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CLONAL PROPAGATION OF CAROB (*CERATONIA SILIQUA* L., FABACEAE)

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Abstract

Mature seeds of carob tree (*Ceratonia siliqua* L.) were germinated on hormone free MS medium. Efforts were made to develop multiple shoots by using axillary buds of *in vitro* grown seedlings on MS medium fortified with different concentrations of BA singly and BA in combination with IAA or GA_3. Axillary buds produced single shoot with a moderate amount of callus at the base of the explant after culturing on MS medium with BA alone. Multiple shoots were regenerated when explants when cultured on MS medium fortified with BA + IAA or BA + GA_3. MS medium supplemented with 1.5 mg/l BA + 0.5 mg/l GA_3 was found more effective in multiple shoot regeneration than all other combinations. Regenerated multiple shoots were excised and cultured on half strength of MS medium containing different concentrations of IBA for root induction. Best root development was obtained in half strength MS medium containing 0.5 mg/l IBA. About 70% of the regenerated plantlets survived in natural conditions.

Introduction

The carob tree, *Ceratonia siliqua* L. (Fabaceae), has been grown since antiquity in most countries of the Mediterranean basin, usually in mild and dry places with poor soils. Its value was recognized by the ancient Greeks who brought it from its native Middle East to Greece and Italy, and by the Arabs, who disseminated it along the North African coast and north into Spain and Portugal.

The carob tree grows as a sclerophyllous evergreen shrub or tree up to 10 m high, with a broad semispherical crown and a thick trunk with brown rough bark and sturdy branches. Carob pods are used as fodder for ruminants (Louca and Papas 1973) and non-ruminants (Sahle *et al.* 1992).The seed of the carob tree contains galactomannon, a commercially valuable carbohydrate that is used as thickener, emulsifier and stabilizer in many commercial products (Lewington 1990). Wood is reported to be used in making furniture and carts. Commercial production of carob pods is estimated currently to be about 310,000 tons per year.

Conventionally carob plants are propagated by seeds. Dried, hard seeds need to be scarified or chipped and then soaked in water or dilute sulfuric or hydrochloric acid solutions until they swell. Germination rate may be only 25%. Seeds usually germinate after six weeks or more. Budding of this plant is done when the stem is at least 1 cm thick. Some common pests, insects (*viz*. the carob moth, *Myelois ceratoniae*) and fungi are main obstructions for the conventional propagation of this plant. It lays eggs on the flowers or newly-formed pods and the larvae bore into the pods and damage them. The larvae of a midge, *Asphondylia gennadii,* cause stunting of pods.

Application of tissue culture method offers rapid multiplication of forest, fruit as well as other crops specially those which are heterozygous and difficult to propagate by conventional means (Litz *et al*. 1983, McCown and McCown 1987, Ahuja 1991). It has already been established that micropropagation of shoots has become an important method for multiplication of fruit trees (Krieken *et al*. 1993). Moreover, micro shoots obtained by this method could be the material source for studying the morphogenetic capacity of different organs and tissues, appearance of somaclonal variants (Besendorfer *et al*. 1989) and resistance to pests or diseases.

Micropropagation of carob using both juvenile and adult tissues has been attempted (Vinterhalter *et al*. 1992, Androulakis 1994, Alorda and Medrano 1996). However, there are no reports of plants from *in vitro* culture being successfully established in the field. So an attempt was made to multiply this plant using *in vitro* culture.

Materials and Methods

The fruits (Fig. 1A) were collected from a 'Haj' pilgrim from Saudi Arabia. Although this tree in not a native of Saudi Arabia but its fruits are readily available there as one of the imported food-stuff. Seed coat of carob is very hard. So it was necessary to scarify seeds (Fig. 1B) with 80% sulphuric acid for 30 minutes with regular shaking and then washed several times with distilled water. Seeds were then surface sterilized with 0.1% mercuric chloride solution for 10 minutes followed by 4-5 washing with sterile distilled water. These seeds were then germinated (Fig. 1C) under axenic condition on MS (Murashige and Skoog 1962) basal medium without growth regulators. Nodal segments containing axillary buds from three to four weeks old seedlings were used as primary explants. Initially explants were cultured on to MS basal medium supplemented with different concentrations of BA singly and a combination treatments of BA + IAA or BA + GA_3. The concentrations of BA were 0.5, 1.0 and 1.5 mg/l while those of of IAA or GA_3 were 0.1, 0.5 and 1.0 mg/l.

For rooting, excised shoots were cultured on half strength of MS medium containing three different concentrations (0.1, 0.5 and 1.0 mg/l) of IBA. Sucrose (3%) and Sigma-agar (0.7%) were added to all the media concentrations. The pH was adjusted to 5.8 before autoclaving (1.05 kg/cm^2 for 20 minutes). Cultures were incubated at a temperature of 25±1° C under the illumination (about 1500 lux) of fluorescent tubes with a light-dark cycle of 16/8 hours. As a potting substrate equal proportion of soil and organic compost was used.

Results and Discussion

Stem segment containing three to four nodes having axillary buds obtained from *in vitro* grown seedlings were cultured on to MS medium containing different concentrations of BA (0.5, 1.0 and 1.5 mg/l) singly and BA in combination with IAA or GA_3 (0.1, 0.5 and 1.0 mg/l). No multiple shoots were produced when explants cultured on MS fortified with different concentrations of BA alone. Single shoot with a moderate amount of callus at the base of the explant were found in these compositions. All the combinations were found to produce a variable response towards multiple shoot induction. However, BA + GA_3 combination was found more effective than BA + IAA combination (Table 1). Among all the treatments of BA + IAA, 1.0 mg/l BA + 0.5 mg/l IAA produced a maximum number of shoots and elongation. On the other hand, BA + GA_3 yielded comparatively higher number of multiple shoots compared to BA + IAA combination. Among all the combinations of BA + GA_3 used, 1.5 mg/l BA + 0.5 mg/l GA_3 produced maximum number of shoots (Fig. 1D). But maximum elongation of shoot was recorded under 1.0 mg/l BA + 1.0 mg/l GA_3 combination. Nodal segments have been demonstrated to be useful materials for mass propagation of woody plants because of high regeneration ability of the explants (Kaur *et al*. 1998, Quraishi and Mishra 1998). The combined effect of BA + GA_3 and BA + IAA on shoot regeneration was also reported earlier workers by Kabir *et al*. (1994), Islam *et al*. (2001), Hoque *et al*. (2007), Karuppusamy and Pullaiah (2007).

In vitro regenerated shoots were excised and cultured on the ½ MS medium supplemented with 0.1, 0.5 and 1.0 (mg/l) of IBA for root induction. Root formation was achieved in all the combinations of IBA but better at 0.5 mg/l in terms of number and length of root compared to other concentrations (Figs 1E and 2). Rooting of higher percentage of excised shoots and complete

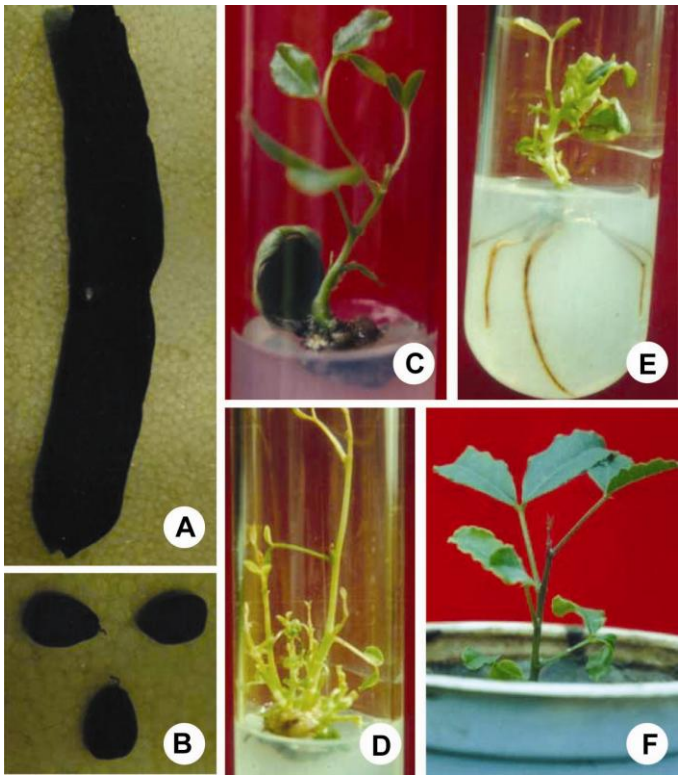

Fig. 1A-F: *Ceratonia siliqua*. A. Fruit, B. Seeds, C. *In vitro* germination of a seed, D. Induction of multiple shoot on MS medium added with 1.5 mg/l BA + 0.5 mg/l GA_3, E. Rooting of excised shoot on half strength MS medium + 0.5 mg/l IBA and F. Acclimatized plant in a small plastic pot.

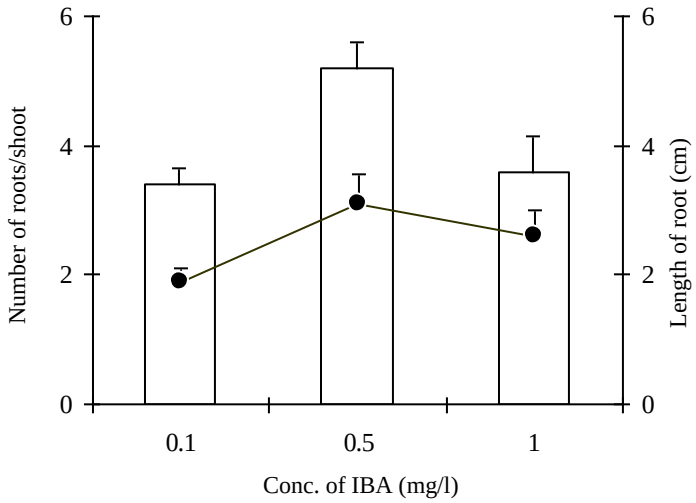

Fig. 2. Effects of different concentrations of IBA on rooting of excised shoot in *Ceratonia siliqua*. n = 12, Bar represents standard error, −●−Root length.

Table 1. Shoot multiplication from axillary bud of *in vitro* raised seedling of *C. siliqua*. Data were recorded after five weeks of culture. n = 12, ± = standard error.

Growth regulators (mg/l)	Number of shoots/ explant	Length of shoot (cm)
0.5 BA + 0.1 IAA	1.65 ± 0.21	2.2 ± 0.26
0.5 BA + 0.5 IAA	2.10 ± 0.31	2.1 ± 0.19
0.5 BA + 1.0 IAA	1.95 ± 0.24	1.9 ± 0.23
1.0 BA + 0.1 IAA	3.10 ± 0.48	2.8 ± 0.34
1.0 BA + 0.5 IAA	3.43 ± 0.21	3.6 ± 0.29
1.0 BA + 1.0 IAA	2.77 ± 0.39	2.7 ± 0.23
1.5 BA + 0.1 IAA	2.18 ± 0.31	3.4 ± 0.31
1.5 BA + 0.5 IAA	2.65 ± 0.46	2.9 ± 0.37
1.5 BA + 1.0 IAA	2.81 ± 0.48	3.1 ± 0.22
0.5 BA + 0.1 GA_3	3.10 ± 0.45	3.1 ± 0.29
0.5 BA + 0.5 GA_3	2.95 ± 0.53	3.8 ± 0.26
0.5 BA + 1.0 GA_3	4.33 ± 0.79	3.6 ± 0.37
1.0 BA + 0.1 GA_3	4.52 ± 0.34	4.3 ± 0.61
1.0 BA + 0.5 GA_3	4.12 ± 0.46	3.9 ± 0.51
1.0 BA + 1.0 GA_3	3.91 ± 0.53	4.7 ± 0.48
1.5 BA + 0.1 GA_3	4.83 ± 0.71	4.4 ± 0.51
1.5 BA + 0.5 GA_3	6.12 ± 1.21	4.2 ± 0.55
1.5 BA + 1.0 GA_3	5.41 ± 1.05	4.1 ± 0.39

complete plant regeneration were accomplished on the above composition. Litz and Jaiswal (1990) reported IBA as the most suitable auxin for induction of root. The successful root initiation using IBA was also described by Chalupa (1983) and Perez and Postigo (1989) in cultures of several forest species. The regenerated plants were transferred to small plastic pots containing soil and compost (Fig. 1F) and subsequently acclimatized to natural environment. After one month, the plantlets were successfully transplanted to the field.

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