Pandanus amaryllifolius in Response to Drought Stress

Subjects: Plant Sciences

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Drought is one of the significant threats to the agricultural sector. However, there is limited knowledge on plant response to drought stress and post-drought recovery. *Pandanus amaryllifolius*, a moderate drought-tolerant plant, is well-known for its ability to survive in low-level soil moisture conditions. Understanding the molecular regulation of drought stress signaling in this plant could help guide the rational design of crop plants to counter this environmental challenge.

antioxidant enzymes | drought stress | Pandanus amaryllifolius | proteomics | stress-responsive proteins | TMT-labelled LCMS/MS

1. Introduction

Drought stress is a significant threat to agricultural productivity worldwide, causing 83% of agricultural economic losses. About US$29 billion was lost from all combined agriculture damages due to natural disasters [1]. Drought has affected the rice grain yield and caused about US$840 million losses in several rice-producing regions in Thailand [2]. In Malaysia, a 12–51% reduction in rice yield due to drought stress was reported from 2007 to 2011 [3]. Moreover, an expanding world population increases pressure on agriculture to use water more efficiently. Hence, it is indispensable to understand the drought response and adaptive mechanisms of plants as it could help to improve crop performance under drought stress conditions.

Drought stress disturbs physiological and biochemical processes in plants, including cell membrane, disrupting transportation of solutes, photosynthesis rate, nutrient uptake, translocation, and causes electron leakage and excessive accumulation of reactive oxygen species (ROS) [4][5]. The impacts of drought stress on plants rely on the severity and the growth period of plants [6]. To cope with these adverse effects, plants have developed intricate responses and adaptive strategies. These include the overproduction of compatible osmolytes, alteration of endogenous hormonal levels, and regulation of physiological and molecular changes [7]. Under moderate drought conditions, plants respond and adapt by altering root architecture [8] and stomatal closure [9], hoping to maintain a balance between stress tolerance and growth. However, if drought conditions become severe, plants tend to activate protection mechanisms against cellular damage, adjust in vivo antioxidant enzyme systems to eliminate excessive ROS, and accumulate proteins to maintain cell turgor, aiming to survive under such conditions [9].

Extensive efforts have been made to understand the plant responsive and adaptive mechanisms to drought stress using different “omics” techniques. Proteomics approaches have been used to determine plants’ proteome responses under drought stress. For instance, Liu et al. determined the protein changes of mulberry in response to drought stress using tandem mass tags (TMT)-label LCMS/MS technique [10]. The authors found that proteins involved in photosynthesis, energy and sugar metabolisms, antioxidant production, hormones, and cell homeostasis were abundantly changed under drought conditions. Using the same technique, Xiao et al. identified 123 differentially changed proteins between 30-d drought-stressed cotton fine roots and control [11]. The number of proteins was increased to 1273 when cotton was exposed to 45-d drought treatment. Goche et al. reported that 237 and 187 root proteins were significantly altered in drought-susceptible and drought-tolerant sorghum varieties, respectively [12]. Other proteomics drought studies have also been reported on chickpea [13], grapevine [14], wheat [15], and banana [16].

Pandanus amaryllifolius is a member of the screw pine family Pandanaceae. This plant is commonly known as fragrant screw pine, pandan (Malaysia and Indonesia), pandan mabango (Philippines), and toei hom (Thailand). *P. amaryllifolius* is widely cultivated in Southeast Asia, such as in Thailand, Indonesia, and Malaysia. The pandan plants grow in clumps with leaf size reaching 40–80 cm tall and a width of about 4.5 cm. Its leaves are dark green with sharp spines on the margins and are commonly used as food flavoring, natural colorants, and herbal medicine. Besides its aromatic value, *P. amaryllifolius* leaves have also been found to contain phenolic compounds that possess health benefits. For instance, Ghasemzadeh and Jaafar reported that gallic acid and cinnamic acid isolated from *P. amaryllifolius* could inhibit 78% of breast cancer MCF-7 cell lines [17]. In addition, metabolic syndromes, such as weight gain, abdominal adipose tissue deposition, and blood pressure, can be reduced after treatment with the leaf extract of *P. amaryllifolius* [18].

Pandanus spp. is a moderate drought-tolerant plant [19]. For example, *P. tectorius* can survive in drought conditions for more than 6 months, whereas *P. odaritissimus* can survive under an area with rainfall of less than 2,000 mm annually [20]. However, there is insufficient information on the extent of the drought tolerance of *P. amaryllifolius* despite its medicinal value. Understanding the plant’s strategy to adapt and survive under drought stress may refine our understanding of drought stress responses in plants and help to develop drought-tolerant crops.

2. Morphological Changes of Drought-Stressed Pandanus amaryllifolius

Our earlier preliminary experiment which exposed Pandanus plants to drought stress conditions for up to one month revealed that the plants could not survive after 14 days (data not shown). Hence, in the current study, we determined the early- to mid-drought response of Pandanus plants. At the morphological level, the changes of Pandanus plants in response to drought stress at several time intervals were determined (**Figure 1**A). The percentage of leaf relative water content (LRWC) for drought-stressed samples was significantly reduced after 4 days of drought treatment, whereas the percentage of relative electrolyte leakage (REL) for drought-stressed samples was significantly increased after 10 days of drought treatment (**Figure 1**B,C). The fresh and dry weights of shoots and roots in well-watered plants were generally higher than drought-stressed Pandanus (**Figure 1**D–G).

Surprisingly, the root dry weight of the 7-day drought-stressed plants was significantly higher compared to well-watered plants (**Figure 1**G). Similarly, the root-to-shoot ratio of the 7-day drought-stress samples was higher than well-watered plants (**Figure 1**H). However, the chlorophyll a, chlorophyll b, total chlorophyll and carotenoid contents of the drought-stress samples were significantly reduced compared to well-watered plants (**Figure 2**A–D). After rewatering, only 7-day drought-stressed Pandanus plants were able to recover (**Figure 3**A). Hence, day 7 of drought treatment was selected for the subsequent experiments.

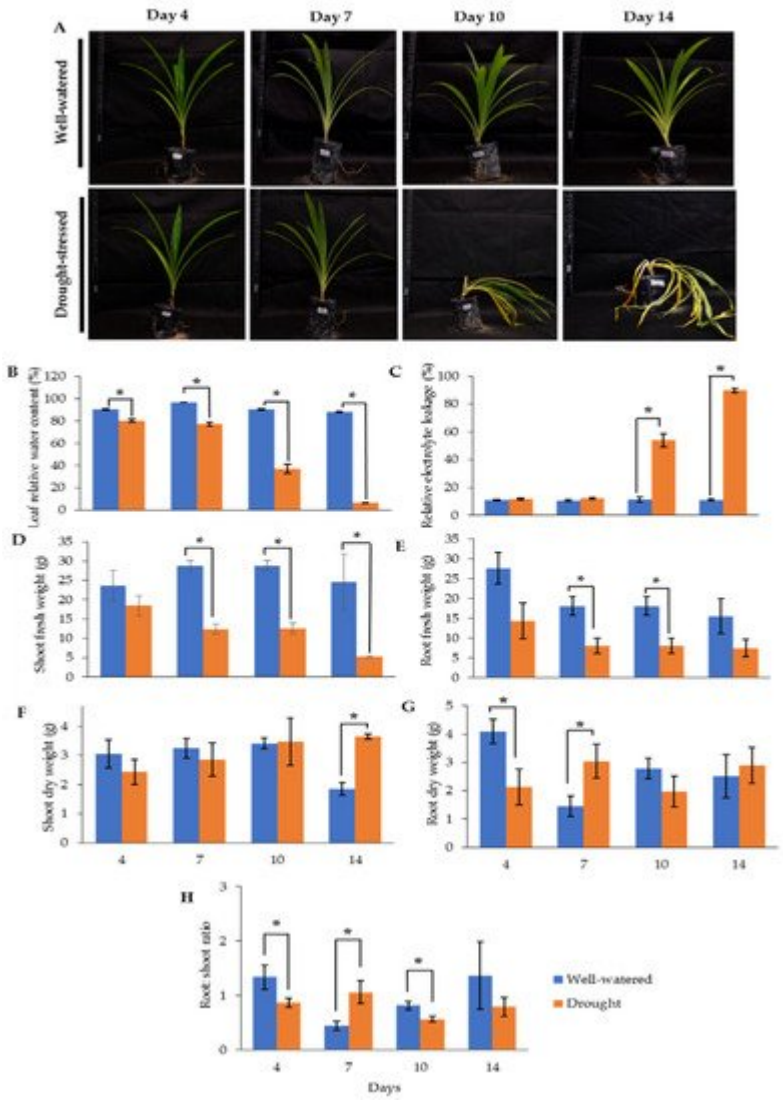

Figure 1. Responses of drought-stressed and well-watered *Pandanus amaryllifolius*. *P. amaryllifolius* plants were subjected to drought stress by withholding water for 4, 7, 10, and 14 days. Well-watered *P. amaryllifolius* was served as control. (**A**) Photographs of *Pandanus* plants were taken at 4, 7, 10, and 14 days. (**B**) The percentage of LRWC of *P. amaryllifolius* leaves at different time points. (**C**) The percentage of REL for each sample at different harvest points. (**D**) Shoot fresh weight of *Pandanus*. (**E**) Root fresh weight of *Pandanus*. (**F**) Shoot dry weight of *Pandanus*. (**G**) Root fresh weight of *Pandanus*. (**H**) Root-to-shoot ratio of *Pandanus* dry weight. Means labeled with asterisk were significantly different based on the Student's *t*-test when its *p*-value < 0.01.

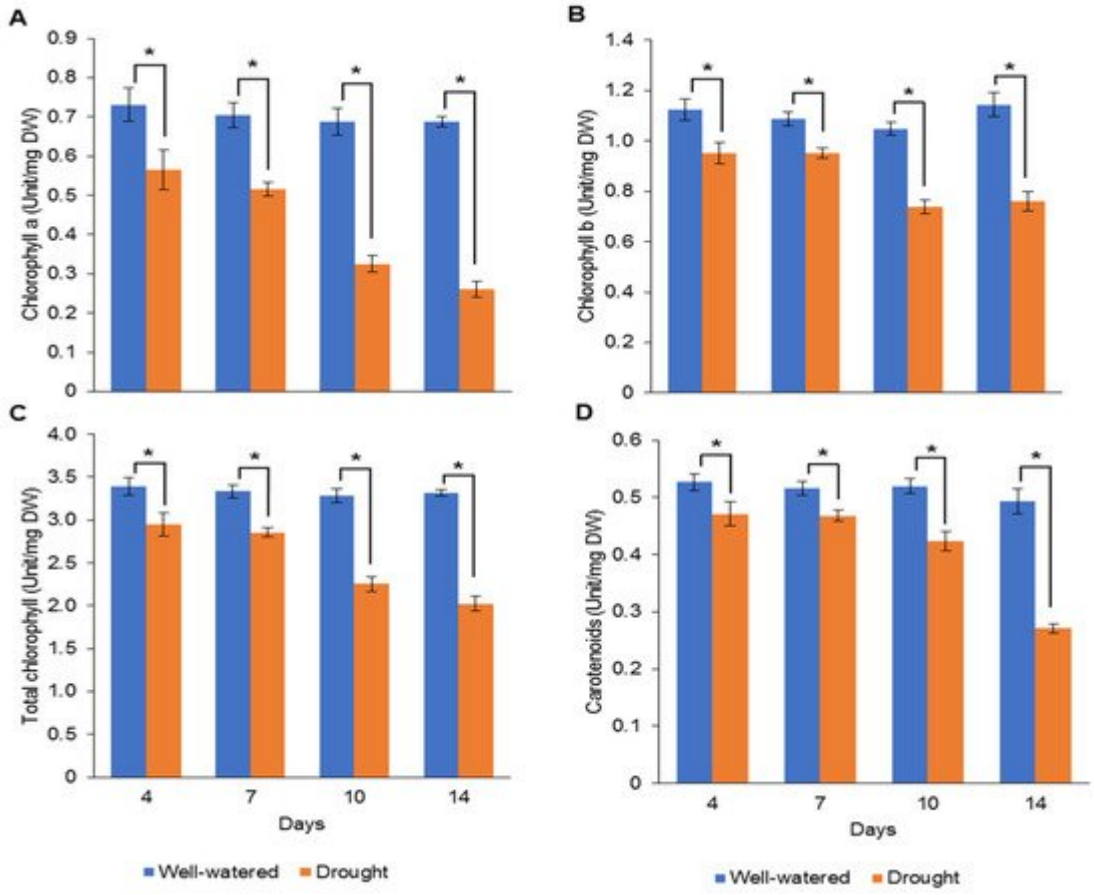

Figure 2. Pigment contents of leaf samples for well-watered and drought-stressed *Pandanus amaryllifolius* at different timepoints. The concentrations of (**A**) chlorophyll a (U mg^{-1} DW), (**B**) chlorophyll b (U mg^{-1} DW), (**C**) total chlorophyll (U mg^{-1} DW), and (**D**) carotenoid (U mg^{-1} DW). Means labeled with asterisk (*) were significantly different based on the Student's *t*-test when its *p*-value < 0.01.

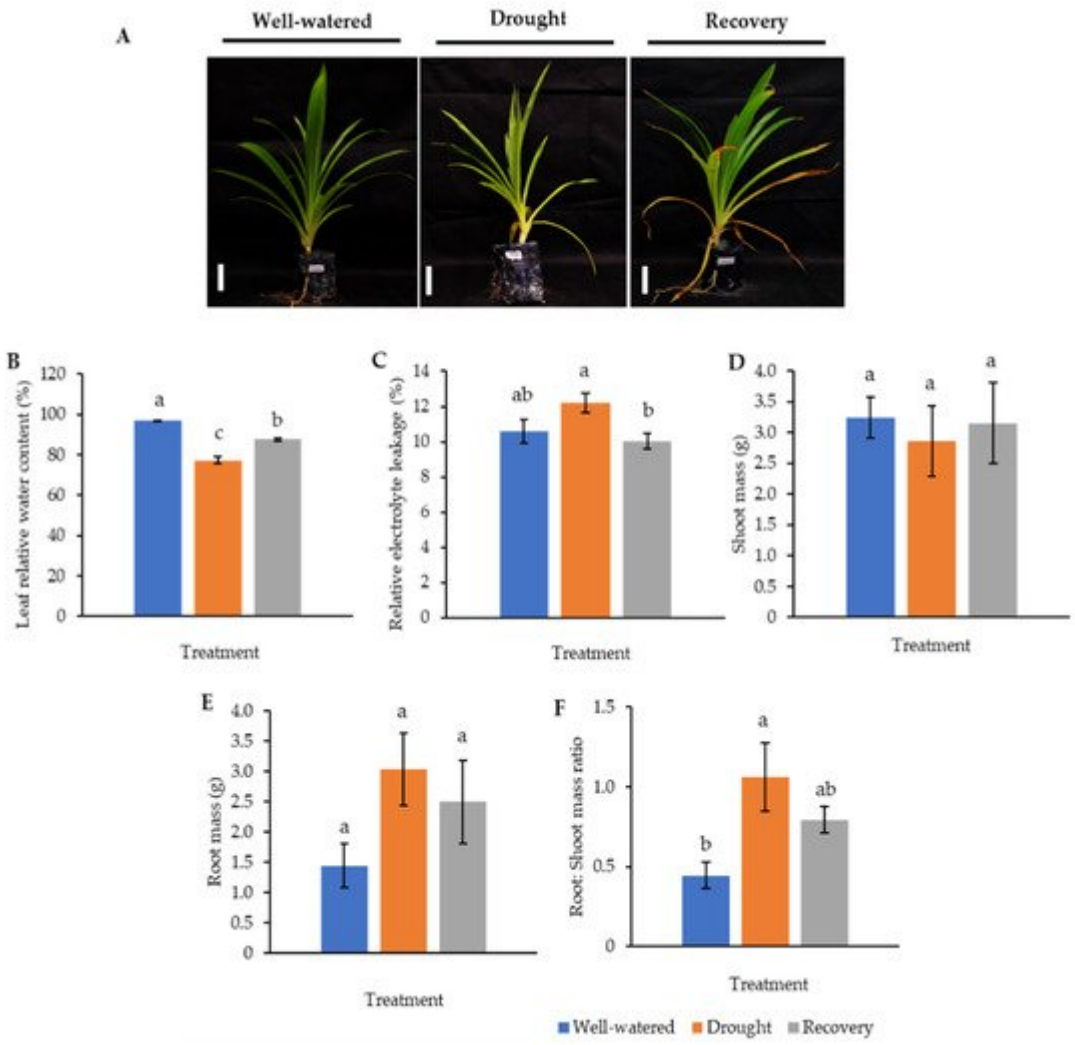

Figure 3. Comparison of well-watered, drought-stressed, and water-recovered *Pandanus amaryllifolius* plants. (**A**) *Pandanus* plants were subjected to drought stress by withholding water for 7 days, whereas the water-recovered plants were rewatered after a 7-day drought treatment and rewatered for 7 days. Well-watered *P. amaryllifolius* served as control. The line bar indicates the scale of the plant = 10 cm. (**B**) The percentage of leaf relative water content of *P. amaryllifolius* leaves. (**C**) The percentage of relative electrolyte leakage for each sample. (**D**) Shoot mass of *P. amaryllifolius*. (**E**) Root mass of *P. amaryllifolius*. (**F**) Root-to-shoot mass ratio of *P. amaryllifolius*. Means labeled with alphabet were significantly different based on the ANOVA followed by post hoc when its p-value < 0.05.

To understand the drought-responsive mechanism of Pandanus plants, a new set of experiments comprising well-watered, 7-day drought-stressed, as well as 7-day drought-stressed and rewatered plants was conducted since 10-day drought-stressed samples were unable to recover after rewatering. These samples were also subjected to morphological, biochemical, and proteomics analysis.

The LRWC in Pandanus plants was decreased with decreasing soil moisture content (**Figure 3** and Figure S1). In particular, the drought-stressed Pandanus plants showed a 20% reduction compared to well-watered plants but recorded comparable LRWC with well-watered plants after rewatering (**Figure 3**B). The REL of the drought-

stressed samples was higher than the well-watered and recovered plants (**Figure 3**C). The imposed drought stress did not affect the mass of both shoots and roots (**Figure 3**D,E). However, the root-to-shoot ratio in drought-stressed Pandanus was significantly higher than other treatments (**Figure 3**F). The pigment content of leaf samples in the drought-stressed Pandanus plants was significantly decreased (**Figure 4**A–D). It is worth mentioning that the leaf chlorophyll and carotenoid contents of the recovered plants were the same as drought-stressed plants, which could be related to insufficient recovery time (**Figure 4**A–D).

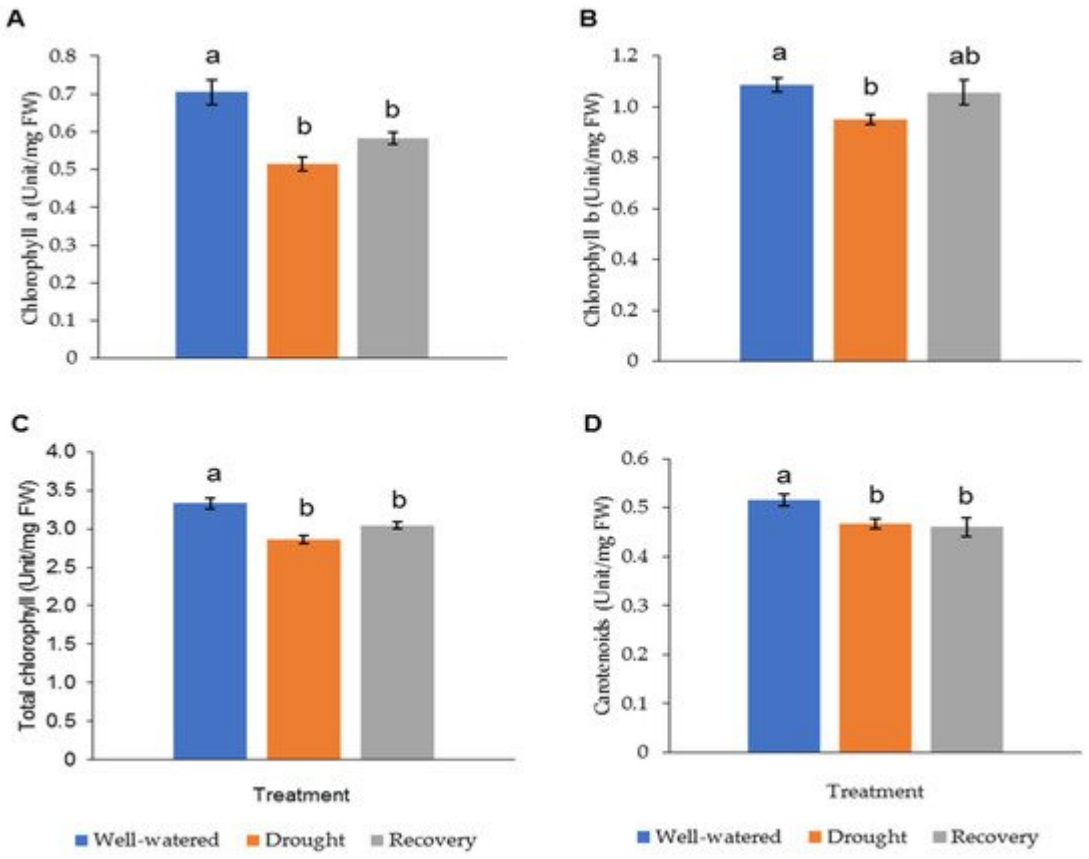

Figure 4. Pigment content of leaf samples for well-watered, drought-stressed, and recovered *Pandanus amaryllifolius*. The concentrations of (**A**) chlorophyll a (U mg^{-1} DW), (**B**) chlorophyll b (U mg^{-1} DW), (**C**) total chlorophyll (U mg^{-1} DW), and (**D**) carotenoids (U mg^{-1} DW). Means labeled with alphabet were significantly different based on the ANOVA followed by post hoc when its p-value < 0.05.

The Pandanus leaves showed slight wilting and clamping after 7 days of drought treatment but recovered after rewatering. However, there was a browning effect observed on the tips of mature leaves (Figure S2A). The yellow-green leaf color pigment pixel percentage in drought-stressed and recovered plants was slightly higher than well-watered plants (Figure S2B).

3. Changes of Proline and Malondialdehyde Contents in Pandanus Plants under Drought Stress

Proline, being an osmoprotectant, is involved in protecting plants from harmful effects caused by environmental stresses. Malondialdehyde (MDA), which results from the breakdown of polyunsaturated fatty acids, is the product of membrane lipid peroxidation. Both proline and MDA serve as an indicator of stress tolerance. In this study, the proline content of the drought-stressed Pandanus plants (1.6 µM g^{-1} FW) was significantly higher than well-watered (1.3 µM g^{-1} FW) and recovered plants (0.8 µM g^{-1} FW) (**Figure 5**A). Similarly, the MDA content of the drought-stressed Pandanus plants was the highest (9.2 nM g^{-1} FW) (**Figure 5**B). The well-watered and recovered samples recorded the same MDA content (7.7 nM g^{-1} FW).

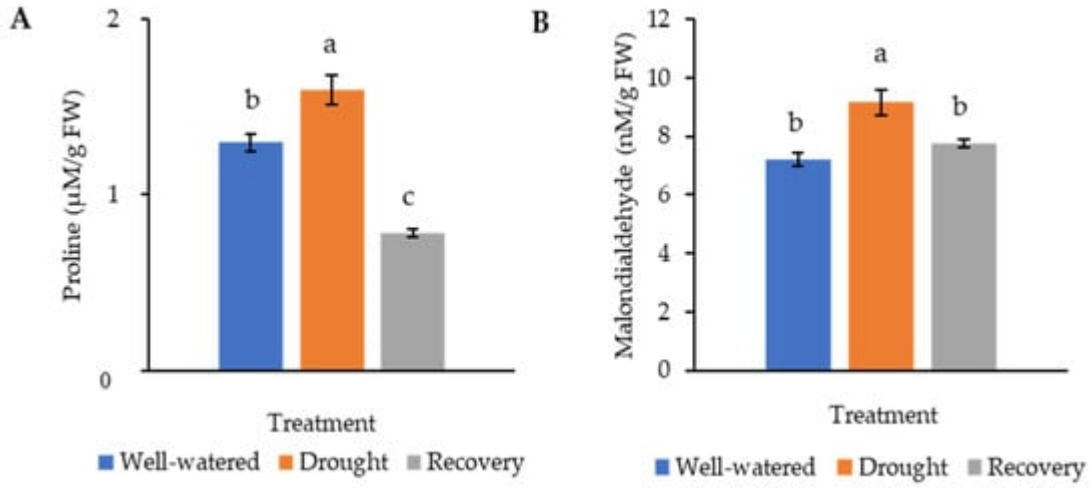

Figure 5. Osmolyte and lipid peroxidation changes of the drought-stressed and well-watered *Pandanus amaryllifolius*. (**A**) Proline content of *P. amaryllifolius* leaves as quantified in µM g^{-1} fresh weight (FW). (**B**) Malondialdehyde (MDA) content of *P. amaryllifolius* leaves as quantified in µM g^{-1} FW. Means labeled with alphabet were significantly different based on the ANOVA followed by post hoc when its *p*-value < 0.05.

4. Antioxidant Enzyme Changes in Pandanus Plants in Response to Drought Stress

Drought stress generally increases the activity of antioxidant enzymes. The highest hydrogen peroxide (H_2O_2) (30.0 µM min^{-1} g^{-1}) and superoxide dismutase (SOD) (462.1 g^{-1} FW) levels were recorded in water-recovered plants (**Figure 6**A,B). Catalase (CAT) and peroxidase (POD) for both well-watered and recovered plants showed significantly higher activity than for drought-stressed plants (**Figure 6**C,D). The highest activity of ascorbate peroxidase (APX) (10.4 M min^{-1} g^{-1}) and glutathione reductase (GR) (126.0 M min^{-1} g^{-1}) was found in drought-stressed samples (**Figure 6**E,F).

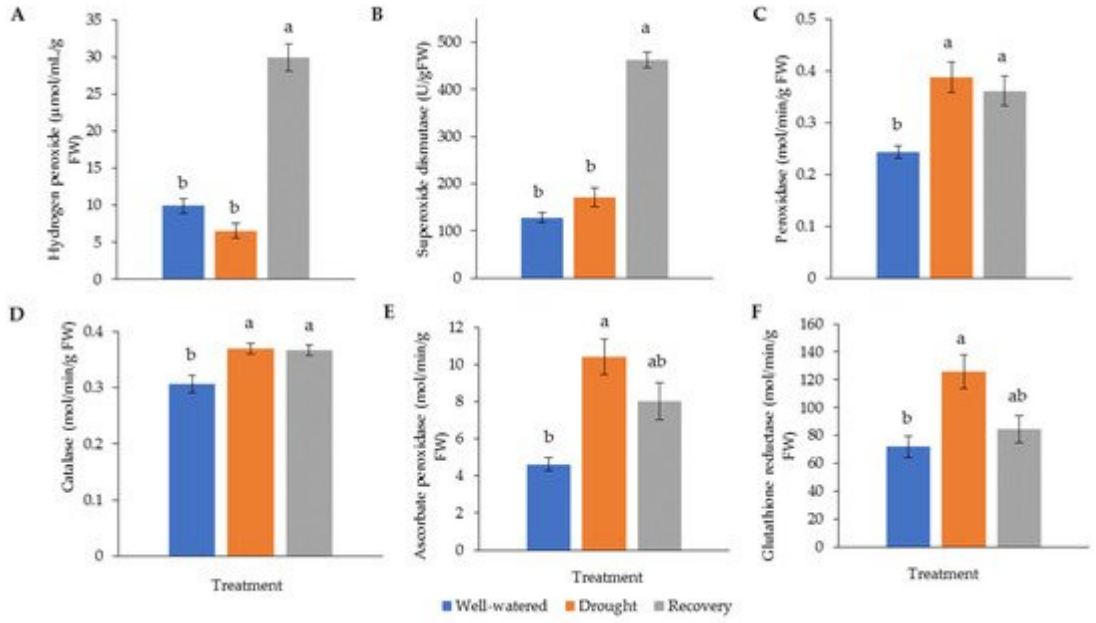

Figure 6. Activity of (**A**) hydrogen peroxide (H_2O_2) and antioxidant enzymes, (**B**) superoxide dismutase (SOD), (**C**) catalase (CAT), (**D**) peroxidase (POD), (**E**) ascorbate peroxidase (APX), and (**F**) glutathione reductase (GR) in *Pandanus amaryllifolius* leaves in response to drought stress and water recovery. Absorbance was measured through a spectrophotometer. H_2O_2 accumulation is shown in μM min^{-1} g^{-1} fresh weight (FW), whereas SOD is shown as U g^{-1} FW based on NBT coloration and inhibition. CAT, POD, APX, and GR are shown in M min^{-1} g^{-1} FW. Means labeled with alphabet were significantly different based on the ANOVA followed by post hoc when its *p*-value < 0.05.

5. Protein Changes in Well-Watered, Drought-Stressed, and Recovered Pandanus Plants

To identify the protein changes of Pandanus plants under drought stress, total protein from well-watered, drought, and water-recovered plants were extracted for nano-LC-MS/MS analysis. Of the 1,415 identified proteins, 74 proteins were found to be significantly altered (**Table 1**). These proteins were visualized with hierarchical clustering (Figures S3A and S4) and clustered into four groups based on the log ratio expression between treatments (Figure S3B). Cluster 1 showed that 12 proteins in the well-watered samples were decreased in abundance when exposed to drought stress (Figure S3B). In contrast, cluster 4 indicated that nine proteins in the recovered samples were increased in abundance when compared to drought-stressed samples (Figure S3B).

Table 1. List of abundantly altered protein profiles between well-watered, drought-stressed, and recovered *Pandanus amaryllifolius*.

Accession	Protein	Biological Process	Function	Cluster [a]
F1SWA0	Zerumbone synthase	Protein synthesis	Oxidoreductase	1

Accession	Protein	Biological Process	Function	Cluster [a]
P49043	Vacuolar-processing enzyme	Cysteine-type endopeptidase	Hydrolase	1
P48711	Ribulose bisphosphate carboxylase large chain	Photorespiration	Magnesium ion binding	1
Q9FLN4	50S ribosomal protein L27, chloroplastic	Ribonucleoprotein	mRNA binding	1
A0A357	30S ribosomal protein S18, chloroplastic	Ribonucleoprotein	rRNA binding	1
A1E9N5	30S ribosomal protein S7, chloroplastic	Ribonucleoprotein	rRNA binding	1
O23760	Caffeic acid 3-O-methyltransferase	Lignin biosynthesis	Methyltransferase	1
B2LMP1	30S ribosomal protein S15, chloroplastic	Ribonucleoprotein	Structural constituent of ribosome	1
A2WXD9	Photosystem II 22 kDa protein 1, chloroplastic	Photosynthesis	Non-photochemical quenching	1
Q9XF91	Photosystem II 22 kDa protein, chloroplastic	Photosynthesis	Non-photochemical quenching	1
Q32RY4	30S ribosomal protein S4, chloroplastic	Ribonucleoprotein	rRNA binding	1
O24461	Ras-related protein Rab7	Protein transport	GTPase activity	1
O22925	Vacuolar-sorting receptor 2	Protein transport	Calcium ion binding	2
Q940M2	Alanine-glyoxylate aminotransferase 2 homolog 1, mitochondrial	Photorespiration	Aminotransferase	2
Q9LUI2	Protein NETWORKED 1A	Cytoskeleton	Actin binding protein	2
P43644	DnaJ protein homolog ANJ1	Stress response	Chaperone	2
P11143	Heat shock 70 kDa protein	Stress response	Chaperone	2
A4QLY6	Photosystem I iron-sulfur center	Photosynthesis (ET)	Oxidoreductase	2
Q05737	GTP-binding protein YPTM2	Protein transport	GTPase activity	2
Q04960	DnaJ protein homolog	Stress response	Chaperone	2

Accession	Protein	Biological Process	Function	Cluster [a]
Q9XIM0	CCG-binding protein 1	Cellular response to hypoxia	Mediator complex binding	3
P81370	Thaumatin-like protein	Plant defence	Pathogenesis	3
Q6DBP4	Pectin acetylesterase 8	Cell wall biogenesis/degradation	Hydrolase	3
Q9FLC0	Peroxidase 52	Hydrogen peroxide	Oxidoreductase	3
Q96520	Peroxidase 12	Hydrogen peroxide	Oxidoreductase	3
P48980	Beta-galactosidase	Carbohydrate metabolism	Glycosidase	3
Q01289	Protochlorophyllide reductase, chloroplastic	Chlorophyll biosynthesis	Oxidoreductase	3
P26792	Beta-fructofuranosidase, insoluble isoenzyme 1	Carbohydrate metabolism	Glycosidase	3
Q0DM51	DEAD-box ATP-dependent RNA helicase 3, chloroplastic	Ribosome biogenesis	Hydrolase	3
F6H7K5	Thiamine thiazole synthase 2, chloroplastic	Thiamine biosynthesis	Transferase	3
Q9LN49	3-ketoacyl-CoA synthase 4	Acyltransferase	Fatty acid biosynthesis	3
Q75LR2	Phospho-2-dehydro-3-deoxyheptonate aldolase 1, chloroplastic	Amino acid biosynthesis	Transferase	3
O82627	Granule-bound starch synthase 1, chloroplastic/amyloplastic	Starch biosynthesis	Glycosyltransferase	3
Q8W0A1	Beta-galactosidase 2	Carbohydrate metabolism	Glycosidase	3
O23787	Thiamine thiazole synthase, chloroplastic	Thiamine biosynthesis	Transferase	3
Q9ZQ94	UDP-glycosyltransferase 73C5	Brassinosteroid metabolism	Glycosyltransferase	3
O80731	Pectin acetylesterase 3	Cell wall biogenesis/degradation	Hydrolase	3
Q9C992	3-ketoacyl-CoA synthase 7	Acyltransferase	Fatty acid	3

Accession	Protein	Biological Process	Function	Cluster [a]
			biosynthesis	
O22436	Magnesium-chelatase subunit ChlI, chloroplastic	Chlorophyll biosynthesis	Ligase	3
Q84P54	Gamma aminobutyrate transaminase 1, mitochondrial	Biotin biosynthesis	Aminotransferase	3
A5JTQ2	Beta-xylosidase/alpha-L-arabinofuranosidase 1 (Fragment)	Carbohydrate metabolism	Glycosidase	3
Q42850	Protochlorophyllide reductase B, chloroplastic	Chlorophyll biosynthesis	Oxidoreductase	3
Q9SD46	Peroxidase 36	Hydrogen peroxide	Oxidoreductase	3
O04931	Alpha-glucosidase	Carbohydrate metabolism	Glycosidase	3
Q08937	29 kDa ribonucleoprotein B, chloroplastic	mRNA processing	Ribonucleoprotein	3
Q5ZE07	Multicopper oxidase LPR1 homolog 2	Phosphate homeostasis	Oxidoreductase	3
A4S6Y4	Lon protease homolog, mitochondrial	Oxidative stress	DNA binding	3
Q40147	Glutamate-1-semialdehyde 2,1-aminomutase, chloroplastic	Chlorophyll biosynthesis	Isomerase	3
Q9LIK0	Plastidial pyruvate kinase 1, chloroplastic	Glycolysis	Kinase	3
Q6STH5	Fe-S cluster assembly factor HCF101, chloroplastic	iron-sulphur cluster assembly	4Fe-4S cluster binding	3
Q0E3C8	Chaperone protein ClpB3, mitochondrial	Stress response	Chaperone	3
Q94LW3	Homeobox protein knotted-1-like 3	Mucilage biosynthesis	DNA binding	3
Q42600	Cytochrome P450 84A1	Phenylpropanoid biosynthesis	Monooxygenase	3
Q56UD0	Beta-fructofuranosidase, insoluble isoenzyme 6	Carbohydrate metabolism	Glycosidase	3
Q8L7S6	Beta-hexosaminidase 3	Carbohydrate metabolism	Glycosidase	3

Accession	Protein	Biological Process	Function	Cluster [a]
Q39613	Peptidyl-prolyl cis-trans isomerase	Protein folding	Chaperone	3
Q9SJ20	Ribonucleoside-diphosphate reductase large subunit	DNA replication	Oxidoreductase	3
Q75GT3	Chaperone protein ClpB2, chloroplastic	Stress response	Chaperone	3
Q9ZUU4	RNA-binding protein CP29B, chloroplastic	mRNA processing	Ribonucleoprotein	3
Q9M591	Magnesium-protoporphyrin IX monomethyl ester [oxidative] cyclase, chloroplastic	Chlorophyll biosynthesis	Oxidoreductase	3
Q9CA67	Geranylgeranyl diphosphate reductase, chloroplastic	Chlorophyll biosynthesis	Oxidoreductase	3
P50246	Adenosylhomocysteinase	One-carbon metabolism	Hydrolase	3
Q6ZIV7	Hypersensitive-induced response protein 1	Potassium ion channel regulation	Histidine kinase binding	3
Q9SI75	Elongation factor G, chloroplastic	Protein biosynthesis	Elongation factor	3
P24846	4-hydroxy-tetrahydrodipicolinate synthase 1, chloroplastic	Amino acid biosynthesis	Allosteric enzyme	3
Q41932	Oxygen-evolving enhancer protein 3-2, chloroplastic	Photosynthesis (ET)	Calcium ion binding	4
P25795	Aldehyde dehydrogenase family 7 member A1	Stress response	Oxidoreductase	4
Q9AXH0	Catalase	Hydrogen peroxide	Oxidoreductase	4
O65660	PLAT domain-containing protein 1	Stress response	Catalase	4
A2YH64	Catalase isozyme B	Hydrogen peroxide	Oxidoreductase	4
Q0E4K1	Catalase isozyme A	Hydrogen peroxide	Oxidoreductase	4
O04932	Probable sucrose-phosphate synthase 1	Glycosyltransferase	Sucrose biosynthesis	4
Q570C8	3-ketoacyl-CoA thiolase 5, peroxisomal	Acyltransferase	Fatty acid biosynthesis	4
Q9SG80	Alpha-L-arabinofuranosidase 1	L-arabinose metabolic	Hydrolase	4

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Figure 7. Heat map of the differentially changed protein classes identified between well-watered, drought-stressed, and water-recovered *Pandanus amaryllifolius*. (**A**) Carbon-related proteins identified between treatments. (**B**)

3 Stressed-related proteins identified between treatments. The intensity scale indicates the range of up-regulation (purple) or down-regulation (yellow) of proteins between treatments.

Clusters 1–4 show the differential accumulation of differentially changed proteins (log ratio expression) between treatments (Figure S3B). Cluster 1 represents the decreasing protein abundance from the well-watered to drought-stressed and recovery samples. Cluster 2 shows the increasing protein abundance pattern from the well-watered to drought-stres … e decreasing protein abund … nt-stressed to recovery sam … y samples.

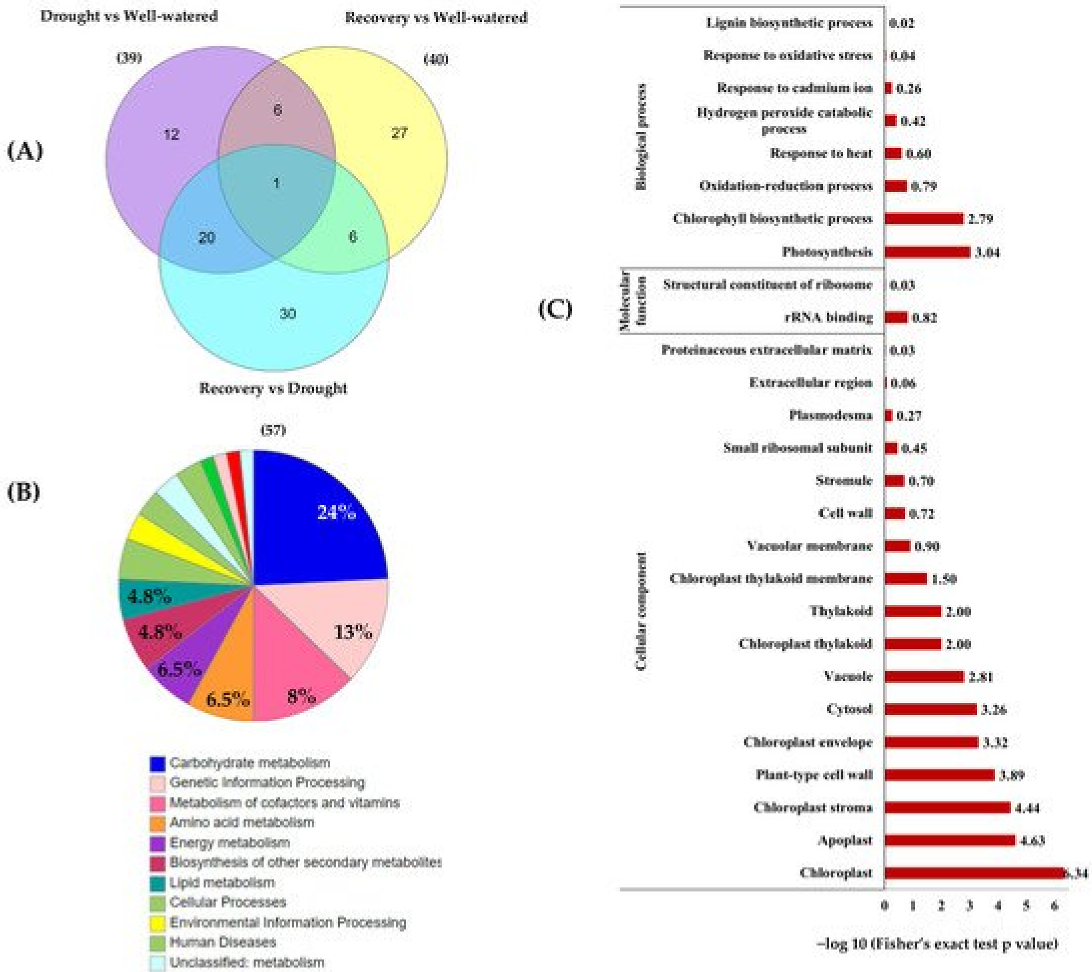
(A)
Drought vs Well-watered
Recovery vs Well-watered
(39)
(40)
12
6
27
1
20
6
30
Recovery vs Drought
(57)
(B)
24%
13%
8%
6.5%
6.5%
4.8%
4.8%
Carbohydrate metabolism
Genetic Information Processing
Metabolism of cofactors and vitamins
Amino acid metabolism
Energy metabolism
Biosynthesis of other secondary metabolites
Lipid metabolism
Cellular Processes
Environmental Information Processing
Human Diseases
Unclassified: metabolism
(C)
Biological process
Lignin biosynthetic process 0.02
Response to oxidative stress 0.04
Response to cadmium ion 0.26
Hydrogen peroxide catabolic process 0.42
Response to heat 0.60
Oxidation-reduction process 0.79
Chlorophyll biosynthetic process 2.79
Photosynthesis 3.04
Molecular function
Structural constituent of ribosome 0.03
rRNA binding 0.82
Cellular component
Proteinaceous extracellular matrix 0.03
Extracellular region 0.06
Plasmodesma 0.27
Small ribosomal subunit 0.45
Stromule 0.70
Cell wall 0.72
Vacuolar membrane 0.90
Chloroplast thylakoid membrane 1.50
Thylakoid 2.00
Chloroplast thylakoid 2.00
Vacuole 2.81
Cytosol 3.26
Chloroplast envelope 3.32
Plant-type cell wall 3.89
Chloroplast stroma 4.44
Apoplast 4.63
Chloroplast 6.34
0 1 2 3 4 5 6
−log 10 (Fisher's exact test p value)

Figure 8. Functional categorization and enrichment of differentially changed proteins between well-watered, drought, and recovery samples. (**A**) The Venn diagram represents the comparison of differentially abundant proteins identified in the leaves of *Pandanus* plants treated with drought stress, well-watered, and recovery; (**B**) KEGG enrichment of differentially changed proteins based on functional category; and (**C**) gene ontology enrichment based on KEGG pathway according to biological processes, molecular functions, and cellular components.

To classify the function of the 74 differentially changed proteins, KEGG pathway enrichment analysis was performed (**Figure 8**). The results showed that 15 of differentially changed proteins were involved in carbohydrate metabolism and another 8 proteins are involved in genetic information processing and cofactors and vitamin metabolism (**Figure 8**B). Based on the gene ontology (GO) functional classification, most of the differentially changed proteins were involved in photosynthesis processes and stress responses (**Figure 8**C).

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