Buletin Teknol. Tanaman, Bil.12, Tambahan.1 (2015): 260-263

The Effect of Different Concentrations of Gibberelic Acid (GA3) on Somatic Embryogenesis of Spinach (Spinacia Oleracea L.)

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Abstract

In order to stablish on efficient system for in vitro plant regeneration of spinach, the explants and optimum culture condition for somatic embryogenesis were investigate. This study was applied conducted to optimize callusing spinach using 4 concentration of 0.01, 0.1, 0.5 and 0.75 µM GA3. In order to callusing used of 4 explants root, cotyledon, hypocotyls and leaf of seedling were used in the ½MS medium containing 0.5 mg/l nicotinic acid, 0.5 mg/l pyridoxine, 0.4 mg/l thiamine, 100 mg/l myo-inosytol, 10 g/l sucrose, 10 µM NAA and 4 different concentrations of 0.01, 0.1, 0.5 and 0.75 µM GA3, 8 g/l Agar and pH: 5/7. Results showed callusing at a concentration of only explants roots and 0.1 µM GA3 accured and at concentrations above and below this value is 0 percent. To regeneration, callus used of two medium, MS and ½Ms medium containing 0.5 mg/l nicotinic acid, 0.5 mg/l pyridoxine, 0.4 mg/l thiamine, 100 mg/l myo-inosytol and 20 mg/l of sucrose. 65 percent of calli induction embryogenesis and 20 percent of embryogenesis induction stems were place in ½MS medium.

Key words: Plant regeneration, Tissue culture, Somatic embryogenesis

Introduction

Spinach (*spinacia oleracea* L.) is one of the major vegetable crops worldwide and known as a rich source of iron, vitamins and minerals. Successful in vitro plant regeneration and transplantation of regenerated plant to soil is essential for biotechnological approaches to plant micropropagation and improvement. Several studies on applying tissue culture methods to spinach plant regeneration have reported that morphogenic pathways of regeneration varied with the type of explants and the culture (Jin *et al.* 2009). For spinach, a dioecious vegetable plant first reported adventitious shoot formation from seedling opical tip calluses on modified MS medium (Murashige*et al.* 1962) supplemented with 4.65 µM of kinetin and 2.89 µM of GA_3 under diffuse light at about 25°C (Neskovic *et al.* 1973). First reported adventitious bud formation from hypocotyl segments (Sasaki *et al*. 1986) and histologically indentified adventitious buds in the regenerants (Sasaki *et al*. 1987). In other research obtained rooted shoots and indicated about 53% of shoots formation from the hypocotyl tissues (Sasaki *et al*. 1989). In addition, the culture system for shoot organogenesis was developed by using a suitable period of culture with a high concentration of 5, 6- dichloro- indole- 3- acetic acid (5, 6- Cl_2- IAA) (Mii *et al*. 1992), and by applying gibberelic acid together with auxin, allowing for adventitious bud formation from hypocotyl (Sasaki *et al*. 1989; Satoh *et al*. 1992) and leaf segments (Al- khayri *et al*. 1991). This group further improved the efficiency of shoot formation (about 57%) by addition of 15% (v/v) coconut water to the modified MS medium supplemented with different concentrations of kinetin, 2- 4- D and GA_3 at 25±°C under 10 h photoperiod (Al- khayri *et al*. 1992).

In the present work, we stablish a system for plant regeneration, investigating the optimum conditions for somatic embryogenesis from root explants of spinach and determined the effect of high concentrations of GA_3 on callus induction and regeneration of spinach.

Materials and Methods

Plant material

The seeds of spinach (*Spinacia oleracea* L.) (provided from seed and plant Improvement Institute of Karaj, Iran) were dipped in 70% (v/v) ethanol for 30s and then surface sterilized for 2 h with a sodium hypochlorite solution (3% active chlorine) containing a few drops of Tween 20 (Figure 1). They were rinsed with sterile distilled water and aseptically placed on an agar- solidified medium containing MS medium with a modification of 20 g/l sucrose. The seeds were incubated at 25°C in the dark.

Culture media

The inorganic media (primary culture medium) mainly used in the study was comprised of half mg/l pyridoxine- HCl, 0.4 mg/l thiamin- HCl, 100 mg/l myo- inositol, 10 g/l sucrose, 10 µM 1- naphthalene acetic acid (NAA), 0.1 µM gibberlic acid (GA_3) and 8 g/l agar. The combinations of growth regulators employed for somatic embryogenesis had been determined in the previous experiments (Sasaki *et al*. 1986). Medium was adjusted to pH: 5/7 with 1N NaOH and autoclaved.

Tissue culture

The cotyledon, hypocotyl and root explants (5 mm in length) were excised from 10 day old seedlings and the leaf explants (5 mm in square) were excised from 30 day old seedlings. The explants of distal and basal portions of cotyledon were obtained by transversely dividing a cotyledon into three parts (distal, middle and basal). For callus formation, the cotyledon and leaf explants were placed on the primary culture medium with the abaxial surface upward and the hypocotyl and root explants were placed horizontally on the culture medium. Cultures were kept at 25°C with a 16 h photoperiod. After 4 weeks of culture, the calli were transferred to a culture the same medium. After 4 weeks, the calli forming embryos was recorded. Plant regeneration obtain after 5 of culture.

Experiments were carried out as factorial based on the complete random design with three replications. Petri dishes carrying 5 explants each were prepared for regeneration.

Results and Discussion

Callugenesis and somatic embryogenesis from root segments

Generally studies on plant regeneration from callus showed that concentrations and combinations of plant hormones for plant regeneration from callus differed with plant species and organ used. In spinach, there are few studies on plant regeneration from plant tissue culture and on embryogenesis (Skoog *et al*. 1975; Smith *et al*. 1982; Mii *et al*. 1987; Sasaki *et al*. 1989; Sasaki *et al*. 1986; Mutoh *et al*. 1990; Ichihashi *et al*. 1991). Callugenesis and somatic embryogenesis just obtain from root explants (Table 1) (Figure 2- 4- A). In other explants percentage of callugenesis and somatic embryogenesis were 0% and obtain in ½MS medium containing 0.5 mg/l nicotinic acid, 0.5 mg/l pyridoxine, 0.4 mg/l thiamine, 100 mg/l myo- inositol, 10 g/l sucrose, 10 µM NAA, 0.1 µM GA_3, 8 g/l Agar and pH: 5/7. In MS medium containing 0.01, 0.5 and 0.75 µM GA_3 and in ½MS medium containing 0.01, 0.5 and 0.75 µM percentage of callugenesis and somatic embryogenesis were 0% (Table 1). Rooting obtain on MS medium without growth regulator containing 30 g/l sucrose 8 weeks ofter culture (Figure 4- B). This result agrees with komai (1996) and Takuma (2014). But in some other similar reports, researchers obtain callugenesis and somatic embryogenesis of cotyledon, hypocotyl and leaf explants. This result was not agree with by my result this study. Embryo formation was in frequently observed on the basal segment of cotyledons and hypocotyls, but these tissues showed a high frequency of callus formation. Calli and embryos were not formed in the cultures of distal adventitious bud formation from hypocotyls (Sasaki *et al*. 1989; Satoh *et al*. 1992; Usman *et al.* 2014). Using an optimal combination of PGR_s modified from a procedure described by Knoll *et al* (1997). The morphogenic pathway achieved in the present study (somatic embryogenesis) is different from that achieved by Knoll *et al*. (1997) (callugenesis). In addition the latter authors demonstrated direct regeneration from proliferating root epidemal and subepidermal cells, without a callus phase. Furthermore, although was also able to obtain SE from root section of the caltivar Jiromaru seedlings using lower levels of the same PGR_s (10 µM NAA, 0.1 µM GA_3), the process of somatic embryogenesis was through a callus phase (Komai *et al*. 1996). In different research with this research the rate of shoot formation from hypocotyl-drived calli is shown and plant regeneration from hypocotyls-drived calli is shown. The calli which were precultured on the medium with 0.1 mg/l IAA exhibited a rate of 40% shoot formation characterized by thick leaves and root formation was not observed (Satoh *et al*. 1992). In other research stem of Ceylon spinach was cultured on MS medium supplemented with various concentrations of 2, 4- D in combination with BA. Within 4 weeks of inoculation in media supplemented with 0.1 µM 2, 4- D incombination with 5 µM BA, stem explants formed highest callus. This result not same with this work. In different research with this work research obtain highest callugenesis of leaf disc explants on BAP and kinetin media (Usman *et al* 2014). In other research same with this work highest regeneration obtain of root explants in medium containing 10 M NAA and 0.1 M GA_3 (Takuma 2014). The present syudy investigations demonstrate that the root segments of spinach have a high capacity for somatic embryogenesis. The systems described here for the establishment of plant regeneration would be useful for micropropagation of spinach plants.

Table 1. Effect of GA_3 concentration (0.01, 0.1, 0.5 and 0.75 mg/l) of root explants spinach on callugenesis, embryogenesis and shooting in MS and ½MS medium.

Medium	Concentration of GA_3 (mg/l)	Characters		
		Callugenesis (%)	Embryogenesis (%)	Shooting (%)
MS	0.01	0	0	0
	0.1	0	0	0
	0.5	0	0	0
	0.75	0	0	0
½MS	0.01	0	0	0
	0.1	90	65	20
	0.5	0	0	0
	0.75	0	0	0

Figure 1. The seeds of spinach provided from seed and plant Improvement Institute of Karaj, Iran.

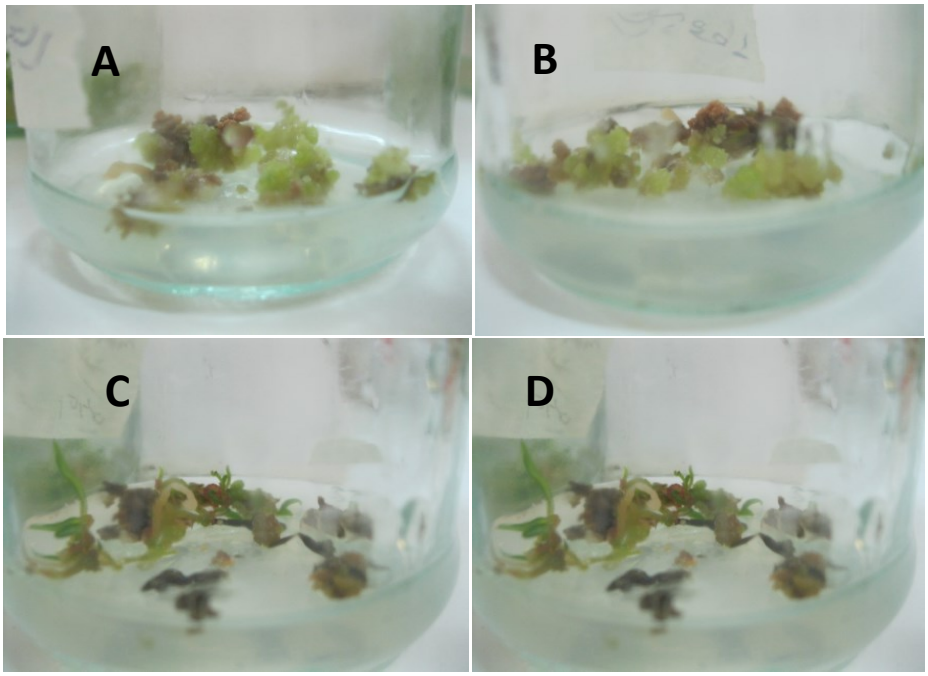


Figure 2. Somatic embryogenesis and plantlet regeneration in spinach (*Spinacia oleracea* L.). **A- B**: Callugenesis and embryogenesis in root explant on the callugenesis medium whit 0.1 mg/l GA_3 (4 weeks after culture); **C- D:** Plantlet regeneration from roots (5 weeks after culture).

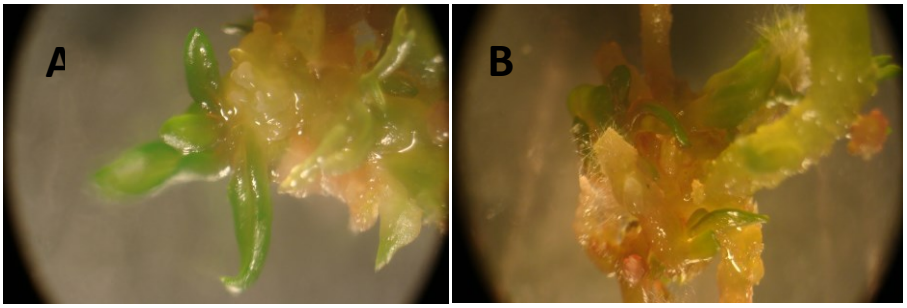
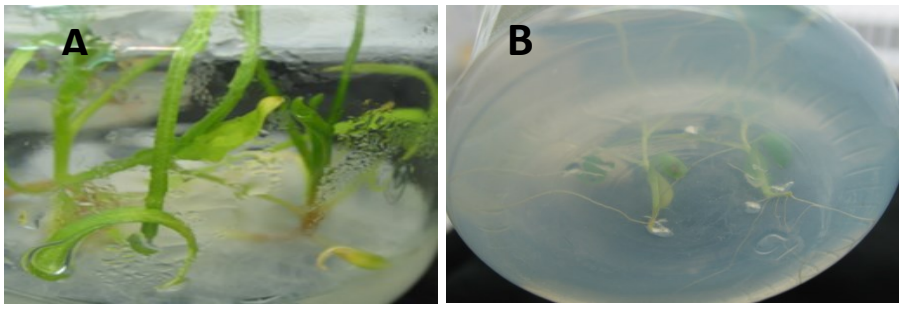


Figure 3. Microscopy observation of calli and shooting. **A- B:** Shoot formation on regeneration calli which were precultured with 0.1 mg/l GA_3 in ½MS medium 4 weeks after culture.



Figur 4. A: Plantlet regeneration from roots on ½MS medium containing 0.1 mg/l GA_3 (6 weeks after culture). **B:** Root regeneration for a complete plantlet on MS medium without growth regulatur containing 30 g/l sucrose 8 week after culture.

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