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OPTIMIZED CALLUS PRODUCTION IN *BALANITES AEGYPTIACA* (L.) DEL.: A POTENTIAL SOURCE OF DIOSGENIN

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Balanites aegyptiaca is an important medicinal tree of desert ecosystem. The fruits and roots of the tree contains a secondary metabolite - diosgenin, which can be used in production of oral contraceptive. Demand of this active component is high in the market. Diosgenin has been reported from callus tissue of *Balanites* and several other plants. A study was carried out using a range of explants to see the effect of NAA and 2,4 D supplemented media on callus induction. It is evident that high biomass yielding calli may have higher diosgenin. Juvenile root segment produced highest biomass on both the phytohormones.

Keywords: Auxins; *Balanites aegyptiaca*; Biomass; Callus; Diosgenin; Hingota.

Introduction

Balanites aegyptiaca (L.) Del. is an important multipurpose tree of the desert ecosystem. This woody genotype belongs to family balanitaceae. It is well distributed in South Africa, India and Egypt. It has economic importance in the field of pharmacology and fuel and fodder. The fruits and roots of this plant contain sapogenins - diosgenin and yamogenin. Diosgenin is an important compound present in the roots (0.2-2.2%) and fruits (0.3-3.8%), which can be used in development of oral contraception drugs. Diosgenin is a steroidal saponin used as the starting point for the commercial source of pregnanolone and progesterone used as the first birth control pills. It is s precursor for the synthesis of a number of steroidal drugs. Effects of culture media and carbon and nitrogen sources on biosynthesis of diosgenin in callus cultures of *B. aegyptiaca* were examined by Suthar *et al.*,[1]. They have observed that a high solute concentrations of MS medium and increased concentration of sucrose raised diosgenin content in callus of *Balanites*. The present study was hence carried out with the aim of studying culture conditions for optimized callus induction and proliferation which can then be used for diosgenin production on a commercial scale.

Materials and Methods

Explant- Seeds, mature stem nodes, mature leaves, young leaves, young thorns, apical buds and floral buds were collected from selected healthy mature tree growing in AFRI campus, Jodhpur. These explants were first washed thoroughly with running tap water followed by washing with 2% phosphate-free detergent solution and rinsed thoroughly with distilled water thrice to remove traces of detergent. These explants were then surface sterilized with 0.1% (w/v) mercuric chloride solution. Mature stem nodes, mature leaves, young leaves and young thorns were exposed to steriliant for 5 minutes, seeds were exposed for 10 minutes while apical buds and floral buds were exposed to steriliant for 3 minutes. Explants were rinsed thrice with autoclaved distilled water to remove the traces of mercuric chloride. Seeds were then inoculated on water-agar (0.8% w/v). These seeds germinated within one to two weeks. Six to eight weeks old seedlings were used as source of explants for juvenile stem nodes and root segments (Fig. 1).

Culture medium and growth conditions- Murashige and Skoog's[2] media was used as a source of nutrient for different experiments. MS media was supplemented with phytohormones, sucrose (3% w/v) and solidified with agar (0.8% w/v). The phytohormones used were α-Naphthalene acetic acid and 2,4 Dichlorophenoxy acetic acid. The pH of the media was adjusted to 5.8 before autoclaving. Fourty milli liter of medium was dispensed per conical flask and in baby food jar. Cotton plugs made up of non-absorbent cotton were used. Glassware, forceps, scalpels, media and distilled water were autoclaved for 20 minutes at 121°C and 15 psi pressure.

The cultures were incubated under 16 hours light (1400-lux light intensity using 40W florescent rods) and 8 hour dark period at a temperature of 26 ± 2°C. The cultures were regularly sub-cultured after every four weeks on fresh medium. Observations were recorded at every one-week interval. Minimum of 25 explants were taken per experiment.

Callus induction and maintenance-Different explants were inoculated on MS media supplemented with different phytohormones. Callus was multiplied by further subculture on same media or on different combination of NAA and BAP or Kinetin (refer tables). Preliminary experiments were conducted to select out the most

Table 1. Effect of NAA (2.5 mg/l) on different explants after 4 weeks of incubation.

Explant	*% Callusing*	*Duration to callus induction*	*Growth*	*Colour*	*Texture*
Root segments	100	1 weeks	+	Creamy white	Compact
Apical buds	33.33	3 weeks	+++	White	Fragile
Mature stem nodes	33.33	2 weeks	Poor	White	Fragile
Juvenile stem nodes	66.67	2 weeks	Poor	White	Fragile
Young thorns	100	2 weeks	Poor	White	Compact
Mature leaves	100	3 weeks	Poor	Creamy white	Fragile
Floral buds	20.00	3 weeks	+++	White	Compact
Young leaves	80.00	3 weeks	Poor	White	Compact

Table 2. Effect of different concentrations of NAA used singly on various explants after 4 weeks of incubation.

Hormone NAA (mg/L)	*Percentage callusing*	*Duration (callus induction)*	*Callus growth*	*Colour*	*Callus texture*	*Fresh weight (g)*	*Dry weight (g)*	*% Biomass*
Root Segment								
0.0	0.0	-	-	-	-	-	-	-
0.5	86.11	2 weeks	++	White	Fragile	1.3274	0.2927	22.05
1.0	85.00	2 weeks	++	White	Fragile	1.7276	0.1252	7.25
2.0	100	2 weeks	++	White	Fragile	2.5708	0.3781	14.71
3.0	75	2 weeks	+	Creamy white	Fragile	1.7801	0.1227	6.89
4.0	52.63	2 weeks	+	Creamy white	Fragile	0.874	0.1048	11.99
5.0	62.50	2 weeks	+	Creamy white	Fragile	0.2575	0.0171	6.64
Apical bud								
0.0	0	-	-	-	-	-	-	-
0.5	28.60	2 weeks	+	White	Fragile	0.0588	0.0064	10.88
1.0	85.18	2 weeks	Poor	Creamy white	Fragile	0.1119	0.0108	9.65
2.0	91.67	2 weeks	+	Creamy white	Fragile	0.1687	0.0167	9.90
3.0	85.71	2 weeks	+	White	Fragile	0.1167	0.0112	9.60
4.0	80.95	2 weeks	+	Creamy white	Fragile	0.0813	0.0062	7.63
5.0	55.56	2 weeks	+	White	Fragile	0.2932	0.0259	8.83
Young leaf								
0.0	0.0	-	-	-	-	-	-	-
0.5	0.0	-	-	-	-	-	-	-
1.0	83.33	3 weeks	Poor	White	Compact	0.0322	0.0021	6.52
2.0	83.33	3 weeks	Poor	White	Compact	0.0741	0.0048	6.48
3.0	66.67	3 weeks	Poor	White	Compact	0.0580	0.0040	6.90
4.0	91.67	3 weeks	Poor	White	Compact	0.9382	0.0251	2.68
5.0	100	3 weeks	Poor	White	Compact	0.2556	0.0223	8.72
Young Thorn								
0.0	-	-	-	-	-	-	-	-
0.5	66.67	2 weeks	Poor	White	Compact	0.0086	0.0007	8.14
1.0	50.00	2 weeks	Poor	White	Compact	0.0413	0.0035	8.47
2.0	50.00	2 weeks	Poor	White	Compact	0.0763	0.0068	8.91
3.0	50.00	2 weeks	Poor	White	Compact	0.0841	0.0051	6.06
4.0	60.00	2 weeks	Poor	White	Compact	0.0817	0.0046	5.63
5.0	62.50	2 weeks	+	White	Compact	0.1218	0.0062	5.09

Table 3. Effect of 2.0 mg/l 2,4 D on explants after 4 weeks of incubation.

Explant	*% Callusing*	*Duration to callus induction*	*Growth*	*Colour*	*Texture*
Root segments	93.33	1 week	+	White	Friable
Apical buds	20.00	3 weeks	+	White	Friable
Young thorns	40.00	3 weeks	+	White	Friable

Table 4. Effect of 2,4 D after 4 weeks of incubation.

Hormone 2,4 D (mg/L)	*Percentage callusing*	*Duration (callus induction)*	*Callus growth*	*Colour*	*Callus texture*	*Fresh weight (g)*	*Dry weight (g)*	*% Biomass*
Root Segment								
0.0	0	-	-	-	-	-	-	-
0.5	100	1 week	+	white	Friable	0.8624	0.0827	9.59
1.0	100	1 week	++	white	Friable	0.7026	0.0769	10.95
2.0	93.33	1 week	+	white	Friable	0.4133	0.0497	12.02
3.0	60.00	1 week	++	white	Friable	0.3271	0.0293	8.97
4.0	66.67	1 week	++	white	Friable	0.3989	0.0245	6.13
5.0	76.67	1 week	++	white	Friable	0.6421	0.0308	4.80
Apical bud								
0.0	0	-	-	-	-	-	-	-
0.5	100	3 weeks	++	white	Friable	0.5604	0.0386	6.89
1.0	100	3 weeks	++	white	Friable	1.8861	0.0863	4.57
2.0	20.00	3 weeks	+	white	Friable	0.2784	0.0185	6.66
3.0	85.71	3 weeks	+	white	Friable	0.4773	0.0332	6.96
4.0	41.67	3 weeks	++	white	Friable	0.5659	0.0367	6.49
5.0	100	3 weeks	+	white	Friable	0.6159	0.0411	6.67

Table 5. Results of incubation of callus derived from floral buds of *Balanites aegyptiaca* on MS+2.5NAA, when transferred to MS media supplemented with following hormonal combinations (25 explants per combination) after 4 weeks.

Hormone			*Colour*	*Texture*	*Growth*	*Fresh weight (g)*	*Dry weight (g)*	*% Biomass*
NAA (mg/L)	*BAP (mg/L)*	*KN (mg/L)*						
0	0	0	White	Very compact	Poor	1.0166	0.1543	15.18
2.5	0	0	White	Compact	++++	0.7498	0.0534	7.12
0.5	0.5	0	Creamy white	Compact	+++	2.9801	0.1599	5.37
0.5	1.0	0	Creamy white	Fragile	++	1.2375	0.0843	6.81
0.5	2.0	0	Creamy white	Compact	++	3.0704	0.1685	5.49
0.5	3.0	0	Creamy white	Compact	Poor	-	-	-
0.5	4.0	0	Creamy white	Compact	No growth	-	-	-
1.0	0.5	0	Creamy white	Compact	Poor	0.6464	0.0360	5.57
1.0	1.0	0	White	Compact spongy	++	1.4645	0.0856	5.84
1.0	2.0	0	White	Compact spongy	++	1.0655	0.0758	7.11
1.0	3.0	0	White	Compact spongy	+	1.6535	0.2749	16.63

Contd...

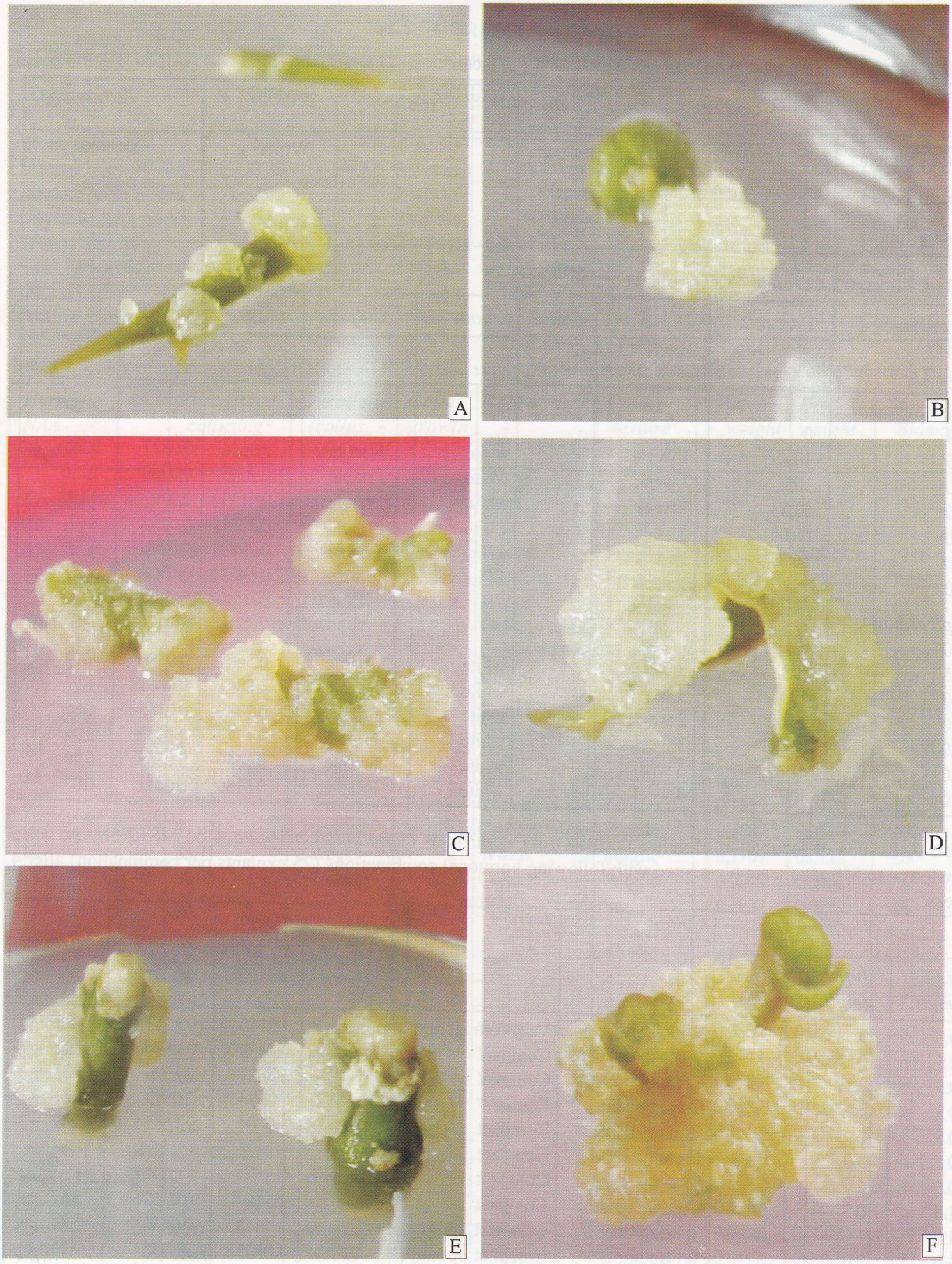

Fig. 1. Callus derived from: A - Young thorn; B – Floral bud; C – Root segment; D – Leaf; E – Stem nodal segment; F – Apical bud in *Balanites aegyptiaca* L. (Del.)

1.0	4.0	0	Creamy white	Fragile watery	+	1.3168	0.0681	5.17
1.0	5.0	0	Creamy white	Compact	+	1.5561	0.0102	0.66
1.0	10.0	0	Creamy	Watery spongy	+	1.5414	0.0996	6.46
1.0	0	2.0	White	Compact	No growth	1.6770	0.0873	5.21
1.0	0	3.0	Creamy white	Compact	No growth	1.6864	0.0934	5.54
1.0	0	4.0	Creamy white	Fragile	No growth	1.9264	0.1024	5.32
2.0	0	2.0	White	Fragile	++	1.6397	0.0837	5.10
1.0	0	10.0	Creamy	Compact	+	1.4723	0.1014	6.89
0	1.0	1.0	Creamy white	Fragile	++	1.1550	0.1037	8.98

responsive explant in terms of callus production with a good multiplication rate. All the explants were initially tested MS media supplemented with 2.5 mg/l NAA (Table 1). Explants reporting higher percentage callusing and/or fast rate of multiplication were then selected for further experimentation on biomass screening. Young thorns and mature leaves were dropped because callus showed poor growth after first sub-culture.

Biomass study: Callus was regularly sampled from cultures and fresh weight was recorded. It was then dried at 65 °C for 48 hours in an electric oven and subsequently dry weight was recorded.

Percentage biomass is calculated as follow:

% Biomass= (Dry weight/ Fresh weight) X 100

Results and Discussion

Results from NAA supplemented MS medium showed good callusing in apical buds, root segments (Table 1 & 2). Apical buds and root segments were then cultured on 2,4-D supplemented medium. It was also observed that young thorn showed 100% callusing but poor growth on NAA supplemented medium (Table 3). Best callusing response was observed from root segments and apical buds on both NAA and 2,4-D supplemented media (Table 4).

Maximum biomass (22.02%) accumulated in callus derived from young leaves on 0.5 mg/l NAA while minimum (2.68%) on root segments cultured on MS medium supplemented with 4.0 mg/l NAA. In case of apical bud explant it was observed that maximum biomass (10.95%) was attained in callus obtained from root segments on 1.0 mg/l 2,4-D supplemented media while minimum (4.57%) on 1.0 mg/l 2,4-D supplemented medium.

Callus derived from floral buds on 2.5 mg/l NAA supplemented media was cultured on different combinations of phytohormones to get a fast growing high biomas-yielding callus. 1.0 mg/l NAA and 3.0 mg/l BAP supplemented media gave moderate growing callus with highest percentage biomass (16.63%). Lowest percentage biomass (0.66%) from floral bud callus was recorded on 1 mg/l NAA and 5.0 mg/l BAP supplemented media which produces moderately growing watery callus.

From the study it appears that the callus percentage biomass decreases with increase in auxin concentration in the media.

The percent biomass of callus derived from root segment has been found to be more than that of all other explant-derived calli that were screened in the present study.It may be possibly due to higher biosynthetic activity of the callus. Over all this study implies that callus produced by root segment yields higher biomass, irrespective of the auxin (2,4-D or NAA) used. It is well known that roots of *Balanites aegyptiaca* synthesizes diosgenin, so there must be a good potential of diosgenin synthesis in the callus derived from root segments[1]. This potential can be explored by conducting further biochemical studies of the callus. Diosgenin has been reported from callus of other species viz. -*Trigonella foenum-graecum* L.[3], *Dioscorea deltoidea*[4], and *Piper betle* L.[5]. Our study recommends the use of root segments of *Balanites aegyptiaca* as the starting material for callus induction.

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