THE DISTRIBUTION OF THE ENZYMES IN RESTING CEREALS *

I. THE DISTRIBUTION OF THE SACCHAROGENIC AMYLASE IN WHEAT, RYE AND BARLEY

by

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In a previous paper (LINDERSTRØM-LANG AND ENGEL [1]) we have described the distribution of the β amylase in the outer layers of germinating barley. It turned out that most of the amylase is located just below the aleurone layer in the so-called subaleurone layer, the first starch-containing cell layer of the endosperm. In the present paper the distribution of the saccharogenic (β) amylase in resting grains of wheat, rye and barley is discussed.

Little is known about the distribution of amylase in these cereals and hitherto no investigations have been made with the help of modern technical equipment. In our earlier paper attention has been paid only to the amylase distribution in the aleurone and starch cell layers in the outer part of the grain. BROWN AND MORRIS [2] and others (for literature see [1]) have been able to demonstrate that the germ part particularly the scutellum contains large amounts of amylase.

According to OHLSON [3] the amylase in resting grain is only partly active. It is possible to activate the inactive enzyme by treating with papain (FORD AND GUTHRIE [4]). In the present paper both active and inactive enzymes have been determined according to the method of MYRBÄCK AND MYRBÄCK [5].

EXPERIMENTAL

1. *Materials.*

Wheat (*Triticum vulgare*) : a good sample of American Manitoba wheat (1939 harvest). Rye (*Secale cereale*) : an inland Dutch sample of Petkus summer rye (1942 harvest). Barley (*Hordeum sativum*) : an inland Dutch sample of Abed Kenia brewers barley (1942 harvest).

2. *Sampling and histological technique.*

These are the same as described by LINDERSTRØM-LANG AND ENGEL [1]. Only slight alterations have been introduced. As we were working on resting grain we did not want frozen sections, so that soaking in water could be avoided. The sections of the dry material were made with an ordinary Sartorius microtome. To avoid curling up of the slices we have used an apparatus on the knife of the microtome as described by LINDERSTRØM-LANG AND MOGENSEN [6]. In studying the amylase distribution in the outer layers of the grain the same sampling has been used as in the previous paper. The diameter of the cylindrical borer was 2 mm. The cylinder is attached with the ventral or crease side of the grain to the microtome table with a very thick viscose solution. A wall of cork may be used round the cylinder to strengthen it.

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In our previous investigation the cylinder had been frozen on the microtome table with a drop of water. This may have been the reason for some discrepancies in that paper. The readily soluble amylase may have shifted to other layers outside its real place in the grain.

The amylase distribution in the rest of the grain has been studied according to the scheme given in Fig. 1 ; for the anatomy of wheat grain and an explanation of the terms we refer to FAIRCLOUGH [10]. The whole grain has been cut in sections of 25 μ thickness.

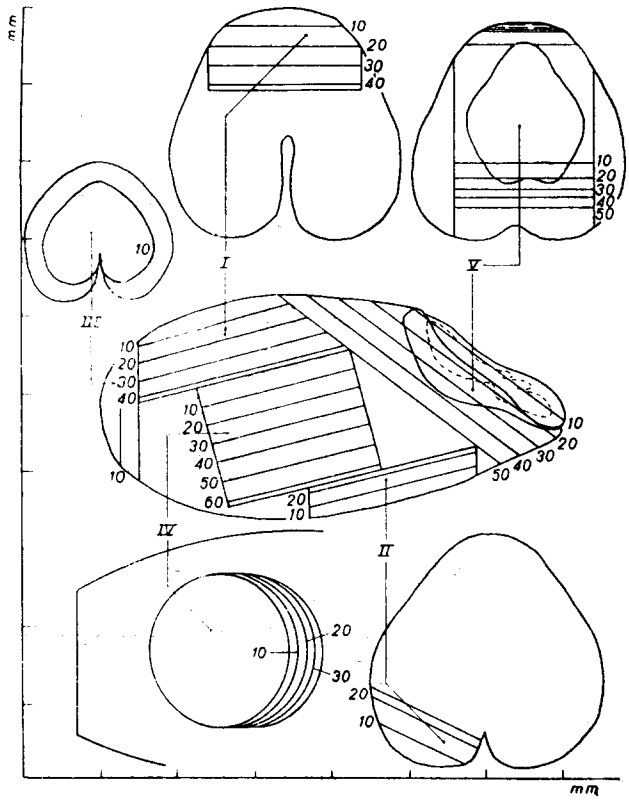

Fig. 1. Scheme of various series of sections used for the estimation of saccharogenic amylase in different parts of the grain.
Series I. Outer layers of the dorsal side. The results of the amylase determinations have been given in the figures 2, 3 and 4.
Series II. Left ventral side. Results in figure 5.
Series III. Outer layers of the upper end. Results in fig. 6.
Series IV. Inner part of the endosperm. Results in Table I.
Series V. Germ part. Results in Figures 7, 8 and 9.
Every tenth section has been ndicated by a numbered line.

Owing to the lack of photographical material a direct projection of the microscopical picture (Magnific. approx. 500×) had to be made on a drawing table. This facilitates careful observation so that the various tissues may be distinguished more exactly.

3. *Extraction and activation.*

Every section has been extracted with 50 μl M/15 phosphate buffer p_H 5.3 during 20 hours at 4° C. After taking 7.9 μl of this extract for the determination of the free active amylase, 7.9 μl of a 2% papain solution (Papayotinum purum „Gehe" 1 : 200) has been added and the activation has been carried out during 20 hours at 30° C. From this extract samples have been taken to carry out the determination of the total amylase amount.

4. *Amylase determination.*

The maltose formed by the action of the amylase has been titrated according to LINDERSTRØM-LANG AND HOLTER [7]. A soluble starch solution of 1,5% in M/45 phosphate buffer p_H 5.3 has been used as a substrate. 7.9 μl of the aqueous or the papain extract have been mixed with 92 μl of the substrate. After mixing hydrolysis has been carried out at 40° C. Of this mixture 17 μl have been titrated after varying times. The enzyme activity has been calculated per μl of tissue and is expressed as mg maltose formed in one hour at 40° C.

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RESULTS AND DISCUSSION

Fig. 2, 3 and 4 give the results of the determinations in the outer layers at the dorsal side of the grains (Fig. 1, I). Curve I represents the enzyme activities of the free amylase in the sections, curve II the amount of amylase after activation with papain, i.e., the total amylase content. Curve III shows the volume percentage of the aleurone cells in the sections. As the bran was completely amylase free, it could be eliminated in the calculation of this percentage. The distance from the surface of the grain is the abscissa in all graphs.

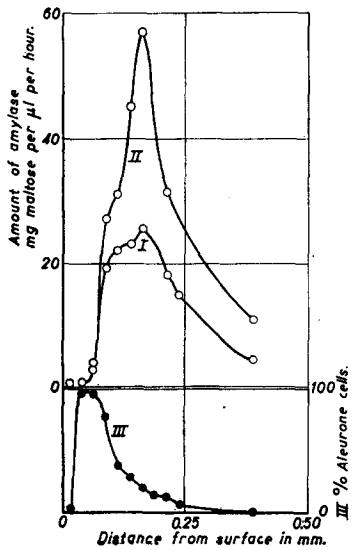

Fig. 2. The distribution of saccharogenic amylase in the outer layers on the dorsal side of grains of Manitoba wheat.
I. Active amylase.
II. Total amylase after activation with papain.
III. Percentage of the aleurone cells in the sections.

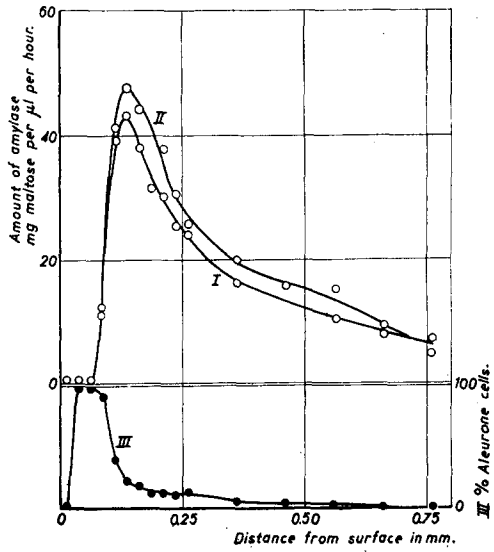

Fig. 3. The distribution of saccharogenic amylase in the outer layers on the dorsal side of grains of Petkus summer rye. Curves as in Fig. 2.

In these three figures a very distinct optimum of the amylase amount may be seen. It is located just below the aleurone cells. Quantitatively, rye has the highest optimum of free active amylase; next follow wheat and barley. With respect to the activation with papain rye contains much less activated amylase (about 10% of the total) than wheat and barley. Wheat and rye show no second optimum, such as occurs in the outer layers of barley; cf. our earlier communication [1]. As may be seen from the figures the aleurone cells do not contain any amylase.

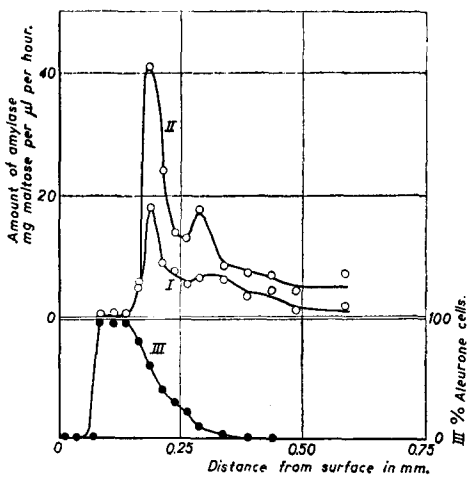

Fig. 4. The distribution of saccharogenic amylase in the outer layers on the dorsal side of grains of Abed Kenia brewers' barley. Curves as in Fig. 2.

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In further experiments we have examined the outer layers of other parts ; viz., the left ventral side and the upper end of the wheat grain.

The series of sections which have all been cut from the same grain are given in Fig. 5 and 6. The location of these sections may be seen in Fig. 1, (II and III). About the same results have been obtained as in Fig. 2.

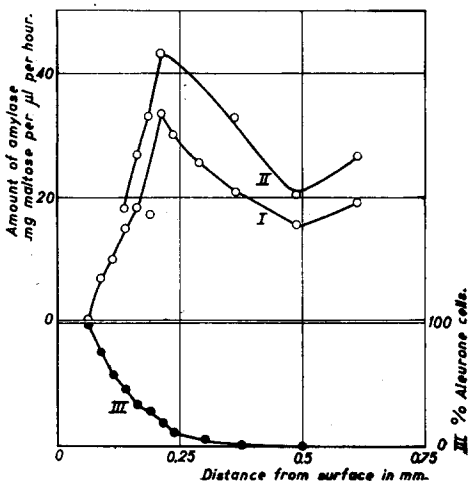

Fig. 5. The distribution of saccharogenic amylase in the outer layers on the left front side of grains of Manitoba wheat. Curves as in Fig. 2.

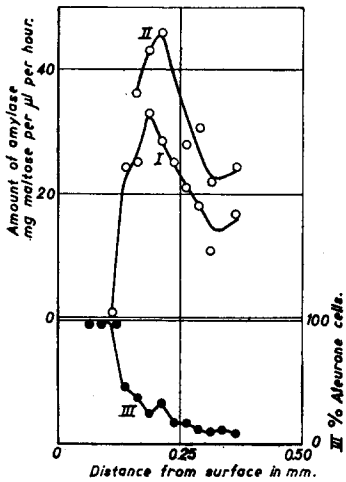

Fig. 6. The distribution of saccharogenic amylase in the outer layers of the upper end of grains of Manitoba wheat. Curves as in Fig. 2.

Considering the inner part of the grain we have turned our attention to the aleurone and subaleurone cells beside the crease. This part of the grain (the same grain as in Fig. 5 and 6) has been cut according to the scheme in Fig. 1 (IV). For some sections of the cylinder the amylase amount has been estimated. In an other grain we have made some amylase determinations in sections containing only starch cells from the inner endosperm. Subaleurone cells have been cut away. The results of these determinations may be found in Table I.

TABLE I

AMYLASE CONTENT OF TISSUE AROUND THE CREASE AND OF THE INNER PART OF THE GRAIN

Location of the sections	Sections numbered	% Aleurone cells	Active amylase expressed as mg maltose per µl per hour
cf. Fig. 1 IV .	20	12	20.0
	28	10	18.4
cf. Fig. 1 I . .	16	0	11.7
	18	0	8.9

From these determinations it follows that the inner endosperm also contains an amount of amylase. In the sections around the crease amounts have been found corresponding with those of the sections in Fig. 2, 5 and 6 with the same percentage of aleurone cells.

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Fig. 2-6 give a fully detailled picture of the distribution of the amylase in the endosperm part of the grain.

The results obtained for the germ part are given in the next Fig. 7, 8 and 9.

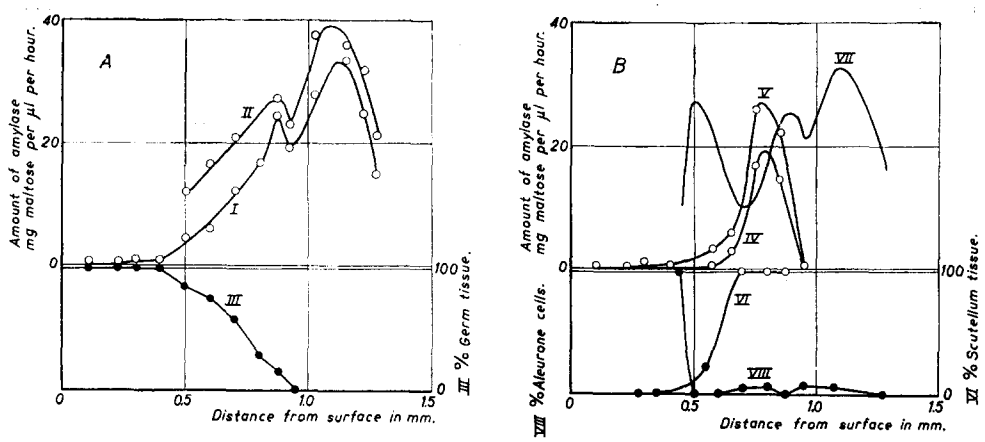

Fig. 7. The distribution of saccharogenic amylase in the germ part of grains of Manitoba wheat.

Curve I : active amylase.
II : total amylase.
III : percentage of germ tissue in the complete sections.
IV : active amylase content of the isolated germ part of the sections.
V : total amylase content of the isolated germ part of the sections.
VI : percentage of scutellum tissue of the germ part of the sections.
VII : active amylase content of the isolated endosperm part of the sections, calculated from curve I, III, and IV.
VIII : percentage aleurone cells of the endosperm part of the sections.

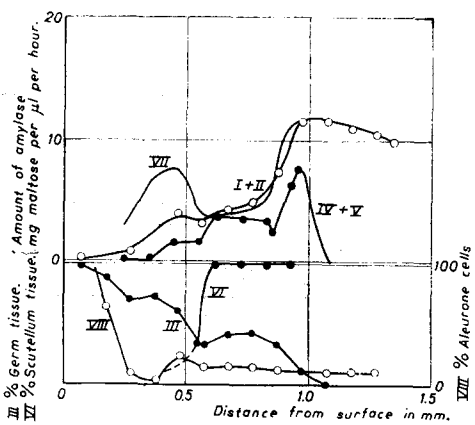

Fig. 8. The distribution of saccharogenic amylase in the germ part of grains of Petkus summer rye. Meaning of curves same as in Figure 7.

The sections have been made according to the scheme in Fig. 1 (V). From the outside to the scutellum parallel cuttings have been made. The abscissa represents the distance from the first section on the root top end in a direction at right angles with the cutting level.

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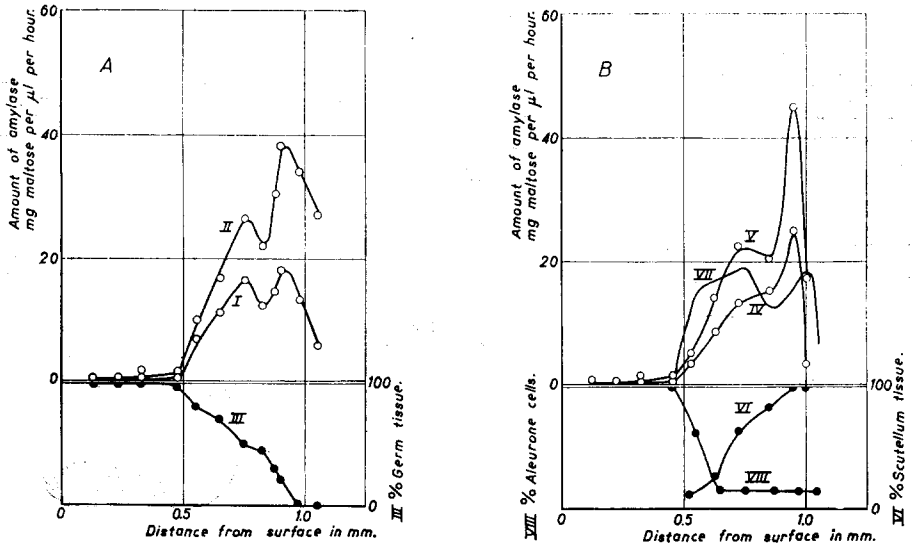

Fig. 9. The distribution of saccharogenic amylase in the germ part of grains of Abed Kenia barley. Meaning of curves same as in Figure 7.

Curves I and II (Fig. 7A, 8 and 9A) give again the experimental results of the amylase determinations. Curves III give the percentage of germ tissue of the sections. Only aleurone, starch and germ cells have been considered in this calculation. In rye (Fig. 8) papain did not increase the amylase amount, so that no inactive amylase is present in the germ part of the grain; accordingly curves I and II coincide.

From the Fig. 7A, 8 and 9A it appears that the outer part ($\pm$0.5 mm) of the germ does not contain amylase.

Curves I and II do not give a decisive answer to the question as to where the amylase is located in the germ part.

In the same series some sections have therefore been made free from the endosperm part by cutting them under a microscope.

The amylase contents of these preparations have been given in the curves IV and V (Fig. 7B, 8 and 9B). In rye (Fig. 8) these curves again coincide, owing to the lack of inactive amylase.

Curves VI (Fig. 7B, 8 and 9B) give the percentage of scutellum tissue of the germ part of the sections. Because of the histological technique these curves imply some inaccuracy as regards the lower part of the curve. However, the upper part of it is correct.

From the curves IV, V and VI it appears that only the scutellum contains a considerable amount of amylase. With the method used no measurable amounts have been found in the rest of the germ.

It would be highly interesting if the amylase amounts in the various parts of the scutellum could be estimated, because according to some histological papers (HORNING [8], GÜNTHER [9]), the epithelial layer against the endosperm has a structure different from the rest. Only by means of finer technique can these estimations be carried out.

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In Fig. 7B, 8 and 9B the curves VII have been obtained by calculation. They represent the amylase amounts in the endosperm part of the sections. They have been derived from curves I, III and IV. Curves VIII give the percentage of the aleurone cells in these endosperm parts. From these curves it appears that in wheat (Fig. 7B) there are two optima in the amylase content of the endosperm parts. The first corresponds with the optimum in the subaleurone layer. The second is broader ($\pm$ 350 μ) and is located inside the grain along the scutellum. In rye (Fig. 8) the situation is the same. In barley (Fig. 9B) the situation differs slightly from that in wheat ; the second optimum in the endosperm part is only small and lies only 50 μ below the scutellum.

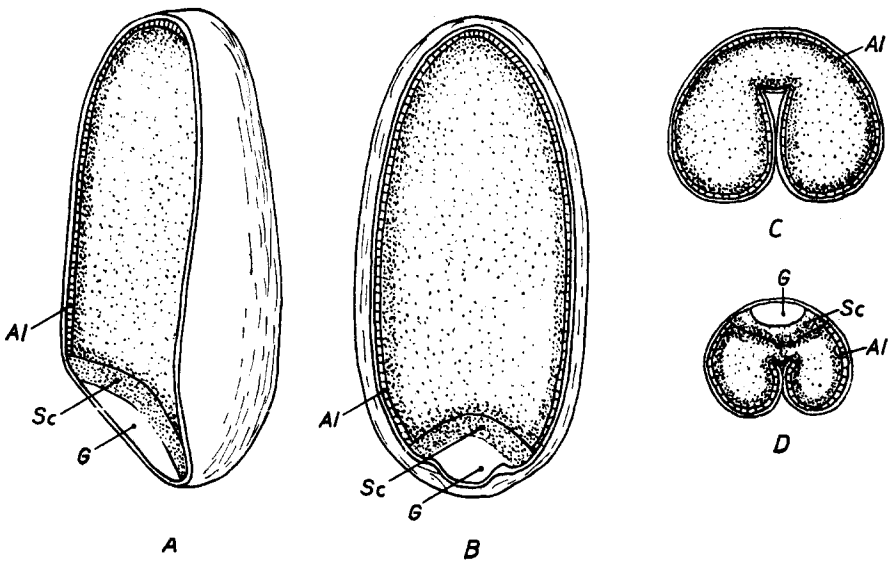

Fig. 10. Scheme of the distribut on of saccharogenic amylase in wheat grain. With some alterations, which may be derived from the given figures, the same scheme may be applied for rye and barley.
Dotted parts = Amylase.
A Longitudinal section along the crease.
B Longitudinal section perpendicular to the crease.
C and D transverse sections of the grain.
G Germ.
Sc Scutellum.
Al Aleurone layer.

The ratio $\frac{\text{total amylase}}{\text{active amylase}}$ is not the same for all parts of the grain. In wheat series in various parts of the same grain have been cut and this ratio has been estimated. See Table II.

The results so far obtained with these resting grains give a general idea of the amylase distribytion in the grain. This is schematically given in Fig. 10.

It would be interesting to find out wether the described distribution of the amylase in the grain coincides with different histological structures in the tissues concerned. HORNING [8] has made a qualitative study of the distribution of the mitochondria in

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TABLE II

THE RATIO $\frac{\text{TOTAL AMYLASE}}{\text{ACTIVE AMYLASE}}$ IN VARIOUS PARTS OF A WHEAT GRAIN

Number of series acc. to Fig. 1 (I—IV)	$\frac{\text{total amylase}}{\text{active amylase}}$
dorsal side I cf. Fig. 2	1.8
ventral side II cf. Fig. 5	1.7
Germ + endosperm V cf. Fig. 7A	1.4
Top end III cf. Fig. 6	1.4
Only germ part V cf. Fig. 7B	2.0

germinating cereals. From his figures it appears that the subaleurone layer, the scutellum and the endosperm layer along the scutellum are particularly rich in mitochondria. It may be that there is a connection between this and the high amylase content of the tissues. Further investigations are necessary to elucidate this problem.

SUMMARY

In resting grains (wheat, rye and barley) the bran and the aleurone cells do not contain any saccharogenic amylase. The first starch containing cell layer (the sub-aleurone) is particularly rich in amylase. The inner endosperm contains moderate quantities but the part which lies close to the germ is also particularly rich. In the germ the amylase is located in the scutellum tissue. There are some differences among the three kinds of grains examined.

RÉSUMÉ

Dans les grains au repos (blé, seigle et orge), le son et l'assise protéique ne renferment pas d'amylase saccharogénique. La première couche de cellules renfermant de l'amidon (le sub-aleurone) est particulièrement riche en amylase. L'intérieur de l'endosperme en contient des quantités modérées, mais la partie voisine du germe est aussi particulièrement riche. Dans le germe, l'amylase est contenue dans le tissu du scutellum. Les trois sortes de céréales examinées accusent quelques différences.

ZUSAMMENFASSUNG

Die Kleie und die Aleuronzellen der ruhenden Getreidekörner (Weizen, Roggen und Gerste) enthalten keine Saccharogen-Amylase. Die erste Zellschicht, welche Stärke enthält (Subaleuron), ist besonders amylasereich. Das Innere des Endosperms enthält mässige Mengen, aber der Teil, der dem Keim am nächsten liegt, ist auch besonders reich. Die im Keim enthaltene Amylase befindet sich im Gewebe des Scutellum. Die drei untersuchten Getreidearten weisen einige Unterschiede auf.

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