Chemical intervention in plant sugar-signalling increases yield and resilience

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The pressing global issue of food insecurity due to population growth, diminishing land and variable climate can only be addressed in agriculture by improving both maximum crop yield potential and resilience.[1, 2] Genetic modification (GM) is one potential solution, but has yet to achieve worldwide acceptance particularly for crops such as wheat.[3] Trehalose-6-phosphate (T6P), a central sugar signal in plants, regulates sucrose use and allocation, underpinning crop growth and development.[4, 5] Here we demonstrate a chemical intervention solution that directly modulates T6P levels *in planta*. Plant-permeable analogues of T6P were designed and constructed based on a 'signalling-precursor' concept for permeability, ready uptake and sunlight-triggered release of T6P *in planta*. For the first time we show that chemical intervention of a potent sugar signal increases grain yield, whereas application to vegetative tissue improves recovery and resurrection from drought. This technology offers an unprecedented and rational means to combine increases in yield with crop stress resilience. Given the generality of the T6P pathway in plants and other small molecule signals in biology, these studies suggest that suitable synthetic exogenous small molecule signal-precursors can be used to directly enhance plant performance and perhaps other organism function.

We designed a signaling-precursor strategy based on release by light (**ED Figure 1**). Light-activated control is a potent strategy in biology, allowing temporal and spatial resolution surpassing that of standard genetic methods;[6] such resolution can be increased when combined with small molecule chemical control.[7-9] Potency is increased further when releasing a signaling molecule with effect amplified several-fold. Hydrophilic or charged molecules do not readily enter plants unless transported.

We designed unnatural precursors (**1**-**4**) of T6P with groups to mask charge, increase hydrophobicity and also be released by light (**Figure 1a**). Their construction (**Figure 1b**) used different phosphorus chemistries: phosphoramidite chemistry[10, 11] to create P(III)-intermediates that were then oxidized to corresponding P(V)-phosphotriesters or direct P(V)-phosphorylation chemistry (**Figure 1b**). Regioselective access to the OH-6 group in trehalose exploited trimethylsilyl (TMS) as a protecting group that is chemically orthogonal to phosphotriester; **12** was prepared on multigram-scale.[13] Phosphorylation (reaction with phosphoramidites **9**-**11**[10, 11] followed by tBuOOH or treatment with $POCl_3$[14] followed by the addition of appropriate alcohol) gave intermediates that were deprotected under mildly acidic conditions (see **Supplementary Methods**). **1**-**4** were all inactive against SnRK1 (**ED Figure 2**).

Mass spectrometry (MS), TLC and NMR (see **Supplementary Methods, Supplementary Table S1** and **ED Figure 1**) revealed release times (95% release, t_{95}) dependent on both light intensity and frequency under a range of conditions. Consistent with design, light-sensitive groups were differently susceptible: precursor **1** gave T6P more rapidly at lower wavelengths; **4** was more reactive at higher. Whilst release with higher light intensity was more rapid (125W / 365 $\mu mol.m^2.s^{-1}$ *cf* 8W / 23 $\mu mol.m^2.s^{-1}$), direct sunlight proved sufficient, in some cases showing t_{95} as brief as 90 min (for **3**). NMR analysis (**Supplementary Methods** and **ED Figure 1ef**) confirmed formation of T6P and generation of potent inhibitory activity against SnRK1 (**ED Figure 2**).

Following successful *in vitro* release, uptake *in planta* was examined. **1**-**4** (to give final concentration 1 mM) were fed to roots of plantlets of *Arabidopsis thaliana* and the aerial (shoot) analyzed over time and with increasing dose (**Figure 2** and **Supplementary Tables S2-S7**). Quantitative MS[16] and HPLC of extract revealed increasing uptake over time (**Figure 2a** and **ED Figure 3**) and dose responses. Consistent with design, structural variation allowed use of altered hydrophobicity to modulate permeability[17] and transport.[18] Importantly, in this way systematic variation of group type and copy number identified (*ortho*-nitrophenyl)ethyl (*o*NPE)-variant **3** (clogP 0.11±0.60, see also **Supplementary Methods** and **Table S17**) as most potent (**Figure 2a**), allowing absorption of ~20% of the after 72 h; **1**,**2** and **4** (clogPs -2.35 to -0.17) were less readily taken up.

Next, light-activated release *in planta* was tested: plants were treated via media, grown for three further days, irradiated, harvested and extracted.[19] T6P-release was confirmed by MSMS (**ED Figure 4**) and determined by quantitative HPLC-MS (2-deoxy-glucose-6-phosphate as internal standard[16], **Figure 2b, ED Figure 4** and **Supplementary Table S10**). Release *in planta* could be controlled and modulated by choice of light source and signalling precursor (**Figure 2b** and **Supplementary Table S10**). Most transgenic approaches alter T6P over a 2-3-fold range only.[4, 5] Using **1**-**4**, levels of up to 900 nmol.g^{-1}FW (100-fold higher than endogenous, 75-fold higher than with genetic methods) were attainable. Consistent with strategy, maximal T6P was released when precursor-treated plants were irradiated with most flux (100 W / 292 μmol.m^2.s^{-1} UV) in all cases. Importantly, and with relevance to field application, under just sunlight all released significantly enhanced T6P (39- 296 nmol.g^{-1}FW) (**Supplementary Table S10**), some ~4-

to-30-fold above endogenous. No significant reduction in fresh weight of plantlets at 1mM (**Supplementary Methods** and **ED Figure 5**) suggested low toxicity. Accumulation of T6P after treatment was revealed through MS-imaging[20] (**Figure 3**) via signature-ion markers[21] in treated leaves of *A. thaliana* seedlings (**Figure 3b**) after 2h of irradiation; the different distributions from **2** and **3** appeared consistent with their measured release rates. Interestingly, increased trehalose was also observed[22] in the same regions (**Figure 3c**), suggestive of metabolism. Moreover, MS-imaging via treatment-specific ions corroborated uptake of precursors into leaves (**Figure 3 d,e**).

The dynamics of this apparently enhanced *in planta* T6P release, and possible consequent metabolic products, were determined not only through both quantitative HPLC-MS and/or enzymatic quantification but also through the use of unnaturally-enriched isotopic labelling of the signalling precursors, allowing unambiguous delineation of their fate (**Figure 4 and ED Figure 6** and **Supplementary Methods**). Thus, in 7-day-old *Arabidopsis* seedlings,[23] 1 mM DMNB-T6P **2** and *o*NPE-T6P **3** (fed for 24 h prior to exposure light, UV 8W, 23 $\mu mol.m^{-2}.s^{-1}$) led to peak T6P after 60 min (229 and 159 nmol g^{-1} FW, respectively), which declined over the following 2 days (**Figure 4a**). Corresponding trehalose levels were also elevated, with peaks at ~2h (up to a maximum of 134 $nmol.g^{-1}$ FW c.f. controls 20 $nmol.g^{-1}$ FW, **Figure 4b**), confirming the metabolism suggested by MS-imaging. Glucose, the next sugar in the pathway, was also increased to a smaller degree, peaking at ~2-4 h (**Figure 4d**). Such levels are consistent with known low metabolic fluxes.[24] Given known interrelationships,[5] sucrose levels were also determined. Strikingly, these increased 2-3-fold over the first 2 h of irradiation (**Figure 4c**) and positively related to T6P for both **2** and **3** (**Figure 4**); fructose was minimally affected (**ED**

Figure 6d). We checked that inhibited growth was not an explanation for sucrose accumulation and found instead that growth was stimulated by T6P (**ED Figure 6e**).

Creation of ^{13}C-isotopically-labeled variant **2*** (**ED Figure 7a**) allowed direct tracking via 'mass-shifts' of the corresponding ions in MS. Treatment with **2*** led to release of ^{13}C-T6P and consequent sequential metabolism (to ^{13}C-trehalose, ^{13}C-glucose) following essentially the same dynamics (**ED Figure 7**). Excitingly, in this way, mass labelling also revealed not only released but also *induced* T6P. Strikingly, this not only accounted for approximately half of T6P measured at 30 min, thereby providing direct evidence of induction of *de novo* T6P synthesis, but this induction continued, giving rise to increased T6P accumulation over time (**ED Figure 7**). This could be due to the large increase in sucrose observed; sucrose induces T6P.[5, 23] Together these data suggested perturbation of T6P levels by two modes-of-action: direct release from the signalling precursor and simultaneously *induced* release by virtue of biosynthesis of T6P by the plant.

Together these data suggested **3** as the plant permeable signalling precursor with the greatest tissue uptake coupled with the greatest temporal control (consistent with its tuned permeability and its fastest release rates), allowing minimal application amounts (0.1 mM) whilst still able to enhance T6P levels some 1.5 to 6.5-fold above endogenous without potentially dramatic disruption of metabolism.[25] Plants were treated with precursor **3** for 72h and then subjected to a single 8 h period under growth lights supplemented with 8W UV (**Supplementary Table S10**, generating ~ 21 nmol. g^{-1}FW T6P) and harvested a day later. The mean starch level (63.2 μmol.g^{-1}FW) determined[26] was significantly higher ($F_{(1,14)}$ =13.59; p = 0.002) than for water-treated plants (40.7 μmol.g^{-1}FW, **ED Figure 5d**).

The release of T6P from **1**-**4** involves fragmentation with concomitant release of side products. Although considered[27] to be non-toxic, we nevertheless tested for any unexpected phenotypic changes. Glucose-6-phosphate (G6P) analogues **14**-**17** of **1**-**4** were synthesized (**Supplementary Methods** and **ED Figure 8**) and compared for their activity; G6P methyl glycoside itself is inactive *in planta* and in all interactions with SnRK1 (**ED Figure 8**) and so its light-activated release from **14**-**17** provided useful controls. **14**-**17** showed similar light-activated release parameters to **1**-**4** (**Supplementary Table S11**) and relative uptake performance similarly dependent on identity of light-sensitive moiety (**ED Figure 8** and **Supplementary Table S12-S14**). No toxicity was observed in any of the plants treated with **14**-**17** up to 0.5 mM (**Supplementary Methods** and **ED Figure 5a-c**), suggesting the benign nature of the light-released moiety. Critically, also, starch was not affected in controls treated with **16**, the G6P-analogue of T6P precursor **3**.

The rate of starch synthesis over a 12h period (**ED Figure 5f,g**) indicated a flux (0.037 μmol.min^{-1}.g^{-1}FW) nearly three times that of water-treated control (0.013 μmol.min^{-1}.g^{-1} FW). T6P is proposed to stimulate starch synthesis through redox-activation[28] of ADP-glucose pyrophosphorylase (AGPase), a rate-limiting enzyme. Whilst not necessarily causal, consistent with this hypothesis, *o*NPE-T6P **3**-treated plants had significantly higher AGPase activity (up 35%, **ED Figure 5e**). AGPase has been previously shown to affect starch turnover.[29]

We then measured transcripts of genes known to be associated with T6P. Firstly, SnRK1 is a proposed target of T6P[15]; SnRK1-induced and -repressed markers *TPS5*, *TPS8*, *bZIP11* and *ASN* responded synchronously to the activation of the precursors in a manner

consistent with known effects of T6P on SnRK1 activity (**ED Figure 9**). However, other markers (e.g. *UDPGDH* and *bGAL4*) showed clear temporal delay (**ED Figure 9b**); observed synchronization of these 'secondary markers' only after a day suggest that these could be later, downstream targets of T6P. Secondly, since starch is not only a proposed target of T6P[28, 30] but also increased upon treatment, expression of starch biosynthetic genes was also examined: transcripts of *APL3*, *SS3*, *BE1* and *GBSS1* were increased up to five-fold (**ED Figure 9c**).

These data from *Arabidopsis* raised the exciting possibility of enhanced starch synthesis in crops, vitally allowing increased yield. Signalling precursors **2** and **3** were applied (0.1, 1, 10 mM) to spring wheat Cadenza, grown in a controlled environment, representative of summer in Northern Europe. Spraying occurred either to ears only or to the whole plant during the grain-filling period (5, 10, 15, 20 days post anthesis (DPA)) at mid-photoperiod. This increased grain yield per plant due to the formation of larger grain in plants treated with 1mM DMNB-T6P **2** and 1mM *o*NPE-T6P **3** particularly (**Figure 5a,b**); in these grains, starch content increased 13-20% (**Figure 5c**). A trend towards higher starch and protein, when expressed as a percentage of component content per gram of grain, was also observed (**Supplementary Table S16**). Dose response showed that yield peaked at 1 mM (**ED Figure 10f**). Minimal spray at just 10 DPA increased yield substantially at 1 and 10 mM doses (**ED Figure 10g**). Plants treated with **2** and **3** stayed greener for longer than plants treated with water, consistent with chlorophyll content (**ED Figure 10a,b**) and prior observations for genetically-enhanced T6P content.[31] T6P release in the wheat grains treated with **2** and **3** was enhanced at 5 DPA (128 $nmol.g^{-1}FW$, 81 $nmol.g^{-1}FW$, respectively) and further at 10 DPA (378 $nmol.g^{-1}FW$, 300 $nmol.g^{-1}FW$, respectively) (**ED**

Figure 10c-e). Trehalose levels were also higher (30-70 nmol.g^{-1}FW *cf* endogenous 13 nmol.g^{-1}FW), consistent with the metabolism observed in *Arabidopsis*.

Next, the effects of signalling precursors upon plant resilience and recovery were tested. Drought is still the biggest global factor limiting crop yields, even in developed countries.[32] When 4-week-old wheat plants were sprayed with **2** or **3** (30 mL, 1 mM, once) after 9 days of drought, the regrowth effects following resumption of watering 1 day post treatment were dramatic (**Figure 6a,b**). Regrowth of new tissue from plants cut-back after drought was also higher in precursor-treated plants (**Figure 6c,d**). This demonstrated both growth of new tissue (resurrection response) and salvage and growth of new tissue (recovery response). T6P solution alone gave identical results to water (**Figure 6**), consistent with inability of T6P to enter directly into plants, further highlighting the design principles of signalling precursors.

In conclusion, we have shown here that a chemical strategy can directly control amounts of an important sugar-signalling molecule *in vivo*. The collected data are consistent with signalling action of released T6P. For example, the mass balance of added signalling precursor appears insufficient to simply act as a carbon source. That said, we do not discount other possible mechanisms behind the exciting traits that we have observed here. The apparent resulting 'biosynthetic amplification' observed from signalling precursors, we believe, is a promising concept; we calculate here up to 50-fold 'molecular amplification' of plant sugar 'product' compared to precursor. One can therefore envisage a self-sustaining production strategy in which a fraction of the additional starch generated by this amplification is used as feedstock chemical for eventual synthesis of the signalling precursors themselves (**Supplementary Discussion** and **Supplementary Table S18**).

We speculate that this chemical approach also offers temporal and strategic flexibility over genetic methods (e.g. a ‘pulse’ to circumvent adaptation effects or in manipulating genetically more complex crops) as well as the prospect of providing an immediate boost to productivity at critical times in plant life cycle (e.g. to allow synchronicity with the sun or to rescue drought-stricken regions, **Supplementary Discussion**) – the potential in managing global food security seems striking and immediate. Given the widespread importance of cell signalling and of carbohydrates in biology, this system, applied here to plants, may also, in principle, have even wider utility.

METHODS

Synthesis of signalling-precursor compounds 1-4. 1*H*-tetrazole solution (0.45 M in CH_3CN) (0.6 mL, 0.24 mmol, 2.0 equiv.) was added into a stirred solution of **12** (100 mg, 0.12 mmol, 1 equiv.) and *bis*-(2-nitrobenzyl)-*N*,*N*-diisopropylphosphoramidite **9** (78.3 mg, 0.18 mmol, 1.5 equiv.) in anhydrous CH_2Cl_2 (5 mL) under an argon atmosphere at 0 ^oC. The resulting reaction mixture was stirred at 0-5^oC and progress of the reaction was monitored by TLC (petroleum ether: ether; 8:2) and mass spectrometry. After complete disappearance of starting material (1h), *t*BuOOH (0.1 mL) was added at 0 ^oC and stirring was continued for another 30 min. After 30 min the reaction mixture was concentrated *in vacuo* and the residue was suspended in methanol (2 mL) and stirred in the presence of 30 mg of Dowex-H^+ resin for 1h at room temperature to globally remove TMS groups. Dowex-H^+ was removed through filtration and the filtrate was concentrated, which on flash chromatography (water: isopropanol: ethyl acetate, 1: 2: 8) purification yielded **1** (70 mg) in 87% isolable yield. Similar reaction protocols were adopted for the synthesis of compounds **2** and **3**. Compound **4** was obtained when a stirred solution of **12** (100 mg, 0.12 mmol) in pyridine (2 mL) at room temperature was treated with $POCl_3$ (0.012 mL, 0.132 mmol) for 10 min followed by addition of 4,5-dimethoxy-2-nitrobenzyl alcohol (76.7 mg, 0.36 mmol) and continuous stirring for 1h. The resulting reaction mixture was concentrated *in vacuo* to yield crude product mixture, which was treated with Dowex-H^+ (30 mg) in methanol (2 mL). After filtration, concentration *in vacuo* and flash chromatography purification yielded **4** (45 mg, 62%) as a pure sticky solid. For additional details see **Supplementary Methods**.

***In planta* uptake of signalling-precursor compounds and release of trehalose-6-phosphate and metabolites.** *In planta* uptake was carried out using *A. thaliana* plantlets. *A. thaliana* (Columbia 0) seeds were surface-sterilised for 10 min in 10% sodium hypochlorite, 0.01% Triton-X-100 and then copiously washed with sterile water and stratified for 3 days at 4 ^{o}C. Seeds were sown onto 0.5 mL solid medium (0.5x Murashige and Skoog (MS) medium with Gamborg's vitamins (Sigma P0404), 0.5% sucrose and 0.5% agar) in 0.5 mL Eppendorf tubes, pierced in the bottom with a tiny hole. The tubes were arrayed in hand-cut polystyrene racks in Phytatrays (Sigma) and floated on liquid medium (same as solid medium but lacking sucrose and agar). Plantlets were grown under the following conditions: 12 h day under Philips master TL-D 840/58W fluorescent lights giving 250 µmol.m^{-2}.s^{-1}, and 23^{o}C day/18 ^{o}C night temperatures. At 18 days after sowing the liquid medium was removed and the tubes were sealed with electrician's tape. All plants were topped up with 0.5 x MS medium with no sucrose.

Plants were treated with compounds by adding 10 µL of 50 mM stock prepared in water or 1% DMSO to the agar medium, avoiding contact with aerial parts. The final concentration of precursor compound in the agar medium was 1mM. After certain period of time (after 24 h, 48 h and 72 h) the aerial part was harvested carefully, weighed and extracted in H_2O:MeOH (1:1) under liquid nitrogen. The crude fresh plant extract thus obtained was analysed by MS and HPLC.

For *in planta* T6P release experiments compound-treated plants were exposed to UV light treatment after 72 h. UV treatments consisted of (a) 8 h exposure to natural daylight, (b) 8 h exposure to a 100 W UV spotlight (BlackRay B-100AP) at a distance of 18 cm, (c) 8 h exposure to an 8 W UV bulb (365 nm, Gelman transilluminator Model 51438) at a distance of 6 cm or (d) exposure for two 8 h periods under 8 W. UV treatments were in addition to normal growth lights. Control plants (except for daylight treatment) were treated under the same conditions but without UV light. At the end of the day of exposure to UV/light, the aerial parts of the plants were quickly harvested, weighed and frozen in liquid nitrogen. For

starch extractions a moderate light regime was selected of 1 × 8 h exposure to 8W UV light. After irradiation plants were returned to the growth room for a further day (day 5) to recover after light treatment and to respond to altered T6P levels before being harvested as above. Frozen tissue was stored at -80 °C until extracted.

Harvested plant material was extracted by liquid/liquid extraction (LLE) followed by solid phase extraction (SPE) for T6P analysis.[19] For LLE/SPE extractions around 25 mg plant tissue was used, pooled from several plants. Samples were reconstituted in 50 µL of H_2O:MeOH (1:1) and 10 µl used for T6P analysis using HPLC-MS (Quattro, Waters) and T6P release determined with quantitative using 2-deoxy-glucose-6-phosphate a suitable calibration internal standard.

Liquid Chromatography Tandem Mass Spectrometry (LC–MS/MS) was used to confirm the identity disaccharide monophosphates via fragmentation pattern analysis performed on a Waters Xevo G2-S QTof (Quadrupole Time-of-flight) Mass Spectrometer coupled to a Waters Acquity Ultra Performance Liquid Chromatography (UPLC), and a Waters Micromass® Quattro micro™ API Mass Spectrometry coupled to a Waters 1525µ binary HPLC pump and a Waters 2777 auto sampler using a SIELC Primesep SB column, solvent A (0.1% formic acid in H_2O) and solvent B (1.0% formic acid in H_2O:CH_3CN (75:25)), were used as the mobile phase at a flow rate of 0.4 mL min^{-1}. For the Xevo G2-S QTof MS, the electrospray (ES) source was operated with a capillary voltage of 2.0 kV and a cone voltage of 30 V. Nitrogen was used as the desolvation gas at a total flow of 800 L/h^{-1}. The intact molecular ion of T6P was detected as m/z 421.0759 ($C_{12}H_{22}O_{14}P$, calculated as 421.0753) in a negative ion-mode. The Time of Flight (ToF) tandem mass spectrum of the parent ion 421.00 was then obtained in a negative ion-mode for the *m*/*z* range from 50 to 500 using optimized collision energy of 20 eV. For the Quattro micro™ API MS, the electrospray (ES) source was operated with a capillary voltage of 3.0 kV and a cone voltage

of 40 V. The quadrupole tandem mass spectra of the parent ion 421.0 of T6P and S6P were obtained in a negative ion-mode for the *m*/*z* range from 50 to 500 using collision energy of 20 eV. With the reference to standard fragmentation patterns, tracking of the fragment ions of T6P in the plant sample was also performed by quadrupole tandem mass spectra. Five most intense m/z peaks recorded in the MS/MS spectrum of T6P, m/z 78.3, 96.4, 138.6, 240.9 and 421.0 (unfragmented) were also selected to perform the Multiple Reaction Monitoring (MRM) and cross-referenced with Selected Ion Recording (SIR) for the intact molecular ion m/z 421.0.

For seedling liquid culture, seeds of *A. thaliana* were grown in liquid culture as described previously.[23] Once the seedlings were 7 days old, *o*NPE-T6P or DMNB-T6P were added to the growth medium to a final concentration of 1mM. Plants were left under growth lights to uptake the compounds for 24 hours. To facilitate precursor release, plants were placed under 23 $\mu mol.m^{-2}.s^{-1}$ UV for 2 hours, after which they were returned to previous environmental conditions. Samples were taken for analysis before addition of compound, 1 day after addition, 30, 60 and 120 minutes during UV treatment, and sampled again at 1 and 2 days post-UV treatment. Samples were weighed, snap frozen and stored at -80°C.

For enzymatic sugar analysis, sugars were extracted from 5-10mg of *A. thaliana* ground under liquid nitrogen, 1mL of 80% was added and sample was heated at 100°C for 1 hour, samples were centrifuged for 10 minutes at 13000g to remove debris. The samples were added to assay buffer.[26] Enzymatic reactions were performed as described previously[33] using hexokinase, glucose-6-phosphate dehydrogenase, phosphoglucose isomerase and invertase from Sigma-Aldrich (H4502, G8404, P5381, I9274 respectively). Two technical replicates were completed for each sample, a total of three biological replicates were analysed. See **Supplementary Methods** for further details.

Extraction and measurement of starch *in planta*. Three to four chemical and UV light treated plantlets were pooled and weighed (fresh weight, 70-100mg) for each biological replicate. Extraction was based on literature methods.[26] Samples were ground in liquid nitrogen to a fine powder in a mortar. The powder was rapidly extracted with 1 mL 80% ethanol at 80°C, followed by 2 × 0.5 mL to rinse, transferred to a 2mL eppendorf at 100°C and heated for 2-3 mins until just boiling. Tubes were transferred to a water bath at 80°C while other samples were accumulated. Samples were centrifuged at 13,000 × *g* for 10 min to collect all solid material. The pellet was extracted twice more with 2 mL hot 80% ethanol. The pellet was washed with 1 mL water, the supernatant removed and 100 μL water added. The pellet was homogenized to a smooth consistency with an Eppendorf micropestle before being made up to 500 μL final volume with water. Samples were heated at 100°C for 10 mins to gelatinize starch granules. Duplicate aliquots (100 μL) were removed and digested with α-amylase (2 U) and amyloglucosidase (6 U) in 0.05 M sodium acetate pH 4.8 for 4 hour at 37°C. Control digests lacking enzyme were also set up. Glucose released from digested starch was measured using an enzymatic assay coupled to the reduction of NADP to NADPH[34] and adapted for microtitre plate reader. Normally 10-20 μL of digest was assayed in triplicate. Starch content is expressed as hexose equivalents per gram fresh weight. See **Supplementary Methods** for further details.

SnRK1 activity. Kinase activities were determined by measuring the incorporation of radiolabeled phosphate into the AMARA peptide substrate and were carried out as described previously.[15]

ADP-glucose pyrophosphorylase activity. Enzyme activity was measured as described previously.[35]

Application of precursors to wheat. Spring wheat (*Triticum aestivum* Cadenza) seeds were sown in Rothamsted standard compost mix and grown in controlled environment conditions with a photoperiod of 16 hours light, 8 hours dark, day/night temperatures of 20°C/16°C, photon flux density of 600µmol m^{-2} s^{-1}, and ambient relative humidity. Once the plants had reached anthesis, solutions of *o*NPE-T6P and DMNB-T6P (0.1, 1 or 10mM) as well as control solutions, water, 1mM T6P and 1mM trehalose were made up in distilled water with 0.1% TWEEN-20. At 5, 10, 15 and 20 days post-anthesis, either the ears, or the whole plant were sprayed individually with the chemicals at volumes of 5mL and 50mL respectively. Leaf samples were taken at 5, 10, 15, 20 and 25 days post anthesis for chlorophyll content analysis, grain was harvested at maturity for analysis. Chlorophyll content of leaves was measured by methanol extraction and spectrophotometry.[36] Starch content of grain was measured enzymatically[26]and protein content was measured by Bradford's assay.[37]

For the drought treatment, Vegetative Cadenza wheat plants were grown in the same compost and environments as above. Once the plants had reached Feekes stage 4, water was withheld for 10 days. On the 9th day, 30ml 1mM solutions of *o*NPE-T6P and DMNB-T6P were applied to all above-ground biomass, on the 10th day the watering schedule was reinstated. Plants were harvested to measure biomass production every 5 days for 30 days after rewatering. Both experiments were completed in replicates of six.

For quantification of T6P, trehalose and sucrose in wheat samples, the harvested wheat grains were weighed, snap frozen and stored at -80 °C. Wheat grain was ground to fine

powder in liquid nitrogen and the sugars were extracted by liquid/liquid extraction (LLE) for T6P, trehalose and sucrose analysis using the same LCMS quantification method as for *Arabidopsis*.

For minimal spray application, spring wheat (*Triticum aestivum* Cadenza) seeds were sown in Rothamsted standard compost mix and grown in controlled environment conditions with a photoperiod of 16 hours light, 8 hours dark, day/night temperatures of 20°C/16°C, photon flux density of 600µmol m^{-2} s^{-1}, and ambient relative humidity. Once the plants had reached anthesis, solutions of oNPE-T6P and DMNB-T6P (1mM and 10mM) and a water control, were made up in distilled water with 0.1% TWEEN-20. At 10 days post anthesis, the top 20cm of above ground biomass encompassing ears and flag leaves were sprayed individually with the chemicals at a volume of 25mL. Grain from individual ears was harvested at maturity for analysis. All wheat experiments were repeated twice, with 3 technical and 6 biological replicates completed at each stage of analysis.

RNA extraction, cDNA synthesis and q-RT-PCR. Total RNA was extracted from 50mg snap-frozen leaf tissue from *A. thaliana* Columbia using the Ribopure™ Kit (Ambion®) according to the manufacturer's instructions. RNA was quantified using a Nanodrop spectrophotometer and integrity of RNA was visualised using denaturing agarose gel electrophoresis.[38] DNA was removed using RQ1 RNase-free DNase (Promega). cDNA was synthesised using SuperScript® III First-Strand Synthesis System (ThermoFisher Scientific) using 2ug of total RNA and oligodT primers according to the manufacturer's instructions. Gene expression was quantified using SYBR Green chemistry on a Real-Time PCR system 7500 (Applied Biosystems). Total reaction size was 20µl containing 10µl SYBR® Green Jumpstart™ Taq ReadyMix™ (Sigma Aldrich), 2µl cDNA and 0.5mM

primers. PCR used an initial denaturation stage of 95°C for 2 min, followed by 40 cycles of 95°C for 15s, 60°C for 1. The specificity of products was confirmed by performing a temperature gradient analysis of products at temperatures ranging from 55°C to 95°C at 0.5°C increments. Two technical replicates were completed for each sample, a total of three biological replicates were analysed. Relative quantification of gene expression was performed using the Livak method using ubiquitin-transferase family protein as the reference gene. Primers utilised for SnRK1 marker gene expression, and starch gene expression are listed in **Supplementary Tables S19 and S20** respectively.

MS Imaging Methods. *Arabidopsis* utilised in ToF-SIMS imaging were grown in Petri dishes on 1/2MS medium with 0.8% agar for 10 days, with a photoperiod of 16 hours light, 8 hours dark, day/night temperatures of 23 °C / 18 °C, photon flux density of 250 µmol m^{-2} s^{-1}. Plants were then transferred to Petri dishes containing the same media supplemented with either 1mM of *o*NPE-T6P or 1mM of DMNB-T6P for 24 hours during which they remained under the previously stated growth conditions. After 24 hours, the plants were exposed to UV light at 23 µmol m^{-2} s^{-1} for 2 hours to facilitate T6P release. Plants were left for 2 hours to recover, frozen and dehydrated in a vacuum chamber before MS-imaging analysis. Reference materials were drop-dried on clean substrates. The ToF-SIMS (time-of-flight secondary ion mass spectrometry) Mass Spectrometry Imaging analysis was performed with the ToF-SIMS IV mass spectrometer (IONTOF, Muenster, Germany) from three leaves, control, oNPE-T6P treated one and DMNB-T6P treated one. A pulsed 25 keV Bi_3^+ primary ion source was used as the analysis beam (pulse width = 23 ns, mass resolution = m/Δm 5000). Mass spectra of the reference material were obtained in positive and negative ion mode at a primary ion dose of 1.1×10^{11} ions.cm^{-2}. The leaf ion images

were also collected in both polarities with a dose of 5.4×10^{10} ions.cm^{-2}. An electron flood gun was employed for charge compensation during the data acquisition. Mass spectral data was analysed in ION-TOF SurfaceLab 6.4 software and further processed in MATLAB and Origin Pro. Known melissic acid markers[21] (*m/z* 435.4, $C_{30}H_{59}O$, $[M\text{-}OH]^-$ and 451.4 $C_{30}H_{59}O_2$, $[M\text{-}H]^-$) were used. See **Supplementary Methods** for further details.

MALDI MS imaging data were acquired using identically prepared leaf samples with a modified QSTAR XL Qq-ToF instrument (Sciex, Ontario, Canada) fitted with a Nd:YAG laser (Elforlight Ltd, Daventry, UK) operated at 1000 kHz in positive ion mode with a fluence of ~ 205 J/m^2 and a pixel size of 200 µm × 200 µm. The QSTAR was operated in continuous raster sampling mode. The sample was affixed to a stainless steel target plate with double sided tape and sprayed with CHCA (5 mg/mL CHCA in 80 % Methanol, 0.1% TFA) using automated spray deposition (TM Sprayer, HTX Technologies, Carrboro, NC). Data were converted from the proprietary .wiff format into .mzML using AB MS Data Converter version 1.3 (Sciex). These mzML files were then converted to .imzML using imzMLConverter[39] and processed in custom made software within MATLAB (version R2014b, Math Works Inc., USA). Images are created by summing across the full-width-half-maximum of the peak of interest to give the intensity within each corresponding pixel. See **Supplementary Methods** for further details.

Statistical methods. ANOVA was applied to data to test for differences between treatments. A natural log transformation was used where necessary to ensure constant variance. The GENSTAT statistical system was used for this analysis (2011, 14th edition, © VSN International Ltd, Hemel Hempstead, UK).

AUTHOR CONTRIBUTIONS

CAG, RS, YG, LFP performed experiments. RS, YG, MKP synthesized compounds. YG, MKP, IG, RS, JB, BGD performed and/or analysed the MS imaging. YG, BGD performed and/or analysed the tandem mass spectrometry. CAG, RS, YG, LFP, MP, BGD designed and analysed the experiments. MP, BGD wrote the paper. All authors read and commented on the paper. A patent has been filed by the University of Oxford and the Rothamsted Research and, if licensed, will afford authors royalties in line with standard university practice.

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STATEMENT ON DATA

Primary data for Figures 2,4,5,6 is provided as spreadsheets. All other data is available on request.

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FIGURE LEGENDS

Figure 1 | Design and synthesis of signalling precursors of T6P. T6P is plant impermeable; synthesis of plant-permeable variants allowed subsequent photoactivated release T6P *in planta*. **(a)** Designed precursors **1**-**4**, their **(b)** syntheses using phosphoramidite chemistry (**1**-**3**) or direct phosphorylation chemistry (**4**) from key intermediate **12**. Universally ^{13}C-labelled **2*** was prepared in essentially the same way (see **ED Figure 7a**).

Figure 2 | *In planta* uptake of signalling precursors and T6P release. (**a**) Uptake of **1**-**4** (1mM in medium) at 24, 48, 72 h (SEM, n= 3). (**b**) T6P released *in planta* (SEM, n= 3). Compounds applied 18d after sowing then irradiated for 72h. *otsA* = *Arabidopsis* over-expressing trehalose-phosphate synthase. GL = 250 µmol.m^{-2}.s^{-1}. UV (8W = 23 µmol.m-^{2}.s-^{1}); 100W (292 µmol.m-^{2}.s-^{1}) = GL supplemented with 365nm. Sunlight (250 µmol.m^{-2}.s^{-1} cloud, 1440 µmol.m^{-2}.s^{-1}. full-sun). ANOVA showed significant differences (P<0.001) between treatments (water or precursor) for each regime. All treatments with precursor and UV showed significance (P<0.001, LSD) *cf* water and UV. For GL * = 0.01<*P*<0.05, ** = 0.001<P<0.01 (LSD, data on natural-log scale). See also **Supplementary Table S10**.

Figure 3 | MS Imaging of treated leaves. For all ion images, pixel intensity scale represents area under corresponding *m/z* peak. **(a-c)** ToF-SIMS **(a)** spectra from surface of *A. thaliana* leaves (top three spectra). Marker ions *m/z* 156.8, 196.8, 212.8 in T6P reference (bottom spectrum). **(b)** T6P (three markers, green) in control (left), *o*NPE-T6P (**3**)-treated (middle) and DMNB-T6P (**2**)-treated leaf (right), anti-colocalized with H_2O (*m/z* 18.0) and silicon substrate (*m/z* 27.9) **(c)** T6P (markers, green, positive mode), trehalose (*m/z* 325.2, 321.2, blue, negative mode) and overlay with known[21] epicuticular wax markers (red, negative mode) in the *o*NPE-T6P (**3**)-treated leaf. **(d-e)** MALDI-MS **(d)** Overlay of mean on-leaf spectra for control, *o*NPE-T6P (**3**)-treated and DMNB-T6P (**2**)-treated leaf. Lower panels show expansions for correlated markers. **(e)** RGB-colour overlay images of marker ions; separate ion images shown on right. Images in **b, c** and **e** are representative of 3 individual images.

Figure 4 | *In planta* T6P release and sugar metabolism over time. 7 day-old Arabidopsis seedlings grown in liquid culture were treated with 1mM of either *o*NPE-T6P **3** or DMNB-T6P **2**; control seedlings treated with water. Seedlings were left under growth lights to uptake the signalling precursors for 24 h, plants were then exposed to 23 $\mu mol.m^{-2}.s^{-1}$ UV for 2 h. Measurements were taken 1 day after uptake (Pre UV), 30 min, 60 min and 120 min post-initiation of UV treatment (23 $\mu mol.m^{-2}.s^{-1}$), 1 day and 2 days post initiation of UV treatment. (**a**) T6P content. (**b**) Trehalose content. (**c**) Sucrose content. (**d**) Glucose content. In all cases n = 3, Statistical significance (Students t-test) are indicated by asterisk: * (p <0.05) and ** (p < 0.01), error bars represent SEM (n=3).

Figure 5. Increased crop yield. (**a**) Increased grain size (20 per tube) after spraying. (**b**) Grain yield per plant with 1mM *o*NPE-T6P (**3**) or DMNB-T6P (**2**)**.** (**c**) Starch content of grain. * = p < 0.05 *cf* water control (student's t-test). Error bars SEM (n=6).

Figure 6. Increased crop resilience. (a) Plants after 20 days recovery following one application (1 mM) *o*NPE-T6P (**3**) or DMNB-T6P (**2**) one day prior to rewatering. **(b)** Dry weight (DWT) biomass from (a). **(c)** Plants after one application (1mM) *o*NPE-T6P (**3**) DMNB-T6P (**2**) one day prior to rewatering, cut at 5 days after rewatering, and left to regrow for 10 days. Cut back point = white arrow and line. **(d)** Fresh weight (FW) biomass of regrowth from (c). In all cases, * = $p < 0.05$ *cf* water control (students t-test, n = 6). Error bars represent SEM (n=6).

EXTENDED DATA FIGURE LEGENDS

Extended Data Figure 1 | The Central Role of T6P in Plants and Design of a Chemical Strategy for its Control

(a) Photosynthesis generates sucrose, which is translocated to growing regions of the plant. Inside the cell it feeds a pool of core metabolites which are substrates for biosynthetic processes that determine growth and productivity. T6P is synthesised from UDPG and G6P by trehalose 6-phosphate synthase (TPS) and therefore reflects the abundance of sucrose. It is broken down by trehalose phosphate phosphatase (TPP). Increasing T6P (i) stimulates starch synthesis and (ii) inhibits SnRK1, a protein kinase central to energy conservation and survival during energy deprivation. Inhibition of SnRK1 by T6P thus diverts carbon skeleton consumption into biosynthetic processes. (b) Trehalose biosynthetic pathway. (c) T6P is plant impermeable. Plant permeable variants allowed subsequent photoactivated release. (d) Generalized mechanism of light-activated release of precursors. (e) Release of T6P by light irradiation from the signalling precursor 1-4 *in vitro*. ^{31}P NMR at different time points of light irradiation confirming the activation of signalling precursors (1-4) and release of T6P. Time points: for 1 (0, 30, 60, 150 and 360min); for 2 (0, 60, 120, 300, 420 and 600 min); for 3 (0, 15, 30, 45 and 60 min); for 4 (0, 60, 120, 240, 360 and 420 min). (f, g) ^{1}H and ^{31}P NMR spectra after complete photolysis of signalling precursor confirming the release of T6P.

Extended Data Figure 2 | Inhibition of SnRK1.

Signalling precursors (**1**-**4**), T6P released from 1-4 (**r1**, **r2**, **r3**, **r4**) and T6P standard (**T6P**) were tested against SnRK1. T6P (0.26 mM) inhibits SnRK1 activity to ~36% of original activity. Signalling precursor compounds show no such inhibition whereas UV-released compounds show identical inhibition to free T6P. SnRK1 activity was determined from level of incorporation of phosphate onto

peptide substrate .min^{-1} .mg $^{-1}$ protein. (s.e.m., n=3). The activities of assays treated with precursors or released T6P were not significantly different from their controls (P<0.001, LSD) following one-way ANOVA of data transformed on the natural log scale.

Extended Data Figure 3 | *In planta* uptake analysis of signalling precursors 1-4.

(**a**) Schematic of protocol used for uptake analysis. (**b**) Calibration curves for *o*NB-T6P **1**, DMNB-T6P **2**, *o*NPE-T6P **3**, mono-DMNB-T6P **4**, respectively. Error bars represent SEM (n = 2) (**c**) HPLC (left) and MS (right) data, $[M+Na]^+$ or $[M-H]^-$, of pure signalling precursors **1-4**. (**d**) HPLC (left) and MS (right) data, $[M+Na]^+$ or $[M-H]^-$, of plant samples afte treatment with signalling precursors **1-4**. In the case of **3** the partially uncaged molecule also accumulated and was detected (coloured light blue).

Extended Data Figure 4 | Extraction and quantification of T6P.

(**a**) Schematic of protocol used for preparation of sample for T6P quantification. LLE = Liquid/liquid extraction, SPE = Solid phase extraction, AEC-MS = Anion exchange chromatography-mass spectrometry. (**b**) Liquid chromatograms of T6P, S6P and 2DG6P separation (top) using conditions optimized in **Supplementary Table S8** (entry 7) and the representative LCMS chromatograms of extraction samples treated with signalling precursors (middle) and water control (bottom). (**c, d**) Liquid chromatograms of variable concentration of T6P (500, 250, 100, 50, 25, 10, 5 µM) with constant concentration (100 µM) of 2DG6P as internal standard. (**e**) Resulting calibration curves of T6P peak area and T6P/2DG6P ratio against T6P concentrations (µM) in water as well as in the plant matrix. (**f-h**) LC-MS/MS analysis of T6P, S6P and the DMNB-T6P **2**-treated plant sample: (**f**) Fragmentation patterns of T6P (top) and S6P (bottom) by Quadrupole Time-of-fight tandem mass spectrometry (QTof-MS/MS) in negative ion mode. (**g**) Fragmentation patterns of T6P (top) and S6P (middle) by Triple quadrupole tandem mass spectrometry (QqQ-MS/MS) in negative ion mode and the T6P fragment ions tracking in the plant matrix (bottom). (**h**) HPLC chromatograms of T6P/S6P by Selected Ion Recording (SIR) of the intact molecular ion (m/z 421.0) and Multiple Reaction

Monitoring (MRM) of the fragment ions give the same retention time for each compound. (**i**) LC-MS quantification method through SIR and LC-MS/MS quantification method through MRM of the T6P level in the DMNB-T6P **2**-treated plant sample. From bottom to top: integration of T6P trace (2661) using SIR of m/z 421.0, integration of T6P trace (2550) using MRM of m/z 78.6, 96.3, 138.7, 241.0 and 421.0, integration for each fragment ion m/z 78.6 (801), m/z 96.3 (868), m/z 138.7 (76), m/z 241.0 (404) and m/z 421.0 (392).

Extended Data Figure 5 | Analysis of *Arabidopsis* plantlets following treatment.

(**a-c**) Phenotype (**a**) Fresh weight of plantlets versus concentration of signalling precursors of T6P (**1-4**) and G6P precursors (14-17) in medium after three days (72 h) of uptake. s.e.m, n=3. Each T6P precursor shown (top) followed by its G6P analogue (bottom). Visual appearance of a typical plantlet was observed for a given concentration of precursors at point of harvest. (**b**) Phenotype of plants at end of light treatments. Plants allowed to take up compounds for 72 h and were then treated the next day with light treatments. Light treatments: GL, growth light irradiance 250 $\mu mol.m^{-2}.s^{-1}$. UV 8 W and UV100 W were GL supplemented with UV light (365 nm). Daylight, part sun/part cloud, irradiance range 250 $\mu mol.m^{-2}.s^{-1}$ under cloud, 1440 $\mu mol.m^{-2}.s^{-1}$ under full sun. Compounds fed to plants to a final concentration of 1 mM. Phenotype of plants fed with *o*NPE-T6P **3** at a reduced final concentration of 0.1 mM shown in right hand panel. Scale, diameter of plastic tube mouth = 10 mm. GL= Growth light, UV= Ultraviolet. (**c**) Typical *A. thaliana* phenotypes in starch experiment. Plants were treated with a final medium concentration of 0.1 mM compound or water for 72 h and then exposed to 8 h 8W UV treatment. The plants were allowed to recover for another 24 h and were harvested at the end of the day and starch content measured. No significant phenotypic differences were observed between treatments. (a) water, (b) *o*NPE-T6P **3** 0.1 mM, (c) *o*NPE-G6P(1-OMe) **16**. Scale, tube diameter = 10 mm.

(**d-g**) Biosynthetic effects of increasing T6P *in planta*. (**d**) Starch level at the end of the day in UV treated (8W, 23 µmol. $m^{-2}.s^{-1}$, 8h) plants fed with *o*NPE-T6P **3** + UV is significantly higher than plants treated with water + UV (n=9, s.e.m). Samples for starch were taken 1 day post-UV

treatment. (**e**) ADP-glucose pyrophosphorylase (AGPase) activity is increased in UV + *o*NPE-T6P **3** plants compared to *o*NPE-T6P only, UV only, water only, UV + water treated plants and *o*NPE-G6P **16** treated plants (n=3, s.e.m.). (**f**) Starch synthesis rate in UV treated (20 µmol. $m^{-2}.s^{-1}$, 8h) oNPE-T6P treated plants (s.e.m., n = 3). (**g**) Starch level at the beginning (SEM n = 3) and at the end (SEM, n = 4) of the day in UV (20 µmol. $m^{-2}.s^{-1}$, 8h) + water-treated (●) and UV + oNPE-T6P-treated (○) plants. *Arabidopsis* used in **e**-**g** were at a light regime of 12h day/12h night, at 250 $\mu mol.m^{-2}.s^{-1}$, and 23 ˚C day/18 ˚C night temperatures, treated with compounds 18 days post sowing, and exposed to UV light 72 hours after addition of compound. Asterisks in **d**-**f** denote statistical significance with ANOVA analysis (p = 0.002). Asterisk in **g** denotes statistical significance by one-way ANOVA (LSD 5% = 11.19).

Extended Data Figure 6 | Quantification of *in planta* metabolites.

(**a**) LCMS chromatograms of trehalose, sucrose, glucose and fructose separation using HILIC column, for details see Supporting Information. (**b**) Liquid chromatograms and peak areas of variable concentration of trehalose (100, 50, 25, 10, 5 µM) and glucose (500, 250, 100, 50, 25, 10, 5 µM). (**c**) Calibration curves of trehalose peak area against the concentrations (µM) and glucose peak area against the concentrations (µM). (**d,e**) As for **Figure 4**, 7 day-old Arabidopsis seedlings grown in liquid culture were treated with 1mM of either *o*NPE-T6P **3** or DMNB-T6P **2** control seedlings were treated with water. Seedlings were left under growth lights to uptake the signalling precursors for 24 h, plants were then exposed to 23 $\mu mol.m^{-2}.s^{-1}$ UV for 2 h. Measurements were taken 1 day after uptake (Pre UV), 30 min, 60 min and 120 min post-initiation of UV treatment (23 $\mu mol.m^{-2}.s^{-1}$), 1 day and 2 days post initiation of UV treatment. See **Figure 4** for T6P content, trehalose content, sucrose content, glucose content; here (**d**) fructose content and (**e**) Fresh weight biomass are shown. In all cases n = 3, Statistical significance (students t-test) are indicated by asterisk: * (p <0.05) and ** (p < 0.01). Error bars represent SEM (n=3).

Extended Data Figure 7 | Dynamics of ^{13}C-T6P, ^{13}C-trehalose, ^{13}C-glucose and T6P in *A. Thaliana* treated by ^{13}C-labelled precursor 2*.

1mM of DMNB-^{13}C-T6P **2*** was added to the growth medium of 7- day-old *Arabidopsi*s seedlings. The plants were left under growth light to uptake for 24 h and the uncaging was performed under 23 μmol.m^{-2}.s^{-1} UV for 2 h. Samples were harvested for analysis at different time points – pre UV, 30 min, 60min and 120 min (after onset of UV irradiation) and 1 day and 2 days after onset of UV irradiation. (a) Synthesis of universally ^{13}C-labelled **2*** in essentially the same manner as for **2**. (**b**) Amount of ^{13}C-T6P released over time *in plant*a. (**c**) Amount of ^{13}C-trehalose accumulated. (**d**) Amount of ^{13}C-glucose accumulated. (**e**) Amount of endogenous T6P. (**f**) Overview of ^{13}C tracking of T6P and metabolites. In all cases SEM n = 3. Asterisks indicate statistical (Students t-test) significance (* 0.01<*P*<0.05, ** 0.001<P<0.01).

Extended Data Figure 8 | Synthesis, *in vitro* SnRK1 inhibition studies and *in planta* uptake analysis of G6P (1-OMe) analogues 14-17.

(**a**) Design and synthesis of *o*NB-G6P **14**, DMNB-G6P **15**, *o*NPE-G6P **16** and mono-DMNB-G6P **17**. (**b**) Lack of inhibition of SnRK1 by G6P (1-OMe) analogues. Bars represent SEM (n=3). (**c**) HPLC and MS data, $[M+Na]^+$ or $[M-H]^-$, of pure G6P (1-OMe) precursors **14-17**. For HPLC conditions see the Supplementary Information section 1. (**d**) HPLC and MS data, $[M+Na]^+$ or $[M-H]^-$, of uptaken G6P(1-OMe) precursors**14-17** *in planta*. (**e**) Calibration curves for DMNB-G6P **15**, *o*NPE-G6P **16**, mono-DMNB-G6P **17**, respectively (n = 2 in all cases).

Extended Data Figure 9 | Transcript abundance of genes involved in starch synthesis and SnRK1 marker genes in response to caged-T6P precursor application of 7- day- old Arabidopsis seedlings in liquid culture.

Seedlings were treated with a final concentration of 1mM of either *o*NPE-T6P **3** or DMNB-T6P **2**, allowed to uptake for 1 day under the growth lights, then treated with 23 μmol.m^{-2}.s^{-1} UV light 2 h to facilitate uncaging. (**a**) Transcript fold change after 60 min of UV treatment and (**b**) 1 day after UV

treatment of SnRK1 marker genes. Marker genes normally down-regulated by SnRK1: *TPS5* (At4g17770), *UDPGDH* (At3g29360) and *bZIP11* (At4g34590), and marker genes normally up-regulated by SnRK1: *TPS8* (At1g70290), *βGAL* (At5g56870), and *ASN* (At3g47340). (**c**) Transcript fold change of starch synthesis genes after 60 min of UV treatment. Genes involved in starch synthesis: *APL3* (At4g39210); *SS3* (At1g11720), *BE1* (At3g20440) and *GBSS1* (At1g32900). (**d**) Transcript fold change after 60 min of UV treatment for starch degradation genes. Genes involved in starch degradation: *BAM1* (At3g23920); *BAM3* (At4g17090); *BAM4* (At5g55700) and *GWD3* (At4g24450). Changes in transcripts for enzymes of degradation were more equivocal with *GWD3* increasing and *BAM* genes showing small changes or decreasing (*BAM3*). All data was normalised to a ubiquitin control. All data are means with SEM of three independent samples

Extended Data Figure 10 | Additional Effects in Wheat.

(a) Chlorophyll content of leaves post anthesis of ear treatments, **(b)** Chlorophyll content of leaves post anthesis of whole plant treatments. **(c-e)** T6P release and metabolism in wheat. Developing wheat grain were treated with either T6P, *o*NPE-T6P 3 or DMNB-T6P 2 (all 1mM) at 5 or 10 days post anthesis (DPA) and harvested 1 day later. **(c)** Amount of T6P in wheat grains (n=3, s.e.m.). **(d)** Trehalose (n=3, s.e.m.). **(e)** Sucrose (n=3, s.e.m.). Asterisks indicate statistical significance with Students T-test (* 0.01<*P*<0.05, ** 0.001<P<0.01). **(f)** Dose response grain yield per plant to T6P precursors (0.1 mM, 1 mM and 10 mM *o*NPE-T6P or DMNB-T6P) and water, T6P and trehalose controls) sprayed to ears (5 ml) or to whole plant (45 ml) at 5, 10, 15 and 20 DPA. Asterisks indicate statistical significance ($p < 0.05$) compared to water control. +- standard error (n=6). **(g)** Grain yield per ear in response to single time point spray (5 ml to ear at 10 DPA). Asterisks in (**f** and **g**) indicate statistical significance ($p < 0.05$) with students t-test in comparison to water control. +/- standard error (n=6).