In vitro propagation and analysis of mixotrophic potential to improve survival rate of Dolichandra unguis-cati under ex vitro conditions

Vineet Soni¹,*¹, Kiran Keswani b, Upma Bhatt a, Deepak Kumar a, Hanwant Singh a

¹ Plant Bioenergetics & Biotechnology Laboratory, Department of Botany, Mohanlal Sukhadia University, Udaipur 313001, Rajasthan, India
² Rajiv Gandhi Institute of IT and Biotechnology, Bharati Vidyapeeth University, Pune 411045, Maharashtra, India

A R T I C L E I N F O

Keywords:
In vitro propagation
Chlorophyll a fluorescence
JIP test
Fv/Fm
Performance index

A B S T R A C T

An efficient and reproducible protocol for in vitro propagation of Dolichandra unguis-cati has been established for the first time from nodal segments. In order to enhance survival rate under ex vitro conditions, photosynthetic potential of in vitro grown plantlets was also studied through JIP test based analysis of polyphasic OJIP chlorophyll a fluorescence OJIP transients, density of active reaction centers, light harvesting efficiency, electron transfer rate, dissipation energy, maximum quantum yield of primary PSII photochemistry and photosynthetic performance index. The best morphogenetic in term of explants response (92.2 %), shoot number (3.43 ± 0.07) and shoot length (4.7 ± 0.31 cm) was obtained on Murashige and Skoog medium supplemented with 0.5 mg l⁻¹ BAP and 1.0 mg l⁻¹ TDZ. The shoots exhibited high frequency rhizogenesis on half strength medium augmented with 2.0 mg l⁻¹ IAA. In vitro plantlets developed highest rate of photosynthesis on day 18 after the initiation of rhizogenesis. High survival rate (96.16%) under ex vitro conditions was observed when in vitro plantlets having high photosynthetic efficiency (Fv/Fm > 0.75) were subjected to hardening and acclimatization process. Plantlets with reduced photosynthetic performance exhibited low survival rate under natural conditions. The developed in vitro protocol will be useful for genetic improvement and multiplication of Dolichandra unguis-cati. The results of this study also show that photosynthetic screening of in vitro developed plantlets is highly essential after the rhizogenesis process to achieve higher survival rate under field conditions.

1. Introduction

Dolichandra unguis-cati (L.) Lohmann (Bignoniaceae) commonly known as ‘cat’s claw’ is a perennial rampant liana which is found in diverse regions of the globe like Brazil, South America, Egypt, Mexico to northern Argentina and western India [1, 2]. The plant has been extensively utilized since time immemorial by the ancient Indian system of medicines for combating several ailments like dysentery and stomach bloating [3], flu, arthrosis, bronchitis, splenosis, headache [4], snake bite, diarrhea, fever, inflammatory reactions [5], rheumatism [6], uterus infection and cysts [7] due to the activities of diverse bioactive molecules like corimboside, vicenin-2, O-flavonol, chlorogenic acid, lupeol, vanillinic acid, quercetin, β-sitosterol, isochlorogenic acid, p-coumaric acid, β-sitosterol glycoside, lapachol, allantoin, decaffeoylacteoside, yoniresinol-3α-O-β-d-glucopyranoside, cirsimarin, cirsimaritin, caffeic acid, ferulic acid, transcinnamic acid, luteolin, apigenin, rosmarinic acid, quercitrin and quercetin ursolic acid [2, 5, 8, 9]. Conventionally, D. unguis-cati is propagated by subterranean tubers and seeds [10, 11]. However, seasonal dependency of seed germination makes it an ineffective way for the conservation of this plant species [12]. Furthermore, development of in vitro protocol is one of the essential steps of genetic improvement and transformation of this high valued plant species. Therefore, immediate attention is highly required for the development of an efficient and reproducible in vitro protocol for mass propagation and genetic improvement of D. unguis-cati.

In vitro propagation of plants is an alternative for rapid and large-scale production of plants under control conditions [13]. The ultimate success of in vitro protocols relies on the ability to transfer the plantlets from in vitro to the ex vitro conditions with a high survival rate [14]. Low survival rate during hardening and acclimatization is the major constraint in the large-scale production of micropropagated plantlets [15]. The high concentration of exogenous sucrose in the nutrient medium induces photosynthetic down-regulation in plantlets raised under in vitro conditions [16, 17]. Therefore, prior to transfer under ex vitro conditions, photosynthetic performance of in vitro developed plantlets should be screened.

* Corresponding author.
E-mail address: vineetsonijn@gmail.com (V. Soni).

https://doi.org/10.1016/j.heliyon.2021.e06101
Received 3 April 2020; Received in revised form 22 June 2020; Accepted 22 January 2021
2405-8440/© 2021 The Author(s). Published by Elsevier Ltd. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).
Chlorophyll fluorescence measurements provide valuable information of the physiological condition of photosystem II and components of photosynthetic electron transport chain [18] and have been widely used to screen photosynthetic performance of plantlets growing under *in vitro* conditions [19, 20, 21]. The JIP-test, based on the rise in polyphasic OJIP chlorophyll fluorescence, provides in depth information on the status and function of PSII reaction centers, antenna, as well as on donor and acceptor sides of PSII [22, 23, 24]. The O–J phase indicates the status of PSII, while the J–I step indicates the performance of the QB, plastoquinone, cytochrome b6f, and plastocyanin. The I–P part of the OJIP induction curve is correlated to the reduction of electron transporters of the PSI acceptor side [25].

Present study was aimed (1) to develop a reproducible protocol for *in vitro* regeneration of *D. unguis-cati* and (2) to perform photosynthetic screening of *in vitro* grown plantlets to achieve high survival rate under ex *vitro* conditions.

2. Materials and methods

2.1. Plant material and surface sterilization

Young shoots of Dolichandra unguis-cati (L.) G.L.Ohmann (Bignoniaceae) were harvested from Botanical Garden of Department of Botany, Mohanlal Sukhadia University, Udaipur, India. The excised shoots were washed thoroughly under running tap water for 3 min to eliminate dust particles and then treated with 0.1% bavistin and rinsed twice with sterile distilled water. Thereafter, surface sterilization of explants was done under a laminar flow chamber with aqueous solution of 0.1% (w/v) HgCl₂ for 3 min. After rinsing with double distilled water, nodal segments were cut into small pieces (2 cm) and used as the explants.

2.2. Culture media and growth conditions

The sterilized nodal explants were placed vertically on solid MS Medium [26] supplemented with 3 % sucrose, 0.8% (w/v) agar (Hi-Media, India) and various combinations/concentrations of plant growth regulators. The pH of the media was adjusted 5.8 before autoclaving at 121 °C for 15 min. All cultures were kept in a growth chamber at 25 ± 2 °C, 65–70% relative humidity with photoperiod of 16-h using a photosynthetic photon flux density (PPFD) of 40 mmol m⁻²s⁻¹ provided by cool white fluorescent tube lights (Philips, India). After 4 weeks of culture response percentage of the explants, numbers of shoots per explant, length of the shoots were evaluated.

2.3. In vitro rhizogenesis

Well developed shoots were subcultured on MS enriched with various concentrations (0.1–5.0 mg ⁻¹) of auxins viz. IBA, IAA and NAA to induce rhizogenesis in vitro. Prior to hardening process, the photosynthetic performance of well rooted plantlets was regularly measured by the analysis of polyphasic chlorophyll a fluorescence kinetics.

2.4. Measurement of photosynthetic performance

Plant Efficiency Analyser, PEA (Hansatech Instruments, U.K.) was used to analyze the photosynthetic potential of *in vitro* developed plantlets. Before the measurements, well developed plantlets were kept to darkness for 1 h. Fluorescence transients were induced over a leaf-lamina area of 4 mm diameter by a red light of 3000 μmol m⁻²s⁻¹ provided by a high intensity LED array of three light emitting diodes. A total measuring time of one second was used thought out the experiments. Fluorescence values were used to calculate phenomenological fluxes (ABS/CSm, ETo/CSm and DIo/CSm), RC/CSm, Fv/Fm (ΦPo) and performance index on cross section basis (PIcs) using following equations (see Eqs. (1), (2), (3), (4), (5), and (6)) of JIP test [27, 28] –

\[
\text{ABS} / \text{CSm} = \text{Fluorescence intensity at 50μs (Fo)}
\]

\[
ETo / \text{CSm} = \PhiPo \times (\text{ABS} / \text{CS})
\]

\[
DIo / \text{CSm} = (\text{ABS} / \text{CS}) - [\PhiPo \times (\text{ABS} / \text{CS})]
\]

\[
\PhiPo = 1 - \frac{(\text{Fo} / \text{Fm}) \text{ or Fv/Fm}}{(\text{Fo} / \text{Fm})}
\]

\[
\text{RC} / \text{CSm} = \PhiPo \times (\text{Vj} / \text{Mo}) \times (\text{ABS} / \text{CSm})
\]

\[
\text{PIcs} = \frac{\text{ABS}}{\text{CS}} \frac{\text{Mo/Vj}}{\text{Fo/Vj}} \frac{\text{Fo}}{\text{Vj}}
\]

where \(\Phi_0\) is calculated as 1- \(V_j\) (Vj is relative variable fluorescence at the J-step and calculated as \(F_{2ms} - F_0)/(F_M - F_0)\), Fv is variable fluorescence between Fm and Fo and \(M_0\) (approximated initial slope of the fluorescence transient) is calculated as \(4 \times (F_{300ms} - F_0)/(F_M - F_0)\).

2.5. Hardening and acclimatization

Only plantlets with functional photosynthetic apparatus and autotrophic potential were transplanted to plastic cups containing autoclaved soil, sand and coco peat (1:2:1) and then kept for 2 weeks in same growth chamber. On the other hand, plantlets with low photosynthetic potential were maintained on nutrient medium until the development of functional photosynthetic apparatus and autotrophic potential. The hardened plantlets were watered once a week. After hardening, the plantlets were subsequently transferred and maintained in the green net house (50% light transparency) with relative humidity 40–50% and 32 ± 2 °C.

2.6. Experimental design and statistical analysis

All tissue culture experiments were conducted with a minimum of 30 replicates per treatment and each experiment was repeated thrice. All data were analyzed statistically using GraphPad Prism 8. Differences were considered significant when the p value was <0.05. Photosynthetic data were analyzed using Biolzyzer Software ver. 3.06 [29].

3. Results and discussion

3.1. *In vitro* establishment and multiplication

BAP at 0.5–5.0 mg l⁻¹ concentrations could not evoke any significant morphogenetic response in nodal explants. After one week of inoculation on MS fortified with 0.5 mg l⁻¹ BAP, explants showed swelling. At elevated concentration of BAP (5.0 mg l⁻¹), formation of single shoot bud followed by the swelling was observed after 11 days of culture (Figure 1a). The shoots emerged on BAP containing medium failed to elongate. Low frequency of shoot bud proliferation was noted on MS augmented with KN at high concentration (5.0 mg l⁻¹) (Figure 1b). In the present studies, KN at low 0.5 mg l⁻¹ was found most effective in multiple shoot bud induction from nodal explants.

TDZ, a cytokinin-like compound, promotes a diverse array of morphogenic responses including shoot bud proliferation [30, 31]. Apart from its cytokinin-like activity, TDZ plays important role in modulation of endogenous hormone levels especially auxin/cytokinin ratio [32]. In the present study, our results revealed that the effect of KN and TDZ combination on multiple shoot bud proliferation is more compared to KN and TDZ when used separately. The best morphogenetic response in the present studies, KIN at low 0.5 mg l⁻¹ was found most effective in multiple shoot bud induction from nodal explants.
3.2. In vitro rhizogenesis

Inadequate rooting is one of the major constraints to the survival rate of plantlets under *ex vitro* conditions [34]. In the present study, Full strength MS singly or in combination with various concentrations of IAA (1.0–3.0 mg l⁻¹) could not evoke significant response in term of rhizogenesis *in vitro*. Half strength MS supplemented with IAA proved best in term of initiating rhizogenesis *in vitro* as compared to full strength MS (Table 2). Half strength MS supplemented with low concentration of IAA (<1.0 mg l⁻¹) could promote rhizogenesis *in vitro* at low frequency. Half strength MS along with IAA 2.0 mg l⁻¹ exhibited highest mean number of roots per shoot (11.40) and increased root length to the maximum (4.03 cm) (Figure 1d), while other concentrations of IAA caused antagonist effect on rhizogenesis in *D. unguis-cati*. Our results are in accordance with the studies carried out on *Cichorium intybus* [35], *Digitalis lanata* [36], *Prosopis laevigata* [37] and *Securidaca longipedunculata* [38]. The superiority of IAA over other auxins for induction of rhizogenesis has also been reported in *Vanda pumila* [39], *Dendrobium chryseum* [40], *Phyllanthus tenellus* [34].

3.3. Chlorophyll *a* fluorescence analysis

After the visible appearance of root primodia on half strength MS + IAA 2.0 mg l⁻¹, the polyphasic chlorophyll *a* fluorescence analysis was done prior to hardening process to evaluate the photosynthetic potential of *in vitro* developed plantlets. Fluorescence parameters ($F_{0}$, $F_{m}$), phenomenological energy fluxes, RC/CSm, $\Phi_{P}$ and Ples remarkably altered with increasing days after root primodia formation (DAR). During the initiation days of rhizogenesis (days 0–6), the plantlets failed to form a complete OJIP curve. On 12 and 18 DAR, *chl a* fluorescence OJIP curve of *in vitro* grown plantlets showed three apparent intermediate phases namely OJ, JI and IP (Figure 2a). Photochemical phase OJ [27]

### Table 1. Influence of KIN and TDZ on explant response (%) and shoot length (cm) after 3 weeks of culture of nodal explants of *D. unguis-cati*. Values within the columns are highly significant at $p < 0.05$ and represented as mean of 30 replicates ± SD. Different characters indicate significant differences among the results ($p < 0.05$).

| Plant growth regulators (mg l⁻¹) | Explant response (%) | Mean shoot length (cm) | Shoots per node |
|-------------------------------|----------------------|------------------------|-----------------|
| KIN  | TDZ    |                |                       |                |
| Control | 0.0 | 0.0 | 0.0 | 0.0 |
| 0.0 | 0.5 | 0.0 | 0.0 | 0.0 |
| 0.0 | 1.0 | 0.0 | 0.0 | 0.0 |
| 0.0 | 2.5 | 0.0 | 0.0 | 0.0 |
| 0.5 | 0.0 | 52.0 ± 3.5 | 2.8 ± 0.02 | 2.66 ± 0.52 |
| 0.5 | 0.5 | 51.21 ± 1.7 | 2.9 ± 0.06 | 2.63 ± 0.65 |
| 0.5 | 1.0 | 92.2 ± 4.4 | 4.7 ± 0.31 | 4.33 ± 0.50 |
| 0.5 | 2.5 | 13.81 ± 2.3 | 0.9 ± 0.06 | 1.41 ± 0.24 |

(Table 1). High cytokinin activity of TDZ inhibits in vitro shoot bud proliferation [33].
and the thermal phase JI [41] are linked to the accumulation of QA and the status of PSII RCs. IP phase displays PQ pool reduction [25, 42]. The minimal (Fo) and maximal (Fm) fluorescence values Fo and Fm, respectively, were significantly increased in plantlets with increasing subculture duration on RIM as ES get exhausted over time. ABS/CSm which represents light harvesting efficiency of active PSII RCs, enhanced progressively and reached to highest level on 18 DAR. Reduced values of ABS/CSm during the emergence of root promidia indicate reduced antenna size and low chl concentration. Similarly, ETo/CSm progressively increased with progression of subculture duration on RIM. Reduced values of ABS/CSm and ETo/CSm during the appearance of root promidia (0 DAR) shows the antagonist effect of higher ES concentration on light harvesting and electron transfer potential in Dolichandra unguis-cati. Decline in DIo/CSm with progression of subculture duration on RIM was associated with reduced light harvesting potential (ABS/CSm). The effects of subculture duration on RIM on phenomenological energy fluxes (ABS/CSm, ETo/CSm and DIo/CSm) are diagrammatically represented through the leaf models.

Dissociated light harvesting complexes [43] and undeveloped photosynthetic apparatus during the initial days of rhizogenesis. The values of Fo apparently increased with increasing the subculture duration or sucrose consumption rate on RIM. Sucrose acts as fuel source for growth and development of in vitro plantlets. The decreased Fm level and disappearance of O-J and J-I phases indicates the presence of dissociated light harvesting complexes [43] and the thermal phase JI [41] are linked to the accumulation of QA and the status of PSII RCs. IP phase displays PQ pool reduction [25, 42]. The minimal (Fo) and maximal (Fm) fluorescence values Fo and Fm, respectively, were significantly increased in plantlets with increasing subculture duration on RIM as ES get exhausted over time. ABS/CSm which represents light harvesting efficiency of active PSII RCs, enhanced progressively and reached to highest level on 18 DAR. Reduced values of ABS/CSm during the emergence of root promidia indicate reduced antenna size and low chl concentration. Similarly, ETo/CSm progressively increased with progression of subculture duration on RIM. Reduced values of ABS/CSm and ETo/CSm during the appearance of root promidia (0 DAR) shows the antagonist effect of higher ES concentration on light harvesting and electron transfer potential in Dolichandra unguis-cati. Decline in DIo/CSm with progression of subculture duration on RIM was associated with reduced light harvesting potential (ABS/CSm). The effects of subculture duration on RIM on phenomenological energy fluxes (ABS/CSm, ETo/CSm and DIo/CSm) are diagrammatically represented through the leaf models.

Dissociated light harvesting complexes [43] and undeveloped photosynthetic apparatus during the initial days of rhizogenesis. The values of Fo apparently increased with increasing the subculture duration or sucrose consumption rate on RIM. Sucrose acts as fuel source for growth and development of in vitro plantlets. The decreased Fm level and disappearance of O-J and J-I phases indicates the presence of dissociated light harvesting complexes [43] and the thermal phase JI [41] are linked to the accumulation of QA and the status of PSII RCs. IP phase displays PQ pool reduction [25, 42]. The minimal (Fo) and maximal (Fm) fluorescence values Fo and Fm, respectively, were significantly increased in plantlets with increasing subculture duration on RIM as ES get exhausted over time. ABS/CSm which represents light harvesting efficiency of active PSII RCs, enhanced progressively and reached to highest level on 18 DAR. Reduced values of ABS/CSm during the emergence of root promidia indicate reduced antenna size and low chl concentration. Similarly, ETo/CSm progressively increased with progression of subculture duration on RIM. Reduced values of ABS/CSm and ETo/CSm during the appearance of root promidia (0 DAR) shows the antagonist effect of higher ES concentration on light harvesting and electron transfer potential in Dolichandra unguis-cati. Decline in DIo/CSm with progression of subculture duration on RIM was associated with reduced light harvesting potential (ABS/CSm). The effects of subculture duration on RIM on phenom

Table 2. Changes in various photosynthetic parameters with increasing the subculture duration after the initiation of root-primodia (days after rhizogenesis-DAR) on RIM. Values within the columns are highly significant at p < 0.05 and represented as mean of 30 replicates ± SD. Different characters indicate significant differences among the results (p < 0.05).

| Media Combinations | % Rooting | Mean no. of root/shoot | Mean root length (cm) |
|-------------------|-----------|-----------------------|----------------------|
| MS full strength  | 0d        | 0d                    | 0d                   |
| MS full strength + 1.0 mg l⁻¹ IAA | 13 ± 3.1 † | 1.16 ± 0.6 † | 1.02 ± 0.4 ⁎       |
| MS full strength + 2.0 mg l⁻¹ IAA | 26 ± 2.6 ^ | 4.16 ± 1.0 ^ | 2.81 ± 0.9 †       |
| MS full strength + 3.0 mg l⁻¹ IAA | 21 ± 3.2 ^ | 4.14 ± 0.8 ^ | 2.87 ± 0.9 †       |
| MS half strength  | 8 ± 1.8 † | 2.82 ± 0.4 † | 1.01 ± 0.4 †       |
| MS half strength + 1.0 mg l⁻¹ IAA | 57 ± 3.2 ° | 4.73 ± 1.0 ° | 1.42 ± 0.3 †       |
| MS half strength + 2.0 mg l⁻¹ IAA | 94 ± 4.8 ° | 11.40 ± 2.4 ° | 4.03 ± 0.8 °       |
| MS half strength + 3.0 mg l⁻¹ IAA | 39 ± 4.1 ° | 4.72 ± 1.8 ° | 1.72 ± 0.6 †       |

Figure 2. Leaf models showing changes in ABS/CSm, ETo/CSm and DIo/CSm and RC/CSm with increasing days after the emergence of root promidia (DAR) on rooting inducing medium (blacks dots represent inactive PSII RCs).
by many researchers [44, 45, 46, 47], and is consistent with Koch’s theory on the inhibitory influence of sugars on photosynthesis [48]. Exogenous sugar inhibits the expression of photosynthetic genes and reduces the activities of enzymes involved in CBB cycle [16]. Therefore, photosynthetic screening of in vitro grown plantlets is highly required before transferring plants from growth chamber to ex vitro conditions.

3.4. Hardening and acclimatization

Rooted plantlets were having high photosynthesis in term of ABS/CSm, ETO/CSm, Dl0/CSm, RC/CSm, ΔΦo and Plcsm showed high survival rate (96.16%) during hardening and acclimatization process (Figure 2e, f). In vitro grown plantlets with reduced photosynthetic potential showed declined rate of survival under natural conditions. Plants produced through in vitro method exhibited similar morphologically to mother plants. The results of this study show that photosynthetic screening of in vitro developed plantlets is highly essential prior to hardening process and the fast Chl a fluorescence transient measurement with high time resolution provide a non-invasive and rapid method to screen the photosynthetic potential of in vitro propagated plantlets to achieve higher survival rate under ex-vitro condition. In future, the developed in vitro protocol can be used for large scale propagation and genetic improvement of D. unguis-cati.

Declarations

Author contribution statement

All authors listed have significantly contributed to the investigation, development and writing of this article.

Funding statement

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Data availability statement

Data will be made available on request.

Declaration of interests statement

The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

References

[1] L.H. Fonseca, S.M. Cabral, M.D. Agra, L.G. Lohmann, Taxonomic revision of Dolichandra (Bignonieae, bignoniaceae), Phytotaxa 301 (1) (2017) 1–70.
[2] J.C. Brondani, F.Z. Reginato, E. da Silva Brum, de Souza, M. Vencato, C.L. Lhamas, C. Viana, M.P. Mandron, Evaluation of acute and subacute toxicity of hydroethanolic extract of Dolichandra unguis-cati L. leaves in rats, J. Ethnopharmacol. 202 (2017) 147–153.
[3] N.I. Hilgert, Plants used in home medicine in the Zenta River basin, Northwest Argentina, J. Ethnopharmacol. 76 (1) (2001) 11–34.
[4] J.A. Duke, Duke’s Handbook of Medicinal Plants of Latin America, CRC Press, Boca Raton (FL), 2008.
[5] E.A. Aboutabl, F.A. Hashem, A.A. Sleem, A.A. Maamoon, Flavonoids, anti-inflammatory activity and cytotoxicity of Macfadyena uniguis-cati L, Afr. J. Tradit. Complement. Altern. Med. 5 (1) (2008) 18–26.
[6] J. Sanz-Biset, J. Campos-de-la-Cruz, M.A. Epipquín-Rivera, S. Gáñigueal, A first survey on the medicinal plants of the Chasura valley (Peruvian Amazon), J. Ethnopharmacol. 122 (2) (2009) 333–362.

[7] A.S.O. Flor, W.L.R. Barbosa, Sabedoria popular no uso de plantas medicinais pelos moradores do bairro do sossego no distrito de Maruã - PA, Rev. Bras. Plantas Med. 17 (4) (2015) 757–766.

[8] D.S. Duarte, M.F. Dolabela, C.E. Salas, D.S. Raslan, A.B. Oliveirais, A. Nenninger, B. Wiedemann, H. Wagner, J. Lombardi, M.T.P. Lopes, Chemical characterization and biological activity of Macfadyenia unguis-cati (Bignoniaceae), J. Pharm. Pharmacol. 52 (2000) 247–252.

[9] L. Chen, D. Chen, Z. Zheng, S. Liu, Q. Tong, J. Xiao, H. Lin, Y. Ming, Cytotoxic and antioxidant activities of Macfadyenia unguis-cati L. aerial parts and bioguided isolation of the antitumor active components, Ind. Crop. Prod. 107 (2017) 531–538.

[10] P.O. Downey, I. Turnbull, The biology of Australian weeds 48.

[11] O.O. Osunkoya, K. Pyle, T. Scharaschkin, K. Dhileepan, What lies beneath? The dissipation of absorbed light energy vs charge separation in lichens, Physiol. 35 (2) (2019) 183–187.

[12] J.C. Buru, J. Dhileepan, O.O. Osunkoya, T. Scharaschkin, Germination biology and origin of each kinetic step, FEBS J. 273 (20) (2006) 4770–4777.

[13] C.A. Espinosa-Leal, C.A. Puente-Garza, S. García-Lara, Biological and origin of each kinetic step, FEBS J. 273 (20) (2006) 4770–4777.

[14] M.S. Shekhawat, M. Manokari, commonly referred to as “acacia” in the literature, J. Ethnopharmacol. 122 (2) (2009) 333–362.

[15] C. Huetteman, J.E. Preece, Thidiazuron: a potent cytokinin for woody plant tissue culture, Plant Cell Tissue Organ Cult. 35 (2) (1999) 243–251.

[16] M.J. Slabaugh, B. Borkowska, A. Fiuk, H. Gawroński, E. Madam, S. Manga, M. Aileni, P. Mamidala, Effects of sucrose concentration on photosynthetic activity of Riccia gangetica (L.) F. Muell., a rare medicinal plant, J. Genetic Eng. Biotechnol. 18 (1) (2017) 1136–1142.

[17] S.K. Talla, E. Madam, S. Manga, M. Aileni, P. Mamidala, Efficient TDZ-induced regeneration from capitulum explants of Gerbera jamesonii Bolus ex Hooker F. L. and origin of each kinetic step, FEBS J. 273 (20) (2006) 4770–4777.

[18] U. Heber, V. Soni, R.J. Strasser, Photoprotection of reaction centers: thermal dissipation of absorbed light energy vs charge separation in lichens, Physiol. Plantarum 142 (2011) 65–78.

[19] R.M. Rodríguez, Biolayer Program to Calculate Fluorescent Transient, Laboratory of Bioenergetics, University of Geneva, Switzerland, 2002.

[20] H. Sallanon, Effects of micropropagation on the medicinal plants of the Chazuta valley (Peruvian Amazon), Plantarum 142 (1) (2011) 65–78.

[21] S. De, H. Lin, Y. Ming, Cytotoxic and antioxidant activities of Macfadyenia unguis-cati L. aerial parts and bioguided isolation of the antitumor active components, Ind. Crop. Prod. 107 (2017) 531–538.

[22] P. Losciale, Frequently asked questions about chlorophyll fluorescence, Photosynthetica 36 (1-2) (1999) 64–76.

[23] U. Bhatt, H. Singh, D. Kumar, V. Soni, Rehydration quickly assembles C. Genoud, A. Coudret, C. Amalric, H. Sallanon, Effects of micropropagation on the medicinal plants of the Chazuta valley (Peruvian Amazon), Plantarum 142 (1) (2011) 65–78.

[24] P.O. Downey, I. Turnbull, “The biology of Australian weeds”, J. Ethnopharmacol. 122 (2) (2009) 333–362.

[25] T. Iwasaki, E. Gaby, The effect of in vitro culture conditions on the pattern of maximum photosynthetic efficiency of photosystem II during acclimatisation of Hellobarua tiger plantelets to ex vitro conditions, Plant Cell Tissue Organ Cult. 125 (3) (2016) 585–593.

[26] V. Soni, R. Kumari, P.L. Swarnkar, High frequency in vitro regeneration system for conservation of Barleria prionitis L., a threatened medicinal shrub, J. Genetic Eng. Biotechnol. 18 (1) (2020) 3.

[27] S.K. Talla, E. Madam, S. Manga, M. Aileni, P. Mamidala, Effects of sucrose concentration on photosynthetic activity of Riccia gangetica (L.) F. Muell., a rare medicinal plant, J. Genetic Eng. Biotechnol. 18 (1) (2020) 3.

[28] H.A. Nikile, K.M. Ntimate, M.R. Shaheen, N.S. Kadam, M.Y. Borde, T.D. Nkam, In vitro propagation, callus culture and bioactive lignin production in Phyllanthus tenuellus Roq.: a new source of phyllanthin, hypophyllanthin and phyllctalin, Sci. Rep. 10 (1) (2020) 1–12.

[29] B. Yucesan, A.U. Turk, E. Gürer, TDZ-induced high frequency plant regeneration through multiple shoot formation in willow chyropy (Chionochloa flabellata), Plant Cell Tissue Organ Cult. 91 (3) (2007) 243–250.

[30] B.P. Blussare, K. John, V.P. Bhatt, T.D. Nkam, In vitro propagation of Digitalis lanata Ehrl. through direct shoot regeneration—A source of cardiotonic glycosides, Ind. Crop. Prod. 121 (2018) 313–319.

[31] T.J. Morales-Dominguez, D.S. de León, C. García-Duarte-Pita, E. Pérez-Molpec-Balch, Germination, in vitro propagation and soil acclimatization of Acacia farnesiana and Prosopis laevigata, South Afr. J. Bot. 124 (2019) 345–349.

[32] T. Ljajle, T. Fidyia, In vitro propagation of Securidaca longipedunculata (Fresem) from shoot tips of an endangered medicinal plant, J. Genetic Eng. Biotechnol. 18 (1) (2020) 3.

[33] S. Maharan, S. Pradhan, B.B. Thapa, B. Pant, In vitro propagation of endangered orchid, Vanda pumila Hook. f. through protocorm culture, Am. J. Plant Sci. 10 (7) (2019) 1220.

[34] S. Maharan, L.S. Thakuri, B.B. Thapa, S. Pradhan, K.K. Pant, G.P. Joshi, B. Pant, In vitro propagation of the endangered orchid Dendrobium chrysanthum Rolfe from protocorms culture, Nepal J. Sci. Technol. 19 (1) (2020) 39–47.

[35] G. Schansker, K.Z. Tió, A.R. Holworth, G. Garab, Chlorophyll a fluorescence: beyond the limits of the QA model, Photosynth. Res. 120 (1-2) (2014) 43–58.

[36] S. Boisvert, D. Joly, R. Carpentier, Quantitative analysis of the experimental O–J–P chlorophyll fluorescence induction kinetics: apparent activation energy and origin of each kinetic step, FERRIS J. 273 (20) (2006) 4770–4777.

[37] Y. Yamane, Y. Kashino, H. Koike, K. Satoh, Inhibitions of fluorescence F64 level and reversible inhibition of photosystem II reaction center by high-temperature treatments in higher plants, Photosynth. Res. 52 (1) (1997) 57–64.

[38] F. Gontes, C. Talavera, C. Oropeza, Y. Desjardins, M.J. Santamaria, Exogenous sucrose can decrease in vitro photosynthesis but improve field survival and growth of coconut (Cocos nucifera L.) in vitro plants, In Vitro Cell. Dev. Biol. Plant 41 (1) (2005) 69–76.

[39] A.K. Lobo, M.M. de Oliveira, M.C. Neto, E.C. Machado, R.V. Ribeiro, J.A. Silveira, Exogenous sucrose supply changes sugar metabolism and reduces photosynthesis of sugarcane through the down-regulation of Rubisco abundance and activity, J. Plant Physiol. 179 (2015) 113–121.

[40] J.J. Bychynsky, B. Borkowska, A. Fiuk, H. Gawrońska, E. Śliwińska, A. Mikula, Effect of sucrose concentration on photosynthetic activity of in vitro cultures of Gentiana kurroo (Rouy) jermolin, Acta Physiol. Plant. 29 (5) (2007) 445–453.

[41] C. Meng, X. Liu, Y. Chai, J. Xu, M. Yue, Another choice for measuring tree photosynthesis in vivo, Peer J. 7 (2019), e5933.

[42] K.E. Koch, Carbohydrate-modulated gene expression in plants, Annu. Rev. Plant Physiol. Plant Mol. Biol. 47 (1996) 509–540.