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# The Heritability of the Amylolytic Activity in Barley

JOSEF NEČAS

Genetics Section of the Department of Plant Physiology, Microbiology and Genetics, Charles University, Praha

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**Souhrn**

V předložené práci byla studována dědičnost $\beta$- i $\alpha$-amylasových aktivit ječmene.

1. Potvrdilo se, že aktivita $\beta$-amylasy je u ječmene dědičně založena a vykazuje v jednotlivých letech pro příslušnou odrůdu charakteristické hodnoty. Vliv měnících se vnějších podmínek na projev znaku v jednotlivých letech je značný. Individuální variabilita znaku má v souborech normální rozdělení. V $F_1$ generaci z křížení odrůd o geneticky různé aktivitě má zpravidla přibližně intermediární charakter s mírnou převahou vyšší aktivity. Reciproká křížení nejsou shodná a projevuje se tu průkazný vliv mateřské odrůdy. Mezi velikostí zrna a aktivitou $\beta$-amylasy je negativní korelace.

2. Aktivita $\alpha$-amylasy je rovněž dědičně založena. Jednotlivé odrůdy po stejně dlouhé době klíčení vykazují za týchž podmínek různý stupeň aktivity. Také dynamika přírůstků $\alpha$-amylasové aktivity během klíčení za stejných podmínek je u jednotlivých odrůd různá a je v positivní korelaci s energií klíčení. Tato vlastnost nemohla být pro zdlouhavou metodiku studována v hybridních souborech.

## Summary

During investigation of amylase activities as continuously variable biochemical features it was found that they are very sensitive to changes in the intensity of vegetative factors. The results of analysis of variance indicate that these characters not only tend to be inheritable themselves, but also the nature and intensity of their reactions to changes in the agro-ecological factors of the external environment show a similar tendency. The actual expression of these features in individuals belonging to a genetically homogeneous group

must be considered as the resultant of all reactions of the realisation process concerned, on stimulatory and inhibitory influences, both internal and external. For the genetical analysis of hybrid groups it is necessary to have the most complete picture possible of this continuous modificability which masks the actual genetical variability.

The results obtained in the present investigation provide evidence of complicated relations involved in the hereditary nature and formation of the features under consideration. In further work on the later generations of reciprocal and back crosses it will be necessary to determine more exactly the relative participation of nuclear and plasmatic components both in the hereditary transmission and in the actual formation of these characters.

The present results, although they are only preliminary, confirm the correctness of the modern view in genetics that genetical analysis should be linked with physiological analysis.

## Introduction

The amylolytic activity of barley has been studied by a number of authors, but for the most part they have devoted themselves to the technological aspects or to practical questions of improvement. Little attention has been paid as yet to explaining the inheritability of these characters, although their varietal differences have often been observed. The genetical approach to the problem of the production of malting barley of high standard as regards these properties has so far been limited for the most part to determination of the linkage groups and the possibility of selection according to other characters in later generations of hybrids. In view of the rather more difficult procedure required, less attention has been devoted so far to tracing the actual transmission of characters from the parent varieties to the hybrid offspring in earlier hybrid generations. An attempt to do this was made in the present work, but unfortunately, although the work promised to be long-term, it had to be ended at the $F_1$ generation, when genetical work was really in its first stages.

## Material and Methods

Material. The original parent varieties were two varieties of two-rowed spring barley, which are classified as high-quality malting barleys — Stupický full-grain (St) and Dětenický hero (D), belonging to the botanical species *Hordeum distichon*, two forms of six-rowed spring barley of the four-rowed type, denoted in this paper as (SoI) — glumed, and (SoII) — naked, belonging to the botanical species *Hordeum vulgare* "form" *tetrastichon* and one six-rowed form of spring barley *Hordeum vulgare* var. *brachyurum* (ALEF.) — (H).

For the sake of simplicity all the established forms will be referred to as varieties and will be denoted by the symbols given in brackets.

Methods. In both years the land chosen for sowing had been under one crop and was prepared in the autumn and spring according to the usual procedure adopted for barley. It was manured with compost and artifical fertilisers in medium doses in accordance with the norms for spring barley. The grain was sown in 10 × 10 cm. drills to a depth of 3·5—4·0 cm. The crop was then left until the harvest without watering or other cultivation. Harvesting was manual, single plants being taken for individual analyses and the whole of each replication for average samples.

In 1955 crossing was carried out in combinations as follows:

| Combinations of crosses | D × H | H × D | D × SoI | SoI × D | D × SoII | SoII × D |
|---|---|---|---|---|---|---|
| No. harvested plants in $F_1$ | 39 | 81 | 28 | 39 | 86 | 74 |
| Combinations of crosses | St × H | H × St | St × SoI | SoI × St | St × SoII | SoII × St |
| No. harvested plants in $F_1$ | 62 | 107 | 49 | 30 | 44 | 121 |
| Combinations of crosses | H × SoI | SoI × H | H × | SoII | SoII × H | |
| No. harvested plants in $F_1$ | 52 | 9 | 51 | | 38 | |

Determination of total $\beta$-amylase activity. The method worked out by KASTNER and JANÁČEK (1956) was used. It was worked out on the basis of SANDEGREN and KLANG's (1950) method for the preparation of barley extracts with total $\beta$-amylase and WINDISCH and KOLBACH'S method (according to PAWLOWSKI and DOEMENS 1938) for iodometric determination of increases in reduction power in a solution of starch paste by amylase action. For the present purpose and under the given laboratory conditions it was necessary to modify the method in certain respects both for analyses of average samples from the different varieties and for the determination of individual variability in the varieties.

Some of the changes were experimentally confirmed and some were carried out on the basis of convincing published data.

A buffer solution (0·3 M $Na_2HPO_4$ and 0·15 M citric acid) was used for extraction, because a freshly prepared solution of basic cystein in HCl could not be neutralised to the pH of the extraction solution in view of the fact that the stability of cystein is too low at this pH. The necessary optimal pH was adjusted after the addition of the extraction solution.

Although lengthy and demanding much care, the preparation of cystein base on each occasion before use has certain advantages as compared with the preparation of cysteinhydrochloride. Cystein base can be better stored, because it is less hygroscopic and is not so liable to be spoilt by oxidation to cystin.

In further elaboration of the method in an adequately equipped laboratory it will be necessary to follow the stability of the redox potential of the extraction mixture in the same way as KASTNER and JANÁČEK (1956) followed the stability of its pH during the whole process of extraction. During rapid extraction the mixture is continually disturbed and its surface is continually in contact with the air.

The experiment with increasing cystein doses showed that greater activity was not achieved.

Table 1. Empirical determination of the effectiveness of the cystein used

| Without cystein | Normal dose 0·35 g./10 g. | 11/2 norm. dose | 2 norm. dose |
|---|---|---|---|
| 83·89 | 133·73 | 133·85 | 108·56 |

The values are given in activity units and are averages of four repetitions.

The double dose reduced activity by producing over-acidity of the extraction mixture. The other data show that a sufficient quantity of cystein is definitely maintained in active form throughout the period of extraction.

Natural potato starch had to be used as substrate because soluble starch was unobtainable at the time. Lower values are obtained with natural starch than with the soluble. For later comparison six repetitions of comparative determination of activity were carried out with

the same sample both with natural starch (average 142·89 activity units) and soluble starch "Lachema" (average 187·41 activity units).

The pH of the substrate was adjusted to 5·0—5·2 with acetate buffer. MYRBÄCK and MYRBÄCK (1933) demonstrated that this pH is really the optimum for $\beta$-amylase and that at a pH of 4·3, which is used in technological analyses, only 80% of true activity is achieved. MYRBÄCK and NEUMÜLLER (1951) also mention pH of 5·0 to 5·2 as optimal. A lower pH is sometimes justified in that it causes the inactivation of accompanying $\alpha$-amylase. Published work supports the fact, confirmed also in the present work, that this amylase is present only in traces in ungerminated barley.

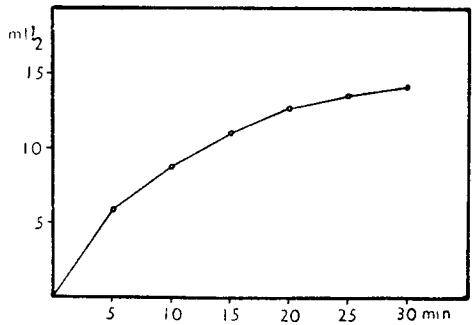



Fig. 1. Influence of the time factor on $\beta$-amylolytic reaction rate of barley extract. Abscissa: reaction time in minutes ordinate: consumption of iodine solution in ml.

The duration of the saccharising reaction was shortened to 15 minutes, because fig. 1, which shows the dependence of $\beta$-amylolytic activity on the reaction time under given conditions, shows clearly that this phase of the reaction curve is the most suitable for comparison of the different samples. Saccharisation took place at a temperature of 25° C.

In order to express the activity of total $\beta$-amylase in the different barley samples units were used analogical to the activity units of Windisch and Kolbach (PAWLOVSKI and DOEMENS 1938), calculated according to the formula:

$$\text{Activity unit} = \frac{(A - B) \,.\, f \,.\, 0{\cdot}0171 \,.\, c \,.\, 100}{s}$$

A = consumption ml. 0·1 N $Na_2S_2O_3$ in control (blind experiment)
B = consumption ml. 0·1 N $Na_2S_2O_3$ in actual experiment
f = analytical factor 0·1 N $Na_2S_2O_3$
c = conversion factor for conversion of amount of extract used to equivalent of 100 g. barley
s = % dry weight
0·0171 = maltose equivalent of one millilitre 0·1 iodine in g.

The conversion factor "c" corresponds to the modifications in the method already mentioned and has no general validity. Thus it is not possible to compare directly the active units mentioned here with units obtained by another method. They are, however, mutually comparable, as shown by empirical checking of the method, which was carried out 24 times by repeated analysis of the same homogenised sample. All values were within the range 129·00—135·00 activity units when $\bar{x} = 131{\cdot}76$, $s_{\bar{x}} = 0{\cdot}17$ and $s_x = 0{\cdot}86$. Thus the reproducibility of the method when carried out as described above in serial application is good. The analysis of variance, the results of which are given in table 2, shows that a greater part is played in the total variability of values from repeated analyses of the same sample by differences between different series worked on concurrently than by differences within these series. This is understandable, for the whole analysis involves a number of operations and it cannot be expected that they will all be reproduced similarly in all details. The distribution of total variability, caused by the above method, made it possible to achieve a more reliable evaluation of the results of genetical analyses. (For a more detailed description of the method see NEČAS 1957.)

It is realised that a marked shortcoming has been the impossibility of carrying out a comparison with the method using papain in combination with cystein as activator. These results would be required for a further confirmation that the total quantity of $\beta$-amylase is really determined and also for throwing light on the question whether varietal differences in activity are in fact caused only by differences in $\beta$-amylase content or perhaps by differences in quality. Differences in proteases in the grain could also have their effect.

Table 2. Analysis of variance of repeated analyses of the same sample on the activity of $\beta$-amylase

| Variability | N | $S(x-\bar{x})$ | V | F | s |
|---|---|---|---|---|---|
| Between series | 3 | 7·95 | 2·65 | 2·84 | |
| Within series | 5 | 3·85 | 0·77 | 0·83 | |
| Uncontrolled influences | 15 | 14·05 | 0·93 | | 0·96 |
| Total | 23 | 25·85 | | | |

Determination of $\alpha$-amylase activity. This determination was carried out only in average samples from the different varieties. The individual variability of this character could not be established, because the method used did not allow of this owing to its lengthiness and the difficulty of getting reproducible results. The method used was that of BLOM and BAK (1938) adapted for measurement with Ostwald's viscosimeter under the given laboratory conditions. Determinations were made of the dynamics of increase in $\alpha$-amylase activity during the germination of the different varieties and $\alpha$-amylase activity following a 96-hour period of germination. (For a more detailed description of procedure see NEČAS 1957.)

For the expression of $\alpha$-amylase activities of the different samples of barley, activity units according to BLOM and BAK (1938) were employed.

$$\text{Activity unit} = \frac{1000}{a \,.\, (t_2 - t_1)}$$

$a$ = dry weight of barley to which the amount of lixivium used corresponds
$t_1$ = time in which the enzyme-hydrolysed starch paste had a viscosity corresponding to an outflow period of 0·90 min.
$t_2$ = time in which it had a viscosity corresponding to an outflow period of 0·40 min.

The values $t_1$ and $t_2$ were obtained by mathematical interpolation of the series of values measured.

The influence of differential thermal inactivity of $\alpha$-amylase on the reduction in viscosity of the paste is shown in fig. 2. From this it is evident that the method is not sensitive enough to record this influence. It was therefore decided to neglect the differential thermal inactivity of the extracts during the serial analyses.

The increase in $\alpha$-amylase activity during germination, indicated by curves of the reduction in starch paste viscosity, is recorded in fig. 3.

Two samples were taken for analysis from each of the tested barley plants and each sample was measured three times. In order to confirm whether the method is capable of registering varietal differences the analysis of variance was performed, the results being presented in table 3.

From the results of this analysis it is evident that varietal differences are recorded quite well by this method. The error of the method is higher than the error in the preparation of extracts.

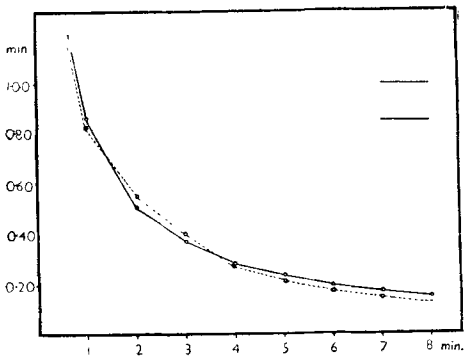



Fig. 2. Influence of differential thermal inactivation of $\beta$-amylase in barley extract on decrease in viscosity of starch paste. Continuous line: non-inactivated extract, broken line: inactivated extract. Abscissa: reaction time in minutes. Ordinate: period of flow of the reacting mixture through the viscosimeter in minutes.

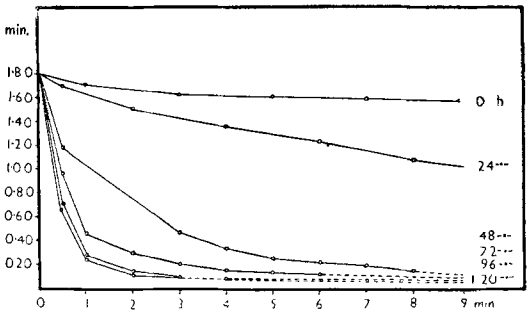
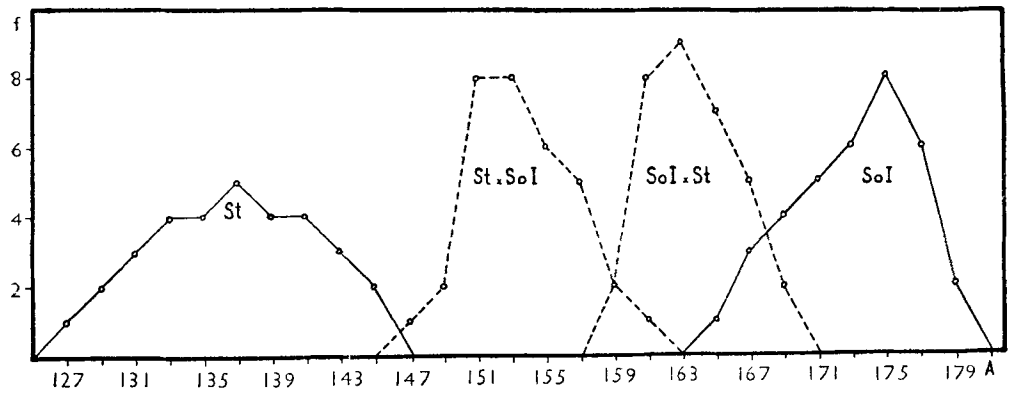



Fig. 3. Increases in $\alpha$-amylase activity during germination. Abscissa: reaction time in minutes. Ordinate: period of flow of the reacting mixture through the viscosimeter in minutes.

Table 3. Analysis of variance of repeated analyses of average samples of the varieties under investigation

| Variability caused | N | $S(x - \bar{x})^2$ | V | F | s |
|---|---|---|---|---|---|
| by varieties | 4 | 14,442,529·96 | 3,610,632·5 | 35·66++ | |
| by parallel samples | 1 | 30,715·62 | 30,715·6 | 0·30 | |
| repeated analyses | 2 | 335,999·56 | 167,999·8 | 1·65 | |
| uncontrolled influences | 22 | 2,227,289·82 | 101,245·0 | | 318·19 |
| Total | 29 | 17,036,634·96 | | | |



## Results

### β-amylase activity

Table 4. Review of the average β-amylase activities of parent varieties in both years from sets of individuals

| Variety | 1955 | | | | 1956 | | | |
|---|---|---|---|---|---|---|---|---|
| | activity units | | | | activity units | | | |
| | n | $\bar{x}$ | $s_{\bar{s}}$ | $s_x$ | n | $\bar{x}$ | $s_{\bar{s}}$ | $s_x$ |
| D | — | — | — | — | 36 | 158·38 | 0·44 | 2·64 |
| St | 50 | 104·24 | 1·02 | 7·24 | 36 | 137·50 | 0,88 | 5·30 |
| SoI | 20 | 139·00 | 0·99 | 4·44 | 35 | 173·17 | 0·60 | 3·60 |
| SoII | — | — | — | — | 24 | 130·43 | 0·71 | 3·50 |
| H | 50 | 155·64 | 1·04 | 7·36 | 36 | 183·38 | 0·56 | 3·40 |

Table 5. Review of average β-amylase activities of parent varieties in 1955, converted to dry weight without glumes

| Variety | D | St | SoI | SoII | H |
|---|---|---|---|---|---|
| Activity units | 139·85 | 117·42 | 157·22 | 110·71 | 179·83 |
| Order | 3 | 4 | 2 | 5 | 1 |

Values for varieties D and SoII are taken from average samples.

Table 6. Review of average β-amylase activities of parent varieties in 1955, converted to dry weight of average 1,000 grains without glumes

| Variety | D | St | SoI | SoII | H |
|---|---|---|---|---|---|
| Activity units | 60·15 | 53·37 | 50·44 | 34·23 | 57·58 |
| Order | 1 | 3 | 4 | 5 | 2 |

Fig. 4. Variability of β-amylase activity in parent varieties and their $F_1$ generation. For an explanation of the marking of varieties and their crosses see text. Abscissa: activity units. Ordinate: frequency in different variation classes, converted to corresponding magnitudes of variation sets.

The empirical distribution curve of individual values for plants of variety St were tested for agreement with normal distribution by Kolmogorov-Smirnov's method (MYSLIVEC 1957). The value of function $\lambda$ was here 0·37 and P = 0·99. Thus, the individual variability of $\beta$-amylase activities in a set of individuals of the same variety is normally distributed and statistical methods for normal distribution can be used for the evaluation of the series.

Table 7. Analysis of variance of $\beta$-amylase activities of average samples of parent varieties in both years

| Year | 1955 | | | | 1956 | | | |
|---|---|---|---|---|---|---|---|---|
| Repl. / Var. | 1 | 2 | 3 | 4 | 1 | 2 | 3 | 4 |
| D | 122·33 | 119·86 | 128·51 | 125·42 | 151·76 | 156·00 | 155·39 | 156·37 |
| St | 112·97 | 109·88 | 107·42 | 111·12 | 140·55 | 146·29 | 147·49 | 149·92 |
| SoI | 134·28 | 137·37 | 141·70 | 132·42 | 171·61 | 177·06 | 178·57 | 168·47 |
| SoII | 121·23 | 105·15 | 110·10 | 106·38 | 157·71 | 168·26 | 168·57 | 162·02 |
| H | 169·82 | 157·47 | 158·71 | 156·24 | 180·50 | 192·38 | 183·88 | 187·62 |

| Variability caused by | N | $S(x - \bar{x})^2$ | V | F | s |
|---|---|---|---|---|---|
| varieties | 4 | 10,256·06 | 2,564·01 | 312·30++ | |
| years | 1 | 13,223·87 | 13,223·87 | 1,610·70++ | |
| replications | 3 | 37·22 | 12·40 | 1·51 | |
| years × rep. | 3 | 233·91 | 77·97 | 9·49++ | |
| varieties × rep. | 12 | 367·43 | 30·62 | 3·72+ | |
| varieties × years | 4 | 754·07 | 188·51 | 22·96++ | |
| uncontrolled infl. | 12 | 98·54 | 8·21 | | 2·86 |
| Total | 39 | 24,971·10 | | | |

Analysis of variance in order makes it possible to delimit the interaction components of the variation of values obtained from analyses of $F_1$ generation and a picture was obtained of the stability of characters and the influence of some factors on their actual expression.

Table 8. Review of average $\beta$-amylase activities in $F_1$ generation

| Combination | n | Activity units | | | | Significance of differences in reciprocal crosses | Order | Conversion to dry weight of 1,000 grains | | Order |
|---|---|---|---|---|---|---|---|---|---|---|
| | | $\frac{P_1+P_2}{2}$ | $\bar{x}$ | $s_{\bar{x}}$ | $s_x$ | | | $\frac{P_1+P_2}{2}$ | $\bar{x}$ | |
| D × H | 24 | 170·38 | 178·25 | 0·58 | 2·86 | $P = 10^{-9}$ | 3 | 58·86 | 63·47 | 1 |
| H × D | 24 | | 185·00 | 0·61 | 3·00 | | 1 | | 58·80 | 3 |
| D × SoI | 18 | 165·77 | 163·78 | 0·73 | 3·12 | $P = 10^{-9}$ | 8 | 55·29 | 39·52 | 13 |
| SoI × D | 6 | | 170·06 | 1·07 | 2·64 | | 6 | | 37·45 | 16 |
| D × SoII | 12 | 144·40 | 140·84 | 0·62 | 2·16 | $P = 10^{-9}$ | 14 | 47·19 | 47·90 | 7 |
| SoII × D | 12 | | 148·67 | 0·40 | 1·42 | | 12 | | 41·29 | 12 |
| St × H | 30 | 160·44 | 171·66 | 0·47 | 2·62 | N | 4 | 55·47 | 59·53 | 2 |
| H × St | 36 | | 172·84 | 0·63 | 3·80 | | 5 | | 56·71 | 4 |
| St × SoI | 21 | 155·33 | 152·72 | 0·41 | 1·92 | $P = 10^{-9}$ | 11 | 51·90 | 52·66 | 5 |
| SoI × St | 6 | | 162·00 | 0·66 | 1·62 | | 9 | | 45·25 | 9 |
| St × SoII | 12 | 133·96 | 137·66 | 0·70 | 2·46 | $P = 0·05$ | 16 | 43·80 | 49·43 | 6 |
| SoII × St | 12 | | 140·00 | 0·73 | 2·56 | | 15 | | 46·66 | 8 |
| H × SoI | 15 | 174·40 | 184·53 | 0·56 | 2·20 | $P = 10^{-9}$ | 2 | 54·01 | 43·31 | 11 |
| SoI × H | 4 | | 164·30 | 0·57 | 1·14 | | 7 | | 37·94 | 15 |
| H × SoII | 14 | 151·59 | 155·85 | 0·65 | 2·44 | $P = 10^{-9}$ | 10 | 45·90 | 45.00 | 10 |
| SoII × H | 12 | | 147·33 | 0·63 | 2·22 | | 13 | | 39·20 | 14 |

Table 9. Intravarietal correlation coefficients of average grain size and average $\beta$-amylase activity

| Varieties 1956 | | | | |
|---|---|---|---|---|
| Variety | n | r | Fluctuation r | Significance |
| D | 24 | — 0·08 | — | N |
| St | 33 | 0·79 | 0·52—0·92 | $P = 10^{-2}$ |
| SoI | 24 | 0·04 | — | N |
| SoII | 24 | — 0·04 | — | N |
| H | 33 | 0·33 | — | N |
| $F_1$ generation 1956 | | | | |
| D × H | 24 | — 0·37 | — | N |
| H × D | 24 | — 0·70 | —0·23 až —0·91 | $P = 10^{-2}$ |
| D × SoI | 18 | — 0·64 | — | N |
| SoI × D | 6 | — 0·39 | — | N |
| D × SoII | 12 | — 0·13 | — | N |
| SoII × D | 12 | 0·55 | — | N |
| St × H | 26 | 0·04 | — | N |
| H × St | 28 | 0·06 | — | N |
| St × SoI | 21 | — 0·03 | — | N |
| SoI × St | 6 | — 0·64 | — | N |
| St × SoII | 12 | 0·08 | — | N |
| SoII × St | 12 | 0·02 | — | N |
| H × SoI | 15 | — 0·16 | — | N |
| H × SoII | 12 | 0·17 | — | N |
| SoII × H | 12 | — 0·54 | — | N |

Table 10. Intervarietal correlation coefficients of average grain size (y) and average $\beta$-amylase activity (x)

| Varieties (n = 5) | |
|---|---|
| r | — 0·44 |
| fluctuation r | — |
| significance | N |
| b xy | — |
| b yx | — |

| $F_1$ generation (n = 16) | |
|---|---|
| r | — 0·64 |
| fluctuation r | 0·00 až — 0·93 |
| significance | $P < 0·05$ |
| b xy | — 2·63 |
| b yx | — 0·16 |

## α-amylase activity

Table 11. Analysis of variance of α-amylase activities in average samples of varieties examined from five replications in 1955

| Variety Paral. sample | D | | St | | SoI | | SoII | | H | |
|---|---|---|---|---|---|---|---|---|---|---|
| Repet. | 1 | 2 | 1 | 2 | 1 | 2 | 1 | 2 | 1 | 2 |
| 1 | 3156 | 3298 | 3390 | 3330 | 2812 | 2739 | 5712 | 5924 | 3721 | 3851 |
| 2 | 2842 | 2867 | 2715 | 2707 | 2805 | 2690 | 5747 | 5870 | 4053 | 4416 |
| 3 | 2802 | 3015 | 2875 | 2969 | 2947 | 3096 | 5994 | 5912 | 4017 | 4428 |
| 4 | 3839 | 3739 | 3421 | 3287 | 2914 | 2943 | 5666 | 5643 | 4261 | 4258 |
| 5 | 3686 | 3822 | 3067 | 3140 | 2694 | 2693 | 5810 | 5910 | 3925 | 3860 |

| Variability caused by | N | $S(x - \bar{x})^2$ | V | F | s |
|---|---|---|---|---|---|
| varieties | 4 | 58,261,900·98 | 14,565,475·24 | 225·57++ | |
| replications | 4 | 557,512·78 | 139,378·19 | 2·15 | |
| parallel determinations | 1 | 44,999·91 | 44,999·91 | 0·69 | |
| uncontrolled influences | 40 | 2,582,812·01 | 64,570·30 | | 254·1 |
| Total | 49 | 61,447,225·68 | | | |

Table 12. Analysis of variation of average α-amylase activities of average samples from all replications with single varieties in both years.

| Variety | D | St | SoI | SoII | H |
|---|---|---|---|---|---|
| 1955 | 4615 | 3524 | 1238 | 4370 | 4175 |
| 1956 | 3243 | 2455 | 1989 | 3853 | 2518 |

| Variability caused by | N | $S(x - \bar{x})^2$ | V | F | s |
|---|---|---|---|---|---|
| varieties | 4 | 7,980,016 | 1,972,504 | 4·36 | |
| years | 1 | 1,493,049 | 1,493,049 | 3·30 | |
| uncontrolled influences | 4 | 1,807,992 | 451,998 | | 672·3 |
| Total | 9 | 11,191,057 | | | |

Table 13. Dynamics of increases in α-amylase activities during germination in both years

| Hours germination | | 0 | | 24 | | 48 | | 72 | | 96 | | 120 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Year | | 1955 | 1956 | 1955 | 1956 | 1955 | 1956 | 1955 | 1956 | 1955 | 1956 | 1955 |
| Variety | Order | | | | | | | | | | | |
| D | 3 | 58 | 72 | 96 | 123 | 704 | 889 | 1800 | 1706 | 4615 | 3243 | 5198 |
| St | 4 | 70 | 57 | 89 | 68 | 674 | 478 | 2223 | 1459 | 3523 | 2455 | 3514 |
| SoI | 5 | 64 | 62 | 96 | 82 | 495 | 454 | 1081 | 1278 | 1238 | 1989 | 2805 |
| SoII | 1 | 66 | 66 | 280 | 235 | 1543 | 1412 | 3968 | 2600 | 4370 | 3853 | 5472 |
| H | 2 | 85 | 62 | 246 | 186 | 1039 | 682 | 3398 | 1651 | 4175 | 2518 | 4431 |

## Discussion

### β-amylase activity

Analyses of average samples of the different varieties chosen, carried out in four repetitions in both years, showed that there are great differences in β-amylase activities. Choice of the varieties was based on published results indicating that six-rowed barley usually has a higher β-amylase activity than two-rowed. (BAMANN and MYRBÄCK 1941, MYRBÄCK and NEUMÜLLEB 1951). The range of barleys in Czechoslovakia had not so far been investigated from this point of view. The review of parent varieties in table 4 shows that six-rowed H barley is in fact the most active when activity is related to the dry weight of the sample. Both the two-rowed varieties are much less active. The relation of activities of both six-rowed barleys of the four-rowed type SoI and SoII is interesting. The first approaches the activity of the six-rowed H, while the second is still less active than little-active two-rowed variety St. In 1956 it showed a rather higher activity in average samples from selected grain, but in analyses of individuals it is definitely the least active. In general the parent varieties can be divided into two groups: 1. highly active varieties showing in 1956 activity within the range 170 to 190 activity units (H, SoI); 2. slightly active varieties with activity in the same year within the range 130 to 160 activity units (D, St, SoII). Both two-rowed varieties are also significantly different. In both years D is more active than St.

The evaluation of these two varieties will contribute to more exact knowledge of the Czechoslovak range of barleys. Comparison of the two-rowed varieties originating from regional barleys and of varieties from the group "Kneifl barleys" will be important for the further improvement of Czechoslovak barleys as regards these characters. Both the newly-selected barleys SoI and SoII could be better evaluated if their origin were known. H barley is not cultivated in Czechoslovakia, it is probably a primitive form in the description of material of the botanical variety mentioned.

The low $\beta$-amylase activity found for SoII is interesting from two points of view. In the type of its ears it belongs to the group of six-rowed barley, but its activity is lower than that of the two-rowed varieties. It shows high increases in $\alpha$-amylase activity during germination and reaches high values within a short time. Some authors have pointed out the possibility of a positive correlation between the activities of the two forms of amylase, but this variety does not bear this out. A new genetical study of this correlation will be necessary. There is also the possibility that this variety behaves like the American

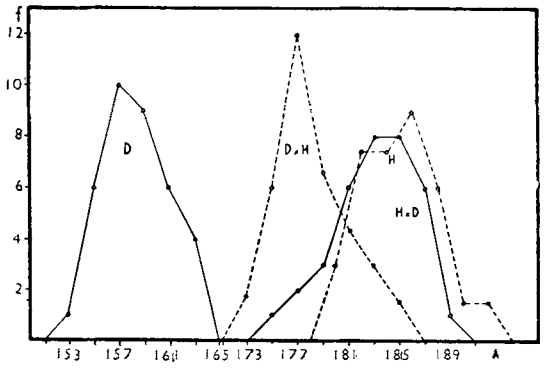



Fig. 5. Variability of $\beta$-amylase activity in parent varieties and their $F_1$ generation. For an explanation of the marking of varieties and their crosses see text. Abscissa: activity units. Ordinate: frequency in different variation classes, converted to corresponding magnitudes of variation sets.

variety Peatland as described by BENDELOW and MEREDITH (1955) and others, that is, that due to some as yet unknown characters it shows a lower activity in the measurements than in fact exists. It is also necessary to take into consideration the fact that from the crossing of highly-active six-rowed forms with slightly-active two-rowed forms there may be produced slightly-active six-rowed forms, as found by DAY, DOWN and FREY (1955). These assumptions, or their more exact formulation, can only be achieved by further work.

Analysis of the variation of values of $\beta$-amylase activities of parent varieties in both years showed that the whole set of agroecological factors of the external environment plays an important part the formation of these characters. Their share in total variability is the highest. 1956 was a very unfavourable year, it was wet and therefore produced a strong deviation. The varietal component also plays an important part in the total variability and points to the genetic nature of the character. The variability component involved in the different replications is insignificant as compared with the others and thus does not interfere with the measurement of other components. Of the interactive components, the most important is the interaction of the influences of varieties and years, which points to the varietal nature of the reaction to changing climatic conditions in the different years. Since the interactive component of variety and replication influence is greater than that of replications alone, there would seem to be also a specific reaction of varieties to soil conditions. The interactive component of the influence of years and replications is also higher than that of replication alone. This is due to change

in the quality of the plots on which the experiment was carried out in the different years.

The modification of the method of individual analysis was found to be so sensitive that it was able to record individual differences. It is, however, necessary to take into acount in relation to the values for each individual the range of fluctuation of values from repeated analyses of the same sample

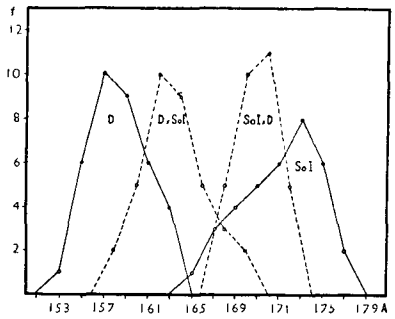



Fig. 6. Variability of $\beta$-amylase activity in parent varieties and their $F_1$ generation. For an explanation of the marking of varieties and their crosses see text. Abscissa: activity units. Ordinate: frequency in different variation classes, converted to corresponding magnitudes of variation sets.

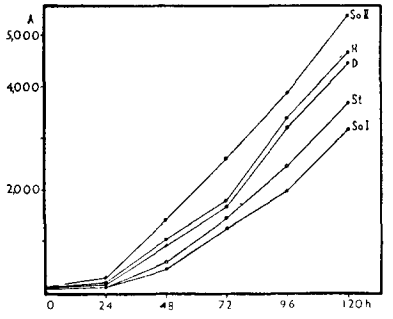



Fig. 7. Dynamics of increases in $\alpha$-amylase activities in the varieties examined during germination. For marking of varieties see text. Abscissa: germination time in hours. Ordinate: activity units.

and the variation curve of the set and to regard this as an approach to the actual curve within the range of the known fluctuation of values caused by errors in the method. Comparison of the variation curve of one variety, obtained from analyses of 50 individuals, with the curve of normal distribution showed satisfactory agreement. It is therefore possible to state that the individual variability of $\beta$-amylase activities is normally distributed.

Satisfactory agreement is also found when comparing the average values calculated from sets of indidividuals separately characterised for $\beta$-amylase activity with the values for the activities of average samples of selected grain of the same varieties. A more marked difference is evident only for the variety SoII. Average samples of selected grain show much higher values than the average for individual analyses. This divergence can be explained as being due to a greater number of very undeveloped grains from lateral flowers in the harvest from single individuals.

The standard errors of the averages are fairly low, so the significance of differences is high. The values of the standard errors corresponded for both years.

In comparing the standard deviations as a measure of variation range it was found that in 1955 there was a greater variation range for the same species than in 1956. It is not possible, however, to draw any conclusions from this, for the samples for individual analyses could not be taken completely at random, but had to be limited to those plants with a total yield

of over 5 g. of grain. In general, the values for standard deviations indicate that the variation range of the character reaches values similar to those for other physiological characters.

It follows from the above analysis of $\beta$-amylase activity of parent varieties that this character can be studied genetically. It has been possible to evaluate only the first filial generation of various combinations of crosses of parent varieties.

It is interesting to note that the $F_1$ generation from reciprocal crosses of the same combination are in most cases significantly conflicting. Their variation curves are mutually shifted on the $x$ axis in the direction of the maternal variety, as can be seen from the respective graphs.

The results obtained must, however, also be judged from another point of view. In some cases, when naked and glumed barleys or barleys with different degrees of gluminess are compared, it is necessary in the interests of accurate measurement to ascertain the gluminess and convert the values of $\beta$-amylase activity to dry weight of grain without glumes (table 5). In the present investigation it was only possible to make this convertion for the varieties in 1955, as the determination of gluminess is very lengthy. From the above-named table it is evident that the order of varieties is not changed, varietal differences are only more emphasised. A completely different picture is obtained, however, when the average activity of the different varieties is converted to the dry weight of 1,000 of their average grains. As can be seen from table 6, varietal differences are eliminated in this conversion and the order of varieties is fundamentally changed. The need for this conversion resulted from the discovery of significant intervarietal negative correlations between the weight of 1,000 average grains and the average activity of $\beta$-amylase. This indicates the advantage offered by such a conversion for improvement work in certain cases. Selection according to activity, related to the weight of 1,000 grains, would at the same time provide selection according to two important characters and, because a negative correlation is involved, the material selected would be narrowed down considerably for further tests. It will, of course, be necessary to examine these relations further, both intra- and intervarietally and also in later hybrid generations. When applying this method of selection it must be borne in mind that a good malting barley can also be of a smaller-grained type, which would be unfavourably classified by this method.

A brief examination of the values converted in this way for the $F_1$ generation shows that not all combinations are similarly affected. There are fundamental deviations in the order for all combinations in which variety SoI and the combinations of two-rowed varieties with SoII are involved. The remainder keep approximately within the same range of values. The reciprocal crosses maintain very significant differences and in the cases where parents are placed in an opposite order by the new evaluation, the $F_1$ generations are usually similarly orientated.

Examination of the correlation relations of $\beta$-amylase activities and the average size of grains provided only two statistically significant correlation coeficients for intra-varietal correlation. Since, in view of the small range of the series examined, it was not possible to prove whether these selected series

also exhibit normal distribution of the average grain sizes and whether they correspond to the character of the original varietal series, their significance is reduced.

Both for the varieties and for the $F_1$ generation a negative intervarietal correlation of both the characters examined was found. The correlation coefficients provide convincing evidence of the actual existence of this relation. This is in agreement with the findings of other authors (ANDERSON, SALLANS and MEREDITH 1941, et al.).

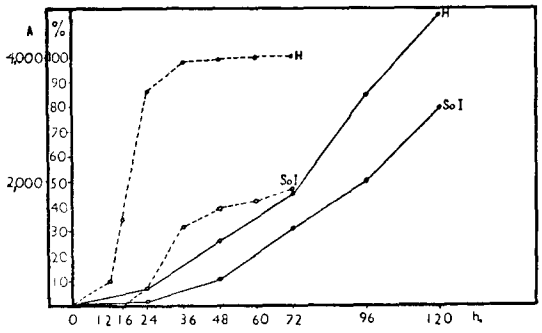



Fig. 8. Relation between germination energy and the dynamics of increases in α-amylase activity. For marking of varieties see text. Abscissa: germination time in hours. Ordinate: activity units and percentage of germinated grains. Continuous line: α-amylase activity. Broken line: germination energy

## α-amylase activity

α-amylase activity could also be studied as a character typical for varieties. This was already confirmed by the analysis of variance made for the purpose of evaluating the method. Further analyses of variance of values relating to this character gave a highly significant share for varieties in the total variability. The variability component for replications is quite insignificant with regard to the varietal component, but higher than the methodical. It is, therefore, also necessary to take into consideration the influence of variations in soil, even though the determination of activity is not yet sufficiently accurate and the extent of the experiment is not great enough to enable this influence to be accurately statistically delimited. The effect of changes in a whole range of agroecological factors in the different years is more evident from the analysis of variance in table 12. Its extent is not sufficient to provide statistically significant values, but can be judged relatively. The influence of varieties preponderates over the influence of external factors. This is in agreement with the behaviour of germination energy with regard to these influences. As has already been demonstrated, the reverse is the case for β-amylase, the behaviour of which in this respect corresponds more closely with grain size.

Comparison of the different varieties as regards the activities of the two amylases is interesting. As has already been mentioned, the variety SoII achieves the highest α-amylase activities most rapidly and shows the lowest β-amylase activity in the ungerminated grain. Variety SoI, which has the slowest development of α-amylase activity during germination, takes second place as regards β-amylase activity. The H variety shows a relatively high

activity for both amylases. Of both the two-rowed varieties, D variety has the higher activity of both amylases. Thus it would be possible to speak of a positive correlation of the activities of both amylases only for the two two-rowed varieties and H variety. This correlation demands further and more thorough genetical study.

From the technological point of view it would be interesting to ascertain whether it is possible to achieve the same malting effect in varieties with similar activities and in those with diverse activities.

Further calculations for α-amylase activities were not made, because the accuracy of the determinations is not great enough to allow of reliable operation with such values.

The relation between α-amylase activity and germination energy, which was also ascertained in the course of this work, is shown for two varieties in graph 8.

Acknowledgment:

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*Address*: Josef Nečas, Prom. Biol., Výzkumný ústav bramborářský ČSAZV, Valečov u Havlíčkova Brodu, Czechoslovakia.

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# Наследственность амилолитической активности ячменя

ЙОЗЕФ НЕЧАС

Отделение генетики кафедры физиологии растений, микробиологии и генетики Карлова Университета, Прага

## Резюме

В данной работе была исследована наследственность активности амилазы ячменя.

1. Было подтверждено, что активность $\beta$-амилазы ячменя заложена наследственно и что соответствующие данные в отдельные годы характерны для каждого сорта. В отдельные годы изменяющиеся внешние условия оказывают очень сильное влияние на проявление данного признака. Индивидуальная изменчивость признака в наборах распределена нормальным образом. В поколении $F_1$ при скрещивании сортов с различной, генетически заложенной активностью, как правило, имеет место приблизительно промежуточный характер этого признака со слабым преобладанием более высокой активности. Реципрокные скрещивания не являются сходными и здесь явственно проявляется влияние материнской породы. Корреляция между величиной зерна и активностью $\beta$-амилазы отрицательна.

2. Активность $\alpha$-амилазы также наследственно заложена. Отдельные сорта, проращиваемые одинаковое время при одинаковых условиях, обладают различной степенью активности. Также динамика прироста активности $\alpha$-амилазы в течение прорастания при одинаковых условиях у отдельных сортов разная и находится в отрицательной корреляции с энергией прорастания. Из-за длительной методики это свойство не могло быть исследовано у наборов гибридов.