Journal of Stress Physiology & Biochemistry, Vol. 7 No. 4 2011, pp. 415-423 ISSN 1997-0838

ORIGINAL ARTICLE

Chemo-protective effect of aqueous extract of the resurrection plant *Selaginella involvens* (Sw.) Spring on UV-tolerance during spore germination of *Pteris argyraea* T. Moore

Sathiyakumar C., Irudayaraj V. and Johnson M.*

Department of Plant Biology and Plant Biotechnology St. Xavier's College (Autonomous) Palayamkottai, INDIA- 627 002

*E-mail: ptcjohnson@gmail.com

Tel: + 91 97 86 92 43 34: Fax: + 91 46 22 25 619 87

Received October 26, 2011

The present study was aimed to know the effect of aqueous extracts of the resurrection plant, *Selaginella involvens* (Sw.) Spring on spore germination in *Pteris argyraea* T. Moore and also to know the ameliorating effect of the extracts on UV-Stress during spore germination of *Pteris argyraea* T. Moore. Based on the present study it is concluded that the extract of *Selaginella involvens*, shows growth promoting effect by enhancing the spore germination in *Pteris argyraea.* Both UV and aqueous extracts of *Selaginella involvens* enhances germination, but the UV stress results in both physical and morphogenetic abnormalities. It is to be noted that in the extract treated spores, the physical abnormalities are in less frequency when compared to the extract- untreated spores.

Key words: *resurrection plant; UV tolerance; Selaginella involvens;Pteris argyraea*

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Key words: resurrection plant; UV tolerance; Selaginella involvens;Pteris argyraea

Each and every organism on the earth require healthy environment for their survival. Today the living organisms on the biosphere, struggle a lot to live in the drastically changed environment. The global climate change is the major problem. The drastic increase in average temperature is mainly due to the green house gas effect and by the increase in intensity of Solar-UV radiation due to the depletion of Ozone filter in the atmosphere. At these extreme environmental conditions several species of plants survive by developing tolerance mechanisms. When exposed to enhanced UV-B radiation, many species of plants can increase the UV-absorbing pigments in their outer leaf tissues. Other adaptations may include increased thickness of leaves that reduces the proportion of inner tissues exposed to UV-B radiation and changes in the protecting waxy layer of the leaves. Several repair mechanisms exist in plants, including repair systems for DNA damage or oxidant injury. The net damage a plant experiences is the result of the balance between damage and protection and repair processes (Turunen and Latola, 2005). There are some UV-B-sensitive species / varieties of crops that experience reductions in yield.

In the meantime, there are also UV-B-tolerant species / varieties, providing the opportunity to utilize their tolerance mechanisms or chemical products. Different plants may synthesize different UV-absorbing pigments, particularly flavonoid pigments, in different quantities. Purified flavonoids naringenin and rutin as well as flavonoid extracts from apple skin prevented the accumulation of DNA damage (Kootstra, 1994). The primitive vascular land plants, 'Pteridophytes' have successfully evolved by facing several biotic and abiotic stresses. Some species of *Selaginella* (*S. involvens, S. wightii*, *S. lepidophylla*) are called resurrection plants due to their drought tolerant capacity. Various species of *Selaginella* synthesize various osmoprotectants like 'Trehalose-sugar' and UV protectants like 'Flavonoid-Pigments'. Several bioactive biflavonoids have been reported in many species of *Selaginella.* There is a recent report that the Indian herb '*Sanjeevani*' (*Selagnella bryopteris*) can promote the growth and protect against heat shock and apoptotic activities of ultra violet and oxidative stress. Sah *et al.,* (2005) have confirmed that the aqueous extract of *S. bryopteris* possesses growth-promoting activity as well as protective action against stress-induced cell death in a number of experimental cell systems including mammalian cells. Treatment of the cells in culture with 10% aqueous extract enhanced cell growth by about 41% in *Sf9* cells and 78% in mammalian cells. Pre-treatment of cells with the *Selaginella* extract (1–2Ч5%) protected against oxidative stress (H_2O_2)-induced cell death. The killing potential of ultra violet (UV) was also significantly reduced when the cells were pre-treated with S*elaginella* extract for 1 h. Thermal radiation suppressed cell growth by about 50%. Pre-treatment of cells with *Selaginella* extract for 1 h afforded complete protection against heat-induced growth suppression. *Selaginella* extract may possess anti-stress and antioxidant activities that could be responsible for such effects. In another case the protective effect of the leaf extract of *Gymnema sylvestre* R. Br. on UV-induced damage in *Salmonella typhi* has been reported (Rachh *et al.,* 2010). Thus it is clear that the various chemicals produced by the stress tolerant plants for photoprotection, chemoprotection and thermoprotection also have more or less the same protective mechanism in other organisms as confirmed by Sah *et al.,* (2005).

Tea catechins at levels 250 - 1000 mg/L- screened off UV-B resulting in less expression of chalcone synthase gene and lighter stress of the UV-B exposed *Arabidopsis thaliana* plants. The application of tea catechins was propitious to the recovery of the UV-B damaged plants, especially at concentration 250 mg L-1. The protective mechanism of tea catechins against UV-B stress is considered to be their sunscreen property instead of their free radical scavenging and antioxidant properties (Lee *et al*., 2008). Thus several plants have UV-stress adaptive mechanisms by producing several UV absorbing chemicals which may be effective on other plants and in human beings. The drought tolerant- resurrection plant *Selaginella involvens* has already been proved for its high degree of immunomodulatory, antioxidant and antilipid peroxidation activities, when compared to *Selaginella delicatula* and *Selaginella wightii* (Gayathri *et al.,* 2005).

The present study has been aimed to know the effect of aqueous extracts of the resurrection plant, *Selaginella involvens* (Sw.) Spring and non resurrection plant, *Selaginella inaequalifolia* (Hook. & Grev.) Spring on spore germination in *Pteris argyraea* T. Moore and also to know the ameliorating effect of the extracts on UV-Stress during spore germination of *Pteris argyraea* T. Moore.

MATERIALS AND METHODS

The plants, *Selaginella involvens* were collected from Upper Kothayar Hills, Kanniyakumari District, Tamil Nadu. The collected plants were air dried and powdered. From the dry powder, 10% stock solution was prepared by soaking 10g of powder in 100ml of distilled water for 24 Hrs. The filtered aqueous extract was used for further experimental studies. For spores, *Pteris argyraea* was also collected from the same locality. The dried spores were presoaked in different concentrations (0.00, 0.25, 0.5, 0.75 & 1.0%) of aqueous extract of *S. involvens*. One set of presoaked spores was irradiated with UV for 10 minutes. Both irradiated and non-irradiated extract-soaked spores were cultured in Half-strength MS medium and the germination was observed under the microscope.

RESULTS

The germination percentage of spores on 15th day has been given in Tables (1-2) and in Histogram (Fig. 3). The germination percentage in spores without UV treatment is comparatively higher in extract soaked spores than in water soaked spores. Thus the germination percentage is just 21 in the water soaked-UV untreated spores. In contrast, maximum germination percentage of 73 has been observed in spores soaked in 0.25% extract without UV treatment.

Table 1: Effect of aqueous extract of *Selaginella involvens* and UV stress (10 minutes) on spore germination in *Pteris argyraea* T. Moore (After 15 days)

Concentration of extract (%)	**Spore germination (%)**	
	Without UV treatment	**With UV treatment**
0.00	21	60
0.25	73	66
0.50	46	**91**
0.75	60	33
01.0	**77**	69

Table 2: Effect of aqueous extract of *Selaginella involvens* and UV stress (15 minutes) on spore germination in *Pteris argyraea* T. Moore after 7 days

Concentration of extract (%)	**Spore germination (%)**	
	Without UV treatment	**With UV treatment**
0.00	3.0	0.5
0.25	0.0	0.0
0.50	0.0	0.0
0.75	0.0	0.5
01.0	0.0	**5.0**

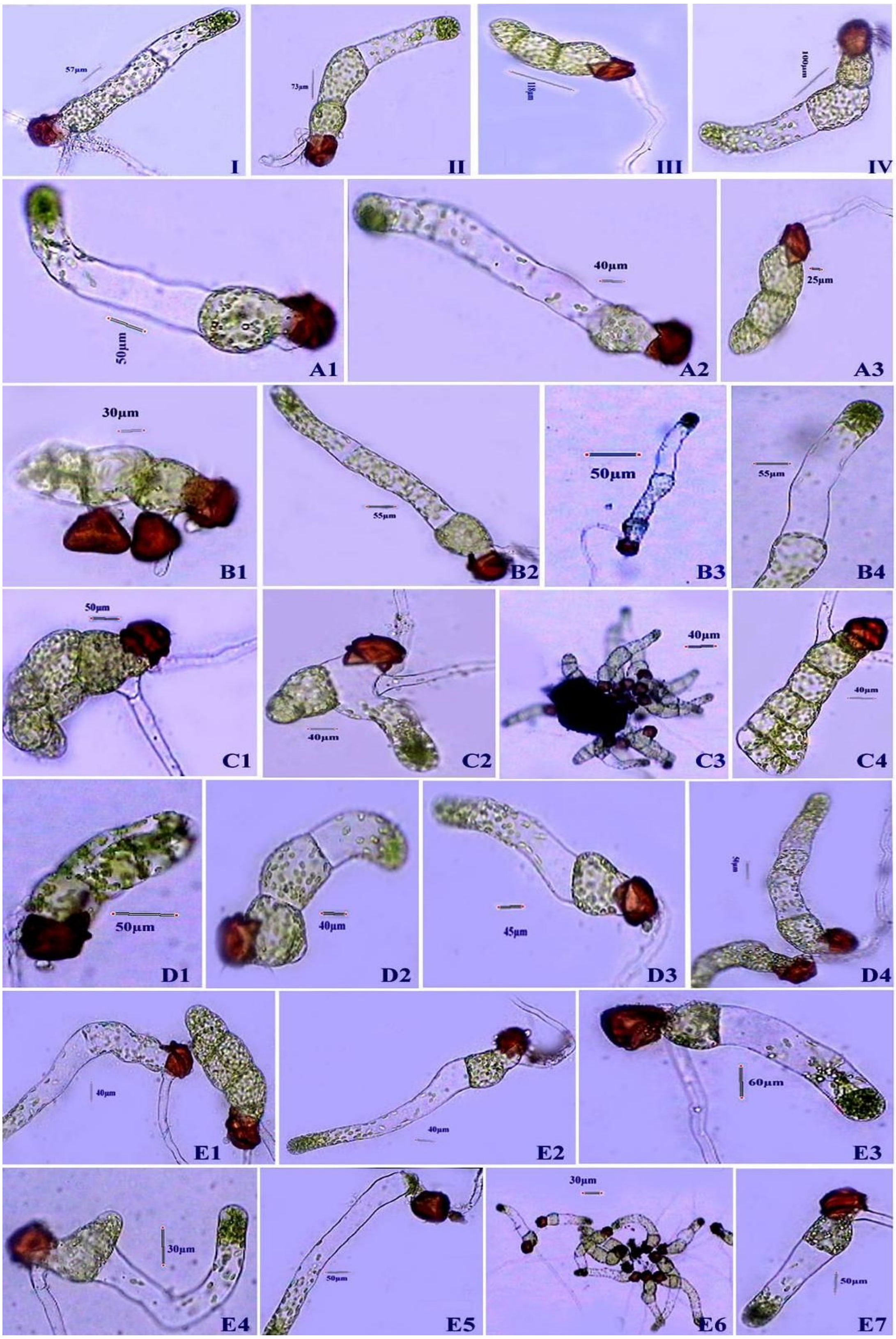

Figure 1. Effect of aqueous extract of *Selaginella involvens* on spore germination of *Pteris argyraea* and ameliorating the effect of UV Stress

Fig. A- E: Germination of pre-soaked and irradiated spores. (A1-3 - 0.00%; B1-B4 – 0.25%; C1-C4 – 0.5%; D1-D4 – 0.75%; E1 – E7 – 1.0%); Fig. I-IV: Germination of pre-soaked and non-irradiated spores (I – 0.0%; II – 0.5%; III – 0.75% and IV – 1.0%)

Figure 2. Germination of pre-soaked and non-irradiated spores (A – 0.0%; B – 0.5%; C – 0.75% and D – 1.0%)

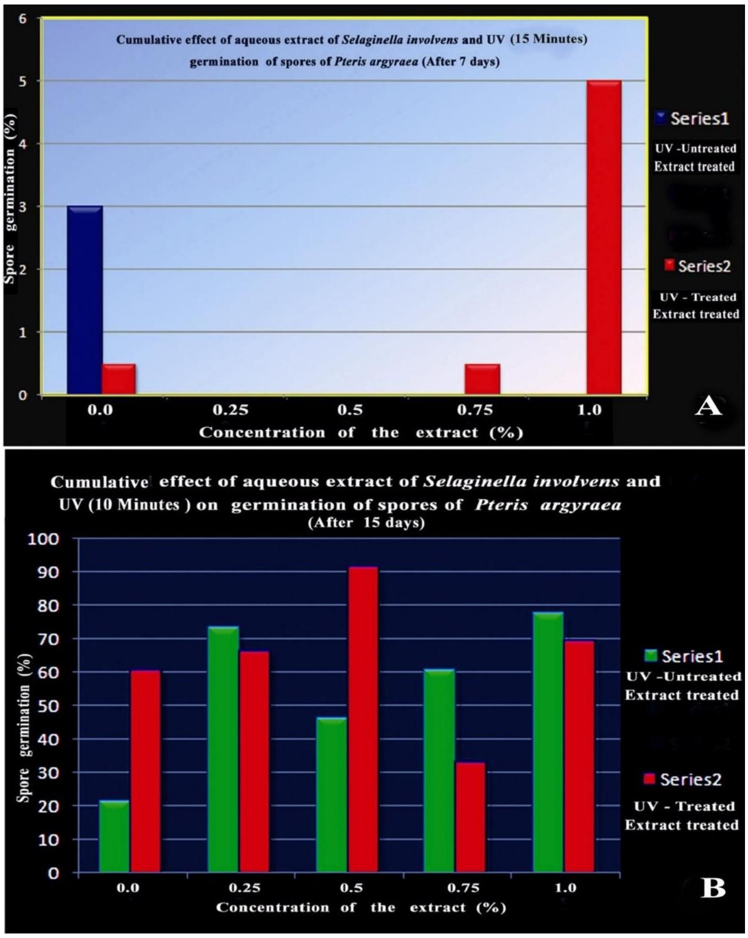

Figure 3. Effect of aqueous extract of *Selaginella involvens* on spore germination of *Pteris argyraea* and ameliorating the effect of UV Stress

In the case of UV and extract treated spores, both higher and lower germination percentage have been observed, in comparison with UV untreated spores. Even in water soaked spores, the UV treatment has enhanced the germination from 21% to 60%. Interestingly, both UV and 0.5% extract treatment drastically enhanced the spore germination from 46% to 93%. All the other lower and higher concentration of the extract slightly decreases the percentage of spore germination in the presence UV treatment. But the extract of *S. involvens* in all the concentration drastically increased the germination without UV treatment (Fig. 1 and 2).

The experiment was repeated with the concentrations 0.0, 0.25, 0.5, 0.75 and 1.0 % of the extract along with fifteen minutes of UV irradiation. On seventh day, there was only 3% spore germination in spores without UV treatment and without extract treatment. In all the other cases of spores treated with extracts but not with UV there is no germination at al. But in the case of UV treated spores, maximum germination of 5% has been observed in 1% extract treated spores (Table 1 and 2, Fig. C). After twenty days, the germination was observed. The percentage of spore germination was comparatively higher in UV and extract treated spores when compared to spores with UV treatment only.

DISCUSSION

From this experiment it is concluded that the extract of *Selaginella involvens* and UV irradiation, stimulate the germination of spores of *Pteris argyraea,* both individually and also in combination. Enhancement in germination of UV treated seeds has also been reported (Kovacs and Keresztes, 2002). Safflower (*Carthamus tinctorious*) seeds with artificial UV-B radiation caused changes in seed germination and seedling growth. In all treatments, UV light sped the germination of these seeds but the subsequent growth of the seedlings was markedly retarded. The hypocotyls become quite short and there is less above shoot biomass (Farokh *et al.,* 2010). As suggested for the rapid germination of UV treated seeds, the higher percentage of germination and higher rate of germination of spores of *Pteris argyraea* T. Moore by UV treatment, in the present study, may be probably due to the fact that UV-B photons (280-320 nm) are more energetic than visible light photons (>400 nm) and hence, have a stronger effect on the surface of plant cells (Kovacs and Keresztes, 2002). This causes the ultimate breakdown of spore wall allowing germination to occur.

But the UV irradiation of the spores resulted in several abnormalities during spore germination. Usually the UV irradiation affects the cell division of the germinating spores. In majority of the cases the protonemal cells become very long and narrow without cross wall formation. The chloroplasts accumulate towards the tip of the terminal cell. The UV radiation also induces the protonema to produce additional protonema in the direction of rhizoids.

Palta and Mehra (2004) have studied the morphogenetic effects of X-Ray on gametophytic generation of the fern *Pteris vittata* L. The X-ray radiation has resulted in the following abnormalities: giant-cell formation, inhibition of cell division and rhizoidal differentiation, effects on spore polarity resulting in ‘rhizoidal protonemata’, ‘protonematic rhizoids’, ‘twin protonemata’ and change in the site of rhizoid formation, degenerative changes in the cytoplasm, and the production of albinos and partial albinos, formation of tumors, and other morphological abnormalities. More or less all the above abnormalities have also been observed during spore germination of *Pteris argyraea* due to UV irradiation.

In the present study also the effect of UV radiation on spore germination resulted in the formation of long, narrow and vacuolated cells without the formation of cross wall and thus indicating the disruption of cell division due to UV radiation. Effect of ultraviolet radiation on cell division and microtubule organization in *Petunia hybrida* protoplasts has been studied by Staxйn *et al.,* (1993). They have found that UV radiation induced breaks in cortical microtubules resulting in shorter fragments with increasing dose. Also, the protoplasts were delayed in their progression through the cell cycle, with G1 and G2 phases being affected as well as the S phase.

Guiragossian and Koning (1986) induced the spore germination in the fern *Schizaea pusilla* by physical and chemical factors. Spores given red light reached half- maximum germination six days earlier than spores under white light. Red light promoted germination while blue light did not. Far-red alight alone could stimulate germination and enhanced the promotive effect of red light. Blue light reduced the effect of red light. Germination of UV-B irradiated spores of the intertidal green alga *Ulva pertusa* Kjellman was significantly lower than in unexposed controls, and the degree of reduction correlated with the UV doses (Wiencke *et al.,* 2006).

In the present study the growth promoting effect and chemophotoprotective effect of aqueous extract of *Selagnella involvens* (Sw.) Spring against UV stress during spore germination in *Pteris argyraea* T. Moore were studied. The extract treatment was given by soaking the spores in the extracts. The germination percentage in spores of *Pteris argyraea* without UV treatment was comparatively higher in extract soaked spores than in water soaked spores. Thus the germination percentage was just 21 in the water soaked-UV untreated spores. In contrast, maximum germination percentage of 73 has been observed in spores soaked in 0.25% extract without UV treatment.

In the case of UV and extract treated spores, both higher and lower germination percentage have been observed, in comparison with UV untreated spores. Even in water soaked spores, the UV treatment has enhanced the germination from 21% to 60%. Interestingly, both UV and 0.5% extract treatment drastically enhanced the spore germination from 46% to 93%. All the other lower and higher concentration of the extract slightly decreases the percentage of spore germination in the presence UV treatment. But the extract of *S. involvens* in all the concentration drastically increased the germination without UV treatment. From this experiment it is concluded that both the extract of *Selaginella* and UV irradiation, stimulate the germination of spores of *Pteris argyraea,* both individually and also in combination. But the UV irradiation of the spores resulted in several abnormalities during spore germination. Usually the UV irradiation affects the cell division of the germinating spores. In majority of the cases the protonemal cells become very long and narrow without cross wall formation. The chloroplasts accumulate towards the tip of the terminal cell. The UV radiation also induces the protonema to produce additional protonema in the direction of rhizoids. It is concluded that the extract of *S. involvens*, show growth promoting activity by enhancing the spore germination in *Pteris argyraea.* Both UV and extract of *Selaginella* enhances germination, but the UV stress results in both physical and morphogenetic abnormalities. It is to be noted that in the extract treated spores, the physical abnormalities are in less frequency when compared to the extract- untreated spores.

The present study along with previous reports show that, the sub lethal dose of UV radiation on plants induces several physiological activities to tolerate and cope with the new environment ultimately resulting in the increase of total dry weight along with the increase in quantity of several useful secondary metabolites. Thus UV radiation should be considered as bad master and good servant to the plants. So in order to understand clearly the physiological activities of the present species of *Selaginella,* detailed phytochemical studies along with detailed studies on physiological activities on other plants should be carried out in future by remembering the real fact that UV radiation is bad master but good servant.

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