Malting Quality of Barley Lines Derived from Tissue Culture

PHIL BREGITZER,[1] M. POULSON,[1] and B. L. JONES[2]

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ABSTRACT

The occurrence of somaclonal (tissue culture-derived) variation in plants regenerated from tissue culture will influence the efficiency with which techniques such as genetic transformation can be used in the development of new barley cultivars. To assess the effect of somaclonal variation on malting quality, 12 families of tissue culture-derived lines from three barley cultivars were analyzed using standard micromalting techniques. Each family was derived from a single regenerated plant that, in turn, was derived from an immature embryo callus culture. Five to six plants from each family were selected in the R_2 generation based on phenotypic similarity to their uncultured parental controls, and advanced to the R_4 and R_5 generations for replicated field tests. The malting quality of the majority of these lines was altered by passage through tissue culture, and most alterations were undesirable. These results suggest that efforts should be made to delineate in vitro (tissue culture) conditions that are less mutagenic to cultured barley cells.

Techniques such as genetic transformation of barley (Wan and Lemaux 1994) have the potential to improve commercially acceptable cultivars by making specific, single trait alterations. Genetic transformation would be particularly useful for improving cultivars used by the malting and brewing industry, because their end-use constraints impose narrow parameters on grain quality and favor small, stepwise changes in cultivar characteristics.

Current methods for genetic transformation of barley and other cereal species require in vitro growth (tissue culture) as a component of the transformation system. The in vitro environment is known to be highly mutagenic to cultured tissues (Kaeppler and Phillips 1993), and can generate spontaneous and heritable genetic changes, termed somaclonal variation (Larkin and Scowcroft 1981). The occurrence of somaclonal variation in transgenic, tissue culture-derived (TCD) plants has serious potential implications for genetic transformation because these plants would contain unselected phenotypic changes in addition to the desired change encoded by the transgene. Severe and widespread somaclonal variation in TCD transgenic plants would preclude the small, stepwise changes that are desired for the rapid development of new malting barley cultivars.

Several studies have examined the morphological and agronomic characteristics of TCD barley lines relative to those of the uncultured parent cultivars (Ullrich et al 1991, Baillie et al 1992, Bregitzer and Poulson 1995). Although some TCD lines were produced that did not have measurable somaclonal variation, many TCD lines showed reductions in agronomic performance or changes in morphological characteristics.

The malting quality of TCD barley lines has not been extensively studied. Our objectives were to determine whether tissue culture generated somaclonal variation for malting quality and to characterize any observed variation.

MATERIALS AND METHODS

The generation of the plant materials has been reported in detail (Bregitzer and Poulson 1995). Briefly, the seed source for all TCD lines and controls was breeders seed used in the USDA-ARS barley breeding program at Aberdeen, ID. The cultivars studied were Klages, Morex, and Piroline. Callus cultures were initiated from immature embryos derived from ≈15 plants of each cultivar. The calli were initiated on MS medium (Murashige and Skoog 1962) supplemented with 3 mg/L 2,4-dichlorophenoxyacetic acid (2,4-D), transferred after four weeks to medium supplemented with 1.5 mg/L 2,4-D and maintained for an additional four weeks, and finally transferred onto MS medium without 2,4-D to induce plant regeneration.

Individual regenerated plants (the R_0 generation) were advanced to the R_2 generation (second self-pollinated generation). One hundred individual R_2 plants (lines) from each R_0-derived family were grown in irrigated field plots. Five or six plants that were visually similar to their uncultured parental controls were selected from each family, and their seed increased to the R_4 and R_5 generations via self-pollination to produce 71 R_2-derived lines from 12 R_0-derived families.

All TCD lines, plus their uncultured parental controls, were planted in four-row plots in a randomized complete block design with four replicates. The test plots were grown in Aberdeen, ID, (irrigated) in 1992 and 1993, and in Nez Perce, ID, (dryland) in 1993. Grain samples used for malting quality analyses consisted of an equal blend from two replicates from each of the three test sites. Samples (170 g) were malted and malting quality analyses were conducted at the USDA-ARS Cereal Crops Research Unit, Madison, WI, using standard micromalting and analysis techniques (Burger and LaBerge 1985).

Analyses of variance were conducted using GLM software (SAS 1988). Each location-year combination was treated as a separate replicate. Comparisons of selections within families, and of families within cultivars versus the uncultured control, were performed using the Tukey's and Dunnett's test procedures, respectively.

RESULTS

The majority of the space-planted R_2 plants were vigorous, uniform, and indistinguishable in phenotype from the parental control plants. Observation of the R_4 and R_5 TCD lines that were developed from the selected plants also did not detect visually identifiable differences from their controls.

Analysis of variance for each trait was conducted separately for each cultivar. Tissue culture-derived lines of all cultivars tested showed significant differences among families for all traits tested, except for extract fine-coarse difference, wort N/malt N ratio, and α-amylase activity for the cultivar Morex (Table I). Selections-

[1]Direct correspondence to: USDA-ARS, National Small Grains Germplasm Research Facility, P. O. Box 307, Aberdeen, ID 83210.
[2]USDA-ARS, Cereal Crops Research Unit, 501 N. Walnut St., Madison, WI 53705.

TABLE I
Sources of Variation for Malting Quality Characteristics of Three Barley Cultivars and Their Tissue Culture-Derived Lines[a]

	Cultivar Source					
	Family			Selections Within Families		
Quality Characteristic	Klages	Morex	Piroline	Klages	Morex	Piroline
Barley protein (%)	***	**	**	NS	NS	NS
Plump barley (%)	***	***	***	NS	NS	NS
Kernel weight (mg)	***	***	***	NS	*	NS
Malt extract (%)	***	***	***	NS	NS	NS
Extract fine-coarse difference (%)	***	NS	**	NS	NS	NS
Ratio wort N/malt N	***	NS	***	NS	NS	NS
Diastatic power (deg)	***	***	***	NS	***	NS
α-amylase (20° units)	***	NS	***	NS	*	NS
β-glucan (%)	***	***	***	*	NS	NS

[a] *,**,*** = Significant at the 0.05, 0.01, and 0.001 probability levels, respectively; NS = not significant.

TABLE II
Mean Malting Quality of the Barley Cultivar Klages and Its Tissue Culture-Derived Lines

Quality Characteristic	Family[a]				
	Control	3518	8132	8922	9673
No. of Selections[b]	...	6	6	6	6
Barley protein (%)	12.7	14.0*[c]	13.9*	13.3	13.2
Plump kernels (%)[d]	62	50*	62	49*	57
Kernel weight (mg)	39.5	36.9*	39.7	38.3	38.1
Malt extract (%)	77.7	76.0*	76.5	77.9	77.5
Extract fine-coarse difference (%)	2.8	3.6	3.5	2.3	3.0
Ratio wort N/malt N	36.2	34.1	37.0	40.0*	36.2
Diastatic power (deg)	109	113	106	118	104
α-amylase (20° units)	39.5	36.1*	37.0	38.0	37.6
β-glucan (%)	0.7	0.8	0.7	0.5*[e]	0.7

[a] Families were derived from individual regenerated plants.
[b] Number of R_2-derived within-family selections tested.
[c] * = Significantly different than the control ($P = 0.5$).
[d] Percent kernels remaining on a 2.4 × 19.1 mm screen.
[e] Selection-within-family variation exists; at least one selction was not different than the control ($P = 0.5$).

TABLE III
Mean Malting Quality of the Barley Cultivar Morex and Its Tissue Culture-Derived Lines

Quality Characteristic	Family[a]			
	Control	430	2169	3665
No. of Selections[b]	...	6	6	6
Barley protein (%)	12.8	13.6	14.0*[c]	14.0*
Plump kernels (%)[d]	80	78	68	66*
Kernel weight (mg)	35.5	34.9	32.8*	33.5*[c]
Malt extract (%)	77.4	77.6	77.1	76.2*
Extract fine-coarse difference (%)	2.1	1.9	2.2	2.5
Ratio wort N/malt N	40.0	40.6	40.1	39.9
Diastatic power (deg)	142	167*[c]	192*	186*
α-amylase (20° units)	44.0	40.5	39.5*	39.4*
β-glucan (%)	0.5	0.4	0.5	0.6

[a] Families were derived from individual regenerated plants.
[b] Number of R_2-derived within-family selections tested.
[c] * = Significantly different than the control ($P = 0.5$).
[d] Percent kernels remaining on a 2.4 × 19.1 mm screen.
[e] Selection-within-family variation exists; at least one selction was not different than the control ($P = 0.5$).

TABLE IV
Mean Malting Quality of the Barley Cultivar Piroline and Its Tissue Culture-Derived Lines

Quality Characteristic	Family[a]					
	Control	2989	8415	9678	10569	10577
No. of Selections[b]	...	6	6	6	6	5
Barley protein (%)	12.2	13.4*[c]	13.7*	13.5*	13.2	13.2
Plump kernels (%)[d]	89	62*	61*	80	80	76*
Kernel weight (mg)	40.7	36.8*	36.8*	39.9	40.1	39.2*
Malt extract (%)	76.7	74.6*	74.8*	76.3	76.7	75.4
Extract fine-coarse difference (%)	2.9	5.2*	4.4*	3.2	3.2	3.3
Ratio wort N/malt N	34.6	28.5*	30.2*	34.4	35.0	30.1
Diastatic power (deg)	115	107	116	124	139*	118
α-amylase (20° units)	34.4	28.7*	29.2*	34.0	35.8	32.0
β-glucan (%)	0.6	0.9*	0.7	0.8	0.9	0.7

[a] Families were derived from individual regenerated plants.
[b] Number of R_2-derived within-family selections tested.
[c] * = Significantly different than the control ($P = 0.5$).
[d] Percent kernels remaining on a 2.4 × 19.1 mm screen.

within-families was a relatively insignificant source of variation and occurred for only four cultivar-trait combinations (Table I).

Means for the measured traits of the 71 TCD lines are therefore presented on a family-within-cultivar basis (Tables II–IV). For some cultivar-trait combinations, no family differences relative to the control were noted (e. g., diastatic power of Klages-derived families) despite the detection of significant variation (Table I). These cases derived from slight overlaps in the ranges of family means with the control performance.

The TCD lines suffered a general reduction in their percentages of plump kernels and in kernel weights. Six of 12 families showed statistically significant reductions for each of these traits. These data reflect the general decline in agronomic performance previously noted for these TCD lines (Bregitzer and Poulson

1995). The TCD lines also had increased protein levels; seven of 12 families showed statistically significant increases. Fewer families showed significant variation for malt extract percentage (four), fine-coarse extract percentage (two), wort N/malt N ratio (three), diastatic power (four), α-amylase activity (five), and β-glucan percentage (two). All significant alterations in diastatic power represented increased enzymatic activities. One TCD family had a lower β-glucan percentage, and one TCD family had a more desirable ratio of wort N/malt N (Klages 8922). All other significant alterations observed for TCD families resulted in reductions in malting quality: higher protein levels, reductions in kernel plumpness and weight, lower extract percentages, higher fine-coarse differences, and reductions in α-amylase activities.

Only one TCD family (Klages 9673) did not show significant alterations in malting quality. Three of 12 families showed reductions in malting quality only for percent protein and for physical kernel characteristics. Two families (Piroline 2989 and 8415) showed particularly poor malting qualities, with relatively large and negative alterations for most measures of malting quality. In general, the families that showed the greatest reductions in malting quality were those families that had previously been shown to have the greatest reductions in agronomic performance (Bregitzer and Poulson 1995).

DISCUSSION

These data indicate that in vitro (tissue) culture of barley generated genetic variability (somaclonal variation) for malting quality. These data also suggest that detectable somaclonal variation for malting quality will be present in the majority of TCD barley plants. It is interesting to note that the increased protein percentages and reductions in agronomic performance observed for most TCD lines (Tables II–IV) (Bregitzer and Poulson 1995) are typical responses of barley to environmental stress. This suggests that the observed somaclonal variation reflected limitations of physiological processes that were critical to normal plant growth and development.

Because the TCD lines in this study were derived from multiple plants grown from breeders seed, varietal heterogeneity (differences among individual plants within a variety) may account for some of the observed variation. However, it is unlikely that this could be a major source of the variation. Random reselections of individual plants within a heterogeneous variety (in this case, via tissue culture) would produce both poorer and better performing lines. Predominantly negative changes in malting quality were observed in this study, and measurements of the agronomic characteristics of these and other TCD lines (including transgenic lines) detected only negative somaclonal variation (Bregitzer and Halbert, *unpublished*; Bregitzer and Poulson 1995).

The efficient development of new malting cultivars is highly dependent on the use of parents (either elite breeding lines or current cultivars) that have good agronomic performance and high malting quality. If transgenic plants were produced that possessed the level of somaclonal variation observed for most of the TCD lines in this study and in previous studies (Bregitzer and Poulson 1995), they would not be desirable parents nor could they be used directly as new cultivars. Thus, the inclusion of genetic transformation in barley breeding programs would be facilitated by developing in vitro methodologies that generate less somaclonal variation.

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