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Desiccation tolerance in Artillery Plant (*Pilea microphylla* (L.) Liebm): A search

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Abstract

Water deficiency is the most significant abiotic stress factor for land plants. Most plants are unable to survive desiccation to the air dry state. There are however a few species from lower plant groups to flowering plants that tolerate desiccation known as resurrection plants. So, the present investigation was undertaken to study the biochemical changes in Pilea microphylla against desiccation (1, 3, 5 and 7 days) and rehydration (30 min) stress. As an initial part of the study total sugar, protein and proline content were analyzed and showed a gradual decline during the stress periods. The SDS-PAGE analysis of protein indicated the disappearance of certain bands in the desiccated and rehydrated samples (7D) when compared to the control indicating the denaturation of proteins during stress. Similarly, the appearance of new bands (15kDa on 3D and 20 and 17kDa on the 5D) were also noticed suggesting the formation of stress related proteins to tide over desiccation. The amount of free amino acids increased in P. microphylla, in pace with desiccation periods. Constitutive increase in the level of proline (the stress amino acid) accumulation is seen when compared with the control i.e., 6 fold higher than that of the control, after exposed to desiccation for 5 D. Increase in proline level relating to exposure time of desiccation stress suggests its role as osmolyte. Similarly, a reduction in chlorophyll level and an increase in carotenoid were also observed during stress. Decrease of total chlorophyll content was observed with duration of desiccation. The decrease in chlorophyll a and b was recovered during rehydration up to 5 D. Further studies are warranted at molecular level in terms of stress proteins and genes involved in desiccation tolerance in this plant.

Keywords: Desiccation, amino acids, SDS-PAGE, rehydration, osmolyte.

Introduction

Most of the crop plants are sensitive to desiccation or drought. Except a small group of vascular angiosperm plants, termed resurrection plants. They have evolved unique mechanisms of desiccation tolerance and thus can tolerate severe water loss, and mostly adjust their water content with the relative humidity in the environment. They have the unique ability to survive months to years without water, lose most of the free water in their vegetative tissues, fall into anabiosis and upon rewatering, quickly regain normal activity. Thus, they are fundamentally different from other drought tolerant plants such as succulents or ephemerals, which cope up with drought by maintaining higher steady state water potential or via a short life cycle, respectively. Desiccation tolerant plants may be subdivided into homoiochlorophyllous and poikilochlorophyllous types[1]. During desiccation homoiochlorophyllous species retain their intact photosynthetic apparatus and chlorophyll content in a readily recoverable form, whereas in poikilochlorophyllous species desiccation results in the loss of chlorophyll, which must be resynthetized following rehydration.

The main goals of the present study are to delineate whether *Pilea microphylla* display an oxidative burst in the time-course response to desiccation-rehydration and to elucidate the protective mechanisms underlying tolerance to drought. *Pilea microphylla* (L.) Liebm. also known commonly as Artillery or Gun powder Plant. It is annual plant native to Florida and belongs to the Urticaceae. The plant grows in extreme conditions of habitats. Direct sunlight causes the leaves to turn brown and fall off, so it prefers filtered light.

Material and Methods

Desiccation treatment: All the experimental *Pilea microphylla* samples were collected from the natural habitat. Before desiccation, the samples were fully hydrated. The samples were desiccated in a desiccator over PEG in a controlled environment chamber. The selected species were subjected to four different desiccation regimes 1 D, 3 D, 5 D and 7 D. After the desiccation exposure a set of desiccated samples were subjected to rehydration for 30 min. The samples were divided into two groups: desiccated and desiccated subsequently rehydrated. Control plants were maintained in an optimal water conditions in each case during the whole experimental period.

Quantification of photosynthetic pigments: Total chlorophylls were estimated[2]. The homogenate was centrifuged at 3000 rpm for 5 min. The aliquots were made up to 3 ml by using 80% acetone and the absorbance was measured at 470, 648 and 664 nm spectrophotometrically against 80% acetone as blank.

Estimation of sugars: Sugar content of leaves was estimated[3].

Quantification of total free amino acids: Total free amino acids were determined[4]. Free proline accumulation was determined using the method of Bates 5.

Estimation of soluble proteins: The soluble proteins were estimated by Lowry method[6].

Polyacrylamide gel electrophoresis (PAGE): Genei mini model slab gel apparatus was used to carry out PAGE[7,8].

Results and Discussion

Photosynthetic pigments: Chlorophylls: The effect of desiccation and rehydration showed varied response on the chlorophyll content of P. microphylla as shown in table-1. Decrease of total chlorophyll content was observed with increasing duration of desiccation. It showed that both chlorophyll a and b decreased and consequently affected chlorophyll a+b and a/b ratio and recovered or showed some recovery during rehydration up to 5 D desiccated plant body.

Plants employ chlorophylls *a* and *b* and carotenoids to capture light for photosynthesis. Most of the pigments serve as an antenna complex and are involved in collecting and transferring light energy to the reaction centres, where chemical reactions occur. Chlorophyll *b* is mainly involved in light harvesting and thus is predominantly found in the chlorophyll *a/b* antenna proteins, whereas chlorophyll *a* is closely associated with the reaction centre complexes[9]. Carotenoids are associated with both antenna and reaction centre proteins, and have multiple functions in photosynthesis. Carotenoids play vital role by masking the chloroplast from photo-oxidative damage caused by high intensity of light. Xanthophylls can achieve photo-protection by quenching the excited state of chlorophyll harmlessly as heat (non-photochemical quenching) as well as scavenging any singlet oxygen, which might have been formed[10]. Concentrations and ratios of photosynthetic pigments, (i.e., chlorophyll *a* and *b*, and carotenoids) are correlated to the irradiance experienced by plants in their natural habitat.

Plants that grow in low light often have greater amounts of chlorophyll *b* and lead to reduced chlorophyll *a* to *b* ratio than plants from high irradiance sites, possibly to increase light capture efficiency[11]. Similarly, higher chlorophyll to carotenoid ratios in plants from shaded habitats when compared with species from well-lit environments, suggests a lesser need for photo-protection. Lower resurrection plants have high levels of light harvesting chlorophyll *a/b* antenna protein complexes in comparison to vascular plants, suggesting shade acclimation[12].

The decrease in Chl *a+b* content was mainly attributed to the destruction of Chl *b*, which is more sensitive to stress than Chl *a*[13]. The desiccation stress can contribute a decrease in total chlorophyll content of the plant, by increasing the activity of Chl degrading enzyme cholorophyllase[14] inducing the destruction of chloroplast structure and instability of pigment protein complexes[15]. Results obtained from this study indicate that chlorophyll *b* is more susceptible to stress than chlorophyll *a* and it will be an ideal marker of drought stress.

Carotenoids: Carotenoid content was gently elevated by desiccation and attained the maximum value on 5 D. This is another indication for antioxidant potential in plants. Compared to the corresponding control, carotenoid contents increased by desiccation in *P. microphylla* table-1. The photosynthetic apparatus in *Pilea microphylla* may be better protected from photo-damage.

Carotenoids protect plants against photo-oxidation, by effectively quenching the excited triplet state of chlorophyll and singlet oxygen. Protection of the photosynthetic apparatus from excess light absorption requires carotenoids (oxygenated)[16]. Similar to other resurrection plants, *P. microphylla* inhabit areas with high irradiances usually have a better developed photo-protective system, illustrated by its chlorophyll to carotenoid ratios[17].

Pilea microphylla studied here have acclimated to the levels of light available, within their habitat. Flowering plants from habitats with low radiation inputs had higher concentrations of photosynthetic pigments (i.e., chlorophyll and carotenoids) and higher chlorophyll to carotenoid ratios, than from sunny environments. Plants from shaded environments usually modify chlorophyll *a/b* protein complexes to increase light harvesting, illustrated by a low chlorophyll *a* to *b* ratio[18].

This species had reduction in the amounts of photosynthetic pigments and chlorophyll *a* to *b* ratio table-1. This illustrates the need for an efficient light harvesting system, to collect all of the available light. The values are in accordance with other species, from extremely shaded environments[17]. The chloroplast distribution in such plants showed some remarkable features. The ventral side of the lamina is unlikely to be exposed to light, yet chloroplasts were found there. Such a feature could afford protection from environmental stress, since it allows preservation of functional chloroplasts on the ventral side, while the chloroplasts on the dorsal side are being damaged.

Total soluble sugar: In *P. microphylla* the level of sugar showed gradual decline from 1 D of desiccation onwards indicating the depletion of stored carbohydrates into soluble sugars and its consumption table-2. Plants accumulate carbohydrates such as starch and fructans as storage substances that can be mobilized during periods of limited energy supply or enhanced energetic demands. While most plant species use starch as their main storage carbohydrate, several angiosperms, mainly from regions with seasonal cold and dry periods, accumulate fructans[19]. Accumulation of fructans might be advantageous, due to their high water solubility, their resistance

to crystallization at freezing temperatures, and the fact that fructan synthesis functions normally under low temperatures[20]. Furthermore, fructans can stabilize membranes and might indirectly contribute to osmotic adjustment upon freezing and dehydration by the release of hexose sugars[21,22]. Many researchers[23,24] has reported that salt and drought stress generally leads to a depletion of starch content and to the accumulation of soluble sugars in leaves. Sugars that accumulate in response to stress can function as osmolytes to maintain cell turgor and have the ability to protect membranes and proteins from stress damage[25].

Total Soluble protein: Soluble protein decreased significantly in both desiccated and also rehydrated plants that are imposed to stress table-3. Protein degradation might be the result of increased activity of protease or other catabolic enzymes, which were activated under desiccation stress or due to fragmentation of proteins by the toxic effects of ROS resulting in reduced protein content[26]. A decrease in the protein concentration would be a typical symptom of oxidative stress and has frequently been observed in drought stressed plants[27].

Table-1

Chlorophyll a, b and carotenoid content (mg g^{-1} FW) of the desiccated (1, 3, 5 and 7 D) and rehydrated plant body of *P. microphylla.* Data points represent means of three replicates and probability level at *P <0.01*

Pigments	Control	1 D	1 R	3 D	3 R	5 D	5 R	7 D	7 R
Chl a	4.2	2.98	3.6	2.26	3.72	1.92	3.67	1.8	2.56
SE	0.96	0.54	0.26	0.27	0.19	0.31	0.26	0.21	0.32
Chl b	1.79	1.73	1.4	1.13	1.52	1.0	1.38	0.92	0.99
SE	0.57	0.55	0.50	0.47	0.56	0.51	0.45	0.44	0.33
Caro	0.80	1.1	1.0	1.2	1.0	1.2	0.98	0.64	0.73
SE	0.90	0.49	0.28	0.57	0.49	0.39	0.50	0.31	0.58
F ratio	1.96**	7.8**	12.6**	14.9**	10.8**	8.9**	7.6**	3.9**	4.8**
CD	1.3	1.4	1.28	1.39	1.46	1.38	1.52	1.28	1.34

Table-2

Influence of desiccation (1, 3, 5 and 7 D; D=days) and rehydration on the levels of total sugar content (µg/g). The values are means of three individual experiments with duplicates and probability level at *P*<0.01

Condition	1 D	3 D	5 D	7D
Control	21.11	19.89	20.67	21.68
SE	2.34	1.21	1.08	1.22
Desiccation	13.17	10.76	6.96	4.6
SE	1.42	1.08	1.07	0.28
Rehydration	17.2	16.04	12.83	12.5
SE	1.04	1.26	1.31	0.78
F ratio	2.85**	3.52**	3.69**	4.85**
CD	1.08	1.24	1.32	1.09

Table-3
Influence of desiccation (1, 3, 5 and 7 D; D=days) and rehydration on the levels of total protein content (mg/g). The values are means of three individual experiments with duplicates and probability level at $P < 0.01$

Condition	1 D	3 D	5 D	7 D
Control	8.97	7.89	8.25	8.55
SE	1.24	0.66	0.92	1.00
Desiccation	6.2	4.3	3.24	1.87
SE	0.08	0.16	0.24	0.13
Rehydration	7.02	8.19	5.74	4.80
SE	1.24	0.29	1.01	0.76
F ratio	1.29**	1.69**	1.99**	2.78**
CD	1.07	0.98	0.29	1.08

Total free amino acids and proline content: Total free amino acids and proline contents in *P.microphylla* are shown in the table-4. The amount of free amino acids increased in *P. microphylla*, in pace with desiccation periods. Although the total amino acids are clearly built up in the species, their accumulation is more obvious during 5D of desiccation. Constitutive level of proline accumulation is also increased when compared with the control i.e., 6 fold higher than that of the control, after exposed to desiccation for 5 D. The proline content progressively enhanced, corresponding to desiccation period up to 5D. Increase in proline level relating to exposure time of desiccation stress suggests its role as osmolyte. The long exposure period (7 D) reduced the proline accumulation. There are three possible reasons for the free proline accumulation under stress: i. stimulation of proline synthesis from glutamic acid[28], which has been found to be dependent on the abscisic acid concentration; ii. inhibition of proline oxidation to other soluble compounds; iii. inhibition of protein synthesis. In contrast to its metabolism, the physiological significance of proline accumulation has been less studied[29].

In halophytes, salt tolerance associates with the capacity to accumulate proline, which acts as compatible solute, involved in osmotic adjustment at the plant cell level[30]. It has been suggested that, the proline accumulation is due primarily to the function of both genes encoding Δ^1-pyrroline-5-carboxylate reductase, and Δ^1-pyrroline-5-carboxylate synthetase[30]. Some reported that decreased protein synthesis and/or increased protein hydrolysis in pearl millet seedling by salinity, could lead to the accumulation of free amino acids and proline[31]. In the present study, proline accumulation is observed in *P. Microphylla* table-4. However, at higher duration of desiccation (above 5D) the plant wilted; this suggests that proline does not help in reducing dehydration damage in this species. Another compatible solute, glycine betaine also functions as an important osmoprotectant between the cytoplasm and vacuole[32]. Furthermore, this compound can reduce lipid peroxidation and protect mitochondrial electron transport reactions from stress damage[32]. Previous studies have reported that, increased glycine betaine contributed to overcome water and salt stress in leguminous plants [33]. For a better understanding of the role of this compatible solute in osmotic maintenance in *P. microphylla*, further studies are warranted.

The free amino acid content has been shown to increase under drought conditions in sorghum[34]. Similar results were obtained in pepper[35], coconut[36], and wheat[37] and ground nut[38]. Free amino acid accumulation is more important to account for most of the changes in osmotic potential. The accumulation of free amino acids under stress at all the experimental species indicates the possibility of their involvement in osmotic adjustment[34]. Osmotic adjustment is one of the important mechanisms alleviating some of the detrimental effects of water stress[39].

Similarly, increased proline accumulation was reported in water stressed sorghum[34], bell pepper[35], wheat[37] and in salt stressed *Catharanthus roseus* [40]. Increased proline in the stressed plants may be an adaptation to overcome the stress conditions. Proline accumulates under stressed conditions supplies energy for growth and survival and thereby helps the plant to tolerate stress[41]. Under abiotic stress like UV light the proline content showed an increase in wheat[42]. NaCl stress showed increased proline content in rice[43] and peanut[33]. Proline accumulation in plants might have a scavenger role of ROS and also act as an osmolyte[44]. The reduced proline oxidase may be another reason for increasing proline accumulation. Many researchers suggested that, proline plays a pivotal role in imparting plants

tolerance to stress that lower the water potential of ambient environment.

Abiotic stresses like salt, heavy metals/drought lead to an increased accumulation of proline in tobacco cells coupled with the γ-glutamyl kinase level[45]. So the induction of proline accumulation, in the present study is may be due to an activation of proline synthesis through the glutamate pathway involving γ-glutamyl kinase, glutamyl phosphate reductase and Δ^1–pyrroline-5-carboxylate reductase activities[46,33,47]. The proline accumulation in desiccation-stressed bryophytes may be attributed to the increased level of γ-glutamyl kinase activity or decreased level of proline metabolizing enzymes, like proline oxidase. The present study coincides with earlier reports concerning water stress in plants[47].

Polypeptide banding pattern in SDS-PAG: Environmental stress affects the protein banding pattern to counteract the stress. In the present study, the desiccation tolerance nature of *P. microphylla* was also evaluated using SDS-PAGE, Figure-1. On the 1 D of desiccation 6 protein bands were formed with molecular mass of 38, 36, 28, 21, 15 and 12 kDa. While on rehydration for 30 mins in the 1 D desiccated plant showed the disappearance of the 15 kDa band and appearance of a new faint band at 60 kDa. Similar studies have been undertaken by earlier workers. Under stress condition the plant synthesize the heat shock proteins. The essential function of heat shock proteins is preventing aggregation and assisting refolding of non-active proteins under both normal and stress conditions[48,49].

In the 3 D desiccated plants only 3 bands were noticed i.e., an intensified 15 kDa band and new bands of 10 and 5 kDa. Meanwhile in the rehydrated plant along with the above 3 bands, 3 new bands at 21, 24 and 32 kDa were observed.

In contrast to the above results, the 5 D desiccated plants showed only two intensified bands at 20 and 17 kDa. The rehydrated plants along with above bands showed two faint bands at 15 and 10 kDa. Some studies[50] showed that the total protein content of the leaves significantly increased when peanut plants were subjected to water stress for 5 to 20 as compared to irrigated controls.

The 7 D desiccated plants generated only a single faint band at 15 kDa. Interestingly the rehydrated plant showed no bands indicating the limitation of the species to drought stress i.e., maximum tolerance is up to 5 D of desiccation. Similarly, some reported that in response to iron stress in *Withania somnifera L.* the SDS-PAGE profile showed a significant increase of protein content in leaves and roots up to a certain limit and then decreased[50].

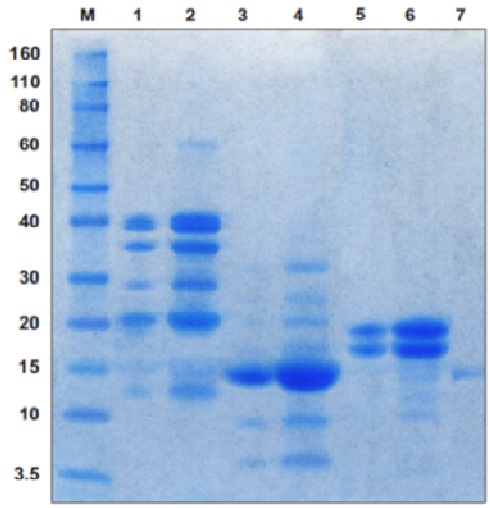

Figure-1
Protein banding pattern in *P. Microphylla* under different regimes of desiccation, M- Marker; i. 1 D desiccated; ii. 1 D rehydrated; iii. 3 D desiccated; iv. 3 D rehydrated; v. 5 D desiccated; vi. 5 D rehydrated; vii. 7 D desiccated

Table-4
Free amino acids, Proline, content of the desiccated (1, 3, 5 and 7 D) and rehydrated plant body of *P.microphylla*. Data points represent means of three replicates and probability level at *P* <0.01

	Control	1 D	1 R	3 D	3 R	5 D	5 R	7 D	7 R
Free amino acids (μg g^{-1} FW)	1.76	4.85	2.1	8.78	2.4	9.69	2.5	8.2	2.6
SE	0.06	0.08	0.04	0.09	0.06	0.07	0.02	0.04	0.08
F ratio	1.3**	1.4**	1.7**	1.9**	1.2**	1.49**	1.8**	1.98**	1.29**
CD	1.03	1.25	1.39	1.21	1.08	1.77	1.67	1.58	1.49
Proline (μg g^{-1} FW)	175.5	323	180.6	556	184.6	1038	190.3	696.5	190.6
SE	0.13	0.29	0.66	0.49	0.78	0.92	0.81	0.67	0.77
F ratio	1.6**	2.4**	1.38**	1.39**	1.66**	1.69**	1.28**	1.39**	1.45**
CD	1.25	1.38	1.42	1.08	1.29	1.65	1.89	1.29	1.66

Conclusion

This study has presented evidence that, *P. microphylla* are equipped to deal with abiotic stress–desiccation and subsequent rehydration environments up to 5 D. Changes in pigment composition and chloroplast thylakoids remained intact or distorted throughout the course of desiccation but regained during rehydration. Further studies are warranted for the characterization of stress related proteins.

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