Acta Physiol Plant (2017) 39:154
DOI 10.1007/s11738-017-2454-1

ORIGINAL ARTICLE

Desiccation-related responses of antioxidative enzymes and photosynthetic pigments in *Brachythecium procumbens* (Mitt.) A. Jaeger

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Received: 13 September 2016 / Revised: 8 June 2017 / Accepted: 17 June 2017 / Published online: 26 June 2017

Abstract Desiccation, a major environmental stress, affects water potential and turgor in the plants leading to physiological imbalance. Though bryophytes have the ability to endure desiccation, the adverse environmental conditions may cause them to dry irreversibly. In the present study, desiccation tolerance mechanism of *Brachythecium procumbens* (Mitt.) A. Jaeger was analysed in terms of its antioxidative response and photosynthetic pigments. Plants of *B. procumbens* were subjected to desiccation stress for varying durations (24–96 h) along with control (0 h) at room temperature. Monitoring was done using antioxidant enzyme activities, photosynthetic pigments, chlorophyll stability index, as well as, relative water content. The antioxidative enzymes—superoxide dismutase and peroxidase—showed higher activity in desiccated plants as compared to control and increased significantly with duration of desiccation. However, the activity of catalase decreased during desiccation. The amount of chlorophyll increased up to 48 h of desiccation treatment as compared to control, whereas in rehydrated samples, relatively lower value was obtained. Majority of bryophytes may withstand a certain level of desiccation for at least a few days, but some are much more tolerant than that. The bryophyte system studied showed basic difference in enzyme activities and chlorophyll under different periods of desiccation. Hence, drought-tolerant genera need to be identified and propagated so that some pioneer colonizers of the ecosystem are naturally conserved.

Keywords *Brachythecium procumbens* · Bryophyta · Drought stress · Desiccation tolerance · Reactive oxygen species · Rehydration

Introduction

Environmental factors, viz., air pollution, heavy metals, heat, cold, UV radiation and chemical compounds cause oxidative stress (Elstner and Osswald 1994). Drought is also a similar type of abiotic stress which may affect the growth and yield of crop plants and can also cause pigment degradation (Hendry et al. 1987) leading to irreversible damage to the photosynthetic system (Clarke et al. 1996). On exposure to abiotic stresses, reactive oxygen species (ROS), viz, superoxide ($O_2^{\cdot-}$), hydroxyl radicals (·OH), hydrogen peroxide (H_2O_2) and singlet oxygen ($^1O_2$) can be produced particularly in chloroplasts and mitochondria (Halliwell 1987). They have the ability to damage lipids, chlorophyll, protein and nucleic acids (Noctor and Foyer 1998). Therefore, plant cells are furnished with enzymatic and non-enzymatic systems to overcome oxidative damage. Catalase (CAT), peroxidase (POD) and superoxide dismutase (SOD) are antioxidant enzymes which play a key role in removing ROSs (Sgherri et al. 2000). In plant cells, superoxide produced due to stress gets converted to H_2O_2 by the action of superoxide dismutase (EC 1.15.1.1). Therefore, SODs act as the first line of defence against different ROS. The second mechanism of H_2O_2 destruction is by peroxidases (EC 1.11.1.7), present in the cell and having a greater affinity for H_2O_2 than CAT. However, CAT (EC 1.11.1.6) converts H_2O_2 to water and O_2. Any

Communicated by H. Peng.

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modulation in the activity of CAT, POD and SOD may serve as an important indicator in plants depicting their ability to the environmental stress (Rensburg and Kruger 1994). Therefore, antioxidative systems help to keep the balance between the increased generation of ROS and their removal and alleviate the oxidative damage (Szalai et al. 2009).

Bryophytes are pioneer colonizers that can survive for long periods under unfavourable conditions. Their response to the external conditions depends on their water content and their capability to counteract drought stress. The evolutionary transactions in land plants have given a critical phylogenetic status to the bryophytes. These plants are ideally compatible as models to understand how primitive plants survived the stress of moving onto the land (Alpert 2006). Although some data regarding antioxidant systems in *Syntrichia ruralis* var. *arenicola* (=*Tortula ruraliformis*), *Dicranella palustris* (Seel et al. 1992a, b; Tuba et al. 1998), *Fontinalis antipyretica* (Roy et al. 1996) and *Polytrichastrum formosum* (=*Polytrichum formosum*) (Pressel et al. 2006) are available, still little is known about the antioxidant activity in desiccated and subsequently rehydrated mosses. Oliver et al. (2000) introduced a bryophytic model for studying stress tolerance mechanisms that offer a great deal of promise for advancing our efforts to understand how plants respond to and survive the severest of stressful environments and also postulate that desiccation tolerance is a primitive and necessary trait for invasion of land. According to Oliver et al. (2005), in bryophytes, two aspects permit their survival: constitutive cellular protection and effective recovery/repair mechanism. Much of the earlier work (Dhindsa and Matowe 1981; Stewart 1990; Seel et al. 1991, 1992a, b; Smirnoff 1992; Oliver and Bewley 1997; Kappen and Valladares 1999) sought simply to establish how long plants would survive in the dry state, while in recent times mechanisms of tolerance are the focus of different studies (Proctor and Smirnoff 2000; Alpert and Oliver 2002; Proctor et al. 2007). It has been assumed that slow drying apparently reduces the oxidative burst by limiting production of ROS. de Carvalho et al. (2012) demonstrated that under slow dehydration, *Fontinalis antipyretica* exhibits low production of ROS upon rehydration, a phenomenon that reduces the cellular damage and increases cell survival.

In this paper, we investigated the role of some important free-radical-scavenging antioxidant enzyme (CAT, POD, SOD) in cells of a West Himalayan moss species: *B. procumbens* to examine physiological responses to water stress and recovery to reveal the method adopted under various levels of water stress and to assess the level to which *B. procumbens* can tolerate drought. Besides, study of photosynthetic pigments (Chl *a*, Chl *b*, total Chl), chlorophyll stability index and relative water content has also been done to serve as bioindicators of stress tolerance, as adverse impact of desiccation increased oxidative stress which altered expression of plant's antioxidant response system as well as photosynthetic pigment systems.

Materials and methods

Source of material

Sample from population of *Brachythecium procumbens* (Mitt.) A. Jaeger was collected from Almora district (29.81°N 79.29°E), Kumaun Hills during winter. Plants were washed under running water to remove adherents. Final rinsing was done with distilled water. The procedure of Mayaba and Beckett (2003), Proctor et al. (2007) and de Carvalho et al. (2012) was used and accordingly after cleaning, apical part of gametophyte (green tissue) was cut from large number of plants and kept on weighed filter paper which was weighed again for fresh weight. Part of the samples was kept in humid conditions and was used as control during the experimental period. The remaining half was desiccated according to Lubaina et al. (2013). Samples were drawn for the study at required time intervals and half of it was rehydrated.

Desiccation and rehydration treatments

The samples were desiccated in desiccators over silica gel under uniform light and temperature conditions. The plants were subjected to four different levels of desiccation, i.e. 24, 48, 72 and 96 h. A set of desiccated samples were rehydrated for 30 min generating two treatment sets at each time interval: desiccated (D) and rehydrated (R). Control plants were treated as non-desiccated samples (0 h) and whole experiment was performed in triplicate.

Antioxidant enzyme activity

CAT activity was estimated according to the modified method of Euler and Josephson (1927) as rate of disappearance of H_2O_2. Extraction and assay of enzyme was done by homogenizing the plant material (100 mg) in 4 ml of distilled water in pre-chilled pestle and mortar under cold condition. The extract was then centrifuged (12,000 rpm for 15 min) and the supernatant was used for the assay of CAT activity. The supernatant was mixed with 1 ml 0.005 M H_2O_2 in potassium phosphate buffer (pH 7.0) and after 5 min, the reaction was stopped by adding 2 N H_2SO_4. The reactants were titrated against 0.1 N $KMnO_4$ till the end point (light pink) was reached.

POD activity was determined by a modified spectrophotometric method of Luck (1963) which was

measured by the change in absorbance of the supernatant at 485 nm. Green plants (50 mg) were ground in 10 ml of distilled water and then centrifuged for 15 min at 12,000 rpm. 5 ml of 0.1 M phosphate buffer (pH 6.0), 1 ml of H_2O_2 and 1 ml of *p*-phenylenediamine were added to 1 ml of extract. After 10 min 5 N H_2SO_4 was added to stop the reaction.

The method of Beauchamp and Fridovich (1971) was used for estimating SOD. Fresh, desiccated and rehydrated moss samples (100 mg) were homogenized in a solution of phosphate buffer (pH 7.0), PVP, EDTA and centrifuged at 2500 rpm for 10 min. The enzyme extract was added to the test tube containing 50 mM phosphate buffer (pH 7.8), 10 mM methionine, 56 mM NBT and 1.17 mM riboflavin and incubated in light. A blank (without enzyme) was maintained for all treatments. After completion of reaction the absorbance of the reaction mixtures was read at 560 nm.

Chlorophyll content and chlorophyll stability index (CSI)

Chlorophyll (Chl *a*, Chl *b* and total Chl) content was determined according to Arnon (1949). Leaves were ground in a mortar in 80% chilled acetone (1:10 w/v). The extract was centrifuged and absorbance of the supernatant was recorded at 645, 652 and 663 nm spectrophotometrically. Chlorophyll stability index (CSI) was calculated according to Sairam et al. (1997).

$$\text{CSI} = (\text{Total Chl under stress}/\text{Total Chl under control}) \times 100.$$

Percent weight loss/gain (WL/WG)

There was an overall loss in plant weight as the desiccation time increased, whereas subsequent rehydration increased the plant weight. Percent of weight loss during desiccation and percent of weight gain during subsequent rehydration was calculated as follows:

$$\text{WL}\ (\%) = [(\text{FW}-\text{DW})/\text{FW}] \times 100$$

$$\text{WG}\ (\%) = [(\text{FW}-\text{RW})/\text{FW}] \times 100$$

where FW, DW and RW were fresh weight, desiccated weight and rehydrated weight, respectively.

Relative water content (RWC)

Relative water content (RWC) was determined using the Castillo's (1996) method and calculated in the plants for each dehydrated and rehydrated period as follows:

$$\text{RWC}\ (\%) = [(\text{FW}-\text{DW})/(\text{TW}-\text{DW})] \times 100$$

$$\text{RWC}\ (\%) = [(\text{FW}-\text{RW})/(\text{TW}-\text{RW})] \times 100$$

where FW, DW, RW and TW were fresh weight, dry weight, rehydrated weight and turgid weight, respectively.

Statistical analysis

The data analysed in triplicate from all the screening methods were subjected to statistical analysis. Values were represented as mean ± standard error (Snedecor and Cochran 1967). One-way analysis of variance (ANOVA) was performed using statistical software SPSS ver. 15.0 for Windows (SPSS Inc., Chicago, Ill., USA) and comparison of means of three biological replicates was performed with Duncan's multiple comparison test and significance was determined at $P \leq 0.05$, 0.01 and 0.001.

Results

Species description

Brachythecium procumbens (Mitt.) A. Jaeger—Pleurocarpous plants are yellowish-green, somewhat glossy, slender to moderate sized, growing in dense tufts. Main stem—creeping, procumbent, giving rise to ascending branches irregularly which may or may not divide again. Leaves—erectopatent, imbricate, dense, concave and plicate, ovate-lanceolate with a long narrow acumen; leaf base—decussate and somewhat auriculate in shape; leaf lamina—1.2–1.4 × 0.6–0.8 mm, acumen 0.7–0.9 mm; leaf margin—smooth, dentate in the upper part and acumen. Costa—single, strong, covering 3/4 of lamina length. Apical and middle leaf cells—elongate, linear–rhomboid, 96–108 × 5–7 µm at apex, 72–87 × 5–7 µm at middle, basal cells less rectangular, 43–54 × 23–27 µm with inflated alar cells.

Catalase (CAT) activity

In the present study, desiccation treatment influenced the activity of CAT and an increment of 39.2% over control was observed during 24-h desiccation treatment (Fig. 1a). As we gradually increased the time of desiccation to 48, 72 and 96 h, it resulted in 25.5, 21.6 and 19.6% increment in CAT activity, respectively, as compared to control. However, in rehydrated samples only 21.6 and 3.9% increment in CAT activity was observed after 24- and 48-h desiccation. After that the activity decreased by 3.9 and 7.8% as compared to control (Table 1) at 72- and 96-h drought stress.

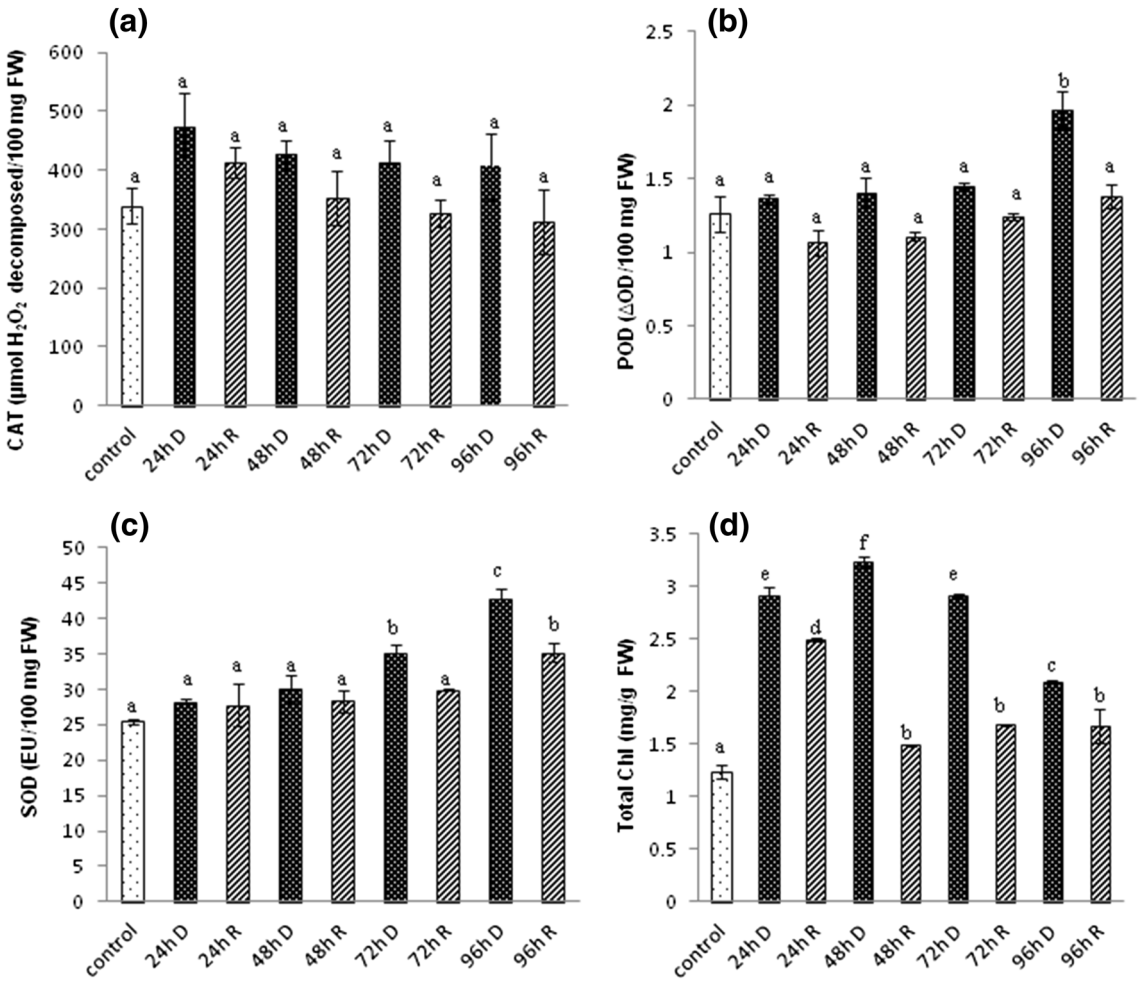

Fig. 1 Effect of dehydration (*D*) and rehydration (*R*) stress on the activity of antioxidant enzyme **a** catalase (CAT), **b** peroxidase (POD), **c** superoxide dismutase (SOD) and **d** total chlorophyll (total Chl) content in *Brachythecium procumbens* (Mitt.) A. Jaeger. Data are presented as mean values ± SE of three replicates and *data with different superscripts* are significantly different at $P \leq 0.05$, as determined by analysis of variance (ANOVA) followed by Duncan's multiple range test (DMRT)

Peroxidase (POD) activity

The data obtained from different desiccation treatments indicated an increment in the activity of POD during drying. As the duration of desiccation increased (24, 48, 72, 96 h) the activity of POD also increased by 8.7, 11.1, 15 and 56.4% respectively, over that of control plants (Fig. 1b), whereas subsequent rehydration showed about 15.1, 11.9 and 1.6% decrement during 24, 48 and 72 h. However, the values became 9.5% higher than control plants (Table 1) in the rehydrated samples after 96-h desiccation.

Superoxide dismutase (SOD) activity

SOD activity was also increased in desiccated plants as compared to control plants and maximum activity was observed at 96 h which was about 67.7% (42.79 EU/100 mg FW) higher than that of control (25.52 EU/100 mg FW), whereas only 10, 18 and 37.7% increment was observed during 24-, 48- and 72-h desiccation regimes (Table 1). Besides, SOD activity in rehydrated samples after 24–96-h desiccation was also increased by about 8.7, 11.1, 17.4 and 38% (Fig. 1c). Statistical study revealed that the correlation between POD and SOD was significant and positive in desiccated plants of *B. procumbens* (Fig. 2a) at $P \leq 0.05$ with r value 0.95.

Photosynthetic pigments

The effect of desiccation on photosynthetic pigments, viz., Chl *a*, Chl *b* and total Chl were also observed. Amount of Chl *a* was maximum at 48-h desiccation (116.7% higher than its control), whereas only 83.3% increment was observed at 24 h as well as 72 h. However, minimum increment (only 40.5%) in Chl *a* was noted at 96-h

Table 1 Effect of dehydration (D) and rehydration (R) stress on the activity of catalase (CAT), peroxidase (POD), superoxide dismutase (SOD) and total chlorophyll content (total Chl) in *Brachythecium procumbens*

Duration	CAT activity (μ mole H_2O_2 decomposed/100 mg FW)	POD activity (ΔOD/ 100 mg FW)	SOD activity (EU/ 100 mg FW)	Total Chl (mg/g FW)
Control	340 ± 30.59[a]	1.26 ± 0.12[a]	25.52 ± 0.36[a]	1.24 ± 0.07[a]
24-h D	473.33 ± 57.03[a] (+39.2%)	1.37 ± 0.03[a] (+8.7%)	28.07 ± 0.54[a] (+10%)	2.92 ± 0.08[e] (+135.5%)
24-h R	413.33 ± 26.69[a] (+21.6%)	1.07 ± 0.08[a] (−15%)	27.73 ± 2.98[a] (+8.7%)	2.49 ± 0.01[d] (+101%)
48-h D	426.67 ± 24.07[a] (+25.5%)	1.4 ± 0.10[a] (+11.1%)	30.09 ± 1.89[a] (+18%)	3.25 ± 0.05[f] (+162.1%)
48-h R	353.33 ± 46.72[a] (+3.9%)	1.11 ± 0.03[a] (−11.9%)	28.36 ± 1.54[a] (+11.1%)	1.49 ± 0.01[b] (+21%)
72-h D	413.33 ± 37.16[a] (+21.6%)	1.46 ± 0.024[a] (+15%)	35.03 ± 1.38[b] (+37.7%)	2.92 ± 0.02[e] (+135.5%)
72-h R	326.67 ± 24.06[a] (−3.9%)	1.24 ± 0.023[a] (−1.6%)	29.97 ± 0.15[a] (+17.4%)	1.69 ± 0.01[b] (+36.3%)
96-h D	406.67 ± 57.02[a] (+19.6%)	1.97 ± 0.13[b] (+56.4%)	42.79 ± 1.43[c] (+67.7%)	2.09 ± 0.01[c] (+70%)
96-h R	313.33 ± 54.63[a] (−7.8%)	1.38 ± 0.08[a] (+9.5%)	35.21 ± 1.33[b] (+38%)	1.68 ± 0.16[b] (+35.5%)

Values in parenthesis represent percent increment (+)/decrement (−) from control. Data are means ± standard error of three replicates. Different alphabets (superscript) are significantly different at $P \leq 0.05$, 0.01

desiccation. During evaluation of Chl *b* concentration in different drying durations, it had been observed that as we increased the desiccation time (24, 48, 72, 96 h) the value became 172.7, 167.3, 165.5 and 98.2% higher in comparison to control, whereas concentration of total chlorophyll subsequently increased at 48-h desiccation (Fig. 1d) from 135.5 to 162.1% as compared to control, but after that it showed only 70% increment during 96 h. Contrary to this, samples rehydrated after desiccation showed relatively low value of photosynthetic pigments than the desiccated plants. The concentration of Chl *a*, Chl *b*, and total Chl was maximum in rehydrated samples after 24-h desiccation and about 57, 127 and 101% higher than their control values (Table 1), respectively. Correlation studies between CAT and Chl *b* showed significant positive correlation in desiccated plants only (Fig. 2b) at $P \leq 0.05$. Besides, positive correlation was obtained between Chl *a*, Chl *b* and total Chl (Fig. 2c, d) which was significant for desiccated and rehydrated plants at $P \leq 0.05$, 0.01.

Chlorophyll stability index (CSI)

In reference to total chlorophyll content, CSI also showed maximum value in 48-h desiccated plants which was about 162% higher than control. However, lowest value of CSI had been reported in 96 h with only 69% higher value than control (Table 2). Correlation studies between CSI and total Chl were positive and significant at all the three probabilities ($P \leq 0.05$, 0.01, 0.001).Data are means ± standard error of three replicates. Different alphabets (superscript) are significantly different at $P \leq 0.05$, 0.01 and 0.001

Percent weight loss/gain (WL/WG)

As the duration of desiccation increased, the percent weight loss also increased and reached maximum at 96-h drying, whereas subsequent rehydrated samples also showed increment in the values during different time period (24, 48, 72, 96 h) and maximum weight gain was seen in the plants facing long desiccation of 96 h (Table 2). No correlation was obtained between Chl *a* and WL (%) (Fig. 2e), whereas positive correlation (Fig. 2f) was observed between Chl *b* and WL (%) which was significant at $P \leq 0.05$ with *r* value 0.89.

Relative water content (RWC)

Desiccation stress led to significant increase in the RWC in all the phases of desiccation (Table 2). Correlation studies between RWC and WL (%) were significant at $P \leq 0.05$, 0.01 and 0.001 (with *r* value 0.99).

Discussion

Water is the most important molecule for all physiological processes taking place in the cells of plants, being responsible for transporting all nutrients, macromolecules and enzymes. In mosses, the turgidity of the one-cell thick leaves is maintained due to the external water coating. In case, the external layer dries, these plants try to establish equilibrium with the surrounding atmosphere (de Carvalho 2013). However, under severe desiccation the ionic strength and pH, crystallization of solutes, increased lipid peroxidation and denaturation of protein are observed at the cellular level (Levitt 1980a, b). As a consequence of these deleterious effects, membranes break and cause leakage of solute. Desiccation-induced free radicals have been reported in plants including bryophytes, and in some cases desiccation tolerance has been correlated with maintenance/ synthesis of enzymes scavenging cytotoxic oxygen

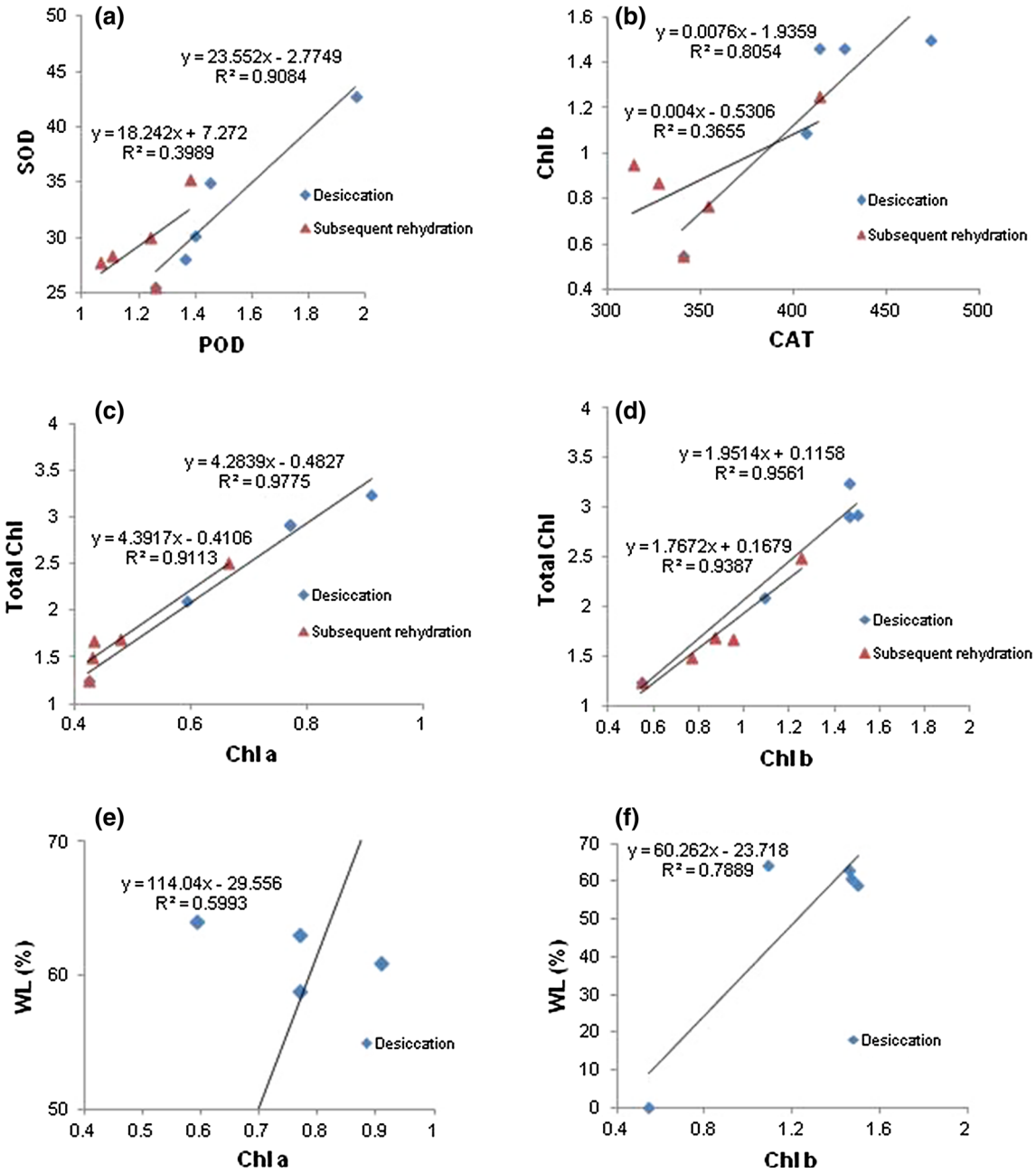

Fig. 2 Graphical representation of correlation studies in *Brachythecium procumbens* (Mitt.) A. Jaeger between **a** superoxide dismutase (SOD) and peroxidase (POD), **b** chlorophyll b (Chl *b*) and catalase (CAT) showing significant differences and positive value in desiccated plants at $P \leq 0.05$. Correlation study between **c** total chlorophyll (total Chl) and chlorophyll *a* (Chl *a*), **d** total chlorophyll (total Chl) and chlorophyll *b* (Chl *b*) was significant for desiccated as well as rehydrated plants at $P \leq 0.05$, 0.01, **e** no correlation between weight loss (WL %) and chlorophyll *a* (Chl *a*), **f** positively significant between weight loss (WL %) and chlorophyll *b* (Chl *b*) at $P \leq 0.05$

species as CAT, POD and SOD (Conteras-Porcia et al. 2011).

During our experiment, desiccation resulted in lowering of CAT activity, as has been reported in some higher plants under stress (Mukherjee and Choudhuri 1983; Schöner and Krause 1990). The extrachloroplastic enzyme CAT was ca. 1.3-folds more active in 24-h desiccated plants than their control plants, but after that desiccation resulted in its gradual decrement. Reduction in protein synthesis due to desiccation (Bewley 1972; Dhindsa 1987) might conceivably affect resynthesis and could be considered responsible for the substantial decrease in CAT activity. Our results indicated that CAT was not involved in overcoming stress as reported by Kang et al. (2012). Moreover, decline in CAT activity could be due to its sensitivity to drought (Jiang and Huang 2000), and to its photoinactivation

Table 2 Chlorophyll stability index (CSI), percent weight loss/gain (WL/WG) and relative water content (RWC) in *B. procumbens* during dehydration (D) and rehydration (R) stress

Duration	CSI	WL (%)	WG (%)	RWC
Control	99.99 ± 5.67[a]	–	–	–
24-h D	235.51 ± 6.18[e]	58.90 ± 3.68[a]	–	12.66 ± 1.71[c]
24-h R	201.48 ± 1.52[d]	–	17.80 ± 5.32[a]	4.39 ± 1.53[a,b]
48-h D	261.68 ± 3.92[f]	60.95 ± 4.16[a]	–	14.25 ± 0.83[c,d]
48-h R	120.72 ± 0.61[b]	–	18.57 ± 2.18[a]	4.82 ± 0.54[a,b]
72-h D	235.12 ± 1.87[e]	61.88 ± 1.05[a]	–	14.86 ± 3.62[c,d]
72-h R	136.06 ± 0.74[b]	–	21.76 ± 2.82[a]	5.54 ± 0.97[b]
96-h D	169.03 ± 1.15[c]	70.35 ± 1.47[b]	–	18.13 ± 1.45[d]
96-h R	135.12 ± 1.29[b]	–	23.86 ± 6.64[a]	6.67 ± 1.30[b]

Data are means ± standard error of three replicates. Different alphabets (superscript) are significantly different at $P \leq 0.05$, 0.01 and 0.001

(Feierabend and Engel 1986; Polle 1997). On the other hand, the activity of POD increased during desiccation. Similar results were observed by Paciolla and Tommasi (2003) for *Brachythecium velutinum, Marchantia polymorpha*, Barón et al. (2009) for *Racomitrium crispipilum* and Lubaina et al. (2013) for *Octoblepharum albidum*. In the present work, increment in the POD activity showed that this enzyme was induced due to dehydration, leading us to infer that $\cdot O_2^-$ and H_2O_2 were generated during desiccation (Platt et al. 1994). Apart from the normal function of POD, it is possible to explain the high level of POD activity in the mosses as the scavenging enzyme for the removal of H_2O_2, the ROS formed in the cell system as a consequence of desiccation stress for its survival or it may play a role in the general defence mechanism against external barriers (Francini et al. 2006). Besides, the SOD activity was also increased throughout dehydration, which indicated that more H_2O_2 was generated in this moss by SOD dismutation and required effective detoxification regime (Mayaba and Beckett 2003). Seel et al. (1992b) also examined the effects of desiccation on SOD activity in *Syntrichia ruralis* var. *arenicola* (=*Tortula ruraliformis*), and *Dicranella palustris* with limited desiccation tolerance. It has been reported by Fan et al. (2009), Liu et al. (2011) and others that species exposed to moderate and/or mild drought stress showed increasing activities of POD and SOD, whereas under severe drought stress, the activity of these enzymes was decreased.

In accordance with our results, the activities of antioxidant enzymes (CAT, POD and SOD) in the rehydrated plants also showed as decreased profile suggesting recovery from stress during rehydration. These results are in harmony with Oliver et al. (2005), who reported that *Tortula ruralis* has a remarkable capacity to rapidly recover its metabolism when rehydrated because protein synthesis recovers within minutes in response to rehydration. The desiccation-tolerant bryophytes on rehydration regain their normal metabolism (Csintalan et al. 1999) probably due to the rapid repair of the cellular damage (Oliver et al. 1993).

Similarly, photosynthetic pigments also serve as effective monitors for assessing the plant's photosynthetic status when under oxidative stress (Millan-Almaraz et al. 2009) as it maintains photosynthesis under drought stress and photosynthesis normally gets reduced before any decrease in chlorophyll content takes place (James et al. 2002). In our experiment a significant increase was observed in Chl *a*, Chl *b* and total Chl in the shorter periods of desiccation stress (24–48 h), but decreased under longer periods (72–96 h) of stress. These results are in harmony with those of Meenakumari et al. (2004) and Manivannan et al. (2007). Several other workers (Kranner and Grill 1997; Gholamin and Khayatnezhad 2011; Ashraf and Harris 2013; Ranganayakulu et al. 2015) also reported a reduction in chlorophyll content and photosynthetic rate as the result of drought stress. Drought stress inhibits chlorophyll biosynthesis enzymes; particularly 5-aminolevulinic acid synthetase (Bharadwaj and Singhal 1981) and according to Surendar et al. (2013), due to the decrease in the activity of antioxidative enzymes, substances that react with thiobarbituric acid accumulate and expedite the decrease of chlorophyll.

During the estimation of RWC, as the dehydration time increased, desiccation stress led to significant increase in RWC. Fu and Huang (2001) have reported decrease in RWC in response to drought stress. Terzi and Kadioglu (2006) also stated that RWC declined as the duration of drought increased but was not related to the length of the drought period. In accordance with our results, the value of RWC was highest at 96-h desiccation. Higher RWC is considered to be a relevant indicator of drought stress tolerance according to Kraus et al. (1995). The statistical comparison of the CSI and RWC also showed positive correlation which was significant. These results are in accordance with the findings of David (2002), where a positive correlation was observed between relative water

content and gas exchange activities thus, subsequently leading to reduction in photosynthesis, transpiration and stomatal conductance.

In conclusion, the protection of desiccated forms of our experimental plants appears to depend on the enzymatic dismutation of superoxides generated, as the activities of POD and SOD were high at all desiccated regimes. Therefore, the present study clearly demonstrated that POD–SOD system plays a vital role in the management of ROS under water stress in *B. procumbens*. Increased activity of SOD has been associated with ability to tolerate stress in plants, which can overcome the effects of treatments which produce and can also be correlated with amount of any superoxide radicals formed due to water scarcity (Clare et al. 1984). Desiccation stress enhanced POD and SOD activities, whereas CAT activity decreased significantly, inferring that POD had a greater ability to decompose H_2O_2 generated by SOD (Kang et al. 2012). Contrary to this, a higher CSI and RWC also enable the plants to withstand stress as higher chlorophyll increases rate of photosynthesis and consequently the productivity. Hence, it can be concluded that dehydration constitutes an organized series of physiological changes which may take place over a long period of time within the plant cells and forms an interesting field of study. This of course needs to be corroborated with the habit and habitat, especially pollution levels, to select plants for magnifying the bryoflora.

Author contribution statement PB performed the experiments, conducted all measurements and laboratory work, interpreted the results and statistical analysis and wrote the first version of the manuscript. AS designed the experiment, supervised its execution, discussed the results and edited the manuscript. All the authors read the manuscript and contributed substantially to its revision and approved the final manuscript.

Acknowledgements We thank the University Grants Commission (U.G.C.), New Delhi for financial assistance. This work was supported by the University Grants Commission [F.4-2/2006(BSR)/BL/13-14/0375].

Compliance with ethical standards

Conflict of interest All the authors contributed equally and declared that there are no conflicts of interest.

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