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The Tom Thumb Dwarfing Gene, *Rht3* in Wheat, I. Reduced Pre-harvest Damage to Breadmaking Quality

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Summary. The effects of the Tom Thumb dwarfing gene, *Rht3*, on the quality and quantity of grain α-amylase produced during germination and by induction with exogenous gibberellic acid are described. In a season conducive to high sprouting damage the gene reduced α-amylase levels in the field by 77%. Selection among random *Rht3* genotypes showed that other genetic factors can be combined with the dwarfing gene to further increase sprouting damage resistance.

Key words Wheat – Quality – Dwarfism – Gibberellin – α-amylase

Introduction

Preharvest sprouting damage in wheat is a world-wide problem, particularly affecting the white-grained North American and Australian crops produced for the Japanese noodle market and the European breadwheat crop (Derera 1980). Severe sprouting can depress yields and viability of grain, however the primary problem is the increase in α-amylase activity which occurs with the onset of germination. This enzyme has deleterious effects on bread and noodle quality, causing hydrolytic loss of dough viscosity during processing (Buchanan and Nicholas 1980; Moss 1980). α-amylase is produced de novo in the germinating grain in response to gibberellins (Yomo and Varner 1971) and the discovery that the 'Tom Thumb' gene, *Rht3*, can inhibit the response of wheat aleurone to gibberellins (Gale and Marshall 1973) provided a new genetic approach to the control of sprouting damage. This paper describes experiments designed to test the feasibility of this method.

Genotypes

Dwarf and tall random lines were produced from the winter wheat cross 'Minister Dwarf' (MD, *Rht3*) × 'Cappelle-Desprez' (CAP, *rht3*). Homozygous dwarf and tall F_3 lines were identified by progeny testing 140 F_2 plants for insensitivity to gibberellic acid (GA), (Gale et al. 1975). Bulked grain from each F_3 line was multiplied at F_4 and sown at F_5 as random F_4 bulks in a field trial and as selected lines in a greenhouse.

Methods

F_4 Field Trial: The parents, 33 dwarf (*Rht3/Rht3*) and 33 tall (*rht3/rht3*) lines were sown as spaced plants in October 1976, with lines randomised within dwarf and tall plots in two blocks. At the end of August 1977, immediately following a ten-day period of wet weather, grain samples from each plot were scored for visible sprouting (per cent of 250 grains showing a ruptured testa) and milled into wholemeals for α-amylase assays.

F_5 Field Trial: The parents, eight dwarf and eight tall lines, were sown as drilled plots (400 plants m^{-2}) in October 1978. The lines were randomised within dwarf and tall groups in three blocks. 100 g grain samples from each plot were milled on a Brabender mill at an extraction rate of 66.5 per cent for estimation of flour α-amylase content.

Artificially Induced Sprouting: A chamber similar to that described by McMaster and Derera (1976) was used to germinate grains on the ear in the dark at 20 °C and 100% r.h. for five days.

Germination: Grains were surface sterilized with $HgCl_2$ (10 mM, 2 min), $NaHClO_3$ (2% available Cl_2, 10 min) and sterile distilled water (2 washes of 5 min) and germinated in the dark at 25 °C on sterile filter paper for five days, with or without 10 μM GA^3.

Incubation of Distal Half Grains: 3 mm distal half grains were cut from $HgCl_2$ sterilized grains and surface sterilized with $NaHClO_3$ as above. These were incubated in batches of four to ten at 25 °C on a slow orbital shaker (100 r.p.m.) in conical flasks containing 0.5 ml/grain Na citrate (0.05 M), $CaCl_2$ (0.02 M), streptomycin (0.01 g l^{-1}) and GA_3 (up to 10 μM) at pH 6.2.

Extraction of α-amylase. Germinated grains and flours were extracted in a solution of 0.34 M NaCl, 1.25 μM Ca acetate at rates of 1 ml $grain^{-1}$ or 2 ml g^{-1} flour. Germinated grains were extracted by grinding in a Polytron blender for 20 sec and flours by shaking at 37 °C for 30 min. Distal half grains were ground in their own incubation medium. Supernatants

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were recovered after centrifugation at 3,000 g for 20 min and stored at – 20 °C for up to one week prior to assay.

Measurement of α-amylase: Falling numbers (FN) of F_4 and F_5 grain samples were measured by the standard method (IACC Standard 107 1967). These values were transformed to liquefaction numbers by the conversion 6000/(FN-50) (Perten 1964).

Very low α-amylase levels and fungal α-amylase standards, used for calibrating the automatic system described below, were assayed using Phadebas (Pharmacia Diagnostics, Uppsala) dye-bound starch tablets.

Most enzyme measurements were made using an automated system (modified from Smith 1970) using β-limit dextrin (Rank, Hovis, MacDougal, UK) as a substrate and continuously recording OD 550 nm after addition of iodine. The results are obtained by calibration against fungal α-amylase (ex *Aspergillus oryza,* Sigma Chemical Co.) where one unit (U) catalyses the hydrolysis of one μmol of glucosidic linkages per minute at 37 °C.

Isoelectric focussing was carried out using pH range 3.5–9.5 gels (LKB Multiphor) as described by Sargeant and Walker (1978).

Results

The experiments described below deal with three aspects of the way in which the Tom Thumb dwarfing gene, *Rht3,* may be exploited to reduce α-amylase levels in sprouted wheat grains; first, a detailed examination of the way in which *Rht3* affects the ability of wheat grains to produce α-amylase; second the way in which *Rht3* reduces sprouting damage in the field; and third, the possibility of combining *Rht3* with other genetic factors affecting the degree of sprouting damage.

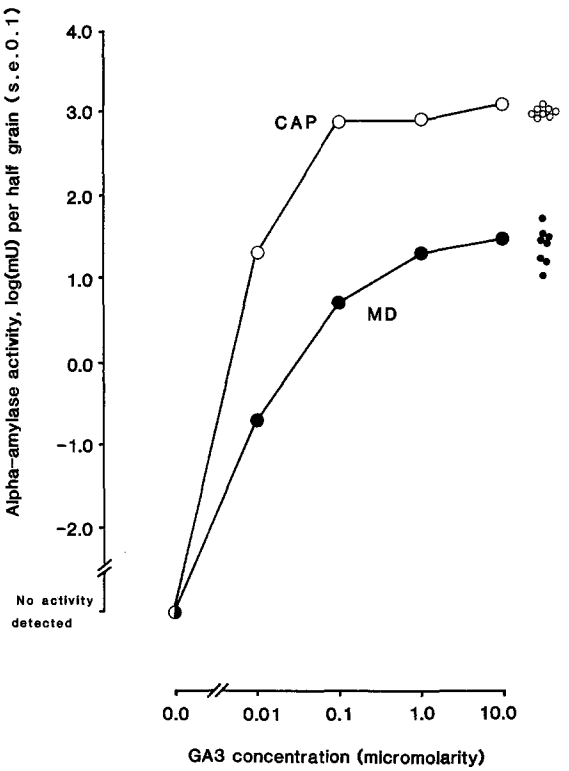

Fig. 1. Dose-response for GA_3 inducible α-amylase in deembryonated half-grains of the parents and random F_5 *Rht3* and *rht3* lines from the cross 'Minister Dwarf' × 'Cappelle-Desprez'. Responses of 'Minister Dwarf' (MD) and 'Cappelle-Desprez' (CAP) were measured over a range of GA_3 levels; respones of random F_5 *rht3* (○) and *Rht3* (●) lines to 10 μM GA_3 are shown on the right of the figure. Enzyme activities were assayed in triplicate samples of four half-grains after incubation with GA_3 at 25 °C for 3 days

Rht3 and α-amylase Production in Wheat Grains

Responses of half grains of the two parents, CAP and MD, to increasing concentrations of GA_3 are shown in Fig. 1. Both varieties showed some response but MD produced only 3 per cent of the maximal CAP enzyme. The 10 μM GA_3 responses for the 16 random *Rht3* and *rht3* F lines are shown on the right of Fig. 1. Clearly the low α-amylase response of MD is inherited in the *Rht3* lines.

Time Course of α-amylase Production in Germinating Whole Grains and GA-treated Distal Half-grains

The extractable α-amylase from grains germinating in water and GA solution (10 μM), and in half grains at the same GA concentration are shown in Fig. 2 for CAP and MD. The effect of GA on germinating grains is apparent only in the tall variety, CAP, over the first two days. Thereafter, and in the 'GA-insensitive' variety MD, there was little difference between the control and GA-treated germinating grains.

In the GA-treated half grains, production of α-amylase was initially less than in whole germinating grains, although later, maximal activity was similar in both systems. From the second day MD produced less enzyme than CAP and by the third day both verieties had reached maximum activity.

The Effect of *Rht3* on the Nature of α-amylase Produced in Germinating Grains

The structural genes encoding for GA-induced grain α-amylases in wheat comprise two triplicate series of genes, *α-Amy-1* on the Group 6 chromosomes and *α-Amy-2* on the Group 7 chromosomes (Nishikawa and Nobuhara 1971; Gale et al. 1982). The isozymes produced by the genes fall into two groups, α-AMY1 with more basic isoelectric points (PI) and α-AMY2 with higher PIs. The relative abilities of these forms of the

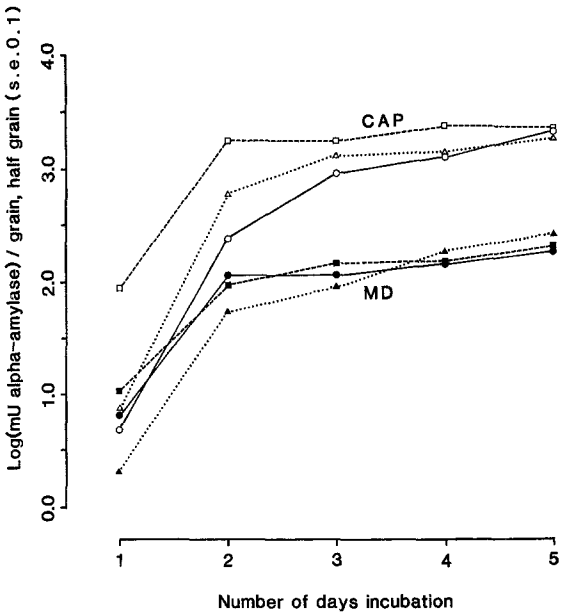

Fig. 2. α-Amylase production during germination and GA_3 induction of 'Cappelle-Desprez' (CAP, hollow symbols) and 'Minister Dwarf' (MD, solid symbols). No activity was detected in control (dry) grains, α-amylase levels in grains germinated with (□, ■) and without (○, ●) 10 μM GA_3 and in half-grains incubated with 10 μM (△, ▲) were assayed in triplicate samples of ten (half) grains. Germination and GA_3 induction were carried out in the dark at 25 °C

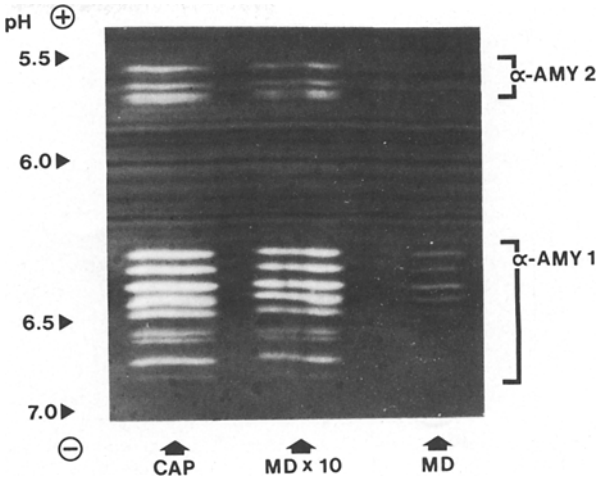

Fig. 3. α-Amylase isozymes from germinated grains of 'Minister Dwarf' (MD) and 'Cappelle-Desprez' (CAP). Light bands of high α-amylase activity are shown after isoelectric focussing and starch/iodine staining. Enzyme extracts from grains germinated for 5 days (CAP 2.22 U/grain, MD 0.22 U/grain) were applied in aliquots equivalent to 0.03 grain (CAP, MD) and 0.30 grain (MD × 10)

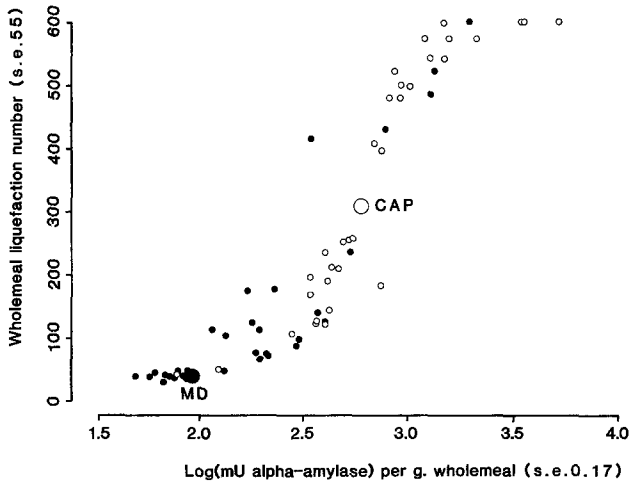

Fig. 4. Dependence of liquefaction number on α-amylase activity in wholemeals from random F_4 *Rht3* and *rht3* lines from 'Minister Dwarf' × 'Cappelle-Desprez'. Scores for each *Rht3* (●) and *rht3* (○) line were averaged over two experimental blocks

enzyme to bind with and digest wheat starch grains (Sargeant and Walker 1978) make it likely that the α-AMY1 isozymes are responsible for most of the deleterious effects of α-amylase on breadmaking quality. Thus it is important to know whether there are differential effects of *Rht3* on isozyme formation.

The effect of *Rht3* in the parents is to reduce isozyme activity generally (Fig. 3). However, when the MD extract is applied to the gel at 10 times concentration, so that the total quantitative activity of the sample is similar to that of CAP, it is clear that the isozyme complement is the same in both varieties.

2 *The Effects of Rht3 on Field α-amylase*

2.1 Field Conditions Conducive to Severe Sprouting Damage

The 1977 UK harvest suffered the most severe sprouting damage of the last decade. The 66 F_4 random dwarf and tall lines were deliberately harvested late in the season so as to maximise the damage in the trial. Visible sprouting was observed in all samples of grain from the experiment, however the *Rht3* and *rht3* populations showed no differences in this parameter, giving values of 17.4 and 20.3±2.7 percent respectively. Nevertheless clear effects of the gene are seen in α-amylase content and Perten liquefaction numbers, both of which measure the damage to breadmaking quality caused by sprouting (Fig. 4).

The scores for *Rht3* lines are generally lower for both measurements, however the relationship between the characters is similar for both genotypic groups. This confirms the isoelectric focussing results discussed above which indicated that *Rht3* affects only the quantity and not the quality of α-amylase.

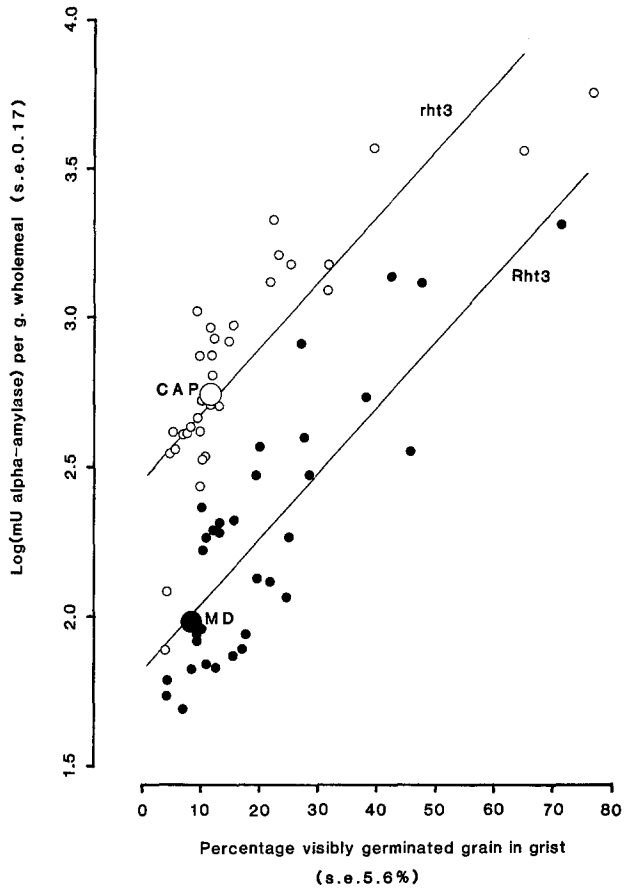

Fig. 5. Regressions of wholemeal α-amylase content onto visible sprouting for random *Rht3* and *rht3* F_4 lines from 'Minister Dwarf' × 'Cappelle-Desprez'. Regression equations: log (mU α-amylase) = 2.469 + 0.022 (% germination) for *rht3* lines (○), = 1.826 + 0.022 (% germination) for *Rht3* lines (●). Data were obtained from the same trial as Fig. 3

The relationship between visible sprouting and α-amylase content in the parents and random F_4 lines (Fig. 5) shows that the enzyme levels are related to visible sprouting in both the dwarfs and the talls. However, over the wide range of visible sprouting, *Rht3* is consistently associated with a 77 per cent reduction in α-amylase.

Field Conditions Conducive to Negligible Sprouting Damage

In 1980 no visible sprouting was observed in the F_5 tall and dwarf random lines, which all produced flour with commercially acceptable falling numbers, but the liquefaction numbers and α-amylase values obtained for *Rht3* lines were much lower than those of *rht3* lines (Table 1). Clearly *Rht3* is associated with reduced levels of α-amylase even when sprouting has not occurred.

Table 1. α-amylase and liquefaction number in flours from *Rht3* and *rht3* unsprouted grain

		α-amylase mU/g flour (s.e.)		Liquefaction number (s.e.)	
Rht 3 group	'Minister Dwarf'	4.0	(0.5)	18.25	(0.16)
	Random F_5 lines	5.1	(1.0)	18.93	(0.49)
rht3 group	'Cappelle-Desprez'	13.6	(3.1)	24.65	(0.60)
	Random F_5 lines	30.5	(10.4)	28.82	(1.61)

Duplicate measurements were made from flours of the parents, 8 random *Rht3* and 8 random *rht3* F_5 lines from 'Minister Dwarf' × 'Cappelle-Desprez' grown in three experimental blocks

Rht3 and Other Genetic Factors Affecting Preharvest Sprouting Damage

The wide range of visible sprouting percentages and α-amylase levels found within the tall and dwarf groups at F_4 indicated the presence of genetic differences not ascribable to the *Rht3/rht3* locus among the lines. The heritability of these differences was examined by selecting three lines from each group, showing low, average and high levels of visible sprouting, and observing their relative abilities to sprout and produce α-amylase under simulated rainfall conditions at F_5.

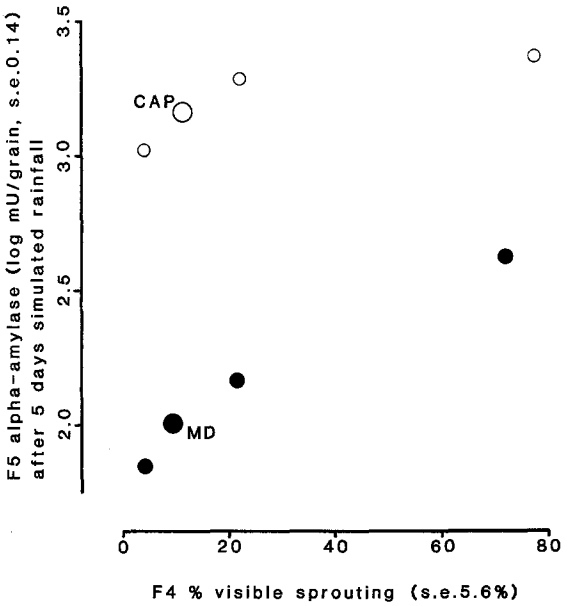

Fig. 6. Heritable variation for sprouting resistance amongst selected *Rht3* and *rht3* lines from 'Minister Dwarf' × 'Cappelle-Desprez'. *Rht3* (●) and *rht3* (○) lines were selected for low, average and high levels of preharvest germination in F_4

Anthesis dates for all the greenhouse grown plants were similar and samples taken at 70 days after flowering were seen to show the largest differences between lines. No α-amylase activity was detected in grain from control (dry) ears, while the artificial sprouting produced the activities shown in Fig. 6.

Visible sprouting in F_4 and α-amylase levels after artificial sprouting in F_5 are highly correlated, having components both associated with and independent from *Rht3*. Thus while *Rht3* consistently reduces the effect of sprouting on breadmaking quality, the level of reduction can be further modified by other genes which probably control the duration of post-harvest ripeness dormancy in the wheat grain and which are segregating in the CAP × MD progeny.

Discussion

The Tom Thumb dwarfing gene has been available to wheat breeders for many years, but has not been used in commercial varieties because of the extreme dwarfism it confers. The discovery of reduced GA-induced α-amylase in Tom Thumb (Gale and Marshall 1973) and the increasing economic importance of sprouting damage in the world crop (Derera 1980) has provoked this evaluation of *Rht3* as a genetic tool to combat the problem.

In Australia Bhatt et al. (1977) have already examined potential use of the *Rht3* variety 'Tordo' in breeding rain-resistant white-grained wheats. However they found that such resistance was strongly associated with undesirably short plant habit in F_2 plants from an *Rht3* × semi-dwarf cross. Certainly extreme dwarfism will be a major problem for wheat breeders attempting to exploit *Rht3*.

Rht3 is carried on chromosome 4A (Morris et al. 1972) and can therefore be transferred to triticale, in which plant height can be maintained at an agronomically acceptable level even in the presence of the dwarfing gene. Low alpha-amylase triticales carrying *Rht3* have been described by Chojnacki et al. (1976) and the gene could be of considerable value in this crop, which is prone to severe sprouting.

Rht3 has similar, but more severe, effects to the agronomically successful 'Norin 10' dwarfing genes *Rht1* and *Rht2* and is related genetically, being allelic to *Rht1* (Gale and Marshall 1976). The insensitivity of germinating *Rht3* grain to GA is, however, not found in 'Norin 10' derived semi-dwarfs. GA release from embryos (Gale and Marshall 1975) and GA uptake by isolated aleurones (Ho et al. 1981) is similar in *Rht3* and *rht3* genotypes. The primary effect of the gene would therefore seem to be a block at an early step common to most GA-mediated developmental systems. Gale and Marshall (1975) noted that release of two other GA-induced hydrolases was blocked and Ho et al. (1981) have shown that a range of other GA-induced processes are blocked. Certainly the several aspects of α-amylase production studied here in the *Rht3* and *rht3* genotypes indicate no differential qualitative effects on the GA-induced enzyme. Only the amount of enzyme produced is affected, while the time course of α-amylase production, the relative amounts of enzyme produced by six α-amylase structural genes and the starch liquefaction capacity of the enzyme as measured by the Falling Number test are all unaffected.

Recently Gibbons (1979) and Okamoto et al. (1980) have suggested that the scutellum rather than the aleurone is the primary site of production of hydrolases during germination. It is of interest to note that α-amylase production is inhibited in both tissues by *Rht3*, as seen by the similar effects of the gene in whole or distal half grains, which lack the scutellum.

The F_4 field trial was grown in 1977, a season which produced losses in grain quality due to sprouting estimated at more than £ 60 M in the UK (Mitchell et al. 1980). The effect of *Rht3* was to reduce α-amylase levels by 77 per cent. This was less than the effect of the gene in distal half grains (97%), but nevertheless represented a reduction in damage to baking quality equivalent to a 29 per cent reduction in visible sprouting. An effect of this magnitude should be large enough to eliminate sprouting as a commercial problem in most seasons, since even in 1977 the average visible sprouting in the South-East of England, where the damage was highest, was 18.8 per cent (Home Grown Cereals Authority 1977).

The observation that *Rht3* also reduced endogenous α-amylase levels in unsprouted grain in the 1980 trial is probably linked to the observation of Marchylo et al. (1980) that the α-AMY1 or 'malt-type' isozymes can appear in the grain late in development but before maturity. It is probable that this phenomenon is GA-mediated and also blocked in the *Rht3* lines.

The differences between the *Rht3* lines in Figure 4 indicate that the gene might not provide sufficient protection in all genetic backgrounds, but that the effect of the gene can be combined with other genetic factors for resistance to visible sprouting. The efficiency of selection in F_4, as measured by the similar ranking of lines in the F_5 artificial rainfall tests, indicates that the combination of *Rht3* with other 'anti-sprouting' factors is both feasible and sufficient to provide protection in most field environments.

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Literature

Bhatt, G.M.; Derera, N.F.; McMaster, G.J. (1977): Utilisation of Tom Thumb source of pre-harvest sprouting tolerance in a wheat breeding programme. Euphytica **26,** 565–572

Buchanan, A.M.; Nicholas, E.M. (1980): Sprouting, α-amylase and breadmaking quality. Cereal Res. Commun. **8,** 23–28

Chojnacki, G.; Bryckzyinski, J.; Tymieniecka, E. (1976): Preliminary information on sprouting in triticale. Cereal Res. Commun. **4,** 111–114

Derera, N.F. (1980): The audit of sprouting. Cereal Res. Commun. **8,** 15–22

Gale, M.D.; Marshall, G.A. (1973): Insensitivity to gibberellin in dwarf wheats. Ann. Bot. **37,** 729–735

Gale, M.D.; Marshall, G.A. (1975): The nature and genetic control of gibberellin insensitivity in dwarf wheat grain. Heredity **35,** 55–65

Gale, M.D.; Marshall, G.A. (1976): The chromosomal location of *Gai1* and *Rht1,* genes for gibberellin insensitivity and semidwarfism, in a derivative of Norin 10 wheat. Heredity **37,** 283–289

Gale, M.D.; Law, C.N.; Chojecki, A.J.; Kempton, R.A. (1982): Genetic control of α-amylase production in wheat. (in preparation)

Gale, M.D.; Law, C.N.; Worland, A.J. (1975): The chromosomal location of a major dwarfing gene from Norin 10 in new British semi-dwarf wheats. Heredity **35,** 417–421

Gibbons, G. (1979): On the localisation and transport of α-amylase during germination and early seedling growth of *Hordeum vulgare.* Carlsberg Res. Commun. **44,** 353–366

Ho, T.H.D.; Nolan, R.C.; Shute, D.E. (1981): Characterization of a gibberellin-insensitive dwarf wheat, D6899: Evidence for a regulatory step common to many diverse responses to gibberellins. Plant Physiol. **67,** 1026–1031

Home Grown Cereals Authority (1977): Cereal quality survey, London: Hamlyn

International Association for Cereal Chemistry (1967): Determination of Falling Number (according to Hagberg-Perten) as a measure of the degree of alpha-amylase activity in grain and flour. IACC Standard 107

Kruger, J.E. (1980): Progress in the chemistry of some quality-affecting enzymes resulting from preharvest sprouting damage. Cereal Res. Commun. **8,** 39–48

McMaster, G.J.; Derera, N.F. (1976): Methodology and sample preparation when screening for sprouting damage in cereals. Cereal Res. Commun. **4,** 251–254

Marchylo, R.H.; LaCroix, L.J.; Kruger, J.E. (1980): Synthesis of α-amylase in specific tissues of the immature wheat kernel. Cereal Res. Commun. **8,** 61–68

Mitchell, B.; Armstrong, C.; Black, M.; Chapman, J. (1980): Physiological aspects of sprouting and spoilage in developing *Triticum aestivum* L. (wheat) grain. In: Seed Production (ed. Hebblethwaite, P. D.), pp. 339–356. London: Butterworths

Morris, R.; Schmidt, J.W.; Johnson, V.A. (1972): Chromosomal location of a dwarfing gene in 'Tom Thumb' wheat derivative by monosomic analysis. Crop Sci. **12,** 247–249

Moss, H.J. (1980): The pasting properties of some wheat starches free of sprouting damage. Cereal Res. Commun. **8,** 297–302

Nishikawa, K.; Nobuhara, M. (1971): Genetic studies of α-amylase isozymes in wheat 1. Location of genes and variation in tetra- and hexaploid wheat. Jpn. J. Genet. **46,** 345–358

Okamoto, K.; Kitano, H.; Akazawa, T. (1980): Biosynthesis and excretion of hydrolases in germinating cereal seeds. Plant Cell Physiol. **21,** 201–204

Perten, H. (1964): Application of the Falling Number method for evaluating α-amylase activity. Cereal Chem. **41,** 127–140

Sargeant, J.G.; Walker, T.S. (1978): Adsorption of wheat alpha-amylase isoenzymes to wheat starch. Stärke **30,** 160–163

Smith, D.B. (1970): An automatic method for the determination of α-amylase activity in cereal extracts. In: Automation in Analytical Chemistry, Technicon Intern. Symposium, 1970

Yomo, H.; Varner, J.E. (1971): Hormonal control in secretory tissue. In: Current Topics in Developmental Biology Vol. 6. (eds. Moscona, A.A.; Monroy, A.) pp. 11–114. New York, London: Acad. Press

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