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In vitro grafting in apple (*Malus domestica.* Borkh) cv. Lal Ambri*

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Abstract: This study was carried out to assess the potential use and applicability of micrografting technique for developing in vitro grafted plantlets. Microshoots of apple cv. Lal Ambri was used as scion and rooted microshoots of Malling-9 as rootstock. Different grafting methods and media formulations were investigated. Main effect of grafting methods and media formulation was found to be significant. Maximum grafting success of 29.01 % was achieved with vertical slit method, which decreased to 21.07 % with horizontal method of grafting. MS semi-solid medium with 3 % sucrose proved best with 29.47 % successful grafts, which decreased to 21.55 % when micrografts were cultured in MS liquid medium plus vermiculite with 6 % sucrose. Interaction effects were significant. Overall, best response of successful micrografts were obtained with a combination of vertical slit method of grafting and culturing micrografts in MS semi solid medium with 3 % sucrose, which showed highest graft success (35.76 %).

Key words: Apple, *In vitro* culture, Micrografting

Introduction

Micrografting is an *in vitro* grafting technique, which involves the placement of a meristem or shoots tip explants onto a decapitated rootstock that has been grown aseptically from seed or micropropagated (Hartmann *et al.,* 2002). Compared to traditional grafting, micrografting has several advantages such as year round plant production, elimination of viruses, conduct compatibility, rejuvenation and correlative relation between rootstocks and scion studies (Hoa *et al.,* 2004; Ribeiro *et al.,* 2008) or use in quarantine as this method has a minimum risk for importing plants (George, 1993). This technique can provide an efficient production of high quality true-to-type plants in a short time period and under controlled and aseptic conditions and enables to produce large quantity of plant material in reduced physical space (Lambardi *et al.,* 1997).

Apple is the principal fruit crop of Jammu and Kashmir region occupying an area of about 1.41 lakh ha out of 3.25 lakh ha under fruit crops, with production of 18.52 lakh metric t out of 22.21 lakh metric t (Anon., 2010). Among apple cultivars grown in Kashmir, Lal Ambri cultivar has gained a prime importance due to its exceptionally high yield potential, excellent fruit and storage quality (Bhat *et al.,* 2005).The work on micrografting of apple has been conducted successfully by various workers in western countries (Abreu *et al.,* 2003; Dobranszki *et al.* 2000; 2005; and Nunes *et al.*, 2005), but no such studies have been carried out in our state for accelerating production of quality planting material. Keeping in view popularity and increased demand of Lal Ambri, present studies were conducted to standardize a micrografting technique in apple using Lal Ambri as scion and Malling-9 as rootstock.

Material and methods

This experiment was conducted in Biotechnology Laboratory, Division of Pomology, Sher-e-Kashmir University of Agricultural Sciences and Technology of Kashmir. Microshoots of M-9 rootstocks were regenerated *in vitro* as per method described earlier by Dalal *et al.* (2006). Proliferated M-9 shoots were cultured in root induction medium (MS + 3.0 mg/l IBA) for one week and then transferred to root development medium (MS hormone free medium) for another one week. Rooted plantlets were taken out and used as root stock for grafting.

Similar protocol as used for M-9 rootstock was used for Lal Ambri scion, but with some modifications. Initially maximum survival of Lal ambri explants was achieved in MS medium fortified with antioxidant combination of ascorbic acid and citric acid at 100 + 150 mg/l, respectively. Highest culture establishment was achieved in MS medium with BAP and IBA at 0.50 and 0.05 mg/l, respectively. Similarly maximum proliferated shoots were induced in MS medium with BAP and IBA each at 0.50 mg/l. Proliferated microshoots of 10 mm length were used for grafting. Before grafting lower end of scions were dipped for one minute in antioxidant solution citric acid 0.5 %.

Three methods of grafting were used:- G_1 = Vertical slit method: The root stock was decapitated to remove all leaves and a vertical slit was made on the stump and the scion base cut into 'V' shape was fitted into slit. G_2= Wedge method: The rootstock was decapitated to remove all leaves. A wedge was cut in the stump and the scion base cut in 'V' shape was gently fitted in to the wedge. G_3= Horizontal method: The rootstock was decapitated to remove all leaves. A scion was cut horizontally and placed gently onto the rootstock after cutting off its top (decapitation).

Six media formulations were tried for the establishment and growth of micrografts. The grafts were individually placed into test tubes with different media formulations as under : - M_1= MS semi-solid + 3 % sucrose; M_2 = MS liquid + 3 % sucrose; M_3=MS liquid + vermiculite + 3 % sucrose; M_4=MS semi-solid + 6% sucrose ; M_5=MS liquid + 6% sucrose; M_6=MS liquid + vermiculite + 6% sucrose. For MS liquid medium a supportive platform was made with Whatman No. 50 filter paper and placed in test tube containing the liquid MS medium. The micrografts were placed individually into test tubes and cultured in these media for 6 weeks at 23±1°C, 16/8 h photoperiod at 3000 lux. Each treatment combination consisted of 10 micrografts

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Stages in micrografting

a) i) M-9 rootstock ii) Lal Ambri scion

b) Stock and Scion grafted by slit method of grafting

c) Micrografted Lal Ambri plantlet developed in MS Semi-solid media

replicated three times. Observations regarding contamination (%), vitrification (%), necrosis incidence (%), graft success (%) was noted after 6 weeks of grafting. The data was recorded and subjected to the analysis of variance in a CRD factorial arrangement (Steel and Torrie, 1984).

Results and discussion

Main effect of grafting methods on contamination and vitrification were non-significant (Fig. 1), whereas main effect of different media formulations on both these parameters were significant (Fig. 2). MS liquid medium and MS liquid medium plus vermiculite showed higher contamination and vitrification at both levels of sugars tested. However, highest level of sucrose showed highest contamination (10.14%) and vitrification (9.22%).

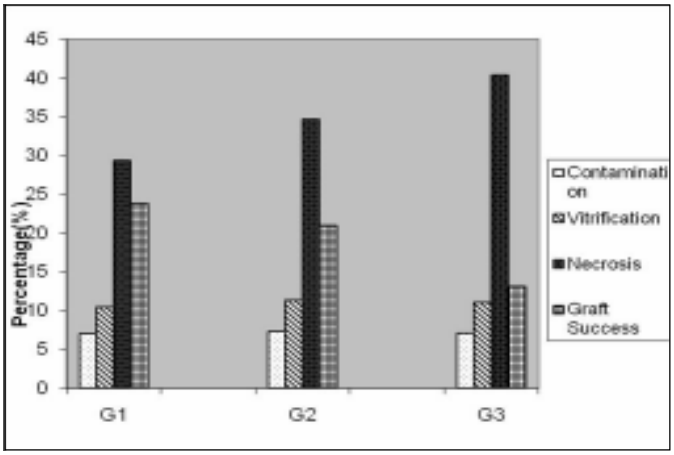

Fig. 1. Effect of different grafting methods on contamination, vitrification, necrosis and graft success of apple cv. Lal ambri grafted on M-9 rootstock

G_1- Vertical Slit ; G_2- Wedge method ;

G_3- Horizontal Method

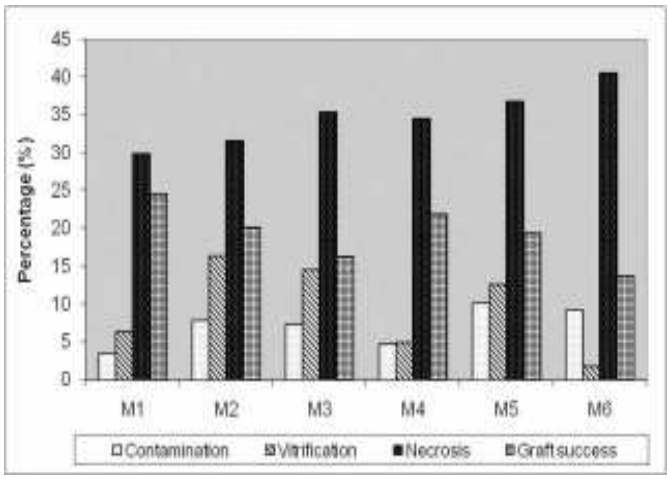

Fig. 2. Effect of different media formulations on contamination, vitrification, necrosis and graft success of apple cv. Lal Ambri on M-9 rootstock.

M_1 = MS semi-solid + 3% sucrose; M_2 = MS liquid + 3% sucrose; M_3 = MS liquid + vermiculite + 3% sucrose; M_4 = MS semi-solid + 6% sucrose; M_5 = MS liquid + 6% sucrose; M_6 = MS liquid + vermiculite + 6% sucrose

MS semi solid media at both levels of sugars showed lowest contamination (3.50 and 4.72% at 3 and 6%, respectively).

Regarding interaction effect (Table 1), the treatment combination of MS semi-solid media with 3 per cent sucrose and vertical slit grafting showed the least contamination (3.33%). Treatment combination of liquid and vermiculite media at 6 per cent sucrose and grafting techniques were non-significant. However, highest contamination (11.00 %) was obtained in treatment combinations of Liquid media with 6 per cent sucrose under wedge grafing. Similar results were reported by

Table 1. Effect of different grafting methods and media formulations on contamination, vitrification, necrosis and graft success of apple cv. Lal Ambri grafted on M-9 rootstock.

Graft method	Media	Contamination (%)	Vitrification (%)	Necrosis (%)	Graft success (%)
G1	M1	3.33	4.89	23.16	34.16
G2	M1	3.58	7.16	29.20	22.00
G3	M1	3.58	6.83	37.15	17.48
G1	M2	8.16	15.50	25.16	22.33
G2	M2	7.83	17.50	32.39	25.00
G3	M2	7.60	15.96	37.28	13.00
G1	M3	6.83	15.41	31.04	19.52
G2	M3	8.00	14.66	35.04	17.21
G3	M3	7.16	13.66	40.15	12.15
G1	M4	4.66	4.50	30.05	28.33
G2	M4	4.50	4.75	34.53	22.66
G3	M4	5.00	5.58	39.06	14.69
G1	M5	9.43	12.25	33.87	23.20
G2	M5	11.00	12.00	34.18	24.32
G3	M5	10.00	13.51	42.16	10.68
G1	M6	9.66	10.08	32.70	15.47
G2	M6	9.00	11.50	42.66	14.81
G3	M6	9.00	10.66	46.38	10.64
CD = p≤ 0.05		2.42	4.30	4.07	4.70

Dziedzic *et al.* (2004), who obtained similar results in cherry micrografts placed on solid medium than on liquid medium because of contamination in liquid medium.

Liquid media with 3 per cent sucrose had 16.32 per cent vitrified plantlets followed by liquid media with vermiculite (14.58%) having similar sucrose level. Semi-solid medium showed less vitrification. However, this media with 6 per cent sucrose had the least (4.94) vitrified shoots. Regarding interaction effect, highest vitrified plantlets (17.50 %) were observed when wedge grafting method was adopted and micrografts cultured in liquid media at 3 per cent sucrose (Table 1). Vitrification in liquid media decreased significantly to 12.00 per cent when sugar concentration increased to 6 per cent. However, lowest vitrified plantlets (4.50 %) were obtained when slit grafting method was adopted and grafts cultured in MS semi-solid media having 6 per cent sucrose. In the present investigation the problem of contamination and vitrification was primarily due to usage of liquid and vermiculite media coupled with matrix potential of the culture medium. The frequency of vitrification increased on medium containing a low sucrose concentration, as sucrose decreases the water potential. Similar findings were also observed by (Ghorbel *et al.*,1998 and Amiri, 2006) in different fruit crops. Dziedzic *et al* (2004) also obtained better results of cherry micrografts placed on solid than on liquid medium because of graft unit vitrification in liquid medium. Deberg *et al.* (1983) reported that vitrification was related to nutrient medium composition. It was especially common when plantlets had too much water.

Main effect of grafting methods and media formulations on explants necrosis was significant (Fig 1 and 2). Highest necrosis of 40.36 per cent was observed under horizontal method of grafting which decreased to 29.33 per cent when vertical slit method of grafting was adopted. Regarding media formulations highest necrosis incidence (40.58%) was observed in MS liquid media plus vermiculite with 6 per cent sucrose. Lowest necrosis (29.83%) was observed in MS semi-solid medium with 3 per cent sucrose. Interaction effect regarding grafting methods and media formulation (Table 1) reveal, that lowest necrosis was obtained when vertical slit method of grafting was used and micrografts were cultured in MS semi-solid media at 3 per cent sucrose whereas, highest necrosis (46.38 %) was achieved when horizantol method of grafting was utilized and micrografts were cultured in MS liquid media plus vermiculite. The drying of shoot tips and low graft integration may be due to small size of shoot tip origin, making problematic the excision, handling, grafting and subsequent maintenance of grafted assembly. Similar results were reported by Ghorbel *et al.*(1998) who reported that in *in vitro* grafting of almond, initial percentage of successful grafts was about 60 per cent, but this decreased to 15 to 20 % at the end of experiment as necrosis hampered the success of grafts. Moreover, in addition to poor development of grafts, larger cicatricial calluses grew at the base of scion leading to its displacement from graft union and contributed to graft failure.

The method of placement of the excised shoot tip onto the rootstock significantly influenced the grafting success, which ranged from 13.10 per cent to 23.83 %. (Fig. 1). Best results were obtained from vertical slit method of grafting showing 23.83 % success, whereas lowest success was achieved under horizontal method showing 13.10% success. Regarding media formulations, highest graft success percentage (24.55 %) was achieved in MS semi-solid medium with 3 per cent sucrose. Lowest graft success (13.64%) was obtained in MS liquid medium plus vermiculite. Regarding interaction effect, highest graft success (34.16%) was obtained when vertical slit method was used and micrografts were cultured in MS semi-solid media having 3 per cent sucrose. Grafting success decreased significantly to 10.64 per cent when horizontal method of grafting was used and micrografts cultured in MS liquid media plus vermiculite. Onay *et al.* (2004) also obtained maximum success under slit method of grafting in Pistachio as this method was easiest and no problem was observed during the union formation as long as the contact surfaces were perfectly smooth.

The results of this study revealed that maximum success was achieved by combination of vertical slit method of grafting and culturing these micrografts in MS semi solid media having 3 per cent sucrose. This technique offers several advantages and greater opportunities of rapid mass propagation for healthy plant material.

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