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A REPORT ON RESURRECTION ACTIVITY OF THE FERN '*Cheilanthes albomarginata* clarke' FROM VARIOUS REGIONS OF HIMACHAL PRADESH, INDIA

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ABSTRACT

Drought is a form of desiccation state typified by a major loss in water content of the plant. Some plants called resurrection plants have a natural ability to tolerate drought stress. Resurrection plants are characterized by the ability to revive or becoming alive after seeming to be dead. In this study, seven different ferns and one clubmoss collected from Solan and adjoining regions of Himachal Pradesh were analyzed for resurrection property in excised fronds. Out of these, one fern namely *Cheilanthes albomarginata* exhibited resurrection activity. Of the eight ferns, relative water content was found to be highest for *Cheilanthes albomarginata*. Of the ten different locations of Himachal Pradesh studied, resurrection activity was detectable in all the samples of *Cheilanthes albomarginata*, with fastest resurrection in Sirmour sample. This study forms the first report of resurrection in *Cheilanthes albomarginata*, and the first study on the exploration of ferns of Himachal Pradesh for resurrection activity.

KEYWORDS: ***Drought stress, resurrection, desiccation, relative water content, ferns, clubmoss, Himachal Pradesh, Cheilanthes albomarginata***

INTRODUCTION

Adverse environmental conditions result in the loss of productivity and limit the range of habitats available to the plants. Additionally, urbanization and elevated population numbers are also a direct cause of increasing water scarcity worldwide, resulting in the loss of productivity of crop plants. The scarcity of water resources from the soil manifests as drought, a physiological form of water stress (Dinakar C et al. 2012). Drought affects the performance of plant and yields of crops, as water is a vital growth component (Yordanov I et al. 2000). Thus, it is imperative to understand the mechanism by which the plants can adapt to the water stress, and able to survive during the extreme drought. A unique class of plants called resurrection plants have the natural ability to tolerate drought stress (Lambers Hans F et al. 2008; Farrant Jill M and Moore JP, 2011; Dinakar C et al. 2012). These plants show adaptive mechanism against drought by adjusting osmotic pressure of the cell, thereby able to survive with very less amount of water as low as of 10% internal water content (Ingle Robert A et al. 2007; Toldi O et al. 2009). Upon dehydration, resurrection plants shrivel up and fold their leaves, until water is available (Farrant Jill M et al. 2007). Examples of some extensively studied resurrection plants include the bryophyte *Tortula ruralis* (Farrant Jill M & Moore JP, 2011), the club mosses *Selaginella lepidophylla* (Yobi A et al. 2013), *S. tamariscina* (Yobi A et al. 2012) and *S. bryopteris* (Pandey V et al. 2010; Deeba F et al. 2009), the dicots *Craterostigma plantagineum* (Lambers Hans F et al. 2008; Rodriguez M et al. 2010), *C. wilmsii* (Bartels D et al. 2011), *Boea hygrometrica* (Mitra J et al. 2013; Gechev Tsanko S et al. 2012), *Myrothamnus flabellifolia* (Gechev Tsanko S et al. 2012; Bartels D et al. 2011), the monocots *Xerophyta viscosa* (Abdalla et al. 2010), *X. humilis* (Bartels D et al. 2011), and *Sporobolus stapfianus* (Oliver M et al. 2011), and the fern *Polypodium vulgare* (Helseth et al. 2005). The ability of these

plants to survive in such fatal condition appears to be multigenic, involving participation of many sets of genes, proteins and metabolites (Dinakar C et al. 2013). The resurrection process in these plants may be one of the strategies to fight against the drought stress. The potential of the resurrection plants to tolerate the extreme conditions of dryness/desiccation can be used to engineer drought tolerant crops. Although resurrection phenomenon is reported across bryophytes to higher plants, pteridophytes, including ferns remain less explored for resurrection. Therefore, the present study was undertaken to identify and characterize resurrection process in ferns and their allies in Himachal Pradesh (H.P.), the hill state of India. Himachal Pradesh, lying at the foothills of Himalayas encompasses diverse vegetation communities and floral assemblages due to wide altitudinal gradient coupled with local variation (Thukral AK, 2012). The major types of vegetation found in the H.P. are tropical, subtropical, wet temperate, dry temperate, subalpine and alpine (Bhagat RM et al. 2009; Ramachandra TV et al. 2012). Around 30 species of ferns and fern allies were reported in H.P. (Thukral AK, 2012). Ferns collected from Solan and adjoining regions of H.P. belonging to 8 genera and one fern ally i.e. *Selaginella kraussiana*, were analyzed for resurrection property. Out of these, only one fern namely *Cheilanthes albomarginata* exhibited resurrection activity. The studies on characterization of resurrection of *C. albomarginata* are presented.

MATERIALS AND METHODS

Collection of ferns

Ferns were collected during the rainy and dry seasons from various regions of Solan and nearby areas, Himachal Pradesh, India. The identity of ferns was verified from herbarium of Punjab University, Chandigarh, India and University of Horticulture and Forestry, Nauni, Himachal Pradesh, India.

Assay for resurrection activity

Fronds were excised from the whole plant and subjected to drying and rehydration to check the resurrection activity. Excised fronds were dried at 40 °C for at least 30 days, and then rehydrated by submerging in beaker containing water. The resurrection activity is defined as the time taken by each frond to open and revive back in green colour (Farrant Jill M et al. 2007).

Determination of relative water content (RWC)

The fronds were weighed at the start (fresh weight) and at regular intervals during the drying time course (dry weight) and the water content was determined at the end of rehydration of fronds (turgid weight) (Gonzalez and Gonzalez-Vilar, 2001). The relative water content was calculated as the absolute water content at any stage in drying rehydration time course, relative to the absolute water content of fully hydrated fronds. RWC was calculated as follows:

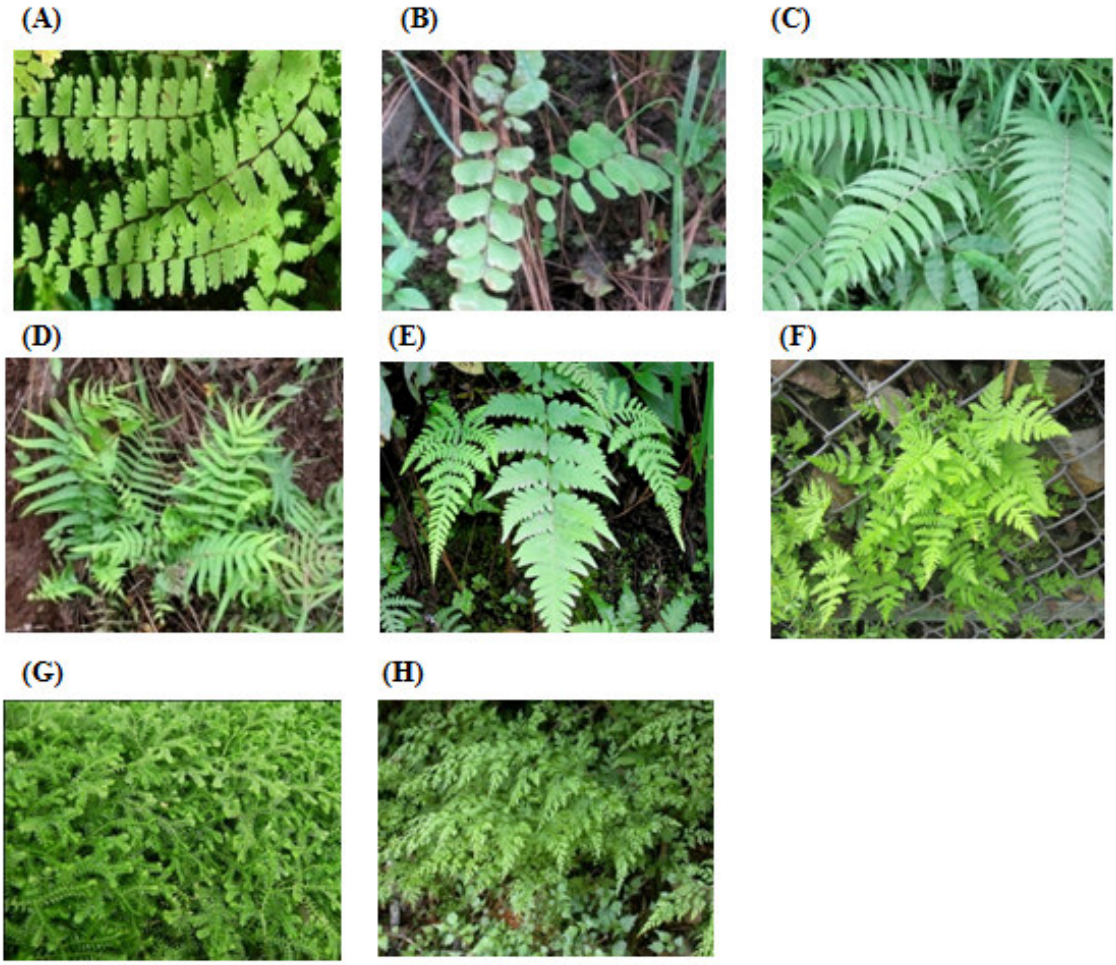
RWC = (FW-DW)/ (TW-DW) x 100

Where, RWC- Relative water content, FW- Fresh weight, DW- Dry weight, TW- Turgid weight.

RESULTS

Collection of various fern samples

The fronds from eight different fern varieties were collected from Solan and its nearby regions in the months of July to August and identified. The photographs of the ferns and club moss in their natural habitat are shown in Fig 1.



The pictures were taken from their natural habitat. The names of collected samples are as follows: (A) Adiantum caudatum (B) Adiantum viridimontanum (C) Christella parasitica (D) Pteris vittata (E) Dryopteris arguta (F) Cheilanthes albomarginata (G) Selaginella krassuiana (H) Onychium japonicum.

Figure 1
The photographs of ferns and club moss used in this study.

Determination of RWC of collected fern samples

RWC is the direct measure of the water content of the plant and thus an assessment indicator for desiccation tolerance in plants, which is an inherent feature of resurrection plants. Therefore, the RWC was measured for each fern sample. The RWC was highest for *C. albomarginata* (92 %) followed by the club moss, *Selaginella kraussiana* (89 %) (Table 1). Thus, RWC values indicated that *C. albomarginata* can survive with very little amount of cellular water, i.e., 8% (Table 1).

Table 1
The list of ferns and fern allies, collected from Solan and adjoining areas, their RWC and resurrection analysis

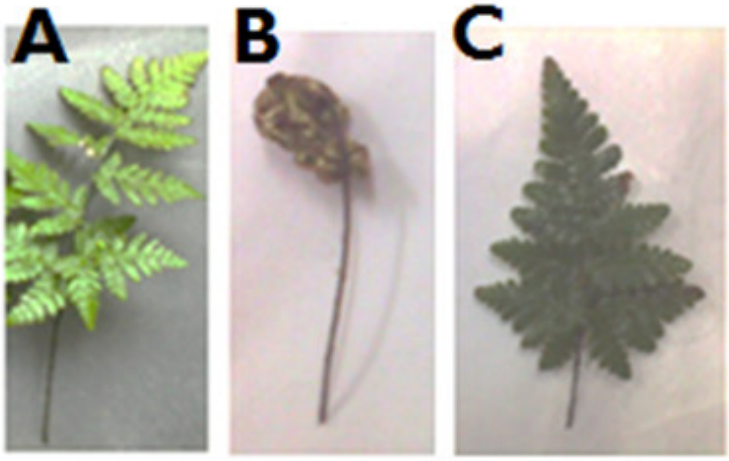
S. N.	Name of the plant	Family	Region	Altitude	(RWC ± SE)* (%)	Resurrection activity
1.	*Adiantum caudatum*	*Pteridaceae*	Bajhol, Solan	1502 m	81 ± 0.015	(-)
2.	*Adiantum viridimontanum*	*Pteridaceae*	Bajhol, Solan	1502 m	83 ± 0.01	(-)
3.	*Christella parasitica*	*Thelypteridaceae*	Bajhol, Solan	1502 m	73 ± 0.025	(-)
4.	*Pteris vittata*	*Pteridaceae*	Bajhol, Solan	1502 m	74 ± 0.01	(-)
5.	*Cheilanthes albomarginata*	*Pteridaceae*	Bajhol, Solan	1502 m	92 ± 0.02	(+)

6.	*Dryopteris Arguta*	*Dryopteridaceae*	Bajhol, Solan	1502 m	57 ± 0.03	(-)
7.	*Selaginella kraussiana*	*Selaginellaceae*	Bajhol, Solan	1502 m	89 ± 0.01	(-)
8.	*Onychium japonicum*	*Pteridaceae*	Bajhol, Solan	1502 m	79 ± 0.01	(-)

**** RWC values are average of two independent experiments. SE: Standard error***
(-) indicates the absence, while (+) indicates the presence of resurrection activity

Cheilanthes albomarginata exhibits resurrection activity

The fronds of seven fern and one club moss samples were analyzed for resurrection activity. It was found that only one fern namely, *Cheilanthes albomarginata* was positive for resurrection activity (Fig 2; Table 1). The resurrection activity is defined as the time taken by each sample to unfold and turn back again in green colour. As shown in Fig 2, the excised frond of *C. albomarginata,* which was tightly curled up after drying (Fig 2B), unfolded and turned back green in color upon rehydration (Fig 2C). On the other hand, none of the other seven samples exhibited resurrection activity (Table 1).



Mature fronds of C. albomarginata (A) were collected and used for assaying resurrection activity by complete dehydration (B) followed by rehydration (C). The fronds were photographed during the fresh, dry and rehydrated stages. A representative photograph of each stage is shown.
A: Fresh and mature frond of C. albomarginata
B: Mature frond of C. albomarginata after complete dryness
C: Mature frond of C. albomarginata after rehydration in water

Figure 2
Assay for the resurrection activity in excised fronds of C. albomarginata.

Analysis of resurrection activity in C. albomarginata collected from regions of H.P., India

Our results indicate that the excised fronds of *C. albomarginata* exhibit resurrection activity. This is consistent with desiccation tolerance behavior of *C. albomarginata* reported earlier. *C. albomarginata* is widely distributed through various regions of H.P. Therefore, resurrection activity was examined in ten different regions of H.P., adjoining Solan, which varied in their climatic conditions (hot/cold) and altitude (range from 373m-2455m) (Table 2). These include Bajhol, Sirmour, Dharampur, Subathu, Kasauli, Shoghi, Mandi, Nalagarh and Shimla to assess their resurrection potential, RWC was estimated for all the ten samples of *C. albomarginata.* As described in Table 2, RWC values varied from 77% (for Bajhol sample) to 92% (for Sirmour sample). Subsequently, the resurrection activity of the ten samples of *C. albomarginata* was analyzed. All the samples of *C. albomarginata* exhibited resurrection activity, albeit to different efficiencies (Table 2). The fastest resurrection was observed with the Sirmour sample (2 h), while the slowest resurrection with Dharampur and Shoghi samples (8 h) of *C. albomarginata*. These results indicate that resurrection potential is an inherent feature of *C. albomarginata.*

Table 2
Comparison of resurrection activity in fronds of C. albomarginata collected from various regions of H.P., India.

S. No.	Place of collection/ district	Altitude (m)	RWC (%)	Resurrection* time (h)
1.	Nalagarh/ Solan	372	83 ± 0.01	6
2.	Sarkaghat/ Mandi	911	80 ± 0.01	3
3.	Nihog/ Sirmour	932	92 ± 0.00	2
4.	Subathu/ Solan	1350	80 ± 0.01	6
5.	Dharampur/ Solan	1483	81 ± 0.02	8
6.	Bajhol/ Solan	1502	77 ± 0.01	4
7.	Kasauli/ Solan	1800	80 ± 0.04	5
8.	Shoghi/ Shimla	1851	85 ± 0.02	8
9.	Chail/ Shimla	2250	88 ± 0.03	4
10.	Shimla/ Shimla	2455	90 ± 0.03	6

**** Average of two independent experiments***

DISCUSSION

Resurrection is a process in which an organism revives after seeming to be dead or of seeming to revive when being in fact dead. The resurrection process is observed in all biological domains by various researchers. It can be said that this may be one of the strategy to fight extreme desiccation condition. The findings of present study revealed the presence of resurrection activity in the excised fronds of fern *Cheilanthes albomarginata* collected from various regions of Himachal Pradesh. *Cheilanthes albomarginata* belongs to the division pteridophytes and family pteridaceae (Anthelme F et al. 2011). It is also called as lip fern. The *Cheilanthes* genus comprises of about 180 species. At the ends of fronds, sporangia, or spore-bearing structures, are found that are protected by leaf margins, which curl over them (Anthelme F et al. 2011). The leaves, often densely covered in trichomes, spring directly from the rootstocks and contain a white coloured substance called farina on the ventral side. Many of them are desert ferns, curling up during dry times and reviving with the availability of moisture. Some members of the genus *Cheilanthes* have been reported to withstand drought (Kappen and Valladares, 2007; Anthelme F et al. 2011; Sprunt SV et al. 2011). However, there are no reports on the resurrection activity in *Cheilanthes albomarginata.* Thus, our study forms the first report of resurrection activity in excised fronds of *C. albomarginata*. The excised fronds curl up during the dry conditions, and revive back to original state upon addition of water, thus exhibiting resurrection. In present study it was found that *Cheilanthes albomarginata* collected from Sirmour region of Himachal Pradesh showed fastest resurrection activity. This could be correlated with the climatic conditions, wherein Sirmour region exhibits a hot climate.

CONCLUSION

In conclusion, we report for the first time, resurrection property of the fern *Cheilanthes albomarginata.* Similar to known resurrection plants, *Cheilanthes albomarginata* remains alive with very less amount of internal water content (~ 8%). Our study adds *Cheilanthes albomarginata* as an additional member to the expanding class of resurrection plants, which can be explored for traits that can be engineered in crop plant for drought tolerance.

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