

Kevin Gallagher
Dr. Graeme Berlyn
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A Review of Organic Biostimulants in the Context of Genetic Engineering

Introduction:

Originally developed in the 1970s as a byproduct of tissue culture biotechnical research, organic biostimulants are non-hormonal mixtures of antioxidants, amino acids, metabolism enhancers, fungi spores, and other bioactive materials that when exogenously applied to plants stimulate growth, increase stress resistance, promote metabolic activity in the rhizosphere, and facilitate optimal yield. These compounds create a self-reinforcing feedback loop, enabling plants to achieve their full physiological capacity and increasing yield in both high and moderate stress environments. Mitigating environmental stressors and understanding the stress response pathways is imperative amid climate change and efforts to increase food production.¹ With a high probability, amplifications of extreme weather events and seasonal temperature fluctuations in tandem with exponential population growth will strain agricultural resources over the next several decades and stress amelioration will become an economic priority. Even today, environmental stressors reduce agricultural production by an estimated 78% (Boyer, 1982), and abiotic stress alone reduces crop yields by about 50% (Hasanuzzaman et al., 2012). Two prominent schools of thought have emerged on how to solve this problem: Genetic engineering and exogenous organic biostimulants.

In the context of the shortcomings of genetic engineering, understanding biostimulants, how their main bioactive components function as stress mitigants, and their larger implications

¹ The major sources of plant stress are climatic, edaphic, physiographic, biotic, and anthropogenic.

will be vital for adept stress management as agriculture evolves amid climate change and elevated consciousness of the sector's high environmental impact. Within this framework, this paper explores recent biostimulant advances in the Berlyn Laboratory of the Yale School of the Environment and uses a literature review of genetic engineering research to identify additional candidates that are likely to exhibit biostimulant properties. Overall, biostimulants hold the potential to increase yields, reduce inorganic fertilizer pollution, and mitigate the adverse impact of climate change on global agriculture.

Biostimulants Overview:

A mixture of stress-mitigating vitamins and coenzymes for higher plants, organic biostimulants have emerged as a rapidly growing field over the past 40 years, with increasingly demonstrated commercial applications. Russo and Berlyn (1990) defined biostimulants as non-fertilizer compounds that when exogenously applied to plants in small quantities, increase stress resistance, promote optimal yield, and reduce required fertilizer by 50% to 100%. (Berlyn and Beck, 1980; Berlyn et al., 1990; Russo and Berlyn, 1990; Russo and Berlyn, 1992; Russo and Berlyn, 1994; Berlyn and Sivaramakrishnan, 1996). Applied either foliarly or via a root drench, these metabolic enhancers can directly promote plant growth and can also stimulate the rhizosphere to a plant's benefit. However, other working definitions of biostimulants exist, some of which include plant hormones, other growth regulators, non-essential trace minerals, and micronutrients. The original interpretation of the concept is used herein.

In small quantities, non-hormonal biostimulants have profound effects on plant growth and total biomass production. Plants are seldomly situated in an ideal environment in which they can achieve their full autotrophic capacity and maximum yield. Even in seemingly optimal environments, stressors are omnipresent, even if they are undetectable (Foyer et al., 1994). By

stimulating metabolic growth processes, biostimulants mitigate these stressors' effect and regulate the response pathways at the molecular level. Also referred to as organic growth enhancers, biostimulants have been demonstrated to increase crop yield, overall plant biomass, plant vigor (Russo and Berlyn, 1990), crop nutritional value (Borsook and Berlyn, 2015), polyphenol content (Berlyn, Shields, and Young, 1995), plant vascular system development, and stress resistance. The effects are self-reinforcing: Increased biomass promotes high nutrient and water uptake, which, in tandem with the elevated hydraulic conductance from increased xylem development, reduces water and nutrient stress levels in leaves, which also benefited from the innate antioxidant capacity of the compounds. The reduced stress and elevated photosynthetic capacity increase the carbon to nitrogen ratio, carbon compounds available for growth, and overall biomass. This is achieved with natural, non-hormonal ingredients.

These compounds achieve the stress reduction predominately via their antioxidant capacity. Under high light fluxes, plants experience photooxidative stress, which is when the light-dependent photosynthesis reactions generate reactive oxygen species (ROS) when the chlorophyll absorbs more energy than it can physically use in photosynthesis. When the photoelectron chain is bottlenecked due to a dearth of NADP or oxidized electron acceptors, the additional electron, usually from photosystem II, reduces an oxygen molecule. The resulting oxygen species, which are energetic molecules with unpaired electrons, can damage lipids, DNA, RNA, proteins, and is exceptionally harmful to chlorophyll. All stressors effect the electron transport chain or its supporting process to a certain degree and will result in ROS. Examples of ROS include singlet oxygen ($^1\text{O}_2$), hydroxyl radical ($\text{HO}\cdot$), hydrogen peroxide (H_2O_2), and superoxide (O_2^-), one of the most detrimental. Extreme damage is irreparable and can result in mutagenesis and cell death. Reactive oxygen species are created by metabolic processes even under optimal conditions and have been shown to function in signaling (Shin et al., 2012). Plants have evolved antioxidant

responses to detoxify these harmful compounds through both enzymatic and non-enzymatic processes that scavenge and neutralize ROS. An enzymatic process, superoxide dismutase (SOD) is an exceptionally powerful antioxidant that reduces ROS into hydrogen peroxide. Hydrogen peroxide is then converted into water and oxygen in either the peroxisome, which is an organelle containing catalase (CAT), or via the ascorbic acid glutathione chain. Since chloroplasts do not contain catalase, it relies on the latter process. Non-enzymatic processes predominately include lower molecular mass antioxidants, which include ascorbic acid, flavonoids, and glutathione (Huang et al., 2019). The organic biostimulant hypothesis is founded on the observation that under stressful conditions, plants cannot produce the optimal amount of these antioxidant compounds and exogenously applying them can increase detoxification capacity and overall productivity (Berlyn and Beck, 1980).

Originally discovered as a byproduct of plant cell culture research, biostimulants increase a plant's antioxidizing capacity to deal with ROS through both the enzymatic and non-enzymatic processes, and bioactive compounds of significant biostimulant ability have been identified. In the Yale biostimulant concept, one of the most bioactive is l-ascorbic acid (AsA), commonly known as vitamin C. AsA functions in the glutathione-ascorbate-SOD oxidation reduction processes, thereby increasing ROS scavenging abilities. AsA also promotes xylem function and keeps iron in its ferrous state, which is required for cell wall synthesis. Berlyn and Russo (1990) demonstrated that it also acts as a phosphorus, nitrogen, potassium, and micronutrient pump. The nutrient is also credited with modulating several other fundamental metabolic functions.

Closely related to AsA, thiamin (vitamin B1), is also a known biostimulant and is essential for cellular respiration. Its active form thiamin pyrophosphate (TPP) is an enzyme cofactor for amino acid synthesis, tricarboxylic acid cycle, and pentose phosphate pathway. It also is an antioxidant indirectly by recycling ascorbic acid via NADPH (nicotinamide adenine dinucleotide

phosphate) synthesis and directly with its O_2^- and OH^- scavenging abilities. However, the precise antioxidant mechanisms are not well understood (Subki et al., 2018). Thiamin is predominately synthesized in a plant's leaves, which can provide sufficient quantities to the roots under low stress conditions. Contrarily, under moderate stress conditions, the leaves cannot supply the roots with an adequate amount of B1, so the exogenous application of the nutrient is necessary to achieve optimal yield and improved plant health.

Glycine and arginine are two other bioactive substances with similar effects. Glycine (Gly) is the simplest amino acid and is found predominately in the form of glycine betaine. It functions in the chloroplast in thylakoid membrane protection, improving photosynthetic efficiency, and is a chlorophyll precursor (Robinson and Jones, 1986; Genard et al., 1991). Mohammadipour et al. (2019) demonstrated that exogenous applications of glycine increased AsA concentration in the leaves of coriander (*Coriandrum sativum*), and the substance is known to promote xylem formation. Glycine rich proteins are a key structural component of plant cell walls (Ringli et al., 2001). In higher plants, Gly is synthesized in the chloroplast from serine, choline, betaine aldehyde, and ethanolamine, and the limiting factor in its production is low choline concentrations and transport, as demonstrated in transgenic plants (Hanson et al., 1980; Rhodes et al., 1993; Nuccio et al., 1998; Huang et al., 2000; McNeil et al., 2000). Plant growth can be improved with exogenous applications of glycine, as proven by the biostimulant concept. The simple amino acid is rapidly taken up by plants relative to other amino acids (Matysiak et al., 2020), but a plant's absorption of the chemical can be improved with surfactants, such as kinetic, lus-50, and sito+ (Subbararo et al., 2001). To this end, including these compounds in a biostimulant formula may promote its uptake efficacy and further promote plant growth. The stimulating ability of glycine is not constant across plants. Although stress response pathways and mechanisms have a wide variety across species, the core cellular responses are generally consistent; However, while most

species exhibit elevated glycine levels as a response to stressors, some species, such as rice (*Oryza sativa*), mustard (*Brassica spp.*), Arabidopsis (*Arabidopsis thaliana*), and tobacco (*Nicotiana tabacum*) do not synthesize the compound but react positively to its application (Ashraf, 1994; Fooland, 1999; Zhu, 2001; Ashraf et al., 2007). Glycine has various levels of demonstrated effectiveness as a biostimulant depending on species and stress factors, but more research is needed to draw definitive conclusions.

Closely related to glycine and with similar biostimulant properties, l-arginine (Arg) is a complex and functionally diverse essential amino acid. In plants, it functions as organic nitrogen storage, is a part of protein synthesis, and is a precursor for polyamines and nitric oxide. In conifers, it is the primary source for nitrogen-containing compounds. Arginine and its derivative polyamines have been shown to act as plant stress response factors. As a biostimulant, arginine is more powerful than glycine likely due to its higher nitrogen levels, but the published comparative studies did not consider testing higher concentrations of glycine to compensate for the nitrogen differential. (Matysiak et al., 2020).

Furthermore, myo-inositol, which is a carbocyclic sugar alcohol ubiquitous in all eukaryotes, exhibits biostimulant properties. The compound is transformed into phytic acid when its hydroxyl groups are esterified with phosphoric acid groups. Phytic acid, also known as inositol hexaphosphate, is the primary phosphorus storage mechanism in most plant tissues and functions as a chelate for calcium, magnesium, iron, and zinc. Myo-inositol interacts with salicylic acid and ethylene-dependent pathways that mediate ROS-induced apoptosis (Hu et al., 2020). It is also an osmotic regulator and critical for cell wall formation (Halls et al., 1974).

The former aqueous carrier for the Yale biostimulant formula was a blend of humic acid and marine algal extracts. This carrier had biostimulant properties but was not the most bioactive component of the formula (Russo and Berlyn, 1992). Humic acid (HA) is a naturally occurring

polymeric organic compound proven to ameliorate nutrient deficiencies via chelate formation (Bohme et al., 1997; Sanchez-Sanchez et al., 2002). This is most profound with the complexing of iron and zinc, increasing the availability of these micronutrients in the soil. The cation exchange capacity of the soil naturally increases as a result. The compound has also been shown to have stimulatory effects similar to that of cytokinin, auxin, and gibberellin (Zhang et al., 2004; Pizzeghello et al., 2001). Comparatively, marine algal extracts promote plant growth, which is attributed to phytohormones (Melo et al., 2020). It has growth regulator-like effects and influences antioxidant capacity and phenolics. While algal extracts remain in the current formula, humic acids have since been removed because they have been demonstrated to rapidly build up in soil, inducing glyphosate toxicity.²

Recent Experiment:

The most recent biostimulant experiments explored coenzyme Q10 (CoQ10) for growth enhancement properties in January of 2020. No statistically significant effect was detected. CoQ10 is a membrane-soluble antioxidant and an electron carrier cofactor in aerobic respiration's electron-transport chain. Without it, cells cannot synthesize ATP. The compound also manages oxidative stress in conjunction with alternative oxidase and potentially functions in the pyrimidine biosynthesis pathway (Stiff et al., 2011).

In the trials, radish plants were grown under optimal greenhouse conditions for 60 days. Five experimental groups were created, with 40 observations per group. Descriptive charts of the total fresh and dry biomass can be found in the appendix. The experimental groups were:

1. Control
2. Inorganic Fertilizer (2.0 grams of Nutraleaf per liter)
3. Core Biostimulant Formula without Fertilizer

² Other biostimulant compounds explored include alpha tocopherol (vitamin E), leucine casein hydrolysate, superoxide dismutase, , mycorrhizae, and iron. Superabsorbent gels were also investigated for yield maximization,

4. Core Biostimulant Formula with Fertilizer and CoQ10
5. Core Biostimulant Formula with no Fertilizer and CoQ10

The group treated with the core biostimulant formula, CoQ10, and inorganic fertilizer had a mean fresh weight 1,165% and 384% higher than that of the control and inorganic fertilizer groups, respectively. These differences were significant at a probability value of 10^{-6} using F and Contrast Tests. The experimental group treated with the core biostimulant formula, CoQ10, and inorganic fertilizer had a mean fresh biomass 3.4% higher than that of the group treated with the core biostimulant formula and inorganic fertilizer. This difference was not statistically significant ($p = 0.45$), meaning that the marginal biostimulant effect CoQ10 has over the core formula was not detectable. However, these experiments were done under optimal conditions and no nutrient stress. CoQ10 may have detectable stress mitigation effects if applied to plants under higher stress levels and soil nutrient deficiencies.

The non-statistically significance marginal stimulating effect of CoQ10 aside, the biostimulant formula's 1,165% and 384% increase in total fresh biomass has profound implications for the agricultural sector. In the trial, the radishes were treated once a week with 25 ml of spray, in which the radishes in the experimental group 'St' received 0.22 grams of biostimulant with each treatment. This weekly 0.22 grams of bioactive substance without additional inorganic fertilizers increasing biomass production by 384% over fertilizer alone has profound implications if biostimulants are adopted by the agricultural sector as a whole. The excess levels of pesticide, fungicide, and inorganic fertilizer use that characterize modern agriculture in the developed world are unsustainable, and this pollution can be decreased while simultaneously increasing yield. Furthermore, the positive impact of fertilizer has reached its upper bound, as a high proportion of inorganic nitrogen is not absorbed by the plants, instead leeching into below-ground aquifers and running off into water tables.

In addition to pollution reduction, the increased yield will have significant benefits for the agricultural center. Holding all other inputs constant, the value of the incremental yield generated by organic biostimulants in excess of the treatment's cost will fall directly to the bottom line. Since the agricultural has low profit margins and produces fungible commodities, this can increase profits and push down food prices over time. For example, Tyree and Berlyn (2011) estimated that replacing hydroponic nutrient solution with organic biostimulant foliar sprays would result in a 1,775% reduction in hydroponic overhead costs. For crops with high commercial value, the rapid adoption is rational. This includes cannabis, industrial hemp, grapes, ornamental bonsai, organic products, vanilla, designer hops, golf course turf, and hydroponics. The impact of biostimulant research and adoption extends past yield augmentation and can mitigate the impact of climate change. Viniculture is a prominent example. Wine grape production area in the United States could decline up to an estimated 81% by the late 21st century, destroying the quality of the produce until vineyards are forced to relocate (White et al., 2006). The tiniest changes in the proportions of grape chemical composition can have substantial effects on wine quality, so the industry has an unmet need for climate change related risk management. Vineyards are already abandoning sensitive rootstocks, such as Riesling and Merlot, in favor of hardier grape varieties. Italy's vineyards have responded by relocating vineyards to higher elevations with cooler microclimates. Across Europe as a whole, the harvest schedule has moved up a month over the past 30 years. By increasing protective phenolic compound levels and bolstering overall stress tolerance, organic biostimulants can preserve grape quality amid changing environmental conditions.

Generally, biostimulants have proved their ability to effectively address plant stress and the evolving needs of modern agriculture.

Advances in Genetic Engineering and Biostimulants:

Plant morphogenesis is regulated by genetic, epigenetic, and environmental factors, and to maximize expected yields, researchers have increased growth through environmental amendments (e.g. inorganic fertilizers, biostimulants, superabsorbent gels) and genetic altercations. Genetically modified organisms (GMOs) are those with altered DNA, and has been used to increase insect, temperature, fungal, viral, bacterial, and general stress resistance. GMOs have also been created with superior yield, aesthetic characteristics, and nutritional value. In 2007, genetically modified crops covered an estimated 114.3 million hectares (Berrie, 2011).

Despite their popularity, GMOs are controversial both in the public and academic spheres. Splicing in the genes from one organism into another can have profound environmental and health impacts: Unintentional cross-pollination can introduce genes into an environment and negatively influence biodiversity, and consumers ingesting GMO crops can experience material adverse side effects. MicroRNA molecules from ingested food have been proven to remain active in the human body after digestion (Campbell, 2000). Genetic transfers from introducing foreign genes into food stock can alter fragile balances and switch genes on and off in unforeseen, potentially disastrous ways. This is not to detract from the significance of GMOs, however. Genetically modified crops have saved large segments of the global the population from starvation.

Increasing crop yields over current levels through further genetic modification is a time consuming and expensive process, and the crops are not guaranteed to be safe for human consumption. A strong argument can be made to equip plants with the tools they need to achieve their full yield potential within their existing genetic framework that took millions of years to evolve. To this end, genes targeted by current genetic engineering efforts and the endogenous compounds that those genes influence can be interpreted as candidate substances for biostimulant effects. For example, plants overexpressing genes for arginine synthesis were created, but

biostimulant experiments have proved that exogenous application can have the same effect. After an extensive literature review, a list of candidates was compiled, and the four most promising compounds are summarized in detail below. They are phenylalanine, silicon, glutathione precursors (selenium, and proline) and boron.

1) Phenylalanine:

Phenylalanine (Phe) is an essential aromatic amino acid, a precursor for l-tyrosine, and is the starting compound used in the synthesis of flavonoids, which are a class of secondary plant phenols.³ Genetically engineered crops have focused on increasing flavonoid concentration due to their protective traits and nutritional value but have not been able to produce commercially viable strains without inhibiting growth (Rojas et al., 2010). Phenylalanine, along with tyrosine and malonate, are the aromatic amino acid building blocks for flavonoids. A large proportion of flavonoids act as antioxidants and scavenge free radicals, including: the flavone luteolin; the flavanols galangin, rutin, myricetin, quercetin, and tiliroside; the flavanone taxifolin; and the flavanols catechin, epicatechin, and gallocatechine. Some have antioxidant capacities superior to that of superoxide dismutase. Flavonoids also protect cell hardware from short wave-length radiation and can function as chelates.

Olivia et al. (2020) demonstrated that exogenously applied phenylalanine on tomato (*Solanum lycopersicum*) was efficiently absorbed and increased Phe, Phe-derived metabolite concentrations in leaves, and pathogen resistance. The application stimulated the general phenylpropanoid pathway, promoting increased phenolic compound production. The paper

³ Tyrosine is a non-essential amino acid required for the biosynthesis of CoQ10, lignin production, and is involved in the stress response pathway.

concluded that the amino acid may “provide a basis for ecologically friendly control of a wide range of plant pathogens.”

Exogenously applied Phe’s demonstrated ability to induce increased pathogen resistance, in tandem with its ROS scavenging abilities, promotion of flavonoid synthesis, and higher flavonoid-induced UV-B tolerance, make it an exceptional biostimulant candidate. The effects may be amplified if applied with alpha tocopherol (vitamin E), which has been demonstrated to increase leaf toughness and pathogen resistance (Berlyn and Sivarmakrishnan, 1996; Neupane and Norris, 1991).

2) Silicon:

The second most abundant chemical in the earth’s crust and making up 31% of soil composition, silicon (Si) is not considered to be an essential nutrient, but it can have profound effects on plants (Sposito, 1989). Depending on the species, silicon is taken up by plants in large amounts through the roots via diffusion or mass flow as monosilicic acid (H_4SiO_4) and is eventually deposited in leaf tissue. Specifically, the monosilicic acid is polymerized, converted into silica gel, and is stored in the cell wall of epidermis and mesophyll cells, where it is immobilized.⁴ Recent studies have shown that silicon reinforces cell wall mechanical strength and increases pathogen resistance, likely due to the increased leaf toughness (Epstein, 1999; Datnoff et al., 2005; Ma et al., 2006). Moraes et al. (2005) demonstrated that silicon application to soil and leaves deterred aphid infestations in corn under greenhouse conditions, and Coors (1987) found a strong correlation between silicon soil availability and resistance of corn to European corn borer.

⁴ The silicic acid can be transformed into silica bodies, as well.

Manivannan et al. (2017) explored the GMO implications of silicon regulating genes, but extensive research of exogenously applied silicon has been produced.

Silicon's growth enhancement characteristics are not consistent in their strength across species, and plants are categorized as low, medium, and high silicon accumulators based on their roots' ability to absorb the substance (e.g. monocots have been demonstrated to store silicon more than dicots, on average). However, silicon has demonstrated stress mitigation capacity even in species with poor uptake abilities, such as tomato (*Solanum lycopersicum*). Silicon is recognized to have the most profound effect in rice, which performs active silicon uptake (Ma et al., 2006). Exogenous applications to this monocot resulted increased plant height, cell wall toughness, stalk rigidity, overall yield, and photosynthetic capacity. Silicic acid fertilization has demonstrated similar effects in numerous species, including corn, wheat, and soybeans (Jang et al., 2018). It has proven to be particularly effective in salt and drought stress responses and recovery (Ahmad et al., 1992).

These positive effects are believed to be the result of several interconnected functions. Primarily, the chemical's role in protection of cell membranes and other structures increases general stress resistance. Furthermore, exogenous silicon has been demonstrated to increase UV stress resistance via antioxidant regulation of ascorbate peroxidase (APX), catalase, and superoxide dismutase. Silicon is also suspected to function as a regulator of osmolytes under drought stress conditions. Si also interacts with known biostimulants: Exogenous application in cucumber (*Cucumis sativus*) showed increased levels of glutathione and AsA, conferring enhanced stress tolerance and growth (Liu et al., 2009). For these reasons, silicon is a promising candidate for probable growth enhancement effects, especially in specialized formulas. For example, the prevailing substrates in commercial floriculture are peat moss and pine bark, which have negligible silicon concentrations. Hydroponic systems lack soil and also have low silicon levels.

Exogenous application through a biostimulant formula could have significant yield implications in both sectors. Epstein et al. (2005) postulated that exogenous silicon application usually only has measurable beneficial effects when the plants are under some stress, so silicon can also be highly beneficial in high stress environments, such as non-freezing resistant spring barley amid temperature fluctuations (Ritonga et al., 2020).

3) Glutathione Precursors:

Glutathione (GHS) is a small thiol molecule that acts as a powerful non-enzymatic antioxidant. It is a key component of the glutathione-ascorbate cycle, which reduces hydrogen peroxide, prevents oxidative denaturation of proteins, assists in chelating toxic metals, reduces lipid peroxidation, and interacts with phytohormones. It also serves as an electron donor during ROS detoxification. The molecule is synthesized in the chloroplast, cytosol, and mitochondria from cysteine, glycine, and glutamic acid. Overexpression of genes related to GHS has been the subject of multiple genetic engineering projects with varying levels of success (Roxas et al., 1997).

Glutathione application has been tested for biostimulant effects on multiple occasions. Kattab (2007), Salama et al. (2009), Hasanuzzman et al. (2011), Wang et al. (2011), Wu et al. (2011), Chen et al. (2012), and Nahar et al. (2015) all showed that exogenously applied GHS can improve abiotic stress tolerance. However, glutathione uptake is low in plants. Kocsy et al. (2001) increased glutathione and its precursors with herbicide safeners, promoting temperature stress resistance. Cutler et. al (2004) focused on l-cysteine derivatives, demonstrating that they serve as effective biostimulants. Exogenous applications of glycine betaine, which is a modified glycine with three methyl groups, and proline have also been demonstrated to increase GHS levels and plant stress resistance (Molla et al., 2014). Selenium, which is an essential trace mineral and

glutathione cofactor, exhibited a similar effect and increased GHS levels when used as a soil amendment (Hasanuzzaman et al., 2017).

The most promising of these precursors for biostimulant effects is proline, which is an amino acid essential for primary metabolism. In higher plants, the compound is synthesized via the glutamate and ornithine pathways. Precursors for proline biosynthesis include glutamic acid, ornithine, and arginine. The compound functions as an antioxidant, osmolyte, and metal chelation. Proline and its genetic mechanisms have been the subject of extensive research. Hyat et al. (2010) summarized the findings of all studies pertaining to low-concentration exogenous application of proline, all of which has significant stress reduction effects. Kaul et al. (2008) demonstrated that exogenous l-proline was an effective ROS scavenger. Furthermore, in addition to its antioxidizing role, proline and its biosynthesis have preventative functions: As a reductive pathway, proline biosynthesis oxidizes NADPH during glutamate reduction, which lowers the NADPH/NADP⁺ ratio, thereby facilitating the uninterrupted flow of the electron transport chain. Under stressful conditions, this translates into preventing the production of ROS. Repeated trials with proline have shown that it is a proven and powerful stress mitigant (Kocsy et al., 2001).

Although exogenous applications of glutathione have muted biostimulant properties due to limited uptake, the application of its precursors (cysteine, glycine, and glutamic acid), proline, or cofactor selenium have demonstrated to increase endogenous GHS levels and will likely have the desired stress mitigation and growth enhancement effects.

4) Boron:

A micronutrient required by plants in trace amounts, boron (B) is vital to plant health and may have biostimulant-like effects. The element is used in the formation and strengthening of cell walls and is a key determinant of agricultural production capacity (Saleem et al., 2011). It is

absorbed through the roots as boric acid and cannot be remobilized through the phloem. In most species, plants do not have a high degree of control over their boron uptake, which is limited by its soil availability and the plant's transpiration rate. Boron has also been demonstrated to be a potassium and phosphorus pump. Transgenic tomato plants with increased boron uptake capacity have been created and have demonstrated increased yield (Ali et al., 2014).

Low boron availability is the most widespread micronutrient deficiency in the world and causes significant quality and yield loss (Shorrocks, 1997). Due to the immobility of the element once fixed, boron must be continuously available in the soil, and adverse effects from deficiencies can manifest rapidly. The importance of boron cannot be overstated. Ali et al. (2014) even claimed, "Boron is the most important constituent of tomato growth and development." Since the element's deficiency is not uncommon in practice and its importance in plant growth, it should be further investigated for biostimulant-like properties. Boron also interacts with other bioactive compounds. Elevated boron levels increased arginine uptake and accumulation in coniferous plants (Chernobrovkina et al., 2010). Boron may amplify arginine's impact in trace amounts. Simultaneous applications of boron and silicon increased plant height, dry weight, yield, amino acid concentration, total soluble sugars, and proline concentrations over a control group and silicon alone. Due to its possible growth enhancement effects, interaction effects, and the prevalence of boron field deficiencies, the often-overlooked element may have biostimulant-like effects when applied in tandem with other compounds.

Conclusion:

Overall, careful examination of genetic engineering literature yields promising candidates for additional biostimulant compounds for future experiments. Even modestly increasing the impact of the biostimulant's self-reinforcing feedback loop through additional factors can greatly

improve efficacy. Other biostimulant candidates were also described in the literature but go beyond the scope of this paper. Candidate osmoprotectants, predominately for salinity stress, include sucrose, polyols, trehalose, and quaternary ammonium compounds, which include alanine betaine, choline *O*-sulfate, hydroxyproline betaine and pipercolate betaine (Rhodes et al., 1993). Genetically engineered crops with elevated osmoprotectant levels has been extensively researched but produced no functional results. Other biostimulant compounds studied include urea, polyamines, putrescine, spermine, spermidine, salicylic acid, hydrogen peroxide, alginic acid, polyphenols, and trehalose (Matysiak et al., 2020). Fulvic acids, lactic acid, fungi (i.e., arbuscular mycorrhizal fungi, AMF, *Trichoderma* spp), and bacteria also have proven biostimulant effects. Other compounds explored ranged from titanium (Basak, 2008) to the extract of the moringa leaf (Yasmeen et al., 2003).

As global temperatures rise and the climate becomes more extreme, solutions for dealing with plant stress will become an agricultural necessity. Today, an estimated 45% of agricultural lands responsible for feeding 38% of the population are subject to continuous or frequent drought (Bot et al., 2000). Dudal (1976) estimated that 90% of arable land experiences major environmental stresses. Sanghera et al. (2011) claims that low temperature damage alone costs global agriculture over \$2 billion annually in lost production. These statistics have real human implications, as elevated environmental stress threatens food security for entire populations. Due to the capital-intensive nature of GMO production and recent controversy surrounding their consumption, it is increasingly likely that biostimulants and stress mitigation techniques will be imperative for adapting to a higher stress environment. The Yale biostimulant concept can also play an integral part in this transformation because current commercial biostimulant products lack the most bioactive compounds in the Yale formula. In total, biostimulants have the potential to increase global crop yield, reduce inorganic fertilizer pollution, and mitigate the effects of climate

change. To this end, biostimulants are a powerful tool to ensure the continued prosperity in agriculture and to promote more effective stewardship of the environment.

Appendix I: CoQ10 Experiment Figures

Figure 1: Total Fresh Biomass by Treatment

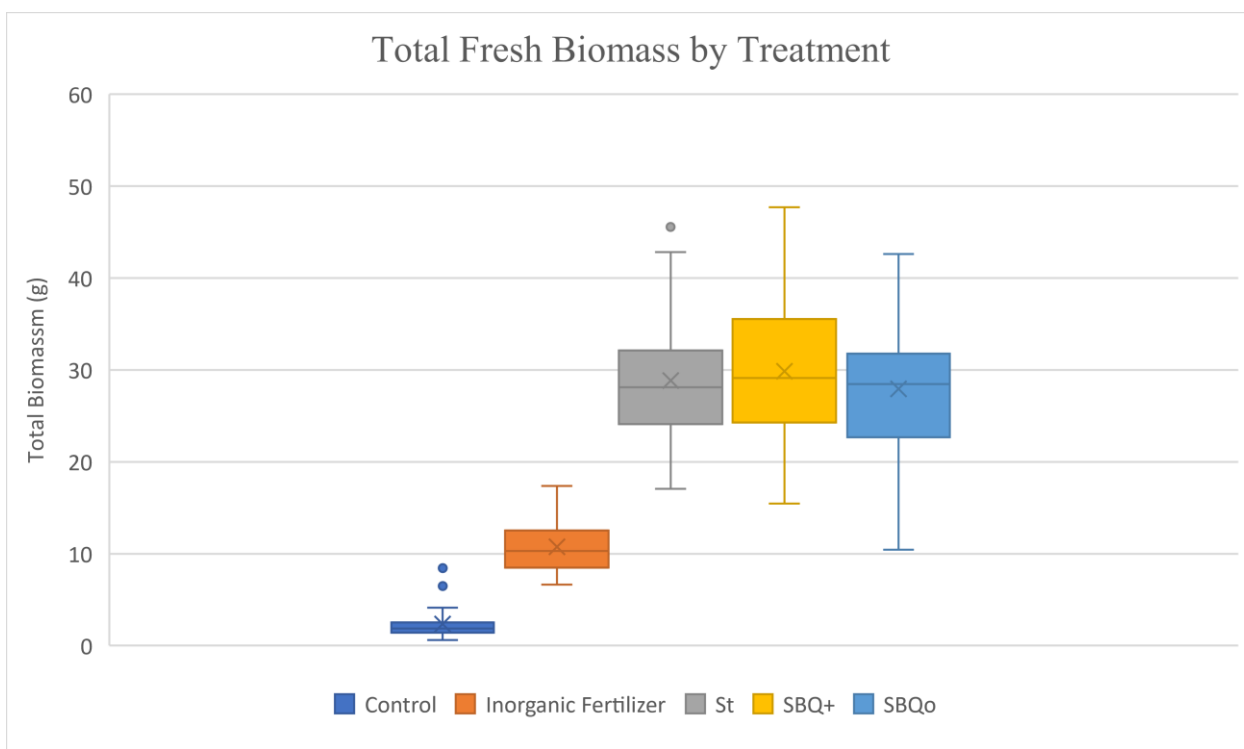


Figure 1: Most recent experimental results (January 2020). The above boxplot illustrates the of total fresh biomass of radish, grown for 60 days (n = 40 per group). The five experimental groups were:

Control: No exogenous nutrients nor fertilizer added

Inorganic Fertilizer: 2.0 grams per liter of Nutraleaf with each treatment

St: The core formula without added inorganic fertilizer

SBQ+: The core formula with inorganic fertilizer and CoQ10

SBQo: The core formula and CoQ10 with no inorganic fertilizer

Each radish was planted in a 25 ml plot with 200 grams of Promix 510. Treatments were applied weekly after seedling establishment by spray on soil. Each plant received 25 ml of spray (1,000 ml per treatment group). Using ANOVA tests, the 'Control' and 'Inorganic Fertilizer' groups had a statistically significant difference of means. The respective means of the three experimental groups 'St,' 'SBQ+,' and 'SBQo' were statistically different from the mean of the groups 'Inorganic Fertilizer' and 'Control.' No statistically significant difference of means existed between the three experimental groups. The average of the group 'SBQ+' was 1,165% and 384% higher than the means of 'Control' and 'Inorganic Fertilizer,' respectively. This shows the large magnitude of the effect of high quality organic biostimulants.

Figure 2: Total Dry Biomass by Treatment

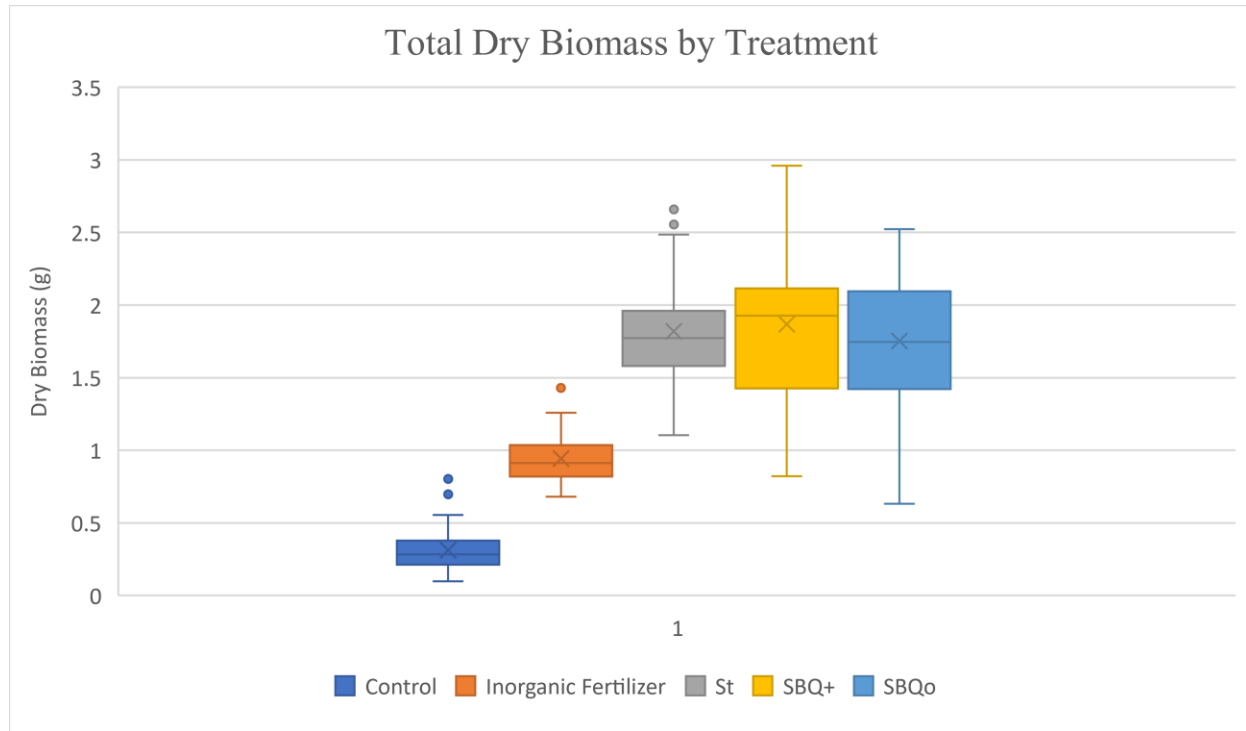


Figure 2: Most recent experimental results (January 2020). The boxplot illustrates the measures of total dry biomass for the same radish experiment. The differentials in group means were less profound, but all differences that were statistically significant for fresh biomass were also significant in the dry biomass measures.

Appendix II: References

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