Abstract

Most plants are unable to survive desiccation to an air-dried state, but a small group of plants known as resurrection plants can tolerate extreme desiccation, and regain normal function after rehydration. Resurrection is the ability of an organism to survive the loss of more than 95% of its cellular water for extended periods and to recover all metabolic competence upon rehydration. Resurrection is widespread in plants, from lower bryophytes to higher vascular angiosperms in both vegetative and reproductive tissues. Himachal Pradesh, the hill state of India, lies in the foothill of Himalayas and harbors a rich and unique diversity of plants; ‘sanjeevani’ cited in the historic epic, Ramayana, was reported from the Himalayas. Resurrection plants have drawn parallels with ‘sanjeevani’.

In the present study, we explored eight different ferns and their allies for resurrection property based on the relative water content, and the ability to revive back upon rehydration. For this, seven ferns (Adiantum caudatum, Adiantum viridimontanum, Christella parasitica, Pteris vittata, Dryopteris arguta, Cheilanthes albomarginata, Onychium japonicum) and one clubmoss (Selaginella kraussiana) samples were collected from Solan and adjoining areas of Himachal Pradesh. Very surprisingly, only one fern namely Cheilanthes albomarginata showed resurrection potential. Analysis of the relative water content (RWC), an indicator of resurrection potential showed that C. albomarginata has highest RWC of 92 ±0.02 %, whereas Dryopteris arguta showed lowest RWC of 57 ± 0.03 %. After one month of desiccation, C. albomarginata fronds showed resurrection potential, in contrast to D. arguta. We further tested C. albomarginata fronds from ten different geographical regions of Himachal Pradesh. Interestingly, all the samples showed resurrection potential with RWC ranging from 77- 92%, and resurrection time ranging from 2-8 hours. Furthermore, we showed that mature fronds (sporulated stage), but not immature fronds (non-sporulated stage) of all the samples of C. albomarginata showed resurrection potential. Interestingly, C. albomarginata fronds collected from Nihog of Sirmour district showed resurrection activity within 2 hours and thus selected for further studies.

Recovery of chlorophyll is another indicator of resurrection potential. We measured chlorophyll levels during different stages of resurrection and compared it
between mature and immature fronds of *C. albomarginata*. It was observed that chlorophyll content was three folds higher in mature fronds as compared to immature *C. albomarginata* fronds. Moreover, the chlorophyll content of mature fronds during desiccation was reduced to 60 % and regained to ~95 % during rehydration as compared to the fresh stage. In contrast, the chlorophyll content of immature fronds during fresh, dry and rehydrated stages remained largely unaltered.

Since light plays a major role in chlorophyll biosynthesis, we studied the effect of light on chlorophyll recovery and resurrection potential. Very interestingly, more than 95 % chlorophyll was recovered in the presence of sunlight as compared to 30 % in the dark during rehydration/resurrection. The resurrection time was delayed to 6 h in the dark as compared to 2 h in the presence of sunlight.

The effect of temperature on resurrection potential showed that the decrease in temperature of water during rehydration to 15 °C increased the resurrection time to ~ 2.5 h, whereas increase in rehydration temperature to 80 °C decreased the resurrection time to 1 h.

To understand the role of metabolites during resurrection, we studied the role of sugar (trehalose), and metabolites (proline, and glycine betaine) as desiccation protectants. The profile of trehalose sugar during resurrection was investigated using TLC, HPLC, FTIR and enzymatic assays. Using the TLC and HPLC approaches, accumulation of trehalose during dehydration, and the disappearance of trehalose during rehydration was observed and confirmed by trehalase enzyme assays, which indicate that the accumulation of trehalose during dehydrated stage, and its utilization/ down-regulation during rehydration. Similarly, the levels of proline and glycine betaine were also analysed by quantitative colorimetric assays, and found to play a critical role in the resurrection. Both proline and glycine betaine accumulation was highest during dry stage and maintained during rehydration of *C. albomarginata* fronds. These findings indicate a protective role for proline and glycine betaine during resurrection.
The correlation analysis of physiological factors affecting resurrection potential indicated positive as well as negative correlation. It was found that RWC showed inverse correlation with both to the dehydrated condition and rehydrated condition whereas trehalose content in dry stage showed inverse correlation with RWC, and the altitude of the location. Similarly, during rehydration condition, trehalose content negatively correlated with RWC (the capacity to obtain full turgidity). The correlation analysis of stress amino acid proline did not indicate any correlation with any of the parameter during fresh stage, whereas during dry stage, accumulation of proline showed a direct correlation with RWC. During rehydration stage, proline content showed a negative correlation with resurrection time. GB content did not show any correlation during fresh stage, dry and rehydrated stages with any of the parameters studied.

To our knowledge, this is the first study to provide insight into the resurrection activity of *C. albomarginata* and its metabolic characterization.