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Effect of temperature on tissue raised shoots of *Swertia chirata* Buch.-Ham. ex Wall: An endangered medicinal plant

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ABSTRACT

Effect of temperature was studied on shoot cultures ofSwertiachirataat normal(25±1 ^{0}C) and low (17^{0}C) temperature levels. Anthocyanin production was stimulated by low temperature and the observations in vitro bore striking similarityto the pigmentation in naturally growing plants of S. chirata in winter season. A decrease in culture room temperature resulted in significant decline in shoot multiplication rate. The observations highlighted the necessity of optimal temperature conditions for efficient in vitro shoot multiplication, production of healthy shoots and probable role in pharmacological property of this important medicinal plant.

Keywords: *Swertiachirata*; in vitro multiplication; cold-acclimation; anthocyanin; pigmentation
Abbreviations: ***MS:*** *Murashige and Skoog (1962);* ***BAP:*** *6, Benzylaminopurine;****Kn:*** *6-Furfurylaminopurine;* ***NAA:*** *α-Naphthalene Acetic Acid;* ***IBA:*** *Indole-3 butyric acid;* ***IAA****: Indole-3 acetic acid;* ***GA$_3$****: Gibberellic acid;* ***Ads:*** *Adenine sulphate*

INTRODUCTION

Foliar pigmentation is a phenomenon observed in a great diversity of plant species across a broad range of environments. This may be attributed to accumulation of anthocyanins often occurring in response to environmental stresses such as nutrient deficiency, drought, low temperature, high light or pathogen attack [1-4]. Anthocyanins are a group of water-soluble flavanoids (glycosides of phenolic aglycans with a flavan C6-C3-C6 skeleton) produced in the cytoplasm and then transported into vacuole [5,6]. Many plant species from temperate regions are able to increase freezing tolerance when exposed to low, non-freezing temperatures in a process called *Cold-Acclimation* (CA). Physiological and biochemical analysis of CA process have revealed that low temperatures induce changes in lipid, protein and carbohydrate composition leading to production of anhocyanins[7-9]. Because CA requires the plant to adjust to a combination of light and low temperature [10] and because photosynthesis is one of the first processes adversely affected by low-temperature exposure [11], it is speculated that anthocyanins as photoprotectant could play an important role in the acquisition of freezing tolerance.

*Swertiachirata*is a pharmaceutically important medicinal plant prized as a cure for an array of ailments viz. chronic fever,anemia, bronchial asthma and liver disorders. The plant finds mention in the Ayurvedic system of medicine, British and American pharmacopoeias. It is an indigenous species of temperate Himalaya, found at an altitude of 1200-3000 m (4,000-10,000 ft.) from Kashmir to Nepal[12]. In Bhutan and Khasi hills it grows at an altitude of 1200-1500 m whereas in Sikkim it is found along 1500-3000 m [13-18]. *S. chirata* can be grown in sub-temperate

regions between 1500 and 2100 m altitudes [19] and is also found in the mountainous regions of Africa, Europe and North America.

S.chirata is primarily utilized for its bitter bioactive compounds including amarogentin, xanthones, iridoid glycosides [20,21], mangiferin and C-glucoflavones[20,22]. The whole plant contains gentiamine alkaloids and the aerial part contains xanthones[23]. The bitterness, antihelmintic, hypoglycemic and antipyretic properties are attributed to amarogentin (most bitter compound isolated till date) [24,25], swertiamarin and other active principles of the herb [26].The plant also contains raisins, tannins, gums, carbonates, phosphates and 4-6 % ash [27].

Since*Swertiachirata*is a pharmaceutically- rich high altitudinal plant species, it can be speculated that low temperature would be playing an important role in plant development and morphogenesis. Henceforth,the present study was undertaken with the objective to monitor the effect of temperature variation on *invitro* shoot culturesof *Swertiachirata*.

EXPERIMENTAL SECTION

In our previous study on *S.chirata* [28]*in vitro* shoots were induced from nodal explants on full strength MS medium and multiplied on MS medium supplemented with 4.44 µM BAP + 2.85 µM IAA + 271.45µM Ads. Multiplied shoots were sub-cultured and maintained on full strength MS medium [3% sucrose, 0.7% agar (w/v)] supplemented with 4.44 µM BAP. The pH of medium was adjusted to 5.8 before adding agar and sterilized by autoclaving at 15lbs (1.8 kg/cm^2) pressure at 121^{0}C for 15 minutes. Cultures were incubated in culture room at 60 - 65% relative humidity under a 16 / 8 hr (light / dark) photoperiod with light supplied by cool-white fluorescent tubes (Philips, India), at an intensity of 50 µmol m^{-2} s^{-1}.

In their natural environment, plants usually experience temperatures which fluctuate widely, especially between day and night. To expedite the growth and morphogenesis *in vitro*, cultures are generally maintained at mean temperatures which are higher than those which would be experienced by the same plants growing *in vivo*. The average constantgrowth room temperature employed in a large sample of experimental reports is 25 ± 2^{0}C. In the present study, the effect of temperature on *in vitro* shoot multiplication was investigated at 25±1^{0}C and 17^{0}C, respectively. Observations pertaining to average number of shoots and average shoot length were recorded after a period of 4 weeks. The experiments were repeated thrice and each treatment consisted of minimum ten replicates. The data was analysed using analysis of variance (ANOVA) of Completely Randomized Design (CRD) in GenStat 5 Edition 3.2 for PC/Windows NT **(**Copyright 1995, Lawes Agricultural Trust (Rothamsted Experimental Station)). The significance level was determined at 5 % ($p < = 0.05$), 1% ($p < = 0.01$) and 0.1% ($p < = 0.001$). Mean values of treatments were compared with Least Significance Difference (LSD).

RESULTS AND DISCUSSION

The study revealed that shoot multiplication was adversely affected with decrease in temperature. A maximum of 8.70 shoots after 4 weeks and 13.90 shoots after 8 weeks was recorded at low temperature (17^{0}C) which was significantly lower than an average of 11.80 and 18.50 shoots obtained at normal culture-room temperature of 25±1^{0}C (Table I, Figure 1 B,C). The decline in length of multiplied shoots was also proportional to decline in temperature. At normal culture-room temperature an average shoot length of 1.94 cm and 2.55 cm could be obtained after a period of 4 weeks and 8 weeks, respectively while at low temperature the mean shoot length attained was restricted to 0.87 cm (after 4 weeks) and 1.26 cm (after 8 weeks). Besides, cultures maintained at a temperature of 17^{0}C for a period of 10 hours exhibited purplish-red pigmentation of shoots, stunted growth and the leaves were observed to have expanded lamina (Figure 1B). The observations are similar to the pigmentation observed in plants of *S. chirata* growing in their natural habitat (Chakrata about 2,300m altitude, district Dehradun Uttarakhand) in cold season (December-February) (Figure 1 A). Suboptimal temperatures experienced either as sudden short-term cold spells or long term seasonal reductions in temperature have been reported to induce *denovo* anthocyanin synthesis resulting in purple/red leaves in nature [29-31]. In the present study, anthocyanin pigmentation in *in vitro* growing shoots of*Swertiachirata*, inducedby a short-spell of low temperature, was similar to the observations in nature in winters. This is also in affirmation with previous studies on *Arabidopsis* where rapid acclimation in response to low temperature was reported [32-33].

Enhanced freezing tolerance is reported to be reversible and can be lost within some days after plants are returned to higher temperature [34]. In consonance to this observation, our study revealed that on reverting the temperature conditions from 17^0C to 25^0C; *in vitro* shoots started regaining normal green pigmentation after a span of 40 days. However, the observations on average shoot number and average shoot length after a period of 4 weeks and 8 weeks suggested that *in vitro* shoot multiplication in cultures given a short-term cold stress of 17^0C was hugely impaired as compared to the shoot multiplication recorded in cultures maintained at optimal temperature of 25^0C. This is in corroboration with studies which suggest that low temperature decreases plant growth rate and prolongs the cell cycle [35-36].

Conclusively, a normal culture room temperature is imperative for optimal *in vitro* shoot multiplication in *S. chirata* which declines significantly with a reduction in temperature.

The study also calls for further studies on biochemical pathways induced by temperature stress in medicinal plants and subsequent effect on active chemical constituents of this valuable medicinal plant. The results thereof shall also be of interests to pharma and drug manufacturing companies.

Table I Effect of temperature on *in vitro* shoot multiplication of *Swertiachirata*

Treatment	MS medium + 4.44 µM BAP + 2.85 µM IAA + 271.45 µM Ads	Mean shoot number		Mean shoot length (cm)	
	Temperature used for maintaining cultures	After 4 wks	After 8 wks	After 4 wks	After 8 wks
1.	25^0 C	11.80	18.50	1.94	2.55
2.	17^0 C	8.70	13.90	0.87	1.26
Grand Mean		10.25	16.20	1.40	1.90
Significance		*	*	***	***
LSD		3.19	4.40	0.36	0.43

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Figure 1 Effect of temperature on in vitro shoot multiplication. MS medium was supplemented with 4.44µM BAP + 2.85 µM IAA + 271.45 µM Adenine Sulphate
(A) Pigmentation in *S. chirata* observed in nature
(B) In vitro shoot multiplication and pigmentation at 17^0C
(C) In vitro shoot multiplication at 25^0C