	79.8	4.3	66.5	48.7	88.1
CDC Goldstar	6467	114	56.5	89.4	81.8	3.6	65.7	46.3	91.0
(2013–2014 mean)									
AC Metcalfe	5846	100	53.9	92.3	84.0	4.5	66.4	46.1	89.1
CDC Copeland	6173	106	55.1	92.8	86.4	4.1	64.7	47.0	90.0
Xena	6678	114	53.0	92.1	81.6	3.7	67.3	49.8	90.5
CDC Goldstar	6549	112	54.7	92.5	83.3	3.1	66.4	47.2	92.2

^a Lodging score was evaluated visually from 1 (strong) to 9 (weak).

Sensory evaluation

The beers were stored at 37°C for 1 week and 30°C for 1 month. The aged beers were scored for three attributes in the sensory evaluation: off-flavor, stale taste, and total staleness. Total staleness is defined as the overall impression of staleness. Trained panelists evaluated the beers based on a scale from 0 (fresh) to 4 (strongly stale) at 0.5 intervals and the data were statistically analyzed with paired t-tests using Microsoft Excel data analysis tools.

Results

Agronomic characteristics

Agronomic characteristic data in the 2RCOOP were collected at 16 sites and 17 sites in Canada during 2013 and 2014, respectively. The 2-year average grain yield of 'CDC Goldstar' was 12% higher than that of 'AC Metcalfe', and 6% higher than that of 'CDC Copeland' (**Table 1**). The average spike of 'CDC Goldstar' was shorter than that of 'CDC Copeland' but longer than that of 'AC Metcalfe' (**Fig. 1A**). 'CDC Goldstar' matured slightly earlier than 'CDC Copeland' and plant height was approximately 3 cm lower than that of 'CDC Copeland', which probably contributed to the lower lodging score of 'CDC Goldstar' compared to the control varieties (**Table 1**, **Fig. 1B**). Furthermore, 'CDC Goldstar' showed a high 1000-grain weight (TGW) and plumpness.

Malt quality

Specific sites were selected in the 2RCOOP to evaluate malt quality by the micro-malting method (**Table 2**). 'CDC Goldstar' malt showed a 0.8-point (%) lower protein content than the control varieties; hence, 'CDC Goldstar' had a higher fine extract in malts. The diastatic power of 'CDC



Fig. 1. (A) spikes, (B) standing plants of 'CDC Goldstar' and other varieties.

Goldstar' malt was similar to that of 'AC Metcalfe' malt, implying a higher amylolytic enzyme profile in this variety. Although total protein content in 'CDC Goldstar' malt was lower than in the control varieties, the Kolbach Index (the ratio of soluble nitrogen to total nitrogen) was 3.7-4.8points (%) higher, whereas soluble nitrogen was comparable to those of 'AC Metcalfe' malt or 'CDC Copeland' malt. Furthermore, 'CDC Goldstar' malt showed a slightly lower wort β -glucan content than the control varieties and an overall superior malt quality.

Brewing performance in pilot-scale brewing trials

Malt quality data for pilot-scale brewing trials are shown in **Table 3**. Target moisture content was set at 42.5% and the desired level of modification was obtained in the tested malts. As observed from malt quality data from the 2RCOOP, 'CDC Goldstar' malt showed slightly higher fine extract, lower total protein, and lower wort β -glucan content than the control variety 'CDC PlatinumStar' malt, which showed almost the same quality profile as the 'CDC Copeland' malt, except for the lack of LOX-1. Although the Kolbach Index was below 40, it was similar in both

 Table 2.
 Malt quality of 'CDC Goldstar' and check varieties from specific sites of the Western Cooperative Two Row Barley Registration

 Trials during 2013–2014

Variety	Steep-out moisture (%)	Malt moisture (%)	Wort colour (°EBC)	Fine extract (%; d.b.)	Apparent final attenu- ation (%)	Total protein (%)	Soluble nitrogen (%)	Kolbach Index	Diastatic power (°WK)	Wort β- glucan (mg/L)
(2013 growing sea	son)									
AC Metcalfe	43.5	4.7	3.9	82.3	81.9	11.8	0.861	45.5	419	74
CDC Copeland	43.4	4.8	4.0	81.5	82.3	11.9	0.856	45.0	372	87
CDC Goldstar	43.4	4.3	5.1	83.0	83.6	10.8	0.842	48.9	407	54
(2014 growing sea	son)									
AC Metcalfe	43.5	4.6	4.6	83.1	85.4	10.0	0.798	49.8	348	29
CDC Copeland	43.4	4.2	4.2	82.8	84.0	9.9	0.765	48.1	284	26
CDC Goldstar	43.4	5.3	5.3	83.5	85.3	9.3	0.805	53.9	348	24
(2013–2014 mean))									
AC Metcalfe	43.5	4.6	4.3	82.7	83.7	10.9	0.830	47.7	384	52
CDC Copeland	43.4	4.5	4.1	82.2	83.2	10.9	0.811	46.6	328	57
CDC Goldstar	43.4	4.8	5.2	83.3	84.5	10.1	0.824	51.4	378	39

Table 3. Malt quality of 'CDC Goldstar' from pilot-scale and commercial scale brewing trials

Variety	Steep-out moisture (%)	Malt moisture (%)	Wort colour (°EBC)	Fine extract (%; d.b.)	Apparent final attenu- ation (%)	Total protein (%)	Soluble nitrogen (%)	Kolbach Index	Diastatic power (°WK)	Wort β- glucan (mg/L)
(Pilot-scale brewing	g)									
CDC PlatinumStar	42.6	6.8	2.9	80.9	81.3	12.9	0.783	37.8	401	183
CDC Goldstar	42.4	5.6	3.1	81.9	84.4	12.5	0.758	37.9	478	138
(Commercial scale	brewing)									
Control malts	_	4.7	4.1	80.9	83.6	11.6	0.830	44.7	410	34
CDC Goldstar	_	4.7	4.9	81.5	84.4	12.1	0.681	35.2	438	49

varieties, and the soluble nitrogen level was within the satisfactory range for malts in North America.

In the fermentation process, the extract and number of yeast cells were monitored, and there was no significant difference among varieties (data not shown). Brewing performance data for pilot-scale brewing trials are shown in **Table 4**. No clear difference was observed between 'CDC Goldstar' beer and 'CDC PlatinumStar' beer for aldehydes, esters, foam stability, THOD, or T2N. Both varieties lacked the LOX-1 enzyme and therefore the beer produced maintained its freshness in these trials, as observed in our previous trials (Hoki *et al.* 2013, 2018a, 2018b).

Brewing performance in commercial-scale brewing trials

Commercial brewing trials were conducted using malts produced in the commercial malting facilities of Prairie Malt. Only single-batch production for 'CDC Goldstar' malt was carried out, and the modification (especially proteolytic modification) was not satisfactory compared to control malts (**Table 3**). Although lower soluble nitrogen in 'CDC Goldstar' malt directly affected the concentration of free amino nitrogen in wort and beer, there was no significant difference between control beer and 'CDC Goldstar' beer in regard to major beer quality-related parameters (**Table 4**).

Sensory evaluation

Total staleness in aged beer (37°C for 1 week, 30°C for 1 month), scored by trained panelists, is shown in **Fig. 2**. The sensory scores for 'CDC Goldstar' beer in pilot-scale brewing trials were lower (meaning "fresher") than those for 'CDC PlatinumStar' beer, although the differences were not statistically significant at the 5% probability level. No difference was observed between control beer and 'CDC Goldstar' beer with regard to sensory evaluation in commercial-scale brewing trials. The sensory scores for normal beers stored 30°C for 1 month are 2.0–3.0, therefore the aged beer tested in the brewing trials were fresher due to the use of LOX-1-less varieties.

Discussion

In 2019, 'CDC Copeland', 'AC Metcalfe', and 'AAC Synergy' dominated the portfolio of malting barley varieties in Western Canada (McMillan *et al.* 2019). 'CDC Copeland' accounted for 44% in cultivated area that year, thereby remaining a leading variety. The 'AAC Synergy' planted area has been increasing since 2014 owing to its excellent agronomic performance and malting quality. In this study, 'CDC Goldstar' showed a 6% higher yield than 'CDC Copeland', which indicates its potential to compete with 'AAC Synergy' (Table 1). Additionally, the yield of 'CDC

Table 4.	Brewing performance	e of 'CDC Goldsta	ir' in p	oilot scale trials a	nd commercial scale trials

Trial variety		Pilot-sc	ale	Commercial scale		
Irial variety		CDC PlatinumStar	CDC Goldstar	Control malts	CDC Goldstar	
(Wort)						
Extract	(%)	11.68	11.64	11.96	12.00	
BU		48.9	47.4	38.2	38.0	
pН		5.51	5.59	5.15	5.16	
Colour	(°EBC)	5.2	4.9	8.4	7.9	
Apparent Extract	(%)	1.89	1.49	1.92	1.84	
Free Amino Nitrogen	(mg/L)	_	_	157	122	
(Beer)						
Original Gravity	(%)	11.16	11.13	11.05	10.96	
Apparent Extract	(%)	1.71	1.39	1.62	1.52	
Alcohol	(vol. %)	4.99	5.14	4.97	4.98	
Colour	(°EBC)	3.7	3.4	5.9	5.4	
BU		27.3	26.3	21.6	21.1	
рН		4.44	4.44	4.24	4.30	
Free Amino Nitrogen	(mg/L)	_	_	73	46	
Acetoaldehyde	(mg/L)	0.9	1.3	0.5	0.6	
Acetone	(mg/L)	0.1	0.1	0.1	0.1	
Ethyl Acetate	(mg/L)	22.1	25.5	15.7	14.3	
Isoamyl Acetate	(mg/L)	1.5	1.9	1.4	1.1	
n-Pronanol	(mg/L)	6.9	6.2	8.9	9.7	
Isobutanol	(mg/L)	7.5	8.0	6.2	6.1	
Isoamyl Alcohol	(mg/L)	45.0	46.0	47	49	
NIBEM	(sec)	271	269	254	252	
THOD	(mg/L)	1.3	1.4	2.5	2.1	
Γ2N						
Fresh beer	(µg/L)	0.04	0.04	0.04	0.03	
$37^{\circ}C \times 1W$	(µg/L)	0.08	0.09	0.10	0.09	
$30^{\circ}C \times 1M$	(µg/L)	0.11	0.11	0.13	0.12	



Fig. 2. Sensory evaluation (total staleness) of aged beers in pilot and commercial scale brewing-trials. n.s.: not significant at the 0.5% probability level.

Goldstar' is comparable to that of 'Xena', a major feed barley variety in Canada. Although there is no direct comparison of yield between 'CDC Goldstar' and other LOX-1-less varieties, the yield of 'CDC PolarStar' is estimated to be 13% lower and that of 'CDC PlatinumStar' is 6% lower than that of 'CDC Goldstar', assuming that the yield of a backcross variety is same as its recurrent parent. The length of a spike in 'CDC Goldstar' tended to be shorter than that in 'CDC Copeland' (**Fig. 1**); however, the larger number of spikes probably contributes to a more stable and higher yield in this variety. Furthermore, 'CDC Goldstar' showed high lodging tolerance and may have an advantage over 'AAC Synergy' in this respect.

As for malt quality, 'CDC Goldstar' showed higher fine extract, higher Kolbach Index, higher diastatic power, and lower wort β -glucan than 'CDC Copeland' (Table 2). One of the reasons brewers in North America prefer 'CDC Copeland' is its craft-like quality profile, which is lower in soluble nitrogen and diastatic power than higher enzymatic varieties (e.g. AC Metcalfe); therefore, it is tempting to speculate whether 'CDC Goldstar' can be used to produce craft-type beers. When we tested the malt quality in a micro-malting system with lower steep-out moisture, 'CDC Goldstar' showed lower soluble nitrogen content than control malts and satisfactory wort β-glucan content (data not shown). Indeed, 'CDC Goldstar' malts used for brewing trials competed well with control malts, although 'CDC Goldstar' showed higher diastatic power. We believe it is worth trying this new variety for craft- and typical lagertype brewing, as 'CDC Goldstar' shows several advantages

with respect to both agronomic characteristics and malt quality.

Sapporo Breweries has released several LOX-1-less malting barley varieties in Australia and Japan, as well as in Canada, and showed that the LOX-1-less trait is effective in all types of genetic background (Hoki et al. 2013, 2018a, 2018b). The earlier LOX-1-less varieties were based on backcross breeding, which implies that the basic characteristics of those varieties were similar to their recurrent parents. However, 'CDC Goldstar' is the first registered variety developed from an original cross and it showed high agronomic and brewing performances, with beer T2N and THOD contents similar to those in 'CDC PlatinumStar'. THOD contents in commercial-scale brewing trials were slightly higher than those in pilot-scale brewing trials, implying more oxidative conditions in commercial brewing trials (Table 4). However, T2N content in aged beers were still low even in commercial-scale brewing trials, ranging from 0.09 to 0.13 μ g L⁻¹. As the threshold value for T2N in beer is 0.1 µg L⁻¹, T2N content of the aged beers in this study were acceptable (Wang and Siebert 1974). As a result, there was no significant difference in sensory evaluation among the beers and the beers produced from 'CDC Goldstar' malt had an enduring fresh taste (Fig. 2).

Currently, 'CDC PlatinumStar' is a major LOX-1-less barley variety available in Canada that is widely acceptable for brewers, as its malt and brewing qualities are almost the same as those in 'CDC Copeland'. However, 'CDC Goldstar' has a higher enzymatic profile and lower wort β glucan content, and proteolytic modification may be controllable. Finally, 'CDC Goldstar' has a higher yield than the current LOX-1-less variety, 'CDC PlatinumStar', with a different malt profile; thus, 'CDC Goldstar' is a promising malting barley variety in Canada.

Author Contribution Statement

MN, YT, TH, WS, and AB jointly bred the new variety. WS made the cross and MN, YT, TH, and AB selected the line in each breeding process. AB also managed the field trial in Canada. RA and TY conducted brewing trials and evaluated brewing quality. NH, NS, and AB managed and supervised the joint breeding program.

Acknowledgments

Sapporo Breweries has released the new variety under the joint breeding program with the University of Saskatchewan and Prairie Malt and are grateful to all participating staff. We also thank our colleagues in Sapporo Breweries for technical assistance in malting, brewing, and quality analysis.

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