Effect of Plantlet Maturity and Explant Type on *In Vitro* Propagation of Radish (*Raphanus sativus* L. Var. Beeralu)

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Abstract

Raphunus sativus* L. commonly known as 'radish' is a popular vegetable crop used by people all over the world for its culinary and medicinal properties. It used as a common pungent ingredient for various abdominal disorders. Almost all parts of the plant including leaves, seeds and roots are utilized in medicinal purposes. An efficient *in vitro* propagation method is required to open genetic improvement of radish because conventional plant breeding methods are much time and labor consuming. Due to limited information of an efficient *in vitro* propagation protocol for *Raphunus sativus*, especially for Sri Lankan varieties, this study was immerged. Study was carried out to find out the effects of different maturity levels and different types of explants from aseptic seedlings on in vitro regeneration of *Raphunus sativus* Variety Beeralu. Five different maturity levels of plants; 5, 10, 15, 20 and 25 days old aseptic leaves, hypocotyl and root explants were cultured on Murashige and Skoog (MS) basal medium with 2.5mg/l BAP (Benzyl Adenine) and 0.1mg/l NAA (1-Naphthaleneacetic Acid). Complete Randomized Design (CRD) with five replicates was used for the study. After 30 days the numbers of regenerated shoots were evaluated. ANOVA (DMRT) test shows there were significant effects at $p<0.05$ level on regeneration of radish with different treatments. Fifteen days age was the best maturity to get maximum regeneration from each explant and fifteen days old hypocotyl was the best explant (6.4 shoots/explant) to regenerate highest number of shoots.

Keywords: *Raphunus sativus, Explants, Hypocotyl, Regenaration, Plant maturity*

INTRODUCTION

Radish (*Raphanus sativus* L.; family Brassicaceae) contains mustard oils like other Brassicas. According to Ghayur *et al.* (2007) *Raphunus sativus*, commonly known as 'radish', is a popular vegetable crop known and used by people all over the world for its culinary properties. Radish, along with peas and turnip, is considered to be the oldest cultivated crop in the world. It is usually eaten as salad, raw, in its original form, cooked, as a snack or as an appetizer. Apart from its use in the kitchen, different parts of this plant such as the leaves, seeds, and roots are prescribed by traditional healers in South Asia. It is a common pungent ingredient used in various abdominal disorders. Almost all parts of the plant including leaves, seeds and roots are utilized in medicine. Therefore, improvements of local radish varieties are required to use local medicinal purposes and experiments. Major genetic improvement of radish has been achieved by conventional plant breeding methods, such as crossing. However, these methods are time and labor 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. 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 (Mohammad *et al.*, 2009). Sri Lankan Radish varieties were not highly focused to plant breeding or genetic engineering techniques. It is necessary to improve Sri Lankan Radish variety through genetic engineering techniques such as doubling chromosome to use for medicinal purposes as well. *In vitro* culture techniques have become a very efficient experimental tool for plant genetic

engineering technique as well as many research purposes. Therefore, present study was carried out to find out effects of maturity and explants types on *in vitro* regeneration ability of *Raphanus sativus* (Radish) variety Beeralu, a Sri Lankan Radish variety to develop an efficient *in vitro* propagation protocol.

MATERIALS AND METHOD

Plant source: Seeds of Radish were purchased from the Seed and Planting Material Division, Department of Agriculture, Sri Lanka.

Establishment of aseptic cultures: Seeds were surface-sterilized by washing tap water, soapy water, immersing in 70% ethanol for 3 minutes, three times from distilled water and soaking in a 20% Clorox for 20 minutes, respectively. Sterilized seeds were then rinsed three times in sterilized distilled water and inoculated on a medium comprised of half-strength MS (Murashige and Skoog, 1962) salts, 3% sucrose without hormones and the medium was solidified with 0.5% agar prior to autoclaving. The seeds were cultured under light for 10 days (Dahanayake *et al.*, 2010).

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 0.8% agar prior to autoclaving at1.4kgcm-2 for 20 minutes.

Regeneration ability of different type of explant in different mediums: Different maturity levels of plants (5, 10,15, 20 and 25days old) leaf, hypocotyl and root explants of aseptic plantlets were cultured on MS basal medium with 0.1mg/l NAA and 2.5mg/l BAP (Manawadu *et al.*, 2013) to investigate the regeneration ability as influenced by age difference. In cultures, leaves were cut into sections ($0.5cm^2$) and placed on media with the adaxial surface toward the media, while hypocotyl and roots were cut into about 5mm and cultured by laying randomly on the media. Five replicates were used from each explant and cultures were kept under light (about 50 μ mol $m^{-2}s^{-1}$).

Data collection and analysis: Experiment was arranged according to the Complete Randomized Design. Regeneration cultures were evaluated 30 days after initiation. Numbers of shoots regenerated from each explant in regeneration and rooting media were recorded. All experiments had five replicates, each with five explants per bottle. Statistical analysis was carried out using the Student Newman-Kuells Means Separation Test of SAS program (9.1.3).

RESULTS AND DISCUSSION

Regeneration ability of explants from different maturity stages: Plantlets from different maturity levels greatly responded for regeneration of shoots from the different explants (Table1). Number of shoots regenerated from all root, hypocotyl and leaf explants increased with increasing plantlet maturity up to 15 days. After that it was decreased. Hypocotyl explants of different maturity levels exhibited much higher regeneration ability than leaf and root explants.

According to Kim *et al.* (2001) to direct regeneration cotyledon explants of *R. sativus* cultivar RS91 (a Korean Radish variety) from four days old seedlings were suitable for the effective induction of shoots on Murashige and Skoog's (MS). But in present study 15 days old hypocotyl explant (5mm hypocotyl segments) was the best explant on MS basal medium with 2.5mg/l BAP.

Mohomad *et al.* (2009) reported that explants of hypocotyl, cotyledon and root were afforded from 6 to 10 day old seedlings of radish cultivars: 'Vermal', 'Chuhong', 'Caudatus' and 'Longipinnatus'. In present study, 5mm hypocotyl explants from 15 day old aseptic seedlings revealed the best regeneration ability in radish variety Beeralu.

As mentioned by Kaviani *et al.* (2011) four week old in vitro plants, obtained from micro cuttings (Shoot tips), showed successful shoot proliferation in *Matthiola incana* species a Brassicaceae plant. But 15 day old hypocotyl explants were the best in the present study.

The best rate of shoot proliferation was induced 15 days after culture on MS mineral medium supplemented with IAA:KN (0.57:13.94μM) from apical bud explants in *Lepidium virginicum* L., family Brassicaceae (Osuna *et al.*, 2006). Similar results observed in the present study 15days old hypocotyl explants were best age for get maximum in vitro shoot regeneration.

Efficient plant regeneration systems are required in this species to propagate unique lines and to improve the quality based on somatic cell genetics and recombinant DNA technology. Maturity of plantlets greatly influenced the regeneration ability of explants excised from garlic root tips, which reached the maximum value (95%) in 15 days old plantlets (Haque *et al.*, 1997). In addition the highest number of shoots per explant was obtained from 30 days old seedlings in *Aeschynomene sensitiva* (Dhahanayake *et al.*, 2010).

Till date, the effect of planting material with plantlets maturity on shoot regeneration has not been reported in *R. sativus* variety Beerlau. It was clear that explant maturity level had a positive effect on shoot induction and also it seemed to be a factor interfering with the level of regeneration. These results agreed with the previous findings in *Lepidium virginicum* (Osuna *et al.*, 2006).

A novel shoot regeneration methodology from root, hypocotyl and leaf explants has been developed for *R. sativus*. This method is promising for rapid multiplication of radish variety Beeralu.

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Table

Table 1: Effects of the maturity of the mother plant on regeneration of shoots from radish seedlings.

Plant maturity (Days)	No. of buds from a leaf explant	No. of buds from a hypocotyl explant	No. of buds from a root explant
5	0^{a}	0.2^{d}	0^{a}
10	0.2^{a}	0.4^{d}	0^{a}
15	0.4^{a}	6.4^{a}	0.4^{a}
20	0^{a}	4.4^{b}	0^{a}
25	0^{a}	2.8^{c}	0^{a}

Means fallowed by the same lower case letters in each column are not significantly different at 5% level in Duncan's Multiple Range Test.