



Energy regulated enzyme and non-enzyme-based antioxidant properties of harvested organic mung bean sprouts (*Vigna radiata*)

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ABSTRACT

The energy-regulated enzyme and non-enzyme-based antioxidant properties of organic mung bean sprouts were studied. H₂O₂ accumulated from 0.04 to 2.09 mmol kg⁻¹ in mung beans during germination for 6 d. Non-enzymatic antioxidants (free amino acid, flavonoid and total phenolics) and antioxidative enzymes maintained the redox equilibrium cooperatively. Exogenous ATP improved the intracellular adenylate by around 25% compared with that in the control (day 4 and 6). Excessive energy supply enhanced the activities of the enzymes by 12.45–38.82% at the early stage of production (day 2 and 4), which resulted in the accumulation of nutritive antioxidants. By contrast, 2, 4-dinitrophenol (DNP) caused energy depletion, resulting in lower activities of the enzymes. Asparagine transformation and polyphenol synthesis metabolisms were elevated under DNP treatment. The massive consumption of antioxidants led to nutritive deficit, although the enzymatic system was activated at the end. In summary, positive energy regulation could be a potential strategy to improve the nutritive values of harvested sprouts.

1. Introduction

Sprouting vegetables (e.g. mung bean, broccoli and radish) have attracted the attention of the public recently because of their convenience and high nutrition (Pająk, Socha, Gałkowska, Rożnowski, & Fortuna, 2014). The development of sprouts from edible seeds leads to increased nutritive values (Aguilera et al., 2013). During seed germination and development, intracellular reactive oxygen species (ROS) such as peroxide (H₂O₂) are accumulated (Job, Rajjou, Lovigny, Belghazi, & Job, 2005). Plants have evolved diverse enzyme and non-enzyme-based antioxidant mechanisms to maintain the cellular ROS balance (Chen et al., 2014b). Activation of the antioxidant enzyme system, including catalase (CAT), superoxide dismutase (SOD), ascorbate peroxidase (APX), and peroxidase (POD) effectively relieves the oxidative damage of plants under different stresses (Lin et al., 2014; Wang, Chen, & Ehlenfeldt, 2011). Non-enzymatic antioxidants, such as phenolics, also notably contribute to the redox equilibrium. These active compounds effectively control the ROS levels and affect the nutritional and functional properties of the germinated seeds (Dueñas et al., 2016; Lazo-Vélez, Guardado-Félix, Avilés-González, Romo-López, & Serna-Saldívar, 2018). Meanwhile, other bioactive compounds also present antioxidant activities (AAs). For instance, many free amino acids (FAAs), such as cysteine, tyrosine, leucine and histidine exhibit

different degrees of AAs (Hur, Lee, Kim, Choi, & Kim, 2014).

Energy metabolism plays a key role in various physiological activities of plants. During germination, stored ingredients, such as polysaccharides, lipids, and proteins, are utilised for development and as energy sources to start the life cycle (Smiri, Chaoui, & El Ferjani, 2009). Recent studies have shown that the energy status is closely related to the nutritive and antioxidant properties of postharvest fruit and vegetables (Yi et al., 2010). Energy depletion or low levels of adenosine triphosphate (ATP) lead to ROS-induced nutritive disorder and senescence of crops (Chen et al., 2014a; Lin et al., 2017; Wang et al., 2013). However, little information about the regulatory role of energy status in antioxidant balance during the development of sprouts is available. Understanding the correlation between energy status and the antioxidant mechanism may provide theory basis for the energy/ATP regulated pre-harvest growth and postharvest preservation of fruit and vegetables. Also, it may help to stimulate new insights to actively control the developmental process and improve the nutritive antioxidant ingredients of crops to meet the increasing demands of food and pharmaceutical industries.

In the present study, the energy regulated enzyme and non-enzyme-based antioxidant properties during the production of mung bean sprouts (*Vigna radiata*) were studied under exogenous treatments with ATP and 2, 4-dinitrophenol (DNP), an inhibitor of ATP production in

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cell mitochondria.

2. Materials and methods

2.1. Plant material, cultivation and treatment

Organic mung bean seeds (*Vigna radiata*) were purchased from a local seed company in Singapore. The seeds were cultivated and treated according to the method of Chen et al. (2015). The mung bean seeds were sterilised in 3% (v/v) sodium hypochlorite for 5 min and washed with distilled (DI) water for several times. They were then soaked in DI water overnight (25 °C). After that, the seeds were divided into three groups of around 100 g seeds in each group. Based on preliminary work, the seeds were immersed in 200 mL ATP (1 mM), DNP (2 mM), and DI water (control), respectively for 5 min (Chen et al., 2018b). After removing the residual chemicals by washing with DI water three times, the treated seeds were spread on a seedling raising plate (30 cm × 22 cm) and germinated in dark for 6 d (25 °C). At the third and fifth days of germination, 20 mL solutions of each treatment were evenly sprayed on the raising plate. At the second day of germination, the germination rate (GR) and germination index (GI) were calculated using the following formulas: $GR = N_2/N_0 \times 100\%$, where N_2 is the number of germinated seeds at day 2, N_0 represents the total number of used seeds; $GI = \sum(G_t/T_t)$, where G_t is the accumulated number of germinated seeds at day t and T_t represents the germination time corresponding to G_t . The GR and GI of each treatment were measured with 150 seeds, which were equally divided into three groups (each group contained 50 seeds) (Shi et al., 2017). At the end of germination (6 d), the shoot length and fresh weight of the sprouts were determined, and the dry weight was measured after drying at 110 °C for 2 d. During germination, the seeds or sprouts of each treatment were collected, cut, frozen, ground, freeze dried, and stored in liquid nitrogen at day 0, 2, 4, and 6 for further analysis.

2.2. Quantification of ATP, ADP, and AMP

The extraction of ATP, ADP, and AMP in the mung bean seeds and sprouts was conducted according to Liu et al. (2015). The extracted sample (20 µL) was injected into a Waters 2695 HPLC system (Alliance 2695, Waters, Millford, MA, USA) equipped with a UV detector (254 nm) with a Luna C18 column. The external standards of ATP, ADP, and AMP (0.5–10 mg L⁻¹, R² > 0.99) were applied to quantify the adenylate contents. The results were expressed as g kg⁻¹ of the freeze-dried samples (Yu & Yang, 2017).

2.3. Nutritive compounds and antioxidant capacities

Soluble sugar and protein contents were measured by the methods of Chen et al. (2018b) and Zhao, Zhang, and Yang (2017). Total FAA was determined by the ninhydrin method (Tian et al., 2010). Briefly, 20 mg of freeze-dried sample was homogenised with 3 mL of acetic acid (10%, v/v) for 5 min and the extract was centrifuged at 12,000 × g for 10 min (4 °C). The supernatant (400 µL) was mixed with 600 µL of ninhydrin reagent and 0.1% (m/v) ascorbic acid solution (20 µL), and then the mixture was boiled at 100 °C for 15 min. The solution was cooled and mixed with 1 mL of 95% (v/v) ethanol. The produced blue-violet solution was diluted with 2 mL of 60% (v/v) ethanol and the absorbance was read at 570 nm. Leucine solution (0–5 mg L⁻¹, R² > 0.99) was applied as a standard. Flavonoid and TPC were tested using the method of Chen et al. (2015). Antioxidant capacities of the mung bean samples were determined by 1,1-Diphenyl-2-picryl-hydrazyl (DPPH), 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS), and ferric reducing antioxidant power assay (FRAP) methods (Chen et al., 2018a; Liu, Tan, Yang, & Wang, 2017).

2.4. Free amino acids profile

The detailed FAA profiles in the mung bean samples were assayed using an amino acid analyser (ARACUS, MembraPure GmbH, Berlin, Germany). The freeze-dried sample (50 mg) was homogenised with 7 mL of ultra-pure water, followed by mixing with 2 mL of 5-sulphosalicylic acid (10%, m/v). The mixed solution was stored at 4 °C (1 h) for precipitation. After centrifugation and filtering through a 0.2-µm filter, the FAA extract (1 mL) was diluted with 1 mL of sample dilution solution (Liyaanarachchi, Mahanama, Somasiri, & Punyasiri, 2018). Two analogue channels of 570 and 440 nm were simultaneously recorded to detect the separated amino acids, and the quantitative calculation was performed using external standards by the software aminoPeak (ARACUS). The results were expressed as g per kg of freeze-dried samples. The data were normalised, and the heatmap and hierarchical cluster of FAA profile were analysed using Heml 1.0 (<http://hemi.biocuckoo.org/>). The principal component analysis (PCA) of the obtained FAA contents was conducted using XLSTAT (Vong, Hua, & Liu, 2018).

2.5. Antioxidative enzyme system

The H₂O₂ and malondialdehyde (MDA) contents were measured according to a previous study (Wang et al., 2017). Enzymatic proteins were extracted by homogenisation of 50 mg of freeze-dried samples with 5 mL of ice-cold 50 mM phosphate buffer (pH 7.5) and centrifugation for 10 min (12,000 × g, 4 °C). The CAT, APX and POD activities were assayed by the method of Chen et al. (2014b). The SOD activity was assayed using a SOD assay kit (Cayman Chemical, Ann Arbor, MI, USA) based on its ability to inhibit the oxidation of hydroxylamine to nitrite by O₂⁻.

2.6. Determination of asparagine and polyphenol metabolism-related genes

The expression patterns of asparagine transformation and polyphenol metabolism-related genes were tested using quantitative real-time PCR (qPCR). The appropriate genes were obtained by comparing the selected genes in *Arabidopsis* and Rye Grain with the whole genome of mung bean in the GenBank database (Gachon, Langlois-Meurinne, Henry, & Saindrean, 2005; Postles et al., 2016). The specific primers for best-matched genes for qPCR in mung bean were designed using Primer 5 software and are listed in Table S1. The RNA extraction and qPCR were conducted by our previous study (Chen et al., 2018b).

2.7. Statistical analysis

Data were analysed statistically using analysis of variance (ANOVA), and means were compared using the least significant difference (LSD) method to assess the effects of energy status on antioxidant properties during mung bean germination. Additionally, differences with $P \leq 0.05$ were considered significant.

3. Results and discussion

3.1. Sprouting of mung beans

The germination of organic mung bean sprouts under different treatments is shown in Fig. S1. The results revealed that, after germination for 2 d, the GRs in three groups were all higher than 90%. However, a significantly higher GI (89.67; $P \leq 0.05$) was recorded in the ATP-treated group. The GI in the control group was 83.33 and the DNP-treated group presented the lowest GI (37.83). Moreover, the highest shoot length, and fresh and dry weights (17.17 cm, 434.39 mg, and 38.56 mg, respectively) were recorded in the ATP-treated mung bean sprouts after germination for 6 d (Figs. S1a and b). By contrast, DNP treatment significantly decreased the shoot length and fresh

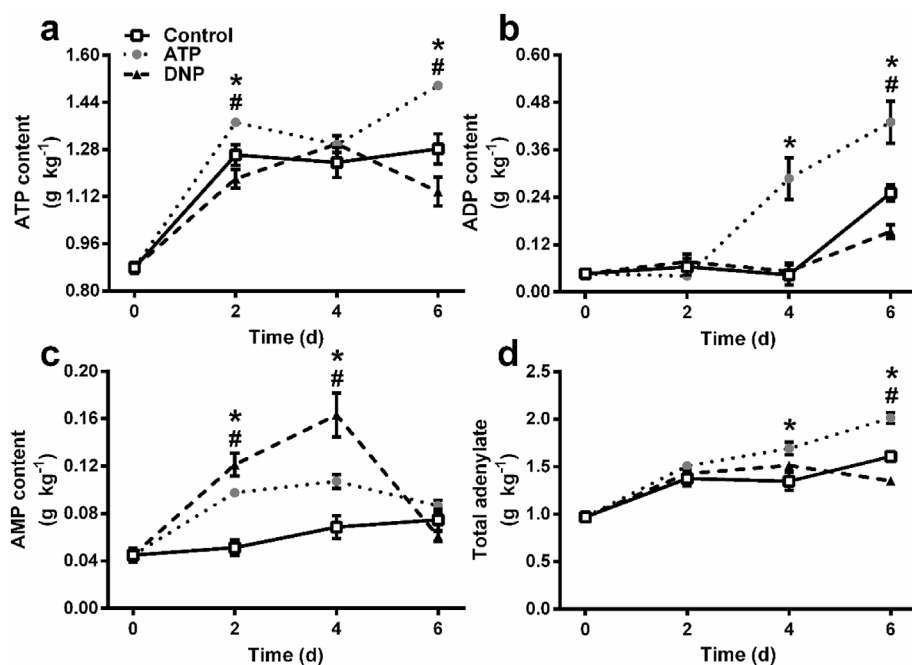


Fig. 1. Effect of ATP and 2, 4-dinitrophenol (DNP) treatments on the contents of ATP, ADP, AMP, and total adenylate during the germination of mung beans. The marks * (ATP treated group) or # (DNP treated group) indicate statistically significant ($P \leq 0.05$) differences compared with the control group at each production time.

weight, by 15.31 and 14.52%, respectively, compared with those in the control group ($P \leq 0.05$; Fig. S1b). The results showed that positive energy regulation effectively increased the biomass yield (increased shoot length, and fresh and dry weights) of mung bean sprouts because of the enhanced GI, which represents the seed vigour and germination speed. A higher GI is positively associated with the better establishment of seedlings (Hu et al., 2014).

3.2. Energy status changes of mung bean sprouts

Fig. 1 shows the changes in adenylate contents in mung beans under different treatments. The results showed that exogenous ATP treatment notably ($P \leq 0.05$) improved the ATP (8.80–16.77%), ADP (71.61–565.77%), AMP (56.45–90.47%) and total adenylate (25.27–25.46%) contents by different degrees compared with those in control. However, DNP significantly ($P \leq 0.05$) lowered the ATP (6.40–11.30%), ADP (39.00%) and total adenylate (15.97%) contents. Both the ATP and DNP treatments induced higher contents of AMP compared with that in the control, which were 0.10 and 0.12 g kg⁻¹ at day 2, 0.11 and 0.16 g kg⁻¹ at day 4, respectively (Fig. 1c). Similar to the conclusion obtained from previous postharvest studies (Chen et al., 2015; Yao et al., 2014), treatment with ATP effectively elevated the intracellular ATP, ADP, AMP, and total adenylate levels (Fig. 1). Moreover, DNP treatment depressed the energy status by decreasing the contents of ATP, ADP, and total adenylates, which was also reported by Lin et al. (2017) in postharvest longan fruit. The results of Fig. S1 and Fig. 1 indicated that active energy control might be a potential strategy to promote sprouting of vegetables.

3.3. Nutritive and antioxidant properties of mung bean

Soluble sugar increased from 134.08 to 412.20 g kg⁻¹ in the control at day 4 and was maintained at 397.97 g kg⁻¹ until day 6. Moreover, FAA levels increased from 5.04 to 76.29 g kg⁻¹. By contrast, soluble protein content decreased continuously, from 54.98 to 44.52 g kg⁻¹, after germination for 6 d in control (Fig. 2a–c). This is because the stored biomacromolecules (proteins, polysaccharides and lipids) in seeds degrade into micromolecules such as oligosaccharides and amino acids which are utilised for energy supply or developmental biosynthesis during germination (Smiri et al., 2009). Exogenous ATP promoted

the accumulation of hydrolysis products (soluble sugars and FAA) and the degradation of proteins, while DNP resulted in opposite results (Fig. 2a–c). In addition to functioning as nutritive and energy sources, FAAs also act as antioxidants in plants. Their chemical structures, such as metal chelating amino acid residues, hydrophobic side chains, phenylalanine, and histidine at the N-terminus may contribute to their antioxidative properties (Hur et al., 2014). The obtained results suggested that positive energy regulation might help to preserve the nutritive substrates of vegetables.

The contents of flavonoids and total phenolics in mung bean (control) increased continuously from 2.50 (flavonoid) and 6.82 (total phenolics) g kg⁻¹ to 9.42 and 14.69 g kg⁻¹, respectively after germination for 6 d (Fig. 2d and e). The results were in accordance with previous studies (Aguilera et al., 2013; Duenas, Hernández, Estrella, & Fernández, 2009). Similar to the results in Fig. 2a, c, ATP improved the contents of antioxidative flavonoids and total phenolics by 20.67–30.31% and 27.48%, respectively compared with control. Lower contents of flavonoids (11.32–27.90%) and TPC (15.39%) were recorded in the DNP-treated group. These results indicated that energy status is a crucial factor to regulate secondary metabolism and affect the contents of these antioxidants.

Various studies suggest that metabolite including FAA, flavonoids, and total phenolics, are closely related to free radical scavenging capacities. For example, a significant correlation between flavonoid contents and AAs was verified by DPPH, ABTS, and FRAP tests (Pajak et al., 2014; h & Yang, 2017). In the present study, the AAs of methanol extracts of mung beans were determined and the results from three different methods showed similar changing trends (Fig. 2f–h). Increased AAs in the control were recorded. Moreover, ATP-treated sprouts exhibited 14.38–24.42, 10.59, and 26.55% higher AAs than those in control when tested by DPPH, ABTS, and FRAP methods, respectively. By contrast, DNP treatment notably ($P \leq 0.05$) lowered the AA levels by 11.43, 11.80–14.39, and 14.33–18.04%, respectively. The linear correlation between antioxidants (FAA, flavonoids, and total phenolics) and AAs assayed by three methods were also statistically calculated (Fig. 2i). Regression coefficients (R) ranging from 0.6187 to 0.7143 between FAA contents and AAs measured by the three methods were recorded. Significant correlation results were also observed for flavonoids (0.9231, 0.8724, and 0.9373) and TPC (0.8596, 0.8514, and 0.8602). The relatively lower R values between FAAs and AAs indicated

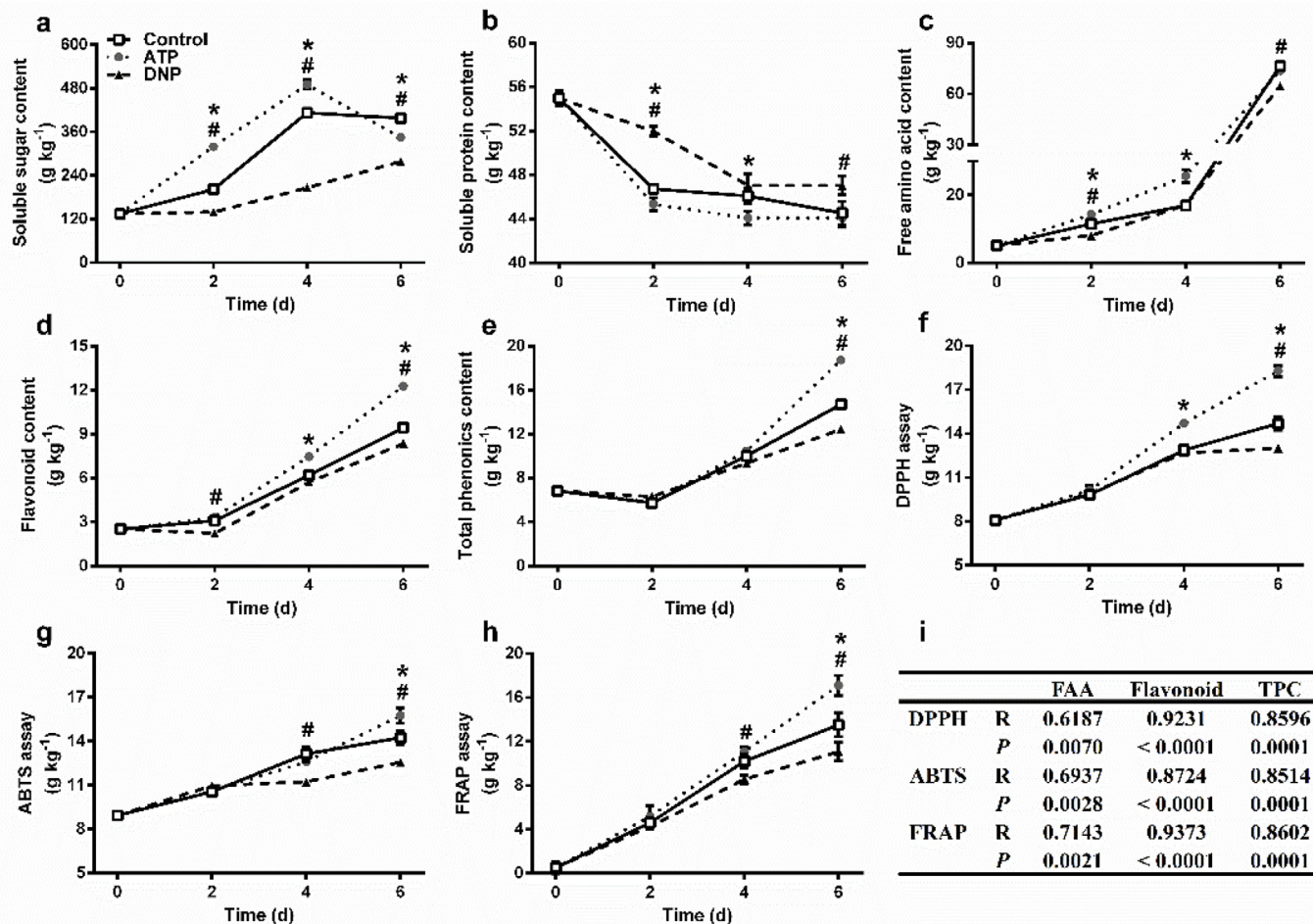


Fig. 2. Effect of ATP and 2, 4-dinitrophenol (DNP) on the contents of soluble sugar, protein, free amino acid (FAA), flavonoid and total phenolics (TPC), antioxidant activities (AAs) assayed by 1,1-Diphenyl-2-picryl-hydrazyl (DPPH), 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS), and ferric reducing antioxidant power assay (FRAP) methods and correlation between antioxidants and AAs. The marks * (ATP treated group) or # (DNP treated group) indicate statistically significant ($P \leq 0.05$) differences compared with the control group at each production time.

that the antioxidant properties of FAAs were not as strong as those of the other two secondary metabolites. However, they still could not be ignored because of their relatively high quantities (Moldes, Medici, Abrahão, Tsai, & Azevedo, 2008). Moreover, all the P values were less than 0.05, which indicated that these metabolites were significantly correlated with the AAs of the extracts (Xin, Chen, Lai, & Yang, 2017).

3.4. Free amino acid metabolism

Protein degradation, which generates amino acids, is an important physiological process during the germination of seeds. The demands for energy and cell developmental material are partly met by the generated amino acids (Hildebrandt, Nesi, Araújo, & Braun, 2015). The detailed FAAs and related metabolites were assayed in this study (Fig. 3; Table S2). Cluster analysis based on hierarchical average linkage allowed the construction of a dendrogram of treatments during the germination process (Fig. 3A). Two large clusters, I (seeds) and II (sprouts), were produced, and cluster II was further divided into three sub-clusters: IIa, IIb and IIc. The sub-clusters presented that control and ATP treated groups showed more similar FAAs patterns than the DNP treatment. The detailed FAA results showed dramatic changes in the quantities and categories of FAA and related metabolites during germination. In seeds (0 d), Hypo (2.21 g kg⁻¹), Arg (1.76 g kg⁻¹), and Glu (1.21 g kg⁻¹) were the dominant FAAs, all of which had levels greater than 1 g kg⁻¹. These three FAAs from glutamine family comprised up to 40.17% of the total FAA content (Fig. 3A; Table S2). The glutamine family is one of

the main nitrogen (N) carriers in plants and it promote the recycling of N, which is crucial during seed germination (Guan, Møller, & Schjørring, 2014).

After the germination of 6 days, the ratios of aspartate and erythrose 4-phosphate/phosphoenolpyruvate families in the control constantly increased to 53.71% and 7.81%, respectively (Fig. 3B; Table S3). Asn became the dominant FAA (69.00 g kg⁻¹), reaching to 45.39% of total FAAs. Free Asn also plays an important role of N storage and transport because of its high ratio of nitrogen/carbon. The accumulation of Asn has been observed during the processes of germination and stress responses in various plants. In addition, free Asn is the predominant FAA in cereal grains under stress conditions (Postles et al., 2016). Compared with the control group, ATP treatment notably ($P \leq 0.05$) induced higher contents of most FAAs. The dominant Asn, Arg, and Val were significantly ($P \leq 0.05$) increased, by 9.09, 18.64, and 16.03%, respectively, at day 6. In the DNP treatment, the major amino acids Asn and Arg were notably ($P \leq 0.05$) reduced, by 8.51 and 8.73%, respectively, compared with the control after germination. The total FAAs in each group were also calculated and the results showed that FAAs in control were continuously elevated from 12.88 to 152.03 g kg⁻¹ (Table S2). Total FAA levels in the ATP-treated group were notably ($P \leq 0.05$) increased at 2 and 6 d. Furthermore, DNP treatment resulted in lower total FAA contents. The changing trends of total FAA content calculated by the FAA profile were generally in accordance with the results obtained in Fig. 2c.

Fig. 3C shows the PCA of the FAA profile in mung beans. Two

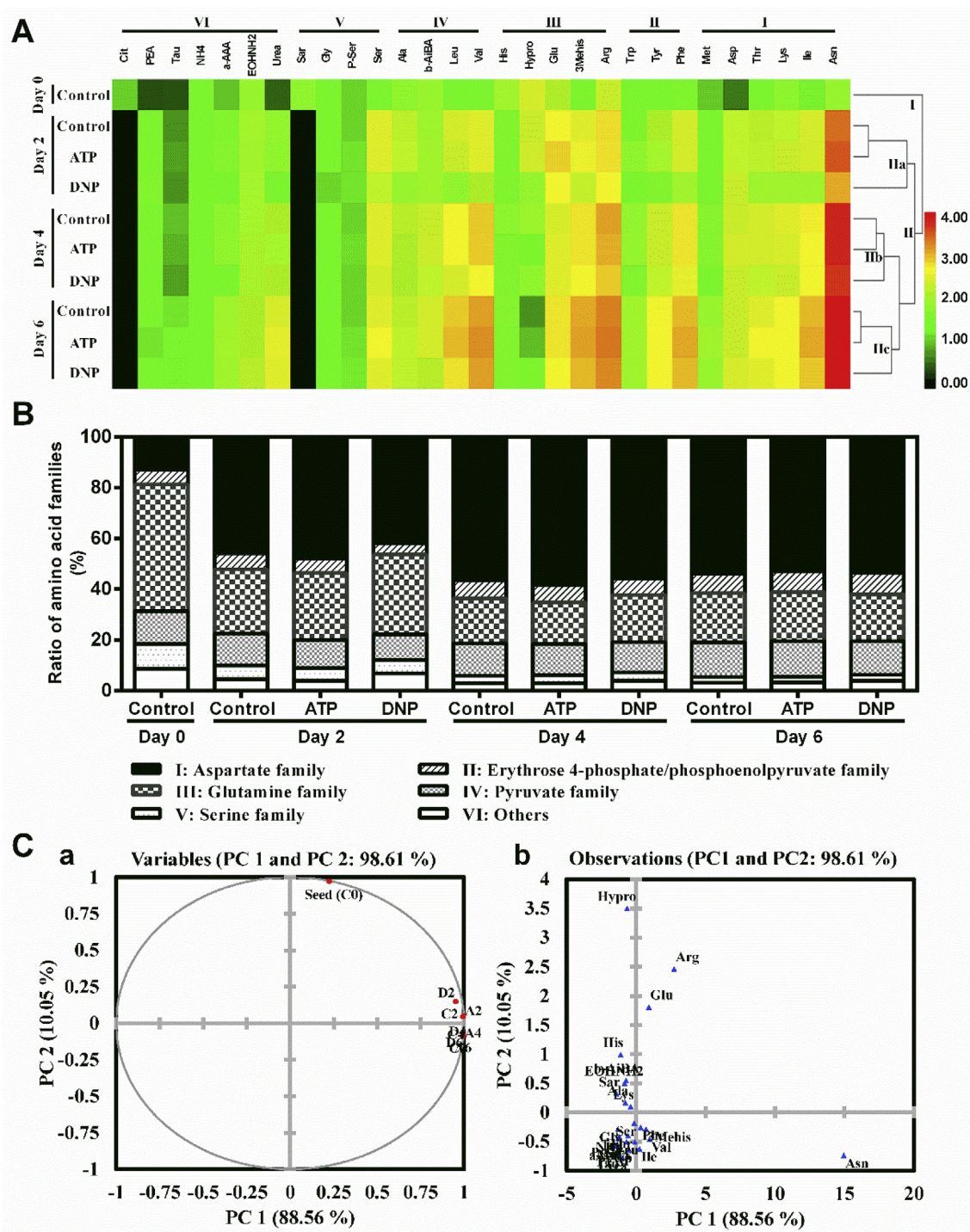


Fig. 3. Heatmap of amino acids and related metabolites during the development of mung beans under different energy conditions. (A) Ratio changes of amino acid families and metabolites in each group. (B) Variables distribution plots (Ca) and observations loading plots (Cb) assayed by principal component analysis (PCA).

Eigenvalues were obtained to produce two principal components (PC1 and PC2) and the proportions of the Eigenvalues were computed as 88.56% (PC1) and 10.05% (PC2) (Table S4). The distribution plots of variables showed that seeds were closely related to PC2 and the other groups were associated with PC1 (Zhang, Chen, Lai, Wang, & Yang, 2018). Plots of observations were utilised to identify the differential FAAs for group discriminations. FAAs including Ile, Asp, Glu, Hypro, and Leu were dominant in PC2, and the Asn was largely affected by PC1 (Table S5). From the variables plot results of PCA (Fig. 3Ca), it could be concluded that the FAA profiles in seeds and sprouts of mung bean were

significantly different. This result also consistent with the cluster analysis obtained in the heatmap (Fig. 3A). Also, the observations loading plots indicated that Asn was mainly affected by PC1, which was also closely associated with the variable of sprouts. Hypro, Arg, and Glu were dominant in PC2, which was related to the seeds (Fig. 3Cb). The results were in accordance with detailed profile of FAAs and related metabolites.

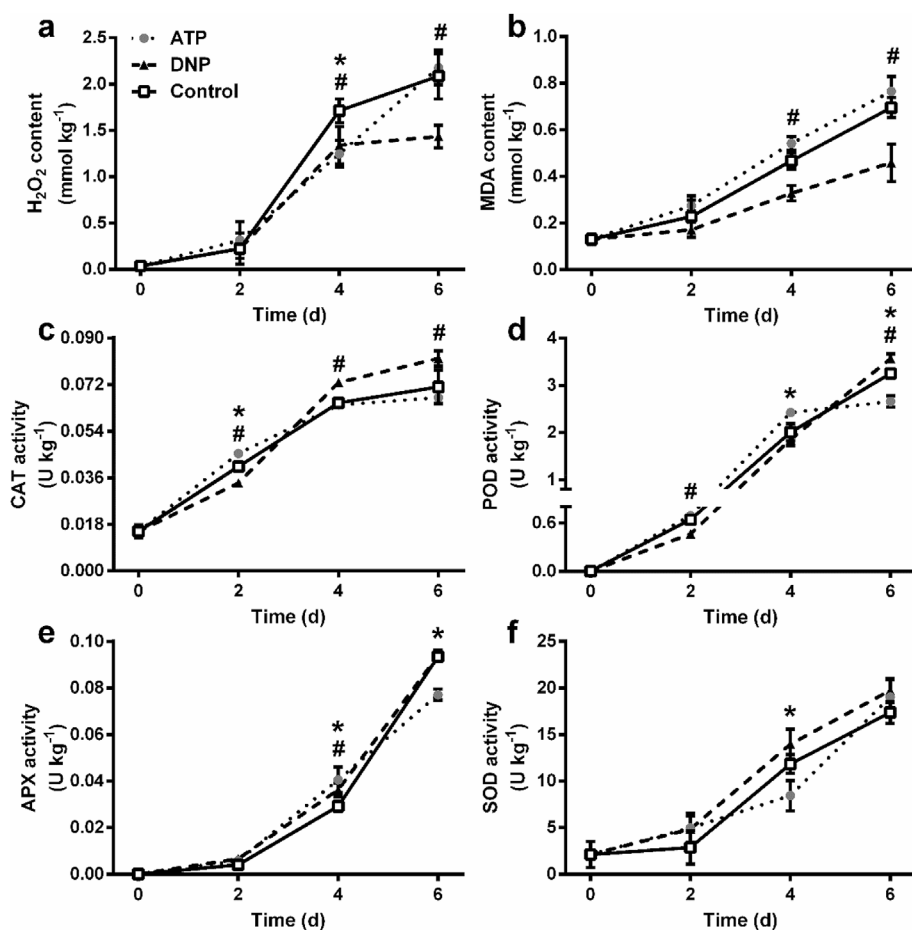


Fig. 4. Effect of ATP and DNP treatment on the contents of hydrogen peroxide (H_2O_2) and malondialdehyde (MDA), activities of catalase (CAT), peroxidase (POD), ascorbate peroxidase (APX) and superoxide dismutase (SOD) during the germination of mung bean. The marks * (ATP treated group) or # (DNP treated group) indicate statistically significant ($P \leq 0.05$) differences compared with the control group at each production time.

3.5. Enzymatic antioxidant activities

Our results showed that H_2O_2 in the control accumulated from 0.04 to $2.09 \text{ mmol kg}^{-1}$ and the MDA was elevated from 0.13 to $0.70 \text{ mmol kg}^{-1}$ during germination (Fig. 4a and b). The results indicated that ROS levels increased as germination proceeded, which was similar to the results of a previous study (Job et al., 2005). The ATP treated samples showed similar results for H_2O_2 and MDA compared to the control group, except that the H_2O_2 content at day 4 was 27.17% lower than that in the control (Fig. 4a and b). Moreover, DNP significantly ($P \leq 0.05$) decreased the H_2O_2 and MDA contents by 21.74–31.25 and 29.77–34.03%, respectively, at day 4 and 6, which indicated that the redox balance was also maintained when energy depletion occurred during the germination. The enzymatic antioxidant system plays an important role to regulate the ROS level (Peng, Yang, Li, Jiang, & Joyce, 2008; Zhang et al., 2018). Our results showed that at a relative early stage, exogenous ATP improved the activities of CAT (day 2), POD (day 4), and APX (day 4) by 12.45, 20.70, and 38.82%, respectively. By contrast, DNP treatment notably lowered the CAT and POD activities by 15.72 and 27.91%, respectively ($P \leq 0.05$), compared with those in control at day 2 (Fig. 4c–f). Moreover, at a relative late stage, activities of CAT, POD, and APX were also induced under conditions of energy depletion. The activities of CAT (day 4 and 6), POD (day 6), and APX (day 4) were enhanced by 12.09–15.44, 9.77, and 24.15%, respectively, under DNP treatment.

Based on the results of non-enzymatic antioxidants in Fig. 2 and the antioxidative enzymes in Fig. 4, we concluded that exogenous ATP treatment induced higher activities of antioxidant enzymes to effectively scavenge the excessive ROS at the early stage and maintain the redox equilibrium. The consumption of non-enzymatic antioxidants (FAA, flavonoids, and total phenolics) was prevented, and the contents

of antioxidants were maintained at relative high levels. By contrast, DNP caused energy starvation, which resulted in lower antioxidant enzyme activities and massive amounts of non-enzymatic compounds were consumed to balance the redox equilibrium at the early stage. Along with the continuous energy deficit stress, both enzyme and non-enzyme-based systems were activated, which contributed to the survival of mung bean sprouts. This result suggested that during the production of crops, positive energy regulation might be crucial to improve the nutritive values through the activation of antioxidant enzymes.

3.6. Expression pattern of related genes

The underlying metabolisms of asparagine and polyphenol under different energy conditions during germination were further tested by qPCR (Fig. 5). GCN is implicated in asparagine synthetase gene expression. AK and GS are involved in the asparagine hydrolysis metabolism: after the decomposition of asparagine, the released aspartate is catalysed by AK to synthesise the aspartate family amino acids, including Met, Lys, and Thr (Hur et al., 2014). Furthermore, the released ammonia is reincorporated into amino acid metabolism by GS (Postles et al., 2016). The results revealed that the asparagine-related genes including GCN, AK and GS were maximally downregulated by 4.19, 4.33, and 1.59-fold, respectively, during germination in the control (Fig. 5a–c). Exogenous ATP had almost no effect on the expression levels of the asparagine related genes. However, DNP notably ($P \leq 0.05$) elevated the expression levels of VrGCN, VrAK, and VrGS by 2.03–2.68, 1.97–2.81, and 1.53–2.53-fold, respectively. These results indicated that the transformation of asparagine was enhanced under energy depletion stress. The results were consistent with the FAA profile (Fig. 3; Table S2), which showed that more asparagine was utilised to synthesis other FAAs. These synthesised FAAs might be further consumed as

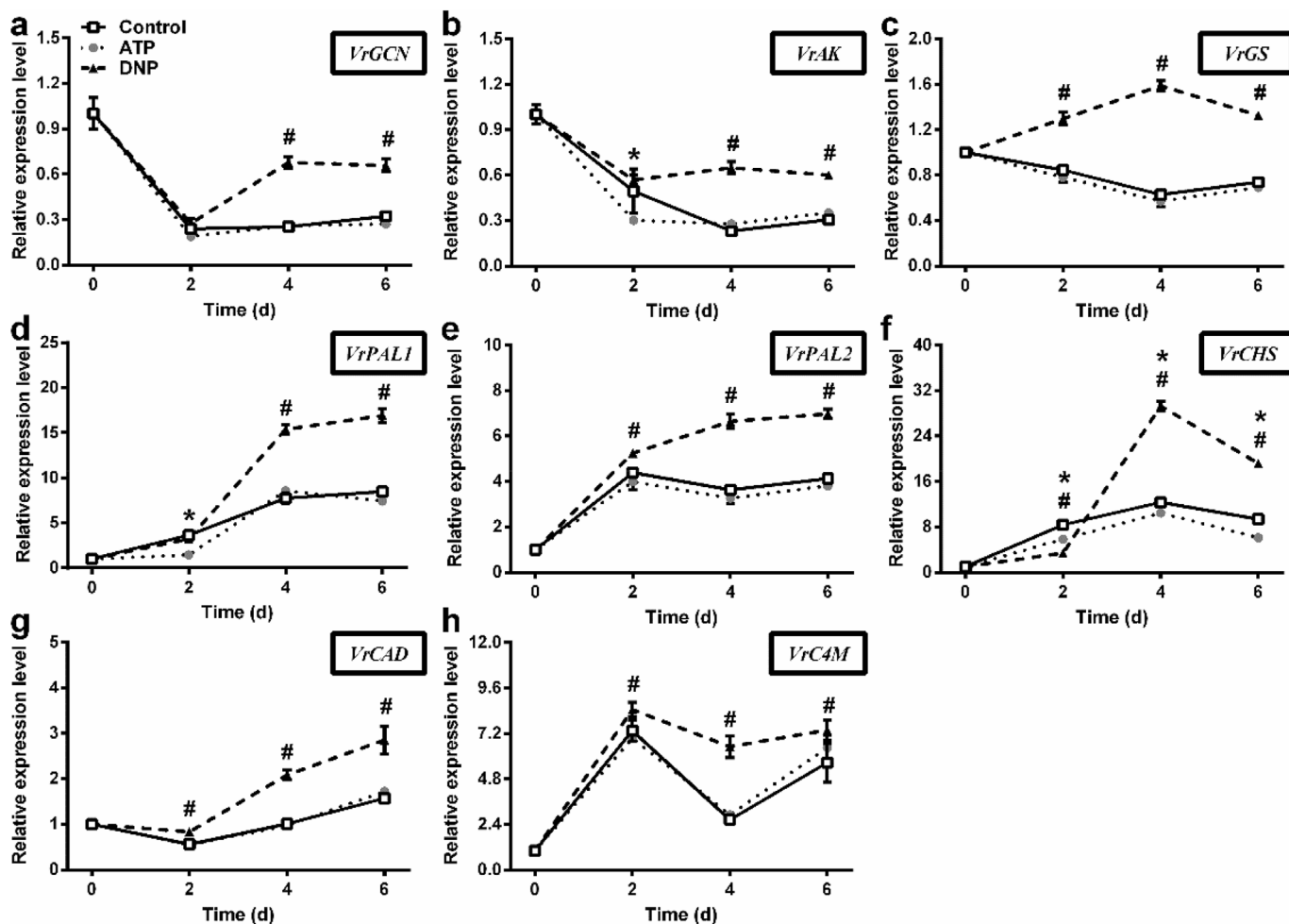


Fig. 5. Effect of ATP and 2, 4-dinitrophenol (DNP) on the relative expression levels of asparagine and polyphenol metabolism-related genes during the germination of mung beans. The marks * (ATP treated group) or # (DNP treated group) indicate statistically significant ($P \leq 0.05$) differences compared with the control group at each production time.

antioxidants.

The metabolism of polyphenols synthesis was also investigated. PAL and C4M are two key enzymes associated with phenylpropanoid metabolism: phenylalanine is deaminated by PAL and the generated cinnamic acid is then catalysed by C4M to form the *p*-coumaric acid. CHS is the entry point enzyme of flavonoid biosynthesis, which contributes to the synthesis of chalcone. In addition, CAD is the key enzyme of monolignol synthesis by catalysing the reduction of hydroxycinnamaldehydes (Wang et al., 2016a). Our transcriptional results showed that the levels of phenylalanine ammonia-lyases (PALs), including *VrPAL1* and *VrPAL2*, in the control were enhanced by 3.63–8.47 and 3.63–4.39-fold, respectively (Fig. 5d and e). ATP significantly ($P \leq 0.05$) decreased *VrPAL1* expression by 1.44-fold at day 2 and showed similar results to the control for *VrPAL2* expression. DNP treatment induced the expression of *VrPAL1* and *VrPAL2*, which were 1.98–1.99 and 1.20–1.83 times higher than those in the control. Similar trends were also observed in the expressions of *VrCHS*, *VrCAD* and *VrC4M*. The results indicated that the phenylpropanoid metabolism was improved overall during the germination of mung beans and these results were consistent with the accumulated contents of flavonoid and total phenolics (Fig. 2d and e). However, the synthesis of flavonoid and total phenolics was significantly enhanced by DNP treatment compared with the other two groups (Fig. 5). The induction of secondary metabolism under energy depletion may have resulted from the massive consumption of antioxidants during ROS scavenging. Overall, the metabolism of energy regulated antioxidant during the germination of

mung bean was summarised in Fig. 6.

4. Conclusion

In conclusion, this study investigated the regulatory role of energy status in the redox balance during the germination of mung bean seeds. Along with seed and sprout development, H_2O_2 constantly accumulated from 0.04 to 2.09 $mmol\ kg^{-1}$. The nutritive antioxidants, including FAA, flavonoids, and total phenolics were synthesised and the activities of antioxidative enzymes were also continuously enhanced. ROS were kept at relative low levels by the cooperative effects of antioxidants and enzymes. Exogenous ATP and DNP significantly ($P \leq 0.05$) increased (8.80–16.77%) or depressed (6.40–11.30%) the intracellular ATP contents, respectively. Excessive energy or ATP supply by exogenous ATP activated the enzymatic antioxidant system at the early stage of germination and prevented the consumption of nutritive antioxidants. By contrast, DNP caused energy depletion, which suppressed the enzyme system at the early germination stage, but promoted the consumption of non-enzymatic antioxidant components (FAA, flavonoids, and total phenolics) to maintain the ROS balance. However, the massive consumption of antioxidants resulted in a nutritive deficit in the mung bean sprouts. The results of this study suggested that positive energy regulation might help to increase the nutritive value of edible sprouts by activating the antioxidant enzyme system.

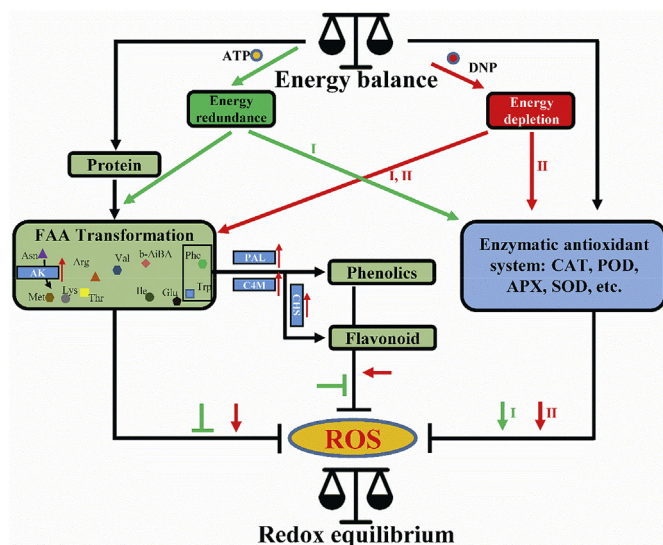


Fig. 6. Synopsis of energy regulated redox balance during the germination of mung bean sprouts. The green symbols represent physiological effects induced by exogenous ATP treatment, while the red symbols represent induced responses by 2, 4-dinitrophenol (DNP) treatment. Symbol I indicates relative early responses at day 2 and 4, while II reveals relatively late responses at day 4 and 6. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.lwt.2019.03.023>.

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