Thermostabilities of grain β-amylase and β-glucanase in Finnish landrace barleys and their putative past adaptedness

HANNU AHOKAS and MARJA-LEENA MANNINEN

Crops and Soil, Agricultural Research Centre, Jokioinen, Finland

Ahokas, H. and Manninen, M.-L. 2000. Thermostabilities of grain β-amylase and β-glucanase in Finnish landrace barleys and their putative past adaptedness.—*Hereditas 132*: 111–118. Lund, Sweden. ISSN 0018-0661. Received November 19, 1999. Accepted March 6, 2000

Thermostability of β-amylase activity was a general feature in a sample of 32 Finnish barley landraces. One of two Finnish landraces probably contributed the thermostability to cv. 'Pirkka' in crosses performed about 70 years ago. The stability is less evolved in β-glucanase activity although the most tolerant types appeared in landraces and in Pirkka with a Finnish landrace background. Selection pressure for thermostability in grains may have been a feature of traditional crop management practices among Finns in the past: drying grain crops, including premature barley, above an oven in a special drying house at temperatures exceeding 55°C, and germination in black, sunlit slash-and-burn soils, with a measured surface temperature of 63°C. A positive, though small correlation between the thermotolerance ratios of the two enzymes may be a remnant of their common long selection pressure ending tens of generations prior to collection in the 1960s and 1970s.

Hannu Ahokas, Crops and Soil, Agricultural Research Centre, Myllytie 10, FIN-31600 Jokioinen, Finland. E-mail: hannu.ahokas@mtt.fi

Samples of Finnish landrace cereals studied previously proved highly variable in several traits, evidently containing a rich variety of genes and combinations of genes. The landrace populations have been mixtures, some approaching mixtures of unique genotypes in the past (AHOKAS 1998; AHOKAS and POUKKULA 1999). The reasons for this variation are evidently historical, environmental and selective (AHOKAS and MANNINEN 2000). Methods of crop and field management prior to about 1930 in Finland may have generated environments which unconsciously selected thermostability of various vital characteristics at germination and harvest. The thermostability of enzymes of malting barley (*Hordeum vulgare* L.) is generally a desired characteristic, and preferred malting barleys apparently have significant β-amylase thermostability (KIHARA et al. 1998). They also show pedigrees with simple inheritance of thermostability (KIHARA et al. 1998). Since high thermostability of β-amylase exceeding 65 % remaining activity seems to be a rare but an inherited characteristic (KIHARA et al. 1998, 1999), there is reason to study thermostability in the sample, probably managed with slash-and-burn culture (see HEIKINHEIMO 1915) and *riihi*-heated drying (see GROTENFELT 1899; TALVE 1961) still some tens of generations earlier.

MATERIAL AND METHODS

Plant material

The material analyzed in this study, which has been described earlier (AHOKAS and POUKKULA 1999), was from the 1996 harvest and of good quality. Most of the Finnish landrace selections have been accessioned by the US Department of Agriculture, Beltsville, MD: PI 349678-PI 349681, PI 415017-PI 415019, PI 467622-PI 467627 and PI 467629-PI 467653.

Sound grains were weighed and hulled partly by hand and further with a 50% H_2SO_4 wash followed by water rinses, and then germinated aseptically on washed, sterile quartz sand in groups of 15 in the dark at 15.5 ± 0.5°C for 120 hours. Thereafter, the germinants were homogenised aseptically in a buffer of pH 4.6 containing 40 mM sodium acetate, 40 mM sodium phosphate and 0.001 % sodium azide as previously described (AHOKAS and POUKKULA 1999). The extract supernatants were stored at -70°C or temporarily at -20°C until used. The assays were replicated and replicates which differed by more than four percentage points were analyzed for a third time. All the results are given as means of the determinations.

Assay of β-amylase

Aliquots (40 µl) of the extract were mixed with 3960 µl of a cold buffer of 50 mM MOPS, pH 7.0 with 1 % BSA (Sigma A-7511). A sample of 200 µl was kept on ice and another heated for 30 min at 56.7°C in a thermostatic circulator (LKB 2219 Multitemp II Thermostatic Circulator using 20 % Shell Antifreeze 402 coolant in the water bath). The actual temperature ranged from 56.3 to 57.0°C during the incubation as measured by the instrument and an external thermocouple (Pt 1000, Knick). Samples of 25 µl of the heated and + 0°C control samples were further diluted with 225 µl of buffer B (100 mM maleic acid, 1 mM EDTA, 0.1% w/v of BSA, NaOH until pH 6.2 was reached and 0.02% sodium azide) and assayed with a *p*-nitrophenyl maltopentaoside substrate containing α-glucosidase purchased from Megazyme.

Table 1. *β-Amylase activity in extracts of germinated grains after 30 min incubation at 56.7°C*

Landrace or reference	Remaining activity (ratio)	Activity without incubation at 56.7°C (arbitrary units for grain mass)
Landrace selections		
HA 22	0.90	0.62
HA 44	0.82	0.74
HA 9-63-4	0.77	0.69
HA 52	0.77	1.63
HA 9	0.76	0.86
HA 70-3	0.76	0.49
HA 20	0.75	1.05
HA 10	0.75	1.13
HA 38	0.75	0.78
HA 37	0.74	1.01
HA 42	0.74	0.54
HA 53	0.74	0.47
HA 17	0.74	0.51
HA 29	0.73	0.98
HA 6-33-02	0.73	0.68
HA 19	0.73	1.12
HA 49	0.73	0.77
HA 18	0.72	1.03
HA 40	0.72	0.64
HA 9-63-8	0.72	0.65
HA 70-2	0.72	0.48
HA 5	0.72	0.89
HA 48	0.72	0.52
HA 11	0.72	0.98
HA 12	0.72	0.81
HA 33	0.72	1.08
HA 9-63-2	0.71	0.81
HA 9-63-1	0.71	0.61
HA 3	0.71	0.89
HA 14	0.70	0.76
HA 146-04-1	0.41	0.24
HA 45	0.39	0.40
Global barleys		
Haruna Nijo	0.87	0.66
Pirkka	0.75	0.68
Noire 2R Montpellier	0.40	0.36
PI 391421	0.38	0.36
Adorra	0.38	0.57
$F_{(6)}$ *lines*[a]		
HA 52 × Adorra	0.65	0.34
HA 52 × Adorra	0.44	0.36
HA 52 × Adorra	0.41	0.30
HA 52 × Adorra	0.40	0.88
HA 52 × Adorra	0.40	0.61

[a] Grains from several F_5 plants.

Table 2. *Spearman coefficients of rank correlation between ratios of β-glucanase thermostability and other independent measurements in the 32 barley landrace samples*[1]

Second variable	r_S	Significance
Activity of β-glucanase without heat treatment (for grain mass)	0.140	NS (P = 0.56)
Activity of β-glucanase without heat treatment (for extract volume)	0.058	NS (P = 0.75)
Thermostability of β-amylase	0.277	P = 0.12
Thermostability of β-amylase excluding two extreme variants (Fig. 2)	0.351	P = 0.057

[1] Without heat treatment, activity of β-glucanase for grain mass vs extract volume, $r_S = 0.944$, $P < 0.001$.

The assay was conducted according to the supplier's instructions and took 10 min at 40°C. The dilutions of the extracts were 1000-fold for the assay, diluting putative endogenous thermoprotecting molecules, e.g. maltose (TAKAHATA et al. 1994), and enzyme inhibitors to insignificant levels.

Assay of β-glucanase

Melted and well-mixed extracts were diluted 3.76-fold with Na-acetate buffer (25 mM, 0.02 % w/v of Na-azide, final pH 4.43) and 1 % w/v of BSA (Sigma A-7511). Samples of 550 µl were either heat-treated for 15 min at + 45.0°C as described or kept on ice. Both the samples were left to stand for 30 min at room temperature, with subsequent assaying of 500 µl at + 30°C for 15 min with a Beta-Glucazyme tablet (Megazyme) based on Azurine-crosslinked barley β-glucan. The reaction was terminated with 6 ml of 1 % w/v Trizma base in water, vortexed twice at 5 min intervals, filtered (Whatman 1, ∅ 9 cm) and absorbances were measured at 590 nm as instructed by the supplier (Megazyme). The final assay pH was 4.8 at 30°C, and was maintained during the heat treatment. In Na-acetate buffer the maximal activity has been observed at pH 5 (KOTAKE et al. 1997). Absorbances were determined in arbitrary units based on the extract volume or the original grain mass.

RESULTS

β-amylase

The activity remaining after heating is presented in Table 1 as the ratio for 32 landrace selections, five global barleys and five lines of the cross HA 52 × 'Adorra'. Among the global barleys, 'Haruna Nijo', known to have thermostable β-amylase based on extracts of ungerminated grains (KIHARA et al. 1998), appeared to have thermostable β-amylase in this study of germinated samples (Table 1). 'Pirkka' (also studied as a4459), known to have highly active β-amylase (SIMBERG 1950; ALLISON and SWANSTON 1974), proved to have highly thermostable β-amylase in this study, while the other global barleys have the lowest ratios, with levels ranging from 0.38 to 0.40. Fifty per cent of the parentage of Pirkka is from two Finnish landraces (SIMBERG 1950; KIVI 1969), the specific parental lines of landraces crossed about 70 years ago not being maintained. The ratio distribution of the landrace samples varies from 0.38 to 0.90 with a mean ± SEM of 0.72 ± 0.09, their total distribution deviating highly significantly from normality ($\chi^2 = 511$, $P \ll 0.001$). The central fraction, landraces with the two highest and two lowest ratios removed, ranges from 0.70 to 0.77 with a mean ± SEM of 0.73 ± 0.003, and fits a normal distribution ($\chi^2 = 2.154$, $P > 0.80$). This suggests that the range of 0.70 to 0.77 is produced by a single allele or several allele types having the same effect. There seem to be other alleles involved, putatively one causing 0.39 to 0.41 ratios, and two others, one giving a ratio of 0.81 and the other giving 0.90 (Table 1).

There is no correlation between the ratio of thermostability and the total unheated β-amylase activity in a given volume of the sample ($r_S = 0.196$, $P > 0.30$), or between the ratio and the activity per unit of grain mass in the sample ($r_S = 0.176$, $P > 0.40$).

β-Glucanase

The activity of β-glucanase is in general less thermostable than that of β-amylase. The activity of the non-heat-treated extracts varied from 0.51 to 1.29 arbitrary units per ml in the samples of the 32 landraces and from 0.75 to 1.21 arbitrary units in the samples of the five global barleys. Relative to grain mass, the variations in activity were 0.80 to 2.24 arbitrary units in the 32 landrace samples and 1.05 to 1.86 arbitrary units in the five global barley samples. The correlation of these activity determinations of the 32 landraces was $r_S = 0.944$ ($P < 0.001$), and that of the five global barleys $r_S = 0.60$ (NS) (Table 2).

The ratios of the activity of the remaining heat-treated (15 min at 45°C) β-glucanase to the original activity varied from 0.52 to 0.81 with a mean of 0.67 in the 32 landraces, from 0.40 to 0.82 with a mean of 0.59 in the five global barleys. Among the global barleys, Pirkka, with a 50% Finnish landrace background, has the highest remaining activity ratio of 0.82, the other ratios being 0.71 (Haruna Nijo), 0.59 (Adorra), 0.45 (Noire 2R Montpellier) and 0.40 (PI 391421).

The distribution of thermostability in the landrace sample (Fig. 1) deviates from normality due to flatness and is bimodal with central modes of 0.61 and 0.73. Various correlations are presented in Table 2: there is a small positive correlation between β-amylase and β-glucanase thermostabilities, $r_S = 0.277$, $P = 0.12$, and if two extreme variants are exluded, $r_S = 0.351$, $P = 0.057$ (Fig. 2).

DISCUSSION

β-amylase

High thermostability of β-amylase in barley cultivars appeared rare and displayed inheritance in the known pedigrees (KIHARA et al. 1998). In these landraces, the total β-amylase activity for grain mass or soluble protein is highly variable (AHOKAS and POUKKULA 1999). The lack of correlation indicates that activity level and thermostability are separate phenomena and probably have a different genetic basis.

The ratios of the five $F_{(6)}$ lines of the cross HA 52 × Adorra, parents with 0.77 and 0.38 ratios, respectively (Table 1), indicate that the thermostability ratio has a simple inheritance as shown by other material (KIHARA et al. 1998). The line with a 0.65 ratio may still have a heterogeneous minority of grains with the low-ratio allele. Allelic differences in the final amino acyl sequence β-amylases have been detected in barley (KREIS et al. 1987; ERKKILÄ et al. 1998) or induced in cloned barley sequencies (OKADA et al. 1995). Amino acyl residue changes have been found to confer thermostability (OKADA et al. 1995; EGLINTON et al. 1998; MIKAMI et al. 1999).

β-Amylases as proteins appear to be multifunctional in various plant species and their different tissues (PAN et al. 1988; AHOKAS and NASKALI 1990; GANA et al. 1998), the enzyme activity not necessarily being the objective of natural selection e.g. under thermostress.

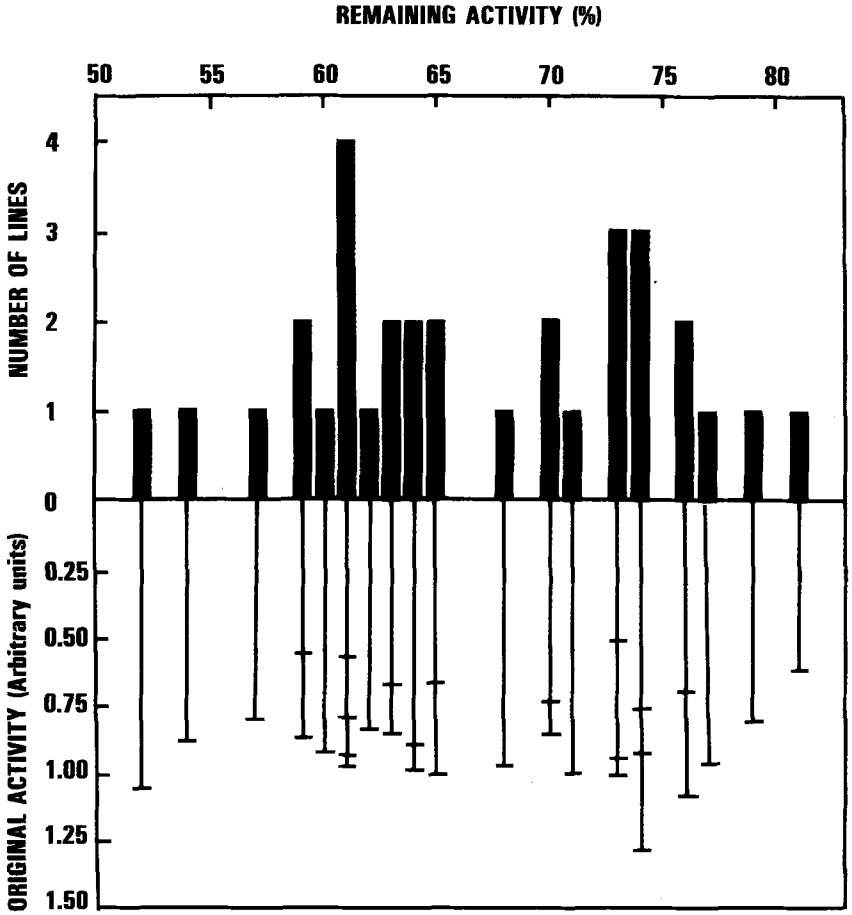

Fig. 1. The bimodal distribution of the remaining activity of β-glucanase percentages among the 32 landrace lines (upper plot), and the original untreated activities (lower plot).

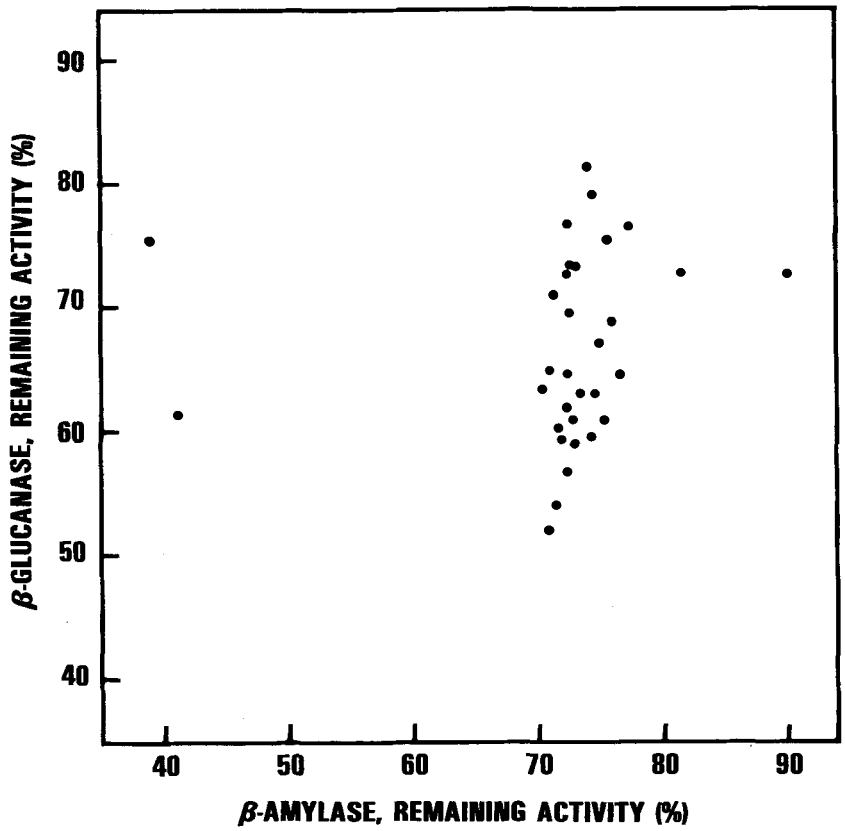

Fig. 2. The distributions of β-amylase and β-glucanase thermostabilities in the 32 landrace lines suggesting an influence of past coadaptation. Spearman coefficients of rank correlation, $r_S = 0.277$, $P = 0.12$ for the whole sample; if the two extreme variants to the left are excluded, $r_S = 0.351$, $P = 0.057$.

β-Glucanase

The activity of unheated β-glucanase showed less variation than beta-amylase or α-amylase (AHOKAS and POUKKULA 1999). This holds true for a wild barley sample of 257 *H. spontaneum* entries (AHOKAS and POUKKULA 1999). Since β-glucanase loosens cellular walls, increasing permeation (HØJ and FINCHER 1995), its excessive activity results in the danger of leakages from the germinating grain may also be a disadvantage. While 1 → 3-β-glucanase has pathogenesis-related effects against fungi, increasing their cell-wall permeability (see e.g. GRENIER et al. 1999), the 1 → 3,1 → 4-β-glucanase makes the cell walls of the wetted and germinating grain tissue more susceptible to invading organisms.

Up to 5 QTLs for finished malt glucanase and 3 QTLs for green malt glucanase were detected (HAN et al. 1995) with two structural genes for isoenzymes of (1 → 3,1 → 4)-β-glucanase (LITTS et al. 1990; WOLF 1992). One of these, EII, is restricted to the aleurone layer of germinated grain, while EI is also transcribed in scutalla on young leaves and roots at germination in addition to aleurone (SLAKESKI et al. 1990; SLAKESKI and FINCHER 1992). The β-glucanase isoenzyme II was found to be glycosylated with 3.6% carbohydrate (WOODWARD and FINCHER 1982). Glycosylation may be the source of thermostability in bacterial β-glucanase (OLSEN and THOMSEN 1991) and many other types of proteins (e.g. GU et al. 1989; NAKAMURA et al. 1998; YÁÑEZ et al. 1998). The level of glycosylation is possibly subject to multigenic variation.

The observed bimodality suggests two alleles, perhaps two types of glycosylation EII gene product, and may also mean a more complicated dependence with two levels of EI activity masking the EII activity levels. The unheated and heated activity did not show any correlation (Table 2), suggesting that thermostability is independent of activity in this landrace sample. The high thermostability found in Pirkka, with a 50% landrace parentage (SIMBERG 1950; KIVI 1969), probably has its origins in Finnish landraces.

This small sample does not necessarily reveal either the extreme activities or the thermostability of the past variation in the Finnish landraces. A genetically modified bacterial β-glucanase has high thermal sta-

bility (JENSEN et al. 1996, 1998). Transgenic barley expressing bacterial β-glucanase has shown stability of the gene over a few generations (JENSEN et al. 1998) and may hence serve as an artificial alternative to the endogeneous resource in barley, although landraces have not yet been throughly screened.

General discussion

Selection of stable protein forms by repeated external heat may have occurred in the landraces. There are two stages during which Finnish landrace cereals were often subjected to heat in the past. The harvested mature and premature straws were frequently dried over a special oven called a *kiuas*, giving off perfusive smoke in a special building called a *riihi* (TALVE 1961). During such drying, crops were commonly subjected to initial temperatures of 55–60°C; the temperatures were later raised, and excessive heating sometimes occurred (GROTENFELT 1899, 1922). Viable grain tissues were sometimes subjected to denaturating heat.

The other stage at which grains may have been subjected to extra heating occurred at germination in black slash-and-burn soils. Different variants of burning as a mode of cultivation (e.g. burn over peatland) did not end in Finland until the 1940s (AHOKAS and MANNINEN 2000) and burning was the prevailing method of field management in the past (HEIKINHEIMO 1915). A dark soil surface, such as that of a burned area, absorbs more solar radiation and thus becomes relatively hot. During different summers in Finland at N latitudes of 61°40′ and 61°52′, respectively, the maximum temperatures measured in the surface layer of burned black soil has been 52.8°C (LIPAS and MÄKI-PETÄYS 1961; VIRO 1974), and up to 63°C on the soil surface (VAARTAJA 1949). In the past, barley commonly germinated in early June, and hence the burned soils served as a heat-selective agent due to their darkness. Soil temperature maxima exceeding 50°C would be exceptional during the germination season even in the subtropical desert habitats of wild barley, but are reached for barley at the seasonal end or post-seasonally (GUTTERMAN 1997). Wild barley has also been a source of thermostability in grain β-amylase (EGLINTON et al. 1998; AHOKAS and NASKALI, unpublished). Due to the apparent multifunction of barley β-amylase, other reasons for the enzyme thermostability cannot be excluded.

The barley enzyme β-glucanase is induced at germination (e.g. BRUNSWICK et al. 1987; SLAKESKI and FINCHER 1992). Therefore, slash-and-burn management putatively provided a thermoselective environment for barley.

The 1→3,1→4-β-glucanase isoenzymes EI and EII are the principle activities expected to appear in samples germinated for five days (BRUNSWICK et al. 1987; LOI et al. 1987; MCFADDEN et al. 1988), with substrate specificity towards mixed-linked 1→3,1→4-β-glucans (HØJ and FINCHER 1995). Malted barley (1→3,1→4)-β-glucanases were found to be thermolabile (BRUNSWICK et al. 1987). The significance for brewing is indicated by the fact that the activity of β-glucanase during malting is positively correlated with malt extract (STUART et al. 1988).

The fate of cereal landraces

It has turned out to be a substantial loss for local plant breeders that the Finnish landraces, themselves a part of the national heritage, have not been adequately maintained. The genetical mixtures of landraces of self-pollinated cereals were lost in about 50 years prior to 1955 (AHOKAS 2000). The endangered state of the national landraces was pointed out by PESOLA (1951) after the topic was discussed at the 8th International Genetic Congress in Stockholm (KIRK 1949), but the urgent collecting and maintainance proposed by PESOLA (1951) remained unrealized.

REFERENCES

Ahokas H, (1998). Megareduction of genetic variation in Finnish cereals by breeding in response to demands of technology and modernization ideology, 1905-1955. In: Plant breeding and forest tree breeding today (ed A-M Niskanen). Dept. Plant Biol., Fac. Agric. Forest., Univ. of Helsinki, p. 6–7.

Ahokas H, (2000). Impacts on agricultural development by Constantin Boije, a missionary and the first plant breeder in Finland. Yliopistopaino, Helsinki.

Ahokas H and Manninen M-L, (2000). Retrospecting genetic variation of Finnish oat (Avena sativa) landraces and observations on revived lines grown prior to 1957. Genet. Resour. Crop Evol. (In press)

Ahokas H and Naskali L, (1990). Geographic variation of α-amylase, β-amylase, β-glucanase, pullulanase and chitinase activity in germinating Hordeum spontaneum barley from Israel and Jordan. Genetica 82: 73–78.

Ahokas H and Poukkula M, (1999). Malting enzyme activities, grain protein variation and yield potentials in the displaced genetic resources of barley landraces of Finland. Genet. Resour. Crop Evol. 46: 251–260.

Allison MJ and Swanston JS, (1974). Relationships between β-amylase polymorphisms in developing, mature and germinating grains of barley. J. Inst. Brew. 80: 285–291.

Brunswick P, Manners DJ and Stark JR, (1987). The development of β-D-glucanases during the germination of barley and the effect of kilning on individual isoenzymes. J. Inst. Brew. 93: 181–186.

Eglinton JK, Langridge P and Evans DE, (1998). Thermostability variation in alleles of barley beta-amylase. J. Cereal Sci. 28: 301–309.

Erkkilä MJ, Leah R, Ahokas H and Cameron-Mills V, (1998). Allele-dependent barley grain β-amylase activity. Plant Physiol. 117: 679–685.

Gana JA, Kalengamaliro NE, Cunningham SM and Volenec JJ, (1998). Expression of β-amylase from alfalfa taproot. Plant Physiol. 118: 1495–1505.

Grenier J, Potvin C, Trudel J and Asselin A, (1999). Some thaumatin-like proteins hydrolyse polymeric β-1,3-glucans. Plant J. 19: 473–480.

Grotenfelt G, (1899). Det primitiva jordbrukets metoder i Finland under den historiska tiden. J. Simelii Arfvingars Boktryckeri, Helsinki (Helsingfors).

Grotenfelt G, (1922). Suomalainen peltokasviviljelys. In: (eds T Arola et al.). Maatalouden tietokirja. Vol. 4: 1–517

Gu J, Matsuda T, Nakamura R, Ishiguro H, Ohkubo I, Sasaki M and Takahashi N, (1989). Chemical deglycosylation of hen ovomucoid: protective effect of carbohydrate moiety on tryptic hydrolysis and heat denaturation. J. Biochem. 106: 66–70.

Gutterman Y, (1997). Spring and summer daily subsurface temperatures in three microhabitats in a flat natural loess area in the Negev Desert, Israel. J. Arid Environ. 36: 225–235.

Han F, Ullrich SE, Chirat S, Menteur S, Jestin L, Sarrafi A, Hayes PM, Jones BL, Blake TK, Wesenberg DM, Kleinhofs A and Kilian A, (1995). Mapping of β-glucan content and β-glucanase activity loci in barley grain and malt. Theor. Appl. Genet. 91: 921–927.

Heikinheimo O, (1915). Kaskiviljelyksen vaikutus Suomen metsiin. German summary: Der Einfluss der Brandwirtschaft auf die Wälder Finnlands. Acta Forest. Fenn. 4 Part 2: 1–264 and appendices.

Høj PB and Fincher GB, (1995). Molecular evolution of plant β-glucan endohydrolases. Plant J. 7: 367–379.

Jensen LG, Olsen O, Kops O, Wolf N, Thomsen KK and Wettstein D von, (1996). Transgenic barley expressing a protein-engineered, thermostable (1,3-1,4)-β-glucanase during germination. Proc. Natl. Acad. Sci. USA 93: 3487–3491.

Jensen LG, Politz O, Olsen O, Thomsen KK and Wettstein D von, (1998). Inheritance of a codon-optimized transgene expressing heat stable (1,3-1,4)-β-glucanase in scutellum and aleurone of germinating barley. Hereditas 129: 215–225.

Kihara M, Kaneko T and Ito K, (1998). Genetic variation of β-amylase thermostability among varieties of barley, Hordeum vulgare L., and relation to malting quality. Plant Breed. 117: 425–428.

Kihara M, Kaneko T, Ito K, Aida Y and Takeda K, (1999). Geographical variation of β-amylase thermostability among varieties of barley (Hordeum vulgare) and β-amylase deficiency. Plant Breed. 118: 453–455.

Kirk LE, (1949). Development of a world cataloguing service for plant breeders and genetics. Hereditas Suppl. (1): 608.

Kivi EI, (1969). Main features of agricultural plant breeding in Finland. Peat Plant News 1: 45–53.

Kotake T, Nakagawa N, Takeda K and Sakurai N, (1997). Purification and characterization of wall-bound exo-1,3-β-D-glucanase from barley (Hordeum vulgare L.) seedlings. Plant Cell Physiol. 38: 194–200.

Kreis M, Williamson M, Buxton B, Pywell J, Hejgaard J and Svendsen I, (1987). Primary structure and differential expression of β-amylase in normal and mutant barleys. Eur. J. Biochem. 169: 517–525.

Lipas E and Mäki-Petäys E, (1961). Kulotuksen vaikutus metsämaan lämpöja kosteusoloihin. Thesis. Dept. of Forest., Univ. of Helsinki.

Litts C, Simmons CR, Karrer EE, Huang N and Rodriguez RL, (1990). The isolation and characterization of a barley 1,3-1,4-β-glucanase gene. Eur. J. Biochem. 194: 831–838.

Loi L, Barton PA and Fincher GB, (1987). Survival of barley (1 → 3, 1 → 4)-β-glucanase isoenzymes during kilning and mashing. J. Cereal Sci. 5: 45–50.

McFadden GI, Ahluwalia B, Clarke AE and Fincher GB, (1988). Expression sites and development regulation of genes encoding (1 → 3,1 → 4)-β-glucanases in germinating barley. Planta 173: 500–508.

Mikami B, Yoon H-J and Yoshigi N, (1999). The crystal structure of the sevenfold mutant of barley β-amylase with increased thermostability at 2.5 Å resolution. J. Mol. Biol. 285: 1235–1243.

Nakamura S, Ogawa M and Nakai S, (1998). Effects of polymannosylation of recombinant cystatin C in yeast on its stability and activity. J. Agric. Food Chem. 46: 2882–2887.

Okada Y, Yoshigi N, Sahara H and Koshino S, (1995). Increase in thermostability of recombinant barley β-amylase by random mutagenesis. Biosci. Biotechnol. Biochem. 59: 1152–1153.

Olsen O and Thomsen KK, (1991). Improvement of bacterial β-glucanase thermostability by glycosylation. J. Gen. Microbiol 137: 579–585.

Pan S-M, Chang T-C, Juang R-H and Su J-C, (1988). Starch phosphorylase inhibitor is β-amylase. Plant Physiol. 88: 1154–1156.

Pesola VA, (1951). Lantstråsädes-och ärtsorternas tidigare och nutida utbredning i Finland, deras egenskaper och betydelse för växtförädlingen samt tillvaratagningen av desamma. English summary: The Finnish country cereal and pea varieties, their distribution, their agronomic characteristics, and their value for plant breeding. J. Sci. Agric. Soc. Finl. 23: 193–210.

Simberg NH, (1950). Undersökning av maltkorn. Department of Chemical Engineering, University of Technology, Helsinki.

Slakeski N, Baulcombe DC, Devos KM, Ahluwalia B, Doan DNP and Fincher GB, (1990). Structure and tissue-specific regulation of gene encoding barley (1 → 3,1 → 4)-β-glucan endohydrolases. Mol. Gen. Genet. 224: 437–449.

Slakeski N and Fincher GB, (1992). Developmental regulation of (1 → 3,1 → 4)-β-glucanase gene expression in barley. Plant Physiol. 99: 1226–1231.

Stuart IM, Loi L and Fincher GB, (1988). Varietal and environmental variations in (1 → 3,1 → 4)-β-glucan levels and (1 → 3,1 → 4)-β-glucanase potential in barley: Relationships to malting quality. J. Cereal Sci. 7: 61–71.

Takahata Y, Noda T and Nagata T, (1994). Effect of β-amylase stability and starch gelatinization during heating on varietal differences in maltose content in sweetpotatoes. J. Agric. Food Chem. 42: 2564–2569.

Talve I, (1961). Den nordosteuropeiska rian. German summary: Die nordosteuropäische Riege. Skrifter Utgivna av Svenska Litteratursällskapet i Finland Syllabication: 387 (Folklivsstudier VI): 1–341.

Vaartaja O, (1949). High surface soil temperatures. On methods of investigation, and thermocouple observations on a wooded heath in the South of Finland. Oikos 1: 6–28.

Viro PJ, (1974). Effects of forest fire on soil. Fire and Ecosystems. In: (eds TT Kozlowski and CE Ahlgren) Academic Press, New York, San Fransisco, London, p. 7–45.

Wolf N, (1992). Structure of the genes encoding Hordeum vulgare (1→3,1→4)-β-glucanase isoenzymes I and II and functional analysis of their promotors in barley aleurone protoplasts. Mol. Gen. Genet. 234: 33–42.

Woodward JR and Fincher GB, (1982). Purification and chemical properties of two 1,3;1,4-β-glucan endohydrolases from germinating barley. Eur. J. Biochem. 121: 663–669.

Yáñez E, Carmona TA, Tiemblo M, Jiménez A and Fernández-Lobato M, (1998). Expression of the Schwanniomyces occidentalis SWA2 amylase in Saccharomyces cerevisiae: role of N-glycosylation on activity, stability and secretion. Biochem. J. 329: 65–71.