SEED PROTEIN CONTENT AND AMINO ACID INFILTRATION OF GRAIN : RELATION TO AND EFFECT ON SUBSEQUENT SEEDLING GROWTH AND YIELD

Thesis for the Degree of Ph. D.

MICHIGAN STATE UNIVERSITY
CONRAD JOSEPH SCHWEIZER

1970

This is to certify that the

thesis entitled

SEED PROTEIN CONTENT AND AMINO ACID
INFILTRATION OF GRAIN: RELATION TO
AND EFFECT ON SUBSEQUENT SEEDLING
GROWTH AND YIELD

presented by

CONRAD JOSEPH SCHWEIZER

has been accepted towards fulfillment
of the requirements for

Ph.D. degree in Horticulture

Stanley K. Ries
Major professor

Date March 10, 1970

O-169

ABSTRACT

SEED PROTEIN CONTENT AND AMINO ACID INFILTRATION OF GRAIN: RELATION TO AND EFFECT ON SUBSEQUENT SEEDLING GROWTH AND YIELD

By

Conrad Joseph Schweizer

High protein seed of oats and several wheat varieties produced significantly larger seedlings and higher yields than low protein seed of the same genotype. There was also a positive correlation between protein content and yield which existed independent of the environmental conditions used to increase the protein content of the parent seed. Weight, per cent moisture and nitrate content of the seed were not correlated with growth or yield in the following generation.

In several experiments, wheat seed vacuum infiltrated for 3 hours in a 1.0 M arginine-HCl solution produced larger seedlings. In a field experiment, plants from oat seed infiltrated with arginine or a mixture of 11 amino acids yielded more grain than controls in 1 of 2 field tests. Etiolated wheat seedlings, from seed infiltrated with arginine, contained higher levels of total amino acids than untreated controls when harvested 6, 9 and 10 days after planting. Aspartate, glutamate and their amides were higher in seedlings developing from infiltrated seed

than in controls, as expected, since they serve as central intermediates in nitrogen metabolism and transport in the germinating seed.

The enhancement of seedling vigor and yield derived from high protein seed may be important in future crop production. The practicality of infiltrating amino acids into seeds to affect subsequent crop production is difficult to evaluate. More importantly, these studies suggest that a genetic alteration in the amino acid composition of storage proteins in seed may be reflected in enhanced seedling growth and yield.

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By

Conrad Joseph Schweizer

A THESIS

Submitted to
Michigan State University
in partial fulfillment of the requirements
for the degree of

DOCTOR OF PHOLOSOPHY

Department of Horticulture

1970

ACKNOWLEDGMENTS

Recognition is extended to my mentor, Dr. S. K. Ries, and to Drs. D. R. Dilley, P. Filner, R. C. Herner, S. Honma, C. J. Pollard, and A. R. Putnam for their efforts relating to this thesis.

I thank Dr. R. J. Laird of the International Maize and Wheat Improvement Center in Mexico and Dr. J. A. Tweedy of Southern Illinois University for supplying the wheat seed used in several experiments.

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INTRODUCTION

A shift in the emphasis of agricultural technology from solely increasing crop yields to enriching the nutritive value of crops, as well as production, is noteworthy throughout the world. Genetic manipulation to improve the protein content and amino acid balance of agronomic crops has gained impetus since 1964 with the discovery of opaque-2 mutant corn, which is high in lysine. Food products are "tailored" with essential amino acids derived from cottonseeds, soybeans, fish protein concentrates and microbial proteins in an effort to amend the caloric intake of some 1.8 billion people. These efforts are directed towards high biological value crops for human and animal consumption (3,7,37,45,54,62).

It is perhaps equally important to consider the effect of alterations in seed composition on subsequent seedling growth and yield. Kidd and West (27, p. 249) in 1919 had a forethought:

> The critical question is therefore- Can we propound a law to the effect that increased vigour of seedling development due to environmental conditions as distinct from hereditary causes, is correlated with increased vigour of growth throughout the life of the plant and with increased yield independently of the subsequent environmental conditions?

They recognized that a relationship existed between internal seed characteristics produced by parental environmental conditions and subsequent seedling vigor. Thus, they alluded to the relationship of seed protein content with subsequent seedling growth and yield. This relationship and the effect and utilization of amino acids infiltrated into seeds on subsequent seedling growth is the nature of the embodied thesis.

LITERATURE REVIEW

Amino Acid Metabolism During Germination and Early Seedling Development

The idea that seed protein could serve as a nitrogen source upon germination was first advanced by Hartig (9) in 1858. It was established that these reserve materials were mobilized upon demand as simpler substances; the first, which was to be investigated, he termed "Gleis." Schulze (49) in the late 19th century found that this substance coincided with an amino acid purified in 1806 by Vauquelin and Robiquet from asparagus juice, called asparagine. He concluded that upon germination protein and carbohydrate degradation occurred yielding peptones, amino acids and possibly ammonia which condensed with organic acids to yield asparagine and in some species glutamine. These are mobilized and accumulate in the growing regions where they are regenerated into new proteins in the presence of soluble carbohydrates. Observing that the nascent proteins had a different composition than those being degraded and that arginine accumulated in *Pinus thunbergii* seedlings, but was scant in various lupine species, he concluded that interconversion of amino acids occurred. Asparagine and glutamine acted as intermediate carriers for nitrogen which existed in the seed largely in protein.

Sure and Tottingham (58) concluded from studies with etiolated pea seedlings that in the shoot region rapid carbohydrate metabolism took place during the early stages of growth. The total nitrogen of the cotyledons decreased with a concomitant increase in ammonia followed by elevated levels of α-amino acids and amides in the shoot region.

Experimenters in the early 1940's quickly re-established that nitrogen in storage protein accumulated as asparagine and glutamine in the growing regions of young seedlings. Glutamic and aspartic acids occupied central positions in protein catabolism and regeneration. The α-keto acids involved in their transamination were supplied by carbohydrate degradation (34,61). The dependence of nitrogen mobilization from the endosperm upon embryo respiration was predicted by Albaum in 1943 (1,2).

Subsequent investigations focused upon the utilization of particular amino acids by excised plant parts. Oat embryos grew as well on casein hydrolyzates or on complete amino acid mixtures as on inorganic nitrogen forms (20). In deletion experiments it was found that DL-valine alone suppressed embryo growth, while in combination with any other amino acid it was stimulatory. L-arginine significantly enhanced root and shoot growth and increased the dry weight of the seedlings, particularly in combination with L-glutamate, L-lysine, DL-valine or glycine. However, in no case did they increase shoot growth when 2mM $NaNO_3$ was added to the basal medium. Without exogenous nitrogen, germinating grain seeds showed marked increases in aspartate, alanine, glycine, lysine, and arginine (15). Concurrently, decreases were noted in glutamine, asparagine, and strikingly in proline. Folkes and Yemm (15) believed that the amino acids liberated in the endosperm were first utilized in embryo proteins, and those in excess went to other amino acids, chlorophylls, and nucleic acid bases.

Roots and sprouts of watermelon seedlings were vacuum infiltrated with labelled ornithine, arginine and urea (25). The majority of ornithine went to amino acids metabolically related, while the label in arginine was found in urea, ornithine, citrulline, lysine, histidine, proline and glutamic acid. Urea was metabolized to CO_2 and NH_4 which were then fixed. Apparently, the imidazole nitrogen of histidine, the guanidino group of arginine, the ε-amino group of lysine, and the indolyl nitrogen of tryptophan may be utilized in the synthesis of other amino acids. Meiss (36) and others (15,31,57) also observed that maximum protein hydrolysis occurred 24-48 hours after water imbibition, and that mobilization of nitrogen from the endosperm was complete after 8-10 days.

Wilson _et al_. (65) in 1954 found that barley seedlings contained transaminases transferring amino groups from each of 17 different amino acids to α-ketoglutarate. Free ammonia was not an intermediate, but existed in pyridoxamine which transferred nitrogen to α-ketoglutarate in the presence of pyridoxal-5-phosphate.

The main storage proteins in _Vicia_ _faba_ L. are legumin and vicillin, rich in basic and dicarboxylic amino acids (30). During germination there is an extensive breakdown of reserve proteins with elevations in all free amino acids except arginine (6). The label in arginine-^{14}C vacuum infiltrated into 10 day-old cotyledons, was found in proline and argininosuccinic acid after 5 minutes; in glutamic acid after 30 minutes; in ornithine, citrulline and asparatic acid after 3 hours; and in 11 other amino acids after 24 hours. Arginase and urease were found in roots, shoots, and cotyledons of _V_. _faba_ (23). Cotyledons of other plant species including beans and peas were found to contain labelled ornithine, proline, urea and CO_2 1.5 hours after infiltration with arginine-^{14}C.

Pea seeds imbibing micromolar solutions of L-citrulline-carbamyl-^{14}C and DL-arginine-5-^{14}C were readily able to convert citrulline to arginine via the enzyme argininosuccinate synthetase (50). Arginine was not extensively metabolized, but incorporated as such or indirectly into proteins. Shargool and Cossins (50) concluded that arginine may serve as a storage form of nitrogen when the external nitrogen supply is high, and may be synthesized and utilized during germination when the external nitrogen supply is limiting.

The nitrogen from L-arginine-U-^{14}C injected into cotyledons of pumpkin seedlings was transported to and utilized by the growing axis (52,53). However, over 80 per cent of the carbon was metabolized to $^{14}CO_2$. This is contrary to evidence presented by Shargool and Cossins who found only 3 per cent $^{14}CO_2$ metabolized from DL-arginine-carbamyl-^{14}C. Arginine enhanced the growth rate of pea seedlings and bud formation in tobacco stems (35). In addition, it increased the growth response of oat coleoptiles to indoleacetic acid. Sugarcane cells rapidly utilized arginine as an organic nitrogen source (35).

Although the metabolism of arginine varies among species, and indeed within species, it is evident that urea cycle intermediates exist in plants and are linked to other metabolic pathways. Thus, arginine either directly or by transmination and oxidative deamination, may give rise to key amino acids localized at the site of protein regeneration. It is possible, and in some species evident (sugarcane cells and maize roots), that arginine and other amino acids (asparagine) may serve a regulatory function in the synthesis of limiting amino acids rather than be metabolized (35,40).

Asparagine, the major amide in "Genesee" wheat seed, decreased sharply upon germination with a corresponding rise in glutamine (63). As the seedling developed, asparagine again began to predominate. Immunochemical studies by Daussant et al. (12) revealed electrophoretic shifts in α-arachin (storage protein of Arachis hypogaea L.) which may have been caused by progressive deamidation of glutamine and asparagine. Thus, the reserve proteins not only provide amino acids but may be the first source of ammonia for the growing seedling. This study also showed that new proteins are synthesized in the cotyledons and roots of germinating seed, not identical to those of the mature seed. Those which are identical indicate resynthesis or hydration of existing proteins. Nevertheless, different proteins characterize different organs during various stages of development which exemplifies the dynamic state of amino acid interconversions (9,12,30,56).

The fate of several amino acids and glucose added to pea cotyledons was given by Larson and Beevers (31). Glutamate and aspartate were rapidly metabolized to neutral and basic amino acids, organic acids, homoserine and CO_2. There was no major metabolic conversion of homoserine. Over 86 per cent of the leucine was directly incorporated into proteins of the cotyledon and later mobilized to the roots and shoots. Glucose was converted to starch, CO_2 and soluble sugars.

The ability of amino acids to provide carbon skeletons for sugar synthesis was elucidated by Stewart and Beevers (57). Malate and fumarate were the principle sinks for aspartate carbon in germinating castor beans. These were readily converted to sugars with some carbon lost as CO_2. Glutamine was the predominant nitrogen form transported to the growing axis. Aspartate, glutamate, alanine, glycine, and serine added to endosperm halves, were converted to sucrose before being transported. Amino acids which occupy terminal positions in nitrogen metabolism were generally transported intact to the growing axis. Others occupying central positions were more readily metabolized and interconverted (24,57).

The conclusions from studies on ribonuclease and protease activity during germination are not clear cut (5,15,22). Similarly, the mechanistic control of transaminases and amino acid interconversions in germinating seedlings is ill defined. Joy and Folkes (24) liken control to that in microorganisms. Amino acids readily metabolized provide no control, whereas those not metabolized are blocks in synthesis. Oaks (40) working on asparagine transport in maize root contended that amino acid requirements at the growing axis may regulate protein degradation, since externally supplied amino acids delayed the disappearance of nitrogen from the endosperm. Asparagine may have been transported according to supply and demand in the root tip. Undoubtedly, the rate of degradation of storage proteins and carbohydrates is in some way under control by the growing axis.

Many species of the family Fabaceae colonize soils deficient in nitrogen (6). Their reserve proteins are high in basic amino acids, particularly arginine. These may supply the basic nitrogen components for other amino acids, nucleic acids, prosthetic groups etc. necessary to maintain the developing photosynthetic and respiratory apparatus of the germinating seed, until the seedling can manufacture its own.

Seed Protein Content: Relationship with Subsequent Growth and Yield

Experimentation directly relating seed protein content with subsequent seedling growth and yield is negligible. Galligar (16) in 1938, observed the growth of root tips in sterile nutrient cultures from seed of sunflower, cotton, pea, castor bean, corn, and soybean which varied in protein and carbohydrate content. Root tips from seed high in starch grew well, whereas those from high protein seeds were less able to maintain growth. Seeds high in sugar and oil had varied root growth among species. It is important to recognize that the negative relationship between the seed protein content and subsequent root growth was observed between plant species and not within a single species. Also, enhanced root growth is not necessarily correlated with favorable shoot growth and yield.

Relationships of seed size with subsequent seedling growth and the effect of seed treatment with trace elements, nitrogenous compounds and other materials on yield, carbohydrate and protein content of grain are well documented (8,10,11,17,18,21,26,28,38,51). In general, larger seeds produce larger plants and higher yields; those where part of the endosperm had been progressively removed, produced smaller seedlings. No correlation was found between the carbohydrate reserves in white mustard seeds and hypocotyl length (55). However, a positive correlation was found between hypocotyl width and carbohydrate content of seeds from the same population. A reciprocal relationship was found between germination ability and protein content in wheat, rye, barley and oat seeds (44).

The alteration of seed protein and carbohydrate content due to fertilization and other environmental conditions is extensively reviewed (11,17,46,47,48,59, 62,64). Those practices which increase crop yields generally lower the protein content of grain. Some workers express doubt that maximum yield and protein can be achieved in a single species through cultural practices and/or breeding (39).

Soaking and coating seeds with various biologically active compounds has produced diversified results in many crops (8,18,21,38,51). Removal of seed coats resulting in the loss of amino acids, carbohydrates and other water soluble materials during imbibition has led to reduced germination and seedling growth (32). Hypothetically, seed coat removal in those species which do not exhibit seed coat dormancy results in rapid imbibition, disrupting membrane integrity, allowing diffusion of soluble solutes.

The nutritive value of seed proteins in animal consumption is abundantly manifest in the literature. Feeding studies often confirm that germinated seeds are higher in biological value than ungerminated seeds (34). Dietary essential amino acids may be synthesized upon germination improving the nutritive value of the seed.

Empirical Generalizations Concerning Seed Protein

1. Upon germination reserve proteins and carbohydrates are degraded to amino and organic acids which are mobilized to sites of reorganization at meristematic regions.

2. In seeds, nitrogen exists mainly in storage proteins which are utilized by the germinating seed, regardless of the external nitrogen supply.

3. Storage proteins of many plant species are rich in basic amino acids, particularly arginine, capable of being oxidatively deaminated or transaminated to α-keto acids. The loss of protein from the endosperm is reciprocally related to protein increases in the embryo.

4. Glutamate, asparatate and their amides occupy central positions in protein degradation and resynthesis.

5. The enzymatic machinery necessary to metabolize exogenously supplied amino acids is evident in germinating seeds. In particular, enzymes and urea cycle intermediates are present in various seed organs incubated with arginine indicating its possible metabolism to other amino acids.

6. A diverse variety of new proteins are present in the germinating seed exemplifying the dynamic state of amino acid metabolism.

7. The degradation of storage materials appears to be controlled by the meristematic axis, possibly involving a type of substrate feedback inhibition.

8. Within a species, larger seeds produce larger seedlings and higher yields.

9. In general, environmental parameters affecting crop yields reciprocally effect their seed protein content.

10. In the limited studies recorded, seed protein content of various plant species is negatively correlated with subsequent root growth and per cent germination.

MATERIALS AND METHODS

Seed Protein Content: Relationship with Subsequent Seedling Growth and Yield

Growth Chamber, Greenhouse and Field Experiments

Winter wheat (*Triticum aestivum* L., cv. Genesee) was obtained from a 1967-68 commercial planting in southwestern Michigan. The enhanced seed protein content was due to postemergence subherbicidal applications of simazine [2-chloro-4,6-bis(ethylamino)-s-triazine] or terbacil [3-tert-butyl-5-chloro-6-methyluracil] (47). Seed was sown in 8 x 10 cm plastic cups in vermiculite in a growth chamber maintained at 10°C during the night and 20°C during the day (16 hours). Each of the 3 field replicates was again replicated 2 times. Equal volumes of Hoagland's solution (Appendix I), with the addition or deletion of supplemental nitrogen, were added to each cup as needed. Hoagland's solution containing 3 mM NO_3^- was added 22 days after planting. Plants not receiving nitrogen were harvested 27 days after planting and those receiving nitrogen were grown for 40 days.

Each of the 3 field replicates was again replicated 3 times in a field experiment in central Michigan on

September 13, 1968 and harvested July 22, 1969. Eighty grams of seed was planted per 1.2 x 5.5 M plot.

Winter wheat ("Ben Hur") used in a greenhouse study was obtained from a field experiment at Southern Illinois University in 1968. The increased protein content of the seed was due to soil surface applications of simazine or atrazine [2-chloro-4-(ethylamino)-6-(isopropylamino)-s-triazine] plus a nitrogen application of 35 kg/ha. Samples from each of the 3 field replicates were again replicated 3 times in a greenhouse maintained at 20-27°C. Seed was planted in 18 cm clay pots in a sandy clay loam. Plants were grown for 50 days and watered as necessary.

In growth chamber and greenhouse experiments, spring wheat ("Ciano") was obtained from a 1968 experiment conducted at Ciudad Obregon, Sonora, Mexico. The varying seed protein content among treatments was in response to nitrogen applications. Treatments were replicated 3 times in the growth chamber and watered as necessary with Hoagland's solution containing 3 mM NO_3^-. Three replicates for each treatment were made in the greenhouse study; other environmental parameters were as previously described. Plants in the growth chamber were harvested after 31 days and those in the greenhouse after 41 days.

Oat seed (Avena sativa L., cv. Rodney) from a 1967 field experiment in central Michigan was planted in the field on April 18, 1968 and the grain harvested July 30.

Forty grams of seed was planted per 0.61 x 9.2 M plot from each control, simazine and terbacil treatment lot, and each seed lot was replicated 3 times. Plots were periodically hand hoed as a weed control measure.

Similar seed was also sown in clay pots in two greenhouse studies each containing 3 replicates. Environmental parameters were as previously defined; one experiment extended 19 days, the other 2 months.

Amino Acid Infiltration: Effect on Subsequent Seedling Growth and Yield

Infiltration Techniques

The source of seed used in these experiments is described in Table 1. Seed was added to L-amino acid solutions at a ratio of 4 grams of seed per 10 ml solution. The pH of the solutions was not determined. Flasks containing the seed and solutions were placed in a dessicator and a vacuum of 730 mm mercury was applied by a water aspirator for 3 hours with 5 periodic releases. Solutions were immediately decanted upon removal from the dessicator, and the seeds rinsed twice with distilled water, blotted dry and placed in a drier at 45°C for 24 hours. Total nitrogen was determined on samples taken from each treatment.

TABLE 1.--Source of seed used in infiltration studies.[1]

Lot Number and Kind	Cultivar	Source and Comments
I - Oats	Garry	MSU Seed Foundation Lab; visually sorted.
II - Winter wheat	Genesee	Untreated controls from 3 replicates of the 1967-68 experiment in southwestern Michigan; visually sorted.
III - Winter wheat	Genesee	Untreated controls from 3 replicates of another 1967-68 field experiment in southwestern Michigan; visually sorted.
IV - Winter wheat	Genesee	Untreated controls from 3 replicates of a 1967-68 experiment in central Michigan; visually sorted.
V - Winter wheat	Genesee	Untreated controls from a 1968-69 experiment in southwestern Michigan; screened through a 6 1/2 / 64 mesh screen, smaller seed discarded.
VI - Spring wheat	Inia	Untreated controls from a 1968-69 experiment at Obregon, Mexico; screened through a 6 1/2 / 64 mesh screen, smaller seed discarded.

[1]Each lot of seed was thoroughly mixed before use.

Growth Chamber, Greenhouse and Field Experiments

Oat seed from lot I (Table 1) was infiltrated with a solution of 0.67 M arginine-HCl and a solution containing alanine, arginine-HCl, asparagine, glycine, glutamine, histidine, leucine, lysine-HCl, methionine, proline and glutamic acid-HCl each at 0.09 M. Samples from each treatment were germinated in vermiculite moistened with distilled water, in a growth chamber at 27°C. After 6 days plants were transferred to nutrient cultures of Hoagland's solution with 2, 8 or 16 mM NO_3^-. Six plants were grown per 200 ml jar lined with aluminum foil, with 3 replicates per treatment, in a growth chamber maintained at 15°C during the night and 20°C during the day (16 hours). Solutions were changed every 2 days and the plants were harvested after 2 weeks.

Seed from the same treatments was used in field experiments at 2 locations in Michigan. Forty grams of seed was planted per 0.61 x 9.2 M plot with a main nitrogen split of 0 and 45 kg/ha, with 3 replicates. In southwestern Michigan, seed was planted April 11, 1969 and harvested July 25; in northern Michigan, seed was planted May 3, 1969 and harvested August 20. Plots were periodically hand hoed as a weed control measure.

Wheat seed from lot II was infiltrated in solutions of amino acids, purine and pyrimidine precursors and grown in a growth chamber for 2 weeks under conditions previously defined. Four replicates per treatment were grown in vermiculite and watered as necessary with Hoagland's solution containing 8 mM NO_3^-.

Solutions of 1.0 M glycine + 1.0 M alanine + 0.25 M glutamic acid-HCl, 0.5 M histidine, 1.0 M arginine-HCl and 0.25 M valine were used in infiltrations of similar wheat seed from lot II. Seed was sown in clay pots and 5 replicates were grown in a greenhouse for 1 month under environmental parameters previously defined.

In another greenhouse experiment, 2 week-old wheat seedlings (from lot II) were sprayed with a mixture of amino acids or urea. The 4 replicates of 10 plants per pot were harvested after 20 days.

Wheat seed from lot III was infiltrated with arginine-HCl and urea for 1, 3 and 9 hours with the vacuum released 5 times during each time interval. Four replicates were grown in a greenhouse under conditions previously defined and harvested after 45 days.

An infiltration solution of 0.3 M methionine was used with wheat seed from lot III. Seed was sown in clay pots in a sandy loam and each treatment was replicated 4 times in a greenhouse. Plants were watered as necessary and harvested after 1 month.

Wheat seed from lots III and IV infiltrated in a 1.0 M arginine-HCl solution was grown in clay pots in a sandy loam in a growth chamber maintained at 10°C during the night and 20°C during the day (16 hours). Plants were watered as necessary with Hoagland's solution containing 3 mM NO_3^- and the 4 replicates were harvested after 42 days.

Wheat seed from lots V and VI was infiltrated in solutions of 1.0 M arginine-HCl and 1.0 M arginine-HCL + 0.5 M sucrose. Seed from lot VI was also infiltrated in solutions of valine, glycine + alanine + glutamic acid-HCL, ammonium phosphate and methionine. Twelve seeds were planted per pot in a clay loam and randomly thinned to 10 seedlings upon emergence. These greenhouse experiments, each employing 4 replicates, were watered as necessary and harvested after 1 month; except one experiment with "Inia" wheat which was grown for 42 days.

Infiltrated seed from lot V was germinated in perlite moistened with distilled water, in a growth chamber at 27°C. After 1 week the seedlings were transplanted to nutrient cultures of Hoagland's solution containing 0, 4, 8, or 16 mM NO_3^-, with 3 replicates. Solutions were changed every 2 days and the plants were grown for 2 weeks in a greenhouse.

Controls infiltrated with distilled water and non-infiltrated controls were planted from each treatment lot in all respective experiments.

Amino Acid Infiltration: Utilization of Arginine During Early Seedling Development

Control and 1.0 M arginine-HCl infiltrated wheat seed from lot V was surface sterilized with 1% sodium hyperchlorite and planted in perlite, moistened with distilled water. Seeds were germinated in a growth chamber maintained at 27°C, in the dark for 6, 9, 10 and 12 days. After 6 days, the top 4 cm of 50 harvested uniform seedlings, including coleoptiles, were immediately ground to homogeneity in 40 ml of distilled water at 4°C with a mortar and pestle and then freeze dried.

Similarly, the top 12 cm of 30 uniform seedlings harvested after 9 days were ground at 4°C and freeze dried. The top 7 cm of 60 uniform seedlings were harvested after 10 days and the top 12 cm of 50 uniform seedlings were harvested after 12 days, similarly ground at 4°C and freeze dried. The tissue harvested from 9, 10 and 12 day-old etiolated seedlings did not include coleoptiles.

Seed was also grown in a greenhouse maintained at 20-27°C, in the light. The top 6 cm of 60 uniform seedlings were harvested after 10 days, similarly ground at 4°C and freeze dried.

Twenty or 40 mg samples from the freeze dried material were extracted 3 times in gently boiling 70% ethanol for 15 minutes. The supernatant solutions were combined, evaporated to dryness, and dissolved in lithium citrate buffer pH 2.8 for subsequent free amino acid determinations on an amino acid analyzer (42). The hydrolyzate of 10 mg of tissue from each of the samples was used in total amino acid analysis.

Twenty grams of ungerminated seed, ground through a 40 mesh screen by a Wiley mill, was also freeze dried and total and free amino acids determined by the outlined procedures.

Homogeneity of the freeze dried material was checked by taking several 10 and 20 mg samples for total nitrogen determinations.

General Procedures

Cultural Practices

Unless otherwise specified, in growth chamber and greenhouse experiments, 20 uniform seeds were planted in each pot and randomly thinned to 15 seedlings upon emergence. In all growth chamber and greenhouse experiments plants were immediately weighed upon harvest and oven dried at 45°C for 72 hours and then reweighed. Field experiments had standard guard rows and alleys which were removed before harvest. Seed heads were threshed with a small plot threshing machine, air dried and cleaned before analysis.

Analytical Procedures

Twenty gram samples ground with a Wiley mill through a 40 or 60 mesh screen were uniformly dried and used for total nitrogen, nitrate and amino acid determinations. Total nitrogen was analyzed by micro-Kjeldahl or automatic procedures (4,13,60). Samples for the nitrogen analyzer were pre-digested in sulfuric acid. Perchloric acid and selenium were added to the mixture prior to digestion and distillation. Ammonia was determined by a color reaction with alkaline phenol and sodium hypochlorite. Total nitrogen was estimated by optical density standardized by micro-Kjeldahl analysis. A factor of 5.7 for wheat and 6.25 for oats was used in the conversion of total nitrogen to protein.

Nitrate was determined according to Lowe and Hamilton (33). Amino acid composition of protein hydrolyzates was determined on an amino acid analyzer (19,42). Samples were hydrolyzed in evacuated tubes containing 6 N HCl (glass distilled) for 22 hours at 110°C (20 psi). After hydrolysis, the samples were evaporated to dryness and dissolved in lithium citrate buffer pH 2.8 for subsequent total amino acid determinations.

To determine seed weight 3 or 5 replicates, each containing 100 or 200 uniform seeds, were oven dried at 45°C for 24 hours and weighed. Per cent seed moisture was determined by drying 3 replicates, each containing 100 uniform seeds, at 102°C for 48 hours.

Experimental Design and Statistical Analysis

Treatments were arranged in randomized complete blocks in all single factor experiments. A split-plot design was employed in all factorial experiments with nitrogen levels as main plots and treatments as sub-plots.

All experimental data was subjected to analysis of variance. Trend comparisons were made with single degrees of freedom. Tukey's test was used to compare means when a significant F value for a factor was obtained. Correlations were made wherever relevant.

RESULTS AND DISCUSSION

Seed Protein Content: Relationship with Subsequent Seedling Growth and Yield

Growth Chamber and Field Experiments with "Genesee" Wheat

The effect of subherbicidal treatments on increasing the seed protein content of legumes and grain crops has been reported (46,47). The protein content of wheat seed as measured by micro-Kjeldahl analysis was positively correlated with subsequent seedling growth (Table 2). Seedling growth was more closely associated with seed protein content when the environmental nitrogen supply was low (Figure 1). There was no significant correlation between seed nitrate and seedling growth, and the per cent dry weight of the forage was not altered.

In a field experiment, the enhanced protein content of the seed (based on Kjeldahl N) due to chemical or nitrogen applications was significantly correlated with subsequent yield (Table 3). The higher seed protein content in the first generation was not reflected in higher seed protein content in the second generation. This is logical, since inheritance of adaptive alterations in response to environmental conditions is unsubstantiated.

TABLE 2.--Relationship between seed protein content of "Genesee" wheat and subsequent seedling growth with and without supplemental nitrogen.[1]

Chemical	Rate (kg/ha)	Seed Analysis		Growth			
				Without N		With N	
		Total Protein (mg/g dry wt)	Nitrate (mμM/g dry wt)	Fresh wt (mg/plant)	Dry wt (%)	Fresh wt (mg/plant)	Dry wt (%)
Control	0	96 a	303	85	27	119 a	26
Simazine	.56	102 ab	337	88	26	126 ab	26
Terbacil	.28	108 b	265	97	27	132 b	26

[1]Means followed by unlike letters are significantly different at the .05 level.

TABLE 3.--Relationship between seed protein content of "Genesee" wheat and subsequent yield.

First Generation				Second Generation	
N level (kg/ha)	Chemical	Rate (kg/ha)	Seed protein (mg/g dry wt)	Yield (kg/ha)[1]	Seed protein (mg/g dry wt)
0	None	0	96	4032	104
	Simazine	.56	102	3731	104
	Terbacil	.28	108	3951	105
	Mean		102	3905 a	104
22	None	0	106	3808	114
	Simazine	.56	119	4140	113
	Terbacil	.28	120	4243	96
	Mean		115	4064 ab	108
45	None	0	128	4301	95
	Simazine	.56	146	4418	106
	Terbacil	.28	139	4409	105
	Mean		138	4376 b	102

[1]Means followed by unlike letters are significantly different at the .05 level. The correlation between seed protein content and subsequent yield is significant at the .01 level ($r = 0.884$).

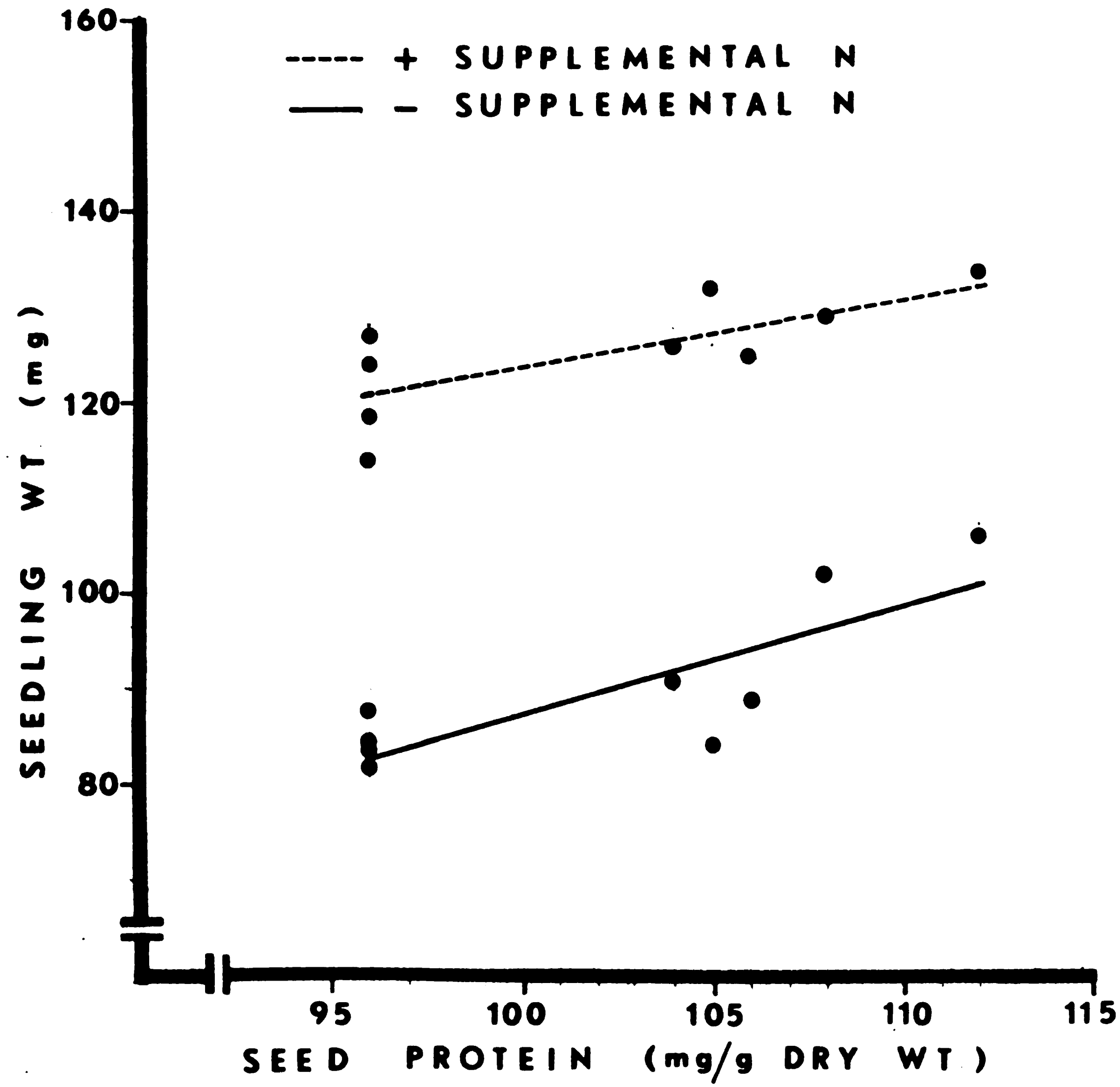

Figure 1.--The correlation of seed protien content with subsequent seedling growth without supplemental nitrogen is significant at the .01 level (r = 0.814), and with supplemental nitrogen at the .05 level (r = 0.756).

Greenhouse Experiment with "Ben Hur" Wheat

Wheat seed containing more protein (based on total amino acids) as a result of previous chemical treatments produced appreciably larger seedlings (Tables 4 and 5). There was no significant difference in the weight or per cent moisture of the seed planted and the per cent dry weight of the forage harvested was not altered. The total protein content per seed can be derived from the data, in which case the correlation between the protein content per seed and subsequent seedling growth is also significant at the .01 level (r = 0.835).

TABLE 4.--Relationship between total amino acid content and subsequent seedling growth of "Ben Hur" wheat.

Chemical	Rate (kg/ha)	Seed Analysis: Wt (mg/seed)	Seed Analysis: Moisture (%)	Seed Analysis: Total amino acids (mg/g dry wt)	Growth-Dry wt: (mg/plant)	Growth-Dry wt: (%)
Control	0	28	7.0	97[1]	62[1]	19.0
Simazine	.56	28	7.1	131	83	19.0
Atrazine	.56	24	7.1	143	83	18.0

[1]F value for treated wheat compared to control is significant at the .01 level for all observations. The correlation of total amino acid content with subsequent seedling growth is significant at the .01 level (r = 0.837).

TABLE 5.--Amino acid composition of protein hydrolyzates from seed of control and chemically treated "Ben Hur" wheat.[1]

Amino acids (mg/g dry wt)	Control	Simazine (.56 kg/ha)	Atrazine (.56 kg/ha)
Aspartic acid	4.9	6.4	6.2
Threonine	2.8	4.3	4.3
Serine	4.8	6.1	7.1
Glutamic acid	30.5	43.0	44.8
Proline	7.6	10.8	12.9
Glycine	4.4	5.7	6.1
Alanine	3.6	4.5	5.1
Valine	4.5	6.0	6.8
Cystine	3.6	4.5	5.3
Methionine	1.4	1.6	2.2
Isoleucine	3.7	4.9	5.6
Leucine	6.7	9.1	9.8
Tyrosine	3.2	4.3	4.8
Phenylalanine	4.8	6.7	7.2
Lysine	3.0	3.7	3.9
Histidine	2.4	3.2	3.5
Arginine	5.1	6.6	7.4
Total	97.0	131.4	143.0

[1]F value for treated wheat compared to control is significant at the .01 level for all observations.

Growth Chamber and Greenhouse Experiments with "Ciano" Wheat

The response from chemicals applied at subherbicidal rates to the first generation suggests that they may accumulate in the maturing seed and thus stimulate growth the following generation. However, the higher protein content of Mexican grown wheat seed can only be attributed to supplemental nitrogen (Table 6). The correlations of total seed protein, based on Kjeldahl nitrogen or total amino acids, with subsequent seedling growth were significant. Total amino acid content per seed is correlated with subsequent seedling growth at the .05 level ($r = 0.634$). The per cent dry weight of the forage and the weight and per cent moisture of the seed planted was unchanged.

At harvest, seedlings from high protein seeds were greener (particularly the lower leaves) with thicker stems (Figure 2).

Greenhouse and Field Experiments with "Rodney" Oats

Seedling growth and yield were significantly correlated with the protein content of the seed planted, based on both total amino acids and Kjeldahl nitrogen (Table 7). The nitrate content of the seed was not significantly correlated with growth or yield; per cent

TABLE 6.--Relationship between seed protein content of Mexican grown "Ciano" wheat and subsequent seedling growth in growth chamber and greenhouse experiments.

Supplemental N (kg/ha)	Seed Analysis				Growth	
	Wt (mg/ seed)	Moisture (%)	Total Protein (mg/g dry wt)		Fresh wt (mg/ plant)[1]	Dry wt (%)
			Kjeldahl	Amino acids		
0	36	7.0	147	113	370 a	16.2
100	35	7.1	157	132	377 ab	16.1
200	35	7.0	161	154	415 b	15.9
300	35	7.0	164	122	407 ab	15.5
400	34	7.0	160	152	455 d	15.5

[1]Means followed by unlike letters are significantly different at the .05 level. F value for interaction of treatments with greenhouse and growth chamber is not significant. The correlation of Kjeldahl protein with seedling growth is significant at the .05 level (r = 0.562). The correlation of total amino acid content with seedling growth is significant at the .01 level (r = 0.688).

Figure 2.--Second generation seedlings from treated "Ciano" wheat seed. Left to right: Control, 200 kg N/ha and 400 kg N/ha.

TABLE 7.--Relationship between seed protein content of "Rodney" oats with growth and yield the following generation.

Chemical	Rate (kg/ha)	First Generation: Seed: Wt (mg)	First Generation: Seed: Moisture (%)	First Generation: Total protein (mg/g dry wt): Kjeldahl	First Generation: Total protein (mg/g dry wt): Amino acids	Second Generation: Seedling fresh wt (mg/plant)	Second Generation: Seed: Yield (kg/ha)	Second Generation: Seed: Kjeldahl (mg/g)
Control	0	29	6.6	136	103	187[1]	3830[1]	117
Simazine	.07	29	6.6	148	132	267	5443	120
Simazine	.28	30	6.7	143	131	282	4905	121
Terbacil	.14	31	6.7	142	127	229	4636	113

[1]F value for control compared to treatments is significant at the .05 level. The correlation of total amino acid content with seedling growth is significant at the .01 level ($r = 0.711$) and with yield at the .05 level ($r = 0.703$). The correlation of Kjeldahl protein with seedling growth is significant at the .05 level ($r = 0.644$) and with yield at the .01 level ($r = 0.750$).

germination, per cent dry weight of the seedlings, seed weight and per cent moisture were not significantly different between treatments. Again, the protein content of the seed harvested was not changed in response to previous chemical treatments.

In this, and in the previous experiment (Table 6) determinations of seed protein content by micro-Kjeldahl or amino acid analysis reveals the relationship between seed protein content and subsequent seedling growth. This suggests that a particular amino acid or peptides in the seed may be of primary consequence in subsequent seedling growth. The amino acid infiltration studies were projected from this hypothesis.

Older seedlings from another greenhouse experiment clearly show a difference in vigor between treatments (Figure 3). Seedling emergence and development during the early stages of growth were independent of treatment. After the second week of growth, plants from protein enriched seeds were greener and had thicker stems.

The dependence of a direct chemical effect on increasing the yield the following generation is remote since high protein seeds induced by nitrogen levels or locations (Tables 3, 6 and 16) produce the same protein-growth relationship. In addition, the seed protein content of the second generation was not altered as it was

Figure 3.--Second generation seedlings from treated "Rodney" oat seed. Left to right: Control, simazine at .07 kg/ha, at .28 kg/ha and terbacil at .14 kg/ha.

in the first generation in response to chemical and/or nitrogen applications (Tables 3 and 7).

Increased seedling growth and yield were not functions of seed weight in these experiments. The seed used in each test was uniform in weight between treatments (Tables 4, 6 and 7). Correlations of seed size with subsequent seedling growth or yield were not significant in any experiment. It is probable, that this positive relationship, reported by other researchers is a manifestation of seed protein content reflected in seed size or weight.

Water loss during seed maturation is indicative of metabolic change preparing the seed for dormancy. Associated with this process are alterations in fine structure such as polysome breakdown and a decrease of endoplasmic reticulum (29,43). However, the exact function of seed moisture content on subsequent seedling vigor is difficult to define. In any case, correlations of per cent seed moisture with subsequent seedling growth or yield were not significant in any experiment (Tables 4, 6 and 7).

Amino Acid Infiltration: Effect on Subsequent Seedling Growth and Yield

Growth Chamber and Field Experiments with "Garry" Oats

The addition of 8 or 16 mM NO_3^- to the nutrient solution produced the largest effect on seedling growth from oat seed infiltrated with amino acids (Table 8). Arginine produced a greater response on seedling growth with 8 mM NO_3^- than controls with 16 mM NO_3^-. The per cent dry weight of the forage harvested was not changed.

TABLE 8.--Effect of external nitrogen levels on the growth of "Garry" oat seedlings from seed infiltrated with amino acids.

Treatment	Conc (M)	N/seed (mg)	Growth (mg dry wt/plant)[2]		
			mM NO_3^-		
			2	8	16
Control	--	0.76	117	119	130
Water	0	0.65	110	115	132
Arginine	0.67	0.95	123	136	129
Amino acids[1]	0.09	0.90	112	124	142

[1]Amino acid mixture: alanine, arginine-HCl, asparagine, glycine, glutamine, histidine, leucine, lysine-HCl, methionine, proline and glutamic acid-HCl each at 0.09 M.

[2]F value for comparison of controls vs. amino acids with quadratic N is significant at the .01 level (CV = 9.2%).

In field experiments, there was a significant overall increase in yield due to amino acid infiltration (Table 9). However, the treatments responded differently to nitrogen levels at the two locations. This indicates that both nitrogen and location affected the response of oats to amino acid fortification. Thus, the exact parameters necessary for increasing the yield cannot be deduced from this experiment.

TABLE 9.--Effect of amino acid infiltration and nitrogen fertilization on subsequent yield of "Garry" oats at two Michigan locations.

Treatment	Conc (M)	N/seed (mg)	Yield (kg/ha)[1]				
			Southwestern		Northern		Mean[2]
			Supplemental N (kg/ha) 0	45	0	45	
Control	--	0.76	954	1349	861	1198	1091
Water	0	0.65	1166	1464	836	1317	1196
Arginine	0.67	0.95	1105	1539	944	1338	1232
Amino acids	0.09	0.90	1051	1532	987	1475	1261

[1]F values for interaction of treatments with N levels or treatments with locations are not significant. F value for interaction of treatments with N at different locations is significant at the .05 level (CV = 8.2%).

[2]F value for comparison of controls vs. treatments is significant at the .01 level.

The concentration of the infiltrating solutions before and after infiltration were identical (Table 10). This suggests a simple diffusion of solutes into the seed, as has been shown with the uptake of phenylalanine by the garden pea (41). Approximately 11 μl of solution would have to be infiltrated into a seed to account for the increase in nitrogen content, assuming simple diffusion. An uptake of this amount is possible; nevertheless, considering these data and the technique of washing the seed after infiltration, the possibility that the amino acids were adsorbed onto some peripheral organ (seed coat, epidermal and cross cells etc.) and not infiltrated into the endosperm, still exists.

TABLE 10.--An estimate of the amount of solution infiltrated into "Garry" oat seed to account for the increase in nitrogen content.

Seed parameter	Treatments		
	Water control	Arginine (0.67 M)	Amino acids (0.09 M)
Dry wt (mg)	36.93	38.95	39.67
N (mg/g dry wt)	17.7	24.4	22.7
N/seed (mg)	0.65	0.95	0.90
Increase in N/seed (mg)	--	0.30	0.25
Solution infiltrated/seed (μl)[1]	--	8.00	10.44
Concentration of solution decanted (M)	--	0.67	0.09

[1]Based on mg of N infiltrated/seed and the concentration of the solutions.

Growth Chamber and Greenhouse Experiments with "Genesee" Wheat (lot II)

Wheat seed infiltrated in solutions of amino acids, purine and pyrimidine precursors produced little effect on seedlings in a growth chamber experiment (Table 11). The solubility of the compounds limited their concentration and consequently nitrogen analysis of the seed planted was not determined. Some of the compounds may not have been appreciably infiltrated into the seed accounting for the lack of response. Nevertheless, anthranilic acid reduced the dry weight of the seedlings compared to untreated controls.

TABLE 11.--Effect of seed infiltration with amino acids, purine and pyrimidine precursors on subsequent seedling growth of "Genesee" wheat.[1]

Treatment	Conc (M)	Growth - Dry wt (mg/plant)	Growth - Dry wt (%)
Control	-	54 a	14.1 a
Water	0	56 a	14.3 a
Anthranilic acid	0.025	41 b	12.6 b
Orotic acid	0.005	58 a	14.3 a
Inosinic acid	0.1	55 a	14.1 a
Pyruvic acid	0.1	58 a	14.2 a
Carbamyl PO4	0.025	57 a	14.0 a
All of the above	as above	59 a	14.0 a
Arginine, methionine and glutamic acid	0.6, 0.15, 0.25	54 a	13.6 ab

[1]Means followed by unlike letters are significantly different at the .01 level.

In a greenhouse experiment, wheat seed infiltrated with various amino acids increased the total nitrogen content of the seed planted (Table 12). However, only arginine produced an increase in subsequent seedling growth without reducing the stand. A mixture of glycine, alanine and glutamic acid infiltrated into the seed significantly reduced their germination. Therefore, growth expressed as mg dry weight/plant would be misleading. Histidine and valine, although infiltrated into the seed, did not significantly alter seedling growth. The per cent dry weight of the forage harvested was not changed.

TABLE 12.--Effect of seed infiltration with amino acids on subsequent seedling growth of "Genesee" wheat.

Treatment	Conc (M)	Seed analysis	Growth			
				Dry wt		
		Total N (mg/g dry wt)	Plants/pot[1]	(mg/pot)[2]	(mg/plant)[1]	(%)
Control	-	19.1	14.8 a	2432 a	164 a	23.2
Water	0	19.8	15.0 a	2428 a	162 a	23.3
Histidine	0.5	22.5	14.8 a	2675 a	181 a	23.2
Arginine	1.0	29.0	14.2 a	2892 b	205 b	23.2
Valine	0.25	20.5	15.0 a	2775 a	185 a	23.5
Glycine,	1.0					
alanine,	1.0					
glutamic acid	0.25	23.4	10.2 b	2331 a	-	21.9

[1]Means followed by unlike letters are significantly different at .01 level.

[2]Means followed by unlike letters are significantly different at .05 level.

A single application of an amino acid mixture or urea on the foliage of 2 week old wheat seedlings produced an increase in both growth and tiller production (Table 13). Each treatment solution contained equivalent amino groups. Foliar sprays of urea have been shown to increase the yield and protein content of "Pawnee" wheat by acting as a nitrogen source (14). Similarly, the amino acid mixture may function as a source of ammonia for the synthesis of proteins at the growing axis.

TABLE 13.--Effect of amino acid and urea spray on seedling growth and tiller production of "Genesee" wheat.[1]

Treatment	Conc (M)	Growth (mg/plant) Fresh wt	Growth (mg/plant) Dry wt	Growth Tillers $\bar{x}$/10 plants
Control	-	836 a	150 ab	1.3 a
Water	0	813 a	146 b	2.0 a
Arginine, methionine and glutamic acid	0.5 0.2 0.3	1126 b	185 ac	12.0 b
Urea	1.25	1180 b	201 c	14.3 b
Urea and amino acids	1/2 above	1080 b	191 c	12.8 b

[1]Means followed by unlike letters are significantly different at the .01 level.

Urea infiltrated into "Genesee" wheat seed however, did not increase the subsequent seedling growth as well as arginine (Table 14).

TABLE 14.--Effect of infiltration time with arginine and urea on subsequent seedling growth of "Genesee" wheat seed.[1]

Treatment	Conc (M)	Infiltration time (hr) 1 N (mg/g dry wt)	1 Fresh wt (g/pot)	3 N (mg/g dry wt)	3 Fresh wt (g/pot)	9 N (mg/g dry wt)	9 Fresh wt (g/pot)
Control	-	15.3	5.62	15.3	5.21	15.3	5.09
Water	0	14.9	5.50	15.8	5.60	13.7	4.97
Arginine	0.5	19.7	5.16	21.4	6.15	22.0	5.53
Arginine	1.0	24.6	5.54	27.0	6.15	24.9	6.03
Urea	1.0	17.9	5.40	19.8	5.61	19.6	5.63
Urea	2.0	21.8	5.94	23.4	5.90	23.4	5.62

[1]F value for interaction of treatment with time is significant at the .05 level (CV = 10.7%).

Growth Chamber and Greenhouse Experiments with "Genesee" Wheat (lots III and IV)

An infiltration period of 3 hours was determined as optimum based on a greenhouse experiment with wheat seed (Table 14). The vacuum was released 5 times during each time interval. The nitrogen content and the subsequent growth of the seeds infiltrated for 9 hours shows little benefit over 3 hours. The low nitrogen content of the water infiltrated control after 9 hours indicates leaching of solutes from the seed. After this period of time the seeds were actively germinating and may exclude further infiltration of solutes. The per cent dry weight of the forage was not altered.

In another greenhouse experiment with wheat seed from lot III, the subsequent seedling growth was reduced due to methionine infiltration (Table 15). The per cent dry weight of the forage was not altered.

TABLE 15.--Effect of seed infiltration with methionine on subsequent seedling growth of "Genesee" wheat.

Treatment	Conc (M)	Seed analysis	Growth-dry wt[1]	
		Total N (mg/g dry wt)	(mg/plant)	(%)
Control	-	15.0	82 a	16.3
Water	0	15.6	86 a	16.5
Methionine	0.3	14.4	63 b	16.2

[1]Means followed by unlike letters are significantly different at .05 level.

The difference in protein content of wheat seed from lots III and IV was due to locations (Table 1). Subsequent seedling growth from low protein seed (lot III) infiltrated with arginine was significantly greater than controls (Table 16). High protein seed (lot IV) similarly infiltrated with arginine did not enhance seedling growth. Arginine infiltration into wheat seed was of little advantage when the inherent protein content was high. It is likely that the smaller, high protein seeds were not able to supply sufficient α-keto acids during the early stages of growth to utilize the additional ammonia from the infiltrated arginine.

TABLE 16.--Effect of 1.0 M arginine infiltration and initial protein level on subsequent seedling growth of "Genesee" wheat seed.

Seed parameter	Low protein seed			High protein seed		
Wt (mg)	33.04			25.50		
Moisture (%)	8.7			7.1		
Total protein/seed (mg)	2.87			3.34		
After infiltration:						
	Control	Water	Arginine	Control	Water	Arginine
Seed N (mg/g dry wt)	15.2	14.2	21.5	23.0	22.7	34.7
Dry wt (mg/plant)[1]	71 a	80 a	103 b	86	81	88
Dry wt (%)	24.9	24.3	25.9	25.2	25.2	24.8

[1]Means followed by unlike letters are significantly different at the .01 level.

These data also show that high protein seeds produce larger seedlings and that this relationship is not a positive function of seed weight or per cent moisture. The per cent dry weight of the forage harvested was not different between treatments.

Greenhouse Experiments with "Genesee" Wheat (lot V)

Sucrose added to an infiltration solution of arginine was less effective in stimulating growth than arginine alone (Table 17). A 1.0 M arginine solution again produced a significant increase in subsequent seedling growth. Sucrose may have competed with arginine for entry into the seed as evidenced by lower total nitrogen per seed. The per cent dry weight of the forage was not changed.

TABLE 17.--Effect of seed infiltration with arginine and sucrose on subsequent seedling growth of "Genesee" wheat.

Treatment	Conc (M)	Seed analysis	Growth[1]	
		Total N (mg/g dry wt)	Fresh wt (mg/plant)	Dry wt (%)
Control	-	16.1	685 a	14.7
Water	0	16.1	723 a	14.7
Arginine	1.0	20.7	816 b	14.5
Arginine and sucrose	1.0 0.5	19.1	743 ab	14.8

[1]Means followed by unlike letters are significantly different at the .01 level.

Arginine infiltrated into wheat seed affected seedling growth when an external nitrogen supply of 8 or 16 mM NO_3^- was supplied (Table 18). The effect was optimum at 8 mM NO_3^-, which was similar to previous results with oats (Table 8).

TABLE 18.--Effect of external nitrogen levels on the growth of "Genesee" wheat seedlings from seed infiltrated with 1.0 M arginine.

Treatment	Conc (M)	Seed analysis: Total N (mg/g dry wt)	Growth (mg dry wt/plant)[1] mM NO_3^-: 0	4	8	16	Mean
Control	-	16.1	27	132	127	130	104 a
Water	0	16.1	27	130	129	127	103 a
Arginine	1.0	20.7	28	132	147	140	112 b
Mean[2]			27 a	131 b	134 b	132 b	

[1]Means followed by unlike letters are significantly different at the .05 level. F value for interaction of linear nitrogen with control vs. arginine is significant at the .05 level (CV = 6.7%).

[2]Means followed by unlike letters are significantly different at the .01 level.

These data indicate that there is a basic nitrogen level necessary for optimum utilization of arginine at a particular stage of growth. At extremely high or low nitrogen levels the effect of arginine on 3 week old seedlings is minimal. This is logical, since arginine cannot be infiltrated into the seed in a quantity necessary to supply all the nitrogen for cellular metabolism throughout the life of the plant. The per cent dry weight of the forage harvested was not changed between treatments within a nitrogen level.

Greenhouse Experiments with "Inia" Wheat

Mexican wheat seed infiltrated with various amino acids, sucrose and ammonium phosphate did not significantly produce larger seedlings than controls, although the total nitrogen content of the seed planted increased due to treatments (Table 19). A mixture of glycine, alanine and glutamic acid did not reduce germination as it did in a previous wheat variety (Table 12).

TABLE 19.--Effect of seed infiltration with amino acids, sucrose and ammonium phosphate on subsequent seedling growth of "Inia" wheat.[1]

Treatment	Conc (M)	Seed analysis: Total N (mg/g dry wt)	Growth-dry wt (mg/plant)	Growth-dry wt (%)
Control	-	18.1	355	20.1
Water	0	18.8	357	19.6
$NH_4H_2PO_4$	0.5	24.6	351	18.9
Arginine	1.0	24.6	363	19.1
Arginine and sucrose	1.0 0.5	24.2	385	19.2
Glycine, alanine and glutamic acid	1.0 1.0 0.25	22.8	354	19.6

[1]F value for difference in treatments is not significant.

Seedling growth from seed infiltrated with valine or methionine did not differ from controls (Table 20). Methionine significantly reduced the growth of "Genesse" wheat in a previous experiment (Table 15). In both experiments with "Inia" wheat the per cent dry weight of the forage harvested was not altered.

TABLE 20.--Effect of seed infiltration with valine and methionine on subsequent seedling growth of "Inia" wheat.

Treatment	Conc (M)	Seed analysis	Growth-dry wt	
		Total N (mg/g dry wt)	(mg/plant)[1]	(%)
Control	-	18.1	216	15.6
Water	0	19.3	204	15.2
Valine	0.25	18.1	210	15.8
Valine	0.50	20.4	206	15.2
Methionine	0.10	20.0	206	15.0
Methionine	0.25	18.6	214	15.1

[1]F value for interaction of treatment with sterilized or unsterilized clay loam is not significant.

The reason "Inia" wheat did not respond to amino acid infiltrations remains elusive, since the mature seed contains a large fraction of its nitrogen in arginine indicating the presence of enzymes necessary to metabolize it into usable substrates (Table 21).

TABLE 21.--Nitrogen distribution in total amino acids of oats and wheat seed.

Amino Acids (µg N/g dry wt)	Oats	Wheat			
	"Rodney"	"Ben Hur"	"Ciano"	"Genesee"	"Inia"
Aspartic Acid	952	516	840	602	633
Threonine	448	329	490	406	447
Serine	644	640	952	616	742
Glutamic Acid	2,226	2,905	2,646	2,870	3,410
Glycine	1,107	821	1,218	966	1,109
Alanine	812	566	826	714	808
Valine	756	538	728	910	1,109
Cystine	392	420	420	56	24
Methionine	98	132	182	105	167
Isoleucine	491	395	518	434	494
Leucine	924	716	994	910	965
Tyrosine	322	248	364	266	272
Phenylalanine	546	407	588	462	504
Lysine	924	575	812	532	694
Histidine	756	650	966	588	790
Arginine	2,520	1,641	2,800	1,456	2,391
Total	13,918	11,499	15,344	11,893	14,559

Amino Acid Infiltration: Utilization of Arginine During Early Seedling Development

A major fraction of the nitrogen in the storage protein of the seed used in the studies relating seed protein content with subsequent seedling growth and yield was in arginine (Table 21). Therefore, enzymes necessary for its utilization in the synthesis of other amino acids and proteins at the growing axis should be present in the germinating seedling. In addition, if these enzymes are present they should function in the utilization of infiltrated arginine.

Six day-old etiolated wheat seedlings from seed infiltrated with 1.0 M arginine had a pronounced increase in total amino acids compared to untreated controls (Table 22). Similar increases were manifested in 9 and 10 day-old etiolated wheat seedlings and in 10 day-old seedlings grown in the light (Tables 22 and 23). Striking increases in aspartic acid were evident. Glutamic acid was higher in treatments than in controls of 6 and 10 day-old etiolated seedlings and 10 day-old seedlings grown in the light. Aspartic and glutamic acid are the prime acceptors for ammonia, however, the resulting amides do not appear in the total amino acid analysis because of the hydrolysis procedure in preparing the sample for total amino acid analysis. Therefore, it is reasonable to conclude from these data that aspartate and to a lesser extent glutamate

TABLE 22.--Amino acid analysis of etiolated "Genesee" wheat seedlings from control and seed infiltrated with 1.0 M arginine.

Amino acids (µMoles/ g dry wt)	Age (days)							
	6				9			
	Total		Free		Total		Free	
	Cont	Arg	Cont	Arg	Cont	Arg	Cont	Arg
Aspartic acid	397	548	7.4	7.5	265	317	6.6	8.0
Threonine	49	85	8.4	13.4	48	64	7.9	7.2
Serine	47	93	9.0	7.1	43	69	6.6	9.4
Asparagine	*	*	277	297	*	*	198	192
Glutamic acid	122	187	31.1	28.6	110	110	9.2	14.1
Glycine	96	161	3.1	2.6	81	100	1.5	1.5
Alanine	93	161	8.0	6.5	84	112	6.3	6.4
Valine	87	149	24.6	19.2	76	91	13.2	13.1
Cystine	*	2	*	*	6	7	*	*
Methionine	12	21	*	*	11	13	*	*
Isoleucine	51	85	12.2	9.9	44	50	6.4	5.1
Leucine	80	133	5.3	4.4	52	74	1.9	2.1
Tyrosine	23	42	2.2	1.8	22	25	1.3	1.7
Phenyl-alanine	35	61	2.0	1.7	32	37	1.5	1.4
Lysine	35	47	3.7	5.1	71	119	6.6	10.2
Histidine	17	16	9.1	6.9	27	34	8.7	7.4
Arginine	29	32	1.2	2.5	45	52	4.5	3.2
Total	1,173	1,823	404	414	1,017	1,274	280	283
Total (mg N/g dry wt)	18.6	28.0	9.9	10.3	18.0	22.7	7.2	7.1

*Present in trace amounts or hydrolyzed in the case of asparagine.

TABLE 23.--Amino acid analysis of ten day-old "Genesee" wheat seedlings grown in the light and in the dark from control and seed infiltrated with 1.0 M arginine.

Amino acids (µMoles/ g dry wt)	Light				Dark			
	Total		Free		Total		Free	
	Cont	Arg	Cont	Arg	Cont	Arg	Cont	Arg
Aspartic acid	242	356	2.3	13.4	544	638	10.1	12.2
Threonine	83	103	2.7	4.9	80	89	8.2	9.0
Serine	79	112	5.6	11.1	94	91	10.6	13.2
Asparagine	*	*	98.2	230	*	*	294	444
Glutamic acid	191	214	24.3	49.4	149	161	34.6	34.8
Glycine	161	198	4.4	12.6	137	146	1.7	2.3
Alanine	164	210	4.9	15.1	119	132	5.9	8.2
Valine	135	182	5.5	9.1	130	133	8.7	12.8
Cystine	28	27	*	*	*	*	*	*
Methionine	1	12	*	*	18	25	*	*
Isoleucine	79	106	2.7	3.6	66	71	1.3	4.2
Leucine	138	203	1.9	2.8	108	128	1.5	1.9
Tyrosine	49	70	0.5	0.6	38	55	4.9	3.7
Phenyl-alanine	72	113	1.1	1.8	55	68	3.3	3.3
Lysine	156	158	1.7	1.9	155	128	7.6	9.0
Histidine	42	43	1.7	1.3	42	38	10.3	12.0
Arginine	128	127	1.4	2.6	120	105	8.3	9.3
Total	1,748	2,234	159	360	1,855	2,008	411	580
Total (mg N/g dry wt)	33.6	40.4	3.7	8.4	34.4	35.4	10.6	15.2

*Present in trace amounts or hydrolyzed in the case of asparagine.

and their amides rise over controls in response to the arginine infiltrated into the seed. This was expected since aspartic and glutamic acid serve as intermediates in transferring nitrogen in storage proteins to amino acid residues used in the de novo synthesis of proteins at the growing axis.

Amino acid analysis of 12 day-old etiolated seedlings was indicative of the preceding results (Table 24). However, the seedlings were beginning to senesce and die (rise in free amino acids) which probably accounted for the subtle differences in total amino acids, aspartic and glutamic acid as noted earlier.

Total and free amino acid analysis of control and infiltrated wheat seed confirmed the increase in nitrogen content as determined by micro-Kjeldahl procedures (Table 24). The increase in total nitrogen in treated seed was due to arginine infiltration.

Relative differences in the free amino acid content of the seedlings were not large and in general corresponded to the differences in total amino acids.

The total mg N/g dry weight, based on amino acid analysis, was higher in the seedlings than in the seed planted (Table 24). This is misleading, but rectifiable. Consider the control of 10 day-old etiolated seedlings which contained 34.4 mg N/g dry weight. Each seedling

TABLE 24.--Amino acid analysis of twelve day-old etiolated "Genesee" wheat seedlings and of seed from control and seed infiltrated with 1.0 M arginine.

Amino acids (μMoles/ g dry wt)	12 day-old				Seed			
	Total		Free		Total		Free	
	Cont	Arg	Cont	Arg	Cont	Arg	Cont	Arg
Aspartic Acid	431	452	5.4	9.1	43	42	0.7	0.4
Threonine	55	58	9.8	9.7	29	28	0.1	0.5
Serine	58	60	11.8	13.5	44	37	0.4	1.1
Asparagine	*	*	343	327	*	*	2.7	0.9
Glutamic acid	118	128	28.0	28.6	205	197	*	*
Glycine	96	106	1.8	1.7	69	65	0.5	0.4
Alanine	99	105	8.8	9.3	51	46	0.6	1.0
Valine	85	94	16.4	18.8	65	59	0.3	0.2
Cystine	*	*	*	*	2	1	*	*
Methionine	15	17	*	*	8	6	*	*
Isoleucine	38	47	5.6	6.2	31	30	0.2	0.1
Leucine	74	79	2.1	2.7	65	60	0.2	0.2
Tyrosine	25	28	3.5	3.8	19	18	0.2	0.1
Phenyl-alanine	36	38	3.8	4.2	33	31	0.2	0.1
Lysine	78	85	22.8	22.9	19	27	*	*
Histidine	38	36	17.7	15.5	14	18	0.1	0.1
Arginine	56	52	10.6	1.2	26	142	*	143
Total	1,302	1,385	491	474	723	807	6	148
Total (mg N/g dry wt)	22.7	23.8	12.9	12.0	11.9	18.2	0.1	8.1

*Present in trace amounts or hydrolyzed in the case of asparagine.

weighed approximately 4 mg, therefore, 0.138 mg N is contained per seedling. The seed planted weighed approximately 38 mg, of which 0.452 mg is N. Therefore, the seedlings do not contain more nitrogen than can be accounted for from the seed itself.

CONCLUSIONS AND SUMMARY

To a large extent, storage proteins serve as a source of nitrogen and amino acids which are utilized in the de novo synthesis of functional proteins at the growing axis. It seems logical that high protein seeds would produce larger seedlings and higher yields than low protein seeds of the same genotype. This relationship has been shown herein, with several wheat varieties and oats. The enhanced protein content of the seed was due to locations or chemical and nitrogen applications. Seed weight, per cent moisture or nitrate content were not related to subsequent seedling growth or yield.

Infiltrating wheat and oat seed with amino acids, urea and various amino acid, purine and pyrimidine precursors produced varied results. In several experiments, wheat seed vacuum infiltrated for 3 hours in a 1.0 M arginine solution produced larger seedlings. In addition, oat seed infiltrated in a 0.67 M arginine solution or in a solution of 11 amino acids yielded more grain than controls in 1 of 2 field tests.

Arginine is a major storage form of nitrogen in the wheat and oat seed used in these studies. The enzymes necessary for arginine metabolism are presumably present in the germinating seed and would enable the utilization of infiltrated arginine. Amino acid analysis of etiolated seedlings during various stages of development, from untreated and infiltrated seed, provides evidence for arginine metabolism. There is a corresponding rise in aspartic and glutamic acids and their amides which serve as central intermediates in the utilization of the guanidino nitrogen of arginine. Further evidence that arginine serves as a nitrogen source was shown when foliar sprays of arginine produced similar effects of seedling growth and tiller production as urea sprays, which are known to supply ammonia.

The amino acid data of etiolated seedlings and the enhanced seedling vigor of infiltrated seeds strongly suggests the internal localization of arginine at a site where it can be metabolized, rather than a peripheral accumulation on the seed coat.

The effect of infiltrating arginine into seed on seedling growth and its utilization by etiolated seedlings provides further evidence for the relationship of seed protein content with subsequent seedling growth and yield. Although the use of high protein seed to enhance future crop production is plausible, the technique of infiltrating arginine or other amino acids into seed may be of limited practical significance. However, the critical question which arises from these studies is:

can the storage protein of seed be altered genetically to contain high levels of basic amino acid residues (i.e. arginine) which can be metabolized upon germination and produce an effect throughout the life of the plant?

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APPENDIX

APPENDIX I

Hoagland's solution[1]

Chemical	Stock solution (g/l)	Final solution (ml stock/l H_2O)
$CaCl_2 \cdot 2H_2O$	147	1.5
K_2SO_4	87	1.0
$MgSO_4 \cdot 7H_2O$	98.6	2.5
KH_2PO_4	27.2	2.5
Fe (Chelate 12%)	16.6	2.5
Minor elements:		1.25
$ZnSO_4 \cdot 7H_2O$	0.088	
H_3BO_4	1.144	
$MnCl_2 \cdot 4H_2O$	0.724	
$CuSO_4 \cdot 5H_2O$	0.032	
$H_2MO_4 \cdot H_2O$	0.008	
KOH	56	adjust pH to 6.3

Equal quantities of 1.0 M stock solutions of KNO_3 and $Ca(NO_3)_2 \cdot 4H_2O$ for the following NO_3 concentrations:

	2 mM	3 mM	4 mM	8 mM	16 mM
ml stock/l H_2O:	0.66	0.99	1.33	2.66	5.32

[1]Modified from Hoagland, D. R. and D. I. Arnon. 1938. The water-culture method for growing plants without soil. Univ. Calif. Agri. Exp. Sta. Circ. 347.