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HARDENING AND *EX VITRO* ESTABLISHMENT OF ROSE PLANTLETS

I). Wilson, N. K. Nayar and P. Sivaprasad
College of Agriculture, Vellayani 695 522, Trivandrum, India

Abstract : Plantlets regenerated by *in vitro* techniques under high humidity and low temperature in an artificial medium did not survive by direct planting out in small pots. An improvised structure made of wooden frame and polyethylene cover helped to maintain high humidity to *ex vitro* plantlets. Attempts made to get successful field establishment with different potting media and nutrient solutions did not give any positive results. Successful hardening and ex vitro establishment of plantlets were achieved by surface inoculation of different species of vesicular arbuscular mycorrhizae (VAM). Inoculation with *Glomus etunicatum* (Becker and Gerd) recorded the highest survival rate of 66.67 per cent, and took minimum days for flowering.

Key words : *Ex vitro* establishment, hardening, rose mycorrhizae.

INTRODUCTION

The most difficult part of *in vitro* propagation is the hardening and *ex vitro* establishment of plantlets. The critical part of acclimatization is the formation of fully functional roots in a potting mixture, while ensuring that the delicate root system is protected against desiccation. The main cause of mortality on transplantation is due to desiccation, since the *in vitro* cultures are maintained at high relative humidity around 100 per cent and comparatively low temperature. A period of humidity acclimatization was considered necessary for the newly transferred plantlets to adapt to the outside environment during which the plantlets undergo morphological and physiological adaptations enabling them to develop typical terrestrial plant water control mechanisms (Grout and Aston, 1977; Sutter *el al.,* 1985, Bhat, 1992).

MATERIALS AND METHODS

The *in vitro* rooted plantlets of rose cv. Folklore having 5 to 10 roots (Fig la) were used to study the *ex vitro* establishment. The rooted plantlets were taken out without injury from the culture vessels using forceps and put in a beaker containing distilled water and shaken thoroughly to remove the adhering pieces of semi-solid medium. The plantlets were planted out in small plastic containers of 7.5 x 6.0 cm containing different sterilized potting media viz., sand, soilrite, vermiculite, sand : soilrite (1:1), sand : vermiculate (1:1), sand : peatmoss (1:1). In order to identify the optimum period of root induction to get successful field establishment the planting out was done in a phased manner, two weeks, three weeks and four weeks after root induction in the root induction medium. An improvised structure made of a wooden frame covered with a polyethylene cover was used to maintain high relative humidity. Cold water (12±2 °C) was sprayed at an interval of 3 h during the day time using a hand sprayer with fine mist nozzle. The nutrient solutions of liquid MS one tenth, half and full strength at the rate of 5 ml per plant were supplied at three days interval to study the response.

The effect of VAM colonization on the growth and survival of plantlets was studied using three species of VAM viz., *Glomus etunicatum*, (Becker and Gerd.), *G. fasciculatum* (Thaxter Sensu Gerd) and *G. constrictum* (Trappe). The plantlets were inoculated with surface sterilized spores of the above fungi. The suspensions containing 50 VAM spores were mixed with top 2 cm layer of sand into which the plantlets were planted. Growth and survival of plantlets were recorded at weekly intervals.

RESULTS AND DISCUSSION

The plantlet survival under different potting mixtures and different period of root induction (Table 1) showed a gradual decline from first

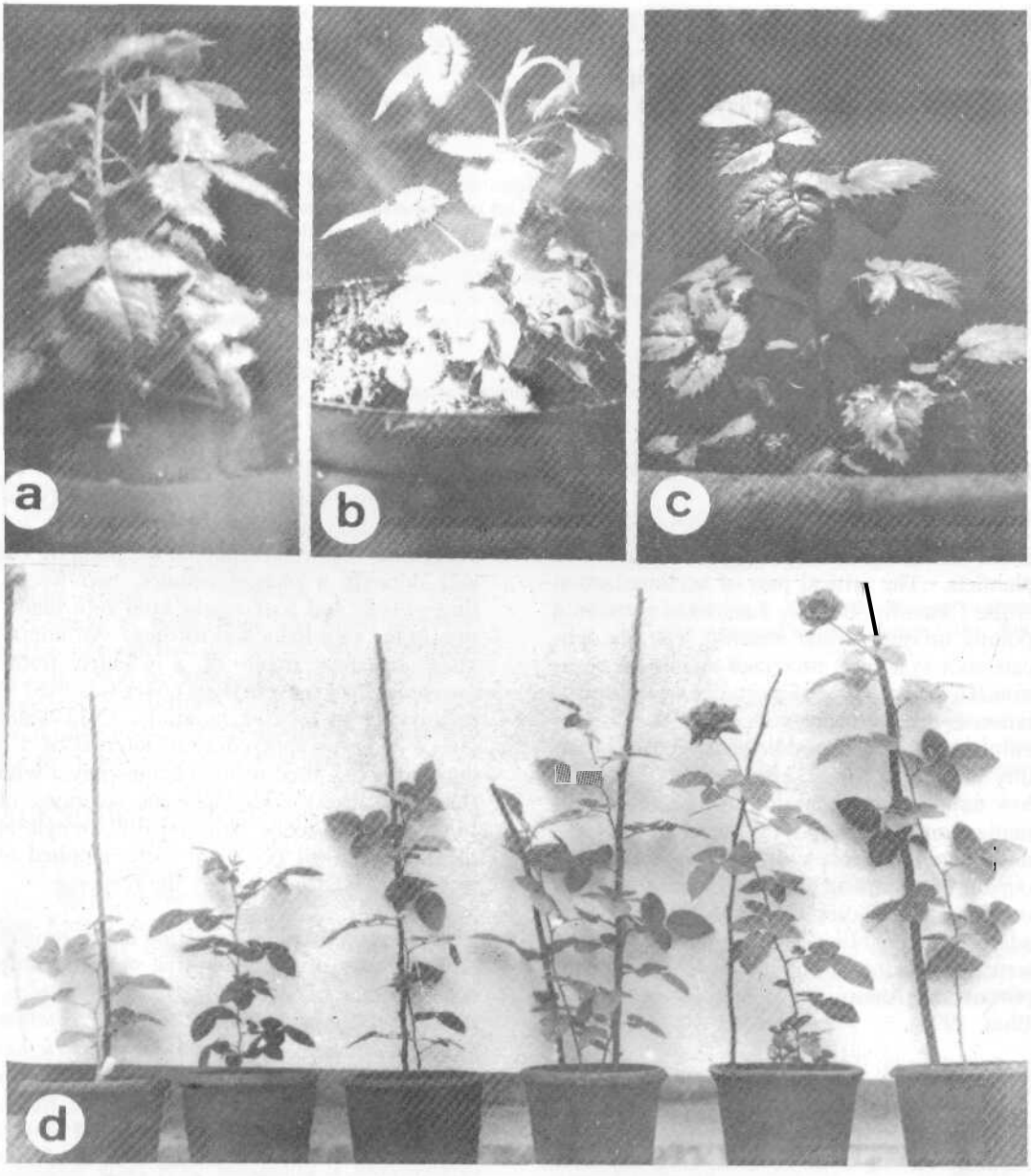

Fig 1. Different stages of *ex vitro* establishment of rose plantlets inoculated with VAM
(a) Plantlets ready for planting out (b) Plantlets 4 weeks after planting out
(c) Plantlets 6 weeks after planting out (d) Plants 16 weeks after planting out in the blooming stage

to third week of planting out. However, when plantlets planted out in sand and soilrite three weeks after root induction, recorded a reasonably high percentage (62.5%) of survival after three weeks. But there was no survival after four weeks.

essential for successful field establishment and survival of *in vitro* derived rose plantlets.

Table 2. Effect of supply of nutrient solution on growth and survival of rose plantlets (cv. Folklore)

Treatment	Quantity ml	Frequency days	Days to emergence of		Plant height, cm		Mean increase in plant height, cm	Survival after three weeks, %
			First leaf	Second leaf	At time of planting out	Three weeks after planting out		
Water	5	3	5.8	11.8	2.96	3.60	0.64	25.00
MS one tenth strength	5	3	5.6	10.4	2.92	3.84	0.92	37.50
MS half strength	5	3	5.8	11.5	3.02	3.90	0.88	12.50
MS full strength	5	3	6.0	12.0	3.00	3.80	0.80	12.50

No. of plants per treatment - 8

Table 3. Effect of mycorrhizae on *ex vitro* establishment of rose (cv. Folklore)

Treatments	Days to emergence		Plant height, cm				
	First leaf	Second leaf	At time of planting out	3 weeks after planting out	Increase in plant height	6 weeks after planting out	Increase in plant height
Control	5.4	10.8	3.9	4.5	0.60	0.0	0.0
G. entunicatum	5.8	11.0	3.5	4.2	0.70	7.5	3.3
G. fasciculatum	4.8	10.6	3.9	4.4	0.50	7.1	2.7
G. constrictum	5.2	10.3	3.8	4.6	0.80	7.2	2.6

No. of plants per treatment = 15

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Table 1. Effect of rooting period and potting mixture on survival of rose plantlets (cv. Folklore)

Potting mixture	Rooting period, week	Survival percentage		
		one week	two week	three week
Sand	Two	87.50	62.50	25.00
	Three	100.00	87.50	62.50
	Four	100.00	50.00	50.00
Soilrite	Two	87.50	37.50	00.00
	Three	100.00	87.50	62.50
	Four	87.50	37.50	37.50
Vermiculite	Two	50.00	25.00	00.00
	Three	75.00	25.00	00.00
	Four	75.00	50.00	12.50
Sand : soilrite (1:1)	Two	100.00	75.00	25.00
	Three	87.50	50.00	25.00
	Four	100.00	75.00	37.50
Sand : peatmoss (1:1)	Two	3750	12.50	00.00
	Three	50.00	50.00	12.50
	Four	25.00	25.00	00.00

No. of plants per treatment = 8

Addition of inorganic salts at different concentrations (Table 2) also did not improve the plantlet survival after three weeks. Although some growth response by way of leaf emergence and slight increase in height was noticed in all the treatments, complete mortality was recorded on fourth week.

There are certain aberrant morphological features reported in the case of *in vitro* raised plantlets. They are leaves with poor or no development of cuticular wax on leaf surface, poor development of palisade and pronounced mesophyll air spaces (Grout and Aston, 1978; Leshem, 1983; Donnelly and Vidaver, 1984). Improved stomatal mechanisms with non-closure of stomata (Brainerd and Fuchigami, 1982) and poor vascular connection between root and shoot due to intervening callus and lack of root hairs are also the problems associated with the *ex vitro* establishment. The failure of the plantlets to establish under ex vitro condition in the present study may be due to these aberrant morphological and physiological characters imparted to the *in vitro* plantlets.

The results of VAM inoculation on growth and survival of plantlets (Fig 1) are presented in Table 3. The plantlets with *Glomus etunicatum* (Becker and Gerd) recorded the highest survival of 66.67 per cent even after six weeks, whereas in un-inoculated treatments, complete mortality was noticed by fourth week. There was no reduction in survival after sixth week. The days taken for flowering ranged from 105 to 130 (Fig 1d). The plantlets inoculated with *G. etunicatum* (Becker and Gerd) took the minimum days (105) for flowering followed by *G. fasciculatum* (Thaxter Sensu Gerd) and *G. constrictum* (Trappe). The mycorrhizal association might have increased the nutrient uptake and their effective utilization and increased stress tolerance might have contributed to the successful establishment and survival of plantlets. Higher photosynthetic efficiency (Sivaprasad and Rai, 1984) and phytochrome production (Allen *et al.*, 1980) have been suggested as the beneficial effects of mycorrhizae in plants. Additionally mycorrhizal inoculation might have helped plantlets to resist environmental stress at transplanting from axenic conditions to normal condition (Barea *et al.*, 1992). The favourable effect of mycorrhizae on *ex vitro* establishment had been reported by many workers. Improved transplant success had been reported using *Glomus* species in *Rubus ideus* and *Paxillus involutus* (Pierik, 1987) jack plantlets (Ramesh, 1990) avacado (Vidal *et al.*, 1992) and strawberry (Chavez and Cerrato, 1990). The present investigation also clearly showed that the mycorrhizal inoculation is

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