Theor Appl Genet (1989) 78: 748–754

Fast-germinating low β-glucan mutants induced in barley with improved malting quality and yield

J. L. Molina-Cano[1], F. Roca de Togores[2], C. Royo[1] and A. Pérez[3]

[1] Institut de Recerca i Tecnologia Agroalimentàries, Centre UPC-IRTA, E-25006 Lleida, Spain
[2] La Cruz del Campo SA, Department of Barley Breeding, E-41007 Sevilla, Spain
[3] Institut de Recerca i Tecnologia Agroalimentàries, Centre Mas Bove, E-Reus, Spain

Received May 1, 1989; Accepted June 27, 1989
Communicated by J. Mac Key

Summary. Mutation breeding has been used to improve the speed of germination in the high-yielding spring barley variety Troubadour. Five mutants were selected which combined fast germination and good agronomic performance. Two of these mutants yielded significantly more than did Troubadour over eight environments, and showed a clear improvement in their malting quality through an increase in extract yield. The improvement in malting quality appeared to be due to a decrease in the β-glucan content, which seemed to enhance the germination speed and thus the starch degradation. The improvement in grain yield is postulated to be due to a better early growth caused by the enhanced germination speed. All the described changes could theoretically be explained by a single mutation event in each of the mutant genotypes, affecting the quantity of β-glucans present in the endosperm.

Key words: Barley – Malting quality – Germination speed – β-Glucans – Mutation breeding

Introduction

Although all genetic variation is ultimately based on mutation, there are clear-cut differences between mutation and cross breeding. The variation of the second process has suffered already the impact of selection (natural and/or human), whereas many mutant genes are produced de novo without any previous kind of selection (Mac Key 1984). While the use of mutation breeding in self-fertilizing species is possible both for simple-inherited and polygenic traits (Brock 1977), there are special examples, all related to barley, where it has proved especially useful: (i) To generate new genetic variability not found within the available germplasm, with the aim of solving qualitative problems, e.g. chill-haze (Von Wettstein et al. 1977) and filterability difficulty in beer (Aastrup 1983), and qualitative plus adaptative problems, such as malting quality and winter hardiness (Enchev 1976). (ii) For correcting a disadvantage, like lodging susceptibility, in an otherwise well-adapted variety, without deeply modifying its genetic background (alternative to backcrossing) (Molina-Cano 1982). (iii) As an alternative to backcrossing to develop near-isogenic lines to be used in breeding or genetic studies, in an attempt to define an ideotype for Mediterranean conditions (Molina-Cano et al. 1989). (iv) To help understand regulatory processes in metabolism such as proline accumulation as a response to water or salt stress (Bright et al. 1981). (v) To develop genetic markers useful in barley genetics and breeding (Persson and Hagberg 1969).

The two-rowed spring barley Troubadour, bred in The Netherlands and released in Spain in 1983, is very high-yielding under Mediterranean environments, especially in southwest Spain, where the domestic crossing programmes have been unable to develop a higher yielding variety. However, its malting quality is fairly poor, giving low extract yields. In fact, it needs to germinate for 1–2 additional days to meet the quality requirements of the brewing industry. Therefore, it seemed that enhancing its germination speed could be a clear breeding goal. The speed of germination was studied in barley by Finlay (1960a, b), who through the study of 800 genotypes found a strong genetic determination of the character.

Thus, to improve the germination speed of Troubadour with mutation breeding seemed to be a logical choice.

The mutagen chosen was sodium azide (N_3Na) because it is known to be very efficient and effective in

barley and does not induce gross chromosomal changes but does induce very high frequencies of point mutations (Nilan 1981). The action of azide in a wide array of organisms, including barley, has been reviewed by Kleinhofs et al. (1978), and a metabolite with mutagenic action, produced after treatment with it of barley embryos and bacterial cells, has been discovered (Owais et al. 1978, 1979).

Materials and methods

Mutagenic treatment

The procedure used was the one described by Molina-Cano (1979) consisting of presoaking 1 kg dry seed of Troubadour screened over a 2.5-mm sieve for 15 h in deionized water at 2 °C, followed by 4 h also in deionized water but with air bubbling at room temperature. The mutagenic treatment itself consisted of soaking the seeds during 2 h in a 10^{-3} *M* solution of N_3Na in phosphate buffer at pH 3, with air bubbling at room temperature. Afterwards, the seeds were rinsed six times in deionized water and sowed directly in moist soil in the field.

Field and screening methods

The handling of the treated material over generations is presented in Table 1.

Germination tests

The M_1 plants were harvested individually, and the main spike from each of them was threshed separately. A grain sample of each spike was subjected to a germination test in a dark growth chamber for two days at 18 °C and 98% relative humidity, with Troubadour seeds as controls. Fast-germinating kernels were sown directly in the field.

Five mutant families were selected in M_3 and again subjected to a germination test. Four lots of 100 seeds from each of the mutant lines, with Troubadour as control, were analyzed under the same environmental conditions as above. Each sample was scored as a percentage of kernels giving: no roots, one root, three roots of up to 3 cm in length or three roots of more than 3 cm in length.

Grain from the same five mutant lines coming from M_5 and M_6 plants were again subjected to a germination test with the same protocol, in M_5 with a single replication and in M_6 replicated twice.

Table 1. Field and screening methods (using Troubadour always as control)

Time	Generation	Operations
Dec. 83	M_1	Sowing of treated seeds (ca. 25,000)
June 84	M_1	Each plant harvested individually and submitted to germination test (2 days, 18 °C, 98% humidity
Dec. 84	M_2	857 fast-germinating seeds selected and transferred to the field
June 85	M_2	50 plants selected for agronomic value and threshed individually
Dec. 85	M_3	50 plant-row progenies sown in the field
June 86	M_3	5 rows (mutants) selected. Germination test.
Dec. 86	M_4	1st yield trial (Granada), 3 replications, plots of 12.6 m^2
Oct.–Nov. 87	M_5	1st micromalting test. Germination test
Dec. 87	M_5	7 yield trials sown at: 2 Sevilla (La Rinconada, Ecija), Granada, 2 Toledo (Rielves, Tembleque), Badajoz (Merida), Lleida (Gimenells). 4 replications each, 12.6 m^2 per plot
Sept.–Oct. 88	M_6	4 micromalting tests. Germination test

Micromalting and malting quality analysis

Micromalting and subsequent analyses were performed with seed lots from one and the same location in 1987 and from three locations in 1988.

The micromalting plant used allows the processing of 32 samples of 200 g each per batch. The micromalting procedure was as follows:

- Steeping time: 57 h (41 h under water and 16 h without water)
- Steeping temperature: 15 °C
- Germination time: 5 days
- Germination temperature: 15 °C
- Air supply to germinating barley: 50 ml/min
- Drying time: 17 h
- Drying temperature: 50 °C
- Kilning time: 2.5 h
- Kilning temperature: 70 °C

Six quality parameters were measured on each malt sample, i.e. extract yield (%), total malt protein (%), soluble malt protein (%), Kolbach Index, apparent final attenuation (%) and viscosity (cp). The analytical procedures used were according to those recommended by the European Brewery Convention (1975). Additionally, the total protein content as well as the β-glucan content of barley were determined as was the β-glucanase activity of malt (McCleary and Glennie Holmes 1985; McCleary and Nurthen 1986; McCleary and Shamerr 1987).

Statistical methods

Chi-square tests were carried out to analyze non-parametric data such as those from the germination trials. Standard analyses of variances and mean separation tests were used for studying the differences among mutant lines and as the control for yield and malting variables. Regression of genotype grain yield on an environmental index, measured as the average yield of all genotypes at each environment (Finlay and Wilkinson 1963), was used to estimate the genotype × environmental interaction, and hence the stability of the different genotypes.

Results

Germination tests

The relevant data are presented in Table 2 and Fig. 1. Four of the five mutants (TR-6, TR-9, TR-43 and TR-60) always germinated faster than the mother variety,

Table 2. Germination after 2 days (%) over 3 environments

Variety	Year (Generation)	Without roots	1 root	3 roots of up to 3 cm	3 roots of more than 3 cm	χ^2 3 *df*
Troubadour	1986	43	36	13	8	–
TR-6	(M_4)	9	45	27	19	59.34**
TR-9		8	38	38	16	84.67**
TR-43		15	50	19	16	34.45**
TR-49		40	44	14	2	6.56
TR-60		5	38	38	19	96.89**
Troubadour	1987	16	32	42	10	–
TR-6	(M_5)	6	9	35	50	183.94**
TR-9		8	22	63	7	18.52**
TR-43		5	18	58	19	27.88**
TR-49		27	49	24	0	34.31**
TR-60		10	7	40	43	130.78**
Troubadour	1988	5	10	27	58	–
TR-6	(M_6)	4	0	28	68	11.96**
TR-9		3	6	15	76	13.32**
TR-43		3	0	16	81	15.40**
TR-49		3	3	29	65	6.69
TR-60		8	3	14	75	17.94**
		Means over 3 environments				
Troubadour		21	26	27	26	–
TR-6		6	18	30	46	28.89**
TR-9		6	22	39	33	18.59**
TR-43		8	23	31	38	14.53**
TR-49		23	32	22	23	2.85
TR-60		8	16	30	46	27.61**

** Significant $p \leq 0.01$
Note: Values listed are means over: 1986, 4 replications; 1987, 1 replication; 1988, 2 replications

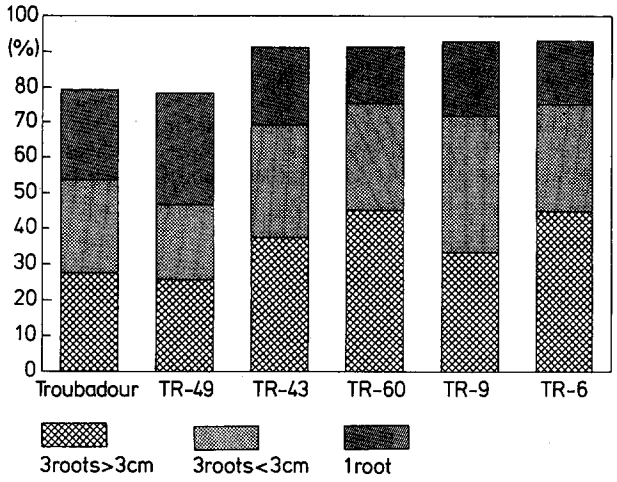

Fig. 1. Mean germination percentage, over three environments, after 2 days

Troubadour, and the fifth one (TR-49) only showed faster germination in 1987, being slower on the average. It is necessary to point out that the germination tests carried out are unequivocal proof of the stability of the mutant phenotypes regarding speed of germination.

Malting quality analyses

The upper part of Table 3 presents the analyses of variance of genotypes versus environments for the nine quality parameters measured, indicating the existence of significant differences among genotypes for barley β-glucans and malt extract yield, Kolbach Index, apparent final attenuation and viscosity. The significant differences existing among environments reflect their dissimilarity which, in turn, improves the degree of discrimination among the studied genotypes.

The lower part of Table 3 shows the mean values of the quality characters studied, averaged over environments. There are three mutants (TR-9, TR-43 and TR-49) with better malting quality than Troubadour, since they show lower barley β-glucan content and better extract yield in malt, and one of them (TR-43) had an even better Kolbach Index. The selected mutant lines demonstrate different quality patterns. Mutant TR-6 has poorer malting quality than Troubadour despite its faster germination pattern, and mutant TR-49 possesses better malting quality than the mother variety but the same germination speed, a reaction that can be explained by a lower β-glucan content.

Table 3. Malting quality over four environments[a]
Analyses of variance (F values)

Source		Barley analyses		Malt analyses						
	df	Total protein (%)[b]	β-glucans (%)[c]	Extract yield (%)	Total malt protein (%)	Soluble malt protein (%)	Kolbach index	Apparent final attenuat (%)	Viscosity (cp)	β-glucanase units/kg (c)
Genotypes	5	1.1	9.52***	6.5***	2.7	2.7	6.3***	16.7***	14.5***	1.49
Environments	3	74.9***	14.44***	19.5***	34.6***	6.7***	7.9***	27.1***	6.2***	7.87***

Mean values

Variety	Barley analyses		Malt analyses						
	Total protein (%)	β-glucans (%)	Extract yield (%)	Total malt protein (%)	Soluble malt protein (%)	Kolbach index	Apparent final attenuat (%)	Viscosity (cp)	β-glucanase units/kg
Troubadour	11.00	3.72	78.97	11.03	3.83	35.00	79.10	1.75	288.50
TR-6	11.20	3.86	78.17	10.95	3.35	30.75*	74.18**	2.17**	259.50
TR-9	10.66	2.70*	80.23*	10.15	3.50	34.75	78.73	1.76	309.00
TR-43	10.43	2.43*	80.52**	9.95	3.83	38.75*	79.10	1.76	284.50
TR-49	10.66	2.27**	80.23*	10.00	3.63	37.00	78.78	1.82	294.50
TR-60	10.83	2.10**	79.95	10.08	3.65	36.25	78.03	1.76	303.00
LSD 0.05	–	0.89	1.08	–	–	3.24	1.41	0.15	–
LSD 0.01	–	1.39	1.49	–	–	4.48	1.94	0.21	–

* Significantly different from Troubadour at the $p \leqslant 0.05$ level
** Significantly different from Troubadour at the $p \leqslant 0.01$ level
*** Significant $p \leqslant 0.01$
[a] List of environments: Granada 87 & 88, Ecija 88, La Rinconada 88 for malt analyses. For barley protein: Granada 88, Ecija 88 and La Rinconada 88. For barley β-glucans and malt β-glucanase: Ecija 88 and La Rinconada 88
[b] For total barley protein, *df* genotypes 5, environments 2
[c] For barley β-glucans and malt β-glucanase, *df* genotypes 5, environments, 1

Table 4. Agronomic performance: days to heading

Variety	Environment			
	Granada 87	La Rinconada 88	Granada 88	Mean
Troubadour	134	89	135	119.3
TR-6	132	86	134	117.3
TR-9	131	87	132	116.7 **
TR-43	132	86	131	116.3**
TR-49	131	85	132	116.0**
TR-60	133	90	138	120.3

** Significantly earlier than Troubadour at $p \leqslant 0.01$ level

Table 5. Agronomic performance: grain yield over eight environments

Variety	Ranking of Varieties	
	Grain yield (Adjusted to 15% moisture)	
	kg/ha	%
TR-9	5,697	108**
TR-43	5,628	107*
TR-49	5,504	104
TR-6	5,429	103
Troubadour	5,279	100
TR-60	5,254	100

Variety	Genotype × environment analysis		
	a (kg/ha)	b	r^2
TR-9	89	1.02	0.99
TR-43	−246	1.07	0.99
TR-49	299	0.95	0.99
TR-6	183	0.96	1.00
Troubadour	−70	0.98	0.99
TR-60	−258	1.00	0.99

$y = a + bx$
y = genotype yield
x = environmental yield
*,** Yields significantly higher than that of Troubadour at the $p \leqslant 0.05$ and $p \leqslant 0.01$ levels, respectively

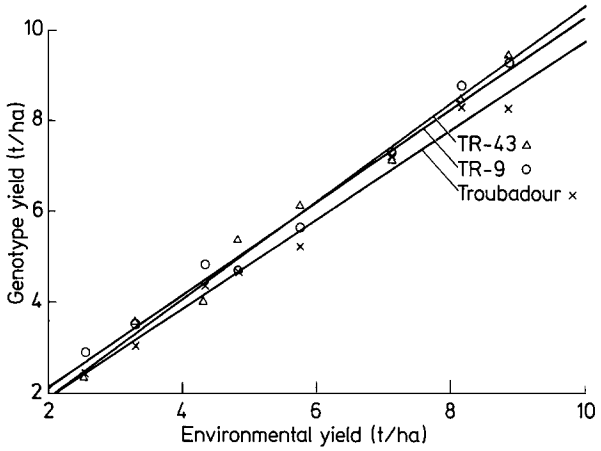

Fig. 2. Genotype × environmental interaction for TR-9 and TR-43 for grain yield over eight environments

Earliness

Table 4 presents the data for days from sowing to heading of the studied genotypes over three environments. Three mutants (TR-9, TR-43 and TR-49) are approximately 3 days significantly earlier than Troubadour.

Grain yield

Before judging the yield results, it should be mentioned that the eight environments explored showed a continuum in yielding capacity from 2.5 to more than 9 tons/ha. A significant ranking must be quite representative under such conditions.

Table 5 gives the ranking in grain yield obtained for the five mutant lines and the control over eight environments (upper part) and the genotype × environment analyses for the same set of observations (lower part). Figure 2 presents the genotype × environment analyses for the two mutants, TR-9 and TR-43, and demonstrates their significantly higher yielding capacity than Troubadour by 8% and 7%, respectively. Mutant TR-9 yielded more than Troubadour in six of the eight environments, and was equal to Troubadour in the remaining. Mutant TR-43 yielded more than the mother variety in five environments, less than the mother variety in 2 and was equal to it in 1. Both mutants, specially TR-43, showed a high-response pattern, i.e. the better the environment, the higher the positive difference in yield above that of Troubadour. Of the other mutants, TR-6 and TR-49 showed a decreasing response, and TR-60 yielded about the same as Troubadour in each environment. The yield stability observed is not a feature generally met for mutants (e.g. Gottschalk and Wolff 1982).

Discussion

The results definitely demonstrate the possibilities for precision and success in mutation breeding. The desired goal of improving faster germination at malting was achieved but, in addition, gains were also made in other quality features as well as in earliness and grain yield. It would be interesting to try to analyze the mutational events behind this. As to the genic changes involved, there are two possible explanations for the facts presented: pleiotropic effects of a single mutation or several

mutational events in closely linked genes (Gottschalk and Wolff 1982).

The faster germination observed for some of the mutants can be explained by a decrease in total β-glucan content in their endosperm cell walls. It is well known that this polysaccharide is a physical barrier to the diffusion of hydrolytic enzymes from the scutellum and the aleurone layer into the endosperm (Palmer and Harvey 1977; Munck et al. 1981; Mac Gregor and Matsuo 1982; Sakurai and Kuraishi 1987). Moreover, it has been shown that poor malting barley genotypes have greater β-glucan and lower starch content in their endosperms than good ones (Smith et al. 1987).

A higher β-glucanase activity in the malt might produce the same result. This enzyme is the first to act during germination by degrading the endosperm cell walls (Palmer 1988), thus facilitating the action of the other hydrolytic enzymes.

No significant differences in β-glucanase activity were induced, although a change could be recorded for content of β-glucans (Table 3). TR-9, TR-43 and TR-49 are examples here upon. Mutant TR-6, although germinating faster than Troubadour, does not change its malting quality, and shows the same levels of extract yield and β-glucans as its mother variety. Mutant TR-60 is also fast-germinating and possesses a somewhat higher extract yield and lower β-glucan content than Troubadour.

The observed improvement in grain yield of TR-9 and TR-43 does not have an easy physiological explanation. Faster germination and seedling emergence will give a vigorous early growth. Such a better start in the Mediterranean climate observed would favour improved grain yield in wheat (Turner and Nicolas 1987) as well as in barley (Ramos et al. 1985). A higher dry matter accumulation before anthesis might augment the real location of energy to the grain. Detailed physiological studies are needed to ascertain such an explanation as to the higher grain yields of TR-9 and TR-43.

Acknowledgements. We gratefully appreciate the suggestions for the improvement of the manuscript made by Prof. J. Mac Key (Uppsala, Sweden). We also thank Docent G. Persson (Svalöf, Sweden) and Prof. I. Romagosa (Lleida, Spain) for reviewing the manuscript, and Ings. A. Rubió and T. Ramo (La Moravia, Lleida, Spain) for their help with the production of malts for the β-glucanase analyses.

References

Aastrup S (1983) Selection and characterization of low β-glucan mutants in barley. Carlsberg Res Commun 48:307–316

Bright SWJ, Kueh JSH, Franklin J, Miflin B (1981) Proline-accumulating barley mutants. In: Asher MJC, Ellis RP, Hayter AM, Whitehouse RNH (eds) Barley genetics IV. Proc 4th Int Barley Genet Symp Edinburgh University Press, Edinburgh, pp 858–863

Brock RD (1977) When to use mutations in plant breeding. In: Manual on mutation breeding, 2nd edn. IAEA, Vienna, pp 213–214

Enchev Y (1976) Induced mutations in winter brewing barley and their use. In: Friedt W, Gaul H, Nilan RA, Whitehouse RNH, Sparrow DHB, Metcalfe DR, Wilson HM (eds) Barley genetics III. Proc 3rd Int Barley Genet Symp. Karl Thiemig, München, pp 190–196

European Brewery Convention (1975) Analytica EBC. 3rd edn. Elsevier, Amsterdam

Finlay KW (1960a) Genetic study of barley germination behaviour. I. Genetic and environmental variation. J Inst Brew 66:51–57

Finlay KW (1960b) Genetic study of barley germination behaviour. II. General and specific combining ability. J Inst Brew 66:58–64

Finlay KW, Wilkinson GN (1963) The analysis of adaptation in a plant breeding programme. Aust J Agric Res 14:742–754

Gottschalk W, Wolff G (1982) Induced mutations in plant breeding. Springer, Berlin Heidelberg New York, pp 105–111 (Monographs genetics 7)

Kleinhofs A, Warner RL, Muehlbauer FJ, Nilan RA (1978) Induction and selection of specific gene mutations in *Hordeum* and *Pisum*. Mutat Res 51:29–35

Mac Gregor AW, Matsuo RR (1982) Starch degradation in endosperms of barley and wheat kernels during initial stages of germination. Cereal Chem 59:210–216

Mac Key J (1984) Selection problems and objectives in mutation breeding. In: IAEA, Selection in mutation breeding. Int Atomic Energy Agency, Vienna, pp 35–48

McCleary BV, Glennie-Holmes M (1985) Enzymic quantification of (1–3), (1–4)-β-D-glucan in barley and malt. J Inst Brew 91:285–295

McCleary BV, Nurthen EJ (1986) Measurement of (1–3), (1–4)-β-D-glucan in malt, wort and beer. J Inst Brew 92:168–173

McCleary BV, Shameer I (1987) Assay of malt β-glucanase using Azo-barley glucan: an improved precipitant. J Inst Brew 93:87–90

Molina-Cano JL (1979) Effect of aeration during sodium azide treatment of barley seeds on the physiological damage in M_1 seedlings. Barley Genet Newsl 9:62–65

Molina-Cano JL (1982) Genetic, agronomic and malting characteristics of a new *Erectoides mutant* induced in barley. Z Pflanzenzuecht 88:34–42

Molina-Cano JL, Ramos JM, García del Moral LF, Roca de Togores F (1989) Agrophysiological analysis of several near-isogenic lines of barley under Mediterranean conditions. An Aula Dei (in press)

Munck L, Gibbons G, Aastrup S (1981) Chemical and structural changes during malting. European Brewery Convention. Proc Copenhagen Congr, pp 11–29

Nilan RA (1981) Recent advances in barley mutagenesis. In: Asher MJC, Ellis RP, Hayter AM, Whitehouse RNH (eds) Barley genetics IV. Proc 4th Int Barley Genet Symp. Edinburgh University Press, Edinburgh, pp 823–831

Owais WM, Zarowitz MA, Gunorich RA, Hodgdon AL, Kleinhofs A, Nilan RA (1978) A mutagenic in vivo metabolite of sodium azide. Mutat Res 50:67–75

Owais WM, Kleinhofs A, Nilan RA (1979) In vivo conversion of sodium azide to a stable mutagenic metabolite in *Salmonella typhimurium*. Mutat Res 68:15–22

Palmer GH (1988) Enzyme development in the aleurone and embryos of Galant and Triumph barleys. J Inst Brew 94:61–63

Palmer GH, Harvey AE (1977) The influence of endosperm structure on the behaviour of barleys in the sedimentation test. J Inst Brew 83:295–299

Persson G, Hagberg A (1969) Induced variation in a quantitative character in barley. Morphology and cytogenetics of *erectoides* mutants. Hereditas 61:115–178

Ramos JM, García del Moral LF, Recalde L (1985) Vegetative growth of winter barley in relation to environmental conditions and grain yield. J Agric Sci Camb 104:413–419

Sakurai N, Kuraishi S (1987) Cell wall polysaccharides and their metabolism in normal and semidwarf barley coleoptiles. In: Yasuda S, Konishi T (eds) Barley genetics V. Proceed 5th Barley Genet Symp. Sankyo Press, Okayama, pp 465–469

Smith DB, Starr C, Parsons DG, Gill AA, Gothard PG, Freeman RL (1987) Barley β-D-glucans. In: Annual report of the plant breeding institute, 1986. Cambridge pp 117–118

Turner NC, Nicolas ME (1987) Drought resistance of wheat for light-textured soils in a Mediterranean climate. In: Srivastava JP, Porceddu E, Acevedo E, Varma S (eds) Drought tolerance in winter cereals. Wiley, New York, pp 203–215

Wettstein D von, Jende-Strid B, Ahrenst-Larsen B, Sorensen JA (1977) Biochemical mutant in barley renders chemical stabilization of beer superfluous. Carlsberg Res Commun 42:341–351