Amino Acids (1994) 7: 165–174

Amino
Acids

Molecular regulation of amino acid biosynthesis in plants

Review Article

B. K. Singh[1] and **B. F. Matthews**[2]

[1] American Cyanamid Company, Princeton, New Jersey, U.S.A.
[2] Plant Molecular Biology Laboratory, USDA/ARS, Beltsville, Maryland, U.S.A.

Accepted December 12, 1993

Summary. Our understanding of amino acid biosynthesis in plants has grown by leaps and bounds in the last decade. It appears that most of the amino acid biosynthesis takes place in the chloroplast. Recent demonstration of glutamine synthetase and DAHP synthase in the vascular tisuue has added a new dimension in the complexity of the nitrogen cycle in plants. Isolation of various genes and transformation of plants with the modified forms of the genes are providing tools for understanding the regulation of various pathways. Plant transformation approaches are also going to provide the food of the future with an improved amino acid composition.

Keywords: Amino acids – Herbicides – Nutritional improvement – Stress tolerance

Introduction

The first crystals of amino acids were obtained from asparagus juice in 1806. These crystals were later identified as asparagine and aspartate (see review by Meister, 1992). Despite this discovery made 188 years ago, most of the progress in understanding amino acid biosynthesis in plants has been made in the last two decades. Approximately 60 enzymes are involved in the biosynthesis of the 20 protein amino acids. Many of these enzymes are present as multiple isoforms. There appear to be alternate routes of synthesis of certain amino acids and the isoforms may be used either in different compartments, different developmental stages or under different environmental conditions. There is a large number of secondary metabolites which are derived from amino acids. Evidence is now emerging that the requirements of plants for these metabolites also regulate the flow of carbon through these pathways. In the 1980's, it was discovered that several classes of highly successful commercial herbicides with excellent environ-

mental and toxicological properties inhibit essential amino acid biosynthesis in plants. These discoveries, combined with the continued interest in improving the nutritional quality of food, increasing the nitrogen use efficiency of crops, etc., have led to an explosion in the literature on amino acid biosynthesis in plants. In this review, we attempt to highlight the most recent progress made on various aspects of amino acid biosynthesis in plants.

Glutamine

Glutamine synthetase (GS) is a key enzyme involved in nitrogen assimilation and distribution. Multiple forms of GS appear to be differently expressed in plants due to their distinctive roles in plant metabolism. An ammonia-regulated soybean gene encoding the cytosolic form of glutamine synthetase (GS) was cloned via complementation in an *Escherichia coli gln* A^- mutant and expressed in transgenic *Lotus corniculatus*. A 3.5 kb promoter fragment of a genomic clone of GS was fused with the *uidA* gene encoding the reporter enzyme, β-glucuronidase (GUS). The gene fusion was expressed in the root apices and vascular bundles. Ammonia increased expression of the gene (Maio et al., 1991).

A full length cDNA encoding the plastidic form of glutamine synthetase was cloned from tobacco leaf RNA by Becker et al. (1992). The cDNA encodes a polypeptide of 432 amino acids, which would have a molecular mass of 47.2 KDa. The protein resembles the GS-2 isoform. Phytochrome mediated light stimulation of expression of the GS-2 gene was noted (Becker et al., 1992).

Glutamine synthetase from *Phaseolus vulgaris* root nodules consists of β and γ subunit polypeptides which are very similar. Antibodies made to small, dissimilar regions within the sequences reacted specifically with each subunit (Cai et al., 1992). These antibodies should be useful tools in further studies of GS subunit expression.

The gene encoding the plastid form of glutamine synthetase was cloned from *P. vulgaris*. A fusion of the upstream 2.3 kb region of the gene with the *uidA* gene encoding reporter enzyme, β-glucuronidase (GUS) allowed promoter regions encoding tissue-specific expression and light regulation to be identified (Cock et al., 1992). Tissue print immunoblots of rice indicate that GS1 is located in large and small vascular bundles of the leaf blade. GS-2 and Fd-GOGAT are located in mesophyll cells. (Kamachi et al., 1992).

A novel glutamine synthetase has been cloned from developing maize kernels (Salazar et al., 1993) by screening a cDNA library made from developing kernels using monoclonal antibodies specific to GS forms expressed in developing kernels.

Aromatic amino acids

Genes encoding 3-deoxyarabinoheptulosonate-7-phosphate (DAHP) synthase (Dyer et al., 1990; Keith et al., 1991), shikimate kinase (Schmid et al., 1992), 5-enolpyruvylshikimate-3phosphate (EPSP) synthase (Barry et al., 1992), chorismate synthase (Schaller et al., 1991), anthranilate synthase (Niyogi and Fink, 1992; Niyogi et al., 1993), phosphoribosyl anthranilate transferase (Rose

et al., 1992), and the β-subunit of tryptophan synthase (Berlyn et al., 1989; Last et al., 1991) have been cloned. All of the isolated genes contain a chloroplast transit peptide sequence which is consistent with the chloroplastic localization of this pathway. Complete and separate pathways of aromatic amino acid biosynthesis in chloroplasts and cytosol have been proposed by Jensen (1986) based on detection of activities of DAHP synthase and chorismate mutase in the two compartments. Since all of the genes isolated thus far contain a putative chloroplast transit peptide sequence, the whole aromatic amino acid pathway should be present exclusively in the chloroplasts. Interestingly, histological studies using antibodies against DAHP synthase did not reveal a significant amount of immunogold label in the chloroplasts. Surprisingly, secondary cell walls of the vascular tissue were intensely labeled (Herrmann et al., 1992). This intriguing observation is similar to the demonstration of glutamine synthetase activities in the vascular tissues (Kamachi et al., 1992). These results may be important in understanding nitrogen metabolism in plants.

Of all the enzymes involved in the aromatic amino acid biosynthetic pathway, 5-enolpyruvylshikimate-3-phosphate (EPSP) synthase is by far the most studied enzyme because it is the target site of glyphosate. Several crop species have been transformed with a bacterial EPSP synthase gene which produces an enzyme with reduced sensitivity to glyphosate or with a gene that produces an enzyme that can metabolize glyphosate (Barry et al., 1992). These transformants are claimed to have commercial levels of tolerance to glyphosate.

Identification of tryptophan synthase mutants have questioned the age-old belief that tryptophan is the sole precursor for indole acetic acid (IAA) (Bandurski and Nonhebel, 1989). Examination of tryptophan and IAA levels in normal and *orp* (orange pericarp) mutant seedlings of maize revealed 50-fold higher levels of IAA in the *orp* mutant despite a 75% reduction in tryptophan content compared to the control (Wright et al., 1991). Additionally, stable isotopes of tryptophan were not incorporated into IAA by the mutant or normal seedlings. Further studies will demonstrate the true biosynthetic pathway for IAA.

Until recently, it was believed that regulation of chorismate mutase and anthranilate synthase at the branch point were the only means of controlling the flux of carbon through this pathway (see the references in Singh et al., 1991). However, it is now clear that carbon flux through this pathway is not only regulated by feedback inhibition of these enzymes but also by controlling the level of expression of pathway enzymes. Recent studies on DAHP synthase (Dyer et al., 1989; McCue and Conn, 1989), chorismate mutase (Kuroki and Conn, 1988), and anthranilate synthase (Niyogi and Fink, 1992; Poulsen et al., 1993) have clearly demonstrated increased expression of these enzymes due to wounding, exposure to fungal elicitors, or high light intensity. Therefore, in order to meet the transient needs for the intermediates or end products of this pathway, plants upregulate the carbon flux by increasing the expression of key enzymes of this pathway. However, feedback inhibition of key enzymes of this pathway may be the means to down regulate the flow of carbon through this pathway. Future studies will reveal the co-ordination of regulation by the mechanisms described above.

Aspartate family

Aspartate is synthesized by aspartate aminotransferase (AAT), which is found in multiple forms located in different compartments of the plant cell. In carrot, several isoforms of AAT are present. A major form present in carrot cell suspension cultures was isolated and examined (Turano et al., 1991). It has the characteristics of a cytosolic AAT. Amino acid sequences obtained from this AAT allowed the cloning of a cDNA encoding AAT (Turano et al., 1992). The clone has high identity to sequences encoding cytoplasmic forms of AAT from other plants and does not appear to have a transit polypeptide, which is consistent with its cytoplasmic localization. It is highly expressed in roots but not in leaves.

In soybean, at least five forms of AAT can be separated on electrophoretic gels (Wadsworth et al., 1993). Genes encoding three of these forms have been cloned, one plastidic (Wadsworth et al. 1993), one mitochondrial (Matthews et al., 1993) and one cytosolic (Matthews et al., 1993). Each has been functionally expressed in *E. coli* and antibody has been prepared to the plastidic and mitochondrial forms.

Similar advances have occurred in elucidating alfalfa aspartate aminotransferase gene families and their location and regulation. Two genes have been cloned encoding alfalfa AAT-1 (Udvardi and Kahn, 1991) and AAT-2 (Gantt et al., 1992), respectively. AAT-1 appears to be the cytosolic form and is induced during nodule development, while AAT-2 is highly homologous to the soybean plastidic AAT and contains a putative plastid-targeting peptide.

Aspartokinase (AK), the first enzyme in the pathway leading to lysine, threonine and methionine biosynthesis, exists as a bifunctional enzyme containing homoserine dehydrogenase (HSDH) activity (Wilson et al., 1991; Weisemann and Matthews, 1993). This parallels the situation in *E. coli*, except that *E. coli* also contains a gene encoding only AK activity. No plant gene encoding only AK activity has been conclusively identified at this time. AK-HSDH genes have been cloned from carrot (Weisemann and Matthews, 1993), *Arabidopsis* (Ghislain et al., in press), maize (Muehlbauer et al., 1993) and soybean (Gebhardt et al., 1993). A small family of genes encoding AK-HSDH is present in soybean, one of which maps close to the Rhg4 locus encoding soybean cyst nematode resistance (Weisemann et al., 1992). Four maize cDNA clones have been isolated which encode different AK-HSDH isoforms. One clone maps to maize chromosome 2, another to chromosome 4 (Muehlbauer et al., 1993).

DHDPS, the branch-point enzyme leading to lysine synthesis, has been cloned recently from wheat (Kaneko et al., 1990), maize (Frisch et al., 1991) and soybean (Silk and Matthews, 1993). The two different wheat clones encode dissimilar, putative chloroplast polypeptides and encode conserved mature portions (94% identical). Only one gene encoding DHDPS has been identified in soybean. It is similar to the genes from monocots in that it also encodes a putative chloroplast polypeptide. The soybean cDNA encoding DHDPS has been functionally expressed in *E. coli* and is more highly sensitive to inhibition by lysine than *E. coli* DHDPS.

Animals obtain essential amino acids from plant sources. One interest in the biochemistry and molecular biology of amino acid production is to learn how

essential amino acids are synthesized and how these pathways are regulated, so the pathways may be engineered to increase the amounts of essential amino acids in edible portions of the plant. Advances in nutritional improvement of the aspartate family of amino acids in crops has been recently reviewed (Matthews and Hughes, 1993). Currently, some of the strategies to increase levels of essential amino acids are being tested. These include mutant selection, modifying seed storage proteins, synthesizing new seed storage proteins, altering genes encoding enzymes regulating the synthesis of essential amino acids and using bacterial genes corresponding to genes encoding key plant enzymes controlling production of essential amino acids. The bacterial genes encode enzymes with regulatory properties different from those found in plants thus allowing over production of certain amino acids.

Recently, the *E. coli dapA* gene encoding DHDPS has been expressed in the chloroplasts of tobacco leaves (Shaul and Galili, 1992; Perl et al., 1993). Free lysine was elevated but no increase in protein-bound lysine was noted. No increase in lysine occurred when DHDPS was targeted to the cytosol, indicating a requirement for DHDPS to be translocated into the chloroplast where the other lysine synthesizing enzymes are found. Similar studies using potato indicate that *E. coli* DHDPS expressed in potato plants can increase free lysine levels (Perl et al., 1992)

A double mutant of *Nicotiana sylvestris* has been isolated which contains AK and DHDPS enzymes with altered feedback regulation. The mutant plant overproduced lysine (Frankard et al., 1992) and had altered morphology and development, probably due to increased free lysine levels.

Branched chain amino acids

The branched chain amino acid biosynthetic pathway is unique in the sense that a set of 4 enzymes carry out reactions in parallel pathways using different substrates leading to the biosynthesis of isoleucine or valine and leucine. A cDNA clone encoding threonine dehydratase (TD), the first enzyme in the isoleucine biosynthetic pathway, has been isolated from tomato (Samach et al., 1991). Surprisingly, the level of TD mRNA was >50-fold higher in sepals and >500-fold higher in the rest of the flower than in roots or leaves. However, the reason for such high levels of expression of biosynthetic TD mRNA in flowers is not understood. TD was believed to be present only as a feedback regulated form of the enzyme in higher plants. However, an isoleucine insensitive form of the enzyme has been identified and characterized in senescing tomato leaves (Szamosi et al., 1993). Isoleucine insensitive TD activity is first noticed when the leaf starts to show the symptoms of senescence. The highest specific activity of the enzyme is observed in the old, yellowish senescing leaves. During senescence, amino acids are liberated due to protein degradation. The new form of recently identified TD will degrade threonine and serine to release ammonia which can be converted to glutamine by glutamine synthetase. Glutamine can then be transported to the growing point or the storage tissue. Appearance of the isoleucine-insensitive enzyme suggests a role for this enzyme in remobilization of nitrogen during plant senescence.

Acetohydroxyacid synthase (AHAS) is the first enzyme common to the synthesis of the branched chain amino acids. cDNAs encoding AHAS have been isolated from several species, e.g. *Arabidopsis*, canola, corn, tobacco etc. (see Keeler et al., 1993 and references cited therein). Messages for AHAS are most prevalent in the dividing cells of developing organs (Keeler et al., 1993) which is consistent with the enzyme activity data (Stidham and Singh, 1991) and the studies monitoring the flux of carbon through this pathway (Singh et al., 1994). A cDNA clone encoding ketoacid reductoisomerase (KARI) has been isolated from a spinach expression library (Dumas et al., 1991). The deduced amino acid sequence shows the "fingerprint" region of NAD(P)H-binding site reported in several NAD(P)H-dependent oxidoreductases. There appears to be a single KARI gene per haploid genome of spinach. A cDNA for 3-isopropylmalate dehydrogenase (IPMDH) from *Brassica napus* has been isolated using functional complementation of yeast (Ellerstorm et al., 1992). All of the cDNAs isolated for AHAS, KARI and IPMDH contain a chloroplast transit peptide sequence, consistent with the chloroplastic localization of the branched chain amino acid biosynthetic pathway.

The branched chain amino acid biosynthetic pathway has received a great deal of attention in the last decade because of the discovery that two classes of highly successful commercial herbicides, imidazolinones and sulfonylureas, inhibit AHAS. Highly favorable environmental properties and high potency of these herbicides have led to the development of herbicide resistant crops (Newhouse et al., 1991, 1992). Imidazolinone resistant lines of corn, the first commercial agricultural biotechnology product, were introduced in 1991.

Phenomenal commercial success of the AHAS inhibiting herbicides has prompted a search for inhibitors of other enzymes of this pathway. Two potent inhibitors of KARI have been reported which have good herbicidal activity (Schulz et al., 1988; Wittenbach et al., 1992). Both inhibitors, Hoe 704 (2dimethylphosphinoyl-2-hydroxyacetate) and N-isopropyl oxalylhydroxamate (IpOHA), are substrate analogs. O-isobutenyl oxalylhydroxamate, which is herbicidal at 400 g/ha, has been identified as an inhibitor of isopropylmalate dehydrogenase (Wittenbach et al., 1992). A herbicidal inhibitor of isopropylmalate isomerase, 1-hydroxy-2-nitro-cyclopentane-1-carboxylic acid, has also been recently reported (Hawkes et al., 1993). Only time will tell if inhibitors of any other enzyme in this pathway will be as commercially successful as the AHAS inhibitors.

Proline biosynthesis

Despite the implicated role of proline in stress tolerance, the proline biosynthetic pathway has only recently been elucidated in higher plants. A cDNA clone that encodes pyrroline-5-carboxylate synthase (P5CS), a bifunctional enzyme containing glutamate kinase and glutamate semialdehyde dehydrogenase activities, has been isolated from *Vigna aconitifolia* (Hu et al., 1992). The bifunctional enzyme is feedback inhibited by proline. In the same laboratory, a cDNA clone for P5C reductase was isolated from a soybean root nodule library (Delauney and Verma, 1990). Two to three copies of this gene were found in the soybean genome and the message was found in nodules, roots and leaves. Salt stress

induced the expression of P5CS and P5CR which indicates a role of these enzymes in proline biosynthesis and osmoregulation in plants. However, a 50-fold overexpression of P5C reductase did not increase the proline content of transgenic tobacco plants suggesting that the reaction catalyzed by this enzyme may not be the rate limiting step for proline biosynthesis (Szoke et al., 1992).

An alternate route of P5C synthesis from ornithine by ornithine aminotransferase (OAT) has been proposed in *Vigna aconitifolia* (Delauney et al., 1993). Under normal physiological conditions, both glutamate and ornithine pathway contribute to proline biosynthesis. Salt stress and nitrogen starvation causes depression of OAT mRNA with a concomitant elevation of P5CS transcripts. In contrast, under excess nitrogen conditions, OAT transcripts were more abundant while P5CS mRNA remained at the constitutive levels. These results indicate the utilization of different pathways of proline biosynthesis under different environmental conditions.

References

Bandurski RS, Nonhebel HM (1989) Auxins. In: Wilkins MB (ed) Advanced plant physiology. John Wiley & Sons, Inc., New York, pp 1–20

Barry G, Kishore G, Padgette S, Taylor M, Kolacz K, Weldon M, Re D, Eichholtz D, Fincher K, Hallas L (1992) Inhibitors of amino acid biosynthesis: strategies for imparting glyphosate tolerance to crop plants. In: Singh BK, Flores HE, Shannon JC (eds) Biosynthesis and molecular regulation of amino acids in plants. American Society of Plant Physiologists, Rockville, pp 139–145

Berlyn MB, Last RL, Fink GR (1989) A gene encoding the tryptophan synthase β subunit of *Arbidopsis thaliana*. Proc Natl Acad Sci 86: 4604–4608

Becker TW, Caboche M, Carrayol E, Hirel B (1992) Nucleotide sequence of a tobacco cDNA encoding plastidic glutamine synthetase and light inducibility, organ specificity and diurnal rhythmicity in the expression of the corresponding genes of tobacco and tomato. Plant Molec Biol 19: 367–379

Cai X, Henry RL, Takemoto LJ, Guikema JA, Wong PP (1992) Antipeptide antibodies that can distinguish specific subunit polypeptides of glutamine synthetase from bean *Phaseolus vulgaris*. Plant Physiol 98: 402–405

Cock JM, Hemon P, Cullimore JV (1992) Characterization of the gene encoding the plastid-located glutamine synthetase of *Phaseolus vulgaris*: regulation of β-glucuronidase gene fusions in transgenic tobacco. Plant Mol Biol 18: 1141–1149

Delauney A, Verma DPS (1990) A soybean pyrroline-5-carboxylate reductase cDNA was isolated by functional complementation in *Escherichia coli* and was found to be osmoregulated. Mol Gen Genet 221: 299–305

Delauney A, Hu CA, Kavi Kishor PB, Verma DPS (1993) Cloning of ornithine δ-aminotransferase cDNA from *Vigna aconitifolia* by trans-complementation in *Escherichia coli* and regulation of proline biosynthesis. J Biol Chem 268: 18673–18678

Dumas R, Lebrun M, Douce R (1991) Isolation, characterization and sequence analysis of a full-length cDNA clone encoding acetohydroxy acid reductoisomerase from spinach chloroplasts. Biochemistry J 277: 469–475

Dyer WE, Henstrand JM, Handa AK, Herrmann KM (1989) Wounding induces the first enzyme of the shikimate pathway in Solanaceae. Proc Natl Acad Sci 86: 7370–7373

Dyer WE, Weaver LM, Zhao J, Kuhn DN, Weller SC, Herrmann KM (1990) A cDNA encoding 3-deoxy-D-arabino-heptulosonate 7-phosphate synthase from *Solanum tuberosum* L. J Biol Chem 265: 1608–1614

Ellerstorm M, Josefsson LG, Rask L, Ronne H (1992) Cloning of a cDNA for rape chloroplast 3-isopropylmalate dehydrogenase by genetic complementation in yeast. Plant Mol Biol 18: 557–566

Frankard V, Ghislain M, Jacobs M (1992) Two feedback-insensitive enzymes of the aspartate pathway in *Nicotiana sylvestris*. Plant Physiol 99: 1285–1293

Frisch DA, Tommey AM, Gengenbach BG, Somers DA (1991) Direct genetic selection of a maize cDNA for dihydrodipicolinate synthase in an *Escherichia coli dapA*-auxotroph. Plant Physiol 96: 444–452

Gantt JS, Larson RJ, Farnham MW, Pathirana SM, Miller SS, Vance CP (1992) Aspartate aminotransferase in effective and ineffective alfalfa nodules. Plant Physiol 98: 868–878

Gebhardt JS, Weisemann JM, Matthews BF (1993) Molecular analysis of the aspartate kinase-homoserine dehydrogenase gene family in soybean. Plant Physiol 102: 69

Ghislain MV, Frankard D, Vandenbossche, Matthews BF, Jacobs M (1994) Molecular analysis of the aspartate kinase-homoserine dehydrogenase gene from *Arabidopsis thaliana*. Plant Mol Biol 24: 835–851

Hawkes TR, Cox JM, Fraser TE, Lewis T (1993) A herbicidal inhibitor of isopropylmalate isomerase. Z Naturforsch 48c: 364–368

Herrmann KM, Zhao JM, Pinto JEBP, Weaver L, Henstrand JM (1992) Regulation of carbon flow into the shikimate pathway. In: Singh BK, Flores HE, Shannon JC (eds) Biosynthesis and molecular regulation of amino acids in plants. American Society of Plant Physiologists, Rockville, pp 12–18

Hu AC, Delauney AJ, Verma DPS (1992) A bifunctional enzyme pyrroline-5-carboxylate synthetase catalyzes the first two steps in proline biosynthesis in plants. Proc Natl Acad Sci 89: 9354–9358

Jensen RA (1986) The shikimate/arogenate pathway: link between carbohydrate metabolism and secondary metabolism. Physiol Plant 66: 164–168

Kamachi K, Yamaya T, Hayakawa H, Mae T, Ojima K (1992) Vascular bundle-specific localization of cytosolic glutamine synthetase in rice leaves. Plant Physiol 99: 1481–1486

Kaneko T, Hashimoto T, Kumpaisal R, Yamada Y (1990) Molecular cloning of wheat dihydrodipicolinate synthase. J Biol Chem 265: 17451–17455

Keeler SJ, Sanders P, Smith JK, Mazur BJ (1993) Regulation of tobacco acetolactate synthase gene expression. Plant Physiol 102: 1009–1018

Keith B, Dong X, Ausubel FM, Fink GR (1991) Differential induction of 3-deoxy-D-arabino-heptulosonate 7-phosphate synthase genes in *Arabidopsis thaliana* by wounding and pathogenic attack. Proc Natl Acad Sci 88: 8821–8825

Kuroki G, Conn EE (1988) Increased chorismate mutase levels as a response to wounding in *Solanum tuberosum* tubers. Plant Physiol 86: 895–898

Last RL, Bissinger PH, Mahoney DJ, Radwanski ER, Fink GR (1991) Tryptophan mutants in *Arabidopsis*: the consequences of duplicated tryptophan synthase β genes. Plant Cell 3: 345–358

Maio GH, Hirel B, Marsolier MC, Ridge RW, Verma DPS (1991) Ammonia-regulated expression of a soybean gene encoding cytosolic glutamine synthetase in transgenic Lotus corniculatus. Plant Cell 3: 11–22

Matthews BF, Hughes CA (1993) Nutritional improvement of the aspartate family of amino acids in edible crop plants. Amino Acids 4: 21–34

Matthews BF, Marmaras SM, Wadsworth GJ (1993) The soybean aspartate amino-transferase gene family. Plant Physiol 102: 69

McCue KF, Conn EE (1989) Induction of 3-deoxy-D-arabino-heptulosonate-7-phosphate synthase activity by fungal elicitor in cultures of *Petroselinum crispum*. Proc Natl Acad Sci 86: 7374–7377

Meister A (1992) Biochemistry of the amino acids. In: Takai K (ed) Frontiers and new horizons in amino acid research. Elsevier, Amsterdam, pp 357–372

Muehlbauer GJ, Somers DA, Matthews BF, Gengenbach BG (1993) Molecular genetics of maize aspartate kinase-homoserine dehydrogenase. Plant Physiol 102: 69

Newhouse KE, Singh BK, Shaner DL, Stidham MA (1991) Mutations in corn (*Zea mays* L.) conferring resistance to imidazolinone herbicides. Theor Appl Gen 83: 65–70

Newhouse KE, Smith WA, Starrett MA, Schaefer TJ, Singh BK (1992) Tolerance to imidazolinone herbicides in wheat. Plant Physiol 100: 882–886

Niyogi KK, Fink GR (1992) Two anthranilate synthase genes in *Arabidopsis*: defense-related regulation of the trypotophan pathway. Plant Cell 4: 721–733

Niyogi KK, Last RL, Fink GR, Keith B (1993) Suppresors of *trp1* fluorescence identify a new *Arabidopsis gene, trp4*, encoding anthranilate synthase β subunit. Plant Cell 5: 1011–1027

Perl A, Shaul O, Galili G (1992) Regulation of lysine synthesis in transgenic potato plants expressing a bacterial dihydrodipicolinate synthase in their chloroplasts. Plant Molec Biol 19: 815–823

Perl A, Galili S, Shaul O, Ben-Tzvi I, Galili G (1993) Bacterial dihydrodipicolinate synthase and desensitized aspartate kinase: Two novel selectable markers for plant transformation. BioTech 11: 715–727

Poulsen C, Bongaerts RJM, Verpoorte R (1993) Purification and characterization of anthranilate synthase from *Cathranthus roseus*. Eur J Biochem 212: 431–440

Rose AB, Casselman AL, Last RL (1992) A phosphoribosylanthranilate transferase gene is defective in blue fluorescent *Arabidopsis thaliana* tryptophan mutants. Plant Physiol 100: 582–592

Salazar RA, Taliercio EW, Muhitch MJ, Chourey PS (1993) Cloning and characterization of a glutamine synthetase cDNA from developing maize kernels. Plant Physiol 102: 68

Samach A, Harevent D, Gutfinger T, Ken-Dror S, Lifschitz E (1991) Biosynthetic threonine deaminase gene of tomato: isolation, structure and upregulation in floral organs. Proc Natl Acad Sci 88: 2678–2682

Schaller A, Schmid J, Leibinger U, Amrhein N (1991) Molecular cloning and analysis of a cDNA coding for chorismate synthase from higher plant *Corydalis sempervirens*. J Biol Chem 266: 21434–21438

Schmid J, Schaller A, Leibinger U, Boll W, Amrhein N (1992) The *in vitro* synthesized tomato shikimate kinase precursor is enzymatically active and is imported by and processed by chloroplasts. Plant J 2: 375–383

Schulz A, Spoenemann P, Koecher H, Wengenmayer F (1988) The herbicidally active experimental compound Hoe704 is a potent inhibitor of the enzyme acetolactate reductoisomerase. FEBS Lett 238: 375–378

Shaul O, Galili G (1992) Increased lysine synthesis in tobacco plants that express high levels of bacterial dihydrodipicolinate synthase in their chloroplasts. Plant J 2: 203–209

Silk GW, Matthews BF (1993) Cloning and expression of the soybean lysine pathway gene *DapA*. Plant Physiol 102: 18

Singh BK, Connelly JA, Siehl DL (1991) Shikimate pathway: why does it mean so much to so many? In: Miflin B (ed) Oxford surveys of plant molecular and cell biology, vol 7. Oxford University Press, Oxford, pp 143–186

Singh BK, Szamosi IT, Shaner DL (1994) Regulation of carbon flow through the branched chain amino acid biosynthetic pathway. In: Wallsgrove RM (ed) Amino acids and their derivatives in higher plants. Cambridge University Press, Cambridge (in press)

Stidham MA, Singh BK (1991) Imidazolinone-acetohydroxyacid synthase interactions. In: Shaner DL, O'Connor SL (eds) The imidazolinone herbicides. CRC Press, Boca Raton, pp 71–90

Szamosi IT, Shaner DL, Singh BK (1993) Identification and characterization of a biodegradative form of threonine dehydratase in senescing tomato (*Lycopersicon esculentum*) leaf. Plant Physiol 101: 999–1004

Szoke A, Miao GH, Hong G, Verma DPS (1992) Subcellular location of pyrroline-5-carboxylate reductase in root/nodule and leaf of soybean. Plant Physiol 99: 1642–1649

Turano FJ, Wilson BJ, Matthews BF (1991) Rapid purification and thermostability of the cytoplasmic aspartate aminotransferase from carrot suspension cultures. Plant Physiol 97: 606–612

Turano FJ, Weisemann JM, Matthews BF (1992) Identification and expression of a cDNA clone encoding aspartate aminotransferase in carrot. Plant Physiol 100: 374–381

Udvardi MK, Kahn ML (1991) Isolation and analysis of a cDNA clone that encodes an alfalfa (*Medicago sativa*) aspartate aminotransferase. Mol Gen Genet 231: 97–105

Wadsworth GJ, Marmaras SM, Matthews BF (1993) Isolation and characterization of a

soybean cDNA clone encoding the plastid form of aspartate aminotransferase. Plant Mol Biol 21: 993–1009

Weisemann JM, Matthews BF (1993) Identification and expression of a cDNA from *Daucus carota* encoding a bifunctional aspartokinase-homoserine dehydrogenase. Plant Molec Biol 22: 301–312

Weisemann JM, Matthews BF, Devine TE (1992) Molecular markers located proximal to the soybean cyst nematode resistance gene, *Rhg4*. Theor Appl Genet 85: 136–138

Wilson BJ, Gray AC, Matthews BF (1991) A bifunctional protein from carrot contains both aspartokinase and homoserine dehydrogenase activities. Plant Physiol 97: 1323–1328

Wittenbach V, Rayner D, Schloss J (1992) Pressure points in the biosynthetic pathway for branched-chain amino acids. In: Singh BK, Flores HE, Shannon JC (eds) Biosynthesis and molecular regulation of amino acids in plants. American Society of Plant Physiologists, Rockville, pp 69–88

Wright AD, Sampson MB, Neuffer MG, Michalczuk L, Slovin JP, Cohen JD (1991) Indole-3-acetic acid biosynthesis: De novo synthesis in the maize mutant orange pericarp, a tryptophan auxotroph. Science 254: 998–1000

Authors' address: Dr. B. K. Singh, American Cyanamid Company, P.O. Box 400, Princeton, NJ 08543 0400, U.S.A.

Received December 1, 1993