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A mutant induced in the malting barley cv Triumph with reduced dormancy and ABA response

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Abstract Induced mutants in the barley cultivar Triumph have been screened for reduced dormancy. One line, which germinated readily 2 weeks after harvest, was classified as ABA-insensitive, since it could tolerate a ten-fold increase in ABA, compared to its parent, before germination was inhibited. This mutant, designated TL43, was genotypically similar to Triumph and phenotypically similar under Scottish growing conditions, except for a slightly reduced grain size. In Spain, it showed considerable reductions in both grain yield and plant height, suggesting that it was less widely adapted than its parent. Levels of α-amylase activity were increased at both sites. The mutant appeared to be different from those with ABA insensitivity or altered dormancy previously documented in either barley or *Arabidopsis*.

Key words Barley · Dormancy · ABA · Malting quality · Germination

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Introduction

Malting barley (*Hordeum vulgare* L.) is a crop of major economic importance in the European Union, with a total production of malt in excess of 7 million tonnes in 1996 (Anonymous 1997). The main malting barley producing countries are Germany, France, Great Britain, Spain, and Denmark. In the more northern growing areas, dormancy can be a severe economic problem. Barley producers and users are required to store the grain for a period of up to 3 months prior to processing. Treatments to release dormancy include a cold treatment of imbibed seeds, which may be very expensive and impractical if grain moisture levels are not optimal (Stowell 1986).

Seed dormancy is defined as the inability of a viable seed to germinate under conditions which are perfectly adequate for germination. The degree of dormancy is influenced by the conditions prevailing during seed development and the duration and conditions of dry storage after harvest (Léon-Kloosterziel et al. 1996). Low temperature and high relative humidity during grain development seem to be the main environmental factors inducing dormancy in barley (Corbineau and Côme 1980, 1982; Barre 1983). Experiments under controlled conditions in a phytotron suggested that plants grown under short-day and cool conditions had higher dormancy levels than those grown under long-day and warm conditions (Schuurink et al. 1992), while previous experiments in controlled environments had shown the dormancy inducing effect of low temperatures during grain filling in barley (Buraas and Skinnes 1985). A full account of dormancy in barley has recently been published by Corbineau and Côme (1996).

The genetics of dormancy is, in general, poorly understood. In the model plant *Arabidopsis thaliana*, mutants have been developed which, besides changes in abscisic acid (ABA) concentration and response, showed either absence of, or alteration in, dormancy

(Koornneef et al. 1984; reviewed in Karssen 1995). The many environmental and genetic factors that determine seed dormancy in *Arabidopsis* have been reviewed by Koornneef and Karssen (1994). The integration of the maturation and germination pathways studied from a genetical perspective, with special emphasis on molecular aspects, has been recently reviewed by McCarty (1995).

In barley, the genetics of dormancy is less understood, despite the economic importance of this trait in the crop. Early investigations by Freistedt (1935), who found that dormancy is controlled by one or two recessive genes, and Moorman (1942), who observed reciprocal differences in crosses involving low- and high-dormancy parents, were continued by Buraas and Skinnes (1984), who suggested a polygenic recessive control, unaffected by cytoplasmic factors, and indicated that dormancy was highly heritable. Recent attempts to study the genetics of dormancy in barley have involved the mapping of quantitative trait loci (QTLs) (Han et al. 1996; Romagosa et al. 1998; Ullrich et al. 1993, 1996; Larson et al. 1996) using doubled haploids derived from the Steptoe (high dormancy) × Morex (low dormancy) cross. Four regions of the barley genome were found to be associated with differences in dormancy. The loci, designated *SD1–SD4*, accounted for, approximately, 50, 15, 5, and 5% of the phenotypic differences, respectively.

Recently, Koornneef et al. (1997) have advocated the use of mutant genotypes and other genetic tools as an optimal approach to understanding complex biological phenomena. This supports previous suggestions that near-isogenic lines (NIL) or induced mutants constitute the most appropriate material to study physiological processes (Molina-Cano et al. 1990; Romagosa et al. 1993). In particular, there is a need for barley genotypes suited to a study the abscisic acid (ABA)/gibberellic acid (GA) system as related to germination behaviour (Jacobsen and Chandler 1987). The best known barley mutant related to hormone response is *slender*, induced by Foster (1977) and characterised by very long leaves and tillers and overproduction of α-amylase by the aleurone. This appears to depend on a GA-receptor function (reviewed in Pollock et al. 1992). Other induced mutants were reported in barley by Ho et al. (1980), including those with altered sensitivity to GA or ABA, but further data on them are not available. From germination studies on a collection of barley mutants Visser et al. (1996) identified a mutant line with a *gigantum* appearance that germinated faster than the parental line in the presence of ABA, but gave no information about its dormancy properties. In addition, several fast-germinating barley mutants, with better malting quality and grain yield, have been induced (Molina-Cano et al. 1989).

The absence of barley mutants with reduced dormancy and sensitivity to ABA during germination, indicated the need for a mutation experiment with the specific objective of developing such genotypes. For both theoretical and practical considerations, the chosen parental cultivar was Triumph, a major malting barley cultivar (cv) in Europe for many years and which is included in the pedigree of most modern malting cvs (Molina-Cano 1991). This genotype, although characterised by superior malting quality and yield and high resistance to lodging and mildew, has the disadvantage of being very prone to dormancy (Schildbach, personal communication), a characteristic that has been frequently inherited by its progeny (Simiand, personal communication).

Materials and methods

Mutagenic treatment

The procedure used was, with slight modifications, the one described by Molina-Cano et al. (1989), consisting of pre-soaking 500 g of dry pure seed of cv Triumph in de-ionised water at 2°C for 15 h, followed by 4 h at room temperature, with aeration. The mutagenic treatment involved soaking the seeds for 2 h in a 10^{-3} M solution of N_3Na in phosphate buffer, pH 3, at room temperature, without aeration. The seeds were then rinsed six-times in de-ionised water and oven dried, prior to sowing them in the field.

Field and screening methods

The handling of the treated material is outlined in Table 1. The sites used for the field work were Dundee (eastern coast of Scotland), where, in most seasons, there is a high incidence of dormancy in susceptible cultivars, and Lleida (north-eastern Spain), where dormancy is infrequent. The germination protocol for seed screened within 1 month after harvest, was that of Analytica EBC (European Brewery Convention 1987), consisting of placing 100 barley seeds between two pieces of Whatman no. 1 filter paper in a 9-cm Petri dish, adding 4 ml of distilled water and incubating for 3 and 5 days at 18–21°C in the dark. A second protocol, for seed screened more than 1 month after harvest, incorporated ABA into the incubation medium.

In parallel with the dormancy tests, phenotypic screening was carried out in the field, and aimed at discarding the poorer looking plants and segregating mutant lines, as well as allowing for a more proper characterisation of the mutants which were finally selected.

Molecular methods to check genotypic identity

At the time the mutagenic experiment reported here was being carried out, we were also selecting within a mutagenised population of cv Alexis, derived from a Triumph cross and phenotypically similar to Triumph except for the lack of dormancy. Given the close similarity between Alexis and Triumph and the possibility of mutation giving rise to male-sterile types, the genotypic identity of all the selected mutants was checked to eliminate the possibility of accidental hybridisation.

Leaf tissue was collected in bulk from approximately 20 seedlings, at the 3–4 leaf stage, of the cvs Alexis and Triumph, and different sodium azide-derived mutants, in both backgrounds. DNA was extracted following a CTAB procedure (Saghai Maroof et al. 1984). The DNA restriction enzymes used for restriction fragment length polymorphism (RFLP) analyses were *Bam*III, *Eco*RI and *Hin*dIII. Agarose-gel electrophoresis, alkaline transfer to nylon membranes,

Table 1 Selection procedure for isolating the barley mutants

Time	Generation	Working place	Operations
1991 (Dec)	M_0	Laboratory	Mutagenic treatment of 500 g pure seed of Triumph barley
1992 (March)	M_1	Field	Sowing M_1 seed in Dundee (Scotland) to obtain M_2 seed with dormancy
1992 (Sep–Nov)	M_2	Laboratory Greenhouse	Selection at laboratory in Lleida (Spain) for germinating non-dormant seeds in 6000 M_2 seeds (60 Petri dishes, 100 seeds each) After 1 month, ABA (10^{-4} M) was included in the germination medium. The 95 germinating grains (67 from water and 28 from ABA containing medium) were transplanted to the pots in the greenhouse
1993	M_3	Field Laboratory	Sowing at Lleida in the field 1 row (22 plants) per each of the 95 putative mutants with bulk seed from the greenhouse. Triumph included as control. Harvested seven spikes/mutant and the remaining seed in bulk. Phenotypic characterisation. Germination tests
1994	M_4	Field Laboratory	Sowing at Lleida in the field 3 rows per mutant with bulk seed from the M_3 row. Triumph included as control. Harvested 12 spikes/mutant and the remaining seed in bulk. Phenotypic characterisation. Sowing at Dundee one row/mutant to check dormancy. Laboratory testing of germination in Dundee
1995	M_5	Field Laboratory	Sowing at Lleida in the field six rows per mutant with single spikes from the M_4 rows, and a solid seeded trial with one replication. Triumph included as control. Purity test (search for non-segregating mutant lines). Phenotypic characterisation. Sowing at Dundee of two rows/mutant with control to check dormancy. Laboratory testing of germination in Dundee
1996	M_6	Field Laboratory	Sowing at Lleida in the field a solid seeded trial with four replications. Agronomic characterisation. Sowing at Dundee in the field a solid seeded trial with one replication. Phenotypic characterisation. Laboratory testing of germination in Dundee and Lleida
1997	M_7	Field Laboratory	Sowing at Lleida in the field a solid seeded trial with three replications. Agronomic characterisation. Sowing at Dundee in the field a solid seeded trial with one replication. Phenotypic characterisation. Laboratory testing of germination in Lleida of samples from both sites, including ABA response test. Micromalting in the presence of ABA

probe labelling and hybridisations followed published protocols (Sambrook et al. 1989). For RAPD analysis PCR was performed in 25-µl reaction volumes containing 0.5 mM primer, 200 mM dNTPs, 2.5 µl of 10× reaction *Taq* buffer, 1U of *Taq* DNA polymerase (Pharmacia Biotech) and 50 ng of template DNA. After the initial denaturation (94°C, 2 min), PCR was run for 40 cycles consisting of a 94°C denaturation step (1 min), a 36°C annealing step (3 min), and a 72°C elongation step (2 min) in a Perkin Elmer model 2400 thermal cycler, followed by a final extension (72°C, 5 min). Amplification products were separated on 2% agarose gels in TAE buffer, stained with ethidium bromide, and photographed under UV light. Duplicate reactions were routinely performed to ensure reproducibility; blanks were included.

Germination tests to check dormancy

The germination protocol was as described above. Although germination tests were carried out in Scotland in all years from 1994 to 1997, only the data of 1995 and 1997 are reported here. Screening in 1994 was primarily aimed at identifying the genotypes for further study and the field layout did not permit the accuracy of comparison with Triumph achieved subsequently. In 1996, post-harvest dormancy was not observed in Triumph at Dundee. In Spain, data were recorded in 1996 and 1997.

Response to exogenous ABA during germination

The germination protocol was as described above but germination was recorded after 3 days. The data are restricted to grain from the 1997 harvest for two reasons: (1) they are from an advanced generation (M7) and, therefore, segregation may be ruled out, and (2) it was possible to design a more comprehensive experiment as seed stocks were in suitable supply. Given the well-known effect of grain size on water uptake in barley (Zila et al. 1942), only grains of the size interval 2.5–2.8 mm were used for the experiment on the response to ABA during germination. This experiment was laid out as a randomized complete block design, as follows:

(1) four replications of 100 seeds of width between 2.5 and 2.8 mm from TL43 and Triumph, and (2) ABA concentrations in the germination medium of 0, 10^{-8}, 10^{-7}, 10^{-6}, 10^{-5}, 10^{-4} and 10^{-3} M [(±)-cis,trans-abscisic acid, Sigma Chemical Co.].

Micromalting and enzyme analysis

For the quantification of α-amylase under conditions similar to those of industrial malting, micromalting was carried out by the protocol of Molina-Cano et al. (1989). Barley samples (40 g) were screened over a 2.2-mm sieve and three replications per "genotype × treatment" combination were laid out in a split-plot design.

The two treatments (ABA vs water) were the main plots and genotype (TL43 and Triumph) the subplot. The ABA treatment [(±)-cis, trans-abscisic acid, Sigma Chemical Co.], was carried out at the end of steeping in the dry period, mixing each sample thoroughly with 4 ml of a 6×10^{-5} M ABA solution in a glass container. The micromalting programme was as follows:

steeping: 57 h (41 under water and 16 of air rest) at 15°C,
germination: 5 days at 15°C,
air supply to germinating barley: 50 ml/min,
drying: 17 h at 50°C,
kilning: 2.5 h at 70°C.

Although a complete set of malt analyses was carried out, data here will be restricted to α-amylase, which may be regarded as a measure of aleurone activity during germination. Assays were carried out by the method of McCleary and Sheehan (1987).

Quantification of ABA in a mature dry grain

ABA concentration was determined as described by Lara and Vendrell (1998). Samples (1 g) of homogenised dried tissue were extracted for 36 h in the dark on a shaker at 4°C with methanol/6.8 mM phosphoric acid (80:20, v/v) and 2,6-di-*tert*.-butyl-4-methylphenol (BHT) as an anti-oxidant (100 mg l^{-1}). The filtrate was adjusted to pH 8.5 and methanol removed under vacuum at 35°C. The aqueous phase was pre-purified by loading onto a Sep-Pack C18 cartridge. The eluted fraction was lyophilised and stored at −20°C until injection into an HPLC system for ABA separation and further quantification. The fraction containing the ABA peak was collected after detection, lyophilised, and stored prior to mass-spectrometric analysis for peak confirmation. Results are expressed as ng ABA g^{-1} DW.

Statistical methods

The statistical analyses used included an analysis of variance (ANOVA) carried out with the SAS System (SAS, 1988), and non-linear curve fitting together with confidence interval determination calculated with TableCurve2D for Windows version 2.03 (Jandel Scientific, Calif., USA, 1994).

Results

Mutant selection and check of genotypic identity

From an original population of 95 putative mutants selected either for absence of dormancy or for germination in the presence of ABA, the four showing the largest effects and designated TL43, TL9, TABA9 and TABA10, respectively, were finally selected in the M_7. The lowest dormancy and susceptibility to ABA during germination was found in TL43, which was the line chosen for a subsequent comparative study with its parent, the cv Triumph.

RAPD analysis was initially used to discriminate between putative Triumph and Alexis mutants. A set of 10-mer random primers was used, one of which clearly distinguished between cvs Triumph and Alexis. As part of another study aimed at characterising the genetic variation among representative barley cvs grown in Spain (Casas et al. 1998), RFLP probes differentiating Alexis and Triumph were identified. Among those, we selected six probes hybridising to four different barley chromosomes: ABG704 (7H), ABG461 (7H), ABG472 (4H), MWG616 (4H), MWG652 (6H), and MWG502 (5H). DNA hybridisation was used to assess the identity of the mutant genotypes. TL-43, showed Triumph banding patterns with all the probes, suggesting its true Triumph NIL nature (data not shown).

Agronomic and qualitative characterisation

The phenotypic characterisation of TL43, compared with the parental cv Triumph, is presented in Fig. 1. Because for grain size (see Fig. 1D) no "genotype × site" interaction was detected in the ANOVA (data not shown), data were averaged over sites (Spain and Scotland). For the remaining characters, data are presented separately for each site.

The length of the vegetative cycle (days from plant emergence to heading) in Scotland in 1995 and in Spain, as an average of 1996 and 1997, is presented in Fig. 1A. TL43 was about 5 days later than Triumph in Spain (autumn sowing: 150 days from emergence to heading) whereas in Scotland (spring sowing: 80 days from emergence to heading) this difference was reduced to about 2 days. Differences in plant height (Fig. 1B) between Triumph and TL43 were not observed in Scotland; however, TL43 was about 30 cm shorter than Triumph in Spain.

Grain yield (Fig. 1C) was investigated only in Spain in 1996 and 1997, due to the limited seed availability. Differences between the mutant and Triumph were large and highly significant, with the TL43 yield only about half that of the parent, due to a reduction in tiller number and spike size (data not shown).

Figure 1D shows grain size distribution averaged over sites and years. The mutant TL43 was characterized by a significantly higher frequency of small grains (<2.2 mm) and a highly significant reduction in the percentage of large kernels (>2.8 mm), whereas the percentages of the two intermediate fractions (2.2–2.5 and 2.5–2.8 mm) were similar in the mutant and parental genotypes. The tendency of the mutant to produce smaller grains was reflected in the 1000-kernel weight (Fig. 1E), obtained in Scotland in 1995, 1996, and 1997, and in Spain in 1996 and 1997. The weight of the mutant grains was significantly and consistently lower than that of Triumph across environments.

The activities of α-amylase, obtained from the micromalting experiment carried out with/without addition of ABA at the end of steeping, with samples harvested in Spain and Scotland in 1997, are shown in Fig. 1F. It is well documented that α-amylase activity

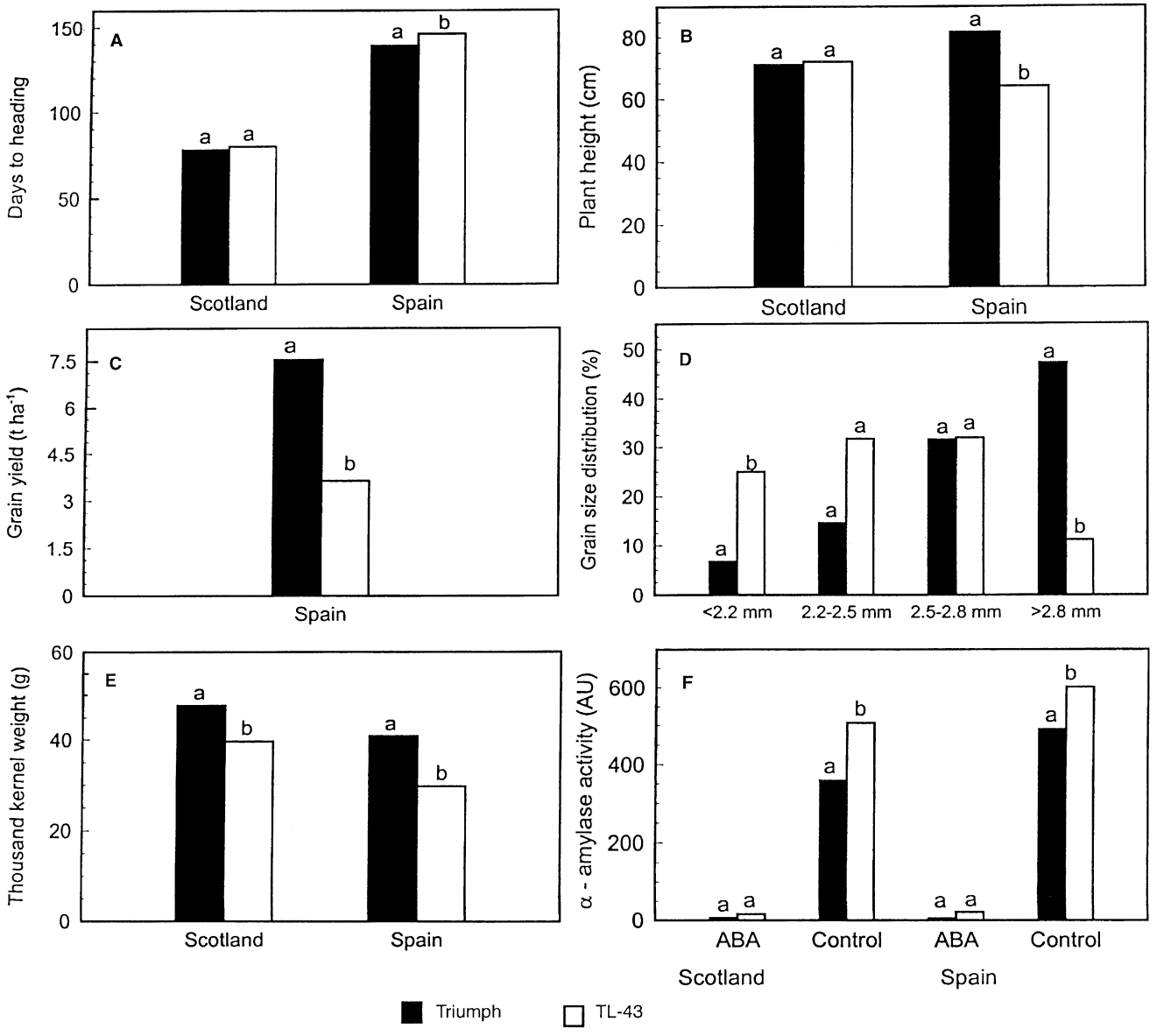

Fig. 1A–F Agronomic and qualitative characterisation of Triumph and TL43. (**A**) Days to heading. (**B**) Plant height (cm). (**C**) Grain yield (t/ha). (**D**) Distribution of grain size (%) as average of grain harvested in Spain and Scotland. (**E**) Thousand-kernel weight (g). (**F**) α-amylase activity. Within a given location, genotypes with *different letters* are different according to an LSD test at $P \leqslant 0.05$

is a standard way to measure the effect of hormones on barley germination (Chandler 1992), also being a measure of starch degradation and, therefore, an important component of extract yield (Briggs 1992). The data presented show a similar effect of ABA on both genotypes at both sites, with the activity of the enzyme reduced to a similar extent. Without ABA, there are significant differences between genotypes, as TL43 had a higher α-amylase activity than Triumph in both Spain and Scotland.

Dormancy behaviour and endogenous ABA concentration

The results of the dormancy assessment carried out 2 weeks after harvest, as is usually recommended by the European Brewery Convention (EBC 1987), are presented in Fig. 2A. Compared with Triumph the mutant had strongly reduced dormancy at all four environments, although the extent of the reduction was influenced by the degree of dormancy. In Scotland 95 and Spain 96, while Triumph showed a germination of 6 and 15%, respectively, the corresponding figures in the mutant were, approximately, 60 and 70%. However, when the level of dormancy in Triumph decreased, as in Scotland 97 and Spain 97, the mutant, though with significantly higher germination percentages, showed a smaller advantage over Triumph.

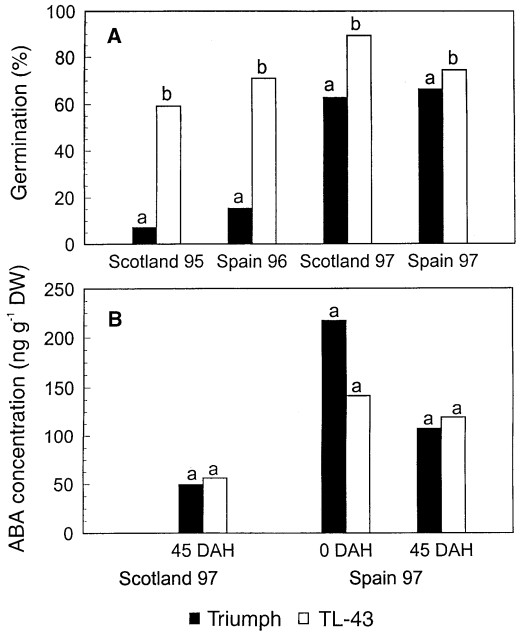

Fig. 2 **A** Germination 2 weeks after harvest. **B** ABA concentration in the grain at harvest (zero days after harvest, 0 DAH) and 45 DAH Within a given environment (site-year combination), genotypes with *different letters* are different according to an LSD test at $P \leqslant 0.05$

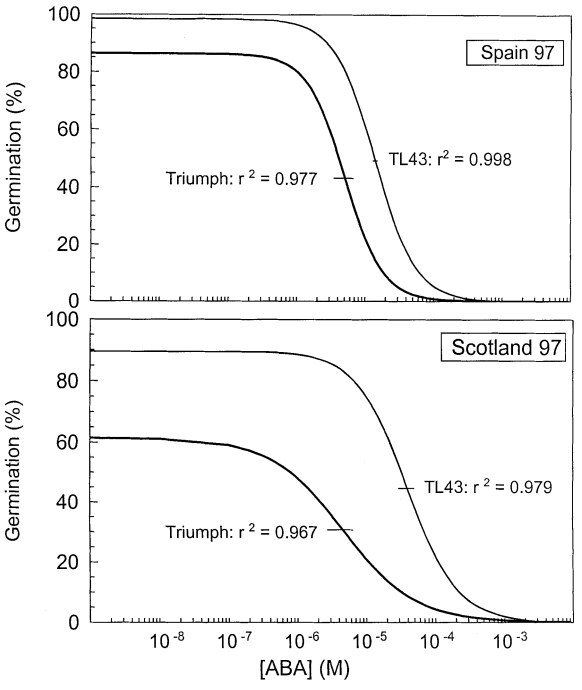

Fig. 3 Genotypic sensitivity of seed to increasing ABA concentrations (logarithmic scale on the X axis) in the germination medium. The curves are logistic dose-response functions with the general equation: Germination $(\%) = a/\{1 + [\log(\mathrm{ABA})/b]c\}$. *Horizontal bars* represent the confidence intervals of the ID50

There were no significant differences between the grains of the parent and mutant genotypes for endogenous ABA concentration in 1997, either in Scotland at 45 days after harvest or in Spain both at harvest and at 45 days later (Fig. 2B). However, as the relative ranking of the Spanish-grown genotypes changed between the two dates, it appears that the decline in endogenous ABA concentration is greater in Triumph. Endogenous ABA concentrations in Scotland were significantly lower than those in Spain, despite very similar levels of dormancy between the two environments. The reasons for this result are currently being investigated, but may depend on the contrasting differences in barley growing conditions existing between the two sites; e.g. (1) length of the barley lifecycle: the autumn (Spain) vs spring (Scotland) growing season, and (2) terminal drought and high temperature (Spain) vs cool-moist weather (Scotland).

Germination response to exogenous ABA

Response curves to different concentrations of ABA in the germination medium are shown in Fig. 3. A logistic dose-response function, germination $(\%) = a/\{1 + [\log(\mathrm{ABA})/b]c\}$, was used to fit the data. Two of these three parameters have a direct biological meaning; a represents the transition height, or germination not affected by ABA, and b the ID50, i.e. the ABA concentration that allows for half the germination of the untreated control; c has no biological meaning. Very good levels of fit were obtained for the dose-response curves (r^2 ranging from 0.967 to 0.998). The ID50 and its 95% confidence interval from each individual curve are also shown in Fig. 3.

Overall, both genotypes showed a lower dormancy level in Spain than in Scotland 45 days after harvest (86 vs 62% for Triumph and 98 vs 89% for TL43). Triumph was more sensitive to ABA than TL43 in both seed lots. Germination was reduced to 50% in Triumph by a lower ABA concentration as compared to TL43. ID50s for Spain and Scotland were similar, 5.1×10^{-6} vs 4.6×10^{-6} for Triumph and 1.4×10^{-5} vs 4.0×10^{-5} for TL43. Thus, because TL43 is able to maintain germination at an ABA concentration ten-times higher than Triumph, it should be considered an ABA-insensitive mutant, according to the definition of Koornneef et al. (1994).

Discussion

A delayed heading, lower grain yield, and smaller grain size and weight, are well-documented side-effects of mutations (reviews in Åastveit 1977; Gaul 1977; Kawai

1977. The mutant TL43 appeared to be later in heading, with shorter stems, smaller and lighter kernels, and lower grain yield than its parental genotype Triumph, when grown in Spain. Although a selection procedure was used to discard the agronomically poorest genotypes, TL43 was retained because of its dormancy response and its acceptable phenotype under Scottish growing conditions.

Two main types of mutants with effects on both ABA concentration and dormancy have been induced in *A. thaliana* (reviews in Karssen 1995; Koornneef et al. 1997). The first group includes ABA-deficient mutants, such as *aba1*, *aba2* and *aba3*, with reduced seed dormancy, a strongly wilty phenotype and enhanced ABA sensitivity. The second group includes ABA-response mutants, such as *abi1*, *abi2* and *abi3*, with reduced seed dormancy and ABA sensitivity but with an endogenous ABA concentration either equal to, or somewhat higher than, the wild-type. Several of them (*abi1*, *abi2*, *abi3* and *aba2*) have been cloned (Koornneef et al. 1998) and shown to encode a protein with distinct regions of homology to that encoded by the viviparous *vp1* gene of maize (McCarty et al. 1991), which seems to be a transcriptional activator.

The *rdo1* and *rdo2 Arabidopsis* mutants (Léon-Kloosterziel et al. 1996) have reduced levels of dormancy, normal levels of ABA, and the same sensitivity to ABA, ethylene, auxin and cytokinins as the wild-type. However, they showed a sensitivity to ABA in germination which was only slightly lower than that of the wild-type. All the evidence presented in the present paper suggests that TL43 is neither similar to the *aba* nor to the *rdo Arabidopsis* mutants, firstly because it is not ABA-deficient, and secondly, because it has an apparently enhanced insensitivity to ABA in germination. It may have some similarity with *Arabidopsis* mutants of the *abi* type, i.e. ABA-insensitive mutants, defined as those able to maintain germination at ABA concentrations ten-times higher than the wild-type (Koornneef et al. 1994). However, the *abi1* and *abi2* mutations inhibit multiple actions of ABA in vegetative tissues (Merlot and Giraudat 1997), display abnormal stomatal regulation and defects in various ABA-mediated morphological and molecular responses to stress, transpiring excessively and wilting (Koornneef et al. 1989). The *abi3* mutant shows numerous defects during embryo development and does not acquire desiccation tolerance, but displays normal water relations (Koornneef et al. 1989). In addition, all *abi* mutants show normal seed weights (Koornneef et al. 1989). At present none of these phenotypes have been observed in TL43, although experiments to characterise dormancy inception and release and ABA concentration during grain development are presently underway. Studies on the water balance of the mutant have not yet been done, although it showed symptoms indicative of stressed conditions, i.e. short straw, small grains, and a much reduced grain yield, only when grown under Spanish conditions. Previous research has shown large differences in patterns of grain filling between Lleida and Dundee (Swanston et al. 1997), which were directly attributed to the hotter and drier Spanish climate in which the grain filled and ripened. However, the appearance of TL43 when grown both under glasshouse and field conditions, in Spain, does not suggest a wilty phenotype.

Hayter and Allison (1976) demonstrated cultivar differences in sensitivity to ABA and were able to induce barley mutants capable of germinating in concentrations of ABA which inhibited germination in the parental cultivar. These mutants were characterised by increased amylase activity which was attributed to a higher endogenous GA level and, generally, by a reduction in height and yield. However, detailed examination of one such mutant (Ellis et al. 1986) indicated the presence of two dwarfing genes, so this mutant was phenotypically very different from TL43. It would seem, therefore, that TL43 is a new type of mutant also different from the *Arabidopsis* mutants of the *aba*, *abi*, and *rdo* types, although with some similarity to *abi3*. Its lack of dormancy in spite of its normal endogenous ABA seed content, could be explained by one or more of the following mechanisms proposed for a fast-germinating barley mutant by Visser et al. (1996): i.e (1) an increased ABA diffusion out of the embryo; (2) a reduction in the ABA sensitivity of the embryo; (3) inhibition of *de novo* ABA synthesis; or (4) an increased ability to degrade extracellular ABA. Another possibility would be a change in the ABA signal transduction pathway, the mutant being, as is *abi3* (Merlot and Giraudat 1997), a putative transcriptional activator.

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