Effects of Explants and Hormone Levels on Regeneration of Radish (*Raphanus sativus* L.) Variety Beeralu Rabu

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Abstract

Radish (*Raphanus sativus* L; Brassicaceae) is an important vegetable crop which produce an edible root with different shapes and sizes. Apart from culinary purposes; radish has some medicinal properties as well. Major genetic improvement of radish has been achieved by conventional plant breeding methods although it is a time and labour consuming process. To overcome this situation, plant genetic engineering is used to improve novel plants with useful agronomic characters. The success in plant genetic engineering is dependent on efficient tissue culture system with high plant regeneration potential. This study was conducted to investigate the effects of different explants and hormones on the plantlet regeneration of Radish (*Raphanus sativus* L; variety Beeralu Rabu). Seven different hormone combinations on MS (Murashige and Skoog's) basal medium with 0.1mg/l NAA + 0.1, 0.5, 1.0, 1.5, 2.0, 2.5, 3.0 mg/l BAPas well as three types of explants: hypocotyl, leaves and root were employed. Completely Randomized Design (CRD) with five replicates was used for the study. Numbers of regenerated shoots were evaluated one month after establishment. Anova (DMRT) test showed that there were significant effects at $p<0.05$ level on radish plantlets regenerated with different treatments. Medium supplemented with 0.1mg/l NAA and 2.5mg/l BAP was the best hormonal combination while hypocotyl explants (5 shoots/explant) was the best explant for the *in vitro* regeneration of Radish variety Beeralu.

Key words: Raphanussativus, Regeneration, Hypocotyl, MS basal medium

Introduction

Radish (*Raphanus sativus* L; Brassicaceae) is an important vegetable crop that is cultivated throughout Asia (Cho, 2008). Roots and leaves with different shapes and sizes are edible parts of this crop and it can be used for medicinal purposes, ornamental as well as culinary purposes. Radish is used as vegetable or salad in Sri Lanka as well as other countries. Radish is one of the vegetables that can be grown in all agro-ecological regions throughout the year if adequate moisture is available in Sri Lanka. There are three main Radish varieties recommended for Sri Lankan conditions as Japan Ball, BeeraluRabu and Table Radish. BeeraluRabu is the recommended variety for low country, while Japan ball has been recommended for the up country region. Sri Lankan radish varieties contain small sizes of tubers. Therefore, those varieties should be able to produce larger tubers to develop the appearance and quality of the tuber to achieve the export market. Genetic improvement is the crucial option for achieve that target. Major genetic improvement of radish has been achieved by conventional plant breeding methods, such as crossing although 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 developed. 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 fundamental option (Mohomad *et al.*, 2009). However, there is limited information on the plant regeneration from cell or tissue cultures of radish especially for Sri Lankan varieties. The aim of this research was to study for the first time, the effects of explant types and culture media on the potential of *in vitro* regeneration of Radish; variety Beeralu.

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Materials and Methods

Preparation of culture media

All the media used were adjusted to a pH value of 5.8 - 6.0 with 1N NaOH or 1N HCl solution, gelled with 0.5% agar prior to autoclaving at 1.4 $kgcm^{-2}$ for 20 minutes.

Regeneration ability of different explants in different media

Leaf, hypocotyl and root explants from aseptic plantlets were cultured on MS basal medium. Six different media supplemented with 0.1 mg/l NAA and 0.1, 0.5, 1.0, 1.5, 2.0, 2.5, 3.0 mg/l BAP were used to investigate the regeneration ability of 15 days old plantlets. Leaves were cut into sections (0.5 cm^2) and placed on media with the adaxial surface toward the media, while hypocotyl and roots were cut into about 5 mm and placed on the media. Five replicates were used from each explant and cultures were kept under light.

Data collection and analysis

Experiment was arranged according to the Completely Randomized Design (CRD). Regeneration was evaluated 30 days after initiation. Numbers of explants with buds in different treatments were recorded. All experiments had five replicates, each with five explants per bottle. Statistical analysis was carried out using the Student Newman-Kuels means separation test using SAS statistical software (ver. 9.1.3).

Results and Discussion

Regeneration ability of different type of explants

The best regeneration ability was revealed in hypocotyl explants among leaf explants and root explants (Figure 1). These shoots were directly regenerated from explants containing vascular tissues. This type of regeneration has also been reported in *Pinus radiate*, *Iris hollandica* and *Altiumsativum* (Kim *et al.*, 2001).

When comparing the means, highest number of shoots per explant (5.0 shoots/explants) was observed in MS basal medium supplemented with 0.1mg/l NAA and 2.5 mg/l BAP (T_6). It is significant from other treatments and can be considered the best medium for regeneration for radish comparing to other treatments. Medium supplemented with 0.1 mg/l NAA and 2.0 mg/l BAP was the second best treatment. Treatment 7 (0.1 mg/l NAA and 3.0 mg/l BAP) and treatment 4 (0.1 mg/l NAA and 1.5 mg/lBAP) gave same results. Treatment 1, 2 and 3 were similar to each other and they were revealed lowest regeneration ability (Table 1). Curtis *et al.* (2004) have used N1B2 medium for hypocotyl explants and Murashige and Skoog (MS) medium with 20 µm benzyl amino purine for cotyledon explants to regenerate shoots of the Korean radish variety 'Jin JuDaePyong'.

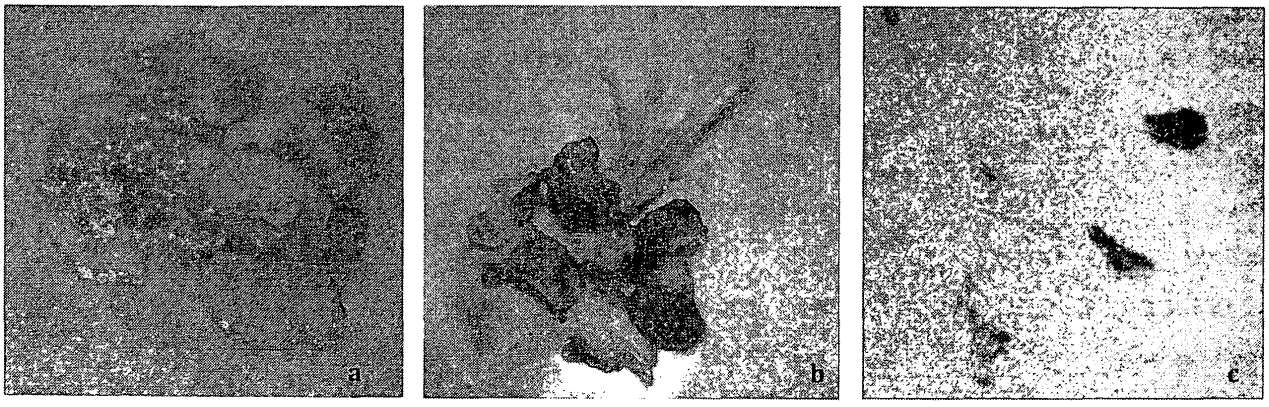

Figure 1. Regeneration of plantlets from explants of 15 days old plants. Photos were taken after 30 days of culture.
a. leaf explants; b. hypocotyl explants; c. root explants.

Table 1. Comparison of the effects of different explants and hormone levels on regeneration of plantlets from radish variety Beeralu.

BAP concentrations + 0.1 NAA (mg/l)	Explants		
	Leaves	Hypocotyl	Roots
0.1(T_1)	0	0^{d}	0
0.5 (T_2)	0	0^{d}	0
1.0 (T_3)	0	0^{d}	0
1.5 (T_4)	0	0.4^{dc}	0
2.0 (T_5)	0	3.0^{b}	0
2.5 (T_6)	0.4	5.0^{a}	0
3.0 (T_7)	0	1.2^{c}	0

Data in the same column followed by different letters are significantly different by Duncan's test at 95% level.

Nevertheless, in the present study all explants were employed in the same medium (MS basal media) with same concentration for every explant. It may be the reason for hypocotyl explant was selected as best explant. If media and concentrations of media was changed, regenerated shoots could have been obtained from other explants as well. The addition of ethylene inhibitors and silver nitrate to the culture media were beneficial towards shoot regeneration from hypocotyl explants (Curtis *et al.*, 2004; Kim *et al.*, 2001). Hypocotyls cultured in the presence of silver nitrate regenerated significantly more shoots compared to N1B2 medium alone. Additives such as silver nitrate supported enhanced shoot regeneration in the present study. However, no other additives were not used in the present study. Only MS basal media with different hormonal concentration were employed.

According to Kim *et al.*, 2001 to direct regeneration from cotyledon explants were suitable for the effective induction of shoots on Murashige and Skoog's (MS) medium containing 3.0 mg/L kinetin. However, in present study, hypocotyl explant was the best explant for plantlets regeneration on MS basal medium with 2.5 mg/l BAP.

As reported by Kim *et al.* (2001), IAA and NAA inhibited shoot regeneration but, in our study, low concentration of NAA (0.1mg/l) promoted good shoot generation.Shoots of present study were directly regenerated shoots from explants containing vascular tissues. This type of regeneration has also been reported in *Pinus radiata*, *Iris hollandica* and *Altium sativum*(Kim *et al.*, 2001).

A Korean radish variety, 'Hannong' were regenerated on a medium supplemented with IAA and kinetin, without NAA and BA, shoots of another Korean radish variety 'Taewang' developed on 2.0 mg/L IAA and 7.0 mg/L kinetin (Kim *et al.*, 2001). These results revealed IAA + kinetin as the best hormone combination for radish regeneration. However, BAP plus NAA have succeeded in the present study.

According to the literature, plant regeneration via organogenesis from hypocotyls showes a poor regeneration frequency. Therefore, studies need to be focused on the use of ethylene inhibitors and polyamines in culture media to improve the shoot production of seedling explants of radish (Kole and Hall, 2008). The present study can be improved using such ethylene inhibitors and polyamines to obtain more shoots from hypocotyl explants.

Maximum regeneration was observed on MS basal medium containing 2.5 mg/l BA with 0.1 mg/l NAA from hypocotyls explant. This study is a baseline to carry further research on radish variety Beralu Rabu for improvement using gene transfer technology to achieve high yielding varieties.

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