Development and Distribution of Wheat Lipase Activity during the Course of Germination[1]

R. DRAPRON, N'GUYEN XUAN ANH, B. LAUNAY, and A. GUILBOT,
Station de Biochimie et Physico-Chimie des Cereales de l' INRA,
CERDIA, 91, Massy, France

ABSTRACT

The development and distribution of lipolytic activity have been studied as a function of plumule growth stage in the course of wheat germination at 15° and 30°C. in darkness and at 30°C. in light, with olive oil as substrate. Lipolytic activity of wheat grain first undergoes a slight decrease, then increases to a level at a determined physiological stage corresponding to a plumule length of about 4 cm., regardless of temperature or light conditions during germination. With germination at 30°C. in darkness, a second increase in activity follows, which reaches a new level at a plumule length of 8 cm. Lipolytic activity is chiefly localized in the coleoptile; rootlets are the next most active fraction, and activity is very low in the residual grain. The lipolytic activity of the different fractions of germinated grain (plumule, rootlets, residual parts of kernel) is all the more important as germination temperature is increased. It is lower in light, under conditions of active photosynthesis, than in darkness, which could indicate that the radiant energy factor plays a part.

Sullivan and Howe (1) in 1933 were the first to show that lipase activity increases considerably during wheat germination, whereas esterase activity remains practically constant. Thirty years later, Rothe's work (2) confirmed and defined these results more accurately. Pomeranz and Shellenberger (3), using Gomori's method for histochemical characterization of free fatty acids, also confirmed this increase in lipolytic activity. MacLeod and White (4) noted that barley lipolytic activity increases twofold after 5 days of germination at 21°C. However, these articles provide little information on germinating conditions and the way in which activity is distributed between the different parts of a kernel. Moreover, the methods used do not always offer the best specificity.

We have taken up this study anew for wheat in order to gain a proper estimate of the development and distribution of lipolytic activity between the different parts of a germinated kernel, as a function of germinating conditions set at 15° and 30°C. in darkness and 30°C. in light.

MATERIALS AND METHODS

Samples

Two Cappelle wheat samples were used, which had been harvested in 1962 (sample 1) and 1964 (sample 2) and stored at 5°C. at approximately 13% moisture. Their germinability was near 98.5%.

Partial Aseptic Treatment

The kernels were immersed for 5 min. in 1% hydrogen peroxide solution under partial vacuum (25 mm. Hg). Normal pressure was then re-established and the grains were left in contact with the solution for another 5-min. period. Finally, the grains

[1]Presented at the 50th Annual Meeting, Kansas City, Mo., April 1965.

were thoroughly washed in cold sterile water. This treatment allowed a large reduction in the number of microorganisms and led to more uniform embryonic development than with hypochlorite or mercuric chloride treatment, and did not disturb the development of lipolytic activity.

Conditions for Germination

Glass beads, 2 mm. in diameter, were washed with water and dried at 180°C., then laid 1 cm. deep in hypochlorite-washed plastic boxes, size 24 by 18 by 10 cm. The surface of each bed was leveled and distilled water was added so that it just covered the top layer of the beads.

The grains were laid, each with its crease facing down on the bed, approximately 1.5 cm. apart to limit contamination by microorganisms still present. The boxes were covered with lids to limit gas exchange and placed at either 15° or 30°C. ± 1° in darkness or 30°C. in light.

Sampling and Storage Techniques

Sampling was carried out as a function of time, with plumule length marking the developmental stage. The grains, on which colonies of microorganisms had developed, were eliminated. After collection, whole plantlets and dissected separate organs (plumule, rootlets, or residual grain) were frozen, ground in the presence of solid carbon dioxide, freeze-dried, and stored at −20°C.

Measurement of Lipolytic Activity

The methods of Desnuelle et al. (5) and Rothe (6) were used for measuring lipolytic activity with crude olive oil (initial free acidity below 1%) as substrate. The lipolysis conditions used with these methods are summarized in Table I. They differed chiefly in the concentration of the enzymatic preparation with regard to substrate, in the water content of the medium, and in the presence of activator and pH, as well as in the hydrolysis period.

The method of Desnuelle et al. allows measurement of the acidity released at a constant pH from an olive oil emulsion stabilized by gum arabic in the presence of biliary salts and calcium ions. A test was carried out with paraffin oil instead of an olive oil emulsion, to establish the correction for acidity of the enzymatic source and for H^+ ions being released by enzymatic systems other than lipase. The determinations were carried out with a pH-Stat recorder. The enzymatic activity was expressed in μeq. of oleic acid released per min. (lipase unit) per mg. of enzymatic preparation on a dry basis. This method, as we have established elsewhere (7), is not used under conditions when lipase activity is very low, as other acidifying processes are too important in comparison with lipase action, but the method is satisfactory when lipase activity increases during germination.

Rothe's method consists in measuring acidity in lipid extracts after incubating a mixture of olive oil and enzymatic preparations, held for 90 hr. at 30°C. and 85% r.h. Traces of acids which exist in the initial substrate were also determined, and a check test was carried out under the same conditions but without a substrate. The difference between acidity measured in the actual test and these interfering acidities thus represented the quantity of acids released under the action of lipases. Activity was expressed in g. oleic acid released in 90 hr. per 10 g. dry product. This method

TABLE I. COMPARISON OF LIPOLYSIS CONDITIONS FOR THE TWO METHODS USED

	Rothe Method	Method of Desnuelle et al.
Concentration of enzymatic preparation with regard to substrate	95/5	25 or 50/1,000
State of medium	Semisolid mixture	Liquid emulsion
Activators	None	Ca^{++}; biliary salts; Cl^- Na^+
Water content, %	20 to 30	90
pH	5 to 6	9.0
Temperature, °C.	30	37
Duration, min.	5,400	10

is particularly well adapted for measuring low activities, and we have used it for defining the development of lipolytic activity during the first stages of germination. However, on account of the low hydration of the medium, which limits the reactant's mobility, the results obtained cannot be compared directly with those obtained by the method of Desnuelle et al.

RESULTS AND DISCUSSION

Since germinations were carried out on wheat from different harvests and under various conditions of temperature and light, we expressed the development of lipolytic activity not as a function of time but as a function of plumule length. This

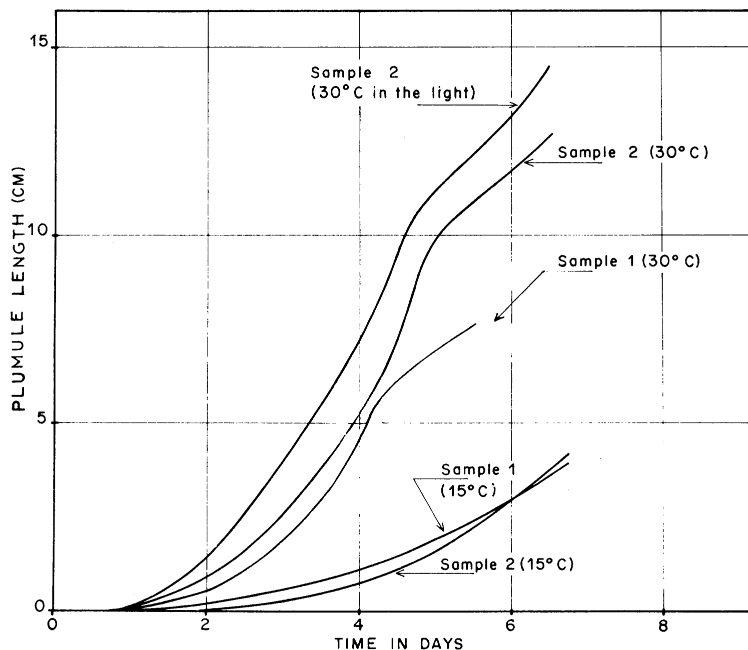

Fig. 1. Plumule growth as a function of time, at 15° and 30°C. in darkness and at 30°C. in light.

TABLE II. DEVELOPMENT OF LIPASE ACTIVITY OF GERMINATING WHEAT (SAMPLE 1) AT 15° AND 30°C. IN DARKNESS (MEASURED BY ROTHE'S METHOD)

Plumule Length	15°C.	30°C.	Plumule Length	15°C.	30°C.
cm.			cm.		
...[a]	0.061	0.061	1.5	0.173	0.210
0.3	0.031	....	2.5	0.209	....
0.5	0.060	0.046	5.0	....	0.360
0.75	0.070	0.098	5.5	0.264	....

[a]Ungerminated wheat.

permitted comparison of activities for the same apparent developmental stage of the plant; therefore, we shall discuss the increase in plumule length as a function of germinating time before discussing the development of lipase activity as a function of plumule length.

Increase in Plumule Length as a Function of Germination Time

Embryo development occurred in less than 10 hr. in 85% of the grains germinated at 30°C. The total of these samples can be taken without risk of errors in selection. On the contrary, at 15°C. more heterogeneous embryo development was observed, a 24-hr. interval being needed before all the kernels showed signs of germination. The percentage of grains reaching the same stage of development in a given time was lower, and under these conditions it is possible that the samples collected during early stages of germination were less representative than those collected later.

Figure 1 shows for both wheat samples the development of plumule growth as a function of time at 15° and 30°C. in darkness and at 30°C. in light. At 15°C. after a lag period of nearly 2 days, a growth increase very similar for samples 1 and 2 was observed. At 30°C. the lag period was perceptibly shorter and the growth rate, similar for both samples, was much higher than at 15°C. However, it appeared that the growth slowed down after the fifth day for sample 2 and on the fourth day for sample 1. The lengthening of the plumule in sample 2 in light appeared similar to that observed in darkness, but slightly greater.

Development of Lipolytic Activity of Germinating Wheat as a Function of Plumule Length

The measurements performed with Rothe's method on sample 1 at 15° and 30°C. showed plainly the existence of an activity minimum in the very first stages of plumule growth (Table II). This minimum seems to testify to some destruction or inhibition of initial activity in the very hydrated medium supplied by germinating grain. The results obtained by MacLeod and White (4) on barley showed the same phenomenon. This confirms Rothe's observations on lipase activity as a function of hydration (2). After the observed minimum, lipase activity increased as a function of plumule length at the two temperatures used. The activity increase was less at 15° than at 30°C.

This increase of lipolytic activity was studied in sample 2 by the method of Desnuelle et al. at later germination times (Fig. 2). In darkness at 15°C., activity

increased to an approximately constant level at about 4-cm. plumule length. In contrast, there was a much greater increase at 30°C. until 4-cm. plumule length was reached, when the activity curve underwent an inflection. The activity then increased again very rapidly until 8-cm. plumule length was reached, when finally the activity became practically constant. Thus, germination temperature seems to have an important effect on lipolytic activity.

When comparing the results obtained at 15° and 30°C. for plumule lengths of 4 and 10 cm., we observed that a temperature increase of 10°C. multiplied lipase activity by 3.5 and 6, respectively. The phenomenon was much less conspicuous in measurements on wheat sample 1 by Rothe's method, probably because this method, carried out in a medium of low hydration on preparations with high activities, gives values which are not proportional to lipolytic activity.

Lipolytic activity developed initially in sample 2 at 30°C. both in light and in darkness. However, the activity with germination in light was lower; it increased only to 4-cm. plumule length and remained constant to at least 10-cm. plumule length (Fig. 2).

Lipase Activity, Localization, and Development in Different Parts of Germinated Wheat

Several experiments were conducted to determine localization of lipolytic activity and its development in the different parts of the wheat kernel. The results obtained by Desnuelle's method are shown in Figs. 3 and 4 for germination at 30°C. in darkness and in light, and in Fig. 5 for germination at 15°C. in darkness.

Under all conditions, lipolytic activity of the grain after removal of plumule and

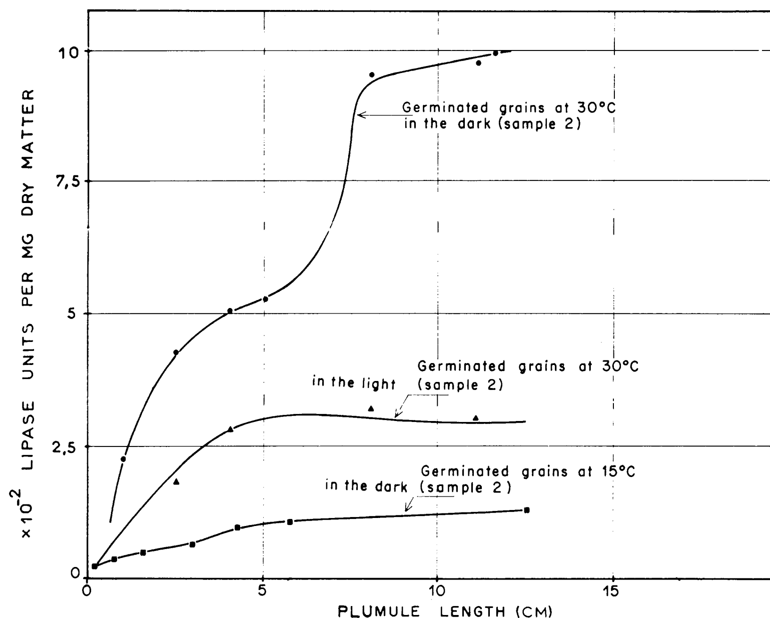

Fig. 2. Development of lipolytic activitv of whole wheat (measured by Desnuelle's method) as a function of plumule length, at 15° and 30°C. in darkness and at 30°C. in light.

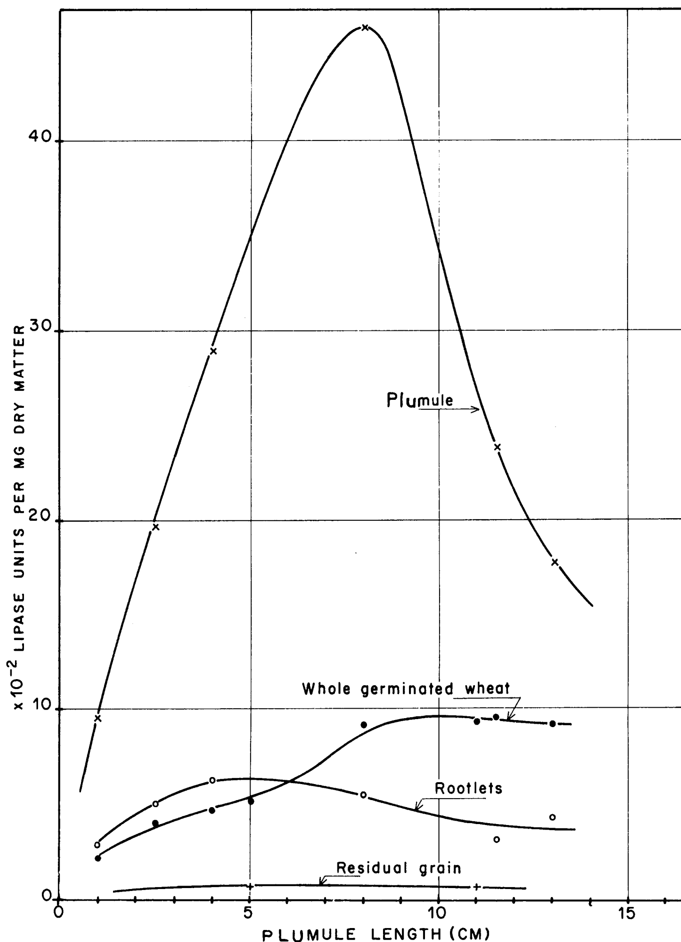

Fig. 3. Development of lipolytic activity (measured by Desnuelle's method) in different parts of the germinated kernel in darkness at 30°C., as a function of plumule length.

rootlets was low. Values were practically constant in each case, and somewhat lower at 15° than at 30°C.

The lipolytic activity of rootlets at 30°C. during germination in the dark, which is relatively high, reached a maximum at 4-cm. plumule length, then decreased very slightly. During germination in light at 30°C. or in darkness at 15°C. the lipolytic activity changed at the same rate as at 30°C. in darkness, but activity was lower.

The major part of the activity appeared to be localized in the plumule, which constitutes material from which lipase can be isolated (8). During germination at 30°C. in darkness the activity increased until it reached a maximum at between 5- and 10-cm. plumule length, then decreased rapidly. During germination at 30°C. in light the lipolytic activity reached a maximum value at between 3- and 5-cm.

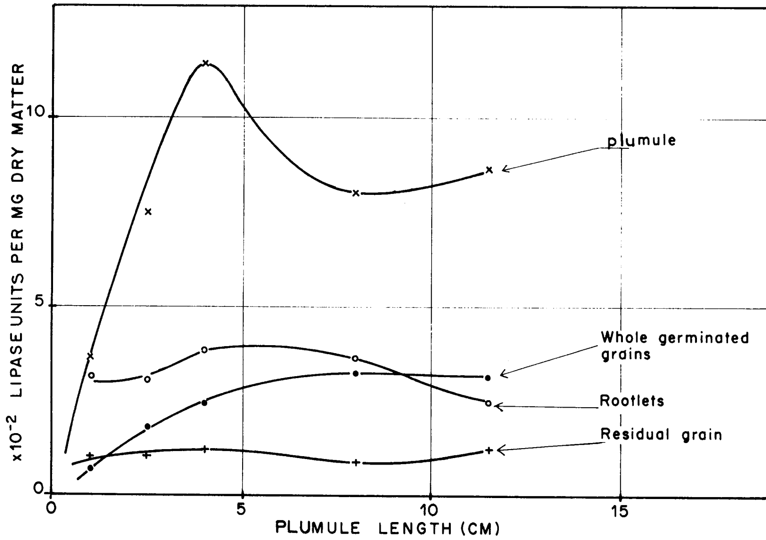

Fig. 4. Development of lipolytic activity (measured by Desnuelle's method) in different parts of the germinated kernel in light at 30°C., as a function of plumule length.

plumule length, then decreased and increased again without ever reaching the activity values observed for the wheat plumule growing in darkness.

The lipolytic activity of the plumule at 15°C. in darkness at first increased at a rapid rate, then more slowly between plumule lengths of 2 and about 4 cm., after which it became constant.

To localize the enzyme inside the plumule we measured the activity of the three morphologically most differentiated parts of the organ (Fig. 6): the cotyledon of the grain (scutellum) (3), the coleoptile (2), and the remainder of the plantlet, the

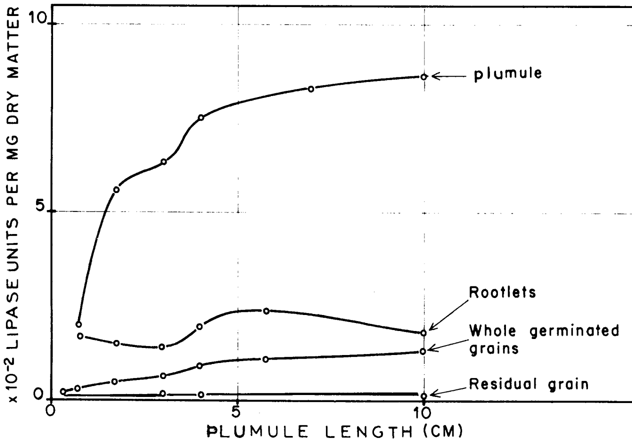

Fig. 5. Development of lipolytic activity (measured by Desnuelle's method) in different parts of the germinated kernel in darkness at 15°C., as a function of plumule length.

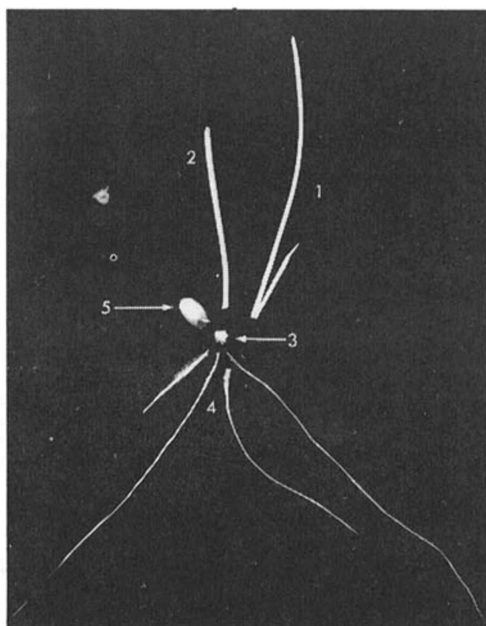

Fig. 6.. Different parts of the germinated kernel of which lipolytic activity was measured. 1: first leaves (4); 2: coleoptile (42.6); 3: scutellum (2.9); 4: rootlets (6.0); 5: residual kernel (0.75). (Numbers in parentheses represent lipolytic activity as 10^{-2} lipase units per mg. dry matter.)

first leaves (1) of which make up its most important fraction by weight. Determinations were made at a developmental stage corresponding to about 5 cm. plumule length in darkness at 30°C. when the first leaf was emerging from the coleoptile. It appeared that lipolytic activity was present chiefly in the coleoptile (42.6×10^{-2} lipase units per mg. dry matter), whereas the scutellum and first leaves had very low lipolytic activity (2.9 and 4.0×10^{-2} lipase units per mg. dry matter respectively).

We were thus able to reveal several particular details in the development of lipolytic activity in the course of wheat germination. Three points seem of special importance. The first and second deal with localization of lipolytic activity in the coleoptile and the influence of temperature on lipase synthesis; the third deals with the influence of light. Measurable lipolytic activity was clearly lower under conditions of active photosynthesis than in darkness. These facts lead to the conclusion that during germination in darkness, degradation of lipids by lipase provides an important source of energy, thus compensating in part for the shortage in radiant energy.

Acknowledgment

This research has been partly financed by a grant made by the U.S. Department of Agriculture under P.L. 480.

The authors wish to express their thanks to Dr. P. Jonard of the Station Centrale de Genetique et d'Amelioration des Plantes de l'INRA, and to Prof. C. Costes of the Ecole Nationale Superieure de Grignon (France) for their suggestions and discussions; to Dr. Majel MacMasters of Kansas State University for editorial assistance.

Literature Cited

1. SULLIVAN, BETTY, and HOWE, MARJORIE A. Lipases of wheat. I. J. Am. Chem. Soc. 55: 320 (1933).
2. ROTHE, M. Biochemische Studien an Gramineen Lipase. Fette, Seifen, Anstrichmittel 11: 905 (1955).
3. POMERANZ, Y., and SHELLENBERGER, J. A. Histochemical characterization of wheat and wheat products. IV. Mapping the free fatty acids in germinating wheat. Cereal Chem. 38: 122 (1961).
4. MacLEOD, ANNA M., and WHITE, H. B. Lipid metabolism in germinating barley. I. The fats. II. Barley lipase. J. Inst. Brewing 38: 487 (1962).
5. DESNUELLE, P., CONSTANTIN, J. M., and BALDY, J. Technique potentiometrique pour la mesure de l'activite de la lipase pancreatique. Bull. Soc. Chim. Biol. 37: 285 (1955).
6. ROTHE, M. Eigenschaften und Reaktionverhalten pflanzlicher Samenlipase in naturlichen Milieu. I. Mitteilung. Aktivitatsbestimmung unter naturlichen Milieubedingungen. II. Mitteilung. Einfluss der Feuchtigkeit auf das Temperaturverhalten. Ernahrungsforschung 3: 21 (1958).
7. DRAPRON, R. La determination de l'activite de la lipase (glycerol ester hydrolase, EC. 3.1.1.3) dans les produits cerealiers. Communication a la Section de Chimie Analytique de la Soc. Chim. de France, Dijon (1965).
8. DRAPRON, R., RITZENTHALER, G., and LAUNAY, B. Sur l'isolement de la lipase (glycerol ester hydrolase E.C. 3.1.1.3) de la gemmule de ble germe. C. R. Acad. Sci. Paris 263: 1168 (1966).

[Received December 12, 1968. Accepted May 20, 1969]