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Indirect, Direct and Secondary Somatic Embryogenesis in *Emblica Officinalis*

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ABSTRACT

A continuously growing callus from cotyledonary leaves was achieved in vitro on MS medium. After 2, 3 sub cultures on the same medium the callus became embryogenic and numerous embryos of different stages of development were observed on the surface of the callus. Direct somatic embryogenesis from cotyledons, and callus mediated (indirect) embryogenesis was observed when mature cotyledons were cultured on BMS medium supplemented with different concentrations ofauxins and cytokinins. 2,4-D alone, as well in combination of Kn led to somatic embryogenesis. BMS supplemented with IAA, IBA, BAP and Kn led to direct embryogenesis without callus formation. IAA and IBA on sub culture give rise to secondary embryos from culture raised embryos. When transferred on MS without hormone these embryos give rise to complete plants Plantlets were regenerated from the embryos 2 months after their transfer to the germination medium without any growth regulators but with the addition of 30 g/l sucrose.

KEYWORDS : direct embryogenesis, indirect embryogenesis, somatic embryogenesis, PGR (plant growth regulators), MS (Murashige and skoog),

INTRODUCTION

Emblicaofficinalis is one of the most economically important fruit trees in the world. Somatic embryogenesis is an efficient method of plant regeneration allowing for rapid production of a large number of healthy plantlets within a short period. Somatic embryogenesis mostly occurs indirectly through an intervening callus phase or directly from initial explants. In direct somatic embryogenesis, the embryo is formed directly from a cell or small group of cells without the production of an intervening callus. Direct somatic embryogenesis is generally rare compared with indirect somatic embryogenesis. Somatic embryos, embryogenic callus, and cell cultures recovered from *in vitro* cultured ovules have also been used to develop cryopreservation strategies for germplasm conservation (1,2) Many studies on *in vitro* somatic embryo regeneration have been done (3,4,5) mainly after callus induction. In this study indirect, indirect as well as secondary embryos were observed.

Somatic embryogenesis, under certain experimental conditions, can occur from any cell of the sporophyte, which behaves like a zygote and replay with a high degree of fidelity a developmental programme leading to the production of embryos like structures. Somatic embryogenesis has been reported in over 71 species and the list is being increased with new reports coming from different plant groups. Embryogenesis has been induced from a variety of explants such as stem, hypocotyl, root, leaves, pedicel, floral parts, excised seed embryos, endosperm and nucellus(6).Organogenic differentiation from callus could take place either into shoot or root, however, shoot and root differentiation is regulated mainly by the hormonal factors besides other chemical and environmental factors. In tobacco root-shoot differentiation was regulated by auxin – cytokinin ratio and the quantitative interaction between auxin and cytokinin (7). Such type of interaction has been reported in a number of plants. In this study, the efficiency of direct and indirect *in vitro* embryogenesis from cotyledonary explants of *Emblicaofficinalis* was studied on culture media with different supplemental growth regulator compounds.

MATERIALS AND METHODS

Cotyledons of *E. officinalis* were taken from the seeds of mature fruits. The cotyledons were directly inoculated on nutrient medium under sterilized conditions of laminar flow bench. Standard techniques of media preparation, inoculation and incubation were followed. Throughout, callus was reared on MS(8) medium supplemented with various plant growth regulators (PGR). Cultures were maintained at 25 ± 2°C in continuous light of 1400 lux intensity. The observations were taken after fixed time intervals.

RESULT AND DISCUSSION

Cotyledons inoculated on auxin or cytokinin alone led to callus induction along with somatic embryogenesis. Inoculation on 2,4-D alone led to good callus induction after about three weeks on all the concentrations along with somatic embryogenesis. Amongst other auxins, like IAA, IBA and NAA; NAA led to callus induction followed by somatic embryogenesis but on IAA and IBA, cotyledons gave rise to somatic embryos directly without callus formation, after some time, on the same PGR (IAA and IBA) somatic embryos gave rise to secondary embryos on subculture. Addition of Kn and BAP alone led to direct embryogenesis from the surface of cotyledons without callus formation. Among combinations of auxin and cytokinin, 2,4-D along with Kn led to somatic embryogenesis with callus formation(Table 1 & 2,Figure 1).

In *Emblicaofficinalis*, embryogenesis has earlier been reported from callus raised from endosperm (3,4). In both these cases the embryos were raised on BMS supplemented with low IAA and BA. (9) raised callus from juvenile explants, but failed to observe embryos from the callus raised from these explants. (5) observed embryos on BMS supplemented with (1-4 mg/l) 2,4-D + (0.05mg/l) Kn and NAA (1-4mg/l) +(0.05 mg/l) Kn. Recently, somatic embryogenesis has been observed from leaf explants of *Emblicaofficinalis*(10). In all these cases somatic embryos originated after callus induction only. Till today no report of secondary embryogenesis was reported in *Emblicaofficinalis* ((Table 3,Figure 1)

Usually, the somatic embryogenic process consists of two main steps, the induction of the process and the expression of the resultant embryos (11,12,13). The process is initiated with somatic cells theoretically from any part of the plant, however, substantial differences in competence are found in practice. The cells which are more

competent for somatic embryogenesis are generally those coming from young tissues, immature zygotic embryos among them. However, stems, roots and leaves may be useful as well. Usually, somatic embryos are induced by simple manipulation of the cultural in vitro conditions. One of the main elements in the culture medium are the growth regulator substances (GRS) such as auxins, cytokinins, abscisic acid and gibberellins among other components. Also, it is important to mention that the hormonal endogenous substances play important roles in the somatic embryogenic process. Auxins are the most important components in the induction of the process (13,14,15,16,17).Somatic cells need the signal for the cell polarization and the asymmetric division given by auxins as it happens in their zygotic counterparts (12,18). The participation of the other GRS is important in the balance of hormonal constituents needed to achieve somatic embryogenesis.The plantlets were regenerated two months after transferring cotyledonary embryos to the germination medium (MS medium supplemented with 3% sucrose and no plant growth regulator.

TABLE- 1: INFLUENCE OF VARIOUS PGRs (AUXIN)ON SOMATIC EMBRYOGENESIS IN *EMBLICA OFFICINALIS* COTYLEDON EXPLANT

S. NO.	MS +3% SUCROSE+(mg/l)PGRs	Response (primary embryos)	With callus formation	Without callus formation	Secondary embryogenesis
1	1.0 2,4-D	+	+	-	-
2	2.0 2,4-D	+	+	-	-
3	1.0 NAA	+	+	-	-
4	2.0 NAA	+	+	-	-
5	1.0 IAA	+	-	+	+
6	2.0 IAA	+	-	+	+
7	1.0 IBA	+	-	+	+
8	2.0 IBA	+	-	+	+

TABLE- 2: INFLUENCE OF VARIOUS PGRs (CYTOKININ) ON SOMATIC EMBRYOGENESIS IN *EMBLICA OFFICINALIS* COTYLEDON EXPLANT

S. NO.	MS +3% SUCROSE+(mg/l)PGRs	Response (primary embryos)	With callus formation	Without callus formation	Secondary embryogenesis
1	1.0 BAP	+	-	+	-
2	2.0 BAP	+	-	+	-
3	1.0 Kn	+	-	+	-
4	2.0 Kn	+	-	+	-

TABLE- 3: INFLUENCE OF VARIOUS PGRs (AUXIN + CYTOKININ) ON SOMATIC EMBRYOGENESIS IN *EMBLICA OFFICINALIS* COTYLEDON EXPLANT

S. NO.	MS +3% SUCROSE+(mg/l)PGRs	Response (primary embryos)	With callus formation	Without callus formation	Secondary embryogenesis
1	1.0 2,4-D + 1.0 BAP	-	-	-	-
2	1.0 2,4-D + 2.0 BAP	-	-	-	-
3	2.0 2,4-D + 1.0 BAP	-	-	-	-
4	2.0 2,4-D + 2.0 BAP	-	-	-	-
5	1.0 2,4-D + 1.0 Kn	+	+	-	-
6	1.0 2,4-D + 2.0 Kn	+	+	-	-
7	2.0 2,4-D + 1.0 Kn	+	+	-	-
8	2.0 2,4-D + 2.0 Kn	+	+	-	-
9	1.0 NAA + 1.0 BAP	-	-	-	-
10	1.0 NAA + 2.0 BAP	-	-	-	-
11	2.0 NAA + 1.0 BAP	-	-	-	-
12	2.0 NAA + 2.0 BAP	-	-	-	-
13	1.0 NAA + 1.0 Kn	-	-	-	-
14	1.0 NAA + 2.0 Kn	-	-	-	-
15	2.0 NAA + 1.0 Kn	-	-	-	-
16	2.0 NAA + 2.0 Kn	-	-	-	-

+ present -absent

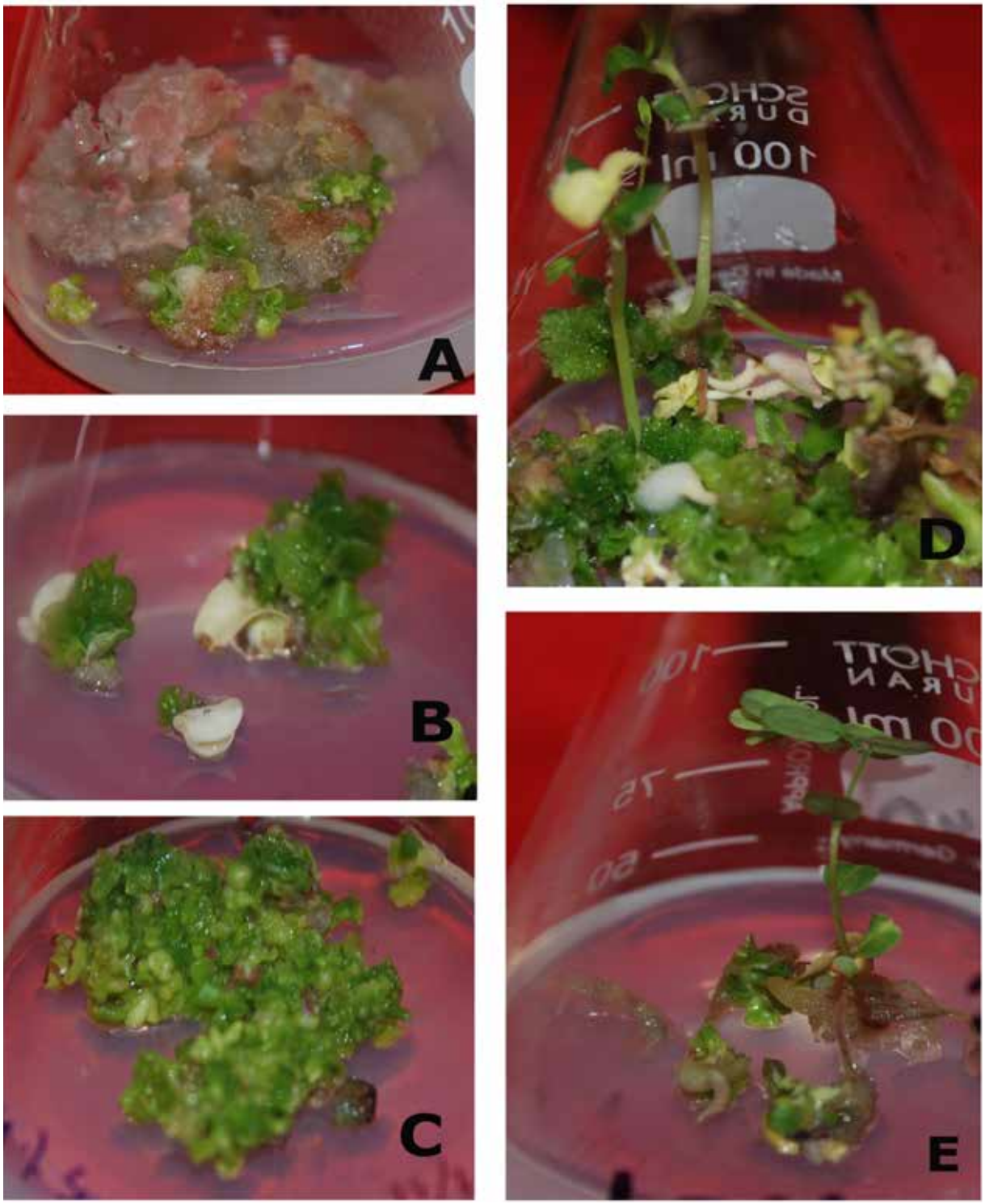

Figure: 1A: Indirect somatic embryogenesis from callus; **B:** Direct embryogenesis from surface of cotyledons; **C:** Secondary embryogensis; **D** and **E**: Plants regenerated from somatic embryos

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