Plant Cell Tiss Organ Cult (2015) 122:57–65
DOI 10.1007/s11240-015-0749-8

CrossMark

ORIGINAL PAPER

Seaweed extract as a novel elicitor and medium for mass propagation and picroside-I production in an endangered medicinal herb *Picrorhiza kurroa*

Neha Sharma[1] · Rajinder Singh Chauhan[1] · Hemant Sood[1]

Received: 26 November 2014 / Accepted: 1 March 2015 / Published online: 14 March 2015
© Springer Science+Business Media Dordrecht 2015

Abstract *Picrorhiza kurroa* is an endangered medicinal herb, used in the preparation of herbal drugs, mainly due to the presence of two iridoid glycosides, picroside-I (P-I) and picroside-II (P-II). Its over exploitation necessitates the development of conservation strategies and enhanced production of secondary metabolites. In present study, effect of seaweed extract (SWE) with and without growth hormones was studied for in vitro propagation and production of P-I in *P. kurroa*. Murashige and Skoog (MS) media supplemented with SWE (MSS) showed 3.23, 1.55, 2.42, 2.52 and 2.41 folds enhancement in total plant biomass, total plant length, number of shoots, root length and number of roots, respectively after 1 month as compared to control C1(MS + sucrose + agar). MSS with growth hormones showed increment of 1.92, 1.14, 1.47, 1.43 and 3.53 folds in total plant biomass, total plant length, number of shoots, root length and number of roots, respectively as compared to control CM (MS + sucrose + growth hormones + agar) after 1 month. SWE media (SWM) showed comparable results and proved to be an economic alternative to MS media. Both MSS and SWM increased P-I accumulation by 3–4 folds and 2–3 folds at 25 ± 2 and 15 ± 2 °C, respectively. Expression of 1-deoxy-D-xylulose 5-phosphate synthase and 3-hydroxy-3-methylglutaryl-CoA reductase, key enzymes of non-mevalonate and mevalonate pathways were up regulated vis-á-vis metabolite content. This is the first report demonstrating the potential of SWE as an elicitor for enhanced growth and P-I production besides being an alternative to MS media for large scale micropropagation of *P. kurroa*.

Keywords Seaweed extract · Biostimulant · *Picrorhiza kurroa* · Picrosides · Expression analysis · HPLC

Abbreviations

P-I	Picroside I
SWE	Seaweed extract
MS	Murashige and Skoog
IBA	Indole-3-butyric acid
KN	Kinetin
SWM	SWE media
MSS	MS media supplemented with SWE
CM	Control medium
DXPS	1-Deoxy-D-xylulose 5-phosphate synthase
HMGR	3-Hydroxy-3-methylglutaryl-CoA reductase
MEP	Non-mevalonate
MVA	Mevalonate

Key message

This study provides the first endeavor for utilizing SWE as a potent elicitor and media for P-I production, multiplication and conservation of *P. kurroa* plants.

Introduction

Picrorhiza kurroa Royle ex Benth (family Scrophulariaceae) is an important perennial medicinal herb endemic to North-Western Himalayan region, found between

Electronic supplementary material The online version of this article (doi:10.1007/s11240-015-0749-8) contains supplementary material, which is available to authorized users.

✉ Hemant Sood
hemant.sood@juit.ac.in

[1] Department of Biotechnology and Bioinformatics, Jaypee University of Information Technology, Waknaghat, Solan 173234, Himachal Pradesh, India

3000 and 5000 m altitudes (Pandit et al. 2012). Picroside-I and picroside-II- the main bioactive constituents of *P. kurroa* are used in herbal formulations such as Picroliv, Katuki, Arogya, Kutaki, Livocare, Livomap, Livomyn, Livplus, Pravekliv and Vimliv for the treatment of liver disorders, fever, asthma, malaria, jaundice, inflammation, allergy, hepatitis-B, etc. (Sah and Varshney 2013; Bhandari et al. 2009). *P. kurroa* has been listed as an endangered medicinal plant species by the International Union for Conservation of Nature and Natural Resources (Nayar and Sastri 1990). Global supply (excluding China and Pakistan) of *P. kurroa* is around 375 tonnes, with India contributing around 70 tonnes. Today price of plant material varies from Rs. 250 to 770 per kg (Shitiz et al. 2013). Owing to its economic importance and depleting population in natural habitat, immediate thrust has to be given for its conservation, micropropagation and in vitro production of secondary metabolites. Different in vitro culture techniques have been employed for micropropagation, conservation and secondary metabolite production in *P. kurroa* (Rawat et al. 2013; Patial et al. 2012; Sood and Chauhan 2010) but limited progress has been made so far with respect to development of rapid and cost effective approach for enhancing secondary metabolite production in this plant species. Till date, no information exists on use of elicitors for in vitro production of picrosides in *P. kurroa,* therefore SWE was tested as a cost effective and potent elicitor for increasing plant biomass and metabolite production.

Seaweeds are macroscopic, multicellular marine alga and their extracts are reported to contain macronutrients, micronutrients, amino acids, vitamins, cytokinins, auxins, gibberellins, carbohydrates, betaines, and abscisic acid-like growth substances (Rengasamy et al. 2015; Stirk et al. 2014; Papenfus et al. 2012; Craigie 2011; Khan et al. 2009). Previous reports have highlighted the importance of SWE and their utilization with significant results on plant growth and development wherein seed germination (Kumar and Sahoo 2011), in vitro mass propagation (Vinoth et al. 2012), early shoot formation (Hurtado et al. 2009), improved yield and fruit quality (Ahmed and Shalaby 2012), deep root development (Anisimov and Chaikina 2014) and enhanced secondary metabolite production (Lola-Luz et al. 2013; Hurtado et al. 2012) have been achieved in various plants species.

The current study evaluates the effect of SWE as a biostimulant with MS medium (MSS) and also as an alternative culture medium (SWM) to MS medium for P-I production and in vitro mass propagation of *P. kurroa.* Expression analysis of 1-deoxy-D-xylulose 5-phosphate synthase (DXPS) and 3-hydroxy-3-methylglutaryl-CoA reductase (HMGR), the rate limiting enzymes of non-mevalonate (MEP) and mevalonate (MVA) pathways vis-á-vis P-I biosynthesis was also studied for the plants grown on MSS and SWM.

Materials and methods

Selection of plant material

One year old *P. kurroa* plants were procured from the nursery of Himalayan Forest Research Institute, Jagatsukh, Manali, H.P., India (1900 m altitude, 20°35.6′–32°6.1′N and 78°57.8′–77°33.7′E) and maintained in greenhouse of Jaypee University of Information Technology, Waknaghat, H.P., India at 25 ± 2 °C, 80–90 % of relative humidity with light irradiance of 100–120 µmol m^{-2} s^{-1} under natural light conditions. Shoot apices of length 0.5–1.0 cm were surface sterilized in 0.5 % Bavistin and 0.1 % Mercuric chloride followed by 4–5 washings in sterile water and cultured on MS media (Murashige and Skoog 1962) supplemented with indole-3-butyric acid (IBA) (3 mg/l), kinetin (KN) (1 mg/l), sucrose (30 g/L) and agar (9 g/L) as a gelling agent. The pH was adjusted to 5.7 using 0.1 N HCl and 0.1 N NaOH and 50 ml of media was dispensed in each jar prior to autoclaving at 121 °C, 15 lb $inch^{-2}$ pressure for 20 min. The cultures were maintained at 25 ± 2 °C, with 70 % relative humidity, 16 h day/8 h night photoperiod at photosynthetic photon flux density of 40 µmol m^{-2} s^{-1} provided by cool white fluorescent tubes (Philips, India) with subculturing after every 4 weeks in a plant tissue culture chamber (Sood and Chauhan 2009b).

Preparation of MS media supplemented with SWE as a biostimulant (MSS)

SWE powder (Supplementary Table 1), provided by Sea6 Energy Pvt Ltd. (Bangalore, India) was obtained from red seaweed *Kappaphycus alvarezii*. Stock solution of SWE was prepared by dissolving 10 g of soluble SWE powder in 10 ml of distilled water by constant stirring with magnetic stirrer for 15 min followed by filter sterilization with 0.22 µm syringe filters. Further, MSS having different concentrations of SWE (0.1, 1.0, 2.0, 3.0 g/L) alone and in combination with growth hormones (IBA: 3 mg/l and KN: 1 mg/l) were prepared (Tables 1 and 2). C1 (MS + sucrose + agar) and CM (MS + sucrose + growth hormones + agar) were used as respective controls for the analysis. *P. kurroa* shoots of 0.5-1.0 cm length were cultured on the above media combinations in two plant tissue culture chambers maintained at different incubation temperatures 25 ± 2 and 15 ± 2 °C and data was recorded for total biomass, total plant length, number of shoots, number of roots and root length after 10th, 20th and 30th day.

Table 1 MS medium supplemented with different concentrations of SWE (g/L), sucrose (30 g/L) and agar (9 g/L)

S. No	Medium name	Medium composition
1.	MSS 1	MS + SWE (0.1) + sucrose + agar
2.	MSS 2	MS + SWE (1.0) + sucrose + agar
3.	MSS 3	MS + SWE (2.0) + sucrose + agar
4.	MSS 4	MS + SWE (3.0) + sucrose + agar
5.	C1	MS + SWE (0.0) + sucrose + agar

Preparation of SWE media (SWM) for *P. kurroa* micropropagation

Shoot apices of *P. kurroa* (0.5–1.0 cm) were taken from green house grown plants, surface sterilized with above mentioned protocol and cultured on 6 different concentrations of SWE (0.01, 0.1, 1.0, 2.0, 3.0, 5.0 g/L), pH 5.7, agar (9 g/L) at 25 ± 2 °C. Optimized concentration (2.0 g/L) of SWE was tested with different combinations of sucrose (30 g/L) and growth hormones (IBA: 3 mg/l, KN: 1 mg/l) on solid (with agar) and liquid (without agar) media (Table 3). Data was recorded for total biomass, total plant length, number of shoots, number of roots and root length by incubating the plants at 25 ± 2 and 15 ± 2 °C on 10th, 20th and 30th day. Results were compared with the similar combinations of MS media.

Hardening of in vitro grown plantlets

The in vitro grown rooted shoots of length 3.0–3.5 cm were gently removed from culture vessels, washed under running tap water and transferred to pots containing sand:soil:vermiculite (1:1:1) for hardening in greenhouse under 100–120 µmol m^{-2} s^{-1} light intensity provided by natural light at 25 ± 2 °C and 80–90 % relative humidity. The plantlets were covered with glass jars for 10–15 days to avoid desiccation. Glass jars were taken off every day for 1–2 h so as to acclimatize them to external environment. Data was recorded for percent survival of plants.

Quantification of P-I

Fresh samples were ground separately in liquid nitrogen and suspended in 80 % methanol. The samples were vortexed and sonicated at room temperature, followed by centrifugation at 10,000 rpm for 15 min. The supernatant was filtered through 0.22 µm filter and the filtrate was diluted 1:10 for estimation of P-I content. P-I was quantified through reverse phase HPLC (Waters) equipped with 515 HPLC Pump, 717 autosampler and 2996 photodiode-array detector set at 270 nm. P-I content was estimated with a Waters Spherisorb® C18, 5 µm (4.6 × 250 mm) reverse phase column. Two solvent systems were used for running the test samples i.e. Solvent A (0.05 % trifluoroacetic acid) and Solvent B (1:1 methanol/acetonitrile mixture) in the ratio of 70:30 (v/v). The column was eluted in the isocratic mode with a flow rate of 1.0 ml/min. The compounds were identified on the basis of their retention time and comparison of UV spectra with P-I standard (ChromaDex, Inc). The corresponding HPLC data was analysed with Empower-2 software.

Table 2 MS medium supplemented with different concentrations of SWE (g/L), sucrose (30 g/L), growth hormones (3 mg/L IBA and 1 mg/L KN) and agar (9 g/L)

S. No	Medium name	Medium composition
1.	MSS 5	MS + SWE (0.1) + sucrose + IBA + KN + agar
2.	MSS 6	MS + SWE (1.0) + sucrose + IBA + KN + agar
3.	MSS 7	MS + SWE (2.0) + sucrose + IBA + KN + agar
4.	MSS 8	MS + SWE (3.0) + sucrose + IBA + KN + agar
5.	CM	MS + SWE (0.0) + sucrose + IBA + KN + agar

Table 3 Different combinations of SWM (SWE 2 g/L) and control media with presence (+) and absence (−) of sucrose (30 g/L), growth hormones (3 mg/L IBA and 1 mg/L KN) and agar (9 g/L)

S. No.	SWM	SWM composition	Control media	Control media composition
1.	SWM 1	SWE − sucrose − growth hormones − agar	MSM1	MS − sucrose − growth hormones − agar
2.	SWM 2	SWE − sucrose − growth hormones + agar	MSM2	MS − sucrose − growth hormones + agar
3.	SWM 3	SWE + sucrose − growth hormones − agar	MSM3	MS + sucrose − growth hormones − agar
4.	SWM 4	SWE + sucrose − growth hormones + agar	MSM4	MS + sucrose − growth hormones + agar
5.	SWM 5	SWE − sucrose + growth hormones − agar	MSM5	MS − sucrose + growth hormones − agar
6.	SWM 6	SWE − sucrose + growth hormones + agar	MSM6	MS − sucrose + growth hormones + agar
7.	SWM 7	SWE + sucrose + growth hormones − agar	MSM7	MS + sucrose + growth hormones − agar
8.	SWM 8	SWE + sucrose + growth hormones + agar	MSM8	MS + sucrose + growth hormones + agar

Expression analysis by qRT PCR

Total RNA was isolated from 100 mg shoots of *P. kurroa* using RaFlex RNA isolation kit (GeNei^{TM}), following manufacturer's instructions. Quality of RNA was checked on 1 % (w/v) ethidium bromide stained agarose gel. cDNA synthesis was done using Verso cDNA synthesis kit (Thermo Scientific, USA) from total RNA (5 µg) as per manufacturer's instructions. cDNA (100 ng) was used as template for qRT PCR reaction using gene specific primers of HMGR and DXPS (Supplementary Table 2) in triplicate on a CFX96 system (Bio-Rad Laboratories; Hercules CA) with the iScript one step RT PCR kit (Bio-Rad). The PCR protocol was as follows: denaturation for 5 min at 94 °C, followed by 40 cycles each of denaturation for 20 s at 94 °C, annealing for 30 s at 55–60 °C, followed by one elongation step for 20 s at 72 °C. To normalize the variance in cDNA input, 26S rRNA was used as internal control. The significant differences between treatments were statistically evaluated by standard deviation.

Statistical analysis

Five replicates of each sample were taken on 10th, 20th, 30th day and experiments were repeated thrice. Descriptive analysis of the data was performed using SPSS 17.0. Analysis of variance (ANOVA) with comparative Duncan's multiple range tests at 5 % was used to determine the significance of differences between replicates.

Results and discussion

Effect of MSS on growth and development of *P. kurroa*

MSS having different concentrations of SWE alone varying from 0.1 to 3.0 g/L (MSS 1–MSS 4; Table 1) and in combination with growth hormones (MSS 5–MSS 8; Table 2) were studied to evaluate the potential of SWE as a biostimulant with MS media (Supplementary Table 3). MSS 3 having 2.0 g/L of SWE was found to be the best medium showing enhancement of 1.5, 2.45, 3.23 folds in biomass, 1.4, 1.55, 1.55 folds in total plant length, 1.69, 2.04, 2.42 folds in number of shoots on 10th, 20th and 30th day, respectively compared to control C1 (Figs. 1 and 2). An increment of 4.45, 2.52 folds in root length and 1.77, 2.41 folds in number of roots was observed on 20th and 30th day, respectively in comparison to control C1 (Fig. 2). Moreover, MSS with growth hormones also improved the plant growth. MSS 7 showed best results with enhancement of 1.8, 1.71, 1.92 folds in total plant biomass, 1.39, 1.5, 1.14 folds in total plant length, 1.81, 1.36, 1.47 folds in number of shoots on 10th, 20th and 30th day, respectively and an increment of 2.3, 1.43 folds in root length and 2.91, 3.5 folds in number of roots on 20th and 30th day, respectively at 25 ± 2 °C as compared to control CM (Fig. 2). Plants grown on MSS 3 showed 1.26, 1.02, 1.07, 1.18 and 2.4 folds increase in total biomass, total plant length, number of shoots, root length and number of roots, respectively after 1 month as compared to control CM (Fig. 2).

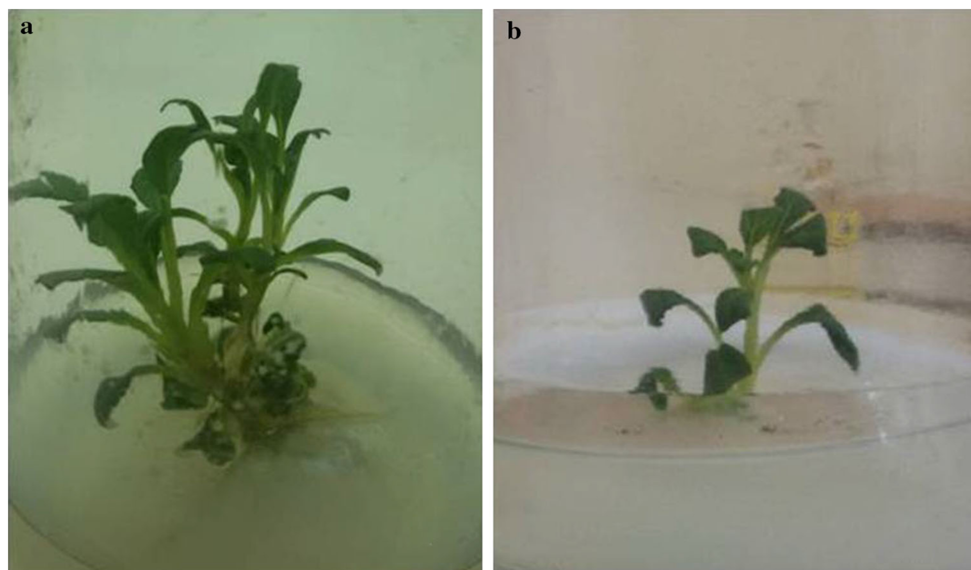

Fig. 1 Shoots grown on **a** MSS 3 (MS + sucrose + SWE + agar), **b** control C1 (MS + sucrose + agar)

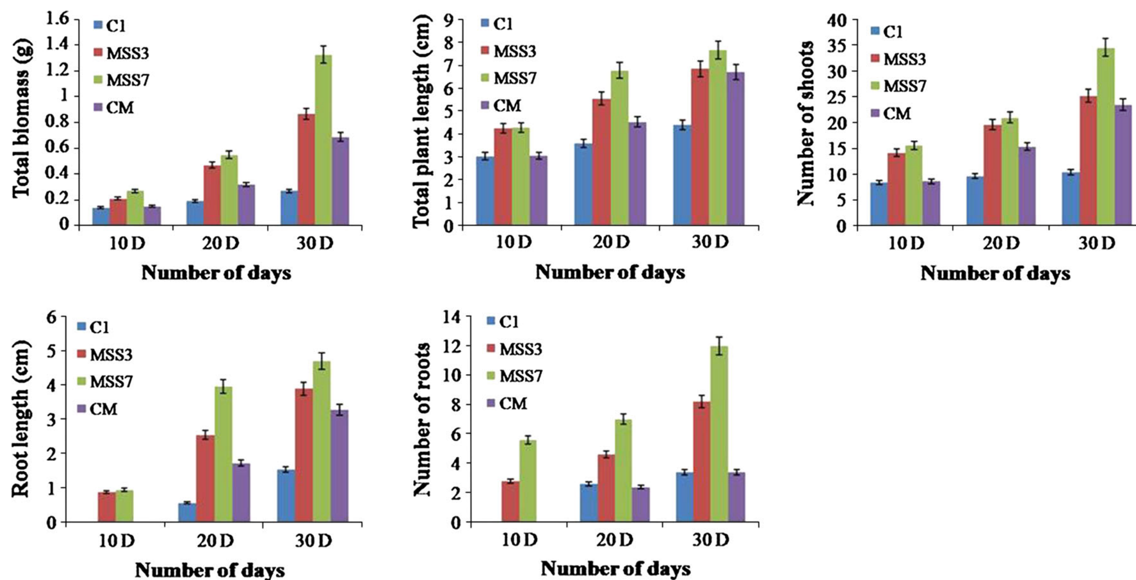

Fig. 2 Growth parameters of *P. kurroa* plants grown on C1 (MS + sucrose + agar), MSS 3 (MS + SWE + sucrose + agar), MSS 7 (MS + SWE + sucrose + IBA + KN + agar) and CM (MS + sucrose + IBA + KN + agar) at 25 ± 2 °C

Results revealed that MSS having SWE with and without growth hormones enhanced biomass, shooting and rooting in *P. kurroa* as compared to control. Previous reports have suggested that SWE contains plant growth regulators like auxins, cytokinins, gibberellins and betains which regulate plant growth and development (Hernández-Herrera et al. 2014; Satish et al. 2014). Our analysis shows a promise for reducing the use of synthetic growth hormones in tissue culture technologies for better and faster growth of plants. Similar studies have shown the effect of SWE in shoot elongation and rooting in *Lycopersicon esculentum* and *Arabidopsis thaliana* (Vinoth et al. 2012; Rayorath et al. 2008).

All media combinations from MSS 1 to MSS 8 were tested on two incubation temperatures for growth enhancement of *P. kurroa*. Plants grown at 15 ± 2 °C showed 5, 2.5 and 1.28 folds increase in leaf length, leaf width and biomass, respectively in comparison to 25 ± 2 °C, although number of shoots and roots remained the same which was in accordance to Sood and Chauhan (2009a).

SWM as an alternative media for *P. kurroa* micropropagation

Out of 6 different tested concentrations of SWE (0.01, 0.1, 1.0, 2.0, 3.0, 5.0 g/L), 2 g/L was found to be the best concentration for shoot multiplication as well as for root induction at 25 ± 2 °C (Fig. 3). Therefore, to estimate the potential of SWM as an alternative to MS media for *P. kurroa*, a comparative study was carried out by replacing

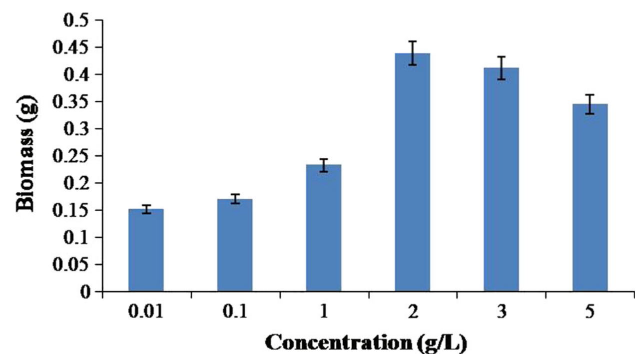

Fig. 3 Total biomass of *P. kurroa* plants grown at different concentrations of SWE (0.01–5 g/L)

MS media with optimized concentration of SWE (2 g/L) in different media combinations (Table 3). Various parameters such as total plant biomass, total plant length, number of shoots, root length and number of roots were analyzed in both solid (with agar) and liquid (without agar) media. Plants grown on liquid SWM could not survive after 20 days which might be due to oxidation and accumulation of phenolic compounds while better growth was observed on solid SWM. SWM 4 (SWE + sucrose + agar) was found to be the best among different media combinations and showed increment of 1.35, 1.63, 1.74 folds in total plant biomass, 2.11, 1.78, 2.26 folds in total plant length, 1.14, 1.42, 1.77 folds in number of shoots on 10th, 20th and 30th day, respectively as compared to control MSM 4 (MS + sucrose + agar). Early rooting (10th day) in SWM 4 with enhancement of 5.45, 4.62 folds in root length and

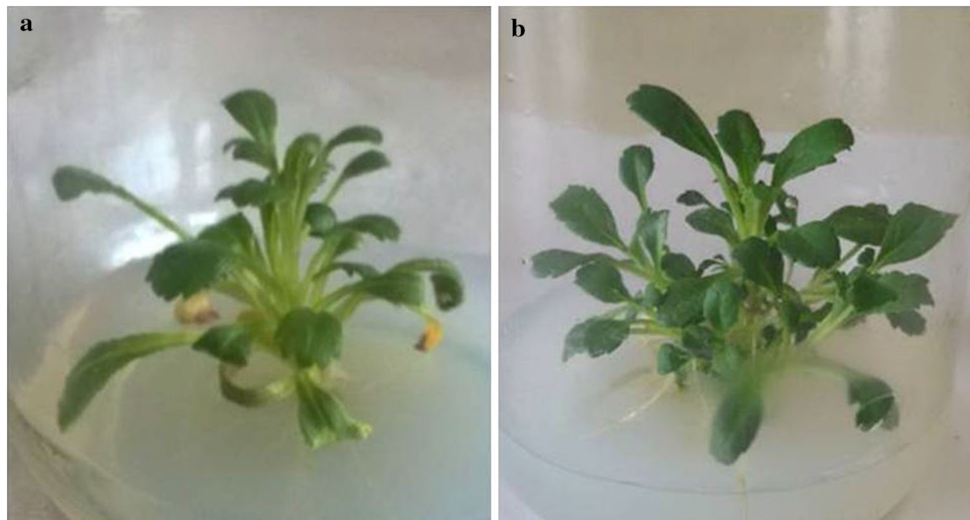

Fig. 4 *P. kurroa* shoots grown on **a** SWM 4 (SWE + sucrose + agar), **b** MSM 8 (MS + sucrose + 3 mg/L IBA + 1 mg/L KN + agar)

2.54, 4.07 folds in root number on 20th and 30th day, respectively was observed at 25 ± 2 °C compared to control MSM 4 (Supplementary Table 4). These results suggest that MS media requires additional supply of growth hormones in comparison to SWM as better growth was observed in SWM 4 than control MSM 4. Moreover, SWM 4 (SWE + sucrose + agar) showed comparable results with MSM 8 (MS + sucrose + growth hormones + agar) till 20th day, thereafter, shoot multiplication was found better in MSM 8 (Fig. 4; Supplementary Table 4). This might be attributed to faster nutrient depletion in SWM 4 as compared to MSM 8, although subculturing the same plants on SWMS 4 (subculturing the shoots again after 20 days on fresh SWM 4) exhibited similar results as obtained with MSM 8 (Fig. 5a). However, number of roots were found to be highest in SWM 4 as compared to MSM 8 (Figs. 5b, 6). Therefore, SWM can be used as an economic alternative for plant growth and micropropagation without addition of growth hormones. Findings of Hurtado et al. (2009) have shown the use of commercially available SWE based medium with and without growth hormones for regeneration of *Kappaphycus* varieties using tissue culture techniques, which is analogous to current study in *P. kurroa*. SWE has also been found to promote growth of shoots and roots in *Arabidopsis thaliana* using DR5:GUS assay which implied that commercially available SWE modulates the concentration and localization of auxins, for the enhanced plant growth (Rayorath et al. 2008).

Well rooted plantlets grown in vitro were transferred to pots containing mixture of sand:soil:vermiculite (1:1:1) in the greenhouse for hardening and showed survival rate of 80 % in the green house conditions (see materials and methods).

In vitro production of P-I

P-I is responsible for various medicinal properties of *P. kurroa*. Previous studies reported the effect of light and temperature on in vitro grown shoots of *P. kurroa* which showed higher P-I content at 15 ± 2 °C as compared to 25 ± 2 °C (Kawoosa et al. 2010; Sood and Chauhan 2010). So, effect of SWE as a biostimulant and as a media was estimated for P–I production at 25 ± 2 and 15 ± 2 °C. One month old plants grown on MSS (MS + sucrose + 2 g/L SWE + agar), SWM (2 g/L SWE + sucrose + agar) and CM (MS + sucrose + 3 mg/L IBA + 1 mg/L KN + agar) at 25 ± 2 and 15 ± 2 °C were subjected to HPLC analysis for the P-I estimation. Our results showed enhanced P-I content at both incubation temperatures. Plants grown on MSS showed highest P-I accumulation with increase of 3.8 and 2.62 folds followed by SWM with 3.0 and 2.4 folds as compared to plants grown on CM at 25 ± 2 and 15 ± 2 °C, respectively (Fig. 7). Our results were also in conjunction with the previous reports where SWE treatment has increased the total phenolic and flavonoid contents in *Brassica oleraceae* and *Kappaphycus* Doty (Lola-Luz et al. 2013; Hurtado et al. 2012). Thus, SWE can be utilized for enhancing P-I production in in vitro cultures of *P. kurroa* for development of economically viable strategies to meet increasing industrial demands.

Expression analysis of rate limiting enzymes vis-á-vis P- I content

HMGR and DXPS are known to act as important control points in MVA and MEP pathways for P-I production in *P.*

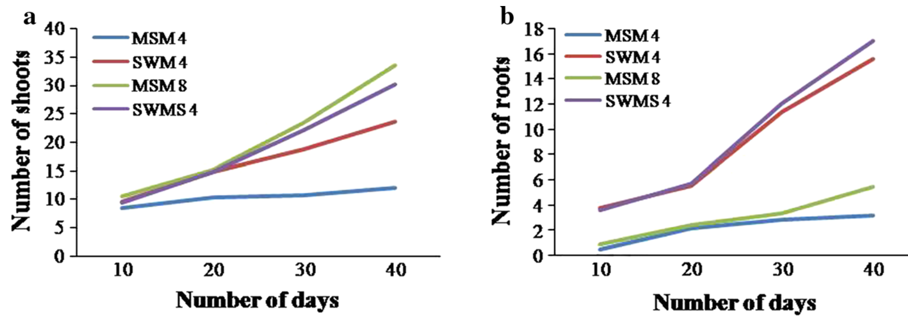

Fig. 5 Effect of different media on **a** number of shoots and **b** number of roots. *P. kurroa* plants grown on MSM 4 (MS + sucrose + agar), SWM 4 (SWE + sucrose + agar), MSM 8 (MS + sucrose + 3 mg/L IBA + 1 mg/L KN + agar), SWMS 4 (Plants subcultured again after 20 days on fresh SWM 4 media)

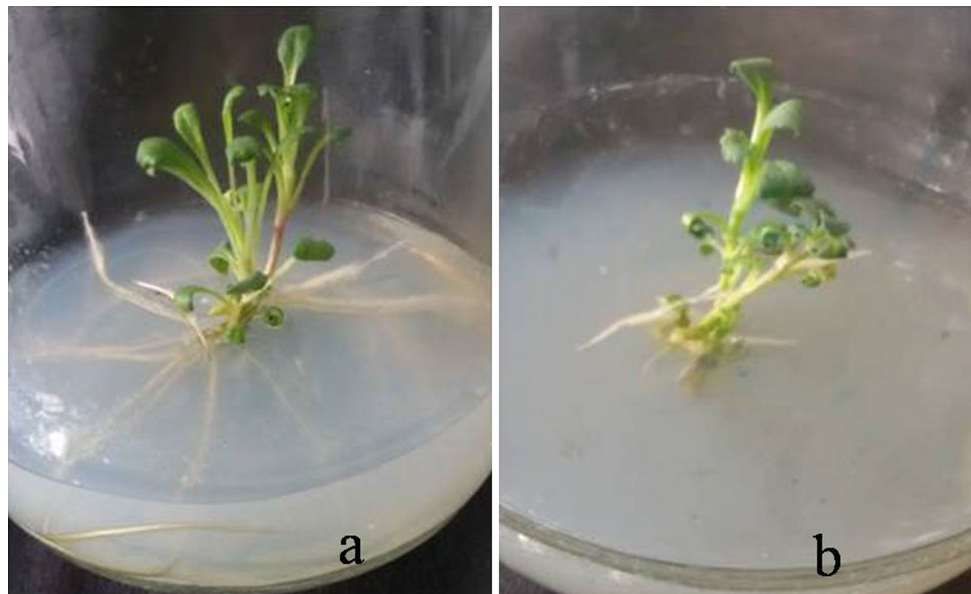

Fig. 6 Rooting of in vitro grown plants on **a** SWM 4, **b** MSM 8

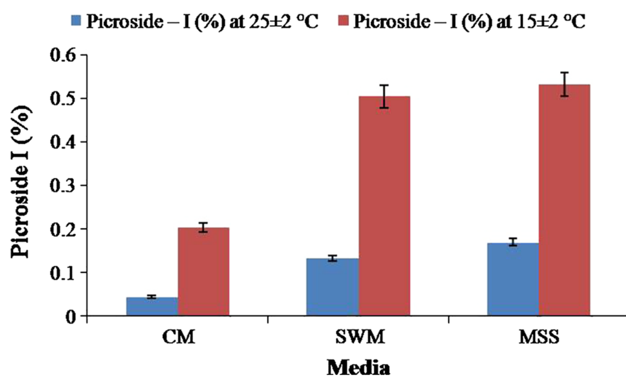

Fig. 7 HPLC quantification of P-I for the plants grown on CM (control media), SWM (SWE media) and MSS (MS media supplemented with SWE) at 25 ± 2 and 15 ± 2 °C

kurroa (Kawoosa et al. 2010). Expression analysis of HMGR and DXPS was done for plants grown on MSS, SWM and CM at two different incubation temperatures, 25 ± 2 and 15 ± 2 °C. DXPS showed higher expression in plants maintained on MSS with 210.5 and 112.98 folds, followed by SWM with 130.69 and 102.53 folds at 25 ± 2 and 15 ± 2 °C, respectively as compared to CM at 25 ± 2 °C. Similarly, HMGR also showed higher expression in plants grown on MSS with 50.21 and 36.13 folds, followed by SWM with 39.12 and 31.56 folds at 25 ± 2 and 15 ± 2 °C, respectively as compared to CM at 25 ± 2 °C (Fig. 8). The expression pattern of these genes was found to be in correlation with P-I content and in agreement with findings of Kawoosa et al. (2010) showing higher expression of genes encoding HMGR and DXPS

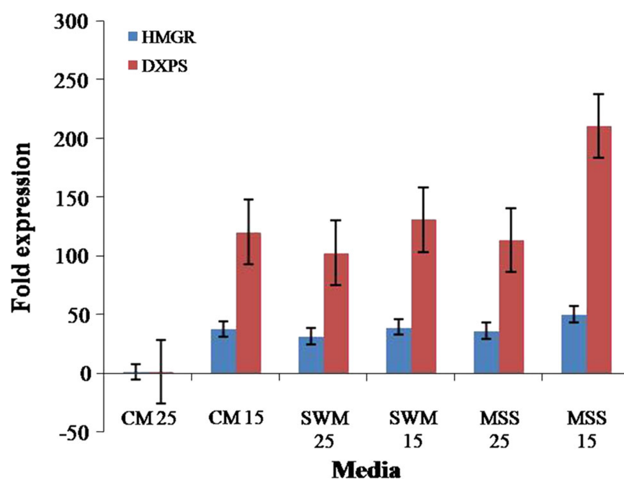

Fig. 8 Expression status of HMGR and DXPS genes of MVA and MEP pathways for the plants grown on CM (control media), SWM (SWE media) and MSS (MS media supplemented with SWE) at two incubation temperatures 25 ± 2 and 15 ± 2 °C

enzymes at 15 °C as compared to 25 °C for P-I production. Similarly, growth hormone such as ethylene has been found to up-regulate HMGR gene activity for diosgenin biosynthesis in in vitro cultures of *Dioscorea zingiberensis* (Diarra et al. 2013).

Cost estimation

The current study was conducted by taking single shoot apex in each jar (50 ml media) in replicates of five and the experiment was repeated 3 times for each treatment. The cost of MSS, SWM and CM was estimated to be Rs. 8.41, 4.31 and 7.46 per jar, respectively. Their components include- MS (HiMedia) Rs. 82/L, sucrose (HiMedia) Rs. 18/L, agar (HiMedia) Rs. 48.27/L, growth hormones (HiMedia) Rs. 1.05/L and SWE (Sea6 Energy) Rs. 20/L. Number of shoots formed in 50 ml of MSS, SWM and CM were 34.6, 18.80 and 23.51, respectively. Therefore, the cost of medium for growing one shoot came out to be Rs. 0.243, 0.229 and 0.317 in MSS, SWM and CM, respectively. These statistics demonstrate that SWE as a biostimulant and as an alternative media offer low cost multiplication of *P. kurroa* plants for industrially important phytochemicals.

Conclusion

Our results revealed that MS media having SWE alone and in combination with growth hormones significantly increased the total biomass production, total plant length, number of shoots, root length and number of roots in *P. kurroa* plants. SWM showed comparable results with CM, therefore it can be used as an alternative to MS media for large scale micropropagation of *P. kurroa.* MSS and SWM increased the P-I production by 2–4 folds at 15 ± 2 and 25 ± 2 °C, thereby, suggesting that SWE can be used as a novel elicitor for enhanced P–I production under in vitro conditions for *P. kurroa*. Up regulated expression of DXPS and HMGR has further validated the role of SWE in enhancing the P–I production. Hence, it can be utilized for commercial production of secondary metabolites and conservation of *P. kurroa* through micropropagation technique. Further studies targeting biochemical and molecular processes can unravel the mechanism behind growth enhancement and metabolite elicitation by SWE.

Acknowledgments The authors are thankful to the Jaypee University of Information Technology for providing the necessary research facilities and Department of Biotechnology, Ministry of Science and Technology, Govt. of India for providing research grant in the form of a programme support on high value medicinal plants to RSC and HS. We are also thankful to the Himalayan Forest Research Institute (HFRI), Manali, India for providing plant material of *P. kurroa*. The authors also gratefully acknowledge Sea6 Energy Pvt Ltd. for providing the seaweed extract powder.

Conflict of interest Authors declare that they have no conflict of interest.

References

Ahmed YM, Shalaby EA (2012) Effect of different seaweed extracts and compost on vegetative growth, yield and fruit quality of cucumber. J Hortic Sci Ornam Plants 4:235–240

Anisimov MM, Chaikina EL (2014) Effect of seaweed extracts on the growth of seedling roots of soybean (*Glycine max* (L.) Merr.) seasonal changes in the activity. Int J Curr Res Acad Rev 2:19–23

Bhandari P, Kumar N, Singh B, Gupta A, Kaul V, Ahuja P (2009) Stability-indicating LC–PDA method for determination of picrosides in hepatoprotective Indian herbal preparations of *Picrorhiza kurroa*. J Chromatogr 69:221–227

Craigie JS (2011) Seaweed extract stimuli in plant science and agriculture. J Appl Phycol 23:371–393

Diarra ST, He J, Wang J, Li J (2013) Ethylene treatment improves diosgenin accumulation in in vitro cultures of *Dioscorea zingiberensis* via up-regulation of CAS and HMGR gene expression. Electron J Biotechnol. doi:10.2225/vol16-issue5-fulltext-9

Hernández-Herrera RM, Santacruz-Ruvalcaba F, Ruiz-López MA, Norrie J, Hernández-Carmona G (2014) Effect of liquid seaweed extracts on growth of tomato seedlings (*Solanum lycopersicum* L.). J Appl Phycol 26:619–628

Hurtado AQ, Yunque DA, Tibubos K, Critchley AT (2009) Use of Acadian marine plant extract powder from *Ascophyllum nodosum* in tissue culture of *Kappaphycus* varieties. J Appl Phycol 21:633–639

Hurtado AQ, Joe M, Sanares RC, Fan D, Prithiviraj B, Critchley AT (2012) Investigation of the application of Acadian marine plant extract powder (AMPEP) to enhance the growth, phenolic content, free radical scavenging, and iron chelating activities of

Kappaphycus Doty (Solieriaceae, Gigartinales, Rhodophyta). J Appl Phycol 24:601–611

Kawoosa T, Singh H, Kumar A, Sharma SK, Devi K, Dutt S, Vats SK, Sharma M, Ahuja PS, Kumar S (2010) Light and temperature regulated terpene biosynthesis: hepatoprotective monoterpene picroside accumulation in *Picrorhiza kurroa*. Funct Integr Genomics 10:393–404

Khan W, Rayirath UP, Subramanian S, Jithesh MN, Rayorath P, Hodges DM, Critchley AT, Craigie JS, Norrie P, Prithiviraj B (2009) Seaweed extracts as biostimulants of plant growth and development. J Plant Growth Regul 28:386–399

Kumar G, Sahoo D (2011) Effect of seaweed liquid extract on growth and yield of *Triticum aestivum* var Pusa Gold. J Appl Phycol 23:251–255

Lola-Luz T, Hennequart F, Gaffney M (2013) Enhancement of phenolic and flavonoid compounds in cabbage (*Brassica oleraceae*) following application of commercial seaweed extracts of the brown seaweed, (*Ascophyllum nodosum*). Agric Food Sci 22:288–295

Murashige T, Skoog F (1962) A revised medium for rapid growth and bioassays with tobacco tissue culture. Physiol Plant 15:473–497

Nayar MP, Sastri ARK (1990) Red data plants of India. CSIR Publication, New Delhi, p 271

Pandit S, Shitiz K, Sood H, Naik PK, Chauhan RS (2012) Expression pattern of fifteen genes of non-mevalonate (MEP) and mevalonate (MVA) pathways in different tissues of endangered medicinal herb *Picrorhiza kurroa* with respect to picrosides content. Mol Biol Rep 48:295–303

Papenfus HB, Stirk WA, Finnie JF, Van Staden J (2012) Seasonal variation in the polyamines of *Ecklonia maxima*. Bot Mar 55:539–546

Patial V, Devi K, Sharma M, Bhattacharya A, Ahuja PS (2012) Propagation of *Picrorhiza kurroa* Royle ex Benth: an important medicinal plant of Western Himalaya. J Med Plant Res 6:4848–4860

Rawat B, Rawat JM, Mishra S, Mishra SN (2013) *Picrorhiza kurrooa*: current status and tissue culture mediated biotechnological interventions. Acta Physiol Plant 35:1–12

Rayorath P, Jithesh MN, Farid A, Khan W, Palanisamy R, Hankins SD, Critchley AT, Prithiviraj B (2008) Rapid bioassays to evaluate the plant growth promoting activity of *Ascophyllum nodosum* (L.) Le Jol. using a model plant, *Arabidopsis thaliana* (L.) Heynh. J Appl Phycol 20:423–429

Rengasamy KRR, Kulkarni MG, Stirk WA, Van Staden J (2015) Eckol—a new plant growth stimulant from the brown seaweed *Ecklonia maxima*. J Appl Phycol 27:581–587

Sah JN, Varshney VK (2013) Chemical constituents of *Picrorhiza* genus: a review. American J Ess Oils Nat Products 1:22–37

Satish L, Rameshkumar R, Rathinapriya P, Pandian S, Rency AS, Sunitha T, Ramesh M (2014) Effect of seaweed liquid extracts and plant growth regulators on in vitro mass propagation of brinjal (*Solanum melongena* L.) through hypocotyl and leaf disc explants. J Appl Phycol. doi:10.1007/s10811-014-0375-6

Shitiz K, Pandit S, Chauhan RS, Sood H (2013) Picrosides content in the rhizomes of *Picrorhiza kurroa* Royle ex. Benth. traded for herbal drugs in the markets of North India. Int J Med Aromat Plants 3:226–233

Sood H, Chauhan RS (2009a) Development of a low cost micropropagation technology for an endangered medicinal herb (*Picrorhiza kurroa*) of North-Western Himalayas. J Plant Sci 4:21–31

Sood H, Chauhan RS (2009b) High frequency callus induction and plantlet regeneration from different explants of *Picrorhiza kurroa*-a medicinal herb of Himalayas. Afr J Biotech 8:1965–1972

Sood H, Chauhan RS (2010) Biosynthesis and accumulation of a medicinal compound, Picroside-I, in cultures of *Picrorhiza kurroa* Royle ex Benth. Plant Cell Tissue Organ Cult 100:113–117

Stirk WA, Tarkowská D, Turečová V, Strnad M, Van Staden J (2014) Abscisic acid, gibberellins and brassinosteroids in Kelpak®, a commercial seaweed extract made from *Ecklonia maxima*. J Appl Phycol 26:561–567

Vinoth S, Gurusaravanan P, Jayabalan N (2012) Effect of seaweed extracts and plant growth regulators on high-frequency in vitro mass propagation of *Lycopersicon esculentum* L (tomato) through double cotyledonary nodal explants. J Appl Phycol 24:1329–1337