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Metabolomic analysis of the roots and shoots of tomato seedlings treated with the commercial seaweed-derived biostimulant Afrikelp

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ABSTRACT

Excessive fertilizer use has had severe environmental and economic consequences globally, posing a major challenge to sustainable agriculture. Biostimulants are naturally-occurring biological products that are used to enhance plant productivity by modulating metabolism. One such biostimulant is Afrikelp, which is derived from the brown alga *Ecklonia maxima* and is purported to enhance plant growth when applied to either the roots or shoots. However, whether a metabolic response is elicited by plants treated with Afrikelp, as well as the manner in which this biostimulant might regulate plant metabolism, has not been scientifically evaluated. In response to this, the present study used gas chromatography-mass spectrometry to explore the changes in primary metabolites between the roots and shoots of tomato (*Lycopersicon esculemtum*) seedlings treated with Afrikelp and those treated with water alone. Principal components analysis separated the samples treated with Afrikelp and the control, confirming a response to treatment at the metabolite level, particularly in the roots. Fold-change analysis identified many metabolites that were increased in abundance (log2 fold-change > 1.5) in both the shoot and root tissues in comparison with the control. Glycerol-3-phosphate, putrescine, and γ-aminobutyric acid were significantly differentially abundant in the root tissues treated with Afrikelp, possibly suggesting mycorrhizal colonization. In the shoot tissues, the most abundant metabolites included tryptophan, sucrose, and galactinol, and, based on pathway analysis, were largely related to energy metabolism, growth, and hormone synthesis, supporting observations that Afrikelp treatment improves crop growth. Metabolic pathway analysis further suggested the enrichment of carbon and nitrogen metabolism pathways in plants treated with the biostimulant. In summary, Afrikelp appears to increase sugar and nitrogen metabolism in the leaves of tomato plants and might encourage mycorrhizal symbiosis in the roots, thereby promoting growth.

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1. Introduction

Inappropriate fertilizer use, primarily excessive nitrogen and phosphate application, has resulted in serious environmental pollution and reduced fertilizer efficacy globally. Improving nitrogen use efficiency thus remains a significant challenge for agriculture. The environmental and economic impacts of excessive nitrogen use have prompted scientists to explore new solutions for maintaining crop yields in a more sustainable manner (Rouphael and Colla, 2020). Biostimulants offer an advantage when used in conjunction with conventional chemical fertilizers, as they are naturally occurring biological products that affect plant productivity by modulating the physiological processes and metabolism of plants and improving aspects relating to the growth, development, or abiotic stress resistance of plants (Bulgari et al., 2019). The term biostimulant is relatively new and is broadly defined as "...any substance or microorganism applied to plants with the aim to enhance nutrition efficiency, abiotic stress tolerance and/or crop quality traits, regardless of its nutrient content" (Du Jardin, 2015). The modes of action of biostimulants differ from those of conventional fertilizers, and biostimulants are typically used in conjunction with traditional crop management strategies, with a specific focus on crop nutrition and protection against abiotic stress (Lötze and Hoffman, 2016).

Six non-microbial and three microbial categories of plant biostimulants have been proposed (Colla and Rhouphael, 2015), one of which includes seaweed extracts. Red, green, and brown macroalgae are the most common seaweed-derived biostimulants used in horticulture and agriculture (Rouphael and Colla, 2020) and have been confirmed to have positive effects on the growth and stress resistance

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(Arthur et al., 2003; Di Filippo-Herrera et al., 2019) of crops. The increased growth of plants treated with seaweed-derived biostimulants has been attributed to increases in net photosynthesis, carbon and nitrogen uptake, and the activity of basal metabolism-related enzymes (González et al., 2013). Seaweed-derived biostimulants have also been shown to increase photosynthetic capacity and improve resistance to fungi and bacteria (Sharma et al., 2014). Biostimulants can be applied to both the leaves and the soil, with the latter application acting on plant metabolism and physiology by improving the soil conditions and specifically enhancing the activity of microflora, as well as promoting root development for nutrient acquisition, thereby positively impacting plant growth (Khan et al., 2009; Nardi et al., 2009). However, the metabolite compositions of the majority of biostimulants, as well as their exact mechanisms of action, are generally poorly understood, as the extracts are complex and the effects on plants are likely the result of the synergy of a combination of compounds (Bulgari et al. 2019; Ertani et al., 2018).

The plant metabolome is composed of an array of small-molecule metabolites that have significant impacts on the biological activities and phenotype of a plant. As metabolites are the end-products of metabolism, studying the metabolites present in a tissue at a particular time offers an excellent indication of the biochemical activity in that tissue. Earlier metabolomics studies that assessed the effects of plant biostimulants on crops indicated that their application results in changes in hormone, sugar, organic acid, and phenolic contents (Ertani et al., 2014; Abou Chehade et al., 2018; Lucini et al., 2018, Barrajón-Catalán et al., 2020). However, metabolomic studies of the effects of biostimulants, and particularly seaweed-based biostimulants, remain rare.

Afrikelp is a commercial biostimulant that is produced from the brown kelp *Ecklonia maxima* (Osbeck) Papenfuss, which occurs along the western and south-western coast of South Africa and Namibia. Like other seaweed-based biostimulants, this kelp-based biostimulant is used to enhance crop growth and yield and can be applied as a foliar spray or dip or can be integrated into irrigation systems to support root and shoot growth and fruit yield (https://afrikelp.com). While Afrikelp is purported to have positive effects on crop growth, the kind of response elicited by plants has not been characterized, nor have the mechanisms of action of the biostimulant on plants been elucidated. Here, we explored the metabolic response of tomato plants treated with Afrikelp, subjecting root and shoot tissues of tomato plants treated with Afrikelp to nontargeted gas chromatography-time of flight-mass spectrometry (GC-TOF-MS) analysis. To our knowledge, this is the first study to profile the primary metabolome of a plant treated with an *E. maxima*-based biostimulant.

2. Materials and methods

2.1. Treatments and growth conditions

Seedlings of the tomato (*Lycopersicon esculemtum* L.; Solanaceae) variety 'Floradade' were purchased from Western Cape Seedlings (Phillipi, Cape Town) and immersed in Afrikelp treatment solution (1% v/v) or water (control) for 30 min to ensure saturation and uptake of the product. They were then individually planted in Universal blend peat moss in pots (9 cm diameter, 500 mL volume) in a growth room at Afrikelp headquarters (Cape Town, South Africa) under a 16 h light/8 h dark light cycle with a temperature of 26°C and a light intensity of 50–70 μmol m^{-2} s^{-1} achieved using Osram 36W/840 cool-white fluorescent tubes. A second Afrikelp treatment was applied via foliar spraying (1% v/v) 7 d later when the seedlings were 15 days old. The pots were arranged in a random block design, with three biological replicates per treatment. Deionized water was applied as the control. Sampling commenced on 25 September 2019 in the morning when the tomato plants were 22 days old. Samples were collected from three biological replicates for each treatment. The shoots and roots were separated and weighed for their biomass. Leaves from same position on each plant were collected and weighed for metabolite analysis. The root samples were collected by immediately rinsing the roots in deionised water to remove the soil and patting dry with paper towel before placing into liquid nitrogen. At least 100 mg of fresh root and shoot material was collected. After being weighed, the tissues were placed into liquid nitrogen and then stored at -80°C. Prior to metabolite extraction, the tissues were dried in a freezer drier and the dry weights were recorded.

2.2. Extraction and metabolomic analysis

The polar fraction of the samples was extracted for primary metabolite analysis at the Max Planck Institute for Molecular Plant Physiology (MPI-MP; Potsdam, Germany) following the protocol detailed in Salem et al. (2016). Briefly, freeze-dried root and shoot tissues (7.3–11.3 mg) were pulverized for 2 min at 25 Hz using a Retsch mill (Haan, Germany) until a fine and homogenous powder was achieved. Fifty microliters of a 1 mg/mL stock solution of ribitol was added as an internal standard. Following drying down in a SpeedVac vacuum concentrator, the samples were resuspended in methoxyamine-hydrochloride/pyridine solution and heated at 37°C for 90 min. The samples were then derivatized in N-methyl-N-trimethylsilyltrifloracetamide (MSTFA) containing a mixture of 13 fatty acid methyl esters (FAMEs) with different chain lengths for use as retention time standards for 30 min at 37°C. One-microliter samples were injected via an autosampler Gerstel Multi-Purpose system (Gerstel GmbH & Co.KG, Mülheim an der Ruhr, Germany) into a 30 m DB-35 column coupled to a time-of-flight mass spectrometer (GC-MS) system (Leco Pegasus HT TOF-MS, LECO Corporation, St. Joseph, MI, USA). Helium was used as carrier gas at a constant flow rate of 2 mL/s. The injection temperature was 230°C and the transfer line and ion source were set to 250°C. The initial temperature of the oven (85°C) increased at a rate of 15°C/min up to a final temperature of 360°C. After a solvent delay of 180 s, mass spectra were recorded at 20 scans s^{-1} within an *m/z* 70–600 scanning range. Blank samples containing no extract or internal standards were injected at the start of the run and at the end of the run.

2.3. Compound identification

The peaks for the GC-MS data were identified by matching the spectra and retention times to an in-house library at MPI-MP using Xcalibur software (ver. 4.1, Thermo Fischer Scientific, Waltham, MA, USA).

2.4. Statistical analysis

The GC-MS data were normalized by the ribitol internal standard and the dry sample weights. The online metabolomics platform MetaboAnalyst 5.0 (https://www.metaboanalyst.ca) was used for the statistical analyses. The normalized dataset was log-transformed prior to statistical analysis to ensure that it followed a Gaussian distribution (Xia and Wishart, 2011). The statistical analyses began by subjecting all the treatments to principal component analysis (PCA) in order to evaluate the structure of the dataset and assess sample groupings. Pairwise root and shoot treatments were thereafter analysed using PCA and fold-change (FC) analysis to identify compounds with high FC values ($\log2FC \geq 1.5$); these being the compounds that differed significantly between the treatments and that could provide insight into the different biochemical responses of the root and shoot tissues to treatment with the biostimulant. The compounds that were identified as having a $\log_2$FC cutoff value of 1.5 in the FC analysis in the treatment groups were mapped to the pathways on the MetaboAnalyst platform using the *Arabidopsis thaliana* L. pathway

library. The pathway *P*-values were computed using a hypergeometric test.

3. Results and discussion

3.1. Sample groupings by PCA

The PCA scores plot for the combined root and shoot samples is shown in Fig. 1. The first principal component (PC) explained 75.4% of the total variation in the dataset and separated the root and shoot samples, strongly indicating the distinctness of the metabolomes of the root and shoot samples. PC2 explained 8.2% of the variance and separated the control and treatment root samples but did not separate the control and treatment shoot samples. The PCA that included only the root samples (Fig. 2a) explained 47% (PC1) and 19.3% (PC2) of the variability, with PC1 showing a clear separation between the two treatments and confirming that Afrikelp elicits a metabolic response in the roots. The PCA plot of the shoot treatments alone (Fig. 2b) showed less of a distinction between the treatments, though the control samples did cluster closely together, whereas the treatment samples indicated far greater spread.

3.2. Differentially abundant metabolites in the root tissues

There were substantially more compounds identified as being higher in abundance in the Afrikelp-treated samples than in the control samples (Table 1). A total of 77 compounds were identified as being significantly more abundant in the root tissues treated with Afrikelp than the control root tissues based on a $\log_2$FC value $\geq$ 1.5, whereas only two compounds were higher in abundance in the control samples than in the treatment samples. Glycerol-3-phosphate (G3P) was the most abundant differential compound in the treated samples ($\log_2$FC 3.67). G3P is a primary component of glycerol-based phospholipids in cellular membranes and has an important role in systemic acquired resistance (SAR), which is activated in response to pathogen infection (Chanda et al., 2011). Similarly, induced systemic resistance (ISR) is a signalling mechanism that is triggered in response to colonization by beneficial microbes (Van Wees et al., 2008). The mycelia of beneficial mycorrhizal fungi absorb soil

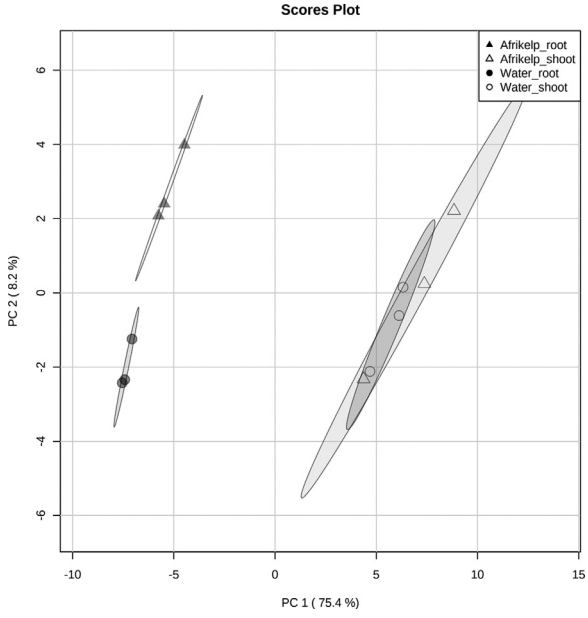

Fig. 1. PCA scores plot based on the compounds detected in the GC-MS analysis of the root and shoot samples treated with Afrikelp or water. The shaded area represents the 95% confidence interval.

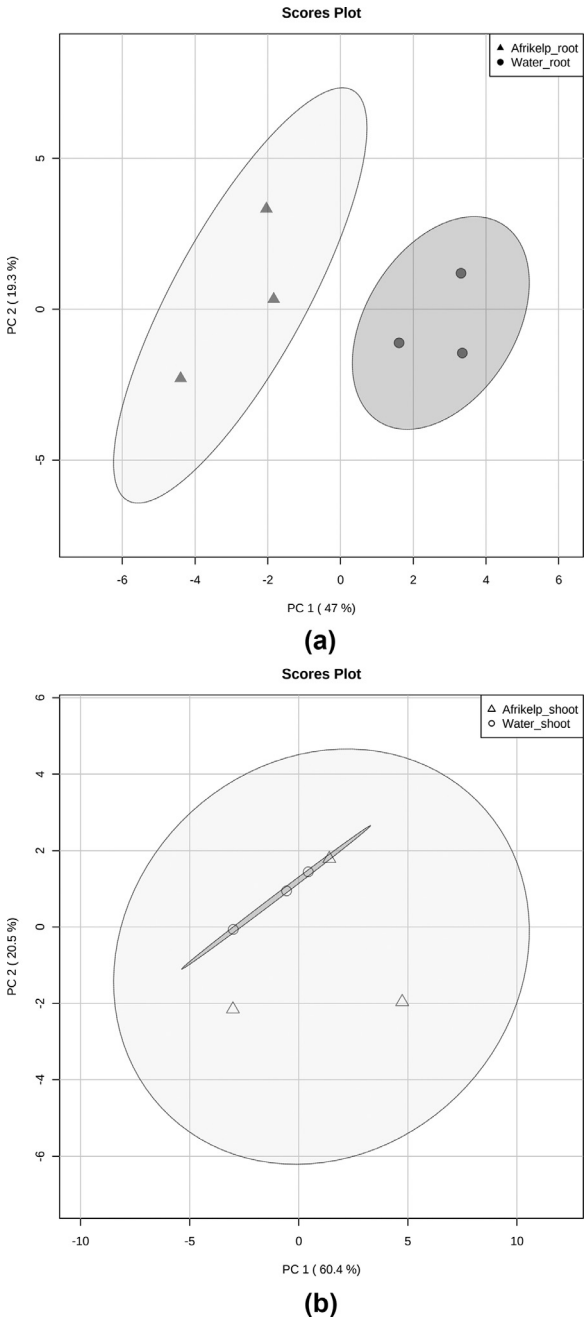

Fig. 2. PCA scores plot based on the compounds detected in the GC-MS analysis. The upper plot (a) includes the root samples and the lower plot (b) includes the shoot samples. The shaded area represents the 95% confidence interval.

nutrients and supply them to plants in exchange for photosynthetic products (Smith and Read, 2008). One study found that G3P is involved in a root-shoot-root signalling mechanism of rhizobia-ISR and is essential for the exclusion of non-desirable nitrogen-fixing rhizobia in the roots of soybean (Shine et al., 2019). Putrescine was the third-most abundant metabolite ($\log_2$FC 2.89) in the root samples treated with Afrikelp. Polyamines such as putrescine, which are essential for cell formation, have been found to increase mycorrhizal infection when applied exogenously to plants (El Ghachtouli et al., 1995; Vassileva and Ignatov, 1999; Qiang-Sheng et al., 2012). In tomato (*Solanum lycospermum* L.) roots colonized with the mycorrhizal fungus *Piriformospora indica,* putrescine was the most significantly induced metabolite, and the putrescine biosynthetic gene *SlADC1* was upregulated in *S. lycopersicum* under *P. indica* infection

Table 1
Differential compounds in the GC-TOF-MS analysis of the root and shoot tissues treated with Afrikelp based on log2 fold-change values. Positive values for compounds indicate upregulated compounds under Afrikelp treatment, whereas negative values indicate downregulated compounds under Afrikelp treatment.

Compound Name	Retention Time (rt)	Log_2FC Value
Root tissues		
Glycerol-3-phosphate	9.31	3.6574
Unknown	16.98	2.9852
Putrescine	8.38	2.8876
Unknown	16.48	2.7788
Phosphoric acid	5.38	2.6915
Unknown	12.3	2.63
Alanine	5.72	2.6137
Ethanolamine	4.58	2.6074
Saccharic acid (or similar)	10.4	2.5487
Trans-4-hydroxy-L-proline	7.26	2.4721
Tryptophan	12.87	2.4654
Lysine	9.98	2.4302
Fumaric acid	6.0	2.3357
Asparagine	8.92	2.3188
GABA	7.32	2.2575
Unknown	17.3	2.1953
Glutamine	7.99	2.1273
Unknown	6.78	2.1156
Tyramine	10.37	2.0511
Citric acid	9.62	2.0317
GABA	5.53	1.9884
Unknown	11.49	1.9577
Unknown	14.29	1.9105
Galactinol	15.31	1.806
Unknown	10.84	1.7792
Aspartic acid	7.41	1.7411
Unknown	14.42	1.7144
Unknown	17.63	1.6958
Unknown	10.33	1.6557
Maltose	14.41	1.6514
Glutamine	9.71	1.6415
Dehydroascorbic acid dimer	10.32	1.6239
Mannose	9.52	1.6205
Unknown	17.18	1.5783
Adenine	11.05	1.5452
Unknown	11.7	1.5331
Glutamic acid	8.23	1.5222
Unknown	15.03	-7.0282
Erythritol	6.60	-2.8111
Shoot tissues		
Tryptophan	12.87	2.0996
Sucrose	13.67	1.9739
Galactinol	15.31	1.9303
Dehydroascorbic acid dimer	10.15	1.789
Maltose	14.15	1.5866
Dehydroascorbic acid dimer	10.32	1.5415
Pyroglutamic acid	8.22	1.5389
2-Oxoglutaric acid	8.5	1.5362

and acted via the arginine decarboxylase (ADC)-mediated pathway (Kundu et al., 2021). We therefore suspect that the apparent ISR in the tomato plants was in response to the initial stages of root colonization by mycorrhizal fungi, as indicated by the increased abundance of G3P and putrescine, which was encouraged by treatment with Afrikelp. Additionally, there is also another possible role for G3P in mycorrhizal infection, as the *RAM2* gene, which encodes a G3P acyl transferase, was found to be required for root colonisation by mycorrhizal fungi in *Medicago truncatula* Gaertn. (Wang et al., 2012). γ-Aminobutyric acid (GABA), which is involved in the regulation of the citrate cycle, was also increased in abundance (FC 2.26) in the Afrikelp-treated samples. Interestingly, rice plants inoculated with *Paenibacillus yonginensis*—a novel plant symbiont that promotes growth via ISR—indicated increased contents of GABA (Kim et al., 2017). GABA is also an important intermediate of nitrogen metabolism and amino acid biosynthesis and might also upregulate nitrate uptake in plants by acting as a long-distance signalling molecule to increase the expression of the nitrate transporter gene *BnNrt2* (Beuve et al., 2004).

Phosphoric acid was high in abundance in the Afrikelp-treated roots (log_2FC 2.7), implying increased uptake of phosphorus by the roots and support for the role of biostimulants in improving plant growth. Fumaric acid was also abundant (log_2FC 2.34) and, as a citrate cycle intermediate, can be metabolized for energy, and its carbon skeletons can be used for the synthesis of other compounds (Chia et al., 2000). Fumaric acid, together with citric acid (which was also more abundant in the treated roots with a log_2FC value of 2.04), may also act as signalling molecules to attract root colonization by beneficial rhizosphere-associated bacterial strains (Zhang et al., 2014). Asparagine was also increased in abundance (log2FC 2.3), and free asparagine is known to have a primary role in nitrogen storage and transport in plants (Lea et al., 2007). Galactinol (log2FC 1.8) was previously found to act as a signalling molecule in the ISR response of cucumber roots colonized by *Pseudomonas chlororaphis* (Kim et al., 2008). Our hypothesis that the application of Afrikelp encourages mycorrhizal symbiosis is in line with previous research that suggests that biostimulants improve soil microbiota and enhance root development for increased nutrient acquisition (Khan et al., 2009; Nardi et al., 2009).

3.3. Differentially abundant metabolites in the shoot tissues

Comparatively fewer compounds (eight) were identified with log_2FC cutoff values of 1.5 between the treatment and control groups of the shoot samples (Table 1). The most abundant metabolite in the Afrikelp-treated shoots was the α-amino acid tryptophan (log_2FC 2.1). Plants utilize the tryptophan biosynthesis pathway to produce the precursors for the synthesis of hormones, such as auxins (Radwanski and Last, 1995), which function in plant growth and development (Teale et al., 2006). Sucrose was the second-most abundant metabolite in the Afrikelp-treated shoot samples (log_2FC 1.97), suggesting increased energy metabolism for plant growth. The abundance of dehydroascorbic acid (log_2FC 1.8), an oxidation product of ascorbate acid, could be related to increased cell growth, as dehydroascorbic acid has been proposed to act as a cell-loosening agent to allow for increased cell expansion (Lin and Varner, 1991). Pyroglutamic acid (5-oxoproline), which had a log_2FC value of 1.54, is a non-protein amino acid derivative that is an intermediate in the glutathione cycle, and 2-oxoglutaric acid, which had a log_2FC value of 1.54, is an intermediate in the Krebs cycle and functions in basal metabolism. These differentially abundant metabolites indicate enhanced energy metabolism, growth, and basal metabolism, supporting previous studies on other biostimulants (Gonzalez et al., 2013; Ertani et al., 2014; Abou Chehade et al., 2018; Lucini et al., 2018, Barrajón-Catalán et al., 2020) as well as general observations that Afrikelp supports plant growth. Furthermore, pyroglutamic acid was previously found to increase the yield of lettuce plants subjected to water deficit stress (Jiménez-Arias et al., 2019).

3.4. Enriched pathways in the root tissues

The top five-most enriched pathways in the root tissues (Table 2), with *P*-values < 0.001, included aminoacyl-tRNA biosynthesis; alanine, aspartate and glutamate metabolism; arginine biosynthesis; tyrosine metabolism; and valine, leucine and isoleucine metabolism, implying significant activation of the metabolite networks associated with amino acid and protein synthesis. Nitrogen is a major limiting factor due to the high nitrogen requirements for the synthesis of nucleic acids and proteins for plant growth and development, and thus the significance of these pathways implies increased nitrogen mobilization for growth, corroborating previous findings on the enhancement of nitrogen metabolism by biostimulants (Ertani et al.,

Table 2
Significantly enriched metabolic pathways in the root and shoot tissues. Only those pathways receiving *P*-values < 0.05 have been shown.

Pathway name	Match score	*P*-value
Root tissues		
Aminoacyl-tRNA biosynthesis	14/46	< 0.001
Alanine, aspartate and glutamate metabolism	9/22	< 0.001
Arginine biosynthesis	6/18	< 0.001
Tyrosine metabolism	5/16	< 0.001
Valine, leucine and isoleucine metabolism	5/22	< 0.001
Isoquinoline alkaloid biosynthesis	3/6	< 0.001
Butanoate metabolism	4/17	0.001
Glyoxylate and dicarboxylate metabolism	5/29	0.001
Citrate cycle	4/20	0.003
Glycine, serine and threonine metabolism	5/33	0.003
Arginine and proline metabolism	5/34	0.003
Glutathione metabolism	5/34	0.007
Glucosinolate metabolism	5/34	0.01
Ascorbate and aldarate metabolism	5/34	0.02
Monobactam biosynthesis	5/34	0.02
Carbon fixation in photosynthetic organisms	5/34	0.02
Phenylalanine, tyrosine and tryptophan metabolism	5/34	0.03
Lysine biosynthesis	5/34	0.03
Galactose metabolism	5/34	< 0.05
Nitrogen metabolism	5/34	< 0.05
Shoot tissues		
Arginine biosynthesis	3/18	0.001
Alanine, aspartate and glutamate metabolism	3/22	0.002
Starch and sucrose metabolism	3/22	0.002
Glutathione metabolism	3/26	0.004
Glyoxylate and dicarboxylate metabolism	3/29	0.005
Glycine, serine and threonine metabolism	3/33	0.008
Arginine and proline metabolism	3/34	0.008
Butanoate metabolism	2/17	0.02
Aminoacyl-tRNA biosynthesis	3/46	0.02
Citrate cycle	2/20	0.03
Phenylalanine, tyrosine and tryptophan metabolism	2/22	0.03
Pantothenate and CoA biosynthesis	2/23	0.03
Galactose metabolism	2/27	< 0.05

2009; González et al., 2013). Of the five most significantly enriched pathways, alanine, aspartate, and glutamate metabolism was the second-most enriched. This further supports the increased synthesis of proteins and amino acids, as glutamate is a primary molecule involved in amino acid metabolism in higher plants, with its α-amino group being directly involved in ammonia assimilation and dissimilation (Forde and Lea, 2007). Arginine biosynthesis was also significantly enriched and has the highest nitrogen to carbon ratio of all proteinogenic amino acids, thereby offering a stable form of organic nitrogen that can be catabolized to release nitrogen stores (Winter et al., 2015). Arginine also regulates putrescine levels in the cells and is a precursor of putrescine biosynthesis (Morris, 2006). Glutathione metabolism was also identified as significant, which might be related to its roles in growth and development, protein synthesis, and antioxidant defence (Hasanuzzaman et al., 2017). Isoquinoline alkaloid biosynthesis was the next-most enriched pathway, suggesting the regulation of amino acid-related alkaloids. The enrichment of ascorbate and aldarate metabolism may be related to increased cell growth, as suggested earlier, or may be related to antioxidant metabolism. The enrichment of monobactam biosynthesis might be related to mycorrhizal symbiosis. The remainder of the pathways were largely associated with nitrogen and carbon metabolism.

3.5. Enriched pathways in the shoot tissues

Various pathways were identified in the shoot tissues treated with Afrikelp (Table 2), many of which were also enriched in the root tissues. The most significant pathway was arginine biosynthesis, followed by alanine, aspartate, and glutamate metabolism and starch and sucrose metabolism, suggesting amino acid and protein synthesis and increased carbohydrate production for plant growth. Another indicator of increased carbohydrate metabolism was the enrichment of the glyoxylate and dicarboxylate metabolism pathway. The following three enriched pathways were also amino acid synthesis-related, and other significant pathways included aminoacyl-tRNA biosynthesis, the citrate cycle, and galactose metabolism, further emphasizing increased protein and energy synthesis in the shoot tissues treated with Afrikelp. These findings overall corroborate earlier reports that seaweed oligosaccharides enhance plant growth by increasing nitrogen assimilation and basal metabolism (González et al., 2013).

4. Conclusions

Though exploratory, our metabolomic findings offer a foundation for understanding the metabolic regulation of Afrikelp on the roots and shoots of tomato seedlings, corroborating previous research that found that biostimulants alter organic acid and sugar contents and regulate carbon and nitrogen metabolism and basal metabolism. Significantly, our research offers preliminary support that treatment with this biostimulant might encourage mycorrhizal symbiosis, which improves nutrient acquisition by plants, thereby enhancing growth. This is inferred based on the increased abundance of G3P, GABA, putrescine, and other metabolites in the root samples treated with Afrikelp, which have been shown by various studies to be indicators of root colonization by mycorrhizal fungi, together with the other indicators of increased nitrogen metabolism discussed herein. This finding is therefore largely speculative and would need to be confirmed via genomics analysis of the mycorrhizal community of the soil and analysis of nitrogen metabolism. In conclusion, the application of Afrikelp elicits a metabolic response from tomato plants, resulting in an increase of beneficial growth- and stress-related metabolites.

Data availability

The data are available from the author.

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Declaration of Competing Interest

Dr Amelia Reddy and Margaret Mandishonha are scientists employed by Afrikelp.

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