# Elicitation of salidroside under tissue culture conditions in the Trans-Himalayan plant *Rhodiola imbricata*

ARCHIT PUNDIR, ANAIDA KAD AND HEMANT SOOD
Department Of Biotechnology And Bioinformatics
Jaypee University Of Information Technology
Waknaghat, District Solan, Himachal Pradesh
India
hemant.sood@juit.ac.in; hemant6sood@gmail.com

*Abstract: Rhodiola imbricata* belonging to Crassulaceae family, is a dioecious perennial plant. It is herb indigenous to the Trans- Himalayan region and exclusively found in Leh-Ladakh valleys of India and border regions of China and Tibet. Various pharmacological activities in *R. imbricata* viz. hepatoprotective, radioprotective, immunomodulatory etc., are attributed to presence of different phytochemicals such as p- tyrosol, salidroside, rosavin and rosin. But the established plant cell cultures of *Rhodiola imbricata* usually encounter problems of low product yields and high cost, which discourage its commercialization. So, in order to enhance the secondary metabolite content in *R. imbricata*, we have studied the effect of different abiotic elicitors on marker compound production. Owing to the previous success of different elicitors on other species of *Rhodiola*, we have performed chemical and physical elicitations on shoot cultures of *Rhodiola imbricata*. The best shoot growth in elicited cultures was reported under Photosynthetic light and data were collected for number of shoots 8±0.33 with average shoot size 6±0.63 cm, number of leaves 31±0.16, leaf size 1±0.28 cm and biomass 10±0.96 g. The highest salidroside content 3.117±0.008 mg/g was obtained by using UV light as an elicitor. Along with elicitations, liquid MS media supplemented with different growth hormones have been tried for improving the biomass of *R. imbricata* under in vitro conditions. The best results were obtained in liquid MS medium supplemented with BAP (1 mg/L) + IBA (2 mg/L) with 87±0.95 g biomass yield in 3 weeks duration of time. The relative enhancement achieved in liquid media in comparison to solid media is 5.35 folds more.

**Keywords:** Elicitation, *Rhodiola imbricata*, suspension culture, saldiroside, HPLC

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## 1. Introduction

*Rhodiola imbricta Edgew*. is a herbaceous, dioecious perennial plant, belonging to Crassulaceae family. There are nearly 1400 species in stone crop family which are distributed in 33 genera with *Rhodiola* present worldwide especially in the regions of South Africa and Northern Hemisphere [1].The *Rhodiola* spp. consists of about 130 species[2] and many *Rhodiola* species have been used traditionally for curing chronic illness and weakness in the regions of Tibet and Western Himalayas belt for over 1000 years [3]. In India, there are six species of *Rhodiola*, which are, *R .tibetica, R. imbricata, R. quadrifida, R. sinuate, R. heterodont, and R. wallichiana* [4].The common names of *Rhodiola imbricata* are Rose root (due to the rose- like fragrance of the fresh cut rootstock), Golden root, Arctic root, Shrolo (as commonly called by the locals of Ladakh region), Solo (by localites of Rohtang- Manali region) and stone crop or Himalayan stone crop in India because it grows along the stones only [5]. *R. imbricata* have an immunomodulatory and anti-cellular potential [6], adaptogenic activity [7], radioprotective efficacy[8], antioxidant potential [9], establish uses in cold, hypoxia and post-stress recovery [10], anti-cancerous and anti- proliferative effects when tested for colon cancer [11], and found to be safe for public use. *R. imbricata* is not only an important traditional medicinal plant, but it is also widely used as a food crop. The roots of *R. imbricata* are used to treat colds, coughs, lung problems, fever and pulmonary diseases in the Tibetan and Amchi traditional medicine system [5]. The plant has also demonstrated its value because of the medicinal properties used to increase physical endurance, treat asthma, impotence, haemorrhage and diseases related gastrointestinal track.

The plant has been extensively used for traditional medicines by the people of Leh and Ladakh. It also finds its mention in Tibetan and Chinese medicines but due to easy availability of *R. rosea* and *R. crenulata*, the potential of *Rhodiola imbricata* has not been completely exploited yet by the pharmaceutical industries. This species contains medicinally important secondary metabolites like Salidroside, Rosavin, Rosin and Tyrosol[13-14]. But plant cell cultures usually encounter problems of low product yields and high cost, which discourage their commercialization. Moreover, the quantitative analysis and HPLC studies of these metabolites proved their concentration to be relatively less in comparison to other species. So, in order to enhance the secondary metabolite content in *R. imbricata*, certain elicitors can be used to provide stress conditions to the plant cells. As there are no reports of *in vitro* shoot cultures and effect of elicitations on shoot cultures of *R. imbricata* for the production of medicinally important secondary metabolites, we aim to study the effect of different abiotic elicitors on secondary metabolite content of this plant. It will not only serve the pharmaceutical industry on large but will also help in increasing India's hold on rosavin and salidroside production in the market due to exclusivity of *R. imbricata* in Trans-Himalayan regions of India.

# 2. Material And Methods

## 2.1 Selection of Plant Material

*R. imbricata* field grown plants were procured from Defence Institute of High Altitude Research (DIHAR), Defence Research and Development Organisation (DRDO), Ministry of Defence, Leh, Jammu Kashmir. The plants were identified by the Botanical Survey of India, Dehradun (Accession number – 117062). These plantlets were maintained in the greenhouse (Figure1) and later tissue cultured in vitro in tissue culture chambers at 15 ± 2 ° C with relative humidity of 70%, a photoperiod of 16 hours per day / 8 hours at 3000 lux of florescent tubes (Philips, India) at Jaypee University of Information Technology, Waknaghat, HP, India [15] (Figure 1).

## 2.2 *In vitro* shoot regeneration

Various MS media combinations were tested for optimization of the culture condition for micropropagation of *R.imbricata.* As methods were previously developed for callus and shoot organogenesis [15], the shoots were regenerated using different concentrations and combinations of 6-Benzylaminopurin (BAP), Indole-3-butyric acid (IBA), Kinetin (KN), Gibberellic acid (GA3) and Thidiazuron (TDZ) with sucrose 3% (w/v) were supplemented with MS media. The pH of each media was adjusted to 5.7 with 10 N HCl, NaOH and 0.8% (w/v) of agar-agar (Plant tissue culture grade) was added as a gelling agent and they were autoclaved at 121°C temperature and 15 lb/in pressure for 15 - 20 min. The cultures were incubated in plant tissue culture chambers maintained at incubation temperatures 15 ± 2 ° C and 25 ± 2 ° C with relative humidity of 70%, a photoperiod of 16 hours per day / 8 hours at 3000 lux of florescent tubes (Philips, India) at Jaypee University of Information Technology, Waknaghat, HP, India. After the growth of plantlets and callus cultures, MS media consisting of different auxins and cytokinins (Table 1) were tested for shoot regeneration under aseptic conditions as mentioned above. The data on days to shoot regeneration, average shoot numbers and shoot length (cm) were recorded. These experiments were performed in triplicates and repeated thrice.

## 2.3 Optimization of Liquid Media for callus induction and shoot regeneration

Cost effective liquid MS media with different concentrations and combinations of indole-3-butyric acid (IBA), 6- Benzylaminopurine (BAP), gibberellic acid (GA3), kinetin (KN) and Thidiazuron (TDZ) with 3% sucrose (w\v) observed for induction and growth of callus and shoot regenration. The pH of each medium was adjusted to 5.7 with 10 N HCl and NaOH and sterilized in an autoclave at 121 ° C and 15 lbs/in pressure for 15-20 minutes.

Fine excisions were made on the leaves of *R .imbricata* for callus induction and were transferred in MS media with different growth hormone concentrations (Table 2). The cultures were incubated in plant tissue culture chambers on a 100 rpm shaker for 8 hours and kept static for 16 hours Biomass of callus was regularly monitored after duration of 1 week.

After callus induction and growth in suspension culture, the tissues were kept in culture room for shoot regeneration under optimized conditions as mentioned before. The data was collected for number of days of callus induction, biomass developed. The data was also collected for number

of shoots formed, weight of biomass, number of leaves, height of shoot, etc.

### 2.4 Elicitations of *Rhodiola imbricata* shoot cultures

In vitro grown shoots were used for carrying out elicitations with chemical and physical elicitors under optimized culture conditios (Table 3) and the data was collected for all the required parameters for control and different elicited cultures.

#### 2.4.1 Chemical elicitors

The in-vitro grown shoots have been cultured on MS media supplemented with growth hormones and different concentrations of chemical elicitors (Table 3). The chemical compounds have been used in mentioned concentrations and supplemented in the media as per the protocol.

#### 2.4.2 Physical Elicitors

**Electric Shocks**

Finely excised shoots submerged in MS media were induced to electric shocks using a DC voltmeter at 5 mA, 10 mA, 20 mA, 30 mA, 50 mA, 75 mA, 100 mA, 125mA, 150mA, 200mA for continuous 2 minutes. The current was measured using an Amp meter. These cultures were later transferred to solid MS media in plant tissue culture chambers in conditions mentioned above.

**Photosynthetic Lights**

Plants transferred on MS media were kept in incubation in a incubater shaker (provided by New Brunswick) integrated with Photosynthetic Growth Lamp of 4000 lux intensity. The control for the same was shoot cultures grown in White Fluorescent Light (WFL) under optimized conditions.

**Ultraviolet Light**

Finely excised plantlets were exposed ultraviolet light kept at 30 cm above in a closed chamber for 10 minutes, 20 minutes, 30 minutes. These plantlets were later transferred to solid MS media and incubated at optimized conditions.

### 2.5 Quantification of Salidroside in *Rhodiola imbricata* tissues

#### 2.5.1 Standard Preparation

A standard stock solution was prepared by dissolving 1 mg of Salidroside (Chromadex) in 1 ml of 80% methanol. These stock solutions were diluted twice to prepare 50 ppm standard working solutions and stored in an HPLC vial at 4 ° C.

#### 2.5.2 Sample Preparation

Fresh tissues in vitro grown in vitro control and tested shoots and callus were taken for salidroside analysis were collected and stored at -80 ° C. Approximately 100 mg of crushed tissue from each sample was collected and filtered with 100% 1:15 methanol (w / v). The Sonicator water bath was used to incubate the mixture at 30 ° C for 15 minutes. The mixture was filtered through a 0.2 µm filter apparatus and the resulting extract was stored in HPLC vials at 4 ° C.

#### 2.5.3 Chromatography Conditions

The analyzes were performed using a Waters HPLC system, equipped with HPLC 515 water pumps, a Waters 717 automatic sampler, a Waters 2996 photodiode array detector and the Empower software. The stationary phase used was the Waters Spherisorb reverse phase C18 column (4.6 mm x 250 mm, 5 µm). The temperature of the column oven was adjusted to 25 ° C. Various mobile phase compositions (methanol, acetonitrile and Milli Q water at pH 5.8) with different flow rates were tested to resolve the standard Salidroside mixture. The diode array detector was used to detect the salidroside (at 225 nm) while the injection volume was maintained at 10 µl. The peak area data and the salidroside retention time were recorded. These experiments were performed in triplicate and repeated three times. The concentration (mg / g) of the compounds was calculated using the formula 1.

## 3. Result

### 3.1. Development of Callus cultures and shoot cultures

Out of tested 10 media combinations (Table 4), callus growth was observed in 4 media combinations *viz.* AA2, AA3, AA4, AA8 at 15 ± 2°C and 25± 2°C. Callus growth was initially observed at 15 ± 2°C (10-30 days) as compared to 25± 2°C (20 -50 days). MS medium supplemented with TDZ (1 mg/L) was found to be the best for regeneration of callus within 10 -1 5 days at 15 ± 2°C with 81-92% of calli from old callus as well as excised leaves and shoots. Leaf explant was found to be the best for initiation of callus in 10-15 days with 91±0.67% of calli. Within 4 weeks of culture, complete callus mass was obtained from leaf explants. The callus mass was maintained by

subculturing on AA2 after 4 -5 weeks. Regeneration was initiated from calli with a green appearance. (Figure 2).

### 3. 2 Regeneration of shoots from callus and its multiplication

Out of tested 10 media combinations (Table 4) used to subculture growing callus cultures, callus was regenerated into shoots in 3 media combination *viz.* AA1, AA3, and AA7 at 15 ± 2°C and 25± 2°C. Shoot regeneration was observed initially at 15 ± 2°C (15-20 days) as compared to 25± 2°C (30-40 days). MS medium supplemented with BAP (1 mg/l) + IBA (2 mg/l) was found to be the best for shoot regeneration from calli within 15 - 20 days at 15 ± 2°C with 15.22±0.01 shoot number and average shoot length of 2.5±0.01 (Figure 3). Regenerated shoots of callus were transplanted in a different combination of media (Table 4). Of the 10 media combinations tested, the shoot was multiplied by 3 media combinations AA1, AA3 and AA7 at 15 ± 2 ° C and 25 ± 2 ° C. The multiplication of shoots was observed before at 15 ± 2 ° C (18-23 days) compared to 25 ± 2 ° C (25-30 days). Furthermore, at 15 ± 2 ° C, the number and duration of the outbreaks were much greater. The MS media supplemented with BAP (1 mg / L) + KN (2 mg / L) was the best for propagating shoots in 18-23 days with 5.74 shoots and an average length of 3.22 at 15 ± 2 ° C (Figure 4).

### 3.3 Optimization of Liquid Media for callus induction and growth and shoot regenration

Out of tested 7 media combinations (Table 5), callus was regenerated in 3 media combinations *viz.* AAL3, AAL5, AAL6 at 25 ± 2°C shaker. MS medium supplemented with BAP (1 mg/L) + IBA (2 mg/L) was found to be the best for initiation and growth of callus within 10 -1 5 days at 25 ± 2°C. Green callus was observed within 15 days without browning of medium and good yield with respect to biomass was collected that is (87±0.95 g) from initial biomass of sub cultured callus 0.43±0.03.Liquid media significantly enhanced plant biomass by 203.1 fold. The average callus biomass obtained in solid media is 16±0.43 g. Thus, the relative enhancement achieved in liquid media in comparison to solid media is 5.35 folds more. The regenerated shoots 21 in number from optimized liquid cultures would used for further multiplication and up scaling experimentation. (Figure 5).

### 3.4 Elicitation and Quantification

Phenotypic effects of different elicitors were noted and HPLC was performed for quantifying salideroside quantification. Different mobile phase compositions (methanol, MilliQ Water and acetonitrile of different pH values) were tested to resolve Salidroside standard mixtures. The optimum resolution could be achieved using MilliQ Water (B), and Acetonitrile (A) as mobile phase with isocratic elution: 15A/85B for Salidroside with flow rate of 10 mL $min^{-1}$. (Figure 6), (Table 6, 7), (Graph 1).

## 5. Discussion

The cultivation of medicinally important Tran-Himalayan plant, *R. imbricata* is very difficult in its natural environment. There are many problems encountered in mass propagation of this plant. The employment of labour is one of the most difficult problems due to expensive availability at high altitudes severe damage to the health of the workers working under these extreme climatic conditions. Even if feasible, the destruction caused to the vulnerable ecosystem would be so drastic that it will simply add the plant varieties to Red list.

Thus, a better way to approach this issue would be the use of biological engineering technologies, such as plant tissue culture and metabolic engineering to obtain medicinally important biological components synthesized by the human intervention. And this is the basic idea behind doing this project.

The rationale behind this study was to develop an *in vitro* system for micropropagation of *R. imbricata* and using this system for enhanced biosynthesis of salidroside using different elicitors. The best medium for callus regeneration was found to be MS medium supplemented with TDZ (1mg/L). Depending upon the endogenous concentration of growth hormones applied to different explants under same in vitro conditions, different responses were noted with respect to plant regeneration [16]. The best growth was reported in MS medium supplemented with BAP (1 mg/l) + IBA (2 mg/l), which is in accordance with results earlier reported [15]. According to the

results obtained in the elicitations, the significant growth enhancement was seen in plants of *R. imbricata* grown in photosynthetic light. The increased plant height, stem girth and multiple shooting was reported for the first time as the effect of being grown in photosynthetic light. The earlier studies find the mention of increased growth and development rate but no morphological changes [17]. For *R. imbricata*, the effect of differently coloured lights was also studies for callus cultures showing enhanced growth in red light and increased accumulation of salidroside in blue light [18]. As for electricity, there was 3 fold increase in the leaf size, which is similar to the findings of Evans Kaimoyo. The results also supported the enhancement of salidroside but not as high as reported earlier [19].

The in vitro grown shoots of *R. imbricata* gave significantly high content of salidroside as compared to salidroside content in field grown root extract. According to the study done by Sahil kapoor *et al* [20], the salidroside content present in field grown parts was 1.08mg/g whereas the amount of salidroside present in *R. imbricata* tissue cultured shoots grown in MS media supplemented with BAP (1 mg/l) + IBA (2 mg/l) was 3.6 mg/g, which is nearly 3.4 folds higher. This finding as it is solves the basic purpose of our study.

In addition to this, the elicited plants also showed enhanced salidroside amount where callus elicited with electric shock gave a positive response. The amount achieved was greater than control callus grown in optimized MS media. But the results achieved were not as impressive as reported by Evans Kaimoyo *et al* in *Pisum sativum* [19]. When the salidroside content of callus cultures grown in Liquid MS media supplemented with similar growth hormones as in optimized solid MS media, slight enhancement was observed. Despite the same media composition, the increased metabolite concentration holds a strong ground for up scaling the research. The similar findings were also reported in suspension cultures of *Rhodiola sachalinensis* by Jay Xu [21]. UV also proved to be an efficient elicitor in enhancing the concentration of salidroside which is in accordance with the research done by Y. K. Bernard [22]**.** In addition to this, the liquid culture optimization to enrich the biomass of the plant was successfully done with 5.35 fold increase in the callus in comparison to callus grown on solid media. The similar findings were also reported by D. Popli *et al.* (2016) [23].

So, the present findings provide platform to upscale the study to bioreactor level. Thus help to achieve desirable plant growth and marker production can be carried out at commercial scale in order to meet the demands of pharmaceutical industries.

# 6. Conclusion

Through this experiment, we have optimized the liquid medium for improving the growth and development of callus. It has enhanced by 5.35 folds within 20-25 days as compared to controls grown in solid media.

Moreover, we have used 9 different elicitors for enhancing the concentration of marker compound that is salidroside under in vitro conditions but photosynthetic light and UV gave some significant elicitation along with good growth and development, whereas some positive morphological changes such as elongated shoot with thick girth were observed in cultures grown in electric shock as an elicitor.

Hence, the current study possesses the robust potential in large scale propagation of this plant and its secondary metabolite production. It also provides a platform for up scaling this research to bioreactor level so that desirable plant growth and marker compound production can be carried out at commercial scale.

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*Figures*

**Figure 1** Plantlets of *R. imbricata* grown and acclimatized in the green house of the Department of Biotechnology and Bioinformatics, Jaypee University of Information Technology, Waknaghat, India.

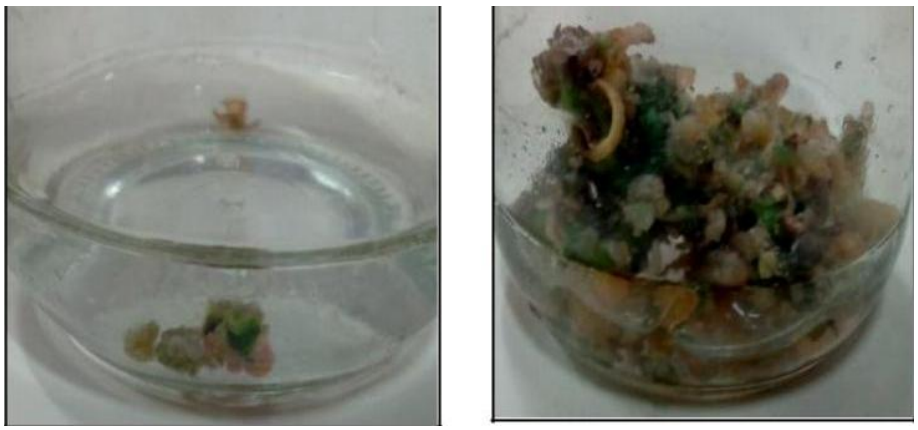
**Figure 2:** *R.imbricata* callus grown in MS media + TDZ (1 mg/L)

**(a)** **(b)** **(c)**

**Figure 3:** Regeneration of shoots from callus of *R.imbricata* (a) After 7 days (b) After 15 days (c) After 30 days of incubation in 15 ± 2°C plant tissue culture chambers.

**(a)** **(b)** **(c)**

**Figure 4:** (a) Initiation of multiple shooting in *R.imbricata* (b) Multiple shooting in *R.imbricata* after 7 days (c) Multiple shooting after 30 days of incubation in 15 ± 2°C plant tissue culture chambers.



**(a)** **(b)**

**Figure 5:** (a) Callus Growth in Liquid media after 5 days (b) Callus Growth in Liquid media after 3 weeks in plant *R. imbricata*

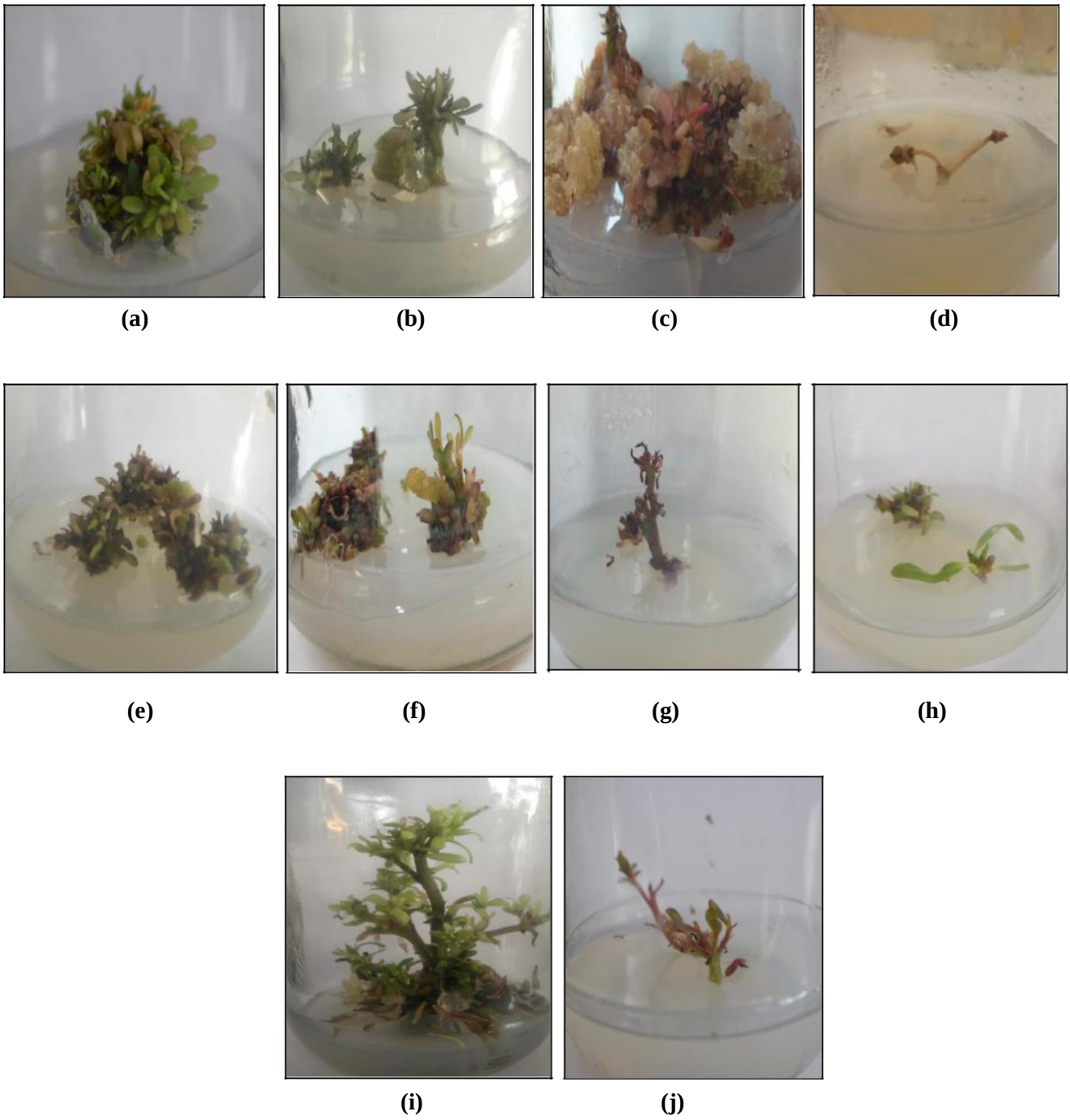



**Figure 6:** In- Vitro grown shoots of *R.imbricata* under different elicitors (a) Control (b) Cinnamic acid (c) Chitin (d) L-Phenylalanine (e) Pectin (f) Methyl Jasmonate (g) Yeast Extract (h) Electric Shocks (i) Photosynthetic Light (j) Ultraviolet Rays

*Tables*

**Table 1:** MS media supplemented used for callus induction and shoot regeneration in *Rhodiola imbricata*.

| S.No. | Medium Name | MS media composition |
|---|---|---|
| **1.** | AA0 | MS |
| **2.** | AA1 | MS + BAP (2 mg/L) + KN (2 mg/L) |
| **3.** | AA2 | MS + TDZ (1 mg/L) |
| **4.** | AA3 | MS + BAP (1 mg/L) + IBA (2 mg/L) |
| **5.** | AA4 | MS + IBA (1 mg/L) + 2,4-D (1.5 mg/L) |
| **6.** | AA5 | MS + BAP (0.5 mg/L) + IBA (2 mg/L) + GA3 (2 mg/L) |
| **7.** | AA6 | MS + IBA (4 mg/L) |
| **8.** | AA7 | MS + BAP (1 mg/L) + KN (2 mg/L) |
| **9.** | AA8 | MS + KN (2 mg/L) + IBA (1 mg/L) |
| **10.** | AA9 | MS + BAP (2 mg/L) + GA3 (2 mg/L) |

**Table 2:** Different liquid media composition for callus induction and regeneration in *R.imbricata* tissue samples.

| S.No. | Medium Name | MS media composition |
|---|---|---|
| **1.** | AAL0 | MS |
| **2.** | AAL1 | MS + BAP (1 mg/L) + KN (1 mg/L) + TDZ (0.5mg/L) |
| **3.** | AAL2 | MS + TDZ (1 mg/L) |
| **4.** | AAL3 | MS + BAP (1 mg/L) + IBA (2 mg/L) |
| **5.** | AAL4 | MS + IBA (1 mg/L) + 2,4-D (1.5 mg/L) |
| **6.** | AAL5 | MS + BAP (0.5 mg/L) + IBA (2 mg/L) |
| **7.** | AAL6 | MS + IBA (0.5 mg/L) + IBA (2 mg/L) + KN (2mg/L) |

**Table 3 :** Chemical and Physical components used as elicitors along with their tested concentrations/ranges in *R.imbricata*.

| S.No | Elicitor (Physical) | Tested factors |
|---|---|---|
| **1** | Cinnamic Acid | 1.0 mM/L, 1.5 mM/L and 2 mM/L |
| **2** | Chitin | 0.5 g/L, 1.5 g/L and 2.0 g/L |
| **3** | L- Phenylalanine | 0.5 mM/L, 1 mM/L and 2 mM/L |
| **4** | Pectin | 0.5 g/L, 1.5 g/L and 2.0 g/L |
| **5** | Methyl Jasmonate | 0.25 mM/L, 0.5 mM/L and 1 mM/L |
| **6** | Yeast Extract | 0.5 g/L, 1.0 g/L and 2.0 g/L |
| **7** | Electric Shocks | 50 mA - 200mA for 2 minutes |
| **8** | Photosynthetic Light | 4000 Lux Intensity |
| **9** | Ultrasound | 20,000 Hz for 1 min, 1.5 min, 2 min |
| **10** | Ultraviolet Rays | 10 minutes, 20 minutes, 30 minutes |

**Table 4:** Effect of MS media supplemented with different growth hormones on callus induction and growth followed by shoot regenration in *R. imbricata*.

| S.No. | Medium Name | Callus Growth (Days) | Callus Colour | Percent Survival | No. of Days For Regeneration | No. of Shoots |
|---|---|---|---|---|---|---|
| **1.** | AA0 | 30 – 35 | Pale Green | 30±0.34 % | -- | -- |
| **2.** | AA1 | -- | -- | -- | 23 – 28 | 4±0.5 |
| **3.** | **AA2** | **7 - 15** | **Dark Green** | **91±0.67 %** | -- | -- |
| **4.** | AA3 | 20 - 25 | Pale Green | 75±0.4 % | **25 - 30** | **8±0.75** |
| **5.** | AA4 | 18 - 25 | Creamy | 45±0.5 % | -- | -- |
| **6.** | AA5 | -- | -- | -- | -- | -- |
| **7.** | AA6 | -- | -- | -- | -- | -- |
| **8.** | AA7 | -- | -- | -- | 18 - 25 | 3±0.5 |
| **9.** | AA8 | 21 - 30 | Yellowish Green | 65±0.5 % | 20 - 25 | 4±0.75 |
| **10.** | AA9 | -- | -- | -- | -- | -- |

**Table 5:** Liquid Media optimized with respect to days of regeneration and Biomass yield of *R.imbricata*

| S.No. | Medium Name | Days For Regeneration | Biomass (g) |
|---|---|---|---|
| **1.** | AAL0 | 28 - 40 | 8±0.93 |
| **2.** | AAL1 | 27 - 35 | 19±0.67 |
| **3.** | AAL2 | -- | -- |
| **4.** | **AAL3** | **20 – 25** | **87±0.95** |
| **5.** | AAL4 | -- | -- |
| **6.** | AAL5 | 23 - 30 | 39±0.38 |
| **7.** | AAL6 | 25 - 31 | 21±0.76 |

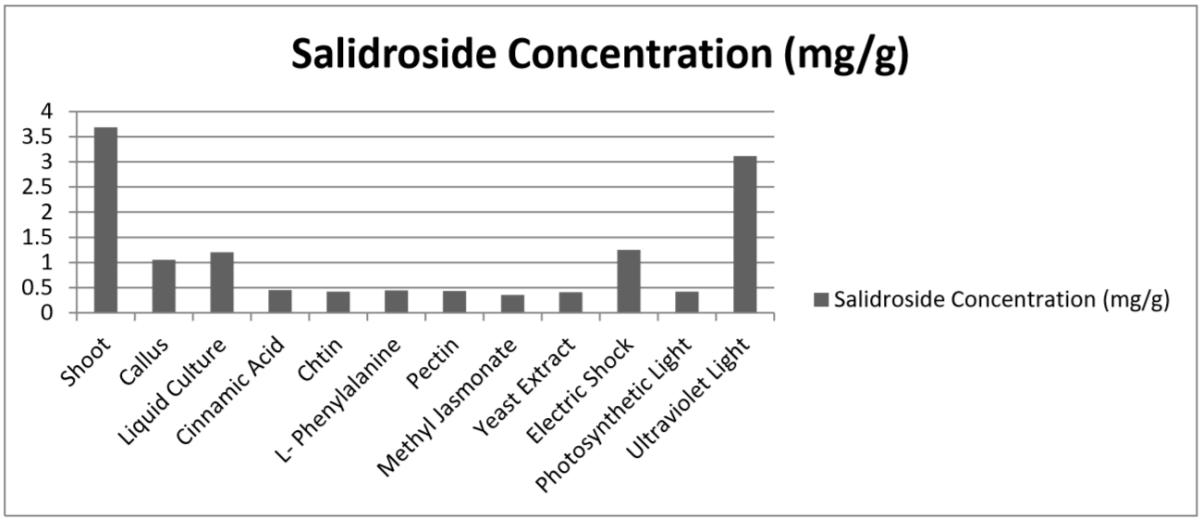
**Table 6: E**ffects of chemical elicitors on in - vitro grown shoots of *R.imbricata* and salidroside quantified.

| S.No | Elicitor | Conc. Tested | Conc. Optimiz Ed | No. of Shoots | Avg. Shoot Size (cm) | No. of Leaves | Average leaf size (cm) | Biomass (g) | Leaf Color | Part Used for extraction | Salidroside Content (mg/g) |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | Cinnamic acid | 1.0 mM/L, 1.5 mM/L and 2 mM/L | 1.5mM/ L | 3±0.67 | 3±0.81 | 20±0.83 | 1±0.22 | 7±0.58 | Dark Green | Shoot + Callus | 0.454 ± 0.03 |
| 2 | Chitin | 0.5 g/L, 1.5 g/L and 2.0 g/L | 2.0 g/L | 1±0.67 | 0.7±0.05 | 5±0.16 | 0.5±0.032 | 5±0.49 | Red, Green | Shoot + Callus | 0.424 ± 0.05 |
| 3 | L-Phenylalanine | 0.5 mM/L, 1 mM/L and 2 mM/L | 1.0 mM/L | 1±0.16 | 0.3±0.06 | 3±0.83 | 1±0.033 | 0.7±0.05 | Pale green | Shoot | 0.444 ± 0.06 |
| 4 | Pectin | 0.5 g/L, 1.5 g/L and 2.0 g/L | 2.0 g/L | 2±0.34 | 0.8±0.05 | 11±0.5 | 0.9±0.062 | 6±0.67 | White, red | Shoot + Callus | 0.433 ± 0.02 |
| 5 | Methyl Jasmonate | 0.25 mM/L, 0.5 mM/L and 1 mM/L | 0.5 mM/L | 1±0.5 | 0.5±0.006 | 12±0.16 | 1±0.33 | 5±0.59 | Green, red, brown | Shoot + Callus | 0.355 ± 0.03 |
| 6 | Yeast Extract | 0.5 g/L, 1.0 g/L and 2.0 g/L | 2.0 g/L | 1±0.16 | 3±0.05 | 8±0.5 | 1±0.033 | 5±0.244 | Red, brown | Shoot | 0.408 ± 0.04 |
| 7 | Electric Shocks | 50 mA - 200mA for 2 minutes | 150 mA for 2 Minute S | 2±0.16 | 1±0.03 | 4±0.85 | 3±0.053 | 5±0.96 | Green | Callus | 1.249 ± 0.09 |
| 8 | Photosynthetic Light | 4000 Lux Intensity | -- | 8±0.33 | 6±0.63 | 31±0.8 | 1±0.28 | 10±0.96 | Green | Shoot + Callus | 0.420 ± 0.05 |
| 9 | Ultraviolet Rays | 10 minutes, 20 minutes, 30 minutes | 30 Minute S | 4±0.16 | 2±0.83 | 3±0.83 | 0.9±0.063 | 4±0.13 | Green, Red | Shoot | 3.117 ± 0.08 |

**Table 7**: Concentration of Salidroside from in-vitro plants of *Rhodiola imbricata*

| S.No | Normal | No. of Shoots | Avg. Shoot size (cm) | No. of Leaves | Average leaf size (cm) | Biomass (g) | Leaf Color | Part Used for extraction | Salidroside Content (mg/g) |
|---|---|---|---|---|---|---|---|---|---|
| 1 | Shoot | 8±0.75 | 5±0.65 | 22±0.83 | 2±0.52 | 6±0.58 | Dark Green | Shoot | 3.687 ± 0.07 |
| 2 | Callus | -- | -- | -- | -- | -- | Green | Callus | 1.054 ± 0.04 |
| 3 | Liquid Culture | -- | -- | -- | -- | -- | Dark Green | Callus | 1.202 ± 0.05 |

*Graphs*




**Graph 1**: Comparative graphical representation of quantified salidroside amount from different tissues of *R. imbricata.*

*Formula*

**Formula 1**

PA of sample/PA of standard) x (1/IV) x (Volume of sample/weight of sample) x dilution factor

Where, PA – Peak Area

IV – Injection Volume