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In vitro floral bud synthesis and multiple shoot regeneration studies in watermelon (*Citrullus lanatus*) from seed explants

R.Chakravarthy*, V.N.Koumudi, D.Daniel Kumar, K.Lavanya

Dept. of Biotechnology, K L University, Green Fields, Vaddeswaram – 522502, Guntur, (INDIA)

E-mail : smgaddad@gmail.com

ABSTRACT

A protocol for invitro flowering and multiple shoot regeneration in watermelon (*Citrillus lanatus* L.) is described. The seeds were excised from mature fruit and cultured on Murashige and Skoog (MS) medium supplemented with Benzyl adenine (BA) 1.0 mg/l. After 3 days seeds were germinated, the sprouted seeds were isolated and cultured on MS medium with Indole Buytric acid (IBA) 0.3mg/l showed efficient rhizogenesis. BA 0.75mg/l + Adenosine (Ads)0.1mg/lt induced proliferated floral buds from invitro grown shoots, which resulted in synthesis of *in vitro* flowers, later developed to multiple shoots originating from single node. The rooted shoots were acclimatized effectively in the field.

KEYWORDS

Citrillus lanatus;
Benzyl adenine (BA);
Murashige and skoog (MS) medium;
Indole buytric acid (IBA);
Adenosine (Ads) and in vitro flowering.

INTRODUCTION

Citrillus lanatus is a creeping vine which bears oval to round shaped edible fruit when ripe. It has a smooth skin and may vary in color from light green to dark green. Some varieties have stripes[8]. The flesh may be white, creamy yellow, pale red, red or dark red. Watermelon flesh is juicy and crunchy. It consists of 90% water. Their seeds are usually black and embedded in the fruit. Citrulline in watermelon rind shows antioxidant effects that protect body from free-radical damage. It gets converted to arginine, an amino acid vital to the heart, circulatory system and immune system. Citrulline helps in treatment for erectile dysfunctioning, also used in regulating blood sugar levels. Lycopene present in this fruit has known antioxidant qualities to keep the skin, heart, and prostate healthy. It is recommended as best food in summer to avoid sun strokes. Seeds of watermelon induces arginine used in treating coronary heart infections also used to regulate breast cancers. The seeds of this fruit contain proteins, B vitamins, minerals and fats which help in reducing bad cholesterols. Seeds are consumed as fruits and salads. Pulp is used in preparation of jellies, jams, sauces, sweets and rind is used in preparation of pickles[1]. Because of its high degree of sterility and polyploidy of the edible varieties, classical breeding is difficult. Due to the decrease agricultural land and prolonged climatic changes due to global warming it is difficult to achieve large production to meet needs of increasing population. Hence techniques of tissue culture come into a force to resolve the aforesaid problems[3].

Nutraceutical values in watermelon

TABLE 1 : Given below is the amount of nutrients present in 100 gm of watermelon.

Calcium	7 mg
Carbohydrate	7.55 gm
Fats	0.15 gm
Fluoride	1.5 mg
Iron	0.24 mg
Magnesium	10 mg
Phosphorus	11 mg
Potassium	112 mg
Protein	0.61 gm
Sodium	1 mg
Sugars	6.20 gm
Total Dietary Fiber	0.4 gm
Total Folate	3 mg
Vitamin A	569 IU
Vitamin C	8.1 mg
Water	91.45 gm
Energy	30 kcal
Other supplements	Lycopene, Phytofluene, Phytoene, Beta-Carotene, Lutein and Neurosporene in minimum quantities

MATERIALS AND METHODS

All the seed explants were first rinsed in sterile distilled water and then sterilized by immersing in 0.1% (w/v) $HgCl_2$ for 3 minutes. After four further rinses in sterile distilled water the explants were cut to the required size and inoculated onto culture medium[4,7]. All the explants were placed horizontally on the medium.

CULTURE MEDIUM AND CONDITIONS

The culture medium used for the explant selection was GR-free Murashige and Skoog[5] medium supplemented with 0.8% (w/v) agar, 3% (w/v) sucrose and enriched with varying hormonal concentrations were used further on to determine optimum growth regulator levels. The pH was adjusted to the range of 5.6 to 5.8 with 1 N NaOH or HCl before molten media were dispensed into petri plates (Borosil, India) and the media were autoclaved at 121 °C at 15 p.s.i pressure for 20 min. The cultures were maintained at 25±2 °C under a 16-h photoperiod of 50μmol m^{-2} s^{-1} irradiance provided by cool white fluorescent tubes.

RESULTS & DISCUSSION

Seeds were extracted from mature fruits and kept for incubation. After two days we found the germination of microscopic nodular bud formation from the seeds. These seeds were supplemented on MS medium with BA (0.25, 0.5, 0.75, 1.0 mg/l) for invitro shoot bud initiation. After 4 days of culture shoot buds were initiated and grown well on BA 1mg/lit concentration, when compared with other concentrations of benzyl adenine. Invitro shoot bud development was found but showed poor growth on BA0.25, 0.5, 0.75 mg/l. The explants inoculated on MS medium with BA 0.25 mg/l showed 60% of shoot regeneration which was comparatively lower than the other tested concentrations. The explants inoculated onto MS medium with BA 0.5 mg/l showed 82% of shoot regeneration where as explants Inoculated onto MS medium with BA 0.75 mg/l showed 90% of shoot growth which can be considered as good rate of regeneration but MS medium with BA 1.0 mg/l gave 98% of shoot regeneration from seed explants[8]. In vitro grown shoot buds were shifted onto MS rooting media with IBA 0.1, 0.3 & 0.5mg/l. The explants with initiated shoots transferred onto MS medium with IBA 0.3mg/l gave very high response in terms of root bud growth after two days of incubation. However the percentage of root initiation was not significant in IBA both at 0.1 mg/l and 0.5 mg/l.

The healthy plantlet regenerated was shifted onto MS medium with BA (0.5, 0.75 & 1.0mg/l) along with adenosine 0.1 mg/l for achieving in vitro flowering. Interestingly our trails with Adenosine gave a remarkable floral bud proliferation resulting in synthesis of in vitro flowers[2,6] after an incubation period of seven days on MS medium with BA 0.75mg/l + Ads 0.1 mg/l (see TABLE 2 & Graph 1). The flower developed showed white colored flowers with hairy green floral stock,(see Figure A & B) on further incubation the plantlet bearing floral buds increased in terms of both shoot and root growth and gave rise to multiple shoots within fifteen days of incubation. On further incubation the flower showed drying and turned brown but gave rise to multiple shoots (see Figure C & D) on further incubation. The multiple shoots showed single nodal origin and were observed to be healthy. They were later hardened for field establishment as shown in (see Figure E).

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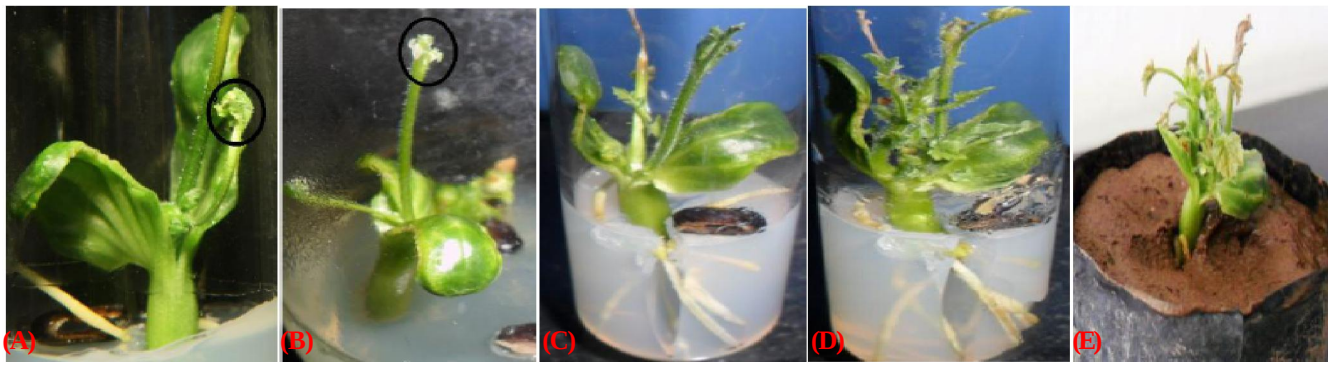
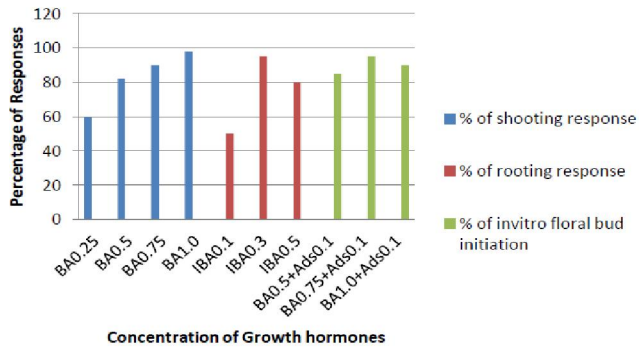


Figure 1 : (A) is showing shoot regeneration and flower bud initiation; (B) is showing invitro flower with white colored petals; (C) shows effective shoot and root regeneration; (D) represents synthesis of multiple shoots with rhizogenesis; (E) pot culture.

TABLE 2 : Effect of growth hormones on induction of shoot, root and floral buds.

S. No	Growth hormones (mg/lit)	% Shoot bud formation	% Root bud initiation	% Invitro floral buds
1	BA0.25	60	-	-
2	BA0.5	82	-	-
3	BA0.75	90	-	-
4	BA1.0	98	-	-
5	IBA0.1	-	50	-
6	IBA0.3	-	95	-
7	IBA0.5	-	80	-
8	BA 0.5+Ads 0.1	-	-	85
9	BA 0.75+Ads 0.1	-	-	95
10	BA 1.0+Ads0.1	-	-	90



Graph 1 : Growth hormone concentrations and its response (%) on invitro shooting, rooting and flowering.

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ABBREVIATIONS

Benzyl adenine –BA
Murashige and Skoog –MS
Indole-3- Butyric acid –IBA
Adenosine –Ads

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