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RESEARCH ARTICLE

Growth, physiological and proteomic responses in field grown wheat varieties exposed to elevated CO_2 under high ambient ozone

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Abstract The present study investigated growth, biochemical, physiological, yield and proteomic changes in 3 wheat varieties exposed to elevated CO_2 (515 ppm) in a background of high ambient ozone in field. Ethylenediurea (EDU) was used as antiozonant. Average ozone concentration was 59 ppb and was sufficient enough to exert phytotoxic effects. Elevated carbon dioxide (eCO_2) and EDU application individually or in combination negated the adverse effects of ozone by modulating antioxidants and antioxidative enzymes. Differential leaf proteomics revealed that at vegetative stage major changes in protein abundance were due to EDU treatment (47, 52 and 41 proteins in PBW-343, LOK1 and HD-2967, respectively). Combined treatment of eCO_2 and EDU was more responsible for changes in 37 proteins during flowering stage of PBW-343 and LOK1. Functional categorization revealed more than 60% differentially abundant protein collectively belonging to carbon metabolism, protein synthesis assembly and degradation and photosynthesis. At both the growth stages, LOK1 was more responsive to eCO_2 and combined treatment (eCO_2 + EDU). HD-2967 was more positively responsive to EDU and combined treatment. eCO_2 in combination of EDU protected these varieties against high ambient O_3.

Keywords Tropospheric ozone · EDU · Elevated CO_2 · Wheat · Yield · 2D-proteomics · Protein abundance · Mass spectrometry

Introduction

Anthropogenic activities are causing accumulation of greenhouse gases (GHGs) of which atmospheric carbon dioxide (CO_2) and tropospheric ozone (O_3) directly affect plant productivity. CO_2 concentration has reached 413.92 ppm (https://www.esrl.noaa.gov) with the further predictions of 936 ppm (RCP8.5) by the year 2100 (IPCC 2014). Similarly, O_3 concentration has doubled since the pre-industrial times (Akimoto 2003; Vingarzan 2004) and still continuously increasing. Assessment Report Five (AR5) suggests O_3 concentration may increase by 40–60% by the end of this century (IPCC 2014). Modelling based studies of ozone risk on Asian agriculture reveals 3.5–20% yield loss for important crops (Avnery et al. 2011; Emberson et al. 2009).

The Indo-Gangetic Plains (IGP), India is one of the most fertile regions and is highly vulnerable to ozone-induced yield loss of important crops (Oksanen et al. 2013). Ozone-induced oxidative stress plays a key role in decreasing crop productivity (Ainsworth 2017). O_3 enters the leaf stomata and alters various physiological processes (Fiscus et al. 2005) such as reduced stomatal conductance and photosynthesis (A) (Morgan et al. 2003) leading to decreased shoot biomass and grain yield (Feng et al. 2008).

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It is known that CO_2 and O_3 exert almost opposite impact on the vegetation. An increase in CO_2 concentration has positive impact on C_3 crop response due to increased photosynthesis and water use efficiency (Ainsworth and Long 2005; Long et al. 2006). Whereas O_3 inhibits photosynthesis and other physiological processes resulting in decreased growth and yield (Gupta et al. 2018; Pandey et al. 2015). It is generally accepted that elevated CO_2 negates the harmful effects of high O_3 (Booker and Fiscus 2005; Wustman et al. 2001). Very few studies have been done so far under natural field conditions to investigate interactive effects of elevated CO_2 and O_3 on crop plants, fewer still in India. Mishra et al. (2013) and Kumari et al. (2015) in wheat studied the effects of elevated CO_2 and O_3 in open top chambers. The drawback of OTC based studies includes chamber effect (McLeod and Long 1999; Morgan et al. 2001), therefore, the comparison of ambient conditions to that of the controlled environmental conditions inside the OTCs requires considerable rationalization. To overcome chamber effect free air concentration enrichment (FACE) facility has been established (Hendrey et al. 1993). FACE facility at CSIR-NBRI has been established and is fully functional since 2013 (Pandey et al. 2017).

To study the impact of high ozone on vegetations in ambient condition, different chemicals, have been used. *N*-[2-(2-oxo-1-imidazolidinyl) ethyl]-*N*′-phenyl urea (EDU) has been widely used to assess O_3 induced acute and chronic injury without appreciable effects on its own (Gupta et al. 2018; Manning et al. 2011; Pandey et al. 2014).

Globally India is the second largest wheat producer and in the year 2017–2018, India contributed 15.36% of global wheat production. The aim of the present study was to understand the basis of physiological, biochemical, proteomic and yield responses of three commonly grown wheat varieties to eCO_2 (under FACE) and high ambient O_3 (with the application of EDU) individually as well as in combination. Our hypotheses were that:

1. eCO_2 will negate the adverse impact of high ambient ozone on wheat growth and yield
2. EDU will further enhance plant performance under eCO_2 condition
3. Wheat varieties will respond differently to eCO_2 and EDU treatments.

Materials and methods

Carbon dioxide enrichment

The Free Air Concentration Enrichment (FACE) system was established at CSIR-National Botanical Research Institute, Lucknow (80° 59′ E, 26° 55′ N, 123 m above sea level), India in 2013 (Pandey et al. 2017). FACE facility consists of six hexagonal CO_2 enrichment rings (3 ambient/non-enrichment/control and 3 elevated/enrichment/treated rings) having a diameter of 10 m. Air compressors were used to pump air mixed with CO_2 in elevated rings. Controlled CO_2 was release inside the ring by solenoid valve and infra-red gas analyser (IRGA) was installed for monitoring of CO_2 concentration inside the ring. Central sensor tower, fitted with six different sensors to monitor CO_2 concentration, temperature, humidity, light intensity, wind speed and wind direction, is placed in each ring. Microprocessor based Supervisory Control and Data Acquisition (SCADA) system is used to control all the sensors and data collection. The system has memory backup, real time clock combination, data logger and single window operation to monitor all necessary parameters.

Meteorological conditions and ozone monitoring

During experiment 24-h weather data of Lucknow city was obtained via online database (https://www.wunderground.com). Ozone Monitor (2B Tech, Model 106-L) was used to monitor ambient ozone at the experimental site from November till March. Accumulated Ozone Threshold 40 ppb (AOT 40) was calculated according to De Leeuw and van Zantvoort (1997).

Plant material, plot preparation and experimental design

Three winter wheat (*Triticum aestivum* L.) varieties viz., PBW-343, LOK1 and HD-2967 were selected for this study. Variety specific characteristics of selected varieties are tabulated in Table S1. Seeds of each variety were sown during mid of November and the treatments began from December beginning. Plants (60 plants) of each variety were grown in two subplots in individual rings and out of these 30 plants were used for EDU treatment. Ambient ring contained one plot of no treatment (control) and one plot of EDU treatment. Similarly, elevated CO_2 ring contained one plot of no treatment (eCO_2 treatment) and one for EDU treatment (eCO_2 + EDU treatment or combined treatment) for each variety. The statistical design of the experiment is provided in Fig. S1. Fertilizers were applied as per recommended doses.

Foliar application of EDU

EDU treatment (200 ppm) began 15 DAG on the whole foliage via mechanical sprayer on treatment plots while on control plots, water was used (Gupta et al. 2018). EDU treatment was done weekly throughout the experiment.

Plant photosynthesis and sample collection

LiCOR 6400 was used to measure physiological parameters. The youngest fully mature leaf at 65 DAG from 5 randomly selected plants was taken for measurement.

Plants were harvested for biomass and biochemical parameters at vegetative (55 DAG) and flowering (95 DAG) stages. Roots and shoots were separated and measured for growth parameters then oven dried at 70 °C. Leaves (5–10) were stored in liquid nitrogen and placed in − 80 °C for further analyses.

Pigments, enzymes and antioxidants estimation

Non-destructive chlorophyll estimation was performed using SPAD-502. Thiobarbituric (TBA) assay was used to measure lipid peroxidation in terms of malondialdehyde (MDA) content (Heath and Packer 1968). The hydrogen peroxide (H_2O_2) was measured using Bernt and Bergmeyer (1974) method. Superoxide dismutase (SOD) activity was assayed using NBT method (Beyer and Fridovich 1987). Catalase (CAT) was assayed by method of Rao et al. (1996). Ascorbate peroxidase (APX) was measured by using method of Chen and Asada 1989. GR activity was assayed according to Smith et al. (1988). Protein content was measured according to Bradford (1976) method. Ascorbate and glutathione were estimated by following the methods of Hodges et al. (1996) and Griffith (1980), respectively.

Total soluble sugar and Starch

Ethanolic extraction of total soluble sugar (Cross et al. 2006) was performed and estimated by anthrone (Yemm and Willis 1954) method. Perchloric acid was used for starch extraction (Whelan 1955) and estimated as per Nguyen et al. (2002).

Yield parameters

Yield parameters were analyzed on the basis of above ground biomass, spike length, spikelet number spike^{-1}, inflorescence weight plant^{-1}, number of seed plant^{-1} from fifteen plants for each treatment. Harvest index and weight of thousand grains were also estimated.

Proteomic studies

Leaf protein extraction

Total leaf proteins were extracted by trichloroacetic acid-Acetone method (Sharma et al. 2017) with few modifications. Extracted proteins were solubilized in solubilisation buffer (7 M urea, 2 M thiourea, 2% CHAPS (w/v), 25 mM DTT and 0.5% immobilized pH gradients buffers (v/v)) at room temperature for 2–3 h and quantified.

Two-dimensional gel electrophoresis and image analysis

Passive rehydration on immobilized pH gradients (IPG) strips of 7 cm length, pH 4–7 (GE Healthcare) was performed overnight in reswelling tray. Ettan IPGphore-3 (GE Healthcare) system was used to conduct Isoelectric focusing (IEF) at 20 °C in 5 step program as follows: Step 1: 50 V for 1 h, Step 2: 500 V for 1 h, Step 3: 1500 V for 2 h (all step and hold), Step 4: 4000 V for 2 h (Gradient) and Step 5: 6000–12,000 V.

Electrophoresis was carried out in Mini Protein tetra cell (Bio-Rad) with Tris–Glycine running buffer and 12% SDS–Polyacrylamide gel (acrylamide: Bis ratio of 29:1) at 70 V for 5 min then 100 V till end using a Power PAC300 (Bio-Rad). Gels were stained with coomassie brilliant blue G (0.5%) and destained (Methanol: Water: Glacial Acetic acid = 45:45:10) and were scanned using gel doc system (Bio-Rad).

Analysis of protein abundance was performed using Image Master 2D Platinum (IMP) 7.0 software (GE Healthcare) on the basis of relative volume (% volume) of protein spots in triplicate images. Three replicates gels from each treatment and control of all three tested wheat variety were analysed. The intensity of protein spots calculated by the software depends on the maximum calibrated pixel intensity within the spot from which minimum pixel value in the spot neighbourhood (background) has been subtracted. The area at 75% of the spot intensity, as measured from the peak of the spot, computed by software and % volume of spot determined Relative volume (% volume) was used to quantify and compare the spots. Relative volume considers the ratio of detected spot pixel density to the sum of all analyzed spot pixel density. Hence, this procedure permitted to normalize experimental variations due to protein loading and staining. Change in % volume more than 1.5-fold was taken as increased abundance and less than 1.5-fold were taken as decreased abundance.

Mass spectrometry and protein identification

After excision of differentially abundant protein spots from the gel, tryptic digestions were performed (Shevchenko et al. 2007). Cut gel pieces were chopped, destained with 50 mM ammonium bicarbonate (ABC) in 50% methanol and were rehydrated, dehydrated with 25 mM ABC and 2:1 (v/v) solution of ACN and 50 mM ABC, respectively. Overnight trypsin digestion was performed at 37 °C in a circulating water bath. Peptides were extracted in 50%

ACN and 1% trifluoroacetic acid (TFA). Samples were spotted on Opti-TOF TM, 384 well target plate in equal volume with the matrix solution (5 mg/ml a-Cyano-4-hydroxycinnamic acid). Mass spectrometry (MS) was performed in 4800 Plus MALDI TOF/TOF analyzer (ABI, USA) to generate monoisotopic peptide masses and were further analyzed by 4000 Series Explorer software version 3.5. Protein identification was done by Mascot software (http://www.matrixscience.com) against NCBInr/Swiss Prot protein database. Search parameters comprised of taxonomy- viridiplantae, fixed modification- carbamidomethylation, variable modification- oxidation, peptide tolerance- ± 100 ppm or 1.2 Da (Dalton), MS/MS tolerance ± 0.2 Da or 0.6 Da respectively and peptide charge + 1.

Statistical analysis

The evaluation of the impact of individual treatments of eCO_2, EDU and combined treatment ("eCO_2", "EDU" and "eCO_2 + EDU" respectively) were analysed with Linear multivariate analysis using SPSS 16.0 (SPSS Inc.) that provides the main effects and interactions of treatments and variety on various experimental parameters. Stages (vegetative, flowering and harvesting) were analysed separately. Venny 2.1 analysis was performed collectively for each variety and separately for different sampling time to obtain treatment specific protein.

Results

FACE, meteorological condition and ozone monitoring

During the experimental period the average elevated CO_2 concentration was 515 ppm (Fig. S2; Table S2). Meteorological data during the experimental period are provided in Table S3. Average ambient ozone concentration during the experiment was 59 ppb. Maximum AOT40 values were obtained during seed germination, flowering and grain filling stages (Fig. 1; Table S4).

Growth, physiology, antioxidants and antioxidative enzymes

Effect of the treatments (eCO_2, EDU and eCO_2 + EDU) on different biochemical parameters and biomass on three wheat varieties (PBW-343, LOK1 and HD-2967) at both stages were revealed in Table 1. Multivariate analysis was performed separately for both the sampling stages (Table S5 and S6). Positive response of biomass to eCO_2 was observed in LOK1 variety at both stages. PBW-343 did not show any significant change in biomass under any treatment. HD-2967 exhibited more positive response to EDU treatment for biomass at vegetative phase (34%) and at flowering phase (24%) (Fig. S3).

Significant changes, observed in *A*, WUE and gs, were due to Cv and Cv × trt (Fig. S4; Table S7). In LOK1 increased *A* (31.7%) was observed due to eCO_2. Similarly, increased *A* (26%) was found due to combined treatment in LOK1. HD-2967 showed significantly increased WUE (62%) with decreased gs (28%) in combined treatment.

Reduced total chlorophyll content was observed in all three varieties at the vegetative stage. PBW-343 and HD-2967 revealed maximum reduction (5% and 4%, respectively) in eCO_2 treatment. Similarly, LOK1 showed maximum decreased (8%) total chlorophyll from individual treatment of eCO_2 and EDU. At flowering stage, total chlorophyll content was increased in all three varieties. The treatment of eCO_2 + EDU enhanced the chlorophyll content in PBW-343 and HD-2967 (7% and 5%, respectively). Similarly, LOK1 showed an increment of 7% and 8% in total chlorophyll in EDU and combined treatment, respectively.

At vegetative stage marked reduction in MDA content was found in PBW-343 (19%), LOK1 (18%) and HD-2967 (14%) under combined treatment. Similarly, decreased H_2O_2 content was found in PBW-343 and LOK1. At flowering stage, decreased MDA and H_2O_2 content were observed in all three treatments (Table 2; Fig. S5).

Among antioxidants (Fig S6; Table S5 and S6), total ascorbate showed contrasting results at both the stages. It was found increased in eCO_2 treated PBW-343 (43%), combined treatment in LOK1 (20%) and in all treatment in HD-2967 (6%, 35% and 40% in eCO_2, EDU and combined treatment, respectively) at vegetative stage. At flowering stage, total ascorbate content was found decreased in all the tested varieties and treatments.

Glutathione content also exhibited contrasting responses during both phases. During vegetative phase, all cultivars demonstrated increased level of total and reduced GSH except PBW-343 in eCO_2 treatment. LOK1 exhibited maximum increment in total and reduced GSH by 94% and 110%, respectively in eCO_2. However, PBW-343 showed 73% (total GSH) and 126% (reduced GSH) increase in EDU treatment while HD-2967 exhibited 43% (total GSH) and 85% (reduced GSH) increase in individual treatments of eCO_2 and EDU during vegetative stage. In flowering stage increment in reduced GSH (39%, 33% and 75% in eCO_2, EDU and combined treatment, respectively) content was observed in PBW-343. LOK1 and HD-2967.

Antioxidative enzymes such as SOD, CAT, APX and GR showed significant activities at vegetative stage due to all factors (Fig. S5; Table S5 and S6). In vegetative phase, maximum increment in APX was found in the combined treatment of all the varieties. At flowering phase, APX was

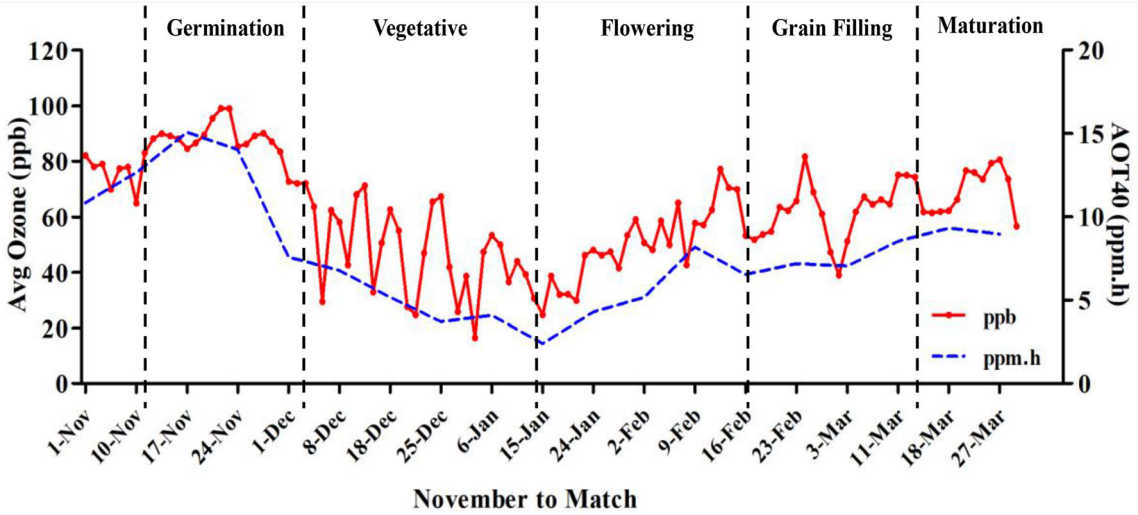

Fig. 1 Average ozone concentration (8 h Daily) and AOT40 (ppm.h) during the study period. The different phases of wheat growth were separated by doted lines

found to be significantly increased by 44% and 33% (eCO_2 treatment) in PBW-343 and HD-2967, respectively. SOD was maximally increased by 19% under eCO_2 in PBW-343, 36% under combined treatment in LOK1 and by 31% under EDU treatment in HD-2967 at vegetative phase. However, in flowering phase, SOD was found only increased in LOK1 by 31, 32 and 91% in eCO_2, EDU and combined treatment, respectively. While it decreased in remaining varieties except significantly increased (61%) under EDU treated HD-2967.

Yield

Yield attributes viz. number of grains plant^{-1} showed maximum increment in LOK1 among all the varieties and treatments followed by PBW-343 (Table 2; Fig. S7; Table S8). Combined treatment resulted in better yield in terms of number of grain plants^{-1} (11% and 21% in PBW-343 and LOK1, respectively), grain weight plants^{-1} (10% and 8% in PBW-343 and LOK1, respectively) and thousand grain weight (4% and 7% in PBW-343 and LOK1, respectively). Surprisingly HD-2967 responded negatively to eCO_2 however positive response to EDU were recorded for number of grain plants^{-1} (12%) and thousand grain weight (2%). Notably, very small decrease in grain weight plants^{-1} in EDU treated HD-2967 was also observed. Non-significant changes in HI were observed in all the varieties and treatments.

Differential leaf protein profiling and functional categorisation

The 2-DGE wheat leaf revealed protein abundance either due to eCO_2 and/or EDU treatment. Representative gels from each treatment within each variety at both sampling stages were analysed (Fig. S9, 10 and 11). Differentially abundant proteins ($P < 0.05$) were identified using IMP-7 software (Table S9). Interestingly, PBW-343 showed least while HD-2967 showed most changes in protein abundance between the sampling stages (Table S9). Detailed information of differentially abundant proteins in each varieties and sampling stages is provided in Table S11.

Venn diagram analysis revealed treatment specific proteins for both sampling stages and collectively for all three varieties (Fig. 2; Table S10). At vegetative stage, 12% proteins were eCO_2 responsive, 24% were EDU responsive and 7% were CO_2 + EDU responsive. Similarly, at flowering stage their percentage were 10%, 17% and 10%, for eCO_2, EDU and combined treatments, respectively.

Elevated CO_2 responsive proteins included: RuBisCO, RuBisCO activase, oxygen evolving enhancer (OEE), glycine decarboxylase, aminomethyltransferase (AMT), malate dehydrogenase (MDH), thioredoxin reductase, thioredoxin-like protein etc. EDU responsive proteins were triosephosphate isomerise (TPI), transketolase (TK), carbonic anhydrase (CA), ATP synthase subunits, peroxiredoxin, thiazole biosynthetic enzyme etc. Proteins found unique in combined treatment included elongation factor, initiation factor, glyceraldehyde-3-phosphate dehydrogenase (G3PDH), phosphor glycerate kinase (PGK), ATPase subunit, ascorbate peroxidase (APX), glutathione S-transferase and S-adenosylmethionine synthase (Fig. 3; Table S10).

Proteins were further categorised according to their putative physiological functions in ten categories namely Protein Synthesis Assembly Degradation (PSAD), Photosynthesis (PHO), Carbon Metabolism (CM), Energy Metabolism (EM), Amino Acid Metabolism (AAM),

Table 1 Effects of eCO_2, EDU and eCO_2 + EDU on growth and biochemical parameters of three wheat varieties

S. nos.	Parameter	PBW-343 (vegetative/flowering)				LOK1 (vegetative/flowering)			
		Control	CO_2 only	EDU only	CO_2 + EDU	Control	CO_2 only	EDU only	CO_2 + EDU
1	Chlorophyll (SPAD unit)	$(44.87 \pm 0.12)^{a}$/ $(48.26 \pm 0.57)^{b}$	$(42.67 \pm 0.07)^{c}$/ $(49.01 \pm 0.39)^{b}$	$(43.70 \pm 0.15)^{b}$/ $(49.04 \pm 0.85)^{b}$	$(44.40 \pm 0.20)^{a}$/ $(51.71 \pm 0.49)^{a}$	$(43.43 \pm 0.09)^{A}$/ $(43.64 \pm 0.54)^{C}$	$(39.87 \pm 0.09)^{C}$/ $(44.98 \pm 0.06)^{B}$	$(39.67 \pm 0.20)^{C}$/ $(47.06 \pm 0.22)^{A}$	$(40.63 \pm 0.29)^{B}$/ $(46.77 \pm 0.37)^{A}$
2	Total ascorbate (mmol g^{-1}freshwt)	$(6.98 \pm 0.02)^{b}$/ $(9.98 \pm 0.03)^{a}$	$(10.01 \pm 0.06)^{a}$/ $(9.76 \pm 0.03)^{b}$	$(6.12 \pm 0.02)^{c}$/ $(9.29 \pm 0.05)^{c}$	$(6.21 \pm 0.09)^{c}$/ $(8.89 \pm 0.02)^{d}$	$(6.41 \pm 0.02)^{B}$/ $(7.81 \pm 0.02)^{A}$	$(6.32 \pm 0.04)^{B}$/ $(6.74 \pm 0.04)^{C}$	$(6.17 \pm 0.03)^{C}$/ $(7.54 \pm 0.05)^{B}$	$(7.73 \pm 0.03)^{A}$/ $(6.88 \pm 0.06)^{C}$
3	Red. ascorbate (mmol g^{-1}freshwt)	$(5.44 \pm 0.00)^{b}$/ $(4.72 \pm 0.00)^{c}$	$(5.85 \pm 0.02)^{a}$/ $(5.41 \pm 0.05)^{b}$	$(4.58 \pm 0.02)^{c}$/ $(4.79 \pm 0.02)^{c}$	$(4.56 \pm 0.01)^{c}$/ $(6.24 \pm 0.06)^{a}$	$(4.75 \pm 0.03)^{D}$/ $(4.86 \pm 0.01)^{B}$	$(5.39 \pm 0.00)^{B}$/ $(4.19 \pm 0.01)^{D}$	$(5.12 \pm 0.03)^{C}$/ $(4.98 \pm 0.01)^{A}$	$(6.43 \pm 0.05)^{A}$/ $(4.49 \pm 0.02)^{C}$
4	DHA (mmol g^{-1}freshwt)	$(1.55 \pm 0.03)^{b}$/ $(5.26 \pm 0.03)^{a}$	$(1.16 \pm 0.04)^{c}$/ $(4.35 \pm 0.03)^{c}$	$(1.53 \pm 0.04)^{b}$/ $(4.50 \pm 0.03)^{b}$	$(1.65 \pm 0.08)^{b}$/ $(2.65 \pm 0.04)^{d}$	$(1.67 \pm 0.04)^{A}$/ $(2.95 \pm 0.03)^{A}$	$(0.93 \pm 0.04)^{C}$/ $(2.55 \pm 0.03)^{B}$	$(1.05 \pm 0.05)^{C}$/ $(2.56 \pm 0.05)^{B}$	$(1.30 \pm 0.03)^{B}$/ $(2.39 \pm 0.04)^{C}$
5	Total glutathione (mmol g^{-1}freshwt)	$(23.92 \pm 0.60)^{c}$/ $(16.14 \pm 0.71)^{c}$	$(23.04 \pm 0.40)^{c}$/ $(21.04 \pm 0.86)^{b}$	$(41.27 \pm 0.34)^{a}$/ $(21.87 \pm 0.93)^{b}$	$(29.97 \pm 0.62)^{b}$/ $(25.39 \pm 0.67)^{a}$	$(26.19 \pm 0.72)^{D}$/ $(18.25 \pm 0.75)^{A}$	$(50.92 \pm 0.96)^{A}$/ $(15.90 \pm 0.60)^{AB}$	$(29.95 \pm 0.44)^{C}$/ $(15.31 \pm 1.11)^{B}$	$(37.88 \pm 0.45)^{B}$/ $(16.27 \pm 0.36)^{AB}$
6	GSSG (mmol g^{-1}freshwt)	$(8.12 \pm 0.20)^{b}$/ $(3.30 \pm 0.13)^{b}$	$(7.10 \pm 0.23)^{c}$/ $(3.23 \pm 0.10)^{b}$	$(5.61 \pm 0.40)^{d}$/ $(4.76 \pm 0.20)^{a}$	$(9.54 \pm 0.10)^{a}$/ (2.90 ± 0.15)b	$(7.06 \pm 0.29)^{B}$/ $(3.48 \pm 0.07)^{C}$	$(10.58 \pm 0.12)^{A}$/ $(2.89 \pm 0.07)^{D}$	$(5.17 \pm 0.40)^{C}$/ $(4.99 \pm 0.15)^{B}$	$(7.87 \pm 0.20)^{B}$/ $(6.38 \pm 0.00)^{A}$
7	GSH (mmol g^{-1}freshwt)	$(15.79 \pm 0.49)^{c}$/ $(12.84 \pm 0.64)^{c}$	$(15.95 \pm 0.47)^{c}$/ $(17.82 \pm 0.76)^{b}$	$(35.66 \pm 0.75)^{a}$/ $(17.11 \pm 0.81)^{b}$	$(20.43 \pm 0.70)^{b}$/ $(22.50 \pm 0.66)^{a}$	$(19.13 \pm 0.92)^{D}$/ $(14.76 \pm 0.80)^{A}$	$(40.34 \pm 1.59)^{A}$/ $(13.01 \pm 0.53)^{A}$	$(24.77 \pm 0.33)^{C}$/ $(10.32 \pm 0.98)^{B}$	$(30.01 \pm 0.27)^{B}$/ $(9.89 \pm 0.36)^{B}$
8	APX (μ mol mg^{-1} $protein^{-1}min^{-1}$)	$(1.02 \pm 0.01)^{b}$/ $(0.84 \pm 0.01)^{b}$	$(1.34 \pm 0.04)^{a}$/ $(1.20 \pm 0.05)^{a}$	$(0.41 \pm 0.02)^{c}$/ $(0.91 \pm 0.03)^{b}$	$(1.27 \pm 0.01)^{a}$/ $(0.86 \pm 0.02)^{b}$	$(0.83 \pm 0.02)^{B}$/ $(1.27 \pm 0.01)^{C}$	$(0.82 \pm 0.02)^{B}$/ $(1.40 \pm 0.01)^{B}$	$(0.81 \pm 0.04)^{B}$/ $(1.95 \pm 0.02)^{A}$	$(0.84 \pm 0.05)^{A}$/ $(1.26 \pm 0.04)^{C}$
9	GR (μmol mg^{-1} $protein^{-1}min^{-1}$)	$(0.07 \pm 0.00)^{a}$/ $(0.17 \pm 0.00)^{b}$	$(0.07 \pm 0.00)^{a}$/ $(0.19 \pm 0.01)^{a}$	$(0.07 \pm 0.00)^{a}$/ $(0.13 \pm 0.00)^{c}$	$(0.06 \pm 0.00)^{b}$/ $(0.18 \pm 0.01)^{ab}$	$(0.07 \pm 0.00)^{B}$/ $(0.16 \pm 0.01)^{A}$	$(0.05 \pm 0.00)^{C}$/ $(0.12 \pm 0.00)^{C}$	$(0.07 \pm 0.00)^{AB}$/ $(0.14 \pm 0.00)^{B}$	$(0.08 \pm 0.00)^{A}$/ $(0.09 \pm 0.00)^{D}$
10	Catalase (nmol mg^{-1} $protein^{-1}min^{-1}$)	$(35.33 \pm 0.58)^{a}$/ $(36.55 \pm 0.75)^{c}$	$(33.88 \pm 0.65)^{a}$/ $(23.69 \pm 0.37)^{d}$	$(33.21 \pm 0.68)^{a}$/ $(58.32 \pm 0.41)^{a}$	$(23.08 \pm 1.49)^{b}$/ $(53.13 \pm 0.67)^{b}$	$(39.01 \pm 0.62)^{B}$/ $(60.13 \pm 0.94)^{C}$	$(33.69 \pm 0.27)^{C}$/ $(57.08 \pm 0.90)^{A}$	$(41.44 \pm 0.90)^{A}$/ $(63.72 \pm 0.85)^{B}$	$(33.08 \pm 0.53)^{C}$/ $(70.83 \pm 1.53)^{A}$
11	SOD (Ug^{-1}freshw.)	$(5.95 \pm 0.11)^{b}$/ $(9.81 \pm 0.28)^{a}$	$(7.07 \pm 0.16)^{a}$/ $(4.67 \pm 0.08)^{bc}$	$(6.18 \pm 0.12)^{b}$/ $(7.12 \pm 0.15)^{ab}$	$(6.24 \pm 0.14)^{a}$/ $(5.84 \pm 0.13)^{c}$	$(5.46 \pm 0.09)^{C}$/ $(4.97 \pm 0.08)^{A}$	$(3.68 \pm 0.07)^{B}$/ $(6.53 \pm 0.20)^{B}$	$(5.36 \pm 0.08)^{C}$/ $(6.6 \pm 0.13)^{A}$	$(7.44 \pm 0.19)^{A}$/ $(9.54 \pm 0.31)^{A}$
12	MDA (nmol g^{-1}fres wt)	$(237.12 \pm 9.78)^{ab}$/ $(102.52 \pm 1.49)^{a}$	$(227.84 \pm 10.32)^{ab}$/ $(93.26 \pm 1.26)^{a}$	$(255.86 \pm 6.07)^{a}$/ $(69.46 \pm 1.97)^{b}$	$(191.83 \pm 9.62)^{b}$/ $(76.27 \pm 1.00)^{b}$	$(221.05 \pm 6.70)^{AB}$/ $(86.74 \pm 2.27)^{B}$	$(215.51 \pm 9.86)^{AB}$/ $(76.95 \pm 0.56)^{C}$	$(246.2 \pm 9.07)^{A}$/ $(72.91 \pm 0.99)^{C}$	$(181.08 \pm 7.55)^{B}$/ $(102.81 \pm 3.83)^{A}$
13	H_2O_2 content (μ mol g^{-1}freshwt)	$(404.25 \pm 23.07)^{a}$/ $(305.13 \pm 4.07)^{a}$	$(381.13 \pm 12.86)^{a}$/ $(215.44 \pm 7.81)^{c}$	$(306.45 \pm 1.83)^{b}$/ $(267.22 \pm 6.73)^{ab}$	$(366.21 \pm 1.87)^{a}$/ $(160.53 \pm 4.14)^{d}$	$(358.30 \pm 10.58)^{A}$/ $(338.41 \pm 3.81)^{A}$	$(100.25 \pm 6.35)^{C}$/ $(176.50 \pm 5.22)^{C}$	$(239.44 \pm 9.5)^{B}$/ $(264.14 \pm 4.39)^{B}$	$(234.77 \pm 1.27)^{B}$/ $(264.29 \pm 4.82)^{B}$
14	Leaf TSS (mg g^{-1}drywt)	$(11.31 \pm 0.20)^{c}$/ $(16.78 \pm 0.12)^{b}$	$(15.34 \pm 0.25)^{a}$/ $(14.86 \pm 0.19)^{c}$	$(13.16 \pm 0.20)^{b}$/ $(16.42 \pm 0.09)^{b}$	$(15.68 \pm 0.39)^{a}$/ $(18.30 \pm 0.08)^{a}$	$(13.16 \pm 0.34)^{B}$/ $(17.85 \pm 0.13)^{A}$	$(14.49 \pm 0.31)^{A}$/ $(12.95 \pm 0.65)^{C}$	$(14.26 \pm 0.24)^{A}$/ $(15.88 \pm 0.30)^{B}$	$(14.15 \pm 0.07)^{A}$/ $(15.51 \pm 0.21)^{B}$
15	Root TSS (mg g^{-1}drywt)	$(3.51 \pm 0.06)^{b}$/ $(5.13 \pm 0.05)^{a}$	$(1.51 \pm 0.02)^{c}$/ $(3.62 \pm 0.05)^{b}$	$(4.38 \pm 0.07)^{a}$/ $(2.14 \pm 0.12)^{c}$	$(1.61 \pm 0.03)^{c}$/ $(2.01 \pm 0.03)^{c}$	$(1.81 \pm 0.06)^{B}$/ $(2.41 \pm 0.02)^{D}$	$(1.98 \pm 0.08)^{AB}$/ $(3.25 \pm 0.02)^{C}$	$(1.87 \pm 0.03)^{AB}$/ $(4.95 \pm 0.16)^{A}$	$(2.09 \pm 0.09)^{A}$/ $(3.54 \pm 0.04)^{B}$
16	Leaf starch (mg g^{-1}drywt)	$(169.73 \pm 0.57)^{c}$/ $(188.58 \pm 1.85)^{a}$	$(175.77 \pm 0.27)^{b}$/ $(187.11 \pm 0.69)^{a}$	$(192.87 \pm 0.63)^{a}$/ $(152.17 \pm 0.80)^{b}$	$(135.06 \pm 0.42)^{d}$/ $(189.65 \pm 2.24)^{a}$	$(95.39 \pm 0.31)^{C}$/ $(139.74 \pm 0.53)^{B}$	$(129.60 \pm 1.17)^{B}$/ $(136.33 \pm 0.48)^{C}$	$(170.4 \pm 1.08)^{A}$/ $(140.05 \pm 0.75)^{B}$	$(132.16 \pm 0.57)^{B}$/ $(145.71 \pm 0.42)^{A}$
17	Root starch (mg g^{-1}drywt)	$(93.52 \pm 0.52)^{a}$/ $(39.73 \pm 0.14)^{d}$	$(87.94 \pm 0.80)^{c}$/ $(57.81 \pm 0.42)^{b}$	$(90.71 \pm 0.92)^{b}$/ $(48.97 \pm 0.15)^{c}$	$(79.06 \pm 0.42)^{d}$/ $(61.12 \pm 0.38)^{a}$	$(69.16 \pm 0.81)^{B}$/ $(34.70 \pm 0.13)^{D}$	$(62.21 \pm 0.27)^{C}$/ $(40.06 \pm 0.27)^{B}$	$(86.40 \pm 0.15)^{A}$/ $(43.12 \pm 0.16)^{A}$	$(62.59 \pm 0.25)^{C}$/ $(38.78 \pm 0.40)^{C}$
18	Shoot length (cm)	$(47.37 \pm 0.76)^{a}$/ $(95.67 \pm 0.15)^{ab}$	$(48.33 \pm 0.54)^{a}$/ $(97.67 \pm 0.39)^{a}$	$(46.17 \pm 0.20)^{a}$/ $(93.33 \pm 0.54)^{b}$	$(51.00 \pm 1.37)^{a}$/ $(95.67 \pm 0.59)^{ab}$	$(67.67 \pm 0.15)^{B}$/ $(102.23 \pm 0.74)^{B}$	$(69.50 \pm 1.02)^{AB}$/ $(107.17 \pm 0.45)^{A}$	$(70.0 \pm 0.26)^{AB}$/ $(104.8 \pm 0.45)^{A}$	$(73.00 \pm 0.68)^{A}$/ $(104.00 \pm 0.93)^{A}$
19	Root length (cm)	$(14.67 \pm 0.49)^{a}$/ $(13.67 \pm 0.15)^{b}$	$(11.83 \pm 0.37)^{a}$/ $(13.67 \pm 0.30)^{b}$	$(14.00 \pm 0.47)^{a}$/ $(13.67 \pm 0.15)^{b}$	$(12.17 \pm 0.61)^{a}$/ $(15.33 \pm 0.15)^{a}$	$(12.67 \pm 0.78)^{A}$/ $(12.67 \pm 0.30)^{A}$	$(12.50 \pm 0.22)^{A}$/ $(13.67 \pm 0.30)^{A}$	$(11.83 \pm 0.32)^{A}$/ $(12.17 \pm 0.27)^{A}$	$(11.67 \pm 0.92)^{A}$/ $(13.67 \pm 0.153)^{A}$
20	Shoot weight (g)	$(1.18 \pm 0.00)^{a}$/ $(11.57 \pm 0.24)^{ab}$	$(1.15 \pm 0.02)^{a}$/ $(8.86 \pm 0.26)^{c}$	$(0.99 \pm 0.02)^{a}$/ $(10.26 \pm 0.21)^{b}$	$(1.17 \pm 0.05)^{a}$/ $(13.03 \pm 0.21)^{a}$	$(1.52 \pm 0.07)^{BC}$/ $(6.59 \pm 0.45)^{A}$	$(2.01 \pm 0.04)^{A}$/ $(7.77 \pm 0.29)^{A}$	$(1.46 \pm 0.02)^{C}$/ $(7.40 \pm 0.180)^{A}$	$(1.85 \pm 0.04)^{AB}$/ $(7.47 \pm 0.12)^{A}$
21	Root weight (g)	$(0.18 \pm 0.01)^{a}$/ $(1.38 \pm 0.13)^{a}$	$(0.21 \pm 0.00)^{a}$/ $(0.79 \pm 0.01)^{b}$	$(0.14 \pm 0.00)^{a}$/ $(1.11 \pm 0.05)^{ab}$	$(0.22 \pm 0.03)^{a}$/ $(1.27 \pm 0.05)^{ab}$	$(0.16 \pm 0.02)^{A}$/ $(0.67 \pm 0.03)^{AB}$	$(0.18 \pm 0.01)^{A}$/ $(0.54 \pm 0.04)^{B}$	$(0.12 \pm 0.00)^{A}$/ $(0.29 \pm 0.01)^{C}$	$(0.16 \pm 0.01)^{A}$/ $(0.82 \pm 0.01)^{A}$

Table 1 continued

S. nos.	Parameter	PBW-343 (vegetative/flowering)				LOK1 (vegetative/flowering)			
		Control	CO_2 only	EDU only	CO_2 + EDU	Control	CO_2 only	EDU only	CO_2 + EDU
22	Total plant weight (g)	$(1.37 \pm 0.01)^{a}$/ $(12.95 \pm 0.34)^{ab}$	$(1.36 \pm 0.02)^{a}$/ $(9.65 \pm 0.27)^{c}$	$(1.13 \pm 0.02)^{a}$/ $(11.37 \pm 0.16)^{b}$	$(1.39 \pm 0.08)^{a}$/ $(14.29 \pm 0.22)^{a}$	$(1.68 \pm 0.08)^{BC}$/ $(7.26 \pm 0.47)^{A}$	$(2.18 \pm 0.05)^{A}$/ $(8.30 \pm 0.27)^{A}$	$(1.59 \pm 0.02)^{C}$/ $(7.69 \pm 0.17)^{A}$	$(2.01 \pm 0.04)^{AB}$/ $(8.30 \pm 0.12)^{A}$

S. nos.	Parameter	HD-2967 (vegetative/flowering)			
		Control	CO_2 only	EDU only	CO_2 + EDU
1	Chlorophyll (SPAD unit)	$(44.80 \pm 0.30)^{\alpha}$/$(44.72 \pm 0.39)^{\beta}$	$(42.87 \pm 0.22)^{\beta}$/$(45.72 \pm 0.39)^{\beta}$	$(44.50 \pm 0.21)^{\alpha}$/$(46.01 \pm 0.39)^{\alpha\beta}$	$(45.03 \pm 0.03)^{\alpha}$/$(47.03 \pm 0.38)^{\alpha}$
2	Total ascorbate (mmol g^{-1}freshwt)	$(5.42 \pm 0.01)^{\delta}$/$(11.18 \pm 0.01)^{\alpha}$	$(5.77 \pm 0.02)^{\gamma}$/$(10.42 \pm 0.11)^{\beta}$	$(7.07 \pm 0.03)^{\beta}$/$(9.81 \pm 0.06)^{\delta}$	$(7.57 \pm 0.02)^{\alpha}$/$(10.10 \pm 0.03)^{\gamma}$
3	Red. ascorbate (mmol g^{-1}freshwt)	$(4.41 \pm 0.03)^{\gamma}$/$(6.10 \pm 0.05)^{\alpha}$	$(3.98 \pm 0.01)^{\delta}$/$(5.22 \pm 0.06)^{\gamma}$	$(5.79 \pm 0.01)^{\alpha}$/$(5.66 \pm 0.08)^{\beta}$	$(5.35 \pm 0.00)^{\beta}$/$(5.99 \pm 0.06)^{\alpha}$
4	DHA (mmol g^{-1}freshwt)	$(1.01 \pm 0.03)^{\delta}$/$(5.08 \pm 0.04)^{\alpha}$	$(1.80 \pm 0.03)^{\beta}$/$(5.20 \pm 0.11)^{\alpha}$	$(1.28 \pm 0.03)^{\gamma}$/$(4.16 \pm 0.08)^{\beta}$	$(2.22 \pm 0.03)^{\alpha}$/$(4.11 \pm 0.09)^{\beta}$
5	Total glutathione (mmol g^{-1}freshwt)	$(19.17 \pm 0.62)^{\beta}$/$(21.22 \pm 0.62)^{\alpha}$	$(27.41 \pm 0.71)^{\alpha}$/$(19.83 \pm 0.20)^{\alpha}$	$(27.40 \pm 0.45)^{\alpha}$/$(17.07 \pm 0.40)^{\beta}$	$(19.71 \pm 1.07)^{\beta}$/$(16.26 \pm 0.40)^{\beta}$
6	GSSG (mmol g^{-1}freshwt)	$(7.17 \pm 0.31)^{\alpha}$/$(4.06 \pm 0.08)^{\alpha}$	$(5.25 \pm 0.11)^{\gamma}$/$(2.20 \pm 0.08)^{\gamma}$	$(5.09 \pm 0.30)^{\gamma}$/$(3.69 \pm 0.20)^{\alpha}$	$(5.99 \pm 0.10)^{\beta}$/$(2.75 \pm 0.07)^{\beta}$
7	GSH (mmol g^{-1}freshwt)	$(12.00 \pm 0.43)^{\beta}$/$(17.16 \pm 0.67)^{\alpha}$	$(22.16 \pm 0.73)^{\alpha}$/$(17.63 \pm 0.25)^{\alpha}$	$(22.31 \pm 0.40)^{\alpha}$/$(13.38 \pm 0.41)^{\beta}$	$(13.72 \pm 1.12)^{\beta}$/$(13.51 \pm 0.37)^{\beta}$
8	APX (μ mol mg^{-1} $protein^{-1}min^{-1}$)	$(0.55 \pm 0.02)^{\gamma}$/$(0.98 \pm 0.02)^{\beta}$	$(0.70 \pm 0.05)^{\beta}$/$(1.30 \pm 0.02)^{\alpha}$	$(0.58 \pm 0.02)^{\gamma}$/$(0.95 \pm 0.02)^{\beta}$	$(0.89 \pm 0.02)^{\alpha}$/$(0.97 \pm 0.01)^{\beta}$
9	GR (μmol mg^{-1} $protein^{-1}min^{-1}$)	$(0.08 \pm 0.00)^{\alpha}$/$(0.09 \pm 0.01)^{\gamma}$	$(0.07 \pm 0.00)^{\beta}$/$(0.11 \pm 0.00)^{\beta}$	$(0.08 \pm 0.00)^{\alpha}$/$(0.13 \pm 0.00)^{\alpha}$	$(0.07 \pm 0.00)^{\alpha\beta}$/$(0.12 \pm 0.00)^{\alpha}$
10	Catalase (nmol mg^{-1} $protein^{-1}min^{-1}$)	$(23.86 \pm 0.94)^{\alpha}$/$(47.75 \pm 0.69)^{\beta}$	$(22.17 \pm 0.74)^{\alpha}$/$(60.20 \pm 0.81)^{\alpha}$	$(16.90 \pm 0.23)^{\beta}$/$(43.26 \pm 0.36)^{\gamma}$	$(7.40 \pm 0.45)^{\gamma}$/$(25.55 \pm 0.64)^{\delta}$
11	SOD (Ug^{-1}freshw.)	$(6.45 \pm 0.18)^{\beta}$/$(4.98 \pm 0.12)^{\beta}$	$(4.48 \pm 0.08)^{\gamma}$/$(4.75 \pm 0.09)^{\gamma}$	$(8.42 \pm 0.23)^{\alpha}$/$(8.00 \pm 0.20)^{\alpha}$	$(6.36 \pm 0.23)^{\beta}$/$(2.71 \pm 0.04)^{\delta}$
12	MDA (nmol g^{-1}fres wt)	$(236.2 \pm 9.83)^{\alpha}$/$(150.20 \pm 2.12)^{\alpha}$	$(186.21 \pm 5.26)^{\beta\gamma}$/$(101.96 \pm 2.92)^{\gamma}$	$(151.39 \pm 11.10)^{\gamma}$/$(115.53 \pm 2.79)^{\beta}$	$(203.60 \pm 3.45)^{\alpha\beta}$/$(104.87 \pm 2.19)^{\beta\gamma}$
13	H_2O_2 content (μ mol g^{-1}freshwt)	$(153.39 \pm 4.0)^{\gamma}$/$(249.81 \pm 3.05)^{\alpha}$	$(187.91 \pm 4.40)^{\beta}$/$(247.43 \pm 7.11)^{\alpha}$	$(246.06 \pm 6.84)^{\alpha}$/$(233.31 \pm 5.94)^{\alpha\beta}$	$(238.30 \pm 9.41)^{\alpha}$/$(184.25 \pm 5.23)^{\beta}$
14	Leaf TSS (mg g^{-1}drywt)	$(15.29 \pm 0.32)^{\beta}$/$(14.46 \pm 0.16)^{\gamma}$	$(13.13 \pm 0.12)^{\gamma}$/$(14.05 \pm 0.09)^{\gamma}$	$(14.93 \pm 0.25)^{\beta}$/$(18.37 \pm 0.16)^{\alpha}$	$(16.06 \pm 0.07)^{\alpha}$/$(15.22 \pm 0.18)^{\beta}$
15	Root TSS (mg g^{-1}drywt)	$(3.36 \pm 0.02)^{\beta\gamma}$/$(2.99 \pm 0.03)^{\alpha}$	$(3.45 \pm 0.06)^{\beta}$/$(2.94 \pm 0.05^{\alpha}$	$(3.24 \pm 0.03)^{\gamma}$/$(2.23 \pm 0.03)^{\beta}$	$(3.66 \pm 0.07)^{\alpha}$/$(2.23 \pm 0.01)^{\beta}$
16	Leaf starch (mg g^{-1}drywt)	$(110.1 \pm 0.98)^{\gamma}$/$(181.47 \pm 1.32)^{\gamma}$	$(111.27 \pm 0.42)^{\beta\gamma}$/$(192.41 \pm 0.89)^{\beta}$	$(145.32 \pm 0.47)^{\alpha}$/$(216.40 \pm 0.72)^{\alpha}$	$(114.08 \pm 1.60)^{\beta}$/$(171.99 \pm 0.58)^{\delta}$
17	Root starch (mg g^{-1}drywt)	$(51.08 \pm 0.26)^{\delta}$/$(46.64 \pm 0.56)^{\alpha}$	$(65.94 \pm 0.32)^{\beta}$/$(37.91 \pm 0.14)^{\gamma}$	$(59.81 \pm 0.37)^{\gamma}$/$(46.33 \pm 0.27)^{\alpha}$	$(85.52 \pm 1.22)^{\alpha}$/$(43.12 \pm 0.41)^{\beta}$
18	Shoot length (cm)	$(44.67 \pm 0.32)^{\alpha}$/$(97.33 \pm 0.75)^{\beta}$	$(47.00 \pm 0.79)^{\alpha}$/$(103.00 \pm 0.52)^{\alpha}$	$(49.17 \pm 0.54)^{\alpha}$/$(100.33 \pm 0.79)^{\alpha\beta}$	$(47.17 \pm 0.58)^{\alpha}$/$(103.67 \pm 0.30)^{\alpha}$
19	Root length (cm)	$(16.33 \pm 0.58)^{\alpha}$/$(13.33 \pm 0.30)^{\beta}$	$(12.83 \pm 0.20)^{\beta}$/$(13.00 \pm 0.26)^{\beta}$	$(14.67 \pm 0.20)^{\alpha\beta}$/$(15.33 \pm 0.30)^{\alpha\beta}$	$(13.17 \pm 0.20)^{\beta}$/$(16.33 \pm 0.30)^{\alpha}$
20	Shoot weight (g)	$(1.01 \pm 0.05)^{\beta}$/$(10.38 \pm 0.51)^{\alpha}$	$(0.98 \pm 0.06)^{\beta}$/$(11.16 \pm 0.24)^{\alpha}$	$(1.42 \pm 0.01)^{\alpha}$/$(12.85 \pm 0.15)^{\alpha}$	$(1.15 \pm 0.02)^{\alpha\beta}$/$(11.34 \pm 0.57)^{\alpha}$
21	Root weight (g)	$(0.18 \pm 0.02)^{\alpha}$/$(0.76 \pm 001)^{\alpha}$	$(0.12 \pm 0.01)^{\alpha}$/$(0.94 \pm 0.06)^{\alpha}$	$(0.18 \pm 0.01)^{\alpha}$/$(0.92 \pm 0.08)^{\alpha}$	$(0.12 \pm 0.00)^{\alpha}$/$(0.62 \pm 0.04)^{\alpha}$
22	Total plant weight (g)	$(1.19 \pm 0.04)^{\beta}$/$(11.15 \pm 0.52)^{\alpha}$	$(1.10 \pm 0.06)^{\beta}$/$(12.11 \pm 0.29)^{\alpha}$	$(1.60 \pm 0.02)^{\alpha}$/$(13.78 \pm 0.17)^{\alpha}$	$(1.27 \pm 0.02)^{\beta}$/$(11.96 \pm 0.53)^{\alpha}$

Values represent mean ± SE; different letters indicate significant differences between treatments within variety ($P < 0.05$)

Table 2 Effect of the treatments on yield parameters of three wheat varieties

S. nos.	Parameter	PBW-343 (vegetative/flowering)				LOK1 (vegetative/flowering)			
		Control	CO_2 only	EDU only	CO_2 + EDU	Control	CO_2 only	EDU only	CO_2 + EDU
1	Above ground biomass (g)	$(14.77 \pm 0.08)^{a}$	$(16.08 \pm 0.09)^{a}$	$(15.78 \pm 0.17)^{a}$	$(16.00 \pm 0.37)^{a}$	$(11.77 \pm 0.55)^{A}$	$(12.17 \pm 0.12)^{A}$	$(11.84 \pm 0.30)^{A}$	$(13.29 \pm 0.47)^{A}$
2	Spike length (cm)	$(17.13 \pm 0.06)^{a}$	$(17.10 \pm 0.25)^{a}$	$(16.93 \pm 0.05)^{a}$	$(18.00 \pm 0.26)^{a}$	$(18.67 \pm 0.15)^{AB}$	$(18.30 \pm 0.08)^{AB}$	$(19.50 \pm 0.22)^{A}$	$(17.17 \pm 0.49)^{B}$
3	Spikelet number spike^{-1}	$(47.67 \pm 0.99)^{b}$	$(53.40 \pm 0.71)^{b}$	$(52.33 \pm 0.41)^{b}$	$(63.33 \pm 0.91)^{a}$	$(32.33 \pm 0.79)^{A}$	$(34.00 \pm 0.93)^{A}$	$(38.33 \pm 1.97)^{A}$	$(35.00 \pm 1.57)^{A}$
4	Inflorescence weight plant^{-1} (g)	$(9.24 \pm 0.19)^{a}$	$(10.30 \pm 0.04)^{a}$	$(10.19 \pm 0.10)^{a}$	$(10.59 \pm 0.29)^{a}$	$(7.51 \pm 0.32)^{A}$	$(8.42 \pm 0.06)^{A}$	$(8.70 \pm 0.34)^{A}$	$(8.10 \pm 0.26)^{A}$
5	Grain number plant^{-1}	$(146.87 \pm 0.91)^{ab}$	$(157.20 \pm 2.00)^{b}$	$(160.40 \pm 0.41)^{a}$	$(163.33 \pm 2.10)^{a}$	$(96.67 \pm 1.27)^{B}$	$(117.33 \pm 2.55)^{A}$	$(110.0 \pm 3.82)^{AB}$	$(117.0 \pm 2.29)^{A}$
6	Grain weight plant^{-1} (g)	$(6.88 \pm 0.07)^{a}$	$(7.50 \pm 0.03)^{a}$	$(7.37 \pm 0.05)^{a}$	$(7.57 \pm 0.17)^{a}$	$(5.18 \pm 0.02)^{A}$	$(5.28 \pm 0.25)^{A}$	$(5.30 \pm 0.20)^{A}$	$(5.58 \pm 0.13)^{A}$
7	Thousand grain weight (g)	$(50.55 \pm 0.78)^{a}$	$(51.19 \pm 0.22)^{a}$	$(51.65 \pm 0.03)^{a}$	$(52.43 \pm 0.30)^{a}$	$(50.48 \pm 0.16)^{AB}$	$(51.85 \pm 0.26)^{B}$	$(51.79 \pm 0.10)^{AB}$	$(54.28 \pm 0.65)^{A}$
8	Harvest Index	$(0.47 \pm 0.00)^{a}$	$(0.47 \pm 0.00)^{a}$	$(0.47 \pm 0.00)^{a}$	$(0.47 \pm 0.00)^{a}$	$(0.43 \pm 0.00)^{C}$	$(0.45 \pm 0.00)^{B}$	$(0.40 \pm 0.00)^{D}$	$(0.47 \pm 0.00)^{A}$
9	Leaf TSS (mg g^{-1}drywt)	$(6.61 \pm 0.18)^{c}$	$(6.37 \pm 0.13)^{c}$	$(7.31 \pm 0.20)^{b}$	$(10.90 \pm 0.24)^{a}$	$(6.64 \pm 0.14)^{B}$	$(6.57 \pm 0.30)^{B}$	$(5.25 \pm 0.50)^{C}$	$(8.37 \pm 0.36)^{A}$
10	Leaf starch (mg g^{-1}drywt)	$(52.00 \pm 0.15)^{a}$	$(50.16 \pm 0.16)^{b}$	$(44.38 \pm 0.14)^{c}$	$(45.27 \pm 0.59)^{c}$	$(65.01 \pm 0.41)^{A}$	$(49.46 \pm 0.41)^{C}$	$(58.58 \pm 0.41)^{B}$	$(49.30 \pm 0.24)^{C}$
11	Grain TSS (mg g^{-1}drywt)	$(11.07 \pm 0.50)^{a}$	$(11.33 \pm 0.48)^{a}$	$(11.33 \pm 0.11)^{a}$	$(11.56 \pm 0.32)^{a}$	$(10.20 \pm 0.16)^{C}$	$(10.58 \pm 0.11)^{BC}$	$(11.04 \pm 0.22)^{AB}$	$(11.37 \pm 0.16)^{A}$
12	Grain starch (mg g^{-1}drywt)	$(595.00 \pm 3.66)^{b}$	$(598.28 \pm 3.01)^{b}$	$(649.97 \pm 3.42)^{a}$	$(632.20 \pm 4.57)^{a}$	$(498.63 \pm 4.4)^{D}$	$(595.88 \pm 1.95)^{B}$	$(676.45 \pm 4.5)^{A}$	$(521.31 \pm 2.56)^{C}$

S. nos.	Parameter	HD-2967 (vegetative/flowering)			
		Control	CO_2 only	EDU only	CO_2 + EDU
1	Above ground biomass (g)	$(16.46 \pm 0.13)^{\alpha}$	$(14.51 \pm 0.17)^{\beta}$	$(16.43 \pm 0.23)^{\alpha}$	$(15.86 \pm 0.38)^{\alpha\beta}$
2	Spike length (cm)	$(16.17 \pm 0.27)^{\alpha}$	$(16.33 \pm 0.15)^{\alpha}$	$(17.50 \pm 0.26)^{\alpha}$	$(16.83 \pm 0.40)^{\alpha}$
3	Spikelet number spike^{-1}	$(54.33 \pm 0.75)^{\alpha\beta}$	$(47.67 \pm 1.07)^{\beta}$	$(56.67 \pm 1.22)^{\alpha}$	$(55.33 \pm 1.16)^{\alpha\beta}$
4	Inflorescence weight plant^{-1} (g)	$(10.04 \pm 0.12)^{\alpha}$	$(8.99 \pm 0.18)^{\alpha}$	$(10.32 \pm 0.13)^{\alpha}$	$(9.95 \pm 0.24)^{\alpha}$
5	Grain number plant^{-1}	$(155.00 \pm 3.5)^{\alpha\beta}$	$(147.67 \pm 3.58)^{\beta}$	$(174.33 \pm 3.62)^{\alpha}$	$(175.67 \pm 1.51)^{\alpha}$
6	Grain weight plant^{-1} (g)	$(7.12 \pm 0.04)^{\alpha}$	$(6.39 \pm 0.06)^{\alpha}$	$(7.12 \pm 0.10)^{\alpha}$	$(7.37 \pm 0.22)^{\alpha}$
7	Thousand grain weight (g)	$(48.42 \pm 0.40)^{\alpha}$	$(47.91 \pm 0.39)^{\alpha}$	$(49.23 \pm 0.39)^{\alpha}$	$(46.80 \pm 0.18)^{\alpha}$
8	Harvest Index	$(0.43 \pm 0.00)^{\beta}$	$(0.44 \pm 0.00)^{\beta}$	$(0.43 \pm 0.00)^{\beta}$	$(0.46 \pm 0.00)^{\alpha}$
9	Leaf TSS (mg g^{-1}drywt)	$(7.22 \pm 0.25)^{\beta\gamma}$	$(8.02 \pm 0.47)^{\alpha\beta}$	$(6.69 \pm 0.18)^{\gamma}$	$(8.31 \pm 0.09)^{\alpha}$
10	Leaf starch (mg g^{-1}drywt)	$(49.03 \pm 0.16)^{\delta}$	$(54.56 \pm 0.14)^{\gamma}$	$(55.73 \pm 0.15)^{\beta}$	$(62.04 \pm 0.31)^{\alpha}$
11	Grain TSS (mg g^{-1}drywt)	$(14.14 \pm 0.03)^{\beta}$	$(14.62 \pm 0.40)^{\beta}$	$(15.57 \pm 0.32)^{\alpha}$	$(14.36 \pm 0.21)^{\beta}$
12	Grain starch (mg g^{-1}drywt)	$(670.28 \pm 3.17)^{\gamma}$	$(716.43 \pm 2.17)^{\alpha}$	$(680.48 \pm 1.22)^{\gamma}$	$(703.06 \pm 3.27)^{\beta}$

Values represent mean ± standard error; different letters indicate significant differences between treatments within each cultivar ($P < 0.05$)

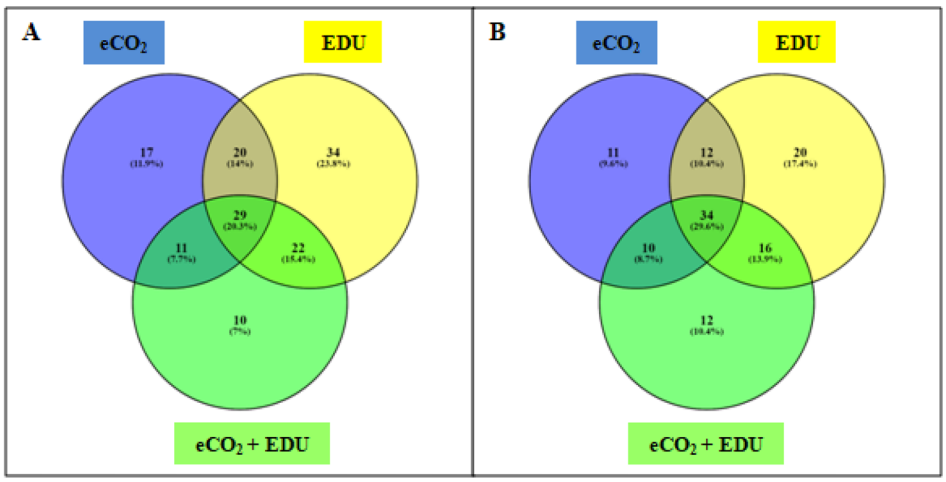

Fig. 2 Venn diagram showing distribution of proteins in different treatments at two developmental stages of wheat leaf. **a** Vegetative stage. **b** Flowering stage

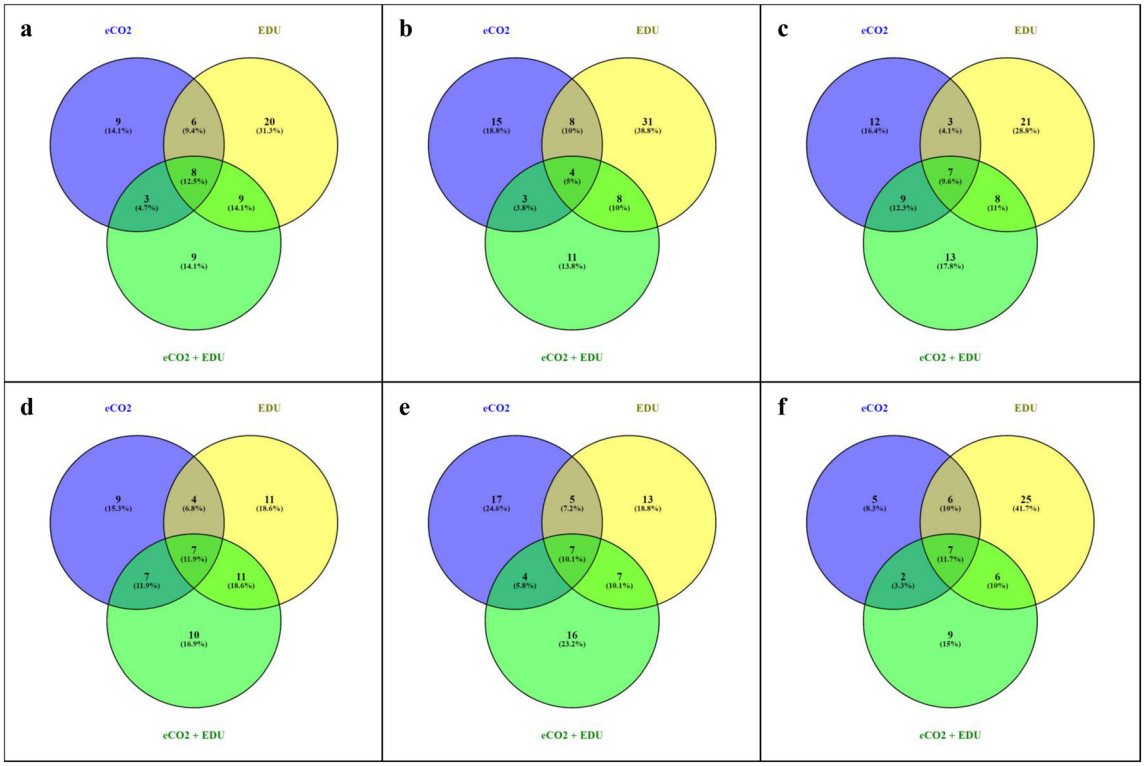

Fig. 3 Venn diagram of individual wheat variety at individual sampling stage. PBW-343 (**a**, **d**), LOK1 (**b**, **e**) and HD-2967 (**c**, **f**) at two developmental stages. Vegetative stage (**a**–**c**), Flowering stage (**d**–**f**)

Signal transduction (ST), Cytoskeleton (CYT), Defense (DF), Lipid biosynthesis (LBS) and Unknown (UK) (Figs. 4, 5). The detailed list of identified proteins from each varieties, treatment and developmental stage are shown in Table 3 (Table S11). All the identified proteins (differentially abundant) and their possible role in different cellular and metabolic pathways are summarized in Fig. S12.

Discussion

Morphological, physiological and biochemical responses

In the present experiment under eCO_2 condition LOK1 showed significantly increased *A* and gs, however, PBW-343 exhibited decreased *A* with the simultaneous reduction in gs. Crop physiological responses to eCO_2 include increased *A*, water use efficiency and reduced gs

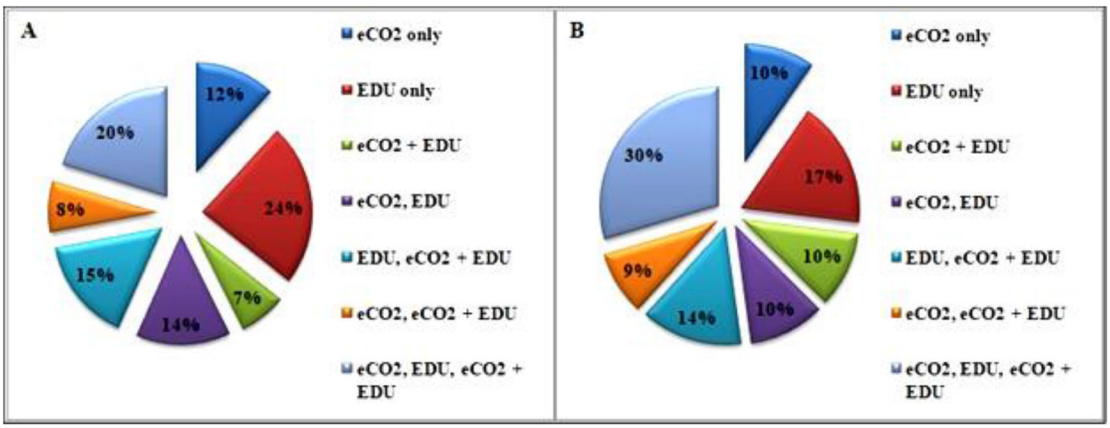

Fig. 4 Percentage distributions of treatment specific proteins at two developmental stages. **a** Vegetative stage. **b** Flowering stage

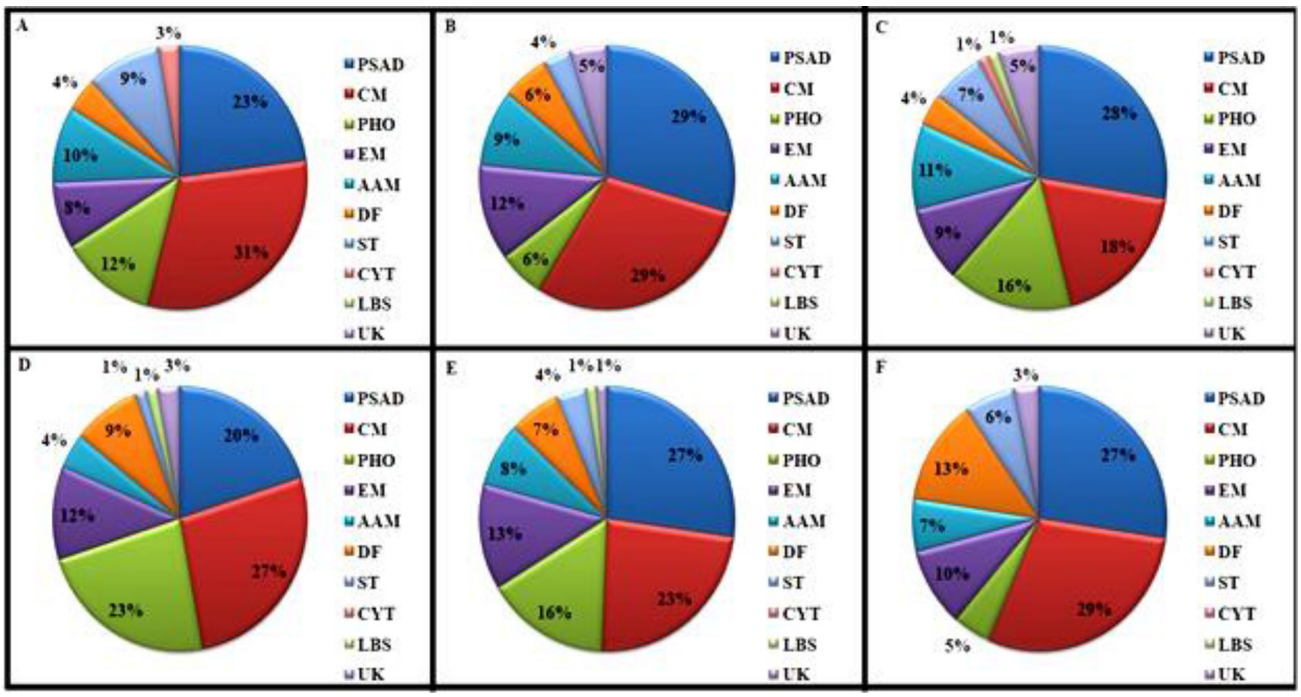

Fig. 5 Stage specific functional characterization of proteins of three wheat varieties at vegetative (**a** PBW-343, **b** LOK1, **c** HD-2967) and flowering stage (**d** PBW-343, **e** LOK1, **f** HD-2967) in leaves. PSAD, protein synthesis, assembly and degradation; CM, carbon metabolism; PHO, photosynthesis; EM, energy metabolism; AAM, amino acid metabolism; DEF, defense; ST, signal transduction; CYT, cytoskeleton; LBS, lipid biosynthesis and UK, unknown

(Ainsworth and Rogers 2007; Pandey et al. 2017; Wang et al. 2012). Response of EDU for all physiological parameters was found insignificant. EDU exerts least impact on physiological parameter (Feng et al. 2010; Gupta et al. 2018). Significantly increased gas exchange parameters in combined treatment was expected, and it was reflected mostly in LOK1 followed by HD-2967.

Decreased H_2O_2 and MDA content were found in all three varieties in vegetative stage, except EDU treated PBW-343 and LOK1. Similarly, in flowering stage beside LOK1 (in combined treatment), all three varieties showed decreased level of H_2O_2 and MDA content. Lesser H_2O_2 and MDA content indicate less membrane damage and improved ROS scavenging activity. eCO_2 mediated reduction in oxidative stress is reported in *Catharanthus roseus* (Singh and Agrawal 2015) and *Vigna radiata* (Mishra and Agrawal 2014). On the contrary, increased levels of MDA in PBW-343 and LOK1 under EDU treatment at vegetative phase and under combined treatment in LOK1 at flowering stage indicated higher sensitivity of these varieties to O_3 or insufficient protection by EDU. EDU mediated protection against membrane damage and

Table 3 List of the treatment—responsive leaf proteins in three wheat varieties

S. nos.	Protein name	PBW-343						LOK1						HD-2967
		Vegetative			Flowering			Vegetative			Flowering			Vegetative stage
		eCO_2	EDU	eCO_2 + EDU	eCO_2	EDU	eCO_2 + EDU	eCO_2	EDU	eCO_2 + EDU	eCO_2	EDU	eCO_2 + EDU	eCO_2
Photosynthesis														
1	psbP domain containing protein 3	$1.58^{a}\downarrow$			$1.52^{b}\downarrow$	$1.75^{b}\downarrow$								
2	Plastocyanin		$1.77^{a}\uparrow$			$1.92^{b}\downarrow$	$1.63^{b}\downarrow$							
3	CytB6-F_Fe-S subunit	$1.65^{a}\downarrow$	$2.21^{a}\downarrow$			$1.57^{b}\downarrow$								
4	Chlorophyll a-b binding protein					$2.32^{b}\downarrow$		$1.60^{a}\downarrow$	$1.64^{a}\downarrow$	$1.71^{a}\downarrow$	$1.71^{b}\downarrow$	$1.59^{b}\downarrow$	$2.30^{b}\downarrow$	
						$1.51^{b}\downarrow$					$1.59^{b}\uparrow$	$2.28^{b}\downarrow$		
5	Photosystem I subunit VII													
6	Ferredoxin-NADP(H) oxidoreductase			$2.44^{a}\uparrow$				$1.57^{a}\downarrow$				$1.67^{b}\downarrow$		$1.54^{a}\uparrow$
				$1.56^{a}\downarrow$										
7	Oxygen evolving enhancer		$1.63^{a}\uparrow$	$2.12^{a}\uparrow$			$1.57^{b}\downarrow$	$2.30^{a}\downarrow$			$2.30^{b}\downarrow$		$1.59^{b}\downarrow$	$1.77^{a}\uparrow$
											$1.54^{b}\uparrow$			
Carbon metabolism														
8	RuBisCO-LSU		$1.81^{a}\uparrow$	$1.58^{a}\uparrow$	$1.64^{b}\uparrow$	$1.73^{b}\uparrow$	$1.50^{b}\downarrow$	$1.61^{a}\downarrow$	$3.50^{a}\downarrow$	$3.35^{a}\downarrow$	$2.02^{b}\downarrow$	$2.19^{b}\downarrow$	$1.53^{b}\downarrow$	$1.58^{a}\uparrow$
			$1.57^{a}\uparrow$			$1.83^{b}\uparrow$	$1.93^{b}\downarrow$	$5.63^{a}\downarrow$	$2.00^{a}\downarrow$	$2.45^{a}\downarrow$		$1.61^{b}\downarrow$	$1.54^{b}\downarrow$	
						$1.58^{b}\uparrow$	$1.86^{b}\downarrow$	$2.39^{a}\downarrow$	$1.84^{a}\uparrow$	$2.18^{a}\downarrow$				
9	RuBisCO-SSU								$1.55^{a}\downarrow$			$1.60^{b}\downarrow$		
									$2.67^{a}\downarrow$					
10	RuBisCO activase A		$1.91^{a}\uparrow$		$3.08^{b}\uparrow$				$2.08^{a}\uparrow$	$1.75^{a}\uparrow$	$1.56^{b}\uparrow$	$1.63^{b}\uparrow$	$1.63^{b}\downarrow$	$1.86^{a}\uparrow$
									$1.74^{a}\downarrow$	$1.53^{a}\uparrow$			$1.52^{b}\uparrow$	
11	RuBisCO activase B		$1.51^{a}\uparrow$	$1.64^{a}\downarrow$		$1.91^{b}\downarrow$					$1.50^{b}\uparrow$			$1.95^{a}\uparrow$
12	Carbonic anhydrase													
13	Phosphoglycerate kinase							$1.56^{a}\uparrow$	$2.64^{a}\downarrow$	$1.63^{a}\uparrow$				
14	Glyceraldehyde-3-phosphate dehydrogenase	$1.79^{a}\downarrow$		$1.67^{a}\downarrow$					$1.56^{a}\downarrow$	$1.80^{a}\uparrow$			$1.76^{b}\uparrow$	
									$2.01^{a}\downarrow$					
15	Fructose-bisphosphate aldolase	$1.61^{a}\downarrow$	$1.88^{a}\downarrow$		$1.68^{b}\uparrow$	$1.72^{b}\uparrow$	$1.86^{b}\uparrow$	$1.75^{a}\downarrow$	$1.91\downarrow$		$1.51^{b}\uparrow$		$1.70^{b}\downarrow$	$1.91^{a}\uparrow$
						$1.65^{b}\downarrow$			$1.55^{a}\uparrow$					$1.51^{a}\uparrow$
16	Fructose-1,6-bisphosphatase								$1.52^{a}\downarrow$				$1.64^{b}\uparrow$	
17	Triosephosphate isomerase		$1.53^{a}\uparrow$		$1.61^{b}\downarrow$	$2.93^{b}\downarrow$	$3.62^{b}\downarrow$					$1.65^{b}\downarrow$		$1.51^{a}\uparrow$
					$2.72^{b}\downarrow$									
18	Transketolase	$1.61^{a}\uparrow$	$1.77^{a}\uparrow$	$2.59^{a}\uparrow$	$1.53^{b}\downarrow$	$2.78^{b}\downarrow$	$2.22^{b}\downarrow$		$1.60^{a}\downarrow$			$1.66^{b}\uparrow$		
					$1.68^{b}\downarrow$	$1.84^{b}\downarrow$	$2.57^{b}\downarrow$							
19	Ribose-5-phosphate isomerase	$1.80^{a}\downarrow$	$2.33^{a}\downarrow$		$1.51^{b}\uparrow$	$1.55^{b}\uparrow$	$2.54^{b}\uparrow$				$1.50^{b}\uparrow$			$2.25^{a}\uparrow$

Table 3 continued

S. nos.	Protein name	PBW-343						LOK1						HD-2967
		Vegetative			Flowering			Vegetative			Flowering			Vegetative stage
		eCO_2	EDU	eCO_2 + EDU	eCO_2	EDU	eCO_2 + EDU	eCO_2	EDU	eCO_2 + EDU	eCO_2	EDU	eCO_2 + EDU	eCO_2
20	Phosphoribulokinase	1.66[a]↓	2.49[a]↓	1.69[a]↓		1.55[b]↑			1.61[a]↑			1.53[b]↑		
21	ADP glucose pyrophosphatase													
22	Plastid ADP-glucose pyrophosphorylase								2.07[a]↑	3.17[a]↑		1.64[b]↑		
23	6-phosphogluconate dehydrogenase	1.53[a]↑												
24	Phosphoglycolate phosphatase					1.72[b]↑	2.81[b]↑							
25	Peroxisomal (S)-2-hydroxy-acid oxidase GLO1		2.18[a]↓	1.85[a]↓					1.94[a]↓					
26	Cytosolic, Fructose bisphosphate aldolase 5								2.14[a]↑ 1.91[a]↑					
27	Cytosolic, Phosphoglycerate kinase	2.08[a]↑ 1.55[a]↑	1.62[a]↑	2.64[a]↑ 2.15[a]↑			1.74[b]↓							
28	2,3-BPG independent phosphoglycerate mutase		1.54[a]↓ 1.80[a]↓			2.40[b]↓ 1.58[b]↓			1.58[a]↑			1.59[b]↓		
29	Enolase		1.93[a]↑ 1.69[a]↑		1.50[b]↓	1.71[b]↓ 1.86[b]↓			1.65[a]↑ -1.53[a]↓	1.82[a]↑	1.79[b]↑ 1.54[b]↑			1.58[a]↑ 1.58[a]↑
30	Cytosolic, Malate dehydrogenase				1.87[b]↑								1.72[b]↑	
31	UTP glucose phosphate uridyltransferase								1.59[a]↑					1.51[a]↑
32	Trehalose-phosphate phosphatase 2	1.76[a]↑		1.52[a]↓	1.55[b]↑		2.31[b]↑		1.66[a]↓			1.65[b]↑	1.66[b]↑	
33	Mito, Malate dehydrogenase	1.63[a]↓		1.51[a]↑										
Protein synthesis, assembly and degradation														
34	50S ribosomal protein L1							2.53[a]↑	1.57[a]↑					1.61[a]↓
35	Glycine rich RNA bp blt801-like	1.27[a]↓	3.22[a]↓ 2.10[a]↓	4.01[a]↓ 5.34[a]↓	1.59[b]↑				1.53[a]↑					1.84[a]↑
36	Chlo, 31 kDa ribonucleoprotein							2.28[a]↑						
37	Chlo stem loop bp 41 kDa													
38	Chlo, 30S ribosomal protein				1.61[b]↓		1.54[b]↓							
39	cp31BHv							2.20[a]↑	1.57[a]↓					

Table 3 continued

S. nos.	Protein name	PBW-343						LOK1						HD-2967
		Vegetative			Flowering			Vegetative			Flowering			Vegetative stage
		eCO_2	EDU	eCO_2 + EDU	eCO_2	EDU	eCO_2 + EDU	eCO_2	EDU	eCO_2 + EDU	eCO_2	EDU	eCO_2 + EDU	eCO_2
40	Eukaryotic IF		$2.22^{a}\uparrow$	$1.65^{a}\uparrow$								$1.79^{b}\downarrow$		
			$1.70^{a}\downarrow$	$1.85^{a}\uparrow$										
41	Eukaryotic EF		$1.88^{a}\downarrow$		$1.63^{b}\downarrow$	$1.70^{b}\downarrow$	$1.65^{b}\downarrow$	$1.62^{a}\uparrow$		$3.81^{a}\downarrow$	$1.72^{b}\uparrow$	$2.20^{b}\uparrow$	$1.77^{b}\uparrow$	$1.63^{a}\uparrow$
					$2.11^{b}\downarrow$			$1.66^{a}\downarrow$			$1.89^{b}\uparrow$		$2.38^{b}\uparrow$	$1.98^{a}\uparrow$
					$2.03^{b}\downarrow$						$1.58^{b}\downarrow$		$1.74^{b}\uparrow$	
42	Chlo, Chaperone protein ClpC		$1.74^{a}\uparrow$	$2.06^{a}\uparrow$				$1.64^{a}\downarrow$	$1.64^{a}\uparrow$					$1.91^{a}\uparrow$
43	Chlo membrane 70 kDa HSP								$2.81^{a}\downarrow$		$1.76^{b}\downarrow$		$1.51^{b}\uparrow$	
44	Stromal 70 kDa HSP-related protein	$1.52^{a}\uparrow$	$2.29^{a}\uparrow$	$2.16^{a}\uparrow$	$1.56^{b}\uparrow$				$6.01^{a}\downarrow$	$1.55^{a}\downarrow$	$1.85^{b}\uparrow$			
		$1.57^{a}\uparrow$	$1.64^{a}\uparrow$		$1.56^{b}\downarrow$									
45	20 kDa chaperonin	$1.59^{a}\uparrow$									$2.36^{b}\uparrow$	$2.21^{b}\uparrow$	$2.83^{b}\uparrow$	
46	Trigger factor							$2.24^{a}\uparrow$	$1.54^{a}\downarrow$		$1.58^{b}\uparrow$			
47	HSP-70								$1.80^{a}\downarrow$				$1.53^{b}\uparrow$	$2.52^{a}\uparrow$
									$2.17^{a}\downarrow$					$2.08^{a}\downarrow$
48	Peptidyl-prolyl cis–trans isomerase CYP20-3													$3.60^{a}\downarrow$
49	Peptidyl-prolyl cis–trans isomerase CYP38							$1.56^{a}\downarrow$						
50	Leucine aminopeptidase 2		$1.58^{a}\uparrow$							$2.24^{a}\downarrow$				
51	RuBisCO LSU bp-α					$1.89^{b}\downarrow$	$1.54^{b}\downarrow$	$2.29^{a}\uparrow$	$1.54^{a}\downarrow$	$2.13^{a}\uparrow$	$1.61^{b}\downarrow$	$3.95^{b}\downarrow$	$2.38^{b}\downarrow$	
							$1.59^{b}\downarrow$				$2.58^{b}\downarrow$			
52	RuBisCO LSU bp-β						$1.98^{b}\uparrow$	$1.92^{a}\uparrow$	$1.10^{a}\uparrow$	$1.66^{a}\uparrow$	$1.52^{b}\uparrow$	$1.59^{b}\uparrow$		
53	Pyridoxal 5′ phosphate synthase PDX1.1								$1.60^{a}\downarrow$	$1.60^{a}\downarrow$	$1.89^{b}\uparrow$	$4.74^{b}\uparrow$	$3.23^{b}\uparrow$	
											$1.58^{b}\uparrow$	$2.72^{b}\uparrow$		
54	Clp protease proteolytic	$1.80^{a}\downarrow$	$2.58^{a}\downarrow$	$2.43^{a}\downarrow$	$1.71^{b}\downarrow$					$1.57^{a}\downarrow$	$1.83^{b}\uparrow$	$3.35^{b}\uparrow$	$3.82^{b}\downarrow$	
											$1.91^{b}\uparrow$		$1.45^{b}\uparrow$	
55	Clp protease ClpA		$2.52^{a}\uparrow$	$2.29^{a}\uparrow$			$1.55^{b}\uparrow$		$1.51^{a}\downarrow$		$1.61^{b}\uparrow$			$1.98^{a}\uparrow$
				$1.69^{a}\uparrow$										$1.59^{a}\uparrow$
56	Presequence protease 1				$1.62^{b}\downarrow$		$1.70^{b}\downarrow$							$1.85^{a}\uparrow$
57	Triticain α		$1.78^{a}\uparrow$	$1.55^{a}\uparrow$				$2.49^{a}\uparrow$	$4.16^{a}\uparrow$				$1.65^{b}\uparrow$	
58	ATP-dependent zinc metalloprotease FTSH 1		$2.08^{a}\uparrow$	$1.53^{a}\uparrow$				$1.74^{a}\downarrow$	$1.67^{a}\downarrow$					
									$1.75^{a}\downarrow$					
59	E3 ubiquitine ligase BRE1										$1.58^{b}\uparrow$	$1.58^{b}\uparrow$		
Amino acid metabolism														

Table 3 continued

S. nos.	Protein name	PBW-343						LOK1						HD-2967
		Vegetative			Flowering			Vegetative			Flowering			Vegetative stage
		eCO_2	EDU	eCO_2 + EDU	eCO_2	EDU	eCO_2 + EDU	eCO_2	EDU	eCO_2 + EDU	eCO_2	EDU	eCO_2 + EDU	eCO_2
60	Glutamine synthetase 2, Plastid	$1.85^{a}\downarrow$	$1.52^{a}\uparrow$ $1.93^{a}\uparrow$						$1.83^{a}\downarrow$					
61	Glutamate decarboxylase								$1.59^{a}\downarrow$					
62	2-isopropyl malate synthase								$1.50^{a}\downarrow$	$1.55^{a}\downarrow$				
63	Aminomethyl transferase													$1.89^{a}\uparrow$
64	Glycine dehydrogenase						$1.65^{b}\downarrow$		$1.51^{a}\uparrow$	$2.60^{a}\downarrow$	$1.67^{b}\uparrow$			
65	Cysteine synthase		$1.54^{a}\uparrow$		$2.13^{b}\uparrow$		$2.67^{b}\uparrow$	$1.82^{a}\downarrow$	$1.58^{a}\downarrow$		$1.87^{b}\uparrow$ $1.80^{b}\uparrow$			
66	S-adenosylmethionine synthase												$1.64^{b}\downarrow$	
67	Cyto, Isocitrate dehydrogenase	$1.72^{a}\uparrow$	$2.61^{a}\uparrow$	$1.60^{a}\uparrow$			$1.55^{b}\downarrow$						$2.26^{b}\downarrow$	$2.34^{a}\downarrow$
68	Glycine decarboxylase	$1.69^{a}\uparrow$				$1.81^{b}\uparrow$						$1.54^{b}\uparrow$	$1.76^{b}\uparrow$	
Defence														
69	Chlo, Thiazole biosynthetic enzyme													
70	Peroxiredoxin-2E − 2													
71	Ascorbate peroxidase			$2.72^{a}\uparrow$		$1.50^{b}\downarrow$	$2.55^{b}\downarrow$			$1.88^{a}\downarrow$				
72	Monodehydroascorbate reductase								$2.23^{a}\downarrow$	$2.12^{a}\uparrow$				
73	Glutathione S-transferase				$2.08^{b}\uparrow$		$2.16^{b}\uparrow$			$1.64^{a}\downarrow$		$1.64^{b}\uparrow$		
74	Chloroplast Cu/Zn SOD					$1.86^{b}\downarrow$		$2.46^{a}\uparrow$	$2.06^{a}\uparrow$	$1.59^{a}\uparrow$				
75	Ubiquitin-like protein				$1.63^{b}\uparrow$									
76	Putative Hydrolase	$2.20^{a}\uparrow$				$1.96^{b}\downarrow$	$1.54^{b}\downarrow$						$1.52^{b}\uparrow$	
77	Germin-like protein 8-14	$1.54^{a}\uparrow$			$2.28^{b}\uparrow$	$1.69^{b}\downarrow$	$2.43^{b}\uparrow$	$1.89^{a}\uparrow$					$1.90^{b}\downarrow$	
Energy metabolism														
78	Vacuolar, proton ATPase A			$1.51^{a}\downarrow$			$2.13^{b}\uparrow$	$1.64^{a}\downarrow$						$2.63^{a}\uparrow$
79	Vacuolar, proton ATPase B1	$1.70^{a}\uparrow$					$1.99^{b}\uparrow$							
80	Chlo, ATP synthase CF1 α	$2.99^{a}\uparrow$	$4.11^{a}\uparrow$	$3.02^{a}\uparrow$	$1.63^{b}\downarrow$	$1.90^{b}\downarrow$	$2.19^{b}\downarrow$			$1.55^{a}\downarrow$	$2.60^{b}\downarrow$	$1.52^{b}\downarrow$	$1.67^{b}\downarrow$	
					$1.76^{b}\uparrow$	$1.76^{b}\uparrow$	$1.72^{b}\uparrow$					$2.03^{b}\uparrow$	$1.95^{b}\downarrow$	
81	Chlo, ATP synthase CF1 β						$1.54^{b}\downarrow$		$2.15^{a}\downarrow$	$1.51^{a}\uparrow$		$1.57^{b}\downarrow$	$1.58^{b}\uparrow$	
										$1.80^{a}\downarrow$		$1.68^{b}\uparrow$	$1.97^{b}\uparrow$	
82	Chlo, ATP synthase γ								$1.53^{a}\uparrow$			$1.53^{b}\downarrow$	$2.20^{b}\downarrow$	
83	Chlo, Soluble inorganic pyrophosphatase 6	$1.88^{a}\downarrow$						$1.74^{a}\downarrow$					$1.80^{b}\downarrow$	

Table 3 continued

S. nos.	Protein name	PBW-343						LOK1						HD-2967
		Vegetative			Flowering			Vegetative			Flowering			Vegetative stage
		eCO_2	EDU	eCO_2 + EDU	eCO_2	EDU	eCO_2 + EDU	eCO_2	EDU	eCO_2 + EDU	eCO_2	EDU	eCO_2 + EDU	eCO_2
84	NADH-ubiquinone oxidoreductase 75 kDa	$2.01^{a}\downarrow$	$1.65^{a}\downarrow$					$1.50^{a}\downarrow$			$2.04^{b}\uparrow$			
85	Mito, ATP synthase α					$1.71^{b}\downarrow$	$1.81^{b}\downarrow$					$2.20^{b}\uparrow$		
86	Mito, ATP synthase β		$1.81^{a}\uparrow$						$1.58^{a}\downarrow$					$1.62^{a}\uparrow$
Signal transduction														
87	NADP Trx reductase C				$1.76^{b}\downarrow$	$2.46^{b}\downarrow$								$1.76^{a}\uparrow$
88	Thioredoxin-like protein CDSP32		$1.56^{a}\uparrow$	$4.07^{a}\uparrow$				$2.39^{a}\uparrow$	$1.90^{a}\uparrow$				$1.82^{b}\downarrow$	$1.74^{a}\uparrow$
89	Nucleoside diphosphate kinase		$2.74^{a}\downarrow$								$1.65^{b}\uparrow$			
90	Receptor protein kinase-like protein ZAR1	$1.70^{a}\uparrow$	$1.9^{a}\uparrow$ $1.84^{a}\uparrow$	$2.69^{a}\uparrow$						$1.52^{a}\downarrow$				
91	Guanine nucleotide bp- β		$1.66^{a}\uparrow$								$1.64^{b}\uparrow$			
Cytoskeleton														
92	Actin-1													$2.00^{a}\uparrow$
93	Translationally-controlled tumor protein homolog	$1.50^{a}\downarrow$	$1.59^{a}\downarrow$	$2.12^{a}\downarrow$										
Lipid biosynthesis														
94	Plastid lipid associated protein					$1.76^{b}\downarrow$						$1.91^{b}\downarrow$		
Unknown														
95	F775_28691				$2.51^{b}\uparrow$	$1.53^{b}\downarrow$	$2.95^{b}\uparrow$							
96	Unknown protein 12					$1.71^{b}\uparrow$	$1.63^{b}\uparrow$			$1.86^{a}\downarrow$ $1.62^{a}\downarrow$				$1.57^{a}\downarrow$
97	Unnamed protein								$1.64^{a}\downarrow$					
98	TRIUR3_22780							$1.55^{a}\uparrow$						
99	Predicted protein											$1.59^{b}\downarrow$		

S. nos.	Protein name	HD-2967					
		Vegetative stage			Flowering		
		eCO_2	EDU	eCO_2 + EDU	eCO_2	EDU	eCO_2 + EDU
Photosynthesis							
1	psbP domain containing protein 3					$2.40^{b}\downarrow$	$-1.77^{b}\downarrow$
2	Plastocyanin		$1.71^{a}\downarrow$	$2.15^{a}\downarrow$			
3	CytB6-F_Fe-S subunit						

Table 3 continued

S. nos.	Protein name	HD-2967					
		Vegetative stage			Flowering		
		eCO_2	EDU	eCO_2 + EDU	eCO_2	EDU	eCO_2 + EDU
4	Chlorophyll a-b binding protein		1.63[a]↓		1.54[b]↓	2.70[b]↓	2.07[b]↓
					2.22[b]↓	1.87[b]↓	1.88[b]↓
5	Photosystem I subunit VII		1.51[a]↓				
6	Ferredoxin-NADP(H) oxidoreductase	1.54[a]↑		1.84[a]↑			
7	Oxygen evolving enhancer	1.77[a]↑		1.86[a]↑			
Carbon metabolism							
8	RuBisCO-LSU	1.58[a]↑	2.02[a]↑	1.90[a]↑	3.14[b]↓	1.65[b]↑	1.77[b]↓
			1.50[a]↓	1.80[a]↓		1.71[b]↓	
			1.79[a]↓	1.55[a]↓		1.68[b]↑	
9	RuBisCO-SSU				1.51[b]↓	1.52[b]↓	
						2.10[b]↓	
10	RuBisCO activase A	1.86[a]↑	1.56[a]↑	1.80[a]↓			
				2.03[a]↓			
11	RuBisCO activase B	1.95[a]↑		1.68[a]↑			2.36[b]↓
12	Carbonic anhydrase		2.79[a]↑		2.13[b]↓		2.37[b]↓
13	Phosphoglycerate kinase			1.63[a]↑			
14	Glyceraldehyde-3-phosphate dehydrogenase		1.51[a]↑				
15	Fructose-bisphosphate aldolase	1.91[a]↑	1.83[a]↑	2.11[a]↑	1.60[b]↓	1.54[b]↑	
		1.51[a]↑	1.50[a]↑				
16	Fructose-1,6-bisphosphatase					1.85[b]↓	1.62[b]↓
17	Triosephosphate isomerase	1.51[a]↑		1.59[a]↑			
18	Transketolase				2.07[b]↑	1.53[b]↑	
						1.62[b]↑	
19	Ribose-5-phosphate isomerase	2.25[a]↑					1.50[b]↓
20	Phosphoribulokinase		1.62[a]↑				
21	ADP glucose pyrophosphatase		1.55[a]↑				
22	Plastid ADP-glucose pyrophosphorylase						
23	6-phosphogluconate dehydrogenase		1.91[a]↑	1.59[a]↑			
24	Phosphoglycolate phosphatase						
25	Peroxisomal (S)-2-hydroxy-acid oxidase GLO1		2.03[a]↑	1.57[a]↑			
				1.52[a]↑			
26	Cytosolic, Fructose bisphosphate aldolase 5						
27	Cytosolic, Phosphoglycerate kinase			1.89[a]↑			1.90[b]↓

Table 3 continued

S. nos.	Protein name	HD-2967					
		Vegetative stage			Flowering		
		eCO_2	EDU	eCO_2 + EDU	eCO_2	EDU	eCO_2 + EDU
28	2,3-BPG independent phosphoglycerate mutase						
29	Enolase	1.58[a]↑		1.72[a]↑		1.59[b]↓	1.59[b]↓
		1.58[a]↑				1.69[b]↑	
30	Cytosolic, Malate dehydrogenase					1.53[b]↑	
31	UTP glucose phosphate uridyltransferase	1.51[a]↑					
32	Trehalose-phosphate phosphatase 2						
33	Mito, Malate dehydrogenase		1.70[a]↑		1.59[b]↑		
Protein synthesis, assembly and degradation							
34	50S ribosomal protein L1	1.61[a]↓	2.32[a]↓	1.86[a]↓		1.78[b]↓	2.11[b]↓
35	Glycine rich RNA bp blt801-like	1.84[a]↑					
36	Chlo, 31 kDa ribonucleoprotein						
37	Chlo stem loop bp 41 kDa			1.56[a]↓			
38	Chlo, 30S ribosomal protein						
39	cp31BHv						
40	Eukaryotic IF		1.55[a]↑	1.81[a]↑			1.74[b]↑
41	Eukaryotic EF	1.63[a]↑	1.53[a]↓	1.55[a]↑		1.69[b]↓	
		1.98[a]↑					
42	Chlo, Chaperone protein ClpC	1.91[a]↑		1.81[a]↑	2.98[b]↑	1.79[b]↑	2.55[b]↑
43	Chlo membrane 70 kDa HSP						
44	Stromal 70 kDa HSP-related protein						
45	20 kDa chaperonin		1.62[a]↓				
46	Trigger factor				1.61[b]↑	1.72[b]↑	
47	HSP-70	2.52[a]↑		2.22[a]↑		1.71[b]↑	
		2.08[a]↓		3.05[a]↓			
48	Peptidyl-prolyl cis–trans isomerase CYP20-3	3.60[a]↓	4.40[a]↓	1.63[a]↓			
49	Peptidyl-prolyl cis–trans isomerase CYP38						
50	Leucine aminopeptidase 2						1.83[b]↑
51	RuBisCO LSU bp-α		1.53[a]↓		1.61[b]↓	1.50[b]↑	
						1.84[b]↓	
52	RuBisCO LSU bp-β					1.52[b]↓	
53	Pyridoxal 5′ phosphate synthase PDX1.1		1.75[a]↑	2.39[a]↓	3.04[b]↓	2.01[b]↓	2.94[b]↓
				2.39[a]↓			
54	Clp protease proteolytic		1.59[a]↑	2.05[a]↑		1.54[b]↓	

Table 3 continued

S. nos.	Protein name	HD-2967					
		Vegetative stage			Flowering		
		eCO_2	EDU	eCO_2 + EDU	eCO_2	EDU	eCO_2 + EDU
55	Clp protease ClpA	1.98[a]↑	2.01[a]↑	1.56[a]↑		1.64[b]↑	
		1.59[a]↑				1.93[b]↑	
56	Presequence protease 1	1.85[a]↑	1.54[a]↑				
57	Triticain α					2.08[b]↓	2.33[b]↓
58	ATP-dependent zinc metalloprotease FTSH 1				1.51[b]↓		
59	E3 ubiquitine ligase BRE1						
Amino acid metabolism							
60	Glutamine synthetase 2, Plastid		1.56[a]↑				
61	Glutamate decarboxylase			1.73[a]↑			
62	2-isopropyl malate synthase						
63	Aminomethyl transferase	1.89[a]↑					
64	Glycine dehydrogenase						
65	Cysteine synthase			1.57[a]↑	1.94[b]↑	7.73[b]↓	2.43[b]↓
						1.53[b]↑	
66	S-adenosylmethionine synthase						
67	Cyto, Isocitrate dehydrogenase	2.34[a]↓	2.70[a]↓	1.78[a]↑		1.79[b]↑	
68	Glycine decarboxylase				1.71[b]↑		1.52[b]↑
Defence							
69	Chlo, Thiazole biosynthetic enzyme		1.62[a]↑				
70	Peroxiredoxin-2E − 2					1.53[b]↓	
71	Ascorbate peroxidase						
72	Monodehydroascorbate reductase					1.91[b]↑	
73	Glutathione S-transferase					1.67[b]↓	
74	Chloroplast Cu/Zn SOD					1.56[b]↑	1.66[b]↓
75	Ubiquitin-like protein		8.64[a]↓	1.58[a]↓			1.63[b]↓
76	Putative Hydrolase						2.45[b]↓
77	Germin-like protein 8-14		1.51[a]↓		1.52[b]↓	2.48[b]↓	1.61[b]↓
Energy metabolism							
78	Vacuolar, proton ATPase A	2.63[a]↑		1.59[a]↑			
79	Vacuolar, proton ATPase B1		1.63[a]↑				
80	Chlo, ATP synthase CF1 α		2.05[a]↓	1.57[a]↑	2.00[b]↓	9.81[b]↓	2.12[b]↓
			1.69[a]↑				
81	Chlo, ATP synthase CF1 β		1.50[a]↓	2.06[a]↓		1.81[b]↑	

Table 3 continued

S. nos.	Protein name	HD-2967					
		Vegetative stage			Flowering		
		eCO_2	EDU	eCO_2 + EDU	eCO_2	EDU	eCO_2 + EDU
82	Chlo, ATP synthase γ						
83	Chlo, Soluble inorganic pyrophosphatase 6				$1.65^{b}\downarrow$	$1.63^{b}\downarrow$	
84	NADH-ubiquinone oxidoreductase 75 kDa						
85	Mito, ATP synthase α					$1.57^{b}\uparrow$	
86	Mito, ATP synthase β	$1.62^{a}\uparrow$				$1.62^{b}\uparrow$	
Signal transduction							
87	NADP Trx reductase C	$1.76^{a}\uparrow$				$1.64^{b}\uparrow$	
88	Thioredoxin-like protein CDSP32	$1.74^{a}\uparrow$	$1.56^{a}\uparrow$	$1.56^{a}\uparrow$	$1.81^{b}\uparrow$		
				$3.44^{a}\uparrow$	$1.72^{b}\uparrow$		
89	Nucleoside diphosphate kinase					$1.58^{b}\uparrow$	
90	Receptor protein kinase-like protein ZAR1			$2.22^{a}\uparrow$			
91	Guanine nucleotide bp- β						
Cytoskeleton							
92	Actin-1	$2.00^{a}\uparrow$	$3.59^{a}\uparrow$	$2.40^{a}\uparrow$			
93	Translationally-controlled tumor protein homolog						
Lipid biosynthesis							
94	Plastid lipid associated protein		$1.74^{a}\downarrow$				
Unknown							
95	F775_28691		$1.55^{a}\uparrow$				
96	Unknown protein 12	$1.57^{a}\downarrow$	$2.03^{a}\downarrow$	$2.33^{a}\downarrow$		$1.53^{b}\uparrow$	
			$3.89^{a}\downarrow$				
97	Unnamed protein			$1.66^{a}\uparrow$			$1.70^{b}\downarrow$
98	TRIUR3_22780						
99	Predicted protein						

Values represent fold changes "↑" represent- increased/more abundance and "↓" represent- decreased/less abundance of protein. In superscript a and b represent sampling stages; vegetative and flowering, respectively

improved ROS scavenging system is previously reported in *Beta vulgaris* (Tiwari and Agrawal 2009), *Brassica juncea* (Pandey et al. 2014) and *Triticum aestivum* (Gupta et al. 2018).

Plants possess efficient enzymatic antioxidant system to cope with oxidative stress. In the present study, at vegetative stage, insignificant response of antioxidative enzymes was observed in eCO_2 condition. But at flowering stage increment in APX and GR activity was detected. Similarly, unaltered enzymatic response in the presence of eCO_2 was reported by Farfan-Vignolo and Asard (2012) and Kumari et al. (2013). EDU caused increased SOD and decreased CAT activity in HD-2967 during vegetative stage. Increased activity of SOD and CAT enzymes in EDU treated *Solanum tuberosum* (Hassan 2006) and *Triticum aestivum* (Singh et al. 2009) has been reported.

Yield response

The grain yield enhancement in eCO_2 might be linked with increment in grain number (Jablonski et al. 2002) and the adverse impact of O_3 on grain yield could be correlated with grain weight rather than grain number (Piikki et al. 2008). Previous studies revealed that EDU can alleviate the negative impact imposed by O_3 on yield attributes (Mishra et al. 2013). All the above arguments support the results of this study. In the present study, out of the three varieties, PBW-343 and LOK1 gave similar response towards all treatments and contributed in yield enhancement. These findings support the initial two hypotheses of our study. Further in HD-2967, eCO_2 did not cause yield improvement which suggests its insensitivity against eCO_2. However, EDU and eCO_2 + EDU caused yield improvement in HD-2967, showing its O_3 sensitive behaviour and support our 3rd hypothesis.

Changes in leaf proteomics

Effect of eCO_2 and/or EDU on proteins of various functional groups revealed contrasting results in all three wheat varieties. Proteins involved in photosynthesis, CM and PSAD were altered more prominently. Leaf protein expression can better demonstrate molecular explanation of adjustment in the treated plants to combat damage caused by increasing level of ambient O_3 and eCO_2 condition.

Photosynthesis related proteins

Enhanced abundance of FNR mediates regeneration of NADPH which could be used in carbon fixation, nitrogen metabolism, and lipid and chlorophyll biosynthesis and redox homeostasis during stress (Das et al. 2015; Kozuleva et al. 2016). Increased abundance of FNR (S.no.6) ensured efficient removal of ROS and regeneration of reducing power (NADPH) under combined treatments and eCO_2 in PBW-343 and HD-2967 during vegetative phase. Yu et al. (2017) reported increased abundance of FNR proteins under eCO_2 in *Cynodon dactylon*. At flowering stage, decreased abundance of FNR protein was observed in EDU treated LOK1. We found decreased abundance of OEE (S.no.7) and Ch a/b binding protein (S.no.4) under eCO_2 treatment and combined treatment in LOK1 during flowering phase. Decreased abundance of OEE (S.no.7) and other membrane proteins were reported against high O_3 exposure response in developing poplar leaves (Bohler et al. 2007).

Carbon metabolism related proteins

Rubisco subunits and Rubisco activase (S.no.8–11) showed mixed abundance at both stages of each variety, it might be the reason we did not get effect of the treatment on photosynthesis. Aranjuelo et al. (2011) and Sarkar et al. (2015) suggested susceptibility of Rubisco protein towards eCO_2 and O_3, respectively. Carbonic anhydrase (S.no.12) is essential for inorganic carbon transport and its assimilation (DiMario et al. 2017). More abundance of CA might be taken as an adaptive strategy of HD-2967 under EDU treatment however less abundance of CA at later stage of development under eCO_2 and in combined treatment could explain loss of net photosynthetic rate under high CO_2 (Porter and Grodzinski 1984). GAPDH (S.no.14) was found more abundant in combined treatment in LOK1 (both stages) and EDU treatment in HD-2967 (vegetative stage). More chloroplastic GAPDH in stress tolerant and less abundance in water and heat stress sensitive plants were reported (Merewitz et al. 2011). Yu et al. (2017) found increased abundance of GAPDH in eCO_2 to overcome heat stress in *Cynodon dactylon*. More abundance of FBA (S.no.15) was observed at flowering stage of PBW-343 (all treatments), LOK1 (eCO_2 treatment) and HD-2967 (EDU treatment). Transcriptional analysis in *Arabidopsis thaliana* shoots for FBA gene showed differential responses to chilling, heat and drought (Lu et al. 2012). More FBA was found to be involved in providing protection in HD-2967 against O_3 under both the developmental phases in EDU treatment. PRK (S.no.20) provided protection specifically against O_3 by increased abundances under EDU treated LOK1, HD-2967 at vegetative stage and PBW-343, LOK1 at flowering stage. EDU treated HD-2967 showed increased level of AGPPase during vegetative stage suggesting sufficiently enhanced level of starch accumulation which was reflected in the form of increased biomass. Smidansky et al. (2002) suggested that the abundance of AGPPase can be associated with increased seed yield. Zhang et al. (2013) demonstrated the negative

impact of ozone in starch biosynthesis and accumulation in wheat. Increased abundance of AGPPase under EDU and combined treatment in LOK1 (both stages) could be a probable reason for increased biomass and increased seed yield. Phosphoglycolate phosphatase (S.no.24), Peroxisomal (S)-2-hydroxy-acid oxidase (S.no.25) are important component of photorespiration, an alternate process from photosynthesizing carbon assimilation. EDU treatment (with or without eCO_2) resulted in more abundance of PGP in PBW-343 (flowering stage) and GLO1 in HD-2967 (vegetative stage), respectively, hence providing an alternate pathway to overcome stress via triggering ROS stress signal (Foyer and Noctor 2009; Sandalio et al. 2013).

Protein synthesis, assembly and degradation related proteins

The RNA binding proteins (RBPs) such as 50S ribosomal proteins L1 (S.no.34), was more abundant in eCO_2 and EDU treated LOK1 (vegetative stage) and less abundant in either of the treatment in HD-2967 at both stages. Li et al. (2018) observed the induced expression of 50S ribosomal proteins in *Vicia faba* exposed to drought and suggests its role in plant protection via restoration protein conformation. The salinity response study on soybean suggest down-regulate 50S ribosomal protein affects protein synthesis and might reduce plant growth (Sobhanian et al. 2010). Lee and Kang (2016) reviewed the role of RBPs in plant growth, development, and stress responses. More abundant Chaperone protein ClpC (S.no.42) protein was also found in HD-2967 under all the treatments specifically during flowering stage. However, in EDU treatment lessen increase was observed and could be an indication of its sensitivity to high O_3. EDU mediated tolerance in HD-2967 is also reflected in the form of better yield in EDU treated HD-2967. The increased abundance of Pyridoxal 5′ phosphate synthase subunit PDX1.1 (S.no.53) in the later stage of LOK1 (all treatment) serves an addon for more improved tolerance and yield among all the three varieties. Bilski et al. (2000) demonstrated the protective role PDX1 towards reactive oxygen species more specifically to singlet oxygen. However, HD-2967 showed diminished PDX1.1 activity. The PDX1.1 least decreased abundance in EDU treatment again suggest the effectiveness of EDU in HD-2967 that helps in better yield response. Chen and Xiong (2005) explains the role of PDX1 in tolerance to osmotic and oxidative stresses. Higher abundance of pre-sequence proteases (S.no.56), known for having chaperone characteristics, under eCO_2 and EDU treatments in HD-2967 during vegetative phase indicated its involvement in substrate recognition, unfolding and translocation activity, collectively its role is protein quality control.

Proteins of amino acid metabolism

Proteins namely glutamine synthetase2 (GS2), glutamate decarboxylase (GAD), 2-isopropyl malate synthase A (IPMS), and mitochondrial aminomethyl transferase (S.no.60–63) were observed only in vegetative stages. GS2 actively incorporate NH_3 into organic compounds, was specifically induced under EDU treatment in HD-2967, and maintains balance between carbon and nitrogen ratio that was reflected in significantly increased biomass (34%) during vegetative stage (Table 1). In combined treatment HD-2967 (vegetative stage) exhibited enhanced abundance of GAD. GAD accumulation plays significant role in GABA metabolism under different environmental stresses Astegno et al. (2015) and maintains C: N balance, pH adjustments in salt stress (Akcay et al. 2012).

Since cysteine homeostasis is important for plant immunity and sulphur homeostasis, increased abundance of cysteine synthase (CS; S.no.65) in EDU treated PBW-343 and eCO_2 + EDU treated HD-2967 during vegetative stage ensured their tolerance to O_3 stresses. In flowering stage PBW-343 (eCO_2 and combined treatment), LOK1 (eCO_2 treatment) and HD-2967 (eCO_2) showed increased abundance of CS thus maintaining a control on sulphur homeostasis in these varieties. However, under EDU treatment the reduced abundance of CS in HD-2967 can be associated with EDU mediated response at flowering stage. Increased abundance of ICDH [NADP] (S.no.67) in vegetative stage of PBW-343 under all treatments and at flowering stage of HD-2967 due to EDU treatment provided evidence of modulating carbon flux, in the form of 2-oxoglutarate, to nitrogen metabolism, helps improved amino acid biosynthesis and further ammonia assimilation via GS.

Proteins related to defense

Defense related proteins and enzymes play crucial role to combat ROS generated during O_3 encounter. Thiazole biosynthetic enzyme and peroxiredoxin-2E−2, (S.no.69–70) were observed in EDU treated HD-2967. More abundance of thiazole biosynthetic enzyme was observed in vegetative stage and less abundance of Peroxiredoxin-2E− 2 in flowering stage. Thiazole biosynthesis enzyme, an active precursor for biosynthesis of thiazole and provides protection to DNA damage. Thus, being specifically induced under EDU treatment, this enzyme provided EDU mediated protection at molecular level against O_3 stress to HD-2967. Peroxiredoxin-2E−2 (Prxs) is thiol peroxidase and help in detoxification even at very low atmospheric O_2 (Dietz 2011; Liebthal et al. 2018) and provide protection in latter stage of HD-2967. APX (S.no.71) was induced in vegetative stage of PBW-343

under combined treatment and could be the reason for reduced MDA level in PBW-343. Enzyme of ASA-GSH cycle; MDHAR (S.no.72) was found in increased abundance in combined treatment of LOK1 at vegetative stage and in HD-2967 under EDU treatment at flowering stage. Increased level of DHAR (S.no.73) was found in PBW-343 (eCO_2 and combined treatment) and LOK1 (EDU treatment) during their flowering stages which was also proved by increased levels of ASA (reduced) and APX during their respective phases (Fig. S3 and S4). The ascorbate–glutathione cycle is directly linked with superoxide dismutase (SOD) mediated detoxification of superoxide radicals (Noshi et al. 2016). Cu/Zn SOD (S.no.74) was found induced during all the treatments of LOK1 but with more abundance under eCO_2 in LOK1 during vegetative phase and specifically under EDU treated HD-2967 during their flowering stage which reflected in the form of increased levels of SOD enzyme (Fig. S3). Increased abundance of Germin like proteins (GLPs, S.no.77) was observed in vegetative stage of PBW-343 and LOK1 (eCO_2) and flowering stage of PBW-343 (eCO_2 and combined treatment). GLPs play an important role for cross linking reaction in the cell wall biochemistry via protein and carbohydrate cross linking (Banerjee and Maiti 2010). In the present study GLP might be protecting the cell wall biochemistry in tested varieties therefore, decreased MDA levels in PBW-343 and LOK1 (under eCO_2) in vegetative phase and under eCO_2 and combined treatment in PBW-343 during flowering phases were observed.

Proteins of energy metabolism

Vacuolar, proton ATPase (S.no.78, 79) is multi subunit, highly conserved H^+ pump that play a crucial role in abiotic stress adaptation (Dong et al. 2011). Their higher abundance under eCO_2 and combined treatments during vegetative and flowering phases, respectively in PBW-343, under all the treatment and more specifically under EDU treatment in HD-2967 allowed these cultivars to efficient molecular trafficking across the vacuolar membrane. Mitochondrial, NADH-ubiquinone oxidoreductase 75 kDa proteins (S.no.84) were exclusively induced in LOK1 under eCO_2 condition during its vegetative stage thus confirming its role in providing energy that drives the various metabolic processes and also helped in maintaining a balanced redox system in the cell. Mitochondrial ATP synthase (S.no.85-86) showed mixed abundance. Both these subunits were found more in EDU treated LOK1 and HD-2967 during flowering phases, thus ensuring their role in providing energy to these varieties under stresses.

Proteins involve in signal transduction

NADP Thioredoxin reductase C (NTRC, S.no.87) was less abundant at flowering stage of PBW-343 in individual treatment of eCO_2 and EDU. In HD-2967, increased abundance NTRC was observed under eCO_2 treatment (vegetative stage) and EDU treatment (flowering stage). NTRC detoxify H_2O_2 in dark, controls non-photochemical quenching of light energy, photosynthetic electron transport, redox regulation of ADP-glucose pyrophosphorylase (starch biosynthesis), and various enzyme activity such as FBA, SBPase, RuBP, CYP20-3, CYP38 and many more (Naranjo et al. 2016; Nikkanen et al. 2016; Pandey et al. 2017). Enhanced abundance of NTRC ensured an efficient cascade of signalling under the stress and plays a key role as a redox switch which can convert reducing power in the form of NADPH, into a redox signal through the thiol groups of thioredoxin domain (Kirchsteiger et al. 2012). Chloroplastic, Nucleoside diphosphate kinase2 (NDPK; S.no.89) was less abundant in vegetative stage of PBW-343 (EDU treatment), increased abundance at flowering stage of LOK1 (eCO_2 treatment) and HD-2967 (EDU treatment). NDPK2 had been originally concerned with phytochrome-mediated cytoplasmic signalling (Choi et al. 1999). Moon et al. (2003) demonstrated the role of NDPK2 mediated pathway to overcome multiple stress in transgenic plants.

Conclusion

Growth, biomass accumulation, yield and proteome of three wheat varieties showed differential response under elevated CO_2, EDU treatment and eCO_2 + EDU at different growth stages. It can be concluded that at both the growth stages, LOK1 was more responsive to eCO_2 and eCO_2 + EDU. While PBW-343 did not respond to any of the treatment. HD-2967 was more positively responded to EDU and combined treatment. This showed that HD-2967 was sensitive to ambient O_3. The identification of EDU-responsive proteins, especially thiazole biosynthetic enzyme and peroxiredoxin, provides not only new insights into ozone stress responses, but also a good starting point for further dissection of EDU mode of action using genetic and other approaches. LOK1 and HD-2967 demonstrated increased HI under combined treatments of eCO_2 + EDU indicating that eCO_2 in combination of EDU protected these varieties against high ambient O_3. Large number of wheat varieties should be screened to determine cultivar specific effects of elevated CO2 and ozone.

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Authors' contribution VP, NP and VKM designed the experiment. VKM, BM and MS did yield and physiological work. VKM and BM did the enzymatic and biochemical analysis. VK, SKG, MS and FD did proteomic work. VKM and VP analysed the data. VKM, NP, FD and VP wrote the paper. All the authors approved the paper.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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