GrowGreen FERTILISER

TECHNICAL REVIEW — BIOSTIMULANTS

Amino Acids in Crop Production

Uptake, Transport, Metabolism, Stress Physiology and Agricultural Application

How plants take up, move and use amino acids, the roles of key amino acids from proline to GABA, and what trials show about application method, rate, timing and reduced nitrogen inputs.

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PUBLISHED

October 2026

LENGTH

35 pages

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October *2026*

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1. Introduction.

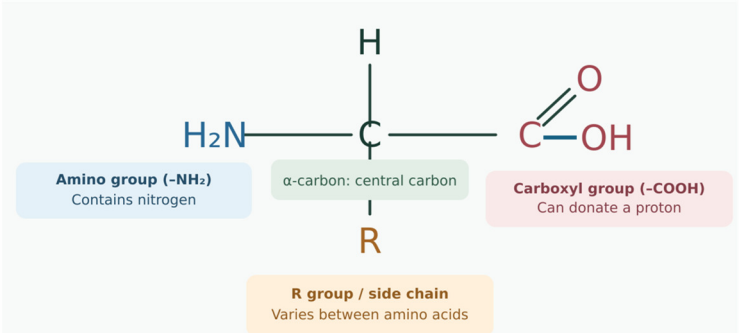
Amino acids are central to plant life. They provide the building blocks for proteins, but their roles extend well beyond protein synthesis: they connect carbon, nitrogen and sulphur metabolism and contribute to the production of other compounds needed for growth (Bady *et al.,* 2026).

Individual amino acids also have different physiological roles. Some contribute to the synthesis of compounds involved in growth and defence, while others participate in responses to environmental stress. Amino acids can also convey information about a plant's nutritional and metabolic state, although the mechanisms of amino-acid signalling are still being investigated (Bady *et al.,* 2026). Their roles depend on the identity of the amino acid, where it is located and how the plant uses it.

2. Definition of amino acids.

An amino acid is an organic molecule containing at least one amino group and one carboxyl group (Figure 1). Amino acids can be described as α-, β- or γ-amino acids according to the position of the amino group relative to the carboxyl group. The amino acids used to build plant proteins are α-amino acids: their amino group and carboxyl group are attached to the same carbon atom, known as the α-carbon. A variable part of the molecule, called the side chain or R group, gives each amino acid its particular chemical properties.

Figure 1. General structure of an α-amino acid.



Plants also contain amino acids outside proteins. These are called free amino acids, and many of them, including proline and glutamate, are α-amino acids. Plants also contain amino acids that are not used as standard building blocks for proteins. One example is γ-aminobutyric acid (GABA), whose amino group is in the γ position. GABA participates in plant metabolism and signalling (Bady *et al.,* 2026).

This distinction is especially important when considering agricultural applications. An amino acid's established function inside a plant does not, by itself, show that applying it to a leaf or root will produce the same effect. Uptake of externally applied amino acids is examined in Chapter 4, their transport and distribution within the plant in Chapter 5, and evidence for effects on crop growth and yield in Chapters 7 and 8.

2.1. General chemical structure of amino acids.

In a typical α-amino acid, the α-carbon is bonded to an amino group, a carboxyl group, a hydrogen atom and a variable side chain, represented by R in Figure 1. The amino and carboxyl groups are shared features of this structure; the side chain distinguishes one amino acid from another. In glycine, for example, the side chain is a hydrogen atom, whereas other amino acids have larger side chains containing groups that can affect their charge or chemical reactivity (Berthon, 1995).

In water, a free amino acid can carry a positive charge on its protonated amino group and a negative charge on its deprotonated carboxyl group. Both groups remain attached to the same molecule; only protons are gained or lost. The proportions of the different charged forms depend on pH (Berthon, 1995).

The side chain contributes additional chemical properties. Depending on its structure and the pH, it may be charged or uncharged, and some side chains can bind mineral ions through atoms such as nitrogen, oxygen or sulphur (Berthon, 1995). These differences are particularly relevant when comparing amino acids as mineral-complexing agents (see Dr Vila's article Amino Acids as Metal-Chelating Agents Properties and Comparison with Conventional Chelators GrowGreen web page).

2.2. Classification of amino acids.

Amino acids can be classified according to whether they are used to build proteins and according to the chemical properties of their side chains. Proteinogenic amino acids are the

20 standard amino acids incorporated into plant proteins. Plants also contain non-proteinogenic amino acids that are not standard protein building blocks but have other metabolic roles. One example is γ-aminobutyric acid (GABA), which also participates in plant signalling (Bady *et al.,* 2026). Either type can occur as a free amino acid, meaning that the molecule is not currently incorporated into a protein.

Table 1 classifies the 20 amino acids used to build plant proteins according to the chemical properties of their side chains. The plant roles listed are examples; amino acids within the same group do not necessarily perform the same functions (Berthon, 1995; Galili *et al.,* 2016).

Table 1. Classification of the 20 proteinogenic amino acids by side-chain properties

Side-chain group	**Amino acids**	**What the structure means**	**Relevant plant roles**
Nonpolar	Glycine, alanine, valine, leucine, isoleucine, proline and methionine	Their side chains generally interact less favourably with water than charged or strongly polar side chains. This affects how they interact with other parts of a protein.	These amino acids help determine protein structure. Valine, leucine and isoleucine also share related biosynthetic and breakdown pathways (Galili *et al.,* 2016).
Aromatic	Phenylalanine, tyrosine and tryptophan	Their ring structures influence how they interact with other molecules and provide chemical starting points for further synthesis. An aromatic ring does not mean all three have the same properties or roles.	Phenylalanine contributes to the synthesis of phenylpropanoid compounds; tryptophan is a precursor in several plant metabolic pathways, including pathways leading to auxin (Galili *et al.,* 2016).
Polar, uncharged	Serine, threonine, asparagine, glutamine and cysteine	Their side chains can form interactions with water and other molecules. Cysteine's thiol group has additional chemical reactivity.	Glutamine is important in nitrogen metabolism; cysteine connects sulphur assimilation with the synthesis of other sulphur-containing compounds (Berthon, 1995; Bady *et al.,* 2026).
Acidic	Aspartate and glutamate	Their side chains usually carry a negative charge near neutral pH, allowing electrostatic interactions	Glutamate has a central role in amino-acid metabolism; aspartate provides a starting point for the synthesis of several

Side-chain group	Amino acids	What the structure means	Relevant plant roles
		and, under suitable conditions, interactions with metal ions.	other amino acids (Berthon, 1995; Galili *et al.,* 2016).
Basic	Lysine, arginine and histidine	Their nitrogen-containing side chains can carry a positive charge. Histidine can gain or lose a proton over a pH range relevant to many biological systems.	Their charge affects interactions within proteins; arginine is also involved in plant nitrogen storage and metabolism. Histidine's side chain can participate in metal binding (Berthon, 1995).

Cysteine and methionine both contain sulphur, but they use it differently. Cysteine has a reactive sulphur-containing group that helps regulate proteins and supports the plant's response to oxidative stress (Bady *et al.,* 2026). Methionine is used to produce other important compounds, including molecules involved in growth regulation and ethylene production (Galili *et al.,* 2016). This is why the two amino acids have different roles in the plant despite both containing sulphur.

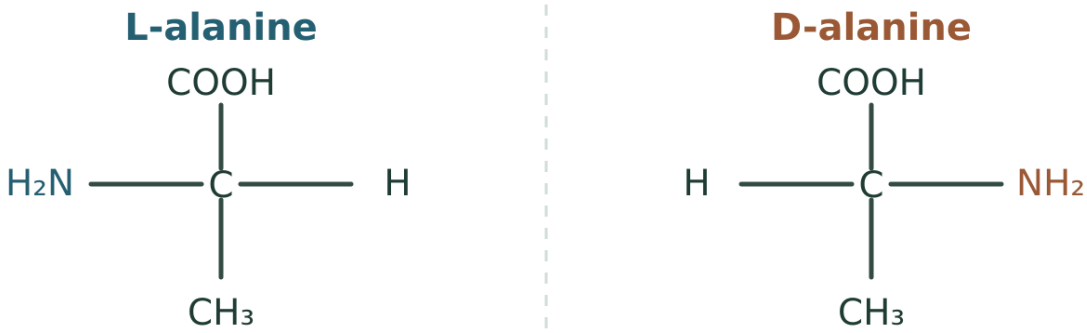
The term *essential amino acids* usually refers to amino acids that humans must obtain from their diet (histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan and valine). Plants can synthesise these amino acids themselves, so "essential" does not mean that they must be applied to the crop. Their concentration in harvested crops is nevertheless important for the nutritional quality of food and feed (Galili *et al.,* 2016).

2.3. L- and D-amino acids.

Most α-amino acids can exist in two forms, designated L and D. They contain the same atoms and bonds, but the groups around the α-carbon have different three-dimensional arrangements, like a left and right hand. These forms are called enantiomers.

Glycine is the exception: its side chain is a hydrogen atom, so its α-carbon has two identical groups and glycine has no L- or D-form. An example of L- and D-alanine can be seen in Figure 2.

Figure 2. L- and D-alanine



Plants use L-amino acids as the standard building blocks of proteins. Roots can also absorb D-amino acids but absorbing one does not mean that the plant inserts it into a protein. Instead, the plant may break it down and use some of its components. For example, Hill *et al.* (2011) found that wheat absorbed D-alanine and released some of its carbon as carbon dioxide during respiration. They also concluded that wheat could use nitrogen supplied as D-alanine. In the same experiment, wheat absorbed L-alanine faster than D-alanine. These results show that the two forms can both enter the plant, but they do not have identical roles or behaviour after uptake (Hill *et al.,* 2011).

Processing can change the balance between L- and D-amino acids in a fertiliser. Chemical hydrolysis commonly uses strong acids or alkalis to break down proteins; under such processing conditions, some L-amino acids can change into D-amino acids through racemisation. Sánchez-Hernández *et al.* (2013) measured substantially more L-to-D conversion in the chemically hydrolysed fertilisers they tested than in those made by enzymatic hydrolysis. This may reduce the proportion of L-amino acids available for the plant's normal protein-synthesis pathways.

3. Amino Acids in Plant Metabolism.

A plant continually makes, uses and breaks down amino acids. Their concentrations are therefore not fixed: they change as nitrogen becomes available, proteins are produced or degraded, and growing tissues require new materials. Amino-acid synthesis is also connected to carbon metabolism, which supplies the carbon frameworks from which these molecules are made (Hildebrandt *et al.,* 2015; Bady *et al.,* 2026).

An amino acid may be incorporated into a protein, remain temporarily in the free amino-acid pool, or be converted into another compound. These routes are regulated rather than interchangeable: the plant adjusts them in response to its nutritional state, development and environment. Changes in some amino-acid pools can also act as signals affecting plant responses, although the mechanisms are not fully understood (Hildebrandt *et al.,* 2015; Bady *et al.,* 2026).

3.1. Amino-acid biosynthesis.

Plants synthesise amino acids by combining nitrogen with carbon compounds produced through metabolism. The nitrogen may come from ammonium absorbed by the roots, nitrate converted to ammonium within the plant, urea converted to ammonium in the soil or within the plant, or nitrogen released when other compounds are broken down (Pratelli and Pilot, 2014; Mérigout *et al.,* 2008). The carbon components come from processes such as photosynthesis and respiration. Amino acid synthesis therefore depends on both the plant's nitrogen supply and its supply of carbon compounds (Pratelli and Pilot, 2014; Bernard and Habash, 2009).

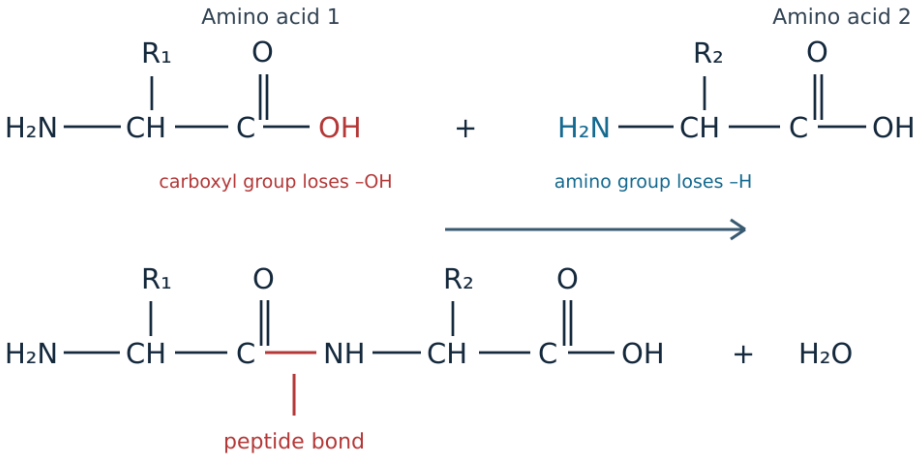
Glutamine and glutamate play a central role in supplying nitrogen for amino-acid synthesis. Glutamine synthetase combines ammonium with glutamate to form glutamine. Glutamate synthase then uses glutamine and a carbon compound called 2-oxoglutarate to produce glutamate. From there, the plant can transfer nitrogen into different pathways that make other amino acids. Each pathway uses its own carbon structure, which helps determine the amino acid produced; the other amino acids do not all begin as glutamine molecules (Bernard and Habash, 2009; Pratelli and Pilot, 2014).

The plant then uses a range of pathways to produce different amino acids. For example, compounds derived from carbohydrate metabolism provide the carbon needed to make alanine, serine and aspartate. Nitrogen can be transferred from glutamate or glutamine into these pathways. Each amino acid has its own sequence of reactions, and the activity of these pathways changes with nitrogen availability, carbon supply and the plant's demand for their products (Pratelli and Pilot, 2014).

3.2. Protein synthesis.

Plants use amino acids to make proteins needed for growth and cellular activity. A ribosome is a small structure inside a cell that assembles a protein. It reads instructions carried by messenger RNA, while transfer RNA brings amino acids to it in the specified order. The ribosome joins neighbouring amino acids with peptide bonds. A peptide bond is the chemical link formed between the carboxyl group of one amino acid and the amino group of the next (Figure 3). As more amino acids are joined, they form a chain that folds into a protein (Chery and Drouard, 2023).

Figure 3. Formation of a peptide bond



Ribosomes use amino acids supplied from the plant's free amino-acid pools, with transfer RNA delivering each amino acid for incorporation into the growing protein chain. These amino acids may be synthesised within the plant, imported from other tissues or recovered through protein breakdown. Protein synthesis occurs in the cytosol and within chloroplasts and mitochondria (Chery and Drouard, 2023; Hildebrandt et al., 2015; Pratelli and Pilot, 2014).

3.3. Free amino-acid pools.

Free amino acids are amino acids that have not been joined into proteins. The term *free amino-acid pool* refers to the amounts of these individual molecules available in a tissue or part of a cell at a given time. It does not describe a single store: different amino acids are present in different amounts, and their concentrations change as the plant makes, uses and transports them (Pratelli and Pilot, 2014; Bady *et al.,* 2026).

Location matters as well as amount. In a study of spinach leaves, Riens *et al.* (1991) found free amino acids in the cytosol, chloroplasts and vacuoles, with different concentrations in each. An amino acid present in one part of a cell may need to cross a membrane before it can be used elsewhere. The amount measured in a whole leaf therefore does not necessarily show how much is available at a particular site of protein synthesis or another metabolic reaction (Riens *et al.,* 1991; Pratelli and Pilot, 2014).

The size and composition of these pools reflect the balance between amino-acid synthesis, protein turnover, transport and metabolic use. This balance changes with growth, nutrient availability and environmental conditions (Pratelli and Pilot, 2014; Bady *et al*., 2026).

3.4. Amino acids as metabolic intermediates.

Free amino acids provide substrates for metabolic pathways beyond protein synthesis. These functions involve both proteinogenic and non-proteinogenic amino acids and connect amino-acid metabolism with the production of other compounds required by the plant (Hildebrandt *et al*., 2015; Pratelli and Pilot, 2014).

For example, free phenylalanine is a starting material for phenylpropanoids, a group of compounds that includes components of lignin in plant cell walls. Free methionine can be converted into S-adenosylmethionine, which participates in the production of ethylene and other compounds. Both phenylalanine and methionine can therefore be used to build proteins or converted into other products (Pratelli and Pilot, 2014; Ravanel *et al.,* 1998). Their individual roles are discussed further in Section 6.

Non-protein-forming amino acids also participate in plant metabolism. GABA, for instance, is produced from glutamate and can be converted into other compounds rather than incorporated as a standard building block of proteins. Amino acids may also be broken down, allowing the plant to reuse their nitrogen while their carbon components enter other pathways. Which route an amino acid follows depends on the plant's needs and conditions (Hildebrandt *et al.,* 2015; Bady *et al.,* 2026).

3.5. Amino acids as signalling molecules.

Some amino acids do more than supply material for proteins or other compounds. Changes in their concentration or location can also trigger a response in the plant. In this context, an amino acid acts as a signal: its presence is detected and helps alter cellular activity. An

amino acid can have both a metabolic role and a signalling role; the two are not mutually exclusive (Bady *et al.,* 2026).

When plant tissue is wounded, glutamate can be released into the spaces around cells. Other cells can detect this glutamate and respond to the injury. This is an example of glutamate acting as a signal, alongside its other roles as an amino acid (Bady *et al.,* 2026).

Glutamine provides a different example. It is formed when the plant incorporates nitrogen into amino acids, so changes in its availability can provide information about the plant's nitrogen status. That information can help the plant adjust its growth and metabolism to the resources available. This does not mean that a rise in glutamine will always increase growth (Bady *et al.,* 2026).

4. Uptake of Exogenous Amino Acids.

Exogenous amino acids are amino acids supplied from outside the plant, including those present in soil or applied to roots or leaves. Before an applied amino acid can take part in plant metabolism, it must reach a plant surface and cross into plant tissue. For root applications, this also depends on what happens to the amino acid in the soil: microorganisms may use it before the roots can absorb it (Näsholm *et al.,* 2009).

Uptake and use are separate steps. Detecting an applied amino acid inside a plant shows that some was absorbed, but it does not establish whether the plant incorporated it into a protein, converted it into another compound or broke it down. The amount absorbed and what happens afterward may also differ between amino acids and between root and foliar application (Näsholm *et al.,* 2009; Pratelli and Pilot, 2014).

4.1. Root uptake.

An amino acid dissolved in soil water can reach the root surface and move with water through spaces in the root cell walls. At this stage, it may be within the root tissue but remains outside the living cells. To enter a root cell, the amino acid must cross its cell membrane, usually with the help of a transport protein. The amount available for this uptake also depends on how quickly soil microorganisms use or transform the amino acid (Lee *et al.,* 2007; Näsholm *et al.,* 2009).

Free amino acids enter root cells mainly through transport proteins in the cell membrane. In *Arabidopsis*, LHT1 contributes to the uptake of neutral amino acids such as alanine and glutamine, and acidic amino acids such as glutamate and aspartate. AAP5 is particularly important for the positively charged amino acids lysine and arginine. Other transporters also contribute to root uptake. These findings do not provide a complete list of transport routes for every amino acid or every crop, and they do not show that an amino acid lacking a named transporter cannot be absorbed (Hirner *et al.,* 2006; Svennerstam *et al.,* 2008; Pratelli and Pilot, 2014).

The uptake mediated by these transporters is generally an active process. Many carry an amino acid into the cell together with a hydrogen ion. The plant uses energy to maintain the difference in hydrogen-ion concentration across the membrane, and this difference helps drive transport. Thus, the transporter does not need to use an ATP molecule directly for each amino acid it carries, but the process still depends indirectly on the plant's energy supply (Hirner *et al.,* 2006).

Roots can also acquire some short peptides. A peptide consists of two or more amino acids joined together; a dipeptide contains two, a tripeptide three and a tetrapeptide four. These molecules require transport routes distinct from those used for free amino acids. In *Arabidopsis*, Komarova *et al.* (2008) showed that AtPTR1 contributes to root uptake of tested dipeptides. This does not mean it carries every possible combination of two amino acids. Soper *et al.* (2011) found that certain peptides containing up to four amino acids could support growth under controlled conditions and detected some supplied peptides intact in plant tissues. Their findings do not identify a transporter for every tetrapeptide or establish a universal size limit for root uptake (Komarova *et al.,* 2008; Soper *et al.,* 2011).

A peptide may also be cut into shorter peptides or free amino acids before entering a root cell. Proteases and peptidases produced by soil microorganisms can cut peptide bonds. Plant roots can contribute too: Adamczyk *et al.* (2009) found protein-cutting enzymes released by leek roots, while Greenfield *et al.* (2020) found that most measured root protease activity in maize and wheat remained at the root surface. These are enzyme-driven processes, rather than peptides simply being dissolved by acid released from roots. The resulting free amino acids may then enter through amino-acid transporters, while some

short peptides may enter intact through peptide transporters (Adamczyk *et al.,* 2009; Greenfield *et al.,* 2020; Komarova *et al.,* 2008).

4.2. Foliar uptake.

Foliar uptake involves two distinct stages: passage across the leaf surface and entry into living leaf cells. The cuticle and pathways associated with stomata provide routes across the surface, whereas transport proteins in cell membranes help amino acids enter the cells. These stages must be distinguished when interpreting experiments: passage through an isolated cuticle demonstrates surface penetration, while uptake by exposed leaf cells demonstrates membrane transport (Arand *et al.,* 2010; Eichert *et al.,* 2008; Hirner *et al.,* 2006).

4.2.1. Passage through the cuticle.

Direct evidence of amino-acid passage through the cuticle comes from experiments with ivy (*Hedera helix*). Arand *et al.* (2010) isolated cuticles from the upper leaf surface, which has no stomata, and measured the passage of glycine, alanine, proline, valine, leucine and phenylalanine. Their molecular masses ranged from 75.1 g mol^{-1} for glycine to 165.2 g mol^{-1} for phenylalanine. All six crossed the isolated cuticles. Because stomata were absent, this experiment demonstrated a cuticular route for these particular free amino acids (Arand *et al.,* 2010).

The authors attributed this movement to diffusion through water-containing, polar pathways within the cuticle. At each pH tested, permeability decreased as amino-acid molecular volume increased. However, size was not the only factor: pH affected both cuticular hydration and the amino acids' charged forms and hydration shells, the water molecules associated with them. Removing cuticular waxes increased amino-acid permeability at pH 6 by approximately 12- to 19-fold, showing that waxes substantially restricted passage even though they did not completely prevent it (Arand *et al.,* 2010).

From the measured passage of amino acids through ivy cuticles, Arand *et al.* (2010) estimated an average radius of approximately 0.6 nm for the water-containing pathways at pH 6, equivalent to an average width of approximately 1.2 nm. This was a calculated estimate, rather than a directly measured opening or a strict molecular-size limit. For comparison, the diffusion-based radii compiled by Gross *et al.* (2025, Table 2) correspond to

effective diameters in water ranging from approximately 0.464 nm for glycine to 0.738 nm for lysine among the 20 standard protein-forming amino acids. These values describe effective size during movement through water, rather than a rigid molecular width, and were obtained at pH 3.5. On this approximate size comparison, lysine is smaller than the estimated average pathway width; it cannot therefore be considered excluded on size alone. However, the different experimental conditions and the influence of molecular charge and hydration prevent this comparison from establishing permeability. Arand *et al.* did not test lysine, so its passage through ivy cuticles remains unconfirmed by that experiment (Arand *et al.,* 2010).

4.2.2. Passage through stomatal pathways.

The studies examined do not directly demonstrate stomatal entry of free amino acids or small peptides. However, experiments using other materials provide information about the size of substances that can enter through this route. Eichert *et al.* (2008) applied fluorescent polystyrene beads to broad-bean (*Vicia faba*) leaves and observed occasional entry of beads approximately 43 nm in diameter through stomata. Entry of the larger beads tested, approximately 1.1 µm in diameter, was not observed. These findings demonstrate a stomatal pathway capable of admitting the smaller beads under the experimental conditions, but do not establish a maximum size or demonstrate amino-acid or peptide entry through that pathway (Eichert *et al.,* 2008).

Particle entry was observed through no more than approximately 10% of stomata. The authors interpreted this movement as diffusion along thin water films lining the stomatal pores. Although both tested particle sizes were smaller than the stomatal openings, only the 43 nm particles entered, indicating that opening width alone did not determine penetration (Eichert *et al.,* 2008).

The broad-bean experiment therefore establishes 43 nm as a demonstrated particle diameter under its experimental conditions, rather than a universal maximum for stomatal entry. It did not test amino-acid peptides of different lengths. Its results cannot establish that every peptide smaller than 43 nm will enter or assign a maximum number of amino acids to a peptide capable of stomatal penetration (Eichert *et al.,* 2008).

4.2.3. Entry into living leaf cells.

Once an amino acid reaches the apoplast, it remains outside living cells and must cross a cell membrane to enter them. Hirner *et al.* (2006) demonstrated that LHT1, the same *Arabidopsis* amino-acid transporter discussed for root uptake, also contributes to uptake into leaf mesophyll cells. These are internal leaf cells involved in photosynthesis. The study located LHT1 at the plasma membrane and found reduced amino-acid uptake in mesophyll cells isolated from plants lacking functional LHT1. Its role therefore concerns passage across the cell membrane, rather than transport through the cuticle or stomatal opening (Hirner *et al.,* 2006).

Experiments expressing LHT1 in yeast also showed that its transport activity depended on an energy supply. Removing glucose reduced uptake to background levels, while disrupting the hydrogen-ion gradient stopped uptake. The authors interpreted these findings as evidence for transport of amino acids together with hydrogen ions. This energy-dependent membrane transport is a separate process from diffusion through the cuticle or along stomatal water films (Hirner *et al.,* 2006).

4.2.4. Peptide uptake and the limits of the size evidence.

Jamaï *et al.* (1996) demonstrated uptake of leucine–leucine, a dipeptide containing two leucine residues, by broad-bean leaf tissue. Importantly, the experiment used peeled leaf discs, incubated with their peeled surface in contact with the solution. It therefore examined uptake by exposed tissue rather than passage across an intact waxy cuticle or through stomata. Uptake varied with concentration and pH and was inhibited by a substance that disrupts the hydrogen-ion gradient, supporting the involvement of membrane transport systems. The study did not identify those systems as the *Arabidopsis* root transporter AtPTR1 (Jamaï *et al.,* 1996).

4.3. Factors affecting amino-acid uptake.

4.3.1. Competition between amino acids.

Amino acids supplied together can compete for the same transporter. In yeast expressing *Arabidopsis* LHT1, Hirner *et al.* (2006) measured labelled proline uptake in the presence of other amino acids at ten times the proline concentration. Cysteine and phenylalanine produced the strongest competition, whereas histidine, lysine and arginine produced the

weakest. This experiment shows that the composition of an amino-acid mixture can influence transport of an individual component. It does not establish the magnitude of this effect in an applied crop formulation (Hirner *et al.,* 2006).

4.3.2. Regulation by nitrogen supply.

Transporter expression can change with the nitrogen supplied to the plant. Hirner *et al.* (2006) found low LHT1 expression in *Arabidopsis* seedlings grown on medium containing 40 mM ammonium nitrate, whereas expression increased when particular amino acids or nitrate were supplied as the sole nitrogen source at 5 mM. Because both nitrogen concentration and composition differed between these treatments, the findings should not be attributed to nitrogen form alone. They demonstrate that LHT1 expression responds to the nitrogen supply, rather than remaining constant under all conditions (Hirner *et al.,* 2006).

4.3.3. Leaf development.

Hirner *et al.* (2006) also found that the pattern of LHT1 expression changed during leaf development. Expression was weaker in young leaves and became widespread in mesophyll cells in mature leaves. This establishes a developmental change in the distribution of a transporter involved in cellular amino-acid uptake. It does not demonstrate that mature leaves absorb more amino acids from a foliar spray, because penetration across the intact leaf surface was not compared between leaf ages (Hirner *et al.,* 2006).

5. Transport and distribution within the plant.

Amino acids absorbed by the plant may be used near the site of application or transported to other organs. Their subsequent use depends on their distribution between tissues and cellular compartments. For amino acid fertilisers, uptake is therefore only the first step: transport through the xylem and phloem helps connect the site of absorption with tissues requiring amino acids for growth, metabolism or storage (Pratelli and Pilot, 2014).

5.1. Xylem transport.

The xylem carries water and dissolved substances from roots towards the shoot. Amino acids present in roots can enter this stream and move to above-ground tissues, particularly leaves, with the water flow associated with transpiration. These amino acids may originate

from uptake from the soil, nitrogen assimilation within roots or redistribution from other tissues (Pratelli and Pilot, 2014; Tegeder, 2014).

For a fertiliser applied to the root zone, this provides a route by which absorbed amino acids can reach tissues beyond the roots. However, the compound transported is not necessarily identical to the one supplied. Roots may metabolise an absorbed amino acid and transfer its nitrogen into other amino acids before export. Studies discussed by Näsholm *et al*. (2009) found that nitrogen supplied as glycine was recovered in several other amino acids, while enrichment of glycine in the xylem also supported transport of a fraction without prior conversion (Näsholm *et al.,* 2009).

Amino acids carried in the xylem can be taken up by surrounding tissues or transferred to the phloem for redistribution to growing organs. Xylem transport therefore connects root uptake with wider distribution but does not mean that every supplied amino acid reaches every organ unchanged or in the same proportion (Pratelli and Pilot, 2014; Tegeder, 2014).

5.2. Phloem transport.

The phloem distributes amino acids from supplying tissues, such as mature leaves, to organs requiring them, including young leaves, roots, fruits and developing seeds. Unlike the predominantly upward movement in the xylem, phloem transport can supply organs above or below the exporting leaf. Movement occurs through sieve tubes, driven by a pressure difference between supplying and receiving tissues (Tegeder and Hammes, 2018).

For amino acids absorbed through a foliar application, the phloem provides a potential route from the treated leaf to other parts of the plant. However, absorption into the leaf does not automatically establish export: the amino acids must become available for loading into the phloem. Loading and subsequent unloading into receiving tissues help determine their distribution (Tegeder and Hammes, 2018).

All standard protein-forming amino acids have been reported in phloem sap, but their concentrations and relative proportions vary with plant metabolism and physiological conditions. Consequently, the composition of a supplied fertiliser should not be assumed to remain unchanged during transport, or to be delivered equally to every organ (Tegeder and Hammes, 2018).

5.3. Source–sink relationships and remobilisation.

A source is a tissue that supplies amino acids for export, whereas a sink receives them to support growth, metabolism or storage. Mature leaves commonly act as sources, while developing leaves, roots, fruits and seeds act as sinks. These roles change during development: a young leaf initially depends on imported nutrients but can later become a supplying tissue as it matures (Tegeder, 2014; Tegeder and Hammes, 2018).

Distribution therefore reflects the changing requirements of different organs. During vegetative growth, developing shoots and roots require amino acids for protein synthesis and other metabolic processes involved in new tissue formation. During seed development, amino acids also provide the building blocks for the formation of storage proteins. Their allocation connects the supply from source tissues with their use in receiving organs (Tegeder and Hammes, 2018).

Remobilisation contributes to this supply by allowing the plant to recover amino acids from existing reserves. During leaf senescence (the ageing process preceding leaf death) proteins are broken down, releasing amino acids that can be exported to other tissues. These amino acids can then contribute to metabolism and the formation of new proteins, including storage proteins in developing seeds. The plant therefore reuses material previously invested in its tissues rather than depending entirely on continued uptake from an external supply (Tegeder, 2014; Tegeder and Hammes, 2018).

6. Physiological Functions of Key Amino Acids.

Beyond their shared role in protein synthesis, individual amino acids participate in different metabolic processes. The following subsections examine the functions of different amino acids and distinguish their roles within the plant from the effects demonstrated when they are supplied externally. This distinction helps assess their relevance as components of fertilisers without assuming that application necessarily enhances every physiological function associated with them (Hildebrandt *et al.,* 2015; Pratelli and Pilot, 2014).

6.1. Proline.

Proline is a protein-forming amino acid that also participates in plant responses to stress. It can accumulate as a compatible solute, helping cells maintain water balance and turgor during drought or salinity without substantially disrupting normal cellular functions. Its

synthesis and breakdown also interact with the reactions that transfer electrons during metabolism. These reactions are important during stress, when disturbances in photosynthesis and respiration can increase the production of reactive oxygen species and contribute to cellular damage (Hildebrandt *et al.,* 2015; Pratelli and Pilot, 2014).

In *Arabidopsis*, the P5CS1 gene encodes an enzyme involved in producing proline from glutamate. Székely *et al*. (2008) found that disrupting this gene reduced proline accumulation during stress and increased sensitivity to salinity, ROS accumulation and membrane lipid damage. Several enzymes involved in removing ROS also showed lower activity in the affected plants. These findings suggest that proline helps preserve the activity or stability of antioxidant enzymes, supporting the plant's ability to control oxidative damage, rather than acting solely by directly neutralising ROS. Proline accumulation can also contribute to osmotic adjustment, helping cells retain water during drought or salinity, although this study found that its contribution to overall leaf water retention was limited. The evidence therefore supports complementary roles in water balance and antioxidant protection, without establishing that externally supplied proline produces the same effects (Székely *et al.,* 2008).

External application has nevertheless produced benefits in particular crop experiments. In young olive trees exposed to salinity, supplied proline mitigated reductions in growth and photosynthetic activity and improved aspects of water status and antioxidant enzyme activity. The response differed between treatments, showing that the benefit depended on the experimental conditions (Ben Ahmed *et al.,* 2010).

Foliar application of proline has also been investigated in tomato plants grown under high temperatures. Tonhati *et al*. (2020) reported reduced levels of hydrogen peroxide, a reactive oxygen species (ROS) that can contribute to oxidative damage when it accumulates excessively. Proline treatment also reduced malondialdehyde, a compound formed during the oxidative breakdown of membrane lipids. Its lower concentration therefore indicated less membrane lipid damage. These reductions occurred without an increase in the measured antioxidant enzyme activities, showing that the protective response was not accompanied by greater activity of those enzymes. The results do not, however, establish the precise mechanism responsible for that protection (Tonhati *et al.,* 2020).

6.2. Glycine.

Glycine is a protein-forming amino acid that also participates in photorespiration. Rubisco, a key enzyme in photosynthesis, normally incorporates carbon dioxide into the pathway used to produce sugars. However, it can also react with oxygen instead of carbon dioxide. This reaction produces a compound that the plant must recycle through photorespiration, converting it back into material usable for sugar production. Glycine and serine are formed during this recycling process. Glycine is an intermediate in this recovery process and is converted into serine. Its involvement therefore supports normal leaf metabolism but does not establish that applying additional glycine increases photosynthesis (Hildebrandt *et al.,* 2015; Pratelli and Pilot, 2014).

Glycine is one of the three amino acids used to form glutathione, alongside glutamate and cysteine. Glutathione is a small peptide consisting of these three amino acids joined together, rather than a single amino acid or a protein. It participates in the plant's antioxidant defence system, helping remove hydrogen peroxide and regenerate other antioxidants. Glycine therefore contributes as a building block for this protective compound; this does not establish that free glycine itself removes ROS or that every glycine application increases antioxidant protection (Hildebrandt *et al.,* 2015).

Foliar application of glycine improved specific growth measurements in lettuce under greenhouse conditions. Noroozlo *et al*. (2019) tested concentrations of 250, 500 and 1000 mg L^{-1} against a control sprayed with water. At 500 mg L^{-1}, glycine significantly increased both shoot fresh and dry weights compared with the control, while 250 mg L^{-1} increased root fresh weight and total leaf chlorophyll. These results demonstrate that glycine application can promote biomass accumulation under the conditions tested, with the effective concentration depending on the characteristic measured. However, the experiment did not establish the mechanism responsible for these improvements: increased chlorophyll alone does not demonstrate a higher photosynthetic rate or explain the increase in biomass (Noroozlo *et al.,* 2019).

Glycine must also be distinguished from glycine betaine, a chemically different compound frequently studied for protection against drought, salinity and temperature stress. Findings from glycine betaine treatments cannot be attributed directly to glycine. For fertilisers containing glycine, the relevant evidence is therefore the response to glycine itself, with its

normal metabolic functions providing context rather than proof of a particular treatment benefit (Pratelli and Pilot, 2014; Noroozlo *et al.,* 2019).

6.3. Glutamate and glutamine.

Glutamate and glutamine are protein-forming amino acids with a central role in incorporating absorbed nitrogen into plant metabolism. Plants first convert nitrate into ammonium, while ammonium taken up directly can enter this process without that conversion. The enzyme glutamine synthetase combines ammonium with glutamate to produce glutamine. A second enzyme, glutamate synthase, transfers nitrogen from glutamine to a carbon-containing molecule, producing glutamate. Together, these reactions allow the plant to incorporate inorganic nitrogen into amino acids that can support growth (Hildebrandt *et al.,* 2015; Pratelli and Pilot, 2014).

Glutamate subsequently supplies amino groups (the nitrogen-containing parts of amino acids) for the formation of many other amino acids. Glutamine also supplies nitrogen for the synthesis of compounds such as nucleotides, the building blocks of DNA and RNA. Their importance therefore extends beyond their own incorporation into proteins: they help provide the materials needed to produce other amino acids and cellular components. Glutamate is also a precursor for proline and contributes to glutathione formation (Hildebrandt *et al.,* 2015), linking it with the protective processes described in section 6.1.

Glutamate can additionally act as a signal. In *Arabidopsis*, Toyota *et al*. (2018) demonstrated that glutamate released following tissue damage activates channels that allow calcium ions to enter cells. The resulting changes in calcium concentration transmit information from the damaged area to other leaves, helping activate defence responses elsewhere in the plant. This illustrates how a free amino acid can participate in coordinating a response to damage, alongside its role in nutrition and protein formation (Toyota *et al.,* 2018).

Evidence for external application must nevertheless be considered separately from these natural functions. In the lettuce experiment described above, foliar glutamine at 250 and 500 mg L^{-1} significantly increased leaf vitamin C compared with the water-sprayed control, while 1000 mg L^{-1} increased total leaf chlorophyll. These findings demonstrate improvements in particular leaf characteristics under the conditions tested, but do not

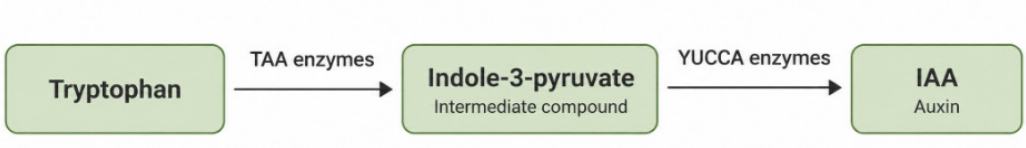
establish that glutamine application increased photosynthesis or that glutamate application would produce the same response (Noroozlo *et al*., 2019).

6.4. Tryptophan.

Tryptophan is a protein-forming amino acid that also provides starting material for the production of indole-3-acetic acid (IAA), a major naturally occurring auxin. Auxins are plant hormones that regulate growth and development, including cell elongation, root formation and the development of different organs. Tryptophan therefore connects amino acid metabolism with hormonal regulation (Mashiguchi *et al.,* 2011; Won *et al.,* 2011).

Experimental work in *Arabidopsis* established a pathway in which tryptophan is converted into IAA through two successive reactions (Figure 4).

Figure 4. Conversion of Tryptophan into IAA (auxin)



These findings demonstrate how plants can use tryptophan to produce auxin, but the amount produced also depends on the activity and regulation of these enzymes. An increase in tryptophan availability alone therefore does not establish a corresponding increase in auxin production (Mashiguchi *et al.,* 2011; Won *et al.,* 2011).

External application has produced measurable benefits in crop experiments. Mustafa *et al*. (2016) tested soil and foliar applications of L-tryptophan in a pot experiment with okra. Foliar application at 10 mg L^{-1} and soil application at 40 mg kg^{-1} soil were the most effective treatments, significantly improving growth and yield compared with untreated plants. The authors reported fruit yield increases of approximately 96% and 47%, respectively, as well as earlier flowering and reduced floral bud drop (the loss of flower buds before they develop further).

6.5. Methionine.

Methionine is a sulphur-containing amino acid used in protein synthesis. It also provides the starting material for S-adenosylmethionine (SAM), a compound involved in several metabolic pathways. SAM supplies methyl groups in reactions that help regulate gene

activity and alter the activity or properties of proteins and other compounds. It also contributes to the production of ethylene and certain polyamines, compounds involved in plant growth, development and responses to stress (Hildebrandt *et al.,* 2015).

Ethylene is a gaseous plant hormone involved in fruit ripening, leaf ageing and responses to environmental conditions. Its production begins when the enzyme SAM synthetase converts methionine into S-adenosylmethionine (SAM). SAM then undergoes further reactions to produce ethylene. Experiments tracing labelled compounds in apple tissue established this pathway, demonstrating that methionine supplies starting material for ethylene formation (Adams and Yang, 1979).

External application has nevertheless produced measurable growth benefits. In hydroponic lettuce, Khan *et al*. (2019) supplied methionine through the nutrient solution and found that low concentrations, particularly 0.02 and 0.2 mg L^{-1}, significantly improved growth measurements, whereas higher concentrations inhibited growth. These findings support a benefit from methionine supplementation under the conditions tested and show why concentration matters. They do not establish that increased ethylene production caused the improvements (Khan *et al.,* 2019).

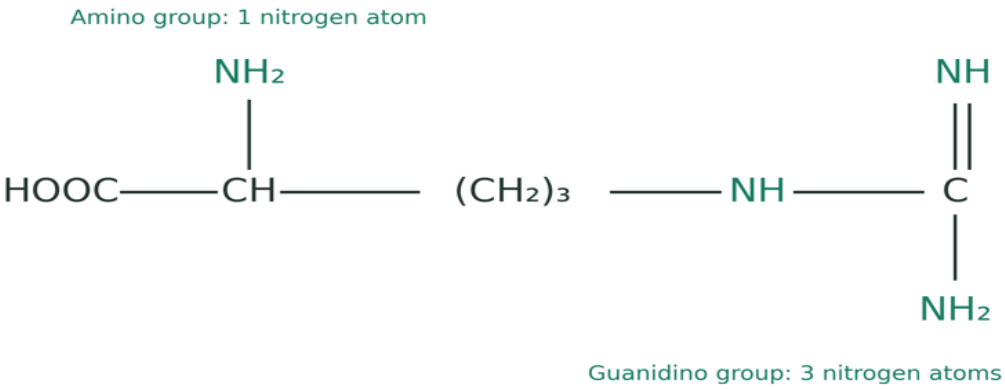
6.6. Arginine.

Arginine is a protein-forming amino acid that also serves as a concentrated reserve of nitrogen. Each molecule contains four nitrogen atoms (Figure 5), giving it a high nitrogen content relative to its carbon content. This makes arginine particularly suitable for storing nitrogen in tissues such as seeds. When these reserves are needed, arginine can be broken down and its nitrogen reused to support the formation of other amino acids and new plant tissue (Winter *et al.,* 2015).

Arginine also supplies starting material for the production of polyamines, small nitrogen-containing compounds involved in growth and responses to stress. One pathway begins with the enzyme arginine decarboxylase and leads to the formation of putrescine, which can subsequently be used to produce other polyamines. In *Arabidopsis*, increasing the activity of this pathway through genetic modification increased putrescine accumulation and improved tolerance to water deficit. This supports a role for arginine metabolism in stress responses,

although it does not demonstrate that applying arginine produces the same effect (Alcázar *et al.,* 2010).

Figure 5. Structure of Arginine. Nitrogen atoms are highlighted in green.



External application has been investigated separately in sugarcane. Silveira *et al*. (2021) sprayed leaves with arginine at 1 mM, approximately 174 mg L^{-1}, and imposed water deficit by adding polyethylene glycol to the nutrient solution, making water less available to the roots. Compared with water-sprayed plants, arginine-treated plants maintained better leaf gas exchange during the stress period and showed stronger antioxidant protection in roots during recovery. Proline synthesis did not increase, showing that the benefits could not simply be attributed to greater proline production. These findings support a protective effect under the experimental conditions, while further evidence is needed to establish the corresponding benefits under field drought (Silveira *et al.,* 2021).

6.7. Cysteine.

Cysteine is a sulphur-containing amino acid used in plant protein synthesis. It also has a central role in sulphur metabolism: plants convert absorbed sulphate into sulphide and incorporate it into cysteine. This connects mineral sulphur nutrition with the production of proteins and other compounds needed for plant growth (Hildebrandt *et al.,* 2015).

Within proteins, cysteine can contribute directly to their structure and function. Its side chain contains a sulphur-bearing group that can form a bond with another cysteine. These disulphide bonds act as molecular links that help stabilise the folded structure of certain proteins. Cysteine does not need to be the most abundant amino acid in a protein to be important: a small number of cysteine residues at particular positions can help maintain its structure or participate in its activity (Hildebrandt *et al.,* 2015).

Cysteine also contributes to glutathione, alongside glutamate and glycine. Its sulphur-bearing group gives glutathione the chemical properties needed to participate in antioxidant reactions. Glutathione additionally provides starting material for phytochelatins, small peptides that bind metals such as cadmium and help limit their harmful effects. In *Arabidopsis*, Howden *et al*. (1995) found that a glutathione-deficient mutant produced fewer phytochelatins and was more sensitive to cadmium. These findings demonstrate the importance of this protective pathway, although they do not establish that external cysteine application produces the same protection (Howden *et al.,* 1995).

6.8. Other agriculturally relevant amino acids.

Other amino acids contribute to processes relevant to crop growth and resilience. Phenylalanine supplies starting material for the production of many phenolic compounds, including flavonoids and lignin. Flavonoids can contribute to protection against ultraviolet radiation and other stresses, while lignin strengthens cell walls and supports the structure of stems and water-conducting tissues. Phenylalanine therefore connects protein synthesis with the formation of structural and protective compounds (Hildebrandt *et al.,* 2015).

Lysine also connects amino acid metabolism with plant defence. Plants can convert it into pipecolic acid, a compound involved in strengthening defence responses following infection. In *Arabidopsis*, Návarová *et al*. (2012) demonstrated that pipecolic acid contributes to systemic acquired resistance, in which an initial infection increases the defensive capacity of tissues elsewhere in the plant. This establishes a role for a product of lysine metabolism, rather than demonstrating that applying lysine itself provides disease protection (Návarová *et al.,* 2012).

Alanine is relevant to conditions where roots receive insufficient oxygen, as can occur in waterlogged soils. It commonly accumulates during oxygen shortage and can be reused when oxygen becomes available again. Experiments in *Arabidopsis* showed that alanine aminotransferase, an enzyme linking alanine with carbon and nitrogen metabolism, contributes to its breakdown during recovery. This illustrates how amino acids can participate in metabolic adjustment during and after stress, without establishing that external alanine application improves waterlogging tolerance (Miyashita *et al.,* 2007).

GABA (gamma-aminobutyric acid) is an amino acid that is not incorporated into proteins. GABA participates in a metabolic pathway known as the GABA shunt, which allows its carbon to enter reactions associated with cellular energy production. Its concentration often increases during stress, linking it with adjustments in metabolism as well as signalling (Hildebrandt *et al.,* 2015). A clear example of its protective role involves the regulation of stomatal opening. In *Arabidopsis*, Xu *et al*. (2021) demonstrated that GABA produced in guard cells (the cells controlling stomatal pores) limits stomatal opening by regulating an ion transporter. This reduces water loss through transpiration and can improve drought resilience. GABA therefore acts as a chemical signal that helps regulate water use, rather than providing this protection through incorporation into a protein. These findings make it relevant to agricultural stress management, although they do not establish that every external GABA treatment will improve crop performance (Xu *et al.,* 2021).

7. Agricultural Application of Amino Acids.

The agricultural value of amino acid applications depends on whether they produce measurable benefits under the conditions in which a crop is grown. Building on the uptake, transport and physiological functions discussed in Chapters 4–6, this chapter examines application methods, concentration and rate, timing and frequency, and their relationship with crop growth, yield and quality. Experimental results provide a basis for assessing practical use, while recognising that an effective treatment in one crop or growing system may not perform equally well in another.

7.1. Application methods.

Amino acids can be supplied through the root zone, as foliar sprays or through seed treatments. Root-zone application includes soil drenches, irrigation and hydroponic nutrient solutions, although soil microorganisms may consume or transform part of the supply before root absorption (Näsholm *et al.,* 2009). Foliar application delivers amino acids directly to leaves, avoiding this initial competition in soil.

Evidence supports measurable benefits from foliar treatments. In greenhouse tomato, Colla *et al*. (2017) applied a legume-derived protein hydrolysate at 3 mL L^{-1}, increasing total fruit yield from 83.0 to 88.8 t ha^{-1}, a significant 7.0% increase compared with untreated plants. In lettuce, foliar glycine at 500 mg L^{-1} significantly increased shoot fresh and dry weight

compared with a water-sprayed control, demonstrating benefits from an individual amino acid applied to leaves (Noroozlo *et al.,* 2019).

Seed treatment can also be effective: Teixeira *et al*. (2018) reported soybean grain productivity increases of at least 22% following seed treatments with individual amino acids or their mixture under field conditions. These studies support foliar and seed applications as useful agricultural options, but do not establish a universal ranking of application methods. Yield and biomass responses demonstrate treatment performance; absorption efficiency requires separate measurements of the proportion absorbed.

7.2. Concentration and application rate.

Concentration describes the amount of amino acid or formulated product in the application solution, whereas application rate describes the amount delivered per plant or hectare. Both are necessary to interpret experimental results: the same concentration can deliver different quantities when spray volume changes, and equal volumes of different products may contain different amounts of amino acids.

Noroozlo *et al*. (2019) compared foliar glycine and glutamine at 250, 500 and 1000 mg L^{-1} in lettuce. Glycine at 500 mg L^{-1} significantly increased shoot fresh and dry weight compared with the water-sprayed control, whereas 250 mg L^{-1} significantly increased root fresh weight. At 1000 mg L^{-1}, shoot dry weight increased significantly, but shoot fresh weight did not differ significantly from the control. These results show that increasing concentration did not consistently improve all growth measurements. Across five applications, each plant received approximately 90–100 mL of solution, equivalent to a calculated 45–50 mg glycine per plant at 500 mg L^{-1} (Noroozlo *et al.,* 2019).

A comparison of product application rates was provided by Calero *et al*. (2025), who tested an amino acid-based formulation at 0.25, 0.50 and 0.75 L ha^{-1} in tomato seedlings. Under weekly application, 0.25 L ha^{-1} produced 19% more shoot dry biomass than 0.50 L ha^{-1} and 39% more than 0.75 L ha^{-1}. The lowest tested rate therefore outperformed the higher rates for this measurement. The formulation's declared amino acids—aspartic acid, arginine, glycine and tryptophan—totalled 7% w/w.

In greenhouse tomato, Colla *et al*. (2017) applied a protein hydrolysate at 3 mL L^{-1} in four foliar applications, delivering calculated product rates of 3.0, 3.6, 5.4 and 6.0 L ha^{-1}, totalling

18 L ha^{-1}. Total marketable yield increased significantly from 83.0 to 88.8 t ha^{-1} (7.0%), while early marketable yield increased from 15.0 to 19.5 t ha^{-1} (30.0%). Marketable fruit number increased from 28.9 to 30.8 fruits per plant, without a significant change in mean fruit weight, indicating that the yield response was associated with greater fruit number. Total soluble solids also increased significantly from 4.00 to 4.57 °Brix. These findings demonstrate benefits for commercial production and fruit quality under the tested programme. However, only one concentration was tested, so the study does not establish an optimum application rate (Colla *et al*., 2017).

Collectively, these experiments show that higher concentrations or product rates do not necessarily produce greater benefits. Meaningful comparisons require the amino-acid composition, concentration, spray volume, rate per application and cumulative amount applied. Rates for individual amino acids, amino acid mixtures and protein hydrolysates should therefore be reported separately rather than treated as interchangeable.

7.3. Application timing and frequency.

Application timing and frequency can influence the response to amino acid treatments. Experiments comparing growth stages or spray intervals provide more direct evidence for scheduling than studies using only one application programme.

In tomato seedlings, Calero *et al*. (2025) compared weekly and fortnightly foliar applications of an amino acid-based formulation at 0.25, 0.50 and 0.75 L ha^{-1}, beginning seven days after sowing. Weekly applications produced significantly greater root and total dry biomass than fortnightly applications at all three rates. At 0.25 L ha^{-1}, weekly treatment increased stem thickness by 24% and root length by 12% relative to fortnightly treatment. These results support more frequent application during seedling establishment under the conditions tested. However, weekly spraying also supplied more product over the experimental period, so frequency and cumulative dose were not separated (Calero *et al.,* 2025).

Growth-stage comparisons show that additional applications do not necessarily improve performance. In a two-season field experiment with rainfed chickpea, Khan *et al*. (2026) compared an amino acid-based formulation applied at 1.25 L ha^{-1} at flower initiation, pod initiation, or both stages. A single application at flower initiation produced the highest seed yield, with reported increases of approximately 50% over the untreated control in both

seasons. Two applications, at flower and pod initiation, increased yield by approximately 30%, despite supplying twice the total product. The result supports flower initiation as the most effective timing among the treatments tested for that crop and formulation, rather than a general recommendation for all amino acid products (Khan *et al.,* 2026).

These experiments demonstrate contrasting responses: weekly application favoured tomato seedling growth, whereas one well-timed application outperformed two applications in chickpea. Application schedules should therefore follow evidence for the crop, developmental stage and formulation. Neither a universal spray interval nor a general advantage from increasing application frequency is established.

7.4. Practical implications and evidence limitations.

The experiments reviewed demonstrate that amino acid-containing treatments can increase crop growth, marketable yield and selected quality characteristics. These improvements provide a practical basis for their inclusion in crop-management programmes and can generate additional revenue that offsets product and application costs.

The commercial opportunity therefore extends beyond supplying nutrients alone: appropriately selected formulations and application programmes can improve the quantity and value of harvested produce. The magnitude of this benefit remains specific to the product, crop and growing conditions, so the results support the programmes tested rather than a claim of profitability for every amino acid-containing product.

8. Amino Acid Fertilisers and Conventional Nitrogen: Comparative Efficiency.

This section examines how much conventional nitrogen fertiliser is needed to achieve a response comparable with amino acid-based treatments. It brings together experiments comparing application rates and their effects on crop yield and quality, including programmes that combine amino acids with reduced mineral nitrogen inputs.

8.1. Comparison with supplementary foliar urea.

El-Sanatawy *et al*. (2021) compared foliar whey-protein hydrolysate at 2 g L^{-1} with urea at 10 g L^{-1}, applied three times using the same spray volumes and background soil-nitrogen treatments. Averaged across soil-nitrogen treatments, the hydrolysate produced grain yields of 6098 and 6078 kg ha^{-1}, compared with 5833 and 5792 kg ha^{-1} for foliar urea in the two

seasons. These statistically significant differences represent yield increases of approximately 4.5% and 4.9%, respectively.

To estimate the nitrogen supplied by these foliar treatments, the following calculation assumes 16% nitrogen by mass in the hydrolysate and 46% nitrogen in urea (the 16% value is a rough average for proteinaceous amino-acid and peptide material; individual amino acids have different nitrogen contents, so it does not describe every amino acid equally).

The calculations below were performed by the author of this report. They are not calculations reported by El-Sanatawy *et al*. (2021), and the assumed 16% is not a verified nitrogen analysis of their hydrolysate formulation.

Estimated nitrogen concentration in the urea spray:

10 g urea L^{-1} × 0.46 = 4.60 g N L^{-1}

Estimated nitrogen concentration in the hydrolysate spray:

2 g hydrolysate L^{-1} × 0.16 = 0.32 g N L^{-1}

Estimated hydrolysate nitrogen as a percentage of urea nitrogen:

(0.32 ÷ 4.60) × 100 = 6.96%

Ratio of nitrogen concentrations:

4.60 ÷ 0.32 = 14.38

Using the assumption of 16% nitrogen in the hydrolysate, the foliar hydrolysate treatment supplied approximately 7% as much nitrogen as the foliar urea treatment, an estimated 93% reduction in supplementary foliar nitrogen, yet produced approximately 4.5–4.9% higher grain yields. Expressed simply, for every 100 units of nitrogen supplied through the urea spray, the hydrolysate supplied an estimated seven units and achieved a higher yield.

This result demonstrates the potential of the hydrolysate to outperform supplementary foliar urea with a much smaller nitrogen contribution. Both treatments received the same background soil-nitrogen programmes, so the comparison concerns the foliar treatments, rather than a 93% reduction in the crop's total nitrogen supply. The estimated nitrogen difference is the author's calculation, based on the assumed hydrolysate composition, rather than a measured replacement ratio established by the study.

8.2. Reduced mineral-nitrogen programmes.

De Luca and De Barreda (2022) compared conventional nitrogen fertilisation with an amino acid-based biostimulant combined with a reduced mineral-fertiliser programme in tall fescue. The biostimulant contained 20% free amino acids and 5.5% total nitrogen. The combined programme supplied 20 kg N ha^{-1} $year^{-1}$ from the biostimulant and 80 kg N ha^{-1} $year^{-1}$ from mineral fertiliser, compared with conventional mineral-fertiliser or urea programmes supplying 200 kg N ha^{-1} $year^{-1}$.

The following calculations were performed by the author of this report using the annual nitrogen rates stated in the study.

Total nitrogen supplied by the combined programme:

20 + 80 = 100 kg N ha^{-1} $year^{-1}$

Reduction in total nitrogen compared with the conventional programme:

200 − 100 = 100 kg N ha^{-1} $year^{-1}$

Percentage reduction in total nitrogen:

(100 ÷ 200) × 100 = 50%

Reduction in nitrogen supplied specifically through mineral fertiliser:

200 − 80 = 120 kg N ha^{-1} $year^{-1}$

Percentage reduction in mineral-fertiliser nitrogen:

(120 ÷ 200) × 100 = 60%

The combined programme therefore supplied half as much total nitrogen and 60% less mineral-fertiliser nitrogen, while maintaining turf quality comparable with conventional programmes during almost the entire experiment. Expressed simply, 80 units of mineral nitrogen plus 20 units of biostimulant nitrogen maintained comparable turf quality to programmes supplying 200 units of conventional nitrogen.

This result supports the combined programme as a means of reducing nitrogen inputs while maintaining turf quality under the tested conditions. It does not establish that 20 units of amino acid-based nitrogen universally replace 120 units of mineral nitrogen: the experiment did not include an 80 kg mineral-N-only treatment to isolate that replacement effect. The

biostimulant alone at 20 kg N ha^{-1} $year^{-1}$ also did not consistently maintain the quality achieved by the higher-nitrogen programmes (De Luca and De Barreda, 2022).

8.3. Internal field trials of Grow Green fertilisation programmes.

Grow Green conducted a field experiment with the Agricultural University Plovdiv in Bulgaria. The study evaluated wheat and canola under local soil and climatic conditions. Its objective was to compare crop responses to GrowGreen programmes with those obtained through conventional nitrogen fertilisation.

Six GrowGreen programmes were tested, using foliar applications alone or combined with soil drenches. Each programme was evaluated alongside ammonium nitrate rates of 0, 100, 200, 300 and 400 kg product ha^{-1}. Treatments receiving ammonium nitrate alone and a control receiving neither ammonium nitrate nor GrowGreen products provided reference comparisons. The experiment used a randomised block design with three replicates per treatment.

Measurements included grain or seed yield, wheat protein and gluten content, and canola protein and oil content. This design allowed assessment of Grow Green programmes both without added ammonium nitrate and in combination with increasing mineral-fertiliser inputs. The following sections focus on the lowest-dose programme, Grow Green 1 (GG1), which comprised two foliar applications totalling 5.9 L ha^{-1} of Grow Green products. Each application included Profert at 1 L ha^{-1}, equivalent to Grow Green's fish emulsion, together with other Grow Green nutrient products. No soil drench was included in GG1.

8.3.1. Wheat.

In the wheat trial, GG1 without added ammonium nitrate produced 202.56 g grain per 0.5 m^2, compared with 202.04 g per 0.5 m^2 following 300 kg ammonium nitrate ha^{-1} alone. Thus, the complete Grow Green programme at 5.9 L ha^{-1} achieved essentially the same observed wheat yield as 300 kg ammonium nitrate ha^{-1}, with a numerical difference of approximately 0.3%.

Based on the supplied product compositions, the estimated nitrogen contribution from the GG1 programme was approximately 0.17 kg N ha^{-1}. Assuming 34% nitrogen in ammonium nitrate, the conventional treatment supplied:

$300 \times 0.34 = 102$ kg N ha^{-1}

The estimated nitrogen-input comparison is:

$(0.17 \div 102) \times 100 = 0.17\%$

$102 \div 0.17 = 600$

On this calculation, the specified GG1 components supplied approximately 1/600 of the fertiliser nitrogen supplied by ammonium nitrate, while the complete programme achieved a closely matching wheat yield.

8.3.2. Canola.

In the canola trial, GG1 without added ammonium nitrate produced 86.82 g seed per five-plant sample, compared with 86.07 g per five-plant sample following 300 kg ammonium nitrate ha^{-1} alone.

Percentage yield difference:

$((86.8167 - 86.0733) \div 86.0733) \times 100 = 0.86\%$

Thus, the complete Grow Green programme at 5.9 L ha^{-1} achieved almost the same observed canola yield as 300 kg ammonium nitrate ha^{-1}, with a numerical difference of approximately 0.9%.

Using the same provisional nitrogen estimate as in Section 8.3.1, the GG1 components with specified nitrogen contents contributed approximately 0.17 kg N ha^{-1}. Assuming ammonium nitrate contained 34% nitrogen:

$300 \times 0.34 = 102$ kg N ha^{-1}

Estimated GG1 nitrogen relative to ammonium nitrate nitrogen:

$(0.17 \div 102) \times 100 = 0.167\%$

Ratio of nitrogen inputs:

$102 \div 0.17 = 600$

On these assumptions, the specified GG1 components supplied approximately 1/600 of the fertiliser nitrogen supplied by ammonium nitrate, while the complete programme produced a closely matching canola yield.

9. Conclusions.

Amino acids are integral to plant growth and development, connecting protein synthesis with carbon, nitrogen and sulphur metabolism. Their functions extend to nutrient storage and remobilisation, signalling, and the formation of compounds involved in growth regulation and defence. Differences in chemical structure and stereochemistry influence their biological use, making amino-acid composition and processing relevant when assessing agricultural formulations.

Plants can absorb externally supplied amino acids through roots and leaves, although their availability and subsequent use depend on several processes. Root uptake is influenced by microbial competition and membrane transport, while foliar uptake requires passage across the leaf surface followed by entry into living cells. Once absorbed, amino acids can be used locally, metabolised or redistributed through the xylem and phloem according to changing source–sink relationships. Absorption therefore provides access to plant metabolism but does not imply that every applied amino acid reaches its destination unchanged.

Individual amino acids contribute to different physiological functions. Glutamate and glutamine are central to nitrogen assimilation; tryptophan and methionine provide precursors for growth-regulating compounds; and proline, arginine, cysteine and GABA participate in processes associated with stress responses and protection. These functions provide a physiological basis for agricultural use. Experiments with external applications demonstrate benefits in particular crops and conditions, while also showing that a molecule's natural role does not automatically predict the response to its application.

The agricultural studies reviewed demonstrate improvements in biomass, marketable yield and selected quality characteristics following treatments with individual amino acids and amino acid-containing formulations. Foliar application offers a practical route that avoids initial competition with soil microorganisms, while root-zone and seed treatments also have demonstrated value. Performance depends on formulation, concentration, application rate, crop stage and frequency. Higher doses or additional applications do not consistently improve results, supporting the use of programmes matched to crop requirements and experimental evidence.

Comparisons with conventional nitrogen fertilisation further demonstrate the potential of amino acid-based programmes to maintain or improve performance with lower fertiliser nitrogen inputs. Published studies support benefits from supplementary foliar hydrolysates and combined programmes using reduced mineral nitrogen.

Taken together, the evidence supports amino acids as useful components of crop nutrition and management programmes. Their value encompasses nutritional and physiological contributions, with the strongest practical recommendations arising from clearly characterised formulations and crop-specific validation of yield, quality and input requirements.

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