AN ABSTRACT OF THE THESIS OF

ARTURO LOPEZ for the MASTER OF SCIENCE
(Name of student) (Degree)

in Farm Crops presented on May 17, 1971
(Major) (Date)

Title: EFFECT OF SEED PROTEIN CONTENT ON PLANT GROWTH OF BARLEY AND WHEAT

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Abstract approved: ______________________
Don F. Grabe

The purpose of this study was to evaluate the effect of seed protein content on plant growth of barley (*Hordeum vulgare* L.) and wheat (*Triticum aestivum* L.). The effects of nitrogen application on chemical and morphological characteristics of the seed were also studied.

Seeds of 'Casbon' winter barley and 'Nugaines' winter wheat with different protein content were obtained by field applications of various amounts of nitrogen at seeding time (fall) and tillering time (spring). The increase in protein content from high nitrogen rates was accompanied by a decrease in seed size and yield. The protein increase occurred primarily in the endosperm. Neither protein content nor size of the embryo was affected by rates of nitrogen application.

The effect of seed protein content on plant growth was evaluated

in terms of imbibition and respiration, speed of germination and subsequent seedling growth, root and shoot elongation at 15, 20 and 25C, and dry matter production when grown in low and high nitrogen soils in the green house.

A positive relationship was found between seed protein content and plant performance. The rates of water absorption and oxygen consumption of germinating barley and wheat seed increased as a result of high protein content. High protein seed had a faster speed of germination and developed into larger seedlings with a higher dry matter content when grown in nitrogen-deficient soil. In nitrogen-enriched soil, seed protein content had little effect on seedling growth.

The beneficial effects of seed protein content are more clearly expressed under stress conditions and are more evident when plant growth depends on the nutrients available from the seed. During initiation of germination, temperature influences the expression of the protein effect. After the seedling has formed and is capable of photosynthesis, the nitrogen availability in the soil influences the effect of seed protein content on plant growth.

The relationship between seed protein content and plant growth can be an important factor in crop production because the increase in protein can be controlled by field application of nitrogen. However, since the high rates of nitrogen necessary to produce high seed protein levels are not consistent with the optimum nitrogen rates for yield, growers would probably be reluctant to apply such high rates unless some type of incentive were given them to do so. For seed production, it could still be important to strive for high protein levels in spite of some loss in yield if improved performance from high protein seeds proves to be of economic value.

The results of this study indicate that producing and planting seeds rich in protein may be an effective method of achieving faster stand establishment of cereal crops. Additional research will determine the effects of high protein on yield.

Effect of Seed Protein Content on Plant Growth of Barley and Wheat

by

Arturo Lopez

A THESIS

submitted to

Oregon State University

in partial fulfillment of
the requirements for the
degree of

Master of Science

June 1972

APPROVED:

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Date thesis is presented May 17, 1971

Typed by Opal Grossnicklaus for Arturo Lopez

ACKNOWLEDGMENTS

To the following persons and institutions, I wish to express my sincere appreciation for their invaluable assistance throughout my graduate program and in the preparation of this thesis.

My special debt of gratitude is owed to Dr. Don F. Grabe, my major professor, for his direction, encouragement and patience throughout my graduate program, the conduction of this research and the preparation of the thesis.

Dr. Te May Ching for her assistance in conducting the laboratory research as well as for her critical review of the thesis.

Mr. Ed Hardin, Mr. Rodger Danielson and all the other members of the Oregon State University Seed Laboratory staff for providing assistance, facilities and supplies throughout my program.

Dr. Richard Converse for his critical review of the thesis. Dr. Te May Ching, Dr. Warren Kronstad, Dr. William Chilcote for serving as members of the graduate committee.

Finally I feel indebted to the Instituto Colombiano Agropecuario (ICA) and the Agency for International Development (AID) for making the study possible.

DEDICATED

To my son, Diego, and to my wife, Teresa, for her encouragement, understanding and patience during my years of graduate work

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INTRODUCTION

High yielding crops depend on good quality seed--vigorous seed that produces highly productive plants. In the absence of good seed, a farmer cannot expect optimum returns from his investment in the production practices necessary for growing a crop.

Several factors are involved in the development and maintenance of seed quality. Loss of seed vigor may be due to the effect of external factors such as mechanical damage, high moisture, diseases and insects. On the other hand, the inherent genetic ability of the seed to produce a strong and highly productive plant is enhanced by favorable conditions during the various stages of seed production, processing and storage.

Several seed attributes such as size, weight, age and the quantity and quality of the food reserves influence seed vigor and have been correlated with plant growth. However, the relative importance of these seed attributes in affecting crop yields is not clear.

Seed protein content has been found to affect plant growth in wheat and oats. Recent reports indicate that seeds with higher protein content developed into larger seedlings which in turn produced higher yields. This positive correlation between seed protein content and plant growth can be an important factor in seed production because the increase in protein can be controlled by application of nitrogen and chemicals such as simazine or terbacil. Hence, it is important to study the factors involved in the production of high protein seed as well as the subsequent performance of the seed.

This research is concerned with the effect of seed protein content on plant growth of barley (Hordeum vulgare L.) and wheat (Triticum aestivum L.). The main objectives were to determine the effects of nitrogen fertilization on chemical and morphological characteristics of the seed and the effect of protein level on seeds and seedlings.

LITERATURE REVIEW

Nitrogen Effect on Plant Growth

Nitrogen is considered to be one of the most important macro-elements in the nutrition of plants. Plants of the Gramineae, including wheat and barley, are very sensitive to insufficient nitrogen and are very responsive to nitrogen fertilization. These species utilize nitrogen during all the different stages of their life cycles. The nitrogen uptake by wheat, for example, occurs from the time the roots begin to function until seed maturity. Nitrogen is used by the plant as a constituent of amino acids, nucleotides and many other essential compounds (25, p. 202).

The nitrogen requirements of plants depend on several external factors as well as on the developmental stage of the plant itself. During plant growth the nitrogenous materials are mainly used in the regions of the plant that are still developing (49, p. 369). Emmerling, cited by Salisbury (49, p. 369), as early as 1900 showed that the nitrogen accumulated by Vicia faba leaves, stems and roots was translocated to the seeds, the latest developing organs.

Most of the seed nitrogen moves into the seed from the vegetative areas of the plant and from the soil and becomes a constituent of the seed grain (38, p. 258). McVickar (37, p. 35) showed that 100 bushels of barley grain contained about 110 pounds of nitrogen and 60 bushels of wheat grain contained approximately 75 pounds of nitrogen.

Nitrogen Utilization in Wheat and Barley

Yield

The principal effect of applied nitrogen, as concluded by Terman et al. (54) is to increase total yield. It has been clearly demonstrated in both wheat and barley, that nitrogen has a marked influence on the relative expression of the different characteristics associated with grain yield and quality.

Rhode (48) pointed out that nitrogen fertilization influenced some of the yield components of wheat such as number of culms, weight of 1000 kernels and number of kernels per head. These effects of nitrogen fertilizer on yield components of wheat are consistent with several other reports (45, 62) which indicate that yield increase as a result of field application of nitrogen is due to increase in number of tillers, number of mature heads per plant, number of kernels per head and increase in kernel weight.

Seed weight is the seed characteristic that most greatly affects yield. This yield component has been reported to be markedly influenced by nitrogen fertilization. The average weight of individual wheat seeds was found to be 14.5 and 33.6 mg following the application of 240 and 60 pounds per acre of nitrogen, respectively (38, p. 264). It seems, according to these results, that seed weight varies inversely with the amount of nitrogen added to the soil. This could explain why high nitrogen applications sometimes result in decreases of yield.

The nitrogen influence on test weight seems to be affected by environmental conditions as well as by external factors such as diseases. According to Olson (38, p. 264), no significant increase or decrease of test weight of hard red winter wheat was found in Kansas when the nitrogen fertilization program was varied. In Oregon, however, excessive nitrogen application in areas subject to yellow dwarf virus disease decreased test weight of barley and increased percent of thin kernels.

Grain Protein

Considerable information has been published concerning the effect of field applications of nitrogen on protein content of cereal kernels.

As stated above, Terman, et al. (54) concluded that the principal effect of applied nitrogen was to increase the total yield when moisture and other growth factors were in the normal range. They concluded also that the increase in protein content of forage and grain occurred only when nitrogen was taken up by the plant in excess of vegetative needs. Hunter, et al. (19), working with pastry-type wheat, also concluded that the protein content of the grain was not raised to objectionably high values until more nitrogen was applied than that required to produce maximum yields. When applied nitrogen increased yield significantly, the yield increased at a greater rate than the protein content. With greater applications than required for maximum yield, protein content increased more rapidly than yield.

Fernandez (11) found in central Mexico that applications of 45 lb/acre of N reduced the protein content of wheat seed percentagewise; 90 lb/acre did not change the protein content of the grain, and only when the nitrogen rate was increased to 135 lb/acre did a large increase in the protein content of the wheat grain occur.

Seth, et al. (51), studied nitrogen utilization in high and low protein wheat varieties in North Carolina. They found that the high protein wheat had the same protein content in the vegetative parts as the low protein varieties. However, when nitrogen was applied to the foliage after heading, protein content increased more rapidly in the heads of high protein varieties. These results indicate that nitrogen translocation from the vegetative areas to the seed is greater in high protein varieties.

Croy and Hageman (10) pointed out that wheat genotypes exist that are superior producers of high protein grain. They studied nitrogen utilization in the varieties Ponca, a high protein hard red winter wheat, and Monon, a low protein soft red winter variety. They found that Monon produced higher yields with supplemental nitrogen while Ponca had grain of higher protein percentage.

Hoenor and Deturk (17), working with high and low protein content corn, found insignificant differences in the vegetative parts of both varieties at the higher (200 ppm) and lower (25 and 50 ppm) nitrogen levels. At intermediate levels (100 ppm), however, the high-nitrogen corn had a higher protein content than the low-nitrogen corn.

The above results show clearly that genetic factors greatly influence nitrogen utilization. This would suggest that different varieties have different physiological bases for utilization and transformation of nitrogenous fertilizers.

Besides the rate of nitrogen fertilization and the ability of the plant to obtain nitrogen in adequate quantities, several other factors affect the responses of the plant to nitrogen. Some of the most important ones, such as time of application, moisture, and soil nitrogen availability, will be reviewed.

It has been found that the time of nitrogen application and the season of the year affect plant responses to nitrogen fertilization. Hobbs (16) found that the protein content of wheat grain was decreased by fall fertilization, whereas spring application significantly increased the protein content of the wheat grain. He concluded that the season of the year had a greater effect on growth characteristics and protein content than did time of application of nitrogen. His results are in agreement with those of Peterson (41) who found that nitrogen is more effective, as far as protein content is concerned, the nearer it is applied to harvest.

Long and Sherbolkoff (29) studied the relation between yield and protein content at three different dates of nitrogen application: November, March, and May. They observed that yield was decreased significantly when the nitrogen application was delayed until May, but the protein content was significantly higher. There were no differences in the protein content of grain following November and March fertilizer applications.

For winter wheat in general, it is concluded that applications of nitrogen made in the spring are more effective than fall applications made at time of seeding. According to Pumphrey (43), nitrogen applied in early spring is less subject to leaching, microbiological tie-up, and denitrification than nitrogen applied at planting time.

The availability of moisture during the growing season is considered to be another critical factor in determining the beneficial effects of nitrogen. Terman, et al. (54) found, while working with wheat under different nitrogen levels and available water, that nitrogen with adequate water increased yield, whereas with severe water deficit, the entire effect was to increase protein content. In intermediate situations, nitrogen increased both yield and protein content.

Leggett (27) pointed out that, in general, for a given soil the greater the supply of available moisture the more nitrogen fertilizer was required to produce maximum yield. In an earlier experiment in different rainfall areas, Leggett and Nelson (28) found that protein content was increased markedly by nitrogen fertilization principally in the drier areas.

The nutritional conditions of the soil also play an important role in nitrogen utilization. In soils deficient in nitrogen, small applications of this element generally result in yield increases with little or no modification in the protein content of the grain, whereas larger applications mainly increase the protein content (4). Macleand and Carson (31) indicated that the percent of protein and nonprotein nitrogen fractions in the barley grain was lower following applications of 100 ppm phosphorous than with 25 and also lower following application of 200 ppm potassium than with 50 ppm. It is reported also in another paper by Macleand (30) that the weight of 1000 kernels of barley decreased with the addition to the soil of 25 ppm of phosphorus plus 100 or 200 ppm nitrogen. With the addition of 100 ppm phosphorus, kernel weight decreased only with the 200 ppm nitrogen treatment.

Protein content has also been found to be affected by crop residue. Hedlin et al. (15) noticed that protein content was low after grass plow down. However, protein content of wheat increased after alfalfa and clover were plowed down. They concluded that crop residues apparently affected rate of nitrification through the growing season. The more rapid the nitrification the higher the grain protein content and yield.

Protein content is reported to vary among the kernels within the head. Alfaf et al. (1) found that grain from the central florets had significantly higher protein content than grain from the top third, and significantly lower protein content than grain from the bottom third of the spike. McNeal and Davis (35), working with spring wheat, found that the protein content of the lateral kernels was higher than that of the central kernels. They also found that the grain in the middle spikelets had a higher protein content than those from the top spikelets. According to their conclusions, the soil N supply was insufficient to enable a maximum protein level to be reached in the later-maturing kernels.

Amino Acids

Waggle et al. (60) reported that total protein and 17 individual amino acids were significantly increased by nitrogen fertilization of grain sorghum. Coic et al. (9) found, in both barley and wheat, that nitrogen fertilization increased glutamic acid, proline and phenyalanine, and decreased lysine, glycine and alanine.

Sosulski _et al._ (52) found that only two amino acids, glutamic acid and proline, were positively correlated with the protein content of the whole wheat kernel, while alanine, arginine, aspartic acid, lysine, serine, threonine and valine were negatively correlated with total protein content. Gunthard and McGinnis (14) found that lysine and argenine varied significantly with protein content in wheat kernels. High protein kernels (16.5%), following field application of nitrogen, contained significantly lower lysine than did the low protein kernels (10.3%). Price (42) concluded that the amino acid nitrogen as a percentage of the total nitrogen tended to be higher in samples of lower total nitrogen content.

Lodging

The most restrictive problem affecting nitrogen fertilization is the lodging of the straw and the excessive growth of straw. Lodging, especially before heading, favors diseases, delays maturity, and reduces yield and test weight (43).

Weibel and Pendleton (61) and Eldredge (12) studied the effect of artificial lodging on winter wheat. They found that lodging greatly decreased yield, test weight, and kernel weight. They reported that the detrimental effects of lodging were more severe when it occurred at heading that at any subsequent stage of growth. They also found that grain from lodged plants was higher in protein content than grain from erect plants.

Loude and Pauli (26) pointed out that the influence of lodging on the protein content of the grain suggested a restricted capacity of the lodged plants to take in or to translocate nutrients or a restricted capacity to synthesize carbohydrates.

Protein Increase by Field Application of Chemicals

Recent research with field application of low rates of herbicides such as simazine indicate that these products might be used to increase grain protein content of some cereals.

Ries *et al.* (46) found that field applications of simazine (2-chloro-4, 6-bis (ethylamino-s-triazine)) increased protein content in bean seed, rice foliage, alfalfa forage and oats. Similar results were found in wheat grain by application of simazine and terbacil (3-tert-butyl-5-chloro-6-methyluracil) at subherbicidal rates (47).

McNeal *et al.* (36) working with spring wheat found that simazine significantly affected both yield and percent protein. They concluded that when simazine was applied in late stages of maturity the increase in protein content was accompanied by a corresponding decrease in yield of grain.

Vergara *et al.* (59) reported that simazine application to flooded soil at flowering time increases the percent protein in the rice grain. They found also that the increase in protein content was accompanied by a decrease in grain yield.

These reports support the observation made by Malloch and Newton (32) that usually there is an inverse relation between yield and protein content, and the results reported by Terman (54) and Hunter et al. (19) working with applications of nitrogen.

Physiological Aspects of Seed Protein Content

Several seed attributes, seed size or weight (24, 63), and lately seed protein content (47, 50) have been correlated with plant growth and yield. All of them but protein content have been carefully studied. There is not, however, agreement in determining which of these attributes is more representative of the ability of the seed to produce a vigorous seedling and consequently a high-yielding crop.

Experiments have shown that some physiological aspects of seed germination, such as imbibition and respiration, are correlated with certain seed characteristics, such as size and protein content.

Imbibition

Keller and Bleack (22) studied seeds of crested wheatgrass (Agropyron desertorum (fish) Schult.) and Fairway wheatgrass (A. cristatum (L) Gaerfn.). They found that the rate of water absorption differed significantly with different strains, seed lots, and age and size of the seed. Some of their results indicated that the largest seed imbibed water at a slow rate between 0 and 90 hours of soaking, whereas the smallest seed had the fastest absorption during the first 40 hours. They also concluded that water absorption was directly proportional to temperature.

Dungan (11) found that the difference in percentage of water taken up by high (15.29%) and low (6.10%) protein corn strains was very great. The low protein corn absorbed water faster. He concluded that high protein levels in the endosperm gave it a hard vitreous texture. Such a a texture of the kernel could contribute to its low absorptive rate.

Mayer and Poljakoff-Mayber (33, p. 39) stated that the chief component which imbibes water in seeds is the protein, whereas starch does not add to the total swelling of the seeds, even when large amounts of starch are present.

Burke (6) concluded that a higher initial rate of absorption was not necessarily associated with a higher rate of germination in wheat varieties. He pointed out that differences in permeability to gases rather than to water were responsible for the differences in their germination rates.

Respiration

Respiration rate of seeds has been associated with emergence and seedling growth rates. Woodstock and Grabe (65) compared the respiration rates of corn during the first 30 hours of germination with seedling growth 3 to 5 days after planting. They concluded that a positive correlation existed between oxygen uptake rate during imbibition and subsequent germination and growth. A similar relationship was established by Kittock and Law (23) for wheat seeds.

Throneberry and Smith (56) pointed out that loss of viability of corn seed appeared to be closely associated with respiratory failure. They concluded that most seeds which failed to germinate had negligible respiratory activity at the 18-hour stage of germination. Hence, the breakdown in respiratory machinery may be an important cause of loss of viability.

Protein Content Effect on Plant Growth and Yield

A positive relationship between plant growth and the protein content of the seed planted was reported by Schweizer and Ries (50) in 1969. They studied the behavior of wheat and oats seeds with different protein content as a result of field application of nitrogen and the herbicides simazine and terbacil. Their results indicated that the seedling growth of wheat and both the seedling growth and yield of oats were positively related to seed protein content. Seeds with higher protein content developed into larger seedlings which in turn yielded 21 to 42 percent more grain in oats.

In 1970 Ries et al. (47) also reported that yield of wheat was directly correlated with seed protein content. Protein content was increased by field application of nitrogen plus simazine and terbacil applied at sub-herbicidal rates. Three experiments were carried out at Michigan and at Obregon, Mexico. High protein content seeds yielded higher the next generation in all of the Michigan field experiments. Yields and protein content were also correlated in the experiments in Mexico; however, the protein-yield relationship was eliminated by 100 lb/acre of nitrogen applied at time of planting.

MATERIALS AND METHODS

Seeds of barley (*Hordeum vulgare* L.) and wheat (*Triticum aestivum* L.) with different protein levels were produced by applying various amounts of nitrogen to the crops. Experiments were conducted to determine the effects of the nitrogen applications on chemical and morphological characteristics of the seeds, physiological aspects of germination, and subsequent seedling growth.

Varieties

'Nugaines' winter wheat and 'Casbon' winter barley were used in these experiments.

Nugaines is a semi-dwarf soft white winter wheat with white chaff, bearded awns, and a common-type head. It has short straw and is very resistant to lodging. Nugaines is considered to have more yield potential than other commercial wheat varieties available for the medium to high rainfall areas. It is adapted in the Pacific Northwest. The baking quality of Nugaines is poor for bread and family flour but is good for crackers and noodles because of its 7.5 to 10 percent protein content (55).

Casbon is a mid-tall, late maturing, six-row feed barley. It was developed from a cross of Cascade X Bonneville. It is adapted to the winter growing areas of the Willamette Valley. Its average yield is 4, 094 lb/acre and its average protein content at the North Willamette Experiment Station is 7.95 percent (39).

Production of Seeds with Different Protein Contents

The barley seed was produced during the crop years 1969-1970 at the North Willamette Experiment Station. Sixteen plots were laid out in a uniform area of a previously established Foundation seed increase field. Individual plots consisted of seven rows 30.5 cm (1 foot) apart and 2.45 m (8 feet) long. Different rates of nitrogen were applied to obtain the desired range of seed protein levels. Urea was used as the source of nitrogen and was applied by hand at the following times and rates:

Treatment	Kg/ha of nitrogen	
	Fall (1969[1/]	Spring (1970)[2/]
N0 (control)	34	0
N1	34	45
N2	34	90
N3	34	180

1/ Seeding time.
2/ Tillering time.

The plots were arranged in a randomized complete block design with four replications. Management practices, except the spring nitrogen applications, were those used for normal Foundation seed production of barley.

The plots were harvested by hand in July 1970. A 1.52 x 2.45 m area (5x8 feet) was harvested from each plot. The sheaves were threshed in a small thresher and the seed cleaned and weighed.

The wheat seed of different protein levels was obtained from fertilizer trials conducted by Dr. Steve Roberts of the Oregon State University Soils Department in 1969. The following rates of nitrogen were applied:

Treatment	Kg/ha of nitrogen	
	Fall (1968)1/	Spring (1969)2/
N0 (control	0	0
N1	28	85
N2	28	131
N3	28	216

1/ Seeding time.
2/ Tillering time.

The plots were harvested and threshed in July 1969 and yield was recorded. The samples were stored at room temperature at the Oregon State University Seed Laboratory until studied.

After plot weights were taken, the seeds of both barley and wheat were screened with hand screens. The very thin and small seeds were discarded while the normal ones were kept at room temperature until the study was made. Barley was studied three months after harvesting, whereas wheat was stored for 15 months before studying.

Seed Characteristics

Protein Content

The protein content of the whole seed, embryo and endosperm was determined. The whole seed protein content was determined by the Udy dye-binding method for all fertilizer treatment. The embryo and endosperm protein was determined by the Micro-Kjeldahl method for samples with highest and lowest protein levels from each crop.

Udy Method. The Udy method was chosen because it is simple, fast and accurate. This method is based on a chemical reaction between the protein and the disulfonic acid dye (Acid Orange 12), at pH 2. 2 to form an insoluble complex. The concentration of unbound dye, as measured colorimetrically using a light filter (470 mμ), provides the estimate of protein content based on a standard chart of known variety of wheat and barley (58).

Twenty-five milliliters of wheat seed and 20 milliliters of barley seed per sample were uniformally milled in a CRC Micro-Miller for 45 seconds. Each ground sample was divided into three sub-samples which were analyzed similarly.

To 970 mg of ground sample, 97 ml of Acid Orange 12 dye was added from an automatic pipette. The mixture was agitated for three minutes on an automatic shaker.

The insoluble complex was filtered through a fiber-glass filter disc and a few drops of filtered dye were used to make the measurement in the color analyzer. The readings on the galvanometer, which were estimated to the nearest .1%, were converted into percent of protein by using Udy wheat and barley conversion tables. A known concentration of dye was used as a reference standard.

Micro-Kjeldahl Method. Representative samples were drawn from samples with the highest and lowest protein content of both wheat (6.6% and 10.8%) and barley (6.5% and 9.4%).

The embryos of the samples described above were dissected from their endosperms by hand using scalpel and forceps. Three sub-samples of ten embryos each were analyzed.

The endosperms were ground and divided into three 30 mg sub-samples for protein determination.

The ten embryos and the ground endosperm were weighed on a micro balance and transferred to digestion flasks. The digestion was made for two hours in an Aminco digestion apparatus. During the distillation process, the powerstat was set at 75. The distillates were collected in 50 ml Erlenmeyers and then titrated with .01 N HCL in an automatic Radiometer titrator set with end point at pH 4.9, proportional band at 1.0 and delay shut-off at 5 sec. Separate blanks were used for the embryo and endosperm protein determination, using the same quantity of reagents and digestion period as for the respective samples.

The percent of nitrogen per sample was calculated by the following equation:

$$\% \text{ N} = \frac{(\text{ml HCL in sample - ml in blank}) \times .01 \times 14 \times 100}{\text{weight sample (mg)}}$$

The protein percent was determined by multiplying the % N times 6.25 (7).

Starch Content

Samples of barley and wheat with the highest and lowest protein contents were analyzed for starch content. Cereal seeds accumulate mainly starch as reserve material, and the relative concentration of this carbohydrate has been found to be affected by field application of nitrogen (57). Furthermore, protein content is directly affected by seed weight.

The starch estimation was made by acid extraction as outlined by Ching (7):

Sample preparation. Twenty-five ml of wheat and 20 ml of barley seed were uniformally milled in a CRC Micro-Miller for 45 seconds. Three replications of 500 mg of the ground seed were put in the oven to dry for 24 hours at 90C.

The original moisture content of the ground wheat and barley seeds was determined on a fresh weight (FW) basis according to the following formula:

$$\% \text{ Moisture} = \frac{\text{Fresh weight - Dry weight}}{\text{Fresh weight}} \times 100$$

Acid Extraction. One hundred mg were weighed out from each dried sub-sample and placed in 50 ml boiling flasks with 20 ml of 0.2 N H_2SO_4. The mixture was then refluxed for 60 minutes in a water bath at 75C. The hot mixture was filtered through Whatman No. 42 filter paper, and the residue was washed with 20 ml of distilled water. The filtrate and wash were then combined and diluted to 100 ml volume with distilled water.

Sugar Determination. The sugar content of the filtered hydrolysate was determined by the Anthrone method using glucose as the standard.

The combined filtrate first diluted to 100 ml was too concentrated for the Anthrone method therefore a 1:25 dilution was made. An aliquot of 1.5 ml of the diluted extract was used for the color reaction and then was read in a DB spectrophotometer at 620 mμ.

Starch Content Calculation. Starch content, expressed both on a fresh weight (FW) and dry weight (DW) basis, was calculated by the following equations:

$$\% \text{Starch (DW)} = \frac{\frac{2500}{1.5} \times \mu g \text{ sugar} \times 0.9 \times 10^{-3}}{100} \times 100$$

$$\% \text{Starch (FW)} = \frac{\frac{2500}{1.5} \times \mu g \text{ sugar} \times 0.9 \times 10^{-3}}{100\,(1 + \frac{\% \text{ moisture}}{100})} \times 100$$

Seed and Embryo Weight

Seeds weights were determined on 1000 seeds per treatment. Embryos weights were determined only in samples with the highest and lowest protein content of both wheat (6.6% and 10.8%) and barley (6.5% and 9.4%). Fifty embryos were dissected from their endosperms, using a scalpel and forceps, and weighed on a micro balance. Endosperm weight was determined by subtracting the weight of the embryo from the weight of the entire seed.

Performance of Seeds

Respiration

The respiration rate of seed samples having the highest and lowest protein content of both wheat and barley was determined.

Oxygen (O_2) uptake and carbon dioxide (CO_2) output during 24 hours of imbibition were measured manometrically at 25C in a Gilson differential respirometer. Samples of 15 seeds surface-disinfected with 5% NaC10 were placed in 17 ml respiration flasks. One ml of distilled water was placed in the main compartment of all the flasks except the two thermobarometers, and 0.2 ml of 4 N KOH was placed in the center well of the flasks for O_2 determination, with a piece of filter paper to provide more surface area for CO_2 absorption. The respiration quotient ($RQ = \frac{CO_2}{O_2}$) was calculated for each reading. Readings were made in triplicate at 2, 4, 6, 12, and 24 hours.

Imbibition

Samples of wheat and barley of highest and lowest protein content were used for this experiment.

Five hundred and sixty seeds were counted out from each sample to measure the water absorption during a 36-hour period. The seeds were exposed to water on a single layer of blotter paper in plastic boxes 24 x 16.5 x 3.8 cm deep. The paper was previously soaked and the seeds were placed in a single layer. A second pre-soaked layer of blotter paper was used to cover the seeds. The total amount of water used, calculated from the difference between the dry and wet blotter papers, was 55 ml. The boxes were placed in a germinator at 25C during the imbibition period.

Water absorption was measured each hour during the first eight hours of imbibition; each two hours from 10 to 24 hours, and each hour during the last interval of imbibition from 25 to 36 hours.

At the intervals indicated above, duplicate sub-samples of ten kernels each were taken out and immediately placed in tared small weighing bottles and weighed to the nearest milligram on an electrometric balance. The seeds in the bottles were then oven-dried for 24 hours at 100C and reweighed. The moisture content of the seeds at each imbibition period was calculated according to the following formula:

$$\% \text{ Moisture} = \frac{\text{Wet weight - Dry weight}}{\text{Wet weight}} \times 100.$$

Duplicate readings were then averaged.

Speed of Germination and Shoot and Root Growth

Speed of germination and root and shoot growth of barley and wheat were measured by incubating the seeds in moist blotter paper in darkness at 15, 20 and 25C.

Twenty-five seeds were taken at random from each sample and placed on a moistened blotter paper and covered with a similarly moistened blotter. Blotters with seeds were placed in plastic dishes 24 x 16.5 x 3.8 cm deep. The plastic dishes were placed at random in germinators at the desired temperatures. Four replications were used.

The germination indexes were determined by adding the quotiens of the daily counts divided by the number of days of germination.

Root and shoot length were measured first when the organs reached 1 cm or more and then every other day for eight days. Average lengths were calculated on the basis of the number of seeds germinated.

Seedling Dry Matter Production in Low and High Nitrogen Soil

Greenhouse experiments were conducted to study the effect of seed protein content on rate of seedling emergence and seedling dry matter production. The studies were conducted in February and March of 1971. One experiment was carried out in high nitrogen soil and a second experiment was conducted in low nitrogen soil.

For each experiment, seeds of each protein content were planted in 38 x 58 x 10 cm wooden flats in randomly selected rows. The rows were spaced 7.5 cm apart and 15 seeds were planted about 2.5 cm apart within each row. The seeds were covered to a depth of 1.5 cm with soil which was packed down firmly. Fermate was applied two days after planting to retard growth of pathogenic fungi. The experiments were laid out in a completely randomized design with four replications. The greenhouse temperature was approximately 22C (72F). The seedlings were harvested 2, 4, and 6 weeks after planting. The plants were cut at ground level with razor blade, dried 24 hours in an oven at 95C, and weighed.

Statistical Analysis

Data were analyzed by ANOV according to procedures outlined in Steel and Torrie (53).

The data from the experiments involving more than two treatments were analyzed as factorials. The least significant difference (LSD) at 5% and 1% levels of significance was the test criterion used for comparison of paired means. The sample with the lowest protein content was considered the check.

The unpaired 't' test was used for the comparisons involving two sample means (embryo and endosperm protein content, total carbohydrate content).

RESULTS AND DISCUSSIONS

Production of Seed with Different Protein Contents

During the process of increasing net protein accumulation by field application of nitrogen, yield and other seed characteristics such as seed weight, embryo weight and starch content were affected.

Seed Yield

Barley seed yield was increased with spring applications of 45 and 90 kg/ha of nitrogen (Table 1). These increases were significant, at the 5% level, only at the 45 kg/ha rate of application. Application of 180 kg/ha actually reduced the yield, but not significantly. The analysis of variance for these data is shown in Appendix Table 1.

The negative effect on yield from the high rate of nitrogen was probably due to lodging. Straw strength of the variety Casbon is classified as mod-stiff (39), but in this experiment, the growth of straw became excessive at the high rate of nitrogen.

Wheat responded somewhat differently to applications of nitrogen in that all nitrogen treatments caused significant increases in yield (Table 2). Maximum yield (6, 997 kg/ha) was achieved with the second increment of nitrogen (28 + 131 kg/ha, fall and spring,

Table 1. Effect of nitrogen applications on seed yield, seed protein content and 1000-seed weight of barley.

Rates of N, kg/ha		Yield	Protein	1000-seed
Fall (1969)	Spring (1970)	kg/ha	%	g
34	0	2792.00	6.33	44.00
34	45	3783.50	6.90	39.50
34	90	3105.00	7.52	40.25
34	180	2590.75	9.08	36.50
LSD (.05)		732.80	.70	2.21
LSD (.01)		1053.40	1.01	2.21
C.V. %		14.98	5.82	3.44

Table 2. Effect of nitrogen applications on seed yield, seed protein content and 1000-seed weight of wheat.

Rates of N, kg/ha		Yield	Protein	1000-seed
Fall (1968)	Spring (1969	kg/ha	%	g
0	0	3926	6.6	40.5
28	85	6975	7.5	35.7
28	131	6997	8.9	33.6
28	216	6232	10.8	30.6
LSD (.05)		840		
LSD (.01)		1134		
C.V. %		10.0		

respectively). The highest nitrogen rate (28 + 216 kg/ha, fall and spring, respectively) reduced the yield somewhat, but it was still significantly higher than the control.

Seed Protein Content

Barley. The data (Table 1) show that all spring nitrogen applications increased seed protein content. The increases were significant at the 5% level for the 45 kg/ha rate, and significant at the 1% level for the 90 and 180 kg/ha rates.

Wheat. Nitrogen fertilization also increased the protein content of wheat (Table 2). As in barley, protein content of the seed increased significantly with increasing rates of nitrogen. These results are consistent with reported data (3, 44).

Yield-Protein Relationships

In barley (Figure 1), there was a negative correlation between seed yield and seed protein content after the first increment of nitrogen applied. Seed protein content increased regularly as the rate of nitrogen was increased, whereas seed yield decreased as the rate of nitrogen was increased from 45 kg/ha to 90 and 180 kg/ha (spring application). Such a negative relationship between yield and protein has also been reported by Terman (54) and Hunter *et al.* (19).

The yield-protein relationship in wheat tended to be positive until the third increment of nitrogen (131 kg N/ha, spring) was

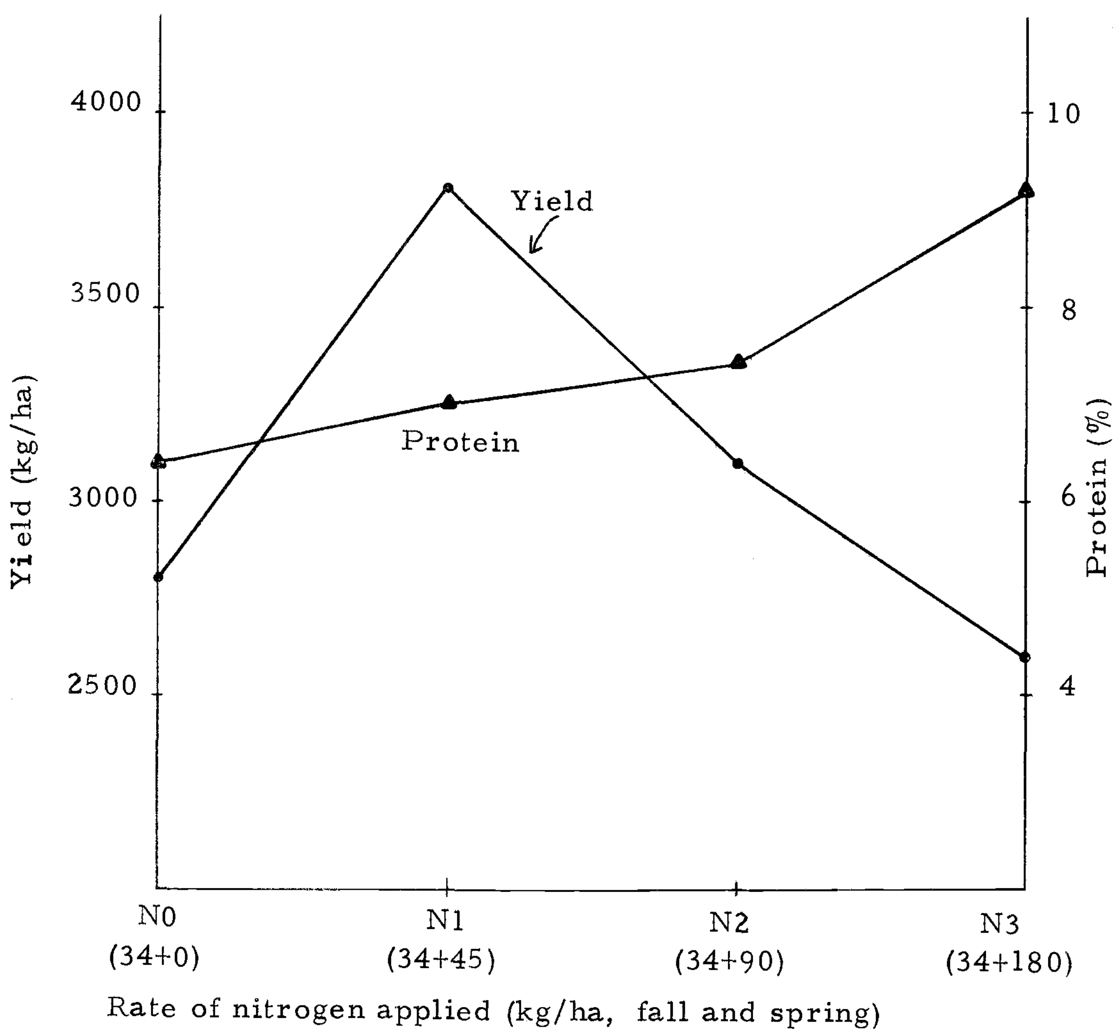


Figure 1. Average seed yield and seed protein content of barley as affected by field application of nitrogen.

applied (Figure 2). At the highest level of nitrogen (216 kg/ha, spring) yield was lowered, but not significantly. These results do not agree completely with those reported by Terman (54) and Hunter, et al. (19). However, in a more recent publication, Hucklesby et al. (18) found a positive correlation between grain yield and grain protein content of wheat. They concluded that such relationships could be achieved with lodging-resistant varieties and with lodging-susceptible varieties also when environmental conditions do not induce lodging.

The results here indicate that with Nugaines, a lodging-resistant variety, both high protein and high yields can be obtained from heavy applications of nitrogen.

The high rates of nitrogen applied in this experiment were higher than normally used in cereal grain production. The higher seed protein levels obtained may be undesirable for processing purposes but may have practical advantages for seeding purposes. The remainder of this thesis is concerned with seed performance attributes as they relate to the protein content of wheat and barley seeds.

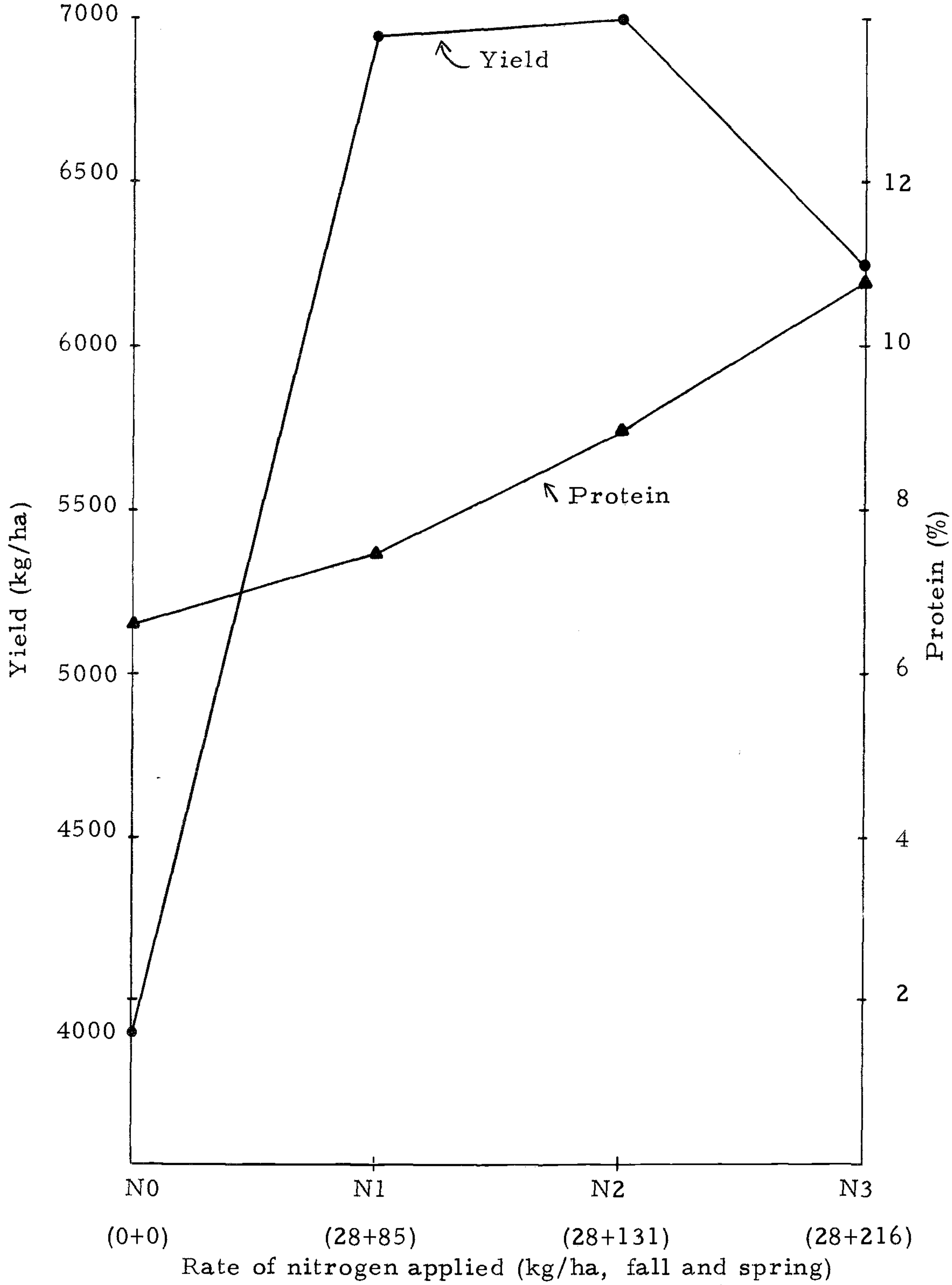


Figure 2. Average seed yield and seed protein content of wheat as affected by field application of nitrogen.

Morophological and Chemical Characteristics of the Seeds

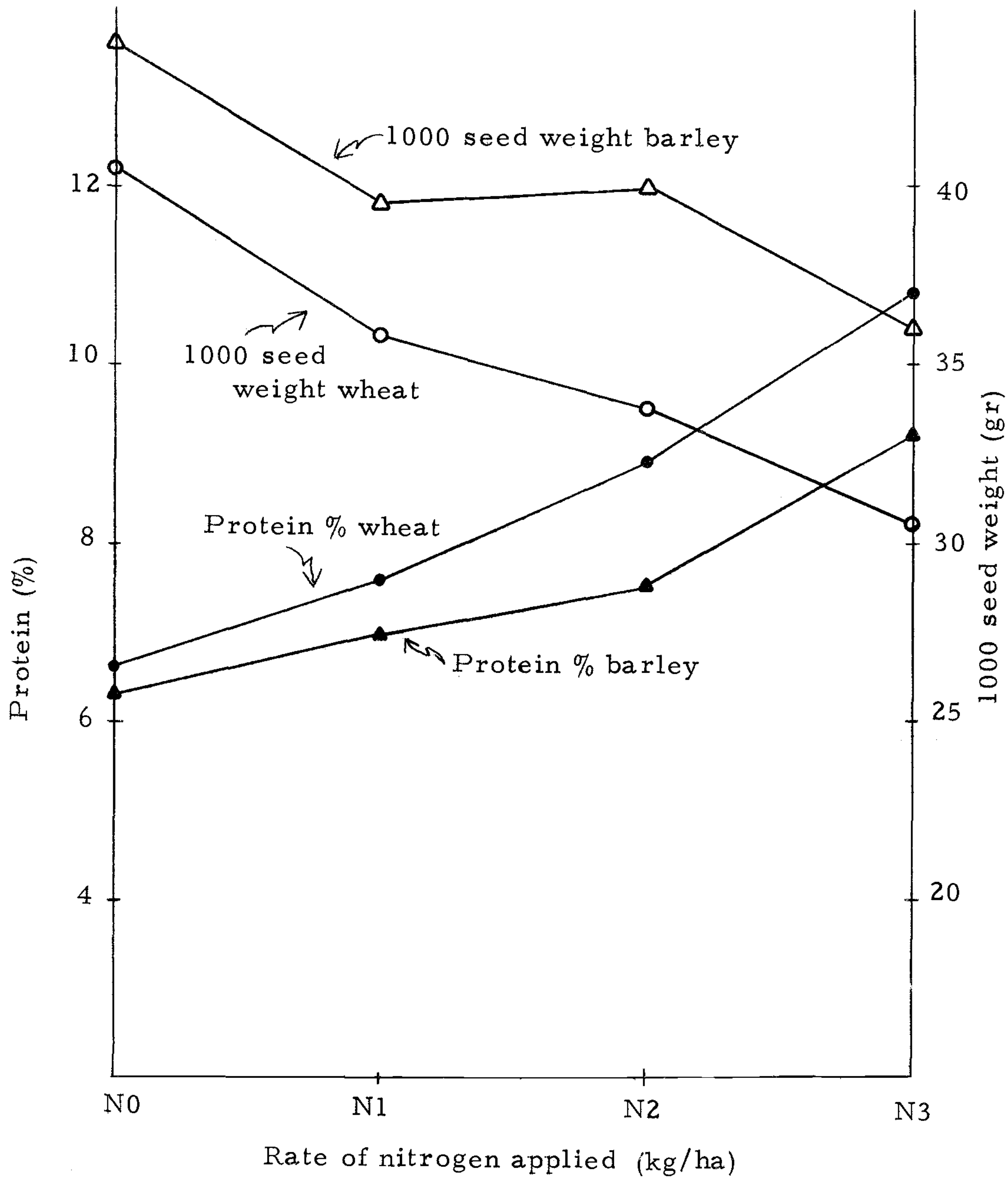
1000-seed weight

Barley. One thousand seed weight decreased as the rate of nitrogen increased (Table 1). These decreases were all significant at the 1% level as shown by the analysis of variance in Appendix Table 3.

The largest increment of nitrogen caused the largest reduction in seed weight, 7.5 gr less than the control. This substantial reduction in seed weight could be the result of lodging. The seeds on the lodged plants were possibly reduced in size because of restricted transport of materials needed for synthesis of storage materials.

Wheat. As in barley, nitrogen fertilization reduced the seed size in wheat (Table 2). The seed weight decreased progressively as the rate of nitrogen applied increased. The weight of 1000 seeds from the plots which received the highest level of nitrogen was 9.9 gr lower than the seeds from the control plots.

The data show that nitrogen fertilization can be expected to increase protein content but decrease the seed size or 1000 seed weight (Figure 3). In this respect, Tweedy *et al*. (57) pointed out that expressing protein as a percentage may be irrelevant if dry



Barley: N0 = 34+0; N1 = 34+45; N2 = 34+90, and N3 = 34+180 kg/ha, fall and spring applications.

Wheat: N0 = 0+0; N1 = 28+85; N2 = 28+131, and N3 = 28+216 kg/ha, fall and spring applications.

Figure 3. Seed protein content and 1000 seed weight of barley and wheat as affected by nitrogen fertilization.

matter is reduced. They concluded that an increase in percentage of protein would be of greatest significance if accompanied by an increase in dry matter, higher or at least equal to the control. This is an interesting point insofar as economic production of high protein food or feed is concerned. As far as seed production is concerned, it is important to establish the importance of the proportion of total protein and starch to the performance of the seed.

Starch Content

Seeds of barley and wheat from the plots which received the lowest and highest nitrogen applications were analyzed for starch content.

Comparisons involving the two samples means were made by the unpaired "t" test at the 5% and 1% levels of significance. The data are shown in Table 3.

Barley. The starch percentage of barley seeds substantially decreased as a result of the 180 kg/ha rate of nitrogen application (Table 3). This difference was significant at the 1% level.

Wheat. The starch percentage of wheat seeds also decreased as a result of nitrogen fertilization. The difference between the control and the 216 kg/ha rate of application was significant at the

5% level (Table 3).

Table 3. Effect of nitrogen application on starch content of barley and wheat seed.

Rates of N, kg/ha		Starch (%)	
Fall	Spring	Fresh weight	Dry weight
Barley			
34	0	68.99	76.86
34	180	55.27	61.36
Difference		13.72**	15.50**
Wheat			
0	0	62.05	69.34
28	216	57.88	64.55
Difference		4.17*	4.79*

** Significant difference at 1% level.
* Significant difference at 5% level.

Embryo and Endosperm Protein Content

The protein contents of dissected embryos and endosperms were determined separately by the Micro-Kjeldahl method.

The data (Table 4) show that the embryos of both wheat and barley contain a high percentage of protein. Nitrogen application, however, did not significantly affect the protein percentage of the embryos in either crop.

The protein content of the endosperm, on the other hand, increased significantly with increased nitrogen applications. This

Table 4. Effect of nitrogen application on protein content and weight of embryo and endosperm of barley and wheat.

Rate of N, kg/ha		Embryo			Endosperm		
Fall	Spring	Weight mg/embryo	Protein mg/embryo	%	Weight mg/endosperm	Protein mg/endosperm	%
Barley							
34	0	.817	.316	38.73	43.2	1.99	5.08
34	126	.830	.329	39.60	35.7	2.36	7.91
Difference				00.82 NS			2.83**
Wheat							
0	0	.363	.154	41.99	39.1	2.62	6.02
28	216	.373	.157	42.04	29.2	3.36	9.56
Difference				00.05 NS			3.54**

**Significant difference at 1% level
NS Nonsignificant.

increase was evident both on an absolute basis and percentagewise, and occurred even though total endosperm weight decreased (Table 4). These results indicate that almost all the increased seed protein resulting from high nitrogen applications was concentrated in the endosperm of barley and wheat seeds.

Performance of Seeds

The effect of protein content on the performance of barley and wheat seed was tested by studying imbibition, respiration, root and shoot growth and dry matter production.

Imbibition

Barley. It is evident from Figure 4 that the barley seed with the highest protein content (9.4%) absorbed water more rapidly than did the low protein (6.4%) seed.

Three different stages were distinguished in the barley imbibition curve (Figure 4). During the first stage, 0 to 12 hours, the amount of water absorbed increased very rapidly; during the second stage, 12 to 18 hours, there was no additional absorption; and during the third stage, after 18 hours, the absorption rate increased rapidly again.

During the third stage of imbibition germination took place. The coleorhiza of the high protein seed started to break the seed

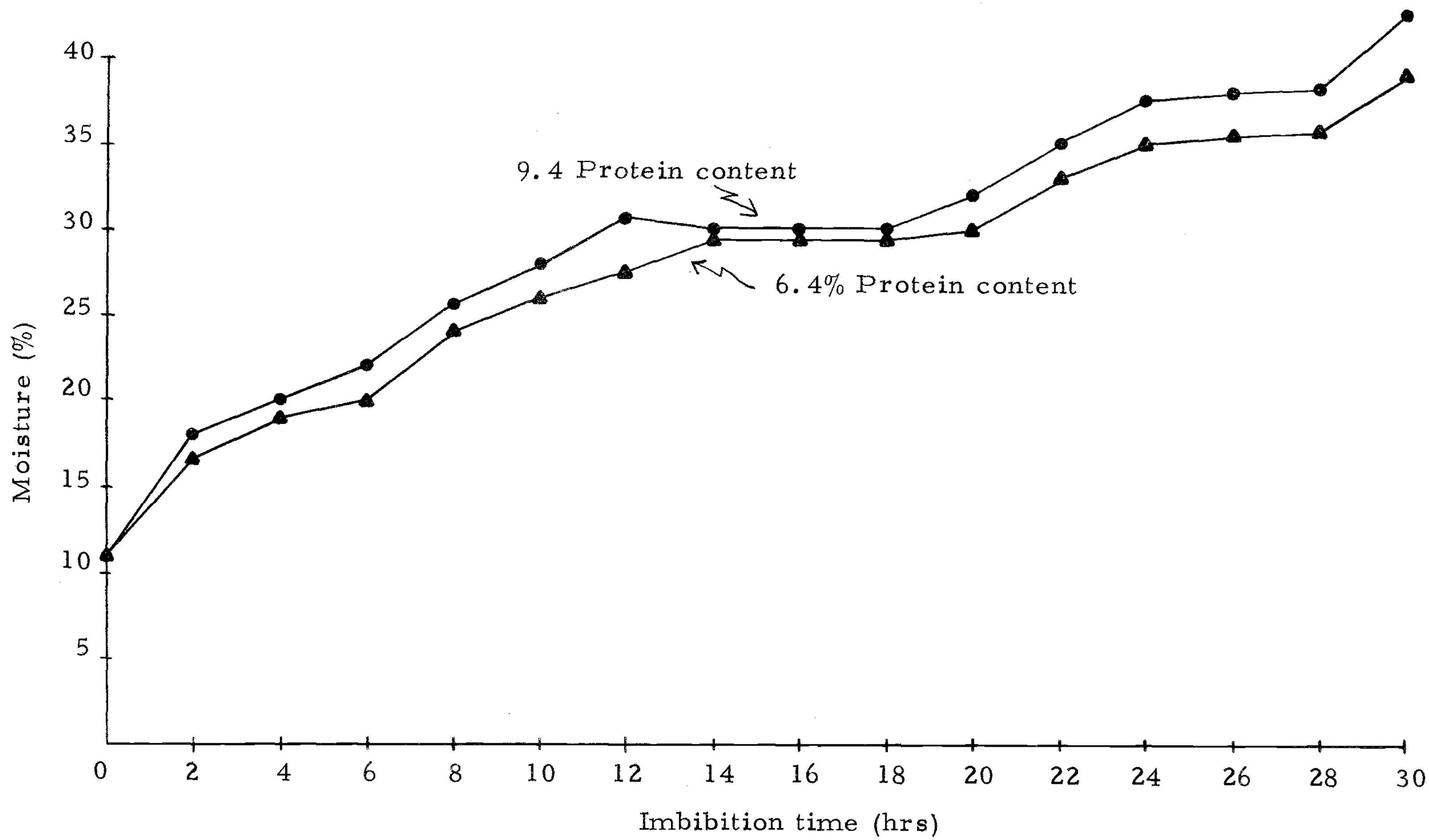


Figure 4. Water absorption by low (6.4%) and high (9.4%) protein barley seed.

coat at about 25 hours after imbibition started; by this time the moisture content of the seed was about 38%. Coleorhiza emergence in the low protein seed started 4 hours later than the high protein seed, but again when the moisture content was 38%.

Wheat. Figure 5 shows that the wheat seed of high protein content (10.8%) absorbed water at a faster rate than did the low protein seed (6.6%).

There were, as in barley, three different stages of imbibition (Figure 5). The first stage of high water absorption rate lasted 10 hours. A lag period of 8 hours then occurred, followed by an increased rate of water uptake again after 18 hours. The high protein seeds started to germinate after 24 hours of imbibition, followed by the low protein seeds 3 hours later. In both cases, the coleorhiza started to break the seed coat when the moisture content of the seed was around 35%.

The curves in Figures 4 and 5 show that seeds with higher protein levels have a higher rate of water uptake. The data suggest also that when incubated at 25C, the higher rate of water absorption was associated with higher speed of germination in both barley and wheat.

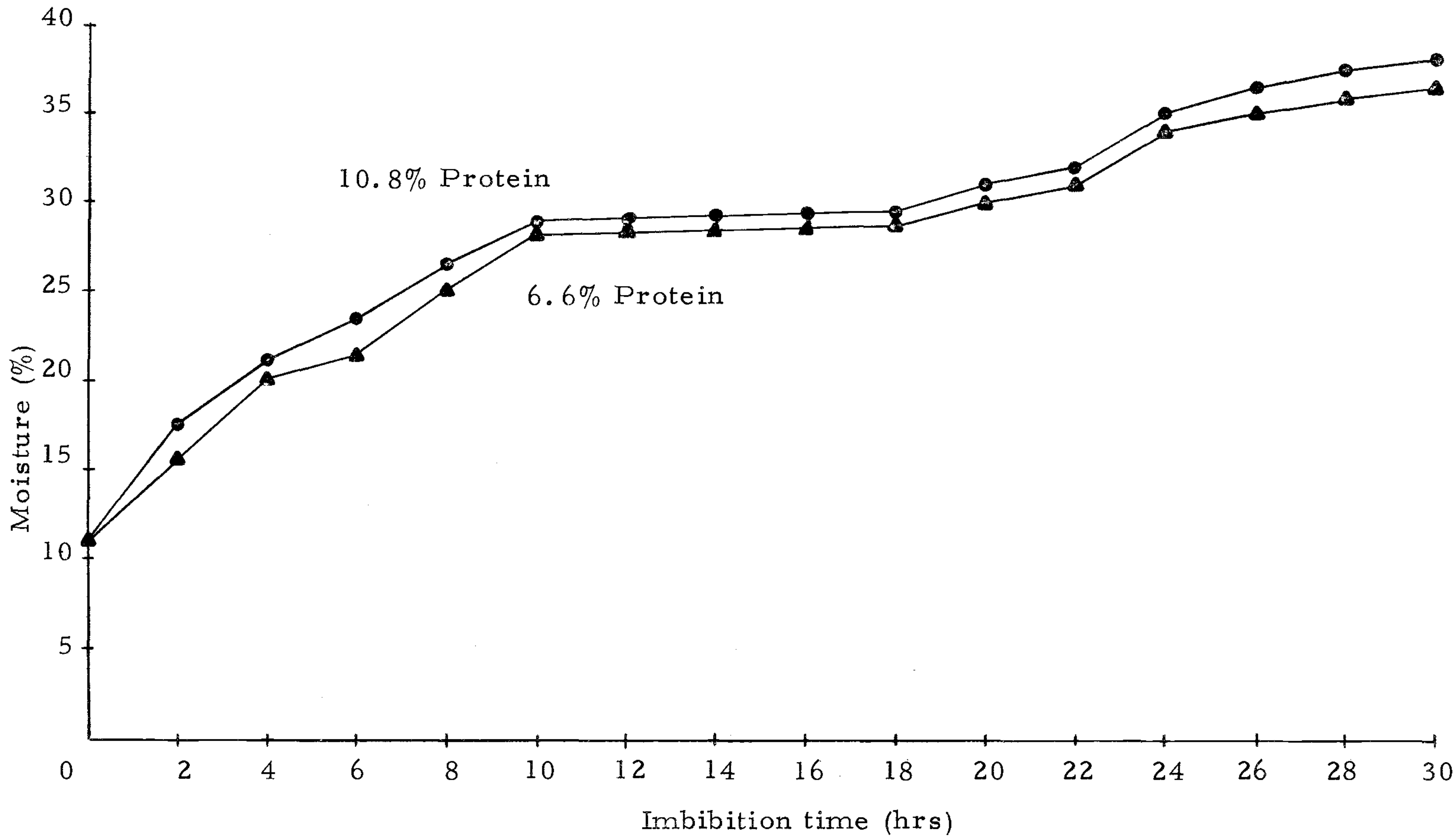


Figure 5. Water absorption by low (6.6%) and high (10.8%) protein wheat seed.

Respiration

Barley. During the first 6 hours of imbibition, there was practically a uniform increase in oxygen uptake. The high protein seed (9.4%) absorbed slightly more oxygen than did the low protein seed (6.4%). After 12 hours of imbibition the rates of absorption became more divergent, and after 24 hours the high protein seed absorbed 3.5 μl/15 seeds/30 min more O_2 than the low protein seed (Figure 6 and Appendix Table 4).

The CO_2 output during the initial 4 and 6 hours of imbibition was slightly higher in the low protein seed. After 12 hours of imbibition the CO_2 output became higher in the high protein seed and the difference became still greater at 24 hours.

Wheat. Differences in O_2 absorption by the low (6.6%) and high (10.8%) protein wheat seed were evident two hours after imbibition. These differences were small until 12 hours, but at 24 hours the high protein seed absorbed 6.6 μl/15 seeds/30 minutes more O_2 than the low protein seed (Figure 7 and Appendix Table 5).

There was practically no difference in the CO_2 output by the low and high protein wheat seed (Figure 7).

Protein content had little effect on the respiratory quotients (RQ) of germinating barley and wheat seed, as shown in Table 5.

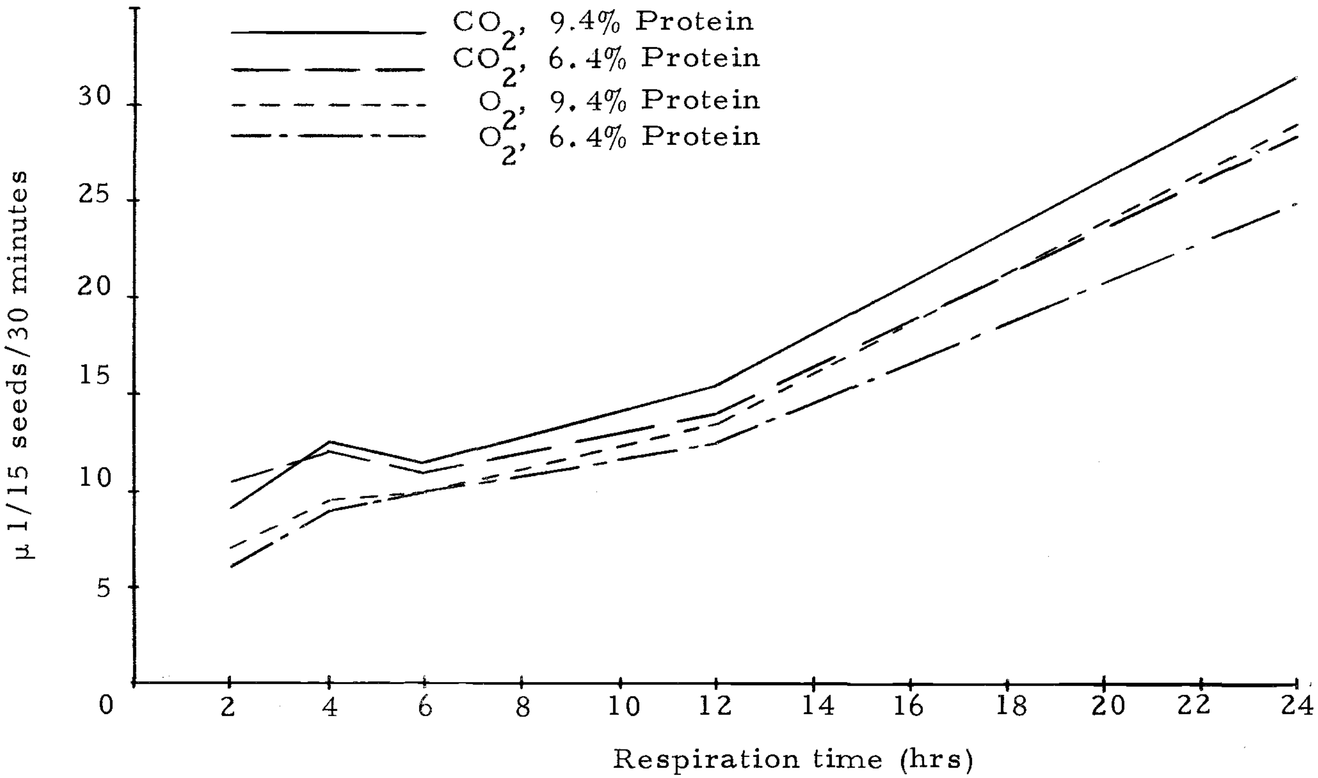


Figure 6. O_2 uptake and CO_2 evolution by low (6.4%) and high (9.4%) protein barley seed.

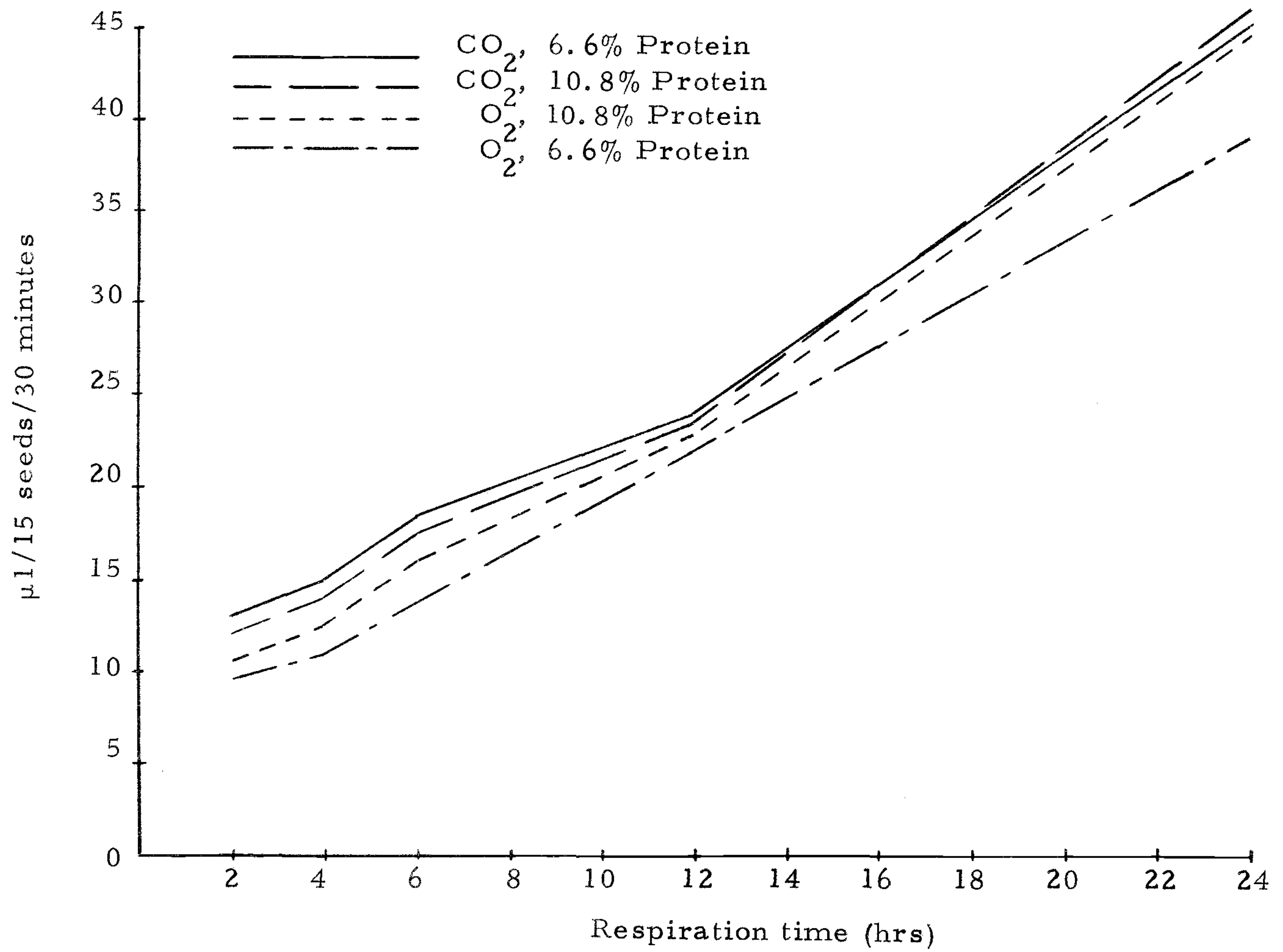


Figure 7. O_2 uptake and CO_2 output by low (6.6%) and high (10.8%) protein wheat seed.

Table 5. Respiratory quotients (RQ) of barley and wheat seed as affected by seed protein content.

Time (hrs)	Respiratory quotient (RQ)			
	Barley		Wheat	
	6.4% Protein	9.4% Protein	6.6% Protein	10.8% Protein
2	1.50	1.38	1.28	1.25
4	1.36	1.24	1.24	1.21
6	1.18	1.15	1.03	1.05
12	1.07	1.14	1.03	1.05
24	1.11	1.08	1.02	1.03

Although carbohydrates characteristically produce an RQ of 1.0 in the presence of sufficient oxygen, the respiratory quotients of both barley and wheat were much above 1.0 during the first 4 hours. This indicates that anaerobic respiration probably occurred in the early stages of imbibition. Mayer and Poljakoff-Mayber (33, p. 138) point out that in seeds with compact tissue fermentation usually occurs initially, resulting in a marked CO_2 output with only a slight O_2 uptake, producing a high RQ. The presence of air inside the seed, intercellular or intertissue spaces, could also alter the CO_2 reading by increasing the pressure in the closed system of the Gilson respirometer. At 12 and 24 hours the RQ of both wheat and barley became very close to the expected value of 1.0.

Speed of Germination

Protein content was clearly reflected in higher speed of germination of both barley and wheat (Tables 6 and 7).

Barley. At 15 and 25 C the speed of germination increased as seed protein content increased. These increases were significant

Table 6. Effect of protein level on the speed of germination indexes of barley at three germination temperatures.

Seed protein content (%)	Germination temperatures		
	15 C	20 C	25 C
6.4 (control)	30.75	36.25	39.50
7.5	37.50	39.25	49.00
8.5	42.00	40.75	52.25
9.4	41.25	41.25	60.00
LSD (.05)	6.21	5.44	5.92
LSD (.01)	8.92	7.82	8.51

Table 7. Effect of protein level on the speed of germination indexes of wheat at three germination temperatures.

Seed protein content (%)	Germination temperatures		
	15 C	20 C	25 C
6.6 control)	28.00	37.25	34.50
7.5	31.00	39.75	39.00
8.9	38.25	41.25	50.50
10.8	45.75	48.75	55.75
LSD (.05)	7.80	8.96	6.24
LSD (.01)	11.22	12.88	8.97

at the 1% level (Table 6). at 20 C, however, no significant differences were found between the germination indexes of the control and high protein seeds.

Wheat. As in barley, high protein resulted in highly significant increases in speed of germination at 15 and 25 C. At 20 C the increases were significant at 5% level (Table 7).

These data indicate that a positive relationship exists between seed protein content and germination speed of barley and wheat, but the expression of these relationships is influenced by temperature.

This temperature effect, greatest in barley, suggests that the influence of protein on germination is probably more important at stress temperatures which are higher or lower than the optimum of 20 C. The analysis of variance of the data are presented in Appendix Tables 6 to 11.

Seedling Growth Rate

Growth rate of the seedling root and shoot was also associated with seed protein content. The higher the protein content, the larger the roots and shoots. The seed protein-growth rate relationships are illustrated in Figures 8 to 13 and in Appendix Tables 12, 13, 20 and 21, and the analyses of variance of the data are presented in Appendix Tables 14 to 19 and 22 to 27.

Barley. Seedling root growth rates of barley at 15, 20 and 25 C are shown in Figures 8, 9 and 10. Each successive increase in seed protein resulted in greater root growth. The relative differences in growth rates were similar at all three temperatures.

Shoot growth rates are shown in Figures 8, 9 and 10. At 15 and 20 C, growth differences were small at first and became progressively greater as the seedlings became older. Growth differences due to protein levels were more apparent at 25 C than at the other two temperatures. In general, it appears that protein

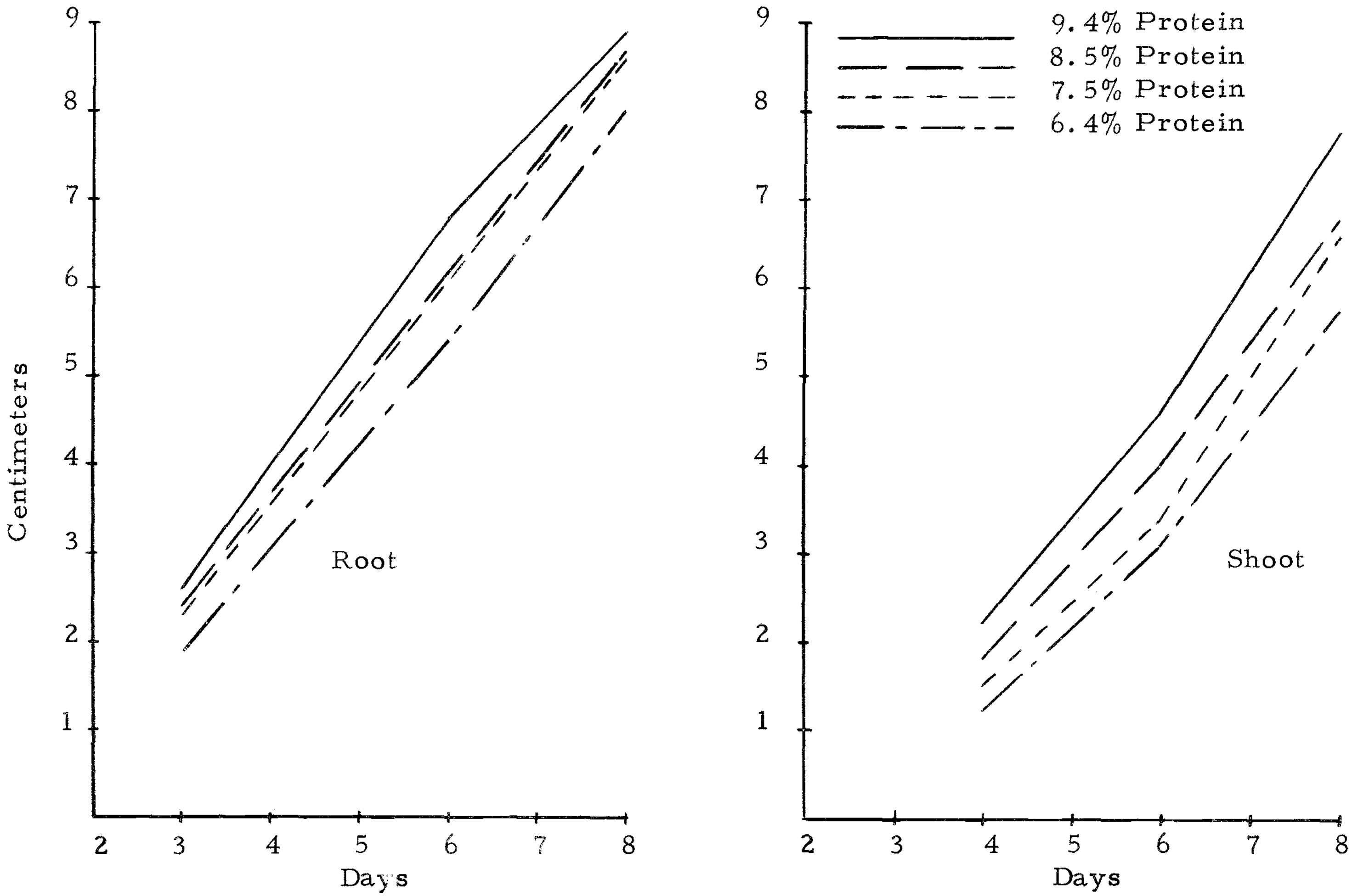


Figure 8. Influence of seed protein content of barley on root and shoot growth at 15 C.

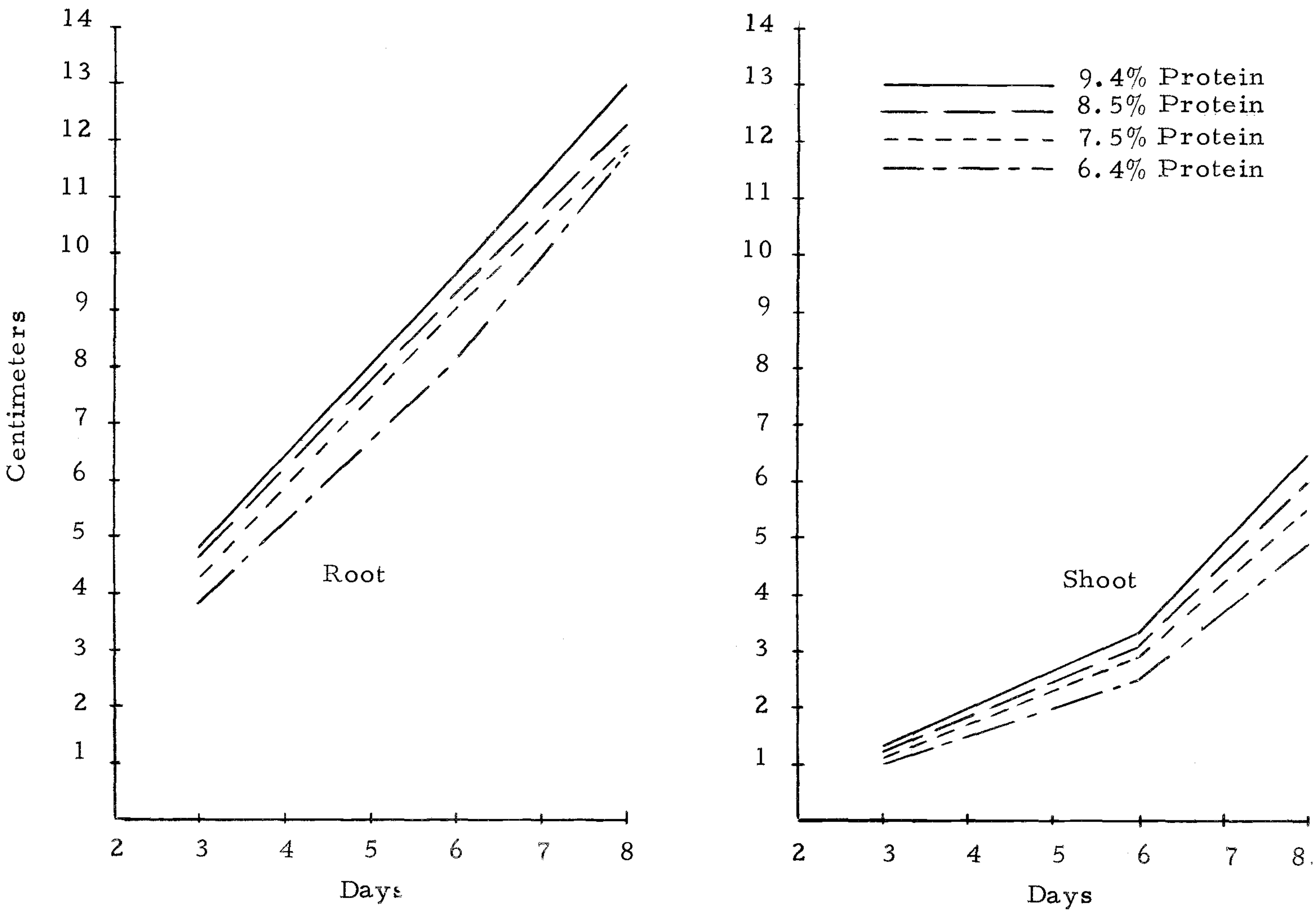


Figure 9. Influence of seed protein content of barley on root and shoot growth at 20 C.

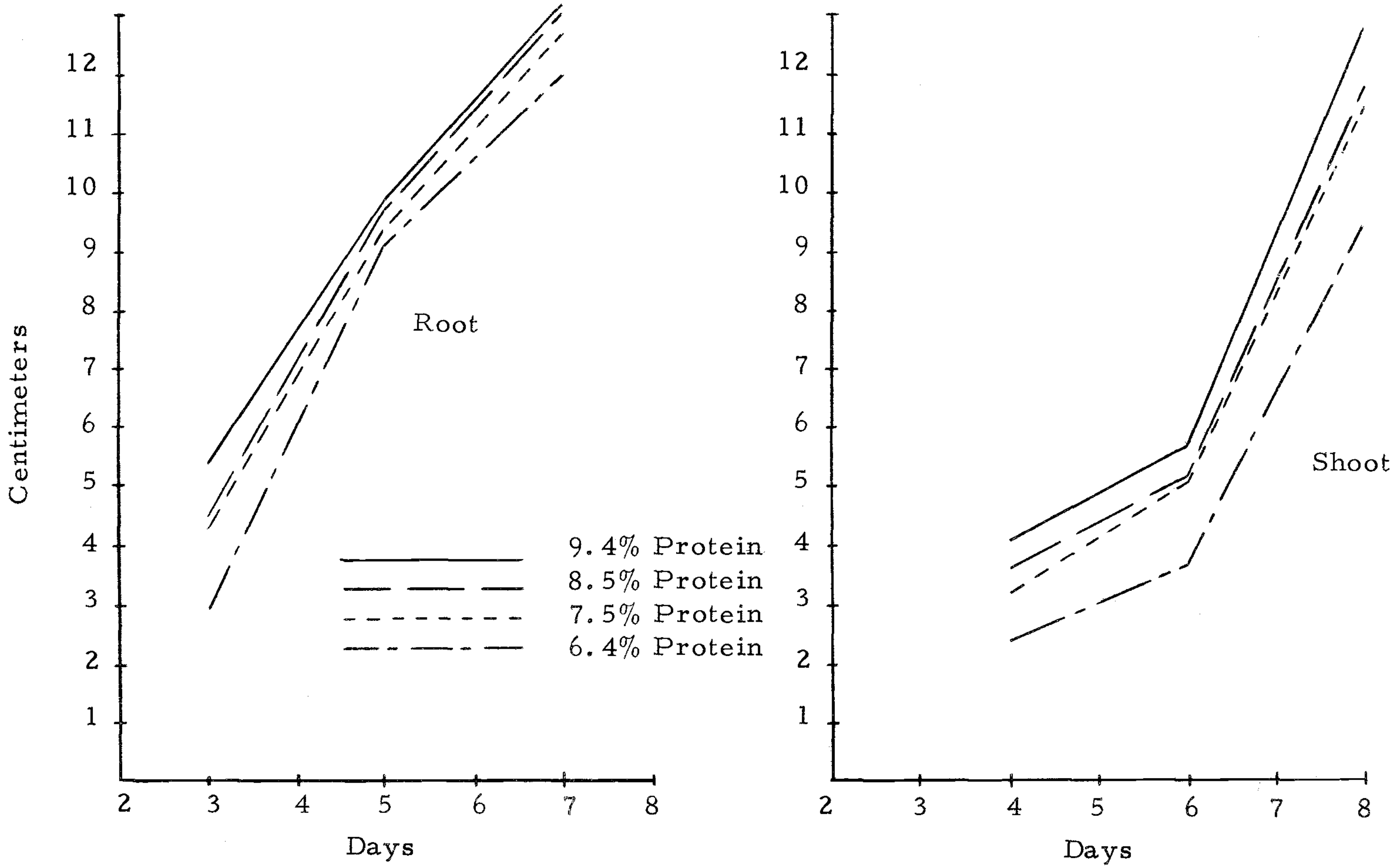


Figure 10. Influence of seed protein content of barley on root and shoot growth at 25 C.

level had a relatively greater effect on shoot growth than on root growth at this early stage of development.

Wheat. Figures 11, 12 and 13 show the root growth of wheat at 15, 20 and 25 C, respectively. As in barley, increased protein resulted in greater root growth rates at all temperatures.

Shoot growth rates are shown in Figures 11, 12 and 13. At all three temperatures, differences due to protein were small at first but became greater with time.

In general, these data indicate that, in both barley and wheat, increased seed protein levels result in larger seedlings at the 8-day stage of development. These results are consistent with the findings of Schweizer and Ries (50).

Seedling Dry Matter Production

Barley. Dry matter production by seedlings grown for six weeks in soils of different nitrogen levels in the greenhouse is shown in Table 8 and Figure 14. The analyses of variance for these data are given in Appendix Tables 28 and 29.

When grown in nitrogen-deficient soil, seedling growth and dry matter production were related to the protein level of the seeds. Thus the beneficial effect of high protein evident in 8-day old seedlings was still apparent in 6-week old plants.

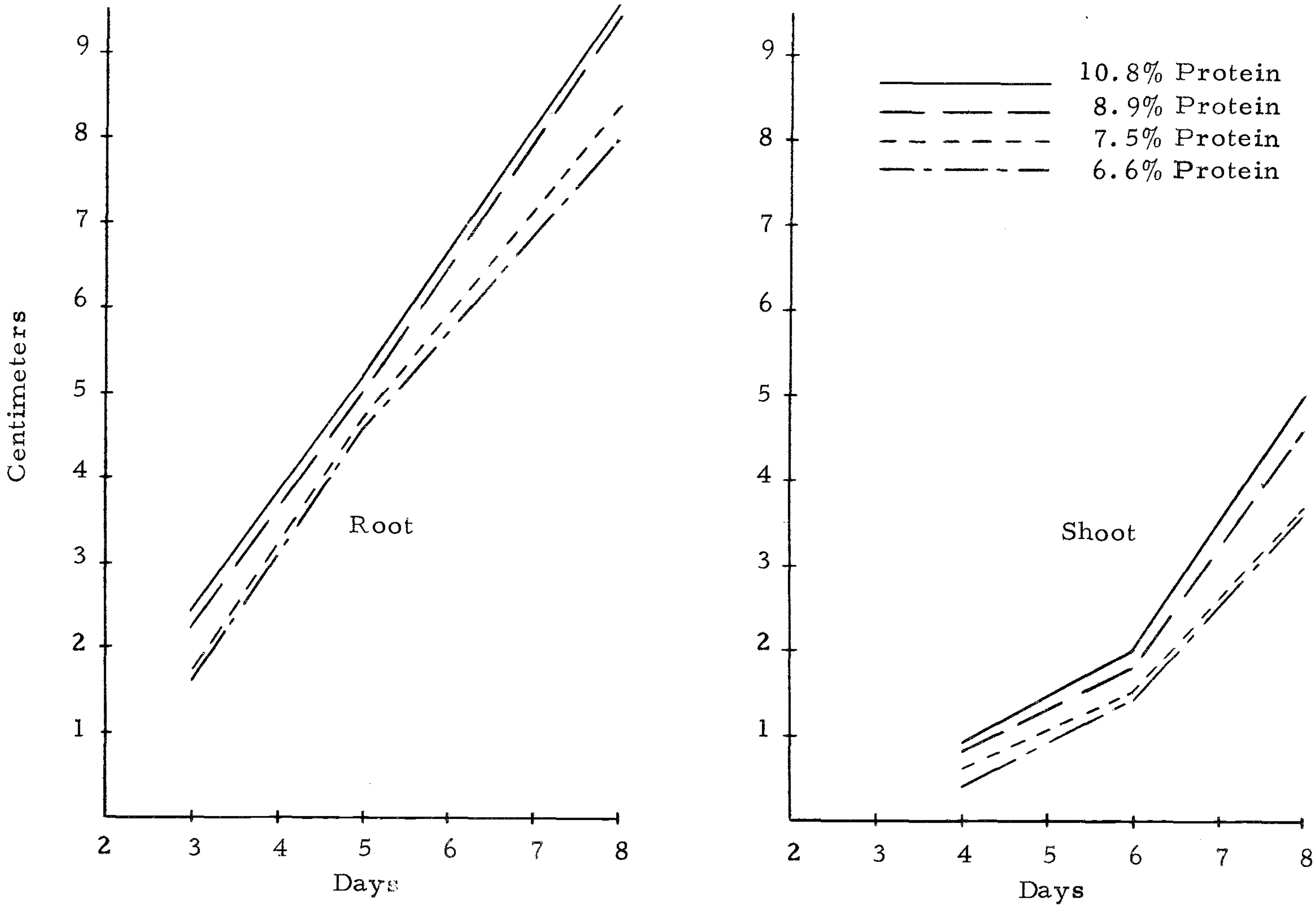


Figure 11. Influence of seed protein content of wheat on root and shoot growth at 15 C.

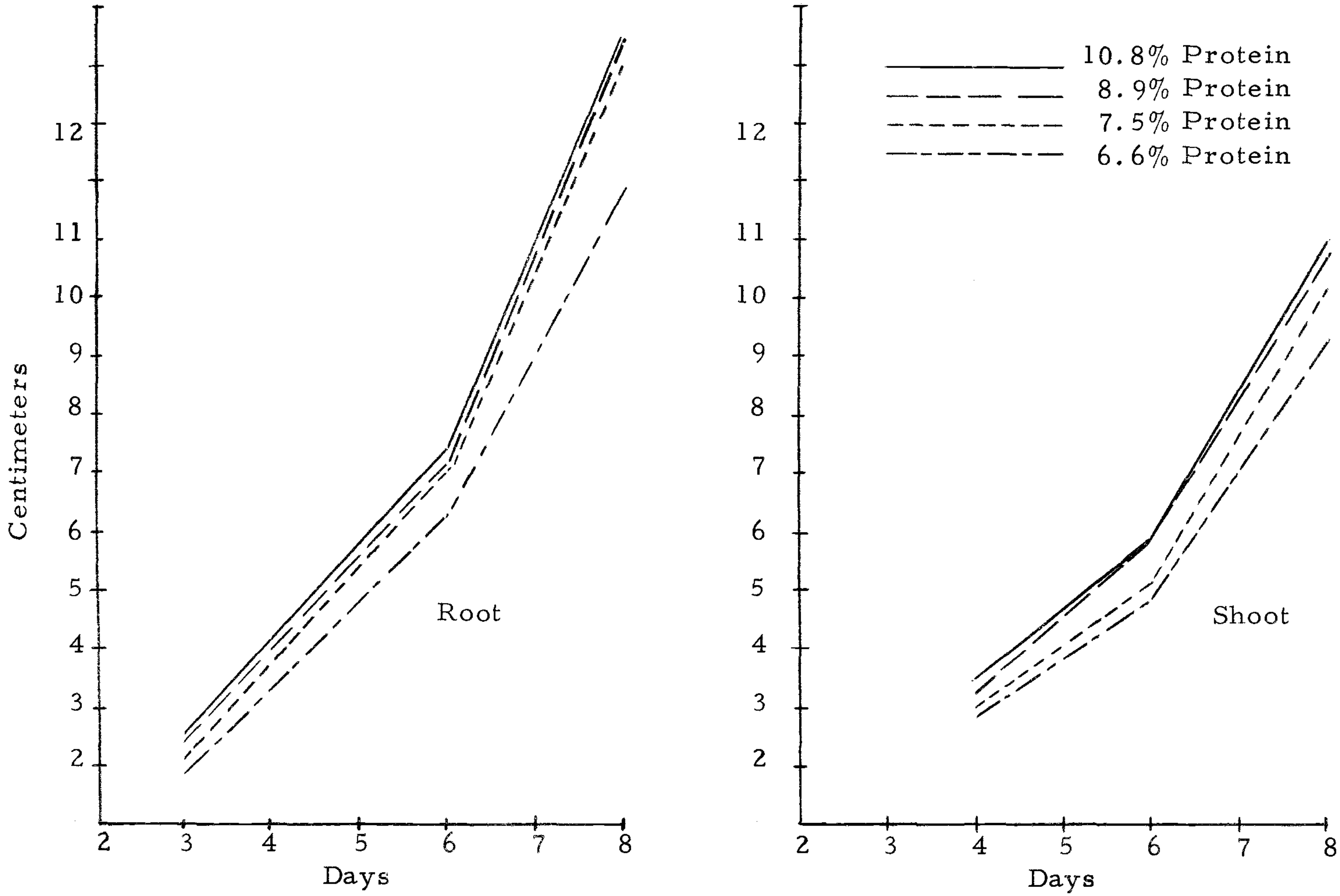


Figure 12. Influence of seed protein content of wheat on root and shoot growth at 20 C.

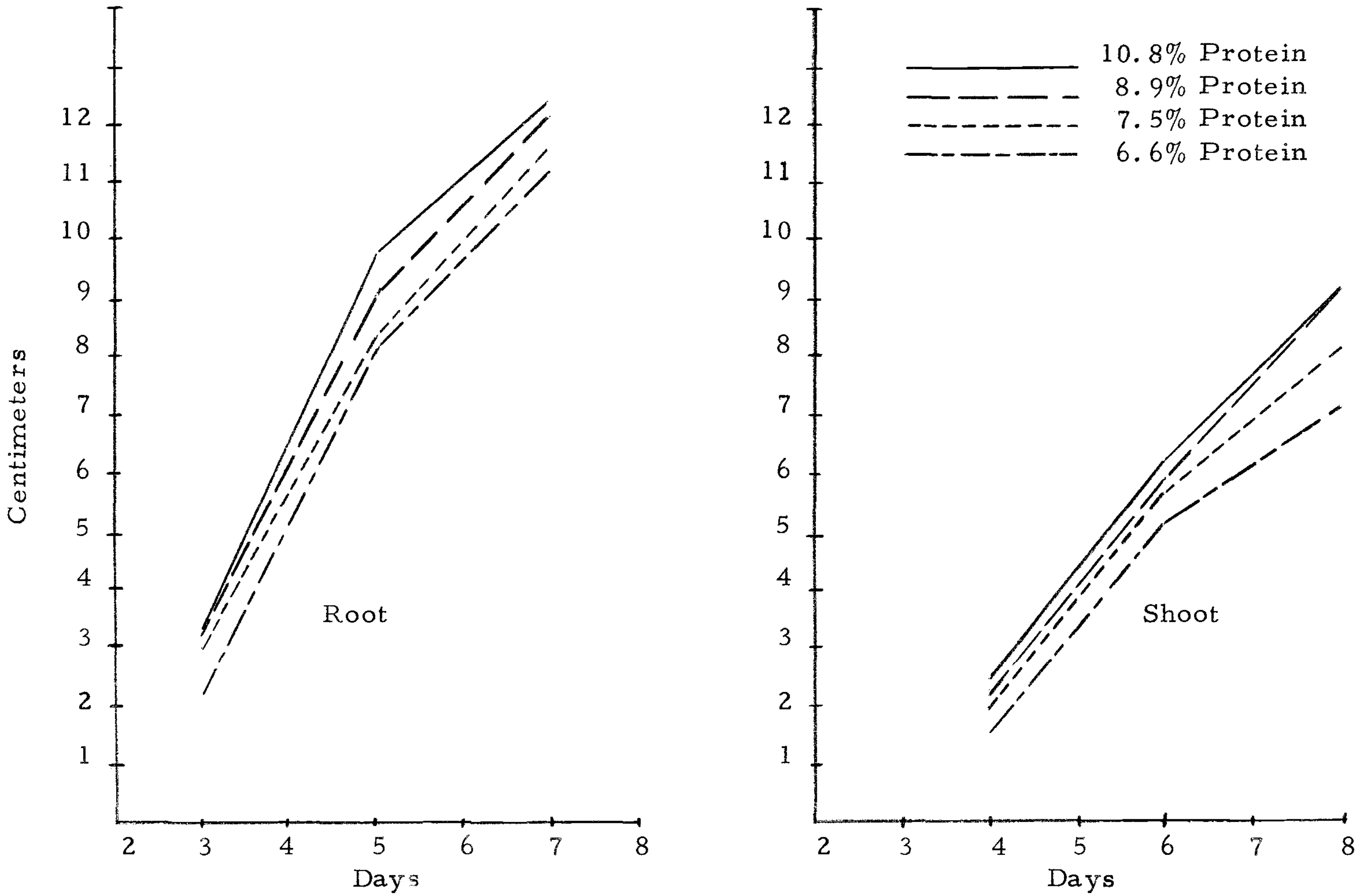


Figure 13. Influence of seed protein content of wheat on root and shoot growth at 25 C.

Table 8. Dry matter production as affected by seed protein content of barley under low and high nitrogen content soils.

Seed protein content (%)	Dry matter production (mg/plant)					
	Low nitrogen soil			High nitrogen soil		
	2 1/	4	6	2	4	6
6.4 (control)	8.75	18.00	24.25	21.50	149.75	454.00
7.5	11.25	21.75	27.25	21.25	155.50	484.75
8.5	11.75	23.25	28.00	20.75	159.00	487.00
9.4	12.25	28.25	32.00	21.50	157.25	486.25
LSD (.05)	.44	3.27	4.09			
LSD (.01)	.67	5.01	6.28			

1/ Weeks after planting.

Table 9. Dry matter production as affected by seed protein content of wheat under low and high nitrogen content soils.

Seed protein content (%)	Dry matter production (mg/plant)					
	Low nitrogen soil			High nitrogen soil		
	2 1/	4	6	2	4	6
6.6	9.75	25.00	50.00	31.00	125.75	339.00
7.5	12.25	31.50	55.00	30.00	131.50	349.25
8.9	13.50	31.00	61.50	32.00	144.25	366.75
10.8	16.75	32.50	62.25	31.75	144.50	372.50
LSD (.05)	.42	4.35	8.55			
LSD (.01)	.64	6.67	10.45			

1/ Weeks after planting.

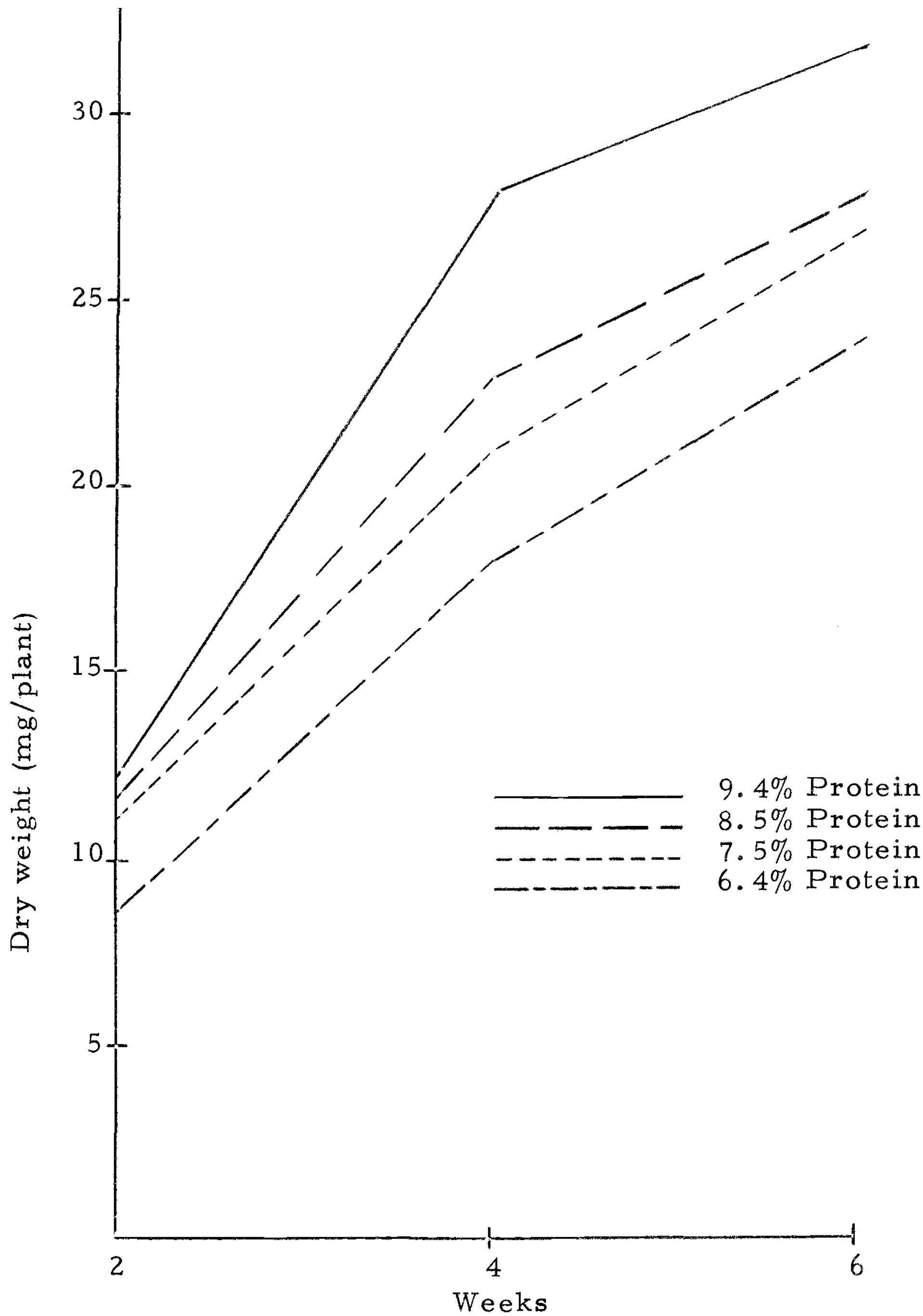


Figure 14. Relationship between seed protein content of barley and dry matter production in low nitrogen soil in the greenhouse.

When grown in nitrogen-enriched soil, however, there was no significant difference in dry matter production due to protein content of the seeds (Table 8).

Wheat. As in barley, dry matter production in nitrogen-deficient soil was positively correlated with seed protein content (Table 9 and Figure 15). In nitrogen-enriched soil, again as in barley, there was no significant difference in dry matter production due to protein content of the seeds (Table 9 and Appendix Tables 30 and 31).

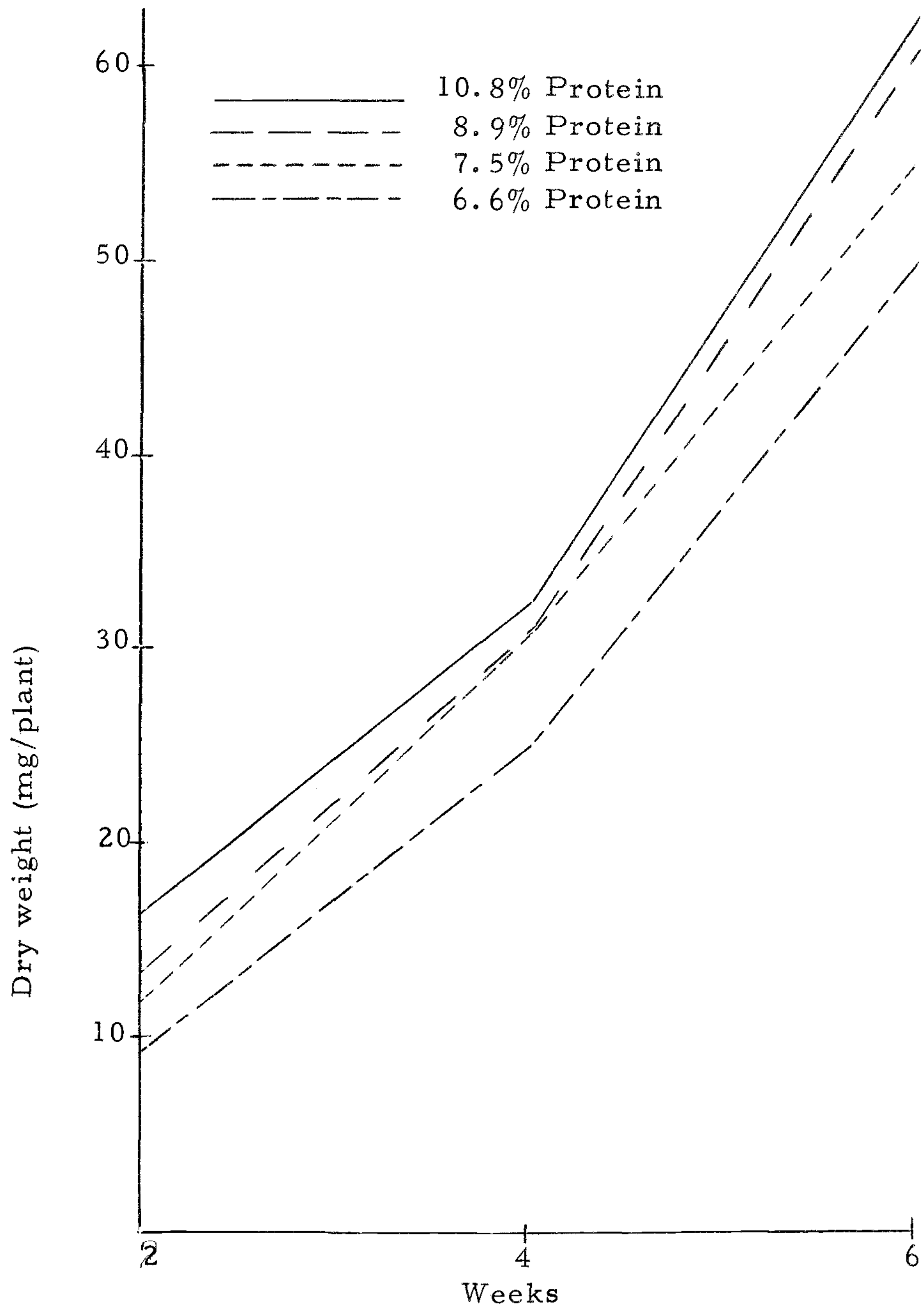


Figure 15. Relationship between seed protein content of wheat and dry matter production in low nitrogen soil in the greenhouse.

GENERAL DISCUSSION

The results of these studies suggest that application of nitrogen to winter wheat and winter barley can increase protein content of seeds, particularly the percentage of endosperm protein. The high rates of nitrogen necessary to produce high seed protein levels are not always consistent with the optimum nitrogen rates for yield. Because of the accompanying yield reductions, growers would probably be reluctant to apply such high rates of nitrogen unless some type of incentive were given them to do so. The degree of yield reduction might be modified, however, with other varieties, more balanced fertilizers, additional moisture, or other environmental conditions. For seed production, it could still be important to strive for high protein levels in spite of some loss in yield if improved performance from high protein seeds proves to be of economic importance.

This work indicates that a positive relationship exists between seed protein content and plant growth. In seed that contained high levels of protein, water imbibition and oxygen consumption were accelerated, the speed of germination was faster, and further development resulted in larger seedlings and greater dry matter production.

Imbibition of water is affected by factors such as permeability of the seed coat, availability of water and composition of the seed. Mayer and Poljakoff-Mayber (33) state that protein is the chief seed component that imbibes water. The results of this work indicate that high protein seed reached the minimum water requirements for germination three to four hours earlier than the low protein seed. This faster absorption of water by the high protein seeds may result in an acceleration of the metabolic processes of germination. These processes would include respiration, enzymatic activity and breakdown of reserve materials, and the transport of those materials to the embryo, resulting in faster cell elongation and synthesis of new material in the growing parts.

The oxygen consumption of the germinating seeds was higher in the high protein seed than in the low protein seed during the first 24 hours of imibition. Several investigators (64, 65) have demonstrated that the rate of respiration during the first 24 hours of germination is correlated with seedling growth and therefore provides a useful indication of seed vigor. In this work, however, the few μl of difference in oxygen consumption between the high and low protein seed may not be enough to establish a positive relationship between respiration rates and seedling growth.

There are hundreds of kinds of protein in seeds. Some of them are storage proteins which generally are not metabolically active themselves but provide substrates for the synthesis of metabolically functional proteins and other biomolecules. Some of them are pre-existing enzymes, structural proteins, and cofactors conserved from the maturation period, all of which are readily activated upon hydration of the seeds during germination. The third group includes proteins that influence the genetic program or developmental patterns of the seed. Histones and acidic chromosomal proteins are some examples of the third group (8). No information is avaialble as to the kind(s) of proteins that were increased by the high nitrogen applications in this study. Therefore it is difficult to explain the increases of germination speed and seedling size obtained at different temperatures. However, some speculation might be given:

Both barley and wheat seeds containing higher protein germinated faster than the seeds with lower protein at 25 and 15 C, but not at 20 C. Since 20 C is the optimum temperature for germination of cereal seeds, 25 and 15 C might be considered as stress conditions. Synergistic effects of added biochemicals often manifest themselves only under stress conditions, and this could also be the case here in germination speed. The positive relationship between high germination speed and high protein content might indicate also that it is the metabolically active proteins that are increased by the added nitrogen.

Seedlings of both crops were larger when produced by seeds with higher protein content at all three temperatures. This might indicate that at least the reserve proteins and metabolically active proteins are increased by the higher fertilizer application. The differences caused by temperature probably are related to the temperature coefficient of metabolic processes. A temperature of 25 C is generally considered optimum for plant growth, and larger seedlings were also obtained at that temperature in this experiment.

When seedlings were grown in the greenhouse, the benefit of protein content was again expressed under the stress condition of nitrogen-deficient soil. In nitrogen-enriched soil, high protein had no apparent effect on seedling growth. These results indicate that the expression of seed protein content on plant growth is evident mainly when plant growth depends on the nutrients available from the seed.

The increase in protein content of barley and wheat seed was accompanied by a reduction in seed size. It was discovered that embryo size was not affected but the size differences were in the endosperm. When germinated, however, the smaller seed (high protein) produced larger seedlings than the larger seeds (low protein). Such results do not agree with published data (5, 20, 21) showing that plants grown from large seeds are superior to those grown from small seeds in rate of seedling growth and in yield. Bremmer et al. (5) pointed out that endosperm size had a considerable effect on growth, whereas embryo size had a negligible effect. They suggest that the relationship between seed size and plant size is governed by the amount of food material present in the seed. It can be concluded from this experiment, however, that the size of the seed in itself may not be the most important attribute determining seed performance, but rather, the composition of the reserve food supplies is the governing factor.

SUMMARY AND CONCLUSIONS

The effect of seed protein content on plant growth was evaluated in Casbon winter barley and Nugaines winter wheat.

Seed of different protein content was obtained by field applications of different rates of nitrogen at seeding time (fall) and tillering time (spring). The increase in protein content from high nitrogen rates was accompanied by a decrease in yield and seed size. The protein increase occurred primarily in the endosperm. Neither protein content of the embryo nor embryo size was affected by the rate of nitrogen application.

The rates of water absorption and oxygen consumption of germinating barley and wheat seed increased as a result of the high protein content. High protein seeds had a faster speed of germination and developed into larger seedlings with a higher dry matter content when grown in nitrogen-deficient soil.

It can be concluded that protein content may have practical advantages for seeding purposes; therefore, it may be important to achieve high protein levels in the seed. However, since increasing protein by application of nitrogen may result in lower yields, it is necessary to develop ways of achieving high protein levels in conjunction with high yields. Time and conditions of nitrogen fertilization, the use of chemicals and lodging resistant varieties could all be important factors to consider.

The benefit of seed protein content is expressed mainly under stress conditions and is more evident when plant growth depends on the nutrients available from the seed. During this period, especially during initiation of germination, temperature may influence the expression of the protein effect. After the seedling has formed and is capable of photosynthesis, the nitrogen availability in the soil may influence the expression of seed protein content on plant growth.

Although the amount of seed protein may be an important factor in seedling growth and perhaps in subsequent yield of the crop, it will be necessary to determine the quality of the protein before a clear explanation of the seed protein content-plant growth relationship can be made.

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APPENDIX

Appendix Table 1. Analysis of variance for seed yield of barley as affected by nitrogen fertilization.

Source of variation	Sums of squares	Degrees of freedom	Mean squares	F
Treatment	3017438.188	3	1005812.729	4.79*
Replication	608111.188	3	202703.729	.97
Error	1889716.563	9	209968.507	
Total	5515264.938	15		

C. V. = 14.98%.
* Significant difference at 5% level.

Appendix Table 2. Analysis of variance of protein content of barley as affected by nitrogen fertilization.

Source of variation	Sums of squares	Degrees of freedom	Mean squares	F
Treatments	16.841	3	5.614	29.55**
Replications	.309	3	.103	94
Error	1.707	9	.190	
Total	18.858	15		

C. V. = 5.82%.
** Significant difference at 1% level.

Appendix Table 3. Analysis of variance of 1000 kernels weight of barley as affected by nitrogen fertilization.

Source of variation	Sums of squares	Degrees of freedom	Mean squares	F
Treatments	114.188	3	38.063	18.52**
Replications	5.188	3	1.729	
Error	17.563	9	1.951	.89
Total	136.938	15		

C. V. = 3.44%
** Significant difference at 1% level.

Appendix Table 4. Oxygen (O_2)uptake, carbon dioxide (CO_2) evolution and respiration quotient (RQ) of barley seed as affected by seed protein content. 1/

Time (hrs)	O_2 uptake 2/		CO_2 evolution 2/		RQ	
	6.4% Protein	9.8% Protein	6.4% Protein	9.8% Protein	6.4% Protein	9.8% Protein
2	6.6	7.3	9.7	10.1	1.50	1.38
4	9.4	9.7	12.8	12.0	1.36	1.24
6	9.8	9.9	11.6	11.4	1.18	1.15
12	12.4	13.6	13.3	15.6	1.07	1.14
24	25.7	29.2	28.6	31.5	1.11	1.08

1/ Temperature = 25C.
2/ µl/15 seeds/30 min.

Appendix Table 5. Oxygen (O_2) uptake, carbon dioxide (CO_2) evolution and respiration quotient (RQ) of wheat seed affected by protein content. 1/

Time (hrs)	O_2 uptake 2/		CO_2 evolution 2/		RQ	
	6.4% Protein	9.8% Protein	6.4% Protein	9.8% Protein	6.4% Protein	9.8% Protein
2	9.8	10.2	13.1	12.3	1.28	1.25
4	11.4	12.2	15.2	13.8	1.24	1.21
6	16.8	17.6	18.2	17.8	1.03	1.05
12	21.7	22.9	23.7	23.0	1.03	1.05
24	38.5	44.2	39.4	45.7	1.02	1.03

1/ Temperature = 25C.
2/ µl/15 seeds/30 min.

Appendix Table 6. Analysis of variance for speed of germination index of barley at 15C.

Source of variation	Sums of squares	Degrees of freedom	Mean squares	F
Protein	317.250	3	105.750	7.01**
Replication	18.750	3	6.250	
Error	135.750	9	15.083	
Total	471.750	15		

C. V. = 10.24%

** Significant difference at 1% level.

Appendix Table 7. Analysis of variance for speed of germination index of barley at 20C.

Source of variation	Sums of squares	Degrees of freedom	Mean squares	F
Protein	69.000	3	23.000	2.00 NS
Replication	13.500	3	4.500	
Error	103.500	9	11.500	
Total	186.000	15		

C. V. = 8.61%.

NS Nonsignificant.

Appendix Table 8. Analysis of variance for speed of germination index of barley at 25C.

Source of variation	Sums of squares	Degrees of freedom	Mean squares	F
Protein	864.688	3	288.229	20.99**
Replication	72.188	3	24.063	
Error	123.563	9	13.729	
Total	1060.438	15		

C. V. = 7.40%.

** Significant difference at 1% level.

Appendix Table 9. Analysis of variance for speed of germination index of wheat at 15C.

Source of variation	Sums of squares	Degrees freedom	Mean squares	F
Protein	755.500	3	251.833	19.57**
Replication	81.000	3	27.000	
Error	214.500	9	23.833	
Total	1051.000	15		

C.V. = 13.61%.
** Significant difference at 1% level.

Appendix Table 10. Analysis of variance for speed of germination index of wheat at 20C.

Source of variation	Sums of squares	Degrees freedom	Mean squares	F
Protein	468.688	3	156.229	4.93*
Replication	26.188	3	8.729	
Error	285.063	9	31.674	
Total	779.938	15		

C.V. = 12.89%.
* Significant difference at 5% level.

Appendix Table 11. Analysis of variance for speed of germination index of wheat at 25C.

Source of variation	Sums of squares	Degrees freedom	Mean squares	F
Protein	1168.188	3	389.396	25.20**
Replication	15.688	3	5.229	
Error	139.063	9	15.451	
Total	1322.938	15		

C.V. = 8.68%.
** Significant difference at 1% level.

Appendix Table 12. Influence of seed protein content of barley on root growth at three different temperatures.

Seed protein content (%)	Root length (cm)								
	15C			20C			25C		
	4[1/]	5	8	3	6	8	3	5	7
6.4	1.92	5.42	8.04	3.95	8.28	11.82	2.87	9.25	12.02
7.5	2.33	6.15	8.15	4.42	9.30	11.94	4.44	9.88	12.76
8.5	2.33	6.19	8.62	4.46	9.49	12.34	4.27	9.52	12.88
9.4	2.53	6.76	8.86	4.63	9.56	12.76	5.36	9.97	12.98
LSD (.05)		.32			.27			.55	
LSD (.01)		.43			.37			.74	

1/ Days after planting.

Appendix Table 13. Influence of seed protein content of barley on shoot growth at three different temperatures.

Seed protein content (%)	Shoot length (cm)								
	15C			20C			25C		
	4[1/]	6	8	3	6	8	4	5	7
6.4	1.07	2.52	4.80	1.25	3.31	5.85	2.41	3.79	9.52
7.5	1.12	2.99	5.40	1.52	3.45	6.71	3.64	5.19	11.51
8.5	1.20	3.10	5.83	1.87	4.00	6.89	3.25	5.03	11.62
9.4	1.18	3.31	6.32	2.16	4.62	7.68	4.17	5.57	12.86
LSD (.05)		.24			.42			.32	
LSD (.01)		.32			.56			.43	

1/ Days after planting.

Appendix Table 14. Analysis of variance for root growth of barley at 15C.

Source of variation	Sums of squares	Degrees of freedom	Mean squares
Protein	5.189	3	1.730**
Days	308.577	2	154.288
P. D.	.850	6	.142
Replication	.407	3	.136
P. R.	.633	9	.070
D. R.	.466	6	.078
P. D. R.	1.439	18	.080
Total	317.562	47	.080

C. V. = 4.9%
** Significant difference at 1% level.

Appendix Table 15. Analysis of variance for root growth of barley at 20C.

Source of variation	Sums of squares	Degrees of freedom	Mean squares
Protein	6.236	3	2.079**
Days	500.119	2	250.059
P. D.	1.287	6	.215
Replication	.595	3	.198
P. R.	.662	9	.074
D. R.	1.321	6	.220
P. D. R.	1.616	18	.090
Total	511.837	47	

C. V. = 3.85%
** Significant difference at 1% level.

Appendix Table 16. Analysis of variance for root growth of barley at 25C.

Source of variation	Sums of squares	Degrees of freedom	Mean squares
Protein	12.210	3	4.070**
Days	583.257	2	291.629
P. D.	4.013	6	.669
Replication	.248	3	.083
P. R.	1.410	9	.157
D. R.	1.176	6	.196
P. D. R.	.941	18	.052
Total	603.255	47	.052

C. V. = 3.61%.
** Significant difference at 1% level.

Appendix Table 17. Analysis of variance for shoot growth of barley at 15C.

Source of variation	Sums of squares	Degrees of freedom	Mean squares
Protein	11.742	3	3.914**
Days	208.619	2	104.309
P. D.	1.169	6	.195
Replication	.327	3	.109
P. R.	.644	9	.072
D. R.	.571	6	.095
P. D. R.	1.450	18	.095
Total	224.522	47	

C. V. = 7.05%.
** Significant differences at 1% level.

Appendix Table 18. Analysis of variance for shoot growth of barley at 20C.

Source of variation	Sums of squares	Degrees of freedom	Mean squares
Protein	4.226	3	1.409**
Days	159.573	2	79.786
P. D.	2.197	6	.366
Replication	.674	3	.366
P. R.	.449	9	.050
D. R.	.284	6	.047
P. D. R.	.214	18	.012
Total	167.618	47	.012

C. V. + 5.25%.
** Significant difference at 1% level.

Appendix Table 19. Analysis of variance for shoot growth of barley at 25C.

Source of variation	Sums of squares	Degrees of freedom	Mean squares
Protein	44.432	3	14.811**
Days	592.618	2	296.309
P. D.	4.104	6	.684
Replication	4.746	3	1.582
P. R.	5.017	9	.557
D. R.	4.284	6	.714
P. D. R.	5.648	18	.314
Total	660.850	47	

C. V. = 3.41%.
** Significant difference at 1% level.

Appendix Table 20. Influence of seed protein content of wheat on root growth at three different temperatures.

Seed protein content (%)	Root growth (cm)								
	15C			20C			25C		
	4 1/	5	8	3	6	8	3	5	8
6.6	1.79	4.64	8.20	1.08	5.39	10.87	2.24	8.20	11.13
7.5	1.65	4.70	8.43	1.30	6.06	13.03	2.97	8.42	11.69
8.9	2.26	5.10	9.35	1.30	6.18	13.48	3.13	9.07	12.63
10.8	2.40	5.21	9.56	1.33	6.45	13.39	3.24	9.75	12.89
LSD (.05)		.32			.27			.55	
LSD (.01)		.43			.37			.74	

1/ Days after planting.

Appendix Table 21. Influence of seed protein content of wheat on shoot growth at three different temperatures.

Seed protein content (%)	Shoot length (cm)								
	15C			20C			25C		
	4 1/	6	8	4	6	8	4	6	8
6.6	.45	1.40	3.60	1.95	3.93	8.34	1.54	5.21	7.33
7.5	.63	1.54	3.79	2.03	4.14	9.27	1.91	5.82	8.23
8.9	.81	1.84	4.71	2.18	4.98	9.71	2.03	5.94	9.13
10.8	.91	1.91	5.06	2.31	4.97	10.04	2.35	6.23	9.17
LSD (.05)		.19			.82			.42	
LSD (.01)		.25			1.11			.56	

1/ Days after planting.

Appendix Table 22. Analysis of variance for root growth of wheat at 15C.

Source of variation	Sums of squares	Degrees of freedom	Mean squares
Protein	6.779	3	2.260**
Days	379.544	2	189.772
P. D.	1.118	6	.186
Replication	.098	3	.033
P. R.	.356	9	.040
D. R.	.538	6	.090
P. D. R.	.697	18	.039
Total	389.129	47	

C. V. = 4.17%.
** Significant difference at 1% level.

Appendix Table 23. Analysis of variance for root growth of wheat at 20C.

Source of variation	Sums of squares	Degrees of freedom	Mean squares
Protein	12.624	3	4.208**
Days	1056.304	2	528.152
P. D.	8.029	6	1.338
Replication	.195	3	.065
P. R.	.542	9	.060
D. R.	.157	6	.026
P. D. R.	.450	18	.025
Total	1078.301	47	.025

C. V. = 2.86%.
** Significant difference at 1% level.

Appendix Table 24. Analysis of variance for root growth of wheat at 25C.

Source of variation	Sums of squares	Degrees of freedom	Mean squares
Protein	14.493	3	4.831**
Days	696.340	2	348.170
P. D.	1.866	6	.311
Replication	.398	3	.133
P. R.	1.921	9	.213
D. R.	.463	6	.077
P. D. R.	2.233	18	.124
Total	717.716	47	

C. V. = 4.78%.

** Significant difference at 1% level.

Appendix Table 25. Analysis of variance for shoot growth of wheat at 15C.

Source of variation	Sums of squares	Degrees of freedom	Mean squares
Protein	5.238	3	1.746**
Days	110.478	2	
P. D.	1.953	6	.326
Replication	.011	3	.004
P. R.	.164	9	.018
D. R.	.164	6	.027
P. D. R.	.187	18	.010
Total	110.195	47	.010

C. V. = 5.85%.

** Significant difference at 1% level.

Appendix Table 26. Analysis of variance for shoot growth of wheat at 20C.

Source of variation	Sums of squares	Degrees of freedom	Mean squares
Protein	13.562	3	4.521**
Days	439.824	2	219.912
P. D.	4.405	6	.734
Replication	1.494	3	.498
P. R.	2.839	9	.315
D. R.	2.010	6	.335
P. D. R.	6.059	18	.337
Total	470.193	47	

C. V. = 10.84%.
** Significant difference at 1% level.

Appendix Table 27. Analysis of variance for shoot growth of wheat at 25C.

Source of variation	Sums of squares	Degrees of freedom	Mean squares
Protein	10.338	3	3.446**
Days	342.523	2	171.262
P. D.	2.333	6	.389
Replication	1.193	3	.398
P. R.	2.554	9	.284
D. R.	.366	6	.061
P. D. R.	1.909	18	.106
Total	361.216	47	.106

C. V. = 7.04%.
** Significant difference at 1% level.

Appendix Table 28. Analysis of variance for dry matter production of barley. Soil low in nitrogen.

Source of variation	Sums of squares	Degrees of freedom	Mean squares
Protein	313.229	3	104.410**
Weeks	2399.625	2	1199.813
P. W.	54.208	6	9.035
Replication	38.896	3	12.965
P. R.	47.021	9	5.225
W. R.	219.542	6	36.590
P. W. R.	71.292	18	3.961
Total	3143.813	47	

C. V. = 15.56%

** Significant difference at 1% level.

Appendix Table 29. Analysis of variance for dry matter production of barley. Nitrogen added to soil.

Source of variation	Sums of quares squares	Degrees of freedom	Mean squares
Protein	1553.083	3	517.694 NS
Weeks	1763717.167	2	881858.583
P. W.	1724.167	6	287.361
Replication	1690.417	3	563.472
P. R.	8277.083	9	919.676
W. R.	5722.333	6	953.722
P. W. R.	10615.667	18	589.759
Total	1793299.915	47	

C. V. - 14.86%

NS Nonsignificant.

Appendix Table 30. Analysis of variance for dry matter production of wheat. Soil low in nitrogen.

Source of variation	Sums of squares	Degrees of freedom	Mean squares
Protein	536.167	3	178.722**
Weeks	15856.292	2	7928.146
P. W.	106.208	6	17.701
Replication	103.000	3	34.333
P. R.	100.500	9	11.167
W. R.	169.875	6	28.313
P. W. R.	267.625	18	14.868
Total	17139.667	47	

C. V. = 14.86%.
** Significant difference at 1% level.

Appendix Table 31. Analysis of variance for dry matter production of wheat. Nitrogen added to the soil.

Source of variation	Sums of squares	Degrees of freedom	Mean squares
Protein	2594.563	3	864.854 NS
Weeks	883883.792	2	441941.896
P. W.	1350.875	6	225.146
Replication	18564.729	3	6188.243
P. R.	3075.354	9	341.706
W. R.	13319.708	6	2219.951
P. W. R.	6604.958	18	2219.951
Total	929393.979	47	

C. V. = 12.32%.
NS Nonsignificant.