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The Effects of Different Culture Media on the Callus Production of Radish *(Raphanus sativus L.)*

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Abstract

This study was conducted to investigate the effects of different genotypes, explants and culture media on the callogenesis, calli growth and regeneration of radish plants. To do this, four varieties of radish ('Vermal', 'Chuhong', 'Caudatus' and 'Longipinnatus'), three culture media with different hormonal combinations based on MS medium as A (without hormone or control), B (2, 4-D 1 mgL^{-1} and BAP 2 mgL^{-1}) and C (2, 4-D 2 mgL^{-1} and BAP 1 mgL^{-1}) as well as three explant types: hypocotyl, cotyledon and root were employed. After one month the callogenesis, diameter, fresh and dry weight of calli and the calli water content were evaluated. The results showed that, the effects of genotype, culture media and explants types were statistically significant for all of the traits. 'Longipinnatus' and 'Vermal' cultivars represented the highest amount of calli growth. Meanwhile, 'Chuhong' and 'Caudatus' had the greatest amount of calli percentage. A and B media had the highest values for calli related traits. These results are indicating the presence of the high internal hormonal concentration in the plants and probably it's inhibiting effects on calli production of the explants in high level hormone enriched media.

Keywords: *Raphanus sativus L.*, Callogenesis, Explants, Genotype, Culture media

Introduction

Radish (*Raphanus sativus* L., 2n=18) is a common vegetable in Asia and in most parts of the world. This plant produces a red colored edible root with different shapes [1]. Apart form culinary purposes, radish has diverse medicinal properties as well [2]. Major genetic improvement of radish has been achieved by conventional plant breeding methods, such as crossing [3]. However, these methods are time and labour consuming.

In recent years, advances in plant genetic engineering have opened a new avenue for crop improvement and various transgenic plants with novel agronomic characteristics have been produced [2,4,5].

The success in plant genetic engineering is dependent upon several factors, from which an efficient tissue culture system, with high plant regeneration potential, is a crucial option.

However, to the best of our knowledge, there is limited information on the plant regeneration from cell and tissue cultures of radish [6,7]. Previous attempts to regenerate plants from leaf protoplasts of radish were not successful [1,6,7]. However, use of radish as one of the donors in the protoplast fusion studies has produced several novel fertile intergeneric somatic hybrids [8,9,10,11].

The aim of this research was to study for the first time the effects of explant types and culture media on the potential of calli production from four cultivars of radish.

Material and Method

In the present study explants of hypocotyl, cotyledon and root were afforded from 6 to 10 days old seedlings of radish cultivars: 'Vermal', 'Chuhong', 'Caudatus' and 'Longipinnatus'. For hypocotyl explants an 8-10mm sample of hypocotyl about 2mm beneath the cotyledonary leaves were used. About cotyledon explants, cotyledons were excised with about 2mm of their petiole and for root explant, a sample of about 1cm of root were used as propagules.

Culture media: Murashige and Skoog [12] medium enriched with L-arginin (0.2 mgL^{-1}), nicotinic acid (0.05 mgL^{-1}), pyridoxin (0.05 mgL^{-1}), and thiamine (0.05 mgL^{-1}) was used for callus production. Three different media were used where, A was control (without hormone), B (2, 4-D 1 mgL^{-1} and BAP 2 mgL^{-1}) and C (2, 4-D 2 mgL^{-1} and BAP 1 mgL^{-1}). Culture media were supplemented with 3% sucrose and 0.8% agar. The pH of the media was adjusted at 5.7 and the prepared media were autoclaved for 20 min at 121°C for sterilization.

Data collection: Cultured explants were incubated in growth chamber with constant temperature of 25±2 °C and day/night period of 16:8. After one month, callogenesis, calli diameter, fresh and dry weight of calli and calli water content were evaluated. Callogenesis was estimated with the proporion of calli producing explants in relation to the total explants. The separated calli were weight, then wrapped in aluminum foil for dry weight measurement in an air forced oven at 70±2°C for 24 hrs. Calli water content was evaluated with this equation:

$$\text{Calli water content} = \frac{\text{Calli fresh weight} - \text{calli dry weight}}{\text{Calli fresh weight}}$$

Statistical analysis: Variance analyses for traits were done with SAS 8.02 statistical software as 4×3×3 factorial design based on CRD with four replications. Duncan's multiple range test at 5% probability level was employed for mean comparison of treatments.

Results and Discussions

Effects of genotype, culture medium and explant type were statistically significant ($p \leq 1\%$) for all traits. Interactive effects were significant as well for all traits except for calli dry weight.

Mean comparison for cultivars showed that 'Chuhong' had the minimum amount of callogenesis.

For calli diameter and fresh and dry weight of calli, 'Longipinnatus' and 'Vermal' cultivars had the greatest values (Table 1).

The highest mean for callogenesis, calli diameter and fresh and dry weight of calli belonged to the B culture medium. On the contrary, B culture medium had the least amount of calli water content (Table 1).

Hypocotyl explant was superior to the other explants in respect to the callogenesis but in contrast, it had the least amount of calli diameter.

For all cultivars root explants had a relative advantage in comparison with other explants. Hypocotyl explants had the least amount of calli fresh and dry weight except for 'Longipinnatus'.

Table1. Mean comparison for calli traits of radish cultivars in different culture media

Cultivar	Culture media	Calli percentage (%)	Calli diameter (mm)	Calli fresh weight (g)	Calli dry weight (g)	Calli water content
'Vermell'	A	88.57ab	6.09ab	0.108b	0.0105b	90.22a
	B	91.43a	5.83b	0.098c	0.0102 b	81.69b
	C	82.66 b	7.12a	0.168a	0.0202a	87.93ab
'Chuhong'	A	85.62b	5.79b	0.092b	0.0097b	88.91ab
	B	76.56c	5.39c	0.073bc	0.0085bc	89.52a
	C	89.75a	6.83a	0.121a	0.0141a	86.47b
'Candutus'	A	84.28b	5.27b	0.097b	0.0101b	92.22ab
	B	79.43bc	4.31c	0.087bc	0.0094bc	91.48b
	C	91.22a	6.87a	0.132a	0.0162a	94.65a
'Longipinnatus'	A	90.41ab	7.77b	0.151b	0.0192b	84.58ab
	B	86.15b	6.69c	0.105c	0.0112 c	85.69a
	C	92.77a	8.85a	0.212a	0.0312 a	82.35b

Different letters in columns show significant difference based on Duncan's multiple range test at P≤0.01
A (without hormone or control), B (2, 4-D 1 mgL^{-1} and BAP 2 mgL^{-1}) and C (2, 4-D 2 mgL^{-1} and BAP 1 mgL^{-1})

Root explant had the greatest values for mean fresh & dry weight of calli in both 'Chuhong' and 'Caudatus' cultivars. About calli water content, hypocotyl explants had the greatest amounts except for 'Longipinnatus' cultivar (Table 2).

Table2. Mean comparison for calli traits of radish cultivars with different explants

Cultivar	Explant	Calli percentage (%)	Calli diameter (mm)	Calli fresh weight (g)	Calli dry weight (g)	Calli water content
'Vermell'	Hypocotyl	80.3a	5.62b	0.09c	0.0083c	91.33a
	Cotyledon	76.2b	6.51a	0.128b	0.0131a	90.01ab
	Root	72.3c	5.98ab	0.101ab	0.0098bc	91.12a
'Chuhong'	Hypocotyl	76.7a	5.36b	0.07c	0.0051c	94.48a
	Cotyledon	71.7b	6.14a	0.129ab	0.0128b	90.22ab
	Root	96.5c	6.31a	0.149	0.0161a	88.91b
'Candutus'	Hypocotyl	69.9a	5.53c	0.08c	0.0077c	92.86a
	Cotyledon	72.8ab	6.26b	0.141b	0.0151b	89.41ab
	Root	70.4b	6.93a	0.194a	0.0188a	86.18b
'Longipinnatus'	Hypocotyl	86.7a	7.48a	0.231a	0.0241a	84.36b
	Cotyledon	78.9ab	6.39b	0.172c	0.0169c	88.56a
	Root	76.6b	7.33a	0.214ab	0.0221ab	85.77ab

Different letters in columns show significant difference based on Duncan's multiple range test at P≤0.01

Mean comparison for callogenesis between cultivars showed that, 'Longipinnatus' and 'Vermal' had the highest and, 'Chuhong' had the lowest amount of callogenesis respectively.

It seems that internal hormone concentrations of two former cultivars was higher than that of others, so that they had better growth in control medium or media with minimum hormonal concentrations. This trend shows that, in cultivars with high potential of internal hormones, calli growth is prohibited in hormone enriched media [13,14,15].

Media with balanced hormonal combinations had the highest percentage of calli production, calli diameter and calli fresh and dry weight. There was root regeneration from callus in the B culture medium. In accordance with our results some plants of *Brassica* genus showed rooting potential from callus as well [13,16].

Sachiko and Hegazi [13] reported plant regeneration from radish callus for the first time. There are contradictory reports on the effects of phytohormones on the callus initiation of different plants [11,13,14]. 2, 4-D is a growth regulator essentially needed for callus initiation and growth in most of the in vitro culture studies but it limits regeneration of plantlets [13,17]. Furthermore, for regeneration purposes callus must be transferred to a 2, 4-D free medium with balanced combinations of cytokinin and one another auxin [18]. In *Raphanus sativus* cultivars 'Morignchi' and 'Nerimashiring', plantlets were regenerated in the first subculture in a 2, 4-D free medium [13,16,18]. Same results were reported for cauliflower [19] and red cabbage [20]. Hypocotyl explants had a relative superiority for callogenesis in comparison with the other explants. There are several reports that diverse explants such as flower, leaf, petiole, stem, cotyledon, shoot-tip and cotyledon have been employed for callus initiation of different plant species [6,7,9]. However hypocotyl explants showed the premium callogenesis in radish. Furthermore, hypocotyl explants were reported as the most suitable explant for in vitro culture purposes especially for callus initiation in broccoli [2,15,21], red cabbage and mustard [20].

In some cases and for some genotypes of radish, concomitant production of callus and plantlets from hypocotyl explants were reported as well [15,22,23]. Shukla and sawny [24] reported that variations in shoot and root production of two rapeseed (*Brassica napus*) cultivars were due to the differences in the internal levels of auxin and cytokinin. In fact differences in tissue culture responses and organogenesis would be dependant on the different potential of explants for internal hormones metabolism [25]. Findings of this experiment are initial steps toward in vitro culture multiplication as well as a trend into enhancing a genetic engineering potential of this plant. However, in the future there is need for novel and accurate protocols for optimization of plant regeneration from these explants.

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