

Germination and Malting Properties of Mutants Derived from Malting Barley cv. Triumph

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ABSTRACT

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Four mutants, demonstrating a range of dormancy, were derived from the malting barley cv. Triumph. Although there were environmental effects on the rate of recovery from dormancy, relative performance of the genotypes was consistent. Recovery from water sensitivity was slower than recovery from dormancy for all genotypes, but a similar ranking of genotypes was observed with two mutants germinating more readily than the parental genotype. Exposure of the grain to the plant hormone abscisic acid (ABA) at the end of each wet phase during steeping had a highly

significant effect on the malting performance of all samples. However, reduction in extract levels was significantly less in the two mutants that demonstrated more rapid recovery from dormancy. None of the mutants exceeded Triumph for hot water extract level after malting in two seasons at sites in Dundee (eastern Scotland) and Lleida (northeastern Spain). However, one mutant combined rapid recovery from dormancy with high extract levels when grown and malted under Scottish conditions and subjected to unithermal hot water extraction.

Molina-Cano et al (1989) derived a system for selecting samples with more rapid germination from a mutated barley population. A number of lines were derived from the cultivar Troubadour and showed malting quality superior to that of their parent (Molina-Cano et al 1989). One particular line, TR49, gave higher extract levels than Troubadour over a range of sites in Spain (Molina-Cano et al 1993) and also over two seasons at a site in eastern Scotland, in addition to one in northeastern Spain (Swanston et al 1995). However, Scottish-grown samples of both genotypes could not be malted for some time after harvest due to dormancy (Swanston et al 1993). For this reason, Scottish growing conditions were used to select samples with modified dormancy from populations derived by mutation breeding in Spain.

Mutants derived from the malting cultivar Triumph were grown in Scotland (Molina-Cano et al 1999) and screened for the capacity to germinate either within one month of harvest or in the presence of the plant hormone abscisic acid (ABA). One mutant, TL43, selected for reduced dormancy, had reduced sensitivity to ABA (Molina-Cano et al 1999) and resembled the *abi3* mutant in *Arabidopsis* (Romagosa et al 2001). TL43 has been the subject of considerable additional investigation, has higher levels of β -amylase than Triumph (Swanston and Molina-Cano 2001), and produces higher levels of α -amylase during malting (Molina-Cano et al 1999). In addition, when observed under scanning electron microscopy, it had a more compacted endosperm structure than its parent and also had higher levels of protein in the grain and differences in the relevant proportions of the hordein polypeptides (Molina-Cano et al 2001).

A number of other mutants with differing expressions of dormancy were also selected, but only TL9 has been studied further. TL9 had significantly greater dormancy than Triumph (Molina-Cano et al 1998) but had higher levels of β -amylase in the grain (Swanston and Molina-Cano 2001). However, from initial observations on TL9 and TL43, it appeared likely that this series of mutations in Triumph would exert effects on malting quality. Therefore we evaluated several mutants as potential parents for malting quality breeding programs in either Spain or Scotland. Although an initial study (Molina-Cano et al 1999) had suggested that α -amylase activity in Triumph and TL43 was similarly affected by ABA applied during malting, we also wanted to see whether response to ABA might provide an explanation for any differences observed in malting properties.

MATERIALS AND METHODS

Four mutants (TL9, TL43, TABA9, and TABA10) were induced in the malting cultivar Triumph as described by Molina-Cano et al (1999). They were included in two sets of experiments. The first set attempted to confirm dormancy ratings under Scottish conditions and to make a preliminary comparison of the malting quality of Spanish and Scottish grown material. The mutants were included along with the parent cultivar in two replicate trials at Lleida (northeastern Spain) and at Dundee (eastern Scotland) in 1997. Following harvest, grain was passed over a series of sieves. To preclude effects of grain size between sites, only the portion 2.5–2.8 mm was retained for subsequent analyses (Swanston and Molina-Cano 2001).

Germinative energy was determined on samples from Dundee, two and four weeks after harvest by adding 4 mL of distilled water to 100 grains as described in the Institute of Brewing recommended methods of analysis (IOB 1982). The percentage of grains germinated after three days (GE3) was recorded. The percentage of grains germinating after five days was also recorded because this character may be under a genetic control different from germinative energy (Thomas et al 1996). However although these authors referred to five-day germination as germinative capacity, it is not the recognized IOB test for this characteristic, so it was designated GE5 in this study.

Malting of grain from the 1997 season was conducted as described by Molina-Cano et al (1999) with European Brewery Convention methods of analysis (EBC 1975) used to determine levels of extract and total and soluble nitrogen. The ratio of soluble to total nitrogen (Kolbach index) was calculated for each sample. A further complete set of samples was malted with grain placed in a solution of $5 \times 10^{-4}M$ ABA at the end of each steep phase (Molina-Cano et al 1999).

In 1999, a more comprehensive study of germination was conducted. In addition to GE3 and GE5 measured at both sites as described above for two and four weeks after harvest, water sensitivity was determined by adding 8 mL of distilled water to 100 grains. Percentage of germination after three days (WS3) and five days (WS5) was again recorded.

To assess the suitability for malting under UK conditions, grain from the 1999 season was malted using the Scottish Crop Research Institute (SCRI) automated micromalting system (Swanston 1997). A steep regime of 8 hr wet, 12 hr dry, 10 hr wet, 10 hr dry, 2 hr wet, at 16°C produced steep moisture contents of 43–45%. This was followed by 96 hr of germination at 16°C. Extract and fermentability levels were determined by scaled-down versions of the IOB recommended method (Swanston and Thomas 1996). Soluble nitrogen content was measured by a spectrophotometric technique (Haselmore and Gill 1995) and Kolbach index calculated as described above.

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RESULTS

Germination, Malting Performance, and Effect of ABA

Germinative energies after three and five days at both two and four weeks after harvest in 1997 for all genotypes are shown in Fig. 1. Analysis of variance (data not shown) revealed significant effects of genotype and also of date after harvest. From Fig. 1, it is clear that the ranking of the cultivars did not change between two and four weeks after harvest. This was confirmed by absence of significant genotype by date interaction.

Hot water extract levels, soluble nitrogen percentages, and Kolbach indices, both with and without ABA treatment, for samples grown in 1997 are presented in Fig. 2. Analysis of variance on the untreated samples showed significant effects of both environment and genotype (Table I), while there was also significant genotype by environment interaction for all three characters. Extract levels from samples grown at Dundee were higher than those from Lleida, which probably reflected the fact that grain protein levels were much closer to optimal for malting at Dundee.

The significant genotype by environment ($G \times E$) interaction for extract level resulted from the change in ranking between sites of TABA10 and TL43. TABA10 and TL9 produced much lower levels of soluble nitrogen and, consequently, lower Kolbach indices

than the other genotypes at Dundee. This led to highly significant $G \times E$ interactions for both characters. TABA10 was the slowest to recover from dormancy at Dundee in 1997 and, although malting did not take place until dormancy was broken, it is possible that there was some residual water sensitivity.

Comparison of malting data with and without ABA (Table I) showed treatment had the largest effect, although differences between genotypes and environments were also significant. In addition to showing the largest reductions in soluble nitrogen and Kolbach index at Lleida, due to ABA treatment, TABA10 and TL9 also showed the largest reduction in extract level over both sites. Triumph showed a much larger reduction in extract level due to ABA at Lleida compared with Dundee, leading to a significant genotype by treatment ($G \times T$) interaction, while TABA9 and TL43 showed less effect on extract level due to ABA than Triumph or the other two mutants. Reductions in soluble nitrogen and Kolbach index due to ABA appeared to be lower in TABA10 and TL9 at Dundee, compared with Lleida and also compared with the other genotypes at Dundee. This resulted in significant ($G \times T$), $E \times T$, and $G \times E \times T$ interactions.

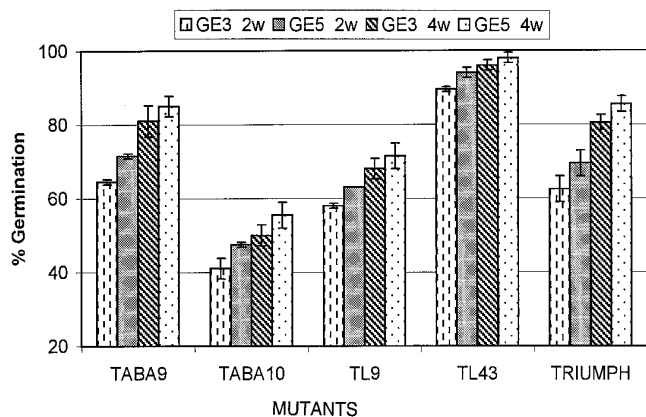


Fig. 1. Mean germinative energy after three days (GE3) and five days (GE5), two weeks (2w), and four weeks (4w) after harvest at Dundee in 1997 in cv. Triumph and four mutants derived from it. Error bars indicate standard deviations.

TABLE I
Analysis of Variance (Mean Square Values)
for Malting Quality Characters in Barleys Grown at Lleida
(Northeastern Spain) and Dundee (Eastern Scotland) in 1997

Sample	df	Extract	Soluble Nitrogen	Kolbach Index ^a
Malted without ABA ^b ($n = 19$)				
Replicate	1	0.65	0.001	5.73
Genotype (G)	4	3.60**c	0.018***	19.54**
Environment (E)	1	13.78***	0.077***	128.53***
$G \times E$	4	2.01*	0.025***	90.68***
Residual	9	0.36	0.000	2.97
All samples (malted with and without ABA) ($n = 39$)				
Replicate	1	2.02	0.000	1.85
Genotype (G)	4	20.03***	0.017***	11.54**
Environment (E)	1	35.72***	0.044***	262.14***
Treatment (T)	1	1,140.62***	1.580***	4,919.32***
$G \times E$	4	7.72**	0.011***	38.47***
$G \times T$	4	25.77***	0.004***	12.35**
$E \times T$	1	0.53	0.033***	0.03
$G \times E \times T$	4	3.96*	0.016***	59.56***
Residual	19	1.23	0.001	2.39

^a Ratio of soluble to total nitrogen.

^b Plant hormone abscisic acid (ABA).

^c *, **, *** = significant at $P < 0.05$, 0.01, and 0.001, respectively.

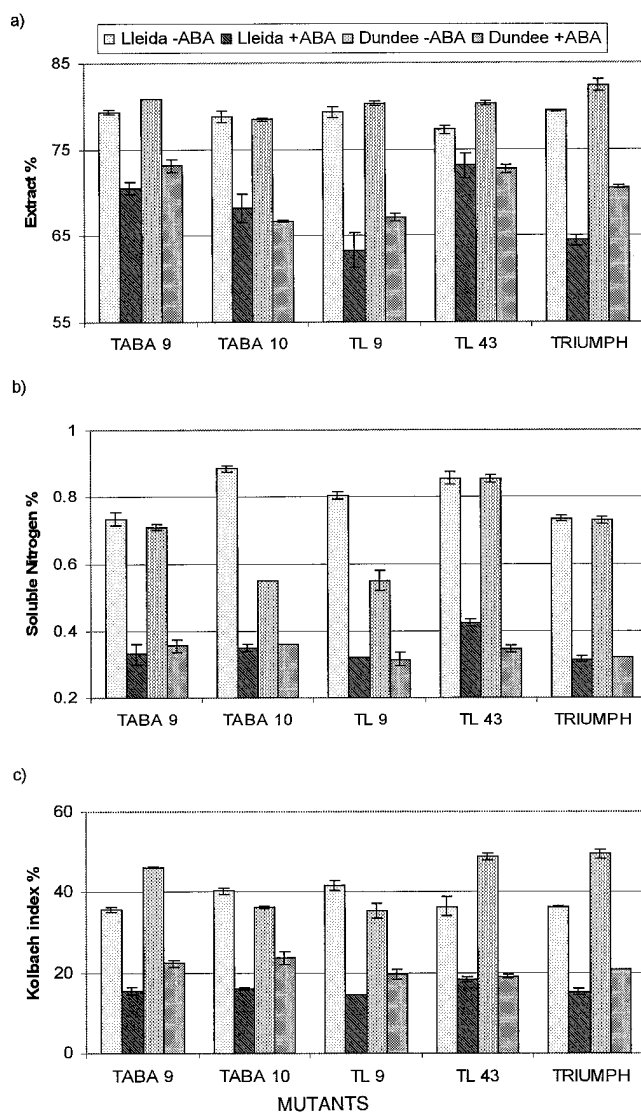


Fig. 2. Malting characters mean values for a) extract level (%), b) soluble nitrogen (%), and c) Kolbach index (%) in cv. Triumph and four mutants derived from it after malting with and without plant hormone abscisic acid (ABA) treatment of seed from the 1997 harvest at Lleida (northeastern Spain) and Dundee (eastern Scotland). Error bars indicate standard deviations.

The combination of all the effects and interactions meant that correlations between extract and protein parameters were very different between environments and treatments (Table II). At Lleida, there was a significant negative correlation between extract level and total malt protein only without ABA in steeping. With ABA, there were significant correlations between extract level and both soluble nitrogen and Kolbach index. At Dundee, the only significant correlation was between extract level and Kolbach index in the absence of ABA.

Germinative Energy and Water Sensitivity

Germinative energy (GE) and water sensitivity (WS) data from two and four weeks after harvest in 1999 are presented in Fig. 3. Analysis of variance (Table III) indicated a highly significant difference between the Dundee and Lleida sites for both GE3 and GE5. As in 1997 there were significant effects of genotype and date after harvest but no genotype \times date ($G \times D$) interaction.

There were, however, significant $G \times E$ and $E \times D$ interactions. Two weeks after harvest, TABA 9 and TL43 showed significantly higher GE3 at Lleida compared with Dundee, while TABA 10 and TL 9 were comparable at both sites. However, all genotypes were higher for GE5 at the Spanish site compared with the Scottish site (Fig. 3). By four weeks after harvest, differences between environments were no longer significant for GE3 in any of the genotypes but, except for TABA 10, there were still differences for GE5, although they were smaller than at two weeks.

TABLE II
Correlations With Extract (r) for Total and Soluble Malt Nitrogen and Kolbach Index in Two Replicates of Five Genotypes ($n = 10$) Grown at Lleida (Northeastern Spain) and Dundee (Eastern Scotland) in 1997 and Malted With or Without (+,-) ABA^a During Steeping

	Dundee		Lleida	
	+ABA	-ABA	+ABA	-ABA
Total nitrogen	0.090	-0.204	0.626	-0.784**b
Soluble nitrogen	0.127	0.498	0.776**	-0.478
Kolbach index ^c	-0.226	0.710*	0.792**	0.264

^a Plant hormone abscisic acid (ABA).

^b *, **, *** = significant at $P < 0.05, 0.01, \text{ and } 0.001$, respectively.

^c Ratio of soluble to total nitrogen.

Samples from both sites in 1999 showed considerable WS values at both two and four weeks after harvest (Fig. 3), with TABA10 and TL9 the most strongly affected. Germination in the Scottish-grown samples was particularly low, but again, it was noticeable that TL43 and TABA9 had higher levels of germination after four weeks than the other genotypes. As with GE, there were significant effects of $G, E,$ and D (Table III). There was no significant $G \times D$ interaction, but $G \times E$ and $E \times D$ interactions were significant for both WS3 and WS5. TABA9 at Lleida showed a considerable increase in germination between two and four weeks after harvest to reach a level similar to that of TL43 after five days of germination, while in Dundee, TABA9 had the lowest WS. However, TL43, which had significantly lower germination than TABA9 at Dundee for two weeks after harvest, gave fairly similar results after four weeks. Germination in 8 mL of water remained very low in the other three genotypes at Dundee.

Extract and Fermentability Levels from 1999 Trials

Although TL43 appeared to have lower extract levels than the other genotypes, particularly at Dundee (Fig. 4), differences between cultivars were not statistically significant, and there was no significant $G \times E$ interaction (Table IV). For all malting parameters, the very large difference between the sites was the dominant effect, although there were significant differences between genotypes for soluble nitrogen, Kolbach index, and fermentability levels. TL43 had higher levels of soluble nitrogen than the other genotypes at both sites and a higher Kolbach index. It therefore had a higher proportion of nitrogenous material in its extract and this probably accounted for the lower fermentability. The higher nitrogen contents of the Spanish samples led to reduced modification, as Kolbach indices were lower than in the Scottish samples and this, in turn, would be likely to reduce extract levels. However, soluble nitrogen levels were still higher at Lleida compared with Dundee, and this would have a deleterious effect on fermentability levels in samples from Spain.

DISCUSSION

The use of mutation followed by screening in an environment that usually fosters substantial levels of dormancy (Molina-Cano et al 1999) has enabled selection of lines in which dormancy is either increased or reduced. While differences between individual

TABLE III
Analysis of Variance (Mean Square Values) for Germinative Energy After Three and Five Days (GE3 and GE5) and Water Sensitivity After Three and Five Days (WS3 and WS5) on Grain Harvested at Lleida (Northeastern Spain) and Dundee (Eastern Scotland) in 1999

Sample ($n = 59$)	df	GE3	GE5	WS3	WS5
Replicate	1	86.5	92.5	6.3	64.1
Genotype (G)	4	3,978.9*** ^a	1,739.3***	1,344.4***	2,113.9***
Environment (E)	1	2,368.8***	10,560.3***	4,788.3***	6,365.4***
Dates (D)	1	10,428.0***	6,489.6***	2,381.4***	2,912.1***
$G \times E$	4	302.0**	161.6**	533.3***	569.9***
$G \times D$	4	42.6	26.8	29.5	50.0
$E \times D$	1	1,066.8***	2,018.4***	264.6**	385.1*
$G \times E \times D$	4	460.0***	252.5***	47.2	74.1
Residual	38	65.5	36.6	32.1	86.0

^a *, **, *** = significant at $P < 0.05, 0.01, \text{ and } 0.001$, respectively.

TABLE IV
Analysis of Variance (Mean Square Values) for Malting Quality Characters in Barleys Grown at Lleida (Northeastern Spain) and Dundee (Eastern Scotland) in 1999

Sample ($n = 29$)	df	Extract	Fermentability	Soluble Nitrogen	Kolbach Index
Replicate	2	0.05	0.10	0.002	6.22
Genotype (G)	4	2.04	16.91*** ^b	0.015***	24.08***
Environment (E)	1	252.88***	61.35***	0.329***	444.76***
$G \times E$	4	1.95	0.71	0.000	1.32
Residual	38	1.38	0.72	0.001	2.84

^a Ratio of soluble to total nitrogen.

^b *, **, *** = significant at $P < 0.05, 0.01, \text{ and } 0.001$, respectively.

genotypes may not be significant in every site or season, there was no evidence of dormancy rankings being significantly altered by environment. This is in agreement with previous findings (Buraas and Skines 1984) that dormancy is highly heritable. However, Thomas et al (1996) noted differences in the genetic factors determining germination after three and five days and also suggested that there were environmental effects on their expression. In this study, the number of grains germinating within 72 hr was similar for both sites four weeks after harvest, although there were differences between genotypes. However, the number beginning to germinate between three and five days was invariably higher at Lleida compared with Dundee.

ABA is generally antagonistic to the effects of gibberellic acid during germination (Skriver and Mundy 1990). Gibberellic acid is the principal regulator of α -amylase mRNA accumulation (Deikman

and Jones 1986) and is also essential for both production and release of protease (Hammerton and Ho 1986), as well as the release of carboxypeptidase (Hammerton and Ho 1986) and β -1,3-glucanase (Jones 1971). Mundy (1984) noted that ABA also enhanced the synthesis of an inhibitor of α -amylase activity. Consequently, ABA would be expected to exert a series of deleterious effects on endosperm modification during malting.

Although TL43 and TABA9 showed faster recovery from dormancy than the other genotypes, and also showed less reduction of extract level when ABA was included at the end of steeping, Kolbach indices suggested that they had no advantage in rate of protein modification. Additionally, Molina-Cano et al (1999) showed α -amylase activity in TL43 was reduced to the same extent as that of Triumph by ABA. In the absence of ABA, TL43 and TABA9 showed no improvement in malting performance compared with

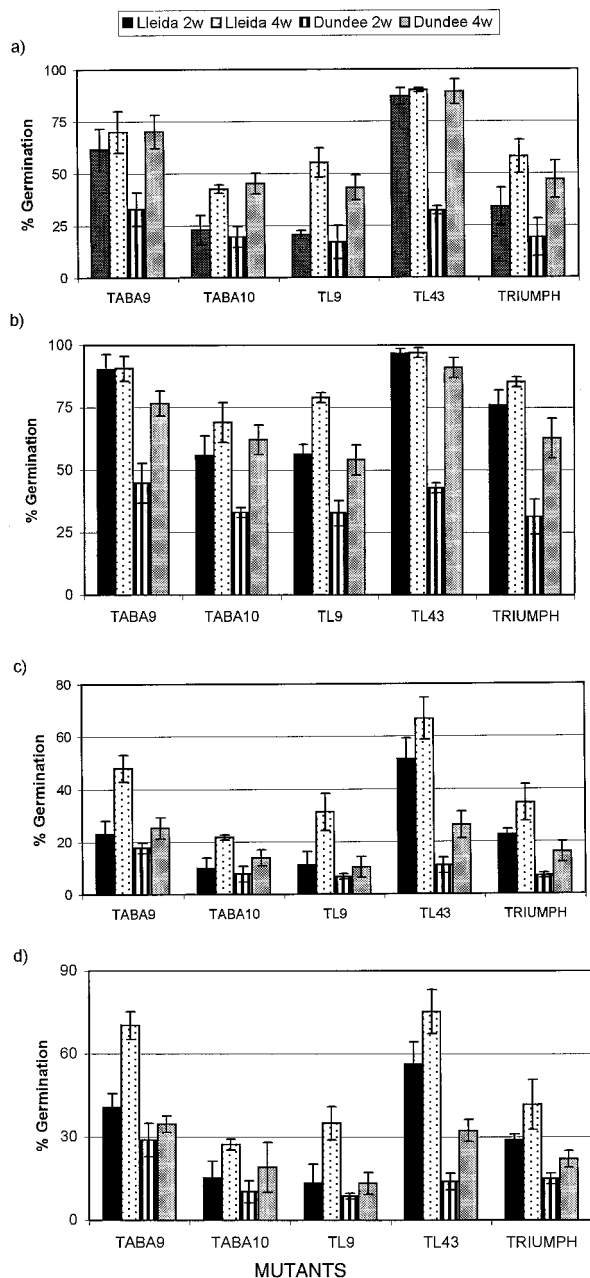


Fig. 3. Mean germinative energy and water sensitivity at two weeks (2w) and four weeks (4w) after harvest at Lleida (northeastern Spain) and Dundee (eastern Scotland) in 1999 in cv. Triumph and four mutants derived from it: **a and b)** germinative energy after three and five days (GE3 and GE5); **c and d)** water sensitivity after three and five days (WS3 and WS5). Error bars indicate standard deviations.

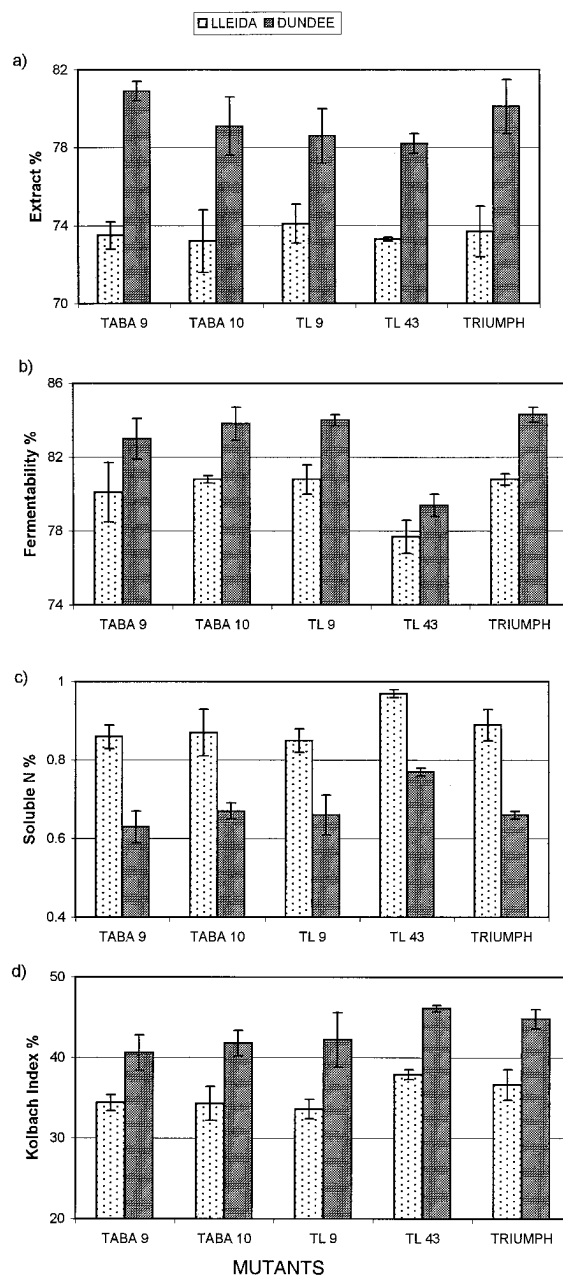


Fig. 4. Malting characters mean values (%) for **a)** extract level, **b)** fermentability level, **c)** soluble nitrogen, and **d)** Kolbach index in cv. Triumph and four mutants derived from it after malting grain from the 1999 harvest at Lleida (northeastern Spain) and Dundee (eastern Scotland). Error bars indicate standard deviations.

Triumph, suggesting that selection for differing responses to ABA may have little effect on hot water extract levels.

None of the lines showed significantly higher extract levels than Triumph using either the EBC or IOB method. This is in contrast to previous observations on mutants derived from cv. Troubadour (Molina-Cano et al 1989, 1993; Swanston et al 1995). These previous comparisons of malting in samples grown at Dundee and Lleida suggested that Kolbach index was both inversely proportional to the level of grain protein (Swanston et al 1997) and highly correlated with extract levels at both sites (Swanston et al 1995). Here, TABA10 and TL43 gave high levels of soluble nitrogen at Lleida, although neither gave particularly high extract levels, and there was no significant correlation between extract level and either soluble nitrogen or Kolbach index. However, the relationship between extent of modification and nitrogen solubilization varies with genotype (Swanston et al 1992). Initial studies were on Troubadour, which is a cultivar of only moderate malting quality, and a mutant derived from it. The mutants described here were induced in a genetic background that was already well-suited to malting.

Significant differences between cultivars in 1999 for soluble nitrogen and fermentability levels were due to the high levels of nitrogenous materials in the extract of TL43, which appeared to behave differently from the other mutants during malting. This will be examined more closely in future studies. Although it has reduced dormancy, its extract and fermentability levels would make it unsuited to malt whisky distilling because it is a combination of these factors that determines the yield of alcohol (Swanston and Thomas 1996). By contrast, TABA9 appears to combine lower dormancy with good malting performance. Therefore, the mutation breeding and selection system assessed here may be a means of developing malting cultivars for areas where dormancy is problematic.

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