Divergent Selection for Amino-nitrogen Concentration in Sugarbeet Roots

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ABSTRACT

Amino-nitrogen is a naturally occurring constituent of sugarbeet that interferes with the extraction of crystallized sucrose during normal factory operations. This study examined 1) the extent amino-nitrogen concentration could be altered by selection within a broad-based germplasm line and 2) the impact selection for amino-nitrogen had on other components of processing quality. Four cycles of mass selection for low amino-nitrogen concentration resulted in a 29% reduction; whereas, selection for high amino-nitrogen concentration increased the concentration by 50%, compared to the parental source. The line selected for low amino-nitrogen concentration had higher concentrations of two other impurity components, sodium and potassium, and a lower sucrose loss to molasses than the line resulting from selection for high amino-nitrogen concentration. Selection for amino-nitrogen concentration, either high or low, did not have a detectable impact on sucrose concentration. Root yield of the line selected for low amino-nitrogen was 9 Mg ha^{-1} greater than the root yield of the line selected for high concentration.

Additional Key Words: *Beta vulgaris*, impurities, potassium, sodium, sucrose, sucrose loss to molasses.

Sugarbeet (*Beta vulgaris* L.) root quality impacts factory efficiency and hence, profitability of the sugarbeet industry. Quality of healthy roots routinely is determined by sucrose concentration and the concentration of naturally occurring compounds, referred to as impurities, which prevent sucrose extraction or slow processing (van den Hil and de Nie, 1989; Campbell, 2002; Dutton and Huijbregts, 2006). For each kg of these impurities, 1.5 kg of sucrose is retained in the molasses. A 1 g kg^{-1} (0.1%) reduction in the loss to molasses across the U.S. would have resulted in a 5-year average annual recovery of an additional 27,723 metric tons of sugar from the 24.3 to 31.9 million metric tons of sugarbeet produced annually since 2008, with little or no increase in processing costs.

Among the impurity components, sodium, potassium, and amino-nitrogen have received the most attention in variety development programs (Campbell, 2005; Hoffman et al., 2011) and are frequently combined with sucrose concentration to calculate payments to growers based upon recoverable sugar per ton. Amino-nitrogen concentration is of special interest not only because of its importance in estimating sucrose loss to molasses (Cariolle and Duval, 2006), but also because of its direct relationship to nitrogen fertility management (Campbell, 2005). Supplemental nitrogen fertilizer frequently is required for optimum productivity; however, excessive nitrogen not only reduces sucrose concentration but also increases the concentration of many of the non-sugar constituents, particularly amino-nitrogen, which interfere with sucrose extraction (Jaggard and Armstrong, 2009; Pocock et al., 1990). Nitrogen management, therefore, is critical, in sugarbeet production: however, complex relationships among environment and production practices complicate nitrogen management decisions (reviewed in Draycott and Christenson, 2003; Cariolle and Duval, 2006). Nevertheless, education, financial incentives, and varietal approval standards based upon processing quality have reduced the amino-nitrogen concentration and increased the sucrose concentration of roots delivered to factories in the United Kingdom (Dutton and Huijbregts, 2006) and the Red River Valley (Hilde et al., 1983).

Based upon an analysis including 52 environments throughout Europe and nine varieties, Hoffman et al. (2009) reported that differences in amino-nitrogen concentration among varieties in environments with low average concentrations were relatively small compared to those observed in environments with high average amino-nitrogen concentrations. Although the magnitude of the differences among varieties was dependent upon the environment, the relative ranking of the varieties was consistent. Based upon a similar analysis encompassing 11 varieties and 23 environments in the Red River Valley, Campbell and Kern (1983) recommended that because of the consistency of relative amino-nitrogen concentration over environments and its relatively large impact on sucrose extraction, amino-nitrogen deserves consideration in attempts to improve sugarbeet quality. A similar consistency of relative rank of amino-nitrogen concentration in diverse locations

also was noted by Owen et al. in a 1960 trial that examined the contribution of six pollinators to hybrid performance. Two cycles of selection for low amino-nitrogen from a heterogeneous population resulted in a 36% reduction in amino-nitrogen concentration. In contrast, selection for high concentrations increased the amino-nitrogen concentration by 93% (Smith and Martin, 1989). Continued selection from this same population resulted in little or no additional change in the amino-nitrogen concentration (Campbell and Fugate, 2012). Smith et al. (1973) observed a predominance of additive gene action for amino-nitrogen and five other nonsucrose components at two contrasting nitrogen fertility levels. Quantitative trait loci that regulate amino-nitrogen concentration have been identified on chromosomes 3 and 4, and two additional Quantitative trait loci that control ion balance have been localized to chromosomes 5 and 9 (Schneider et al., 2002).

In this report, we examine the extent amino-nitrogen concentration can be altered by mass selection within a broad-based germplasm line and the impact almost exclusive selection for amino-nitrogen has on other impurity components, sucrose concentration, and root yield.

MATERIALS AND METHODS

F1028 (PI 668026) and F1029 (PI 668027) were selected almost exclusively for low and high amino-nitrogen concentration, respectively, from F1010 (PI 535818). F1010 is a high-sucrose heterogeneous multigerm germplasm selected from a broad-based population formed by intermating selected accessions from the USDA-ARS *Beta* germplasm collection (Campbell, 1989; 1990).

Selection was based upon the amino-nitrogen concentration of individual roots relative to other roots within a grid. Individual cells of the grid were 10 m long and two rows wide with a row-spacing of 56 cm. Plants on the ends of the rows were not harvested. Initially, the amino-nitrogen concentrations of 250 F1010 roots were determined, and 43 roots with low concentration and 41 roots with high amino-nitrogen concentration were selected. Each group was allowed to interpollinate, seed was harvested in bulk, and subsequent selection was within a group. Moderate-size roots typical of the parental population were chosen for the individual amino-nitrogen measurements during each of four mass selection cycles. Samples for analysis were obtained by collecting the tissue removed diagonally from the taproot with a 3.2 cm wood bit and an electric drill (~ 10 cm long). Sampled roots remained viable and were used as mother roots to produce seed for additional selection cycles. Relatively low selection intensities (24 to 42%) were employed to retain much of the genetic diversity within F1010. The number of plants selected within a group and selection cycle ranged from 30 to 51. Selected roots from the fourth cycle provided seed for replicated field trials between 2008 and 2012.

The experimental design for the 2008 to 2012 performance trials

was a randomized complete block with four replicates. Individual experimental units were two-row by 10-m plots with rows 56 cm apart. Trials were planted near Fargo, ND during the first 2 weeks of May and harvested during the last 2 weeks of September. Weeds were controlled with herbicides, cultivation, and hand weeding, as needed. In addition to F1010, F1028, and F1029, the field trials included a commercial hybrid, ACH-817 (Crystal Beet Seed, Moorhead, MN). Root yield was the weight of all roots from a single plot at harvest expressed as Mg ha^{-1}. Sucrose, sodium, potassium, and amino-nitrogen concentrations were based upon brei samples from a composite random sample of 10-12 roots from each plot that had been passed through a beet saw.

The brei from each field sample or individual root tissue sample was mixed and quickly frozen for later analysis. Sucrose was determined polarimetrically (Autopol 880, Rudolph Research Analytical, Flanders, NJ) using aluminum sulfate-clarified brei samples (McGinnis, 1982). The aluminum sulfate-clarified filtrate used to determine sucrose concentration also was used to measure sodium, potassium, and amino-nitrogen concentrations. Sodium and potassium concentrations were determined by flame-photometry (Corning 410C, Cole-Parmer Instrument Co., Chicago, IL). Amino-nitrogen concentration was determined with a spectrophotometer (Spectronic-21D, Milton Roy Co., Ivyland, PA) using the copper method and a wavelength of 610 nm (International Commission for Uniform Methods of Sugar Analysis, 2007). Aliquots of an L- glutamine solution were used to establish a standard curve. Only root weight and sucrose and amino-nitrogen concentrations were determined for the individual root tissue samples.

Sucrose loss to molasses (LTM), an estimate of the sucrose that will be contained in the molasses after normal extraction is completed, was calculated using the equation American Crystal Sugar Company (Moorhead, MN) uses when calculating payments to individual growers:

$$LTM = 1.5 \times [(Na \times 3.5) + (K \times 2.5) + (AmN \times 9.5)] / 1100.$$

Sodium (Na), potassium (K) and amino-nitrogen (AmN) concentrations are expressed as mg kg^{-1} (parts per million) and LTM as g kg^{-1}. Root yield and the concentrations of all other variables are reported on a fresh weight basis. The SAS GLM procedure (ver. 9.1, SAS Institute, Inc., Cary, NC) was used for the analysis of variance with $\alpha = 0.05$. Years were assumed to be random effects and treatments fixed effects (McIntosh, 1983). The "estimate" function of the SAS GLM procedure was used to quantify differences between the 5-year means of F1028 and F1010, F1029 and F1010, and F1029 and F1028.

RESULTS

Only a few selection cycles were completed before substantial differences between the high and low amino-nitrogen sub-populations were apparent. The amino-nitrogen concentration of the high popula-

Table 1. Changes in amino-nitrogen concentration, sucrose concentration, and root weight in response to four cycles of selection for low and high amino-nitrogen concentration in F1010, Fargo, ND. 2001-2006.

Year: Selection cycle	Amino-nitrogen			Sucrose (SE)	Root weight (SE)
	Mean (SE)	Range Low	Range High		
	---------- *mg kg^{-1}* ----------			*g kg^{-1}*	*g root^{-1}*
2001: No prior selection					
F1010	1295(40)†	154	3744	163(1)	1055(27)
2003: First cycle					
Low amino-N	1523(26)	771	3158	147(1)	1211(31)
High amino-N	2015(51)	771	5579	151(1)	970(26)
2004: Second cycle					
Low amino-N	635(18)	212	1651	132(1)	806(24)
High amino-N	1054(46)	302	3952	106(1)	838(27)
2005: Third cycle					
Low amino-N	285(9)	72	577	119(1)	748(22)
High amino-N	506(20)	190	1368	129(1)	619(19)
2006: Fourth cycle					
Low amino-N	254(14)	21	814	161(1)	1199(34)
High amino-N	819(44)	150	2444	165(1)	902(31)

†Numbers in parenthesis are the standard errors of the mean.

Table 2. Amino-nitrogen, sodium, potassium, sucrose loss to molasses, sucrose concentration, and root yield of F1028 (selected for low amino-nitrogen), F1029 (selected for high amino-nitrogen), F1010 (the parental population), and an adapted hybrid (ACH-817), in field trials at Fargo, ND, 2008-2012.

Year	F1028	F1010	F1029	ACH-817	Year mean
			Amino-nitrogen (mg kg^{-1})		
2008	329 a[†]	361 a	370 a	375 a	359 C
2009	412 b	527 b	1041 a	511 b	623 B
2010	264 a	362 a	487 a	373 a	372 C
2011	289 a	485 a	633 a	628 a	509 BC
2012	681 b	1057 b	1802 a	1040 a	1145 A
Mean	395 C	558 B	867 A	585 B	601
			Sodium (mg kg^{-1})		
2008	805 ab	850 ab	675 b	895 a	806 B
2009	785 a	515 b	430 b	575 ab	576 C
2010	310 b	300 b	300 b	440 a	338 D
2011	1165 a	1010 a	912 a	822 a	978 A
2012	430 a	530 a	495 a	440 a	474 C
Mean	699 A	641 AB	562 B	634 AB	634
			Potassium (mg kg^{-1})		
2008	2090 a	1740 b	1840 ab	1885 ab	1889 C
2009	1835 a	1620 a	1670 a	1735 a	1715 D
2010	2145 a	1925 b	2040 ab	2090 a	2050 B
2011	2340 a	2160 ab	2075 ab	1905 b	2120 B
2012	2665 a	2245 b	2480 ab	2340 ab	2432 A
Mean	2215 A	1938 B	2021 B	1991 B	2041
			Sucrose loss to molasses (g kg^{-1})		
2008	15 a	15 a	14 a	16 a	15 CD
2009	15 b	15 b	21 a	15 b	17 BC
2010	12 a	13 a	15 a	14 a	13 D
2011	17 a	18 a	20 a	18 a	18 B
2012	20 b	24 b	34 a	24 b	25 A
Mean	16 B	17 B	21 A	17 B	18

Year	F1028	F1010	F1029	ACH-817	Year mean
			Sucrose (g kg^{-1})		
2008	163 a	162 a	158 a	171 a	163 B
2009	151 b	155 b	151 b	165 a	156 C
2010	152 b	164 ab	156 ab	167 a	160 BC
2011	131 b	139 b	135 b	151 a	139 D
2012	189 b	177 c	182 bc	202 a	187 A
Mean	157 B	159 B	156 B	171 A	161
			Root yield (Mg ha^{-1})		
2008	65 a	57 a	45 b	60 a	57 A
2009	44 a	37 b	31 b	49 a	40 B
2010	55 b	52 b	49 b	64 a	55 A
2011	37 ab	34 b	28 c	42 a	35 C
2012	34 b	39 b	37 b	58 a	42 B
Mean	47 B	44 C	38 D	55 A	46

†Differences among genotypes within a year followed by the same lower case letter are not significant, according to Fisher's Protected $LSD_{0.05}$; differences among main effect means followed by the same upper case letter are not significant (P = 0.05).

tion (2015 mg kg^{-1}) was 1.3 times that of the low amino-nitrogen population (1523 mg kg^{-1}) in progeny of the individual roots initially selected from F1010 (Table 1). Amino-nitrogen concentrations of progeny of the first, second, and third high-concentration selection cycles were 1.7, 1.8, and 3.2 times that of the low amino-nitrogen selections, respectively. The 3.2 multiple observed in 2006 was greater than the magnitude (1.1 to 2.6) of any of the subsequent multiples observed in the 5 years of yield trials (Table 2). In all selection cycles, the amino-nitrogen concentration range of the progeny of the high amino-nitrogen selections included individual roots with amino-nitrogen concentrations that exceeded the concentration of all progeny of the low amino-nitrogen selections (Table 1). Likewise, in all years except one (2003), the range of the progeny of the low amino-nitrogen selections included roots that were lower than any individual progeny selected for high amino-nitrogen concentration. In all except the second cycle (2004), the mean sucrose concentration of the high amino-nitrogen selections exceeded the sucrose concentration of the low selections and the mean root weight was less than that of the low selections. For the population (F1010) prior to selection (2001),

Table 3. Differences between average amino-nitrogen, sodium, potassium, sucrose loss to molasses, sucrose concentration, and root yield of the parental line (F1010) and lines selected for low (F1028) and high (F1029) amino-nitrogen concentration and differences between the two selected lines, F1028 and F1029, in field trials at Fargo, ND, 2008-2012.

	F1028 minus F1010†	F1029 minus F1010†	F1029 minus F1028‡
Amino-nitrogen (mg kg^{-1})	-163 *	309 **	472 **
Sodium (mg kg^{-1})	58 ns	-79 ns	-137 **
Potassium (mg kg^{-1})	277 **	83 ns	-194 **
Loss to Molasses (g kg^{-1})	-1 ns	4 **	5 **
Sucrose (g kg^{-1})	-2 ns	-3 ns	-1 ns
Root yield (Mg ha^{-1})	3 *	-6 **	-9 **

* and ** indicate difference is significant and the 0.05 and 0.01 probability levels, respectively; ' ns' indicates difference was not significant ($P < 0.05$).
†The difference is equal to the average of the selected line minus the average of the parental line (F1010).
‡The difference is equal to the average of the line selected for high amino-nitrogen (F1029) minus the average of the line selected for low amino-nitrogen (F1028).

the correlations between amino-nitrogen and sucrose (-0.22; $P<0.001$) and between sucrose and root weight (-0.46; $P<0.001$) were negative and the correlation between amino-nitrogen and root weight (0.24; $P<0.001$) was positive.

The analysis of the performance trials including all 5 years and the four genotypes indicated the amino-nitrogen concentration of F1029 was greater than the amino-nitrogen concentration of F1010 and F1028 and that the concentration of F1028 was lower than that of F1010 (Table 2). Selecting for high amino-nitrogen concentration resulted in an average increase of 309 mg kg^{-1} (Table 3), or 55%, compared to the parental population, F1010. In each of the 5 years of trials,

the amino-nitrogen concentration of F1028 was lower than the unselected parental population, F1010, and the 5-year average difference of 163 mg kg^{-1} (Table 3) between F1028 and F1010 was significant. The difference between the mean amino-nitrogen concentration of F1028 and ACH-817 was significant; whereas, the difference between F1010 and ACH-817 was not (Table 2). Sixty-five percent of the 472 mg kg^{-1} difference between F1028 and F1029 appears to have resulted from selecting for high amino-nitrogen concentration with the remaining 35% the result of selecting for low concentration (Table 3). A significant genotype X year interaction for amino-nitrogen concentration was due to small differences in the relative concentration of ACH-817 compared to the other genotypes, particularly F1010, and to differences in the magnitude but not the order of the differences among F1010, F1028, and F1029 (Table 2).

With the exception of the difference between F1028 and F1029, differences among F1010, F1028, F1029, and ACH-817 in sodium concentration were not significant (Table 2). The 137 mg kg^{-1} difference between F1028 and F1029 resulted from a reduction of 79 mg kg^{-1} in F1029 and an increase of 58 mg kg^{-1} in F1028 in conjunction with selection for high and low amino-nitrogen concentration, respectively (Table 3). Differences in potassium concentration among F1010, F1029, and ACH-817 were small and not significant in all years except 2010; however, the 5-year average potassium concentration of all three was lower than the potassium concentration of F1028 (Table 2). F1028, the line selected for low amino-nitrogen concentration, had 277 mg kg^{-1} more potassium than the unselected parental population, F1010 (Table 3); a 14% increase in potassium.

Selecting for increased amino-nitrogen concentration (F1029) resulted in an increase in the loss to molasses (Tables 2 & 3), compared to the parental population (F1010); however, selecting for low amino-nitrogen concentration (F1028) did not reduce the loss to molasses. This was primarily due to a 14% increase in potassium and a small increase in sodium concentration that accompanied selection for low amino-nitrogen concentration. The loss to molasses for F1028 and F1010 was similar to the loss to molasses of ACH-817. Differences in sucrose concentration among F1010, F1028, and F1029 were small and did not follow a discernible pattern, indicating that selecting for amino-nitrogen concentration had little or no impact on sucrose concentration. The sucrose concentration of F1010, F1028, and F1029 was approximately 90% of the sucrose concentration of ACH-817. The average root yield of F1028 was 7% greater than the root yield of F1010. In contrast, the average root yield of F1029 was 14% less than the root yield of F1010. Root yields of F1028 were significantly higher than the root yields of F1029 in 3 of 5 years and in only 1 year (2012) was the root yield of F1029 slightly higher than the root yield of F1028. The root yield of F1010 was approximately 80% of the root yield of ACH-817.

DISCUSSION

The 29% reduction in amino-nitrogen concentration resulting from selection for low amino-nitrogen is similar to the 24% reduction achieved through selection for low amino-nitrogen concentration in an unrelated heterogeneous population (Campbell and Fugate, 2012). Selection for increased amino-nitrogen concentration resulted in a greater change in concentration than selecting for low concentration in this trial and the trial conducted by Smith and Martin (1989). The asymmetrical response to divergent selection in this study is consistent with the observation by Smith and Martin (1989) that a greater variance for higher selection might be expected as past selection within the parental populations may have reduced the variance for reduced amino-nitrogen concentration, and other impurities. The magnitude of the difference in amino-nitrogen concentration between F1028 and F1029 increased as the average concentration for the year, or F1010, increased; a relationship also noted by Hoffman et al. (2009) in an extensive analysis of European yield trials.

The significant differences between F1028 and F1029 for sodium, potassium, and loss to molasses indicated the change in amino-nitrogen affected the concentration of the other two impurity components and loss to molasses. F1028 had higher concentrations of sodium and potassium and a lower loss to molasses than F1029. The loss to molasses difference between F1028 and F1010 was not significant, suggesting that the increased sodium and potassium in F1028 at least partially compensated for the reduced amino-nitrogen concentration of F1028. In an unrelated line also selected for low amino-nitrogen concentration, F1027 (PI 665410), the loss to molasses was significantly lower than the loss to molasses of the parental population (Campbell and Fugate, 2012).

The reduced amino-nitrogen concentration of F1028 did not result in a decrease in the loss to molasses and hence, did not increase the value of recently harvested roots. However, the combination of reduced amino-nitrogen and increased sodium and potassium may increase sucrose extraction from stored roots during the processing campaign. As amino-nitrogen concentrations increase, the acidity of the thick juice also increases, creating a need for additional soda ash (Na_2CO_3). The sodium from the Na_2CO_3 increases the sodium concentration of the juice, resulting in an increase in the sugar retained in the molasses. Higher concentrations of sodium and potassium in the root increase the natural alkalinity of the juice and reduce the soda ash requirement (Junghans et al., 1998).

Selection for amino-nitrogen concentration, either high or low, did not have a detectable impact on sucrose concentration. Selection for high amino-nitrogen reduced root yields by 6 Mg ha^{-1}. In contrast, selecting for low amino-nitrogen increased root yields by 3 Mg ha^{-1}. In four of the five years of trials, the root yield of the low selection (F1028) was greater than the root yield of F1029, the high selection (Table 2).

Inherent differences among adapted varieties in amino-nitrogen concentration have been documented (Campbell and Kern, 1983; Hilde et al., 1983; van den Hil and de Nie, 1989; Hoffman et al., 2009). Substantial reductions in amino-nitrogen concentration have been achieved with only a few selection cycles from a heterogeneous parental source (Smith and Martin, 1989). Interactions among the impurity components have complicated efforts to improve sucrose extraction rates by altering a single impurity component (Owen, et al., 1960; Campbell, 2005). Some of these relationships may be dependent upon the population examined. Tsialtas and Maslaris (2009) concluded that in some environments, sugarbeet nitrogen nutrition and therefore, amino-nitrogen concentration may be affected by a genotype's ability to exclude sodium from the root. In addition to quantitative trait loci that regulate amino-nitrogen concentration, quantitative trait loci that regulate ionic balance in the root also have been detected (Schneider et al., 2002). Additional research to determine how the impurity components interact with each other, with the environment, and with sucrose concentration and root yield will facilitate progress in improving inherent sugarbeet processing quality.

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