

From plant biology research to technology transfer and knowledge extension: improving food quality and mitigating environmental impacts

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Abstract

Academic scientists face an unpredictable path from plant biology research to real-life application. Fundamental studies of γ -aminobutyrate and carotenoid metabolism, control of *Botrytis* infection, and the uptake and distribution of mineral nutrients illustrate that most academic research in plant biology could lead to innovative solutions for food, agriculture, and the environment. The time to application depends on various factors such as the fundamental nature of the scientific questions, the development of enabling technologies, the research priorities of funding agencies, the existence of competitive research, the willingness of researchers to become engaged in commercial activities, and ultimately the insight and creativity of the researchers. Applied research is likely to be adopted more rapidly by industry than basic research, so academic scientists engaged in basic research are less likely to participate in science commercialization. It is argued that the merit of Discovery Grant applications to the Natural Sciences and Engineering Research Council (NSERC) of Canada should not be evaluated for their potential impact on policy and (or) technology. Matching industry funds in Canada rarely support the search for knowledge. Therefore, NSERC Discovery Grants should fund basic research in its entirety.

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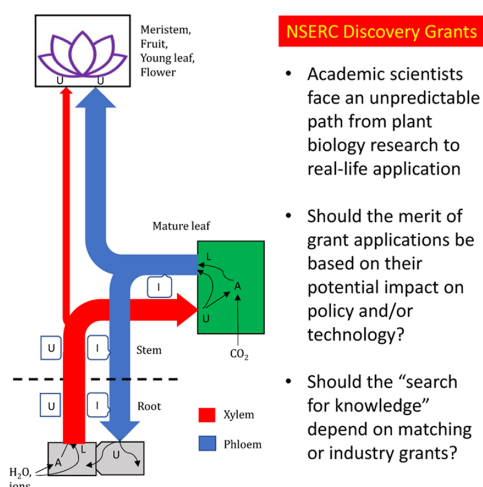
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Introduction

In 1984, I joined the Department of Horticultural Science in the Ontario Agricultural College at the University of Guelph. This was the opportunity that I was looking for: a department focused on the generation of novel horticultural products and optimization of production practices and with a growing interest in using physiological and biochemical strategies to advance horticultural research. I was expected to develop a research program in plant nutrition. From the beginning, my graduate students were trained in both basic and applied aspects of plant biology, always with the goal of understanding the foundational science underlying real-life applications.

Almost all academic research in Canada has been supported by the Discovery Grants Program (as it is now known) at the Natural Sciences and Engineering Research Council (NSERC) of Canada, though the program supports basic research only, and scientists are generally discouraged from overlapping basic research with more applied goals. Academic scientists who work closely with industry face different issues. This research targets real-life applications of the natural sciences, is directed at solving specific practical problems or developing new technologies or techniques, generally employs empirical methodologies, and is often a collection of projects. Unfortunately, funding for this type of research generally does not support “the acquisition of new knowledge or the expansion of existing knowledge” (Natural Sciences and Engineering Research Council of Canada 2022). Consequently, I managed two distinct research programs for almost 15 years. The first was funded by the Discovery Grants Program and had long-term goals related to amino acid metabolism. The second was funded by the Ontario Ministry of Agriculture Food and Rural Affairs (OMAFRA as it is now known) and industry with a focus on two essential plant elements: nitrogen (N) because it is often applied in excessive amounts for horticultural crop production, leading to environmental contamination, and boron (B) because of its uncertain function and involvement in many physiological disorders in important horticultural plants.

Over the last decade or so, there has been a shift in science policy with respect to Discovery Grants. While these grants still “recognize the creativity and innovation that are at the heart of all research advances”, the merit of the research is now being evaluated, in part, from its “impact on policy and (or) technology” (Natural Sciences and Engineering Research Council of Canada 2022). Furthermore, they are now considered “grants in aid of research”, and “are not meant to support the full costs of a research program” (Natural Sciences and Engineering Research Council of Canada 2022). In Canada, at least in principle, this means that funding for basic research is no longer based solely on the “search for knowledge”. In practice, funding for basic research has been on the decline (Gingras 2019), so a “successful” academic scientist at a research-intensive university, even one that historically does not conduct applied research, must seek and gain support from more applied funding programs, including those offered by NSERC, which by design require “matching” or “collaborative” industry support.

University research advances in plant biology may be translated to industry in two ways: patenting and licensing or spin-offs (i.e., technology transfer); and agricultural outreach (i.e., knowledge extension). In today’s research climate, proposals submitted to both government and industry must predict the commercial functionalities to be realized. However, the functionalities often lie dormant for various reasons including: the hypothesis is false; the need for further research; the priorities of the funding agencies change; and simultaneous discovery by another research team (Garud et al. 2018). Alternatively, novel functionalities or technologies may emerge, rendering latent scientific

discoveries with commercial interest. Despite our best efforts, and contrary to a carefully formulated research plan, the path from scientific discovery to real-life application is often unpredictable.

Context: Translating scientific discoveries and knowledge into real-life applications is not a straight path

In this paper, several cases are discussed to illustrate how unpredictable the path from scientific discovery to real-life application can be. The first case describes a lengthy journey involving many research teams, from the discovery of the nonprotein amino acid, γ - or 4-aminobutyrate (GABA), to the generation of GABA-enriched, plant-based foods with potential human health benefits, and to the use of exogenous GABA to improve plant health under stress conditions. The remaining cases describe journeys of shorter duration, but they are just as fraught with highlights and disappointments. The second case describes the generation and utility of novel elite corn (*Zea mays* L.) lines with enriched levels of carotenoids for ameliorating macular degeneration in humans. The third case deals with the development of an eco-friendly method for ameliorating *Botrytis cinerea* infection in plants. Finally, the fourth case discusses how understanding of nutrient uptake and redistribution in plants evolved, eventually leading to a sustainable nutrient delivery strategy in floriculture operations, thereby minimizing environmental and economic costs.

Plant GABA

Improving human and plant health

Functional foods containing GABA are believed to be able to prevent and (or) treat different diseases, especially hypertension, diabetes, and neurological disorders (Abe et al. 1995; Yoshimura et al. 2010; Diana et al. 2014; Boonstra et al. 2015; Ngo and Vo 2019; Oketch-Rabah et al. 2021). Furthermore, GABA can accumulate in plants in response to both abiotic and biotic stress conditions (e.g., Kinnersley and Turano 2000; Shelp et al. 2017b). Thus, there has been increasing interest in generating GABA-enriched crops for human consumption, and in using exogenous GABA to mitigate stress-induced losses in plant yield and quality (Gramazio et al. 2020; Shelp et al. 2021a).

Elucidating metabolic pathways and function

GABA was first identified in potato tuber (*Solanum tuberosum* L.) in 1949 (Steward et al. 1949); however, most research on GABA over the next 50 years focused on its role as an inhibitory neurotransmitter in mammals (Ngo and Vo 2019). Our initial interest in GABA metabolism was triggered by difficulty in tagging the biosynthetic pathway for arginine, an important storage protein amino acid in developing soybean cotyledons, using radiolabelled glutamate (Glu); surprisingly, Glu was rapidly and predominantly metabolized to CO₂ and GABA (Micallef and Shelp 1989a, 1989b). Consequently, the focus shifted, and several key findings were reported shortly thereafter: Glu- and pyridoxal phosphate-dependent Glu decarboxylase (GAD) is the primary enzyme generating the GABA (Tuin and Shelp 1994, 1996), and GAD is cytosolic, whereas the second and third enzymes of the GABA shunt (GABA transaminase or GABA-T, succinic semialdehyde dehydrogenase or SSADH) are mitochondrial (Breitkreuz and Shelp 1995; Busch and Fromm 1999; Clark et al. 2009a) (Fig. 1). Perhaps most significant was the finding that plant GAD, unlike bacterial and mammalian GADs, binds calcium-calmodulin (Ca²⁺-CaM) to its C-terminal autoinhibitory domain, resulting in activation of the enzyme at neutral pH, but not at its acidic pH optimum (Baum et al. 1993; Ling et al. 1994; Arazi et al. 1995; Snedden et al. 1995). At that time, CaM was receiving attention for its role in stress signaling (Zelinski 1998).

During the late 1990s, the study of enzyme function transitioned from plant species such as tobacco (*Nicotiana tabacum* L.), which provide abundant plant material for enzyme extraction and organelle

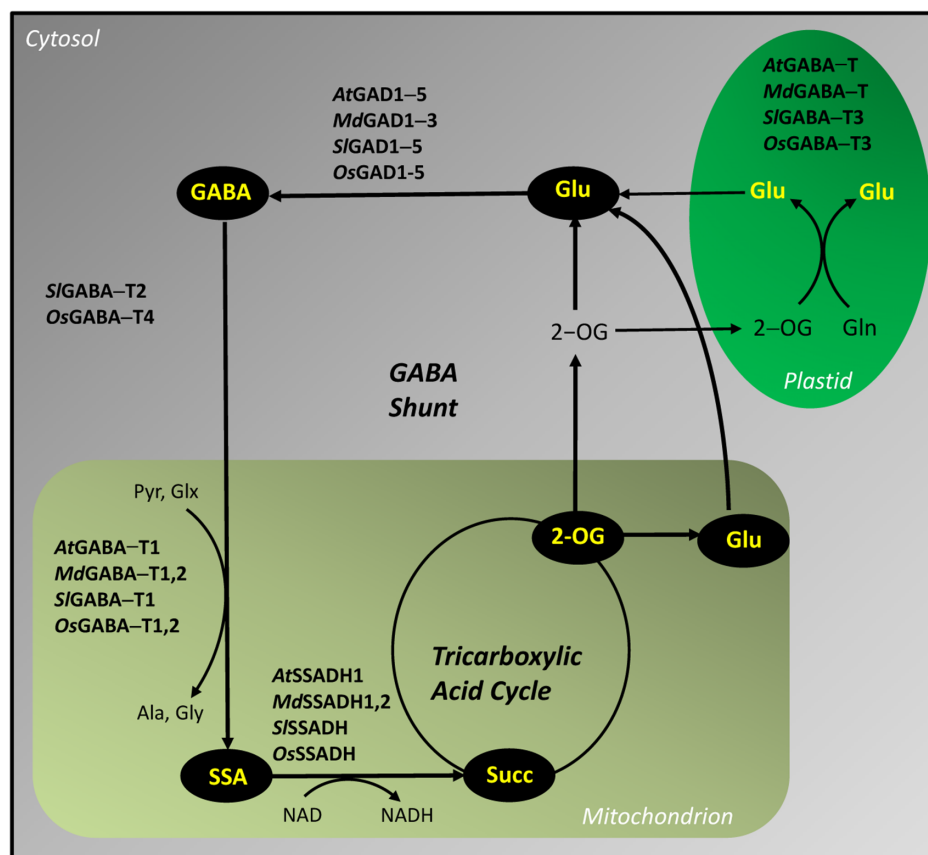


Fig. 1. Compartmentation of multiple isoforms of the GABA Shunt enzymes in *Arabidopsis*, tomato, rice, and apple. The shunt consists of three enzymes: (1) glutamate decarboxylase (GAD), which catalyses the decarboxylation of glutamate (Glu) to γ -aminobutyrate (GABA); (2) GABA transaminase (GABA-T), which converts GABA to succinic semialdehyde (SSA); and (3) succinic semialdehyde dehydrogenase (SSADH), which catalyses the oxidation of succinic semialdehyde (SSA) to succinate (Succ). The GABA Shunt is represented by the bold arrows. Succinate is a component of the GABA shunt, the tricarboxylic acid cycle, and a substrate of the mitochondrial electron transport chain. (Based on Shelp et al. 2021a.) Additional abbreviations: Ala, alanine; Gln, glutamine; Glx, glyoxylate; Gly, glycine; 2-OG, 2-oxoglutarate; Pyr, pyruvate.

fractionation, to *Arabidopsis thaliana*, the first model plant for genomic and molecular research (e.g., Van Cauwenberghe and Shelp 1999, Van Cauwenberghe et al. 2002). This facilitated identification of the genes encoding GAD (*AtGAD1-5*), GABA-T (*AtGABA-T*) and SSADH (*AtSSADH*) and enabled their biochemical characterization as recombinant proteins generated in bacterial expression systems (e.g., Shelp et al. 2012a). Together, *AtGABA-T* and *AtSSADH* catalyze the conversion of GABA to SSA and then succinate. Notably, plant GABA-T and SSADH have different amino acceptor (pyruvate and glyoxylate versus 2-oxoglutarate) and co-factor (NAD^+ versus NADP^+) requirements than the bacterial and mammalian forms, respectively.

Complementary studies demonstrated that three of the five *Arabidopsis* genes possess the CaM-binding domain (Shelp et al. 1999, 2012a) (Fig. 1). *AtGAD1-5* also exhibit different expression profiles. For example, *AtGAD2* is strongly expressed and widely distributed throughout the shoot and roots (Shelp et al. 2012b); the GABA level in the shoot of the *atgad2* mutant is less than 25% of that in the wild type (WT), but the level in the roots is unaffected (Xu et al. 2021). In contrast,

AtGAD1 is strongly and widely distributed in roots (Shelp et al. 2012b), and the GABA level in roots of the *atgad1* mutant is only 15% of the WT (Bouché et al. 2004). *AtGAD4* expression is negligible in both shoot and roots, but is strongly induced by various stresses, including hypoxia (Miyashita and Good 2008). The *atgaba-t* mutant is phenotypically normal, except for lower seed production, and the leaf GABA level increases up to 16-fold. The *atssadh* mutant overaccumulates GABA and H₂O₂ by two- and four-fold, respectively (Bouché et al. 2003; Fait et al. 2005). The product of the SSADH reaction, succinate, contributes to the production of carbon skeletons and NADH via the tricarboxylic acid cycle and to the generation of ATP via the mitochondrial electron transport chain, which prevents the accumulation of reactive O₂ species (Tuin and Shelp 1994; Bouché et al. 2003).

Baum et al. (1996) demonstrated that transgenic tobacco plants constitutively overexpressing a mutant petunia (*Petunia x hybrida* Vilm.) GAD lacking the CaM-binding domain (*GADΔC*) have elevated levels of GABA and are stunted and infertile. Yet, McLean et al. (2003) were able to select transgenic tobacco plants overexpressing *NtGAD* or *NtGADΔC* and exhibiting a normal phenotype; the GABA levels were up to 3-fold higher in the shoot, and up to 28-fold higher in the root than in the WT. The high-GABA plants exhibit resistance to the root-knot nematode and the oblique-banded leafroller (MacGregor et al. 2003; McLean et al. 2003). Overexpression of the petunia *GADΔC* under control of a developmentally regulated seed promoter increases the GABA level in mature *Arabidopsis* seeds by approximately 50-fold compared to the WT (Fait et al. 2011).

In summary, fundamental inquiry into the role of GABA in plants led to the elucidation of its metabolic route and how it might be manipulated in important economic crops. In the late 1990s there was considerable interest protecting intellectual property related to gene discovery and utility. Consequently, we began to prepare a patent application that included GABA-T and high-GABA plants, but the university, ever aware of the costs associated with the patenting process, looked for an industry partner to share the burden. In the end, the potential partner initiated its own application and neither party completed the process. Furthermore, at the time the safety of consuming elevated levels of GABA in plant-derived foodstuffs was unknown. Nonetheless, the pace of plant-GABA research continued to increase over time (251 publications prior to 1997 versus 3342 papers published from 1997 to 2016, an 883% increase over a 20-year period) and changes in GABA levels were realized by cultural management and genetic engineering strategies. Below, several case studies are discussed of GABA enrichment in economic crops (i.e., tomato (*Solanum lycopersicum* L.) fruit, rice (*Oryza sativa* L.) grain, sprouting grains and seeds, controlled atmosphere-stored apple (*Malus x domestica* Borkh.) fruit, with emphasis on research from the main scientists contributing to these advances.

Managing the hyperaccumulation of GABA in sprouts of cereal grains and legume seeds

Sprouting/germination is a useful processing step for improving the nutritional and potential disease-prevention qualities of cereal grains and legume seeds (Gani et al. 2012; Gu et al. 2017; Idowu et al. 2020; Munarko et al. 2022). For example, it reduces the level of phytic acid, which interferes with the absorption of minerals by the body, and the level of gluten, a protein of concern to celiacs (Kim et al. 2012). Furthermore, sprouting increases the GABA level in grains and seeds of various crops, including brown rice, buckwheat, waxy wheat, oat, adzuki bean, kidney bean, lentil, lupin, sesame, soybean, and pea (Cho and Lim. 2016; Gan et al. 2017).

Based on earlier reviews of the literature, it was first suggested that arginine, derived from the mobilization of protein reserves in germinating seeds, is degraded to urea and ornithine, with the carbon and nitrogen from ornithine being converted via Glu to GABA, and then reported that GABA synthesis can be elevated by environmental stimuli (Bown and Shelp 1989, 1997). As might be predicted from the biochemical properties of the plant GAD, an acidic pH of 5–6, a temperature of

approximately 30–40 °C, and the addition of exogenous Glu and Ca^{2+} /CaM are optimal for generating GABA in sprouting grain and germinating seeds (Scott-Taggart et al. 1999; Oh 2003; Chung et al. 2009; Techo et al. 2018; Jiang et al. 2021). Hypoxia also increases the GABA level (Chung et al. 2009; Lin et al. 2015; Shen et al. 2015; Thuwapanichayanan et al. 2015; Ding et al. 2016; Techo et al. 2018).

The accumulation of GABA in germinated brown rice, quinoa (*Chenopodium quinoa* Willd.) and adzuki bean (*Vigna angularis* (Willd.) Ohwi & H. Ohashi) is also associated with elevated GAD activity and expression of the various GAD genes (Liu et al. 2005; Zhao et al. 2017; Hussain et al. 2020; Jiang et al. 2021; Zhang et al. 2021). Elucidation of the precise role(s) of the various GADs, as well as GABA-Ts and SSADHs, requires additional study using RNAi or gene editing technologies. Nonetheless, germinated cereal grains and legume seeds are now being commercially marketed as a rich source of GABA. For example, germinated brown rice (GBR) has 15 times more GABA than standard brown rice, and 10 times the level in germinated white rice (Coconuts Bangkok 2019). GBR was developed for marketing in Japan in 1995. GBR products were developed and marketed first by Domer Co. (Ueda City, Nagano Pref.) and the city government, Mino-cho of Kagawa Pref., was one of the earliest organizations engaged in the production of GBR (Patil and Khan 2011).

Breeding and genetic engineering to hyperaccumulate GABA in tomato fruits and rice grains

Breeding and genetic engineering of GABA-enriched tomato fruit was spearheaded by Professor Hiroshi Ezura and co-workers at the University of Tsukuba in Japan (Gramazio et al. 2020). Early research indicated that fruit of *Solanum pennellii* Correll, a wild tomato relative, contains higher GABA levels than fruit of the domesticated species (Table 1). Typically, GABA increasingly accumulates in fruits of modern tomato cultivars such as Micro-Tom from flowering to the mature green stage, then decreasing during ripening (Rolin et al. 2000; Akihiro et al. 2008). In contrast, the GABA-enriched cultivar DC03-9 does not show a rapid decline in GABA after the breaker stage (Table 1). Three cytosolic CaM-dependent SIGADs are present in the fruit, though *SIGAD2* and *SIGAD1* are expressed during the first two-thirds of fruit development, and *SIGAD1* is expressed during the second half (Akihiro et al. 2008). GABA levels in red fruit are not reduced in RNAi-interference (RNAi)-*SIGAD1* lines, whereas they are reduced by 28%–77%, 72%–95%, and 64%–93%, respectively, in RNAi-*SIGAD2*, RNAi-*SIGAD3*, and RNAi-*SIGADall* lines, with little effect on overall plant growth, indicating that the expression of *SIGAD2,3* is correlated with GABA accumulation in red fruit (Takayama et al. 2015).

Three pyruvate/glyoxylate-dependent SIGABA-Ts are present in tomato fruit, but only SIGABA-T1 is mitochondrial. SIGABA-T2 and SIGABA-T3 are cytosolic and plastidial, respectively (Akihiro et al. 2008; Clark et al. 2009b) (Fig. 1). The expression of all three GABA-Ts is similar during the first half of fruit development, but the expression of GABA-T2,3 declines thereafter (Akihiro et al. 2008). Loss-of-function analyses confirmed that pyruvate and glyoxylate-dependent GABA-T activity is essential for GABA catabolism in tomato fruit and showed that *SIGABA-T1* is the major gene responsible (Koike et al. 2013; Li et al. 2018). RNAi plants with suppressed *SIGABA-T* expression are dwarf and infertile. Notably, in DC03-9 the expression of GABA-T1,2 remains steady during the breaker and red stages of development, but GABA-T activity increases (Saito et al. 2008). A single mitochondrial NAD^+ -dependent *SSADH* is present in the fruit and its expression is steady throughout development (Akihiro et al. 2008). Thus, the enriched GABA level in DC03-9 fruit is likely due to elevated GABA synthesis rather than catabolism.

Fruit of transgenic tomato plants constitutively overexpressing *SIGAD3* exhibit a normal phenotype and possess up to five-fold higher GABA levels than the WT (Takayama et al. 2015) (Table 1). Fruit-ripening-specific overexpression of *SIGAD3ΔC* increases the fruit GABA levels even more, but

Table 1. Natural variation, breeding and genetic engineering/genome editing strategies influence GABA levels in ripe tomato fruits and rice grain.

Strategy	GABA enrichment, -fold	References
Tomato fruits		
Comparison of <i>Solanum pennellii</i> fruit with <i>S. lycopersicum</i> fruit	3.5	Schauer et al. 2005
Comparison of DGO3-9 with House Momotaro	10	Saito et al. 2008
Fruit-ripening-specific RNAi suppression of <i>SIGABA-T1</i> expression	7	Koike et al. 2013
Constitutive overexpression of <i>SIGAD3</i>	up to 5	Takayama et al. 2015
Fruit-ripening-specific overexpression of <i>SIGAD3ΔC</i> mutant lacking full CaM-binding domain	up to 18	Takayama et al. 2017
Expression of <i>SIGAD2ΔC</i> or <i>SIGAD3ΔC</i> lacking full or partial CaM-binding domain in Micro-Tom using CRISPR/Cas9	7–15	Nonaka et al. 2017
Hybrid F1 lines derived from Aichi First and Micro-Tom <i>SIGAD3ΔC</i> mutant lacking autoinhibitory CaM-binding domain using CRISPR/Cas9	3	Lee et al. 2018
Suppression of <i>SIGABA-TP1</i> and <i>SIGABA-TP1,3</i> in Micro-Tom and Ailsa Craig using multiplex CRISPR/Cas9	3.5	Li et al. 2018
Expression of <i>SIGADΔC</i> lacking CaM-binding domain in Sicilian Rouge using CRISPR/Cas9	4–5	Waltz 2021
Rice grain		
Suppression of <i>OsGABA-T1</i> expression using RNAi in subspecies <i>japonica</i> Ningjing	20	Zhou et al. 2015
Seed-specific overexpression of <i>OsGAD2ΔC</i> and RNAi suppression of <i>OsGABA-T1</i> in subspecies <i>japonica</i> Koshihikari	45	Akama et al. 2009; Shimajiri et al. 2013b; Kowaka et al. 2015
Expression of <i>OsGAD3ΔC</i> in subspecies <i>japonica</i> Nipponbare using CRISPR/Cas9	7	Akama et al. 2020

the fruit never turn red (Takayama et al. 2017). Thus, sufficient GABA could be accumulated for practical use, but strict regulations for genetically modified organisms (GMOs) at the time limited their subsequent utilization.

CRISPR/Cas9 technology-mediated deletion of the C-terminal autoinhibitory domain in *SIGAD2* and *SIGAD3* also increases fruit GABA levels (Table 1), but plant growth, flowering, and fruit yield are reduced in the former case, suggesting that *SIGAD3* is a suitable CRISPR/Cas9 target for increasing GABA levels in tomato fruit (Nonaka et al. 2017). This was followed by the production of F₁ hybrid lines with elevated GABA levels by crossing the MicroTom *SIGAD3* mutant created with CRISPR/Cas9 to a WT cultivar (Lee et al. 2018). Subsequently, Li et al. (2018) exploited a multiplex CRISPR/Cas9 system to demonstrate that suppression of *SIGABA-TP1* and *SISSADH* increases leaf and fruit GABA levels and reduces plant height and fruit set. Interestingly, leaf GABA accumulation is associated with the inhibition of GAD activity and leaf necrosis in the SSADH mutant with the accumulation of reactive O₂ species. More recently, the Sanatech Seed Company of Japan released a commercial tomato cultivar with elevated GABA levels; this was created by expressing a mutant GAD gene lacking the CaM-binding domain (presumably *SIGAD3ΔC*) using CRISPR/Cas9 (Waltz 2021) (Table 1). Notably, CRISPR/Cas9-engineered plant materials are being considered genome edited, rather than genetically modified, thereby reducing some opposition to their commercialization.

Professor Kazuhito Akama at Shimane University in Japan led efforts to genetically engineer rice grain with elevated GABA levels. Five *GAD* genes are present in maturing rice seed. Four of these

possess the C-terminal CaM binding domain; the C-terminus of OsGAD2 does not bind CaM, but is still autoinhibitory (Akama et al. 2001; Akama and Takaiwa 2007). *OsGAD1* shows the highest expression, followed by *OsGAD5*, *OsGAD4* and *OsGAD2,3* (Zhao et al. 2017). The expression of all five *OsGADs* tends to increase with seed maturation. Four GABA-Ts are present in maturing rice seed, with GABA-T1,2 located in mitochondria, OsGABA-T3 in plastids, and OsGABA-T4 in the cytosol (Shimajiri et al. 2013a) (Fig. 1). The three organelle-located GABA-Ts utilize pyruvate and glyoxylate, but not 2-oxoglutarate, as amino acceptors. The activities of recombinant OsGABA-T3 and OsGABA-T1, respectively, are approximately 200- and 50-fold that for recombinant OsGABA-T2. The expression of *OsGABA-T1* is higher than that for *GABA-T2,3,4* in maturing seeds; *OsGABA-T1,2,4* expression increases throughout seed maturation, whereas *OsGABA-T3* expression declines (Shimajiri et al. 2013b; Zhao et al. 2017).

RNA interference of *OsGABA-T1* suppresses the expression of *OsGABA-T1*, as well as *OsGABA-T2*, and increases GABA levels in brown and white rice by approximately 20 and 10 times and 10 and five times, respectively, after 10 days and 4 months of storage (Zhou et al. 2015) (Table 1). Seed-specific overexpression of *OsGAD2ΔC* and RNAi suppression of GABA-T leads to sustained and high GABA levels in brown rice (Akama et al. 2009; Shimajiri et al. 2013b, Kowaka et al. 2015). CRISPR/Cas9 technology-mediated deletion of the C-terminal autoinhibitory domain in *OsGAD3* increases the seed GABA level by seven-fold and this is accompanied by higher grain weight and protein content than WT brown rice (Akama et al. 2020).

Using exogenous GABA to mitigate postharvest deterioration and crop stress

During postharvest storage, horticultural commodities are often exposed to multiple abiotic stresses (i.e., low temperature, low O₂, elevated CO₂) to reduce ethylene production and respiration, so that ripening/senescence is delayed and nutritional and sensory quality is preserved (Lum et al. 2016; Aghdam et al. 2022). Overall, these studies indicated that improvements in the marketability of horticultural products are generally associated with the promotion of GABA biosynthesis and GABA shunt activity, with or without the accumulation of GABA. During prolonged storage, the onset of senescence-related injury may also be associated with the accumulation of GABA.

Our research, conducted in collaboration with Dr. Gale Bozzo at the University of Guelph and Dr. Jennifer DeEll at OMAFRA, focused on apple fruits, since they are stored, more than any other botanical fruit, under controlled atmosphere conditions. Furthermore, gene sequences for the key steps in GABA metabolism in apple fruit were identified by comparison to the *Arabidopsis* genome (Brikis et al. 2018; Aghdam et al. 2022), allowing elucidation of the biochemical properties and subcellular location of multiple isoforms of the encoded proteins (Fig. 1), as well as their transcript abundance. Exogenous GABA was never considered to be an effective protection against the multiple abiotic stresses imposed during postharvest storage because its penetration through the apple skin was presumed to be low. However, many other researchers demonstrated that exogenous GABA significantly alleviates chilling injury, bacterial/fungal decay, and loss of quality in postharvest fruits, vegetables and cut flowers during storage (see references in Aghdam et al. 2022).

With the current rate of global warming, the temperature is expected to rise by 1.5–2.4 °C by 2050, field-grown crops will be subjected to more extreme weather events and concurrent and multiple climate changes (e.g., drought, waterlogging/O₂ deprivation, salinity), which reduce crop yields (Shelp et al. 2021a). Because of the positive effects of endogenous and exogenous GABA on the levels of reactive O₂ species, membrane stability, non-enzymatic and enzymatic antioxidants, and crosstalk among phytohormones, exogenous application of GABA was recently posited to be an effective and sustainable strategy against multiple stress under both open and closed environments. For example,

GABA binding to receptors can interfere directly with ion transport in stomata and root epidermal cells, and with the activity of phytopathogens, thereby enhancing tolerance to drought, waterlogging (hypoxia), and disease (Shelp et al. 2021a). And promoting the activity of the GABA pathway can restore or partially restore stress-induced losses in respiration and energy generation.

Novel elite corn lines with enriched carotenoid levels

Carotenoids are acquired in the diet and associated with protection from many chronic diseases, including an eye disease known as age-related macular degeneration (ARMD). ARMD is associated with loss of lutein and zeaxanthin from the macular region of the eye (see references in Burt et al. 2013). Currently, it is the leading cause of vision loss in people over 50, affecting approximately 2.5 million Canadians (Fighting Blindness Canada 2020).

It was known that egg carotenoids are readily absorbed into the human body, and that the dietary intake of lutein by humans could be enhanced by supplementing the diet of laying hens with marigold (*Tagetes erecta* L.) petal extracts (Delgado-Vargas et al. 1998). Professor Elizabeth Lee and co-workers at the University of Guelph posited and confirmed that the carotenoid levels, as well as the zeaxanthin:lutein balance, in field corn could be improved by allele mining of the Orange Flint race using traditional breeding techniques (Burt et al. 2010, 2011a, 2011b). Subsequently, the feasibility of using high-carotenoid corn to generate high-lutein and (or) high-zeaxanthin eggs, and the utility of using the high-carotenoid lines as donors to develop elite high-carotenoid inbred lines with high carotenoids were demonstrated (Burt et al. 2013). Accordingly, the future to apply high-carotenoid corn seemed bright. Furthermore, a leading agriculture company was interested in introgressing the high carotenoid content into sweet corn for human consumption. In the meantime, however, a global ingredient manufacturer in the eye health space generated marigold lines with elevated levels of both lutein and zeaxanthin, which reduced the interest of corn producers in commercializing the high-carotenoid corn.

Eco-friendly control of *Botrytis* infection

Botrytis cinerea, the causal agent of the gray mold disease, is the most economically important necrotrophic fungal plant pathogen in the world (see refs in Seifi et al. 2019). While conventional fungicides against the gray mold disease are available, there are situations where they do not offer complete control, and negative health and environmental consequences have been ascribed to some of them. Therefore, the development of novel resistance-enhancing and eco-friendly controls for such an impactful disease seemed important for production of horticultural and field crops, as well as organically grown crops.

Many natural compounds are known to activate defense responses against a certain type of biotic or abiotic stress. Recently, we demonstrated that exogenous application of spermine (Spm), unlike other polyamines, induces strong resistance against *B. cinerea* in tomato, common bean, and *Arabidopsis* plants (Seifi et al. 2019). Furthermore, co-application of Spm with salicylic acid, a priming agent against a broad spectrum of phytopathogens, suppressed disease in a tomato-*B. cinerea* pathosystem in a synergistic interaction. Since there are many other important pathogens with a similar necrotrophic mode of infection (e.g., *Sclerotinia sclerotiorum* and *Alternaria solani*), this finding appeared to be a significant step towards establishing novel eco-friendly methods to control a wide range of pathogens (Seifi and Shelp 2019). A patent application was submitted, but our industry partner was unable to replicate the synergistic interaction in greenhouse and field trials with various crop species, so the application was abandoned.

Sustainable greenhouse floriculture

Improving plant, human, and environment health

The limited mobility of B and the excessive use of N, typically in the form of nitrate, are associated with physiological disorders in many horticultural crops, especially brassicas such as broccoli (*Brassica oleracea* var. *italica* Plenck), cauliflower (*B. oleracea* L. var. *botrytis*), radish (*Raphanus sativus* L.) and rutabaga (*B. napus* ssp. *rapifera* (Metzg.) Sinsk). Nitrate accumulation in the plant is harmful to the health of humans, especially babies, when consumed ([Committee on Nitrate Accumulation 1972](#); [Liang and Zhang 2020](#)), and nitrate in the environment is the main source of nitrous oxide, a major greenhouse gas and contributor to global climate change.

Across Canada, greenhouse growers have come under scrutiny as a possible source of unacceptable nutrient levels in local freshwater resources (e.g., [Ontario Ministry of the Environment 2012](#); [Maguire et al. 2018](#)). Closed irrigation systems (e.g., drip irrigation and subirrigation), which recycle and reuse the nutrient solution, have been adopted to assist in meeting the increasingly stringent regulations set by environmental agencies. Opportunity exists for optimizing the nutrient use efficiency (NUE) in floricultural systems.

Understanding nutrient uptake and distribution

Broccoli is one of the few temperate vegetable plants that bleed phloem exudate spontaneously, and because it is susceptible to the hollow stem disorder, it became my model horticultural plant for studying plant nutrition ([Shattuck and Shelp 1987](#); [Shelp 1987](#)). Our research with this plant and other horticultural brassicas such as rutabaga, radish and cauliflower established that even though the phloem mobility of B in temperate plants is limited, it can undergo both direct and indirect xylem-to-phloem transfer in sufficient amounts to satisfy the demands of developing sink regions that do not readily transpire ([Shelp and Shattuck 1987a, 1987b](#); [Shelp et al. 1987, 1998](#); [Shelp 1988](#); [Liu et al. 1993](#); [Marentes et al. 1997](#)) ([Fig. 2](#)). Notably, in species that produce significant amounts of polyols in source leaves, B is readily translocated as B-polyol complexes ([Brown and Shelp 1997](#)). “Boron is thus unique among the essential plant nutrients in that it has restricted mobility in many plant species and is freely mobile in others. No other element is known to vary so greatly in mobility” ([Brown and Shelp 1997](#), p. 85).

Since both nitrate and chloride appear to serve important osmotic roles in the vacuole, we posited that exogenous chloride can induce the mobilization of nitrate from the vacuole, thereby improving the efficiency of nitrogen use ([Flowers 1988](#); [Liu and Shelp 1995](#)). The mass of broccoli plants increases by two- to threefold during flower development, and substitution of nitrate with chloride at the onset of flowering decreases yield by 13% and shoot nitrate contents by approximately 60% during the flowering period ([Liu and Shelp 1995](#)). Chloride application over the entire growing period does not affect the final shoot yield and total-N contents but does decrease shoot-nitrate and increase total-N in phloem exudate ([Liu and Shelp 1996](#)). These findings suggested that nitrate absorption by the roots is not inhibited by chloride, and the decrease in shoot nitrate accumulation is consistent with a chloride-induced mobilization and reduction of vacuolar nitrate and (or) a decrease in vacuolar nitrate storage. While this research was discontinued due to a lack of funding, it is now clear that nitrate accumulation in vacuoles is regulated by chloride channels and relies on a H⁺ gradient established by vacuolar ATPase and vacuolar pyrophosphatase ([Liang and Zhang 2020](#)), and chloride accumulates at macronutrient levels in many plant species, thereby significantly improving the use of the absorbed nitrate ([Rosales et al. 2020](#)). Clearly, more research on the use of chloride to improve N use efficiency would have been warranted.

Young plants absorb nutrients through the root system, but that process declines with the onset of flowering ([Pate 1986](#); [White 2012](#)). Consequently, previously acquired and stored nutrients are very

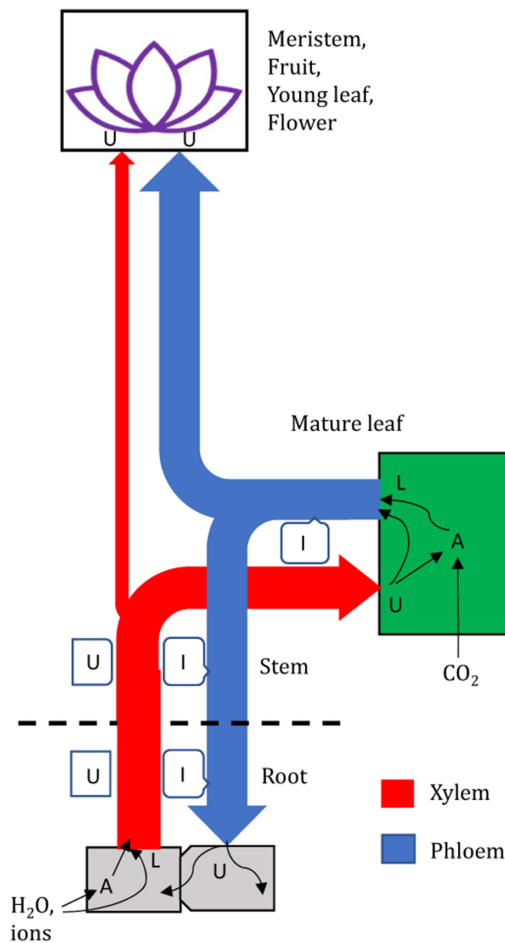


Fig. 2. Basic elements of the circulatory system in chrysanthemum. Roots function in the absorption and as a source of nutrients for the remainder of the plant, whereas mature leaves, as well as the fruit, young leaf, and flower function as sinks for nutrients. Nutrients are transported from the roots to the sinks via two pathways: the xylem and the phloem. Xylem is the principal source of nutrients for mature leaves, which lose water readily. In contrast, phloem supplies the younger tissues and roots, which do not readily lose water. Three generalizations are possible: nutrient uptake by roots is less during reproductive growth than during vegetative phase; reproductive growth often relies on nutrients acquired and stored during vegetative growth; and the level and activity of high-affinity nutrient transporters in roots are often stimulated by low-sufficiency nutrient supply. Abbreviations: I, interchange; assimilation; L, loading; U, unloading. (This figure is based on information in [Pate 1986](#); [Shelp et al. 2017a, 2020](#); [Sutton et al. 2019](#)).

important for flower and fruit development ([Fig. 2](#)). Recent studies have demonstrated that the efficiency of nutrient absorption by the root and redistribution by the shoot can be induced by strategically lowering the nutrient supply so that plant yield and quality are unaffected ([Shelp et al. 2017a, 2020](#); [Sutton et al. 2019](#)).

Optimizing nutrient delivery

This research was initiated in the mid-1990s following the success of the field experiments described above for broccoli, but its latent value to the floricultural industry was not fully realized until new funding from a combination of government and industry sources became available. The funding provided the opportunity to better understand how the plant's inherent capacity and attributes can inform the decision-making processes about fertilizer application in an indoor floriculture setting.

Subirrigation (also known as capillary irrigation) was of particular interest because the composition of the recycled nutrient solution is essentially unchanged, though its use is limited to smaller pot sizes (MacDonald et al. 2013; Ferrarezi et al. 2015; Semananda et al. 2018). Two practical strategies were combined for improving NUE in greenhouse-grown pot chrysanthemum, which is well suited to subirrigation: (i) removal of nutrient supply during reproductive growth to stimulate remobilization of nutrients from vegetative organs and (ii) reduction in the nutrient supply during vegetative growth to enhance uptake efficiency (Fig. 2). Research and commercial greenhouse experiments demonstrated that the entire macronutrient and micronutrient supplies can be removed during reproductive growth, and only 25%–50% of the levels of macronutrients and micronutrients currently recommended by the industry is required during the vegetative period, without any adverse effects on crop yield and flower quality (MacDonald et al. 2014; Shelp et al. 2017a, 2020, 2021a, 2021b; Sutton et al. 2019; Duncan Stephens et al. 2021; Donetz et al. 2022; Teeter-Wood, K., unpublished data). Thus, the nutrient requirements over the crop cycle can be reduced by approximately 75%–87.5%. It is also of interest to optimize nutrient delivery with drip irrigation, another common method for irrigating indoor ornamentals (Lévesque et al. 2009). Unlike subirrigation, drip irrigation is scalable to any size, and a reduction in salt accumulation in the root medium obtained with a lower nutrient supply could reduce the need for over irrigation. Collaboration is continuing with the industry, farmers and fertilizer manufacturers to reduce the amount of fertilizer applied to chrysanthemum and other ornamentals. This innovative low-input nutrient delivery strategy should reduce the costs of fertilizer and treatment of spent nutrient solution and improve the environmental sustainability of the greenhouse industry.

Conclusions

The case studies presented here illustrate that most academic research in plant biology has the potential for translation into innovative solutions for food, agriculture, and the environment. However, the time frame for its application depends on various factors such as the fundamental nature of the questions under consideration, the development of new enabling technologies, the research priorities of funding agencies and availability of continued funding, the existence of competitive research, the willingness of researchers to become engaged in commercial activities, and ultimately the insight and creativity of the researchers. By its very nature, applied research in plant biology may be more rapidly adopted by industry than basic research, but it does not appear to generate as many patenting and licensing opportunities (Garud et al. 2018; Hmielewski and Powell 2018).

Three of the cases studies target the production of novel plant products that could improve human health. GABA-enriched tomato fruits and rice grains were generated approximately seven decades after the discovery of GABA in plants and required elucidation of the biochemical pathway, identification of the genes responsible for encoding the enzymes involved, development of plant transformation and gene/genome editing technologies, and involvement of several research teams. Enrichment of GABA in germinating cereal grains and legume seeds was based on a fundamental understanding of the biochemical regulation of glutamate decarboxylase, the primary enzyme for GABA biosynthesis. While the breeding of high carotenoid corn for animal and human consumption using exotic genetic resources was certainly feasible, more rapid advances by competitors limited the commercial opportunities for this product.

Three cases targeted improvements in crop production. The suggestion to use exogenous GABA to improve the tolerance of horticultural and agronomic crops to multiple abiotic and biotic stresses was based on the (partial) restoration of losses in respiration and energy, as well as quality, under the adverse environmental condition present in postharvest storage or because of changes in global climate. Advances in the commercial production of GABA, as well the safety of GABA for human

consumption, are still required before this application can truly be realized (Shelp et al. 2021a, 2021b; Aghdam et al. 2022). Spermine was efficacious in activating novel plant defense mechanisms under controlled conditions, but unfortunately, it was not possible to demonstrate a synergistic interaction between Spm and salicylic acid under real-world conditions. The optimization of nutrient delivery in floricultural crop production depended on an enhanced understanding of nutrient uptake, utilization, and distribution, as well as renewed interest from government and the floricultural industry in minimizing environmental risks. Adoption of this technology is underway.

Together, these case studies illustrate that most academic research in plant biology and perhaps other natural sciences as well, will eventually have real-life application. Consequently, academic research might be better distinguished by the anticipated time from discovery to real-life application (e.g., long- and short-term), rather than as basic or applied.

Hmielecki and Powell (2018) have argued that connection with industry contacts and a focus of academic scientists on applied, rather than basic, research is associated with engagement in commercialization activities. This suggests that academic scientists engaged in basic research are less likely to participate in science commercialization. It therefore seems inappropriate for the merit of NSERC Discovery Grants applications to be evaluated from their potential impact on policy and (or) technology, as is currently done (Natural Sciences and Engineering Research Council of Canada 2022). Since matching industry funds in Canada rarely support the search for knowledge, it also seems appropriate that NSERC Discovery Grants should fund basic research in its entirety, rather than being considered as a grant-in-aid. If indeed, Canada is interested in the search for scientific knowledge then a plan should be devised for wholly funding such programs.

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Author contributions

BJS conceived and designed the study and wrote the manuscript.

Competing interests

The author declares that there is no conflict of interest regarding the publication of this paper.

Data availability statement

All relevant data are within the paper.

Competing interests

The authors have declared that no competing interests exist.

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