VNU Journal of Science: Natural Sciences and Technology, Vol. 32, No. 1S (2016) 242-246

Direct Adventitious Shoot Regeneration from Triploid Watermelon Cotyledons

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Received 02 June 2016
Revised 02 August 2016; Accepted 09 Septeber 2016

Abstract: An *in vitro* regeneration system was established for cotyledon explants of triploid watermelon (*Citrullus lanatus* Thumb.). Full strength MS medium supplemented with 0.2 mg.L^{-1} IAA in combination with different concentrations of BAP was employed for investigating adventitious shoot formation. Medium containing 1.5 mg.L^{-1} BAP and 0.2 mg.L^{-1} IAA was suitable for direct shoot induction from cotyledon explants. Adventitious shoots were elongated for 3 weeks on medium containing 0.5 mg.L^{-1} GA3 before transferring into rooting medium. Frequency of root induction achieved approximately 88% with an average of 4.3 root per shoot on half strength MS medium supplemented with 0.5 mg.L^{-1} IBA. Rooted shoots were successfully acclimatized in potted soil mixture with survival rate of 82.6%.

Keywords: Triploid, *Citrullus lanatus*, regeneration.

1. Introduction

Watermelon (*Citrullus lanatus* Thumb.) is an economically important cucurbitaceous species grown in tropical and semitropical regions of the world. Originating from Africa, watermelon has been cultivated worldwide, especially in Asia, Caribbean and Southern parts of the United State of America [1]. This fruit crop is widely planted in many provinces in the Southern Vietnam with annual products of over 500 thousand tons in total [2]. Watermelon flesh is rich source of carbohydrates, minerals and vitamins [3]. The fiber content in watermelon may be useful to lessen cholesterol and heart disease. Its flesh also contains a significant amount of lycopene, a carotenoid pigment, which is highly efficient on reducing free radical, preventing risk to cancer of the pancreas, prostate and stomach [4, 5].

Most watermelon cultivars are propagated from F1 hybrid diploid and thus exhibit great vigor (heterosis). Seeds saved from these hybrid populations normally are not used for next propagation. The excessive number of hard seeds in the flesh is an undesirable trait for consumers [6]. In recent years, seedless watermelon (triploid) production has meaningfully increased in Vietnam and other countries. However, growing seedless watermelon is more difficult than growing seeded varieties (diploid) [7]. Due to sterile cultivars, triploid watermelon plants are mainly propagated from seeds produced by crossing diploid and tetraploid lines. In addition, triploid

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watermelon can also be multiplied through endosperm culture or propagating triploid plantlets *in vitro*. The objective of the study was to establish an efficient direct regeneration system of triploid watermelon plantlets *in vitro* from cotyledon explants. This regeneration system can be applied as a prerequisite step for genetic transformation and other experiments.

2. Materials and Methods

Plant materials and surface sterilization

Triploid watermelon (*Citrullus vulgaris* Thunb.) cv Famers Wonderful was employed as explant source for establishment of aseptic materials *in vitro*. Initially, mature seeds were shortly soaked in 70% (v/v) ethanol and removed seed coats. Subsequently, decoated seeds were immersed in 1.0% NaClO solution for 15 minutes. To remove any trace of chlorine, decoated seeds were rinsed several times in sterile distilled water before being placed onto germination medium. The cultures were initially maintained in darkness to promote germination of seeds.

Culture medium conditions

Media were modified from the full strength MS medium (Murashige and Skoog, 1962) [8] supplemented with 3% (w/v) sucrose, 10% (v/v) coconut milk, different kinds of vitamins and plant growth regulators. The pH of media was adjusted to pH prior to being solidified with 7.0 $g.L^{-1}$ agar and autoclaved at 121^{o}C for 15 minutes. Cultures were maintained at 25 ± 1^{o}C with a photoperiod of 12h under an illumination of cool white fluorescent lamps at approximately 35 $\mu mol.m^{-2}.s^{-1}$.

Direct adventitious shoot induction

Cotyledon explants were derived from 5-day-old seedlings of triploid cultivars. Cotyledons were excised into small segments with sizes of 0.5 × 1.0 cm and cultured on modified MS medium (as described above) supplemented with various concentrations of benzyl amino purine (BAP) and 0.2 $mg.L^{-1}$ indole-3-acetic acid (IAA).

Shoot elongation and root formation

Shoots were separated from adventitious clumps and transferred to new medium containing gibberellic acid-3 (GA3) and various vitamins for the elongation process. After 3 weeks growing on elongation medium, shoots longer than 2.0 cm were disjoined and placed on root induction medium. To enhance root formation, mineral levels of root induction media were half strength (50%) of MS medium. These media were supplemented with indole-3-acetic acid (IBA) to stimulate root formation. Plantlets with well-developed roots were transplanted to small pots under high humidity prior to being maintained under ambient humidity.

Data analysis

Experiments were arranged in a randomized design and performed in 3 replicates for each treatment. The data was subject to analysis of variance using SPSS (17.0) software. Significant means were separated at $p = 0.05$ using least significant test.

3. Results and Discussion

Adventitious bud formation from cotyledon explants

Shoot regeneration from cotyledon explants can be occurred through direct or indirect pathway depending on the species and culture conditions. In this experiment, explants from cotyledons were cultured on MS medium supplemented with different concentration of BAP in combination with 0.2 $mg.L^{-1}$ IAA to induce adventitious bud formation (Table 1). Adventitious buds were clearly observed after 3

weeks of culture and developed into shoots at the end of 3rd week. On medium W9 containing 1.5 mg.L^{-1} BAP and 0.2 mg.L^{-1} IAA, the shoot induction rate and shoot number were recorded at 81.67% and 7.1 shoots per explant, respectively. Higher concentration of BAP in medium also stimulated shoot formation as indicated in medium W10 with the induction rate of approximately 83% and 7.3 shoots per explant. Therefore, the concentration of 1.5 mg.L^{-1} BAP and 0.2 mg.L^{-1} IAA could be optimal levels for shoot formation of triploid watermelon cultivar. The results suggested that the combination of high level of BAP and low level of IAA might be suitable for shoot induction from cotyledons. The results were in accordance with the findings of Zhao et al on diploid watermelon [9]. They observed that high percentage of shoot regeneration was induced on medium supplemented with 2.0 mg.L^{-1} BAP and 0.2 mg.L^{-1} IAA. Shalaby and co-worker (2008) found that MS medium supplemented with BAP from 1.0 to 5.0 mg.L^{-1} could be used for shoots induction from triploid watermelon cotyledons [7].

Table 1. Effect of BAP on shoot formation from cotyledon explants

Medium code	BAP (mg.L^{-1})	Regeneration frequency (%)	Mean no. of shoots/ explant
W0	0.0	0.00	0.00
W7	0.5	35.00	3.3 ± 0.28
W8	1.0	56.62	5.6 ± 0.32
W9	1.5	81.67	7.1 ± 0.36
W10	2.0	83.33	7.3 ± 0.41

Shoot elongation

Adventitious shoots induced from cotyledon explants remained compact with short internodes and were unsuitable for rooting. To promote growth, adventitious shoots were separated from each other and transferred to elongation medium. The culture was maintained for 3 weeks at the same photoperiod and temperature as those applied for shoot induction. As shown in Table 2, shoots cultured on medium WE3 containing 0.5 mg.L^{-1} GA3 could elongate to approximately 3.6 cm in comparison to shoot length on control medium WE0. Growth on medium WE4, shoots could reach 4.4 cm in height with longer internodes. Our results were similar to data from Islam et al (1999). They cultured adventitious shoots of diploid watermelon with GA3 at concentration of 0.5 mg.L^{-1} to promote shoot elongation [10].

Table 2. Effect of GA3 and BAP on shoot elongation

Mediumcode	GA3 (mg.L^{-1})	Mean of shoot length (cm)
WE0	0.00	2.1 ± 0.14
WE1	0.10	2.5 ± 0.22
WE2	0.25	3.2 ± 0.31
WE3	0.50	3.6 ± 0.35
WE4	1.00	4.4 ± 0.38

Rooting of shoots

Triploid shoots reached over 2.0 cm in height on elongation media were excised and transferred to root induction media. These media were modified from MS basal medium with haft strength salt concentration. After 10 days growth on root induction media, root primordia were formed and expanded rapidly. Medium WR0 without IBA can promote root formation of triploid shoots up to 21.66% with average of 1.1 roots per shoot (Table 3). However, media containing IBA improved significantly root formation. Medium WR3 supplemented with 0.5 mg.L^{-1} IBA promoted approximately 88.33% root formation compared to control medium WR0. These results agreed with reported by Li et al (2011) that IBA increased efficient root formation in watermelon [3]. Badr-elden and co-worker (2012) used haft strength MS medium supplemented with 0.5 mg.L^{-1} IBA to induce root formation of diploid watermelon cultivar Giza1 [11].

Table 3. Effect of IBA on root formation

Medium code	IBA (mg.L^{-1})	Frequency of root formation (%)	Mean no. of roots/ shoot
WR0	0.00	21.66	1.1 ± 0.12
WR1	0.10	38.35	2.7 ± 0.24
WR2	0.25	56.67	3.2 ± 0.28
WR3	0.50	88.33	4.3 ± 0.31
WR4	1.00	91.67	4.4 ± 0.45

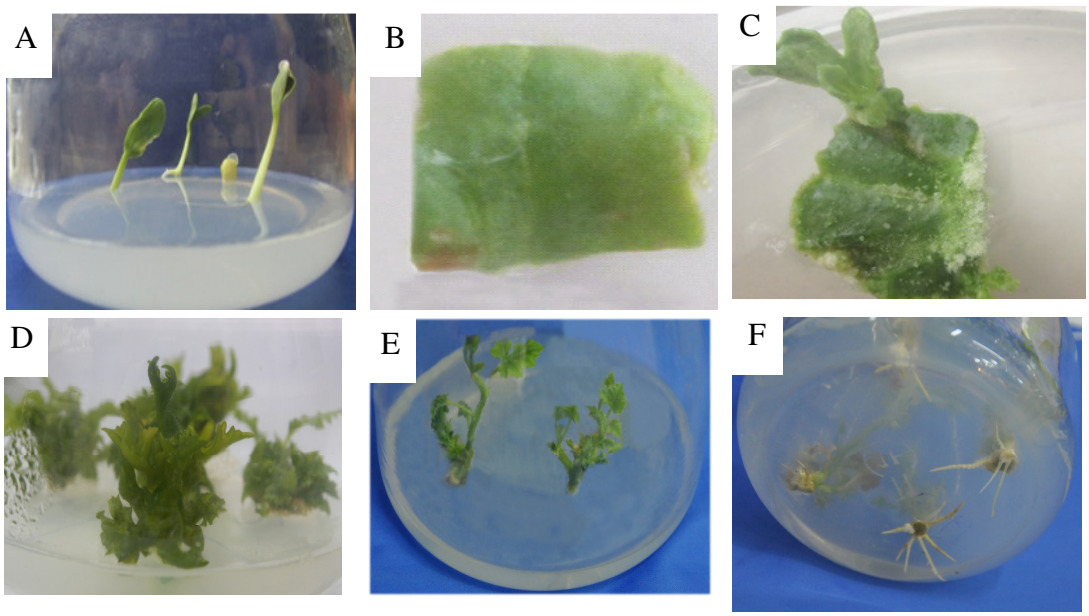

Figure 1. Direct regeneration of shoots from cotyledon explants of triploid watermelon.

A. Triploid seed germination *in vitro*; B. Cotyledon explant on medium containing BAP and IAA; C. Shoot regeneration from cotyledon explant; D. Adventitious shoots derived from cotyledon explant; E. Shoot elongation; F. Rooted shoots.

In conclusion, we have successfully established media for the direct induction of shoot formation from triploid watermelon cotyledons. Adventitious shoots were induced on medium containing 1.5 mg.L^{-1} BAP and 0.2 mg.L^{-1} IAA and subsequently elongated on medium with 0.5 mg.L^{-1} GA3. Elongated triploid shoots were rooted on haft strength MS medium supplemented with 0.5 mg.L^{-1} IBA. Approximately 82.6% of rooted shoots were successfully acclimatized in potted soil mixture.

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Nghiên cứu tái sinh chồi bất định trực tiếp từ lá mầm dưa hấu tam bội

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Tóm tắt: Nghiên cứu xây dựng các môi trường nuôi cấy nhằm tái sinh chồi dưa hấu tam bội (*Citrullus lanatus* Thumb.). Môi trường cơ bản MS có bổ sung các vitamin, BAP và IAA ở các nồng độ khác nhau để đánh giá khả năng tạo chồi. Môi trường có chứa 1,5 mg.L^{-1} BAP và 0,2 mg.L^{-1} IAA là phù hợp cho tái sinh chồi trực tiếp từ mảnh lá mầm dưa hấu tam bội. Các chồi tái sinh được nuôi cấy kéo dài trên môi trường có 0,5 mg.L^{-1} GA3 để tăng kích thước chồi trước khi tiến hành tạo rễ. Môi trường MS có hàm lượng khoáng giảm đi 50% và bổ sung thêm 0,5 mg.L^{-1} IBA đã cho tỷ lệ tạo rễ 88,33% với tỷ lệ trung bình 4,3 rễ/ chồi. Sau 10 ngày chuyển sang trồng trên giá thể đất, tỷ lệ sống sót của cây con đạt được xấp xỉ 82,60%.

Từ khóa: Tam bội, *Citrullus lanatus*, tái sinh.