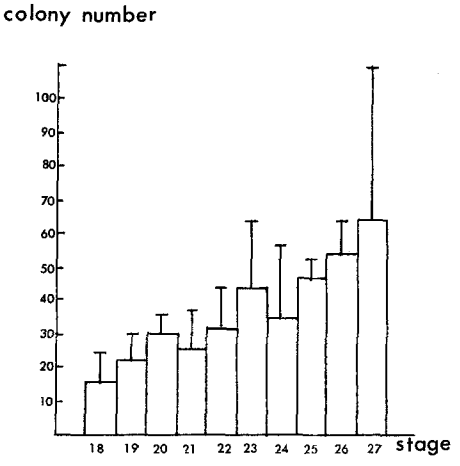

Figure 2. Effect on colony number of increasing developmental stage. 2 wing buds were cultured in each dish. Each value is the mean of between 3 and 10 determinations. Error bars represent standard deviations.

Effect of additional growth factors on stage 19 tail bud conditioned medium. Values given are the mean number of colonies formed over five determinations, followed by the standard deviation in brackets. Two similar experiments gave the same pattern of outcome.

	No additives	4 ng/ml EGF	2 ng/ml TGF-β	4 ng/ml TGF + 2 ng/ml TGF-β
Control	0 (0)	322 (26)	2 (1)	467 (51)
Conditioned	7 (3)	356 (25)	5 (2)	749 (35)

though there was some variability. During this time, however, the size of the limb bud is also increasing markedly [10], so relative transforming ability per µg protein is either remaining constant or falling. Tail bud conditioned medium induced formation of colonies (table). Addition of TGF-β (final volume 2 ng/ml) to conditioned medium had little effect. Addition of EGF (final volume 4 ng/ml) to conditioned medium led to a non-significant increase over controls with the same EGF level. Addition of both EGF and TGF-β to conditioned medium leads to a significant increase in colony number over the equivalent controls ($p < 0.001$ by t-test analysis).

The responsiveness of the assay system to fixed doses of EGF varies from day to day and it is therefore difficult to establish standard dose response curves which give exact results. However, 2 limb buds have an equivalent effect to the presence of EGF in the range 0.01–0.05 ng/ml. If colony stimulation by conditioned medium was entirely due to an EGF-like molecule at these doses, addition of TGF-β should enhance it [11], but this was not the case (table). From these results, the conditioned medium could contain small amounts of both EGF-like activity and TGF-β like activity, or molecules with so far unidentified interactive characteristics.

The technique described here opens up the possibility of mapping a single embryo for its distribution of transforming growth factors, using only the simplest equipment. It is hoped that this procedure will reveal local sites of production, and hence suggest roles for these molecules in development.

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Variability of β-amylase isoenzymes within a collection of inbred lines of rye (*Secale cereale* L.)

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Summary. Within one collection of 18 inbred rye lines, seven distinct isoenzymatic patterns of β-amylase were found, and five of them formed a group of similar patterns shifting to distinct positions in the gel. It was proved that the shift of the pattern was due to genetic factors. In crossed immunoelectrophoresis, drastic differences were shown in the quantity of the different β-amylase constituents. No antigenic differences could be demonstrated between the analyzed isoenzymes.
Key words. Secale cereale; β-amylase; variability; immunochemical characteristics.

β-Amylase, isolated from mature kernels of cereals such as hexaploid wheat (*Triticum aestivum* L.) and barley (*Hordeum vulgare* L.) exhibits a significant polymorphism. Foundations of the latter have not been clarified completely; as suggested by available literature data, they may involve numerous structural genes [1–3] and also post-translational modifications [4–6].

In this report we describe the β-amylase isoenzymatic variability within a collection of inbred lines by means of isoelectric focusing and crossed immunoelectrophoresis. The report

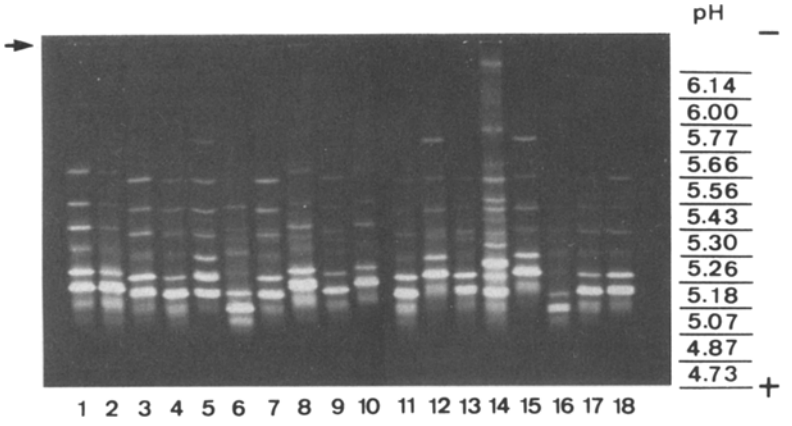

Figure 1. Isoelectric focusing demonstrated slight shifting of the entire β-amylase isoenzymatic pattern in the polyacrylamide gel for most inbred lines of rye. 1–18 = inbred line numbers. Arrow shows the place of protein extract application.

presents the first stage of our studies attempting to clarify a) foundations of β-amylase polymorphism and b) some aspects of the regulation of β-amylase gene expression.

Materials and methods. Inbred lines. Kernel samples of 18 rye lines, originating from 20–22 generations of inbreeding, were obtained from the Laboratory of Rye Genetics, Academy of Agriculture, Cracow. The following inbred lines of rye were included in the studies: Dańkowskie sel. 131 (No. 1), Horton (No. 2), Horton C5 (No. 3), Imperial E (No. 4), Kazimierskie B4 (No. 5), Kazimierskie C3 (No. 6), Kazimierskie D (No. 7), Ludowe D1 (No. 8), Mikulicki Wcz. R (No. 9), Rogalińskie F (No. 10), Rogalińskie Pa (No. 11), Uniwersalne 145 (No. 12), Wielkopolskie C (No. 13), Wierzbieńskie B1 (No. 14), Wierzbieńskie C (No. 15). Włoszanowskie C (No. 16), Zeelandzkie E (No. 17), Zeelandszkie G (No. 18).

Protein extraction. Flour of individual kernels was extracted in the ratio 1:15 (w/v). The conditions of the extraction were described previously [7].

Isoelectric focusing (IEF). IEF separation was carried out as described previously [7] with some modifications. Gels contained 4% Ampholines pH 4–6 and 1% Ampholines pH 6–8. Proteins were electrofocused for 4 h at 200–600 V. Separation gels were placed on polyacrylamide gels containing 0.66% soluble starch. After 30 min incubation the starch-containing gels were stained with iodine solution [8].

Crossed IEF-immunoelectrophoresis. After isoelectric focusing performed as above, a strip of polyacrymamide gel containing separated proteins was excised and crossed electrophoresis was performed into 1.2% agarose gel containing 0.75% anti-wheat β-amylase serum. The immunoelectrophoresis was run for 8 h, at 4 °C and 5 V per cm. Subsequently, the gel was dried and stained with Serva violet 49.

Results. a) Determination of β-amylase isoenzyme variability using IEF. Basically, two β-amylase electrophoretic patterns were encountered within 18 inbred lines, the typical and the untypical pattern. Within each line, individual kernels always showed the same isoenzymatic variant.

The typical zymogram consisted of 7 basic bands and was noted in all studied lines, except for line No. 14 (fig. 1). Band patterns, and their specific activities, were similar in the 17 lines. Within the lines showing the same zymogram, 5 groups could be distinguished, differing in gel position of the zymogram. Group I included lines Nos. 1, 2, 8, 10, group II – lines Nos. 3, 4, 5, 7, 9, 11, 13, 17, 18, group III – line No. 6, group IV – lines Nos. 12 and 15, group V – line No. 16.

The zymogram of line No. 14 contained a higher number of isoenzymatic bands as compared to the typical zymogram.

b) Conditioning of β-amylase zymogram shift. We compared isoenzymatic patterns obtained for line No. 10 (group I) and line No. 17 (group II), mixed extracts of lines Nos. 10 and 17 and an extract of F_1 hybrids obtained by reciprocal crossing of lines Nos. 10 and 17 (fig. 2). For the extract mixture, double bands were obtained (fig. 2A) corresponding to fused patterns of the original line extracts, which excluded gel artefacts as a reason for band shifting. On the other hand, the double bands for extracts of F_1 kernels (fig. 2, F_1) showed the genetic conditioning of the effect of β-amylase zymogram shifting.

c) Immunochemical characteristics. Crossed immunoelectrophoresis was applied for further characterization of zymograms and for quantitative estimation of individual isoenzymes. The immunoferograms of the typical and untypical isoenzymatic patterns showed one single precipitation line only, common for all isoenzymatic forms (fig. 3a and b). Comparison of the height of the precipitation line peaks indicated a significant disproportion in the content of individual isoenzymes. In the cathodal part of the IEF gel, bands of either the typical or untypical zymograms had an enzy-

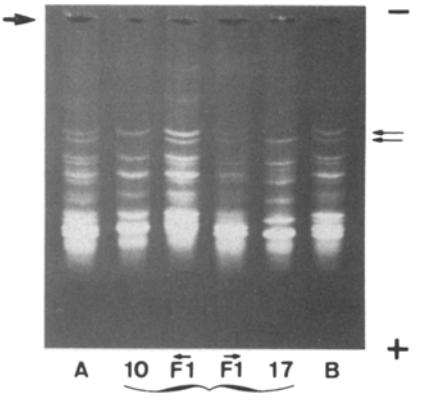

Figure 2. Double isoenzymatic bands were obtained for extracts of F_1 hybrids and for the mixture of parental line extracts showing genetic conditioning of β-amylase pattern shifting.
A, extract mixture (1:1) of lines Nos. 10 and 17.
10 and 17, line numbers.
$\overleftarrow{F1}$, F_1 hybrid exract of line No. 10 (♀) × line No. 17 (♂) cross.
$\overrightarrow{F1}$, F_1 hybrid extract of line No. 17 (♀) × line No. 10 (♂) cross.
B, extract mixture (1:1) of $\overleftarrow{F1}$ and $\overrightarrow{F1}$.
Bigger arrow (on the left) indicates the place of protein extract application. Smaller arrows (on the right) show one of the isoenzymatic doublets resulting from line crossing or extract mixing.

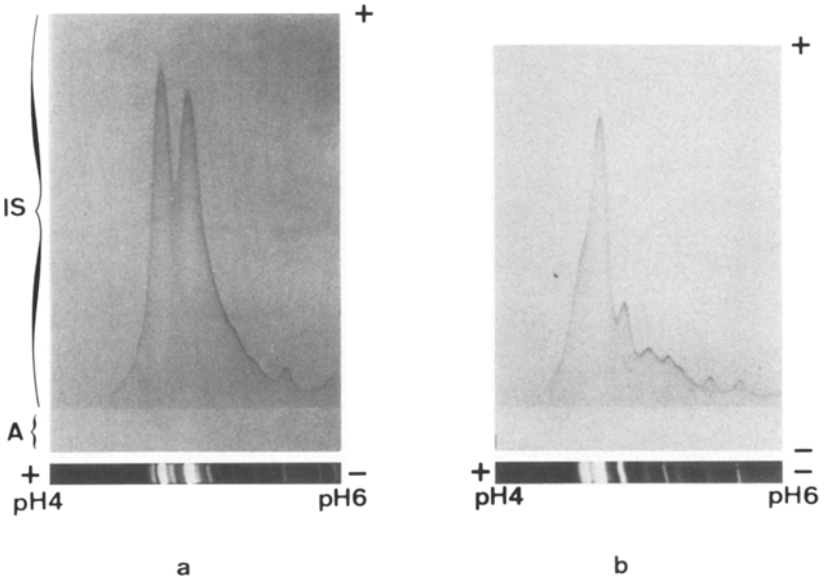

Figure 3. Immunochemical quantitation of β-amylase isoenzymes showed significant differences in contribution of individual isoenzymes to the β-amylase zymogram. *a* Immunoferogram for line No. 14 (untypical β-amylase zymogram). *b* Immunoferogram for line No. 11 (typical β-amylase zymogram). A, Agarose gel on which was deposited a strip of polyacrylamide gel containing proteins separated by IEF. IS, Agarose gel containing anti-wheat β-amylase serum.

matic protein content many times lower as compared to bands of the anodal gel part. A basic difference between typical and untypical zymograms involved the presence of a single principal precipitation peak for the anodal part of the typical zymogram (fig. 3 b) as compared to two such peaks for the untypical zymogram (fig. 3 a).

Discussion. The studies were performed on kernels of rye lines originating from an inbreeding of many years. Analysis of individual kernels within each line demonstrated the homozygous character of the material in respect to β-amylase variants (data not shown). The studied collection of inbred lines was selected from various breeding materials in order to provide a collection with a wide variety of many traits [9]. The extensive variability of traits within the collection of rye lines was confirmed in isoelectric focusing analysis of esterases, in which each of 18 lines demonstrated a distinct zymogram of esterases (Sadowski, unpublished data). The similarity of the polymorphic β-amylase pattern in 17 rye lines (those showing the typical isoenzymatic pattern) in respect to the number of isoenzymes, was rather unexpected. The position of the entire zymogram in the gel provided evidence for a certain variability within the 17 rye lines. The results demonstrated that the shifting of the zymogram pattern in the gel is not a gel artefact. The analysis of F_1 kernels in which the isoenzymatic pattern corresponded to patterns of the paternal lines in accordance with the codominant inheritance fashion provided evidence for genetic conditioning of the shift.

Concerning the immunochemical study, the applied antiserum was shown by immunoabsorption [10] to be specific for β-amylase of rye extract. Immunochemical characterization of zymograms presented in this study suggests an antigenic identity of individual isoenzymes of either the typical or untypical zymograms. β-Amylases of the same zymogram behave as a single antigen (no characteristic spur of the immunoprecipitation line was observed, see figs 3 a and b), which exhibits polymorphism in respect to the isoelectric point. Isoenzymes of the anodal gel part prevailed quantitatively. The observation raises the question of the origin of these isoenzymes, which showed a multifold higher amount compared to the isoenzymes of the cathodal gel part. It cannot be excluded that the minor cathodic constituents result from a proteolytic process or other post-translational modifications.

Further studies will aim at determining the extent to which rye β-amylase polymorphism is determined by structural genes on the one hand and by post-translational modifications on the other.

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