Chapter 11
Nitrogen Fixation and Assimilation

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Abstract New crop plants suited to grow in semiarid environments will be fundamental to the future of agriculture. The interactions between nitrogen supply and water availability that determine yield and quality in crops grown in semiarid environments are being elucidated. Tools for analyzing the metabolic changes associated with enhanced nitrogen assimilation under drought have been generated. Here is summarized the crop and other plants that have altered NUE, yield performances, and metabolic profiles caused by *in planta* expression of 31 different transgenes generated in the past two decades. The change in nitrogen concentration has profound effects on plant metabolisms. The metabolic changes resulted in phenotypic changes that included increases in mean plant biomass production in dry soils, tolerance to the herbicide phosphinothricin, tolerance to both severe and mild water deficit, and resistance to rotting necrotrophs. Leaves, and grain had higher nutritional value and higher yield, indicating improved NUE and WUE by some of the transgenes. Therefore, in view of global climate change, continued efforts to alter nitrogen fixation and assimilation by transgenesis and mutation should be pursued through technology stacking.

11.1 Introduction

Nitrogen is an essential component in cellular physiology with only oxygen, carbon, and hydrogen being more abundant (Marchner 1995; Andrews et al. 2004). Nitrogen is present in numerous essential compounds including nucleoside

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C. Kole (ed.), *Genomics and Breeding for Climate-Resilient Crops*, Vol. 2,
DOI 10.1007/978-3-642-37048-9_11,

phosphates and amino acids that form the building blocks of nucleic acids and proteins, respectively. In plants, nitrogen is used in large amounts in photosynthetic pigments, defense chemicals, and structural compounds. However, inorganic N is difficult to assimilate. Dinitrogen in the atmosphere is very inert. Reduction to ammonium requires the energy of a lightning bolt and two adenosine triphosphates (ATPs) or energy from petrochemicals or 12 ATP dephosphorylations per molecule within a nodule or other anaerobic environment. However, these ammonium fertilizers are prone to escape from the cell as ammonia gas. Photorespiration releases tenfold more ammonium than is assimilated from the environment and plants only re-assimilate 98 % of it. Consequently, a haze of ammonia gas is found floating above a photosynthetic canopy. That ammonia may be lost on the wind or returned to the plant or soil by rains or dew falls. Any improvements to these nitrogen cycles (Table 11.1; Tercé-Laforgue et al. 2004a,b; Seebauer et al. 2010; de Carvalho et al. 2011) can have a massive positive impact on the efficiency of agriculture, reduce its carbon footprint, and over geological time scales reverse some of the anthropogenic contributors to global warming.

The assimilation of ammonium has a second major problem associated with it. Ammonia is assimilated releasing one acidic proton per molecule (Marchner 1995). There is enough flux to reduce the pH of even well-buffered soils to concentrations that inhibit plant growth, both directly and by the release of toxic concentrations of micronutrients (Al and Mn in particular). Reduction within a nodule or other anaerobic environment compounds this problem by releasing two protons per ammonium produced (Indrasumunar et al. 2011, 2012). Soil acidification is a worldwide problem on a massive scale.

Nitrates and nitrites provide a solution to the acidification problem, as their reduction to ammonium absorbs 3–4 protons (Marchner 1995). So a pH-balanced fertilizer should theoretically be a 4 to 1 mixture of ammonium and nitrates. Nitrates and nitrites are the ions produced by those lightning bolts that provide about 10 % of the world's reduced nitrogen a year. However, they are not without costs and problems. Nitrite is highly toxic to photosynthesis and respiration and so must be immediately reduced to ammonium. Plants produce massive amounts of nitrite reductase for this purpose. Nitrate is benign, easy to store and transport, and consequently is the major form of inorganic N found in plants. However, plants still produce tenfold more nitrate reductase than is absolutely needed for assimilation, growth, and yield (Kleinhofs et al. 1980; Wang et al. 2012). Why? That is still unclear.

The major problem with nitrates in the environment is that they are water soluble and so rapidly leached from soils (Lee and Nielsen 1987; David et al. 1997). So much is lost from agricultural soils, industrial activity, and human waste treatments that the world's rivers, lakes, and oceans are significantly fertilized (Cherfas 1990; Burkholder et al. 1992). The algae are the microorganisms that benefit the most. Unfortunately, they run low on other nutrients (P, K) and so produce toxins to kill other organisms to obtain the limiting nutrients by their decomposition. In addition, they absorb much of the water's oxygen (at night) killing even toxin-resistant

Table 11.1 Selected transgenic and mutant lines with effects on N fixation, N transport, primary N assimilating genes, and secondary N metabolism (adapted from Pathak et al. 2008; Lightfoot 2009)

Gene and paralog/mutant allele number	Gene source	Promoter(s)	Target plant	Phenotype observed	
Nrt1.1—high-affinity nitrate transporter 1	*A. thaliana*	CaMV 35S	*A. thaliana*	Increase in constitutive nitrate uptake but not the induced form	Liu et al. (1999)
Nrt2.1—high-affinity nitrate transporter 2	*N. plumbaginifolia*	CaMV 35S, rolD	*N. tabacum*	Increased nitrate influx under low N conditions	Fraisier et al. (2000)
				Three- to fourfold drop in NR protein and activity, no change in NR transcript	Vincentz and Caboche (1991)
NR—nitrate reductase isoform 1	*N. plumbaginifolia*	CaMV 35S	*N. tabacum*	Increased NR activity, biomass, drought stress	Ferrario-Mery et al. (1998)
	N. tabacum	CaMV 35S	*L. sativa*	Reduced nitrate content, chlorate sensitivity	Curtis et al. (1999)
	N. tabacum	CaMV 35S	*N. plumbaginifolia*	Nitrite accumulation in high nitrate supply	Lea et al. (2004)
	H. vulgaris	NMS mutation	*H. vulgaris*	No phenotype, with just 10 % of NR activity	Kleinhofs et al. (1980), Oh et al. (1980)
	Porphyra sp.	RAB17	*Z. mays*	Improved NUE, yield with N limitation	Loussaert et al. (2008)
NR—nitrate reductase isoform 2	*N. tabacum*	CaMV 35S	*S. tuberosum*	Reduced nitrate levels	Djannane et al. (2002)
NiR—nitrite reductase	*N. tabacum*	CaMV 35S	*N. plumbaginifolia*	Increased NiR activity, no phenotypic difference	Crete et al. (1997)
	N. tabacum	CaMV 35S	*A. thaliana*	Increased NiR activity, no phenotypic difference	Crete et al. (1997)
	S. oleracea	CaMV 35S	*A. thaliana*	Higher NiR activity, higher nitrite accumulation	Takahashi et al. (2001)
AMT—ammonium transporters	*A. thaliana*	RAB17	*Z. mays*	Increased NUE	Gupta et al. (2008, 2011)
	Z. mays	UBI	*G. max*	Increased NUE	Gupta et al. (2008, 2011)

(continued)

Table 11.1 (continued)

Gene and paralog/mutant allele number	Gene source	Promoter(s)	Target plant	Phenotype observed	
GS2—chloroplastic glutamine synthetase	*O. sativa*	CaMV 35S	*N. tabacum*	Improved photorespiration capacity and increased resistance to photooxidation	Kozaki and Takeba (1996)
	O. sativa	CaMV 35S	*O. sativa*	Enhanced photorespiration, salt tolerance	Hoshida et al. (2000)
	N. tabacum	Rbc SSU1	*N. tabacum*	Enhanced growth rate	Migge et al. (2000)
	P. sativa	CaMV 35S	*N. tabacum*	Co-suppression reduced growth rate	Migge et al. (2000)
Fd-GOGAT—ferredoxin-dependent glutamate synthase	*N. tabacum*	CaMV 35S	*N. tabacum*	Diurnal changes in NH_3 assimilation	Ferrario-Mery et al. (2002)
GS1—cytosolic glutamine synthetase	*P. vulgaris*	CAMV 35S:: MITATPase	*N. tabacum*	Growth inhibited, insoluble GS in mitochondria	Hemon et al. (1990)
	P. vulgaris	CAMV 35S	*N. tabacum*	Increased herbicide tolerance	Hemon et al. (1990)
	G. max	CaMV 35S	*L. corniculatus*	Accelerated senescence	Vincent et al. (1997)
	G. max	rolD	*L. japonicus*	Decrease in biomass	Limami et al. (1999)
	P. vulgaris	Rbc SSU1	*T. aestivum*	Enhanced capacity to accumulate nitrogen	Habash et al. (2001)
	M. sativa	CaMV 35S	*N. tabacum*	Enhanced growth under N starvation	Fuentes et al. (2001)
	M. sativa	CaMV 35S	*N. tabacum*	Herbicide tolerance	Donn et al. (1984)
	G. max	CaMV 35S	*M. sativa*	No increase in GS activity	Ortega et al. (2001)
	P. sativa	CaMV 35S	*N. tabacum*	Enhanced growth, leaf-soluble protein, ammonia levels	Oliveira et al. (2002)
	G. max	CaMV 35S	*P. sativum*	No change in whole-plant N	Fei et al. (2003)
	M. sativa	CaMV 35S	*L. japonicas*	Higher biomass and leaf proteins	Suarez et al. (2003)
	P. sylvestris	CaMV 35S	*Populus sp.*	Enhanced growth rate, leaf chlorophyll, total soluble protein	Gallardo et al. (1999)

	O. sativa	CaMV 35S	*O. sativa*	Increased protein, amino acids, and nitrogen content	Cai et al. (2009)
	E. coli	CaMV 35S	*O. sativa*	Decreased salt, cold and drought tolerance; seed yield and amino acid content	Cai et al. (2009)
NADH-GOGAT–NADH-dependent glutamate synthase	*O. sativa*	CaMV 35S	*O. sativa*	Enhanced grain filling, increased grain weight	Yamaya et al. (2002)
	M. sativa	CaMV 35S	*M. sativa*	Higher total C and N content, increased dry weight	Schoenbeck et al. (2000)
GDH—glutamate dehydrogenases *gdhA* microbial NADPH dependent	*E. coli*	CaMV 35S	*N. tabacum*	Increased biomass, nutritional value	Ameziane et al. (2000)
				N assimilation, NUE, WUE, herbicide tolerance	Mungur et al. (2005, 2006)
				Amino acid and sugar content	Lightfoot et al. (1999)
	E. coli	*Os*UBI	*Z. mays*	Increased N assimilation, biomass, and sugar content	Lightfoot et al. (2007)
				Herbicide tolerance, grain biomass, amino acids	Lightfoot et al. (1999)
	E. coli	CaMV 35S	*A. thaliana*	Increased biomass and herbicide tolerance	Lightfoot (unpublished)
	C. sorokiniana	CaMV 35S	*T. aestivum*	Increased biomass	Schmidt and Miller (2009)
	C. sorokiniana	CaMV 35S	*Z. mays*	Increased biomass	Schmidt and Miller (2009)
	A. nidulans	CaMV 35S	*L. esculentum*	Higher amino acid concentrations	Kisaka and Kida (2005, 2007)
	A. niger	CaMV 35S	*O. sativa*	Increase in dry weight, nitrogen content, and yield with high N	Abiko et al. (2010)

(continued)

Table 11.1 (continued)

Gene and paralog/mutant allele number	Gene source	Promoter(s)	Target plant	Phenotype observed	
GDH1–nonmicrobial NADH dependent	*A. thaliana*	CaMV35S	*A. thaliana*	Increased abiotic stress tolerance	Coruzzi and Brears (1999)
	L. esculentum	CaMV 35S:: MIT	*L. esculentum*	Twice GDH activity, higher mRNA abundance, and twice	Kisaka et al. (2007)
AS1–glutamine-dependent asparagine synthetase	*A. thaliana*	CaMV 35S	*A. thaliana*	Enhanced seed protein	Lam et al. (2003)
	P. sativum	CaMV 35S	*N. tabacum*	Slightly increased biomass and increased level of free asparagines, plants required N source	Harrison et al. (2003)
	E. coli	CaMV 35S	*L. sativa*	Increased leaf mass, amino acids, proteins lower NO_3 and organic acids	Sobolev et al. (2010)
*Asp*AT–mitochondrial aspartate aminotransferase	*S. bicolor*	CaMV 35S	*N. tabacum*	Increased AspAT, PEPCase activity	Sentoku et al. (2000)
AlaAT–alanine aminotransferase	*H. vulgaris*	btg26	*B. napus*	Yields constant to 50% less N fertilizer	Good et al. (2007)
			S. bicolor	Increased NUE	
		OsANT1	*O. sativa*	Excess N increased biomass, grain yield metabolites, and total nitrogen content, indicating increased nitrogen uptake efficiency	
iGluR–glutamate receptors	*A. thaliana*	Mutants	*A. thaliana*	Growth inhibited or enhanced, kainate resistant	Coruzzi et al. (1998)
	A. thaliana	RAB17	*A. thaliana*	Increased NUE	
	Z. mays	RAB17	*Z. mays*	Increased NUE and yield	Dotson et al. (2009)
MMP1–S-methylmethionine permease1	*S. cerevisiae*	AAP1	*P. sativum*	Increased plant growth and seed number, S, N, and protein content	Tan et al. (2010)
POT–proton-dependent oligopeptide transport (PTR class)	*A. thaliana*	CaMV 35S	*A. thaliana*	Increased NUE, biomass and protein in seed, MSX resistance in mutants	Schneeberger et al. (2008)
		Null mutant			

HMP–flavohemoglobin	*E. coli*	OsACT1	*Z. mays*	Decreased NO, increased NUE, biomass, chlorophyll, and yield in the field	Basra et al. (2007)
		AtACT7	*G. max*		
ANR1–MADS transcription factor	*A. thaliana*	CaMV 35S	*A. thaliana*	Lateral root induction and elongation	Zhang and Forde (1998)
GLB1–PII regulatory protein	*A. thaliana*	CaMV 35S	*A. thaliana*	Growth rate, increased anthocyanin production in low N	Zhang et al. (2003)
Dof1–transcription factor	*Z. mays*	35S C4PDK	*A. thaliana*	Enhanced growth rate under N-limited conditions, increase	Yanagisawa et al. (2004)
	Z. mays	35S C4PDK	*O. sativa*	In amino acid content	Kurai et al. (2011)
bZIP–transcription factor, MYB–transcription factor, glycosyl hydrolase family 9	*A. thaliana*	CaMV 35S	*A. thaliana*	Improved NUE, plant size, vegetative growth, growth rate, light-inducible seedling vigor, and/or biomass	Nadzan et al. (2007)
Zinc finger C3HC4, NF-YB–plant nuclear factor Y	*A. thaliana*	CaMV 35S	*A. thaliana*	Improved NUE and WUE	Nelson et al. (2007)
	Z. mays	OsRACT	*Z. mays*	Improved NUE and WUE	
HAP3–transcription factor and interacting 14:3:3 proteins	*A. thaliana*	CaMV 35S	*Z. mays*	Improved NUE and seed yield	Dotson et al. (2009)
			G. max	Improved NUE and WUE	
			G. hirsutum	Improved NUE, WUE, and yield	
SnRK1–transcription factor	*M. hupehensis*	CaMV 35S	*L. esculentum*	Increased nitrogen uptake	Wang et al. (2012)
FUM2–fumarase	*A. thaliana*	Mutant	*A. thaliana*	Enhanced growth on high N	Pracharoenwattana et al. (2010)
IPT–cytokinin synthesis	*A. tumefaciens*	SARK	*N. tabacum*	Increased assimilation on low N	Rubio-Wilhelmi Mdel et al. (2012)
GCL–glyoxylate carboligase (EC 4.1.1.47)	*E. coli*	CaMV 35S	*N. tabacum*	Increased amino acid content	de Carvalho et al. (2011)

aerobes. Finally, they bloom, blocking the light needed for photosynthesis by submerged organisms. Millions of acres of oceans are affected.

The major problem with nitrates in the human diet (water or food) is that they are metabolized to potent carcinogens (nitrosamines) in the acid of the human stomach (Tannenbaum et al. 1978; Moller et al. 1990; Mirvish 1985; Duncan et al. 1998). High nitrate and so nitrosamine amounts in human diets are associated with many different cancers as well as fertility problems. However, nitrates are naturally excreted in human and animal saliva for the purpose of producing some nitrosamines in the gut. This is because the combination of acid and nitrosamine effectively kills many human and animal pathogens resistant to one or the other. *Helicobacter pylori* is one example. This microbe causes stomach ulcers that left untreated often become cancerous. *H. pylori* is endemic and became more abundant as lifestyles became more stressful. Consequently, several epidemiological studies found diets high in nitrate to be healthful in the 1990s and beyond, whereas before that they were significantly unhealthful. Clearly, then the healthiest option is a low-nitrate diet and low-stress lifestyle. However where lifestyle change is not an option, for *H. pylori* and like pathogens, the lesions they cause are better treated with drugs than nitrosamines.

However produced and applied, microbes in the soil take up the bulk of all fertilizers before the plant can (Jahns and Kaltwasser 2000; Jahns et al. 1999; Trenkel 1997; Cabello et al. 2004; Garcia-Teijeiro et al. 2009; Koivunen et al. 2004). Microbes pass the N molecules through about seven microbial cells before plants absorb them. Ammonium can be assimilated or oxidized to nitrite, nitrate, nitrous oxide, or dinitrogen by microbial activities. Plants have to absorb N from microbes by force using highly efficient enzymes or by trade through symbiosis [reviewed by Ferguson and Indrasumunar (2011)]. In symbiosis the microbes are provided with sugars in return for ammonium. The microbes may be free living in the rhizosphere or housed in specialized structures like nodules. Symbiotic microbes produce a variety of chemical signals to elicit the delivery of sugars from the plants. These systems are ripe for manipulation by biotechnology approaches.

11.2 Plant Assimilations

Because soil particles do not naturally have many N-containing minerals, and because N can be readily lost from the rooting environment, it is the nutrient element that most often limits plant growth and so agricultural yields (Marchner 1995; Specht et al. 1999; Duvick 2005). As noted above, nitrogen is found in the environment in many forms and comprises about 80 % of the earth's atmosphere as triple-bonded nitrogen gas (N_2). However, this large fraction of N is not directly accessible by plants and must be bonded to one or more of three other essential nutrient elements including oxygen and/or hydrogen through N-fixation processes and carbon through N-assimilation processes. Plants are able to absorb a little NH_3

from the atmosphere through stomata in leaves, but this is dependent upon air concentrations. The ions NO_3^- and NH_4^+ are the primary forms for uptake in by plants. The most abundant form that is available to the plant roots is NO_3^-, and the most abundant form in leaves is NH_4^+. The process of nitrification by soil bacteria readily converts fertilizer NH_4^+ to NO_3^- (Trenkel 1997; Zhao et al. 2005). Relative nitrogen uptake is also dependent on soil conditions. Ammonium uptake is favored by a neutral pH and NO_3^- uptake is favored by low pH. Nitrate also does not bind to the negatively charged soil particles; therefore, it is more freely available to plant roots especially through mass flow of soil water than is NH_4^+, which binds to negatively charged soil particles and so moves primarily by diffusion. As noted above, the assimilation of NH_4^+ by roots causes the rhizosphere to become acidic, while NO_3^- causes the rhizosphere to become more basic.

11.2.1 Uptake of Nitrogen

Nitrogen uptake and assimilation summates a series of vital processes controlling plant growth and development (Godon et al 1996; Krouk et al 2010; Meyer et al. 2006). Nitrate, nitrite, and ammonium uptakes (and reuptakes following losses) occur against massive concentration gradients that require lots of energy to generate and maintain. Happily in agriculture, plants are spaced sufficiently that they have an excess of captured light energy relative to the N and C supplies. Transgenic plants overexpressing low-affinity nitrate uptake transporter Nrt1 increased the constitutive but not the induced nitrate uptake (Table 11.1; Liu et al. 1999). Equally, plants transgenic with Nrt2.1 the high-affinity nitrate transporter 2 increased nitrate influx under low-N conditions (Fraisier et al. 2000). Transgenic plants expressing an ammonium transporter increased nitrogen use efficiency (NUE; Gupta et al. 2008). Glutamate receptors in transgenic plants provided better growth. Equally the uptake of short peptides had positive effects. All these transport-associated phenotypes would be desirable in agricultural production systems directed toward greater efficiency and lower environmental impacts. A stack of the three transgenes would be of interest.

11.2.2 Nitrate Reduction

Nitrate acquired in the roots can be reduced in the shoot or the root or even stored in vacuoles in the root or shoot for later assimilation. However, nitrate must be reduced to a useable form. This occurs via a two-step process catalyzed by the enzymes nitrate reductase and nitrite reductase to form NH_4^+. Both enzymes are produced in massive excess compared to flux needed through the pathway so that mutants that reduce their amounts by 90 % do not have phenotypes (Table 10.1; Kleinhofs et al. 1980). Equally some transgenic plants overexpressing nitrate

reductase (NR) increased nitrate reduction but were not altered in phenotypes (Crete et al. 1997; Curtis et al. 1999; Djannane et al. 2002; Lea et al. 2004). However, two studies of NR overexpressing transgenic plants did record altered phenotypes including increased biomass, drought stress (Ferrario-Mery et al. 1998), and improved NUE and yield during N limitation (Loussaert et al. 2008). These phenotypes would be desirable in agricultural crops. Coupling of NR to photosynthesis should be possible by the transformation of plants with a ferredoxin-dependent NR from cyanobacteria.

11.2.3 Dinitrogen Fixation

The ability to fix dinitrogen is restricted to the bacterial world but widespread among microbes (Ferguson and Indrasumunar 2011; Valentine et al. 2011; Reid et al. 2011). Many different *nif* gene families exist suggesting selections for variation have been favorable for species. The need for an anaerobic environment for nif activity means that transferring the enzymes to plants will be difficult. To date, transgenics in this field are bacterial, as in hydrogenase-enhanced microbes, or if plant they are designed to improve the chances of nodule occupancy by improved bacterial strains. Strains that are most likely to set up nodule occupancy are rarely the most efficient nitrogen fixers. Plants also often fail to maintain effective nodules through flowering and pod set (Sinclair et al. 2007). Soybean and common bean, for example, have senescent nodules by flowering. Some species do have indeterminate nodules, and it would be a valid goal of biotechnology to transfer this trait to major legume crops.

11.2.4 Ammonium Assimilation

The N acquired as NH_4^+ does not require reduction upon uptake into the root, thus providing some energy savings to the plant over that of the NO_3^- form reviewed by Marchner (1995). However, it does require assimilation to avoid loss and at high concentrations (>10 mM) toxicity to the plant. Various studies have shown that under conditions of excessive NH_4^+ uptake, most plant species will transport this N source to the shoot, which is more sensitive to ammonium ions (Marchner 1995).

One important process to build key macromolecules in any living organism is the acquisition and utilization of inorganic forms of nitrogen during metabolism (Lea and Miflin 2011). Plants use amino acids and their precursors and catabolic products for important metabolic activities. Various other roles of amino acids include nitrogen storage and transport and the production of a very large number of secondary compounds including structural lignin compounds, light-absorbing

pigments, phenolics, and plant hormones. Plants convert the available inorganic nitrogen into organic compounds through the process of ammonium assimilation, which occurs in plants by two main pathways. The first and primary pathway involves a reaction with glutamate to form glutamine, which is catalyzed by glutamine synthetase (GS, EC 6.3.1.2) and requires an energy source of ATP. There are two isoenzymes of GS based on their location in the plant, either in the cytosol (GS1) or in the root plastids or shoot chloroplasts (GS2). Expressed in germinating seeds or in the vascular bundles of roots and shoots, the cytosolic form (GS1) produces glutamine for intracellular nitrogen transport. GS2 located in root plastids produces amide nitrogen for local consumption, while GS2 in the shoot chloroplasts re-assimilates photorespiratory ammonium (Lam et al. 1996). GS1 is encoded by a set of 3–6 paralogs in different crop species, so hetero-hexamers can form. However, the affinity for the substrates hardly differs. Amino acid identity is very high even to GS2. GS2 has a short peptide extension at the C terminus that might be involved in regulation by phosphorylation. Alleles of the GS1 and GS2 encoding genes do exist that differ in their regulation. Alleles of GS appear to underlie quantitative trait loci (QTL) determining NUE and seed yield (Cañas et al. 2009, 2010; Coque et al. 2008). Transgenic analyses have been made of GS2 but not GS1 (Table 11.1). Among the 12 studies in nine plant species, the phenotypes reported included enhanced accumulation of N, growth under N starvation, herbicide (PPT) tolerance, leaf-soluble protein, ammonia, amino acids, and chlorophyll. Some genes and constructs though decreased growth; salt, cold, and drought tolerance; seed yield; and amino acid content. Therefore, the use of GS transgenics in agriculture will be useful and desirable but only with careful attention to regulation and expression.

11.2.5 Transaminases

The glutamine molecules produced by GS are used by a whole series of transaminases to produce the 20 protein amino acids and some nonprotein amino acids. Cardinal among the transaminases is the reaction catalyzed by glutamate synthase (GOGAT, EC 1.4.14) to form glutamate. There are two common isoenzymes of GOGAT including a ferredoxin-dependent GOGAT (Fdx-GOGAT) and an NADH-dependent GOGAT (NADH-GOGAT). While both forms are plastidic, the Fdx-GOGAT enzyme is predominately found in photosynthetic organs, and the NADH-GOGAT enzyme is found more in non-photosynthetic tissues, such as in roots and the vascular bundles of developing leaves (Schoenbeck et al. 2000: Yamaya et al. 2002). An NADPH-dependent GOGAT can be found in certain organs and in many bacteria. Plants transgenic with the NADH-dependent plant GOGAT have been reported. Phenotypes included enhanced grain filling, grain weight, total C and N content, and dry weight (Table 10.1). Phenotypes were very similar to the benefits reported from alanine dehydrogenase and asparagines synthase suggesting that transaminases are acting on a common pathway.

11.2.6 Glutamate Dehydrogenases

The second pathway for ammonium assimilation also results in the formation of glutamate through a reversible reaction catalyzed by glutamate dehydrogenase (GDH, EC 1.4.1.2), with a lower energy requirement than GS/GOGAT. There are also at least two forms of GDH that occur in plants that include an NADH-dependent form found in the mitochondria and an NADPH-dependent form localized in the chloroplasts of photosynthetic organs. In addition, there are enzymes capable of aminating reactions that resemble GDH (Turano Personal communication). GDHs present in plants serve as a link between carbon and nitrogen metabolism due to the ability to assimilate ammonium into glutamate or deaminate glutamate into 2-oxoglutarate and ammonium (Forde and Lea 2007). However, due to the reversibility of this reaction, the assimilatory role of GDH is severely inhibited at low concentrations of ammonium. Additionally, GDH enzymes have a low affinity for ammonium compared with GS, which further limits their assimilatory effectiveness. It has been suggested that the NAD-requiring form of GDH may be involved in carbon rather than nitrogen metabolism, (Coruzzi and Brears 1999; Kisaki et al. 2007; Nadzan et al. 2007) with glutamate catabolism providing carbon skeletons both for the TCA cycle and energy production during carbon or energy deficit. Alternate functions for GDH have also been proposed in which it has been assigned the role of re-assimilating excess ammonium, due to the limited ability of the GS/GOGAT cycle, during specific developmental stages (Loulakakis et al. 2002), such as during germination, seed set, and leaf senescence (Coruzzi and Brears 1999; Kisaka et al. 2007).

In contrast to plant GDHs, those found in microbes are very active in the assimilation of ammonium (Lightfoot et al. 1999; 2001). Plants did not have the opportunity to incorporate this type of NADPH-dependent GDH because the bacterial lines that gave rise to chloroplasts do not contain *gdhA* genes. The few cyanobacteria with GDH activity have acquired genes by transgenesis or cellular fusions. Transgenic plants in six crop species have been produced that express *gdhA* genes from three microbes (Ameziane et al. 2000; Lightfoot et al. 2007). Phenotypes in plants include increased biomass, water deficit tolerance, nutritional value, herbicide resistance, N assimilation, NUE, water use efficiency (WUE), amino acid, and sugar content (Mungur et al. 2005, 2006; Lightfoot 2008; Lightfoot and Fakhoury 2010; Nolte et al. 2004; Nolte 2009; Table 11.1). GDH genes used in this way are being evaluated for commercialization.

One problem faced by this and the alanine dehydrogenase transgenics (Good et al. 2005; Shrawat et al. 2008) is a dependence on soil type for some of the beneficial effects. GDH seems to provide a growth advantage on silty-loam clay soils common in the southern Midwest. In contrast, the alanine dehydrogenase transgenics seem to work best on sandy soils. Combining the technologies or altering their regulation might provide stable beneficial effects in many soil types and locations.

11.2.7 Other Aminases

A variety of other enzymes exist that are capable of aminating reactions. Each will be a candidate for overexpression in transgenic plants. Phenylalanine ammonia lyase has been used in many transgenic plants. Equally, the enzymes of cyanide assimilations (cysteine metabolism) might be more active than previously thought and could be manipulated. Alteration of the enzymes of heme and chlorophyll biosynthesis might be tried again. The *Escherichia coli hemA* gene was functional, but *hemB* became insoluble in plant chloroplasts (Denhart, Lightfoot and Gupta Unpublished). In this same pathway, the protoporphyrinogen oxidases are targets of increasingly used and useful selective herbicides. Another major sink of amines are the lignins and lignols. Emerging research suggests that transgenic manipulation of these pathways will alter NUE and therefore WUE (Castiglioni et al. 2008; Century et al. 2008; Goldman 2009; Vidal 2010; Jung et al. 2011). These enzymes might also be usefully manipulated in stacks with transgenes to improve NUE, WUE, and other traits including herbicide tolerance.

11.3 Conclusions

The assimilation of inorganic nitrogen is a key process in the productivity of crop plants. There are many steps at which metabolic improvements can be made. In the future, the chance to provide active nodules to nonlegumes will provide an impetus for biotechnology. In addition, the combination of existing transgenes and new promoter for their regulation will provide for new avenues in crop improvement.

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