DEVELOPMENT OF A METHOD FOR RAPID DETERMINATION OF GERM DAMAGE IN CEREALS

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TABLE OF CONTENTS

THIS BOOK CONTAINS NUMEROUS PAGES WITH DIAGRAMS THAT ARE CROOKED COMPARED TO THE REST OF THE INFORMATION ON THE PAGE.

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INTRODUCTION

The development of the modern grain processing industries made standards necessary for efficient grain trading. The Grain Standards Act established the Production and Marketing Administration, Grain Branch, of the United States Department of Agriculture. The present agency responsible for grain standards and grain inspection is the Consumer and Marketing Service, Grain Division. The standards and methods of grain evaluation are concerned with inspection of the physical properties of grain. The methods for grain inspection have changed little since the conception of grain standards.

Germ damage is one of the most difficult damages to evaluate. In corn, wheat, barley, rye, and sorghum germ damage is added to other factors related to total damage. Total damage is composed of heat damaged, sprouted, frosted, badly ground damaged, badly weather damaged, moldy, diseased, germ damaged, and otherwise materially damaged kernels. In oat evaluation, germ damage is related to determination of sound cultivated oats. Diseased oats, other grains and other damages causes the percent of sound cultivated oats to be lowered and thus the grain grade.

The evaluation of germ damage is very time consuming because it requires the manual removal of the grain pericarp, or hull in the case of oats and barley. This process becomes very tedious and requires a large amount of patience by the grain inspector. Because of the time required to evaluate germ damage, small samples of two to ten grams may be used for the determination. These small samples may not be representative of the large grain shipment. Mustiness is often associated with this damage, and a grain inspector may not check for germ damage if an odor is not present. Thus grain samples which have germ damage but no noticeable odor may slip by without being inspected for germ damage

This study was involved with finding a method which would improve on the speed and accuracy of the present method for germ damage evaluation. Six of the more difficult cereals (wheat, corn, sorghum, barley, rye, and oats) to evaluate for germ damage were involved in the study. Many mechanical and chemical procedures were found in the literature. However, it is only possible at this time to discuss the finding in the area of chemical procedures.

REVIEW OF LITERATURE

Moisture and Temperature: Bailey as early as 1917 pointed out the importance of low moisture levels for storage of grain. It was reported by Bailey and Gurjar (1918) and later by Bottomley et al. (1952) that moisture increases in grain are followed by a gradual increase in respiratory rate until a certain "critical" moisture level is reached. The critical moisture level is the moisture level at which grain heating starts. The critical moisture level was determined by Snow (1944) to be 14.6% for wheat and 13.7% for corn.

Enzyme activation which must occur before the onset of heating and other damages is also affected by moisture levels. Swanson, in 1934, was one of the first to study the relationship of moisture content and enzyme activity in wheat. Swanson (1934) reported that moisture levels of 27.0% were needed for activation of amylases. However, this did not explain the deterioration of grain at moisture levels as low as 14.0% to 15.0%. Linko and Milner (1959) working on enzymes present in wheat embryos found that glutamic acid decarboxlase and glutamic acid alanine transaminase were activated at moisture levels as low as 14.0% and 15.0%. In a later publication Linko (1960b) pointed out that enzymes which are activated at low moisture levels induce the

accumulation of vital metabolic intermediates. The critical moisture content for glutamic acid decarboxylase, transaminases and proteases is between 13.0% and 16.0%. Other enzyme systems such as the amylases and dehydrogenases are activated only when the proteins become saturated with water at about 25.0% or more.

Low temperature is also important in maintaining sound grain. High temperature causes chemical reaction rates to increase. High temperatures will cause protein denaturation. Linko (1960b) suggested that 38 ^{o}C would be needed for enzyme inactivation at moisture levels below 15%.

Moisture and temperature are also related to the growth of microorganisms and insects in grain. Since most germ damage is due to the action of fungi, low moisture and low temperatures are important for proper storage. Gilman and Barron (1930) observed that moisture requirements for certain saprophytic fungi coincide with the critical moisture level. Cotton et al. (1953) working on the determination of the rates of insect reproduction in grain showed that temperatures of 85 ^{o}F to 90 ^{o}F and moisture levels above 12% were necessary for the insects to reproduce.

Microflora and Germ Damage: Duvel (1909) and Shanahan (1910) were two of the first workers to describe deterioration of grain by fungi. As early as 1912, Darsie noted that moldy seeds produce more heating than non-moldy seeds. However, the relation between germ damage and mold contamination was not known to exist until much later. Thomas (1937) believed that germ damage resulted from toxic compounds produced by grain fungi. Thomas showed that grain germination percentages decreased after treating grain samples with filtrates of pure cultures of thirteen species and strains of fungi which commonly occur on grain. Aspergillus flavus had the greatest effect on decreasing the viability of grain.

Later research proved that growth of certain fungi contributed to the decrease of grain viability. Christensen (1955) showed that mold growth preceded decreases in germination and increases in sick wheat.

In an attempt to control fungal growth numerous studies were executed to discover the types of microflora and the condition required for their growth. Milner et al. (1947) in a microflora analysis of the fungi present on commercial sick wheat found 60% Aspergillus glaucus, 20% Penicillium, and the remaining 20% as A. niger, A. flavus, A. candidus, Nigrospora, Rhizopus, Trichoderm, and several unidentified bacteria. The results of microflora analysis on sound grain samples were 90% Alternaria, 5% Fusariun, and 5% Helminthosporium. Bacteria which require 95% relative humidity were found to cause decreases in viability at a 18% moisture. It would not be possible for bacteria to survive with lower moisture levels. Christensen (1955) writing in Cereal Chemistry described the microflora present in 26 samples of commercial grain containing 5 to 55% germ damage. Aspergillus restrictus, A. repeus, A. candidus, and A. flavus were the main species of fungi present on the grain. Papavizas and Christensen (1957) working with spring wheat samples inoculated with storage fungi found that A. restricutus cause rapid reduction in germination at 14.7% moistures. A. candidus and A. restricutus were both effective at 15 to 16% moisture levels in increasing germ damage and sick wheat. The importance of the A. restrictus ability to grow was not clearly shown until the work of Christensen and Linko (1963). Their paper reports the finding of small moisture differences on mold growth and compared moisture meters to the standard air drying method. Their findings indicate that certain moisture meters may give moisture values as much as 1% below the standard air oven drying method. Thus A. restrictus which may invade grain at 13.5% moisture

stored for 16 months may appear in grain shipment thought to be safe for storage. A recent study, by Christensen (1967b) with Durum wheat, gave rise to a better understanding of A. restrictus invasion of wheat. This study showed that invasion of durum wheat by A. restutus and A. hulophilicus at moisture levels of 13.4 to 13.6% at 25°C over a period of 493 days produced no decrease in germination percentage and brown embryo formation. Grain which was stored at moistures above 14.2% produced brown germ.

Christensen (1967b) reports that very little variation exists between the action of fungi on durum and other classes of wheat. Lack of variation also exists between different cereals. In a study of commercially graded Number Two Corn stored at various moistures and temperatures (Christensen 1967a), damage occurred only at moisture above 14.5% and at 25°C storage temperature. These conditions are very similar for damage to occur in wheat.

Observations of Germ Damages Not Resulting from Microflora: Swanson as early as 1934 observed that wheat which was stored in a partial vacuum and treated with ethyl mercury phosphate still developed sick wheat. Baking quality was also deleteriously effected by this type of damage. Carter and Young (1945) also produced "sick" wheat without fungi or bacteria being present in the grain mass. Samples of Fulcater wheat were sealed in containers at various moistures and temperatures for periods as long as 687 days. Temperatures of 104°F and moisture levels of 12.2% in the grain stored for a year produced 40% discoloration of the germ in the grain sample. Milner et al. (1947) in a storage study using various atmosphere showed the Carter and Young experiment to be correct. Milner (1948) writing about the research of Carter and Young stated that moisture levels as low as 12.2% would not possibly support the growth of molds and bacteria. Glass et al. (1959) working with wheat stored

in atmospheres of oxygen or nitrogen at temperatures of 20°C and 30°C, and moistures from 13% to 18% found that fungal activity was curtailed in a nitrogen atmosphere. However, discoloration of the embryo and loss of the seed viability did occur in the nitrogen atmosphere at moisture levels of 18% and temperatures of 30°C. Viability of the grain stored under nitrogen remained fairly high for 16 to 24 weeks even at 18% moisture content while grain stored in air deteriorated very quickly. The researchers concluded that inert storage of damp wheat at 30°C without damage is not possible due to active seed metabolism.

It is not fully understood what occurs during the deterioration of grain and the development of dark germ. An understanding of the environmental factors such as moisture and temperature was established by Roberts (1960). Roberts developed mathematical equations to predict the period of seed viability in wheat. He believed that similar equations could be developed for oats and barley. In later work Roberts (1961) reported the effect of age on seed viability. Grain samples sealed in glass tubes were found in the foundation stone of the Nuremberg City Theater in 1955. A document in the city archives stated that the seeds were placed there in 1832. Germination and moisture determinations of the 132-year-old seeds are shown in Table 1.

Table 1. The percentages of moisture and germination for three different cereals stored for 132 years.

Type of Grain	Moisture %	Germination %
Wheat	8.28	0.0
Oats	8.04	21.9
Barley	7.30	12.0

The mean monthly temperatures at a soil depth of 50 cm varied from 1.3°C in January to 17.6°C in July or an effective soil temperature of 10.6°C. These findings would indicate that even if grain is stored at relatively low moistures and temperatures grain viability is a decreasing function of age.

Nonenzymic Browning and Biochemical Changes: Milner, Christensen, and Geddes (1947) speculated on the possibility of nonenzymic browning as an explanation of darkening of germ. Linko and Milner (1959) found that considerable changes in composition occur immediately after wetting grain. Changes observed in the wet grain were a rapid decrease of the quantity of free glutamic acid and a corresponding increase in free gamma aminobutyric acid. An earlier observation reported by Jones and Gersdorff (1941) indicated the protein nitrogen decreased and the amino acid nitrogen increased after prolonged storage of wheat. Linko (1961a) found that as the viability of wheat decreased the glutamic acid content also decreased. Linko and Sogn (1960) working on the measurement of glutamic acid decarboxylase found a correlation coefficient r of .921 to exist between the enzyme activity and germ damage. The zone electrophoresis studies of Linko (1961b) also indicated that if a high glutamic acid peak was accompanied by a high basic amino acid peak, deterioration is very extensive and the wheat had been dead for a long time.

Montgomery and Smith (1956) noted that the quantity and composition of soluble carbohydrates in wheat is effected by the storage temperature. Linko, Cheng, and Milner (1960) found the changes in the compositions of wheat germ after long periods of storage were marked by an increase in reducing sugars a decrease in non-reducing carbohydrates. The formation of reducing sugars preceded the appearance of browning which in turn was followed by an increase in fluorescence at moisture levels of 15%. Increases in reducing sugars of

stored grain have been observed by many research workers. The level of glucose and fructose, measured after browning, did not show as large an increase as did the increase of sucrose and raffinose. It was believed that the low levels of glucose and fructose were caused by these sugars being involved in the browning reaction.

The above research would indicate that browning in grain may result from non-enzymic browning. Maillard (1912) first described the reaction as a condensation of carbohydrates and protein material. He suggested that the active group of the sugar reacts with the amino group of protein or amino acids to produce a shiffs base. Successive decarboxylations and dehydrations caused the formation of dark pigments called "melanoidins". Recent studies of model systems containing glycine and glucose have produced increases in light absorbance at pH levels of 5.5 to 5.6. Darkening of the glycine-glucose solution occurred in five to 10 days at 55°C (Song et al., 1966). A kinetic study by Song and Chichester (1966) of the glucose-glycine model has produced a possible mechanism for nonenzymic browning. In browning of germ a nonenzymic process is very likely to occur.

Changes in lipids have long been used to measure the deterioration of grain. An article that appeared in Northwest Miller (Anon., 1929) noted that an increase in fat acidity of grain occurred after unfavorable storage. The numerous storage studied on fungi described earlier recorded the increase in fat acidity. Geddes (1935) showed that fat acidity increased with increases in mold growth. Fat acidity results from the actions of lipase on lipid. Daftary and Pomeranz (1965) found that grain deterioration was accompanied by formation of four unidentified fluorescence compounds. It was also noted that

breakdown of polar lipids is far more rapid than the formation of free fatty acids or the disappearance of triglycerides.

Greaves and Hirst (1925) found that, during the storage, inorganic phosphorus increases at a slower rate in whole grain than it does in milled products. Most phosphorus is found in grain in the form of phytin, a calcium-magnesium salt of inositol phosphoric acid which is not utilized by man. Phytin is acted upon by phytase which releases the phosphorus. Glass and Geddes (1959) measured both phytic acid phosphorus and inorganic phosphorus in sound and damaged wheat. Addition of the percentages of different phosphorus forms revealed that inorganic phosphorus results from the hydrolysis of phytic acid. Measurements of inorganic phosphorus and fat acidity showed that inorganic phosphorus developed a week before fat acidity. Samples which contained A. restrictus or A. flavus developed higher levels of inorganic phosphorus.

Methods for the Evaluation of Germ Damage: Acidity measurement has been used for many years to evaluate deterioration of grain. The second chapter of the Anderson-Alcock book (Zeleny, 1954) on storage describes three types of acidity as fat acidity, amino acid acidity, and phosphate acidity. The increase of amino acids in grain or cereal products is determined by extraction of amino acids and then titration with a base of known normality. Snell and Snell (1954) have described a method for inorganic phosphorus and phytin phosphorus. Present studies would tend to indicate that fat acidity and inorganic phosphorus are the better indexes of grain condition. A method for fat acidity described by Baker et al. (1957) decreased the old extraction time from four hr to six min. Correlation with cob rot in corn and fat acidity was $r = 0.97$. Baker (1961) developed a colorimetric method for the determination of fat acidity in grain. The method employed the Stein mill extraction process

with cupric acetate which produces a color complex with fatty acids which can be measured colorimetrically. A recent colorimetric method for the evaluation of fat acidity was developed by Mackenzie et al. (1967) using a dye, Rhodamine B, which was described earlier by Fegiel (1956) as a spot test for organic acids. The method was used for a rapid evaluation of fat acidity in animal tissue. Studies of damages in 245 samples of corn (Zeleny and Coleman, 1938) showed that damaged kernels as determined with % grading damage resulted in a correlation coefficient of 0.90 between the fat acidity reading and the % of damaged kernels. Fat acidity compared with the logarithm of the percent of germination for 209 samples was -0.85. The relationship between phosphate acidity and germination correlates better than it does with amino acid acidity.

The relation of fluorescence to germ damage was established by Cole and Milner (1953). Extraction of whole wheat and germ with .2N HCl produced liquids with increasing optical density and fluorescence properties. A correlation coefficient of 0.748 was reported for graded germ damage with fluorometric values. Germination also correlated well with fluorescence determination with an r value of 0.775.

Measurement of certain enzymes has also been shown to be successful in determination of grain damage. Linko (1961a) outlined the procedure for determination of glutamic acid decarboxylase. A method was developed to determine the enzyme activity by measurement of the CO_2 produced in 30 min. Correlation of glutamic acid decarboxylase activity with germination was 0.920 as found by Bautista (1962).

Chemical dyes have also been used on grain to make damages more visually apparent. Perhaps the best known dye for determination of viability is 2,3,5-

triphenyl tetrazolium chloride which was described by Lakon (1940) for the detection of the living parts of grain. Baird (1950) et al. provided the research for establishing a chemical grain viability test. Correlation of the tetrazolium dye method with standard germination tests is very high with an r value of 0.95. The red formazan form of the dye results from the presence of a reducing enzyme succino dehydrogenase (Jambor, 1954). Schenk (1957) found that high amounts of mold may cause false values to result from the test. Weith (1959) found the method difficult to use in testing viability of heat damaged grain.

Wierzbowski (1964) working with 2,6-dichloroquinonechlorimide found that damaged grains were colored brown to greenish-brown. Mold mycellium and sprouted grain were also colored by the dye. Color usually appeared after 60 minutes of soaking in an .5% solution of the dye.

Christensen and Quasen (1959) described a chemical procedure for revealing germ damage in corn and wheat. The method employed a 2% solution of sodium hypochlorite to bleach the germ covering to enable a quick visual inspection of the wet kernels. Their results showed good correlation between the conventional method of grading and the bleaching method.

MATERIALS AND METHODS

Materials: Commercial grain samples having various degrees of damaged grain were obtained from the Federal Grain Inspection Laboratory in Kansas City, Missouri. Additional samples of oats and sorghum were obtained from the State of Kansas Inspection Stations at Atchison and Salina. Two rye samples having germ damages were obtained from the Department of Agronomy at Kansas State University. Sprouted kernels were prepared in the laboratory at the Department of Grain Science and Industry by placing kernels in moist paper for 2-4 days.

Wierzbowski Dye Method: A solution of .5% 2,6-dichloroquinonechlorimide was prepared by dissolving .5g of the dye in 80 ml of 95% ethanol. The alcoholic dye solution is stabilized with 20 ml of 10% NaCl solution. The kernels tested were placed in a small beaker and covered with the dye solution. After an hour of soaking the kernels were removed from the dye and examined. The dye may be reused if its color is not overly dark.

Bleaching Procedure Using an Open Container: The method is applicable for use in a hood or well-ventilated room and is a modified procedure of the Christensen Qasen method.

A grain samples of 20 to 30 g was placed in a 1000 ml beaker and covered with 75 ml of a 6% solution of sodium hypochlorite. The beaker was transferred to a magnetic-stirring hot plate which had warmed for 15 min. and was able to bring 250 ml of water at 25 ^{o}C to a rolling boil in 10 min. After heating for 3 min., the beaker was removed and the grain was washed with cold tap water 3 times. The grain was dried by spreading it in a bread pan and heating for 5 min. in a 150 ^{o}C air oven.

Bleaching Procedure for Oats and Barley: The open container bleaching method was used except the grain was first treated with 100 ml of 15% HCl. After the mixture boils for 2 min., the acid is poured off. A cold tap water wash helps remove excess acid and hulls. The wet grain is then bleached using the open container method. Oats and barley hulls may also be removed by allowing the grain to set in 6% commercial bleach for 18 hr.

Bleaching Procedure for Closed Container: This procedure is similar to the open container procedure and is applicable for use in a room with poor ventilation. A special flask and assembly, as shown in Fig. 1, was used for this procedure. The 5/8" side arm, on the 1000 ml Erlenmeyer flask, was attached so that 150 ml of solution could be contained without over flow. The grain sample and a sufficient amount of bleach to cover the grain were added using the funnel. The tubing clamp under the funnel was closed and the hot plate was moved under the flask after the tap water was turned on to produce a partial vacuum. The water line going to the drain was pushed down into the drain to avoid the whiplash of the tubing. After the foam subsided ($2\frac{1}{2}$ to 3 min.) the hot plate was removed and both tubing clamps were opened and the grain was washed. The flask was supported by a clamp in order to let the flask tilt 45^{o} to wash out all the grain into the wire basket. The same drying procedure as described in the open container procedure was used.

Standard Chemical Grain Analysis: Grain samples were ground in a Wiley Mill using a 30 mesh screen. Samples were ground not more than 12 hours before undergoing analysis. Screw cap sample bottles were used for sample storage before and after analysis. Moisture was determined using the air oven procedure as outlined in AACC method 44-19 (1962). If analysis of fat was to be determined the vacuum oven method was applied as described in AACC method

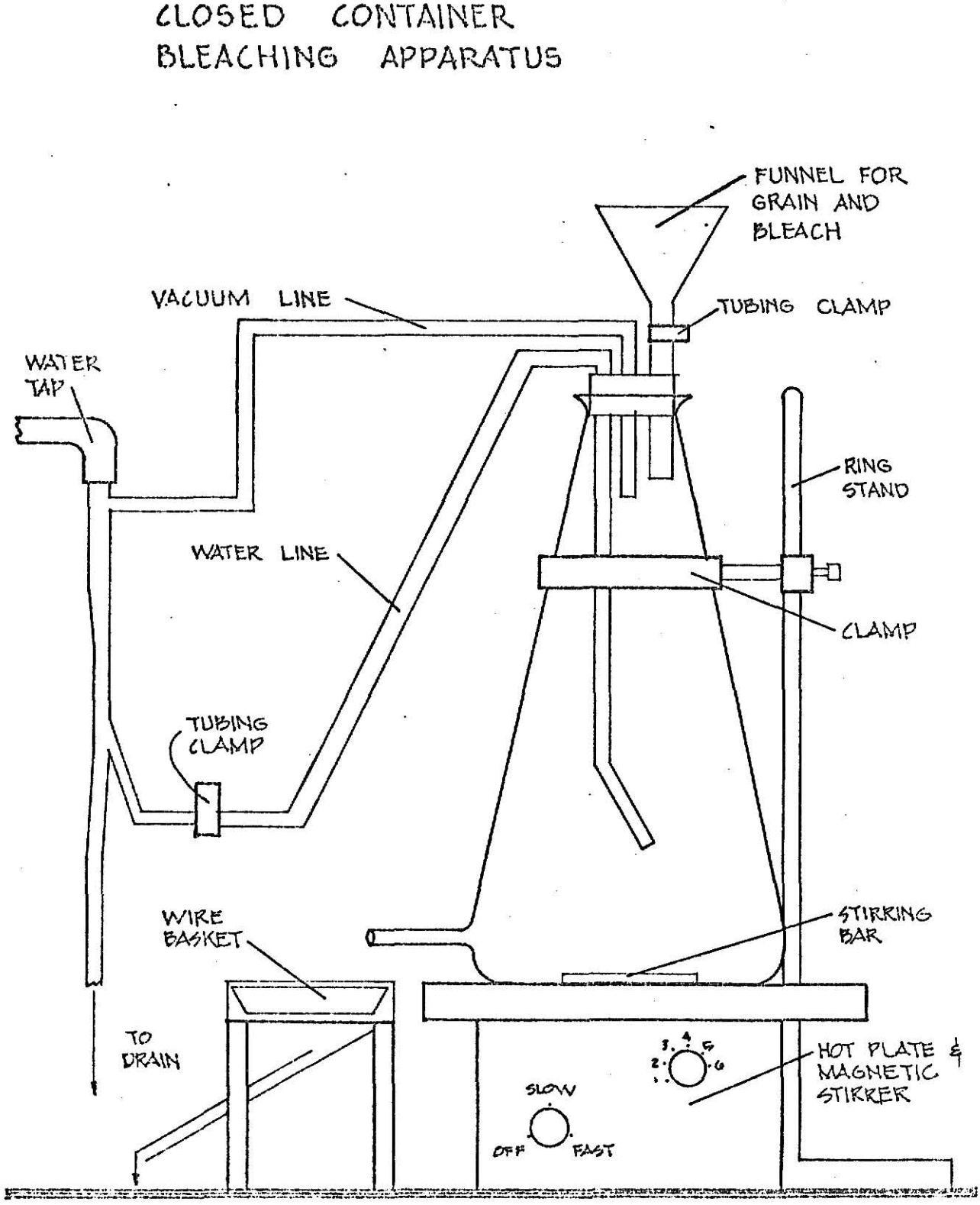


Fig. 1. Closed container bleaching apparatus for more efficient grain washing to be used in area of poor ventilation.

44-40. Crude or Kjeldahl protein was determined using the procedure as described in AACC method 46-10. The procedure used for evaluation of total ash was AACC method 08-01. Crude fat was determined on a 2 g sample which was dried 5 hr. in the vacuum oven for moisture determination. AACC method 30-20 was followed for the crude fat determination. Crude fiber was determined on the residue after moisture and fat analysis. AACC method 32-15 was used for the crude fiber analysis.

Grain Grading Procedures: All samples used in the grading studies were cut by a 12 spout multiple divider (Great Western) or by the Burrows Grain Divider. Samples which were to make up bleached and unbleached pairs were cut from one portion of grain using the Burrows Grain Divider. Sample sizes ranged from 10-30 g and small plastic bags were used for sample containers. Grain samples which had visible mold growth on the germ were not used for grading.

Various grading procedures were used in trying to establish a correlation between the bleaching procedure and the standard grading method now in use. The two methods used in obtaining data for correlation were the known sample pair and the unknown sample pair. In early studies the known sample pair method was used, in which the grain inspector knew the bleached sample that corresponded to the unbleached sample because of their identical markings. The unknown sample pair procedure removed the possibility of error due to the grader anticipating the sample damage and not accurately evaluating the grain. Samples of grain were not numbered as pairs but were numbered individually and randomly from 1 to n number of plastic bags of bleached and unbleached grain. Instructions for grading were for the grader to grade only for germ damage, to grade as would normally be done in commercial practice and to divide samples

using a divider if smaller sample sizes than that contained in the plastic bag were used.

Method of Statistical Analysis: Analysis of variance and regression analysis were the two statistical techniques used in this study. Raw data to be used for analysis of variance was coded by adding 1.0 to each value. Simple analysis of variance and tests for interaction assuming normal population and homogeneous variance (Fryer, 1968) were determined using a desk calculator.

Correlation and regression analysis values were determined using the IBM 360 computer. The multiple regression program was supplied by the Kansas State University Department of Statistics. A number of statistical values were obtained from the analysis. The coefficient of linear correlation (r) and coefficient of determination (r^2) were determined using this program. The r^2 value gives an indication of the scatter of points around the regression line. The percent of $1-r^2$, obtained by multiplying $1-r^2$ by 100, gives an indication of unaccounted for variance (error). The program provides for an F test of the r^2 value which tests the null hypothesis (H_o) that no relationship exists between the independent and dependent variables versus the alternative hypothesis (H_a) that a relationship does exist. If the table F value is exceeded by the calculated F then the null hypothesis is rejected and the alternative hypothesis is accepted and the relationship is believed to be significant. The regular coefficient (B) is the slope of the regression line given by the equation $y = Bx + A$. The t test is designed to evaluate a null hypothesis that $B = 0$ versus an alternative hypothesis that $B \neq 0$. Thus, if the table value of the t is exceeded by the absolute value of the calculated t then the B is considered significantly different from zero and the

correlation does not result from chance. Significance for the r values was determined from table 8 of Statistics: An Introductory Analysis (Yamane, 1967). Levels of significance are marked with 1, 2, or 3 asterisks for levels of .05, .01, and .001 respectively.

Germination Test: Samples of 100 kernels were surface sterilized with .1% mercuric chloride solution for 2 min. The kernels were washed 5 times with tap water and then placed crease down on paper towels. The paper towels were folded to prevent the grain from falling out, rolled and soaked in water. The paper towels holding the kernels were placed in a 600 ml beaker and covered. The beakers were stored at room temperature. Germinated seeds were removed every 2 days for a period of seven days before terminating the test.

Glutamic Acid Decarboxylase (GADA): A modified Linko (1961a) procedure was used for this determination. Grain samples were ground in a Wiley Mill using a 20 mesh screen and stored in screw cap bottles. Sandstedt-Blish pressure meters were employed having ethyl lactate in the manometers instead of mercury. Thirty gram samples of ground grain were mixed with .1M glutamic acid in .067M phosphate buffer in the pressure meter cups. The pressure meters were assembled and placed in a 30°C water bath. After a 5 min. equilibration the manometers were adjusted to zero and pressure readings were recorded after 30 min.

Waring Blendor Dehulling Process: The Freeman and Watson (1969) procedure for peeling sorghum was adapted for removing the hulls of oats and barley. The Waring Blendor blades were covered with rubber tubing which had been placed in boiling water in order to facilitate slipping the tubing over the blades. Twenty g of oats or barley were placed in a blendor and covered with 250 ml of hot water (140°F). A variable voltage supply was used to start the blendor at a low speed to avoid loss of water. Blendor speed was increased from 0 to

15,000 rpm in 5 sec. and left at full speed for 30 sec. of mixing. The hulls floated to the surface and were removed by hand after the blendor was stopped. After the removal of the hulls, the blendor was run an additional 5 sec. at full speed using a 5 sec. interval for increasing the speed. The hulls were floated off with hot water and the kernels were dried at 150° C for 5 min.

RESULTS AND DISCUSSION

Wierzbowski Dye Method: The top row of boxes in Plate I shows various grain samples after treatment with .5% 2,6-dichloroquinonechlorimide. Soaking time in the dye was varied for various samples but did not exceed 2 hr. in any test.

The first section of Plate I shows samples of sound sorghum and wheat before and after treatment with the dye. In the case of each grain the first column is untreated. Note that the dye's main visible effect on the grain is to darken the kernel color. Soaking time for these kernels was one hour and 30 min.

Section 2 shows the effect of the dye on hulled and unhulled oats. The first column of grain is sound untreated oats which is followed by a column of kernels of the same oats sample after an hour of dye treatment. Again the visual distinction between the dyed and undyed grain is the darker color. The third column of kernels in the second section shows sound groats which were dyed as whole oats and then dehulled by hand. The final columns of the second section shows grain which was hulled using the Waring Blendor. In this process kernels are sometimes broken or bruised from the blade impact. Black coloration occurs not only on bruised areas of the groats but also on the germ. Kernels which have been flattened are more extensively colored than the

kernels near the top of the column which have had less physical punishment. The number of dyed or black germs would indicate that the dye does not show germ damage but indicates damage to the hull which covers the germ. This same occurrence was observed with hulled and unhulled barley. The germs of corn, sorghum, and wheat are also colored by the dye if the pericarp covering the embryo is broken. Scraping of the germ face of wheat after soaking in the dye for an hour will show germ coloration only in an irregular pattern characteristic of liquid seepage into the germ area.

Section 3 of the top row of Plate I shows the effects of soaking kernels of wheat, which were previously bleached in hydrogen peroxide and sodium hypochlorite for 2 hr. in the dye solution. The darkening of the germs is again seen in this case. The kernels which were pretreated by 6% sodium hypochlorite were all sound but were blackened by the dye. The hydrogen peroxide bleached grains, shown on the left, were believed sound, but bleaching with this bleach does not give a good indication of damage. Note that the germs of the hydrogen peroxide bleached grain did not color as well as did the sodium hypochlorite bleached grain.

Section 4 shows the effect of the dye on sprouted grain. The darkening of the sprout results from the dye treatment. Mold growth on grain is also shown by the darkening of the grain, but is not pictured.

Tests on the rapidity of dye coloration showed that wheat which had the germ covering removed was blackened in 15 minutes. Moldy grain, sprouted grain and sound samples of the six types of grain under study were dyed after 35 min. Longer periods of soaking of the grain in the dye solution produced darker coloration.

Bleaching Factors: The elevated boiling temperature of the bleaching solution is predicted by Roults Law. Roults Law states that boiling point elevation is related to the product of a solution constant and the number of moles of solute ions present in the solution. Thus a 12% solution of sodium hypochlorite should boil at a higher temperature than does a 6% bleaching solution. A series of boiling point determinations showed that 75 ml of water placed in a 1000 ml beaker with 20 g of yellow grain sorghum boiled in 2 min. 25 sec. at 97°C. Under the same conditions the boiling point was reached in 2 min. 30 sec. at 99.5°C with 6% commercial bleach. These standard conditions were again used for 12% commercial bleach to produce boiling in 2 min. 45 sec. at 105°C. Prolonged heating of the bleach and grain will result in a decrease in temperature after 4 to 5 min. of heating. The maximum heat point is marked by the visual formation of large amounts of foam which will raise to the 800 or 900 ml mark of a 1000 ml beaker. Limited testing would seem to indicate reduction of heat output of the hot plate would slow the foam formation.

Results for the time studies are shown pictorially in the second row of Plate I. Kernels treated with bleach for 1 min. do not seem to display any of the characteristics of bleached grain as do the kernels which received 3 min. or more of bleach treatment. After 2 min. of treatment bleach characteristics are visible. However, coloration is still darker than those samples shown in "sample treated after 3 to 4 min." The kernels treated in hot bleaching solution for 20 min. show some germ deterioration and irregular coloration. The prolonged effect of bleach treatment is shown in the sample which was soaked in 6% commercial bleach for 24 hr. at room temperature. Long periods and higher bleach percentages will cause greater kernel deterioration, leaving only the center portion of the kernel.

Bleach concentration is also an important factor in achieving a desirably treated sample. Christensen reported using a 2% solution of NaOCl in bleaching grain. However, the concentration series shown in Plate I would indicate that a better job of bleaching can be accomplished with bleaches of higher percent of sodium hyperchlorite. Note that some bleached kernels do not show the characteristics of bleached grain and appear more like frostbitten wheat than like the polished kernels resulting from the treatment by a higher percent bleaching solution. Increased concentrations of sodium hypochlorite produce fewer of the dull-appearing kernels which are not bleached. The bleach concentration series would seem to indicate that an adequate concentration of sodium hypochlorite is about 6%, since visual determination of germ damage is not hampered by the presence of pericarp above the germ. Bleach concentrations of 6% or above show acceptable results and basically the same coloration except for the 12% bleach.

Different Bleaches: The sodium hypochlorite bleaching action on grain was found to be unique among the other bleaches tested. Exploratory tests with sorghum and wheat showed that chlorine gas produced from a chemical generator causes severe blackening of the grain. Solutions of various concentrations of calcium hypochlorite which were heat bleach tested on grain produced bleached grain with a chalky appearance.

Plate I, row 5 shows wheat treated with 30% hydrogen peroxide using a 3 min. heat treatment. Hydrogen peroxide provided excellent whitening of wheat kernels but little, if any, indication of damages below the germ covering. A sample of wheat having known damages was bleached with H_2O_2, dried, and then bleached with NaOCl which did show the germ damage. The dyed bleached kernels also indicated the difference between the H_2O_2 bleach and the NaOCl bleach.

In row 1, section 3, the hydrogen peroxide kernels (shown on the left) do not show darkening of the germ or kernel surface as do the kernels treated with sodium hypochlorite. It should be remembered that all the sodium hypochlorite kernels were shown to be sound after bleaching.

Sections 2 and 3 of the last row of Plate I show kernels bleached with 6% NaOCl and 6% commercial bleach containing sodium hypochlorite. The almost identical appearance between kernels bleached with 6% sodium hypochloride and 6% commercial bleach show that performance quality of the bleach is not significantly affected by additional agents added to the bleach. This also means use of commercial bleaches makes a savings of $5.00/gal. of bleach. Section 4 of the last row of Plate I shows the results of using an old 12% commercial bleach on wheat. The same 12% bleach 6 months earlier produced a bleached wheat which is shown in section 4 of row 4.

Chemical Analysis: It is believed that the bleaching process is actually a combined process of whitening and chemical dehulling. Table 2 shows the results of chemical analysis of untreated grain, grain bleached in 6% commercial sodium hypochlorite and grain which was allowed to soak for 24 hr. at room temperature. Bleached samples were dried for 5 min. at 130°C in an air oven before grinding. Certain patterns are present in each triplet of analytical results. Moisture and ash increase in each group of three with the greatest difference between the untreated grain and the grain bleached for 24 hours. The increase in ash is due to the absorption and adsorption of chemical compounds present in the bleach. Protein content in most grains treated with bleach decreases: .6% to .7% in wheat, .2% to .9% in sorghum, and .7% in the rye sample. In one corn sample there was a decrease of .1% and in the other sample there was a .2% increase. No significant difference is apparent

Table 2. Chemical Analysis of Bleached and Unbleached Grain.

Sample	14% Moisture Basis Moisture	Ash	Protein	Fat	Fiber
Rye					
Unbleached	9.88	1.92	12.6	1.50	2.55
3-minute bleach	13.16	1.93	12.8	1.45	2.31
24-hour bleach	14.51	3.78	11.9	1.35	1.94
White Corn					
Unbleached	13.42	1.25	7.9	3.62	2.24
3-minute bleach	13.60	1.25	8.0	3.98	1.58
24-hour bleach	16.71	2.37	8.1	3.62	1.39
White Sorghum					
Unbleached	11.37	1.39	8.9	2.97	2.13
3-minute bleach	13.67	1.52	8.8	2.88	2.28
24-hour bleach	17.39	3.70	8.7	3.03	1.54
Hard Red Winter Wheat #1					
Unbleached	11.37	1.73	13.1	1.32	2.48
3-minute bleach	12.90	1.78	13.3	1.27	1.74
24-hour bleach	14.34	3.01	12.5	.97	1.30
Hard Red Winter Wheat #2					
Unbleached	10.35	1.69	11.1	1.42	2.28
3-minute bleach	10.62	1.61	11.2	1.58	1.41
24-hour bleach	13.65	3.32	10.4	1.49	1.32
Yellow Corn					
Unbleached	10.18	1.34	8.1	3.68	2.03
3-minute bleach	11.00	1.35	8.2	3.97	1.37
24-hour bleach	21.19	4.13	8.0	3.50	1.15
Yellow Sorghum					
Unbleached	11.60	1.46	7.2	2.49	2.11
3-minute bleach	14.16	1.43	7.1	2.64	1.86
24-hour bleach	13.26	4.25	6.3	2.81	1.14

Explanation of Plate I

Row 1. Grain treated in 2,6-dichloroquinonechlorimide .

Section 1. Sorghum and wheat before (left) and after (right) the dye treatment.

Section 2. Dye action on whole and hulled oats.

Section 3. Dye action on grain previously bleached with 30% H_2O_2 (left) and 6% NaOCl (right).

Section 4. Results of dye treatment on sprouted grain.

Row 2 and half of row 3. The results of treating wheat with 12% commercial bleach (NaOCl) using constant heat for various times.

Last half of row 3 and row 4. The results of bleaching wheat in various concentration of commercial bleach (NaOCl) for 3 min. using a constant heat source.

Row 5. The action of different bleaches on wheat.

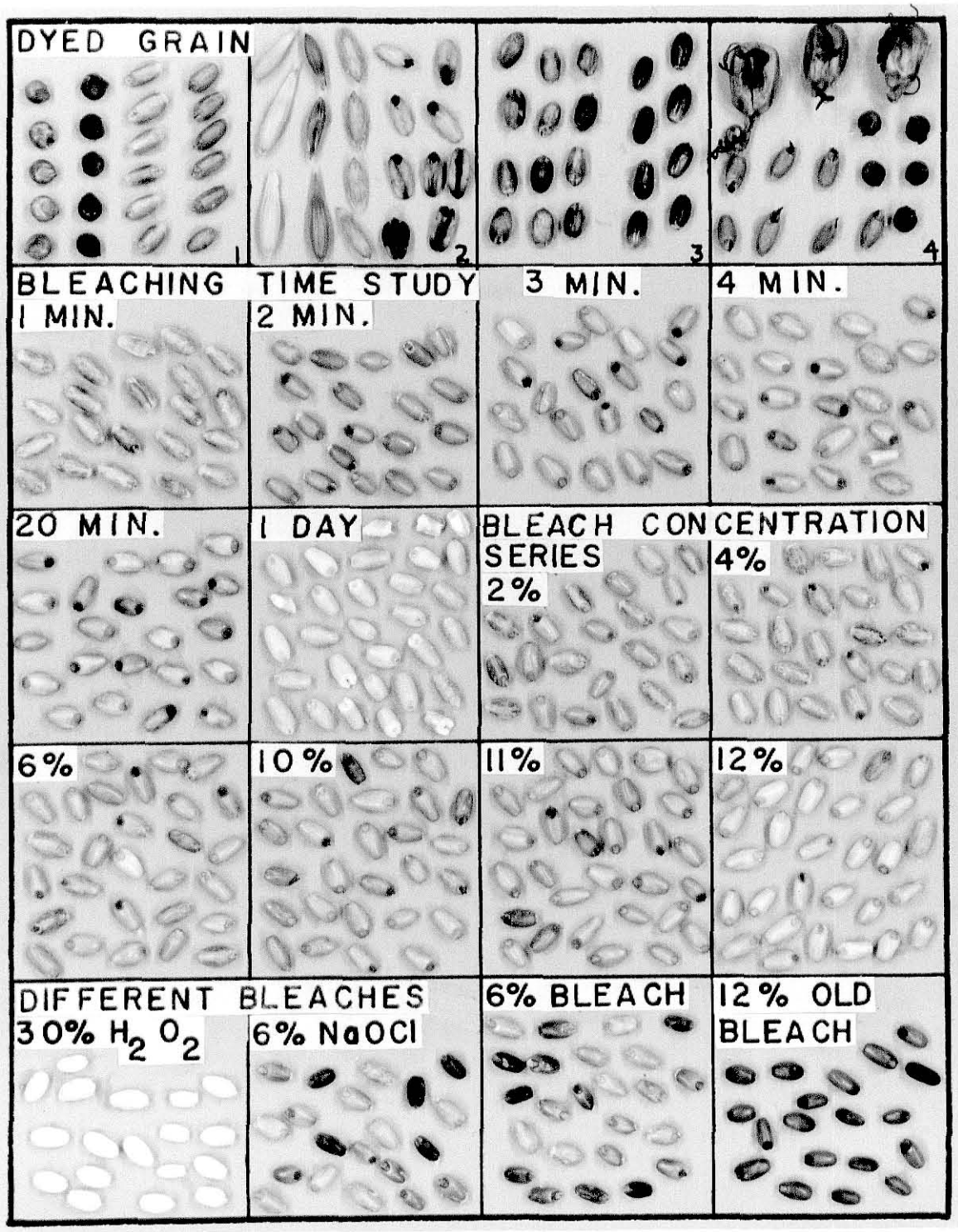
Section 1. Results of a 3 min. heat bleach using 30% H_2O_2.

Section 2. Results of a 3 min. heat bleach using a chemically prepared solution of 6% NaOCl (National Formulary).

Section 3. Results of a 3 min. heat bleach of 6% commercial bleach (NaOCl).

Section 4. The results of a 3 min. heat bleach using an old 12% commercial bleach (NaOCl).

Plate I


DYED GRAIN
1
2
3
4
BLEACHING TIME STUDY
1 MIN.
2 MIN.
3 MIN.
4 MIN.
20 MIN.
1 DAY
BLEACH CONCENTRATION SERIES
2%
4%
6%
10%
11%
12%
DIFFERENT BLEACHES
30% H_2O_2
6% NaOCl
6% BLEACH
12% OLD BLEACH

between the percentages of crude fat in any particular group. The results of the crude fiber analysis show a very strong decreasing trend in each sample group. A decreased crude fiber percentage was found to exist between all untreated grain samples and 3 min. bleaches except in the case of white sorghum. This sample was not rechecked and this discrepancy could result from analysis error since the analysis did show a .59% decrease of fiber in the sample treated for 24 hr. The fiber loss occurring in wheat was about 1% for 24 hr. treatment. These analytical results confirm the visual observation of hull and bee wing being removed by the bleach solution.

Bleaching All Grain: Plate II shows the results of bleaching wheat and corn with 6% commercial bleach. In the case of wheat, the germ damage is readily apparent as dark or black colored germs. Sound germ is marked by light to tan colored germ. Heat damage is also much more apparent as may be seen by the dark brown kernels which contrast greatly with the sound white or light tan colored kernels. Generally, light colored grain such as white wheat will bleach better than dark colored wheats such as red winter wheats.

There is little difference in appearance between the samples of bleached and unbleached corn when compared to the striking difference in other grains. Those differences which do appear are a lightness of color and damage is more discernible in the bleached corn sample. Corn is not as difficult to evaluate for germ damage as is wheat.

Sorghum presents a very difficult germ grading problem because of its small kernel size and the well concealed germ. Bleaching greatly aids in revealing the germ (Plate III). Variation in bleaching ease is also observed in sorghum.

Rye also may be bleached with 6% sodium hypochlorite to reveal the germ damage. Bleached and unbleached rye samples are shown in the second row of Plate III.

Grain having heavy hulls such as oats and barley have always presented difficulties in grading. The electric barley pearler has long been used as part of the regular barley grading procedure. Once the hull is removed from barley, heat damage and germ damage can be easily evaluated. However, oats are not successfully dehulled using the barley pearler and must be dehulled by hand. Germ and heat damage evaluation is made by determining the percent damage by weighing both the damaged kernel and its hull. Note that the bleaching process is not sufficient in some cases to remove the hulls as is shown in the bottom row of Plate III by the two kernels with partially remaining hulls. Bleached barley is shown in the top row of Plate IV.

Since germ damage is never considered alone unless it is the single grading factor, the effect of bleach must be considered on other types of damage. Black tip fungus which is visible on wheat kernels in the top row of Plate II is totally removed by the bleach action. Sprout shown in the second row of Plate IV is also removed in part or totally in some cases. Surface mold as shown on the bottom row of Plate IV is completely removed from the corn. Other surface damages would also be difficult if not impossible for the grader to evaluate. This would mean that evaluation of germ damage must be made on a separate sample and then added to other percents of damage found in an untreated sample.

Early Correlation Studies: The early studies aided in establishing a relationship between the bleached and unbleached method. The Grain Division of

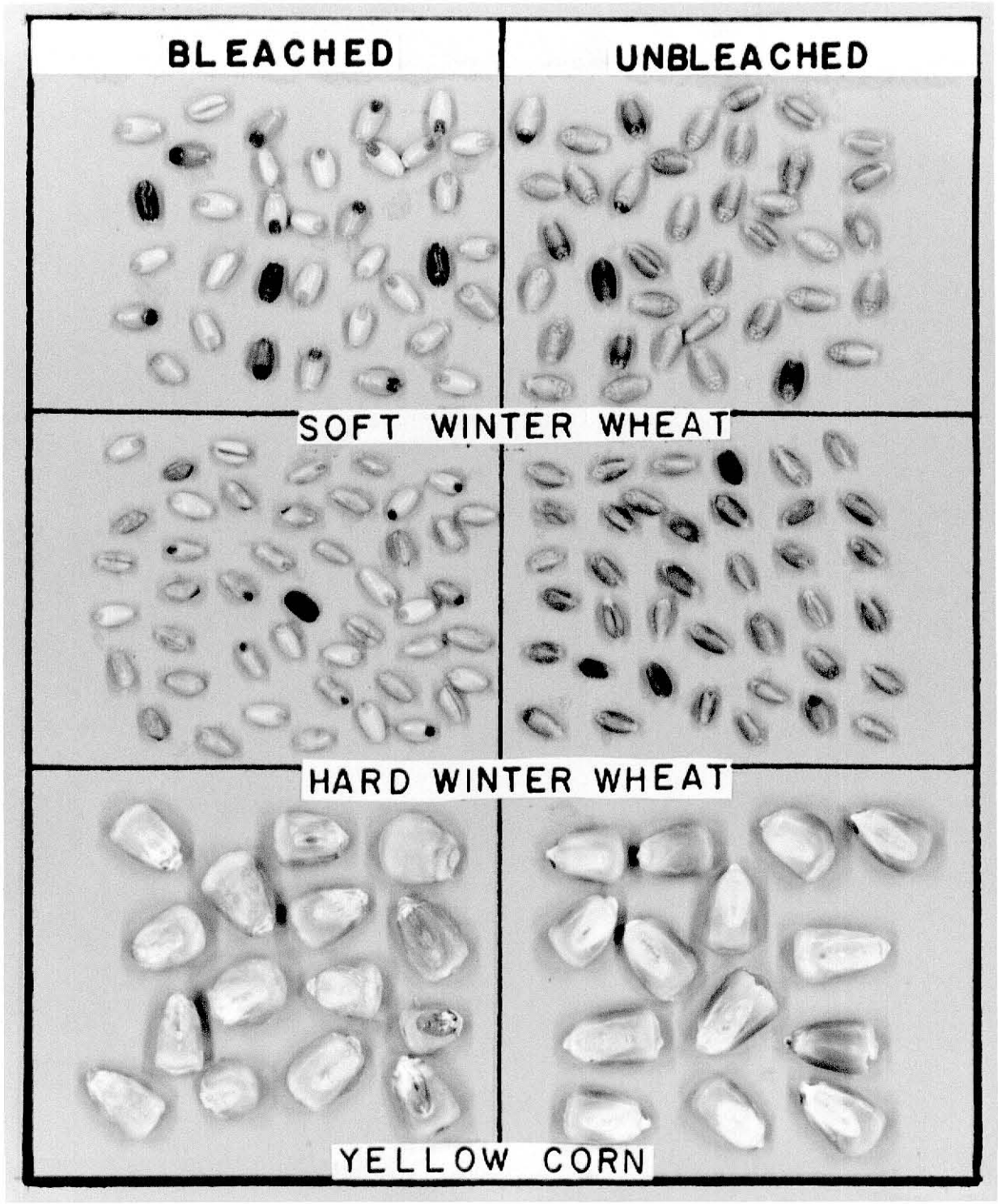
Explanation of Plate II

Row 1. Soft winter wheat bleached with 6% commercial bleach (NaOCl) and unbleached wheat.

Row 2. Hard winter wheat bleached with 6% commercial bleach (NaOCl) and unbleached wheat.

Row 3. Yellow corn bleached with 6% commercial bleach (NaOCl) and unbleached corn.

Plate II


BLEACHED
UNBLEACHED
SOFT WINTER WHEAT
HARD WINTER WHEAT
YELLOW CORN

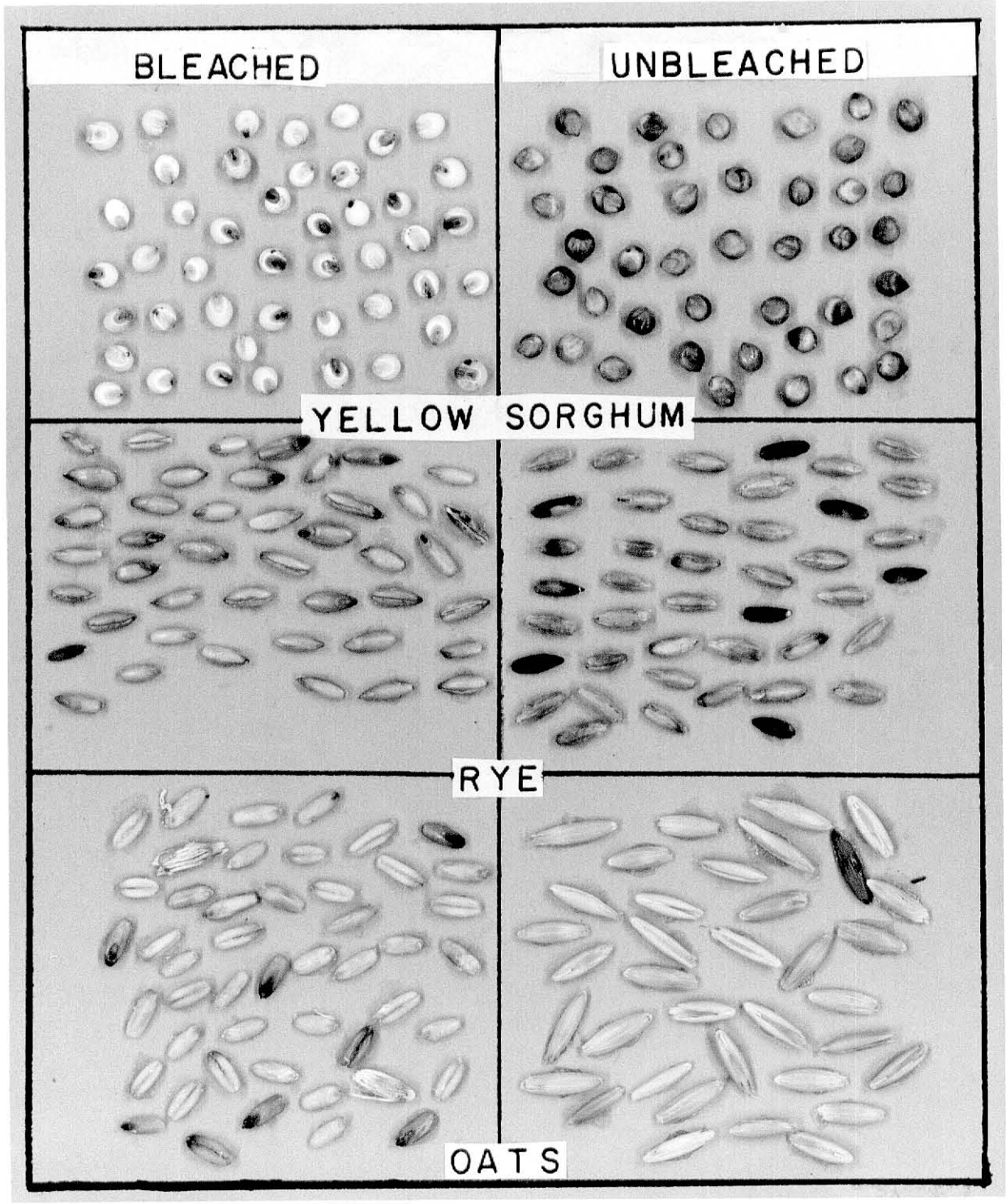
Explanation of Plate III

Row 1. Yellow sorghum bleached in 6% commercial bleach (NaOCl) and unbleached sorghum.

Row 2. Rye bleached with 6% commercial bleach (NaOCl) and unbleached rye.

Row 3. Oats treated with 15% HCl and 6% commercial bleach (NaOCl) and sound untreated oats.

Plate III



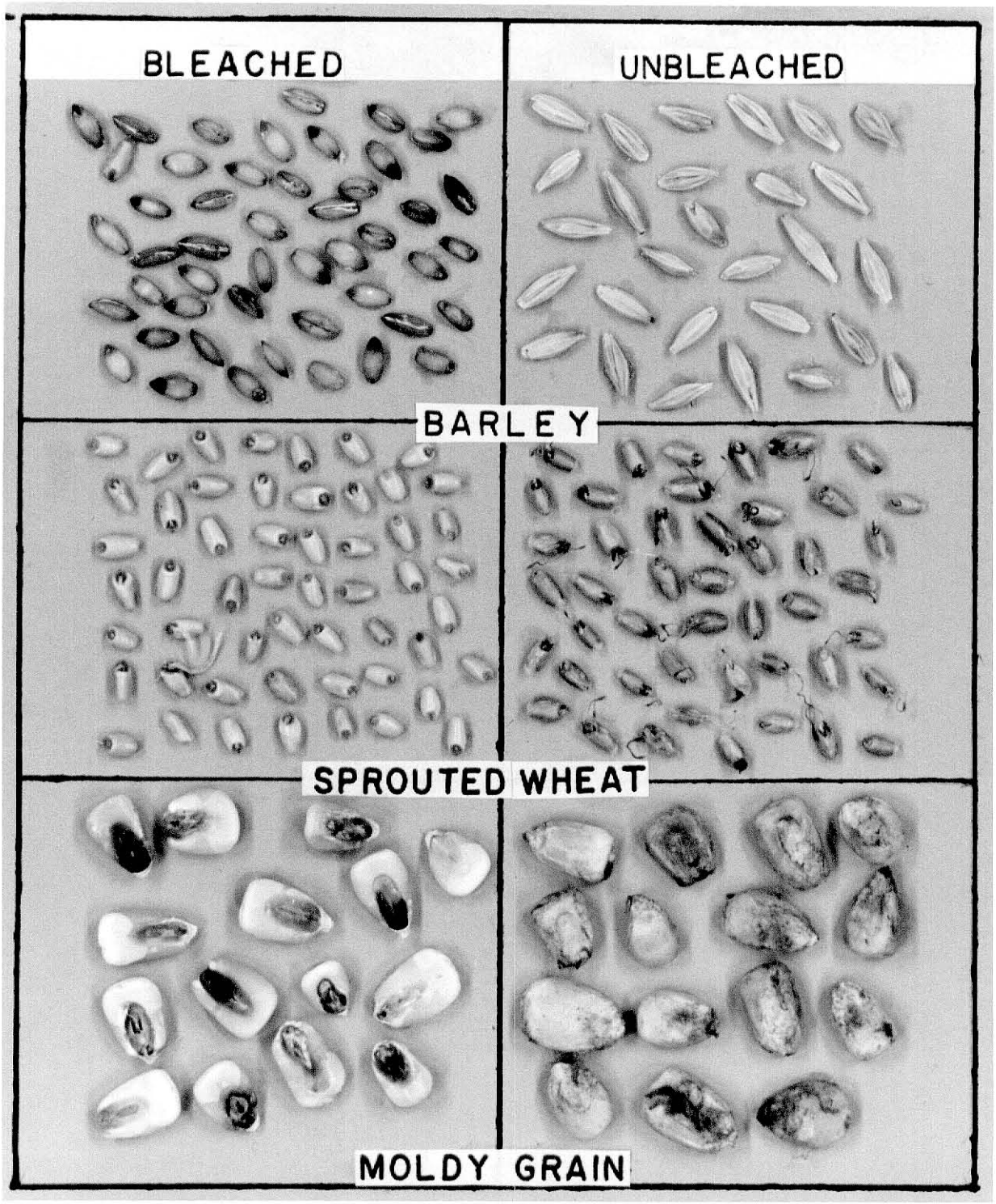
Explanation of Plate IV

Row 1. Barley treated with 15% HCl and 6% commercial bleach (NaOCl) and untreated barley.

Row 2. Sprouted wheat before (right) and after (left) bleach treatment.

Row 3. Moldy corn before (right) and after (left) bleach treatment.

Plate IV



the Consumer and Marketing Service, U.S.D.A., at Kansas City, Missouri, graded 15 known sample pairs of wheat and 10 known sample pairs of sorghum. Statistical analysis of the data is shown in Table 3 and graphic analysis is shown in Figures 2 and 3.

Table 3. Statistical Analysis for 15 Observations on Wheat and 10 Observations on Sorghum Using Known Sample Pair.

Sample	r	r^2	F	Table F	% $1-r^2$	Reg. Coef.	Std Dev of B	T	Table T
Wheat	.9871***	.9745	496.04	4.67	2.55	.8936	.0401	22.47	1.77
Sorghum	.9007***	.8113	34.39	5.32	18.87	.8442	.1440	5.86	1.86

wheat:n = 15

Table value for F and T are at .05 level sorghum:n = 10

Correlation Studies at Kansas Stations: The State of Kansas Grain Inspection Department was also asked to help evaluate the bleaching method. Visits to six of the state stations were helpful in learning the station practices as well as gaining additional data for better appraisal of the new germ damage evaluation procedures. It was also necessary to discuss with the grain inspectors the guidelines for determination of germ damage of bleached grain. Variations in germ grading practices were observed in sample sizes graded and in methods used to remove the pericarp covering the germ. Sample sizes for evaluation of germ damage varied from 2 to 10 g. Some inspectors scrape every kernel while others scraped only kernels that did not appear to be sound. Most graders use pointed forceps for removing the germ covering while razor blades or knives were used in other cases.

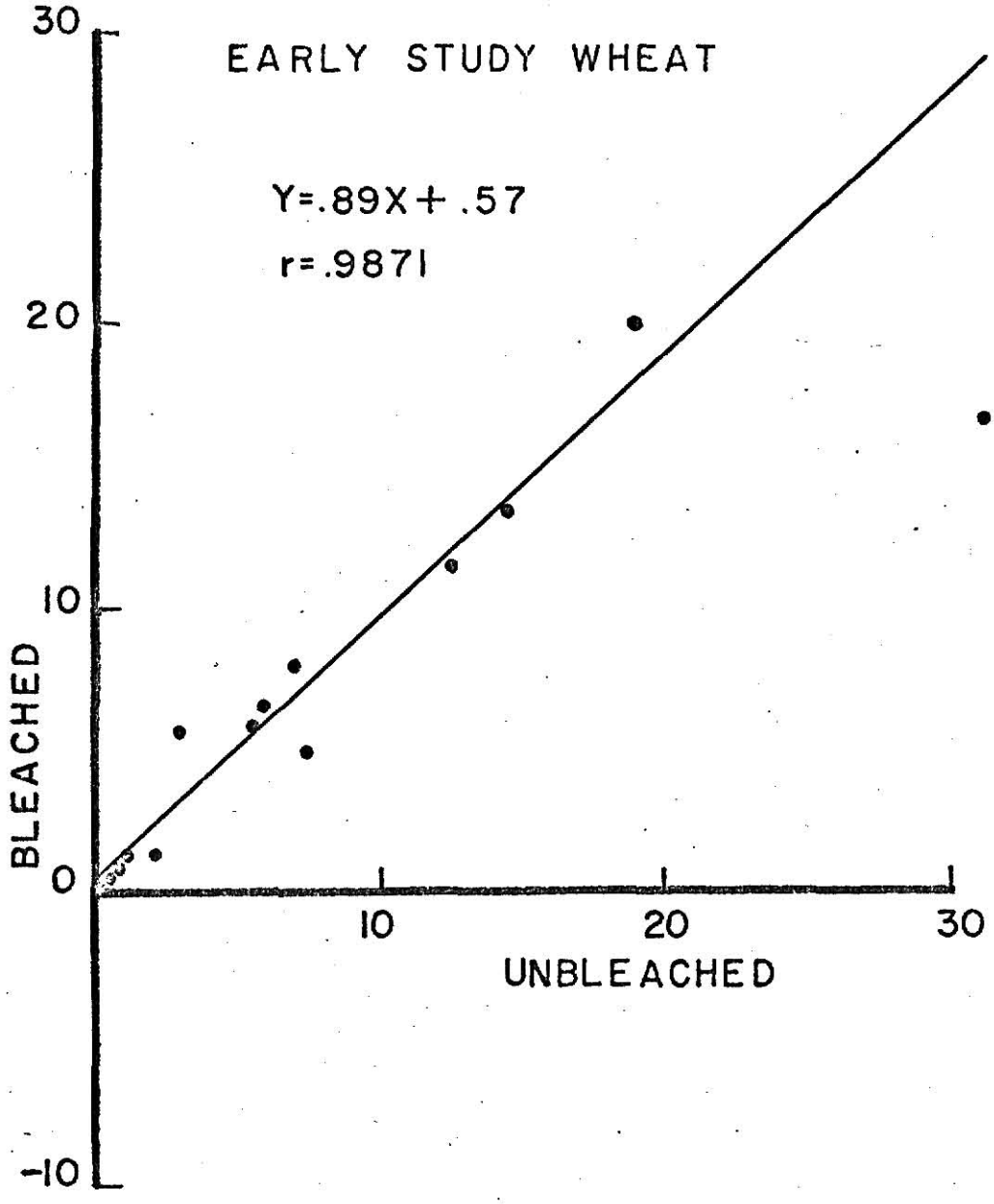


Fig. 2. The % germ damage using the standard method versus the % germ damage using the bleached method for samples of wheat with the known pair method.

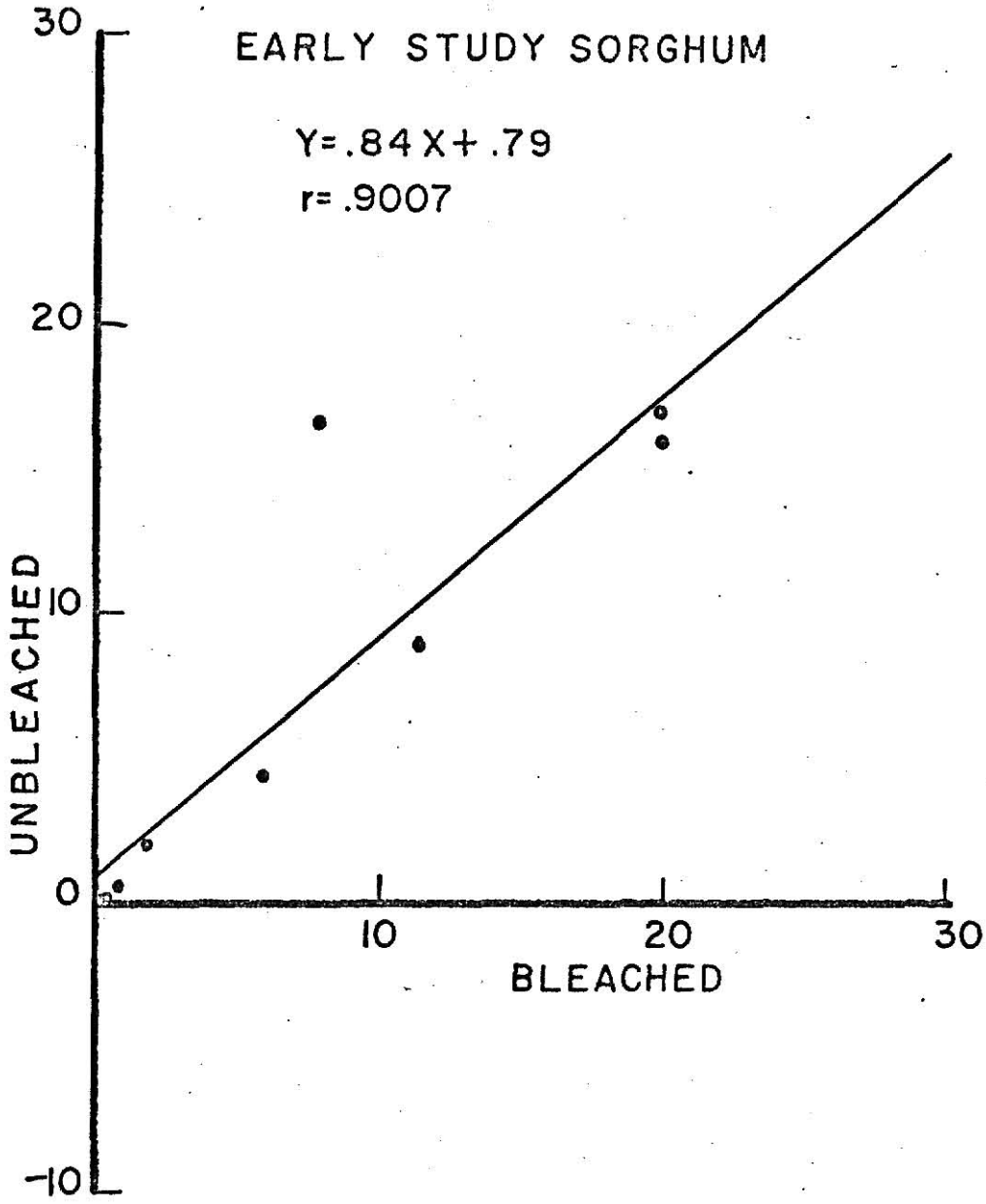


Fig. 7. The % germ damage using the bleaching method versus the % germ damage using the bleached method for samples of sorghum with the known pair method.

The preliminary study consisted of visiting each of the 6 stations to discuss the new procedure and have the inspectors grade 8 samples of bleached and unbleached grain marked as unknown pairs. Percent germ damage, sample size and grading time were recorded for each sample. Table 4 shows the percent germ damage for each sample of the 8 samples at stations A through F. The correlation value (r) was determined on the equivalent samples which were cut from the same sample.

Table 4. Percentage of Germ Damage at Stations A through F for Eight Samples of Bleached and Unbleached Grain.

Sample Description	Stations							
	A	B	C	D	E	F	Total	x
No. 1 Sorghum Bleached	1.0	0.0	0.0	0.0	0.0	1.0	2.0	.33
No. 2 Rye Bleached	0.0	0.0	0.0	---	0.0	.5	.5	.09
No. 3 Sorghum Bleached	0.0	3.0	1.4	1.4	0.0	2.0	7.8	1.3
No. 4 Sorghum Unbleached	1.1	0.0	.6	.7	0.0	.5	2.9	.48
No. 5 Sorghum Unbleached	.2	0.0	1.0	1.3	1.0	0.0	3.5	.58
No. 6 Rye Unbleached	0.0	0.0	0.0	---	0.0	.6	.6	.10
No. 7 Sorghum Bleached	17.5	24.0	8.0	8.5	6.0	12.3	77.3	12.88
No. 8 Sorghum Unbleached	11.2	21.0	6.5	1.4	13.0	13.0	66.0	11.00

Equivalent Samples			
No. 1 Sorghum Bleached	.33	No. 4 Sorghum Unbleached	.48
No. 2 Rye Bleached	.09	No. 6 Rye Unbleached	.10
No. 3 Sorghum Bleached	1.3	No. 5 Sorghum Unbleached	.58
No. 7 Sorghum Bleached	12.88	No. 8 Sorghum Unbleached	11.00

r = .990

Variations in the percent damage reported is rather large as is demonstrated in the unbleached sorghum sample No. 8 with damages reported at 1.4 to 13.0 percent. Variation in the same sample which was bleached (No. 7 Sorghum Bleached) varied from 6.0 to 12.3 percent. This is a large variation. However, the bleaching method was new to the grain inspectors and some variation would probably exist until proficiency is attained with the new procedure. A simple one way analysis of variance of the data (Table 5) showed that a larger variation existed within a station than between stations. The analysis of variance also would indicate that values at one station are not significantly different from values at another station.

Table 5. One Way Analysis of Variance Using Values from Table 4.

Source of Variation	DF	Sum Squares	Mean Squares	F Ratio
Between stations	5	91.16	18.23	.492
Within stations	42	1556.89	37.07	
Total	47	1648.05		

Table Value of $F_{.05}(5,42) = 3.99$

$.492 < 3.99$ Accept Hypothesis $\bar{Y}_A = \bar{Y}_B = Y_C = \ldots = Y_F$

Table 6. Analysis of Variance to Check the Interaction of Method With Station Using Sorghum Samples.

Source of Variation	DF	Sum of Squares	Mean Square	F Ratio
Station	5	1.08	.22	$F_{(5,12)}=.46$
Method	1	.48	.48	$F_{(1,12)}=.65$
Method X Station	5	3.71	.74	$F_{(5,12)}=1.00$
Error	12	8.95	.74	
Total	23	14.02		

Table Value of $F_{.05}(5,12) = 6.07 > .46$ Thus $\tau_i = 0$

Table Value of $F_{.05}(1,12) = 11.75 > .65$ Thus $\beta_j = 0$

Table Value of $F_{.05}(5,12) = 6.07 > 1.00$ Thus $(\tau\beta)_{ij} = 0$

Since sorghums 1,3,4, and 5 were all cut from the same sample, an additional test for interaction of station and method is possible. Table 6 shows that no interaction of sample and station was found.

The results of the grading time study (Table 7) shows the merit of the bleaching procedure. To express these results better, it would take 50.85 min. to grade a 30 g sample of unbleached sorghum and 18.99 min. to grade the bleached sorghum sample. Thus the grading time saved in evaluation of germ damage in sorghum is 31.86 min. The results for the sample of rye would show a time savings of only 3.19 min. for grading a 30 g sample.

Table 7. Grading Time Expressed g/min. for Station A Through F and Samples 1 to 8.

Sample Description	Stations							
	A	B	C	D	E	F	Total	X
No. 1 Sorghum Bleached	1.01	2.38	1.96	1.44	2.87	1.13	10.79	1.80
No. 2 Rye Bleached	1.09	3.85	.79	----	6.90	3.00	15.63	3.13
No. 3 Sorghum Bleached	1.73	.71	.76	----	2.20	1.25	6.65	1.33
No. 4 Sorghum Unbleached	.68	.50	.47	.92	.29	.75	3.61	.60
No. 5 Sorghum Unbleached	.66	.47	.64	.67	.46	.75	3.65	.61
No. 6 Rye Unbleached	.93	7.50	.49	----	2.20	.62	11.72	2.35
No. 7 Sorghum Bleached	1.27	.42	1.20	1.17	4.15	1.43	9.64	1.61
No. 8 Sorghum Unbleached	.42	.71	.50	.60	.48	.62	3.33	.56

Sample	Average Grams Graded/Minute
Unbleached Sorghum	.59
Bleached Sorghum	1.58
Unbleached Rye	2.35
Bleached Rye	3.13

Expanded Study for Kansas Stations: The success of the preliminary tests gave encouragement to further evaluation of the bleaching procedure. In this experiment each station received 12 numerically marked samples and one sample of bleached wheat marked control. The raw data is shown in Table 8, which also shows the type of grain graded at each station. The graphic results (Figures 4-9) of all stations shows the 10 points plotted for each station including the results of the preliminary study. Summation of the data and statistical analysis is described in Table 9.

Table 8. Results of the Expanded Test at the Kansas Grading Stations Giving Type of Grain Graded and % Germ Damage for Bleached and Unbleached Grain.

Station	Type of Grain	Percent Germ Damage		Difference in Percent
		Bleached	Unbleached	
Station A	Corn	3.0	1.9	1.1
	Corn	11.6	22.4	11.2
	Corn	52.8	53.3	.5
	Corn	23.3	14.0	9.2
	Rye	39.4	36.9	2.5
	Rye	26.5	26.5	0.0
	Control	9.8	Below Average 4.6	
Station B	Wheat	4.0	4.0	0.0
	Wheat	8.0	6.0	2.0
	Wheat	12.0	6.0	6.0
	Wheat	26.0	18.0	8.0
	Sorghum	21.0	10.0	11.0
	Sorghum	17.0	5.0	12.0
	Control	20.0	Above Average 5.6	
Station C	Corn	33.9	27.2	6.7
	Corn	28.1	46.4	18.3
	Rye	6.9	12.0	5.1
	Rye	35.5	28.0	7.5
	Rye	23.4	22.0	1.4
	Rye	43.3	30.9	12.4
	Control	10.2	Below Average 4.2	

Table 8 (Continued)

Station	Type of Grain	Percent Germ Damage Bleached	Unbleached	Difference in Percent
Station D	Corn	6.7	2.8	3.9
	Corn	5.1	3.5	1.6
	Corn	14.5	17.8	3.3
	Corn	7.4	28.0	20.6
	Wheat	22.6	11.5	11.1
	Wheat	41.0	25.0	16.0
	Control	10.0	Below Average 4.4	
Station E	Sorghum	20.0	13.8	6.2
	Sorghum	16.0	20.0	4.0
	Sorghum	15.0	6.0	9.0
	Rye	10.0	40.0	30.0
	Rye	20.0	32.5	12.5
	Rye	30.0	10.0	20.0
	Control	20.0	Above Average 5.6	
Station F	Wheat	66.3	64.2	2.1
	Wheat	75.8	70.1	5.7
	Wheat	36.9	20.3	16.6
	Wheat	21.4	13.4	.2
	Sorghum	3.9	.5	3.4
	Sorghum	21.6	7.6	4.0
	Control	16.2	Above Average 1.8	

Control Average: 14.4

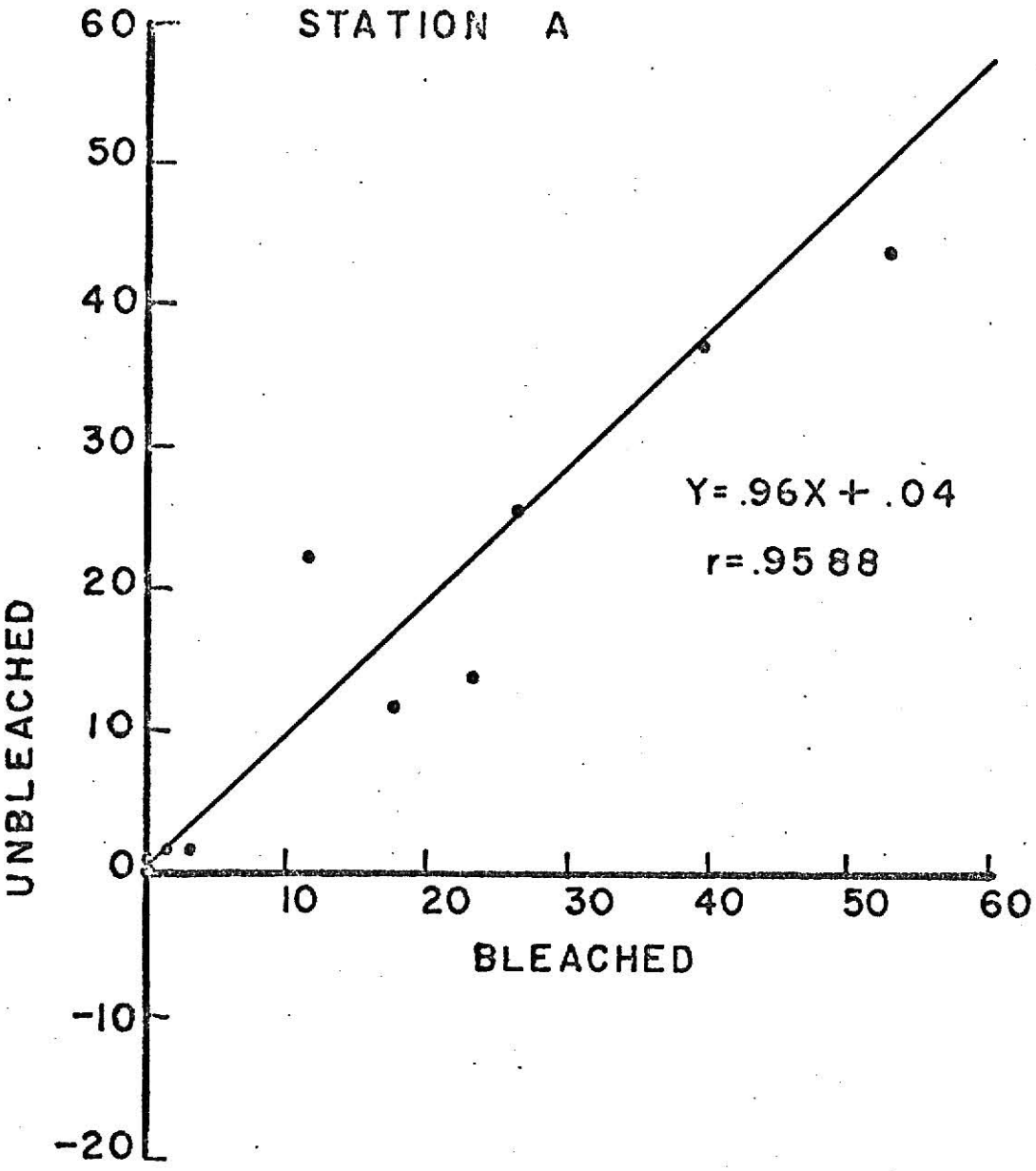


Fig. 4. The % germ damage for bleached grain (X) versus the % germ damage for unbleached grain (Y) resulting from 10 observations at station A.

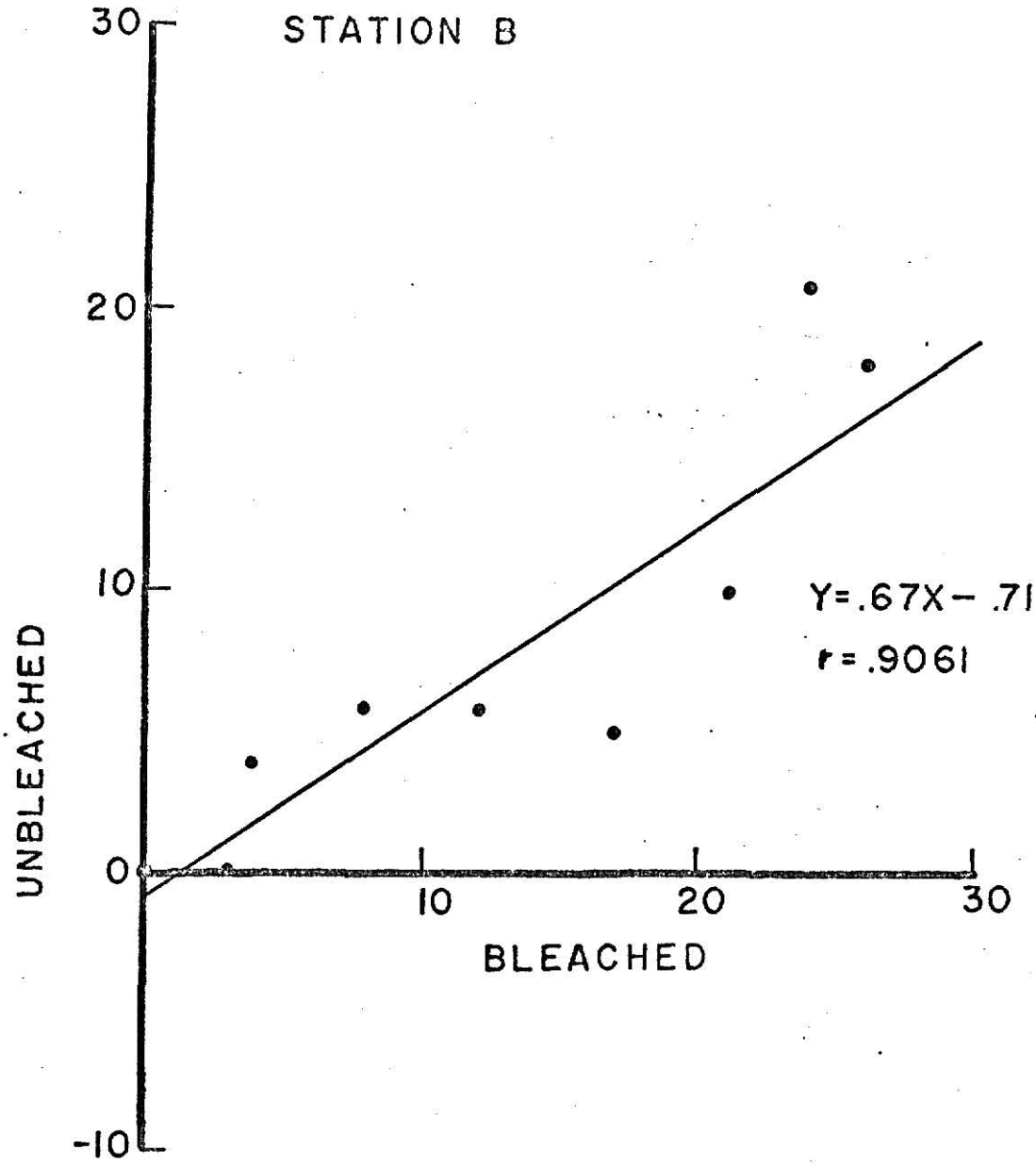


Fig. 5. The % germ damage for bleached grain (X) versus the % germ damage for unbleached grain (Y) resulting from 10 observations at station B.

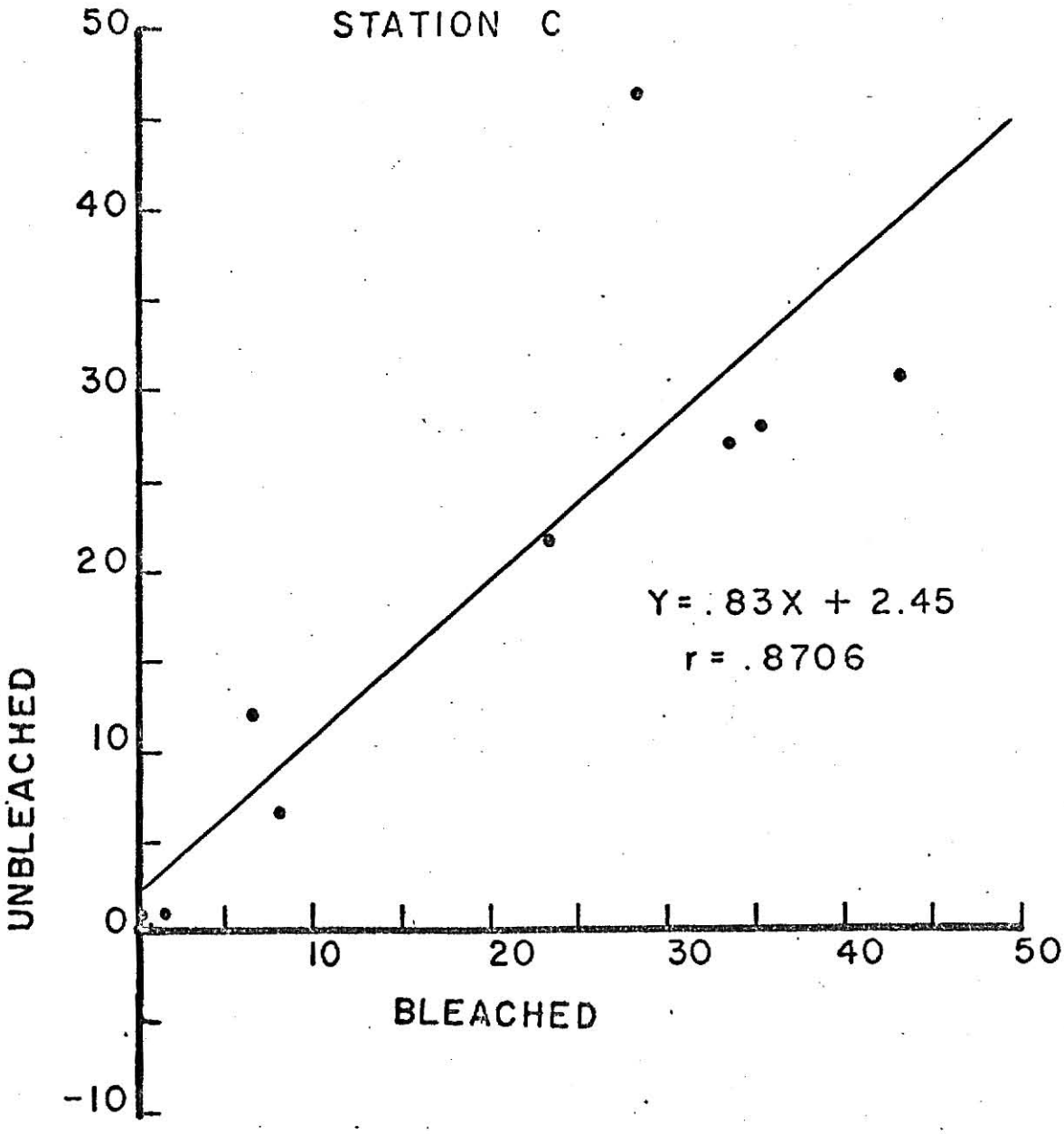


Fig. 6. The % germ damage for bleached grain (X) versus the % germ damage for unbleached grain (Y) resulting from 10 observations at station C.

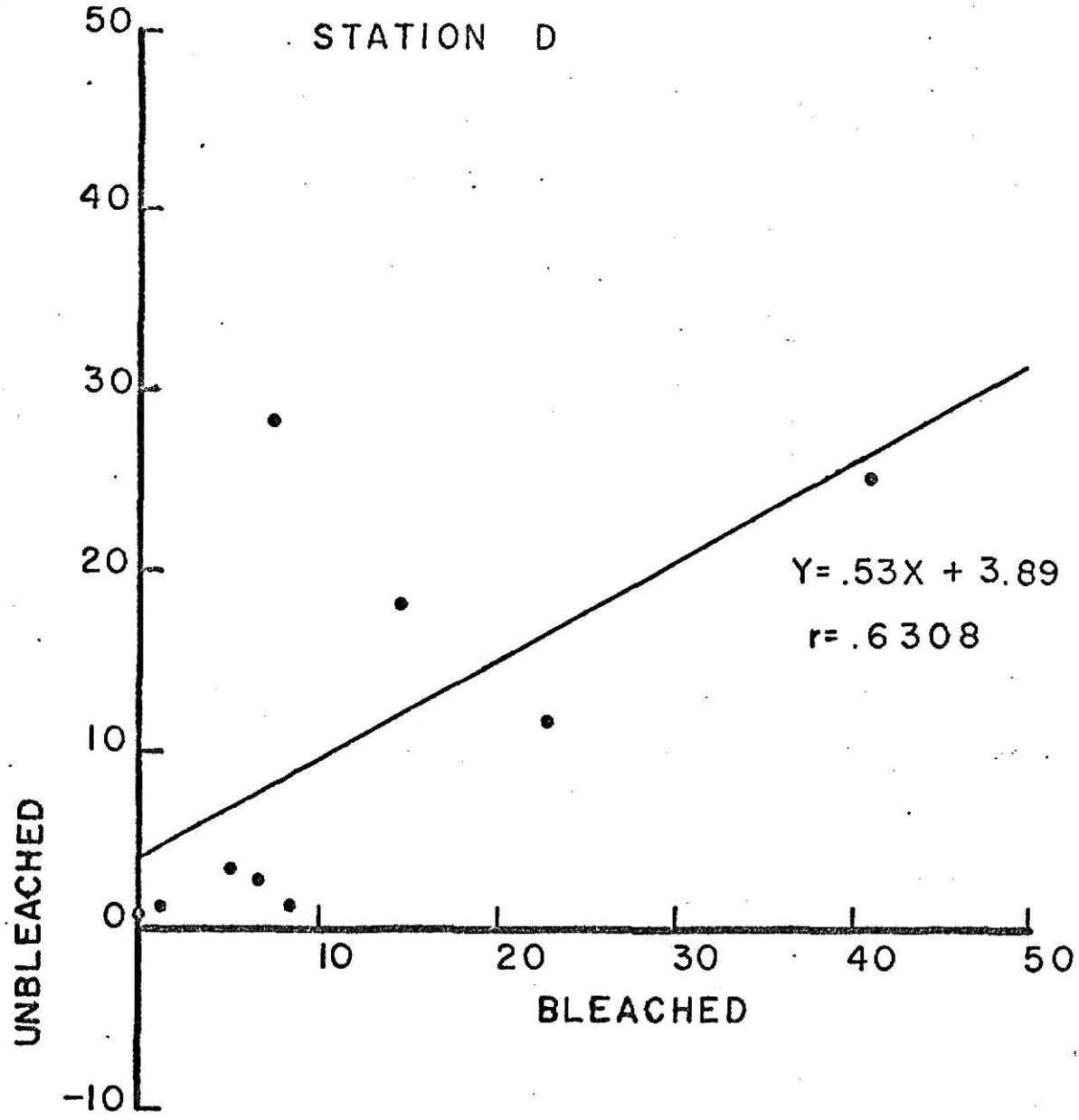


Fig. 7. The % germ damage for bleached grain (X) versus the % germ damage for unbleached grain (Y) resulting from 9 observations at station D.

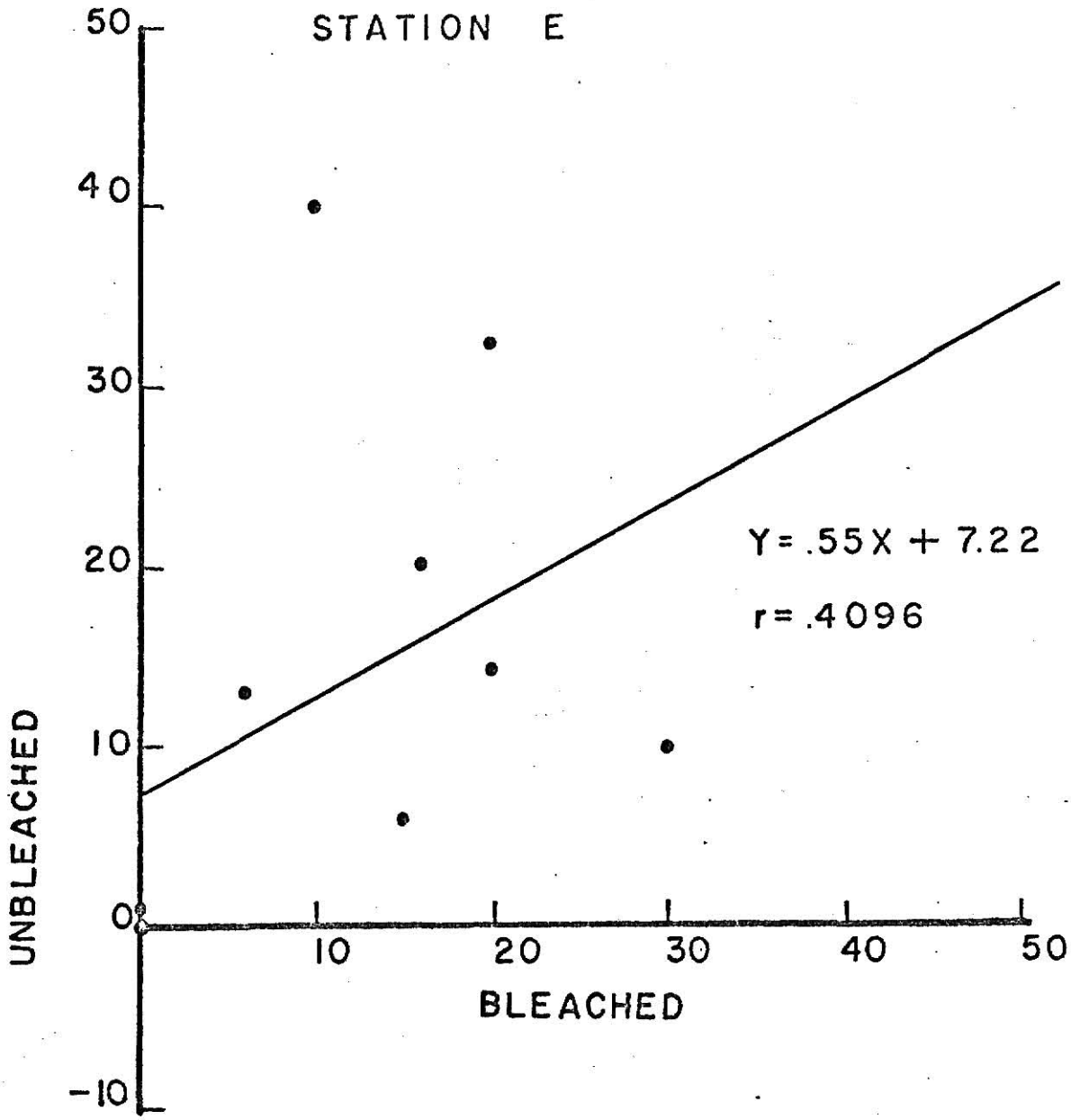


Fig. 8. The % germ damage for bleached grain (X) versus the % germ damage for unbleached grain (Y) resulting from 10 observations at station E.

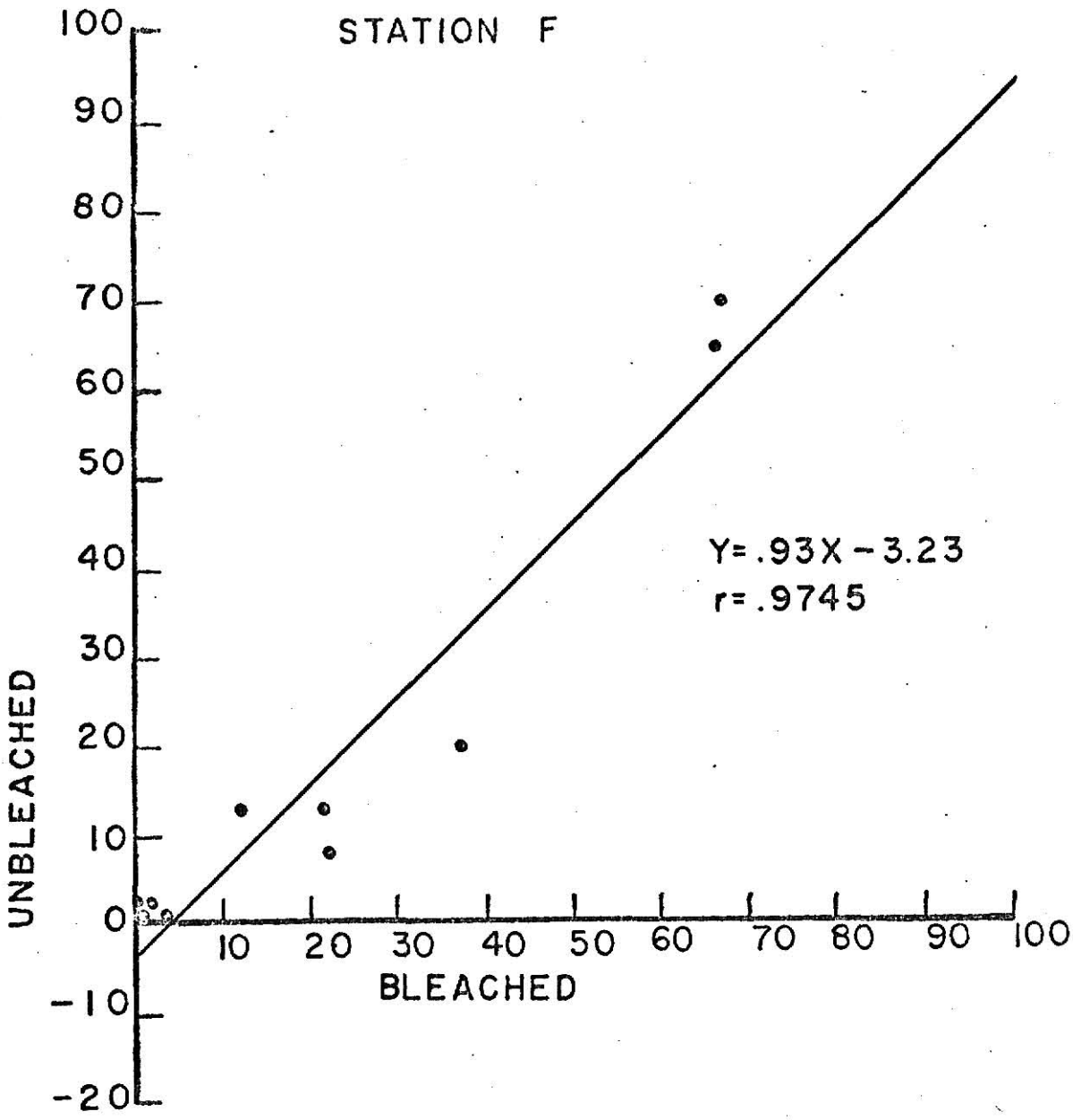


Fig. 9. The % germ damage for bleached grain (X) versus the % germ damage for unbleached grain (Y) resulting from 10 observations at station F.

Table 9. Statistical analysis for all data obtained at Kansas stations.

Station	r	r^2	F	Table F	$\frac{1-r_2}{1-r}$	Reg. Coef.	Std Dev of B	T	Table T
A	.9588***	.9193	91.07	5.32	9.07	.9580	.1004	9.54	1.86
B	.9061***	.8211	36.71	5.32	17.89	.6700	.1106	6.06	1.86
C	.8706***	.7579	25.04	5.32	24.21	.8310	.1661	5.00	1.86
D	.6308**	.3979	4.63	5.59	60.21	.5318	.2473	2.15	1.90
E	.4096	.1678	1.61	5.32	83.22	.5477	.4312	1.27	1.86
F	.9745***	.9497	150.97	5.32	5.03	.9300	.0757	12.29	1.86

A, B, C, E, F: n = 10

D: n = 9

Table values for F and T are at the .05 level.

Variations in the correlation results are very evident from the graphs and the statistical data of Table 9. In accounting for these variations a number of factors must be considered. The main factor results from the different types of grain sent to each station. Note that stations which had high r values had more samples of wheat (stations B and F). Wheat is not only more commonly evaluated but also easier to evaluate than is sorghum as is shown by the earlier studies. Another factor related to variation in correlation is the quality of grading at each station. Station E which has the lowest correlation is also the station where only 2 g. samples were evaluated by some graders. Grain inspectors may not have evaluated these samples as well as they would have with samples for trade and some undue pressure may have resulted due to increased load near the wheat harvest.

Time Studies at Kansas City: Additional time study samples were graded by inspectors at the Grain Division of the Consumer and Marketing Service,

U.S.D.A. Samples of corn, rye, and wheat numbered as unknown pairs were used to determine the saving in time acquired by using the bleaching method. Table 10 shows the coefficients of linear correlation between bleached and unbleached samples as well as grading speeds. A better understanding of the results may be obtained from Table 11 which shows the time needed to grade a 30 g. sample of grain. Figure 10 shows the graphic results of the time study for all 12 samples using the bleaching procedure and the four samples of oats using the Waring Blendor method which will be described in a later section. The r for the 16 values is higher than any of the correlation coefficients determined for the Kansas stations.

Table 10. Correlation and grading speed at the U.S.D.A. resulting from test grain inspection lab at Kansas City.

Type of Grain	Number of Samples	r	Grams Graded per Minute Bleached	Unbleached
Corn	4	.9947***	10.49	9.40
Rye	4	.9882***	.43	.23
Wheat	4	.9998***	3.14	1.13

Table 11. Time in minutes required to grade 30 g. of grain.

Type of Grain	Bleached	Unbleached	Time Saved
Corn	2.86	3.19	.33
Rye	69.77	130.43	60.66
Wheat	9.55	26.55	17.00

A proper comparison of grading time for bleached and unbleached samples must include the time required for the bleaching process. Processing time for the bleaching method is 8 to 10 min. depending on the speed of the worker and

the moisture content desired. It may be possible to work with a wetter grain than 12% to 13% (Table 1) which results after 5 min. at 150°C. Table 12 gives the time required to grade a 30-g. sample with an 8 min. bleach-processing time added to each value. Only corn is graded more quickly by the standard grading practice now in use. The bleaching method would enable a grain inspector to grade a larger sample than is presently being graded and thus increase the statistical soundness of the determination. The increased productivity of the grader may also decrease the tediousness of the work and grain inspector fatigue, which increases grading errors.

Table 12. Average time in minutes to process and grade 30 g. of grain.

Type of Grain	Bleached	Unbleached	Difference in Time Shorter (+) or Longer (-)
Sorghum	26.99	50.85	+23.86
Wheat	17.55	26.55	+ 9.00
Corn	10.86	3.19	- 7.67
Rye	77.77	130.43	+52.66

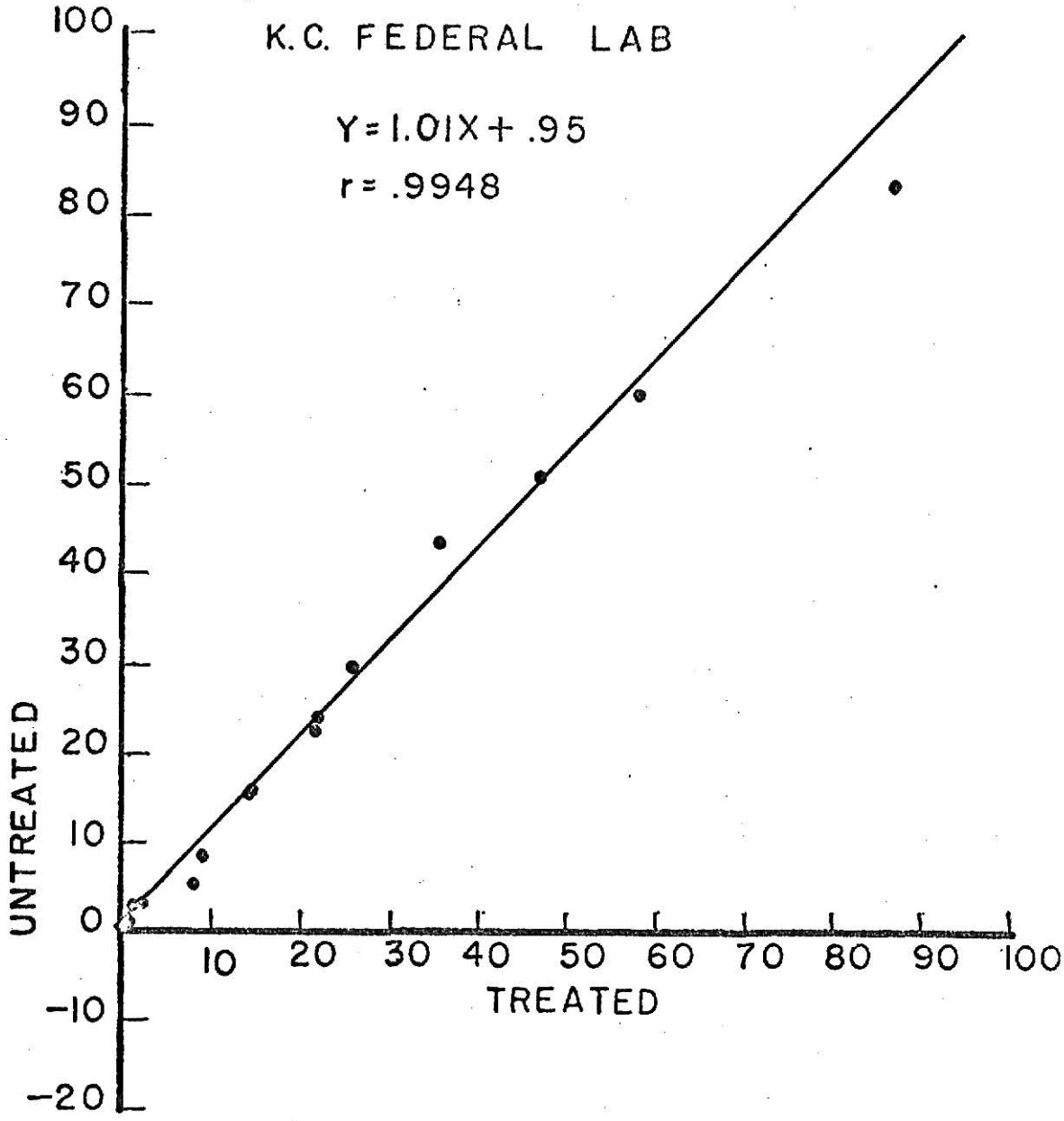


Fig. 10. The % germ damage of grain after treatment with bleach or Waring Blendor versus % germ damage found in untreated grain resulting from 16 observations.

Results for Each Type of Grain: The total results of all tests for correlation on wheat, sorghum, rye, and corn are shown graphically in Figures 11-14. Figure 15 shows the graphic results for combining the data on each grain. More detailed statistical information is included in Table 13 along with the statistical information on the Christensen-Qasen data. The graphic analysis of the Christensen-Qasen data for wheat is shown in Figure 16.

Christensen and Qasen did not apply statistical analysis to the 10 observations on wheat and the 10 observations on corn. To evaluate better and make comparisons easier, statistical analysis of the data was necessary. Note that in the case of the Christensen-Qasen data as well as for data gathered in this study, correlation is extremely high. The significance of this data is demonstrated by the large margin by which the calculated F value exceeds the table value of F at 1 and n-2 degrees of freedom. If the calculated F value is less than the table value, no relation exists between the bleached and unbleached values for germ damage. The r^2 values give an indication of closeness of fit, or indicates the degree of scattering of the points around the regression line. Significance for wheat and corn are shown by both the present studies and the Christensen-Qasen data. Christensen and Qasen, however, in evaluation of corn, separate dark germ and ochre germ (questionable germ damage). This is not possible in actual grading and may account for the .13-.14 variation in r values between the Christensen-Qasen data and the data resulting from the present studies. Methods for evaluation of the data were not explained in the article. This may also be instrumental in causing the variation. The significant results on sorghum and rye should also be noted as significant in relation to time savings. It should be noted that rye is not a grain commonly graded by grain inspectors involved with these tests, and the same is true for

sorghum to a lesser extent. Attempts were made to overcome this problem by giving a station the type of grain its inspectors had most frequently graded. However, this was not always possible.

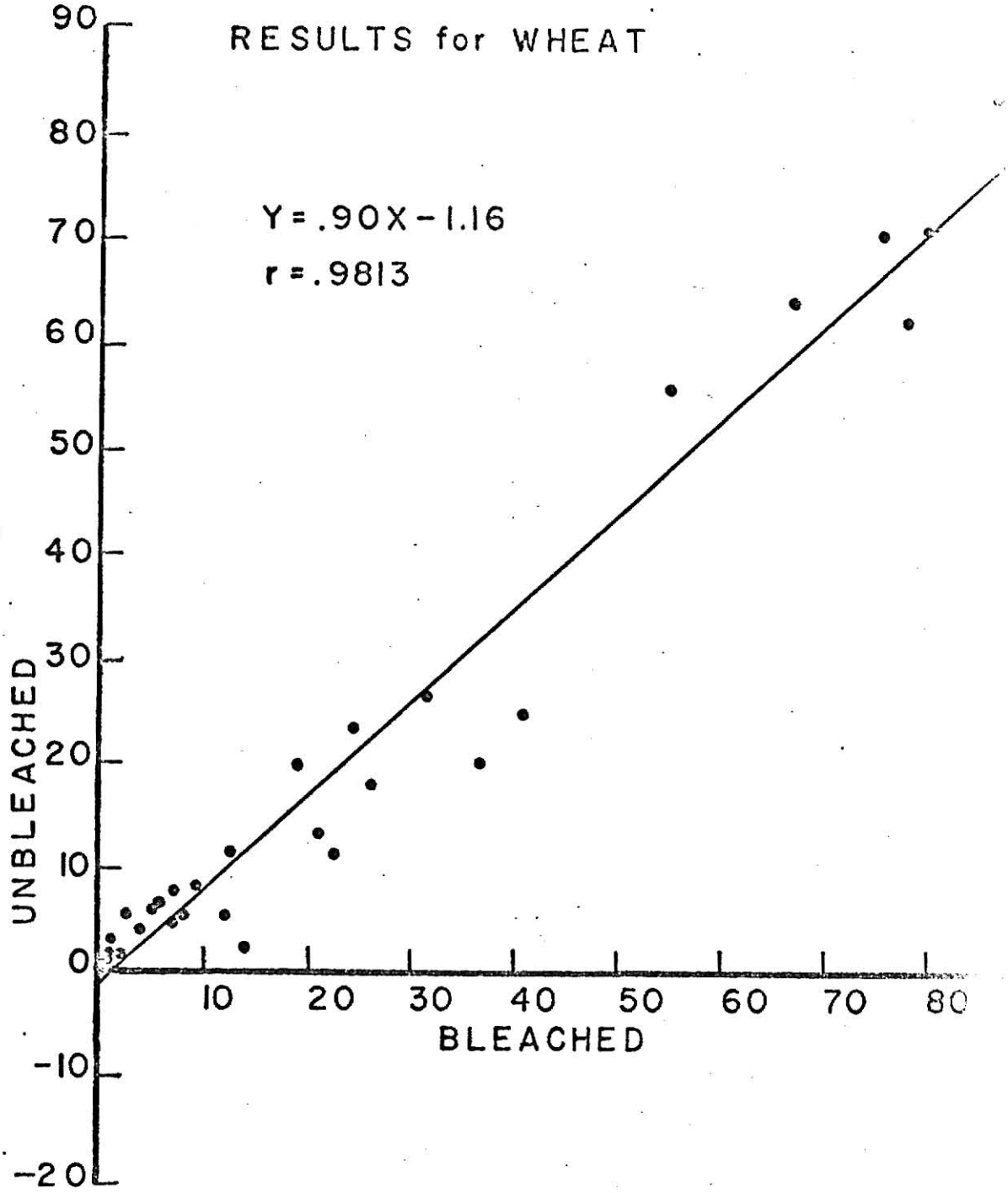


Fig. 11. The % germ damage found using the bleaching method (X) versus the % germ damage found using the unbleached method (Y) for all samples of wheat (n = 34).

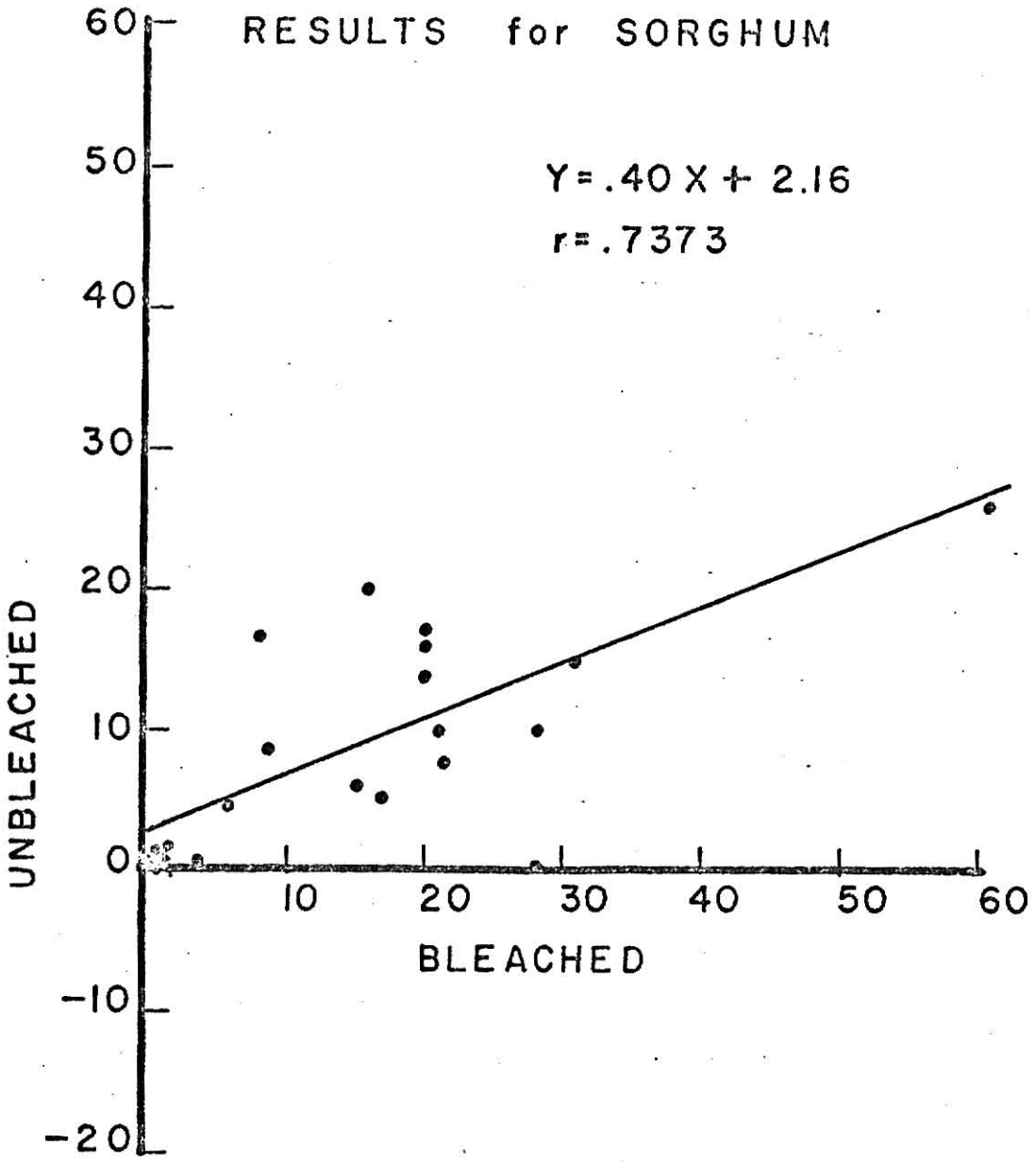


Fig. 12. The % germ damage found using the bleaching method (X) versus the % germ damage found using the unbleached method (Y) for all samples of sorghum (n = 25).

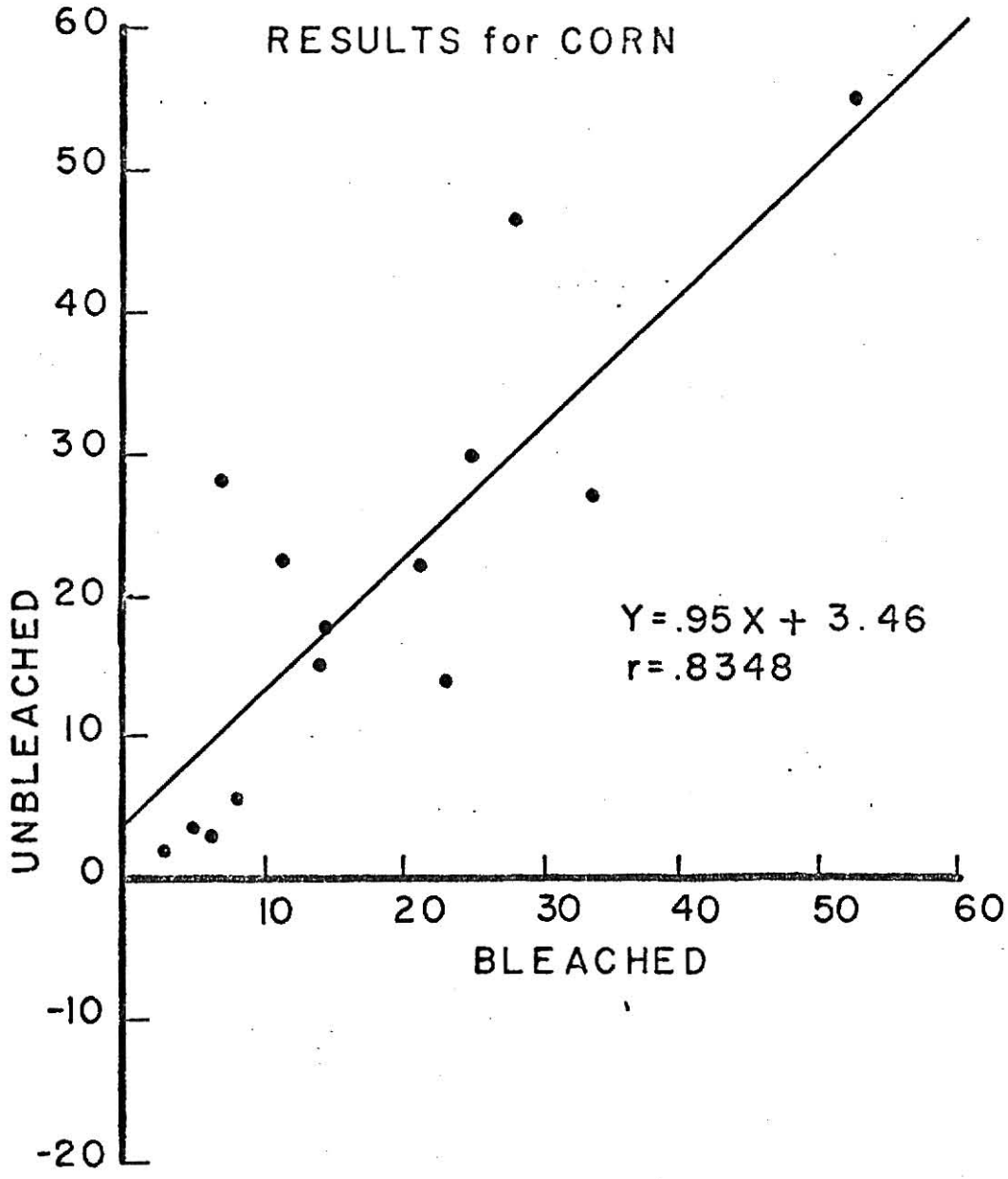


Fig. 13. The % germ damage found using the bleaching method (X) versus the % germ damage found using the unbleached method (Y) for all samples of corn (n = 14).

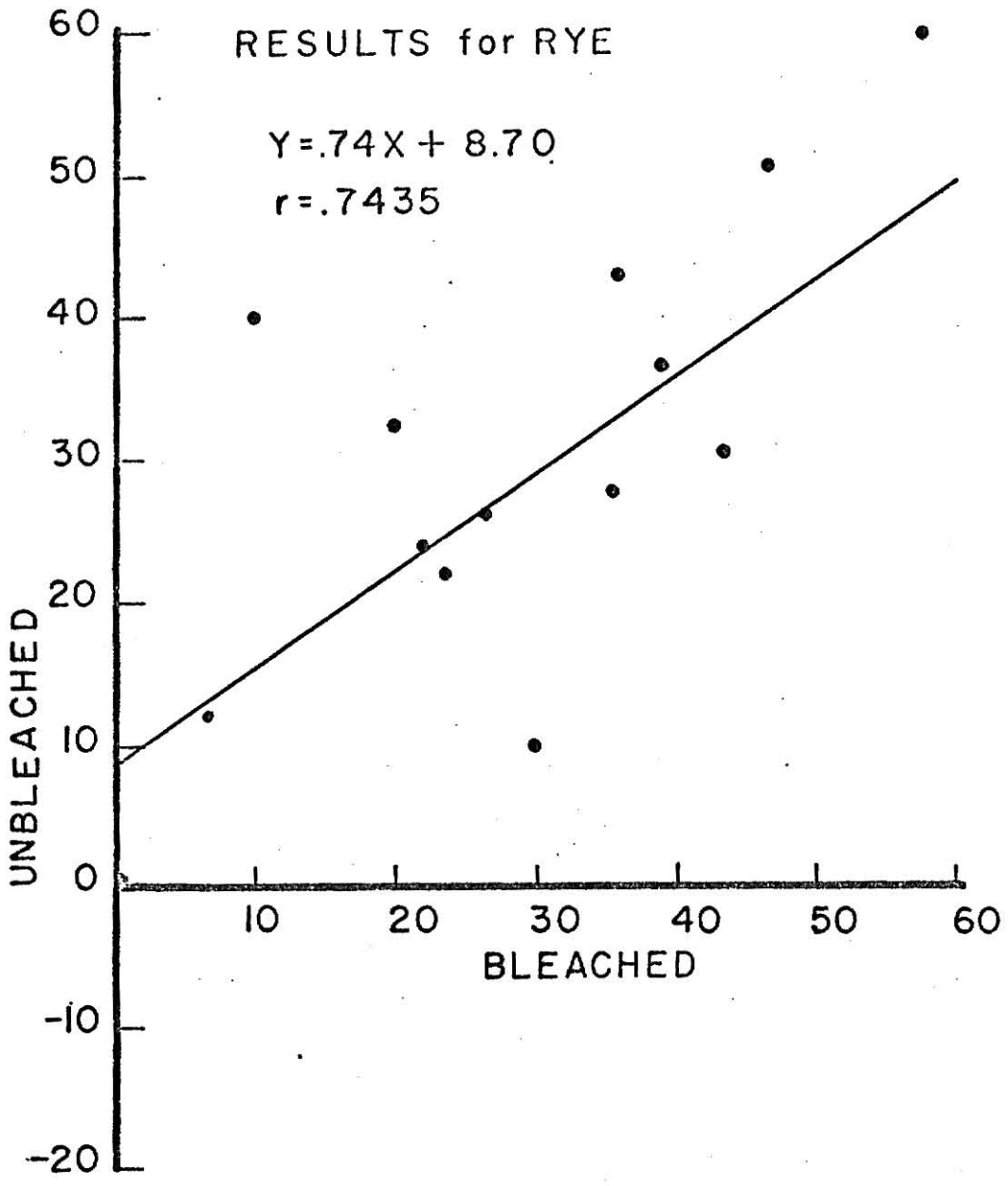


Fig. 14. The % germ damage found using the bleaching method (X) versus the % germ damage found using the unbleached method (Y) for all samples of rye (n = 14).

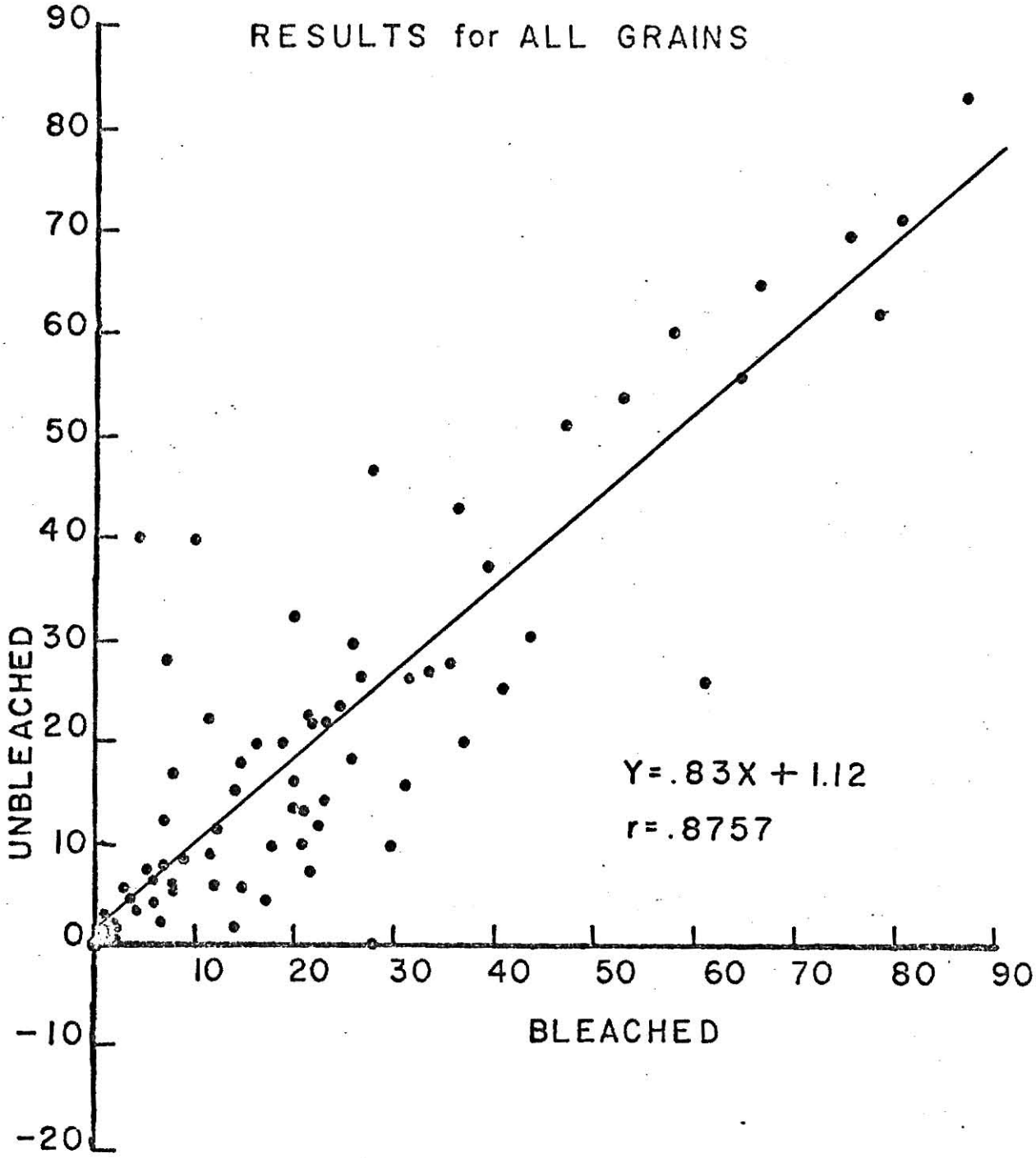


Fig. 15. The % germ damage found using the bleaching method (X) versus the % germ damage found using the unbleached method (Y) for all types of grain tested (n = 87).

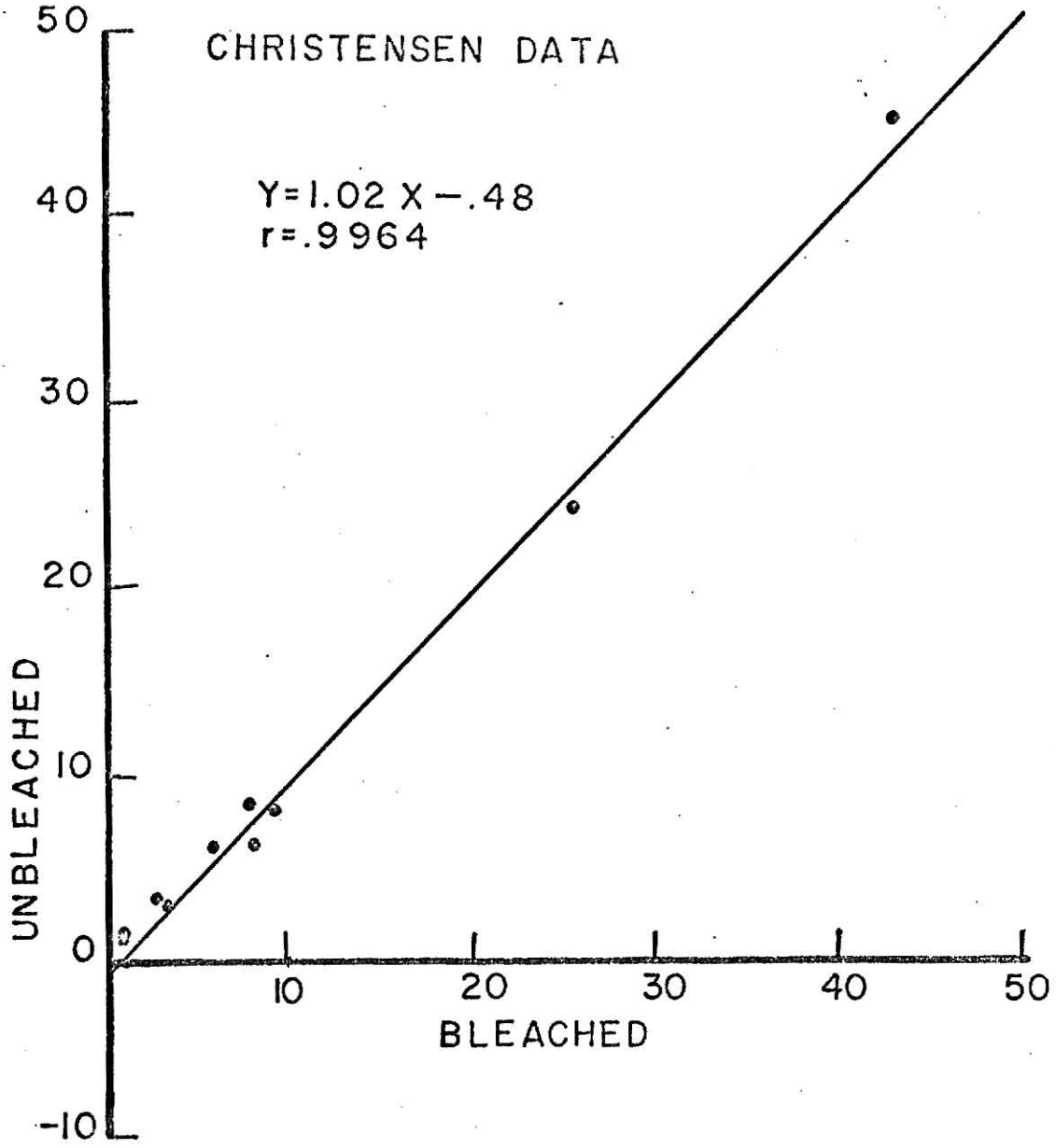


Fig. 16. The % germ damage found in bleached wheat versus the % germ damage found in unbleached wheat from data obtained by Christensen and Qasen (n = 10).

Table 13. Statistical analysis for all samples of each type of grain and statistical analysis of the Christensen-Qasen data.

Wheat: n = 34. Sorghum: n = 25. Corn: n = 14. Rye: n = 14. All Grain: n = 87.

Sample	r	r^2	F	Table F	% $1-r^2$	Reg Coef	Std Dev of B	T	Table T
Wheat	.9813***	.9630	833.39	4.15	3.7	.8959	.0310	28.87	1.70
Sorghum	.7373**	.5436	27.40	4.28	45.64	.3953	.0755	5.23	1.72
Corn	.8348***	.6968	27.58	4.75	30.32	.9496	.1808	5.25	1.77
Rye	.7435***	.5528	14.84	4.75	44.72	.7413	.1925	3.85	1.77
All Grain	.8757***	.7668	279.58	3.96	23.32	.8325	.0498	16.72	1.66
		CHRISTENSEN-QASEN DATA			Wheat: n = 10.	Corn: n = 10.			
Wheat	.9964***	.9927	1091.45	5.32	.73	1.0249	.0310	33.04	1.86
Corn Dark Germ	.9638***	.9290	104.63	5.32	7.10	1.1375	.1112	10.23	1.86
Corn Ochre Germ	.9896***	.9793	380.02	5.32	2.07	.8893	.0456	19.49	1.86

Table values for F and T are at the .05 level of significance.

Germination and Glutamic Acid Decarboxylase (GADA): Thus far the search for evaluation of the bleaching method has relied on correlation studies of reported germ damage between bleached and unbleached samples. Since germ damage evaluation is basically an indication of embryo condition it is only reasonable that other methods for evaluation of germ condition could be used. Glutamic acid decarboxylase and germination were chosen as standards to evaluate the bleaching method and standard method of germ damage determination.

Five samples of sorghum and five samples of wheat were mechanically divided into two samples of 30 g. for grading evaluation and two samples of 80 g. for glutamic acid decarboxylase and germination. The results of the germination test of 5 sorghum and 5 wheat samples are shown graphically for unbleached grading in figure 17. A similar graphic presentation is shown for the bleaching method (figure 18). A graphic display of the GADA results for only wheat for the unbleached and bleached tests are shown in figures 19 and 20 respectively. Statistical analysis of all results on wheat and sorghum are shown in table 14. Correlation in all cases is higher for the results obtained from the bleaching procedure. Correlation was much higher for wheat than for sorghum. Only the correlation check between bleached sorghum and GADA results are significant in F and T tests. Significant T tests were also obtained for bleached and unbleached samples. The T test shows significance if the absolute value of the calculated T exceeds the table value.

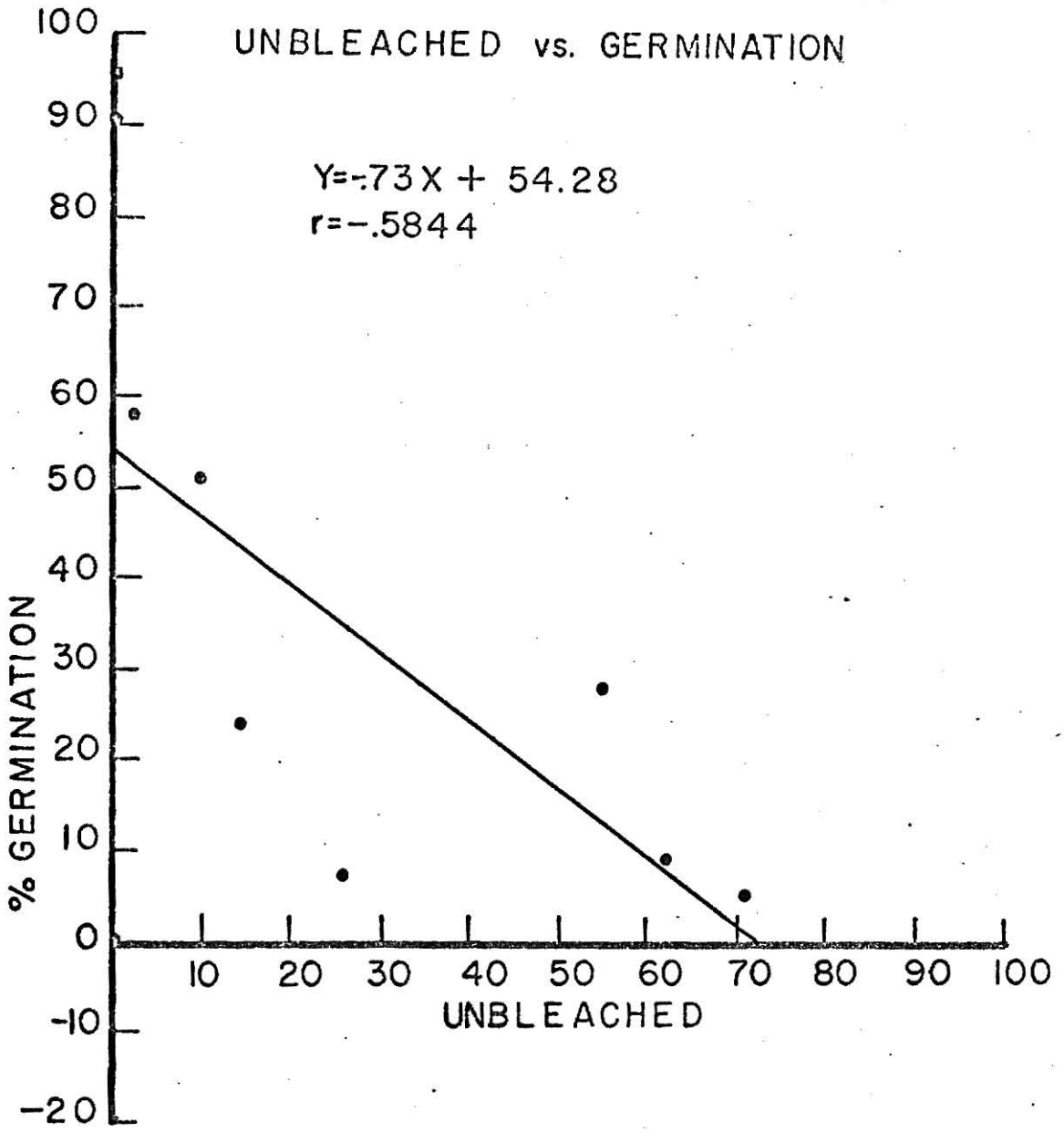


Fig. 17. The % of germ damage determined on unbleached samples of wheat and sorghum versus the % germination on the same samples (n = 10).

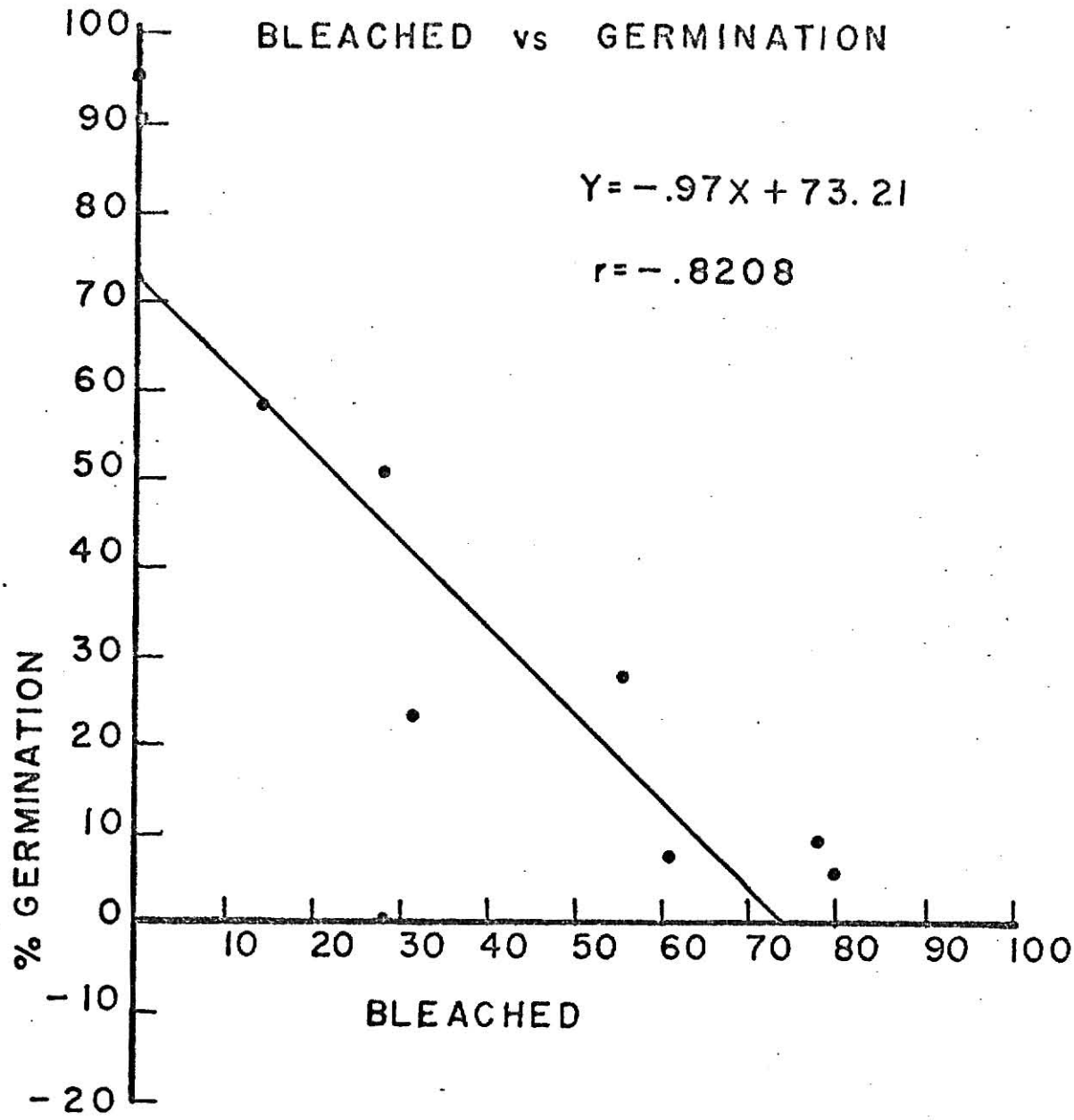


Fig. 18. The % germ damage as determined on bleached samples of wheat and sorghum versus the % germination on the same samples of untreated grain (n = 10).

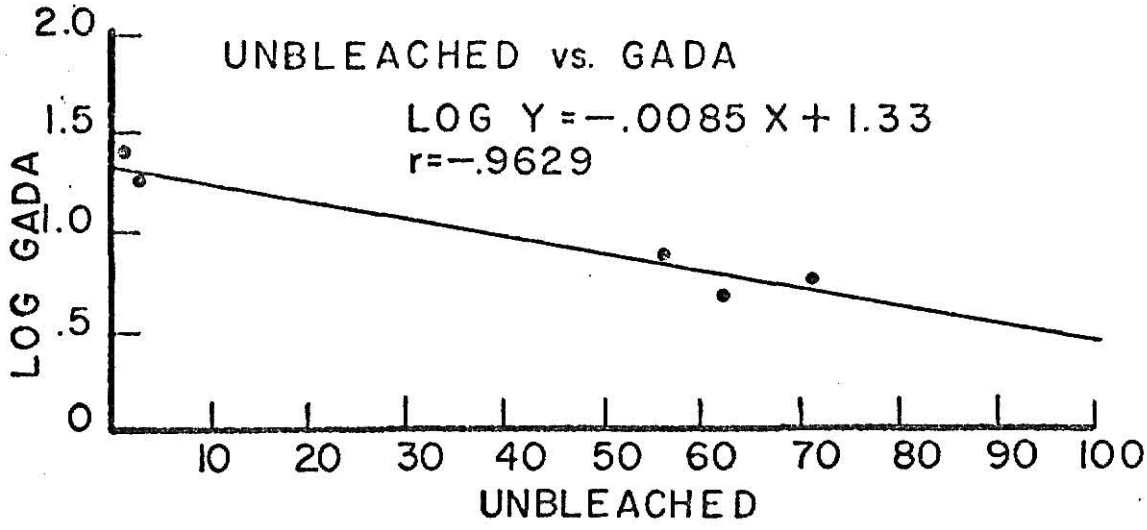


Fig. 19. The % germ damage as determined on unbleached wheat versus the Log of the CO_2 pressure (mm of ethyl lactate).

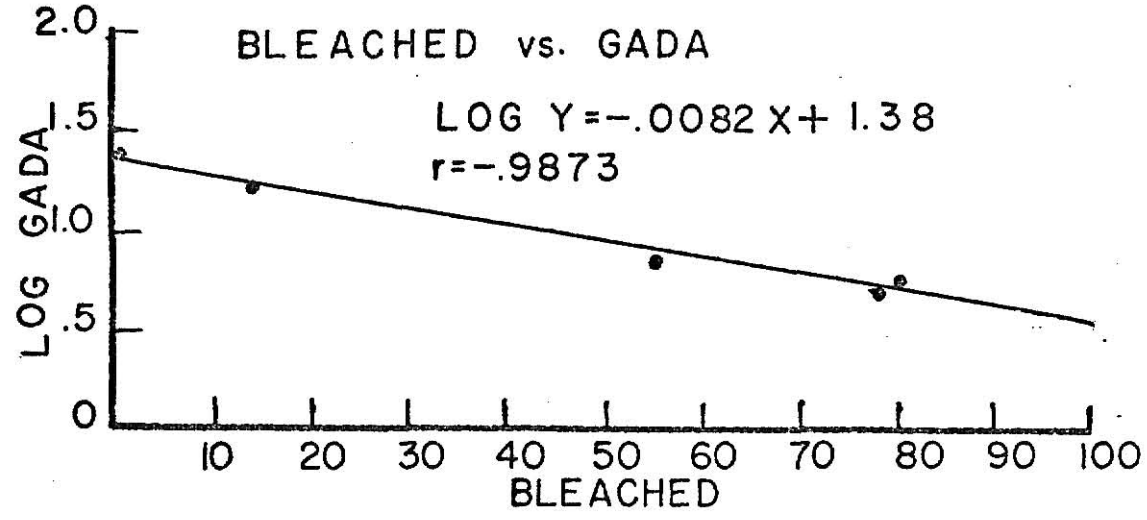


Fig. 20. The % germ damage as determined on bleached wheat versus the $\log_{10}$ of the CO_2 pressure (mm of ethyl lactate).

Table 14. Statistical analysis on germination and GADA when compared with % germ damage found in bleached and unbleached grain sample of wheat and sorghum.

Values Compared	r	r^2	Wheat F	$\%\ 1-r^2$	Reg Coef	Std Dev of B	T
Bleached, Unbleached	.9802***	.9607	73.33	3.93	.9173	.1071	8.56
Bleached, Germination	.9749***	.9505	57.61	4.95	- .9987	.1316	- 7.59
Bleached, GADA	.9873***	.9747	115.72	2.53	.0007606	.0008	-10.76
Unbleached, Germination	.9369**	.8777	21.53	12.23	-1.0255	.2210	- 4.64
Unbleached, GADA	.9629***	.9273	38.25	7.27	- .0085	.0013	- 6.18
			Sorghum				
Bleached, Unbleached	.8679*	.7532	9.15	24.68	.4393	.1452	3.03
Bleached, Germination	-.7808*	.6096	4.68	39.04	-1.3257	.6124	- 2.16
Bleached, GADA	-.8844**	.7820	10.77	21.80	- .0085	.0026	- 3.28
Unbleached, Germination	-.4327	.1872	.69	81.28	-1.4514	1.7459	- .83
Unbleached, GADA	-.5633	.3173	1.39	68.70	- .0108	.0091	- 1.18

Table values for all tests:

$F_{.05}(1,3) = 10.13$

$T_{.05\ 3} = 2.35$

Waring Blendor Method: A total of nine sample pairs were graded in a correlation check for evaluation of the new hull removing process. Four samples were used in the time study and all samples were graded at the Grain Division Consumer and Marketing Service, U.S.D.A., in Kansas City. The time studies showed that a 30-g. sample of oats hulled by the blender can be graded in 17.54 min. while the unhulled oats take 20.68 min. Sample preparation time is 1 to 2 minutes for dehulling and 4 to 5 minutes in 150°C air oven for drying or a minimum of 6 min. Thus, if the process time is included, a total time loss of 3.86 min. would be experienced. Figure 21 shows the plot for 9 observations and table 15 shows the statistical analysis of the data.

Table 15. Statistical analysis of Waring Blendor method for oats.

% $1-r^2$	r	r^2	F	Table F	Reg Coef	Std Dev of B	T	Table T
44.22	.7468	.5578	8.829	5.59	.5828	.1961	2.99	1.90

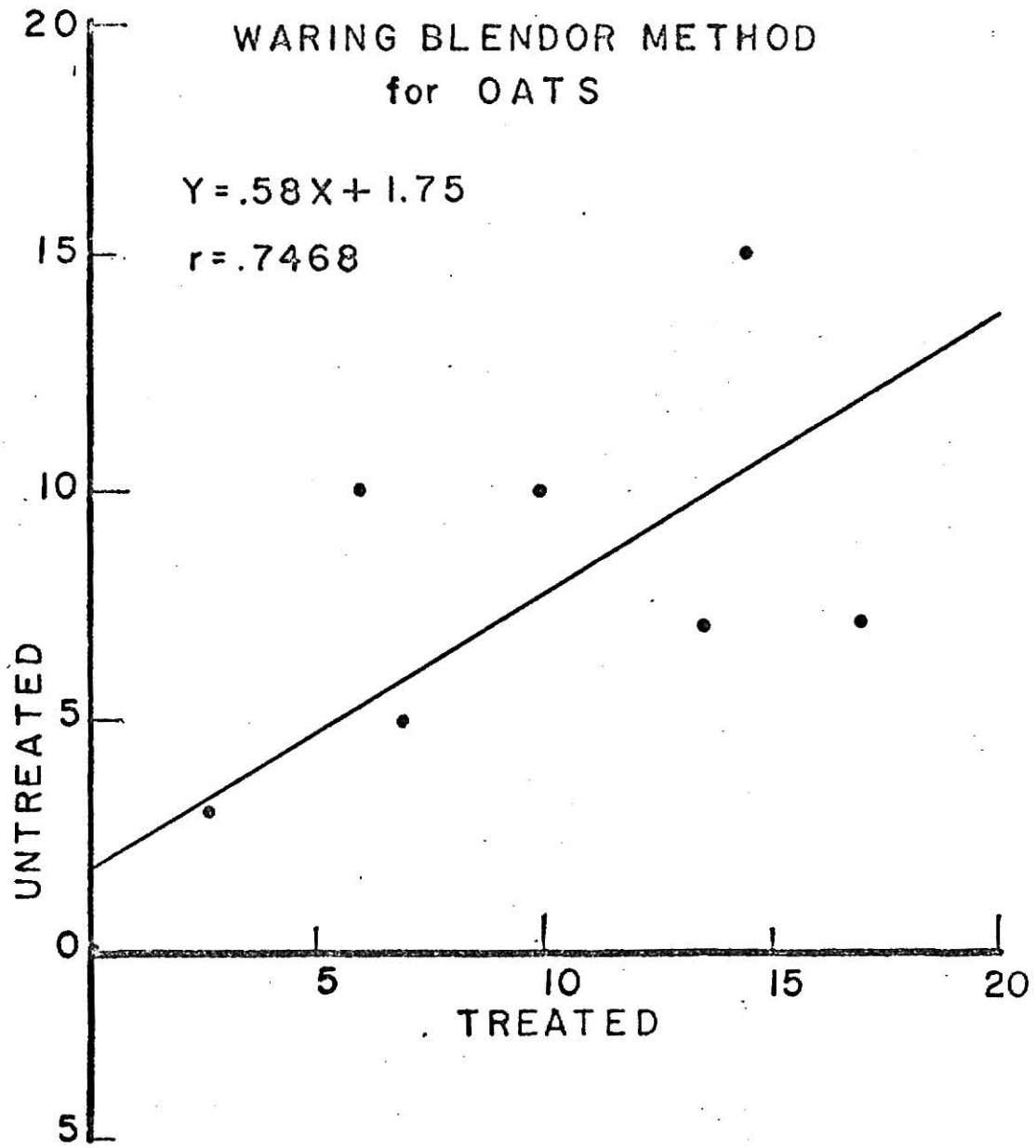


Fig. 21. The % germ damage as found using the Waring Blendor method (X) versus the % germ damage using the standard grading procedure (Y) for oats.

SUMMARY

The Wierzhowski dye method will cause black to brown coloration to occur in grain if the pericarp covering the embryo is broken. Tests confirmed the finding of Wierzbowski (1964) that dye will color surface mold, sprout damage and mechanically damaged grains.

The bleaching method for wheat was modified from the Christensen-Qasen (1959) procedure by increasing bleach strength and drying the grain before grading. This technique was also found to work not only for wheat and corn but also for sorghum and rye. Additional chemical treatment was needed for dehulling oats and barley. Tests with chlorine gas, hydrogen peroxide, calcium hypochlorite and sodium hypochlorite showed that 6% to 12% solutions of sodium hypochlorite are best for revealing germ damage in cereal grains.

Chemical analysis of bleached grain showed a loss of protein and fiber values after bleaching. Protein losses were greatest for wheat with a decrease of about .6%. Fiber losses for the 3 min. bleaching treatments were significant. After the 24 hr. treatment, losses as large as 1% were recorded. Chemical analysis and the action of .5% 2,6-dichloroquinonechlorimide on bleached grain would indicate that the bleach action acts as a whitener and hull-remover.

Time studies for grading 30 g. of grain showed a time saving of 23.86 min. for sorghum, 9 min. for wheat, and 52.66 min. for rye using the bleaching method. Grading time for bleached corn was 7.67 min. longer than was grading time for the unbleached. Correlation studies between the bleaching method and the standard method resulted in good correlation ($r = 0.8757$) for the summation results and varying degrees of correlation for different types of grain (wheat $r = 0.9813$ and sorghum $r = 0.7373$).

A study of the relation between % germination and glutamic acid decarboxylase activity showed better correlation with germ damage determined by the bleaching method than the standard procedure. Higher levels of significance were found to exist in the 5 observations of wheat than in the 5 observations of sorghum.

The Waring Blendor method for dehulling oats was found to correlate ($r = 0.7468$) with the standard grading procedure. Grading time was shortened but the total process time makes the procedure longer by 3.86 min.

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DEVELOPMENT OF A METHOD FOR RAPID DETERMINATION OF GERM DAMAGE IN CEREALS

by

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Accurate evaluation of germ damage has long been a difficult problem for grain inspectors. Germ damage most often results from the action of fungi which requires minimum moisture levels of 13.5% (A. restrictus) or above. However, germ damage may also result in grain stored in inert atmospheres where mold growth is inhibited. Loss of grain viability is believed to occur due to products formed by enzyme action within the kernel. Nonenzymic browning of germ is indirectly caused by enzymes which free amino acids and reducing sugars. The formation of dark pigments take vital material away from biochemical pathways which result in the loss of kernel viability. Significant correlation values have been found for enzyme activity and germ damage.

Chemical evaluation for germ damage has resulted in a number of techniques. Fat acidity and measurement of fluorescence of acidic extracts of grain has shown some correlation with germ damage. Enzyme activity has also been used in the evaluation of germ damage. Glutamic acid decarboxylase activity measurement and triphenyl tetrazolium chloride dye procedure produced good results for showing germ damage in .5 hr. or more.

Soaking of grain in .5% of 2,6-dichloroquinonechlorimide for 60 min. causes darkening of sprouted, moldy and mechanically damaged grain. This was found not to be an accurate measure of germ damage since a broken germ covering does not always indicate a loss of viability.

Bleaching grain for germ damage evaluation is best accomplished by using a 6% to 12% solution of NaOCl. A temperature of 105^{o}C occurs in a heat bleach treatment using 12% commercial bleach after 3-4 min. The procedure using a 6% bleach can also be used in bleaching rye and sorghum as well as corn and wheat. Bleaching can also be accomplished by soaking grain in 6% bleach for 12 hr. A hot 15% HCl treatment is needed before the regular bleaching process for oats and barley.

The bleaching method correlated well with the standard grading procedure r = 0.8757 for 87 observations. Correlation for certain types of grains showed some variations with a high for wheat of r = 0.9813 and a low for sorghum of r = 0.7373. Time studies for grading 30 g. of grain showed a time saving of 23.86 min. for sorghum, 9 min. for wheat and 52.66 min. for rye by using the bleaching method. Grading time plus process time for corn was 7.67 min. longer for bleached grain than for unbleached grain.

A study of the correlation between % germination and glutamic acid decarboxylase (GADA) with % germ damage using the bleaching and standard methods gave higher r values with the bleaching method. Significantly higher r values were found for wheat samples tested than for sorghum samples tested with germination and GADA.

The Waring Blendor method for dehulling oats compared to the standard grading procedure gave an r value of 0.7468 for 9 samples. Grading time is shortened but the total process time makes the procedure 2.86 min. longer than the present method.