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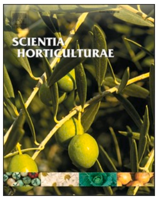

Research Paper

Supplementation of fertiliser with the biostimulant molasses enhances hemp (*Cannabis sativa*) seed functional food antioxidant capacity by induction of stress responses

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ABSTRACT

Hemp is low-tetrahydrocannabinol (THC) *Cannabis sativa* that is cultivated globally for food and fibre. Hemp seeds and seed-oil are popularly consumed individually or utilised within cooking, due to their flavour, nutrition, and functional food benefits. The functional food properties of hemp seed include its beneficial fatty acid (FA) profile and antioxidant activity, which are associated with reduced inflammation that benefits chronic conditions including cardiovascular disease and cancer. Maximising these functional food properties can offer extensive health benefits. Biostimulants – such as molasses – are fertiliser additives that are associated with improvements to plant growth, yield, and biochemical composition; however, they have been scarcely applied to hemp. Furthermore, due to the complex composition of biostimulants, understanding their modes of action poses a significant challenge, impeding the optimization of their usage and the realization of associated benefits. Accordingly, this study aimed to characterise the impact of molasses on the functional food properties of hemp seeds and explore biostimulant-induced biochemical changes as indicators of modes of effect. Although molasses treatment did not significantly alter the FA profile ($p = 0.960$ for ω-6:ω-3 FA ratio), the antioxidant capacity (as measured by ABTS) was significantly increased (3.8-fold increase, $p = 0.008$). Metabolite profiling and statistical modelling indicated that this change was likely associated with increases in several lipophilic antioxidant metabolites, including hydroxycinnamic acid amides and cannabisins. Comparison of root, leaf, seed, and sugar leaf tissue changes in phytohormones and metabolites indicated that the growth of hemp with molasses was predominantly associated with the induction of the plant's endogenous stress response within the roots. These

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Abbreviations: ABA, abscisic acid; ABTS, 2,2-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt; AMF, arbuscular mycorrhizal fungi; BHT, butylated hydroxytoluene; CA, cinnamic acid; CAA, cellular antioxidant activity; CBDA, cannabidiolic acid; CBG, cannabigerolic acid; CVD, cardiovascular diseases; DCF, 2′,7′-dichlorofluorescein; DCFDA, 2′,7′-dichlorodihydrofluorescein diacetate; DPPH, 2,2-diphenyl-1-picrylhydrazyl; EC, electrical conductivity; FA, fatty acids; FAME, fatty acid methyl ester; FBS, fetal bovine serum; F-C, Folin-Ciocalteu; FID, flame ionisation detector; GA3, gibberellin A3; GA4, gibberellin A4; GAE, gallic acid equivalent; GC, gas chromatography; HCAA, hydroxycinnamic acid amide; HEPES, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid buffer; IAA, indole-3-acetic acid; IBA, indole-3-butyric acid; ICA, indole-3-carboxylic acid; ID, inner diameter; JA, jasmonic acid; JA-Ile, jasmonic acid-isoleucine; LCQTOF MS, liquid chromatography quantitative time-of-flight mass spectrometry; L:D, light:dark; Methyl-IAA, methyl-indole-3-acetic acid; Methyl-JA, methyl-jasmonic acid; NEAA, non-essential amino acids; OPDA, 12-oxo-phytodienoic acid; PBS, phosphate buffered saline; PCA, principle component analysis; PGPB, plant growth promoting bacteria; P metabolite, polar metabolite; PNP metabolites, polar and non-polar metabolites; PPF, photosynthetic photon flux; PUFA, polyunsaturated fatty acids; RH, relative humidity; ROS, reactive oxygen species; RPMI-1640, Roswell Park Memorial Institute-1640; SA, salicylic acid; TE, Trolox equivalent; TEAC, Trolox equivalent antioxidant capacity; THC, tetrahydrocannabinol; TPC, total phenolic content.

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results indicate that the biostimulant molasses is a beneficial fertiliser additive for enhancing the functional food (antioxidant) potential of hemp seeds, thereby improving the health-benefits imparted to consumers.

1. Introduction

Cannabis sativa (cannabis) is a historic crop, which has been utilised by various cultures as a water-efficient crop for the production of fibre, herds, flowers, and seeds (Bonini et al., 2018; Wise et al., 2023a). In recent years, global shifts in legal and cultural perspectives towards cannabis have revitalised the plant as a significant source of modern food and medicine. The species *Cannabis sativa* is broadly split into two varieties: those that are high in the psychoactive compound tetrahydrocannabinol (THC) and are utilised as a recreational drug and medicine; and those that are low in THC (hemp) which may be utilised as a fibre or food source for humans and livestock (Small, 2015). The seeds and seed oils of hemp plants have become popular foods due to their flavour and nutritional qualities, however, they also have added health benefits including antioxidant and anti-inflammatory activity, and as such are considered a 'functional food' (Rupasinghe et al., 2020; Cerino et al., 2021). Clinical trials comparing diets rich in antioxidants, indicate an association with reduced oxidative stress in individuals with hypertension, diabetes, obesity, or dyslipidaemia, wherein oxidative stress is considered a factor in the associated metabolic disease (Ávila-Escalante et al., 2020). Similarly, diets with increased anti-inflammatory foods have been associated with reduced systemic inflammation which is also a contributor to chronic health conditions (Mukherjee et al., 2023). Accordingly, the consumption of antioxidant rich foods such as hemp seeds are associated with improvements to health and metabolism which may reduce the risk of chronic diseases.

Various components of hemp seed are associated with functional properties, such as the phenolics, tocopherols, lignanamides, and peptides, which impart both antioxidant (Girgih et al., 2014; Yan et al., 2015; Andre et al., 2016) and anti-inflammatory activities (Zhou et al., 2018; Martinez et al., 2020; Rodriguez-Martin et al., 2020). Additionally, the anti-inflammatory effects are also imparted through the hemp seed fatty acids (FA), which are present as a beneficial 3:1 ratio of ω-6:ω-3 polyunsaturated fatty acids (PUFA) (Leizer et al., 2000; Kaushal et al., 2020). It is recommended to reduce the dietary intake of ω-6 FAs, which are associated with increased inflammation and chronic disease, while increasing the intake of ω-3 FAs, which are associated with anti-inflammatory and anti-cancer effects (De Lorgeril and Salen, 2012). Furthermore, as dietary sources of ω-3 PUFA are limited to fish (wild caught) and their oils, and select plant seeds and their oils including chia, flax, and hemp (Saini and Keum, 2018), the low ratio of ω-6:ω-3 in hemp seed is considered relatively uncommon. Therefore, hemp seeds are a valuable source of plant-based ω-3 PUFA, which also impart antioxidant and anti-inflammatory benefits when consumed. Consumption of hemp seeds is thus promoted as a strategy for improving health and reducing the risks of various chronic conditions. From a public health perspective, increasing these functional benefits of hemp seeds and other commonly consumed functional foods (fruits and vegetables), is a strategy to improving population health without significant intervention.

Cultivation conditions and practices are known to impact plant growth, yield, and quality (Krüger and Josuttis, 2012). For example, crop growing season (and the associated environmental difference) was found to be more impactful to hemp seed antioxidant and nutritional content than genotype (Menga et al., 2022). Biostimulants are cultivation additives that impart benefits to plant growth, greater than can be attributed to their nutritional value alone (du Jardin, 2015). Biostimulants may be derived from diverse sources, including plant-derivatives that have been shown to impact various measures including yield, biochemical composition, and stress tolerance, through unknown modes of action (Yakhin et al., 2017; Wise and Selby-Pham, 2023; Wise et al., 2023c). The plant-derived biostimulant molasses is a dark brown, viscous liquid that is a by-product of sugar refinement from sugar cane and beetroot (Palmonari et al., 2020). Application of molasses (both beetroot- and sugar cane-derived) to plants has been associated with a range of benefits, including: increased yield and sugar content of beetroot under saline conditions (Nadeeka and Seran, 2020; Aljabri et al., 2021), increased yield in spinach (Pyakurel et al., 2019), increased biomass in maize (Shahzad et al., 2018), and increased salinity tolerance of thyme (Koźmińska et al., 2021), as well as benefits to soil ecosystems and productivity (Welbaum et al., 2004). Furthermore, the combined application of molasses with plant growth-promoting bacteria (PGPB) has been shown to benefit root and shoot growth in sorghum (Suliasih and Widawati, 2016), and when combined with other plant-derived biostimulants, molasses has been shown to enhance the yield and functional food value of strawberry (Wise et al., 2024b) and tomato (Wise and Selby-Pham, 2024).

Another waste product in sugar refinement is the molasses distillery effluent, which is diluted molasses from the cleaning of distillery equipment. Application of molasses distillery effluent to plants has been associated with increases in sugar cane yield (Srivastava et al., 2012), banana yield (Thakare et al., 2013), sweet pepper yield (Gaafar et al., 2019), nutrient uptake for radish (Hatano et al., 2016), improved growth and development of rapeseed (Li et al., 2020), and increased heavy metal uptake by common reed and sedge, which is a potential bioremediation strategy (Nagy et al., 2020). Furthermore, the application of molasses distillery effluent has been shown to impact the antioxidant activity of cabbage (Bimova and Pokluda, 2009) and black bean (Elayaraj, 2014), indicating the potential for this biostimulant to impact functional food potential. However, the effects of molasses on cannabis seeds are as yet unknown, and the mode of action of molasses (and biostimulants in general) is poorly understood. Understanding how biostimulants impart benefits is a key step in optimising their usage and benefits. Accordingly, this study aimed to identify the effects of molasses on hemp seed functional food value, and to characterise molecular changes within the plant to better understand the mechanism by which molasses imparts these benefits.

2. Materials and methods

2.1. Materials

The biostimulant utilised was 7% v/v (10% w/v) aqueous molasses solution, produced from cane molasses generously provided by Nutrifield Pty Ltd (Melbourne, VIC, Australia), which had 10% ash content, 40% sucrose, and phytohormone contents as presented in Table S1. The plant-nutritient (elemental) composition of molasses was as follows, 0.65% w/v N, 0.06% w/v P, 0.65% w/v K, 1.2% w/v Ca, 0.45% w/v Mg, 0.37% w/v S, 0.17% w/v Si, and 0.05% w/v Fe. Analytical grade methanol, Folin-Ciocalteu (F-C) reagent, sodium carbonate, gallic acid, 2,2-diphenyl-1-picrylhydrazyl (DPPH), 2,2-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS), potassium persulfate and Trolox were sourced from Sigma-Aldrich (St. Louis, MO, USA). Roswell Park Memorial Institute-1640 (RPMI-1640) medium (with 300 mg/L L-glutamine), fetal bovine serum (FBS), penicillin/streptomycin, phosphate buffered saline (PBS) without calcium and magnesium, TrypLE Select, pyruvate and 4-(2-hydroxyethyl)−1-piperazineethanesulfonic acid buffer (HEPES) was purchased from Gibco (Life Technologies, ThermoFisher Scientific). Phenol-red free RPMI-1640 medium (with 300 mg/L L-glutamine), non-essential amino acids (NEAA), and trypan blue dye were purchased from Sigma Aldrich Corporation. Cell culture flasks, flat bottom 96-well plates, 96-well black/

clear bottom plates and all other cell culture and assay consumables and chemicals were purchased from Costar-Corning via Sigma Aldrich, unless otherwise indicated.

2.2. Propagation

All plants utilised in experiments and for root and leaf sample analyses were produced from a hermaphrodite genotype of *Cannabis sativa* (cv. Fairnsfield) cuttings, propagated as per Wise et al. (2020a) with the following adaptations: leaves were cut to 75% their size, and cuttings were treated (by dipping the bottom 5 mm of stem into the gel) with 3 g/L indole-3-butyric acid (IBA) propagation gel (Clonex Purple, Growth Technology, O'Connor, WA) and maintained for 2 weeks (until mature) in aeroponic propagators under full spectrum 40 W horticultural LED bars supplying a photosynthetic photon flux (PPF) of 100–120 μmol/s.

2.3. Biostimulant effect on roots and leaves of young plants

Eight mature cuttings ($n = 4$ per treatment) were provided with 7 days of treatment (control nutrients or 2 mL/L molasses solution added to the nutrients) within aeroponic propagators, and then sampled (roots and leaves) on liquid nitrogen as per the method described in Wise et al. (2024a).

2.4. Biostimulant effect on seeds and sugar leaves of mature plants

2.4.1. Growth conditions

Cannabis plants were grown hydroponically to maturity with supplementation of the biostimulant to produce seeds and sugar leaves for analysis. Thirty-two mature cuttings were planted into 15 cm pots in Coco perlite substrate and provided with Coco A&B nutrients (Nutrifield Pty Ltd., Melbourne, VIC) prepared to an electrical conductivity (EC) of 0.8 and a pH of 5.8. The nutrient profile of the concentrated liquid nutrients was as follows, for part-A: 1.0% w/v N, 1.7% w/v P, 1.7% w/v K, 0.8% w/v S, 1.0% w/v Mg, 0.009% w/v B, 0.003% w/v Cu, 0.01% w/v Mn, 0.001% w/v Mo, 0.008% w/v Zn, and 0.015% w/v Si, and for part-B: 3.0% w/v N, 0.4% w/v K, 4.5% w/v Ca, and 0.04% w/v Fe.

After 1 week, 24 plants of consistent height were transferred into 27 L plastic pots with a coir perlite substrate and grown in a controlled environment for 11 weeks as per Wise et al. (2024c). During weeks 4–11 inclusive (photoperiod-induced flowering phase), 12 of the plants were randomly selected to receive 2 mL/L of the molasses solution, mixed into the nutrient solution.

2.4.1. Seed and sugar leaf collection

At the end of the 11 weeks of growth, sugar leaves were sampled from each plant using a scalpel, immediately frozen in liquid nitrogen, and then transported on dry ice to a −80 °C freezer for storage prior to analyses. The plants were then cut at the base, covered in fruit fly netting (1.5 mm aperture), and hung to dry in the grow room in the dark for 14 days, with environmental conditions at 50% relative humidity (RH) and 18 °C. Once the plants were dry, the seeds were collected in the fly netting by manually agitating the plants. The seeds were manually separated from the dry leaves and chaff before analyses. The seed yields from each plant were weighed ($n = 12$), and subsets of between 10 and 12 seeds per plant were weighed to calculate the average individual seed weight ($n = 4$).

2.5. Analysis of antioxidant measures

2.5.1. Extraction

Whole seeds were finely ground in a mortar and pestle, and then 0.1 g of powder was combined with 1 mL of methanol. The mixture was extracted in an ultrasonic bath (FXP 14, Unisonics, Australia) at room temperature for 60 min before centrifugation at 2223 x g, 4 °C, for 10 min (Allegra X-12R, Beckman Coulter, USA). The supernatant (extract) was analysed for total phenolic content (TPC), antioxidant capacity using the ABTS, DPPH, and cell-based assays, and polar metabolite (P metabolite) analyses.

2.5.2. Total phenolic content (TPC)

The total phenolic content of hemp seed extracts was determined ($n = 12$) using the F-C method, following the protocols by Ainsworth and Gillespie (2007) and Singleton et al. (1965), with some adaptations. Specifically, 20 μL sample extract was added to 180 μL Milli-Q water, followed by 1 mL of 0.2 N F-C reagent, and 800 μL of 7.5% (w/v) sodium carbonate solution and then incubated at 37 °C for 60 min. The absorbance was measured at 765 nm using the Multiskan GO Microplate Reader (Thermo Fisher Scientific, Australia). Gallic acid (50–500 μg/mL) in methanol was used as the standard reference and the results were expressed as mg gallic acid equivalent (GAE) per 100 g seeds.

2.5.3. DPPH assay

The free radical scavenging activity of hemp seed extracts was assessed ($n = 6$) using the DPPH radical decolourisation assay described by Thaipong et al. (2006), with modification. In this study, DPPH stock solution was prepared by dissolving 24 mg DPPH in 100 mL of methanol. The working DPPH solution was then prepared by mixing 10 mL of the DPPH stock solution with 13 mL methanol to obtain an absorbance of 1.10 ± 0.02 units at 515 nm. For the assay, 100 μL sample extract was added to 900 μL working DPPH solution. The solution was mixed well and left for 2 h in the dark, and then absorbance was measured at 515 nm using the Multiskan GO Microplate Reader. Trolox solution (50–500 μM) in methanol was used as the standard reference and the results were expressed as mg Trolox equivalents (TE) per 100 g seeds.

2.5.4. ABTS assay

The free radical scavenging activity of hemp seed extracts was assessed ($n = 5$) using the ABTS radical cation decolourisation assay described by Thaipong et al. (2006), with modification. In this study, the ABTS solution was prepared by mixing 7.4 mM ABTS$^{\bullet+}$stock solution with 2.6 mM potassium persulfate solution in equal volumes and leaving the solution at room temperature in the dark for 12 h. After that, to prepare the ABTS solution, 1 mL of the resulting solution was mixed with 14 mL methanol to obtain an absorbance of 1.10 ± 0.02 units at 734 nm. For the assay, 100 μL sample extract was added to 900 μL ABTS solution. The solution was mixed well and left for 2 h in the dark, and then absorbance at 734 nm was measured using the Multiskan GO Microplate Reader. Trolox solution (50–500 μM) in methanol was used as the standard reference and the results were expressed as mg TE per 100 g seeds.

2.5.5. Cell-based assays

2.5.5.1. Cell culture. The human hepatocellular carcinoma Hep-G2 cell line was kindly provided by the Microbiology and Immunology Laboratory at RMIT University, Melbourne, Australia. This adherent cell line was maintained in T-75 culture flasks within a humidified incubator at standard growth conditions (37 °C and 5% CO_2/95% air). The complete culture medium consisted of RPMI-1640 media (with L-glutamine), 10% FBS (v/v), 1% pyruvate (v/v), 100 IU/mL penicillin and 100 μg/mL streptomycin. To maintain log phase growth, cells were passaged at 80–90% confluence with the media being changed every 3 days. Subculturing consisted of washing the attached cells with PBS before adding 5 mL TrypLE Select and incubating for 5 min to aid cell detachment from culture flasks. Once all cells were detached, the cells were then washed in PBS and centrifuged at 200 x *g* before discarding the supernatant. The remaining cells were then resuspended in fresh media and split 1:5 into new T-75 culture flasks.

2.5.5.2. In vitro cell viability assays. The CellTiter 96 Aqueous Non-

Radioactive Cell Proliferation Assay (Promega) (also known as the 'MTS' Assay) was used to screen cell viability. This assay measures the metabolic activity of viable cells by assessing the ability of mitochondrial dehydrogenases to reduce the yellow tetrazolium salt (3-(4,5-dimethylthiazol-2-yl)−5-(3-carboxymethoxyphenyl)−2-(4-sulfophenyl)−2H-tetrazolium, 'MTS') to a blue formazan product that can be measured via spectrophotometry at 490–500 nm (Adan et al., 2016). Briefly, cells were seeded into Cellstar® 96-well polystyrene, flat-bottomed microplates (96-well plate) (Greiner Bio-One, Cat. No. 655 180; Greiner) at a density of 10,000 cells/mL and left to attach for 16 h. After this period, the cells were washed with PBS before adding 100 μL of fresh media. The cells were then treated with 100 μL of media containing each plant extract (as per Section 2.5.1) and left to incubate under standard growth conditions for 22 h. After this incubation period, 40 μL of MTS reagent was added to each well and the extent of formazan formation after 2 h of incubation was compared to that of an untreated control group by measuring the absorbance at a wavelength of 490 nm using a plate reader (FlexStation 3 Microplate Reader, Waltham, CA, USA). Cell-free wells containing each extract were also monitored to control for any intrinsic reducing effects that each plant extract may have on the MTS reagent, and these values were subtracted from the experimental readings. The experiment was repeated three times in triplicate and the exposed cell viability results were expressed as a percentage of the untreated control cell viability. The standard biologically significant threshold of a 20% reduction in cell viability compared to untreated cells was utilised for the assessment of *in vitro* cytotoxicity (O'Keefe et al., 2016).

2.5.5.3. Monitoring intracellular reactive oxygen species (ROS) generation. To measure the extent of ROS generation in exposed cells, a spectrofluorometric assay utilising a fluorescein probe was used. This assay is performed in cells loaded with non-fluorescent 2′,7′-dichlorodihydrofluorescein (H_2DCF) via pre-incubation with 2′,7′-dichlorodihydrofluorescein diacetate (DCFDA) (Sigma, St Louis, MO, USA). Generation of intracellular ROS (predominantly peroxides) is detected via the oxidation of H_2DCF to 2′,7′-dichlorofluorescein (DCF), which is highly fluorescent with peak excitation and emission wavelengths of 498 nm and 522 nm, respectively (O'Keefe et al., 2016).

Cells were first seeded into opaque clear bottom 96-well plates at a density of 10,000 cells per well and left to incubate for 16 h to aid attachment. To prevent the premature hydrolysis of the DCFDA probe, cells were first washed once with PBS to remove endogenous esterases that may be present within the complete culture medium. Next, under low light conditions, the cells were loaded with the DCFDA probe at 100 μM and incubated under normal growth conditions in the dark for 30 min. After which, the cells were washed with PBS and then 100 μL of fresh RPMI-1640 (phenol-red free complete culture media) was added. Each well was then treated with 100 μL of media containing the plant extract and the plate was placed back into the incubator to begin the 24 h exposure period. After 24 h, peroxide generation was determined by reading fluorescence on a plate reader (FlexStation 3 Microplate Reader) at excitation and emission wavelengths of 485 and 530 nm, respectively. Cell-free wells containing each extract were also monitored to control for any autofluorescence, which was subtracted from the final reading. The experiment was repeated three times in triplicate and the fluorescence readings obtained from the treated cells were expressed as a ratio to the untreated control cells.

2.5.5.4. Antioxidant activity of extracts. To assess the antioxidant activity of each extract, the DCF assay was again utilised. In this case, the same procedure was performed as outlined in Sections 2.5.5.3, except the cells were incubated for 30 min with each extract before treatment with the pro-oxidant hydrogen peroxide (H_2O_2) at 125 μM. Each plate was then returned to the incubator and left under standard growth conditions for 24 h. After which, the antioxidant activity of each extract was determined by comparing the fluorescence of treated groups to that which was seen in cells treated with H_2O_2 alone. The experiment was repeated three times in triplicate and the fluorescence readings obtained from the treated cells were expressed as a ratio to the control cells.

2.6. Phytohormone, bioassay, and metabolite analyses

Phytohormone profiling (12-oxo-phytodienoic acid (OPDA), abscisic acid (ABA), brassinolide, cinnamic acid (CA), gibberellin A3 (GA3), gibberellin A4 (GA4), indole-3-acetic acid (IAA), IBA, indole-3-carboxylic acid (ICA), jasmonic acid (JA), jasmonic acid-isoleucine (JA-Ile), methyl-indole-3-acetic acid (Methyl-IAA), methyl-jasmonic acid (Methyl-JA), salicylic acid (SA), and zeatin), protein content, hydrogen peroxide, peroxidase, chitinase activity, and metabolite profiling (using an extract solvent ratio of 1:2:2 of water:methanol: chloroform to extract "polar and non-polar metabolites", PNP metabolites) were performed for seed, sugar leaf, root, and leaf samples ($n = 4$ for all assays and samples), as per the method described in Wise et al. (2024a). Polar metabolite (P metabolite) profiling was performed on seed extracts (using 100% methanol as per Section 2.5.1, $n = 6$) by liquid chromatography quantitative time-of-flight mass spectrometry (LCQTOF MS) in positive mode as per Leonard et al. (2021b).

2.7. Seed fatty acid (FA) extraction, and fatty acid methyl ester (FAME) profiling

The lipid extraction ($n = 4$) and GC analysis were conducted according to the method described in Gupta et al. (2015) with modifications. Seed powder (50 mg, dried) was subjected to solvent extraction using 600 μL of chloroform and methanol (2:1 ratio, v/v). The solvent mixture was vortexed for 2 min, followed by centrifugation at 10,000 x *g* for 10 min to obtain the supernatant (lipid extract). This extraction was repeated three times, and the extracts were combined and filtered through 0.22 μm filter. The lipid extract was dried, and the lipid yield was determined gravimetrically.

Further, extracted lipids were transesterified by acid-catalysed transesterification to obtain fatty acid methyl esters (FAMEs). One millilitre of toluene was added to the extracted lipids, followed by the addition of 10 μL of C19:0 as an internal standard (50 mg of C19:0 in 10 mL of toluene) and 200 μL butylated hydroxytoluene (BHT) as an antioxidant (100 mg of BHT in 100 mL of toluene). Next, 500 μL of acidic methanol was added to the solution, which was then incubated overnight at 50 °C. After incubation, 1 mL of 5% (w/v) sodium chloride was added, and the fatty acid methyl esters were extracted into hexane (1 mL of hexane, 2 times). The hexane layer was collected and washed with a 1 mL potassium bicarbonate stock solution (2% w/v in Milli-Q water). The final hexane layer was collected and dried over sodium sulfate (anhydrous). Hexane was evaporated using nitrogen gas to concentrate the FAMEs if needed.

FAME analysis was performed on a Shimadzu Gas chromatograph (GC, 2090N, Shimadzu, Kyoto, Japan) equipped with a flame ionisation detector (FID) and connected to a BID 2030 unit using a FAMEWAX column (30 *m* × 0.32 mm ID (inner diameter)). The inlet was held at 25 °C with a constant column flow rate of 5 mL/min with split injection (1/150). The oven program was held at 150 °C (5 min hold), ramped to 250 °C at a rate of 10 °C per min. and held at 250 °C for 1 min. Fatty acid esters were identified by comparison of peak areas of FAMEs standards (CRM47885, Sigma Aldrich, Castle Hill, NSW).

2.8. Trolox equivalent antioxidant capacity (TEAC) modelling

A model to predict TEAC (as measured by ABTS assay) was developed using a data set (Table S15) of 118 compounds, with average values sourced from Cai et al. (2006), Miller and Rice-Evans (1997), Plumb et al. (1997), Raudonis et al. (2012), Rice-Evans (1995), Rice-Evans et al. (1995), and Thuong et al. (2010) using the method presented in Wise et al. (2020b) with the following changes: the physicochemical terms utilised were sourced from ChemMine Tools (https://chemminetools.ucr.edu/, accessed March 2022), which included the JoeLib descriptors Number_of_HBD_1 (nHBD1), and Number_of_aliphatic_OH_groups (nAliOH), the ChemmineR Properties RCOR, and the Smarts Search result for [$([CX3] = [CX3])] (vinylic carbon, VC). Then beginning with all combinations of terms up to 3rd order, an automated step-wise term selection was utilised with the alpha to enter and remove set to 0.15. Model validation was performed using k-fold cross validation with k-folds = 10. The results for TEAC prediction were limited to the positive domain, to match the domain of the training data.

2.9. Data analyses

Treatment effects for measures with variation in both control and treatment were assessed by students *t*-test, while measures with variation in only one treatment were assessed by 1-sample *t*-test with the value of the non-varied group utilised for the associated single-tailed hypothesis test, performed in Minitab 19 statistical software package (Minitab Inc., State College, PA). Profile analyses (principal component analysis; PCA) were performed using the web-tool Metaboanalyst 5.0 (Chong et al., 2019), which required the following data transformations to achieve normalisation prior to analyses: for root phytohormone profiles, root metabolite profiles, and leaf metabolite profiles–cube root transformation and Pareto scaling, for sugar leaf metabolite

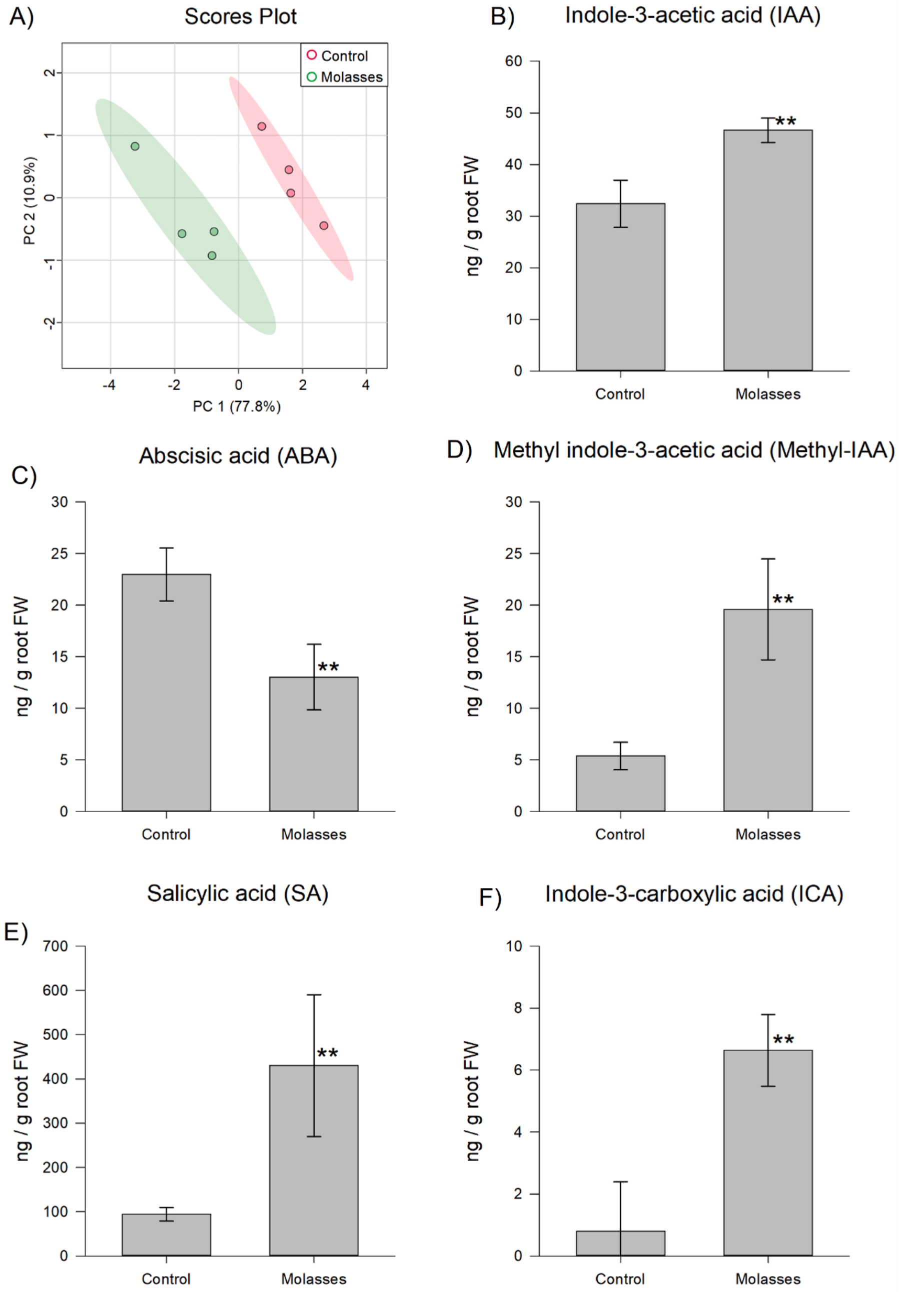

Fig. 1. Changes to root biochemistry from growth with molasses. A) Principal component analysis (PCA) of root metabolite profiles from 4 biological replicates for control and molasses. Root concentrations of B) indole-3-acetic acid (IAA), C) abscisic acid (ABA), D) methyl indole-3-acetic acid (Methyl-IAA), E) salicylic acid (SA), and F) indole-3-carboxylic acid (ICA), following 1 week of control or molasses treatments. B–F) Data presented as mean ± standard deviation for 4 biological replicates. Significant differences are indicated as ** for $p < 0.01$ calculated by Student's *t*-test.

profiles–square root transformation and auto-scaling, for seed PNP metabolite profiles, and fatty acid profiles–cube root transformation and auto-scaling, and for seed P metabolite profiles–log transformation and Pareto scaling.

3. Results

3.1. Yield and nutritional value

Yield and nutritional measures were not significantly different between treatments (Table S2) for seed yield ($p = 0.669$), seed size ($p = 0.256$), oil content ($p = 0.529$), or protein content ($p = 0.783$).

3.2. Molecular changes

Phytohormone levels within the leaf (Table S3), sugar leaf (Table S4), and seed (Table S5) were not significantly different between treatment and control. However within roots, the molasses treatment was associated with a significant change to the phytohormone profile (Fig. 1, Table S6), and individually significant increases to IAA (1.4-fold increase, $p = 0.002$), Methyl-IAA (3.7-fold increase, $p = 0.002$), ICA (8.3-fold increase, $p = 0.001$), and SA (4.5-fold increase, $p = 0.006$), and a significant decrease to ABA (0.6-fold decrease, $p = 0.003$).

Hydrogen peroxide levels were not significantly different between treatment and control for the seed ($p = 0.474$), leaf ($p = 0.420$), or sugar leaf ($p = 0.985$), and was not detected in the root (Table S7). Peroxidase activity was not significantly different between the treatment and control for the root ($p = 0.488$), or sugar leaf ($p = 0.356$), and was not detected in the leaves, however, there was a marginally significant ($p = 0.058$) 4.3-fold increase in the seeds (Table S8). Chitinase activity (Table S9) was not significantly different between treatments for all tissues: roots ($p = 0.887$), leaf ($p = 0.570$), sugar leaf ($p = 0.486$), or seed ($p = 0.409$).

Profile analyses of the metabolites within the root, leaf, and sugar leaf (Fig. S1) revealed that the treatment did not significantly change any of the tissue sample metabolite profiles, as indicated by the overlapping 95% confidence regions within the PCAs. Comparison of individual root metabolites identified 5 peaks with significant or marginally significant changes, of which 3 metabolites were identified (Table S10): methyl (17beta)−3,6-dimethoxyestra-1,3,5(10),6,8-pentaene-17-carboxylate (0.68-fold decrease, $p = 0.068$), 1-(3-acetyl-2,4,6-trihydroxyphenyl)−1,5-anhydrohexitol (8.15-fold increase, $p = 0.077$), and genistein (3.14-fold increase, $p = 0.094$). No individual metabolite was significantly changed from the treatment within the leaf samples. Within the sugar leaves, 59 metabolite peaks were significantly changed (or had marginally significant changes), which included 19 that were identified (Table S11). Of particular note were the compounds: 2-[4-(hydroxymethyl)cyclohexyl]−2-propanyl hexopyranoside (0.67-fold decrease, $p = 0.042$), 3-[3-(beta-D-glucopyranosyloxy)−2-methoxyphenyl]propanoic acid (0.54-fold decrease, $p = 0.050$), caffeic acid 3-glucoside (0.75-fold decrease, $p = 0.051$), cannabigerolic acid (0.67-fold decrease, $p = 0.033$), L-glutamic acid (0.51-fold decrease, $p = 0.090$), N-feruloylglycine (0.64-fold decrease, $p = 0.026$), pyrogallol-2-O-glucuronide (0.68-fold decrease, $p = 0.045$), salicylic acid (0.63-fold decrease, $p = 0.062$), and 1-sinapoyl-D-glucose (0.77-fold decrease, $p = 0.028$).

Profile analyses of the seed PNP metabolites indicated that the biostimulant treatment did not significantly alter the metabolite profile of seeds, as represented by the overlapping regions of 95% confidence within the PCA (Fig. S2). A comparison of individual metabolites identified 29 peaks which were significantly changed (or had marginally significant changes) between treatments, of which 15 were identified plant metabolites (Table 1).

Table 1
Seed PNP metabolite profile comparison from biostimulant treatment.

Metabolite	Fold-change[a]	p-value
3b-Hydroxy-6b-angeloyloxy-7(11)-eremophilen-12,8b-olide	1.38	0.094
Azelaic acid	1.94	0.078
Cannabidiolic acid (CBDA)	1.35	0.096
Citpressine II	0.73	0.033
Dehydrochorismic acid	3.67	0.072
Dihydroferulic acid 4-glucuronide	1.60	0.080
Haplodimerine	0.71	0.061
Herbarumin II	1.49	0.083
Tricoumaroyl spermidine	1.45	0.097
Uridine	1.42	0.021
Veranisatin C	1.77	0.068
Wighteone	1.37	0.032

[a] Compared to untreated control.

Profile analyses of seed P metabolites exhibited separation of 95% confidence regions within the PCA (Fig. 2), indicating that the seed metabolite profiles were significantly different. Comparison of individual metabolites within treatment groups identified six metabolites that were changed significantly or with marginal significance (Tables 2, S13).

3.3. TEAC model

Polynomial regression was utilised to generate a model from four physicochemical measures across 7 terms (Table S16), to predict TEAC with $R^2 = 0.8576$ and 10-fold $R^2 = 0.8103$ (Eq. (1)). After the domain restriction, $TEAC_{Predicted}$ achieved an $R^2 = 0.\ 8617$ (Eq. (2), Fig. 3).

$$\begin{aligned} TEAC\ model = {} & 0.244 + 0.157\ \times VC + 0.396\ \times nHBD1 - 0.703 \\ & \times nAliOH - 0.832\ \times RCOR + 0.0426\ \times nHBD1^2 \\ & + 0.243\ \times RCOR^2 - 0.00161 \\ & \times nHBD1^3\ \left(R^2 = 0.8576,\ 10 - fold\ R^2 = 0.8103\right) \end{aligned} \tag{1}$$

$$TEAC_{predicted} = \begin{cases} TEAC\,model,\ for\,TEAC\,model\ >\ 0 \\ 0,\ \ for\,TEAC\,model \leq 0 \end{cases} \quad \left(R^2 = 0.8617\right) \tag{2}$$

3.4. Functional food value

Profile analyses indicated that seed FA profiles were not significantly different between treatments, due to their overlapping regions of 95% confidence within the PCA (Fig. S3). Categorisation of FAs based on their saturation, or the terminal carbon-carbon double bond position (ω) indicated that there were no significant changes to the saturated ($p = 0.263$), unsaturated ($p = 0.162$), or ω-3 ($P = 0.542$) FA contents (Table S19). A marginally significant change was observed to the total ω-6 contents, reducing from 43% in control to 41% in plants grown with molasses (0.95-fold change, $p = 0.057$), however, no corresponding change was observed to the ratio of ω-6:ω-3 FA ($p = 0.960$). Comparison of individual FAs revealed that out of 35 FAs included in the FAME standards, 15 were not detected in any seed samples, 18 were detected in seeds from both treatments, and two (C15:0 Methyl pentadecanoate, $p = 0.196$ and C23:0 Methyl tricosanoate, $p = 0.091$) were detected in seeds from molasses-treated plants only (i.e. not in untreated control seeds). Of the 20 FAs detected (Table S19), C16:1 n7 Methyl palmitoleate was the only FA that was significantly different between treatments, accounting for 0.32% of FAs in the control group, and 0.24% of the FAs in the molasses-treated group (0.75-fold change, $p = 0.031$).

Several assays were utilised to measure the presence of functional compounds or antioxidant capacity (Table S21). The F-C assay found no significant difference in TPC ($p = 0.531$) following molasses treatment. Similarly, no significant difference was found for antioxidant capacity as measured by the DPPH assay ($p = 0.334$), or antioxidant activity as measured by the cell-based assay ($p = 0.367$). Furthermore, the cell-based prooxidant assay was also not significantly different between

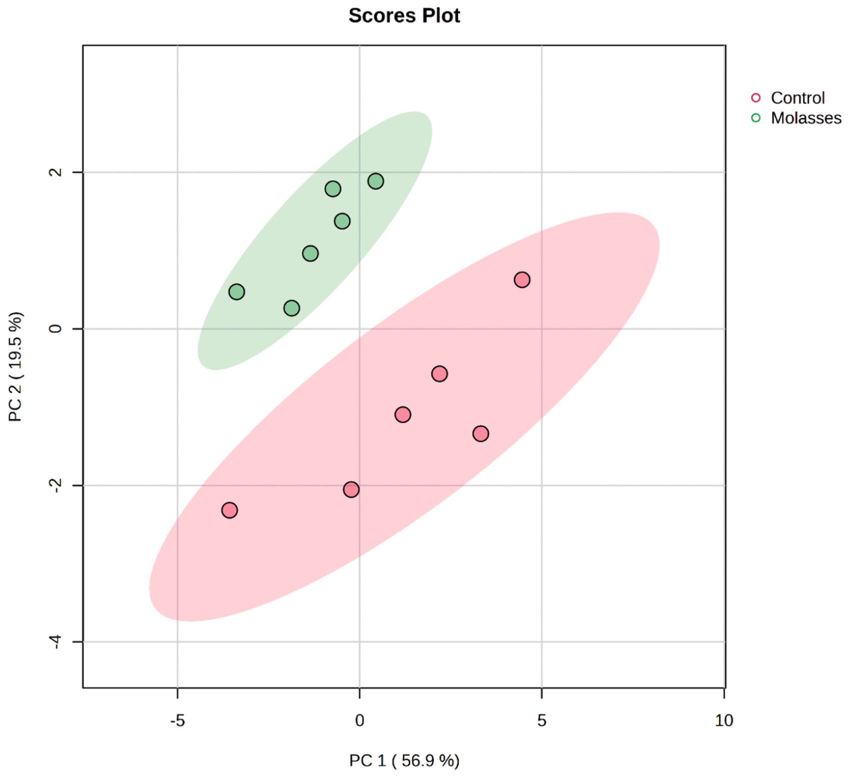

Fig. 2. Changes to seed metabolite profile from growth with molasses. Principal component analysis (PCA) of seed polar metabolite (P metabolite) profiles from 6 biological replicates for control and molasses-treated plants.

Table 2

Seed P metabolite profile comparison from biostimulant treatment.

Metabolite	Fold-change[a]	p-value
Cannabisin B	7.42	<0.001
Cannabisin C	1.57	0.028
Cannabisin H	6.67	0.001
N-feruloyltyramine	1.49	0.067
N-transcaffeoyltyramine	8.31	<0.001
N-trans-coumaroyltyramine	16.05	<0.001

[a] Compared to untreated control.

treatments ($p = 0.943$). The cell-based cytotoxicity assay showed a 5% decrease in cell viability following exposure to the seed extract from molasses-treated plants as compared to control (Table S21, $p = 0.019$), however, this was not deemed to be biologically significant, being < 20% decrease in cell viability compared to untreated control cells. Antioxidant capacity as measured by the ABTS assay found that seed from molasses-treated plants had 3.8-fold greater Trolox equivalent capacity as compared to control plants ($p = 0.008$, Fig. 4).

The TEAC model (Eqs. (1), and (2)) was utilised to predict the TE antioxidant capacity (TEAC) of metabolites across both the polar and non-polar (PNP) metabolite, and the polar (P) metabolite datasets (Table 3). Of the compounds where the fold-change was significant or marginally significant, eleven metabolites were estimated to have $\text{TEAC}_{\text{Predicted}} > 0$ (Table 3). Across the metabolites in both datasets, nine compounds were predicted to have antioxidant capacity with predicted $\text{TEAC}_{\text{Predicted}} > 1$, which were CBDA, tricoumaroyl spermidine, wighteone, cannabisin B, cannabisin C, cannabisin H, N-feruloyltyramine, N-transcaffeoyltyramine, and N-trans-coumaroyltyramine.

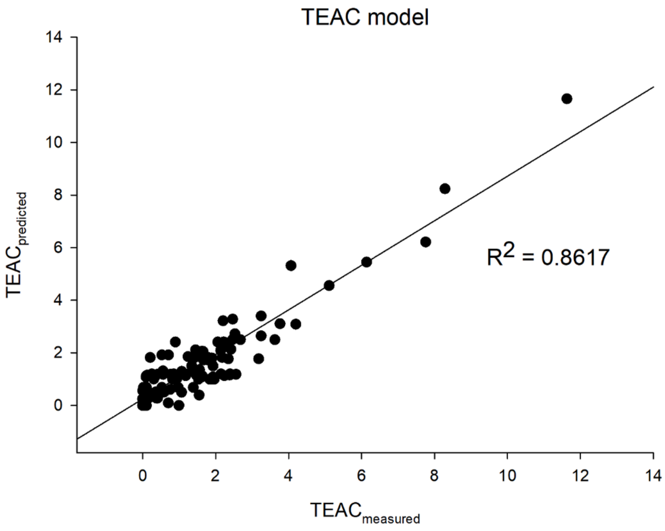

Fig. 3. Trolox equivalent antioxidant capacity (TEAC) model. Polynomial regression analysis with 10-fold cross-validation to predict TEAC from physicochemical measures ($n = 118$).

4. Discussion

4.1. *Molasses impact on hemp seed functional food potential*

Herein, the growth of cannabis with molasses did not alter the seed nutritional or yield measures (Table S2), and both treatments produced seeds with typical (Callaway, 2004) ω-6:ω-3 ratios of 2.7:1 (Table S20). The only statistically significant change was a decrease in palmitoleic acid (Table S19), which is a less common ω-7 fatty acid. Palmitoleic acid has been proposed as potentially beneficial to obesity, liver health, and

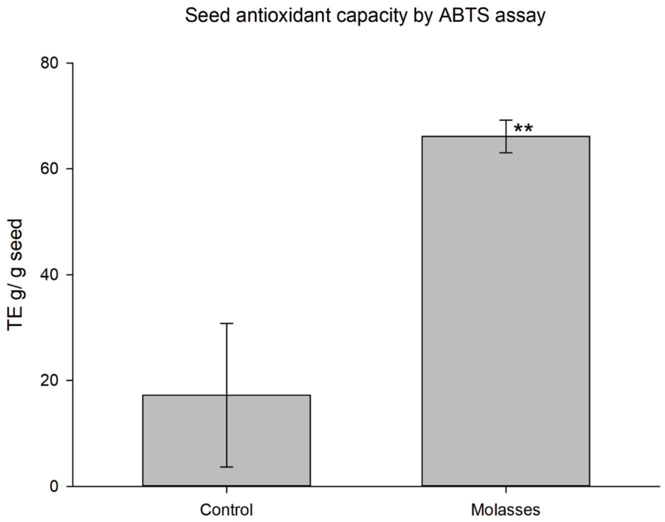

Fig. 4. Seed antioxidant capacity from growth with molasses. Antioxidant capacity measured by ABTS assay for seed samples from 5 biological replicates (plants) for control and molasses-treated plants. Data presented as mean ± standard error of the mean, with significant differences indicated by ** for $p < 0.01$ calculated by Student's *t*-test.

Table 3
Predicted antioxidant capacity of seed metabolites altered by growth with molasses.

Name	Fold-change[a]	p-value	$TEAC_{Predicted}$ (mM)
PNP metabolite dataset			
3b-Hydroxy-6b-angeloyloxy-7(11)-eremophilen-12,8b-olide	1.38	0.094	0.61
Azelaic acid	1.94	0.078	0.24
Cannabidiolic acid (CBDA)	1.35	0.096	1.82
Citpressine II	0.73	0.033	0.09
Dehydrochorismic acid	3.67	0.072	0.99
Haplodimerine	0.71	0.061	0.68
Tricoumaroyl spermidine	1.45	0.097	4.03
Uridine	1.42	0.021	0.30
Wighteone	1.37	0.032	1.50
P metabolite dataset			
Cannabisin B	7.42	<0.001	5.47
Cannabisin C	1.57	0.028	4.71
Cannabisin H	6.67	0.001	1.84
N-Feruloyltyramine	1.49	0.067	1.93
N-Transcaffeoyltyramine	8.32	<0.001	2.56
N-Trans-coumaroyltyramine	16.05	<0.001	1.93

[a] Compared to untreated control.

cardiovascular health, however more clinical studies are needed to support this association (Frigolet and Gutiérrez-Aguilar, 2017). Furthermore, palmitoleic acid contents reported for hemp seed herein were relatively low (0.32% in control vs. 0.24% in molasses-treated plants, $p = 0.031$), compared to endogenous synthesis of palmitoleic acid from the palmitic acid (11.45% in control vs. 9.54% in molasses, $p = 0.102$), which is considered the predominant source of palmitoleic acid in humans (de Souza et al., 2018). Accordingly, molasses treatment had negligible impact on the hemp seed fatty acid profile and therefore did not significantly affect this aspect of hemp seed functional food value.

The impact of molasses treatment during plant growth on hemp seed functional food antioxidant properties was explored through a range of chemical-based assays, *in vitro* cell-based assays, and *in silico* modelling. Plants produce antioxidants to regulate ROS that are produced naturally during photosynthesis, metabolism, and abiotic and biotic stress responses, and may similarly regulate ROS in humans when consumed as part of a plant-rich diet (Andre et al., 2010). Whilst endogenous antioxidant systems are present in humans, sustained presence or impaired regulation of ROS is associated with chronic health conditions such as diabetes and CVD, which may be mediated by increased dietary antioxidants (Ilich et al., 2014; Kumar et al., 2015). Hemp seeds are one of many plant-derived foods that are rich in antioxidants and so are therefore associated with having functional food capacity (Selby-Pham et al., 2017b; Rupasinghe et al., 2020). Growth of cannabis with molasses was shown to increase the seed antioxidant capacity when analysed by the ABTS assay, but not DPPH, cell-based assay, or by TPC (Table S21). The review by Munteanu and Apetrei (2021) compared differences between common antioxidant assays, and explains that ABTS and DPPH assays are both neutralising reactions of free radical cations, with reactivity to a large variety of substances. However, the ABTS assay is known to be suitable for both hydrophilic and lipophilic compounds, such as carotenoids and tocopherols (Munteanu and Apetrei, 2021), and has been shown to detect antioxidant capacity over a broader range of fruits and vegetables compared to the DPPH assay (Floegel et al., 2011). The TPC assay measures the reducing capacity of the F-C reagent, to estimate the total phenolic contents, compared to a standard phenolic compound such as gallic acid. This assay is aqueous and non-specific, with interference reported from sugars and amino acids, and may therefore overestimate phenolic contents (Munteanu and Apetrei, 2021). Unlike ABTS, DPPH, and TPC which measure reducing capacity, the cell-based antioxidant assay–adapted from the cellular antioxidant activity (CAA) assay–measures antioxidant activity through the disrupted production (by oxidation) of DCF within human HepG2 cells (López-Alarcón and Denicola, 2013). The cell-based assay results were consistent with the other *in vitro* assays, confirming the hemp seed extracts exhibited antioxidant activity, however, no significant difference was observed between the treatment extracts. Accordingly, as the same polar extract was analysed for all the antioxidant assays, the increase as measured by ABTS alongside no change for DPPH, TPC, or the cell-based assay, suggests a change to the more lipophilic fraction of this extract.

Metabolite profiling of the same extract analysed for the antioxidant assays (P metabolites), identified significant or marginally significant increases in six compounds (Table 2). To explore if these metabolites were likely associated with the measured increase in ABTS-antioxidant capacity, the TEAC model (Eqs. (1) and (2)) was applied to predict the antioxidant capacity (TEAC, as determined by ABTS) of these compounds (Table 3). The six compounds were predicted to have TEAC ranging between 1.84 mM for cannabisin H to 5.47 mM for cannabisin B (Table 3). Whilst ABTS-experimentally determined TEAC values for these individual compounds were not available, antioxidant capacity has been reported for cannabisin B, cannabisin C, N-feruloyltyramine, N-transcaffeoyltyramine, and N-trans-coumaroyltyramine by other *in vitro* methods or by ABTS in non-TEAC units (Table S18). Accordingly, the prediction of these compounds as having antioxidant capacity (TEAC, as determined by ABTS) is consistent with the experimental results from other antioxidant assays of these compounds, whilst the predicted antioxidant capacity for cannabisin H is novel and may suggest further exploration of this compound by *in vitro* study. Furthermore, the predicted ABTS antioxidant capacity of these metabolites suggests that increases in their abundances following the molasses treatment is a contributor to the increased ABTS antioxidant capacity of the hemp seed.

The lipophilicities of the six altered P metabolites (Table 2) were explored by comparing their LogP (Table S14), which indicated that all were lipophilic based on their positive LogP values (Selby-Pham et al., 2017a). More specifically, cannabisin H, N-feruloyltyramine, N-trans-caffeoyltyramine, and N-trans-coumaroyltyramine had similar lipophilicities with LogP between 2.1–2.7, while cannabisin B and cannabisin C had the highest lipophilicities with LogP values of 4.2 and 4.6, respectively (Table S14). The ABTS assay (as compared to DPPH and TPC assays) is most appropriate for the detection of antioxidant capacity from lipophilic compounds (Munteanu and Apetrei, 2021). As such, the detection of increased antioxidant capacity by ABTS but not DPPH or TPC (Table S21), may relate to the increase in seed lipophilic antioxidant metabolites (Table 3).

All the increased seed P metabolites (Table 2) are proposed to be downstream biosynthetic products of the tyrosine and phenylalanine pathways (Leonard et al., 2021a). N-feruloyltyramine, N-trans-caffeoyltyramine, and N-trans-coumaroyltyramine are hydroxycinnamic acid amides (HCCAs) (Facchini et al., 2002; Macoy et al., 2015), which are 'coupled' to form the lignanamide cannabisins (Chen et al., 2012; Leonard et al., 2021a). These compounds (HCCAs and cannabisins) have previously been sourced from defatted hemp seed (Di Palo et al., 2022), and in particular, cannabisin B and N-transcaffeoyltyramine were shown to be abundant phenolics within the hulls of hemp seeds (Pojić et al., 2014). Various potential health benefits have been associated with dietary supplementation with HCCAs and cannabisins, including antioxidant and anti-inflammatory potential (Farinon et al., 2020). Other benefits also include: antiproliferative activity against hepatoblastoma disease for cannabisin B (Chen et al., 2013), benefits to neural cells associated with Alzheimer's disease from cannabisin B and N-trans-caffeoyltyramine (Di Palo et al., 2022), reduced weight in mice fed with a high-fat diet and reduced liver-inflammation (Veeriah et al., 2022) and beneficially upregulated expression of genes relating to inflammatory bowel disease (Lee et al., 2022) for N-transcaffeoyltyramine, and reversed inflammation-impaired gut barrier function from N-trans caffeoyltyramine and N-transferuloyltyramine (Bolster et al., 2022). Accordingly, the increases to these compounds in hemp seeds grown with molasses (up to 16-fold increase, Table 2), is a strong indication of improved functional food value from growth with this biostimulant.

Profiling of PNP metabolites identified changes to various metabolites (Table 1), which indicate potential improvements to the functional food value of hemp seeds grown with the molasses biostimulant. Most notably, azelaic acid, uridine, and wighteone were increased (Table 1) and have been associated with health benefits when consumed by humans or animal models. Azelaic acid is a bioactive compound commonly sourced from wheat, barley, and rye and utilised as a pharmaceutical dermatological agent for the treatment of acne (Anastassakis, 2022). However, azelaic acid may also be beneficial when consumed, as demonstrated by the protection of mice from high-fat diets, which was attributed to the modulation of endogenous antioxidant and ROS scavenging mechanisms and improved insulin sensitivity (Muthulakshmi and Saravanan, 2013; Muthulakshmi et al., 2015; Chae et al., 2023). Similarly, uridine consumption has also been associated with benefiting high-fat diets, by promoting weight loss in mice through alterations to lipid and carbohydrate metabolism (Liu et al., 2019). Another mouse study associated dietary uridine with improved motor function and gastrointestinal function in a Parkinson's disease model (Perez-Pardo et al., 2018), while in gerbils, Holguin et al. (2008) demonstrated an association between dietary uridine and enhanced learning and brain function. These neuroprotective effects from dietary uridine are consistent with human studies showing improvements in brain atrophy and cognition in Alzheimer's disease patients from a uridine-enriched multi-nutrient (Baumel et al., 2021). Finally, wighteone demonstrated liver-protection effects in rats (Lin et al., 1996), and reduced the proliferation of human breast cancer cells *in vitro* (Lin et al., 1996). The antioxidant activity of these and the other affected metabolites (Table 1) was explored through the TEAC model presented herein. Although azelaic acid has been shown to exhibit antioxidant capacity as determined by cell-based assays (Zhang et al., 2020), the $\mathrm{TEAC_{Predicted}}$ (by ABTS) was 0.24 mM, which was relatively low compard to the capacities predicted for the other changed metabolites (Table 3). Uridine also had a similarly low $\mathrm{TEAC_{Predicted}}$ of 0.30 mM, indicating that whilst these metabolites (azelaic acid and uridine) may provide protective benefits (discussed above), they are unlikely to be significant contributors to hemp seed antioxidant capacity. By contrast, wighteone was predicted to have $\mathrm{TEAC_{Predicted}}$ of 1.50 mM, which is consistent with other non-ABTS *in vitro* studies indicating that this compound has antioxidant potential (Erasto et al., 2004). Of the other changed metabolites, the minimally studied metabolite tricoumaroyl spermidine had a particularly high $\mathrm{TEAC_{Predicted}}$ of 4.03 (Table 3), and may therefore be a compound of interest in future explorations of antioxidant capacity in hemp. Accordingly, the changes to the PNP metabolites in hemp seed from growth with molasses are indicative of benefits to the functional food value, including potential benefits to antioxidant capacity, neuroprotection, cancer, and liver health.

4.2. *Molasses biostimulant mode of action*

The utility of molasses as a biostimulant to benefit hemp seed functional food value was explored, with demonstrated increases in antioxidant activity (Fig. 4, Table 3), and enhanced functional metabolite profile (Tables 1 and 2). As biostimulant modes of action remain largely uncharacterised, a range of molecular changes were explored in cannabis roots–as indicative of changes from direct interaction with the molasses–and in the leaves, sugar leaves, and seeds–which indicate signal transduction throughout the plant. Although various changes were observed in the root phytohormone and metabolite profiles, no changes were observed for any molecular measures (phytohormone, chitinase, hydrogen peroxide, and metabolites) within the associated leaves, indicating a lack of signal transduction (Cheung et al., 2020) from the roots to the leaves following 7 days of root exposure to molasses. By contrast, the application of molasses to mature plant roots over 8 weeks during flower and seed development, resulted in molecular changes in the sugar leaf metabolites (Table S11) indicating that signal transduction did occur in these plants after a longer treatment, or that the signal transduction occurred in a tissue-specific way.

4.3. *Stimulation of beneficial microbes*

Molasses is a thick viscous liquid produced as a byproduct during cane or beet sugar refinement and used as a food source for ruminant livestock. This study utilised cane molasses containing 40% sucrose, which is similar to an average cane molasses composition of 76.8% dry matter, 62.3% total sugar, 48.8% sucrose, 8.07% fructose, 5.29% glucose, and other sugars, as well as organic acids, complex carbohydrates, minerals, and melanoidins (Chandra et al., 2008; Palmonari et al., 2020). Simple sugars such as sucrose are generally not included in fertilisers, as they are not bioavailable to plants, however, plant roots secrete a range of compounds (including sucrose) which are associated with promoting symbiotic relationships to beneficial microbes (Tian et al., 2021). Accordingly, the provision of low concentrations of sugars and other plant-derived compounds may function as artificial root exudates, and thereby stimulate beneficial microbial growth to benefit plant growth (Sun et al., 2021). This was particularly demonstrated in Tian et al. (2022), wherein fertiliser supplementation with different levels of sucrose and plant growth-promoting microbes (PGPM) was explored for their effects on rhubarb growth, and it was found that low concentrations of sucrose (0.015%) were beneficial to root growth and reduced soil pathogen presence. This concentration of sucrose (0.015%) is similar to those in the nutrient solutions applied herein (0.008%), which may indicate that stimulation of beneficial microbes could be a potential mechanism of molasses to benefit plant growth.

The growth of plants with beneficial microbes has been shown to positively impact antioxidant phytochemical contents and antioxidant capacity of pea seeds (Jain et al., 2014), chickpeas (Singh et al., 2014), spinach (Khalid et al., 2017), the *Brassica* species (Petrić et al., 2022), and antioxidant enzyme activity in soybeans (Osman et al., 2021). Furthermore, the application of plant extracts has been associated with the growth stimulation of beneficial microbial populations, along with improvements in plant growth (Luziatelli et al., 2019). Accordingly, the provision of molasses (plant-derived, and high in sucrose) to the rhizosphere may be inducing changes to the microbiome or stimulating plant-microbe interactions, which are impacting plant growth.

When exploring changes to sugar leaf metabolites, it was found that 1-sinapoyl-D-glucose decreased (Table S11), which was also demonstrated in tomato roots following colonization with beneficial arbuscular mycorrhizal fungi (AMF) (Casarrubias-Castillo et al., 2020). The sugar leaf metabolite N-feruloylglycine was also observed to decrease, which is opposite to what was observed in rice roots following growth with PGPB (Valette et al., 2020). Furthermore, exploration of seed metabolites identified an increase in CBDA (Table 2), which is similar to the results of Tanney (2023), which demonstrated increases in hemp inflorescence CBDA contents from growth with PGPB. Therefore, the observed changes to plant metabolites in response to growth with the biostimulant molasses may indicate the promotion of beneficial microbes, which may in turn have contributed to the increased antioxidant capacity observed in the produced hemp seed.

4.4. *Induction of stress response*

Plants produce antioxidants within seeds in response to stressors, to protect the seed during the subsequent germination (Govindaraj et al., 2017). Furthermore, biostimulant treatments are associated with both increased antioxidant activity and improvements in stress tolerance, as demonstrated for kelp treatment of soybean (Kocira et al., 2018), and maize, and in propolis plant extract treatment of faba bean (Desoky et al., 2021), and various other plant species and biostimulants (Drobek et al., 2019). Priming of plants with various abiotic stressors is known to be beneficial to inducing a heightened response upon subsequent stress (Ouhibi et al., 2014; Wang et al., 2014; Li et al., 2015; Ru et al., 2022). A similar effect has been demonstrated in hemp plants utilising pathogen-related compounds such as chitosan to prime against – and increase defence against – subsequent biotic infection (Suwanchaikasem et al., 2022, 2023a, 2023b). Cell wall fragments and degradation products are also associated with induced stress responses, as their exogenous detection may be indicative of damage (Wu et al., 2014). Noting the imperfect refinery process of molasses, it is common for soluble and insoluble-suspended non-sugar plant-derived compounds and degradation products to be present (Olbrich, 1963), which may function to signal damage when detected by roots within the rhizosphere. Accordingly, a hypothesised mode of action of the molasses biostimulant and its impacts to increase cannabis seed antioxidant activity is through induction of a stress response (in the absence of a true stressor).

The molasses utilised herein contained several phytohormones (Table S1), which may elicit effects when applied to plant roots. Phytohormones are complex signalling molecules involved in many aspects of plant growth and regulating responses to environmental stimuli. Endogenously, the phytohormones OPDA, JA, JA-Ile, ABA, SA, and CA (SA precursor) are often associated with plant stress and defence responses (Dave and Graham, 2012; Boatwright and Pajerowska-Mukhtar, 2013; Lv et al., 2021; Gilroy and Breen, 2022). Similarly, when applied to plants exogenously, these phytohormones – along with the lesser studied ICA – have been observed to mitigate stress or improve plant growth (Chen et al., 2006; Sun et al., 2012; Hussain et al., 2014; Gamir et al., 2018; Khan et al., 2020; Wang et al., 2020). Other phytohormones present in the molasses were auxins (IAA and methyl-IAA), wherein exogenous application of IAA is associated with increased stress tolerance, photosynthetic activity, and antioxidant defence (Kosakivska et al., 2022). Similarly, the cytokinin zeatin was present (Table S1), which has benefited drought tolerance, yield, nutrient uptake, and other metabolic activities in wheat when applied exogenously (Zaheer et al., 2019; Raza et al., 2020). However, across the studies exploring exogenous applications of phytohormones mentioned above, the concentrations of application tend to be in the μM–mM range, whereas the hormone levels in the nutrient solutions applied to plants herein were calculated to be in the low or sub nM range (Table S1). Therefore, the low levels of exogenous phytohormones provided to plants from molasses herein seem an unlikely driver of the molecular response observed.

Root molecular changes were explored as indicative of the most direct effects of the molasses treatment, which was applied to the rhizosphere. Various biochemical changes were identified within the roots, which suggest that the molasses treatment may have induced a stress response. While root auxins IAA, methyl-IAA, and ICA were found to increase (Table S6), and auxins are generally associated with root growth (Saini et al., 2013; Goto et al., 2022; Shen et al., 2022), they have also been associated with stress and pathogen responses (Pan et al., 2008; Gamir et al., 2012; Bielach et al., 2017, 2018). Additionally, SA and ABA were also changed (Table S6), which are also often associated with defence/stress pathways (Lv et al., 2021). As both stress-related and auxin phytohormones were present within the exogenously applied molasses, their detection by roots may have stimulated endogenous mechanisms resulting in these observed changes. Regarding the metabolic changes in roots, molasses treatment was associated with increased genistein, which is an isoflavonoid associated with roots and root-nodules in legumes (Zhang and Smith, 1996; Kneer et al., 1999), and is increased in lupin embryos following exogenous sugar application (60 mM sucrose, compared to 0.23 mM applied from molasses herein) and in response to fusarium (pathogen) infection (Formela et al., 2014). The other root metabolite change that is potentially indicative of stress induction, is the increase in 1-(3-acetyl-2,4,6-trihydroxyphenyl)−1, 5-anhydrohexitol. This minimally studied metabolite was also observed to increase in cannabis roots following treatment with a fish emulsion biostimulant, which was hypothesised to be part of an induced pathogen response (Wise et al., 2024a). However, the application of the fish hydrolysate was accompanied by increases in chitinase, and peroxidase (Wise et al., 2024a), which are typically associated with pathogens and wounding responses (Kasprzewska, 2003; Veitch, 2004) but were not observed herein (no change to root chitinase, or peroxidase). Therefore, across all the molecular changes within the roots, it appears that a stress (but not pathogen) response was induced by the molasses treatment, which is consistent with the provision of hormones within the molasses.

Sugar leaves are the small leaves surrounding the female flowers within the cannabis inflorescence (Spitzer-Rimon et al., 2019; Das et al., 2022). Although the sugar leaves are not a yield component (of seed or flower harvest), their proximity within the inflorescence provides insights into the metabolic changes occurring in the terminal parts of the plant and may provide insights into inflorescence-quality-related properties such as odour or inhaled pharmacokinetics (Wise et al., 2019, 2023b). Within the sugar leaves, decreases were observed in various phenolic compounds, including cannabisin B, cannabigerolic acid (CBG), n-feruloylglycine, pyrogallol-2-O-glucuronide, and caffeic acid 3-glucoside, which have antioxidant activity and are associated with biotic and abiotic responses (Kumar et al., 2020; Pratyusha, 2022). The precursor cannabinoid CBG has been observed to be unchanged from wounding, decreased from herbivory and heat stress, and increased from drought stress in early flowering hemp plants (Park et al., 2022). Therefore, the change in CBG may represent an altered stress state of the plant. N-feruloylglycine reduction may also reflect an induced stress response, as this metabolite was shown to decrease in rice roots from the presence of a bacterial pathogen (Valette et al., 2020). Caffeic acid 3-glucoside is a derivative of caffeic acid, which has been shown to be involved in various plant stress responses (Riaz et al., 2019), and the glucoside derivatives specifically may exhibit *in vitro* antifungal action (Davidson and Taylor, 2007). Accordingly, the reduction in the potentially antifungal caffeic acid 3-glucoside, indicates an altered plant stress response but does not suggest induction of a pathogenic response. Decreases were also observed in other metabolites including L-glutamic acid, 3-[3-(beta-D-Glucopyranosyloxy)−2-methoxyphenyl]propanoic acid, SA, and 2-[4-(hydroxymethyl)cyclohexyl]−2-propanyl hexopyranoside (Table S11). L-Glutamic acid is a non-essential amino acid, involved in various plant systems including protein synthesis, and functions as a signalling molecule (translocated via the phloem) as part of wound, pathogen, and abiotic stress responses (Qiu et al., 2020). The reduction in 3-[3-(beta-D-Glucopyranosyloxy)−2-methoxyphenyl] propanoic acid is opposite to the change observed in Dandlen et al. (2023), wherein this metabolite increased in citrus following viral infection. Finally, exploration of the reductions to SA and 2-[4-(hydroxymethyl)cyclohexyl]−2-propanyl hexopyranoside, indicates that this change was opposite to what was observed as part of the hypothesised pathogen response in cannabis roots from fish hydrolysate application in Wise et al. (2024a), as SA is associated with induction of the plant defence response (Innes, 2018). Therefore, the decrease in sugar leaf metabolites, which are positively associated with the plant defence response, supports the inference that growth with molasses has resulted in an altered stress state of the plants, appearing as a reduced stress status within the sugar leaves.

Seed formation and metabolism occur while the seed is on the parent plant, wherein after maturation and drying the seed transitions into a metabolically inactive state (Kermode, 2017). Accordingly, the environmental factors of the parent plant during seed formation are shown to be impactful to the seed metabolism and germination success (He et al., 2014). Plant seeds contain specialised secondary metabolites to support successful subsequent seedling establishment, which includes phenolic and flavonoid metabolites in the seed coat that are associated with protection against, and transcriptionally regulated by, environmental factors (Xu et al., 2015; Corso et al., 2020). Accordingly, variations in metabolite contents of seeds may be indicative of the environmentally induced altered metabolic state of the parent plant during seed formation. Various metabolic changes were detected in response to growth with the molasses biostimulant, including increased levels of HCCAs (e.g. N-feruloyltyramine, N-transcaffeoyltyramine, and N-trans-coumaroyltyramine, Table 2) and their downstream biosynthetic products, cannabisins (Leonard et al., 2021a). Noting that HCCA levels have been associated with strengthening plant defences in response to fungus and pathogens in barley, onion, potato, and tomatoes (Facchini et al., 2002), this increase within the hemp seeds may reflect the induction of a pathogen defence response. Other metabolic changes in seeds included increases to CBDA (Table 2), which is the precursor to cannabidiol (CBD) – one of the major therapeutic cannabinoids associated with cannabis inflorescence (Sarma et al., 2020). Whilst the biological role of cannabinoids in plants remains unknown, various studies have suggested their connection to the plant defence response (Biteznik et al., 2023), and specifically herbivory (MacWilliams et al., 2023). Although cannabinoid accumulation is largely associated with the inflorescence, the detection of increased CBDA within the seeds may further support an induced defence response. Furthermore, seed levels of azelaic acid were also observed to increase from growth with the molasses biostimulant (Table 1). A similar change to azelaic acid was reported in cannabis roots, following treatment with a fish hydrolysate biostimulant, which was hypothesised to be associated with a pathogen response (Wise et al., 2024a). It is postulated that azelaic acid is a stress indicator for lipid peroxidation in *Arabidopsis* (Zoeller et al., 2012), and is involved in microbe-associated systemic acquired resistance (Cheval and Faulkner, 2018). Accordingly, this increase in azelaic acid and the other metabolic changes within the harvested hemp seeds is supportive of altered metabolism within the parent plant grown with molasses that reflects induction of a defence response.

5. Conclusion

Herein growth of hemp plants with supplementation of the fertiliser with molasses is shown to beneficially impact the antioxidant capacity of the produced seeds. Metabolite profiling and predictive modelling indicate that this change is likely associated with increases in hydroxycinnamic acid amide and cannabisin metabolites, which are lipophilic compounds with predicted antioxidant capacity. Various other beneficial metabolites were also increased within seeds, including azelaic acid, uridine and wighteone, which are associated with neuroprotection, reduced cancer risk, and improved liver health, further supporting that the produced hemp seeds had enhanced functional food potential. Exploration of molecular changes identified tissue-specific changes to phytohormone and metabolite contents that are indicative of an induced stress response, predominantly occurring within the roots, at the site of the molasses application. Utilisation of molasses during hemp cultivation is therefore recommended to enhance the quality of the produced seed and impart additional health benefits when consumed. These findings are promising developments in the field of biostimulant research and support the exploration in future studies of molasses impacts on other antioxidant functional foods.

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CRediT authorship contribution statement

Kimber Wise: Writing – original draft, Visualization, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Luke B. Williams:** Writing – review & editing, Methodology, Investigation, Data curation. **Sophie Selby-Pham:** Writing – review & editing, Methodology, Investigation, Data curation. **Paul F.A. Wright:** Writing – review & editing, Supervision, Methodology, Formal analysis. **Tomer Simovich:** Writing – review & editing, Supervision. **Harsharn Gill:** Writing – review & editing, Supervision. **Adarsha Gupta:** Writing – review & editing, Methodology, Investigation, Data curation. **Munish Puri:** Writing – review & editing, Supervision. **Jamie Selby-Pham:** Writing – review & editing, Supervision, Methodology, Formal analysis, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Available data is presented in the supplemental files.

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Supplementary materials

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References

Adan, A., Kiraz, Y., Baran, Y., 2016. Cell proliferation and cytotoxicity assays. Curr. Pharm. Biotechnol. 17, 1213–1221.

Ainsworth, E.A., Gillespie, K.M., 2007. Estimation of total phenolic content and other oxidation substrates in plant tissues using Folin–Ciocalteu reagent. Nat. Protoc. 2, 875–877.

Aljabri, M., Alharbi, S., Al-Qthanin, R.N., Ismaeil, F.M., Chen, J., Abou-Elwafa, S.F., 2021. Recycling of beet sugar byproducts and wastes enhances sugar beet productivity and salt redistribution in saline soils. Environ. Sci. Pollut. Res. 28, 45745–45755.

Anastassakis, K., 2022. Azelaic Acid, Androgenetic Alopecia From A to Z: Vol. 2 Drugs, Herbs, Nutrition and Supplements. Springer, pp. 151–154.

Andre, C.M., Hausman, J.F., Guerriero, G., 2016. *Cannabis sativa*: the plant of the thousand and one molecules. Front. Plant Sci. 7, 19 https://doi.org/10.3389/fpls.2016.00019.

Andre, C.M., Larondelle, Y., Evers, D., 2010. Dietary antioxidants and oxidative stress from a human and plant perspective: a review. Curr. Nutr. Food Sci. 6, 2–12.

Ávila-Escalante, M.L., Coop-Gamas, F., Cervantes-Rodríguez, M., Méndez-Iturbide, D., Aranda-González, I.I., 2020. The effect of diet on oxidative stress and metabolic diseases-Clinically controlled trials. J. Food Biochem. 44, e13191.

Baumel, B.S., Doraiswamy, P.M., Sabbagh, M., Wurtman, R., 2021. Potential neuroregenerative and neuroprotective effects of uridine/choline-enriched multinutrient dietary intervention for mild cognitive impairment: a narrative review. Neurol. Ther. 10, 43–60.

Bielach, A., Hrtyan, M., Tognetti, V.B., 2017. Plants under stress: involvement of auxin and cytokinin. Int. J. Mol. Sci. 18, 1427.

Bimova, P., Pokluda, R., 2009. Impact of organic fertilizers on total antioxidant capacity in head cabbage. Hortic. Sci. 36, 21–25.

Biteznik, L., Dermastia, M., Trdan, S., 2023. Secondary metabolites of hemp (*Cannabis sativa* L.) and their role in defence against pests and pathogens. Acta Biol. Slov. 66, 78.

Boatwright, J.L., Pajerowska-Mukhtar, K., 2013. Salicylic acid: an old hormone up to new tricks. Mol. Plant Pathol. 14, 623–634.

Bolster, D., Chae, L., van Klinken, J.W., Kalgaonkar, S., 2022. Impact of selected novel plant bioactives on improvement of impaired gut barrier function using human primary cell intestinal epithelium. J. Food Bioact. 20, 11–16.

Bonini, S.A., Premoli, M., Tambaro, S., Kumar, A., Maccarinelli, G., Memo, M., Mastinu, A., 2018. *Cannabis sativa*: a comprehensive ethnopharmacological review of a medicinal plant with a long history. J. Ethnopharmacol. 227, 300–315.

Cai, Y.Z., Sun, M., Xing, J., Luo, Q., Corke, H., 2006. Structure–radical scavenging activity relationships of phenolic compounds from traditional Chinese medicinal plants. Life Sci. 78, 2872–2888.

Callaway, J., 2004. Hempseed as a nutritional resource: an overview. Euphytica 140, 65–72.

Casarrubias-Castillo, K., Montero-Vargas, J.M., Dabdoub-González, N., Winkler, R., Martinez-Gallardo, N.A., Aviles-Arnaut, H., Délano-Frier, J.P., 2020. Distinct gene expression and secondary metabolite profiles in *suppressor of prosystemin-mediated responses2* (*spr2*) tomato mutants having impaired mycorrhizal colonization. PeerJ. 8, e8888.

Cerino, P., Buonerba, C., Cannazza, G., D'Auria, J., Ottoni, E., Fulgione, A., Di Stasio, A., Pierri, B., Gallo, A., 2021. A review of hemp as food and nutritional supplement. Cannabis Cannabinoid Res. 6, 19–27.

Chae, M., Wu, C., Shin, J., Lee, S.J., 2023. Azelaic acid induces cholecystokinin secretion and reduces fat-rich food preference via activation of Olfr544. J. Funct. Foods 105, 105577.

Chandra, R., Bharagava, R.N., Rai, V., 2008. Melanoidins as major colourant in sugarcane molasses based distillery effluent and its degradation. Bioresour. Technol. 99, 4648–4660.

Chen, C.W., Yang, Y.W., Lur, H.S., Tsai, Y.G., Chang, M.C., 2006. A novel function of abscisic acid in the regulation of rice (*Oryza sativa* L.) root growth and development. Plant Cell Physiol. 47, 1–13.

Chen, T., Hao, J., He, J., Zhang, J., Li, Y., Liu, R., Li, L., 2013. Cannabisin B induces autophagic cell death by inhibiting the AKT/mTOR pathway and S phase cell cycle arrest in HepG2 cells. Food Chem. 138, 1034–1041.

Chen, T., He, J., Zhang, J., Li, X., Zhang, H., Hao, J., Li, L., 2012. The isolation and identification of two compounds with predominant radical scavenging activity in hempseed (seed of *Cannabis sativa* L.). Food Chem. 134, 1030–1037.

Cheung, A.Y., Qu, L.J., Russinova, E., Zhao, Y., Zipfel, C., 2020. Update on receptors and signaling. Plant Physiol. 182, 1527–1530.

Cheval, C., Faulkner, C., 2018. Plasmodesmal regulation during plant–pathogen interactions. New Phytol. 217, 62–67.

Chong, J., Wishart, D.S., Xia, J., 2019. Using MetaboAnalyst 4.0 for comprehensive and integrative metabolomics data analysis. Curr. Protoc. Bioinform. 68, e86.

Corso, M., Perreau, F., Mouille, G., Lepiniec, L., 2020. Specialized phenolic compounds in seeds: structures, functions, and regulations. Plant Sci. 296, 110471.

Dandlen, S.A., Da Silva, J.P., Miguel, M.G., Duarte, A., Power, D.M., Marques, N.T., 2023. Quick decline and stem pitting *Citrus tristeza virus* isolates induce a distinct metabolomic profile and antioxidant enzyme activity in the phloem sap of two citrus species. Plants 12, 1394.

Das, P.C., Vista, A.R., Tabil, L.G., Baik, O.D., 2022. Postharvest operations of cannabis and their effect on cannabinoid content: a review. Bioengineering 9, 364.

Dave, A., Graham, I.A., 2012. Oxylipin signaling: a distinct role for the jasmonic acid precursor cis-(+)-12-oxo-phytodienoic acid (cis-OPDA). Front. Plant Sci. 3, 42.

Davidson, P., Taylor, T., 2007. Chemical peservatives and natural antimicrobial compounds. In: Doyle, M.P., Beuchat, L.R. (Eds.), Food Microbiology: Fundamentals and Frontiers. ASM Press, United Kingdom, pp. 713–745.

De Lorgeril, M., Salen, P., 2012. New insights into the health effects of dietary saturated and omega-6 and omega-3 polyunsaturated fatty acids. BMC Med. 10, 1–5.

de Souza, C.O., Vannice, G.K., Rosa Neto, J.C., Calder, P.C., 2018. Is palmitoleic acid a plausible nonpharmacological strategy to prevent or control chronic metabolic and inflammatory disorders? Mol. Nutr. Food Res. 62, 1700504.

Desoky, E.S.M., Elrys, A.S., Mansour, E., Eid, R.S., Selem, E., Rady, M.M., Ali, E.F., Mersal, G.A., Semida, W.M., 2021. Application of biostimulants promotes growth and productivity by fortifying the antioxidant machinery and suppressing oxidative stress in faba bean under various abiotic stresses. Sci. Hortic. 288, 110340.

Di Palo, A., Siniscalchi, C., Crescente, G., De Leo, I., Fiorentino, A., Pacifico, S., Russo, A., Potenza, N., 2022. Effect of cannabidiolic acid, N-trans-caffeoyltyramine and cannabisin B from hemp seeds on microRNA Expression in human neural cells. Curr. Issues Mol. Biol. 44, 5106–5116.

Drobek, M., Frąc, M., Cybulska, J., 2019. Plant biostimulants: importance of the quality and yield of horticultural crops and the improvement of plant tolerance to abiotic stress-a review. Agronomy 9, 335.

du Jardin, P., 2015. Plant biostimulants: definition, concept, main categories and regulation. Sci. Hortic. 196, 3–14.

Elayaraj, B., 2014. Impact of sugar factory effluent on germination, biochemical and antioxidant enzyme activity of Black gram (*Vigna mungo* (L.) Hepper) variety Vamban-3. Int. J. Currr. Res. 3, 84–89.

Erasto, P., Bojase-Moleta, G., Majinda, R.R.T., 2004. Antimicrobial and antioxidant flavonoids from the root wood of *Bolusanthus speciosus*. Phytochemistry 65, 875–880.

Facchini, P.J., Hagel, J., Zulak, K.G., 2002. Hydroxycinnamic acid amide metabolism: physiology and biochemistry. Can. J. Bot. 80, 577–589.

Farinon, B., Molinari, R., Costantini, L., Merendino, N., 2020. The seed of industrial hemp (*Cannabis sativa* L.): nutritional quality and potential functionality for human health and nutrition. Nutrients 12, 1935.

Floegel, A., Kim, D.O., Chung, S.J., Koo, S.I., Chun, O.K., 2011. Comparison of ABTS/DPPH assays to measure antioxidant capacity in popular antioxidant-rich US foods. J. Food Compos. Anal. 24, 1043–1048.

Formela, M., Samardakiewicz, S., Marczak, Ł., Nowak, W., Narożna, D., Bednarski, W., Kasprowicz-Maluśki, A., Morkunas, I., 2014. Effects of endogenous signals and *Fusarium oxysporum* on the mechanism regulating genistein synthesis and accumulation in yellow lupine and their impact on plant cell cytoskeleton. Molecules 19, 13392–13421.

Frigolet, M.E., Gutiérrez-Aguilar, R., 2017. The role of the novel lipokine palmitoleic acid in health and disease. Adv. Nutr. 8, 173S–181S.

Gaafar, M.S., EL-Shimi, N.M., Helmy, M., 2019. Effect of foliar and soil application of some residuals of sugar cane products (molasses and vinasses) with mineral fertilizer levels on growth, yield and quality of sweet pepper. Minoufia J. Plant Prod. 4, 353–373.

Gamir, J., Pastor, V., Cerezo, M., Flors, V., 2012. Identification of indole-3-carboxylic acid as mediator of priming against *Plectosphaerella cucumerina*. Plant Physiol. Biochem. 61, 169–179.

Gamir, J., Pastor, V., Sánchez-Bel, P., Agut, B., Mateu, D., García-Andrade, J., Flors, V., 2018. Starch degradation, abscisic acid and vesicular trafficking are important elements in callose priming by indole-3-carboxylic acid in response to *Plectosphaerella cucumerina* infection. Plant J. 96, 518–531.

Gilroy, E., Breen, S., 2022. Interplay between phytohormone signalling pathways in plant defence–other than salicylic acid and jasmonic acid. Essays Biochem. 66, 657–671.

Girgih, A.T., He, R., Malomo, S., Offengenden, M., Wu, J., Aluko, R.E., 2014. Structural and functional characterization of hemp seed (*Cannabis sativa* L.) protein-derived antioxidant and antihypertensive peptides. J. Funct. Foods 6, 384–394.

Goto, T., Soyano, T., Liu, M., Mori, T., Kawaguchi, M., 2022. Auxin methylation by IAMT1, duplicated in the legume lineage, promotes root nodule development in *Lotus japonicus*. Proc. Natl. Acad. Sci. USA 119, e2116549119.

Govindaraj, M., Masilamani, P., Albert, V.A., Bhaskaran, M., 2017. Role of antioxidant in seed quality-a review. Agric. Rev. 38, 180–190.

Gupta, A., Abraham, R.E., Barrow, C.J., Puri, M., 2015. Omega-3 fatty acid production from enzyme saccharified hemp hydrolysate using a novel marine thraustochytrid strain. Bioresour. Technol. 184, 373–378.

Hatano, K.i., Kanazawa, K., Tomura, H., Yamatsu, T., Tsunoda, K.i., Kubota, K., 2016. Molasses melanoidin promotes copper uptake for radish sprouts: the potential for an accelerator of phytoextraction. Environ. Sci. Pollut. Res. 23, 17656–17663.

He, H., de Souza Vidigal, D., Snoek, L.B., Schnabel, S., Nijveen, H., Hilhorst, H., Bentsink, L., 2014. Interaction between parental environment and genotype affects plant and seed performance in *Arabidopsis*. J. Exp. Bot. 65, 6603–6615.

Holguin, S., Martinez, J., Chow, C., Wurtman, R., 2008. Dietary uridine enhances the improvement in learning and memory produced by administering DHA to gerbils. FASEB J. 22, 3938.

Hussain, S., Saleem, M.F., Iqbal, J., Ibrahim, M., Atta, S., Ahmed, T., Rehmani, M., 2014. Exogenous application of abscisic acid may improve the growth and yield of sunflower hybrids under drought. Pak. J. Agric. Sci. 51, 49–58.

Ilich, J.Z., Kelly, O.J., Kim, Y., Spicer, M.T., 2014. Low-grade chronic inflammation perpetuated by modern diet as a promoter of obesity and osteoporosis. Arh. Hig. Rada Toksikol. 65, 139–147.

Innes, R., 2018. The positives and negatives of NPR: a unifying model for salicylic acid signaling in plants. Cell 173, 1314–1315.

Jain, A., Singh, A., Chaudhary, A., Singh, S., Singh, H.B., 2014. Modulation of nutritional and antioxidant potential of seeds and pericarp of pea pods treated with microbial consortium. Food Res. Int. 64, 275–282.

Kasprzewska, A., 2003. Plant chitinases-regulation and function. Cell. Mol. Biol. Lett. 8, 809–824.

Kaushal, N., Gupta, M., Kulshreshtha, E., 2020. Hempseed (*Cannabis sativa*) lipid fractions alleviate high-fat diet-induced fatty liver disease through regulation of inflammation and oxidative stress. Heliyon 6, e04422.

Kermode, A.R., 2017. Regulatory mechanisms in the transition from seed development to germination: interactions between the embryo and the seed environment. Seed Development and Germination. Routledge, pp. 273–332.

Khalid, M., Hassani, D., Bilal, M., Asad, F., Huang, D., 2017. Influence of bio-fertilizer containing beneficial fungi and rhizospheric bacteria on health promoting compounds and antioxidant activity of *Spinacia oleracea* L. Bot. Stud. 58, 1–9.

Khan, N., Bano, A., Ali, S., Babar, M.A., 2020. Crosstalk amongst phytohormones from planta and PGPR under biotic and abiotic stresses. Plant Growth Regul. 90, 189–203.

Kneer, R., Poulev, A.A., Olesinski, A., Raskin, I., 1999. Characterization of the elicitor-induced biosynthesis and secretion of genistein from roots of *Lupinus luteus* L. J. Exp. Bot. 50, 1553–1559.

Kocira, S., Szparaga, A., Kocira, A., Czerwińska, E., Wójtowicz, A., Bronowicka-Mielniczuk, U., Koszel, M., Findura, P., 2018. Modeling biometric traits, yield and nutritional and antioxidant properties of seeds of three soybean cultivars through the application of biostimulant containing seaweed and amino acids. Front. Plant Sci. 9, 388.

Kosakivska, I.V., Vedenicheva, N.P., Babenko, L.M., Voytenko, L.V., Romanenko, K.O., Vasyuk, V.A., 2022. Exogenous phytohormones in the regulation of growth and development of cereals under abiotic stresses. Mol. Biol. Rep. 49, 617–628.

Koźmińska, A., Hanus-Fajerska, E., Halecki, W., Ciarkowska, K., 2021. Beet molasses enhance salinity tolerance in *Thymus serpyllum* - a study under greenhouse condition. Plants 10, 1819.

Krüger, E., Josuttis, M., 2012. Effects of growing and climate conditions on berry yield and nutritional quality. In: Proceedings of the X International Symposium on Vaccinium and Other Superfruits 1017, pp. 351–362.

Kumar, S., Abedin, M.M., Singh, A.K., Das, S., 2020. Role of phenolic compounds in plant-defensive mechanisms. In: Lone, R., Shuab, R., Kamili, A.N. (Eds.). In: Plant Phenolics in Sustainable Agriculture, 1. Springer Singapore, Singapore, pp. 517–532.

Kumar, V., Khan, A.A., Tripathi, A., Dixit, P.K., Bajaj, U., 2015. Role of oxidative stress in various diseases: relevance of dietary antioxidants. J. Phytopharm. 4, 126–132.

Lee, S.H., Veeriah, V., Levine, F., 2022. A potent HNF4α agonist reveals that HNF4α controls genes important in inflammatory bowel disease and Paneth cells. PLoS ONE 17, e0266066.

Leizer, C., Ribnicky, D., Poulev, A., Dushenkov, S., Raskin, I., 2000. The composition of hemp seed oil and its potential as an important source of nutrition. J. Nutraceuticals Funct. Med. Foods 2, 35–53.

Leonard, W., Zhang, P., Ying, D., Fang, Z., 2021a. Lignanamides: sources, biosynthesis and potential health benefits – a minireview. Crit. Rev. Food Sci. Nutr. 61, 1404–1414.

Leonard, W., Zhang, P., Ying, D., Xiong, Y., Fang, Z., 2021b. Extrusion improves the phenolic profile and biological activities of hempseed (*Cannabis sativa* L.) hull. Food Chem. 346, 128606.

Li, S., Zhao, X., Ye, X., Zhang, L., Shi, L., Xu, F., Ding, G., 2020. The effects of condensed molasses soluble on the growth and development of rapeseed through seed germination, hydroponics and field trials. Agriculture 10, 260.

Li, X., Topbjerg, H.B., Jiang, D., Liu, F., 2015. Drought priming at vegetative stage improves the antioxidant capacity and photosynthesis performance of wheat exposed to a short-term low temperature stress at jointing stage. Plant Soil 393, 307–318.

Lin, C.C., Lee, H.Y., Chang, C.H., Namba, T., Hattori, M., 1996. Evaluation of the liver protective principles from the root of *Cudrania cochinchinensis* var. *gerontogea*. Phytother. Res. 10, 13–17.

Liu, Y., Zhang, Y., Yin, J., Ruan, Z., Wu, X., Yin, Y., 2019. Uridine dynamic administration affects circadian variations in lipid metabolisms in the liver of high-fat-diet-fed mice. Chronobiol. Int. 36, 1258–1267.

López-Alarcón, C., Denicola, A., 2013. Evaluating the antioxidant capacity of natural products: a review on chemical and cellular-based assays. Anal. Chim. Acta 763, 1–10.

Luziatelli, F., Ficca, A.G., Colla, G., Baldassarre Švecová, E., Ruzzi, M., 2019. Foliar application of vegetal-derived bioactive compounds stimulates the growth of beneficial bacteria and enhances microbiome biodiversity in lettuce. Front. Plant Sci. 10, 60.

Lv, Z.Y., Sun, W.J., Jiang, R., Chen, J.F., Ying, X., Zhang, L., Chen, W.S., 2021. Phytohormones jasmonic acid, salicylic acid, gibberellins, and abscisic acid are key mediators of plant secondary metabolites. World J. Tradit. Chin. Med. 7, 307–325.

Macoy, D.M., Kim, W.Y., Lee, S.Y., Kim, M.G., 2015. Biosynthesis, physiology, and functions of hydroxycinnamic acid amides in plants. Plant Biotechnol. Rep. 9, 269–278.

MacWilliams, J., Peirce, E., Pitt, W.J., Schreiner, M., Matthews, T., Yao, L., Broeckling, C., Nachappa, P., 2023. Assessing the adaptive role of cannabidiol (CBD) in *Cannabis sativa* defense against cannabis aphids. Front. Plant Sci. 14, 1223894.

Martinez, J.R., Montserrat-de la Paz, S., De la Puerta, R., García-Giménez, M.D., Fernández-Arche, M.Á., 2020. Characterization of bioactive compounds in defatted hempseed (*Cannabis sativa* L.) by UHPLC-HRMS/MS and anti-inflammatory activity in primary human monocytes. Food Funct. 11, 4057–4066.

Menga, V., Garofalo, C., Suriano, S., Beleggia, R., Colecchia, S.A., Perrone, D., Montanari, M., Pecchioni, N., Fares, C., 2022. Phenolic acid composition and antioxidant activity of whole and defatted seeds of Italian hemp cultivars: a two-year case study. Agriculture 12, 759.

Miller, N.J., Rice-Evans, C.A., 1997. The relative contributions of ascorbic acid and phenolic antioxidants to the total antioxidant activity of orange and apple fruit juices and blackcurrant drink. Food Chem. 60, 331–337.

Mukherjee, M.S., Han, C.Y., Sukumaran, S., Delaney, C.L., Miller, M.D., 2023. Effect of anti-inflammatory diets on inflammation markers in adult human populations: a systematic review of randomized controlled trials. Nutr. Rev. 81, 55–74.

Munteanu, I.G., Apetrei, C., 2021. Analytical methods used in determining antioxidant activity: a review. Int. J. Mol. Sci. 22, 3380.

Muthulakshmi, S., Chakrabarti, A.K., Mukherjee, S., 2015. Gene expression profile of high-fat diet-fed C57BL/6J mice: in search of potential role of azelaic acid. J. Physiol. Biochem. 71, 29–42.

Muthulakshmi, S., Saravanan, R., 2013. Protective effects of azelaic acid against high-fat diet-induced oxidative stress in liver, kidney and heart of C57BL/6J mice. Mol. Cell. Biochem. 377, 23–33.

Nadeeka, P.W., Seran, T.H., 2020. The effects of goat manure and sugarcane molasses on the growth and yield of beetroot (*Beta vulgaris* L.). J. Agric. Sci. 65, 321–335 (Belgr.).

Nagy, A., Magyar, T., Juhász, C., Tamás, J., 2020. Phytoremediation of acid mine drainage using by-product of lysine fermentation. Water Sci. Technol. 81, 1507–1517.

O'Keefe, S.J., Feltis, B.N., Piva, T.J., Turney, T.W., Wright, P.F., 2016. ZnO nanoparticles and organic chemical UV-filters are equally well tolerated by human immune cells. Nanotoxicology 10, 1287–1296.

Olbrich, H., 1963. The molasses. Biotechnologie-Kempe GmbH, Berlin.

Osman, H.S., Gowayed, S.M., Elbagory, M., Omara, A.E.D., El-Monem, A.M.A., El-Razek, A., Usama, A., Hafez, E.M., 2021. Interactive impacts of beneficial microbes and Si-Zn nanocomposite on growth and productivity of soybean subjected to water deficit under salt-affected soil conditions. Plants 10, 1396.

Ouhibi, C., Attia, H., Rebah, F., Msilini, N., Chebbi, M., Aarrouf, J., Urban, L., Lachaal, M., 2014. Salt stress mitigation by seed priming with UV-C in lettuce plants: growth, antioxidant activity and phenolic compounds. Plant Physiol. Biochem. 83, 126–133.

Palmonari, A., Cavallini, D., Sniffen, C.J., Fernandes, L., Holder, P., Fagioli, L., Fusaro, I., Biagi, G., Formigoni, A., Mammi, L., 2020. Characterization of molasses chemical composition. J. Dairy Sci. 103, 6244–6249.

Pan, X., Welti, R., Wang, X., 2008. Simultaneous quantification of major phytohormones and related compounds in crude plant extracts by liquid chromatography–electrospray tandem mass spectrometry. Phytochemistry 69, 1773–1781.

Park, S.H., Pauli, C.S., Gostin, E.L., Staples, S.K., Seifried, D., Kinney, C., Vanden Heuvel, B.D., 2022. Effects of short-term environmental stresses on the onset of cannabinoid production in young immature flowers of industrial hemp (*Cannabis sativa* L.). J. Cannabis Res. 4, 1–13.

Perez-Pardo, P., Dodiya, H.B., Broersen, L.M., Douna, H., van Wijk, N., Lopes da Silva, S., Garssen, J., Keshavarzian, A., Kraneveld, A.D., 2018. Gut–brain and brain–gut axis in Parkinson's disease models: effects of a uridine and fish oil diet. Nutr. Neurosci. 21, 391–402.

Petrić, I., Šamec, D., Karalija, E., Salopek-Sondi, B., 2022. Beneficial microbes and molecules for mitigation of soil salinity in *Brassica* species: a Review. Soil Syst. 6, 18.

Plumb, G.W., Price, K.R., Modes, M.J., Williamson, G., 1997. Antioxidant properties of the major polyphenolic compounds in broccoli. Free Radic. Res. 27, 429–435.

Pojić, M., Mišan, A., Sakač, M., Dapčević Hadnađev, T., Šarić, B., Milovanović, I., Hadnađev, M., 2014. Characterization of byproducts originating from hemp oil processing. J. Agric. Food Chem. 62, 12436–12442.

Pratyusha, S., 2022. Phenolic compounds in the plant development and defense: an overview, In: Hasanuzzaman, M., Nahar, K. (Eds.), Plant Stress Physiology-Perspectives in Agriculture. IntechOpen, pp. 125–140.

Pyakurel, A., Dahal, B.R., Rijal, S., 2019. Effect of molasses and organic fertilizer in soil fertility and yield of spinach in Khotang, Nepal. Int. J. Appl. Sci. Biotechnol. 7, 49–53.

Qiu, X.M., Sun, Y.Y., Ye, X.Y., Li, Z.G., 2020. Signaling role of glutamate in plants. Front. Plant Sci. 10, 1743.

Raudonis, R., Raudone, L., Jakstas, V., Janulis, V., 2012. Comparative evaluation of post-column free radical scavenging and ferric reducing antioxidant power assays for screening of antioxidants in strawberries. J. Chromatogr. A 1233, 8–15.

Raza, M.A.S., Zaheer, M.S., Saleem, M.F., Khan, I.H., Ahmad, S., Iqbal, R., 2020. Drought ameliorating effect of exogenous applied cytokinin in wheat. Pak. J. Agric. Sci. 57, 725–733.

Riaz, U., Kharal, M.A., Murtaza, G., uz Zaman, Q., Javaid, S., Malik, H.A., Aziz, H., Abbas, Z., 2019. Prospective roles and mechanisms of caffeic acid in counter plant stress: a mini review. Pak. J. Agric. Res. 32, 8.

Rice-Evans, C.A., 1995. Plant polyphenols: free radical scavengers or chain-breaking antioxidants? Biochemical Society Symposia. Portland Press Limited, pp. 103–116.

Rice-Evans, C.A., Miller, N.J., Bolwell, P.G., Bramley, P.M., Pridham, J.B., 1995. The relative antioxidant activities of plant-derived polyphenolic flavonoids. Free Radic. Res. 22, 375–383.

Rodriguez-Martin, N.M., Montserrat-de la Paz, S., Toscano, R., Grao-Cruces, E., Villanueva, A., Pedroche, J., Millan, F., Millan-Linares, M.C., 2020. Hemp (*Cannabis sativa* L.) protein hydrolysates promote anti-inflammatory response in primary human monocytes. Biomolecules 10, 803.

Ru, C., Hu, X., Chen, D., Wang, W., Song, T., 2022. Heat and drought priming induce tolerance to subsequent heat and drought stress by regulating leaf photosynthesis, root morphology, and antioxidant defense in maize seedlings. Environ. Exp. Bot. 202, 105010.

Rupasinghe, H.V., Davis, A., Kumar, S.K., Murray, B., Zheljazkov, V.D., 2020. Industrial hemp (*Cannabis sativa* subsp. sativa) as an emerging source for value-added functional food ingredients and nutraceuticals. Molecules 25, 4078.

Saini, R.K., Keum, Y.S., 2018. Omega-3 and omega-6 polyunsaturated fatty acids: dietary sources, metabolism, and significance - a review. Life Sci. 203, 255–267.

Saini, S., Sharma, I., Kaur, N., Pati, P.K., 2013. Auxin: a master regulator in plant root development. Plant Cell Rep. 32, 741–757.

Sarma, N.D., Waye, A., ElSohly, M.A., Brown, P.N., Elzinga, S., Johnson, H.E., Marles, R. J., Melanson, J.E., Russo, E., Deyton, L., 2020. Cannabis inflorescence for medical purposes: USP considerations for quality attributes. J. Nat. Prod. 83, 1334–1351.

Selby-Pham, S.N., Miller, R.B., Howell, K., Dunshea, F., Bennett, L.E., 2017a. Physicochemical properties of dietary phytochemicals can predict their passive absorption in the human small intestine. Sci. Rep. 7, 1931.

Selby-Pham, S.N.B., Cottrell, J., Dunshea, F., Ng, K., Bennett, L.E., Howell, K., 2017b. Dietary phytochemicals promote health by enhancing antioxidant defence in a pig model. Nutrients 9, 758.

Shahzad, H., Iqbal, M., Bashir, S., Farooq, M., 2018. Relative efficacy of organic substrates on maize root proliferation under water stress. Biosci. J. 35, 101–114.

Shen, Y., Fan, K., Wang, Y., Wang, H., Ding, S., Song, D., Shen, J., Li, H., Song, Y., Han, X., 2022. Red and blue light affect the formation of adventitious roots of tea cuttings (*Camellia sinensis*) by regulating hormone synthesis and signal transduction pathways of mature leaves. Front. Plant Sci. 13, 943662.

Singh, A., Jain, A., Sarma, B.K., Upadhyay, R.S., Singh, H.B., 2014. Beneficial compatible microbes enhance antioxidants in chickpea edible parts through synergistic interactions. LWT Food Sci. Technol. 56, 390–397.

Singleton, V.L., Joseph, A., Rossi, J., 1965. Colorimetry of Total Phenolics with Phosphomolybdic-Phosphotungstic Acid Reagents. Am. J. Enol. Vitic. 16, 144–158.

Small, E., 2015. Evolution and classification of *Cannabis sativa* (marijuana, hemp) in relation to human utilization. Bot. Rev. 81, 189–294.

Spitzer-Rimon, B., Duchin, S., Bernstein, N., Kamenetsky, R., 2019. Architecture and florogenesis in female *Cannabis sativa* plants. Front. Plant Sci. 10, 350.

Srivastava, P., Singh, R., Srivastava, P., Shrivastava, M., 2012. Utilization of molasses based distillery effluent for fertigation of sugarcane. Biodegradation 23, 897–905.

Suliasih, S., Widawati, S., 2016. Effect of plant growth promoting rhizobacteria and molasses on seed germination and seedling growth of *Sorghum bicolor* l. Moench. In: Proceedings of the 1st SATREPS Conference. Bogor, Indonesia.

Sun, H., Jiang, S., Jiang, C., Wu, C., Gao, M., Wang, Q., 2021. A review of root exudates and rhizosphere microbiome for crop production. Environ. Sci. Pollut. Res. 28, 54497–54510.

Sun, W.J., Nie, Y.X., Gao, Y., Dai, A.H., Bai, J.G., 2012. Exogenous cinnamic acid regulates antioxidant enzyme activity and reduces lipid peroxidation in drought-stressed cucumber leaves. Acta Physiol. Plant. 34, 641–655.

Suwanchaikasem, P., Idnurm, A., Selby-Pham, J., Walker, R., Boughton, B.A., 2022. Root-TRAPR: a modular plant growth device to visualize root development and monitor growth parameters, as applied to an elicitor response of *Cannabis sativa*. Plant Methods 18, 1–20.

Suwanchaikasem, P., Nie, S., Idnurm, A., Selby-Pham, J., Walker, R., Boughton, B.A., 2023a. Effects of chitin and chitosan on root growth, biochemical defense response and exudate proteome of *Cannabis sativa*. Plant Environ. Interact. 4, 115–133.

Suwanchaikasem, P., Nie, S., Selby-Pham, J., Walker, R., Boughton, B.A., Idnurm, A., 2023b. Hormonal and proteomic analyses of southern blight disease caused by *Athelia rolfsii* and root chitosan priming on *Cannabis sativa* in an *in vitro* hydroponic system. Plant Direct 7, e528.

Tanney, C.A., 2023. The Effect of Microbial Inoculation and Nutrient Regimes on Cannabis Stalked Trichome Development, Growth, and Cannabinoid Profiles. McGill University, Montreal, Canada.

Thaipong, K., Boonprakob, U., Crosby, K., Cisneros-Zevallos, L., Hawkins Byrne, D., 2006. Comparison of ABTS, DPPH, FRAP, and ORAC assays for estimating antioxidant activity from guava fruit extracts. J. Food Compost. Anal. 19, 669–675.

Thakare, P., Chaudhary, M., Pokal, W., 2013. Physico-Chemical characterisation of molasses and its effects on the growth of *Abelmoschus esenlentus* (Lady's Finger). World Appl. Sci. J. 21, 869–872.

Thuong, P.T., Hung, T.M., Ngoc, T.M., Ha do, T., Min, B.S., Kwack, S.J., Kang, T.S., Choi, J.S., Bae, K., 2010. Antioxidant activities of coumarins from Korean medicinal plants and their structure-activity relationships. Phytother. Res. 24, 101–106.

Tian, T., Sun, B., Shi, H., Gao, T., He, Y., Li, Y., Liu, Y., Li, X., Zhang, L., Li, S., 2021. Sucrose triggers a novel signaling cascade promoting *Bacillus subtilis* rhizosphere colonization. ISME J. 15, 2723–2737.

Tian, Y., Liu, Y., Yue, L., Uwaremwe, C., Zhao, X., Zhou, Q., Wang, Y., Wang, R., 2022. Bacterial inoculant and sucrose amendments improve the growth of *Rheum palmatum* L. by reprograming its metabolite composition and altering its soil microbial community. Int. J. Mol. Sci. 23, 1694.

Valette, M., Rey, M., Gerin, F., Comte, G., Wisniewski-Dyé, F., 2020. A common metabolomic signature is observed upon inoculation of rice roots with various rhizobacteria. J. Integr. Plant Biol. 62, 228–246.

Veeriah, V., Lee, S.H., Levine, F., 2022. Long-term oral administration of an HNF4α agonist prevents weight gain and hepatic steatosis by promoting increased mitochondrial mass and function. Cell Death Dis. 13, 89.

Veitch, N.C., 2004. Structural determinants of plant peroxidase function. Phytochem. Rev. 3, 3–18.

Wang, J., Song, L., Gong, X., Xu, J., Li, M., 2020. Functions of jasmonic acid in plant regulation and response to abiotic stress. Int. J. Mol. Sci. 21, 1446.

Wang, X., Cai, J., Liu, F., Dai, T., Cao, W., Wollenweber, B., Jiang, D., 2014. Multiple heat priming enhances thermo-tolerance to a later high temperature stress via improving subcellular antioxidant activities in wheat seedlings. Plant Physiol. Biochem. 74, 185–192.

Welbaum, G.E., Sturz, A.V., Dong, Z., Nowak, J., 2004. Managing soil microorganisms to improve productivity of agro-ecosystems. Crit. Rev. Plant Sci. 23, 175–193.

Wise, K., Baziotopoulos, E., Zhang, C., Leaming, M., Shen, L.H., Selby-Pham, J., 2023a. Comparative study of water requirements and water footprints of fibre crops hemp (*Cannabis sativa*) and cotton (*Gossypium hirsutum* L.). J. Agrometeorol. 25, 392–396.

Wise, K., Gill, H., Selby-Pham, J., 2019. Inhalation absorption prediction (IAP) model for predicting medicinal cannabis phytochemical pharmacokinetics. Pharmacogn. Commun. 9, 85–90.

Wise, K., Gill, H., Selby-Pham, J., 2020a. Willow bark extract and the biostimulant complex Root Nectar® increase propagation efficiency in chrysanthemum and lavender cuttings. Sci. Hortic. 263, 109108.

Wise, K., Phan, N., Selby-Pham, J., Simovich, T., Gill, H., 2023b. Utilisation of QSPR ODT modelling and odour vector modelling to predict *Cannabis sativa* odour. PLoS ONE 18, e0284842.

Wise, K., Selby-Pham, J., 2023. Enhancement of tomato functional food value through nutrient supplementation with fish emulsion biostimulant. J. Hortic. Sci. 18, 351–356.

Wise, K., Selby-Pham, J., 2024. Enhancement of tomato hedonic quality through fertiliser supplementation with a biostimulant complex comprising molasses, *Aloe vera* extract and fish-hydrolysate. NZ J. Crop Hortic. Sci. 1–10.

Wise, K., Selby-Pham, J., Chai, X., Simovich, T., Gupta, S., Gill, H., 2024a. Fertiliser supplementation with a biostimulant complex of fish hydrolysate, *Aloe vera* extract, and kelp alters cannabis root architecture to enhance nutrient uptake. Sci. Hortic. 323, 112483.

Wise, K., Selby-Pham, J., Simovich, T., Gill, H., 2023c. Enhancement of capsicum (*Capsicum annuum* L.) functional food value through nutrient supplementation with a biostimulant complex comprising triacontanol, phosphate, and potassium. NZ J. Crop Hortic. Sci. 1–12.

Wise, K., Selby-Pham, J., Simovich, T., Gill, H., 2024b. A biostimulant complex comprising molasses, Aloe vera extract, and fish-hydrolysate enhances yield, aroma, and functional food value of strawberry fruit. Adv. Hortic. Sci. 38, 47–62.

Wise, K., Selby-Pham, S.N.B., Selby-Pham, J., Gill, H., 2020b. Development of intestinal bioavailability prediction (IBP) and phytochemical relative antioxidant potential prediction (PRAPP) models for optimizing functional food value of *Cannabis sativa* (hemp). Int. J. Food Prop. 23, 1287–1295.

Wise, K., Simovich, T., Gill, H., Selby-Pham, J., 2024c. Bioaccumulation of silicon and enhancement of cannabis inflorescence yield from fertiliser supplementation with the biostimulants silicate and phosphite. NZ J. Crop Hortic. Sci. 1–13.

Wu, S., Shan, L., He, P., 2014. Microbial signature-triggered plant defense responses and early signaling mechanisms. Plant Sci. 228, 118–126.

Xu, W., Dubos, C., Lepiniec, L., 2015. Transcriptional control of flavonoid biosynthesis by MYB–bHLH–WDR complexes. Trends Plant Sci. 20, 176–185.

Yakhin, O.I., Lubyanov, A.A., Yakhin, I.A., Brown, P.H., 2017. Biostimulants in plant science: a global perspective. Front. Plant Sci. 7, 2049.

Yan, X., Tang, J., dos Santos Passos, C., Nurisso, A., Simões-Pires, C.A., Ji, M., Lou, H., Fan, P., 2015. Characterization of lignanamides from hemp (*Cannabis sativa* L.) seed and their antioxidant and acetylcholinesterase inhibitory activities. J. Agric. Food Chem. 63, 10611–10619.

Zaheer, M.S., Raza, M.A.S., Saleem, M.F., Erinle, K.O., Iqbal, R., Ahmad, S., 2019. Effect of rhizobacteria and cytokinins application on wheat growth and yield under normal vs drought conditions. Commun. Soil Sci. Plant Anal. 50, 2521–2533.

Zhang, D., Luo, Z., Jin, Y., Chen, Y., Yang, T., Yang, Q., Wu, B., Shang, Y., Liu, X., Wei, Y., Zhou, F., 2020. Azelaic acid exerts antileukemia effects against acute myeloid leukemia by regulating the Prdxs/ROS signaling pathway. Oxid. Med. Cell. Longev. 2020, 1295984.

Zhang, F., Smith, D.L., 1996. Genistein accumulation in soybean (*Glycine* max [L.] Merr.) root systems under suboptimal root zone temperatures. J. Exp. Bot. 47, 785–792.

Zhou, Y., Wang, S., Lou, H., Fan, P., 2018. Chemical constituents of hemp (*Cannabis sativa* L.) seed with potential anti-neuroinflammatory activity. Phytochem. Lett. 23, 57–61.

Zoeller, M., Stingl, N., Krischke, M., Fekete, A., Waller, F., Berger, S., Mueller, M.J., 2012. Lipid profiling of the *Arabidopsis* hypersensitive response reveals specific lipid peroxidation and fragmentation processes: biogenesis of pimelic and azelaic acid. Plant Physiol. 160, 365–378.