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Review

Genetics of nitrogen metabolism and physiological/biochemical selection for increased grain crop productivity *

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Contents

Summary. It is necessary to increase protein productivity of grain crops to meet present and future world protein requirements. Conventional plant breeding methodology has been to select genotypes with enhanced yield or grain protein concentration. In addition to this determination of end product, we suggest measurements of a number of physiological and biochemical processes of nitrogen (N) metabolism which precede plant maturity as selection criteria for enhanced N metabolism and grain crop productivity. The measurement across the growing season of genotypic variation in components of N metabolism would constitute a physiological/biochemical selection program to be incorporated with the determination of harvestable end product. A properly designed physiological/biochemical selection program would integrate the effects of plant genotype, environment, and their interactions allowing identification of the factors limiting productivity of particular genotypes, and would also estimate end product. Our review of literature pertinent to whole plant N metabolism suggests that such a selection program initially include NO_3^- uptake, N_2 fixation, N accumulation, N remobilization, seed protein synthesis, and Nitrogen Harvest Index.

Key words: Genetics – Nitrogen – Grain crops – Selection – Plant breeding

1 Introduction

Seed production by a grain crop is the result of a series of genetically controlled metabolic events. The challenge facing plant scientists is to understand the mechanisms of these metabolic events and to discover how they interrelate and interact with diverse environments. Such information should aid plant geneticists to manipulate crops predictably for the creation of genotypes with increased yield potential and enhanced nutritional quality of the grain.

Nitrogen (N) is essential for plant growth and the production of plant protein. Similarly, N in the form of protein is important to human nutrition (FAO/WHO 1973). Altschul (1976) described the current debate among human nutritionists regarding the presence and

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degree of protein deficiency in the diets of peoples in some parts of the less developed world. It is unclear whether these human nutritional problems can be resolved with more food or by encouraging dietary shifts toward foods with improved protein quality. In addition to these problems, it is essential to improve crop productivity in order to be able to provide food to satisfy the need of an expanding world population. A variety of elements of plant N metabolism significantly affect the productivity of crops, suggesting that the quantity and quality of grain production may be influenced by manipulating these elements.

Modification of growth and reproductive processes to control plant N metabolism and stimulate enhanced grain crop productivity can be accomplished with two general approaches. The growth environment may be altered or the plant genotype may be improved to achieve higher grain productivity. The approach of the plant geneticist is to modify the genotype through plant breeding consequently changing crop response to the environment. It has been clearly established that many components of plant N metabolism depend upon the supply of energy or reductant from photosynthesis (McKee 1962; Hageman and Flesher 1960; Hardy et al. 1976). Genetic modification of plant photosynthesis may affect plant N metabolism. Therefore, an examination of processes of N metabolism in plants may be incomplete without simultaneously considering photosynthesis. However, possible effects of components of photosynthesis on plant productivity are outside the scope of this review. Our aim will be to consider genetic approaches having the potential to enhance the efficiency of plant N metabolism thereby increasing the quality and quantity of grain production.

2 A comparison of nitrogen flux in some major grain crops

A comparison of the seasonal N flux in three major U.S. grain crops, soybean [*Glycine max* (L.) Merrill], wheat [*Triticum aestivum* (L.) em. Thell], and maize (*Zea mays* L.) gives an estimate of the extent of genetic variation present among these species. We have summarized data from the few reported field studies with sufficient information to determine seasonal N accumulation profiles in soybean (Fig. 1), wheat (Fig. 2), and maize (Fig. 3). The profiles for each crop are generally similar although the summarized data were collected over a range of geographic areas, soil types, fertility and moisture conditions, and crop cultivars.

The lag phase of N accumulation in maize appears to be relatively short (Fig. 4). The long lag phase in wheat is to be expected as this crop is planted early in spring, and as is the case with winter wheat, plant growth initiates under cool conditions. Soybean usually encounters early season temperatures similar to maize. Therefore, the longer lag phase for soybean is probably not induced environmentally, but represents a physiological difference between the two species. Early season carbon assimilation in maize may be enhanced by C_4 photosynthesis resulting in greater energy availability and N accumulation than in soybean with C_3 photo-

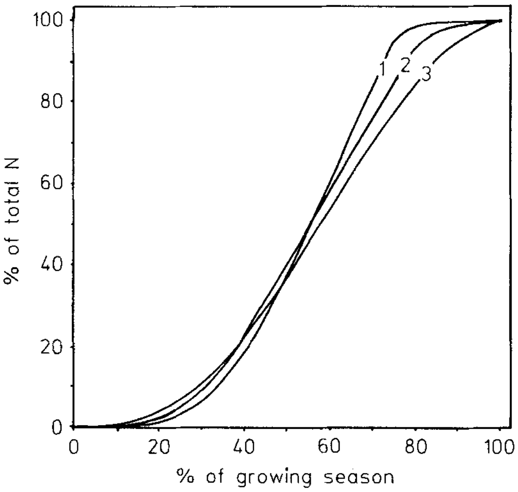

Fig. 1. Estimated seasonal nitrogen accumulation in field-grown soybean. *1* Calculated from Hanway and Weber (1971 a, b) using the variety 'Hawkeye' grown in 1961 and 1963; *2* data from Hammond et al. (1951) using 'Richland' soybean grown on Webster soil; and *3* data from Borst and Thatcher (1931) using 'Manchu' soybean grown in 1925

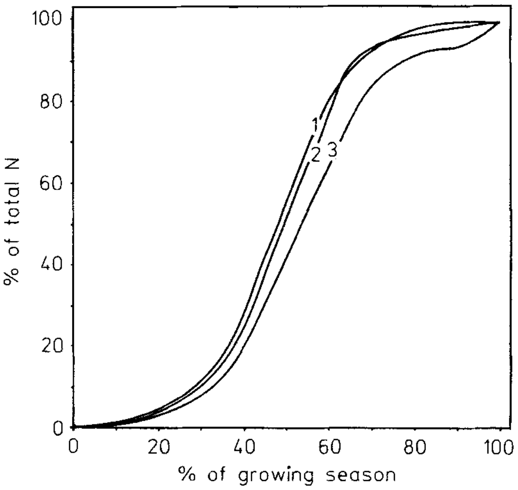

Fig. 2. Estimated seasonal nitrogen accumulation in field-grown wheat. *1* Data from Knowles and Watkin (1931) including spring growth only (estimated to have begun April 1); *2* data from Lal and Sharma (1973) based upon the two-year mean of the variety 'Kalyan Sona'; and *3* data from McNeal et al. (1966)

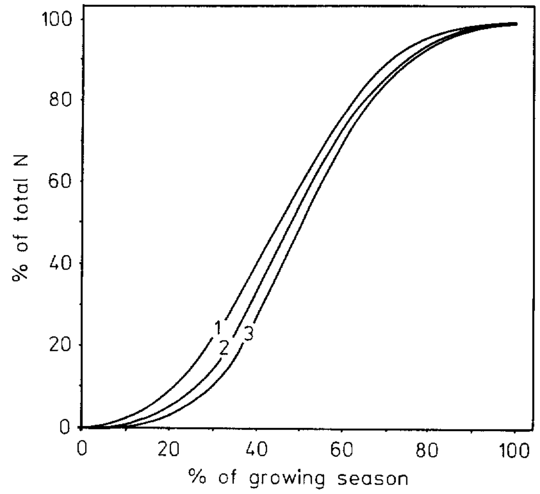

Fig. 3. Estimated seasonal nitrogen accumulation in field-grown maize. *1* Data from Sayre (1948); *2* data from Hanway (1962a, b) based upon treatment (Plot 1004) in which no apparent nutrient deficiencies existed; and *3* data from Hay et al. (1953)

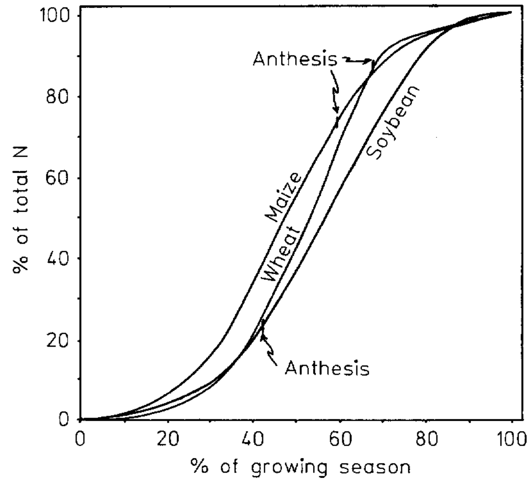

Fig. 4. Estimates of mean seasonal nitrogen accumulation of field-grown soybean, wheat, and maize, derived from Figs. 1–3, respectively

synthesis. Alternatively, early seasonal N accumulation in soybean via nitrate uptake and reduction may function less efficiently than in maize.

After the initial lag phase, the relative rate of N accumulation by wheat is higher than in either maize or soybean, suggesting that a large proportion of the total seasonal N accumulation occurs in a relatively shorter time in wheat than in maize or soybean. The relative rates of N accumulation by maize and soybean after the initial lag phase are approximately equal, indicating that the proportion of total seasonal N accumulated by the two crops during this part of their growth cycles is similar.

Of the three crops, the soybean is unique in that N is derived from both N_2 fixation and combined N from the soil. Each contributes N for plant development with N_2 fixation predominating later in the growing season (Harper and Hageman 1972; Thibodeau and Jaworski 1975). The linear phase of N accumulation in soybean terminates after about 78–80% of the season has elapsed, compared to 65 and 70% in maize and wheat, respectively (Fig. 4). The ability of soybean to utilize atmospheric N_2 may cause N to accumulate until later in the growing season than is possible in either wheat or maize. Studies of seasonal N_2 fixation in field-grown soybeans suggest that maximal rates occur after about 75% of the season has elapsed (Fig. 5). The end of the linear phase of N accumulation (Fig. 1) would correspond with, or closely follow the peak of N_2 fixation (Fig. 5).

Another major difference between the three crops is the time of anthesis (Fig. 4). The soybean varieties used in these studies were generally indeterminate genotypes similar to the varieties grown in the northern United States. Anthesis in these varieties occurs when approximately 25% of the total season N has accumulated in the crop and when vegetative growth is less than half complete (Egli and Leggett 1973; Hanway and Weber 1971a, b). This suggests that reproductive and vegetative growth compete for nutrients during more than half of the life cycle of soybean. Anthesis in wheat and maize occurs when about 85% and 70% of the total season N has accumulated in these two crops, respectively. The occurrence of anthesis in maize and wheat after the major portion of crop N has accumulated in-

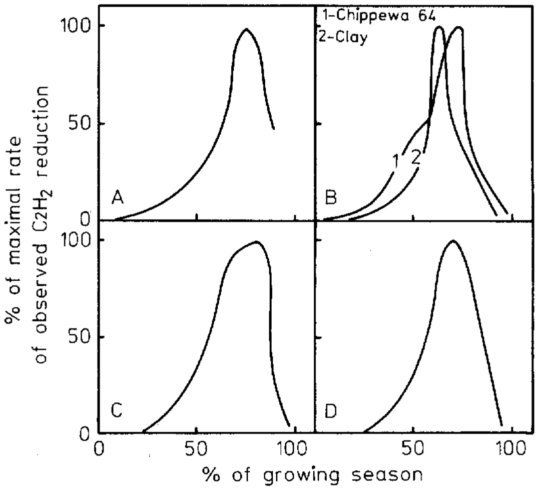

Fig. 5. Seasonal N_2 fixation measured with acetylene reduction in field-grown soybeans. The figure was derived from data reported by A – Harper and Hageman (1972); B – Lawn and Brun (1974); C – Hardy et al. (1971); D – Thibodeau and Jaworski (1975)

Table 1. Estimates of nitrogen harvest index and nitrogen remobilization efficiency in field-grown soybean, wheat, and maize

Crop and reference	Nitrogen harvest index (%)	Nitrogen remobilization efficiency[a] (%)
Soybean		
Borst and Thatcher (1931)[b]	66	47
Hammond et al. (1951)[c]	80	–
Hanway and Weber (1971 b)[d]	66	54[e]
Jeppson et al. (1978)[f]	74	–
Mean	72	51
Wheat		
Austin et al. (1977)	68	–
Austin et al. (1980)[g]	75	–
Boatwright and Haas (1961)	68	–
Desai and Bhatia (1978)[h]	76	–
Dubois and Fossati (1981)	74	–
Knowles and Watkin (1931)	74	72
Lal and Sharma (1973)	–	78[i]
Lal et al. (1978)	–	57
McNeal et al. (1966)[j]	64	59
McNeal et al. (1968)[k]	71	62
Mean	71	66
Maize		
Hanway (1962 a, b)[l]	62	42
Hay et al. (1953)[m]	68	53[n]
Jones and Huston (1914)	65	41
Sayre (1948, 1955)	55	39
Mean	63	44

[a] Nitrogen remobilization efficiency =

$$\frac{\text{Maximum vegetative N} - \text{Final vegetative N}}{\text{Maximum vegetative N}} \times 100\%$$

[b] Data from 'Manchu' soybeans grown in 1925

[c] Includes only 'Richland' soybean grown on Webster soil

[d] Mean of seven soybean genotypes grown in 1963

[e] Calculated from Hanway and Weber (1971 b), Fig. 1

[f] From Jeppson et al. (1978), 1976 sampling experiment

[g] Mean of 12 genotypes at two locations

[h] Mean of 15 durum wheat (*Triticum durum* Desf.) genotypes

[i] Mean of two varieties for two years

[j] Mean of five spring wheat varieties under dryland conditions

[k] Mean of seven varieties under dryland and irrigated conditions

[l] Estimated from Plot 1004 (no apparent nutrient deficiencies)

[m] Mean of two hybrids

[n] Calculated from Hay et al. (1953) Table 2

dicates that competition for reduced N between vegetative and reproductive growth in these crops is less than in soybean.

Nitrogen Harvest Index (NHI) is the proportion of the total N yield contained in the grain at maturity (Canvin 1976; Austin et al. 1977) and is of relevance to the productivity of grain crops. The NHI of wheat and soybean is higher than maize (Table 1). Wheat accumulated 85% and soybean 25% of total season N before anthesis (Fig. 4), although the NHI of the two crops is similar (Table 1). Therefore, relative to soybean, a larger proportion of the N accumulated in wheat is remobilized and translocated to the developing grain. We suggest that the measure of the ability of a crop to remove N from the vegetative tissue (McNeal et al. 1966) be termed Nitrogen Remobilization Efficiency (NRE). The NRE of wheat is higher than that of both maize and soybean (Table 1), which further indicates that wheat remobilizes an exceptionally large proportion of vegetative N. In soybean, the NRE is lower and the NHI is higher than in wheat, suggesting that a large proportion of the assimilated N is used by the developing soybean seed immediately, rather than first being incorporated into leaf or stem proteins as in wheat. The incorporation of N into leaf and stem proteins and its subsequent remobilization and translocation to the developing grain may be an inefficient process and therefore consideration should be given to the possible development of genotypes in which a greater proportion of N is used directly by the developing seed.

While it must be recognized that the environment influences N accumulation (McNeal et al. 1968; Deckard et al. 1973; Lal et al. 1978), NHI (Spratt and Gasser 1970), and NRE (Beauchamp et al. 1976), the evidence indicates that seasonal N flux in soybean, wheat, and maize is dissimilar. Interspecific comparisons suggest possible approaches to improve the N economy of crop plants, but intraspecific genetic variability is the prerequisite for genetic improvement with present technology. An outstanding example of such intraspecific genetic variability is the classical selection experiments begun in 1896 at the Illinois Agricultural Experiment Station which involve continuous selection for high and low grain protein (Fig. 6)

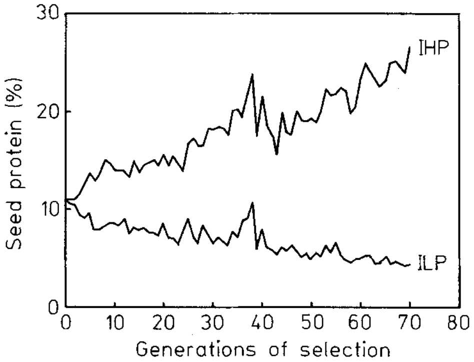

Fig. 6. Mean protein content of Illinois High Protein (IHP) and Illinois Low Protein (ILP) maize after 70 generations of selection. Data are from Dudley et al. (1974)

in maize from one original foundation stock (Hoener and DeTurk 1938; Winter 1929; Woodworth et al. 1952). The continuous response to selection and the presence of residual genetic variability after many cycles of selection (Dudley and Lambert 1969; Leng 1962) demonstrate the involvement of a large number of genes in the control of grain protein concentration. A number of genetically controlled steps of N metabolism were probably modified during these classical selection experiments suggesting that genetic improvements of individual components of N metabolism may be a useful addition to selection based upon end product.

3 Genetic control of nitrogen metabolism

The genetic improvement of the quality and quantity of seed protein in many grain crops has received much attention. This effort has focused on seed composition but less consideration has been given to the genetics of metabolic events which antecede seed formation. Studies of the metabolic events preceding seed formation usually have examined only one aspect of N metabolism. The limited amount of literature and the necessarily narrow scope of most reports make it difficult to identify the genetically controlled factors that limit N metabolism in a germplasm pool. Nevertheless, elements of N metabolism with the potential to affect productivity and, therefore, suitable for genetic improvement may include: nitrate (NO_3^-) uptake, N translocation, NO_3^- reduction, N_2 fixation, phloem loading, N remobilization, phloem unloading, and protein synthesis in the developing grain. Evidence for genetic variability in NO_3^- uptake, NO_3^- reduction, N_2 fixation, N remobilization, and protein synthesis in the developing grain has been reported.

3.1 Nitrate uptake

Nitrate is the principal source of N utilized by nonleguminous crops grown in well-aerated soils (Meeker et al. 1974; Johnson et al. 1976; Mengel and Kirkby 1978). Goodman (1979) indicated that the root uptake of soil N was a function of the size and "activity" of the root. Differences in the concentration at which root NO_3^- absorption is half the maximum rate (K_m) indicate the presence of interspecific genetic variation for root NO_3^- absorption (Table 2) in barley (*Hordeum vulgare* L.), perennial ryegrass (*Lolium perenne* L.), and maize. The relatively high K_m observed in barley is evidence that root affinity for NO_3^- in barley is lower than in maize or perennial ryegrass. According to Rao and Rains (1976) the low affinity for NO_3^- in barley might be due to intensive breeding for high response to fertilizer N with resultant low efficiency for NO_3^- uptake.

Table 2. Reported concentration values at half maximum NO_3^- uptake (Km) from nutrient solution by three crops

Crop and reference	Temperature (C °)	pH	Km (μM)
Barley			
Rao and Rains (1976)	30	6.0	110
Perennial ryegrass			
Lycklama (1963)	25	6.2	33
Maize			
van den Honert and Hooymans (1955)	25	6.0	21

Intraspecific genetic differences in removal of NO_3^- from solution were observed in wheat by Rao et al. (1977) in short-term experiments (4 or 6 h), but differences were not apparent in long-term experiments. Genetic differences in root NO_3^- uptake were observed within a group of four maize inbreds, within a set of six F_1 hybrids derived from the inbreds, and between the inbreds and hybrids (Chavalier and Schrader 1977). The rate of NO_3^- absorption, although related to root dry weight, was not constant, suggesting that physiological activity and root affinity for NO_3^- exert some control of NO_3^- absorption in maize roots. Furthermore, the evidence indicates that these factors are under genetic control and subject to modification.

3.2 Nitrate reductase

Nitrate reductase is the first enzyme associated with the assimilation of NO_3^- and its activity (NRA) in nonlegumes is considered by many to be the rate-limiting step in N assimilation (Hageman et al. 1967; Beevers and Hageman 1969). It has been suggested that NO_3^- availability is the limiting factor in N assimilation because plant growth is closely related to soil NO_3^- availability and NRA is induced by the presence of NO_3^-. However, Hageman et al. (1976), indicated that measurements of NRA were more useful than tissue NO_3^- concentration to index grain production and that genotypic differences in NRA levels are independent of tissue NO_3^- concentration. This evidence has led to the suggestion that NRA would be useful as a measurement to select plant genotypes with enhanced NO_3^- assimilation properties.

A number of reports indicate the presence of genetic variation in grain crops for NRA (Table 3). Further, the presence of heritable variation in NRA (Duffield et al. 1972; Eck et al. 1975) suggests that genotypes with enhanced ability to reduce NO_3^- should be obtainable with appropriate breeding methods.

Table 3. Research reports of genetic variation in nitrate reductase activity (NRA) in crop plants

Crop and reference	Summary of results
Sorghum	
Eck et al. (1975)	Significant additive and non-additive genetic variation. Broad-sense heritability = 0.9, narrow-sense = 0.41
Mishra et al. (1981)	NRA differences among parents and hybrids at three growth stages
Wheat	
Croy and Hageman (1970)	Little variation in mean seasonal NRA among 32 winter wheats
Dalling et al. (1975)	Differences in average seasonal NRA
Dalling and Lyon (1977)	More than three-fold differences in seedling NRA
Deckard et al. (1982)	Identified chromosome arms upon which genes controlling NRA are located
Maize	
Hageman et al. (1961)	Significant difference in the NRA of two hybrids
Hageman et al. (1963)	Up to three-fold differences in NRA of inbreds
Schrader et al. (1966)	NRA differences among inbreds and hybrids
Warner et al. (1969)	Two inbreds of a hybrid differed by two loci controlling nitrate reductase synthesis and decay

Theoretically such genotypes would possess improved properties of NO_3^- assimilation and attain higher levels of N accumulation and/or grain and grain protein yield. Deckard and Busch (1978) attempted the use of NRA measurements to predict the grain or grain protein yield of wheat lines. They reported that mid-parental NRA of the second leaf in growth chamber grown seedlings correlated with the mean yield of 21 F_6 lines from each of 25 crosses. However, the NRA measurements did not distinguish the highest from the lowest yielding line within each cross, nor were they related to total plant N accumulation. Deckard and Busch (1978) concluded that the genotypes tested had differing ratios of NRA estimated N input to total N accumulation and/or unequal Nitrogen Harvest Indices which may have been partially responsible for the failure of NRA to identify lines with high yield or grain protein.

Genotypic differences in the ratio of NRA estimated N input to total N accumulation have been reported in other crop species. In the above-ground tissue of six maize genotypes this ratio varied from 2.2 to 4.1 (Deckard et al. 1973) and varied from 1.07 to 1.82 among 11 grain sorghum hybrids (Eck et al. 1975). Dalling et al. (1975) reported regressions of N content on total plant NRA across the season and observed differences between the regression coefficients of seven wheat varieties. The regression coefficients of the wheat varieties fell into two distinct classes, one with a high ratio of total N accumulation to NRA estimated N input and the other with a low ratio. These reports of genotypic differences of NRA estimated N input to total N accumulation suggest a potential difficulty in the use of NRA as a criterion for selecting plant genotypes with high N assimilatory properties. Despite reports of genetic variation for NRA in a number of crop species, we conclude that there is little justification for the use of NRA to identify plant genotypes with properties of enhanced N accumulation or improved seed or seed protein yield. Our conclusion is supported by reports in which NRA does not relate to total N accumulation (Deckard and Busch 1978; Eck et al. 1975); the reported close relationship between leaf NO_3^- content and NRA measured on an individual leaf rather than a canopy basis (Meeker et al. 1974) suggesting environmental rather than genetic control of NRA; and the reported genotypic differences in the ratio of NRA estimated N input to total N accumulation.

3.3 Nitrogen fixation

3.3.1 Legumes

Apart from assimilating combined inorganic N from the soil, legumes have the ability to derive their N requirements from atmospheric N_2 by the active participation of *Rhizobium* in the root nodules. Genetic enhancement of N_2 fixation could conceivably result from alteration of the legume host and the *Rhizobium* microsymbiont and/or the establishment of particularly desirable host-microsymbiont combinations.

Host plant genetic variability. Vincent (1980) has suggested a number of characteristics of the legume host which may affect the symbiosis. The photosynthetic capability, the influence upon hydrogen evolution, and the transport and assimilation of fixed N are characters of the host genotypes which may vary the "physiological environment" provided for N_2 fixation. Furthermore, the host legume is a major factor in determining nodule persistence and thus the duration of N_2 fixation (Vincent 1980). Genetic variability in these traits may be exploited to enhance N_2 fixation and/or plant productivity by developing genotypes characterized by prolonged duration of N_2 fixation, higher rates of N_2 fixation, and reduced energy requirement per unit N_2 fixed.

Genotypic differences in the nodulation and N_2 fixation associated with the host plant have been reported in a number of grain legumes including chick pea (*Cicer arietinum* L.), soybean, *Phaseolus vulgaris* L., pea (*Pisum sativum* L.), *Vicia faba* L., and cowpea [*Vigna unguiculata* (L.) Walp] (Table 4). Qualitative genetic factors may have been responsible for a proportion of the observed variability. For example, in soybean no nodule formation occurs with specific *R. japonicum* strains when the Rj_2 (Caldwell 1966) and Rj_4 (Vest and Caldwell 1972) alleles are present in the host. Similarly, Lie et al. (1976) observed little or no nodule formation with particular host-*Rhizobium* strain combinations in pea. Ineffective symbiosis (nodulation without N_2 fixation) was described with all but one cultivated soybean genotype inoculated with fast-growing strains of *R. japonicum* (Keyser et al. 1982). We suggest that these examples of host genetic variability are very specific in nature and are distinct from continuous variability resulting from differences in the "physiological environment" that the host provides for N_2 fixation. Reported measurements of N_2 fixation using plants nodulated with a mixture of rhizobial genotypes or with a variable indigenous population (Wacek and Brill 1976; Westerman and Kolar 1978; Zary et al. 1978; Rai and Singh 1979; Cregan et al. 1980) rather than with one specific strain support the suggestion of continuous genetic variation in the host for N_2 fixation. Host genetic variation for N_2 fixation in grain legumes is yet to be exploited to develop germplasm with enhanced N_2 fixation potential.

Table 4. Research reports of genetic variation in nitrogen fixation and related traits in grain legumes

Crop and reference	Summary of results
Chick pea	
Rai and Singh (1979)	Genetic variation in nodule mass and nodule leghemoglobin
Soybean	
Cregan et al. (1980)	Consistently high nodulation and acetylene reduction activity in some genotypes
Wacek and Brill (1976)	Varietal differences in acetylene reduction activity of young plants
Phaseolus vulgaris	
Graham and Rosas (1977)	Genotypic differences in acetylene reduction activity and activity/unit nodule weight
Westerman and Kolar (1978)	Genotypic differences in seasonal acetylene reduction activity
Pea	
Lie et al (1976)	Wide genotypic differences in nodule number and nodule mass
Vicia faba	
El-Sherbeeny et al. (1977b)	Variation in total N yield of lines grown in the absence of reduced N
Cowpea	
Zary et al. (1978)	Greater than three-fold differences in acetylene reduction activity among varieties tested

Symbiotic variability in the Rhizobium microsymbiont. Abel and Erdman (1964) reported significant *Rhizobium* strain effects upon seed yield, seed protein and oil concentration, as well as nodule weight per plant in field-grown 'Lee' soybean. In similar field studies, soybeans nodulated with particular strains of *R. japonicum* produced significantly higher seed yields than soybeans inoculated with other strains (Caldwell and Vest 1970). Differences in nitrogenase activity have been observed in greenhouse and field-grown peanut (*Arachis hypogaea* L.) nodulated with a range of strains of *Rhizobium* (Elkan et al. 1980; Wynne et al. 1980). Large differences in the N_2 fixation of a *V. faba* genotype were reported with 20 randomly selected field isolates of *R. leguminosarum* derived from the indigenous soil population (El-Sherbeeny et al. 1977a). The authors observed that effectiveness of the 20 isolates followed a normal distribution and that inoculation with the best strain produced over eight times as much plant N than the poorest. Evidently the genotype of the *Rhizobium* is an important factor controlling quantitative differences in N_2 fixation.

The presence or absence of hydrogenase in nodules for the recapture of electrons lost to protons through the operation of nitrogenase may partly explain genetic differences among strains of *Rhizobium*. Strains of *Rhizobium* with hydrogen uptake (Hup^+) and without this capability (Hup^-) have been identified. Greenhouse-grown soybean plants inoculated with Hup^+ *R. japonicum* accumulated greater total N than plants inoculated with Hup^- strains (Albrecht et al. 1979), and field-grown soybeans inoculated with Hup^+ *R. japonicum* produced seed with greater N concentration than plants inoculated with Hup^- strains (Hanus et al. 1981). These results may be explained by the suggestion that Hup^+ strains are energetically more efficient in the fixation of N (Shubert and Evans 1976). The potential for a greater magnitude or efficiency of N_2 fixation may be realized following nodulation by strains of *Rhizobium* which have been characterized to be particularly effective.

Legume host × Rhizobium interactions. To ascertain the relative importance of variation associated with the legume host, strain of *Rhizobium*, and host × strain interaction, Mytton et al. (1977) inoculated each of six *V. faba* varieties individually with six strains of *R. leguminosarum*. The *V. faba* host, *Rhizobium*, and host ×

strain variance components accounted for 8.9, 11.8, and 73.8% of the total variation in plant dry weight respectively. The larger variation associated with the host × strain interaction indicates the potential to increase productivity through the use of optimal host-strain combinations in agriculture. In a similar study significant host × strain interactions for grain yield and nodule mass were detected in field-grown chick pea (Rai and Singh 1979). Peanut host × strain interactions for N_2-ase activity and total N were reported in a greenhouse experiment (Wynne et al. 1980), but similar interactions were not observed in a field study (Elkan et al. 1980). In a field-experiment, Vest and Caldwell (1970) also reported no soybean host × strain interactions but did detect yield differences attributable to both soybean host and strain of *Rhizobium.* These reports indicate that host × strain interactions, although frequently detected, are not observed in all cases. Interactions may be less prevalent or more difficult to detect in the field than in the greenhouse where the population of *Rhizobium* and available soil N can be carefully controlled. This conclusion is especially supported by the dissimilar results between field and greenhouse studies with peanuts in North Carolina (Elkan et al. 1980; Wynne et al. 1980).

Nodulation by specific strains of Rhizobium. Identification of legume genotypes, strains of *Rhizobium,* or host-strain combinations with the potential to maximize N_2 fixation and plant growth offer the promise of enhanced productivity. Genotypes with an improved capability to support N_2 fixation may be used commercially. However, the commercial use of superior strains or of efficacious host-strain combinations is more difficult in soils where a population of *Rhizobium* is present (Ham 1976; Ham 1980). In these soils, the frequency of root nodules formed by an introduced strain inoculated at normal commercial rates would range from 5–20% (Johnson et al. 1965; Caldwell and Vest 1970; Kuykendall and Weber 1978). Therefore, the challenge is to establish a selected *Rhizobium* in the soil followed by high rates of nodulation.

Brockwell (1980) listed 10 criteria other than symbiotic competence useful in the selection of a superior strain of *Rhizobium.* Included were competitive ability with other strains for nodule sites, persistence in the soil, and ability to migrate and colonize soil away from host roots. Selected *Rhizobium* with these characteristics would allow for future increases in the proportion of nodules occupied by the introduced strains.

Two other approaches to achieve nodulation by an introduced strain of *Rhizobium* have been proposed and studied in soybeans. Devine and Weber (1977) suggested the use of a soybean which would exclude nodulation by indigenous *Rhizobia* but would nodulate with an introduced strain of exceptionally high N_2 fixation efficiency. The non-nodulating soybean genotype (rj_1rj_1) (Williams and Lynch 1954) meets the first requirement of effectively excluding nodulation by most strains of *Rhizobium.* However, strains of *Rhizobium* with high N_2 fixation efficiency able to nodulate the rj_1rj_1 soybean genotype have not been obtained as yet. Kvien et al. (1981) proposed that soybean genotypes be screened to find lines which would form a large proportion of nodules with an introduced efficient strain rather than with the predominant indigenous strains. A selected soybean host would thus function to preferentially accept nodulation by superior genotypes of *Rhizobium.* Both proposals seem promising genetic approaches to enhance N_2 fixation in soybeans and possibly other legume species.

3.3.2 Associative nitrogen fixation in grasses

Although grasses mainly assimilate inorganic N for growth they may stimulate N_2 fixation by bacteria associated with their roots (Greenland 1977; van Berkum and Bohlool 1980). The aim of identifying associative N_2 fixation by heterotrophic bacteria in grasses is ultimately to use this characteristic for the production of crops. Many fundamental aspects of associative N_2 fixation in grasses need to be established before it will be possible to predict whether this process can be genetically manipulated using plant breeding techniques including genetic engineering approaches. Atlhough associative N_2 fixation research has suffered from highly optimistic inferences, more recent research offers some hope that grain grasses may one day rely in part upon N derived from N_2 fixation (van Berkum et al. 1982). Many of the programs endeavoring to demonstrate genetic variability for N_2 fixation in grasses have depended upon existing techniques for the measurement of N_2 fixation. The lack of rapid, simple, and meaningful methods to quantify associative N_2 fixation in field grown grain grasses has hindered any real progress in these programs (van Berkum and Bohlool 1980).

3.4 Nitrogen accumulation

There is evidence that high N accumulation is an important contributor to high grain and grain protein yield. Desai and Bhatia (1978) observed that of 15 durum wheats (*Triticum durum* Desf.) the genotype which accumulated the most N (g/m^2) produced the highest grain protein yield and near maximal grain yield. Also, they reported a correlation of $r = 0.81$ ($P \leqq 0.01$) between plant N accumulation and grain yield. Higher N accumulation and grain yield were observed in more recently developed short statured cultivars and experimental wheat lines than in older cultivars in environments with both high and low N fertility (Austin et al. 1980). Pollmer et al. (1979) suggested that high grain protein yield in maize would result from more 'intensive' N uptake and prolonged N accumulation. Therefore, N accumulation and plant productivity appear to be closely associated. Furthermore, N accumulation integrates the effects of difficult to quantify plant metabolic activities and the measurement of total N may be accomplished with established techniques.

Genetic differences in total above-ground N accumulation have been reported in a number of crop species (Table 5). The genotypes used in these studies were selected to maximize differences in N metabolism and often represented extremes in seed protein concentration, geographic origin, or agronomic adaption. In contrast, Dubois and Fossati (1981) used 12 adapted winter wheats which they selected because of high yield potential rather than anticipated differences in N metabolism. Differences in grain yield and grain protein concentration, but no significant differences in measured N accumulation were observed. This study suggests that genetic variabiliy for N accumulation is limited in agronomically adapted high yielding germplasm. However, genotypic variation in N accumulation may be larger when selection is applied for both enhanced N metabolism and superior agronomic performance. For example, a number of maize hybrids produced from inbreds selected for yield, other agronomic traits, and high grain protein concentration produced grain yields equal to or exceeding standard hybrids and accumulated more above-ground N and produced more seed protein per unit area than standard hybrids (Pollmer et al. 1979). The study by Pollmer et al. (1979) indicates that genetic variation for N accumulation exists even in adapted high yielding germplasm.

Table 5. Research reports of genetic variation in above-ground nitrogen accumulation in grain crops

Crop and reference	Results
Soybean	
Jeppson et al. (1978)	Differences in N accumulation of non-nodulating soybean genotypes
Barley	
Kirby (1968)	Differences in total N accumulation among five barley cultivars
Grain sorghum	
Eck et al. (1975)	Significant differences among the N accumulation of 11 sorghum varieties
Wheat	
Austin et al. (1977)	Greater than three-fold difference in N accumulation among wheats of diverse geographic origin and taxonomic classification
Desai and Bhati (1978)	More than two-fold differences among genotypes in total plant N accumulation
McNeal et al. (1966)	One wheat accumulated greater N than did four others tested
McNeal et al. (1968)	Genetic differences in N accumulation in an irrigated experiment but not in a similar non-irrigated trial
Maize	
Horner and DeTurk (1938)	Illinois High Protein maize accumulated more N from a high NO_3^- concentration hydroponic culture than did Illinois Low Protein maize

Reports by Johnson et al. (1967) and McNeal et al. (1972) have indicated that selection for high seed protein concentration is usually effective. However, according to the data presented by Johnson et al. (1967) grain protein yield in these selections was no better than standard cultivars. Also grain protein yield in selections based upon high seed protein concentration may be inferior to that of genotypes selected for low seed protein concentration (McNeal et al. 1972). In this instance, low seed protein yield in the selected genotypes may have resulted from poor N accumulation, and/or inferior N remobilization. It is possible that increased seed protein yield may be obtained more readily by selecting for higher N accumulation rather than higher grain N concentration. However, selection for higher N accumulation must be accomplished without reducing Nitrogen Harvest Index (NHI). We suggest that increased grain N yields should be obtained by selecting for higher N accumulation while maintaining NHI because it appears that N uptake is independent of N remobilization and translocation to the developing grain (McNeal et al. 1966; Johnson et al. 1967; Desai and Bhatia 1978).

3.5 Nitrogen remobilization

Apparently Adorjan (1902) was the first to report the translocation of N from vegetative tissue to the developing grain. Nitrogen redistribution or remobilization has been reviewed (Williams 1955; Ohlrogee 1960) and Seth et al. (1960) suggested the presence of genetic variation for Nitrogen Remobilization Efficiency (NRE). It was reported that of four wheat varieties those with high seed protein percentage either absorbed more N from the soil or translocated a greater proportion of vegetative N to the kernel (Seth et al. 1960). Higher than normal grain protein concentration in wheat lines derived from crosses with 'Atlas 66' was reportedly due to more complete translocation of N from the foliage to the grain (Johnson et al. 1968).

Peterson et al. (1975) observed that two oat (*Avena sativa* L.) genotypes with high groat protein concentration remobilized a greater amount of vegetative N to the developing panicle than did four lines with lower groat protein. Data reported by Pollmer et al. (1979) suggest good general combining ability for NRE in several maize inbreds selected for high grain protein concentration and yield. Significant differences for NRE were not detected among a group of 11 nodulating and non-nodulating soybean genotypes (Jeppson et al. 1978). However, sets of four lines previously classified as efficient or inefficient based upon NHI, as a group mobilized 60% and 51% of accumulated N from leaflets, respectively, and 53% and 43% of petiole N, respectively. Stem NRE was 80% for the efficient and 73% for the inefficient genotypes. In wheat, the mean NRE of five varieties ranged from 49.5% for the spike chaff to 82.7% for the lower leaves (Lal et al. 1978). Varietal differences in the NRE of the culm, flag leaf, lower leaves and spike chaff were also observed among the five varieties.

Although the presence of genetic variability for N remobilization has been established in a number of crops, its exploitation for plant improvement has yet to be reported. The nature of genetic control or magnitude of the heritability of N remobilization is not known. In part this may be due to difficulty in the measurement of N remobilization, which requires N determinations at a number of times during the plant growth cycle to estimate maximum and final vegetative N. The development of a rapid biochemical technique to estimate N remobilization would facilitate the use of NRE in selection programs. Proteases are enzymes responsible for the degradation of polypeptides into amino acids and high protease activity may be associated with plant genotypes with a high potential for N remobilization from the vegetative tissue to the grain. The relationship between protease activity and N remobilization is indicated by the reported higher post anthesis protease levels in the leaf blades of a high grain protein wheat as compared to a lower grain protein wheat (Rao and Croy 1972). Similarly, in rice (*Oryza sativa* L.) the high grain protein variety 'IR480' was reported to have almost twice the leaf protease activity of the lower seed protein variety 'IR8' (Perez et al. 1973). Estimates of N remobilization from the measurement of protease activity assume that enzyme activity is the rate limiting step of N movement from the vegetative tissue. A high correlation between observed N loss from the vegetative tissue and the rate of protease activity has been reported in two wheat varieties (Dalling et al. 1976). Therefore, a close relationship between protease activity and the rate of N loss from the vegetative tissue is indicated. The work of Dalling et al. (1976) is especially useful because it shows that a quantitative relationship between protease activity and N movement from leaf tissue exists.

Cagampang et al. (1971) demonstrated genetic variability for N remobilization in seven pairs of rice lines each pair consisting of a high and low seed protein genotype. They measured the amino-N concentration in the bleeding sap collected from the internode below the panicle at ten days after anthesis. The sap collected over a 12-h period from the seven low and high protein lines contained means of 65 μg and 90 μg amino N/10 tillers, respectively. Therefore, remobilization potential may be estimated from the measurement of amino-N concentration of sap.

Increased productivity of protein per unit land area requires enhanced translocation of vegetative N to the seed thereby leaving less N in the unharvested crop residue. Although the genetic control of N remobilization is poorly defined, available evidence suggests that genetic improvement is possible. Protease activity measurements in the vegetative tissue or estimates of amino N or total N movement to the developing grain could be useful to identify genotypes having enhanced remobilization potential.

3.6 Protein synthesis in the developing grain

Remobilization of vegetative N provides the developing grain with a source of N for the synthesis of seed storage proteins. The affinity of the developing seed for N could be an important factor to achieve maximum N movement from vegetation to seed, but few research reports have dealt with genetic variation in the affinity of the developing grain for translocated nitrogenous substances. Differences in the seed protein concentration of wheat varieties have been observed to be related to the rate of protein synthesis in the developing grain (Seth et al. 1960). However, it is unclear whether the observed differences in the rate of kernel protein synthesis were due to amino acid supply or actually reflected the ability of the grain to synthesize protein. Brunori et al. (1980) reported wide variation in rate and duration of N deposition in wheat kernels and suggested the possibility of using these characteristics as criteria in the selection of genotypes with the potential for high seed protein accumulation.

A detailed study of differences in the biochemistry of the developing rice kernel showed that rapid protein accumulation occurred in the seed from 4 to 16 days after flowering with no further increase after 16 days (Cruz et al. 1970). Of three varieties, levels of soluble amino N, protein, and RNA were higher in the variety with high seed protein. Cruz et al. (1970) compared high and low seed protein segregants from crosses of low × high seed protein varieties and found that free amino acid content in the developing kernel and the rate of amino acid incorporation were the two factors which consistently distinguished high from low protein varieties. Yet, it was suggested that free amino acids in the developing kernel do not induce storage protein synthesis. Therefore, enhanced grain protein production will not result merely from increased availability of amino acids to the developing grain but also depends upon separate genetic improvement in the activity of enzymes associated with storage protein synthesis.

3.7 Nitrogen harvest index

Nitrogen Harvest Index (NHI), similar to N accumulation, integrates the effects of a number of genetically controlled metabolic activities and their interaction with the environment. The NHI may be useful to measure the N economy of a grain crop because the genetics of many components of N metabolism may be reflected by NHI. Nitrogen Harvest Index has been the subject of several research reports due to the relative ease with which it is determined. However, to our knowledge no investigation has comprehensively evaluated NHI as a criterion for plant selection, although, the presence of inter- and intraspecific genetic variation in NHI has been indicated (Table 6).

Table 6. Research reports of genetic variation in nitrogen harvest index (NHI)

Crop and reference	Results
Soybean	
Hanway and Weber (1971 b)	NHI varied from 61.8 to 69.1% in seven genotypes
Jeppson et al. (1978)	NHI varied from 67 to 80% among seven non-nodulating lines
Wheat	
Austin et al. (1977)	Broad-sense heritability of 61% in a group of 47 winter wheats
Desai and Bhati (1978)	NHI ranged rom 57 to 86% among a diverse group of durum wheats
Dubois and Fossati (1981)	No significant NHI difference among a group of adapted high yielding wheat genotypes
McNeal et al. (1966)	Similar NHI among five spring wheats
McNeal et al. (1968)	Varietal differences in NHI were particularly large under irrigated conditions
Maize	
Pollmer et al. (1979)	In six hybrids (ear N/whole plant N) × 100% varied from 59.5 to 73.2 in highly fertile soil and from 71.6 to 76.5 in less fertile soil

4 Selection for improved nitrogen metabolism

Measurements of harvestable end products such as seed yield, protein yield, or seed protein concentration have been the criteria used to select grain crop genotypes with increased productivity. Selection based upon the harvestable end product has the virtue of integrating the effects of genotype, environment, and the interaction of the two, over the entire crop season. Although this approach is a good measure of genetic advance, no insight into the physiological and biochemical basis of crop improvement is provided. Such insight may be essential to identify factors limiting further advance. It must be recognized that a measurement of one physiological trait at predetermined intervals during the growth cycle will not reliably estimate the potential for harvestable end product. For example, in five wheat genotypes a highly significant correlation between total season NRA per plant and total N accumulation per plant was found but no correlation was observed between grain N and total seasonal NRA (Dalling et al. 1975). The absence of a correlation between grain N and total seasonal NRA was due to large genotypic differences in NHI rather than the inability to estimate reduced N accumulation with NRA measurements. Hageman et al. (1976) also found no correlation between grain protein production and NRA in a series of 45 maize hybrids. However, when the hybrids were divided into groups, each group with one parent in common, correlations of NRA with grain yield and grain protein were found within most groups. This observation suggests that limiting the amount of genetic diversity decreases the confounding effects of other genetic differences. Therefore the measurement of only one physiological or biochemical trait is probably not a reliable estimate of the harvestable end-product especially in the presence of a wide spectrum of genetic variability. The limited usefulness of measuring one physiological or biochemical trait is of relevance to the objective of plant breeding for more efficient N metabolism, which is best served through maximal diversity rather than minimizing the genetic variability in a screening program.

The measurement throughout the growing season of genotypic variation in pertinent components of N metabolism would be the basis of a physiological/biochemical selection program. This approach would estimate end product; integrate the effects of genotype, environment, and their interaction; and allow the identification of factors limiting productivity. It has been suggested that a strategy of selection based upon simultaneous measurement of a number of traits would require the determination of the major controlling enzymes involved in the processes of growth and yield and particularly "the optimum levels of activity of each such enzyme in combinations with specified levels of the other enzymes" (Hageman et al. 1967). A flexible approach of enzyme analyses combined with measurements of other traits, as for example, total N accumulation, Nitrogen Remobilization Efficiency, Nitrogen Harvest Index, constitutes an integrated physiological/biochemical approach and would seem more appropriate.

An understanding of the exploitable genetic variation, the physiology of a crop, and its production environment is necessary to decide which physiological and enzyme measurements would be included in a physiological/biochemical selection program. A decision may be aided by an approach similar to that of Austin et al. (1980) who compared recently developed wheat lines with those grown over the past 70 years to identify physiological changes associated with genetic improvements in yield. The diversity of materials included in a selection scheme will be a major factor determining the magnitude of potential for genetic improvement. The limited genetic variability among cultivated varieties of most major grain crops grown in the United States of America (National Academy of Science, 1972) indicates that the prospect of large amounts of measurable and exploitable genetic variation may not be great. Genetic progress can only occur in the presence of genetic variation, therefore, it is important to use unique germplasm sources and perhaps wild relatives of crop plants. Exotic crop germplasm collections or variability developed in tissue culture or induced by mutagenesis potentially offer the availability of a wide range of genetic material. Although the use of exotic germplasm is detrimental to the rapid development of agronomically acceptable germplasm, its use may yield otherwise unavailable physiological variation and could produce important long term improvement.

A physiological/biochemical selection scheme should be developed by carefully taking into consideration the environment in which the program is to operate. Halloran and Lee (1979) showed that the expression of traits, as for example, N translocation from the vegetative tissue to the developing grain can be significantly affected by growth under artificial conditions. Most aspects of N metabolism are probably under multigenic control and their expression susceptible to environment modification. Therefore, we suggest that physiological and biochemical determinations be made on plants grown under typical production conditions.

5 Conclusions

To our knowledge no breeding program to date has used integrated physiological/biochemical selection as we propose for the enhancement of N metabolism and plant productivity. Our approach combines the traditional methods of estimating harvestable end product together with measurements of the interacting genetically controlled physiological processes which precede plant maturity. A plant breeding program of this nature would result in genetically enhanced plant productivity together with an understanding of the physiological events leading to the improvement. An integrated physiological/biochemical program of plant breeding must provide flexibility to accommodate changes in selection procedure which may result from the identification of traits determined to limit productivity.

The information we have assembled indicates that an integrated physiological/biochemical selection program may have to include an evaluation of, for example, NO_3^- uptake, N_2 fixation, N accumulation, N remobilization, seed protein synthesis, and NHI. The aspects of genetic variation in NO_3^- uptake which probably should be evaluated are the rapidity of the initiation, peak rate, and duration of uptake as well as the rate per unit root surface. In legumes, the variation of the host to provide a favorable physiological environment for early initiation and greater duration of N_2 fixation should be appraised. Genetic variation in the *Rhizobium* micro-symbiont must be determined and optimal host-*Rhizobium* combinations should be identified. Genetic variation in N accumulation must be determined because it provides an integrated measure of NO_3^- uptake and/or N_2 fixation, as well as transport and assimilation of N. Nitrogen remobilization can be estimated by measuring the difference between maximal and final vegetative N. However, the duration of N removal or the sequence of vegetative tissues from which N is removed should also be considered. Measurements of leaf protease activity and/or amino acid movement from vegetative to seed tissue may prove useful to characterize genetic variation in N remobilization. Genetic variability of protein synthesis in developing seed should be appraised. Finally, screening should include determination of genetic variation in NHI to identify genotypes in which a minimal proportion of total N remains in the unharvested vegetative tissue.

An integrated physiological/biochemical selection program will define harvestable end product, delimit the range of genetic variation available in each trait, and identify those factors which limit N metabolism. This approach would permit the identification of parents with exceptional levels of specific parameters to be followed by hybridization and continued selection to combine these traits into unique genotypes with the potential for more efficient N metabolism and higher productivity. In the short-term, unanticipated physiological difficulties may arise as a result of combining traits from divergent genetic backgrounds, and therefore, physiological/biochemical selection is unlikely to produce immediate increases in crop productivity. Also, selections based upon physiological and biochemical criteria may possess agronomic, disease resistant, and grain quality characteristics not fully acceptable for commercial production. However, the advantage of integrated physiological/biochemical selection is that germplasm developed should possess defined physiological variability which would be otherwise unavailable and, indeed, in the long-term, should benefit traditional plant breeding programs by increasing useful genetic variation.

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