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Physiological, molecular, and genetic mechanism of action of the biostimulant Quantis™ for increased thermotolerance of potato (*Solanum tuberosum* L.)

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Abstract

Background Raising global temperatures limit crop productivity and new strategies are needed to improve the resilience of thermosensitive crops such as potato (*Solanum tuberosum L.*). Biostimulants are emerging as potential crop protection products against environmental stress, however their mechanism of action remains largely unknown, hindering their wider adoption. We used comprehensive physiological, molecular, and mass spectrometry approaches to develop understanding of the mechanism of plant thermotolerance exerted by the biostimulant, Quantis™, under heat stress. Using orthologues gene mutations in *Arabidopsis thaliana* we report heat-defence genes, modified by Quantis™, which were also investigated for potential overlapping functions in biotic stress defence to *Sclerotinia sclerotiorum* and *Rhizoctonia solani*.

Results Quantis™ enhanced PSII photochemical efficiency and decreased thermal dissipation of potato grown under heat stress. These effects were associated with upregulation of genes with antioxidant function, including PR10, flavonoid 3′-hydroxylase and β-glucosidases, and modulation of abscisic acid (ABA) and cytokinin (CK) activity in leaves by Quantis™. The biostimulant modulated the expression of the heat-defence genes, PEN1, PR4 or MEE59, with functions in leaf photoprotection and root thermal protection, but with no overlapping function in biotic stress defence. Protective root growth under heat stress, following the biostimulant application, was correlated with enhanced CK signalling in roots. Increased endogenous concentrations of ABA and CK in potato leaves and significant upregulation of *StFKF1* were consistent with tuberisation promoting effects. Quantis™ application resulted in 4% tuber weight increase and 40% larger tuber size thus mitigating negative effects of heat stress on tuber growth.

Conclusions Quantis™ application prior to heat stress effectively primed heat tolerance responses and alleviated temperature stress of *S. tuberosum* L. and *A. thaliana* by modulating the expression and function of PR4 and MEE59 and by regulating CK activity above and below ground, indicating that the mechanism of action of the biostimulant is conserved, and will be effective in many plant species. Thus, a biostimulant application targeting the most susceptible crop developmental stages to heat disorders can be effectively integrated within future agronomy practices to mitigate losses in other thermosensitive crops.

Keywords *Solanum tuberosum*, Biostimulant, Thermotolerance, Abscisic acid, Cytokinins, Gibberellins, Heat stress, *penetration1*, *Pathogenesis related 4*, *Maternal effect embryo arrest 59*

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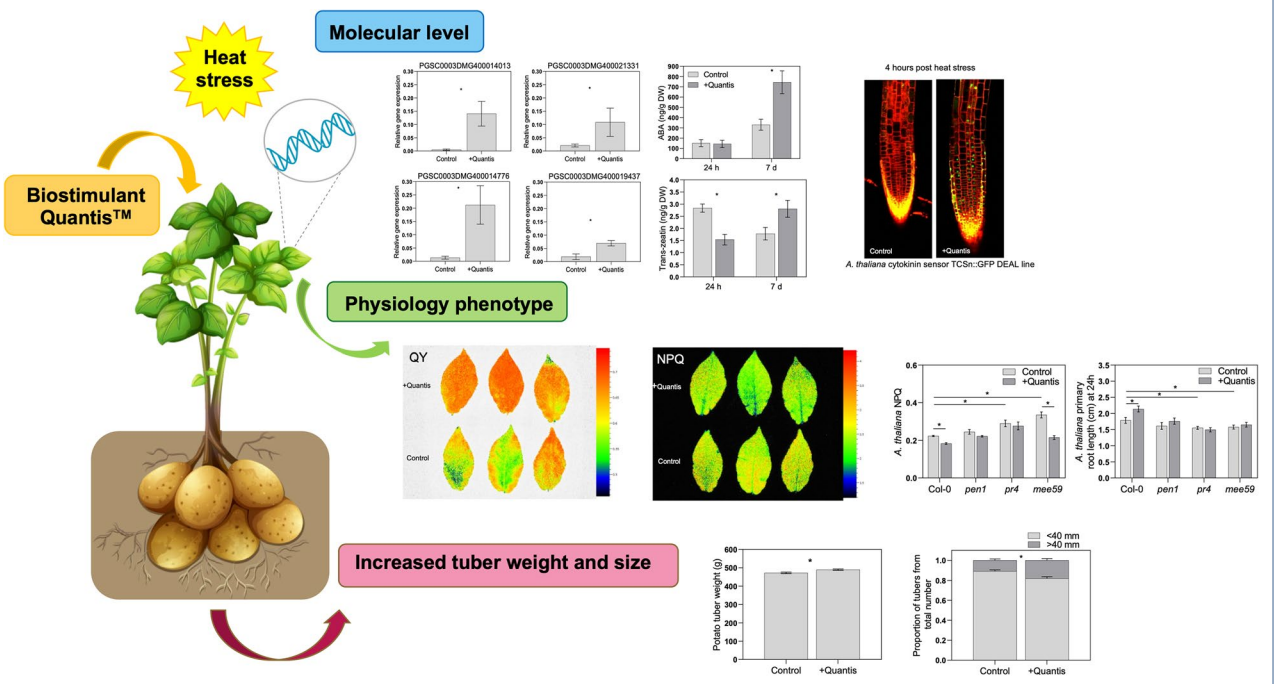
Graphical Abstract



Background

Potato (*Solanum tuberosum L.*) is the fifth most important crop for global food security with an estimated production of 376 million tons worldwide [1]. Potato cultivars have been adapted to grow under temperate climates with optimum temperatures for biomass and tuber formation between 20 and 25 °C and 15–20 °C, respectively [2]. The crop is thus inherently sensitive to heat stress, with temperature extremes due to climate change expected to pose significant challenges for potato productivity in many regions of the world [3, 4]. Prolonged heat stress caused by temperatures exceeding 28 °C is associated firstly with chlorophyll and CO_2 fixation losses in potato leaves followed by a disruption of the translocation of photo assimilates such as sucrose from source (leaves) to sink organs (stolons; tubers) in the plant [5, 6]. The plant is most susceptible to heat stress disorder at the tuber initiation and tuberisation stages when symptoms manifest as delayed tuber formation and development, reduced tuber size and weight and/or tuber necrosis and deformities [4, 7]. Additional quality losses, associated with reduced accumulation of starch and sugar in tubers include undesirable changes in flavour and texture reducing crop marketability and price [8]. Therefore, there is a significant need, and opportunity, to develop new crop protection strategies to mitigate the effects of alleviated temperatures on potato production and increase the environmental resilience of the crop.

Biostimulants are emerging as a new class of multi-compounds of heterogenous nature that are increasingly being integrated into crop management practices to enhance environmental stress tolerance, improve plant health, and maintain yields under changing climate [9–12]. Whilst there is compounding evidence that biostimulants can promote plant growth/yield as well as prime or condition plant defence against environmental stress [13–17], the molecular or genetic mechanisms of action behind their biological function remain largely unknown, thus hindering their wider adoption for crop environmental protection.

In this study, we investigated the effectiveness of the biostimulant Quantis™ (Syngenta, UK) in alleviating the effects of heat stress in potato. Quantis™ is a natural by-product of the fermentation of sugar cane and yeast, enriched with potassium and calcium (Table 1). The recommended application by the manufacturer is at the early stages of tuber initiation and development when the crop is most susceptible to heat stress. Under high leaf temperature or light, plants deploy photoprotective mechanisms to avoid irreversible damage to photosystem II (PSII) in the chloroplast caused by excessive energy generated by light-harvesting complexes. The

Table 1 Nutrient composition of Quantis™ showing minimum (guaranteed content) and maximum (content in specification) % w/w of amino acids, organic carbons, potassium, and calcium

Nutrients	Min[a] guaranteed content (% w/w)	Min[a] oxide (% w/w)	Max[b] value in specification (% w/w)	Max[b] oxide (% w/w)
Organic carbon (C)	12.75 (23.2 on dry matter)		19.25 (38.5 on dry matter)	
Organic matter calculated	13.10 (26.2 on dry matter)		18.40 (33.4 on dry matter)	
Potassium (K)	6.70	8.05 K_2O[c]	10.20	12.30 K_2O[c]
Calcium (Ca)	0.75		1.70	
Total nitrogen (N)	0.60		1.10	
Organic nitrogen (N)	0.60		0.90	
Total amino acids[d]	1.80		2.20	

[a] Minimum

[b] Maximum

[c] Potassium oxide

[d] Total amino acids include glutamic acid, aspartic acid, alanine, and proline

primary, rapidly activated mechanism to thermally dissipate un-used energy for photochemistry is non-photochemical quenching (NPQ) [18]. Simultaneously, defence and secondary metabolic pathways are activated under genetic control modulated by phytohormone signalling and crosstalk to balance plant growth against environmental stress response. The phytohormones, abscisic acid (ABA), gibberellins (GAs), auxin (indole acetic acid, IAA), cytokinins (CKs), salicylic acid (SA) and jasmonic acid (JA) are the major environmental stress response regulators, whilst in potato CKs, GAs, and IAA also control tuber induction, development, and tuberisation [19].

We used physiological, molecular and genetic approaches to develop understanding of the underpinning mechanism of action of Quantis™ on PSII efficiency, plant photosynthesis, and potato productivity under heat stress. We report and validate here key candidate genes modulated by Quantis™ in the plant thermotolerance response and test the hypothesis that these abiotic stress-defence genes have overlapping functions in biotic stress defence to the fungal pathogens, *Sclerotinia sclerotiorum* and *Rhizoctonia solani*.

Materials and methods

Plant material and growth conditions

The potato (*Solanum tuberosum L.*) cultivar Maris Piper obtained from Syngenta Crop Protection, UK was firstly chitted under growth room conditions of 10 °C and 16-h photoperiod for 4 weeks until four sprouts of 1 cm were allowed to form on each potato seed prior to planting in 5-L pots containing John Innes 3 compost. The potato plants were then grown under glasshouse conditions with 16-h photoperiod, humidity set at 70%, and watering maintained at 40% field capacity. There were no pesticide or nutritional treatments applied to the potato plants.

An experiment with completely randomised design consisting of 14 replicates per treatment of water (control) or the biostimulant Quantis™ was performed twice. Quantis™ was applied using a hand-held sprayer at a rate of 0.2 ml m^{-2} (equivalent of field rate of 2 l ha^{-1}) at tuber initiation stage of potatoes, confirmed visually by the formation of tubers at the stolon tip, at 58 and 39 days post-sowing for experiment 1 and 2, respectively (time 0 h in Additional file 1: Figure S1). In both experiments, heat stress in the first 24 h post-biostimulant application was achieved by shutting the air vents in the glasshouse at 12 h following treatment. The ambient temperature in individual experiments varied between 20 and 36 °C with the highest temperatures of 35 °C and 38 °C recorded 12 h and 24 h post-treatment application in experiment 1 and 2, respectively (Additional file 1: Figure S1). Potato plants were grown to maturity, occurring at 101 and 81 days following sowing of chitted seed for experiment 1 and 2, respectively.

Seeds of *Arabidopsis thaliana* were obtained from NASC, UK except for TCSn::GFP DEAL line which was provided by Dr Ranjan Swarup, University of Nottingham, UK. The seeds were surface sterilised using 25% sodium hypochlorite (parazone, Jeyes Ltd, UK) and 0.2% Tween-20 prior to washing them three times with sterile distilled water. The seeds were plated on 50% Murashige and Skoog (MS) Basal Medium (pH 5.8) (Sigma-Aldrich, UK) with 1% agar and cold stratified for 2 days at 4 °C. The plates were placed vertically in a controlled environment with a 16-h photoperiod at 21 °C for 1 week prior to confocal microscopy and root length measurements. For functional validation, *A. thaliana* mutants were grown in plates as described above, before being transplanted in 3×4-well trays with M3 compost (Levington, Everris Limited, UK). The trays were kept in a controlled environment with a 16-h photoperiod at 22 °C.

Leaf gas exchange (GE) and chlorophyll fluorescence (CF) measurements

Infrared gas analyser (IRGA) and LI6800 (LI-COR, USA) were used to measure gas exchange (GE) and chlorophyll fluorescence (CF) simultaneously on the fully expanded terminal leaflet ($n=7$) at 0 h post-application (hpa) of water or the biostimulant (prior to heat stress), 12 h and 24 hpa. These measurements were performed on both experiments under glasshouse conditions. Leaves were dark adapted for 20 min by wrapping them in aluminium foil. The leaf was further dark adapted for 5 min in the cuvette prior to actinic light application. The fluorescence parameters, PSII maximum efficiency in the light (F_v'/F_m'), non-photochemical quenching (NPQ), quantum yield of PSII photochemistry (ΦPSII) were automatically calculated using LI-COR® software (LI-COR, USA) alongside gas exchange measurements [20].The IRGA conditions to measure photosynthesis traits were gas flow rate of 500 µmol air s^{-1} with a cuvette temperature of 21 °C, CO_2 concentration of 400 µmol mol^{-1}, relative humidity of 60% and actinic light of 1200 µmol m^{-2} s^{-1} PPFD or in darkness.

A hand-held portable fluorometer (FluorPen FP100, PSI, Czech Republic) was used on the same plants at 48 hpa to measure CF on the fully expanded terminal leaflet ($n=7$). Measurement of maximum fluorescence in light (F_m') was accomplished through a saturating light of 3000 µmol m^{-2} s^{-1}. CF parameters included (F_v'/F_m'), quantum yield (QY) and calculated ratios with JIP-test for dissipation energy flux (DIo/RC) and absorbed photon flux (ABS/RC) from OJIP florescence transient kinetics [21, 22]. At 7 dpa CF measurements were made again on the detached, fully expanded, terminal leaves for each treatment ($n=7$) using a closed 800C FluorCam imaging fluorometer (PSI, Czech Republic). Detached leaves were placed adaxial side up between two 1-cm-thick glass plates containing a damp filter paper and dark adapted for 1 h into the FluorCam imaging chamber. A saturating light pulse (1000 µmol m^{-2} s^{-1} photosynthetic photon flux density (PPFD), 800 ms duration) was applied to measure non-photochemical quenching (NPQ) and QY [23]. The FluorCam was also used to measure NPQ and QY on *A. thaliana* wild type Columbia-0 (Col-0) and the mutants, *penetration1* (*pen1*), *maternal effect embryo arrest 59* (*mee59*) and *pathogenesis related 4* (*pr4*) ($n=6$) at 24 hpa with heat stress of 42 °C for 4 h at 12 hpa. Measurements were carried out on the whole Arabidopsis plants (GS 1.07) in 3 × 4-well trays following dark adaptation for 1 h in the imaging chamber prior to application of a saturating pulse (1000 µmol m^{-2} s^{-1} PPFD).

Tuber yield components

Total tuber weight from the water-control and the biostimulant-treated plants ($n=14$) in each experiment was determined at crop maturity. Tuber size (mm) was measured using a potato grading square (Syngenta, UK), allowing classification of potato tubers into size of less or more than 40 mm of diameter.

Transcriptomics and gene expression of *Solanum tuberosum L.*

Potato leaf samples (100 mg) were collected at 24 hpa (following heat stress for 12 h) ($n=7$) and snap frozen in liquid nitrogen for RNA extraction using RNeasy Plant kit (Qiagen) along with RNase-free DNase kit (Qiagen) to remove residual DNA from the samples. RNA samples were sent to a commercial sequencing provider (Novogene, Cambridge, UK) for mRNA sequencing. The sequencing libraries were generated using a NEBNext® Ultra™ RNA library preparation kit for Illumina® (NEB, USA). Messenger RNA was purified from total RNA using poly-T oligo-attached magnetic beads. After fragmentation, the first strand cDNA was synthesised using random hexamer primers, followed by the second strand cDNA synthesis using either dUTP for directional library or dTTP for non-directional library. The library was checked with Qubit and real-time PCR for quantification and bioanalyser for size distribution detection. The clustering of the index-coded samples was performed according to the manufacturer's instructions. After cluster generation, the library preparations were sequenced on an Illumina platform and paired-end reads were generated on an Illumina® NovaSeq 6000 sequencing system which employs a paired-end 150 bp short read sequencing carried out with 20 million reads per sample. Raw data (raw reads) of FASTQ format were firstly processed through fastp. In this step, clean data (clean reads) were obtained by removing reads containing adapter and poly-N sequences and reads with low quality from raw data. At the same time, Q20, Q30 and GC content of the clean data were calculated. All the downstream analyses were based on the clean data with high quality. Reference genome and gene model annotation files were downloaded from genome website browser (ensemblplants_solanum_tuberosum_soltub_3_0_gca_000226075_1) directly. Paired-end clean reads were mapped to the reference genome using histat2 v2.05. HISAT2 uses a large set of small GFM indexes that collectively cover the whole genome. Differential expression analysis between biostimulant-treated and water-treated plant groups (seven biological replicates per treatment) was performed using DESeq2 R package [24]. DESeq2 provides statistical routines for determining differential

expression in digital gene expression data using a model based on negative binomial distribution. The resulting P values were adjusted using the Benjamini and Hochberg's approach for controlling False Discovery Rate (FDR). Genes with an adjusted *P* value < 0.05 and log2-fold change > 0 found by DESeq2 were assigned as significantly differentially expressed. Biological functions or pathways that are significantly associated with differentially expressed genes (DEGs) were investigated through Gene Ontology (GO) and Kyoto Encyclopaedia of Genes and Genomes (KEGG) enrichment analysis implemented by clusterProfiler R package [25], in which gene length bias was corrected. GO and KEGG terms with padj < 0.05 were considered significantly enriched by DEGs. The top significant DEGs genes from the RNA-seq data were also validated using gene expression analysis. Strand cDNA was synthesised according to manufacturer's protocol using iScript cDNA synthesis kit (Bio-Rad). Quantitative reverse transcription PCR (RT-qPCR) was conducted with Sybr Green (Bio-Rad) in a CFX96 Touch Real-Time PCR Detection System (BioRad). The protocol consisted of pre-denaturation for 5 min at 94 °C, followed by 40 cycles of 94 °C for 15 s and 54 °C for 30 s. Primers used for target gene expression are shown in Additional file 5: Table S2. Relative quantification for untreated (water control) and treated (Quantis™) was calculated using $2^{-\Delta\Delta C}{}_{T}$ method [26] where gene expression was normalised to the mean Ct values of reference genes *EF1α* and *CUL3A*.

Candidate gene validation in *Arabidopsis thaliana*

From the top 5 DEGs with *p* adjust value < 0.05 and with a log2-fold change of ≥ + 0.5, only gene orthologues of *Arabidopsis thaliana* with > 40% identity (At3G11820, AT3G04720 and AT4G37300) with potato genes (PGSC0003DMG400021331, PGSC0003DMG400019437, PGSC0003DMG400019435, PGSC0003DMG400020844) were identified through Ensembl Plants database. The corresponding gene mutants (*pen1*, *pr4* and *maternal effect embryo arrest 59* (*mee59*)) were used for downstream functional analysis (Table 2). *A. thaliana* gene orthologue, AT3G04720, for transcript IDs PGSC0003DMG400019437, PGSC0003DMG400019435 is the same.

Heat stress experiments with *A. thaliana*

A. thaliana Col-0 and mutants *pen1*, *pr4* and *mee59* ($n = 8$) were surface sterilised and grown in 50% MS basal medium for 1 week prior to mist application of Quantis™ at 0.2 ml m^{-2} or sterile distilled water as control. Root length was measured using ImageJ with SmartRoot plugin [27] on photographs taken at 0, 1, 2 and 5 dpa with heat stress (42 ℃) at 12 hpa for 4 h. The experiment was repeated twice. The data from 24 hpa, 48 hpa and 5 dpa were converted to area under root growth curve (AURGC) calculated as cumulative root growth using the formula [28] below:

$$AURGC = \sum [(x_{i+1} + x_i)/2](t_{i+1} - t_i),$$

where x_i = root growth at the ith time of root measurement; x_{i+1} = root growth at the $i+1$ root measurement time; $t_{i+1} - t_i$ = the number of time points between ith and $i+1$ root growth measurement time.

Fungal pathogen inoculation and disease assessment

Rhizoctonia solani (*R. solani*) anastomosis group (AG) 2–1 of known pathogenicity to *Arabidopsis thaliana* ([29]; isolate 1934, University of Nottingham isolate

Table 2 Top significant differentially expressed genes (DEGs) in *Solanum tuberosum* following application of Quantis™ shown with their *A. thaliana* gene orthologues, and respective *A. thaliana* mutants. Percentage (%) identity to the transcript (*S. tuberosum*) gene ID is included

Solanum tuberosum gene ID	Description	log_2fold change	Padj[a]	% Query cover	% identity[b]	*A. thaliana* orthologue	NASC ID[c]
PGSC0003DMG400014013	Flavonoid 3-hydroxylase	1.54	4.11E-02	99	37.81	AT4G36220	N575876
PGSC0003DMG400021331	Penetration 1	1.43	4.49E-02	84	76.98	AT3G11820	N666629
PGSC0003DMG400014776	Tyramine hydroxycinnamoyl transferase	1.41	6.72E-03	94	36.06	AT2G39030	N567947
PGSC0003DMG400019437	Pathogen- and wound-inducible antifungal protein CBP20	1.32	1.14E-02	84	73.55	AT3G04720	N666544
PGSC0003DMG400019435	Wound-induced protein WIN1	0.84	1.20E-03	96	66.15	AT3G04720	N666544
PGSC0003DMG400020844	Seed dormancy, maternal effect	0.58	3.04E-05	96	40	AT4G37300	N664015

[a] *p* adjust value

[b] % identity allows filtering of hits by degree of identity

[c] Nottingham Arabidopsis Stock Centre

collection) and *Sclerotinia sclerotiorum* (University of Nottingham isolate collection) were used to determine the functionality of candidate genes in response to biotic stress. Both fungal pathogens were cultured on potato dextrose agar (PDA) (Sigma Aldrich, UK) at 20 °C for 6 days. Seeds of *A. thaliana* Col-0, *pen1*, *pr4* and *mee59* were surface sterilised and grown in 50% MS basal medium for 1 week as described above prior to transplanting seedlings into 3×4 trays with M3 compost (Levington, Everris Limited, UK). At GS 1.08, seedlings were inoculated with *S. sclerotiorum* by placing three (1 plug per leaf) 4-mm-diameter PDA plugs (*S. sclerotiorum* colonised or non-inoculated) per plant on adaxial side of each of the three leaves. *R. solani* AG2-1 inoculation was performed by placing ten 6 mm diameter PDA plugs (AG2-1 colonised or non-inoculated) per tray well 3 cm from the top of the compost prior to transplanting seedlings from the MS basal media plates. A completely randomised design for genotype (Col-0, *pen1*, *pr4* or *mee59*) with 6 replications was used for pathogen experiments repeated twice. The plants were kept in a controlled environment with a 16-h photoperiod at 22 °C for 7 days. Photographs for disease assessments were taken using a Nikon D600 DSLR camera at 7 days post-inoculation (dpi). Leaf blight lesions by *R. solani* AG2-1 and chlorosis/necrosis lesions by *S. sclerotiorum*, were determined by measuring, using ImageJ [27], diseased plant area and expressing it as percentage from the whole plant leaf area (GS 3.20).

Analysis of endogenous phytohormones

The endogenous content of the phytohormones gibberellin A4 (GA4), trans-zeatin (Z), abscisic acid (ABA), auxin (IAA), salicylic acid (SA) and jasmonic acid (JA) were quantified using liquid chromatography coupled to electrospray ionisation tandem mass spectrometry (LC/ESI–MS/MS) as described by Müller and Munné-Bosch [26]. Freeze-dried leaf samples (100 mg) of each plant ($n=7$) used for the RNA-sequencing were repeatedly extracted with a mixture of methanol:isopropanol:acetic acid, 50:49:1 (v/v/v) using ultra-sonication (Branson 2510 ultrasonic cleaner, Bransonic, Danbury, CT, USA). Deuterium-labelled plant hormones were added to the initial extract to estimate recovery rates and correct for potential hormone losses during extraction. The resulting extracts were vortexed and centrifuged at 4 °C for 10 min at 1300 rpm, and supernatants were collected, pooled and filtered using 0.22-µm hydrophobic PTFE Syringe Filters (Phenomenex, Torrance, CA, USA) prior to injection into the UHPLC/ESI–MS/MS system. All hormones were analysed using negative ion mode, except cytokinins, which were measured using positive ion mode with conditions set as described by [30].

Microscopy and imaging of *A. thaliana* TCSn::GFP DEAL

A. thaliana TCSn::GFP DEAL seeds ($n=3$) were surface sterilised as described above and grown in MS basal medium for 1 week prior to mist application of Quantis™ at 0.2 ml m^{-2} or sterile distilled water as control. At 12 hpa, the seedlings were heat stressed in a controlled environment for 4 h at 42 °C and seedlings roots were imaged with the Leica SP5 Confocal microscope (Leica Microsystems, Germany). This experiment was repeated twice. Relative GFP-fluorescence on primary root tip was quantified by measuring the green pixels present at 4 h post-heat stress (hphs) using ImageJ [27].

Statistical analysis

GenStat v. 21.1 (VSN International Ltd, UK) was used for all data analysis. There were no interactions between individual experiments and treatment and therefore experiments were used as replicates in statistical analysis. Two-sample Student's t-test was used for comparisons in the data with normal distribution of residuals, with treatment effects considered significantly different at $P<0.05$. Generalised linear model (GLM) with binomial distribution, logit link function and treatment included as a fitted term was used to analyse the tuber size data. GLM with Poisson distribution, logarithm function and treatment and rep included as fitted terms was used for tuber weight data.

Results

Quantis™ application increases the efficiency of PSII photochemistry, tuber weight and size of potato under heat stress

Application of Quantis™ followed by heat stress at 12 h post-application (hpa) resulted in significantly higher operating efficiency of photosystem (PS) II photochemistry (F_v'/F_m') and quantum yield of electron transport, measured as ΦPSII at 24 hpa, in treated plants compared to the water control (Fig. 1a, b). In contrast, thermal dissipation of excitation energy in the light-harvesting complexes of PSII, estimated as non-photochemical quenching (NPQ), was significantly reduced by Quantis™ (Fig. 1c). There were no differences between treatments for stomatal conductance, and although photosynthesis was higher in Quantis™-treated plants the difference with the control plants was not statistically significant ($p=0.10$) (Fig. 1d,e). Increased quantum yield (QY) and F_v'/F_m' (Fig. 2a, b), consistent with reductions in absorption (ABS/RC) and dissipation (DIo/RC) energy fluxes per reaction centre (Fig. 2c,d) were measured at 48 hpa of the biostimulant. At 7 days post-application (dpa), Quantis™-treated plants exhibited significantly higher QY (Fig. 3a, b) and lower NPQ (Fig. 3c,d) compared to the control.

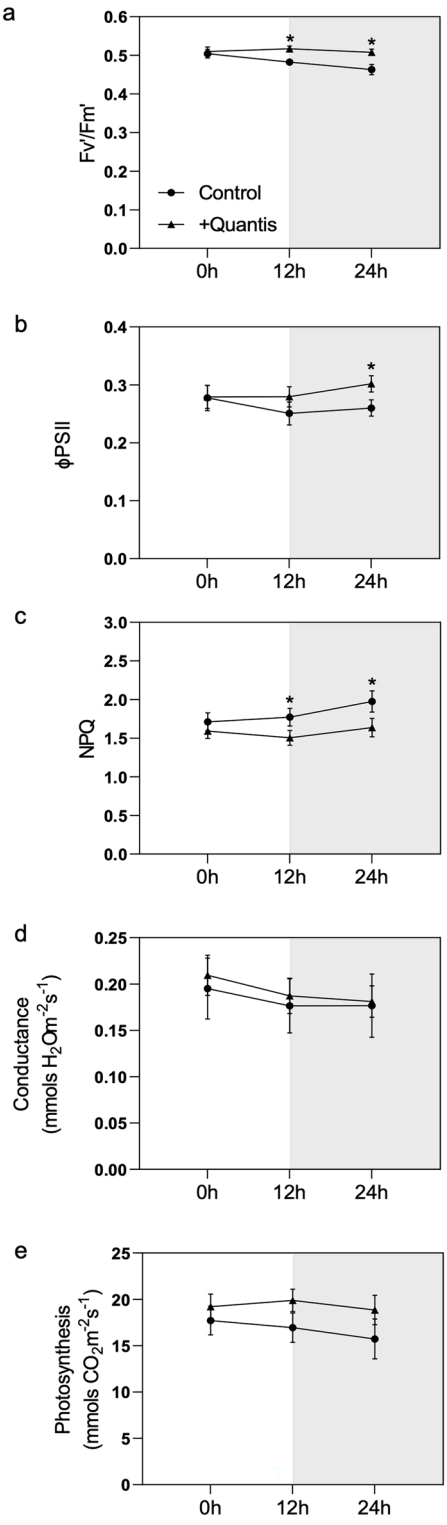


Fig. 1 Chlorophyll fluorescence and photosynthetic activity of Quantis™-treated and untreated (water-control) potato plants from 0 h to 24 h post-Quantis.™ application and 12 h post-heat stress. **a** PSII maximum efficiency (F_v'/F_m'), **b** quantum yield of PSII photochemistry (ΦPSII), **c** non-photochemical quenching (NPQ), **d** stomatal conductance, and **e** rate of photosynthesis. Ambient temperature varied from 20–36 ℃, with highest temperature of 35 ℃ and 38 ℃ recorded at 12 h and 24 h post-application. Grey shading a–e shows period of heat stress from 12 h post-application. Data represent mean ± *SEM* of seven biological replicates per treatment with two experimental replicates. Asterisk indicates significant difference according to Student's *t* test, $p < 0.05$

A significantly higher (4%) potato tuber weight (Fig. 4a) and greater percentage of tubers (40%) with sizes of > 40 mm (Fig. 4b) yielded from the biostimulant-treated plants compared with the water control.

Gene expression profiling shows significantly enriched molecular functions and transduction pathways for environmental stress response upon Quantis™ treatment

To investigate the molecular mechanism of Quantis™ action on potato plants under heat stress, we performed RNA-sequencing analysis on leaf samples taken at 24 hpa and heat stress of 12 h. The results showed 198 and 283 genes that were uniquely expressed in the control or Quantis™ treated samples, respectively, whilst 16689 genes were common and expressed in both samples (Fig. 5a).

The overall distribution of differentially expressed genes (DEGs) (Additional file 4: Table S1) showed 782 upregulated and 462 downregulated genes due to treatment and heat stress (Fig. 5b). From all DEGs, only 11 were significant at padj < 0.05 (Additional file 4: Table S1). These genes included just one downregulated (− 0.25 log2fold change) orphan potato gene with unknown function (PGSC0003DMG402023118). Ten genes were upregulated (padj < 0.05) by Quantis™ (Additional file 4: Table S1) and included three conserved genes with unknown functions (PGSC0003DMG400016462, PGSC0003DMG400014284, PGSC0003DMG400020844), one gene (PGSC0003DMG400001632) encoding Translocon-associated protein, alpha subunit with duplicated blocks on *S. tuberosum* chromosomes, the circadian clock-associated *StFKF1* gene, (PGSC0003DMG400019971), regulating positively flower development in potato, and wound-induced protein WIN1 (PGSC0003DMG400019435) involved in the plant defence response. The remaining top four significant, differentially expressed genes (Table 2), flavonoid 3-hydroxylase (PGSC0003DMG400014013), PEN1 syntaxin, (PGSC0003DMG400021331), tyramine hydroxycinnamoyl transferase (PGSC0003DMG400014776) and pathogen- and wound-inducible antifungal protein

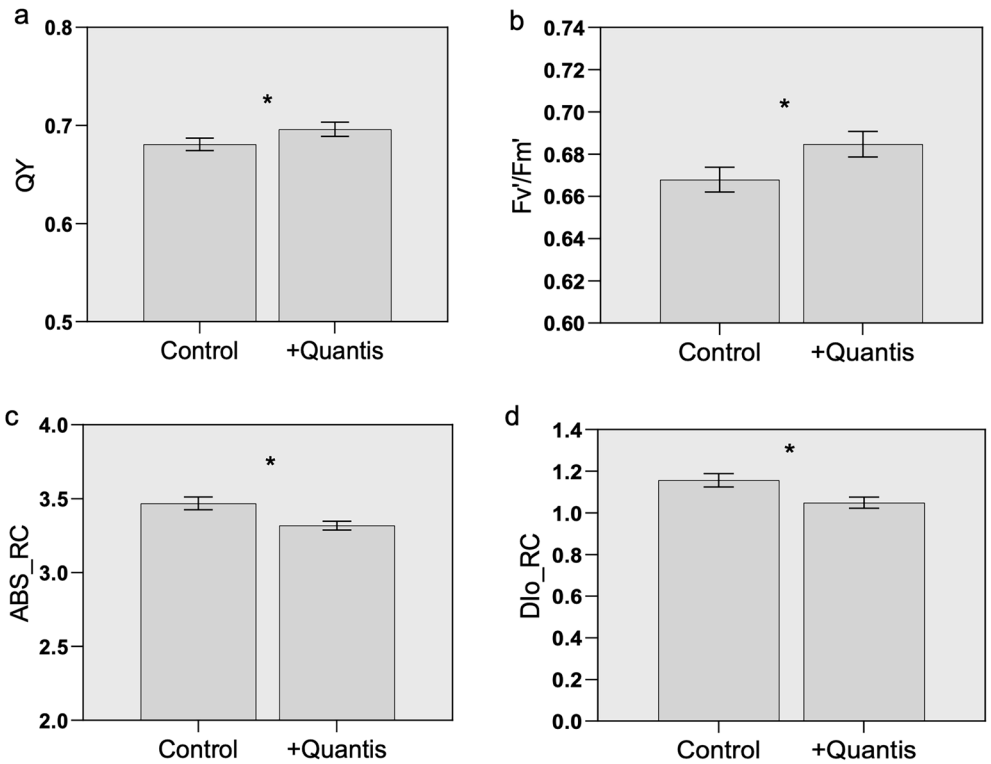


Fig. 2 Chlorophyll fluorescence of Quantis™-treated and untreated (water-control) potato (cv. Maris Piper) plants at 48 h post-Quantis™ application (36 h post-heat stress) measured with modulated fluorometer FluorPen (PSI, Czech Republic). **a** Quantum yield (QY), **b** PSII maximum efficiency (F_v'/F_m'), **c** absorbed photon flux per reaction centre (ABS/RC) and **d** dissipation energy flux per reaction centre (Dio/RC) measured on the fully expanded terminal leaflet. Data represent mean ± *SEM* of seven biological replicates per treatment with two experimental replicates. Asterisk indicates significant difference according to Student's *t* test, $p < 0.05$

CBP20 (PGSC0003DMG400019437), with the highest log2-fold change in expression (Additional file 4: Table S1) due to biostimulant treatment compared to the control were used for validation using qRT-PCR (Additional file 5: Table S2) because they all encoded molecules with known functions in plant defence to environmental stress. Flavonoid 3′-hydroxylase (F3′H) catalyses hydroxylation at the 3′-position of dihydrokaempferol to yield dihydroquercetin downstream of naringenin, directing flavonoid biosynthesis towards some of the most potent biological compounds with antioxidant function including anthocyanins [31]. Hevein-like preproprotein WIN1 and pathogen- and wound-inducible antifungal protein CBP20 were identified as the pathogenesis protein 4 (PR4) orthologue in Arabidopsis, encoding a chitinase of Class II. PEN1 encoding syntaxin is known to function in defence to biotic stress [32]. Tyramine hydroxycinnamoyl transferase is responsible for the synthesis of phenolamides, associated with cell wall fortification [33] and ROS scavenging under environmental stress [34]. qRT-PCR results showed that all four genes were indeed significantly upregulated following Quantis™ application (Fig. 6a–d) in agreement with the RNA-seq results.

GO and KEGG analyses were performed with all DEGs to identify significant plant cellular, molecular, or biological functions, and metabolic or signalling pathways enriched by differential gene expression of heat-stressed plants treated with Quantis™. DEGs upregulated in Quantis™-treated plants in comparison to the control enriched significantly GO terms (Additional file 6: Table S3) including ribosome and structural constituent of ribosome ($n = 39$), structural molecule activity ($n = 41$), defence response ($n = 29$), chitinase activity ($n = 5$), isoprenoid binding ($n = 4$), glucosidase activity ($n = 4$), abscisic acid binding ($n = 4$), enzyme inhibitor activity ($n = 17$), oxidoreductase activity acting on paired donors, with oxidation of a pair of donors resulting in the reduction of molecular oxygen to two molecules of water ($n = 6$), alcohol binding ($n = 4$) and carbon–carbon lyase activity ($n = 12$) (Fig. 7a). There were no significantly enriched GO terms with downregulated DEGs. Significant KEGG pathway terms (Additional file 7: Table S4) included ribosome ($n = 37$), MAPK signalling ($n = 24$), plant–pathogen

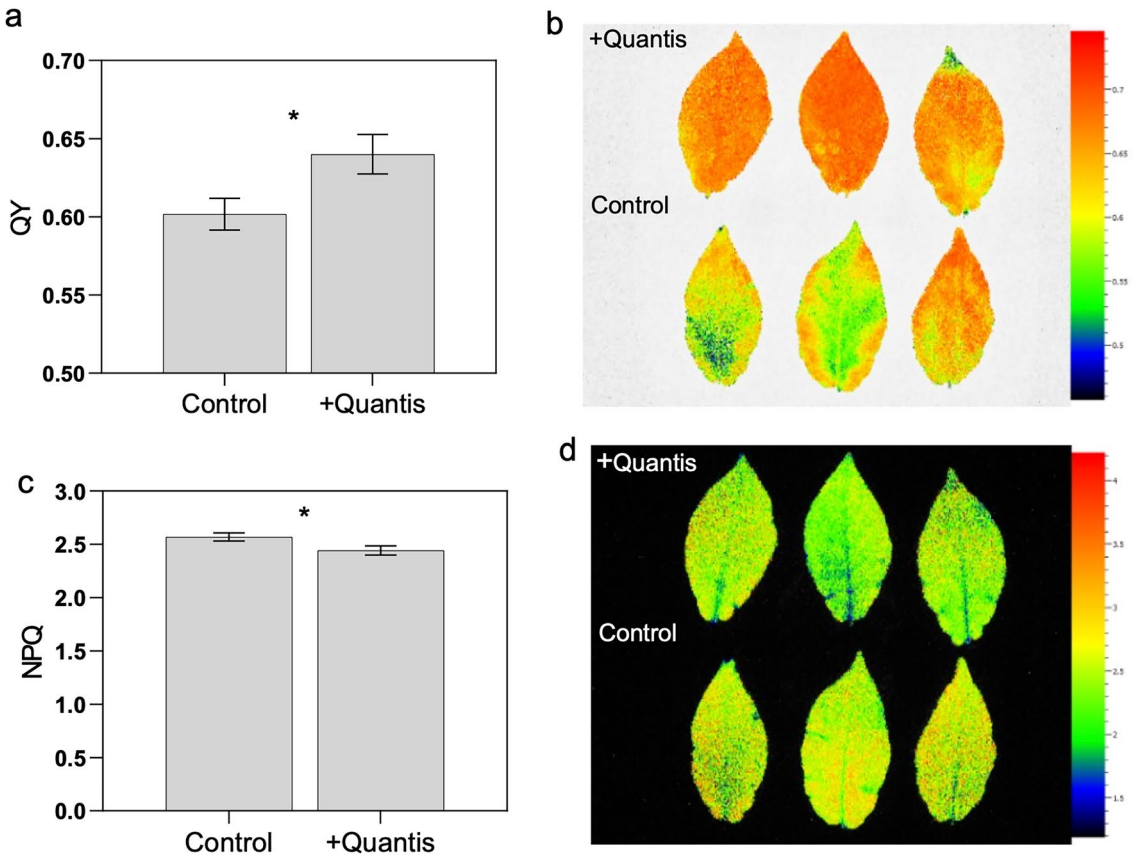


Fig. 3 Chlorophyll fluorescence of Quantis™-treated and untreated (water-control) detached fully expanded terminal potato (cv. Maris Piper) leaves. **a**, **b** Quantum yield (QY), (c,d) non-photochemical quenching (NPQ) measured at 7 days post-application using a closed 800C FluorCam imaging fluorometer (PSI, Czech Republic). Data represent mean ± *SEM* of seven biological replicates per treatment with two experimental replicates. Asterisk indicates significant difference according to Student's *t* test, $p < 0.05$

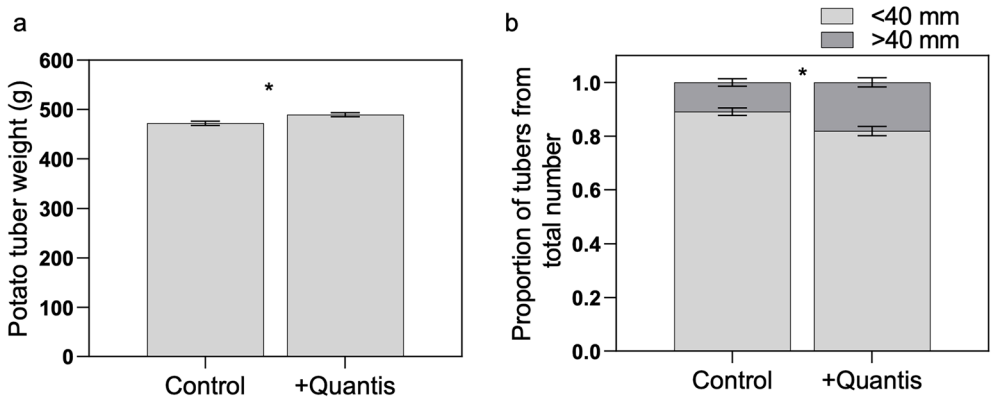


Fig. 4 **a** Mean tuber weight per plant and **b** proportion of tubers from the total number of tubers of (water-control) and Quantis™-treated potatoes (cv. Maris Piper) graded as less or more than 40 mm at maturity. Back-transformed means shown ± *SEM* of 14 biological replicates per treatment from two experimental replicates. Generalised linear model with Poisson and log function with treatment included as fitted term used for tuber weight analysis. Generalised linear model with binomial distribution, logit function with treatment and rep included as fitted terms used for tuber size analysis. Chi pr < 0.001 is indicated by an asterisk

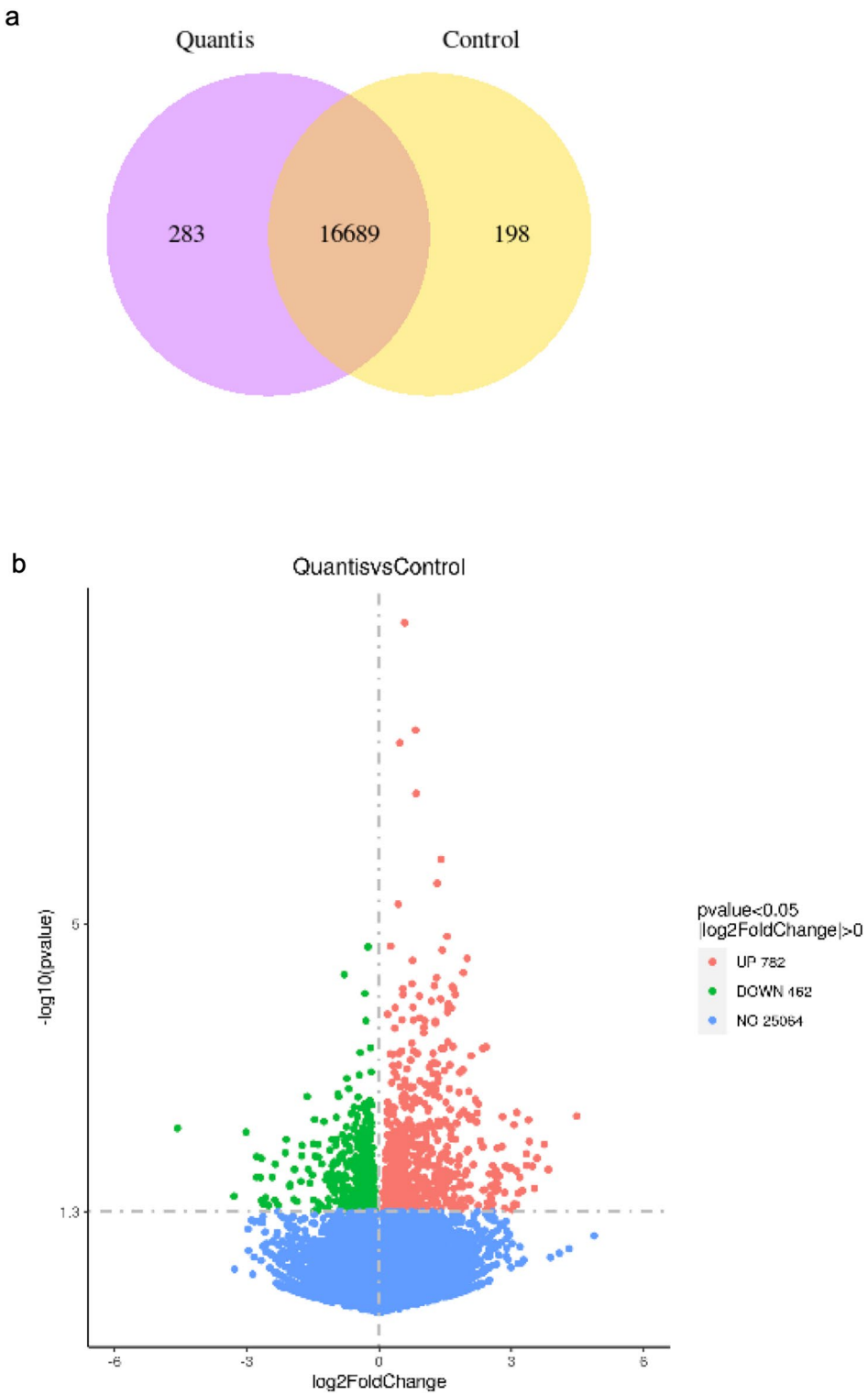


Fig. 5 Transcriptome profiling using RNA-seq of potato (cv. Maris Piper) leaf samples collected at 24 h post-application (following heat stress for 12 h). **a** Venn diagram showing number of differentially expressed and co-expressed genes for the control (water) and Quantis.™ and **b** volcano plot showing upregulated and downregulated genes. Full list of annotated DEGs for treatment comparisons is contained within Additional file 4: Table S1

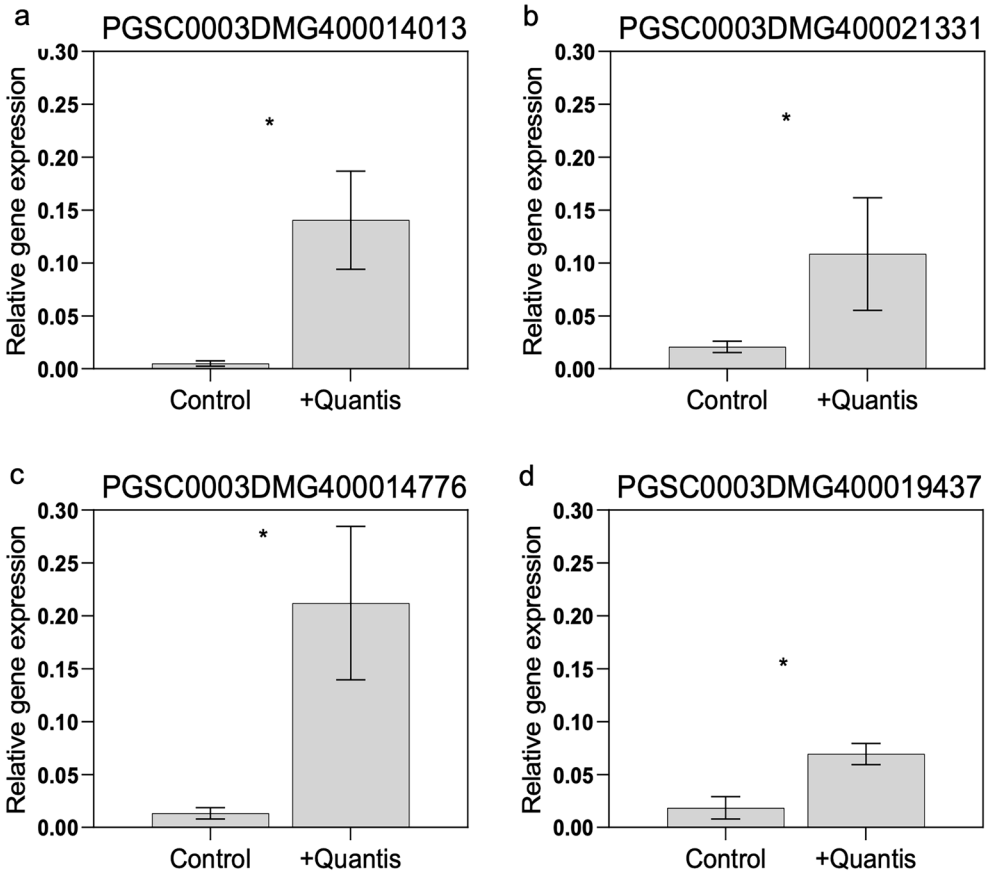


Fig. 6 Quantitative gene expression using qRT-PCR for the top significant differentially expressed genes by Quantis™, **a** PGSC0003DMG400014013, **b** PGSC0003DMG400021331, **c** PGSC0003DMG400014776 and **d** PGSC0003DMG400019437. RNA extraction from potato (cv. Maris Piper) leaf samples collected at 24 h post-application. Data represent mean ± *SEM* of seven biological replicates per treatment. Asterisk indicates significant difference according to Student's *t* test, $p < 0.05$

interaction ($n = 25$) and zeatin biosynthesis ($n = 12$) (Fig. 7b). From these KEGG terms, all were enriched with upregulated DEGs following treatment with Quantis™ apart from zeatin biosynthesis.

Quantis™ increases plant tolerance to heat stress by regulating the expression and functionality of *PEN1*, *PR4* and *MEE59*

To functionally validate the plant genetic control by Quantis™ under heat stress, we identified gene orthologues in *A. thaliana* with at least 40% identity to the candidate genes of *S. tuberosum* with log2-fold change > 0.5 and padj < 0.05 (Table 2 and Additional file 4: Table S1). Based on this, PEN1, PR4 and MEE59 were chosen as Arabidopsis orthologues of the potato candidate genes for further analysis (Table 2).

To confirm the above ground CF phenotype, previously identified in potato plants, following Quantis™ treatment, and to functionally validate the Quantis™-candidate genes for their effect on PSII efficiency and NPQ, we measured CF of the *A. thaliana* mutants, *pen1*, *pr4* and *mee59* (Table 2) following exposure to heat stress of 42 ℃. *A. thaliana* Col-0 exhibited the same CF phenotype observed in potato, following Quantis™ treatment and heat stress, manifesting as significantly increased QY and decreased NPQ in comparison to the control (Fig. 8a, b). Compared to Arabidopsis Col-0 under mock (water) treatment, *mee59* had significantly lower QY, whilst both *mee59* and *pr4* had higher NPQ suggesting a function for both PR4 and MEE59 in photoprotection (Fig. 8a, b). There were no significant QY or NPQ responses of the mutants, *pen1* and *pr4*, to Quantis™, however we measured lower NPQ of *mee59* following the biostimulant treatment indicating that Quantis™ may downregulate NPQ in the absence of functional MEE59 (Fig. 8a, b).

To investigate below ground functions of Quantis™-gene candidates we carried out additional heat stress experiments to phenotype root growth under heat stress. Stress exerted on *A. thaliana* seedlings from increasing ambient temperature from 22 °C to 30 °C

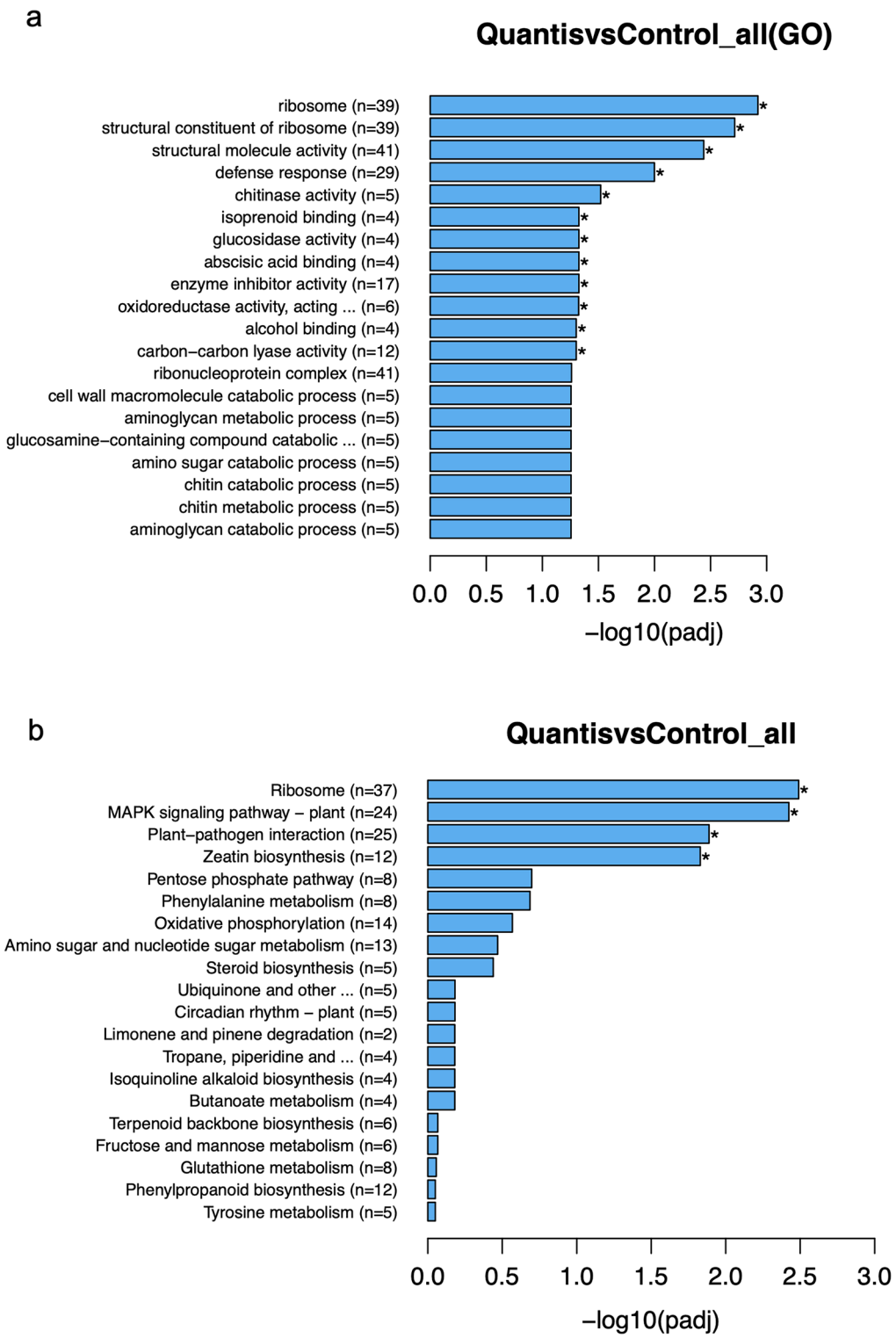


Fig. 7 Transcriptome profiling of potato leaf samples at 24 h post-treatment (following heat stress for 12 h) showing **a** Gene Ontology (GO) enrichment analysis using differential gene expression with significant pathway terms indicated by an asterix, and **b** Kyoto Encyclopaedia of Genes and Genomes (KEGG) enrichment analysis using differential gene expression with significant pathway terms indicated by an asterisk. Differential gene expression data were subjected to a screening with a false discovery rate (FDR) of < 1 and considered significantly differentially expressed with adjusted *p* adjust value < 0.05. Full list of potato transcripts in GO and KEGG analyses are contained in Additional file 6: Tables S3 and Additional file 7: Table S4, respectively

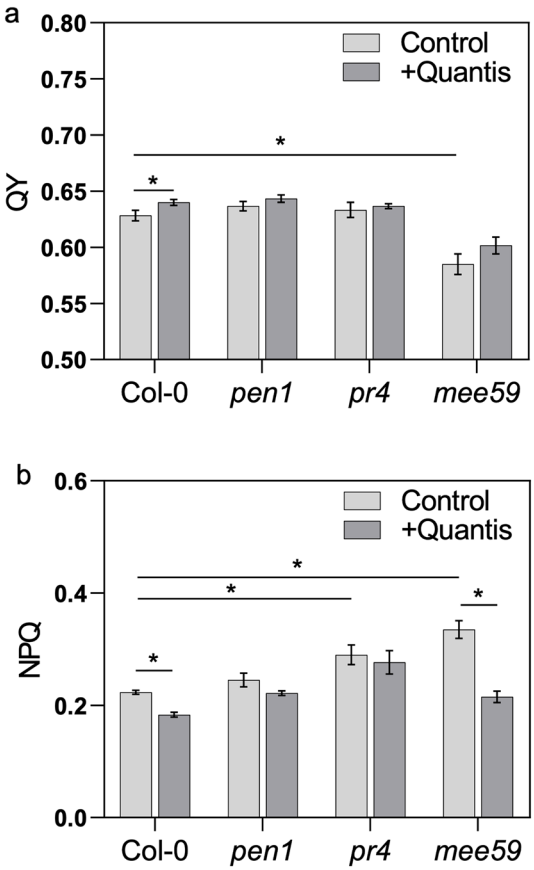


Fig. 8 Chlorophyll fluorescence **a** quantum yield (QY) and **b** non-photochemical quenching (NPQ) of Quantis™-treated and untreated (water-control) *A. thaliana* WT-type Columbia-0 (Col-0), *penetration1* (*pen1*), *pathogenesis related 4* (*pr4*) and *maternal effect embryo arrest 59* (*mee59*) measured at 24 h post-application with heat stress of 42 °C was applied 12 h post-application for 4 h. Data represent mean ± *SEM* of six biological replicates per treatment. Two experiments were performed. Asterisk indicates significant difference according to Student's *t* test, $p < 0.05$

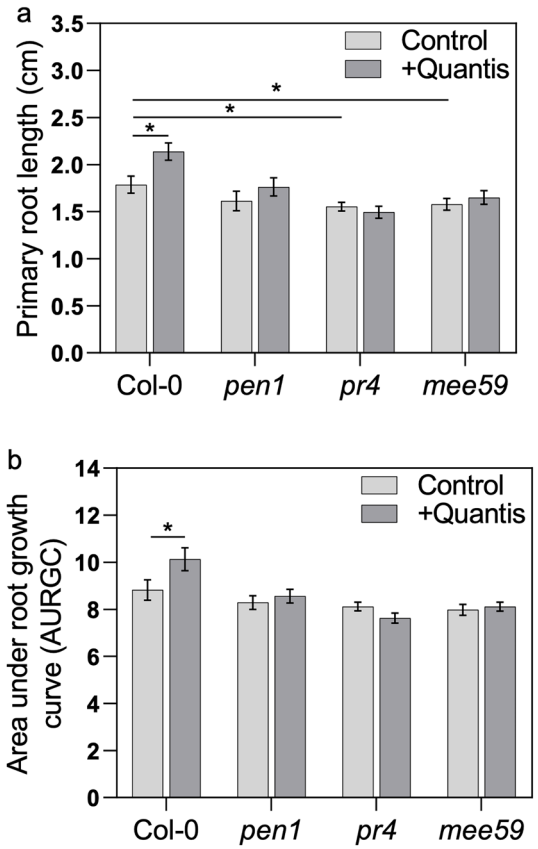


Fig. 9 Primary root length (cm) measured **a** 12 h following heat stress and **b** area under root growth curve from 24 h to 5 days of untreated (water-control) and Quantis™-treated *A. thaliana* WT-type Columbia-0 (Col-0), *penetration1* (*pen1*), *pathogenesis related 4* (*pr4*), *maternal effect embryo arrest 59* (*mee59*). Heat stress of 42 ℃ was applied 12 h post-Quantis™ application for a period of 4 h. Data represent mean ± *SEM* of eight biological replicates per treatment. Two experiments were performed. Asterisk indicates significant difference according to Student's *t* test, $p < 0.05$

results in significant inhibition of root growth of up to 50% [35]. Our results showed that following 4 h of heat stress, Quantis™ increased root length of Col-0 by 18% but failed to increase the root length of the mutants, *pen1*, *pr4* and *mee59* (Fig. 9a). Furthermore, *pr4* and *mee59* exhibited significantly shorter roots compared to Col-0 under mock (water) treatment (Fig. 9a) suggesting a root protective function of PR4 and MEE59 under elevated temperature. To determine the effects on root development over the time of 24 h to 5 days we calculated area under the root growth curve (AURGC) (Fig. 9b). The AURGC of Arabidopsis Col-0 increased by 12% following Quantis™ application in contrast to the mutants, which failed to respond to treatment (Fig. 9b). There were no significant differences between Col-0, *pen1*, *pr4* and *mee59* for AURGC under untreated conditions suggesting that the protective function of the Quantis™-gene candidates was exerted in the first 24 h following heat stress (Fig. 9b).

Collectively these results show that Quantis™ positively regulated above and below ground heat stress tolerance in plants exhibited as increased plant PSII operating capacity and protective root growth via modulation of the expression and functionality of PEN1, PR4 or MEE59.

PEN1, PR4 and MEE59 modulated by Quantis™ have no function in defence to *Sclerotinia sclerotiorum* and *Rhizoctonia solani* AG2-1

To investigate PEN1, PR4 and MEE59 functionality in defence to fungal pathogen infection, the mutants *pen1*, *pr4* and *mee59* along with Col-0 as the control were used for characterisation of Sclerotinia and

Damping-off disease phenotypes. There were no significant differences for disease severity by *Sclerotinia sclerotiorum* or *R. solani* AG2-1 at 7 dpi between the wild type *A. thaliana* Col-0 and the mutants suggesting that these genes do not function in increasing resistance to *Sclerotinia sclerotiorum* or *R. solani* AG2-1 (Additional file 2: Figure S2).

Quantis™ modulates leaf endogenous ABA, GA and CK content and enhances CK activity in roots under heat stress

To investigate hormonal variations caused by Quantis™ in potato plants under heat stress, we performed hormonal profiling of potato leaves at 24 hpa and 7dpa. The results showed 66% and 46% reduction of GA4 and trans-zeatin, respectively, at 24hpa due to Quantis™ treatment (Fig. 10a, b). However, at 7 dpa, trans-zeatin and ABA increased by 37% and 55%, respectively, in Quantis™-treated plants compared

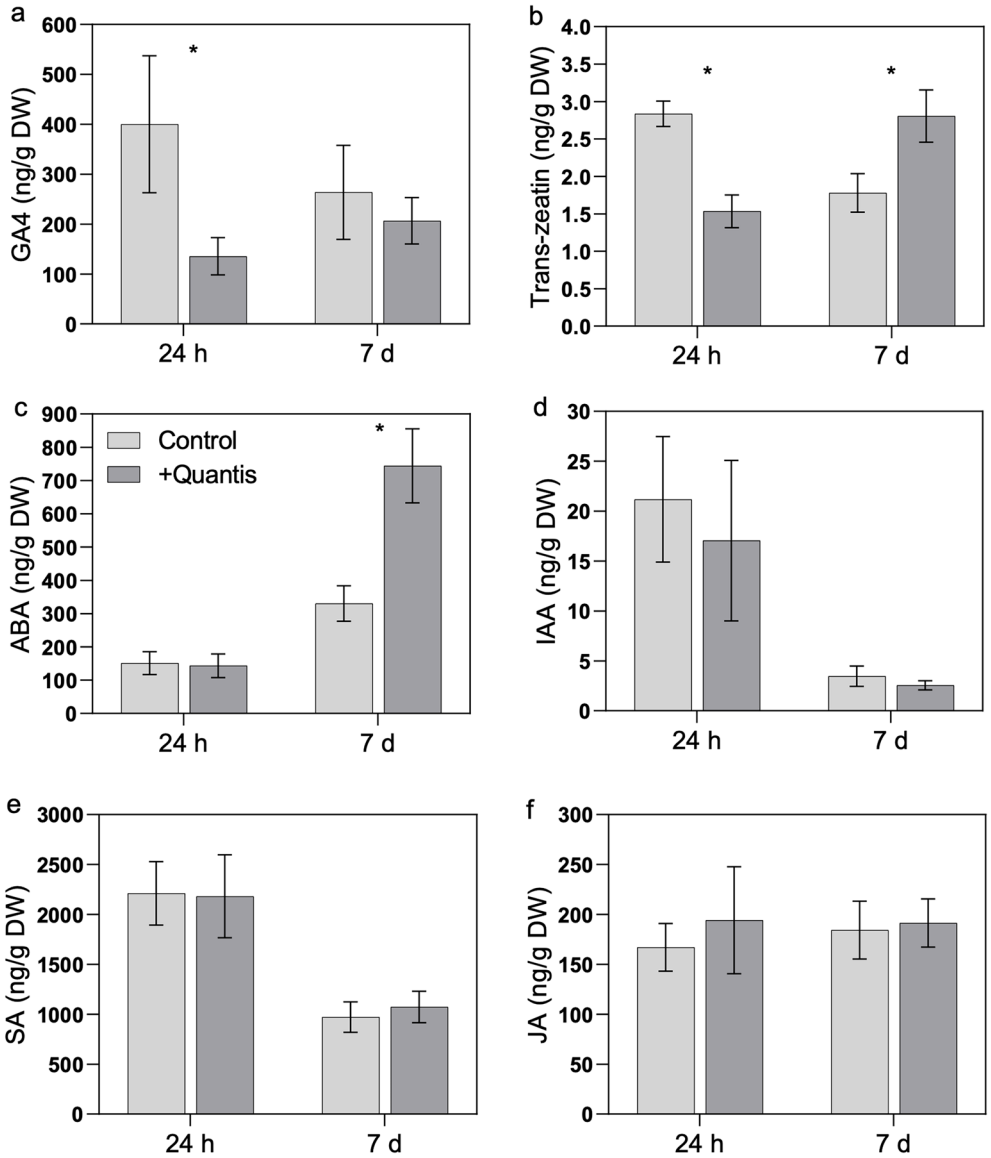


Fig. 10 Hormonal profiling of leaf samples of Quantis.™-treated and untreated (water-control) potato (cv. Maris Piper) collected at 24 h post-application (following heat stress for 12 h) and 7 days post-application. **a** Gibberellin A4 (GA4), **b** trans-zeatin (Z), **c** abscisic acid (ABA), **d** auxin (IAA), **e** salicylic acid (SA), **f** jasmonic acid (JA) Concentrations of endogenous phytohormones were determined by liquid chromatography coupled to electrospray ionisation tandem mass spectrometry (LC/ESI–MS/MS). Data represent mean ± *SEM* of seven biological replicates per treatment. Asterisk indicates significant difference according to Student's *t* test, $p < 0.05$

to the water control (Fig. 10b, c). There were no significant differences between treated and untreated plants for IAA, SA and JA content at 24 hpa or 7 dpa (Fig. 10d–f). Results from the hormonal profiling and the KEGG enriched zeatin biosynthesis pathway suggested that endogenous cytokinin content in plant leaves is modified by the biostimulant application under heat stress. Under heat stress cytokinin synthesis, transport and signalling in plant roots and shoots are inhibited resulting in increased leaf senescence and reduced root viability and growth [36]. To investigate if Quantis™ maintains cytokinin signalling in plant roots under heat stress, we performed confocal microscopy on *A. thaliana* cytokinin sensor TCSn::GFP DEAL line which was either treated with the biostimulant or with water, and exposed to temperature of 42 °C 12 hpa for 4 h. We visually confirmed (Fig. 11a, Additional file 3: Figure S3) and quantified (Fig. 11b) a significant increase in relative GFP-fluorescence in response to Quantis™ application under heat stress. These results suggest that Quantis modifies cytokinin content in leaves and positively regulates cytokinin activity in plant roots under heat stress.

Discussion

We show here that the application of Quantis™ prior to heat stress effectively primed heat tolerance responses and alleviated temperature stress of *Solanum tuberosum* L. resulting in 4% tuber weight increase and 40% larger tuber size. Furthermore, increased thermotolerance exerted by Quantis™ was confirmed in *Arabidopsis thaliana* demonstrating that the mechanism of action of the biostimulant is consistent in different plant species.

The photosynthetic apparatus in the chloroplast is the primary target of heat damage by the overproduction of reactive oxygen species (ROS) causing oxidative damage to biomolecules including pigments, proteins, lipids and DNA leading to cellular injuries and cell death [37]. PSII is the most sensitive component of the photosynthetic machinery to excessive ROS damage, thus plants activate photoprotective mechanisms with one of them being upregulation of non-photochemical quenching (NPQ) causing depression of PSII activity to avoid damage to PSII reaction centres [38]. Here, we observed enhanced PSII photochemical efficiency, measured as Fv'/Fm', ΦPSII and QY, which was consistent with decreased thermal dissipation indicated by lower NPQ and DIo/RC induced by the biostimulant application under heat stress

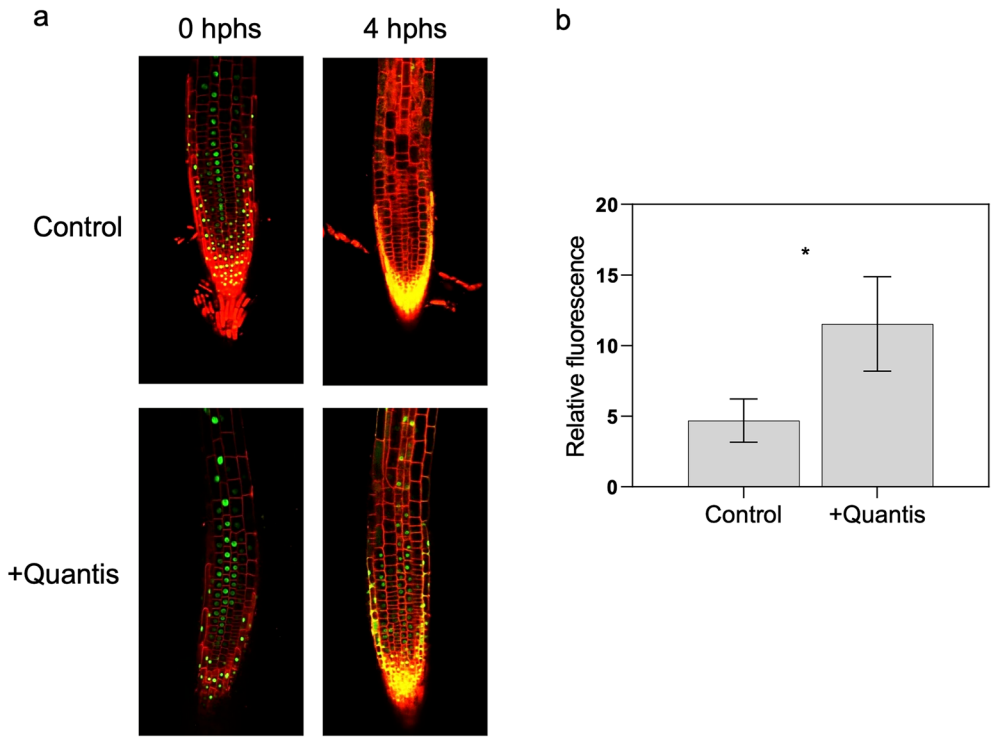


Fig. 11 GFP-fluorescence **a** visualised by confocal microscopy and **b** quantified as relative fluorescence on roots of *A. thaliana* cytokinin sensor TCSn::GFP DEAL line following heat stress of 42 ºC applied 12 h post-application of Quantis™ or water for a period of 4 h. Data represent mean ± *SEM* of three biological replicates per treatment with two experimental replicates. Asterisk indicates significant difference according to Student's *t* test, $p < 0.05$

in potato for up to 7 days. Decrease in NPQ is often associated with activation of ROS-mediated signalling to upregulate thermal defence response in plants [37]. This is in agreement with the simultaneous reduction of absorption flux ABS/RC suggesting rapid adjustment of light harvesting operational capacity of PSII in response to temperature acclimation following biostimulant application. In both plant species, excitation energy from PSII remained effectively regulated to drive photochemical processes indicating potential deployment of alternative photoprotective mechanisms activated by Quantis™. Indeed, the enrichment with upregulated DEGs in potato for increased activity of isoprenoids, and flavonoids (phenylpropanoids) suggested modulated functionality of non-enzymatic antioxidants together with plant hormones [39] as the primary strategy exerted by Quantis™ to impart increased thermotolerance. This agrees with the observed significant upregulation of flavonoid 3′-hydroxylase (F3'H) by the biostimulant application in our RNA-seq data. Overexpression of F3'H results in increased concentrations of flavanol glycosides and anthocyanins in plants, acting to reduce ROS accumulation, and enhance tolerance to various environmental stresses, including salt stress in rice and tobacco [40–42], drought stress in alfalfa [43] and *A. thaliana* [44], and temperature stress in *A. thaliana* [45], potato [46] and apple [47].

Quantis™ specifically upregulated, Hevein-like preproprotein WIN1 and pathogen- and wound-inducible antifungal protein CBP20, and PEN1-encoding syntaxin. These genes, and one more with unknown function in potato, but with an identifiable orthologue in *A. thaliana*, MEE59, with function in seed dormancy were selected for further functional validation using mutants of *A. thaliana*. Our results showed that PR4 and MEE59 functioned in both leaf photoprotection and in root thermal protection of *A. thaliana*, but not in biotic stress defence to *S. sclerotiorum* and *R. solani* AG2-1. Therefore, Quantis™ increased above and below ground thermotolerance by specifically modulating the expression and functionality of heat-stress defence genes. This agrees with previous work in monocotyledonous plants showing that induction of *OsPR4* and *ZmPR4* contributes positively to abiotic (drought, salt, cold, heat shock, and UV light) stress responses [48], although this is the first report for a role of PEN1 and MEE59 in abiotic stress tolerance. The RNA-seq analysis showed enrichment with numerous other genes with clear functions in environmental defence that were upregulated by Quantis™. For example, a gene for tyramine hydroxycinnamoyl transferase encoding an enzyme responsible for the synthesis of hydroxycinnamic acid amides of tyramine which is specifically induced in Solanaceous plants in response to abiotic stress [34], including ozone (tobacco) or UV-C light (pepper), wounding or pathogen attack (potato) [33, 49–51]. We observed enrichment with genes encoding endochitinases and chitinases belonging to PR protein classes known to be induced during saline and cold-adaptation tolerance in tobacco [52] and winged bean [53], thermotolerance and drought tolerance in Arabidopsis [54, 55]. Whilst the main role of plant chitinases is to degrade chitin of fungal or insect origin during the plant defence response [56], chitin fragments such as chito-oligosaccharides released by plants themselves can act as a general elicitor of common defence pathways that are functional for increased tolerance to biotic and abiotic stress [57]. Similarly, in our study, the most enriched KEGG pathways were mitogen-activated protein kinase (MAPK)-signalling and plant–pathogen interactions enriched with upregulated transcripts for StWRKY8, StWRKY9, StWRKY11, PR1, ethylene signalling mediated by ERF1 and ERF4 and Ca^{2+} signalling towards cell reinforcement and general defence-related gene induction.

Phytohormone signalling pathways in response to abiotic stress have been shown to be activated by β-glucosidases, demonstrated for hydrolysis of gibberellin (GA) glucosides in rice [58], cytokinins (CKs) in maize [59] and abscisic acid (ABA) glucose conjugate hydrolysis in barley [60] and Arabidopsis [61]. Here, we observed significant enrichment with upregulated genes by Quantis™ for β-glucosidases and modulation of GAs, CKs and ABA in potato leaves by the biostimulant. GAs, CKs and ABA play major roles in source to sink signalling and sink strength determination in plants under optimum and abiotic stress conditions [30], but in potato these hormones also regulate tuberisation and tuber development [62]. The phytohormone analysis of potato leaf samples collected at 24 hpa showed significant reductions of GA4 due to the biostimulant treatment. Although the exact regulatory functions of leaf-derived GA-dependant signalling and conversion events in repressing or promoting tuber growth remain not fully understood, reduced rate of biosynthesis of GAs in the plant leaves of potatoes has been shown to correlate positively with higher rates of GA20 to GA1 conversion in the shoot favouring tuberisation transition [62]. In agreement with significant KEGG term enrichment with downregulated transcripts for zeatin biosynthesis, the most active cytokinin, trans-zeatin, was first reduced at 24 hpa by Quantis™ before increasing in concentration together with ABA at 7 dpa. Both ABA and CKs have been shown to exert positive effects on photosynthesis and photoprotection in plants exposed to environmental stress [30]. Furthermore, endogenous CKs have been shown to enhance both leaf and root thermotolerance associated with enhanced

regulation of cytokinin transport between root and shoot for maintenance of leaf photosynthetic activity and root viability [63]. We showed here that Quantis™ not only modulated CK content in leaves, but also increased CK activity in roots under heat stress. This agrees with published evidence of increased thermotolerance, and temperature acclimation associated with elevated CKs action in Arabidopsis roots under high temperature stress [64]. The potato RNA-seq analysis also showed significant GO terms for alcohol binding, ABA binding and defence response that were most enriched with upregulated transcripts for pathogenesis proteins PR10 with some of them homologous to pru av 1, bet v 1 allergens that have been previously characterised in cherry and birch, respectively [65]. These ribonuclease-like proteins have been shown to have specific cytokinin binding affinity, high binding capacity for fatty acids and flavonoids and ability to induce ABA responsive genes [40]. In potato, exogenous application of ABA can promote tuberisation leading to higher number of tubers, whilst CKs have been shown to function in tuber enlargement and growth rather than to signal tuber transition. Tuber transition in turn is regulated not just by phytohormonal action, but also by photoperiod-mediated flowering transition [66]. Although the photoperiod response of potato is stronger under high temperature, the effect of elevated temperature is to inhibit tuber formation [62]. In potato, the blue light receptor FLAVIN-BINDING, KELCH REPEAT, F-BOX 1 (*StFKF1*) binds the circadian clock controlled GIGANTEA (*StGI*) in the light, thus promoting proteasomal degradation of transcriptional repressor CYCLING DOF FACTOR1 (*StCDF1*) enabling the activation in the leaf of CONSTANS (*StCO*) transcription and the mobile tuberisation signal FLOWERING LOCUS T (FT) paralog SELF PRUNING 6A (*StSP6A*). In our data, *StFKF1* was significantly upregulated by Quantis™ suggesting tuberisation promoting effects. Overexpression of the Arabidopsis LOV KELCH REPEAT PROTEIN 2 (*LKP2*) of the same FKF family of F-box/blue light receptor proteins, has been shown to promote potato tuber formation [67].

Conclusions

Quantis™ positively regulated heat stress tolerance in plants, exhibited as increased plant PSII operating capacity above ground and increased root growth below ground, via upregulation of genes with antioxidant function, including PR10, F3′H and β-glucosidases, together with modulation of cytokinin activity in leaves and roots, and expression of PEN1, PR4 or MEE59. Significant upregulation of *StFKF1* consistent with tuberisation promoting effects and increase in endogenous concentration of ABA and CKs in potato leaves modulated by Quantis™ resulted in significantly increased potato tuber weight and size, thus mitigating negative effects of heat stress on potato tuber growth.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s40538-023-00531-3.

Additional file 1: Figure S1. Recorded temperature (°C) in glasshouse conditions under 16 h light and 8 h darkness for the two experimental replicates. Temperature shown at 0, 12, 24, 48 hours and 3 - 7 days post-application of Quantis™.

Additional file 2: Figure S2. Disease severity (%) by (a) *Rhizoctonia solani* AG2-1 and (b) *Sclerotinia sclerotiorum* assessed at 7 days post-inoculation of seedlings of *A. thaliana* WT-type Columbia-0 (Col-0), *penetration1* (*pen1*), *pathogenesis related 4* (*pr4*), *maternal effect embryo arrest 59* (*mee59*) (growth stage 3.20). Data represent mean ± *SEM* of six biological replicates per treatment with two experimental replicates. Asterisk indicates significant difference according to Student's *t* test, $p < 0.05$.

Additional file 3: Figure S3. Confocal microscopy images showing GFP-fluorescence of *A. thaliana* cytokinin sensor TCSn::GFP DEAL line following heat stress of 42 ℃ applied 12 hours post-application of Quantis™ (+Quantis) or water (control) for a period of 4 hours in 6 replications. Images used to calculate relative fluorescence shown in Fig. 11.

Additional file 4. Table S1. Differentially expressed genes due to treatment and heat stress with gene description, padj value and log2-fold change in expression.

Additional file 5. Table S2. List of primers used for target gene expression using qRT-PCR.

Additional file 6. Table S3. Significantly enriched (padj<0.05) gene ontology (GO) terms in all categories showing gene ratios and gene IDs.

Additional file 7. Table S4. Significantly enriched (padj<0.05) kyoto encyclopaedia of genes and genomes (KEGG) terms in all categories with gene ratios, gene and kegg IDs.

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Author contributions
RR, RS and DJ conceived the experiments. RR and DJ analysed the results and wrote the manuscript. DJ carried out GE and CF measurements and heat stress experiments with *A. thaliana*. DA and DJ carried out CF imaging at the University of Nottingham. RS advised on drought experiments and confocal microscopy carried out by DJ. SMB performed hormonal quantification on samples. CD and DJ carried out inoculation experiments. All authors reviewed and edited the manuscript.

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Availability of data and materials
All data are available upon request to the corresponding author.

Declarations

Ethics approval and consent to participate
Not applicable.

Consent for publication
Not applicable.

Competing interests
The authors have no conflicts of interest.

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