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Effect of antioxidants and associate changes in antioxidant enzymes in controlling browning and necrosis of proliferating shoots of elite *Jatropha curcas* L

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ABSTRACT

A high yielding elite plant of *Jatropha curcas* was established under aseptic condition from field-grown nodal explants. Shoots were proliferated in MS medium supplemented with 0.5 mg dm^{-3} benzyladenine and 0.1 mg dm^{-3} indolebutyric acid along with 10 mg dm^{-3} adenine sulphate and a combination of 15 mg dm^{-3} each of L-glutamine and L-arginine. However, within 15–20 d of culture incubation, tissue browning/necrosis leading to poor plant regeneration *in vitro* was observed. A set of different antioxidants, namely, reduced glutathione, ascorbic acid, tocopherol and cysteine were used in the medium individually and in combination to solve the problem of tissue browning and necrosis. The addition of antioxidants proved beneficial for the growth of the shoots. The optimum medium comprised of 25 mg dm^{-3} reduced glutathione and 10 mg dm^{-3} ascorbic acid, where proliferating shoots having highest leaf canopy area, remained fresh, green and regenerative up to 40 d of culture incubation without any subculture. The activities of antioxidant enzymes, such as superoxide dismutase was higher in control shoots, indicating that tissue browning/necrosis was associated with oxidative stress which was further supported by higher contents of H_2O_2 and phenolics in control shoots compared to the other treatments. Similarly glutathione reductase, ascorbate peroxidase and guiacol peroxidase was higher in treated shoots than control indicating that these shoots have developed antioxidant enzymatic protective system which determine the ability to survive in oxidative stress and up regulation of these enzymes would help to reduce the built up of reactive oxygen species.

Abbreviations: AdS, adenine sulphate; APX, ascorbate peroxidase; AA, ascorbic acid; BA, 6-benzyladenine; BSA, bovine serum albumin; Cys, cysteine; EDTA, ethylene diaminetetraacetic acid; GPX, guiacol peroxidase; GR, glutathione reductase; GSH, glutathione, reduced form; GSSG, glutathione, oxidized form; H_2O_2, hydrogen peroxide; IBA, indole-3-butyric acid; MS, Murashige and Skoog's (1962) medium; NADPH, β-nicotinamide adenine dinucleotide phosphate, reduced form; NBT, nitroblue tetrazolium; OH, hydroxyl radicals; PVP, polyvinylpyrrolidone; PMSF, phenazine methosulfate; ROS, reactive oxygen species; SDS, sodium dodecyl sulphate; SOD, superoxide dismutase; Toco, α-tocopherol; TCA, trichloroacetic acid.

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1. Introduction

Biofuel is a locally-available source of energy that not only can provide energy to meet the increased energy demand derived from the economic development of developing countries, but also contributes to climate change mitigation and rural development. *Jatropha curcas* (Fam. Euphorbiaceae) plant has been acknowledged as the preferred crop for the purpose which is drought resistant, perennial and fast growing on poor soil [1]. It produces oil from the seeds, which can be combusted as fuel without being refined [2]. It burns with clear smoke-free flame, tested successfully as fuel for simple diesel engine [3]. Although there are other sources available as biofuel crops, but being a non food crop *Jatropha* cultivation as biodiesel crop ranked first among all, considering social, economic and humanitarian reasons [4]. *Jatropha* has a lot of interspecific genetic diversity [5] as well as diversity among different accessions of Indian varieties [6]. The inter-variety spread of oil yield between *Jatropha* varieties ranges between 30 and 37%. Therefore, use of elite variety for planting material can assure a 20% more biodiesel yield. The present study overcomes hitherto problem of browning of *in vitro* cultures of *Jatropha* [7] and achieves a robust protocol for large scale propagation of tissue raised plants of its elite variety. There are few reports on *in vitro* shoot regeneration from different seedling explants [8,9], nodal explants [10] and from leaf explants [11]. In most of the reports *in vitro* clonal propagation was achieved through organogenesis [1,10,12], but few are through somatic embryogenesis [13,14]. Although shoot multiplication and their growth is not a problem in *J. curcas*, but the regenerating shoots turned brown/necrosed within 15–20 d of culture incubation. Due to browning of tissue in *Jatropha*, it becomes difficult to continue long-term cultures. Tissue browning generally inhibits the growth and adventitious shoot formation [15]. The problem of browning in callus cultures of *Jatropha* has recently been published [7]. Reactive oxygen species such as superoxide radical, H_2O_2 and OH radicals have a role in lipid peroxidation, membrane damage and consequently in leaf senescence. It has been shown that during leaf senescence, proteins, phospholipids and pigments may be degraded by free radicals as free radical scavenging declines [16]. Free radicals have been involved in programmed cell death, both in animal and in plant cells [17]. Antioxidant protection involves compounds such as carotenoids, ascorbic acid, *a*-tocopherol, glutathione, cysteine, phenolics and flavonoids [18] and a number of enzymes including superoxide dismutase (SOD), guiacol peroxidase (GPX) and ascorbate peroxidase (APX), catalase (CAT) and glutathione reductase (GR) [19]. SOD is believed to play a crucial role in the antioxidant systems as it catalyses the dismutation of O_2- into H_2O_2 and O_2 [20]. The conversion of H_2O_2 into water is carried out in cytosol and chloroplasts by ascorbate–glutathione cycle, which involves APX and GR [21].

In the present communication control of browning leading to necrosis of shoots has been solved with the addition of antioxidants in the medium. The objective of the present study was to study changes in antioxidant enzyme activities, pigment (chlorophylls and carotenoids) concentrations and total phenolic compounds in relation to the growth and development of shoots.

2. Material and methods

2.1. In vitro-*proliferation of shoots*

A high yielding, C2 accession of *J. curcas* (2 years of age) procured from Central Salt and Marine Chemicals Research Institute, Bhavnagar (Institute of CSIR), India, growing in NBRI campus had been selected as elite tree for taking field-grown nodal explants for establishment under aseptic condition. Shoots were proliferated in MS [22] medium containing 15 mg dm^{-3} each of L-glutamine and L-arginine in the basal media. A combination of 0.5 mg dm^{-3} benzyladenine (BA) and 0.1 mg dm^{-3} indole-3-butyric acid (IBA) along with 10 mg dm^{-3} adenine sulphate (AdS) was supplemented in the medium for proliferation and growth of regenerated shoots.

2.2. *Effects of antioxidants on growth of cultures*

Initially, different antioxidants, like reduced glutathione, tocopherol, ascorbic acid, and cysteine (two concentrations

Table 1 – Effect of antioxidants on browning of proliferating shoots of *J. curcas*.

Antioxidant concentration mg dm^{-3}		Period after which shoots started necrosis (d)	*Mean of number of shoots ± SE	*Mean of height of shoots ± SE (cm)	*Mean of number of leaves $shoot^{-1}$ ± SE	Induced callus
Control		15	1.7 ± 0.09	2.2 ± 0.1	7.8 ± 0.3	++
AA	10	20	2.3 ± 0.12	2.3 ± 0.2	6.2 ± 0.3	+
	20	17	2.0 ± 0.10	2.5 ± 0.3	8.6 ± 0.1	+
GSH	25	25	2.5 ± 0.08	2.8 ± 0.2	10.2 ± 0.6	–
	40	25	3.3 ± 0.09	3.1 ± 0.2	12.0 ± 0.8	+
Toco	40	25	2.3 ± 0.08	2.5 ± 0.3	11.3 ± 0.1	–
	60	20	2.7 ± 0.03	3.0 ± 0.4	10.8 ± 0.3	+
Cyst	10	20	3.0 ± 0.06	2.2 ± 0.3	12.0 ± 0.5	–
	20	15	2.0 ± 0.03	2.0 ± 0.1	10.8 ± 0.4	+

Culture incubation: 30 d.
*Mean of 5 replicate shoots.
AA – ascorbic acid; GSH – glutathione reduced; Toco – tocopherol; Cyst – cysteine.
'+' sign denotes increasing amount, while '–' sign denotes no callus present.