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Micrografting: A technique to shorten the hardening time of micropropagated shoots of tea *(Camellia sinensis (L) O. Kuntze)*

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ABSTRACT

A micrografting technique was developed for tea where *in vitro* raised shoots were grafted on 6 months old tea seedling. The survival rate was found to be as high as 98%. Moreover, the growth of grafted plants was much higher than ungrafted *in vitro* raised plants of the same cultivar, so that the grafted plants were ready to transfer to the field within 6 months. Using this technique, 3000 micrografted plants were transferred to the field. Experiments are underway to evaluate the field performance of micro grafted plants.

Key words: micrografting, tea, India, hardening

INTRODUCTION

Although, conventional tea breeding is well established and contributed much for tea improvement over the past several decades, this method has some limitations owing to following reasons: (1) perennial nature, (2) long generation cycle, (3) high inbreeding depression, (4) self-incompatibility, (5) unavailability of distinct mutants of different biotic and abiotic stress, (6) lack of proper selection criteria, (7) low success rate of hand pollination, (8) long duration for seed maturation (12-18 month) and (10) clonal difference of flowering time and fruit bearing capability of some clones. Though vegetative propagation is an effective method of tea propagation, yet it is limited by several factors such as: (i) requirement of high plant propagule as maximum of 200-250 cuttings/plant/annum can be produced (personal communication with Mr. C.K. Mohan, R and D Department, Tata Tea Ltd, Munnar, India) (ii) unavailability of suitable planting materials due to winter dormancy in certain tea growing areas such as North-East India and drought in most of the tea growing regions of India, (iii) poor survival rate in the nursery due to poor root formation of some clones and (iv) seasonal dependent rooting ability of the cuttings.

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Tissue culture is therefore certainly be a better alternative for conventional propagation and reduction in breeding time. However, once the *in vitro* plants are produced, the success depends on hardening and subsequent establishment of hardened plants in the field, specially for woody plants. In tea, although a number of reports are available on tissue culture, only little information is available about time taken and subsequent survival percentage during hardening (Mondal, 2003a and Mondal *et al.*, 2003). Specifically in tea, *ex vitro* rooting of *in vitro* raised tea plants depends upon several factors such as genotypes, pH and composition of the soil and potting mixture, season which ultimately results the survival percentage (Mondal, 2003b). To overcome these limitations, various approaches namely CO_2 enrichment, specially designed hardening chamber (Sharma *et al.*, 1999), micrografting (Prakash *et al.*, 1999) and biological hardening (Pandey *et al.*, 2000) have been developed. Micrografting is an old technique and has been successfully reported in a range of horticultural plants such as citrus, cherry, kiwifruit, pistachio, stone fruits, apple, grapes, forest trees (*Larix decidua* and *Picea* spp (Druart, 2003; Hang and Millikaan, 1988; Jonard, 1986; Negueroles and Jones 1979; Onay, 2003; Troncosco *et al.*, 1999). But all these studies aimed either to establish the micropropagated plant by overcoming the *ex vitro* rooting problem or to produce the virus-free plants. However, no attention has been paid for reducing the hardening time, which generally account for 12-18 months for slow growing woody plants such as tea before transferring to the field (data not shown). Therefore, as an alternative approach, grafting to reduce the hardening time of micropropagated tea shoots, was investigated.

MATERIALS AND METHODS

In this study, micropropagated shoots were grafted on seedlings as per the protocol described by Prakash *et al.* (1999). The nodal segments with axillary buds from field grown plants of cultivar RD/1/101, TTL-4, TTL-5, N/17/17 were micropropagated as described by Rajshekaran and Mohankumar (1992). The basal end of 4-5 cm micropropagated shoots of 3-4 month old were used as scion and given a pointed cut before grafting on 6-month old tea seedling which was used as the roots stock. The graft union was tied with polythene tape and kept under a poly-tunnel inside the greenhouse. For control, micropropagated shoots (without roots) of the same cultivar, size (4-5 cm) and age (3-4 month) were transferred to greenhouse initially for 3 months to induce *ex vitro* rooting and subsequently transferred to polythene sleeve (22 cm length and 12.5 cm wide and 150 gauge) filled with virgin soil and kept in a nursery (Mondal et al., 1998).

RESULTS AND DISCUSSION

Prakash *et al.* (1999) reported the enhancement of graft union and better scion development with plant growth regulator (PGR) treatment during grafting of micropropagated tea shoots. The study was mainly confined to evaluate the effect of PGR on graft union, assessment of compatibility of root stock, effect of age of rootstock and season on graft union. They also observed a higher rate of survival in auto-graft than

in hetero-graft. However, our results differ in two important aspects from the earlier report of Prakash *et al.* (1999). Firstly, we achieved a higher rate of survival (92-98 %) with out any PGR treatment (Table 1). Therefore it can be concluded that the use of PGR can be avoided for micro-grafting. This was an absolute necessity for large scale use of the technique to eliminate the cost of PGR. Secondly, we found that the growth of grafted plants was much higher than ungrafted *in vitro* raised plants of same cultivar (Table1).

Table 1: Description of different graft combination and their performance*

Graft combination		Survival (%)	Height (cm)
Scion	Stock		
TTL-4	B/6/61 X C 6017	91.5	48.6
TTL-4	B/6/61 X TRI 2025	92.0	44.0
TTL-4	Micropropagated Plant (Control)	82.0	25.2
TTL-5	B/4/142 X TRI 2026	95.0	47.3
TTL-5	Micropropagated Plant (Control)	87.5	35.5
N/17/17	B/4/142 X TRI 2026	92.0	66.1
N/17/17	Micropropagated Plant (Control)	85.0	17.0
RD/1/101	UPASI-9 X C6017	94.8	45.2
RD/1/101	Micropropagated Plant (Control)	83.0	36.0

Data (Mean $\pm$ S.E) pooled from three independent experiments each with three replications.
*Data collected from 12 months old plants.

The reason for better growth of grafted plants may be due to the higher root volume (Fig. 1 A) which perhaps help former to absorb more water and nutrients from the soil. It also may be partly due to the pre-existing tap root system of the seedling, which was used as the root stock. Practically, this is of great importance to reduce the hardening period of tissue culture plants. Micropropagated tea plants of the same cultivar which required 12-18 months (data not shown) in the hardening phase, but the same cultivar when micrografted took 6-8 months, and it virtually reduced the hardening period by 6-10 months. Therefore it plays an important role in tea breeding because the work is slow due to perennial nature of the tea plant. As grafting is done on seedlings the grafted plants will perform better during drought period due to the presence of a tap root system. Drought is a major abiotic stress problem in most of the tea growing areas in India. Further, using this technique, two different combinations such as quality and yield or

diseases tolerance and yield can be combined in the composite plant. However, using this technique 3000 micrografted plants were produced and experiments are underway to evaluate the field performance of the micrografted plants (Fig 1. B).

Fig. 1. A. Root biomass development in Tea plants of same age (left: grafted and right: non-grafted plants. Arrow indicates the graft union)

Fig 1. B. Photograph of 2 years old field grown micrografted tea plants

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