Biomass and Bioenergy 83 (2015) 501–510

Contents lists available at ScienceDirect

Biomass and Bioenergy

journal homepage: http://www.elsevier.com/locate/biombioe

Research paper

Micropropagation of elite genotype of *Jatropha curcas* L. through enhanced axillary bud proliferation and *ex vitro* rooting

Mangal S. Rathore*, Sonam Yadav, Pawan Yadav [1], Jasminkumar Kheni [2], Bhavanath Jha**

CSIR-Central Salt and Marine Chemicals Research Institute (CSIR-CSMCRI), G.B. Marg, Bhavnagar, 364002, Gujarat, India

ARTICLE INFO

Article history:
Received 1 September 2015
Received in revised form
26 October 2015
Accepted 27 October 2015
Available online 12 November 2015

Keywords:
Acclimatization
Cloning
ex vitro
Mature
Micropropagation
Rooting

ABSTRACT

We developed simple, improved and reproducible micropropagation process for elite and mature genotype of *Jatropha curcas* L. through enhanced axillary bud proliferation. On 7 g L^{-1} agar-gelled Murashige and Skoog's (MS) medium containing 2.0 mg L^{-1} 6-benzylaminopurine (BAP) and 0.1 mg L^{-1} indole-3-acetic acid (IAA), 96.67 ± 3.33% explants produced 1.73 ± 0.07 shoot buds of 1.27 ± 0.04 cm per explant. Shoots were multiplied (9.33 ± 0.09 shoot buds of 2.18 ± 0.01 cm per nodal segment) by subculturing the *in vitro*-derived nodal segments on MS medium containing 0.5 mg L^{-1} each BAP and IAA. On half-strength of MS salt with 2 g L^{-1} activated charcoal (AC) and 3.0 mg L^{-1} indole-3-butyric acid (IBA), 73.33 ± 3.33% of the shoots rooted *in vitro*. Alternatively the bases of microshoots were treated with 500 mg L^{-1} IBA for 3 min and transferred onto sterile mixture of soilrite and soil (1:1 v/v). 70.00 ± 5.77% of shoots rooted *ex vitro*, which could be acclimatized simultaneously. The rooted plantlets were acclimatized by slow and gradual exposure from high (75–85%) relative humidity (RH) and low (24–26 °C) temperature to low (40–50%) RH and high (30–32 °C) temperature. The cloning procedure described is superior to methods reported earlier and has potential applications for large-scale true-to-type propagation of *J. curcas* to supply planting propagules for promotion of commercial plantation.

1. Introduction

Jatropha curcas L. (member of *Euphorbiaceae*) commonly called physic nut rapidly emerged as a potential biodiesel crop as the crude oil of *J. curcas* can be easily converted into biodiesel meeting US and European standards [1,2]. The plant species is being studied and researched across the globe. The major achievements on *J. curcas* till date include development of regeneration protocols [3–9]; transformation protocols [5,10,11]; development of transgenic *J. curcas* using S-*DREB2A* [12] and *SbNHX1* [13] gene; development of molecular markers for genotyping [14–18]; transcriptome analysis of the oil-rich seed [19]; determination of complete nucleotide sequence of chloroplast genome [20]; analysis of the genome sequence [21]; proteomic analysis of oil mobilization in seed germination and post germination development [22] and functional characterization of different genes. Beside a considerable progress towards achieving goal, still research on *J. curcas* to meet future energy requirements is awaited [23,24]. Agro-technologies have been developed for *J. curcas* for diverse environmental conditions [25].

As an increase in scale of *J. curcas* cultivation, there is need to develop improved varieties. Various techniques for breeding of new *J. curcas* varieties have been reviewed [23]. Among various techniques of breeding, *in vitro* propagation would play a very important role for germplasm improvement through non-transgenic approach. With advancement of *J. curcas* biotechnology and generation of base line data on different aspects of *J. curcas* including consistent seed yield data from mature progenies, there would be need for a simple, efficient and cost effective method for rapid and mass-scale true-to-type propagation of mature and elite genotypes of *J. curcas* for supply of quality planting propagules for large-scale cultivation. Being monoecious heterozygous system, propagation through seeds is not a viable option for multiplication of elite genotypes. The most common practice to multiply the elite genotypes of *J. curcas* is through vegetative cuttings. Generation of large-number of cuttings from

* Corresponding author.
** Corresponding author.
E-mail addresses: mangalrathore@csmcri.org, mangalrathore@gmail.com (M.S. Rathore), bjha@csmcri.org (B. Jha).
[1] Present address: Department of Biotechnology, School of Life Science, Central University of Rajasthan (CUR), Bandarsindhri 305801, Rajasthan, India.
[2] Present address: Department of Biotechnology, Junagadh Agricultural University, Junagadh 362002, Gujarat, India.

http://dx.doi.org/10.1016/j.biombioe.2015.10.023
0961-9534/

an elite genotype of *J. curcas* has its own limitations including diminishment of seed yield of so called elite genotype in subsequent season. This also causes the carry-over of disease-causing pathogens from one generation to the next. *In vitro* techniques are the basis for various crop improvement programs through both GMO and non-GMO technologies and have great potential for rapid and mass-scale multiplication of a selected genotype. Various workers have attempted establishment of tissue cultures of *J. curcas* using different explants and biotechnological advancements in *J. curcas* have been reviewed [8,9,12,26–29]. From literature survey *J. curcas* tissue culture propagation appears simple and promising. However, reproducibility of the results has been great challenge thus creating confusion. To the best of our knowledge none of the available reports pointed the practical constraints/difficulties in large-scale multiplication of a selected genotype of *J. curcas*; there remains a huge gap in the available information on tissue culture of *J. curcas* and utilization. During mass-scale long-term tissue culture of *J. curcas*, leaf drying in healthy cultures (Fig. 1a), drying of main shoot bud along with regeneration of new shoot buds (Fig. 1b), complete drying of cultures (Fig. 1c), abnormal growth of shoot buds (Fig. 1d) and subsequent drying, difficult to handle yellow colour bacterial contamination without killing the cultures (Fig. 1e) and sudden outbreak of fungal contamination in cultures and on tissue surface were observed. Also repeated sub-culture of a shoot for longer-time causes *in vitro* flowering (Fig. 1f) and use of larger culture vessels i.e. phyta-jar or glass bottles to obtain more numbers of shoot buds lead hyper-hydration of cultures (Fig. 1g, h) and further drying of tissues in cultures. Occurrence and control of endophytic bacterial contamination using augmentin® in long-term proliferating shoot cultures of elite *J. curcas* has been reported [30,31]. Periodic occurrence of abnormal growth, senescence and leaf fasciation in cultures of *J. curcas* were also observed [5]. These problems may vary from one laboratory to other and one genotype to other genotype; and seriously limit the applicability of *in vitro* techniques.

Earlier the genetic and epi-genetic status of *in vitro* regenerates of *J. curcas* at different generations were reported [32,33] using cytological and molecular tools. In present report, our intension is to point out the problems in large-scale long-term tissue culture of *J. curcas* for true-to-type propagation and to address these. We present experimental results and discuss here simple and reproducible micropropagation system though enhanced axillary shoot bud proliferation and *ex vitro* root regeneration in *J. curcas*.

2. Material and methods

2.1. Plant material and culture conditions

The elite genotypes were selected and marked based on consistency of seed yield during last few years (4–5 years) in CSIR-CSMCRI-Jatropha Experimental Plot Nesvad, Bhavnagar (Gujarat) India. These marked plants served as mother plant and used as source of plant material for establishment of *in vitro* cultures. The mother plants were watered regularly and supplied with proper doses of inorganic fertilizers and farm yard manure (FYM). Alternatively, cuttings of elite plants were raised in earthen pots and maintained under greenhouse conditions. Murashige and Skoog's (MS) basal medium [34] was used throughout the experiments. The media was gelled with 7 g L^{-1} agar (Spectrochem Pvt. Ltd.) and supplied 30.0 g L^{-1} sucrose as carbohydrate source. The pH of medium was adjusted to 5.77 ± 0.05 before adding agar and prior to sterilization. The cultures were maintained in a culture room at 26 ± 2 °C, 55–60% relative humidity (RH), under 12 h (h) photoperiod with a light intensity of 35–40 μmol m^{-2} s^{-1} spectral flux photon (SFP) of photo-synthetically active (460–700 nm) radiations.

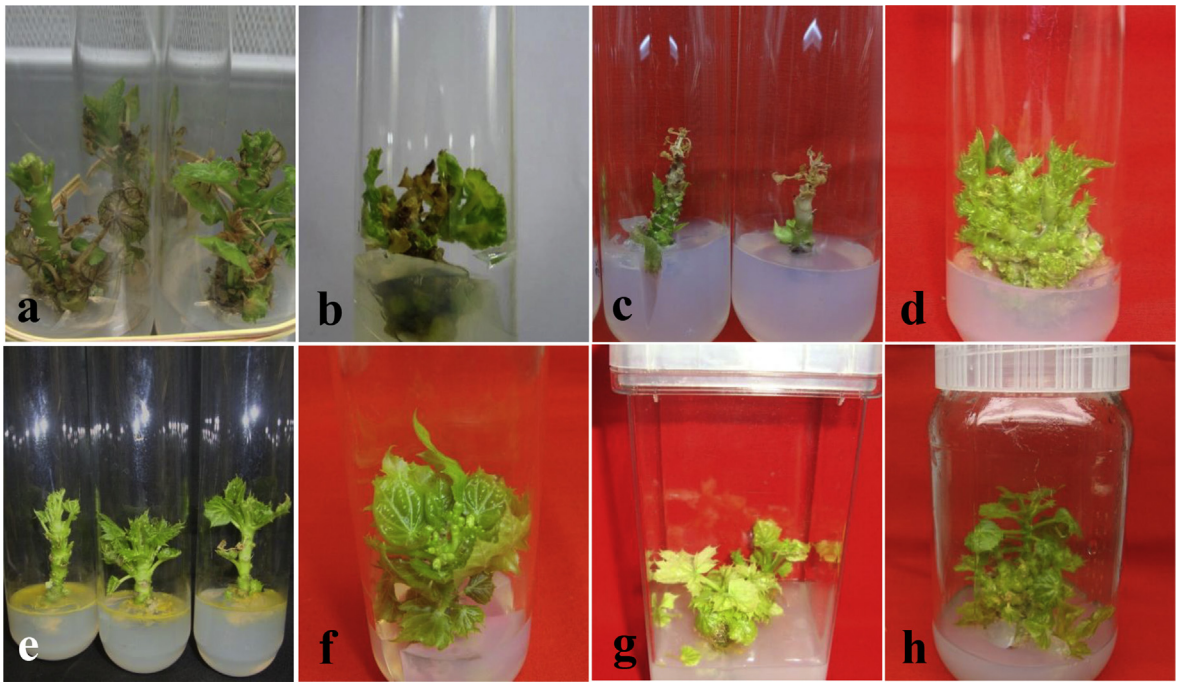

Fig. 1. Different kinds of constraints in establishment of long-term tissue culture of *J. curcas*: leaf drying in healthy cultures (a); drying of main shoot along with regeneration of new shoot buds (b); drying of shoot tips in cultures (c); abnormal growth of shoot buds (d); difficult to handle yellow colour bacterial contamination (e); *in vitro* flowering (f) in cultures; hyper-hydration of shoots buds in larger culture vessels (g and h). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)