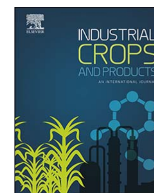
Industrial Crops & Products 111 (2018) 544–554

Contents lists available at ScienceDirect

Industrial Crops & Products

journal homepage: www.elsevier.com/locate/indcrop



Development of enhanced hypericin yielding transgenic plants and somaclones: High throughput direct organogenesis from leaf and callus explants of *Hypericum perforatum*

Shamshad A. Khan*, Priyanka Verma, Akshata Arbat, Sushma Gaikwad, Varsha A. Parasharami

CSIR-National Chemical Laboratory, Division of Biochemical Sciences, Dr. Homi Bhabha Road, Pashan, Pune, Maharashtra 411008, India

ARTICLE INFO

Keywords:
Direct regeneration
Indirect regeneration
ISSR
Somaclones
Hypericum perforatum
Hypericin
Transgenic plants

ABSTRACT

Hypericum perforatum is well known for its anti-depressent properties due to the presence of hypericins and hyperforins. The direct regeneration protocol from leaf explants and *Agrobacterium tumefaciens* mediated genetic transformation is a prerequisite for gene transfer studies in this valuable plant system as *H. perforatum* has shown its recalcitrance to these studies previously. The aim of generating transgenic shoots or somaclones in the present study is to select high hypericine yielding *H. perforatum* plants. High throughput direct bud organogenesis from leaf explants has been achieved for the first time in this plant system on media combination of MS + 5.0 mg/l BAP + 1.0 mg/l. This medium interestingly also worked as the indirect regeneration media for generation of somaclones from callus cultures. Thus far optimized single media can be used for direct and indirect regeneration in *H. perforatum*. The direct regeneration frequency of plantlets from leaf explants was found to be 80% while 100% was the regeneration frequency noted for indirect plantlet regeneration from callus cultures. The optimized direct regeneration protocol from leaf explants was utilized for *A. tumefaciens* (harbouring p*CAMBIA* 1301) mediated genetic transformation studies with the aim of establishing transgenic lines of *H. perforatum*. The resultant study successfully gave rise to kanamycin resistant GUS positive shoots with transformation efficiency of 33.36 ± 3.6. A total of four transgenic *H. perforatum* clones namely T2, T3, T5 and T8 with different growth parameters were established, where highest of fivefold increase in hypericin content was recorded by T5 (276.8 ± 9.2 µg/g dry wt.) in comparison to control non transformed plants (35.6 ± 2.7 µg/g dry wt.). Interestingly the presence of serotonin and melatonin was also detected in transgenic plants on TLC basis. The transgenic nature of the plants was confirmed by PCR with amplification of *uidA* gene. Apart from it, from indirect regeneration from callus, total of ten somaclones were generated. The ISSR profiling of somaclones scored a total of 156 bands, among which 118 were polymorphic in nature. The glasshouse established somaclones showed up to twofold increase in hypericin content on HPLC analysis.

1. Introduction

The St. Jonh's wort (*Hypericum perforatum*) is amongst the oldest of the plants and dates its history back to 2000 years (Family: Clusiaceae). It has found immense application in folk medicines since ancient times as it has anti- inflammation (Hammer and Birt, 2014), antiviral (Birt et al., 2009), sedatives, diuretic and wound healing abilities (Wolfle et al., 2014). Since past two decades *H. perforatum* and its related species have been explored due to its herbal antidepressant activities and it's different extracts have been used in treating moderate to severe depression as the use of this plant as anti depressent has very less side effects associated with it. The use of this medicinal herb as an antidepressant has been well recognized as recently monograph of the Herbal Medicinal Product Committee (HMPC) belonging to European Medicines Agency accepted the attributes of this herb (Galeotti, 2017). The ever increasing number of case of depression and interest of people towards herbal drugs has resulted in *H. perforatum* being used as one of the highly consumed herb in the world (Ekor, 2014). More than 450 species of *Hypericum* have been reported and plants are mostly known to be perennial, herbaceous deciduous shrubs which find its habitat in Northern America, Europe and Asia. The herb is tetraploid in nature ($2n = 4x = 32$) although variation in chromosome numbers have also been observed. The secondary metabolite concentration in this plant is varied as different plant segment contains different kinds and class of secondary metabolites. Different kinds of xanthones, biflavones, flavanols, procyanidins, essential oils as well as naphthodianthrones and

* Corresponding author.
E-mail address: shamshad_k@rediffmail.com (S.A. Khan).

https://doi.org/10.1016/j.indcrop.2017.11.032
Received 10 July 2017; Received in revised form 26 September 2017; Accepted 16 November 2017
0926-6690/ © 2017 Elsevier B.V. All rights reserved.

phloroglucinols are found in *H. perforatum*. Among the different secondary metabolites the *H. perforatum* plants contain, naphthodianthrones, flavonoids and phloroglucinols which are majorly responsible for antidepressant activities of this herbal shrub. The major naphthodianthrones found in this plant is known as hypericin which is responsible for its antidpresent activities. The aerial parts of the plants mainly flowers and leaves are known to contain hypericins but the yield is quite low in leaves (0.03–0.3 dw) as compared to flower (1–14% dw). The concentration of secondary metabolite especially hypericin and hyperforin is very less in *H. perforatum* plants in their native environment. The environmental conditions such as biotic stress also affect the concentration of the compounds as well as the genotype of the plants itself (Filippini et al., 2010).

The *in vitro* biotechnological approaches is a prerequisite in this plant system because of two reasons (i) There is an utmost requirement of an alternate source of production of *H. perforatum* plants as *the* plants itself contains small sized seeds which are difficult to be cultured by the conventional methods and harvesting of these plants is leading to diminishing of genetic variability. Apart from it the studies of this plant at different *in vitro* level such as callus phase, suspension phase and whole plant level could lead to a new variety development which may contain desirable amounts of antidepressant molecules. (ii) The genetic level manipulations are required in this prized medicinal plant because the pathway level understandings are not complete. At *in vitro* levels the desirable study is to establish a direct regeneration protocol from leaf explants in *H. perforatum* as the successful studies of this aspect may lead to easier gene transfer studies with stable gene transfer through the use of *A. tumefaciens* mediated genetic transformations. A direct regeneration protocol has been always desirable from the leaf explants as adventitious shoot bud induction from leaf could lead to transformants with less chances of forming chimera when using gene transfer studies for transgenic plant production (Khan et al., 2014).

Although *Agrobacterium* based genetic transformations have been performed in this plant system but with different species of *Hypericum*. The *in vitro* plant tissue culturing has provided a platform from selection of variants among a population to transgenic studies by helping in selection of recombinant cell lines. The phenomenon of somaclonal variation is very famously known as the result of artifact of plant tissue culture since the time *in vitro* regeneration studies have taken place. The induced somaclonal variation through indirect regeneration may lead to an array of variants amongst which the most desirable variant in terms of growth or secondary metabolite yield can be selected and propagated further which may result in a new variety development. The inter-simple sequence repeat polymerase chain reaction (ISSR-PCR) profiling of the somaclonal variants provides further confirmation on how much these somaclonal variation have affected the genome of the parent plants as ISSR technique uses the microsatellite DNA sequences for raising the multi locus markers. Previously a restricted study on this plant system and its tissue culture studies has been performed majorly with other varieties of *Hypericum* plants such as *H. bupleuroides H. maculatum, H. erectum* and *H. perforatum* (Oluk et al., 2010). For efficient multiplication of *H. perforatum* plants leaf explants have been used in previous studies (Wojcik and Podstolski, 2007) and bioactivity potentials of this plant have also been assessed (Cirak et al., 2007). The gene transfer studies in *H. perforatum* has not been achieved completely because of the recalcitrant nature of the plant system, although indirect and particle bombardment methods were applied previous in *H. perforatum* (Koperdacova et al., 2011) The particle bombardment study using plasmid vector pCAMBIA1301 encoding an intron-containing b-glucuronidase (gus A) and hygromycin phosphotransferase (hpt) genes showed the successful integration of GUS genes but transgenic plants were not assessed phytochemically for hypericin and hyperforins (Franklin et al., 2007).

The *A. tumefaciens* mediated genetic transformation studies in *H. perforatum* has not been very fruitful as the secondary metabolites present in the plant itself were reported to inhibit the growth of the bacteria at the time of co- cultivation (Hou et al., 2016).

The transgenic research in *H. perforatum* requires the *A. tumefaciens* mediated genetic transformation studies because the already characterized genes such as HPPKS1, HPPKS2 and Hyp1 (Karppinen, 2010; Karppinen et al., 2016) could be understood better in respect of their function and role in the biosynthetic pathway of hypericin. The gene transfer protocol establishment through *A. tumefaciens* mediated genetic transformation studies could help in manipulation of genes at *in vitro* level for a better understanding of the pathway.

Present work deals with the above discussed problems associated with *H. perforatum* and aims to establish of high hypericine yielding transgenic lines or somaclones. The study establishes efficient direct regeneration protocol from the leaf explants and transgenic plant production mediated through *A. tumefaciens* mediated studies in *H. perforatum* for the first time. Apart from it the report also discusses the indirect regeneration protocol establishment from callus cultures in *H. perforatum* leading to somaclone generation followed by their molecular and phytochemical analysis.

2. Material and methods

2.1. In vitro plant establishment

The seeds of *H. perforatum* were cleaned properly by running tap water followed by their washing with Tween 20 detergent. The surface sterilization of the explants was done by using 0.1% mercuric chloride for 45 s followed by washing with autoclaved distilled water. For germination sterilized seeds were plated on MS (Murashige and Skoog, 1962) basal medium. Germinated seedlings were transferred to multiplication medium consisted of MS basal medium fortified by 2.5 mg/l Kinetin (Kn). The plant growth and response of the explants were assessed after 6 weeks and data were generated by taking the mean of the triplicates of the experiments.

2.2. Indirect regeneration and somaclone development

Leaf explants from *in vitro* raised shoot cultures were placed on 7.0 mg/l NAA + 1.0 mg/l BAP containing MS basal and WPM (Lloyd and McCown, 1980) basal medium separately. The callus was subsequently multiplied on the callusing medium by regular sub-culture every 4–5th weeks. Two-week-old callus was transferred on various media combinations comprising of BAP + NAA for differentiation and regeneration. Eight to ten small pieces of 1 cm^2 callus explants per 90-mm radiation-sterilized plastic petri-plates were placed. The experiment was set up in triplicates. After 30-day incubation period, shoot buds obtained were transferred to standardized multiplication medium. Each obtained shoot was maintained as separate clone on standardized multiplication medium. The 20 day old shoots were placed on one fourth, half and full strengths of MS basal medium alone as well as supplemented with IBA, and IAA for induction of roots. The media was optimized on the basis of number of roots, root length and root thickness.

2.3. Direct shoot bud regeneration from leaf explants and transgenic plant development

The leaves from one month old *in vitro* grown plants were used as explants for direct regeneration study. The leaf explants were placed on a full strength MS medium fortified with combinations of BAP and NAA. Each combination was tested in triplicates. *A. tumefaciens* strain ATCC15955 with pCAMBIA 1301 having GUS reported gene was tested for genetic transformation studies using the protocol for direct shoot bud regeneration from leaf explants. The loop full of bacterial strain was inoculated in 50 ml YEP media with 20 mg/l kanamycin. The culture was incubated at 28 °C overnight under dark condition on a shaker with 200 rpm. Leaf explants were manually pricked with sterile

Download English Version:

https://daneshyari.com/en/article/8880987

Download Persian Version:

https://daneshyari.com/article/8880987

Daneshyari.com