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Comparative Study Of Saussurea Costus Using Kinetin Hormone

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ABSTRACT

Saussurea costus is a high value of medicinal plant facing the pressure of commercial exploitation in Himalayan region. Therefore, is a need for the development of reliable seed germination protocol of the species. In the present investigation, the seeds were potent and the phytohormone (kinetin) showed better response for the propagation of the species. The findings study showed that the best suitable temperature for in vitro seed germination of Saussurea costus is 35°C. The given temperature has the highest mean value of germination percentage ie., 21.66 ± 2.72. In case of in vitro germination via MS medium, the seeds without any Kinetin treatment showed best results as compared to those seeds which were provided kinetin treatment at different time intervals. The seeds from control has the highest mean value of germination percentage i.e., 79 ± 0.47. The findings of the present study may be helpful to the germination of this valuable plant of Himalayan region.

Keywords: Saussurea costus, phytohormone (kinetin), germination

INTRODUCTION

Medicinal plants have been a resource for healing in local communities around the world for thousands of years. Still, it remains of contemporary importance as primary healthcare mode for approximately 85% of the world's population (Pesic, 2015) and as a resource for drug discovery, with 80% of all synthetic drugs deriving from them (Bauer & Bronstrup, 2014). Concurrently, the last few hundred years has been a prolific rise in the introduction, development, and advancement of herbal substances analysis. Humans have been identifying and selecting medicinal plants and foods based on organoleptic assessment of suitability and quality for thousands of years, but it is only in the span of the last seven decades since the invention of basic analytical techniques, e.g, paper chromatography, that has seen rapid development from sight, touch, and smell to using sophisticated instrumentation (Fitzgerald et al., 2020).

A medicinal plant is any plant that, in one or more of its organs, contains substances that can be used for therapeutic purposes or which are precursors for the synthesis of useful drugs. Several plants have been used in traditional medicine for many years (Sofowora et al., 2013).

The *Saussurea*, a genus of high-altitude medicinal plants in the Himalayan region, includes about 433 species which is named after a Swiss philosopher Horace Benedict de Saussure (1740-1799) (TPL, 2018). Around 27 species of *Saussurea* are known to have medicinal uses in diseases like cough, leucorrhea, ulcer, liver and heart problems, etc. (Butola and Samant, 2010). It is distributed throughout the Holarctic regions: Asia,

Europe, and North America but found concentrated in Asia (Kita et al., 2004). *Saussurea* species are habitat-specific, distributed in a narrow geographical range, and most of them are in the Himalayas at high altitudes. The maximum diversity of *Saussurea* DC. Reported from the Sino-Himalayan region, however, this genus extended its distribution to Europe, Asia, and North America (Chopra and Vishwakarma, 2018). Indian boundary encompassed 61 species and most of these species restrict their distribution to Alpine Himalayas (Pusalkar and Singh, 2009) and also evident that 37 species have been reported from the Himalayas (Chopra and Vishwakarma, 2018).

Saussurea costus (Falc.) Lipschitz, syn *Saussurea lappa* C.B. Clarke, one of the best-known species within this genus, is commonly known as costus in English and has different vernacular names in India like Kut (Gujarati), Kur (Bengali), Postkhai (Kashmiri), Sepuddy (Malayalam), Kot (Punjabi), Kushta (Sanskrit), Kostum (Tamil), Kustom (Telugu), Kushta (Marathi), Koshta (Kannada) and Kuth (Hindi) (Kirtikar and Basu, 2001). *Saussurea costus* (root oil and roots) has become an important drug in the international market.

Saussurea costus is a perennial rhizomatous herbaceous plant, commonly called just had a wide application in drug discovery programs and is traditionally known for its medicinal usage in the Indian system of medicine. The wide distribution of *S. costus* is recorded from the Himalayas and Indo-Myanmar hotspot biodiversity region, however, the majority of the population of *S. costus* is restricted to Western Himalayas in India, China, and Pakistan (Butola & Samant, 2010). This species is found growing in close association with Betula forests on hill slopes of the Himalayas and grows well in the moist slopes between the elevation ranges of 2000-4000m MSL (Hajra et al., 1995). According to Chopra and Vishwakarma (2018), this species is reported naturally growing in Rajouri of Jammu, Bhaderwah of Doda, Pir Panjal Mountain range of Shopian, Kishanganga valley of Kashmir, Chenab valley, and Suru valley of Ladakh, Nanda Devi National Park, and Valley of Flowers National Park in Uttarakhand and Church in Himachal Pradesh. In India, Butola and Samant (2010) reported the cultivation of *S. costus* in Himachal Pradesh and Uttarakhand, and in addition to these, Kokate et al. (2002) reported this species cultivated in Tamil Nadu and Uttar Pradesh to meet the commercial demand of the market. Kuniyal et al. (2005) indicated Indo-China and Vietnam are also places for commercial multiplication of this species on a large scale for industries and pharmaceutical sectors.

2. MATERIALS AND METHODS

2.1 Seed Collection

The seeds were provided by the department for the comparative study of *Saussurea costus*. A total of 315 seeds were taken into consideration. The given seeds were divided into two categories:

(i) For seed germination via soil (180 seeds)
(ii) For seed germination via MS medium (135 seeds)

2.2 Glassware and Apparatus

Different laboratory apparatus and glassware were used for in-vitro seed germination, comprising test tubes, beakers, measuring cylinders, flasks, Petri dishes, forceps, spatula, electronic weighing machine, hot plate stirrer, and horizontal laminar flow cabinet. Before the experiment, all glassware was thoroughly brushed with mild liquid detergent and washed up with normal tap water. After air drying, all glassware were incubated in a bacteriological incubator for about 30 minutes at 80 °C. Along with the glassware, were kept the cotton plugs for test tubes and flasks, prepared from non-absorbent cotton wrapped in muslin cloth.

2.3 Medium for growth

Two different media were selected for the experiment.

(i) Mountain soil (autoclaved at 121°C, 15 p.s.i., for 20 minutes)
(ii) MS medium (autoclaved at 121°C, 15 p.s.i., for 25 minutes)

2.4 Method for soil germination

The experiment began on the 6th of May, 2022. For germination via soil, 180 seeds were taken into consideration. The seeds were then categorized into three sets for different temperatures. The temperatures were

(i) 4°C – via refrigeration
(ii) 25°C – via seed germination chamber
(iii) 35°C – via seed germinator

All seeds were first thoroughly washed with Tween 20, a mild detergent, and then treated with Crosstin, a systemic fungicide for about 20 minutes. Then, they were again thoroughly washed with distilled water.
A set of nine Petri plates were taken for the experiment. Each category of temperature was given three Petri plates with a total of 60 seeds i.e., 20 seeds per Petri plate.
Seeds were kept in these petri plates containing moistened Whatman No. 1 and then labeled according to the category of temperature mentioned above.
After 7 days, the seeds were observed.

Seed	in 4°C	in 25°C	in 35°C
Saussurea costus	0/60	11/60	12/60

Again after 7 days, the seeds were observed.

Seed	In 4°C	In 25°C	In 35°C
Saussurea costus	0/60	12/60	13/60

After the completion of two weeks, the root lengths, as well as shoot lengths of seedlings, were taken.
After the measurements were taken, these replicates were transferred into small disposable cups containing autoclaved mountain soil.

Calculations:

(i) Germination percentage: $\frac{\textbf{Number of germinated seeds}}{\textbf{Total number of seeds taken}}$ **X 100**

(ii) Mean germination time (MGT) was calculated by the equation,

$$\mathbf{MGT = \sum (n_i \times d_i) / N}$$

Where n_i = number of seeds germinated at day i
d_i= number of days from the beginning of the experiment
N = total number of seeds germinated in the experiment

The seeds germinated through seed germination in the petri plate method did not show a significant increase in seed germination. So, further, the study was conducted in MS medium through the treatment of kinetin hormone to increase the germination percentage.

2.5 Method for germination via MS medium

The experiment began on the 14th of May, 2022. For in vitro germination, 135 seeds were taken into consideration. Kinetin, a type class of plant hormone cytokinin, was selected for the experiment. The seeds were then categorized into five sets for different time intervals, they were soaked in the kinetin solution. The time intervals taken for the experiment were
(i) Control
(ii) For 3 hours
(iii) For 6 hours
(iv) For 12 hours
(v) For 24 hours

All seeds were first thoroughly washed with Tween 20, a mild detergent, and then treated with Crosstin, a systemic fungicide for about 20 minutes. Then, they were again thoroughly washed with distilled water.

2.5. (i) Preparation of MS medium

MS medium (Murashige & Skoog, 1962) supplemented with 3% sucrose and gelled with 0.8% agar was used as a culture and nutrient medium. The medium was autoclaved at 121°C and 15 p.s.i. for 20 minutes before it was dispensed in culture vessels. Different stock solutions of macronutrient, micronutrient, iron source, vitamins, and various plant growth hormones/regulators of different concentrations were made in advance and stored in a freezer before mixing them up to form the medium.

2.5. (ii) Preparation of kinetin solution

For each time interval mentioned above, 0.003125 g of commercial kinetin powder was added to 62.5 ml of distilled water. Then the solution was kept on a hotplate magnetic stirrer for about 20 minutes. The solution was then kept aside to cool down. The seeds were then soaked in the solution for 3 hours, 6 hours, 12 hours, and 24 hours respectively.

Before inoculating the seeds from each time interval, the seeds were surface-sterilized with the help of $HgCl_2$, a seed surface sterilizer for about 3 minutes. Then, they were washed three times with distilled water.

Calculations:

a) Germination percentage: $\frac{\textbf{Number of germinated seeds}}{\textbf{Total number of seeds taken}}$ **X 100**

b) Mean germination time (MGT) was calculated by the equation,

$$\textbf{MGT} = \sum (n_i \times d_i) / N$$

Where n_i = number of seeds germinated at day i

d_i= number of days from the beginning of the experiment

N = total number of seeds germinated in the experiment

RESULTS

Table 1. Measurements of plants grown in vitro (25°C)

Petri plates	Replicates	Root length (In cm.)	Shoot length (In cm.)	O.P.L. (In cm.)
Petri plate I	R1	1.6	2.3	3.9
	R2	2.5	2.4	4.9
	R3	1.9	2.9	4.8
	R4	1.6	1.3	2.9
	R5	1.2	0.7	1.9
Petri plate II	R1	2.3	2.1	4.4
	R2	1.0	2.2	3.2
	R3	1.3	2.4	3.7
	R4	1.6	1.2	2.8
Petri plate III	R1	1.1	1.5	2.6
	R2	0.7	0.8	1.5
	R3	1.4	1.3	2.7

Table 2. Measurements of plants grown in vitro (35°C)

Petri plates	Replicates	Root length (in cm.)	Short length (in cm.)	O.P.L. (in cm.)
Petri plate I	R1	3.0	3.5	6.5
	R2	3.4	2.6	6.0
	R3	1.0	2.5	3.5
	R4	1.8	2.4	4.2
	R5	0.7	1.4	2.1
Petri plate II	R1	1.1	1.3	2.4
	R2	0.3	0.6	0.9
	R3	0.6	1.1	1.7
	R4	1.3	3.3	4.6
	R5	3.6	2.9	6.5
Petri plate III	R1	0.5	1.7	2.2
	R2	0.4	1.3	1.7
	R3	0.4	1.5	1.9

No germination was observed in the case of the 4°C temperature category.

Table 3. Effect of various temperatures in different replicates

Temperature (in °C)	Total seeds taken (per replicate)	Total number of seeds germinated	Germination Percentage (%)	Mean value of Germination percentage
4°C	20	0	0.0 %	
	20	0	0.0 %	0
	20	0	0.0%	
25°C	20	5	25.0 %	
	20	4	20.0 %	20 ± 2.35
	20	3	15.0 %	
35°C	20	5	25.0 %	
	20	5	25.0 %	21.66 ± 2.72
	20	3	15.0 %	

Table 4. Mean value of germination

Temperature (in °C)	Mean value of germination
4°C	0
25°C	8.60 ± 0.27
35°C	13.97 ± 3.31

Table 5. Effect of Kinetin hormone on seeds soaked for different time intervals

Concentration of Kinetin (in ppm)	Time	Mean value of germination %
50 ppm	Control	79 ± 0.47
50 ppm	3 hours	65.66 ± 5.66
50 ppm	6 hours	55 ± 1.41
50 ppm	12 hours	41.66 ± 4.90
50 ppm	24 hours	61.33 ± 1.51

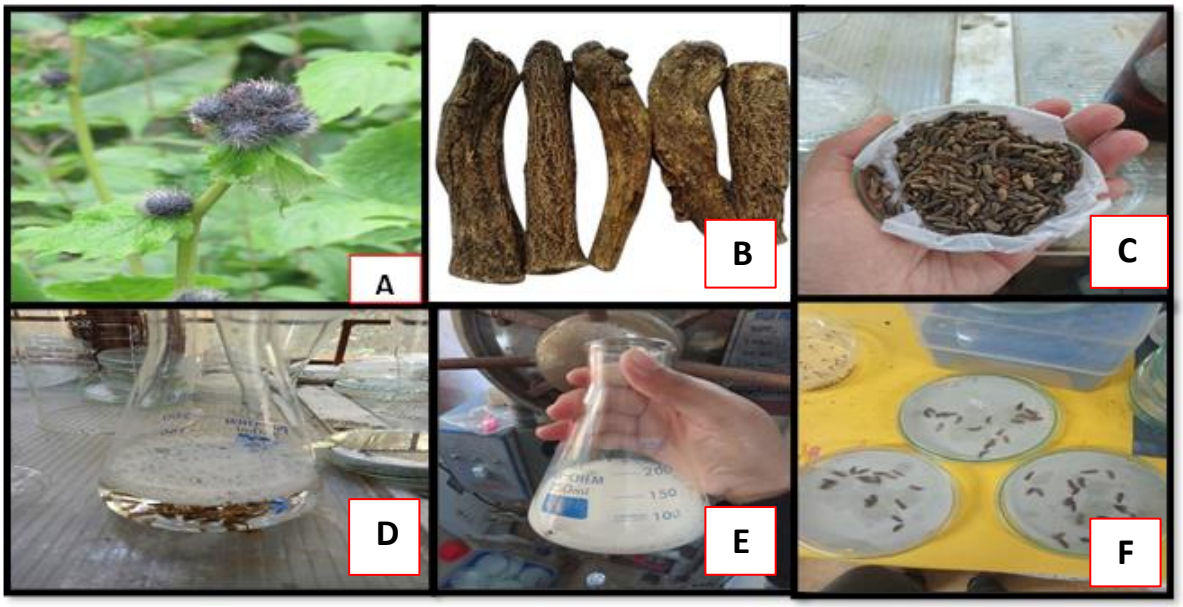

Figure 1. (A) Saussurea costus with leaves and flowers, (B) Roots of S. costus, (C) Seeds of S. costus provided by the department, (D) Seeds washed with Tween 20, (E) Seeds treated with Crosstin, (F) Seeds kept in petri plates after sterilization.

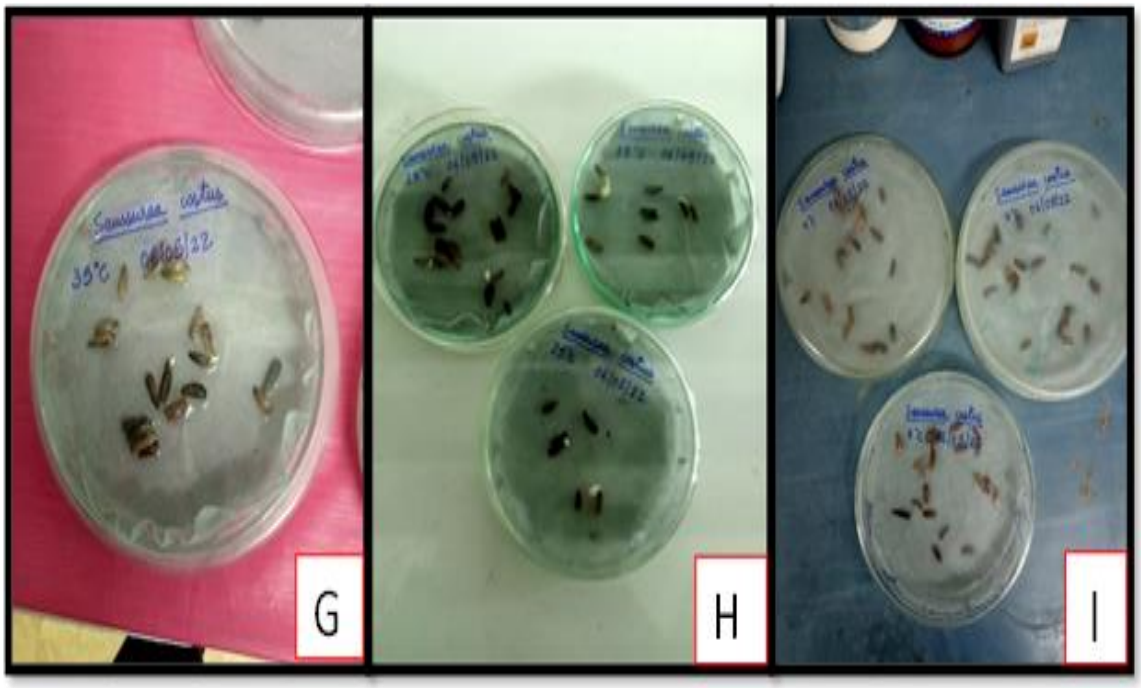

Figure 2. (G, H) Seeds kept at 35°C and 25°C showing germination, (I) Seeds kept at 4°C showing no germination.

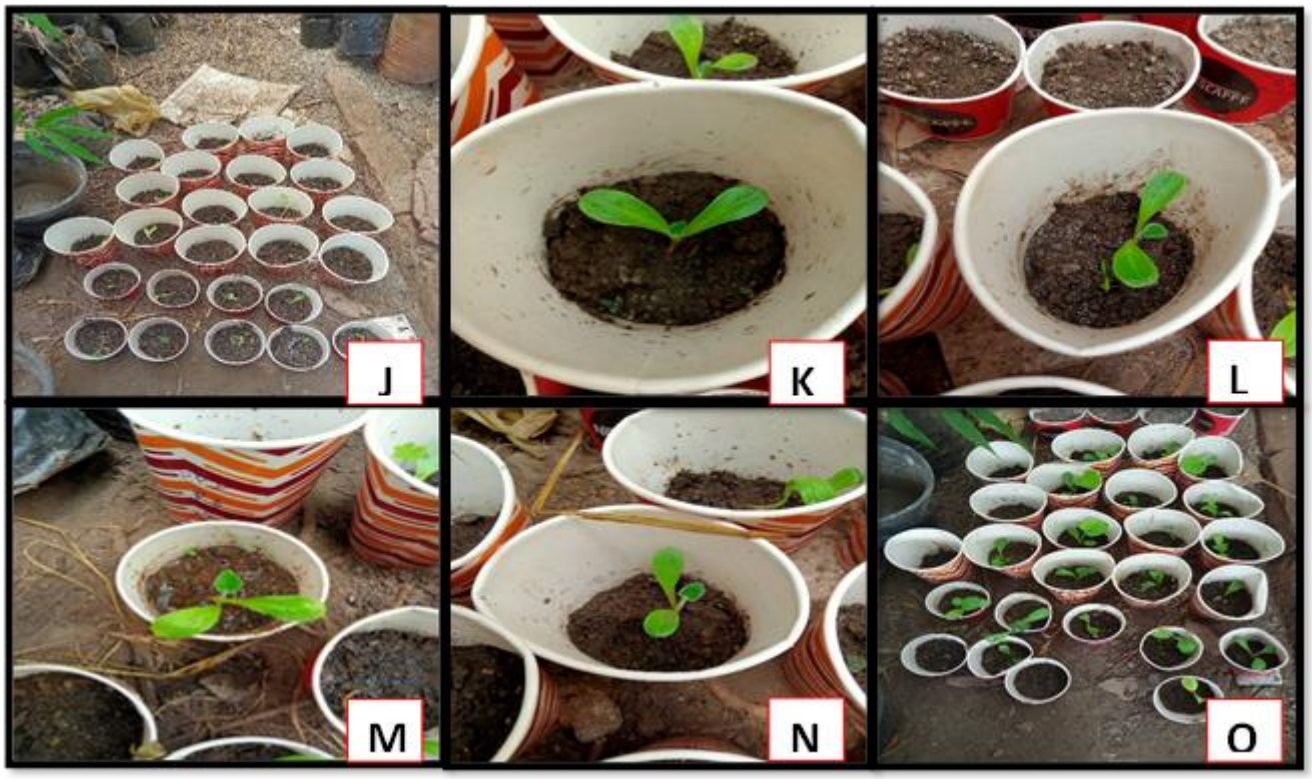

Figure .3. (J) Germinated seeds transferred into the soil, (K, L, M, N) Plantlets showing initiation of 3rd leaf after 3 weeks, (O) Plantlets after 4 weeks of transfer.

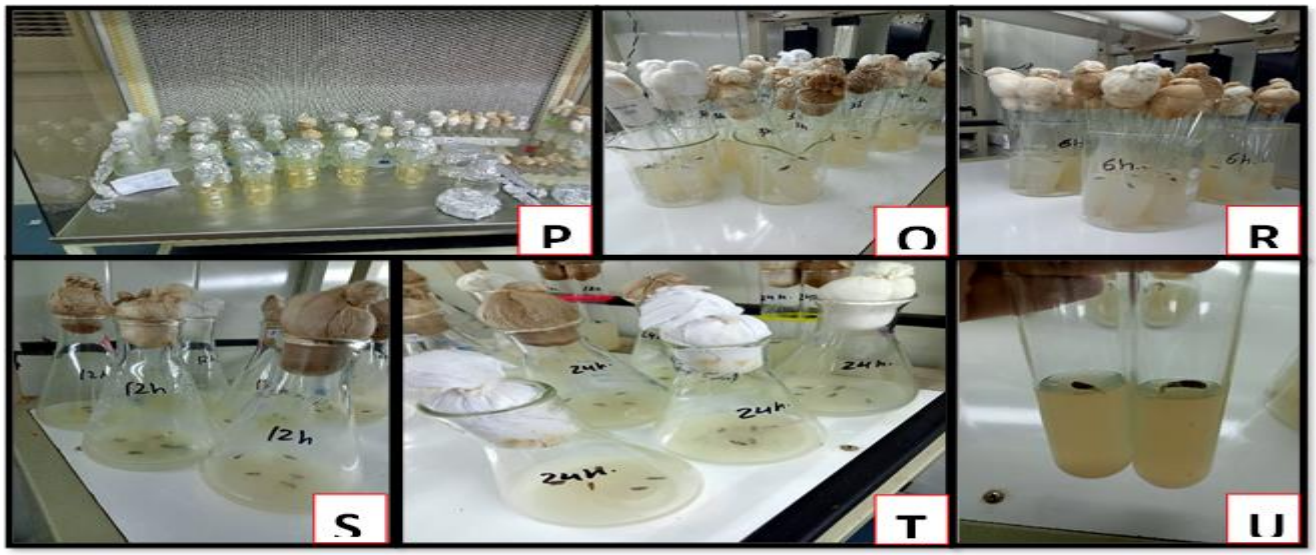

Figure .4. (P) Prepared media for seed germination via MS medium with all required materials in laminar flow chamber, Soaked and sterilized seeds inoculated in MS media of different time intervals – (Q) 3 hours, (R) 6 hours, (S) 12 hours, and (T) 24 hours, (U) Initiation of seed germination after a week of inoculation, in seeds of 3 hours' time interval.

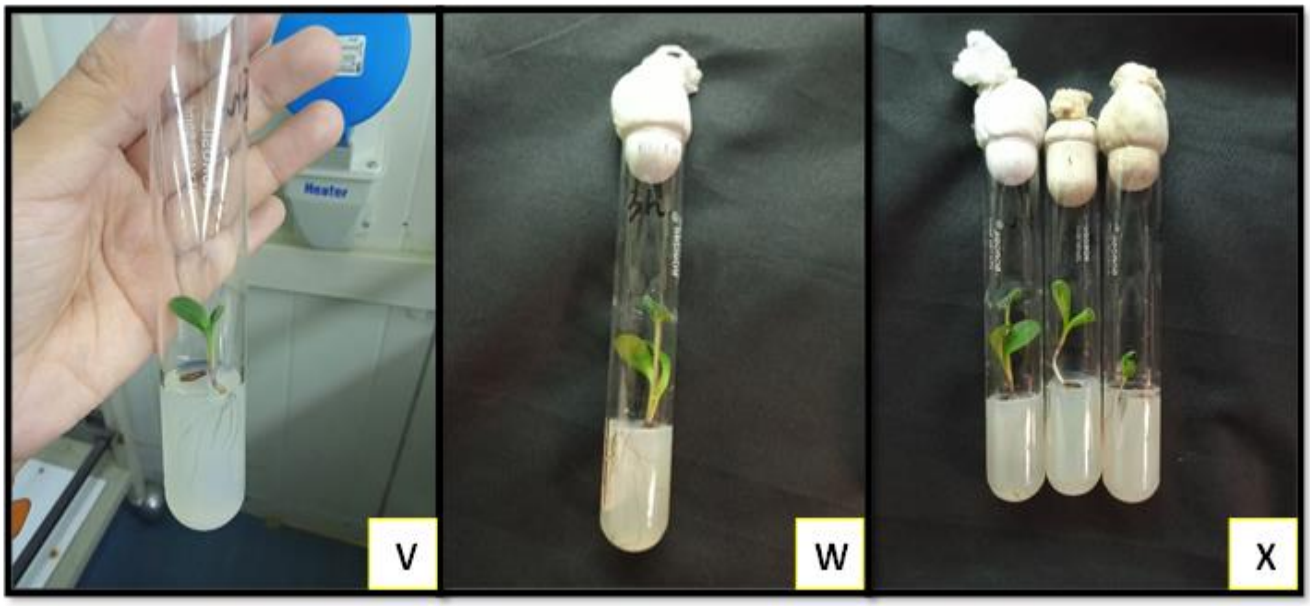

Figure 5. (V) S. costus plant showing developing stem, leaves and roots after three weeks of inoculation, (W) S. costus plant showing emergence of 3rd leaf after six weeks of inoculation, (X) S. costus plants showing emergence of plumule to 3rd leaf.

Figure 6. S. costus plants showing various stages of seed germination

DISCUSSION

The role of medicinal plants is particularly important in the Himalayan region, as it is the major source of medication for a wide range of ailments. Therapeutic effects of medicinal plants are associated with their chemical peculiarities, which are in reality components of the defence strategies of plants (Parveen et al., 2013). Populations of some high-value medicinal plants are decreasing due to overexploitation and environmental changes. Despite several policies and attempts by the government of illegal harvesting of medicinal plants in the Himalayas still persist (Kala, 2005). The development of a suitable seed germination protocol for the high-value species can serve as a viable conservation strategy, considering this; it is an attempt to seed germination capacity of *Saussurea costus*.

Seed germination is a crucial stage in the life cycle of plants. Next generation of plant begins with the seed. According to EH Roberts (1988), temperature can affect the percentage and rate of germination through at least three separate physiological processes. 1. Seeds continuously deteriorate and, unless in the meanwhile they are germinated, they will ultimately die. The rate of deterioration depends mainly on moisture content and temperature. 2. Most seeds are initially dormant. Relatively dry seeds continuously lose dormancy at a rate which is temperature-dependent. 3. Once seeds have lost dormancy their rate of germination (reciprocal of the time taken to germinate) shows a positive linear relation between the base temperature (at and below which the rate is zero) and the optimum temperature (at which the rate is maximal); and a negative linear relation between the optimal temperature and the ceiling temperature (at and above which the rate is again zero).

Kinetin is a cytokinin-like synthetic compound that regulates cell growth in plants. It was shown to naturally exist in DNA of organisms including humans and various plants. (https://www.clinisciences.com/en/buy/cat-plant-growth-regulators-cytokinins-4829.html#:~:text=Kinetin%20is%20often%20used%20in,including%20humans%20and%20various%20plants.)

At temperature 35°C, the mean germination percentage was observed to be highest (21.66%) as compared to that at 25°C and 4°C (20.0% and 0% respectively). Although according to Bano et al., (2018), the most practical and useful pre-treatment for propagation of species (S. costus) on a large scale is the chilling treatment for a period of 50 days at low temperature (3-4°C) as well as treatment of seeds with lower concentration of gibberellin. Seeds without kinetin treatment were observed to have highest germination percentage (79.0%) as compared to the seeds soaked for 6 hours, having lowest germination percentage (41.66%). Kinetin and 2-iP are not useful for proliferation, but better suited for conservation purposes. Shoot formation is independent of kinetin concentration starting from 0.5 mg L–1. Therefore, an unnecessary increase of concentration can be avoided. Higher concentrations of kinetin promoted rooting but decreased root length. Treatment with 2-iP is suitable for root formation and at low concentration promotes rooting

(Gailite et al., 2010). Kinetin and IBA showed maximum shooting and rooting response both in terms of number of shoots, roots, shoot and root length from subcultured calli (Bhardwaj et al., 2016).

CONCLUSION

Saussurea costus is a high value of medicinal plant facing the pressure of commercial exploitation in Himalayan region. Therefore, is a need for the development of reliable seed germination protocol of the species. In the present investigation, the seeds were potent and the phytohormone (kinetin) showed better response for the propagation of the species. The findings of the present study may be helpful to the germination of this valuable plant of Himalayan region.

The above studies showed that the best suitable temperature for in vitro seed germination of Saussurea costus is 35°C. The given temperature has the highest mean value of germination percentage ie., 21.66 ± 2.72. In case of in vitro germination via MS medium, the seeds without any Kinetin treatment showed best results as compared to those seeds which were provided kinetin treatment at different time intervals. The sseds from control has the highest mean value of germination percentage i.e., 79 ± 0.47.

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