

## ***In vitro* Conservation of Rose Coloured Leadwort: Effect of Mannitol on Growth of Plantlets**

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### **ABSTRACT**

*In vitro* study of manitol on conserved rose coloured leadwort (*Plumbago indica* Linn.) was conducted. Nodal segments containing one axillary bud were cultured on Murashige and Skoog (MS) medium + 30 g/l sucrose. The results of different addition of mannitol (0, 20, 40 and 60 g/l) to the media with plant growth regulator (PGR) (0.5 mg/l BA + 0.16 mg/l IAA) or without PGR were compared. The nodal segments were *in vitro* conserved under 50  $\mu\text{mol m}^{-2}\text{s}^{-1}$  light intensity and 12 h photoperiod at 25°C without subculture. It was found that mannitol added to the media could reduce the growth of plantlets derived from buds of nodal segments especially the height of plantlets was reduced up to 83.1% in media with PGR and 93.3 % in media without PGR. Mannitol at concentrations of 40 and 60 mg/l had tended to reduce the number of shoots per plantlet. The plantlets could survive on 20 g/l mannitol until eight months of conservation without any dead plantlet and the plantlets were still vigorous. The survival plantlets from every medium could regenerate new shoots after subculture onto the fresh medium.

**Key words:** conservation, mannitol, *Plumbago indica* Linn, rose coloured leadwort, slow growth

### **INTRODUCTION**

Rose coloured leadwort (*Plumbago indica* Linn.) is one of the valuable medicinal plants. It is an important composition in many recipes of Thai traditional medicine (Saralamp, 1992) and also provides plumbagin, plumbaginol, sitosterol and stigmasterol (Dinda and Chel, 1992; Dinda *et al.*, 1994) for pharmaceutical uses. Rose coloured leadwort is an endemic plant found in all part of Thailand. Due to the increasing of demand, the roots of rose coloured leadwort are heavily collected from wild. Consequently, loss of plant resources in the future will occur. Such conventional method of conservation needs high cost of land and labours, moreover there are risks of deterioration from

nature disaster and plant pests during conservation. *In vitro* slow growth culture is now an alternative method for plant conservation that can eliminate the obstacles of field conservation (Golmirzaie and Toledo, 1999). For safety from loss that may occur, *in vitro* slow growth culture method was decided to support our conservation of rose coloured leadwort.

There was a report on micro-propagation of *Plumbago rosea* Linn. (Harikrishnan and Hariharan, 1996) but none was conducted on *in vitro* conservation. Sugar alcohol such as mannitol and sorbitol have often been used as metabolically inert osmotica in plant cell culture (Thompson *et al.*, 1986) and in slow growth media (Golmirzaie and Toledo, 1999). This investigation attended to

study the effect of mannitol on growth of rose coloured leadwort for *in vitro* conservation.

## MATERIAL AND METHODS

### Plant materials

*In vitro* culture of rose coloured leadwort (*Plumbago indica* Linn.) from Kanchanaburi location was used in this study. Stock cultures were maintained on solidified Murashige and Skoog (MS) (Murashige and Skoog, 1962) medium supplemented with 30 g/l sucrose, 6.5 g/l agar, pH 5.6 under 50  $\mu\text{mol m}^{-2}\text{s}^{-1}$  light intensity, 12 h photoperiod at 25°C. The 5 mm-nodal segments with one axillary bud dissected from 1-month-old stock cultures, were used as a started plant materials for slow growth storage.

### Medium composition

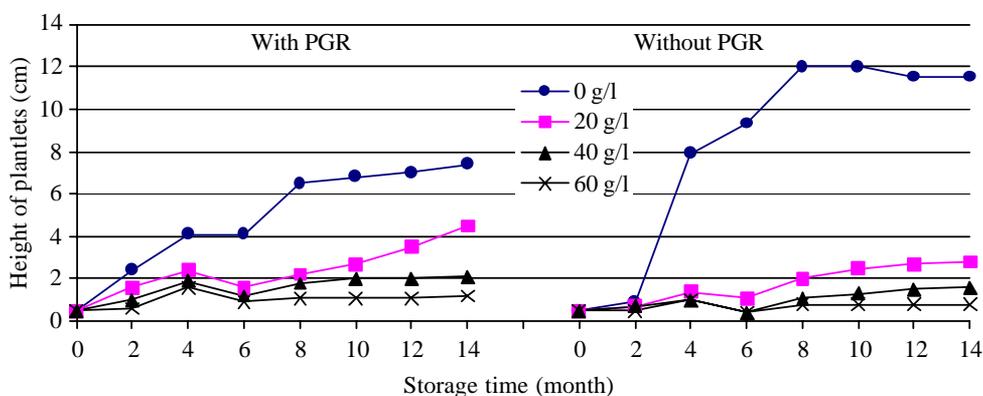
The nodal segments were stored on solidified MS medium supplemented with 30 g/l sucrose and 7 g/l agar, pH 5.6. The growth and survival of storage plantlets were observed comparing among the different addition of mannitol 0, 20, 40 and 60 g/l and with plant growth regulator (PGR) (0.5 mg/l 6-benzylamino purine; BA + 0.16 mg/l Indole acetic acid; IAA) or without PGR to

the culture medium. The cultures were maintained under 50  $\mu\text{mol m}^{-2}\text{s}^{-1}$  light intensity, 12 h photoperiod at 25°C without subculture.

Twenty nodal segments were used in each medium composition. Height of plantlets, shoot multiplication, root regeneration and percentage of dead plantlets were recorded every two months interval. Finally, the survival plantlets were subcultured onto fresh culture medium (MS medium + 0.5 mg/l BA + 0.16 mg/l IAA + 30 g/l sucrose + 6.5 g/l agar, pH 5.6). Regeneration rate and number of shoot multiplication were observed.

## RESULTS AND DISCUSSION

In our preliminary study, *in vitro* cultures of rose coloured leadwort were well developed on MS medium containing 30 g/l sucrose with 0.5 mg/l BA and 0.16 mg/l IAA (modified from Harikrishnan and Hariharan, 1996). Addition of mannitol to the culture media could reduce the growth of rose coloured leadwort plantlets in term of stem height. The higher concentration of mannitol added to the medium, the higher reduction of stem height was observed (Figure 1). After 8 months of storage, the plantlets showed the average stem height reduction of 66.1 to 83.1% in the



**Figure 1** Effect of mannitol on height of plantlets cultured on MS medium + 30 g/l sucrose + 0, 20, 40 and 60 g/l mannitol + PGR (0.5 mg/l BA + 0.16 mg/l IAA) or without PGR after culture for 14 months.

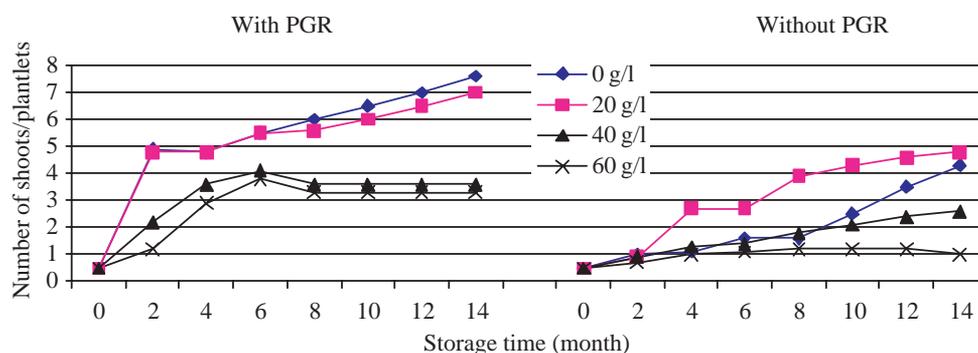
culture medium with PGR and 83.3 to 93.3% in the culture medium without PGR, depended on the increasing of mannitol added concentration. After 8 months of storage, the growth of plantlets was quite stable though the storage time was prolonged to 14 months.

The number of shoot multiplication during storage depended on the PGR supplemented to the culture medium. The plantlets cultured on PGR added media developed more shoot multiplication than those on media without PGR. However, the culture media added with mannitol 40 and 60 g/l have tended to reduce the shoot multiplication of

plantlets in both media with and without PGR (Figure 2).

The plantlets began rooting at the second month of storage on the culture media without PGR and after 4, 6 and 8 months of storage on the PGR added media depended on the mannitol concentrations of 0, 20 and 40 g/l, respectively. The plantlets cultured on the medium with PGR + 60 g/l mannitol did not form root until died. The growth of root was shown in Table 1 by ranking number. The roots could develop better on the medium without PGR than on PGR added media.

The death of plantlets was observed on the



**Figure 2** Effect of mannitol on shoot multiplication of plantlets from nodal segments cultured on MS medium + 30 g/l sucrose + mannitol (0, 20, 40 and 60 g/l) + PGR (0.5 mg/l BA + 0.16 mg/l IAA) or without PGR after cultured for 14 months.

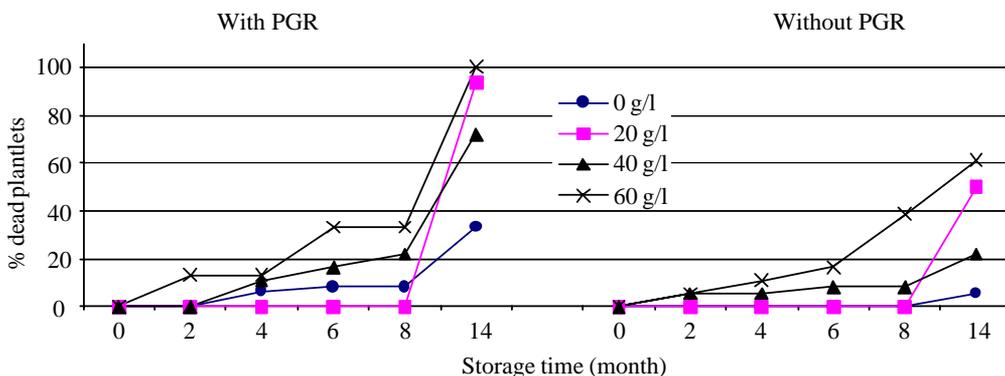
**Table 1** Root development of plantlets cultured on the medium supplemented with PGR (0.5 mg/l BA + 0.16 mg/l IAA) and without PGR after 8 months of storage.

Mannitol concentration	With PGR		Without PGR	
	Rate of root growth <sup>1</sup>	Month <sup>2</sup>	Rate of root growth <sup>1</sup>	Month <sup>2</sup>
0 g/l	1.8	fourth	2.2	second
20 g/l	1.1	sixth	2.7	second
40 g/l	0.4	eighth	1.6	second
60 g/l	0	-	0.5	second

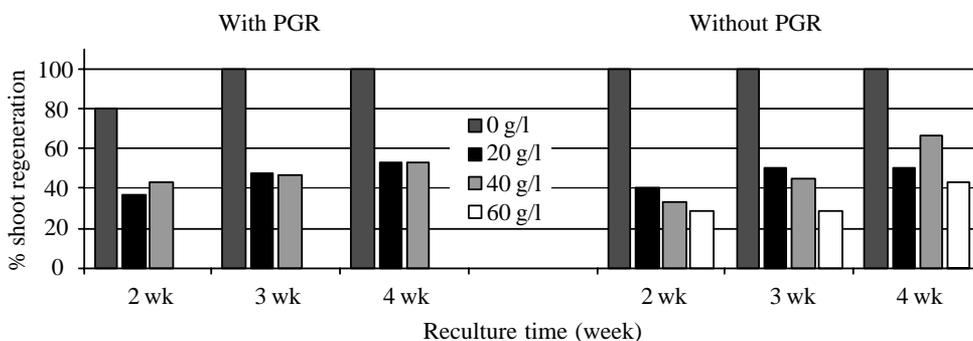
1: Ranking number; 0: no root regeneration, 0.1-1: poor growth, 1.1-2: moderately growth, 2.1-3: well growth  
2: Recording time at which root regeneration was observed.

media containing 40 and 60 g/l mannitol after two months of storage and was highest in percentage when the plantlets were cultured on 60 g/l mannitol added medium after storage for eight months (Figure 3). Most of the plantlets could survive longer on the media contained 0 and 20 g/l mannitol. However, after prolonging the storage time until 14 months, the plantlets cultured on the mannitol added media died 72 to 100 % on the media supplemented with PGR and 22 to 61 % on the media without PGR. The surviving plantlets were subcultured onto fresh MS medium supplemented with 0.5 mg/l BA, 0.16 mg/l IAA, 30 g/l sucrose

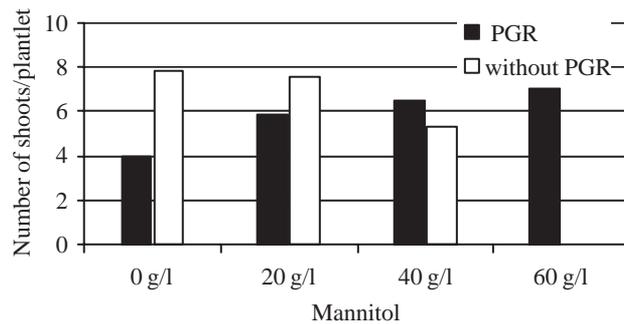
and 6.5 g/l agar at pH 5.6. The plantlets from mannitol added media could regenerate new shoots after subculture but slower than those from non-mannitol added media in both with and without PGR supplementation (Figure 4). The numbers of shoot multiplication are shown in Figure 5. The number of shoots per plantlet was higher when the plantlets were subcultured from PGR added media with higher concentrations of mannitol. In contrast, the plantlets from the media without PGR produced higher number of shoots per plantlet at lower concentrations of mannitol.



**Figure 3** Effect of mannitol on the death of plantlets cultured on MS medium + 30 g/l sucrose + mannitol (0, 20, 40 and 60 g/l) + PGR (0.5 mg/l BA+ 0.16 mg/l IAA) or without PGR after culture for 14 months.



**Figure 4** Regeneration ability of survival plantlets after storage for 14 months and recultured on MS medium + 30 g/l sucrose + 0.5 mg/l BA + 0.16 mg/l IAA for 4 weeks.



**Figure 5** Shoot multiplication ability of survival plantlets after storage for 14 months and recultured on MS medium + 30 g/l sucrose + 0.5 mg/l BA + 0.16 mg/l IAA for 4 weeks.

Minerals dissolved in water are introduced into cells through differences in osmotic pressures of the cells. The inclusion of sugar in medium increases the osmotic potential, thus reducing the uptake of minerals from the medium by cells. As a consequence, plant growth is delayed (Golmirzaie and Toledo, 1999). High concentration of mannitol added to the media produced osmotic effect which resulted in the reduction of growth of rose coloured leadwort plantlets. However, the plantlets on PGR free media rooted in the early of storage time. Consequently, plantlets obtained minerals via roots functioned and survived by photosynthesis. Mannitol can be absorbed into plant cell (Thompson *et al.*, 1986). Too high concentration of mannitol added to the media might be harmful and caused the death of the storage rose coloured leadwort plantlets. Sarkar (1998) reported that 20 or 40 g/l mannitol in combination with sucrose could enhance survival of *in vitro* conservation of potato but not with 60 g/l mannitol. The combination of BA and IAA supplemented to the medium affected the shoot multiplication of plantlets, subsequently the number of shoots per plantlet obtained was more than the increasing of plantlets height. The other effect of PGR was the prolongation of the root initiation that might be related to the rate of deterioration of storage plantlets.

## CONCLUSION

The study reveals that mannitol supplemented culture media could reduce the growth of rose coloured leadwort plantlets especially in stem height. BA and IAA added medium enhanced shoot multiplication, however, with 40 and 60 g/l mannitol have tended to reduce number of shoots per plantlet. Mannitol 20 g/l added to the culture medium could reduce the growth of plantlets and prolong subculture time to 8 months without an appearance of dead plantlet. However, after prolonging the storage time up to 14 months all survived plantlets could grow after subculture onto fresh culture medium.

## ACKNOWLEDGEMENTS

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