

Vitazyme biostimulant increases specialty crop production and contains active brassinosteroids; a spectrofluorometric, chemical (LCMS), and bioassay determination

Roberto Bogomolni

i-Cultiver, Inc

Theresa McLaughlin

Stanford University Mass Spectrometry, Stanford University

Robert Reed

i-Cultiver, Inc

Samir Adhikari

i-Cultiver, Inc

Brandon K. Mendoza

i-Cultiver, Inc

Rosangelica O. Lopez

i-Cultiver, Inc

Kent F. McCue

USDA

Paul Syltie

Vital Earth Resources, Inc

Scott Hammer

Vital Earth Resources, Inc

Rajnish Khanna

raj@i-cultiver.com

i-Cultiver, Inc

Research Article

Keywords: Biostimulant, Brassinosteroid, Spectrofluorometric analysis, Specialty Crop yield, Sustainability

Posted Date: September 19th, 2025

DOI: <https://doi.org/10.21203/rs.3.rs-7474445/v1>

License:  This work is licensed under a Creative Commons Attribution 4.0 International License.
[Read Full License](#)

Additional Declarations: No competing interests reported.

Version of Record: A version of this preprint was published at BMC Plant Biology on December 24th, 2025. See the published version at <https://doi.org/10.1186/s12870-025-07934-4>.

Abstract

Biostimulants are agricultural inputs that enhance the ability of plants to absorb and utilize nutrients more efficiently through natural processes. There exists a gap in understanding how various biostimulant fertilizers act in improving crop yields. This knowledge is critical to help growers make educated decisions to optimize their costs and use of different fertilizers to improve food quality and production, sustainably. Vitazyme, a commercially available biostimulant, is stated to contain phytohormone brassinosteroids (BR) and other plant growth promoting substances. In this study, Vitazyme was tested for its influence on promoting yield of greenhouse-grown specialty crops, and for the presence and activity of BR. Treatment with Vitazyme significantly increased broccoli weight by 46% and number of tomatoes by 52%. Overall, there was a positive trend in all crops tested, with increased number of pea pods, and total harvested weight of daikon radish, plum purple radish, kale, peas, and tomato. These trials are continuing over multiple seasons to establish Vitazyme efficacy in specialty crop production. Detection and quantification of brassinosteroids poses several challenges due to their low concentrations and over 70 known BR family members. Previously, derivatization of BR with a fluorescent label followed by spectrofluorometric analysis has been shown to be effective in detecting BR. However, sugars carry similar geminal hydroxyl groups as BR, are derivatized similarly and interfere in spectrofluorometric assays. We modified the procedures and show that fluorescence profiles of derivatized sugar are distinct from derivatized BR standards, and that this new method can indeed be used to detect total BR in agricultural inputs. These spectrofluorometric assays were used to detect total BR, followed by mass spectrometric analysis to identify and quantify 28-homobrassinolide as one of the BR in Vitazyme. To confirm that this complex fertilizer mixture contains active BR, a bioassay was developed using *Arabidopsis* mutant *de-etiolated2* (*det2*), defective in BR biosynthesis. Application of Vitazyme recovered the short growth phenotype in dark-grown *det2* mutant, confirming that Vitazyme contains BR activity. This study provides a framework of utilizing multiple approaches to investigate and advance our knowledge of how complex fertilizer mixtures act in improving crop production.

Background

There exists a critical need to better understand the mechanisms of activity of fertilizers and amendments that are used in agricultural applications, with direct implications on improving food quality and safety. There is a significant gap in our knowledge of how most of the agricultural product's act, even though they are used widely to grow our foods. Deeper understanding of underlying mechanisms will help growers make better informed decisions in choosing optimal products to grow their crops sustainably. According to CDFA (California Department of Food & Agriculture), plant biostimulants include any substance or microorganism (or mixtures thereof) that are not nutrient based fertilizers, but they support plant's natural nutrition processes by improving nutrient uptake, or use efficiency, leading to increased plant growth, development, quality, or yield. Global biostimulants market is growing rapidly with a compound annual growth rate of over 11% and is projected to reach over USD 9.7 billion by 2032 (Fortune Business Insights). In this study, a commercially available biostimulant, Vitazyme (Vital Earth

Resources, Inc., Gladewater, Texas, USA) was assessed for the presence of phytohormone brassinosteroid as the active component.

Discovery and activity of brassinosteroid (BR) in plants

A novel class of phytohormone, Brassins, shown to promote plant growth, were isolated from 400 pounds of rape seed (Mandava et al., 1973), followed by identification of the active compound, Brassinolide (BL) from *Brassica napus* pollen (Grove et al., 1979). Soon after, methods to synthesize BL and other related compounds were developed (Thompson et al., 1979; Fung and Sidall, 1980; Ishiguro et al., 1980; Sakakibara et al., 1982). Subsequently, BL and related compounds were detected in various plant species with the development of Gas chromatography/mass spectrometry (GCMS) and Liquid chromatography/mass spectrometry LCMS) methods (Takatsuto et al., 1982; Takatsuto, 1994; Gamoh and Takatsuto, 1994). More than 70 naturally occurring compounds have been detected with structures related to “3-oxygenated (20b)-5a-cholestane-22a,23a-diols with alkyl or oxy substitutions in the Brassinosteroid (BR) family from plant species ranging from algae to angiosperms (Zullo, 2018; Zullo and Adam, 2002). BR play critical regulatory roles throughout plant growth and development as well as in responses to biotic and abiotic stresses. Molecular and genetic analysis have revealed BR signaling mechanisms involving regulation of plant gene expression, protein modification and crosstalk with other phytohormones (Clouse et al., 1991; Gregory and Mandava, 1982; Meudt et al., 1983; Schlagnhauser et al., 1984a and 1984b). Gene mutations disrupting either BR-signaling or BR-biosynthesis cause dwarfism in Arabidopsis (Chory et al., 1991; Li and Chory, 1997) and garden pea (Nomura et al., 1997). In Arabidopsis, the *DE-ETIOLATED2* (*DET2*) gene encodes a steroid 5 α -reductase (S5R), which catalyzes the reduction of a double bond, a rate-limiting step in steroid hormone metabolism (Li and Chory, 1997). There is functional conservation between mammalian and plant S5R, suggesting a common evolutionary origin of steroid hormone biosynthesis pathways (Li and Chory, 1997). Recessive mutations in *DET2* gene result in dark-grown (etiolated) seedlings showing characteristics of light-grown seedlings, including hypocotyl growth inhibition and cotyledon expansion (Chory et al., 1991). Application of active BRs, such as BL abolish the abnormal growth phenotypes in *det2* mutants (Chory et al., 1991; Li et al., 1996). Recovery of normal hypocotyl growth in dark-grown *det2* mutant can be used to develop bioassays to confirm BR activity in complex biostimulant mixtures, such as Vitazyme.

Brassinosteroid based applications for crop improvement

Genes involved in BR biosynthesis and signaling pathways have been identified in various crop species, particularly in cereal crops such as rice (*Oryza sativa*), wheat (*Triticum aestivum*), maize (*Zea mays*), and barley (*Hordeum vulgare*) (Dockter et al., 2014; Fang et al., 2020; Gruszka et al., 2011; Hartwig et al., 2011; Kir et al., 2015; Liu D. et al., 2021; Nakamura et al., 2006). Several BR-related genes have been targeted for genetic modifications, including gene editing approaches to improve agronomic traits in cereal crops (Liu et al., 2021; Zolkiewicz et al., 2025). For example, transgenic expression of transcription factor, *BRASSINAZOLE RESISTANT 1* (*OsBZR1*) driven by a bundle-sheath specific promoter in rice

increased chloroplast area and chloroplast number (Cackett et al., 2025). Three major traits that influence grain yield in cereal crops are panicle density, grain number and grain weight (Xing and Zhang, 2010). BR is involved in modulating all three of these developmental traits and regulates plant architecture (Yin et al., 2025), providing the potential to develop semi-dwarf, high-yielding cereal varieties with resistance to lodging (Song et al., 2023). While improving traits through BR related gene manipulation provides specific and targeted solutions for future of crop production, these technologies face longer development and commercialization tracks. An alternative is exogenous application of BR to improve plant growth, yield and quality. Application of 1 pM and 10 nM 28-Homobrassinolide (HBL), a BR analog, was shown to increase alga *Chlorella vulgaris* growth, as well as total amounts of nucleic acid and protein (Bajguz, 2000). HBL application (0.12 g ha^{-1}) increased tomato fruit quality and yield (Sridhara et al., 2021). The potential benefits of exogenous BR applications have been well presented; however, literature searches reveal there are only few published studies on the effects of commercially available BR fertilizers on crop improvement. Vitazyme is a commercially available organic biostimulant. According to the manufacturer, it contains BRs and other plant growth stimulant substances, providing benefits of Vitazyme application on plant growth (Syltie, 1985), improved germination and seedling vigor in rice, tomato and cotton (Umesha et al., 2009).

Specialty crops are defined by the United States Department of Agriculture (USDA) as fruits and vegetables, tree nuts, dried fruits and horticulture and nursery crops, including floriculture. The global specialty crop market size in 2024 was estimated to be over USD 1.6 trillion (Business Research Insights). Prior to performing chemical analysis for the presence of BR in Vitazyme, we tested the effects of Vitazyme treatment on broccoli, radish, kale, peas and tomato to establish benefits of this fertilizer on specialty crops.

Chemical detection of BRs

Chemical detection of BRs in plant tissues and plant derived fertilizers has been difficult due to the extremely small amount of these compounds in the samples. Chromatographic approaches followed by mass spectroscopy detection were successful in the purification and quantitation of small amounts of BRs after previous derivatization of the hormones with boronic acid compounds that aided with the detection (Gamoh, 1989; Gamoh, 1990; Gamoh and Takatsuto, 1994; Svatoš et al., 2004). Since most agricultural fertilizer producers may not have access to mass spectroscopy facilities, a simpler BR spectroscopic detection and quantitation method was needed. The specific labeling of BR with fluorescent boronic acid for their detection and quantitation has been a useful addition to the tools currently available (Gamoh, 1989; Gamoh, 1990; Tangtreamjitmun and Chindaphan, 2015). Unfortunately, reliable quantitation of BR from these efforts is questionable because of possible interference of plant sugars, that due to their similarities in chemical structure are subject to the same derivatization reaction as authentic BR and contribute to the resulting fluorescence used for detection (Luis et al., 1998; Peng and Qin, 2008). In this study, we have developed a spectrofluorometric method for the determination of BR and applied it to study a commercial biostimulant fertilizer.

Methods

Specialty crop trials and Vitazyme treatments

All specialty crop plants were started from seeds and germinated in Sunshine mix #1 (Sun Gro®) soil pots in the greenhouse. All plants were grown to 3–4 inches tall and then transplanted into larger pots, placed in randomized order on benches until harvest in a single greenhouse room. The greenhouse used supplemental lights to maintain long days and cooling to control high temperature fluctuations. All plants received liquid nutrient supplementation program consisting of Peters Professional 20/20/20 water soluble fertilizer (1:64 ppm) applied once per week, as well as a disease suppression program consisting of Floramite and Decathlon at a rate of ¼ tsp per gallon of water, mixed/agitated, and applied through a controlled sprayer at the rate of 1–2 gal per 100 plants. Vitazyme (Vital Earth Resources, Inc., Gladewater, Texas, USA) was applied according to manufacturer's instructions, at 1:100 dilution in water, sprayed on to leaves (drenching point) with PB Mistlers ULTRA with Pressure Release. Vitazyme treatments were started soon after transplanting and continued every other week for a total of three treatments. During Vitazyme treatments, plants were covered inside a cardboard box with open bottom and top to prevent any spray on to Control plants in the randomized placement setting. These treatments were done carefully to maintain treated (Vitazyme) and untreated (Control) plants with minimal plant handling. Control plants were treated with the same amount of water without Vitazyme using similar sprayers at the time of treatments. All plants were grown to maturity and were harvested on the same day. The specialty crops tested in the greenhouse included daikon radish (*Raphanus sativus* var. Longipinnatus), broccoli (*Brassica oleracea* var. Italica), kale (*Brassica oleracea* var. Acephala), peas (*Pisum sativum* L.), plum purple radish (*Raphanus sativus*) and tomato (*Solanum lycopersicum* var. Microtom). Between 6–17 plants per treatment were tested for each specialty crop as indicated in Table 1.

Brassinosteroids spectrofluorometric assay

The chemical addition reaction of the fluorescent label 3-(Dansylamino)phenylboronic acid (DAPBA) with brassinosteroids is possible because their structure contains geminal hydroxyl groups in *cis* configuration that are attacked by the boronic acid moiety. Since practically all brassinosteroids contain this structural feature the fluorescence method is not restricted to any single BR, it extends to all brassinosteroids contained in the samples. Therefore, the result of the quantitation using this fluorescence approach should be interpreted to represent the concentration of all brassinosteroids present. Any standard brassinosteroid can be used as reference concentration standard, and the result is reported as brassinosteroids expressed as the concentration of the used brassinosteroid standard. The reaction protocol presented here was modified from previously reported methods (Luis et al., 1998; Gamoh et al., 1990; Peng et al, 2008) for the derivatization of brassinosteroids with a fluorescent label to aid in their detection and quantitation using a spectrofluorimetric approach. Several aspects of the protocol do not follow identical technology presented in the references, particularly solvents used and

derivatization reaction conditions that were optimized for further detection and purification of the derivatized brassinosteroids using LCMS approaches. The use of different solubilization solvents than those used in the listed references resulted in slight differences in the spectral parameters.

Reagents used were DAPBA, 95% purity (B148825, BenchChem), BL, > 99% purity (HY-N0273, MedChemExpress), and HBL, > 96% purity (HY-N9435, MedChemExpress). Note, HBL is also known as (22R,23R)-28-homobrassinolide. BL molecular formula: $C_{28}H_{48}O_6$; HBL molecular formula: $C_{29}H_{50}O_6$. Stock solutions (2 mg/ml) were prepared in Dimethyl Sulfoxide (DMSO) with final stock concentrations of DAPBA (5.4 mM), BL (4.16 mM), HBL (4.04 mM). The DMSO stocks were tested to be stable for at least 5-weeks at -20 °C and 3-months at -80 °C, provided they are kept away from light, particularly the DAPBA stock. Derivatization reactions were performed in 5 mM aqueous solution of DiSodium Hydrogen Phosphate dodeca hydrate ($Na_2HPO_4 \cdot 12H_2O$), pH 7.4. This buffer solution will be referred to in the following text as PBS (Phosphate Buffer Solution). The derivatization Reaction was performed in a 2ml vial with 50µl standard (BL or HBL) stock solution, 50 µL DAPBA stock solution and 1.9 mL of 5 mM PBS to make a 2 mL total volume reaction. Under these conditions, the final DAPBA, BL or HBL concentrations in the reaction volume are 135 µM, 104 µM and 101 µM, respectively, in approximately 5% DMSO in 0.5 mM PBS, pH 7.4. To detect brassinosteroids in Vitazyme, the reaction volumes were modified to 1 mL Vitazyme, 100µL of 1.08 mM DAPBA and 1 mL of 5 mM PBS pH 7.4. For glucose analysis (Fig. 4), the final concentrations in the 2 ml reaction were DAPBA (106 µM) with glucose (100 µM). For spectrometric measurements, a Cary Eclipse Spectrofluorometer with WIN FLR acquisition software (Agilent Technologies), and a diode array UV-Vis spectrophotometer (Agilent HP 8452) with 190–820 nm spectral range were used.

The reaction mixture was incubated for 20 minutes at 40°C, or for 30 min at 20°C. The different times and temperatures do not make significant changes in the resulting product. Measurements of fluorescence emission spectrum were performed at 342 nm at slit bandwidths of 10 nm for excitation and 5 nm for emission. The excitation spectrum (emission at 505 nm) was measured with the same slit setup. The result is shown in Fig. 1. The wavelengths reported for this reaction previously in Gamoh et al., 1990 are: excitation max 345 nm and emission max 515 nm, whereas our values in DMSO/PBS are 342 nm and 505 nm respectively, this results from their use of a different solvent, which instead of 5% DMSO in 5 mM PBS was aqueous acetonitrile. The different dielectric solvent properties cause this small red shift. This reaction product is stable in -20°C freezer for 3 weeks. In our protocol we use a spike of BL as the standard for quantitation. The BL spike approach consists of the addition of a known amount of the standard BL to the same reaction mixture (under identical reaction conditions) as those experienced by the BL present in the test sample. This requires the presence of a slight excess of the DAPBA label, so that the spike addition can be derivatized as well. The additional fluorescence generated by the spike addition is the calibrating fluorescence intensity for computation of the pre-existing BL concentration in the unknown sample (Fig. 3). Note that the reaction volume of Vitazyme is ½ of total reaction volume. This results in a 1:1 dilution with respect to the original Vitazyme biostimulant solution.

Brassinosteroids LCMS assay

The LCMS assay was performed at the Stanford University Mass Spectrometry (SUMS) facility. The derivatized samples were analyzed by LC-ESI/MS on a Waters Acquity UPLC and Thermo Exploris 240 Orbitrap mass spectrometer. The derivatized analyte was HBL-DAPBA ($C_{47}H_{65}N_2O_8SB$). The derivatized Vitazyme sample was diluted 1:10 with acetonitrile and vortexed for 2 minutes at 10,000 to remove precipitate. The standard HBL-DAPBA was diluted with acetonitrile to several different concentrations for calibration as described (Supplementary Fig. 2). The LCMS method was based on Svatoš et al., 2004. The column was an Agilent Zorbax SB-C18 2.7u 2.1x50mm with isocratic mobile phase 25% A (0.1% formic acid in water) / 75% B (0.1% formic acid in acetonitrile). The column temperature was 40°C, flow rate 0.2mL/minute and injection volume 5uL. Targeted SIM mass spectra were acquired in positive ion mode with center mass m/z 815.4471 and 829.4627, Scan Width 2, RF 70, and resolution 60,000. Easy-IC internal mass calibration was enabled in Run Start mode.

Arabidopsis seedling BR bioassay

Arabidopsis thaliana Columbia ecotype (Col-0), or *det2* (Chory et al., 1991) seeds were surface sterilized (Khanna et al., 2006) and plated on half strength MS (Murashige & Skoog Basal Salt Mixture, PhytoTech Labs) salts, buffered with 1 g/L MES (2-(N-Morpholino) Ethane Sulfonic Acid, Research Organics, Inc.) at pH 5.8 with KOH, plus 0.7% Agar Noble (Difco Laboratories). The media did not contain any added sugars. Either Brassinosteroid standard or Vitazyme solution was added at various amounts as indicated (Fig. 6). The HBL standard stock (5 µg/µl) solution was prepared in acetonitrile and diluted to (20 ng/µl) in sterilized di-water. Both, HBL standard and Vitazyme treatments were applied as 200 µl solutions evenly spread on the top layer of agar plates using sterilized glass beads. The experiments were repeated 3 times consistently by stratifying the plates for 4 days at 4°C, germination was induced by 3-hours of white light treatment in a growth chamber at 21°C, followed by continuous placement in the growth chamber in darkness for 7-days. Hypocotyl lengths were measured using ImageJ software (National Institutes of Health). Seedlings were photographed using iPhone 16 (Apple) camera.

Results

Vitazyme, a commercial fertilizer, was tested for its benefits in crop production. The manufacturer's website (<https://vitalearth.com/vitazyme/>) contains an extensive list of studies performed with multiple crops spanning over 15 years of trials. However, searches of peer-reviewed journals revealed only four publications on Vitazyme in promoting growth of pear millet, bermudagrass and seed quality of rice, tomato and cotton (Deepak et al., 2004; Rogers, 2017; Syltie, 1985; Umesha et al., 2009). This is a common gap due to the challenges faced by industry in performing independent basic research to establish product activity and performance. It is critical to fill this gap in fundamental knowledge of how agricultural products act to ensure future food security and sustainability. To this end, Vitazyme was analyzed for its crop benefits and underlying activity. Vitazyme is manufactured through fermentation of

plant materials. According to the manufacturer's label, Vitazyme contains Homobrassinolide as the active ingredient.

Vitazyme promoted production of specialty crops in the greenhouse

Most of the previously published reports have linked BR benefits to cereal production and seed quality (see above). Here we tested Vitazyme application on specialty crops grown under controlled environment in a greenhouse. Vitazyme treatments significantly (P value = 0.004) increased total harvested weight of broccoli (N = 6 plants) by over 46% and significantly (P value = 0.02) increased the total number of tomatoes (N = 16 plants) by over 52% (Table 1). The number of pea pods showed an increase (42.42%, P value = 0.24), and overall, there was an increase in the harvested weight of daikon radish (16%), kale (6%), peas (29%), plum purple radish (12%), and tomato (20%) (Table 1). These data showed a positive trend in the influence of Vitazyme on crop production (Table 1; Supplementary Fig. 1). Future trials over multiple seasons are ongoing.

Development of spectrofluorimetric assay to detect BRs in Vitazyme

Based on the data above, Vitazyme was selected for further analysis to validate manufacturer's claim of its BR activity. DAPBA was used to derivatize BR standards and any BR present in Vitazyme, followed by spectrofluorimetric assays for detection. DAPBA alone in 5% DMSO in 0.5 mM PBS, pH 7.4 (see Methods) showed fluorescence excitation peak at 327 nm and emission peak at 555 nm (Fig. 1A and 1B). DAPBA absorption spectrum is shown (Fig. 1C). Next, BR standards, namely BL (Fig. 2A, 2B) and HBL (Fig. 2C, 2D) were derivatized with DAPBA for fluorescence measurement with excitation at 342 nm, both standards showed maximum emission peaks at 505 nm (Fig. 2). Note that all fluorescence parameters for BL and HBL are identical, as expected from their near identical structures, differing only in one carbon atom in a position remote from the Boronic acid reaction site.

For derivatization of a sample containing unknown quantities of brassinosteroids a preliminary study to determine the approximate content is necessary to adjust properly the DAPBA concentrations to get full stoichiometric derivatization of both the pre-existing and the added spiked brassinosteroids. We carried out this preliminary phase by doing a series of reactions with increasing amounts of DAPBA until any further increase did not result in an increase in 505 nm emission fluorescence of the reaction product (data not shown). For the Vitazyme sample this study resulted in an estimate that the total brassinosteroid content did not exceed 30 μ M. With this result the reaction conditions were adjusted as in Methods. Figure 3 shows DAPBA products in Vitazyme, with identical excitation (with max at 342 nm) and emission (max at 505 nm) as BL and HBL standards in Fig. 2. These data indicate that Vitazyme contains brassinosteroids. To further confirm the presence of derivatized BRs in Vitazyme, we spiked the Vitazyme/DAPBA reaction with BL standard. The emission spectra profiles of Vitazyme/DAPBA reaction were identical before and after addition of 40 μ M BL standard, with a notable increase in peak intensity

at max 505 nm (Fig. 3B). Different amounts of BL addition (either 10 μ M or 40 μ M), resulted in a linear increase in fluorescence at 505 nm (Fig. 3C). These data strongly supported that 505 nm peak observed in DAPBA derivatized Vitazyme were due to the presence of one or more BRs.

Because all BRs would give identical fluorescence parameters and most likely nearly identical fluorescence intensities (this is assumed, however our result with BL and HBL strongly supports this assumption) the identification of the different derivatized brassinosteroids can be accomplished with additional analytical work using LCMS, which we carried out through the Stanford University Mass Spectrometry facility (Stanford University), as presented below.

Reaction of sugars with DAPBA

In this study, we show the sugar derivatization product with DAPBA has distinct fluorescence properties from those of BR. The product of glucose and DAPBA shows excitation peak at 327 nm and an emission peak at 535 nm (Fig. 4), distinct from the BR/DAPBA products (Figs. 2 and 3). Note, fluorescence profiles of DAPBA alone and glucose/DAPBA product, both show excitation peak at 327 nm, with a shift in the emission peak in DAPBA fluorescence from 555 nm to 535 nm in the presence of sugar (Figs. 1 and 4). Fructose yielded the same results (data not shown). Vitazyme does not contain any added sugars, it is produced through fermentation of plant materials, therefore it lacks sugars (Vital Earth Resources, Inc.). The fluorescence profiles of BL, HBL and Vitazyme (Figs. 3 and 4) indicate that Vitazyme contains BR, and this assay can be performed to distinguish between BR/DAPBA and sugar/DAPBA products.

Quantification of derivatized brassinosteroids by LCMS

Using the BL/DAPBA and HBL/DAPBA reaction products as reference the LCMS approach accomplished the Liquid Chromatographic purification of both products in the standards as well in the Vitazyme solution and the assignment of distinct specific mass/charge ratio peaks in the Mass Spectroscopy data (Fig. 5). Derivatized HBL standard was used to generate linear concentration relationships (Supplementary Fig. 2) to determine the amount of HBL in derivatized Vitazyme. HBL (between 1.08 μ M to 1.26 μ M) was detectable in Vitazyme in three independent batches of the product (Table 2). This unequivocally shows the presence of HBL in the Vitazyme biostimulant product.

Table
2

Seedling bioassay to determine whether BR in Vitazyme is active

The Arabidopsis *det2*-mutant is deficient in BR biosynthesis and exhibits a defective hypocotyl-elongation phenotype in dark-grown seedlings. Exogenous treatment of *det2*-mutant seedlings with the BR-biostimulant Vitazyme was able to rescue the hypocotyl elongation, indicating that BR are in its active components (Fig. 6). The Col-0 seedlings grow normally in the dark, but *det2* seedlings exhibit short hypocotyl phenotype, which could be recovered with the addition of either HBL or Vitazyme on top of the

agar plates (Fig. 6). These results confirm that Vitazyme contains BR activity. This simple seedling bioassay can be used to perform a direct BR activity test for fertilizers.

Discussion

This study was performed to evaluate a commercially used biostimulant, Vitazyme, for its efficacy in improving crop production. According to the manufacturer, BR are an active component in this fertilizer. BR are known to provide growth benefits to plants, however, there is a need to systematically perform mode of action studies with commercial fertilizers to test their performance in greenhouse and field environments to help growers make informed choices, and to identify sustainable options in the agricultural industry. This study was performed independently through the Biotechnology Education and Specialized Training (BEST) internship program (i-Cultiver, Inc.) to develop deeper understanding of fertilizers used in food production through advanced mode-of-action studies, as previously reported by us with other types of fertilizers (Mehlferber et al., 2025).

We report here the spectrofluorometric determination of brassinosteroids in the commercial biostimulant fertilizer Vitazyme, based on a specific derivatization reaction with the fluorescent label DAPBA and its quantitation with authentic BR standards. It is assumed that all BR yield similar reaction products with DAPBA because of their similar structures in the reactive molecular group. Previous reports have shown DAPBA reaction results with BL and castasterone, showing nearly identical DAPBA products (Takatsuto et al., 1990). We tested this assumption by carrying out the reaction with pure brassinosteroids, showing that all yielded similar fluorescent derivatives, having nearly identical absorption and fluorescent properties. LCMS was used to quantify HBL amounts in Vitazyme. Given that over 70 different BR have been reported already the fluorescence assay approach is a much better representation of the hormone content in samples. While the LCMS approach alone does report only those BR that have been separated chromatographically, the fluorescence derivatization approach reports the sum of all BR present (but does not specify which ones they are), However when combined with LCMS, as we have done it becomes the most powerful tool available to characterize the BR contained in an unknown sample.

Previous use of the fluorescence technique by Tangtreamjitmun, et al., 2015, reported much larger amounts of BR in four different fertilizers, up to 282 μM concentration in one of the products. None of these were Vitazyme. BR act at low concentrations, generally between 0.001 nM to 10 nM range, with poor solubility in aqueous solutions up to 5 μM . The previously reported concentrations would require a very large dilution factor of fertilizer to achieve the optimally active range. It is possible that the previous analysis included sugar/DAPBA reaction products, inflating the quantitative determination. We show here that glucose, a sugar that reacts with DAPBA by virtue of the presence of geminal hydroxyl groups in its structure, like in most sugars, serves as an example for all members of this chemical family (Fig. 4). We show that sugar fluorescence excitation and emission wavelengths are different, but they are broad enough to overlap with the BR/DAPBA product fluorescence resulting in an over-estimate of the BR content if the sugar contribution is ignored. Presence of sugar fluorescence interference in samples

needs to be determined when performing BR spectrofluorometric assay. Vitazyme did not show any evidence of sugar/DAPBA reaction (Fig. 3). Note that the spiked fluorescence showed identical fluorescence emission peak and shape as the original reaction fluorescence (Fig. 3). This is not shown in any of the previous papers, particularly in Tangtreamjitmun, et al., 2015, reporting quantitative BR determination. Furthermore, we performed LCMS on the standard (HBL) and Vitazyme derivatized samples and detected in Vitazyme the presence of both BR supporting our contention that our result represents the sum content of various BR present in the biostimulant.

With increased use of phytohormone based fertilizers, there is a need for a simple and accurate chemical assay of brassinosteroids in commercial fertilizers and biostimulant products. The emerging fluorescent labeling spectroscopic approach provides a good solution to detect total BR. Stability of active BR in complex fertilizers is another consideration. It is important to confirm the chemical analytical results with biological assays showing the presence of the brassinosteroid hormone physiological activity. Crop productivity trials is a common way to test fertilizer efficacy, however, to establish the activity of components in complex fertilizer mixtures requires specific functionality tests. Here we used an *Arabidopsis det2* mutant, known to lack BR, to specifically test for BR activity in Vitazyme (Fig. 6). Based on these results, BR activity is likely to be an important component of Vitazyme. Further studies to understand mode of action of Vitazyme and other fertilizers will significantly improve grower's ability to optimize inputs to sustainably increase agricultural productivity.

Conclusions

Vitazyme biostimulant improves specialty crop production and it contains active BR, including homobrassinolide. The spectrofluorometric assay presented here can serve as a relatively easier method to detect total brassinosteroids present in fertilizer mixtures, taking into consideration the presence of any sugars. Bioactivity seedling assay provides a functional confirmation of brassinosteroid activity in Vitazyme.

Abbreviations

BL
Brassinolide
BR
Brassinosteroid, Brassinosteroids
DAPBA
3-(Dansylamino)phenylboronic acid
DET2
DE-ETIOLATED2
DMSO
Dimethyl Sulfoxide
HBL

Homobrassinolide
LCMS
Liquid chromatography/mass spectrometry
PBS
Phosphate Buffer Solution

Declarations

Ethics approval and consent to participate

Not applicable

Consent for publication

Not applicable

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Competing interests

Rajnish Khanna is the founder of i-Cultiver, an independent company providing consultation and research assistance to food and agricultural industries. All aspects of this study were performed by independent researchers. Roberto Bogomolni and Rajnish Khanna are co-Founders of LOVnod Biosciences, a chemical analysis company. Paul Syltie is the Director of Research and Scott Hammer is the President at Vital Earth Resources, Inc., the company that manufactures Vitazyme. Paul provided product application guidelines. The authors declare that they have no competing interests.

Disclaimer: Mention of trade names or commercial products in this publication is solely for the purpose of providing specific information and does not imply recommendation or endorsement.

Funding

The authors declare that this work was funded and performed through i-Cultiver, Inc., which independently provides consultation services to agricultural product manufacturers; this includes Vital Earth Resources, Inc., which manufactures the Vitazyme product. This funding provided for research costs associated with this study. It did not influence the factual reporting of findings herein. LOVnod Biosciences provides chemical analysis support to i-Cultiver. Robert, Brandon, Samir and Rosangelica were supported by i-Cultiver's Biotechnology Education and Specialized Training (B.E.S.T.) internship program in collaboration with Dr. Katie Krolikowski, (Contra Costa Community College, Richmond, CA) and Dr. Zhiyong Wang (Carnegie Institution for Science at Stanford University, CA).

Authors' contributions

R.B. conceived, designed and performed all spectrofluorometric determinations; R.K. and R.B. collaborated on LCMS analysis, which was performed by T.M. at the Stanford University Mass Spectrometry, Stanford CA.; R.K. conceived and designed the seedling bioassay study; R.K. and K.M. conducted the experiments, obtained seedling images and data with support in planting and measurements from R.R, S.A., B.M., R.L; R.K. performed statistical analysis and produced graphical figures; P.S. and S.H. provided product-specific details in the study design. R.B. and R.K. drafted the manuscript supplemented with technical and editorial comments from all authors.

Acknowledgements

The authors thank Dr. Zhiyong Wang (Carnegie Institution for Science at Stanford University, CA) for providing the *det2*-mutant, collaboration on BR detection and quantification, and for reviewing the manuscript.

References

1. Bajguz A. Effect of brassinosteroids on nucleic acids and protein content in cultured cells of *Chlorella vulgaris*. *Plant Physiol Biochem*. 2000;38:209–15.
2. Cackett L, Luginbuehl LH, Hendron R-W, Plackett ARG, Stanley S, Hua L, Wang N, Kelly S, Hibberd JM. Increased chloroplast area in the rice bundle sheath through cell-specific perturbation of brassinosteroid signaling. *Plant Physiol*. 2025;197:kiaf108. <https://doi.org/10.1093/plphys/kiaf108>.
3. Chory J, Nagpal P, Peto CA. Phenotypic and genetic analysis of *det2*, a new mutant that affects light-regulated seedling development in *Arabidopsis*. *Plant Cell*. 1991;3:445–59.
4. Clouse SD, Zurek D. Molecular analysis of brassinolide action in plant growth and development. *ACS Symp. Ser. Am. Chem. Soc*. 1991;122–140.
5. Deepak SA, Syltie PW, Shetty CNP, Shetty HS. Vitazyme promotes growth in pearl millet. *Crop Manage*. 2004;3:1–6. <https://doi.org/10.1094/CM-2004-0803-01-RS>.
6. Dockter C, Gruszka D, Braumann I, Druka A, Druka I, Franckowiak J, Gough SP, Janeczko A, Kurowska M, Lundqvist J, Lundqvist U, Marzec M, Matyszczyk I, Müller AH, Oklestkova J, Schulz B, Zakhrebekova S, Hansson M. Induced variations in brassinosteroid genes define barley height and sturdiness, and expand the green revolution genetic toolkit. *Plant Physiol*. 2014;166:1912–27. <https://doi.org/10.1104/pp.114.250738>.
7. Fang J, Zhu W, Tong Y. Knock-down the expression of brassinosteroid receptor TaBRI1 reduces photosynthesis, tolerance to high light and high temperature stresses and grain yield in wheat. *Plants*. 2020;9:840. <https://doi.org/10.3390/plants9070840>.
8. Fung S, Sidall JB. Stereoselective synthesis of Brassinolide: A plant growth promoting steroidal lactone. *J Am Chem Soc*. 1980;102:6580–1.

9. Gamoh K. A boronic acid derivative as a highly sensitive fluorescence derivatization reagent for brassinosteroids in liquid chromatography. *Anal Chim Acta*. 1989;222:201–4.
10. Gamoh K. Determination of traces of natural brassinosteroids as dansylaminophenylboronates by liquid chromatography with fluorimetric detection. *Anal Chim Acta*. 1990;228:101–5.
11. Gamoh K, Takatsuto S. Liquid chromatographic assay of brassinosteroids in plants. *J Chromatogr A*. 1994;658:17–25.
12. Gregory LE, Mandava NB. The activity and interaction of brassinolide and gibberellic acid in mung bean epicotyls. *Physiol Plant*. 1982;54:239–43.
13. Grove MD, Spencer GF, Rohwedder WK, Mandava N, Worley JF, Warthen JD, Steffens GL, Flippen-Anderson JL, Cook JC. Jr. Brassinolide, a plant growth-promoting steroid isolated from *Brassica napus* pollen. *Nature*. 1979;281:216–7.
14. Gruszka D, Szarejko I, Maluszynski M. New allele of *HvBR1* gene encoding brassinosteroid receptor in barley. *J Appl Genet*. 2011;52:257–68. <https://pubmed.ncbi.nlm.nih.gov/21302020/>.
15. Hartwig T, Chuck GS, Fujioka S, Klempien A, Weizbauer R, Potluri DPV, Choe S, Johal GS, Schulz B. Brassinosteroid control of sex determination in maize. *Proc. Natl. Acad. Sci. USA*. 2011;108:19814–19819. <https://doi.org/10.1073/pnas.1108359108>
16. Ishiguro M, Takatsuto S, Morisaki M, Ikekawa N. Synthesis of Brassinolide, a steroidal lactone with plant-growth promoting activity. *J Chem Soc Chem Comm*. 1980;20:962–4.
17. Khanna R, Shen Y, Toledo-Ortiz G, Kikis EA, Johannesson H, Hwang YS, Quail PH. Functional profiling reveals that only a small number of phytochrome-regulated early-response genes in Arabidopsis are necessary for optimal deetiolation. *Plant Cell*. 2006;18:2157–71.
18. Kir G, Ye H, Nelissen H, Neelakandan AK, Kusnandar AS, Luo A, Inzé D, Sylvester AW, Yin Y, Becraft PW. RNA interference knockdown of *BRASSINOSTEROID INSENSITIVE1* in maize reveals novel functions for brassinosteroid signaling in controlling plant architecture. *Plant Physiol*. 2015;169:826–39. <https://doi.org/10.1104/pp.15.00367>.
19. Li J, Chory J. A putative leucine-rich repeat receptor kinase involved in brassinosteroid signal transduction. *Cell*. 1997;90:929–38.
20. Li J, Nagpal P, Vitart V, McMorris CT, Chory J. *Science*. 1996;272:398–401.
21. Liu D, Yu Z, Zhang G, Yin W, Li L, Niu M, Meng W, Zhang X, Dong N, Liu J, Yang Y, Wang S, Chu C, Tong H. Diversification of plant agronomic traits by genome editing of brassinosteroid signaling family genes in rice. *Plant Physiol*. 2021;187:2563–76. <https://doi.org/10.1093/plphys/kiab394>.
22. Luis GP, Granda M, Badía R, Díaz-García R. Selective fluorescent chemosensor for fructose. *Analyst*. 1998;123:155–8.
23. Mandava NB, Sidwell BA, Mitchell JW, Worley JF. Production of Brassins from rape pollen. A convenient preparatory method. *Ind Eng Chem Prod Res Dev*. 1973;12:138–9.
24. Mehlferber E, McCue KF, Bi Y, Reed R, Ferrel J, Khanna R. Azomite, a volcanic ash-based fertilizer modulates gene expression during photomorphogenesis through phyB-dependent and independent

- pathways. *Plant Gene*. 2025;43:100523. <https://doi.org/10.1016/j.plgene.2025.100523>.
25. Meudt WJ, Thompson MJ, Bennett HW. Investigations on the mechanism of the brassinosteroid response. III. Techniques for potential enhancement of crop production. *Proc. Annu. Meet. Plant Growth Regul. Soc. Am.* 1983;10:312–318.
 26. Nakamura A, Fujioka S, Sunohara H, Kamiya N, Hong Z, Inukai Y, Miura K, Takatsuto S, Yoshida S, Ueguchi-Tanaka M, Hasegawa Y, Kitano H, Matsuoka M. The role of *OsBRI1* and its homologous genes, *OsBRL1* and *OsBRL3*, in rice. *Plant Physiol.* 2006;140:580–590. <https://doi.org/10.1104/pp.105.072330>
 27. Nomura T, Nakayama M, Reid JB, Takeuchi Y, Yokota T. Blockage of brassinosteroid biosynthesis and sensitivity causes dwarfism in garden pea. *Plant Physiol.* 1997;113:31–7.
 28. Peng B, Qin Y. Lipophilic polymer membrane optical sensor with a synthetic receptor for saccharide detection. *Anal Chem.* 2008;80:6137–41.
 29. Rogers JK, Crop. *Forage Turfgrass Manage.* 2017;3:1–3. <https://doi.org/10.2134/cftm2017.03.0020>. cftm2017.03.0020.
 30. Sakakibara M, Mori K. Facile synthesis of (22*R*,23*R*)-homobrassinolide. *Agric Biol Chem.* 1982;46:2769–79.
 31. Schlagnhauser C, Arteca RN, Yopp JH. A brassinosteroid-cytokinin interaction on ethylene production by etiolated mung bean segments. *Physiol Plant.* 1984;60(a):347–50.
 32. Schlagnhauser C, Arteca RN, Yopp JH. Evidence that brassinosteroid stimulates auxin-induced ethylene synthesis in mung bean hypocotyls between S-adenosylmethionine and 1-aminocyclopropane-1-carboxylic acid. *Physiol Plant.* 1984(b):61:555–558.
 33. Song L, Liu J, Cao BL, Liu B, Zhang XP, Chen ZY, Dong CQ, Liu XQ, Zhang ZH, Wang WX et al. (2023). Reducing brassinosteroid signalling enhances grain yield in semi-dwarf wheat. *Nature.* 2023;617:118–124.
 34. Sridhara S, Ramesh N, Gopakkali P, Paramesh V, Tamamc N, Abdelbacki AMM, Elansary HO, El-Sabroun AM, Abdelmohsen SAM. Application of homobrassinolide enhances growth, yield and quality of tomato. *Saudi J Biolog Sci.* 2021;28:4800–6.
 35. Svatoš A, Antonchick A, Schneider B. Determination of brassinosteroids in the sub-femtomolar range using dansyl-3-aminophenylboronate derivatization and electrospray mass spectrometry. *Rapid Commun Mass Spectrom.* 2004;18:816–21.
 36. Syltje PW. Effect of very small amounts of highly active biological substances on plant growth. *Bio Agric Hort.* 1985;2:245–69.
 37. Takatsuto S, Brassinosteroids. Distribution in plants, bioassays and microanalysis by gas chromatography-mass spectrometry. *J Chromatogr A.* 1994;658:3–15.
 38. Takatsuto S, Omote K, Gamoh K, Ishibashi M. Identification of brassinolide and castasterone in buckwheat (*Fagopyrum esculentum* Moench) pollen. *Agric Bioi Chern.* 1990;54:757–62.

39. Takatsuto S, Ying B, Morisaki M, Ikekawa N. Microanalysis of brassinolide and its analogs by gas chromatography and gas chromatography-mass spectrometry. *J Chromatogr A*. 1982;239:233–41.
40. Tangtreamjitmun N, Chindaphan K. Spectrofluorimetric determination of brassinosteroids plant hormones in bio-extract samples. *Malaysian J Anal Sci*. 2015;19:557–64.
41. Thompson MJ, Mandava NB, Flippen-Anderson JL, Worley JF, Dutky SR, Robbins WE, Lusby W. Synthesis of Brassino Steroids: New plant-growth-promoting steroids. *J Org Chem*. 1979;44:5002–4.
42. Umesha S, Hariprasad P, Deepak SA, Girish ST, Syltie P. Vitazyme treatment improves seed quality parameters of paddy, tomato and cotton. *Asian Jr Microbiol Biotech Env Sc*. 2009;11:223–30.
43. Xing YZ, Zhang QF. Genetic and molecular bases of rice yield. *Annu Rev Plant Biol*. 2010;61:421–42.
44. Yin W, Dong N, Li X, Yang Y, Lu Z, Zhou W, Qian Q, Chu C, Tong H. Understanding brassinosteroid-centric phytohormone interactions for crop improvement. *J Integr Plant Biol*. 2025;67:563–81.
45. Zolkiewicz K, Ahmar S, Gruszka D. Genetic manipulations of brassinosteroid-related genes improve various agronomic traits and yield in cereals enabling new biotechnological revolution: Achievements and perspectives. *Biotech. Advances*. 2025;81:108556.
<https://doi.org/10.1016/j.biotechadv.2025.108556>.
46. Zullo MAT. Brassinosteroids and related compounds, 1st ed.; Lambert Academic Publishing: Beau Bassin, Mauritius, 2018; ISBN 978-3-330-34627-7.
47. Zullo MAT, Adam G. Brassinosteroid phytohormones-Structure, bioactivity and applications. *Braz J Plant Physiol*. 2002;14:143–81.

Tables

Table 1 Vitazyme treatment improved specialty crop production. All plants were grown in a greenhouse as described in methods. Number of plants/treatment: The number of plants tested either without Vitazyme (Control) or with (Vitazyme). The number and weight of specialty crop products harvested is shown. Increase in the number and weight harvested is shown (% increase), *P*-values are indicated by *= <0.1 and **=<0.05 (highlighted).

Specialty Crop	Number of plants/ treatment	Number harvested (Control)	Number harvested (Vitazyme)	Increase in number harvested	<i>P</i> -value	Weight (g) harvested (Control)	Weight (g) harvested (Vitazyme)	Increase in weight harvested	<i>P</i> -value
Broccoli	6	6	6	-	NA	1209.50	1773.40	46.62%	0.0040**
Daikon Radish	17	19	19	-	NA	1589.80	1845.80	16.10%	0.5439
Kale	8	8	8	-	NA	1265.00	1340.80	5.99%	0.1882
Peas	8	33	47	42.42%	0.2436	35.60	46.10	29.49%	0.4115
Plum Purple Radish	9	9	9	-	NA	1781.00	1996.60	12.11%	0.4847
Tomato	16	116	177	52.59%	0.0209**	462.60	555.20	20.02%	0.2159

Table 2

Quantification of HBL in derivatized Vitazyme by LCMS. Table shows RT (Retention Time) and Area used to calculate concentrations of HBL in Vitazyme, based on linear concentration curve established with pure HBL standard (Supplemental Fig. 2). Final calculated concentration of HBL in Vitazyme is shown in μM . Three independent batches of Vitazyme were tested for quantification HBL.

Sample	Analysis Type	RT	Area	Concentration	Units
Derivatized Vitazyme- 1	HBL-DAPBA	5.0618	38349184	1.26	μM
Derivatized Vitazyme- 2	HBL-DAPBA	5.0382	35745562	1.17	μM
Derivatized Vitazyme- 3	HBL-DAPBA	5.0927	32769747	1.08	μM

Figures

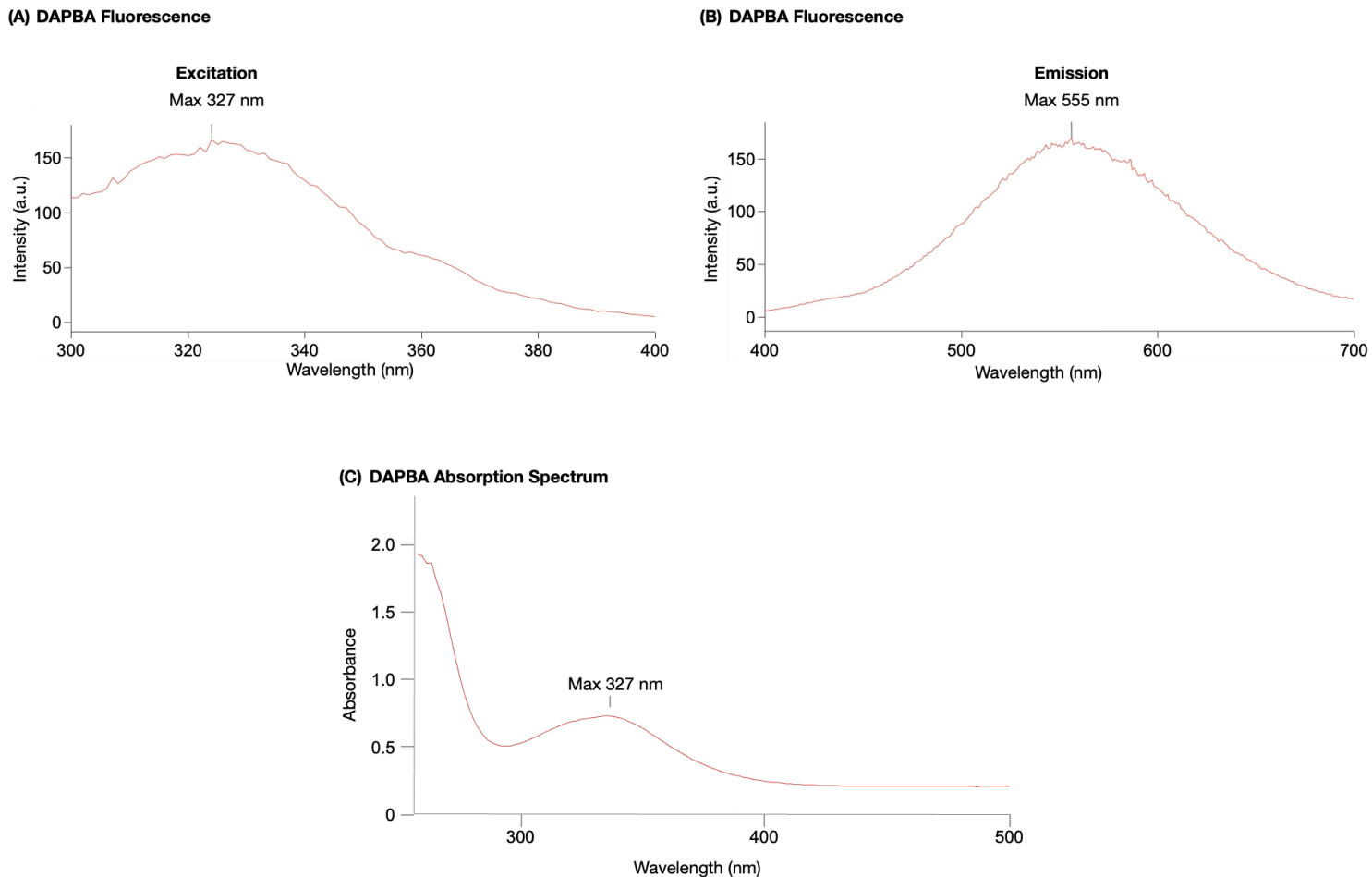
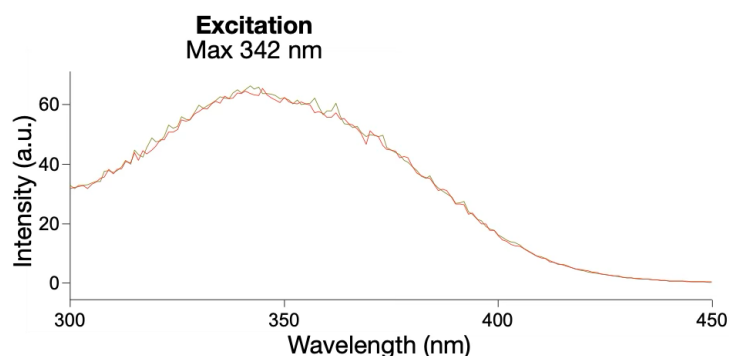


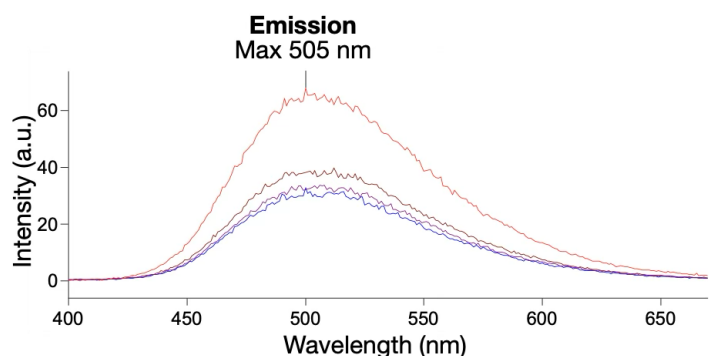
Figure 1

Fluorescence associated with DAPBA. (A) Fluorescence excitation (Max peak, 327 nm) with emission at 555 nm of DAPBA in 5% DMSO. (B) Fluorescence emission (Max peak, 555 nm) with excitation at 327 nm of the same sample. (C) Absorption spectrum of a 20 mM solution of DAPBA.

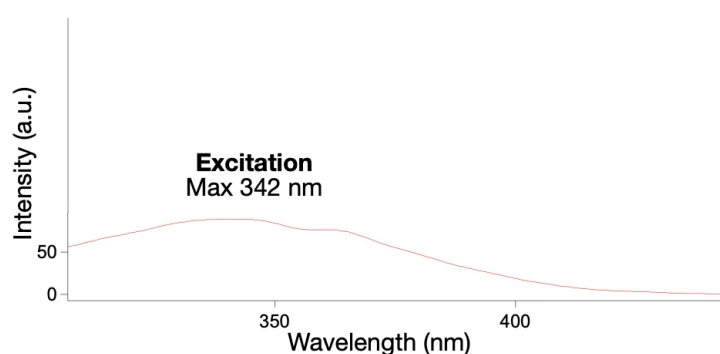
(A) Brassinolide / DAPBA Reaction



(B) Brassinolide / DAPBA Reaction



(C) Homobrassinolide / DAPBA Reaction



(D) Homobrassinolide / DAPBA Reaction

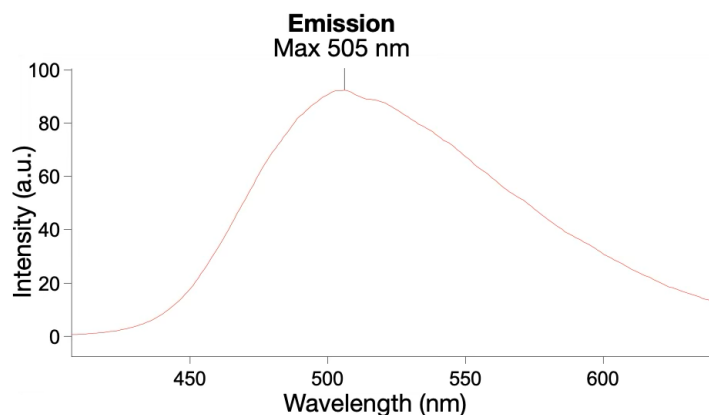
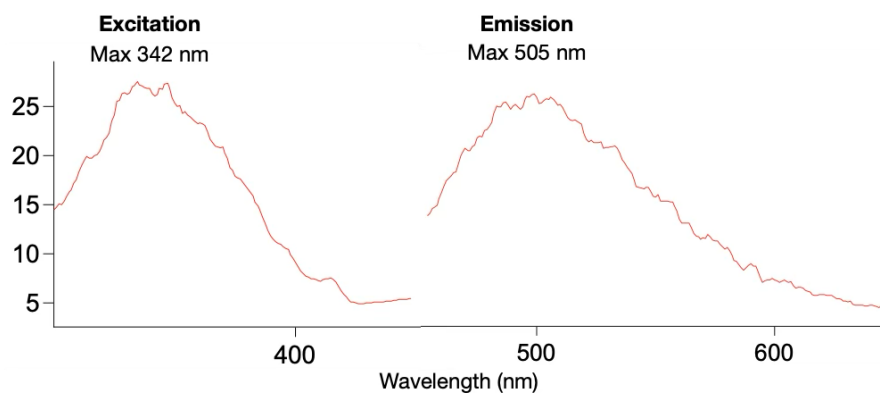


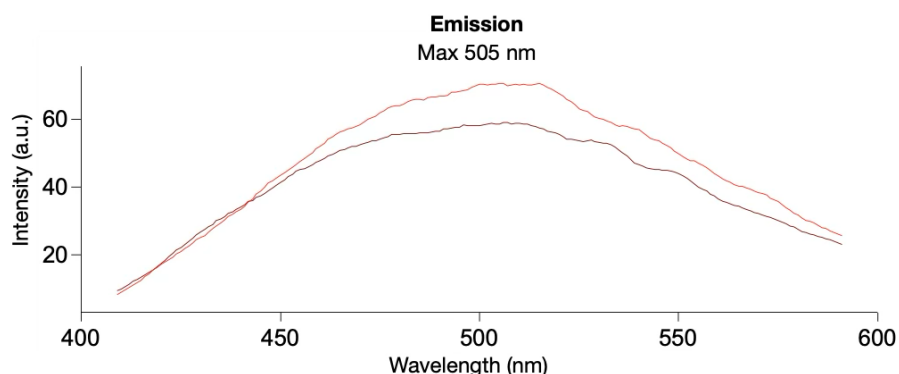
Figure 2

Fluorescence changes associated with the chemical reaction between BR standards and DAPBA. (A) Fluorescence excitation spectra for standard, Brassinolide / DAPBA (Max peak, 342 nm) for the emission recorded at 505 nm after 30 minutes of reaction. (B) From lowest to highest intensity the traces show the fluorescence emission spectra for standard, Brassinolide / DAPBA (Max peak, 505 nm) increase over time upon formation of the BL/DAPBA product at 5, 8, 10, and 30 minutes from the time BL (100 μ M) was mixed with DAPBA (160 μ M), with 342 nm excitation. (C) Fluorescence excitation spectrum for Homobrassinolide / DAPBA (Max peak, 342 nm) for the emission recorded at 505 nm. (D) Fluorescence emission spectrum for Homobrassinolide / DAPBA (Max peak, 505 nm) of the HBL/DAPBA product after 30 minutes from the time HBL (101 μ M) was mixed with DAPBA (160 μ M), with 342 nm excitation.

(A) DAPBA Products in VITAZYME



(B) Total Brassinosteroids in VITAZYME



(C) Brassinolide-Spiked Increase in Fluorescence

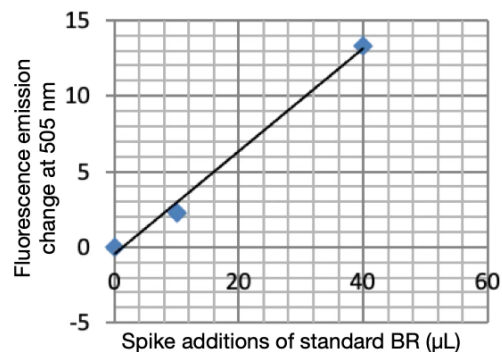


Figure 3

Detection of DAPBA products in Vitazyme. (A) Left; Fluorescence excitation (Max peak, 342 nm) with emission at 505 nm, and right; fluorescence emission (Max peak, 505 nm) with excitation at 342 nm of the Vitazyme/DAPBA reaction product. Increase in Vitazyme fluorescence with BL spike (B) BR spike reaction: The Vitazyme/DAPBA reaction was spiked with 0, 10, or 40 μL of Brassinolide standard solution (100 μM). The upper trace shows the fluorescence emission scans for 40 μL addition and lower trace for 0 μL addition (without BR spike). (C) Plot of fluorescence intensity increase at 505 nm after different spike additions.

Glucose / DAPBA Reaction

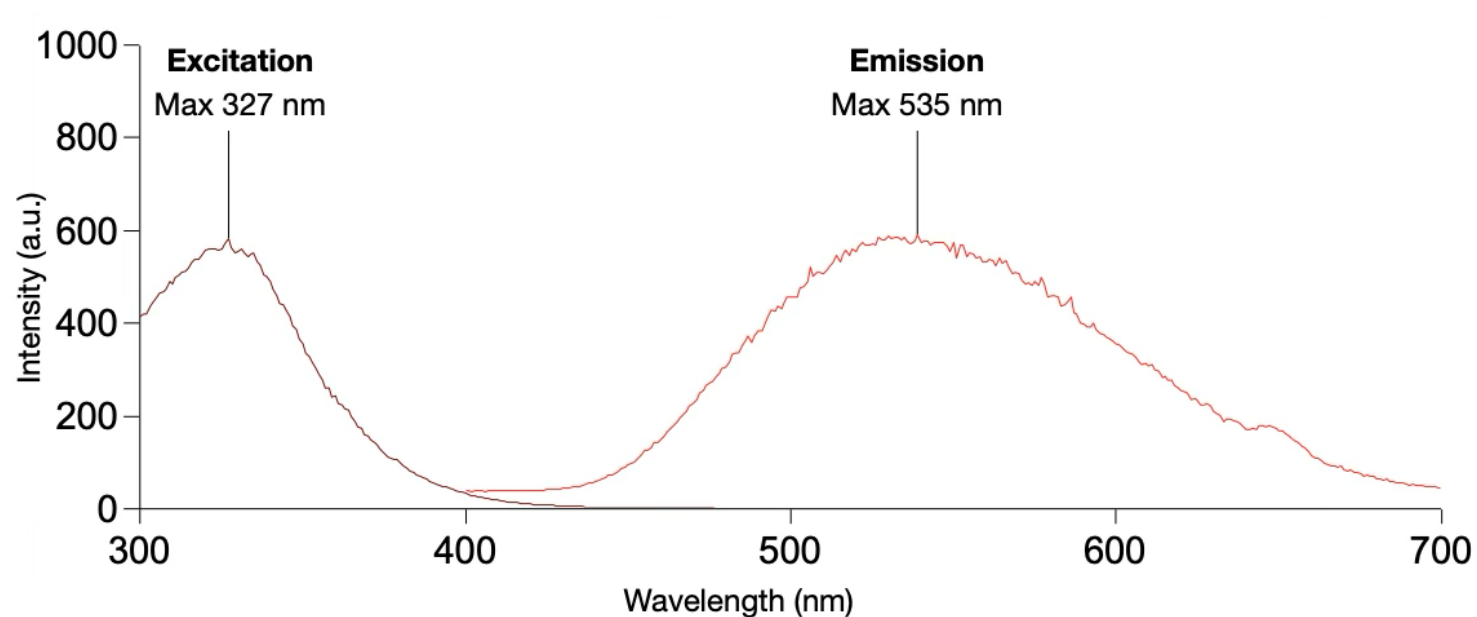


Figure 4

Fluorescence changes associated with the chemical reaction between DAPBA and glucose. The traces show the fluorescence excitation and emission spectra of the reaction product of DAPBA (106 μM) with glucose (100 μM).

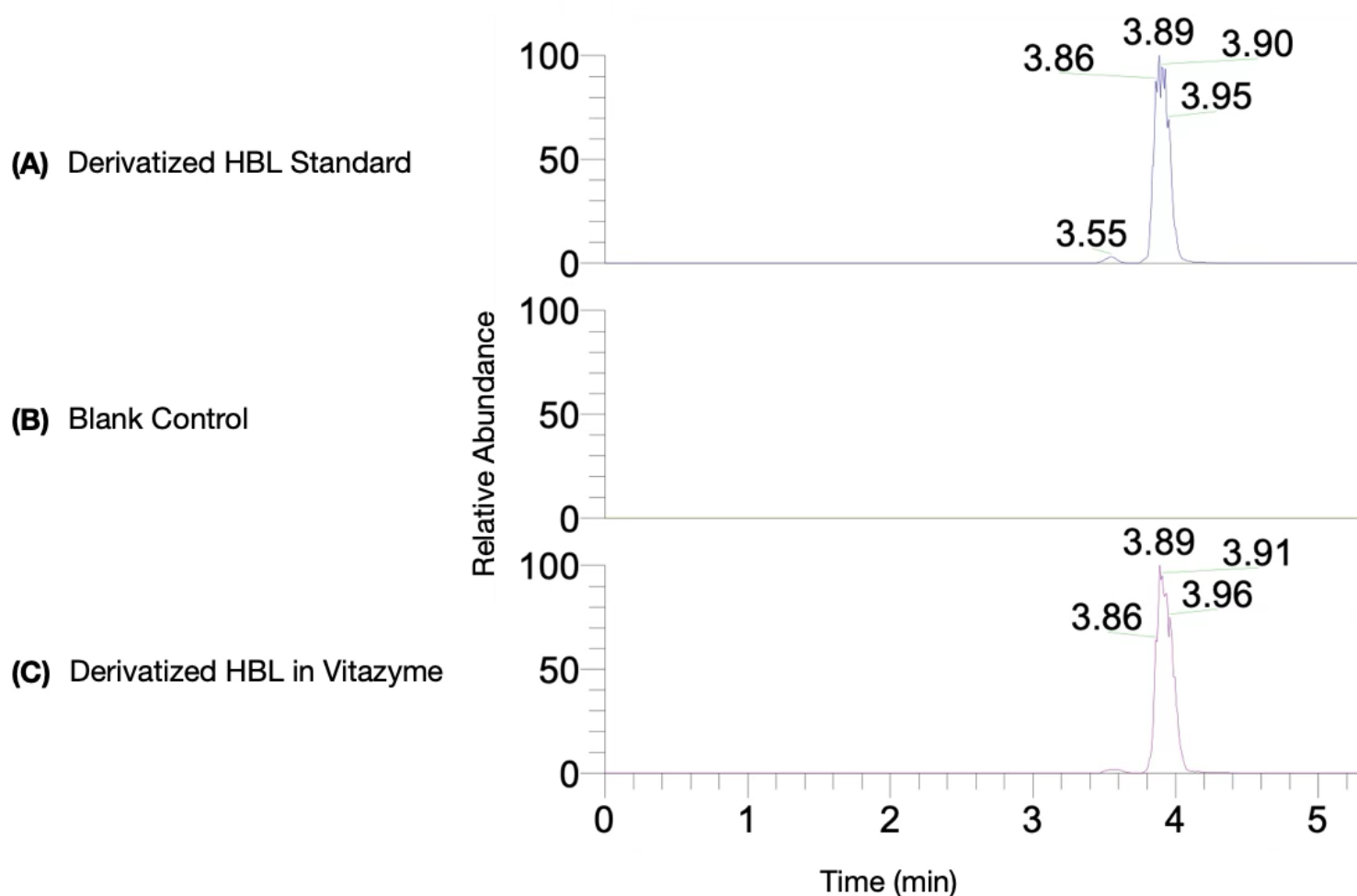


Figure 5

LCMS analysis of derivatized Brassinosteroids in Vitazyme biostimulant. DAPBA-derivatized standard (HBL) was used to compare with derivatized Vitazyme in LCMS analysis. Blank Control (without standard or Vitazyme) was used as negative control. Peaks observed are marked by their appearance in Time (min). Representative plot shows HBL peaks at 3.89 min. The other peaks are expected to be background in the solutions. The samples were run independently of each other and are not quantitatively comparable with each other.

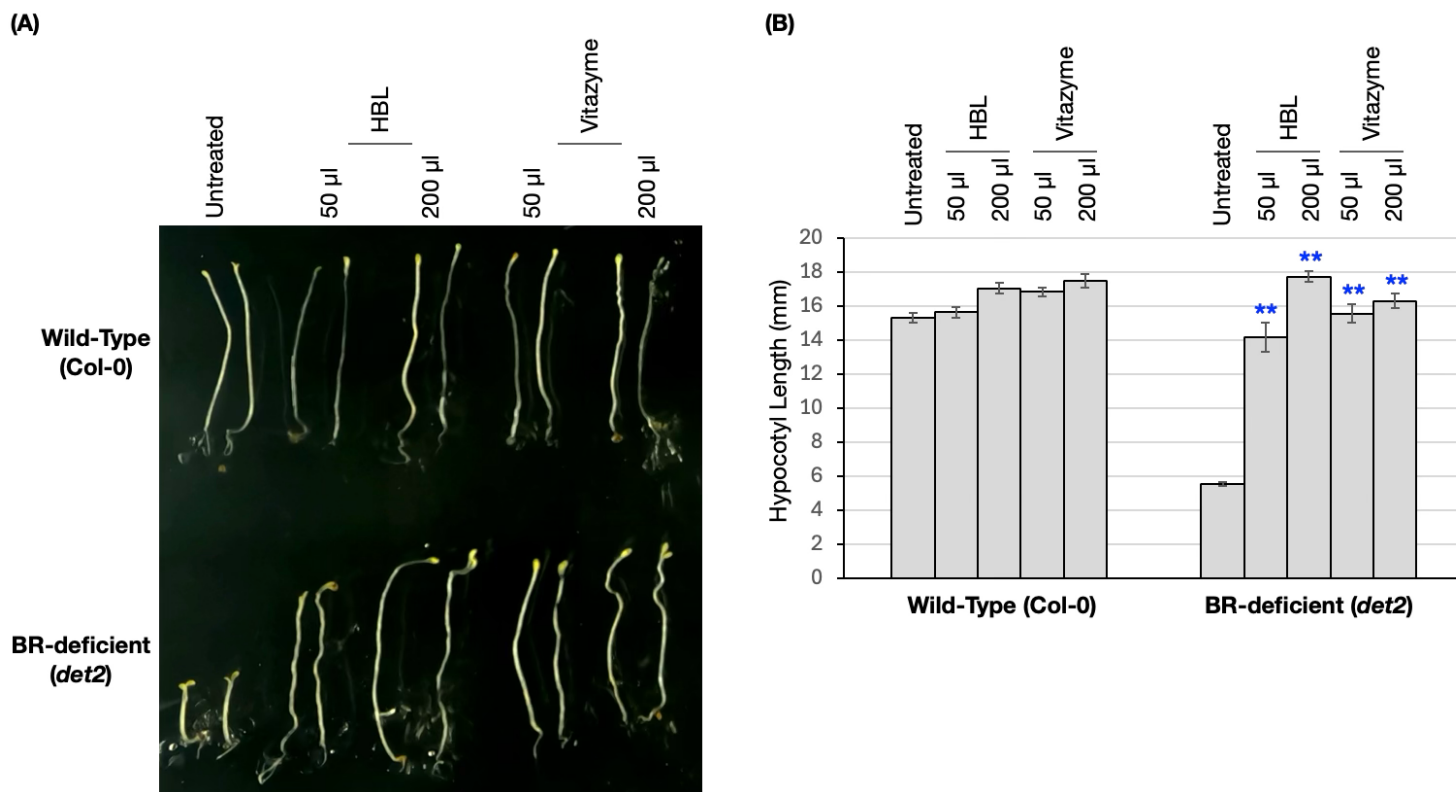


Figure 6

Vitazyme treatment rescued the hypocotyl growth defect of *det2* seedlings. (A) Images of representative wild-type (Col-0) and BR-deficient (*det2*-mutant) seedlings are shown. All seedlings were grown in darkness for 7-days on agar plates (Untreated), or with the addition of either 50 µl or 200 µl (applied as a 200 µl aqueous solutions) of HBL standard or Vitazyme on top of the agar, before applying the seeds. (B) Average hypocotyl lengths of wild-type (Col-0) seedlings, and *det2*-mutant seedlings grown in darkness for 7-days. The *det2* seedlings showed increased hypocotyl growth when treated with Vitazyme. Data are representative of three independent trials with 10-30 seedlings per trial. HBL standard solution was prepared as described in Methods. Standard Error are shown, and *P*-values are indicated by **=<0.05.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [SupplementaryFigures.docx](#)