CHANGES IN CARBOHYDRATE CONCENTRATION

AND

AMYLOLYTIC ACTIVITY IN GERMINATING MAIZE.

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Text.

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I N D E X.

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SUMMARY.

Changes in the concentration of some carbohydrates and in amylolytic activity have been followed during germination of Zea mays L. var. Hickory King and var. Early Pearl. Assay techniques have been developed which permitted assay of individual grains. Thus during the investigation both groups and individual grains were used as samples. The use of groups permitted control of assay technique. Length of radicle, coleoptile and lateral roots were recorded in order to permit quantitative estimation of correlation between growth and the concentration of the various carbohydrates.

Initially, during the study of changes in the carbohydrate concentration in Hickory King grains, total reducing sugar, sucrose and dextrin concentrations were estimated. However, the results obtained for changes in dextrin concentration, although reproduceable, thereby indicating reliable assay technique, presented a confusing picture and, in view of the apparent importance of sucrose and reducing sugar concentration, assay of dextrin concentration was discontinued in subsequent study of Early Pearl. Instead changes in total reducing sugar, sucrose and glucose concentrations were followed.

The results revealed that there is very considerable variability in physiological activity between grains subjected to the same germination conditions. However, all, irrespective of variety, follow the same basic metabolic pattern during germination.

The trends observed were:

(i) Reducing sugar accumulates slowly during the first 72-96 hours germination, but thereafter accumulation is very rapid, although concentration may decrease towards the end of the germination period.

(ii) Glucose follows a similar pattern to reducing sugar, accumulating slowly during the early stages of germination, followed by a period of rapid increase in concentration, which may decrease towards the end of the germination period.

(iii) Sucrose concentration in dormant grains is fairly high, but it decreases markedly during the first

96 hours germination. This is followed by a phase of sucrose accumulation.

(iv) Dextrin concentration shows two peaks. Initial level is low, but it accumulates rapidly during the first 72 hours. The level decreases between 72 and 120 hours but increases when the germination period is increased to 192 hours, after which there is a marked decrease. It was impossible, from the data relating to the study of individual grains, to discern a trend in dextrin concentration.

With the exception of dextrin, about which there is little information, the results are in general agreement with the literature.

Investigation of correlation between the various carbohydrates and between these and growth revealed that:

(i) reducing sugar concentration and growth are positively correlated;

(ii) glucose concentration and growth are positively correlated;

(iii) sucrose and reducing sugar concentrations are negatively correlated during the initial stages of germination;

(iv) sucrose and glucose concentrations are negatively correlated during early germination;

(v) glucose and reducing sugar are positively correlated;

(vi) in general, correlation between growth and concentration of the carbohydrates studied, decreases during the later periods of germination.

These observations suggested that growth was, at least during the early stages of germination, dependent on the level of reducing sugar, and more particularly on the level of glucose, and that sucrose is the principal source of reducing sugar during this period.

The relationship between amylase activity (total alpha- and beta-amylase activity) and reducing sugar concentration tends to

be curvilinear, which suggests that amylolytic activity produces relatively little reducing sugar during early germination, even though amylase activity and growth may be positively correlated.

The results suggest, contrary to the observations of previous workers, that alpha-amylolytic activity may be present in dormant grains and that maize is not characterised by low levels of beta-amylase activity during germination.

From the observations it is concluded that the initial accumulation of reducing sugar is the result of sucrose hydrolysis, and therefore sucrose is an important metabolite during early germination. Amylolytic activity contributes little reducing sugar during the initial stages of germination but that after approximately 72 hours it represents the major source of reducing sugar.

CHAPTER 1.

INTRODUCTION AND REVIEW OF THE PERTINENT LITERATURE.

I N T R O D U C T I O N.

Cereals, because of their economic importance, have been the subject of sustained research. The industries which have developed, using both germinated and ungerminated grain, have stimulated investigation into the chemical changes that take place during germination. In particular, changes in carbohydrate concentration and the systems which bring about these changes, have received attention. Considerable information of this nature, on a wide variety of cereals is available to research workers and, indeed, unless some new aspect is to be studied, little useful purpose is achieved by repeating this work.

In germinating grain, changes in carbohydrate concentration and amylolytic activity have their significance in the growth of the embryo; indeed, it has been said that in maize, the embryo contributed fats and the endosperm supplied carbohydrates during germination (Malhotra 1934). It would seem, therefore, that correlating chemical changes occuring in the grain with growth and development of the shoot and root system could improve our understanding of germination. It is surprising that, to my knowledge, this approach has not been previously used, especially so since, as early as 1924, Toole remarked that, in maize 'the correlation of the appearance of reducing sugar in the embryo with the first growth of the tissue in which such appearance occurs and the sharp localisation of these changes, illustrate the dependence of growth on internal changes.'

Trends in carbohydrate concentration and amylolytic activity during germination are most easily observed when groups of grain are used as the sample unit, because the procedure decreases variation and permits replication. However, measurement of radicle, coleoptile and lateral root length in large groups of grain is impractical and, indeed, undesirable since, unless single grains are used as the sample unit, no information can be obtained as to

the conformity of behaviour of individual grains. That they do exhibit considerable physiological variability has been emphasised by Dure (1960b). Even though maize, because of its large grain size, is suited to investigations in which the individual grain is the sample unit, the sample is still too small to permit replication from the same grain. Reliable techniques had, therefore, to be developed using small groups (10 grains) as the sample unit. After these techniques were shown to be reliable when working with a small sample weight - approximately equivalent to that of a single grain, they could be applied to investigations on individual grains without replication but with some degree of confidence.

The desirability of determining the concentration of as many carbohydrates as possible in each sample cannot be over emphasised since such data permit comment on relative concentrations of different carbchydrates at the same stage of germination. However, where the sample size is small, the number of different carbohydrates that could be determined quantitatively is limited and only the major carbohydrates, as evidenced by their concentration, could be investigated. Starch concentration was, however, not assessed, since, being present in large amounts in the endosperm, it is unlikely to influence the growth of the embryo directly.

This work, therefore, followed three phases:

(i) Qualitative and semi-quantitative procedures designed to select the carbohydrates for further investigation.

(ii) Investigation of changes in the concentration of some carbohydrates during germination.

(iii) Investigation of changes in amylolytic activity during germination.

It is beyond the scope of this work to review all the literature on the above topics. However, a review of literature pertinent to the research is useful.

PERTINENT LITERATURE.

(i) Literature pertinent to techniques adopted in chromatographic analysis.

The application of paper chromatography to the analysis of carbohydrates was first reported by Partridge (1946). He utilised this technique for the qualitative analysis of polysaccharides, identifying the sugar components by reference to known sugars (Partridge 1948). In 1947, Flood et al and Hawthorn established a technique for quantitative estimation of sugar after chromatography. However, the degree of accuracy was only approximately 5 per cent.

Jermyn and Isherwood (1949) developed quantitative methods for the estimation of sugars. It is interesting that, in order to obtain better separation, they utilised the technique of allowing the solvent to drip from the end of the paper, stating Rf values as the ratio of movement of an unknown to that of some reference compound.

Fink et al (1963) have compiled a table of correlated Rf values for several classes of compounds in a wide variety of solvent systems. These data are most useful in the selection of solvent systems as well as supplying a guide to other compounds which might overlap or interfere.

Many methods have been developed for quantitative estimation of sugars on the paper. These involve chromogenic agents and the unknown is compared with standard solutions which have been run on the same sheet. Concentration can be determined as transmittance with a densitometer or as optical density after elution. However, elution of the chromagen is often problematical. Indeed, methods have been developed which involve the formation of a chromagen after elution (Jermyn and Isherwood 1949). Serious error is always possible where marker strips are used, a feature which is avoided where elution is after formation of the chromagen.

Although these techniques can be organised on a routine basis, they are limited in that the range of concentrations of sugars that can be determined is narrow. Investigations which involve samples containing considerable differences in concentration of

a variety of carbohydrates and where there is a wide range of concentrations of any particular carbohydrate between different samples, cannot easily be adapted to a routine quantitative method of this nature.

Semi-quantitative techniques in which the concentration of sugar is given a value, according to an arbitrary scale, have been developed for investigations of this nature (Drennan 1962). Such a technique is particularly useful where some idea of relative concentrations and general trends is required.

This type of procedure was adopted in the present work to establish which carbohydrates should be investigated quantitatively and the trends could be anticipated, thus facilitating the problem of estimating extraction volumes prior to quantitative estimation.

(ii) Literature pertinent to investigation of changes in carbohydrate concentration.

The earliest work on the chemical composition and changes that take place during germination in maize (Hopkins 1898, Hopkins et al 1903, Adronescu 1919 and Toole 1924) were limited in that suitable quantitative methods had not been developed. It is interesting, however, that as early as 1924, Toole noted that, although the utilisation of fats in the early stages of germination was limited in the attached embryo, in the excised embryo considerable growth occurs at the expense of the stored fats. He concluded that fat was relatively unimportant in normal germination and that 'in maize the starch of the endosperm would seem to be a much more readily available source of food for the developing plantlet than the fat of the embryo itself'.

He thus interpreted the presence of sucrose in the embryo (also demonstrated by Lampe 1931) as a translocation form of carbohydrate in maize. Whilst this is probably true (Edelman et al 1959) it seems that the presence of sucrose in the scutellum is established during development of the grain (Bernstein 1943) and not during the initial stages of germination. Indeed, sucrose concentration has been shown to decrease during the early stages of germination (Bond and Glass 1963). Its presence in the embryo of ungerminated grain and its early decrease suggests that sucrose may be an important metabolite in the early stages of germination

as has been demonstrated in barley by the extensive work of McLeod et al (1951, 1952, 1953 a & b, 1960). Hellebust and Forward (1962) have demonstrated that invertase activity is high in growing maize roots.

The hypothesis that sucrose is active during early stages of germination is supported by the observations of Andronescu (1919) and subsequently Dure (1960a) who demonstrated that isolated embryos could develop into normal vigorous plants when supplied with sucrose. It is interesting that glucose was shown to be the most satisfactory in this regard. Further support is the evidence that the products of starch hydrolysis do not appear during the first two or three days of germination. Toole (1924) observed that there is an increase in the reducing sugar concentration in the embryo before the appearance of reducing sugars in the endosperm. Dure (1960a) concluded that early growth of the axis is dependent on the scutellar food reserves since the growth of the axis, when the embryo is cultured in a non-nutrient medium, is identical with the growth of the intact kernel axis for the first five days. Dure (1960b) demonstrated that amylolytic activity is low over the first three days and thus as Drennan (1962) has suggested in Avena, it seems likely that the metabolism of germinating maize is not initially dependent on starch-derived materials. Fats and some of the sugars were suggested as likely alternatives for respiratory substrates. The importance of fats during the early stages of germination in maize has been demonstrated (Malhotra 1934, Dure 1960a).

Taufel et al (1960) reported on the levels of simple sugars (raffinose, sucrose, glucose, maltose and fructose) in germinating corn. Of these sugars only maltose was absent in the ungerminated grains. However, it appeared during germination but remained at a considerably lower level than glucose. Sucrose, glucose, maltose and fructose increased during germination. Raffinose disappeared during the early stages of germination. It is interesting that sucrose, which is the principal non-reducing sugar, was not shown to decrease during the early stages of germination. Bottomley et al (1950-1952) have shown that when corn is brought to a moisture content of 30 per cent there is a

marked decrease in non-reducing sugars.

Bond and Glass (1963) identified raffinose, sucrose, glucose, fructose, myoinositol and glycerol in ungerminated corn. Maltose appeared after 48 hours of germination. Glucose remained fairly constant for 48 hours then increased. Sucrose showed a small but definite decrease in the first two days and then increased rapidly. These authors observed that sucrose and glucose comprised the largest part of the sugars studied. The high concentration of glucose relative to that of maltose suggests that it might be more meaningful to express reducing sugar as glucose rather than maltose.

The general trends that have been observed in maize are similar to those in other cereals. Bond and Glass (1963) have noted that 'Carbohydrate metabolism in germinating corn appears to resemble in a general way that of other cereals which have been studied. The precise nature and mechanism of the gross changes observed remain to be established'.

(iii) Literature pertinent to determination of amylase activity in maize.

Starch is the principal storage carbohydrate in the endosperm of cereals and, on hydrolysis, yields sugars which constitute the chief source of energy in the early development of the plant. The enzymes involved in the degradation of starch and their specific function have been extensively reviewed (Hopkins 1946, Myrback and Neumuller 1950, Bernfeld 1951, Peat 1951, Sumner and Somers 1953 and Badenhuizen 1959). The major part of mobilisation of the endosperm, in cereals, depends on the action of alpha and beta-amylase, other hydrolytic enzymes Z, D and R which cleave linkages other than the 1-4 glucosidic link, provide relatively little soluble carbohydrate (Peat 1951, Baldwin 1957, Pigman 1957, Badenhuizen 1959).

Although both alpha and beta-amylase cleave the 1-4 alpha-glucosidic link in starch, their action is different. Beta-amylase hydrolyses the polymer from the non-reducing ends, and in so doing, produces maltose units (Hopkins 1946, Myrback and Neumuller 1950, Bailey and Whelan 1957 and Badenhuizen 1959).

Although starches more generally contain 75-80% amylopectin, some waxy starches contain so little amylose that they fail to give a reaction to iodine (Crocker and Barton 1953). Since beta-amylase is incapable of hydrolysing the 1-6 glucosidic link, its action produces residues termed limit dextrins. Unlike beta-amylase, alpha-amylase can act at any 1-4 glucosidic link in the polymer (Myrback and Neumuller 1950, Bernfeld 1951, Pigman 1957 and Badenhuizen 1959). However, its action on maltotriose, to produce maltose and glucose is slow (Bernfeld 1951). Alpha-amylase, like beta-amylase, cannot hydrolyse the 1-6 glucosidic link. The difference between the mode of action of these two enzymes implies that they would be complementary in their action on starch, in that the action of alpha-amylase would prevent the formation of limit dextrins, whilst beta-amylase would produce the bulk of the reducing sugar.

In view of the importance of amylase activity during malting, considerable research has been directed towards methods of measuring total amylolytic activity and the activity of alpha and beta-amylase independently. Wildmer (1958) in a review of the subject cites some 150 methods for the determination of amylase activity. Most are based on one of the manifestations of amylase action:

(a) Measurement of increase in reducing sugars i.e. saccharification.
(b) Observation of change in the starch-iodine colour reaction i.e. dextrinisation or,
(c) Measurement of decrease in viscosity of starch during hydrolysis.

Since in extracts from germinating cereals both alpha and beta-amylases are present, it has become necessary to devise procedures which involve the selective inhibition of one or other amylase so that their activity can be determined independently. If the activity of alpha and beta-amylase are to be expressed in the same units, then clearly (since their mode of action is different) it is necessary to demonstrate that their actions are additive in for example, either saccharification or

dextrinisation.

In the earlier procedures, it was assumed that a dextrinising method measured alpha-amylase activity exlusive of beta-amylase activity. Subsequent work, however, showed that this was not the case and Sandstedt, Kneen and Blish (1939) modified the proccdure to measure dextrinisation in an excess of beta-amylase. Under such conditions, changes in activity are attributed solely to the action of alpha-amylase. Thus the standard American Society of Brewing Chemists (1958) procedure for the determination of alpha-amylase activity, measures the rate of dextrinisation of beta limit dextrins, as measured by the starch iodine reaction. The dextrinisation units may also be converted to saccharification units (Kneen and Sandstedt 1941, Olson, Evans and Dickson 1944). In this way, beta-amylase activity can be determined by difference between total saccharifying activity and alpha-amylase activity expressed in saccharification units. By use of this conversion, Kneen and Sandstedt (1941) established that alpha and beta-amylase saccharogenic activities were additive in combination over a wide range of relative proportions of the enzymes.

Saccharifying methods, which measure the production of reducing sugars as a result of amylolytic activity, are in general use. Various procedures have been used for the estimation of reducing sugars, among them 3, 5 dinitrosalicylic acid (Waldt 1952, Kirsop 1953, Bernfeld 1955, Dure 1960b and Bendelow 1963). Although satisfactory results have been obtained with this method, it has the disadvantage that the graph relating colour yield and sugar concentration does not go the origin (Kirsop 1953, Muller 1959). However, copper reduction colorimetric methods also have disadvantages when used on crude extracts (Briggs 1967).

Bernfeld (1955) and Dure (1960b) have used saccharogenic activity in the assay of alpha and beta-amylase separately. The separation of the two enzymes involved selective inhibition of one and assaying for the other. Dure (1960b) in his investigations on amylolytic activity in germinating maize, assumed that, since cereal amylases have similar physical properties (Kneen 1945), these properties would hold true for maize amylases. He thus utilised the heat inactivation of beta-amylase (Ohlsson 1922 and 1930, Kneen, Miller and Sandstedt 1942, Myrback and Neumuller

1950 Bernfeld 1959) for the assay of alpha-amylase and pH inactivation of alpha-amylase (Ohlsson 1922 and 1930, Geddes 1946, Myrback and Neumuller 1950) for the assay of beta-amylase. There is, however, clear evidence that neither of these two procedures are really satisfactory since in both, the treatments are not selective. Thus Dure (1960b) was, in some instances, only able to recover 40% of the total amylolytic activity in separate determinations of alpha- and beta-amylase activity.

Bendelow (1963) developed a routine procedure for the assay of alpha-amylase activity, in the presence of phenyl mercury chloride, which has been shown to cause loss of beta-amylase activity while having little effect on alpha-amylase action (Weill and Caldwell 1945). Briggs (1967) has observed that phenyl mercury chloride has no effect on alpha-amylase activity when dextrinising activity is followed after heating at 70°C.

Whilst the results obtained by Bendelow (1963) showed a close correlation (0.99) with those determined by the official American Society for Brewing Chemists method, there is some doubt that the activity of beta-amylase determined by difference between total saccharogenic and alpha-saccharogenic activity, is always accurate. Meredith (1965) has shown that when the ratio of beta- to alpha-amylase is between 1:1 and 1:3, there is an excess saccharification over that determined for the beta-amylase independently. This excess saccharification varied between 6% and 12%.

Since in maize, beta-amylase to alpha-amylase saccharification activity is usually less than 1:1, except in the early stages when activity is low, the error involved in the estimation of beta-amylase activity by difference is likely to be considerably less than 12%. This suggests that the method is as reliable, if not more so, than those involving thermal and pH inactivation. A further advantage of this method (Bendelow 1963) lies in its simplicity, making routine analysis less tedious.

Where activity of alpha- and beta-amylase is to be determined and more particularly where the activity is high, special precautions have to be observed to maintain first order kinetics. It has been shown that alpha-amylase activity decreases as the average length of the polymer drops below 10 glucose residues (Hopkins 1946, Schwimmer 1950). Beta-amylase activity is thought

to decrease as a 1-6 alpha branch in the polymer is approached (Badenhuizen 1959). Dure (1960b) developed a procedure in which for each assay of amylolytic activity the concentration of tissue extract at which substrate became limiting, was determined. The concentration of tissue for the determination was kept well below this value thereafter. This procedure is not suited to routine analysis and a simpler method has been devised and is described under Materials and Methods.

CHAPTER 2.

MATERIALS AND METHODS.

The grains used in these studies were certified varieties as supplied by Messrs. Starke-Ayres Ltd. of Cape Town. Two varieties were investigated, Zea mays L., var. Hickory King and var. Early Pearl. The former was selected first because of the large grain size which simplified the task of assaying single grains. 'Early Pearl' was selected because, unlike 'Hickory King' which is a starchy grain, it is considered a sweet grain. Work on two grains of this type would supply some idea on the conformity, or otherwise, of physiological behaviour during germination.

GERMINATION.

Undamaged grains were taken at random and weighed. After surface sterilising for 5 minutes with 0.1% mercuric chloride containing a little detergent as a wetting agent, they were thoroughly rinsed with distilled water, and placed to germinate scutellum downwards (Toole 1924) on moist filter paper in an oven at 25°C. The filter paper was draped over glass slats with the ends dipping into troughs of distilled water. Germination was in the dark, except for short periods when grains were removed from the oven.

After increasing periods of germination, the grains were removed and weighed. Measurements of radicle length, coleoptile length, and length of lateral roots arising from the cotyledonary node were recorded. Subsequent treatment varied according to whether the sample was to be assayed for carbohydrates or amylolytic activity.

CHROMATOGRAPHIC ANALYSIS.

Grains were removed from the oven at 24 hour intervals and dried at 80°C for 48 hours, after which they were ground in a Casella mill, with a 0.5 mm. sieve. The meal was then ground further with a pestle and mortar, producing a meal that would pass

a 100 mesh sieve. 4 gm. samples were extracted thrice with 50 cc. volumes of 80% ethanol. Each extraction consisted of 20 minutes mechanical shaking followed by decantation through Whatman's No. 1 filter paper. After the final extraction, the residue and filter paper were thoroughly washed with 80% ethanol. The filtrate was evaporated to dryness at 45°C under reduced pressure, and the residue redissolved in 5.0 ml. of 80% ethanol. 0.04 ml. aliquots were drop-loaded, using an Agla microsyringe, onto Whatman's No. 1 chromatography sheets. Marker spots of xylose, fructose, glucose, sucrose, maltose, and in some instances maltotriose and maltotetraose, were included on the sheets.

Two solvent systems were selected; Ethyl acetate, acetic acid, formic acid, water (36:6:2:3) which gave good separation of glucose, sucrose, maltose and fructose, and isopropyl alcohol, ethyl acetate, water (6:1:3) which gave good separation of maltotriose and maltotetrose, a feature not obtained with the former solvent system.

The chromatograms were run at room temperature for up to 36 hours in the case of ethyl acetate, acetic acid, formic acid, water, and until the solvent front had travelled approximately 35 cms. in the case of isopropyl alcohol, ethyl acetate, water. After running the chromatograms were air-dried and then sprayed with p.anisidine hydrochloride and placed in a ventilated oven at 105°C for 10 mins. Chromatograms were examined with both visible and ultraviolet light. Except when concentration was high, as in the standards, maltotetrose was only visible under ultraviolet light.

CARBOHYDRATE ASSAY.

Grains were removed from the oven, and, after the necessary measurements had been recorded, they were dried in a ventilated oven at 80°C for 24 hours. The dried grains, after cooling, were weighed and then ground in a Cassella mill with a 0.5 mm. sieve. This was followed by further grinding with a pestle and mortar to yield a fine meal that would pass a 100 mesh sieve. Known weights of this meal were used in assay for reducing sugar, sucrose, glucose and dextrin concentration. The procedure is summarised

in figure 1.

The sample was extracted thrice with 30 ml. volumes of 80% ethanol. Each extraction consisted of 20 minutes mechanical shaking followed by decantation through Whatman's No. 1 filter paper. After the final extraction the residue and filter paper were thoroughly washed with 80% ethanol. The filter paper containing the residue was returned to the ventilated oven at 80°C for a further 24 hours. This sample was used for dextrin analysis.

30 ml. of distilled water was added to the filtrate, which was then concentrated under reduced pressure at 46°C, until the volume had been reduced to approximately 30 ml. This alcohol free extract was then washed into a 50 ml. volumetric flask and made up to volume with distilled water and 0.5 ml. saturated aqueous solution of lead acetate. Lead acetate aids in clarification (Loomis and Shull 1937 and Horwitz 1955). The extract was then filtered through Whatman's No. 1 filter paper onto an excess of potassium oxalate to de-lead the extract. This precipitate was removed by further filtration through Whatman's No. 1 filter paper. No washing was required during the latter two filtrations because the extract was made to standard volume after concentration.

This clear extract was used for the determination of reducing sugar, sucrose and glucose concentration.

(1) Determination of reducing sugar concentration.

Reducing sugar concentration was determined twice for each extract, and the mean of the two values, which in most instances corresponded satisfactorily, was used in the calculation. Depending on concentration, either a 5 ml. or a 2 ml. aliquot made up to 5 ml. with distilled water, was used for reducing sugar determination. The aliquot was added to 1 ml. of Nelson's copper reagent (Nelson 1944 and Bell 1955) and placed in a boiling water bath for 20 minutes. After cooling in cold water, 2 ml. of arseno-molybdate reagent was added, with shaking, and the total volume was made up to 25 ml. with distilled water.

Optical density was determined at 500 mμ. with a Beckman D.B. Spectrophotometer. Reducing sugar concentration was determined by reference to a maltose calibration graph (fig. 2) and the result expressed as a percentage of the dry weight.

(ii) Determination of sucrose concentration.

Sucrose concentration was determined by the increase in the reducing power after hydrolysis with the enzyme invertase (Loomis and Shull 1937, and Horwitz 1955). 20 ml. aliquots of clear extract were hydrolysed for two hours with 4 drops of 1% Invertase in the presense of 2 drops of 10% acetic acid and one drop of methyl red. After dilution to appropriate volume, Nelson's test for reducing sugars was performed on 5 ml. aliquots. The use of controls established that no measurable quantity of reducing sugar was introduced with the invertase. The increase in reducing sugar, as a result of the formation of invert sugar from sucrose, was determined by reference to a glucose calibration curve (fig. 3) and was expressed as a percentage of the dry weight.

(iii) Determination of glucose concentration.

The procedure adopted was a modification of that proposed by Keston (1956), which involved the simultaneous use of the enzymes glucose oxidase and peroxidase coupled with the chromagenic oxygen acceptor - o- dianisidine. Glucose oxidase has a high degree of specificity (Keilin and Hartree 1948 and 1952) but D-fructose may act as a substrate to a small degree (McComb et al 1957).

1 ml. of the clear extract, diluted appropriately to ensure the optical density would be within the range, was added to 0.1 ml. of 0.25% o- dianisidine dihydrocholoride and 6.0 ml. of enzyme preparation (Sigma Technical Bulletin No. 510). Digestion proceded for 30 minutes at 37°C after which tubes were removed from the water bath and optical density was determined at 450 mμ. within 30 minutes. Concentration was determined by reference to a calibration graph (fig. 4) and expressed as percentage dry weight. Standards were run concurrently to ensure accuracy.

(iv) Determination of Dextrin concentration.

Dextrin concentration was determined as increase in reducing power of a 10% ethanol extract after acid hydrolysis (Loomis and Shull, 1937). The residue after extraction with 80% ethanol, and after drying at 80°C for 24 hours (page 13) was removed from the filter paper and weighed. The sample was extracted thrice with 25 ml. samples of 10% ethanol. The extraction consisted of mechanical shaking for 20 minutes, followed by decantation through Whatman's No. 1 filter paper. After the final extraction, the filter paper and residue were thoroughly washed with 10% ethanol and made up to 100 ml. with distilled water. 25 ml. aliquots were hydrolysed with 2 ml. of concentrated hydrocholoric acid in a boiling water bath for 2½ hours, after which the sample was nearly neutralised with sodium hydroxide and made up to 50 ml. with distilled water (Horwitz 1955). The reducing power was determined on 5 ml. aliquots using Nelson's method. Concentration was determined by reference to a glucose calibration graph (fig. 3) and expressed as a percentage dry weight.

ASSAY OF AMYLASE ACTIVITY.

The procedure adopted was a modification of that proposed by Bendelow (1963). Amylase activity was determined as total amylolytic activity (considered to be due to the combined action of alpha and beta-amylase), alpha-amylase activity, and beta-amylase activity by difference. Since increase in reducing sugar concentration as a result of amylase activity was used to determine activity, it was necessary and desirable to determine reducing sugar concentration in the samples. The procedure adopted is summarised in fig. 5.

(i) Preparation of extract.

Germinated grains were ground, with an automatic pestle and mortar, with a little 0.5% aqueous NaCl, to produce a fine paste which was then washed into a volumetric flask (25 or 50 ml. in the case of individual grains and 250 or 500 in the case of

groups of grain). After making to volume with 0.5% NaCl, the brei was allowed to stand at room temperature for twenty minutes. 25 ml. aliquots were centifuged at approximately 2,000 r.p.m. for five minutes, and the supernatant collected for assay. Single treatments were used for the assay of individual grains, but treatments were replicated three times in the assay of groups of grain.

(ii) Determination of reducing sugar concentration.

5 ml. of the supernatant was diluted to 25 ml., in a volumetric flask, with distilled water. A 2 ml. aliquot was added to 2 ml. dinitrosalicylic acid reagent and 1 ml. of 0.1% soluble starch (Bendelow 1963). The tubes were then placed in a boiling water-bath for exactly five minutes, after which they were cooled rapidly. The contents were diluted with 20 ml. of distilled water. Optical density was determined at 505 mμ. against a reference prepared as above except that 2 ml. of distilled water replaced the 2 ml. of extract. By reference to a calibration graph (fig. 6), optical density was converted to milligrams of maltose and expressed as milligrams of maltose per grain.

(iii) Determination of total or alpha and beta-amylase activity.

2 ml. of the diluted extract prepared for the determination of the reducing sugar concentration was placed in a boiling tube and equilibrated at 25°C. for 20 minutes. At time zero, 1 ml. of 0.1% starch (previously attemperated) was rapidly blown into the extract, and the digest shaken for 15 seconds. All pipettes were plugged with cotton wool to avoid contamination by salivary amylase. Starch was added to other digests at ½ minute intervals. After the required digest period, depending on the activity of the extract, the reaction was stopped by the addition of 2 ml. dinitrosalicylic acid reagent. Subsequent treatment was as outlined for reducing sugar determination. The difference between reducing power

of the digest and that obtained in the determination of background reducing sugars, represents that produced as a result of the combined action of all the starch hydrolysing enzymes in the extract. These are, however, principally alpha and beta-amylase (Dure 1960b). This activity was expressed as milligrams maltose per minute per grain, to facilate comparison between groups and individual grains.

As has been indicated in the review of the literature (page 9), it is important to ensure that, in the measurement of amylase activity, substrate does not limit the reaction. The following procedure was adopted to avoid substrate becoming limiting. An active extract was prepared in the normal way for the assay of total amylase activity (fig. 5), and a progress curve of increase in reducing power (milligrams of maltose) against time was constructed (fig. 7). The amount of reducing sugar produced as a result of amylolytic activity, when the velocity (given by the slope) was linear, could be determined. In all subsequent determinations, if the increase in reducing sugar concentration in the digest period was greater than that produced in the same period as established from fig. 7, then the enzyme was diluted and the assay was repeated. In this way, by reference to values obtained when substrate was not limiting, it was possible to ensure that the observed activities, as given in the tables, were not obtained when substrate was limiting. This method is suited to investigations which involve large numbers of determinations, since it is less tedious than that adopted by Dure (1960b) and Drennan and Berrie (1962), who prepared progress curves for each digest.

(iv) Determination of alpha-amylase activity.

Alpha-amylase activity was determined after selective inhibition of beta-amylase by phenyl mercury chloride (Bendelow 1963). In order to check the effect of phenyl mercury chloride on the activity of alpha- and beta-amylase, progress curves of optical density against time were constructed for the following digests:

(a) alpha-amylase in the absence of phenyl mercury chloride (PMC).

(b) alpha-amylase in the presence of PMC.

(c) beta-amylase in the absence of PMC.

(d) beta-amylase in the presence of PMC.

The results (fig. 8) indicate clearly that PMC exerted no noticeable effect on alpha-amylase activity, whereas it causes complete inactivation of beta-amylase activity. Although the alpha-amylase used was not derived from cereals, the result taken in conjunction with the evidence in the literature (Bendelow 1963 and Briggs 1967) implied that the method was satisfactory. Certainly inactivation of beta-amylase, which was obtained from barley, was complete in the presence of PMC.

The procedure outlined by Bendelow (1963) was adopted with some modifications. 5 ml. of the supernatant, after centrifugation was added to 5 ml. of a saturated aqueous solution of PMC and allowed to stand for 20 minutes. The volume was made up to 25 ml. with distilled water, and 2 ml. of the extract was pippetted into a boiling tube and attemperated at 25°C for 20 minutes. The digestion procedure followed has been outlined above. The difference between the reducing potential of the digest and that obtained for the background reducing sugar represents the reducing sugar produced by the action of the amylolytic enzymes other than beta-amylase, principally alpha-amylase. Activity was expressed as milligrams maltose per minute per grain.

(v) Determination of beta-amylase activity.

Beta-amylolytic activity was determined indirectly, as the difference between total amylolytic activity and alpha-amylase activity.

STATISTICAL ANALYSIS.

(i) Analysis of variance.

The procedure for factorial experiments was adopted to compare the variation between individual factors (germination, sample and replication) with error variation (Steel and Torrie 1960). Within-factor variation was assesed by computation of values for 5% least significant difference.

(ii) Regression analysis.

The relationship existing between any two sets of data (e.g. radicle length and reducing sugar concentration) was assesed by the procedure for linear correlation of two variables (Croxton 1959). Although growth follows a sigmoid pattern, non-linear regression was not examined since it was felt that correlation was likely to be more evident during the initial stages of germination and was, therefore, likely to be linear. Scatter plots of the data supported this opinion, but, even in non-linear regression, the curves can, over short distances (i.e. short germination periods), be approximated by straight lines. The procedure of selecting periods over which correlation is to be studied is subjective, and since, 'the presence of correlation between two sets of data does not necessarily mean that causation is present' (Croxton 1959), it can lead to erroneous conclusions. However, provided the correlation can be explained satisfactorily in a physiological sense and in the context of germination it is unlikely to be purely fortuitous.

CHAPTER 3.

RESULTS AND DISCUSSION.

I. CHROMOTOGRAPHIC ANALYSIS.

Results.

Examination of Plate 1 reveals that sucrose, glucose and fructose are the sugars present in the greatest concentration in germinating maize. Ungerminated grains contain marked quantities of sucrose, which decrease initially until the third day, after which the concentration increases steadily with germination period. It is doubtful whether glucose is present to any extent in ungerminated grain but it appears rapidly within the first day. There is some evidence that the concentration may be less on the second and third days. Glucose level increases steadily after the second day. Fructose is apparently absent in the dry grain. Faint spots are visible on the first and second days and concentration increases with germination period. Maltose spots are faint and only appear on the third or fourth day after which there is a steady increase. Maltose is, however, apparently consistently at a lower level than glucose. These results are summarised in Table 1.

Chromatographic separation using ethyl acetate, isopropyl alcohol, water (6:1:3) showed that maltotetraose did not appear until the fourth day and, by the eighth day, was still at a low level (Plate 2). Maltotriose was apparently absent. An unidentified compound with a low Rf. value was present in the ungerminated grain and increased slightly during germination.

Discussion and Conclusions.

The decrease in sucrose concentration during the first three days of germination is significant and supports the observations of Bond and Glass (1963). Since amylolytic activity has been shown to be low during the first three days of germination (Dure 1960b) it seems that this sugar might be

an important metabolite during early growth of the embryo, as has been suggested in other cereals (MacLeod et al 1951-1960 and Drennan 1962).

The marked increase in glucose within the first day, when amylolytic activity is low (Dure 1960b) suggests that it may arise as a result of sucrose hydrolysis. Indeed, if this is so, then quantitative assays should show a negative correlation between sucrose and reducing sugar or glucose concentration during the initial germination period. The fructose present within the first one or two days may also represent one of the products of sucrose hydrolysis. It is interesting that Hellebust and Forward (1962) have demonstrated the importance of invertase activity in growing radicles of corn.

The appearance of maltose after the third or fourth day suggests that amylolytic activity is low during the initial stages of germination. It is interesting that maltose appears to be present at consistently lower levels than glucose, supporting the contention of Bond and Glass (1963) who suggested that it might be more realistic to express reducing sugar in glucose units rather than maltose units when considering maize.

In view of these results, it was decided to investigate changes in sucrose and reducing sugar concentration. Dextrin concentration was investigated during the work on Hickory King but was subsequently abandoned in favour of determining both reducing sugar and glucose.

II. CHANGES IN CARBOHYDRATE CONCENTRATION.

A. HICKORY KING.

1. Reducing sugar concentration.

(i) Groups of grain.

Three ten-grain samples were investigated after each 24 hour interval, over a period of 240 hours. The quantitative changes occuring in reducing sugar, during germination, are given in Table 2 and the analysis of variance in Table 3a.

Ungerminated grains contain little reducing sugar, approximately 0.06% of their dry weight. During the first 48 hours of germination, the concentration increases significantly to about 0.246% of the dry weight but decreases markedly between 48 and 72 hours. This decrease is observed in all the samples and corresponds with the visible appearance of growth (table 4, fig. 9). Increasing the germination period after 96 hours results in a marked increase in the level of reducing sugar in the grains but the rate of accumulation decreases slightly after 144 hours. The concentration of reducing sugar increases approximately 22 times (although in one sample there was a forty-two-fold increase) during the 240 hour germination period. The effect of germination period on level of reducing sugar is, therefore, highly significant (tables 2 and 3a). This implies that the variability is such that the means derived from the assay of ten-grain samples, taken at random, are not necessarily representative of the population as a whole. However, the germination period means (table 2) are derived from the observations on three samples, each of ten grains and therefore the general trend in reducing concentration outlined should approach that for the population. It is noteworthy that variability in concentration of reducing sugar is not related to the fresh weight of the grain (table 5, fig. 10). There is a significant interaction between sample number and germination period (table 3a). This indicates that the response of reducing sugar concentration to germination period is not the same for each sample, i.e. the trend in level of reducing sugar over the 240 hour germination period is different in each sample. This is, perhaps, due in part, to the significant variation between samples receiving the same treatment. Especially so, since the means within each of the first two germination periods i.e. 0 and 24 hours, are not very different but, as the germination time increases, the variability between samples receiving the same treatment increases.

Variation between the means for replication, which provides a measure of the reliability of the assay procedure, was not significant. The evidence suggests, therefore, that the techniques can be applied to the assay of single grains, without replication

but with some degree of confidence.

Growth of radicle, coleoptile and lateral roots are closely correlated with the level of reducing sugar, especially during the initial 178 hours of germination (table 3b, figs. 11, 12 and 13). In general, low levels of reducing sugar are associated with least growth. As the concentration of reducing sugar increases in the grain, there is an increase in the development of the shoot and root system.

(ii) Individual grains.

Ten grains were assayed for reducing sugar concentrations at 24 hour intervals during germination period of 240 hours. Concentrations, expressed as percentage of the dry weight, are presented in table 6. Increasing the period of germination exerts a significant effect on the level of reducing sugar in the grain (table 7a). Reducing sugar concentration is low (approximately 0.05% of the dry weight) in ungerminated grain, with the range being between 0.013% and 0.143%. There is, therefore a significant variation between the concentration in individual grains. This variation is not related to the fresh weight of the grain (table 8, fig. 15).

Although the general trend is an increase in reducing sugar concentration during the initial 48 hours, the increase is not significant. After 48 hours, there is a rapid accumulation of reducing sugar. However, in general, the rate of accumulation of reducing sugar decreases after 144 hours (fig. 14). The overall increase in concentration is of the order 25-fold although one grain contained 5.46% reducing sugar, indicating that the concentration had increased over 100 times, assuming the original concentration to be similar to that of the mean for ungerminated grains. As germination proceeds, the variability between grains receiving the same treatment increases. So great is the variability that it is doubtful whether random ten-grain samples can be considered representative. However, the variability is important in investigations of the relationship of physiological activities to development of the shoot and root system.

The lengths of radicles, coleoptiles and lateral roots are shown in table 9. During the initial 100 hours of germination, low levels of reducing sugar are, in general, associated with least growth. As the level of reducing sugar in the grain increases during germination, extension of the radicle, coleoptile and lateral roots occurs (table 7b, figs. 16, 17, and 18). Radicle and coleoptile length do not show much dependence on reducing sugar concentration after 168 hours. However, the rate of growth of these organs decreases after 168 hours. Growth of lateral roots shows some correlation with reducing sugar between 168 and 240 hours but the correlation coefficient (+0.653) is not high enough to be considered conclusive (table 7b).

2. Sucrose Concentration.

(i) Groups of grain.

The results of analyses for sucrose concentration are shown in Table 10. Sucrose comprises approximately 1% of the dry weight of ungerminated grain. Concentration decreases, significantly, to about 0.2% of the dry weight during the initial 96 hours germination. With further increases in the germination period up to 192 hours, sucrose accumulates in the grain but the concentration never attains the level originally present. After 192 hours, there is evidence of a significant decrease in sucrose concentration (table 10). The general trend in sucrose concentration is summarised in fig. 9.

The means for ~~each~~ samples receiving the same treatment are frequently significantly different, the variation becoming increasingly apparent with greater germination time. This variability between the means of similar treatment is significant (table 11a). A random sample of ten grains, where each individual is extremely variable is, therefore, not necessarily representative. The significant interaction between sample and germination period may be a function of the variability of the individual samples which would result in different response curves for each sample (tables 10 and 11a).

There is no significant difference between the means for replication, thus the procedure adopted in the assay of sucrose concentration is considered satisfactory.

Considering the germination period as a whole, there is no evidence of linear correlation of sucrose concentration and length of radicle, coleoptile or lateral roots (table 11b, figs. 19, 20 and 21). However, radicle length, in particular, is negatively correlated with sucrose concentration during the first 96 hours. This correlation is even more marked between 48 and 96 hours, possibly because there is no measurable growth before 48 hours (Table 4). Thus, during the initial period of germination, high levels of sucrose are associated with short radicles. As sucrose concentration decreases, extension of the radicle takes place (fig. 20). It is noteworthy that although during the first 96 hours correlation of sucrose concentration and length of coleoptile and lateral roots is low, (table 11b) it is none-the-less negative, which suggests that growth, in general, is dependent on a decrease in the concentration of sucrose. There is no evidence that sucrose level and growth are correlated when the germination period is increased beyond 96 hours. Thus, for the development of the shoot and root system, the level of sucrose is mainly of importance during the initial stages of germination.

During the first 96 hours germination, sucrose concentration is negatively correlated with reducing sugar concentration (table 12a, fig. 22). High levels of sucrose are associated with low levels of reducing sugar, and as sucrose concentration decreases, the level of reducing sugar increases. There is no evidence that with germination periods greater than 96 hours, the concentrations of sucrose and reducing sugar are related.

(ii) Individual grains.

Sucrose concentrations in individual grains are given in table 13. The concentration of sucrose in ungerminated grains is high, constituting approximately 1% of the dry weight. However, there is considerable variability, and the grain having the highest level of sucrose contained almost twice as much sucrose as that with the lowest concentration. The level of sucrose decreases

markedly during the first 72 hours, after which there is a steady accumulation of sucrose in the seedling. However, during the 240 hour period investigated sucrose never again attained the level originally present in the fruit (table 13, fig. 14). Variability in physiological behaviour is such however, that some grains (grain 1 after 240 hours, table 13) may accumulate sucrose to the extent of 2.9% of their dry weight. The eleven-fold variation between highest and lowest sucrose concentration after 240 hours germination, implies that the mean drived from ten individual assays on single grains is not necessarily representative of the population. The interpretation of trends is therefore tentative. The rate of accumulation of sucrose, indicated by the slope (fig. 14), decreases slightly towards the end of the germination period. The effect of germination period on the level of sucrose is highly significant (table 14a).

Sucrose concentration does not show any marked correlation with the development of the shoot and root system (table 14b, figs. 23,24 and 25). The highest degree of correlation was shown to be between sucrose level and radicle length, during the initial period of germination. This correlation (coefficient of -0.463) could however only explain approximately 46% of the variation between sucrose level and radicle length. It is noteworthy, however, that irrespective of the very low levels of correlation, the level of sucrose is always negatively related to the length of the organs measured (table 14b). In spite of the considerable variability, there is a tendency for high levels of sucrose to be associated with least growth, and decreased sucrose concentration to be associated with extension of the shoot and root system.

During the initial 96 hours of germination, sucrose and reducing sugar concentrations are negatively related. Low levels of reducing sugar tending to be associated with high levels of sucrose. As the concentration of sucrose decreases during the first 96 hours there is an increase in the concentration of reducing sugar (fig. 26, table 12b). However the degree of correlation is low and only half the variability can be ascribed to these two variables. There is a marked correlation between sucrose concentration and level of reducing sugar after 96 hours,

indicating that the accumulation of sucrose is related to an increase in the concentration of reducing sugar.

3. Dextrin concentration.

(i) Groups of grain.

The results of the dextrin analyses are given in table 15. Germination period exerts a significant effect on the concentration of dextrin in the grain (table 16a). Dextrin constitutes a small fraction (approximately 0.15%) of the dry weight of ungerminated grain. During the first 72 hours of germination there is a significant accumulation of dextrin. The notable decrease in concentration of dextrin between 72 and 120 hours corresponds with the appearance of measurable growth (table 4, 15, fig. 9). Further increase in the germination period to 192 hours results in an increase in the level of dextrin. The concentration of dextrin decreases after 192 hours.

Although samples receiving the same treatment are, in general, not significantly different, there is a highly significant interaction between sample and germination period (table 16a). The trend in dextrin concentration is not the same in the three samples. There is, however, no significant difference between the means of replicates.

There is no evidence of linear regression between dextrin concentration and the development of the shoot and root system (table 16b). Analysis of regression during the period of maximum extension, i.e. 48 - 168 hours in the case of the radicle and 72 - 168 hours for the coleoptile and lateral roots does not reveal a high degree of correlation.

(ii) Individual grains.

The results of analyses of single grains are presented in table 17. Although germination period exerts a significant effect on the level of dextrin in the grain (table 18), it is impossible from a study of the means, to detect any general trend. However, the lowest concentrations of dextrin were observed after 48 hours (0.053%) and 144 hours (0.275), which do not correspond

with the periods showing least dextrin in the analysis of groups of grain.

There is no evidence of linear correlation between dextrin concentration and the development of the shoot and root system (table 19).

4. Discussion of results, and conclusions.

It is important, at the outset, to justify the use of single assays, i.e. assays without replication, in the investigation of carbohydrate content of single grains under varying germination conditions, since, without such justification, the results of the study of single grains could not be considered reliable.

The reliability of the assay techniques may be established by replicate assay of small quantities of meal, approximately equal to the weight of meal from one grain, derived from the same sample. Whilst significant differences between replicates do occur (tables 2, 10 and 15), the variation between the means for replication is not significant (tables 3a, 11a and 16a). There is therefore, conformity between replicates, in spite of the assay of samples having widely different carbohydrate levels. It is interesting to compare the differences between replicates with the differences between observations on individual grains receiving the same treatment. The variation in concentration is considerably greater between single grains than between replicates. For example, replicates which are chosen because of their large variation in reducing sugar concentration (table 2, sample 2 after 168 hours germination) have a difference in reducing sugar concentration of 0.275%, whereas, in the same germination period, the maximum difference between concentrations in individual grains was 2.098% (table 6). Thus although the difference between any two replications may, occasionally, be significant, the magnitude of the variation is a fraction of that apparent between individual grains receiving the same treatment.

The variation in concentration of various carbohydrates studied in individual grains can, therefore, be confidently ascribed to differences in their physiological behaviour during germination, and not to chance variation inherent in the techniques adopted.

The trend in reducing sugar concentration, elucidated by the investigation of individual grains, is similar to that demonstrated by the analysis of groups of grain (figs. 9 and 14). The trend, a low level of reducing sugar in ungerminated grains, with a gradual increase during the initial period (0 - 72 hours), followed by a phase of rapid accumulation, is in general agreement with the literature. Similarly the trend in sucrose concentration during germination does not conflict with the observations of other workers (Toole 1924, Lampe 1931, Malhotra 1934, Taufel et al 1960 and Bond and Glass 1963). The concentration of sucrose decreases markedly during the first 96 hours of germination, after which there is an accumulation. The observations on individual and groups of grains conform satisfactorily.

There has been little investigation into the changes that occur in the concentration of dextrin during germination. Indeed, as far as I am aware, no quantative assay has been made in maize. Whilst the assay procedure in the present work yielded reproduceable results the variation in behaviour of grains receiving different germination treatments are such that on inspection of the data for individual grains (table 17) it is impossible to formulate a general trend. Similarly the trend observed in the data derived from the analysis of groups of grain, is difficult to explain physiologically. Perhaps the results should be regarded with suspicion, because of the danger of assessing normal variation as being the result of changed germination period. However, since more than one germination period is associated with each increase or decrease in dextrin concentration (fig. 9) the results may be discussed tentatively in relation to the germination process.

Changes in carbohydrate concentration during germination are related to the establishment of the young plant. Consequently trends referred to above should be discussed in the general context of growth of the embryo.

Dure (1960a) reported that growth of isolated embryos is stimulated, in particular, by the presence of glucose in the nutrient medium. It would seem reasonable therefore to expect germination to be dependent, to some extent, on the availability of reducing sugars, especially so in cereals, where the endosperm is comprised

principally of starch. Indeed, Toole (1924) demonstrated that elongation of the coleorhiza was accompanied by the appearance of reducing sugar.

Reducing sugar is only present in low concentration in ungerminated grain, and cannot, therefore, be considered as a carbohydrate reserve of any significance. Since amylolytic activity is low during the first three days of germination (Dure 1960b), it is unlikely that reducing sugar production as a result of amylolytic activity in the endosperm is important during early germination. Furthermore, Dure (1960a) has clearly shown that initial development must depend on scutellar reserves, because embryos, isolated from the endosperm and grown on non-nutrient medium, do not differ significantly from intact grains. Thus there are two phases in germination: the initial growth period when the degradation products of the endosperm are not available and the period after three or four days when sugars are formed rapidly as a result of mobilisation of the endosperm.

The major reserves in the scutellum are fats and sucrose. There is no doubt that fat is an important respiratory substrate during early growth (Dure 1960a), but the role of sucrose has not been clearly established. However its presence in the embryo (Toole 1924 and Lampe 1931), together with its importance in the extension of corn radicles (Hellebust and Forward 1963) suggest that it is an important metabolite during early germination.

Sucrose yields, on hydrolysis, equal quantities of the two reducing sugars glucose and fructose. The increase in reducing sugars observed during the initial periods of germination (while growth is still dependent on scutellar reserves) could be the result of sucrose hydrolysis, and, since glucose stimulates growth, this raising of the level of glucose (and fructose) may be responsible for the extension of the radicle i.e. the reducing sugar necessary for the initial growth of the embryo is derived, principally, from the sucrose present in the scutellum.

Prerequisites of such an hypothesis are that:

(i) there should be a positive correlation between growth and concentration of reducing sugar,

(ii) there should be a negative correlation between sucrose and reducing sugar concentration and,

(iii) sucrose should, indirectly, be negatively correlated with growth.

The results give clear evidence that growth of the radicle, coleoptile and lateral roots are closely correlated with the level of reducing sugar (tables 3b and 7b, figs. 11, 12, 13, 16, 17 and 18). That this correlation is especially marked during the initial 168 hours is not surprising, since, during this period, it is probable that low levels of reducing sugar limit the general metabolism. This is likely to continue during the initial stages of endosperm mobilisation. However, during the later stages of germination, the degree of correlation is less. This may be attributed to the decreased growth rate so that reducing sugar accumulates. The lag between accumulation of reducing sugars and increased growth suggests that the increased level of reducing sugar stimulated the growth of the embryo (figs. 9 and 14). The notable decrease in reducing sugar concentration after 48 hours (fig. 9), at the time of first measurable growth, suggests that the growth stimulated by the initial increase in reducing sugar concentration, utilises the reducing sugar more rapidly than it is produced. If during this stage reducing sugar formation is from sucrose, then the rate of formation would be relatively low because of the low sucrose level.

Although, during early germination, a negative correlation between sucrose and reducing sugar concentration is demonstrated (tables 12a and 12b), it is important to note that not all the reducing sugar is derived from sucrose, since,

(i) reducing sugar is present in ungerminated grain,

(ii) the trisaccharide raffinose, which is present in ungerminated grain, dissappears during the first three days with the formation of sucrose and melibiose (Bond and Glass 1963) and,

(iii) there is some amylolytic activity during early germination (Dure 1960b).

The correlation (-0.772) obtained with groups of grain can, therefore, be considered fairly conclusive evidence that the major part of the reducing sugar present during early germination is derived from sucrose. The correlation demonstrated in the studies on individual grains (-0.519), although less convincing, supports the general hypothesis. Further evidence is the marked increase in glucose and fructose demonstrated chromatographically and also by Bond and Glass (1963). The low level of maltose present during early germination implies that the source of reducing sugar is not starch (plate 1).

Since the relationship between sucrose concentration and growth is likely to be indirect, and because not all the reducing sugar is derived from sucrose, a high degree of correlation would not necessarily be expected. Indeed the correlation coefficient -0.846 (table 11a) for correlation of sucrose and radicle length during the first 96 hours germination is surprisingly high. The lower degrees of correlation demonstrated between sucrose and coleoptile and between sucrose and lateral roots may be due to the fact that these organs develop later, possibly when other sources of reducing sugar are becoming available. The data for individual grains show poor correlation but, significantly, the relationship is always negative (table 14b) i.e. maximal growth is associated with lowest sucrose concentration. There is, therefore, sufficient evidence to suggest that growth, of the radicle in particular, is dependent on the hydrolysis of sucrose with concomitant reducing sugar formation.

It is interesting that in the data derived from groups of grain, no correlation between sucrose and reducing sugar concentration was demonstrated after 96 hours (table 12a). The results of analyses of individual grains, however, reveal a positive correlation (+0.752, table 12b). Although it seems likely that the level of reducing sugar could influence the synthesis of sucrose, the absence of the linear regression in the group data makes the observation inconclusive.

Alpha-amylase is a dextrinising enzyme and it was thought that changes in the level of dextrin could be of some importance during germination. Whilst this is probably true, the observations are not readily explained with the information available.

Amylolytic activity is low during the early stages of germination (Dure 1960b) and, therefore, are probably not responsible for the apparent increase in dextrin concentration during the first 72 hours (fig. 9). Lampe (1931) reports the presence of 'globules of liquid dextrin' in developing maize kernels, and it is possible that with the onset of germination these dextrins become more easily extracted. If this is so then they may act as the first substrate on which the amylases act, thereby resulting in a decrease in dextrin concentration.

High levels of amylase activity, in particular alpha-amylase activity, could be responsible for the increase in level of dextrin observed between 120 and 168 hours. Amylase activity tends to decrease after the eighth day (Dure 1960b), a feature which would result in decreased dextrin concentration.

In view of the discrepancy between the data derived from the investigation of groups and that from individual grains, and because of the apparent importance of the sucrose reducing sugar relationship, it was decided that in subsequent work it might be more meaningful to investigate changes in glucose rather than in dextrin concentration.

Dure (1960b), because of the marked variability between grains receiving the same treatment, selected grains having the same 'physiological age' in order to replicate treatments. This study of individual grains emphasises the differences in the physiology of germination between individual grains, of the same population, receiving the same treatment. During the early periods of germination the variation is, to a large extent, an expression of the concentration and mobilisation of sucrose. It is however, impossible at this stage, to conclude that grains originally having a high concentration of sucrose in the scutellum germinate more vigorously than those with a low level. This is certainly an interesting problem awaiting investigation. After the initial growth, variability is probably a result of the degree of initial development, since this affects water and salt uptake, and of the differences in the mobilisation of the endosperm, as this represents the source of reducing sugars during later germination.

From this study the following conclusions may be drawn:

(i) Growth of the embryo is dependent on the availability of reducing sugars, both during the initial stages when development is dependent on scutellar reserves, and after the mobilisation of the endosperm has started.

(ii) Sucrose is an important metabolite during the early stages of germination, since it is mainly responsible for the production of reducing sugars during this period.

(iii) After mobilisation of the endosperm has begun, sucrose ceases to be of any importance in the subsequent growth of the seedling.

(iv) Variability in the germination and development of the seedling is to some extent determined initially by the mobilisation of sucrose and later by mobilisation of the endosperm.

(v) Dextrin, as such, is of relatively minor significance to the growth and development of the young plant.

B. EARLY PEARL.

1. Reducing sugar concentration.

(i) Groups of grain.

Ungerminated grains contain little reducing sugar, about 0.002% of their dry weight (table 20). The concentration increases markedly during the first 48-72 hours, reaching a level of approximately 0.68% of the dry weight. Between 72 and 96 hours there is a significant decrease in the level of reducing sugar which is evident in all three samples and corresponds with a notable increase in growth rate, as shown by the slope of the growth curve (fig. 28). Increasing the germination period after 96 hours results in a very marked increase in reducing sugar concentration. However,

the rate of accumulation of reducing sugar decreases after 168 hours. The final concentration of reducing sugar is some 3,000 times that originally present. There is evidence that in some samples the level of reducing sugar decreases significantly towards the end of the germination period. The effect of germination period on reducing sugar concentration is highly significant (table 21a).

During the early germination periods (0-24 hours) the means for groups receiving the same treatment are not significantly different (table 20). However, with increased germination period, the levels of reducing sugar in different groups are frequently significantly different i.e. The between sample variability increases during germination and the variation between the sample means is significant (table 21a). This variability between samples is, perhaps, responsible for the significant interaction between samples and germination period, because the response of level of reducing sugar to changes in the germination period is not the same for each sample. Although individual ten-grain samples cannot be considered representative, the general trend demonstrated by the germination period means, derived from observations on three groups of ten grains, should approach that for the population as a whole.

The between means variation for replication, which tests the variability due to assay procedure, is not significant (table 21a). Differences between individual replicates are, however, occasionally significant (table 20). Since the weight of meal extracted in each replicate was approximately equal to that obtained from a single grain, the techniques could be applied to the assay of single grains, without replication but with some confidence.

Growth of radicle, coleoptile and lateral roots (table 22) are closely correlated with the concentration of reducing sugar in the grain. Raising the level of reducing sugar results in increased growth (table 21b, figs. 29,30 and 31).

(ii) Individual grains.

The quantitative changes occuring in reducing sugar during germination are given in table 23. Increasing the germination period exerts a significant effect on the concentration of reducing sugar in the grain (table 24a). The level is low initially, and

none of the ten grains assayed contained a measureable quantity. Reducing sugar concentration increases during the first 144 hours of germination, in particular between 96 and 144 hours where the average concentration increases from 1.062 - 7.653% (dry weight). Although the level increases steadily between 0 and 96 hours, the level attained is only approximately 1%. There is, however, considerable variability; after 24 hours, the concentration varies between 0.006% and 0.340% and the maximum variation observed was 1.625% to 15.810% after 168 hours germination (table 23). This variability between grains of a certified variety is so great that it is not surprising that 10 grain samples are not necessarily representative (table 21a).

The general trend, a slow increase in reducing sugar concentration during the first 96 hours, followed by a marked increase after 96 hours, is similar to that obtained in the assay of 10-grain samples (figs. 28 and 32). However, there is evidence that reducing sugar concentration decreases significantly after 192 hours (table 23).

The lengths of radicle , coleoptile and roots are shown in table 25. Growth and reducing sugar concentration are not linearly related over the whole germination period (table 24b, figs. 33,34 and 35). However, during the first 96 hours, low levels of reducing sugar are, in general, associated with least growth of radicle and coleoptile. As the level of reducing sugar in the grain increases during germination, extension of the radicle and coleoptile occurs. The decrease in accumulation of reducing sugar during the latter germination periods is probably partly responsible for the independence of growth and reducing sugar concentration after 96 hours. It may also be partly due to the very high levels of reducing sugar which consequently do not limit growth since the concentration exceeds requirements.

2. Glucose concentration.

(i) Groups of Grain.

Glucose constitutes approximately 0.1% of the dry weight of ungerminated grains (table 26). The concentration increases significantly during the first 96 hours and with further increase in the germination period up to 192 hours, the rate of accumulation

is very marked. After 192 hours, there is evidence that the concentration may decrease (fig. 28).

The means for samples receiving the same treatment are significantly different, the variation being increasingly evident with longer germination period (table 26). The variability is such that the between sample variance is significant (table 27a) and consequently, a random ten-grain sample is not necessarily representative. Such marked variability results in different response curves of reducing sugar during germination for different samples and is, therefore, probably responsible for the significant sample/germination period interaction (table 27a).

Occasionally replicates may be significantly different (e.g. table 26, sample 3 after 144 hours germination). However, the variance between replicate means is not significant (table 27a). Therefore, single assays can be regarded as yielding reliable results.

Linear correlation between glucose concentration and growth of the shoot and root system is very marked over the 240 hour germination period investigated (table 27b). High levels of glucose are associated with long radicles, coleoptiles and lateral roots (figs. 36,37 and 38). Extension of the shoot and root system follows increased glucose concentration in the grain.

The marked linear correlation between reducing sugar and glucose concentration (table 27a, fig. 39) implies that glucose is the principal reducing sugar in the grain during germination and also, indirectly, that both assay techniques are reliable since they yield similar results.

(ii) Individual grains.

Glucose concentrations, expressed as a percentage of the dry weight are presented in table 28. The effect of increasing germination period on the accumulation of glucose in the grain is highly significant (table 29a). The concentration is low in ungerminated grains, constituting approximately 0.013% of the dry weight, although in some grains the level is too low to permit estimation with the present technique. The level increases steadily until 96 hours, after which glucose accumulates markedly

attaining a level of approximately 5% after 168 hours. The level decreases significantly after 192 hours. The general trend is, therefore, a slow initial increase, followed by a period of rapid accumulation and then, finally, by a phase during which glucose utilisation exceeds production (fig. 32). This trend is very similar to that observed in groups of grain (fig. 28).

There is considerable variability between grains receiving the same treatment, especially during the higher germination periods.

Growth does not appear to be markedly correlated with level of glucose during germination. The highest linear correlation, which only explains approximately 60% of the variation, was observed during the initial 192 hours of germination (table 29b, figs. 40, 41 and 42). In general, greater growth is associated with higher level of glucose in the grain.

Glucose concentration shows a marked positive linear correlation with level of reducing sugar (table 29b, fig. 43). The very significant interdependence of glucose and reducing sugar over the whole germination period suggests that glucose comprises the major part of the reducing sugar fraction and also that the assay procedures for both total reducing sugars and glucose are reliable.

3. Sucrose concentration.

(i) Groups of grain.

The concentration of sucrose in groups of grain during germination is given in table 30. Sucrose comprises approximately 0.5% of the dry weight of ungerminated grains. The concentration decreases significantly to about 0.03% of the dry weight during the first 96 hours of germination. Increasing the germination period after 96 hours results in a marked accumulation of sucrose in the grain. The rate of accumulation decreases after 192 hours and there is some evidence that the concentration may decrease after 216 hours. (fig. 28).

Generally the means for samples subjected to the same germination treatment are significantly different (table 30) and the overall between sample variance is significant (table 31a). This suggests

that ten-grain samples are not large enough to average the variability between grains. The highly significant interaction between sample and germination period may be a function of the variability of individual samples, which results in different response curves (tables 30 and 31a).

Replicates are occasionally significantly different (table 30, sample 1, ungerminated), but the overall between mean variance for replication is not significant (table 31a). The variability between replicates is a small fraction of that between samples receiving the same treatment and, therefore, the procedure adopted was considered satisfactory.

There is no evidence of linear regression between sucrose concentration and development of the shoot and root system over the 240 hour germination period studied (table 31b). However, radicle and coleoptile in particular, show marked negative correlation with sucrose concentration during the first 96 hours. Thus, during this period, least growth of radicle and coleoptile is associated with high levels of sucrose. Decrease in sucrose concentration results in increased development of the radicle and coleoptile (table 31b, figs. 44 and 45). Although length of lateral roots and sucrose concentration are negatively correlated during the first 96 hours, the degree of correlation is not high (table 31b, fig. 46). This may be because the lateral roots develop after the radicle and coleoptile and are, therefore, not dependent solely on the products of sucrose hydrolysis (table 22). Growth after 96 hours does not appear to be dependent on sucrose concentration (table 31b).

During the first 96 hours of germination, sucrose concentration is negatively correlated with level of reducing sugar. High levels of sucrose are, in general, associated with low concentrations of reducing sugar and as sucrose concentration decreases, the reducing sugar increases. There is no evidence that sucrose and reducing sugar are positively correlated when the germination period is greater than 96 hours (table 32a, fig. 47).

A marked negative linear regression is evident between sucrose and glucose concentrations during the initial 96 hours germination. Low levels of glucose tend to be associated with high levels of sucrose. As sucrose is hydrolysed there is a corresponding increase in glucose concentration. No linear relationship is evident between

glucose and sucrose when the germination period is increased after 96 hours (table 32a, fig.48).

(ii) Individual grains.

The concentration of sucrose in ungerminated grains is fairly high, constituting approximately 0.4% of the dry weight. There is a marked increase during the first 24 hours, which is followed by a steady decrease until 96 hours. Increasing the germination period further, results in a rapid accumulation of sucrose in the grain. Sucrose concentration decreases significantly after 144 hours, but then remains fairly constant at about 0.85% of the dry weight (table 33, fig. 32). The overall effect of germination period on the level of sucrose is significant (table 34a).

There is considerable variability between grains receiving the same treatment. This is especially evident when the germination period exceeds 120 hours, or when the sucrose concentration is high. Some grains may contain as much as seven times more sucrose than others subjected to the same germination conditions (e.g. grains 7 and 10 after 168 hours germination, table 33).

Sucrose concentration does not show any marked degree of linear regression with growth of the shoot and root system (table 34b, figs. 49, 50 and 51). Highest correlation was demonstrated between sucrose and radicle length, and between sucrose and coleoptile length, during the initial 96 hours of germination. However, the correlation coefficients are low and only 50% of the observed variability could be explained. None the less it is significant that the level of sucrose is always negatively related to the length of the organs measured. There is therefore, a tendency for high levels of sucrose to be associated with least growth and decreased sucrose concentration to be associated with extension of the shoot and root sytem.

Sucrose and reducing sugar concentrations do not appear to be markedly correlated (table 32b, fig. 52). The overall positive regression (0-240 hours) is to be regarded with suspicion in view of the negative correlation during the early germination period 0-96 hours). Although the latter correlation is low (-0.517) it

suggests that low levels of sucrose may tend to be associated with high levels of reducing sugar.

There is no evidence of linear regression between sucrose and glucose concentration (table 32b, fig. 53). Correlation during the first 96 hours, although low, is negative.

4. Discussion and conclusions.

The reliability of the assay procedures adopted for the determination of total reducing sugar and sucrose has been established (page 28). The results of the work on Early Pearl support the contention that the assay procedures yield reliable results even though only single assays were made in the investigation of single grains. In no instance was the variance between replicate means significant (tables 21a and 31a), and although replicates were occasionally significantly different, the difference is a fraction of the variation between grains receiving the same treatment. Thus, for example, the largest difference between replicates of sample 1 after 168 hours germination was 0.326% (table 20) whereas individual grains, after 168 hours, had a maximum variability of 14.185% (table 23). There is, therefore, satisfactory conformity between replicates, in spite of assaying samples with widely differing concentrations.

The largest difference between replicates during the assay of glucose is 0.426% (table 26, sample 1 after 192 hours germination), whereas the maximum variation between individual grains after 192 hours was 7.290% (table 28). Error in the assay procedure cannot account for the large variation observed between individual grains. The variations are therefore a reflection of real differences between individual grains in their physiological behaviour during germination.

The general trend in reducing sugar concentration, is low level in ungerminated grains with a gradual increase during the initial 96 hours, followed by a phase of rapid accumulation, is similar to that observed in Hickory King grain. Similarly the initial high level of sucrose, with a marked decrease during the first 96 hours, after which sucrose accumulates in the grain, is similar to that

observed in Hickory King (figs. 9, 14, 28 and 32). These trends are in general agreement with the literature (Toole 1924, Lampe 1931, Malhotra 1934, Taufel et al 1960 and Bond and Glass 1963).

Trends in glucose concentration are not very different from those observed in reducing sugar data. During the initial 96 hours accumulation is slow but after 96 hours glucose concentration increases rapidly, attaining a maximum after 192 hours. This trend is similar to that reported by Taufel et al (1960) and Bond and Glass (1963).

The results must be considered in the general context of germination and more specifically in relation to the conclusions reached as a result of the work on Hickory King grains (page 34). Since analyses of glucose concentration have been made, it is important to restate the prerequisites of the hypothesis (formulated on page 30) which relates to the significance of sucrose and reducing sugar as metabolites during early germination. These are that:

(i) there should be a positive correlation between growth and concentration of reducing sugar,

(ii) there should be a positive correlation between growth and concentration of glucose,

(iii) there should be a negative correlation between sucrose and reducing sugar concentration,

(iv) there should be a negative correlation between sucrose and glucose concentrations,

(v) sucrose should, indirectly, be negatively correlated with growth,

(vi) glucose and reducing sugar should be positively correlated.

In the data pertaining to groups of grain there is very clear evidence that growth of the shoot and root system is correlated with the concentration of reducing sugar (table 21b, figs. 29, 30 and 31). This marked positive correlation is evident over the whole 240 hour germination period, which suggests that growth is stimulated by increased reducing sugar concentration. In investigations on single

grains correlation was only demonstrated during the initial 96 hour germination period (table 24b, figs. 33, 34 and 35). This is not surprising, since, during the initial 96 hours when the level of reducing sugar is low, it is likely that the development of the radicle and coleoptile is limited by the available reducing sugar. During the later stages of germination, after mobilisation of the endosperm has started, reducing sugar production probably exceeds utilisation. In the data for groups of grain there is a marked increase in the rate of accumulation of reducing sugar between 48 and 72 hours, and a sharp decrease in concentration between 72 and 96 hours (fig. 28). This is not evident in the data for individual grains, although there is some evidence that the rate of accumulation decreases slightly between 48 and 96 hours (fig. 32). This trend is followed, after a short lag, by the appearance of measureable growth. It seems likely, therefore, that the growth stimulated by the initial increase in reducing sugar concentration utilises the available reducing sugar as rapidly, or more so, than it is produced. This is not surprising, since, after 48 hours, the concentration of sucrose has decreased significantly (tables 30 and 31) and, therefore, reducing sugar production, as a result of sucrose hydrolysis, would undoubtedly be reduced. These results, a positive correlation between growth and reducing sugar concentration, fulfil the first prerequisite of the hypothesis (page 42).

There is a marked linear regression between glucose concentration and the development of the shoot and root system (table 27b, figs. 36, 37 and 38). Thus high levels of glucose apparently stimulate growth of radicle, coleoptile and lateral roots Correlation between glucose and growth is low in the data derived from the study of individual grains (table 29b, figs. 40, 41 and 42), maximum correlation being evident during the first 192 hours of germination. However, since growth is unlikely to be dependent solely on the level of glucose, because other sugars e.g. fructose undoubtedly also enter into the general metabolism involved in growth, this relatively low degree of correlation is not surprising. Indeed the correlation is high enough to support the observation of Dure (1960a) that glucose is an important reducing sugar during early germination, either because of its high concentration relative to other reducing sugars

(Bond and Glass 1963) or because it is more readily metabolised. The second requirement of the hypothesis (page 42) is, therefore, satisfied.

There is some discrepancy between the concentrations of reducing sugar and glucose. Since glucose is not the only reducing sugar present in germinating grains (Taufel et al 1960 and Bond and Glass 1963), the concentration of the reducing sugar should always be equal to, or exceed that for glucose. The discrepancy is mainly the result of expressing the reducing fraction in terms of maltose. This is aggravated by the greater sensitivity of the glucose assay procedure, evidenced by the inability of the reducing sugar assay procedure to detect reducing sugar in some grains where glucose was shown to be present (tables 23 and 28). In groups of grain there is more reducing sugar than glucose between 24 and 72 hours germination (fig. 28). This is probably mainly the result of formation of fructose and glucose during sucrose hydrolysis. Generally after 96 hours the level of reducing sugar is greater than that for glucose. After 192 hours glucose constitutes approximately 65% of the total reducing sugar. This proportion would be considerably higher if the results were not expressed in maltose units, i.e. if the actual concentration of each reducing sugar was known. Indeed Bond and Glass (1963) have shown that glucose may comprise 80% of the total reducing sugar fraction. However, since a marked correlation between glucose and reducing sugar was demonstrated (tables 27a, 29a and figs. 39 and 43), the sixth requirement of the hypothesis (page 42) is fulfiled.

As has been explained (page 31) sucrose is not the sole source of reducing sugar during the early periods of germination and, therefore, although negative linear regression may be expected during the first 96 hours, a fairly low level of correlation would not detract from the hypothesis. On the other hand, the degree of correlation observed (-0.703, table 32a, fig. 47) is high enough to suggest that sucrose is the major source of reducing sugar during this period. The low correlation revealed by the study of individual grains (-0.517, table 32b, fig. 52), although less convincing does offer some support for the general hypothesis, and thereby fulfils the third prerequisite of the hypothesis (page 42).

There is, therefore, sufficient evidence to suggest that, during the first 96 hours of germination, sucrose hydrolysis yields the major proportion of the reducing sugar. This would be supported if negative regression was demonstrated between sucrose and glucose concentrations, because, apart from melibiose, which is present in low concentration (Bond and Glass 1963), the only other major source of reducing sugar would be the result of amylolysis. There is evidence however, that only trace amounts of glucose are formed by the latter process (Hopkins 1946 and Bernfeld 1951). Although there is evidence (Edelman et al 1959) that reducing sugar formed in the endosperm may be converted to sucrose during transport into the scutellum, a negative regression between sucrose and glucose should still be demonstrable.

The data for groups of grain revealed a marked negative correlation between sucrose and glucose concentrations during the initial 96 hours (table 32a, fig. 48). However, the correlation observed in single grains was low and is therefore not conclusive (-0.373, table 32b, fig. 53). However, it seems reasonable to suggest that the results satisfy the fourth requirement of the hypothesis (page 42).

In both groups and individual grains there was a marked increase in the sucrose concentration during the first 24 hours of germination. It is possible that this increase may be the result of raffinose breakdown, since Bond and Glass (1963) have demonstrated that the disappearance of raffinose is accompanied by the appearance of sucrose and melibiose.

Since growth is stimulated by the presence of some reducing sugars and principally glucose (Dure 1960a), the relationship between sucrose and growth is indirect. Indeed the negative correlation, that exists between sucrose and the development of the shoot and root system during the initial 96 hours germination, is surprisingly high (table 31b, figs. 44, 45 and 46). The data for individual grains show a lower degree of correlation but significantly, the relationship is always negative during the first 96 hours (table 34b, figs. 50, 51 and 52). Therefore, maximal growth is usually associated with lowest sucrose concentration during this period. Growth does not seem to be related to the concentration of

sucrose when the germination period exceeds 96 hours. These results satisfy the fifth prerequisite of the hypothesis (page 42).

The study of individual grains does not always yield as conclusive evidence as that derived from groups of grain. This may be the result of the smaller overall sample during assay of individual grains, and also of intrinsic differences between grains which are high lighted by the assay of single grains. Undoubtedly part of the cause is the lack of replication, since there is evidence that single assays are not always reliable. However, because there is no conflict in the results, the assay of individual grains is worthwhile. Certainly, it emphasises the considerable physiological variability between grains from the same population. Equally important however, is that the results imply that, irrespective of the variability, the grains conform to a general metabolic pattern during germination and that, during the initial stages, sucrose is of fundamental importance.

It is striking that even though Hickory King and Early Pearl grains differ morphologically and physiologically, they both depend on the same metabolic pathway during germination. Thus the conclusions reached as a result of investigations of Hickory King grains have received support from the study of Early Pearl. Indeed the assay of changes in glucose concentration during germination has not only led to improved understanding of the early germination process but has also supported the general hypothesis.

From the study of these two grain types the following conclusions may be drawn:

(i) Growth of the embryo is dependent on the availability of reducing sugar, in particular glucose, during both the initial stages, when development is dependent on scutellar reserves and after mobilisation of the endosperm.

(ii) The importance of glucose may be incidental because of its relatively high concentration or because it is more readily metabolised. It seems likely that, at least during the first 48 hours, fructose may be of some importance because its concentration should

be equal to, or somewhat less than, glucose during this period.

(iii) Sucrose is an important metabolite during the early stages of germination since it is mainly responsible for the production of reducing sugar during this period. During the first 24 hours it is possible that, at least in Early Pearl, sucrose may be formed as a result of raffinose hydrolysis. Some sucrose may also be synthesised as reducing sugar is transferred from the endosperm to the scutellum.

(iv) After mobilisation of the endosperm has begun to supply large amounts of reducing sugar, sucrose is produced more rapidly than it is utilised and accumulates in the grain.

(v) Variability in germination and development of the young plant is, to some extent, determined initially by the level and mobilisation of sucrose and subsequently by the mobilisation of the endosperm.

(vi) Dextrin, as such, is of relatively minor significance to the growth and development of the young plant.

(vii) It seems reasonable to suggest that most varieties of maize are likely to follow the same basic metabolic pattern that has been elucidated by the study of Hickory King and Early Pearl.

III. CHANGES IN AMYLOLYTIC ACTIVITY.

A. HICKORY KING

1. Reducing sugar concentration.

Since increase in the reducing power was used as the index of amylase activity, it was necessary to determine the "background" reducing potential of the extract before assaying for amylase activity. This potential is expressed as milligrams maltose per grain. Whilst substances other than reducing sugars certainly influence the reducing potential in crude extracts (Briggs 1967), the major part of the reducing fraction is comprised of reducing sugars, principally glucose (Bond and Glass 1963).

(i) Groups of grain.

The concentration of reducing sugar is low in dormant grains and single grains contain, on the average, 4 mgms. (table 35). The level of reducing sugar increases slowly during the first 24-48 hours, after which reducing sugar accumulates rapidly, the level increasing more than two-fold between 48 and 96 hours (fig. 54). After 144 hours, the rate of accumulation decreases notably, although reducing sugar accumulates during the whole 240 hour period.

Measureable growth only appears after 48 hours and then exhibits a typical sigmoid form: slow between 48 and 96 hours, followed by a phase of rapid growth (96-144 hours) after which the growth rate decreases (table 36, fig. 54).

There is no significant variation between the sample means for reducing sugar (table 37a), although the means for samples receiving the same treatment are frequently significantly different (table 35). In ungerminated grains the sample having the highest concentration contains more than nine times that present in the sample with the lowest concentration, and after 72 hours of germination the difference between highest and lowest was approximately 9 mgms., a difference of approximately 20%. This very considerable variation between samples receiving the same treatment results in different response

curves for each sample during germination, and thus the sample/germination period interaction is significant (table 37a).

The between means variation for replication is not significant (table 37a) and in no instance was there a significant difference between replicates (table 35), thus the assay procedure adopted could be applied to the investigation of individual grains.

Length of radicle, coleoptile and lateral roots are closely correlated with the level of reducing sugar in the grain. In general low levels of reducing sugar are associated with least growth. As the concentration increases there is an increase in the development of the shoot and root system (table 37b, figs. 55, 56 and 57).

(ii) Individual grains.

The results for analyses of reducing sugar concentration are presented in table 38. Three of the ten ungerminated grains assayed contained no measureable quantity of reducing sugar, although one grain contained almost 12 mgms. The mean level of reducing sugar per grain was approximately 6.6 mgms. Reducing sugar concentration increases slowly during the first 24 hours, but then accumulates rapidly until 96 hours, after which there is a notable decrease in the rate of accumulation (fig. 58). The overall effect of germination period on reducing sugar concentration is significant (table 39a).

There is considerable variability between grains, especially with increased germination period. Thus, after 216 hours, one grain (grain 9, table 38) contained almost twice as much reducing sugar as grain 8, which received the same treatment. The difference being approximately 64 mgms.

Linear correlation between reducing sugar concentration and length of radicle, coleoptile and lateral roots is not very marked over the 240 hour germination period. If, however, the first 48 hours of germination are ignored, because during this period there is no measureable growth (table 40), the correlation is even less marked (table 39b, figs. 59, 60 and 61). Thus, in general, the study of individual grains does not reveal much linear correlation between growth and level of reducing sugar.

2. Total amylolytic activity.

Since in cereals, alpha- and beta-amylase are responsible for the bulk of starch hydrolysis, the increase in reducing potential observed in the experimental digests is considered to be due to the combined action of the two enzymes. Thus total amylolytic activity is considered to be the same as the sum of the action of alpha and beta. For convenience and to avoid confusion, the combined action has been designated as $\alpha\beta$ amylase activity. The assay procedure adopted (page 17) ensured that during the estimation of activity, substrate did not limit the rate of reaction. The values obtained therefore represent the activity in unlimited substrate.

(i) Groups of grain.

Amylolytic activity is low in dormant grains. Although activity increases significantly during the first 24 hours the level remains low (approximately 0.6 mgms. maltose per minute per grain). Between 24 and 144 hours, increase in amylase activity is almost directly proportional to germination time, but with germination periods exceeding 144 hours, the rate of increase in activity decreases as activity tends to a maximum (table 41, fig. 62). The overall effect of increasing germination period on amylase activity is significant (table 42a).

Individual samples vary considerably in their activity, with the variation frequently being as much as 5 mgms of maltose per minute per grain, which is more than 35% of the mean maximum activity observed (i.e. after 240 hours). The variation is such that both the variance between sample means and the sample/germination period interaction are significant (table 42a).

Occasionally replicates are significantly different (e.g. table 41, replicates of sample 1 after 168 hours). However, the variation between replicate means is not significant (table 42a) and therefore the variation, as a result of the assay procedure, is small enough to permit assay of individual grains without replication, but with some confidence.

Amylolytic activity and length of radicle, coleoptile and lateral roots show a high degree of linear correlation over the 240 hour germination period studied. A marked correlation is also evident

betwen amylolytic activity and concentration of reducing sugar, especially during the first 96 hours of germination (table 42b, figs. 63, 64, 65, and 66). Total amylolytic activity and level of reducing sugar are not linearly related between 120 and 240 hours. The apparent curvilinear relationship between total amylolytic activity and reducing sugar concentration is of some significance and is discussed on page 60.

(ii) Individual grains.

The development of total amylolytic activity follows a sigmoid pattern: activity is low in resting grains (0.057 mgms. maltose / min./ grain) and increases slowly during the initial 96 hour germination period. Between 96 and 144 hours there is a very marked increase in activity followed by a reduction in the rate of increase in activity as a maximum activity is approached. There is no evidence that activity decreases during the 240 hour germination period (table 43, fig. 67). Activity increases approximately 220 times and the overall effect of germination period on amylolytic activity is significant (table 44a).

Grains receiving the same treatment vary considerably in their amylolytic activity. Frequently the grain with the highest activity is more than five times as active as the grain which has the lowest activity (table 43).

The data (table 43) do not reveal any correlation between amylolytic activity and the development of the shoot and root systems, but a marked correlation, especially during the initial 96 hours, is evident between amylase activity and reducing sugar concentration (table 44b, figs. 68, 69, 70 and 71). In general the relationship appears curvilinear, a feature which is discussed on page 60.

3. Alpha-amylase activity.

(i) Groups of grain.

Alpha-amylase activity is low in ungerminated grain, and the three samples varied between 0.078 and 0.291 mgms maltose/min/grain.

Activity increases significantly during the first 24 hours, but the general trend is a slow increase until 72 hours, after which there is a very marked development of alpha-amylase activity. Activity tends to a maximum when the germination period is increased after 144 hours and there is some evidence that activity may decrease towards the end of the germination period studied (table 45, fig. 62).

Ten-grain samples receiving the same treatment vary considerably in the alpha-amylase activity and consequently the between means variance for sample was shown to be significant (table 46a). Since samples vary considerably, the pattern of development of alpha-amylase activity, shown by the samples, is not the same. This is probably responsible for the significant sample/germination period interaction (table 46a). Although ten-grain samples cannot be considered representative of the population as a whole, it seems likely that the trend observed in the germination period mean data (table 45, fig. 62) should approach that for the population.

Although there is some variability between replicates, in only one instance (table 45, sample 1 after 120 hours germination) was there a significant difference between individual replicates, and the between means variance for replication was not significant (table 46a). It is reasonable therefore, to consider the assay procedure sufficiently reliable to be applied to the assay of individual grains without replication.

Alpha-amylase activity shows a considerable degree of linear correlation with the development of the shoot and root system. Measureable growth is not evident before 72 hours (table 36) and, if the period 72-240 hours is considered, the degree of correlation is less marked (table 46b, figs. 72, 73, and 74).

Reducing sugar concentration and alpha-amylase activity are correlated, especially during the first 96 hours (table 46b, fig. 75). There is no evidence that a linear correlation exists when the germination period is increased after 120 hours. During the early germination periods, therefore, low levels of reducing sugar are generally associated with low alpha-amylase activity. The overall relationship may be described as curvilinear; the significance of this result is discussed on page 60.

(ii) Individual grains.

50% of the ungerminated grains assayed had no measureable alpha-amylase activity and those, in which activity was demonstrated, ranged between 0.028 and 0.219 mgms. maltose/min. A slow, steady increase in activity characterises the initial 96 hours, which is followed by a phase of rapid development of alpha-amylase activity between 96 and 192 hours; after which activity tends to a maximum. There is some evidence that activity may decrease towards the end of the germination period (table 47, fig. 67).

Grains receiving the same treatment vary considerably in their activity, sometimes by as much as 17.5 mgms. maltose/min/grain (table 47, grains 4 and 6 after 240 hours). Although this was the maximum difference between grains receiving the same treatment, the relative difference was smaller than that observed after 120 hours i.e. 16 fold as opposed to 300 fold difference. With such great variability in physiological activity, it is not surprising that the between means variance for ten-grain samples was significant (table 46a).

There is no evidence that linear regression exists between the level of amylase activity and development of the shoot and root system. The correlation coefficients are even less when the period of measureable growth is considered (table 48b, figs. 76, 77 and 78). There is some evidence of linear correlation between alpha-amylase activity and concentration of reducing sugar. It does not appear that this correlation is more marked during the initial 96 hours of germination possibly because the relationship is curvilinear. Low levels of amylase activity are generally associated with low levels of reducing sugar in the grain (table 48b, fig. 79).

4. Beta-amylase activity.

(i) Groups of grain.

Beta-amylase activity is only responsible for approximately 30% of the total amylolytic activity in resting grains. In the three samples investigated, the proportion of beta-amylase varied between 20% and 38% and, thus in all three, alpha-amylase was the

dominant amylase (table 49). Beta-amylase activity does not increase significantly during the first 24 hours germination, but thereafter activity increases markedly, tending to a maximum after 168 hours (table 49, fig. 62). However, the very high values observed after 240 hours make interpretation difficult. It is interesting that high values for beta-amylase activity were observed in all three samples (table 49).

During the first 48 hours alpha- and beta-amylase activity increase at almost the same rate, thus, after 48 hours, beta-amylase is still only responsible for 30% of the total activity. When the germination period is increased further, alpha-amylase activity increases more rapidly than beta-amylase, and consequently beta-amylase contributes proportionately less to the total amylolytic activity. After 144 hours, beta-amylase represents only 27% of the total activity. However, alpha-amylase activity tends tc a maximum after 144 hours, whereas beta-amylase activity does not, thus beta-amylase contributes an increasing proportion to the total activity. The high values obtained for beta-amylase activity after 240 hours make interpreation of the trend after 192 hours difficult. If, however, beta-amylase activity does tend to decrease after 192 hours, then it would tend to contribute uniformly to the total amylolytic activity during the later periods of germination. The results suggest, therefore, that alpha-amylase is always the dominant amylase, but that beta-amylase may, during the early stages of germination, be responsible for almost half the total amylolytic activity.

There is some evidence that the development of alpha- and beta-amylase activity may be correlated during the initial periods of germination (table 50b, fig. 80). During the first 96 hours, low levels of alpha-amylase tend to be associated with low levels of beta-amylase activity.

Although there is considerable variability between samples, and indeed, samples subjected to the same germination conditions can be significantly different, notably when the germination period exceeds 144 hours, the between means variance for samples is not significant (tables 49 and 50a).

A significant difference between replicates was only observed in one instance (table 49, sample 1 after 168 hours germination) and,

therefore, the method adopted to determine beta-amylase activity, although only by difference between total and alpha-amylolytic activity, can be considered reliable.

Beta-amylolytic activity shows a marked degree of linear correlation with the development or the shoot and root system (table 50b, figs. 81, 82, and 83). Low levels of activity tend to be associated with least development of the radicle, coleoptile and lateral roots.

During the first 96 hours beta-amylase activity shows a very high degree of correlation with the level of reducing sugar. This correlation is not evident when the germination period exceeds 120 hours. Thus, only during the early stages of germination, do low concentrations of reducing sugar appear to be associated with low levels of beta-amylase activity (table 50b, fig. 84), however the relationship appears curvilinear (page 60).

(ii) Individual grains.

In the investigations on ungerminated grains beta-amylase activity was only demonstrated in 20% of the grains, and in all cases, except grain 9 (table 51), alpha-amylase was the dominant amylolytic enzyme. Alpha-amylase contributed approximately 75% of the total amylolytic activity. Within the first 48 hours, however, beta-amylase activity increased more rapidly than alpha-amylase activity and, therefore, contributed proportionately more to the total amylolytic activity i.e. approximately 47%. When the germination period was increased beyond 96 hours, alpha-amylolytic activity increases more rapidly than beta-amyolytic activity, so that beta-amylolytic activity contributed less to the total amylolytic activity, although it represents approximately 40% of the activity between 144 and 192 hours. When the germination period exceeds 192 hours, the trends become difficult to establish, although there is some evidence that beta-amylase activity does not tend to a maximum as early as alpha-amylase and, therefore, towards the end of the 240 hour germination period studied, alpha- and beta-amylase may be equal in their saccharifying activity (table 51, fig. 67).

Individual grains subjected to the same germination conditions vary considerably in their beta-amylase activity. This variation is especially evident as the germination period increases (table 51).

Beta-amylase activity does not seem to show any linear relationship to the growth of radicle, coleoptile and lateral roots. Even when the period of measurable growth is considered (72-240 hours) the correlation is low (table 52b, figs. 85, 86 and 87). However, beta-amylolytic activity may be correlated with the level of reducing sugar during the initial 96 hours of germination (table 52b, fig. 88). Thus during early germination reducing sugar concentration increases with the development of beta-amylolytic activity. The relationship is, however, curvilinear (page 60).

During the initial 96 hours of germination low levels of alpha-amylolytic activity tend to be associated with low levels of beta-amylolytic activity. However, as the germination period increases after 96 hours, the development of alpha- and beta-amylase seem to be independent (table 52b, fig. 89).

5. Discussion and conclusions.

In the investigations of carbohydrate concentration the reliability of the assay procedure was established by replicate assay of small quantities of meal, approximately equal to the weight of meal obtained from one grain, derived from the same sample. This approach was not possible in the assay of amylolytic activity since a dry meal, from which sub-samples could be taken, could not be prepared. All grains in the sample were ground together to form one brei from which aliquots were taken. There is therefore, no check on the extraction of the amylases or reducing sugars and the usefulness of the replicates is limited in that it supplies an index of the reliability of the actual determination of amylolytic activity. However, since cereal amylases are soluble when active (Myrback and Neumuller 1950), the supernatant should contain the active amylases and it is reasonable to suppose that extraction is complete and reproduceable. Especially so since Dure (1960b) has

shown that the spun-down pellet from such brei shows no amylolytic activity. Further support for this contention is that the general trends observed in the data for groups and single grains show close agreement, as do the levels of amylolytic activity.

In no instance is the between means variance for replication significant and, although replicates are occasionally different, the difference is usually small by comparison with the overall variation. Thus, for example, in the data for total amylolytic activity (tables 41 & 43) the maximum variation between replicates was 2.375 mgms. maltose per minute per grain (sample 1 after 168 hours germination), whereas the maximum variation between individual grains after the same germination period was 8.484 mgm. maltose per minute per grain. Therefore, in general, the assay procedure is sufficiently reproduceable to yield reliable results in the study of single grains where there was no replication of assay. However, some of the observed variation is undoubtedly the result of error introduced by the assay procedure.

The general patterns of development of amylase activity in the data for both groups and individual grains, are in agreement with numerous workers (Kneen 1944, Myrback and Neumuller 1950, Bernfeld 1951, Peat 1951, Pigman 1957 and Dure 1960b). Amylolytic activity is low in resting grains and during germination both alpha- and beta-amylase activities increase, but alpha-amylase becomes the chief amylolytic enzyme. This finding supports the observations of many other workers (Kneen 1944, Peat 1951, Crocker and Barton 1953, Pigman 1957 and Dure 1960b).

Two aspects of the results are, however, not in general agreement with the literature. These are the level of alpha-amylase activity in resting grains and the relative contributions of alpha- and beta-amylase to the total amylolytic activity.

Many workers have observed that all of the amylolytic activity in ungerminated grain is attributable to beta-amylase (Kneen 1944, Myrback and Neumuller 1950, Bernfeld 1951, Peat 1951, Pigman 1957 and Dure 1960b). However Dure (1960b) notes that there is 'very little alpha-amylase activity in the scutellum of the resting grain'.

The procedure Dure (1960b) adopted for the separation of alpha- and beta-amylase activity relied on thermal inactivation of beta-amylase, according to the method of Bernfeld (1951). Cereal beta-amylase is rapidly inactivated at 70°C whereas cereal alpha-amylase is much more stable at this temperature (Ohlsson 1930, Kneen, Miller and Sandstedt 1942, Myrback and Neumuller 1950, Bernfeld 1951 and Dure 1960b), although after five minutes at 70°C alpha-amylolytic activity may be decreased by as much as 50% (Dure 1960b). It seems likely therefore, that since the selective inhibition of beta-amylase with phenyl mercury chloride does not appear to reduce alpha-amylase activity in any way (Bendelow 1963, Briggs 1967 and page 18) the determination of alpha-amylase activity by this method is likely to yield more reliable results. If this is so, then it is reasonable to suggest that the present results, which demonstrate that alpha-amylase may not only be present in the ungerminated grains, but can be responsible for 70% of the total amylolytic activity, do not contradict previous observations but merely amplify them by virtue of the greater accuracy of the assay procedure.

It has been said that the relatively slight increase in beta-amylase activity during germination is a characteristic of maize and some other cereals (Peat 1951). The present results suggest that beta-amylolytic activity increases substantially during germination and may, during the period of maximum amylolytic activity, be responsible for approximately 30% of the total amylolytic activity. Dure (1960b) notes that beta-amylase accounts for only 1/10 of the total activity during germination. Whilst different grains do probably vary, it seems possible that this low value may, in part at least, be the result of the assay procedure, since low pH inactivation may reduce beta-amylase activity by as much as 50% in barley (Myrback and Neumuller 1950). Undoubtedly the procedure adopted in the present work also introduces error in the estimation of beta-amylase activity, since the values are arrived at by difference between total and alpha-amylolytic activity. However, it is reasonable to suggest that the procedure adopted in this work results in more accurate determination of the relative contributions of alpha- and beta-amylase to the total amylolytic activity.

In cereal malts there is clear evidence that the activity of both alpha- and beta-amylase may be artificially increased by the addition of proteases, which suggest that net synthesis of these two enzymes does not occur during germination, but rather, that they become freed from a bound form (Geddes 1946, Myrback and Neumuller 1950, Sumner and Somers 1953 and Dure 1960b). Geddes (1946) has, however, reported a net synthesis of alpha-amylase during germination of barley. The linear regression demonstrated between alpha- and beta-amylase suggests that these two enzymes are related in their development. There is conjecture regarding the site of origin of the amylases in germinating cereals. Many workers have considered the scutellar epithelium to be the source of the amylases because microscopical examination has revealed an apparent migration of particles from the scutellum into the endosperm (Toole 1924 and Horning and Petrie 1927), and because mitochondria isolated from corn scutellum contain amylolytic activity (Hageman and Hanson 1955). However, Bernstein (1943), on the basis of genetic studies, suggested that each amylase had a separate origin; the inheritance of alpha-amylase being a characteristic of the embryo, whilst beta-amylase originated in the endosperm. Thus, as has been pointed out by Dure (1960b), most workers agree that some or all of the amylase originates in the scutellum. The finding that alpha-amylase activity may develop in the isolated scutellum and beta-amylase activity in the isolated endosperm (Dure 1960b) suggests that these two enzymes are independent in their origin and it is doubtful, therefore, whether the activity of either alpha- or beta-amylase controls the activity of the other. Thus it seems likely that the positive linear regression between alpha- and beta-amylase activity is the result of response to some common factor in germination, and not because their development is causally related.

Reducing sugar comprises the major portion of the total reducing-fraction and therefore, in view of the previous evidence, that reducing sugar and growth are correlated, it is not surprising that a high degree of correlation is demonstrated between growth and total reducing fraction (table 37b, figs. 55, 56, and 57). The low levels of correlation observed in the data for individual grains may, at least in part, be because the determinations were

carried out on a crude extract and also because of the absence of replicates.

It is interesting that amylolytic activity and level of reducing sugar show a marked degree of linear correlation, in particular during the initial 96 hours of germination (tables 42b, 44b, 46b, 48b, 50b and 52b). However, the results suggest that the relationship between amylase activity (total, alpha- and beta-amylase) and reducing sugar concentration is curvilinear (figs. 66, 72, 75, 79, 84 and 88). Thus when the level of reducing sugar is low i.e. during the initial 96 hours germination, reducing sugar concentration increases more rapidly than amylase activity. This suggests that, during this period, reducing sugar and level of amylase activity are not interdependent: the marked increase in reducing sugar with relatively little increase in the level of amylase activity implies that, during the first 96 hours, the major proportion of the reducing sugar is not the result of amylolytic activity. This supports the contention that at least during the early periods of germination, some other carbohydrate, probably sucrose, serves as the major source of reducing sugar.

If amylolytic activity resulted in a large part of the reducing fraction formed during the early stages of germination, it would be reasonable to expect a high degree of correlation between amylase activity and growth, because growth is related to reducing sugar concentration, especially during this period. Although there is evidence, in the group data (tables 42b, 46b and 50b), that amylase activity and growth may be correlated, there is no evidence of correlation in the data from individual grains (tables 44b, 48b and 52b). In view of the curvilinear relationship between amylase activity and reducing sugar concentration it seems possible that the correlation between amylase activity and growth is probably, to some extent at least, the result of independent response to the general conditions of germination, especially during the early stages of germination.

There seems little doubt that amyolytic activity supplies some reducing sugar during early germination. However, it is necessary to consider the extent of its contribution.

If, during early germination, growth is mainly related to

lipid (Dure 1960a) and to the formation of reducing sugar as a result of sucrose hydrolysis, then it seems unlikely that it would be causally related to the degree of amylolytic activity. This is especially so since:

(i) alpha-amylolytic activity is very low during the early periods of germination and therefore non-reducing ends of polymers, on which beta-amylase acts to produce maltose units, are not produced rapidly. Furthermore alpha-amylase originates in the scutellum and there is very slow secretion of alpha-amylase during the early stages of germination, because 'an interaction between the scutellum and the endosperm must take place before the scutellum will secrete alpha-amylase into the endosperm' (Dure 1960b).

(ii) beta-amylase activity is low during the first 48 hours and therefore is unlikely to contribute significantly to the reducing fraction. This is supported to some extent by the appearance of maltose only after 3 to 4 days germination.

(iii) if the scutellum acts to absorb carbohydrate from the endosperm and convert it to sucrose (Edelman et al 1959), then the level of sucrose might supply, indirectly, some idea of the amount of reducing sugar formed as a result of amylolytic activity. If amyolytic activity produces substantial amounts of reducing sugar during early germination, it might be expected that the level of sucrose would at least remain fairly constant. The observed decrease implies that sucrose is utilised more rapidly than it is replaced by the formation of reducing sugar in the endosperm which is subsequently converted to sucrose. Since sucrose concentration only increases after approximately 96 hours, it seems likely that reducing sugar production in the endosperm only exceeds the metabolic demand after about 96 hours germination. Thus it is reasonable to

> suggest that reducing sugar formation as a result of amyolytic activity is of minor significance during the first 24 to 48 hours germination, but that, as the germination period increases, so amylolytic activity contributes an increasing proportion of the total reducing sugar fraction, until after about 96 hours, when amylolytic activity represents the major source of reducing sugar of grains germinating in the dark.

It seems reasonable to suggest, therefore, that the positive correlation between amylase activity and both growth and reducing sugar concentration during the early periods of germination arises because both variables are being influenced by the same cause i.e. germination period, and not because of any direct cause and effect relationship existing between them. Certainly the action of alpha- and beta-amylases does produce reducing sugars during early germination, but it is likely that they represent a small proportion of the total reducing sugar fraction.

The studies on changes in the carbohydrate concentration during germination led to the conclusion that sucrose is the major source of reducing sugar during early germination. It seems likely that this reducing sugar is supplemented to a small extent by amylolytic activity during the first 48 hours but thereafter amylolytic activity supplies an increasing proportion of the reducing sugar until approximately 96 hours when it represents the major source. After 96 hours germination, amylolytic activity produces more reducing sugar than is utilised metabolically and this results in an accumulation of sucrose.

B. EARLY PEARL.

1. Reducing sugar concentration.

(i) Groups of grain.

Reducing sugar concentration (total reducing fraction expressed as milligrams maltose per grain) in groups of grain during germination is given in table 53. Dormant grains are characterised by having a low level of reducing sugar, single grains containing, on the average, 10 mgms. The rate of accumulation of reducing sugar (given by the slope of the curve, fig. 90) increases during the first 72 hours germination, reaching a maximum between 72 and 96 hours (0.78 mgms. maltose/hour). When the germination period is increased after 96 hours, the concentration of reducing sugar in the grain tends to a maximum, which is attained after 144 hours germination (approximately 70 mgms./grain). There is evidence that the level of reducing sugar may decrease when the germination period exceeds 168 hours.

During the initial 192 hours germination, there is no significant difference between replicates, but when the germination period exceeds 192 hours, replicates may be significantly different (table 53). However, the between means variance is not significant (table 54a). Thus, although there is some error in the assay of individual grains when there is no replication, the results should be sufficiently reliable to permit some comment on the variability between grains.

Variance between sample means is only significant at the 5% probability level (table 54a), but the variation is sufficiently large to result in different response curves for samples during germination, thereby causing the interaction between sample and germination to be significant (table 54a). The actual difference between samples increases with germination period. Thus, for example, sample 1 (table 53, 79.375 mgms.) contained approximately 35 mgms. more reducing sugar than sample 3 (table 53, 44.375 mgms) after 192 hours germination. This is a difference of about 80%.

Measurable growth is only apparent after 48 hours germination (table 55) and growth rate is slow during the first 72 hours, after which growth is rapid. There is no evidence that growth rate decreases towards the end of the germination period (fig. 90).

Concentration of reducing sugar and growth of radicle, coleoptile and lateral roots are not linearly related over the whole germination period. However, there is evidence that, during the initial 72 hours, as the level of reducing sugar rises, extension of the shoot and root system takes place (table 54b, figs. 91,92 and 93).

(ii) Individual grains.

Reducing sugar concentration in resting grains varies between 12.5 and 40.6 mgms. (grains 1 and 9, table 56), and the mean 19.437 mgms. is somewhat higher than that derived from the study of groups of grain (10.7 mgms., table 53). Reducing sugar concentration apparently decreases during the first 24 hours, but the variation between grains is such that the decrease is considerably less than the value for the least significant difference between means (table 56). Reducing sugar probably accumulates slowly during the first 24 hours germination (0.29 mgms. maltose/hour/grain) but the rate of accumulation (given by the slope, fig. 94) increases markedly between 24 and 48 hours, during which period the rate is 1.17 mgms. maltose/hour/grain. As the germination period approaches 144 hours, the concentration of reducing sugar tends to a maximum attaining a level of approximately 87 mgms. maltose/grain (fig. 94). When the germination period exceeds 144 hours the level of reducing sugar decreases.

There is considerable variability between grains receiving the same treatment, especially as the germination period increases. Thus, after 240 hours, one grain (grain 2, table 56) contained more than twice as much reducing sugar as grain 8, which had been subjected to the same germination conditions. Such large variation, approximately 70 mgms., must represent a real difference in physiological activity and cannot be the result of error caused by the lack of replication.

Growth of radicle and coleoptile are linearly related to the concentration of reducing sugar in the grain, especially during the first 96 hours. As the level of reducing sugar increases, extension of the radicle and coleoptile takes place. There is

no evidence that growth and reducing sugar concentration are related when the germination period exceeds 96 hours (table 57b, figs. 95, 96 and 97). Growth data are presented in table 58.

2. Total amylolytic activity.

(i) Groups of grain.

Amylolytic activity is low in ungerminated grains and one sample had no measureable activity (table 59). Activity increases steadily, although not significantly, during the first 48 hours, after which activity increases rapidly, tending to a maximum after 192 hours germination (fig. 98). However, the variation between samples is so great that the means for the germination periods do not follow a definite sequence. Interpretation of the trend is therefore somewhat tentative and indeed, plotting a general trend results in the sum of alpha- and beta-amylase sometimes being apparently greater than the total amylolytic activity. The overall effect of increasing germination period on amylase activity is significant (table 60a).

Although the between means variance for sample is only significant at the 5% probability level (table 60a), the difference in total amylolytic activity between groups receiving the same treatment may be as much as 3 mgms. maltose/min./grain, which represents approximately 30% of the maximum activity observed (sample 1 after 216 hours, table 59). Variation of this magnitude is undoubtedly responsible for the significant sample/germination interaction, since it results in different response curves for each sample during germination (table 60a).

Occasionally replicates are significantly different (e.g. replicates of sample 1 after 192 hours, table 59), but the variation between replicate means is not significant (table 60a) and therefore the error introduced into the assay of individual grains, because of the absence of replicates, is likely to be small.

Total amylolytic activity and level of reducing sugar are linearly related over the whole germination period studied (table 60b, fig. 99). However, inspection of fig. 99 reveals that the

overall relationship is best described as curvilinear; at low levels of amylase activity, increase in the reducing sugar concentration is independent of the level of amylase activity. Conversely, when the level of amylase activity is high, further increase in the amylase activity has little or not effect on the level of reducing sugar.

Although there is some evidence of linear regression between total amylolytic activity and growth, the relationship is not very marked, and the highest degree of correlation was demonstrated between amylase activity and length of coleoptile (table 60b, figs. 100, 101 and 102). Correlation is least when the germination period exceeds 96 hours.

(ii) Individual grains.

Amylolytic activity is low in dormant grains and of the ten grains assayed only three had measureable activity. With such variability, it is not surprising that the level of amylolytic activity apparently decreases during the first 24 hours even though after 24 hours germination all grains showed amylase activity (table 61). Activity increases slowly during the first 48 hours, which is followed by a phase of rapid increase in activity, which tends to a maximum after approximately 144 hours germination (fig. 103).

Grains subjected to the same germination conditions vary considerably in their amylolytic activity. The greatest variability was shown after 168 hours, when the grain with the maximum activity (grain 7, table 61) was seven times as active as grain 9, which showed the lowest activity. Such variability cannot be ascribed to the assay procedure and must be a reflection of real differences in physiological activity.

Total amylolytic activity and level of reducing sugar are positively correlated, especially during the first 96 hours. Thus, during this period, low levels of reducing sugar are usually associated with low amylolytic activity. However the relationship between amylolytic activity and level of reducing sugar tends to be curvilinear (table 62b, fig. 104). Thus when the germination period is short and amylase activity is low, the level of reducing sugar

increases rapidly relative to increase in amylase activity, but, when the germination period are increased, and when both level of reducing sugar and amylolytic activity is increased, then amylase activity tends to increase independently of reducing sugar.

Except during the early stages of germination, when length of radicle and level of amylolytic activity may be correlated, there is no evidence that growth and amylase activity are linearly related (table 62b, figs. 105, 106, and 107).

3. Alpha-amylase activity.

(i) Groups of grain.

None of the three samples of ungerminated grain had any measureable alpha-amylase activity, although activity was evident in all three samples after 24 hours germination (table 63). Activity increases slowly during the first 72 hours germination, after which it increases rapidly, reaching a maximum after about 168 hours (fig. 98). There is some evidence that activity may decrease towards the end of the germination period. Activities observed in successive germination periods vary considerably and make interpretation of the general trend difficult. During the early periods of germination, the germination period means are probably representative of the population because samples subjected to the same germination conditions do not vary significantly (table 63). However variability increases with increase in the germination period and this is probably responsible for the significant sample/germination interaction (table 64a).

Although there is variability between replicates, in general, the variations are only occasionally significant and the between means variance for replication is not significant (tables 63 and 64a). Thus it seems reasonable to consider the assay procedure sufficiently reliable to be applied to the assay of individual grains without replication.

Although alpha-amylase activity and level of reducing sugar appear to be linearly correlated, the relationship is curvilinear and, during the early periods of germination, reducing sugar

concentration increases considerably with little change in amylase activity (table 64b, fig. 108).

A linear relationship is evident between alpha-amylase activity and growth of radicle and coleoptile, but not with the development of the lateral roots (table 64b, figs. 109,110 and 111). The results suggest, therefore, that, over the whole germination period, low levels of alpha-amylase activity are associated with least growth of radicle and coleoptile and that as activity increases, extension of the radicle and coleoptile takes place. It is interesting that, when the early and later periods of germination are considered separately, there is no evidence of linear correlation (table 64b, figs. 109, 110 and 111).

(ii) Individual grains.

Only one of the ten ungerminated grains assayed had any measureable alpha-amylase activity, and even after 24 hours 40% of the grains had not developed detectable alpha-amylase activity (table 65). Activity increases slowly during the first 48 hours, which is followed by a phase of rapid development of alpha-amylolytic activity between 48 and 120 hours. Maximum activity is attained after approximately 144 hours and there is some evidence that activity may decrease with further increase in the germination period (fig. 103).

Grains receiving the same treatment vary considerably in their alpha-amylase activity, so much so, that it is doubtful whether the means are really representative. After 144 hours germination, for example, the grain with the highest activity (grain 8, table 65) was more than seven times as active as the grain with the lowest activity. It is interesting that two grains, after 168 hours germination did not have any detectable alpha-amylase activity.

The results suggest that the level of alpha-amylase activity and concentration of reducing sugar in the grain may be linearly related during the first 96 hours of germination, but the level of correlation is not very high (table 66b, fig. 112). It is noteworthy that the relationship between alpha-amylase activity and reducing sugar concentration is curvilinear and that, during the early periods of

germination, a considerable increase in reducing sugar occurs without much increase in amylase activity. This suggests that, during this stage, these two variables are not related (fig. 112).

Alpha-amylase activity and growth of the shoot and root system do not appear to be linearly correlated and it would seem, therefore, that growth is not directly dependent on the level of amylase activity (table 66b, figs. 113, 114 and 115).

4. Beta-amylase activity.

(i) Groups of grain.

Beta-amylase activity was demonstrated in two of the three groups of dormant grains assayed, but activity was very low, approximately 0.013 mgms. maltose/min./grain (table 67, fig. 98). Although the increase in activity between 0 and 24 hours is not significant, it represents a ten-fold increase and all three groups had measureable beta-amylase activity. Activity increases slowly during the first 72 hours, but between 72 and 144 hours there is a marked increase, tending to a maximum when the germination period approaches 192 hours. There is some evidence that the level of beta-amylase activity may decrease towards the end of the germination period (table 67, fig. 98).

In the ungerminated grains beta-amylase is the major amylolytic enzyme, since no alpha-amylase activity was demonstrated. However, after 24 hours germination, beta-amylase contributes somewhat less than half of the total amylolytic activity, and, as the germination period increases to 144 hours, beta-amylase contributes proportionately less to the total activity. Thus after 144 hours approximately 62% of the total amylolytic activity results from the action of alpha-amylase and 38% from beta-amylase. However, since beta-amylase activity does not appear to decrease as much as alpha-amylolytic activity during later germination, each enzyme contributes almost equally to the activity when the germination period approaches 240 hours (fig. 98). The results suggest that, after the initial period of 24 hours, alpha-amylase is the dominant amylase. Beta-amylase may be responsible for all the activity in ungerminated grains and for

approximately 40% of the total activity when amylolytic activity is at its maximum.

Alpha- and beta-amylolytic activities appear to be positively correlated (table 68b, fig. 116). Thus low levels of alpha- and beta-amylase tend to occur together, and, as the level of activity of alpha--amylase increases, there is an increase in beta-amylase activity.

In general, replicates are not significantly different, and the between means variance for replication is not significant (table 67 and 68a). Therefore, even though beta-amylase activity was only determined by difference between total and alpha-amylolytic activity, the assay procedure can be considered reliable.

Samples subjected to the same germination conditions show considerable variability in their beta-amylolytic activity, especially during the later stages of germination. After 168 hours germination, the sample having the highest activity was twice as active as that with the lowest activity (samples 2 and 3, table 67). Samples behave differently in their pattern of development of beta-amylase activity, a feature which is probably responsible for the significant sample/germination interaction (table 68a).

There is no evidence that beta-amylase activity is linearly correlated with growth of the shoot and root system (table 68b, figs. 117, 118 and 119). The highest degree of correlation was + 0.646, between reducing sugar concentration and radicle length over the whole 240-hour germination period.

Although the analysis of the data for linear regression between beta-amylase activity and reducing sugar concentration (tables 53 and 67) revealed high degrees of positive correlation, even when the whole germination period was considered (table 68b), the distribution of the points in the scatter plot (fig. 120) suggests that the relationship over the 240-hour period is better described as curvilinear. Thus, during early germination, the level of reducing sugar increases markedly with little increase in beta-amylase activity. Consequently it may be suggested that beta-amylase activity and reducing sugar concentration are not necessarily causally related during early germination, and that most of the reducing sugar produced during this period originates other than by beta-amylase activity.

(ii) Individual grains.

In the investigation of ungerminated grains, beta-amylase activity was only demonstrated in 30% of the grains, and, with the exception of grain 6, where alpha- and beta-amylase had equal activities, beta-amylase was the dominant amylolytic enzyme (tables 65 and 69). In most instances beta-amylase was the sole amylolytic enzyme. There is no noticeable increase in the level of beta-amylolytic activity during the first 24 hours, in fact the mean was lower than that for ungerminated grains, even though, after 24 hours germination, all grains contained measureable quantities of beta-amylase. This emphasises the very considerable variability between grains. Alpha-amylase soon becomes the dominant amylase and, after 48 hours, as the germination period increases beta-amylase contributes proportionately less to the total activity, until alpha-amylase activity decreases when the germination period exceeds 144 hours (fig. 103). Beta-amylolytic activity, which reaches a maximum after about 144 hours germination, does not decrease in activity as rapidly as does alpha-amylase activity. Thus towards the end of the germination period studied, alpha- and beta-amylase contribute almost equally to the total amyolytic activity. It is noteworthy, however, that during the period of maximum amylolytic activity, beta-amylase is responsible for approximately 47% of the total activity. In view of the difficulty in determining the general trend, the sum of alpha- and beta-amylolytic activities may be apparently greater than the total amylolytic activity. However, this was never the case in practice, since, beta-amylase activity was determined by difference between total and alpha-amylolytic activity.

Although the degree of positive linear regression between alpha- and beta-amylase is not very high, there is a tendency for low levels of alpha-amylase activity to be associated with low levels of beta-amylase activity and that increased activity of one enzyme tends, in general, to be associated with increased activity of the other (table 70b, fig. 121).

During the early stages of germination (0-96 hours), beta-amylase activity is linearly related to growth of radicle and coleoptile, thus, during this period, low levels of activity tend to be associated with least growth of radicle and coleoptile. As the

level of beta-amylase activity increases, extension of radicle and coleoptile takes place (table 70b, figs. 122 and 123). There is no evidence that beta-amylase activity and length of lateral roots are linearly related (table 70b, fig. 124).

Beta-amylolytic activity and level of reducing sugar show a high degree of positive linear regression during the first 96 hours germination, indicating that, during this period, an increase in beta-amylolytic activity results in increased reducing sugar (table 70b, fig. 125). However, although this is the case, large increases in reducing sugar occur with very little increase in amylolytic activity and it seems doubtful, therefore, that these two variables are causally related, especially since the overall relationship is apparently curvilinear (fig. 125).

5. Discussion and conclusions.

The reliability of the procedures adopted was established during the assay of amylolytic activity in Hickory King grains. The studies on Early Pearl confirm that the assay procedures are reliable and can, with some confidence, be applied to the study of individual grains.

The general patterns of development of amylase activity in Early Pearl grains are similar to those observed in Hickory King and are in agreement with the literature (Kneen 1944, Myrback and Neumuller 1950, Bernfeld 1951, Peat 1951, Crocker and Barton 1953, Pigman 1957 and Dure 1960b). Amylolytic activity is low in resting grains and both alpha- and beta-amylase increase in activity during germination, although alpha-amylase becomes the chief amylolytic enzyme.

Whilst the results show marked similarities with the observations on Hickory King, one feature, the almost complete absence of alpha-amylolytic activity in resting grains, does not conform. It is interesting that previous workers (Kneen 1944, Myrback and Neumuller 1950, Bernfeld 1951, Peat 1951, Pigman 1957 and Dure (1960b) have observed that all of the amylolytic activity in resting grains is attributable to beta-amylase activity. Although these results suggest that in Early Pearl beta-amylolytic activity is the major amylolytic enzyme in dormant grains, this is not always the case

since one grain at least (grain 6, table 69) had equal alpha- and beta-amylolytic activity.

A striking feature of the results is the appearance of alpha-amylolytic activity during the first 24 hours. Although none of the ungerminated samples, and only one of the individual grains had measurable alpha-amylase activity (tables 63 and 65), after 24 hours germination, all three samples and 60% of the individual grains had developed alpha-amylolytic activity. This suggests that alpha-amylolytic activity appears more rapidly than has been suggested by Dure (1960b), who was unable to detect activity within the first three days of germination. However, since Dure used a thermal inactivation technique, which may decrease the observed alpha-amylase activity by as much as 50% (page 58), it seems likely that the apparent absence of alpha-amylolytic activity during this period is the result of limitations in assay technique.

The marked increase in beta-amylolytic activity during germination is in agreement with the observations on Hickory King, but is not in general agreement with the literature (Peat 1951 and Dure 1960b). Since the use of low pH inactivation of alpha-amylase for the separate determination of beta-amylase activity does not always yield reliable results (page 58), it seems likely that the apparently higher beta-amylase activity is the result of more accurate assay and not because either Hickory King or Early Pearl grains are inherently different to other maize grains. It seems reasonable that beta-amylase activity should increase markedly during germination because alpha- and beta-amylase are complementary in their action on starch; alpha-amylase being principally responsible for dextrinisation and producing little reducing sugar, whereas beta-amylolytic activity produces individual maltose units. Alpha-amylolytic action is essential in view of the relatively high proportion of amylopectin in starch (Crocker and Barton 1963). Beta-amylase hydrolyses the 1,4 glucosidic link in the polymer, from the non-reducing end, but it cannot hydrolyse the 1,6 glucosidic link, thus beta-amylase action on amylopectin results in the production of limit dextrins and small quantities of reducing sugar.

Although the results suggest that alpha- and beta-amylase activity are related in their development, it seems likely that this is fortuitous and is the response of both, individually, to the

general process of germination, because as Dure (1960b) has shown, not only do these two enzymes have different sites of origin but they develop independently in isolated endosperm (beta-amylase) and scutellum (alpha-amylase).

In general the results confirm the observation (page 34) that growth is stimulated by an increase in the level of reducing sugar. Thus low levels of reducing sugar tend to be associated with least growth, and, as the level of reducing sugar increases, there is a marked increase in the growth of the shoot and root system (tables 54b and 57b). Since the reducing sugar determinations were carried out on a crude brei and other substances are known to influence the determination of reducing sugar under such conditions (Briggs 1967), the degree of correlation is surprisingly high.

Although the level of reducing sugar and activity of amylase (total, alpha- and beta-) may be apparently linearly related, especially during the initial 96 hours germination, the overall relationship is always curvilinear (tables 60b, 62b, 64b, 66b, 68b and 70b, figs. 99, 104, 108, 112, 120 and 125). Thus during the early periods of germination, in particular between 0-72 hours, the level of reducing sugar in the grains may increase markedly with little or no change in amylase activity. These two variables are therefore probably independent during this period, even though they may be apparently linearly related. Similarly in the later stages of germination, amylase activity may change with little or no corresponding change in reducing sugar concentration. Thus it seems likely that amylase activity and reducing sugar concentration are probably only related or interdependent for a short period, during the mid-germination period between approximately 72 and 96 hours. This observation suggests that during the early periods the principal source of reducing sugar is not starch and that between 72 and 120 hours growth is increasingly dependent on reducing sugar produced by amylolytic activity. When the germination period exceeds 96 hours it is likely that reducing sugar production is far in excess of metabolic requirement and the observed accumulation of sucrose after 96 hours, is probably an expression of the increased level of reducing sugar. These results are very similar to those obtained with Hickory King, and they support the contention that during the

early periods some carbohydrate other than starch, probably sucrose, serves as the major source of reducing sugar.

The results suggest, therefore, that the two major carbohydrate reserves in maize grains are sucrose and starch (including amylopectin) and that sucrose is mobilised rapidly to supply the reducing sugars essential for the initial growth of the coleoptile and radicle. The reserve of sucrose is, however, small and within 96 hours the level decreases considerably. Thus, the rate of formation of reducing sugar, by hydrolysis of sucrose, probably decreases markedly sometime before 96 hours. Since there is no evidence that growth becomes seriously restricted or that the level of reducing sugar drops markedly during the first 96 hours (although both growth rate and rate of accumulation of reducing sugar may decrease slightly as 96 hour germination is approached), it seems likely that amylolytic activity starts early during germination and steadily increases so that after 96 hours it represents the major source of reducing sugar. This is supported by the marked accumulation of sucrose and reducing sugar after 96 hours, which suggests that reducing sugar produced exceeds metabolic demand and that some reducing sugar is converted to sucrose.

CHAPTER 4.

CONCLUDING REMARKS.

Some of the problems originally visualised have been, at least partially, resolved but, as is the case with most research, many more arise to take their place.

Thus although it seems certain that sucrose serves as the major source of the reducing sugar essential for growth during the early stages, it is probably supplemented by reducing sugar originating, from the endosperm, by amylolytic activity. The convenient division of germination into two phases, the first during which growth is dependent solely on the reserves present in the scutellum and the second when products of starch hydrolysis becomes readily available is difficult to establish. This will be especially so if the apparently higher levels of alpha- and beta-amylase activity, which have been observed, are shown to be a feature of most grains. However it may well be that the presence of alpha-amylase activity in ungerminated Hickory King grains was because the grains were fresh and had only been stored for a short time, whereas the Early Pearl grains were obtained some months after the annual supply became available at the suppliers. With the accuracy of estimation inherent in the phenyl mercury chloride inhibition technique, the effect of storage time on the presence of residual amylase activity and on the development of amylase activity during germination warrants further study.

Even though amylase activity is present during the early periods of germination, it is important to establish how much reducing sugar is formed as a result of its action on the endosperm. Certainly this is likely to be less than in an artificial system where available substrate is not limited i.e. the values for amylolytic activity recorded are the theoretical maximum activities, and not the effective activity in viva. This taken together with a possible time lag between uptake of reducing sugar from the endosperm and subsequent transport to the growing regions, suggests that, at least during the first 72 hours of germination, growth may be entirely dependent on the scutellar reserves and be independent of the endosperm. Indeed

Dure (1960a) has demonstrated that there is no significant difference between the growth of isolated and attached embryos during the first five days.

The decrease in sucrose concentration is most marked within the first 72 hours germination and this suggests that invertase becomes active very rapidly. Since a marked decrease may even occur within the first 24 hours, it seems reasonable to suggest that the germination conditions activate or free the enzyme from some bound form. It is doubtful whether a net synthesis of invertase would occur in such a short period. It would certainly be worthwhile to locate the precise distribution of sucrose and invertase in the embryo/scutellum complex. In view of the fact that a large part of the decrease in sucrose occurs before emergence of the radicle and coleoptile through the pericarp, a more detailed study of the development of invertase activity and of disappearance of sucrose in relation to growth of the radicle and coleoptile before emergence through the pericarp, might improve our understanding of the relative contributions of the scutellar and endosperm reserves to the total reducing sugar complex during early germination.

There is no doubt that different grains from the same population vary considerably in their physiological activity, but it is most striking that all appear to conform to the same fundamental metabolic pattern and that, especially during the early periods of germination, growth is directly related to activity. This suggests that, during this period, growth is limited by the available respiratory substrate, mainly reducing sugar. Since the reducing sugar arises principally by hydrolysis of sucrose, it would be worthwhile to establish the significance of sucrose concentration in the ungerminated grain in relation to the final establishment of the seedling, and further, whether varieties that have very high levels of sucrose (varieties used in the canning industry) differ in any fundamental way from Early Pearl and Hickory King. The results suggest that most varieties should conform to the same general pattern.

It may be concluded, therefore, that the respiratory substrates during early germination are lipid and reducing sugar which is derived mainly from sucrose and to a lesser extent from raffinose and starch, and that as germination proceeds the activity of alpha- and beta-

amylase increases so that, as a result of their hydrolytic action on the endosperm, they contribute increasingly to the respiratory substrate. After 96 hours, reducing sugar production exceeds respiratory utilisation and this results in an accumulation of sucrose.

REFERENCES.

Adronescu, D.I., 1919. Germination and further development of the embryo of Zea mays separated from the endosperm. Amer. J. Bot., 6,443-452.

American Society of Brewing Chemists, 1958. Methods of analysis, 6th edition.

Badenhuizen, N.P., 1959. Chemistry and biology of the starch granule. Protoplasmatologia II, B, 2, b, delta. Springer-verlag, Vienna.

Bailey, J.M. and Whelan, W.J., 1957. Mechanism of carbohydrase action. 3. The action pattern of beta-amylase. Biochem. J., 67,540-547.

Baldwin, E., 1957. Dynamic aspects of biochemistry. Cambridge University press.

Bell, D.J., 1955. In modern methods of plant analysis Vol. II, page 20. Edited by Paech, K., and Tracey, M.V. Springer-verlag, Berlin, Göttingen and Heidelberg.

Bendelow, V.M., 1963. Modified procedure for the determination of diastatic activity and alpha-amylase activity. J. Inst. Brew., 69,467-472.

Bernfeld, P., 1951. Enzymes of starch degradation and synthesis. Advances in Enzymol., II, 380-424.

Bernstein, L., 1943. Amylases and carbohydrates in developing maize endosperm. Amer. J. Bot., 30, 517-526.

Bond, A.B., and Glass, R.L., 1963. The sugars of germinating corn (Zea mays). Cereal Chem., 40,459-466.

Bottomley, R.A., Christensen, C.M. and Geddes, W.F., 1950. Grain storage studies. IX. The influence of various temperatures, humidities, and oxygen concentrations on mold growth and biochemical changes in stored yellow corn. Cereal Chem., 27,271-296.

Bottomley, R.A., Christensen, C.M. and Geddes, W.F., 1952. Grain storage studies. X. The influence of aeration, time, and moisture content on fat acidity, non-reducing sugars, and mold flora of stored yellow corn. Cereal Chem., 29,53-64.

Briggs, D.E., 1967. Modified assay for alpha-amylase in germinating barley. J. Inst. Brew., 73,361-370.

Croxton, F.E., 1959. Elementary statistics with applications in medicine and the biological sciences. Dover Publications, Inc. New York.

Drennan, D.S.H., 1962. Physiological studies of germination in the genus Avena. II. Changes in some metabolites during the germination of grains of A. sativa. New. Phytol., 61,261-265.

Drennan, D.S.H. and Berrie, A.M.M., 1962. Physiological studies of germination in the genus Avena. I. The development of amylase activity. New. Phytol., 61,1-9.

Dure, L.S., 1960a. Gross nutritional contributions of maize endosperm and scutellum to germination growth of maize axis. Plant Physiol., 35,919-925.

Dure, L.S., 1960b. Site of origin and extent of activity of amylases in maize germination. Plant Physiol., 35,925-934.

Edelman, J., Shibko, S.I. and Keys, A.J., 1959. Role of the scutellum of cereal seedlings in the synthesis and transport of sucrose. J. Expt. Bot., 10,178-189.

Fink, K., Cline, R.E., and Fink, R.M., 1963. Paper chromatography of several classes of compound: correlated Rf. values in a variety of solvent systems. Anal. Chem., 35,389-398.

Flood, A.E., Hirst, E.L., and Jones, J.K.N., 1947. Quantitative estimation of mixtures of sugars by the paper chromatogram method. Nature, 160,86-87.

Geddes, N.F., 1946. The amylases of wheat and their significance in milling and baking technology. Advances in Enzymol., 6,416-463.

Hageman, R.H. and Hanson, J.B., 1955. Carbohydrase activity of cytoplasmic particles prepared from corn scutellum. Plant Physiol., 30 Suppl.:iv.

Hawthorn, J.R., 1947. Micro-estimation of sugars separated on the filter paper chromatogram. Nature, 160,714.

Hellebust, J.A., and Forward, D.F., 1962. The invertase of the corn radicle and its activity in successive stages of growth. Can. J. Bot., 40,113-126.

Hopkins, C.G., 1898. The Chemistry of the corn kernel. Ill. Agr. Expt. Sta. Bull. 53.

Hopkins, R.H., 1946. The action of the amylases. Advances in Enzymol., 6,386-412.

Hopkins, C.G., Smith, L.H., and East, E.M., 1903. The structure of the corn kernel and the composition of its different parts. Ill. Agr. Expt. Sta. Bull. 87.

Horning, E.S. and Petrie, A.H.K., 1927. The enzymatic function of mitochondria in the germination of cereals. Proc. Royal Society, B, 102,188-206.

Horwitz, W., 1955. Official methods of analysis of the association of official agricultural chemists. Editor, Horwitz, W. Eighth edition.

Jermyn, M.A., and Isherwood, F.A., 1949. Improved separation of sugars on the paper partition chromatogram. Biochem. J., 44,402-407.

Keilin, D., and Hartree, E.F., 1948. Properties of glucose oxidase (Notatin). Biochem. J., 42,221-229.

Keilin, D., and Hartree, E.F., 1952. Specificity of glucose oxidase (Notatin). Biochem. J., 50,331.

Kirsop., B.H., 1953. Rapid method of estimating the activity of beta-amylase in barley malts. J. Inst. Brew., 59,378-381.

Kneen, E., 1945. Sorghum amylase. Cereal Chem., 22, 112-134.

Kneen, E., Miller, B.S., and Sandstedt, R.M., 1942. Influence of temperature on the development of amylase in germinating wheat. Cereal Chem., 19,11-27.

Kneen, E., and Sandstedt, R.M., 1941. Beta-amylase activity and its determination in germinated and ungerminated cereals. Cereal Chem., 18,237-252.

Lampe, L., 1931. A microchemical and morphological study of the developing endosperm of maize. Bot. Gaz., 91,337-376.

Loomis, W.E., and Shull, C.A., 1937. Methods in plant physiology. McGraw-Hill, New York.

MacLeod, A.M., 1951. Sugars and amino acids of the barley grain. J. Inst. Brew., 57, 163-169.

MacLeod, A.M., 1952. Studies on the free sugars of the barley grain. I. Historical survey. J. Inst. Brew., 58,270-276.

MacLeod, A.M., 1953b. Studies on the free sugars of the barley grain. IV. Low molecular fructosans. J. Inst. Brew., 59,462-469.

MacLeod, A.M., 1960. Barley carbohydrate metabolism in relation to malting. Wallerstein Labs. Communs., 23,87-98.

MacLeod, A.M., Travis, D.C., and Wreay, D.G., 1953a. Studies on the free sugars of the barley grain. III. Changes in sugar content during malting. J. Inst. Brew., 59,154-165.

Malhotra, R.C., 1934. Chemistry of corn seed germination. Cereal Chem., 11,105-109.

McComb, R.B., Yushok, W.D , and Batt, W.G., 1957. 2-de-oxy-d-glucose, a new substrate for glucose oxidase (Glucose aero dehydrogenase). J. Franklin Inst., 263,161-165.

Meredith, W.O.S., 1965. Combined action of alpha- and beta-amylases in saccharification. American Society of Brewing Chemists Proceedings, 1965, 5-10.

Miller, G.L., 1959. Use of dinitrosalicylic acid reagent for determination of reducing sugar. Anal. Chem., 31,426-428.

Myrback, K., and Neumuller, G., 1950. Amylases and the hydrolysis of strarch and glycogen. In : The Enzyme, J.B. Sumner and K. Myrback Eds. Volume 1, 1st. edition. Academic Press, New York.

Nelson, N., 1944. A photometric adaptation of the Somogyi method for the determination of glucose. J. Biol. Chem., 153,375-380.

Ohlsson, E., 1922. Sur L'existence de deux ferments amylolytiques dans la diastase du malt. Compt. rend soc. biol., 87,1183-1184.

Ohlsson, E., 1930. Uber die beiden Komponenten der malzdiastase, besonders mit Rüchsicht auf die Mutarotation der bei der Hydrolyse der Stärke gebildeten produkte. Zeits. Physiol. Chem., 189,17-63.

Olson, W.J., Evans, R., and Dickson, A.D., 1944. A modification of the Kneen Sandsted method for determination of alpha- and beta-amylase in barley malt. Cereal Chem., 21,533-539.

Partridge, S.M., 1946. Application of the paper partition chromatogram to the qualitative analysis of reducing sugars. Nature, 158,270.

Partridge, S.M., 1948. Filter paper partition chromatography of sugars. 2. An examination of the blood group of a specific substance from Hog Gastric Mucin and the specific Polysaccaride of Bacterium dysenteria (Shiga). Biochem. J., 42,241-253.

Peat, S., 1951 The biological transfomations of starch. Advances in Enzymol., II,339-373.

Pigman, W., 1957. The Carbohydrates. Academic Press, New York.

Sandstedt, R.M., Kneen, E., and Blish, M.A. 1939. A standardised Wohlgemuth procedure for alpha-amylase activity. Cereal Chem., 16,712-723.

Schwimmer, S., 1950. Kinetics of malt alpha-amylase action. J. Biol. Chem., 186,181-193.

Sigma Technical Bulletin No. 510, 1967. The enzymatic/colorimetric determination of glucose in whole blood, plasma or serum at 425-510 mμ. Sigma Chemical Company, St. Louis, U.S.A.

Steel, R.G.D., and Torrie, J.H., 1960. Principles and Procedures of Statistics. McGraw-hill Book Company Inc., New York.

Sumner, J.B., and Somers, G.F., 1953. The chemistry and methods of enzymes. Academic Press, New York.

Taufel, K., Steinbach, K.J., and Hartman, B. 1960. The lower saccharides of corn and their behaviour during storage and germination. Nahrung, 4,452-465.

Toole, E., 1924. The transformations and the course of development of germinating maize. Amer. J. Bot., 11,325-350.

Waldt, L.M., 1952. Instrumental approach to diastatic power determination. Proc. A.M. Amer. Soc. Brew. Chem., 17-20.

Weill, C.E., and Caldwell, M.L., 1945. Essential groups of beta-amylase. II. Sulfhydryl groups. J. Amer. Chem. Soc., 67,214-217.

Wildner, H. 1958. Methoden zur messung der enzymatischen amylolyse. Hans. Carl, Nürnberg.

CHANGES IN CARBOHYDRATE CONCENTRATION AND AMYLOLYTIC ACTIVITY IN GERMINATING MAIZE.

VOLUME II

Tables and Figures.

A dissertation presented to Rhodes University for the degree of Doctor of Philosophy.

Charles M. Breen

December, 1969.

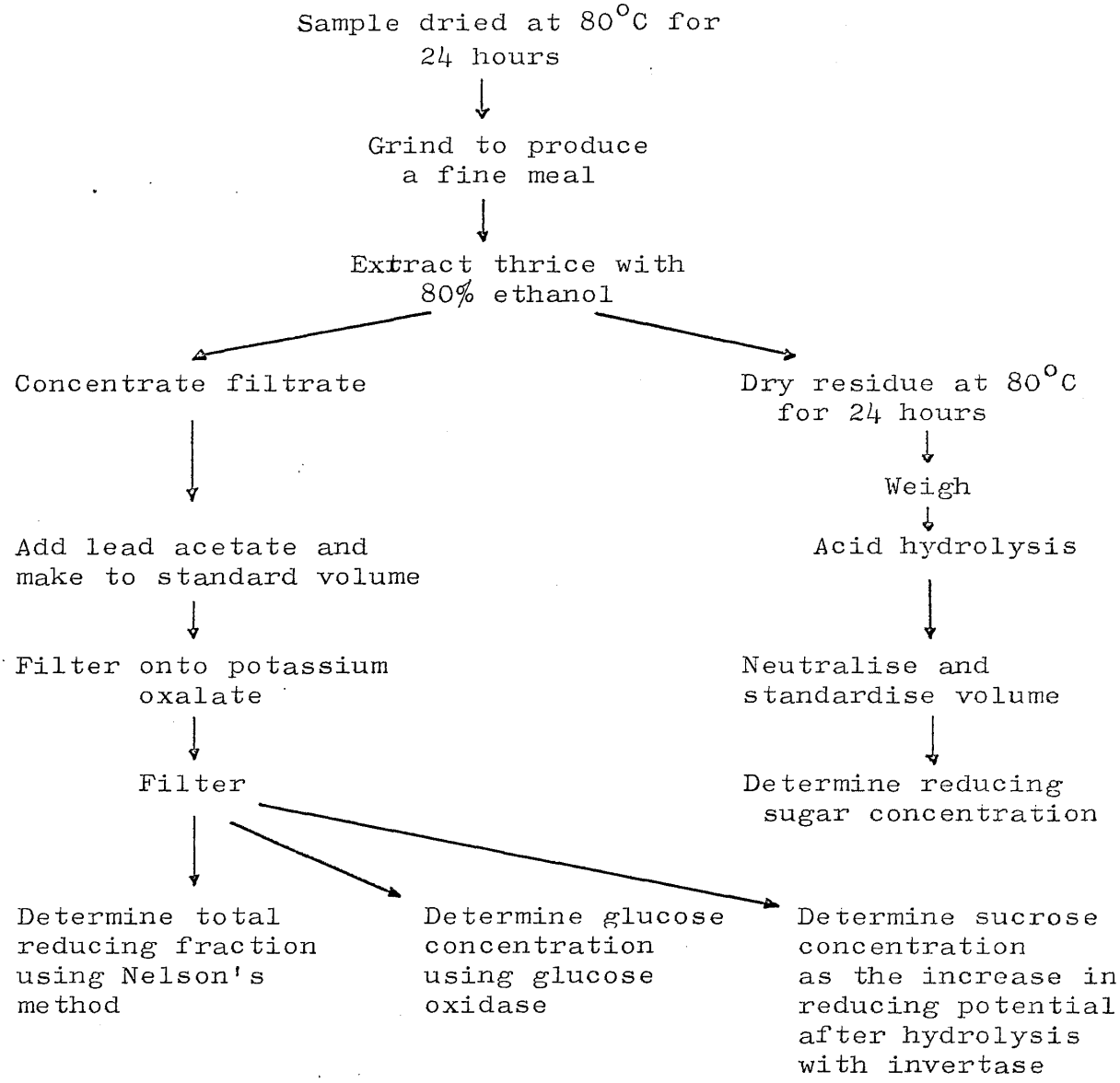

Fig.1. Flow sheet of the procedure adopted to determine reducing sugar, glucose, sucrose and dextrin concentration in germinating maize.

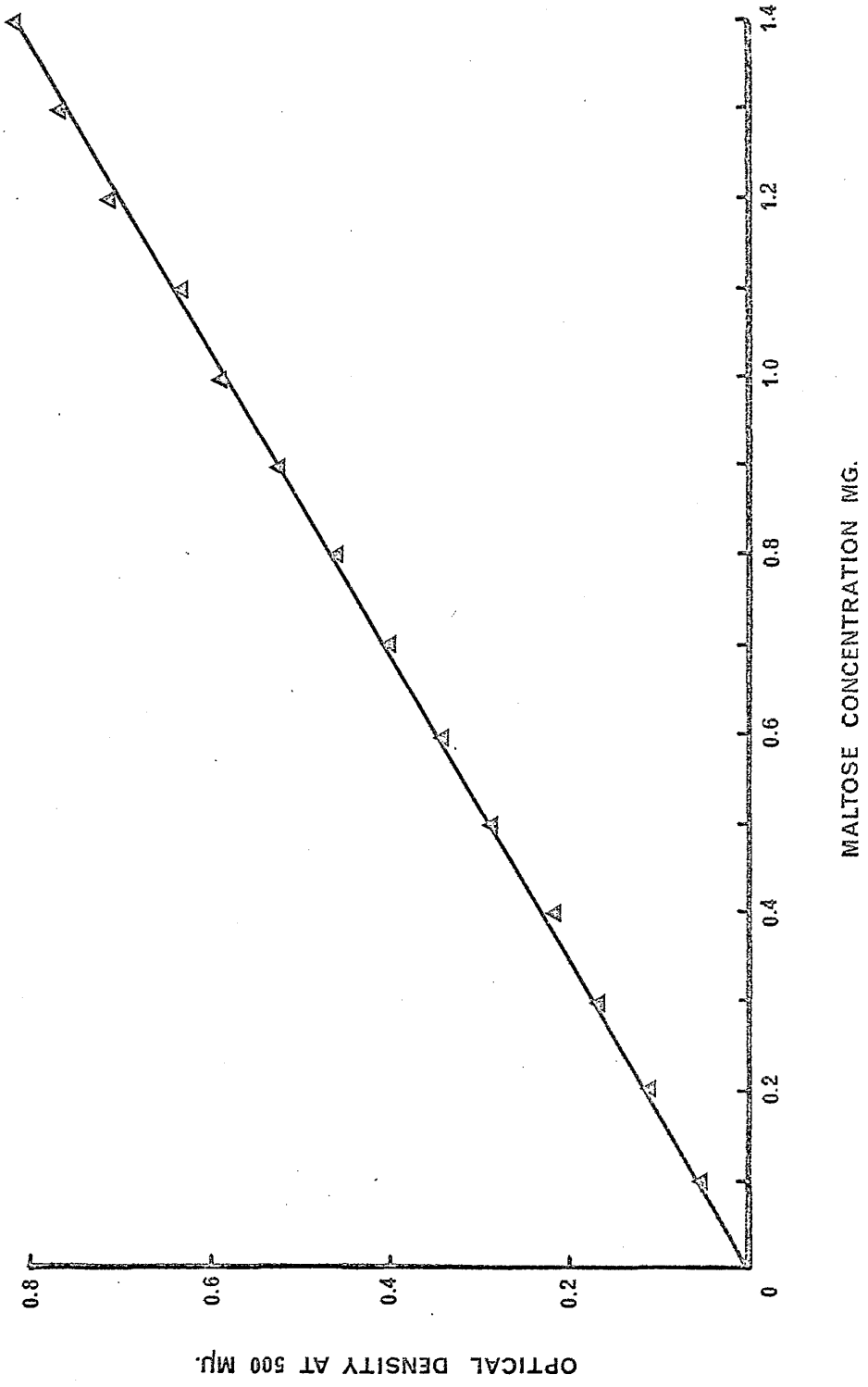

Fig. 2. Calibration graph relating maltose concentration (mgms.) and optical density using Nelson's method.

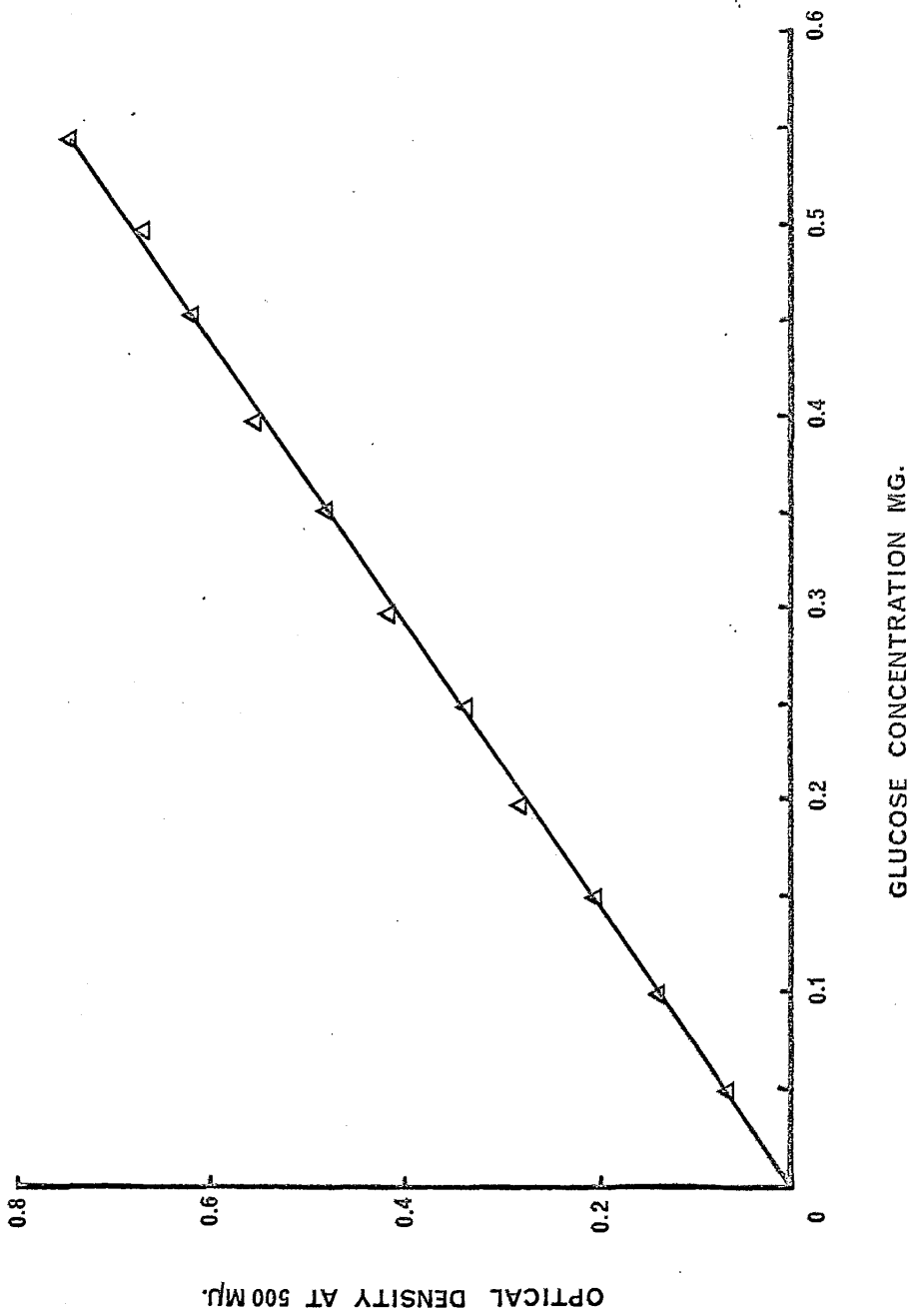

Fig. 3. Calibration graph relating glucose concentration (mgms.) and optical density using Nelson's method.

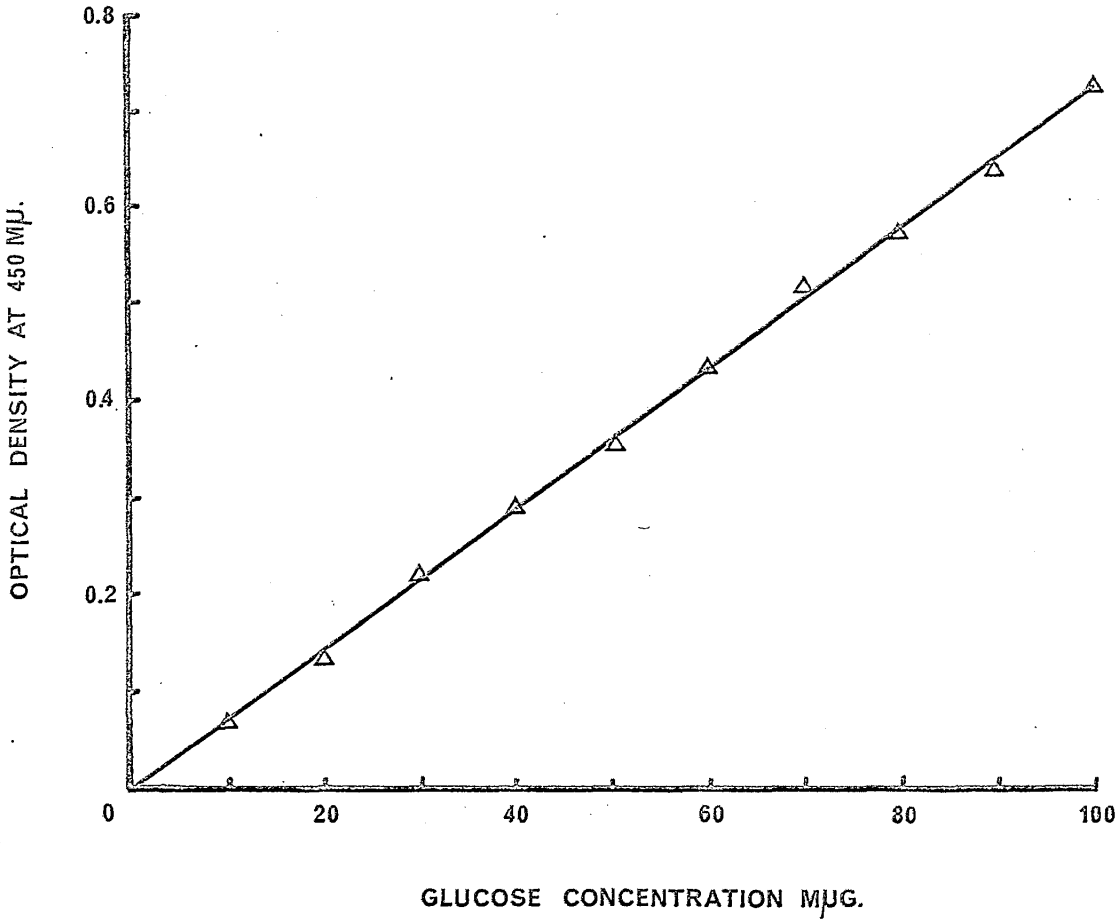

Fig. 4. Calibration graph relating glucose concentration (micrograms) and optical density using glucose oxidase method.

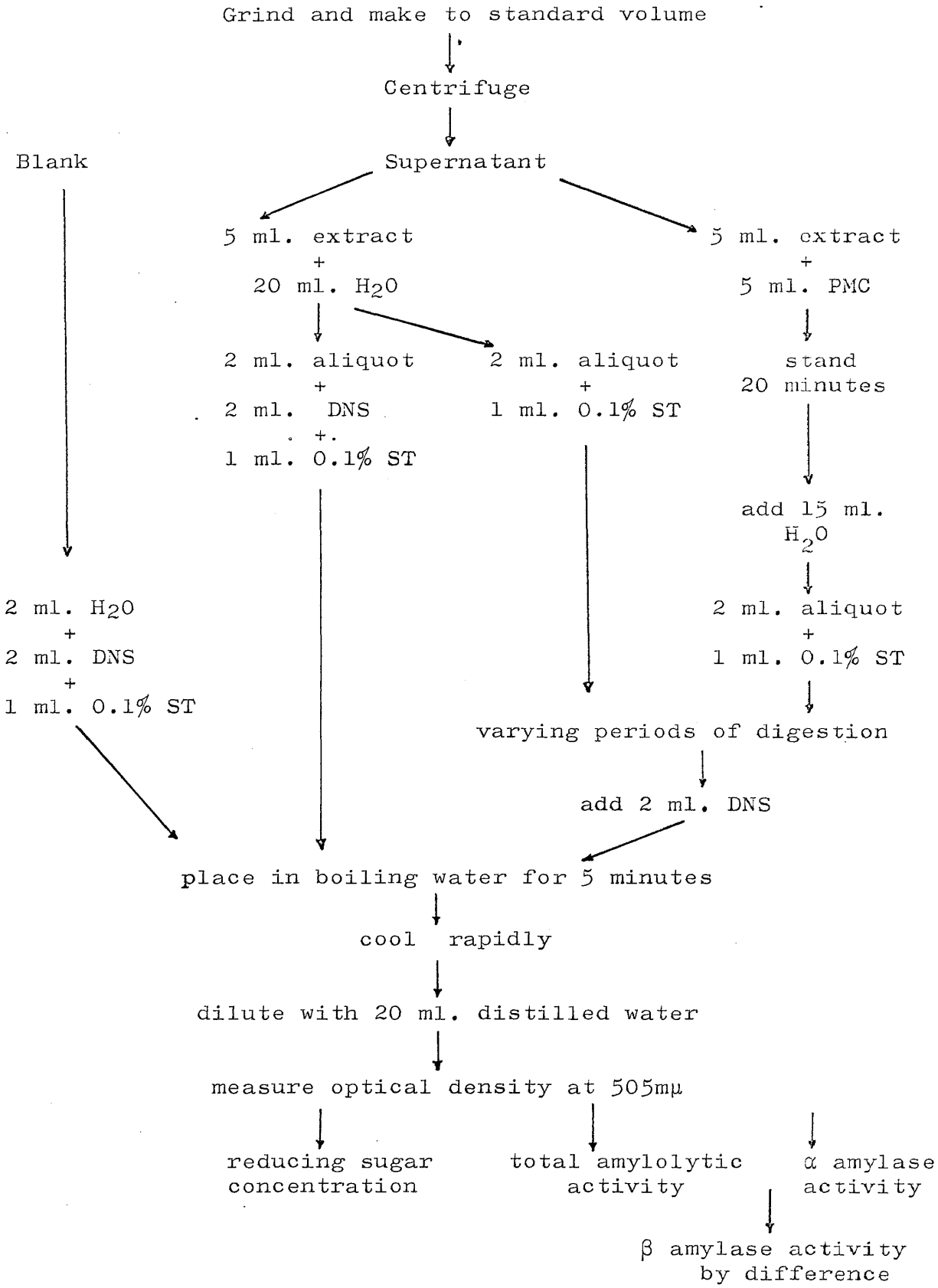

Fig.5 Flow sheet of procedure adopted to determine reducing sugar concentration, total amylolytic activity, α amylase activity and β amylase activity by difference. PMC phenyl mercury chloride, ST starch, DNS dinitrosalicylic acid.

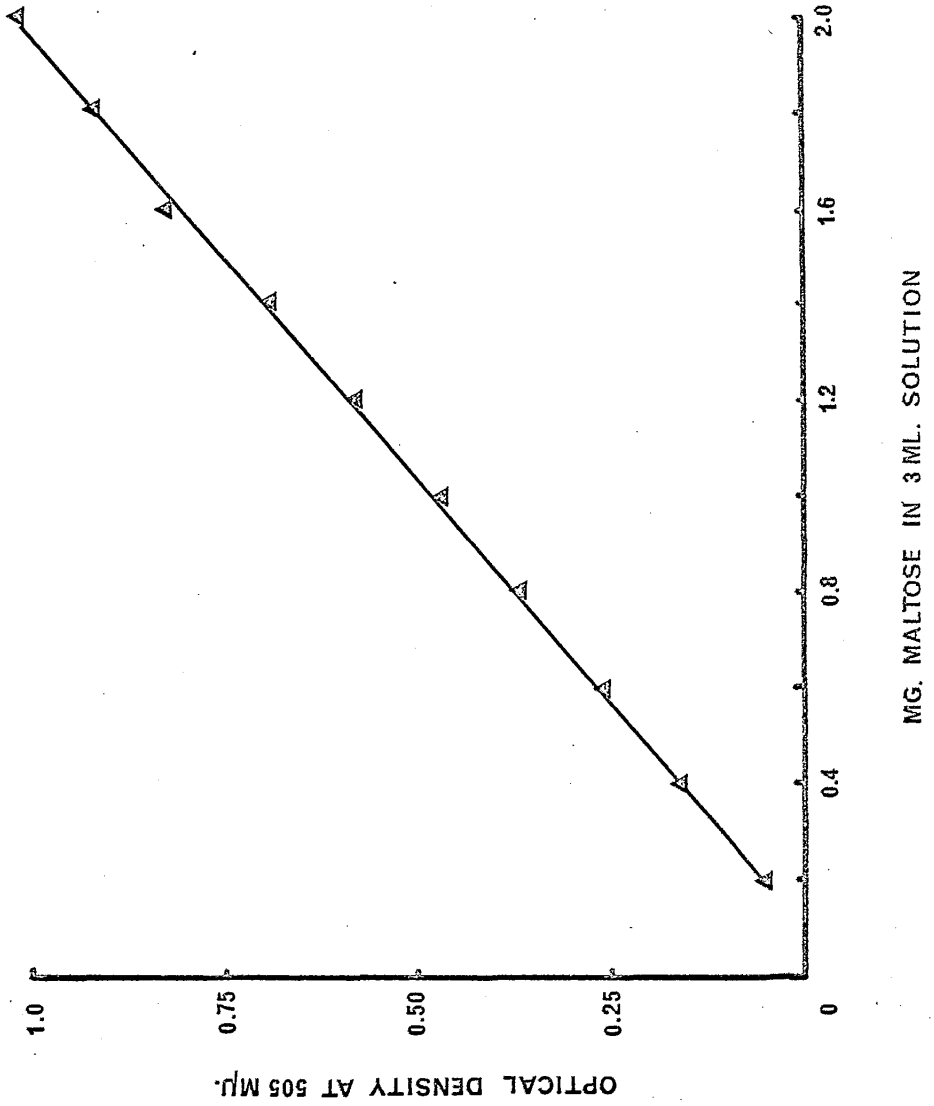

Fig. 6. Calibration graph relating maltose concentration (mgms.) and optical density using the dinitrosalicylic acid method.

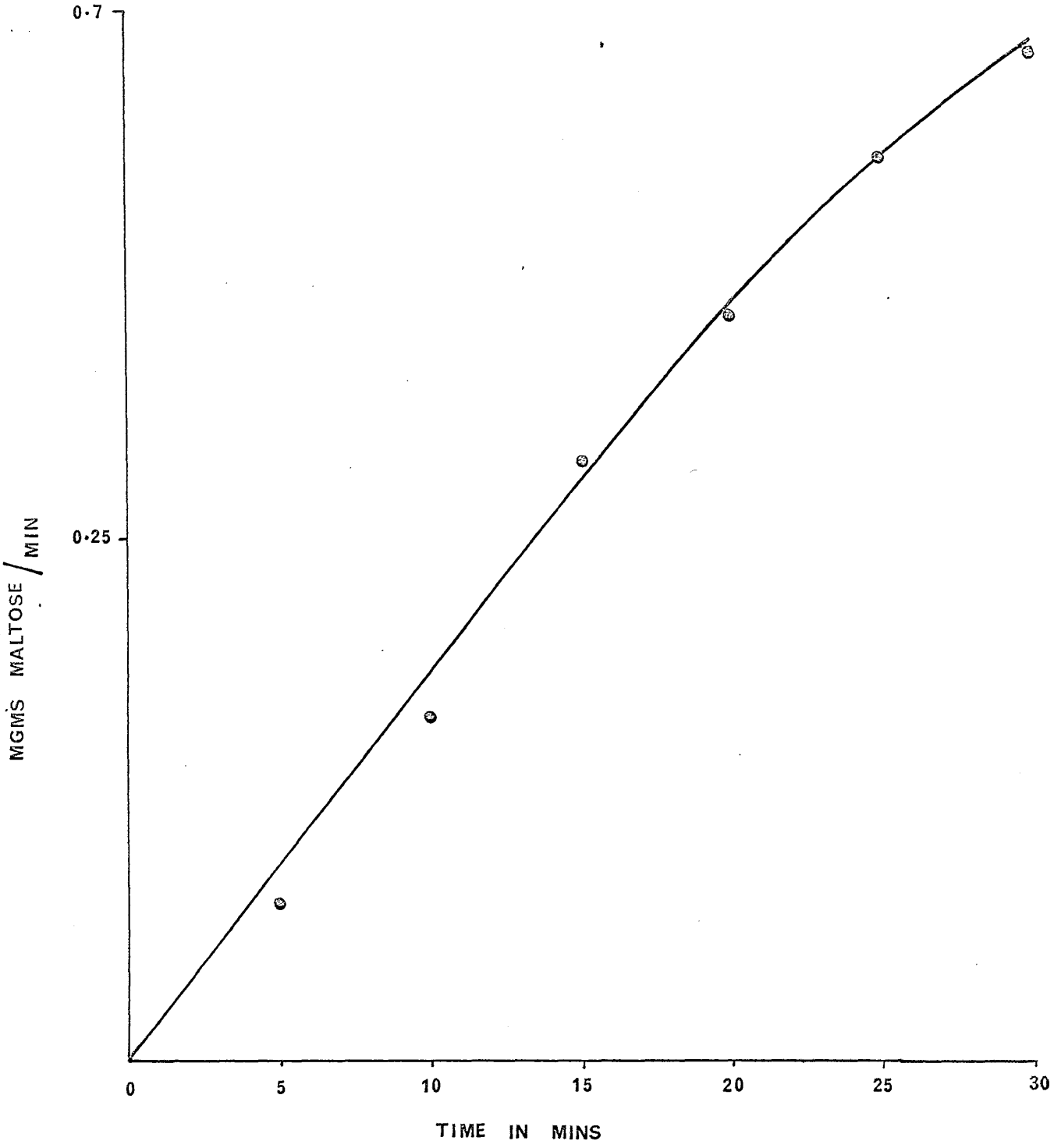

Fig. 7. Progress curve of mgms. maltose produced with time. Substrate becomes limiting after approximately 20 mins. Provided the rate of maltose production did not exceed 0.02 mgms. maltose/min. (rate calculated from initial velocity), it was assumed the substrate was not limiting.

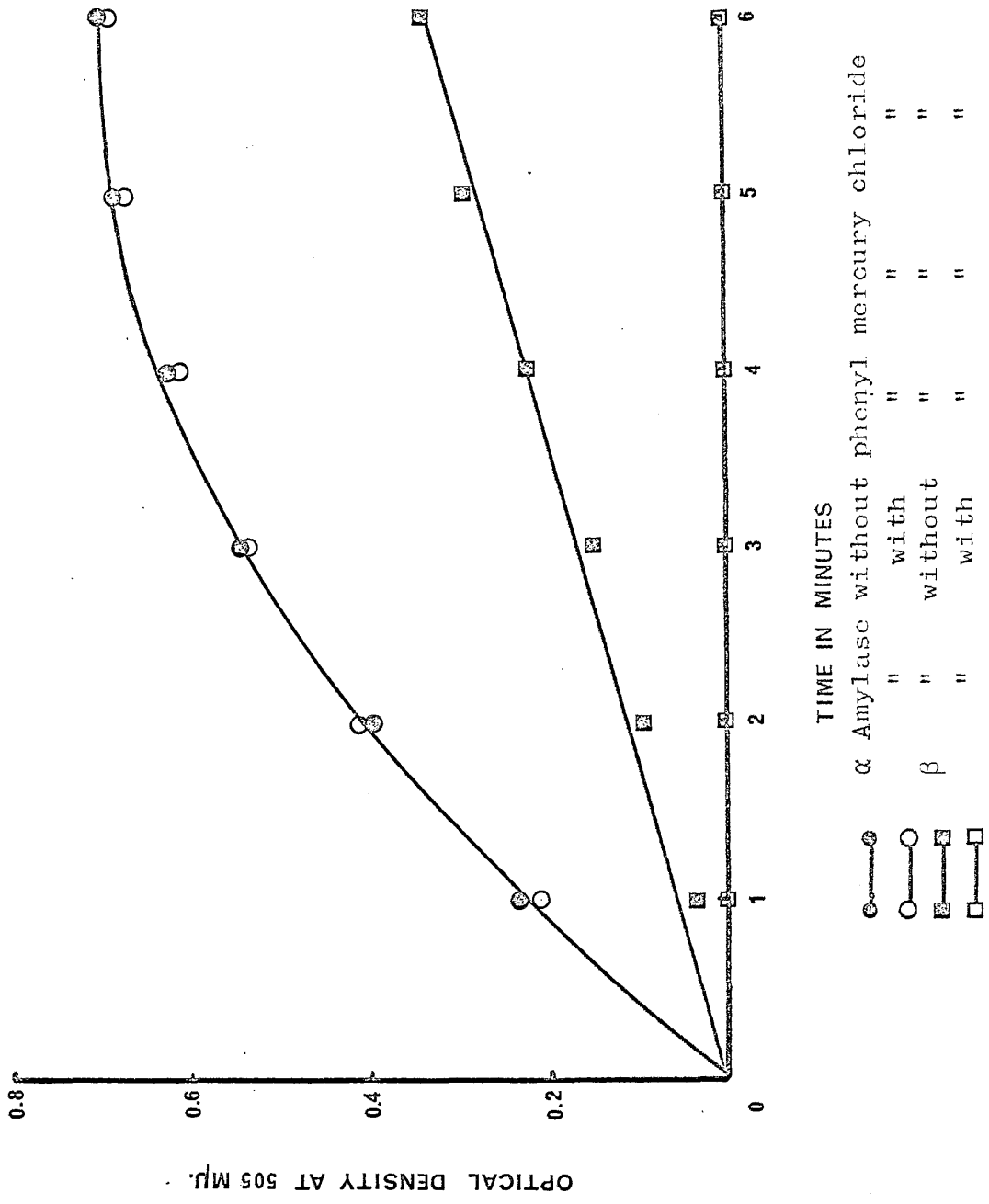

Fig. 8. The effect of phenyl mercury chloride on the action of bacterial alpha- and cereal beta-amylase. Phenyl mercury chloride inhibits beta-amylase but has no measureable effect on alpha-amylase activity.

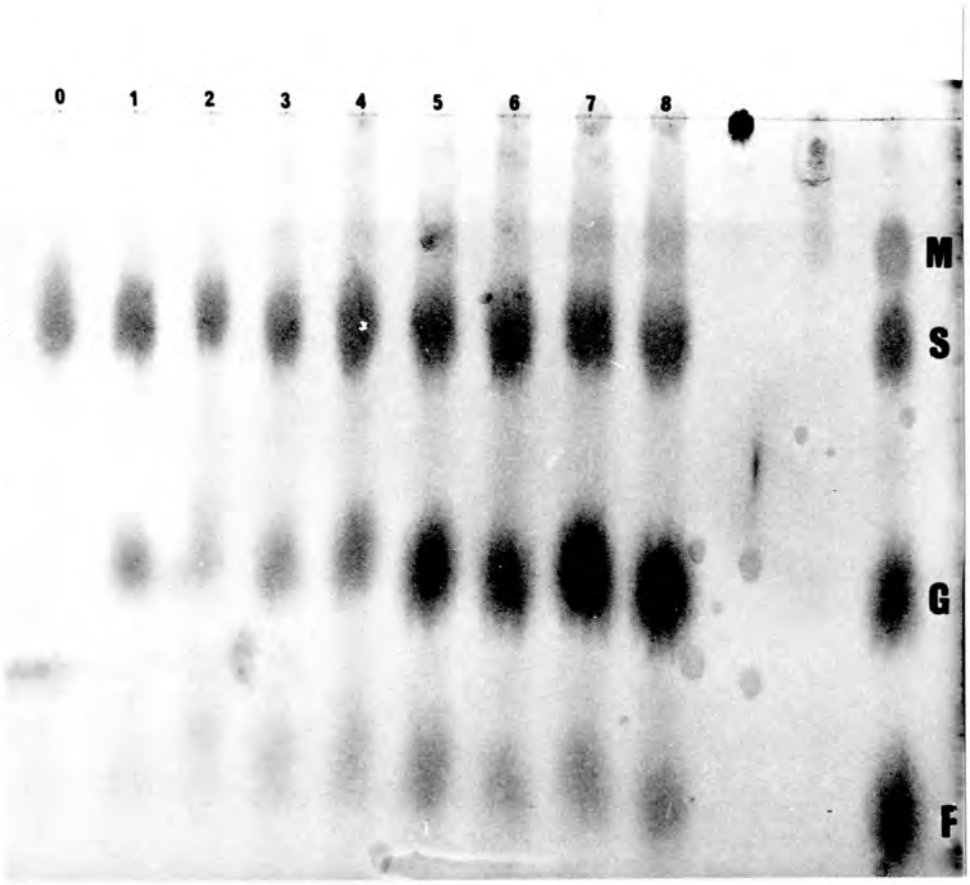

Plate 1. Composition of 80% ethanol extract of grains of Zea mays L. var. Hickory King, during the first 8 days of germination. Chromatogram run with ethyl acetate, acetic acid, formic acid, water (36:6:2:3). M-maltose, S-sucrose, G-glucose, F-fructose.

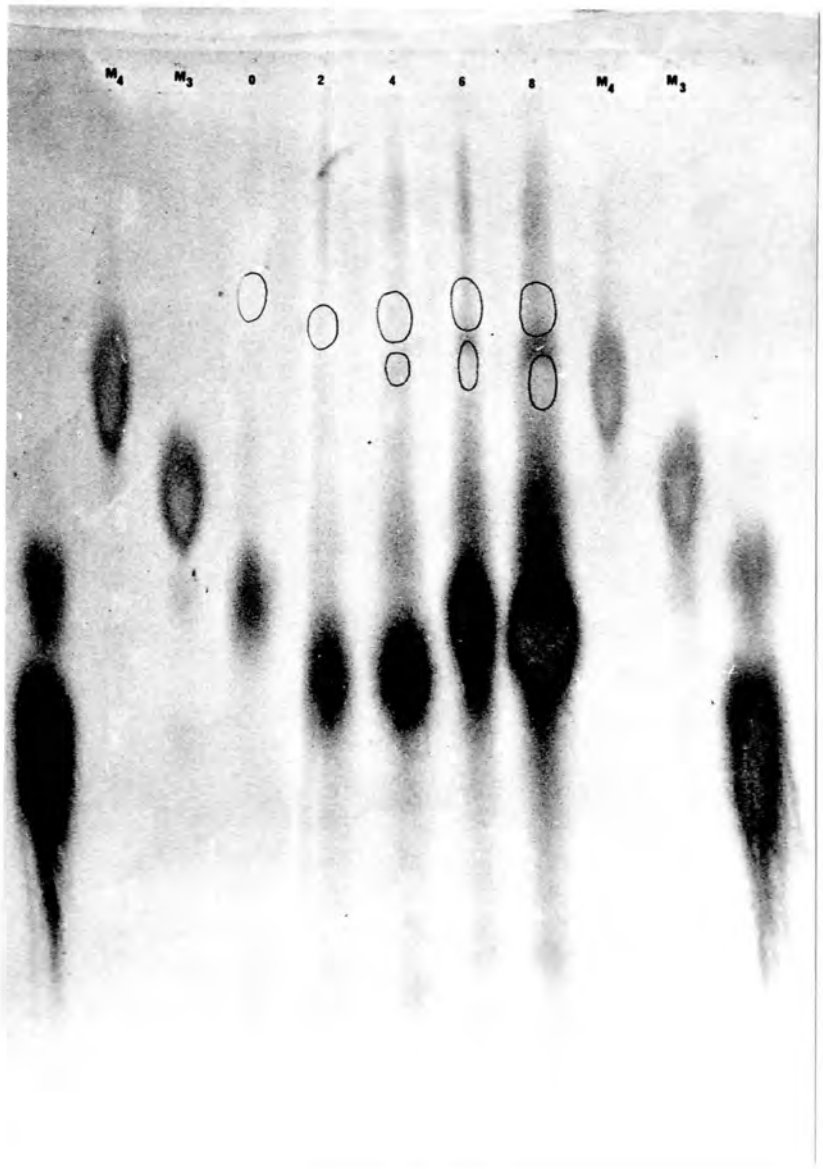

Plate 2. Composition of 80% ethanol extract of grains of Zea mays L. var Hickory King, during the first 8 days of germination. Chromatogram run with ethyl acetate, isopropyl alcohol, water (6:1:3). M4 - maltotetraose, M3 - maltotriose.

TABLE 1

COMPOSITION OF 80% ETHANOL EXTRACTS OF GRAINS OF ZEA MAYS L. VAR HICKORY KING, DURING THE FIRST EIGHT DAYS OF GERMINATION

Marker Sugars	Rf* Values	Days Germinated 0	1	2	3	4	5	6	7	8
Fructose	1.000	-	+	+	+	++	++	+++	+++	+++
Glucose	0.688	+?	++	+	+	++	+++	+++	+++	+++
Sucrose	0.322	+++	++	++	+++	+++	+++	+++	+++	+++
Maltose	0.183	-	-	-	+?	+	++	++	++	++

\- No visible spot

\+ ++ +++ Increasing density of spots

* Rf Expressed as $\frac{\text{Distance Travelled}}{\text{Distance Travelled by fructose.}}$

TABLE 2

REDUCING SUGAR CONCENTRATION (% DRY WEIGHT) IN GROUPS OF HICKORY KING GRAIN DURING GERMINATION. ASSAY WAS REPLICATED THREE TIMES ON EACH GROUP.

Germination Period (Hrs)	Replication Number 1	2	3	Mean	Germination Period Mean
0	0.072	0.083	0.069	0.075	
0	0.052	0.042	0.055	0.050	0.061
0	0.052	0.059	0.062	0.058	
24	0.131	0.092	0.122	0.115	
24	0.110	0.110	0.106	0.109	0.118
24	0.113	0.163	0.119	0.132	
48	0.186	0.174	0.178	0.179	
48	0.261	0.266	0.245	0.257	0.246
48	0.312	0.298	0.291	0.300	
72	0.096	0.087	0.114	0.099	
72	0.119	0.124	0.143	0.129	0.138
72	0.227	0.186	0.144	0.186	
96	0.284	0.255	0.241	0.260	
96	0.241	0.238	0.230	0.236	0.246
96	0.225	0.257	0.241	0.241	
120	0.628	0.654	0.619	0.634	
120	0.823	0.734	0.658	0.738	0.685
120	0.720	0.630	0.695	0.682	
144	0.745	0.792	0.792	0.776	
144	1.193	1.192	1.233	1.206	0.934
144	0.802	0.838	0.819	0.820	
168	1.543	1.570	1.567	1.560	
168	1.088	1.003	0.813	0.968	1.240
168	1.140	1.186	1.247	1.191	
192	0.813	0.809	0.809	0.811	
192	1.159	1.038	1.199	1.132	0.956
192	0.906	0.934	0.934	0.924	
216	0.866	0.957	0.928	0.917	
216	1.025	1.026	1.019	1.024	1.064
216	1.251	1.250	1.257	1.253	
240	1.062	1.106	1.142	1.103	
240	1.021	0.997	0.993	1.004	1.374
240	1.983	2.018	2.049	2.017	

L. S. D. Between Observations at 0.05 P. L. ± 0.012

" " " " Means " " " " ± 0.075

" " " " Germination Period Means " " ± 0.036

TABLE 3a

ANALYSIS OF VARIANCE DATA FOR REDUCING SUGAR CONCENTRATION IN GROUPS OF HICKORY KING GRAIN.

Source	Degrees of Freedom	Sum of Squares	Variance	Variance Ratio(F)	Observations
Germination	10	21.942371	2.194237	1282.861	* * *
Replication	2	0.000214	0.000107	0.063	N. S.
Sample	2	0.239217	0.119609	69.929	* * *
Sample / Rep.	4	0.010109	0.002527	1.478	N. S.
Rep / Germ.	20	0.022577	0.001129	0.660	N. S.
Sample / Germ.	20	2.892714	0.144636	85.561	* * *
Error	40	0.068417	0.001710		

* * * Significant at 0.1% Probability Level.

TABLE 3b

RESULTS OF LINEAR REGRESSION OF REDUCING SUGAR CONCENTRATION AND LENGTH OF RADICLE, COLEOPTILE AND LATERAL ROOTS IN GROUPS OF HICKORY KING GRAINS.

Factor	Germination Period Hours.	Correlation Coefficient	t	Significance
Reducing Sugar Radicle Length	0-240	+ 0.895	11.170	* * *
	0-168	+ 0.939	12.805	* * *
	48-168	+ 0.918	9.340	* * *
	192-240	+ 0.661	-	-
Reducing Sugar Coleoptile Length	0-240	+ 0.850	8.948	* * *
	0-168	+ 0.954	15.090	* * *
	72-168	+ 0.940	9.934	* * *
	192-240	+ 0.415	-	-
Reducing Sugar Lateral Root Length	0-240	+ 0.859	9.339	* * *
	0-168	+ 0.950	14.271	* * *
	72-168	+ 0.936	9.583	* * *
	192-240	+ 0.415	-	-

* * * Significant at 0.1% Probability Level.

TABLE 4

LENGTH (MM.) OF RADICLE, COLEOPTILE AND LATERAL ROOTS (ARISING FROM THE COTYLEDONARY NODE) IN GROUPS OF TEN HICKORY KING GRAINS DURING GERMINATION.

Germination Period Hrs.	Radicle	Coleoptile	Laterals	Growth Index	Mean Radicle	Mean Coleoptile	Mean Laterals	Mean Growth Index
48	-	-	-	-				
48	3	-	-	0.003	3.7	-		0.011
48	8	-	-	0.008				
72	20	5	1	0.026				
72	36	9	-	0.045	39	13	1	0.059
72	61	25	2	0.088				
96	91	44	111	0.246				
96	193	41	172	0.406	151	50	131	0.201
96	169	65	111	0.345				
120	289	82	213	0.584				
120	268	102	402	0.772	268	94	284	0.679
120	249	98	238	0.585				
144	397	204	747	1.348				
144	543	289	962	1.794	437	236	828	0.942
144	371	216	774	1.361				
168	446	268	959	1.673				
168	442	183	670	1.295	454	242	854	2.467
168	473	275	934	1.682				
192	627	299	1609	2.535				
192	675	381	1181	2.237	629	326	1199	2.769
192	584	298	806	1.688				
216	519	398	1272	2.189				
216	492	496	1599	2.587	626	458	1450	2.234
216	868	481	1480	2.829				
240	1029	574	1825	3.428				
240	746	611	2017	3.374	923	571	1953	3.069
240	994	528	2018	3.540				

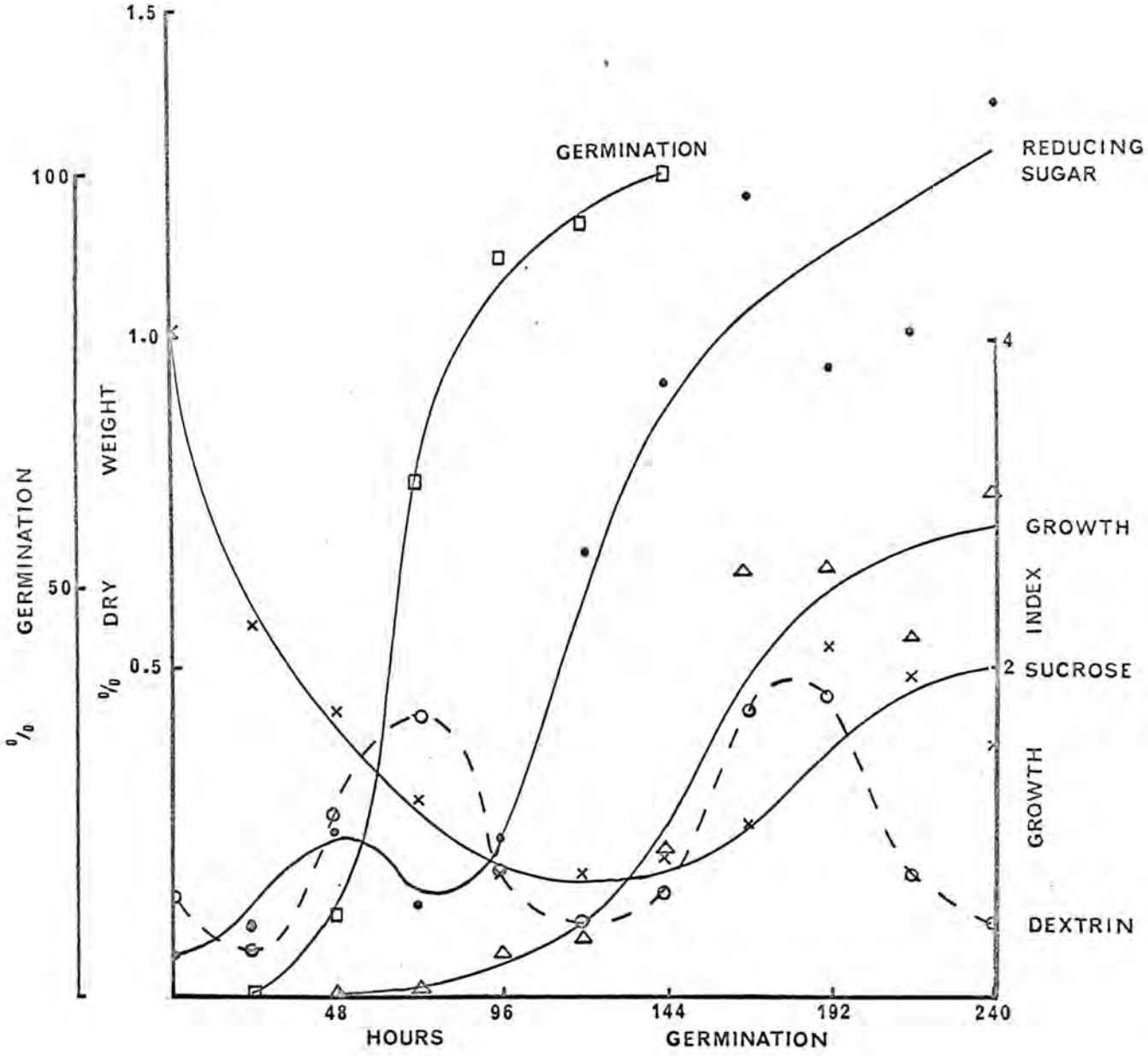

Fig. 9. Changes in reducing sugar, sucrose and dextrin concentrations during germination of groups of Hickory King grain. Growth index expressed as $\frac{\text{sum of lengths of organs measured}}{1000}$

L. S. D. between means for reducing sugar at 0.05 P.L. ± 0.036%
" " " " " " sucrose at 0.05 P.L. ± 0.052%
" " " " " " dextrin at 0.05 P.L. ± 0.064%.

TABLE 5

RESULTS OF LINEAR REGRESSION (CORRELATION COEFFICIENT r) BETWEEN FRESH GRAIN WEIGHT (GRAMS) AND REDUCING SUGAR CONCENTRATION (% DRY WEIGHT) OF GROUPS OF HICKORY KING GRAIN DURING GERMINATION. THREE GROUPS OF GRAIN WERE INVESTIGATED AT EACH GERMINATION PERIOD.

Germination Period Hrs	Grain Weight			Reducing Sugar		
	1	2	3	1	2	3
0	7.27654	6.62649	6.67627	0.075	0.050	0.058
24	6.98059	6.84388	7.10123	0.115	0.109	0.132
48	6.91987	6.59470	6.58299	0.179	0.257	0.300
72	9.06412	9.30341	8.78237	0.099	0.129	0.186
96	7.30477	6.68807	6.70020	0.260	0.236	0.241
120	6.99898	6.75269	6.81125	0.634	0.738	0.682
144	7.05188	7.35524	7.59117	0.776	1.206	0.820
168	7.09407	6.45029	6.86767	1.560	0.968	1.191
192	6.99980	6.57871	7.24432	0.811	1.132	0.924
216	6.65183	6.33114	7.07830	0.917	1.024	1.253
240	6.75708	6.50620	6.53642	1.103	1.004	2.017

Correlation Coefficient	0 - 48 Hours	0 - 120 Hours	0 - 240 Hours
	- 0.326	- 0.627	+ 0.477

NOTE: Because of the small number of observations at each germination period, the results are inconclusive.

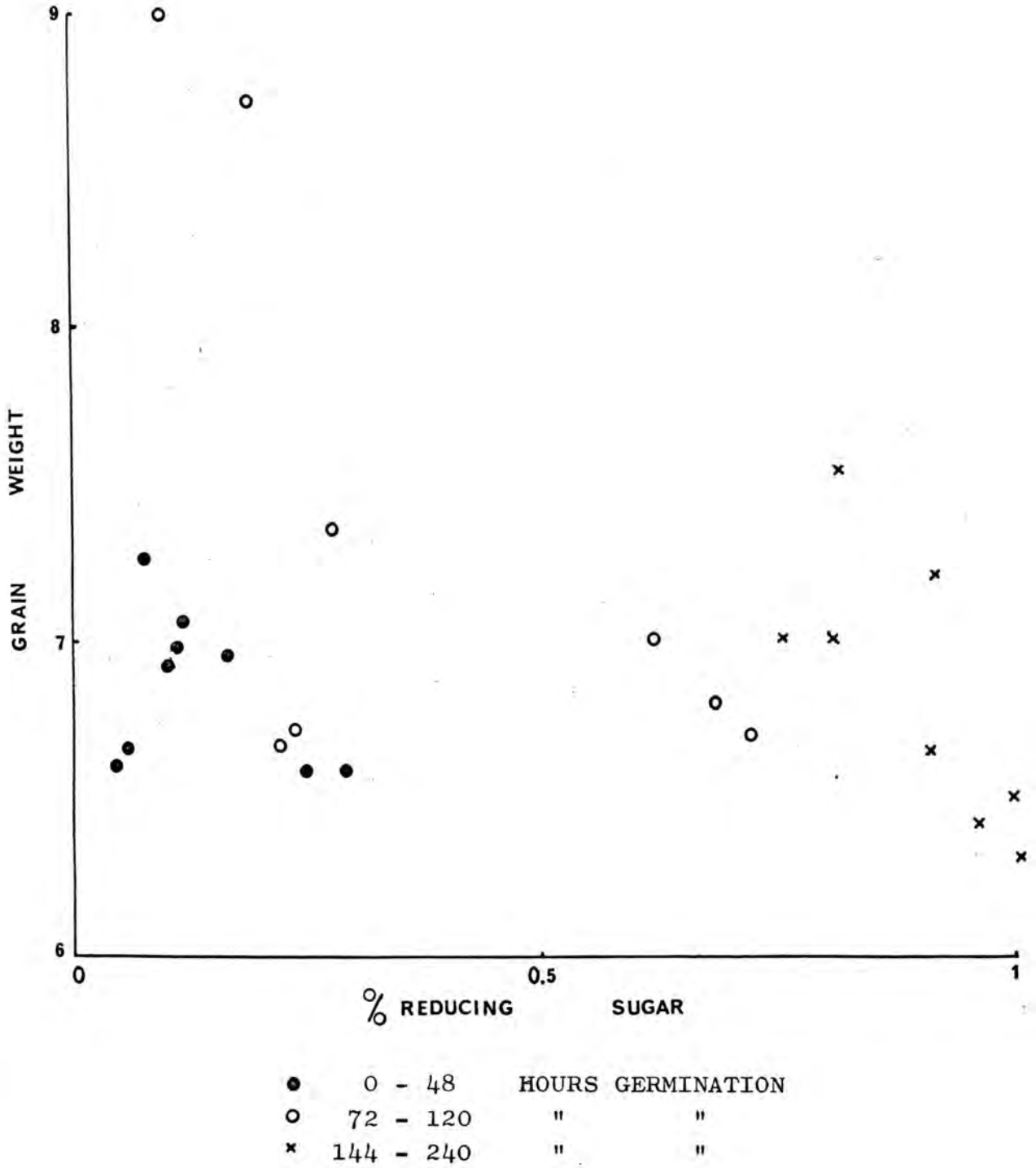

Fig. 10. Scatter plot illustrating correlation between grain weight and reducing sugar concentration (% dry weight) in groups of Hickory King grain. Although a negative correlation may exist, the number of observations are too small to make the results conclusive.

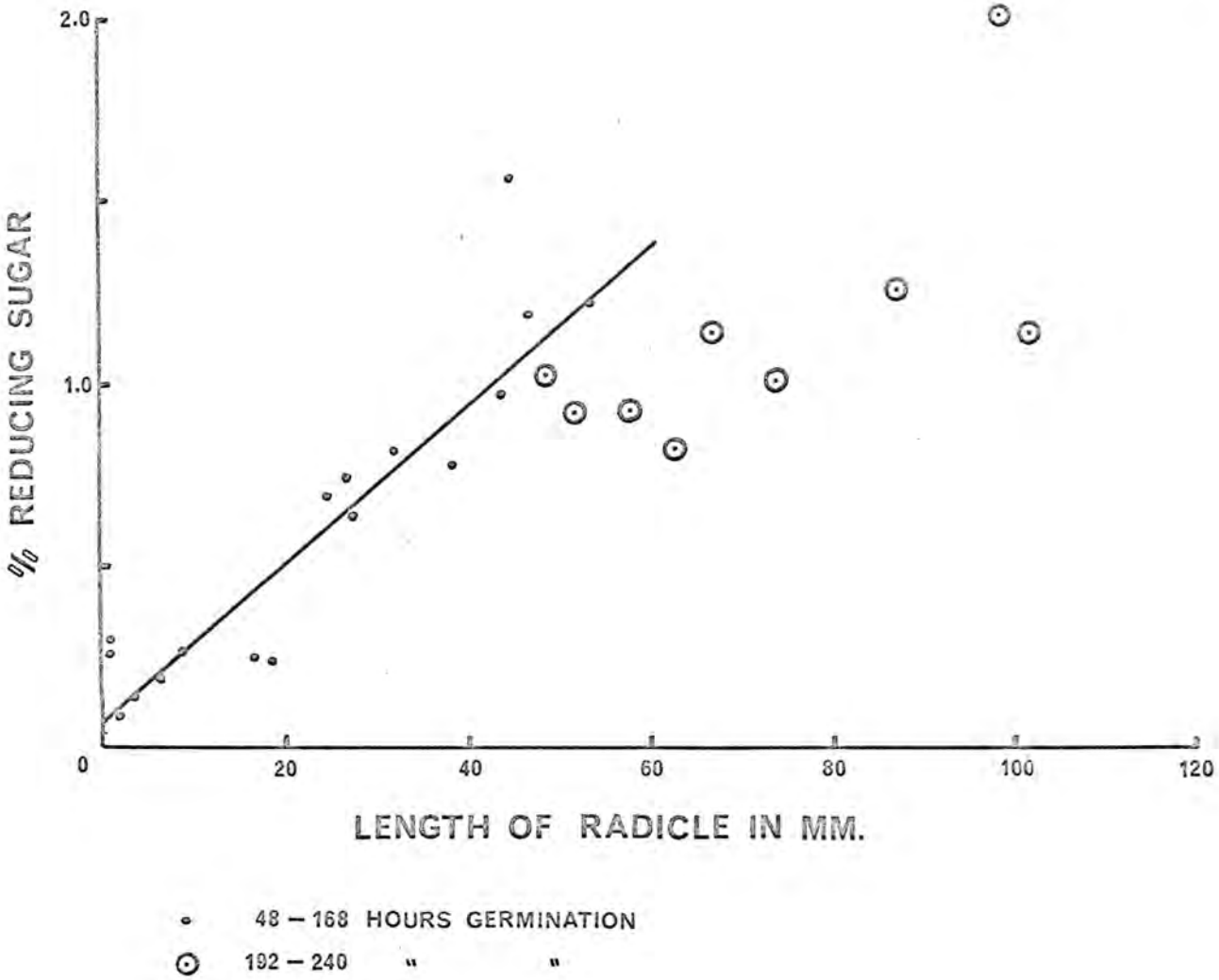

Fig. 11. Scatter plot illustrating correlation between reducing sugar concentration and radicle length in groups of Hickory King grain. Correlation coefficient for 0-168 hours. + 0.939.

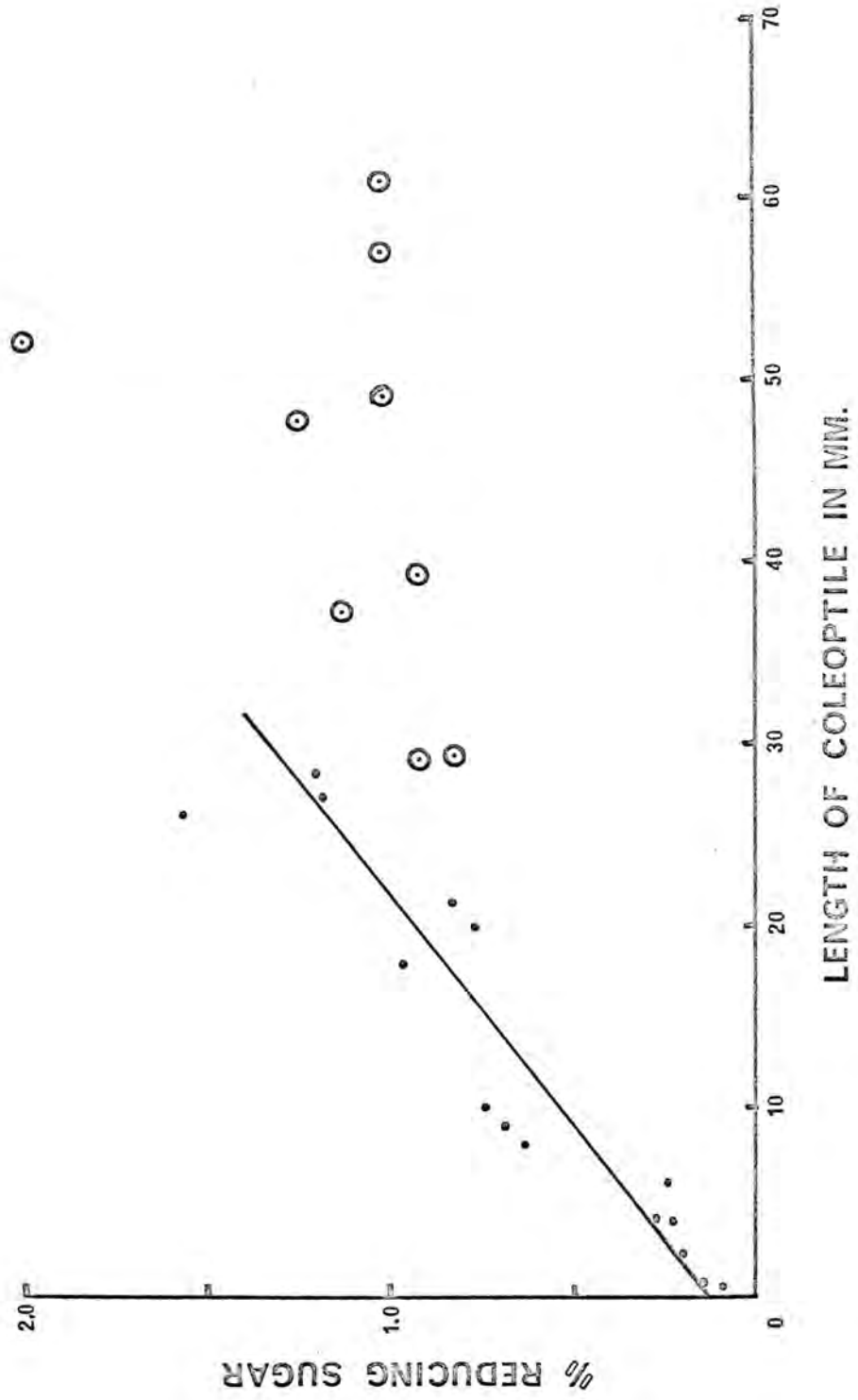

Fig. 12. Scatter plot illustrating correlation between reducing sugar concentration and radicle length in groups of Hickory King grain. Correlation coefficient for 0-168 hours. + 0.954.

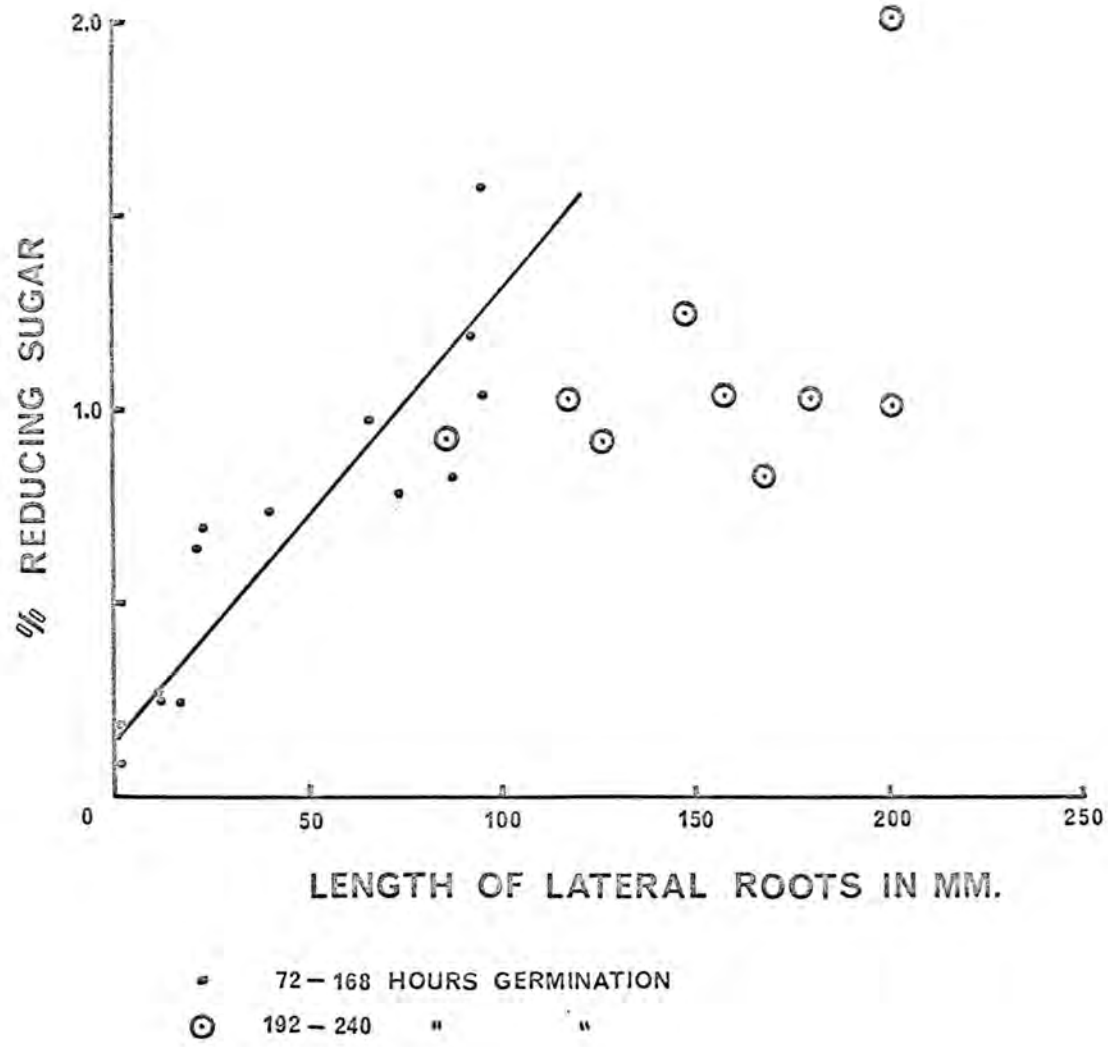

Fig. 13. Scatter plot illustrating correlation between reducing sugar concentration and lateral root length in groups of Hickory King grain. Correlation coefficient for 0-168 hours. + 0.950.

TABLE 6

REDUCING SUGAR CONCENTRATION (% DRY WEIGHT) IN INDIVIDUAL HICKORY KING GRAINS DURING GERMINATION.

(Growth Index = $\frac{\text{length of coleoptile, radicle and lateral roots}}{100}$)

Germination Period (hrs)	1	2	3	4	5	6	7	8	9	10	Mean	% Germination	Growth Index
0	0.013	0.075	0.025	0.143	0.037	0.043	0.103	0.020	0.055	0.030	0.054	0	0
24	0.088	0.135	0.128	0.135	0.074	0.068	0.138	0.172	0.167	0.039	0.114	0	0
48	0.148	0.019	0.011	0.150	0.026	0.330	0.259	0.216	0.018	0.165	0.134	20	0.003
72	0.342	0.251	0.174	0.236	0.275	0.296	0.152	0.253	0.321	0.233	0.253	70	0.059
96	0.583	0.392	0.968	0.822	0.440	0.468	0.626	0.450	0.674	0.151	0.557	90	0.201
120	0.501	0.385	1.330	0.360	0.456	0.527	0.569	0.616	0.711	0.629	0.608	100	0.679
144	0.161	0.240	0.124	0.521	0.127	0.200	0.357	0.607	0.453	0.177	0.297	100	0.942
168	0.421	0.462	1.198	1.214	2.348	0.364	0.538	0.410	0.275	0.539	0.977	100	2.467
192	0.705	0.647	2.048	0.933	3.590	0.735	0.260	2.048	2.279	0.746	1.398	100	2.769
216	0.693	0.768	0.538	0.522	1.009	0.352	3.288	0.796	0.734	0.413	0.911	100	2.234
240	5.460	0.944	0.649	2.016	1.266	0.660	1.367	0.460	0.672	2.612	1.611	100	3.069

NOTE. - In this and all subsequent tables, the data has been corrected to three decimal places after calculation of the mean. The mean is, therefore, not necessarily the mean of the values in the table.

Least Significant Difference Between Observations at 0.05 Probability Level ± 0.184
" " " " Means " " ± 0.610

TABLE 7a

ANALYSIS OF VARIANCE DATA FOR REDUCING SUGAR CONCENTRATION IN INDIVIDUAL HICKORY KING GRAINS.

Source	Degree of Freedom	Sum of Squares	Variance	Variance Ratio	Observations
Germination	10	28.542	2.854	6.067	* * *
Grain Number	9	2.083	0.231	0.492	N. S.
Error	90	42.338	0.470		

* * * Significant at 0.1% Probability Level.

TABLE 7b

RESULTS OF LINEAR REGRESSION OF REDUCING SUGAR CONCENTRATION AND LENGTH OF RADICLE, COLEOPTILE AND LATERAL ROOTS IN INDIVIDUAL HICKORY KING GRAINS.

Factor	Germination Period Hours	Correlation Coefficient	t	Observations
Reducing Sugar	0 - 240	+ 0.510	6.161	* * *
Radicle Length	0 - 120	+ 0.675	6.967	* * *
	144 - 240	+ 0.267	-	N. S.
Reducing Sugar	0 - 240	+ 0.661	9.154	* * *
Coleoptile Length	0 - 120	+ 0.802	10.225	* * *
	144 - 240	+ 0.520	4.218	N. S.
Reducing Sugar	0 - 240	+ 0.730	11.100	* * *
Length of Lateral Roots	0 - 120	+ 0.616	5.955	* * *
	144 - 240	+ 0.653		* * *

* * * Significant at 0.1% Probability Level.

TABLE 8

RESULTS OF LINEAR REGRESSION (CORRELATION COEFFICIENT r) BETWEEN FRESH GRAIN WEIGHT (GRAMS.) AND REDUCING SUGAR CONCENTRATION (% DRY WEIGHT) OF INDIVIDUAL HICKORY KING GRAINS DURING GERMINATION.

Germination Period (Hours)	Grain Weight			Reducing Sugar Concentration		
	0	24	48	0	24	48
	0.70500	0.73410	0.97244	0.013	0.088	0.148
	0.97165	0.55681	0.49328	0.075	0.135	0.019
	0.65908	0.58776	0.75523	0.025	0.128	0.011
	0.66258	0.58390	0.82957	0.143	0.135	0.150
	0.59500	0.63599	0.60273	0.037	0.074	0.026
	0.60328	0.53627	0.91705	0.043	0.068	0.330
	0.58679	0.66866	0.71909	0.103	0.138	0.259
	0.52152	0.41408	0.64292	0.020	0.172	0.216
	0.70972	0.75832	0.52472	0.055	0.167	0.018
	0.62187	0.61644	0.71920	0.030	0.039	0.165

Correlation Coefficient	0 Hours	0 - 24 Hours	0 - 48 Hours
	+ 0.016	+ 0.047	+ 0.328

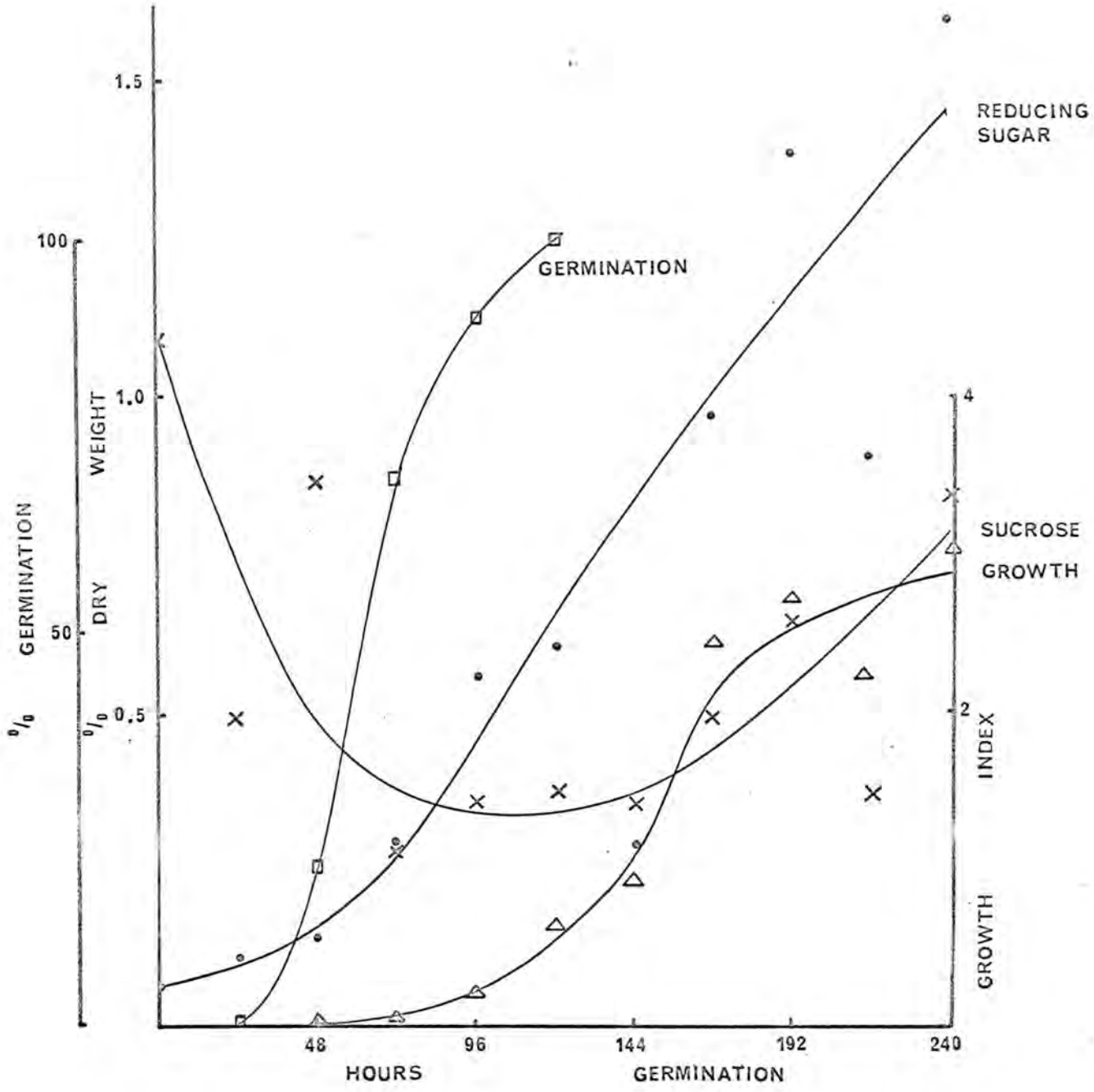

Fig. 14. Changes in reducing sugar and sucrose concentration in groups of Hickory King grain during germination. Growth index expressed as $\frac{\text{sum of lengths of organs measured}}{1000}$

L.S.D. between means for reducing sugar at 0.05 P.L. ± 0.610%
" " " " " " sucrose " 0.05 P.L. ± 0.299%.

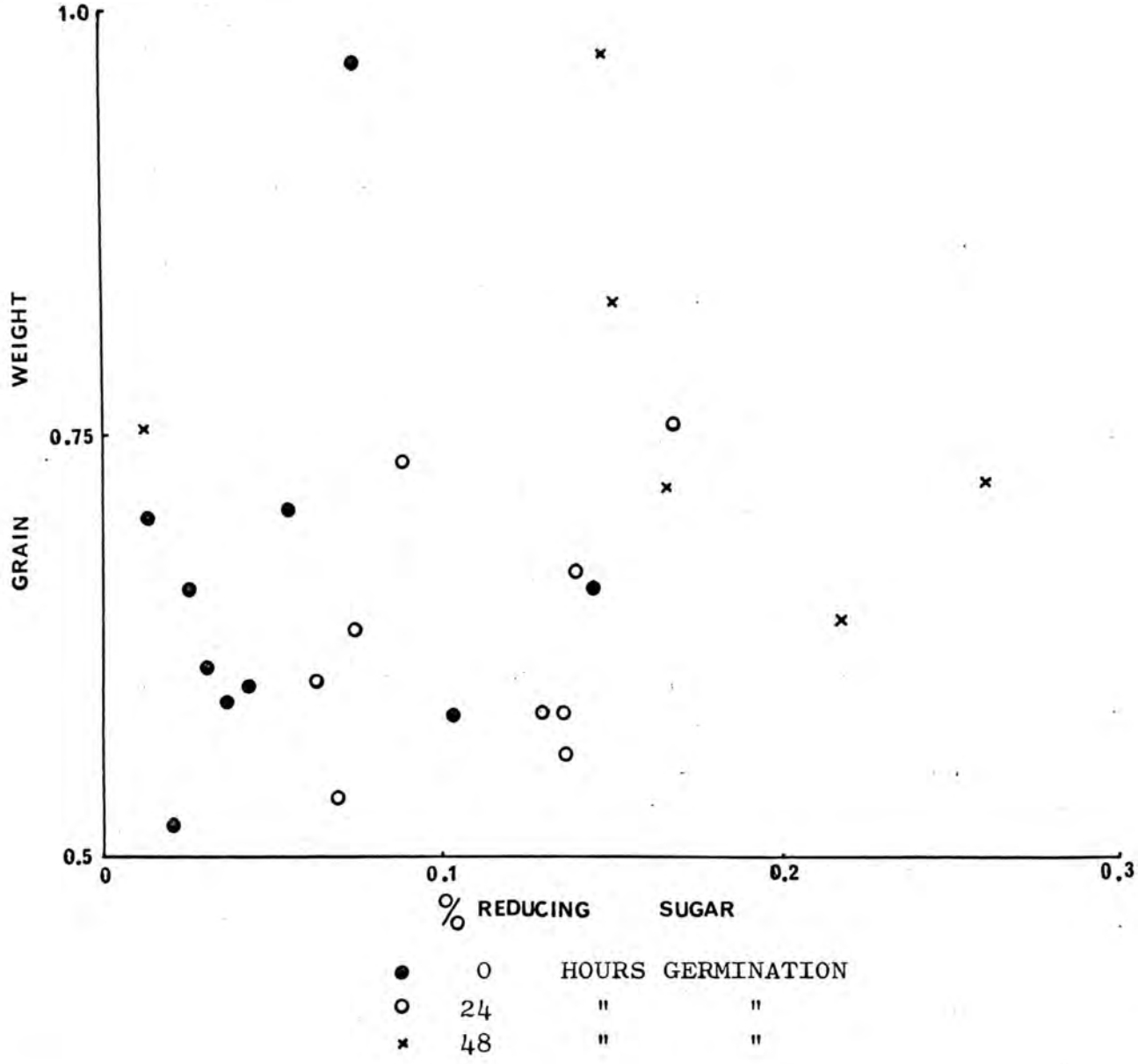

Fig. 15. Scatter plot illustrating correlation between grain weight (grams) and reducing sugar concentration (% dry weight) in individual Hickory King grains.

TABLE 9

LENGTH OF RADICLE, COLEOPTILE AND LATERAL ROOTS (ARISING FROM THE COTYLEDONARY NODE) OF INDIVIDUAL HICKORY KING GRAINS AFTER DIFFERENT PERIODS OF GROWTH.

Grain Number	GERMINATION PERIOD IN HOURS																											
	48			72			96			120			144			168			192			216			240			
	Radicle	Coleoptile	Laterals	Radicle	Coleoptile	Laterals	Radicle	Coleoptile	Laterals	Radicle	Coleoptile	Laterals	Radicle	Coleoptile	Laterals	Radicle	Coleoptile	Laterals	Radicle	Coleoptile	Laterals	Radicle	Coleoptile	Laterals	Radicle	Coleoptile	Laterals	
1	-	-	-	6	-	-	24	15	39	32	11	52	20	3	5	56	112	149	94	20	71	76	63	160	25	91	337	
2	-	-	-	9	-	-	10	3	17	-	6	21	57	6	11	57	15	58	59	35	53	77	41	63	46	35	93	
3	1.0	-	-	8	-	-	49	14	39	42	19	48	31	7	32	64	34	67	174	30	195	40	10	65	52	93	210	
4	-	-	-	13	-	4	20	8	6	16	4	-	75	29	53	91	37	194	38	37	129	17	27	120	198	45	160	
5	-	-	-	4	-	-	7	4	-	21	-	-	17	5	16	72	39	218	91	52	400	18	48	280	-	23	88	
6	-	-	-	-	-	-	12	-	-	43	7	15	59	12	13	86	44	162	24	21	83	-	15	109	50	36	179	
7	-	-	-	-	-	-	-	-	-	-	9	28	40	10	53	94	63	154	105	63	228	72	72	358	147	72	141	
8	2.0	-	-	5	7	-	22	3	-	13	5	-	77	4	71	61	25	94	37	55	179	46	36	119	27	74	228	
9	-	-	-	-	-	-	34	9	6	-	6	-	39	31	90	39	52	325	141	27	66	54	30	118	88	53	123	
10	-	-	-	3	-	-	49	8	16	31	14	-	46	9	21	57	18	30	52	24	186	12	13	75	50	46	259	
Mean	0.3	-	-	4.8	0.7	0.4	6.4	1.4	12.3	19.8	8.1	40	46.1	11.6	36.5	67.7	33.9	145.1	81.5	36.4	159.0	41.2	35.5	146.7	68.3	56.8	181.8	
Growth Index	0.003			0.059			0.201			0.679			0.942			2.467			2.769			2.234			3.069			

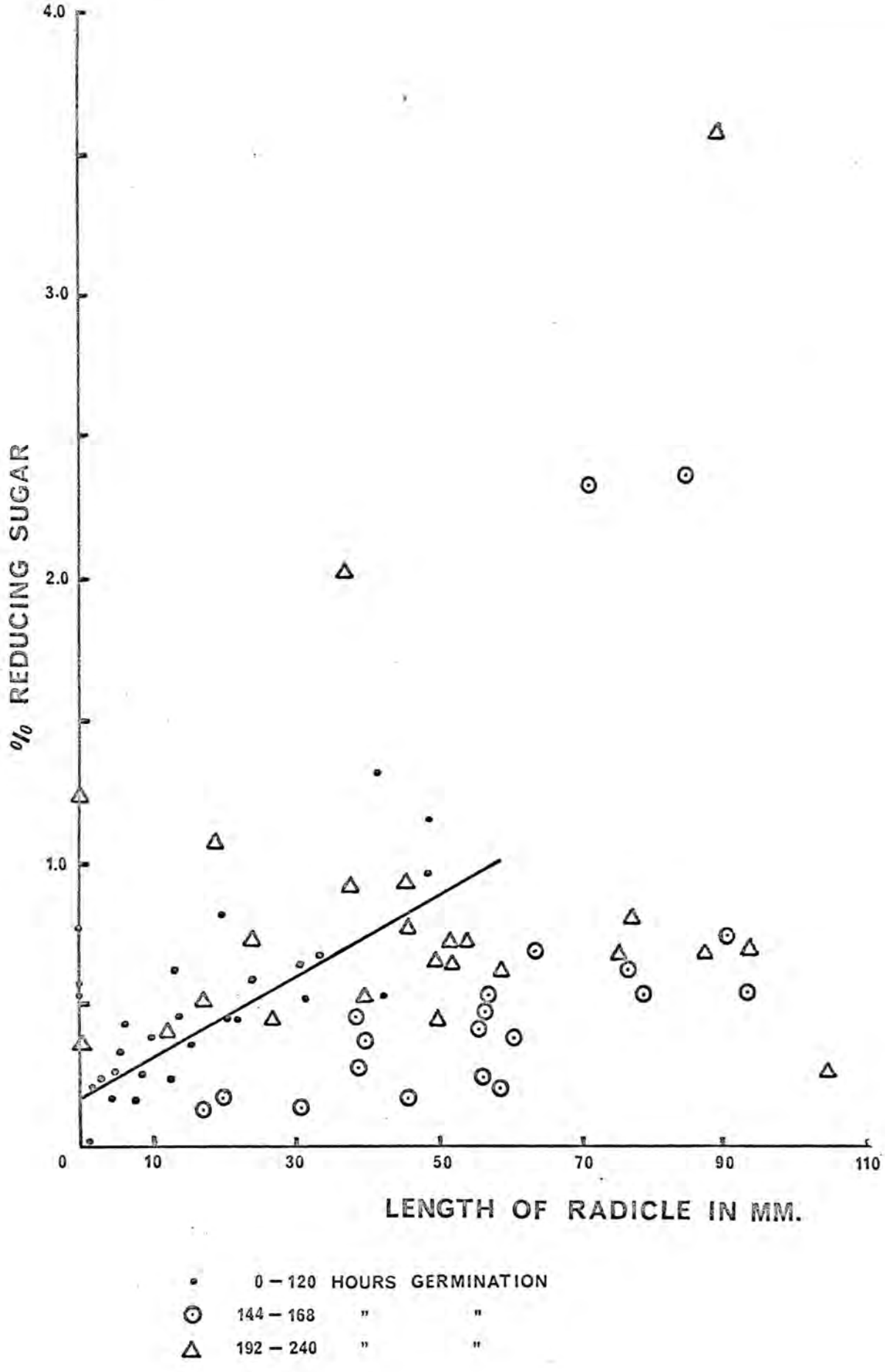

Fig. 16. Scatter plot illustrating correlation between reducing sugar concentration and radicle length in individual Hickory King grains. Correlation coefficient for 0-120 hours. + 0.675.

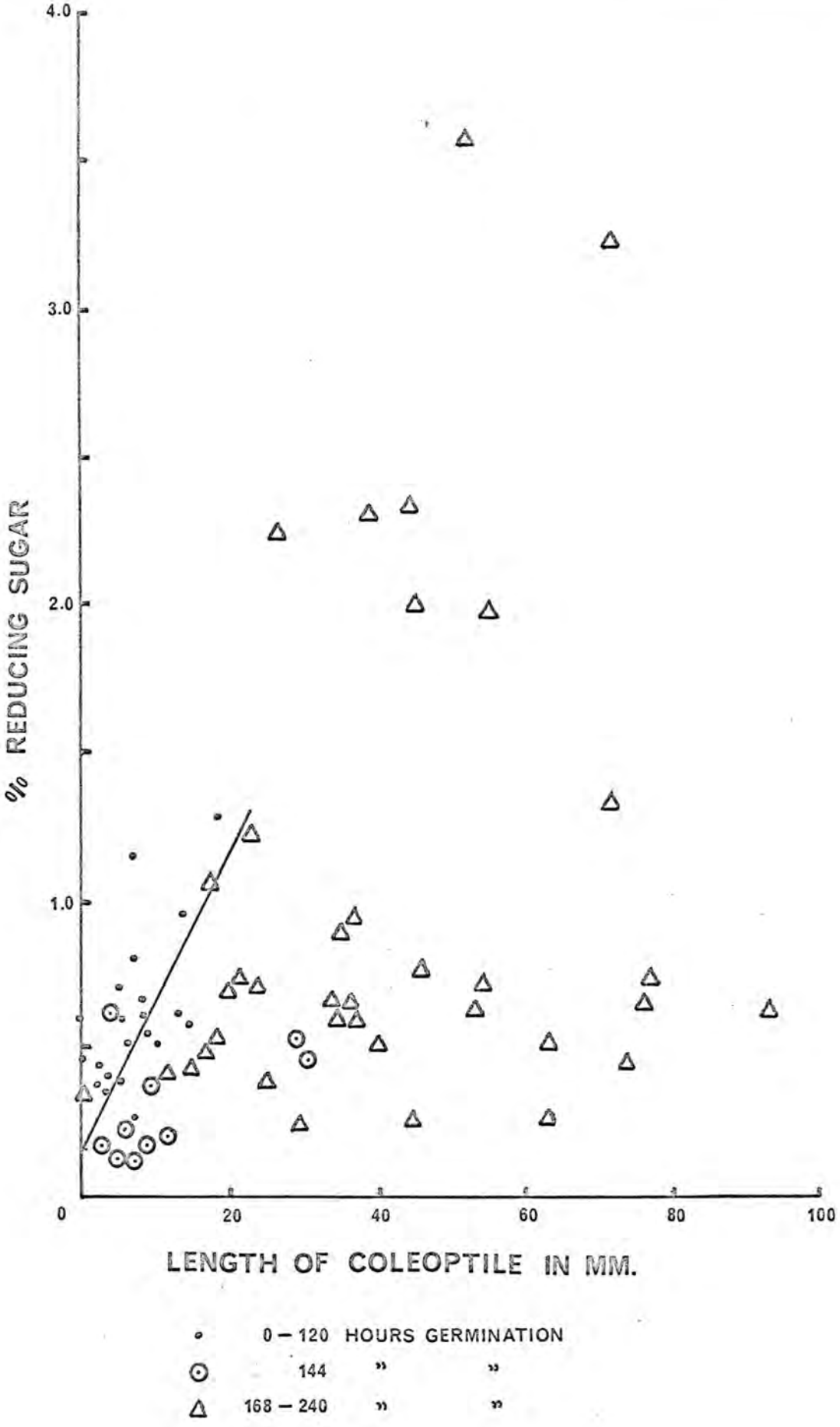

Fig. 17. Scatter plot illustrating correlation between reducing sugar concentration and coleoptile length in individual Hickory King grains. Correlation coefficient for 0-120 hours. + 0.802.

Fig. 18. Scatter plot illustrating correlation between reducing sugar concentration and lateral root length in individual Hickory King grains.

TABLE 10

SUCROSE CONCENTRATION (% DRY WEIGHT) IN GROUPS OF HICKORY KING GRAIN DURING GERMINATION. ASSAY WAS REPLICATED THREE TIMES ON EACH GROUP.

Germination Period (hours)	Replication Number 1	2	3	Mean	Germination Period Mean
0	0.915	1.075	0.923	0.971	
0	1.247	1.288	1.260	1.265	1.101
0	1.192	1.013	0.996	1.067	
24	0.680	0.730	0.672	0.694	
24	0.692	0.828	0.759	0.760	0.721
24	0.753	0.639	0.736	0.709	
48	0.440	0.457	0.475	0.457	
48	0.410	0.469	0.496	0.458	0.433
48	0.357	0.393	0.400	0.384	
72	0.357	0.273	0.310	0.314	
72	0.365	0.262	0.345	0.324	0.314
72	0.302	0.293	0.315	0.303	
96	0.165	0.169	0.197	0.177	
96	0.242	0.202	0.194	0.213	0.189
96	0.176	0.176	0.177	0.176	
120	0.202	0.178	0.201	0.194	
120	0.205	0.138	0.180	0.174	0.195
120	0.225	0.236	0.186	0.216	
144	0.230	0.216	0.242	0.229	
144	0.142	0.152	0.109	0.134	0.217
144	0.287	0.278	0.302	0.289	
168	0.287	0.167	0.212	0.222	
168	0.316	0.320	0.308	0.315	0.272
168	0.285	0.283	0.267	0.278	
192	0.470	0.530	0.605	0.535	
192	0.596	0.705	0.445	0.582	0.527
192	0.502	0.417	0.472	0.464	
216	0.288	0.165	0.272	0.242	
216	0.827	0.793	0.778	0.799	0.477
216	0.385	0.417	0.367	0.390	
240	0.168	0.275	0.286	0.243	
240	0.198	0.247	0.235	0.227	0.352
240	0.629	0.478	0.655	0.587	

L. S. D. Between Observations at 0.05 P. L. ± 0.157
" " " " Means " " ± .091
" " " " C. P. Means " " ± .052

TABLE 11a

ANALYSIS OF VARIANCE DATA FOR SUCROSE CONCENTRATION IN GROUPS OF HICKORY KING GRAINS.

Source	Degrees of Freedom	Sum of Squares	Variance	Variance ratio (F)	Observations
Germination	10	6.746191	0.674619	216.332	* * *
Replication	2	0.001139	0.000570	0.183	N. S.
Sample	2	0.131052	0.065526	21.012	*
Sample/Replication	4	0.014826	0.003707	1.189	N. S.
Replication/Germination	20	0.035862	0.001793	0.575	N. S.
Sample/Germination	20	0.848311	0.042416	13.601	* * *
Error	40	0.124738	0.003118		

* * * Significant at 0.1% Probability Level.
* " " 5% " "

TABLE 11b

RESULTS OF LINEAR REGRESSION OF SUCROSE CONCENTRATION AND LENGTH OF RADICLE, COLEOPTILE AND LATERAL ROOTS IN GROUPS OF HICKORY KING GRAINS.

Factor	Germination Period Hours	Correlation Coefficient	t	Observations
Sucrose	0 - 240	- 0.293	-	-
	0 - 96	- 0.627	2.903	*
Radicle	48 - 96	- 0.846	4.197	* * *
Length	120 - 240	+ 0.353	-	-
Sucrose	0 - 240	- 0.212	2.877	* *
	0 - 168	- 0.524	-	-
Coleoptile	72 - 168	- 0.179	-	-
Length	192 - 240	- 0.281	-	-
Sucrose	0 - 240	- 0.186	-	-
	72 - 168	- 0.485	-	-
Lateral Root	192 - 240	- 0.114	-	-

* * * Significant at 0.1% Probability Level.
* * " " 1.0% " "
* " " 5.0% " "

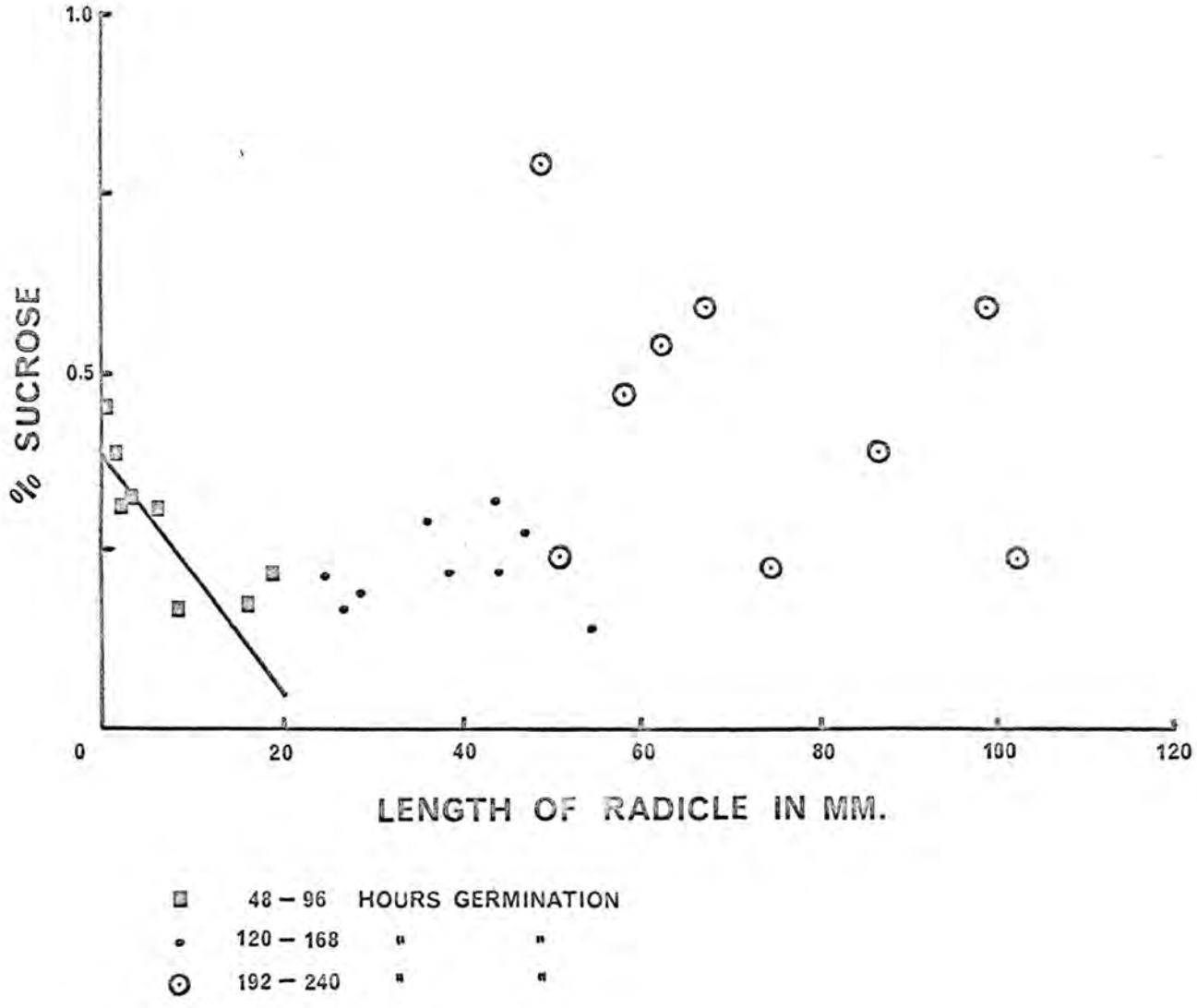

Fig. 19. Scatter plot illustrating correlation between sucrose concentration and radicle length in groups of Hickory King grains. Correlation coefficient for 0-96 hours. - 0.627.

Fig. 20. Scatter plot illustrating correlation between sucrose concentration and coleoptile length in groups of Hickory King grain. Correlation coefficient for 0-168 hours - 0.524.

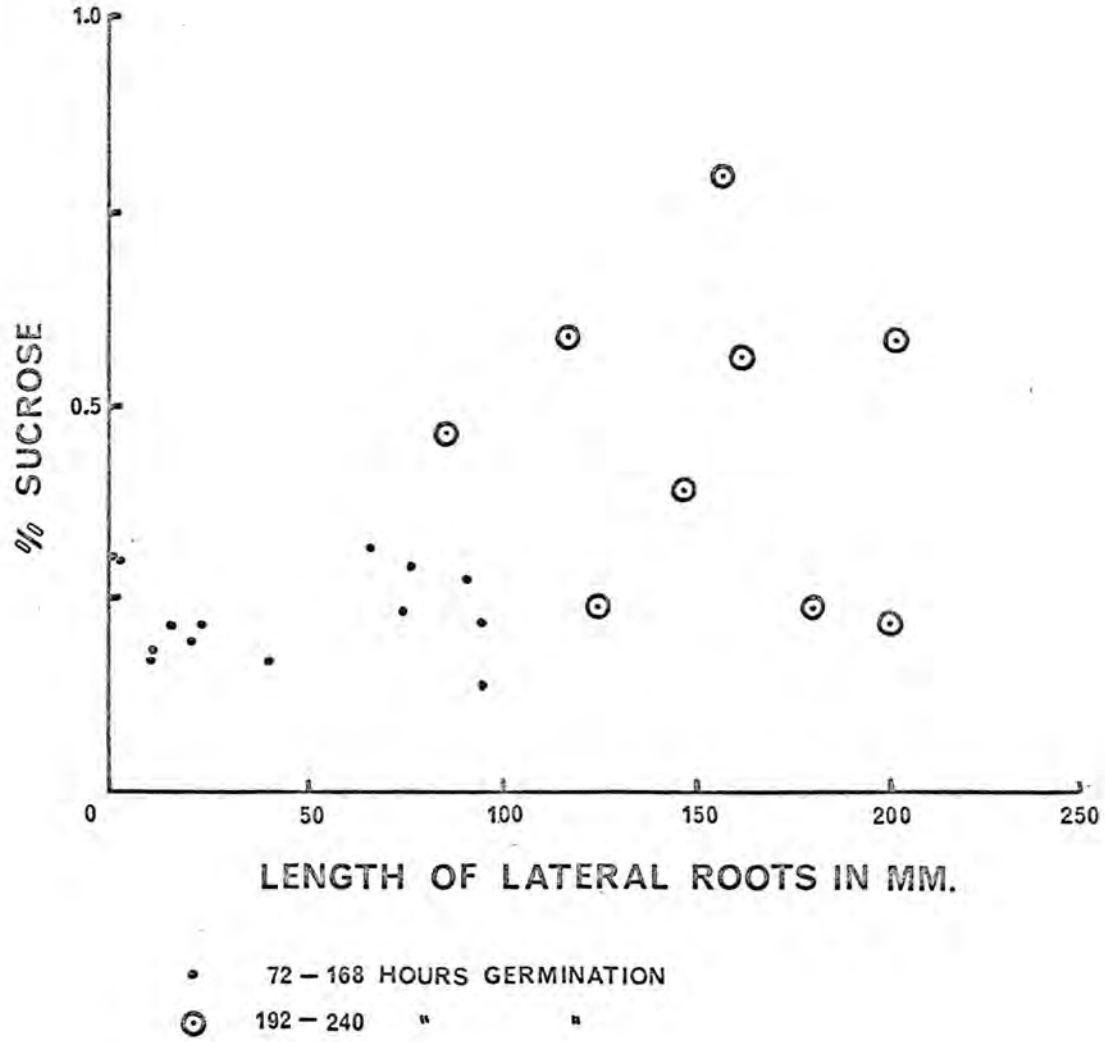

Fig. 21. Scatter plot illustrating correlation between sucrose concentration and lateral root length in groups of Hickory King grain.

TABLE 12a

LINEAR REGRESSION OF SUCROSE AND REDUCING SUGAR CONCENTRATION IN GROUPS OF HICKORY KING GRAINS.

Factor	Germination Period Hours	Correlation Coefficient	t	Observations
Sucrose	0-240	- 0.327	-	
	0-96	- 0.772	4.379	* * *
Reducing Sugar	120-240	+ 0.298	-	

* * * Significant at 0.1% Probability Level.

TABLE 12 b

LINEAR REGRESSION OF SUCROSE AND REDUCING SUGAR CONCENTRATION IN INDIVIDUAL HICKORY KING GRAINS.

Factor	Germination Period	Correlation Coefficient	t	Observations
Sucrose	0-240	+ 0.411	4.660	* * *
	0-96	- 0.519	4.250	* * *
Reducing Sugar conc.	120-240	+ 0.752	8.688	* * *

* * * Significant at 0.1% Probability Level

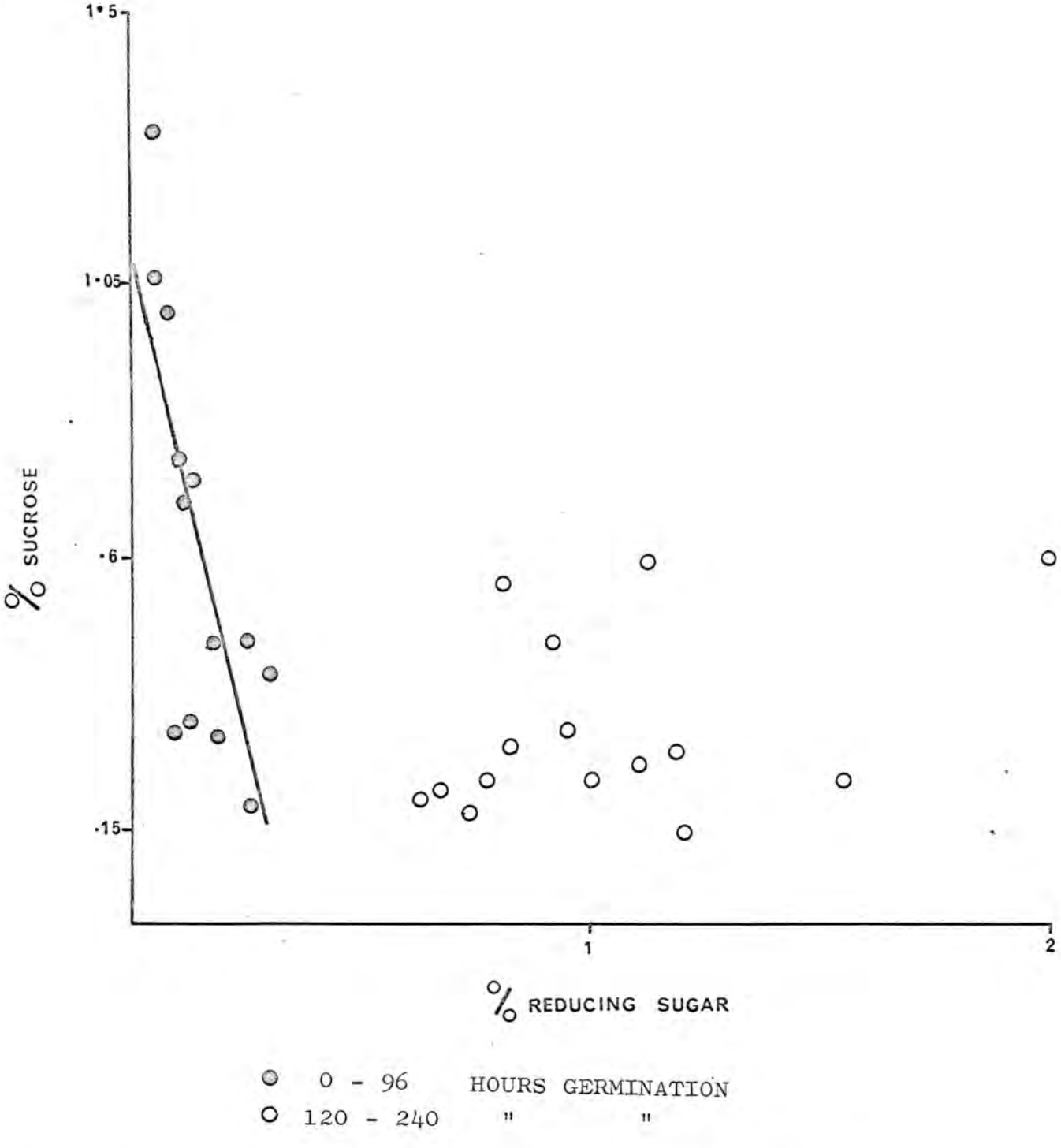

Fig. 22. Scatter plot illustrating correlation between sucrose and reducing sugar concentrations in groups of Hickory King grain. Correlation coefficient for 0-96 hours - 0.772.

TABLE 13

SUCROSE CONCENTRATION (% DRY WEIGHT) IN INDIVIDUAL HICKORY KING GRAINS DURING GERMINATION.

Germination Period (hrs)	GRAIN NUMBER 1	2	3	4	5	6	7	8	9	10	Mean	% Germination	Growth Index
0	0.972	1.096	1.307	1.107	0.989	0.744	1.394	1.552	0.826	0.833	1.082	0	0
24	0.508	0.401	0.416	0.361	0.483	0.476	0.269	0.493	1.052	0.343	0.480	0	0
48	0.799	0.882	0.727	0.970	0.943	0.658	0.948	0.802	1.207	0.625	0.856	20	0.003
72	0.238	0.327	0.148	0.386	0.340	0.290	0.339	0.239	0.185	0.316	0.281	70	0.059
96	0.352	0.560	0.202	0.273	0.544	0.371	0.309	0.374	0.356	0.211	0.355	90	0.201
120	0.360	0.802	0.320	0.414	0.447	0.214	0.402	0.534	0.095	0.128	0.372	100	0.679
144	0.434	0.377	0.469	0.251	0.564	0.338	0.347	0.133	0.217	0.373	0.350	100	0.942
168	0.054	0.093	0.536	0.523	1.015	1.770	0.132	0.260	0.078	0.448	0.491	100	2.467
192	0.578	0.549	0.781	0.408	0.848	0.430	0.963	0.568	0.784	0.499	0.641	100	2.769
216	0.209	0.421	0.452	0.421	0.100	0.246	0.688	0.336	0.455	0.363	0.369	100	2.234
240	2.934	0.612	0.247	1.280	0.639	0.598	0.738	0.339	0.565	0.452	0.840	100	3.069

Least Significant Difference Between Observations at 0.05 Probability Level ± 0.093

" " " " Means " " " " ± 0.299

TABLE 14a

ANALYSIS OF VARIANCE DATA FOR SUCROSE CONCENTRATION IN INDIVIDUAL HICKORY KING GRAINS.

Source	Degrees of Freedom	Sum of Squares	Variance	Variance Ratio (F)	Observations
Germination	10	6.663	0.666	5.534	* * *
Grain Number	9	0.376	0.042	0.347	N. S.
Error	90	10.836	0.120		

* * * Significant at 0.1% Probability Level.

TABLE 14b

RESULTS OF LINEAR REGRESSION OF SUCROSE CONCENTRATION AND LENGTH OF RADICLE, COLEOPTILE AND LATERAL ROOTS IN INDIVIDUAL HICKORY KING GRAINS.

Factor	Germination Period (hours)	Correlation Coefficient	t	Observations
Sucrose	0 - 240	+ 0.025	-	-
	0 - 96	- 0.420	3.350	* *
	48 - 96	- 0.463	2.673	*
Radicle Length	120 - 240	+ 0.250	-	-
Sucrose	0 - 240	+ 0.133	-	-
	0 - 96	- 0.349	-	-
	48 - 96	- 0.372	-	-
Coleoptile Length	120 - 240	+ 0.392	-	-
Sucrose	0 - 240	+ 0.145	-	-
	0 - 96	- 0.261	-	-
	48 - 96	- 0.269	-	-
Lateral Root Length	120 - 240	+ 0.368	-	-

* * Significant at 0.5% Probability Level.
* " " 2.0% " "

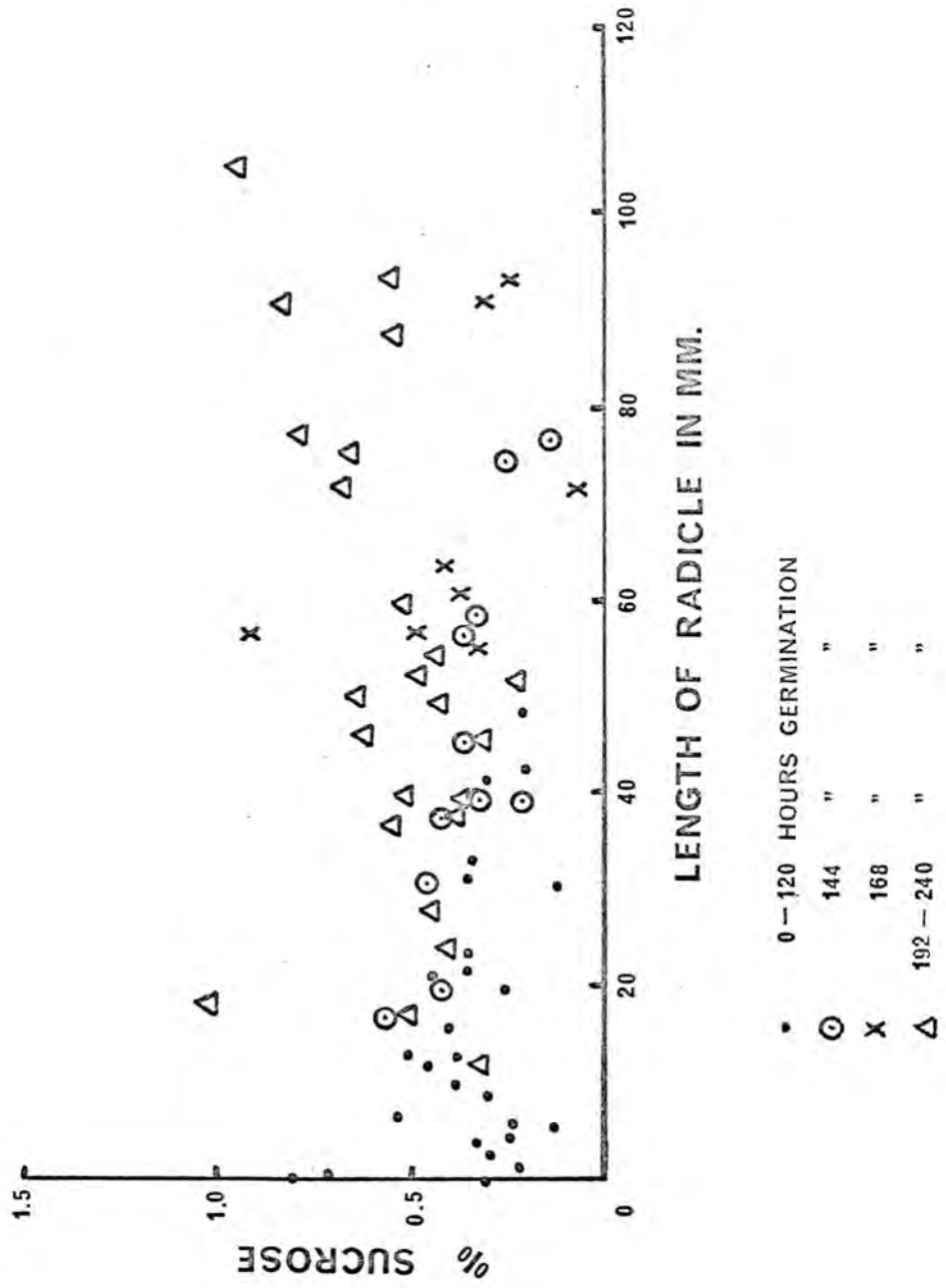

Fig. 23. Scatter plot illustrating correlation between sucrose concentration and radicle length in individual Hickory King grains.

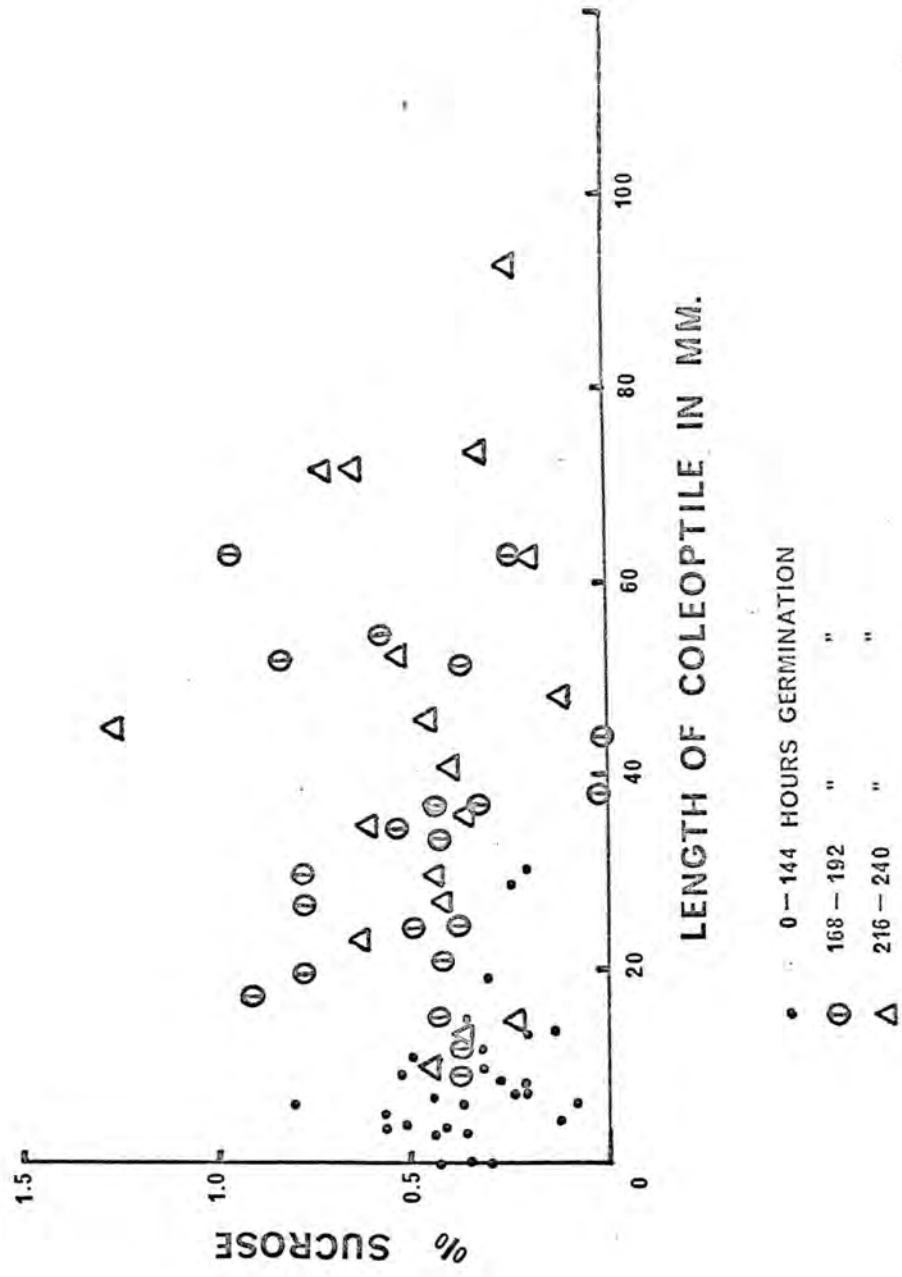

Fig. 24. Scatter plot illustrating correlation between sucrose concentration and coleoptile length in individual Hickory King grains.

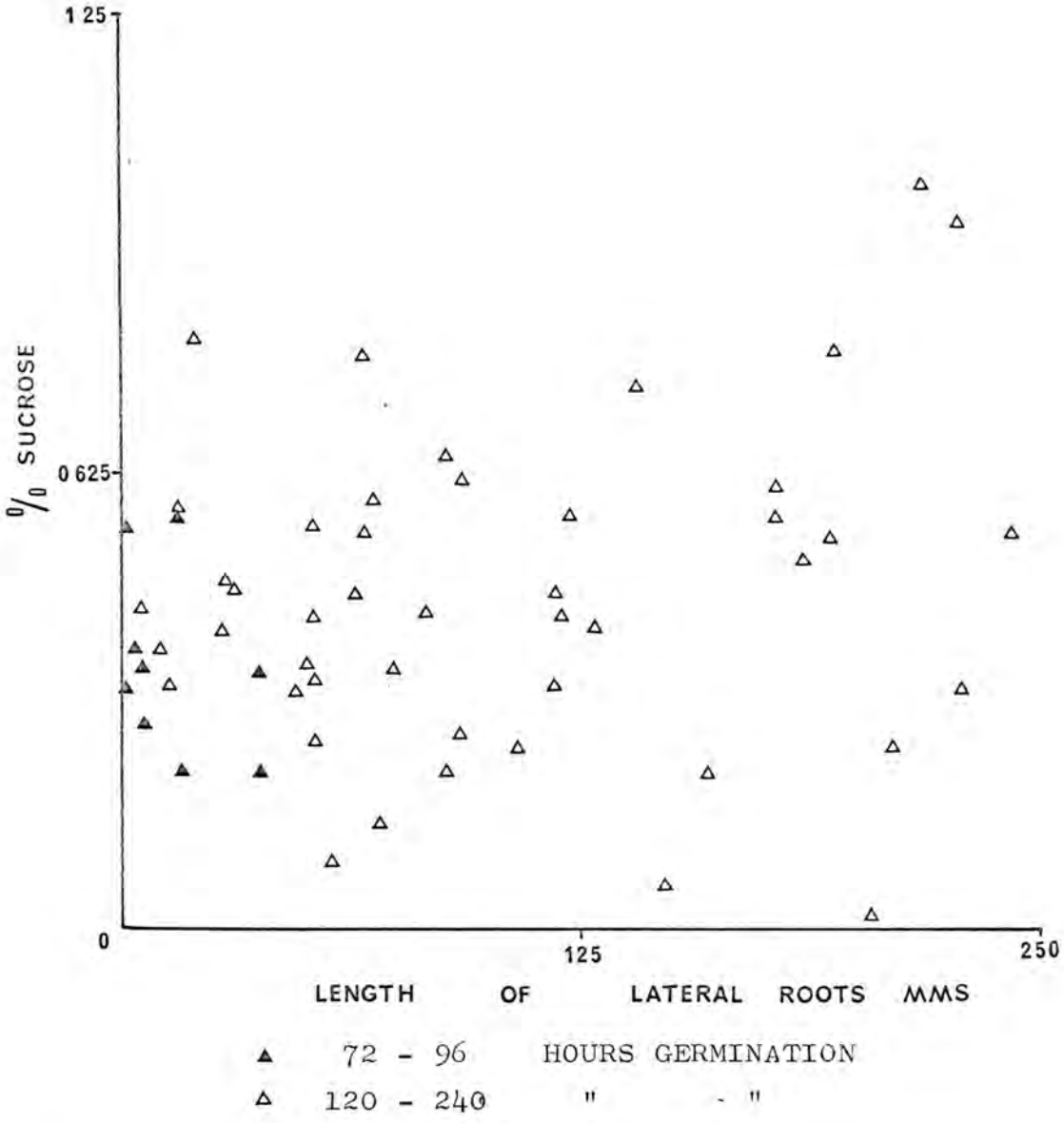

Fig. 25. Scatter plot illustrating correlation between sucrose concentration and lateral root length in individual Hickory King grains.

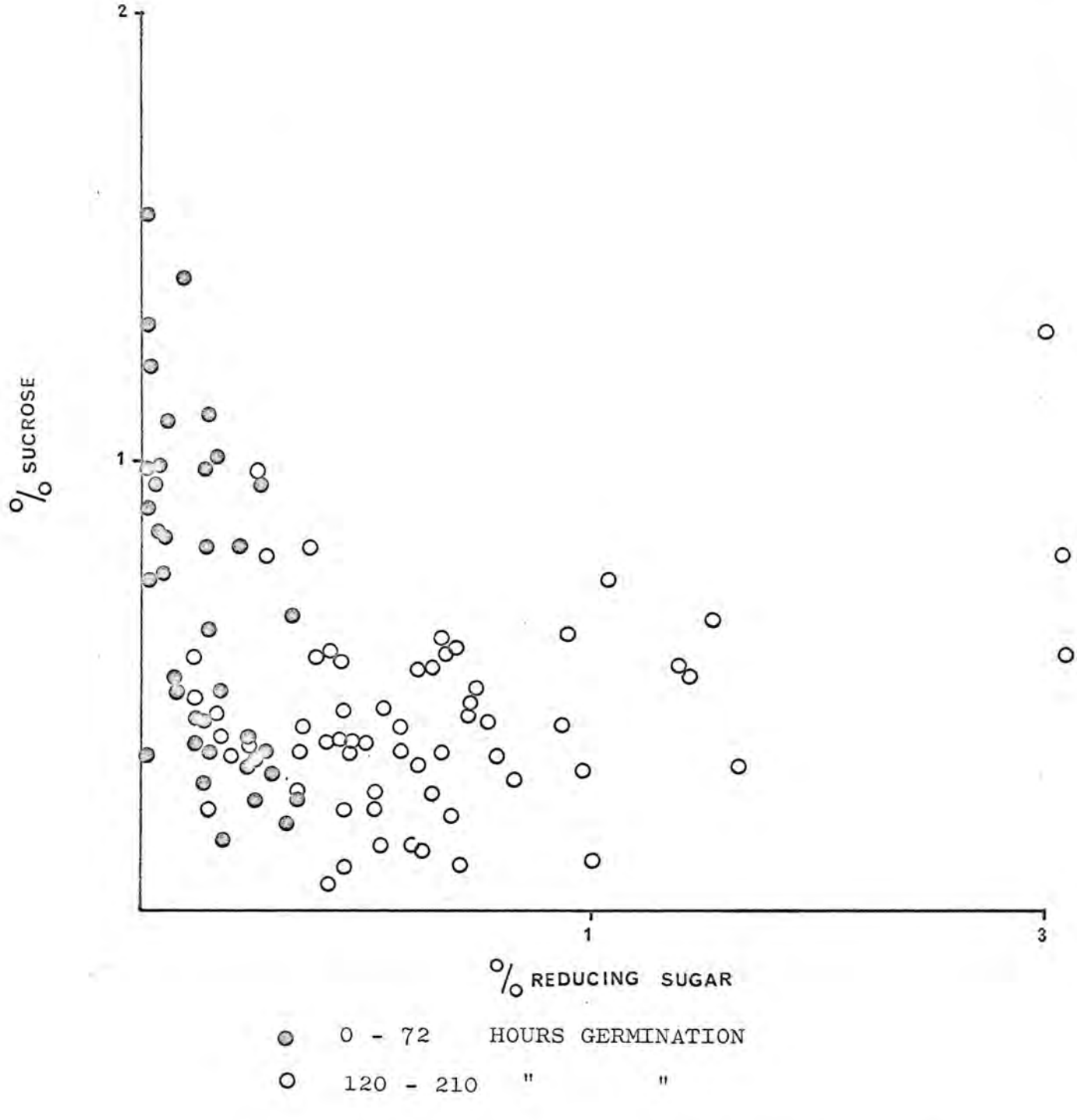

Fig. 26. Scatter plot illustrating correlation between sucrose and reducing sugar concentrations in individual Hickory King grains.

TABLE 15.

DEXTRIN CONCENTRATION (% DRY WEIGHT) IN GROUPS OF HICKORY KING GRAIN. ASSAY WAS REPLICATED THREE TIMES ON EACH GROUP.

Germination Period (hrs)	Replication Number 1	2	3	Mean	Germination Period Mean
0	0.093	0.139	0.126	0.120	
0	0.214	0.205	0.194	0.204	0.138
0	0.126	0.053	0.097	0.092	
24	0.054	0.058	0.018	0.043	
24	0.081	0.096	0.080	0.086	0.065
24	0.078	0.057	0.067	0.067	
48	0.304	0.209	0.251	0.255	
48	0.246	0.170	0.187	0.201	0.261
48	0.308	0.319	0.359	0.328	
72	0.362	0.368	0.389	0.373	
72	0.452	0.418	0.460	0.443	0.415
72	0.401	0.420	0.467	0.429	
96	0.165	0.212	0.355	0.244	
96	0.154	0.152	0.153	0.153	0.189
96	0.133	0.206	0.172	0.171	
120	0.086	0.092	0.093	0.090	
120	0.091	0.114	0.095	0.100	0.110
120	0.123	0.179	0.114	0.139	
144	0.092	0.130	0.109	0.111	
144	0.201	0.246	0.319	0.255	0.159
144	0.121	0.130	0.084	0.112	
168	0.459	0.451	0.500	0.470	
168	0.411	0.367	0.474	0.417	0.427
168	0.403	0.412	0.363	0.393	
192	0.256	0.357	0.369	0.324	
192	0.602	0.565	0.507	0.558	0.450
192	0.470	0.479	0.455	0.468	
216	0.251	0.174	0.155	0.193	
216	0.209	0.166	0.174	0.183	0.194
216	0.184	0.212	0.219	0.205	
240	0.109	0.069	0.064	0.086	
240	0.045	0.063	0.046	0.051	0.102
240	0.205	0.102	0.202	0.170	

L. S. D. Between Observations at 0.05 P. L. ± 0.112
" " " " Means " " " ± 0.064
" " " " G. P. Means " " " ± 0.037

TABLE 16a

ANALYSIS OF VARIANCE DATA FOR DEXTRIN CONCENTRATION IN GROUPS OF HICKORY KING GRAINS.

Source	Degrees of Freedom	Sum of Squares	Variance	Variance Ratio (F)	Observation
Germination	10	1.777641	0.177764	119.456	* * *
Replication	2	0.001704	0.000852	0.573	N. S.
Sample	2	0.017931	0.008966	6.025	N. S.
Sample/Replication	4	0.001612	0.000403	0.271	N. S.
Replication/Germination	20	0.025780	0.001289	0.866	N. S.
Sample/Germination	20	0.211903	0.010595	7.120	* * *
Error	40	0.059525	0.001488		

* * * Significant at 0.1% Probability Level.

TABLE 16b.

RESULTS OF LINEAR REGRESSION OF DEXTRIN CONCENTRATION AND LENGTH OF RADICLE, COLEOPTILE AND LATERAL ROOTS IN GROUPS OF HICKORY KING GRAINS.

Factor	Germination Period Hours	Correlation Coefficient
Dextrin	0 -240	+ 0.085
Radicle	48 -168	- 0.075
Dextrin	0 -240	+ 0.032
Coleoptile	48 -168	+ 0.054
Dextrin	0 -240	+ 0.029
Lateral Roots	48 -168	+ 0.054

TABLE 17

DEXTRIN CONCENTRATION (% DRY WEIGHT) IN INDIVIDUAL HICKORY KING GRAINS DURING GERMINATION.

GRAIN	GERMINATION PERIOD IN HOURS										
NO.	0	24	48	72	96	120	144	168	192	216	240
1	0.182	0.558	0.105	0.501	0.563	0.320	0.315	0.054	0.211	0.080	1.966
2	0.256	0.644	0.019	0.117	0.245	0.410	0.429	0.093	0.000	0.127	1.003
3	0.322	1.378	0.041	0.111	0.817	0.807	0.162	0.536	0.974	0.126	0.868
4	0.446	0.775	0.043	0.275	0.499	0.689	0.240	0.523	0.564	0.201	1.484
5	0.424	0.912	0.067	0.565	0.674	0.460	0.105	1.015	1.613	0.225	1.123
6	0.440	1.026	0.042	0.511	0.505	0.534	0.170	1.770	0.302	0.340	1.628
7	0.311	0.690	0.025	0.427	0.536	0.365	0.452	0.132	1.573	1.625	0.802
8	0.330	0.879	0.119	0.264	0.785	0.705	0.454	0.260	1.180	0.517	0.854
9	0.359	0.728	0.008	0.559	0.646	0.437	0.289	0.078	1.518	0.629	1.061
10	0.349	0.697	0.062	0.593	0.866	0.293	0.133	0.448	0.520	0.250	1.387
MEAN	0.342	0.829	0.053	0.392	0.614	0.502	0.275	0.491	0.845	0.412	1.218

Least Significant Difference Between Observation at 0.05 Probability Level ± 0.993

" " " " Means " " " " ± 0.314

TABLE 18.

ANALYSIS OF VARIANCE DATA FOR DEXTRIN CONCENTRATION IN INDIVIDUAL HICKORY KING GRAINS.

Source	Degrees of Freedom	Sum of Squares	Variance	Variance Ratio(F)	Observation
Germination	10	8.072	0.807	6.588	* * *
Grain Number	9	1.291	0.143	1.171	N. S.
Error	90	11.028	0.123		

* * * Significant at 0.1% Probability Level.

TABLE 19

RESULTS OF LINEAR REGRESSION OF DEXTRIN CONCENTRATION AND LENGTH OF RADICLE, COLEOPTILE AND LATERAL ROOTS IN INDIVIDUAL HICKORY KING GRAINS.

Factor	Germination Period	Correlation Coefficient.
Dextrin	0 -240	+ 0.380
Radicle	0 -96	+ 0.279
	120 -240	+ 0.350
Dextrin	0 -240	+ 0.476
Coleoptile	0 -96	+ 0.237
	120 -240	+ 0.513
Dextrin	0 -240	+ 0.502
	0 -96	+ 0.186
Lateral Roots	120 -240	+ 0.549

TABLE 20.

REDUCING SUGAR CONCENTRATION (% DRY WEIGHT) IN GROUPS OF EARLY PEARL GRAIN DURING GERMINATION. ASSAY WAS REPLICATED TIMES ON EACH GROUP.

Germination Period (Hrs)	1	2	3	Mean	Germination Period Mean
0	0.002	0.001	0.002	0.002	
0	0.002	0.002	0.005	0.003	0.002
0	-	0.001	0.003	0.001	
24	0.095	0.123	0.130	0.116	
24	0.193	0.205	0.193	0.197	0.106
24	0.003	0.004	0.005	0.004	
48	0.448	0.436	0.460	0.448	
48	1.000	0.931	0.881	0.937	0.684
48	0.650	0.679	0.672	0.667	
72	0.871	0.900	0.925	0.899	
72	0.630	0.604	0.605	0.613	0.699
72	0.571	0.577	0.607	0.585	
96	0.568	0.566	0.540	0.558	
96	0.619	0.595	0.598	0.604	0.495
96	0.321	0.320	0.330	0.324	
120	1.838	1.854	1.839	1.844	
120	1.169	1.120	1.149	1.146	1.780
120	2.400	2.446	2.209	2.352	
144	2.376	2.370	2.390	2.379	
144	4.949	4.724	4.871	4.848	4.020
144	4.628	5.022	4.854	4.835	
168	8.263	8.233	7.937	8.144	
168	4.600	4.638	4.658	4.632	5.773
168	4.581	4.442	4.605	4.543	
192	3.974	4.115	4.113	4.067	
192	4.055	4.545	4.589	4.396	6.024
192	9.626	9.610	9.586	9.607	
216	6.486	6.486	6.316	6.429	
216	4.975	4.954	4.849	4.926	4.586
216	2.374	2.456	2.381	2.404	
240	9.087	10.042	9.912	9.681	
240	6.638	6.945	6.871	6.818	6.048
240	1.581	1.690	1.667	1.646	

L	S	D	Between Observations at 0.05 P.	L. ± 0.325
"	"	"	" Means " " " "	± 0.188
"	"	"	" Germination Period Means " "	± 0.108

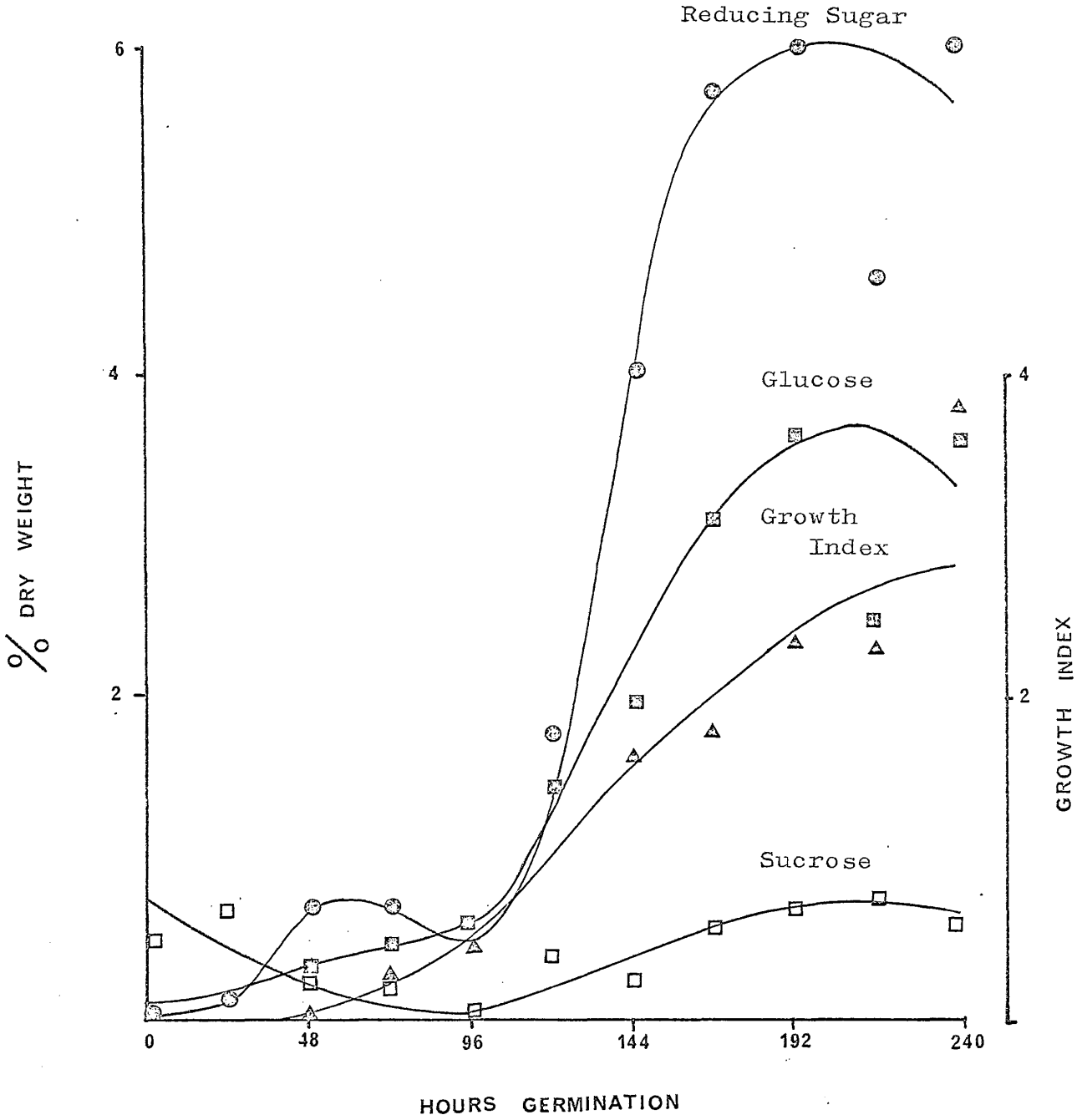

Fig. 28. Changes in reducing sugar, glucose and sucrose concentration in groups of Early Pearl grain during germination. Growth index expressed as $\frac{\text{sum of lengths of organs measured}}{1000}$

L.S.D. between means for reducing sugar at 0.05 P.L. ± 0.108%

" " " " " " sucrose " 0.05 P.L. ± 0.017%

" " " " " " glucose " 0.05 P.L. ± 0.080%.

TABLE 21a

ANALYSIS OF VARIANCE DATA FOR REDUCING SUGAR CONCENTRATION IN GROUPS OF EARLY PEARL GRAIN.

Source	Degrees of Freedom	Sum Squares	Variance	Variance Ratio(F)	Observations
Germination	10	582.858250	58.285825	4365.979	* * *
Replication	2	0.064940	0.032470	2.432	N. S.
Sample	2	8.366790	4.183395	313.363	* * *
Sample/Replication	4	0.015640	0.003910	0.293	N. S.
Replication/Germination	20	0.433960	0.021698	1.625	N. S.
Sample/Germination	20	214.286610	10.714331	802.572	* * *
Error	40	0.534000	0.013350		

* * * Significant at 0 1% Probability Level.

TABLE 21b

RESULTS OF LINEAR REGRESSION OF REDUCING SUGAR CONCENTRATION AND LENGTH OF RADICLE, COLEOPTILE AND LATERAL ROOTS IN GROUPS OF EARLY PEARL GRAINS.

Factor	Germination Period Hours	Correlation Coefficient	t	Significance
Reducing Sugar Radicle	0 - 240	+ 0.756	6.429	* * *
Reducing Sugar Coleoptile	0 - 240	+ 0.809	7.661	* * *
Reducing Sugar Lateral Roots	0 - 240	+ 0.803	7.502	* * *

* * * Significant at 0.1% Probability Level.

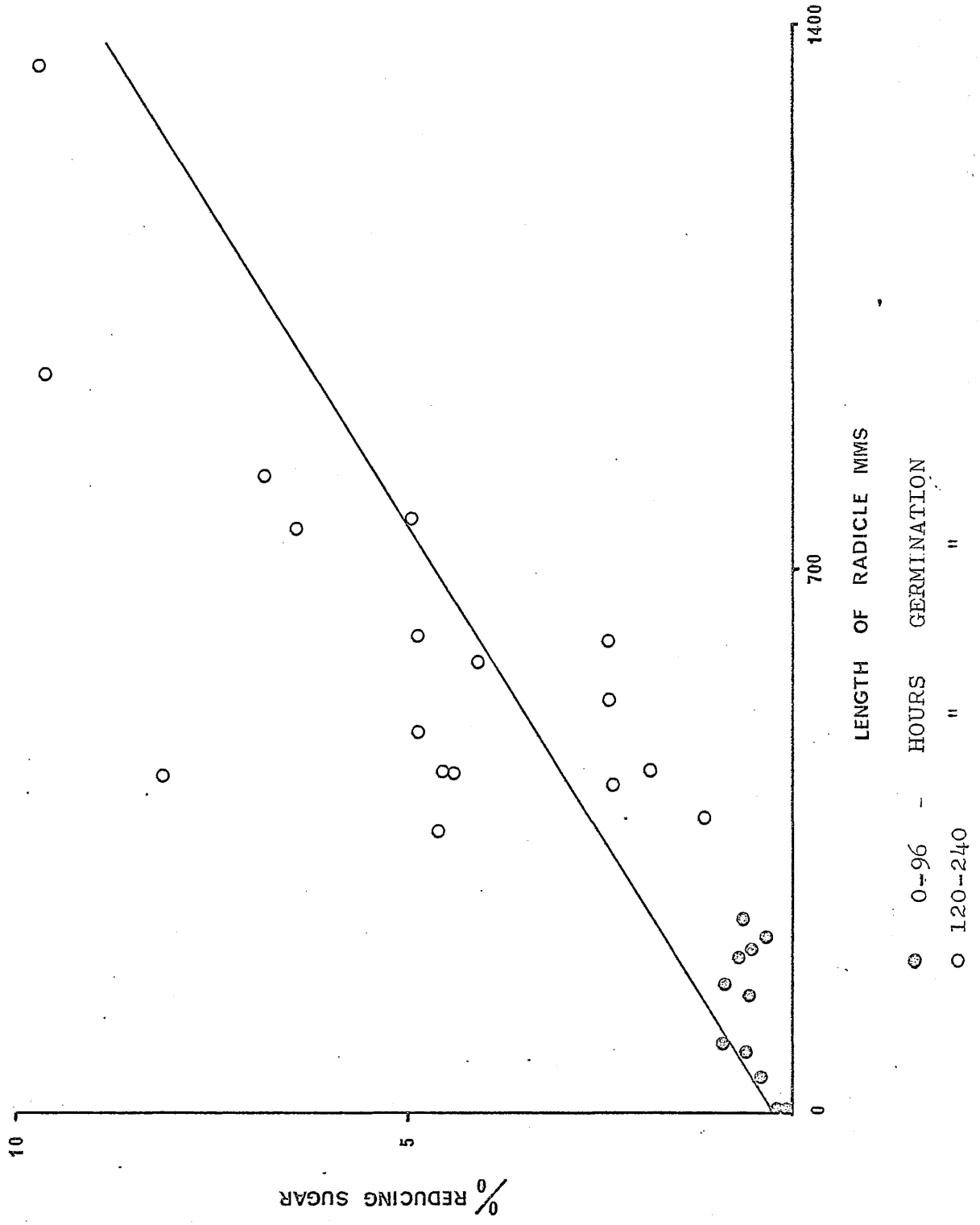

Fig. 29. Scatter plot illustrating correlation between reducing sugar concentration and radicle length in groups of Early Pearl grain. Correlation coefficient for 0-240 hours + 0.756.

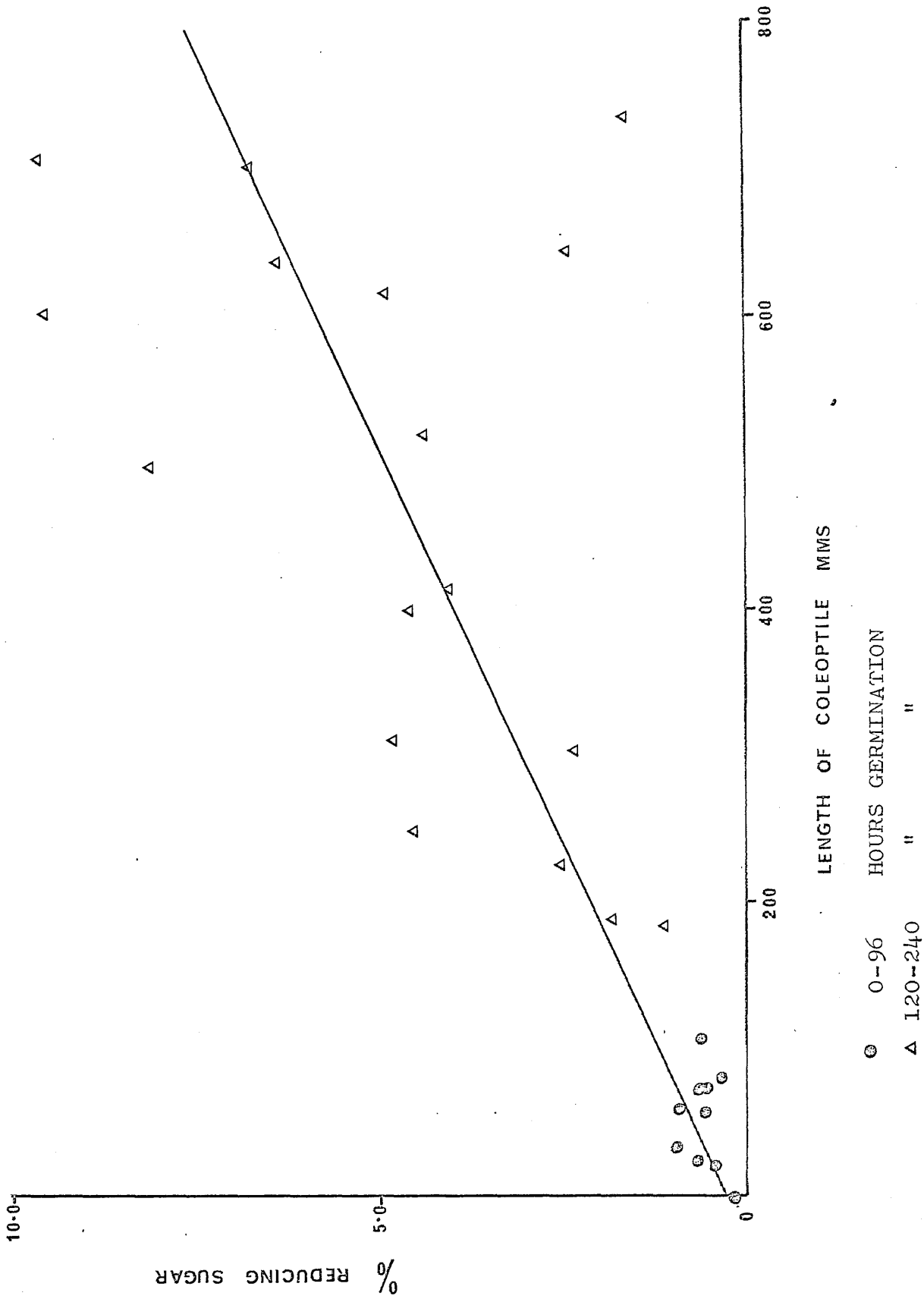

Fig. 30. Scatter plot illustrating correlation between reducing sugar concentration and coleoptile length in groups of Early Pearl grain. Correlation coefficient for 0-240 hours + 0.809.

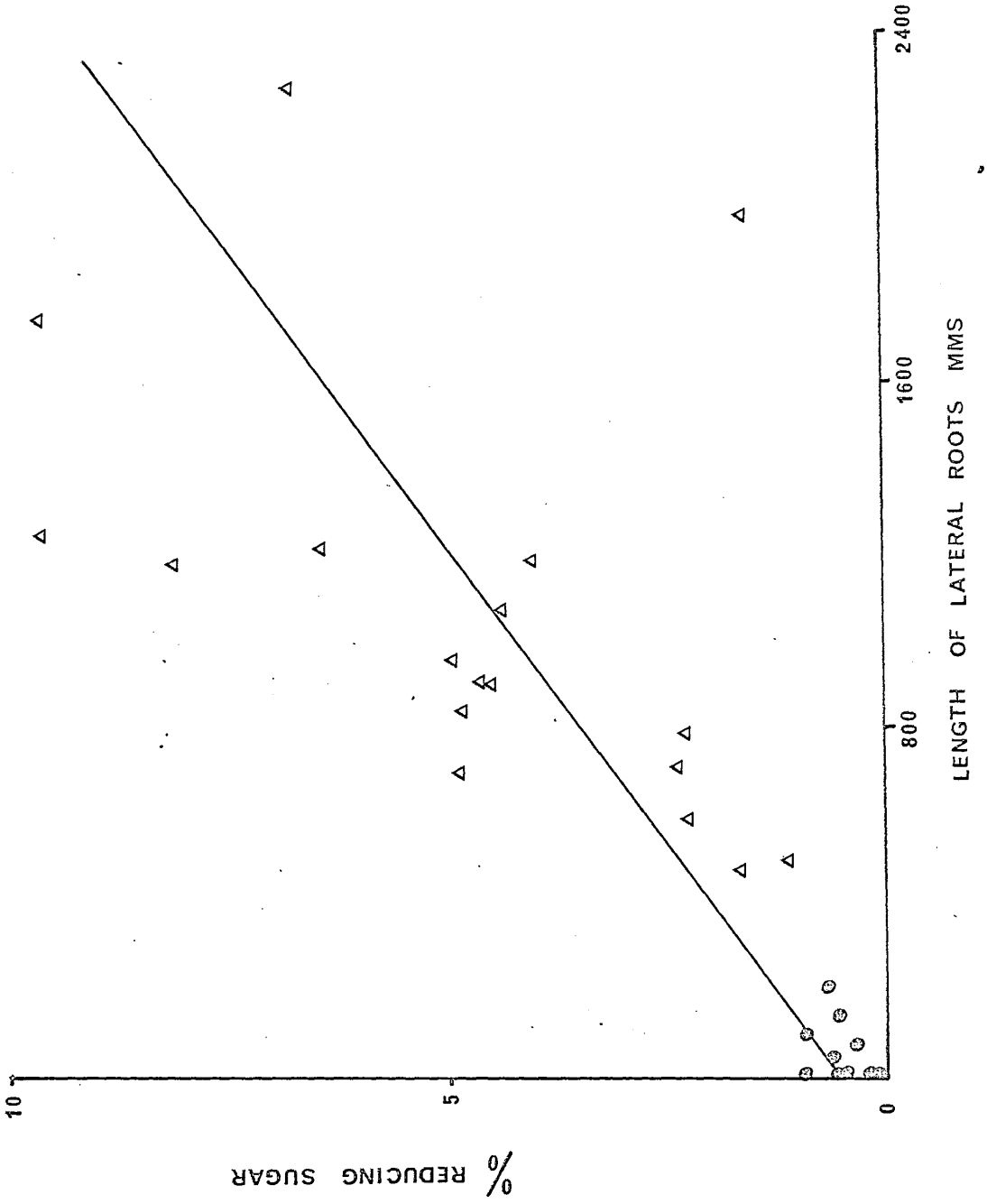

Fig. 31. Scatter plot illustrating correlation between reducing sugar concentration and lateral root length in groups of Early Pearl grain. Correlation coefficient for 0-240 hours % 0.803.

TABLE 22

LENGTH (MMS.) OF RADICLE, COLEOPTILE AND LATERAL ROOTS OF GROUPS OF EARLY PEARL GRAINS DURING GERMINATION.

	Radicle	Coleoptile	Laterals	Growth Index	Mean Radicle	Mean Coleoptile	Mean Laterals	Mean Growth Index
48	47	22	-	0.069				
48	88	34	5	0.127	71.7	26.7	1.7	0.100
48	80	24	-	0.104				
72	167	62	117	0.346				
72	200	76	49	0.325	172.6	65.3	58.0	0.296
72	151	58	8	0.217				
96	212	75	142	0.429				
96	249	107	210	0.566	228.3	81.7	139.3	0.455
96	224	81	66	0.371				
120	446	189	488	1.123				
120	381	186	490	1.057	415.7	199.7	522.7	1.138
120	420	224	590	1.234				
144	531	304	788	1.623				
144	617	308	701	1.626	546.7	306.3	776.7	1.630
144	492	307	841	1.640				
168	431	496	1171	2.098				
168	360	399	916	1.675	410.0	381.0	1001.0	1.792
168	439	248	916	1.603				
192	580	414	1175	2.169				
192	438	518	1070	2.026	654.3	510.7	1165.3	2.330
192	945	600	1251	2.796				
216	751	637	1214	2.602				
216	761	613	958	2.332	705.7	632.0	962.0	2.300
216	605	646	714	1.965				
240	1342	705	1747	3.794				
240	815	700	2279	3.794	1151.3	713.7	2001.0	3.866
240	1297	736	1977	4.010				

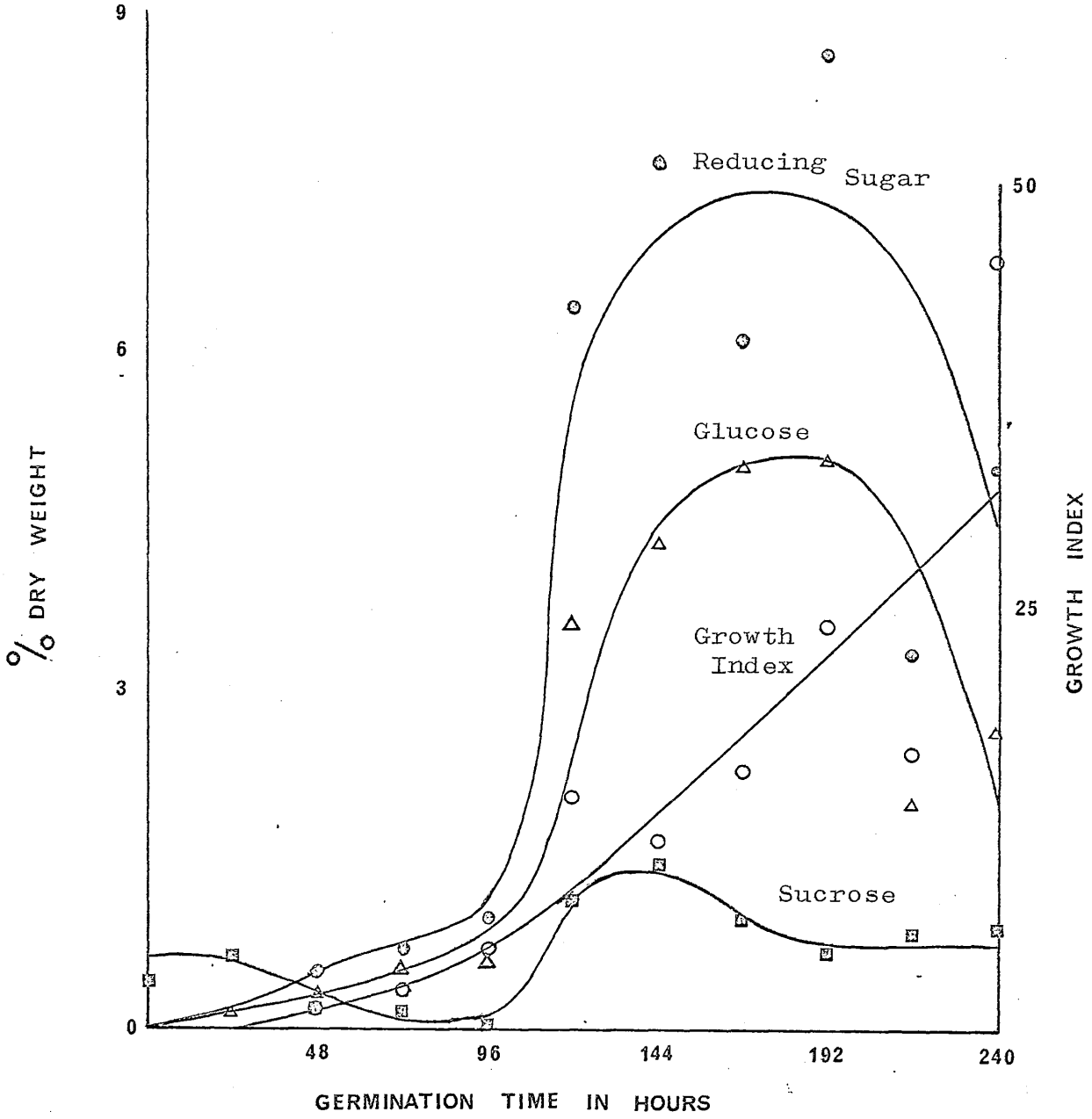

Fig. 32. Changes in reducing sugar, glucose and sucrose concentration in individual Early Pearl grains during germination. Growth index expressed as $\frac{\text{sum of lengths of organs measured}}{100}$

L.S.D. between means for reducing sugar at 0.05 P.L. ± 0.308%
" " " " " " glucose " 0.05 P.L. ± 1.715%
" " " " " " sucrose " 0.05 P.L. ± 0.068%.

TABLE 23

CONCENTRATION OF REDUCING SUGAR (% DRY WEIGHT) IN INDIVIDUAL EARLY PEARL GRAINS DURING GERMINATION.

Germination Period (Hrs)	Grain Number 1	2	3	4	5	6	7	8	9	10	Mean	% Germination	Growth Index
0	-	-	-	-	-	-	-	-	-	-	-	-	-
24	0.006	0.200	0.300	0.097	0.247	0.158	0.156	0.340	0.197	0.142	0.155	-	-
48	0.333	0.300	0.517	0.548	1.238	0.464	0.234	0.297	0.273	0.980	0.518	100	0.100
72	0.484	0.443	0.759	0.479	0.464	0.608	1.502	0.753	0.293	1.535	0.732	100	0.207
96	1.400	0.990	2.346	0.681	0.842	0.760	1.092	0.964	0.762	0.783	1.062	100	0.453
120	7.221	5.154	7.094	5.852	3.934	7.762	8.451	6.938	5.726	6.032	6.416	100	1.388
144	10.223	5.490	7.816	9.869	9.679	6.756	9.616	4.687	9.119	3.274	7.653	100	1.113
168	5.127	2.139	2.336	1.796	4.671	15.810	1.625	2.082	12.339	13.388	6.131	100	1.526
192	7.550	10.749	2.978	8.210	7.013	12.995	1.582	11.877	12.012	11.561	8.653	100	2.374
216	0.631	1.027	6.68	6.799	6.363	4.768	2.963	2.810	0.625	0.882	3.302	100	1.609
240	3.845	10.146	1.097	0.475	4.372	10.667	0.423	7.890	6.815	4.000	4.973	100	4.549

Least Significant Difference Between Observations at 0.05 Probability Level ± 7.295

" " " " Means " " " " ± 2.308

TABLE 24a

ANALYSIS OF VARIANCE DATA FOR REDUCING SUGAR CONCENTRATION IN INDIVIDUAL EARLY PEARL GRAINS.

Source	Degrees of Freedom	Sum Squares	Variance	Variance Ratio (F)	Observations
Germination	10	1072.379	107.238	15.900	* * *
Grain Number	9	70.918	7.880	1.168	N. S.
Error	90	607.020	6.745		

* * * Significant at 0.1% Probability Level.

TABLE 24b

RESULTS OF LINEAR REGRESSION OF REDUCING SUGAR CONCENTRATION AND LENGTH OF RADICLE, COLEOPTILE AND LATERAL ROOTS IN INDIVIDUAL EARLY PEARL GRAINS.

Factor	Germination Period hours	Correlation Coefficient	t	Significance
Reducing Sugar	0 - 240	+ 0.494	-	-
Radicle	0 - 96	+ 0.801	9.269	* * *
	120 - 240	+ 0.198	-	-
Reducing Sugar	0 - 240	+ 0.335	-	-
Coleoptile	0 - 96	+ 0.798	9.174	* * *
	120 - 240	+ 0.103	-	-
Reducing Sugar	0 - 240	+ 0.465	-	-
Lateral Roots	0 - 96	+ 0.525	-	-
	120 - 240	+ 0.029	-	-

TABLE 25

LENGTH (MMS.) OF RADICLE, COLEOPTILE AND LATERAL ROOTS OF INDIVIDUAL EARLY PEARL GRAINS DURING GERMINATION.

Grain Number	GERMINATION PERIOD 48			72			96			120			144			168			192			216			240		
	Radicle	Coleoptile	Laterals	Radicle	Coleoptile	Laterals	Radicle	Coleoptile	Laterals	Radicle	Coleoptile	Laterals	Radicle	Coleoptile	Laterals	Radicle	Coleoptile	Laterals	Radicle	Coleoptile	Laterals	Radicle	Coleoptile	Laterals	Radicle	Coleoptile	Laterals
1	14	4	-	15	3	-	35	18	18	44	45	43	52	7	95	58	45	110	23	14	275	21	30	35	51	117	63
2	11	3	-	17	5	-	17	18	40	48	22	125	54	10	58	17	20	37	39	24	115	31	10	34	339	160	182
3	10	4	-	16	7	8	40	14	36	39	7	52	37	15	96	49	36	122	199	53	117	45	55	201	28	89	194
4	12	-	-	1	6	-	14	7	-	81	9	61	44	12	57	28	17	46	27	21	96	30	61	77	56	106	147
5	5	1	-	13	4	-	24	6	-	48	23	81	27	11	24	25	12	43	54	11	59	45	106	255	123	68	366
6	5	2	-	22	5	-	13	6	-	55	20	165	32	11	79	125	53	63	86	39	173	24	21	48	128	53	51
7	3	-	-	1	6	-	12	7	-	27	8	23	37	9	76	24	39	84	47	15	104	75	61	34	189	133	336
8	-	2	-	15	6	-	17	10	41	83	22	123	29	15	61	17	18	54	60	25	125	41	21	66	169	48	432
9	-	3	-	6	6	-	15	7	-	16	5	47	24	8	34	60	46	42	193	45	175	10	7	69	58	78	214
10	16	5	-	33	12	-	15	7	26	32	8	26	37	9	53	26	31	179	43	21	96	14	37	43	179	77	315
Mean	7.6	2.4	-	13.9	6.0	0.8	20.2	9.0	16.1	47.3	16.9	74.6	37.3	10.7	63.3	42.9		78.0		26.8		33.8		86.2		92.9	
																	31.7		77.1		133.5		40.9		132.0		230.0
Growth Index		0.100			0.207			0.453			1.388			1.113			1.526			2.374			1.609			4.549	

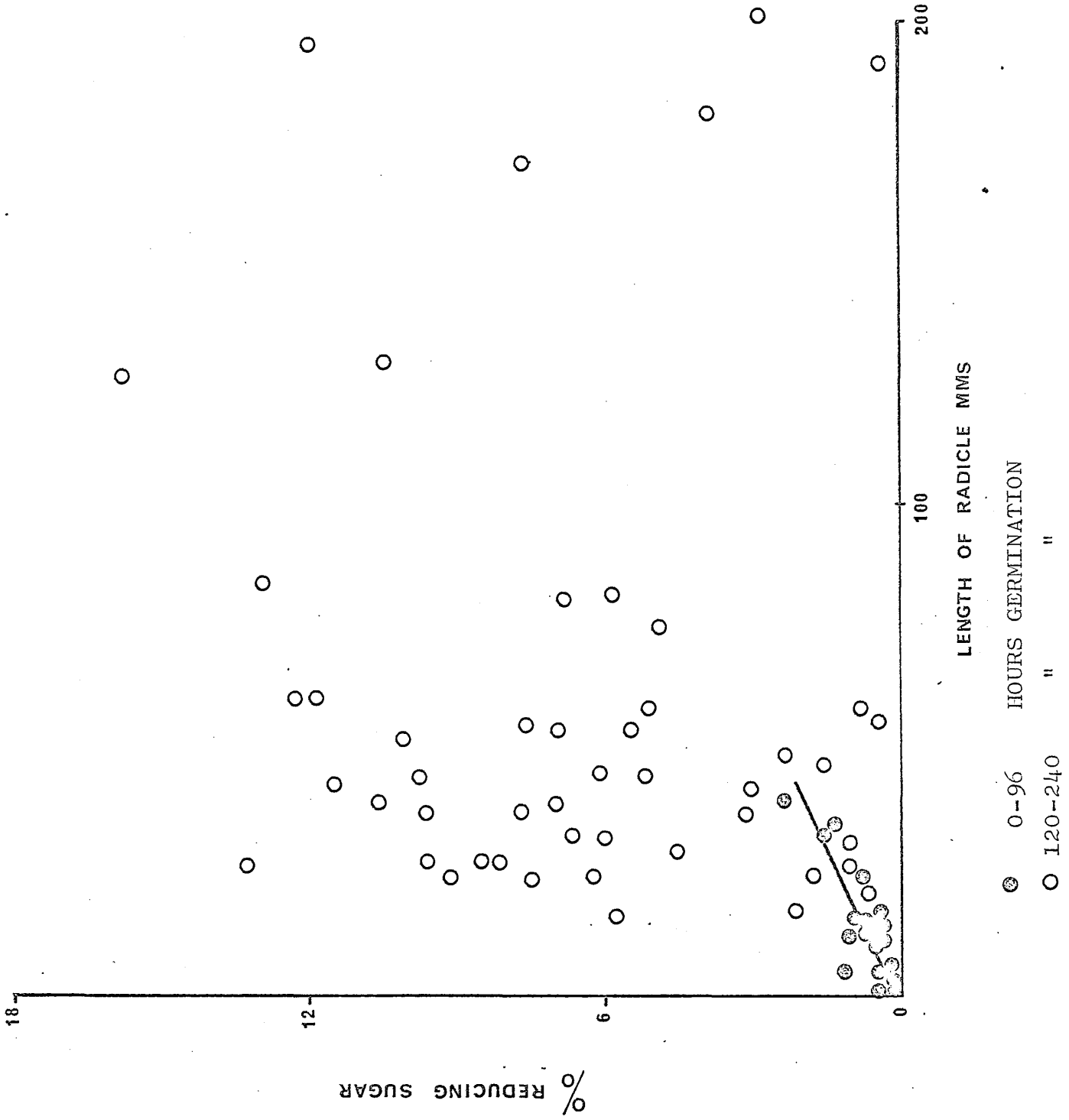

Fig. 33. Scatter plot illustrating correlation between reducing sugar concentration and radicle length in individual Early Pearl grains. Correlation coefficient for 0-96 hours + 0.801.

Fig. 34. Scatter plot illustrating correlation between reducing sugar concentration and coleoptile length in individual Early Pearl grains. Correlation coefficient for 0-96 hours. + 0.798.

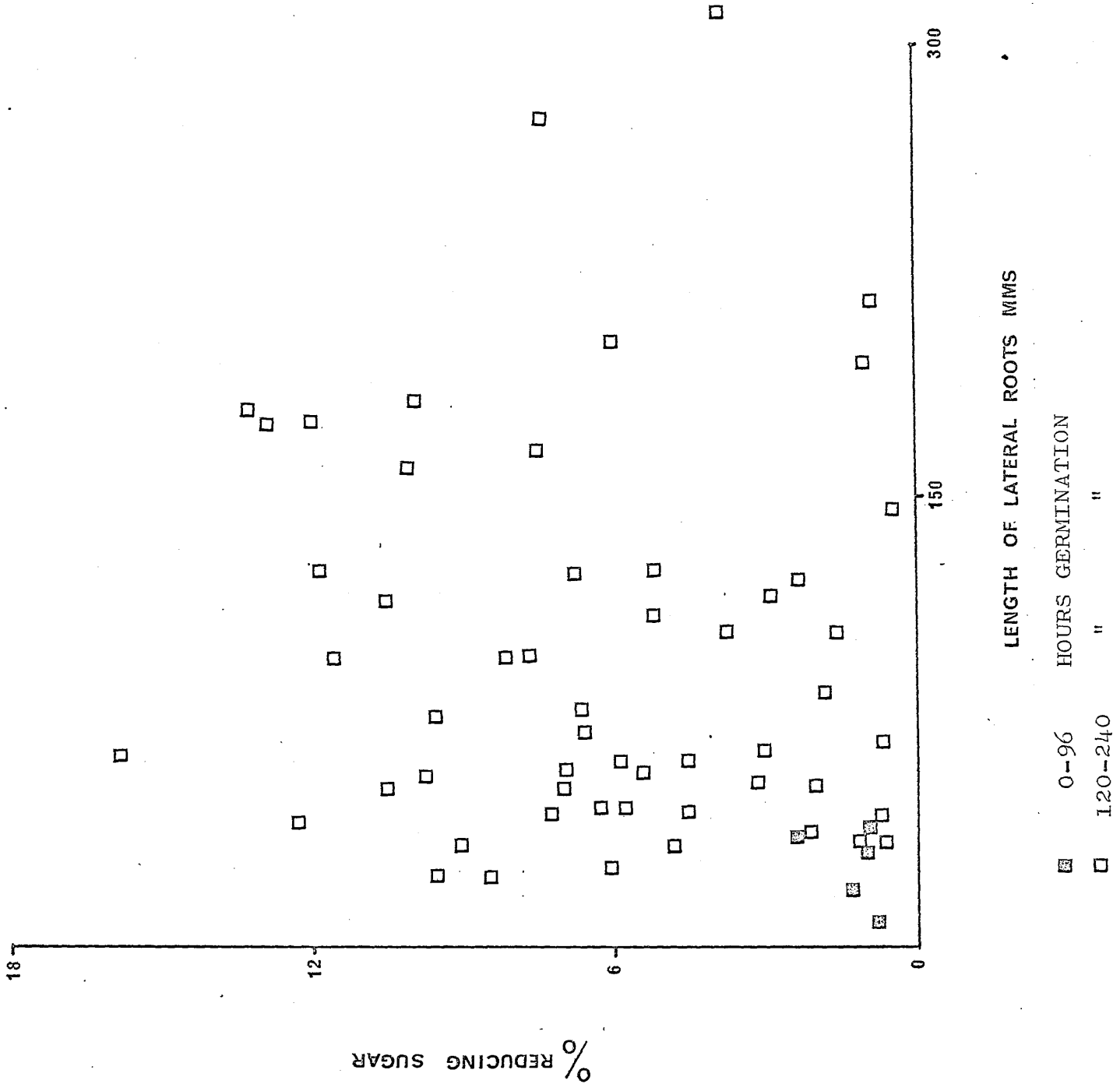

Fig. 35. Scatter plot illustrating correlation between reducing sugar concentration and lateral root length in individual Early Pearl grains.

TABLE 26

GLUCOSE CONCENTRATION (% DRY WEIGHT) IN GROUPS OF EARLY PEARL GRAIN DURING GERMINATION.

Germination Period (Hrs)	1	2	3	Mean	Germination Period Mean
0	0.051	0.052	0.051	0.051	
0	0.058	0.070	0.127	0.085	0.117
0	0.233	0.269	0.257	0.253	
24	0.154	0.166	0.166	0.162	
24	0.194	0.206	0.205	0.202	0.152
24	0.149	0.148	0.134	0.144	
48	0.236	0.243	0.316	0.265	
48	0.425	0.363	0.440	0.409	0.309
48	0.348	0.358	0.361	0.356	
72	0.529	0.604	0.601	0.578	
72	0.468	0.471	0.446	0.461	0.452
72	0.488	0.479	0.438	0.468	
96	0.712	0.746	0.717	0.725	
96	0.839	0.808	0.847	0.831	0.600
96	0.457	0.440	0.430	0.442	
120	1.452	1.457	1.453	1.454	
120	1.322	1.400	1.300	1.341	1.434
120	1.953	2.038	1.964	1.985	
144	1.310	1.302	1.386	1.333	
144	2.695	2.540	2.607	2.614	1.995
144	2.981	2.476	2.651	2.703	
168	3.991	4.212	4.394	4.199	
168	3.170	3.134	3.273	3.192	3.105
168	3.095	2.844	2.937	2.959	
192	2.623	3.049	2.929	2.867	
192	4.332	4.242	4.177	4.250	3.559
192	4.813	4.812	4.613	4.746	
216	3.366	3.044	3.405	3.272	
216	3.253	3.176	3.117	3.182	2.446
216	1.749	1.719	1.629	1.699	
240	4.019	4.129	4.188	4.112	
240	2.442	2.651	2.686	2.593	3.609
240	5.370	5.394	5.210	5.325	

L. S. D. Between Observations at 0.05 P. L. ± 0.240

" " " " Means " " " " ± 0.138

" " " " Germination Period Means " " ± 0.080

TABLE 27a

ANALYSIS OF VARIANCE DATA FOR GLUCOSE CONCENTRATION IN GROUPS OF EARLY PEARL GRAIN.

Source	Degrees of Freedom	Sum Squares	Variance	Variance Ratio(F)	Observations
Germination	10	214.609250	21.460925	2954.016	* * *
Replication	2	0.002620	0.001310	0.180	N. S.
Sample	2	0.722470	0.361235	49.723	*
Sample/Rep.	4	0.108260	0.027065	3.725	N. S.
Replication/ Germination	20	0.199030	0.009952	1.370	N. S.
Sample/Germ.	20	28.106280	1.405314	193.436	* * *
Error	40	0.290600	0.007265		

* * * Significant at 0.1% Probability Level.
* " " 5% " "

TABLE 27b

RESULTS OF LINEAR REGRESSION OF GLUCOSE CONCENTRATION AND LENGTH OF RADICLE COLEOPTILE, LATERAL ROOTS AND REDUCING SUGAR IN GROUPS OF EARLY PEARL GRAIN.

Factor	Germination Period Hours	Correlation Coefficient	t	Significance.
Glucose/Radicle	0 -240	+ 0.860	9.222	* * *
Glucose/Coleoptile	0 -240	+ 0.892	10.984	* * *
Glucose/Lateral Roots	0 -240	+ 0.884	10.574	* * *
Glucose/Reducing Sugar	0 -240	+ 0.836	8.480	* * *

* * * Significant at 0.1% Probability Level.

Fig. 36. Scatter plot illustrating correlation between glucose concentration and radicle length in groups of Early Pearl grain. Correlation coefficient for 0-240 hours + 0.860.

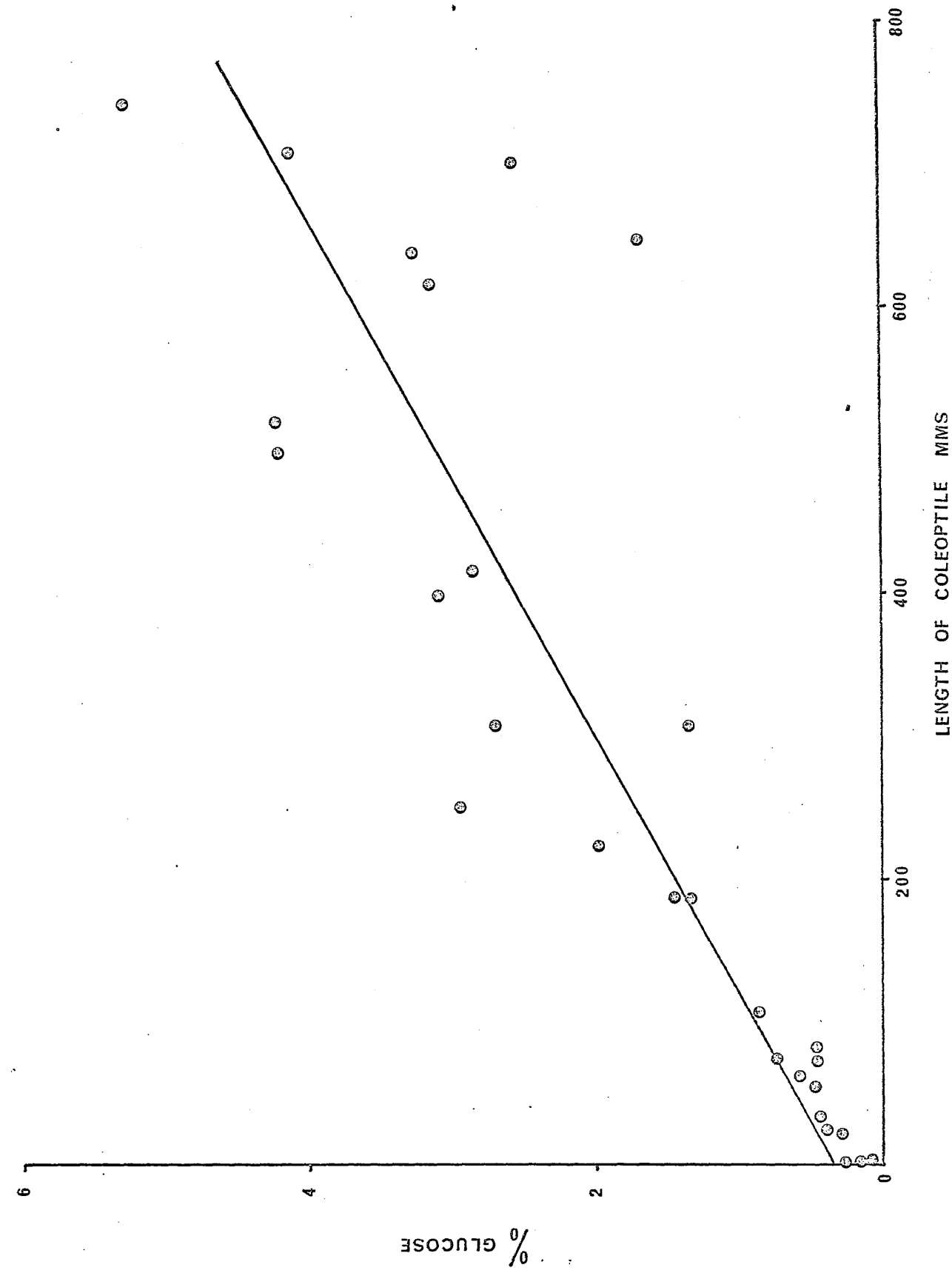

Fig. 37. Scatter plot illustrating correlation between glucose concentration and coleoptile length in groups of Early Pearl grain. Correlation coefficient for 0-240 hours + 0.892.

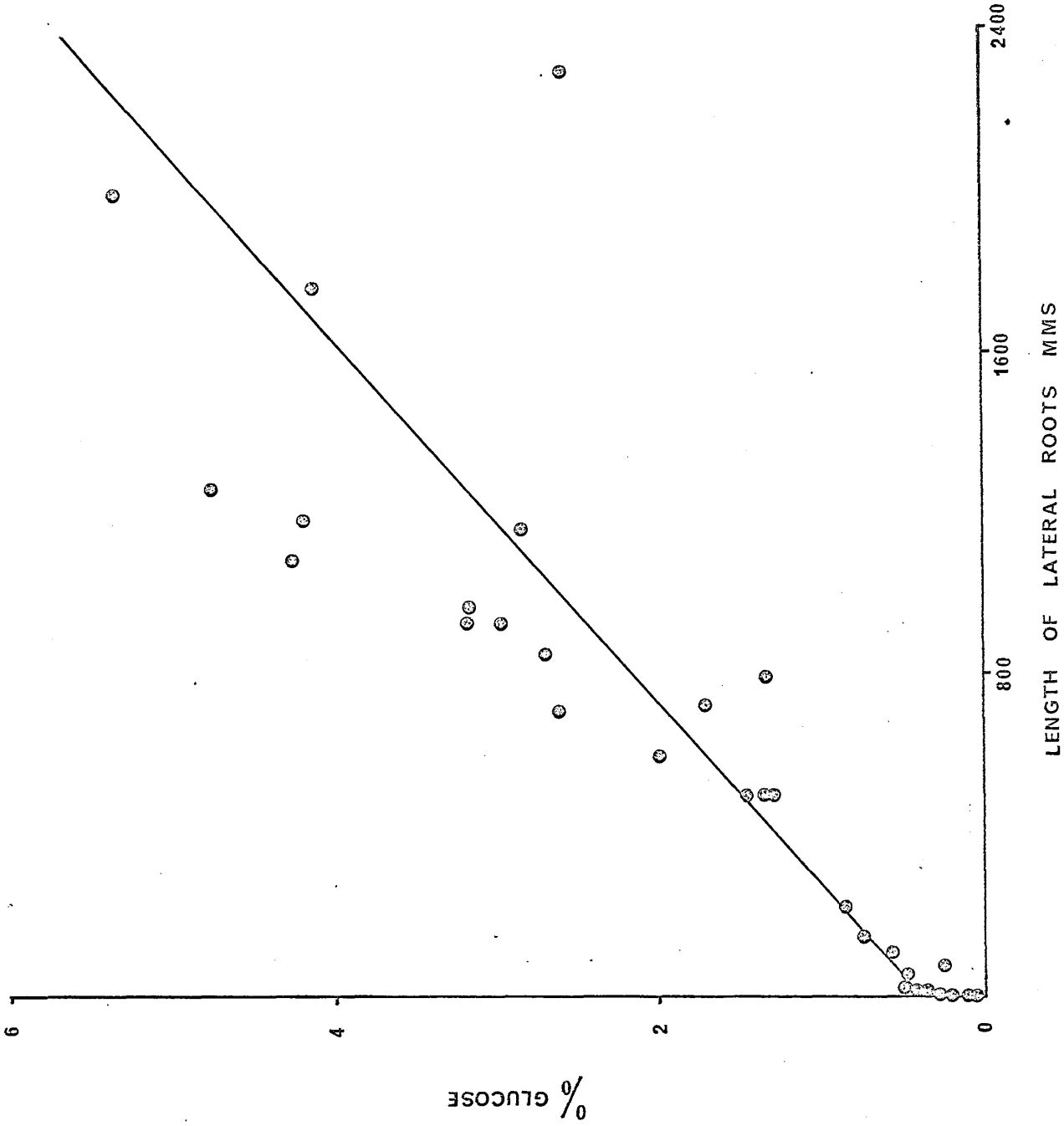

Fig. 38. Scatter plot illustrating correlation between glucose concentration and lateral root length in groups of Early Pearl grain. Correlation coefficient for 0-240 hours + 0.884.

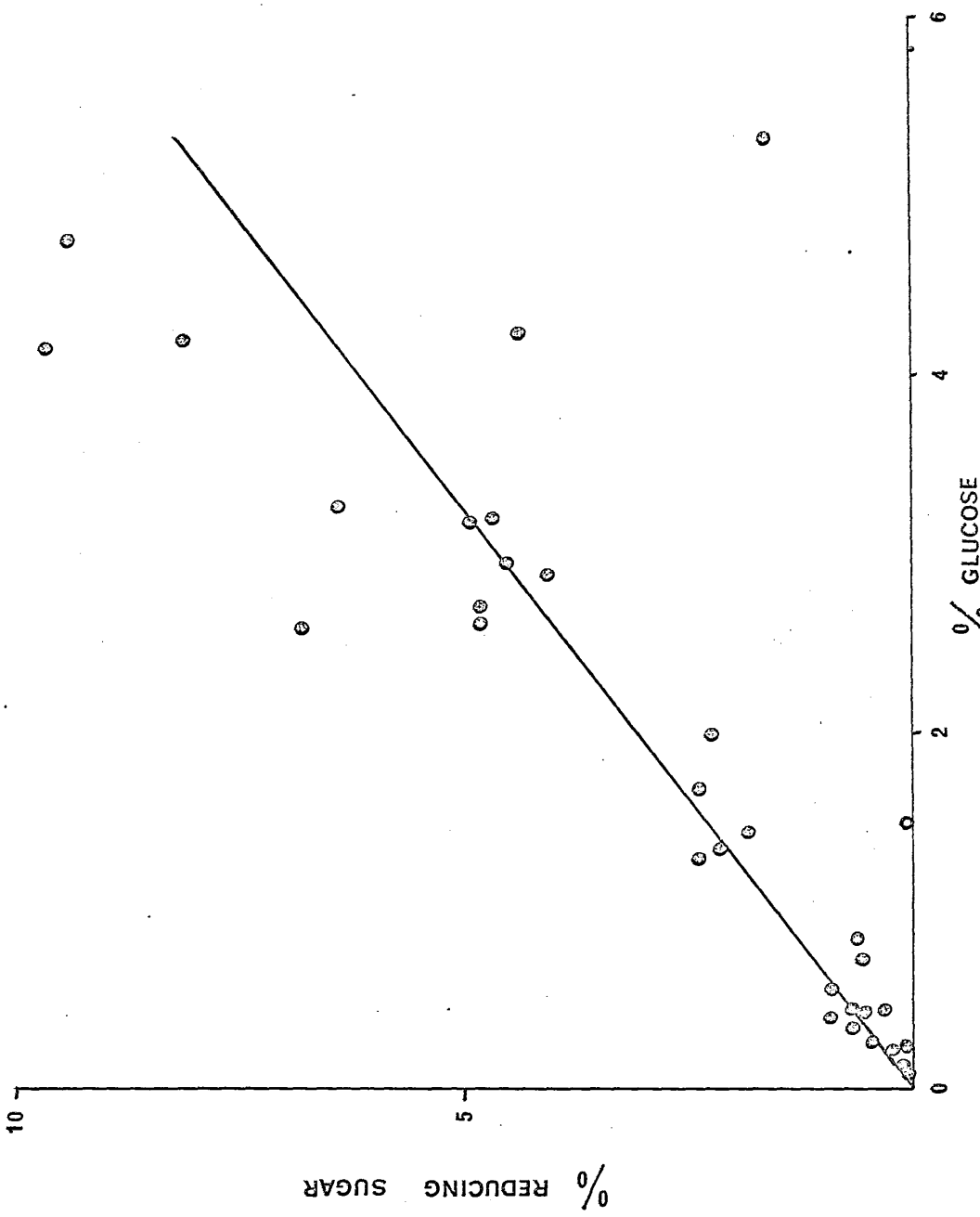

Fig. 39. Scatter plot illustrating correlation between reducing sugar and glucose concentration in groups of Early Pearl grain. Correlation coefficient for 0-240 hours + 0.836.

TABLE 28

GLUCOSE CONCENTRATION (% DRY WEIGHT) IN INDIVIDUAL EARLY PEARL GRAINS DURING GERMINATION.

Germination Period (Hrs)	Grain Number 1	2	3	4	5	6	7	8	9	10	Mean	% Germination	Growth Index
0	0.029	0.044	0.049	-	-	-	-	-	0.013	-	0.013	-	-
24	0.159	0.162	0.159	0.127	0.226	0.139	0.179	0.194	0.145	0.131	0.162	-	-
48	0.254	0.206	0.272	0.292	0.767	0.320	0.260	0.254	0.282	0.546	0.345	100	0.100
72	0.474	0.404	0.392	0.315	0.386	0.576	1.343	0.443	0.190	0.809	0.533	100	0.207
96	0.607	0.393	0.929	0.352	0.331	0.563	0.589	1.991	0.049	-	0.580	100	0.453
120	3.868	2.891	3.855	3.518	1.905	4.684	4.908	3.880	3.412	3.438	3.636	100	1.388
144	6.009	2.971	3.908	5.731	5.084	3.113	5.701	3.255	4.711	2.425	4.293	100	1.113
168	4.170	1.160	1.738	1.200	3.324	15.096	3.404	2.018	10.772	10.562	5.244	100	1.526
192	6.883	6.411	1.636	4.166	4.383	8.609	1.319	7.315	7.696	4.841	5.326	100	2.374
216	0.156	0.690	4.503	4.887	4.160	3.492	2.221	2.662	0.194	0.137	2.310	100	1.609
240	1.626	4.340	0.487	0.158	2.484	7.004	0.634	4.649	3.548	2.143	2.707	100	4.549

Least Significant Difference Between Observations at 0.05 Probability Level ± 5.423

" " " " Means " " " " ± 1.715

TABLE 29a

ANALYSIS OF VARIANCE DATA FOR GLUCOSE CONCENTRATION IN INDIVIDUAL EARLY PEARL GRAINS.

Source	Degrees of Freedom	Sum Squares	Variance	Variance Ratio (F)	Observations
Germination	10	434.356	43.436	11.639	* * *
Grain Number	9	46.818	5.202	1.394	N. S.
Error	90	335.870	3.732		

* * * Significant at 0.1% Probability Level.

TABLE 29b

RESULTS OF LINEAR REGRESSION OF GLUCOSE CONCENTRATION AND LENGTH OF RADICLE, COLEOPTILE, LATERAL ROOTS AND REDUCING SUGAR CONCENTRATION IN INDIVIDUAL EARLY PEARL GRAINS.

Factor	Germination Period (Hrs)	Correlation Coefficient	t	Significance
Glucose	0 -240	+ 0.437	-	-
Radicle	0 -192	+ 0.606	7.146	* * *
Glucose	0 -240	+ 0.306	-	-
Coleoptile	0 -192	+ 0.691	8.966	* * *
Glucose	0 -240	+ 0.433	-	-
Lateral Roots	0 -192	+ 0.690	8.942	* * *
Glucose	0 -240	+ 0.954	33.065	* * *
Reducing Sugar				

* * * Significant at 0.1% Probability Level.

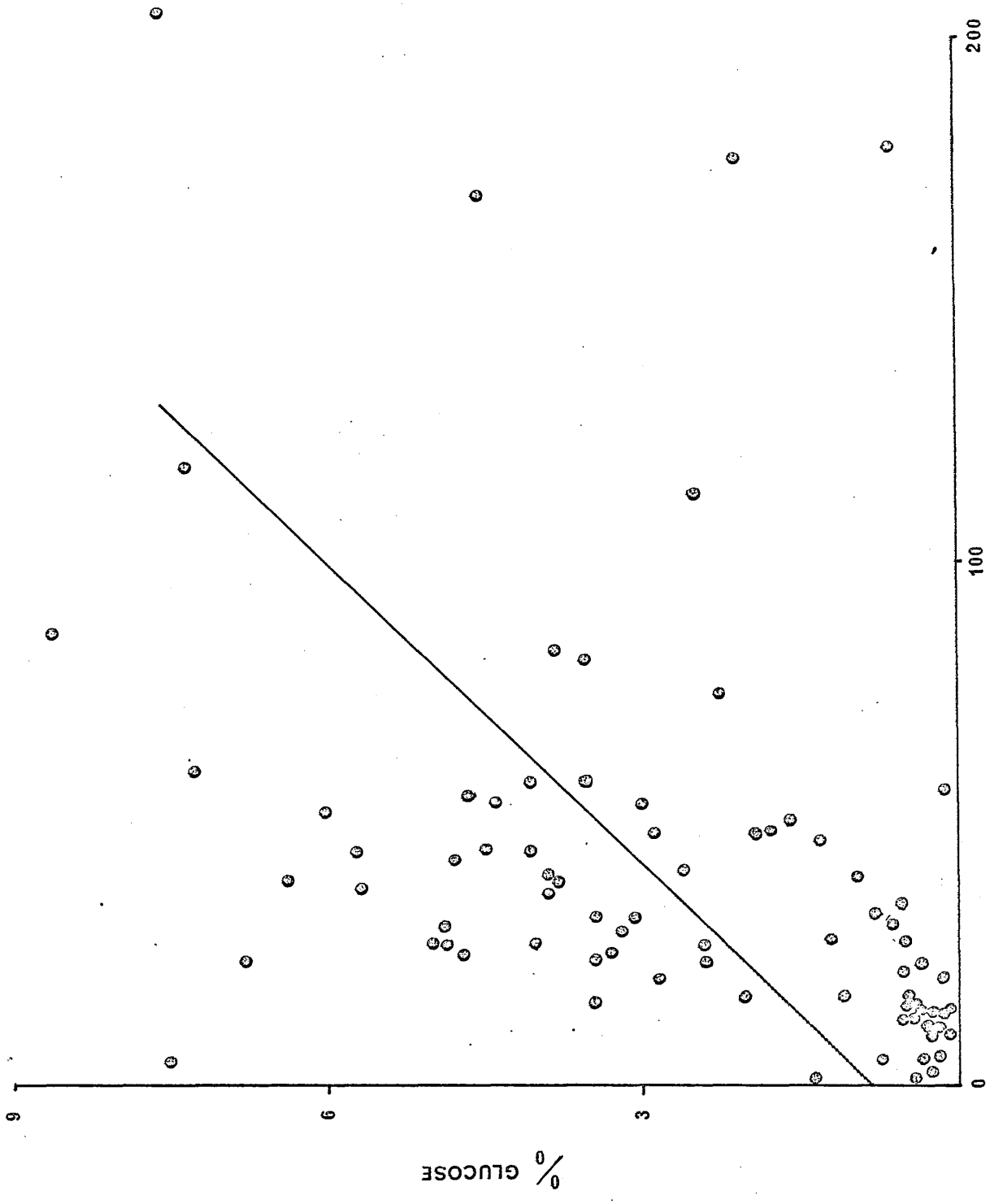

Fig. 40. Scatter plot illustrating correlation between glucose concentration and radicle length in individual Early Pearl grains. Correlation coefficient for 0-192 hours + 0.606.

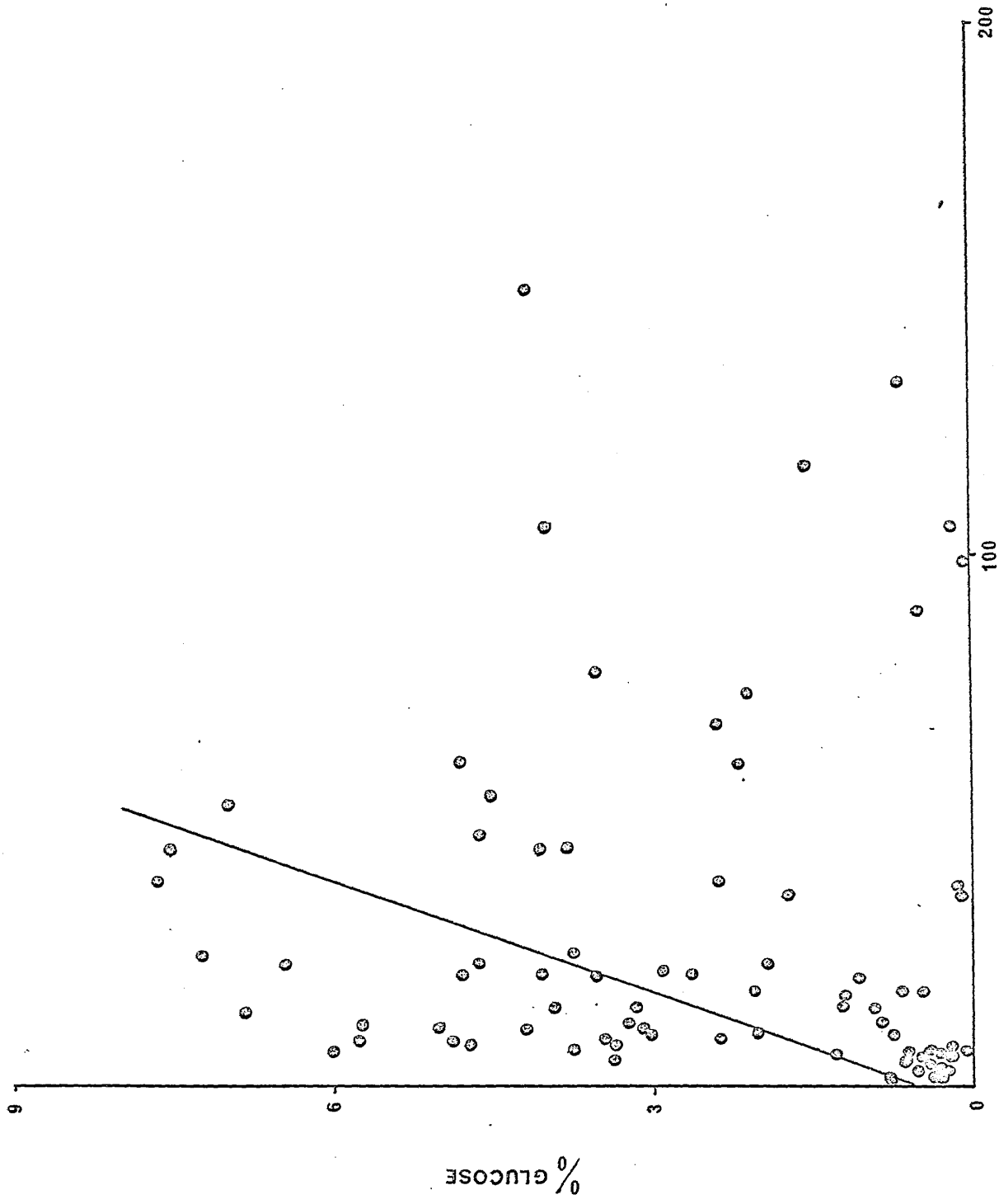

Fig. 41. Scatter plot illustrating correlation between glucose concentration and coleoptile length in individual Early Pearl grains. Correlation coefficient for 0-192 hours + 0.691.

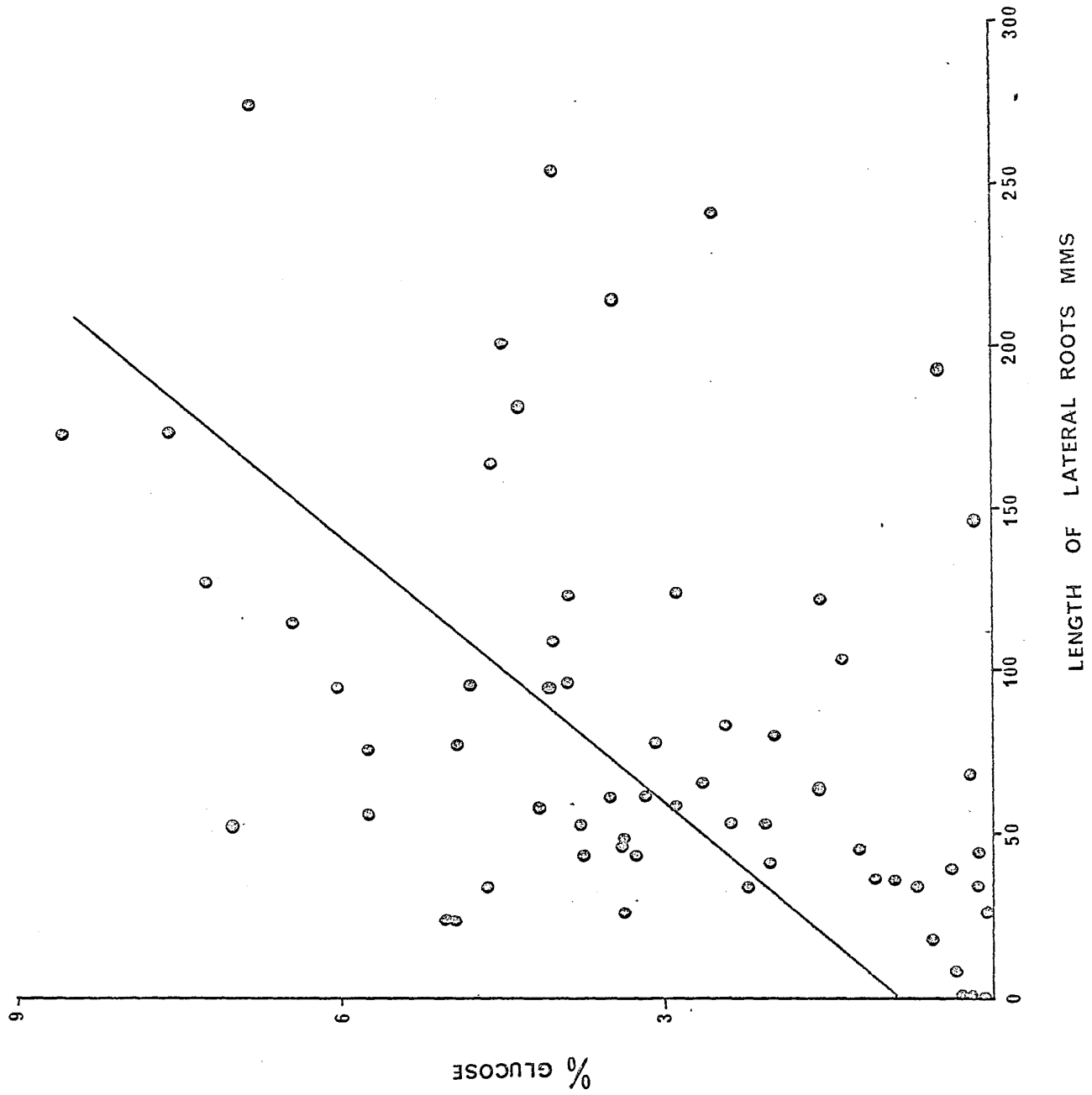

Fig. 42. Scatter plot illustrating correlation between glucose concentration and lateral root length in individual Early Pearl grains. Correlation coefficient for 0-192 hours + 0.690.

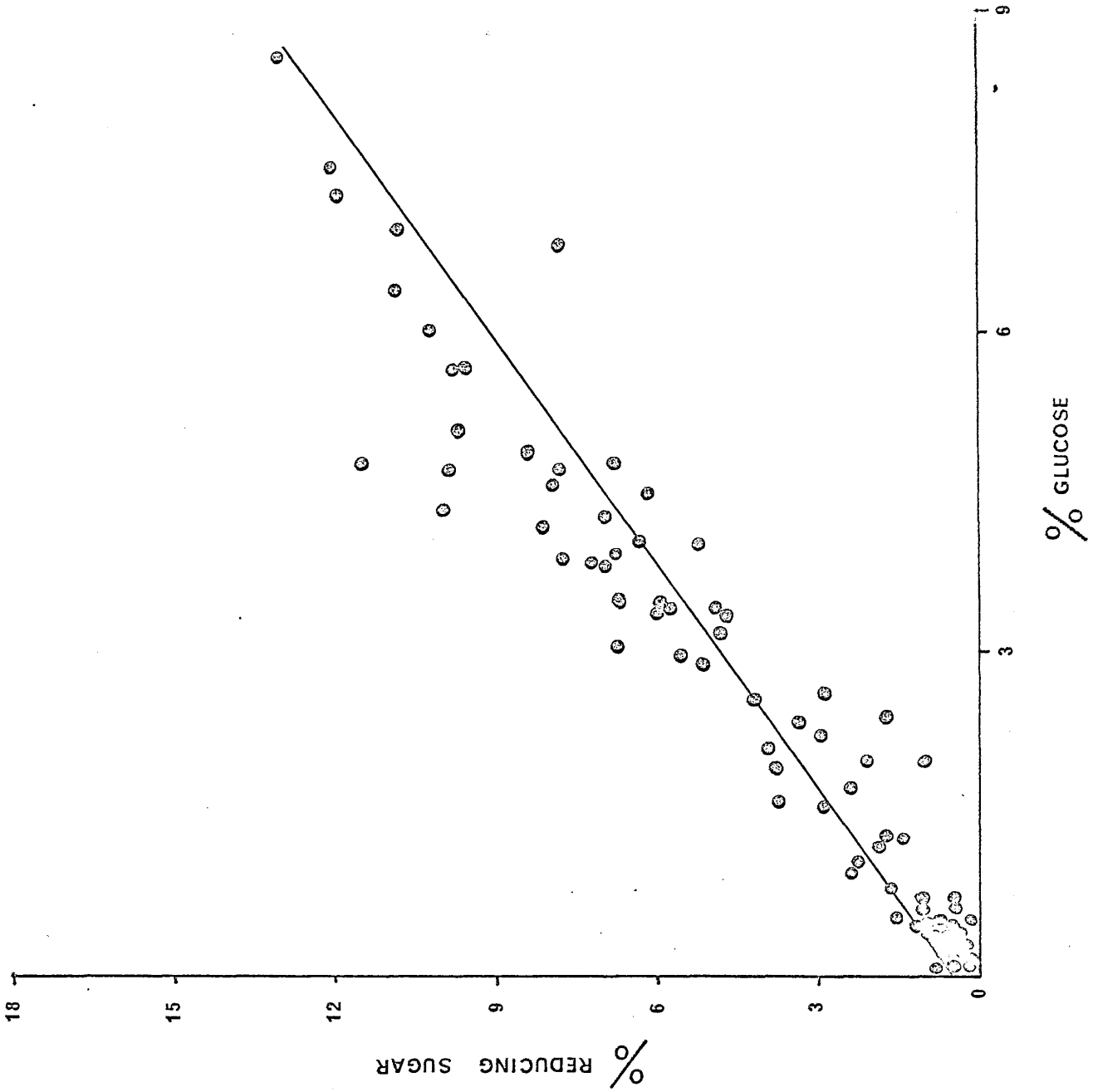

Fig. 43. Scatter plot illustrating correlation between reducing sugar and glucose concentrations in individual Early Pearl grains. Correlation coefficient for 0-240 hours + 0.954.

TABLE 30

CONCENTRATION (% DRY WEIGHT) OF SUCROSE IN GROUPS OF EARLY PEARL GRAIN DURING GERMINATION.

Germination Period (Hrs)	1	2	3	Mean	Germination Period Mean
0	0.617	0.562	0.542	0.574	
0	0.558	0.641	0.567	0.589	0.486
0	0.289	0.288	0.313	0.297	
24	0.617	0.626	0.637	0.627	
24	0.667	0.681	0.673	0.674	0.676
24	0.787	0.672	0.728	0.729	
48	0.184	0.209	0.182	0.192	
48	0.159	0.164	0.158	0.160	0.218
48	0.294	0.323	0.293	0.303	
72	0.250	0.249	0.202	0.234	
72	0.217	0.192	0.201	0.203	0.191
72	0.131	0.148	0.130	0.136	
96	0.022	0.034	0.034	0.030	
96	0.019	0.018	0.014	0.017	0.035
96	0.051	0.058	0.062	0.057	
120	0.327	0.321	0.394	0.347	
120	0.502	0.568	0.494	0.521	0.396
120	0.290	0.345	0.326	0.320	
144	0.198	0.202	0.221	0.207	
144	0.209	0.187	0.196	0.197	0.202
144	0.203	0.195	0.206	0.201	
168	0.326	0.363	0.333	0.341	
168	0.637	0.643	0.654	0.645	0.542
168	0.635	0.668	0.617	0.640	
192	0.664	0.643	0.663	0.657	
192	0.682	0.663	0.665	0.670	0.663
192	0.670	0.665	0.652	0.662	
216	0.785	0.795	0.823	0.701	
216	0.896	0.893	0.900	0.896	0.735
216	0.617	0.605	0.603	0.608	
240	0.641	0.627	0.623	0.630	
240	0.489	0.499	0.545	0.511	0.576
240	0.573	0.642	0.549	0.588	

L. S. D. Between Observations at 0.05 P. L. ± 0.052

" " " " Means " " " " ± 0.030

" " " " Germination Period Means " ± 0.017

TABLE 31a

ANALYSIS OF VARIANCE DATA FOR SUCROSE CONCENTRATION IN GROUPS OF EARLY PEARL GRAINS.

Source	Degrees of Freedom	Sum Squares	Variance	Variance Ratio (F)	Observations
Germination	10	5.201828	0.520183	773.190	* * *
Replication	2	0.000700	0.000350	0.520	N. S.
Sample	2	0.045448	0.022724	33.777	*
Sample/Rep.	4	0.000729	0.000182	0.271	N. S.
Replication/Germ.	20	0.008621	0.000431	0.641	N. S.
Sample/Germ.	20	0.588763	0.029438	43.756	* * *
Error	40	0.026911	0.000673		

* * * Significant at 0.1% Probability Level.
* " " 5.0% " "

TABLE 31b

RESULTS OF LINEAR REGRESSION OF SUCROSE CONCENTRATION AND LENGTH OF RADICLE, COLEOPTILE AND LATERAL ROOTS IN GROUPS OF EARLY PEARL GRAINS.

Factor	Germination Period (Hrs)	Correlation Coefficient	t	Significance
Sucrose	0 -240	+ 0.339	-	-
	0 -96	- 0.849	5.793	* * *
Radicle	120 -240	+ 0.328	-	-
Sucrose	0 -240	+ 0.474	-	-
	0 -96	- 0.846	5.725	* * *
Coleoptile	120 -240	+ 0.561	-	-
Sucrose	0 -240	+ 0.418	-	-
	0 -96	- 0.614	2.804	*
Lateral Roots	120 -240	+ 0.316	-	-

* * * Significant at 0.1% Probability Level.
* " " 2.0% " "

TABLE 32a

RESULTS OF LINEAR REGRESSION OF SUCROSE CONCENTRATION AND REDUCING SUGAR AND GLUCOSE CONCENTRATIONS IN GROUPS OF EARLY PEARL GRAINS.

Factor	Germination Period (Hrs)	Correlation Coefficient	t	Significance
Sucrose	0 -240	+ 0.364	-	-
	0 -96	- 0.703	3.564	* *
Reducing Sugar	120 -240	+ 0.227	-	-
Sucrose	0 -240	+ 0.428	-	-
	0 -96	- 0.841	5.603	* * *
Glucose	120 -240	+ 0.421	-	-

* * * Significant at 0.1% Probability Level.
* * " " 0.5% " "

TABLE 32b

RESULTS OF LINEAR REGRESSION OF SUCROSE CONCENTRATION AND REDUCING SUGAR AND GLUCOSE CONCENTRATIONS IN INDIVIDUAL EARLY PEARL GRAINS.

Factor	Germination Period (Hrs)	Correlation Coefficient	t	Significance
Sucrose	0 -240	+ 0.624	8.297	* * *
	0 -96	- 0.517	4.184	* * *
Reducing Sugar	120 -240	+ 0.332	-	-
Sucrose	0 -240	+ 0.578	7.338	* * *
	0 -96	- 0.373	-	-
Glucose	120 -240	+ 0.283	-	-

* * * Significant at 0.1% Probability Level.

Fig. 44. Scatter plot illustrating correlation between sucrose concentration and radicle length in groups of Early Pearl grain. Correlation coefficient for 0-96 hours = 0.849.

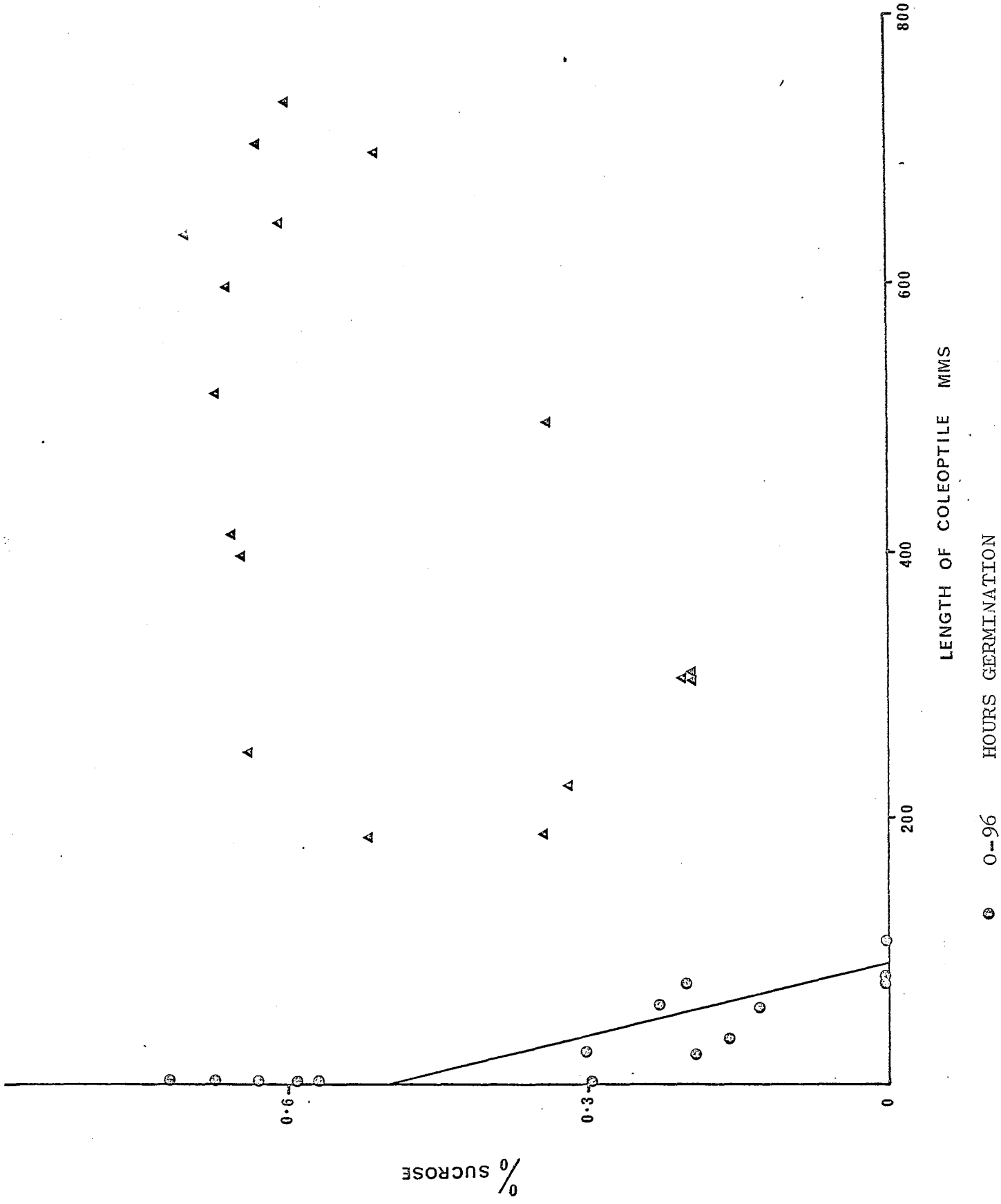

Fig. 45. Scatter plot illustrating correlation between sucrose concentration and coleoptile length in groups of Early Pearl grain. Correlation coefficient for 0-96 hours = 0.846.

Fig. 46. Scatter plot illustrating correlation between sucrose concentration and lateral root length in groups of Early Pearl grain.

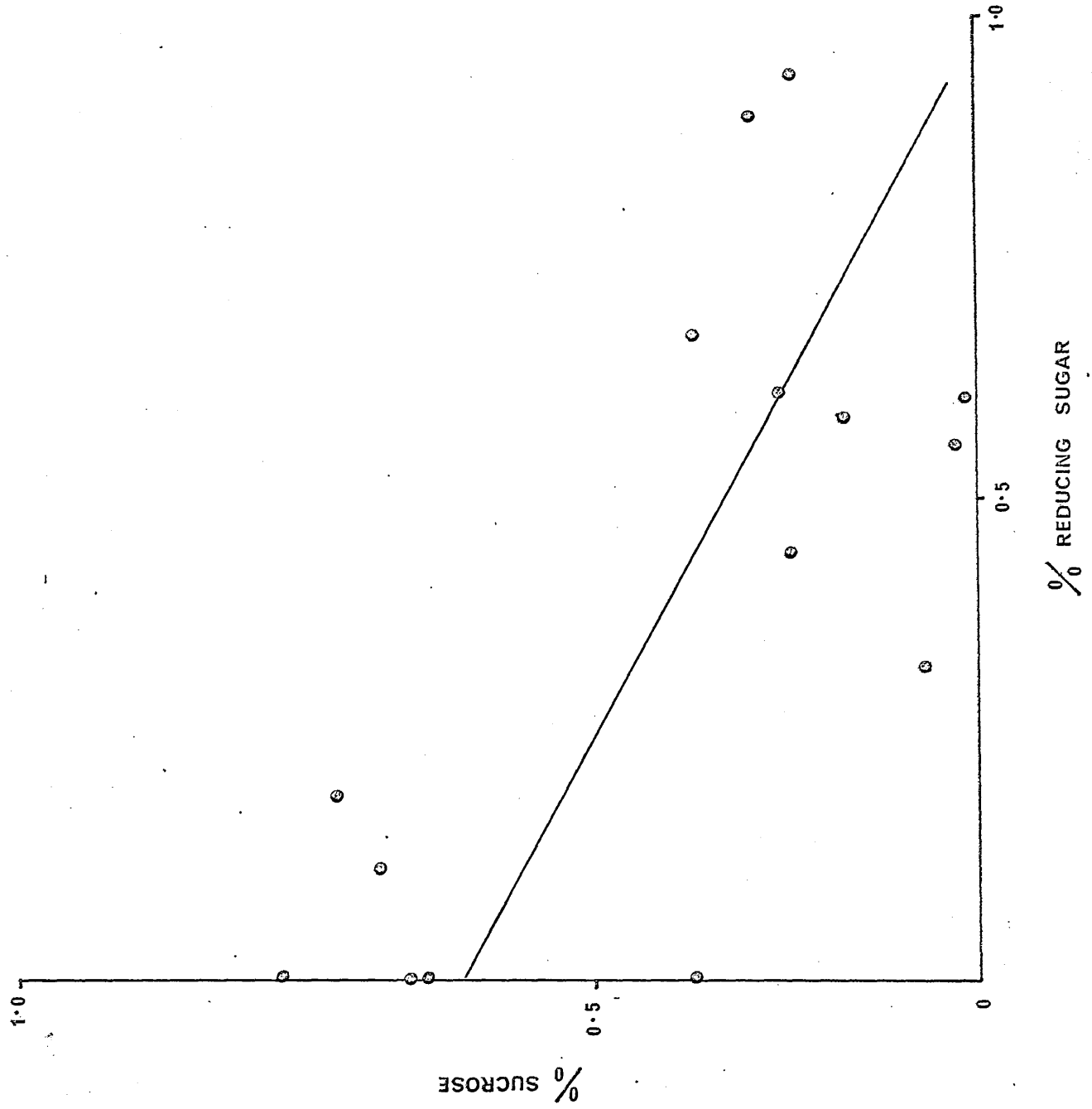

Fig. 47. Scatter plot illustrating correlation between sucrose and reducing sugar concentration during the first 96 hours germination in groups of Early Pearl grain. Correlation coefficient for 0-96 hours = 0.703.

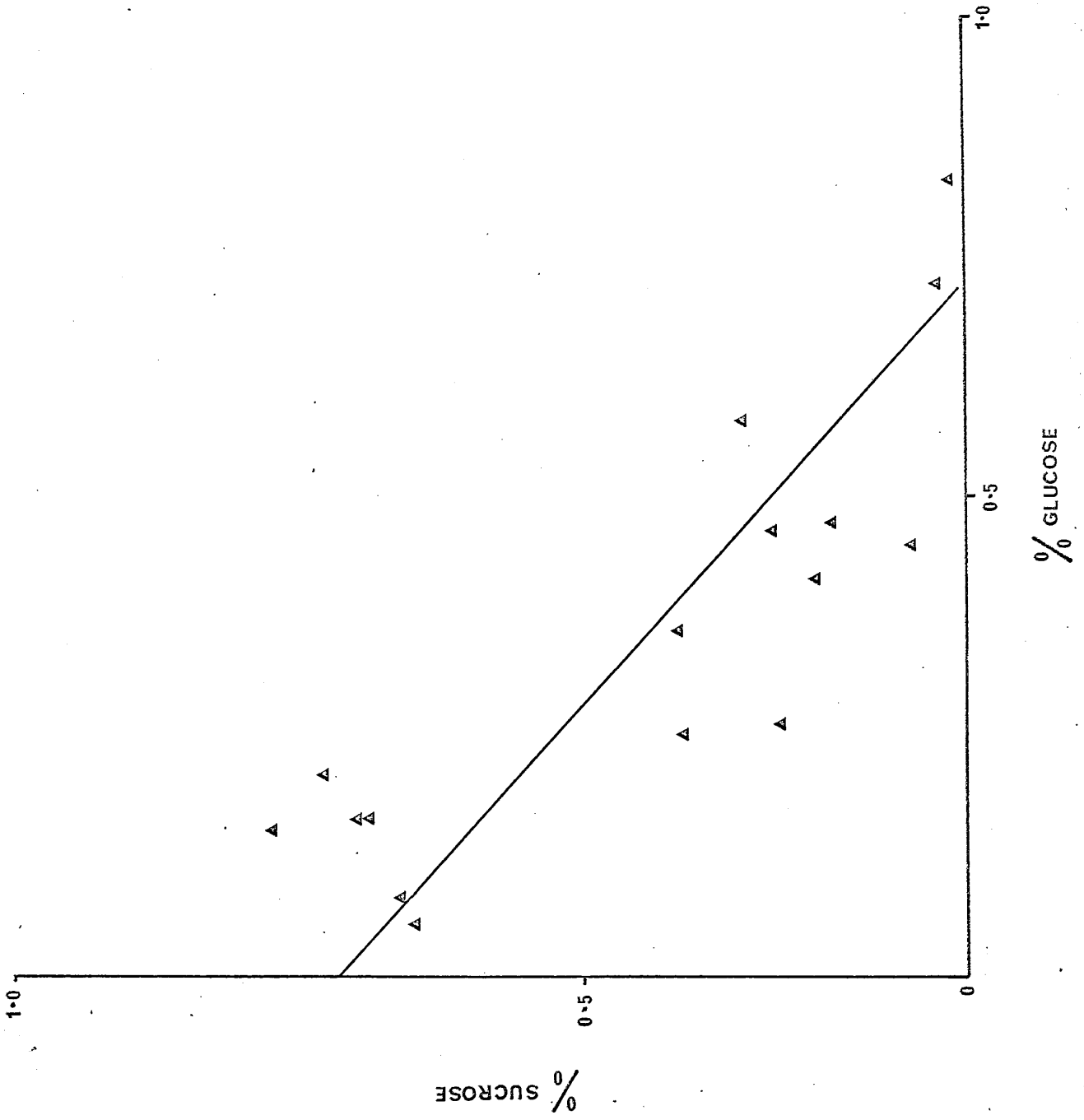

Fig. 48. Scatter plot illustrating correlation between sucrose and glucose concentrations during the first 96 hours germination in groups of Early Pearl grain. Correlation coefficient for 0-96 hours = 0.841.

TABLE 33

CONCENTRATION OF SUCROSE (% DRY WEIGHT) IN INDIVIDUAL EARLY PEARL GRAINS DURING GERMINATION.

Germination Period (Hrs)	Grain Number 1	2	3	4	5	6	7	8	9	10	Mean	% Germination	Growth Index
0	0.258	0.495	0.395	0.499	0.324	0.379	0.316	0.486	0.321	0.356	0.383	-	-
24	0.282	0.750	0.580	0.316	0.936	0.818	0.660	1.026	0.442	0.659	0.647	-	-
48	0.121	0.285	0.244	0.301	0.299	0.260	0.257	0.223	0.218	0.143	0.235	100	0.100
72	0.131	0.595	0.070	0.206	0.157	0.261	0.043	0.071	0.056	0.197	0.179	100	0.207
96	0.018	0.010	0.097	0.023	0.166	0.119	0.011	0.112	0.047	0.024	0.063	100	0.453
120	0.602	1.028	1.201	1.087	0.854	1.526	1.433	1.419	1.505	0.940	1.159	100	1.388
144	1.379	1.804	1.416	1.875	1.854	1.037	1.652	1.637	1.411	0.887	1.495	100	1.113
168	1.221	0.588	1.059	0.912	0.948	1.395	1.626	0.996	0.780	0.228	0.975	100	1.526
192	0.883	0.664	0.841	0.303	1.047	0.895	0.401	0.550	0.891	0.522	0.699	100	2.374
216	0.118	0.315	1.711	1.534	1.348	0.923	0.816	1.011	0.343	0.183	0.830	100	1.609
240	0.899	1.324	0.324	0.421	0.975	1.514	0.375	0.857	1.492	0.787	0.897	100	4.549

Least Significant Difference Between Observations at 0.05 Probability Level ± 0.847

" " " " Means " " " " ± 0.268

TABLE 34a

ANALYSIS OF VARIANCE DATA FOR SUCROSE CONCENTRATION IN INDIVIDUAL EARLY PEARL GRAINS.

Source	Degrees of Freedom	Sum Squares	Variance	Variance Ratio (F)	Observations
Germination	10	19.707	1.971	21.603	* * *
Grain Number	9	1.350	0.150	1.645	N. S.
Error	90	8.210	0.091		

* * * Significant at 0.1% Probability Level.

TABLE 34b

RESULTS OF LINEAR REGRESSION OF SUCROSE CONCENTRATION AND LENGTH OF RADICLE, COLEOPTILE AND LATERAL ROOTS IN INDIVIDUAL EARLY PEARL GRAINS.

Factor	Germination Period (Hrs)	Correlation Coefficient	t	Significance
Sucrose	0 -240	+ 0.349	-	-
	0 -96	- 0.539	4.433	* * *
Radicle	120 -240	+ 0.031	-	-
Sucrose	0 -240	+ 0.287	-	-
	0 -96	- 0.628	5.590	* * *
Coleoptile	120 -240	- 0.122	-	-
Sucrose	0 -240	+ 0.364	-	-
	0 -96	- 0.335	-	-
Lateral Roots	120 -240	- 0.112	-	-

* * * Significant at 0.1% Probability Level.

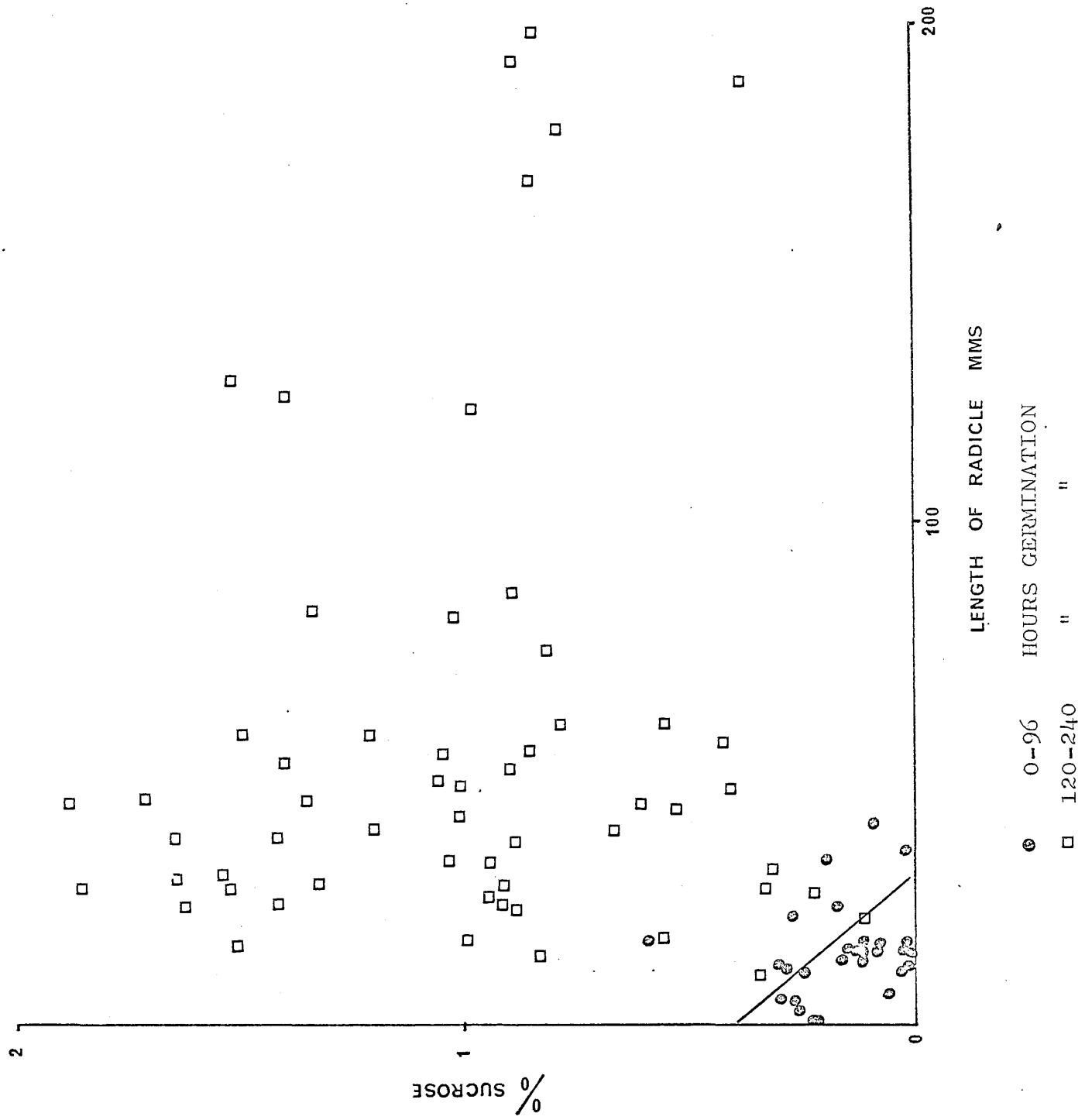

Fig. 49. Scatter plot illustrating correlation between sucrose concentration and radicle length in individual Early Pearl grains.

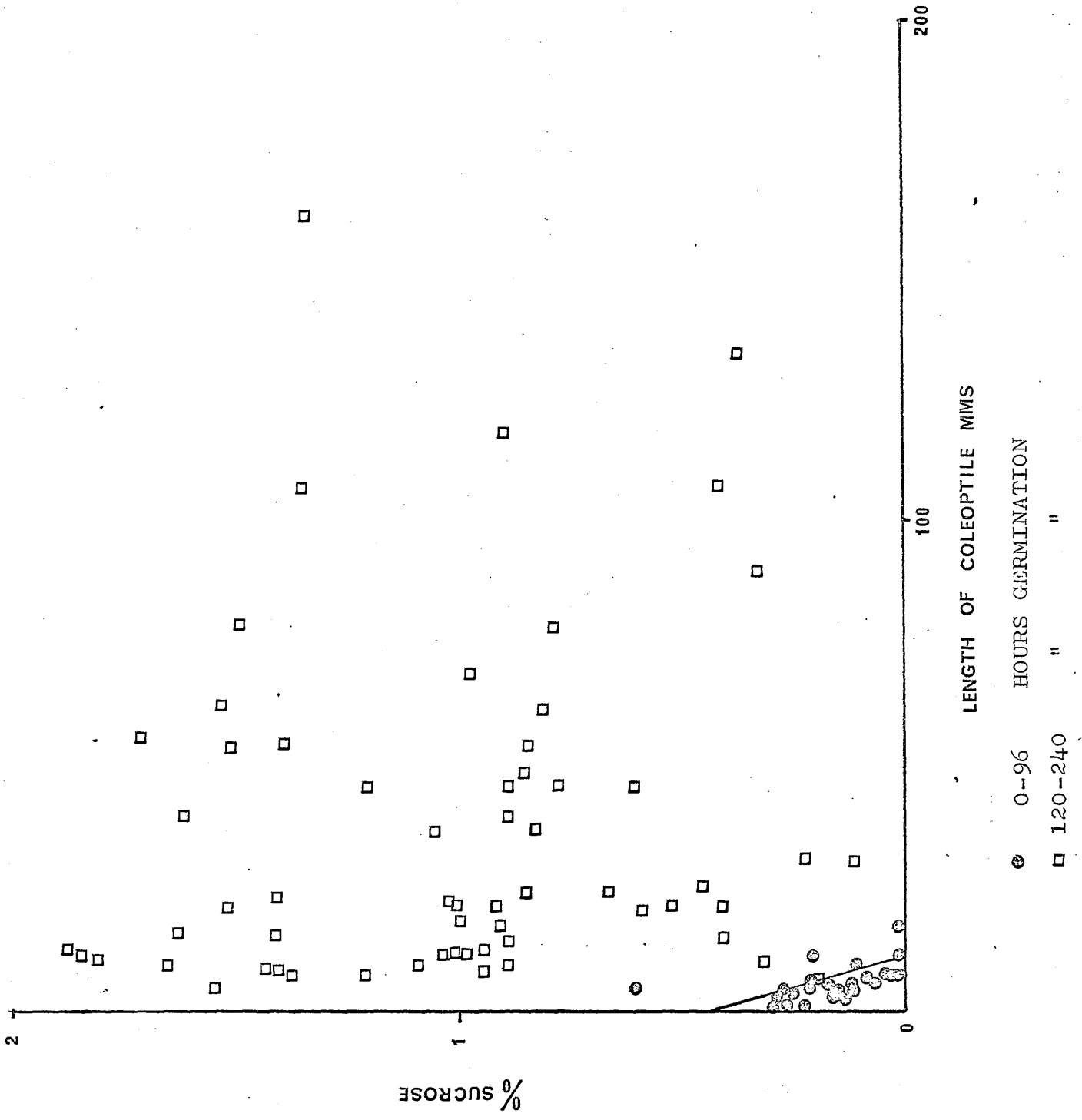

Fig. 50. Scatter plot illustrating correlation between sucrose concentration and coleoptile length in individual Early Pearl grains.

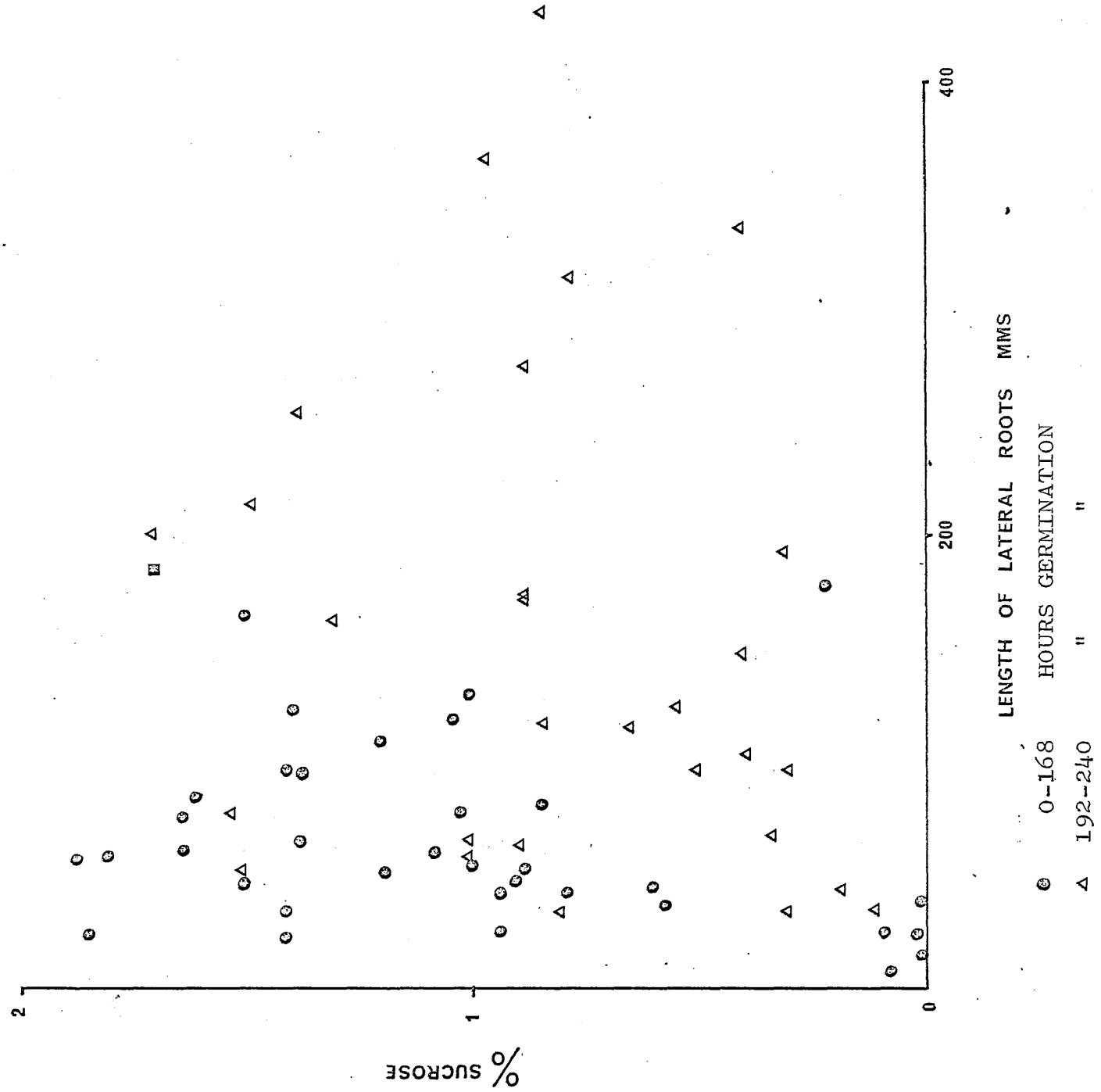

Fig. 51. Scatter plot illustrating correlation between sucrose concentration and lateral root length in individual Early Pearl grains.

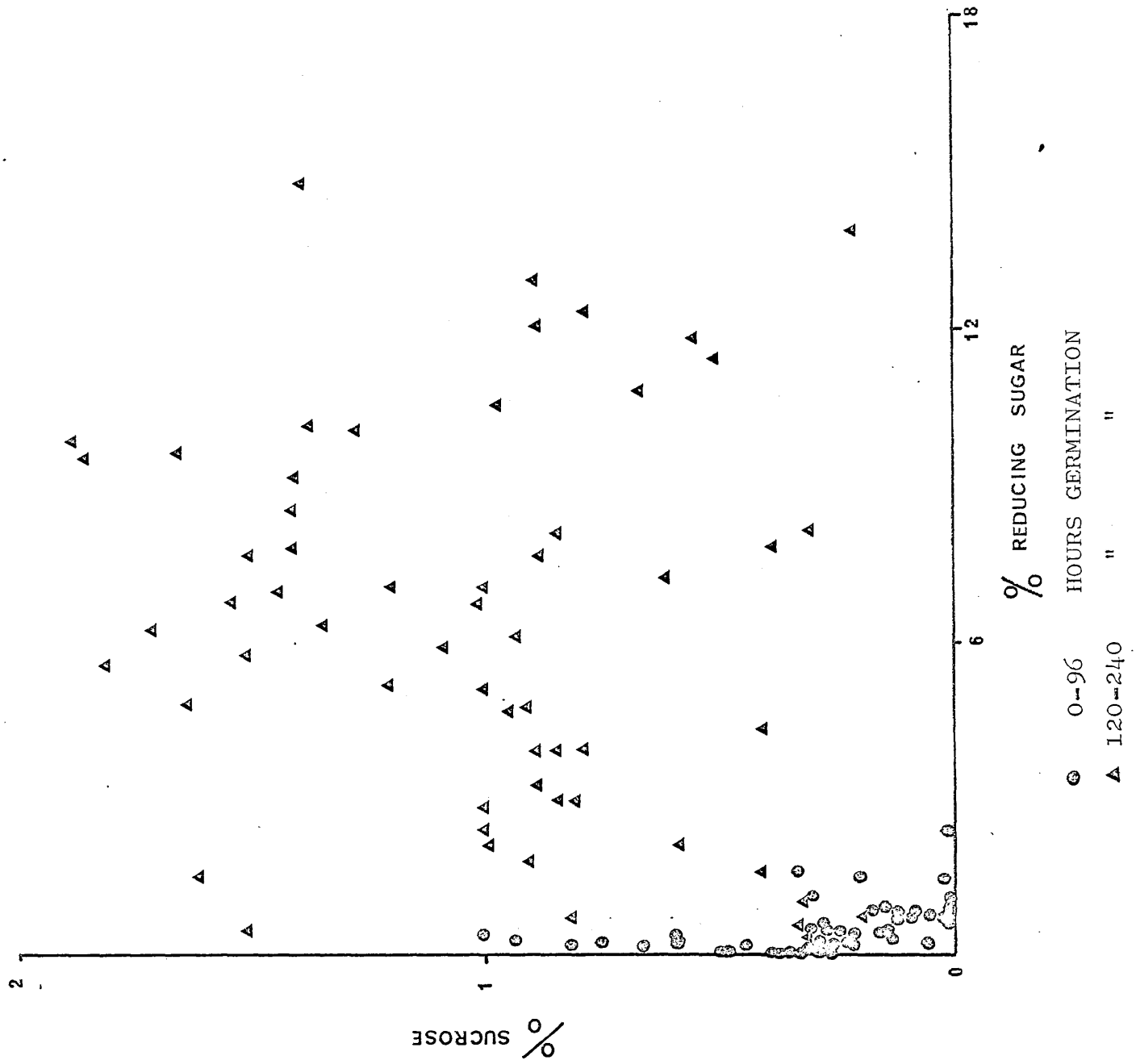

Fig. 52. Scatter plot illustrating correlation between sucrose and reducing sugar concentrations in individual Early Pearl grains.

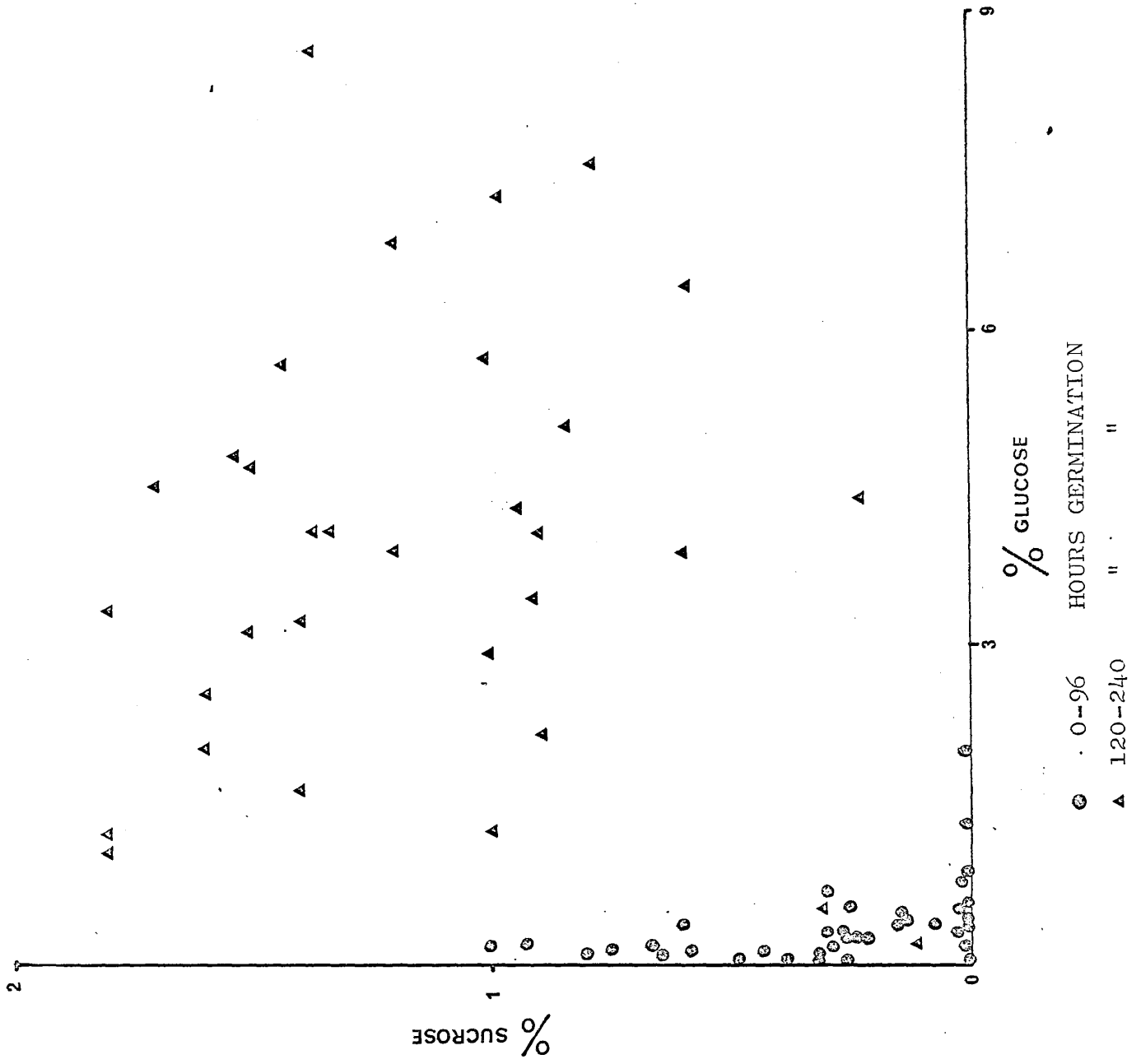

Fig. 53. Scatter plot illustrating correlation between sucrose and glucose concentration in individual Early Pearl grains.

TABLE 35

REDUCING SUGAR CONCENTRATION (MGMS. PER GRAIN) IN GROUPS OF HICKORY KING GRAIN DURING GERMINATION. ASSAY WAS REPLICATED THREE TIMES ON EACH GROUP.

Germination Period (hrs)	Replication Number 1	2	3	Mean	Germination Period Mean
0	7.500	8.125	7.500	7.708	
0	6.250	6.250	0.938	4.479	4.340
0	0.938	0.625	0.938	0.834	
24	10.026	10.110	10.125	10.087	
24	6.875	6.317	6.096	6.429	8.681
24	9.875	9.500	9.208	9.528	
48	35.313	35.625	35.625	35.521	
48	22.500	22.438	22.438	22.459	31.243
48	35.750	35.688	35.813	35.750	
72	60.000	61.250	61.250	60.833	
72	76.250	76.000	76.500	76.250	68.097
72	66.875	67.000	67.750	67.208	
96	73.750	74.250	74.750	74.250	
96	73.750	73.750	72.125	73.208	47.764
96	62.500	62.250	62.750	62.500	
120	65.000	63.125	63.750	63.958	
120	58.125	58.250	57.500	57.958	64.167
120	70.000	70.875	70.875	70.583	
144	72.354	71.826	71.915	72.032	
144	72.813	72.625	72.750	72.729	71.420
144	69.125	69.875	69.500	69.500	
168	83.750	84.000	84.250	84.000	
168	76.000	76.875	76.250	76.375	78.778
168	75.125	76.250	76.500	75.958	
192	71.250	73.750	73.500	72.833	
192	78.125	78.125	78.375	78.208	79.236
192	86.250	86.000	87.750	86.667	
216	116.875	116.250	116.815	116.647	
216	116.250	115.875	116.250	116.125	113.340
216	107.500	107.375	106.875	107.250	
240	92.500	91.875	91.000	91.792	
240	91.250	94.750	95.875	93.958	95.497
240	101.250	101.375	99.600	100.742	

L. S. D. Between Observations at 0.05 Probability Level ± 2.826

L. S. D. " Means " " " " ± 1.636

L. S. D. " Germination Period Means " " " ± 0.944

TABLE '36

LENGTHS (MMS.) OF RADICLE, COLEOPTILE AND LATERAL ROOTS OF GROUPS OF HICKORY KING GRAINS DURING GERMINATION.

Germination Period (Hrs)	Radicle	Coleoptile	Laterals	Total	Mean Radicle	Mean Coleoptile	Mean Laterals	Growth Index
72	75	13	-	88				
72	77	30	35	142	76.3	25.7	12.3	0.11
72	77	34	2	113				
96	150	66	200	416				
96	369	92	475	936	240.0	73.3	316.0	0.41
96	201	62	273	536				
120	311	151	666	1128				
120	350	105	615	1070	354.3	134.0	641.0	1.13
120	402	146	642	1190				
144	354	200	1009	1563				
144	449	368	2156	2973	446.0	276.7	1562.7	2.29
144	535	262	1523	2320				
168	437	344	1979	2760				
168	586	325	1513	2424	524.7	341.7	1717.0	2.58
168	551	356	1659	2566				
192	682	328	1501	2511				
192	731	381	1650	2762	648.3	340.0	1715.3	2.70
192	532	311	1995	2838				
216	620	523	2459	3602				
216	1025	404	1298	2727	754.0	494.3	2007.3	3.26
216	617	556	2265	3438				
240	495	420	1690	2605				
240	530	634	2469	3633	569.0	588.3	2190.0	3.35
240	682	711	2411	3804				

TABLE 37a

ANALYSIS OF VARIANCE RESULTS FOR TOTAL REDUCING FRACTION IN GROUPS OF HICKORY KING GRAINS.

Source	Degrees of Freedom	Sum Squares	Variance	Variance Ratio (F)	Observations
Germination	10	104811.520	10481.152	12265.830	* * *
Replication	2	0.640	0.320	0.374	N. S.
Sample	2	18.940	9.470	11.083	N. S.
Sample/Rep.	4	1.590	3.975	0.465	N. S.
Replication/Germ.	20	12.990	0.649	0.760	N. S.
Sample/Germ.	20	2003.550	100.177	117.235	* * *
Error	40	34.180	0.854		

* * * Significant at 0.1% Probability Level.

TABLE 37b

RESULTS OF LINEAR REGRESSION OF TOTAL REDUCING FRACTION AND LENGTH OF RADICLE, COLEOPTILE AND LATERAL ROOTS IN GROUPS OF HICKORY KING GRAINS.

Factor	Germination Period (Hrs)	Correlation Coefficient	t	Significance
Reducing Sugar	0 -240	+ 0.836	8.483	* * *
Radicle	72 -240	+ 0.681	4.362	* * *
Reducing Sugar	0 -240	+ 0.797	7.347	* * *
Coleoptile	72 -240	+ 0.778	5.808	* * *
Reducing Sugar	0 -240	+ 0.776	6.850	* * *
Lateral	72 -240	+ 0.677	4.314	* * *

* * * Significant at 0.1% Probability Level.

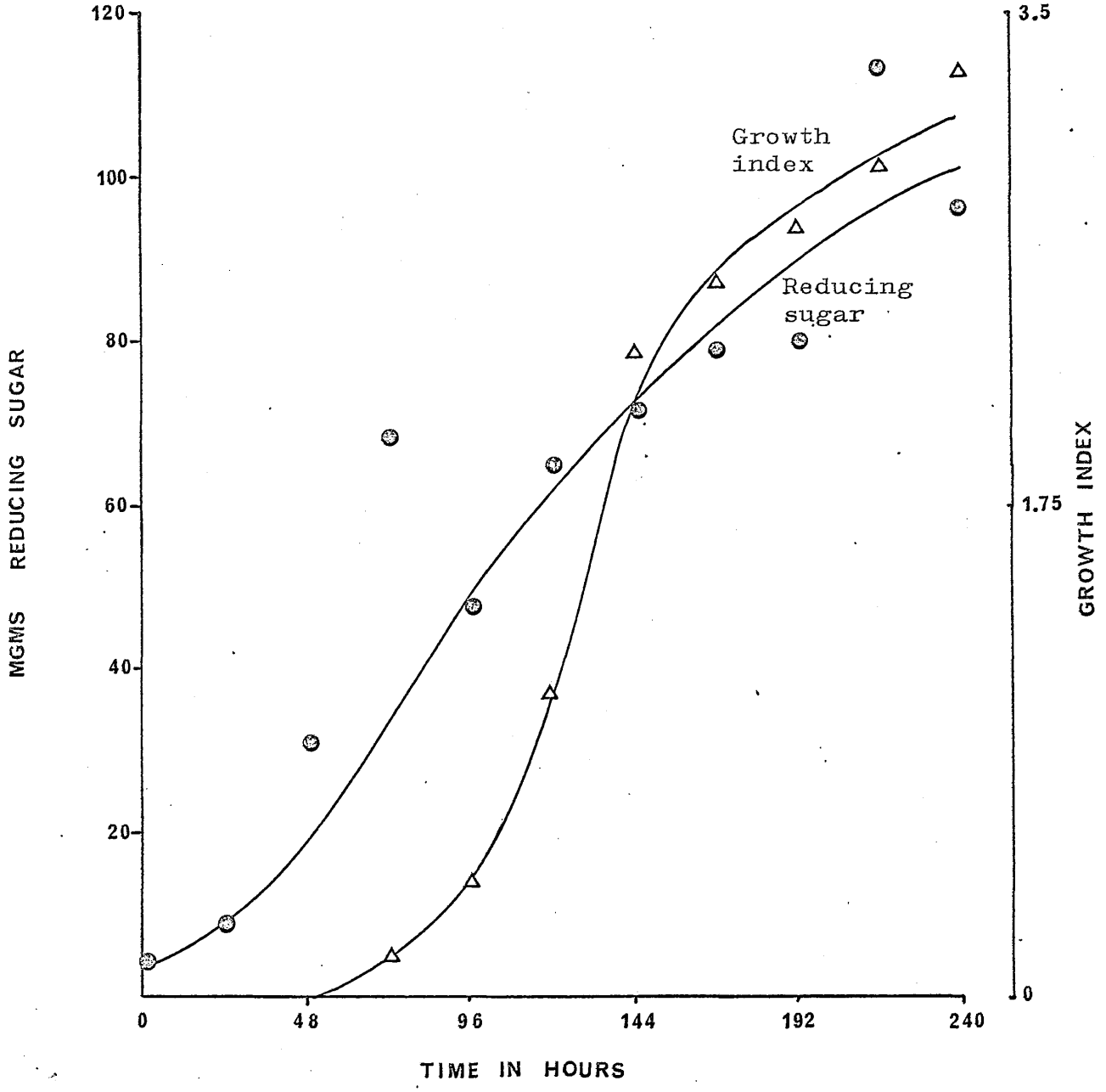

Fig. 54. Changes in total reducing fraction (expressed as mgms. maltose) in groups of Hickory King grain during germination. Growth index expressed as $\frac{\text{sum of lengths of organs measured}}{1000}$

L.S.D. between means for reducing sugar at 0.05 P.L. ± 0.944 mgms.

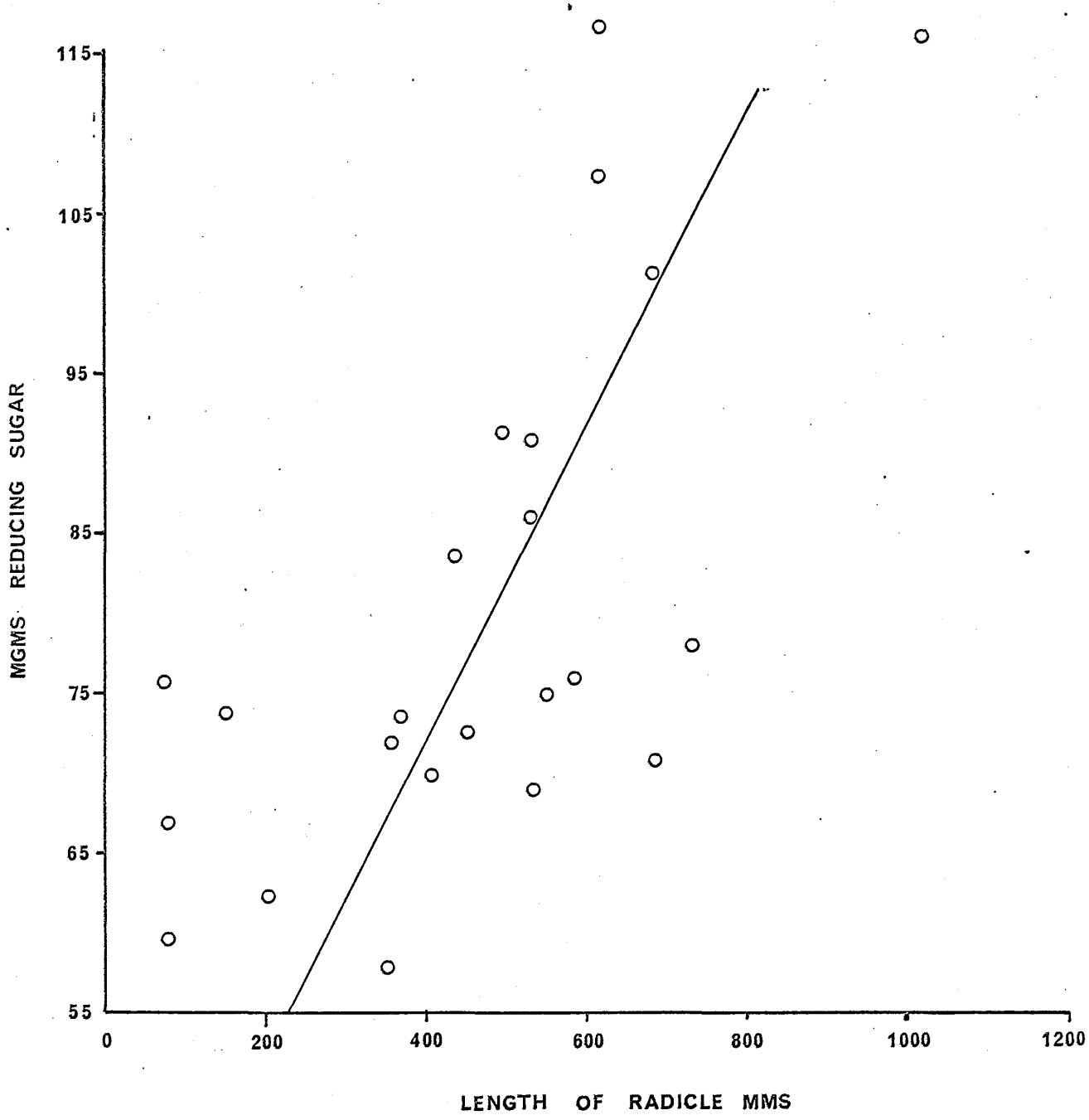

Fig. 55. Scatter plot illustrating correlation between reducing sugar concentration and radicle length in groups of Hickory King grain. Correlation coefficient for 0-240 hours + 0.836.

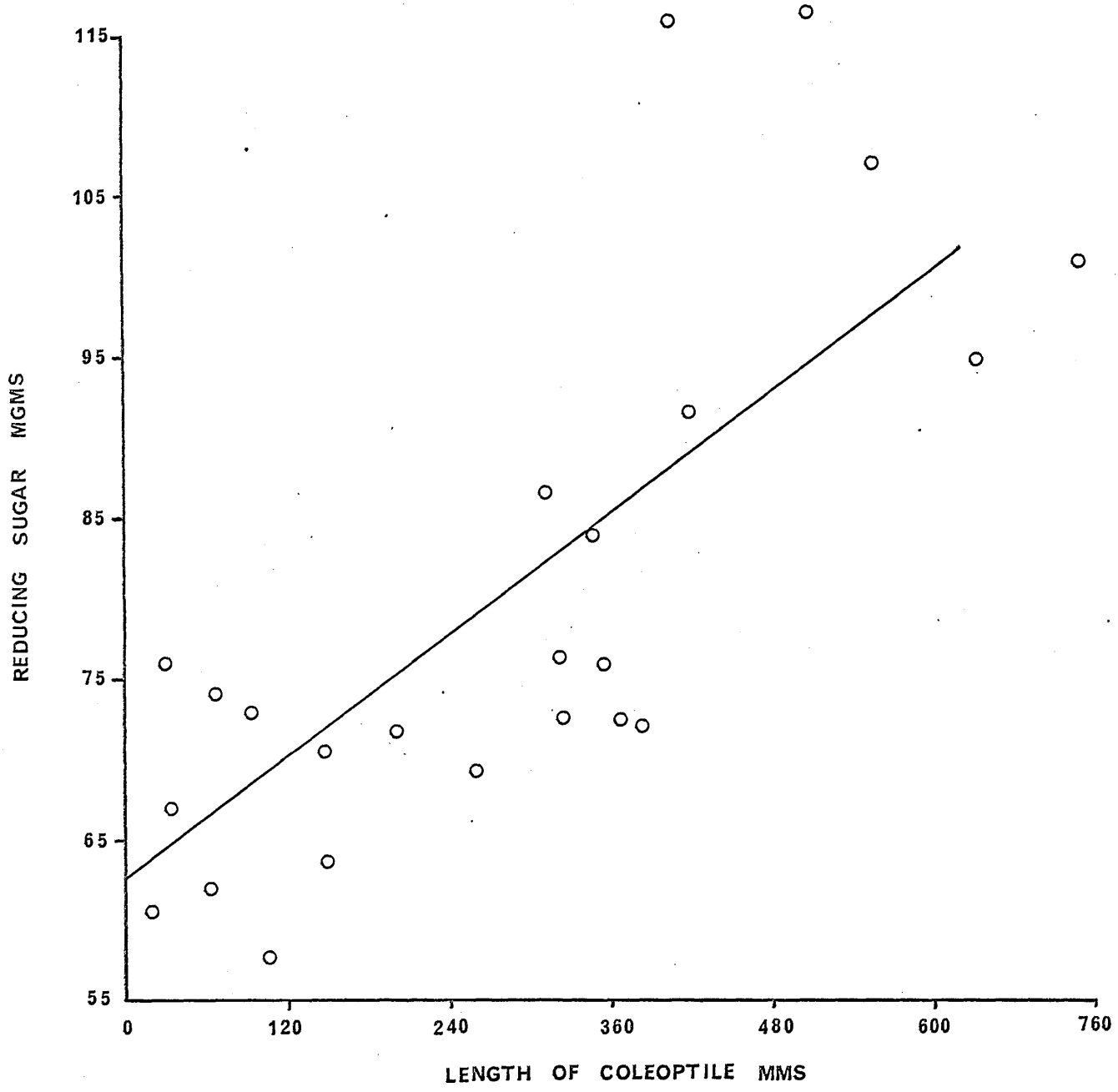

Fig. 56. Scatter plot illustrating correlation between reducing sugar concentration and coleoptile length in groups of Hickory King grain. Correlation coefficient for 0-240 hours + 0.797.

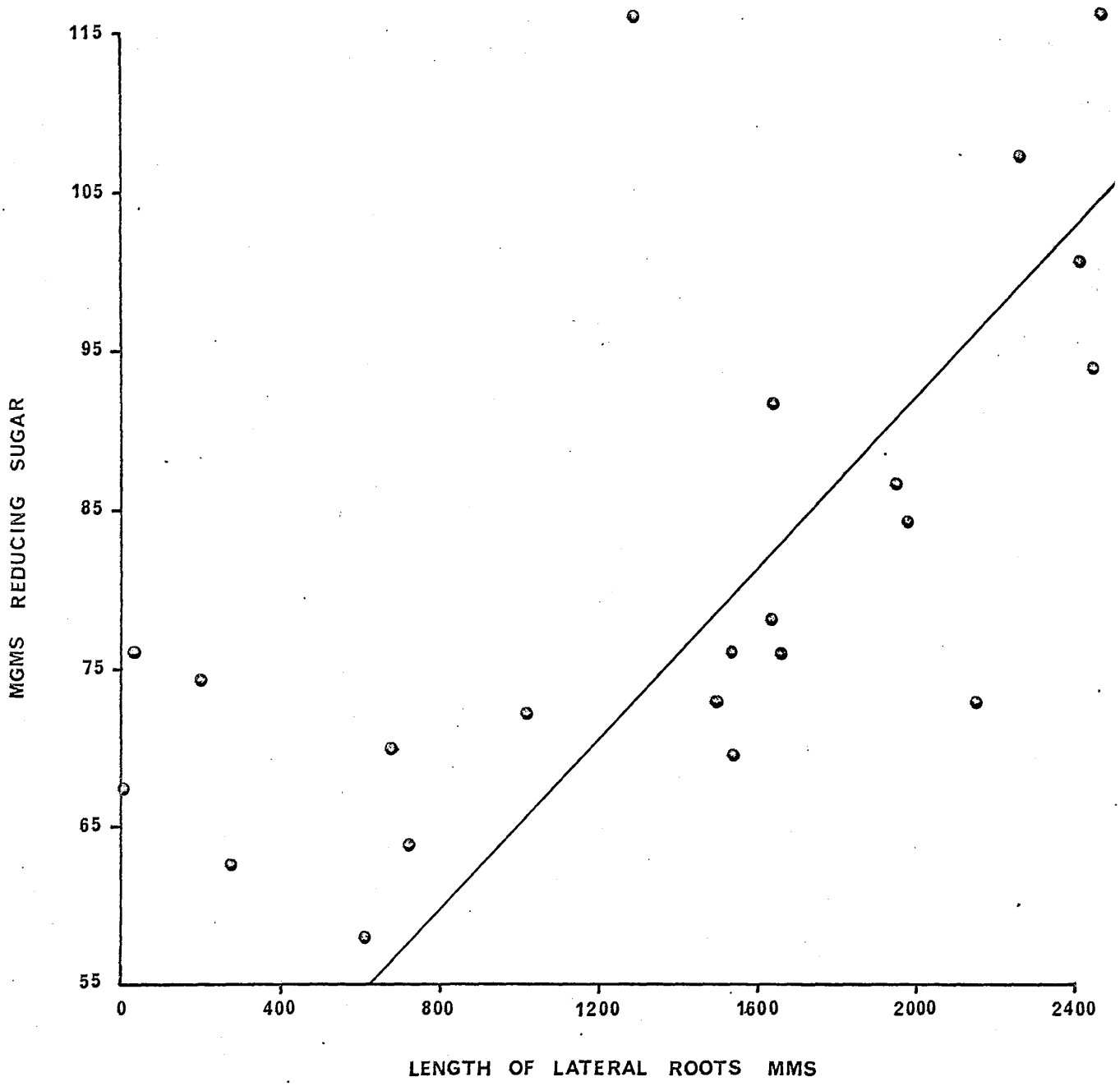

Fig. 57. Scatter plot illustrating correlation between reducing sugar concentration and lateral root length in groups of Hickory King grain. Correlation coefficient for 0-240 hours + 0.776.

TABLE 38

REDUCING SUGAR CONCENTRATION (MGMS) IN INDIVIDUAL HICKORY KING GRAINS DURING GERMINATION.

Germination Period (hrs)	Grain Number 1	2	3	4	5	6	7	8	9	10	Mean	% Germination	Growth Index
0	9.687	-	8.875	-	6.250	11.875	9.563	9.000	10.625	-	6.587	-	-
24	8.325	10.144	12.065	7.056	10.256	9.864	12.630	8.643	6.045	11.902	9.693	-	-
48	56.250	58.750	41.750	56.875	60.250	30.937	43.437	32.812	45.125	56.000	48.219	-	-
72	71.750	78.125	92.500	65.625	42.875	44.750	57.500	84.750	43.875	47.875	62.962	90	0.119
96	51.250	58.750	60.000	63.500	85.625	57 500	40.875	72.750	44.875	50.250	58.501	100	0.483
120	36.125	45.000	66.875	68.750	61.000	65.000	35.625	54.375	40.000	54.875	52.762	100	1.489
144	93.125	74.000	85.000	72.500	73.375	100.000	106.250	52.500	47.250	79.625	78.362	100	2.230
168	81.000	83.750	89.750	61.250	32.500	27.500	79.750	77.750	80.000	57.000	67.025	100	2.712
192	138.125	101.500	79.750	105.375	111.500	92.125	90.000	105.000	112.500	83.125	101.900	100	1.932
216	118.750	84.625	88.375	158.750	115.375	120.000	100.375	68.750	132.500	93.500	108.100	100	2.131
240	109.625	122.125	82.625	127.875	101.000	47.875	109.125	96.250	87.750	122.625	100.687	100	2.568

Least Significant Difference Between Observations at 0.05 Probability Level ± 48.098

" " " " Means " " " " ± 15.210

TABLE 39a

ANALYSIS OF VARIANCE RESULTS FOR TOTAL REDUCING FRACTION IN INDIVIDUAL HICKORY KING GRAINS.

Source	Degrees of Freedom	Sum Squares	Variance	Variance Ratio (F)	Observations
Germination	10	115866.870	11586.687	39.499	* * *
Sample	9	2520.100	280.011	0.955	N. S.
Error	90	26400.420	293.338		

* * * Significant at 0.1% Probability Level.

TABLE 39b

RESULTS OF LINEAR REGRESSION OF TOTAL REDUCING FRACTION AND LENGTH OF RADICLE COLEOPTILE AND LATERAL ROOTS IN INDIVIDUAL HICKORY KING GRAINS.

Factor	Germination Period (Hrs)	Correlation Coefficient	t	Significance
Reducing Sugar Radicle	0 -240	+ 0.623	8 222	* * *
	0 -96	+ 0.422	-	-
	72 -240	+ 0.352	-	-
Reducing Sugar Coleoptile	0 -240	+ 0.510	-	-
	0 -96	+ 0.422	-	-
	72 -240	+ 0.201	-	-
Reducing Sugar Laterals	0 -240	+ 0.551	-	-
	0 -96	+ 0.153	-	-
	72 -240	+ 0.269	-	-

* * * Significant at 0.1% Probability Level.

TABLE 40

LENGTHS (MMS.) OF RADICLE, COLEOPTILE AND LATERAL ROOTS OF INDIVIDUAL HICKORY KING GRAINS DURING GERMINATION.

	GERMINATION PERIOD IN HOURS																								
	72			96			120			144			168			192			216			240			
Grain Number	Radicle	Coleoptile	Laterals	Radicle	Coleoptile	Laterals	Radicle	Coleoptile	Laterals	Radicle	Coleoptile	Laterals	Radicle	Coleoptile	Laterals	Radicle	Coleoptile	Laterals	Radicle	Coleoptile	Laterals	Radicle	Coleoptile	Laterals
1	6	3	-	27	9	16	6	7	8	62	38	104	44	24	64	64	26	105	59	53	122	38	37	171
2	13	5	12	-	6	11	44	29	169	72	45	155	38	37	144	43	30	86	38	30	178	42	40	163
3	21	2	-	14	8	8	25	17	53	38	35	122	49	79	243	42	40	214	62	42	297	31	64	181
4	12	3	-	2	5	11	43	16	69	23	32	136	78	37	204	57	59	160	63	17	102	62	43	131
5	-	4	5	27	10	8	46	36	156	5	13	53	97	69	256	50	14	79	43	33	38	58	-16	143
6	-	4	-	14	5	-	16	6	59	62	61	226	58	37	66	62	43	134	23	26	50	31	87	102
7	2	4	-	1	7	13	31	18	81	37	13	89	29	74	212	33	34	137	27	53	150	57	15	174
8	16	4	-	2	6	-	50	25	194	44	40	83	28	17	120	29	9	59	39	54	152	43	62	180
9	-	-	-	1	5	38	13	20	72	41	43	157	39	62	241	22	20	151	42	46	124	22	61	161
10	3	-	-	68	29	132	24	23	142	87	73	241	32	41	193	26	13	91	38	18	112	43	44	266
Mean	7.3	2.9	1.7	15.6	9.0	23.7	29.8	19.7	100.3	47.1	39.3	136.6	49.2	47.7	174.3	42.8	28.8	121.6	43.4	37.2	132.5	42.7	46.9	167.2
Growth Index	0.119			0.483			1.498			2.230			2.712			1.932			2.131			2.568		

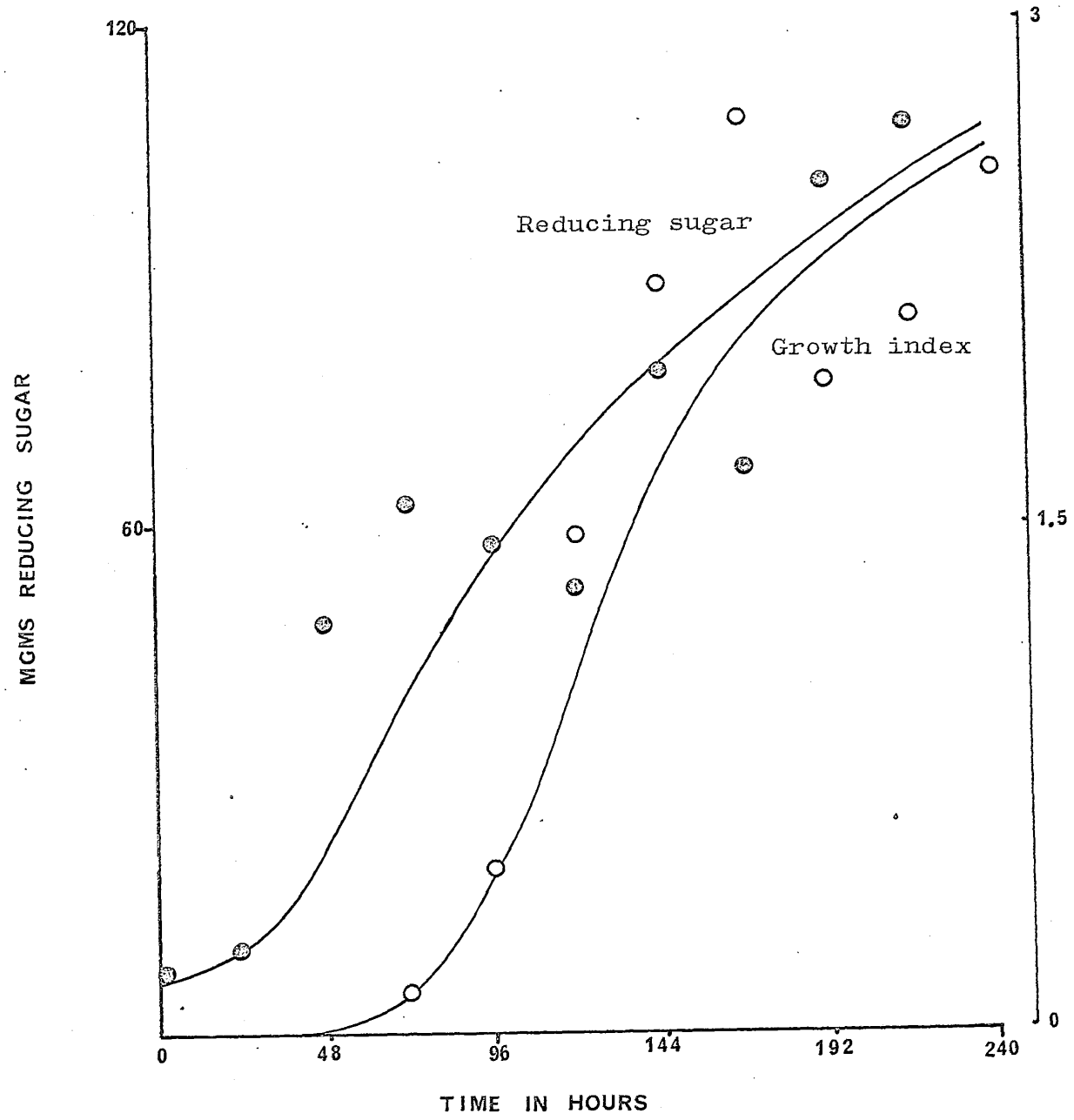

Fig. 58. Changes in total reducing fraction (expressed as mgms. maltose) in individual Hickory King grains during germination. Growth index expressed as $\frac{\text{sum of lengths of organs measured}}{1000}$

L.S.D. between means for reducing sugar at 0.05 P.L. ± 15.210mgms.

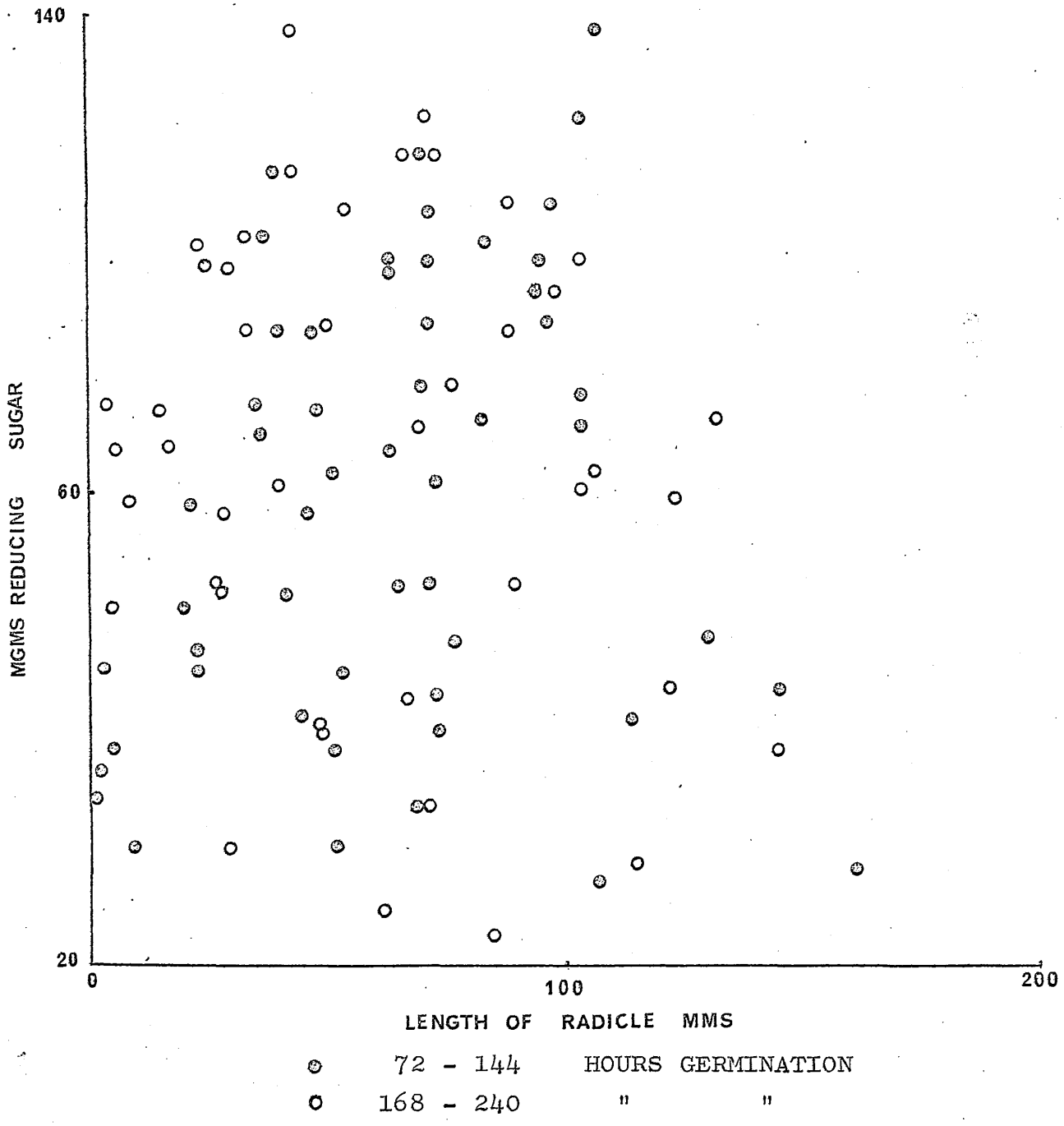

Fig. 59. Scatter plot illustrating correlation between reducing sugar concentration and radicle length in individual Hickory King grains.

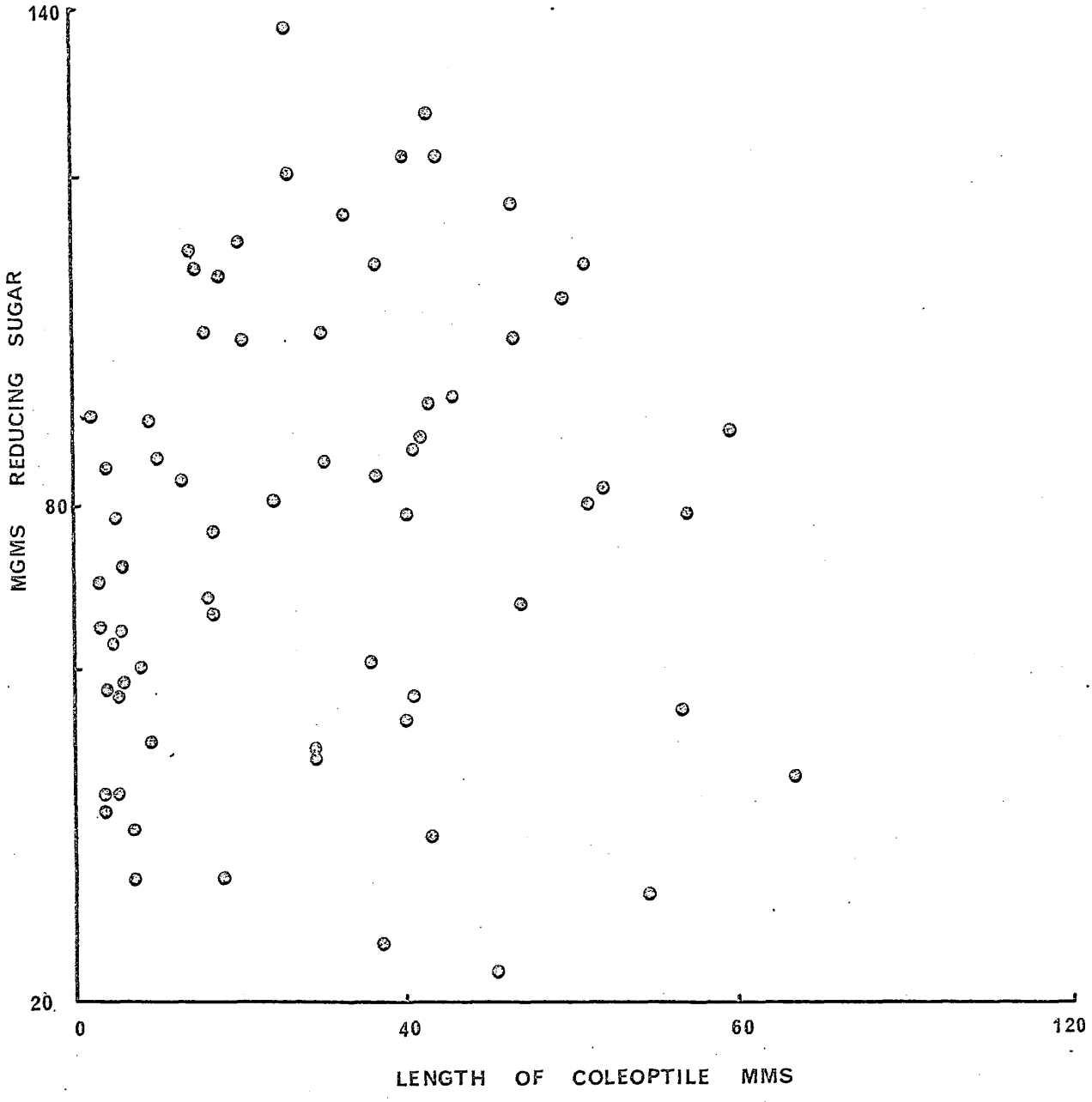

Fig. 60. Scatter plot illustrating correlation between reducing sugar concentration and coleoptile length in individual Hickory King grains.

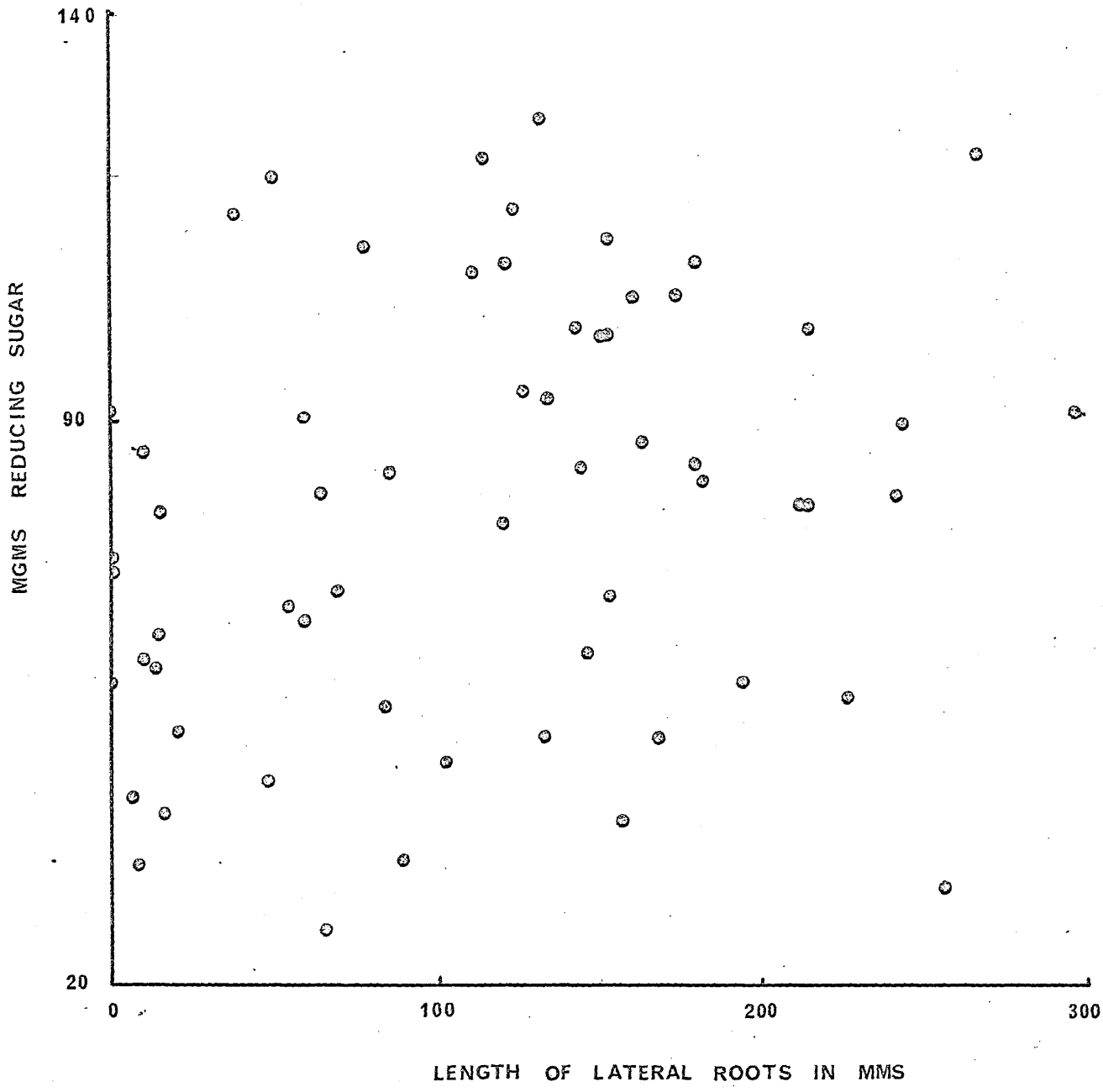

Fig. 61. Scatter plot illustrating correlation between reducing sugar concentration and lateral root length in individual Hickory King grains.

TABLE 41

TOTAL AMYLOLYTIC ACTIVITY (MGMS MALTOSE/MIN/GRAIN) IN GROUPS OF HICKORY KING GRAIN DURING GERMINATION. ASSAY WAS REPLICATED THREE TIMES ON EACH GROUP.

Germination Period (Hrs)	Replication Number 1	2	3	Mean	Germination Period Mean
0	0.133	0.109	0.141	0.128	
0	0.195	0.172	0.297	0.221	0.245
0	0.383	0.388	0.383	0.385	
24	0.385	0.378	0.381	0.381	
24	0.613	0.681	0.678	0.657	0.599
24	0.768	0.775	0.725	0.756	
48	1.657	1.644	1.648	1.650	
48	1.719	1.736	1.728	1.728	1.925
48	1.385	1.389	1.382	1.385	
72	4.302	4.391	4.391	4.361	
72	3.820	3.809	3.813	3.814	3.695
72	2.874	2.902	2.956	2.911	
96	5.125	5.208	5.209	5.181	
96	4.584	4.250	4.823	4.552	4.355
96	3.281	3.354	3.365	3.333	
120	8.611	8.473	8.681	8.588	
120	9.375	9.084	9.375	9.278	8.590
120	7.986	7.778	7.950	7.904	
144	10.375	10.209	10.591	10.392	
144	11.410	11.438	10.775	11.208	10.981
144	11.396	11.584	11.048	11.343	
168	13.750	13.917	11.542	13.070	
168	14.625	14.584	14.625	14.611	14.795
168	16.709	16.646	16.750	16.702	
192	14.000	13.175	12.975	13.383	
192	12.125	10.825	11.975	11.642	11.092
192	8.750	8.050	7.950	8.250	
216	14.500	14.625	14.375	14.500	
216	12.500	12.575	12.500	12.525	12.048
216	9.125	9.100	9.125	9.117	
240	14.063	14.594	14.907	14.521	
240	12.969	11.625	11.344	11.979	14.021
240	15.625	15.125	15.938	15.563	

L. S. D. Between Observations at 0.05 P.L. ± 1.008
" " " " Means " " " " ± 0.609
" " " " Germination Period Means " ± 0.339

TABLE 42a

ANALYSIS OF VARIANCE RESULTS FOR TOTAL AMYLOLYTIC ACTIVITY (ALPHA- AND BETA- AMYLASE) IN GROUPS OF HICKORY KING GRAIN.

Source	Degrees of Freedom	Sum Squares	Variance	Variance Ration(F)	Observations
Germination	10	2719.6768	271.9677	2120.151	* * *
Replication	2	0.4374	0.2187	1.705	N. S.
Sample	2	9.8856	4.9428	38.532	* *
Sample/Rep.	4	0.3285	0.0821	0.640	N. S.
Replication/Germ.	20	2.6534	0.1327	1.034	N. S.
Sample/Germ.	20	129.2765	6.4638	50.389	* * *
Error	40	5.1311	0.1283		

* * * Significant at 0.1% Probability Level.
* * " " 5.0% " "

TABLE 42b

RESULTS OF LINEAR REGRESSION OF TOTAL AMYLOLYTIC ACTIVITY (ALPHA- AND BETA- AMYLASE) AND TOTAL REDUCING FRACTION RADICLE, COLEOPTILE AND LATERAL ROOT LENGTH IN GROUPS OF HICKORY KING GRAINS.

Factor	Germination Period (Hrs)	Correlation Coefficient	t	Observations
Amylase	0 -240	+ 0.817	7.889	* * *
	0 -96	+ 0.951	11.090	* * *
Reducing Sugar	120 -240	+ 0.334	-	-
Amylase	0 -240	+ 0.891	10.927	* * *
Radicle	72 -240	+ 0.766	5.571	* * *
Amylase	0 -240	+ 0.865	9.598	* * *
Coleoptile	72 -240	+ 0.785	5.943	* * *
Amylase	0 -240	+ 0.880	10.315	* * *
Lateral	72 -240	+ 0.801	6.276	* * *

* * * Significant at 0.1% Probability Level.

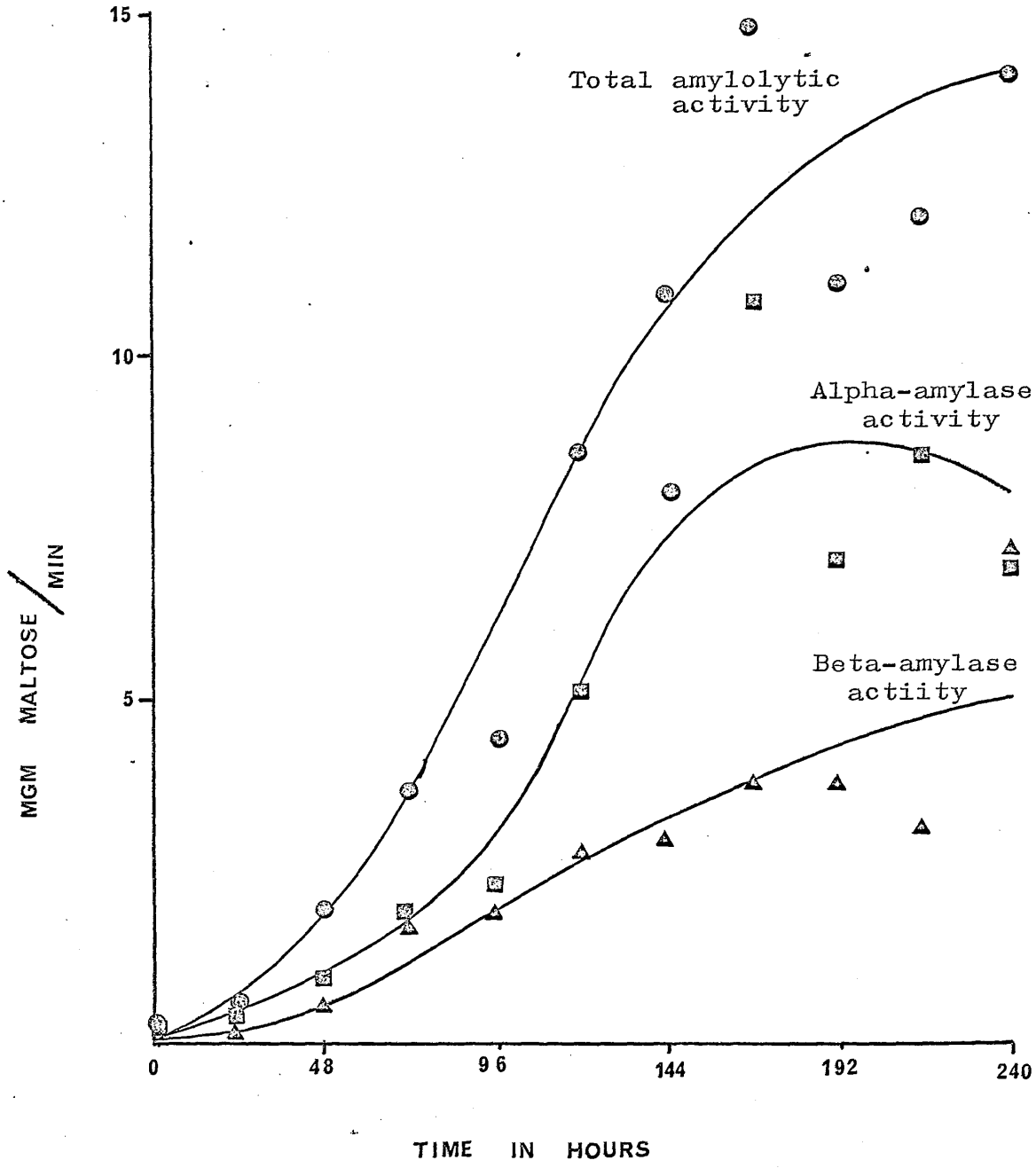

Fig. 62. Changes in amylolytic activity (mgms. maltose/min./grain) in groups of Hickory King grain during germination.

L.S.D. between means for total amylolytic activity at 0.05 P.L. ± 0.339.
" " " " " " alpha-amylolytic activity at 0.05 P.?. ± 0.298.
" " " " " " beta-amylolytic activity at 0.05 P.L. ± 0.432.

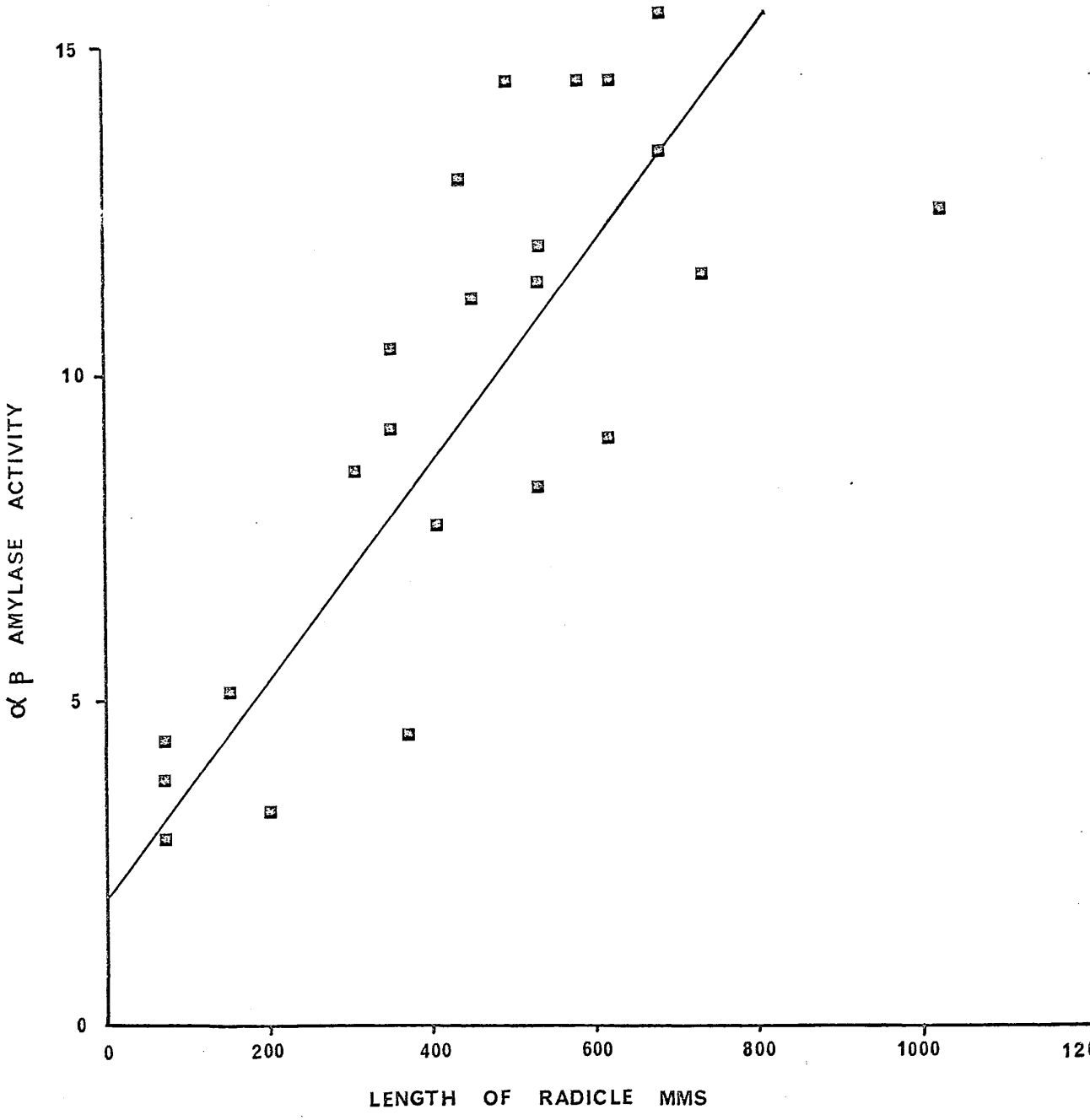

Fig. 63. Scatter plot illustrating correlation between total amylolytic activity (alpha- and beta- amylase) and radicle length in groups of Hickory King grain. Correlation coefficient for 0-240 hours + 0.891.

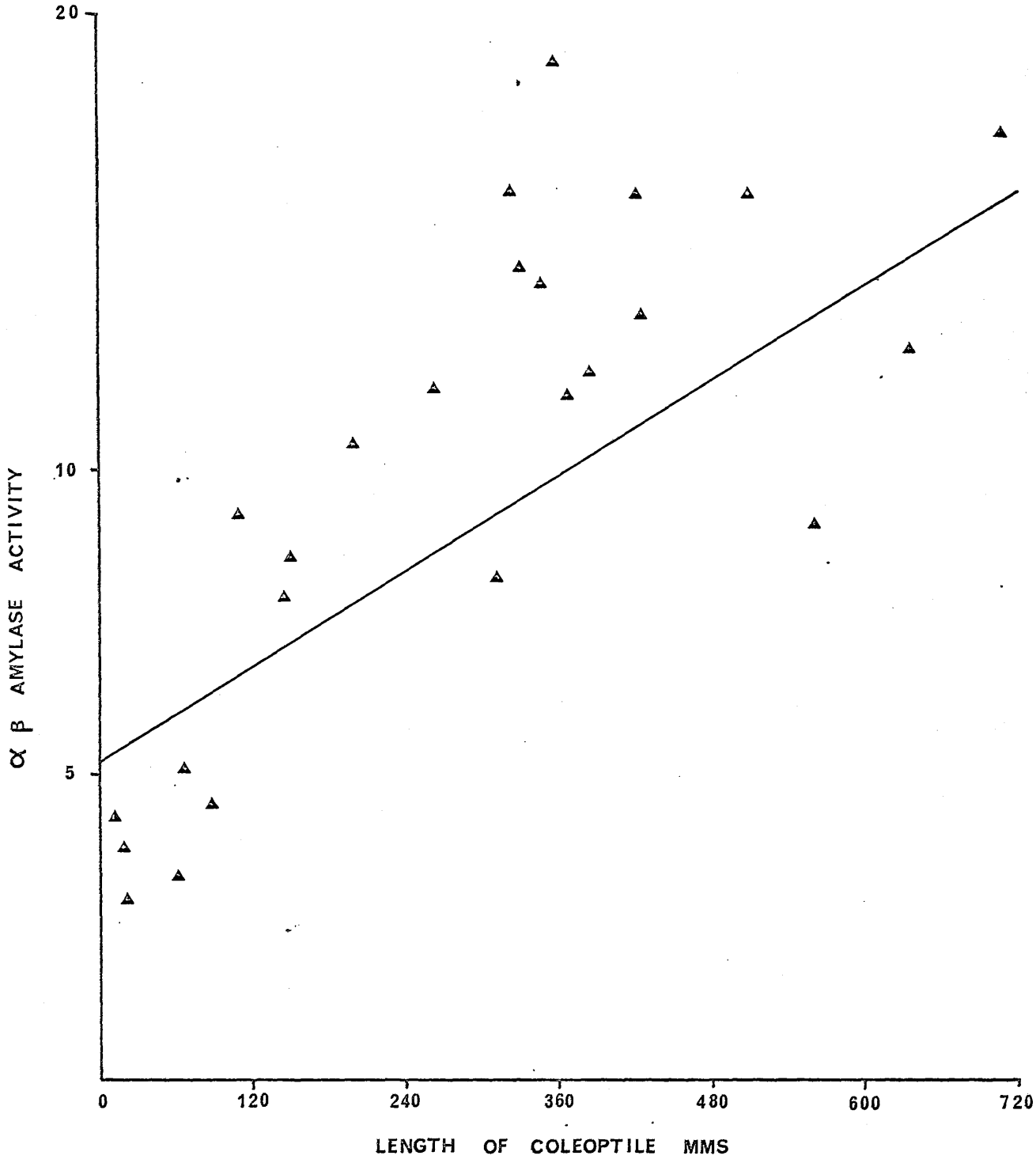

Fig. 64. Scatter plot illustrating correlation between total amylolytic (alpha- and beta-amylase) activity and Coleoptile length in groups of Hickory King grain. Correlation coefficient for 0-240 hours + 0.865.

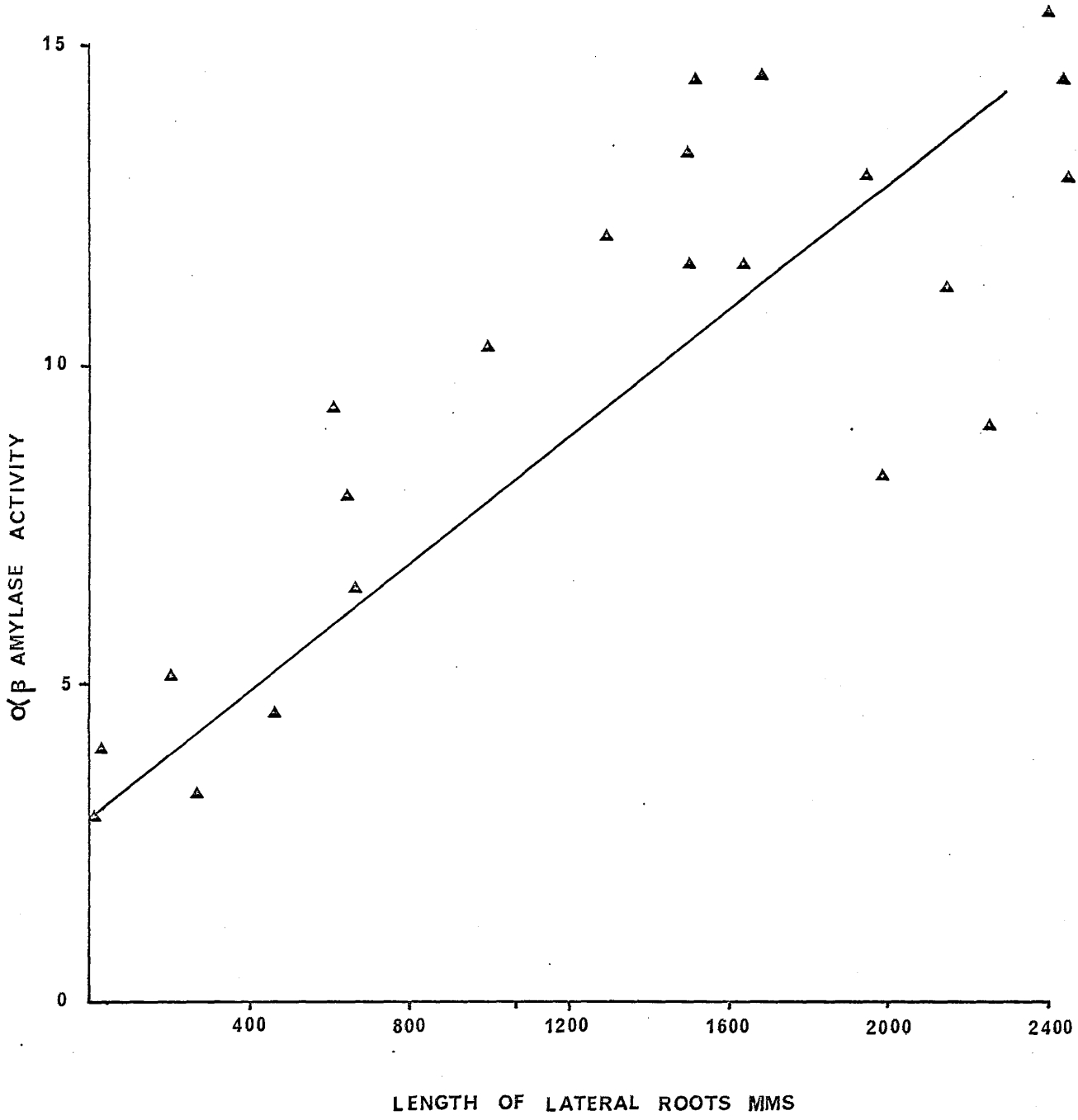

Fig. 65. Scatter plot illustrating correlation between total amylolytic (alpha- and beta-amylase) activity and lateral root length in groups of Hickory King grain. Correlation coefficient for 0-240 hours + 0.880.

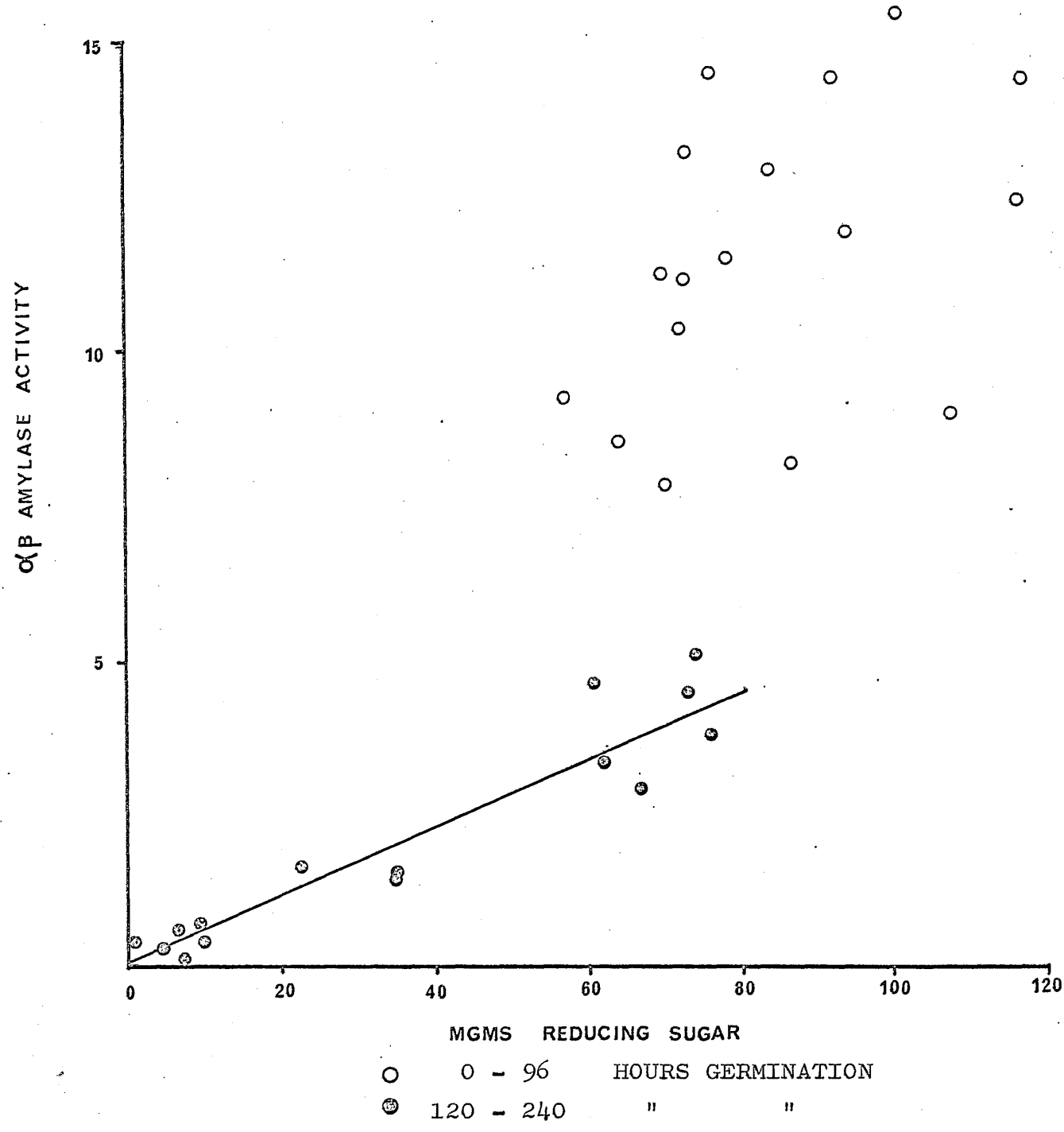

Fig. 66. Scatter plot illustrating correlation between total amylolytic activity (alpha- and beta-amylase) and reducing sugar concentration in groups of Hickory King grain. Correlation coefficient for 0-96 hours + 0.951.

TABLE 43

TOTAL AMYLOLYTIC ACTIVITY (MGMS. MALTOSE/ MIN.) IN INDIVIDUAL HICKORY KING GRAINS DURING GERMINATION.

Germination Period (hrs)	Grain Number 1	2	3	4	5	6	7	8	9	10	Mean	% Germination	Growth Index
0	-	-	0.028	0.219	-	0.125	-	0.056	0.141	-	0.057	-	-
24	0.221	0.381	0.719	0.250	0.453	0.662	0.698	0.384	0.297	0.671	0.474	-	-
48	1.687	1.562	0.753	1.045	0.961	0.902	1.312	0.805	1.528	0.975	1.153	-	-
72	3.883	4.667	4.187	2.812	1.946	1.017	2.837	3.717	1.946	2.017	2.908	90	0.119
96	1.170	2.241	1.384	1.571	1.188	1.625	2.661	4.473	1.991	2.375	1.738	100	0.483
120	0.698	2.708	2.188	5.937	1.844	1.979	1.094	4.271	1.427	1.500	2.365	100	1.489
144	8.125	3.450	4.225	0.875	4.662	7.550	4.975	3.750	8.025	6.062	5.130	100	2.230
168	6.594	2.766	6.906	6.453	5.375	6.719	11.250	10.000	5.937	6.656	6.766	100	2.712
192	16.875	7.400	3.325	8.925	11.650	6.375	10.950	12.950	26.375	10.000	11.482	100	1.932
216	14.200	6.825	12.450	30.750	8.300	27.100	9.675	3.875	24.500	9.250	14.692	100	2.131
240	10.187	14.375	7.469	34.281	10.687	3.187	11.781	7.812	7.219	16.844	12.084	100	2.568

Least Significant Difference Between Observations at 0.05 Probability Level ± 12.553

" " " " Means " " " " ± 3.968

TABLE 44a

ANALYSIS OF VARIANCE RESULTS FOR TOTAL AMYLOLYTIC ACTIVITY (ALPHA- AND BETA- AMYLASE) IN INDIVIDUAL HICKORY KING GRAINS.

Source Source	Degrees of Freedom	Sum of Squares	Variance	Variance Ratio(F)	Observations
Germination	10	2717.053	271.705	13.814	* * *
Sample	9	189.639	22.182	1.128	N. S.
Error	90	1770.235	19.669		

* * * Significant at 0.1% Probability Level.

TABLE 44b

RESULTS OF LINEAR REGRESSION OF TOTAL AMYLOLYTIC ACTIVITY AND TOTAL REDUCING FRACTION, RADICLE COLEOPTILE AND LATERAL ROOT LENGTH IN INDIVIDUAL HICKORY KING GRAINS.

Factor	Germination Period (Hrs)	Correlation Coefficient	t	Significance
Amylolytic	0 -240	+ 0.779	12.914	* * *
	0 -96	+ 0.829	10.269	* * *
Reducing Sugar	120 -240	+ 0.803	8.803	* * *
Amylolytic	0 -240	+ 0.532	-	-
	0 -96	+ 0.362	-	-
Radicle	72 -240	+ 0.354	-	-
Amylolytic	0 -240	+ 0.420	-	-
	0 -96	+ 0.406	-	-
Coleoptile	72 -240	+ 0.218	-	-
Amylolytic	0 -240	+ 0.475	-	-
	0 -96	+ 0.195	-	-
Laterals	72 -240	+ 0.291	-	-

* * * Significant at 0.1% Probability Level.

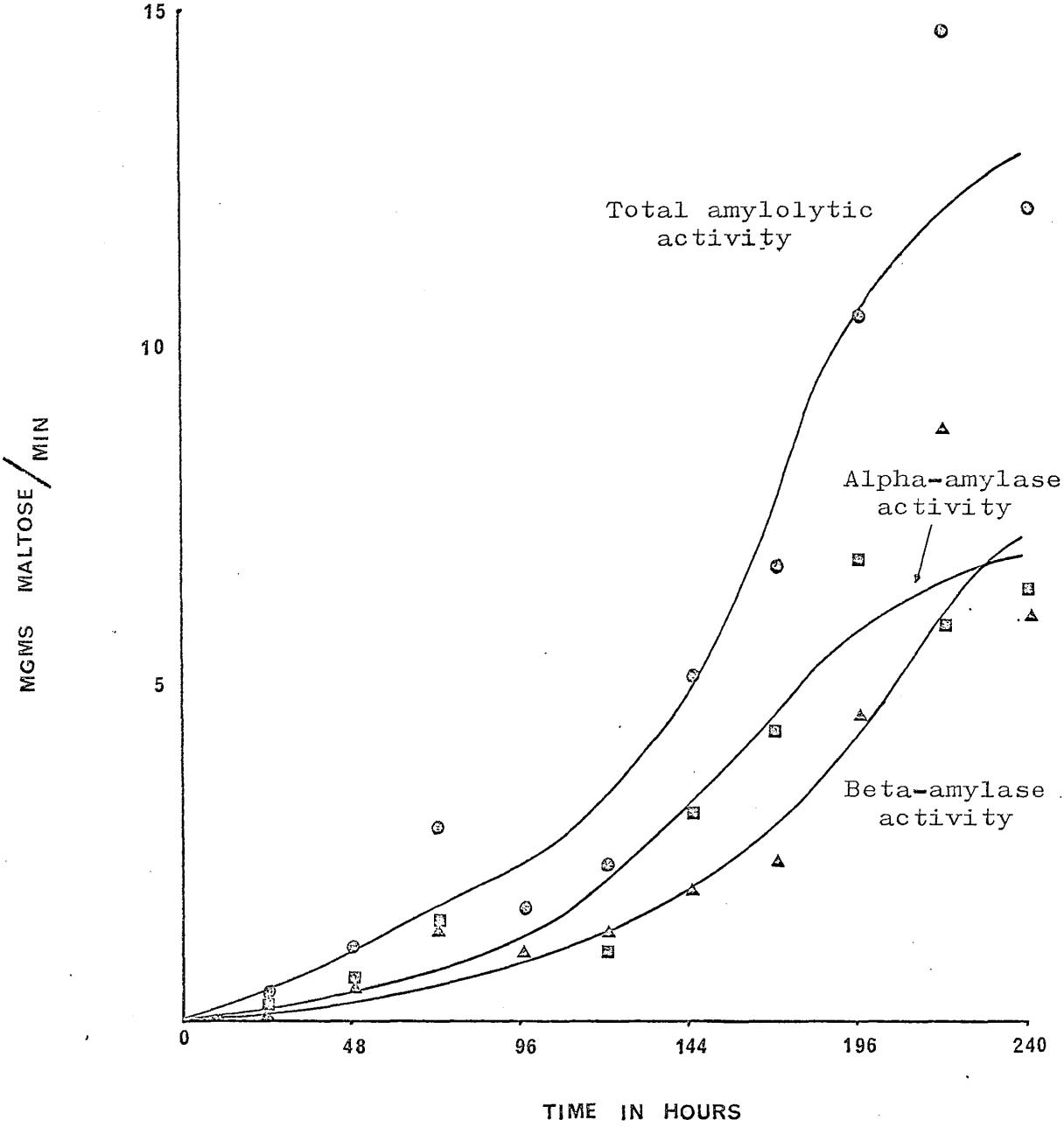

Fig. 67. Changes in amylolytic activity (mgms. maltose/min./grain) in individual Hickory King grains during germination.

L.S.D. between means for total amylolytic activity at 0.05 P.L. ± 3.968.

" " " " " " alpha-amylolytic activity at 0.05 P.L. ± 0.380.

" " " " " " beta-amylolytic activity at 0.05 P.L. ± 0.325.

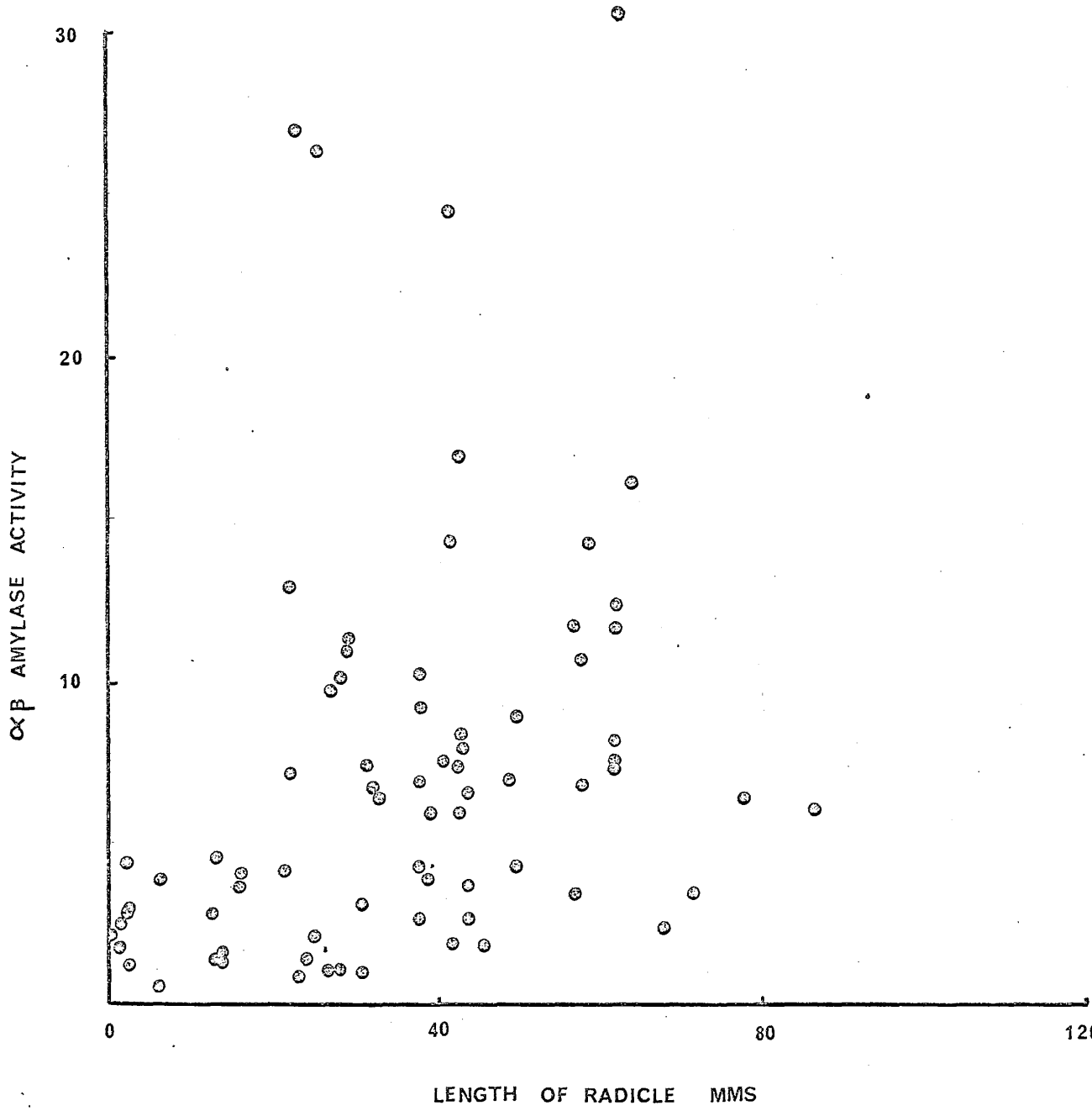

Fig. 68. Scatter plot illustrating correlation between total amylolytic activity (alpha- and beta-amylase) and radicle length in individual Hickory King grains.

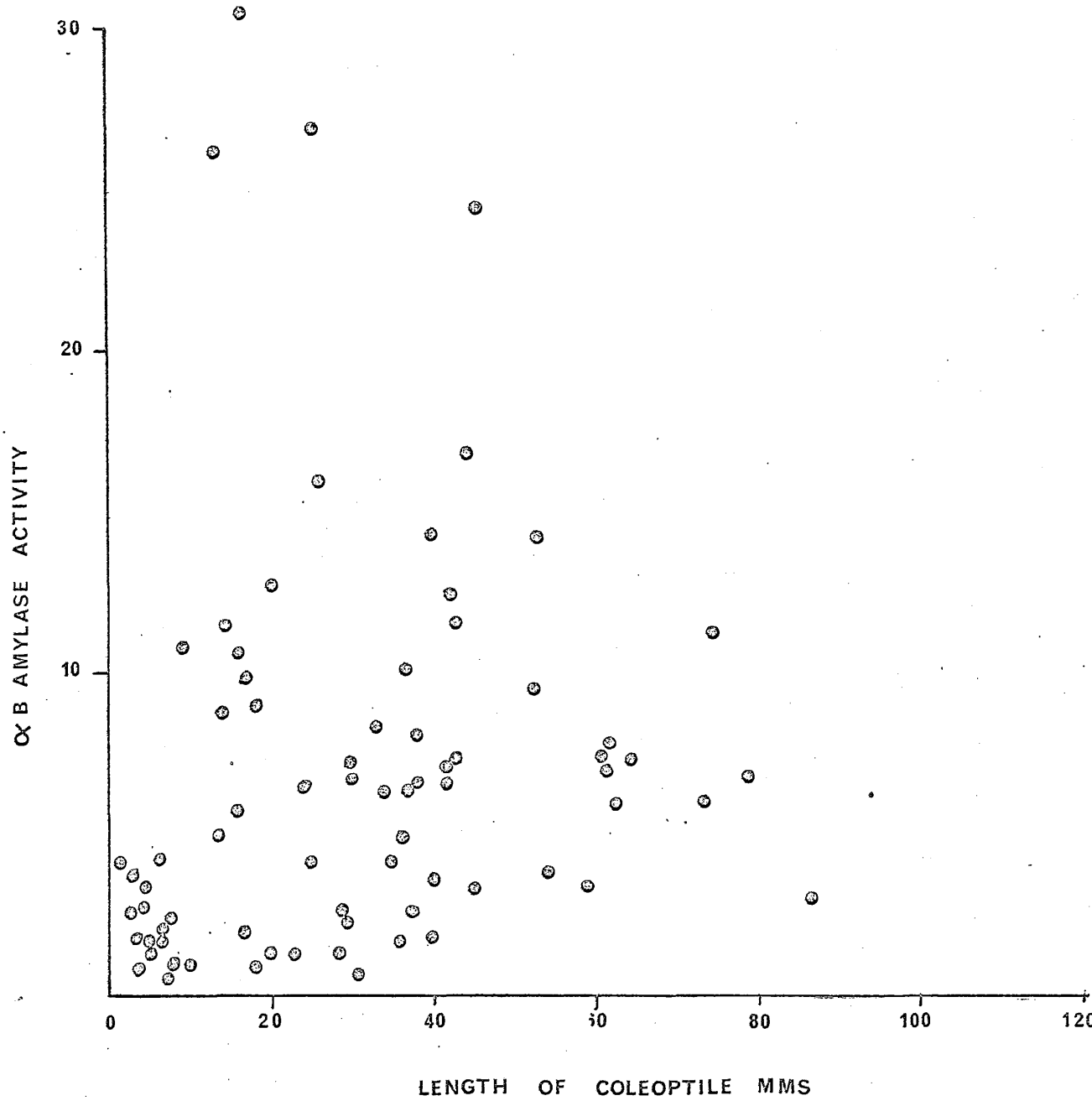

Fig. 69. Scatter plot illustrating correlation between total amylolytic activity (alpha- and beta-amylase) and coleoptile length in individual Hickory King grains.

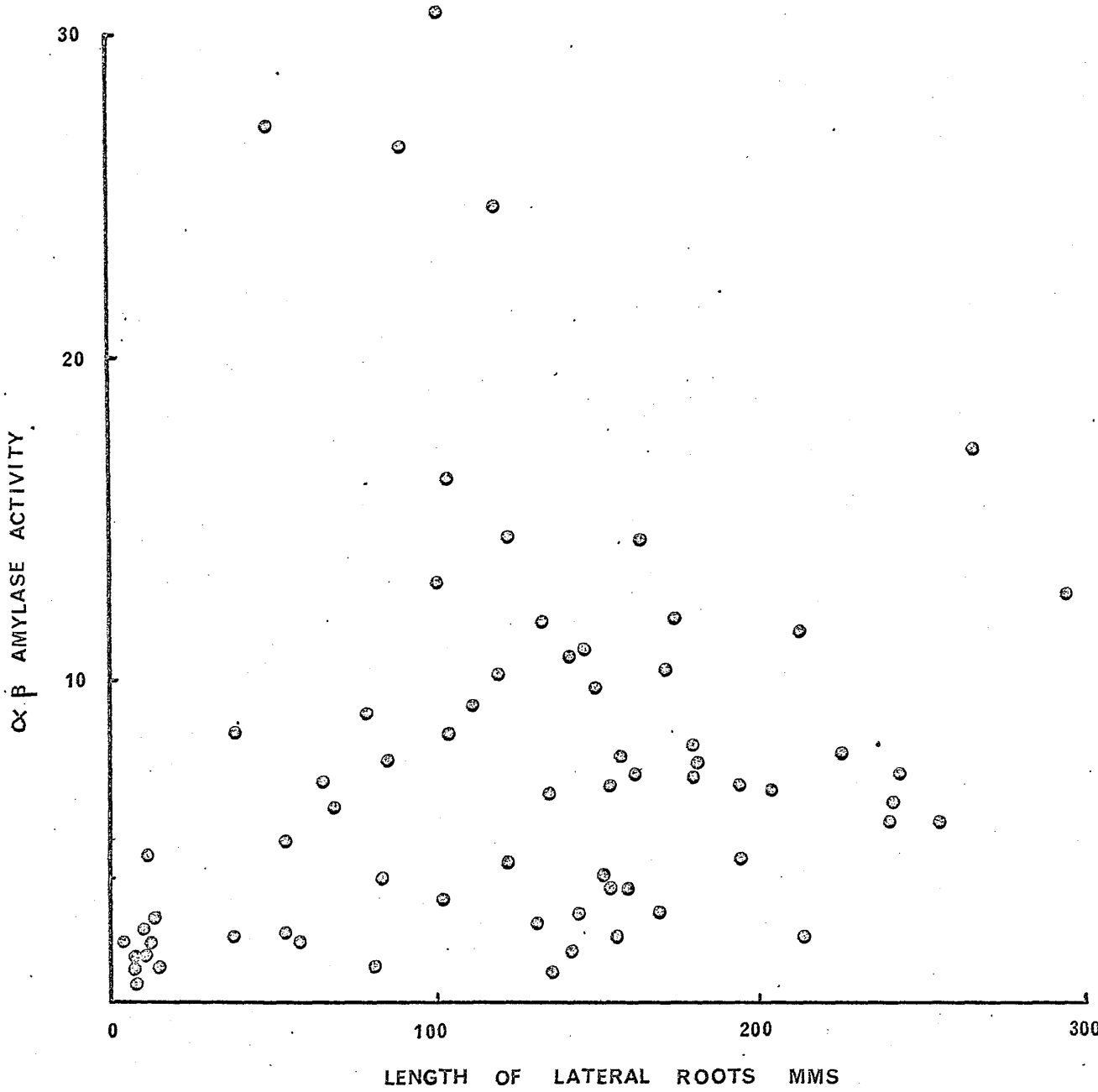

Fig. 70. Scatter plot illustrating correlation between total amylolytic activity (alpha- and beta-amylase) and lateral root length in individual Hickory King grains.

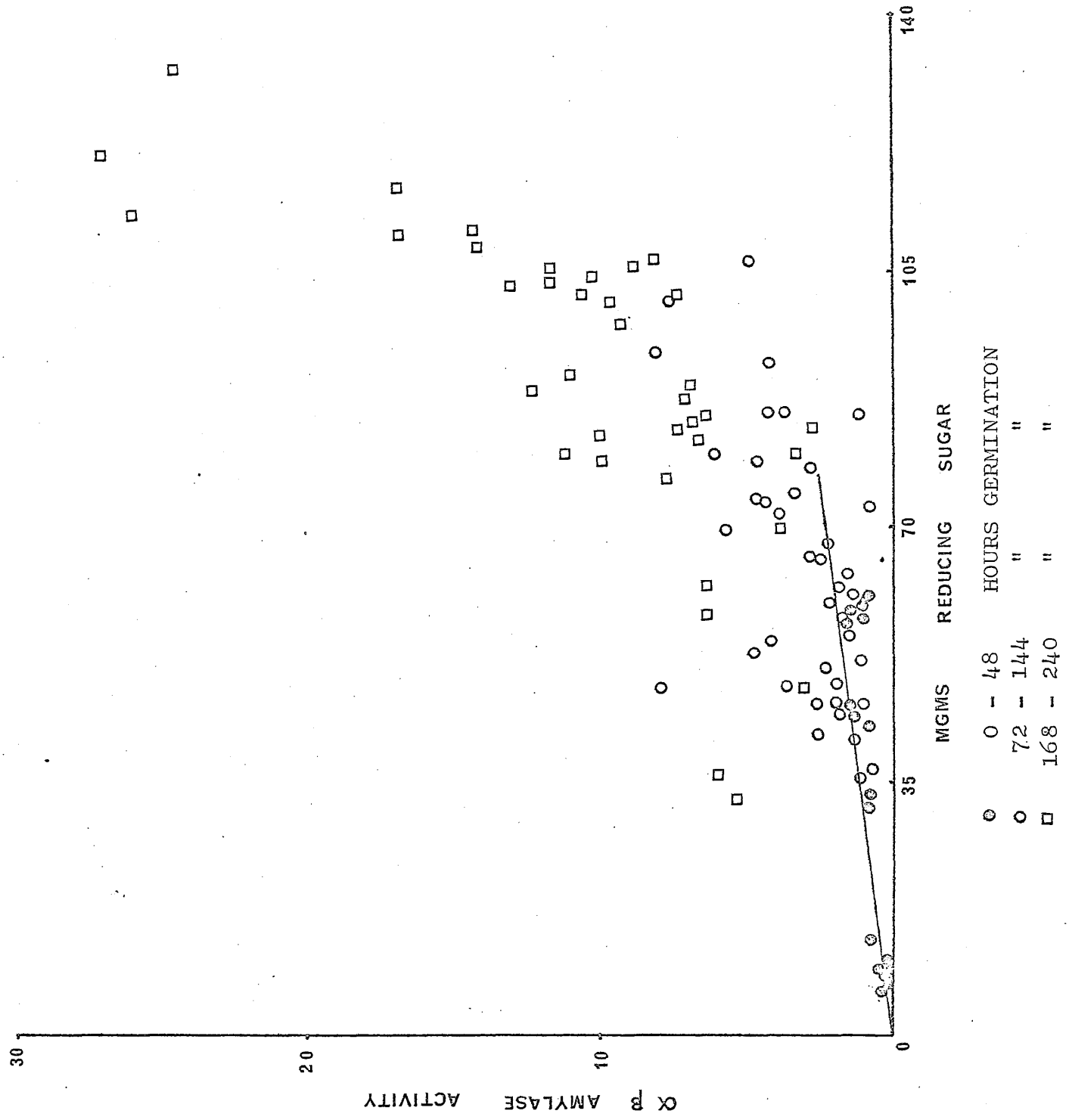

Fig. 71. Scatter plot illustrating correlation between total amylolytic activity (alpha- and beta- amylase) and reducing sugar concentration in individual Hickory King grains. Correlation coefficient for 0-96 hours + 0.829.

TABLE 45

ALPHA-AMYLOLYTIC ACTIVITY (MGMS. MALTOSE/ML./GRAIN) IN GROUPS OF HICKORY KING GRAIN DURING GERMINATION. ASSAY WAS REPLICATED THREE TIMES FOR EACH GROUP.

Germination Period (hrs)	Replication Number 1	2	3	Mean	Germination Period Mean
0	0.094	0.062	0.080	0.078	
0	0.148	0.122	0.258	0.176	0.182
0	0.288	0.300	0.286	0.291	
24	0.325	0.348	0.333	0.335	
24	0.495	0.488	0.486	0.490	0.404
24	0.383	0.389	0.393	0.388	
48	0.985	0.969	0.836	0.930	
48	1.302	1.305	1.199	1.269	0.992
48	0.809	0.764	0.761	0.778	
72	2.427	2.392	2.410	2.406	
72	1.803	1.792	1.778	1.791	1.901
72	1.499	1.514	1.503	1.505	
96	3.593	3.354	3.459	3.469	
96	2.396	2.604	2.584	2.528	2.353
96	1.063	1.094	1.031	1.063	
120	6.639	6.875	4.306	5.940	
120	6.389	6.306	6.320	6.338	5.230
120	5.000	4.848	4.889	3.412	
144	7.391	7.792	7.644	7.609	
144	8.075	8.375	8.209	8.220	8.051
144	8.312	8.625	8.035	8.324	
168	10.417	10.292	10.333	10.347	
168	11.292	11.063	11.292	11.216	10.923
168	11.188	11.334	11.084	11.202	
192	10.125	9.375	9.300	9.600	
192	7.075	6.750	7.450	7.092	7.033
192	4.375	4.675	4.175	4.408	
216	11.000	11.125	10.025	10.717	
216	9.050	9.100	8.900	9.017	8.658
216	6.250	6.225	6.250	6.243	
240	6.719	6.782	7.032	6.844	
240	5.250	6.063	5.500	5.604	6.938
240	8.375	8.094	8.625	8.365	

I - S - D Between Observations at 0.05 P. L. ± 0.893

" " " " Means at 0.05 P. L. ± 0.536

" " " "Germination Period Means at 0.05 P. L. ± 0.298

TABLE 46a

ANALYSIS OF VARIANCE RESULTS FOR ALPHA-AMYLASE ACTIVITY IN IN GROUPS OF HICKORY KING GRAINS.

Source	Degrees of Freedom	Sum Squares	Variance	Variance Ratio (F)	Observations
Germination	10	1285.8526	128.5853	1349.870	* * *
Replication	2	0.3459	0.1729	1.816	N. S.
Sample	2	16.0430	8.0215	84.209	* * *
Sample/Rep.	4	0.5882	0.1470	1.544	N. S.
Rep. /Sample	20	1.8512	0.0926	0.972	N. S.
Sample/Germ.	20	82.7014	4.1351	43.409	* * *
Error	40	3.8103	0.0953		

* * * Significant at 0.1% Probability Level.

TABLE 46b

RESULTS OF LINEAR REGRESSION OF ALPHA-AMYLASE ACTIVITY AND TOTAL REDUCING FRACTION, RADICLE, COLEOPTILE AND LATERAL ROOT LENGTH IN GROUPS OF HICKORY KING GRAIN.

Factor	Germination Period (Hrs)	Correlation Coefficient	t	Observations
Amylase	0 -240	+ 0.750	6.313	* * *
	0 -96	+ 0.858	6.023	* * *
Reducing Sugar	120 -240	+ 0.184	-	-
Amylase	0 -240	+ 0.857	9.259	* * *
Radicle	72 -240	+ 0.716	4.811	* * *
Amylase	0 -240	+ 0.786	7.079	* * *
Coleoptile	72 -240	+ 0.639	-	-
Amylase	0 -240	+ 0.833	8.383	* * *
Laterals	72 -240	+ 0.712	4.756	* * *

* * * Significant at 0.1% Probability Level.

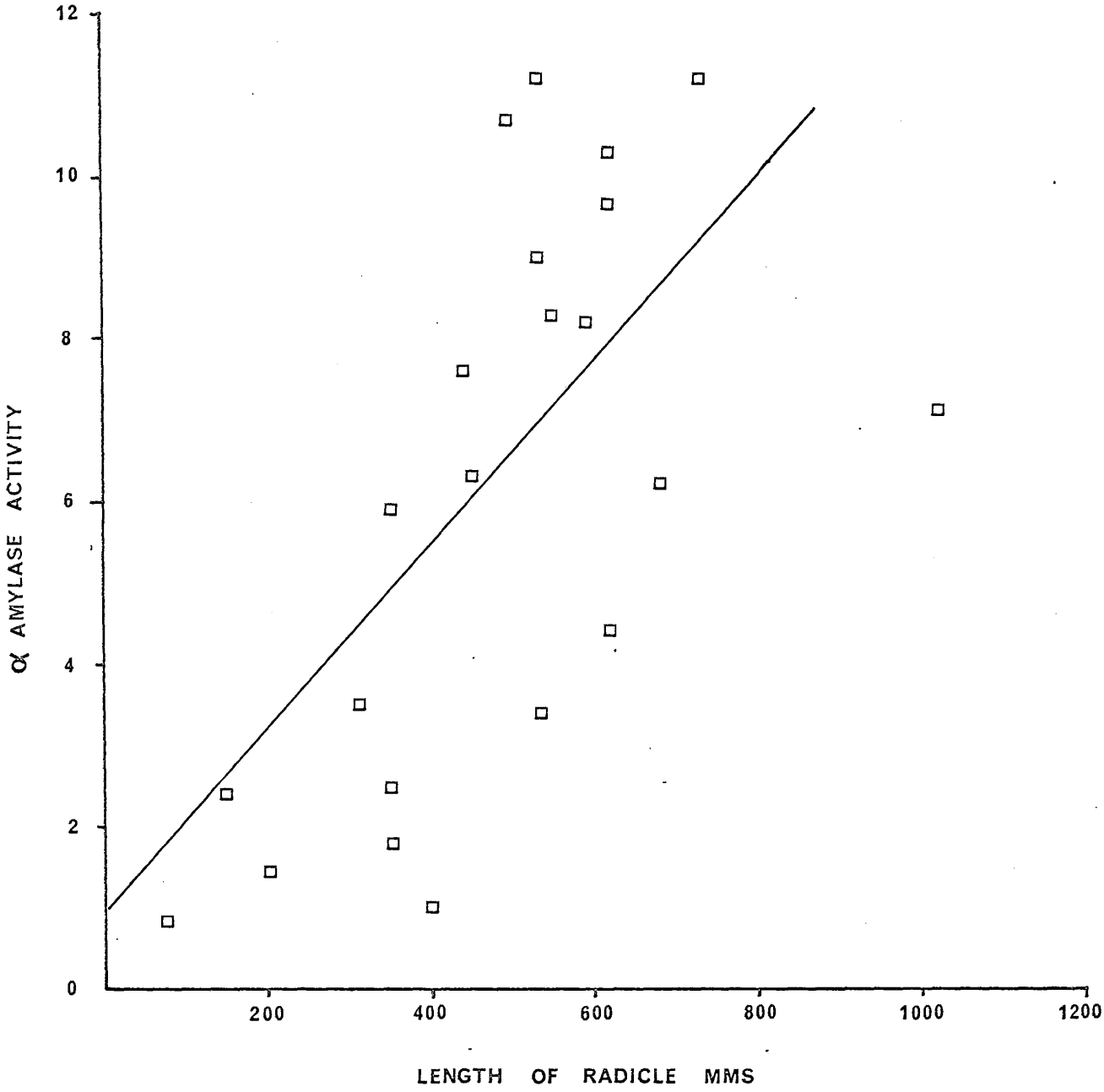

Fig. 72. Scatter plot illustrating correlation between alpha-amylolytic activity and radicle length in groups of Hickory King grain. Correlation coefficient for 0-240 hours + 0.857.

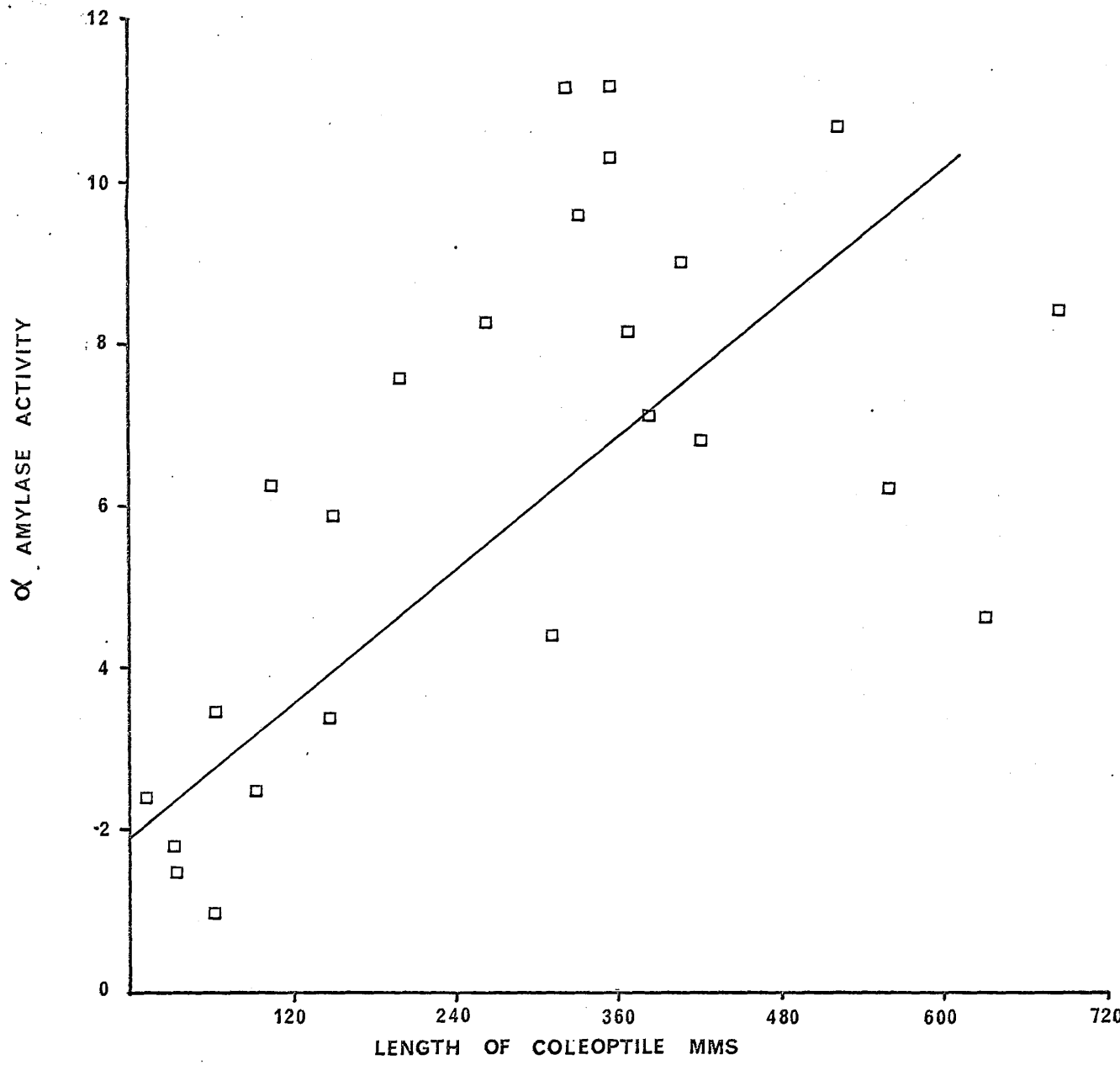

Fig. 73. Scatter plot illustrating correlation between alpha-amylolytic activity and coleoptile length in groups of Hickory King grain. Correlation coefficient for 0-240 hours + 0.786.

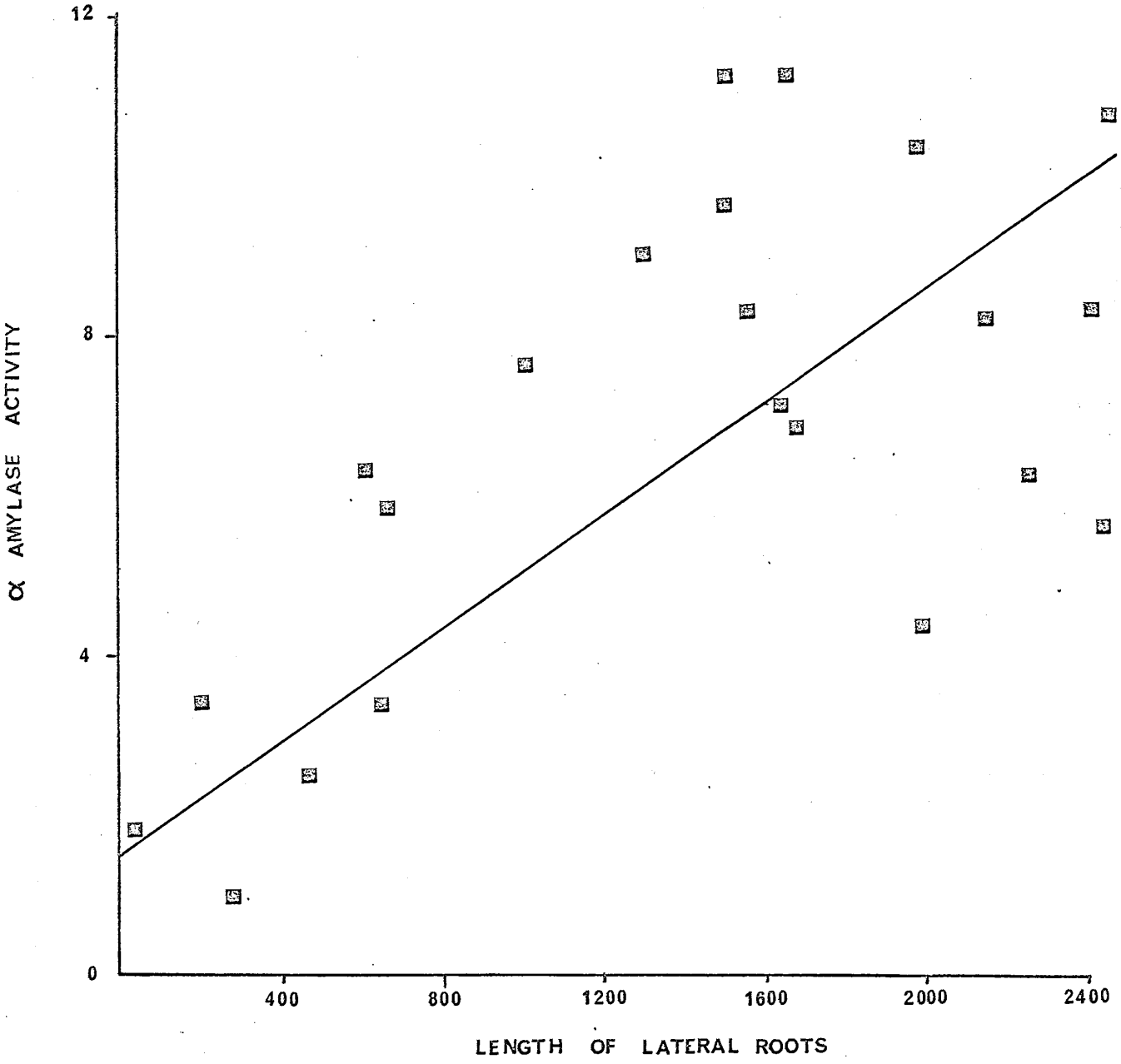

Fig. 74. Scatter plot illustrating correlation between alpha- amylolytic activity and lateral root length in groups of Hickory King grain. Correlation coefficient for 0-240 hours + 0.833.

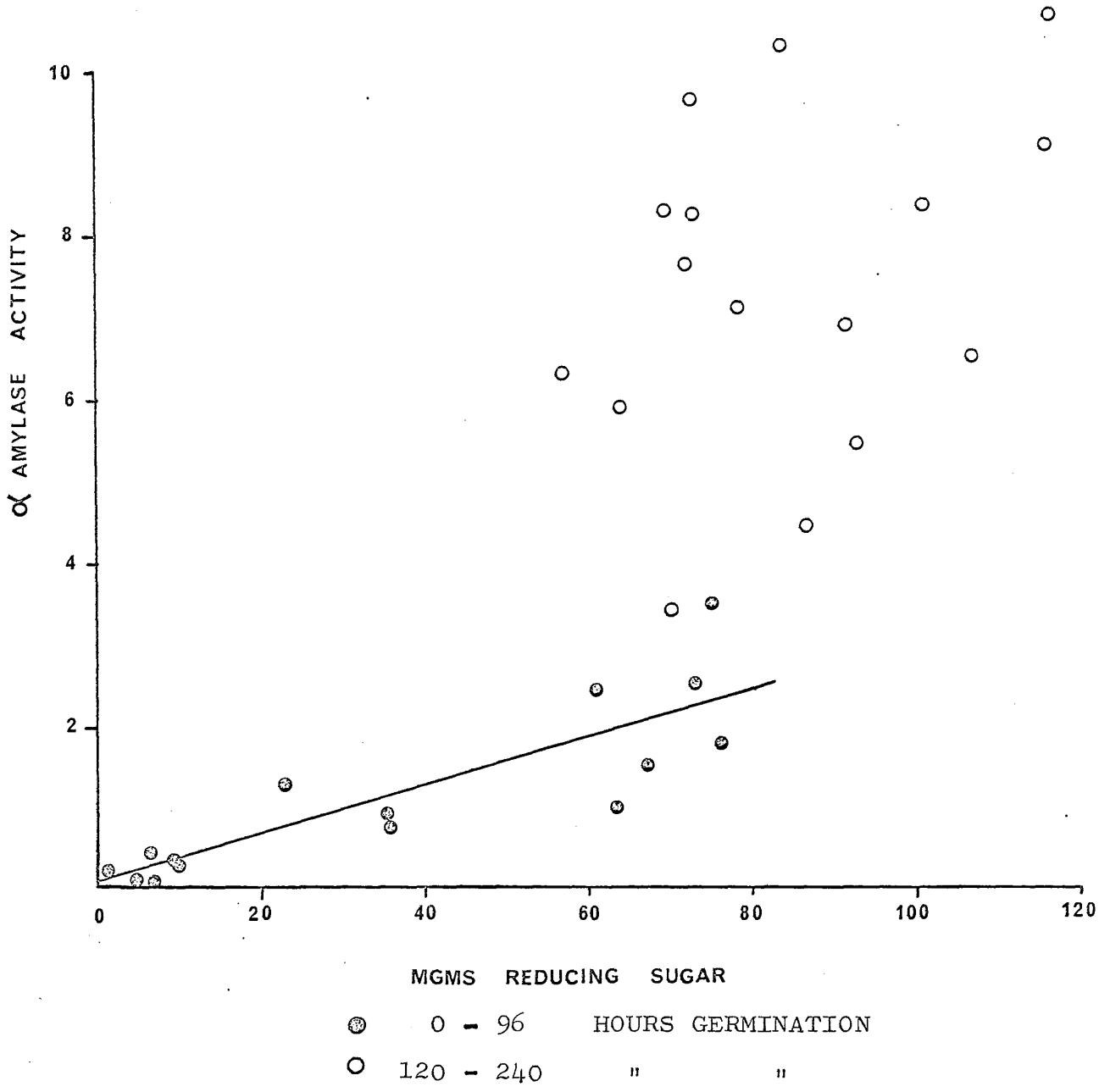

Fig. 75. Scatter plot illustrating correlation between alpha-amylolytic activity and reducing sugar concentration in groups of Hickory King grain. Correlation coefficient for 0-96 hours + 0.858.

TABLE 47

ALPHA-AMYLOLYTIC ACTIVITY (MGMS. MALTOSE/MIN.) IN INDIVIDUAL HICKORY KING GRAINS DURING GERMINATION.

Germination Period (hrs)	Grain Number 1	2	3	4	5	6	7	8	9	10	Mean	% Germination	Growth Index
0	-	-	0.028	0.219	-	0.078	-	0.056	0.047	-	0.043	-	-
24	0.132	0.193	0.440	0.125	0.230	0.213	0.395	0.295	0.169	0.572	0.276	-	-
48	1.183	1.023	0.612	0.453	0.236	0.484	0.805	0.145	0.841	0.334	0.612	-	-
72	2.379	2.308	2.279	1.433	1.079	0.550	1.542	1.196	1.237	1.021	1.502	90	0.119
96	1.071	0.964	0.884	0.134	0.134	0.804	0.464	2.259	0.723	1.902	0.934	100	0.483
120	0.114	1.458	1.219	3.042	0.958	1.792	0.156	1.281	0.437	0.010	1.047	100	1.489
144	5.812	3.150	2.637	0.700	0.850	2.475	3.500	2.937	5.337	4.425	3.182	100	2.230
168	3.937	1.359	4.406	4.781	3.016	3.906	6.281	9.828	1 594	3.656	4.276	100	2.712
192	12.250	3.150	0.800	5.700	6.750	3.450	6.000	7.000	16.250	6.500	6.785	100	1.932
216	6.750	2.825	4.700	19.750	4.425	7.575	4.050	1.050	11.125	5.900	5.815	100	2.131
240	5.406	6.656	4.656	18.687	3.187	1.156	3.906	3.375	7.219	9.594	6.384	100	2.568

Least Significant Difference Between Observations at 0.05 Probability Level ± 1.201

" " " " Means " " " " ± 0.380

TABLE 48a

ANALYSIS OF VARIANCE RESULTS FOR ALPHA-AMYLASE ACTIVITY IN INDIVIDUAL HICKORY KING GRAINS.

Source	Degrees of Freedom	Sum Squares	Variance	Variance Ratio (F)	Observations
Germination	10	819.567	81.957	10.803	* * *
Sample	9	93.960	10.440	1.376	N. S.
Error	90	682.752	7.586		

* * * Significant at 0.1% Probability Level.

TABLE 48b

RESULTS OF LINEAR REGRESSION OF ALPHA-AMYLASE ACTIVITY, AND TOTAL REDUCING FRACTION, RADICLE, COLEOPTILE AND LATERAL ROOT LENGTH IN INDIVIDUAL HICKORY KING GRAINS.

Factor	Germination Period Hours	Correlation Coefficient	t	Observations
Amylase	0 -240	+ 0.738	11.336	* * *
	0 -96	+ 0.728	7.357	* * *
Reducing Sugar	120 -240	+ 0.515	-	-
Amylase	0 -240	+ 0.551	-	-
	0 -96	+ 0.452	-	-
Radicle	72 -240	+ 0.398	-	-
Amylase	0 -240	+ 0.384	-	-
	0 -96	+ 0.439	-	-
Coleoptile	72 -240	+ 0.185	-	-
Amylase	0 -240	+ 0.460	-	-
	0 -96	+ 0.287	-	-
Laterals	72 -240	+ 0.286	-	-

* * * Significant at 0.1% Probability Level.

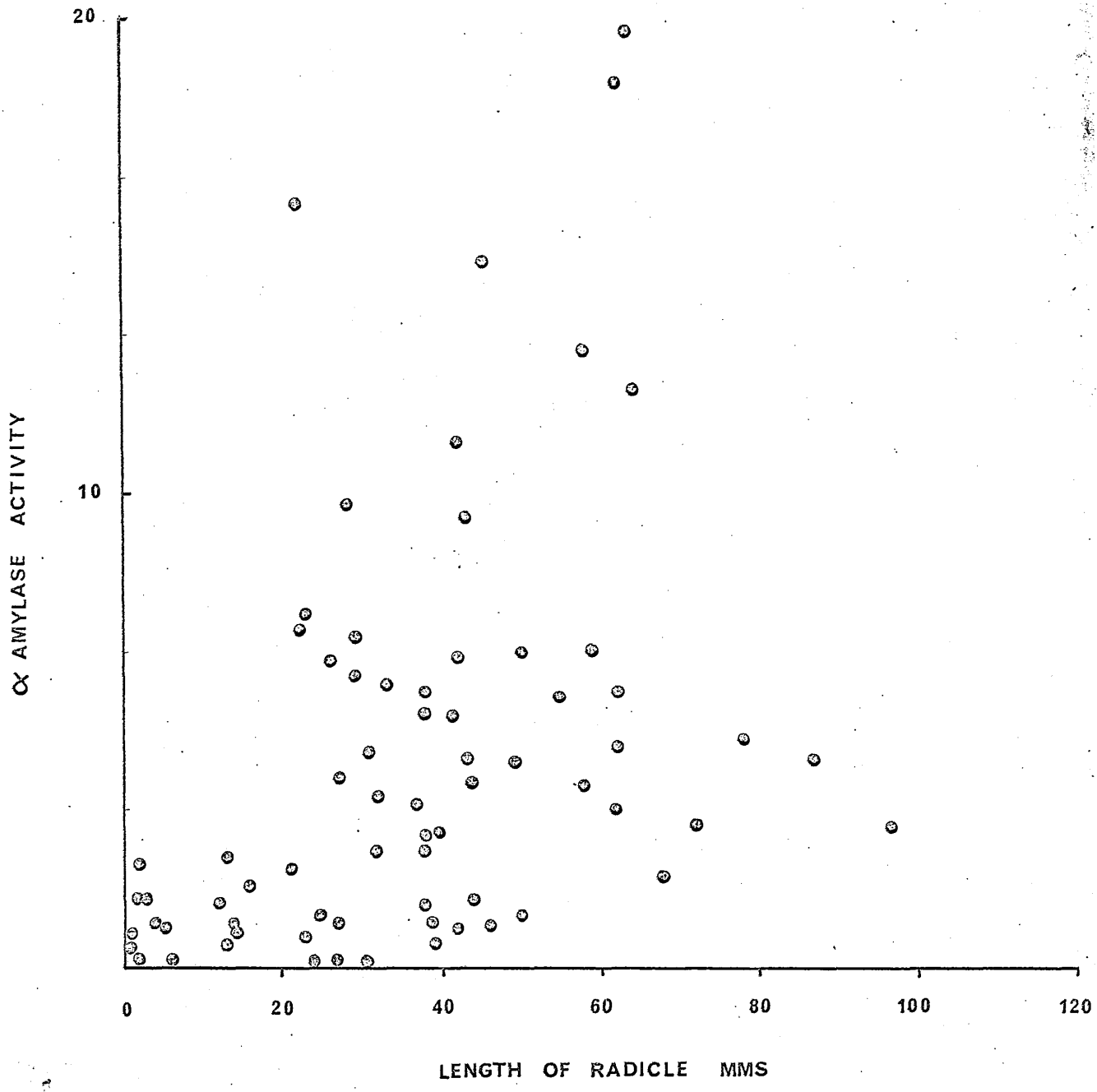

Fig. 76. Scatter plot illustrating correlation between alpha-amylolytic activity and radicle length in individual Hickory King grains.

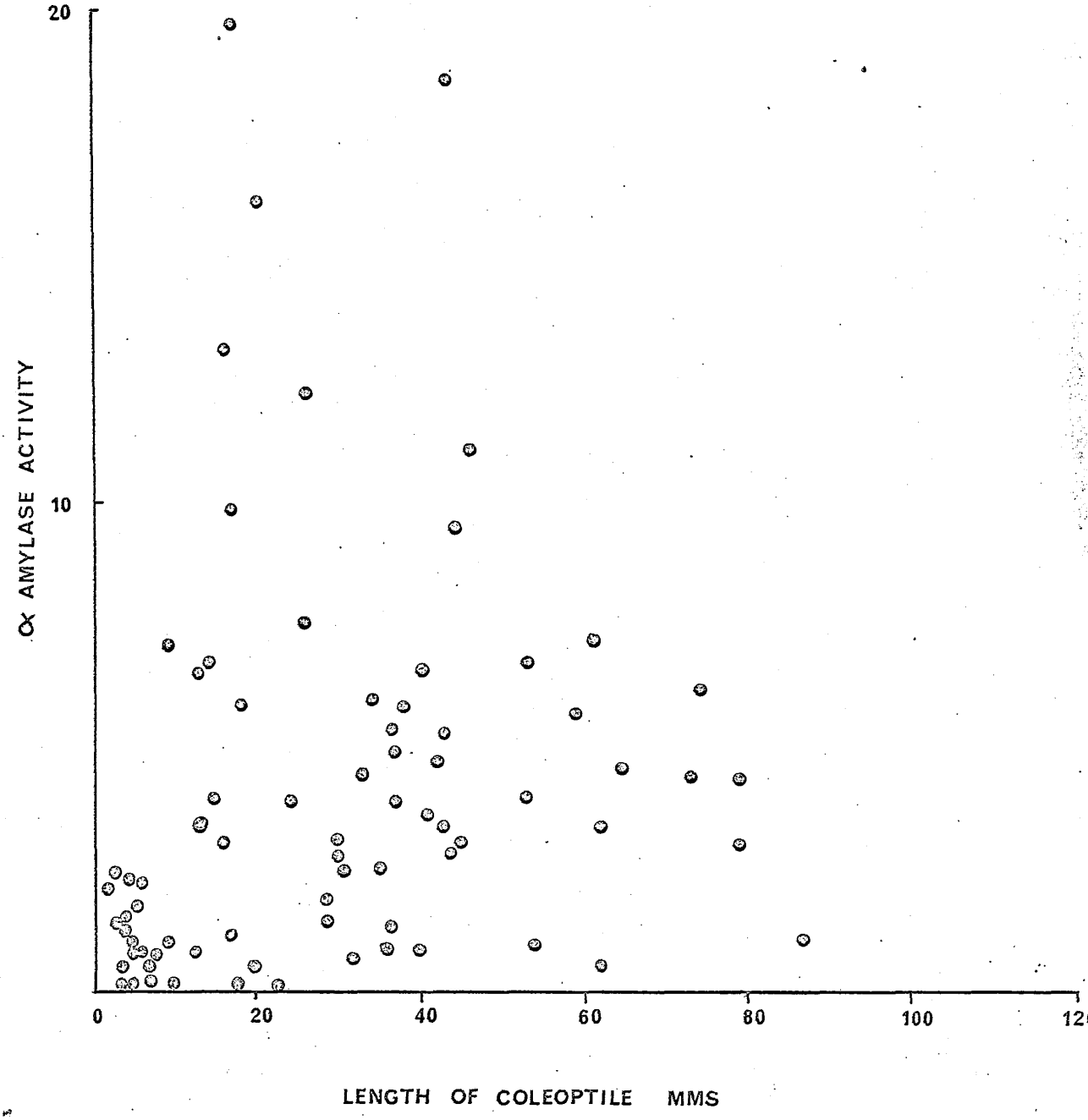

Fig. 77. Scatter plot illustrating correlation between alpha-amylolytic activity and coleoptile length in individual Hickory King grains.

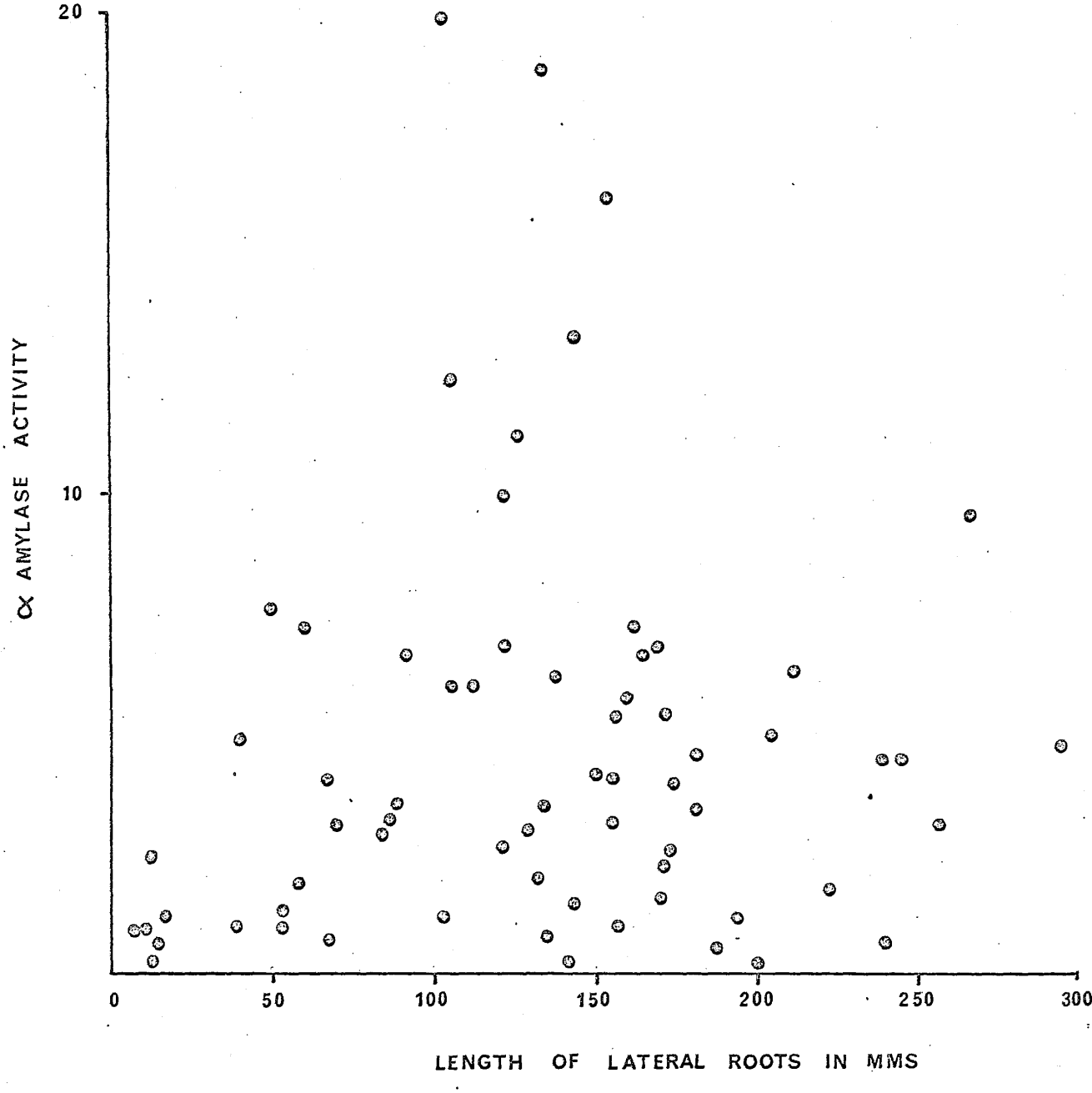

Fig. 78. Scatter plot illustrating correlation between alpha-amylolytic activity and lateral root length in individual Hickory King grains.

Fig. 79. Scatter plot illustrating correlation between alpha-amylolytic activity and reducing sugar concentration in individual Hickory King grains. Correlation coefficient for 0-96 hours + 0.728.

TABLE 49

BETA-AMYLOLYTIC ACTIVITY (MGMS. MALTOSE/MIN./GRAIN) IN GROUPS OF HICKORY KING GRAIN DURING GERMINATION. ASSAY WAS REPLICATED THREE TIMES ON EACH GROUP.

Germination Period (hrs)	Replication Number 1	2	3	Mean	Germination Period Mean
0	0.039	0.047	0.061	0.049	
0	0.047	0.050	0.039	0.045	0.063
0	0.095	0.088	0.099	0.094	
24	0.060	0.030	0.048	0.046	
24	0.118	0.199	0.192	0.170	0.194
24	0.385	0.386	0.332	0.368	
48	0.672	0.675	0.812	0.720	
48	0.417	0.431	0.429	0.426	0.584
48	0.575	0.625	0.621	0.607	
72	1.875	1.999	1.981	1.952	
72	2.017	2.017	2.035	2.023	1.793
72	1.375	1.388	1.453	1.405	
96	1.532	1.584	1.750	1.622	
96	2.188	1.646	2.239	2.024	1.962
96	2.218	2.260	2.234	2.237	
120	1.972	1.598	4.375	2.648	
120	2.986	2.778	3.055	2.940	2.860
120	2.986	2.930	3.061	2.992	
144	2.984	2.407	2.947	2.779	
144	3.335	3.063	2.566	2.988	3.040
144	3.084	3.959	3.013	3.352	
168	3.333	3.625	1.209	2.272	
168	3.333	3.521	3.333	3.396	3.873
168	5.521	5.312	5.666	5.500	
192	3.875	3.800	3.625	3.767	
192	5.050	4.075	4.525	4.550	3.997
192	3.875	3.375	3.775	3.675	
216	3.500	3.550	3.850	3.633	
216	3.450	3.475	3.100	3.342	3.283
216	2.875	2.875	2.875	2.875	
240	7.344	7.812	7.875	7.677	
240	7.719	5.562	5.844	6.375	7.083
240	7.250	7.031	7.313	7.198	

Least Significant Difference Between Observations at 0.05 P. L. $\pm$ 1.298

" " " " Means at 0.05 P. L. $\pm$ 0.750

" " " " Germination Period Means at 0.05 P. L. $\pm$ 0.432

TABLE 50a

ANALYSIS OF VARIANCE RESULTS FOR BETA-AMYLASE ACTIVITY IN GROUPS OF HICKOYR KING GRAIN.

Source	Degrees of Freedom	Sum Squares	Variance	Variance Ratio (F)	Observations
Germination	10	375.7057	37.5706	179.223	* * *
Replication	2	0.2327	0.1163	0.555	N. S.
Sample	2	1.0697	0.5348	2.551	N. S.
Sample/Rep.	4	0.6719	0.1680	0.801	N. S.
Replication/Germ.	20	3.9822	0.1991	0.950	N. S.
Sample/Germ.	20	18.6669	0.9333	4.452	* * *
Error	40	8.3852	0.2096		

* * * Significant at 0.1% Probability Level.

TABLE 50b

RESULTS OF LINEAR REGRESSION OF BETA-AMYLASE ACTIVITY AND TOTAL REDUCING FRACTION, ALPHA-AMYLASE ACTIVITY, RADICLE, COLEOPTILE AND LATERAL ROOT LENGTH IN GROUPS OF HICKORY KING GRAIN.

Factor	Germination Period (Hrs)	Correlation Coefficient	t	Observations
Beta-Amylase	0 -240	+ 0.769	6.698	* * *
	0 -96	+ 0.951	11.090	* * *
Reducing Sugar	120 -240	+ 0.297	-	-
Beta-Amylase	0 -240	+ 0.714	5.678	* * *
	0 -96	+ 0.787	4.599	* * *
Alpha-Amylase	120 -240	+ 0.002	-	-
Beta-Amylase	0 -240	+ 0.774	6.806	* * *
Radicle	72 -240	+ 0.539	-	-
Beta-Amylase	0 -240	+ 0.850	8.984	* * *
Coleoptile	72 -240	+ 0.752	5.351	* * *
Beta-Amylase	0 -240	+ 0.800	7.424	* * *
Laterals	72 -240	+ 0.651	4.023	* * *

* * * Significant at 0.1% Probability Level.

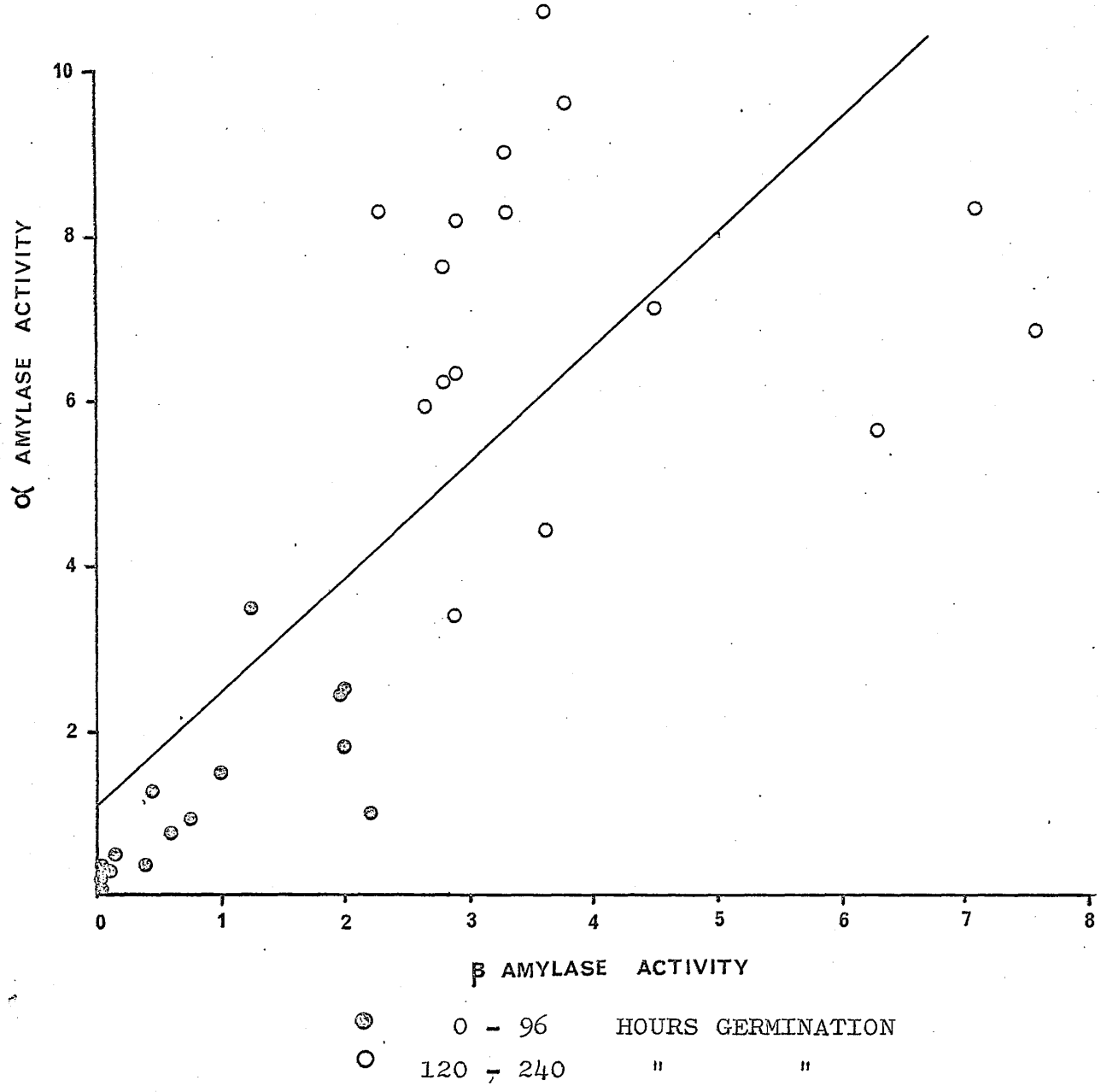

Fig. 80. Scatter plot illustrating correlation between alpha- and beta-amylase activity (mgms. maltose/min.) in groups of Hickory King grain. Correlation coefficient for 0-240 hours + 0.714.

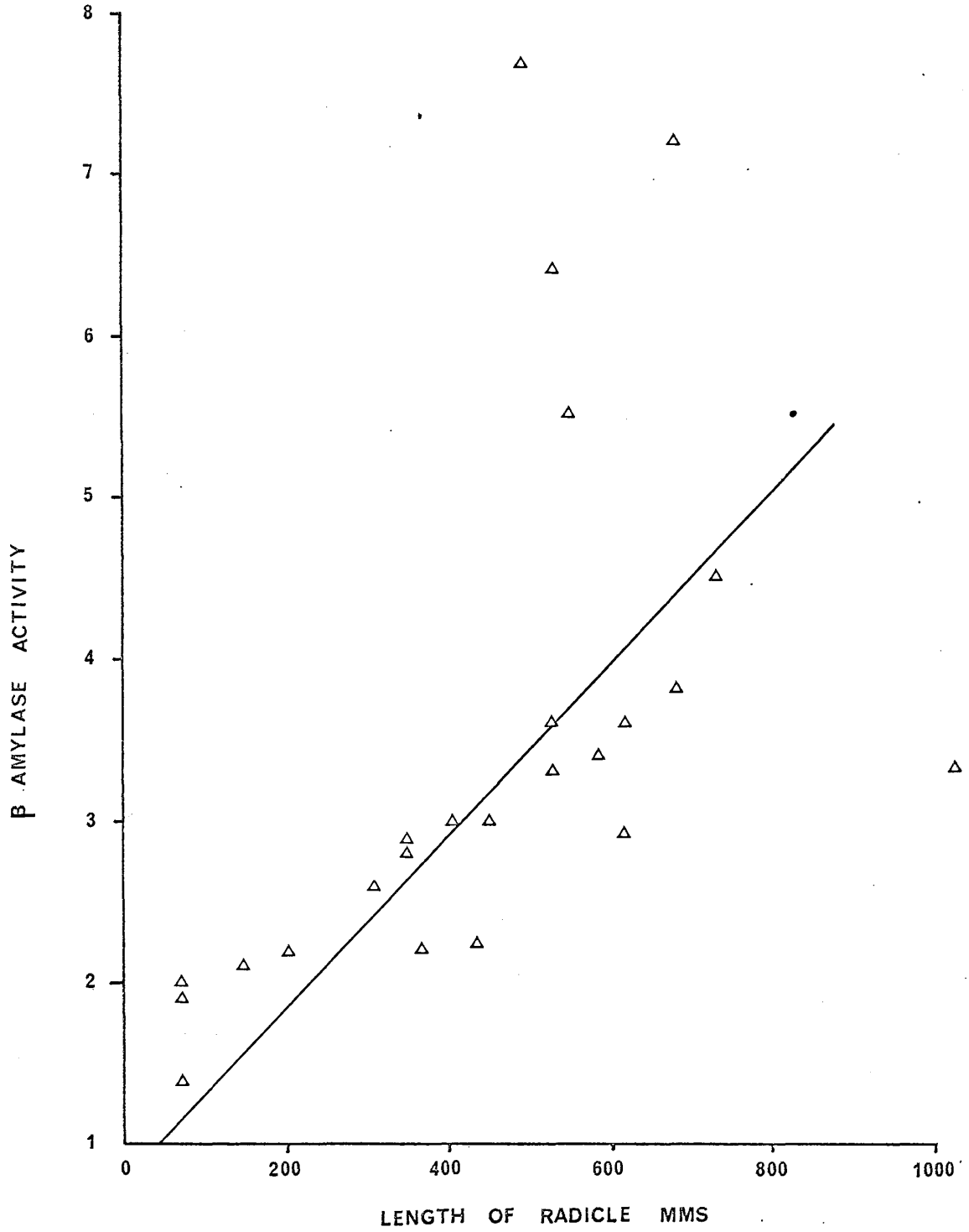

Fig. 81. Scatter plot illustrating correlation between beta-amylase (mgms. maltose/min.) activity and radicle length in groups of Hickory King grain. Correlation coefficient for 0-240 hours + 0.774.

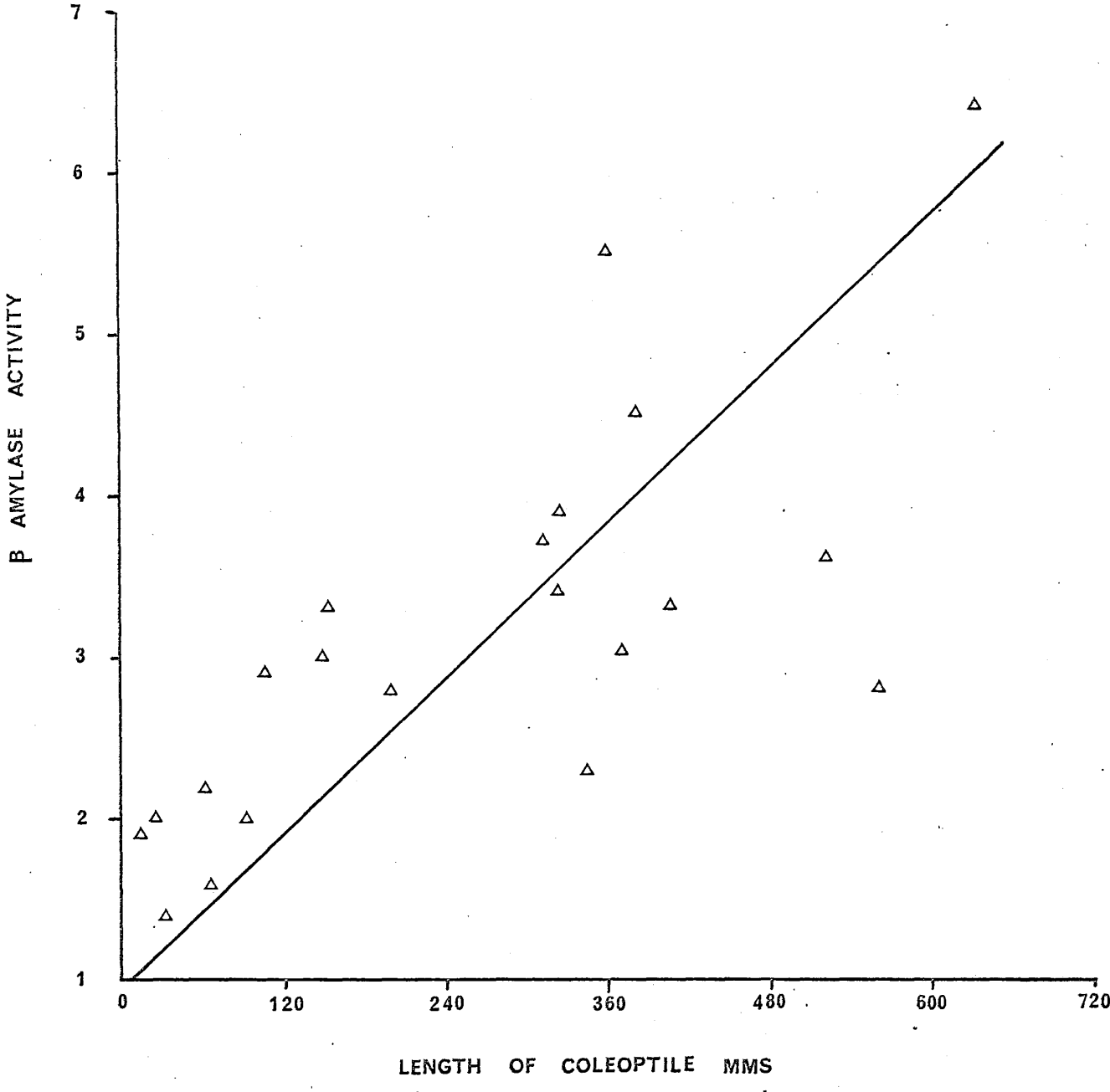

Fig. 82. Scatter plot illustrating correlation between beta-amylase activity (mgms. maltose/min.) and coleoptile length in groups of Hickory King grain. Correlation coefficient for 0-240 hours + 0.850.

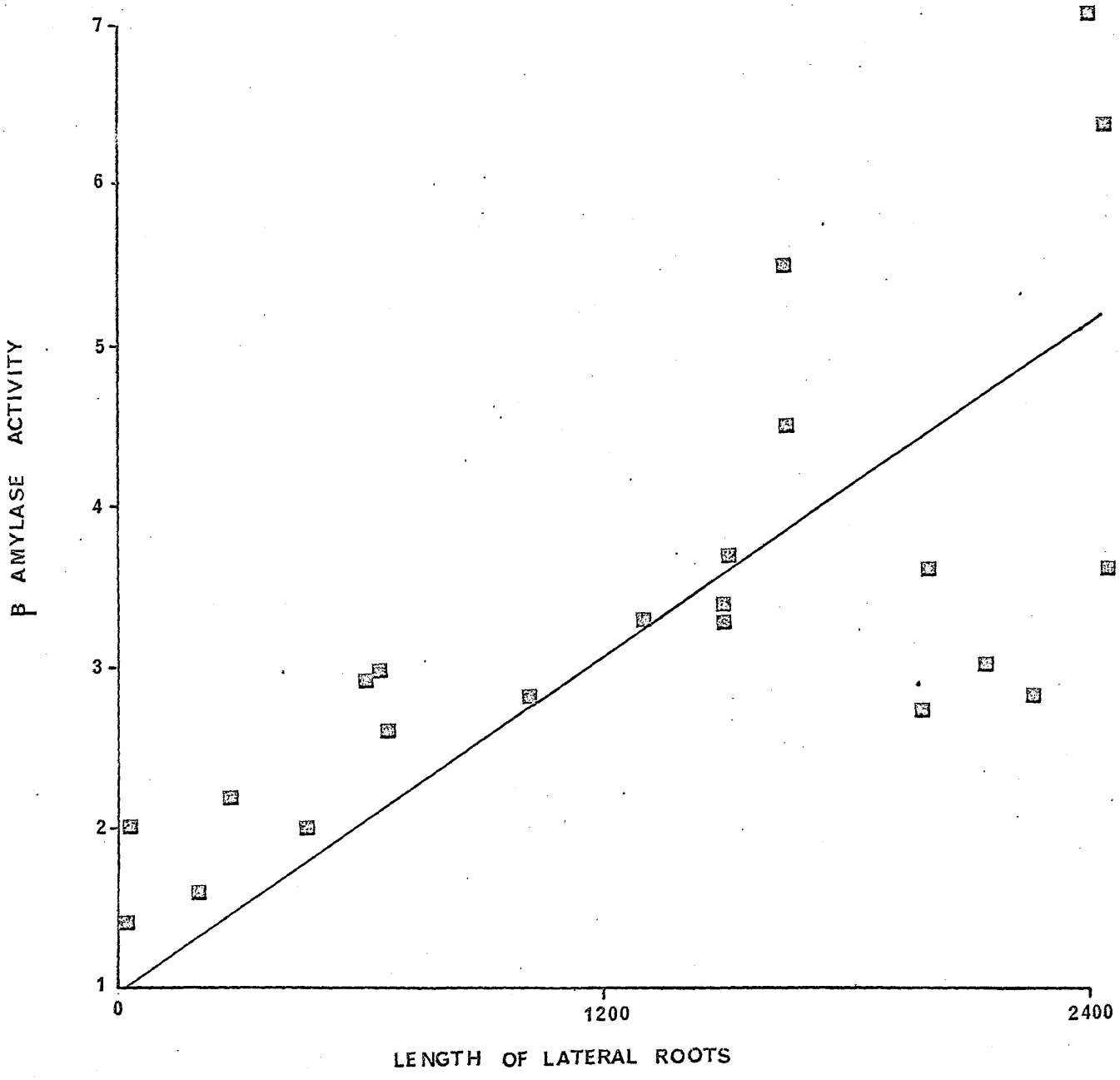

Fig. 83. Scatter plot illustrating correlation between beta-amylase activity (mgms. maltose/min.) and lateral root length in groups of Hickory King grain. Correlation coefficient for 0-240 hours + 0.800.

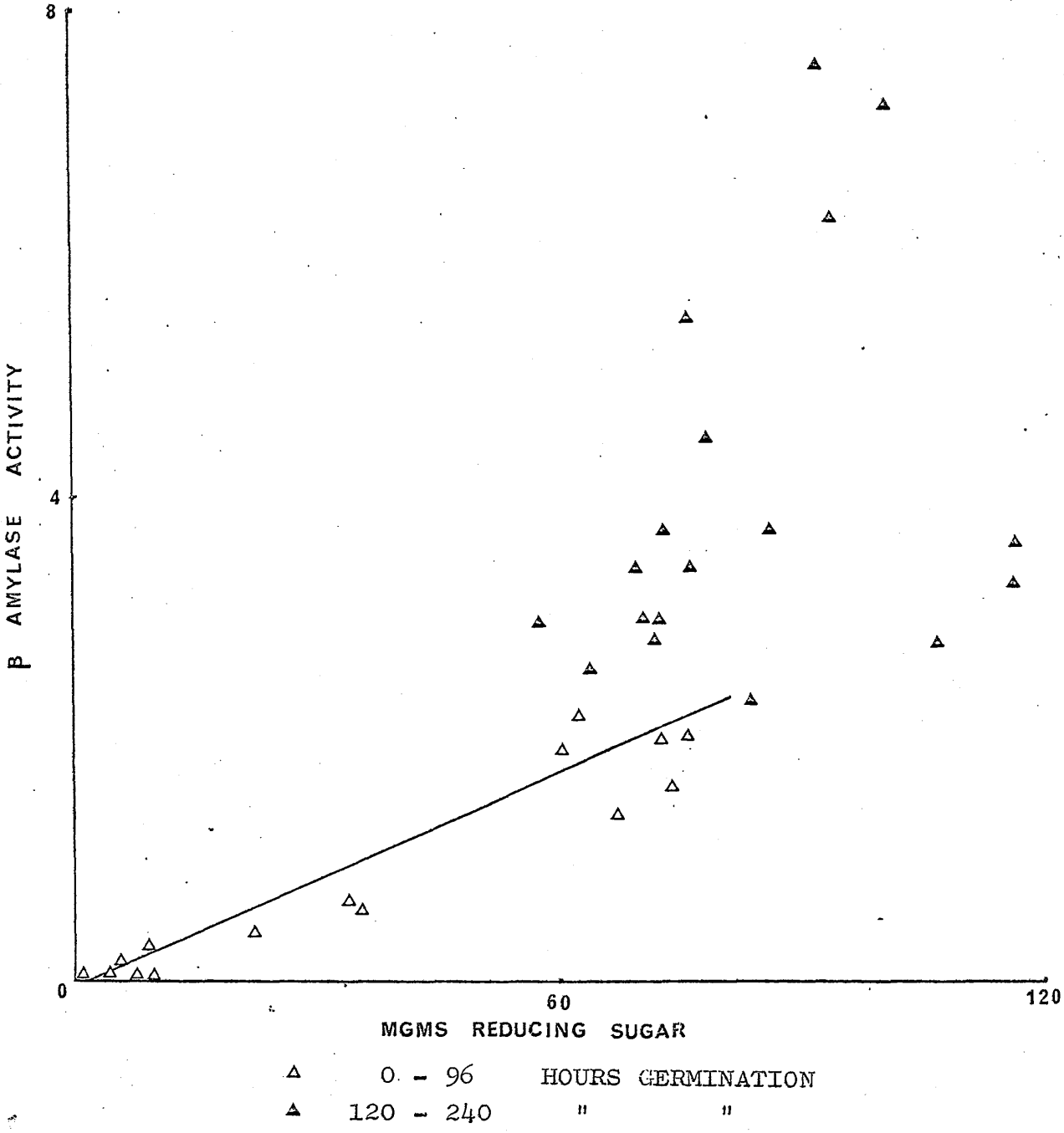

Fig. 84. Scatter plot illustrating correlation between beta-amylase activity (mgms. maltose/min.) and reducing sugar concentration in groups of Hickory King grain. Correlation coefficient for 0-96 hours + 0.951.

TABLE 51

BETA-AMYLOLYTIC ACTIVITY (MGMS. MALTOSE/MIN.) IN INDIVIDUAL HICKORY KING GRAINS DURING GERMINATION

Germination Period (hrs)	Grain Number 1	2	3	4	5	6	7	8	9	10	Mean	% Germination	Growth Index
0	-	-	-	-	-	0.047	-	-	0.094	-	0.014	-	-
24	0.089	0.188	0.279	0.125	0.223	0.449	0.303	0.089	0.128	0.099	0.197	-	-
48	0.504	0.539	0.141	0.592	0.725	0.418	0.507	0.660	0.687	0.641	0.541	-	-
72	1.504	2.359	1.908	1.379	0.867	0.467	1.345	2.521	0.709	0.996	1.405	90	0.199
96	0.099	1.277	0.500	1.437	1.054	0.821	2.197	2.214	1.268	0.473	1.134	100	0.483
120	0.584	1.250	0.969	2.895	0.886	0.187	0.938	2.990	0.990	1.490	1.318	100	1.489
144	2.313	0.300	1.588	0.175	3.812	4.675	1.475	0.813	2.688	1.637	1.948	100	2.230
168	2.657	1.407	2.500	1.672	2.359	2.813	4.969	0.172	4.343	2.000	2.489	100	2.712
192	4.625	4.250	2.525	3.225	4.900	2.925	4.950	3.950	10.125	3.500	4.697	100	1.932
216	7.450	4.000	7.750	11.000	3.875	19.525	5.625	2.825	23.375	3.350	8.877	100	2.131
240	4.781	7.719	2.813	15.594	7.500	2.031	7.875	4.437	0.000	7.250	6.000	100	2.568

Least Significant Difference Between Observations at 0.05 Probability Level ± 1.028

" " " " Means " " " " ± 0.325

TABLE 52a

ANALYSIS OF VARIANCE RESULTS FOR BETA-AMYLASE ACTIVITY IN INDIVIDUAL HICKORY KING GRAINS.

Source	Degrees of Freedom	Sum of Squares	Variance	Variance Ratio (F)	Observations
Germination	10	777.223	77.722	10.437	* * *
Sample	9	51.838	5.760	0.773	N. S.
Error	90	670.217	7.447		

* * * Significant at 0.1% Probability Level.

TABLE 52b

LINEAR REGRESSION OF BETA-AMYLASE ACTIVITY AND TOTAL REDUCING FRACTION, ALPHA-AMYLASE ACTIVITY, RADICLE, COLEOPTILE AND LATERAL ROOT LENGTH IN INDIVIDUAL HICKORY KING GRAINS.

Factor	Germination Period Hours	Correlation Coefficient	t	Observations
Beta-Amylase	0 -240	+ 0.688	9.852	* * *
	0 -96	+ 0.795	9.080	* * *
Reducing Sugar	120 -240	+ 0.361	-	-
Beta-Amylase	0 -240	+ 0.734	11.232	* * *
	0 -96	+ 0.689	6.586	* * *
Alpha-Amylase	120 -240	+ 0.640	6.343	* * *
Beta-Amylase	0 -240	+ 0.421	-	-
	0 -96	+ 0.216	-	-
Radicle	72 -240	+ 0.244	-	-
Beta-Amylase	0 -240	+ 0.365	-	-
	0 -96	+ 0.310	-	-
Coleoptile	72 -240	+ 0.188	-	-
Beta-Amylase	0 -240	+ 0.394	-	-
	0 -96	+ 0.074	-	-
Laterals	72 -240	+ 0.293	-	-

* * * Significant at 0.1% Probability Level.

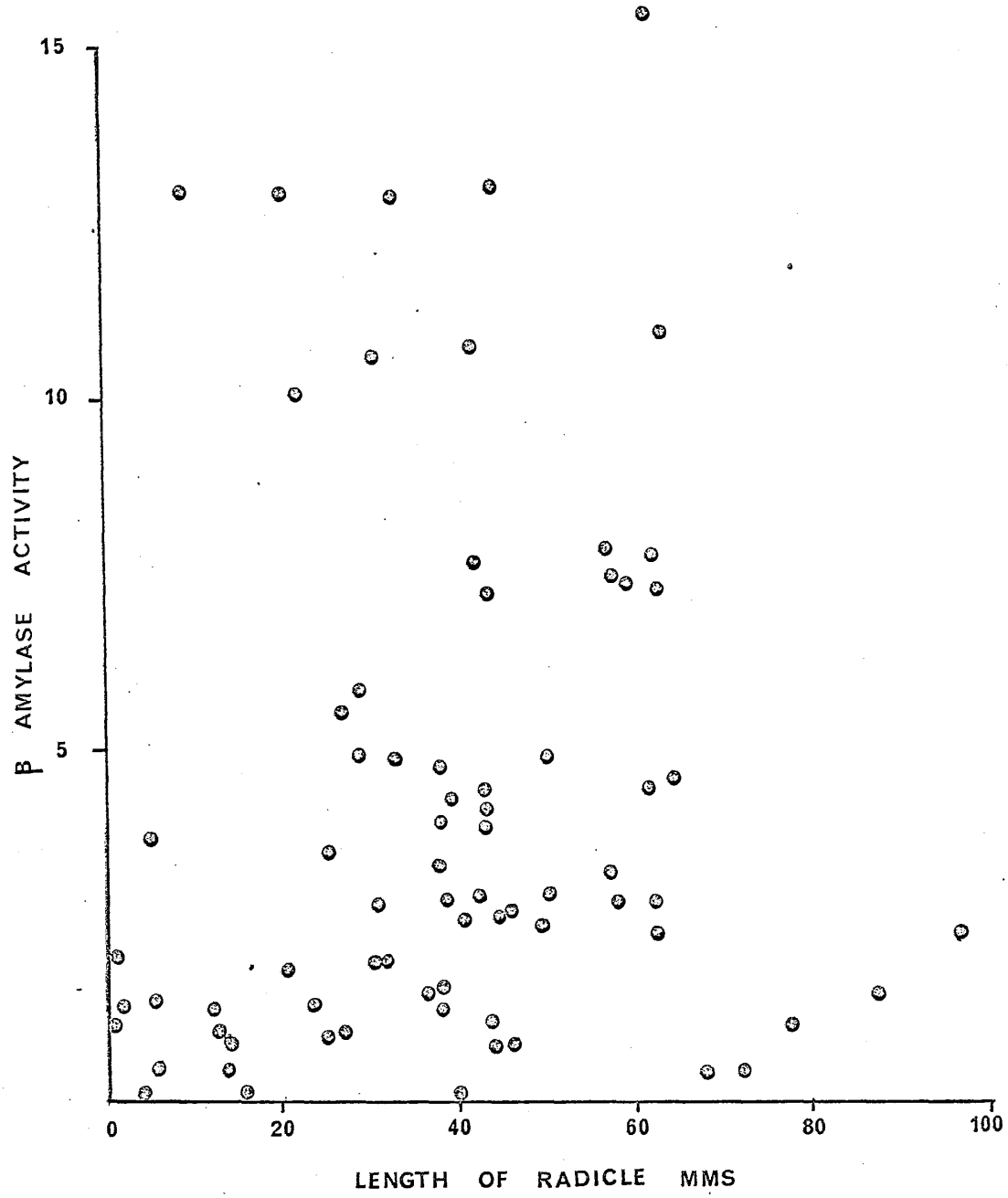

Fig. 85. Scatter plot illustrating correlation between beta-amylase activity (mgms. maltose/min.) and reducing sugar concentration in groups of Hickory King grain. Correlation coefficient for 0-96 hours + 0.951.

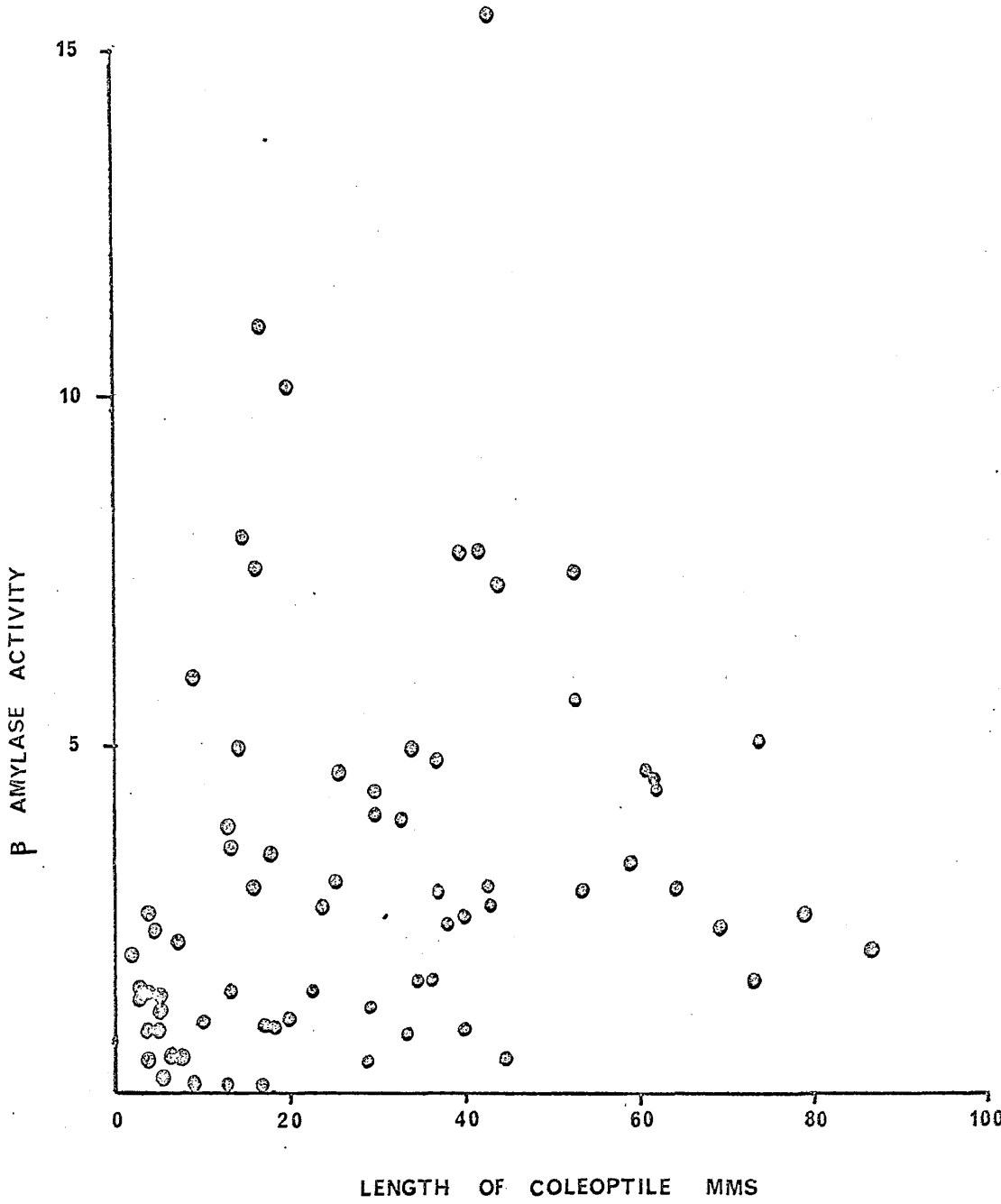

Fig. 86. Scatter plot illustrating correlation between beta-amylase activity (mgms. maltose/min.) and Coleoptile length in individual Hickory King grains.

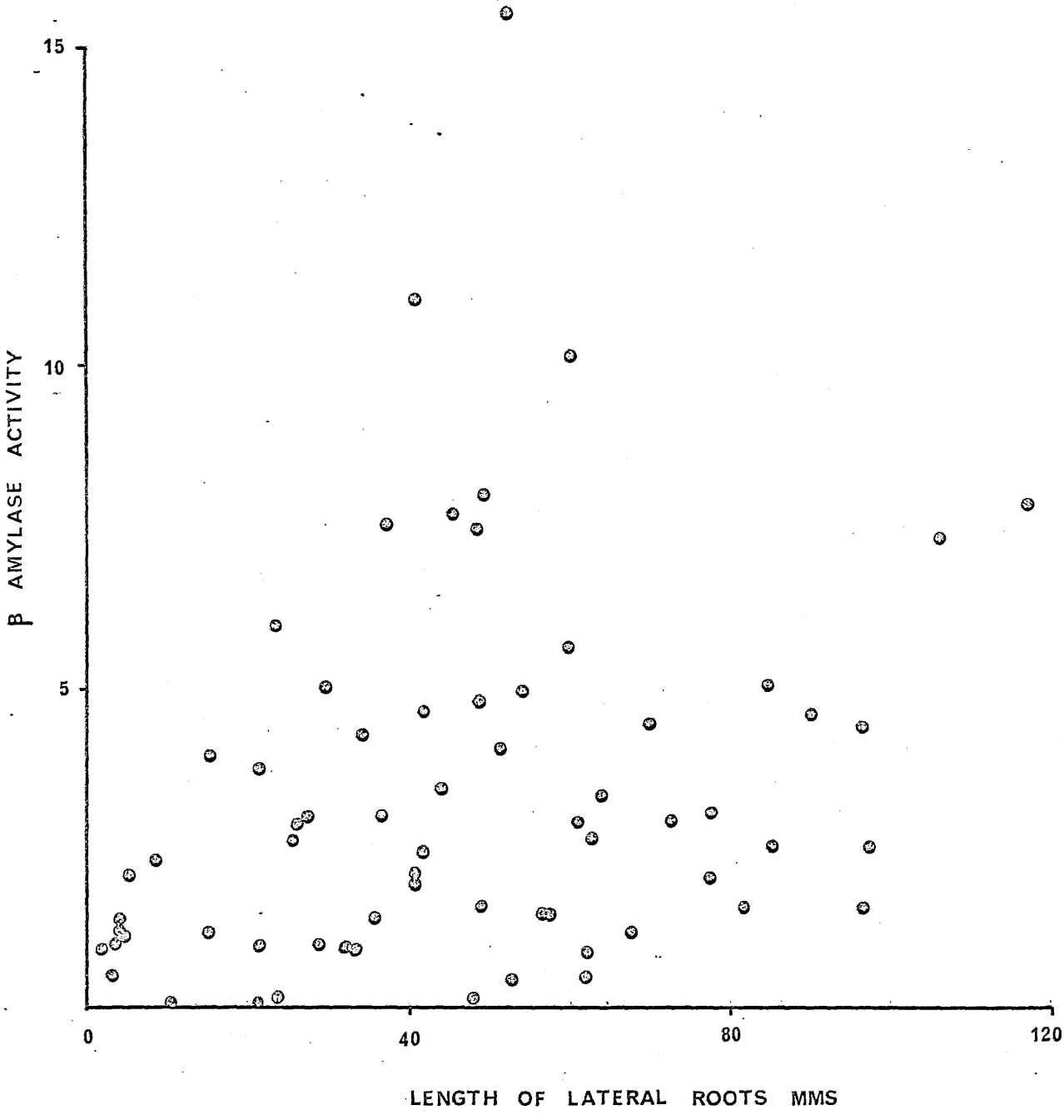

Fig. 87. Scatter plot illustrating correlation between beta-amylase activity (mgms. maltose/min.) and lateral root length in individual Hickory King grains.

Fig. 88. Scatter plot illustrating correlation between beta-amylase activity (mgms. maltose/min.) and reducing sugar concentration in individual Hickory King grains. Correlation coefficient for 0-96 hours + 0.795.

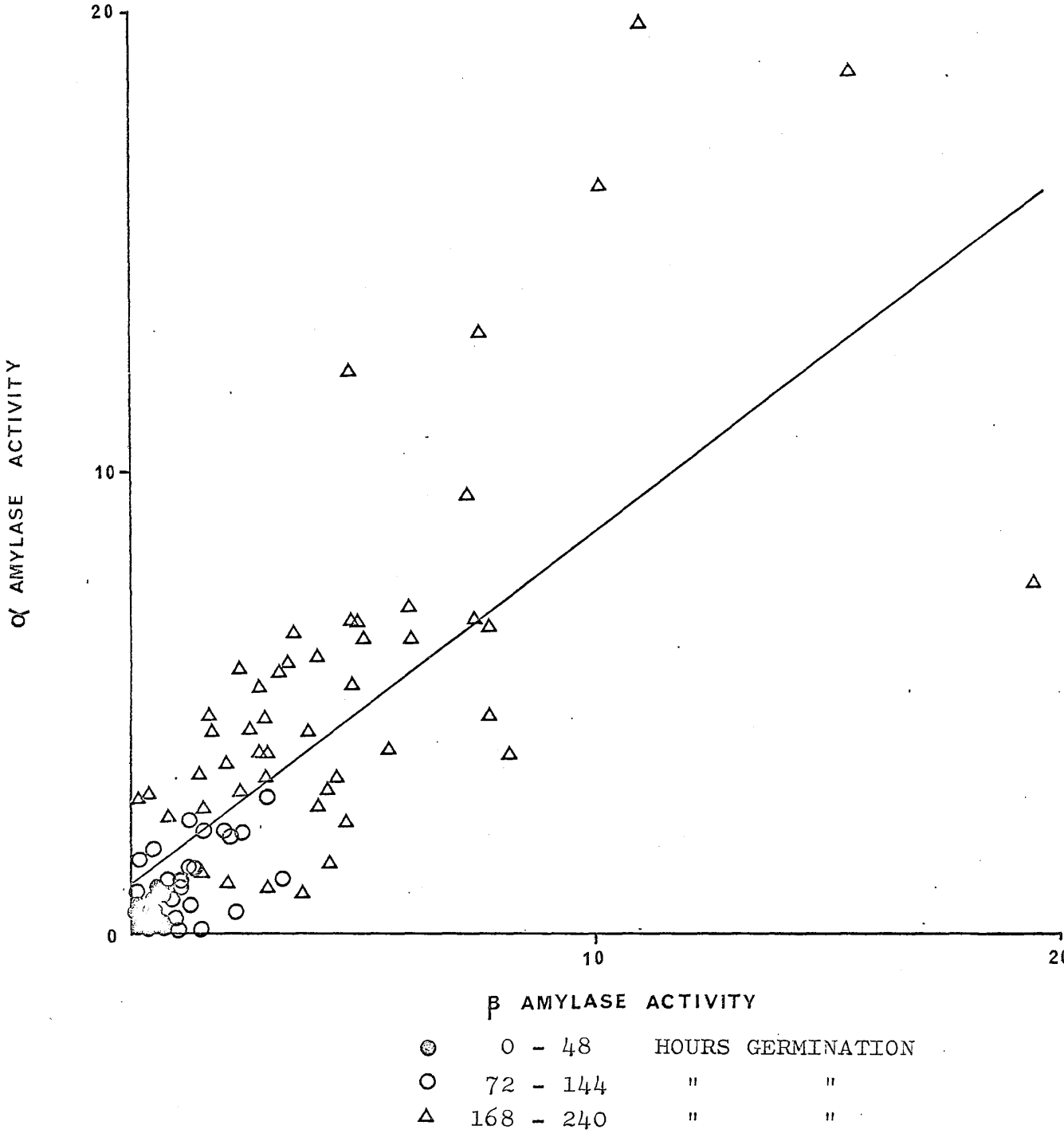

Fig. 89. Scatter plot illustrating correlation between beta-amylase and alpha-amylase activities (mgms. maltose/min. in individual Hickory King grains.

TABLE . 53

REDUCING SUGAR CONCENTRATION (MGMS.) IN GROUPS OF EARLY PEARL GRAIN DURING GERMINATION. ASSAY WAS REPLICATED THREE TIMES ON EACH GROUP.

Germination Period (Hrs)	Replication Number 1	2	3	Mean	Germination Period Mean
0	9.062	8.750	9.062	8.958	
0	11.250	12.817	12.817	12.295	10.659
0	10.937	11.250	11.250	11.146	
24	17.187	17.500	17.500	17.396	
24	16.250	16.562	16.875	16.562	17.187
24	17.187	17.500	18.125	17.604	
48	29.375	28.437	28.750	28.854	
48	28.750	27.187	27.500	27.812	27.083
48	24.687	24.687	24.375	24.583	
72	41.250	41.250	42.500	41.667	
72	33.750	31.875	31.250	32.292	33.021
72	25.312	25.000	25.000	25.104	
96	71.250	70.625	70.625	70.833	
96	68.125	67.500	68.125	67.917	68.472
96	67.500	65.500	67.500	66.667	
120	78.750	88.125	88.125	85.000	
120	81.250	82.500	83.125	82.292	81.944
120	77.500	80.625	77.500	78.542	
144	64.375	65.625	66.250	65.417	
144	54.375	52.500	54.375	53.750	54.648
144	44.337	43.750	46.250	44.779	
168	58.125	66.875	66.875	63.958	
168	40.000	38.750	49.375	42.708	55.000
168	55.625	60.000	59.375	58.333	
192	79.375	101.875	79.375	86.875	
192	76.250	75.000	72.500	74.583	72.708
192	44.375	62.500	63.125	56.667	
216	63.750	48.125	63.750	58.542	
216	42.500	42.500	45.625	43.542	49.375
216	35.625	51.250	51.250	46.042	
240	72.500	71.250	73.125	72.292	
240	66.250	84.375	76.250	75.625	68.958
240	54.375	60.625	61.875	58.958	

L. S. D. Between Observations at 0.05 P. L. ± 12.646

" " " " Means " " " " ± 7.300

" " " " Germination Period Means " " ± 1.21

TABLE 54a

ANALYSIS OF VARIANCE RESULTS FOR TOTAL REDUCING FRACTION IN GROUPS OF EARLY PEARL GRAIN.

Source	Degrees of Freedom	Sum Squares	Variance	Variance Ratio (F)	Observations
Germination	10	51299.820	5129.982	284.446	* * *
Replication	2	144.580	72.290	4.008	N. S.
Sample	2	1730.720	865.360	47.982	* *
Sample/Rep.	4	33.580	8.395	0.465	N. S.
Replication/Germ.	20	420.790	21.039	1.167	N. S.
Sample/Germ.	20	2431.180	121.559	6.740	* * *
Error	40	721.400	18.035		

* * * Significant at 0.1% Probability Level.
* * " " 5% " "

TABLE 54b

RESULTS OF LINEAR REGRESSION OF TOTAL REDUCING FRACTION AND LENGTH OF RADICLE, COLEOPTILE AND LATERAL ROOTS IN GROUPS OF EARLY PEARL GRAIN.

Factor	Germination Period(Hrs)	Correlation Coefficient	t	Significance.
Reducing Sugar Radicle	0 -240	+ 0.731	5.964	* * *
	0 -72	+ 0.744	3.521	* * *
	96 -240	- 0.051	-	-
Reducing Sugar Coleoptile	0 -240	+ 0.670	-	-
	0 -72	+ 0.682	2.949	* *
	96 -240	- 0.133	-	-
Reducing Sugar Lateral Roots	0 -240	+ 0.540	-	-
	0 -72	+ 0.746	3.542	* * *
	96 -240	- 0.119	-	-

* * * Significant at 0.1% Probability Level.
* * " " 2% " "

TABLE 55

LENGTH (MMS.) OF RADICLE, COLEOPTILE AND LATERAL ROOTS IN GROUPS OF EARLY PEARL GRAINS DURING GERMINATION.

Germination Period (hrs)	Radicle	Coleoptile	Laterals	Growth Index	Mean Radicle	Mean Coleoptile	Mean Laterals	Mean Growth Index
48	48	3	-	0.051				
48	23	3	-	0.026	44.6	2.6	-	0.05
48	63	2	-	0.065				
72	251	54	96	0.401				
72	250	56	50	0.356	259.3	57.3	67.0	0.38
72	277	62	55	0.394				
96	446	145	444	1.035				
96	539	267	680	1.486	472.0	230.6	574.0	1.28
96	431	280	598	1.309				
120	637	377	860	1.874				
120	583	315	831	1.729	270.0	347.3	822.6	1.44
120	590	350	777	1.717				
144	749	381	1522	2.652				
144	699	364	796	1.859	573.6	303.0	887.3	1.76
144	273	164	351	0.788				
168	571	343	514	1.428				
168	461	389	752	1.602	440.6	313.3	563.3	1.32
168	290	208	424	.922				
192	622	450	890	1.962				
192	539	464	685	1.688	612.0	476.6	823.0	1.91
192	675	516	894	2.085				
216	879	574	155	1.608				
216	868	721	1810	3.399	893.6	655.3	2027.0	3.58
216	934	671	2720	4.325				
240	912	705	2251	3.868				
240	845	688	2003	3.036	1[illegible]3	720.3	209.3	3.82
240	1247	768	2025	4.040				

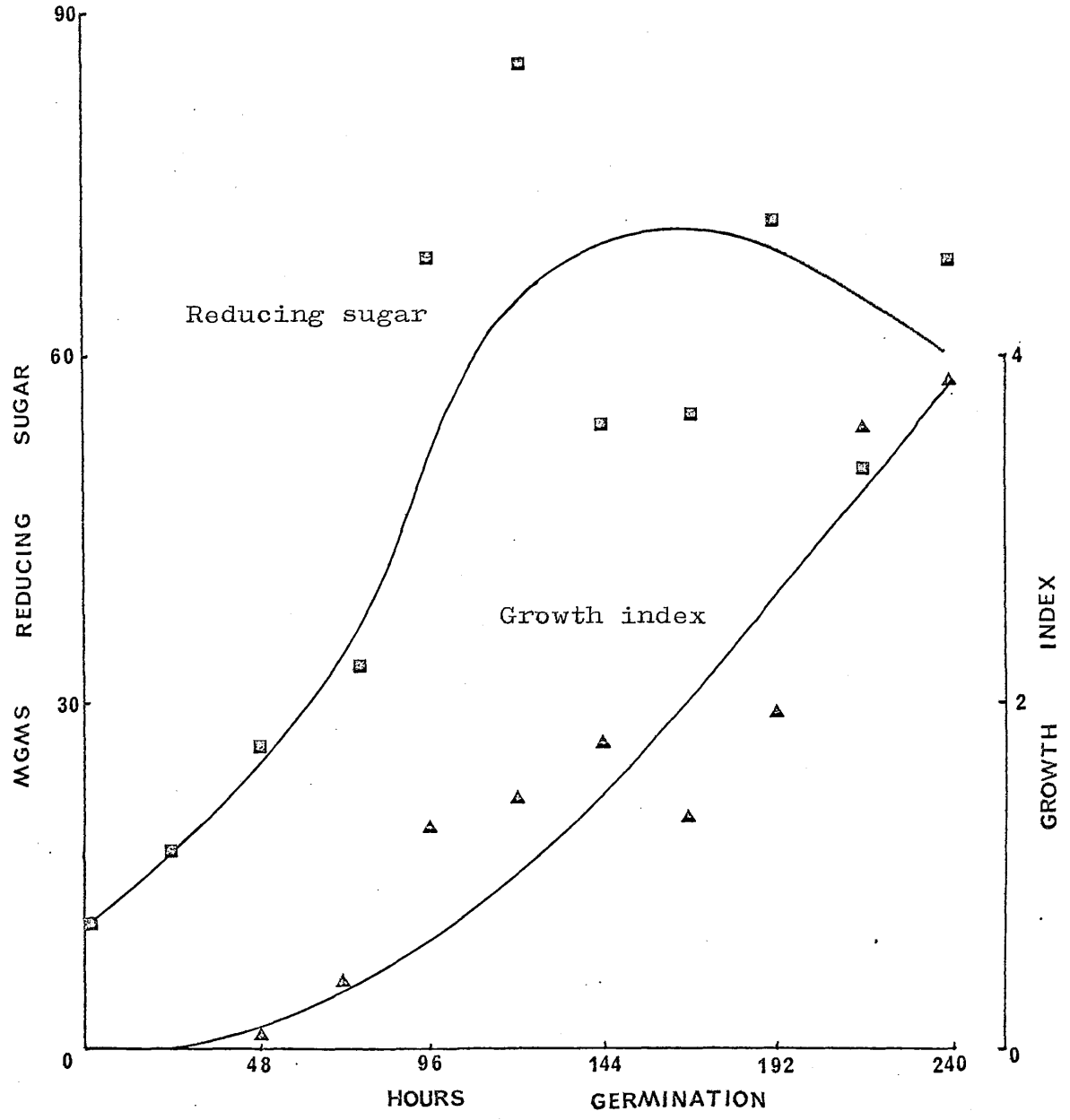

Fig. 90. Changes in total reducing fraction (expressed as mgms. maltose) in groups of Early Pearl grain during germination. Growth index expressed as $\frac{\text{sum of lengths of organs measured}}{1000}$

L.S.D. between means for reducing sugar at 0.05 P.L. 4.214 mgms.

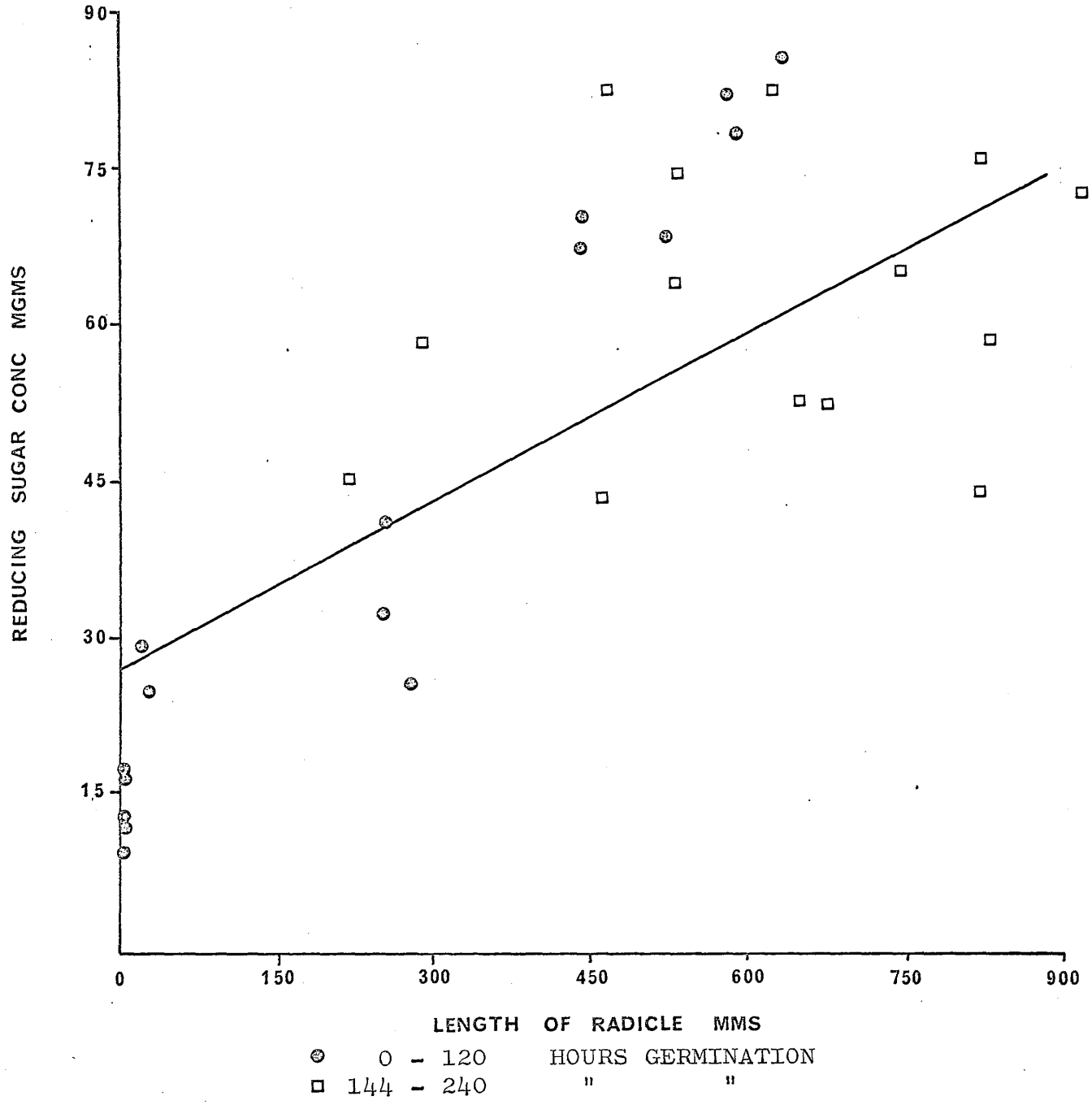

Fig. 91. Scatter plot illustrating correlation between reducing sugar concentration and radicle length in groups of Early Pearl grain. Correlation coefficient for 0-240 hours + .731.

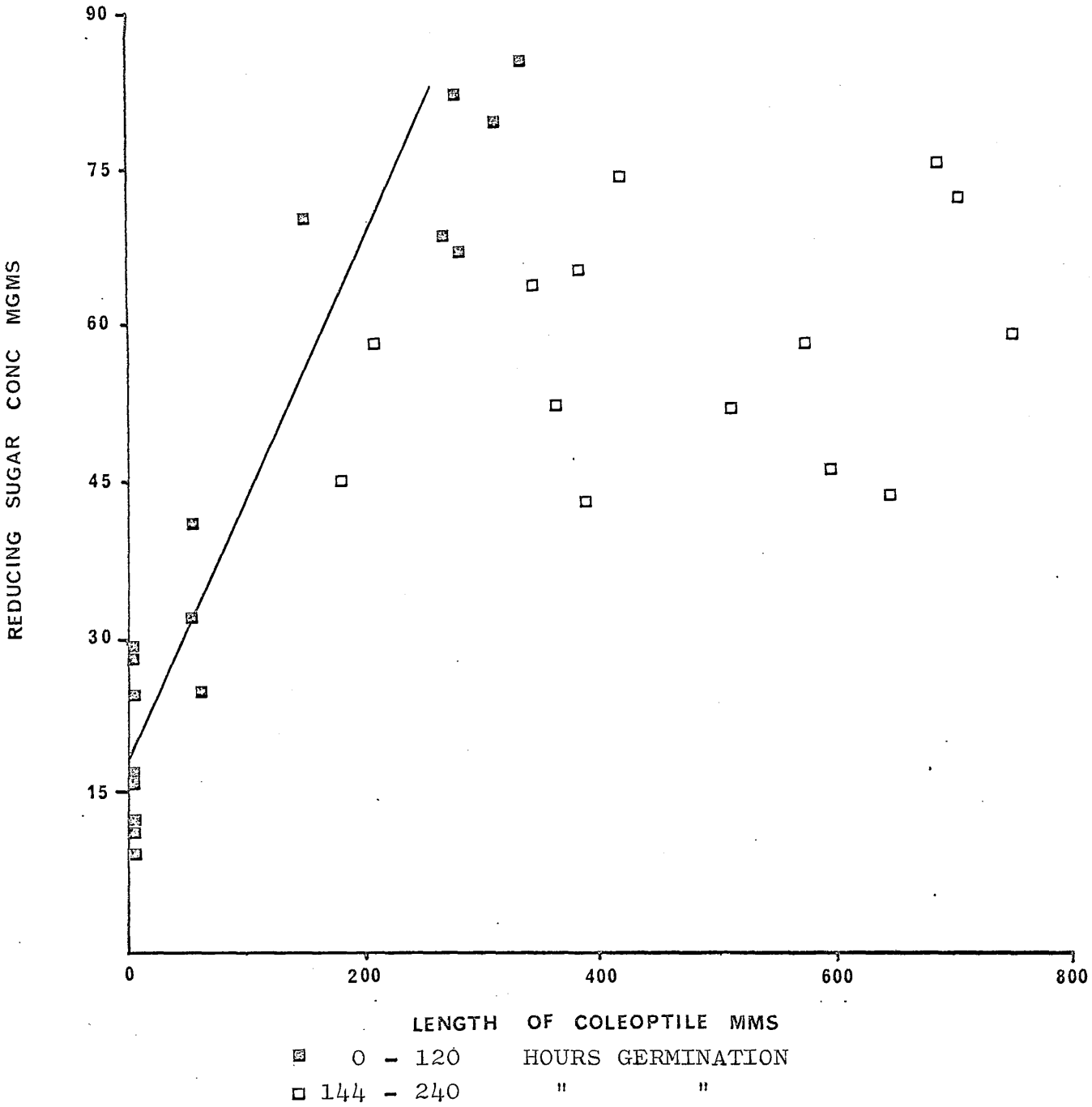

Fig. 92. Scatter plot illustrating correlation between reducing sugar concentration and coleoptile length in groups of Early Pearl grain. Correlation coefficient for 0-240 hours + 0.682.

Fig. 93. Scatter plot illustrating correlation between reducing sugar concentration and lateral root length in groups of Early Pearl grain.

TABLE 56

REDUCING SUGAR CONCENTRATION (MGMS.) IN INDIVIDUAL EARLY PEARL GRAINS DURING GERMINATION.

Germination Period (Hrs)	Grain Number 1	2	3	4	5	6	7	8	9	10	Mean	% Germination	Growth Index
0	18.750	40.625	22.500	18.125	18.750	13.125	17.500	15.000	12.500	17.500	19.437	-	-
24	14.735	19.375	17.500	13.125	13.125	17.500	26.875	17.500	19.375	15.000	15.925	-	-
48	46.875	41.875	60.625	35.000	36.250	55.000	45.000	42.500	31.250	38.750	43.312	90	0.091
72	90.625	71.875	104.687	63.473	69.062	57.000	62.500	63.750	68.437	57.187	70.906	100	0.375
96	91.250	92.500	95.000	115.000	70.000	72.500	86.250	70.000	86.250	35.000	81.375	100	0.992
120	91.250	88.750	105.000	123.750	78.750	72.500	93.750	58.750	60.000	93.750	86.625	100	1.366
144	86.250	105.000	115.000	117.500	[illegible]	[illegible]	[illegible]	137.500	72.500	95.000	96.875	100	1.273
168	92.500	108.750	115.000	97.500	86.250	100.000	118.750	86.250	70.000	50.000	92.500	100	1.674
192	93.750	83.750	63.750	103.750	58.750	61.250	68.750	106.250	80.000	65.000	78.500	100	1.873
216	58.750	78.750	68.750	51.250	67.500	90.000	77.500	83.750	41.250	60.000	67.750	100	2.465
240	78.750	120.000	98.750	118.750	100.000	108.750	110.000	50.000	66.000	55.000	90.600	100	2.377

Least Significant Difference Between Observations at 0.05 Probability Level ± 43.561

" " " " Means " " " " ± 13.775

TABLE 57a

ANALYSIS OF VARIANCE RESULTS FOR TOTAL REDUCING FRACTION IN INDIVIDUAL EARLY PEARL GRAINS.

Source	Degrees of Freedom	Sum Squares	Variance	Variance Ratio(F)	Observations
Germination	10	89253.530	8925.353	37.249	* * *
Sample	9	8202.240	911.360	3.803	* *
Error	90	21565.190	239.613		

* * * Significant at 0.1% Probability Level.
* * " " 5% " "

TABLE 57b

RESULTS OF LINEAR REGRESSION OF TOTAL REDUCING FRACTION AND LENGTH OF RADICLE COLEOPTILE AND LATERAL ROOTS IN INDIVIDUAL EARLY PEARL GRAINS.

Factor	Germination Period (Hrs)	Correlation Coefficient	t	Significance
Reducing Sugar	0 -240	+ 0.659	9.105	* * *
	0 -96	+ 0.825	10.114	* * *
Radicle	120 -240	+ 0.058	-	-
Reducing Sugar	0 -240	+ 0.518	-	-
	0 -96	+ 0.781	8.664	* * *
Coleoptile	120 -240	- 0.017	-	-
Reducing Sugar	0 -240	+ 0.468	-	-
	0 -96	+ 0.607	-	-
Lateral Roots	120 -240	- 0.046	-	-

* * * Significant at 0.1% Probability Level.

TABLE 58

LENGTH (MMS.) OF RADICLE, COLEOPTILE AND LATERAL ROOTS OF INDIVIDUAL EARLY PEARL GRAINS DURING GERMINATION.

Grain Number	GERMINATION PERIOD IN HOURS																											
	48			72			96			120			144			168			192			216			240		
	Radicle	Coleoptile	Laterals	Radicle	Coleoptile	Laterals	Radicle	Coleoptile	Laterals	Radicle	Coleoptile	Laterals	Radicle	Coleoptile	Laterals	Radicle	Coleoptile	Laterals	Radicle	Coleoptile	Laterals	Radicle	Coleoptile	Laterals	Radicle	Coleoptile	Laterals
1	4	2	-	37	20	10	49	15	52	37	22	103	50	15	61	50	31	92	55	97	88	34	59	99	37	69		117
2	8	3	-	6	6	-	66	9	28	91	44	54	47	14	98	73	61	99	-	7	58	64	27	86	37	70		170
3	3	-	-	53	23	36	56	20	13	38	17	117	42	24	112	54	60	62	65	4	79	48	50	100	34	39		168
4	13	3	-	22	6	10	63	25	55	31	32	75	46	19	74	24	21	39	107	63	165	29	29	50	51	64		147
5	4	2	-	7	4	-	31	15	70	89	28	4	34	25	93	43	38	76	57	57	57	27	47	110	28	59		172
6	17	7	2	9	3	-	28	11	46	18	11	50	37	18	45	36	9	107	44	36	61	31	28	209	74	67		223
7	12	3	-	13	9	26	18	8	35	29	12	51	27	15	83	81	24	96	61	53	52	48	64	151	40	49		60
8	-	-	-	29	5	-	72	28	84	61	7	27	67	43	-	39	29	66	59	40	43	87	98	181	37	47		67
9	2	3	-	6	3	-	33	4	5	26	18	48	28	8	44	43	51	187	66	53	171	56	58	360	64	69		244
10	1	2	-	26	6	-	22	15	16	59	49	118	57	19	28	-	25	58	57	57	61	62	65	108	89	5		-
Mean	6.4	2.5	0.2	20.8	8.5	8.2	43.8	15.0	40.4	47.9	24.0	64.7	43.5	20.0	63.8	44.3	34.9	88.2	46.7	57.1	48.6	83.5	145.4	52.5	53.8	49.1		134.8
Growth Index		0.091			0.375			0.992			1.366			1.273			1.674			1.873			2.465			2.377	

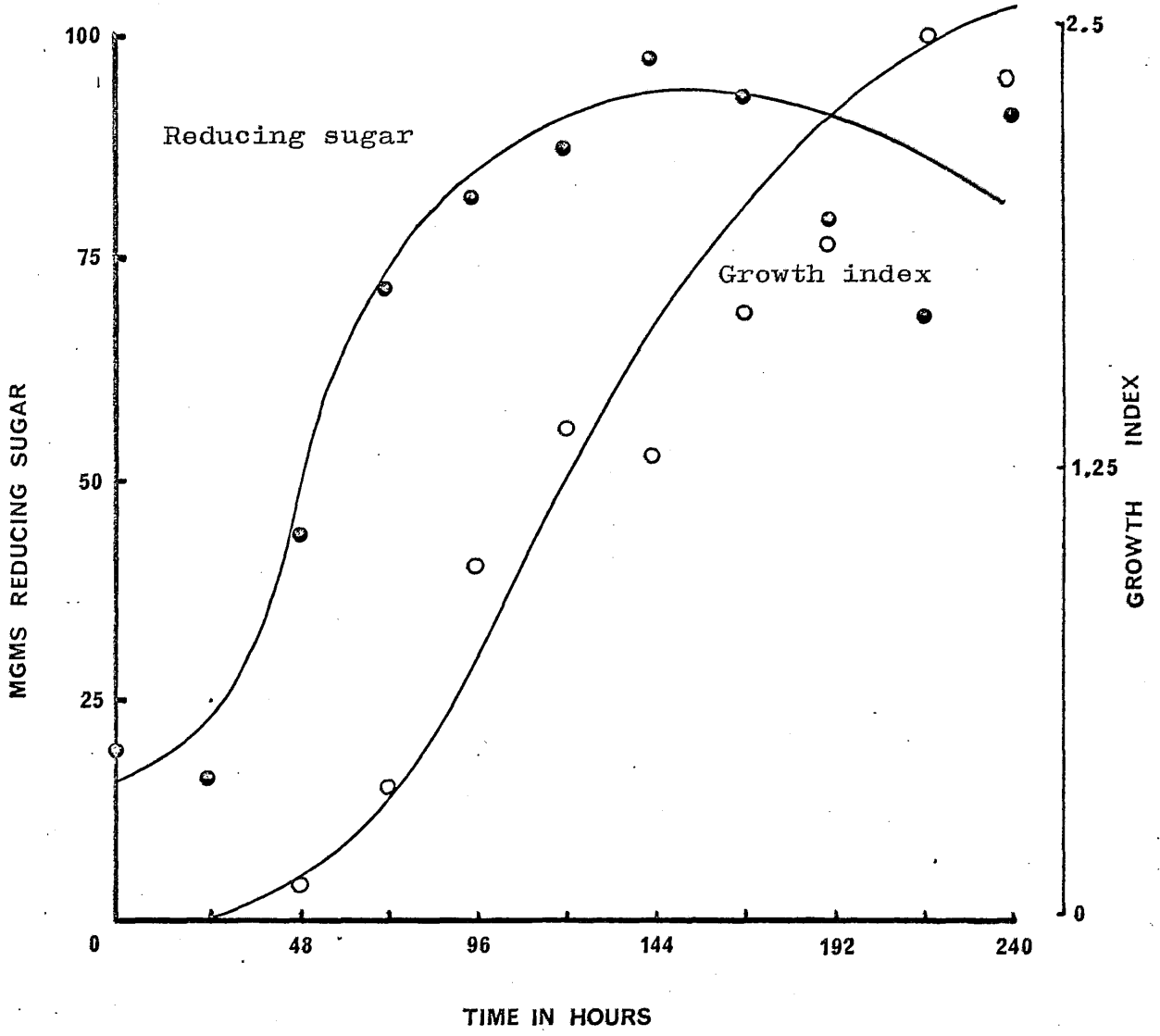

Fig. 94. Changes in total reducing fraction (expressed as mgms. maltose) in individual Early Pearl grains during germination. Growth index expressed as $\frac{\text{sum of lengths of organs measured}}{1000}$

L.S.D. between means for reducing sugar at 0.05 P.L. ± 13.775.

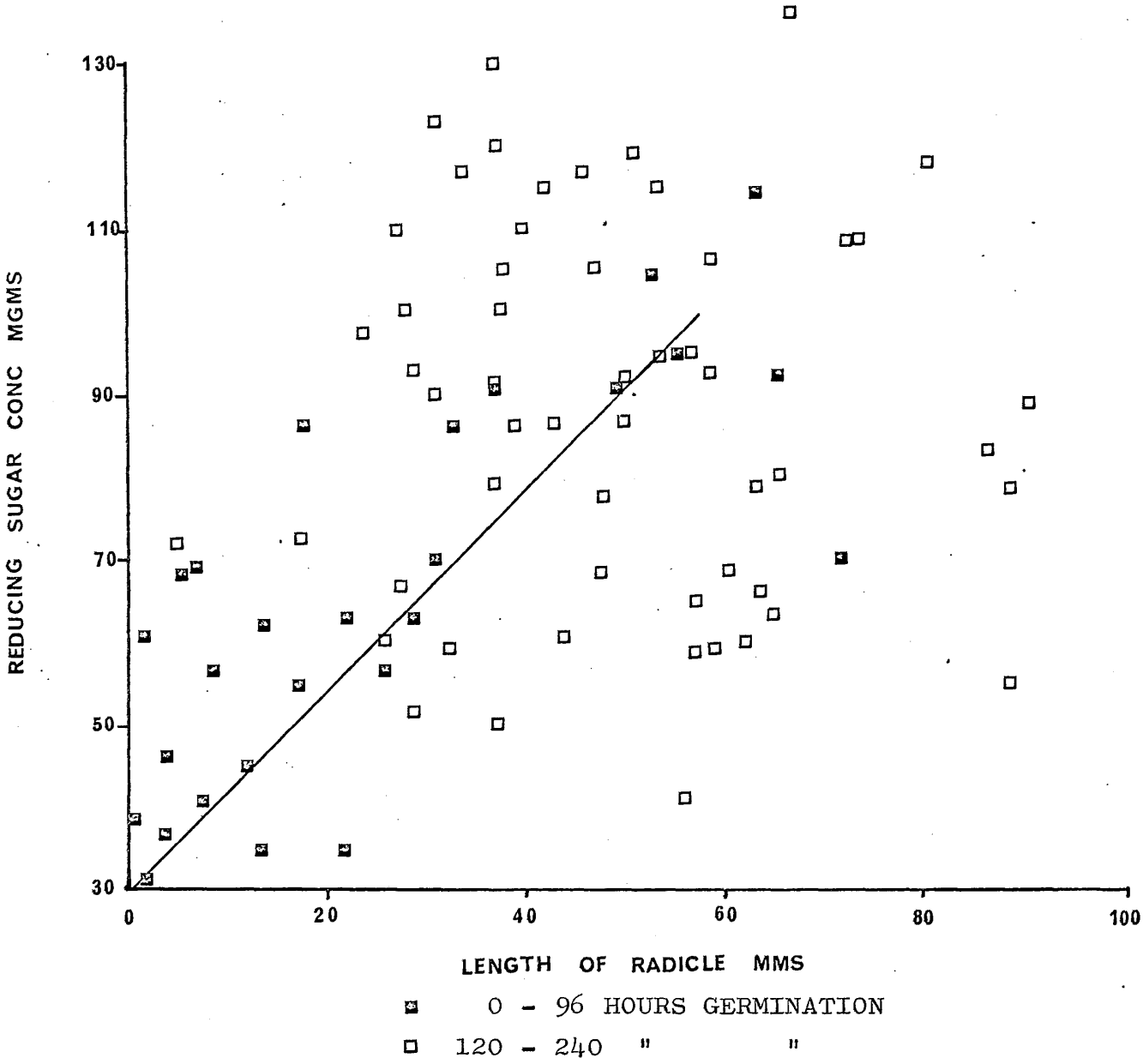

Fig. 95. Scatter plot illustrating correlation between reducing sugar concentration and radicle length in individual Early Pearl grains. Correlation coefficient for 0-96 hours + 0.825.

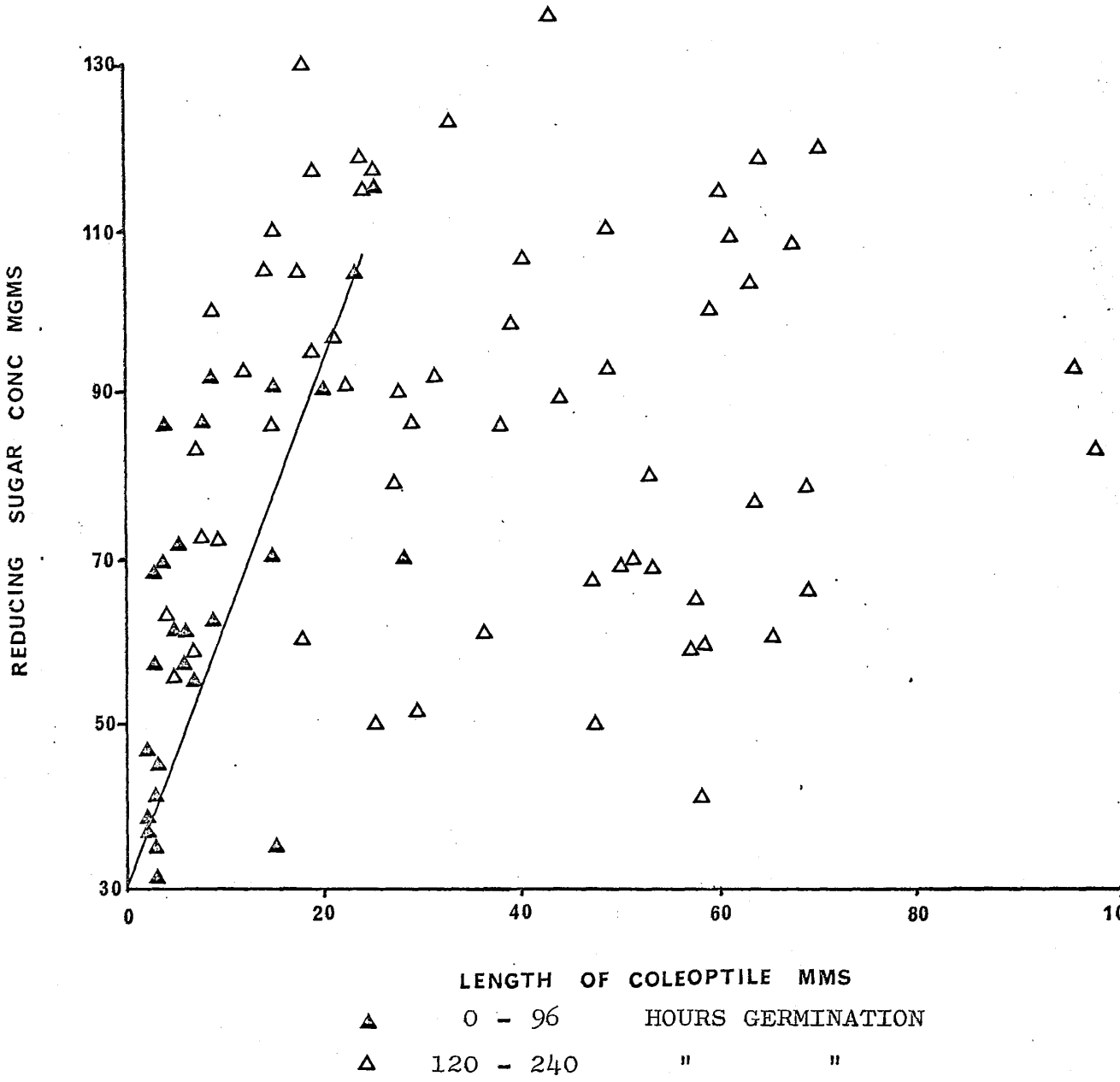

Fig. 96. Scatter plot illustrating correlation between reducing sugar concentration and coleoptile length in individual Early Pearl grains. Correlation coefficient for 0-96 hours + 0.781.

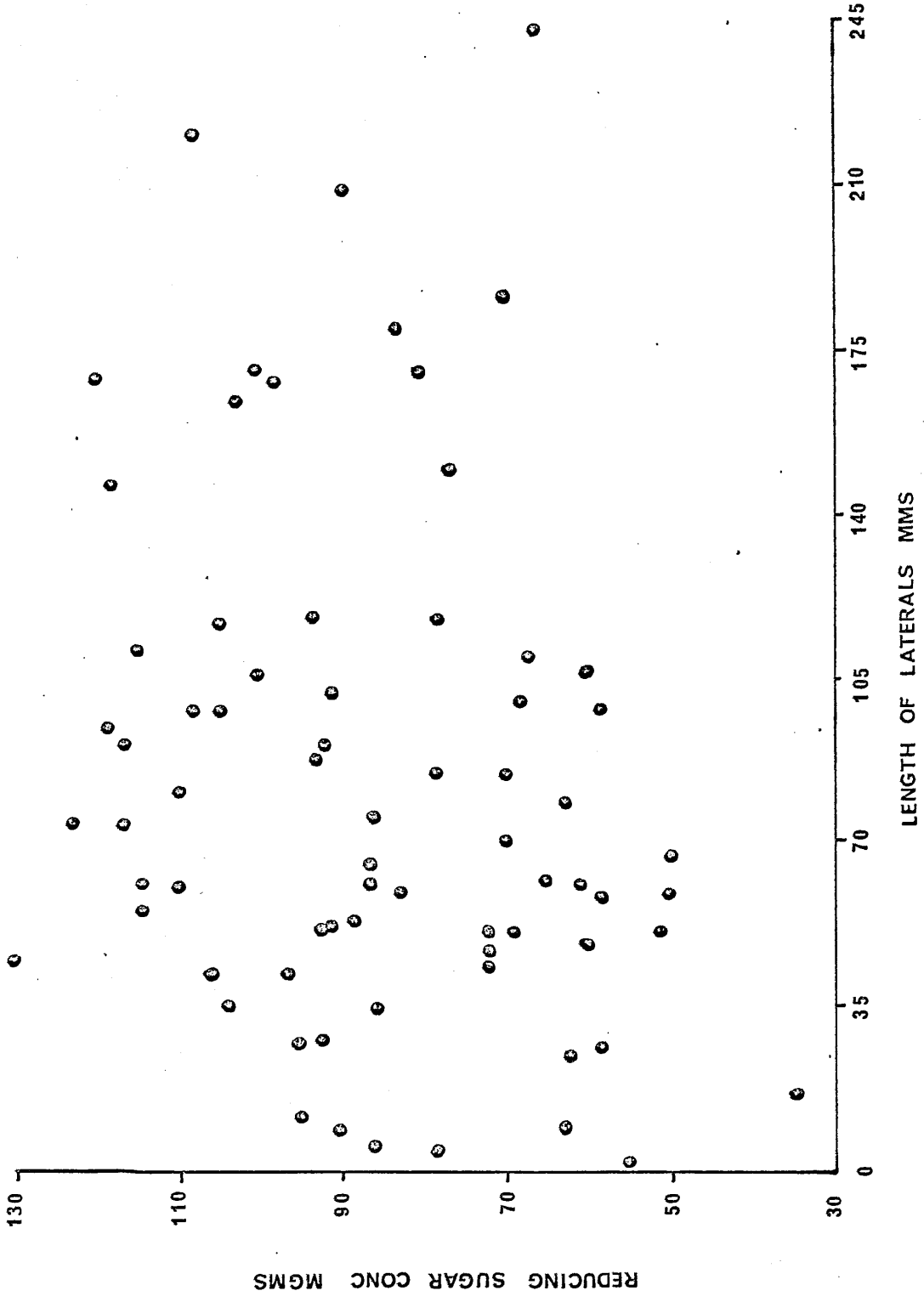

Fig. 97. Scatter plot illustrating correlation between reducing sugar concentration and lateral root length in individual Early Pearl grains.

TABLE 59

TOTAL AMYLOLYTIC ACTIVITY (MGMS. MALTOSE/ MIN./ GRAIN) IN GROUPS OF EARLY PEARL GRAIN DURING GERMINATION. ASSAY WAS REPLICATED THREE TIMES ON EACH GROUP.

Germination Period (Hrs)	Replication Number 1	2	3	Mean	Germination Period Mean
0	-	-	-	-	
0	0.040	0.020	0.025	0.028	0.013
0	0.010	0.015	0.010	0.012	
24	0.262	0.250	0.262	0.258	
24	0.287	0.262	0.275	0.275	0.267
24	0.312	0.300	0.275	0.296	
48	0.291	0.333	0.325	0.316	
48	0.533	0.542	0.558	0.544	0.432
48	0.425	0.416	0.467	0.436	
72	0.575	0.545	0.550	0.557	
72	0.220	0.183	0.191	0.198	0.465
72	0.642	0.642	0.642	0.642	
96	5.916	6.000	6.000	5.972	
96	6.750	6.708	6.666	6.708	5.972
96	3.875	4.833	5.500	5.236	
120	9.937	9.062	9.062	9.354	
120	9.000	8.562	11.437	9.666	8.868
120	7.687	7.312	7.750	7.583	
144	5.446	5.357	5.357	5.387	
144	3.839	4.107	3.571	3.839	4.156
144	3.393	3.214	3.125	3.274	
168	5.893	4.553	4.732	5.059	
168	3.661	3.928	2.143	3.244	4.422
168	5.268	4.553	5.089	4.970	
192	12.411	8.839	12.411	11.220	
192	9.286	9.315	9.553	9.385	8.720
192	7.411	4.553	4.643	5.536	
216	11.250	12.437	10.875	11.521	
216	8.687	8.750	8.562	8.666	9.402
216	8.937	7.625	7.500	8.021	
240	6.312	6.437	6.312	6.354	
240	7.750	6.125	6.812	6.896	5.728
240	4.375	3.750	3.687	3.937	

L. S. D. Between Observations at 0.05 P. L. ± 1.812

" " " " Means " " " " ± 1.046

" " " " Germination Period Means " ± 0.604

TABLE '60a

ANALYSIS OF VARIANCE RESULTS FOR TOTAL AMYLOLYTIC ACTIVITY (ALPHA- AND BETA-AMYLASE) IN GROUPS OF EARLY PEARL GRAIN.

Source	Degrees of Freedom	Sum Squares	Variance	Variance Ratio(F)	Observations
Germination	10	1218.2537	121.8254	337.981	* * *
Replication	2	2.4704	1.2352	3.472	N. S.
Sample	2	35.6886	17.8443	49.506	* *
Sample/Rep.	4	0.7665	0.1916	0.532	N. S.
Replication/Germ.	20	9.4807	0.4740	1.315	N. S.
Sample/Germ.	20	75.3703	3.7685	10.455	* * *
Error	40	14.418	0.3604		

* * * Significant at 0.1% Probability Level.
* * " " 5% " "

TABLE 60b

RESULTS OF LINEAR REGRESSION OF TOTAL AMYLOLYTIC ACTIVITY (ALPHA- AND BETA-AMYLASE) AND TOTAL REDUCING FRACTION, RADICLE, COLEOPTILE AND LATERAL ROOT LENGTH IN GROUPS OF EARLY PEARL GRAIN.

Factor	Germination Period(Hrs)	Correlation Coefficient	t	Significance
Amylase	0 -240	+ 0.860	9.583	* * *
	0 -72	+ 0.728	3.358	* *
Reducing Sugar	96 -240	+ 0.522	-	-
Amylase	0 -240	+ 0.758	6.470	* * *
	0 -72	+ 0.562	-	-
Radicle	96 -240	+ 0.202	-	-
Amylase	0 -240	+ 0.764	6.593	* * *
	0 -72	+ 0.505	-	-
Coleoptile	96 -240	+ 0.253	-	-
Amylase	0 -240	+ 0.568	3.835	* * *
	0 -72	+ 0.507	-	-
Lateral Roots	96 -240	+ 0.004	-	-

* * * Significant at 0.1% Probability Level.
* * " " 2% " "

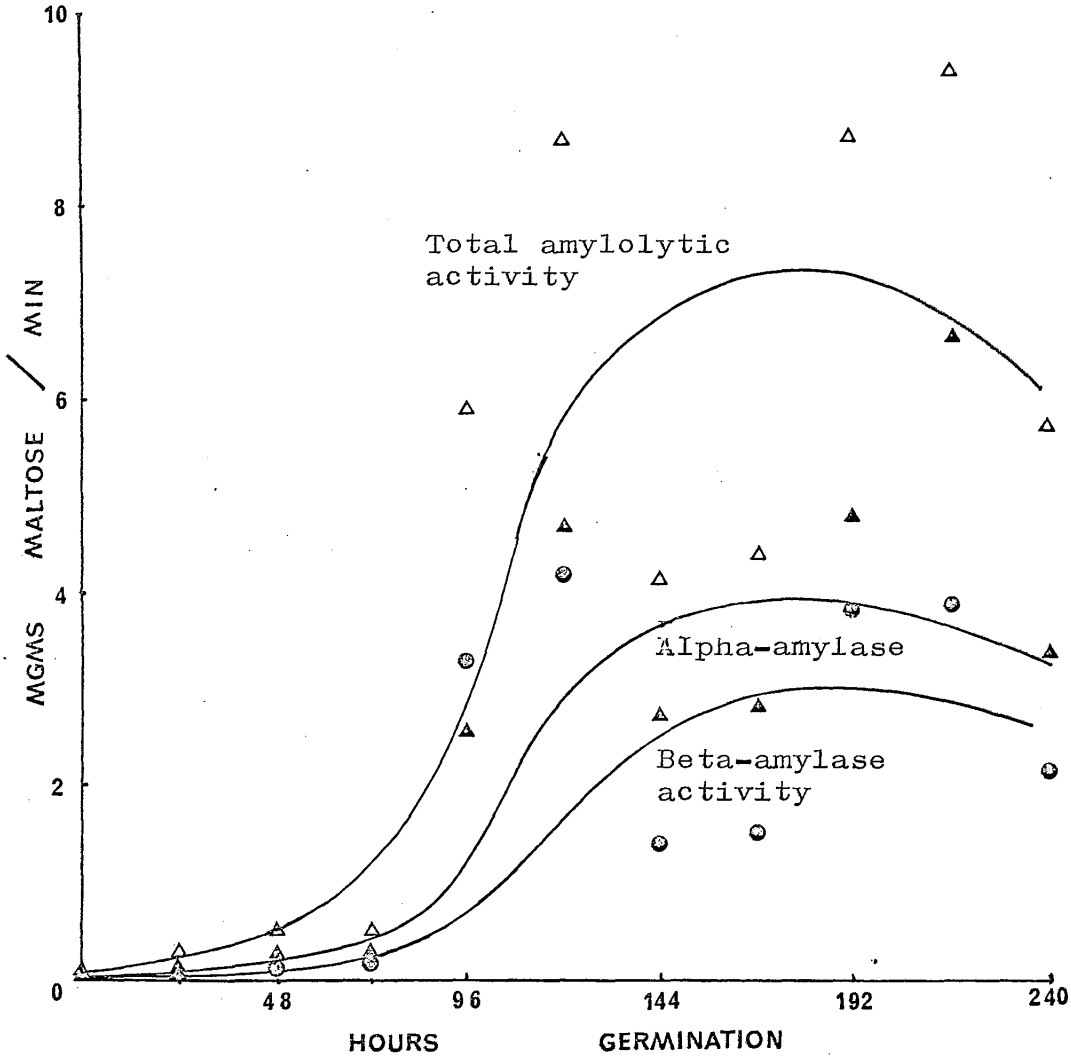

Fig. 98. Changes in amylolytic activity (mgms. maltose/min./grain) in groups of Early Pearl grain during germination.

L.S.D. Between means for total amylolytic activity at 0.05 P.L. ± 0.604 mgms.
" " " " " " alpha-amylolytic activity at 0.05 P.L. ± 0.762 mgms.
" " " " " " beta-amylolytic activity at 0.05 P.L. ± 0.380 mgms.

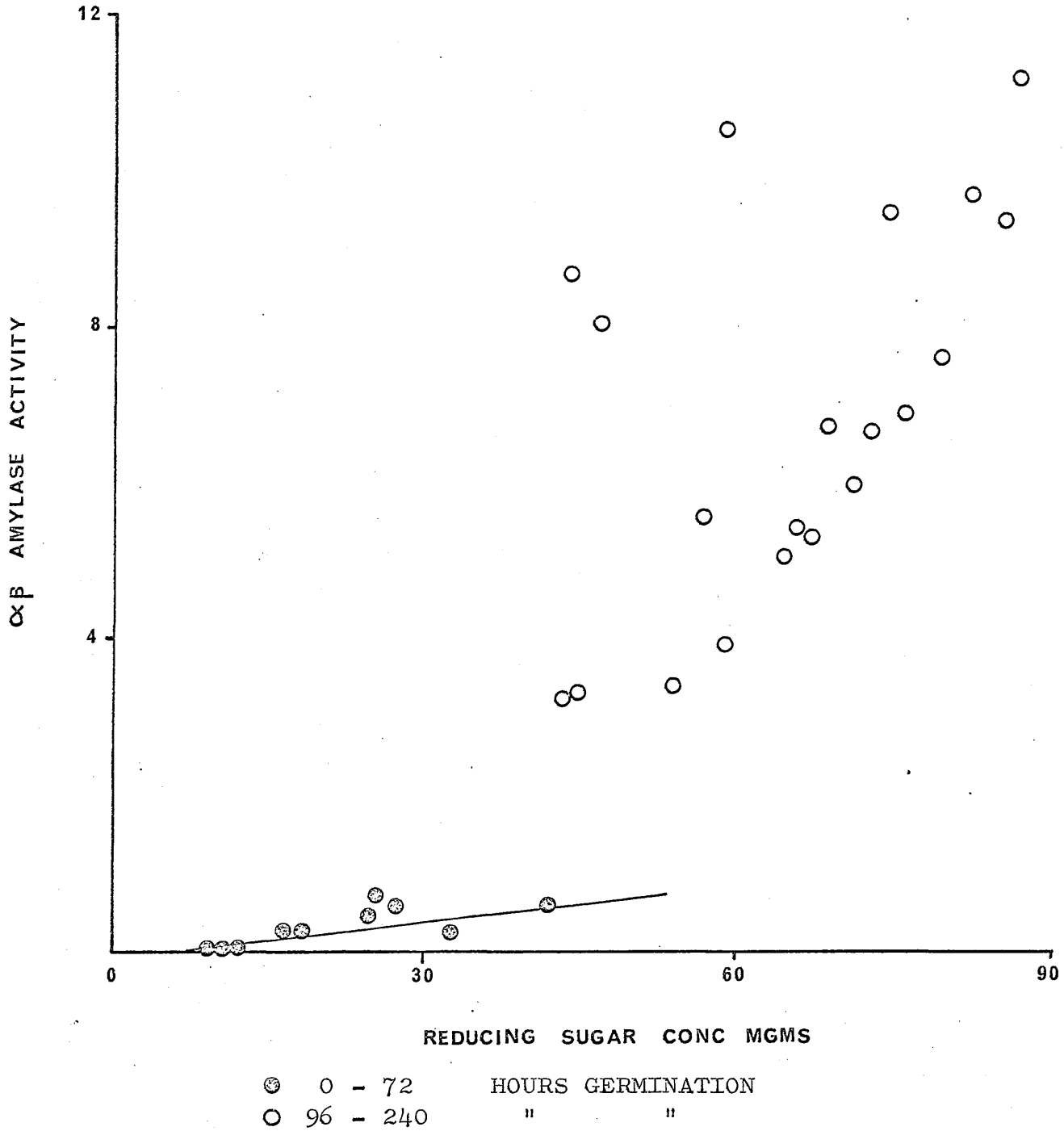

Fig. 99. Scatter plot illustrating correlation between total amylolytic activity (alpha- and beta-amylase) and reducing sugar concentration in groups of Early Pearl grain. Correlation coefficient for 0-72 hours + 0.728.

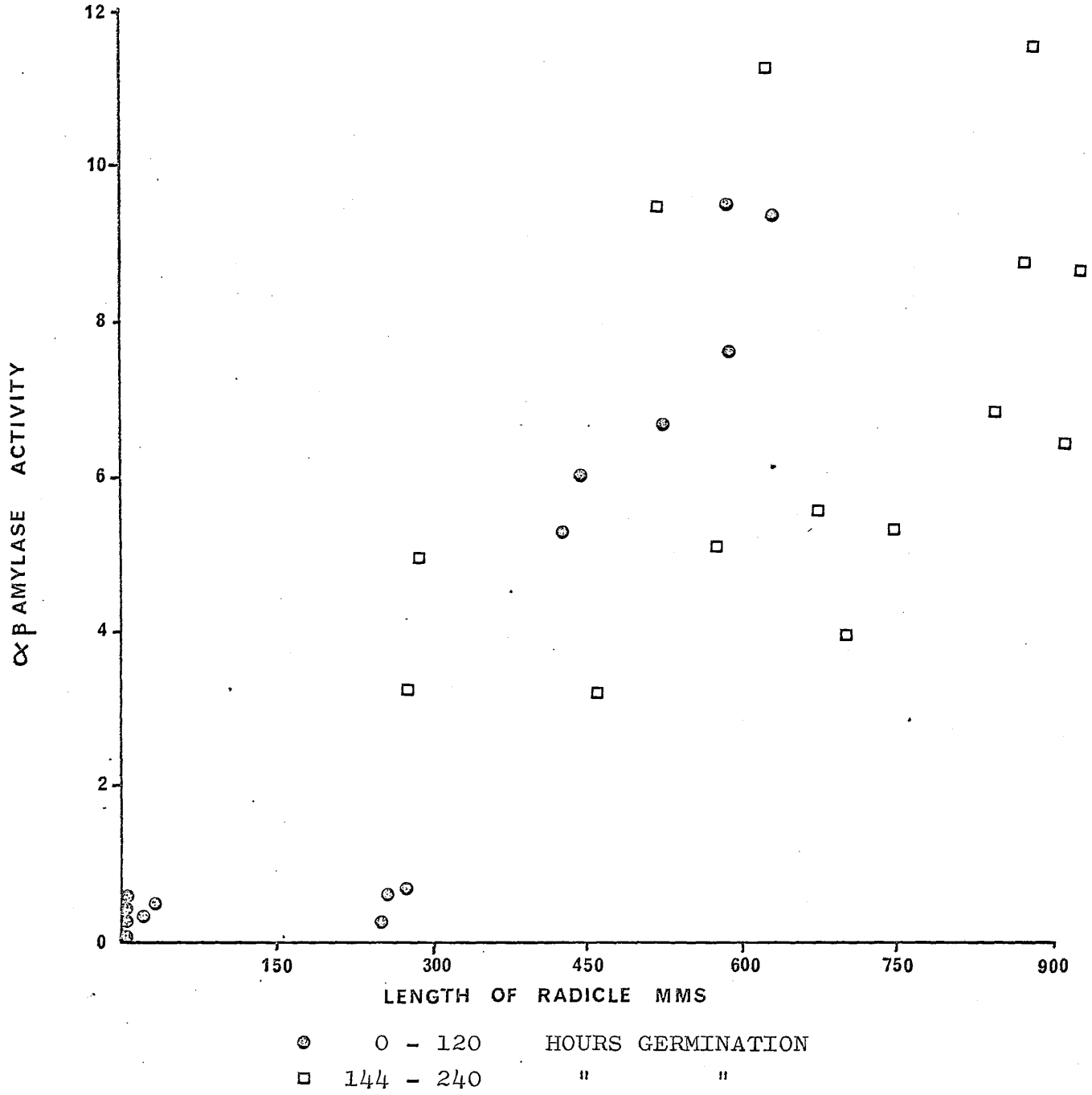

Fig. 100. Scatter plot illustrating correlation between total amylolytic activity (alpha- and beta-amylase) and radicle length in groups of Early Pearl grain.

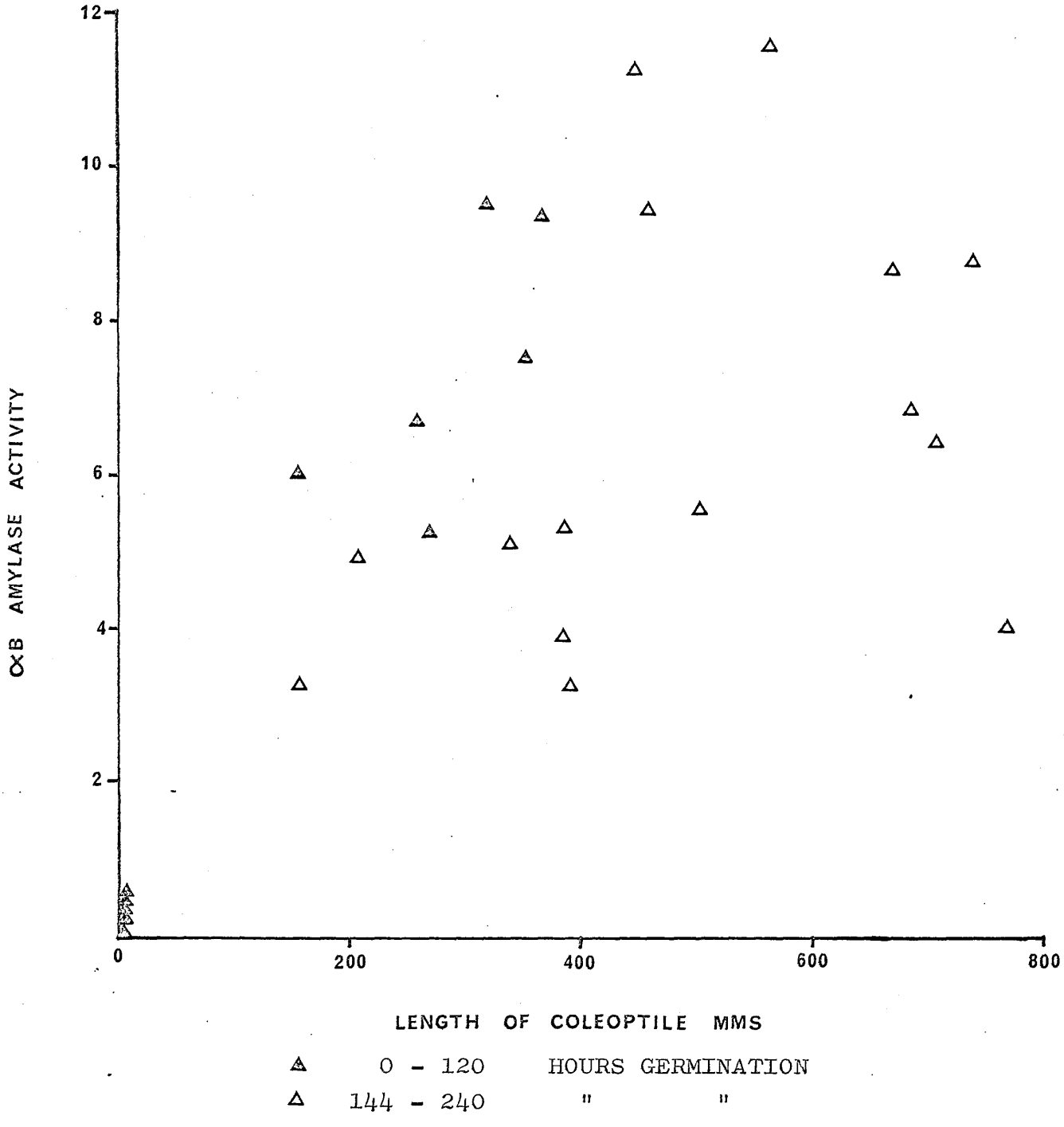

Fig. 101. Scatter plot illustrating correlation between total amylolytic activity (alpha- and beta-amylase) and coleptile length in groups of Early Pearl grain.

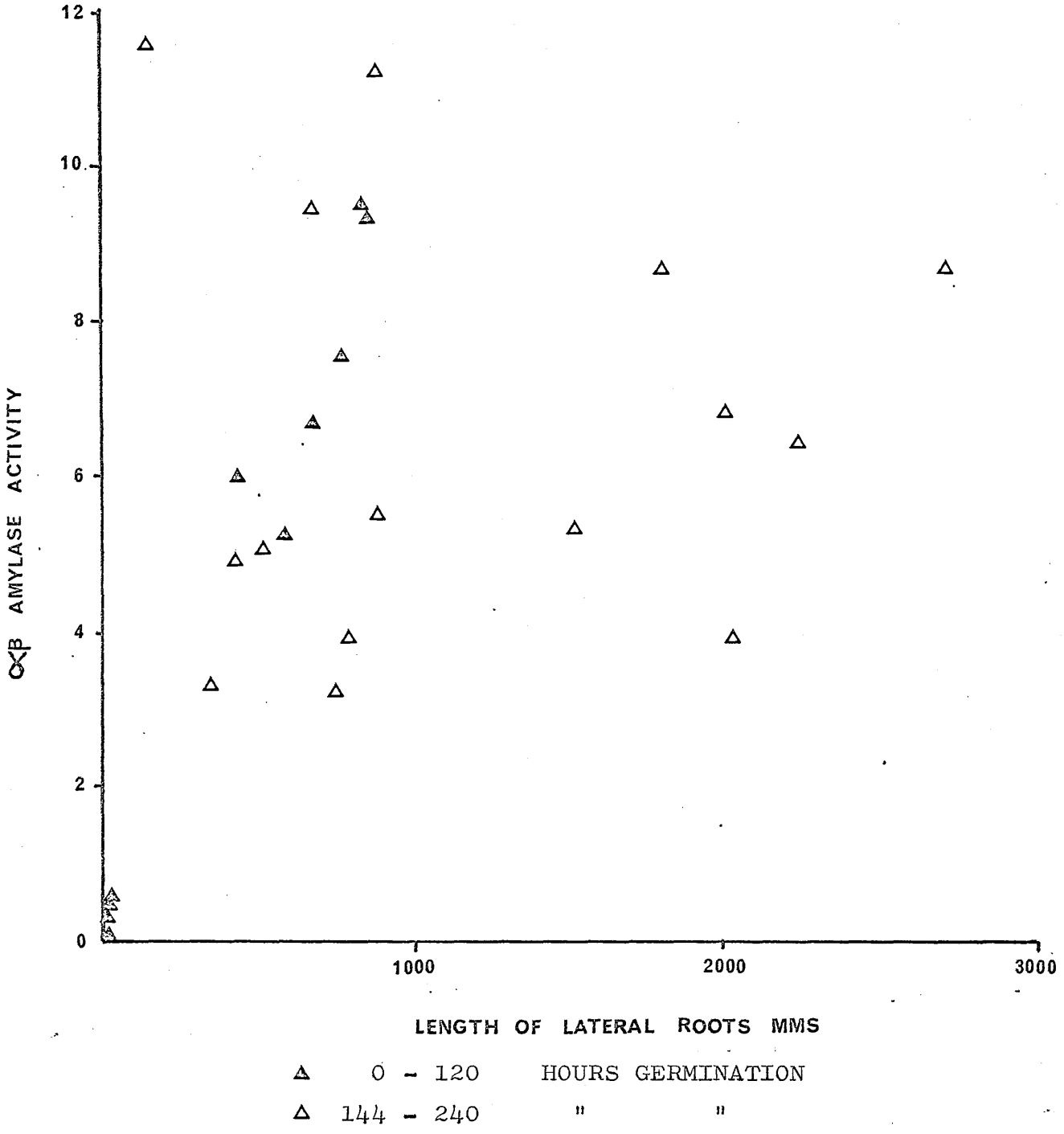

Fig. 102. Scatter plot illustrating correlation between total amylolytic activity (alpha- and beta-amylase) and lateral root length in groups of Early Pearl grain.

TABLE 61

TOTAL AMYLOLYTIC ACTIVITY (MGMS MALTOSE / MIN.) IN INDIVIDUAL EARLY PEARL GRAINS DURING GERMINATION.

Germination Period (Hrs)	Grain Number 1	2	3	4	5	6	7	8	9	10	Mean	% Germination	Growth Index
0	-	1.250	-	-	-	1.250	-	-	1.875	-	0.437	-	-
24	0.250	0.562	0.062	0.219	0.187	0.312	0.281	0.625	0.187	0.125	0.281	-	-
48	0.958	1.125	1.542	0.708	0.917	1.708	0.833	1.083	0.583	1.458	1.091	90	0.091
72	2.430	2.630	6.693	1.693	4.114	1.250	1.562	1.094	2.005	1.276	2.475	100	0.375
96	7.083	5.833	2.833	4.167	3.500	4.000	7.083	5.000	6.083	1.167	4.675	100	0.992
120	7.625	5.125	14.250	12.125	6.625	5.000	6.625	3.625	3.250	9.750	7.400	100	1.366
144	12.679	6.429	10.000	10.357	11.071	12.321	9.107	8.214	2.321	4.107	8.655	100	1.273
168	17.083	8.542	5.833	8.542	6.667	2.500	13.333	5.625	1.875	0.625	7.062	100	1.674
192	3.875	5.250	8.125	12.125	3.250	2.000	1.125	5.750	1.875	3.250	4.662	100	1.873
216	3.281	4.687	9.844	3.125	4.219	7.812	7.500	3.750	2.500	6.406	5.312	100	2.465
240	3.750	17.000	12.250	7.375	11.625	5.125	11.500	1.500	5.875	5.000	8.100	100	2.377

Least Significant Difference Between Observations at 0.05 Probability Level ± 7.988

" " " " Means " " " " ± 2.527

TABLE 62a

ANALYSIS OF VARIANCE RESULTS FOR TOTAL AMYLOLYTIC ACTIVITY (ALPHA- AND BETA-AMYLASE) IN INDIVIDUAL EARLY PEARL GRAINS.

Source	Degrees of Freedom	Sum Squares	Variance	Variance Ratio(F)	Observations
Germination	10	959.483	95.948	11.878	* * *
Sample	9	162.522	18.058	2.236	* *
Error	90	726.985	8.078		

* * * Significant at 0.1% Probability Level.
* * " " 5% " "

TABLE 62b

RESULTS OF LINEAR REGRESSION OF TOTAL AMYLOLYTIC ACTIVITY (ALPHA-, BETA-AMYLASE) AND TOTAL REDUCING FRACTION, RADICLE, COLEOPTILE AND LATERAL ROOT LENGTH IN INDIVIUAL EARLY PEARL GRAINS.

Factor	Germination Period(Hrs)	Correlation Coefficient	t	Significance
Amylase	0 -240	+ 0.788	13.301	* * *
	0 -96	+ 0.838	10.640	* * *
Reducing Sugar	120 -240	+ 0.658	-	-
Amylase	0 -240	+ 0.534	-	-
	0 -96	+ 0.764	8.204	* * *
Radicle	120 -240	+ 0.034	-	-
Amylase	0 -240	+ 0.426	-	-
	0 -96	+ 0.681	-	-
Coleoptile	120 -240	- 0.094	-	-
Amylase	0 -240	+ 0.492	-	-
	0 -96	+ 0.704	-	-
Lateral	120 -240	+ 0.070	-	-

* * * Significant at 0.1% Probability Level.

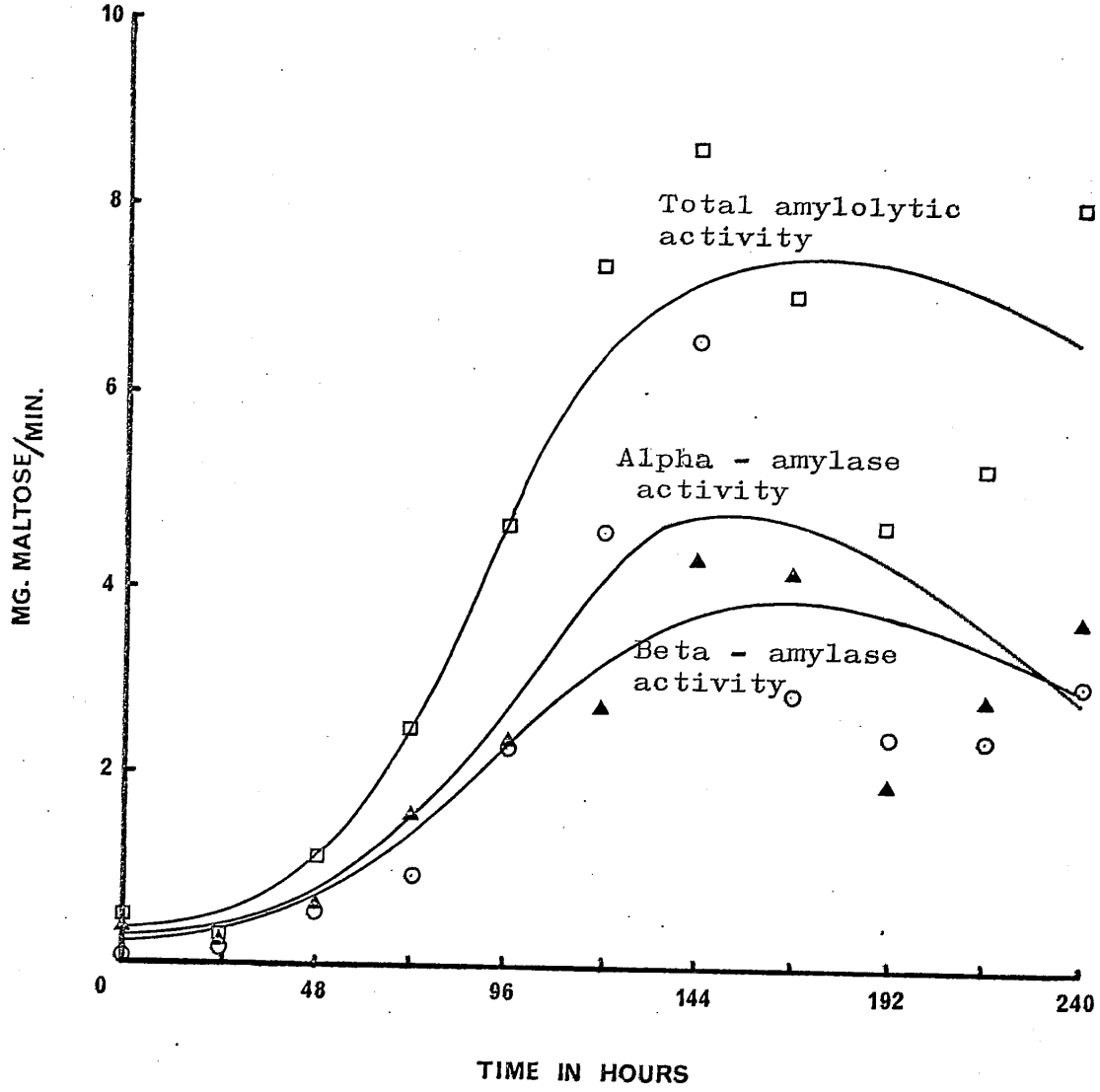

Fig. 103. Changes in amylolytic activity (mgms. maltose/min./grain) in individual Early Pearl grains during germination.

L.S.D. between means for total amylolytic activity at 0.05 P.L. ± 2.527 mgms.

" " " " " " alpha-amylolytic activity at 0.05 P.L. ± 1.601 mgms.

" " " " " " beta-amylolytic activity at 0.05 P.L. ± 1.238.mgms.

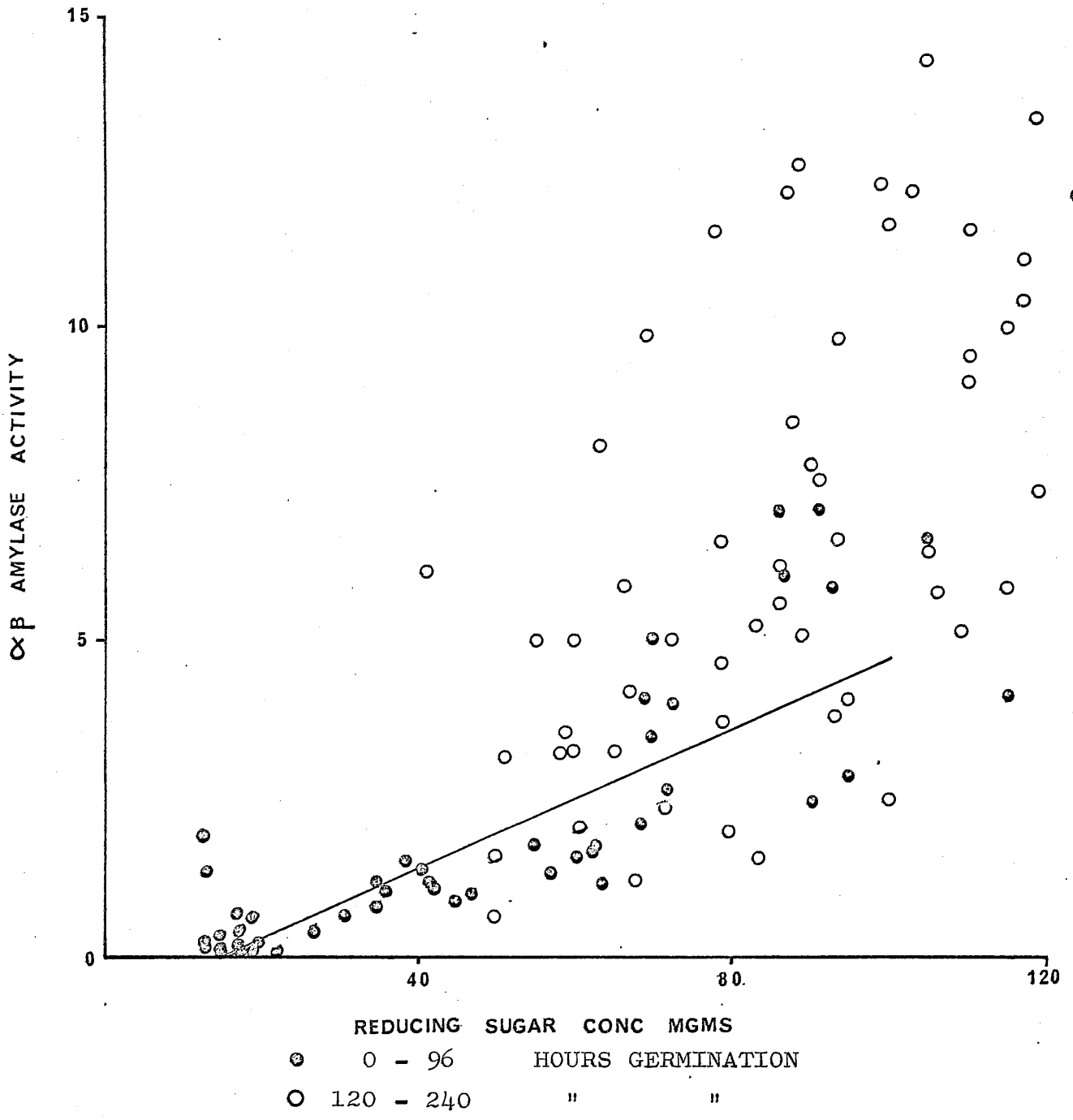

Fig. 104. Scatter plot illustrating correlation between total amylolytic activity (alpha- and beta-amylase) and reducing sugar concentration in individual Early Pearl grains. Correlation coefficient for 0-96 hours + 0.838.

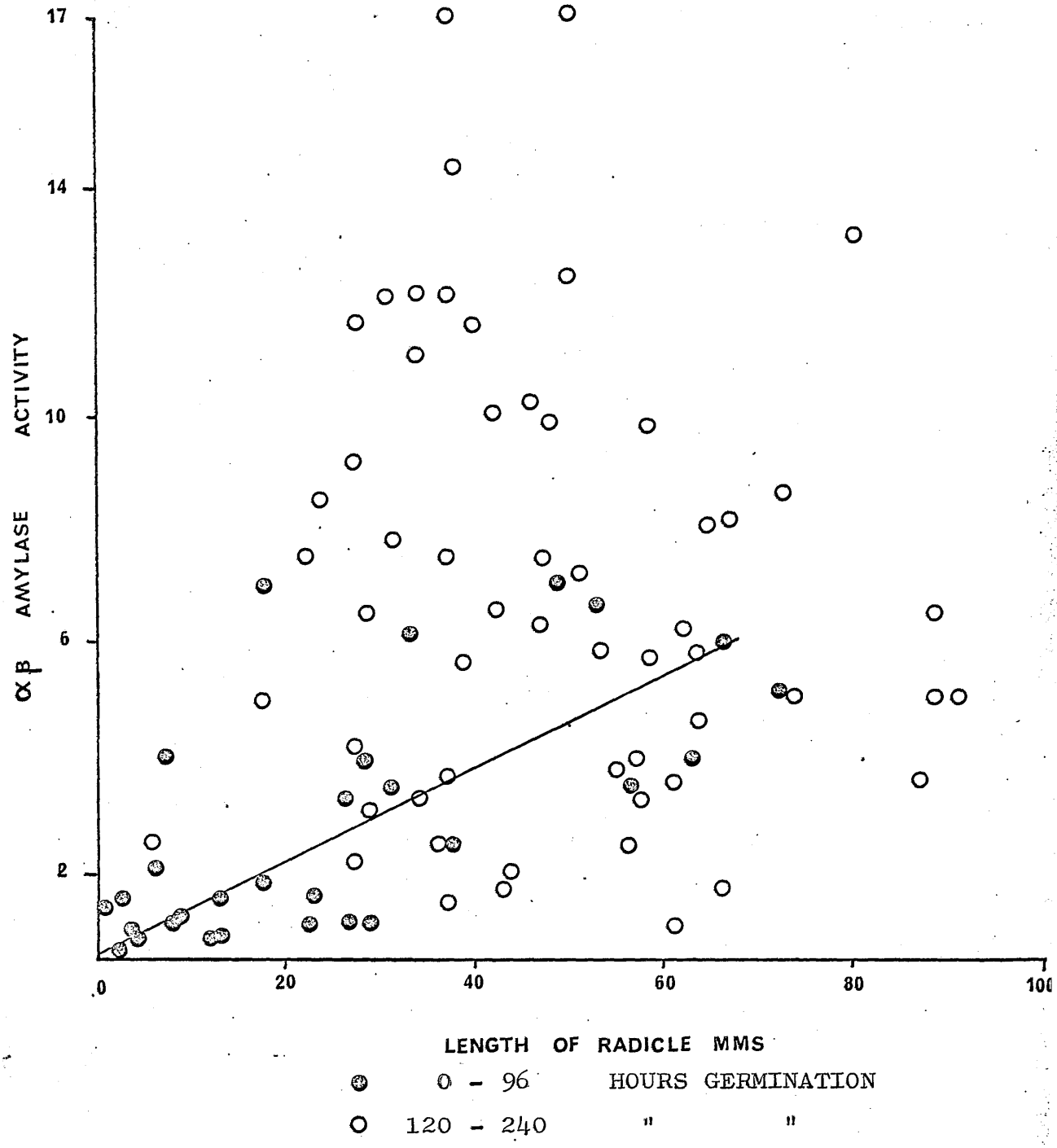

Fig. 105. Scatter plot illustrating correlation between total amylolytic activity (alpha- and beta-amylase) and radicle length in individual Early Pearl grains. Correlation coefficient for 0-96 hours + 0.764.

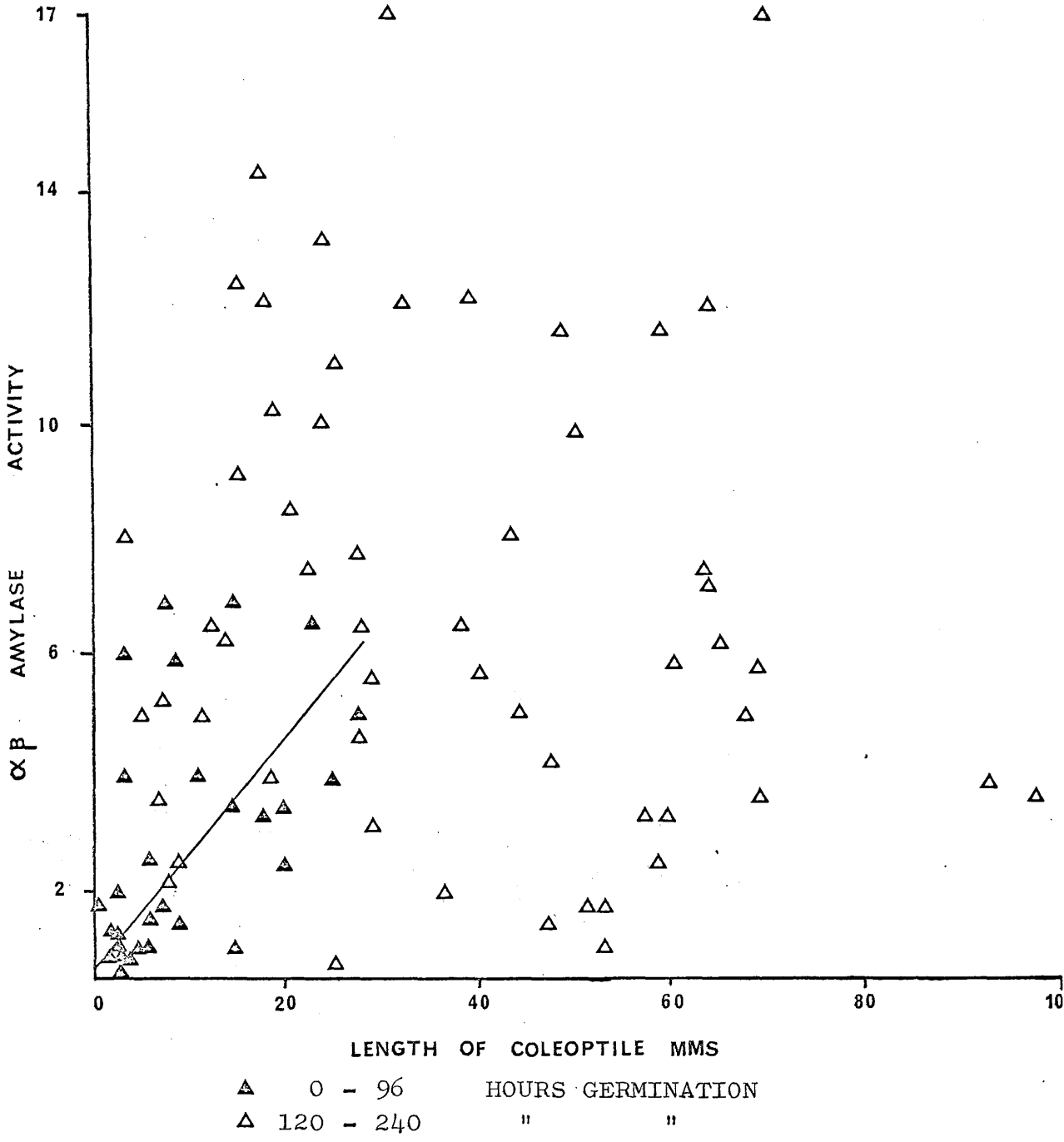

Fig. 106. Scatter plot illustrating correlation between total amylolytic activity (alpha- and beta-amylase) and coleoptile length in individual Early Pearl grains.

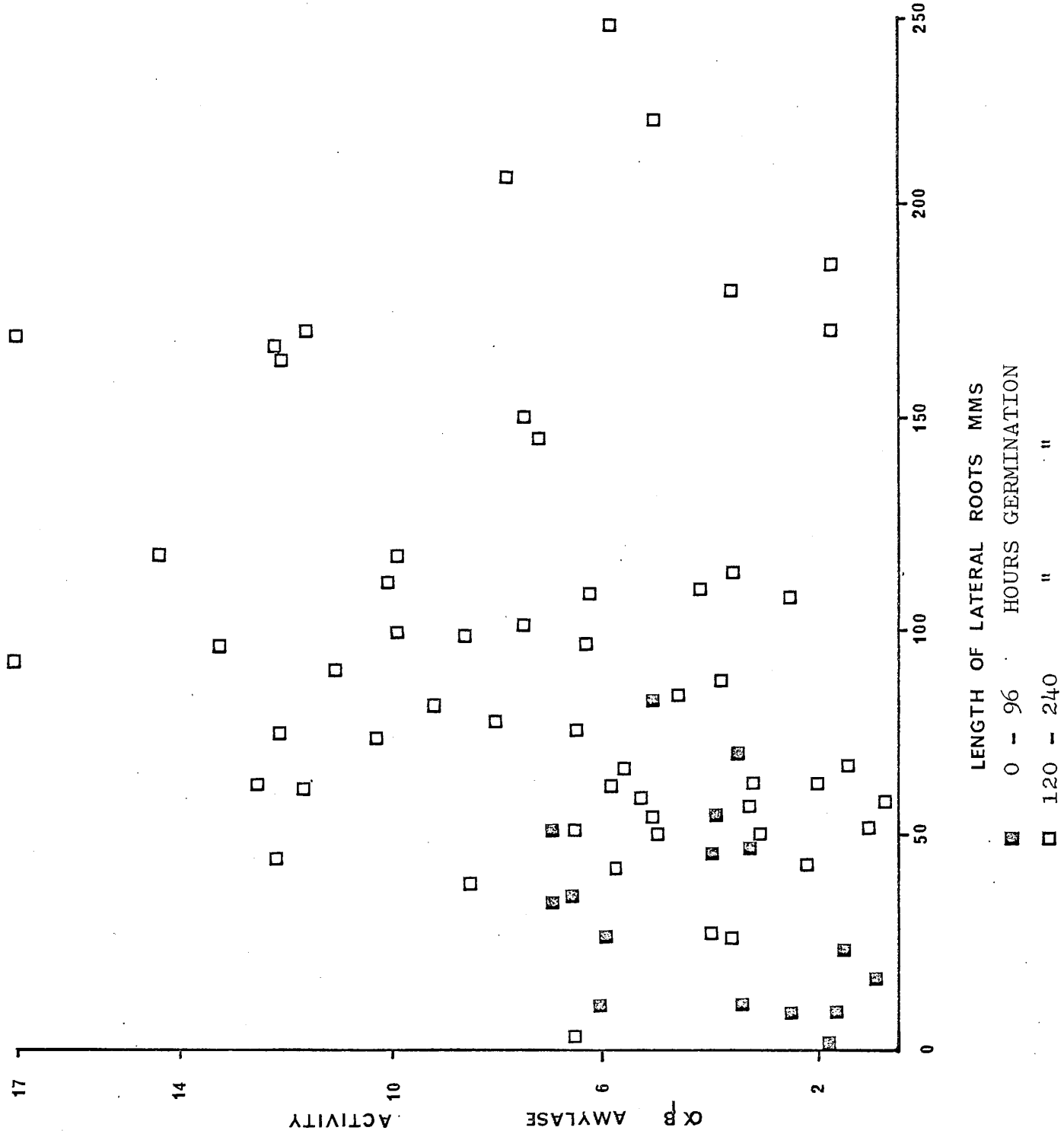

Fig. 107. Scatter plot illustrating correlation between total amylolytic activity (alpha- and beta-amylase) and lateral root length in individual Early Pearl grains.

Table 63

ALPHA-AMYLOLYTIC ACTIVITY (MGMS. MALTOSE/ MIN./ GRAIN) OF GROUPS OF EARLY PEARL GRAIN DURING GERMINATION. ASSAY WAS REPLICATED THREE TIMES ON EACH GROUP.

Germination Period (Hrs)	Replication Number 1	2	3	Mean	Germination Period Mean
0	-	-	-	-	
0	-	-	-	-	-
0	-	-	-	-	
24	0.162	0.150	0.062	0.125	
24	0.162	0.137	0.150	0.150	0.139
24	0.150	0.150	0.125	0.142	
48	0.133	0.142	0.150	0.143	
48	0.333	0.391	0.367	0.364	0.253
48	0.267	0.242	0.250	0.253	
72	0.192	0.200	0.192	0.195	
72	0.192	0.183	0.192	0.189	0.260
72	0.400	0.392	0.400	0.397	
96	2.833	2.833	2.916	2.861	
96	3.208	2.625	2.625	2.819	2.601
96	2.125	2.041	2.208	2.125	
120	6.000	4.875	5.125	5.333	
120	4.687	4.437	4.187	4.437	4.645
120	4.312	3.937	4.250	4.166	
144	3.928	3.839	3.750	3.839	
144	2.411	2.857	2.321	2.530	2.748
144	1.875	2.143	1.607	1.875	
168	3.839	2.678	2.768	3.095	
168	3.393	3.393	2.143	2.976	2.877
168	3.036	2.232	2.411	2.560	
192	7.053	3.661	7.143	5.952	
192	4.821	5.178	5.178	5.059	4.801
192	5.268	2.589	2.321	3.393	
216	6.875	8.437	6.750	7.354	
216	6.125	5.875	5.687	5.896	6.708
216	6.312	4.750	9.562	6.875	
240	4.062	4.125	4.062	4.083	
240	5.250	3.375	4.375	4.333	3.465
240	2.375	1.812	1.750	1.979	

L. S. D. Between Observations at 0.05 P. L. ± 2.288

" " " " Means " " " " ± 1.320

" " " " Germination Period Means " " ± 0.762

TABLE 64a

ANALYSIS OF VARIANCE RESULTS FOR ALPHA-AMYLASE ACTIVITY IN GROUPS OF EARLY PEARL GRAIN.

Source	Degrees of Freedom	Sum Squares	Variance	Variance Ratio(F)	Observations
Germination	10	454.9955	49.4995	84.554	* * *
Replication	2	2.2251	1.1126	2.068	N. S.
Sample	2	11.6116	5.8058	10.789	N. S.
Sample/Rep.	4	0.7917	0.1979	0.368	N. S.
Replication/Germ.	20	8.3576	0.4179	0.777	N. S.
Sample/Germ.	20	21.7646	1.0882	2.022	* *
Error	40	21.5245	0.5381		

* * * Significant at 0.1% Probability Level.
* * " " 5% " "

TABLE 64b

RESULTS OF LINEAR REGRESSION OF ALPHA-AMYLASE ACTIVITY AND TOTAL REDUCING FRACTION, RADICLE, COLEOPTILE AND LATERAL ROOT LENGTH IN GROUPS OF EARLY PEARL GRAIN.

Factor	Germination Period(Hrs)	Correlation Coefficient	t	Significance
Amylase	0 -240	+ 0.760	6.511	* * *
	0 -72	+ 0.623	-	-
Reducing Sugar	96 -240	+ 0.168	-	-
Amylase	0 -240	+ 0.800	7.424	* * *
	0 -72	+ 0.548	-	-
Radicle	96 -240	+ 0.388	-	-
Amylase	0 -240	+ 0.828	8.122	* * *
	0 -72	+ 0.500	-	-
Coleoptile	96 -240	+ 0.486	-	-
Amylase	0 -240	+ 0.665	-	-
	0 -72	+ 0.373	-	-
Lateral Roots	96 -240	+ 0.270	-	-

* * * Significant at 0.1% Probability Level.

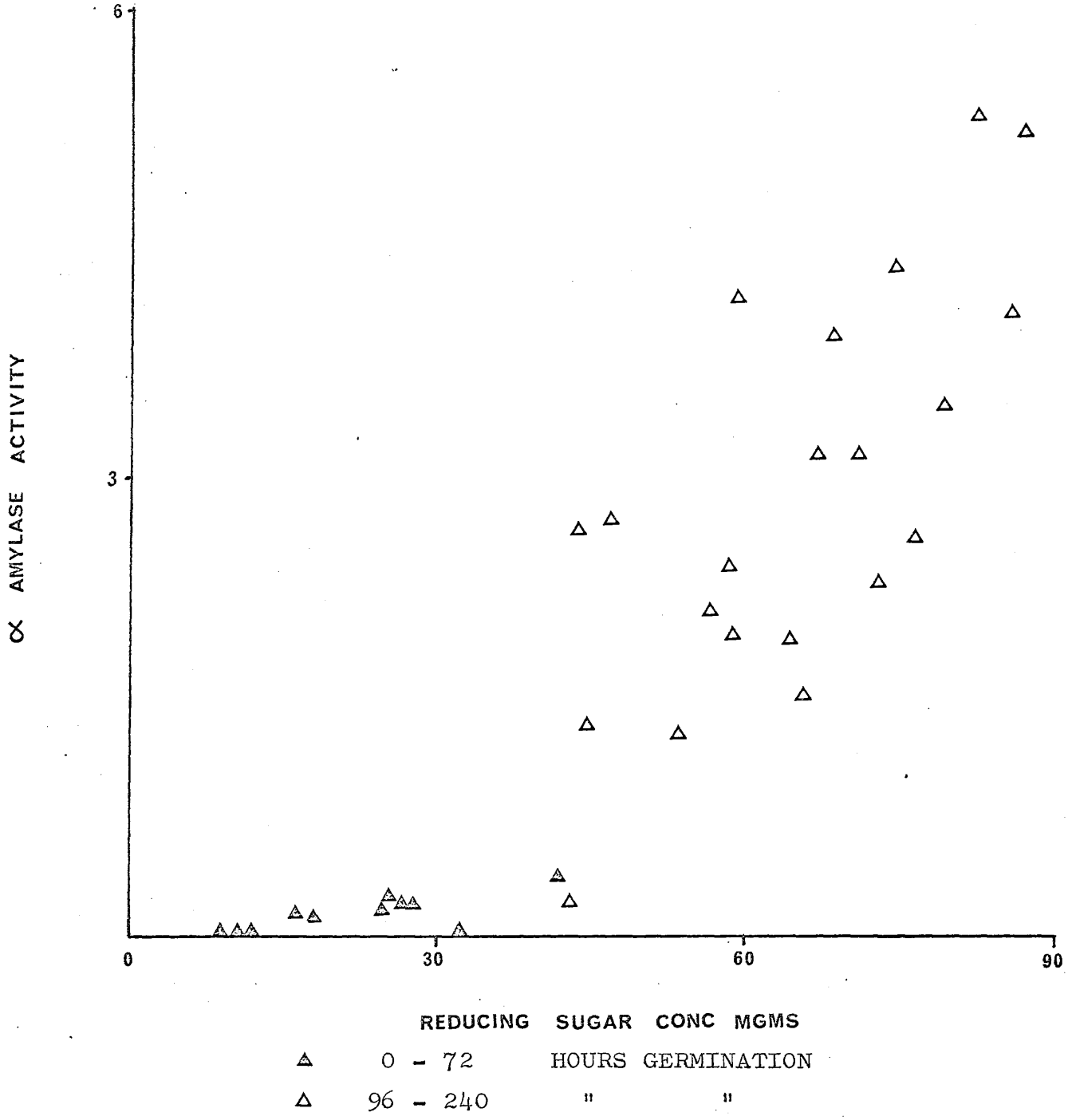

Fig. 108. Scatter plot illustrating correlation between alpha-amylolytic activity (alpha- and beta-amylase) and reducing sugar concentration in groups of Early Pearl grain.

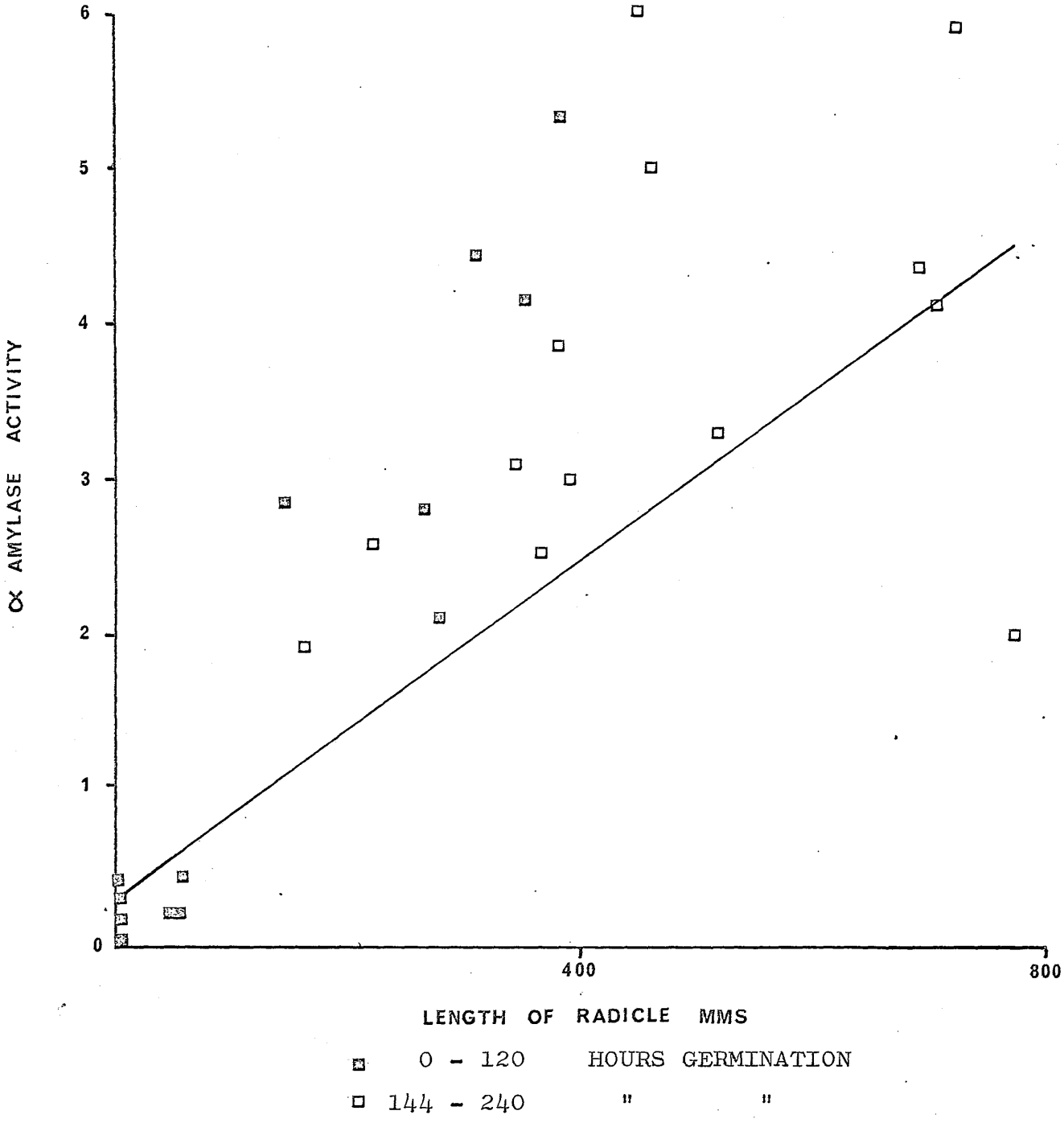

Fig. 109. Scatter plot illustrating correlation between alpha-amylolytic activity and radicle length in groups of Early Pearl grain. Correlation coefficient for 0-240 hours + 0.800.

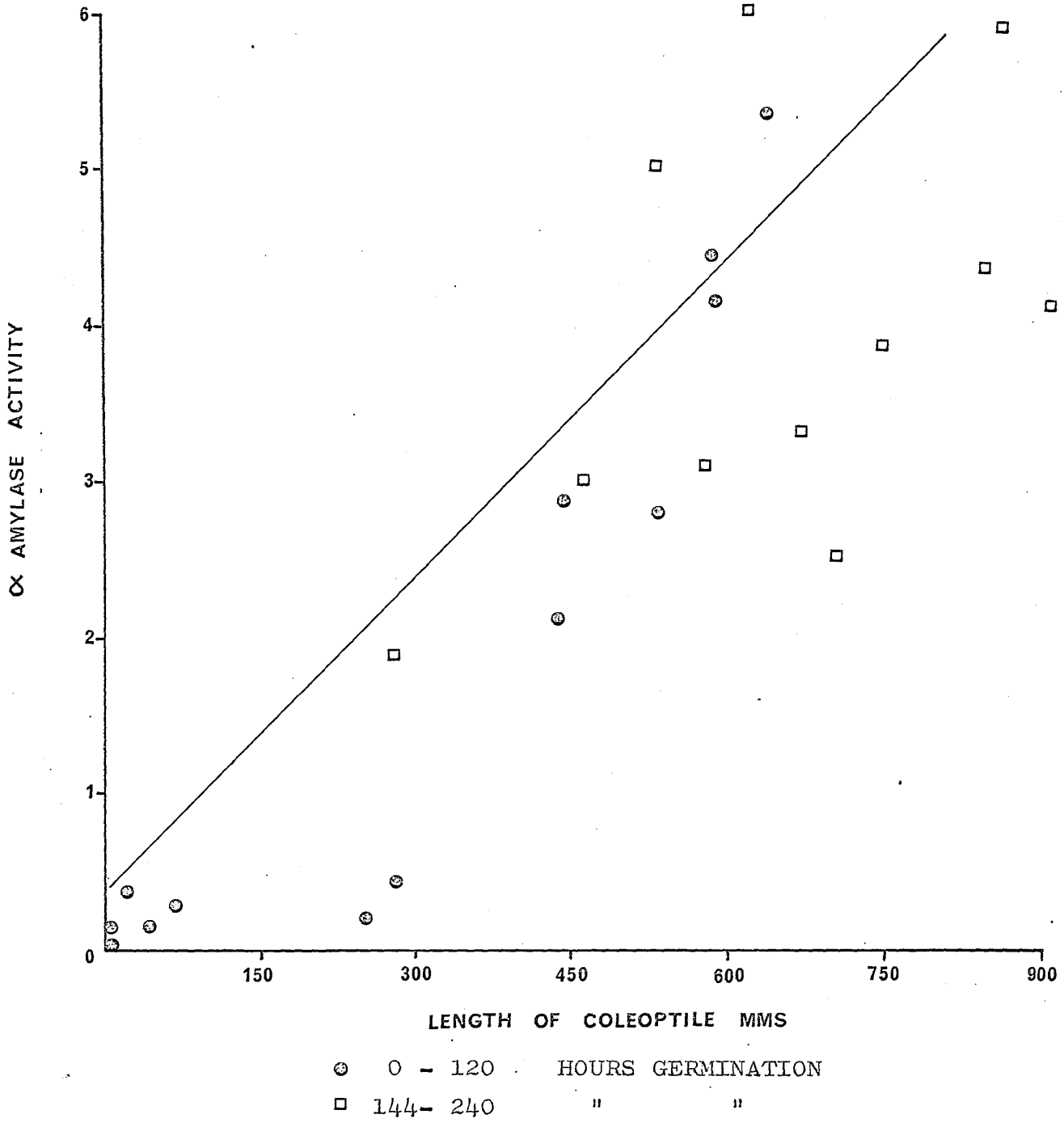

Fig. 110. Scatter plot illustrating correlation between alpha-amylolytic activity and coleoptile length in groups of Early Pearl grain. Correlation coefficient for 0-240 hours + 0.828

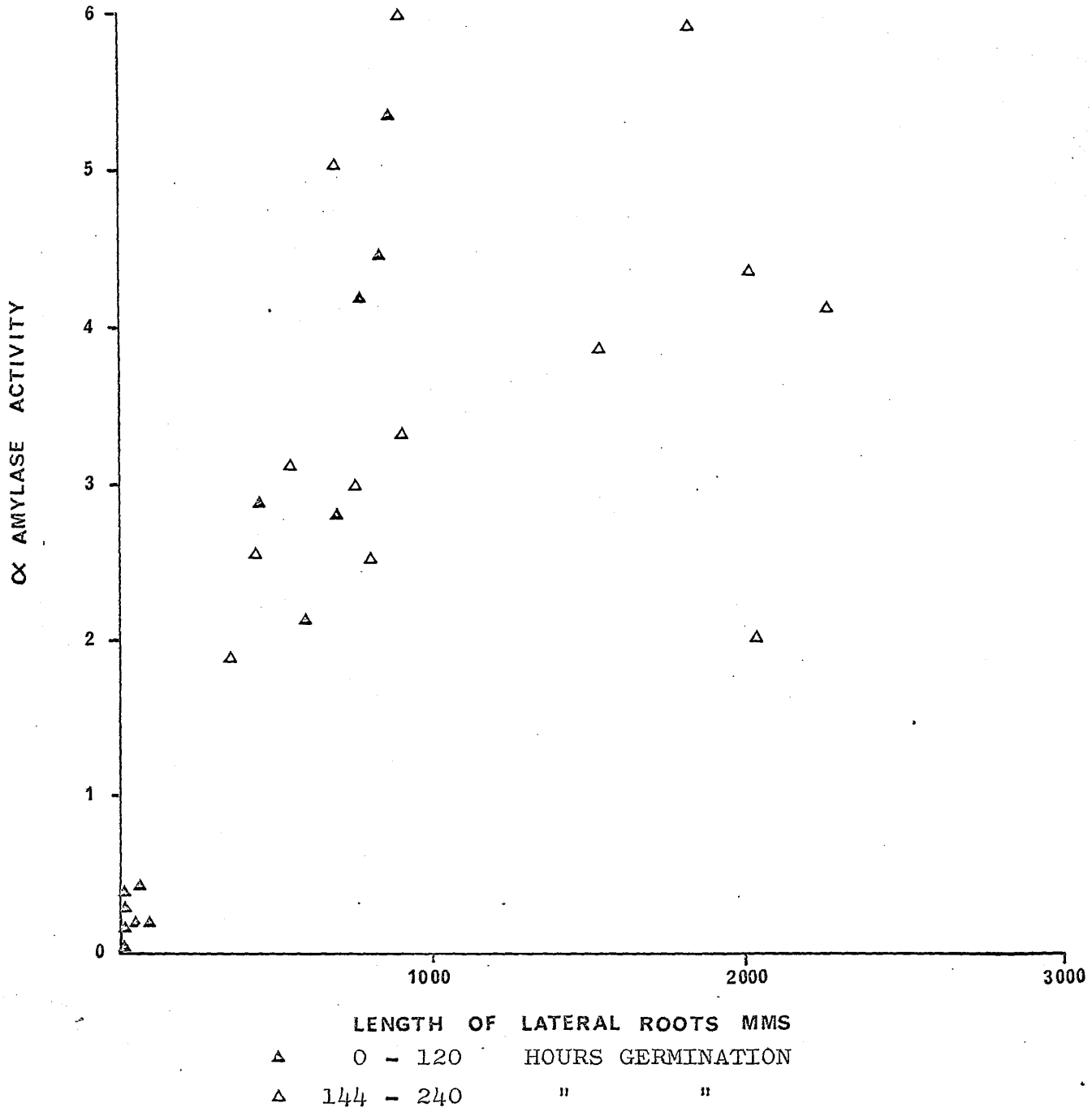

Fig. 111. Scatter plot illustrating correlation between alpha-activity and lateral root length in groups of Early Pearl grain.

TABLE 65

ALPHA-AMYLOLYTIC ACTIVITY (MGMS MALTOSE / MIN) IN INDIVIDUAL EARLY PEARL GRAINS DURING GERMINATION.

Germination Period (Hrs)	Grain Number 1	2	3	4	5	6	7	8	9	10	Mean	% Germination	Growth Index
0	-	-	-	-	-	0.625	-	-	-	-	0.062	-	-
24	0.094	0.281	-	0.094	0.031	0.187	0.031	-	-	-	0.072	-	-
48	0.333	0.583	0.833	0.167	0.375	0.958	0.333	0.375	0.458	0.917	0.533	90	0.091
72	1.042	1.406	2.838	0.469	2.109	0.469	0.234	-	0.495	-	0.906	100	0.375
96	3.917	2.583	0.500	1.083	2.583	2.250	4.250	2.167	3.333	0.250	2.292	100	0.992
120	4.875	2.875	12.250	6.875	3.375	2.625	4.250	2.250	1.250	5.500	4.612	100	1.366
144	8.036	3.750	3.214	4.821	5.375	6.964	5.179	2.679	1.071	2.500	6.607	100	1.273
168	3.542	3.958	2.292	2.083	2.708	-	6.875	1.875	0.208	-	2.854	100	1.674
192	1.250	2.625	5.250	6.250	2.125	1.375	0.625	3.000	1.000	1.250	2.475	100	1.873
216	0.156	2.656	4.531	1.250	2.187	3.750	4.062	2.031	1.850	2.187	2.466	100	2.465
240	2.625	9.500	6.875	4.125	7.000	2.125	6.375	0.500	3.000	2.000	4.412	100	2.377

Least Significant Difference Between Observations at 0.05 Probability Level ± 5.064

" " " " Means " " " " ± 1.601

TABLE 66a

ANALYSIS OF VARIANCE RESULTS FOR ALPHA-AMYLASE ACTIVITY IN INDIVIDUAL EARLY PEARL GRAINS.

Source	Degrees of Freedom	Sum Squares	Variance	Variance Ratio(F)	Observations
Germination	10	217.799	21.780	11.948	* * *
Sample	9	27.440	3.049	1.673	N. S.
Error	90	164.061	1.823		

* * * Significant at 0.1% Probability Level.

TABLE 66b

RESULTS OF LINEAR REGRESSION OF ALPHA-AMYLASE ACTIVITY AND TOTAL REDUCING FRACTION, RADICLE COLEOPTILE AND LATERAL ROOT LENGTH IN INDIVIDUAL EARLY PEARL GRAINS.

Factor	Germination Period(Hrs)	Correlation Coefficient	t	Significance
Amylase	0 -240	+ 0.699	-	-
	0 -96	+ 0.722	7.230	* * *
Reducing Sugar	120 -240	+ 0.555	-	-
Amylase	0 -240	+ 0.463	-	-
	0 -96	+ 0.614	-	-
Radicle	120 -240	+ 0.019	-	-
Amylase	0 -240	+ 0.360	-	-
	0 -96	+ 0.547	-	-
Coleoptile	120 -240	- 0.124	-	-
Amylase	0 -240	+ 0.476	-	-
	0 -96	+ 0.670	-	-
Lateral Roots	120 -240	+ 0.108	-	-

* * * Significant at 0.1% Prabability Level.

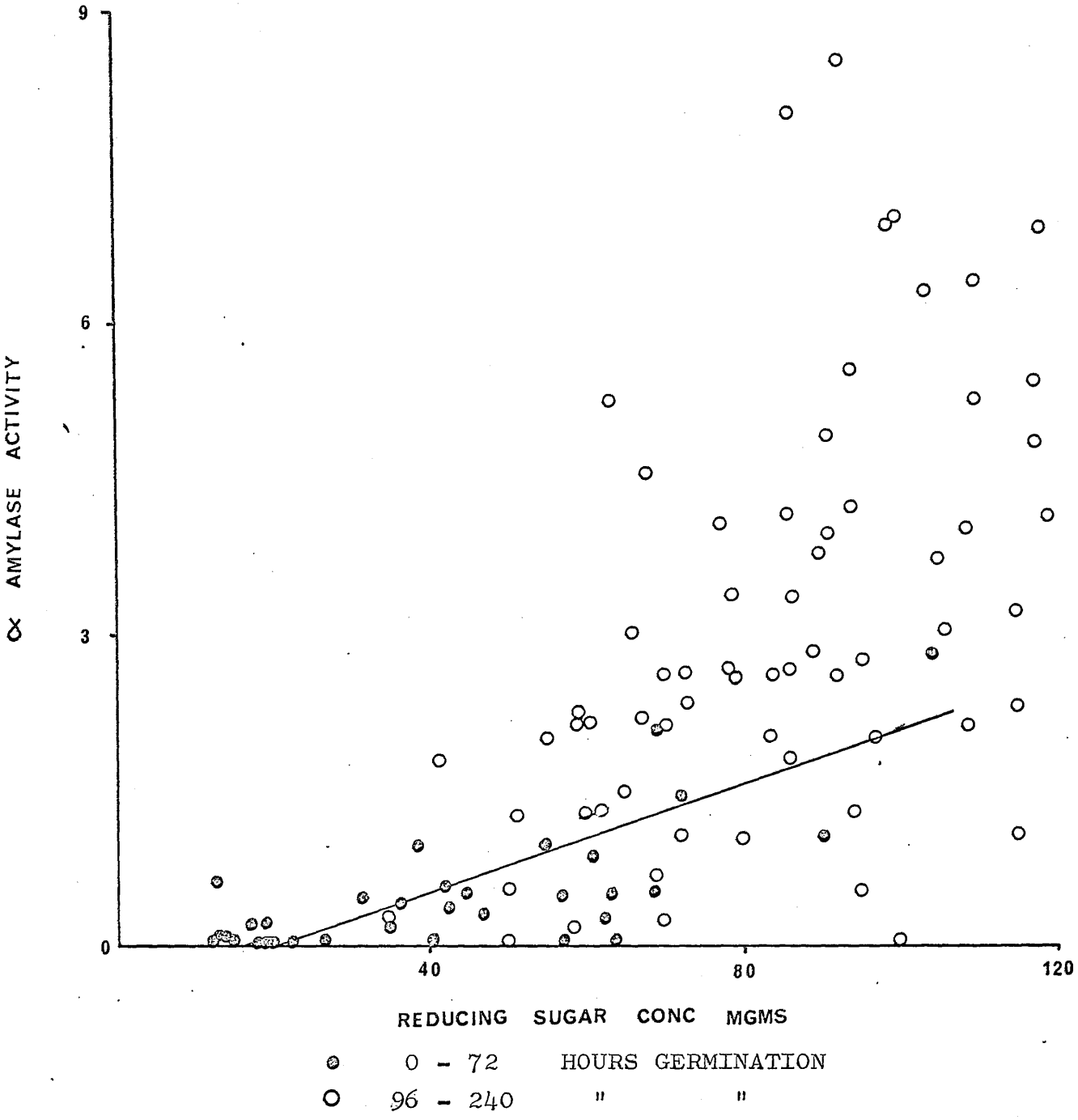

Fig. 112. Scatter plot illustrating correlation between alpha-amylolytic activity and reducing sugar concentration in individual Early Pearl grains. Correlation coefficient for 0-96 hours.

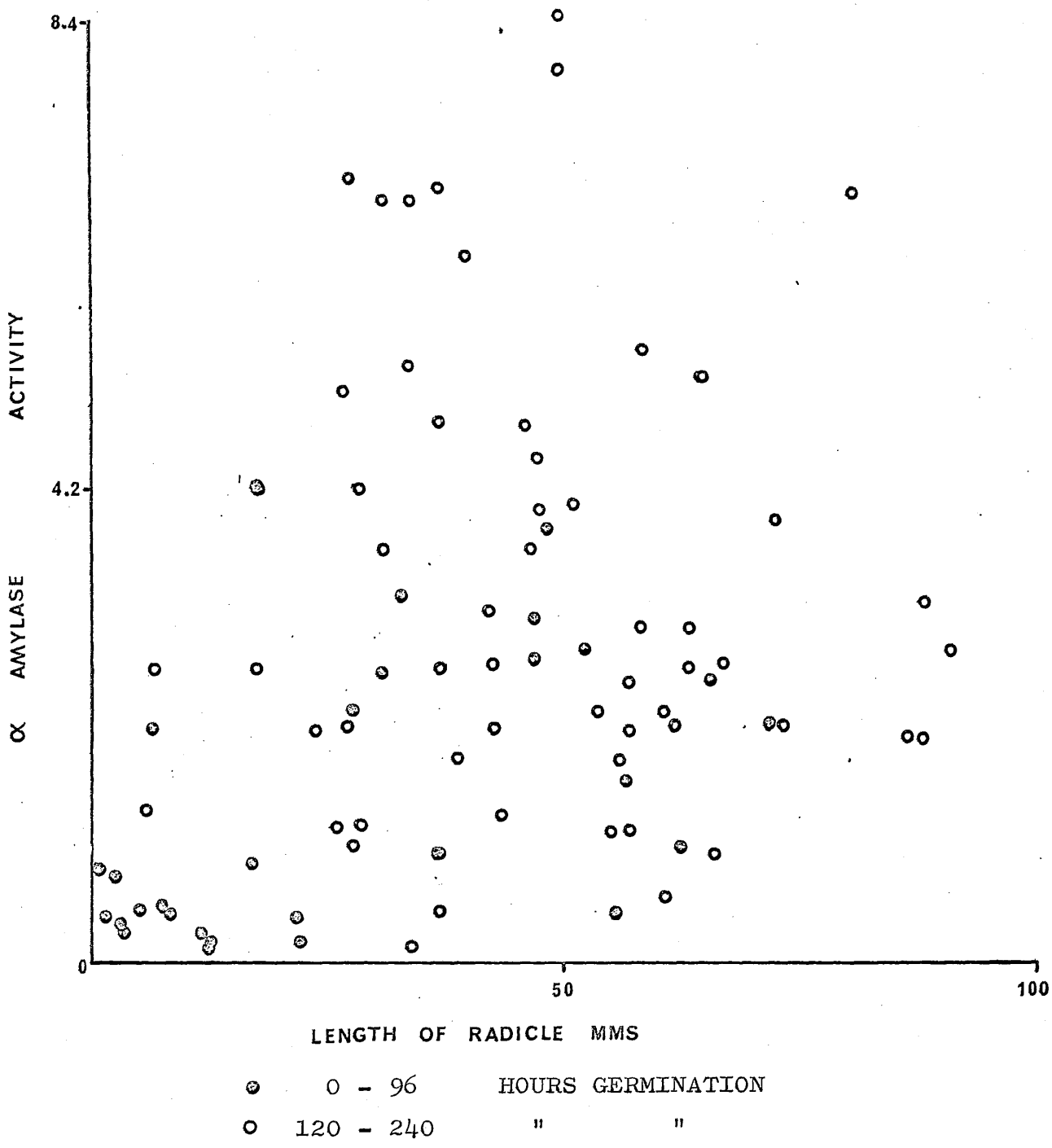

Fig. 113. Scatter plot illustrating correlation between alpha-amylolytic activity and radicle length in individual Early Pearl grains.

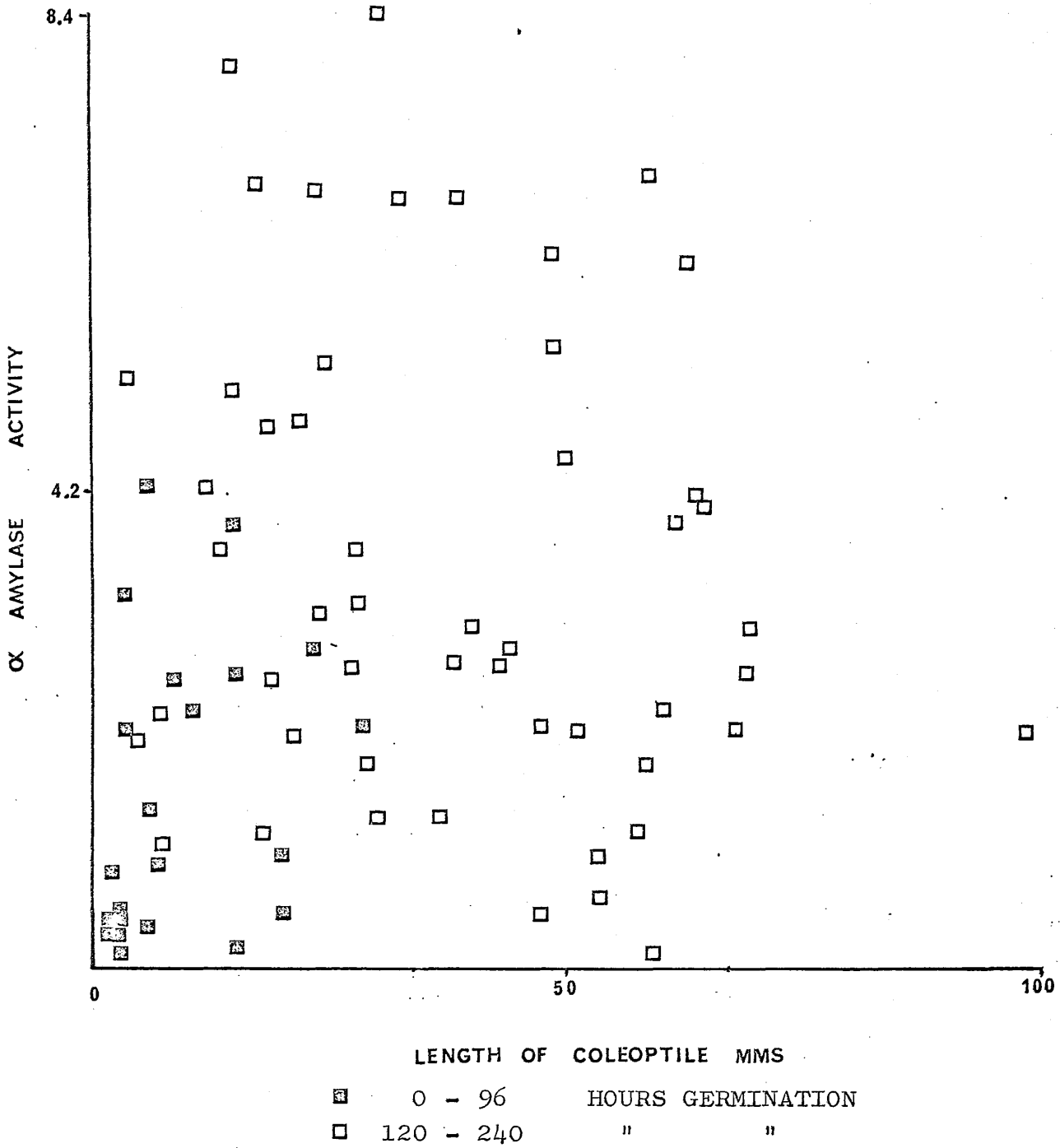

Fig. 114. Scatter plot illustrating correlation between alpha-amylolytic activity and coleoptile length in individual Early Pearl grains.

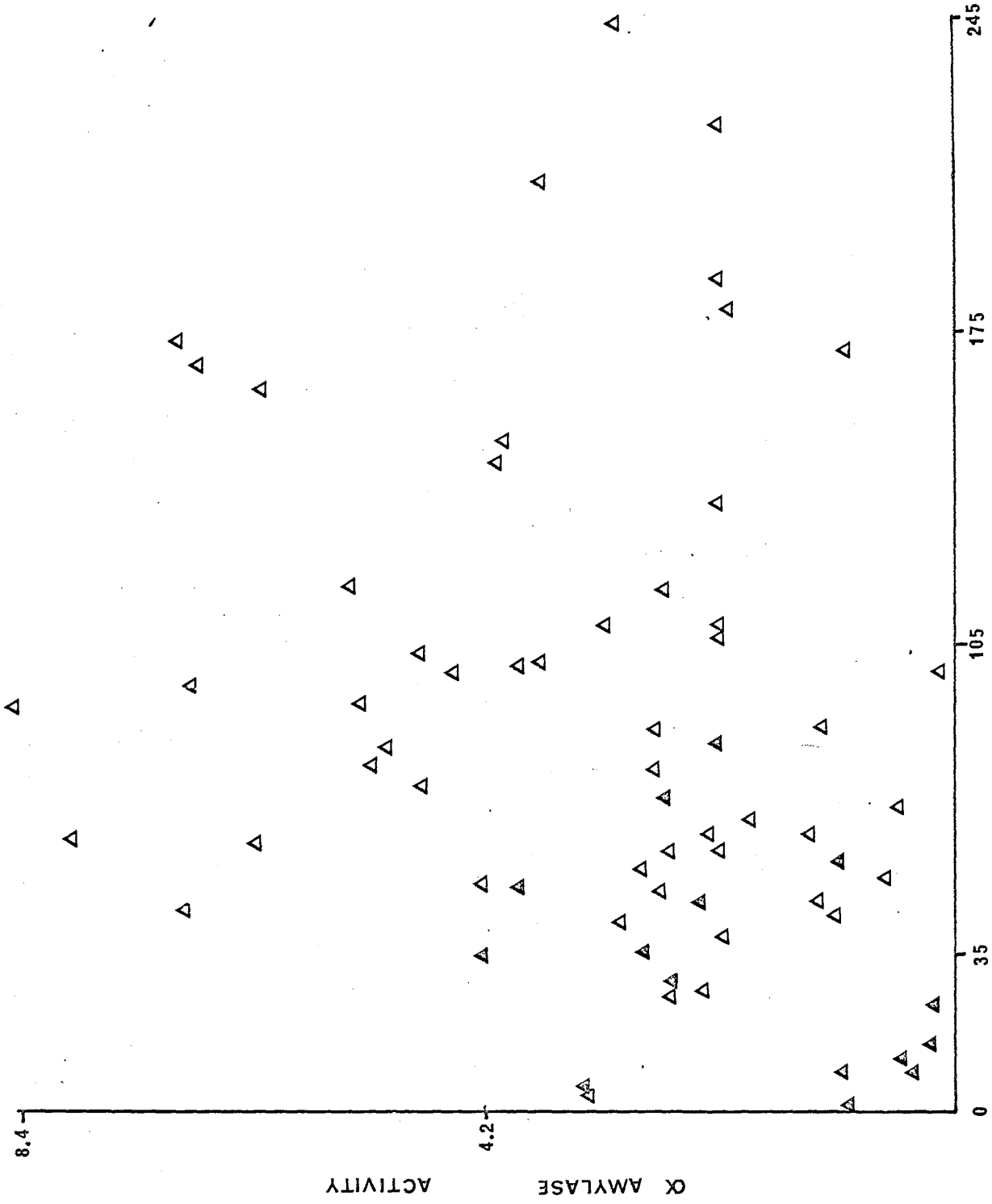

Fig. 115. Scatter plot illustrating correlation between alpha-amylolytic activity and lateral root length in individual Early Pearl grain.

TABLE 67

BETA-AMYLOLYTIC ACTIVITY (MGMS. MALTOSE/MIN/ GRAIN) IN GROUPS OF EARLY PEARL GRAIN DURING GERMINATION. ASSAY WAS REPLICATED THREE TIMES ON EACH GROUP.

Germination Period (Hrs)	Replication Number 1	2	3	Mean	Germination Period Mean
0	-	-	-	-	
0	0.040	0.020	0.025	0.028	0.013
0	0.010	0.015	0.010	0.012	
24	0.100	0.100	0.200	0.133	
24	0.125	0.125	0.125	0.125	0.137
24	0.162	0.150	0.150	0.154	
48	0.158	0.191	0.175	0.174	
48	0.200	0.151	0.191	0.181	0.179
48	0.158	0.174	0.217	0.183	
72	0.383	0.345	0.358	0.362	
72	0.028	-	-	0.009	0.205
72	0.242	0.250	0.242	0.245	
96	3.083	3.166	3.083	3.111	
96	3.541	4.083	4.000	3.875	3.365
96	3.250	2.791	3.291	3.111	
120	3.937	4.187	3.937	4.020	
120	4.312	4.125	7.250	5.229	4.222
120	3.375	3.375	3.500	3.417	
144	1.518	1.518	1.607	1.548	
144	1.428	1.250	1.250	1.309	1.409
144	1.518	1.071	1.518	1.369	
168	2.053	1.875	1.964	1.964	
168	0.268	0.536	-	0.268	1.547
168	2.232	2.321	2.678	2.413	
192	5.375	5.178	5.268	5.274	
192	4.464	4.196	4.373	4.344	3.918
192	2.143	1.964	2.321	2.143	
216	4.375	4.000	4.125	4.167	
216	2.562	2.875	2.625	2.687	3.979
216	2.750	2.875	2.687	2.771	
240	2.250	2.312	2.250	2.271	
240	2.500	2.750	2.437	2.562	2.264
240	2.000	1.937	1.937	1.958	

L. S. D. Between Observations at 0.05 P. L. ± 1.140
L. S. D. " Means " " " " ± 0.658
" " " " Germination Period Means " ± 0.380

TABLE 68a

ANALYSIS OF VARIANCE RESULTS FOR BETA-AMYLASE ACTIVITY IN GROUPS OF EARLY PEARL GRAIN.

Source	Degrees of Freedom	Sum Squares	Variance	Variance Ratio(F)	Observations
Germination	10	236.8006	23.6801	200.093	* * *
Replication	2	0.2637	0.1318	1.114	N. S.
Sample	2	3.7707	1.8854	15.931	N. S.
Sample/Rep.	4	0.2572	0.0643	0.543	N. S.
Replication/Germ.	20	2.0396	0.1020	0.862	N. S.
Sample/Germ.	20	30.6666	1.5333	12.956	* *
Error	40	4.7338	0.1183		

* * * Significant at 0.1% Probability Level.
* * " " 5% " "

TABLE 68b

RESULTS OF LINEAR REGRESSION OF BETA-AMYLASE ACTIVITY AND TOTAL REDUCING FRACTION, ALPHA-AMYLASE ACTIVITY, RADICLE, COLEOPTILE AND LATERAL ROOT LENGTH IN GROUPS OF EARLY PEARL GRAIN.

Factor	Germination Period(Hrs)	Correlation Coefficient	t	Significance
Beta-Amylase	0 -240	+ 0.840	8.620	* * *
	0 -72	+ 0.651	-	-
Alpha-Amylase	96 -240	+ 0.542	-	-
Amylase	0 -240	+ 0.646	-	-
	0 -72	+ 0.466	-	-
Radicle	96 -240	- 0.018	-	-
Amylase	0 -240	+ 0.623	-	-
	0 -72	+ 0.410	-	-
Coleoptile	96 -240	- 0.040	-	-
Amylase	0 -240	+ 0.445	-	-
	0 -72	+ 0.565	-	-
Radicle	96 -240	- 0.179	-	-
Amylase	0 -240	+ 0.882	10.421	* * *
	0 -72	+ 0.710	3.188	* *
Reducing Sugar	96 -240	+ 0.713	4.432	* * *

* * * Significant at 0.1% Probability Level.
* * " " 1% " "

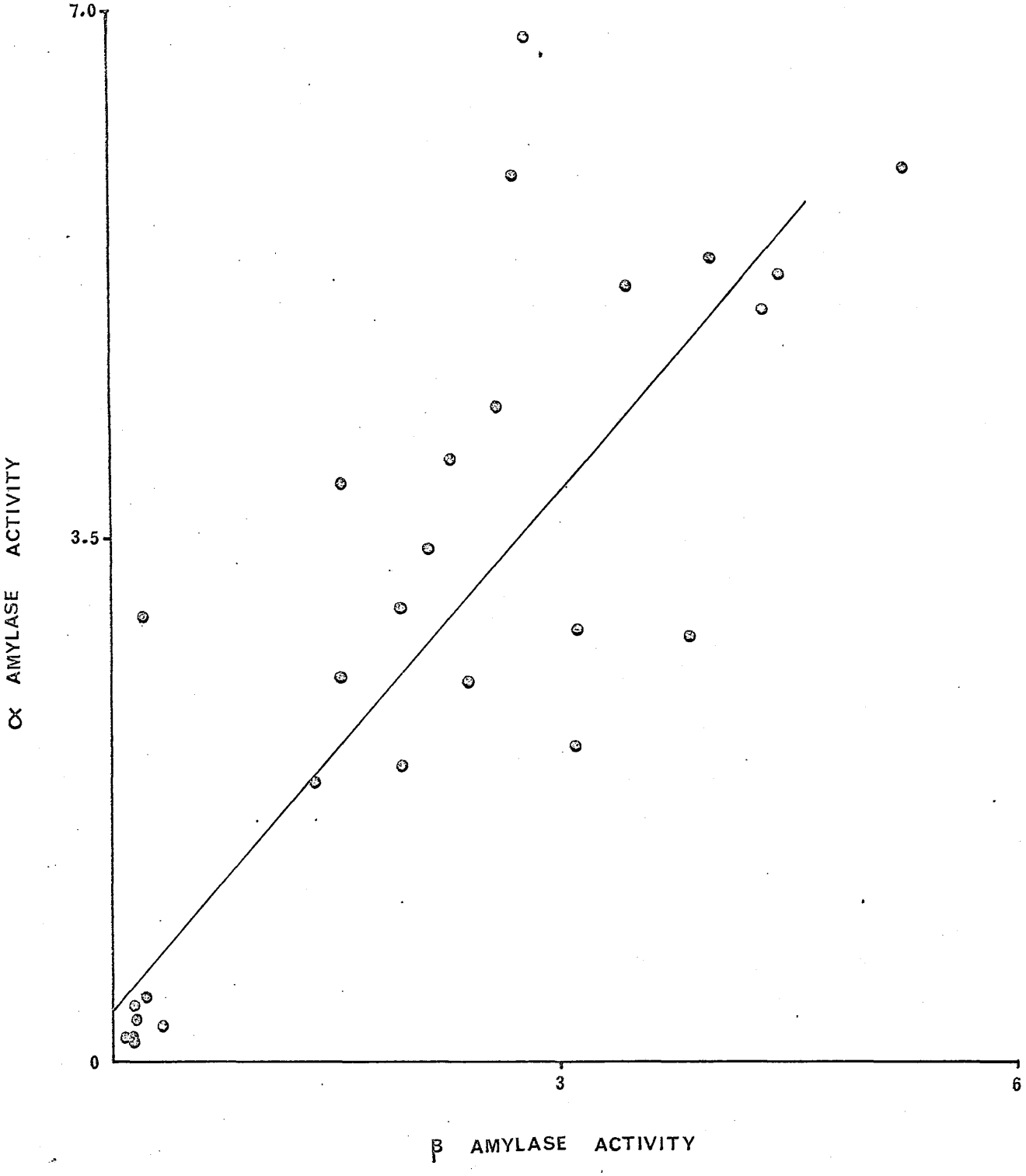

Fig. 116. Scatter plot illustrating correlation between alpha- and beta-amylase activity (mgms. maltose/min.) in groups of Early Pearl grain. Correlation coefficient for 0-240 hours + 0.840.

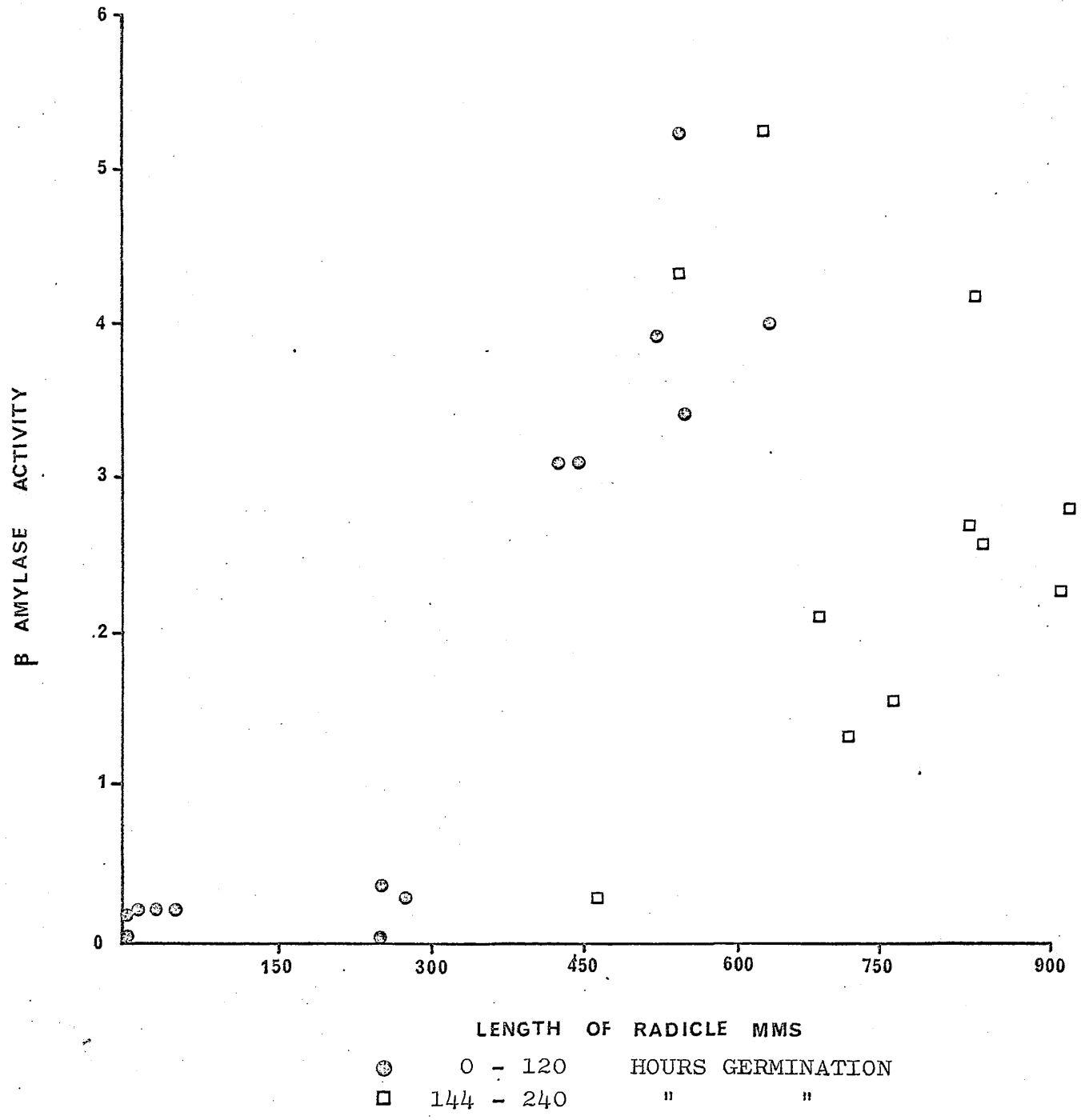

Fig. 117. Scatter plot illustrating correlation between beta-amylase activity (mgms. maltose/min.) and radicle length in groups of Early Pearl grain.

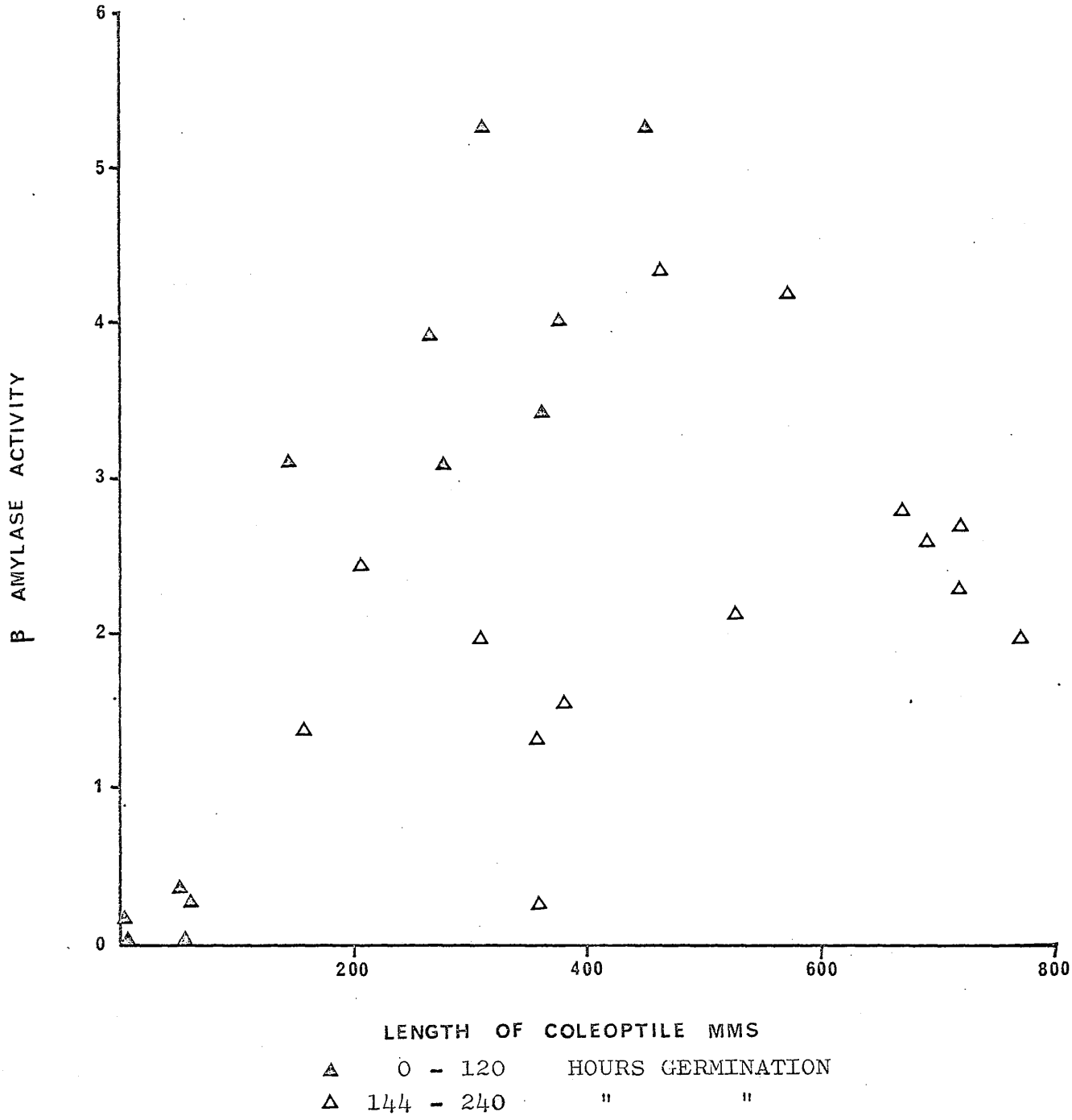

Fig. 118. Scatter plot illustrating correlation between beta-amylase activity (mgms. maltose/min.) and coleoptile length in groups of Early Pearl grain.

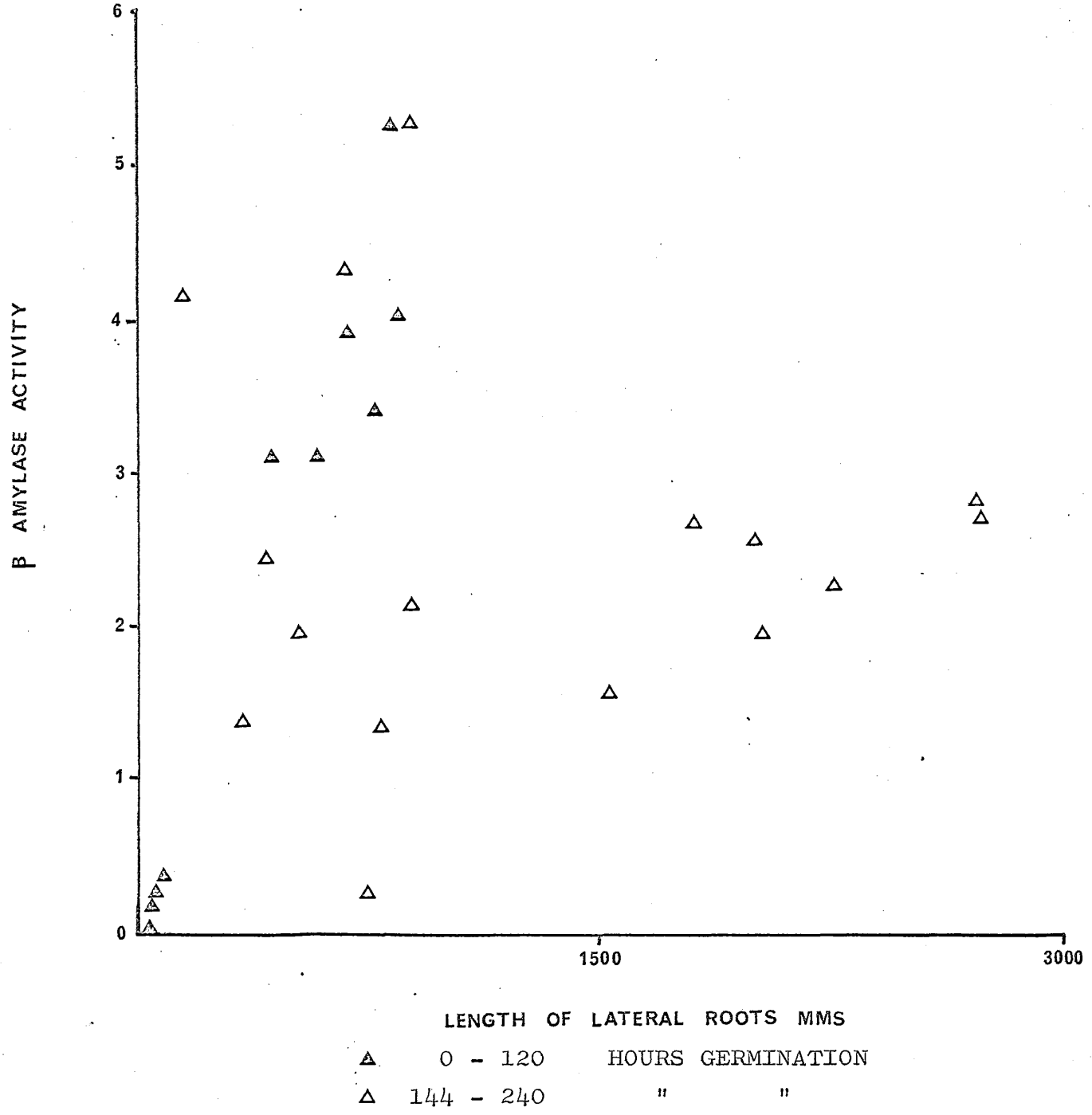

Fig. 119. Scatter plot illustrating correlation between beta-amylase activity (mgms. maltose/min.) and lateral root length in groups of Early Pearl grain.

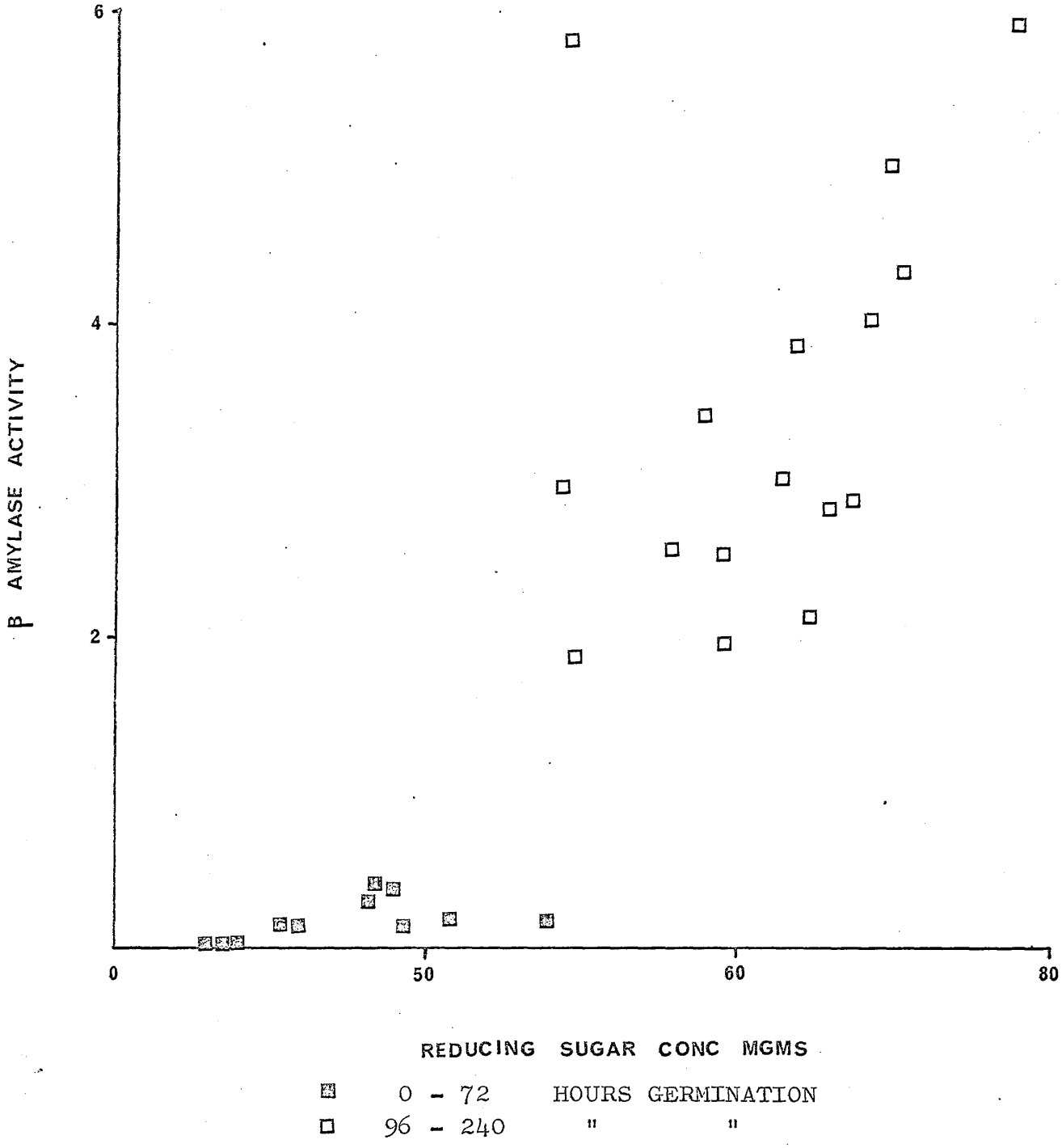

Fig. 120. Scatter plot illustrating correlation between beta-amylase activity (mgms. maltose/min.) and reducing sugar concentration in groups of Early Pearl grain. Correlation coefficient for 0-72 hours + 0.710.

TABLE 69

BETA-AMYLOLYTIC ACTIVITY (MGMS MALTOSE / MIN.) IN INDIVIDUAL EARLY PEARL GRAINS DURING GERMINATION.

Germination Period (Hrs)	Grain Number											% Germination	Growth Index
	1	2	3	4	5	6	7	8	9	10	Mean		
0	-	1.250	-	-	-	0.625	-	-	1.875	-	0.375	-	-
24	0.156	0.281	0.062	0.125	0.156	0.125	0.250	0.625	0.187	0.125	0.209	-	-
48	0.625	0.542	0.709	0.541	0.542	0.750	0.500	0.708	0.125	0.541	0.558	90	0.091
72	1.388	1.224	3.855	1.224	2.005	0.781	1.328	1.094	1.510	1.276	1.568	100	0.375
96	3.166	3.250	2.333	3.084	0.917	1.750	2.833	2.833	2.750	0.917	2.383	100	0.992
120	2.750	2.250	2.000	5.250	3.250	2.375	2.375	1.375	2.000	4.250	2.787	100	1.366
144	4.643	2.679	6.786	5.536	5.714	5.357	3.928	5.535	1.250	1.607	4.303	100	1.273
168	8.542	4.584	3.541	6.459	3.959	2.500	6.458	3.750	1.667	0.625	4.208	100	1.674
192	2.625	2.625	2.875	5.875	1.125	0.625	0.500	2.750	0.875	2.000	1.987	100	1.873
216	3.125	2.031	5.313	1.875	2.032	4.062	3.438	1.719	0.650	4.219	2.846	100	2.465
240	1.125	7.500	5.375	3.250	4.625	3.000	5.125	1.000	2.875	3.000	3.687	100	2.377

Least Significant Difference Between Observations at 0.05 Probability Level ± 3.914

" " " " Means " " " " ± 1.238

TABLE '70a

ANALYSIS OF VARIANCE RESULTS FOR BETA-AMYLASE ACTIVITY IN INDIVIDUAL EARLY PEARL GRAINS.

Source	Degrees of Freedom	Sum Squares	Variance	Variance Ratio(F)	Observations
Germination	10	294.443	29.444	9.094	* * *
Sample	9	62.502	6.945	2.145	* *
Error	90	291.389	3.238		

* * * Significant at 0.1% Probability Level.
* * " " 5% " "

TABLE 70b

RESULTS OF LINEAR REGRESSION OF BETA-AMYLASE ACTIVITY AND TOTAL REDUCING FRACTION, ALPHA-AMYLASE ACTIVITY, RADICLE COLEOPTILE AND LATERAL ROOT LENGTH IN INDIVIDUAL EARLY PEARL GRAINS.

Factor	Germination Period(Hrs)	Correlation Coefficient	t	Significance
Beta-Amylase	0 -240	+ 0.768	12.462	* * *
	0 -96	+ 0.780	8.636	* * *
Alpha-Amylase	120 -240	+ 0.628	-	-
Beta-Amylase	0 -240	+ 0.552	-	-
	0 -96	+ 0.834	10.472	* * *
Radicle	120 -240	+ 0.046	-	-
Beta-Amylase	0 -240	+ 0.453	-	-
	0 -96	+ 0.744	7.714	* * *
Coleoptile	120 -240	- 0.033	-	-
Beta-Amylase	0 -240	+ 0.447	-	-
	0 -96	+ 0.657	-	-
Lateral Roots	120 -240	+ 0.003	-	-
Beta-Amylase	0 -240	+ 0.795	13.619	* * *
	0 -96	+ 0.864	11.889	* * *
Reducing Sugar	120 -240	+ 0.651	-	-

* * * Significant at 0.1% Probability Level.

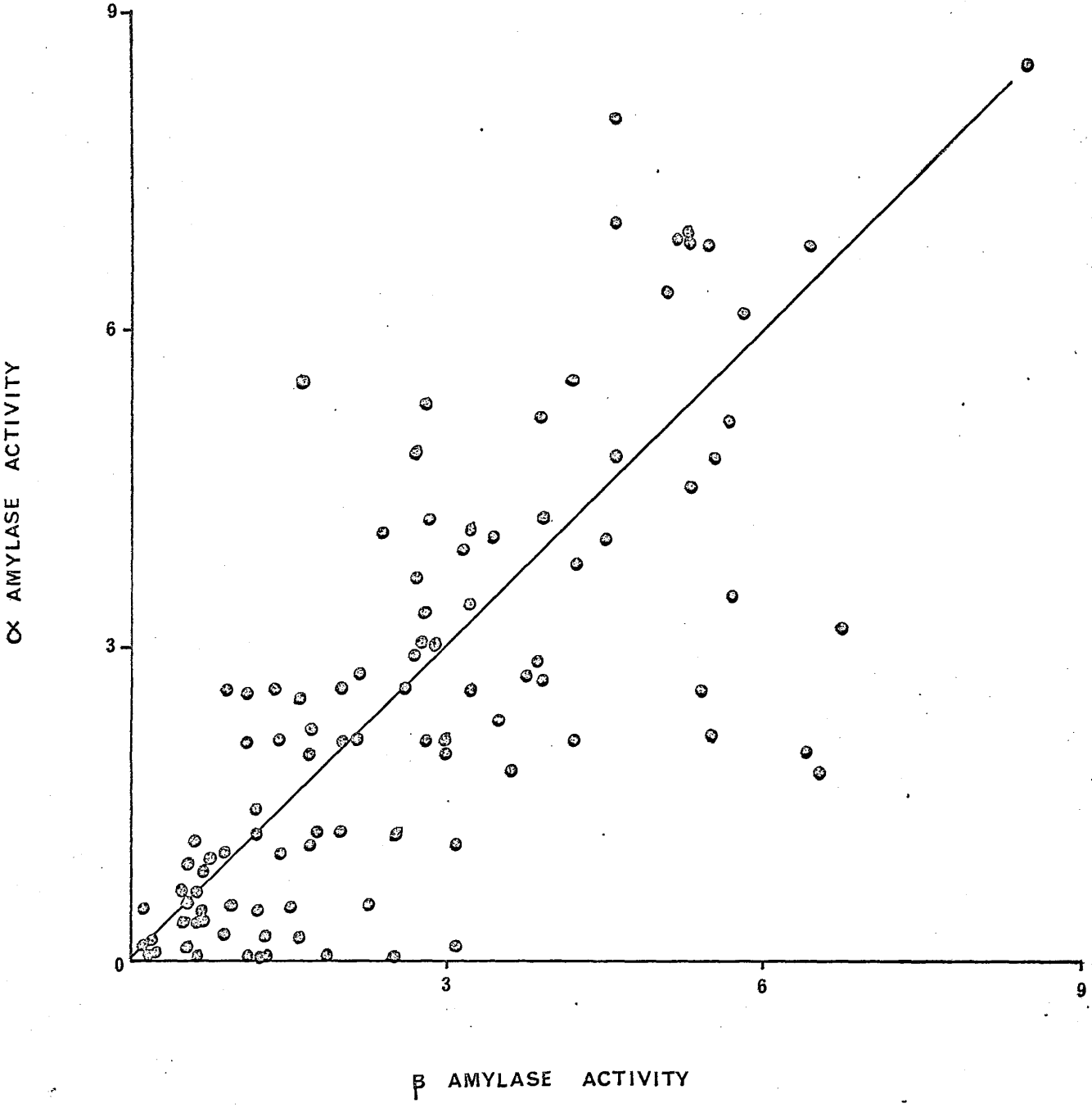

Fig. 121. Scatter plot illustrating correlation between beta-amylase activity and alpha-amylase activities (mgms. maltose/min.) in individual Early Pearl grains. . Correlation coefficient for 0-240 hours + 0.768.

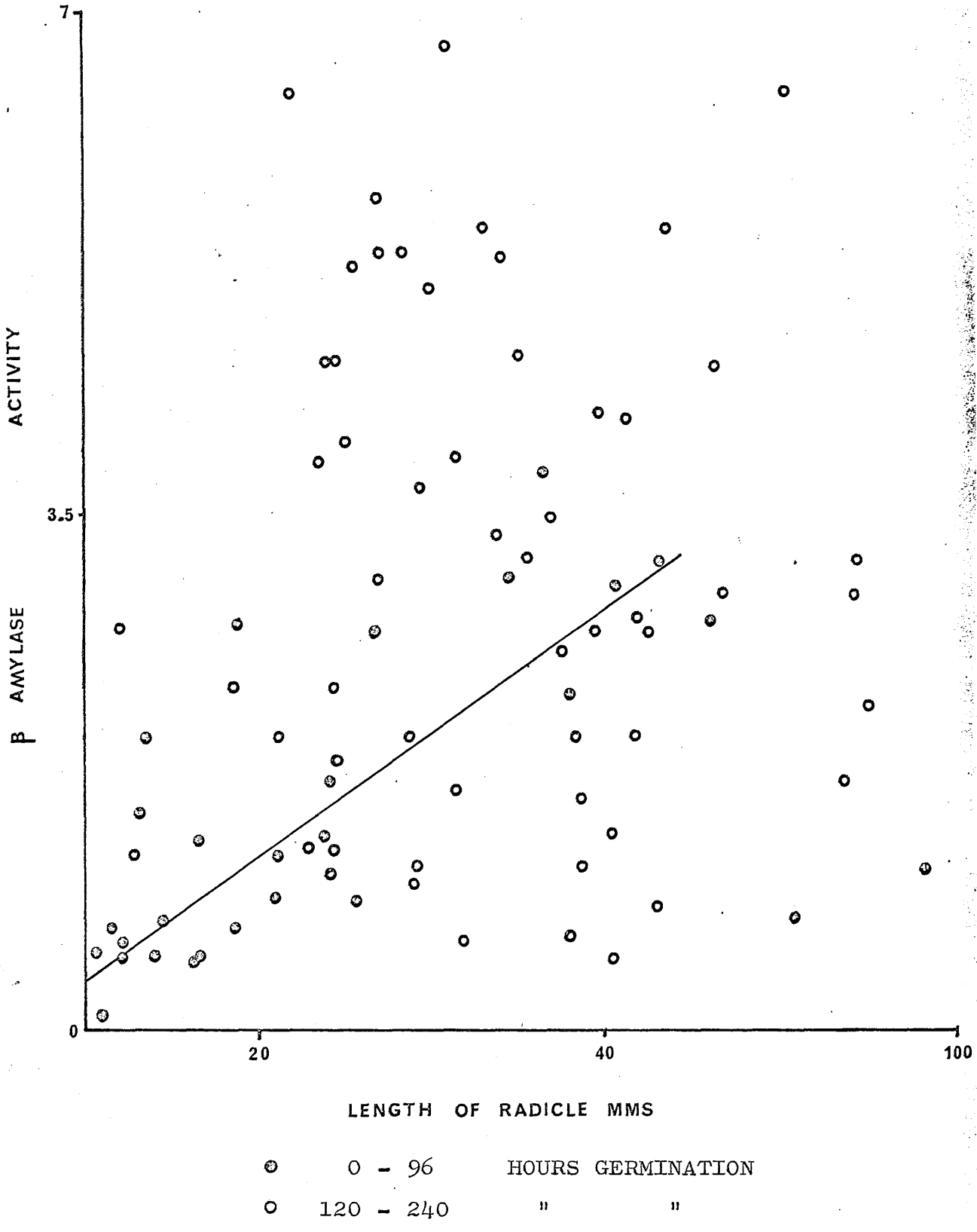

Fig. 122. Scatter plot illustrating correlation between beta-amylase activity (mgms. maltose/min.) and radicle length in individual Early Pearl grains. Correlation coefficient for 0-96 hours + 0.834.

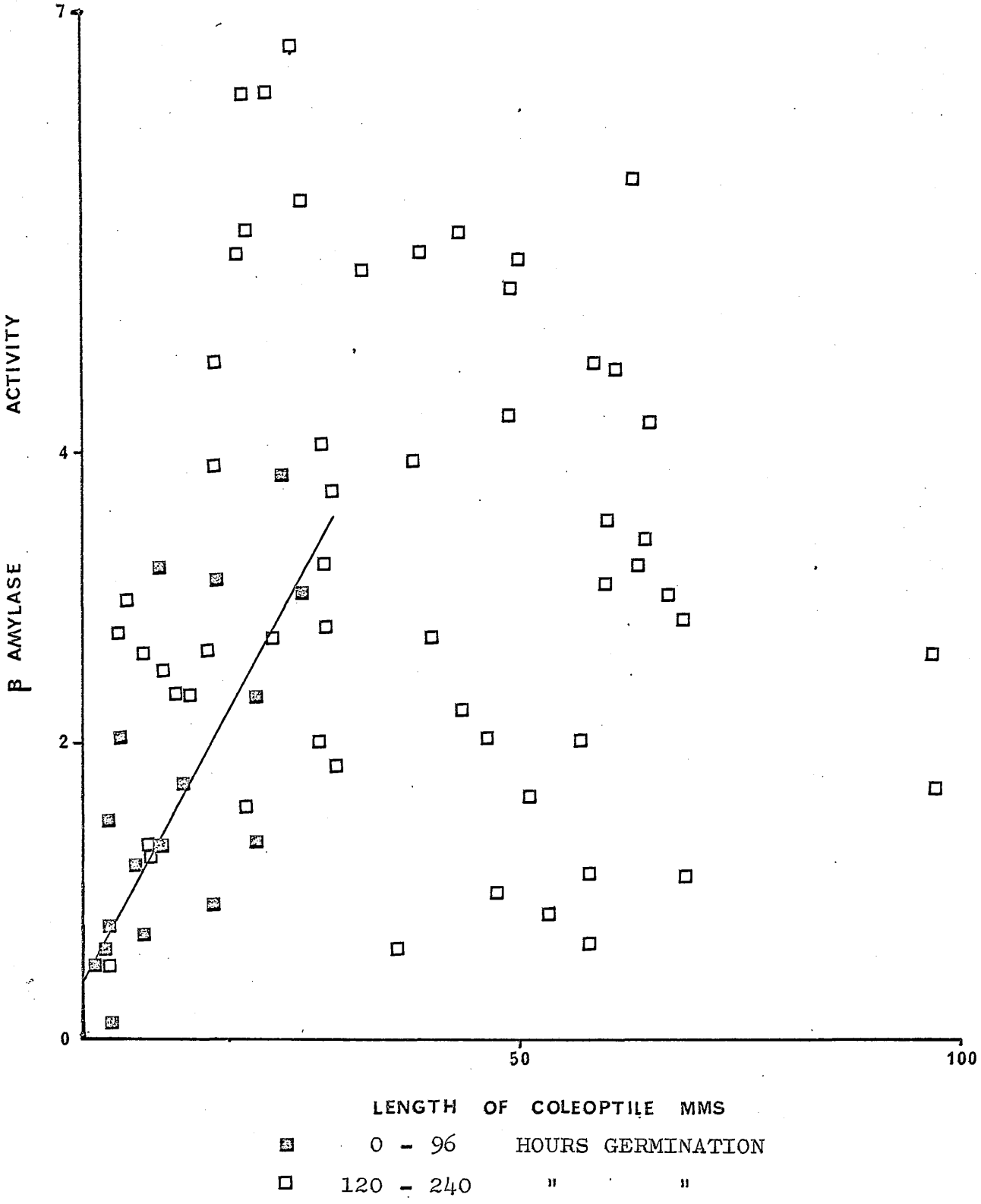

Fig. 123. Scatter plot illustrating correlation between beta-amylase activity (mgms. maltose/min.) and Coleoptile length in individual Early Pearl grains. Correlation coefficient for 0-96 hours + 0.744.

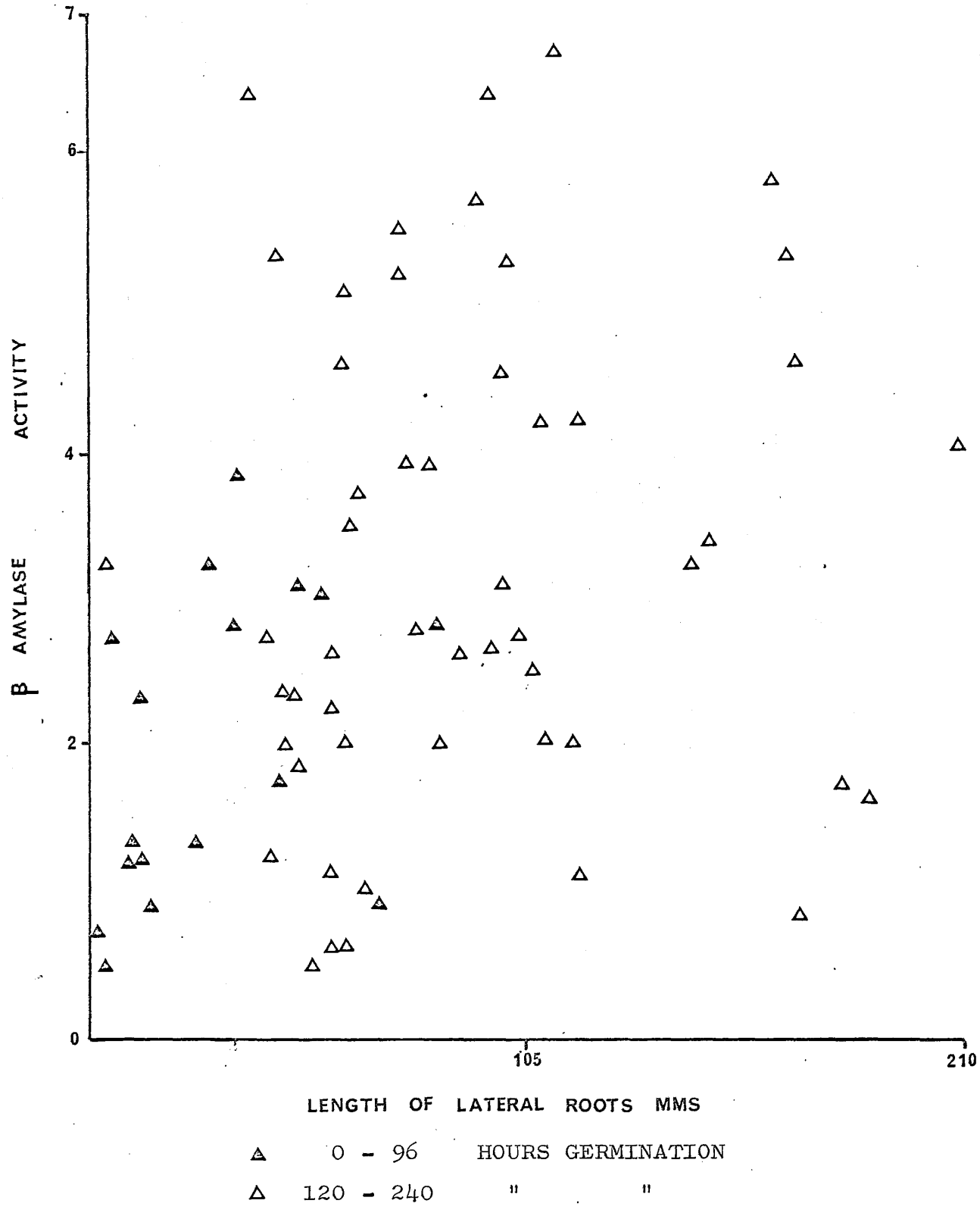

Fig. 124. Scatter plot illustrating correlation between beta-amylase activity (mgms. maltose/min.) and lateral root length in individual Early Pearl grains.

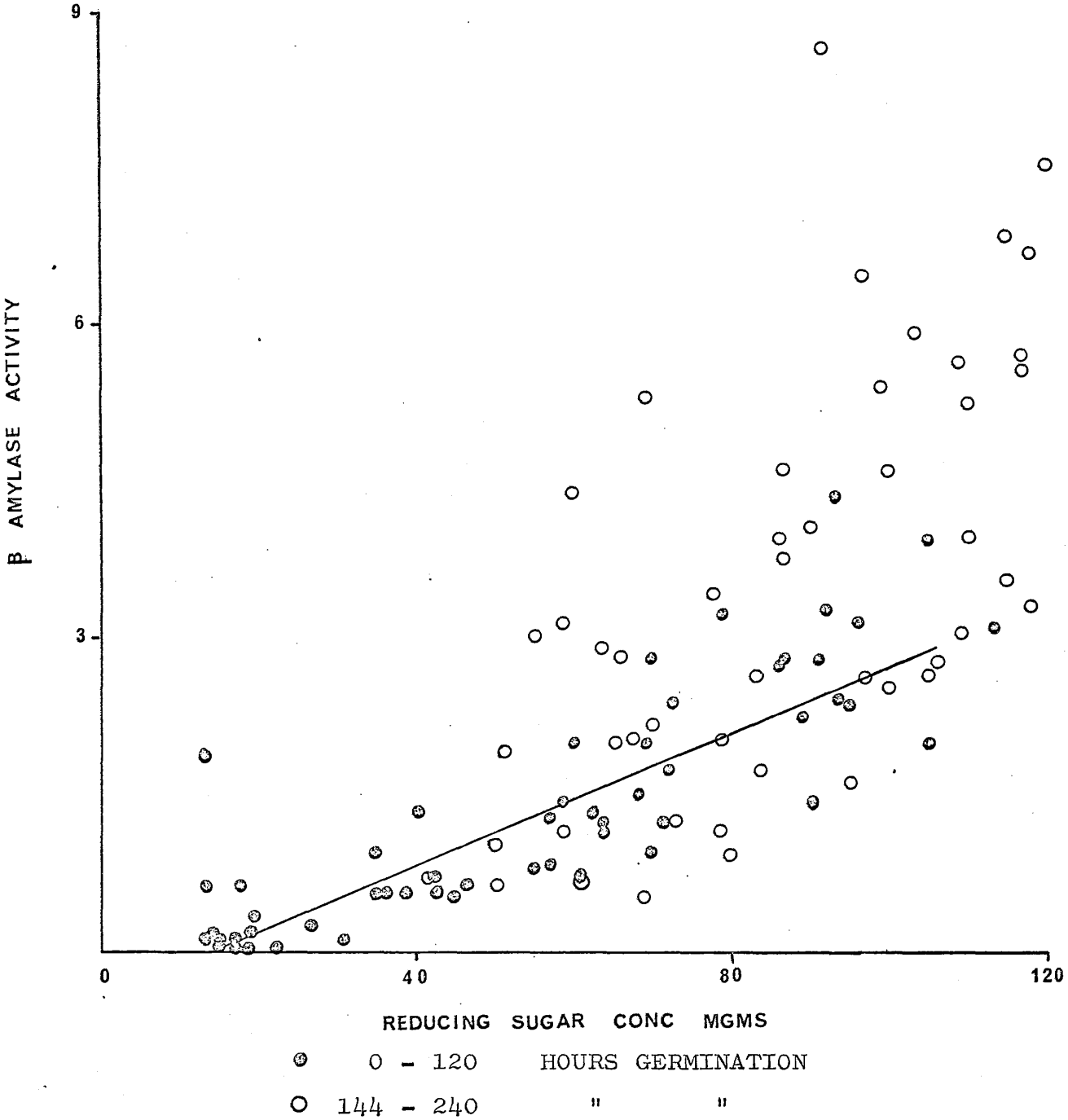

Fig. 125. Scatter plot illustrating correlation between beta-amylase activity (mgms. maltose/min.) and reducing sugar concentration in individual Early Pearl grains. Correlation coefficient for 0-96 hours + 0.864.